Synthesis and Evaluation of Norcantharidin and Acrylonitrile Derivatives as Potential Anti-Cancer Agents

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B.Sc. (Hons)

Thesis Presented for the Degree of
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THE UNIVERSITY OF NEWCASTLE AUSTRALIA

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I hereby certify that this thesis is submitted in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each senior co-author; and endorsed by the Faculty Assistant Dean of Research Training, attesting to my contribution to the joint publications.

Mark Tarleton

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List of publications included as part of the thesis

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Contents

Chapter 5 – Appendix

List of Abbreviations

Adenosine Diphosphate	**ADP**
Asialoglycoprotein receptor	**ASGP-R**
Adenosine Triphosphate	**ATP**
Cdk-activating kinase	**CAK**
Cyclin-dependant kinases	**Cdks**
Deoxyribonucleic Acid	**DNA**
Estrogen Receptor	**ER**
Growth Inhibition 50	**GI_{50}**
Dissociation constant for an enzyme inhibitor complex	**K*i***
Lethal Dose 50	**LD_{50}**
National Cancer Institute	**NCI**
Protein 16	**p16**
Protein 21	**p21**
Protein 27	**p27**
Protein 53	**p53**
Protein Phosphatases	**PPs**
Protein Phosphatase 1	**PP1**
Protein Phosphatase 2A	**PP2A**
Protein Phosphatase 2B	**PP2B**
Protein Phosphatase 2C	**PP2C**
Protein Phosphatase 4	**PP4**
Protein Phosphatase 6	**PP6**
Protein Phosphatase 7	**PP7**
Retinoblastoma protein	**Rb**
Structure Activity Relationships	**SAR**
Tumour Suppression Genes	**TSG**

Breast carcinoma	**MCF-7 (ER +ve), MDA-MB231 (ER –ve)**
Colon carcinoma	**HCT116, HT29, WiDr, SW480, HCT-8**
Glioblastoma	**SJ-G2**
Haematopoietic carcinoma	**L1210, HL60**
Hepatocellular carcinoma	**Hep-3B, Hep-1**
Kidney carcinoma	**G401**
Leukaemia	**K-562, KG1a**
Liver carcinoma	**Be17402, SMMC-7721**
Lung carcinoma	**H460, A549**
Neck and head carcinoma	**KB**
Neuroblastoma	**BE2-C**
Oesophageal carcinoma	**ECA109**
Osteosarcoma	**143B**
Ovarian carcinoma	**A2780, ADDP, HO-8910**
Pancreatic carcinoma	**Panc-1**
Prostate carcinoma	**DU145**
Skin carcinoma	**A431**
Stomach carcinoma	**SGC-7901**

Abstract

Treating cancer by targeting protein phosphatases, namely protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A) is a novel 'fighting fire with fire' strategy. There are numerous small molecule inhibitors known to achieve this. Most important to this study, cantharidin, and its demethylated analogue, norcantharidin, offer the simplest structure for subsequent modification. The added benefit of effective membrane permeability, makes these compounds ideal candidates for further development. In contrast to most other anticancer drugs, these compounds stimulate the production of white blood cells by bone marrow, while other anticancer drugs that have the unwanted side effect of inducing myelosuppression.

Cantharidin displays kidney toxicity which has prevented its use in mainstream oncology. However, norcantharidin is void of kidney toxicity allowing its development for the treatment of cancer. These biologically active compounds have been shown to have multiple uses such as the treatment of warts. Norcantharidin analogues have also been shown to display anti-parasitic activity against nematode *Haemonchus contortus*, the barbers pole worm, an intestinal parasite that affects livestock industries.

Preliminary analysis of a norcantharidin derivative with a single reduced carbonyl group displayed selectivity towards HT29 (colon; GI_{50} = 14μM) and SJ-G2 (glioblastoma; GI_{50} = 15μM) when tested against the NCI 60 cell line panel. Intrigued by this finding, multiple small focused libraries were synthesised and assessed in order to compile structure activity relationships (SAR). Interestingly an analogue with an isopropyl ether showed promise with strong selectivity towards HT29 (colon; GI_{50} = 19μM) and SJ-G2 (glioblastoma; GI_{50} = 21μM) cell lines but completely void of activity (>100μM) against all seven remaining carcinoma cell lines tested.

Norcantharidin analogues were also tested for anti-parasitic activity against *Haemonchus contortus*, the barbers pole worm, with multiple analogues displayed activity against *Haemonchus contortus* with associated LD_{50s} between 25-40 μM. The observed hit-rate of 5.6% associated with this screening of norcantharidin analogues is far higher than conventionally used drug screening methods usually employed. As part of a toxicity pre-filter, all new anti-parasitic compounds are screened against a panel of ten cancer cell lines to ensure the end user was not subjected to toxic compounds being applied in a non-ideal environment such as farming communities. Surprisingly, analogues from a small acrylonitrile library, originally used as an internal standard, displayed high levels of cytotoxicity.

Subsequent focused library development based on the acrylonitrile scaffold produced multiple broad spectrum cytotoxic compounds with average GI_{50} values of 1.1-2.1 μM across the nine carcinoma cell lines examined. Interestingly, some acrylonitrile compounds showed a high degree of specificity towards MCF-7 (breast carcinoma) cells of up to 543 fold over the other carcinoma cell lines tested. Some of these compounds were further shown to selectively target estrogen dependent MCF-7 cells that over express the estrogen receptor (ER+ve) over estrogen receptor negative carcinoma (MDA-MB231) and non-malignant breast tissue (MCF10A) up to 268 and 126 fold respectively.

With the high throughput of synthesised analogues, flow chemistry methodologies were developed in order to alleviate some of the associated issues with synthetic medicinal chemistry such as reproducibility between batches and difficulty in scale up for live animal studies. Along with effectively producing specific acrylonitrile derivatives in high purity and yield, these procedures were further developed in other projects leading to the discovery of the most potent protein phosphatase inhibitors developed within the research group.

Chapter 1 – Literature Introduction

1.1 Introduction

Protein phosphorylation and dephosphorylation is a fundamental process regulating virtually all cellular signalling pathways. The control of phosphorylation levels is a delicate balance between phosphatases and kinases (Figure 1.1). Kinases transfer a phosphate from ATP to a protein, typically at a serine, threonine or tyrosine residue. Phosphatases remove the phosphate group, i.e. dephosphorylate the protein. At present there are around 518 known kinases (about 2% of the human genome) in contrast to the relatively few known protein phosphatases. However, the regulation of protein phosphorylation requires the coordinated control of both kinases and phosphatases.

The phosphorylation-dephosphorylation cycle can be regarded as a molecular "on-off" switch, but is more accurately thought of as a regulator capable of subtly modulating protein activity. The end result of these associated pathways is the regulation of many different cellular processes, as diverse as glycogen metabolism, calcium transport, muscle contraction, gene expression, protein synthesis, intracellular transport, phototransduction, cell cycle progression and apoptosis.[1-3]

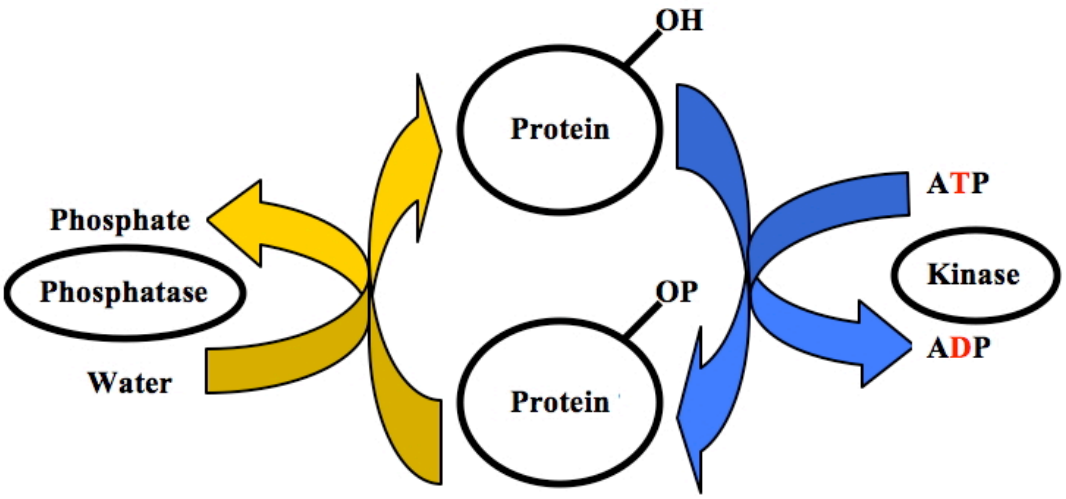


Figure 1.1: Schematic representation of phosphorylation/dephosphorylation process. ATP = adenosine triphosphate; ADP = adenosine diphosphate; P = phosphate.

1.2 The Cell Cycle

In healthy cells the cell cycle is a highly regulated process. It allows the reproduction of cells by duplication of their contents, followed by division into two identical daughter cells. In eukaryotic cells, division occupies only a small part of the cell cycle, during the rest of the time, cells are in interphase.[4] It is during interphase that DNA replication and other processes fundamental to cell cycle progression, including cell differentiation, gene expression and protein synthesis occur.[5-7] For a cell to proceed successfully through the cell cycle it is important that each stage of the cell cycle be completed prior to entry into the next. This is regulated through a series of checkpoints.[5,8,9]

The cell cycle is divided into four distinct phases (Figure 1.2); the first gap phase (G_1) followed by DNA replication (S), a second gap phase (G_2), and finally mitosis (M), which involves cell division into two identical daughter cells. When a cell enters G_1, normal cellular activity returns, including gene expression and protein synthesis.[9,10] Many of the new cells formed differentiate and do not undergo further division. For cells continuing through the cycle, DNA replication occurs in the S phase, during which DNA content doubles and chromosomes are replicated.[4,9,10] These cells then enter the G_2 phase before progressing to mitosis. During M phase, the replicated chromosomes condense and the sister chromatids separate.[4.9.10] It is here that division occurs and mitosis is complete when two daughter cells have formed.

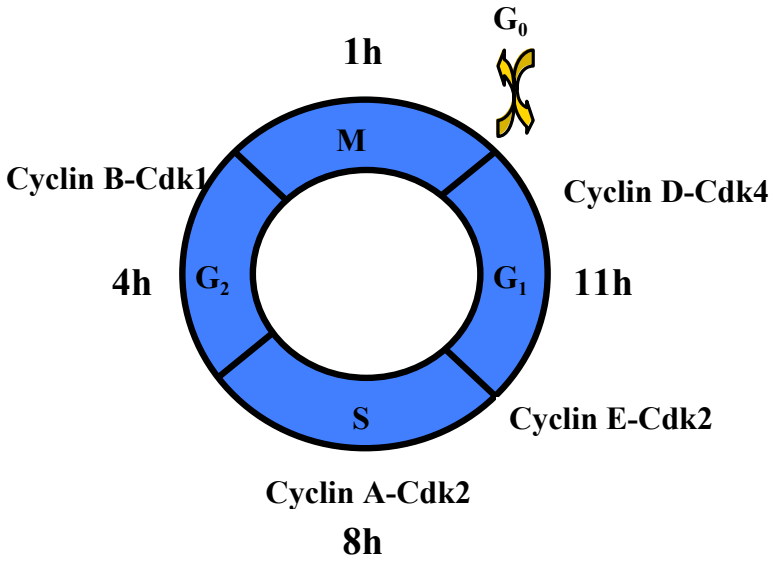


Figure 1.2: A schematic representation of *the Cell Cycle* highlighting the key enzymes (the cyclins and cyclin dependent kinases) that are associated with progression through each phase. The cell cycle is divided into four distinct phases, firstly the gap phase (G_1) followed by DNA replication (S), a second gap phase (G_2), and finally mitosis (M), which involves cell division into two identical daughter cells. Average time spent in each phase based on average cell 24 hour cycle.

1.2.1 Cell Cycle Regulation

The Kinases

Normal cell progression through each phase of the cell cycle is primarily controlled by the cyclin-dependant kinases (cdks). These kinases have similar structures (Figure 1.3), and comprise a regulatory (cyclin) and catalytic (cdk) subunit. They allow a cell to pass from one phase to the next, as highlighted by the cyclin-cdk complexes (Figure 1.2), and ultimately through a set of checkpoints that monitor completion of critical events such as DNA replication and spindle formation. There are two major checkpoints that regulate cell cycle progression. Firstly, at the end of G_1, known as the restriction point, where a cell decides on the viability of progression through the cell cycle and, secondly, at the G_2/M interphase, where DNA replication is examined for completeness.[7,9,10]

Additionally, regulation of the G_1 and G_2/M checkpoints are also governed by cdk inhibitors, especially p16, p21, p27, and the tumour suppression genes (TSG), primarily Rb and p53.[11] The cyclin-cdk complexes are responsible for the phosphorylation of inactive enzymes required for the activation of the relative cell cycle events. Specific cyclin-cdk protein complexes are required to push cells through specific checkpoints. The cyclin D-cdk4 complex pushes cells through the G_1 phase while the cyclin E-cdk2 complex allows cells through late G_1. The cyclin A-cdk2 complex (Figure 1.3), regulates S-phase entry, and the cyclin B-cdk1 complex controls the G_2/M checkpoint (Figure 1.2).[7,9,11] If a cellular defect is noted, the activation of the appropriate cyclin-cdk complex is not completed and the cell cycle is halted. If no defect is detected, the cyclin-cdk complex is activated via phosphorylation. This leads to the activation of a transcription factor by the removal of an inhibitory phosphate present within the transcription factor. Transcription of the specific genes necessary for the next phase of the cell cycle occurs, including the respective cyclin and kinases genes.[9,10]

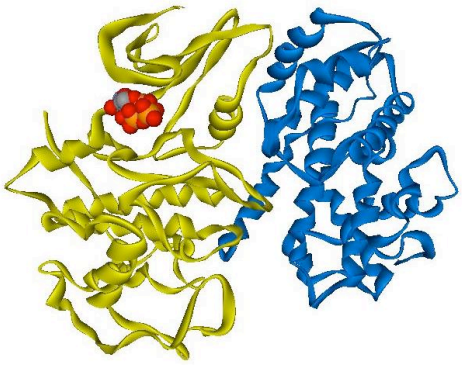


Figure 1.3: 3D representation of cyclin A (blue) and the cdk2 subunit (yellow) with ATP highlighted as a space filled model within the active site of cdk2 (PDB 1FIN). The atoms within the ATP molecule are colour coded. [12]

1.3 The Protein Phosphatase Family of Enzymes

Whilst phosphorylation plays a crucial role in cell viability, protein dephosphorylation is of equal importance. There are three distinct classes of protein phosphatases: tyrosine-specific, serine/threonine-specific, and dual-specificity phosphatases. Each class of phosphatases has distinct functions.[13-16]

Protein phosphatases (PPs) that catalyse dephosphorylation of serine and threonine residues are further classified into four subtypes based on their biological characteristics, sensitivities to specific inhibitors, and substrate specificity. They include PP1, PP2A, PP2B (calcineurin, Ca^{2+}/calmodulin dependent protein phosphatase), and PP2C (ATP / Mg^{2+} dependent protein phosphatase). Multiple isoforms of each protein phosphatase exist.[1,3,17-20] The primary amino acid sequences of PP1, PP2A, and PP2B are similar, with PP1 and PP2A sharing 43% sequence identity. PP2C is structurally distinct, belonging to a completely different gene family.[17-20]

Other protein phosphatases have been identified including PP4, PP5, PP6 and PP7.[15] PP4 and PP6 are structurally related to PP2A, sharing 65 and 57% amino acid homology respectively.[15,21] PP4 is 45% similar to PP1, but only in the catalytic domains. PP5 contains a catalytic domain common to PP1, PP2A, PP2B, PP4 and PP6. PP7 contains a catalytic core domain similar to the other phosphatases, but has unique N- and C-terminal regions and shares <35% identity with other known protein phosphatases.[21] Other recent additions to these classes include relatives of PP1: PPZ1, PPZ2, PPQ; relatives of PP2A: PPV, PPG; and a new PP2B, rdgC.[15,21,22] Despite this wide range of protein phosphatases, the majority of phosphatase activity within the cell is attributable to PP1 and PP2A.[22,23]

1.3.1 The Structure and Function of PP1 and PP2A

Both PP1 and PP2A are structurally complex molecules. They consist of three protein subunits forming a heterotrimer with a bimetallic active site within the catalytic domain.[24,25] The catalytic subunits (PP1c, 37kDa and PP2Ac 36kDa) are structurally related sharing 50% amino acid identity. PP1 is highly conserved across species boundaries with ca. 90% sequence identity across mammalian and drosophila species, representing one of the most conserved classes of enzymes known.[26-28] The catalytic subunits also associate with additional regulatory units that reduce the substrate specificity of PP1 and PP2A, giving rise to specific sub-cellular localisations. PP2A typically exists as a heterotrimer comprising three subunits: A, B and C (Figure 1.4).[29]

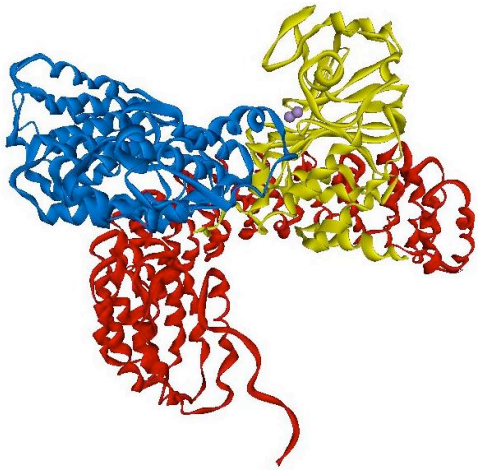


Figure 1.4: The structure of Aα-B56γ-Cα PP2A heterotrimer complex comprised of a scaffold Aα subunit (red), the regulatory B56γ subunit (blue), the catalytic Cα subunit (yellow), and the catalytic domain Mn atoms (purple) (PDB 2IAE).[29]

A number of different A and C units have been described in the literature. Additionally, several B units have also been identified and these units are believed responsible for the distinct substrate specificity observed. The crystal structure of subunit B56γ**,** the final unknown piece of PP2A was recently solved giving mechanistic insight into heterotimer assembly from the three separate subunits.[30] PP1 and PP2A are also strictly regulated by a number of endogenous protein inhibitors, e.g. PP1-inhibitor-1, PP1-inhibitor-2, dopamine and cAMP-regulated phosphoprotein (DARPP-32), and nuclear inhibitor of protein phosphatase 1 (NIPP-1).[15,28-31]

The physiological roles of PP1 and PP2A have been primarily discovered *via* mutation studies using yeast and drosophila.[32] In particular it is known that PP2A mediates regulatory control of the cell cycle by modulating the activity of the cdks and the TSG Rb.[33] The activity of the cyclin-cdk complexes is dependant on cyclin kinase binding, their phosphorylation level, and their interactions with appropriate inhibitory proteins (Figure 1.5). Activation of the cyclin-kinase complexes requires phosphorylation of the threonine residue (Thr-161) by cdk-activating kinase (CAK), followed by removal of inhibitory phosphates on tyrosine (Y15) and threonine (T14).[11,34] The addition of phosphates to Y15 and T14 is catalysed by Wee 1 and Mik 1, while cdc25A, B, or C phosphatases facilitate their removal.[11,34] PP2A plays a pivotal role in regulating this process by stimulating Wee 1 activity, inhibiting cdc25 activity, and/or by directly dephosphorylating Thr 161 on the cyclin-cdk complex.[11,34]

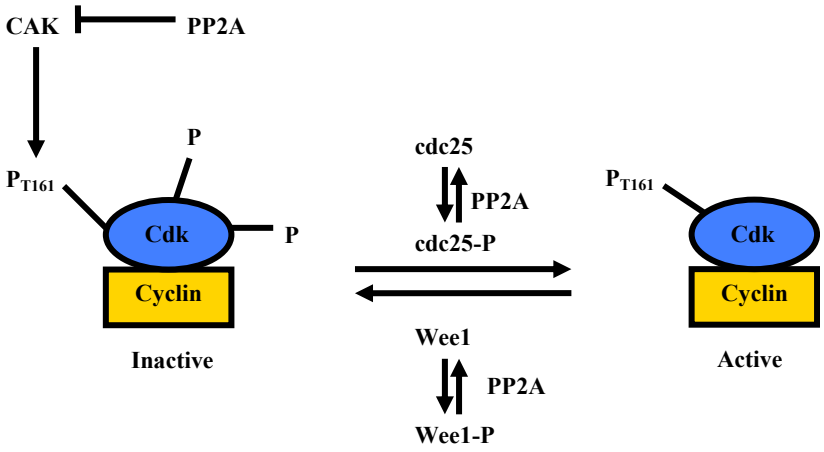


Figure 1.5: PP2A and the Cyclin-cdk Activation pathway. Activation of this pathway requires phosphorylation of Thr-161 by CAK and removal of two inhibitory phosphates by cdc25. PP2A regulates this process by stimulating Wee 1 activity, inhibiting cdc25 activity, and/or by directly dephosphorylating Thr 161.[11,34]

The G_1/S transition checkpoint or restriction point of the cell cycle is regulated by the cyclin D-cdk4 and cyclin E-cdk2 complexes. These complexes mediate their effects by phosphorylating and inactivating the TSG, pRb (Figure 1.6).[11] The pRb protein controls the movement of cells into the S-phase. Phosphorylation of pRb inhibits interaction with the S-phase transcription factor E2F facilitating transcription of proteins required for DNA synthesis and hence continuation of the cell cycle into, and through, the S-phase.[11] Dephosphorylation of phospho-Rb (pRb-P) by PP1 blocks continuation of the cell cycle. Hence, PP1 and PP2A are negative regulators of the cell cycle.

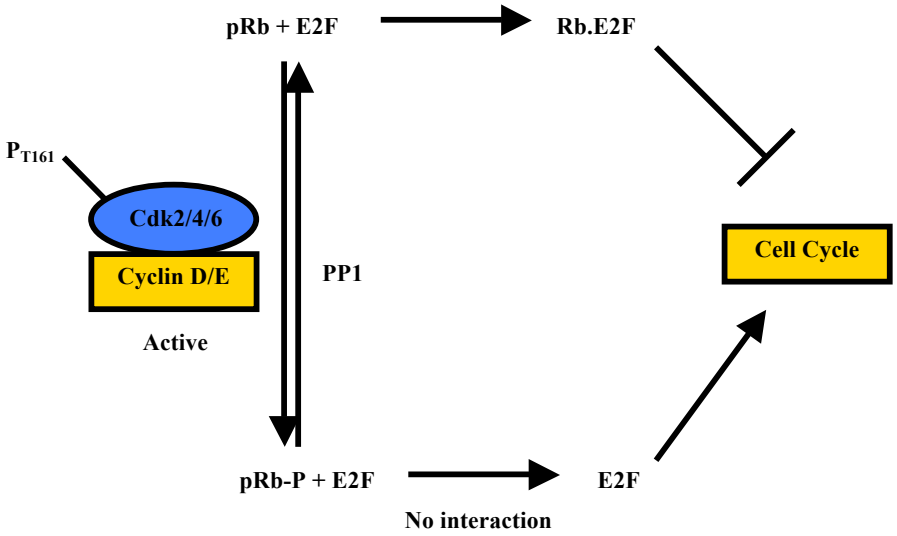


Figure 1.6: PP1 and phosphorylation of the retinoblastoma protein. Phosphorylation of pRb inhibits interaction with E2F allowing continuation of the cell cycle into S-phase. PP1 regulates this process by dephosphorylating pRb, allowing interaction with E2F which stops continuation of the cell cycle.[11]

1.3.2 Therapeutic Potential of PP1 and PP2A Inhibition

Given the roles of both PP1 and PP2A in the cell cycle described above, a number of research teams have targeted the inhibition of PP1 and PP2A as a potentially novel anti-cancer strategy.[35-40] These research teams believe that the inhibition of protein phosphatases offers an opportunity to move away from the traditional treatment approaches for the eradication of cancer. Most traditional treatments of malignancy (cancer) rely on therapeutics that prevent uncontrolled cellular replication, by targeting rapidly dividing cells or by inducing a fatal flaw in the DNA of the dividing cell. These traditional approaches are grouped together as with radiation therapy (radiotherapy) and chemical therapy (chemotherapy). Both approaches apply treatment regimes specifically designed to destroy tumour cells. However there are often difficulties in applying a sufficiently high dose to kill tumour cells without destroying the surrounding normal tissue. Additionally, there are numerous solid tumours that become resistant to radiation therapy such as prostate and cervical cancers.[41-43]

Whilst the development of chemotherapy revolutionised cancer treatment, a significant number of these drugs also target normal cells. Usually, cancer cells divide faster and take up more available drug making them more susceptible, while normal cells usually have the ability to recover better than tumour cells. There are of course exceptions to this, such as slow growing colon and solid tumours. However, chemotherapeutics target other healthy rapidly dividing cells found in the mouth, hair, stomach, and fingernails.[44-47]

In contrast to these traditional cancer treatment paradigms designed to stop the cell cycle of tumours, a "fighting fire with fire" approach may also be employed. Inhibition of PP1 and PP2A may result in the abrogation of the G_1 and G_2/M checkpoints forcing cells through the cell cycle prematurely, resulting in uncontrolled mitosis promoting the apoptotic cell death pathway.[48,49] Hence, inhibition of PP1 and PP2A could potentially provide a new therapeutical target for cancer.

The role of PP1 and PP2A as negative regulators of the cell cycle is exemplified by the ability of various inhibitors including okadaic acid (**1**) from the marine sponge *Halichondria okadai*, calcyculin A (**2**) from the marine sponge *Discodermia calyx*, microcystin-LR (**3**) from the cyanobacteria *Microsystis sp.*, fostriecin (**4**) from the bacteria *Streptomyces pulveraceus*, and cantharidin (**5**) from a range blister beetles of the *Meloidae* family, to accelerate and enhance cell cycle progression (Figure 1.7 and 1.8).[48,50] Okadaic acid (**1**), calyculin A (**2**), microcystin-LR (**3**) are classified as tumour promoters.[51] However, some PP1 and PP2A inhibitors are also potent anticancer compounds and mediate their effects by prematurely activating cell cycle progression and inhibiting vital mitotic processes.[52-54]

Of particular relevance to this current research program are fostriecin (**4**) (Figure 1.7), cantharidin (**5**) (Figure 1.8) and analogues thereof, which employ the 'fire with fire' approach of treating malignancy. Both cantharidin and fostriecin have been used clinically for the treatment of cancers.[55-60]

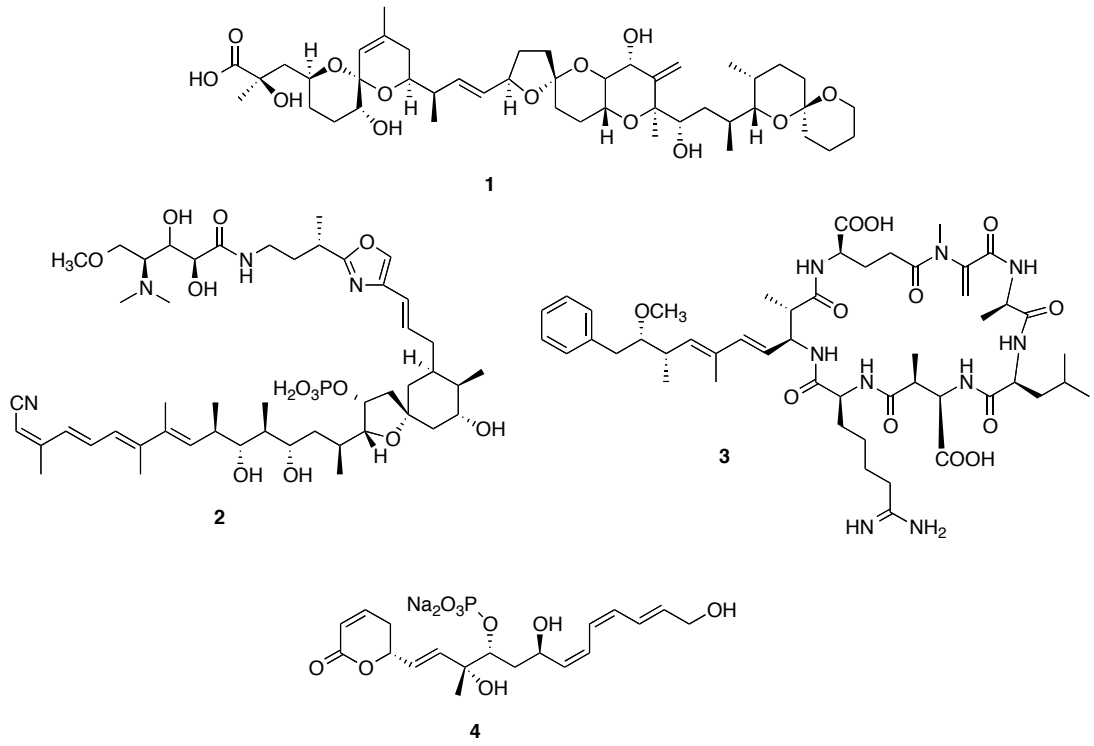


Figure 1.7: Chemical structures of the natural product okadaic acid (**1**), calyculin A (**2**), microcystin-LR (**3**), and fostriecin (**4**). These natural products are potent protein phosphatase inhibitors.

1.4 Small Molecule PP1 and PP2A Inhibitors

A number of protein phosphatase inhibitors have been isolated from natural sources. However, quantitative biological evaluation of the efficacy of these small molecule protein phosphatase inhibitors such as fostriecin (**4**),[50] cantharidin (**5**),[61] norcantharidin (**6**),[61] thyrsferyl 23-acetate (**7**),[62] isopalinurin (**8**),[63] and dragmacidin E (**9**),[64] (Figures 1.7 and 1.8) is limited within the literature (Table 1.1).

Table 1.1: Inhibition of PP1 and PP2A by selected small molecule inhibitors (**4-9**). [a] IC_{50} (μM) values are the compound concentration that inhibits enzyme activity by 50% relative to an untreated control; [b] reported as a protein phosphatase inhibitor without inhibition data.

Compound	PP1 IC_{50} (μM)[a]	PP2A IC_{50} (μM)
fostriecin (**4**)[50]	4	0.040
cantharidin (**5**)[84]	1.78	0.26
norcantharidin (**6**)[84]	1.98	0.37
thyrsferyl 23-acetate (**7**)[62]	n.r[b]	4-16
isopalinurin (**8**)[63]	n.r	n.r
dragmacidin E (**9**)[64]	n.r	n.r

Some of these molecules, such as cantharidin and fostriecin show significant promise for selective inhibition of specific protein phosphatases and even anticancer activity. This is particularly evident with fostriecin, which is reported to be 40,000 fold PP2A selective over PP1.[65] Although the physiological processes regulated by PP1 and PP2A are diverse, PP2A directly mediates regulatory control of the cell cycle. Selective inhibition of PP2A is therefore relevant to the 'fire with fire' approach. Selective inhibition of PP2A may also result in reduced toxicity by not affecting biological processes regulated primarily by PP1.

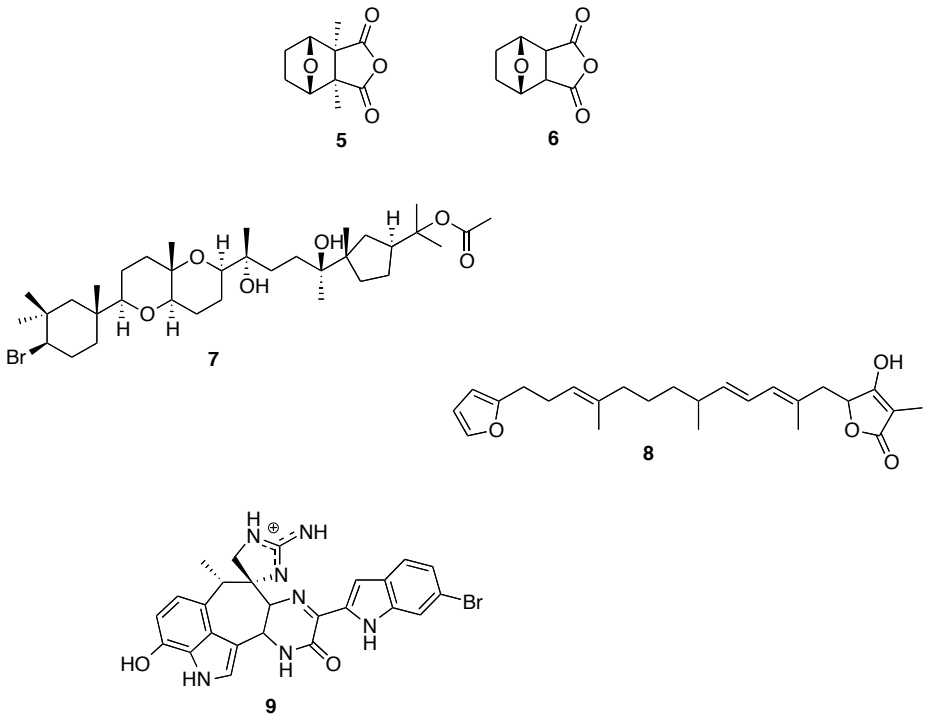
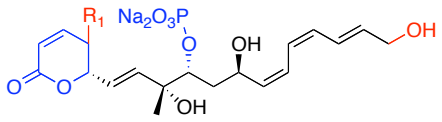


Figure 1.8: Chemical structures of cantharidin (**5**), norcantharidin (**6**), thyrsferyl 23-acetate (**7**), isopalinurin (**8**), and dragmacidin E (**9**). These compounds are potent protein phosphatase inhibitors, however, minimal quantitative biological data is reported for (**7**), (**8**), and (**9**).

Fostriecin

Selected modifications of the fostriecin scaffold have been carried out, however, no phosphatase inhibition data has yet been reported.[59,66,67] Data pertaining to its potency as an anti-tumour agent against L1210 (leukaemia) and HCT-8 (colon) cells was recorded, with GI_{50} values of ~0.4 and ~5.0 μM respectively.[59,66,67] Preliminary SAR evaluation indicates that the unsaturated lactone and phosphate ester moieties are required for anticancer activity, while ring hydroxylation or removal of the terminal hydroxyl group had only modest effects on activity (Figure 1.9). The fostriecin scaffold is complex and subsequent changes to form new analogues are difficult and time consuming.[59,66-68] Combined with a low stability and low plasma half-life, making it difficult to attain a therapeutic level in humans, fostrecin is a poor candidate for further development.[69]



4

Figure 1.9: Fostriecin (**4**) scaffold highlighting areas required for anticancer activity (blue) and areas having modest effects on anticancer activity (red).

Cantharidin

Of all the known inhibitors of serine/threonine protein phosphatases, cantharidin (**5**) offers the simplest structure for subsequent modification, and as such there have been a number of reports of synthetic modifications. However, this data is difficult to rationalise due to the varying inhibition values (IC_{50} values not K*i*) reported for cantharidin, which for PP2A range from 40 nM to 8 μM. A combination of differing assay methodologies, variation in enzyme preparation (recombinant, chicken, mouse), protein concentration, purity, substrate concentration and/or the purity of the material being assayed is responsible for the observed differences in the inhibition reported with cantharidin.[55,70]

Cantharidin-rich extracts from the *Meloidae* family of *Coleoptera* (beetles) have been utilised in natural Chinese remedies for Melina, with ancient transcriptions (306–168 BC) referring to the use of *Mylabris* for the treatment of furuncles and piles. Further, cantharidin has been used topically (0.7%) in the treatment of warts, whilst the anti-cancer potential of the molecule was identified over 700 years ago with the first recorded use of cantharidin as a tumour suppressant reported in 1264.[3,57]

Cantharidin (**5**) (Figure 1.8) reduces cell viability in a time-, concentration- and cell line-dependent manner with GI_{50} values generally in the low μM range. For example in neuroblastoma cells, the GI_{50} value after 24h incubation was 4.5μM, improving progressively to

1.3 μM by 120h.[71] Our group has reported similar values of 6-11 μM after 72h in ovarian, bone and colon cancer cell lines.[51] Cantharidin is also active in ovarian, melanoma, and epidermoid carcinoma biopsies, but less active in cancer biopsies of lung, adenocarcinoma, pancreas, breast, cervix, mesothelioma or sarcoma tumours.[72] Clinical trials involving cantharidin have shown this agent to stimulate the production of white blood cells by bone marrow, contrasting most other anticancer drugs that have the unwanted side effect of inducing myelosuppression, which leaves the patients more susceptible to infection.[73] Although cantharidin is cytotoxic to cancer cells and stimulatory on the bone marrow, the kidney toxicity of this drug has prevented its use in mainstream oncology.[73,74]

Similarly norcantharidin (**6**) (Figure 1.8), the demethylated analogue of cantharidin, also possesses anticancer activity and stimulates production of white blood cells by bone marrow. It however displays no kidney toxicity.[57,70] Norcantharidin is active *in vitro* with GI_{50} values of 13-47 μM against several tumour cell lines including hepatoma (L1210, HL60), ovarian (A2780, ADDP), colon (HT29), osteocarcinoma (143B), and leukaemia cell lines (K-562).[55,57,70] However, our own studies have shown norcantharidin to be approximately 10 fold less cytotoxic than cantharidin in many of these cell lines. Norcantharidin has also been used *in vivo* in the treatment of primary hepatoma, oesophageal, gastric and cardia carcinomas.[57] Preliminary data from our research group has suggested that norcantharidin derivatives also display anti-parasitic activity against nematode *Haemonchus contortus*, barbers pole worm, an intestinal parasite that affects many livestock industries.[75,76]

1.5 Current Norcantharidin Structure Activity Relationships (SAR)

As previously stated, norcantharidin possesses numerous unique qualities that make it amenable for anti-cancer drug development. This includes membrane permeability, consequently not requiring an active transport mechanism to enter cells. It does not induce myelosupression but instead had been shown to induce haematopoiesis in human studies, and finally as an extract it has been used clinically in China for the treatment of cancer since 1264 and for the topical treatment of warts since the 1970s.[11,57]

The catalytic domain in human serine/threonine protein phosphatases and binding site for cantharidin and norcantharidin analogues is common in PP1, PP2A, PP2B, PP4, PP5, and PP6. It is a highly conserved domain, with residues coordinating to two metal ions within the active site. Cantharidin and norcantharidin have been shown to interact with this active site in their corresponding hydrolysed dicarboxylic acid forms. Hydrolysis of the anhydride may occur upon binding, giving rise to one ligand confirmation. Alternatively it can be hydrolysed before

binding allowing the possibility of another completely different binding pose (Figure 1.10).[24,77,78]

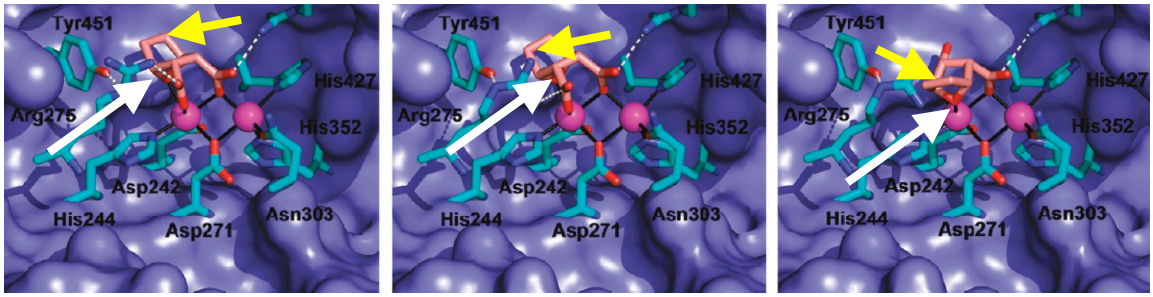
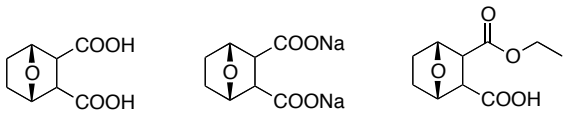


Figure 1.10: Norcantharidin binding within the di metallic active site of Mn_2PP5. The conformation of norcantharidin in the dicarboxylic acid form hydrolysed upon binding (left). The two possible conformations of norcantharidin introduced as the dicarboxylic acid (centre) and (right). Arrows highlighting the 7-oxo bridge head (white) and 5,6-ethyl bridge (yellow) for comparison.[24]

The presence of the 7-oxo bridgehead and a single carboxylate are crucial for binding within the di metallic phosphatase active site. The norcantharidin dicarboxylic acid (**10**), bis-sodium salt (**11**), and acid ethyl ester (**12**) show only negligible differences in PP1 and PP2A inhibition (Figure 1.11).[11,78,79-82]



10 **11** **12**

Figure 1.11: Ring opened norcantharidin derivatives. Dicarboxylic acid (**10**), bis-sodium salt (**11**), and acid ethyl ester (**12**) have only negligible difference in PP1 and PP2A inhibition.[80-82]

However, phosphatase inhibition *in vitro* attributed to a particular analogue cannot be the sole method of analysis in the search for a viable anticancer drug candidate. Variation to the norcantharidin scaffold affects the ability of the drug to reach the targeted binding pocket in the active ring opened dicarboxylic acid form. Cell permeability and half-live within living tissue, along with varying concentrations of protein phosphatases within different cells all effect anticancer activity. As a result of this, cytotoxicity data taken from cell cultures is primarily used to assess the large number of norcantharidin analogues in order to develop structure activity relationships (SAR).

There are a number of potential regions suitable for rapid alteration associated with the norcantharidin scaffold (Figure 1.12). Numerous alterations have been made at the anhydride bridge (**A**), the carbonyl group (**B**), the bridgehead (**C**), and the tail section (**D**) in an effort to improve activity both as protein phosphatase inhibitors and anti-cancer agents. This has led to a wide variety of new compounds with varying success in anticancer cell and protein phosphatase assays. A summary of the SAR for these four regions will be discussed in turn.

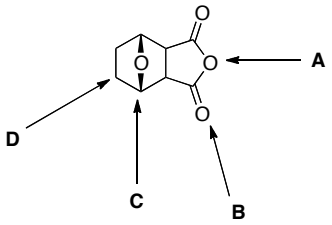


Figure 1.12: Sites of alterations on norcantharidin backbone which includes; the anhydride bridge (**A**), the carbonyl group (**B**), the bridgehead (**C**), and the tail section (**D**).

1.5.1 Region A – Anhydride Bridge

By far, the most significant work on the norcantharidin scaffold has been carried out at the anhydride bridge (Region A). A wide range of analogues have been synthesised including the development of both the ring opened and ring closed structures (Figure 1.13).

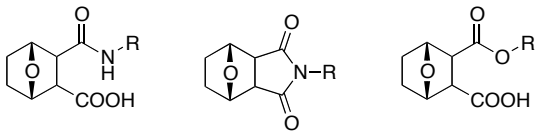
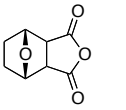
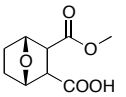
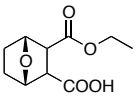
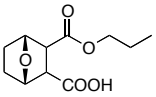
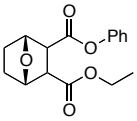


Figure 1.13: Generic structure of ring opened acid/amide (left), ring closed norcantharimide (centre), and ring opened acid/ester (right) norcantharidin analogues.

Early work at the anhydride moiety produced a library of ring opened esters varying in aliphatic chain length (**13**-**15**) (Table 1.2).[83] Against all cell lines tested, none of these analogues displayed enhanced activity relative to norcantharidin, despite having good PP1 and PP2A inhibition activity. Aromatic esters were also tested but again none displayed potent activity, with the exception of compound (**16**), containing a phenyl ethyl di-ester, which produced comparable or improved anticancer activity across all tested cell lines tested.[83-85]

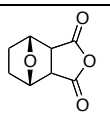
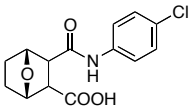
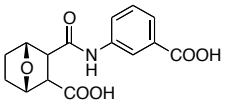
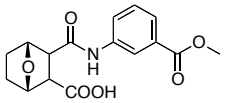
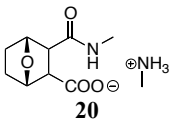
Table 1.2: Anticancer activity of norcantharidin and most active ring opened acid/ester analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	A2780[a]	ADDP[a]	143B[b]	HCT116[c]	HT29[c]	L1210[d]	HL60[d]	WiDr[c]	SW480[c]
 **6**	50±0	47±3	43±9	24±4	33±7	13±0.3	25±2	36±5	33±7
 **13**	100±10	180±8	118±8	76±14	105±5	263±19	117±9	257±34	150±17
 **14**	185±5	330±39	437±37	226±9	243±30	443±92	333±88	450±60	310±20
 **15**	110±10	315±65	450±50	75±5	150±45	313±3	270±15	33±6	118±13
 **16**	16±3	-	-	18±5	24±4	23±4	-	38±9	38±6

[a] Ovarian carcinoma (A2780, ADDP), [b] Osteosarcoma (143B), [c] Colon carcinoma (HCT116, HT29, WiDr, SW480), [d] Haematopoietic carcinoma (L1210, HL60), [e] (-) not determined.

With little success from straight chain aliphatic, a subsequent ring opened library incorporated aromatic amines possessing functional groups such as halogens (**17**), carboxylic acids (**18**), esters (**19**), and amine salts (**20**) (Table 1.3). However, none of these analogues displayed potent cytotoxicity or potent phosphatase inhibition.[86]

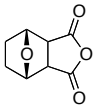
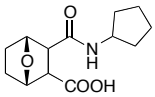
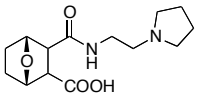
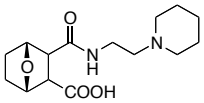
Table 1.3: Anticancer activity of norcantharidin and ring opened acid/amide analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	A2780[a]	G401[b]	HT29[c]	H460[d]	L1210[e]
 **6**	39±5	35±2.3	33±7	50±4	13±0
 **17**	87±7.3	90±5.8	>100	>100	>100
 **18**	82±10	>100	>100	>100	>100
 **19**	90±0.1	>100	>100	>100	>100
 **20**	88±7.3	>100	89±0.8	>100	>100

[a] Ovarian carcinoma (A2780), [b] Kidney carcinoma (G401), [c] Colon carcinoma (HT29), [d] Lung carcinoma (H460), [e] Haematopoietic carcinoma (L1210).

A series of ring opened norcantharidin analogues, possessing substituted heterocyclic and cyclic amines have also been reported.[87] A cyclopentylamine derivative (**21**) (Table 1.4), showed promising cytotoxicity with comparable or improved activity compared to norcantharidin. However, alkyl chain extension (**22**, **23**) between the amide and the heterocyclic tail was not tolerated, suggesting that the proximity of the heterocyclic ring to the amide significantly influences activity (Table 1.4).

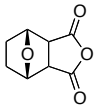
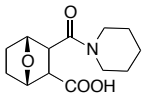
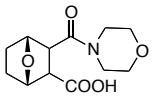
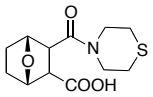
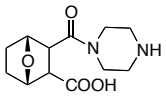
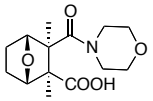
Table 1.4: Anticancer activity of norcantharidin and selected ring opened acid/amide analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **6**	33±7	33±7	64±3.4	50±0	45±1.8	33±0.8	30±1.1	56±1.8	35±1.1
 **21**	44±3.0	27±4.5	48±2.5	56±1.0	29±4.3	32±2.0	33±4.0	31±2.0	31±1.5
 **22**	>100	>100	>100	>100	>100	>100	>100	>100	>100
 **23**	>100	>100	>100	>100	>100	>100	>100	>100	>100

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2).

Data obtained on the screening of subsequent libraries support the hypothesis of heterocyclic ring proximity to the amide influences activity. All analogues possessing shortened linkers displayed potent activity (Table 1.5). Making simple bioisosteric replacements at C4 of the piperidine ring with heteroatoms (**24-27**), further improvements in cytotoxicity were observed. Combining this successful modification, the morpholine ring, with the parent cantharidin backbone produced the most active analogue (**28**) to date across all cancer lines tested with an average GI_{50} of 3.3 μM and is the only cantharidin analogue ever reported to be a more active phosphatase inhibitor than the lead compound.[87]

Table 1.5: Anticancer activity of norcantharidin and selected ring opened acid/amide analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

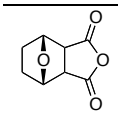
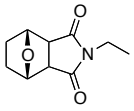
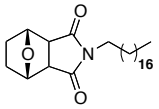
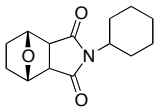
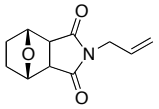
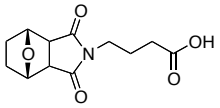
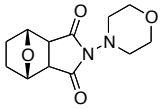
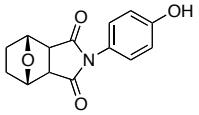
Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **6**	33±7	33±7	64±3.4	50±0	45±1.8	33±0.8	30±1.1	56±1.8	35±1.1
 **24**	14±1.2	9.3±0.4	14±0.9	12±1.0	19±1.7	14±0.2	16±0.3	14±0.3	18±1.8
 **25**	7.4±2.1	5.5±0.8	7.7±1.8	8.3±0.4	18±2.8	8.5±1.3	14±0.4	6.0±0.6	11±0.6
 **26**	7.7±1.6	5.3±0.6	7.0±0.1	6.2±0.1	13±1.2	8.5±0.7	11±1.0	5.6±0.2	12±0.9
 **27**	17±0.0	17±0.9	20±1.7	24±2.5	21±1.6	23±3.0	29±1.5	20±0.7	13±3.0
 **28**	2.8±0.0	3.9±0.1	5.8±0.2	4.9±0.4	3.3±0.1	2.9±0.1	1.7±0.0	2.5±0.3	2.1±0.1

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2).

Additional series of norcantharidin analogues are the ring closed analogues known as norcantharimides, exemplified by compound (**29**). It is important to note that the introduction of the cyclic imide moiety renders these analogues incapable of ring opening, effectively leaving all such analogues devoid of protein phosphatase inhibition, and thus any growth inhibition noted is unlikely to directly result from protein phosphatse inhibition of an unmodified norcantharidin. It is possible that in vivo these analogues are metabolised to a form that is protein phosphatase active. However, most of these derivatives to date have typically reduced activity against all cancer lines tested.

Early research commenced with simple short chained primary amine derivatives to form a norcantharimide library.[88] All initial compounds resulted in poor percentage growth inhibition at 100 μM concentration recording values of <10% across all cell lines (Table 1.6). Attempts to further increase the cytotoxicity of these analogues included extension and branching of the alkyl chain, terminal unsaturation of the alkyl chain, epoxidation and dihydroxylation of terminal alkenes, addition of terminal carboxylic acids, addition of heterocycles, and aromatic tails (**29-35**) (Table 1.6). Unfortunately the majority of the aforementioned derivatives displayed reduced activity across all cell lines tested.[88]

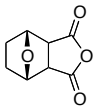
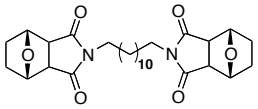
Table 1.6: Anticancer activity of norcantharidin and inactive ring closed norcantharimides. Values in normal text are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control. Values in *italics* are percentage growth inhibition (%) after continuous exposure to 100μM drug solution for 72 hours.

Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **6**	33±7	33±7	64±3.4	50±0	45±1.8	33±0.8	30±1.1	56±1.8	35±1.1
 **29**	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
 **30**	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
 **31**	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
 **32**	*19±9*	*50±26*	*80±12*	*67±12*	*20±8*	*32±5*	*15±17*	*62±5*	*<10*
 **33**	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*	*<10*
 **34**	*29±5*	*29±1*	*12±4*	*<10*	*18±0*	*30±5*	*<10*	*39±8*	*<10*
 **35**	*47±8*	*21±3*	*22±4*	*15±3*	*12±1*	*27±0*	*<10*	*14±6*	*44±4*

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2).

The most promising norcantharimide analogue in this initial library was the dodecyl linked norcantharimide dimer (**36**) (Table 1.7). This compound was significantly more active against HT29 (colon) cells than the parent norcantharidin with a GI_{50} of 8.3±0.7 μM vs. 33±7 μM respectively. This norcantharimide dimer is also comparable or better across the remainder of the cell lines when compared to norcantharidin with average associated GI_{50}s of 29μM and 43 μM respectively.[88]

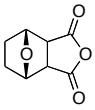
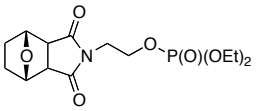
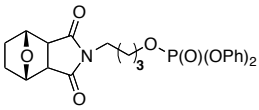
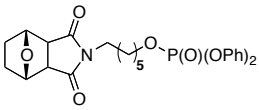
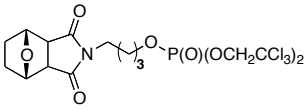
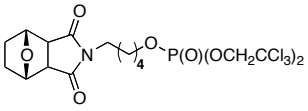
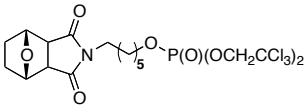
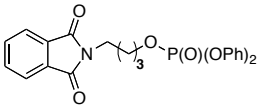
Table 1.7: Anticancer activity of norcantharidin and an active ring closed norcantharimide dimer analogue. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **6**	33±7	33±7	64±3.4	50±0	45±1.8	33±0.8	30±1.1	56±1.8	35±1.1
 **36**	8.3±0.7	24±4	18±0	19±1	31±7	18±4	60±6	17±4	43±10

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2).

In contrast to the majority of ring closed analogues, norcantharimide analogues containing terminal phosphate esters have been shown to display modest to good levels of cytotoxicity (Table 1.8).[89] From this research, a trend between the ease of phosphate ester hydrolysis and cytotoxicity was observed with the most labile esters corresponding to the most biologically active analogues. Numerous analogues displayed comparable cytotoxicity to norcantharidin across all cell lines examined (**37-41**) as well as one analogue (**42**) that was up to five fold more potent than norcantharidin with an average GI_{50} of 11 μM. The length of aliphatic chain between the norcantharimide and phosphate ester moieties influenced cytotoxicity. Of the active analogues, longer chains were more potent in analogues that contained biphenyl (**38**, **39**) and bis-trichloroethyl (**40 - 42**) phosphate esters. Compound (**43**), shown to be inactive compared to its norcantharimide (**38**), demonstrates the presence of both the norcantharidin and a labile phosphate ester moieties are necessary to retain cytotoxicity (Table 1.8). However, these analogues are not phosphatase inhibitors, suggesting a different mechanism of action.[89]

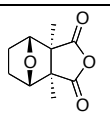
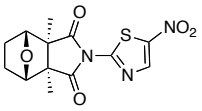
Table 1.8: Anticancer activity of norcantharidin and phosphate ester analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **6**	33±7	33±7	64±3.4	50±0	45±1.8	33±0.8	30±1.1	56±1.8	35±1.1
 **37**	33±1.0	18±0.4	28±1.3	15±0.6	49±5	27±1.5	21±3.3	-[i]	43±4.1
 **38**	14±0.2	28±1.2	19±1	17±1	64±4.8	21±1.9	13±0.6	25±3.2	17±2
 **39**	5.4±0	14±0.3	9.1±1	11±0.8	25±3.7	13±0	7±0.2	14±0.3	14±0
 **40**	16±1.5	32±1.7	28±0	26±1	56±1	31±1.9	49±0.3	40±2	48±0.3
 **41**	15±0.9	17±0.3	16±0.2	16±0.9	29±0.7	18±1	26±1	22±0.7	19±1.7
 **42**	11±0.3	10±0.7	12±0.9	7.7±0.3	13±0.3	12±0.3	13±0.9	9.3±0.1	14±0.3
 **43**	>100	>100	>100	>100	>100	>100	>100	>100	>100

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2), [i] (-) not determined.

Additional series of ring closed analogues include *N*-thiazolyl- and *N*-thiadiazolyl cantharidin derivatives. Of the ten analogues reported, only one showed promise (**44**) (Table 1.9), the 2-*N*-5-nitrothiazole, which produced significantly higher degree of cytotoxicity over both of the tested cell lines than the parent cantharidin.[90]

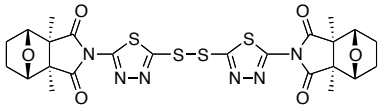
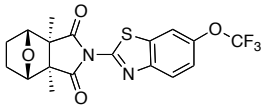
Table 1.9: Anticancer activity of cantharidin and a ring closed cantharimide analogue. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	Hep-3B[a]	Hep-1[a]
 **5**	2	4
 **44**	0.4	1.25

[a] Hepatocellular carcinoma (Hep-3B, Hep-1)

Kok *et al.* also reported the synthesis and cytotoxicity of some additional norcantharimide analogues with a *N*-thiazolyl ring closed backbone were also synthesised.[91-93] Two such analogues were the cantharimide dimer (**45**) and the 2-*N*-6-(trifluoromethyl)benzo[α]thiazole (**46**), which displayed specific toxicity towards the Hep3B and KG1a cell lines at a concentration of 25 μg/mL with close to complete cell death achieved after 48 hours for the latter cell line (Table 1.10).

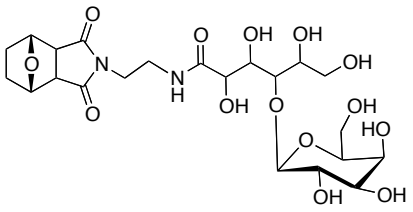
Table 1.10: Anticancer activity of ring closed cantharimide analogues. Values are % survival values, the percentage of cells that survive when exposed to a 25 μM solution of the corresponding cantharimide analogue for 48 hours relative to an untreated control.

Structure	A549[a]	Hep-3B[b]	MDA-MB231[c]	KG1a[d]	K562[d]
 **45**	45	10	50	0	-[e]
 **46**	-	-	-	20	5

[a] Lung carcinoma (A549), [b] Hepatocellular carcinoma (Hep-3B), [c] Breast carcinoma (MDA-MB231), [d] Myelogenous leukaemia (KG1a, K562), [e] (-) not determined.

Recently reports have described the design and synthesis of norcantharimide analogues with the specific intent to enhance carcinoma targeting and cell permeability. The first of these analogues is a norcantharidin/lactose acid analogue (**47**) designed to specifically target hepatocellular carcinoma (Figure 1.14).[94] This derivative was designed to target and inhibit the asialoglycoprotein receptor (ASGP-R) on the surface of liver tumour cells. In doing so, it was believed that the *N*-lactose norcantharimide would be concentrated on the surface of the target cells which display a high concentration of this receptor, ideally enhancing treatment and

reducing associated side effects by limiting these compounds reaching other active sites throughout the body.[94] However, it is not mentioned how these compounds enter the cell once accumulated at the cell surface. To date, this approach, while interesting, has not been substantiated by the corresponding biological data.



47

Figure 1.14: Structure of norcantharidin and lactose acid analogue (**47**). Stereochemistry not reported.[94]

Other groups have reported the incorporation of norcantharidin, in its acid-amide form, into side arms of the chitosan backbone. The result of this is a stable water soluble norcantharidin analogue (Figure 1.15). Preliminary animal study results with ring opened norcantharidin attached to a chitosan backbone *via* an amide linkage show that at a dose of 20 mg/kg, these norcantharidin-chitosan analogues can reduce the weight of tumours in mice by up to 61% when compared to an untreated control. When compared to norcantharidin and chitosan separately, producing tumour reductions of 25 and 21% respectively at the same dosage level, the norcantharidin incorporated chitosan analogue significantly enhances inhibition of tumour growth while also increasing the white blood cell count, a trait unique to these anticancer compounds.[95]

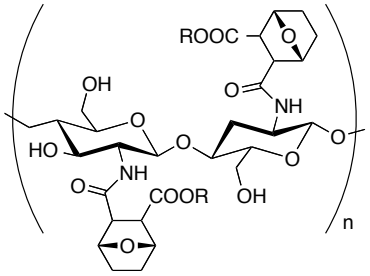


Figure 1.15: Structure of norcantharidin and chitosan analogues. (n) repeating chitosan backbone.[95]

Norcantharidin, in its dicarboxylic acid form, has been added to other biologically active compounds in an effort to circumvent observed resistance or lack of associated cell specificity. When added to a platinum based cisplatin scaffold, dicarboxylic norcantharidin derivatives were shown to retain anti-tumour activity against cisplatin-resistant L1210 (leukaemia) and SK-Hep-1 (hepatocellular carcinoma) cell lines. In aqueous solutions, dicarboxylic norcantharidin is released from the platinum scaffold and is believed to be responsible for the retention of activity in cisplatin resistant cells. The released norcantharidin ligand inhibits PP2A and consequently

the nucleotide excision repair mechanism, an important factor attributed to cisplatin resistance.[96-98]

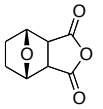
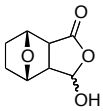
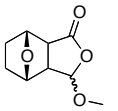
The addition of norcantharidin to illudin M also retains the cytotoxicity associated with illudin M against Panc-1 (pancreatic carcinoma) and HT29 (colon carcinoma) cell lines. However, these analogues showed much improved specificity towards these tumour cells over HF (non-malignant fibroblasts) when compared to the parent illudin M, a non specific cytotoxin.[99,100]

1.5.2 Region B – Carbonyl group

The modification of a single carbonyl group (region B), exemplified by compound (**49**), was also tested to determine its role in anticancer activity. This successful alteration produced a new series of compounds, the Novo series (Table 1.11). The parent compound (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**48**), showed an increase in activity against colon cancer (HT29) relative to norcantharidin (**6**). Interestingly, compound (**48**) displayed poor activity across all other cell lines tested showing a degree of selectively to target HT29 over other preliminary cancer cell lines.[84]

The methyl ether analogue (**49**), as with compound (**48**), displayed no activity against all other tested cell lines but showed comparable activity against cell line HT29 to that of norcantharidin (**6**). Further extension of the aliphatic chain (ethyl, propyl) did not result in any increase in activity across the cell lines tested, however comparable activity against HT29 cells was retained.[84]

Table 1.11: Anticancer activity of norcantharidin and selected carbonyl modified analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	A2780[a]	ADDP[a]	143B[b]	HCT116[c]	HT29[c]
 **6**	50±0	47±3	43±9	24±4	33±7
 **48**	333±55	275±56	450±50	78±7	14±0.3
 **49**	538±83	323±40	>1000	143±23	28±1

[a] Ovarian carcinoma (A2780, ADDP), [b] Osteosarcoma (143B), [c] Colon carcinoma (HCT116, HT29).

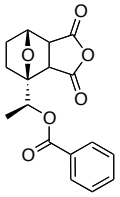
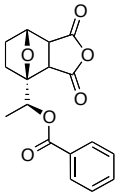
1.5.3 Region C – Bridgehead

Early work at region C involved making alterations to or removal of the oxygen bridge head. In all cases, structural alterations to the bridge head were not tolerated with all compounds incorporating these changes displaying no activity.[85,86,101]

Subsequent modifications to the bridge head involved extending a side chain from the base of the bridge, exemplified by compound (**50a**). These modifications incorporated varying functional groups such as electron withdrawing acetyl, alkyl nitrile, amide, and an electron donating hydroxyl group. Unfortunately all of these additions were not tolerated resulting in no increase in cytotoxicity.[86]

However, other research suggests that addition of a methyl benzoate ester at the norcantharidin bridge head (**50a, 50b**) (Table 1.12) could be of use and may increase activity as multiple enantiomers of this compound have been reported to be a modest selective inhibitor of PP2B.[102]

Table 1.12: Inhibition of PP1, PP2A and PP2B by norcantharidin analogues. [a] Values are % inhibition values, the percentage of cells inhibited when exposed to a 100 μM solution of the corresponding analogue for 72 hours relative to an untreated control.

Structure	PP1 (%)	PP2A (%)	PP2B (%)
 **50a**	0	1	25
 **50b**	0	3	12

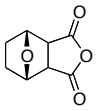
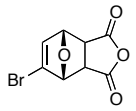
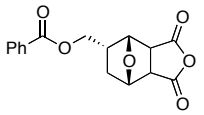
1.5.4 Region D - Tail

To date, previous work at region D has shown that limited structural modifications are tolerated with all derivatives synthesised showing reduced activity over all cancer cell lines tested.[79,85,86,103] The presence of a double bond (5,6-ene) at this location results in a loss of activity against all lines tested consisting of ovarian, breast, colon, and leukaemia, caner cells.[86,87] The presence of the 5,6-double bond has also been shown to reduce the stability of these analogues.

Subtle alteration to this region of the scaffold such as mono bromo addition to the 5,6 double bond (**51**) was not tolerated. However, a methyl benzoate ester analogue (**52**) displayed activity

against the A2780 (ovarian) cell line, and similar activity against H460 (lung) cell line (Table 1.13).[85,86]

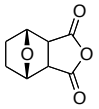
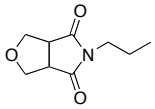
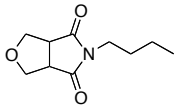
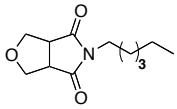
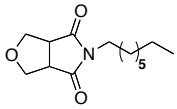
Table 1.13: Anticancer activity of norcantharidin and tail modified norcantharidin analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	A2780[a]	G401[b]	HT29[c]	H460[d]	L1210[e]
 **6**	39±5	35±2.3	33±7	50±4	13±0
 **51**	-[f]	-	>100	-	>100
 **52**	30±8.0	53±7	57±7.7	43±1.5	49±5.8

[a] Ovarian carcinoma (A2780), [b] Kidney carcinoma (G401), [c] Colon carcinoma (HT29), [d] Lung carcinoma (H460), [e] Haematopoietic carcinoma (L1210), [f] (-) not determined.

The effect of 5,6-ethyl bridge removal has been biologically assessed against nine cell lines.[103] In the majority of analogues, the removal of the 5,6-ethyl bridge resulted in a complete loss in activity (**53-55**). However, a derivative with an eight carbon chain, 5-octyldihydro-1*H*-furo[3,4-*c*]pyrrole-4,6(5*H*,6a*H*)-dione (**56**), produced comparable cytotoxicity to norcantharidin across all cell lines (Table 1.14).[79,103]

Table1.14: Anticancer activity of Δ-5,6-ethyl bridge removed norcantharidin analogues. Values in normal text are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control, Values in *italics* are % inhibition values, the percentage of cells inhibited when exposed to a 100 μM solution of the corresponding analogue for 72 hours relative to an untreated control.

Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **6**	33±7	33±7	64±3.4	50±0	45±1.8	33±0.8	30±1.1	56±1.8	35±1.1
 **53**	*<10*	*<10*	*<10*	*11±1*	*<10*	*<10*	*<10*	*<10*	*<10*
 **54**	*<10*	*<10*	*<10*	*12±1*	*<10*	*<10*	*<10*	*<10*	*<10*
 **55**	*<10*	*11±3*	*10±5*	*17±1*	*<10*	*<10*	*<10*	*<10*	*<10*
 **56**	21±1	29±2	22±4	30±0	71±1	36±1	33±2	59±4	28±0

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2).

1.5.5 Alterations in Multiple Regions

While rarer, analogues possessing simultaneous structural modifications in multiple regions on the norcantharidin scaffold have been reported (Figure 1.16).[104]

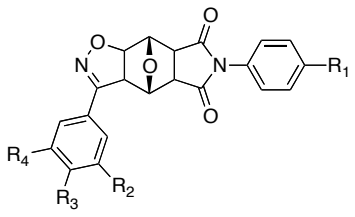


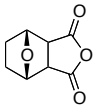
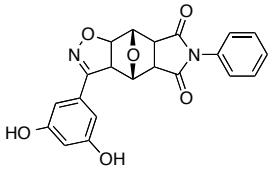
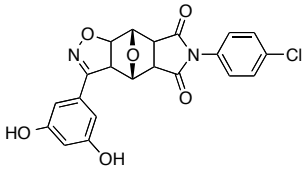
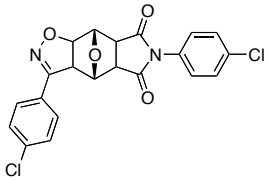
Figure 1.16: Isoxazoline/norcantharidin backbone.[104]

Structural changes have included incorporation of electron donating aromatic groups off the isoxazoline moiety but this structural change was generally not tolerated with activity lost across the entire cell range evaluated. Notable exceptions to this were analogues (**57**) and (**58**) (Table 1.15) which both showed selectivity towards the HL60 cells producing GI_{50} values superior to that of norcantharidin. Compound **58** also showed significantly higher cytotoxicity

against HO8910 cells with a GI_{50} of 16.5 μM. Norcantharidin displayed no activity against this cell line.[104]

In an additional series of norcantharidin / isoxazoline based molecules, a series of derivatives with electron withdrawing arenes attached to the isoxazoline ring was also produced.[104] Attaching a *para*-chloro aromatic moiety to the isoxazoline ring, produced 3 analogues that were significantly more active than norcantharidin against HL60 cells. The most active of these, (**59**), also had a second *para*-chloro aromatic ring present off the imide nose (Region A) of the structure. The final library incorporated a *para*-sulfoxide benzyl tail off the isoxazoline ring but this was poorly tolerated with no increase in activity gained.[104]

Table 1.15: Anticancer activity of norcantharidin and selected isoxazoline analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	KB[a]	SGC-7901[b]	HL60[c]	Bel7402[d]	HO-8910[e]	ECA109[f]
 **6**	32.2	79.0	5.0	24.0	>100	>100
 **57**	>100	>100	1.6	>100	>100	>100
 **58**	>100	>100	1.6	>100	16.5	>100
 **59**	>100	>100	0.36	>100	>100	>100

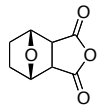
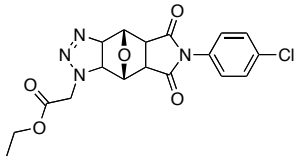
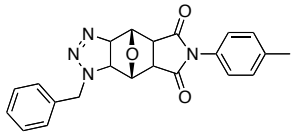
[a] Neck & Head carcinoma (KB), [b] Stomach carcinoma (SGC-7901), [c] Haematopoietic carcinoma (HL60), [d] Liver carcinoma (Be17402), [e] Ovarian carcinoma (HO-8910), [f] Oesophageal carcinoma (ECA109).

Deng *et al.* further explored this norcantharidin / isoxazolone class of hybrid molecules with the synthesis of analogues possessing an ethyl acetate tail linked to a 1, 2, 3-triazole ring.[105] These derivatives generally resulted in no activity across all cell lines but notably compound (**60**) (Table 1.16), again with a *para*-chloro arene at the anhydride bridge showed promise with an increase in potency against ECA109 and HO8910 lines and comparable potency against K562

cells compared to the parent norcantharidin. Analogue (**61**) was also active against the same series of cancer cell lines.

The final structural change attempted was to add a *para*-nitro benzyl group to the 1, 2, 3-triazole backbone. This change was not tolerated, with all derivatives shown to be not active.[105]

Table 1.16: Anticancer activity of selected 1, 2, 3-triazole norcantharidin analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	KB[a]	SMMC-7721[b]	SGC-7901[c]	ECA109[d]	HO-8910[e]	K562[f]
 **6**	2.80±0.1	66.8±0.3	13.8±0.1	316±3.5	256±2.0	20.9±1.0
 **60**	17.7±1.5	102.0±2.3	74.5±1.5	72.8±2.0	94.7±2.3	22.6±1.2
 **61**	27.4±0.8	19.0±0.2	28.5±1.4	21.8±1.2	4.36±0.1	14.2±0.2

[a] Neck & Head carcinoma (KB), [b] Liver carcinoma (SMMC-7721), [c] Stomach carcinoma (SGC-7901), [d] Oesophageal carcinoma (ECA109), [e] Ovarian carcinoma (HO-8910), [f] Myelogenous leukaemia (K562).

Other multiple site norcantharidin analogues have incorporated the previously shown success of isoxazole and pyrazole rings at the 5,6 position (Figure 1.17).[104,105] Unfortunately this was only carried out as a synthetic exercise and the effect of the new isoxazole and pyrazole dimers was not biologically evaluated.[106-108]

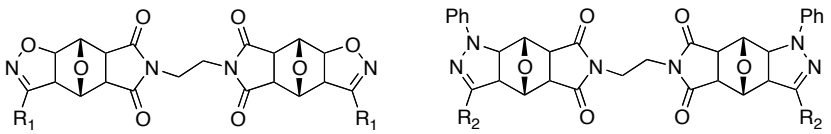
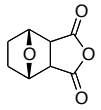
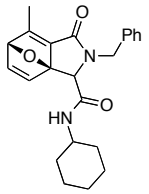
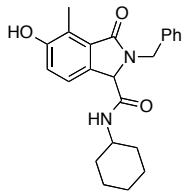


Figure 1.17: Isoxazole (left) and pyrazole (right) norcantharidin dimer backbones.[106-108]

Alterations at multiple sites have also been reported by our research team. Basing new compound libraries on the scaffold of cantharidin and norcantharidin in an effort to mimic the associated cytotoxicity, a small library was synthesised utilising a four component Ugi reaction followed by an intramolecular Diels-Alder cycloaddition. This resulted in a synthetic method that provided rapid access to highly complex molecules containing a high degree of structural rigidity.[109,110] Although this was originally carried out as a synthetic exercise, (3*R*,3a*S*,6*R*)-2-(benxyl)-1,2,3,6-tetrahydro-7-methyl-1-oxo-*N*-(cyclohexyl)-3a,6-epoxy-3a*H*-isoindole-3-

carboxamide (**62**) produced excellent biological data, producing cytotoxicity an order of magnitude higher that the parent norcantharidin across all nine cell lines (Table 1.17). Compound (**62**) was further synthetically modified through treatment of a protic ionic liquid that converted the bicyclic ring into an aromatic oxoisoindoline in high yields.[110] This synthetic modification resulted in compound (**63**) having comparable cytotoxicity to norcantharidin, but an order of magnitude lower than (**62**), against all tested cell lines (Table 1.17). This strongly support a crucial role for the bicycle[2.2.1]heptane skeleton in eliciting the observed cytotoxicity of the norcantharidin classes of compounds.

Table 1.17: Anticancer activity of norcantharidin and Ugi/Diels-Alder analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **6**	33±7	33±7	64±3.4	50±0	45±1.8	33±0.8	30±1.1	56±1.8	35±1.1
 **62**	3.2±0.1	3.2±0.1	7.5±0.4	4.4±0.3	3.3±0.2	2.9±0.2	2.1±0.3	3.7±0.6	1.7±0.1
 **63**	23±1	25±1	26±1	24±2	32±0	31±1	52±9	53±2	41±11

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2).

1.5.6 Summary of SAR

As outlined herein there have been numerous structural modifications to the norcantharidin scaffold. To date, few of these changes have been biologically successful; with only seven analogues (Figure 1.18) increasing potency compared to norcantharidin over all carcinoma cell lines tested.

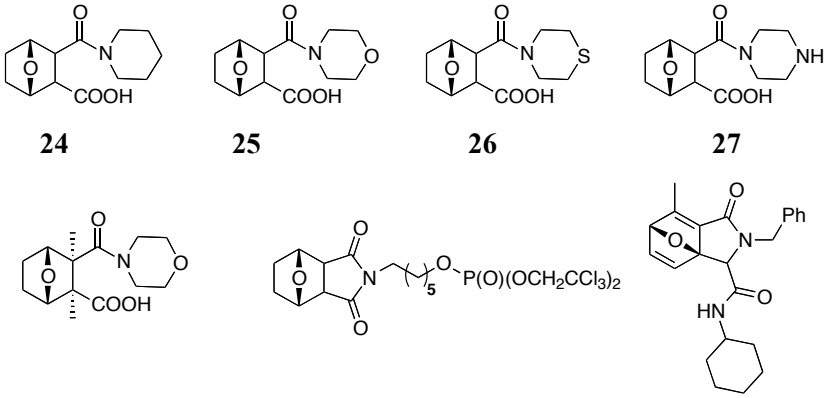


Figure 1.18: Norcantharidin analogues (**24, 25, 26, 27, 28, 42, 62**) more potent than norcantharidin across all carcinoma cell lines tested.

Interestingly, five of the seven successful analogues share common characteristics of ring opened acid/amides with a cyclohexyl heterocycle moiety. This suggests that these ring opened norcantharidin analogues are able to successfully reach the phosphatase catalytic domain forming a favourable interaction. It also suggests that only one free carboxylic acid is necessary and that the heterocycle moiety is also beneficial for phosphatase inhibition and anticancer activity. It is not critical however, as ring closed analogues (**42**) and (**62**) remain active, suggesting a different action mechanism to phosphatase inhibition.

Some structural modifications have highlighted areas of the norcantharidin scaffold that are not only beneficial but also essential. The area receiving the most research, the anhydride nose (Region A), indicates that the majority of active analogues contain the anhydride nose in the 'ring opened' conformation or are capable of undergoing a facile ring opening. However, a small number of 'ring closed' analogues have also been discovered suggesting this alteration of the norcantharidin scaffold can be beneficial for activity but not essential. This is most probably due to a change in mechanism of action. However, all data to date suggests that the 7-oxo bridgehead (Region C) is the most important part of the scaffold and crucial for activity. Direct evidence of this is illustrated by removing this moiety from active analogues resulting in derivatives void of cytotoxicity. The presence of the 5,6-ethyl bridge (Region D) is also highly beneficial for activity. There are very limited examples showing addition at region D can result in equipotent analogues, however, the high number of inactive analogues without the 5,6-ethyl

bridge indicate its as important as the 7-oxo bridgehead. An overall summary of norcantharidin SAR is shown below (Figure 1.19).

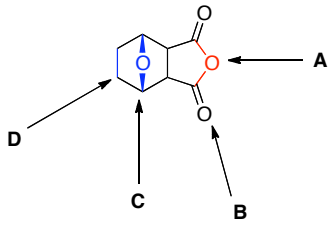


Figure 1.19: Norcantharidin scaffold highlighting areas crucial for anticancer activity (blue), consisting of the 5,6-ethyl bridge (Region D) and the 7-oxo bridgehead (Region C). Areas beneficial for anticancer activity (red) include the anhydride nose in a conformation allowing hydrolysis to yield dicarboxylic acids (Region A).

1.7 Project Aims

It has been established that norcantharidin and its analogues have potential as anti-cancer lead compounds. The synthetically simple scaffold is easily accessed, making structural analogues readily available, a beneficial trait in structure based drug design. There is a great potential for the development of cell line specific norcantharidin analogues, as opposed to the current broad-spectrum norcantharidin inhibitors.

There has been limited work carried out at the carbonyl group (Region B) thus far making it difficult to access any SAR data in detail. However, of the analogues produced at this site (n=3 to date), selectivity towards colon carcinoma (HT29) has been observed. Some analogues have been shown to be more potent against these cell lines than norcantharidin and inactive against all other cell lines. The presence of this selectivity shows great promise in the field of norcantharidin based drug design as selectivity towards the target carcinoma is highly desired to reduce side effects on the human body during treatment.

However, treating cancer is not the only observed use for norcantharidin. Previous reports of norcantharidin used for the treatment of warts, boils, and piles suggest that there are other avenues to explore with norcantharidin analogues. Preliminary data has shown a norcantharidin analogue (Figure 1.20) displays activity against the nematode *Haemonchus contortus* (the barbers pole worm), an intestinal parasite that affects many livestock industries. This parasite has developed an increased resistance to all three main classes of anthelmintics (benzimidazole, levamisole, and ivermectin) currently used within the primary agriculture industry, presenting another biological target and a great potential for future norcantharidin analogue development.[111,112]

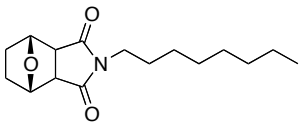


Figure 1.20: Norcantharidin derivative with activity towards *Haemonchus contortus*.

Future development of the synthetic processes used to generate norcantharidin analogues must also be considered. Successful analogues will require subsequent scale up in a quick and efficient manner to facilitate their use in live animal or clinical studies. In order to achieve this, certain flow chemistry methodologies will be developed in a bid to eliminate such issues as variance between large batches, loss of unreacted starting materials, and time consuming manual purification.

This research project will address the following aims:

- Initial focus on the development of norcantharidin analogues at the carbonyl group (Region B) in an attempt to further understand the parameters necessary to achieve desired colon carcinoma (HT29) specificity observed.
- Synthesised analogues in addition to being assessed for anticancer applications will also be tested against *Haemonchus contortus* in an attempt to expand the use of these novel biologically active compounds.
- Develop flow chemistry methodologies to produce norcantharidin analogues on a larger scale in a quick and efficient manner.

1.7 References

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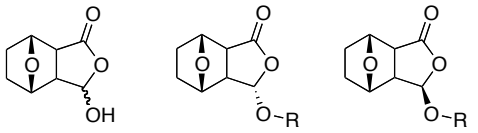
Chapter 2 - Summary of Papers

This thesis is based on eight journal publications, all of which have been accepted for or have actually been published. The complete list of publications is given below in chronological order:

- Campbell, B. E., Tarleton, M., Gordon, C. P., Sakoff, J. A., Gilbert, J., McCluskey, A, Gasser, R. B. (**2011**), Norcantharidin analogues with nematocidal activity in *Haemonchus contortus*. *Bioorganic and Medicinal Chemistry Letters*, 21, 3277-3281.
- Tarleton, M., Gilbert, J., Robertson, M. J., McCluskey, A., Sakoff, J. A. (**2011**), Library synthesis and cytotoxicity of a family of 2-phenylacrylonitriles and discovery of an estrogen dependent breast cancer lead compound. *Medicinal Chemistry Communications*, 2, 31-37.
- Tarleton, M., McCluskey, A. (**2011**), A flow chemistry route to 2-phenyl-3-(1*H*-pyrrol-2-yl)propan-1-amines. *Tetrahedron Letters*, 52, 1583-1586.
- Tarleton, M., Young, K. A., Unicomb, E., McCluskey, S. N., Robertson, M. J., Gordon, C. P., McCluskey, A. (**2011**), A flow chemistry approach to norcantharidin analogues, *Letters in Drug Design and Discovery*. 8, 568-574.
- Tarleton, M., Bernhardt, P. V., McCluskey, A. (**2012**), Crystal structures of (3*R*,3a*R*,4*S*,7*R*,7a*S*)-3-(allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one and (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-[(*E*)-but-2-en-1-yloxy]hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one: confirmation of NMR predicted stereocentre geometry. *Journal of Chemical Crystallography*, 42, 639-644.
- Tarleton, M., Gilbert, J., Sakoff, J. A., McCluskey, A. (**2012**), Synthesis and anticancer activity of a series of norcantharidin analogues. *European Journal of Medicinal Chemistry*, 54, 573-581.
- Tarleton, M., Dyson, L., Gilbert, J., Sakoff, J. A., McCluskey, A. (**2013**), Focused library development of 2-phenylacrylamides as broad spectrum cytotoxic agents. *Bioorganic and Medicinal Chemistry*, 21, 333-347.
- Tarleton, M., Gilbert, J., Sakoff, J. A., McCluskey, A. (**2012**), Cytotoxic 2-phenylacrylnitriles, the importance of the cyanide moiety and discovery of potent broad spectrum cytotoxic agents. *European Journal of Medicinal Chemistry*, 57, 65-73.

2.1 Paper I

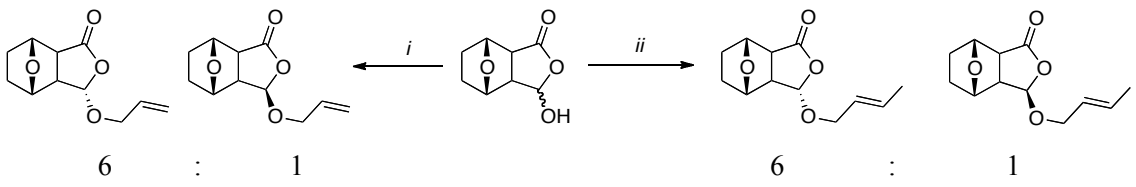
Initially, this project carried on from what had been previously achieved by our research group and others on the medicinal chemistry of norcantharidin. Our particular interest was in the anticancer properties of these molecules. At the commencement of this thesis, only three 'Novo' series analogues had been synthesised with structural modifications of one of the anhydride carbonyl moieties. Although only limited SAR data was obtained, these norcantharidin analogues, named the 'Novo' series (Figure 2.1) displayed selective cytotoxicity towards HT29 (colon) carcinoma cells. This toxicity was confirmed by screening against the NCI 60 cancer cell line panel, where only toxicity towards colon based cell lines was observed. Intrigued by this finding, five focused Novo compound libraries were synthesised and the cytotoxicity against nine human carcinoma cell line assessed and submitted for publication as **Paper I, [**Tarleton, M., Gilbert, J., Sakoff, J. A., McCluskey, A. Synthesis and anticancer activity of a series of norcantharidin analogues. *In Press European Journal of Medicinal Chemistry* (**2012**)]. These libraries consisted of analogues incorporating chain unsaturation, simple alkyl chains (linear and branched), epoxides, and phosphate esters into the Novo scaffold at the lactone position shown below (Figure 2.1).



(**48**) (**64**) (**65**)

Figure 2.1: Structures of Novo parent molecule (**48**) and both possible isomers (**64** and **65**) of Novo derivatives. Analogues varied at position 'R'.

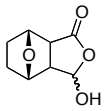
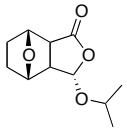
Analogues possessing ether linked allyl and crotyl chains were initially synthesised. Subsequent purification allowed isolation of the stereoisomers as shown (Scheme 2.1). The structure of each isomer was assigned by ^{1}H NMR analysis, and screened separately to determine the difference, if any, in biological activity. Both diastereomers of Novo analogues with unsaturated chains showed no difference in cytotoxicity. All analogues from this library were shown to be less active than the parent norcantharidin derivative (**48**).



Scheme 2.1: Reagents and conditions (i) THF, pTsOH, prop-2-en-1-ol, MW 80 °C, 180 W, 1 hour; (ii) THF, pTsOH, but-2-en-1-ol, MW 80 °C, 180 W, 1 hour. Ratio of isolated epimers for reference.

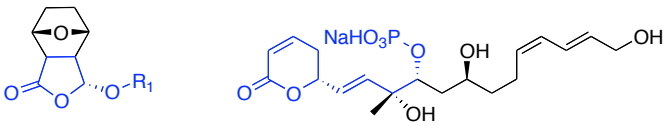
Based on the initial findings of no difference in biological activity between the diastereomers, only the major isomer of each subsequent analogue was assessed in the remaining focus libraries. Aliphatic chain extension at the same position of the Novo scaffold resulted in a drop in activity, however, the introduction of an isopropyl tail moiety (**65**) produced a compound with greater selectivity towards HT29 (colon) and (SJ-G2) (gioblastoma) cells than that shown by the parent Novo analogue (**48**) (Table 2.1) and was devoid of any activity against the remaining tumour cell lines, defined herein as $GI_{50} > 100\mu M$.

Table 2.1: Anticancer activity of Novo and Novo analogue with an isopropyl tail. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **48**	23 ± 4	41 ± 4	34 ± 3	27 ± 1	>100	72 ± 3	90 ± 10	36 ± 0	23 ± 5
 **65**	19 ± 0.9	94 ± 0.8	>100	>100	>100	>100	>100	44 ± 5.4	21 ± 2.6

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2).

As cantharidin and norcantharidin were known protein phosphatase 1 and 2A inhibitors, we examined the protein phosphatase literature and noted that fostrecin (**4**) was the most potent small molecule phosphatase inhibitor thus far reported. Given this and the chemical similarity between the hydroxyl lactone of the Novo scaffold and the α-hydroxy lactone of Fostrecin (Figure 2.2), we designed a simple series of Novo-Fostrecin hybrid analogues in an effort to determine if the introduction of a simple phosphate tail onto the Novo scaffold would improve cytotoxicity.



4

Figure 2.2: Structural similarity between Novo (left) and Fostrecin (**4**) (right) scaffolds highlighted in blue.

Attempts to mimic the activity associated with fostrecin, were however also unsuccessful. Introduction of an epoxide moiety into the ether tail resulted in a reduction in activity compared to the Novo parent. The final structural modification incorporated terminal phosphate esters to the ether tail of the Novo scaffold. Although analogues were produced with a greater structural

similarity to that of Fostrecin, these modifications did not result in any further increases in cytotoxicity.

2.2 Paper II

The publication of **Paper II, (**Tarleton, M., Bernhardt, P. V., McCluskey, A. (**2012**), Crystal structures of (3*R*,3a*R*,4*S*,7*R*,7a*S*)-3-(allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one and (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-[(*E*)-but-2-en-1-yloxy]hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one: confirmation of NMR predicted stereocentre geometry. *Journal of Chemical Crystallography*, 42, 639-644), addressed the issue associated with the Novo series of analogues within this thesis, that is the stereochemistry at C3 (Figure 2.3). While the initial Novo analogues had been screened for PP1, PP2A, and anticancer activity, and previously reported, the C3 stereochemistry of the most active compounds was not determined and thus hampered the rational drug design process with this series of compounds. In this thesis, the stereochemistry at C3 was assigned *via* ^{1}H NMR analysis of the coupling constant assigned to the hydrogen at C3 that couples with the hydrogen at C3a, with coupling constants in the order of 1.7 Hz (major isomer) and 6.8 Hz (minor isomer) observed. Apart from this slight difference, the spectrum between each isolated diastereomeric pair was almost identical.

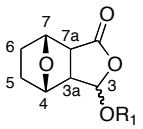
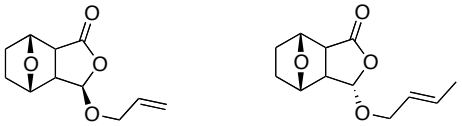


Figure 2.3: Chemical structure and nomenclature of Novo series scaffold.

During the course of this thesis we were able, for the first time, to grow crystals suitable for X-ray crystallographic analysis. Crystals of both diastereomers with different ^{1}H coupling assigned to the C3 hydrogen were submitted for X-ray crystallographic analysis. Compounds (3*R*,3a*R*,4*S*,7*R*,7a*S*)-3-(allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**66**) and (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-((*E*)-but-2-en-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**67**) (Figure 2.4) enabled an illustrative example of each diastereomeric pairings crystal structure to be solved. This data correlated well with our original ^{1}H NMR determination. Based on this, all subsequent stereochemical assignment was based on ^{1}H NMR analysis.



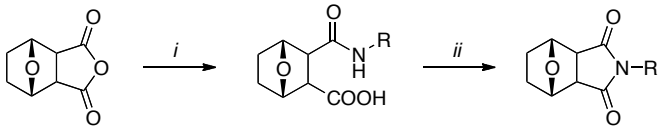
Minor	Major
J = 6.8 Hz	J = 1.7 Hz
angle = 11°	angle = 109°
66	**67**

Figure 2.4: Differences in Minor and Major isomer spectral data including ^{3}J (Hz) and Dihedral angles (°) and Chemical structures of (3*R*,3a*R*,4*S*,7*R*,7a*S*)-3-(allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**66**) and (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-((*E*)-but-2-en-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**67**).

The structural determination of each possible diastereomer also assisted in the explanation of the observed 6:1 isomer ratio. Knowing which diastereomer was favoured, it was concluded that these reactions proceed via an oxonium-stabilised intermediate and followed an S_N1 like mechanism. With the stabilised carbocation located at C3, the corresponding nucleophile, in this case an alcohol, is able to attack from either above the plane from the side of the oxo bridgehead, or alternatively from below the plane of the norcantharidin scaffold. In this case, attack from below the plane is far more likely as it is far less sterically hindered, lower polar surfaces, and gives rise to the more thermodynamically stable isomer in the observed 6:1 ratio.

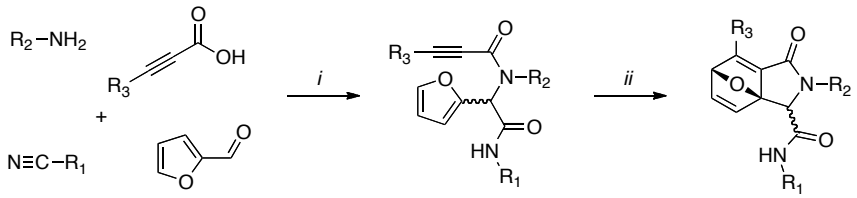
2.3 Paper III

Paper III, (Campbell, B. E., Tarleton, M., Gordon, C. P., Sakoff, J. A., Gilbert, J., McCluskey, A, Gasser, R. B. (**2011**), Norcantharidin analogues with nematocidal activity in *Haemonchus contortus*. *Bioorganic and Medicinal Chemistry Letters*, 21, 3277-3281), involved the design and synthesis of norcantharidin based analogues to be biologically assessed as potential anti-parasitic agents. The first library of anhydride ring opened norcantharidin analogues, primarily added aliphatic chains and substituted aromatic ring moieties through the anhydride nose *via* amide linkages (Scheme 2.2). Subsequent heating of these analogues resulted in the formation of a second library of norcantharidin analogues, with these derivatives possessing a *N*-substituted cyclic imide moiety (Scheme 2.2).



Scheme 2.2: Reagents and conditions (i) THF, 1.1eq amine, R.T, 12 hours; (ii) $PhCH_3$, sealed tube, 140 °C, 36 hours.

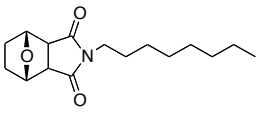
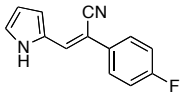
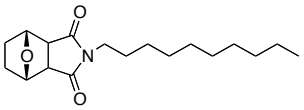
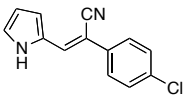
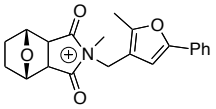
The final focus library of this publication consisted of producing analogues via a four-component Ugi reaction followed by an intramolecular Diels-Alder cycloaddition (Scheme 2.3). However, due to issues of reproducibility within the anti-parasitic biological assay, a library of compounds with known activity was synthesised and sent as an internal standard along with the three norcantharidin based focus libraries of compound.



Scheme 2.3: Reagents and conditions (i) MeOH, alkynoic acid, furan carbaldehyde, R_2-NH_2, isocyanide, R.T, 30 minutes; (ii) $PhCH_3$, sealed tube, 200 °C, 36 hours.

Of the 54 compounds analysed for anti-parasitic activity, three analogues displayed activity against *Haemonchus contortus* with associated LD_{50s} between 25-40 μM (Table 2.2). Interestingly, all three of the active analogues came from the same norcantharimide library, two with long aliphatic chians (**68**) (**69**) and one with an aromatic furan linked tail (**70**). The success rate of 5.6% associated with this screening is far higher than conventionally used drug screening methods usually employed. These three active compounds are also well suited for further development as they are synthesised in two steps, are low cost, and of high purity without further purification.

Table 2.2: Antiparasitic activity of three active norcantharimide analogues (**68**, **69**, and **70**) and two internal standard acrylonitriles (**71** and **72**) against *Haemonchus contortus*. Values are LD_{50} (μM) and LD_{90} (μM) values, the concentration that is a lethal dose to 50% and 90% of the biological population respectively relative to an untreated control.

Structure	LD_{50} (μM)	LD_{99} (μM)	Structure	LD_{50} (μM)	LD_{99} (μM)
 **68**	40	80	 **71**	10	50
 **69**	30	90	 **72**	10	25
 **70**	25	100			

As part of a toxicity pre-filter, all new anti-parasitic compounds are screened against a panel of ten cancer cell lines to ensure the end user is not subjected to toxic compounds being applied in a non-ideal environment such as farming communities.

2.4 Paper IV

Surprisingly, analogues from the small acrylonitrile library, originally used as an internal standard, were shown to be highly cytotoxic warranting further investigation. The first section of these further investigations was published in **Paper IV**, (Tarleton, M., Gilbert, J., Robertson, M. J., McCluskey, A., Sakoff, J. A. (**2011**), Library synthesis and cytotoxicity of a family of 2-phenylacrylonitriles and discovery of an estrogen dependent breast cancer lead compound. *Medicinal Chemistry Communications*, 2, 31-37). In this work we identified a novel cytotoxic scaffold in which we determined three key structural features. This consisted of aromatic moiety one (Ar_1) corresponding to the aldehyde component added in the Knoevenagel condensation part of the synthesis, aromatic moiety two (Ar_2) corresponding to the benzyl nitrile used, and the conjugated cyano linker region between them (Figure 2.5).

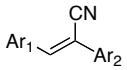
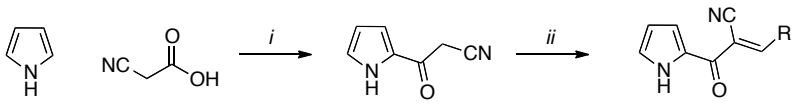


Figure 2.5: Generic structural representation of the α,β-unsaturated acrylonitrile scaffold.

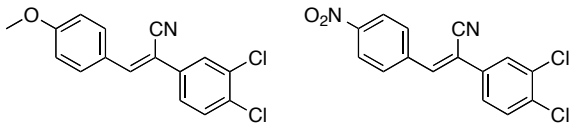
To explore the structure activity relationships (SAR) associated with cytotoxicity of this class of compounds, a series of focused compound libraries were synthesised. The first of these libraries examined the effect of removing the alkene bond and consequently conjugation between both aromatic moieties. Thus, flow hydrogenation approaches were developed and the reduced compounds evaluated for cytotoxicity. In all instances, double bond removal voided all activity.

Next, we examined the effect of removing the aromatic moiety (Ar_1). This was achieved *via* the introduction of an aliphatic chain in place of an aromatic ring in this position. Using the most active nitrile, 2-(3,4-dichlorophenyl)acetonitrile as aromatic moiety (Ar_2), the incorporation of aliphatic chians at (Ar_1) resulted in all activity being lost indicating the need for extended conjugation throughout the molecule in order to retain cytotoxicity. To further test this, a carbonyl spacer was introduced between Ar_1 and the alkene bond. Keeping Ar_1 as a pyrrole ring, a small focused library was synthesised (Scheme 2.4). Although this resulted in loss in activity, it further highlighted the importance of a conjugated system between two aromatic groups is for biological activity.



Scheme 2.4: Reagents and conditions (i) Ac_2O, 75 °C, 35 minutes; (ii) EtOH, piperidine (cat), RCHO, reflux, 2 hours.

With a wide range of aldehydes available, the final synthetic modification assessed was the reintroduction of an aromatic moiety at the Ar_1 position. This produced multiple compounds of interest as both broad spectrum as well as cell line specific inhibitors. Of particular note was 2-(3,4-dichlorophenyl)-3-(4-methoxyphenyl)acrylonitrile (**73**) returning GI_{50} values of 0.52-3 μM across all cell lines tested (Figure 2.6). Another analogue, 2-(3,4-dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**74**) (Figure 2.6), displayed up to 543 fold selectivity with a GI_{50} of 0.127 ± 0.0043 μM towards estrogen receptor positive human breast cancer cell line, MCF-7.

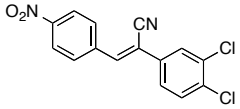


73 **74**

Figure 2.6: Chemical structures of 2-(3,4-dichlorophenyl)-3-(4-methoxyphenyl)acrylonitrile (**73**) and 2-(3,4-dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**74**).

Further examination of (**74**) against an additional estrogen receptor negative breast cancer cell line, (MDA-MB231), and a normal non-tumourigenic breast epithelial cell line, (MCF-10A), resulted in poor growth inhibition of 34 ± 2 and 16 ± 4 μM respectively showing a ~268 and ~126 fold preference for MCF-7 estrogen dependent breast cancer cells (Table 2.3).

Table 2.3: Cytotoxicity of (**74**) against a panel of breast cell lines. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	MCF-7[a]	MDA-MB231[b]	MCF10A[c]
 **74**	0.127 ± 0.043	34 ± 2	16 ± 4

[a] ER+ve Breast carcinoma (MCF-7), [b] ER-ve Breast carcinoma (MDA-MB231), [c] Breast epithelial (MCF-10A).

2.5 Paper V

Paper V, (Tarleton, M., McCluskey, A. (**2011**), A flow chemistry route to 2-phenyl-3-(1*H*-pyrrol-2-yl)propan-1-amines. *Tetrahedron Letters*, 52, 1583-1586), details the methods in which the nitrile analogues, presented in Paper IV, with varying stages of reduction were synthesised from the corresponding α,β-unsaturated acrylonitriles. Traditionally hydrogenations of this manner were carried out in a number of ways. Both the Parr pressurised reaction

apparatus and hydrogen balloon and septum methods employ the use of a hydrogen atmosphere sourced from a hydrogen gas cylinder and a heterogeneous catalyst stirred throughout the solution. The major disadvantages of these methods is that they require a bulky equipment setup, including a hydrogen gas cylinder, suffer from varying product yields between batches, and more importantly require handling and isolation of hazardous catalysts from the desired reaction mixture, a process that reduces isolated product yield and increases reaction workup periods.

With only milligram quantities originally needed of new analogues for full characterisation and biological screening, gram scale hydrogenations were initially inappropriate, however the potential to produce desired analogues on this scale was still required to meet synthetic requirements for any subsequent animal studies.

The ThalesNano H-cube® flow hydrogenator alleviated all of the issues normally associated with hydrogenations. Conditions such as flow rate, temperature and pressure are precisely controlled resulting in far less side reactions and undesired product formation, catalysts are isolated within prepacked cartridges and can be replaced and changed without exposure to the catalyst. Importantly, the potential to scale up to any amount of desired product is present by simply allowing the system to process the flow stream for longer time frames.

The initial substituted acrylonitrile library contained two separate sites applicable for reduction, the olefin double bond, and the nitrile moiety (Figure 2.7). A series of optimisation experiments changing the pressure (bar) and temperature (°C) produced a set of experimental conditions that selectively and quantitatively hydrogenated the olefin double bond. By further changing the catalyst, solvent, and reaction conditions quantitative hydrogenation of both the olefin and nitrile moieties was achieved yielding the corresponding saturated primary amines.

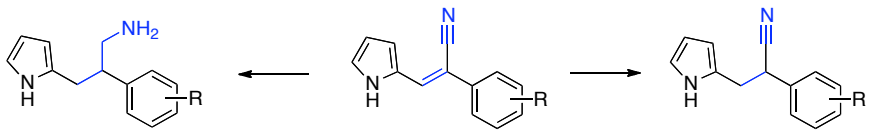


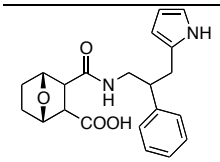
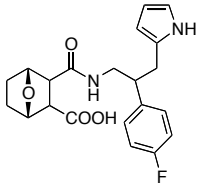
Figure 2.7: Location of the two sites applicable for reduction within the α,β-unsaturated acrylonitriles.

2.6 Paper VI

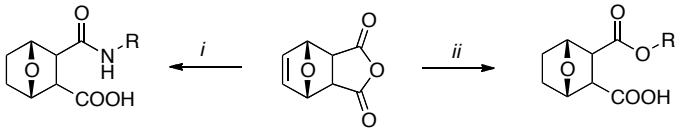
The versatility of these techniques illustrated in Paper V were further developed and expanded to rapidly synthesise norcantharidin based analogues using a flow reactor apparatus. This was achieved in **Paper VI**, (Tarleton, M., Young, K. A., Unicomb, E., McCluskey, S. N., Robertson, M. J., Gordon, C. P., McCluskey, A. (**2011**), A flow chemistry approach to norcantharidin

analogues, *Letters in Drug Design and Discovery*, 8, 568-574), and involved the *in-situ* hydrogenation and addition of amines including those formed in Paper V, with another synthetic scaffold, norcantharidin, resulting in the formation of novel protein phosphatase 1 and 2A inhibitors (**75**) and (**76**) (Table 2.4).

Table 2.4: Inhibition of PP1 and PP2A by nitrile and norcantharidin hybrid analogues. [a] IC_{50} (μM) values are the compound concentration that inhibits enzyme activity by 50% relative to an untreated control.

Structure	PP1 IC_{50} (μM)[a]	PP2A IC_{50} (μM)[a]
 **75**	14.1 ± 7.90	18.5 ± 3.5
 **76**	4.5 ± 1.55	7.25 ± 1.25

This synthetic technique was not only limited to the addition of amines to 5,6-dehydronorcanatharidin (**78**) to form the corresponding amide ring opened analogues (**77**). It could also adapted to add low boiling point alcohols when used as the solvent to form the corresponding ester ring opened analogues (**79**) (Scheme 2.5).



77 **78** **79**

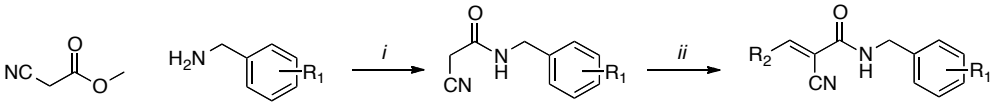
Scheme 2.5: Reagents and conditions (i) 0.055M solution of RNH_2 added to 0.05M solution of (**78**), H-cube, 50 bar, 50 °C, 1 mL/min, 10% Pd/C; (ii) 0.05M solution of (**78**) in ROH, H-cube, 50 bar, 50 °C, 1 mL/min, 10% Pd/C.

2.7 Paper VII

Paper VII, **[**Tarleton, M., Dyson, L., Gilbert, J., Sakoff, J. A., McCluskey, A., Focused library development of 2-phenylacrylamides as broad spectrum cytotoxic agents. Submitted to *Bioorganic and Medicinal Chemistry* (**2012**)], followed the preliminary investigation of Paper IV by further examining the effect of interrupting the conjugation between both aromatic moieties. The synthesis of two libraries, the first with a pyrrole ring as Ar_1, the second with an indole ring in the same location were synthesised, adding a carbonyl group between Ar_1 and the cyano alkene. This structural change largely resulted in the elimination of all biological activity, however the bis-indole analogue produced excellent broad spectrum activity with an average

GI_{50} of 11.0 μM across all carcinoma cell lines indicating that some degree of spacing and conjugation at this location is tolerated.

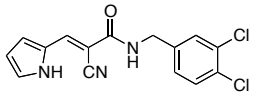
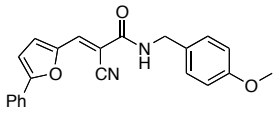
Next, interrupting the conjugation between the acrylonitrile and Ar_2 was investigated. Initially evaluating pyrrole and furan analogues, multiple focused compound libraries were developed with an amide linkage employed to achieve the desired conjugation interruption (Scheme 2.6).



Scheme 2.6: Reagents and conditions (i) MeOH, MW 120 °C, 200W, 15 minutes; (ii) EtOH, piperidine (cat), RCHO, MW 120 °C, 200W, 15 minutes.

This structural manipulation produced two areas of interest. Firstly two compounds (**80**) and (**81**) showed excellent selectivity towards the MCF-7 (breast) cell line returning GI_{50} values of 6.0 ± 1 and 3.0 ± 1 μM respectively (Table 2.6). These compounds are inactive against the remaining tested cell lines returning GI_{50} values of >50 μM. With significant structural variance between these two analogues it suggests that the presence and positioning of electron density on both Ar_1 and Ar_2 affects the ability to target MCF-7 (breast) selectively.

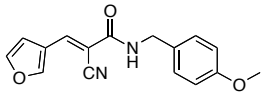
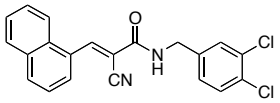
Table 2.6: Anticancer activity of MCF-7 selective acrylamide analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **80**	>50	>50	6±1	>50	>50	>50	>50	>50	>50
 **81**	>50	>50	3±1	>50	>50	>50	>50	>50	>50

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2).

A further two compounds of interest, (**82**) and (**83**), displayed excellent broad spectrum biological activity with average GI_{50} values of 7.0 and 9.6 μM respectively (Table 2.7). The development of these amide derivatives again demonstrates that the cytotoxicity of these analogues is not entirely due to electron withdrawing effects with other characteristics such as orientation of electron density and lone electron pairs also being of importance.

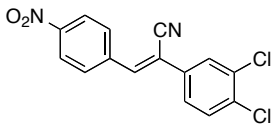
Table 2.7: Anticancer activity of active acrylamide analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **82**	6±1	8±0	7±1	6±0	8±0	5±0	7±0	7±0	8±0
 **83**	7±0	11±0	8±1	7±0	7±0	8±0	12±1	7±0	13±1

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2).

2.8 Paper VIII

The final publication **Paper VIII**, **[**Tarleton, M., Gilbert, J., Sakoff, J. A., McCluskey, A. Cytotoxic 2-phenylacrylnitriles, the importance of the cyanide moiety and discovery of potent broad spectrum cytotoxic agents. Submitted to *European Journal of Medicinal Chemistry* (**2012**)] also follows the initial research carried out in Paper IV based on the acrylonitrile scaffold. Using the most selective inhibitor of estrogen receptor positive (ER +ve) human breast cancer cell line MCF-7 (**74**) (Figure 2.8), as a new compound lead, new analogues were designed and synthesised to further test the structural tolerance of the acrylonitrile library.

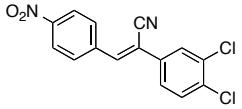
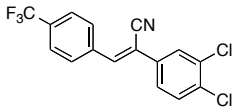
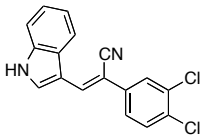


74

Figure 2.8: Chemical structure of 2-(3,4-dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**74**).

Initially, increasing electronegativity and electron density on aromatic moiety one (Ar_1) was assessed. A reduction in activity due to the presence of a trifluoromethyl moiety at this position (**80**) showed there was a limit to the degree of electronegativity required. Interestingly, the introduction of an indole ring in the same location produced the most potent broad spectrum inhibitor to date (**81**) with an average GI_{50} of 1.4 μM (Table 2.8). Synthetic manipulation afforded two derivatives with the cyano group replaced with a carboxylic acid. This resulted in a loss in all activity further highlighting the pivotal nature of the conjugated acrylonitrile moiety.

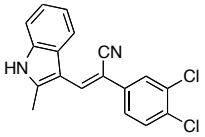
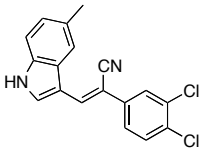
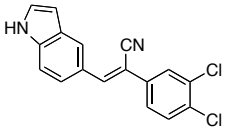
Table 2.8: Anticancer activity of active acrylonitrile analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **74**	3.1±1.8	8.4±3.3	0.127 ±0.043	12±1	69±2	7.1±0.1	18±3	8.9±0.7	14±1
 **80**	35±6	49±13	31±6	41±9	36±1	53±14	45±13	43±9	41±10
 **81**	0.85 ±0.1	2.1±0.1	1.3±0.0	0.9±0.1	1.0±0.1	0.8±0.0	3.1±0.1	1.0±0.1	1.7±0.1

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2).

With previous success attained from incorporating substituted furan carbaldehydes and now the discovery of the indole acrylonitrile derivative (**81**), two final focused libraries were developed based on these two scaffolds. In all furan derivatives assessed no noteworthy percentage inhibition was reached at 25 μM that warranted full GI_{50} determination. However, derivatives based on the indole scaffold were highly cytotoxic. Methylation of the indole scaffold at positions 2 (**82**) and 5 (**83**) were tolerated returning average GI_{50} values of 2.9 and 4.0 μM respectively (Table 2.9). The most interesting result of the library came from the C5-substituted indole (**84**), which returned the most potent average GI_{50} value of 0.53 μM across all carcinoma cell lines (Table 2.9). This suggests that both the positioning of electron density and the lone pair of electrons capable of participating in hydrogen bonding interactions on Ar_1 is of importance for broad spectrum cytotoxicity.

Table 2.9: Anticancer activity of selected active indole acrylonitrile analogues. Values are GI_{50} (μM) values, the concentration that reduces cell growth by 50% relative to an untreated control.

Structure	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]
 **82**	1.9±0	3.8±0.4	2.8±0.2	2.2±0.4	2.2±0.2	2.1±0.1	5.6±0.6	2.0±0	4.0±0.5
 **83**	2.8±0.3	4.7±0.6	3.7±0.2	3.2±0.2	3.0±0.1	3.0±0.1	7.4±1	3.4±0.2	5.3±0.5
 **84**	0.36±0.0	0.67±0.0	0.23±0.0	0.41±0.0	0.39±0.0	0.37±0.1	1.04±0.3	0.33±0.1	0.83±0.2

[a] Colon carcinoma (HT29, SW480), [b] Breast carcinoma (MCF-7), [c] Ovarian carcinoma (A2780), [d] Lung carcinoma (H460), [e] Skin carcinoma (A431), [f] Prostate carcinoma (DU145), [g] Neuroblastoma (BE2-C), [h] Glioblastoma (SJ-G2).

2.9 Overall Conclusions

In conclusion, research undertaken within this thesis has led to the discovery of multiple novel anticancer drug lines containing multiple lead compounds for further study and development.

Initially libraries based on the small molecule anticancer lead, norcantharidin, were synthesised and screened, however at the commencement of this thesis, the exact stereochemistry of these analogues was assumed but unconfirmed. The synthetic pathway utilised for these analogues produced two possible products and, depending on the substituent added, could be isolated and evaluated separately. The ratio between the two possible isomers was observed experimentally to be 6:1 in all cases, which correlated with the primary findings of others. This suggested that in the reaction mechanism, there was a clear preference to one reaction pathway over another.

Solving a crystal structure of each possible isomer alleviated the stereochemical identity issue, and the chemical structure of subsequent analogues could be assigned based on the correlation between the solved crystal structure and ^{1}H NMR analysis. Once the true identity of these analogues was determined, the difference in biological activity between isomers was assessed. In all cases analysed, there was no significant difference in biological activity between the two stereoisomers. Knowing this, only the major isomer was assessed with future derivatives allowing acceleration of the purification process and production of SAR data much faster on a largely untouched area of the norcantharidin scaffold.

Of the different structural modifications examined on the Novo/norcantharidin lead compound, the most potent analogue resulted from the addition of an ether linked isopropyl moiety (**65**). This analogue displayed greater activity against HT29 (colon) and SJ-G2 (glioblastoma) cells than the parent Novo analogue with GI_{50} values of 19 ± 0.9 and 21 ± 2.1 μM respectively against these lines (Table 2.1). Of greatest interest was that this compound was completely void of biological activity against all the other carcinoma cell lines examined showing improved cell line selectivity relative to the parent Novo molecule.

Initially, this thesis was based on the synthesis and anticancer evaluation of norcantharidin analogues. As norcantharidin analogues have been shown to trigger other types of biological activity, they were also tested against *Haemonchus contortus* in an attempt to expand the use of these novel biologically active compounds. As with all new compounds within the research group, they are always evaluated for cytotoxicity to avoid any ultimate end users from being exposed to potentially dangerous compounds. This resulted in the serendipitous discovery of a new family of lead compounds. Used as internal standards in an anti-parasitic bioassay, the first library of α,β-unsaturated acrylonitrile compounds were highly cytotoxic with selected analogues showing selectivity towards estrogen receptor positive MCF-7 (breast) carcinoma.

Focused library development based on the initial acrylonitrile scaffold, produced six compounds with a high degree of selectivity towards the MCF-7 cells of up to 543 fold over all nine other carcinoma cell lines tested against (Figure 2.9). Some of these compounds were further shown to selectively target estrogen dependent MCF-7 cells that over express the estrogen receptor (ER+ve) over estrogen receptor negative carcinoma (MDA-MB231) and non-malignant breast tissue (MCF10A) up to 268 and 126 fold respectively.

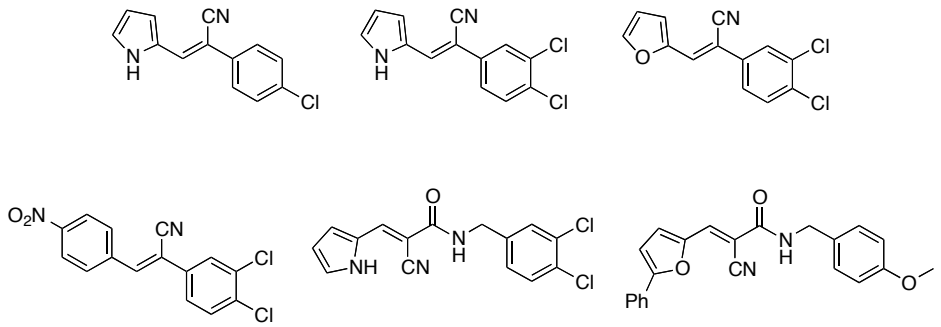


Figure 2.9: Chemical structures of the six compounds displaying selectivity towards the MCF-7 breast cancer cell line.

The acrylonitrile compound library development component of this thesis also produced five highly cytotoxic broad spectrum compounds with average GI_{50} values of 0.53-2.1 μM across all carcinoma cell lines examined (Figure 2.10).

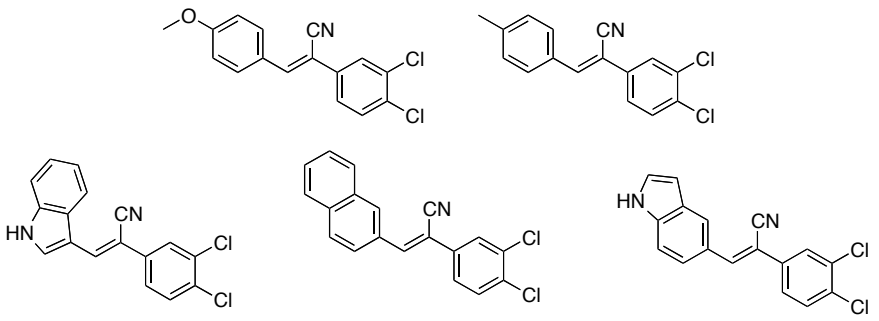


Figure 2.10: Chemical structures of the five acrylonitrile analogues displaying broad spectrum cytotoxicity against the panel of nine cancer cell lines evaluated in this thesis.

These eleven compounds also show that there is some degree of structural variation tolerated in certain positions of the acrylonitrile scaffold suggesting that there are still multiple areas that can be modified and explored in the future.

The flow reactor based synthetic methods employed were a fundamental component of this research project. The use of the ThalesNano H-cube® flow hydrogenator alleviated the associated issue with traditional methods of batch wise hydrogenations. Making simple changes in pressure, temperature, and catalyst led to the rapid optimisation of reaction conditions that produced the highest yield of the desired product. These conditions were used on a larger scale to produce a desired amount of each acrylonitrile derived analogue for biological testing by simply passing through more reagent solution through the self contained catalyst bed. This resulted in the novel selective reduction of one or both possible sites on the acrylonitrile compounds and ten new derivatives for biological evaluation.

Some of these novel reduced compounds were further developed in other projects leading to the discovery of novel protein phosphatase (PP1 and PP2A) inhibitors developed within the research group. The development of these flow reduction chemistry techniques along with the discovery of multiple compound leads, indicates that the research within this project has a range of applications that can be incorporated and developed further in multiple new research projects in the future.

Chapter 3 – Publications

3.1 Paper I

European Journal of Medicinal Chemistry 54 (2012) 573–581

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journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and anticancer activity of a series of norcantharidin analogues

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ABSTRACT

Cantharidin (**1**) and norcantharidin (**2**) display high levels of anticancer activity against a broad range of tumour cell lines. Synthetic manipulation of norcantharidin yields (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**), which also displays a high level of anticancer activity against tumour cells but interestingly, shows selectivity towards HT29 (colon; $GI_{50} = 14$ μM) and SJ-G2 (glioblastoma; $GI_{50} = 15$ μM) cell lines. Substitution at the hydroxyl group of the cyclic lactone within (**3**) produces a diasteromeric pair of products that have no difference in cytotoxicity over the cell lines tested. Incorporation of an isopropyl tail at this position (**16**) produced the most promising compound of this series to date, with strong selectivity towards HT29 (colon; $GI_{50} = 19$ μM) and SJ-G2 (glioblastoma; $GI_{50} = 21$ μM) cell lines but completely void of any activity against the remaining tumour cell lines ($GI_{50} > 100$ μM), as per the parent molecule. We also discovered that the introduction of a terminal phosphate moiety (**28**) at the same position produced a different trend in cytotoxicity with strong activity in BE2-C (neuroblastoma; $GI_{50} = 9$ μM) cells; suggestive of an alternate mode of action.

1. Introduction

Cantharidin, *exo*,*exo*-bicyclo[2.2.1]heptane 2,3-dicarboxylic acid anhydride (**1**) is found in approximately 1500 species of Mylabris (blister beetles) (Fig. 1). The dried body of blister beetles can comprise up to 6% by weight of cantharidin. Upon contact with the skin, cantharidin induces rapid blistering, giving rise to their common name, blister beetles. For the past 2000 years cantharidin in the form of Mylabris has been used in Chinese traditional medicine. Cantharidin has been used for the treatment of a number of conditions, but arguably the most important is its reported use as a treatment of hepatoma and oesophageal carcinoma with the first recorded incidence of this usage in 1264. In Western society cantharidin is known as the active ingredient of the purported aphrodisiac "Spanish fly" and used topically for the treatment of warts. Generally, however Western medicine has decreed cantharidin too toxic for internal use due to the induction of nephrotoxicity. Interestingly cantharidin does not induce myleosuppression, a toxicity commonly seen with most anticancer chemotherapies. Such toxicity is dose limiting and significantly reduces the efficacy of chemotherapy treatment [1–4].

Norcantharidin (**2**), the demethylated analogue of cantharidin also possesses anticancer activity and stimulates the bone marrow, however, the nephrotoxicity associated with cantharidin treatment is absent (Fig. 1) [1,5]. Norcantharidin is active *in vitro* against several tumour cell lines including cervical, hepatoma, ovarian, laryngocarcinoma, colon, osteocarcinoma, and leukaemia cell lines [1,5,6]. However, our own studies have shown norcantharidin to be approximately 10 fold less cytotoxic than cantharidin in many of these cell lines. Norcantharidin has also been used *in vivo* in the treatment of primary hepatoma, oesophageal, gastric and cardiac carcinomas [1].

The anticancer activity of cantharidin (**1**) and norcantharidin (**2**) is thought to come from the inhibition of protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A), two phosphatases that are known to be involved in many different cellular processes, including cell cycle progression and control of mitotic events [2,7–10].

While there are many other naturally derived compounds known to inhibit PP1 and PP2A, fostriecin (**4**) is most important as it displays broad spectrum anticancer activity and has undergone phase I clinical trial assessment. These trials however, produced poor results because of its susceptibility to oxidative degradation resulting in a short plasma half life of 30 min [8,11–14].

In an effort to mimic some of these desired attributes shown by fostriecin, synthetic modification of norcantharidin to yield (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (Novo-6; **3**), a cyclic lactone that shares some structural similarity with fostriecin was examined (Fig. 2). The introduction of a cyclic lactone moiety adversely affects the ability of this new lead,

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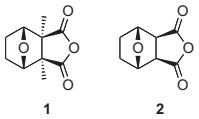
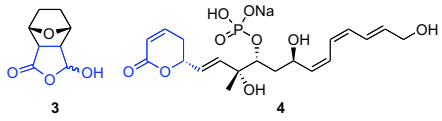


1 2

Fig. 1. Chemical structure of cantharidin (**1**) and norcantharidin (**2**).



3 4

Fig. 2. Chemical structure of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**) and fostriecin (**4**).

3, to ring open to the dicarboxylic acid rendering it PP1 and PP2A inactive. This is in keeping with our and Bertini et al. reports on the importance of anhydride ring opening in relation to the PP1 and PP2A inhibition by the (nor)cantharidin series of compounds [15,16]. This new lead compound is also applicable for further chain extension, in an effort to produce more stable cytotoxic derivatives based on fostriecin.

Previous efforts using the norcantharidin backbone in producing cytotoxic compounds has had limited success with few exceptions [6,17–28]. Our initial screening of **3** showed a surprising degree of colon cancer cell line (HT29) selectivity [6,29], we now show that (**3**) is also potent in glioblastoma cells (SJ-G2) (Table 1).

We thus set about examining the effect of varying the side chain and the cytotoxicity of this class of norcantharidin analogues [6,17].

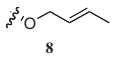
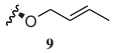
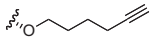
2. Chemistry

The synthesis of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**) from its precursor (3a*R*,4*S*,7-*R*,7a*S*)-3a,4,7,7a-tetrahydro-4,7-epoxyisobenzofuran-1,3-dione (**5**) proceeded as a one step reduction in wet ethanol in the presence of catalytic Pd/C under 4 bar of H_2 as shown in Scheme 1. Subsequent filtration through a celite bed and recrystallisation afforded (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**) in moderate to good yields (40–65%). While this is a robust method for the synthesis of **3**, the isolated yield was variable, a consequence of changes in the water content of the hydrogenation solvent.

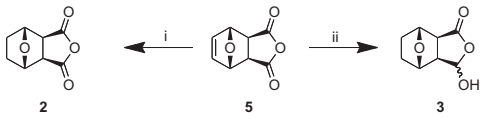
With the cyclic lactone (**3**) in hand, we initially investigated the development of three focused libraries by treatment of **3** with corresponding commercially available alcohols that consisted of either unsaturated (Library A), straight chained (Library B) or branched (Library C) tails as shown in Scheme 2.

We have previously shown that modification in this manner of the norcantharidin backbone can be achieved via an S_N2 directed substitution reaction between an alcohol and (**3**) in the presence of catalytic pTsOH at reflux with the alcohol acting as both the reagent and solvent for the reaction. However this synthetic route was only effective with low boiling point alcohols that could be separated from the desired analogues *in vacuo*. Hence, a new methodology was employed where a solution of **3**, the desired alcohol and pTsOH in THF was heated under microwave radiation (see experimental) to afford the desired norcantharidin analogue with only residual

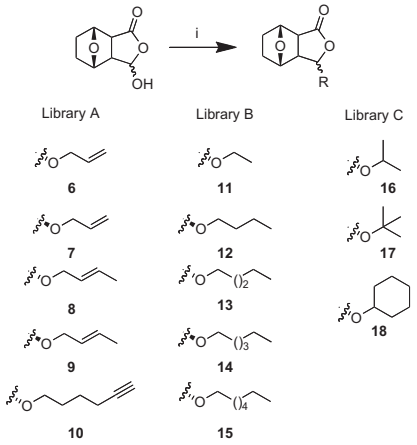
Table 1
Anticancer activity of norcantharidin (**2**), (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**) and unsaturated analogues (**6**–**10**). Values in are GI_{50} (μM) concentrations.

Compound	HT29[a]	SJ-G2[b]	BE2-C[c]	SW480[a]	MCF-7[d]	A2780[e]	H460[f]	A431[g]	DU145[h]
Norcantharidin **2**	23 ± 4	23 ± 5	36 ± 0	41 ± 4	34 ± 3	27 ± 1	>100	72 ± 3	90 ± 10
Novo-6 **3**	14 ± 3	15 ± 4	28 ± 2	>100	>100	>100	>100	>100	>100
 **6**	nd	nd	nd	nd	>100	nd	>100	>100	>100
 **7**	nd	nd	>100	>100	>100	>100	>100	>100	>100
 **8**	42 ± 5	55 ± 3	84 ± 5	93 ± 8	77 ± 10	57 ± 5	>100	>100	>100
 **9**	31 ± 4	35 ± 4	63 ± 11	>100	>100	>100	>100	>100	>100
 **10**	51 ± 1	55 ± 0	>100	>100	>100	>100	>100	>100	>100

nd = not determined.
[a] Colon carcinoma.
[b] Glioblastoma.
[c] Neuroblastoma.
[d] Breast carcinoma.
[e] Ovarian carcinoma.
[f] Lung carcinoma.
[g] Skin carcinoma.
[h] Prostate carcinoma.



Scheme 1. Reagents and conditions: (i) dry acetone, 4 bar H_2, 10% Pd/C 48 h; (ii) Wet EtOH, 4 bar H_2, 10% Pd/C, 48 h.

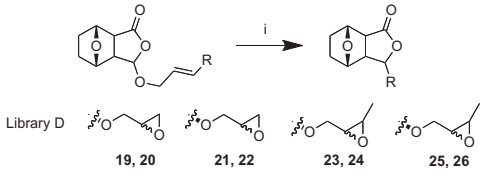


Scheme 2. Reagents and conditions. (i) ROH, cat. p-TsOH, THF, 80 °C, MW – see experimental for details.

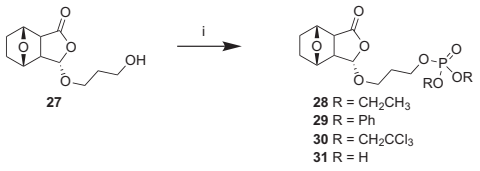
amounts of the used alcohol remaining which was removed by subsequent chromatography.

Analogues with unsaturated tails were also modified to incorporate an epoxide ring. This was carried out in an attempt to achieve some of the broader spectrum anticancer activity shown by other small molecule natural products such as Fostriecin. Reaction of the unsaturated analogues (**6**–**9**) with *m*-chloroperbenzoic acid as shown in Scheme 3, produced epoxide analogues (Library D) in moderate to excellent overall yield (26–85%).

Analogues with a terminal phosphate moiety were also synthesised in a final attempt to mimic the biological activity associated with Fostriecin. Reaction of a terminal hydroxyl norcantharidin analogue (**27**) with various chlorophosphates in the presence of dibutyltin oxide produced the desired analogues (Library E) in good yields (50–78%). Further synthetic manipulation of analogue (**30**) produced a final analogue with the phosphate esters cleaved resulting in a terminal free phosphoric acid moiety (**31**) (Scheme 4).



Scheme 3. Reagents and conditions. (i) m-CPBA, CH_2Cl_2, 0 to rt °C, 16 h.



Scheme 4. Reagents and conditions: (i) Bu_2SnO, CH_2Cl_2, Cl—P(OR)$_2$, TEA.

3. Cytotoxicity

We are interested in the development of anti-cancer agents that show specificity towards a particular cancer type, such as **3**, which displayed high levels of colon cancer specificity, as well as agents that show a broad range of toxicities across an array of cancer cell lines. We have a particular interest in cancers with poor prognosis and have developed an in-house panel of cancer cell lines to identify compounds that may prove beneficial in the treatment of such cancers. With the desired analogues synthesized, we examined their cytotoxicity against a panel of nine human tumour cell lines: HT29 and SW480 (colon carcinoma), MCF-7 (breast carcinoma), A2780 (ovarian carcinoma), H460 (lung carcinoma), A431 (skin carcinoma), DU145 (prostate carcinoma), BE2-C (neuroblastoma) and SJ-G2 (glioblastoma). All analogues were initially screened at a dose of 100 μM and those that induced appreciable growth inhibition underwent full dose response analysis in order to obtain a GI_{50} value.

4. Results and discussion

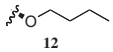
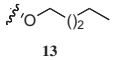
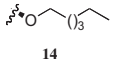
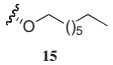
Evaluation of the cytotoxicity data indicated that no particular library of analogues produced significantly different levels of activity than any other. However, there were some compounds that showed comparable activity to the parent norcantharidin derivative (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**3**) or in some cases greater activity in particular cell lines.

The first library assessed, Library A, consisting of compounds with unsaturation within the newly formed tail produced some interesting data in its own right (Table 1). With these compounds being the first to be synthesized, both possible isomers were isolated and separately tested to determine if there was a difference in cytotoxicity attributed to the molecular orientation of the stereocentre. Both isomers comprising of an allyl tail (**6**, **7**) produced a similar growth inhibition profile with slight preference towards the HT29 and SJG2 cells lines, however, neither analogue displayed noteworthy potency.

The second pairing of compounds consisting of a crotyl tail (**8**, **9**) showed initial promise with percentage inhibition at 100 μM deemed high enough to proceed to the second level of screening and obtain GI_{50} values. Interestingly again, both compounds showed similar activity and preference towards HT29 (colon) and SJ-G2 (glioblastoma) cells with GI_{50} values of (42 ± 5 and 55 ± 3 μM) (**8**) and (31 ± 4 and 35 ± 4 μM) (**9**) respectively with activity being completely void against the other cell lines tested. The final compound of Library A (**10**) consisting of a hexyl carbon chain and a terminal alkyne showed minimal activity, suggesting a tail of this length is not advantageous; however, this analogue still presented with some selectivity towards the HT29 and SJ-G2 cells.

The introduction of simple aliphatic chains produced a completely different trend in the cytotoxicity data (Table 2). As previous data suggested, there was no significant difference in cytotoxicity between the *R* and *S* isomers of any one compound,

Table 2
Anticancer activity of straight chain analogues (**11**–**15**). Values in are GI_{50} (μM) concentrations.

Compound	HT29[a]	SJ-G2[b]	BE2-C[c]	SW480[a]	MCF-7[d]	A2780[e]	H460[f]	A431[g]	DU145[h]
 **11**	77 ± 12	49 ± 15	>100	>100	>100	>100	>100	>100	>100
 **12**	50 ± 6	32 ± 2	93 ± 7	>100	>100	>100	>100	>100	>100
 **13**	32 ± 3	37 ± 6	38 ± 2	39 ± 4	27 ± 6	20 ± 1	>100	68 ± 5	>100
 **14**	nd	nd	>100	>100	>100	>100	>100	>100	>100
 **15**	40 ± 7	45 ± 10	51 ± 4	49 ± 8	36 ± 5	28 ± 2	>100	72 ± 3	86 ± 8

nd = not determined.
[a] Colon carcinoma.
[b] Glioblastoma.
[c] Neuroblastoma, MCF-7.
[d] Breast carcinoma.
[e] Ovarian carcinoma.
[f] Lung carcinoma.
[g] Skin carcinoma.
[h] Prostate carcinoma.

thus only the major isomer, formed in excess, was isolated and screened. Compounds (**11**) and (**12**) comprising of ethyl and butyl chains respectively showed limited cytotoxicity along with (**14**), having a hexyl tail. This is consistent with results shown by (**10**), indicating a six carbon chain at this position of these analogues is detrimental to activity and is causing an unfavourable interaction within the binding pocket. However, (**13**) and (**15**) with pentyl and octyl chains produced comparable cytotoxicity to (**2**) across all cell lines tested, showing no selectivity towards HT29 (colon) or SJ-G2 (glioblastoma) cells as previously observed with these derivatives.

Adding alcohols with varying degrees of branching again produced interesting results. Analogue **16** consisting of an isopropyl tail produced the most notable data of the series. This analogue showed similar potency to the lead (**3**) and maintained selectivity against HT29 (colon) and SJ-G2 (glioblastoma) cells, with GI_{50}s of 19 ± 0.9 and 21 ± 2.1 μM respectively (Table 3). A similar selectivity profile was observed with further branching to the tertiary butyl tail as with **17** and cyclohexyl **18**; however potency was compromised.

The effect of introducing an epoxide ring via modification of derivatives (**6**–**9**) was also carried out to produce compounds (**19**–**26**) (Table 4). In each case, two products were isolated and tested separately with almost identical ^{1}H and ^{13}C NMR spectra. Numerous attempts at crystallisation of the formed clear oils were unsuccessful and as a result, the absolute stereochemistry of each isolated product is still unknown. Although, none of these analogues, irrespective of stereocentre configuration, displayed noteworthy potency in growth inhibition the selectivity towards the HT29 and SJ-G2 cells was still apparent.

The final structural modification assessed was the introduction of terminal phosphate moieties to the tail of novo analogues via modification of a novo analogue with a terminal hydroxyl group (**27**) (Table 5). All esterifications proceeded in good yields. Interestingly **28** produced a different trend in cytotoxicity with significant growth inhibition in BE2-C cells (GI_{50} = 9.2 μM) through to a negligible effect in H460 cells (GI_{50} > 100 μM). This is in contrast to analogues **29** and **30** that maintained the selectivity towards HT29 and SJ-G2 cells, albeit at relatively low potency. We also examined the effect of a free terminal phosphoric acid moiety. Accordingly we generated analogue **31**; however this analogue displayed low levels of cytotoxicity being active against only the HT29 (GI_{50} = 42 ± 4 μM), MCF-7 (45 ± 5 μM) and A2780 (33 ± 4 μM) cell lines. This is in keeping with previous findings in our laboratory that long alkyl chains afford broad spectrum activity in the ring-opened norcantharidin analogues and the norcantharimides [22,23,30].

5. Conclusion

A series of norcantharidin cyclic lactone derivatives were prepared, by microwave assisted organic synthetic approaches, in moderate to good yields and their ability to inhibit the growth of a panel of human cancer cell lines was examined. Interestingly, there was no observed difference in cytotoxicity across any of the cancer cell lines tested between the two possible diastereoisomers produced for each analogue during synthesis.

Overall the most interesting data collected was attributed to the isopropyl substituted derivative **16**, with strong selectivity shown towards HT29 and SJ-G2 cell lines returning GI_{50} values of 19 ± 0.9 and 21 ± 2.1 μM respectively while being completely inactive against all other cell lines. We also discovered a new cytotoxicity trend following the introduction of a terminal phosphoric acid moiety giving rise an analogue with strong selectivity towards BE2-C cells and the greatest anti-growth activity (GI_{50} 9 μM) observed in this study. These analogues add considerably to the SAR

Table 3
Anticancer activity of branched analogues (**16**–**18**). Values in are GI_{50} (μM) concentrations.

Compound	HT29[a]	SJ-G2[b]	BE2-C[c]	SW480[a]	MCF-7[d]	A2780[e]	H460[f]	A431[g]	DU145[h]
 **16**	19 ± 1	21 ± 3	44 ± 5	94 ± 1	>100	>100	>100	>100	>100
 **17**	52 ± 5	48 ± 7	>100	>100	>100	>100	>100	>100	>100
 **18**	45 ± 4	41 ± 11	>100	>100	>100	>100	>100	>100	>100

nd = not determined.
[a] Colon carcinoma.
[b] Glioblastoma.
[c] Neuroblastoma, MCF-7.
[d] Breast carcinoma.
[e] Ovarian carcinoma.
[f] Lung carcinoma.
[g] Skin carcinoma.
[h] Prostate carcinoma.

surrounding this class of compounds and hold promise for future development of novel anti-cancer agents.

6. Experimental

6.1. Biology

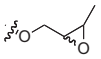
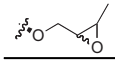
6.1.1. Cell culture and stock solutions

Stock solutions were prepared as follows and stored at −20 °C: Cantharidin (Biomol, USA) as a 30 mM solution in dimethylsulphoxide (DMSO); norcantharidin as a 30 mM solution in water and norcantharidin analogues as 40 mM solutions in DMSO. All cell lines were cultured at 37 °C, under 5% CO_2 in air and were maintained in Dulbecco's modified Eagle's medium (Trace Biosciences, Australia) supplemented with 10% foetal bovine serum, 10 mM sodium bicarbonate penicillin (100 IU/mL), streptomycin (100 μg/mL), and glutamine (4 mM).

6.1.2. In vitro growth inhibition assay

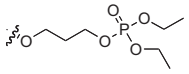
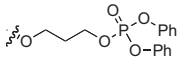
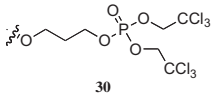
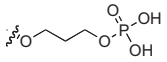
Cells in logarithmic growth were transferred to 96-well plates. Cytotoxicity was determined by plating cells in duplicate in 100 mL medium at a density of 2500–4000 cells/well. On day 0, (24 h after plating) when the cells were in logarithmic growth, 100 μL medium with or without the test agent was added to each well. After 72 h drug exposure growth inhibitory effects were evaluated using the MTT (3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay and absorbance read at 540 nm. Percentage growth inhibition was determined at a fixed drug concentration of 100 μM. A value of 100% is indicative of total cell growth inhibition. Those analogues showing appreciable percentage growth inhibition underwent further dose

Table 4
Anticancer activity of epoxide analogues (**19**–**26**). Values recorded are the percentage growth inhibition at 100 μM compound concentration.

Compound		HT29[a]	SJ-G2[b]	BE2-C[c]	SW480[a]	MCF-7[d]	A2780[e]	H460[f]	A431[g]	DU145[h]
	**19**	69 ± 1	75 ± 1	42 ± 5	32 ± 1	37 ± 2	46 ± 4	11 ± 3	35 ± 2	33 ± 2
	20	52 ± 2	59 ± 3	42 ± 4	16 ± 3	43 ± 4	83 ± 7	7 ± 3	18 ± 2	21 ± 4
	**21**	68 ± 2	73 ± 1	35 ± 5	23 ± 5	33 ± 2	42 ± 4	7 ± 2	22 ± 3	25 ± 2
	22	74 ± 1	76 ± 2	38 ± 3	28 ± 3	40 ± 2	42 ± 3	14 ± 1	28 ± 3	30 ± 2
	**23**	75 ± 1	88 ± 2	54 ± 13	41 ± 12	61 ± 13	55 ± 16	24 ± 5	35 ± 6	37 ± 3
	24	68 ± 3	79 ± 1	53 ± 21	37 ± 13	46 ± 12	58 ± 16	9 ± 4	17 ± 2	20 ± 2
	**25**	70 ± 2	80 ± 2	23 ± 5	17 ± 0	27 ± 2	26 ± 3	9 ± 1	18 ± 0	17 ± 5
	26	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd = not determined.
[a] Colon carcinoma.
[b] Glioblastoma.
[c] Neuroblastoma, MCF-7.
[d] Breast carcinoma.
[e] Ovarian carcinoma.
[f] Lung carcinoma.
[g] Skin carcinoma.
[h] Prostate carcinoma.

Table 5
Anticancer activity of terminal phosphate esters and phosphoric acid analogues (**28**–**31**). Values in are GI_{50} (μM) concentrations.

Compound	HT29[a]	SJ-G2[b]	BE2-C[c]	SW480[a]	MCF-7[d]	A2780[e]	H460[f]	A431[g]	DU145[h]
 **28**	17 ± 1	43 ± 3.4	9 ± 0.2	9 ± 7	20 ± 1	22 ± 2	>100	87 ± 12	84 ± 3
 **29**	40 ± 4	40 ± 10	>100	>100	>100	>100	>100	>100	>100
 **30**	37 ± 10	41 ± 10	>100	>100	89 ± 6	81 ± 1	>100	>100	>100
 **31**	42 ± 4	57 ± 2	67 ± 4	65 ± 4	45 ± 5	33 ± 4	6 ± 3	52 ± 2	65 ± 6

nd = not determined.
[a] Colon carcinoma.
[b] Glioblastoma.
[c] Neuroblastoma, MCF-7.
[d] Breast carcinoma.
[e] Ovarian carcinoma.
[f] Lung carcinoma.
[g] Skin carcinoma.
[h] Prostate carcinoma.

response analysis allowing for the calculation of a GI_{50} value. This value is the drug concentration at which cell growth is 50% inhibited based on the difference between the optical density values on day 0 and those at the end of drug exposure [29].

6.2. Chemistry

6.2.1. General experimental – general methods

All reagents were purchased from Sigma–Aldrich, Matrix Scientific or Lancaster Synthesis and were used without purification. With the exception of THF (anhydrous > 99%) obtained from Sigma–Aldrich, all solvents were re-distilled from glass prior to use.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance™ AMX 300 MHz spectrometer at 300.1315 and 75.4762 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) measured relative to the internal standards, and coupling constants (J) are expressed in Hertz (Hz). Mass spectra were recorded on a Shimadzu LCMS 2010 EV using a mobile phase of 1:1 acetonitrile:H_2O with 0.1% formic acid.

Melting points were recorded on a Stuart Scientific melting point apparatus (UK) and are uncorrected. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} pre-coated aluminium plates with a thickness of 0.2 mm. Column chromatography was performed under 'flash' conditions on Merck silica gel 60 (230–400 mesh) or using the Biotage SP4 flash purification system with a 100 g pre-packed snap column.

A CEM Discover® BenchMate microwave (120 °C, 200 W, 1 h) was used to perform several refluxes. Hydrogenations were performed using the H-Cube® continuous-flow hydrogenation reactor utilizing a palladium-carbon (CatCart®) catalyst, a flow rate of 1 ml/min, 40 bar of pressure and column temperature 40 °C.

6.2.2. General microwave procedure

An alcohol derivative (1.07 mmol, 1 eq) was added to a magnetically stirred solution of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (1.18 mmol, 1.05 eq) (**3**) in anhydrous THF (5 mL). To this, a catalytic amount of 4-toluene sulfonic acid (10 mg) was added and the resulting solution was treated with microwave radiation at 80 °C for 1 h at 150 W. The resulting solution was subjected to silica chromatography (2:8 EtOAc:hexane) to afford the desired derivative.

6.2.3. (3S,3aR,4S,7R,7aS)-3-(Allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (6) and (3R,3aR,4S,7R,7aS)-3-(allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (7)

Synthesised as per the general microwave procedure above from **3** and allyl alcohol to afford the title compounds (6:1) as a white solid (R_f 0.81) (26%), m.p. 36–38 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.89 (m, 1H), 5.30 (d, J = 1.7 Hz, 1H), 5.28 (m, 2H), 4.83 (d, J = 4.6 Hz, 1H), 4.69 (d, J = 4.6 Hz, 1H), 4.30 (m, 1H), 4.07 (m, 1H), 2.92 (d, J = 8.0 Hz, 1H), 2.53 (dd, J = 1.7, 8.0 Hz, 1H), 1.85–1.46 (m, 4H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.5, 132.3, 118.0, 105.8, 79.6, 79.0, 69.7, 50.3, 49.7, 28.0, 27.3; IR (KBr) ν_{max}/cm^{-1}: 2988 (C–H), 1747 (C=O), 1638 (C=C), 1150 (C–O); m/z (APCI M + H) 211. HRMS calculated for M + H; $C_{11}H_{15}O_4$; 211.0965.

6.2.4. (3R,3aR,4S,7R,7aS)-3-(Allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (7)

Isolated as a white crystalline solid (R_f 0.62) (8%), m.p. 100 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.87–5.60 (m, 1H), 5.56 (d, J = 6.8 Hz,

1H), 5.37–5.23 (m, 2H), 5.13 (d, J = 4.8 Hz, 1H), 4.92 (d, J = 4.8 Hz, 1H), 4.46–4.40 (m, 1H), 4.17–4.10 (m, 1H), 2.89 (d, J = 8.4 Hz, 1H), 2.72–2.68 (m, 1H), 1.86–1.71 (m, 2H), 1.57–1.43 (m, 2H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 174.8, 132.5, 117.7, 102.4, 78.7, 76.0, 70.8, 51.1, 46.6, 27.6, 27.5; IR (KBr) ν_{max}/cm^{-1}: 2988 (C–H), 1747 (C=O), 1638 (C=C), 1150 (C–O); m/z (APCI M + H) 211. HRMS calculated for M + H; $C_{11}H_{14}O_4$; 211.0965.

*6.2.5. (3S,3aR,4S,7R,7aS)-3-((E)-But-2-en-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**8**) and (3R,3aR,4S,7R,7aS)-3-((E)-but-2-en-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**9**)*

Synthesised as per the general microwave procedure above from **3** and but-2-en-1-ol to afford the title compounds (6:1) as a white crystalline solid (R_f 0.81) (42%), m.p. 78 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.78 (dq, J = 15.2, 6.4 Hz, 1H), 5.55 (dt, 15.2, 6.0 Hz, 1H), 5.30 (d, J = 1.1 Hz, 1H), 4.83 (d, J = 4.5 Hz, 1H), 4.69 (d, J = 4.5 Hz, 1H), 4.24 (dd, J = 6.0, 0.8 Hz, 1H), 4.01 (dd, J = 7.1, 0.8 Hz, 1H), 2.91 (d, J = 7.9 Hz, 1H), 2.50 (dd, J = 7.9, 1.1 Hz, 1H), 1.80–1.45 (m, 4H), 1.73 (d, J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.5, 131.2, 125.0, 105.5, 79.7, 79.0, 69.6, 50.4, 49.8, 28.0, 27.2, 17.3; IR (KBr) ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1617 (C=C), 1153 (C–O); m/z (APCI M + H) 225. HRMS calculated for M + H; $C_{12}H_{16}O_4$; 225.1121.

Isolated as a white solid (R_f 0.62) (9%), m.p. 116–118 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.76 (dq, J = 15.3, 6.4 Hz, 1H), 5.57 (m, 1H), 5.56 (d, J = 6.8 Hz, 1H), 5.11 (d, J = 4.8 Hz, 1H), 4.90 (d, J = 4.8 Hz, 1H), 4.35 (m, 1H), 4.06 (m, 1H), 2.88 (d, J = 8.3 Hz, 1H), 2.67 (dd, J = 6.8, 8.3 Hz, 1H), 1.85–1.38 (m, 4H), 1.73 (d, J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.0, 130.6, 125.3, 102.1, 78.7, 76.0, 70.6, 51.2, 46.6, 27.6, 27.5, 17.2; IR (KBr) ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1617 (C=C), 1153 (C–O); m/z (APCI M + H) 225. HRMS calculated for M + H; $C_{12}H_{17}O_4$; 225.1121.

*6.2.6. (3S,3aR,4S,7R,7aS)-3-(Hex-5-yn-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**10**)*

Synthesised as per the general microwave procedure above from **3** and hex-5-yn-1-ol to afford the title compound as a clear oil (80%); ^{1}H NMR (Acetone-d_6) (300 MHz): δ 5.32 (d, J = 1.5 Hz, 1H), 4.73 (d, J = 4.2 Hz, 1H), 4.65 (d, J = 4.2 Hz, 1H), 3.79–3.71 (m, 1H), 3.62–3.55 (m, 1H), 3.00 (d, J = 7.9 Hz, 1H), 2.53 (dd, J = 7.9, 1.5 Hz, 1H), 2.36–2.31 (m, 1H), 2.23–2.14 (m, 3H), 2.08–2.02 (m, 1H), 1.74–1.51 (m, 6H). ^{13}C NMR (Acetone-d_6) (75 MHz): δ 175.2, 106.6, 83.2, 79.5, 78.8, 68.7, 68.1, 50.0, 49.2, 27.8, 27.5, 27.0, 24.4, 17.0; IR (film) ν_{max}/cm^{-1}: 3325 (C≡C–H), 2933 (C–H), 2145 (C≡C), 1779 (C=O), 1124 (C–O); m/z (APCI M + H) 251. HRMS calculated for M + H; $C_{14}H_{19}O_4$; 251.1278.

*6.2.7. (3S,3aR,4S,7R,7aS)-3-Ethoxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**11**)*

Synthesised as per the general microwave procedure above from **3** and ethanol to afford the title compound as a white solid (80%), m.p. 71–72 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.27 (d, J = 1.5 Hz, 1H), 4.84 (d, J = 4.7 Hz, 1H), 4.70 (d, J = 4.7 Hz, 1H), 3.87 (m, 1H), 3.60 (m, 1H), 2.91 (d, J = 8.0, 1H), 2.50 (dd, J = 1.5,8.0 Hz, 1H), 1.81–1.76 (m, 2H), 1.60–1.50 (m, 2H), 1.23 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.6, 106.7, 79.7, 79.0, 64.9, 50.5, 49.8, 28.1, 27.3, 14.4; IR (KBr) ν_{max}/cm^{-1}: 2979 (C–H), 2881 (C–H), 1752 (C=O), 1190 (C–O); m/z (APCI M + H) 199. HRMS calculated for M + H; $C_{10}H_{15}O_4$; 199.0965.

*6.2.8. (3S,3aR,4S,7R,7aS)-3-Butoxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**12**)*

Synthesised as per the general microwave procedure above from **3** and butan-1-ol to afford the title compound as pale yellow solid (23%), m.p. 46–47 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.26 (d, J = 1.7 Hz, 1H), 4.84 (d, J = 4.5 Hz, 1H), 4.70 (d, J = 4.5 Hz, 1H), 3.80 (m, 1H), 3.53 (m, 1H), 2.91 (d, J = 8.0 Hz, 1H), 2.50 (dd, J = 1.7, 8.0 Hz, 1H), 1.79–1.33 (m, 8H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.9, 107.0, 79.7, 79.0, 69.2, 50.4, 49.8, 30.9, 28.0, 27.3, 18.6, 13.2; IR (KBr) ν_{max}/cm^{-1}: 2952 (C–H), 2870 (C–H), 1772 (C=O), 1122 (C–O); m/z (APCI M + H) 227. HRMS calculated for M + H; $C_{12}H_{19}O_4$; 227.1278.

*6.2.9. (3S,3aR,4S,7R,7aS)-3-(Pentyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**13**)*

Synthesised as per the general microwave procedure above from **3** and pentan-1-ol to afford the title compound as a yellow oil (53%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.22 (d, J = 1.7 Hz, 1H), 4.79 (d, J = 4.6 Hz, 1H), 4.66 (d, J = 4.6 Hz, 1H), 3.75 (m, 1H), 3.48 (m, 1H), 2.88 (d, J = 7.9 Hz, 1H), 2.46 (dd, J = 1.7, 7.9 Hz, 1H), 1.75–1.25 (m, 10H), 0.86 (t, J = 6.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.6, 106.9, 79.7, 79.0, 69.5, 50.4, 49.8, 28.5, 28.0, 27.5, 27.2, 21.8, 13.4; IR (film) ν_{max}/cm^{-1}: 2938 (C–H), 1777 (C=O), 1122 (C–O); m/z (APCI M + H) 241.0. HRMS calculated for M + H; $C_{13}H_{21}O_4$; 241.1434.

*6.2.10. (3S,3aR,4S,7R,7aS)-3-(Hexyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**14**)*

Synthesised as per the general microwave procedure above from **3** and hexan-1-ol to afford the title compound as a yellow oil (31%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.24 (d, J = 1.8 Hz, 1H), 4.82 (d, J = 4.7 Hz, 1H), 4.68 (d, J = 4.7 Hz, 1H), 3.78 (m, 1H), 3.50 (m, 1H), 2.90 (d, J = 8.0 Hz, 1H), 2.48 (dd, J = 1.8, 8.0 Hz, 1H), 1.85–1.22 (m, 12H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.5, 106.9, 79.7, 79.0, 69.5, 50.4, 49.8, 28.8, 28.0, 27.3, 25.0, 21.9, 20.4, 13.4; IR (film) ν_{max}/cm^{-1}: 2932 (C–H), 1780 (C=O), 1124 (C–O); m/z (APCI M + H) 255. HRMS calculated for M + H; $C_{14}H_{23}O_4$; 255.1591.

*6.2.11. (3S,3aR,4S,7R,7aS)-3-(Octyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**15**)*

Synthesised as per the general microwave procedure above from **3** and octan-1-ol to afford the title compound as a dark yellow oil (26%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.25 (d, J = 1.7 Hz, 1H), 4.83 (d, J = 4.6 Hz, 1H), 4.69 (d, J = 4.6 Hz), 3.80 (m, 1H), 3.51 (m, 1H), 2.90 (d, J = 8.0 Hz, 1H), 2.49 (dd, J = 1.7, 8.0 Hz, 1H), 2.04–1.25 (m, 16H), 0.88 (t, J = 6.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.4, 106.9, 79.7, 79.0, 69.5, 50.4, 49.8, 31.2, 28.8, 28.7, 28.6, 28.0, 27.3, 25.4, 22.1, 13.5; IR (film) ν_{max}/cm^{-1}: 2938 (C–H), 1782 (C=O), 1123 (C–O); m/z (APCI M + H) 283. HRMS calculated for M + H; $C_{16}H_{27}O_4$; 283.1904.

*6.2.12. (3S,3aR,4S,7R,7aS)-3-Isopropoxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**16**)*

Synthesised as per the general microwave procedure above from **3** and isopropanol to afford the title compound as a white solid (49%), m.p. 74–76 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.36 (d, J = 1.6 Hz, 1H), 4.83 (d, J = 4.3 Hz, 1H), 4.68 (d, J = 4.3 Hz, 1H), 3.98 (sept, J = 6.2 Hz, 1H), 2.91 (d, J = 8.0 Hz, 1H), 2.47 (dd, J = 1.6, 8.0 Hz, 1H), 1.79–1.75 (m, 2H), 1.59–1.46 (m, 2H), 1.22–1.21 (m, 6H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.7, 105.2, 79.7, 79.0, 71.5, 50.8, 50.0, 28.1, 27.6, 22.7, 21.0; IR (KBr) ν_{max}/cm^{-1}: 2964 (C–H), 2984 (C–H), 1732 (C=O), 1124 (C–O); m/z (APCI M + H) 212. HRMS calculated for M + H; $C_{11}H_{17}O_4$; 213.1121.

*6.2.13. (3S,3aR,4S,7R,7aS)-3-(Tert-butoxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**17**)*

Synthesised as per the general microwave procedure above from **3** and *tert*-butanol to afford the title compound as a white solid (76%), m.p. 127–128 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.47 (d, J = 1.3 Hz, 1H), 4.83 (d, J = 3.9 Hz, 1H), 4.64 (d, J = 3.5 Hz, 1H), 2.88 (d, J = 8.0 Hz, 1H), 2.45 (dd, J = 1.3, 7.0 Hz, 1H), 1.76–1.27 (m, 13H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.7, 102.2, 79.6, 79.0, 76.9 51.7, 50.0, 28.2, 28.0, 27.1; IR (KBr) ν_{max}/cm^{-1}: 2985 (C–H), 2878 (C–H), 1759

(C=O), 1121 (C–O); *m/z* (APCI M + H) 227. HRMS calculated for M + H; $C_{12}H_{19}O_4$; 227.1278.

*6.2.14. (3S,3aR,4S,7R,7aS)-3-(Cyclohexyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**18**)*

Synthesised as per the general microwave procedure above from **3** and cyclohexanol to afford the title compound as a yellow solid (26%), m.p. 100–102 °C; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.41 (d, J = 1.8 Hz, 1H), 4.84 (d, J = 4.5 Hz, 1H), 4.68 (d, J = 4.5 Hz, 1H), 3.65 (m, 1H), 2.92 (d, J = 8.0 Hz, 1H), 2.49 (dd, J = 1.8, 8.0 Hz, 1H), 1.85–1.22 (m, 14H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.9, 105.1, 79.7, 79.0, 50.8, 50.0, 32.7, 30.1, 28.1, 27.2, 24.9, 23.3; IR (KBr) ν_{max}/cm^{-1}: 2972 (C–H), 2853 (C–H), 1748 (C=O), 1193 (C–O); *m/z* (APCI M + H) 253. HRMS calculated for M + H; $C_{14}H_{21}O_4$; 253.1434.

6.3. General epoxidation procedure

m-Chloroperbenzoic acid (0.74 g, 77% in water, 3.31 mmol, 2 eq) was added to a magnetically stirred solution of the corresponding unsaturated Novo derivative (1.66 mmol, 1 eq) in anhydrous DCM (10 mL) at 0 °C. The resulting solution was warmed to room temperature, and stirred for 16 h before being diluted with CH_2Cl_2 (20 mL) and washed with $NaHCO_3$ (3 × 10 mL, sat solution). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The formed clear oil was subjected to silica chromatography (EtOAc:hexanes 3:7) to afford both isomers of the desired epoxide.

*6.3.1. (3S,3aR,4S,7R,7aS)-3-(Oxiran-2-ylmethoxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**19**, **20**)*

Synthesised as per the general epoxidation procedure above from (**6**) to afford the title compounds as clear oils (1:1) combined yield (74%); ^{1}H NMR ($CDCl_3$) (300 MHz) (**19**): δ 5.31 (d, J = 1.5 Hz, 1H), 4.82 (d, J = 4.6 Hz, 1H), 4.70 (d, J = 4.6 Hz, 1H), 4.07 (dd, J = 2.7, 11.4 Hz, 1H), 3.39 (dd, J = 7.1, 11.4 Hz, 1H), 3.16 (m, 1H), 2.91 (d, J = 7.9 Hz, 1H), 2.82 (t, J = 4.4 Hz, 1H), 2.58 (m, 2H), 1.82–1.69 (m, 2H), 1.59–1.46 (m, 2H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.3, 106.6, 79.5, 79.0, 70.5, 50.3, 49.7, 49.6, 43.9, 28.0, 27.2; IR (film) ν_{max}/cm^{-1}: 2992 (C–H), 1742 (C=O), 1230 (C–O), 1150 (C–O); *m/z* APCI (M + H) 227.

^{1}H NMR ($CDCl_3$) (300 MHz) (**20**): δ 5.27 (d, J = 1.6 Hz, 1H), 4.80 (d, J = 4.5 Hz, 1H), 4.68 (d, J = 4.5 Hz, 1H), 3.78 (m, 2H), 3.14 (m, 1H), 2.89 (d, J = 7.9 Hz, 1H), 2.79 (t, J = 4.5 Hz, 1H), 2.66 (m, 1H), 2.52 (dd, J = 1.6, 7.9 Hz, 1H), 1.79–1.66 (m, 2H), 1.57–1.43 (m, 2H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.3, 106.6, 79.6, 79.0, 68.5, 50.2, 49.6, 49.3, 43.7, 28.0, 27.2; IR (film) ν_{max}/cm^{-1}: 2992 (C–H), 1742 (C=O), 1230 (C–O), 1150 (C–O); *m/z* APCI (M + H) 227. HRMS calculated for M + H; $C_{11}H_{15}O_4$; 227.0914.

*6.3.2. (3R,3aR,4S,7R,7aS)-3-(Oxiran-2-ylmethoxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**21**, **22**)*

Synthesised as per the general epoxidation procedure above from (**7**) to afford the title compounds as clear oils (1:1) combined yield (68%); ^{1}H NMR ($CDCl_3$) (300 MHz) (**21**): δ 5.59 (d, J = 6.8 Hz, 1H), 5.12 (d, J = 4.7 Hz, 1H), 4.91 (d, J = 4.7 Hz, 1H), 4.12 (dd, J = 2.6, 11.4 Hz, 1H), 3.40 (dd, J = 7.4, 11.4 Hz, 1H), 3.26 (m, 1H), 2.87 (m, 2H), 2.73 (dd, J = 6.8, 8.2 Hz, 1H), 2.58 (m, 1H), 1.86–1.71 (m, 2H), 1.57–1.40 (m, 2H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 174.7, 103.3, 78.7, 76.9, 76.5, 76.0, 72.0, 51.1, 49.8, 46.6, 43.8, 28.8, 27.6, 27.4; IR (film) ν_{max}/cm^{-1}: 2994 (C–H), 1742 (C=O), 1230 (C–O), 1150 (C–O); *m/z* APCI (M + H) 227.

^{1}H NMR ($CDCl_3$) (300 MHz) (**22**): δ 5.52 (d, J = 6.8 Hz, 1H), 5.03 (d, J = 3.8 Hz, 1H), 4.87 (d, J = 3.8 Hz, 1H), 3.90 (m, 2H), 3.18 (m, 1H), 2.75 (m, 4H), 1.75–1.69 (m, 2H), 1.55–1.32 (m, 2H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 174.7, 103.4, 78.7, 76.0, 68.7, 51.0, 49.4, 46.5, 43.7, 27.5, 27.4; IR (film) ν_{max}/cm^{-1}: 2994 (C–H), 1742 (C=O), 1230 (C–O), 1150 (C–O); *m/z* APCI (M + H) 227. HRMS calculated for M + H; $C_{11}H_{15}O_5$; 227.0914.

*6.3.3. (3S,3aR,4S,7R,7aS)-3-((3-Methyloxiran-2-yl)methoxy) hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**23**, **24**)*

Synthesised as per the general epoxidation procedure above from (**8**) to afford the title compounds as clear oils (1:1) combined yield (85%); ^{1}H NMR ($CDCl_3$) (300 MHz) (**23**): δ 5.30 (d, J = 1.6 Hz, 1H), 4.82 (d, J = 4.6 Hz, 1H), 4.70 (d, J = 4.6 Hz, 1H), 4.01 (dd, J = 2.9, 11.3 Hz, 1H), 3.42 (dd, J = 6.8, 11.3 Hz, 1H), 2.89 (m, 3H), 2.56 (dd, J = 1.6, 7.9 Hz, 1H), 1.85–1.70 (m, 2H), 1.59–1.46 (m, 2H), 1.32 (d, J = 5.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.4, 106.8, 79.7, 79.0, 70.1, 56.7, 51.9, 50.3, 49.6, 28.0, 27.2, 16.6; IR (film) ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1226 (C–O), 1153 (C–O); *m/z* (APCI M + H) 241.

^{1}H NMR ($CDCl_3$) (300 MHz) (**24**): δ 5.27 (d, J = 1.6 Hz, 1H), 4.82 (d, J = 4.6 Hz, 1H), 4.70 (d, J = 4.6 Hz, 1H), 3.80 (m, 2H), 2.93 (m, 3H), 2.52 (dd, J = 1.6, 7.9 Hz, 1H), 1.84–1.70 (m, 2H), 1.59–1.45 (m, 2H), 1.32 (d, J = 5.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.3, 106.6, 79.7, 79.0, 68.1, 56.3, 51.5, 50.2, 49.7, 28.0, 27.2, 16.6; ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1226 (C–O), 1153 (C–O); *m/z* (APCI M + H) 241. HRMS calculated for M + H; $C_{12}H_{17}O_5$; 241.1071.

*6.3.4. (3R,3aR,4S,7R,7aS)-3-((3-Methyloxiran-2-yl)methoxy) hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**25**, **26**)*

Synthesised as per the general epoxidation procedure above from (**9**) to afford the title compounds as clear oils (1:1) combined yield (26%); ^{1}H NMR ($CDCl_3$) (300 MHz) (**25**): δ 5.52 (d, J = 6.8 Hz, 1H), 5.05 (d, J = 4.4 Hz, 1H), 4.89 (d, J = 4.4 Hz, 1H), 4.03 (dd, J = 3.5, 12.0 Hz, 1H), 3.84 (dd, J = 2.7, 12.0 Hz, 1H), 3.09 (m, 1H), 2.89 (m, 2H), 2.70 (dd, J = 6.8, 8.2 Hz, 1H), 1.84–1.69 (m, 2H), 1.56–1.41 (m, 2H), 1.34 (d, J = 5.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): δ 174.8, 103.4, 78.7, 76.0, 68.2, 56.5, 51.4, 51.0, 46.6, 27.6, 27.4, 16.7; IR (film) ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1226 (C–O), 1153 (C–O); *m/z* (APCI M + H) 241.

^{1}H NMR ($CDCl_3$) (300 MHz) (**26**): 5.50 (d, J = 6.8 Hz, 1H), 5.05 (d, J = 4.4 Hz, 1H), 4.89 (d, J = 4.4 Hz, 1H), 3.79 (m, 2H), 2.98 (m, 3H), 2.70 (dd, J = 6.8, 8.2 Hz, 1H), 1.84–1.68 (m, 2H), 1.56–1.41 (m, 2H), 1.34 (d, J = 5.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) (75 MHz): 174.7, 103.2, 78.7, 76.1, 66.3, 56.1, 51.1, 51.0, 46.6, 27.6, 27.4, 16.7; IR (film) ν_{max}/cm^{-1}: 2985 (C–H), 1752 (C=O), 1226 (C–O), 1153 (C–O); *m/z* (APCI M + H) 241. HRMS calculated for M + H; $C_{12}H_{17}O_5$; 241.1071.

*6.3.5. (3S,3aR,4S,7R,7aS)-3-Propan-3-oloxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**27**)*

1,3-Propanediol (11.8 mmol, 10 eq) was added to a magnetically stirred solution of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (1.18 mmol, 1 eq) in anhydrous THF (5 mL). To this, a catalytic amount of 4-toluene sulfonic acid (10 mg) was added and the resulting solution was treated with microwave radiation at 80 °C for 1 h at 150 W. The resulting solution was subjected to silica chromatography (2:8 EtOAc:Hexane) to afford the desired analogue as a clear oil (80%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.22 (d, J = 1.5 Hz, 1H), 4.74 (d, J = 4.5 Hz, 1H), 4.64 (d, J = 4.5 Hz, 1H), 3.84 (m, 1H), 3.64 (m, 3H), 2.86 (d, J = 7.9 Hz, 1H), 2.70 (br, 1H), 2.44 (dd, J = 1.5, 7.9 Hz, 1H), 1.74 (m, 4H), 1.48 (m, 2H); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 177.8, 107.1, 79.7, 79.0, 66.6, 58.9, 50.3, 49.8, 31.6, 28.0, 27.2; IR (film) ν_{max}/cm^{-1}: 3535 (O–H), 2943 (C–H), 1773 (C=O), 1039 (C–O); *m/z* (APCI M + H) 229. HRMS calculated for M + H; $C_{11}H_{17}O_5$; 229.1071.

6.4. General phosphoesterification procedure

Dibutyltin oxide (0.19 mmol, 0.2 eq) was added to a magnetically stirred solution of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-propan-3-oloxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**27**) (0.95 mmol, 1 eq) in

anhydrous CH_2Cl_2 (10 mL) and stirred for 30 min at room temperature under a nitrogen atmosphere. To this, the desired chlorophosphate (0.95 mmol, 1 eq) and triethylamine (3.41 mmol, 3.6 eq) were added and left to stir for a further 18 h at room temperature under a nitrogen atmosphere. The resulting solution was then quenched with water (15 mL). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The formed clear oil was subjected to silica chromatography (EtOAc:hexanes 1:1) to afford the desired phosphate analogue.

*6.4.1. Phosphoric acid diethyl ester 3-(5-oxo-4,10-dioxa-tricyclo [5.2.1.0]dec-3-yloxy)-propyl ester (**28**)*

Synthesised using the general procedure as above, from diethylchlorophosphate to afford (**28**) as a clear oil (50%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.22 (d, J = 1.4 Hz, 1H), 4.78 (d, J = 4.3 Hz, 1H), 4.66 (d, J = 4.3 Hz, 1H), 4.09 (m, 6H), 3.86 (m, 1H), 3.62 (m, 1H), 2.86 (d, J = 7.9 Hz, 1H), 2.46 (dd, J = 1.4, 7.9 Hz, 1H), 1.92 (m, 2H), 1.73 (m, 2H), 1.52 (m, 2H), 1.30 (t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.4, 106.9, 79.6, 79.0, 65.1, 63.4, 63.3, 63.2, 50.3, 49.6, 29.6, 28.0, 27.2, 15.6, 15.5; IR (film) ν_{max}/cm^{-1}: 2943 (C–H), 1773 (C=O), 1269 (P=O), 1038 (C–O); m/z (APCI M + H) 365. HRMS calculated for M + H; $C_{15}H_{26}O_8P$; 365.1360.

*6.4.2. Phosphoric acid 3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0]dec-3-yloxy)-propyl ester diphenyl ester (**29**)*

Synthesised using the general procedure as above, from diphenylchlorophosphate to afford (**29**) as a clear oil (61%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.34 (m, 4H), 7.20 (m, 6H), 5.16 (d, J = 1.7 Hz, 1H), 4.81 (d, J = 4.8 Hz, 1H), 4.60 (d, J = 4.8 Hz, 1H), 4.33 (m, 2H), 3.86 (m, 1H), 3.59 (m, 1H), 2.86 (d, J = 7.9 Hz, 1H), 2.44 (dd, J = 1.7, 7.9 Hz, 1H), 1.97 (m, 2H), 1.72 (m, 2H), 1.48 (m, 2H); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.4, 150.1, 129.3, 124.9, 119.7, 106.8, 79.6, 79.0, 65.1, 64.8, 50.2, 49.6, 29.1, 28.0, 27.3; IR (film) ν_{max}/cm^{-1}: 2961 (C–H), 1774 (C=O), 1590 (Ar), 1489 (Ar), 1222 (P=O), 1190 (C–O); m/z (ACPI M + H) 461. HRMS calculated for $C_{23}H_{26}O_8P$; 461.1360.

*6.4.3. Phosphoric acid 3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0]dec-3-yloxy)-propyl ester bis-(2,2,2-trichloro-ethyl) ester (**30**)*

Synthesised using the general procedure as above, from bis(2,2,2-trichloroethyl)phosphochloridate to afford (**30**) as a clear oil (78%); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.28 (d, J = 1.7 Hz, 1H), 4.85 (d, J = 4.7 Hz, 1H), 4.72 (d, J = 4.7 Hz, 1H), 4.66 (m, 4H), 4.34 (m, 2H), 3.94 (m, 1H), 3.71 (m, 1H), 2.92 (d, J = 7.9 Hz, 1H), 2.51 (dd, J = 1.7, 7.9 Hz, 1H), 2.06 (m, 2H), 1.81 (m, 2H), 1.55 (m, 2H); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.9, 107.3, 94.7, 94.6, 80.2, 79.6, 66.1, 66.0, 65.3, 50.9, 50.2, 30.1, 30.0, 28.6, 27.8; IR (film) ν_{max}/cm^{-1}: 2954 (C–H), 1775 (C=O), 1235 (P=O), 918 (C–Cl); m/z (APCI M + H) 571. HRMS calculated for $C_{15}H_{20}Cl_6O_8P$; 568.9021.

*6.4.4. Phosphoric acid mono-[3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0] dec-3-yloxy)-propyl] ester (**31**)*

Freshly prepared activated Zn–Cu couple consisting of ~16% Cu (0.88 mmol, 2 eq) and acetylacetone (4.38 mmol, 10 eq) was added to a magnetically stirred solution of phosphoric acid 3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0^{2,6}]dec-3-yloxy)-propyl ester bis-(2,2,2-trichloro-ethyl) ester (**30**) (0.44 mmol, 1 eq) in anhydrous DMF (3 mL) under a nitrogen atmosphere. The resultant solution was heated for 2 h at 55 °C during which the red Zn–Cu couple dissolved and turned green. At the conclusion of this period, Chelex resin (10 mL settled volume in 15 mL water: 30 mL MeOH) was added and stirred for a further hour. The reaction was filtered through celite and washed with a small amount of water. The solvent was then removed *in vacuo* to afford the desired phosphate analogue (**31**) as a clear oil (68%); ^{1}H NMR (D_2O) (300 MHz): δ 5.49 (d, J = 1.6 Hz, 1H), 4.82 (d, J = 4.8 Hz, 1H), 4.78 (d, J = 4.8 Hz, 1H), 3.86–3.61 (m, 4H), 3.15 (d, J = 8.1 Hz, 1H), 2.64 (dd, J = 1.6, 8.1 Hz, 1H), 1.75–1.72 (m, 2H), 1.66–1.49 (m, 4H); ^{13}C NMR (D_2O) (75 MHz): δ 176.5, 106.9, 79.6, 78.4, 65.2, 63.8, 51.3, 49.8, 29.9, 28.7, 27.9; IR (thin film) cm^{-1}: 3510 (O–H), 1773 (C=O), 1243 (P=O), 1082 (C–O); LRMS (APCI M-2): 306. HRMS calculated for $C_{11}H_{18}O_8P$; 309.0734.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.06.010.

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3.2 Paper II

J Chem Crystallogr
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COMMUNICATION

Crystal Structures of (3*R*,3a*R*,4*S*,7*R*,7a*S*)-3-(Allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one and (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-((*E*)-But-2-en-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one: Confirmation of NMR Predicted Stereocentre Geometry

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Received: 1 August 2011 / Accepted: 17 January 2012

Abstract Crystal structures of two isomeric norcantharidin derivatives (3R,3aR,4S,7R,7aS)-3-(allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**7b**), and (3S,3aR,4S,7R,7aS)-3-((*E*)-but-2-en-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**8a**) have been determined. In both instances the equivalent enantiomer was also obtained. The crystal structures of these compounds clarify the stereochemistry inferred only by NMR analysis before.

Keywords Norcantharidin · Conformation · Crystal structure · Stereocentre

Introduction

Cantharidin (**1**) (Fig. 1) is a naturally occurring toxin found in over 1,000 species of blister beetles. Used by the Chinese as a natural remedy for the past 2,000 years, cantharidin has a long history as a therapeutic agent [1, 2]. The anti-cancer potential of cantharidin (**1**), was first recorded in 1,264 [1, 3]. Structurally simple, cantharidin (**1**) displays a number of features amenable to lead development including exhibiting no myelosuppresion and not being a substrate for the P-glycoprotein efflux pump. Despite this, the dose limiting nephrotoxicity has prevented cantharidin's entry into Western medicine [1, 2, 4]. Notwithstanding this, norcantharidin (**2**) (Fig. 1), the demethylated analogue, displays the favourable anti-cancer properties of cantharidin but displays little to no nephrotoxicity [1, 5]. In addition to the anti-cancer properties, both cantharidin and norcantharidin are potent inhibitors of the serine/threonine protein phosphatases PP1 and PP2A. Cantharidin is a 1.78 and 0.26 μM potent PP1 and PP2A inhibitor, respectively, while norcantharidin returns IC_{50} values of 1.98 and 0.37 μM for PP1 and PP2A, respectively [6].

Over the past decade we and others have expended considerable effort in the development of a better understanding of the key structural features that are required for both protein phosphatase inhibition and the anti-cancer effects of these analogues [6–22]. In the course of one such study we re-discovered, Novo-6 (**3**) (Fig. 1), a product that arose form the hydrogenation of 5,6-dehydronorcantharidin first reported by Eggelte [23]. Novo-6 (**3**) is phosphatase inactive, but does display remarkable anti-cancer selectivity with preferential cell death of colon cancer derived cell lines [6]. Excited by this observation, we have been keen to develop these analogues further, but our rational drug design approaches have been significantly hampered by the unknown stereochemistry at the C3-OH. The synthetic chemistry utilised in the preparation of Novo-6 suggests that there are two possible diastereomers, **3a** and **3b** (Scheme 1, enantiomers shown in boxed section).

Our initial efforts revealed only one diastereomer by TLC whereas those of Eggelte et al. showed the presence of two diastereomers by TLC [23]. This suggested that we could separate **3a** (anti) from **3b** (syn) by flash chromatography. We also note that our and Eggelte's efforts also suggest the presence of a major and minor product [23]. This is in keeping with the expected approach of the anhydride C=O to the surface of the Pd–C catalyst where the 7-O bridgehead would prefer to be distal with respect to the catalyst surface allowing a closer approach of the

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anhydride C=O to the surface. This occurs with the 7-O up (Fig. 2). Approach with the 7-O down places the anhydride C=O more distant to the surface thus disfavouring hydrogenation (Fig. 2). Our analysis suggests a 6:1 ratio of major:minor isomers (the actual ratios vary depending on the nature of the ether substituent). The positive identity of the major and minor diastereomers has been accomplished by derivatisation as an ether and crystallisation.

Experimental

Materials

All starting materials were purchased from Aldrich Chemical Co. and Lancaster Synthesis. Solvents were bulk, and distilled from glass prior to use, with the exception of THF which was freshly distilled from sodium-benzophenone. Reaction progress was monitored by TLC, on aluminium plates coated with silica gel with fluorescent indicator (Merck 60 F_{254}) and flash chromatography was conducted on Merck silica gel 60 (230–400 mesh). ^{1}H and ^{13}C spectra were recorded on a Bruker Avance AMX 300 MHz spectrometer at 300.13 and 75.48 MHz, respectively. Spectra were recorded using deuterated chloroform ($CDCl_3$) and chemical shifts are relative to TMS as internal standard. Melting points were recorded using a Büchi melting point M-565 apparatus and are uncorrected.

Synthesis

Compounds **7a** and **7b** (Scheme 2) were prepared by adding allyl alcohol (0.06 g, 1.07 mmol) to a magnetically stirred solution of Novo-6 (**3**, 0.20 g, 1.18 mmol) and catalytic *p*-TsOH (0.01 g) in anhydrous THF. The resulting solution was treated under microwave radiation for 1 h at 80 °C and 150 W. The reaction was then concentrated in vacuo and the residue was purified by flash chromatography (2:8 EtOAc:Hexanes) to yield an unseparated mixture of the diastereomers **7a** and **7b**. Slow evaporation of the eluate led to preferential crystallisation of **7b**. Analogue **7a** was obtained by concentration of the mother liquor.

7a: yield 59 mg, 26%, mp 36–38 °C, ^{1}H NMR ($CDCl_3$): δ 5.89 (m, 1H), 5.30 (d, $J = 1.7$ Hz, 1H), 5.28 (m, 2H), 4.83 (d, $J = 4.6$ Hz, 1H), 4.69 (d, $J = 4.6$ Hz, 1H), 4.30 (m, 1H), 4.07 (m, 1H), 2.92 (d, $J = 8.0$ Hz, 1H), 2.53 (dd, $J = 1.6$, 8.0 Hz, 1H), 1.85–1.46 (m, 4H) ppm; ^{13}C NMR ($CDCl_3$): δ 175.5, 132.3, 118.0, 105.8, 79.6, 79.0, 69.7, 50.3, 49.7, 28.0, 27.3 ppm.

7b: yield 18 mg, 8%, mp 100 °C, ^{1}H NMR ($CDCl_3$): δ 5.87–5.60 (m, 1H), 5.56 (d, $J = 6.8$ Hz, 1H), 5.37–5.23 (m, 2H), 5.13 (d, $J = 4.8$ Hz, 1H), 4.92 (d, $J = 4.8$ Hz, 1H), 4.46–4.40 (m, 1H), 4.17–4.10 (m, 1H), 2.89 (d, $J = 8.4$ Hz, 1H), 2.72–2.68 (m, 1H), 1.86–1.71 (m, 2H), 1.57–1.43 (m, 2H) ppm; ^{13}C NMR ($CDCl_3$): δ 174.8, 132.5, 117.7, 102.4, 78.7, 76.0, 70.8, 51.1, 46.6, 27.6, 27.5 ppm.

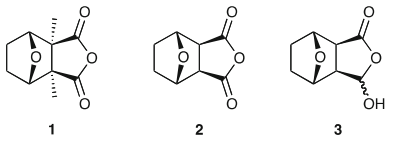


Fig. 1 Chemical structures of cantharidin (**1**), norcantharidin (**2**), and Novo-6 (**3**)

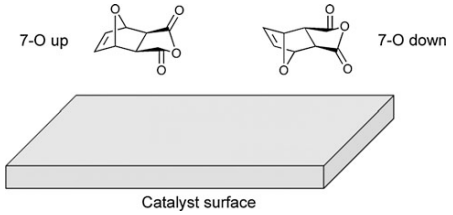
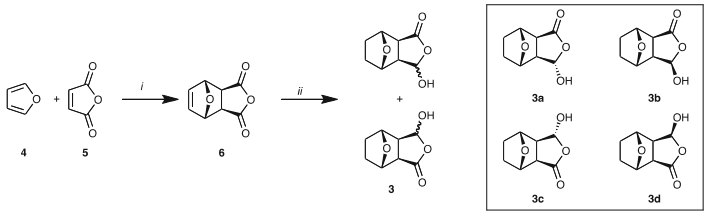
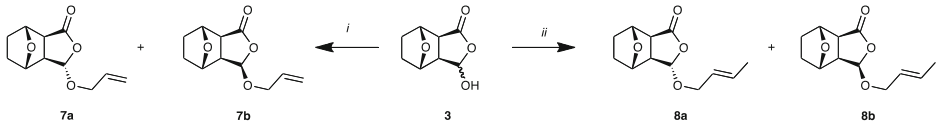


Fig. 2 Schematic representation of the approach of the two most likely approach configurations of **6** to the catalyst surface



Scheme 1 Reagents and Conditions: *i* Et_2O, rt, 48 h; *ii* Pd–C, H_2 (4 atm), wet EtOH



Scheme 2 Reagents and conditions *i* THF, pTsOH, allyl alcohol, MW 80 °C, 180 W, 1 h; *ii* THF, pTsOH, crotyl alcohol, MW 80 °C, 180 W, 1 h

Compounds **8a** and **8b** were prepared by adding crotyl alcohol (0.08 g, 1.07 mmol) to a magnetically stirred solution of Novo-6 (0.20 g, 1.18 mmol) and catalytic p-TsOH (0.01 g) in anhydrous THF. The resulting solution was treated under microwave radiation for 1 h at 80 °C and 150 W. The reaction was then concentrated in vacuo and the residue was purified by flash chromatography (1:9 EtOAc:Hexanes) to yield the corresponding isomers. Slow evaporation of the eluting solvents achieved preferential crystallisation of **8a** in this case. Isomer **8b** was obtained by concentration of the mother liquor.

8a: yield 100 mg, 42%, mp 78 °C, ^{1}H NMR ($CDCl_3$): δ 5.78 (dq, $J = 15.2$, 6.4 Hz, 1H), 5.55 (dt, 15.2, 6.0 Hz, 1H), 5.30 (d, $J = 1.1$ Hz, 1H), 4.83 (d, $J = 4.5$ Hz, 1H), 4.69 (d, $J = 4.6$ Hz, 1H), 4.24 (dd, $J = 6.0$, 0.8 Hz, 1H), 4.01 (dd, $J = 7.1$, 0.8 Hz, 1H), 2.91 (d, $J = 7.9$ Hz, 1H), 2.50 (d, $J = 7.9$ Hz, 1H), 1.80-1.45 (m, 4H), 1.73 (d, $J = 6.4$ Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$): δ 175.5, 131.2, 125.0, 105.5, 79.7, 79.0, 69.6, 50.4, 49.8, 28.0, 27.2, 17.3 ppm.

8b: yield 21 mg, 9%, mp 116–118 °C, ^{1}H NMR ($CDCl_3$): δ 5.76 (dq, $J = 15.3$, 6.4 Hz, 1H), 5.57 (m, 1H), 5.56 (d, $J = 6.8$ Hz, 1H), 5.11 (d, $J = 4.8$ Hz, 1H), 4.90 (d, $J = 4.7$ Hz, 1H), 4.35 (dt, $J = 5.5$, 1.3 Hz, 1H), 4.06 (m, 1H), 2.88 (d, $J = 8.4$ Hz, 1H), 2.67 (dd, $J = 6.9$, 8.1 Hz, 1H), 1.85–1.38 (m, 4H), 1.73 (d, $J = 6.5$ Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$): δ 175.0, 130.6, 125.3, 102.1, 78.7, 76.0, 70.6, 51.2, 46.6, 27.6, 27.5, 17.2 ppm.

X-Ray Crystallography

Crystallographic data (MoKα, $2\theta_{max} = 50°$) were collected on an Oxford Diffraction Gemini S Ultra CCD diffractometer at 293 K. Data reduction and empirical absorption corrections were carried out with the CrysAlis Pro program (Oxford Diffraction vers. 171.33.42). The structure was solved by direct methods with SHELXS86 and refined with SHELXL97 [24]. All non-H-atoms were refined aniostropically and H-atoms were constrained at their estimated positions using a riding model. The thermal ellipsoid diagrams were generated with ORTEP3 [25]. All crystallographic calculations were carried out within the WinGX graphical user interface [26]. The crystal and instrumental parameters used in the unit-cell determination and data collection are summarized in Table 1.

Table 1 Crystal data and refinement details for (**7b**) and (**8a**)

	(**7b**)	(**8a**)
Formula	$C_{11}H_{14}O_4$	$C_{12}H_{16}O_4$
Formula weight	210.22	224.25
Temperature (K)	298	298
Wavelength (Å)	0.71073	0.71073
Crystal system	Triclinic	Monoclinic
Space group	$P\bar{1}$ (No. 2)	$P2_1/c$ (No. 14)
Unit cell dimensions		
a (Å)	4.8787(5)	16.926(1)
b (Å)	9.6866(8)	8.4182(4
c (Å)	11.1979(9)	8.1004(5)
α (°)	89.843(6)	
β (°)	79.465(7)	94.128
γ (°)	85.653(7)	
Volume (Å^3)	518.74(8)	1151.2(1)
Z	2	4
Density (calculated) (g cm^{-3})	1.346	1.294
Absorption coefficient (mm^{-1})	0.102	0.097
F(000)	224	480
Crystal size (mm)	0.3 × 0.2 × 0.08	0.4 × 0.4 × 0.1
θ range for data collection (°)	3.70–25.00	3.42–24.99
Reflections collected	3,289	4,181
Independent reflections	1,828	2,020
Observed reflections	979	944
R_{int}	0.0249	0.0367
Number of parameters	136	145
Goodness-of-fit on F^2	0.797	0.773
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0360$, $wR_2 = 0.0609$	$R_1 = 0.0423$, $wR_2 = 0.0773$
R indices[all data]	$R_1 = 0.0876$, $wR_2 = 0.0673$	$R_1 = 0.178$, $wR_2 = 0.0871$
CCDC deposition no.	826792	826791

Results and Discussion

Our primary interest with Novo-6 was in the development of new structure activity relationship data through the synthesis of focused compound libraries, and we rationalised that this approach may also be used to simplify the separation of the diastereomers identified in Scheme 1. We hoped that the introduction of a hydrophobic tail would simplify chromatographic resolution. The C3-OH moiety results in a highly polar material with no R_f difference observed between isomers (0.35, 1:1 EtOAc:Hexanes). However, given the additional synthetic step to form the desired ether analogues, consideration must be given to the mechanism of addition. If the reaction proceeds via an acid catalysed S_N2 mechanism, then we would expect inversion of configuration, however if the reaction proceeded via an oxonium stabilised carbocation, no conclusion as to the stereochemistry of Novo-6 could be drawn. Regardless, obtaining crystals of the resultant products would allow assignment of the relative stereochemistry of the Novo-ether analogues, which in turn could assist in the design of new, more potent analogues.

In a typical experiment, the *p*-toluene sulfonic acid mediated substitution reaction produced both desired isomers as shown in Scheme 2. NMR examination of the crude reaction mixture showed the presence of two isomers in a 6:1 ratio. Flash silica chromatography allowed isolation of each isomer pairing (**7a**:**7b** and **8a**:**8b**).

Characterisation of the relative stereochemistry of each isomer was originally carried out by ^{1}H NMR analysis. Although the spectra were very similar, a change in coupling constant assigned to the hydrogen at position C3 was observed between isomers. The major isomer was always found to have a higher R_f (TLC, 0.81, 1:1 EtOAc:Hexanes) and produced a characteristic doublet with a coupling constant of $J = 1.7$ Hz whereas the minor isomer, with a lower R_f (0.62, 1:1 EtOAc:Hexanes), produced a doublet with a coupling constant, $J = 6.8$ Hz. This was originally explained by the varying bond angle and orbital overlap between the hydrogens at position **3** and **3a** between the two isomers depicted in Fig. 3. In order to confirm these observations, crystal structures of **7b** (Fig. 4) and **8a** (Fig. 5) each as racemates were determined.

The conformation of **7b**, shown in Fig. 4, indicates that the allyloxy substituent is syn with respect to the furan O-atom (O2). The allyl group in this conformation is close to the bridgehead O-atom (O4), which based on an acid catalysed S_N2 mechanism is less favourable than the orientation shown for the major product **7a**, making this the less favoured isomer which correlates well with the observed yield of this isomer. The H–C3–C3a–H dihedral angle obtained from this crystal structure is 10.4° and supports the previously assigned NMR structure based on

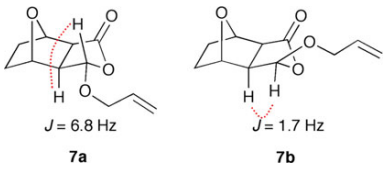


Fig. 3 Dihedral angle (°) and ^3J (Hz) difference between **7a** (110.7°) and **7b** (10.4°)

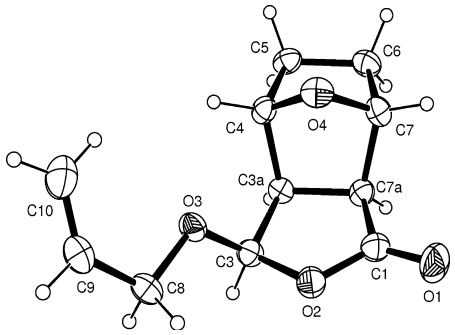


Fig. 4 ORTEP 3 view of **7b** (30% ellipsoids shown)

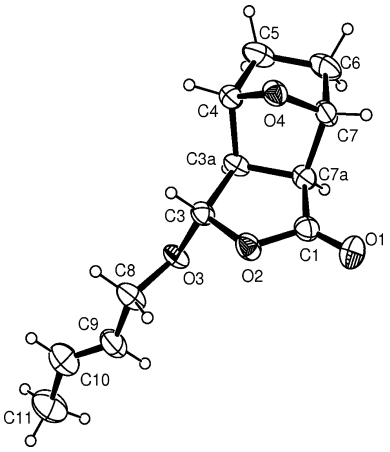


Fig. 5 ORTEP 3 view of **8a** (30% ellipsoids shown)

dihedral angle and orbital overlap between hydrogens at positions **3** and **3a**.

Compound **8a** was also crystallised. In this case the crotyl ether substitutent is anti with respect to the bridgehead O-atom. Of note is that the H-C3-C3a-H dihedral angle is 110.7°. This further confirms the observed ratio of anti*:*syn of 6:1 and stereochemical assignment from the structure of **7b**. Nucleophilic attack on C3 by the corresponding alcohol is far less hindered from below the plane compared to attack from above the plane of the Novo backbone due to the presence of large polar groups situated at the bridgehead and lactone group of the molecule.

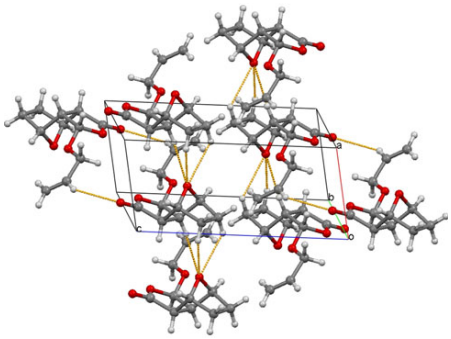


Fig. 6 Mercury (vers. 2.4) representation of the packing in **7b** highlighting non-classical H-bonding contacts

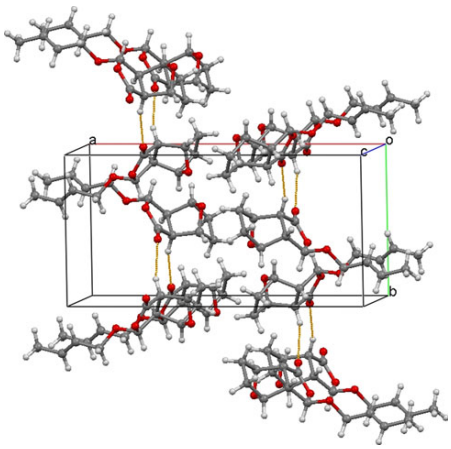


Fig. 7 Mercury (vers. 2.4) representation of the packing in **8a** highlighting non-classical H-bonding contacts

The packing diagram of **7b** is presented in Fig. 6 (generated with Mercury vers. 2.4) showing a number of weak non-classical C–H···O bonds (none closer than 2.6 Å).

A somewhat different packing is seen in **8a** and in this case the carbonyl O-atom is the only potential acceptor involved in significant non-classical H-bonds (C5–H···O2′ 2.54 Å: symmetry x, $3/2 - y$, $z - 1/2$). The oxa bridgeheads of neighbouring molecules are pointing towards each other and the ether tails are pointing in the same direction, in contrast to the anti-parallel arrangement in **7b**.

In summary, two unsaturated Novo-6 analogues, **7** and **8**, have been synthesised, separated into their corresponding epimers and one example of each has been structurally characterised. The difference in configuration at position **3** (atom C8 in Fig. 4, 5) results in an observable change in ^{1}H NMR specifically the ^{3}J coupling between hydrogens at positions **3** and **3a**, respectively due to their very different dihedral angles. The ratio of isomers has been experimentally shown to be 6:1. The major isomer, with the ether tail orientated below the plane of the lactone ring, has far less orbital overlap and a ^{3}J coupling constant of $J = 1.7$ Hz corresponding to the hydrogens at positions **3** and somewhat different packing is seen in, respectively. The minor isomer with the ether tail orientated above the plane of the lactone ring, has a higher degree of orbital overlap resulting in a higher ^{3}J coupling constant of 6.8 Hz between hydrogens at positions **3** and **3a**, respectively (Fig. 7).

Acknowledgment The authors acknowledge the financial support of the Australian Research Council, the Australian Cancer Research and Ramaciotti Foundations, and John Morris Scientific, Australia. MT acknowledges the UNI-PRS postgraduate funding from the University of Newcastle.

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3.3 Paper III

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Norcantharidin analogues with nematocidal activity in *Haemonchus contortus*

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ABSTRACT

With the major problems with resistance in parasitic nematodes of livestock to anthelmintic drugs, there is an urgent need to develop new nematocides. In the present study, we employed a targeted approach for the design of a series of norcantharidin analogues ($n = 54$) for activity testing against the barber's pole worm (*Haemonchus contortus*) of small ruminants in a larval development assay (LDA) and also for toxicity testing on nine distinct human cell lines. Although none of the 54 analogues synthesized were toxic to any of these cell lines, three of them (*N*-octyl-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (**B2**), *N*-decyl-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (**B3**) and 4-[(4-methyl)-3-ethyl-2-methyl-5-phenylfuran-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (**B21**) reproducibly displayed 99–100% lethality to *H. contortus* in LDA, with LD_{50s} of 25–40 μM. The high 'hit rate' (5.6%) indicates that the approach taken here has advantages over conventional drug screening methods. A major advantage of norcantharidin analogues over some other currently available anthelmintics is that they can be produced in one to two steps in large amounts at low cost and high purity, and do not require any additional steps for the isolation of the active isomer. This positions them well for commercial development.

In spite of their massive impact, parasites of humans and other animals are seriously neglected in terms of funding for research and development (R&D) of drugs, vaccines and diagnostics. The current production losses caused by parasites to agriculture worldwide have a major adverse impact on farm profitability and exacerbate the global food shortage. For instance, nematodes of livestock cause major production losses to farmers due to poor productivity, failure to thrive and deaths.[1–5] In particular, strongylid nematodes are of paramount importance as pathogens of sheep, goats, cattle and pigs, causing gastrointestinal diseases and associated complications, often leading to death in severely affected animals.[5] Currently, these nematodes are controlled predominantly through the use of anthelmintics, but widespread resistance against a range of compounds (of three main classes) has compromised their efficacy.[6–11] Thus, there is an urgent need to work toward identifying new drug targets and developing new nematocides.

We have been pursuing the molecular characterization of a number of gender- and/or stage-enriched molecules in parasitic nematodes (Strongylida) using *C. elegans* as a reference organism, with a perspective on predicting novel drug targets.[12–30] Through a number of studies,[16,24,31] we have provided insights into genes encoding protein phosphatases (PPs) for selected strongylid nematodes. These studies have shown that selected serine–threonine phosphatases (STPs) are quite conserved between parasitic and free-living nematodes and have inferred that they play key roles in pathways required for the growth, development, survival and/or reproduction.[25] In addition, phylogenetic analysis has indicated that such STPs are specific to nematodes, clustering, with strong support, to the exclusion of related molecules in other invertebrates and vertebrates.[24]

Current literature indicates that inhibitors, such as cantharidin (1) (from the blister beetle, *Mylabris*)[32–36] and a number of analogues with the same pharmacophoric units, most notably some derived from norcantharidin (2), have no adverse toxic effects on well-defined, cultured human cells[34,35,37–39] but were considered to have unique potential for the development of nematocides (Fig. 1).[25] The former characteristic is important, as the focus should be on identifying compounds that have no adverse effect on mammalian cells (representing the host animal) but are lethal to parasitic nematodes or block their reproduction. Some norcantharidin analogues are known to display excellent STP (i.e., PP1 and PP2A) inhibitory activity.[34,35,40,41] Preliminary work conducted by us showed that some norcantharidin analogues, which had no toxic effect on human cell lines, killed larvae of the trichostrongylid nematodes *Trichostrongylus vitrinus* and/or *Haemonchus contortus*.[25] Homology modelling and in silico docking suggested that that

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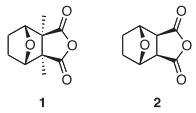


Figure 1. The chemical structures of cantharidin (**1**) and norcantharidin (**2**).

some prototype molecules designed bind specifically to active sites in selected STPs of these parasites.[25] These initial findings indicated an opportunity for the discovery of a novel class of nematocides and significant biotechnological outcomes. In the present study, we designed a series of norcantharidin analogues and then tested their nematocidal effect on *H. contortus* in a larval development assay (LDA).

Three families of norcantharidin analogues were synthesized: the ring-opened acid amides (library A, **A1**–**A24**), the ring-closed norcantharimides (library B, **B1**–**B22**) and the tetrahydroepoxyisoindole carboxamides (library C). These compounds were accessed through a series of robust in house-developed and generic approaches reported previously.[34,35,42–45] These approaches facilitated the rapid installation of a range of substituents for structure–activity relationship (SAR) studies.

All chemicals were tested for cytotoxicity in vitro against nine different human cancer cell lines: HT29 (colon), SW480 (colon), MCF-7 (breast), A2780 (ovarian), H460 (lung), A431 (skin), DU145 (prostate), BE2-C (neuronal) and SJ-G2 (brain), as previously described.[32,35,40,41] Following cytotoxicity testing, chemicals were tested in a larval development assay (LDA). *H. contortus* (Haecon 5 strain) was raised in helminth-free lambs (Merino crosses; 24 weeks of age), as described by Nikolaou et al.[46]

The present study employed a targeted approach for the design of a series of norcantharimide analogues for toxicity testing on nine different human cancer cell lines and for subsequent testing for nematocidal activity in LDA against *H. contortus*. A total of 54 analogues was synthesized (see Supplementary data). For both the ring-opened acid amide (library A) and ring-closed norcantharimide analogues (library B), the ease of synthesis typically related to the nucleophilicity of the amine used to develop each focused library member. The more nucleophilic amines favoured ring-closing to the corresponding norcantharimide, non-nucleophilic amines required prolonged heating to effect this transformation (path B in Scheme 1). Most notable was the reaction of *N*-methyl-1-(5-phenylfuran-3-yl)methanamine which, instead of affording the expected ring-opened analogue (path A in Scheme 1), underwent a very facile ring-closing to the quaternary ammonium norcantharimide (**B21**).

The synthesis of the tetrahydroepoxyisoindole carboxyamides was more challenging, but the products typically represented good yields (Scheme 2, library C).

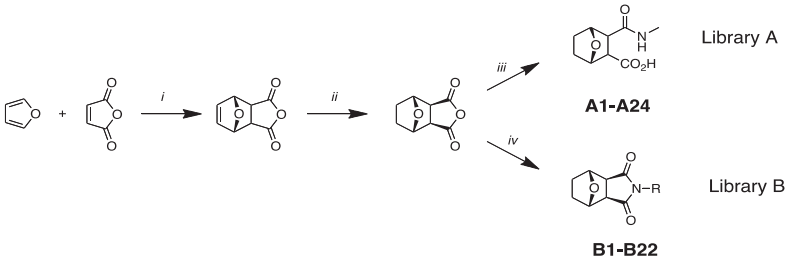
As a series of internal assay-validation standards were also synthesized, using a phase transfer catalyst approach, selected (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile derivatives, which had been reported previously to be active against both *H. contortus* and the cat flea, *Ctenocephalides felis*.[47,48] The library compounds listed are those inhibitors that passed our toxicity pre-filter by displaying low levels, defined as a mean GI_{50} value >75 μM, of cell death in nine distinct human cancer cell lines (HT29, SW480, MCF-7, A2780, H460, A431, DU145, BE2-C and SJ-G2).[32,34,35,40,41] As reported previously,[34,35] the ring-opened acid amine analogues displayed higher levels of cytotoxicity (10–60 μM) than the corresponding ring-closed norcantharimides (>75 μM). All 54 analogues that passed the 'toxicity filter' were evaluated for nematocidal activity against *H. contortus* in LDA.

Based on our previous studies,[34–37,40,41] we anticipated our library A analogues to be the most potent (as we had shown protein phosphatase inhibition by some of these analogues). To our surprise, none of the analogues from library A returned notable parasite lethality. Analogues were tested over a drug concentration range of 12.5–100 μM.

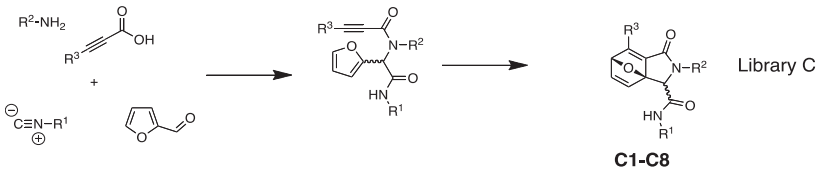
Subsequently, we examined the library B analogues representing the norcantharimide that we have shown previously to be devoid of protein phosphatase inhibition. To our surprise, three analogues (library B; **B2**, **B3** and **B21**; Fig. 2) from this library achieved 99–100% lethality of *H. contortus* in LDA (Table 1). All three compounds were retested on four separate occasions, achieving the same result. Progression to a full-dose response evaluation, at concentrations between 10 and 100 μM, revealed that each of these analogues had LD_{50} values in the range of 25–40 μM (Table 1). Interestingly, both **B2** and **B3** possess a long alkyl chain, which may enhance their bioavailability and assist their transport across the parasite cuticle and into tissues and cells, allowing access to the target in the parasite. Given our previous findings in relation to protein phosphatase inhibition by this class of norcantharidin analogue, we believe that it is unlikely that the ultimate target is a serine–threonine protein phosphatase. The highly hydrophobic nature of the phenylfuran moiety of **B21** most likely also assists transport through cell membranes. We had hoped that further elongation of the alkyl tail of **B2** and **B3** may improve uptake, but the dodecyl **B4**, tetradecyl **B5** and octadecyl **B6** were inactive. Presumably, this lack of activity is a consequence of poor water solubility.

Given the activity of **B2** and **B3**, we specifically tailored the synthesis of library C to include hydrophobic groups. However no compound from library C displayed any noteworthy nematocidal activity at the initial screening doses (12.5–100 μM).

Libraries A and B are related via a simple ring closing which effectively removes an acid and an amide moiety from the inactive pharmacophore. This, in turn, suggested that either the ultimate protein target disfavours the presence of these hydrogen bond



Scheme 1. Reagents and conditions: (i) Et_2O, rt, 48 h; (ii) acetone, 10% Pd–C, H_2 (g) 50 psi, 18 h; (iii) RNH_2, $PhCH_3$, Δ, 24–36 h.



Scheme 2. Reagents and conditions: (i) R^1NC, alkynoic acid, furan carbaldehyde, R^2NH_2, CH_3OH, rt 30 min; (ii) $PhCH_3$, sealed tube 200 °C, 36 h.

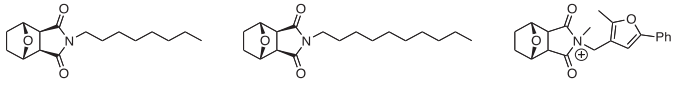
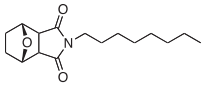
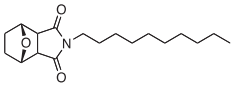
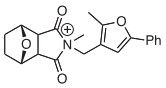
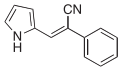
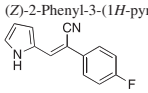
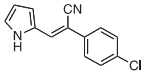
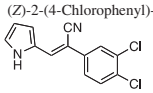


Figure 2. The three compounds (**B2**, **B3** and **B21**; ring closed norcantharimide analogues) from library B which consistently killed 99–100% of *Haemonchus contortus* in the larval development assay (LDA).

Table 1
Of 54 compounds synthesized (see Supplementary data, and Figs. 1 and 2) tested against *Haemonchus contortus* in a larval development assay (LDA), three norcantharimide analogues from library B reproducibly killed *H. contortus*. Included in the LDA were four control compounds (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile, (*Z*)-2-(4-fluorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile, (*Z*)-2-(4-chlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile, (*Z*)-2-(3,4-dichlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile)[47,48]

Compounds	Concentration range (μM) tested	LD_{50} (μM)	LD_{99} (μM)
Test compounds with nematocidal activity:			
 **B2**	10–100	40	80
 **B3**	10–100	30	90
 **B21**	10–100	25	100
Control compounds:			
 (*Z*)-2-Phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile	6.25–100	6	100
 (*Z*)-2-(4-Fluorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile	6.25–100	10	50
 (*Z*)-2-(4-Chlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile	6.25–100	10	25
 (*Z*)-2-(3,4-Dichlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile	6.25–100	7	12.5

The LD_{50} and LD_{99} values for each compound are indicated.

donor–acceptor groups or that these groups prevent the penetration of the tissues or cuticle of the parasite, or that analogues with the library A pharmacophore are substrates for an active efflux or degradation mechanism. Which mechanism is operational is currently unknown. Library C differs in the relative spatial presentation of the key pharmacophoric moieties (relative to library B), suggesting that the position of these groups is crucial to eliciting the observed lethality of *H. contortus* for **B2**, **B3** and **B21**. Although an ovicidal effect has been reported for some commercially available anthelmintics, such as benzimidazoles,[49,50] there was no evidence of this effect for any of the analogues tested herein.

Until relatively recently, the search for novel drugs against parasites has usually been carried out using approaches which are decades old, such as the screening of many thousands of chemicals for inhibition or disruption of parasite growth and/or development in vitro. Today, genomic, proteomic, bioinformatic and/or chemoinformatic technologies are increasingly being used to assist the search for new compounds.[30,51–57] A major goal of current genomic and transcriptomic studies of parasites is the inference of novel candidate drug targets, guided by essentiality and genetic interaction studies.[16–23,27,28] However, the major challenge is not only to identify potential targets, but, importantly, to prioritize them, such that available resources can be focused on those most likely to lead to effective treatments. The length of time and the prohibitive costs associated with bringing a new drug to market, together with the knowledge that most lead-compounds fail at some stage in the development process, have deterred most pharmaceutical companies from investing in the discovery of entirely novel targets and classes of anthelmintics using integrated genomic–bioinformatic–chemoinformatic platforms. However, the recent success in developing monepantel through to a commercial product[58–64] provides fresh hope for the discovery of novel classes of anthelmintic compounds.[65]

In the present investigation, we were guided by a range of previous studies[16,24,25,31] showing that selected serine–threonine phosphatases (i.e., PP1 and PP2A) might represent suitable targets for strongylid nematodes, including *H. contortus*, because they are: (i) known to be essential for growth, development, survival and/or reproduction, (ii) conserved between these nematodes and *C. elegans* but (ii) divergent from related molecules in other invertebrates and vertebrates (including mammalian hosts).[25] That some norcantharidin derivatives display exquisite PP1 and PP2A inhibitor activity[34,35,40,41] suggested that a series of analogues, with no or limited toxicity to mammalian cell lines, could be designed and produced to specifically inhibit serine–threonine phosphatases of *H. contortus*. Three of the 54 analogues synthesized displayed almost complete lethality to *H. contortus* in LDA, achieving a 'hit rate' that exceeded (by at least five times) that reported previously for traditional screening methods.[66]

Although norcantharidin is known as a phosphatase inhibitor,[40,41,34–37,67] some of the novel analogues synthesized and tested herein (and which no longer closely resemble the original 'backbone molecule') might have molecular targets other than PP1s and/or PP2As. Currently, we are exploring new approaches to facilitate and determine the target(s) of these compounds. In addition, further work should also focus on improving the LD_{50} and bioavailability of the three compounds. A major commercial advantage of these chemicals over some other currently available anthelmintics is that they can be produced in one to two steps in large amounts at low cost and high purity, and do not require any additional steps for the isolation of the active isomer. By contrast, monepantel (an aminoacetonitrile derivative),[56] for example, needs to be synthesized in multi-step chemical reaction pathways, followed by the isolation of the active optical isomer. Given that the present norcantharimide analogues display a lack of toxicity to mammalian cells, they should now be tested directly in vivo (in sheep) against *H. contortus* and also for activity in vitro and in vivo against other parasitic nematodes. In future, compounds that are not toxic to mammalian cell lines and have failed screens on plant parasites and/or, for example, cancer cells should be screened for activity and lethality against parasitic nematodes of animals and humans.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.031.

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3.4 Paper IV

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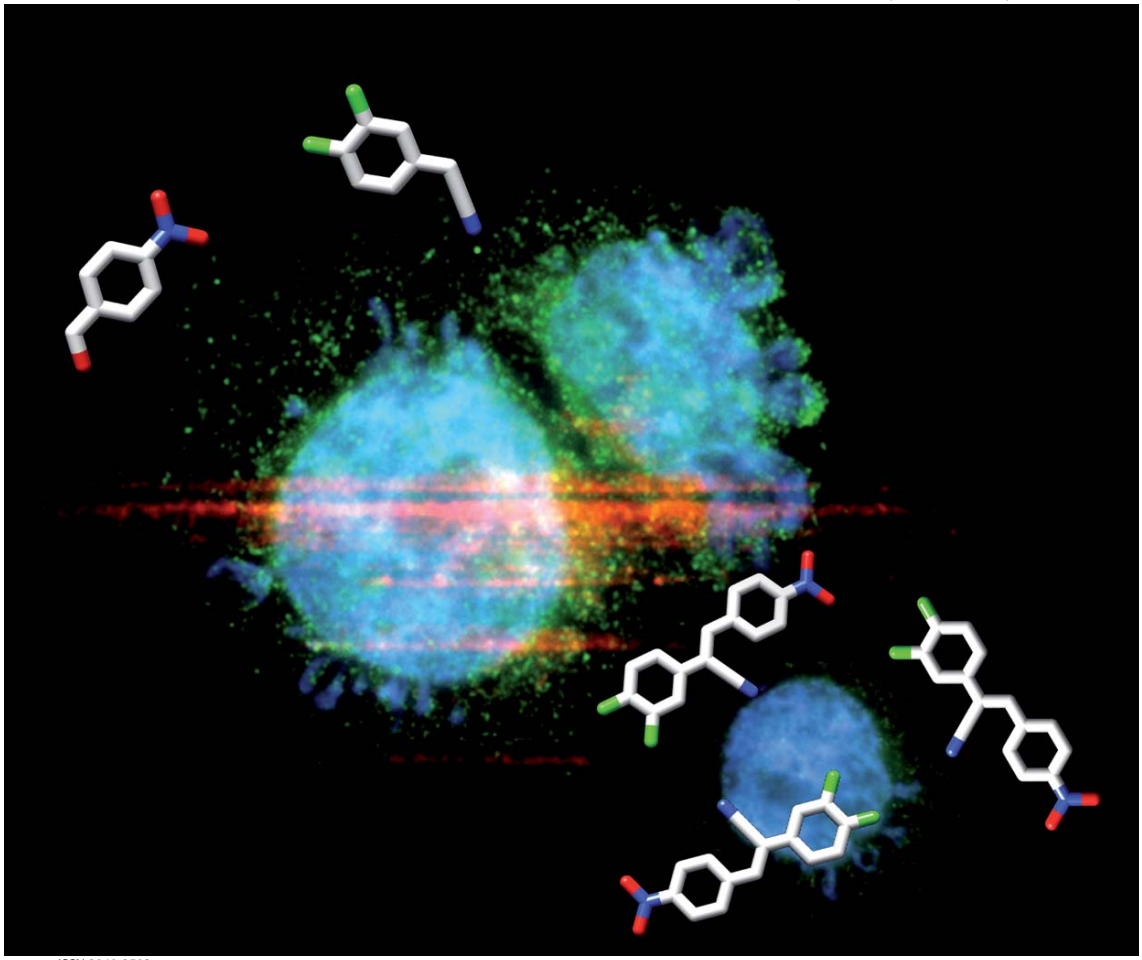

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CONCISE ARTICLE
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CONCISE ARTICLE

Library synthesis and cytotoxicity of a family of 2-phenylacrylonitriles and discovery of an estrogen dependent breast cancer lead compound†

Mark Tarleton,[a] Jayne Gilbert,[b] Mark J. Robertson,[a] Adam McCluskey*[a] and Jennette A. Sakoff[b]

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In our efforts to prevent highly toxic compounds progressing through our anti-parasitic drug development program, we serendipitously discovered a family of 2-phenylacrylonitriles with excellent growth inhibition of a panel of ten human cancer cell lines. Focused library approaches facilitated the identification of a simple pharmacophore, comprising two terminal aromatic moieties linked *via* a conjugated cyano (acrylonitrile) moiety. Efforts that perturbed this pharmacophore resulted in a significant drop in growth inhibition. Multiple libraries led to the discovery of two key lead compounds. The first, (*Z*)-2-(3,4-dichlorophenyl)-3-(4-methoxyphenyl)acrylonitrile (**31**) exhibits broad spectrum growth inhibition with GI_{50} values of 0.52–3 μM (HT29 and BE2-C cancer cell lines respectively; average = 1.6 μM). Of greater note is (*Z*)-2-(3,4-dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**28**), a 0.127 ± 0.043 μM growth inhibitor of the estrogen receptor positive (ER+ve) human breast cancer cell line, MCF-7. Analogue **28** displays up to 543 fold selectivity towards MCF-7 cells compared with nine other non-breast derived cancer cell lines. Further screening of **28** against one human, ER−ve breast cancer cell line (MDA-MB231) and one normal non-tumourigenic breast epithelial cell line (MCF-10A) returned poor growth inhibition values of 34 ± 2 and 16 ± 4μM, demonstrating *ca.* ∼268 and∼126 fold preference for the MCF-7 estrogen dependent breast cancer cells.

Introduction

Over the past decade, our group has invested considerable effort in the development of highly focused compound libraries in the search for new biologically active molecules. We have an active interest in the development of anti-cancer and anti-parasitic agents.[1] In this latter regard, we were initially keen to ensure the internal validity of our anti-parasitic screening approach, and as such were keen to include anti-parasitic agents of known activity. Our preference was for a small library of agents with divergent activities and ease of synthetic access. The (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile derivatives reported by Ali *et al.*, fulfilled both of these criteria (Fig. 1).[2]

One additional requirement that we imposed on our development of anti-parasitic agents was a toxicity pre-filter to ensure that the ultimate end user would not be subjected to highly toxic agents that would be applied in a non-ideal environment, *e.g.* rural farming communities. We thus chose to routinely examine the cytotoxicity of all new compounds in our anti-parasite program *via* cytotoxicity screening against a panel of ten human cancer cell lines: HT29 and SW480 (colon carcinoma), MCF-7 (breast carcinoma), A2780 (ovarian carcinoma), H460 (lung carcinoma), A431 (skin carcinoma), DU145 (prostate carcinoma), BEC-2 (neuroblastoma), SJ-G2 (glioblastoma), MIA (pancreatic carcinoma). The first series of compounds examined in this panel of cell lines were the known (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitriles (**1–5**, Fig. 1).[2] To our surprise, these

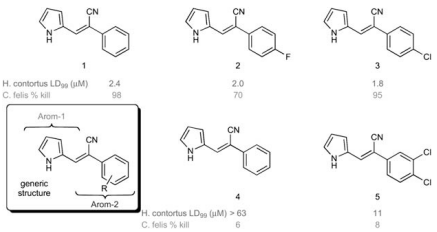

Fig. 1 Generic structural representation, and selected examples, of the compounds reported by Ali *et al.* as active against *Haemonchus contortus* (*H. contortus*) and *Ctenocephalides felis* (*C. felis*).[2]

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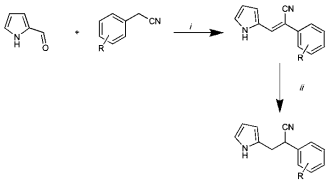


Scheme 1 Reagents and conditions: (*i*) H_2O, 40% $PhCH_2NMe_3(OH)$, 50 °C, 5 h; (ii) 0.05M solution in acetone, ThalesNano H-cube™, 10% Pd/C catalyst at 1 mL min^{-1} at 50 °C and 50 bar H_2 pressure.

analogues were highly cytotoxic (see below). Intrigued by these findings we set about developing a series of highly focused libraries in an effort to improve on the cell death and cell line specificity that we noted. Our efforts are reported herein.

Results and discussion

Our drug development paradigm is a simple one, relying on the application of robust reliable chemistries in the synthesis of highly focused compound libraries that are then subjected to rapid biological screening. This approach allows for the development of multiple library generations in a short period of time. The original synthesis of (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile derivatives reported by Ali *et al.*, fits well within our paradigm.[2] Library access was readily accomplished, in excellent yields, by the simple condensation of pyrrole-2-carboxaldehyde (**Arom-1** in Fig. 1) with a family of phenylacetonitrile derivatives (Scheme 1).[3–5]

In our initial investigations, *Library A* comprised the five analogues shown in Fig. 1, retaining the pyrrole moiety with variations of the aromatic substituent (**Arom-2** in Fig. 1). *Library A* was screened for growth inhibition against our panel of ten human cancer cell lines (Table 1).

With the exception of the parent phenyl substituted (**1**), which is a modest inhibitor of cell growth, all *Library A* members displayed excellent levels of growth inhibition across all cell lines examined. Two *Library A* members stand out with the 4-chlorophenyl (**3**) and 3,4-dichlorophenyl (**5**) analogues returning the best growth inhibition with average GI_{50} values of 22 μM and 19 μM respectively. Both analogues **3** and **5** also displayed a considerable degree of specificity for the breast cancer cell line MCF-7 with GI_{50} values of 4.0 ± 0.5 and 0.56 ± 0.03 μM respectively. Introduction of the second chlorine atom (**3** → **5**) also notably enhanced cell death in the H460 (lung carcinoma) and A431 (skin carcinoma) cell lines (GI_{50}'s = 5.7 ± 0.7 and 3.2 ± 0.1 μM respectively). Thus the type and number of electronegative moieties attached to the phenyl ring appears to have a role in the growth inhibition of key cancer cell lines.

Based on the biological data from *Library A* it appeared that an extended conjugated system with one end possessing a high level of electronegative atoms was beneficial to activity. Working on this hypothesis we utilized **5** as the lead compound in the design and subsequent synthesis of *Library B*, which comprised the same five compounds as in *Library A* lacking only the alkene double bond of the acrylonitrile moiety, which we believed would have an adverse effect on cell death. *Library B* analogues were accessed *via* simple flow hydrogenation approaches (see experimental) (Scheme 1). We note that these conditions resulted in reduction of the $-NO_2$ to $-NH_2$ (**9**). The growth inhibition screening data for *Library B* analogues are shown in Table 2.

The effect of conjugation removal from *Library B* is quite evident, with a significant reduction in growth inhibition across all compounds and cell lines examined, supporting our

Table 1 Evaluation of the cytotoxicity, GI_{50} (μM) values, of *Library A* (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile derivatives **1–5**, against a panel of ten human cancer cell lines. GI_{50} is the concentration of drug that reduces cell growth by 50%.[6,7]

Ar (compound)	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]
 **1**	41 ± 6	26 ± 4	17 ± 1	25 ± 2	81 ± 11	57 ± 5	> 100	61 ± 7	46 ± 7	81 ± 11
 **2**	31 ± 4	24 ± 2	15 ± 1	25 ± 0	52 ± 2	37 ± 3	59 ± 10	43 ± 7	30 ± 4	63 ± 5
 **3**	18 ± 1	22 ± 0	4.0 ± 0.5	18 ± 0	19 ± 1	23 ± 3	37 ± 3	21 ± 1	20 ± 1	38 ± 2
 **4**	25 ± 1	24 ± 1	18 ± 1	18 ± 1	32 ± 0	27 ± 1	26 ± 1	22 ± 1	24 ± 1	34 ± 1
 **5**	15 ± 1	23 ± 1	0.56 ± 0.03	16 ± 0	5.7 ± 0.7	3.2 ± 0.1	41 ± 8	25 ± 1	20 ± 1	46 ± 7

[a] HT29 and SW480 (colon carcinoma). [b] MCF-7 (breast carcinoma). [c] A2780 (ovarian carcinoma). [d] H460 (lung carcinoma). [e] A431 (skin carcinoma). [f] DU145 (prostate carcinoma). [g] BEC-2 (neuroblastoma). [h] SJ-G2 (glioblastoma). [i] MIA (pancreatic carcinoma).

Table 2 Evaluation of the cytotoxicity, GI_{50} (μM) values, of *Library B* (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)propanenitrile derivatives **6–10**, against a panel of ten human cancer cell lines. GI_{50} is the concentration of drug that reduces cell growth by 50%. Values in parentheses are percent growth inhibition at 100 μM drug concentration.[6,7]

Ar (compound)	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]
 **6**	(39 ± 8)	(40 ± 14)	(47 ± 9)	(76 ± 10)	(15 ± 7)	(20 ± 4)	(7 ± 5)	(43 ± 6)	(27 ± 5)	(30 ± 5)
 **7**	73 ± 7	78 ± 8	65 ± 5	69 ± 4	88 ± 12	89 ± 11	91 ± 9	75 ± 3	86 ± 11	90 ± 10
 **8**	37 ± 7	53 ± 3	26 ± 2	26 ± 3	58 ± 2	58 ± 1	60 ± 5	45 ± 6	52 ± 5	58 ± 6
 **9**	67 ± 18	70 ± 5	37 ± 7	29 ± 3	64 ± 21	78.0 ± 7	90 ± 10	65 ± 9	87 ± 11	82 ± 11
 **10**	(51 ± 4)	(34 ± 2)	(84 ± 1)	(63 ± 1)	(55 ± 1)	(44 ± 1)	(24 ± 3)	(42 ± 3)	(33 ± 4)	(20 ± 2)

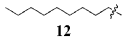
[a] HT29 and SW480 (colon carcinoma). [b] MCF-7 (breast carcinoma). [c] A2780 (ovarian carcinoma). [d] H460 (lung carcinoma). [e] A431 (skin carcinoma). [f] DU145 (prostate carcinoma). [g] BEC-2 (neuroblastoma). [h] SJ-G2 (glioblastoma). [i] MIA (pancreatic carcinoma).

hypothesis. The unconjugated analogues **7–9** exhibited a three fold potency reduction when compared with their conjugated counterparts, analogues **2–4**, with average GI_{50} values of 80 (**7**), 47 (**8**) and 67 μM (**9**). A more significant potency reduction was noted for the dichloro analogue (**10**), with this analogue being essentially inactive with an average growth inhibition of 45% at 100 μM drug concentration. It is also of note, that none of these *Library B* analogues exhibit any degree of cell line specificity, again differing from their conjugated counterparts, which display breast carcinoma cell line specificity (MCF-7). This strongly suggests a role for conjugation from the phenyl ring through to the pyrrole moiety, *i.e.* conjugation that spans the entire analogue. To further evaluate the conjugation requirement we evaluated two additional focused libraries.

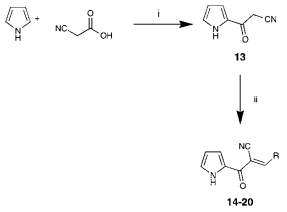
Within *Library C* we reintroduced the acrylonitrile moiety, but removed the **Arom-1** pyrrole ring, removing the potential for full extension of the conjugated pharmacophore from **Arom-1** to **Arom-2** (Fig. 1). *Library C* comprised two analogues retaining the active 3,4-dichlorophenyl pharmacophore from *Library A*, replacing **Arom-1** with a simple alkyl chain (C_4 and C_9). Synthesis was effected as shown in Scheme 1, replacing pyrolle-2-carboxyaldehyde with pentanal and decanal. With both analogues a significant reduction in growth inhibition relative to **5** was observed (Table 3). The C_9-**12** was essentially inactive with minimal growth inhibition noted even at 100 μM drug concentration, whilst the C_4-**11** was two fold less potent than the lead, **5**.

To confirm the pivotal nature of both **Arom-1** and **Arom-2** along with the nature of the conjugated system, we developed *Library D* in which the aromatic moieties were further separated by the introduction of a carbonyl spacer moiety (Scheme 2). Access to *Library D* was effected by treatment of pyrrole with cyanoacetic acid affording 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile **13** in good

Table 3 Evaluation of the cytotoxicity, GI_{50} (μM) values, of *Library C* (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile derivatives **11** and **12**, against a panel of ten human cancer cell lines. GI_{50} is the concentration of drug that reduces cell growth by 50%. Values in parentheses are percent growth inhibition at 100 μM drug concentration.[6,7]

Ar (compound)	HT29[a]	SW480[a]	MCF-7[b]	A2780c	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]
 **11**	53 ± 0	68 ± 2	49 ± 2	24 ± 1	100	88 ± 1	87 ± 3	73 ± 14	70 ± 6	95 ± 4
 **12**	(40 ± 3)	(53 ± 6)	(79 ± 4)	(72 ± 14)	(26 ± 5)	(29 ± 4)	(28 ± 12)	(48 ± 3)	(21 ± 1)	(22 ± 5)

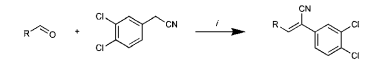
[a] HT29 and SW480 (colon carcinoma). [b] MCF-7 (breast carcinoma). [c] A2780 (ovarian carcinoma). [d] H460 (lung carcinoma). [e] A431 (skin carcinoma). [f] DU145 (prostate carcinoma). [g] BEC-2 (neuroblastoma). [h] SJ-G2 (glioblastoma). [i] MIA (pancreatic carcinoma).



Scheme 2 Reagents and conditions. (i) Ac_2O, 35 min; (ii) RCHO (see table for details), piperidine (cat), EtOH reflux 2 h.

yield. The required carbonyl spacer was introduced *via* a Knoevenagel condensation,[8–13] with selected aldehydes (see Table 4 for details). These analogues, **14–20** were specifically designed such that the final products largely mirrored the analogues found in *Libraries A–C*.

As can be seen from the data presented in Table 4 relating to *Library D*, all analogues are essentially inactive, strongly suggesting the pivotal nature of the conjugated pharmacophore. Whilst the carbonyl spacer moiety does not directly block the conjugated system, it does allow for system elongation and the electron deficient nature of the C=O also perturbs the electron



Scheme 3 RCHO (see table for details), piperidine (cat), EtOH reflux 2 h.

flow, disrupting the flow of electron density from **Arom-1** to **Arom-2**. Regardless, it is clear that for activity no disruption of the conjugated system is permitted.

Having established the pivotal nature of the **Arom-1**, **Arom-2** and conjugated moieties we next turned our attention to modifications of **Arom-1** with the synthesis of *Library E*. *Library E* retained the 3,4-dichlorophenyl moiety identified in *Library A* as the most active pharmacophore. Synthesis of *Library E* was as per Scheme 1, replacing pyrrole-2-carboxaldehyde with a family of aromatic aldehydes (see Scheme 3 and Table 5 for details).

Library E analogues were evaluated for their growth inhibition capabilities against ten human cancer cell lines, these data are shown in Table 5.

Replacement of the pyrrole **Arom-1** moiety with a simple phenyl ring results in **21**, an analogue which displayed only modest growth inhibition at 100 μM drug concentrations. The equivalent 4-methylphenyl, **22**, and the 2-naphthyl, **23**, analogue are both a highly potent broad spectrum agents returning GI_{50} values in the 1–2.3 μM range against all the cancer cell lines

Table 4 Evaluation of the cytotoxicity of *Library D* (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-carbonyl)acrylonitrile derivatives **14–20**. All values are percentage growth inhibition at 100 μM drug concentration, against a panel of ten human cancer cell lines.[6,7]

R_1	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]
 **14**	< 10	< 10	12 ± 5	< 10	< 10	< 10	< 10	< 10	< 10	< 10
 **15**	14 ± 3	14 ± 3	39 ± 6	19 ± 11	11 ± 4	< 10	< 10	11 ± 3	20 ± 4	27 ± 2
 **16**	54 ± 9	68 ± 15	84 ± 17	75 ± 27	38 ± 5	31 ± 8	32 ± 10	63 ± 15	53 ± 9	59 ± 13
 **17**	< 10	< 10	19 ± 16	19 ± 16	< 10	< 10	< 10	19 ± 5	< 10	< 10
 **18**	< 10	< 10	14 ± 2	< 10	< 10	< 10	< 10	14 ± 4	10 ± 4	< 10
 **19**	< 10	< 10	15 ± 5	< 10	< 10	< 10	< 10	< 10	< 10	11 ± 2
 **20**	< 10	< 10	22 ± 8	< 10	24 ± 20	13 ± 8	< 10	< 10	< 10	< 10

[a] HT29 and SW480 (colon carcinoma). [b] MCF-7 (breast carcinoma). [c] A2780 (ovarian carcinoma). [d] H460 (lung carcinoma). [e] A431 (skin carcinoma). [f] DU145 (prostate carcinoma). [g] BEC-2 (neuroblastoma). [h] SJ-G2 (glioblastoma). [i] MIA (pancreatic carcinoma).

Table 5 Evaluation of the cytotoxicity, GI_{50} (μM) values, of *Library E* (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile derivatives **21–35**, against a panel of ten human cancer cell lines. GI_{50} is the concentration of drug that reduces cell growth by 50%. Values in parentheses are percent growth inhibition at 100 μM drug concentration.[6,7]

Ar (compound)	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]
 **21**	(56 ± 1)	(67 ± 1)	(79 ± 1)	(62 ± 2)	(36 ± 4)	(71 ± 1)	(36 ± 3)	(47 ± 12)	(72 ± 9)	(61 ± 3)
 **22**	1.6 ± 0.09	2.3 ± 0.03	2.2 ± 0.1	2.0 ± 0.0	2.1 ± 0.1	1.8 ± 0.1	2.2 ± 0.2	2.2 ± 0.1	2.1 ± 0.1	2.0 ± 0.1
 **23**	1.6 ± 0.2	2.3 ± 0.1	1.5 ± 0.2	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.2	2.7 ± 0.4	1.9 ± 0.0	2.3 ± 0.0	2.1 ± 0.1
 **24**	–[j]	–[j]	–[j]	–[j]	–[j]	–[j]	–[j]	–[j]	–[j]	–[j]
 **25**	9.3 ± 2.4	5.6 ± 0.6	6.5 ± 1.0	9.5 ± 1	10 ± 0	10 ± 0	18 ± 2	13 ± 2	16 ± 1	11 ± 1
 **26**	2.5 ± 0.3	5.0 ± 1.4	4.3 ± 0.9	4.0 ± 0.6	4.8 ± 1.0	4.3 ± 0.6	12 ± 4	4.1 ± 0.8	6.1 ± 0.6	4.0 ± 0.6
 **27**	20 ± 1	22 ± 1	16 ± 0	20 ± 1	21 ± 2.0	20 ± 0	26 ± 2	21 ± 1	22 ± 1	21 ± 1
 **28**	3.1 ± 1.8	8.4 ± 3.3	0.127 ± 0.043	12 ± 1	69 ± 2	7.1 ± 0.1	18 ± 3	8.9 ± 0.7	14 ± 1	27 ± 4
 **29**	8.4 ± 0.2	8.9 ± 0.5	7.2 ± 0.2	11 ± 1	40 ± 6	16 ± 3	55 ± 7	12 ± 2	12 ± 1	15 ± 1
 **30**	30 ± 3	36 ± 5	23 ± 3	20 ± 1	26 ± 2	21 ± 1	45 ± 11	22 ± 1	21 ± 2	28 ± 2
 **31**	0.52 ± 0.05	1.4 ± 0.1	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0	0.6 ± 0.1	1.4 ± 0.1	2.7 ± 2.0	1.5 ± 0.2	0.7 ± 0.0
 **32**	18 ± 1	27 ± 1	25 ± 1	18 ± 1	33 ± 3	25 ± 1	47 ± 11	22 ± 1	21 ± 2	38 ± 7
 **33**	18 ± 1	18 ± 2	15 ± 1	18 ± 1	9.4 ± 1.6	26 ± 1	24 ± 1	16 ± 3	22 ± 1	22 ± 2
 **34**	–[j]	–[j]	–[j]	–[j]	–[j]	–[j]	–[j]	–[j]	–[j]	–[j]
 **35**	>100	90 ± 6	28 ± 14	93 ± 3	>100	88 ± 6	>100	>100	95.0	>100

[a] HT29 and SW480 (colon carcinoma). [b] MCF-7 (breast carcinoma). [c] A2780 (ovarian carcinoma). [d] H460 (lung carcinoma). [e] A431 (skin carcinoma). [f] DU145 (prostate carcinoma). [g] BEC-2 (neuroblastoma). [h] SJ-G2 (glioblastoma). [i] MIA (pancreatic carcinoma). [j] Not soluble in testing medium.

examined. This suggests the presence of a hydrophobic binding pocket. Introduction of a halogen to the phenyl ring of **21** afforded 4-F (**25**), 4-Cl (**26**) and 4-Br (**27**) (GI_{50}s 3–26 μM) with increased growth inhibition relative to **21** but a decrease relative to **22** and **23**. In this series the 4-Br **27** was least potent (GI_{50}s 16–26 μM) with the 4-Cl **26** the most potent (GI_{50}s 3–12 μM). This was clearly an effect of increasing electronegativity at this point of the molecule, which suggested that the introduction of a 4-F substituent would show an increase in cytotoxicity. However, the 4-F **25** displayed a slight reduction in potency. The potency reduction with **25** (GI_{50}s 5.6–18 μM) was most probably due to the dual electron withdrawing and inductive effects known to be associated with F substituents. The 3-Cl analogue **29** was significantly less potent than the corresponding 4-Cl **26** with GI_{50}s 7–55 μM, showing a preference for these moieties to be in the 4-position. Collectively, both the nature and positioning of the electron-withdrawing moiety is crucial for good levels of growth inhibition. While *Library E* showed an overall increase in growth inhibition across all cell types, selectivity towards and against specific tumour types was also apparent. In this regard, analogue **29** displayed good activity against all cell lines except H460 (lung carcinoma) and DU145 (prostate carcinoma) cell lines with GI_{50} values of 40 ± 6 and 55 ± 7 μM respectively. This level of activity, against H460 and DU145, is five times lower than against the other cell lines examined.

Furthermore, the introduction of the 4-NO_2 with **28** had the simple effect of increasing potency against MCF-7 carcinoma cells whilst effecting a reduction in potency against the other cancer cell lines. Analogue **28** displayed an impressive level of potency and selectivity for the breast carcinoma cell line, MCF-7 with a $GI_{50} = 0.127 \pm 0.043$ μM, which is 543 times more potent against the H460 ($GI_{50} = 69 \pm 2$ μM) lung carcinoma cell line and 25 times more potent against the next best examined (HT29; $GI_{50} = 3 \pm 1.8$ μM).

Of the other variations in the **Arom-1** moiety examined, only the introduction of a 4-OCH_3 moiety, **31**, had any noteworthy impact on growth inhibition. The 4-OCH_3 analogue (**31**) is the most potent broad spectrum analogue generated in this study with the majority of the GI_{50} values being at or below 1 μM. Interestingly neither the free 4-OH (**30**) nor the ethyl ester (**32**) showed any noteworthy inhibition or selectivity suggesting that the potency enhancements were a combination of the presence of the oxygen lone pairs of electrons and the additional steric component afforded by the introduction of a methyl group.

Returning to analogues **5** and **28** we were intrigued with the level of specificity of these analogues with the inhibition of the MCF-7 breast carcinoma cell line. As this cell line is known to be estrogen dependent and over expresses the estrogen receptor (ER+ve) we chose to examine the effect of **5** and **28** on one ER negative (ER−ve) breast carcinoma cell line (MDA-MB231) together with one normal breast cell line (MCF10A) that was derived from non-malignant breast epithelial tissue. The growth inhibition results presented in Table 6 clearly show that **5** and **28** are up to 268 fold more potent in the estrogen dependent MCF-7 cells (ER+ve) compared with the estrogen independent carcinoma cell line MDA-MB-231 (ER−ve) and up to 126 fold more potent in MCF-7 cells than in the non-tumourigenic breast epithelial cell line MCF10A. The ability to specifically target estrogen dependent tumour cells, while having little or no effect on normal breast cells, or on cells derived from other tumour types is a significant and unique finding of this study.

Table 6 Evaluation of the cytotoxicity, GI_{50} (μM) values, of **5** and **28** against a panel of breast cell lines. GI_{50} is the concentration of drug that reduces cell growth by 50%.[6,7]

Compound	MCF-7[a]	MDA-MB-231[b]	MCF10A[c]
5	0.56 ± 0.03	46 ± 4	28 ± 5
28	0.127 ± 0.043	34 ± 2	16 ± 4

[a] MCF-7 (ER+ve breast carcinoma). [b] MDA-MB-231 (ER−ve breast carcinoma). [c] MCF10A (non-tumourigenic breast epithelial cell line).

Conclusions

Our efforts to minimize progression of highly toxic compounds into our anti-parasitic drug development program led to the synthesis of a series of known (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile derivatives (Fig. 1, **1–5**). To our surprise these analogues displayed high levels of growth inhibition against our panel of ten human cancer cell lines. Subsequent focused library development identified **Arom-1** to **Arom-2** conjugation as important for activity, with efforts to modify this feature having a significant effect on the levels of growth inhibition observed. The requirement for both aromatic rings was confirmed on examination of the aliphatic analogues **11** (GI_{50} = 24–100 μM) and **12** (inactive). The parent pyrrole moiety as **Arom-1** was not required to maintain good levels of growth inhibition with activity increased by the introduction of simple substituted benzene analogues. Of particular note was the introduction of a 4-OCH_3 substituent in analogue **31** which afforded the most active broad spectrum analogue with an average $GI_{50} = 1.6$ μM, and modest selectivity for the HT29 human colon carcinoma cell line ($GI_{50} = 0.52 \pm 0.05$ μM). More interestingly, the introduction of a 4-NO_2 substituent with analogue **28** afforded up to 543-fold MCF-7 (human breast carcinoma) cell line specificity relative to cell lines derived from other tumour types. Examination of two additional human breast cell lines: MDA-MB-231 (ER−ve carcinoma) and MCF-10A (normal epithelial), demonstrated that **28** preferentially inhibited the growth of MCF-7 cells (268 and 126 fold respectively Although somewhat less potent, a similar preference was also noted for analogue **5**. The retention of the 3,4-dichlorophenyl (**Arom-2**) moiety appears to be important for imparting the observed selectivity for breast cancer cell lines compared to the other nine cancer cell lines examined. The MCF-7 human breast carcinoma cell line is estrogen dependent while the MDA-MB-231 line is not suggesting that hormonal status may play a role in the improved efficacy of **28** and **5**. The exact nature of this phenomenon is currently under investigation and will be reported in due course.

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3.5 Paper V

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A flow chemistry route to 2-phenyl-3-(1*H*-pyrrol-2-yl)propan-1-amines

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ABSTRACT

The Knoevenagel condensation of pyrrole-2-carboxaldehyde (**1**) with a range of substituted benzyl nitriles (**2a**–**e**) afforded rapid access to a family of α,β-unsaturated nitriles (**3a**–**e**) in good yields (67–78%). Flow hydrogenation (ThalesNano H-cube™) at 60 °C, 50 bar H_2 pressure, 1.0 mL/min through a 10% Pd-C catalyst selectively, and quantitatively, hydrogenated the olefin double bond (**4a**–**e**). Use of a Raney Nickel catalyst at 70 °C, 70 bar H_2 pressure and flow rates of 0.5–1.0 mL/min afforded quantitative conversion into the corresponding saturated amines with the reduction of both the olefin and nitrile bonds (**5a**–**e**). The versatility of this approach was further exemplified by reaction of **5a** and **5c** with norcantharidin to afford acid amide norcantharidin analogues **7** and **8** as novel protein phosphatase 1 and 2A inhibitors.

The current focus of our medicinal chemistry team is in the development of protein phosphatase 1 and 2A and dynamin GTPase inhibitors.[1–3] While targets change, all medicinal chemistry programmes require access to novel building blocks to explore new chemical space.[4] These new building blocks are initially only required in milligram quantities. As the drug development programme progresses, increased quantities of the drug are required. Thus there is a pressing need to develop elegant approaches amenable to the rapid production of novel building blocks, but also production scale-up to meet the demands of whole cell, and ultimately whole animal studies. In our case, this has demanded the development of approaches capable of delivering building blocks for libraries of **12**–**24** compounds and subsequent scale-up from 10 mg to 10 g.

Until recently, synthetic re-scaling required laborious re-optimisation of methods developed to access the small quantities of reagents required at the initial library synthesis stage. However, the introduction of flow chemistry has had a significant impact on our ability to deliver the required compound quantities both at the initial building block stage and the animal testing stages of our programmes.[5–9] Recently, we had cause to require rapid access to a range of novel amines of the type shown in Figure 1.[10,11]

We rationalised that access to the required amines should be attainable by a two-step process commencing from pyrrole-2-carboxaldehyde (**1**) and a range of substituted benzyl nitriles **2a**–**e** via a Knoevenagel condensation.[10–19] Reduction would then afford the desired analogues. In our subsequent synthetic efforts we also imposed additional constraints requiring flexible approaches that allow generation of mg to gram quantities of each of the library components. In the initial Knoevenagel approaches this was a relatively trivial proposition, and one that has been addressed by us, and others elsewhere.[10–19] However, the hydrogenation requirement stalls rapid library development in a standard laboratory environment, typically limiting this step to a batch-wise approach with the quantity of each batch limited by the available volume of the hydrogenating equipment. This is clearly a disadvantage not only in regards to high throughput, but with reaction scale-up. In efforts to accelerate both analogue development and reaction scale-up, our team has invested heavily in flow chemistry technology. Having access to the ThalesNano H-cube™ (H-cube) flow hydrogenator we turned our attention to the synthesis of Type A (**4a**–**e**) and Type B (**5a**–**e**) analogues as shown in Scheme 1.

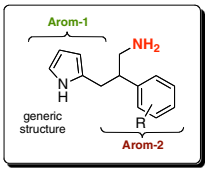

Figure 1. Generic structure of the target bis-aromatic amines.

In a typical experiment, the benzyltrimethylammonium hydroxide [$PhCH_2NMe_3(OH)$] mediated Knoevenagel condensation allowed direct access to the desired α,β-unsaturated nitriles (Scheme 1) in good yields (67–78%).[10,11,20]

With these nitriles in hand we next turned our attention to the flow hydrogenation using the H-cube. Flow chemistry approaches

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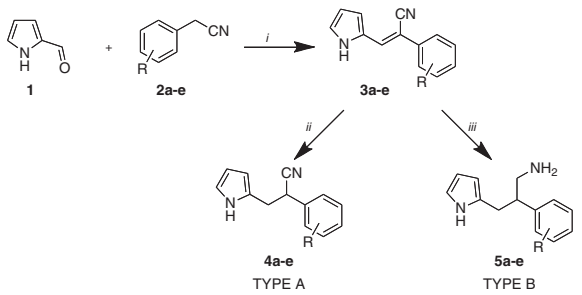


Scheme 1. Reagents and conditions: (i) H_2O, $PhCH_2NMe_3(OH)$, 50 °C, 5 h; (ii) 0.05 M **3a–e** (acetone), 10% Pd-C, 50 °C, 50 bar H_2, 1.0 mL/min, H-cube; (iii) 0.05 M **3a–e** (1 M NH_3 in MeOH), Raney Ni, 70 °C, 70 bar H_2, 0.5 mL/min, H-cube.

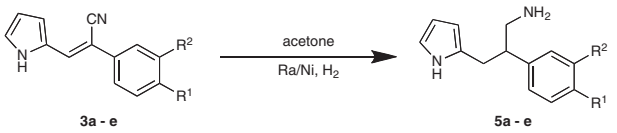
Table 1
Optimisation of temperature and H_2 pressure, for the reduction of **3a** to **5a** using a Raney Ni hydrogenation catalyst at a 1.0 mL/min flow rate. Reactions were conducted for 10 min

Entry	*P* (bar)	*T* (°C)	Conversion (%)
1	90	100	30
2	80	100	30
3	70	100	30
4	60	100	30
5	50	100	25
6	40	100	25
7	100	90	25
8	100	80	15
9	100	70	12
10	100	60	5
11	100	50	0
12	100	40	0

have facilitated rapid modification, and reaction optimisation. As only sufficient sample was required for analysis, for example, by HPLC or MS, incredibly short reaction times were feasible allowing multiple runs in a single day.[21] This greatly enhanced our ability to survey a range of reaction conditions from temperature, catalyst residence time (flow rate), stoichiometry and in this instance, hydrogen pressure. The H-cube uses pre-packed catalyst cartridges and in situ hydrogen generation from deionised water alleviating safety concerns normally associated with hydrogen gas and handling of catalyst materials.[22]

Our first series of optimisation reactions involved passing 0.05 M solutions of (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile (**3a**) (in acetone) through each of three hydrogenation catalyst cartridges: 5% Pd-C; 10% Pd-C and Raney Nickel (Ra/Ni).[22] To best maximise the potential reduction in each instance our initial examinations were conducted at 100 °C and 100 bar H_2 pressure (H-cube 'controlled mode'), flow rate was 1.0 mL/min. No reaction was observed with the 5% Pd-C catalyst under these conditions, but complete reduction of the olefinic double bond was accomplished with the 10% Pd-C catalyst (100%),[23] and reduction of both the olefin and the nitrile moieties was accomplished with the Raney Nickel (Ra/Ni) catalyst (95%). The reduced isolated yield (95% vs 100%) with the Ra/Ni catalyst was attributed to issues with product stability and work-up. With both the catalysts we noted 100% consumption of the starting material, but with the Ra/Ni catalyst, the product was contaminated with ca. 5% of an unidentified highly polar by-product. Filtration through a silica gel plug removed this material, but afforded a slightly reduced isolated yield. The amine produced via the Ra/Ni-catalysed reduction also rapidly discoloured on exposure to air. In this latter case the amino analogue was only obtained after the feeder solution was allowed to re-circulate until no starting material was detected by mass spectrometry. While we did not explore the flow hydrogenation of the olefinic double bond in great detail, all the evaluated analogues

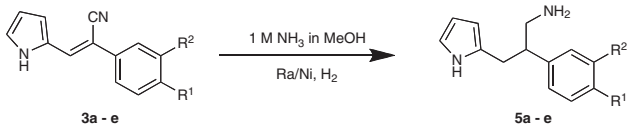
Table 2
Flow hydrogenation of acrylonitrile analogues **3a–e** at 60 °C and 50 bar H_2 pressure at 1.0 mL/min



Entry	R^1	R^2	Pressure (bar)	Product	Isolated yield (%)
1	H	H	50	**5a**	100
2	H	H	100	**5a**	100
3	$NO_2(NH_2)$[a]	H	50	**5b**	23
4	F	H	50	**5c**	66
5	Cl	H	50	**5d**	32
6	Cl	Cl	50	**5e**	30

[a] The NO_2 was reduced to NH_2.

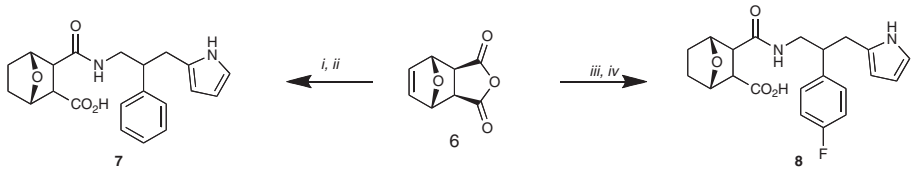
Table 3
Isolated yield of amines **5a**–**e** obtained by the flow reduction of **3a**–**e** using a Raney Ni hydrogenation catalyst at 70 °C and 70 bar H_2 pressure



3a - e **5a - e**

Entry	R^1	R^2	Flow rate (mL/min)	Product	Yield (%)
1	H	H	0.5	**5a**	100
2	$NO_2(NH_2)$[a]	H	1.0	**5b**	100
3	F	H	0.5	**5c**	100
4	Cl	H	0.5	**5d**	100
5	Cl	Cl	0.5	**5e**	100

[a] The NO_2 was reduced to NH_2.



7 6 **8**

PP2A inhibition = 18.5 ± 3.50 μM
PP1 inhibition = 14.1 ± 7.90 μM

PP2A inhibition = 7.25 ± 1.25 μM
PP1 inhibition = 4.5 ± 1.55 μM

Scheme 2. Reagents and conditions: (i) 0.05 M solution of **6** (acetone), 50 °C, 50 bar H_2, 1.0 mL/min (H-cube); (ii) 0.05 M solution of **5a** (1 M NH_3 in MeOH); (iii) 0.05 M solution of **6** (acetone), 50 °C, 50 bar H_2, 1.0 mL/min (H-cube); (iv) 0.055 M solution of **5c** (1 M NH_3 in MeOH), 50 °C, 50 bar H_2, 1.0 mL/min (H-cube). Protein phosphatase activity (IC_{50} values) of **7** and **8**.

were smoothly and cleanly converted into the saturated nitrile analogues, **4a**–**e**.

Having established that the 10% Pd-C and Ra/Ni catalysts afforded two different products, and that we were unable to determine reaction conditions under which only the nitrile group was reduced (data not shown), we sought to optimise the flow hydrogenation conditions that would most expediently allow the synthesis of the desired bis-aromatic amines (see Fig. 1). In this evaluation process we did not isolate the reduced product, but conducted a rapid scan of multiple reaction conditions. With our initial efforts conducted at 100 °C, we commenced our evaluation at this temperature and reduced the H_2 pressure. The reaction flow rate was fixed at 1.0 mL/min. Table 1, entries 1–6; stepped the hydrogen pressure downwards in 10 bar increments at a constant 100 °C, and it is clear from the data presented that all H_2 pressures evaluated gave the desired product. Next we fixed the H_2 pressure at 100 bar and stepped the reaction temperature down in 10 °C increments from 90 to 40 °C. Here no product was observed at 40 or 50 °C, but a low conversion of 5% was noted at 60 °C (Table 1, entries 7–12).

Having rapidly surveyed the hydrogenation condition requirements for the phenyl analogue (**5a**), we applied the mildest full conversion conditions of 60 °C and 50 bar H_2 pressure to the series of acrylonitrile analogues of interest (**3a**–**e**; see Scheme 1 and Table 2 for details). In this series of experiments the reaction was allowed to continue uninterrupted for ca. 20 min, which ensured the collection of sufficient sample for purification, identification and yield calculations. As can be seen from Table 2, only the parent (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile (**3a**) was converted quantitatively into the desired 2-phenyl-3-(1*H*-pyrrol-2-yl)propan-1-amine (**5a**) under these conditions. Poor conversion of the other analogues was noted, ranging from 23% (Table 2, entry 3) to 66% (Table 2, entry 4). We also noted simultaneous reduction of the aromatic NO_2 moiety to NH_2 under these conditions (Table 2, entry 3). The failure to achieve complete conversion into the bis-aromatic amines significantly complicated product isolation and purification.

Given the poor yields observed and the low conversion rates we initiated an additional evaluation and optimisation cycle. Repeating the flow reduction of **3a**–**e** (as 0.05 M solutions) in 1 M NH_3/MeOH at 70 bar H_2 pressure, 70 °C and 1.0 (or 0.5) mL/min allowed complete conversion of the α,β-unsaturated nitriles into the desired bis-aromatic amines (**5a**–**e**, Table 3).[24]

To demonstrate further the versatility of our approach, bis-aromatic amines **5a** and **5c** were dissolved in acetone (as 0.05 M solutions) and subjected to flow hydrogenation conditions in the presence of 5,6-dehydronorcantharidin (**6**) which gave the corresponding ring-opened acid amides **7** and **8** (Scheme 2). These compounds proved to be effective protein phosphatase 1 and 2A inhibitors (see Scheme 2 for data). This demonstrated the versatility of flow chemistry approaches to the rapid development of biologically active molecules.

In conclusion we have reported an elegantly simple flow chemistry approach to a series of bis-aromatic amines **5a**–**e** that have been further utilised in the development of novel norcantharidin derivatives **7** and **8**. Judicious choice of hydrogenation catalyst allows the reduction of either the olefin double bond (10% Pd-C) (**4a**–**e**) or reduction of the α,β-unsaturated nitrile (Raney Nickel) (**5a**–**e**). The

use of flow chemistry approaches allowed rapid surveying of the reaction conditions and the ability to up-scale the quantity of the product produced by simply increasing the reaction time.

Acknowledgements

The authors acknowledge the financial support of the Australian Research Council, the Australian Cancer Research and Ramaciotti Foundations, and John Morris Scientific, Australia. M.T. acknowledges the UNI-PRS postgraduate funding from the University of Newcastle. Protein phosphatase inhibition data for 7 and 8 were determined by Drs Jennette Sakoff and Jayne Gilbert, Calvary Mater Hospital, Newcastle Australia.

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20. **Example synthesis of pyrrole-2-ylacrylonitriles**: *(Z)-2-Phenyl-3-(1H-pyrrol-2-yl)acrylonitrile* (**3a**):[10] 1*H*-Pyrrole-2-carbaldehyde (**1**) (165 mg, 1.74 mmol) was added to vigorously stirred H_2O (10 mL) and heated to 50 °C upon which it dissolved. Phenylacetonitrile (**2a**) (193 mg, 1.65 mmol) was then slowly added forming a suspension. Heating was continued at 50 °C and once a clear solution was evident, typically 5–10 min, 40% $PhCH_2NMe_3(OH)$ (7 mL) was added dropwise. The reaction vessel was sealed and the mixture stirred at 50 °C for 5 h, the solution filtered hot, washed with warm H_2O and dried under suction and recrystallised from EtOH to afford **3a** as a brown solid; 73%; mp 94–96 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.61–7.57 (m, 2H), 7.45–7.40 (m, 2H), 7.42 (s, 1H), 7.35–7.30 (m, 1H), 7.08–7.06 (m, 1H), 6.73 (dd, J = 1.4, 3.7 Hz, 1H), 6.37 (dd, J = 1.4, 3.7, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 133.4, 130.7, 128.5, 127.6, 127.2, 124.4, 123.5, 120.1, 118.5, 110.3, 100.8.
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23. **Example reduction of the olefin moiety**: *2-Phenyl-3-(1H-pyrrol-2-yl)propanenitrile* (**4a**):[11] (*Z*)-2-Phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile (**3a**) (990 mg, 5.1 mmol) was dissolved in sufficient freshly distilled dry acetone (100 mL) to form a 0.05 M solution. This solution was hydrogenated using the ThalesNano H-cube™ using a 10% Pd/C catalyst at 1 mL/min flow rate, 50 °C and 50 bar H_2 pressure. The acetone was removed in vacuo and the crude oil was subjected to flash silica chromatography (1:1 $CHCl_3$/hexanes) to afford **4a** as a brown oil; 98%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.03 (br s, 1H), 7.42–7.35 (m, 3H), 7.29–7.26 (m, 2H), 6.69–6.67 (m, 1H), 6.15–6.13 (m, 1H), 6.03–6.02 (m, 1H), 4.01 (t, J = 7.4 Hz, 1H), 3.28–3.14 (m, 2H). ^{13}C NMR ($CDCl_3$,75 MHz): δ 134.5, 128.6, 127.8, 126.8, 125.7, 120.4, 117.3, 108.1, 107.4, 38.4, 34.0.
24. **Example reduction of the olefin and nitrile moieties**: *2-(4-Fluorophenyl)-3-(1H-pyrrol-2-yl)propan-1-amine* (**5c**): a solution of (*Z*)-2-(4-fluorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile **(3c)** (0.05 M, 990 mg, 4.6 mmol) in 1 M NH_3 in MeOH (100 mL) was hydrogenated using the ThalesNano H-cube™ using a Ra/Ni catalyst at 0.5 mL/min flow rate, 70 °C and 70 bar H_2 pressure. The solvent was removed in vacuo and the crude oil subjected to flash silica chromatography (0.05:0.95 MeOH/CH_2Cl_2) to afford **5c** as a clear oil; 100%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.47 (br s, 1H), 7.15–7.10 (m, 2H), 7.04–6.98 (m, 2H), 6.59–6.58 (m, 1H), 6.08–6.06 (m, 1H), 5.84–5.83 (m, 1H), 2.97–2.85 (m, 5H), 2.41 (br s, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 162.8, 159.5, 138.0, 129.2, 128.7, 116.0, 115.1, 114.9, 107.5, 105.9, 47.9, 46.2, 32.0; HRMS calculated for ($M+H^+$): $C_{13}H_{16}FN_2$, 219.1298; found 219.1307.

3.6 Paper VI

Letters in Drug Design & Discovery, 2011, *8*, ???-??? 1

A Flow Chemistry Approach to Norcantharidin Analogues

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Abstract: Acid-ester and acid-amide norcantharidin derivatives are prepared using a 'one-pot' synthetic procedure utilizing the ThalesNano H-cube™ flow hydrogenator. Traditionally, rapid library generation and reaction scale up of these analogues was limited by the batch wise hydrogenation of 5,6-dehydronorcantharidin. This was resolved with the use of flow chemistry. With no associated scale up issues, a method was devised to produce norcantharidin, along with acid-ester and acid-amide analogues on any scale necessary for biological screening.

Keywords: Cantharidin, Norcantharidin, Flow hydrogenation, Flow chemistry, Protein phosphatase inhibition.

INTRODUCTION

The process of drug development is a long and arduous one. From lead discovery and compound library development the synthetic chemistry requirements change from the production of milligram to grams and at time kilograms quantities of active compound(s) [1]. This places considerable pressure on the robustness of the chemistry utilised [2]. One of our teams primary areas of research has been in the development of small molecule inhibitors, based on cantharidin (**1**) and norcantharidin (**2**) (Fig. **1**), of the serine / threonine protein phosphatases 1 and 2A (PP1 and PP2A) [3-5].

1 **2**

Fig. (1). Chemical structures of cantharidin (**1**) and norcantharidin (**2**).

Our initial interest was sparked by the structural simplicity of **1** and **2** relative to the archetypal protein phosphatase inhibitors such as okadaic acid (**3**) and microcystin-LR (**4**) (Fig. **2**) [6,7]. We, and others, have developed a number of highly focused norcantharidin derived compound libraries [4,8-11]. Our efforts in this area have been rewarded by the development of new classes of inhibitor with a broad range of protein phosphatase inhibition levels and PP1/PP2A selectivity [4]. We have also explored the effect of these inhibitors potential as anti-malarial, anti-parasitic and anticancer drugs [4,12].

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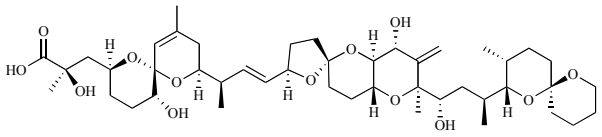
While simple approaches to multiple analogues of **2** are available commencing with the Diels–Alder addition of furan and maleic anhydride followed by hydrogenation to **2** (Scheme **1**). The direct synthesis of **1** is more complex due to the presence of two methyl groups preventing simple Diels-Alder approaches [13], but its total synthesis has been greatly accelerated by the use of high pressure chemistry approaches [14]. Additionally the presence of these methyl groups has been deemed responsible for the known nephrotoxicity of **1**. Hence **2** is the more promising lead compound in a drug development program as it retains most of the PP1 / PP2A inhibition and anti-cancer properties of **1** including the lack of myelosuppresion, with greatly reduced nephrotoxicity [3-5].

Notwithstanding the ease of synthesis of **2**, the need for hydrogenation in the second synthetic step stalls rapid library development in a standard laboratory environment. Typically this step is limited to a batch wise approach, with the quantity of each batch limited by the volume of the hydrogenating system. This also introduces batch-to-batch variations in the amount and quality of **2** produced. This is a disadvantage not only in regards to library development but to reaction scale up.

In efforts to accelerate both analogue development and reaction scale up, our team has invested heavily in flow chemistry technology *via* the Australian Cancer Research Foundation Centre for Kinomics. Having access to the ThalesNano H-cube™ (H-cube) flow hydrogenator we turned our attention to the synthesis of, initially, norcantharidin (**2**) and later the 'one-pot' synthesis of norcantharidin analogue libraries.

Typically our batch hydrogenation of **5** to **2** is conducted using a Parr hydrogenator: 10% Pd-C, 4 bar H_2, 24 hours on a 5 g scale. Our initial evaluation of this reaction under flow hydrogenation conditions was conducted with a 0.05M solution of **5** in acetone at 1 mL/min at 50 ºC and 50 bar H_2 pressure [15]. These conditions were chosen as being at the mid-range of the capability of the H-cube (upper limit is 100 ºC, 100 bar H_2 and a flow rate of 3 mL/min) [16]. In this in-



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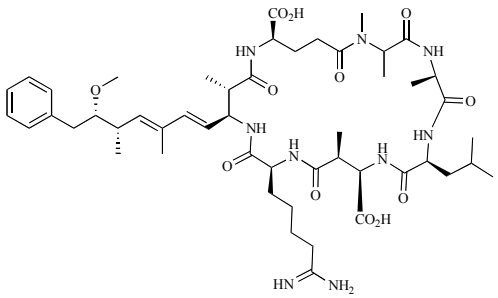

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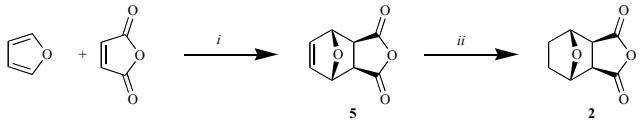
Fig. (2). Okadaic acid (**3**) and microcystin LR (**4**), two archetypal members of the okadaic acid class of compounds.



5 **2**

Scheme 1. Reagents and Conditions: *i*) Et_2O, rt, 24-48 hrs; (ii) Dry Acetone, 10% Pd-C; H_2, 4 Bar, 24 hrs, rt.

stance we observed 100 % conversion of **5** to **2** (Table **1**, entry 1). Subsequent optimisation studies commenced with changes in the H_2 pressure, and as can be seen from the data presented in Table **1**, all conditions excepting entry 2, effected complete conversion of **5** to **2**. Controlled H_2 mode at 0 bar (atmospheric pressure), at room temperature, only afforded a 60% conversion at 1 mL/min.

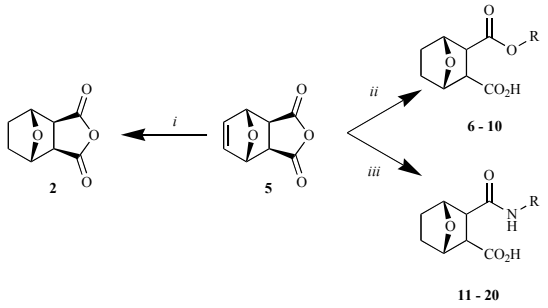
The continuous production of **2** from **5** using the conditions outlined in Table **1**, entry 1, affords the desired compound at a rate of 12g/day which compares very favourably with 5g/day *via* our batch hydrogenation approaches. Additionally, hydrogen is produced "on demand" from deionized water and the catalyst cartridges are supplied pre-packaged, which eliminates the safety concerns associated with flammable gases and active catalyst disposal are negated [16,17].

In previous studies we have shown that simple stirring of **2** in the presence of amines or alcohols afforded excellent yields of ring opened acid-amides or acid-ester analogues [8,18]. Having successfully converted our batch hydrogenation synthesis of **2** to a flow approach, we examined the possibility of coupling this process in a 'one-pot' manner with the aforementioned reactions with simple aliphatic alcohols. We rationalised that such an approach would permit access to the required quantities of analogues for biological screening in a rapid manner, and facilitate compound scale up should the biological data suggest a favourable outcome [19].

Thus we modified the synthetic procedure used for the flow synthesis of **2** from **5** with the simple incorporation of

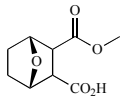
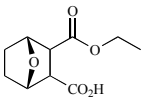
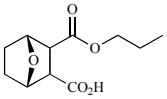
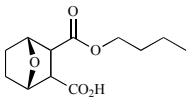
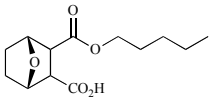
Table 1. Optimisation of the Flow Hydrogenation of 5,6-dehydronorcantharidin (5) to Norcantharidin (2)

Entry	Catalyst	H_2 Pressure (bar)	Temperature (°C)	Yield (%)
1	10% Pd/C	50	50	100
2	10% Pd/C	0 (atmospheric pressure)	rt	60
3	10% Pd/C	30	30	100
4	10% Pd/C	40	40	100



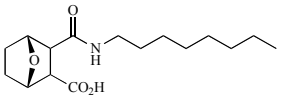
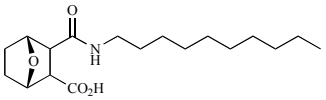
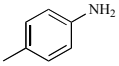
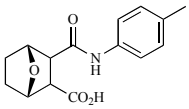
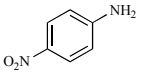
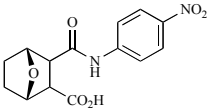
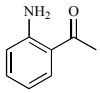
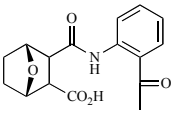
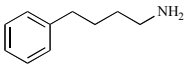
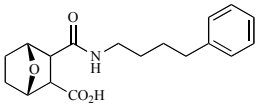
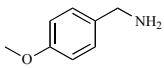
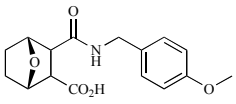
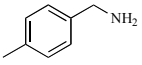
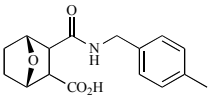
Scheme 2. Reagents and Conditions: i) 0.05M solution of **5** in acetone, 50 bar, 50°C, 1 mL/min, 10% Pd/C; (ii) 0.05M solution of **3** in ROH, 50 bar, 50°C, 1 mL/min, 10% Pd/C; (iii) 0.055M (or 0.03M) solution of RNH_2 in acetone added to 0.05M solution of **3** in acetone, 50 bar, 50°C, 1 mL/min, 10% Pd/C.

Table 2. A 'One-Pot' Coupled Flow Hydrogenation and Ring Opening Synthesis of Norcantharidin Ring Opened Acid-Esters and Acid-Amides (Protein Phosphatase Inhibition Data Taken from Refs. [8, 15, 18])

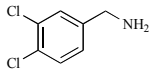
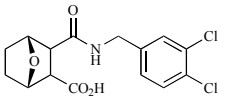
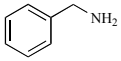
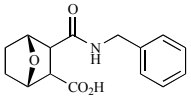
Entry	Alcohol	Product	Yield (%)	PP1 IC_{50} (μM)	PP2A IC_{50} (μM)
1	CH_3OH	 **6**	100	4.71	0.41
2	CH_3CH_2OH	 **7**	100	2.96	0.45
3	$CH_3(CH_2)_2OH$	 **8**	100	4.82	0.47
4	$CH_3(CH_2)_3OH$	 **9**	0%	>100	>100
5	$CH_3(CH_2)_4OH$	 **10**	0%	>100	>100

either an alcohol or an amine into the reagent stream that contained **5**. A series of solutions of **5** (0.05M) in methanol (Table **2**, entry 1), ethanol (Table **2**, entry 2) and propanol (Table **2**, entry 3) were subjected to flow hydrogenation conditions of 1 mL/min reagent flow, 50 bar H_2 at 50ºC. The outcomes of these reactions are tabulated in Table **2**.

Table 3. A 'One-Pot' Coupled Flow Hydrogenation and Ring Opening Synthesis of Norcantharidin Ring Opened Acid-Amides (Protein Phosphatase Inhibition Data Taken from Ref. [8, 15, 18])

Entry	Amine	Product	Yield (%)	PP1 IC_{50} (μM)	PP2A IC_{50} (μM)
1	$CH_3(CH_2)_7\mathbf{NH_2}$	 **11**	63	56 ± 9	25 ± 5
2	$CH_3(CH_2)_9\mathbf{NH_2}$	 **12**	58	25 ± 7	8.6 ± 1.5
3		 **13**	0%	n.d.[a]	n.d.
4		 **14**	0%	74 ± 13	23 ± 1.5
5		 **15**	0%	n.d.	n.d.
6		 **16**	55	(68 ± 3)[b]	(87 ± 2)[b]
7		 **17**	100	35 ± 5	13 ± 1
8		 **18**	100[c]	n.d.	n.d.

(Table 3). Contd…..

Entry	Amine	Product	Yield (%)	PP1 IC_{50} (μM)	PP2A IC_{50} (μM)
9		 **19**	52	>100	>100
10		 **20**	91	35 ± 0	12 ± 0

[a]n.d. = not determined; [b]percentage inhibition at 100 μM drug concentration; [c]reagents were recycled through the catalyst bed until MS showed complete consumption of the starting anhydride **5**.

The data presented in Table **2** shows that for short chain alcohols the ring opening of the anhydride by the alcohol and reduction of the 5,6-double bond of **5** occurs in an excellent yield. The longer chain alcohols failed to give any observable product under these conditions (Table **2**, entries 4 and 5), nor as 0.05M solutions in dry acetone (data not shown). We have previously noted that the reaction of **2** with longer chain alcohols is sluggish and difficult to drive to completion this is reflected when conducting a two-step process *via* flow hydrogenation [18].

In the development of our protein phosphatase inhibitor libraries we have previously reported that the acid amide analogue is more potent than the equivalent acid ester analogue [18,20]. Thus, an acetone solution of **5** (0.05M) and an amine (0.055M, see Table **3** for details) were subjected to flow hydrogenation conditions of 1 mL/min reagent flow, 50 bar H_2 at 50ºC. The outcomes of these reactions are tabulated in Table **3**.

As we had observed no conversion to the acid ester with issues with long chain alcohols above, we first evaluated the addition of octyl and decylamine to **5** (Table **3**, entries 1 and 2). As can be seen the addition proceeded smoothly with yields of 63% and 58% respectively. While quantitative conversion was not obtained, the outcome was more favourable than that noted with alcohols. This is most likely a function of the amine versus alcohol nucleophilicity. In a more thorough investigation of this flow hydrogenation/nucleophilic anhydride ring opening reaction sequence we specifically targeted products that were of interest to our protein phosphatase inhibition program [3-5, 8, 12, 18, 20].

The attempted addition of three anilines (Table **3**, entries 7-9) failed. For example, under the conditions used, addition of the 0.055 M aniline solution to the 0.05 M solution of **5** resulted in the immediate precipitation of the (1*S*,4*R*)-3-(anilino)-7-oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid. The precipitation of products during a flow chemistry transformation is a limitation of this technology, but one that is simply overcome by a 'solvent-switch' approach [21]. Solvent switching was not required in this instance with dilution of the feeder solution to 0.03 M (in acetone) sufficient to prevent the product from precipitating and thus, with 4-methylbenzylamine afforded quantitative conversion to the desired product (Table **3**, entry 8). This process was repeated for the other amines that precipitated and also effected quantitative conversion to the desired products (data not shown). The other amines evaluated (Table **3**, entries 6, 7, 9 and 10) all proceed to afford the desired products in good to excellent yields (52-100%).

An additional advantage flow chemistry here was the ability to loop the reagents stream, i.e. in the event of incomplete conversion the post-catalyst eluant was delivered back to the reagent vessel and the reaction continued until complete conversion observed (by MS). The effect of such reagent looping is shown with **17** (Table **3**, entry 7).

In conclusion herein we have reported the combined reduction and nucleophilic anhydride ring opening of a focused library of norcantharidin analogues. This reaction sequence works best with amine nucleophiles but due care and consideration must be given to the product solubility as flow chemistry has a low tolerance for materials that precipitate in the reaction lines.

This two-step procedure was conducted in excellent yields, typically quantitative, allowing for the rapid development of novel potentially biologically active analogues. The flow chemistry approaches developed herein are independent of scale and facilitate both the initial screening stages of a drug design and discovery program, and the increase in compound quantity required as such a program progresses through to in cell and animal studies.

EXPERIMENTAL

General Experimental

All starting materials were purchased from Aldrich Chemical Co. and Lancaster Synthesis. Solvents were bulk, and distilled from glass prior to use. Reaction progress was monitored by TLC, on aluminium plates coated with silica gel with fluorescent indicator (Merck 60 F_{254}) and flash chromatography was conducted utilising SNAP Biotage KP-SIL columns. ^1H and ^{13}C spectra were recorded on a Bruker

Advance AMX 300 MHz spectrometer at 300.13 and 75.48 MHz, respectively. Chemical shifts are relative to TMS as internal standard. All compounds returned satisfactory Mass spectra were obtained using a micromass liquid chromatography Z-path (LCZ) platform spectrometer. Mass to charge ratios (*m/z*) are stated with their peak intensity as apercentage in parentheses. All mass spectra were obtained *via* the ES method thus fragmentation patterns were not observed. The University of Wollongong, Australia, Biomolecular Mass Spectrometry Laboratory, analysed samples for HRMS. The spectra were run on a micromass QTof2 spectrometer using polyethylene glycol or polypropylene glycol as the internal standard.

In a Typical Synthesis of Acid-Ester Analogues [18]

A mixture of 5,6-dehydronorcantharidin (**5**) (0.05 M in methanol) was stirred until dissolved. The corresponding solution was then passed through the H-cube flow hydrogenation apparatus under the conditions of 50 bar, 50°C, 1 mL/min, controlled H_2, 10% Pd/C. The reaction eluant was collected, and the solvent removed *in vacuo* to leave a white solid which did not require further purification.

(1S,4R)-3-(Methoxycarbonyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (6)

^{1}H NMR (acetone-d_6) (300 MHz): δ 4.78-4.77 (m, 2H), 3.55 (s, 3H), 3.10-3.00 (m, 2H), 1.70-1.55 (m, 4H); ^{13}C NMR (acetone-d_6) (75 MHz): δ 170.9, 170.7, 77.8, 77.4, 51.5, 50.9, 50.1, 28.2, 27.2; MP 140-141°C [18].

(1S,4R)-3-(Ethoxycarbonyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (7)

^{1}H NMR ($CDCl_3$) (300 MHz): δ 8.88 (bs, 1H), 4.93 (d, *J* = 2.9 Hz, 1H), 4.87 (d, *J* = 2.9 Hz, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.03-2.95 (m, 2H), 1.83-1.79 (m, 2H), 1.55-1.48 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 175.7, 170.3, 78.0, 77.8, 60.5, 51.8, 51.7, 28.5, 28.4, 13.4; MP 112-113°C [18].

In a Typical Synthesis of Acid-Amide Analogues [8,20]

A mixture of 5,6-dehydronorcantharidin (**5**) (0.05M in acetone) was stirred until dissolved. To this, a solution of amine (0.055M in acetone) was added and the resulting solution was passed through the H-cube flow hydrogenation apparatus under the conditions of 50 bar, 50°C, 1 mL/min, controlled H_2, 10% Pd/C. The reaction was collected, and the solvent removed *in vacuo* to leave an off white precipitate. This was purified by either adding ether (10 mL) and collecting the observed precipitate under suction or subjected to flash silica chromatography (~5% MeOH/DCM) to afford the desired norcantharidin acid amide.

(1S,4R)-3-(4-Methoxybenzylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (17)

^{1}H NMR (DMSO-d_6) (300 MHz): δ 7.68 (t, *J* = 5.5 Hz, 1H), 7.17 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 4.69 (s, 1H), 4.46-4.44 (m, 1H), 4.19 (dd, *J* = 14.9, 5.5 Hz, 1H), 4.01 (dd, *J* = 14.9, 5.5 Hz, 1H), 3.71 (s, 3H), 2.82 (d, *J* = 9.8 Hz, 1H), 2.72 (d, *J* = 9.8 Hz, 1H), 1.71-1.38 (m, 4H); ^{13}C NMR (DMSO-d_6) (75 MHz): δ 173.1, 171.2, 158.0, 131.3, 128.4, 113.5, 78.6, 77.5, 54.9, 53.9, 44.9, 28.7, 27.9, 21.1; MP 143-144°C [8].

(1S,4R)-3-(Benzylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (20)

^{1}H NMR (DMSO-d_6) (300 MHz): δ 7.97 (t, *J* = 5.7 Hz, 1H), 7.62-7.59 (m, 1H), 7.43-7.37 (m, 2H), 7.31-7.23 (m, 2H), 4.73 (s, 1H), 4.49 (d, *J* = 4.1 Hz, 1H), 4.28-4.14 (m, 2H), 2.95 (d, *J* = 9.6 Hz, 1H), 2.85 (d, *J* = 9.6 Hz, 1H), 1.63-1.41 (m, 4H); ^{13}C NMR (DMSO-d_6) (75 MHz): δ 173.0, 172.2, 130.9, 128.1, 127.6, 126.2, 78.9, 77.1, 53.1, 51.9, 42.4, 28.0, 27.7; MP 170°C [8].

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3.7 Paper VII

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Focused library development of 2-phenylacrylamides as broad spectrum cytotoxic agents

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ABSTRACT

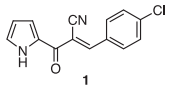
With our lead compound (*E*)-3-(4-chlorophenyl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (**1**) inducing 50% growth inhibition of 11 cancer cell lines at 27–61 μM, potency enhancements were rapidly established through the synthesis of a series of focused compound libraries. Six highly focused libraries (46 compounds in total) were synthesised. Each library allowed the identification of a new lead compound, viz *Library A* identified (*E*)-3-(pentafluorophenyl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (**11**) and (*E*)-3-(1*H*-indol-3-yl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (**13**) as inhibitors with improved cytotoxicity. Synthesis of discrete libraries of amidoacrylamide analogues (Ar–C═C(CN)–Ar✠Ar–C═C(CN)–C(═O)NH)–Ar) resulted in a series of analogues significantly more potent that the lead, **1**. Three furan three analogues: (*E*)-3-(5-chlorofuran-2-yl)-2-cyano-*N*-(4-methoxybenzyl)acrylamide (**33**), (*E*)-3-(5-bromofuran-2-yl)-2-cyano-*N*-(4-methoxybenzyl)acrylamide (**34**) and (*E*)-2-cyano-3-(furan-3-yl)-*N*-(4-methoxybenzyl)acrylamide (**37**) returned broad spectrum growth inhibition (GI_{50} values of 5–16 μM). Replacement of the furan moiety with simple aromatics gave an additional three analogues: (*E*)-2-cyano-*N*-(4-methoxybenzyl)-3-phenylacrylamide (**39**), (*E*)-3-(4-chlorophenyl)-2-cyano-*N*-(4-methoxybenzyl)acrylamide (**41**) and (*E*)-2-cyano-*N*-(4-methoxyphenyl)-3-(naphthalen-1-yl)acrylamide (**45**) with GI_{50} values of 7–24 μM. The final library retained the aromatic substituents but introduced a 3,4-dichlorbenzylamine moiety to afford the 1-naphthyl substituted **52**, which was the most potent broad spectrum cytotoxic analogue produced here in with an average GI_{50} = 8.6 μM. This represents a fivefold potency enhancement relative to **1** and a new cytotoxic scaffold suitable for further development.

1. Introduction

Cancer is a debilitating, life-threatening disease that will affect 1 in 3 people during the course of their lives.[1] Current treatment strategies for cancer have limited efficacy, especially in the common malignancies such as breast, colon and lung cancer. Since the 1960's cancer drug discovery development has provided about 100 approved products for the treatment of malignancy. While major advances have been made in the chemotherapeutic management of patients, one-half of all cancer patients either, do not respond to therapy or relapse following initial response, and ultimately die from their metastatic disease. Better targeted therapies are clearly needed in the fight against cancer.

We have multiple research programs targeting novel modes of action for anti-cancer drugs including protein phosphatase inhibitors that accelerate the cell cycle leading to cell death and dynamin inhibitors that affect cell cycle progression at the abscission stage.[2–12] Most recently our anti-cancer drug development program facilitated the development of a family of (*Z*)-2-phenylacrylonitriles. In this latter report we observed modest levels of cytotoxicity with the related (*E*)-2-(1*H*-pyrrole-2-carbonyl)-3-(aromatic) acrylonitriles such as **1** (Fig. 1).[13]

Over the past decade there has been an increasing prevalence in the number of biologically active compounds that contain an acrylonitrile moiety similar to those reported herein.[14–23] The acrylonitrile pharmacophore is active across multiple cellular pathways known to be involved in the production of cytotoxic effects in cell line models of cancer. These pathways include tubulin polymerisation inhibition,[14–16,18] cell death via apoptosis,[20–22] and



1

Figure 1. (*E*)-3-(4-Chlorophenyl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (**1**) inhibits the growth of eleven human cancer cell lines displaying a 31–84% growth inhibition at 100 μM drug concentration. See Table 1 for details of cell lines examined were.

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tyrosine kinase inhibition.[17] Indeed it could be argued that our leads are structurally related to the monomeric tyrphostins, which are known kinase inhibitors. There are also reports of selectivity between cytotoxicity and antifungal activity.[19]

Building on our medicinal chemistry paradigm of iterative focused library synthesis, biological screening, re-design and library synthesis,[2–13] we have identified (*E*)-3-(4-chlorophenyl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (**1**) as a synthetically accessible anti-cancer lead compound. Our lead acrylonitrile (**1**) was accessed in two simple steps from pyrrole, cyanoacetic acid and *p*-chlorobenzaldehyde. The commercial availability of a wide range of aldehydes allowed for rapid exploration of structure activity relationships (SAR). Herein we report an exploration of the SAR associated with **1** via the synthesis and biological evaluation of a series of focused compound libraries.

2. Results and discussion

Our focused compound library development commenced with the synthesis of (*E*)-3-(4-chlorophenyl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (**1**). In a typical synthesis, cyanoacetic acid was mixed with acetic anhydride followed by the addition of pyrrole and the mixture heated at 75 °C for 35 min, extractive work up and silica gel chromatography afforded 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile (**4**). Treatment of **4** in ethanol with *p*-chlorobenzaldehyde and a catalytic quantity of piperidine, after work up gave **1** in a 39% yield (two steps) (Scheme 1).[24]

In the development of our first focused library (*Library A*), **4** was treated with ten aldehydes (see Table 1 for details),[13,25–31] and the resultant compounds (**1**, **5**–**13**) were screened for growth inhibition against a panel of eleven cancer cell lines: HT29 and SW480 (colon carcinoma), MCF-7 (breast carcinoma), A2780 (ovarian carcinoma), H460 (lung carcinoma), A431 (skin carcinoma), DU145 (prostate carcinoma), BEC-2 (neuroblastoma), SJ-G2 (glioblastoma), MIA (pancreatic carcinoma) and SMA (spontaneous murine astrocytoma).[6] In vitro cytotoxicity assays have been used for decades as a tool to understand hypothesis driven questions regarding drug action. Our efforts in this area do not include the use of normal cell lines as a single in vitro screening assay as normal cell lines fail to provide a 'go/no-go' step in the drug development process. Indeed, when used in a prospective manner, they have not been highly predictive of in vivo toxicity.[32] A single in vitro screening platform is unlikely to provide the data required to evaluate risk and predict human toxicity. In this regard, it is widely accepted that additional models using a tiered toxicity screening approach are required in order to define and predict clinical toxicity. As part of our drug development strategy small molecules that satisfy specific drugability characteristics as well as cytotoxicity will undergo further biological evaluation in animal models.

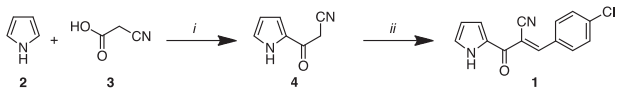
Initial cytotoxicity efficacy was determined at 25 μM drug concentration. Two of the ten *Library A* analogues were sufficiently active at (or near) our screening threshold (90% growth inhibition at 25 μM drug concentration, see also Supplementary data Tables S1–S6) to warrant the determination of GI_{50} values. These data are presented in Table 1. The lead 4-Cl (**1**) and pentafluorophenyl (**11**) returned average GI_{50} values of 40 and approximately 70 μM, respectively across the 11 cell lines examined. This suggested that the presence of an electron withdrawing group may be important and imparted favourable growth inhibition. Of the other *Library A* analogues only the indole substituted **13** showed even a moderate level of potency at the initial 25 μM concentration, and most notably a ~50% growth inhibition against the SW480, MCF-7 and BE2-C cell lines (Supplementary data, Table S1). This level of growth inhibition while low was deemed encouraging enough to continue our studies through the development of *Library B*.

Given the modest level potency, but apparent cell line specificity, noted with (**13**) we chose to examine the effect of a simple pyrrole to indole replacement through application of the same chemistry (commencing with indole) and the same family of aldehydes as described in Scheme 1. This led to the synthesis of *Library B* comprising the indolylcarbonylacrylamide analogues **14**-**23**. The growth inhibiton screening data is presented in Table 2.

From *Library B*, three analogues, the 4-NO_2 (**18**), pentafluoro (**21**) and bis-indole (**23**) proceeded to GI_{50} determination. The introduction of the indole nucleus saw a significant reduction in cytotoxicity previously observed with the pyrroles (Table S2, Supplementary data), cf. indoles **15** and **14** compared with pyrroles **1** and **5.** As with the pyrrole analogue, the pentafluorophenyl substituted **21** was the most active in this library, and we note that the 4-NO_2 substituted **18** was also active. As with *Library A*, we note that electron withdrawing groups on the phenyl ring conferred higher levels of cell growth inhibition compared with the indolylacrylamide analogues of *Library B*.

We next turned our attention to the possible effect of introducing additional functionality to the linker chain with the synthesis of *Library C* which introduced an amide moiety. In designing our next library we combined the most favourable properties of the pyrrole *Library A* with the 4-OCH_3 and 3,4-dicholoro phenyl substituents that afforded high levels of activity in our original phenylacrylonitrile compound series.[13] Accordingly we set about developing an expedient route to simple analogues that combined theses characteristics with the additional requirement of introducing an amide moiety. Treatment of 4-methoxybenzylamine and 3,4-dichlorobenzylamine with methyl cyanoacetate afforded the cyanoamides **25** and **26** in excellent yields. Subsequent treatment of **25** and **26** with 1*H*-pyrrole-2-carbaldehyde and furfural afforded *Library C* analogues **27**–**30** with the desired amide moiety installed.[25–31]

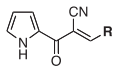
Cytotoxicity screening of **27**–**30** (*Library C*, Table 3) gave rise to a number of interesting outcomes. Firstly the 4-OCH_3 furan analogue **29** returned the highest level of activity noted thus far with GI_{50} values of 16–33 μM. Secondly, analogues **27**, **28** and **30** showed activity in specific cell lines rather than broad anti-cancer activity across all cell lines. Analogue **27** returned GI_{50} values of 36 and 47 μM for the MCF-7 and the BE2-C cell lines, respectively. Against all other cell lines **27** was inactive (GI_{50} >50 μM). Pyrrole **28** showed a similar cytotoxicity profile to **27**, however, this analogue displayed significant selectivity towards the MCF-7 breast cancer cell line; GI_{50} = 6 μM, six times more active than the 4-OCH_3 analogue, **27**. This was the most potent analogue thus far and represented a fivefold increase relative to the lead (**1**) and 15-fold MCF-7 selectivity. Of equal note was that the simple bioisosteric replacement of the pyrrole NH to the furan O atom gave two analogues with good activity against the cell lines examined. Furan **30** showed a similar selectivity profile to that observed with



2 3 4 1

Scheme 1. Reagents and conditions: (i) Ac_2O, 85 °C, 5 min; (ii) RCHO (see table for details), piperidine (cat), EtOH reflux 2 h.

Table 1
Evaluation of the cytotoxicity (GI_{50} (μM)) of *Library A* derivatives **1**, and **5**–**13** against a panel of eleven cancer cell lines



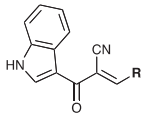
Compound	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **1**	36 ± 5	34 ± 3	27 ± 3	35 ± 4	43 ± 7	52 ± 11	61 ± 10	34 ± 3	37 ± 7	48 ± 17	38 ± 6
 **5**	–[k]	–	–	–	–	–	–	–	–	–	–
 **6**	–	–	–	–	–	–	–	–	–	–	–
 **7**	–	–	–	–	–	–	–	–	–	–	–
 **8**	–	–	–	–	–	–	–	–	–	–	–
 **9**	–	–	–	–	–	–	–	–	–	–	–
 **10**	–	–	–	–	–	–	–	–	–	–	–
 **11**	224	>100	23 ± 14	64 ± 24	31 ± 10	64 ± 9	>100	14 ± 3	23 ± 13	25 ± 4	>100
 **12**	–	–	–	–	–	–	–	–	–	–	–
 **13**	–	43%[l]	56%[l]	–	–	–	–	49%[l]	–	–	–

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).
[k] '–' = Insufficient growth inhibition at 25 μM drug concentrations to proceed to full dose response evaluation, percentage growth inhibition values are tabulated in Table S1 (Supplementary data).
[l] Percentage growth inhibition at 25 μM drug concentration.

28. It thus appeared that the 3,4-dicholorophenyl moiety played a role in imparting cancer cell line specificity. Overall the furans **29** and **30** are 2- to 3-fold more potent than **1**.

Given the potency we noted with the furans **29** and **30** we expanded the *Library C* to developed a new focused library, *Library D*, in which we retained the 4-OCH_3Ph moiety of **29** as it had given

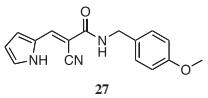
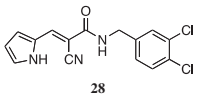
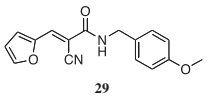
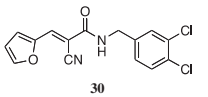
Table 2
Evaluation of the cytotoxicity (GI_{50} (μM)) of *Library B* analogues **14**–**23** against a panel of eleven cancer cell lines



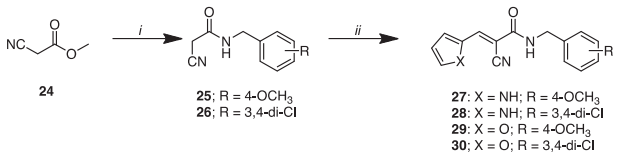
Compound	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **14**	–[k]	–	–	–	–	–	–	–	–	–	–
 **15**	–	–	–	–	–	–	–	–	–	–	–
 **16**	–	–	–	–	–	–	–	–	–	–	–
 **17**	–	–	–	–	–	–	–	–	–	–	–
 **18**	41 ± 1	42 ± 3	35 ± 3	48 ± 6	47 ± 4	46 ± 6	59 ± 0	40 ± 5	37 ± 4	46 ± 9	60 ± 6
 **19**	–	33%[l]	42%[l]	32%[l]	–	–	–	33%[l]	–	–	–
 **20**	–	–	–	–	–	–	–	–	–	–	–
 **21**	18 ± 3	18 ± 3	13 ± 4	11 ± 0	35 ± 7	19 ± 1	30 ± 1	10 ± 2	14 ± 1	21 ± 5	20 ± 1
 **22**	–	–	40%[l]	–	–	–	–	–	–	–	–
 **23**	6.0 ± 0.1	8.3 ± 0.4	11 ± 1	9.3 ± 0.2	7.6 ± 0.3	7.6 ± 0.1	17 ± 1	12 ± 0	13 ± 0	14 ± 0	15 ± 1

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).
[k] '—' = Insufficient growth inhibition at 25 μM drug concentrations to proceed to full dose response evaluation, percentage growth inhibition values are tabulated in Table S2 (Supplementary data).
[l] Percentage growth inhibition at 25 μM drug concentration.

Table 3
Evaluation of the cytotoxicity (GI_{50} (μM)) of *Library C* analogues **27**–**30** against a panel of eleven cancer cell lines

Compound	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **27**	>50	>50	34 ± 6	>50	>50	>50	>50	47 ± 2	>50	>50	>50
 **28**	85 ± 12	>50	6 ± 1	>50	>50	>50	>50	43 ± 4	>50	>50	>50
 **29**	22 ± 5	31 ± 2	27 ± 6	24 ± 4	32 ± 1	16 ± 1	29 ± 1	24 ± 4	31 ± 1	34 ± 3	33 ± 2
 **30**	20 ± 6	>50	20 ± 5	>50	>50	>50	24 ± 7	>50	>50	>50	38 ± 7

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).



Scheme 2. Reagents and conditions: (i) MeOH, MW, 200 W, 120 °C, 15 min; (ii) 1*H*-pyrrole-2-carbaldehyde or furfural, piperidine (cat), EtOH, MW, 200 W, 120 °C, 15 min.

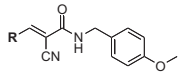
rise to the broad spectrum cytotoxicity and modified the furan nucleus. *Library D* was prepared as per Scheme 2, and screened for cytotoxicity as before and these data are presented in Table 4.

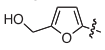
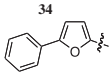
On examination of the data presented in Table 4 it was immediately apparent that bulky or electron donating 5-substituted-(furan-2yl)acrylamide derivatives (**31**, **32**, and **35**) lost the broad spectrum activity of the furan lead, **29**. Furan **31** was largely inactive, but did show a toxicity profile similar to indole **13**. Analogue **35** showed a 20-fold specificity towards the MCF-7 breast cancer cell line (GI_{50} = 3 ± 1 μM). Fusing the phenyl ring in the form of the benzofuran analogue **38** resulted in a loss of MCF-7 selectivity with **38** inactive across all cell lines examined. Electron withdrawing substituents were well tolerated with the 5-Cl (**33**) and 5-Br (**34**) highly cytotoxic with average GI_{50} values of 12 and 13 μM, respectively. Interestingly the 4-bromo (**36**) (cf. 5-bromo **34**) saw a marked reduction in potency from an average GI_{50} = 13 μM to ⩾53 μM, indicating a high level of substituent pattern dependence. This positional dependence was also apparent with the furan-3-ylacrylamide analogue (**37**) which was the most active analogue generated thus far with an average GI_{50} = 7 μM, four times more potent than the equivalent furan-2-ylacrylamide (**29**, Table 3).

Having established that multiple variations in the furan moiety were permitted we extended the scope of these studies and explored replacement of the furan ring with other simple aromatic systems while retaining the 4-OCH_3Ph moiety, using the same chemistry as in Scheme 2 to afford *Library E* analogues **39**–**45**, which were subsequently screened against our panel of eleven cancer cell lines. These data are presented in Table 5.

As can be seen from the data presented in Table 5, again the position and type of substituent on the newly introduced aromatic ring had a pronounced effect on the observed cytotoxicity. Those analogues containing a small electron withdrawing group or positioned the electron density at the C2′/C3′ region returned a significantly higher level of broad spectrum cytotoxicity than those lacking such a substituent. This is particularly evident on comparison of the C4′-Cl (**41**) with the C4′-OCH_3 (**43**) with the former returning an average GI_{50} value of 11 μM, while the latter was essentially inactive. Also of note again, was the positional dependence of a fused aromatic system such as the C1′- and C2′-naphthyl analogues **45** and **44** with average GI_{50} values of ⩾51 and 15 μM, respectively. The C1' analogue **45** is roughly threefold more potent than the corresponding C2′ **44**. These findings are in keeping with

Table 4
Evaluation of the cytotoxicity(GI_{50} (μM)) of *Library D* analogues **31**–**38** against a panel of eleven cancer cell lines



Compound	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **31**	92 ± 6	29 ± 2	21 ± 1	60 ± 11	>100	58 ± 11	39 ± 6	21 ± 4	75 ± 14	51 ± 4	69 ± 14
 **32**	–[k]	–	–	–	–	–	–	–	–	–	–
 **33**	9 ± 1	13 ± 0	9 ± 1	9 ± 1	15 ± 0	9 ± 1	12 ± 1	11 ± 0	13 ± 1	15 ± 0	14 ± 0
 **34**	10 ± 1	14 ± 1	11 ± 2	12 ± 0	16 ± 1	9 ± 1	12 ± 1	13 ± 1	13 ± 0	16 ± 0	14 ± 0
 **35**	56 ± 8	>100	3 ± 1	>100	>100	>100	>100	>100	>100	>100	>100
 **36**	36 ± 8	>100	20 ± 2	28 ± 6	>100	14 ± 4	61 ± 17	36 ± 11	23 ± 0.3	65 ± 3	>100
 **37**	6 ± 1	8 ± 0	7 ± 1	6 ± 0	8 ± 0	5 ± 0	7 ± 0	7 ± 0	8 ± 0	8 ± 0	7 ± 0
 **38**	–	–	40%	–	–	–	–	–	–	34%	–

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).
[k] '—' = insufficient growth inhibition at 25 μM drug concentrations to proceed to full dose response evaluation, percentage growth inhibition values are tabulated in Table S4 (Supplementary data).

the general trends observed with the substituted furan analogues shown in Table 5.

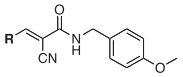
From the data obtained thus far a pharmacophore of broad spectrum cytotoxicity for these acrylamides has emerged that has identified the acrylamide moiety as essential, but permits the introduction of an amide spacer, considerably extending the scope for the development of these analogues. This pharmacophore was applied in the design and development of our final focused library, *Library F*, in which the 3,4-dichlorophenyl moiety was re-introduced. Note that with the parent pyrrole series this 3,4-dichlorophenyl moiety afforded high levels of MCF-7 specificity, but with this extended pharmacophore we anticipated more broad spectrum activity would be observed. As anticipated, the data in Table 6 shows four analogues (**46**, **48**, **51** and **52**) with average GI_{50} values ranging from ca. 10 to 70 μM. The trends in activity matched that predicted, viz the most active analogue was C1'-naphthyl (**52**), with an average GI_{50} value of 8.6 μM.

3. Conclusion

Replacement of the 4-ClPh moiety of the lead **1**, typically removed all vestiges of activity against the panel of eleven cell lines examined herein. Only the pentafluorophenyl substituted **11** saw a similarly modest level of potency, but not across all the cell lines examined. Pentafluoro **11** was inactive against the SW480, DU145 and the SMA cell lines. Indole **13** showed modest specificity towards the SW480, MCF-7 and BE2-C cell lines. Replacement of the pyrrole moiety with an indole gave three analogues; **18**, **21** and **23**; with average GI_{50} values of 46, 19 and 11 μM, respectively.

The introduction of an amide moiety was explored with the synthesis of *Library C*, and this produced an immediate increase in potency with the furan analogue **29** displaying good broad spectrum activity with an average GI_{50} value of 27 μM. Within *Library C* the pyrrole analogues displayed preferential activity towards the

Table 5
Evaluation of the cytotoxicity (GI_{50} (μM)) of *Library E* analogues **39**–**45**, against a panel of eleven cancer cell lines



Compound	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **39**	9 ± 1	14 ± 0	18 ± 2	9 ± 1	18 ± 1	9 ± 1	13 ± 2	15 ± 0	17 ± 1	24 ± 3	16 ± 1
 **40**	36 ± 3	34 ± 3	36 ± 2	33 ± 2	41 ± 4	26 ± 5	34 ± 3	35 ± 3	39 ± 6	38 ± 6	42 ± 5
 **41**	7 ± 0	10 ± 0	11 ± 1	7 ± 0	14 ± 1	6 ± 0	7 ± 1	12 ± 0	11 ± 0	16 ± 1	17 ± 0
 **42**	–[k]	–	–	–	–	–	–	–	–	–	–
 **43**	–	–	–	–	–	–	–	–	–	–	–
 **44**	54 ± 9	41 ± 1	33 ± 3	40 ± 2	>100	37 ± 3	80 ± 16	42 ± 2	40 ± 4	49 ± 8	43 ± 4
 **45**	13 ± 1	14 ± 1	16 ± 0	16 ± 0	15 ± 0	15 ± 1	16 ± 0	14 ± 2	15 ± 1	15 ± 1	15 ± 0

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
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[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).
[k] '—' = insufficient growth inhibition at 25 μM drug concentrations to proceed to full dose response evaluation, percentage growth inhibition values are tabulated in Table S5 (Supplementary data).

MCF-7 cell line with pyrrole **27** a 34 μM and **28** a 6 μM potent inhibitor of cell growth.

Exploration of the furan moiety with *Library D* afforded three highly potent analogues. Furans **33**, **34** and **37** were 12, 13 and 7 μM potent across the cell lines examined. The 3-furanyl **37**, was the most active furan analogue in this series. The phenyl substituted was MCF-7 specific returning the best activity yet noted with $GI_{50MCF-7}$ = 3 μM. Replacement of the furan moiety with *Library E* gave five analogues that proceeded to GI_{50} determination. The most noteworthy analogues in this library were the phenyl **39**, 4-chlorphenyl **41** and 1-naphthyl **45** with average GI_{50} values of 15, 11 and 15 μM, respectively. In the final library introduction of the 3,4-dichlorobenzyl moiety produced a further increase in potency for the 1-naphthyl substituted **52** to 8.6 μM.

Herein from six focused libraries comprising a total of 46 analogues we have identified eleven analogues with GI_{50} <15 μM; an average of threefold more active than the lead. The most potent analogue were **28** (6 μM), **37** (7 μM) and **52** (8.6 μM). Of the remaining analogues, the furan **35** displayed the highest level of specificity towards the MCF-7 cell line (3 μM). The development of the analogues herein clearly demonstrated that the cytotoxicity of these acrylamide analogues was not derived solely from the electron withdrawing effect, with other characteristics such as orientation of electron density and lone electron pairs having a pronounced effect.

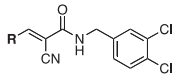
While some of our molecules show potent in vitro cytotoxicity in a range of cancer cell lines additional biological models will need to be utilised in order to define the in vivo efficacy and toxicity of these small molecules. These findings will be reported in due course.

4. Experimental section

4.1. Materials

All starting materials were purchased from Aldrich Chemical Co. and Lancaster Synthesis. Solvents were bulk, and distilled from glass prior to use. Reaction progress was monitored by TLC, on alu-

Table 6
Evaluation of the cytotoxicity (GI_{50} (μM)) of *Library F* analogues **46**–**52** against a panel of eleven cancer cell lines



Compound	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **46**	70 ± 15	49 ± 7	29 ± 5	49 ± 6	61 ± 6	43 ± 6	71 ± 15	32 ± 1	43 ± 2	47 ± 2	43 ± 8
 **47**	–[k]	–	–	–	–	–	–	–	–	–	–
 **48**	33 ± 1	30 ± 0	21 ± 1	32 ± 1	33 ± 0	30 ± 0	30 ± 1	31 ± 0	30 ± 0	31 ± 1	33 ± 2
 **49**	–	–	–	–	–	–	–	–	–	–	–
 **50**	>100	95 ± 0	45 ± 7	86 ± 11	>100	90 ± 8	70 ± 6	93 ± 2	>100	79 ± 11	>100
 **51**	44 ± 2	50 ± 4	29 ± 1	44 ± 4	>100	50 ± 6	77 ± 7	40 ± 0	49 ± 8	44 ± 1	64 ± 20
 **52**	7 ± 0	11 ± 0	8 ± 1	7 ± 0	7 ± 0	8 ± 0	12 ± 1	7 ± 0	13 ± 1	8 ± 0	7 ± 0

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).
[k] '–' = insufficient growth inhibition at 25 μM drug concentrations to proceed to full dose response evaluation, percentage growth inhibition values are tabulated in Table S2 (Supplementary data).

minium plates coated with silica gel with fluorescent indicator (Merck 60 F254) and flash chromatography was conducted utilizing SNAP Biotage KP-SIL columns. ^{1}H and ^{13}C spectra were recorded on a Bruker Advance AMX 300 MHz spectrometer at 300.13 and 75.48 MHz, respectively. Chemical shifts are relative to TMS as internal standard. All compounds returned satisfactory mass spectra were obtained using a micromass liquid chromatography Z-path (LCZ) platform spectrometer. Mass to charge ratios (m/z) are stated with their peak intensity as a percentage in parentheses. All mass spectra were obtained via the ES method thus fragmentation patterns were not observed. The University of Wollongong, Australia, Biomolecular Mass Spectrometry Laboratory, analyzed samples for HRMS. The spectra were run on a micromass QTof2 spectrometer using polyethylene glycol or polypropylene glycol as the internal standard. Compound purity was confirmed by a combination of LC–MS (HPLC), micro and/or high resolution mass spectrometry and NMR analysis. All analogues are ⩾95% purity.

4.1.1. Cell culture and stock solutions[22,23]

Stock solutions were prepared as follows and stored at −20 °C: drugs were prepared as 40 mM solutions in DMSO. All cell lines were cultured at 37 °C, under 5% CO_2 in air and were maintained in Dulbecco's modified Eagle's medium (Trace Biosciences, Australia) supplemented with 10% foetal bovine serum, 10 mM sodium bicarbonate penicillin (100 μg mL^{-1}), streptomycin (100 μg mL^{-1}), and glutamine (4 mM).

4.1.2. In vitro growth inhibition assays

Cells in logarithmic growth were transferred to 96-well plates. Cytotoxicity was determined by plating cells in duplicate in medium (100 μL) at a density of 2500–4000 cells/well. On day 0 (24 h after plating), when the cells were in logarithmic growth, medium (100 μL) with or without the test agent was added to each well. After 72 h of drug exposure, growth inhibitory effects were evaluated using the MTT (3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay and their absorbance was read at

540 nm. Percentage growth inhibition was determined at a fixed drug concentration of 25 μM. A value of 100% is indicative of total cell growth inhibition. Those analogues showing appreciable percentage growth inhibition underwent further dose response analysis to allow the calculation of GI_{50} values. The GI_{50} value is defined as the drug concentration at which cell growth is 50 % inhibited based on the difference between the optical density values on day 0 and those at the end of drug exposure.[33]

4.2. Chemistry

4.2.1. General methods

THF was freshly distilled from sodium–benzophenone. Flash chromatography was carried out using silica gel 200–400 mesh (60 Å). ^{1}H and ^{13}CNMR were recorded at 300 and 75 MHz, respectively using a Bruker Avance 300 MHz spectrometer in $CDCl_3$, acetone-d_6 and DMSO-d_6. GCMS was performed using a Shimadzu GCMS-QP2100. The instrument uses a quadrupole mass spectrometer and detects samples via electron impact ionization (EI). The Children's Medical Research Institute, Cell Signalling Unit Mass Spectrometry Laboratory analyzed samples for HRMS. The spectra were run on the VG Autospec-oa-tof tandem high resolution mass spectrometer using CI (chemical ionization), with methane as the carrier gas and PFK (perfluorokerosene) as the reference. All samples returned satisfactory analyses.

4.2.2. (*E*)-3-(4-Chlorophenyl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (1)

Cyanoacetic acid (1.360 g, 16 mmol) was added to Ac_2O (8 mL) and the resultant suspension was stirred and heated to 50 °C upon which the solid material dissolved. Pyrrole (1.073 g, 16 mmol) was then added and the solution was heated at 75 °C for 35 min. The solution was then diluted with EtOAc (20 mL) and washed with 0.1 M NaOH (3 × 10 mL). The organic layer was then collected and dried using $MgSO_4$. The solvent was then removed under vacuum and the residue purified by flash silica chromatography (1:10 EtOAc/Hexanes to 1:1 EtOAc/Hexanes) to afford 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile, 70%. Next, to an ethanolic solution (10 mL) of 4-methylbenzaldehyde (1.57 mmol) was added an ethanolic solution (10 mL) of 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile (1.49 mmol). This mixture was heated to 70 °C at which time, piperidine (2 drops) was added, and the solution was then heated under reflux for an additional 2 h. After this time, the solution was cooled and the solvent removed in vacuo to afford a brown oil which was purified by flash chromatography (1:10 EtOAc/Hexanes) to afford (**1**), as a yellow solid; 39%; 192–194 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 11.30 (br, NH), 8.28 (s, 1H, CH═C), 8.13–8.11 (m, 2H, Ar H2 + Ar H6), 7.66–7.63 (m, 2H, Ar H3 + Ar H5), 7.45–7.44 (m, 1H, Pyr H5), 7.35–7.34 (m, 1H, Pyr H3), 6.38–6.36 (m, 1H, Pyr H4);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 173.6, 151.1, 137.4, 131.7 (2 × Ar), 130.8, 128.8 (2 × Ar), 126.9, 119.7, 118.7, 116.6, 110.4, 109.5;

IR (KBr) cm^{-1}: 3284 (NH), 2211 (CN), 1627 (C═O);

LRMS: (APCI M+1) 257. HRMS: Calcd for $C_{14}H_9ClN_2O$: 256.0403, found (ACPI M+1) 257.0478.

4.2.3. (*E*)-2-(1*H*-Pyrrole-2-carbonyl)-3-*p*-tolylacrylonitrile (5)

Synthesized using the general procedure as for (**1**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-chlorobenzaldehyde to afford (**5**) as a yellow solid; 81%; 242–244 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.22 (br, NH), 8.24 (s, 1H, CH═C), 7.95 (d, *J* = 8.0 Hz, 2H, Ar H2 + Ar H6), 7.37 (d, *J* = 8.0 Hz, 2H, Ar H3 + Ar H5), 7.29–7.26 (m, 2H, Pyr H3 + Pyr H5), 6.33–6.30 (m, 1H, Pyr H4), 2.37 (s, 3H, CH_3);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 174.8, 153.2, 143.5, 130.6 (2 × Ar), 129.7 (2 × Ar), 129.3, 129.0, 127.9, 119.3, 117.7, 110.8, 107.8, 21.2;

IR (KBr) cm^{-1}: 3291 (NH), 2210 (CN), 1622 (C═O);

LRMS: (APCI M+1) 237. HRMS: Calcd for $C_{15}H_{12}N_2O$; Exact mass: 236.0950, found (ACPI M+1) 237.1028.

4.2.4. (*E*)-3-(4-Hydroxyphenyl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (6)

Synthesized using the general procedure as for (**1**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-hydroxybenzaldehyde to afford (**6**) as an orange solid; 43%; 240–243 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ = 12.07 (br, NH), 8.68 (br, OH), 8.18 (s, 1H, CH═C), 7.99 (d, *J* = 8.7 Hz, 2H, Ar H2 + Ar H6), 7.28–7.23 (m, 2H, Pyr H5 + Pyr H3), 6.94 (d, *J* = 8.7 Hz, 2H, Ar H3 + Ar H5), 6.30–6.27 (m, 1H, Pyr H4);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 175.0, 162.3, 153.3, 133.6 (2 × Ar), 129.2, 127.3, 123.1, 118.6, 118.4, 116.2 (2 × Ar), 110.5, 104.1;

IR (KBr) cm^{-1}: 3419 (OH), 3290 (NH), 2218 (CN), 1617 (C═O), 1603 (Ar);

LRMS: (APCI M+1) 239. HRMS: Calcd for $C_{14}H_{10}N_2O_2$; Exact mass: 239.0829; found (ACPI M+1) 239.0829.

4.2.5. (*E*)-3-(4-Methoxyphenyl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (7)

Synthesized using the general procedure as for (**1**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-methoxybenzaldehyde to afford (**7**) as a yellow solid; 83%; 166–168 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.14 (br, NH), 8.24 (s, 1H, CH═C), 8.08 (d, *J* = 8.9 Hz, 2H, Ar H2 + Ar H6), 7.28–7.25 (m, 2H, Pyr H5 + Pyr H3), 7.13 (d, *J* = 8.9 Hz, 2H, Ar H3 + Ar H5), 6.31 (s, 1H, Pyr H4), 3.85 (s, 3H, OCH_3);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 174.9, 163.0, 153.0, 133.1 (2 × Ar), 129.1, 127.5, 124.6, 118.9, 118.2, 114.8 (2 × Ar), 110.6, 105.5, 55.6;

IR (KBr) cm^{-1}: 3306 (NH), 2209 (CN), 1617 (C═O), 1507 (Ar);

LRMS: (APCI M+1) 253. HRMS: Calcd for $C_{15}H_{12}N_2O_2$; Exact mass: 252.0899, found (ACPI M+1) 253.0979.

4.2.6. (*E*)-3-(4-Nitrophenyl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (8)

Synthesized using the general procedure as for (**1**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-nitrobenzaldehyde to afford (**8**) as a purple solid; 37%; 199–200 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.32 (br, NH), 8.40–8.38 (m, 3H, Ar H3 + Ar H5 + Pyr H5), 8.24–8.21 (m, 2H, Ar H2 + Ar H6), 7.24 (m, 2H, Pyr H5 + Pyr H3), 6.34 (s, 1H, Pyr H4);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 174.2, 150.6, 148.8, 138.2, 131.3 (2 × Ar), 130.6, 128.7, 124.0 (2 × Ar), 120.3, 116.6, 113.1, 111.1;

IR (KBr) cm^{-1}: 3308 (NH), 2228 (CN), 1633 (C═O), 1517 (NO) 1343 (NO);

LRMS: (APCI M+1) 268. HRMS: Calcd for $C_{14}H_9N_3O_3$; Exact mass: 267.0644, found (ACPI M+1) 268.0717.

4.2.7. (*E*)-3-(3,4-Dichlorophenyl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (9)

Synthesized using the general procedure as for (**1**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 3,4-dichlorobenzaldehyde to afford (**9**) as a yellow solid; 66%; 178–181 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 11.31 (br, NH), 8.29–8.27 (m, 2H, Ar H5 + CH═C), 8.11–8.08 (m, 1H, Ar H6), 7.84–7.81 (m, 1H, Ar H2), 7.45–7.36 (m, 2H, Pyr H5 + Pyr H3), 6.39–6.37 (m, 1H, Pyr H4);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 173.4, 149.6, 135.0, 132.4, 132.1, 131.7, 130.8, 129.3, 127.2, 127.0, 119.0, 116.3, 111.0, 110.5;

IR (KBr) cm^{-1}: 3310 (NH), 2222 (CN), 1632 (C=O);

LRMS: (APCI M+1) 290. HRMS: Calcd for $C_{14}H_8Cl_2N_2O$; Exact mass: 290.0014, found (ACPI M+1) 291.0079.

4.2.8. (*E*)-2-(1*H*-Pyrrole-2-carbonyl)-3-(4-(trifluoromethyl) phenyl)acrylonitrile (10)

Synthesized using the general procedure as for (**1**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-trifluoromethylbenzaldehyde to afford (**10**) as a yellow solid; 41%; 160–162 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.30 (br, 1H), 8.37 (s, 1H, CH=C), 8.19 (d, J = 8.1 Hz, 2H, Ar H2 + Ar H6), 7.94 (d, J = 8.1 Hz, 2H, Ar H3 + Ar H6), 7.32 (s, 2H, Pyr H5 + Pyr H3), 6.34 (s, 1H, Pyr H4);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 174.4, 151.4, 136.0, 131.2, 130.8 (2 × Ar), 128.8, 128.5 (2 × Ar), 125.9, 125.8, 120.1, 116.8, 112.1, 111.0;

IR (KBr) cm^{-1}: 3295 (NH), 2228 (CN), 1637 (C=O), 1561 (Ar), 1325 (C–F);

LRMS: (ESI M−1) 289. HRMS: Calcd for $C_{15}H_9F_3N_2O$; Exact mass: 290.0667, found (ESI M−H) 289.0673.

4.2.9. (*E*)-3-(Pentafluorophenyl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (11)

Synthesized using the general procedure as for (**1**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 2,3,4,5-pentafluorobenzaldehyde to afford (**11**) as a white solid; 22%; 178–180 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 11.01 (br, NH), 7.35–7.32 (m, 1H, Pyr H5), 7.22–7.19 (m, 1H, Pyr H3), 6.41–6.38 (m, 1H, Pyr H4), 5.67 (s, 1H, CH=C);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 174.3, 154.3, 142.5, 135.0, 134.7, 124.6 (2 × Ar), 120.2, 118.4, 114.7 (2 × Ar), 110.5, 106.3, 106.1;

IR (KBr) cm^{-1}: 3367 (NH), 2209 (CN), 1656 (C=C), 1608 (C=O);

LRMS: ESI (M−1) 311. HRMS: Calcd for $C_{14}H_5F_5N_2O$; Exact mass: 312.0322, found (ESI M−H) 311.1706.

4.2.10. (*E*)-3-(Naphthalen-2-yl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (12)

Synthesized using the general procedure as for (**1**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 2-naphthaldehyde to afford (**12**) as a brown solid; 35%; 140–142 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.27 (br, NH), 8.57 (s, 1H, Ar H1), 8.44 (s, 1H, CH=C), 8.23–8.21 (m, 1H, Ar H5), 8.11–7.99 (m, 3H, Ar H3 + Ar H4 + Ar H8), 7.70–7.60 (m, 2H, Ar H7 + Pyr H5), 7.35–7.31 (m, 2H, Ar H6 + Pyr H3), 6.35–6.34 (m, 1H, Pyr H4);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 174.8, 153.2, 134.4, 133.4, 132.4, 129.7, 129.0, 128.9, 128.8, 128.7, 128.0, 127.7, 127.2, 124.7, 119.5, 119.2, 110.8, 109.1;

IR (KBr) cm^{-1}: 3291 (NH), 2215 (CN), 1617 (C=O);

LRMS: (ACPI M+1) 273. HRMS: Calcd for $C_{18}H_{12}N_2O$; Exact mass: 272.0950, found (ACPI M+1) 273.1028.

4.2.11. (*E*)-3-(1*H*-Indol-3-yl)-2-(1*H*-pyrrole-2-carbonyl)acrylonitrile (13)

Synthesized using the general procedure as for (**1**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 1*H*-indole-3-carbaldehyde to afford (**13**) as an orange solid; 95%; 300–302 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.49 (br, NH), 12.00 (br, NH), 8.67 (s, 1H, CH=C), 8.63 (s, 1H, Ind H2), 7.92–7.89 (m, 1H, Ind H7), 7.59–7.56 (m, 1H, Ind H4), 7.36–7.20 (m, 4H, Ind H5 + Ind H6 + Pyr H3 + Pyr H5), 6.31–6.29 (m, 1H, Pyr H4);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 174.4, 145.7, 136.2, 131.9, 129.7, 127.2, 126.4, 123.5, 121.9, 120.3, 118.4, 117.5, 112.8, 110.4, 110.3, 99.4;

IR (KBr) cm^{-1}: 3260 (NH), 2212 (CN), 1611 (C=O);

LRMS: (ESI M−1) 260. HRMS: Calcd for $C_{16}H_{11}N_3O$; Exact mass: 261.0902, found (ESI M−H) 260.0807.

4.2.12. (*E*)-3-(4-Chlorophenyl)-2-(1*H*-indole-3-carbonyl)acrylonitrile (14)

Cyanoacetic acid (0.363, 4.26 mmol) was added to Ac_2O (8 mL) and the resultant solution was stirred and heated to 50 °C upon which the solid material dissolved. Indole (0.5 g, 4.26 mmol) was then added and the solution was heated at 85 °C for 5 min. The solution was cooled to 0 °C and the solid was collected under suction and washed with ice cold MeOH (2 × 5 mL) to afford 3-(1*H*-indol-3-yl)-3-oxopropanenitrile in a 76% yield. Next, to an ethanolic solution (10 mL) of 4-chlorobenzaldehyde (1.14 mmol) was added an ethanolic solution (10 mL) of 3-oxo-3-(1*H*-indol-3-yl)-3-oxopropanenitrile (1.08 mmol). This mixture was heated to 70 °C at which time, piperidine (2 drops) was added, and the solution was then heated under reflux for an additional 2 h. After this time, the solution was cooled and the solvent removed in vacuo to yield a yellow solid. The crude solid was then recrystallised from EtOH to afford (**14**) as a yellow solid; 80%; 230–232 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 11.31 (br, NH), 8.54 (s, 1H, Ind H2), 8.34–8.30 (m, 1H, Ind H4), 8.22 (s, 1H, CH=C), 8.11 (d, J = 8.7 Hz, 2H, Ar H2 + Ar H6), 7.66–7.57 (m, 3H, Ar H3 + Ar H5 + Ind H7), 7.32–7.27 (m, 2H, Ind H5 + Ind H6);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 180.0, 149.8, 137.0, 136.3, 134.3, 131.4 (2 × Ar), 131.0, 128.7 (2 × Ar), 126.1, 123.2, 122.0, 121.4, 116.9, 113.9, 111.9, 111.6;

IR (KBr) cm^{-1}: 3175 (NH), 2211 (CN), 1636 (C=C), 1600 (C=O), 822 (Ar–Cl);

LRMS: (ESI M−1) 305. HRMS: Calcd for $C_{18}H_{11}ClN_2O$; Exact mass: 306.0560, found (ESI M−H) 305.0533.

4.2.13. (*E*)-2-(1*H*-Indole-3-carbonyl)-3-*p*-tolylacrylonitrile (15)

Synthesized using the general procedure as for (**14**), from 3-oxo-3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 4-chlorobenzaldehyde to afford (**15**) as a yellow solid; 88% 216–219 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.26 (br, NH), 8.43 (s, 1H, Ind H2), 8.18–8.16 (m, 2H, Ind H4 + CH=C), 7.94 (d, J = 8.1 Hz, 2H, Ar H2 + Ar H6), 7.55–7.52 (m, 1H, Ind H7), 7.38 (d, J = 8.1 Hz, 2H, Ar H3 + Ar H5), 7.30–7.21 (m, 2H, Ind H5 + Ind H6), 2.37 (s, 3H, OCH_3);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 181.3, 152.1, 142.9, 136.6, 135.7, 130.3 (2 × Ar), 129.7 (2 × Ar), 126.0, 123.4, 122.4, 122.3, 121.3, 117.8, 113.5, 112.4, 110.1, 21.2;

IR (KBr) cm^{-1}: 3262 (NH), 2216 (CN), 1636 (C=C), 1623 (C=O);

LRMS: (ESI M+1) 287. HRMS: Calcd $C_{19}H_{14}N_2O$; Exact mass: 286.1106, found (ESI M+H) 287.1210.

4.2.14. (*E*)-3-(4-Hydroxyphenyl)-2-(1*H*-indole-3-carbonyl)acrylonitrile (16)

Synthesized using the general procedure as for (**14**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 4-hydroxybenzaldehyde to afford (**16**) as a yellow solid; 90%; 252–253 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.17 (br, NH), 8.40 (s, 1H, Ind H2), 8.17–8.13 (m, 2H, Ind H4 + CH=C), 7.98 (d, J = 8.8 Hz, 2H, Ar H2 + Ar H6), 7.53–7.51 (m, 1H, Ind H7), 7.27–7.23 (m, 2H, Ind H5 + Ind H6), 6.94 (d, J = 8.8 Hz, 2H, Ar H3 + Ar H5);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 181.5, 162.0, 152.3, 136.5, 135.0, 133.2 (2 × Ar), 126.1, 123.3, 122.1, 121.7, 121.3, 118.6, 116.1 (2 × Ar), 113.7, 112.3, 106.4;

IR (KBr) cm^{-1}: 3351 (OH), 3232 (NH), 1606 (C=O);

LRMS: (ESI M+1) 289. HRMS: Calcd for $C_{18}H_{12}N_2O_2$; Exact mass: 288.0899, found (ESI M+H) 289.0929

4.2.15. (*E*)-2-(1*H*-Indole-3-carbonyl)-3-(4-methoxyphenyl)acrylonitrile (17)

Synthesized using the general procedure as for (**14**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 4-methoxybenzaldehyde to afford (**17**) as a yellow solid; 92%; 256–258 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.21 (br, NH), 8.42 (s, 1H, Ind H2), 8.18–8.16 (m, 2H, Ind H4 + CH=C), 8.07 (d, J = 8.9 Hz, 2H, Ar H2 + Ar H6), 7.54–7.52 (m, 1H, Ind H7), 7.26–7.24 (m, 2H, Ind H5 + Ind H6), 7.14 (d, J = 8.9 Hz, 2H, Ar H3 + Ar H6), 3.86 (s, 3H, OCH_3);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 181.4, 162.6, 151.9, 136.5, 135.3, 132.7 (2 × Ar), 126.2, 124.8, 123.4, 122.2, 121.3, 118.3, 114.7 (2 × Ar), 113.6, 112.3, 107.9, 55.6;

IR (KBr) cm^{-1}: 3220 (NH), 2221 (CN), 1591 (C=O), 1176 (C–O);

LRMS: (ESI M−1) 301. HRMS: Calcd for $C_{19}H_{14}N_2O_2$; Exact mass: 302.1055, found (ESI M−H) 301.1074.

4.2.16. (*E*)-2-(1*H*-Indole-3-carbonyl)-3-(4-nitrophenyl)acrylonitrile (18)

Synthesized using the general procedure as for (**14**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 4-nitrobenzaldehyde to afford (**18**) as a red solid; 75%; 286–288 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.37 (br, NH), 8.50 (s, 1H, Ind H2), 8.40 (d, J = 8.8 Hz, 2H, Ar H3 + Ar H5), 8.34 (s, 1H, CH=C), 8.23–8.16 (m, 3H, Ar H2 + Ar H6 + Ind H4), 7.56–7.53 (m, 1H, Ind H7), 7.30–7.27 (m, 2H, Ind H5 + Ind H6);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 180.7, 149.3, 148.6, 138.6, 136.8, 136.7, 131.0 (2 × Ar), 125.9, 123.9 (2 × Ar), 123.7, 122.5, 121.2, 116.7, 115.0, 113.3, 112.5;

IR (KBr) cm^{-1}: 3331 (NH), 2219 (CN), 1625 (C=C), 1599 (Ar), 1344 (N–O);

LRMS: (ESI M−1) 316. HRMS: Calcd for $C_{18}H_{11}N_3O_3$; Exact mass: 317.0800, found (ESI M−H) 316.0791.

4.2.17. (*E*)-2-(1*H*-Indole-3-carbonyl)-3-(3,4-dichlorophenyl) acrylonitrile (19)

Synthesized using the general procedure as for (**14**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 3,4-dichlorobenzaldehyde to afford (**19**) as a yellow solid; 88%; 288–290 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.34 (br, NH), 8.46 (s 1H, Ar1 H2), 8.24–8.20 (m, 3H, Ar1 H4 + Ar1 H7 + CH=C), 8.05–8.01 (m, 1H, Ar2 H5), 7.89–8.86 (m, 1H, Ar2 H2), 7.54–7.52 (m, 1H, Ar2 H6), 7.34–7.25 (m, 2H, Ar1 H5 + Ar1 H6);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 180.8, 149.0, 136.7, 136.6, 134.3, 133.0, 132.0, 131.7, 131.2, 129.4, 125.9, 123.6, 122.5, 121.2, 116.9, 116.8, 113.3, 112.4;

IR (KBr) cm^{-1}: 3230 (NH), 2223 (CN), 1635 (C=C), 1594 (C=O), 755 (Ar–Cl);

LRMS: (ESI M−1) 339. HRMS: Calcd for $C_{18}H_{10}Cl_2N_2O$; Exact mass: 340.0170, found (ESI M−H) 339.0198.

4.2.18. (*E*)-2-(1*H*-Indole-3-carbonyl)-3-(4-(trifluoromethyl) phenyl)acrylonitrile (20)

Synthesized using the general procedure as for (**14**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 4-(trifluoromethyl)benzaldehyde to afford (**20**) as a yellow solid; 73%; 242–243 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.30 (br, NH), 8.48 (s, 1H, Ind H2), 8.31 (s, 1H, CH=C), 8.20–8.18 (m, 3H, Ar H2 + Ar H6 + Ind H4), 7.95 (d, J = 7.8 Hz, 2H, Ar H3 + Ar H5), 7.57–7.53 (m, 1H, Ind H7), 7.31–7.26 (m, 2H, Ind H5 + Ind H6);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 180.8, 150.0, 136.7, 136.5, 136.4, 130.6 (2 × Ar), 125.9 (2 × Ar), 125.8, 125.7, 125.6, 123.6, 122.5, 121.2, 116.9, 114.2, 113.4, 112.6;

IR (KBr) cm^{-1}: 3211 (NH), 2223 (CN), 1596 (C=O), 1336 (C–F);

LRMS: (ESI M−1) 339. HRMS: Calcd for $C_{19}H_{11}F_3N_2O$; Exact mass: 340.0823, found ESI (M−H) 339.0829.

4.2.19. (*E*)-2-(1*H*-Indole-3-carbonyl)-3-(perfluorophenyl)acrylonitrile (21)

Synthesized using the general procedure as for (**14**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 2,3,4,5,6-pentafluorobenzaldehyde to afford (**21**) as a yellow solid; 71%; 240–242 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.18 (br, NH), 8.39-8.38 (m, 1H, Ind H2), 7.97–7.94 (m, 1H, Ind H4), 7.56–7.52 (m, 1H, Ind H7), 7.31–7.17 (m, 3H, Ind H5 + Ind H6 + CH=C);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 159.9, 138.2, 136.8, 136.0, 134.9, 130.4, 124.5, 123.9, 122.9, 122.7 (2 × Ar), 121.5 (2 × Ar), 121.2, 120.6, 119.2, 112.6, 105.1;

IR (KBr) cm^{-1}: 3283 (NH), 2212 (CN), 1655 (C=C), 1596 (C=O), 1207 (C–F);

LRMS: (ESI M−1) 361. HRMS: Calcd for $C_{18}H_7F_5N2O$; Exact mass: 362.0479, found (ESI M−H) 316.0465.

4.2.20. (*E*)-2-(1*H*-Indole-3-carbonyl)-3-(naphthalen-2-yl)acrylonitrile (22)

Synthesized using the general procedure as for (**14**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 2-naphthaldehyde to afford (**22**) as a yellow solid; 72%; 302–304 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.29 (br, NH), 8.54–8.50 (m, 2H, Ind H2 + Ind H4), 8.38 (s, 1H, CH=C), 8.24–8.20 (m, 2H, Ar H5 + Ar H8), 8.11–7.99 (m, 3H, Ind H7 + Ar H1 + Ar H4), 7.70–7.54 (m, 3H, Ar H3 + Ar H6 + Ar H7), 7.32–7.24 (m, 2H, Ind H5 + Ind H6);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 181.3, 152.0, 136.6, 135.9, 134.3, 132.8, 132.4, 130.0, 128.9, 128.6, 128.5, 127.7, 127.2, 126.1, 124.8, 123.5, 122.4, 121.3, 117.8, 112.5, 112.4, 111.4;

IR (KBr) cm^{-1}: 3174 (NH), 2214 (CN), 1597 (C=O), 1516 (Ar);

LRMS: (ESI M−1) 321. HRMS: Calcd for $C_{22}H_{14}N_2O$; Exact mass: 322.1106, found (ESI M−H) 321.1104.

4.2.21. (*E*)-3-(1*H*-Indol-3-yl)-2-(1*H*-indole-3-carbonyl)acryloni trile (23)

Synthesized using the general procedure as for (**14**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile 1*H*-indole-3-carbaldehyde to afford (**23**) as a yellow solid; 79%; 292–294 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.14 (br, 2H), 8.63 (s, 1H, Ind H2), 8.60 (s, 1H, CH=C), 8.47 (s, 1H, Ind2 H2), 8.24–8.22 (m, 1H, Ind H4), 7.94–7.91 (m, 1H, Ind2 H4), 7.59–7.53 (m, 2H, Ind H7 + Ind2 H7), 7.31–7.21 (m, 4H, Ind H5 + Ind H6 + Ind2 H5 + Ind2 H6);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 180.7, 145.0, 136.3, 136.1, 133.7, 131.2, 127.2, 126.4, 123.3, 123.1, 121.9, 121.7, 121.5, 120.5, 118.5, 114.3, 112.7, 112.2, 110.3, 101.9;

IR (KBr) cm^{-1}: 3262 (NH), 2200 (CN), 1612 (C=O);

LRMS: (ESI M−1) 310. HRMS: Calcd for $C_{20}H_{13}N_3O$; Exact mass: 311.1059, found (ESI M−H) 310.0987.

4.2.22. (*E*)-2-Cyano-*N*-(4-methoxybenzyl)-3-(1*H*-pyrrol-2-yl) acrylamide (27)

Methyl 2-cyanoacetate (**24**) (0.72, 7.28 mmol) was added to a solution of (4-methoxybenzylamine (1.0 g, 7.28 mmol) in MeOH (4 mL). The resultant solution was heated under microwave radiation for 15 min at 200 W and 120 °C. After this period, the reaction was cooled in the freezer for 30 min upon which a crystalline solid was formed. The solid was collected by filtration, washed with ice cold MeOH (2 × 5 mL) and dried under vacuum to afford *N*-(4-methoxybenzyl)propionamide (25); 65%. Next, *N*-(4-methoxybenzyl)propionamide (3.77 mmol) was added to an ethanolic solution (4 mL) of 1*H*-pyrrole-2-carbaldehyde (4.15 mmol) and piperidine (2 drops). This mixture was heated under microwave radiation for 15 min at 200 W and 120 °C. After this period, the solution was cooled and the solvent removed in vacuo to yield a brown crude solid, which was purified by flash chromatography (2:8

EtOAc/Hexanes) to afford (**27**) as a brown solid; 81%; mp 203–204 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 10.94 (br, NH), 8.18 (s, 1H, CH=C), 7.65 (br, NH), 7.33–7.28 (m, 4H, Ar H2 + Ar H6 + Pyr H3 + Pyr H5), 6.87 (d, J = 8.7 Hz, 2H, Ar H3 + Ar H5), 6.43 (m, 1H, Pyr H4), 4.49 (d, J = 6.0 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 160.7, 158.4, 139.6, 131.3, 130.7, 128.4 (2 × Ar), 125.7, 117.1, 116.5, 113.1 (2 × Ar), 111.6, 94.8, 54.0, 42.4;

IR (KBr) cm^{-1}: 3360 (NH), 3234 (NH), 2202 (CN), 1650 (C=O);

LRMS: (ESI M+1) 282. HRMS: Calcd for $C_{16}H_{15}N_3O_2$; Exact mass: 281.1164, found (ESI M+H) 282.1208.

4.2.23. (*E*)-2-Cyano-*N*-(3,4-dichlorobenzyl)-3-(1*H*-pyrrol-2-yl) acrylamide (28)

Synthesized using the general procedure as for (**27**), from *N*-(3,4-dichlorobenzyl)propioamide and 1*H*-pyrrole-2-carbaldehyde to afford (**28**) as a yellow solid; 80%; 234–235 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 11.88 (br, NH), 8.67 (t, J = 5.9 Hz, NH), 8.05 (s, 1H, CH=C), 7.58–7.53 (m, 2H, Ar H5 + Ar H2), 7.30–7.26 (m, 3H, Ar H6 + Pyr H5 + Pyr H3), 6.42–6.40 (m, 1H, Pyr H4), 4.36 (d, J = 5.9 Hz, 2H, CH_2NH);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 161.8, 141.1, 140.5, 140.3, 130.7, 130.4, 129.3, 127.7, 126.5, 126.3, 117.7, 115.5, 112.5, 94.3, 42.0;

IR (KBr) cm^{-1}: 3342 (NH), 3232 (NH), 2209 (CN), 1643 (C=O), 749 (Ar–Cl);

LRMS: (ESI M−1) 318. HRMS: Calcd for $C_{15}H_{11}Cl_2N_3O$; Exact mass: 319.0279, found (ESI M−H) 318.0198.

4.2.24. (*E*)-2-Cyano-3-(furan-2-yl)-*N*-(4-methoxybenzyl)acryl amide (29)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and furan-2-carbaldehyde to afford (**29**) as a orange solid; 67%; 119–120 °C.

^{1}H NMR (Acetone-d$_6$) (300 MHz): δ 8.03 (s, 1H, CH=C), 7.94 (d, J = 1.6 Hz, 1H, Fur H5), 7.86 (br, NH), 7.36 (d, J = 3.6 Hz, 1H, Fur H3), 7.30 (d, J = 8.6 Hz, 2H, Ar H2 + Ar H6), 6.88 (d, J = 8.6 Hz, 2H, Ar H3 + Ar H5), 6.77 (dd, J = 1.6 Hz, 3.6 Hz, 1H, Fur H4), 4.50 (d, J = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 159.7, 158.5, 148.6, 147.4, 135.7, 130.4, 128.5 (2 × Ar), 120.1, 115.3, 113.2 (2 × Ar), 113.0, 100.6, 54.1, 42.4;

IR (KBr) cm^{-1}: 3325 (NH), 2225 (CN), 1659 (C=O);

LRMS: (ESI M+1) 283. HRMS: Calcd for $C_{16}H_{14}N_2O_3$; Exact mass 282.1004, found (ESI M+H) 283.0989.

4.2.25. (*E*)-2-Cyano-*N*-(3,4-dichlorobenzyl)-3-(furan-2-yl)acryl amide (30)

Synthesized using the general procedure as for (**27**), from *N*-(3,4-dichlorobenzyl)propioamide and furan-2-carbaldehyde to afford (**30**) as a light yellow solid; 76%; 198–199 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 8.92 (t, J = 5.9 Hz, NH), 8.12 (d, J = 1.6 Hz, 1H, Fur H5), 7.99 (s, 1H, CH=C), 7.58 (d, J = 8.3 Hz, 1H, Ar H5), 7.55 (d, J = 2.0 Hz, 1H, Ar H1), 7.37 (d, J = 3.6 Hz, 1H, Fur H3), 7.29 (dd, J = 2.0, 8.3 Hz, 1H, Ar H6), 6.81 (dd, J = 1.6, 3.6 Hz, 1H, Fur H4), 4.37 (d, J = 5.9 Hz, 2H, CH_2NH);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 161.0, 148.7, 148.3, 140.1, 136.1, 130.7, 130.4, 129.4, 127.7, 121.8, 116.0, 113.8, 103.4, 100.3, 42.1;

IR (KBr) cm^{-1}: 3358 (NH), 2216 (CN), 1671 (C=O), 757 (Ar–Cl);

LRMS: (ESI M−1) 319. HRMS: Calcd for $C_{15}H_{10}Cl_2N_2O_2$; Exact mass: 320.0119, found (ESI M−H) 319.0039.

4.2.26. (*E*)-2-Cyano-*N*-(4-methoxybenzyl)-3-(5-methylfuran-2-yl)acrylamide (31)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 5-methylfuran-2-carbaldehyde to afford (**31**) as a white solid; 92%; 146–147 °C.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.94 (s, 1H, CH=O), 7.83 (br, NH), 7.32–7.26 (m, 3H, Ar H2 + Ar H6 + Fur H3), 6.88 (d, J = 8.7 Hz, 2H, Ar H3 + Ar H5), 6.43–6.42 (m, 1H), Fur H4), 4.49 (d, J = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3);

^{13}C NMR ($CDCl_3$) (75 MHz): δ 160.0, 158.4, 147.3, 135.4, 130.6, 128.5 (2 × Ar), 122.3, 115.6, 113.1 (2 × Ar), 110.0, 99.9, 98.3, 54.0, 42.5, 12.5;

IR (KBr) cm^{-1}: 3364 (NH), 2214 (CN), 1671 (C=O);

LRMS: (ESI M+1) 297. HRMS: Calcd for $C_{17}H_{16}N_2O_3$; Exact mass: 296.1161, found (ESI M+H) 297.1206.

4.2.27. (*E*)-2-Cyano-3-(5-(hydroxymethyl)furan-2-yl)-*N*-(4-methoxybenzyl)acrylamide (32)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 5-(hydroxymethyl)furan-2-carbaldehyde to afford (**32**) as a white solid; 45%; 130–132 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 7.98 (s, 1H, CH=O), 7.82 (br, NH), 7.33–7.29 (m, 3H, Ar H2 + Ar H6 + Fur H3), 6.88 (d, J = 8.7 Hz, 2H, Ar H3 + Ar H5), 6.62 (d, J = 3.5 Hz, 1H, Fur H4), 4.65 (s, 2H, CH_2OH), 4.50 (br, OH), 4.49 (d, J = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 161.1, 159.8, 158.5, 147.8, 135.7, 130.5, 128.5 (2 × Ar), 121.1, 115.4, 113.2 (2 × Ar), 109.9, 99.6, 56.1, 54.1, 42.6;

IR (KBr) cm^{-1}: 3347 (NH), 3235 (OH), 2215 (CN), 1667 (C=O), 1248 (C–O);

LRMS: (ESI M+1) 313. HRMS: Calcd for $C_{17}H_{16}N_2O_4$; Exact mass: 312.1110, found (ESI M+H) 313.1122.

4.2.28. (*E*)-3-(5-Chlorofuran-2-yl)-2-cyano-*N*-(4-methoxyben zyl)acrylamide (33)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 5-chlorofuran-2-carbaldehyde to afford (**33**) as a yellow solid; 71%; 164–165 °C.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.99 (s, 1H, CH=C), 7.25 (d, J = 8.5 Hz, 2H, Ar H2 + Ar H6), 7.20–7.19 (m, 1H, Fur H3), 6.88 (d, J = 8.5 Hz, 2H, Ar H3 + Ar H6), 6.55 (br, NH), 6.44–6.43 (m, 1H, Fur H4), 4.51 (d, J = 5.6 Hz, 2H, CH_2NH), 3.81 (s, 3H, OCH_3);

^{13}C NMR ($CDCl_3$) (75 MHz): δ 159.3, 158.8, 147.9, 142.5, 135.6, 128.8 (2 × Ar), 128.6, 122.0, 115.8, 113.7 (2 × Ar), 110.1, 99.4, 54.8, 43.5;

IR (KBr) cm^{-1}: 3342 (NH), 2217 (CN), 1666 (C=O), 1251 (C–O);

LRMS: (ESI M+1) 317. HRMS: Calcd for $C_{16}H_{13}ClN_2O_3$; Exact mass: 316.0615, found (ESI M+H) 317.0666.

4.2.29. (*E*)-3-(5-Bromofuran-2-yl)-2-cyano-*N*-(4-methoxyben zyl)acrylamide (34)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 5-bromofuran-2-carbaldehyde to afford (**34**) as a yellow solid; 84%; 160–162 °C.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 8.01 (s, 1H, CH=C), 7.25 (d, J = 8.6 Hz, 2H, Ar H2 + Ar H6), 7.16 (d, J = 3.7 Hz, 1H, Fur H3), 6.89 (d, J = 8.6 Hz, 2H, Ar H3 + Ar H5), 6.58-6.57 (m, 2H, Fur H4 + NH), 4.51 (d, J = 5.7 Hz, 2H, CH_2NH), 3.80 (s, 3H, OCH_3);

^{13}C NMR ($CDCl_3$) (75 MHz): δ 159.3, 158.7, 150.2, 135.5, 129.1, 128.8 (2 × Ar), 128.6, 122.0, 115.7, 115.1, 113.7 (2 × Ar), 99.5, 54.8, 43.5;

IR (KBr) cm^{-1}: 3348 (NH), 2219 (CN), 1672 (C=O), 1247 (C–O), 807 (Ar–Br);

LRMS: (ESI M+1) 361. HRMS: Calcd for: $C_{16}H_{13}BrN_2O_3$; Exact mass: 360.0110, found (ESI M+H) 361.0145.

4.2.30. (*E*)-2-Cyano-*N*-(4-methoxybenzyl)-3-(5-phenylfuran-2-yl)acrylamide (35)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 5-phenylfuran-2-carbaldehyde to afford (**35**) as a yellow solid; 85%; 203–205 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 8.81 (t, J = 5.9 Hz, NH), 7.99 (s, CH═C), 7.90 (d, J = 7.3 Hz, 2H, Ar2 H2 + Ar2 H6), 7.53–7.48 (m, 2H, Ar2 H3 + Ar2 H5), 7.44–7.42 (m, 2H, Ar2 H4 + Fur H3), 7.34–7.33 (m, 1H, Fur H4), 7.23 (d, J = 8.6 Hz, 2H, Ar H2 + Ar H6), 6.88 (d J = 8.6 Hz, 2H, Ar H3 + Ar H5), 4.32 (d, J = 5.9 Hz, 2H, CH_2NH), 3.71 (s, 3H, OCH_3);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 160.9, 158.2, 157.7, 147.7, 135.1, 130.9, 129.5, 129.1 (2 × Ar), 128.7 (2 × Ar), 128.6, 124.7 (2 × Ar), 124.6, 116.6, 113.6 (2 × Ar), 109.6, 99.7, 54.9, 42.6;

IR (KBr) cm^{-1}: 3362 (NH), 2209 (CN), 1666 (C═O), 1607 (Ar), 1247 (C–O);

LRMS: (ESI M+1) 359. HRMS: Calcd for $C_{22}H_{18}N_2O_3$; Exact mass: 358.1317, found (ESI M+H) 359.1489.

4.2.31. (*E*)-3-(4-Bromofuran-2-yl)-2-cyano-*N*-(4-methoxybenzyl)acrylamide (36)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 4-bromofuran-2-carbaldehyde to afford (**36**) as a white solid; 70%; 169–170 °C.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 8.04 (s, 1H, CH═C), 7.68 (s, 1H, Fur H5), 7.26–7.23 (m, 3H, Ar H2 + Ar H6 + Fur H3), 6.88 (d, J = 8.6 Hz, 2H, Ar H3 + Ar H5), 6.59 (br, NH), 4.51 (d, J = 5.7 Hz, 2H, CH_2NH), 3.80 (s, 3H, OCH_3);

^{13}C NMR ($CDCl_3$) (75 MHz): δ 159.0, 158.8, 148.9, 144.8, 136.1, 128.8 (2 × Ar), 128.5, 121.7, 115.6, 113.7 (2 × Ar), 102.6, 101.1, 54.8, 43.6;

IR (KBr) cm^{-1}: 3332 (NH), 2223 (CN), 1660 (C═O), 1254 (C–O), 926 (Ar–Br);

LRMS: (ESI M+1) 361. HRMS: Calcd for $C_{16}H_{13}BrN_2O_3$; Exact mass: 360.0110, found (ESI M+H) 361.0195.

4.2.32. (*E*)-2-Cyano-3-(furan-3-yl)-*N*-(4-methoxybenzyl)acrylamide (37)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and furan-3-carbaldehyde to afford (**37**) as a brown solid; 60%; 122–124 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 8.32 (s, 1H, Fur H2), 8.21 (s, 1H, CH═C), 7.84 (br, NH), 7.78-7.77 (m, 1H, Fur H5), 7.30 (d J = 8.7 Hz, 2H, Ar H2 + Ar H6), 7.23–7.22 (m, 1H, Fur H4), 6.88 (d, J = 8.7 Hz, 2H, Ar H3 + Ar H5), 4.49 (d, J = 6.0 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 159.7, 158.5, 149.4, 145.0, 141.6, 130.4, 128.5 (2 × Ar), 120.4, 115.8, 113.2, (2 × Ar), 107.3, 103.8, 54.1, 42.5;

IR (KBr) cm^{-1}: 3332 (NH), 2213 (CN), 1661 (C═O), 1611 (Ar), 1513 (Ar), 1251 (C–O). LRMS: (ESI M+1) 283;

HRMS: Calcd for Chemical Formula: $C_{16}H_{14}N_2O_3$; Exact mass: 282.1004, found (ESI M+H) 283.1032.

4.2.33. (*E*)-3-(Benzofuran-2-yl)-2-cyano-*N*-(4-methoxybenzyl)acrylamide (38)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and benzofuran-2-carbaldehyde to afford (**38**) as a white solid; 87%; 150–153 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 8.96 (t, J = 5.8 Hz, NH), 8.14 (s, 1H, CH═C), 7.83–7.80 (m, 1H, Ar2 H4), 7.76 (s, 1H, Ar2 H3), 7.68–7.65 (m, 1H, Ar2 H7), 7.54–7.49 (m, 1H, Ar2 H6), 7.38–7.32 (m, 1H, Ar2 H5), 7.25 (d, J = 8.5 Hz, 2H, Ar H2 + Ar H6), 6.88 (d, J = 8.5 Hz, 2H, Ar H3 + Ar H5), 4.34 (d, J = 5.8 Hz, 2H, CH_2NH), 3.72 (s, 3H, OCH_3);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 160.4, 158.2, 155.3, 149.6, 136.3, 130.7, 128.8 (2 × Ar), 128.5, 127.3, 124.1, 123.0, 117.3, 115.7, 113.6 (2 × Ar), 111.6, 104.1, 55.0, 42.6;

IR (KBr) cm^{-1}: 3342 (NH), 2225 (CN), 1672 (C═O), 1513 (Ar), 1251 (C–O);

LRMS: (ESI M+1) 333. HRMS: Calcd for $C_{20}H_{16}N_2O_3$; Exact mass: 322.1161, found (ESI M+H) 333.1231.

4.2.34. (*E*)-2-Cyano-*N*-(4-methoxybenzyl)-3-phenylacrylamide (39)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and benzaldehyde to afford (**39**) as a white solid; 63%; 117–118 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 8.27 (s, 1H, CH═C), 8.00 (m, 32H, Ar2 H2 + Ar2 H6, NH), 7.57 (m, 3H, Ar2 H3 + Ar2 H4 + Ar2 H5), 7.32 (d, J = 8.7 Hz, 2H, Ar H2 + Ar H6), 6.89 (d, J = 8.7 Hz, 2H, Ar H3 + Ar H5), 4.52 (d, J = 5.9 Hz, 2H, CH_2NH), 3.78 (s, 3H, OCH_3);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 159.7, 158.5, 150.7, 131.8 (2 × Ar), 130.9, 130.3, 129.7 (2 × Ar), 128.6 (2 × Ar), 115.6, 113.2 (2 × Ar), 105.4, 104.4, 54.0, 42.6;

IR (KBr) cm^{-1}: 3336 (NH), 2223 (CN), 1663 (C═O), 1259 (C–O);

LRMS: (ESI M+1) 293. HRMS: Calcd for $C_{18}H_{16}N_2O_2$; Exact mass: 292.1212, found (ESI M+H) 293.1215.

4.2.35. (*E*)-2-Cyano-*N*-(4-methoxybenzyl)-3-p-tolylacrylamide (40)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 4-methylbenzaldehyde to afford (**40**) as a yellow solid; 70%; 115–117 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 8.22 (s, 1H, CH═C), 7.90 (d, J = 8.2 Hz, 2H, Ar2 H2 + Ar 2 H6), 7.38 (d, J = 8.2 Hz, 2H, Ar 2 H3 + Ar2 H5), 7.32 (d, J = 8.8 Hz, 2H, Ar H2 + Ar H6), 6.88 (d, J = 8.8 Hz, 2H, Ar H3 + Ar H5), 4.51 (d, J = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 159.9, 158.4, 150.7, 142.8, 130.4, 129.9 (2 × Ar), 129.3 (2 × Ar), 129.1, 128.6 (2 × Ar), 115.7, 113.2 (2 × Ar), 103.9, 54.0, 42.6, 20.2;

IR (KBr) cm^{-1}: 3339 (NH), 2220 (CN), 1662 (C═O), 1259 (C–O);

LRMS: (ESI M+1) 307. HRMS: Calcd for $C_{19}H_{18}N_2O_2$; Exact mass: 306.1368, found (ESI M+H) 307.1435.

4.2.36. (*E*)-3-(4-Chlorophenyl)-2-cyano-*N*-(4-methoxybenzyl)acrylamide (41)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 4-chlorobenzaldehyde to afford (**41**) as a yellow solid; 28%; 134–135 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 8.25 (s, 1H, CH═C), 8.01 (d, J = 8.8 Hz, 2H, Ar2 H2 + Ar2 H6), 7.60 (d, J = 8.8 Hz, 2H, Ar2 H3 + Ar2 H5), 7.31 (d, J = 8.7 Hz, 2H, Ar H2 + Ar H6), 6.89 (d, J = 8.7 Hz, 2H, Ar H3 + Ar H5), 4.51 (d, J = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 159.5, 158.5, 149.3, 137.1, 131.3 (2 × Ar), 130.5, 130.3, 128.8 (2 × Ar), 128.6 (2 × Ar), 115.4, 113.2 (2 × Ar), 106.0, 54.1, 42.6;

IR (KBr) cm^{-1}: 3361 (NH), 2216 (CN), 1664 (C═O), 1252 (C–O), 819 (Ar–Cl);

LRMS: (ESI M+1) 327. HRMS: Calcd for $C_{18}H_{15}ClN_2O_2$; Exact mass: 326.0822, found (ESI M+H) 327.0846.

4.2.37. (*E*)-2-Cyano-3-(4-hydroxyphenyl)-*N*-(4-methoxybenzyl)acrylamide (42)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 4-hydroxybenzaldehyde to afford (**42**) as a light yellow solid; 20%; 197–198 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 8.16 (s, 1H, CH═C), 7.94 (d, J = 8.8 Hz, 2H, Ar H3 + Ar H5), 7.81 (br, NH), 7.31 (d, J = 8.6 Hz, 2H, Ar2 H2 + Ar2 H6), 7.00 (d, J = 8.8 Hz, 2H, Ar H2 + Ar H6), 6.88 (d, J = 8.6 Hz, 2H, Ar2 H3 + Ar2 H5), 4.50 (d, J = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 161.0, 160.2, 150.5, 132.5 (2 × Ar), 130.6, 128.5 (2 × Ar), 123.4, 116.4, 115.6 (2 × Ar), 113.1 (2 × Ar), 102.8, 100.8, 54.0, 42.5;

IR (KBr) cm^{-1}: 3342 (NH), 3153 (OH), 2212 (CN), 1645 (C=O), 1172 (C–O);

LRMS: (ESI M+1) 309. HRMS: Calcd for $C_{18}H_{16}N_2O_3$; Exact mass: 308.3312, found (ESI M+H) 309.1234.

4.2.38. (*E*)-2-Cyano-*N*-(4-methoxyphenyl)-3-(4-methoxybenzyl) acrylamide (43)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 4-methoxybenzaldehyde to afford (**43**) as a yellow solid; 34%; 138–139 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 8.19 (s, 1H, CH=C), 8.01 (d, J = 8.9 Hz, 2H, Ar2 H2 + Ar2 H6), 7.84 (br, NH), 7.31 (d, J = 8.5 Hz, 2H, Ar H2 + Ar H6), 7.10 (d, J = 8.9 Hz, 2H, Ar2 H3 + Ar2 H5), 6.89 (d, J = 8.5 Hz, 2H, Ar H3 + Ar H5), 4.50 (d, J = 6.0 Hz, 2H, CH_2NH), 3.91 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 162.7, 160.2, 158.5, 150.3, 132.1 (2 × Ar), 130.5, 128.5 (2 × Ar), 124.3, 116.5, 114.1 (2 × Ar), 113.1 (2 × Ar), 101.6, 54.6, 54.0, 42.5;

IR (KBr) cm^{-1}: 3420 (NH), 2200 (CN), 1667 (C=O), 1250 (C–O), 1178 (C-O);

LRMS: (ESI M+1) 323. HRMS: Calcd for $C_{19}H_{18}N_2O_3$; Exact mass: 322.1317, found (ESI M+H) 323.1378.

4.2.39. (*E*)-2-Cyano-*N*-(4-methoxybenzyl)-3-(naphthalen-2-yl) acrylamide (44)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 2-naphthaldehyde to afford (**44**) as a yellow solid; 45%; 143–144 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 8.49 (s, 1H CH=C), 8.43 (s, 1H, Ar2 H1), 8.20 (dd, J = 1.7, 8.7 Hz, 1H, Ar2 H8), 8.09–7.99 (m, 4H, Ar2 H5 + Ar2 H4 + Ar2 H6 + NH), 7.70–7.60 (m, 2H, Ar2 H5 + Ar2 H3), 7.34 (d, J = 8.7 Hz, 2H, Ar H2 + Ar H6), 6.90 (d, J = 8.7 Hz, 2H, Ar H3 + Ar H5), 4.54 (d, J = 5.9 Hz, 2H, CH_2NH), 3.78 (s, 3H, OCH_3);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 160.9, 158.2, 150.5, 134.2, 132.6, 132.3, 130.7, 129.4, 128.9 (2 × Ar), 128.8, 128.7, 128.6, 127.7, 127.2, 124.5, 116.4, 113.6 (2 × Ar), 106.1, 55.0, 42.6;

IR (KBr) cm^{-1}: 3371 (NH), 2212 (CN), 1676 (C=O), 1253 (C–O);

LRMS: (ESI M+1) 343. HRMS: Calcd for $C_{22}H_{18}N_2O_2$; Exact mass: 342.1368, found (ESI M+H) 343.1527.

4.2.40. (*E*)-2-Cyano-*N*-(4-methoxybenzyl)-3-(naphthalen-1-yl) acrylamide (45)

Synthesized using the general procedure as for (**27**), from *N*-(4-methoxybenzyl)propioamide and 1-naphthaldehyde to afford (**45**) as a clear oil; 70%.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 9.03 (s, 1H, CH=C), 8.14–8.07 (m, 4H, Ar2 H5 + Ar2 H8 + Ar2 H4 + NH), 8.02–7.99 (m, 1H, Ar2 H2), 7.67–7.58 (m, 3H, Ar2 H3 + Ar H6 + Ar2 H7), 7.37 (d, J = 8.7 Hz, 2H, Ar H2 + Ar H6), 6.90 (d, J = 8.7 Hz, 2H, Ar H3 + Ar H5), 4.57 (d, J = 6.0 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 159.7, 158.6, 148.7, 133.1, 131.6, 130.9, 130.3, 128.7 (2 × Ar), 128.4, 127.0, 126.7, 126.2, 125.3, 124.8, 122.8, 115.3, 113.2 (2 × Ar), 109.3, 54.1, 42.7;

IR (KBr) cm^{-1}: 3357 (NH), 2227 (CN), 1676 (C=O), 1237 (C–O);

LRMS: (ESI M+1) 343. HRMS: Calcd for $C_{22}H_{18}N_2O_2$; Exact mass: 342.1368, found (ESI M+H) 343.1448.

4.2.41. (*E*)-2-Cyano-*N*-(3,4-dichlorobenzyl)-3-phenylacrylamide (46)

Synthesized using the general procedure as for (**27**), from *N*-(3,4-dichlorobenzyl)propioamide and benzaldehyde to afford (**46**) as a yellow solid; 57%; 159–160 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 8.29 (s, 1H, CH=C), 8.22 (br, NH), 8.02–7.99 (m, 2H, Ar2 H2 + Ar2 H6), 7.61–7.51 (m, 5H, Ar2 H3 + Ar2 H4 + Ar2 H5 + Ar H1 + Ar H5), 7.38 (dd, J = 2.0, 8.3 Hz, 1H, Ar H6), 4.60 (d, J = 6.0 Hz, 2H, CH_2NH);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 160.2, 151.2, 139.6, 131.9, 131.7, 131.1, 129.9, 129.8 (2 × Ar), 129.5, 129.3 (2 × Ar), 128.6, 127.3, 115.5, 104.9, 42.1;

IR (KBr) cm^{-1}: 3364 (NH), 2215 (CN), 1678 (C=O), 679 (Ar–Cl);

LRMS: (ESI M−1) 329. HRMS: Calcd for $C_{17}H_{12}Cl_2N_2O$; Exact mass: 330.0327, found (ESI M−H) 329.0273.

4.2.42. (*E*)-2-Cyano-*N*-(3,4-dichlorobenzyl)-3-p-tolylacrylamide (47)

Synthesized using the general procedure as for (**27**), from *N*-(3,4-dichlorobenzyl)propioamide and 4-methylbenzaldehyde to afford (**47**) as a light yellow solid; 62%; 161–162 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 8.99 (t, J = 5.8 Hz, NH), 8.16 (s, 1H, CH=C), 7.85 (d, J = 8.1 Hz, 2H. Ar2 H2 + Ar2 H6), 7.59–7.56 (m, 2H, Ar H5 + Ar H1), 7.36 (d, J = 8.1 Hz, 2H, Ar2 H3 + Ar2 H6), 7.31 (dd, J = 1.9, 8.3 Hz, 1H, Ar H6), 4.40 (d, J = 5.8 Hz, 2H, CH_2NH), 2.37 (s, 3H, OCH_3);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 161.2, 150.9, 143.0, 140.1, 130.8, 130.4, 130.1, 129.8 (2 × Ar), 129.7 (2 × Ar), 129.4, 129.1, 127.8, 116.4, 104.5, 42.1, 21.1;

IR (KBr) cm^{-1}: 3366 (NH), 2217 (CN), 1681 (C=O), 813 (Ar–Cl);

LRMS: (ESI M−1) 343. HRMS: Calcd for $C_{18}H_{14}Cl_2N_2O$; Exact mass: 344.0483, found (ESI M−H) 343.0446.

4.2.43. (*E*)-3-(4-Chlorophenyl)-2-cyano-*N*-(3,4-dichlorobenzyl)acrylamide (48)

Synthesized using the general procedure as for (**27**), from *N*-(3,4-dichlorobenzyl)propioamide and 4-chlorobenzaldehyde to afford (**48**) as a yellow solid; 30%; 156–158 °C.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 8.27 (s, 1H, CH=C), 8.24 (br, NH), 8.02 (d, J = 8.5 Hz, 2H, Ar2 H2 + Ar2 H6), 7.63–7.60 (m, 3H, Ar2 H3 + Ar2 H5 + Ar H5), 7.53 (d, J = 8.3 Hz, 1H, Ar H1), 7.38 (dd, J = 2.0, 8.3 Hz, 1H, Ar H6), 4.60 (d, J = 6.1 Hz, 2H, CH_2NH);

^{13}C NMR (Acetone-d_6) (75 MHz): δ 159.9, 149.7, 139.5, 138.3, 137.2, 131.3 (2 × Ar), 131.1, 130.5, 129.9, 129.3, 128.8, 127.3 (2 × Ar), 124.6, 115.3, 42.2;

IR (KBr) cm^{-1}: 3384 (NH), 2212 (CN), 1673 (C=O);

LRMS: (ESI M−1) 363. HRMS: Calcd for $C_{17}H_{11}Cl_3N_2O$; Exact mass: 363.9937, found (ESI M−H) 363.9459.

4.2.44. (*E*)-2-Cyano-*N*-(3,4-dichlorobenzyl)-3-(4-hydroxyphenyl)acrylamide (49)

Synthesized using the general procedure as for (**27**), from *N*-(3,4-dichlorobenzyl)propioamide and 4-hydroxybenzaldehyde to afford (**49**) as a yellow solid; 61%; 230–232 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 10.57 (br, OH), 8.86 (t, J = 5.8 Hz, NH), 8.08 (s, 1H, CH=C), 7.87 (d, J = 8.7 Hz, 2H, Ar2 H2 + Ar2 H6), 7.58–7.54 (m, 2H, Ar H1 + Ar H5), 7.29 (dd, J = 2.0, 8.3 Hz, 1H, Ar H6), 6.92 (d, J = 8.7 Hz, 2H, Ar H3 + Ar H5), 4.38 (d, J = 5.8 Hz, 2H, CH_2NH);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 161.8, 161.7, 150.8, 140.3, 132.9 (2 × Ar), 130.7, 130.4, 129.4, 129.3, 127.7, 122.8, 117.0, 116.1 (2 × Ar), 100.6, 42.1;

IR (KBr) cm^{-1}: 3350 (NH), 3140 (OH), 2217 (CN), 1642 (C=O), 837 (Ar–Cl);

LRMS: (ESI M−1) 345. HRMS: Calcd for $C_{17}H_{12}Cl_2N_2O_2$; Exact mass: 346.0276, found (ESI M−H) 345.0249.

4.2.45. (*E*)-2-Cyano-*N*-(3,4-dichlorobenzyl)-3-(4-methoxyphenyl)acrylamide (50)

Synthesized using the general procedure as for (**27**), from *N*-(3,4-dichlorobenzyl)propioamide and 4-methoxybenzaldehyde to afford (**50**) as a light yellow solid; 58%; 169–170 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 8.91 (t, J = 5.8 Hz, 1H, NH), 8.14 (s, 1H, CH═C), 7.96 (d, J = 8.9 Hz, 2H, Ar2 H2 + Ar2 H6), 7.59–7.56 (m, 2H, Ar H1 + Ar H5), 7.30 (dd, J = 1.9, 8.2 Hz, 1H, Ar H6), 7.11 (d, J = 8.9 Hz, 2H, Ar2 H3 + Ar2 H5), 4.39 (d, J = 5.8 Hz, 2H, CH_2NH), 3.84 (s, 3H, OCH_3);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 162.6, 161.5, 150.5, 140.2, 132.5 (2 × Ar), 130.7, 130.4, 129.4, 129.4, 127.7, 124.3, 116.8, 114.7 (2 × Ar), 102.1, 55.5, 42.1;

IR (KBr) cm^{-1}: 3368 (NH), 2208 (CN), 1673 (C═O), 1182 (C–O), 832 (Ar–Cl);

LRMS: (ESI M−1) 359. HRMS: Calcd for $C_{18}H_{14}Cl_2N_2O_2$; Exact mass: 360.0432, found (ESI M−H) 359.0397.

4.2.46. (*E*)-2-Cyano-*N*-(3,4-dichlorobenzyl)-3-(naphthalen-2-yl)acrylamide (51)

Synthesized using the general procedure as for (**27**), from *N*-(3,4-dichlorobenzyl)propioamide and 2-naphthaldehyde to afford (**52**) as a light yellow solid; 40%; 180–181 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 9.08 (t, J = 5.6 Hz, NH), 8.45 (s, 1H, CH═C), 8.36 (s, 1H, Ar2 H1), 8.14–7.97 (m, 4H, Ar2 H5 + Ar2 H8 + Ar2 H4 + Ar H5), 7.69-7.58 (m, 4H, Ar2 H6 + Ar2 H7 + Ar2 H3 + Ar H1), 7.33 (dd, J = 1.8, 8.3 Hz, 1H, Ar H6), 4.43 (d, J = 5.6 Hz, 2H, CH_2NH);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 161.2, 151.0, 140.0, 134.3, 132.7, 132.3, 130.8, 130.4, 129.5, 129.4, 129.0, 128.8, 128.6, 127.8, 127.8, 127.7, 127.2, 124.5, 116.4, 105.7, 42.2;

IR (KBr) cm^{-1}: 3370 (NH), 2212 (CN), 1686 (C═O), 1257 (C–O), 739 (Ar–Cl);

RMS: (ESI M−1) 379. HRMS: Calcd for $C_{21}H_{14}Cl_2N_2O$; Exact mass: 380.0483, found (ESI M−H) 379.0541.

4.2.47. (*E*)-2-Cyano-*N*-(3,4-dichlorobenzyl)-3-(naphthalen-1-yl)acrylamide (52)

Synthesized using the general procedure as for (**27**), from *N*-(3,4-dichlorobenzyl)propioamide and 1-naphthaldehyde to afford (**52**) as a light yellow solid; 57%; 173–175 °C.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 9.25 (t, J = 5.7 Hz, NH), 8.90 (s, 1H, CH═C), 8.14–8.02 (m, 4H, Ar2 H5 + Ar2 H4 + Ar2 H8 + Ar2 H2), 7.68–7.59 (m, 5H, Ar2 H3 + Ar2 H6 + Ar2 H7 + Ar H1 + Ar H5), 7.37 (dd, J = 1.7, 8.3 Hz, 1H, Ar H6), 5.73 (d, J = 5.7 Hz, 2H, CH_2NH);

^{13}C NMR (DMSO-d_6) (75 MHz): δ 161.0, 148.9, 140.0, 132.9, 131.9, 130.8, 130.7, 130.4, 129.5, 129.4, 129.2, 128.7, 127.8, 127.5, 127.2, 126.8, 125.4, 123.6, 115.9, 110.3, 42.1;

IR (KBr) cm^{-1}: 3370 (NH), 2214 (CN), 1680 (C═O), 779 (Ar–Cl);

LRMS: (ESI M−1) 379. HRMS: Calcd for $C_{21}H_{14}Cl_2N_2O$; Exact mass: 380.0483, found (ESI M−H) 379.0469.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.10.003.

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3.8 Paper VIII

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Short communication

Cytotoxic 2-phenyacrylnitriles, the importance of the cyanide moiety and discovery of potent broad spectrum cytotoxic agents

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ABSTRACT

We previously reported the discovery of a simple conjugated cyano pharmacophore which had led to the development of (*Z*)-2-(3,4-dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**1**), as a selective inhibitor of oestrogen receptor positive (ER+ve) human breast cancer cell line, MCF-7. Further exploration though modification of the acrylonitrile and aromatic substituents has highlighted key structural components necessary for broad spectrum cytotoxicity. The acrylic acid derivates (*Z*)-2-(3,4-dichlorophenyl)-3-(4-nitrophenyl)acrylic acid (**8**) and (*Z*)-2-(3,4-dichlorophenyl)-3-(4-methoxyphenyl)acrylic acid (**9**) were inactive; confirming the importance of the cyanide moiety. The most potent 2-phenylacrylonitriles synthesized were (*Z*)-2-(3,4-dichlorophenyl)-3-(1*H*-indol-3-yl)acrylonitrile (**3**) and (*Z*)-2-(3,4-dichlorophenyl)-3-(1*H*-indol-5-yl)acrylonitrile (**20**) with an average GI_{50} values of 1.4 and 0.53 μM respectively. Five additional (*Z*)-2-(3,4-dichlorophenyl)-3-(indolyl)acrylonitriles also displayed average GI_{50} values of ≤8.4 μM. In the case of indole **20**, this represents a 32-fold increase in broad spectrum cytotoxicity relative to the lead (**1**).

1. Introduction

In 2010 over 100,000 Australians were diagnosed with cancer and more than 43,000 died of this disease. Without better targeted therapies cancer will remain one of the leading causes of death in Australia [1]. Cancer is a family of diseases, that despite five decades of intense research still affects 1 in 3 people during the course of their lives. Current treatment strategies for cancer have limited efficacy, especially in the common malignancies such as breast, colon and lung cancer. Despite notable successes with new targeted therapies such as kinase inhibitors (particularly with haematologic malignancies), one-half of all cancer patients either, do not respond to therapy or relapse following initial response, and ultimately die from their metastatic disease [2–5]. For these patients the sole hope for survival lies in the development of better anticancer agents, both as targetted growth inhibitors of specific cancer types, e.g. oestrogen positive (ER+ve) breast cancer, but also as broad spectrum agents that are active across a panel of cancer types.

We recently reported a family of 2-phenylacrylonitriles that displayed novel anticancer activity [6]. With these 2-phenylacrylonitriles we developed a basic pharmacophore hypothesis to explain the observed SAR. This pharmacophore described an extended conjugation spanning two terminal aromatic rings with one of these rings containing an electron withdrawing group as important for the maintenance of cytotoxicity. Central to this conjugation was the presence of an acrylonitrile (cyanide) moiety (Fig. 1). Analogues such as **1** displayed high levels of specificity, ~500 fold relative to the other cell lines examined, towards the ER+ve breast cancer cell line MCF-7, but no single analogue displayed high potency against our panel of eleven cancer cell lines against which we routinely screen for cytotoxicity. In this work we further explore the SAR associated with this class of cytotoxic agents seeking to develop broad spectrum cytotoxic agents.

2. Chemistry

Our approach to lead compound development relies on the synthesis of highly focused small compound libraries combined with a robust biological screen. Herein each library was designed in an effort to answer specific questions about our original acrylonitrile pharmacophore and with compounds typically accessed via a facile Knoevenagel condensation between a phenylacrylonitrile and an appropriately substituted aldehyde (Scheme 1). In a typical synthesis 3,4-dichlorophenylacetonitrile was treated with one

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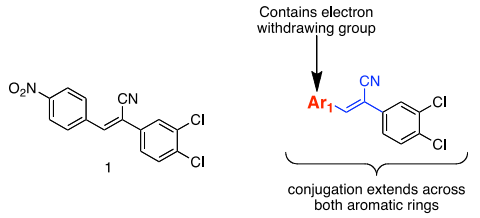


Fig. 1. (*Z*)-2-(3,4-Dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**1**), a 0.127 ± 0.043 μM growth inhibitor of the oestrogen receptor positive (ER+ve) human breast cancer cell line, MCF-7 [6].

equivalent of an aromatic aldehyde in water with added 40% $PhCH_2NMe_3(OH)$ at 50 °C for 5 h, after which time the desired product was collected either by filtration and recrystallization or by extractive work up and flash chromatography (Scheme 1) [6,8–21]. *Library A* modified the Ar_1 moiety and explored the effect of the electron-withdrawing moiety in the second aromatic moiety, retaining the 3,4-dichlorophenylacrylo moiety present in **1** (Fig. 1).

Library B was specifically designed to determine the effect of −CN to $-CO_2H$ bioisosteric modification on the observed cytotoxicity of the two most potent compounds (**1** and **7**, Table 1) from our original series of acrylonitrile analogues and was accessed from the Knoevenagel condensation of 3,4-dichlorophenylacetic acid with 4-nitro- and 4-methoxy-benzaldehyde to afford the acrylic acid analogues **8** and **9** respectively.

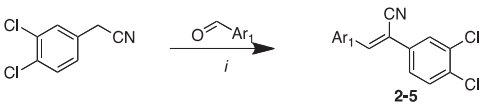
Library C was accessed using the same chemistry as described in Scheme 1 using four furan carboxaldehydes to yield substituted furans **10**–**13** (Table 3).

The final library, *Library D*, retained the 3,4-dichlorophenyl moiety from **1**, but modified the second aromatic ring through reaction with a family of indole carboxaldehydes (Table 4). As before all analogues were accessed in good to excellent yields.

3. Cytotoxicity

With the desired analogues synthesized, we examined their cytotoxicity against a panel of eleven human tumour cell lines: HT29 and SW480 (colon carcinoma), MCF-7 (breast carcinoma), A2780 (ovarian carcinoma), H460 (lung carcinoma), A431 (skin carcinoma), DU145 (prostate carcinoma), BE2-C (neuroblastoma), SJ-G2 (glioblastoma), MIA (pancreatic carcinoma) and SMA (spontaneous murine astrocytoma). All analogues were initially screened at a drug concentration of 25 μM. Those analogues returning good to excellent percentage growth inhibition across all cell lines, or those displaying cell line specificity were subjected to full dose response, GI_{50}, evaluation. These data are shown in Tables 1–4.

As part of our drug development program we do not screen against normal cell lines, as a single *in vitro* assay is a poor measure by which to evaluate risk and predict human toxicity. These approaches fail to provide a "go/no-go" step in the drug development process and when used in a prospective manner, they have not been highly predictive of *in vivo* toxicity [22]. A single *in vitro*



Scheme 1. Reagents and conditions: (i) Ar_1CHO (see Table 1 for details), 40% $PhCH_2NMe_3(OH)$ (cat), H_2O 50 °C 5 h.

screening platform is unlikely to provide the data required to evaluate risk and predict human toxicity. It is widely accepted that additional models using a tiered toxicity screening approach are required in order to define and predict clinical toxicity. As part of our drug development strategy small molecules that satisfy specific druggability characteristics as well as cytotoxicity will undergo further biological evaluation in animal models.

4. Results and discussion

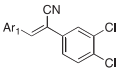
Using an approach of focused library synthesis, biological screening, re-design and library synthesis we commenced our 2-phenylacrylonitrile SAR investigations with the synthesis of a discrete four component library (*Library A*) [6,7]. Initially we sought to confirm the effect of increasing electron-withdrawing groups (EWG) on the first aromatic substituent (Ar_1, Fig. 1). The importance of the EWG was probed via the synthesis of **2**–**5** (Table 1, Scheme 1).

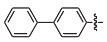
Of the *Library A* analogues, the 4-Ph (**5**) was insoluble in the testing media precluding evaluation. In *Library A* the 4-CF_3 moiety of **2** most closely mimicked the electron withdrawing effects of the 4-NO_2 moiety of **1**, and returned modest levels of growth inhibition with an average GI_{50} ~ 41 μM (c.f. **1**, average GI_{50} = 16.8 μM). The 4-CF_3 (**2**) was equipotent with furan (**4**) (average GI_{50} = 39 μM), but a 3-fold reduction in activity relative to **1**. Indole (**3**) was highly potent displaying excellent broad spectrum growth inhibition, with an average GI_{50} value of 1.4 μM. Indole (**3**) was 12 fold more potent than **1**. Of the analogues shown in Table 1, the 4-OCH_3 (**7**) displayed the highest level of broad spectrum cytotoxicity with an average GI_{50} value of 1.08 μM. The 4-OCH_3 (**7**) was 16 fold more potent than **1**. The indole (**3**), furan (**4**), phenyl (**5**) and 4-OCH_3 (**7**) lack the electron withdrawing capability, but do place a considerable degree of electron density in a similar region to the $-CF_3$ and $-NO_2$ moieties of (**1**) and (**2**) which may account for the observed activity.

While the 4-CF_3 (**2**) was equipotent with the furan (**4**) its activity compared very poorly to both the lead (**1**) and our previously reported 4-F analogue (**6**; average GI_{50} value of 11 μM). The enhanced potency of **7** with the electron donating $-OCH_3$ moiety strongly suggested that the enhanced cytotoxicity was not one purely associated with the EWG effects at C4′, but that there was also possibly a steric component. This was reinforced on examination of the cytotoxicity of (**3**) and (**4**) which despite possessing no Ar_1-EWG returned excellent and modest average GI_{50} values of 1.4 and 36 μM respectively. We note that indole (**3**) was significantly more cytotoxic than furan (**4**). This may be due to the relative orientation of the lone pairs of electrons and/or the increased steric bulk associated with the indole moiety. Regardless, (**3**) and (**4**) showed clear evidence of growth inhibition which is contrary to our initial 2-phenylacrylonitrile hypothesis [6]. From within *Library A* only (**4**) displayed any selectivity towards the MCF-7 breast cancer cell line, and this was a modest 5-fold. Indole (**3**) and 4-OCH_3 (**7**) were the most potent acrylonitriles to emerge from our laboratory thus far, but failed to display any cell line specificity, unlike the lead acrylonitrile (**1**).

To investigate the role of the cyanide moiety within the acrylonitrile unit, two acrylic acid analogues were synthesized (**8** and **9**) from 3,4-dichlorophenylacetic acid and 4-methoxybenzaldehyde and 4-nitrobenzaldehyde (Scheme 2). Cytotoxicity screening showed that neither of the two acrylic acid derivatives (**8**) and (**9**) was active at 25 μM drug concentration (*Library B*, Table 2). This compared very poorly with the corresponding acrylonitrile analogues (**1**) and (**7**) (Table 1) which, against our original panel of 10 human cancer cell lines, returned an average GI_{50} value of 11 and

Table 1
Evaluation of the cytotoxicity, GI_{50} values, of (*Z*)-2-(3,4-dichlorophenyl)-3-(aromatic)acrylonitriles derivatives **2**–**5** (*Library A*), against a panel of eleven cancer cell lines. GI_{50} is the concentration of drug that reduces cell growth by 50%.



Ar_1	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **1**	3.1 ± 1.8	8.4 ± 3.3	0.127 ± 0.04	12 ± 1	69 ± 2	7.1 ± 0.1	18 ± 3	8.9 ± 0.7	14 ± 1	27 ± 4	n.d.[k]
 **2**	35 ± 6	49 ± 13	31 ± 6	41 ± 9	36 ± 1	53 ± 14	45 ± 13	43 ± 9	41 ± 10	35 ± 8	45 ± 13
 **3**	0.85 ± 0.1	2.1 ± 0.1	1.3 ± 0.0	0.90 ± 0.1	1.0 ± 0.1	0.8 ± 0.0	3.1 ± 0.1	1.0 ± 0.1	1.7 ± 0.1	1.6 ± 0.2	1.3 ± 0.2
 **4**	36 ± 0	45 ± 4	7 ± 0	38 ± 2	52 ± 24	39 ± 4	42 ± 6	38 ± 1	50 ± 6	46 ± 6	36 ± 0
 **5**	–	–	–	–	–	–	–	–	–	–	–[l]
 **6**	9.3 ± 2.4	5.6 ± 0.6	6.5 ± 1.0	9.5 ± 1	10 ± 0	10 ± 0	18 ± 2	13 ± 2	16 ± 1	11 ± 1	–6
 **7**	0.52 ± 0.05	1.4 ± 0.1	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	1.4 ± 0.1	2.7 ± 2.0	1.5 ± 0.2	0.7 ± 0.0	–6

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).
[k] n.d. = not determined.
[l] Insoluble in testing media.

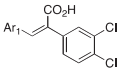
1.1 μM respectively [6]. While it was possible that this reduction in cytotoxicity was merely a reflection of the change in cell permeability, but with other carboxylate containing cytotoxics we have not experienced such changes in cell permeability [23,24]. We believe that these data strongly suggested a pivotal role for the −CN moiety in preventing cell growth.

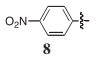
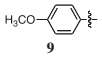
Of the *Library A* active analogues we next examined the effects of subtle modifications of the furan moiety. Synthesis was conducted as per Scheme 1. This new library, *Library C*, comprised four furan analogues (**10**–**13**) and these were screened against our panel of eleven cancer cell lines. These data are presented in Table 3. As can be seen from the data presented, none of these furan analogues (**10**–**13**) returned growth inhibition at 25 μM drug concentration at a level that warranted determination of a full GI_{50} determination. Of the two most active furans (**12** and **13**) the 5-methyl (**13**) is marginally the most potent (Table 3). However the level of activity in *Library C* was not deemed promising enough to warrant the synthesis of additional members, rather our attention turned to the most active compound produced thus far, the indole (**3**).

Taking indole (**3**) as our new lead compound we developed *Library D* in which the 3,4-dichlorophenyl moiety was retained and modifications were made to the indole nucleus. Synthesis was conducted as per Scheme 1 and the results of cytotoxicity screening are presented in Table 4.

With the exception of the 5-cyanoindole (**18**) all *Library D* analogues returned excellent levels of growth inhibition with average GI_{50} values between 0.53 and 8.4 μM. The 2-methylindole (**14**) was marginally more potent (average GI_{50} = 2.9 μM) than the corresponding 5-methylindole (**15**) (average GI_{50} = 4.0 μM), suggesting a positional preference for a small alkyl moiety. Examination of the 5-Cl, 5-Br and 5-CN indoles (**16**–**18**) showed a clear trend of diminishing potency as the size/EWG potential of the 5-moiety increased, with average GI_{50} values of 8.2 and 8.4 μM for (**16**) and (**17**) with (**18**) essentially inactive. Interestingly the benzo[*g*]indole (**19**) which positions the indole substituents at C6/C7, saw a restoration of activity with an average GI_{50} = 5.3 μM, demonstrating that 5-substituents are less well tolerated and like our initial *Library A* EWGs are not as well

Table 2
Evaluation of the cytotoxicity of (*Z*)-2-(3,4-dichlorophenyl)-3-(aromatic)acrylic acid derivatives **8** and **9** against a panel of eleven cancer cell lines. Values are the % growth inhibition measured at 25 μM drug concentration.



Ar_1	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **8**	28 ± 1	<10	11 ± 5	<10	<10	<10	<10	<10	<10	<10	<10
 **9**	11 ± 1	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

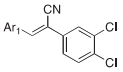
[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).

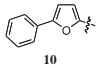
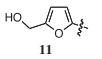
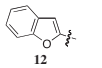
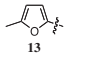
tolerated as our original hypothesis suggested. To further evaluate this we synthesized indole (**20**), (*Z*)-2-(3,4-dichlorophenyl)-3-(1*H*-indol-5-yl)acrylonitrile saw an inversion of the indole moiety relative to lead (**3**), with the addition to the acrylonitrile moiety occurring via C5 of the indole (with **3**, the addition occurs via the indole C3) (Fig. 2). This minor change gave rise to the most potent broad spectrum cytotoxic analogue in the libraries developed to date with an average GI_{50} = 0.53 μM. This is a 32 fold increase in cytotoxicity relative to **1**.

5. Conclusion

In our initial series of 2-phenylacrylonitriles we demonstrated high levels of specificity towards the ER+ve breast cancer cell line,

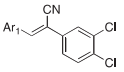
Table 3
Cell death of (*Z*)-2-(3,4-dichlorophenyl)-3-(furanyl)acrylonitriles derivatives **10**–**13** (*Library B*) against a panel of eleven cancer cell lines. Values are the % growth inhibition measured at 25 μM drug concentration.



Ar_1	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **10**	15 ± 2	<10	<10	13 ± 1	11 ± 2	<10	<10	<10	<10	<10	<10
 **11**	41 ± 4	<10	27 ± 4	25 ± 6	30 ± 5	<10	<10	<10	27 ± 1	<10	16 ± 2
 **12**	43 ± 5	43 ± 1	40 ± 2	49 ± 4	36 ± 3	54 ± 5	14 ± 4	20 ± 8	46 ± 24	47 ± 1	55 ± 18
 **13**	58 ± 3	47 ± 1	68 ± 0	43 ± 3	40 ± 2	46 ± 5	<10	50 ± 2	32 ± 2	36 ± 1	33 ± 3

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).

Table 4
Evaluation of the cytotoxicity, GI_{50} values, of (*Z*)-2-(3,4-dichlorophenyl)-3-(indolyl)acrylonitriles derivatives **14**–**20** (*Library D*), against a panel of eleven cancer cell lines. GI_{50} is the concentration of drug that reduces cell growth by 50%. Values in parentheses and italics are the % growth inhibition measured at 25 μM drug concentration.

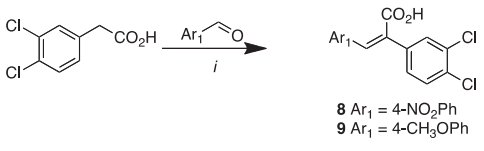


Ar_1	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **14**	1.9 ± 0.06	3.8 ± 0.35	2.8 ± 0.20	2.2 ± 0.37	2.2 ± 0.20	2.1 ± 0.07	5.6 ± 0.61	2.0 ± 0.03	4.0 ± 0.50	2.4 ± 0.22	2.9 ± 0.24
 **15**	2.8 ± 0.26	4.7 ± 0.58	3.7 ± 0.23	3.2 ± 0.20	3.0 ± 0.12	3.0 ± 0.10	7.4 ± 1.03	3.4 ± 0.23	5.3 ± 0.58	3.7 ± 0.09	4.1 ± 0.27
 **16**	7.1 ± 0.19	8.8 ± 1.33	2.3 ± 1.46	7.4 ± 0.49	8.7 ± 0.73	7.7 ± 0.20	12 ± 0.50	8.6 ± 0.69	9.7 ± 0.88	11 ± 0.17	6.7 ± 0.15
 **17**	7.0 ± 0.12	8.2 ± 1.01	6.9 ± 0.94	6.8 ± 0.68	7.5 ± 0.29	7.3 ± 0.38	13 ± 1.00	7.0 ± 0.52	10 ± 0.29	9.0 ± 0.52	9.2 ± 0.73
 **18**	(*57 ± 5*)	(*43 ± 2*)	(*38 ± 8*)	(*47 ± 4*)	(*33 ± 3*)	(*34 ± 2*)	(*18 ± 7*)	(*50 ± 5*)	(*44 ± 2*)	(*43 ± 2*)	(*44 ± 7*)
 **19**	4.2 ± 0.40	7.5 ± 0.31	4.0 ± 0.15	3.7 ± 0.27	5.0 ± 0.69	3.0 ± 0.50	8.7 ± 1.20	3.8 ± 0.80	6.8 ± 0.72	6.1 ± 0.90	5.9 ± 0.55
 **20**	0.36 ± 0.01	0.67 ± 0.03	0.23 ± 0.02	0.41 ± 0.04	0.39 ± 0.02	0.37 ± 0.08	1.04 ± 0.28	0.33 ± 0.09	0.83 ± 0.18	0.50 ± 0.08	0.74 ± 0.06

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).

MCF-7, but limited broad spectrum efficacy [6]. Preliminary conclusions based on focused libraries developed herein suggest that EWGs on Ar_1 are not pivotal to cytotoxicity in this class of compound. We hypothesize that the presence and positioning of a lone pair of electrons capable of participating in hydrogen bonding interactions may be of importance.

Of particular note were the two indole analogues (**3**) and (**20**) which return average GI_{50} values of 1.4 and 0.53 μM respectively. Indoles (**3**) and (**20**) lack a powerful EWG but are, 12 and 32 fold respectively, more active than the lead (**1**). Moreover we have also demonstrated the pivotal nature of the –CN moiety (of the acrylonitrile) as conversion to the corresponding –COOH (acrylic acid) gave compounds bereft of activity against the eleven human cancer cell lines evaluated. These new indolylacrylonitriles hold considerable promise as broad spectrum anticancer agents and we will report new developments in due course.



Scheme 2. Reagents and conditions: (i) 4-$CH_3OPhCHO$ or 4-NO_2PhCHO, Et_3N, Ac_2O, N_2, 140 °C.

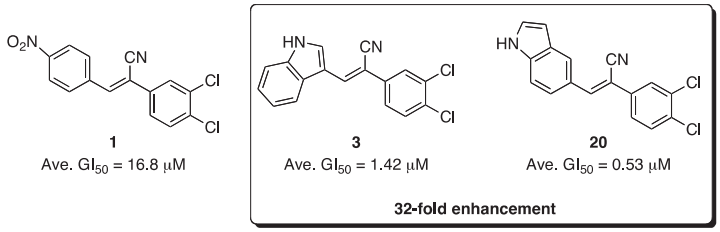


Fig. 2. Chemical structures of the acrylonitrile lead (**1**) and indoles (**3**) and (**20**) and their average GI_{50} values across the cell lines evaluated herein. Simple modifications have affected a 32-fold potency enhancement.

6. Experimental

6.1. Biology

6.1.1. Cell culture and stock solutions

Stock solutions were prepared as follows and stored at −20 °C: drugs were stored as 40 mM solutions in DMSO. All cell lines were cultured at 37 °C, under 5% CO_2 in air and were maintained in Dulbecco's modified Eagle's medium (Trace Biosciences, Australia) supplemented with 10% foetal bovine serum, 10 mM sodium bicarbonate penicillin (100 IU/mL), streptomycin (100 μg/mL), and glutamine (4 mM).

6.1.2. In vitro growth inhibition assay

Cells in logarithmic growth were transferred to 96-well plates. Cytotoxicity was determined by plating cells in duplicate in 100 mL medium at a density of 2500–4000 cells/well. On day 0, (24 h after plating) when the cells were in logarithmic growth, 100 μL medium with or without the test agent was added to each well. After 72 h drug exposure growth inhibitory effects were evaluated using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay and absorbance read at 540 nm. Percentage growth inhibition was determined at a fixed drug concentration of 25 μM. A value of 100% is indicative of total cell growth inhibition. Those analogues showing appreciable percentage growth inhibition underwent further dose response analysis allowing for the calculation of a GI_{50} value. This value is the drug concentration at which cell growth is 50% inhibited based on the difference between the optical density values on day 0 and those at the end of drug exposure [25,26].

6.2. Chemistry

6.2.1. General experimental – general methods

All reagents were purchased from Sigma–Aldrich, Matrix Scientific or Lancaster Synthesis and were used without purification. With the exception of THF (anhydrous > 99%) obtained from Sigma–Aldrich, all solvents were re-distilled from glass prior to use.

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance™ AMX 300 MHz spectrometer at 300.13 and 75.48 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) measured relative to the internal standards, and coupling constants (J) are expressed in Hertz (Hz). Mass spectra were recorded on a Shimadzu LCMS 2010 EV using a mobile phase of 1:1 acetonitrile:H_2O with 0.1% formic acid. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values.

Melting points were recorded on a Stuart Scientific melting point apparatus (UK) and are uncorrected. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} pre-coated aluminium plates with a thickness of 0.2 mm. Column chromatography was performed under 'flash' conditions on Merck silica gel 60 (230–400 mesh) or using the Biotage SP4 flash purification system with a 100 g pre-packed snap column.

A CEM Discover® BenchMate microwave (120 °C, 200 W, 1 h) was used to perform several refluxes.

6.2.2. (Z)-2-(3,4-Dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**1**)

4-Nitrobenzaldehyde (263 mg, 1.74 mmol), was added to a vigorously stirring solution of water (10 mL) and heated to 50 °C. Once the aldehyde was seen to dissolve, 3,4-dichlorophenylacetonitrile (307 mg, 1.65 mmol) was slowly added resulting in the formation of a suspension and the reaction mixture was stirred for a further 10 min. After this time, 40% *N,N,N*-trimethyl(phenyl)methanammonium hydroxide, [$PhCH_2NMe_3$(OH)], in water (7 mL) was added drop-wise to the reaction mixture and once addition was complete, the reaction vessel was sealed and stirring was continued at 50 °C for 5 h. After this period, the solution was filtered hot, washed with warm water and dried under suction to yield a brown solid. The crude solid was subsequently recrystallized from EtOH to afford (**1**) as a purple solid (75%), m.p. 133–134 °C; ^1H NMR ($CDCl_3$): δ 8.34 (d, J = 8.8 Hz, 2H, H-3′ and H-5′), 8.03 (d, J = 8.8 Hz, 2H, H-2′ and H-6′), 7.81–7.80 (m, 1H, H-5), 7.58–7.56 (m, 2H, H-2 and H-6), 7.26 (s, 1H, CH=C); ^{13}C NMR ($CDCl_3$) (75 MHz): δ; 148.6, 140.1, 138.9, 134.7, 133.9, 133.3, 131.3, 130.1, 128.0, 125.4, 124.3, 116.4, 113.7; IR (KBr) ν_{max}/cm^{-1}: 2215 (CN), 1674 (C=C), 1592 (Ar), 1513 (NO), 1345 (NO); m/z (APCI M + H) 289 (NO_2 → NH_2); HRMS (APCI M + H): Calculated for $C_{15}H_{10}Cl_2N_2$; Exact Mass: 288.0221, found: 289.0263. Anal. $C_{15}H_{10}Cl_2N_2$ (C, H, N).

6.2.3. (Z)-2-(3,4-Dichlorophenyl)-3-(4-(trifluoromethyl)phenyl) acrylonitrile (**2**)

Synthesized using the general procedure as for (**1**), from 4-(trifluoromethyl)benzaldehyde and 3,4-dichlorophenylacetonitrile to afford (**2**) as a white solid (89%), m.p. 120–122 °C; ^1H NMR (DMSO-d_6): δ 8.27 (s, 1H, CH=C), 8.09–8.05 (m, 3H, H-2′, H-6′ and H-5), 7.90 (d, J = 8.2 Hz, 2H, H-3′ and H-5′), 7.79–7.70 (m, 2H, H-2 and H-6); ^{13}C NMR (DMSO-d_6): δ 142.9, 137.2, 133.8, 132.8, 132.2, 131.2, 130.0, 129.8 (2× Ar), 127.5, 126.3, 125.8 (2× Ar), 125.7, 116.7, 110.6; IR (KBr) ν_{max}/cm^{-1}: 2215 (CN), 1618 (C=C), 1478 (Ar); m/z (APCI M − H) 341; HRMS (ESI M − H): Calculated for $C_{16}H_8Cl_2F_3N$; Exact Mass: 340.9986, found: 341.0126. Anal. $C_{16}H_8Cl_2F_3N$ (C, H, N).

6.2.4. (Z)-2-(3,4-Dichlorophenyl)-3-(1H-indol-3-yl)acrylonitrile (**3**)

Synthesized using the general procedure as for (**1**), from 1*H*-indole-3-carbaldehyde to afford (**3**) as a yellow solid (48%), m.p. 225–226 °C; ^1H NMR (DMSO-d_6): δ 12.03 (br, NH), 8.39 (s, 1H, CH=C), 8.34 (s, 1H, H-2′), 8.12–8.09 (m, 1H, H-4′), 8.03–8.02 (m, 1H, H-5), 7.70–7.61 (m, 2H, H-5′ and H-6′), 7.52–7.49 (m, 1H, H-7′), 7.26–7.16 (m, 2H, H-2 and H-6); ^{13}C NMR (DMSO-d_6): δ 136.3, 135.7, 135.2,

131.9, 130.9, 129.8, 127.8, 127.2, 126.1, 125.0, 122.8, 120.7, 119.2, 118.9, 112.2, 110.7, 99.6; IR (KBr) ν_{max}/cm^{-1}: 3311 (NH), 2212 (CN), 1628 (C═C), 1574 (Ar), 732 (Ar–Cl); *m/z* (APCI M − H) 311; HRMS (ESI M − H): Calculated for $C_{17}H_{10}Cl_2N_2$; Exact Mass: 312.0221, found: 311.0160. Anal. $C_{17}H_{10}Cl_2N_2$ (C, H, N).

*6.2.5. (Z)-2-(3,4-Dichlorophenyl)-3-(furan-2-yl)acrylonitrile (**4**)*

Synthesized using the general procedure as for (**1**), from furan-2-carbaldehyde to afford (**4**) as a light orange solid (75%), m.p. 126–128 °C; ^{1}H NMR ($CDCl_3$): δ 7.72 (s, 1H, H-5′), 7.63 (s, 1H, CH═C), 7.48 (m, 2H, H-5 and H-2), 7.36 (s, 1H, H-6), 7.23 (d, J = 3.4 Hz, 1H, H-3′), 6.61 (dd, J = 3.2, 1.4 Hz, 1H, H-4′); ^{13}C NMR ($CDCl_3$): δ 149.6, 145.6, 133.7, 133.1, 130.9, 129.2, 128.9, 127.2, 124.7, 117.0, 116.4, 113.0, 105.1; IR (KBr) ν_{max}/cm^{-1}: 2215 (CN), 1617 (C═C), 1466 (Ar); *m/z* (APCI M − H) 363; HRMS (ESI M − H): Calculated for $C_{13}H_7Cl_2NO$; Exact Mass: 262.9905, found: 263.0243. Anal. $C_{13}H_7Cl_2NO$ (C, H, N).

*6.2.6. (Z)-3-(Biphenyl-4-yl)-2-(3,4-dichlorophenyl)acrylonitrile (**5**)*

Synthesized using the general procedure as for (**1**), from biphenyl-4-carbaldehyde to afford (**5**) as a white solid (61%), m.p. 230–232 °C; ^{1}H NMR (DMSO-d_6): δ 8.23 (s, 1H, CH═C), 8.08–8.05 (m, 3H, H-2, H-3′ and H-5′), 7.89 (d, J = 8.2 Hz, 2H, H-2′ and H-6′), 7.81–7.73 (m, 4H, H-2″, H-3″, H-5″ and H-6″), 7.53–7.49 (m, 2H, H-5, H-4″), 7.45–7.42 (m, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 144.6, 143.0, 139.3, 135.0, 132.8, 132.6, 132.2, 131.8, 130.5, 129.6, 128.7, 127.8, 127.6, 127.3, 126.6, 118.0, 107.9; IR (KBr) ν_{max}/cm^{-1}: 2216 (CN), 1593 (Ar), 1474 (Ar), 1448 (Ar); *m/z* (APCI M − H) 349; HRMS (ESI M − H): Calculated for $C_{21}H_{13}Cl_2N$; Exact Mass: 349.0425, found: 349.0397. Anal. $C_{21}H_{13}Cl_2N$ (C, H, N).

*6.2.7. (Z)-2-(3,4-Dichlorophenyl)-3-(4-fluorophenyl) acrylonitrile (**6**)*

Synthesized using the general procedure as for (**1**), from 4-fluorobenzaldehyde and 4-chlorophenyl acetonitrile to afford (**6**) as a white solid (94%), m.p. 156–157 °C (Lit. [4] 155 °C); ^{1}H NMR ($CDCl_3$): δ 7.93–7.88 (m, 2H, H-2′ and H-6′), 7.75–7.74 (m, 1H, H-5), 7.51–7.48 (m, 3H, H-3′, H-5′ and CH═C), 7.20–7.15 (m, 2H, H-2 and H-6); ^{13}C NMR ($CDCl_3$): δ 141.5, 133.7, 133.0 (2× Ar), 132.9, 131.1, 131.0, 130.5, 128.9, 127.1, 124.6 (2× Ar), 116.6, 115.9, 115.7; IR (KBr) ν_{max}/cm^{-1}: 2213 (CN), 1636 (C═C), 1596 (Ar), 809 (Ar–Cl); *m/z* (APCI M − H) 291; HRMS (APCI M − H): Calculated for $C_{15}H_8Cl_2FN$; Exact Mass: 291.0018, found: 291.0029. Anal. $C_{15}H_8Cl_2FN$ (C, H, N).

*6.2.8. (Z)-2-(3,4-Dichlorophenyl)-3-(4-methoxyphenyl) acrylonitrile (**7**)*

Synthesized using the general procedure as for (**1**), from 4-methoxybenzaldehyde and 3,4-dichlorophenylacetonitrile to afford (**7**) as a yellow solid (79%), m.p. 166–167 °C (Lit. [4] 142 °C); ^{1}H NMR (DMSO-d_6): 8.10 (s, 1H, CH═C), 8.00–7.96 (m, 3H, H-5, H-2′ and H-6′), 7.77–7.75 (m, 1H, H-2), 7.70–7.67 (m, 1H, H-6), 7.13 (d, J = 8.9 Hz, 2H, H-3′ and H-5′), 3.85 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6): δ 162.1, 144.8, 135.4, 132.5, 132.0 (2× Ar), 131.7, 131.6, 127.4, 126.3, 126.2, 118.3, 115.1 (2× Ar), 104.9, 55.6; IR (KBr) ν_{max}/cm^{-1}: 2212 (CN), 1638 (C═C), 1609 (Ar), 1593 (Ar), 1513 (Ar); *m/z* (APCI M − H) 303; HRMS (APCI M − H): Calculated for $C_{16}H_{11}Cl_2NO$; Exact Mass: 303.0296, found: 303.0299. Anal. $C_{16}H_{11}Cl_2NO$ (C, H, N).

*6.2.9. (Z)-2-(3,4-Dichlorophenyl)-3-(4-nitrophenyl)acrylic acid (**8**)*

Triethylamine (1.51 g, 15 mmol) was added to a solution of 4-nitrobenzaldehyde (1.51 g, 10 mmol) and 2-(3,4-dichlorophenyl) acetic acid (2.29 g, 11 mmol) in Ac_2O (5 mL). The solution was heated at 140 °C under a nitrogen atmosphere for 1 h. After this period, the solvent was removed *in vacuo* to yield a yellow oil which was purified by flash silica chromatography (1:9 EtOAc:Hexanes) to afford (**8**) as a yellow solid (1.76 g, 52%), m.p. 151–153 °C; ^{1}H NMR (DMSO-d_6): δ 8.15 (d, J = 8.8 Hz, 2H, H-3′ and H-5′), 7.90 (d, J = 8.8 Hz, 2H, H-2′ and H-6′), 7.83 (m, 1H, H-5), 7.61 (m, 2H, H-2 and H-6), 6.73 (s, 1H, CH═C); ^{13}C NMR (DMSO-d_6): δ 170.5, 145.6, 145.2, 143.9, 139.1, 131.0, 130.4, 130.0, 128.9 (2× Ar), 128.0, 126.4, 123.2 (2× Ar), 119.1; IR (KBr) ν_{max}/cm^{-1}: 3347 (OH), 1594 (C═O), 1337 (N–O); *m/z* (APCI M − H) 336; HRMS (ESI M − H): Calculated for $C_{15}H_9Cl_2NO_4$; Exact Mass: 335.9836, found: 335.9919. Anal. $C_{15}H_9Cl_2NO_4$ (C, H, N).

*6.2.10. (Z)-2-(3,4-Dichlorophenyl)-3-(4-methoxyphenyl)acrylic acid (**9**)*

Synthesized using the general procedure as for (**8**), from 4-methoxybenzaldehyde to afford (**9**) as a yellow solid (15%), m.p. 221–223 °C; ^{1}H NMR (DMSO-d_6): δ 7.75 (s, 1H, CH═C), 7.63 (d, J = 8.2 Hz, 1H, H-5), 7.46 (d, J = 2.0 Hz, 1H, H-2), 7.15 (dd, J = 2.0, 8.2 Hz, 1H, H-6), 7.04 (d, J = 8.9 Hz, 2H, H-2′ and H-6′), 6.82 (d, J = 8.9 Hz, 2H, H-3′ and H-5′), 3.71 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6): δ 167.7, 160.1, 139.9, 137.5, 132.1 (2× Ar), 132.0, 131.6, 131.1, 130.7, 130.1, 128.1, 126.1, 114.0 (2× Ar), 55.1; IR (KBr) ν_{max}/cm^{-1}: 3448 (OH), 1663 (C═O), 1172 (C–O); *m/z* (APCI M − H) 321; HRMS (ESI M − H): Calculated for $C_{16}H_{12}Cl_2O_3$; Exact Mass: 321.0091, found: 321.0184. Anal. $C_{16}H_{12}Cl_2O_3$ (C, H, N).

*6.2.11. (Z)-2-(3,4-Dichlorophenyl)-3-(5-phenylfuran-2-yl) acrylonitrile (**10**)*

Synthesized using the general procedure as for (**1**), from 5-phenyl-2-furaldehyde and 3,4-dichlorophenylacetonitrile to afford (**10**) as a yellow solid (94%), m.p. 141–143 °C; ^{1}H NMR (DMSO-d_6): δ 8.00 (s, 1H, CH═C), 7.95–7.94 (m, 1H, H-2″), 7.90–7.87 (m, 2H, H-6″ and H-3″), 7.72–7.62 (m, 2H, H-5″ and H-5), 7.51–7.36 (m, 3H, H-4″, H-2, and H-6), 7.27–7.25 (m, 1H, H-4′), 7.18–7.17 (m, 1H, H-3′); ^{13}C NMR (DMSO-d_6): δ 156.3, 148.6, 134.2, 132.0, 131.1, 131.1, 131.0, 129.0, 128.9, 128.8, 126.6, 125.5, 124.4, 121.6, 117.5, 109.2, 102.2; IR (KBr) ν_{max}/cm^{-1}: 2214 (CN), 1630 (C═C), 1578 (Ar), 1507 (Ar), 1448 (Ar), 798 (Ar–Cl); *m/z* (APCI M − H) 339; HRMS (APCI M − H): Calculated for $C_{19}H_{11}Cl_2NO$; Exact Mass: 339.0218, found: 339.0219. Anal. $C_{19}H_{11}Cl_2NO$ (C, H, N).

*6.2.12. (Z)-2-(3,4-Dichlorophenyl)-3-(5-(hydroxymethyl)furan-2-yl)acrylonitrile (**11**)*

Synthesized using the general procedure as for (**1**), from 5-(hydroxymethyl)-2-furaldehyde and 3,4-dichlorophenylacetonitrile to afford (**11**) as a yellow solid (78%), m.p. 145–147 °C; ^{1}H NMR ($CDCl_3$): δ 7.70–7.69 (m, 1H, H-2), 7.51–7.42 (m, 2H, H-5 and H-6), 7.32 (s, 1H, CH═C), 7.14 (d, J = 3.5 Hz, 1H, H-4′), 6.50 (d, J = 3.5 Hz, 1H, H-3′), 4.71 (s, 2H, CH_2), 2.49 (br, OH); ^{13}C NMR ($CDCl_3$): δ 157.1, 148.8, 133.1, 133.0, 132.5, 130.5, 128.2, 126.6, 124.1, 117.1, 116.6, 110.2, 104.3, 56.9; IR (KBr) ν_{max}/cm^{-1}: 3425 (OH), 2213 (CN), 1625 (C═C), 1513 (Ar), 796 (Ar–Cl); *m/z* (APCI M − H) 293; HRMS (ESI M − H): Calculated for $C_{14}H_9Cl_2NO_2$; Exact Mass: 293.0010, found: 293.0013. Anal. $C_{14}H_9Cl_2NO_2$ (C, H, N).

*6.2.13. (Z)-3-(Benzofuran-2-yl)-2-(3,4-dichlorophenyl) acrylonitrile (**12**)*

Synthesized using the general procedure as for (**1**), from 1-benzofuran-2-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**12**) as a yellow solid (60%), m.p. 203–205 °C; ^{1}H NMR (DMSO-d_6): δ 8.21 (s, 1H, H-3′), 8.06 (s, 1H, CH═C), 7.81–7.64 (m, 4H, H-7′, H-4′, H-2, H-6), 7.55 (s, 1H, H-5), 7.50–7.45 (m, 1H, H-6′), 7.36–7.31 (m, 1H, H-5′); ^{13}C NMR (DMSO-d_6): δ 155.0, 150.7, 133.9, 132.1, 131.9, 131.3, 130.2, 127.7, 127.5, 127.2, 126.0, 124.0, 122.7, 116.7, 114.4, 111.5, 106.5; IR (KBr) ν_{max}/cm^{-1}: 2220 (CN), 1632 (C═C), 1578 (Ar), 1509 (Ar), 790 (Ar–Cl); *m/z* (APCI M − H) 313; HRMS (APCI M − H): Calculated for $C_{17}H_9Cl_2NO$; Exact Mass: 313.0061, found: 313.0064. Anal. $C_{17}H_9Cl_2NO$ (C, H, N).

*6.2.14. (Z)-2-(3,4-Dichlorophenyl)-3-(5-methylfuran-2-yl) acrylonitrile (**13**)*

Synthesized using the general procedure as for (**1**), from 5-methyl-2-furaldehyde and 3,4-dichlorophenylacetonitrile to afford (**13**) as a yellow solid (95%), m.p. 154–156 °C; ^{1}H NMR (acetone-d_6): δ 7.91–7.89 (m, 1H, H-2), 7.77 (s, 1H, CH=C), 7.68–7.67 (m, 2H, H-5 and H-6), 7.15–7.14 (m, 1H, H-3′), 6.39–6.37 (m, 1H, H-4′), 2.42 (s, 3H, CH_3); ^{13}C NMR (acetone-d_6): δ 156.4, 148.2, 134.4, 132.1, 131.1, 130.6, 128.7, 126.3, 124.7, 118.6, 116.5, 109.3, 101.8, 12.4; IR (KBr) ν_{max}/cm^{-1}: 2212 (CN), 1629 (C=C), 1545 (Ar), 1520 (Ar), 785 (Ar–Cl); *m*/*z* (APCI M − H) 277; HRMS (APCI M − H): Calculated for $C_{14}H_9Cl_2NO$; Exact Mass: 277.0061, found: 277.0065. Anal. $C_{14}H_9Cl_2NO$ (C, H, N).

*6.2.15. (Z)-2-(3,4-Dichlorophenyl)-3-(2-methyl-1H-indol-3-yl) acrylonitrile (**14**)*

Synthesized using the general procedure as for (**1**), from 2-methyl-1*H*-indole-3-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**14**) as a yellow solid (78%), m.p. 217–218 °C; ^{1}H NMR (acetone-d_6): δ 11.10 (br, NH), 8.50 (1, 1H, CH=C), 8.31 (s, 1H, H-2), 7.96 (d, J = 2.2 Hz, H-4′), 7.81 (m, 1H, H-4′), 7.72 (dd, J = 8.5, 2.2 Hz, 1H, H-5′), 7.63 (d, J = 8.5 Hz, 1H, H-6′), 7.44 (d, J = 8.3 Hz, 1H, H-5), 7.09 (dd, J = 8.3, 1.2 Hz, 1H, H-6), 2.45 (s, 3H, CH_3); ^{13}C NMR (acetone-d_6): δ 135.3, 135.1, 133.9, 132.0, 130.4, 130.0, 129.8, 127.4, 127.2, 125.9, 124.3, 124.2, 118.6, 117.5, 111.3, 110.4, 99.8, 20.3; IR (KBr) ν_{max}/cm^{-1}: 3333 (NH), 2196 (CN), 1639 (C=C), 1578 (Ar), 1507 (Ar), 790 (Ar–Cl); *m*/*z* (ESI M − H) 325; HRMS (ESI M − H): Calculated for $C_{18}H_{12}Cl_2N_2$; Exact Mass: 326.0378, found: 326.0378. Anal. $C_{18}H_{12}Cl_2N_2$ (C, H, N).

*6.2.16. (Z)-2-(3,4-Dichlorophenyl)-3-(5-methyl-1H-indol-3-yl) acrylonitrile (**15**)*

Synthesized using the general procedure as for (**1**), from 5-methyl-1*H*-indole-3-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**15**) as a yellow solid (51%), m.p. 207–209 °C; ^{1}H NMR (DMSO-d_6): δ 11.96 (br, NH), 8.37 (s, 1H, CH=C), 8.34 (s, 1H, H-2′), 8.07 (m, 1H, H-4′), 7.93 (s, H-2), 7.72–7.66 (m, 2H, H-6′ and H-7′), 7.41 (d, J = 8.3 Hz, 1H, H-5), 7.08 (dd, J = 8.3, 1.0 Hz, 1H, H-6), 2.45 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 136.3, 135.2, 134.1, 131.9, 130.9, 129.8, 129.7, 127.8, 127.5, 126.1, 125.0, 124.5, 119.5, 118.5, 112.0, 110.3, 99.1, 21.3; IR (KBr) ν_{max}/cm^{-1}: 3333 (NH), 2207 (CN), 1633 (C=C), 1577 (Ar), 1507 (Ar), 1471 (Ar), 786 (Ar–Cl); *m*/*z* (ESI M − H) 325; HRMS (ESI M − H): Calculated for $C_{18}H_{12}Cl_2N_2$; Exact Mass: 326.0378, found: 326.0378. Anal. $C_{18}H_{12}Cl_2N_2$ (C, H, N).

*6.2.17. (Z)-3-(5-Chloro-1H-indol-3-yl)-2-(3,4-dichlorophenyl) acrylonitrile (**16**)*

Synthesized using the general procedure as for (**1**), from 5-chloro-1*H*-indole-3-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**16**) as a yellow solid (21%), m.p. 273–274 °C; ^{1}H NMR (DMSO-d_6): δ 12.22 (br, NH), 8.46 (s, 1H, CH=C), 8.40 (s, 1H, H-2′), 8.31 (d, J = 1.8 Hz, 1H, H-2), 8.13 (s, 1H, H-4′), 7.74–7.67 (m, 2H, H-6′ and H-7′), 7.54 (d, J = 8.6 Hz, 1H, H-5), 7.25 (dd, J = 8.6, 1.8 Hz, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 135.8, 134.9, 134.2, 131.9, 131.0, 130.0, 129.0, 128.5, 126.2, 125.8, 125.2, 122.9, 119.1, 118.5, 113.9, 110.4, 100.3; IR (KBr) ν_{max}/cm^{-1}: 3299 (NH), 2207 (CN), 1636 (C=C), 1591 (Ar), 1510 (Ar), 1471 (Ar) 792 (Ar–Cl); *m*/*z* (ESI M − H) 345; HRMS (ESI M − H): Calculated for $C_{17}H_9Cl_3N_2$; Exact Mass: 345.9831, found: 345.9839. Anal. $C_{17}H_9Cl_3N_2$ (C, H, N).

*6.2.18. (Z)-3-(5-Bromo-1H-indol-3-yl)-2-(3,4-dichlorophenyl) acrylonitrile (**17**)*

Synthesized using the general procedure as for (**1**), from 5-bromo-1*H*-indole-3-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**17**) as a yellow solid (30%), m.p. 268–270 °C; ^{1}H NMR (DMSO-d_6): δ 12.14 (br, NH), 8.45–8.34 (m, 3H, CH=C, H-2′, and H-2), 8.12 (s, 1H, H-4′), 7.72–7.69 (m, 2H, H-6′ and H-7′), 7.50 (d, J = 8.6 Hz, 1H, H-5), 7.37 (dd, J = 8.6, 1.8 Hz, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 135.7, 134.9, 134.5, 131.9, 130.9, 130.0, 129.1, 128.8, 126.2, 125.5, 125.2, 121.5, 119.2, 114.3, 113.8, 110.3, 100.4; IR (KBr) ν_{max}/cm^{-1}: 3334 (NH), 2211 (CN), 1654 (C=C), 1592 (Ar); *m*/*z* (ESI M − H) 389; HRMS (ESI M − H): Calculated for $C_{17}H_9BrCl_2N_2$; Exact Mass: 389.9326, found: 389.9396. Anal. $C_{17}H_9BrCl_2N_2$ (C, H, N).

*6.2.19. (Z)-3-(2-Cyano-2-(3,4-dichlorophenyl)vinyl)-1H-indole-5-carbonitrile (**18**)*

Synthesized using the general procedure as for (**1**), from 3-formyl-1*H*-indole-5-carbonitrile and 3,4-dichlorophenylacetonitrile to afford (**18**) as a yellow solid (60%), m.p. >300 °C; ^{1}H NMR (DMSO-d_6): δ 8.73 (s, 1H, CH=C), 8.51 (s, 1H, H-2′), 8.39 (s, 1H, H-2), 8.09 (s, 1H, H-4′), 7.71–7.63 (m, 3H, H-6′, H-7′, and H-5), 7.55 (dd, J = 8.4, 1.3 Hz, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 137.5, 135.1, 134.6, 132.0, 131.0, 130.4, 129.8, 127.1, 126.3, 125.5, 125.3, 124.9, 120.3, 118.9, 113.7, 111.1, 102.9, 101.7; IR (KBr) ν_{max}/cm^{-1}: 3296 (NH), 2222 (CN), 2207 (CN), 1623 (C=C), 1473 (Ar), 809 (Ar–Cl); *m*/*z* (ESI M − H) 336; HRMS (ESI M − H): Calculated for $C_{18}H_9Cl_2N_3$; Exact Mass: 337.0174, found: 337.0223. Anal. $C_{18}H_9Cl_2N_3$ (C, H, N).

*6.2.20. (Z)-3-(1H-Benzo[g]indol-3-yl)-2-(3,4-dichlorophenyl) acrylonitrile (**19**)*

Synthesized using the general procedure as for (**1**), from 1*H*-benzo[g]indole-3-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**19**) as a yellow solid (24%), m.p. 254–256 °C; ^{1}H NMR (DMSO-d_6): δ 8.46–8.39 (m, 3H, CH=C, H-6′, and H-9′), 8.24 (d, J = 8.8 Hz, 1H, H-7′), 8.11 (s, 1H, H-2′), 7.99–7.97 (m, 1H, H-8′), 7.75–7.57 (m, 4H, H-2, H-5, H-4′, and H-5′), 7.50–7.45 (m, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 136.4, 135.0, 132.0, 131.1, 130.6, 130.4, 130.1, 128.4, 126.3, 126.0, 125.3, 125.2, 124.5, 123.6, 121.7, 121.5, 120.6, 119.3, 118.8, 112.4, 100.7; IR (KBr) ν_{max}/cm^{-1}: 3316 (NH), 2196 (CN), 1639 (C=C), 1588 (Ar), 1516 (Ar), 1473 (Ar), 744 (Ar–Cl); *m*/*z* (ESI M − H) 361; HRMS (ESI M − H): Calculated for $C_{21}H_{12}Cl_2N_2$; Exact Mass: 362.0378, found: 362.0399. Anal. $C_{21}H_{12}Cl_2N_2$ (C, H, N).

*6.2.21. (Z)-2-(3,4-Dichlorophenyl)-3-(1H-indol-5-yl)acrylonitrile (**20**)*

Synthesized using the general procedure as for (**1**), from 1*H*-indole-5-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**20**) as a yellow solid (70%), m.p. 185–186 °C; ^{1}H NMR (acetone-d_6): δ 10.7 (br, NH), 8.31 (s, 1H, H-4′), 8.10 (s, 1H, CH=C), 7.96–7.89 (m, 2H, H-6′ and H-7′), 7.75–7.57 (m, 3H, H-2, H-5, and H-6), 7.47 (d, J = 3.2 Hz, 1H, H-2′), 6.63 (d, J = 3.2 Hz, 1H, H-3′); ^{13}C NMR (acetone-d_6): δ 145.7, 135.5, 132.0, 130.9, 130.5, 126.6, 126.1, 124.9, 124.5, 123.2, 122.2, 117.6, 111.4, 111.3, 102.3, 102.2; IR (KBr) ν_{max}/cm^{-1}: 3338 (NH), 2207 (CN), 1653 (C=C), 1569 (Ar), 1474 (Ar); *m*/*z* (ESI M − H) 311; HRMS (ESI M − H): Calculated for $C_{17}H_{10}Cl_2N_2$; Exact Mass: 312.0221, found: 312.0286. Anal. $C_{17}H_{10}Cl_2N_2$ (C, H, N).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.09.019.

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Chapter 4 – Synthesis and Experimental

4.1 General Notes

4.1.1 Reactants, Reagents, Solvents, and Glassware

All reagents were purchased from Sigma-Aldrich with the exception of the following: 2-propanol (Lancaster synthesis), 1-octanol (Fluka), benzaldehyde (Ajax Fine Chemicals), 4-chlorobenzaldehyde (Merck), and 4-methoxybenzaldehyde (Riedel-de Haen). All reagents were used without further purification with the exception of all furan-based reagents, which were freshly distilled prior to use.

THF was purified and dried using an LC Technology Solutions Inc. apparatus. ACN was purchased from Merck. DMF was purchased from Sigma Aldrich. All other solvents were freshly distilled from glass. Glassware was cleaned with water then acetone and oven dried at 130 °C prior to use.

4.1.2 Purification

Filtration was aided with Celite 500 fine where specified. Organic extracts were dried over anhydrous $MgSO_4$. TLC analyses were carried out on precoated silica plates with added fluorescent indicator (Merck 60 F_{254}). Visualisation was achieved by UV light (254 nm) where applicable and/or phosphomolybdic acid dip [2.5% H_2SO_4, 2% $H_3PO_3MoO_4$, 1% $Ce(SO_4)_2$]. Column chromatography was carried out under flash conditions and performed using Grace flash silica with a particle size of 40-63 microns or on a Teledyne Isco CombiFlash®Rf system using Grace Reveleris or Teledyne Isco RediSep®Rf pre-packed flash silica cartridges.

4.1.3 Characterisation and Instrumentation

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR experiments were recorded on a Bruker Avance 300 NMR spectrometer. All spectra were recorded at 20 °C in deuterated solvents; chloroform ($CDCl_3$), acetone (Acetone-d_6), deuterium oxide (D_2O), and dimethylsulfoxide (DMSO-d_6) as specified. Chemical shifts (δ_H and δ_C) are reported in parts per million (ppm) relative to internal standard tetramethylsilane (TMS), and coupling constants are expressed in Hertz (Hz). Splitting patterns in spectra are designated as s (singlet), br (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), sept (septet), and m (multiplet).

Infrared spectra were recorded as potassium bromide (KBr) discs for solids or as thin films on sodium chloride plates for oils on a Perkin Elmer Spectrum BX FT-IR System using Perkin Elmer Spectrum Software version 5.3.1.

Melting points were determined using a Büchi Melting Point M-565 instrument.

Microwave reactions were carried out using a CEM Discover microwave system at atmospheric pressure.

Low resolution mass spectra (LRMS) ionised *via* electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) were recorded on a Shimadzu LCMS-2010 EV instrument in positive mode using a solvent system of 1% formic acid in ACN/water (1:1). All samples were analysed using ESI unless otherwise indicated. Analyses of LRMS were performed on Shimadzu LCMS-solution software.

High resolution mass spectra (HRMS) were obtained from a VG Autospec-oa-tof tandem high resolution mass spectrometer using chemical ionization (CI) with methane as the carrier gas and perfluorokerosene (PFK) as the reference. HRMS samples were analysed either in the Biomolecular Mass Spectrometry Laboratory at the University of Wollongong, Australia or The Children's Medical Research Institute, Cell Signalling Unit Mass Spectrometry Laboratory, Sydney, Australia.

Hydrogenations were performed using a Parr pressure reaction apparatus or a ThalesNano H-cube® flow hydrogenator as described.

4.1.4 Biological testing

Cell culture and stock solutions

Stock solutions were prepared as follows and stored at -20 °C: drugs were prepared as 40 mM solutions in DMSO. All cell lines with the exception of MCF10A were cultured at 37 °C, under 5% CO_2 in air and were maintained in Dulbecco's modified Eagle's medium (Trace Biosciences, Australia) supplemented with 10 % foetal bovine serum, 10 mM sodium bicarbonate penicillin (100 μg/mL), streptomycin (100 μg/mL), and glutamine (4 mM). MCF10A were cultured as above and further supplemented with insulin (2 mg/ml), hydrocortisone (0.25 mg/ml), cholera toxin (1 mg/ml), and epidermal growth factor (100 μg/ml).

In vitro growth inhibition assays

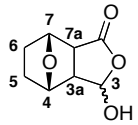
Cells in logarithmic growth were transferred to 96-well plates. Cytotoxicity was determined by plating cells in duplicate in medium (100 mL) at a density of 2500-4000 cells/well. On day zero (24 hours after plating), when the cells were in logarithmic growth, medium (100 mL) with or without the test agent was added to each well. After 72 hours of drug exposure, growth inhibitory effects were evaluated using the MTT (3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay and their absorbance was read at 540 nm. Percentage growth inhibition was determined at a fixed drug concentration of 100 μM. A value of 100% is indicative of total cell growth inhibition. Those analogues showing appreciable percentage growth inhibition underwent further dose response analysis to allow the calculation of GI_{50} values. The GI_{50} value is defined as the drug concentration at which cell growth is 50%

inhibited based on the difference between the optical density values on day zero and those at the end of drug exposure.

4.2 Synthesis of Novo Derivatives

4.2.1 Chain Branching

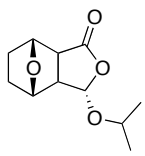
*(3S,3aR,4S,7R,7aS)-3-Hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**1**)*[1]



Furan (34.70 g, 500 mmol) was added to a magnetically stirred solution of maleic anhydride (10.00 g, 100 mmol) in ether (50 mL) and the reaction mixture was stirred at room temperature for 48 hours. The formed white precipitate was collected under suction and washed with ice cold ether (2 x 10 mL) to afford 4,10-dioxatricyclo[5.2.1.0]decane-3,5-dione (11.96 g, 72%) as a white solid which was used directly without further purification; m.p. 113-114 °C (Lit.[1] 120-121 °C).

To a solution of 4,10-dioxatricyclo[5.2.1.0]decane-3,5-dione (4.00 g, 24.1 mmol) in distilled EtOH (50 mL) was added 10% Pd/C (0.40 g) and the reaction mixture was shaken under 4 atms of H_2 at room temperature for 17 hours. After this time, the reaction mixture was filtered through celite and concentrated *in vacuo*, to give an off-white solid that subsequently was recrystallised (EtOH:EtOAc:Hexanes 3:2:1) to afford a white crystalline solid (2.58 g, 63%), m.p. 178-179 °C (Lit.[1] 175-176 °C); δ_H (Acetone d_6) (300 MHz): 5.49 (br, 1H, H-3), 4.71 (s, 1H, H-7), 4.59 (d, *J* = 3.7 Hz, 1H, H-4), 3.30 (br, 1H, OH), 3.02 (d, *J* = 7.6 Hz, 1H, H-7a), 2.37 (d, *J* = 7.6 Hz, 1H, H-3a), 1.60-1.43 (m, 4H, H-5a, H-5b , H-6a and H-6b); δ_C (Acetone d_6) (75 MHz): 175.4, 101.5, 79.6, 78.8, 51.3, 49.8, 27.6, 27.0; IR (KBr) ν_{max}/cm^{-1}: 3266 (OH), 2964 (C-H), 1732 (C=O), 1228 (CO-O), 1124 (C-O); m/z (APCI M-H) 169.

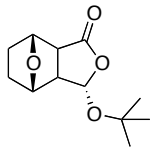
*(3S,3aR,4S,7R,7aS)-3-Isopropoxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**2**)*



2-Propanol (64 mg, 1.07 mmol) was added to a magnetically stirred solution of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**1**) (200 mg, 1.18 mmol) in anhydrous THF (5 mL). A catalytic amount of 4-toluene sulfonic acid (10 mg) was subsequently added and the reaction mixture was treated with microwave radiation at 80 °C for 1 hour at 150 W. Concentration under reduced pressure gave a white residue with was further purified by column chromatography eluting with ethyl acetate and hexane (2:8) to afford (**2**) as a white solid (111 mg, 49%), m.p. 74-76 °C; δ_H ($CDCl_3$) (300 MHz): 5.36 (d, *J* = 1.6 Hz, 1H, H-3), 4.83 (d, *J* = 4.3 Hz, 1H, H-7), 4.68 (d, *J* = 4.3 Hz, 1H, H-4), 3.98 (sept, *J* = 6.2 Hz, 1H, OCH), 2.91 (d, *J* = 8.0 Hz, 1H, H-7a), 2.47 (dd, *J* = 1.6, 8.0 Hz, 1H, H-3a), 1.79-1.75 (m, 2H, H-5a and H-6a), 1.59-1.46 (m, 2H, H-5b and H-6b), 1.22-1.21 (m, 6H, $(CH_3)_2$); δ_C ($CDCl_3$) (75

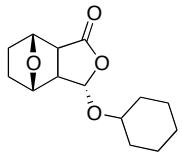
MHz): 175.7, 105.2, 79.7, 79.0, 71.5, 50.8, 50.0, 28.1, 27.6, 22.7, 21.0; IR (KBr) ν_{max}/cm^{-1}: 2964 (C-H), 2984 (C-H), 1732 (C=O), 1124 (C-O); m/z (APCI M+H) 212.

*(3S,3aR,4S,7R,7aS)-3-(tert-Butoxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**3**)*



Synthesized using the general procedure as for (**2**), from 2-methyl-2-propanol and (**1**) to afford (**3**) as a white solid (76%), m.p. 127-128 °C; δ_H ($CDCl_3$) (300 MHz): δ 5.47 (d, *J* = 1.3 Hz, 1H, H-3), 4.83 (d, *J* = 3.9 Hz, 1H, H-7), 4.64 (d, *J* = 3.5 Hz, 1H, H-4), 2.88 (d, *J* = 8.0 Hz, 1H, H-7a), 2.45 (dd, *J* = 1.3, 7.0 Hz, 1H, H-3a), 1.76-1.27 [m, 13H, H-5a, H-5b, H-6a, H-6b and $C(CH_3)_3$]; δ_C ($CDCl_3$) (75 MHz): δ 175.7, 102.2, 79.6, 79.0, 76.9 51.7, 50.0, 28.2, 28.0, 27.1; IR (KBr) ν_{max}/cm^{-1}: 2985 (C-H), 2878 (C-H), 1759 (C=O), 1121 (C-O); m/z (APCI M+H) 227.

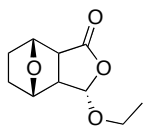
*(3S,3aR,4S,7R,7aS)-3-(Cyclohexyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**4**)*



Synthesized using the general procedure as for (**2**), from cyclohexanol and (**1**) to afford (**4**) as a yellow solid (26%) m.p. 100-102 °C; δ_H ($CDCl_3$) (300 MHz): 5.41 (d, *J* = 1.8 Hz, 1H, H-3), 4.84 (d, *J* = 4.5 Hz, 1H, H-7), 4.68 (d, *J* = 4.5 Hz, 1H, H-4), 3.65 (m, 1H, OCH), 2.92 (d, *J* = 8.0 Hz, 1H, H-7a), 2.49 (dd, *J* = 1.8, 8.0 Hz, 1H, H-3a), 1.85-1.22 (m, 14H, H-5a, H-5b, H-6a, H-6b and $CH_2CH_2CH_2CH_2CH_2$); δ_C ($CDCl_3$) (75 MHz): 175.9, 105.1, 79.7, 79.0, 50.8, 50.0, 32.7, 30.1, 28.1, 27.2, 24.9, 23.3; IR (KBr) ν_{max}/cm^{-1}: 2972 (C-H), 2853 (C-H), 1748 (C=O), 1193 (C-O); m/z (APCI M+H) 253.

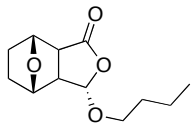
4.2.2 Chain Elongation

*(3S,3aR,4S,7R,7aS)-3-Ethoxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**5**)*[2]



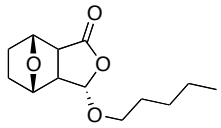
Synthesized using the general procedure as for (**2**), from ethanol and (**1**) to afford (**5**) as a white solid (80%), m.p. 71-72 °C (Lit. not reported); δ_H ($CDCl_3$) (300 MHz): 5.27 (d, *J* = 1.5 Hz, 1H, H-3), 4.84 (d, *J* = 4.7 Hz, 1H, H-7), 4.70 (d, *J* = 4.7 Hz, 1H, H-4), 3.87 (m, 1H, OCH), 3.60 (m, 1H, OCH), 2.91 (d, *J* = 8.0, 1H, H-7a), 2.50 (dd, *J* = 1.5,8.0 Hz, 1H, H-3a), 1.81-1.76 (m, 2H, H-5a and H-6a), 1.60-1.50 (m, 2H, H-5b and H-6b), 1.23 (t, *J*= 7.1 Hz, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz): 175.6, 106.7, 79.7, 79.0, 64.9, 50.5, 49.8, 28.1, 27.3, 14.4; IR (KBr) ν_{max}/cm^{-1}: 2979 (C-H), 2881 (C-H), 1752 (C=O), 1190 (C-O); m/z (APCI M+H) 199.

*(3S,3aR,4S,7R,7aS)-3-Butoxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**6**)*



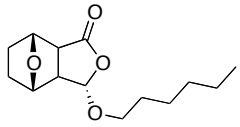
Synthesized using the general procedure as for (**2**), from butanol and (**1**) to afford (**6**) as a yellow solid (23%), m.p. 46-47 °C; δ_H ($CDCl_3$) (300 MHz): 5.26 (d, *J* = 1.7 Hz, 1H, H-3), 4.84 (d, *J* = 4.5 Hz, 1H, H-7), 4.70 (d, *J* = 4.5 Hz, 1H, H-4), 3.80 (m, 1H, OCH), 3.53 (m, 1H, OCH), 2.91 (d, *J* = 8.0 Hz, 1H, H-7a), 2.50 (dd, *J* = 1.7, 8.0 Hz, 1H, H-3a), 1.79-1.33 (m, 8H, H-5a, H-5b, H-6a, H-6b and CH_2CH_2), 0.92 (t, *J* = 7.3Hz, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz): 175.9, 107.0, 79.7, 79.0, 69.2, 50.4, 49.8, 30.9, 28.0, 27.3, 18.6, 13.2; IR (KBr) ν_{max}/cm^{-1}: 2952 (C-H), 2870 (C-H), 1772 (C=O), 1122 (C-O); m/z (APCI M+H) 227.

*(3S,3aR,4S,7R,7aS)-3-(Pentyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**7**)*



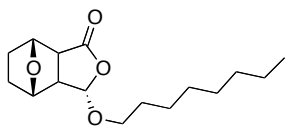
Synthesized using the general procedure as for (**2**), from pentanol and (**1**) to afford (**7**) as a yellow oil (53%); δ_H ($CDCl_3$) (300 MHz): 5.22 (d, *J* = 1.7 Hz, 1H, H-3), 4.79 (d, *J* = 4.6 Hz, 1H, H-7), 4.66 (d, *J* = 4.6 Hz, 1H, H-4), 3.75 (m, 1H, OCH), 3.48 (m, 1H, OCH), 2.88 (d, *J* = 7.9 Hz, 1H, H-7a), 2.46 (dd, *J* = 1.7, 7.9 Hz, 1H, H-3a), 1.75-1.25 (m, 10H, H-5a, H-5b, H-6a, H-6b and $CH_2CH_2CH_2$), 0.86 (t, *J* = 6.3 Hz, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz): 175.6, 106.9, 79.7, 79.0, 69.5, 50.4, 49.8, 28.5, 28.0, 27.5, 27.2, 21.8, 13.4; IR (film) ν_{max}/cm^{-1}: 2938 (C-H), 1777 (C=O), 1122 (C-O); m/z (APCI M+H) 241.

*(3S,3aR,4S,7R,7aS)-3-(Hexyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**8**)*



Synthesized using the general procedure as for (**2**), from hexanol and (**1**) to afford (**8**) as a yellow oil (31%); δ_H ($CDCl_3$) (300 MHz): 5.24 (d, *J* = 1.8 Hz, 1H, H-3), 4.82 (d, *J* = 4.7 Hz, 1H, H-7), 4.68 (d, *J* = 4.7 Hz, 1H, H-4), 3.78 (m, 1H, OCH), 3.50 (m, 1H, OCH), 2.90 (d, *J* = 8.0 Hz, 1H, H-7a), 2.48 (dd, *J* = 1.8, 8.0 Hz, 1H, H-3a), 1.85-1.22 (m, 12H, H-5a, H-5b, H-6a, H-6b and $CH_2CH_2CH_2CH_2$), 0.88 (t, *J* = 6.6 Hz, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz): 175.5, 106.9, 79.7, 79.0, 69.5, 50.4, 49.8, 28.8, 28.0, 27.3, 25.0, 21.9, 20.4, 13.4; IR (film) ν_{max}/cm^{-1}: 2932 (C-H), 1780 (C=O), 1124 (C-O); m/z (APCI M+H) 255.

*(3S,3aR,4S,7R,7aS)-3-(Octyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**9**)*

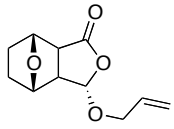


Synthesized using the general procedure as for (**2**), from octanol and (**1**) to afford (**9**) as a yellow oil (26%); δ_H ($CDCl_3$) (300 MHz): 5.25 (d, *J* = 1.7 Hz, 1H, H-3), 4.83 (d, *J* = 4.6 Hz, 1H, H-7), 4.69 (d, *J* = 4.6 Hz, H-4), 3.80 (m, 1H, OCH), 3.51 (m, 1H, OCH), 2.90 (d, *J* = 8.0 Hz, 1H, H-7a), 2.49 (dd, *J* = 1.7, 8.0 Hz, 1H, H-3a), 2.04-1.25 (m, 16H, H-5a, H-5b, H-6a, H-6b

and $CH_2CH_2CH_2CH_2CH_2CH_2$), 0.88 (t, *J* = 6.2 Hz, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz): 175.4, 106.9, 79.7, 79.0, 69.5, 50.4, 49.8, 31.2, 28.8, 28.7, 28.6, 28.0, 27.3, 25.4, 22.1, 13.5; IR (film) ν_{max}/cm^{-1}: 2938 (C-H), 1782 (C=O), 1123 (C-O); m/z (APCI M+H) 283.

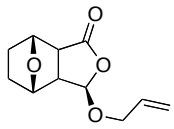
4.2.3 Chain Unsaturation

*(3S,3aR,4S,7R,7aS)-3-(Allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**10**)*



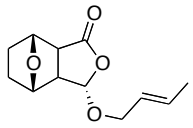
Synthesized using the general procedure as for (**2**), from prop-2-en-1-ol and (**1**) to afford (**10**) as a white solid (26%), m.p. 36-38 °C; δ_H ($CDCl_3$) (300 MHz): 5.89 (m, 1H, CH=), 5.30 (d, *J* = 1.7 Hz, 1H, H-3), 5.28 (m, 2H, $=CH_2$), 4.83 (d, *J* = 4.6 Hz, 1H, H-7), 4.69 (d, *J* = 4.6 Hz, 1H, H-4), 4.30 (m, 1H, OCH), 4.07 (m, 1H, OCH), 2.92 (d, *J* = 8.0 Hz, 1H, H-7a), 2.53 (dd, *J* = 1.7, 8.0 Hz, 1H, H-3a), 1.85-1.46 (m, 4H, H-5a, H-5b, H-6a and H-6b); δ_C ($CDCl_3$) (75 MHz): 175.5, 132.3, 118.0, 105.8, 79.6, 79.0, 69.7, 50.3, 49.7, 28.0, 27.3; IR (KBr) ν_{max}/cm^{-1}: 2988 (C-H), 1747 (C=O), 1638 (C=C), 1150 (C-O); m/z (APCI M+H) 211.

*(3R,3aR,4S,7R,7aS)-3-(Allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**11**)*



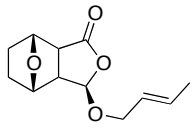
Synthesized using the general procedure as for (**2**), from prop-2-en-1-ol and (**1**) to afford (**11**) as a crystalline white solid (8%), m.p. 100 °C; δ_H ($CDCl_3$) (300 MHz): 5.87-5.60 (m, 1H, CH=), 5.56 (d, *J* = 6.8 Hz, 1H, H-3), 5.37-5.23 (m, 2H, $=CH_2$), 5.13 (d, *J* = 4.8 Hz, 1H, H-7), 4.92 (d, *J* = 4.8 Hz, 1H, H-4), 4.46-4.40 (m, 1H, OCH), 4.17-4.10 (m, 1H, OCH), 2.89 (d, *J* = 8.4 Hz, 1H, H-7a), 2.72-2.68 (m, 1H, H-3a), 1.86-1.71 (m, 2H, H-5a and H-6a), 1.57-1.43 (m, 2H, H-5b and H-6b); δ_C ($CDCl_3$) (75 MHz): 174.8, 132.5, 117.7, 102.4, 78.7, 76.0, 70.8, 51.1, 46.6, 27.6, 27.5; IR (KBr) ν_{max}/cm^{-1}: 2988 (C-H), 1747 (C=O), 1638 (C=C), 1150 (C-O); m/z (APCI M+H) 211.

*(3S,3aR,4S,7R,7aS)-3-(E)-But-2-en-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**12**)*



Synthesized using the general procedure as for (**2**), from (2*E*)-but-2-en-1-ol and (**1**) to afford (**12**) as a crystalline white solid (42%), m.p. 78 °C; δ_H ($CDCl_3$) (300 MHz): 5.78 (dq, *J* = 15.2, 6.4 Hz, 1H, $=CHCH_3$), 5.55 (dt, 15.2, 6.0 Hz, 1H, $CH_2CH=$), 5.30 (d, *J* = 1.1 Hz, 1H, H-3), 4.83 (d, *J* = 4.5 Hz, 1H, H-7), 4.69 (d, *J* = 4.5Hz, 1H, H-4), 4.24 (dd, *J* = 6.0, 0.8 Hz, 1H, OCH), 4.01 (dd, *J* = 7.1, 0.8 Hz, 1H, OCH), 2.91 (d, *J* = 7.9 Hz, 1H, H-7a), 2.50 (dd, *J* = 7.9, 1.1 Hz, 1H, H-3a), 1.80-1.45 (m, 4H, H-5a, H-5b, H-6a and H-6b), 1.73 (d, *J* = 6.4 Hz, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz): 175.5, 131.2, 125.0, 105.5, 79.7, 79.0, 69.6, 50.4, 49.8, 28.0, 27.2, 17.3; IR (KBr) ν_{max}/cm^{-1}: 2985 (C-H), 1752 (C=O), 1617 (C=C), 1153 (C-O); m/z (APCI M+H) 225.

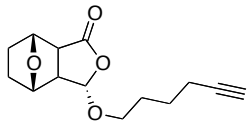
(3R,3aR,4S,7R,7aS)-3-(E)-But-2-en-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one



(13)

Synthesized using the general procedure as for (**2**), from (2*E*)-but-2-en-1-ol and (**1**) to afford (**13**) as a white solid (9%), m.p. 116-118 °C; δ_H ($CDCl_3$) (300 MHz): 5.76 (dq, *J* = 15.3, 6.4 Hz, 1H, =$CHCH_3$), 5.57 (m, 1H, CH_2CH=), 5.56 (d, *J* = 6.8 Hz, 1H, H-3), 5.11 (d, *J* = 4.8 Hz, 1H, H-7), 4.90 (d, *J* = 4.8 Hz, 1H, H-4), 4.35 (m, 1H, OCH), 4.06 (m, 1H, OCH), 2.88 (d, *J* = 8.3 Hz, 1H, H-7a), 2.67 (dd, *J* = 6.8, 8.3 Hz, 1H, H-3a), 1.85-1.38 (m, 4H, H-5a, H-5b, H-6a and H-6b), 1.73 (d, *J* = 6.4 Hz, 3H); δ_C ($CDCl_3$) (75 MHz): 175.0, 130.6, 125.3, 102.1, 78.7, 76.0, 70.6, 51.2, 46.6, 27.6, 27.5, 17.2; IR (KBr) ν_{max}/cm^{-1}: 2985 (C-H), 1752 (C=O), 1617 (C=C), 1153 (C-O); m/z (APCI M+H) 225.

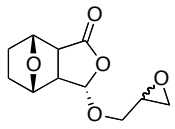
*(3S,3aR,4S,7R,7aS)-3-(Hex-5-yn-1-yloxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**14**)*



Synthesized using the general procedure as for (**2**), from 5-heyn-1-ol and (**1**) to afford (**14**) as a clear oil (80)%; δ_H ($CDCl_3$) (300 MHz): 5.32 (d, *J* = 1.5 Hz, 1H, H-3), 4.73 (d, *J* = 4.2 Hz, 1H, H-7), 4.65 (d, *J* = 4.2 Hz, 1H, H-4), 3.79-3.71 (m, 1H, OCH), 3.62-3.55 (m, 1H, OCH), 3.00 (d, *J* = 7.9 Hz, 1H, H-7a), 2.53 (dd, *J* = 7.9, 1.5 Hz, 1H, H-3a), 2.36-2.31 (m, 1H, CH_2≡), 2.23-2.14 (m, 3H, C≡H, H-5a and H-6a), 2.08-2.02 (m,1H, CH_2≡), 1.74-1.51 (m, 6H, H-5b, H-6b and CH_2CH_2); δ_C ($CDCl_3$) (75 MHz): 175.2, 106.6, 83.2, 79.5, 78.8, 68.7, 68.1, 50.0, 49.2, 27.8, 27.5, 27.0, 24.4, 17.0; IR (film) ν_{max}/cm^{-1}: 3325 (C≡C-H), 2933 (C-H), 2145 (C≡C), 1779 (C=O), 1124 (C-O); m/z (APCI M+H) 251.

4.2.4 Epoxidation

(3S,3aR,4S,7R,7aS)-3-(Oxiran-2-ylmethoxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one



(15a) (15b)

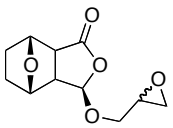
m-Chloroperbenzoic acid (740 mg, 77% in water, 3.31 mmol) was added to a magnetically stirred solution of (3S,3aR,4S,7R,7aS)-3-(allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**10**) (350 mg, 1.66 mmol) in anhydrous DCM (10 mL) at 0 °C. The resulting solution was warmed to room temperature, and stirred for 16 hours before being diluted with DCM (20 mL) and washed with saturated $NaHCO_3$ (3 x 10 mL). The organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The formed clear oil was subjected to silica chromatography (EtOAc:Hexanes 3:7) to afford (**15a**) and (**15b**) as clear oils (1:1) with a combined yield (278 mg, 74%); δ_H ($CDCl_3$) (300 MHz) (**15a**): 5.31 (d, *J* = 1.5 Hz, 1H, H-3),

4.82 (d, *J* = 4.6 Hz, 1H, H-7), 4.70 (d, *J* = 4.6 Hz, 1H, H-4), 4.07 (dd, *J* = 2.7, 11.4 Hz, 1H, OCH), 3.39 (dd, *J* = 7.1, 11.4 Hz, 1H, OCH), 3.16 (m, 1H, CH), 2.91 (d, *J* = 7.9 Hz, 1H, H-7a), 2.82 (m, 1H, H-3a), 2.58 (m, 2H, CH_2-O), 1.82-1.69 (m, 2H, H-5a and H-6a), 1.59-1.46 (m, 2H, H-5b and H-6b); δ_C ($CDCl_3$) (75 MHz) (**15a**): 175.3, 106.6, 79.5, 79.0, 70.5, 50.3, 49.7, 49.6, 43.9, 28.0, 27.2; IR (film) ν_{max}/cm^{-1}: 2992 (C-H), 1742 (C=O), 1230 (C-O), 1150 (C-O); m/z APCI (M+H) 227.

δ_H ($CDCl_3$) (300 MHz) (**15b**): 5.27 (d, *J* = 1.6 Hz, 1H, H-3), 4.80 (d, *J* = 4.5 Hz, 1H, H-7), 4.68 (d, *J* = 4.5 Hz, 1H, H-4), 3.78 (m, 2H, OCH_2), 3.14 (m, 1H, CH), 2.89 (d, *J* = 7.9 Hz, 1H, H-7a), 2.79 (m, 1H, CH_2-O), 2.66 (m, 1H, CH_2-O), 2.52 (dd, *J* = 1.6, 7.9 Hz, 1H, H-3a), 1.79-1.66 (m, 2H. H-5a and H-6a), 1.57-1.43 (m, 2H, H-5b and H-6b); δ_C ($CDCl_3$) (75 MHz) (**15b**): 175.3, 106.6, 79.6, 79.0, 68.5, 50.2, 49.6, 49.3, 43.7, 28.0, 27.2; IR (film) ν_{max}/cm^{-1}: 2992 (C-H), 1742 (C=O), 1230 (C-O), 1150 (C-O); m/z APCI (M+H) 227.

(3R,3aR,4S,7R,7aS)-3-(Oxiran-2-ylmethoxy)hexahydro-4,7-epoxyisobenzofuran-1(3H)-one

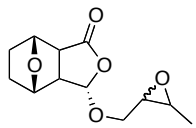
(16a) (16b)



Synthesized using the general procedure as for (**15**), from (3R,3aR,4S,7R,7aS)-3-(allyloxy)hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**11**) to afford (**16a**) and (**16b**) as clear oils (1:1) with a combined yield (68%); δ_H ($CDCl_3$) (300 MHz) (**16a**): 5.59 (d, *J* = 6.8 Hz, 1H, H-3), 5.12 (d, *J* = 4.7 Hz, 1H, H-7), 4.91 (d, *J* = 4.7 Hz, 1H, H-4), 4.12 (dd, *J* = 2.6, 11.4 Hz, 1H, OCH), 3.40 (dd, *J* = 7.4, 11.4 Hz, 1H, OCH), 3.26 (m, 1H, CH), 2.87 (m, 2H, H-7a and CH_2-O), 2.73 (dd, *J* = 6.8, 8.2 Hz, 1H, H-3a), 2.58 (m, 1H, CH_2-O), 1.86-1.71 (m, 2H, H-5a and H-6a), 1.57-1.40 (m, 2H, H-5b and H-6b); δ_C ($CDCl_3$) (75 MHz) (**16a**): 174.7, 103.3, 78.7, 76.9, 76.5, 76.0, 72.0, 51.1, 49.8, 46.6, 43.8, 28.8, 27.6, 27.4; IR (film) ν_{max}/cm^{-1}: 2994 (C-H), 1742 (C=O), 1230 (C-O), 1150 (C-O); m/z (APCI M+H) 227.

δ_H ($CDCl_3$) (300 MHz) (**16b**): 5.52 (d, *J* = 6.8 Hz, 1H, H-3), 5.03 (d, *J* = 3.8 Hz, 1H, H-7), 4.87 (d, *J* = 3.8 Hz, 1H, H-4), 3.90 (m, 2H, OCH_2), 3.18 (m, 1H, CH), 2.75 (m, 4H, H-7a, H-3a and CH_2-O), 1.75-1.69 (m, 2H, H-5a and H-6a), 1.55-1.32 (m, 2H, H-5b and H-6b); δ_C ($CDCl_3$) (75 MHz) (**16b**): 174.7, 103.4, 78.7, 76.0, 68.7, 51.0, 49.4, 46.5, 43.7, 27.5, 27.4; IR (film) ν_{max}/cm^{-1}: 2994 (C-H), 1742 (C=O), 1230 (C-O), 1150 (C-O); m/z (APCI M+H) 227.

(3S,3aR,4S,7R,7aS)-3-[(3-Methyloxiran-2-yl)methoxy]hexahydro-4,7-epoxyisobenzofuran-1(3H)-one (17a) (17b)

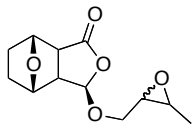


Synthesized using the general procedure as for (**15**), from (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-[(*E*)-but-2-en-1-yloxy]hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**12**) to afford (**17a**) and (**17b**) as clear oils (1:1) with a

combined yield (85%); δ_H ($CDCl_3$) (300 MHz) (**17a**): 5.30 (d, *J* = 1.6 Hz, 1H, H-3), 4.82 (d, *J* = 4.6 Hz, 1H, H-7), 4.70 (d, *J* = 4.6 Hz, 1H, H-4), 4.01 (dd, *J* = 2.9, 11.3 Hz, 1H, OCH), 3.42 (dd, *J* = 6.8, 11.3 Hz, 1H, OCH), 2.89 (m, 3H, H-7a, CH and CH), 2.56 (dd, *J* = 1.6, 7.9 Hz, 1H, H-3a), 1.85-1.70 (m, 2H, H-5a and H-6a), 1.59-1.46 (m, 2H, H-5b and H-6b), 1.32 (d, *J* = 5.0 Hz, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz) (**17a**): 175.4, 106.8, 79.7, 79.0, 70.1, 56.7, 51.9, 50.3, 49.6, 28.0, 27.2, 16.6; IR (film) ν_{max}/cm^{-1}: 2985 (C-H), 1752 (C=O), 1226 (C-O), 1153 (C-O); m/z (APCI M+H) 241.

δ_H ($CDCl_3$) (300 MHz) (**17b**): 5.27 (d, *J* = 1.6 Hz, 1H, H-3), 4.82 (d, *J* = 4.6 Hz, 1H, H-7), 4.70 (d, *J* = 4.6 Hz, 1H, H-4), 3.80 (m, 2H, CH and OCH), 2.93 (m, 3H, OCH, CH and H-7a), 2.52 (dd, *J* = 1.6, 7.9 Hz, 1H, H-3a), 1.84-1.70 (m, 2H, H-5a and H-5b), 1.59-1.45 (m, 2H, H-5b and H-6b), 1.32 (d, *J* = 5.2 Hz, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz) (**17b**): 175.3, 106.6, 79.7, 79.0, 68.1, 56.3, 51.5, 50.2, 49.7, 28.0, 27.2, 16.6; IR (film) ν_{max}/cm^{-1}: 2985 (C-H), 1752 (C=O), 1226 (C-O), 1153 (C-O); m/z (APCI M+H) 241.

(3R,3aR,4S,7R,7aS)-3-[(3-Methyloxiran-2-yl)methoxy]hexahydro-4,7-epoxyisobenzofuran-1(3H)-one ***(18a) (18b)***

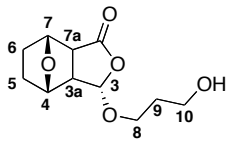


Synthesized using the general procedure as for (**15**), from (3*R*,3a*R*,4*S*,7*R*,7a*S*)-3-[(*E*)-but-2-en-1-yloxy]hexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**13**) to afford (**18a**) and (**18b**) as a clear oils (1:1) with a combined yield (26%); δ_H ($CDCl_3$) (300 MHz) (**18a**): 5.52 (d, *J* = 6.8 Hz, 1H, H-3), 5.05 (d, *J* = 4.4 Hz, 1H, H-7), 4.89 (d, *J* = 4.4 Hz, 1H, H-4), 4.03 (dd, *J* = 3.5, 12.0 Hz, 1H, OCH), 3.84 (dd, *J* = 2.7, 12.0 Hz, 1H, OCH), 3.09 (m, 1H, CH), 2.89 (m, 2H, CH and H-7a), 2.70 (dd, *J* = 6.8, 8.2 Hz, 1H, H-3a), 1.84-1.69 (m, 2H, H-5a and H-6a), 1.56-1.41 (m, 2H, H-5b and H-6b), 1.34 (d, *J* = 5.3 Hz, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz): 174.8, 103.4, 78.7, 76.0, 68.2, 56.5, 51.4, 51.0, 46.6, 27.6, 27.4, 16.7; IR (film) ν_{max}/cm^{-1}: 2985 (C-H), 1752 (C=O), 1226 (C-O), 1153 (C-O); m/z (APCI M+H) 241.

δ_H ($CDCl_3$) (300 MHz) (**18b**): 5.50 (d, *J* = 6.8 Hz, 1H, H-3), 5.05 (d, *J* = 4.4 Hz, 1H, H-7), 4.89 (d, *J* = 4.4 Hz, 1H, H-4), 3.79 (m, 2H, CH and OCH), 2.98 (m, 3H, OCH, CH and H-7a), 2.70 (dd, *J* = 6.8, 8.2 Hz, 1H, H-3a), 1.84-1.68 (m, 2H, H-5a and H-5b), 1.56-1.41 (m, 2H, H-5b and H-6b), 1.34 (d, *J* = 5.2 Hz, 3H, CH_3) δ_C ($CDCl_3$) (75 MHz) (**18b**): 174.7, 103.2, 78.7, 76.1, 66.3, 56.1, 51.1, 51.0, 46.6, 27.6, 27.4, 16.7; IR (film) ν_{max}/cm^{-1}: 2985 (C-H), 1752 (C=O), 1226 (C-O), 1153 (C-O); m/z (APCI M+H) 241.

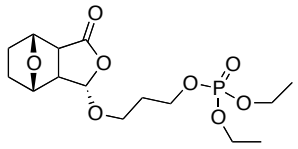
4.2.5 Phosphate Esters

*(3S,3aR,4S,7R,7aS)-3-Propan-3-oloxyhexahydro-4,7-epoxyisobenzofuran-1(3H)-one (**19**)*



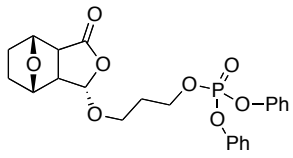
1,3-Propanediol (890 mg, 11.8 mmol) was added to a magnetically stirred solution of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-hydroxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**1**) (200 mg, 1.18 mmol) in anhydrous THF (5 mL). A catalytic amount of 4-toluene sulfonic acid (10mg) was subsequently added and the resulting solution was treated with microwave radiation at 80 °C for 1 hour at 150 W. The resulting solution was subjected to silica chromatography (2:8 EtOAc:Hexane) to afford (**19**) as a clear oil (215 mg, 80%); δ_H ($CDCl_3$) (300 MHz): 5.22 (d, *J* = 1.5 Hz, 1H, H-3), 4.74 (d, *J* = 4.5 Hz, 1H, H-7), 4.64 (d, *J* = 4.5 Hz, 1H, H-4), 3.84 (m, 1H, H-8), 3.64 (m, 3H, H-8 and H_2-10), 2.86 (d, *J* = 7.9 Hz, 1H, H-7a), 2.70 (br, 1H, OH), 2.44 (dd, *J* = 1.5, 7.9 Hz, 1H, H-3a), 1.74 (m, 4H, H-5a, H-6a and H_2-9), 1.48 (m, 2H, H-5b and H-6b); δ_C ($CDCl_3$) (75 MHz): 177.8, 107.1, 79.7, 79.0, 66.6, 58.9, 50.3, 49.8, 31.6, 28.0, 27.2; IR (film) ν_{max}/cm^{-1}: 3535 (O-H), 2943 (C-H), 1773 (C=O), 1039 (C-O); m/z (APCI M+H) 229.

*Phosphoric acid diethyl ester 3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.$0^{2,6}$]dec-3-yloxy)-propyl ester (**20**)*



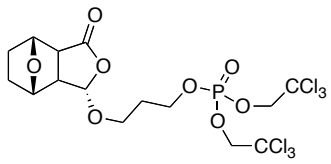
Dibutyltin oxide (47 mg, 0.19 mmol) was added to a magnetically stirred solution of (3*S*,3a*R*,4*S*,7*R*,7a*S*)-3-propan-3-oloxyhexahydro-4,7-epoxyisobenzofuran-1(3*H*)-one (**19**) (216 mg, 0.95 mmol) in anhydrous DCM (10 mL) and stirred for 30 minutes at room temperature under a nitrogen atmosphere. Diethylchlorophosphate (250 mg, 0.95 mmol) and triethylamine (345 mg, 3.41 mmol) were subsequently added and stirring was continued for a further 18 hours at room temperature under a nitrogen atmosphere. After this time, the reaction mixture was quenched with water (15 mL) and the organic layer was collected, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting clear oil was subjected to silica chromatography (EtOAc:Hexanes 1:1) to afford (**20**) as a clear oil (692 mg, 50%); δ_H ($CDCl_3$) (300 MHz): 5.22 (d, *J* = 1.4 Hz, 1H, H-3), 4.78 (d, *J* = 4.3 Hz, 1H, H-7), 4.66 (d, *J* = 4.3 Hz, 1H, H-4), 4.09 (m, 6H, H_2-10, and 2 x CH_2), 3.86 (m, 1H, H-8), 3.62 (m, 1H, H-8), 2.86 (d, *J* = 7.9 Hz, 1H, H-7a), 2.46 (dd, *J* = 1.4, 7.9 Hz, 1H, H-3a), 1.92 (m, 2H, H_2-9), 1.73 (m, 2H, H-5a and H-6a), 1.52 (m, 2H, H-5b and H-6b), 1.30 (t, *J* = 7.1 Hz, 6H, 2 x CH_3); δ_C ($CDCl_3$) (75 MHz): 175.4, 106.9, 79.6, 79.0, 65.1, 63.4, 63.3, 63.2, 50.3, 49.6, 29.6, 28.0, 27.2, 15.6, 15.5; IR (film) ν_{max}/cm^{-1}: 2943 (C-H), 1773 (C=O), 1269 (P=O), 1038 (C-O); m/z (APCI M+H) 365.

*Phosphoric acid 3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0^{2,6}]dec-3-yloxy)-propyl ester diphenyl ester (**21**)*



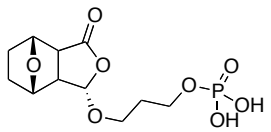
Synthesized using the general procedure as for (**20**), from diphenylchlorophosphate to afford (**21**) as a clear oil (61%); δ_H ($CDCl_3$) (300 MHz): 7.34 (m, 4H, 2x Ar-H3 and 2x Ar-H5), 7.20 (m, 6H, 2 x Ar-H2, 2 x Ar-H4 and 2 x Ar-H6), 5.16 (d, *J* = 1.7 Hz, 1H, H-3), 4.81 (d, *J* = 4.8 Hz, 1H, H-7), 4.60 (d, *J* = 4.8 Hz, 1H, H-4), 4.33 (m, 2H, H_2-10), 3.86 (m, 1H, H-8), 3.59 (m, 1H, H-8), 2.86 (d, *J* = 7.9 Hz, 1H, H-7a), 2.44 (dd, *J* = 1.7, 7.9 Hz, 1H, H-3a), 1.97 (m, 2H, H_2-9), 1.72 (m, 2H, H-5a and H-6a), 1.48 (m, 2H, H-5b and H-6b); δ_C ($CDCl_3$) (75 MHz): 175.4, 150.1, 129.3, 124.9, 119.7, 106.8, 79.6, 79.0, 65.1, 64.8, 50.2, 49.6, 29.1, 28.0, 27.3; IR (film) ν_{max}/cm^{-1}: 2961 (C-H), 1774 (C=O), 1590 (Ar), 1489 (Ar), 1222 (P=O), 1190 (C-O); m/z (ACPI M+H) 461.

*Phosphoric acid 3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0^{2,6}]dec-3-yloxy)-propyl ester bis-(2,2,2-trichloro-ethyl) ester (**22**)*



Synthesized using the general procedure as for (**20**), from bis(2,2,2-trichloroethyl)phosphochloridate to afford (**22**) as a clear oil (78%); δ_H ($CDCl_3$) (300 MHz): 5.28 (d, *J* = 1.7 Hz, 1H, H-3), 4.85 (d, *J* = 4.7 Hz, 1H, H-7), 4.72 (d, *J* = 4.7 Hz, 1H, H-4), 4.66 (m, 4H, 2x CH_2), 4.34 (m, 2H, H_2-10), 3.94 (m, 1H, H-8), 3.71 (m, 1H, H-8), 2.92 (d, *J* = 7.9 Hz, 1H, H-7a), 2.51 (dd, *J* = 1.7, 7.9 Hz, 1H, H-3a), 2.06 (m, 2H, H_2-9), 1.81 (m, 2H, H-5a and H-6a), 1.55 (m, 2H, H-5b and H-6b); δ_C ($CDCl_3$) (75 MHz): 175.9, 107.3, 94.7, 94.6, 80.2, 79.6, 66.1, 66.0, 65.3, 50.9, 50.2, 30.1, 30.0, 28.6, 27.8; IR (film) ν_{max}/cm^{-1}: 2954 (C-H), 1775 (C=O), 1235 (P=O), 918 (C-Cl); m/z (APCI M+H) 571.

*Phosphoric acid mono-[3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0^{2,6}]dec-3-yloxy)-propyl] ester (**23**)*



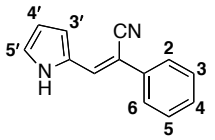
Freshly prepared activated Zn-Cu couple consisting of ~16% Cu (57 mg, 0.88 mmol) and acetylacetone (438 mg, 4.38 mmol) was added to a magnetically stirred solution of phosphoric acid 3-(5-oxo-4,10-dioxa-tricyclo[5.2.1.0^{2,6}]dec-3-yloxy)-propyl ester bis-(2,2,2-trichloro-ethyl) ester (**22**) (250 mg, 0.44 mmol) in anhydrous DMF (3 mL) under a nitrogen atmosphere. The resultant solution was heated for 2 hours at 55 °C during which the red Zn-Cu couple dissolved and turned green. At the conclusion of this period, Chelex resin (10 mL settled volume in 15 mL water: 30 mL MeOH) was added and stirred for a further hour. The reaction was filtered through celite and washed with a small amount of water. The solvent was then removed *in vacuo* to

afford (**23**) as a clear oil (92 mg, 68%); δ_H (D_2O) (300 MHz): 5.49 (d, *J* = 1.6 Hz, 1H, H-3), 4.82 (d, *J* = 4.8 Hz, 1H, H-7), 4.78 (d, *J* = 4.8 Hz, 1H, H-4), 3.86-3.61 (m, 4H, H_2-8 and H_2-10), 3.15 (d, *J* =8.1 Hz, 1H, H-7a), 2.64 (dd, *J* = 1.6, 8.1 Hz, 1H, H-3a), 1.75-1.72 (m, 2H, H_2-9), 1.66-1.49 (m, 4H, H-5a, H-6a H-5b and H-6b); δ_C (D_2O) (75 MHz): 176.5, 106.9, 79.6, 78.4, 65.2, 63.8, 51.3, 49.8, 29.9, 28.7, 27.9; IR (film) ν_{max}/cm^{-1}: 3510 (O-H), 1773 (C=O), 1243 (P=O), 1082 (C-O); m/z (APCI M-2H) 306.

4.3 Synthesis of First Generation Nitrile Derivatives

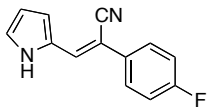
4.3.1 Condensation of 1*H*-pyrrole-2-carbaldehyde and acetonitriles

*(Z)-2-Phenyl-3-(1H-pyrrol-2-yl)acrylonitrile (**24**)*[3]



1*H*-Pyrrole-2-carbaldehyde (165 mg, 1.74 mmol), was added to a vigorously stirring solution of water (10 mL) and heated to 50 °C. Once the aldehyde was seen to dissolve, 2-phenylacetonirile (193 mg, 1.65 mmol) was slowly added resulting in the formation of a suspension and the reaction mixture was stirred for a further 10 minutes. After this time, 40% *N,N,N*-trimethyl(phenyl)methanammonium hydroxide, [$PhCH_2NMe_3(OH)$], in water (7 mL) was added drop-wise to the reaction mixture and once addition was complete, the reaction vessel was sealed and stirring was continued at 50 °C for 5 hours. After this period, the solution was filtered hot, washed with warm water and dried under suction to yield a brown solid. The crude solid was subsequently recrystallised from EtOH to afford (**24**) as a brown crystalline solid (234 mg, 73%), m.p. 94-96 ºC (Lit. not reported); δ_H ($CDCl_3$) (300 MHz): 9.81 (br, 1H, NH), 7.61-7.57 (m, 2H, H-2 and H-6), 7.45-7.40 (m, 2H, H-3 and H-5), 7.42 (s, 1H, HC=C), 7.35-7.30 (m, 1H, H-4), 7.08-7.06 (m, 1H, H-5′), 6.73 (dd, *J* = 1.4, 3.7 Hz, 1H, H-3′), 6.37 (dd, *J* = 1.4, 3.7, 1H, H-4′); δ_C ($CDCl_3$) (75 MHz): 133.4, 130.7, 128.5 (2 x Ar), 127.6, 127.2, 124.4 (2 x Ar), 123.5, 120.1, 118.5, 110.3, 100.8; IR (KBr) ν_{max}/cm^{-1}: 3396 (NH), 2205 (CN), 1683 (C=C), 1601 (Ar), 1589 (Ar), 1496 (Ar); m/z (APCI M+H) 195; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_{10}N_2$; Exact Mass: 195.0922, found: 195.0921.

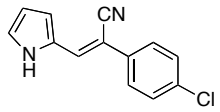
*(Z)-2-(4-Fluorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile (**25**)*[3]



Synthesized using the general procedure as for (**24**), from 1*H*-pyrrole-2-carbaldehyde and 4-fluorophenylacetonitrile to afford (**25**) as a yellow solid (78%), m.p. 115-116 ºC (Lit. not reported); δ_H ($CDCl_3$) (300 MHz): 9.82 (br, 1H, NH), 7.56-7.51 (m, 2H, H-2 and H-6), 7.32 (s, 1H, HC=C), 7.13-7.06 (m, 3H, H-3, H-5 and H-5′), 6.71-6.70 (m, 1H, H-3′), 6.36-6.34 (m, 1H, H-4′); δ_C ($CDCl_3$) (75 MHz): 162.0 (d, *J* = 248.8 Hz, C-F), 130.7, 129.6 (d, *J* = 2.5 Hz, C-F), 127.0, 126.2 (d. *J* =

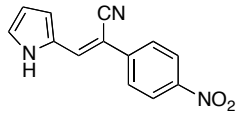
8.1 Hz, C-F), 123.5, 119.9, 118.5, 115.6 (d, *J* = 22.1 Hz, C-F), 110.3, 99.7; IR (KBr) ν_{max}/cm^{-1}: 3401 (NH), 2205 (CN), 1641 (C=C), 1597 (Ar), 1507 (Ar); m/z (APCI M+H) 213; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_9FN_2$; Exact Mass: 213.0828, found: 213.0826.

*(Z)-2-(4-Chlorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile (**26**)*[3]



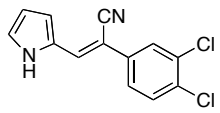
Synthesized using the general procedure as for (**24**), from 1*H*-pyrrole-2-carbaldehyde and 4-chlorophenylacetonitrile to afford (**26**) as a yellow solid (67%), m.p. 112-114 ºC (Lit. not reported); δ_H ($CDCl_3$) (300 MHz): 9.78 (br, 1H, NH), 7.51-7.49 (m, 2H, H-2 and H-6), 7.38-7.35 (m, 3H, H-3, H-5 and HC=C), 7.08 (s, 1H, H-5′), 6.72 (d, *J* = 2.7 Hz, 1H, H-3′), 6.36 (s, 1H, H-4′); δ_C ($CDCl_3$) (75 MHz): 133.4, 131.9, 130.9, 128.7 (2 x Ar), 126.9, 125.6 (2 x Ar), 123.8, 119.7, 118.9, 110.4, 99.5; IR (KBr) ν_{max}/cm^{-1}: 3380 (NH), 2213 (CN), 1636 (C=C), 1603 (Ar), 741 (Ar-Cl); m/z (APCI M+H) 229; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_9ClN_2$; Exact Mass: 229.0533, found: 229.0536.

*(Z)-2-(4-Nitrophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile (**27**)*[3]



Synthesized using the general procedure as for (**24**), from 1*H*-pyrrole-2-carbaldehyde and 4-nitrophenyl acetonitrile to afford (**27**) as a dark green solid (70%), m.p. 130-134 ºC (Lit. not reported); δ_H ($CDCl_3$) (300 MHz): 9.80 (br, 1H, NH), 8.23-8.26 (m, 2H, H-3 and H-5), 7.74-7.70 (m, 2H, H-2 and H-6), 7.56 (s, 1H, HC=C), 7.18-7.17 (m, 1H, H-5′), 6.84 (dd, *J* = 1.3, 3.8 Hz, 1H, H-3′), 6.42 (dd, *J* = 1.3, 3.8 Hz, 1H, H-4′). δ_C ($CDCl_3$) (75 MHz): 133.1, 132.0, 129.3, 126.9, 125.4, 124.7 (2 x Ar), 124.0 (2 x Ar), 123.0 121.0, 119.3, 111.1; IR (KBr) ν_{max}/cm^{-1}: 3398 (NH), 2205 (CN), 1636 (C=C), 1602 (Ar), 1578 (Ar), 1508 (Ar) 1331 (NO); m/z (APCI M+H) 210 ($NO_2 \rightarrow NH_2$); HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_9N_3O_2$; Exact Mass: 240.0775, found: 240.0777.

*(Z)-2-(3,4-Dichlorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile (**28**)*[3]

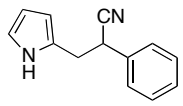


Synthesized using the general procedure as for (**24**), from 1*H*-pyrrole-2-carbaldehyde and 3,4-dichlorophenyl acetonitrile to afford (**28**) as a dark yellow solid (72%), m.p. 140-142 ºC (Lit. not reported); δ_H ($CDCl_3$) (300 MHz): 9.78 (br, 1H, NH), 7.88 (s, 1H, HC=C), 7.78 (d, *J* = 2.1 Hz, 1H, H-5), 7.64-7.56 (m, 2H, H-2 and H-6), 7.26-7.24 (m, 1H, H-5′), 7.21-7.20 (m, 1H, H-3′), 6.39-6.37 (m, 1H, H-4′); δ_C ($CDCl_3$) (75 MHz): 135.0, 132.7, 132.0, 130.5, 130.1, 127.1, 125.7, 124.1, 123.9, 117.8, 114.4, 110.8, 98.3; IR (KBr) ν_{max}/cm^{-1}: 3415

(NH), 2199 (CN), 1636 (C=C), 1604 (Ar), 1588 (Ar); m/z (APCI M+H) 263; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_8Cl_2N_2$; Exact Mass: 263.0144, found: 263.0146.

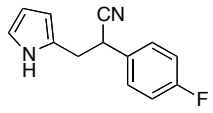
4.3.2 Reduced acrylonitriles

*2-Phenyl-3-(1H-pyrrol-2-yl)propanenitrile (**29**)*



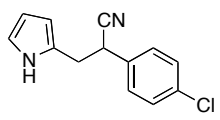
(*Z*)-2-Phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile (**24**) (990 mg, 5.1 mmol) was dissolved into sufficient freshly distilled dry acetone to form a 0.05 M solution. This solution was hydrogenated using the ThalesNano H-cube™ using a 10% Pd/C catalyst at 1 mL/min at 50 ºC and 50 bar H_2 pressure. After completion of the reaction, the solvent was removed *in vacuo* and the resulting crude oil was subjected to flash silica chromatography (1:1 $CHCl_3$:Hexanes) to afford (**29**) as a brown oil (980 mg, 98%); δ_H ($CDCl_3$) (300 MHz): 8.03 (br, 1H, NH), 7.42-7.35 (m, 3H, H-3, H-4 and H-5), 7.29-7.26 (m, 2H, H-2 and H-6), 6.69-6.67 (m, 1H, H-5′), 6.15-6.13 (m, 1H, H-3′), 6.03-6.02 (m, 1H, H-4′), 4.01 (t, J = 7.4 Hz, 1H, CH), 3.28-3.14 (m, 2H, CH_2); δ_C ($CDCl_3$) (75 MHz): 134.5, 128.6 (2 x Ar), 127.8, 126.8, 125.7 (2 x Ar), 120.4, 117.3, 108.1, 107.4, 38.4, 34.0; IR (film) ν_{max}/cm^{-1}: 3384 (NH), 2242 (CN), 1597 (Ar); m/z (APCI M+H) 197; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_{12}N_2$; Exact Mass: 197.1079, found: 197.1082.

*2-(4-Fluorophenyl)-3-(1H-pyrrol-2-yl)propanenitrile (**30**)*



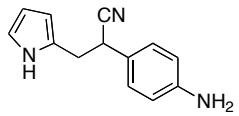
Synthesized using the general procedure as for (**24**), from (Z)-2-(4-fluorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**25**) to afford (**30**) as a light brown oil (95%); δ_H ($CDCl_3$) (300 MHz): 8.06 (br, 1H, NH), 7.24-7.19 (m, 2H, H-2 and H-5), 7.09-7.03 (m, 2H, H-3 and H-5), 6.69 (d, J = 1.4 Hz, 1H, H-5′), 6.15-6.12 (m, 1H, H-3′), 5.98 (s, 1H, H-4′), 4.00 (t, J = 6.8 Hz, 1H, CH), 3.25-3.14 (m, 2H, CH_2); δ_C ($CDCl_3$) (75 MHz): 163.6 (d, J = 246.3 Hz, C-F), 130.2, 128.6 (d, J = 8.3 Hz, C-F), 125.3 (d, J = 2.6 Hz, C-F), 120.2, 117.4, 115.4, 108.2 (d, J = 21.8 Hz), 107.6, 37.6, 34.0; IR (film) ν_{max}/cm^{-1}: 3404 (NH), 2244 (CN), 1602 (Ar), 1509 (Ar); m/z (APCI M+H) 215; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_{11}FN$; Exact Mass: 215.0985, found: 215.0986.

*2-(4-Chlorophenyl)-3-(1H-pyrrol-2-yl)propanenitrile (**31**)*



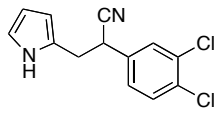
Synthesized using the general procedure as for (**24**), from (*Z*)-2-(4-chlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**26**) to afford (**31**) as a light yellow oil (76%); δ_H ($CDCl_3$) (300 MHz): 8.04 (br, 1H, NH), 7.38-7.32 (m, 2H, H-3 and H-5), 7.19-7.16 (m, 2H, H-2 and H-6), 6.70-6.68 (m, 1H, H-5′), 6.15-6.12 (m, 1H, H-3′), 5.99-5.98 (m, 1H, H-4′), 3.99 (t, *J* = 6.7 Hz, 1H, CH), 3.25-3.12 (m, 2H, CH_2); δ_C ($CDCl_3$) (75 MHz): 132.9, 128.7 (2 x Ar), 128.6, 128.2, 126.8 (2 x Ar), 229.9, 117.4, 108.2, 107.7, 37.8, 33.9; IR (film) ν_{max}/cm^{-1}: 3398 (NH), 2215 (CN), 1598 (Ar), 1511 (Ar); m/z (APCI M+H) 231; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_{11}ClN_2$; Exact Mass: 231.0690, found: 231.0693.

*2-(4-Aminophenyl)-3-(1H-pyrrol-2-yl)propanenitrile (**32**)*



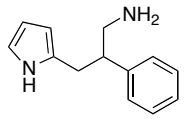
Synthesized using the general procedure as for (**24**), from (Z)-2-(4-nitrophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**27**) to afford (**32**) as a dark brown oil (12%); δ_H ($CDCl_3$) (300 MHz): 7.96 (br, 1H, NH), 7.04-7.01 (m, 2H, H-2 and H-6), 6.68-6.64 (m, 3H, H-3, H-5 and H-5′), 6.14-6.10 (m, 1H, H-3′), 6.01 (s, 1H, H-4′), 3.88 (t, *J* = 7.2 Hz, 1H, CH), 3.75 (br, 2H, NH_2), 3.22-3.09 (m, 2H, CH_2); δ_C ($CDCl_3$) (75 MHz): 145.9, 127.8 (2 x Ar), 126.1, 124.1, 120.8, 117.2, 114.8 (2 x Ar), 107.9, 107.3, 37.6, 34.1; IR (film) ν_{max}/cm^{-1}: 3434 (NH), 3402 (NH), 2235 (CN), 1602 (Ar), 1505 (Ar); m/z (APCI M+H) 212; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_{11}N_3O_2$; Exact Mass: 212.1188, found: 212.1286.

*2-(3,4-Dichlorophenyl)-3-(1H-pyrrol-2-yl)propanenitrile (**33**)*



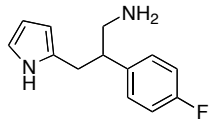
Synthesized using the general procedure as for (**24**), from (Z)-2-(3,4-dichlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**28**) to afford (**33**) as a yellow oil (65%); δ_H ($CDCl_3$) (300 MHz): 8.10 (br, 1H, NH), 7.45-7.33 (m, 2H, H-2 and H-5), 7.07-7.04 (m, 1H, H-6), 6.72-6.70 (m, 1H, H-5′), 6.16-6.13 (m, 1H, H-3′), 5.98 (s, 1H, H-4′), 3.97 (t, *J* = 6.6Hz, 1H, CH), 3.25-3.12 (m, 2H, CH_2); δ_C ($CDCl_3$) (75 MHz): 134.4, 132.7, 132.2, 130.4, 128.8, 126.2, 124.7, 119.4, 117.6, 108.3, 107.9, 37.6, 33.7; IR (film) ν_{max}/cm^{-1}: 3392 (NH), 2221 (CN), 1600 (Ar); m/z (APCI M+H) 267; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_{10}Cl_2N_2$; Exact Mass: 265.0300, found: 265.0303.

*2-Phenyl-3-(1H-pyrrol-2-yl)propan-1-amine (**34**)*



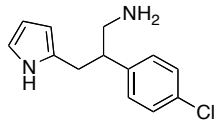
(Z)-2-Phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile (**24**) (894 mg, 4.6 mmol) was dissolved into sufficient 1M NH_3 in MeOH to form a 0.05 M solution. This solution was hydrogenated using the ThalesNano H-cube™ using a Ra/Ni catalyst at 0.5 mL/min flow rate at 70 ºC and 70 bar H_2 pressure. After completion of the reaction, the solvent was removed *in vacuo* and the resulting crude oil subjected to flash silica chromatography (0.05:0.95 MeOH: CH_2Cl_2) to afford (**34**) as a clear oil (920 mg, 100%); δ_H (DMSO-d_6) (300 MHz): 10.53 (br, 1H, NH), 7.31-7.16 (m, 5H, H-2, H-3, H-4, H-5 and H-6), 6.54 (s, 1H, H-5′), 8.54 (s, 1H, H-3′), 5.63 (s, 1H, H-4′), 2.97-2.81 (m, 2H, Pyr-CH_2), 2.78-2.70 (m, 3H, CH, NH_2CH_2), 2.59 (br, 2H, NH_2); δ_C (DMSO-d_6) (75 MHz): 144.1, 130.0, 128.1 (2 x Ar), 127.8 (2 x Ar), 126.0, 115.7, 107.0, 105.2, 48.9, 47.0, 31.4; IR (film) ν_{max}/cm^{-1}: 3363 (NH), 3092 (NH), 2924 (CH), 2851 (CH), 1574 (Ar), 1494 (Ar), 1452 (Ar); m/z (ESI M+H) 201; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{13}H_{16}N_2$; Exact Mass: 201.1392, found: 201.1389.

*2-(4-Fluorophenyl)-3-(1H-pyrrol-2-yl)propan-1-amine (**35**)*



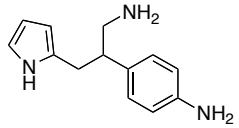
Synthesized using the general procedure as for (**34**), from (Z)-2-(4-fluorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**25**) to afford (**35**) as a clear oil (100%); δ_H ($CDCl_3$) (300 MHz): 8.47 (br, 1H, NH), 7.15-7.10 (m, 2H, H-2 and H-6), 7.04-6.98 (m, 2H, H-3 and H-5), 6.59-6.58 (m, 1H, H-5′), 6.08-6.06 (m, 1H, H-3′), 5.84-5.83 (m, 1H, H-4′), 2.97-2.85 (m, 5H, Pyr-CH_2, CH and NH_2CH_2), 2.41 (br, 2H, NH_2); δ_C ($CDCl_3$) (75 MHz): 162.8 (d, J = 247.6 Hz, C-F), 159.5, 138.0 (d, J = 2.8 Hz, C-F), 129.0 (d, J = 8.2 Hz, C-F), 116.0, 115.0 (d, J = 22.0 Hz, C-F), 107.5, 105.9, 47.9, 46.2, 32.0; IR (film) ν_{max}/cm^{-1}: 3360 (NH), 3248 (NH), 2956 (CH), 1612 (Ar), 1495 (Ar); m/z (ESI M+H) 219; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{13}H_{16}FN_2$; Exact Mass: 219.1298, found: 219.1301.

*2-(4-Chlorophenyl)-3-(1H-pyrrol-2-yl)propan-1-amine (**36**)*



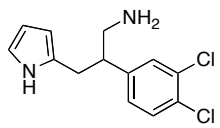
Synthesized using the general procedure as for (**34**), from (*Z*)-2-(4-chlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**26**) to afford (**36**) as a light yellow oil (100%); δ_H (DMSO-d_6) (300 MHz): 10.69 (br, 1H, NH), 7.35-7.22 (m, 4H, H-2, H-3, H-5 and H-6), 6.55 (s, 1H, H-5′), 5.83 (s, 1H, H-3′), 5.63 (m, 3H, H-4′ and NH_2), 3.23-3.15 (m, 1H, CH), 3.00-2.78 (m, 4H, Pyr-CH_2 and NH_2CH_2); δ_C (DMSO-d_6) (75 MHz): 129.8, 129.0, 128.4, 127.8, 126.6, 116.0, 107.1, 105.5, 45.4, 44.2, 31.7; IR (film) ν_{max}/cm^{-1}: 3361 (NH), 2978 (CH), 1495 (Ar), 701 (Ar-Cl); m/z (ESI M+H) 235; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{13}H_{15}ClN_2$; Exact Mass: 235.1003, found: 235.1001.

*4-(1-Amino-3-(1H-pyrrol-2-yl)propan-2-yl)aniline (**37**)*



Synthesized using the general procedure as for (**34**), from (Z)-2-(4-nitrophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**27**) to afford (**37**) as a brown oil (100%); δ_H (DMSO-d_6) (300 MHz): 10.47 (br, 1H, NH), 6.87-6.85 (m, 2H, H-2 and H-6), 6.54-6.44 (m, 4H, H-3, H-5 and Ar-NH_2), 5.83-5.78 (m, 1H, H-5′), 5.61 (s, 1H, H-3′), 5.45 (s, 1H, H-4′), 4.83 (br, 2H, NH_2), 2.84-2.79 (m, 3H, CH and Pyr-CH_2), 2.69-2.58 (m, 2H, NH_2CH_2); δ_C (DMSO-d_6) (75 MHz): 141.9, 126.4 (2 x Ar), 125.3, 122.8, 116.8, 112.1 (2 x Ar), 107.6, 106.8, 47.9, 46.5, 31.2; IR (film) ν_{max}/cm^{-1}: 3353 (NH), 3215 (NH), 2918 (CH), 1615 (Ar), 1519 (Ar); m/z (ESI M+H) 216; HRMS (ESI M+H): Calculated for Chemical Formula:$C_{13}H_{17}N_3$; Exact Mass: 216.1501, found: 216.1499.

*2-(3,4-Dichlorophenyl)-3-(1H-pyrrol-2-yl)propan-1-amine (**38**)*

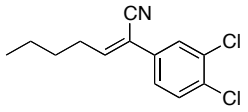


Synthesized using the general procedure as for (**34**), from (Z)-2-(3,4-dichlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**28**) to afford (**38**) as a yellow oil (100%); δ_H (DMSO-d_6) (300 MHz): 10.51 (br, 1H, NH), 7.52-7.43 (m, 2H, H-5 and H-2), 7.21-7.18 (m, 1H, H-6), 6.53 (s, 1H, H-5′), 5.82-5.81 (m, 1H, H-3′), 5.60 (s, 1H, H-4′), 3.18 (br, 2H, NH_2), 3.03-2.82 (m, 2H, Pyr-CH_2), 2.71-2.62 (m, 3H, CH and NH_2CH_2); δ_C (DMSO-d_6) (75 MHz): 130.6, 130.1, 130.0, 129.9, 129.3, 128.5, 128.3, 116.0, 107.1, 105.4, 47.6, 46.1, 31.1; IR (film) ν_{max}/cm^{-1}: 3359 (NH), 2958 (CH), 1495 (Ar), 728 (Ar-Cl); m/z (ESI M+H) 270; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{13}H_{14}C_{l2}N_2$; Exact Mass: 269.0613, found: 269.0610.

4.4 Synthesis of Second Generation Nitrile Derivatives

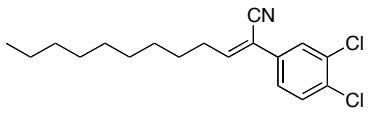
4.4.1 Aliphatic aldehydes + 2-(3,4-dichlorophenyl)acetonitrile

*(Z)-2-(3,4-Dichlorophenyl)hept-2-enenitrile (**39**)*



To a stirring solution of 2-(3,4-dichlorophenyl)acetonitrile (199 mg, 1.07 mmol) in EtOH (10 mL) was added a solution of pentanal (97 mg, 1.13 mmol) in EtOH (10 mL). The resultant solution was stirred and heated to 70 °C upon which piperidine was added (2 drops). The solution was then heated under reflux for 2 hours. After this time, the reaction mixture was cooled on an ice bath to 0 °C and the solvent removed *in vacuo* to yield a crude oil which was subjected to flash chromatography (1:19 EtOAc:Hexanes) to afford (**39**) as a clear oil (95 mg, 35%); δ_H ($CDCl_3$) (300 MHz): 7.62-7.61 (m, 1H, H-5), 7.48-7.45 (m, 1H, H-2), 7.38-7.34 (m, 1H, H-6), 6.85 (t, *J* = 7.7 Hz, 1H, HC=C), 2.60 (q, *J* = 7.7 Hz, 2H, CH_2CH=C), 1.60-1.33 (m, 4H, CH_2CH_2), 0.96 (t, *J* = 7.2 Hz, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz): 148.4, 132.8, 132.7, 132.5, 130.3, 126.8, 124.3, 115.3, 113.4, 31.5, 30.0, 21.8, 13.3; IR (film) ν_{max}/cm^{-1}: 2958 (CH), 2957 (CH), 2870 (CH), 2218 (CN), 1615 (C=C), 1473 (Ar); m/z (APCI M-H) 252; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{13}H_{10}Cl_2N$; Exact Mass: 252.0346, found: 252.0348.

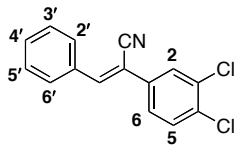
*(Z)-2-(3,4-Dichlorophenyl)dodec-2-enenitrile (**40**)*



Synthesized using the general procedure as for (**39**), from decanal and 2-(3,4-dichlorophenyl)acetonitrile to afford (**40**) as a clear oil (21%); δ_H ($CDCl_3$) (300 MHz): 7.62-7.61 (m, 1H, H-5), 7.49-7.46 (m, 1H, H-2), 7.37 (dd, *J* = 8.4, 2.2 Hz, 1H, H-6), 6.87-6,82 (m, 1H, CH=C), 2.63-2.52 (m, 4H, CH_2CH_2CH=C), 2.34-2.14 (m, 4H, CH_2CH_2), 1.59-1.52 (m, 6H, $CH_2CH_2CH_2$), 0.91-0.85 (m, 5H, CH_2CH_3); δ_C ($CDCl_3$) (75 MHz): 148.4, 132.8, 132.5, 130.3, 126.1, 125.2, 124.9, 115.3, 113.4, 31.9, 31.3, 31.2, 29.1, 28.8, 28.7, 28.0, 22.2, 13.5; IR (film) ν_{max}/cm^{-1}: 2957 (CH), 2930 (CH), 2860 (CH), 2219 (CN), 1619 (C=C), 1482 (CH); m/z (APCI M-H) 322; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{18}H_{23}Cl_2N$; Exact Mass: 322.1129, found: 322.1127.

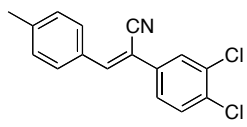
4.4.2 Aromatic aldehydes + 2-(3,4-dichlorophenyl)acetonitrile

*(Z)-2-(3,4-Dichlorophenyl)-3-phenylacrylonitrile (**41**)*[4]



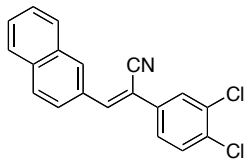
Synthesized using the general procedure as for (**24**), from benzaldehyde and 3,4-dichlorophenylacetonitrile to afford (**41**) as a white solid (89%), m.p. 146-147 ºC (Lit.[4] 144 ºC); δ_H ($CDCl_3$) (300 MHz): 7.91-7.87 (m, 2H, H-2 and H-5), 7.77-7.76 (m, 1H, H-6), 7.53-7.47 (m, 6H, H-2′, H-3′, H-4′, H-5′, H-6′ and CH=C); δ_C ($CDCl_3$) (75 MHz): 143.0, 133.9, 133.0, 132.8, 132.6, 130.6, 130.4, 128.9 (2 x Ar), 128.5 (2 x Ar), 127.2, 124.6, 116.7, 108.8; IR (KBr) ν_{max}/cm^{-1}: 2212 (CN), 1636 (C=C), 1590 (Ar), 1568 (Ar), 1496 (Ar), 676 (Ar-Cl); m/z (APCI M-H) 273; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{15}H_9Cl_2N$; Exact Mass: 272.0033, found: 272.0033.

*(Z)-2-(3,4-Dichlorophenyl)-3-p-tolylacrylonitrile (**42**)*



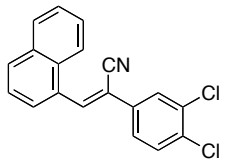
Synthesized using the general procedure as for (**24**), from 4-methylbenzaldehyde and 2-(3,4-dichlorophenyl)acetonitrile to afford (**42**) as a yellow solid (71%), m.p. 164-165 ºC; δ_H ($CDCl_3$) (300 MHz): 7.80 (d, *J* = 8.1 Hz, 2H, H-2′ and H-6′), 7.74 (m, 1H, H-5), 7.50-7.7.49 (m, 3H, H-2, H-6 and CH=C), 7.28 (d, *J* = 8.1 Hz, 2H, H-3′ and H-5′), 2.42 (s, 3H, Ar-CH_3); δ_C ($CDCl_3$) (75 MHz): 143.0, 141.4, 134.1, 132.9, 132.5, 130.4, 129.8, 129.3 (2 x Ar), 129.0 (2 x Ar), 127.0, 124.5, 117.0, 107.4, 21.1; IR (KBr) ν_{max}/cm^{-1}: 2214 (CN), 1637 (C=C), 1594 (Ar), 1509 (Ar), 811 (Ar-Cl); m/z (APCI M-H) 288; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{16}H_{11}NCl_2$; Exact Mass: 286.0190, found: 286.0192.

*(Z)-2-(3,4-Dichlorophenyl)-3-(naphthalen-2-yl)acrylonitrile (**43**)*



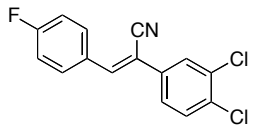
Synthesized using the general procedure as for (**24**), from 2-naphthaldehyde and 3,4-dichlorophenyl acetonitrile to afford (**43**) as a yellow solid (71%), m.p. 170-171 ºC; δ_H (DMSO-d_6) (300 MHz): 8.42 (s, 1H, H-1′), 8.32 (s, 1H, CH=C), 8.12-7.97 (m, 5H, H-3′, H-5′, H-6′, H-7′ and H-8′), 7.76 (m, 2H, H-4′ and H-5), 7.61 (m, 2H, H-2 and H-6); δ_C (DMSO-d_6) (75 MHz): 144.7, 134.5, 133.7, 132.4, 132.0, 131.7, 131.2, 130.9, 128.8, 128.7, 128.6, 128.0, 127.7, 127.3, 127.1, 126.1, 124.7, 117.4, 107.9; IR (KBr) ν_{max}/cm^{-1}: 2210 (CN), 1626 (C=C), 1598 (Ar), 1591 (Ar), 1477 (Ar), 1466 (Ar), 809 (Ar-Cl), 743 (Ar-Cl); m/z (APCI M-H) 323; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{19}H_{11}Cl_2N$; Exact Mass: 322.0190, found: 322.0089.

*(Z)-2-(3,4-Dichlorophenyl)-3-(naphthalen-1-yl)acrylonitrile (**44**)*



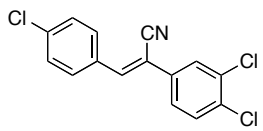
Synthesized using the general procedure as for (**24**), from 1-naphthaldehyde and 3,4-dichlorophenylacetonitrile to afford (**44**) as a yellow solid (72%), m.p. 218-219 ºC; δ_H (DMSO-d_6) (300 MHz): 8.84 (s, 1H, CH=C), 8.19-8.18 (m, 2H, H-4′ and H-8′), 8.10-7.99 (m, 3H, H-2′, H-5′ and H-7′), 7.82-7.81 (m, 2H, H-3′ and H-6′), 7.67-7.61 (m, 3H, H-2, H-5 and H-6); δ_C (DMSO-d_6) (75 MHz): 143.5, 134.0, 133.0, 132.0, 131.9, 131.1, 130.9, 130.8, 128.5, 127.8, 127.0, 126.8, 126.6, 126.5, 125.3, 125.2, 124.3, 117.0, 112.3; IR (KBr) ν_{max}/cm^{-1}: 2216 (CN), 1636 (C=C), 1508 (Ar), 1474 (Ar), 776 (Ar-Cl); m/z (APCI M-H) 323; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{19}H_{11}Cl_2N$; Exact Mass: 322.0190, found: 322.0188.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-fluorophenyl)acrylonitrile (**45**)*[4]



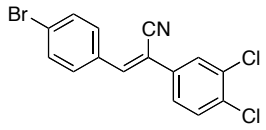
Synthesized using the general procedure as for (**24**), from 4-fluorobenzaldehyde and 4-chlorophenyl acetonitrile to afford (**45**) as a white solid (94%), m.p. 156-157 ºC (Lit.[4] 155 ºC); δ_H ($CDCl_3$)(300 MHz): 7.93-7.88 (m, 2H, H-2′ and H-6′), 7.75-7.74 (m, 1H, H-5), 7.51-7.48 (m, 3H, H-3′, H-5′ and CH=C), 7.20-7.15 (m 2H, H-2 and H-6); δ_C ($CDCl_3$)(75 MHz): 163.5 (d, *J* = 253.9 Hz, C-F), 141.5, 133.7, 132.9 (d, *J* = 5.5 Hz, C-F), 131.1 (d, *J* = 8.6 Hz, C-F), 130.5, 128.9, 129.8, 127.1, 124.6, 116.6, 115.8 (d, *J* = 21.9 Hz, C-F), 99.6; IR (KBr) ν_{max}/cm^{-1}: 2213 (CN), 1636 (C=C), 1596 (Ar), 809 (Ar-Cl); m/z (APCI M-H) 291; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{15}H_8Cl_2FN$; Exact Mass: 289.9939, found: 289.9942.

*(Z)-3-(4-Chlorophenyl)-2-(3,4-dichlorophenyl)acrylonitrile (**46**)*[5]



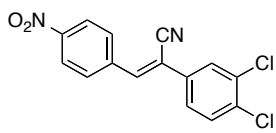
Synthesized using the general procedure as for (**24**), from 4-chlorobenzaldehyde and 3,4-dichlorophenyl acetonitrile to afford (**46**) as a white solid (66%), m.p. 167-168 ºC (Lit. not reported); δ_H ($CDCl_3$) (300 MHz): 7.80 (d, *J* = 8.1Hz, 2H, H-2′ and H-6′), 7.75-7.74 (m, 1H, H-5), 7.51-7.49 (m, 3H, H-2, H-6 and CH=C), 7.29 (d, *J* = 8.1Hz, 2H, H-3′ and H-5′); δ_C ($CDCl_3$) (75 MHz): 143.0, 141.4, 134.1, 132.9, 132.6, 130.4, 129.9, 129.3 (2 x Ar), 129.0 (2 x Ar), 127.1, 124.5, 117.0, 107.5; IR (KBr) ν_{max}/cm^{-1}: 2214 (CN), 1637 (C=C), 1594 (Ar), 1478 (Ar), 810 (Ar-Cl); m/z (APCI M-H) 307; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{15}H_8Cl_3N$; Exact Mass: 305.9643, found: 305.9646.

*(Z)-3-(4-Bromophenyl)-2-(3,4-dichlorophenyl)acrylonitrile (**47**)*



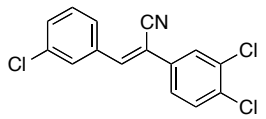
Synthesized using the general procedure as for (**24**), from 4-bromobenzaldehyde and 3,4-dichlorophenyl acetonitrile to afford (**47**) as a yellow solid (85%), m.p. 120-121 ºC; δ_H ($CDCl_3$) (300 MHz): 7.75 (m, 3H, H-2′, H-6′ and CH=C), 7.61 (d, *J* = 8.56 Hz, 2H, H-3′ and H-5′), 7.50 (m, 2H, H-2 and H-5, 7.46 (d, *J* = 8.45 Hz, 1H, H-6); δ_C ($CDCl_3$) (75 MHz): 141.8, 134.1, 133.6, 133.6, 132.2, 131.9, 131.0, 130.7 (2 x Ar), 127.6 (2 x Ar), 125.6, 125.1, 116.9, 110.1; IR (KBr) ν_{max}/cm^{-1}: 2214 (CN), 1635 (C=C), 1598 (Ar); m/z (APCI M-H) 351; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{15}H_8BrCl_2N$; Exact Mass: 349.9137, found: 349.9140.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**48**)*



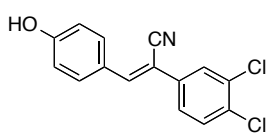
Synthesized using the general procedure as for (**24**), from 4-nitrobenzaldehyde and 3,4-dichlorophenyl acetonitrile to afford (**48**) as a purple solid (75%), m.p. 133-134 ºC; δ_H ($CDCl_3$) (300 MHz): 8.34 (d, *J* = 8.8Hz, 2H, H-3′ and H-5′), 8.03 (d, *J* = 8.8Hz, 2H, H-2′ and H-6′), 7.81-7.80 (m, 1H, H-5), 7.58-7.56 (m, 2H, H-2 and H-6), 7.26 (s, 1H, CH=C); δ_C ($CDCl_3$) (75 MHz): 139.6, 138.3, 133.3, 132.8, 132.1, 130.7, 129.5 (2 x Ar), 129.1, 127.4, 124.9, 123.7 (2 x Ar), 115.8, 113.2; IR (KBr) ν_{max}/cm^{-1}: 2215 (CN), 1674 (C=C), 1592 (Ar), 1513 (NO), 1345 (NO); m/z (APCI M+H) 289 ($NO_2 \rightarrow NH_2$); HRMS (APCI M+H): Calculated for Chemical Formula: $C_{15}H_{10}Cl_2N_2$; Exact Mass: 289.0300, found: 289.0297.

*(Z)-3-(3-Chlorophenyl)-2-(3,4-dichlorophenyl)acrylonitrile (**49**)*[4]



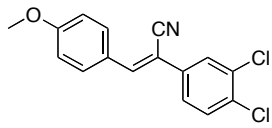
Synthesized using the general procedure as for (**24**), from 3-chlorobenzaldehyde and 3,4-dichlorophenyl acetonitrile to afford (**49**) as a white solid (65%), m.p. 138-140 ºC (Lit.[4] 142 ºC); δ_H ($CDCl_3$) (300 MHz): 7.84-7.81 (m, 2H, H-2′ and H-6′), 7.76 (s, 1H, CH=C), 7.52-7.51 (m, 2H, H-4′ and H-5′), 7.45-7.42 (m, 3H, H-2, H-5 and H-6); δ_C ($CDCl_3$) (75 MHz): 141.0, 134.6, 134.2, 133.4, 133.3, 133.1, 130.5, 130.4, 129.8, 128.9, 127.3, 126.6, 124.7, 116.2, 110.5; IR (KBr) ν_{max}/cm^{-1}: 2212 (CN), 1636 (C=C), 1601 (Ar), 677 (Ar-Cl); m/z (APCI M-H) 307; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{15}H_8Cl_3N$; Exact Mass: 305.9643, found: 305.9646.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-hydroxyphenyl)acrylonitrile (**50**)*



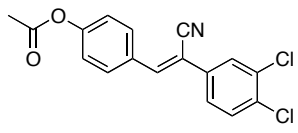
Synthesized using the general procedure as for (**39**), from 4-hydroxybenzaldehyde and 2-(3,4-dichlorophenyl)acetonitrile to afford (**50**) as a yellow solid (90%), m.p. 153-154 ºC; δ_H ($CDCl_3$) (300 MHz): 7.90-7.89 (m, 4H, H-2′, H-6′, CH=C and H-5), 7.67-7.66 (m, 2H, H-2 and H-6), 6.97-6.94 (m, 2H, H-3′ and H-5′), 5.98 (br, 1H, OH); δ_C ($CDCl_3$) (75 MHz): 161.1, 143.6, 135.3, 132.0, 131.4 (2 x Ar), 130.9, 130.5, 126.5, 124.8, 123.9, 117.3, 115.6 (2 x Ar), 103.0; IR (KBr) ν_{max}/cm^{-1}: 3467 (OH), 2210 (CN), 1580 (Ar); m/z (APCI M-H) 288; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{15}H_9Cl_2NO$; Exact Mass: 287.9982, found: 287.9985.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-methoxyphenyl)acrylonitrile (**51**)*[4]



Synthesized using the general procedure as for (**24**), from 4-methoxybenzaldehyde and 3,4-dichlorophenylacetonitrile to afford (**51**) as a yellow solid (79%), m.p. 166-167 ºC (Lit.[4] 142 ºC); δ_H ($CDCl_3$) (300 MHz): 7.89 (d, *J* = 8.9 Hz, 2H, H-2′ and H-6′), 7.73 (m, 1H, H-5), 7.49-7.44 (m, 3H, H-2, H-6 and CH=C), 6.99 (d, *J* = 8.9 Hz, 2H, H-3′ and H-5′), 3.88 (s, 3H, OCH_3); δ_C ($CDCl_3$) (75 MHz): 161.4, 142.5, 134.4, 132.9, 132.3, 130.9 (2 x Ar), 130.3, 126.9, 125.4, 124.4, 117.2, 114.0 (2 x Ar), 105.7, 54.9; IR (KBr) ν_{max}/cm^{-1}: 2212 (CN), 1638 (C=C), 1609 (Ar), 1593 (Ar), 1513 (Ar); m/z (APCI M-H) 303; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{16}H_{11}Cl_2NO$; Exact Mass: 302.0139, found: 302.0137.

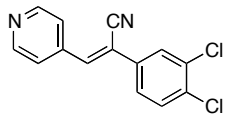
*(Z)-4-(2-cyano-2-(3,4-dichlorophenyl)vinyl)phenyl acetate (**52**)*



To a stirred solution of (*Z*)-2-(3,4-dichlorophenyl)-3-(4-hydroxyphenyl)acrylonitrile (**50**) (290 mg, 1.0 mmol) in acetic anhydride (5 mL) was added conc. H_2SO_4 (3 drops) and stirring was continued overnight. After this time the reaction mixture was quenched with water (10 mL) and the resultant precipitate was collected under suction, and recrystallised from EtOH to afford (**52**) as a yellow solid (113 mg, 35%), m.p. 154-155 ºC; δ_H ($CDCl_3$) (300 MHz): 7.92 (d, *J* = 8.7 Hz, 2H, H-2′ and H-6′), 7.75 (m, 1H, H-5), 7.51-7.49 (m 3H, H-2, H-6 and CH=C), 7.23 (d, *J* = 8.7 Hz, 2H, H-3′ and H-5′), 2.33 (s, 3H, $COCH_3$); δ_C ($CDCl_3$) (75 MHz): 168.4, 152.0, 141.7, 133.8, 133.0, 132.9, 131.1, 130.4, 130.2 (2 x Ar), 127.1, 124.6, 121.8 (2 x Ar), 116.6, 108.8, 20.6; IR

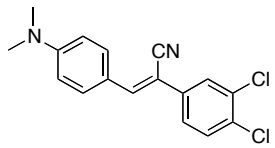
(KBr) ν_{max}/cm^{-1}: 2210 (CN), 1764 (C=O), 1597 (Ar), 1507 (Ar), 1221 (CO); m/z (APCI M-H) 331; HRMS (APCI M-H): Calculated for Chemical Formula: $C_{17}H_{11}Cl_2NO_2$; Exact Mass: 330.0088, found: 330.0090.

*(Z)-2-(3,4-Dichlorophenyl)-3-(pyridin-4-yl)acrylonitrile (**53**)*



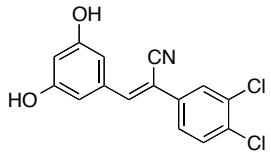
Synthesized using the general procedure as for (**24**), from 4-pyridinecarboxaldehyde and 3,4-dichlorophenyl acetonitrile to afford (**53**) as a white solid (66%), m.p. 188-189 ºC; δ_H ($CDCl_3$) (300 MHz): 8.77 (d, *J* = 6.2 Hz, 2H, H-3′ and H-5′), 7.79 (m, 1H, H-5), 7.69 (d, *J* = 6.2 Hz, 2H, H-2′ and H-6′), 7.55-7.54 (m, 2H, H-2 and H-6), 7.47 (s, 1H, CH=C); δ_C ($CDCl_3$) (75 MHz): 150.3 (2 x Ar), 139.6, 139.4, 134.1, 133.3, 132.7, 130.7, 127.5, 124.9, 122.0 (2 x Ar), 115.6, 113.7; IR (KBr) ν_{max}/cm^{-1}: 2217 (CN), 1636 (C=C), 816 (Ar-Cl); m/z (APCI M+H) 275; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{14}H_8Cl_2N$; Exact Mass: 275.0144, found: 275.0141.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(dimethylamino)phenyl)acrylonitrile (**54**)*[6]



Synthesized using the general procedure as for (**24**), from 4-*N,N*-dimethylaminobenzaldehyde and 3,4-dichlorophenylacetonitrile to afford (**54**) as a yellow solid (40%), m.p. 210-212 ºC (Lit.[6] 213-214); δ_H ($CDCl_3$) (300 MHz): 7.86 (d, *J* = 9.0 Hz, 2H, H-2′ and H-6′), 7.71- 6.69 (m, 1H, H-5), 7.46-7.45 (m, 2H, H-2 and H-6), 7.37 (s, 1H, CH=C), 6.72 (d, *J* = 9.0 Hz, 2H, H-3′ and H-5′), 3.08 (s, 6H, $N(CH_3)_2$); δ_C ($CDCl_3$) (75 MHz): 151.5, 143.0, 135.2, 134.2, 132.6, 131.1 (2 x Ar), 130.2, 126.4, 124.0, 120.4, 118.3, 111.0 (2 x Ar), 101.1, 29.5; IR (KBr) ν_{max}/cm^{-1}: 2208 (CN), 1614 (C=C), 1580 (Ar), 805 (Ar-Cl); m/z (APCI M+H) 317; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{17}H_{14}Cl_2N_2$; Exact Mass: 317.0613, found: 317.0616.

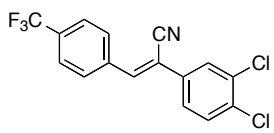
*(Z)-2-(3,4-Dichlorophenyl)-3-(3,5-dihydroxyphenyl)acrylonitrile (**55**)*



Synthesized using the general procedure as for (**39**), from 3,5-dihydroxybenzaldehyde and 2-(3,4-dichlorophenyl)acetonitrile to afford (**55**) as a brown solid (25%), m.p. >300 ºC; δ_H ($CDCl_3$) (300 MHz): 8.76 (br, 2H, 2 x OH), 7.92-7.91 (m, 1H, H-5), 7.82 (s, 1H, CH=C), 7.69-7.67 (m, 2H, H-2 and H-6), 6.99 (m, 2H, H-2′ and H-5′), 6.52-6.51 (m, 1H, H-4′); δ_C ($CDCl_3$) (75 MHz): 158.3 (2 x Ar), 144.0, 134.7, 132.1, 131.7, 130.5, 129.7, 127.7, 127.0, 125.3, 116.5 (2 x Ar), 107.5, 105.2; IR (KBr) ν_{max}/cm^{-1}: 3437 (OH), 2218 (CN), 1582 (Ar), 804 (Ar-Cl); m/z (APCI M-H) 304; HRMS (APCI M-H):

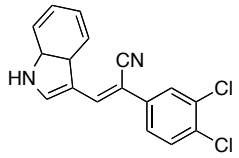
Calculated for Chemical Formula: $C_{15}H_9Cl_2NO_2$; Exact Mass: 303.9931, found: 303.9933.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(trifluoromethyl)phenyl)acrylonitrile (**56**)*



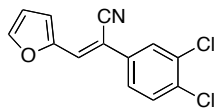
Synthesized using the general procedure as for (**24**), from 4-(trifluoromethyl)benzaldehyde and 3,4-dichlorophenylacetonitrile to afford (**56**) as a white solid (89%), m.p. 120-122 ºC; δ_H (DMSO-d_6) (300 MHz): 8.27 (s, 1H, CH=C), 8.09-8.05 (m, 3H, H-2′, H-6′ and H-5), 7.90 (d, *J* = 8.2 Hz, 2H, H-3′ and H-5′), 7.79-7.70 (m, 2H, H-2 and H-6); δ_C (DMSO-d_6) (75 MHz): 142.9, 137.2, 133.8, 132.8, 132.2, 131.2, 130.0, 129.8 (2 x Ar), 127.5, 126.3, 125.8 (2 x Ar), 125.7, 116.7, 110.6; IR (KBr) ν_{max}/cm^{-1}: 2215 (CN), 1618 (C=C), 1478 (Ar); m/z (APCI M-H) 340; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{16}H_8Cl_2F_3N$; Exact Mass: 339.9907, found: 339.9910.

*(Z)-2-(3,4-Dichlorophenyl)-3-(1H-indol-3-yl)acrylonitrile (**57**)*



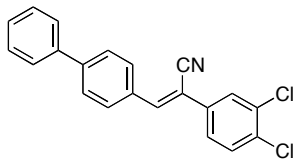
Synthesized using the general procedure as for (**24**), from 1*H*-indole-3-carbaldehyde to afford (**57**) as a yellow solid (48%), m.p. 225-226 °C; δ_H (DMSO-d_6) (300 MHz): 12.03 (br, NH), 8.39 (s, 1H, CH=C), 8.34 (s, 1H, H-2′), 8.12-8.09 (m, 1H, H-4′), 8.03-8.02 (m, 1H, H-5), 7.70-7.61 (m, 2H, H-5′ and H-6′), 7.52-7.49 (m, 1H, H-7′), 7.26-7.16 (m, 2H, H-2 and H-6); δ_C (DMSO-d_6) (75 MHz): 136.3, 135.7, 135.2, 131.9, 130.9, 129.8, 127.8, 127.2, 126.1, 125.0, 122.8, 120.7, 119.2, 118.9, 112.2, 110.7, 99.6; IR (KBr) ν_{max}/cm^{-1}: 3311 (NH), 2212 (CN), 1628 (C=C), 1574 (Ar), 732 (Ar-Cl); m/z (APCI M-H) 311; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{17}H_{10}Cl_2N_2$; Exact Mass: 311.0142, found: 311.0140.

*(Z)-2-(3,4-Dichlorophenyl)-3-(furan-2-yl)acrylonitrile (**58**)*



Synthesized using the general procedure as for (**24**), from furan-2-carbaldehyde to afford (**58**) as a light orange solid (75%), m.p. 126-128 °C; δ_H ($CDCl_3$) (300 MHz): 7.72 (s, 1H, H-5′), 7.63 (s, 1H, CH=C), 7.48 (m, 2H, H-5 and H-2), 7.36 (s, 1H, H-6), 7.23 (d, *J* = 3.4 Hz, 1H, H-3′), 6.61 (dd, *J* = 3.2, 1.4 Hz, 1H, H-4′); δ_C ($CDCl_3$) (75 MHz): 149.6, 145.6, 133.7, 133.1, 130.9, 129.2, 128.9, 127.2, 124.7, 117.0, 116.4, 113.0, 105.1; IR (KBr) ν_{max}/cm^{-1}: 2215 (CN), 1617 (C=C), 1466 (Ar); m/z (APCI M-H) 262; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{13}H_7Cl_2NO$; Exact Mass: 261.9916, found: 261.9917.

*(Z)-3-(biphenyl-4-yl)-2-(3,4-dichlorophenyl)acrylonitrile (**59**)*

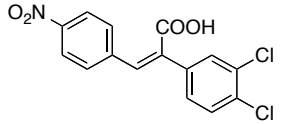


Synthesized using the general procedure as for (**24**), from biphenyl-4-carbaldehyde to afford (**59**) as a white solid (61%), m.p. 230-232 °C; δ_H ($CDCl_3$) (300 MHz): 7.98 (d, *J* = 8.2 Hz, 2H, H-3′ and H-5′), 7.78 (s, 1H, CH=C), 7.72 (d, *J* = 8.2 Hz, 2H, H-2′ and H-6′), 7.65 (d, *J* = 7.3 Hz, 2H, H-2′′ and H-6′′), 7.57-7.37 (m, 6H, H-2, H-5, H-6, H-3′′, H-4′′ and H-5′′); δ_C ($CDCl_3$) (75 MHz): 143.8, 142.9, 139.6, 134.4, 133.4, 133.2, 131.9, 130.9, 130.0, 128.9, 128.1, 127.5, 127.0, 125.0, 117.4, 108.8; IR (KBr) ν_{max}/cm^{-1}: 2216 (CN), 1593 (Ar), 1474 (Ar), 1448 (Ar); m/z (APCI M-H) 349; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{21}H_{13}Cl_2N$; Exact Mass: 348.0346, found: 348.0347.

4.5 Synthesis of Third Generation Nitrile Derivatives

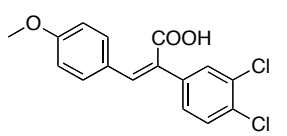
4.5.1 Aromatic aldehydes + 2-(3,4-dichlorophenyl)acetic acid

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-nitrophenyl)acrylic acid (**60**)*



Triethylamine (1.51g, 15 mmol) was added to a solution of 4-nitrobenzaldehyde (1.51g, 10 mmol) and 2-(3,4-dichlorophenyl)acetic acid (2.29g, 11 mmol) in Ac_2O (5 mL). The solution was heated at 140 °C under a nitrogen atmosphere for 1 hour. After this period, the solvent was removed *in vacuo* to yield a yellow oil which was purified by flash silica chromatography (1:9 EtOAc:Hexanes) to afford (**60**) as a yellow solid (1.76 g, 52%), m.p. 151-153 °C; δ_H (DMSO-d_6) (300 MHz): 8.15 (d, *J* = 8.8 Hz, 2H, H-3′ and H-5′), 7.90 (d, *J* = 8.8 Hz, 2H, H-2′ and H-6′), 7.83 (m, 1H, H-5), 7.61 (m, 2H, H-2 and H-6), 6.73 (s, 1H, CH=C); δ_C (DMSO-d_6) (75 MHz): 170.5, 145.6, 145.2, 143.9, 139.1, 131.0, 130.4, 130.0, 128.9 (2 x Ar), 128.0, 126.4, 123.2 (2 x Ar), 119.1; IR (KBr) ν_{max}/cm^{-1}: 3347 (OH), 1594 (C=O), 1337 (N-O); m/z (APCI M-H) 336; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{15}H_9Cl_2NO_4$; Exact Mass: 335.9830, found: 335.9829.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-methoxyphenyl)acrylic acid (**61**)*

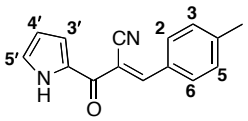


Synthesized using the general procedure as for (**60**), from 4-methoxybenzaldehyde to afford (**61**) as a yellow solid (15%), m.p. 221-223 °C; δ_H (DMSO-d_6) (300 MHz): 7.75 (s, 1H, CH=C), 7.63 (d, *J* = 8.2 Hz, 1H, H-5), 7.46 (d, *J* = 2.0 Hz, 1H, H-2), 7.15 (dd, *J* = 2.0, 8.2, Hz, 1H, H-6), 7.04 (d, *J* = 8.9 Hz, 2H, H-2′ and H-6′), 6.82 (d, *J* = 8.9 Hz, 2H, H-3′ and H-5′), 3.71 (s, 3H, OCH_3); δ_C (DMSO-d_6) (75 MHz): 167.7, 160.1, 139.9,

137.5, 132.1 (2 x Ar), 132.0, 131.6, 131.1, 130.7, 130.1, 128.1, 126.1, 114.0 (2 x Ar), 55.1; IR (KBr) ν_{max}/cm^{-1}: 3448 (OH), 1663 (C=O), 1172 (C-O); m/z (APCI M-H) 321; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{16}H_{12}Cl_2O_3$; Exact Mass: 321.0084, found: 321.0084.

4.5.2 Aromatic aldehydes + 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile

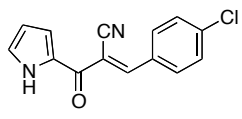
*(E)-2-(1H-Pyrrole-2-carbonyl)-3-p-tolylacrylonitrile (**62**)*



Cyano acetic acid (1.36g, 16 mmol) was added to acetic anhydride (8 mL) and the resultant suspension was stirred and heated to 50 °C until complete dissolution was observed. Pyrrole (1.07g, 16 mmol) was subsequently added and the reaction mixture was heated at 75 °C for 35 minutes. After this time, EtOAc (20 mL) was added and the reaction mixture was washed with 0.1M NaOH (3 x 10 mL). The organic layer was collected, dried ($MgSO_4$) and evaporated under reduced pressure. The resulting residue was purified by flash silica chromatography (1:10 EtOAc:Hexanes to 1:1 EtOAc:Hexanes) to afford 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile (1.50 g, 70%).

To a stirring solution of freshly prepared 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile (200 mg, 1.49 mmol) in EtOH (10 mL) was added a solution of 4-methylbenzaldehyde (189 mg, 1.57 mmol) in EtOH (10 mL). Stirring was continued and the reaction mixture was heated to 70 ºC and piperidine (2 drops) was added. After addition of the piperidine, the reaction mixture was heated under reflux for 2 hours. After this time, the reaction mixture was cooled and the solvent removed *in vacuo* to afford a brown oil which was purified by flash chromatography (1:10 EtOAc:Hexanes) to afford (**62**) as a yellow solid (285 mg, 81%), m.p. 242-244 ºC; δ_H ($CDCl_3$) (300 MHz): 12.22 (br, NH), 8.24 (s, 1H, CH=C), 7.95 (d, *J* = 8.0Hz, 2H, H-2 and H-6), 7.37 (d, *J* = 8.0Hz, 2H, H-3 and H-5), 7.29-7.26 (m, 2H, H-3′ and H-5′), 6.33-6.30 (m, 1H, H-4′), 2.37 (s, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz): 174.8, 153.2, 143.5, 130.6 (2 x Ar), 129.7 (2 x Ar), 129.3, 129.0, 127.9, 119.3, 117.7, 110.8, 107.8, 21.2; IR (KBr) νmax/cm^{-1}: 3291 (NH), 2210 (CN), 1622 (C=O); m/z (APCI M+H) 237; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{15}H_{12}N_2O$; Exact Mass: 237.1029, found: 237.1028.

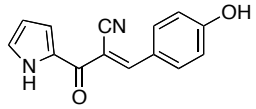
*(E)-3-(4-Chlorophenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**63**)*



Synthesized using the general procedure as for (**62**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-chlorobenzaldehyde to afford (**63**) as a yellow solid (39%), m.p. 192-194 °C; δ_H (Acetone-d_6) (300

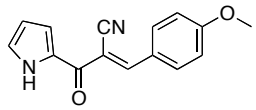
MHz): 11.30 (br, NH), 8.28 (s, 1H, CH=C), 8.13-8.11 (m, 2H, H-2 and H-6), 7.66-7.63 (m, 2H, H-3 and H-5), 7.45-7.44 (m, 1H, H-5′), 7.35-7.34 (m, 1H, H-3′), 6.38-6.36 (m, 1H, H-4′); δ_C (Acetone-d_6) (75 MHz): 173.6, 151.1, 137.4, 131.7 (2 x Ar), 130.8, 128.8 (2 x Ar), 126.9, 119.7, 118.7, 116.6, 110.4, 109.5; IR (KBr) ν_{max}/cm^{-1}: 3284 (NH), 2211 (CN), 1627 (C=O); m/z (APCI M+H) 257; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{14}H_9ClN_2O$; Exact Mass: 257.0482, found: 257.0479.

*(E)-3-(4-Hydroxyphenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**64**)*



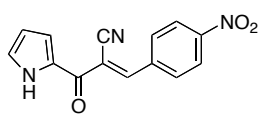
Synthesized using the general procedure as for (**62**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-hydroxybenzaldehyde to afford (**64**) as an orange solid (43%), m.p. 240-243 °C; δ_H (DMSO-d_6) (300 MHz): 12.07 (br, NH), 8.68 (br, OH), 8.18 (s, 1H, CH=C), 7.99 (d, *J* = 8.7Hz, 2H, H-2 and H-6), 7.28-7.23 (m, 2H, H-5′ and H-3′), 6.94 (d, *J* = 8.7Hz, 2H, H-3 and H-5), 6.30-6.27 (m, 1H, H-4′); δ_C (DMSO-d_6) (75 MHz): 175.0, 162.3, 153.3, 133.6 (2 x Ar), 129.2, 127.3, 123.1, 118.6, 118.4, 116.2 (2 x Ar), 110.5, 104.1; IR (KBr) ν_{max}/cm^{-1}: 3419 (OH), 3290 (NH), 2218 (CN), 1617 (C=O), 1603 (Ar); m/z (APCI M+H) 239; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{14}H_{10}N_2O_2$; Exact Mass: 239.0821, found: 239.0823.

*(E)-3-(4-Methoxyphenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**65**)*



Synthesized using the general procedure as for (**62**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-methoxybenzaldehyde to afford (**65**) as a yellow solid (83%), m.p. 166-168 °C; δ_H (DMSO-d_6) (300 MHz): 12.14 (br, NH), 8.24 (s, 1H, CH=C), 8.08 (d, *J* = 8.9Hz, 2H, H-2 and H-6), 7.28-7.25 (m, 2H, H-5′ and H-3′), 7.13 (d, *J* = 8.9Hz, 2H, H-3 and H-5), 6.31 (s, 1H, H-4′), 3.85 (s, 3H, OCH_3); δ_C (DMSO-d_6) (75 MHz): 174.9, 163.0, 153.0, 133.1 (2 x Ar), 129.1, 127.5, 124.6, 118.9, 118.2, 114.8 (2 x Ar), 110.6, 105.5, 55.6; IR (KBr) ν_{max}/cm^{-1}: 3306 (NH), 2209 (CN), 1617 (C=O), 1507 (Ar); m/z (APCI M+H) 253; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{15}H_{12}N_2O_2$; Exact Mass: 253.0978, found: 253.0979.

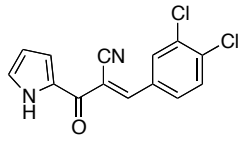
*(E)-3-(4-Nitrophenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**66**)*



Synthesized using the general procedure as for (**62**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-nitrobenzaldehyde to afford (**66**) as a purple solid (37%), m.p. 199-200 °C; δ_H (DMSO-d_6) (300 MHz): 12.32 (br, NH), 8.40-8.38 (m, 3H, H-3, H-5 and CH=C), 8.24-8.21 (m, 2H, H-2 and H-6), 7.24 (m, 2H, H-5′ and H-3′), 6.34 (s, 1H, H-4′); δ_C (DMSO-d_6) (75 MHz): 174.2, 150.6, 148.8, 138.2, 131.3 (2 x Ar), 130.6, 128.7, 124.0 (2 x Ar), 120.3, 116.6, 113.1, 111.1;

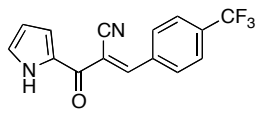
IR (KBr) ν_{max}/cm^{-1}: 3308 (NH), 2228 (CN), 1633 (C=O), 1517 (NO) 1343 (NO); m/z (APCI M+H) 268; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{14}H_9N_3O_3$; Exact Mass: 268.0723, found: 268.0720.

*(E)-3-(3,4-Dichlorophenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**67**)*



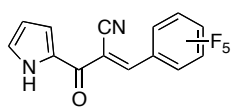
Synthesized using the general procedure as for (**62**), from 3-oxo-3-(1H-pyrrol-2-yl)propanenitrile and 3,4-dichlorobenzaldehyde to afford (**67**) as a yellow solid (66%), m.p. 178-181 °C; δ_H (Acetone-d_6) (300 MHz): 11.31 (br, NH), 8.29-8.27 (m, 2H, H-5 and CH=C), 8.11-8.08 (m, 1H, H-6), 7.84-7.81 (m, 1H, H-2), 7.45-7.36 (m, 2H, H-5′ and H-3′), 6.39-6.37 (m, 1H, H-4′); δ_C (Acetone-d_6) (75 MHz): 173.4, 149.6, 135.0, 132.4, 132.1, 131.7, 130.8, 129.3, 127.2, 127.0, 119.0, 116.3, 111.0, 110.5; IR (KBr) ν_{max}/cm^{-1}: 3310 (NH), 2222 (CN), 1632 (C=O); m/z (APCI M+H) 290; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{14}H_8Cl_2N_2O$; Exact Mass: 291.0093, found: 291.0090.

*(E)-2-(1H-Pyrrole-2-carbonyl)-3-(4-(trifluoromethyl)phenyl)acrylonitrile (**68**)*



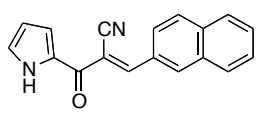
Synthesized using the general procedure as for (**62**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-(trifluoromethyl)benzaldehyde to afford (**68**) as a yellow solid (41%), m.p. 160-162 °C; δ_H (DMSO-d_6) (300 MHz): 12.30 (br, 1H), 8.37 (s, 1H, CH=C), 8.19 (d, *J* = 8.1Hz, 2H, H-2 and H-6), 7.94 (d, *J* = 8.1Hz, 2H, H-3 and H-6), 7.32 (s, 2H, H-5′ and H-3′), 6.34 (s, 1H, H-4′); δ_C (DMSO-d_6) (75 MHz): 174.4, 151.4, 136.0, 131.2, 130.8 (2 x Ar), 128.8, 128.5 (2 x Ar), 125.9, 125.8, 120.1, 116.8, 112.1, 111.0; IR (KBr) ν_{max}/cm^{-1}: 3295 (NH), 2228 (CN), 1637 (C=O), 1561 (Ar), 1325 (C-F); m/z (ESI M-H) 289; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{15}H_9F_3N_2O$; Exact Mass: 289.0588, found: 289.0585.

*(E)-3-(Perfluorophenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**69**)*



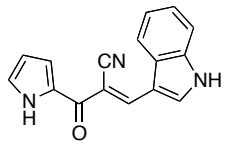
Synthesized using the general procedure as for (**62**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 2,3,4,5-pentafluorobenzaldehyde to afford (**69**) as a white solid (22%), m.p. 178-180 °C; δ_H (Acetone-d_6) (300 MHz): 11.01 (br, NH), 7.35-7.32 (m, 1H, H-5′), 7.22-7.19 (m, 1H, H-3′), 6.41-6.38 (m, 1H, H-4′), 5.67 (s, 1H, CH=C); δ_C (Acetone-d_6) (75 MHz): 174.3, 154.3, 142.5, 135.0, 134.7, 124.6 (2 x Ar), 120.2, 118.4, 114.7 (2 x Ar), 110.5, 106.3, 106.1; IR (KBr) ν_{max}/cm^{-1}: 3367 (NH), 2209 (CN), 1656 (C=C), 1608 (C=O); m/z (ESI M-H) 311; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{14}H_5F_5N_2O$; Exact Mass: 311.0243, found: 311.0240.

*(E)-3-(Naphthalen-2-yl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**70**)*



Synthesized using the general procedure as for (**62**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 2-naphthaldehyde to afford (**70**) as a brown solid (35%), m.p. 140-142 °C; δ_H (DMSO-d_6) (300 MHz): 12.27 (br, NH), 8.57 (s, 1H, H-1), 8.44 (s, 1H, CH=C), 8.23-8.21 (m, 1H, H-5), 8.11-7.99 (m, 3H, H-3, H-4 and H-8), 7.70-7.60 (m, 2H, H-7 and H-5′), 7.35-7.31 (m, 2H, H-6 and H-3′), 6.35-6.34 (m, 1H, H-4′); δ_C (DMSO-d_6) (75 MHz): 174.8, 153.2, 134.4, 133.4, 132.4, 129.7, 129.0, 128.9, 128.8, 128.7, 128.0, 127.7, 127.2, 124.7, 119.5, 119.2, 110.8, 109.1; IR (KBr) ν_{max}/cm^{-1}: 3291 (NH), 2215 (CN), 1617 (C=O); m/z (APCI M+H) 273; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{18}H_{12}N_2O$; Exact Mass: 273.1029, found: 273.1028.

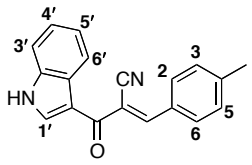
*(E)-3-(1H-Indol-3-yl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**71**)*



Synthesized using the general procedure as for (**62**), from 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 1*H*-indole-3-carbaldehyde to afford (**71**) as an orange solid (95%), m.p. 300-302 °C; δ_H (DMSO-d_6) (300 MHz): 12.49 (br, NH), 12.00 (br, NH), 8.67 (s, 1H, CH=C), 8.63 (s, 1H, H-2), 7.92-7.89 (m, 1H, H-7), 7.59-7.56 (m, 1H, H-4), 7.36-7.20 (m, 4H, H-5, H-6, H-3′ and H-5′), 6.31-6.29 (m, 1H, H-4′); δ_C (DMSO-d_6) (75 MHz): 174.4, 145.7, 136.2, 131.9, 129.7, 127.2, 126.4, 123.5, 121.9, 120.3, 118.4, 117.5, 112.8, 110.4, 110.3, 99.4; IR (KBr) ν_{max}/cm^{-1}: 3260 (NH), 2212 (CN), 1611 (C=O); m/z (ESI M-H) 260; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{16}H_{11}N_3O$; Exact Mass: 260.0823, found: 260.0821.

4.5.3 Aromatic aldehydes + 3-oxo-3-(1*H*-indol-3-yl)-3-oxopropanenitrile

*(E)-2-(1H-Indole-3-carbonyl)-3-p-tolylacrylonitrile (**72**)*

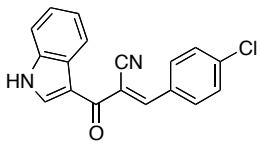


Cyanoacetic acid (363 mg, 4.26 mmol) was added to acetic anhydride (8 mL) and the resultant solution was stirred and heated to 50 °C upon which the solid material dissolved. Indole (500 mg, 4.26 mmol) was subsequently added and the reaction mixture was heated at 85 °C for 5 minutes. After this time, the reaction mixture was cooled to 0 °C and the resulting precipitate was isolated by filtration and washed with ice cold MeOH (2 x 5 mL) to afford 3-(1*H*-indol-3-yl)-3-oxopropanenitrile (596 mg, 76%).

To a stirring solution of freshly prepared 3-oxo-3-(1*H*-indol-3-yl)-3-oxopropanenitrile (200 mg, 1.08 mmol) in EtOH (10 mL) was added a solution of 4-methylbenzaldehyde (137 mg, 1.14 mmol) in EtOH (10 mL). Stirring was continued and the reaction mixture was

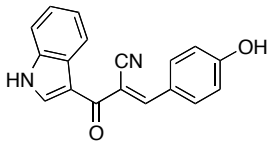
heated to 70 ºC and piperidine (2 drops) was added. After addition of the piperidine, the reaction mixture was heated under reflux for 2 hours. After this time, the reaction mixture was cooled and the solvent removed *in vacuo* to yield a yellow solid. The crude solid was subsequently recrystallised from EtOH to afford (**72**) as a yellow solid (272 mg, 88%), m.p. 216-219 °C; δ_H (DMSO-d_6) (300 MHz): 12.26 (br, NH), 8.43 (s, 1H, H-2′), 8.18-8.16 (m, 2H, H-4′ and CH=C), 7.94 (d, *J* = 8.1 Hz, 2H, H-2 and H-6), 7.55-7.52 (m, 1H, H-7′), 7.38 (d, *J* = 8.1 Hz, 2H, H-3 and H-5), 7.30-7.21 (m, 2H, H-5′ and H-6′), 2.37 (s, 3H, OCH_3); δ_C (DMSO-d_6) (75 MHz): 181.3, 152.1, 142.9, 136.6, 135.7, 130.3 (2 x Ar), 129.7 (2 x Ar), 126.0, 123.4, 122.4, 122.3, 121.3, 117.8, 113.5, 112.4, 110.1, 21.2; IR (KBr) ν_{max}/cm^{-1}: 3262 (NH), 2216 (CN), 1636 (C=C), 1623 (C=O); m/z (ESI M+H) 287; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{19}H_{14}N_2O$; Exact Mass: 287.1185, found: 287.1182.

*(E)-3-(4-Chlorophenyl)-2-(1H-indole-3-carbonyl)acrylonitrile (**73**)*



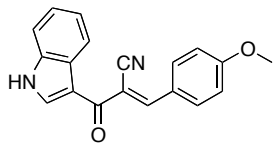
Synthesized using the general procedure as for (**72**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 4-chlorobenzaldehyde to afford (**73**) as a yellow solid (80%), m.p. 230-232 °C; δ_H (Acetone-d_6) (300 MHz): 11.31 (br, NH), 8.54 (s, 1H, H-2′), 8.34-8.30 (m, 1H, H-4′), 8.22 (s, 1H, CH=C), 8.11 (d, *J* = 8.7 Hz, 2H, H-2 and H-6), 7.66-7.57 (m, 3H, H-3, H-5 and H-7′), 7.32-7.27 (m, 2H, H-5′ and H-6′); δ_C (Acetone-d_6) (75 MHz): 180.0, 149.8, 137.0, 136.3, 134.3, 131.4 (2 x Ar), 131.0, 128.7 (2 x Ar), 126.1, 123.2, 122.0, 121.4, 116.9, 113.9, 111.9, 111.6; IR (KBr) ν_{max}/cm^{-1}: 3175 (NH), 2211 (CN), 1636 (C=C), 1600 (C=O), 822 (Ar-Cl); m/z (ESI M-H) 305; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{18}H_{11}ClN_2O$; Exact Mass: 305.0481, found: 305.0479.

*(E)-3-(4-Hydroxyphenyl)-2-(1H-indole-3-carbonyl)acrylonitrile (**74**)*



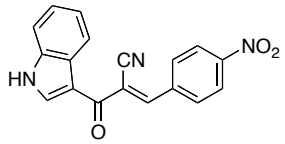
Synthesized using the general procedure as for (**72**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 4-hydroxybenzaldehyde to afford (**74**) as a yellow solid (90%), m.p. 252-253 °C; δ_H (DMSO-d_6) (300 MHz): 12.17 (br, NH), 8.40 (s, 1H, H-2′), 8.17-8.13 (m, 2H, H-4′ and CH=C), 7.98 (d, *J* = 8.8Hz, 2H, H-2 and H6), 7.53-7.51 (m, 1H, H-7′), 7.27-7.23 (m, 2H, H-5′ and H-6′), 6.94 (d, *J* = 8.8Hz, 2H, H-3 and H-5); δ_C (DMSO-d_6) (75 MHz): 181.5, 162.0, 152.3, 136.5, 135.0, 133.2 (2 x Ar), 126.1, 123.3, 122.1, 121.7, 121.3, 118.6, 116.1 (2 x Ar), 113.7, 112.3, 106.4; IR (KBr) ν_{max}/cm^{-1}: 3351 (OH), 3232 (NH), 1606 (C=O); m/z (ESI M+H) 289; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{18}H_{12}N_2O_2$; Exact Mass: 289.0978, found: 289.0979.

*(E)-2-(1H-Indole-3-carbonyl)-3-(4-methoxyphenyl)acrylonitrile (**75**)*



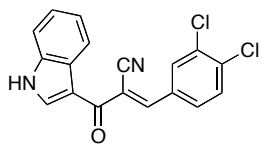
Synthesized using the general procedure as for (**72**), from 3-1*H*-indol-3-yl)-3-oxopropanenitrile and 4-methoxybenzaldehyde to afford (**75**) as a yellow solid (92%), m.p. 256-258 °C; δ_H (DMSO-d_6) (300 MHz): 12.21 (br, NH), 8.42 (s, 1H, H-2′), 8.18-8.16 (m, 2H, H-4′ and CH=C), 8.07 (d, *J* = 8.9Hz, 2H, H-2 and H-6), 7.54-7.52 (m, 1H, H-7′), 7.26-7.24 (m, 2H, H-5′ and H-6′), 7.14 (d, *J* = 8.9Hz, 2H, H-3 and H-6), 3.86 (s, 3H, OCH_3); δ_C (DMSO-d_6) (75 MHz): 181.4, 162.6, 151.9, 136.5, 135.3, 132.7 (2 x Ar), 126.2, 124.8, 123.4, 122.2, 121.3, 118.3, 114.7 (2 x Ar), 113.6, 112.3, 107.9, 55.6; IR (KBr) ν_{max}/cm^{-1}: 3220 (NH), 2221 (CN), 1591 (C=O), 1176 (C-O); m/z (ESI M-H) 301; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{19}H_{14}N_2O_2$; Exact Mass: 301.0976, found: 301.0974.

*(E)-2-(1H-Indole-3-carbonyl)-3-(4-nitrophenyl)acrylonitrile (**76**)*



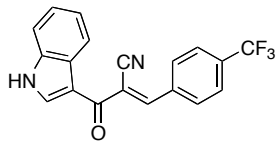
Synthesized using the general procedure as for (**72**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 4-nitrobenzaldehyde to afford (**76**) as a red solid (75%), m.p. 286-288 °C; δ_H (DMSO-d_6) (300 MHz): 12.37 (br, NH), 8.50 (s, 1H, H-2′), 8.40 (d, *J* = 8.8 Hz, 2H, H-3 and H-5), 8.34 (s, 1H, CH=C), 8.23-8.16 (m, 3H, H-2, H-6 and H-4′), 7.56-7.53 (m, 1H, H-7′), 7.30-7.27 (m, 2H, H-5′ and H-6′); δ_C (DMSO-d_6) (75 MHz): 180.7, 149.3, 148.6, 138.6, 136.8, 136.7, 131.0 (2 x Ar), 125.9, 123.9 (2 x Ar), 123.7, 122.5, 121.2, 116.7, 115.0, 113.3, 112.5; IR (KBr) ν_{max}/cm^{-1}: 3331 (NH), 2219 (CN), 1625 (C=C), 1599 (Ar), 1344 (N-O); m/z (ESI M-H) 316; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{18}H_{11}N_3O_3$; Exact Mass: 316.0721, found: 316.0721.

*(E)-2-(1H-Indole-3-carbonyl)-3-(3,4-dichlorophenyl)acrylonitrile (**77**)*



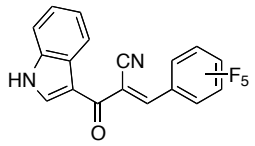
Synthesized using the general procedure as for (**72**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 3,4-dichlorobenzaldehyde to afford (**77**) as a yellow solid (88%), m.p. 288-290 °C; δ_H (DMSO-d_6) (300 MHz): 12.34 (br, NH), 8.46 (s 1H, H-2′), 8.24-8.20 (m, 3H, H-4′, H-7′ and CH=C), 8.05-8.01 (m, 1H, H-5), 7.89-8.86 (m, 1H, H-2), 7.54-7.52 (m, 1H, H-6), 7.34-7.25 (m, 2H, H-5′ and H-6′); δ_C (DMSO-d_6) (75 MHz): 180.8, 149.0, 136.7, 136.6, 134.3, 133.0, 132.0, 131.7, 131.2, 129.4, 125.9, 123.6, 122.5, 121.2, 116.9, 116.8, 113.3, 112.4; IR (KBr) ν_{max}/cm^{-1}: 3230 (NH), 2223 (CN), 1635 (C=C), 1594 (C=O), 755 (Ar-Cl); m/z (ESI M-H) 339; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{18}H_{10}Cl_2N_2O$; Exact Mass: 339.0091, found: 339.0094.

*(E)-2-(1H-Indole-3-carbonyl)-3-(4-(trifluoromethyl)phenyl)acrylonitrile (**78**)*



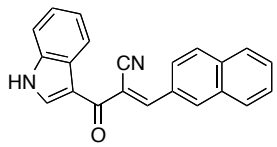
Synthesized using the general procedure as for (**72**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 4-(trifluoromethyl)benzaldehyde to afford (**78**) as a yellow solid (73%), m.p. 242-243 °C; δ_H (DMSO-d_6) (300 MHz): 12.30 (br, NH), 8.48 (s, 1H, H-2′), 8.31 (s, 1H, CH=C), 8.20-8.18 (m, 3H, H-2, H-6 and H-4′), 7.95 (d, *J* = 7.8Hz, 2H, H3 and H-5), 7.57-7.53 (m, 1H, H-7′), 7.31-7.26 (m, 2H, H-5′ and H-6′); δ_C (DMSO-d_6) (75 MHz): 180.8, 150.0, 136.7, 136.5, 136.4, 130.6 (2 x Ar), 125.9 (2 x Ar), 125.8, 125.7, 125.6, 123.6, 122.5, 121.2, 116.9, 114.2, 113.4, 112.6; IR (KBr) ν_{max}/cm^{-1}: 3211 (NH), 2223 (CN), 1596 (C=O), 1336 (C-F); m/z (ESI M-H) 339; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{19}H_{11}F_3N_2O$; Exact Mass: 339.0744, found: 339.0746.

*(E)-2-(1H-Indole-3-carbonyl)-3-(perfluorophenyl)acrylonitrile (**79**)*



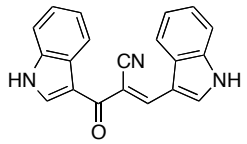
Synthesized using the general procedure as for (**72**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 2,3,4,5,6-pentafluorobenzaldehyde to afford (**79**) as a yellow solid (71%), m.p. 240-242 °C; δ_H (DMSO-d_6) (300 MHz): 12.18 (br, NH), 8.39-8.38 (m, 1H, H-2′), 7.97-7.94 (m, 1H, H-4′), 7.56-7.52 (m, 1H, H-7′), 7.31-7.17 (m, 3H, H-5′, H-6′ and CH=C); δ_C (DMSO-d_6) (75 MHz): 159.9, 138.2, 136.8, 136.0, 134.9, 130.4, 124.5, 123.9, 122.9, 122.7 (2 x Ar), 121.5 (2 x Ar), 121.2, 120.6, 119.2, 112.6, 105.1; IR (KBr) ν_{max}/cm^{-1}: 3283 (NH), 2212 (CN), 1655 (C=C), 1596 (C=O), 1207 (C-F); m/z (ESI M-H) 361; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{18}H_7F_5N_2O$; Exact Mass: 361.0400, found: 316.0403.

*(E)-2-(1H-Indole-3-carbonyl)-3-(naphthalen-2-yl)acrylonitrile (**80**)*



Synthesized using the general procedure as for (**72**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 2-naphthaldehyde to afford (**80**) as a yellow solid (72%), m.p. 302-304 °C; δ_H (DMSO-d_6) (300 MHz): 12.29 (br, NH), 8.54-8.50 (m, 2H, H-2′ and H-4′), 8.38 (s, 1H, CH=C), 8.24-8.20 (m, 2H, H-5 and H-8), 8.11-7.99 (m, 3H, H-7′, H-1 and H-4), 7.70-7.54 (m, 3H, H-3, H-6 and H-7), 7.32-7.24 (m, 2H, H-5′ and H-6′); δ_C (DMSO-d_6) (75 MHz): 181.3, 152.0, 136.6, 135.9, 134.3, 132.8, 132.4, 130.0, 128.9, 128.6, 128.5, 127.7, 127.2, 126.1, 124.8, 123.5, 122.4, 121.3, 117.8, 112.5, 112.4, 111.4; IR (KBr) ν_{max}/cm^{-1}: 3174 (NH), 2214 (CN), 1597 (C=O), 1516 (Ar); m/z (ESI M-H) 321; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{22}H_{14}N_2O$; Exact Mass: 321.1027, found: 321.1025.

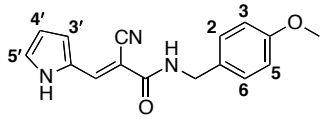
*(E)-3-(1H-Indol-3-yl)-2-(1H-indole-3-carbonyl)acrylonitrile (**81**)*



Synthesized using the general procedure as for (**72**), from 3-(1*H*-indol-3-yl)-3-oxopropanenitrile and 1*H*-indole-3-carbaldehyde to afford (**81**) as a yellow solid (79%), m.p. 292-294 °C; δ_H (DMSO-d_6) (300 MHz): 12.14 (br, 2H), 8.63 (s, 1H, H-2′), 8.60 (s, 1H, CH=C), 8.47 (s, 1H, H-2), 8.24-8.22 (m, 1H, H-4′), 7.94-7.91 (m, 1H, H-4), 7.59-7.53 (m, 2H, H-7′ and H-7), 7.31-7.21 (m, 4H, H-5′, H-6′, H-5 and H-6); δ_C (DMSO-d_6) (75 MHz): 180.7, 145.0, 136.3, 136.1, 133.7, 131.2, 127.2, 126.4, 123.3, 123.1, 121.9, 121.7, 121.5, 120.5, 118.5, 114.3, 112.7, 112.2, 110.3, 101.9; IR (KBr) ν_{max}/cm^{-1}: 3262 (NH), 2200 (CN), 1612 (C=O); m/z (ESI M-H) 310; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{20}H_{13}N_3O$; Exact Mass: 310.0980, found: 310.0983.

4.5.4 Aromatic aldehydes + *N*-(4-methoxybenzyl)propionamide

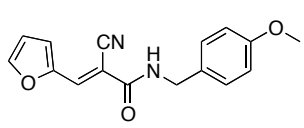
*(E)-2-cyano-N-(4-methoxybenzyl)-3-(1H-pyrrol-2-yl)acrylamide (**82**)*



Methyl 2-cyanoacetate (0.72 g, 7.28 mmol) was added to a solution of 4-methoxybenzylamine (1.00 g, 7.28 mmol) in MeOH (4 mL) and the resulting reaction mixture was heated under microwave radiation for 15 minutes at 200 W and 120 °C. After this period, the reaction mixture was cooled in the freezer for 30 minutes upon which a crystalline solid was seen to form. The solid was isolated by filtration, washed with ice cold MeOH (2 x 5 mL) and dried under vacuum to afford *N*-(4-methoxybenzyl)propionamide (966 mg, 65%).

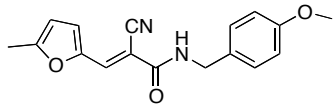
Freshly prepared *N*-(4-methoxybenzyl)propionamide (770 mg, 3.77 mmol) was added to an ethanolic solution (4 mL) of 1*H*-pyrrole-2-carbaldehyde (400 mg, 4.15 mmol) and piperidine (2 drops). The reaction mixture was subsequently heated under microwave radiation for 15 minutes at 200 W and 120 °C. After this period, the reaction mixture was cooled and the solvent removed *in vacuo* to yield a brown crude solid which was purified by flash chromatography (2:8 EtOAc:Hexanes) to afford (**82**) as a brown solid (859 mg, 81%), m.p. 203-204 ºC; δ_H (Acetone-d_6) (300 MHz): 10.94 (br, NH), 8.18 (s, 1H, CH=C), 7.65 (br, NH), 7.33-7.28 (m, 4H, H-2, H-6, H-3′ and H-5′), 6.87 (d, *J* = 8.7 Hz, 2H, H-3 and H-5), 6.43 (m, 1H, H-4′), 4.49 (d, *J* = 6.0 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3); δ_C (Acetone-d_6) (75 MHz): 160.7, 158.4, 139.6, 131.3, 130.7, 128.4 (2 x Ar), 125.7, 117.1, 116.5, 113.1 (2 x Ar), 111.6, 94.8, 54.0, 42.4; IR (KBr) ν_{max}/cm^{-1}: 3360 (NH), 3234 (NH), 2202 (CN), 1650 (C=O); m/z (ESI M+H) 282; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{16}H_{15}N_3O_2$; Exact Mass: 282.1243, found: 282.1245.

*(E)-2-Cyano-3-(furan-2-yl)-N-(4-methoxybenzyl)acrylamide (**83**)*



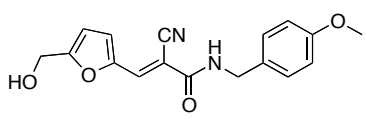
Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and furan-2-carbaldehyde to afford (**83**) as a orange solid (67%), m.p. 119-120 °C; δ_H (Acetone-d_6) (300 MHz): 8.03 (s, 1H, CH=C), 7.94 (d, *J* = 1.6 Hz, 1H, H-5′), 7.86 (br, NH), 7.36 (d, *J* = 3.6 Hz, 1H, H-3′), 7.30 (d, *J* = 8.6 Hz, 2H, H-2 and H-6), 6.88 (d, *J* = 8.6 Hz, 2H, H-3 and H-5), 6.77 (dd, *J* = 1.6 Hz, 3.6 Hz, 1H, H-4′), 4.50 (d, *J* = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3); δ_C (Acetone-d_6) (75 MHz): 159.7, 158.5, 148.6, 147.4, 135.7, 130.4, 128.5 (2 x Ar), 120.1, 115.3, 113.2 (2 x Ar), 113.0, 100.6, 54.1, 42.4; IR (KBr) ν_{max}/cm^{-1}: 3325 (NH), 2225 (CN), 1659 (C=O); m/z (ESI M+H) 283; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{16}H_{14}N_2O_3$; Exact Mass: 283.1083, found: 283.1085.

*(E)-2-Cyano-N-(4-methoxybenzyl)-3-(5-methylfuran-2-yl)acrylamide (**84**)*



Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 5-methylfuran-2-carbaldehyde to afford (**84**) as a white solid (92%), m.p. 146-147 °C; δ_H ($CDCl_3$) (300 MHz): 7.94 (s, 1H, CH=O), 7.83 (br, NH), 7.32-7.26 (m, 3H, H-2, H-6 and H-3′), 6.88 (d, *J* = 8.7 Hz, 2H, H-3 and H-5), 6.43-6.42 (m, 1H), H-4′), 4.49 (d, *J* = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3); δ_C ($CDCl_3$) (75 MHz): 160.0, 158.4, 147.3, 135.4, 130.6, 128.5 (2 x Ar), 122.3, 115.6, 113.1 (2 x Ar), 110.0, 99.9, 98.3, 54.0, 42.5, 12.5; IR (KBr) ν_{max}/cm^{-1}: 3364 (NH), 2214 (CN), 1671 (C=O); m/z (ESI M+H) 297; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{17}H_{16}N_2O_3$; Exact Mass: 297.1240, found: 297.1238.

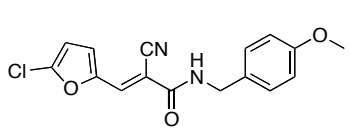
*(E)-2-Cyano-3-(5-(hydroxymethyl)furan-2-yl)-N-(4-methoxybenzyl)acrylamide (**85**)*



Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 5-(hydroxymethyl)furan-2-carbaldehyde to afford (**85**) as a white solid (45%), m.p. 130-132 °C; δ_H (Acetone-d_6) (300 MHz): 7.98 (s, 1H, CH=O), 7.82 (br, NH), 7.33-7.29 (m, 3H, H-2, H-6 and H-3′), 6.88 (d, *J* = 8.7 Hz, 2H, H-3 and H-5), 6.62 (d, *J* = 3.5 Hz, 1H, H-4′), 4.65 (s, 2H, CH_2OH), 4.50 (br, OH), 4.49 (d, *J* = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3); δ_C (Acetone-d_6) (75 MHz): 161.1, 159.8, 158.5, 147.8, 135.7, 130.5, 128.5 (2 x Ar), 121.1, 115.4, 113.2 (2 x Ar), 109.9, 99.6, 56.1, 54.1, 42.6; IR (KBr) ν_{max}/cm^{-1}: 3347 (NH), 3235 (OH), 2215 (CN), 1667 (C=O), 1248 (C-O); m/z (ESI M+H) 313; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{17}H_{16}N_2O_4$; Exact Mass: 313.1189,

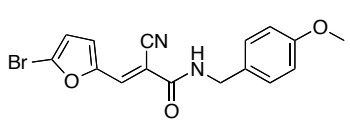
found: 313.1192.

*(E)-3-(5-Chlorofuran-2-yl)-2-cyano-N-(4-methoxybenzyl)acrylamide (**86**)*



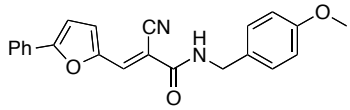
Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 5-chlorofuran-2-carbaldehyde to afford (**86**) as a yellow solid (71%), m.p. 164-165 °C; δ_H ($CDCl_3$) (300 MHz): 7.99 (s, 1H, CH=C), 7.25 (d, *J* = 8.5 Hz, 2H, H-2 and H-6), 7.20-7.19 (m, 1H, H-3′), 6.88 (d, *J* = 8.5 Hz, 2H, H-3 and H-6), 6.55 (br, NH), 6.44-6.43 (m, 1H, H-4′), 4.51 (d, *J* = 5.6 Hz, 2H, CH_2NH), 3.81 (s, 3H, OCH_3); δ_C ($CDCl_3$) (75 MHz): 159.3, 158.8, 147.9, 142.5, 135.6, 128.8 (2 x Ar), 128.6, 122.0, 115.8, 113.7 (2 x Ar), 110.1, 99.4, 54.8, 43.5; IR (KBr) ν_{max}/cm^{-1}: 3342 (NH), 2217 (CN), 1666 (C=O), 1251 (C-O); m/z (ESI M+H) 317; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{16}H_{13}ClN_2O_3$; Exact Mass: 317.0694, found: 317.0691.

*(E)-3-(5-Bromofuran-2-yl)-2-cyano-N-(4-methoxybenzyl)acrylamide (**87**)*



Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 5-bromofuran-2-carbaldehyde to afford (**87**) as a yellow solid (84%), m.p. 160-162 °C; δ_H ($CDCl_3$) (300 MHz): 8.01 (s, 1H, CH=C), 7.25 (d, *J* = 8.6 Hz, 2H, H-2 and H-6), 7.16 (d, *J* = 3.7 Hz, 1H, H-3′), 6.89 (d, *J* = 8.6 Hz, 2H, H-3 and H-5), 6.58-6.57 (m, 2H, H-4′ and NH), 4.51 (d, *J* = 5.7 Hz, 2H, CH_2NH), 3.80 (s, 3H, OCH_3); δ_C ($CDCl_3$) (75 MHz): 159.3, 158.7, 150.2, 135.5, 129.1, 128.8 (2 x Ar), 128.6, 122.0, 115.7, 115.1, 113.7 (2 x Ar), 99.5, 54.8, 43.5; IR (KBr) ν_{max}/cm^{-1}: 3348 (NH), 2219 (CN), 1672 (C=O), 1247 (C-O), 807 (Ar-Br); m/z (ESI M+H) 361; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{16}H_{13}BrN_2O_3$; Exact Mass: 361.0189, found: 361.0186.

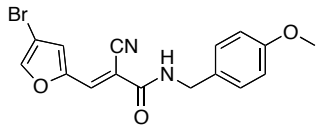
*(E)-2-Cyano-N-(4-methoxybenzyl)-3-(5-phenylfuran-2-yl)acrylamide (**88**)*



Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 5-phenylfuran-2-carbaldehyde to afford (**88**) as a yellow solid (85%), m.p. 203-205 °C; δ_H (DMSO-d_6) (300 MHz): 8.81 (t, *J* = 5.9 Hz, NH), 7.99 (s, CH=C), 7.90 (d, *J* = 7.3 Hz, 2H, H-2″ and H-6″), 7.53-7.48 (m, 2H, H-3″ and H-5″), 7.44-7.42 (m, 2H, H4″ and H-3′), 7.34-7.33 (m, 1H, H-4′), 7.23 (d, *J* = 8.6 Hz, 2H, H-2 and H-6), 6.88 (d *J* = 8.6 Hz, 2H, H-3 and H-5), 4.32 (d, *J* = 5.9 Hz, 2H, CH_2NH), 3.71 (s, 3H, OCH_3); δ_C (DMSO-d_6) (75 MHz): 160.9, 158.2, 157.7, 147.7, 135.1, 130.9, 129.5, 129.1 (2 x Ar), 128.7 (2 x Ar), 128.6, 124.7 (2 x Ar), 124.6, 116.6, 113.6 (2 x Ar), 109.6, 99.7, 54.9, 42.6; IR (KBr) ν_{max}/cm^{-1}: 3362

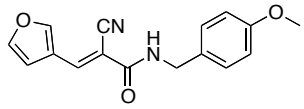
(NH), 2209 (CN), 1666 (C=O), 1607 (Ar), 1247 (C-O); m/z (ESI M+H) 359; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{22}H_{18}N_2O_3$; Exact Mass: 359.1396, found: 359.1394.

*(E)-3-(4-Bromofuran-2-yl)-2-cyano-N-(4-methoxybenzyl)acrylamide (**89**)*



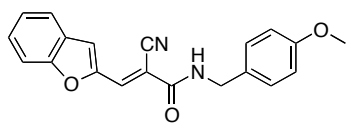
Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 4-bromofuran-2-carbaldehyde to afford (**89**) as a white solid (70%), m.p. 169-170 °C; δ_H ($CDCl_3$) (300 MHz): 8.04 (s, 1H, CH=C), 7.68 (s, 1H, H-5′), 7.26-7.23 (m, 3H, H-2, H-6 and H-3′), 6.88 (d, *J* = 8.6 Hz, 2H, H-3 and H-5), 6.59 (br, NH), 4.51 (d, *J* = 5.7 Hz, 2H, CH_2NH), 3.80 (s, 3H, OCH_3); δ_C ($CDCl_3$) (75 MHz): 159.0, 158.8, 148.9, 144.8, 136.1, 128.8 (2 x Ar), 128.5, 121.7, 115.6, 113.7 (2 x Ar), 102.6, 101.1, 54.8, 43.6; IR (KBr) ν_{max}/cm^{-1}: 3332 (NH), 2223 (CN), 1660 (C=O), 1254 (C-O), 926 (Ar-Br); m/z (ESI M+H) 361; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{16}H_{13}BrN_2O_3$; Exact Mass: 361.0189, found: 361.0192.

*(E)-2-Cyano-3-(furan-3-yl)-N-(4-methoxybenzyl)acrylamide (**90**)*



Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and furan-3-carbaldehyde to afford (**90**) as a brown solid (60%), m.p. 122-124 °C; δ_H (Acetone-d_6) (300 MHz): 8.32 (s, 1H, H-2′), 8.21 (s, 1H, CH=C), 7.84 (br, NH), 7.78-7.77 (m, 1H, H-5′), 7.30 (d *J* = 8.7 Hz, 2H, H-2 and H-6), 7.23-7.22 (m, 1H, H-4′), 6.88 (d, *J* = 8.7 Hz, 2H, H-3 and H-5), 4.49 (d, *J* = 6.0 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3); δ_C (Acetone-d_6) (75 MHz): 159.7, 158.5, 149.4, 145.0, 141.6, 130.4, 128.5 (2 x Ar), 120.4, 115.8, 113.2, (2 x Ar), 107.3, 103.8, 54.1, 42.5; IR (KBr) ν_{max}/cm^{-1}: 3332 (NH), 2213 (CN), 1661 (C=O), 1611 (Ar), 1513 (Ar), 1251 (C-O); m/z (ESI M+H) 283; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{16}H_{14}N_2O_3$; Exact Mass: 283.1083, found: 283.1080.

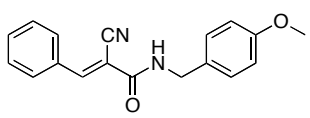
*(E)-3-(Benzofuran-2-yl)-2-cyano-N-(4-methoxybenzyl)acrylamide (**91**)*



Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and benzofuran-2-carbaldehyde to afford (**91**) as a white solid (87%), m.p. 150-153 °C; δ_H (DMSO-d_6) (300 MHz): 8.96 (t, *J* = 5.8 Hz, NH), 8.14 (s, 1H, CH=C), 7.83-7.80 (m, 1H, H-4′), 7.76 (s, 1H, H-3′), 7.68-7.65 (m, 1H, H-7′), 7.54-7.49 (m, 1H, H-6′), 7.38-7.32 (m, 1H, H-5′), 7.25 (d, *J* = 8.5 Hz, 2H, H-2 and H-6), 6.88 (d, *J* = 8.5 Hz, 2H, H-3 and H-5), 4.34 (d, *J* = 5.8 Hz, 2H, CH_2NH), 3.72 (s, 3H, OCH_3); δ_C (DMSO-d_6) (75 MHz):

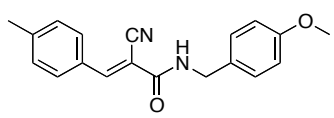
160.4, 158.2, 155.3, 149.6, 136.3, 130.7, 128.8 (2 x Ar), 128.5, 127.3, 124.1, 123.0, 117.3, 115.7, 113.6 (2 x Ar), 111.6, 104.1, 55.0, 42.6; IR (KBr) ν_{max}/cm^{-1}: 3342 (NH), 2225 (CN), 1672 (C=O), 1513 (Ar), 1251 (C-O); m/z (ESI M+H) 333; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{20}H_{16}N_2O_3$; Exact Mass: 323.1240, found: 333.1239.

*(E)-2-Cyano-N-(4-methoxybenzyl)-3-phenylacrylamide (**92**)*[7]



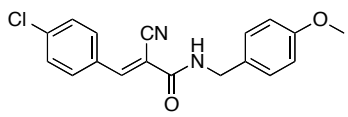
Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and benzaldehyde to afford (**92**) as a white solid (63%), m.p. 117-118 °C (Lit. not reported); δ_H (Acetone-d_6) (300 MHz): 8.27 (s, 1H, CH=C), 8.00 (m, 3H, H-2′, H-6′ and NH), 7.57 (m, 3H, H-3′, H-4′ and H-5′), 7.32 (d, *J* = 8.7 Hz, 2H, H-2 and H-6), 6.89 (d, *J* = 8.7 Hz, 2H, H-3 and H-5), 4.52 (d, *J* = 5.9 Hz, 2H, CH_2NH), 3.78 (s, 3H, OCH_3); δ_C (Acetone-d_6) (75 MHz): 159.7, 158.5, 150.7, 131.8 (2 x Ar), 130.9, 130.3, 129.7 (2 x Ar), 128.6 (2 x Ar), 115.6, 113.2 (2 x Ar), 105.4, 104.4, 54.0, 42.6; IR (KBr) ν_{max}/cm^{-1}: 3336 (NH), 2223 (CN), 1663 (C=O), 1259 (C-O); m/z (ESI M+H) 293; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{18}H_{16}N_2O_2$; Exact Mass: 293.1291, found: 293.1289.

*(E)-2-Cyano-N-(4-methoxybenzyl)-3-p-tolylacrylamide (**93**)*



Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 4-methylbenzaldehyde to afford (**93**) as a yellow solid (70%), m.p. 115-117 °C; δ_H (Acetone-d_6) (300 MHz): 8.22 (s, 1H, CH=C), 7.90 (d, *J* = 8.2 Hz, 2H, H-2′ and H-6′), 7.38 (d, *J* = 8.2Hz, 2H, H-3′ and H-5′), 7.32 (d, *J* = 8.8 Hz, 2H, H-2 and H-6), 6.88 (d, *J* = 8.8 Hz, 2H, H-3 and H-5), 4.51 (d, *J* = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3); δ_C (Acetone-d_6) (75 MHz): 159.9, 158.4, 150.7, 142.8, 130.4, 129.9 (2 x Ar), 129.3 (2 x Ar), 129.1, 128.6 (2 x Ar), 115.7, 113.2 (2 x Ar), 103.9, 54.0, 42.6, 20.2; IR (KBr) ν_{max}/cm^{-1}: 3339 (NH), 2220 (CN), 1662 (C=O), 1259 (C-O); m/z (ESI M+H) 307; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{19}H_{18}N_2O_2$; Exact Mass: 307.1447, found: 307.1445.

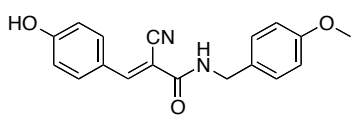
*(E)-3-(4-Chlorophenyl)-2-cyano-N-(4-methoxybenzyl)acrylamide (**94**)*[7]



Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 4-chlorobenzaldehyde to afford (**94**) as a yellow solid (28%), m.p. 134-135 °C (Lit. not reported); δ_H (Acetone-d_6) (300 MHz): 8.25 (s, 1H, CH=C), 8.01 (d, *J* = 8.8 Hz, 2H, H-2′ and H-6′), 7.60 (d, *J* = 8.8 Hz, 2H, H-3′ and H-5′), 7.31 (d, *J* = 8.7 Hz, 2H, H-2 and H-6), 6.89 (d, *J* = 8.7 Hz, 2H, H-3 and H-5), 4.51 (d, *J* = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3);

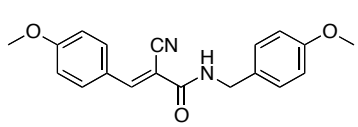
δ_C (Acetone-d_6) (75 MHz): 159.5, 158.5, 149.3, 137.1, 131.3 (2 x Ar), 130.5, 130.3, 128.8 (2 x Ar), 128.6 (2 x Ar), 115.4, 113.2 (2 x Ar), 106.0, 54.1, 42.6; IR (KBr) ν_{max}/cm^{-1}: 3361 (NH), 2216 (CN), 1664 (C=O), 1252 (C-O), 819 (Ar-Cl); m/z (ESI M+H) 327; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{18}H_{15}ClN_2O_2$; Exact Mass: 327.0901, found: 327.0904.

*(E)-2-Cyano-3-(4-hydroxyphenyl)-N-(4-methoxybenzyl)acrylamide (**95**)*[7]



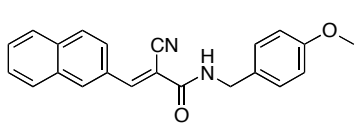
Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 4-hydroxybenzaldehyde to afford (**95**) as a light yellow solid (20%), m.p. 197-198 °C (Lit. not reported); δ_H (Acetone-d_6) (300 MHz): 8.16 (s, 1H, CH=C), 7.94 (d, *J* = 8.8Hz, 2H, H-3 and H-5), 7.81 (br, NH), 7.31 (d, *J* = 8.6 Hz, 2H, H-2′ and H-6′), 7.00 (d, *J* = 8.8 Hz, 2H, H-2 and H-6), 6.88 (d, *J* = 8.6 Hz, 2H, H-3′ and H-5′), 4.50 (d, *J* = 5.9 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3); δ_C (Acetone-d_6) (75 MHz): 161.0, 160.2, 150.5, 132.5 (2 x Ar), 130.6, 128.5 (2 x Ar), 123.4, 116.4, 115.6 (2 x Ar), 113.1 (2 x Ar), 102.8, 100.8, 54.0, 42.5; IR (KBr) ν_{max}/cm^{-1}: 3342 (NH), 3153 (OH), 2212 (CN), 1645 (C=O), 1172 (C-O); m/z (ESI M+H) 309; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{18}H_{16}N_2O_3$; Exact Mass: 309.1240, found: 309.1238.

*(E)-2-Cyano-3-(4-methoxyphenyl)-N-(4-methoxybenzyl)acrylamide (**96**)*[7]



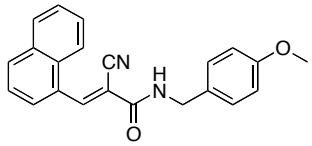
Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 4-hydroxybenzaldehyde to afford (**96**) as a light yellow solid (20%), m.p. 197-198 °C (Lit. not reported); δ_H (Acetone-d_6) (300 MHz): 8.19 (s, 1H, CH=C), 8.01 (d, *J* = 8.9 Hz, 2H, H-2′ and H-6′), 7.84 (br, NH), 7.31 (d, *J* = 8.5 Hz, 2H, H-2 and H-6), 7.10 (d, *J* = 8.9 Hz, 2H, H-3′ and H-5′), 6.89 (d, *J* = 8.5 Hz, 2H, H-3 and H-5), 4.50 (d, *J* = 6.0 Hz, 2H, CH_2NH), 3.91 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3); δ_C (Acetone-d_6) (75 MHz): 162.7, 160.2, 158.5, 150.3, 132.1 (2 x Ar), 130.5, 128.5 (2 x Ar), 124.3, 116.5, 114.1 (2 x Ar), 113.1 (2 x Ar), 101.6, 54.6, 54.0, 42.5; IR (KBr) ν_{max}/cm^{-1}: 3420 (NH), 2200 (CN), 1667 (C=O), 1250 (C-O), 1178 (C-O); m/z (ESI M+H) 323; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{19}H_{18}N_2O_3$; Exact Mass: 323.1396, found: 323.1393.

*(E)-2-Cyano-N-(4-methoxyphenyl)-3-(naphthalen-2-yl)acrylamide (**97**)*



Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 2-naphthaldehyde to afford (**97**) as a yellow solid (45%), m.p. 143-144 °C; δ_H (Acetone-d_6) (300 MHz): 8.49 (s, 1H CH=C), 8.43 (s, 1H, H-1′), 8.20 (dd, *J* = 1.7, 8.7 Hz, 1H, H-8′), 8.09-7.99 (m, 4H, H-5′, H-4′, H-6′ and NH), 7.70-7.60 (m, 2H, H-5′ and H-3′), 7.34 (d, *J* = 8.7 Hz, 2H, H-2 and H-6), 6.90 (d, *J* = 8.7 Hz, 2H, H-3 and H-5), 4.54 (d, *J* = 5.9 Hz, 2H, CH_2NH), 3.78 (s, 3H, OCH_3); δ_C (Acetone-d_6) (75 MHz): 160.9, 158.2, 150.5, 134.2, 132.6, 132.3, 130.7, 129.4, 128.9 (2 X Ar), 128.8, 128.7, 128.6, 127.7, 127.2, 124.5, 116.4, 113.6 (2 x Ar), 106.1, 55.0, 42.6; IR (KBr) ν_{max}/cm^{-1}: 3371 (NH), 2212 (CN), 1676 (C=O), 1253 (C-O); m/z (ESI M+H) 343; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{22}H_{18}N_2O_2$; Exact Mass: 343.1447, found: 343.1445.

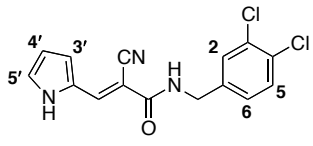
*(E)-2-Cyano-N-(4-methoxyphenyl)-3-(naphthalen-1-yl)acrylamide (**98**)*



Synthesized using the general procedure as for (**82**), from *N*-(4-methoxybenzyl)propioamide and 1-naphthaldehyde to afford (**98**) as a clear oil (70%); δ_H (Acetone-d_6) (300 MHz): 9.03 (s, 1H, CH=C), 8.14-8.07 (m, 4H, H-5′, H-8′, H-4′ and NH), 8.02-7.99 (m, 1H, H-2′), 7.67-7.58 (m, 3H, H-3′, H-6 and H-7′), 7.37 (d, *J* = 8.7 Hz, 2H, H-2 and H-6), 6.90 (d, *J* = 8.7 Hz, 2H, H-3 and H-5), 4.57 (d, *J* = 6.0 Hz, 2H, CH_2NH), 3.77 (s, 3H, OCH_3); δ_C (Acetone-d_6) (75 MHz): 159.7, 158.6, 148.7, 133.1, 131.6, 130.9, 130.3, 128.7 (2 x Ar), 128.4, 127.0, 126.7, 126.2, 125.3, 124.8, 122.8, 115.3, 113.2 (2 x Ar), 109.3, 54.1, 42.7; IR (KBr) ν_{max}/cm^{-1}: 3357 (NH), 2227 (CN), 1676 (C=O), 1237 (C-O); m/z (ESI M+H) 343; HRMS (ESI M+H): Calculated for Chemical Formula: $C_{22}H_{18}N_2O_2$; Exact Mass: 343.1447, found: 343.1448.

4.5.5 Aromatic aldehydes + *N*-(3,4-dichlorobenzyl)propioamide

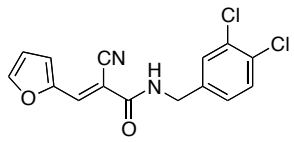
*(E)-2-Cyano-N-(3,4-dichlorobenzyl)-3-(1H-pyrrol-2-yl)acrylamide (**99**)*



Synthesized using the general procedure as for (**82**), from *N*-(3,4-dichlorobenzyl)propioamide and 1*H*-pyrrole-2-carbaldehyde to afford (**99**) as a yellow solid (80%), m.p. 234-235 °C; δ_H (DMSO-d_6) (300 MHz): 11.88 (br, NH), 8.67 (t, *J* = 5.9 Hz, NH), 8.05 (s, 1H, CH=C), 7.58-7.53 (m, 2H, H5 and H2), 7.30-7.26 (m, 3H, H6, H5′ and H3′), 6.42-6.40 (m, 1H, H4′), 4.36 (d, *J* = 5.9 Hz, 2H, CH_2NH); δ_C (DMSO-d_6) (75 MHz): 161.8, 141.1, 140.5, 140.3, 130.7, 130.4, 129.3, 127.7, 126.5, 126.3, 117.7, 115.5,

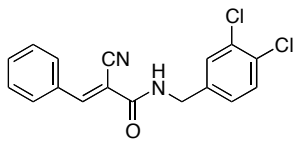
112.5, 94.3, 42.0; IR (KBr) ν_{max}/cm^{-1}: 3342 (NH), 3232 (NH), 2209 (CN), 1643 (C=O), 749 (Ar-Cl); m/z (ESI M-H) 318; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{15}H_{11}Cl_2N_3O$; Exact Mass: 318.0200, found: 318.0198.

*(E)-2-Cyano-N-(3,4-dichlorobenzyl)-3-(furan-2-yl)acrylamide (**100**)*



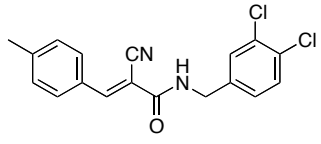
Synthesized using the general procedure as for (**82**), from *N*-(3,4-dichlorobenzyl)propioamide and furan-2-carbaldehyde to afford (**100**) as a light yellow solid (76%), m.p. 198-199 °C; δ_H (DMSO-d_6) (300 MHz): 8.92 (t, *J* = 5.9 Hz, NH), 8.12 (d, *J* = 1.6 Hz, 1H, H-5′), 7.99 (s, 1H, CH=C), 7.58 (d, *J* = 8.3 Hz, 1H, H-5), 7.55 (d, *J* = 2.0 Hz, 1H, H-1), 7.37 (d, *J* = 3.6 Hz, 1H, H-3′), 7.29 (dd, *J* = 2.0, 8.3 Hz, 1H, H-6), 6.81 (dd, *J* = 1.6, 3.6 Hz, 1H, H-4′), 4.37 (d, *J* = 5.9 Hz, 2H, CH_2NH); δ_C (DMSO-d_6) (75 MHz): 161.0, 148.7, 148.3, 140.1, 136.1, 130.7, 130.4, 129.4, 127.7, 121.8, 116.0, 113.8, 103.4, 100.3, 42.1; IR (KBr) ν_{max}/cm^{-1}: 3358 (NH), 2216 (CN), 1671 (C=O), 757 (Ar-Cl); m/z (ESI M-H) 319; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{15}H_{10}Cl_2N_2O_2$; Exact Mass: 319.0040, found: 319.0039.

*(E)-2-Cyano-N-(3,4-dichlorobenzyl)-3-phenylacrylamide (**101**)*



Synthesized using the general procedure as for (**82**), from *N*-(3,4-dichlorobenzyl)propioamide and benzaldehyde to afford (**101**) as a yellow solid (57%), m.p. 159-160 °C; δ_H (Acetone-d_6) (300 MHz): 8.29 (s, 1H, CH=C), 8.22 (br, NH), 8.02-7.99 (m, 2H, H-2′ and H-6′), 7.61-7.51 (m, 5H, H-3′, H-4′, H-5′, H-1 and H-5), 7.38 (dd, *J* = 2.0, 8.3 Hz, 1H, H-6), 4.60 (d, *J* = 6.0 Hz, 2H, CH_2NH); δ_C (Acetone-d_6) (75 MHz): 160.2, 151.2, 139.6, 131.9, 131.7, 131.1, 129.9, 129.8 (2 x Ar), 129.5, 129.3 (2 x Ar), 128.6, 127.3, 115.5, 104.9, 42.1; IR (KBr) ν_{max}/cm^{-1}: 3364 (NH), 2215 (CN), 1678 (C=O), 679 (Ar-Cl); m/z (ESI M-H) 329; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{17}H_{12}Cl_2N_2O$; Exact Mass: 329.0248, found: 329.0250.

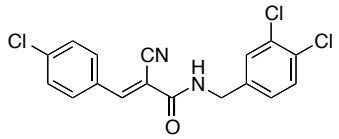
*(E)-2-Cyano-N-(3,4-dichlorobenzyl)-3-p-tolylacrylamide (**102**)*



Synthesized using the general procedure as for (**82**), from *N*-(3,4-dichlorobenzyl)propioamide and 4-methylbenzaldehyde to afford (**102**) as a light yellow solid (62%), m.p. 161-162 °C; δ_H (DMSO-d_6) (300 MHz): 8.99 (t, *J* = 5.8 Hz, NH), 8.16 (s, 1H, CH=C), 7.85 (d, *J* = 8.1 Hz, 2H, H-2′ and H-6′), 7.59-7.56 (m, 2H, H-5 and H-1), 7.36 (d, *J* = 8.1 Hz, 2H, H-3′ and H-6′), 7.31 (dd,v*J* = 1.9, 8.3 Hz, 1H, H-6), 4.40 (d, *J* = 5.8 Hz, 2H, CH_2NH), 2.37 (s, 3H, OCH_3); δ_C

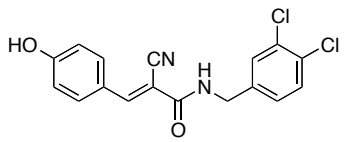
(DMSO-d_6) (75 MHz): 161.2, 150.9, 143.0, 140.1, 130.8, 130.4, 130.1, 129.8 (2 x Ar), 129.7 (2 x Ar), 129.4, 129.1, 127.8, 116.4, 104.5, 42.1, 21.1; IR (KBr) ν_{max}/cm^{-1}: 3366 (NH), 2217 (CN), 1681 (C=O), 813 (Ar-Cl); m/z (ESI M-H) 343; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{18}H_{14}Cl_2N_2O$; Exact Mass: 343.0404, found: 343.0406.

*(E)-3-(4-Chlorophenyl)-2-cyano-N-(3,4-dichlorobenzyl)acrylamide (**103**)*



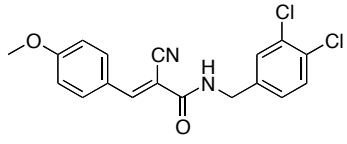
Synthesized using the general procedure as for (**82**), from *N*-(3,4-dichlorobenzyl)propioamide and 4-chlorobenzaldehyde to afford (**103**) as a yellow solid (30%), m.p. 156-158 °C; δ_H (Acetone-d_6) (300 MHz): 8.27 (s, 1H, CH=C), 8.24 (br, NH), 8.02 (d, *J* = 8.5 Hz, 2H, H-2′ and H-6′), 7.63-7.60 (m, 3H, H-3′, H-5′ and H-5), 7.53 (d, *J* = 8.3 Hz, 1H, H-2), 7.38 (dd, *J* = 2.0, 8.3 Hz, 1H, H-6), 4.60 (d, *J* = 6.1 Hz, 2H, CH_2NH); δ_C (Acetone-d_6) (75 MHz): 159.9, 149.7, 139.5, 138.3, 137.2, 131.3 (2 x Ar), 131.1, 130.5, 129.9, 129.3, 128.8, 127.3 (2 x Ar), 124.6, 115.3, 42.2; IR (KBr) ν_{max}/cm^{-1}: 3384 (NH), 2212 (CN), 1673 (C=O); m/z (ESI M-H) 363; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{17}H_{11}Cl_3N_2O$; Exact Mass: 362.9858, found: 362.9860.

*(E)-2-Cyano-N-(3,4-dichlorobenzyl)-3-(4-hydroxyphenyl)acrylamide (**104**)*



Synthesized using the general procedure as for (**82**), from *N*-(3,4-dichlorobenzyl)propioamide and 4-hydroxybenzaldehyde to afford (**104**) as a yellow solid (61%), m.p. 230-232 °C; δ_H (DMSO-d_6) (300 MHz): 10.57 (br, OH), 8.86 (t, *J* = 5.8 Hz, NH), 8.08 (s, 1H, CH=C), 7.87 (d, *J* = 8.7 Hz, 2H, H-2′ and H-6′), 7.58-7.54 (m, 2H, H-1 and H-5), 7.29 (dd, *J* = 2.0, 8.3 Hz, 1H, H-6), 6.92 (d, *J* = 8.7 Hz, 2H, H-3′ and H-5′), 4.38 (d, *J* = 5.8 Hz, 2H, CH_2NH); δ_C (DMSO-d_6) (75 MHz): 161.8, 161.7, 150.8, 140.3, 132.9 (2 x Ar), 130.7, 130.4, 129.4, 129.3, 127.7, 122.8, 117.0, 116.1 (2 x Ar), 100.6, 42.1; IR (KBr) ν_{max}/cm^{-1}: 3350 (NH), 3140 (OH), 2217 (CN), 1642 (C=O), 837 (Ar-Cl); m/z (ESI M-H) 345; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{17}H_{12}Cl_2N_2O_2$; Exact Mass: 345.0197, found: 345.0199.

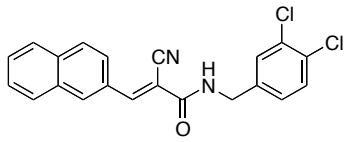
*(E)-2-Cyano-N-(3,4-dichlorobenzyl)-3-(4-methoxyphenyl)acrylamide (**105**)*



Synthesized using the general procedure as for (**82**), from *N*-(3,4-dichlorobenzyl)propioamide and 4-methoxybenzaldehyde to afford (**105**) as a light yellow solid (58%), m.p. 169-170 °C; δ_H (DMSO-d_6) (300 MHz): 8.91 (t, *J* = 5.8 Hz, 1H, NH), 8.14 (s, 1H, CH=C), 7.96 (d, *J* = 8.9 Hz, 2H, H-2′ and H-6′),

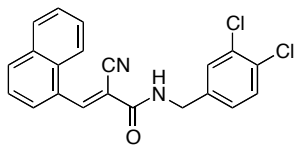
7.59-7.56 (m, 2H, H-1 and H-5), 7.30 (dd, *J* = 1.9, 8.2 Hz, 1H, H-6), 7.11 (d, *J* = 8.9 Hz, 2H, H-3′ and H-5′), 4.39 (d, *J* = 5.8 Hz, 2H, CH_2NH), 3.84 (s, 3H, OCH_3); δ_C (DMSO-d_6) (75 MHz): 162.6, 161.5, 150.5, 140.2, 132.5 (2 x Ar), 130.7, 130.4, 129.4, 129.4, 127.7, 124.3, 116.8, 114.7 (2 x Ar), 102.1, 55.5, 42.1; IR (KBr) ν_{max}/cm^{-1}: 3368 (NH), 2208 (CN), 1673 (C=O), 1182 (C-O), 832 (Ar-Cl); m/z (ESI M-H) 359. HRMS (ESI M-H): Calculated for Chemical Formula: $C_{18}H_{14}Cl_2N_2O_2$; Exact Mass: 359.0353, found: 359.0355.

*(E)-2-Cyano-N-(3,4-dichlorobenzyl)-3-(naphthalen-2-yl)acrylamide (**106**)*



Synthesized using the general procedure as for (**82**), from *N*-(3,4-dichlorobenzyl)propioamide and 2-naphthaldehyde to afford (**106**) as a light yellow solid (40%), m.p. 180-181 °C; δ_H (DMSO-d_6) (300 MHz): 9.08 (t, *J* = 5.6 Hz, NH), 8.45 (s, 1H, CH=C), 8.36 (s, 1H, H-1′), 8.14-7.97 (m, 4H, H-5′, H-8′, H-4′ and H-5), 7.69-7.58 (m, 4H, H-6′, H-7′, H-3 and H-1), 7.33 (dd, *J* = 1.8, 8.3 Hz, 1H, H-6), 4.43 (d, *J* = 5.6 Hz, 2H, CH_2NH); δ_C (DMSO-d_6) (75 MHz): 161.2, 151.0, 140.0, 134.3, 132.7, 132.3, 130.8, 130.4, 129.5, 129.4, 129.0, 128.8, 128.6, 127.8, 127.8, 127.7, 127.2, 124.5, 116.4, 105.7, 42.2; IR (KBr) ν_{max}/cm^{-1}: 3370 (NH), 2212 (CN), 1686 (C=O), 1257 (C-O), 739 (Ar-Cl); m/z (ESI M-H) 379; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{21}H_{14}Cl_2N_2O$; Exact Mass: 379.0404, found: 379.0401.

*(E)-2-Cyano-N-(3,4-dichlorobenzyl)-3-(naphthalen-1-yl)acrylamide (**107**)*



Synthesized using the general procedure as for (**82**), from *N*-(3,4-dichlorobenzyl)propioamide and 1-naphthaldehyde to afford (**107**) as a light yellow solid (57%), m.p. 173-175 °C; δ_H (DMSO-d_6) (300 MHz): 9.25 (t, *J* = 5.7 Hz, NH), 8.90 (s, 1H, CH=C), 8.14-8.02 (m, 4H, H-5′, H-4′, H-8′ and H-2′), 7.68-7.59 (m, 5H, H-3′, H-6′, H-7′, H-2 and H-5), 7.37 (dd, *J* = 1.7, 8.3 Hz, 1H, H-6), 5.73 (d, *J* = 5.7 Hz, 2H, CH_2NH); δ_C (DMSO-d_6) (75 MHz): 161.0, 148.9, 140.0, 132.9, 131.9, 130.8, 130.7, 130.4, 129.5, 129.4, 129.2, 128.7, 127.8, 127.5, 127.2, 126.8, 125.4, 123.6, 115.9, 110.3, 42.1; IR (KBr) ν_{max}/cm^{-1}: 3370 (NH), 2214 (CN), 1680 (C=O), 779 (Ar-Cl); m/z (ESI M-H) 379; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{21}H_{14}Cl_2N_2O$; Exact Mass: 379.0404, found: 379.0405.

4.6 References

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[7] McCluskey, A. Robinson, P. J., Hill, T., Scott, J. L., Edwards, J. K., *Tetrahedron Letters,* **2002**, 43(17), 3117-3120.

Chapter 5 – Appendix

5.1 Paper III Supplementary Data

Supplementary Data

Norcantharidin analogues with nematocidal activity in *Haemonchus contortus*

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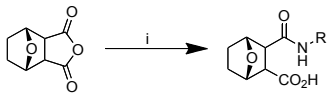
Library A: Preparation of 7-Oxabicyclo(2.2.1)heptane-2-carboxylic acid derivatives

General Synthetic Scheme for carboxylic acid derivatives

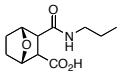
Synthesis of ring-opened acid amide norcantharidin analogues (*Library A)*: In a typical synthesis, a solution of maleic anhydride (4.90 g, 0.05 mol) dissolved in ether (50 mL) and furan (17.01 g, 0.25 mol) was allowed to stir overnight at room temperature (22-24 °C). The white solid produced was then filtered and dried under suction before being dissolved in ethyl acetate (EtOAc) (25 mL) and hydrogenated for 16 h using a palladium catalyst (0.500 g, 10% Pd-C). The catalyst was then filtered from the solution, and the ethyl acetate was removed by rotary evaporation to yield 7.2 g of white crystalline norcantharidin (86%).

The 7-oxabicyclo(2.2.1)heptane-2-carboxylic acid derivatives were prepared by treating norcantharidin directly with amines. In a typical procedure the norcantharidin (168 mg, 1 mmol) was dissolved in tetrahydrofuran (20 mL) and to this the requisite amine (1.1 equiv) added portionwise (for solids) or dropwise (in tetrahydrofuran (5 mL) for liquids. After complete reaction (TLC) the solid was filtered, or the solvent evaporated (in those cases when no precipitate was evident) and the product purified by flash chromatography (hexanes : ethyl acetate).



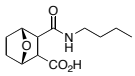
Scheme S1. Reagents and conditions: (i) RNH_2, THF, rt.

*3-Propylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A1**)*



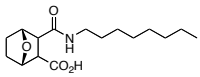
MP 125-126 °C; ^{1}H NMR (300 MHz) (DMSO-d_6): δ 0.80 (3H, t, *J* = 7.5 Hz), 1.34-1.36 (2H, m), 1.41-1.51 (4H, m), 2.81 (2H, s), 2.90 (1H, q, *J* = 5.8 Hz), 4.45 (1H, d, *J* = 3.1 Hz), 4.71 (1H, d, *J* = 2.0 Hz), 7.23 (1H, d); ^{13}C NMR (75 MHz) (DMSO-d_6): δ 11.0, 22.2, 28.3, 28.7, 40.3, 51.4, 53.1, 76.7, 78.7, 170.2, 172.2.

*3-(Butylcarbamoyl)-7-oxabicyclo(2.2.1)heptane-2-carboxylic acid (**A2**)*



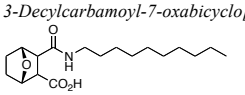
MP 101-104 °C; ^{1}H NMR (300 MHz) (DMSO-d_6): δ 0.84 (3H, t, *J* = 7.2 Hz), 1.26-1.34 (4H, m), 1.46-1.52 (4H, m), 2.81 (2H, s), 2.96 (2H, q, *J* = 5.9 Hz), 4.45 (1H, d, *J* = 4.0 Hz), 4.71 (1H, d, *J* = 3.6 Hz), 7.24 (1H, brs); ^{13}C NMR (75 MHz) (DMSO-d_6): δ 13.5, 19.4, 28.3, 28.7, 31.0, 51.5, 53.1, 76.7, 78.7, 170.2, 172.2.

*3-Octylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A3**)*



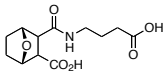
MP 131-132 ºC; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 0.84 (3H, t, *J* = 5.6 Hz), 1.22 (12H, bs), 1.51-1.31 (4H, m), 2.80 (2H, bs), 2.94 (2H, q, *J* = 5.6 Hz), 4.43 (1H, d, *J* = 3.9 Hz), 4.71 (1H, d, *J* = 2.6 Hz), 7.34 (1H, t, *J* = 5.6 Hz), 11.81 (1H, bs); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 13.9, 22.0, 26.4, 28.3, 28.6, 28.7, 28.8, 28.9, 31.2, 38.3, 51.3, 52.9,76.6, 78.7, 170.2, 172.3.

*3-Decylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A4**)*



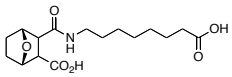
MP 110-111 °C; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 0.84 (3H, t, *J* = 5.6 Hz), 1.22 (16H, bs), 1.54-1.28 (4H, m), 2.80 (2H, bs), 2.94 (2H, t, *J* = 5.6 Hz), 4.43 (1H, d, *J* = 3.9 Hz), 4.71 (1H, d, *J* = 2.6 Hz), 7.33 (1H, t, *J* =5.6 Hz), 12.01 (1H, bs); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 13.8, 22.0, 26.4, 28.3, 28.6, 28.7, 28.8, 28.9, 31.2, 38.4, 51.3,52.9, 76.2, 78.7, 170.2, 172.3.

*3-(3-Carboxpropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A5**)*



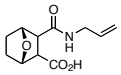
MP 135-137 °C; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.52 (4H, m), 2.16 (2H, t), 2.78 (2H, t), 3.02 (2H, m), 4.47 (1H, d, *J* = 2.4 Hz), 4.72 (1H, d, *J* = 2.5 Hz), 7.25 (1H, t, *J* = 5.6 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 24.8, 28.3, 28.7, 31.7, 37.9, 52.3, 53.3, 76.9, 78.3, 170.9, 172.8, 174.7.

*3-(7-Carboxyheptylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A6**)*



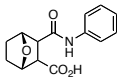
MP 125-126 °C; ^{1}H NMR (300 MHz) (DMSO-d_6): δ 1.23 (6H, m), 1.51-1.46 (4H, m), 2.17 (2H, m), 2.81 (2H, m), 2.96 (2H, m), 4.45 (1H, d), 4.70 (1H, d), 7.27 (1H, m); ^{13}C NMR (75 MHz) (DMSO-d_6): δ 24.4, 26.2, 28.4, 28.7, 28.8, 33.6, 38.4, 51.4, 53.0, 76.7, 78.7, 170.2, 172.3, 174.4.

*3-Allylcarbamoyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A7**)*



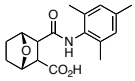
MP 105-107 °C; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.46-1.52 (4H, m), 2.91 (1H, d, *J* = 5.1 Hz), 2.16 (1H, d, *J* = 5.1 Hz), 3.72 (2H, m), 4.67 (2H, t, *J* = 5.2 Hz), 5.06-5.13 (2H, m), 5.76-5.82 (1H, m), 8.14 (1H, brs); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 24.5, 29.0, 50.0, 51.8, 77.6, 79.6, 115.0, 135.2, 168.9, 173.0, 173.8.

*3-(Phenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A9**)*



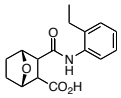
^{1}H NMR (300 MHz) (DMSO-d_6): 1.48-1.59 (4H, m), 2.92 (1H, d, J = 9.4 Hz), 3.03 (1H, d, J = 9.4 Hz), 4.62 (1H, d, J = 3.9 Hz), 4.76 (1H, s), 7.01 (1H, t, J = 6.7 Hz), 7.25 (1H, t, J = 7.6 Hz, 7.50 (1H, d, J = 7.6 Hz), 9.64 (1H, s). ^{13}C NMR (75 MHz) (DMSO-d_6): 29.3, 29.8, 52.6, 54.4, 77.8, 79.6, 120.1 (2C), 123.8, 129.4 (2C), 140.1, 170.1, 173.1.

*3-(2',4',6'-Trimethylphenylcarbamoyl)-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (**A10**)*



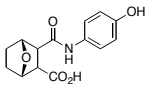
^{1}H NMR (300 MHz) (DMSO-d_6): δ 1.51-1.66 (4H, m), 2.08 (6H, s), 2.19 (3H, s), 2.88 (1H, d, J = 9.4 Hz), 3.12 (1H, d, J = 9.4 Hz), 4.58 (1H, d, J = 4.5 Hz), 4.77 (1H, s), 6.82 (2H, s), 8.87 (1H, s), 11.85 (1H, brs); ^{13}C NMR (75 MHz) (DMSO-d_6): δ 17.9 (2C), 18.0, 28.5, 29.0, 50.9, 52.9, 76.6, 79.3, 128.0, 132.4, 134.9, 135.0, 168.8, 172.2.

*3-(2-Ethylphenylcarbamoyl)-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (**A11**)*



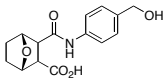
MP 171-172 °C; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 1.12 (3H, t, J = 5.8 Hz), 1.53-1.67 (4H, m), 2.55 (2H, q, J = 5.8 Hz), 3.05 (1H, d, J = 9.6 Hz), 3.10 (1H, d, J = 9.6 Hz), 4.72 (1H, d, J = 3.6 Hz), 4.84 (1H, d, J = 2.8 Hz), 7.18-7.01 (3H, m), 7.65 (1H, d, J = 8.8 Hz), 8.72 (1H, bs), 12.07 (1H, bs); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 14.0, 23.5, 28.38, 28.43, 51.7, 54.1, 77.0, 79.0, 123.2, 124.4, 125.8, 128.2, 135.1, 135.7, 169.4, 172.2.

*3-(4-Hydroxyphenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A12**)*



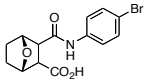
MP 154-156 °C; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 1.15-1.47 (4H, m), 2.90 (1H, d, J = 9.6 Hz), 2.99 (1H, d, J = 9.6 Hz), 4.59 (1H, d, J = 4.0 Hz), 4.76 (1H, d, J = 2.8 Hz), 6.65 (2H, d, J = 8.6 Hz), 7.27 (2H, d, J = 8.6 Hz), 9.08 (1H, bs), 9.29 (1H, bs), 11.68 (1H, bs); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 172.2, 168.6, 153.1, 130.8, 120.9, 114.8, 78.6, 76.7, 53.3, 51.5, 28.9, 28.3.

*3-(4'-Benzylalcoholcarbamoyl)-7-oxabicyclo(2.2.1)heptane-2-carboxylic acid (**A13**)*



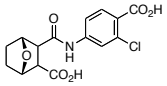
MP 137-138 °C; ^{1}H NMR (DMSO-d_6): 1.50-1.65 (4H, m), 2.97 (2H, dd, *J* = 26.4 Hz), 4.39 (2H, d, *J* = 3.9 Hz), 4.61 (1H, d, *J* = 3.9 Hz), 4.75 (1H, d, *J* = 2.2 Hz), 5.07 (1H, brs), 7.19 (1H, d, *J* = 8.3 Hz), 7.45 (1H, d, *J* = 8.3 Hz), 9.62 (1H, s); ^{13}C NMR (75 MHz) (DMSO-d_6): 29.3, 29.9, 52.6, 54.5, 63.6, 77.8, 79.6, 119.9 (2C), 127.7 (2C), 138.0, 138.7, 170.1, 173.1.

*3-(4-Bromophenylcarbamoyl)-7-Oxabicyclo(2.2.1)heptane-2-carboxylic acid (**A14**)*



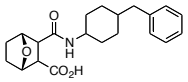
MP 187-189°C; ^{1}H NMR (DMSO-d_6): 1.45-1.60 (4H, m), 2.93 (1H, d, *J* = 9.3Hz), 3.03 (1H, d, *J* = 9.6Hz), 4.63 (1H, d, *J* = 3.1Hz), 4.76 (1H, d, *J* = 3.3Hz), 7.42-7.51 (4H, q, *J* = 8.7Hz), 9.78 (1H, s), 11.96 (1H, s); ^{13}C NMR (75 MHz) (DMSO-d_6): 28.3, 28.9, 51.6, 53.3, 76.8, 78.5, 114.3, 121.0, 131.3, 138.5, 169.4, 172.0.

*3-(4-Carboxy-3-chlorophenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A15**)*



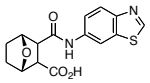
MP 216-217 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.48-1.56 (4H, m), 2.96 (1H, d, *J* = 9.6 Hz), 3.04 (1H, d, *J* = 9.6 Hz), 3.36 (1H, bs), 4.65 (1H, d, *J* = 3.6 Hz), 4.76 (1H, d, *J* = 2.8 Hz), 7.42 (1H, dd, *J* = 8.6, 2.0 Hz), 7.80 (1H, d, *J* = 8.6 Hz), 7.87 (1H, d, *J* = 1.9 Hz), 10.17 (1H, s), 12.56 (1H, bs); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 28.3, 28.8, 51.7, 53.2, 76.9, 78.4, 116.8, 120.1, 124.0, 132.2, 133.0, 142.8, 165.8, 170.1, 172.0.

*3-(1-Benzylpiperidin-4-ylcarbamoyl]-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A16**)*



MP 124-125 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56-1.52 (2H, m), 1.67-1.75 (45H, m), 1.94-2.01 (2H, m), 2.69-2.79 (2H, m), 2.90 (1H, d, *J* = 9.9 Hz), 2.95 (1H, d, *J* = 9.9 Hz), 3.12-3.26 (2H, m), 3.35 (1H, s), 3.72-3.81 (1H, m), 4.00 (2H, s), 4.58 (1H, d, *J* = 4.5 Hz), 4.90 (1H, d, *J* = 4.5 Hz), 7.39-7.44 (5H, m), 12.56 (1H, bs); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 27.9, 28.2, 43.7, 49.8, 50.0, 53.8, 54.3, 60.1, 77.8, 78.7, 127.9, 128.1, 129.9, 131.6, 172.6, 175.0.

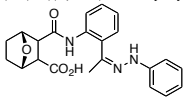
*3-(Benzo[d]thiazol-5-ylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A17**)*



MP 159-160 °C; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 1.49-1.61 (4H, m), 2.98 (1H, d, *J* = 9.8 Hz), 3.11 (1H, d, *J* = 9.6 Hz), 4.67 (1H, d, *J* = 2.9 Hz), 4.79 (1H, d, *J* = 2.6 Hz), 7.52 (1H, dd, *J* = 8.8, 2.6 Hz), 7.98 (1H, d, *J* = 8.8 Hz), 8.47 (1H,

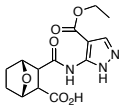
d, *J* = 2.6 Hz), 9.23 (1H, s), 10.0 (1H, bs), 12.00 (1H, bs); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 28.3, 28.9, 51.7, 53.3, 76.8, 78.6, 111.4, 118.7, 122.7, 134.1, 136.9, 148.8, 154.3, 169.6, 172.1.

(3-(2-((Z)-1-(2-Phenylhydrazono)ethyl)phenylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid ***(A18)***



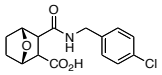
MP 102 °C; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 1.29-1.49 (4H, m), 2.29 (3H, s), 2.85 (1H, d, *J* = 9.7 Hz), 2.94 (1Hd, *J* = 9.7 Hz), 4.62 (1H, d, *J* = 2.6 H z), 4.71 (1H, d, *J* = 3.1 Hz), 6.08-6.77 (1H, m), 7.09 (1H, t, *J* = 7.4 Hz), 7.21-7.26 (5H, m), 7.46 (1H, d, *J* = 7.8 Hz), 8.13 (1H, d, *J* = 8.1 Hz), 9.22 (1H, bs), 10.69 (1H, bs), 12.01 (1H, bs); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 15.9, 28.3, 30.5, 51.9, 54.3, 76.8, 78.2, 112.9, 119.2, 121.5, 123.0, 127.6, 128.2, 128.4, 128.8, 135.8, 143.1, 145.7, 169.1, 171.9.

3-[4-(Ethoxycarbonyl)-1H-pyrazol-3-ylcarbamoyl]-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid ***(A19)***



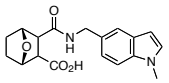
MP 173-175 °C; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 1.28 (3H, t, *J* = 7.09 Hz), 1.56-1.69 (4H, m), 3.16 (2H, bs), 4.18-4.26 (2H, m); 4.79 (1H, d, *J* = 4.2 Hz), 4.90 (1H, d, *J* = 3.6 Hz), 7.75 (1H, bs), 9.86 (1H, bs), 12.07 (1H, bs); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 14.1, 28.0, 28.5, 52.1, 54.2, 59.5, 77.2, 78.6, 98.8, 137.6, 141.6, 162.5, 169.5, 171.8.

3-(4-Chlorobenzylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid ***(A20)***



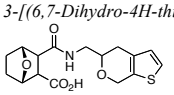
MP: 183-185ºC; ^{1}H NMR (DMSO-d_6): 1.41-1.56 (4H, m), 2.87 (2H, q, *J* = 9.0 Hz), 4.19 (2H, m), 4.50 (1H, d, *J* = 4.0Hz), 4.73 (1H, d *J* = 2.8 Hz), 7.25-7.41 (4H, q), 7.96 (1H, t, *J* = 5.6 Hz). ^{13}C NMR (75 MHz) (DMSO-d_6): 28.4, 28.8, 41.4, 51.4, 52.9, 76.8, 78.7, 128.0, 129.0, 138.6, 170.6, 172.3.

3-[(1-Methyl-1H-indol-4-yl)methylcarbamoyl]-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid ***(A21)***



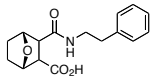
MP 161-162 °C; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 1.44-1.51 (4H, m), 2.83 (1H, d, *J* = 9.6 Hz), 2.65 (1H, d, *J* = 9.6 Hz), 4.45 (3H, m), 3.77 (3H, s), 4.74 (1H, d, *J* = 5.2 Hz), 6.48 (1H, d, *J* = 2.9 Hz), 6.94 (1H, d, *J* = 7.2 Hz), 7.08 (1H, t, *J* = 8.6 Hz), 7.29 (1H, d, *J* = 2.9 Hz), 7.32 (1H, d, *J* = 7.2 Hz), 7.88 (1H, t, *J* = 5.2 Hz), 12.08 (1H, bs); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 28.3, 28.8, 32.5, 40.5, 51.1, 52.9, 76.7, 79.0, 98.6, 108.5, 117.6, 120.8, 126.5,129.2, 130.3, 136.2, 170.2, 172.4.

*3-[(6,7-Dihydro-4H-thieno[3,2-c]pyran-4-yl)methylcarbamoyl]-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A22**)*



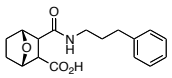
MP 159-160 °C; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 1.44-1.51 (4H, m), 2.48 (1H, dd, *J* = 5.3, 1.7 Hz), 2.74 (1H, bs), 2.81 (1H, d, *J* = 9.6 Hz), 2.91 (1H, d, *J* = 9.7 Hz), 3.03- 3.12 (1H, m), 3.54-3.70 (2H, m), 4.04-4.11 (1H, m), 4.33 (1H, d, *J* = 3.7 Hz), 4.58-4.60 (1H, m), 4.71 (1H, d, *J* = 2.9 Hz), 6.86 (1H, d, *J* = 5.2 Hz), 7.31 (1H, d, *J* = 5.2 Hz), 7.64 (1H, t, *J* = 5.6 Hz), 11.78 (1H, bs); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 24.9, 28.3, 28.8, 42.6, 51.2, 52.7, 62.3, 74.0, 78.8, 76.6, 123.0, 123.9, 133.3, 134.7, 170.7, 172.3.

3-(Phenethylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A23**)



MP 135-137ºC; ^{1}H NMR (300 MHz) (DMSO-d_6): 1.43-1-56 (4H, m), 1.64 (2H, quin *J* = 7.3Hz), 2.52 (2H, t *J* = 7.5Hz), 2.98 (2H, q *J* = 6.2Hz), 4.47 (1H, d *J* = 4.3Hz), 4.71 (1H, d *J* = 2.3Hz), 7.15-7.28(5H, m), 7.34 (1H, t *J* = 5.3Hz). ^{13}C NMR (75 MHz) (DMSO-d_6): 28.6, 28.8, 30.7, 32.5, 38.9, 51.5, 53.0, 76.7, 78.6, 125.6, 128.1, 128.2, 141.7, 170.3, 172.2.

*3-(3-Phenylpropylcarbamoyl)-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid (**A24**)*



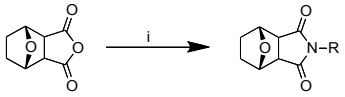
MP 105-107ºC; ^{1}H NMR (DMSO-d_6): 1.35-1.60 (8H, m), 2.51 (2H, quin *J* = 7.2 Hz), 2.98 (2H, q), 4.43 91H, d), 4.69 (1H, d, *J* = 2.3 Hz), 7.15-7.29 (5H, m), 7.30 (1H, t, *J* = 5.2 Hz); ^{13}C NMR (75 MHz) (DMSO-d_6): 28.3, 28.5, 34.7, 38.2, 49.3, 52.3, 53.3, 77.0, 78.5, 125.6, 128.2, 142.1, 170.7, 172.7.

Library B: Preparation of 7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide derivatives

General Synthetic Scheme for dicarboxide derivatives

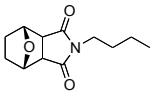
Synthesis of ring closed cantharimide analogues (*Library B*): A thick-walled pressure tube containing a solution of norcantharidin (0.1505 g, 0.9 mol) in anhydrous toluene (10 ml), an equimolar amount of the amine (0.9 mol) and triethylamine (1 ml) was degassed, and then sealed and heated (CAUTION: this step must be conducted behind a safety screen) in an oil bath at 200 ºC for 24 h. After cooling to room temperature, the reaction mixture was taken up in ethyl acetate (25 ml) and then washed with saturated $NaHCO_3$ (2 x 25 mL). The pH of the aqueous layer was adjusted to pH 2 by the cautious addition of concentrated HCl. The acidic layer was extracted with ethyl acetate (3 x 25 mL), dried over $MgSO_4$ and the solvent removed *in vacuo*, in order to yield the crude norcantharimide, which was either a solid or an oil. This crude product was purified by flash chromatography, eluting with ethyl acetate:methanol (19:1). The desired products were obtained with yields in the range of 15-75%. All reagents were of commercial quality and were used as

received (Aldrich). Solvents were dried and purified using standard techniques.[68] Reactions were monitored by thin layer chromatography (TLC) on aluminium plates coated with silica gel employing a fluorescent indicator (Merck 60 F_{254}).



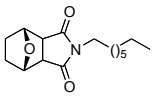
Scheme S2. Reagents and conditions: (i) RNH_2, $PhCH_3$, sealed tube, 140 ºC, 36 h.

N-Butyl-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide ***(B1)***



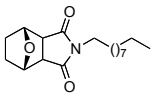
MP 82-83 °C; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 0.90 (3H, t, *J* = 7.2 Hz), 1.28 (2H, sep, *J* = 7.8 Hz), 1.49-1.59 (4H, m), 1.82-1.85 (2H, m), 2.83 (2H, s), 3.45 (2H, t, *J* = 7.3 Hz), 4.85 (2H, q, *J* = 0.7 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 12.9, 19.3, 28.0 (2C), 29.0, 38.2, 49.3 (2C) 78.4, 176.6 (2C).

*N-Octyl-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (****B2****)*



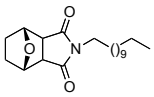
MP 36-37 °C; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 0.85 (3H, t, *J* = 6.9 Hz), 1.24 (10H, s), 1.52-1.59 (4H, m), 1.81-1.84 (2H, m), 2.82 (2H, s), 3.43 (2H, t, *J* = 7.5 Hz), 4.84 (2H, q, *J* = 1.0 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 13.4, 22.0, 21.9, 26.1, 27.0, 28.0 (2C), 28.4, 28.5, 31.1, 38.5, 49.3 (2C) 78.4, 176.5 (2C).

N-Decyl-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (***B3***)



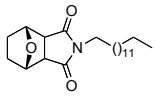
MP 30-31 °C; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 0.88 (3H, t, *J* = 6.7 Hz), 1.26 (14H, s), 1.52-1.59 (4H, m), 1.82-1.85 (2H, m), 2.81 (2H, s), 3.44 (2H, t, *J* = 7.3 Hz), 4.84 (2H, t, *J* = 2.1 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 14.0, 22.7, 26.7, 27.6, 28.7 (2C), 29.1, 29.3, 29.5, 32.0, 39.2, 50.5 (2C), 79.1 (2C), 177.2 (2C).

*N-Dodecyl-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (****B4****)*



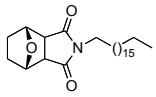
MP 35-37 ºC; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 0.87 (4H, m), 1.24 (20H, s), 1.85-1.82 (2H, m), 1.60-1.50 (4H, m), 2.83 (2H, s), 3.44 (2H, t *J* = 7.3 Hz), 4.85 (2H, q *J* = 2.3 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 13.5, 22.1, 26.1, 27.0, 28.0, 28.5, 28.8, 28.9, 29.0, 38.5, 49.3, 78.5, 176.7 (2C).

*4-Tetradecyl-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (**B5**)*



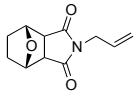
MP 45-46 °C; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 0.86 (2H, m), 1.23 (20H, s), 1.56 (2H, m), 1.83 (2H, quin, *J* = 4.5 Hz), 2.82 (2H, s), 3.42 (2H, t, *J* = 7.3 Hz), 4.84 (2H, q, *J* = 2.2 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 13.5, 22.1, 26.1, 28.0, 28.5, 29.0, 29.1, 38.5, 49.3, 78.5, 176.7 (2C).

*4-Octadecyl-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (**B6**)*



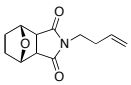
MP 62-64 ºC; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.58 (2H, m), 1.83-1.91 (4H, m), 2.86 (2H, s), 2.33 (2H, t, *J* = 7.4 Hz), 3.54 (2H, t, *J* = 6.8 Hz), 4.86 (2H, q, *J* = 2.3 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 22.0, 28.0, 30.4, 37.5, 49.3, 78.5, 176.7, 177.1 (2C).

*N-Allyl-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (**B7**)*



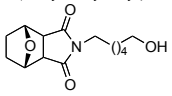
MP 116-117 ºC; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.55-1.62 (2H, m), 1.85-1.88 (2H, m), 2.89 (2H, s), 4.08 (2H, dd, *J* = 4.0, 1.3 Hz), 4.88-4.90 (2H, m), 5.16-5.22 (2H, m), 5.71-5.76 (1H, m); ^{13}C NMR (75 MHz) ($DMSO-d_6$): δ 28.0 (2C), 40.3, 49.4, 78.4, 116.9, 129.8, 176.0 (2C).

*4-But-3-enyl-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (**B8**).*



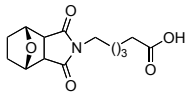
MP 64–65 ºC: ^{1}H NMR (300 MHz) ($DMSO-d_6$): δ 1.61 (4H, brs), 2.17 (2H, q, *J* = 6.9 Hz), 3.00 (2H, s), 3.37 (2H, t, *J* = 6.9 Hz), 4.66 (2H, s, 2H, 1 x CH), 4.99 (2H, m), 5.65 (1H, m); ^{13}C NMR (75 MHz) ($DMSO-d_6$): δ 28.8 (2C), 32.2, 38.2, 50.2 (2C), 79.2, 79.2, 117.9, 135.5, 170.1 (2C).

*4-(6-Hydroxyhexyl)-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (**B9**)*



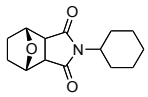
MP 56-57 ºC; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.27 (4H, m), 1.48 (2H, m), 1.57 (2H, m), 2.15 (2H, m), 2.82 (2H, s), 3.41 (2H, m), 3.54 (2H, m), 4.80 (2H, m); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 24.5, 25.6, 26.8, 27.9, 31.8, 38.3, 49.3, 61.9, 78.5, 176.8 (2C).

*N-Hexanoic acid-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (**B10**)*



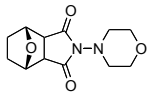
MP 110-112 °C; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.30 (2H, m), 1.60 (6H, m), 1.83 (2H, m), 2.32 (2H, t, J = 7.44 Hz), 2.86 (2H, s), 3.45 (2H, t, J = 7.23 Hz), 4.86 (2H, q, J = 2.2 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 23.5, 25.4, 26.6, 28.0, 33.2, 38.2, 49.3, 78.5, 176.8, 178.6.

*4-Cyclohexyl-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (**B11**)*



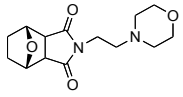
MP 97-99 °C; ^{1}H NMR (300 MHz) (DMSO-d_6): δ 1.16 (4H, m), 1.53 (6H, m), 1.77 (4H, m), 2.05 (2H, m), 2.73 (2H, s), 3.86 (1H, m), 4.78 (2H, m); ^{13}C NMR (75 NHz) (DMSO-d_6): δ 25.0, 25.8, 28.5, 28.6, 49.4, 51.9, 79.1, 177.3 (2C).

*4-Morpholin-4-yl-7-Oxabicyclo(2.2.1)heptane-2,3-dicarboximide (**B12**)*



MP 169-171 °C; ^{1}H NMR (300 MHz) (DMSO-d_6): δ 1.53 (2H, m), 1.78 (2H, m), 2.73 (2H, s), 3.18 (4H, t, J = 4.5 Hz), 3.71 (4H, t, J = 4.7 Hz), 4.78 (2H, m); ^{13}C NMR (75 MHz) (DMSO-d_6): δ 28.5, 47.9, 51.2, 66.7, 79.1, 175.1.

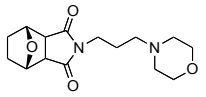
*4-(2-Morpholin-4-ylethyl)-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide Dione (**B13**)*



10

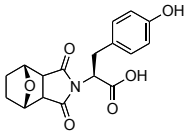
MP 109-111 °C; ^{1}H NMR (300 MHz) (DMSO-d_6): δ 1.56 (2H, m), 1.80 (2H, m), 2.43-2.48 (6H, m), 2.84 (2H, s), 3.55 (2H, t, *J* = 4.5 Hz), 3.60 (4H, t, *J* = 4.5 Hz), 4.82 (2H, m); ^{13}C NMR (75 MHz) (DMSO-d_6): δ 28.6, 36.6, 49.9, 53.4, 55.1, 67.0, 79.0, 177.1 (2C).

*4-(3-Morpholin-4-ylpropyl)-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (**B14**)*



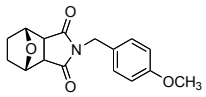
^{1}H NMR (300 MHz) (DMSO-d_6): δ 1.55 (2H, m), 1.70 (2H, s), 1.81 (2H, m), 2.81 (2H, s), 2.30 (2H, t, *J* = 4.5 Hz), 2.37 (4H, m), 3.48 (2H, t, *J* = 4.5 Hz), 3.65 (4H, t, *J* = 4.7 Hz), 4.82 (2H, m); ^{13}C NMR (75 MHz) (DMSO-d_6): δ 24.2, 28.5, 37.2, 49.9, 51.3, 53.4, 55.8, 66.8, 79.0, 177.2 (2C).

*2-(3,5-Dioxo-10-oxa-4-azatricyclo[5.2.1.02,6]dec-4-yl)-3-(4-hydroxyphenyl)propionic acid (**B15**)*



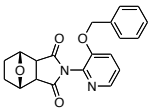
MP 158-161 °C; ^{1}H NMR (300 MHz) (DMSO-d_6): δ 1.56 (4H, m), 2.89 (1H, d, *J* = 7.2 Hz), 2.98 (1H, d, *J* = 7.2 Hz), 3.10 (2H, d, *J* = 11 Hz), 3.30 (2H, m), 4.50 (1H, m), 4.65 (1H, m), 4.73 (1H, q, *J* = 4.6, 11 Hz), 7.09 (2H, d, *J* = 7.3 Hz), 7.18 (2H, d, *J* = 7.3 Hz); ^{13}C NMR (75 MHz) (DMSO-d_6): δ 27.8, 28.0, 33.2, 126.4, 48.9, 49.0, 53.4, 78.1, 128.1, 128.9, 137.0, 169.3, 176.5 (2C).

*4-(4-Methoxybenzyl)-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide (**B16**)*



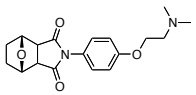
MP 83-85 ºC; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.60 (2H, m), 1.85 (2H, m), 2.84 2H, s), 3.77 (3H, s), 4.56 (2H, s), 4.87 (2H, q *J* = 2.1Hz), 6.81 (2H, d *J* = 6.7Hz), 7.25 (2H, d, *J* = 6.6Hz); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 28.0, 41.4, 49.5, 54.6, 78.5, 113.4, 127.3, 129.1, 158.7, 176.2 (2C).

*3-[3-(Benzyloxy)pyridin-2-yl]-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione (**B17**)*



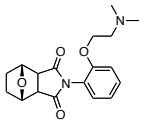
MP 99-102 °C; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 1.52-1.49 (4H, m), 2.89 (2H, s), 4.65 (2H, t, *J* = 2.4 Hz), 5.10 (2H, s), 6.46 (1H, dd, *J* = 7.7, 5.0 Hz), 7.06 (1H, dd, *J* = 7.8, 1.2 Hz), 7.34 (4H, m), 7.48 (2H, m); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 28.5, 51.5, 68.9, 77.6, 111.9, 116.7, 127.4, 127.7, 128.3, 136.8, 138.3, 140.4, 150.8, 172.4.

4-{4-[2-(Dimethylamino)ethoxy]phenyl}-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione ***(B18)***



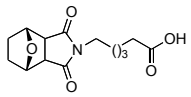
MP 145-146 °C; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 1.52-1.49 (4H, m), 2.27 (6H, s), 2.68 (2H, t, *J* = 5.8 Hz), 2.85 (2H, s), 3.91 (2H, t, *J* = 5.8 Hz), 4.65 (2H, t, *J* = 2.4 Hz), 6.48 (2H, d, *J* = 8.8 Hz), 6.64 (2H, d, *J* = 8.8 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.6, 142.5, 115.3, 114.8, 78.2, 65.6, 57.4, 52.0, 45.0, 149.5, 172.7.

4-{2,[2-(Dimethylamino)ethoxy]phenyl}-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione ***(B19)***



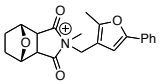
MP 113-115 °C; ^{1}H NMR (300 MHz) (DMSO-d_6) δ 1.53-1.45 (4H, m), 2.24 (6H, s), 2.71 (2H, t, *J* = 5.2 Hz), 3.96 (2H, s), 4.20 (2H, t, *J* = 5.2 Hz), 4.67 (2H, t, *J* = 2.4 Hz), 6.98 (1H, t, *J* = 8.1 Hz), 7.12 (1H, d, *J* = 8.0 Hz), 7.35 (2H, m). ^{13}C NMR (75 MHz) (DMSO-d_6) δ 28.9, 44.1, 53.1, 56.9, 65.1, 79.2, 113.0, 120.8, 123.1, 130.2, 130.5, 156.5, 173.7.

N-Hexanoic acid-7-oxabicyclo(2.2.1)heptane-2,3-dicarboximide ***(B20)***



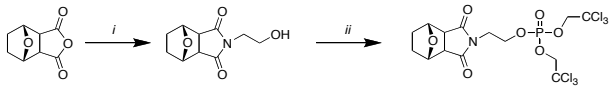
MP 110-112 °C; ^{1}H NMR ($CDCl_3$): δ 1.30 (2H, m), 1.60 (6H, m), 1.83 (2H, m), 2.32 (2H, t, *J* = 7.44 Hz), 2.86 (2H, s), 3.45 (2H, t, *J* = 7.23 Hz), 4.86 (2H, q, *J* = 2.2 Hz); ^{13}C NMR ($CDCl_3$): δ 23.5, 25.4, 26.6, 28.0, 33.2, 38.2, 49.3, 78.5, 176.8, 178.6.

4-[(4-Methyl)-3-ethyl-2-methyl-5-phenylfuran-10-oxa-4-azatricyclo[5.2.1]decane-3,5-dione ***(B21)***



MP 191 ºC; 1H NMR (300 MHz) (DMSO-d_6): δ 1.48 (4H, m), 2.24 (3H, s), 2.45 (3H, s), 3.92 (2H, s), 2.75 (2H, s), 4.65 (2H, d, *J* = 2.4 Hz), 6.37 (1H, s), 7.42 (5H, m); ^{13}C NMR (75 MHz) (DMSO-d_6): δ 13.2, 28.9, 33.2, 44.1, 53.1, 79.1, 109.4, 116.1, 125.7, 127.5, 128.7, 130.0, 148.3, 151.1, 173.9.

*4-Aza-4-(2-{bis(2,2,2-trichloroethyl)phosphate}ethyl)-10-oxatricyclo[5.2.1.0$^{(2,6)}$]decane-3,5-dione (**B22**)*



Scheme S3. Synthessis of **B22**. Reagents and Conditions. (i) $H_2NCH_2CH_2OH$, $PhCH_3$, Et_3N, D; (ii) $ClP(O)(OCCl_3)_2$, n-Bu_2O, Et_3N, rt.

To norcantharidin (168 mg, 1 mmol) in toluene (10 mL) was added 2-aminoethanol (67 mg, 1.1 mmol, 1.1 equiv) and triethylamine (202 mg, 2.0 equiv). The resultant solution was heated at reflux for 18 h and evaporated to dryness. The desired compound was obtained via flash column chromatography using EtOAc as the solvent as a white solid (73%), MP 164–166 ºC. ^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.61 (m, 2H), 1.84 (2H, m), 2.89 (2H, s), 3.01 (2H, br s), 3.70 (2H, t, *J* = 4.8 Hz), 3.64 (2H, q, *J* = 3.6 Hz), 4.86 (2H d, *J* = 2.1 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 27.9, 41.3, 49.4, 59.5, 78.6, 177.1.

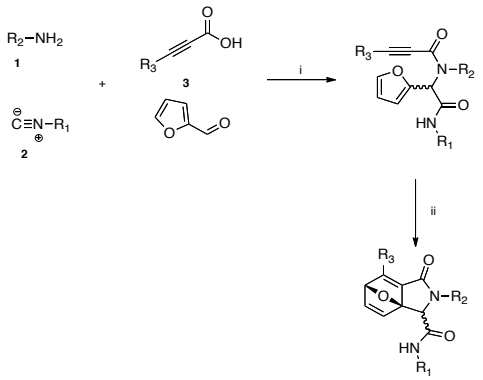
To a solution of the above compound, 4-aza-4-(2-hydroxyethyl)-10-oxatricyclo[5.2.1.0]decane-3,5-dione (211 mg, 1 mmol) in anhydrous CH_2Cl_2 (10 mL) was added n-Bu_2SnO (50 mg, 0.2 mmol, 0.2 equiv) and the resulting mixture stirred at room temperature for 30 min under a N_2 atmosphere. Next diethyl chlorophosphate (172.5 mg, 1 mmol, 1.0 equiv) and triethylamine (500 μL, 3.6 mmol, 3.6 equiv) was then added and the mixture stirred for 18 h. The reaction was then quenched with H_2O (10 mL). The organic layer was then separated, dried ($MgSO_4$) and evaporated to dryness. The crude product was then purified via flash column chromatography using 4:1 EtOAc/hexanes as the solvent to yield the title compound as a colourless oil (60%).

^{1}H NMR (300 MHz) ($CDCl_3$): δ 1.55 (2H, m), 1.75 (2H, m), 3.75 (2H, t, *J* = 4.8 Hz), 4.28 (2H, t, *J* = 8.8, 5.1 Hz), 4.57 (2H, dd, *J* = 9.4, 4.7 Hz), 4.80 (2H, dd, *J* = 3.0, 2.2 Hz), 5.25 (2H, s); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 13.6, 27.9, 38.2, 49.5, 59.7, 64.3, 78.4, 176.4.

LIBRARY C COMPOUNDS

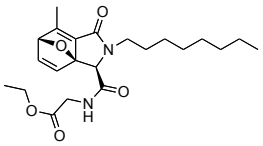
Library C: General Procedure.

A solution of amine (1 eq, 1.2 mmol), 2-furaldehyde (1 eq, 1.2 mmol) and anhydrous MeOH (10.0 mL) was stirred at room temperature for 0.5 h. 2-butynoic acid (1.2 eq, 1.4 mmol) was added and the resulting mixture was stirred for 0.5 h prior to the addition of an isocyanide (1 eq, 1.2 mmol). The mixture was stirred at room temperature for 2 h, quenched with 1 M NaOH (100 mL), extracted with CH_2Cl_2 (2 x 50 mL), dried (magnesium sulphate), and concentrated *in vacuo*. A sealed tube was charged with the crude mixture, toluene (80 mL), degassed, and heated (250 °C) for 36 h. The resulting mixture concentrated *in vacuo* and subjected to flash silica gel column chromatography.



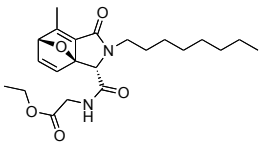
Scheme S3. Reagents and Conditions: (i) **1, 2, 3**, furfural, CH_3OH, room temperature, 30 min; (ii) $PhCH_3$, sealed tube 200 ºC 36 h.

*(3S,3aS,6R)-2-(octyl)-1,2,3,6-tetrahydro-7-methyl-1-oxo-N-(2-ethoxy-2-oxoethyl)-3a,6-epoxy-3aH-isoindole-3-carboxamide (**C1**-46)*



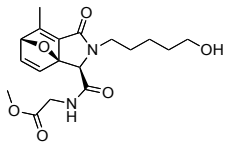
MP 72-73 ºC; ^{1}H NMR (300 MHz) (acetone-d_6): δ 0.75 (3H, t, *J* = 6.7 Hz), 1.25-1.08 (4H, m), 1.54-1.31 (8H, m), 2.00 (3H, s), 2.87 (2H, m), 3.66 (2H, m), 4.01-3.92 (2H, m), 4.04 (2H, m), 5.22 (1H, s), 5.27 (1H, d, *J* = 2.1 Hz), 7.02 (1H, dd, *J* = 5.5, 2.1 Hz), 7.10 (1H, d, *J* = 5.5 Hz), 8.44 (1 H, t, *J* = 5.2 Hz); ^{13}C NMR (75 MHz) (acetone-d_6): δ 12.5, 12.9, 13.1, 21.8, 25.8, 26.1, 26.2, 26.4, 28.4, 31.1, 40.4,41.0, 60.1, 62.0, 91.0, 130.6, 136.7, 141.2, 142.6, 157.8, 167.6, 168.7.

*(3R,3aS,6R)-2-(octyl)-1,2,3,6-tetrahydro-7-methyl-1-oxo-N-(2-ethoxy-2-oxoethyl)-3a,6-epoxy-3aH-isoindole-3-carboxamide (**C2**)*



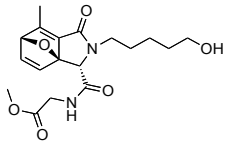
^{1}H NMR (300 MHz) (acetone-d_6): δ 0.88 (3H, t, *J* = 6.9 Hz), 1.24 (2H, m), 1.31 (4H, m), 1.60 (8H, m), 2.07 (3H, s), 3.00 (2H, ddd, *J* = 13.7, 8.1, 5.4 Hz), 3.79 (2H, qd, *J* = 14.0, 8.0 Hz), 3.95 (2 H, m), 4.14 (1H, q, *J* = 7.1 Hz), 4.99 (1H, s), 5.36 (1H, d, *J* = 2.1 Hz), 7.0 (1H, d, *J* = 5.5 Hz), 7.21 (1H, dd, *J* = 5.5, 2.1 Hz), 7.64 (1H, t, *J* = 5.4 Hz); ^{13}C NMR (75 MHz) (acetone-d_6): δ 12.5, 12.9, 13.0, 21.8, 26.2, 26.5, 28.4, 28.5, 31.1, 40.3, 40.4, 41.2, 60.0, 61.4, 90.8, 91.3, 141.9, 144.8, 155.9, 162.6, 165.3, 168.7.

*(3S,3aS,6R)-2-(5-Pentanol)-1,2,3,6-tetrahydro-7-propyl-1-oxo-N-(2-methoxy-2-oxoethyl)-3a,6-epoxy-3aH-isoindole-3-carboxamide (**C3**)*



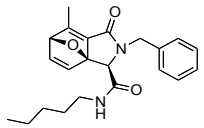
MP 76-78 ºC; ^{1}H NMR (300 MHz) (acetone-d_6): δ 1.22 (2H, m), 1.49-1.32 (6H, m), 1.96 (3H, s), 2.44 (1H, bs), 2.89 (1H, ddd, *J* = 13.7, 8.3, 5.2 Hz), 3.41 (1H, t, *J* = 6.4 Hz), 3.57 (3H, s), 3.74-3.60 (1H, m), 4.07-3.87 (1H, m), 4.46 (1H, s), 5.29 (1H, d, *J* = 2.0 Hz), 7.03 (1H, dd, *J* = 5.4, 2.0 Hz), 7.08 (1H, d, *J* = 5.4 Hz), 8.31 (1H, t, *J* = 5.8 Hz); ^{13}C NMR (75 MHz) (acetone-d_6): δ 15.0, 24.9, 28.3, 34.0, 42.5, 43.4, 53.3, 63.1, 64.4, 93.4, 95.2, 143.6, 144.9, 145.0, 160.5, 165.2, 170.1, 171.6.

*(3R,3aS,6R)-2-(5-pentanol)-1,2,3,6-tetrahydro-7-propyl-1-oxo-N-(2-methoxy-2-oxoethyl)-3a,6-epoxy-3aH-isoindole-3-carboxamide (**C4**)*



MP 85-87 ºC; ^{1}H NMR (300 MHz) (acetone-d_6): δ 1.26 (2H, m), 1.59-1.35 (4H, m), 1.95 (3H, s), 2.66 (1H, bs), 2.88 (1H, ddd, *J* = 13.6, 7.8, 5.5 Hz), 3.42 (2H, t, *J* = 6.3 Hz), 3.66 (1H, td, *J* = 14.0, 7.9 Hz), 3.55 (3H, s), 3.72-3.75 (2H, m), 4.87 (1H, s), 5.23 (1H, d, *J* = 1.9 Hz), 7.06 (1H, dd, *J* = 5.3, 1.9 Hz), 7.09 (1H, d, *J* = 5.3 Hz), 7.55 (1H, t, *J* = 5.1 Hz); ^{13}C NMR (75 MHz) (acetone-d_6): δ 15.0, 25.0, 28.8, 34.3, 42.6, 43.6, 53.2, 63.2, 63.8, 93.3, 93.8, 144.4,145.1, 147.3, 158.3, 165.2, 167.9, 171.7.

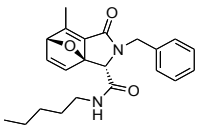
*(3S,3aS,6R)-2-(Benzyl)-1,2,3,6-tetrahydro-7-methyl-1-oxo-N-(pentyl)-3a,6-epoxy-3aH-isoindole-3-carboxamide (**C5**)*



MP: 76-77 °C; ^{1}H NMR (300 MHz) ($CDCl_3$): δ 0.85 (3H, t, *J* = 6.9 Hz), 1.35-1.13 (4H, m), 1.42 (2H, td, *J* = 14.2, 7.2, Hz), 2.11 (3H, s), 3.20 (2H, dp, *J* = 13.1, 7.0 Hz), 3.93 (1H, d, *J* = 14.9 Hz), 4.08 (3H, s), 5.17 (1H, d, *J* = 14.9 Hz), 5.17 (1H, d, *J* = 1.8 Hz), 6.63 (1H, t, *J* = 5.7 Hz), 6.96 (1H, d, *J* = 5.4 Hz), 7.00 (1H, dd, *J* = 5.5, 1.9 Hz), 7.50-6.78 (1H, m), 7.57-6.85 (3H, m); ^{13}C NMR (75 MHz) ($CDCl_3$): δ 13.4, 13.7, 22.0, 28.4, 28.5, 39.1, 45.1, 61.4, 91.2, 92.9, 127.3, 128.1, 128.2, 135.0, 141.1, 142.0, 142.4, 158.1, 163.1, 166.4.

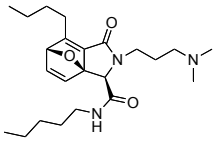
(3R,3aS,6R)-2-(benzyl)-1,2,3,6-tetrahydro-7-methyl-1-oxo-N-(pentyl)-3a,6-epoxy-3aH-isoindole-3-carboxamide

(C6)



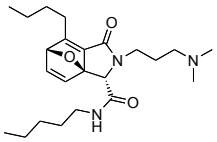
MP: 82-84 °C; ^{1}H NMR (DMSO-d_6): δ 0.84 (3H, t, *J* = 6.8 Hz), 1.28-1.12 (4H, m), 1.43-1.28 (2H, m), 2.06 (3H, s), 3.21-2.83 (2H, m), 3.34 (3H, s), 3.72 (1H, d, *J* = 15.4 Hz), 4.53 (1H, s), 5.02 (1H, d, *J* = 15.4 Hz), 5.42 (1H, d, *J* = 2.0 Hz), 7.08 (1H, d, *J* = 5.3 Hz), 7.19 (1H, dd, *J* = 5.6, 1.9 Hz), 7.40-7.26 (2H, m), 8.07 (1H, t, *J* = 5.5 Hz); ^{13}C NMR (DMSO-d_6): δ 13.78, 13.83, 21.7, 28.3, 28.5, 38.6, 44.8, 60.6, 90.7, 91.4, 127.4, 127.5, 128.7, 136.3, 142.51, 142.54, 145.3, 156.9, 163.2, 164.2.

*(3S,3aS,6R)-2-(4-N,N-Dimethylaminopropyl)-1,2,3,6-tetrahydro-7-pentyl-1-oxo-N-(pentyl)-3a,6-epoxy-3aH-isoindole-3-carboxamide (**C7**)*



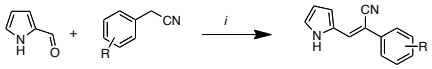
^{1}H NMR (300 MHz) (acetone-d_6): δ 0.89 (3H, t, *J* = 6.9 Hz), 1.24-1.41 (5H, m), 1.51-1.77 (10H, m), 2.12 (9H, s), 4.38 (1H, s), 2.21 (1H, ddd, *J* = 13.4, 7.0, 5.5 Hz), 3.05 (3H, m), 3.32 (2H, q, *J* = 6.8 Hz), 3.45-3.63 (3H, m), 5.08 (1H, d, *J* = 0.8 Hz), 5.22 (2H, dd, *J* = 10.6, 1.1 Hz), 6.34 (1H, d, *J* = 5.8 Hz), 6.51 (1H, dd, *J* = 5.8, 1.8 Hz), 8.08 (1H, t, *J* = 5.4 Hz); ^{13}C NMR (75 MHz) (acetone-d_6): δ 13.0, 19.7, 21.6, 24.1, 24.7, 28.2, 28.4, 28.5, 30.1, 38.7, 39.6, 44.2, 50.2, 55.8, 63.0, 82.4, 91.2, 106.8, 132.3, 135.7, 141.6, 167.0, 170.8.

*(3R,3aS,6R)-2-(4-N,N-dimethylaminopropyl)-1,2,3,6-tetrahydro-7-pentyl-1-oxo-N-(pentyl)-3a,6-epoxy-3aH-isoindole-3-carboxamide (**C8**)*



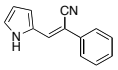
MP 67-68 ºC; ^{1}H NMR (300 MHz) (acetone-d_6): δ 0.87 (3H, t, *J* = 6.9 Hz), 1.30 (4H, m), 1.51 (2H, m), 1.57-1.82 (4H, m), 2.22 (4H, m), 2.13 (6H, s), 3.08 (3H, m), 3.23 (2H, m), 3.75 (3H, m), 4.81 (1H, s), 5.07 (1H, d, *J* = 0.8 Hz), 5.22 (2H, dd, *J* = 10.6, 1.1 Hz), 6.48 (1H, dd, *J* = 5.7, 1.8 Hz), 6.65 (1H, d, *J* = 5.7 Hz), 7.12 (1H, t, *J* = 5.4 Hz); ^{13}C NMR (75 MHz) (acetone-d_6): δ 12.9, 13.6, 19.7, 21.5, 24.1, 28.7, 30.1, 38.2, 38.4, 39.7, 44.2, 44.8, 50.9, 56.4, 61.4, 82.4, 90.3, 106.3, 133.5, 135.3, 141.6, 164.7, 170.7.

Synthesis of (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile derivatives



Scheme S4. Reagents and Conditions: (*i*) H_2O, 40% $PhCH_2NMe_3(OH)$, 50ºC, 5 h or RCHO (see table for details), piperidine (cat), EtOH reflux 2 h.

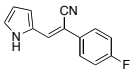
(Z)-2-Phenyl-3-(1H-pyrrol-2-yl)acrylonitrile



1*H*-pyrrole-2-carbaldehyde (165 mg, 1.74 mmol), was added to a vigorously stirred solution of water (10 mL) and heated to 50 °C up on which it dissolved. 2-Phenylacetonirile (193 mg, 1.65 mmol) was then slowly added forming a suspension. Once a clear solution was evident, typically 5-10 minutes, 40 % $PhCH_2NMe_3(OH)$ (7 mL) was added dropwise. After complete addition, the reaction vessel was sealed and stirred at 50 °C for 5 hours. After this period, the solution was filtered hot, washed with warm water and dried under suction to yield a solid. The crude solid was then recrystallised from EtOH to afford **1** as a brown solid; 73%; MP 94–96 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 9.81 (br, 1H, NH), 7.61-7.57 (m, 2H, Ar H2; Ar H6), 7.45-7.40 (m, 2H, Ar H3; Ar H5), 7.42 (s, 1H, HC=C), 7.35-7.30 (m, 1H, Ar H4), 7.08-7.06 (m, 1H, Pyr H-5), 6.73 (dd, *J* = 1.4, 3.7 Hz, 1H, Pyr H3), 6.37 (dd, *J* = 1.4, 3.7, 1H (Pyr H4); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 100.8, 110.3, 118.5, 120.1, 123.5, 124.4 (2 x Ar), 127.2, 127.6, 128.5 (2 x Ar), 130.7, 133.4.

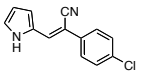
*2-(4-Fluorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile **(2)***



Synthesized using the general procedure as for **1**, from 1*H*-pyrrole-2-carbaldehyde and 4-fluorophenylacetonitrile to afford **2** as a yellow solid; 78%; mp 115-116 ºC.

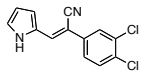
^{1}H NMR ($CDCl_3$) (300 MHz): δ 6.34-6.36 (1H, m), 6.71 (1H, m), 7.06-7.13 (3H, m), 7.32 (1H, s), 7.51-7.56 (2H, m), 9.82 (1H, br); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 99.7, 110.3 (2 x Ar), 115.4, 115.7, 118.5, 119.9, 123.5, 126.1 (2 x Ar), 127.0, 129.6, 130.7.

*(Z)-2-(4-Chlorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile **(3)***



MP 112–114 ºC; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 6.36 (1H, s), 6.72 (1H, d, *J* = 2.7 Hz), 7.08 (1H, s), 7.35-7.38 (3H, m), 7.49-7.51 (2H, m), 9.78 (1H, br); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 99.5, 110.4, 118.9, 119.7, 123.8, 125.6 (2 x Ar), 126.9, 128.7 (2 x Ar), 130.9, 131.9, 133.4.

*(Z)-2-(3,4-dichlorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile). (Z)-2-(3,4-Dichlorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile **(5)***



MP 140–142 ºC; ^{1}H NMR ($CDCl_3$) (300 MHz): δ 6.37-6.39 (1H, m), 7.20 (1H, m), 7.24-7.26 (1H, m), 7.56-7.64 (2H, m), 7.78 (1H, d, *J* = 2.1 Hz), 7.88 (1H, s), 9.78 (1H, br); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 98.3, 110.8, 114.4, 117.8, 123.9, 124.1, 125.7, 127.1, 130.1, 130.5,132.0, 132.7,135.0.

Cytotoxicity Evaluation

HT29 (colon), SW480 (colon), MCF-7 (breast), A2780 (ovarian), H460 (lung), A431 (skin), DU145 (prostate), BE2-C (neuronal) and SJ-G2 (brain) cell lines were cultured at 37 ºC under 5% CO_2 in air and were maintained in Dulbecco's modification of Eagle's medium (DMEM; Trace Biosciences, Australia) supplemented with 10% foetal bovine serum, 10 mM sodium bicarbonate penicillin (100 IU/ml), streptomycin (100 mg/ml) and glutamine (4 mM). [All solutions were prepared as follows and stored at -20 ºC: cantharidin (Biomol, USA) as a 30 mM solution in dimethylsulphoxide (DMSO), norcantharidin as a 30 mM solution in H_20, and norcantharidin analogues as 40 mM solutions in DMSO.] In a logarithmic phase of growth, cells were transferred to 96-well plates. Cytotoxicity was determined by plating cells in duplicate in 100 µl of medium at a density of 2,500-4,000 cells per well. On day 0 (24 h after plating), when the cells were in logarithmic growth, 100 µl of medium with or without the test compound were added to each well. After 72 h, growth inhibitory effects due to drug exposure were evaluated using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, and the absorbance was read at 540 nm. Growth inhibition (%) was determined at a drug concentration of 100 µM. A value of 100% was indicative of total cell growth inhibition. Analogues showing an appreciable percentage of growth inhibition underwent a further dose response analysis, allowing for the calculation of a GI_{50} value. This value is the drug concentration at which cell growth is inhibited by 50%, based on the difference between the optical density value on day 0 and that at the end of drug exposure.

Screening Compounds for Nematocidal Activity

H. contortus (Haecon 5 strain) was raised in helminth-free lambs (Merino crosses; 24 weeks of age), as described by Nikolaou et al.[46] Lambs were infected by intra-ruminal inoculation with 7,000 third-stage larvae (L3s). The patency of infection (21-35 days) was determined by the examination of faeces for the presence of strongylid eggs.[69] Faeces from infected sheep were collected for the immediate isolation of *H. contortus* eggs. Faeces (10 g) were homogenized in 100 ml of sucrose solution (specific gravity: 1.15) and sieved (mesh size: 1 mm). The solution was then placed into a flat dish and transparency film (code PP100C; NOBO) placed on the surface. The sheets were left for 45 min to allow the eggs to stick and then to be removed. The eggs were washed from the sheets with H_20 into a 50 ml centrifuge tube and then diluted further. The tube was then centrifuged at 1,000 x g for 10 min and the sedimented eggs then suspended in 0.5 ml of H_20. Eggs were enumerated by serial dilution, and the number was adjusted to 200 eggs per 20 µl of H_20. The larval development assay (LDA) was conducted as described by Gill et al.[70], with the following modifications. Compounds were tested at 12.5, 25, 50 and 100 mM. Moxidectin® (cydectin, Fort Dodge) was used (at the same concentrations) as a positive control in each experiment. Also MT-CN compounds[47] were used as positive controls in each assay, as these compounds had been shown previously to have a toxic effect on *H. contortus*. Aliquots (10 ml) of dilutions of each compound were made in 1.5 ml microcentrifuge tubes, 1 ml of molten agar added, the tube vortexed and the agar aliquotted (150 µl) into the wells of a 96-well microtitre plate. DMSO (1%; i.e. 10 µl of a 100% solution per 1 ml of agar) was used as a solvent-only control in each assay. Two to three replicates were performed for each concentration of each compound. Eggs (n = 200) in 20 µl of water were added to each well and then incubated for 16 h at 27 °C. The number of unhatched eggs in each well was determined, and 15 ml of nutritive medium were then added to each well. Nutritive medium was prepared as follows: 1 g of yeast extract was added to 90 ml of physiological saline and autoclaved for 20 min at 121 °C. Three ml of 10 x Earle's balanced salt solution [EBSS; potassium chloride (KCl) 53 mg/l, sodium bicarbonate ($NaHCO_3$) 261.9 mg/l, sodium chloride (NaCl) 1172.4 mg/l, sodium phosphate monobasic ($NaH_2PO_4.H_2O$) 10.1 mg/ml] were added to 27 ml of yeast extract solution, and the pH adjusted to 5.5 with bicarbonate. Following 6 days of incubation (27 °C), the number of L3s that had developed in each well was counted microscopically at 20-times magnification. Dose-response curves were established for compounds shown to consistently kill *H. contortus* in LDA, by testing concentrations (in 10 µM increments) between 10 and 100 µM. The dose-response assays were repeated at least three times for each compound. The reproducibility of results (i.e. nematocidal activity) for each compound was assessed on different days. The resultant data were expressed as a percentage of mortality against $\log_{10}$ of the concentrations of the compound tested. For each compound, the LD_{50} value (concentration at which 50% of the larvae were killed) was estimated based on the line of best fit to the dose-response curve.

References and notes

68. Armareggio, W.L.F; Chai, L.L. Elsevier, 2003, 5th Edition.
69. MAFF, 1977, Technical Bulletin 18, Her Majesty's Stationary Office, London, UK.
70. Gill, J. H.; Redwin, J. M.; Van Wyk, J. A.; Lacey, E. Int. J. Parasitol. 1995, 25, 463.

5.2 Paper IV Supplementary Data

Supplementary Material (ESI) for Medicinal Chemistry Communications

Library synthesis and cytotoxicity of a family of 2-phenylacrylonitriles and discovery of an estrogen dependent, breast cancer lead compound

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Supplementary Data

Experimental Section

Materials

All starting materials were purchased from Aldrich Chemical Co. and Lancaster Synthesis. Solvents were bulk, and distilled from glass prior to use. Reaction progress was monitored by TLC, on aluminium plates coated with silica gel with fluorescent indicator (Merck 60 F_{254}) and flash chromatography was conducted utilizing SNAP Biotage KP-SIL columns.

Cell culture and stock solutions

Stock solutions were prepared as follows and stored at -20 °C: drugs were prepared as 40 mM solutions in DMSO. All cell lines with the exception of MCF10A were cultured at 37 °C, under 5 % CO_2 in air and were maintained in Dulbecco s modified Eagle s medium (Trace Biosciences, Australia) supplemented with 10 % foetal bovine serum, 10 mM sodium bicarbonate penicillin (100 µg mL^{-1}), streptomycin (100 µg mL^{-1}), and glutamine (4 mM). MCF10A were cultured as above and further supplemented with insulin (2mg/ml), hydrocortisone (0.25mg/ml), cholera toxin (1mg/ml), and epidermal growth factor

(100µg/ml).

In vitro growth inhibition assays

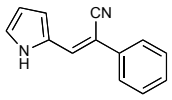
Cells in logarithmic growth were transferred to 96-well plates. Cytotoxicity was determined by plating cells in duplicate in medium (100 µL) at a density of 2500-4000 cells/well. On day 0 (24 h after plating), when the cells were in logarithmic growth, medium (100 µL) with or without the test agent was added to each well. After 72 h of drug exposure, growth inhibitory effects were evaluated using the MTT (3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay and their absorbance was read at 540 nm. Percentage growth inhibition was determined at a fixed drug concentration of 100 µM. A value of 100 % is indicative of total cell growth inhibition. Those analogues showing appreciable percentage growth inhibition underwent further dose response analysis to allow the calculation of GI_{50} values. The GI_{50} value is defined as the drug concentration at which cell growth is 50 % inhibited based on the difference between the optical density values on day 0 and those at the end of drug exposure.[1,2]

Chemistry

General methods

THF was freshly distilled from sodium–benzophenone. Flash chromatography was carried out using silica gel 200–400 mesh (60 Å). ^{1}H and ^{13}CNMR were recorded at 300 MHz and 75 MHz respectively using a Bruker Avance 300 MHz spectrometer in $CDCl_3$ and DMSO-d_6. GCMS was performed using a Shimadzu GCMS-QP2100. The instrument uses a quadrupole mass spectrometer and detects samples via electron impact ionization (EI). The University of Wollongong, Australia, Biomolecular Mass Spectrometry Laboratory analyzed samples for HRMS. The spectra were run on the VG Autospec-oa-tof tandem high resolution mass spectrometer using CI (chemical ionization), with methane as the carrier gas and PFK (perfluorokerosene) as the reference.

*(Z)-2-Phenyl-3-(1H-pyrrol-2-yl)acrylonitrile (**1**)*[3]

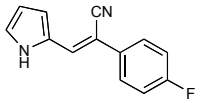


1*H*-pyrrole-2-carbaldehyde (165 mg, 1.74 mmol), was added to a vigorously stirred solution of water (10 mL) and heated to 50 °C up on which it dissolved. 2-

Phenylacetonirile (193 mg, 1.65 mmol) was then slowly added forming a suspension. Once a clear solution was evident, typically 5-10 minutes, 40 % $PhCH_2NMe_3(OH)$ (7 mL) was added dropwise. After complete addition, the reaction vessel was sealed and stirred at 50 °C for 5 hours. After this period, the solution was filtered hot, washed with warm water and dried under suction to yield a solid. The crude solid was then recrystallised from EtOH to afford **1** as a brown solid; 73%; 94–96 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 9.81 (br, 1H, NH), 7.61-7.57 (m, 2H, Ar H2; Ar H6), 7.45-7.40 (m, 2H, Ar H3; Ar H5), 7.42 (s, 1H, HC=C), 7.35-7.30 (m, 1H, Ar H4), 7.08-7.06 (m, 1H, Pyr H-5), 6.73 (dd, *J* = 1.4, 3.7 Hz, 1H, Pyr H3), 6.37 (dd, *J* = 1.4, 3.7, 1H (Pyr H4); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 133.4, 130.7, 128.5 (2 x Ar), 127.6, 127.2, 124.4 (2 x Ar), 123.5, 120.1, 118.5, 110.3, 100.8; υ_{max}(KBr)/cm^{-1}: 3396 (NH), 2205 (CN), 1683 (C=C), 1601 (Ar), 1589 (Ar), 1496 (Ar); LRMS (APCI M+1) 195.

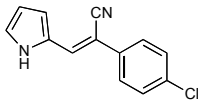
*(E)-2-(4-Fluorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile (**2**)*[3]



Synthesized using the general procedure as for **1**, from 1*H*-pyrrole-2-carbaldehyde and 4-fluorophenylacetonitrile to afford **2** as a yellow solid; 78%; mp 115-116 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 9.82 (br, 1H, NH), 7.56-7.51 (m, 2H, Ar H2; Ar H6), 7.32 (s, 1H, HC=C), 7.13-7.06 (m, 3H, Ar H3; Ar H5; Pyr H5), 6.71-6.70 (m, 1H, Pyr H3), 6.36-6.34 (m, 1H, Pyr H4); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 130.7, 129.6, 127.0, 126.1 (2 x Ar), 123.5, 119.9, 118.5, 115.7, 115.4, 110.3 (2 x Ar), 99.7; υ_{max}(KBr)/cm^{-1}: 3401 (NH), 2205 (CN), 1641 (C=C), 1597 (Ar), 1507 (Ar); LRMS (APCI M+1) 213.

*(Z)-2-(4-Chlorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile (**3**)*[3]

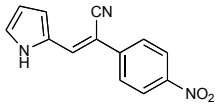


Synthesized using the general procedure as for **1**, from 1*H*-pyrrole-2-carbaldehyde and 4-chlorophenylacetonitrile to afford **3** as a yellow solid; 67%; mp 112–114 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 9.78 (br, 1H, NH), 7.51-7.49 (m, 2H, Ar H2; Ar H6), 7.38-7.35 (m, 3H, Ar H3; Ar H5; HC=C), 7.08 (s, 1H, Pyr H5), 6.72 (d, *J* = 2.7 Hz, 1H, Pyr H3), 6.36 (s, 1H, Pyr H4); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 133.4, 131.9, 130.9, 128.7 (2 x

Ar), 126.9, 125.6 (2 x Ar), 123.8, 119.7, 118.9, 110.4, 99.5; υ_{max}(KBr)/cm^{-1}: 3380 (NH), 2213 (CN), 1636 (C=C), 1603 (Ar), 741 (Ar-Cl); LRMS (APCI M+1) 229.

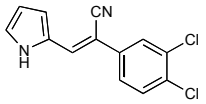
*(Z)-2-(4-Nitrophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile (**4**)*[3]



Synthesized using the general procedure as for **1**, from 1*H*-pyrrole-2-carbaldehyde and 4-nitrophenylacetonitrile to afford **4** as a dark green solid; 70%; mp 130–134 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 9.80 (br, 1H, NH), 8.23-8.26 (m, 2H, Ar H3; Ar H5), 7.74-7.70 (m, 2H, Ar H2; Ar H6), 7.56 (s, 1H, HC=C), 7.18-7.17 (m, 1H, Pyr H5), 6.84 (dd, *J* = 1.3, 3.8 Hz, 1H, Pyr H3), 6.42 (dd, *J* = 1.3, 3.8 Hz, 1H, Pyr H4); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 133.1, 132.0, 129.3, 126.9, 125.4, 124.7 (2 x Ar), 124.0 (2 x Ar), 123.0 121.0, 119.3, 111.1; υ_{max}(KBr)/cm^{-1}: 3398 (NH), 2205 (CN), 1636 (C=C), 1602 (Ar), 1578 (Ar), 1508 (Ar) 1331 (NO); LRMS (APCI M+1) 210.

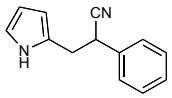
*(Z)-2-(3,4-Dichlorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile (**5**)*[3]



Synthesized using the general procedure as for **1**, from 1*H*-pyrrole-2-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford **3** as a dark yellow solid; 72%; mp 140–142 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 9.78 (br, 1H, NH), 7.88 (s, 1H, HC=C), 7.78 (d, *J* = 2.1 Hz, 1H, Ar H5), 7.64-7.56 (m, 2H, Ar H2; Ar H6), 7.26-7.24 (m, 1H, Pyr H5), 7.21-7.20 (m, 1H Pyr H3), 6.39-6.37 (m, 1H Pyr H4); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 135.0, 132.7, 132.0, 130.5, 130.1, 127.1, 125.7, 124.1, 123.9, 117.8, 114.4, 110.8, 98.3; υ_{max} KBr)/cm^{-1}: 3415 (NH), 2199 (CN), 1636 (C=C), 1604 (Ar), 1588 (Ar); LRMS (APCI M+1) 263.

*2-Phenyl-3-(1H-pyrrol-2-yl)propanenitrile (**6**)*

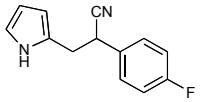


(*Z*)-2-Phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile (**1**) (5.1 mmol) was dissolved into sufficient freshly distilled dry acetone to form a 0.05 M solution. This solution hydrogenated using

the ThalesNano H-cube™ using a 10% Pd/C catalyst at 1 mL/min at 50 ºC and 50 bar H_2 pressure. The solvent was then removed *in vacuo* and the crude oil was subjected to flash silica chromatography (1:1 $CHCl_3$:Hexanes) to afford **6** as a brown oil; 98%.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 8.03 (br, 1H, NH), 7.42-7.35 (m, 3H, Ar H3; Ar H4; Ar H5), 7.29-7.26 (m, 2H, Ar H2; Ar H6), 6.69-6.67 (m, 1H, Pyr H5), 6.15-6.13 (m, 1H, Pyr H3), 6.03-6.02 (m, 1H, Pyr H4), 4.01 (t, *J* = 7.4 Hz, 1H, CH), 3.28-3.14 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) (75 MHz): 134.5, 128.6 (2 x Ar), 127.8, 126.8, 125.7 (2 x Ar), 120.4, 117.3, 108.1, 107.4, 38.4, 34.0; υ_{max}(film)/cm^{-1}: 3384 (NH), 2242 (CN), 1597 (Ar); LRMS (APCI M+1) 197; HRMS (ESI M+H) for $C_{13}H_{12}N_2$, calculated 197.1079; found 197.1083

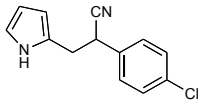
2-(4-Fluorophenyl)-3-(1H-pyrrol-2-yl)propanenitrile (7)



Synthesized using the general procedure as for **6**, from (*Z*)-2-(4-fluorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**2**) to afford **7** as a light brown oil; 95%.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 8.06 (br, 1H, NH), 7.24-7.19 (m, 2H, Ar H2; Ar H5), 7.09-7.03 (m, 2H, Ar H3; Ar H5), 6.69 (d, *J* = 1.4 Hz, 1H, Pyr H5), 6.15-6.12 (m, 1H, Pyr H3), 5.98 (s, 1H, Pyr H4), 4.00 (t, *J* = 6.8 Hz, 1H, CH), 3.25-3.14 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 163.6, 130.2, 128.6 (2 x Ar), 125.3, 120.2, 117.4, 115.4, 108.2 (2 x Ar), 107.6, 37.6, 34.0; υ_{max}(film)/cm^{-1}: 3404 (NH), 2244 (CN), 1602 (Ar), 1509 (Ar); LRMS (APCI M+1) 215; HRMS (ESI M+H) for $C_{13}H_{11}FN_2$, calculated 215.0985; found 215.0986

2-(4-Chlorophenyl)-3-(1H-pyrrol-2-yl)propanenitrile (8)

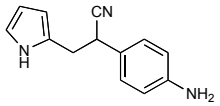


Synthesized using the general procedure as for **6**, from (*Z*)-2-(4-chlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**3**) to afford **7** as a light yellow oil; 76%.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 8.04 (br, 1H, NH), 7.38-7.32 (m, 2H, Ar H3; Ar H5), 7.19-7.16 (m, 2H, Ar H2; Ar H6), 6.70-6.68 (m, 1H, Pyr H5), 6.15-6.12 (m, 1H, Pyr H3), 5.99-5.98 (m, 1H, Pyr H4), 3.99 (t, *J* = 6.7 Hz, 1H, CH), 3.25-3.12 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 132.9, 128.7 (2 x Ar), 128.6, 128.2, 126.8 (2 x Ar), 229.9, 117.4,

108.2, 107.7, 37.8, 33.9; υ_{max}(KBr)/cm^{-1}: 3398 (NH), 2215 (CN), 1598 (Ar), 1511 (Ar); LRMS (APCI M+1) 231; HRMS (ESI M+H) for $C_{13}H_{11}ClN_2$, calculated 231.0689; found 231.0694.

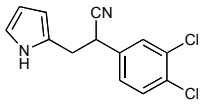
*2-(4-Aminophenyl)-3-(1H-pyrrol-2-yl)propanenitrile (**9**)*



Synthesized using the general procedure as for **6**, from (*Z*)-2-(4-nitrophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**4**) to afford **9** as a dark brown oil; 12%.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.96 (br, 1H, NH), 7.04-7.01 (m, 2H, Ar H2; Ar H6), 6.68-6.64 (m, 3H, Ar H3; Ar H5; Pyr H5), 6.14-6.10 (m, 1H, Pyr H3), 6.01 (s, 1H, Pyr H4), 3.88 (t, *J* = 7.2 Hz, 1H, CH), 3.75 (br, 2H, NH_2), 3.22-3.09 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 145.9, 127.8 (2 x Ar), 126.1, 124.1, 120.8, 117.2, 114.8 (2 x Ar), 107.9, 107.3, 37.6, 34.1; υ_{max}(KBr)/cm^{-1}: 3434 (NH), 3402 (NH), 2235 (CN), 1602 (Ar), 1505 (Ar); LRMS (APCI M+1) 212; HRMS (ESI M+H) for $C_{13}H_{11}N_3O_2$, calculated 242.0930; found 242.0933.

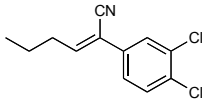
*2-(3,4-Dichlorophenyl)-3-(1H-pyrrol-2-yl)propanenitrile (**10**)*



Synthesized using the general procedure as for **6**, from (*Z*)-2-(3,4-dichlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**2**) to afford **10** as a yellow oil; 65%.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 8.10 (br, 1H, NH), 7.45-7.33 (m, 2H, Ar H2; Ar H5), 7.07-7.04 (m, 1H, Ar H6), 6.72-6.70 (m, 1H, Pyr H5), 6.16-6.13 (m, 1H, Pyr H3), 5.98 (s, 1H, Pyr H4), 3.97 (t, *J* = 6.6Hz, 1H, CH), 3.25-3.12 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 134.4, 132.7, 132.2, 130.4, 128.8, 126.2, 124.7, 119.4, 117.6, 108.3, 107.9, 37.6, 33.7; υ_{max}(KBr)/cm^{-1}: 3392 (NH), 2221 (CN), 1600 (Ar); LRMS (APCI M+1) 267; HRMS (ESI M+H) for $C_{13}H_{10}Cl_2N_2$, calculated 265.0299; found 265.0305.

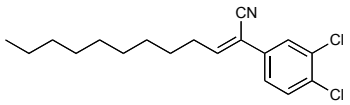
*(Z)-2-(3,4-Dichlorophenyl)hept-2-enenitrile (**11**)*



Pentanal (97 mg, 1.13 mmol) was dissolved in distilled ethanol (10 mL) to this was added 2-(3,4-dichlorophenyl)acetonitrile (199 mg, 1.07 mmol) in ethanol (10 mL). The resultant solution was stirred and heated to 70 °C upon which piperidine was added (2 drops). The solution was then heated under reflux for 2 hours. After this time, the solution was cooled in the to 0 °C and the solvent removed *in vacuo* to yield an oil. Subsequent flash chromatography (1:19 EtOAc:Hexanes) to afford **11** as a clear oil; 35%.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.62-7.61 (m, 1H, Ar H5), 7.48-7.45 (m, 1H, Ar H2), 7.38-7.34 (m, 1H, Ar H6), 6.85 (t, *J* = 7.7 Hz, 1H, HC=C), 2.60 (q, *J* = 7.7 Hz, 2H, CH_2CH=C), 1.60-1.33 (m, 4H, CH_2CH_2), 0.96 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 148.4, 132.8, 132.7, 132.5, 130.3, 126.8, 124.3, 115.3, 113.4, 31.5, 30.0, 21.8, 13.3; υ_{max}(film)/cm^{-1}: 2958 (CH), 2957 (CH), 2870 (CH), 2218 (CN), 1615 (C=C), 1473 (Ar); LRMS (APCI M-1) 252; HRMS (ESI M+H) for $C_{13}H_{10}N_2$, calculated 254.0503; found 254.0501.

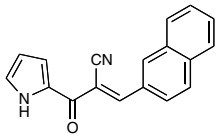
*(E)-2-(3,4-Dichlorophenyl)dodec-2-enenitrile (**12**)*



Synthesized using the general procedure as for **11**, from decanal and 2-(3,4-dichlorophenyl)acetonitrile to afford **12** as a clear oil; 21%.

^{1}H NMR ($CDCl_3$) (300 MHz): 7.62-7.61 (m, 1H, Ar H5), 7.49-7.46 (m, 1H, Ar H2), 7.37 (dd, *J* = 8.4, 2.2 Hz, 1H, Ar H6), 6.87-6,82 (m, 1H, CH=C), 2.63-2.52 (m, 4H, CH_2CH_2CH=C), 2.34-2.14 (m, 4H, CH_2CH_2), 1.59-1.52 (m, 6H, $CH_2CH_2CH_2$), 0.91-0.85 (m, 5H, CH_2CH_3); ^{13}C NMR ($CDCl_3$) (75 MHz): 148.4, 132.8, 132.5, 130.3, 126.1, 125.2, 124.9, 115.3, 113.4, 31.9, 31.3, 31.2, 29.1, 28.8, 28.7, 28.0, 22.2, 13.5; υ_{max}(film)/cm^{-1}: 2957 (CH), 2930 (CH), 2860 (CH), 2219 (CN), 1619 (C=C), 1482 (CH); LRMS (APCI M-1) 322 HRMS (ESI M+H) for $C_{18}H_{23}Cl_2N_2$, calculated 324.1286; found 324.1289.

*(E)-3-(Naphthalen-2-yl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**14**)*

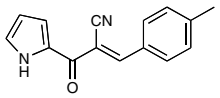


Cyano acetic acid (1.360g, 16 mmol) was added to Ac_2O (8 mL) and the resultant suspension was stirred and heated to 50 °C upon which the solid material dissolved. Pyrrole (1.073g, 16 mmol) was then added and the solution was heated at 75 °C for 35 minutes. The solution was then diluted with EtOAc (20 mL) and washed with 0.1M NaOH (3 x 10 mL). The organic layer was then collected and dried using $MgSO_4$. The solvent was then removed under vacuum and the residue purified by flash silica chromatography (1:10 EtOAc:Hexanes to 1:1 EtOAc:Hexanes) to afford 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile, 70%.

Next, to an ethanolic solution (10 mL) of 2-naphthaldehyde (1.56 mmol) was added an ethanolic solution (10 mL) of 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile (1.56 mmol). This mixture was heated to 70 ºC at which time, piperidine (2 drops) was added, and the solution was then heated under reflux for an additional 2 hours. After this time, the solution was cooled and the solvent removed *in vacuo* to afford a brown oil which was purified by flash chromatography (1:10 EtOAc:Hexanes) to afford **14** as a brown solid; 35%; mp 140-142 ºC.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.27 (br, 1H, NH), 8.57 (s, 1H, Ar H1), 8.44-8.42 (s, 1H, CH=C), 8.23-8.21 (m, 1H, Ar H5), 8.11-7.99 (m, 3H, Ar H3; Ar H4; Ar H8), 7.70-7.60 (m, 2H, Ar H7; Pyr H5), 7.35-7.31 (m, 2H, Ar H6; Pyr H3), 6.35-6.34 (m, 1H, Pyr H4); ^{13}C NMR (DMSO-d_6) (75 MHz): δ 174.8, 153.2, 134.4, 133.4, 132.4, 129.7, 129.0, 128.9, 128.8, 128.7, 128.0, 127.7, 127.2, 124.7, 119.5, 119.2, 110.8, 109.1; υ_{max}(KBr)/cm^{-1}: 3291 (NH), 2215 (CN), 1617 (C=O); LRMS (APCI M+1) 273; HRMS (ESI M+H) for $C_{18}H_{12}N_2$, calculated 272.0950; found 272.0954.

*(E)-2-(1H-Pyrrole-2-carbonyl)-3-p-tolylacrylonitrile (**15**)*

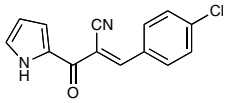


Synthesized using the general procedure as for **14**, from 4-methylbenzaldehyde to afford **15** as a yellow solid, 81%; mp 242-244 ºC.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.22 (br, 1H, NH), 8.24 (s, 1H, CH=C), 7.95 (d, *J* = 8.0Hz, 2H, Ar H2; Ar H6), 7.37 (d, *J* = 8.0Hz, 2H, Ar H3; Ar H5), 7.29-7.26 (m, 2H, Pyr H5; Pyr H3), 6.33-6.30 (m, 1H, Pyr H4), 2.37 (s, 3H, $ArCH_3$); ^{13}C NMR (DMSO-d_6) (75

MHz): δ 174.8, 153.2, 143.5, 130.6 (2 x Ar), 129.7 (2 x Ar), 129.3, 129.0, 127.9, 119.3, 117.7, 110.8, 107.8, 21.2; υ_{max}(KBr)/cm^{-1}: 3291 (NH), 2210 (CN), 1622 (C=O); LRMS (APCI M+1) 237; HRMS (ESI M+H) for $C_{14}H_{12}N_2$, calculated 209.1079; found 209.1082.

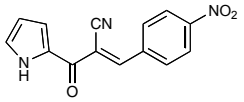
*(E)-3-(4-Chlorophenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**16**)*



Synthesized using the general procedure as for **14**, from 4-chlorobenzaldehyde to afford **16** as a yellow solid, 39%; mp 192-194 ºC.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 11.30 (br, 1H, NH), 8.28 (s, 1H, CH=C), 8.13-8.11 (m, 2H, Ar H2; Ar H6), 7.66-7.63 (m, 2H, Ar H3; Ar H5), 7.45-7.44 (m, 1H, Pyr H5), 7.35-7.34 (m, 1H, Pyr H3), 6.38-6.36 (m, 1H, Pyr H4); ^{13}C NMR (Acetone-d_6) (75 MHz): δ 173.6, 151.1, 137.4, 131.7 (2 x Ar), 130.8, 128.8 (2 x Ar), 126.9, 119.7, 118.7, 116.6, 110.4, 109.5; υ_{max}(KBr)/cm^{-1}: 3284 (NH), 2211 (CN), 1627 (C=O), 760 (Ar-Cl); LRMS (APCI M+1) 257; HRMS (ESI M+H) for $C_{13}H_9ClN_2$, calculated 229.0533; found 229.0537.

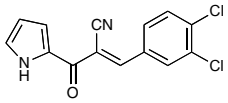
*(E)-3-(4-Nitrophenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**17**)*



Synthesized using the general procedure as for **14**, from 4-nitrobenzaldehyde to afford **17** as a purple solid, 37%; mp 199-200 ºC.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.32 (br, 1H, NH), 8.40-8.38 (m, 3H, Ar H3; Ar H5; Pyr H5), 8.24-8.21 (m, 2H, Ar H2; Ar H6), 7.24 (m, 2H, Pyr H5; Pyr H3), 6.34 (s, 1H, Pyr H4); ^{13}C NMR (DMSO-*d6*) (75 MHz): δ 174.2, 150.6, 148.8, 138.2, 131.3 (2 x Ar), 130.6, 128.7, 124.0 (2 x Ar), 120.3, 116.6, 113.1, 111.1; υ_{max}(KBr)/cm^{-1}: 3308 (NH), 2228 (CN), 1633 (C=O), 1517 (NO) 1343 (NO); LRMS (APCI M+1) 238; HRMS (ESI M+H) for $C_{13}H_9N_3O_2$, calculated 240.0773; found 240.0777.

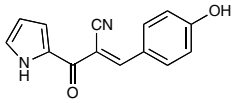
*(E)-3-(3,4-Dichlorophenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**18**)*



Synthesized using the general procedure as for **14**, from 3,4-dichlorobenzaldehyde to afford **18** as a yellow solid; 66%; mp 178-181 ºC.

^{1}H NMR (Acetone-d_6) (300 MHz): δ 11.31 (br, 1H, NH), 8.29-8.27 (m, 2H, Ar H5; CH=C), 8.11-8.08 (m, 1H, Ar H6), 7.84-7.81 (m, 1H, Ar H2), 7.45-7.36 (m, 2H, Pyr H5; Pyr H3), 6.39-6.37 (m, 1H, Pyr H4); ^{13}C NMR (Acetone-d_6) (75 MHz): δ 173.4, 149.6, 135.0, 132.4, 132.1, 131.7, 130.8, 129.3, 127.2, 127.0, 119.0, 116.3, 111.0, 110.5; υ_{max}(KBr)/cm^{-1}: 3310 (NH), 2222 (CN), 1632 (C=O); LRMS (APCI M+1) 290; HRMS (ESI M+H) for $C_{13}H_8Cl_2N_2$, calculated 263.0143; found 263.0144.

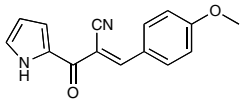
*(E)-3-(4-Hydroxyphenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**19**)*



Synthesized using the general procedure as for **14**, from 4-hydroxybenzaldehyde to afford **1** as a orange solid; 43%; mp 240-243 ºC.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.07 (br, 1H, NH), 8.68 (br, 1H, OH), 8.18 (s, 1H, CH=C), 7.99 (d, J = 8.7Hz, 2H, Ar H2; Ar H6), 7.28-7.23 (m, 2H, Pyr H5; Pyr H3), 6.94 (d, J = 8.7Hz, 2H, Ar H3; Ar H5), 6.30-6.27 (m, 1H, Pyr H4); ^{13}C NMR (DMSO-d_6) (75 MHz): δ 175.0, 162.3, 153.3, 133.6 (2 x Ar), 129.2, 127.3, 123.1, 118.6, 118.4, 116.2 (2 x Ar), 110.5, 104.1; υ_{max}(KBr)/cm^{-1}: 3419 (OH), 3290 (NH), 2218 (CN), 1617 (C=O), 1603 (Ar); LRMS (APCI M+1) 239; HRMS (ESI M+H) for $C_{13}H_{10}N_2O$, calculated 211.0871; found 211.0875.

*(E)-3-(4-Methoxyphenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (**20**)*

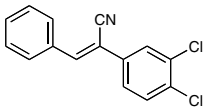


Synthesized using the general procedure as for **14**, from 4-methoxybenzaldehyde to afford **20** as a yellow solid; 83%; mp 166-168 ºC.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 12.14 (br, 1H, NH), 8.24 (s, 1H, CH=C), 8.08 (d, J = 8.9Hz, 2H, Ar H2; Ar H6), 7.28-7.25 (m, 2H, Pyr H5; Pyr H3), 7.13 (d, J = 8.9Hz, 2H, Ar H3; Ar H5), 6.31 (s, 1H, Pyr H4), 3.85 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) (75 MHz): δ 174.9, 163.0, 153.0, 133.1 (2 x Ar), 129.1, 127.5, 124.6, 118.9, 118.2, 114.8 (2 x Ar), 110.6, 105.5, 55.6; υ_{max}(KBr)/cm^{-1}: 3306 (NH), 2209 (CN), 1617 (C=O), 1507 (Ar); LRMS

(APCI M+1) 253; HRMS (ESI M+H) for $C_{14}H_{12}N_2O$, calculated 225.1028; found 225.1029.

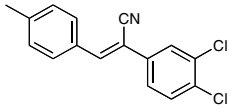
*(Z)-2-(3,4-Dichlorophenyl)-3-phenylacrylonitrile (**21**)*[4]



Synthesized using the general procedure as for **1**, from benzaldehyde and 3,4-dichlorophenylacetonitrile to afford **21** as a white solid; 89%; mp 146-147 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.91-7.87 (m, 2H, Ar H2; Ar H5), 7.77-7.76 (m, 1H, Ar H6), 7.53-7.47 (m, 6H, Ar2 H2; Ar2 H3; Ar2 H4; Ar2 H5; Ar2 H6; CH=C); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 143.0, 133.9, 133.0, 132.8, 132.6, 130.6, 130.4, 128.9 (2 x Ar), 128.5 (2 x Ar), 127.2, 124.6, 116.7, 108.8; υ_{max}(KBr)/cm^{-1}: 2212 (CN), 1636 (C=C), 1590 (Ar), 1568 (Ar), 1496 (Ar), 676 (Ar-Cl); LRMS (APCI M-1) 273.

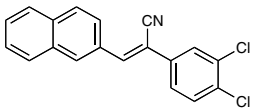
*(Z)-2-(3,4-dichlorophenyl)-3-p-tolylacrylonitrile (**22**)*



Synthesized using the general procedure as for **1**, from 4-methylbenzaldehyde and 2-(3,4-dichlorophenyl)acetonitrile to afford **22** as a yellow solid; 71%; mp 164-165 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.80 (d, *J* = 8.1 Hz, 2H, Ar2 H2; Ar2 H6), 7.74 (m, 1H, Ar H5), 7.50-7.7.49 (m, 3H, Ar H2; Ar H6; CH=C), 7.28 (d, *J* = 8.1 Hz, 2H, Ar2 H3; Ar2 H5), 2.42 (s, 3H, $ArCH_3$); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 143.0, 141.4, 134.1, 132.9, 132.5, 130.4, 129.8, 129.3 (2 x Ar), 129.0 (2 x Ar), 127.0, 124.5, 117.0, 107.4, 21.1; υ_{max}(KBr)/cm^{-1}: 2214 (CN), 1637 (C=C), 1594 (Ar), 1509 (Ar), 811 (Ar-Cl); LRMS (APCI M-1) 287; HRMS (ESI M+H) for $C_{16}H_{11}NCl_{22}$, calculated 288.0347; found 288.0350.

*(Z)-2-(3,4-dichlorophenyl)-3-(naphthalen-2-yl)acrylonitrile (**23**)*

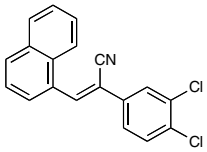


Synthesized using the general procedure as for **1**, from 2-naphthaldehyde and 3,4-dichlorophenylacetonitrile to afford **23** as a yellow solid; 71%; mp 170-171 ºC.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 8.42 (s, 1H, Ar2 H1), 8.32 (s, 1H, CH=C), 8.12-7.97

(m, 5H, Ar2 H3; Ar2 H5; Ar2 H6; Ar2 H7; Ar2 H8), 7.76 (m, 2H, Ar2 H4; Ar H5), 7.61 (m, 2H, Ar H2; Ar H6); ^{13}C NMR (DMSO-d_6) (75 MHz): δ 144.7, 134.5, 133.7, 132.4, 132.0, 131.7, 131.2, 130.9, 128.8, 128.7, 128.6, 128.0, 127.7, 127.3, 127.1, 126.1, 124.7, 117.4, 107.9; υ_{max}(KBr)/cm^{-1}: 2210 (CN), 1626 (C=C), 1598 (Ar), 1591 (Ar), 1477 (Ar), 1466 (Ar), 809 (Ar-Cl), 743 (Ar-Cl); LRMS (APCI M-1) 323; HRMS (ESI M+H) for $C_{19}H_{11}Cl_2N$, calculated 324.0347; found 325.0353.

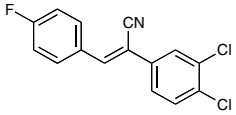
*(Z)-2-(3,4-dichlorophenyl)-3-(naphthalen-1-yl)acrylonitrile (**24**)*



Synthesized using the general procedure as for **1**, from 1-naphthaldehyde and 3,4-dichlorophenylacetonitrile to afford **24** as a yellow solid; 72%; mp 218-219 ºC.

^{1}H NMR (DMSO-d_6) (300 MHz): δ 8.84 (s, 1H, CH=C), 8.19-8.18 (m, 2H, Ar2 H4; Ar2 H8), 8.10-7.99 (m, 3H, Ar2 H2; Ar2 H5; Ar2 H7), 7.82-7.81 (m, 2H, Ar2 H3; Ar2 H6), 7.67-7.61 (m, 3H, Ar H2; Ar H5 Ar H6); ^{13}C NMR (DMSO-d_6) (75 MHz): δ 143.5, 134.0, 133.0, 132.0, 131.9, 131.1, 130.9, 130.8, 128.5, 127.8, 127.0, 126.8, 126.6, 126.5, 125.3, 125.2, 124.3, 117.0, 112.3; υ_{max}(KBr)/cm^{-1}: 2216 (CN), 1636 (C=C), 1508 (Ar), 1474 (Ar), 776 (Ar-Cl); LRMS (APCI M-1) 323; HRMS (ESI M+H) for $C_{19}H_{11}Cl_2N$, calculated 324.0347; found 324.03523.

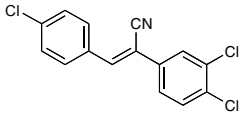
*(E)-2-(3,4-dichlorophenyl)-3-(4-fluorophenyl)acrylonitrile (**25**)*[5]



Synthesized using the general procedure as for **1**, from 4-fluorobenzaldehyde and 4-chlorophenylacetonitrile to afford **25** as a white solid; 94%; mp 156-157 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.93-7.88 (m, 2H, Ar2 H2; Ar2 H6), 7.75-7.74 (m, 1H, Ar H5), 7.51-7.48 (m, 3H, Ar2 H3; Ar2 H5; CH=C), 7.20-7.15 (m 2H, Ar H5; Ar H6); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 141.5, 133.7, 133.0 (2 x Ar), 132.9, 131.1, 131.0, 130.5, 128.9, 127.1, 124.6 (2 x Ar), 116.6, 115.9, 115.7; υ_{max}(KBr)/cm^{-1}: 2213 (CN), 1636 (C=C), 1596 (Ar), 809 (Ar-Cl); LRMS (APCI M-1) 291.

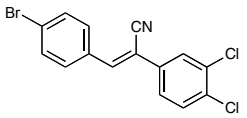
*(Z)-3-(4-chlorophenyl)-2-(3,4-dichlorophenyl)acrylonitrile (**26**)*[6]



Synthesized using the general procedure as for **1**, from 4-chlorobenzaldehyde and 3,4-dichlorophenylacetonitrile to afford **26** as a white solid; 66%; mp 167-168 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.80 (d, *J* = 8.1Hz, 2H, Ar2 H2; Ar2 H6), 7.75-7.74 (m, 1H, Ar H5), 7.51-7.49 (m, 3H Ar H2; Ar H6; CH=C), 7.29 (d, *J* = 8.1Hz, 2H, Ar2 H3; Ar2 H5); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 143.0, 141.4, 134.1, 132.9, 132.6, 130.4, 129.9, 129.3 (2 x Ar), 129.0 (2 x Ar), 127.1, 124.5, 117.0, 107.5; υ_{max}(KBr)/cm^{-1}: 2214 (CN), 1637 (C=C), 1594 (Ar), 1478 (Ar), 810 (Ar-Cl); LRMS (APCI M-1) 307.

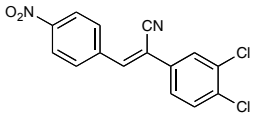
*(Z)-3-(4-bromophenyl)-2-(3,4-dichlorophenyl)acrylonitrile (**27**)*



Synthesized using the general procedure as for **1**, from 4-bromobenzaldehyde and 3,4-dichlorophenylacetonitrile to afford **27** as a yellow solid; 85%; mp 120-121 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.75 (m, 3H, Ar2 H2; Ar2 H6; CH=C), 7.61 (d, *J* = 8.56 Hz, 2H, Ar2 H3; Ar2 H5), 7.50 (m, 2H, Ar H2; Ar H5), 7.46 (d, *J* = 8.45 Hz, 1H, Ar H6); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 141.8, 134.1, 133.6, 133.6, 132.2, 131.9, 131.0, 130.7 (2 x Ar), 127.6 (2 x Ar), 125.6, 125.1, 116.9, 110.1; υ_{max}(KBr)/cm^{-1}: 2214 (CN), 1635 (C=C), 1598 (Ar); LRMS (APCI M-1) 351; HRMS (ESI M+H) for $C_{15}H_8BrCl_2N$, calculated 351.9295; found 351.9298.

*(Z)-2-(3,4-dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**28**)*

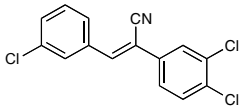


Synthesized using the general procedure as for **1**, from 4-nitrobenzaldehyde and 3,4-dichlorophenylacetonitrile to afford **28** as a purple solid; 75%; mp 133-134 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 8.34 (d, *J* = 8.8Hz, 2H, Ar2 H3; Ar2 H5), 8.03 (d, *J* = 8.8Hz, 2H, Ar2 H2; Ar2 H6), 7.81-7.80 (m, 1H, Ar H5), 7.58-7.56 (m, 2H, Ar H2 + Ar H6),

7.26 (s, 1H, CH=C), ; ^{13}C NMR ($CDCl_3$) (75 MHz): δ 139.6, 138.3, 133.3, 132.8, 132.1, 130.7, 129.5 (2 x Ar), 129.1, 127.4, 124.9, 123.7 (2 x Ar), 115.8, 113.2; υ_{max}(KBr)/cm^{-1}: 2215 (CN), 1674 (C=C), 1592 (Ar), 1513 (NO), 1345 (NO); LRMS (APCI M+1) 289; HRMS (ESI M+H) for $C_{15}H_8Cl_2N_2O_2$, calculated 319.0041; found 319.0048.

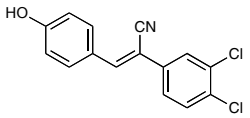
*(Z)-3-(3-chlorophenyl)-2-(3,4-dichlorophenyl)acrylonitrile (**29**)*[5]



Synthesized using the general procedure as for **1**, from 3-chlorobenzaldehyde and 3,4-dichlorophenylacetonitrile to afford **29** as a white solid; 65%; mp 138-140 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.84-7.81 (m, 2H, Ar2 H2; Ar2 H6), 7.76 (s, 1H, CH=C), 7.52-7.51 (m, 2H, Ar2 H4; Ar2 H5), 7.45-7.42 (m, 3H, Ar H2; Ar H5; Ar H6); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 141.0, 134.6, 134.2, 133.4, 133.3, 133.1, 130.5, 130.4, 129.8, 128.9, 127.3, 126.6, 124.7, 116.2, 110.5; υ_{max}(KBr)/cm^{-1}: 2212 (CN), 1636 (C=C), 1601 (Ar), 677 (Ar-Cl); LRMS (APCI M-1) 307.

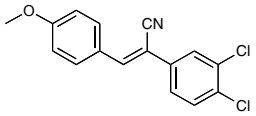
*(Z)-2-(3,4-dichlorophenyl)-3-(4-hydroxyphenyl)acrylonitrile (**30**)*



Synthesized using the general procedure as for **11**, from 4-hydroxybenzaldehyde and 2-(3,4-dichlorophenyl)acetonitrile to afford **30** as a yellow solid; 90%; mp 153-154 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.90-7.89 (m, 4H, Ar2 H2; Ar2 H6; CH=C; Ar H5), 7.67-7.66 (m, 2H, Ar H2; Ar H6), 6.97-6.94 (m, 2H, Ar2 H3; Ar2 H5), 5.98 (br, 1H, OH); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 161.1, 143.6, 135.3, 132.0, 131.4 (2 x Ar), 130.9, 130.5, 126.5, 124.8, 123.9, 117.3, 115.6 (2 x Ar), 103.0; υ_{max}(KBr)/cm^{-1}: 3467 (OH), 2210 (CN), 1580 (Ar); LRMS (APCI M-1) 288; HRMS (ESI M+H) for $C_{15}H_9Cl_2NO$, calculated 290.0139; found 290.0144.

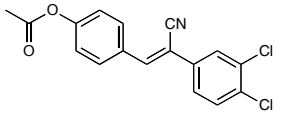
*(Z)-2-(3,4-dichlorophenyl)-3-(4-methoxyphenyl)acrylonitrile (**31**)*[6]



Synthesized using the general procedure as for **1**, from 4-methoxybenzaldehyde and 3,4-dichlorophenylacetonitrile to afford **31** as a yellow solid; 79%; mp 166-167 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.89 (d, *J* = 8.9 Hz, 2H, Ar2 H2; Ar2 H6), 7.73 (m, 1H, Ar H5), 7.49-7.44 (m, 3H, Ar H2; Ar H6; CH=C), 6.99 (d, *J* = 8.9 Hz, 2H, Ar2 H3; Ar2 H5), 3.88 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 161.4, 142.5, 134.4, 132.9, 132.3, 130.9 (2 x Ar), 130.3, 126.9, 125.4, 124.4, 117.2, 114.0 (2 x Ar), 105.7, 54.9; υ_{max}(KBr)/cm^{-1}: 2212 (CN), 1638 (C=C), 1609 (Ar), 1593 (Ar), 1513 (Ar); LRMS (APCI M-1) 303.

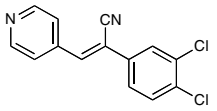
*(Z)-4-(2-cyano-2-(3,4-dichlorophenyl)vinyl)phenyl acetate (**32**)*



(*Z*)-2-(3,4-dichlorophenyl)-3-(4-hydroxyphenyl)acrylonitrile (**30**) (290 mg, 1.0 mmol) was dissolved in Ac_2O (5 mL), and 3 drops of conc. H_2SO_4 were added and the solution stirred overnight. The reaction was quenched with H_2O (10 mL) and the resultant precipitate was collected under suction, and recrystallised from EtOH to afford **32** as a yellow solid; 35%; mp 154-155 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.92 (d, *J* = 8.7 Hz, 2H, Ar2 H2; Ar2 H6), 7.75 (m, 1H, Ar H5), 7.51-7.49 (m 3H, Ar H2; Ar H6; CH=C), 7.23 (d, *J* = 8.7 Hz, 2H, Ar2 H3; Ar2 H5), 2.33 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 168.4, 152.0, 141.7, 133.8, 133.0, 132.9, 131.1, 130.4, 130.2 (2 x Ar), 127.1, 124.6, 121.8 (2 x Ar), 116.6, 108.8, 20.6; υ_{max}(KBr)/cm^{-1}: 2210 (CN), 1764 (C=O), 1597 (Ar), 1507 (Ar), 1221 (CO); LRMS (APCI M-1) 331; HRMS (ESI M+H) for $C_{17}H_{11}Cl_2NO_2$, calculated 332.0245; found 332.0249.

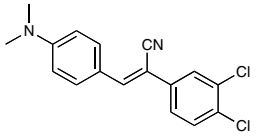
*(Z)-2-(3,4-dichlorophenyl)-3-(pyridin-4-yl)acrylonitrile (**33**)*



Synthesized using the general procedure as for **1**, from 4-pyridinecarboxaldehyde and 3,4-dichlorophenylacetonitrile to afford **33** as a white solid; 66%; mp 188-189 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 8.77 (d, *J* = 6.2 Hz, 2H, Ar2 H3; Ar2 H5), 7.79 (m, 1H, Ar H5), 7.69 (d, *J* = 6.2 Hz, 2H, Ar2 H2; Ar2 H6), 7.55-7.54 (m, 2H, Ar H2; Ar H6), 7.47 (s, 1H, CH=C); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 150.3 (2 x Ar), 139.6, 139.4, 134.1, 133.3, 132.7, 130.7, 127.5, 124.9, 122.0 (2 x Ar), 115.6, 113.7; υ_{max}(KBr)/cm^{-1}: 2217 (CN), 1636 (C=C), 816 (Ar-Cl); LRMS (APCI M+1) 275; HRMS (ESI M+H) for $C_{14}H_8Cl_2N_2$, calculated 275.0143; found 275.0144.

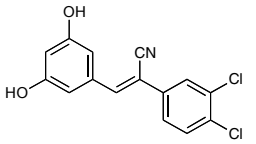
*(Z)-2-(3,4-dichlorophenyl)-3-(4-(dimethylamino)phenyl)acrylonitrile (**34**)*[6]



Synthesized using the general procedure as for **1**, from 4-*N,N*-dimethylaminobenzaldehyde and 3,4-dichlorophenylacetonitrile to afford **34** as a yellow solid; 40%; mp 210-212ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): δ 7.86 (d, *J* = 9.0 Hz, 2H, Ar2 H2; Ar2 H6), 7.71- 6.69 (m, 1H, Ar H5), 7.46-7.45 (m, 2H, Ar H2; Ar H6), 7.37 (s, 1H, CH=C), 6.72 (d, *J* = 9.0 Hz, 2H, Ar2 H3; Ar2 H5), 3.08 (s, 6H, $N(CH_3)_2$); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 151.5, 143.0, 135.2, 134.2, 132.6, 131.1 (2 x Ar), 130.2, 126.4, 124.0, 120.4, 118.3, 111.0 (2 x Ar), 101.1, 29.5; υ_{max}(KBr)/cm^{-1}: 2208 (CN), 1614 (C=C), 1580 (Ar), 805 (Ar-Cl); LRMS (APCI M+1) 317.

*(Z)-2-(3,4-dichlorophenyl)-3-(3,5-dihydroxyphenyl)acrylonitrile (**35**)*



Synthesized using the general procedure as for **11**, from 3,5-dihydroxybenzaldehyde and 2-(3,4-dichlorophenyl)acetonitrile to afford **35** as a brown solid; 25%; mp >300 ºC.

^{1}H NMR ($CDCl_3$) (300 MHz): 8.76 (br, 2H, 2 x OH), 7.92-7.91 (m, 1H, Ar H5), 7.82 (s, 1H, CH=C), 7.69-7.67 (m, 2H, Ar H2; Ar H5), 6.99 (m, 2H, Ar2 H2; Ar2 H5), 6.52-6.51 (m, 1H, Ar2 H6); ^{13}C NMR ($CDCl_3$) (75 MHz): 158.3 (2 x Ar), 144.0, 134.7, 132.1, 131.7, 130.5, 129.7, 127.7, 127.0, 125.3, 116.5 (2 x Ar), 107.5, 105.2; υ_{max}(KBr)/cm^{-1}: 3437 (OH), 2218 (CN), 1582 (Ar), 804 (Ar-Cl); LRMS (APCI M-2) 304; HRMS (ESI M+H) for

$C_{15}H_9Cl_2NO_2$, calculated 306.0089; found 306.0095.

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5.3 Product Codes

Code	Page	Book	Molar Mass		Code	Page	Book	Molar Mass
MT-NOVO-00	41	013	170.16		MT-NOVO-11b	101	013	224.25
MT-NOVO-01	38	013	198.21		MT-NOVO-13a	44	015	226.23
MT-NOVO-02	39	013	212.24		MT-NOVO-13b	44	015	226.23
MT-NOVO-03	43	013	226.26		MT-NOVO-13c	51	015	226.23
MT-NOVO-04	42	013	226.26		MT-NOVO-13d	51	015	226.23
MT-NOVO-05a	76	013	240.29		MT-NOVO-14a	46	015	240.25
MT-NOVO-06	78	013	282.37		MT-NOVO-14b	46	015	240.25
MT-NOVO-07	116	013	210.22		MT-NOVO-14c	47	015	240.25
MT-NOVO-07a	116	013	210.22		MT-NOVO-15	131	015	250.29
MT-NOVO-08	75	013	252.30		MT021B016	21	016	228.24
MT-NOVO-09a	77	013	254.32		MT024B016	24	016	364.33
MT-NOVO-11a	101	013	224.25		MT023B016	23	016	460.41
MT030B016	30	106	308.22		MT027B016	27	016	571.00

Code	Page	Book	Molar Mass		Code	Page	Book	Molar Mass
MT-CN-01	94	013	194.23		MT-CN-19	49	014	308.59
MT-CN-03	89	013	212.22		MT-CN-20	53	014	308.59
MT-CN-05	93	013	228.68		MT-CN-21	77	014	292.11
MT-CN-06	88	013	239.23		MT-CN-23	159	015	353.04
MT-CN-07	90	013	263.12		MT-CN-24	56	014	304.17
MT-CN-09	95	013	196.25		MT-CN-25	61	014	290.14
MT-CN-10	19	014	214.24		MT-CN-26	63	014	306.14
MT-CN-11	21	014	230.69		MT-CN-27	66	014	288.17
MT-CN-12	14	014	211.26		MT-CN-28	59	014	317.21
MT-CN-13	22	014	265.14		MT-CN-29	68	014	332.18
MT-CN-14	45	014	274.14		MT-CN-31	71	014	254.16
MT-CN-15	48	014	324.20		MT-CN-32	73	014	324.29
MT-CN-16	60	014	324.20		MT-CN-33	11	016	264.11
MT-CN-17	67	014	275.13		MT-CN-34	MR	MR	350.24
MT-CN-18	47	014	319.14					

Code	Page	Book	Molar Mass		Code	Page	Book	Molar Mass
MT-CN-35	114	014	313.18		MT-CN-210	34	015	272.30
MT-CN-36	17	015	342.14		MT-CN-211	5	015	306.75
MT-CN-201	2	105	256.69		MT-CN-212	6	015	341.19
MT-CN-202	3	015	291.13		MT-CN-213	7	015	317.30
MT-CN-203	8	015	267.24		MT-CN-214	12	015	302.33
MT-CN-204	13	015	252.27		MT-CN-215	15	015	340.30
MT-CN-205	14	015	290.24		MT-CN-216	19	015	362.25
MT-CN-206	18	015	312.19		MT-CN-217	22	015	311.34
MT-CN-207	23	015	261.18		MT-CN-218	30	015	286.33
MT-CN-208	36	015	236.27		MT-CN-219	31	015	288.30
MT-CN-209	37	015	238.24		MT-CN-220	29	015	322.36

Code	Page	Book	Molar Mass		Code	Page	Book	Molar Mass
MT-CA-06	93	015	323.17		MT-BA-14	113	015	381.25
MT-CA-07	70	015	338.14		MT-BA-15	114	015	381.25
MT-BA-01	80	014	281.31		MT-BA-16	122	015	365.64
MT-BA-02	76	014	282.29		MT-BA-18	121	015	347.20
MT-BA-03	97A	015	292.33		MT-BA-19	120	015	345.22
MT-BA-04	97B	015	342.29		MT-BA-20	119	015	361.22
MT-BA-05	98A	015	342.29		MT-FA-01	94	014	312.32
MT-BA-06	103	015	326.78		MT-FA-02	95	014	282.29
MT-BA-08	108	015	308.33		MT-FA-03	99	014	358.39
MT-BA-09	107	015	306.36		MT-FA-04	100	014	296.32
MT-BA-10	104	015	322.36		MT-FA-05	101	014	361.19
MT-BA-11	105	015	320.17		MT-FA-06	102	014	332.35
MT-BA-12	106	015	321.16		MT-FA-07	105	014	316.74
MT-BA-13	110	015	331.20		MT-FA-08	106	014	361.19