Small Molecule Inhibitors of Mdm2 E3 Ubiquitin Ligase Activity

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‘All truths are easy to understand once they are discovered; the point is to discover them’

Galileo Galilei

Abstract

'Small Molecule Inhibitors of Mdm2 E3 Ubiquitin Ligase Activity'

Half of cancers retain wild type p53 but have alterations in the pathways involved in p53 regulation. Murine double minute 2 (Mdm2) regulates p53 by acting as an E3 ubiquitin ligase, which tags p53 for degradation through the proteasome. A small molecule inhibitor, a 5-deazaflavin analogue, has previously been identified by high throughput screening to inhibit Mdm2 E3 ubiquitin ligase activity, thereby reactivating apoptotic function of p53 selectively in cancer cells.

Ninety 5-deazaflavin analogues have been synthesised by an optimized existing method and a novel method of synthesis, using the required 6-anilinouracil and 2-*p*-toluenesulfonyloxybenzaldehyde.The biological ability of the 5-deazaflavin analogues to act as inhibitors of Mdm2 E3 ubiquitin ligase activity to reactivate p53 has been ascertained. A new quantitative biological assay was developed, by scientists based at the Beatson Institute, for 5-deazaflavin compounds, showing excellent inhibition of Mdm2 E3 ubiquitin ligase activity on the previous qualitative biological assay, to yield IC_{50} data.

The biological results have established a clear and logical structure-activity relationship comprising of an electron-withdrawing hydrophobic substituent at the nine position and the N_{10} phenyl being a prerequisite for activity as a Mdm2 inhibitor. Also *meta* substitution of the N_{10} phenyl improves activity against Mdm2 E3 ubiquitin ligase activity. Hit optimization has occurred with 10-(3-chlorophenyl)-9-trifluoromethyl-5-deazaflavin being thirty times more active than the previous identified hit compound, 10-(4-chlorophenyl)-7-nitro-5-deazaflavin.

Using the X-ray crystal structure of the Mdm2/MdmX heterodimer, an improved understanding of how Mdm2 acts as an E3 ubiquitin ligase is described and used to form a hypothesis of how 5-deazaflavin analogues function as inhibitors of Mdm2.

The work suggests the principle that small molecular weight compounds can inhibit E3 ubiquitin ligases as a possible anti-cancer therapy, and provide the foundation and framework for additional studies and investigation in a new and developing field of medicinal chemistry.

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Publications

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Abbreviations

^{3}J	Coupling constants through 3 bonds during *meta* coupling
3T3	3 Day transfer, inoculum 3 x 10^5 cells
^{4}J	Coupling constants through 4 bonds due to *meta* coupling
aq	Aqueous
ARF	Alternate reading frame
ARF-BP1	ARF-Binding Protein 1
ATP	Adenosine-5'-triphosphate
br s	Broad singlet
calcd	Calculated
CARP1/2	Caspases-8/10 associated RING proteins 1 and 2
$CDCl_3$	Deuterated chloroform
CDK	Cyclin-dependent kinase
CH	Tertiary carbon
CH_2	Secondary carbon
CH_3	Primary carbon
COP1	Constitutively photomorphogenic 1
Cq	Quaternary carbon
d	Doublet
Daxx	Death domain associated protein
dd	Doublet of doublets
ddd	Doublet of doublets of doublets
DEAD	Diethylazodicarboxylate
dec	Decomposed
DIBAL-H	Di-isobutylaluminum hydride
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DUBs	Deubiquitinating enzymes
E1	Ubiquitin activating enzyme
E2	Ubiquitin conjugating enzyme
E3	Ubiquitin ligase
E6-AP	E6-associated protein
ESI	Electrospray ionisation
FDA	Food and Drug Administration
FT-IR	Fourier Transform Infra-Red
G_1	Gap 1 phase of the cell cycle
G_2	Gap 2 phase of the cell cycle
G76	Carboxyl-terminal glycine of ubiquitin
GST	Glutathione *S*-transferase

Hdm2	Human double minute 2
HECT	Homologous to E6-AP carboxyl terminus
HLI	Hdm2 ligase inhibitor
HPLC	High performance liquid chromatography
HPV	Human Papilloma Virus
HRMS	High Resolution Mass Spectrum
IC_{50}	Half maximal inhibitory concentration
IUPAC	International Union of Pure and Applied Chemistry
J	Coupling constants
K48	Ubiquitin subunits linked though lysine 48
kDa	Kilo Dalton
L11	Ribosomal protein 11
L23	Ribosomal protein 23
L5	Ribosomal protein 5
M	Mitosis phase of the cell cycle
m	Multiplet
m.pt	Melting point
MDa	Mega dalton
Mdm2	Murine double minute 2
Mdm4	Murine double minute 4
MdmX	MIPS digital media extension
mRNA	Messenger ribonucleic acid
MTBP	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein
N^3	Third position of the 5-deazaflavin pharmacophore
NMR	Nuclear magnetic resonance
OTs	*p*-Toluenesulfonyloxide method of 5-deazaflavin synthesis
p21	Protein 21
p53	Protein 53
PAI-1	Plasminogen activator inhibitor-1
Pd/C	Palladium on carbon
PIRH2	p53 induced protein with RING-H2 domain
ppm	Parts per million
PUMA	*p*53 unregulated modulator of apoptosis protein
q	Quadruplet
Redox	Reduction and oxidation
RING	Really Interesting New Gene
RPE	Retinal Pigment Epithelium
S	Synthesis phase of the cell cycle
s	Singlet
SAR	Structure-activity relationship
t	Triplet
TMS	Tetramethylsilane
TOPORS	Human topoisomerase I and p53 binding protein

TR3	Nerve growth factor IB
Ub	Ubiquitinylation
UV	Ultra violet light
δ	Chemical shifts
π	Substituent hydrophobicity constant
σ	Hammet substituent constant

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Compound Naming

In the main body of text the trivial names will be used for the compounds, for ease of reading, but in the experimental section the International Union of Pure and Applied Chemistry (IUPAC) systematic nomenclature will be used.

Hence in my thesis, the final compounds are called 5-deazaflavins but in the experimental chapter these compounds are given their full name of 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-diones. The same nomenclature system will be applied to the intermediates of 5-deazaflavin synthesis. For example, in the main text of this thesis the intermediate is called 6-anilinouracil but in the experimental section this compound is given its full name of 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione.

A final compound describes the last compound to be synthesised using a stated reaction pathway that will be biologically tested as an inhibitor of Mdm2 E3 ubiquitin ligase activity.

Introduction

Cancer

Cancer is defined as a collection of diseases with the common feature of uncontrolled and unregulated cell growth. Cancer cells grow regardless of normal controls and have the ability to invade other tissue, either by direct growth into adjacent tissue through invasion or by implantation into distant sites by metastasis [1].

In the UK, cancer is the cause of more than a quarter (26%) of all deaths with more than 284,000 people diagnosed with cancer each year [2]. There were 154,484 cancer deaths in the UK in 2007 [3]. The risk of developing cancer tends to increase with age and in 2007, 76% of cases diagnosed in the UK were in people aged over 65 [4-6]. This statistic proves that age is one of the biggest pre-disposing risk factors in cancer.

Cancer is the second leading cause of death in America behind cardiovascular disease. Overall 1 in 4 deaths in the USA are due to cancer with 1,479,350 new cases of cancer predicted in 2009 [7]. Of that number, 38% or 562,340 people are predicted to die of the disease [8].

The total economic burden of cancer to the US economy in 2008 was estimated to be $192.4 billion, broken down as $72.1 billion on direct medical cost including health spending and $120.4 billion on indirect cost of lost productivity due to premature death [9].

Each year 10.9 million people worldwide are diagnosed with cancer and there are 6.7 million deaths from the disease. It is estimated that there are 24.6

million people alive who have received a diagnosis of cancer in the last five years [10].

Cancer is and will continue to be a global public health issue as the world population increases and developed countries, such as the USA and the UK, have ageing populations [11], the number of people diagnosed with cancer and the scale of the problem will increase.

What Causes Cancer?

Cancer is caused by mutations to genes that encode for proteins involved in cell growth, division, homeostasis and programmed cell death (apoptosis) leading to unregulated and uncontrolled cell growth. In the majority of cancers, multiple mutation events are required to transform a normal cell into a cancerous cell [12, 13].

Genetic mutations found in cancer affect two classes of genes: oncogenes, where a gain of function (or expression) mutation drives a cell towards cancer, and tumour suppressor genes, where a loss of function (or expression) mutation leads to cancer [14, 15].

Normal cellular genes that can be converted by mutation into oncogenes are called proto-oncogenes. Proto-oncogenes are normal genes that code for proteins that are vital for cell function, growth, repair, regulation and survival. Mutations in proto-oncogenes create oncogenes which causes the gene to over express itself, increasing the amount or activity of the protein that the gene codes for [16]. There are three types of genetic mutation that can make a proto-oncogene mutate into an oncogene [Figure 1] [17].

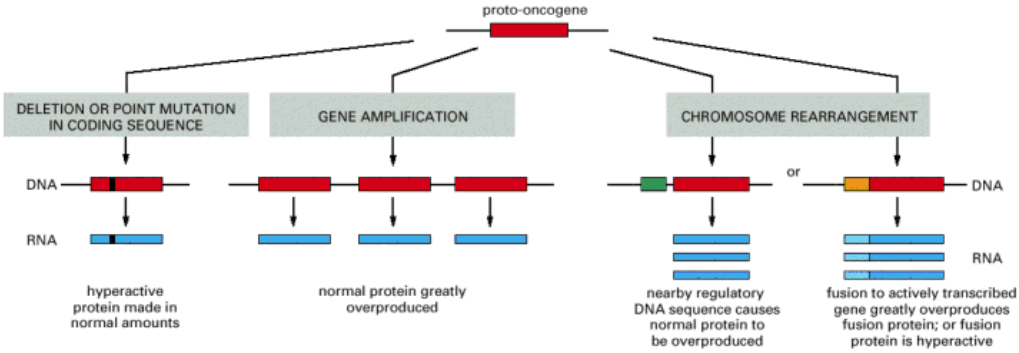


Figure 1. The three ways in which a proto-oncogene can be converted into an oncogene. The gene may be changed by a small scale alteration in sequence such as point mutation or by a large scale alteration such as partial deletion. Gene amplification events caused by errors in DNA replication may over express the cancer critical gene because of the presence of extra copies of the gene. Chromosome rearrangement that involves the breakage and rejoining of the DNA helix can cause cancer proto-oncogenes to mutate into oncogenes. These changes can occur in adjacent control regions of the DNA so that the gene is simply expressed at concentrations that are much higher than normal or the change can occur in the protein coding region so as to yield a hyperactive protein [17, 18]. Taken from [17].

Tumour suppressor genes code for tumour suppressor proteins which generally control cell growth or promote apoptosis. The function of these proteins can be lost by mutations to the related tumour suppressor gene or to genes that code for regulator or activating proteins of the tumour suppressor protein. The loss of tumour suppressor protein function leads to increased cell growth with decreased apoptosis of a cell and potentially cancer [19-22].

The complexity of cancer as a disease can be understood in a logical manner by a small number of underlying principle or hallmarks [23]. The seven hallmarks of cancer cells are:

1) Self sufficiency in growth signals.

2) Insensitivity to antigrowth signals.

3) Evading apoptosis.

4) Limitless replicative potential or evading senescence [24-26].

5) Sustained angiogenesis [27].

6) Tissue invasion and metastasis [28].

7) Genome instability [12, 13, 18].

Cancer cell formation is a multi-step process, which requires the occurrence of many different mutations relating to the hallmarks of cancer [23], with each genetic mutation guiding the progressive transformation of normal human cells into cancerous cells. In other words cancer development continues in a similar fashion to Darwinian evolution, with a succession of genetic changes, each giving one or more type of growth advantage, from the hallmarks [23], leads to the conversion of normal cells to cancerous cells [29]. Cancer is caused by the propagation of genetic mutation from one cell to another until the cell has achieved a growth advantage causing uncontrolled cell growth.

Genetic mutations occur regularly in the human body with an estimated rate of one mutation for every twenty million gene cell divisions [30]. There are approximately 10^{14} target cells in the average human being and a large number of genes involved in regulating cell expansion, it is remarkable that cancer is not more common. The small number of mutations yet the high number of potential targets highlights the efficiency of the body's antitumourigenic mechanisms in protecting itself from genetic mutations [31]. Cancer only prevails when these antitumourigenic mechanisms fail [32]. A protein highly involved in the body's antitumourigenic mechanisms is p53, whose primary function is to prevent the propagation of genetic mutations.

p53

p53 is a 393 amino acid [33, 34] tumour suppressor protein known as the 'Guardian of the Genome' [35] or 'Cellular Gatekeeper' [36] because of its critical role in coordinating cellular response to carcinogenic or genotoxic stress. p53 functions as a transcription factor [37] which is a protein that binds

to specific DNA sequences and thereby controls the transcription of genetic information from DNA to mRNA [38]. p53 plays a central role in the cell's defence against tumour development [35, 36, 39]. p53 induces cell cycle arrest so that the cell can undergo genetic repair. If however the cell is damaged beyond repair, p53 induces apoptosis [40-42].

p53 is named after its initially overestimated molecular mass of 53kDa. The correct molecular mass of p53 is actually 43.7kDa . Initial overestimation was due to the presence of proline rich regions that slows down p53 migration in gels used to estimate its mass [43].

p53 was first discovered in 1979 as a cellular partner of simian virus 40 large T-antigen, the oncoprotein of this tumour virus were found by several research groups working independently [44-50]. Further research, in the 1980s, has seen the cloning of p53 [51-56] and the understanding that p53 was not an oncogene as first thought but a tumour suppressor protein [57-59]. The functions of p53 as a transcriptional factor [60-64] involved in apoptosis [65, 66], cell cycle arrest [67] and senescence [68] were discovered in the 1990s. More recent research work has shown new functions of p53 in metabolism [69] and embryo implantation [70]. All of these discoveries contribute to the understanding of p53 function in the human body. The next big step in p53 research will be the transfer of the wealth of knowledge on p53 function into applications in cancer treatment and prevention [43, 71].

p53 is activated by a variety of post-translational modifications which results in p53 concentration to increase causing an increased ability of p53 to bind to DNA, mediating transcriptional activation [72]. Transcriptional activation of

p53 can initiate cell cycle arrest allowing time for DNA repair, initiate removal of the damaged cells by apoptosis or the prevention of the cell from growing at all in senescence. The response to p53 depends partly on which p53 responsive genes are activated following induction of p53. Many p53 inducible genes play a role in mediating the different responses to p53 [73]. However p53 is induced, the response is to prevent the propagation of genetic mutations by either cell cycle arrest, apoptosis or senescence.

p53 in Cell Cycle Arrest

The cell cycle [Figure 2] is the orderly sequence of events by which cells reproduce thus regulating cell growth. The cell cycle can be divided into 4 distinct phases; cells enter the cycle, due to external and internal growth signals, at gap 1 phase (G_1) and proceed around the cycle in a clockwise direction. The G_1 phase is required for cell growth and preparation of DNA synthesis. The synthesis phase (S), is where DNA is replicated. The gap 2 phase (G_2) is needed for cell growth and preparation for mitosis. Interphase consists of the G_1, S and G_2 where the cell grows continuously. In the last phase mitosis (M), the nucleus divides by mitosis then the cytoplasm divides by cytokinesis. At the end of the cell cycle there are two genetically identical daughter cells, so one new cell is produced hence cell division [17, 73-76].

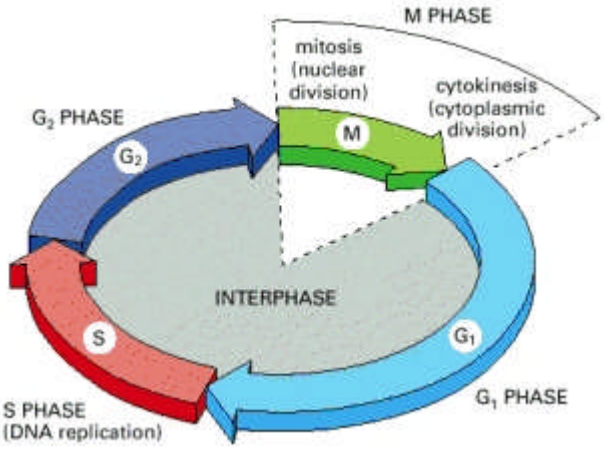


Figure 2. The cell cycle. The cell grows continuously in interphase, which consists of 3 phases: DNA replication is confined to the S phase; G_1 is the gap between M phase and S phase, while G_2 is the gap between S phase and M phase. In M phase, the nucleus and then the cytoplasm divide. Taken from [17].

Progression through the cell cycle is tightly regulated by cell cycle checkpoints that detect possible defects during DNA synthesis and chromosome segregation. Regulation at these checkpoints ensures that critical events in a particular phase of the cell cycle are completed before a new phase is initiated. A variety of mechanisms are involved in the regulation of the checkpoints. These mechanisms are controlled by proteins with the most important group/class of regulator proteins being the cyclin-dependent kinases (CDKs). CDKs allow progression through the different phases of the cell cycle by the phosphorylation of substrates. Their kinase activity is dependent on the presence of activating subunits known as cyclins. There are many different CDKs and cyclins involved in the many different cell cycle checkpoints [74-77].

p53 initiates cell cycle arrest by stimulating transcription of p21. p21 is a CDK inhibitor that inhibits the CDK2/cyclin E and CDK2/cyclin A kinases, preventing these kinases from promoting cell cycle progression at the G_1/S and S cell cycle check point [78].

Cell cycle arrest prevents the propagation of genetic mutation by allowing the cell to undergo genetic repair. If however the cell is damaged beyond repair, as previously mentioned, p53 induces apoptosis or depending on the type of DNA damage, p53 induces senescence.

p53 in Apoptosis

Apoptosis is an active, tightly regulated, energy-dependent process of cellular suicide that has been conserved throughout evolution [79]. The name 'apoptosis' was first used in 1972, to refer to cells undergoing cell death with defined morphological observations [80, 81]. Apoptotic cells undergo a typical series of morphological changes; cell shrinkage, membrane blebbing, nuclear chromatin condensation and DNA fragmentation [81, 82]. The cell breaks down into cellular fragments, known as apoptotic bodies that are engulfed, through phagocytosis [83].

The developmental role of apoptosis has been highly conserved throughout evolution; for example the maturation of the lungs in foetuses [84]. Apoptosis occurs in adult organisms to maintain normal cellular homeostasis. This includes regulating a response to infectious agents [85] and eliminating cells that have acquired DNA damage [86] or genetic mutation. Insufficient apoptosis can lead to cancer [87].

p53 triggers apoptosis by directly activating transcription of a large array of proteins involved in the activation, maintenance and progression of apoptosis such as the *p*53 unregulated modulator of apoptosis protein (PUMA) [88].

p53 in Senescence

Senescence can be described as irreversible cycle arrest. The word senescence derives from *senex*, a Latin word meaning old man or old age and was first termed to describe cells that ceased to divide in culture [89]. Senescence is characterised by the inability of cells to grow despite the presence of abundant nutrients and by the maintenance of cell viability and metabolic activity [26]. Senescence in normal cells is caused by telomere shortening [90, 91] following extensive cell division. Telomeres are basically the ends of linear chromosomes [92]. Certain cells can undergo senescence independently of telomere shortening [93-95] due to stress, with the nature of this type of senescence not well understood [24-26].

Senescence is triggered through the p53 pathway similar to cell cycle arrest but the maintenance and induction of senescence by p53 is not well understood, in comparison to cell cycle arrest or apoptosis. One method by which p53 maintains senescence is through regulation of plasminogen activator inhibitor-1 (PAI-1) expression, which is a marker of senescent cells [68, 96]. p53 stabilizes PAI-1 mRNA through direct binding [97]. Senescent mouse and human cells have been shown to escape senescence when PAI-1 is down-regulated [98, 99].

Cells that fail to senesce and continue to grow despite dysfunctional telomeres develop chromosomal mutations that can lead to cancer [100]. Cellular senescence is an important tumour suppression mechanism, which plays a role in the body's defence against cancer formation [101].

p53 in Cancer

Loss of the tumour suppressor activity of p53 can lead to uncontrolled cell growth and tumour formation causing cancer. The importance of p53 as a tumour suppressor is highlighted by the fact that most, if not, all human cancers show a loss of normal p53 function [39, 102], with 50% of these being due to a direct mutation of the p53 gene [102, 103]. This leads to the expression of mutant p53 protein, defective in its tumour suppression properties [104]. The remaining 50% of human cancers retain wild type p53, but have mutations to genes encoding for proteins involved in p53 regulation or activation [105].

p53 in Other Diseases and Biological Functions

p53 senescence activity contributes to the development of insulin resistance in diabetes [106]. p53 also plays a role in neuro-degeneration such as Alzheimer's [107], Parkinson's [108] and Huntington's [109] disease.

Recent research shows that p53 plays a role in other biological functions such as metabolism [69], sun tanning [110], and contributes to ageing [111-113]. p53 can also promote aerobic respiration which becomes important for endurance during exercise [114].

p53 Regulation

p53 has strong growth suppressive activity and therefore must be tightly regulated to allow normal cells to live and grow. This is achieved to a large extent by a protein called murine double minute 2 (Mdm2) [115, 116].

Mdm2

The Mdm2 gene was first discovered in DNA associated with paired acentric chromatin bodies, termed double minutes, in spontaneously transformed mouse 3T3 fibroblasts [117]. The corresponding human protein is sometimes referred to as Human double minute 2 (Hdm2) but in this thesis the abbreviation Mdm2 will be used regardless of species.

Mdm2 regulates p53 stability, function and concentration in three different ways:

1) Mdm2 binds via protein-protein interactions to the N-terminal transcription activation domain of p53 preventing transcription of p53 [118, 119].

2) Mdm2 is an E3 ubiquitin ligase, and thus promotes p53 degradation by ubiquitin dependent proteasomal degradation [120-124].

3) Mdm2 causes nuclear export of p53 into the cytoplasm of the cell, moving p53 away from its site of action [125].

Mdm2 is a negative regulator of p53 and forms an autoregulatory feedback loop with p53 [126, 127]. Mdm2 regulates p53 protein levels [114, 115, 117-122] while p53 transcribes Mdm2 [128, 129]. Mdm2 can also undergo auto-ubiquitinylation thereby self targeting itself for degradation [130] with certain kinases regulating this process [131]. Daxx (death domain associated protein) regulates the switch between Mdm2 ubiquitinylation of p53 and Mdm2 auto-ubiquitinylation [132]. Another protein, MTBP (Mdm2, transformed 3T3 cell double minute 2, p53 binding protein), has been shown to promote Mdm2 E3 ubiquitin ligase activity causing p53 ubquitination and degradation in

unstressed cells [133, 134]. The E3 ubiquitin ligase activity of Mdm2 towards p53 is significantly enhanced by heterodimerization with MdmX [135].

MdmX (also known as Mdm4) is a non redundant homologue of Mdm2 that also regulates p53 [136] and is overexpressed in many cancers [137]. Unlike Mdm2, however, MdmX expression is not regulated by p53 and MdmX is thus not part of the negative feedback loop with p53. MdmX also lacks intrinsic ubiquitin ligase activity but is itself a target for Mdm2 ubiquitinylation. It forms heterodimers with Mdm2, which enhances the ability of Mdm2 to induce p53 degradation [138]. MdmX binds p53 at the same site and with similar affinity as Mdm2 and in so doing blocks p53 transcriptional activity.

The negative regulation of p53 by Mdm2 is interrupted in several different ways depending on the nature of the genotoxic or non genotoxic stress. The suppression of Mdm2 regulation of p53 allows normal functions of p53 to resume hence inducing cell cycle arrest, apoptosis or senescence.

Most importantly, the functions of Mdm2 in p53 suppression are inhibited upon association with the ARF protein [112, 132]. ARF is a tumour suppressor protein which induces p53 mediated apoptosis by associating with Mdm2 to inhibit the ubiquitinylation and degradation of p53 [115, 139]. Mdm2 is also regulated by proteins involved in ribosome assembly and function such as L5, L11 and L23 [140-143]. Furthermore, Mdm2 is regulated through post translational modifications, including auto-ubiquitinylation [144] and multi-site phosphorylations by a range of kinases, in particular the DNA damage-induced kinases [139-145].

An example of post translational regulation of Mdm2 is by TR3 (Nerve Growth Factor IB) a novel negative regulator of Mdm2 by mediating with p53. TR3 down regulates p53 transcription activity by blocking acetylation of p53 by directly interacting with p53 but not with Mdm2 therefore inhibiting Mdm2 expression. Acetylation of p53 by TR3 down regulates transcriptional activity of p53 while Mdm2 has been shown to be inactivated when acetylated [145]. Also TR3 protects p53 from Mdm2 induced proteasome degradation [146].

The ability of Mdm2 to act as an E3 ubiquitin ligase leading to p53 protein degradation by ubiquitinylation is important in regulating p53 concentration levels in the cell. So what is an E3 ubiquitin ligase and what is ubiquitinylation?

Mdm2 E3 Ubiquitin Ligase Activity

Mdm2 E3 ubiquitin ligase activity is responsible for the attachment of ubiquitin onto p53. Ubiquitin is a 76 amino acid protein of 8 kDa in size, identified in 1975 [147]. Ubiquitin can be covalently attached to other proteins, such as p53, in a process called ubiquitinylation [Figure 3] which is an inducible and reversible process [148]. Ubiquitin tags a protein for degradation into its constituent amino acids by the 26S proteasome in an ATP dependent mechanism [149-151].

Ubiquitinylation of p53 involves three enzymes in a sequential reaction. Ubiquitin activating enzyme (E1) binds to ubiquitin in an ATP dependent manner, forming a thiol ester bond between its active site cysteine and the carboxyl-terminal glycine (G76) of ubiquitin [152]. The activated ubiquitin is then transferred from the E1-ubiquitin complex to ubiquitin conjugating

enzyme (E2) by transthioesterification forming a thioester bond between E2's active site cysteine and the G76 of ubiquitin. The final step involves Mdm2 which is already bound to p53 and acts as an E3 ubiquitin ligase. Mdm2 causes ubiquitin to be directly transferred from E2 to p53, forming an isopeptide linkage between the terminal G76 of ubiquitin and the ε amine group of an internal lysine of p53 [153]. Once p53 has been mono-ubiquitinylated, a further ubiquitin can be attached to the first ubiquitin to form a poly-ubiquitin chain by the same process. A poly-ubiquitin chain of four or more ubiquitin subunits linked though lysine 48 (K48) [154] is required to identify p53 for degradation into its constituent amino acids by the 26S proteasome with ubiquitin being recycled [149-151, 155-161].

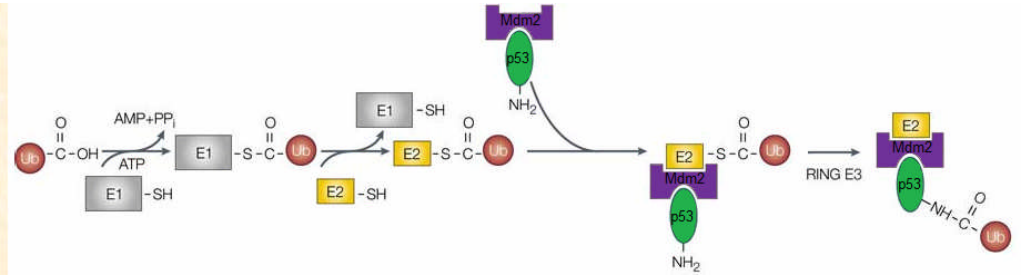


Figure 3. Ubiquitinylation of p53. Where ubiquitin (ub, red), ubiquitin activating enzyme (E1, grey), ubiquitin conjugating enzyme (E2, yellow), Mdm2 (purple) acting as an E3 ubiquitin ligase and p53 (green), the substrate, are shown. Adapted from [150].

E3 ubiquitin ligases provide the specificity and selectivity for substrate recognition of the ubiquitinylation process. There are known to be 1000 different E3 ubiquitin ligases in the human body and around 20 different E2 enzymes while there is just a single E1 [150, 155]. E3 ubiquitin ligases can be classified into two major types based on their domain structure and role in the ubiquitinylation process.

Homologous to E6-AP carboxyl terminus (HECT) are a class of E3 ubiquitin ligases that all function in a similar mannar. The first HECT domain E3

ubiquitin ligases was discovered during the identification of the E6-associated protein (E6-AP) responsible for E6-dependent ubiquitinylation of p53 [162, 163] by the Human Papilloma Virus (HPV). Later studies showed homology between the carboxyl terminal of E6-AP and a number of unrelated proteins [164] functioning as E3 ubiquitin ligases, hence a new class of ligases was discovered. The HECT domain is 350 amino acids long with a conserved active site cysteine, 35 amino acids away from the carboxyl terminal [165]. HECT E3 ubiquitin ligases transfer ubiquitin from E2 to an active site cysteine on itself, forming a thioester intermediate. This is then followed by transfer of ubiquitin to the substrate [Figure 4] [166, 167].

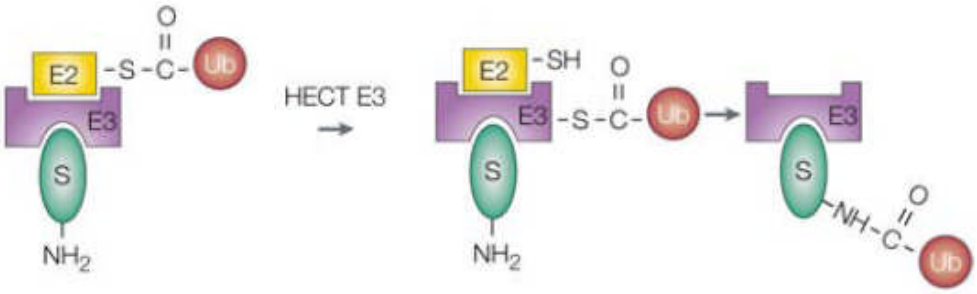


Figure 4. HECT E3 ubiquitin ligase mode of action in ubiquitinylation. Where ubiquitin (ub, red), ubiquitin conjugating enzyme (E2, yellow**),** HECT E3 ubiquitin ligase (purple) and the substrate (green) are shown. Adapted from [150].

The second type of E3 ubiquitin ligases are the Really Interesting New Gene (RING) domain [168]. Mdm2 is one of these ligases. RING domain E3 ubiquitin ligases are structurally defined by active site histidine and cysteine residues bound to two zinc atoms, in a cross branched system [169]. The NMR solution structure of Mdm2 RING domain is known [170]. RING E3 ubiquitin ligases allow ubiquitin to be transferred directly from E2 to substrate. In the case of Mdm2, p53 is the substrate. Mdm2 can also undergo auto-ubiquitinylation therefore self targeting itself for degradation [130].

Other E3 Ubiquitin Ligases to p53

Apart from Mdm2, other proteins can act as E3 ubiquitin ligases towards p53. p53 induced protein with RING-H2 domain (PIRH2) is a protein that negatively regulates p53 by binding to p53 and acting as a RING domain E3 ubiquitin ligase independently of Mdm2. Like Mdm2, PIRH2 is transcribed by p53 causing an autoregulatory feedback loop that controls p53 function [171]. Constitutively photomorphogenic 1 (COP1) acts independently of Mdm2 as a RING E3 ubiquitin ligase to p53 [172]. ARF-Binding Protein 1 (ARF-BP1) acts as a HECT E3 ubiquitin ligase towards p53 [173]. Caspases-8/10 associated RING proteins 1 and 2 (CARP1/2) physically interact with and ubiquitinate p53 independently of Mdm2, targeting it for degradation in the absence of Mdm2 but more uniquely CAPR acts as an E3 ubiquitin ligase towards serine 20 or 15-phosphorylated p53 [174]. p53 is phosphorylated at the 15 and 20 serine residues by DNA damage induced kinases due to genotoxic stress, such as DNA damage or UV light damage. Also CARP acts as an E3 ubiquitin ligase towards caspases [175], which are a family of cysteine proteases that plays essential roles in apoptosis. The human topoisomerase I and p53 binding protein (TOPORS) also acts as an E3 ubiquitin ligase towards p53 [176]. The roles of these E3 ubiquitin ligases in p53 regulation with or without Mdm2 are not well understood, and there is no evidence that exists to suggest that any of these p53 E3 ubiquitin ligases can replace Mdm2 in the regulation of p53 stability [177].

Once Mdm2 has acted as an E3 ubiquitin ligase and attached four ubiquitin proteins onto p53. p53 is then broken down by the 26S proteasome into its constituent amino acids.

26S Proteasome

The 26S proteasome, also known as the proteasome holoenzyme, is a 2.5 MDa multicatalytic protease that degrades polyubiquinated proteins to small peptides. The structure of the 26S proteasome can be divided into two major subcomplexes; the 20S core particle contains the protease subunits and the 19S particle that regulates the function of the proteasome [178, 179]. The 20S core particle is a barrel shaped structure made up of four stacked heptagonal rings [180]. The two inner rings contain the proteolytic active sites facing inward into the chamber. The 19S component is comprised of at least 18 different subunits and can assemble at either end of the 20S, and is proposed to form a lid and base substructure, which may specifically recognise ubiquitinylated protein [181]. The mechanism of how the proteasome recognises ubiquinated proteins has been partly described with the identification of an ubiquitin binding subunit called Rpn10/Mcb1 located in the 19S component [182]. In most cases the proteasome cleaves protein substrates into small peptides, usually 3-22 amino acid residues in length [183]. The ubiquitin molecules are cleaved off the protein by deubiquitinating enzymes (DUBs) (thiol proteases) and recycled. There are at least five different structural classes of DUBs, and a wide range of substrate specificities and functions have been reported [184].

Small Molecule Inhibitors of 26S Proteasomal Degradation

A drug named Bortezomib, **1**, functions as a selective and reversible inhibitor of the 26S proteasome thus inhibiting the degradation of proteins critically involved in regulation of cell proliferation and survival [185]. Inhibition of the 26S proteasome prevents the degradation of key proteins and affects multiple signalling cascades within the cell, ultimately leading to cell death by apoptosis

[186]. Bortezomib, **1**, also known as VELCADE© (and originally known as PS-341) is a first in class proteasome inhibitor for the treatment of multiple myeloma, a form of cancer [187]. It received FDA approval in 2003 [188, 189]. Bortezomib, **1**, is a modified dipeptidyl boronic acid analogue [Figure 5] that binds reversibly and with high affinity to the 26S proteasome β-subunit [190, 191], with other different inhibitors of the 26S proteasome currently being researched [192].

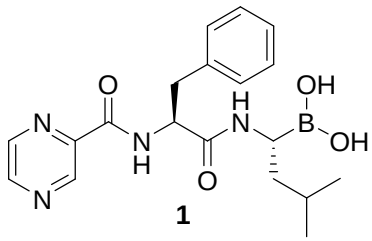


Figure 5. The chemical structure of Bortezomib, 1.

Bortezomib, **1**, proves that the proteasome system and the ubiquitinylation process can be targeted by drugs for the treatment of cancer. Furthermore a compound called Ubistatin can inhibit proteasome recognition by blocking the recognition of proteins with K48 linked poly ubiquitin chains for degradation [193, 194]. Also an E1 ubiquitin activating ligase inhibitor, PYR-41, a pyrazone derivative [195], has been discovered that blocks proteasomal degradation of p53 leading to the reactivation of p53, and in transformed cells containing wild type p53 causes apoptosis [195, 196]. The potential problems of E1 inhibitors are their limited specificity as these inhibitors can affect any protein undergoing ubiquitinylation within the cell.

These three examples Bortezonmib, **1**, Ubistatins and PYR-41 show proof of concept that small molecules can be used to inhibit the degradation of proteins to cause a reactivation of the apoptotic response by p53 in the treatment of

cancer. Hence the inhibition of Mdm2 regulation of p53 could be a potential drug target.

Small Molecule Inhibitors of Mdm2

Small molecule inhibitors of Mdm2 regulatory activity of p53 provide a possible therapeutic target in the treatment of cancer. About a half of all cancers retain wild-type p53 [197] and in these the normal regulation of p53 is sometimes disrupted through direct overexpression of Mdm2 (in *ca.* 7% of cancers [198]). Mdm2 overexpression due to gene amplification is especially frequent (*ca.* 30%) in human osteogenic sarcomas and soft tissue sarcomas [199].

Because of the central role of p53 in tumour suppression, non genotoxic therapeutic strategies that activate p53 in one way or another are highly desirable. Depending on p53 status this is achievable in various ways. For example, proof-of-concept studies have shown that mutant p53 might be able to be stabilised or otherwise reactivated pharmacologically [200-202]. In tumours that retain a functional p53 pathway, on the other hand, preventing p53 degradation is an attractive option.

Inhibiting the p53 regulatory activity of Mdm2 liberates stabilised p53 and reactivates the p53 pathway to growth arrest and apoptosis. The increase in p53 levels would be seen in cancerous and non-cancerous cells. The selectivity of p53 in inducing cell cycle arrest or apoptosis is questionable and needs to be answered, but there is the suggestion that activation of p53 may cause tumour specific cell death as transformed cells are more sensitive to p53 induced

apoptosis than their normal counterparts [203]. Mdm2 as a cancer drug target has been validated by many studies [204-207].

There are two classes of compound that target Mdm2 to reactivate p53 that have potential to be used in cancer therapy. The first class of compounds inhibit Mdm2/p53 protein-protein interactions at the p53 N-terminal transcription domain, while the second class inhibits Mdm2 E3 ubiquitin ligase activity.

Inhibitors of Mdm2/p53 Protein-Protein Interaction

A large array of small molecule inhibitors of the Mdm2/p53 protein-protein interaction have been discovered [208-213] because this interaction was pioneering in proving that a protein-protein interaction could be targeted by drugs. It was previously thought that protein–protein interactions could not be effectively inhibited with drug-like small molecules [214]. The X-ray crystal structure of a complex between the N-terminal domain of Mdm2 and a 12mer peptide, encompassing residues 16-27 of the p53 transactivation domain, showed that the bulk of the p53/Mdm2 interaction in fact involved just three lipophilic residues of p53, buried in a well-defined hydrophobic surface cleft in Mdm2, of a size that could clearly be fully occupied by a small molecule [118]. The Mdm2/p53 interaction was further studied with peptides [215-217] that helped define the pharmacophore model and Mdm2 as a target [218], which provided the platform for subsequent development of non peptide small molecule inhibitors. The best known Mdm2/p53 class of inhibitors are the cis-imidazoline derivatives called Nutlins [219] as these were the first potent and

selective inhibitors of the Mdm2/p53 protein-protein interaction [177] and have progressed to early phase clinical trials [220].

Mdm2 E3 Ubiquitin Ligase Inhibitors

The first report on Mdm2 E3 ligase inhibitors dates back to 2002 and concerns the arylsulfonamide, **2**, bisarylurea, **3**, and acylimidazolone, **4**, compounds [Figure 6] which were discovered in an Mdm2-mediated p53 ubiquitinylation screen of a chemical library [221]. It was shown that all three compounds behaved as simple reversible inhibitors of Mdm2 *in vitro*, that they bound to Mdm2 in a mutually exclusive manner, and that inhibition was non competitive with respect to both E2 and p53 substrates. Furthermore, the compounds were selective, as they did not inhibit E3 ligases other than Mdm2, and, interestingly, did not inhibit Mdm2 auto-ubiquitinylation.

It is known that while the isolated Mdm2 RING domain that includes the extreme C-terminus of Mdm2 retains E3 ligase activity, ubiquitinylation of p53 by Mdm2 also requires the N-terminal domain, where the main p53 recruitment site resides, as well as the central acidic domain, which contains a secondary p53-binding site [222]. One could therefore imagine that the above compounds might prevent p53 ubiquitinylation not at the level of the Mdm2 E3 catalytic activity but by preventing p53 binding. A lack of effects of the compounds on the physical interaction between Mdm2 and p53 was demonstrated, however, suggesting that the mode of inhibition may be allosteric, perhaps by blocking a structural rearrangement of Mdm2 necessary for p53 ubiquitinylation but not for Mdm2 auto-ubiquitinylation [223].

Regardless of the mechanism of Mdm2 inhibition, the selectivity towards p53 ubiquitinylation as opposed to Mdm2 auto-ubiquitinylation by the arylsulfonamide, **2**, bisarylurea, **3**, and acylimidazolone, **4**, compounds would be desirable from a therapeutic viewpoint, since inhibition of both activities might lead to accumulation of Mdm2, which in turn would be expected to limit inhibition of p53 ubiquitinylation and subsequent degradation. However, no cellular or *in vivo* activity data were presented for these compounds, and apparently there has not been any follow-up since the original report [214], [221].

Another p53 selective Mdm2 E3 ligase inhibitor in the public domain concerns a compound (of undisclosed structure) that was identified in a high-throughput chemical library screen using more than 600,000 compounds in an Mdm2-mediated p53 ubiquitinylation assay, as well as an Mdm2 auto-ubiquitinylation counter-screen [224]. It was observed that although most of the numerous screening hits identified showed similar activity in the p53 and auto-ubiquitinylation assays, a few chemotypes displayed some selectivity. The most selective compound (structure not given) inhibited p53 ubiquitinylation with an IC_{50} value of 8 μM but was inactive at concentrations up to 100 μM in the auto-ubiquitinylation assay.

Using the same high-throughput screen [224], five alkaloid extracts from the sea squid (*ascidian Lissoclinum cf. badium*), collected from the coast of Papua New Guinea, have been identified to inhibit Mdm2 E3 ubiquitin ligase activity. The two previously known alkaloids diplamine B, **5** [225-227] [Figure 6], and lissoclinidine B, **6** [228] [Figure 6], were shown to stabilize Mdm2 and p53 in

cells. Moreover, lissoclinidine B, **6**, was shown to selectively kill wild-type p53 expressing transformed cells [229]. Other alkaloids have been identified by high-throughput screening to inhibit Mdm2 E3 ubiquitin ligase activity. Most notably, NSC354961, **7** [Figure 6], has the potential to stabilize p53 but shows significant toxicity to non-transformed cells, so should not be considered as a potential drug candidate [195].

Sempervirine, **8** [Figure 6], another natural product, was discovered as an inhibitor of Mdm2 E3 ubiquitin ligase activity in a high-throughput screen [230]. Sempervirine, **8**, was observed to inhibit both Mdm2-dependent p53 ubiquitinylation and Mdm2 auto-ubiquitinylation. Treatment of cancer cells harbouring wild-type p53 with this compound induced stabilisation of p53 and apoptosis. The structurally unusual [231] plant alkaloid sempervirine, **8**, can be extracted from a US coastal plant called Yellow Jessamine and has long been known to possess anticancer activities [232], and perhaps inhibition of Mdm2 E3 ligase activity contributes to these.

Acridine derivatives, **9** [Figure 6], have been shown to stabilise p53 protein levels by blocking p53 ubiquitinylation through a different mechanism to that occurring following DNA damage p53 stabilisation. Acridine derivatives, **9**, induce p53 dependent cell death and induce p53 transcriptional activity in tumour cells *in vivo* [233]. The mechanism of p53 ubiquitinylation inhibition is unknown, and further tests need to be carried out to determine if the acridine derivatives inhibit Mdm2 E3 ubiquitin ligase activity. Nevertheless, these compounds are an example of how p53 ubiquitinylation can be inhibited by small molecules.

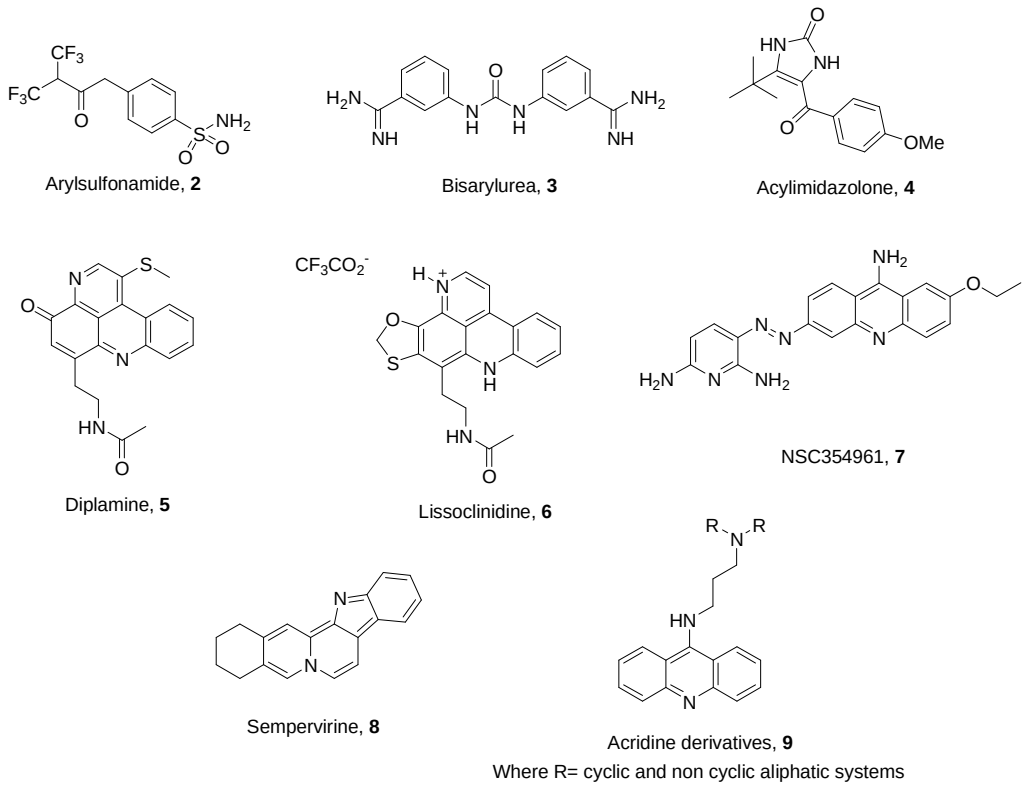


Figure 6. The chemical structures of small molecule inhibitors of Mdm2 E3 ubiquitin ligase activity.

Computer modelling has been used to identify Mdm2 E3 ubiquitin ligase inhibitors by virtual screening [234] but the Mdm2 RING domain X-ray crystal structure is not known. Even though the NMR dimer structure of Mdm2 RING domain is known [170], the protein biology and mode of action of Mdm2 RING domain is not understood well enough to produce accurate computer models. At the moment, computer models are a very basic guide for searching for Mdm2 E3 ubiquitin ligase inhibitors, whereas high throughput screening is currently more useful in identifying potential Mdm2 E3 ubiquitin ligase inhibitors [214]. After further biological research computer modelling could become a more accurate tool for the future.

Reactivation of p53 by inhibiting Mdm2 E3 ubiquitin ligase activity is an attractive therapeutic goal. Several examples or members of this new class of potential anti-cancer therapeutic agents have been developed (as previously

described), but require hit optimisation to improve their potency and selectivity due to the high concentrations required. The development of Mdm2 E3 ubiquitin ligase inhibitors is hampered by the biological complexity of the ubiquitinylation process and there is a real need for more research to be carried out. The lack of understanding of the Mdm2 RING domain mode of action as an E3 ubiquitin ligase is also detrimental for the development of inhibitors [214]. Researchers should not be down hearted; to quote a Journal of National Cancer Institute news review. ‘‘Ligases are today where kinases were 10 to 15 years ago. It’s probably going to take a real visionary group that’s willing to take huge risk…to make a breakthrough’’ [235].

There is one more class of compounds that inhibit Mdm2 E3 ubiquitin ligase activity that so far have not been mentioned. These are the HLI (Hdm2 ligase inhibitor) compounds which form the background, framework and basis of my work. The remainder of this thesis is devoted to the development of second generation HLI compounds to inhibit Mdm2 E3 ubiquitin ligase activity to reactivate p53.

HLI Inhibitors of Mdm2 E3 Ubiquitin Ligase Activity

A family of closely related 7-nitro-5-deazaflavin compounds called HLI98 compounds, **10-12** [Figure 7], have been identified as inhibitors of Mdm2 E3 ubiquitin ligase activity by high throughput screening of Mdm2 auto-ubiquitinylation [236]. Using cell-based assays, the hit compound HLI98C, **11**, was demonstrated to inhibit selectively Mdm2 E3 ubiquitin ligase activity and p53 ubiquitinylation, to increase Mdm2 and p53 protein levels, to reactivate p53 function, and to induce p53 dependent apoptosis in cancer cells. On the

downside, these compounds have low potency and have clear p53 independent toxicity. They do, however, succeed in showing proof of principle that small molecules can inhibit Mdm2 E3 ubiquitin ligases, having potential for use as a cancer therapy [237].

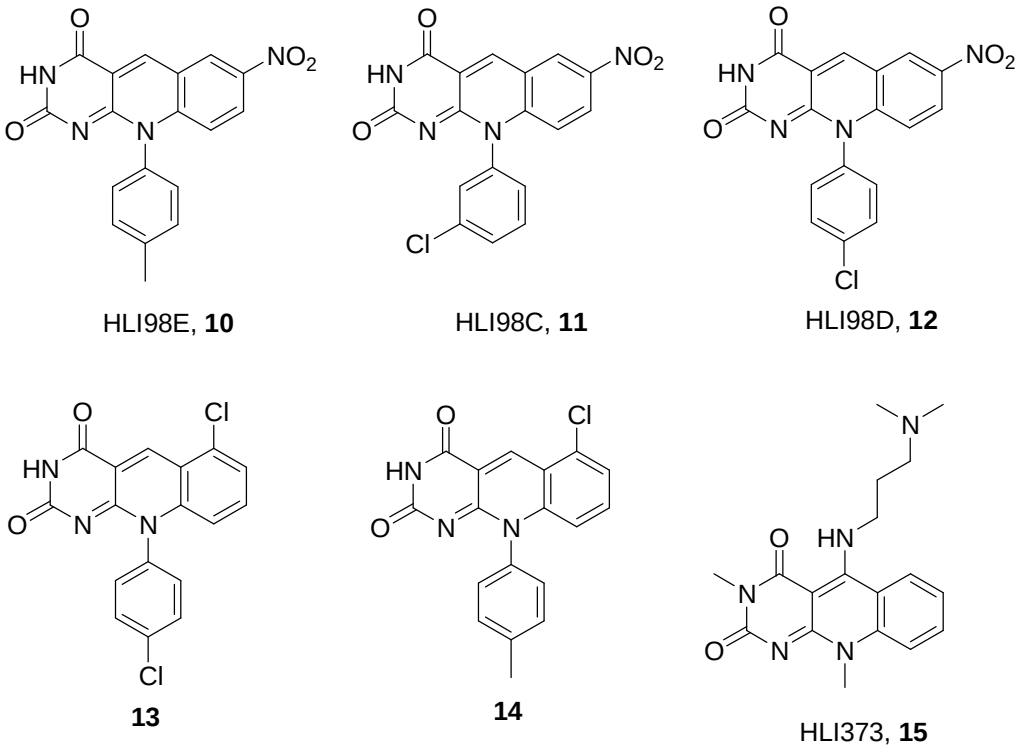


Figure 7. The chemical structures of the HLI or 5-deazaflavin inhibitors of Mdm2 E3 ubiquitin ligase activity.

A potential problem of the HLI98 compounds, **10**-**12**, results from the presence of the nitro group which is susceptible to one electron reduction leading to generation of the nitro anion radical. The planar heteroaromatic system of the HLI98 compounds, **10**-**12** , can intercalate with DNA [238] and the presence of the reactive radical can then result in cytotoxicity through DNA damage [239, 240].

Further literature research work investigated the ability of 5-deazaflavin analogues to stabilise and activate p53. Results show that the nitro group present in HLI98 compounds, **10**-**12**, is not essential for 5-deazaflavins to

reactivate p53. Thus the 6-chloro-5-deazaflavin analogues, **13** & **14** [Figure 7], were found to increase p53 levels to the same degree as HLI98 compounds, **10**-**12**. Removal of the 7-nitro group therefore reduces the risk of cytotoxicity, without affecting activity. No other structure activity relationships could be deduced from the biological data obtained [241].

Recently a more water soluble and potent 5-deazaflavin, HLI373, **15** [Figure 7], was discovered [242] which has a 5-dimethylaminopropylamino side chain but lacks the 10-aryl group of HLI98 compounds, **10**-**12**. HLI373, **15**, was determined to have high solubility and was more potent at inhibiting Mdm2-p53 ubiquitinylation than the HLI98 compounds, **10**-**12**, resulting in an increase in Mdm2 and p53 protein levels, causing apoptosis. Furthermore, HLI373, **15**, was shown to induce apoptosis in a variety of different cancer cell lines containing wild type p53 [243].

Previous research into 5-deazaflavins has investigated the redox chemistry of the compound and its derivatives [244, 245]. The discovery of a naturally occurring 8-hydro-5-deazaflavin (also called coenzyme F_{420}), that functions as a coenzyme with important electron transfer function [246] in anaerobic methanogenic bacteria [247] generated the interest in 5-deazaflavin compounds. F_{420} was first synthesised in 1970 by Cheng and co-workers [248]. Methanogenic bacteria produce methane as a by-product of metabolism in low levels of oxygen [249] by a different method of energy production than most other living organisisms. More recent research has used 5-deazaflavins, 5-deazaflavin analogues and their derivatives to potentially inhibit protein

kinaseC (PKC) and tyrosine kinases as antiproliferative agents with antitumour activity against different tumour cell lines [250-254].

Aims

To continue the work already underway in finding a small molecule inhibitor, based on the 10-phenyl-5-deazaflavin template [Figure 8], of Mdm2 E3 ubiquitin ligase activity to reactivate p53, to be used as a potential therapy for cancers that retains wild type p53.

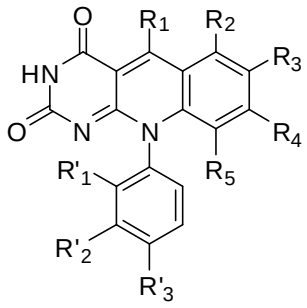


Figure 8. Chemical structure of 10-phenyl-5-deazaflavin.

The aim of the work was:

- To build a more complete structure-activity relationship (SAR) of the 5-deazaflavin pharmacophore as an inhibitor of Mdm2 E3 ubiquitin ligase activity to reactivate p53.
- To undertake hit optimisation of the 5-deazaflavin pharmacophore investigated to produce a more potent inhibitor of Mdm2 E3 ubiquitin ligase ability.
- To gain an improved understanding of how Mdm2 acts as an E3 ubiquitin ligase, to overcome a problem which hinders the development of ligase inhibitors at present.
- To gain a better understanding about the mode of action of how 5-deazaflavin acts as an inhibitor of Mdm2 E3 ubiquitin ligase activity.

Synthesis

5-Deazaflavin Analogues to be Synthesised

Recap of Previous Research

Previous literature research had identified 5-deazaflavin analogues as inhibitors of Mdm2 [237], **10**-**15**, and had shown that the undesirable nitro group can be replaced by a chloro group [241]. Limited research has investigated the SAR of the 5-deazaflavin pharmacophore as an inhibitor of Mdm2 E3 ubiquitin ligase activity.

From the previous research [237, 241], having a chloro group at the six position of 5-deazaflavin [R_2 - Figure 9] or a nitro group at the seven position of 5-deazaflavin [R_3 - Figure 9] with either a methyl or chloro group on the N_{10} phenyl [D Ring - Figure 9] provides 5-deazaflavins that inhibit Mdm2 E3 ubiquitin ligase activity.

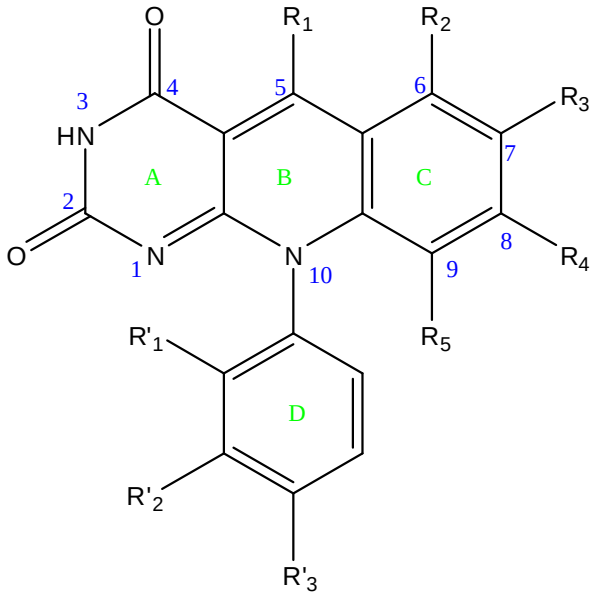


Figure 9. The labelled structure of 10-phenyl-5-deazaflavin. The blue numbers relate to the carbon atom number and the green letters relate to the heterocyclic or homocyclic ring. The substituents are labelled R_1 to R_5 on the exposed quinoline ring (C ring) and R'_1 to R'_3 on the N_{10} phenyl (D ring).

Initial Plan

The initial plan was to synthesise a library of closely related 5-deazaflavin analogues, **16-66** [Table 1], to obtain biological information on how well these compounds inhibit Mdm2 E3 activity. These compounds would provide information of how the structure of the 5-deazaflavin template relates to potency as an Mdm2 E3 ubiquitin ligase inhibitor. The 5-deazaflavin analogues synthesised, **16-66**, probe the different substituents and substitution pattern on the exposed quinoline ring [C Ring - Figure 9] while keeping the substituents on the N_{10} phenyl constant. To achieve this, the nitro, chloro, trifluoromethyl and methyl groups were 'moved around' the exposed quinoline ring whilst keeping the N_{10} phenyl constant as unsubstituted, or being an *ortho*-fluoro or *para*-chloro group [Table 1].

The nitro and chloro groups were chosen to be 'moved around' the exposed quinoline ring of 5-deazaflavin to investigate the discrepancy between position and substituent from the previous research [237, 241]. The trifluoromethyl group was chosen as an iso-electronic replacement group of the nitro group, as it has similar electronic properties but without the undesirable effect of undergoing one electron reduction which in 5-deazaflavin can be cytotoxic to cells. Similar nitro-5-deazaflavin compounds with the nitro group at the six, seven, eight or nine positions [R_2, R_3, R_4 and R_5 - Figure 9] which have long chain alkyl groups at the N_{10} position have been shown to have cytotoxic properties [255]. The cytotoxic effect of these compounds, however, was eradicated when the nitro group was not present or replaced with the isoelectronic replacement group, trifluoromethyl.

5-Deazaflavin compounds contain a redox system [Figure 10] where facile hydride transfer reduction can occur producing the 1,5-dihydro species, which in the presence of oxygen are readily oxidised back [256]. The electrophilic nature of the system is undesirable due to the possible physiological redox system interference leading to poor bioavailability and potential toxicity in the body. Indeed, flavins which are structurally similar to 5-deazaflavin apart from having a nitrogen atom at the five position [Figure 10] and are even more redox reactive than 5-deazaflavin, have been shown to have antimalarial activity with drug-like properties [257]. To reduce the redox system reactivity, neutral or electron-donating substituents such as the methyl group in the exposed quinoline ring of the 5-deazaflavin should make the compound more stable, while electron-withdrawing substituents on the exposed quinoline ring enhance the reactivity of the redox system [258]. Therefore a methyl substituent on the exposed quinoline ring will stabilise the redox system of 5-deazaflavin, and was chosen to see how the stabilisation of the redox system will affect the compound's ability to be an inhibitor of Mdm2 E3 ligase activity.

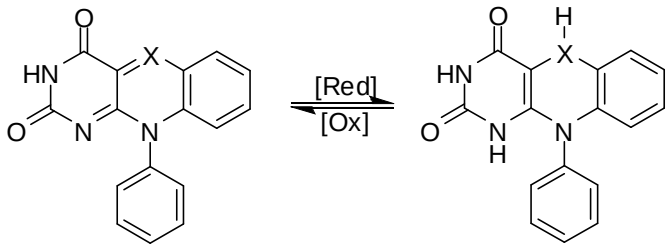


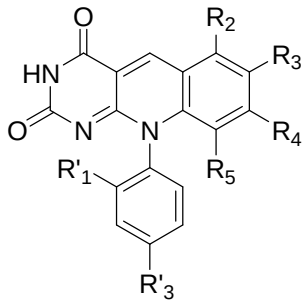
Figure 10. The redox system of the 5-deazaflavin and flavin compounds. X is a carbon atom for the 5-deazaflavins compound or a nitrogen atom for the flavin compound. Red stands for reduction. Ox stands for oxidation.

The *ortho*-fluoro and *para*-chloro were selected as substituents on the N_{10} phenyl as from previous work [241] these substituents were a common theme in active inhibitors. The unsubstituted N_{10} phenyl series of compounds were

selected for synthesis as they could be used to compare the effect of no substitution with substitution of the N_{10} phenyl of 5-deazaflavin compounds on the inhibitors' activity on Mdm2 E3 ubiquitin ligase ability.

Analogues of 5-deazaflavin with no substituents on the exposed quinoline ring but with the N_{10} phenyl constant as unsubstituted, or with an *ortho*-fluoro or *para*-chloro group will be synthesised and tested. These 5-deazaflavin analogues were synthesised and tested to see the effect of substituents on the N_{10} phenyl and no substituents on the exposed quinoline ring has on the inhibition of Mdm2.

Table 1



Compound	R_2	R_3	R_4	R_5	R'_1	R'_3
16	NO_2					
17		NO_2				
18			NO_2			
19				NO_2		
20	NO_2				F	
21		$^{*-}NO_2$			F	
22			NO_2		F	
23				NO_2	F	
24	NO_2					Cl
25		$^{*+}NO_2$				Cl
26			NO_2			Cl
27				NO_2		Cl
28	Cl					
29		Cl				
30			Cl			
31				Cl		
32	$^{*+}$ Cl				F	
33		Cl			F	
34			Cl		F	

Compound	R_2	R_3	R_4	R_5	R'_1	R'_3
35				Cl	F	
36	$^{*-}$ Cl					Cl
37		Cl				Cl
38			Cl			Cl
39				Cl		Cl
40	CF_3					
41		CF_3				
42			CF_3			
43				CF_3		
44	CF_3				F	
45		$^{*+}$ CF_3			F	
46			CF_3		F	
47				$^{*-}$ CF_3	F	
48	CF_3					Cl
49		$^{*-}$ CF_3				Cl
50			CF_3			Cl
51				CF_3		Cl
52	Me					
53		Me				
54			Me			
55				Me		
56	Me				F	
57		Me			F	
58			Me		F	
59				Me	F	
60	Me					Cl
61		Me				Cl
62			Me			Cl
63				Me		Cl
64						
65					F	
66						Cl

Table 1. 5-Deazaflavin analogues to be synthesised and tested as inhibitors of Mdm2 E3 ubiquitin ligase activity. $*^+$ represents compound already reported to have been synthesised, tested and found to reactivate p53 [241]. $*^-$ represents a compound already reported to have been synthesised, tested and found not to reactivate p53 [241]. Empty squares represent hydrogen atoms.

Development of 5-Deazaflavin Synthetic Route

To synthesise the desired analogues of 5-deazaflavin, a three step synthesis pathway [Figure 11] was designed [241, 259-268]. The first step of this scheme involves the reaction between 2,4,6-trichloropyrimidine, **67**, and sodium hydroxide by base-catalysed hydrolysis [269] to synthesise 6-chlorouracil, **68**, [Figure 12] [262, 266, 267]. The next reaction involves the corresponding

anilines, which are all commercially available, fusing with 6-chlorouracil, **68**, at melt temperature to give the desired 6-anilinouracil analogues [259, 260, 263, 265]. The final step of the process was to react the desired 6-anilinouracil analogue with the commercially available desired substituted 2-halobenzaldehyde by the Yoneda method of cyclisation condensation [241, 261, 265, 270], to synthesise the required 5-deazaflavin analogue.

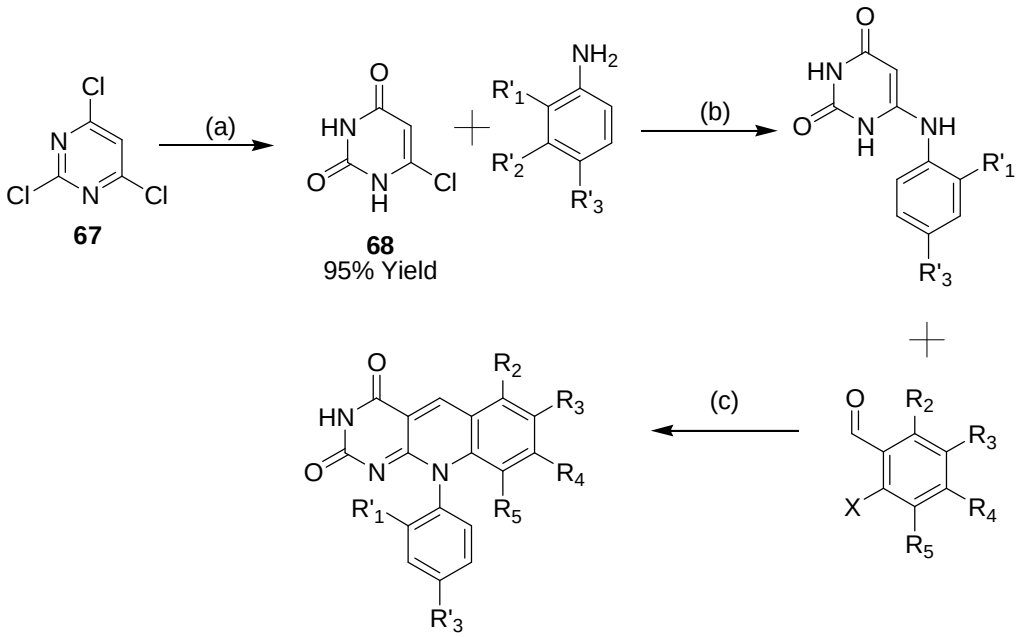


Figure 11. Synthesis of 5-deazaflavin analogues. (a) NaOH (aq) and 100 ^{O}C. (b) Δ. (c) DMF and 160 ^{O}C where X = F or Cl [241, 259-268].

The advantage of this method of 5-deazaflavin synthesis, in comparison to other methods [270-274], is the high yields produced, good availability of starting materials and the versatility of introducing substituents onto the exposed quinoline and the N_{10} phenyl.

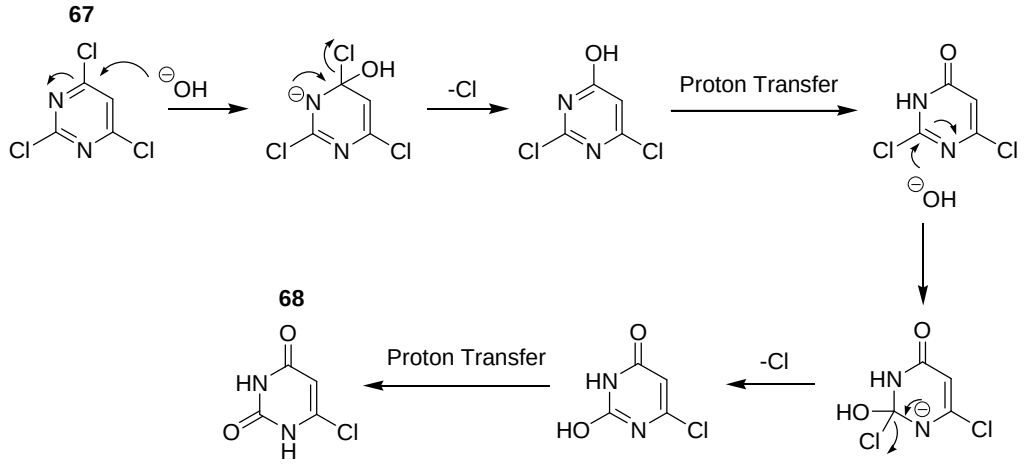


Figure 12. Mechanism of base catalysed hydrolysis to produce 6-chlorouracil, 68.

The trial synthesis of the totally unsubstituted 6-anilinouracil, **69**, was initially performed in the microwave reactor [259] and due to lower than predicted yields, the separation methods of washing with diethyl ether [260] and hydrochloric acid [259] were compared. The different separation techniques had no effect on the yields which were approximately 50% for both.

Due to the low yields and the impracticality of using the microwave reactor for larger volumes, 6-anilinouracil, **69**, was synthesised, in a high yield, by reflux conditions and using diethyl ether for separation. The mechanism for the formation of 6-anilinouracil, **69**, from 6-chlorouracil, **68**, and aniline, **70**, is by nucleophilic substitution [Figure 13].

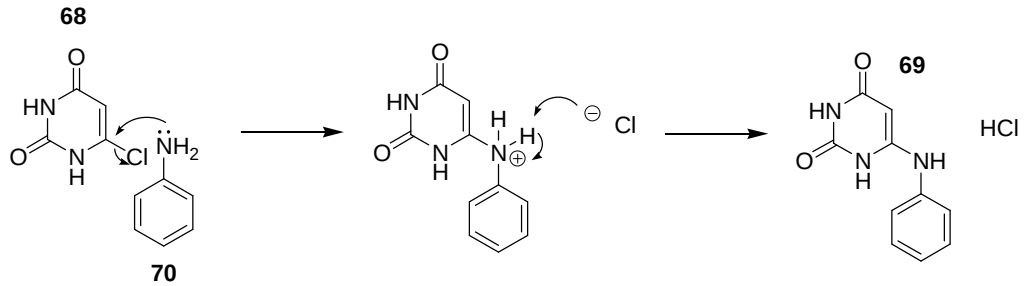


Figure 13. The mechanism of 6-anilinouracil, 69, formation.

The unsubstituted analogue, the 10-phenyl-5-deazaflavin, **64**, was synthesised by the Yoneda method of cyclisation condensation [241, 261, 265, 270] using 6-anilinouracil, **69**, and 2-chlorobenzaldehyde in DMF. During the trial

synthesise of 10-phenyl-5-deazaflavin, **64**, a new method of purification was developed, using 95% dichloromethane and 5% methanol in dry column flash chromatography instead of other methods of purification. The previous methods of purification were recrystallisation from DMF [261, 265, 270] or no purification at all but the addition of water to the reaction mixture with the 5-deazaflavin precipitating out and being collected by filtration [241]. The dry column flash chromatography method was more reliable than the previous two purification methods. Another development discovered during the Yoneda reaction was that a microwave reactor can be used, thus reducing the reaction time from four hours under reflux, to thirty minutes in the microwave reactor. Therefore the proposed pathway to synthesise 5-deazaflavins has been proven, and there was now a general procedure to synthesise the further analogues of 5-deazaflavin.

Mechanism of the Yoneda Reaction of 5-Deazaflavin Synthesis

The mechanism proposed by the Yoneda group involves the condensation of the required 6-anilinouracil and 2-halobenzaldehyde to initially form the aryl-bis(6-anilinouracil-5-yl) methane, **71a**, which undergoes thermal fragmentation to give both the 2-halobenzylidene-6-anilinouracil, **72**, and the starting material 6-anilinouracil. Further cyclisation occurs to the 2-halobenzylidene-6-anilinouracil, **72**, by intramolecular nucleophilic attack to form the desired 5-deazaflavin, [Figure 14] [261, 264, 268].

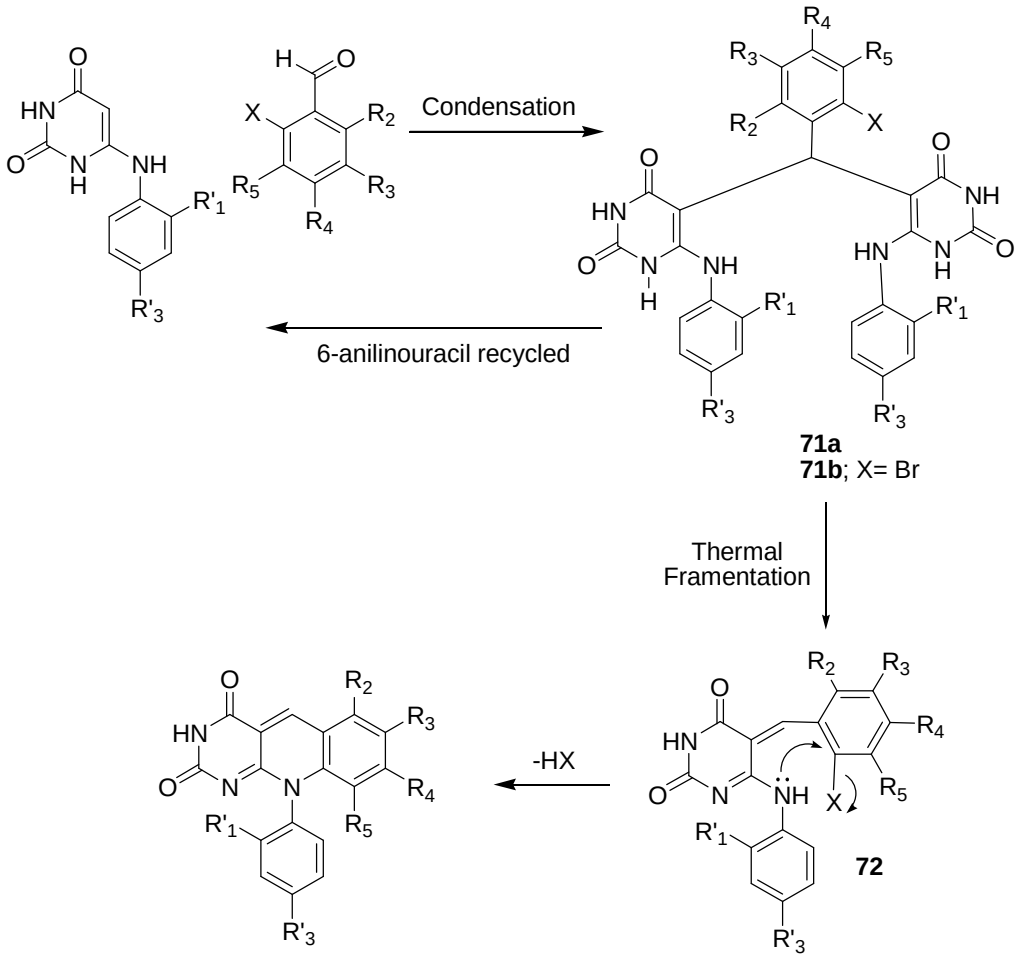


Figure 14. Proposed mechanism of Yoneda reaction of 5-deazaflavin synthesis. Where X = F or Cl.

The evidence to support this is the intermediary *o*-bromophenylbis(6-anilino-3-methyluracil-5-yl) methane, **71b**, has been isolated and easily converted into the 5-deazaflavin upon further heating [261]. In my opinion, there is little evidence to support the proposed Yoneda mechanism. Furthermore, even if one takes recycling of the 6-anilinouracil into account, the expected reaction would involve two equivalents of the 6-anilinouracil to one equivalent of 2-halobenzaldehyde but it does not. Actually there is a slight excess of the 2-halobenzaldehyde required for the reaction. Another mechanism could involve just a dehydration to form the intermediate 2-halobenzylidene-6-anilinouracil, **72** [Figure 15]. Whatever the mechanism, the Yoneda method of 5-deazaflavin

synthesis was successful for the synthesis of the most basic 5-deazaflavin analogue required for the initial work plan.

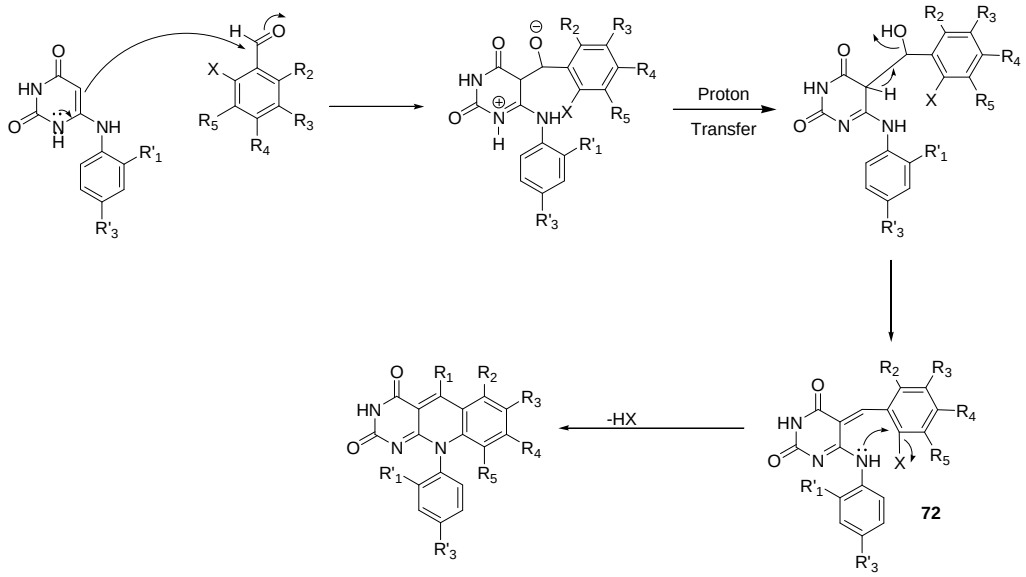


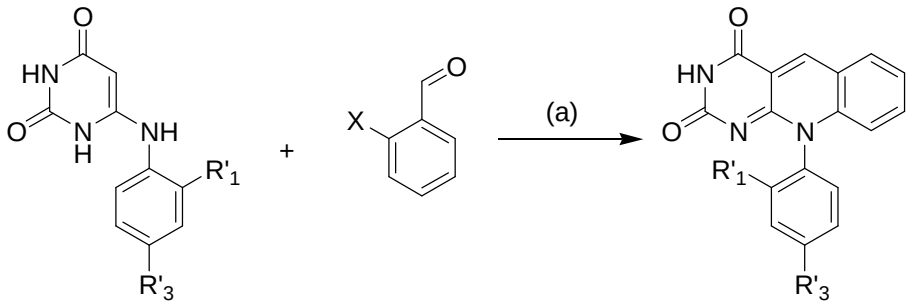
Figure 15. Another possible mechanism for the synthesis of 5-deazaflavin. Where X = F or Cl.

Conformation and Further Comparison of the Yoneda Method of 5-Deazaflavin Synthesis

The Yoneda reaction was further confirmed as a synthetic pathway by the synthesis of 10-(4-chlorophenyl)-5-deazaflavin, **66**, and 10-(2-fluorophenyl)-5-deazaflavin, **65**. During the synthesis of these two trial 5-deazaflavin analogues, the method of heating by using a microwave reactor and conventional reflux was compared. There was little effect on the yield of 5-deazaflavin synthesised so either method of heating was used to synthesise the required analogues of 5-deazaflavin depending on time constraints.

A further comparison of reagents was carried out investigating the difference between using 2-fluoro- and 2-chloro-benzaldehydes synthesising the required 5-deazaflavin analogues with similar percentage yields after column chromatography purification [Table 2]. Therfore both reagents will be used in the Yoneda reaction to synthesise the required analogue.

Table 2



Compound	X	R'_1	R'_3	Yield (%)
64	Cl			90
65	Cl	F		91
66	Cl		Cl	46
64	F			81
65	F	F		70
66	F		Cl	58

Table 2. Comparing the use of 2-fluoro and 2-chloro benzaldehyde as reagents for the synthesis of 5-deazaflavin analogues using the Yoneda method. (a) DMF and 160 ^{O}C where X = F or Cl. Empty squares represent hydrogen atoms. The yield of the reaction shown is after purification using flash chromatography with 95% dichloromethane and 5% methanol.

The Yoneda method of 5-deazaflavin synthesis has been successful in synthesising three analogues of 5-deazaflavin, **64**-**66** required for the initial plan to confirm SAR of the 5-deazaflavin template as potential inhibitors of Mdm2 E3 ubiquitin ligase activity to reactivate p53. The synthesis process was optimised and shown to work with the two possible reagents, the 2-chloro or 2-fluoro benzaldehyde, that will be used in the next stage of work, the analogue synthesis.

Synthesis of 5-Deazaflavin Analogue

The commercially available 2-halobenzaldehydes were reacted with the required 6-anilinouracil intermediates by the Yoneda method to produce thirty three 5-deazaflavin analogues, **17**, **21**, **25**, **28**-**53**, **56**, **57**, **60** & **61**, from the initial plan of action.

The *para*-chloro substituted N_{10} phenyl 5-deazaflavin analogues, **25**, **36**-**39**, **48**-**51**, **60** & **61**, were synthesised with lower yields than the comparable

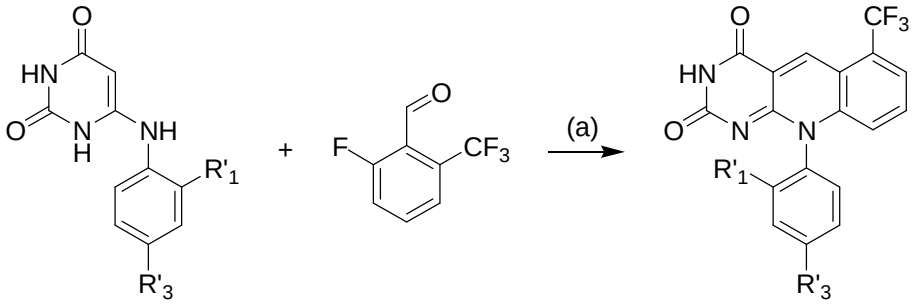
unsubstituted and *ortho*-fluoro N_{10} phenyl 5-deazaflavin analogues, **17**, **21**, **28**-**35**, **40**-**47**, **52**, **53**, **56** & **57**. This observation may be due to the electron-withdrawing effect of the chloro group at the *para* position making the 2-halobenzylidene-6-anilinouracil, **72**, reaction transition state, anilino nitrogen, less nucleophilic. Therefore, it does not react as well in the intramolecular nucleophilic substitution reaction step. The *ortho*-fluoro 5-deazaflavin derivatives, **32**-**35**, **44**-**47**, **56** & **57**, do not follow this trend even though fluoro is electron-withdrawing as the *ortho* position is not as activating as the *para* position so does not affect the lone pairs on the nitrogen of the reaction intermediate, 2-halobenzylidene-6-anilinouracil, **72**.

The 6-methyl-5-deazaflavin analogues, **52**, **56** & **60**, were originally synthesised in very low yield, and could not be tested as inhibitors of Mdm2 E3 ubiquitin ligase activity due to the small quantities synthesised. This could be due to the electron-donating effect of the 6-methyl substituents on the benzaldehyde causing the carbon on the carbonyl to be less susceptible to nucleophilic attack. Another explanation for the low yields of the 6-methyl-5-deazaflavin analogues, **52**, **56** & **60**, could be the methyl group size causes steric hindrance to occur in the reaction intermediates of the Yoneda method. To overcome the low yields, the reaction for these analogues was scaled up using more reagents and repeated, which produced the 6-methyl-10-phenyl-5-deazaflavin analogues, **52**, **56** & **60**, in sufficient amounts for testing.

The 6-trifluoromethyl-5-deazaflavin analogues, **40**, **44** & **48**, were synthesised in low yields. The trifluoromethyl electron-withdrawing group at the six position of the 2-halobenzaldehyde makes the carbonyl carbon more electro-

positive so the reaction of 5-deazaflavin synthesis would occur better/faster than for the other analogues synthesised. A possibility of the low yields could be steric hindrance by the trifluoromethyl group in the reaction intermediates, or that the reaction was being overheated therefore degrading the 6-trifluoro-5-deazaflavin analogues, **40**, **44** & **48**, through prolonged heating. Therefore a small scale study was carried out investigating the reaction time of the 6-trifluoro-5-deazaflavin analogues, **40**, **44** & **48**, and yield [Table 3]. The study was inconclusive and not investigated further, as there were sufficient quantities of 6-trifluoro-5-deazaflavin analogues, **40**, **44** & **48,** to test as inhibitors of Mdm2 E3 ubiquitin ligase activity.

Table 3



Compound	R'$_1$	R'$_3$	Time (min)	Yield (%)
44	F		60	26
44	F		30	52
44	F		20	10
44	F		10	18
48		Cl	360	11
48		Cl	60	12
48		Cl	30	18
48		Cl	20	40
48		Cl	10	17
40			60	30
40			30	15
40			20	9
40			10	13

Table 3. Yields of the 6-trifluoromethyl-5-deazaflavin analogues at different reaction times. (a) DMF and 160 ^{O}C. Empty squares represent hydrogen atoms. The yield of the reaction shown is after purification using flash chromatography with 95% dichloromethane and 5% methanol.

Another development was that the use of 3-chloro-2-fluorobenzaldehyde and 4-chloro-2-fluorobenzaldehyde as reagents in the Yoneda synthesis produced the 9-chloro-5-deazaflavin, **31**, **35** & **39**, and 8-chloro-5-deazaflavin, **30**, **34** & **38** analogues instead of the 9-fluoro or 8-fluoro 5-deazaflavin derivatives due to the fluoride atom being displaced rather than the chloride atom. An explanation of this observation is that fluorine atoms are more electronegative than chlorine atoms and a decrease in the electron density at the carbon fluorine bond results in faster attack by the internal nucleophile compared to the carbon chlorine bond [241, 275, 276].

The fifteen remaining 5-deazaflavin analogues, **16**, **18**-**20**, **22**, **23**, **24**, **26** **27**, **54**, **55**, **58**, **59**, **62** & **63**, required to be synthesised for the initial plan to probe the SAR of the 5-deazaflavin pharmacophore could not readily be synthesised using the Yoneda method of synthesis. This is due to the fact that the 2-halobenzaldehydes required [Figure 16] are not commercially available. A new alternative method of 5-deazaflavin synthesis would have to be devised to produce the remaining 5-deazaflavin analogues, or the individual 2-halobenzaldehydes would have to be synthesised and used in the Yoneda method, to produce the remaining 5-deazaflavin analogues, **16**, **18**, **20**, **22**-**24**, **26**, **27**, **54**-**59**, **62**, **63**.

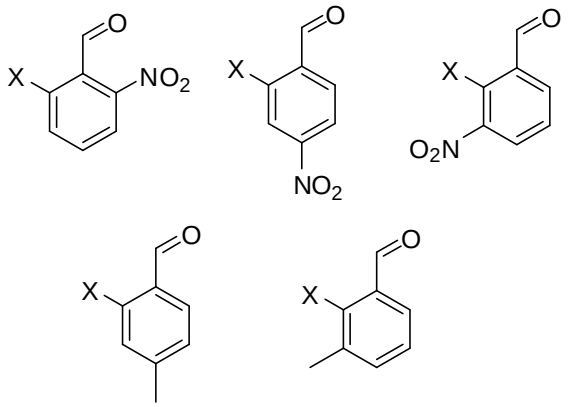


Figure 16. The commercially unavailable 2-halobenzaldehyde reagents for the Yoneda method of 5-deazaflavin synthesis. Where X = F, Cl or Br.

Alternative Synthesis of 5-Deazaflavin Analogues

Two alternative methods of synthesising the remaining 5-deazaflavin, **16**, **18**, **20**, **22**-**24**, **26**, **27**, **54**-**59**, **62**, **63**, from the initial plan were investigated.

Diethylazodicarboxylate (DEAD) Method of 5-Deazaflavin Synthesis

The first is similar to the Yoneda method and actually devised by the same research group uses diethylazodicarboxylate, **73** (DEAD), in an oxidative cleavage reaction [Figure 17] [261, 264]. The mechanism of the reaction [Figure 18] involves the formation of 6-phenylamino-5-benzylideneuracil, **74**, from the required 6-anilinouracil and benzaldehyde in the presence of acetic acid. Then oxidative coupling of DEAD, **73**, to 6-phenylamino-5-benzylideneuracil, **74**, followed by cyclisation, with the elimination of 6-amino-5-(1,2-bisethoxycarbonylhydrazino)uracil, **75**, to form the 1,5-dihydro-5-deazaflavin, **76**, which undergoes dehygrogenation by DEAD, **73**, to produce the desired 5-deazaflavin [261, 264]. The DEAD method's major disadvantage is that there is no selectivity of substituent position on the exposed quinoline ring. Due to this potential problem and the success of the other alternative method, this work was not continued.

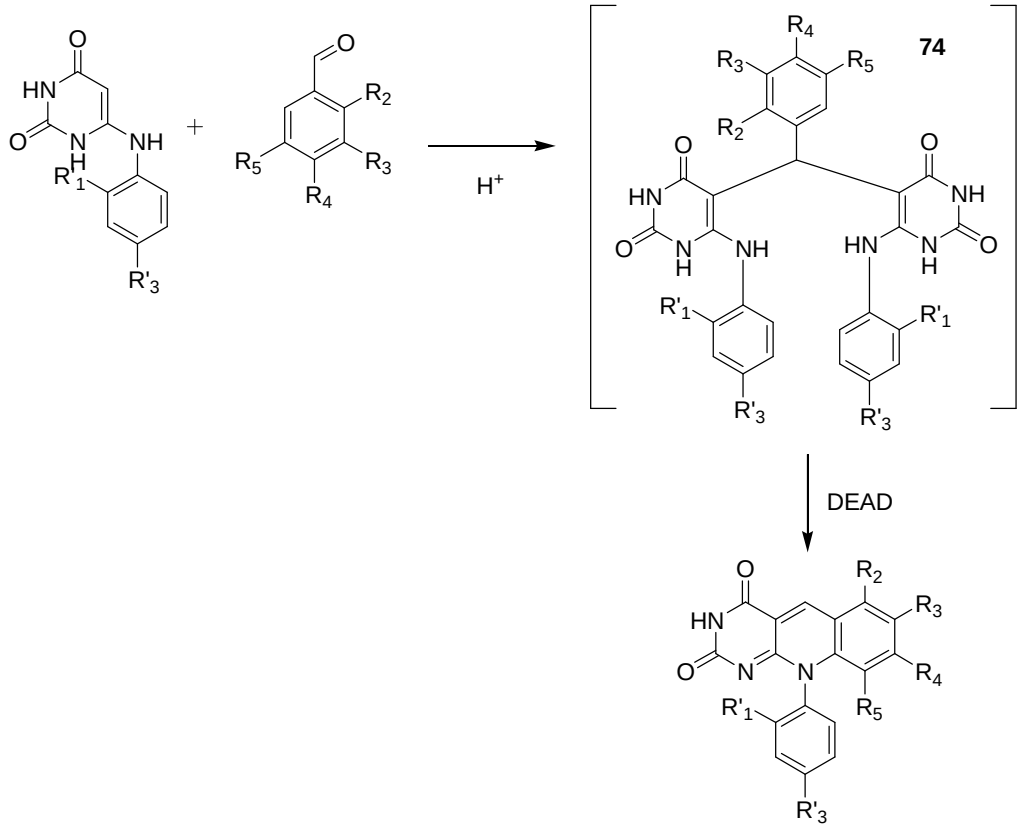


Figure 17. The DEAD synthesis of 5-deazaflavin. A potential alternative synthesis of the remaining fifteen 5-deazaflavin analogues from the initial plan [261, 264].

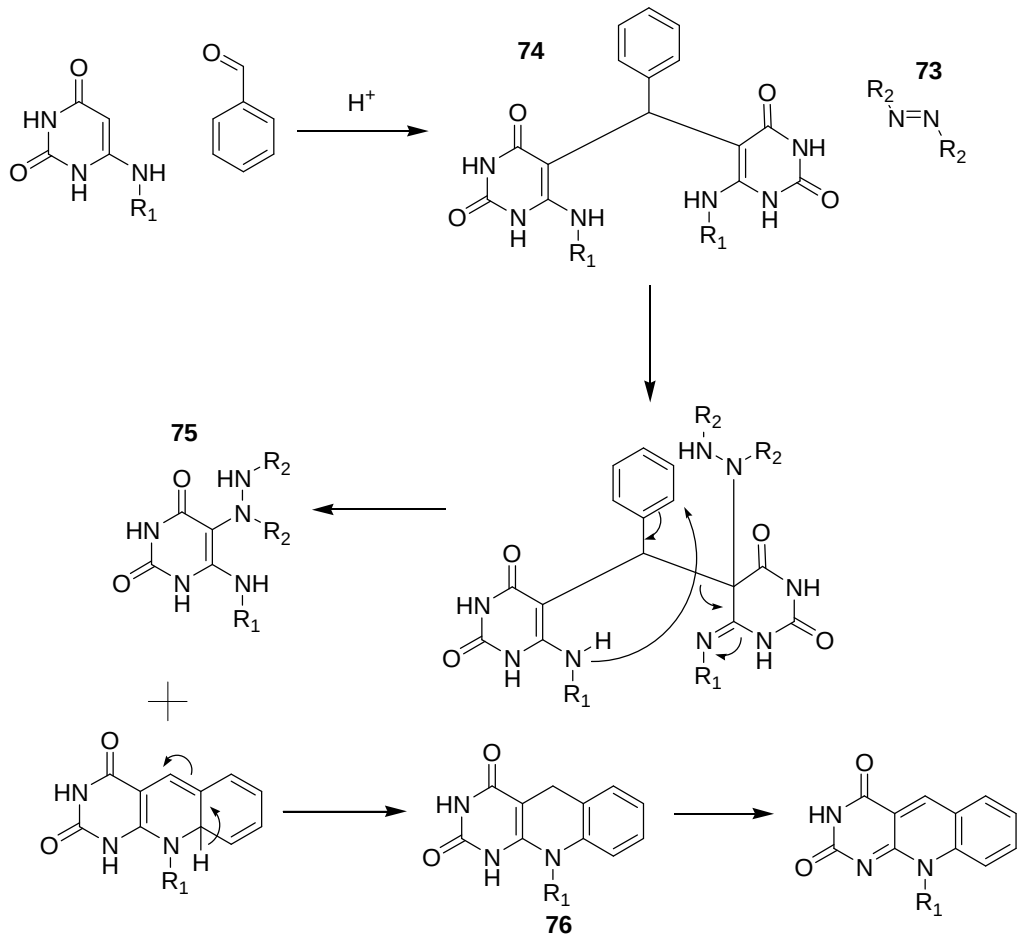


Figure 18. The mechanism of the DEAD method of 5-deazaflavin synthesis. Where R_1 = Ph and $R_2 = CO_2C_2H_5$.

p-Toluenesulfonyloxy Method of 5-Deazaflavin Synthesis

The second method or *p*-toluenesulfonyloxy (shortened to OTs) method [Figure 19] was trialed successfully for the synthesis of the unsubstituted 10-phenyl-5-deazaflavin analogue, **64**. In this method the *p*-toluenesulfonyloxy group is used as the leaving group instead of a halogen in the Yoneda method.

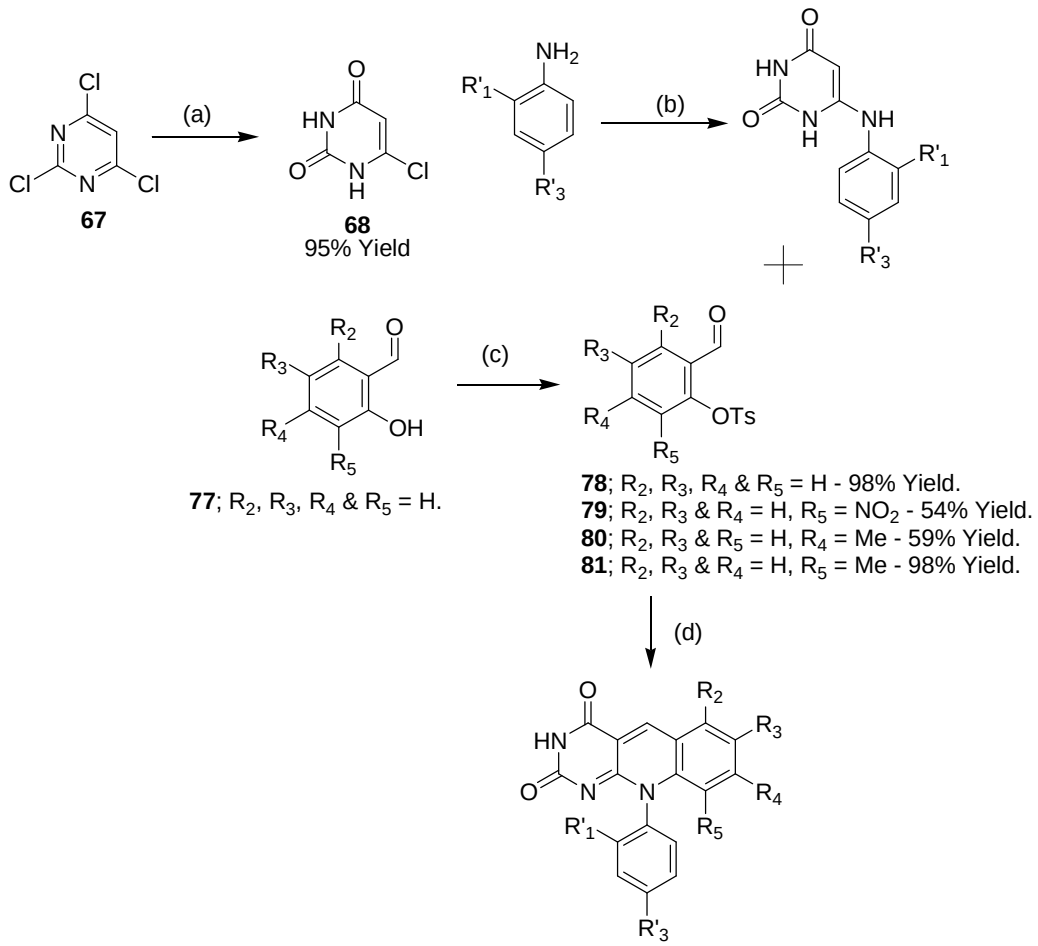


Figure 19. The OTs method of 5-deazaflavin synthesis. (a) NaOH (aq) and 100 ^{O}C. (b) Δ. (c) Acetone, Na_2CO_3, TsCl and 60 ^{O}C. (d) DMF and 160 ^{O}C.

For the trial synthesis, salicylaldehyde, **77**, was stirred in acetone with sodium carbonate to deprotonate the hydroxy group which then reacts with the added *p*-toluenesulfonyl chloride to form 2-*p*-toluenesulfonyloxybenzaldehyde, **78**. This intermediate is used with 6-anilinouracil, **69**, under the same conditions as for the Yoneda method to synthesise 10-phenyl-5-deazaflavin, **64**.

The three commercially available 2-hydroxybenzaldehydes [Figure 20] were converted to the required 2-*p*-toluenesulfonyloxybenzaldehyde analogues, **79**-**81**, under the same conditions as the synthesis of 2-*p*-toluenesulfonyloxybenzaldehyde, **78**. The 2-*p*-toluenesulfonyloxybenzaldehyde analogues, **79**-**81**, were used with the required 6-anilinouracil, under the same conditions as for the Yoneda method

to synthesise 10-phenyl-5-deazaflavin, **64**, to form the eight 5-deazaflavin analogues, **23**, **27**, **54**-**59**, **62** & **63**, for the initial plan of work.

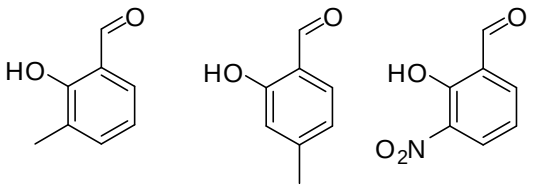


Figure 20. The three commercially available 2-hydroxybenzaldehydes used to synthesise 5-deazaflavin analogues by the OTs method.

The six remaining 5-deazaflavin analogues from the initial work, **16**, **18**, **20**, **22**, **24** & **26**, could not be synthesised using the OTs method as the 2-hydroxybenzaldehydes required were not commercially available. Therefore, the 2-halobenzaldehydes were synthesised to produce the required starting materials of the Yoneda method to synthesise the remaining 5-deazaflavin analogues, **16**, **18**, **20**, **22**, **24** & **26**, for the initial plan.

Synthesis of 2-Fluoro-6-nitrobenzaldehyde Starting Reagent, 82

2-Fluoro-6-nitrobenzaldehyde, **82**, was synthesised by the literature method [Figure 21] [277-279]. Pure 2-fluoro-6-nitrobenzyl bromide, **83**, was synthesised by free radical halogenation using N-bromosuccinimide as the halogen radical source. Nucleophilic substitution of 2-fluoro-6-nitrobenzyl bromide, **83**, by pyridine produced the bromide salt, 2-fluoro-6-nitrobenzyl pyridinium bromide, **84**. The reagent *p*-nitrosodimethylaniline hydrochloride, **85**, is synthesized by concentrated hydrochloric acid reacting with sodium nitrite to form the electrophile NO^+, which then reacts with N,N-dimethylaniline, **86**, in a similar fashion to aromatic nitration of benzene with the dimethylamino group being *para* directing. *p*-Nitrosodimethylaniline hydrochloride, **85**, and 2-fluoro-6-nitrobenzyl pyridinium bromide, **84**, are reacted by nucleophilic displacement to produce N-(*p*-dimethylaminobenzyl)-

α-(6-fluoro-o-nitrophenyl) nitrone, **87**, which in the presence of concentrated sulphuric acid undergoes intramolecular rearrangement to produce 2-fluoro-6-nitrobenzaldehyde, **82**.

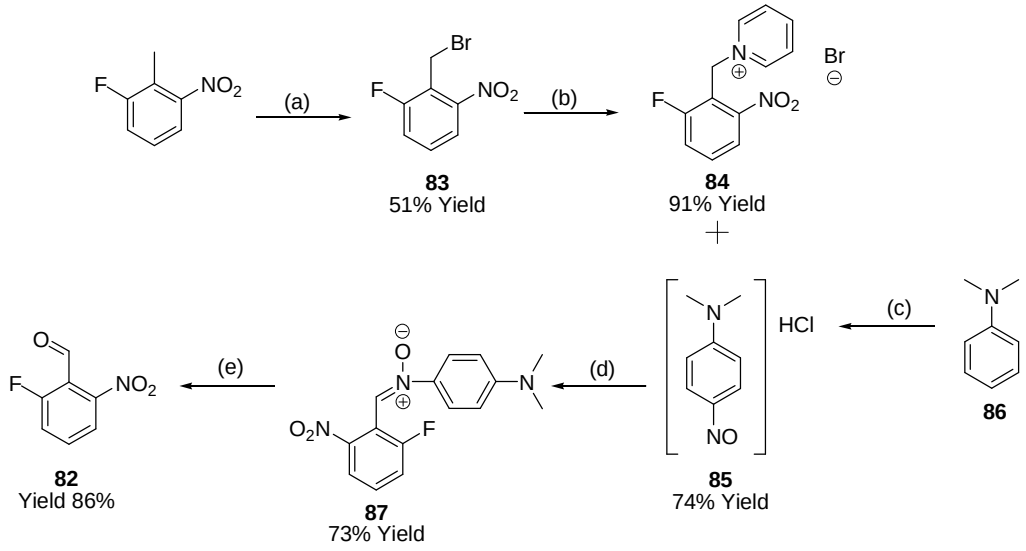


Figure 21. The Synthesis of 2-Fluoro-6-nitrobenzaldehyde, 82. (a) N-bromosuccinimide, benzoyl peroxide, CCl_4 and 80 ^{O}C. (b) EtOH, pyridine and 80 ^{O}C. (c) sodium nitrite and conc HCl. (d) EtOH and NaOH. (e) Conc H_2SO_4 [277-279].

The 2-fluoro-6-nitrobenzaldehyde, **82**, synthesised was then used in the Yoneda method to produce the three 6-nitro-5-deazaflavin analogues, **16**, **20** & **24**, required for the initial plan of the work. Now only the 8-nitro-5-deazaflavin analogues, **18**, **22** & **26**, were required to be synthesised to complete the initial SAR study.

Synthesis of 2-Chloro-4-nitrobenzaldehyde Starting Reagent, 88

2-Chloro-4-nitrobenzaldehyde, **88**, was synthesised by a literature method, by reduction of the methyl ester derivative, methyl-2-chloro-4-nitrobenzoate, **89**, by di-isobutylaluminum hydride (or DIBAL-H) [280, 281] [Figure 22]. Di-isobutylaluminum hydride acts as a source of hydrogen anions in the reduction.

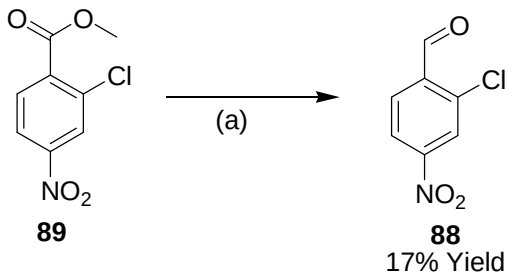


Figure 22. Synthesis of 2-chloro-4-nitrobenzaldehyde, 88. (a) DIBAL, N_2 atms, toluene, $-78^{O}C$ [280, 281].

Using the 2-chloro-4-nitrobenzaldehyde, **88**, reagent, the remaining three 8-nitro-5-deazaflavin analogues, **18**, **22** & **26**, were synthesised by the Yoneda method thus finishing the synthesis stage of the planned initial work. The 5-deazaflavin final compounds synthesised, **16**-**66**, were tested as potential inhibitors of Mdm2 E3 ubiquitin ligase activity to reactivate p53.

Biological Activity

The fifty 5-deazaflavin analogues, **16**-**66**, synthesised for the initial plan were sent to the Beatson Institute in Glasgow to obtain biological data.

Qualitative Biological Test

The biological test originally carried out on the compounds was an *in vitro* test on the inhibition of p53 ubiquitinylation. The test can be described as having E1, E2, Mdm2 (to act as an E3), p53, ATP (as ubiquitinylation is an energy depent biochemical reaction) and ubiquitin present with a known concentration of compound added in DMSO. Mdm2 was pre-bound to glutathione *S*-transferase (GST) and tripeptide glutathione assay beads. After 1 hour, at 37 ^{O}C under continuous shaking, the ubiquitinylation reaction was stopped by dipping the assay into ice. The queched assay was washed, which removed the E1, E2, unbound Mdm2 and free ubiquitin, and probed with a p53 identifying antibody to produce a Western Blot for the tested compound yielding qualitative data as excellent, good, reasonable or inactive [282] [Figure 23]. The qualitative biological data were evaluated by a visual comparison of the Western Blot for the tested compound with a negative and positive control. The negative control contained the same proteins as before except the E2 and compound to be tested were not present, so no ubiquitinylated or polyubiquitinylated forms of p53 could be seen. The positive control contains the same proteins as before except the compound to be tested was not present, so the ubiquitinylated or polyubiquitinylated forms of p53 could be seen [Figure 23]. p53 connected to a chain, of up to four, ubiquitin subunits linked though lysine 48 [154] is referred to as the polyubiquitinylated forms of p53.

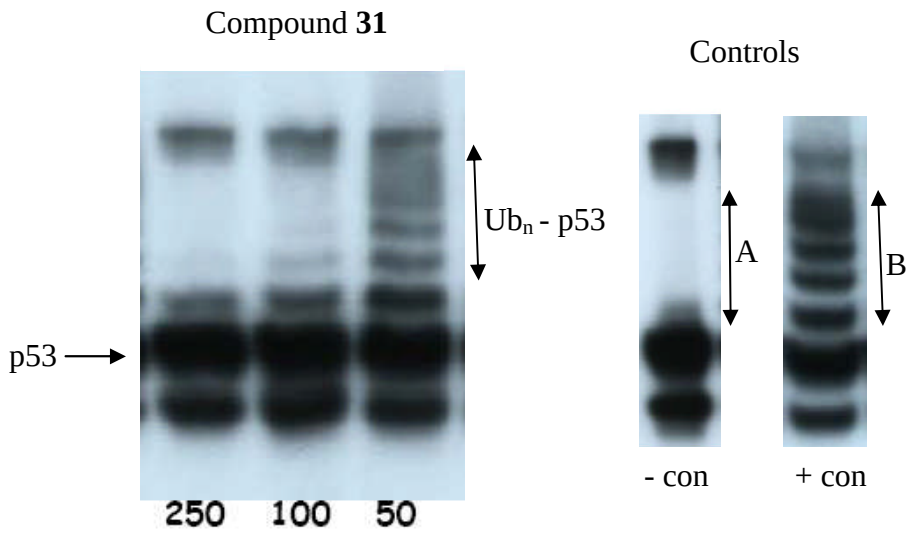


Figure 23. An example of the *in vitro* qualitative biological test on the inhibition of p53 ubiquitinylation for 31. The *in vitro* assay Western Blot of **31** at 250 μM, 100 μM and 50 μM concentration compared with the negative control (no ubiquitinylation of p53 seen and labelled as - con) and the positive control (ubiquitinylated or polyubiquitinylated forms of p53 seen and labelled as + con). A - represents no ubiquitinylated or polyubiquitinylated forms of p53 therefore no ubiquitinylation has occurred. B - represents ubiquitinylated or polyubiquitinylated forms of p53 therefore ubiquitinylation has occurred. All bands represent p53 protein, as identified by an anti-p53 antibody, with p53 or either the monoubiquitinylated or polyubiquitinylated, with different ubiquitin branching, forms of p53 (Ub_n – p53) shown and labeled. Compound **31** and the control blots shown from different Western Blots to ease comparison. At 250 μM concentration, the compound is stated as an excellent inhibitor. At 100 μM concentration, the compound is stated as a reasonable inhibitor. At 50 μM concentration, the compound is stated as inactive as an inhibitor of p53 ubiquitinylation [282].

The compounds were also tested on retinal pigment epithelial (RPE) cells to investigate p53 reactivation. p53 and p21 protein levels were compared to a control [241]. The control was a Western Blot of p53 and p21 protein of cells without any compound to be tested added. Again qualitative data were obtained and was deemed to be excellent, good and reasonable against the control [Figure 24]. p21 is a down stream target of p53 involved in cell cycle arrest. For the cell based assay, lower concentrations of the 5-deazaflavin analogue to be tested were used, than for the *in vitro* assay as the pharmacological effects occur at a lower concentration in the cell based assay than in the biochemical p53 ubiquitinylation (*in vitro*) assay [237, 241].

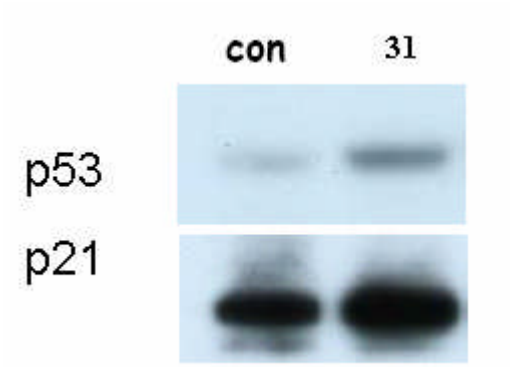
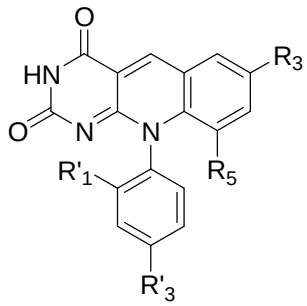


Figure 24. An example of the cell based qualitative biological test on p53 reactivation and p21 up-regulation for 31. RPE cells were used with a concentration of 10 μM of compound. The control (labelled con) is a Western Blot of cells with no compound added. Both p53 and p21 protein levels are reactivated by **31** [241 & 282].

Biological Results

The results show that six 5-deazaflavin analogues, **23**, **27**, **39**, **43**, **47** & **51**, are more potent inhibitors of Mdm2 ubiquitinylation of p53 than the previously identified hit compound, HLI98D, **12** [237] [Table 4] (See Appendix for full table of results). Compound HLI98D, **12**, was synthesised as 10-(4-chlorophenyl)-7-nitro-5-deazaflavin, **25** (from now on just labelled as **25**), and a visual comparison of results confirm that these six compounds, **23**, **27**, **39**, **43**, **47** & **51**, [Table 4] are more potent inhibitors. These six compounds, **23**, **27**, **39**, **43**, **47** & **51**, also reactivate p53 and up-regulate p21 in the cell based biological test [Table 5]. All the other 5-deazaflavin compounds, **16**-**22**, **24**-**26**, **28**-**38**, **40**-**42**, **44**-**46**, **48**-**50** & **52**-**66**, showed very low to no activity at inhibiting Mdm2 ubiquitinylation of p53, reactivating p53 and up-regulating p21.

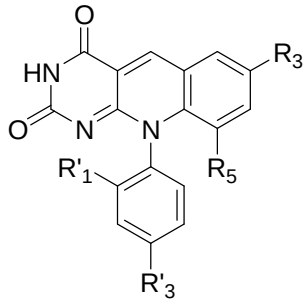
Table 4



Compound	R_3	R_5	R'_1	R'_3	Inhibition of Ub of p53 at		
					250 μM	100 μM	50 μM
43		CF_3			+++	+++	++
47		CF_3	F		+++	+++	+++
51		CF_3		Cl	+++	+++	+++
39		Cl		Cl	+++	++	+
23		NO_2	F		+++	+	-
27		NO_2		Cl	+++	++	-
25	NO_2			Cl	ND	+	-
31				Cl	+++	-	-

Table 4. The *in vitro* results for the six 5-deazaflavin analogues, 23, 27, 39, 43, 47 & 51, that are more potent inhibitors of Mdm2 than the previously identified hit compound, 10-(4-chlorophenyl)-7-nitro-5-deazaflavin, 25. Empty squares represent hydrogen atoms. Ub stands for ubiquitinylation. Plusses and minuses indicates level of inhibition of p53 ubiquitinylation at the stated concentration with the following representing; +++ excellent, ++ good, + reasonable, - inactive. ND stands for not determined. Compound **31** results are shown to aid comparison of the tabulated results to the *in vitro* qualitative biological data provided [Figure 23] [282].

Table 5



Compound	R_3	R_5	R'_1	R'_3	Reactivation of p53 at			Up-regulation of p21 at		
					10 μM	5 μM	1 μM	10 μM	5 μM	1 μM
43		CF_3			+++	+++	++	+++	++	++
47		CF_3	F		+++	++	+	++	ND	ND
51		CF_3		Cl	ND	+++	++	ND	ND	++
39		Cl		Cl	+++	++	+	+++	++	++
23		NO_2	F		+	+	-	++	-	-
27		NO_2		Cl	+	-	-	++	++	-
25	NO_2			Cl	++	-	-	++	ND	ND
31				Cl	+	-	-	+	ND	ND

Table 5. The cell based qualitative biological results for p53 reactivation and p21 up-regulation for the six 5-deazaflavin analogues, 23, 27, 39, 43, 47 & 51, that are more potent inhibitors of Mdm2 than the previously identified hit compound, 10-(4-chlorophenyl)-7-nitro-5-deazaflavin, 25. Empty squares represent hydrogen atoms. Plusses and minuses indicates level of reactivation of the protein at the stated concentration with the following representing; +++ excellent, ++ good, + reasonable, - inactive. ND stands for not determined. Compound **31** results are shown to aid comparison of the tabulated results to the cell based qualitative biological data provided [Figure 24] [241 & 282].

SAR

A common feature of these six compounds, **23**, **27**, **39**, **43**, **47** & **51**, is the presence of an electron-withdrawing hydrophobic group at the nine position [R_5 - Figure 9] of 5-deazaflavin. Another SAR feature observed was that substitution of the 5-deazaflavin template is required for activity as the unsubstituted 10-phenyl-5-deazaflavin, **64**, is inactive. Substitution on the exposed quinoline ring at the nine position is a prerequisite for activity with the 5-deazaflavin analogues with no substitution on the exposed quinoline ring the 10-(4-chlorophenyl)-5-deazaflavin, **66**, and 10-(2-fluorophenyl)-5-deazaflavin, **65**, analogues or analogues with different substitution patterns on the exposed quinoline ring, **16**-**22**, **24**-**26**, **28**-**38**, **40**-**42**, **44**-**46**, **48**-**50**, **52**-**66**, being inactive.

All the methyl containing 5-deazaflavin analogues, **52-63**, whatever the position on the exposed quinoline ring and substituents on the N_{10} phenyl were inactive. All the other 5-deazaflavin compounds, **16-22**, **24-26**, **28-38**, **40-42**, **44-46**, **48-50**, **64-66**, synthesised showed very low to no activity.

Unfortunately, the qualitative nature of the biological data was starting to hinder interpretation, as comparisons of the six most potent inhibitorsof Mdm2 E3 ubiquitin ligase, **23**, **27**, **39**, **43**, **47** & **51**, could not be made.

Quantitative Biological Test

Description

A new biological *in vitro* assay which obtains quantitative data in the form of IC_{50} (half maximal inhibitory concentration**)** for the inhibition of p53 ubiquitinylation by Mdm2, was developed by the collaborators at the Beatson Institute.

IC_{50} represents the concentration of a drug that is required for 50% inhibition, of a particular biological function, *in vitro*. The IC_{50} is a measure of the effectiveness of a compound in inhibiting a biological or biochemical function with the quantitative measure thus indicating how much of a particular inhibitor is needed to inhibit a given biological process by 50% of control [283].

To obtain IC_{50} data for inhibition of p53 ubiquitinylation, the new assay contained E1, E2, Mdm2 (bound to GST and assay beads), p53, ATP and fluorescently labelled ubiquitin as a tracer, with fluorescence measured [Figure 25] [282].

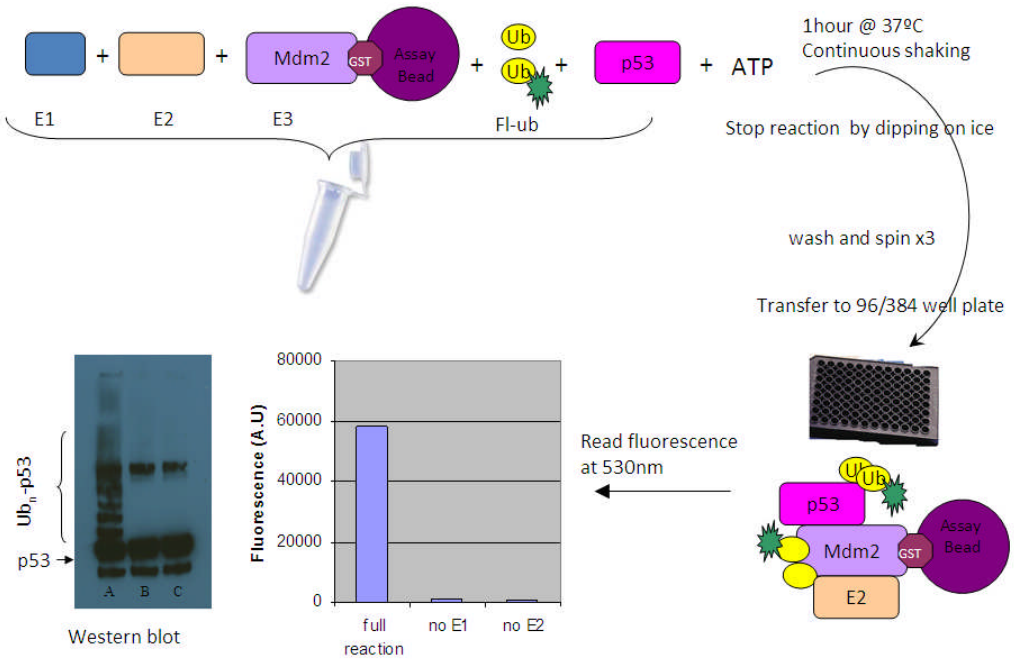


Figure 25. A diagram representing the new quantitative *in vitro* assay used to obtain IC_{50} data for inhibition of p53 ubiquitinylation. Where the E1 (blue square), E2 (cream square), Mdm2 (the E3, lilac square), GST (claret octagon), assay bead (purple circle), ubiquitin (yellow oval), fluorescently labelled ubiquitin (Fl-ub, green explosion connected to ubiquitin) and p53 (fluorescent pink) are shown. The graph shows the full biochemical reaction and two negative controls, where ubiquitinylation could not occur do to the lack of E1 or E2 protein. The controls are preformed to calculate background fluorescents (or noise). The Western Blot of the washed and p53 labeled (by an anti-p53 antibody) assay is also shown for the full reaction (A), no E1 (B) and no E2 (C) controls. Ub_n – p53 stands for ubiquitin bound to p53 by either the monoubiquitinylated or polyubiquitinylated (with different ubiquitin branching) forms. Fluorecent obtained was converted to % inhibition of Mdm2 and used to yield IC_{50} data depending on whom carried out the biological assay (see data provided by the biological collabators in the Appendix tittled IC_{50} *In Vitro* Assay Data 1, Mdm2 Auto-ubiquitinylation IC_{50} *In Vitro* Assay Data and IC_{50} *In Vitro* Assay Data 2). Diagram kindly provided by Dr Mezna from the Beatson Institute for Cancer Research [282].

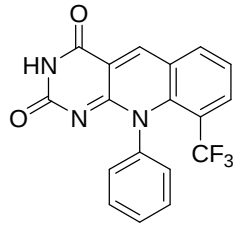
The assay was performed two to four times per compound at six different concentraions with % inhibition of Mdm2 determined from the fluorescent signal. The IC_{50} value was obtained from a graph of average % inhibition of Mdm2 against concentration of compound tested. The new assay was used to obtain results for four of the six most active inhibitors of Mdm2 E3 ubiquitin ligase activity, **39**, **43**, **47** & **51** [Table 6] (see data provided by the biological collabators in the Appendix tittled IC_{50} *In Vitro* Assay Data 1). The two 9-nitro-5-deazaflavin analogues, **23** & **27**, were not selected for testing, as from the past qualitative biological test or pre-screen from visual comparison these compounds were not as active as the other four tested compounds, **39**, **43**, **47** &

51. Also for the two most active inhibitors of Mdm2, **39** & **51**, the inhibition of Mdm2 auto-ubiquitinylation IC_{50} data were obtained using a newly developed assay [Table 6], similar to the assay used to gain data for inhibition of p53 ubiquitinylation [282]. The difference was that p53 was not present in the Mdm2 auto-ubiquitinylation assay. The assay was performed four times per compound at eight different concentraions with % inhibition of Mdm2 auto-ubiquitinylation determined from the fluorescent signal. The IC_{50} for Mdm2 auto-ubiquitinylation value was obtained by using the average % inhibition of Mdm2 auto-ubiquitinylation, in a graph, against concentration of compound tested (see data provided by the biological collaborators in the Appendix titled Mdm2 Auto-ubiquitinylation IC_{50} *In Vitro* Assay Data).

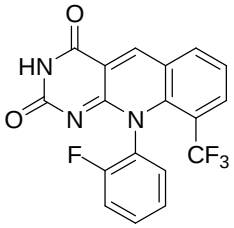
Biological Results

Table 6

Compound Number	IC_{50} for Inhibition of Ub of p53 (μM)	IC_{50} for Inhibition of Mdm2 auto-Ub (μM)
43	27.8 ± 5.0	ND
47	18.7 ± 3.5	ND
39	11.9 ± 5.0	20.8 ± 6.2
51	8.0 ± 4.3	12.9 ± 7.2
25	75.0 ± ND	ND



43



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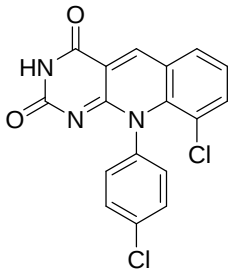
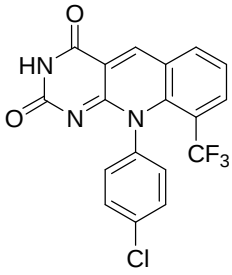
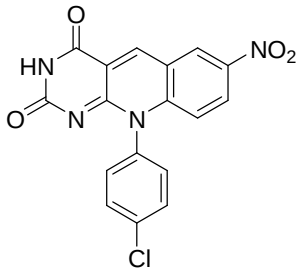


39



51



25

Table 6. IC_{50} data for inhibiting ubiquitinylation of p53 and Mdm2 auto-ubiquitinylation. Ub stands for ubiquitinylation and ND stands for not determined with compound structures shown. For the IC_{50} raw data see Appendix titled IC_{50} *In Vitro* Assay Data 1 and Mdm2 Auto-ubiquitinylation IC_{50} *In Vitro* Assay Data.

The previously identified hit compound 10-(4-chlorophenyl)-7-nitro-5-deazaflavin, **25**, IC_{50} value was also obtained at 75 μM [Table 6], using the newly developed assay. Therefore the current hit compound, **51**, is 9.4 times more active than the previously identified hit compound, **25**.

SAR

Furthermore these results also prove that the undesirable nitro group present in the HLI98 compounds, **10**-**12**, [241] was not essential for 5-deazaflavins function as inhibitors of Mdm2 E3 ubiquitin ligase activity. Removal of the 7-

nitro group therefore reduces the risk of off target cytotoxicity, without affecting Mdm2 inhibitor activity and actually increasing inhibitor potency.

The results have further confirmed the previous SAR that an electron-withdrawing hydrophobic group is required at the nine position of the 5-deazaflavin. These results also show that substitution of the N_{10} phenyl improves activity. The unsubstituted N_{10} phenyl analogue 10-phenyl-9-trifluoromethyl-5-deazaflavin, **43**, is the poorest inhibitor but with the addition of a *para*-chloro group in 10-(4-chlorophenyl)-9-trifluoromethyl-5-deazaflavin, **51**, inhibitor activity is increased three fold.

Summary of Biological Results

Analysis of these results allows a deduction of SAR, relating the structure of the compounds to their ability to inhibit Mdm2 E3 ubquitin ligase activity. Electron-withdrawing hydrophobic groups at the nine position confer activity, as shown by the inactivity of the 9-methyl-5-deazaflavin analogues, **52-63**, and analogues with no or other substitution patterns on the exposed quinoline ring, **16**-**22**, **24**-**26**, **28**-**38**, **40**-**42**, **44**-**46**, **48**-**50**, **64**-**66**. Substitution of the N_{10} phenyl improves activity. Clear hit optimization was observed with the new hit compound 10-(4-chorophenyl)-9-trifluoromethyl-5-deazaflavin, **51**, being 9.4 times more potent than the previously identified hit compound 10-(4-chlorophenyl)-7-nitro-5-deazaflavin, **25**.

To conclude, fifty 5-deazaflavin analogues, **16**-**66**, have been synthesised and tested. Six of these compounds, **23, 27, 39, 43, 47** & **51**, were more potent than the previous hit compound, **25**. IC_{50} data were obtained for the

most promising compounds, **39**, **43**, **47** & **51**, using a newly developed assay which can be interpreted to give SAR. It has been shown that the nitro group in HLI98D, **25**, is not required for inhibitor activity, removing the potential off target cytotoxic risk. The principle that small molecules can inhibit E3 ubiquitin ligase has been confirmed.

Medicinal Chemistry

10-Substituted-5-Deazaflavin Analogue Synthesis and Biological Results

Rationale, Synthesis and Biological Testing

The previous work undertaken shows that substitution of the N_{10} phenyl improves activity. The next set of 5-deazaflavin analogues, **90-95** were synthesised to investigate if the phenyl group on N_{10} is a requirement for 5-deazaflavin analogue activity as inhibitors of Mdm2.

To investigate the N_{10} posititon of 5-deazaflavin, six 10-substituted-5-deazaflavin analogues, **90-95** [Table 7], with hydrogen, methyl and benzyl groups at the N_{10} position with unsubstitution or nine trifluoromethyl on the exposed quinoline ring were synthesised by the Yoneda method [Figure 26]. Benzyl was chosen as a substituent at the N_{10} position to see how the addition of flexibility around the nitrogen phenyl carbon bond affects activity.

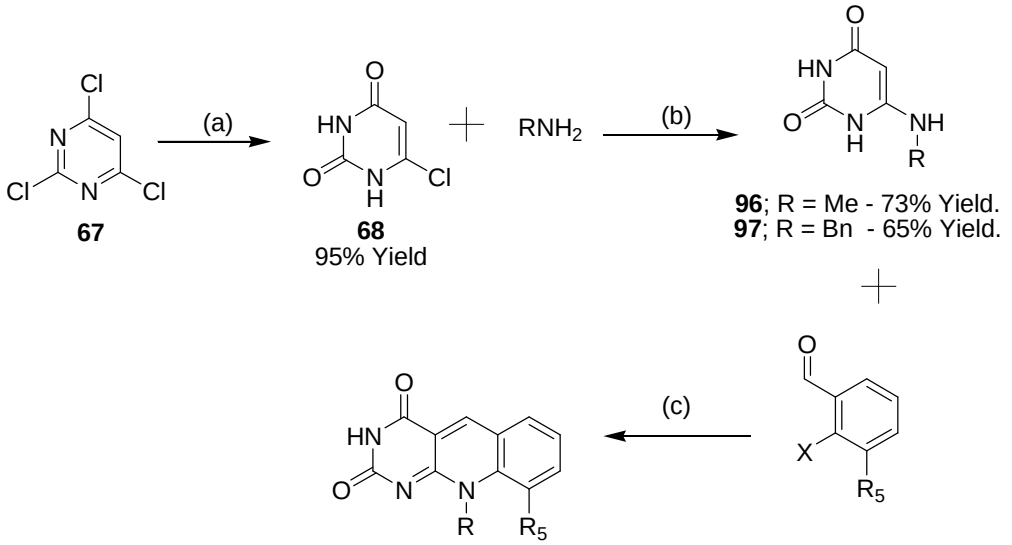


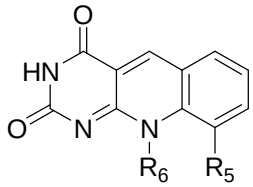
Figure 26. Synthesis of 10-substituted-5-deazaflavins analogues. (a) NaOH (aq) and 100 ^{O}C. (b) Δ. (c) DMF and 160 ^{O}C. Where R = H, Me or Bn group, R_5 = H or CF_3 and X = F or Cl [241, 259-268].

The two intermediates 6-methylaminouracil, **96**, and 6-benzylaminouracil, **97**, were synthesised. These were then used to synthesise the six required 5-deazaflavin analogues, **90-95**.

The six 10-substituted-5-deazaflavin analogues, **90-95**, were tested first in the qualitative *in vitro* biological test of inhibition of p53 ubiquitinylation, as a pre-screen at 250 μM concentration [Table 7]. If the compounds showed sufficient activity, they were then tested in the quantitative biological test to obtain IC_{50} values as inhibitors of Mdm2 E3 ubiquitin ligase active [Table 7].

Biological Results

Table 7



Compound	R_5	R_6	Pre-Screen	Inhibition of Ub of p53 (μM)
90			Inactive	ND
91	CF_3	H	Inactive	ND
92		Me	Inactive	ND
93	CF_3	Me	Inactive	ND
94		Bn	Inactive	ND
95	CF_3	Bn	Active	129.2 ± ND

Table 7. Biological results of 10-substituted-5-deazaflavin analogues, 90-95, synthesised. Pre-screen data obtained using 250 μM concentration of compound and IC_{50} data for inhibiting ubiquitinylation of p53 by Mdm2. Empty squares represent hydrogen atoms. Ub stands for ubiquitinylation. ND stands for not determined.

SAR

All of the 10-substituted-5-deazaflavin analogues with the exposed quinoline ring unsubstituted, **90**, **92** & **94**, were inactive, which is predictable as having an electron-withdrawing hydrophobic group at the nine position is a prerequisite for activity. An interestingly development, was that the N_{10} unsubstituted and methyl 9-trifluoromethyl-5-deazaflavin analogues, **91** & **93**, were inactive as inhibitors of Mdm2. The only active compound was 10-

benzyl-9-trifluoromethyl-5-deazaflavin, **95**, with an IC_{50} of 129 μM which is approximately 16 times less active than the current hit compound, 10-(4-chlorophenyl)-9-trifluoromethyl-5-deazaflavin, **51**. Also 10-benzyl-9-trifluoromethyl-5-deazaflavin, **95**, is four times less active than the comparable 10-phenyl-9-trifluoromethyl-5-deazaflavin, **43**. These results show that the N_{10} phenyl is a requirement for activity, as shown by the inactivity of N_{10} unsubstituted and methyl 5-deazaflavin analogues, **91** & **93**, and the low potency of the N_{10} benzyl active compound, **95**.

10-Pyridinyl-5-deazaflavin Analogues

The 10-pyridinyl-5-deazaflavin analogues were initially required to be synthesised to probe the N_{10} position. The 6-(4-N-pyridinyl)-aminouracil intermediate was synthesised, but when used in the Yoneda method of 5-deazaflavin synthesis, no reaction occurred. A lot of time and effort was invested to synthesise these analogues but due to the biological evidence proving that the N_{10} phenyl was required for activity, this work was discontinued.

Summary of 10-Substituted-5-Deazaflavin Biological Results

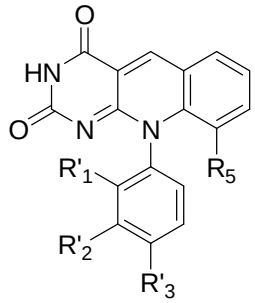
These biological results show that the N_{10} phenyl is required for activity as well as an electron-withdrawing hydrophobic group at the nine position. The importance of substituent and substituent position on the N_{10} phenyl affects activity as an inhibitor of Mdm2 E3 ubiquitin ligase activity required investigation.

N_{10}-Phenyl Substituent and Substitution Pattern 5-Deazaflavins Analogues Synthesis and Biological Results

From previous research work performed, the N_{10} phenyl group was shown to be required for activity with different substituent and substituent position causing an increase in activity as inhibitors of Mdm2. To probe which substituent and position provides the best inhibitors, twenty two 5-deazaflavin analogues, **98-119**, [Table 8] were synthesised using the Yoneda method. These analogues were tested by the biological collaborators using, firstly, the qualitative *in vitro* biological test of inhibition for p53 ubiquitinylation at 250 μM concentration [Table 8]. If the compound showed activity, the quantitative biological assay [282] was conducted to obtain IC_{50} data of p53 ubiquitinylation by Mdm2 [Table 8]. The assay was performed once per compound at nine different concentrations with the fluorescence of the assay measured. The IC_{50} values for inhibiting p53 ubiquitinylation by Mdm2 were obtained from using a graph of fluorescence against concentration of compound tested (see data provided by the biological collaborators in the Appendix titled IC_{50} *In Vitro* Assay Data 2). The 50% fluorescence was generated from the fluorescence signal of the negative control (no inhibitor, therefore ubiquitinylation and polyubiquitinylation forms of p53 present) minus the fluorescence signal of the positive control (no E1 present, therefore no ubiquitinylation and polyubiquitinylation forms of p53 present) hence removing background signal (noise).

Biological Results

Table 8



Compound	R_5	R'_1	R'_2	R'_3	Pre-Screen	IC_{50} (μM)
98		Cl			Inactive	ND
99			Cl		Inactive	ND
66*				Cl	Inactive	ND
65*		F			Inactive	ND
100			F		Inactive	ND
101				F	Inactive	ND
102		Me			Inactive	ND
103			Me		Inactive	ND
104				Me	Inactive	ND
105	Cl	Cl			Inactive	ND
106	Cl		Cl		Active	>100
36*	Cl			Cl	Active	>100
32*	Cl	F			Inactive	ND
107	Cl		F		Active	>250
108	Cl			F	Inactive	ND
109	Cl	Me			Inactive	ND
110	Cl		Me		Inactive	ND
111	Cl			Me	Inactive	ND
112	CF_3	Cl			Inactive	ND
113	CF_3		Cl		Active	2.5
51*	CF_3			Cl	Active	8.0
47*	CF_3	F			Active	18.7
114	CF_3		F		Active	13
115	CF_3			F	Active	14
116	CF_3	Me			Inactive	ND
117	CF_3		Me		Active	4.5
118	CF_3			Me	Active	95
119	CF_3		Cl	Cl	Active	10.1

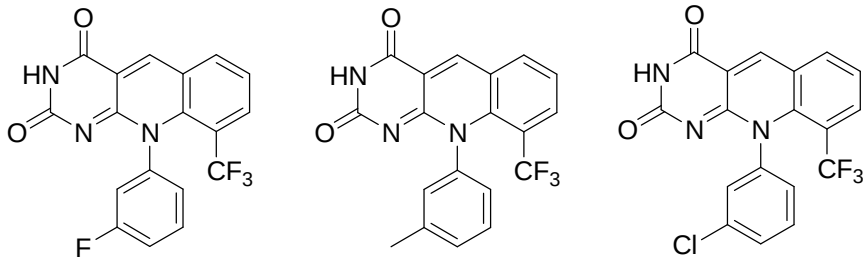
Table 8. The biological results of the twenty two 5-deazaflavin analogues, 98-119, synthesised to investigate the N_{10} phenyl substituent and substituent position. Pre-screen data obtained using 250μM concentration of compound and IC_{50} data for inhibiting ubiquitinylation of p53 by Mdm2. Empty squares represent hydrogen atoms. ND stands for not determined. * represents 5-deazaflavins compounds already synthesised and tested but shown in the table to aid comparison. For the IC_{50} raw data see Appendix titled IC_{50} *In Vitro* Assay Data 2.

SAR

Observation of these results allowed a deduction of SAR, for inhibition of Mdm2 E3 ubiquitin ligase activity. Again, these results further prove that the presence of electron-withdrawing hydrophobic group at the nine position confer activity, as shown by the inactivity of all of the unsubstituted exposed quinoline ring 5-deazaflavin analogues, **98**-**104**. In particular, the trifluoromethyl group is the important substituent at the nine position for inhibitor activity (as shown by **47**, **51** & **114**-**118**) with the chloro group less active by comparison (as shown by **32**, **36** & **105**-**108**). The best position on the N_{10} phenyl for inhibiting Mdm2 activity is the *meta* position. As shown by all of the comparable methyl, fluoro and chloro substituted N_{10} phenyl 9-trifluoromethy 5-deazaflavins analogue, **47**, **51** & **114**-**118**, the *meta* substituted analogues, **113**, **114** & **117**, are the most active inhibitors of Mdm2. The best substituent on the *meta* N_{10} phenyl for activity is the chloro group (as shown by **113**), possibly due to the increased hydrophobic nature of the compounds [Table 9]. There is a link between activity as an Mdm2 inhibitor and partition coefficient (a measure of hydrophilicity, as illustrated in [Table 9]). 10-(3,4-Dichlorophenyl)-9-trifluoromethyl-5-deazaflavin, **119**, has chloro groups at the *meta* and *para* positions of the N_{10} phenyl, is active but not as active as the singly substituted *meta*- or *para*-chloro N_{10} phenyl 9-trifluoromethyl-5-deazaflavin analogues 10-(3-chlorophenyl)-9-trifluoromethyl-5-deazaflavin, **113**, and 10-(4-chlorophenyl)-9-trifluoromethyl-5-deazaflavin, **51**.

Table 9

Compound	Inhibition of Ub of p53 (μM)	CLog P
114	13	4.2
117	4.5	4.5
113	2.5	4.6



114 **117** **113**

Table 9. The link between compound activity as an inhibitor of Mdm2 E3 ubiquitin ligase activity to Log P. CLog P calculated from Chem Draw Pro 10.0. With IC_{50} data for inhibiting ubiquitinylation of p53. Ub stands for ubiquitinylation**.**

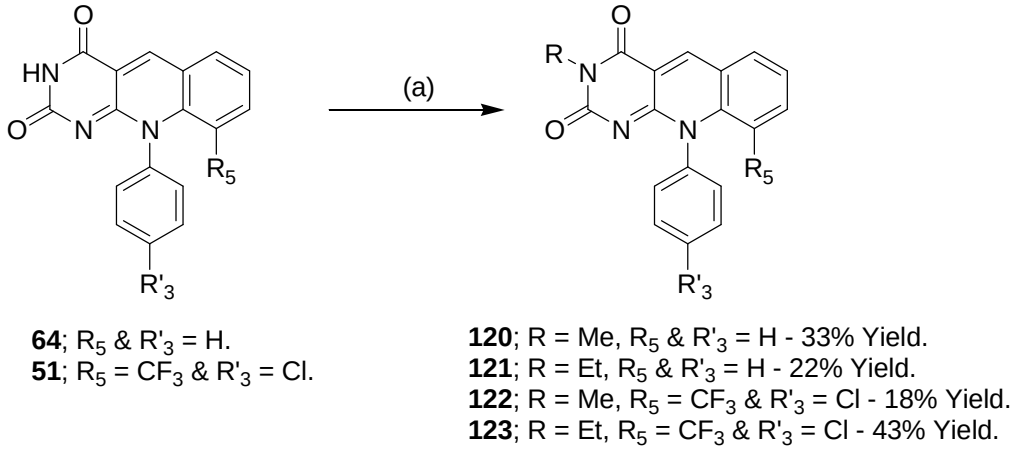
A noticeable exception to the SAR rule that the 9-trifluoromethyl group is a prerequisite for activity, is for the *ortho*-chloro and *ortho*-methyl 9-trifluoromethyl-5-deazaflavin analogues, **112** & **116**, possibly due to the size of the substituent at the *ortho* position forcing the 5-deazaflavins into a conformation than cannot act in the site of action of inhibiting Mdm2. 10-(2-Fluorophenyl)-9-trifluoromethyl-5-deazaflavin, **47** act as an inhibitor of Mdm2 (even with the *ortho* position substituted) possibly due to the smaller size of the fluorine atom, leading to the compound having the required conformation to act as an inhibitor.

These biological results also show clear hit optimization, with the current hit compound, 10-(3-chlorophenyl)-9-trifluoromethyl-5-deazaflavin, **113**, being 30 times more active than the previously identified hit compound, 10-(4-chlorophenyl)-7-nitro-5-deazaflavin, **25**.

3-Substituted-5-Deazaflavin Analogues Synthesis and Biological Results

To investigate the role of the third position (N^3) [Figure 9] of the 5-deazaflavin pharmacophore, four 5-deazaflavin analogues, **120**-**123** [Figure 27], were

synthesized, containing a methyl or ethyl group at the N^3 position with either the exposed quinoline ring unsubstituted, **120** & **121**, or 9-trifluoromethyl and *para*-chloro substitution of the N_{10} phenyl, **122** & **123**.



64; R_5 & R'_3 = H.
51; R_5 = CF_3 & R'_3 = Cl.

120; R = Me, R_5 & R'_3 = H - 33% Yield.
121; R = Et, R_5 & R'_3 = H - 22% Yield.
122; R = Me, R_5 = CF_3 & R'_3 = Cl - 18% Yield.
123; R = Et, R_5 = CF_3 & R'_3 = Cl - 43% Yield.

Figure 27. Synthesis of 3-substituted-5-deazaflavin analogues. (a) ROH, NaOR, RI and Δ. Where R = Me or Et.

The N^3 methyl and ethyl 5-deazaflavin analogues, **120** & **121**, were inactive at 250 μM concentration on the qualitative *in vitro* biological assay as inhibitors of Mdm2. Due to the fact of these analogues, **120** & **121**, not having the required trifluoromethyl group at the nine position. The N^3 methyl or ethyl-10-(4-chlorophenyl)-9-trifluoromethyl-5-deazaflavin analogues, **122** & **123**, have not, at the time of writing, been tested on the qualitative *in vitro* biological assay.

These results will be interesting as they will establish if the N^3 position is required for inhibition of Mdm2 E3 ubiquitin ligase ability. If the N^3 position is not required for inhibitor activity, this would allow the addition of acidic and basic solubilising groups to be added to the N^3 position to improve the poor aqueous solubility of the 5-deazaflavin compounds.

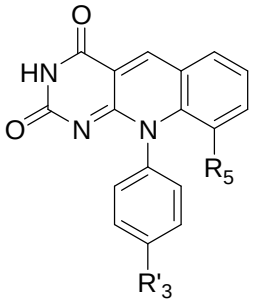
9-Substituted-5-Deazaflavin Analogues Synthesis and Biological Results

Rationale

At present having an electron-withdrawing and hydrophobic substituent at the nine position of 5-deazaflavin is a prerequisite for inhibitor activity, with the most active substituent being the trifluoromethyl group. The majority of 5-deazaflavin analogues synthesised and tested contain an electron-withdrawing and hydrophobic substituent at the nine position. Apart from the 9-methyl-5-deazaflavin analogues, **52-63**, which have electron-donating and hydrophobic substituent and are inactive as inhibitors of Mdm2 E3 ubiquitin ligase activity. Other substituents with different electronic and hydrophobic character at the nine position of 5-deazaflavin require further investigation.

To this end, the fluoro and bromo substituents at the nine position of 5-deazaflavin with the N_{10} phenyl either unsubstituted, **124** & **125** [Figure 28], the least active substituent pattern, or bearing *para*-chloro, **126** & **127** [Figure 28], at the time the most active substituent pattern, were synthesised. These compounds, **124-127** [Figure 28], will compare the three different halogen atoms as substituents at the nine position of 5-deazaflavin on inhibitor activity towards Mdm2. Using Craig plots [284] the hydroxy and cyano substituents were selected at the nine position of 5-deazaflavin as these substituents have different electronic and hydrophilic character to the previous substituents at the nine position. A Craig plot compares the Hammet substituent constant (σ), a measure of electronic effects of a substituent and the substituent hydrophobicity constant (π) [285]. The hydroxy substituent is electron-donating and hydrophilic while the cyano group is electron-withdrawing and hydrophilic. These 5-deazaflavin analogues with different nine substituents

will have the N_{10} phenyl either unsubstituted, **128** & **129** [Figure 28], the least active substituent pattern, or *para*-chloro, **130** & **131** [Figure 28], at the time of synthesis the most active substituent pattern.

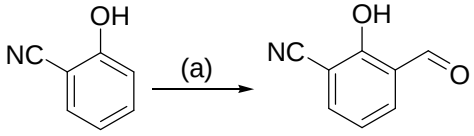


124; R_5 = F & R'_3 = H.
125; R_5 = Br & R'_3 = H.
126; R_5 = F & R'_3 = Cl.
127; R_5 = Br & R'_3 = Cl.
128; R_5 = OH & R'_3 = H.
129; R_5 = CN & R'_3 = H.
130; R_5 = OH & R'_3 = Cl.
131; R_5 = CN & R'_3 = Cl.

Figure 28. The 9-Substituted-5-Deazaflavin Analogues, 124-131.

Synthesis

The 9-hydroxy-5-deazaflavin and 9-fluoro-5-deazaflavin analogues, **128**, **130**, **124** & **126**, were synthesised by the Yoneda method whilst the 9-bromo-5-deazaflavin analogues, **125** & **127**, were synthesised using the OTs method. The synthesis of the 9-cyano-5-deazaflavin analogues, **129** & **131**, was more problematic as the starting material for the Yoneda or OTs methods were not commercially available. Therefore the 3-cyano-2-hydroxy-benzaldehyde, **132**, starting material was synthesised by the Reimer-Tiemann Reaction [Figure 29] [286, 287] in low yields.



133 **132**
3% Yield

Figure 29. Synthesis of 3-cyano-2-hydroxybenzaldehyde, 132. (a) NaOH, Chloroform, 70 ^{O}C, mechanical stirring [286, 287].

The mechanism of the Reimer-Tiemann reaction involves the generation of the dichlorocarbene from chloroform on treatment with sodium hydroxide [286]. The generation of the carbene occurs in the rate limiting step by unimolecular elimination of a chloride ion from the trichloromethyl anion. The dichlorocarbene formed reacts rapidly with water to form carbon monoxide which slowly hydrolyses into sodium formate in the alkaline solution [286] [Figure 30]. These two steps of the hydrolysis of carbene consumes sodium hydroxide and compete with the next step of the reaction, the synthesis of 3-cyano-2-hydroxybenzaldehyde, **132** [286].

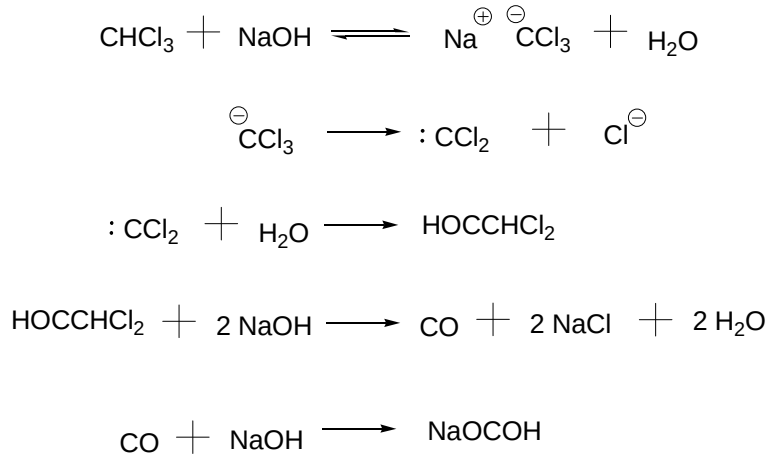


Figure 30. The generation of the reactive dichlorocarbene from chloroform and sodium hydroxide for the synthesis of 3-cyano-2-hydroxybenzaldehyde, 132.

The dichlorocarbene generated acts as an electrophile to the resonant forms of the phenoxide anion of the 2-hydroxybenzonitrile, **133**, leading to the synthesis of the desired 3-cyano-2-hydroxybenzaldehyde, **132** [Figure 31] [286].

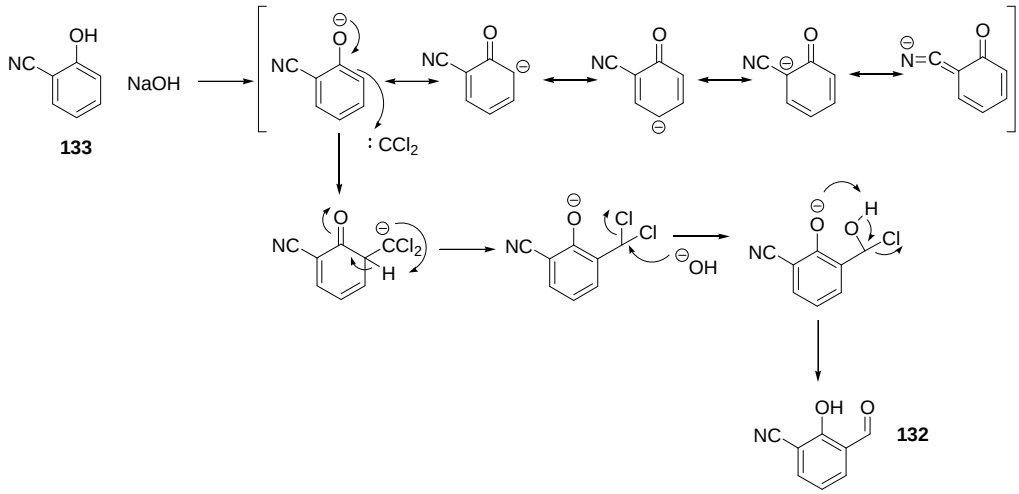


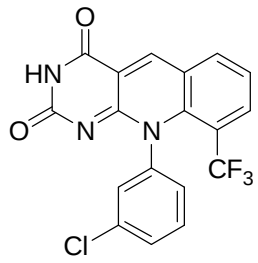
Figure 31. The mechanism of the Reimer-Tiemann reaction to synthesise 3-cyano-2-hydroxybenzaldehyde, 132.

The low yields of 3-cyano-2-hydro-benzaldehyde, **132**, were seen because:

- Neutralisation of the sodium hydroxide by the side products of dichlorocarbene generation competes with the formation of 3-cyano-2-hydrobenzaldehyde, **132** [286].
- Side reactions occur with the phenoxide anion of the 2-hydroxybenzononitrile leading to the synthesis of undesirable side products and again competing with the synthesis of 3-cyano-2-hydroxybenzaldehyde, **132** [286].
- The problems of phase transfer, even with using mechanical stirring, as the phenoxide anion substrate remains in the aqueous layer while the dichlorocarbene is present in the organic layer of chloroform [286].

3-Cyano-2-hydroxybenzaldehyde, **132**, was then used to synthesise the required 9-cyano-5-deazaflavin analogues using the OTs method. 10-(4-Chlorophenyl)-9-cyano-5-deazaflavin, **131**, was synthesised to compare with the other analogues investigating the nine position of the 5-deazaflavin, **124-128** & **130**, as inhibitors of Mdm2 E3 ubiquitin ligase activity. 10-(3-

Chlorophenyl)-9-cyano-5-deazaflavin, **134** [Figure 32], was synthesised to compare with the most active compound 10-(3-chlorophenyl)-9-trifluoromethyl-5-deazaflavin, **113**. 10-(Phenyl)-9-cyano-5-deazaflavin, **129**, was not synthesised due to time constraints.



134

Figure 32. 10-(3-Chlorophenyl)-9-cyano-5-deazaflavin, 134.

Biological Results

Both of the 9-hydroxy-5-deazaflavin analogues, **128** & **130**, synthesised were inactive at inhibiting Mdm2 from the qualitative *in vitro* biological assay at 250 μM concentration. These results together with the inactivity of the 9-methyl-5-deazaflavin analogues, **52-63**, are proof that an electron-donating substituent at the nine position of 5-deazaflavin do not produce active 5-deazaflavin compounds towards Mdm2 and that electron-withdrawing groups at the nine position are a prerequisite for activity. Both the 9-fluoro-5-deazaflavin analogues, **124** & **126**, were inactive. 10-Phenyl-9-bromo-5-deazaflavin, **125**, was inactive with IC_{50} data of greater than 100μM. 10-(4-Chlorophenyl)-9-bromo-5-deazaflavin, **127**, biological results has not yet been determined but it would be interesting to compare against 10-(4-chlorophenyl)-9-chloro-5-deazaflavin, **39**, to see which halogen is the most active at the nine position of 5-deazaflavin as a Mdm2 inhibitor. The 9-cyano-5-deazaflavin analogues synthesised, **131** & **134**, at the time of writing have not yet been tested as potential inhibitors of Mdm2 E3 ubiquitin ligase activity. These compounds,

131 & **134**, would confirm what type of hydrophilic properties the nine position substituent of 5-deazaflavin is required for inhibitor activity of Mdm2. Because the cyano substituent is hydrophilic whilst the trifluoromethyl substituent, which is present in the most active nine substituted 5-deazaflavin compounds, **47**, **51**, **113-115**, **117** & **118**, is hydrophobic, with both substituents being electron-withdrawing, is a prerequisite for activity against Mdm2 activity.

Mode of Action Hypothesis

Introduction

The main problem in developing inhibitors of Mdm2 E3 ubiquitin ligase activity is the lack of understanding of how Mdm2 acts as an E3 ubiquitin ligase. Another major hindrance is the limited knowledge of 5-deazaflavin analogues mode of action as inhibitors of Mdm2 E3 ubiquitin ligase activity.

Using an auto-ubiquitinylation assay, the RING domain of Mdm2 has been shown to be inhibited by a 5-deazaflavin analogue, HLI98C, **11**, to a similar degree as the full length Mdm2 protein [237]. This suggests that the 5-deazaflavin pharmacophore affects only the RING domain of Mdm2 to act as an inhibitor of Mdm2 E3 ubiquitin ligase activity. The Mdm2 RING domain structure has been characterised by NMR, and shows two zinc atoms in a hydrophobic pocket bound to cysteine and histidine amino acid residues [Figure 33] [170].

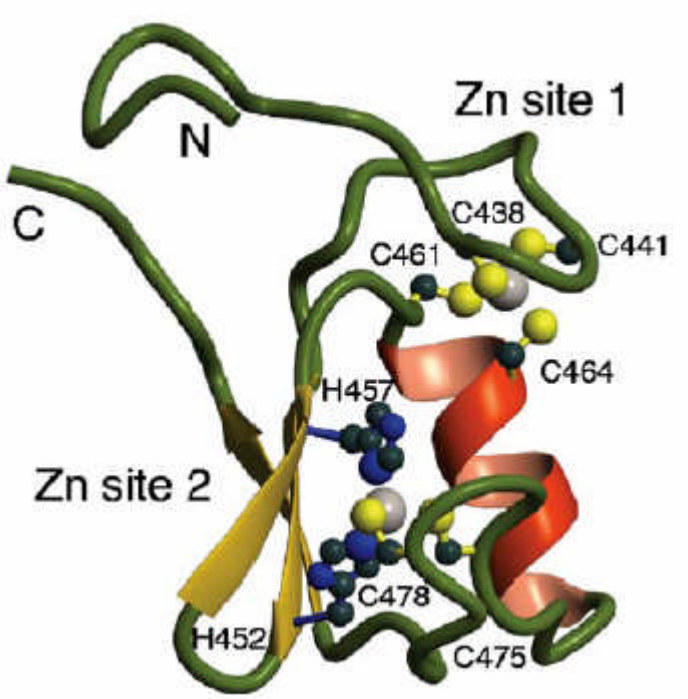


Figure 33. A ribbon representation of the lowest energy structure of Mdm2 RING domain. The diagram shows the distribution of regular secondary structure elements and the location of two Zn^{2+}-binding sites. The side chains of the zinc ligands are shown as spheres, coloured black (C), blue (N) or yellow (S) Where C = cysteine and H = histidine with the number meaning the number of the amino acid of the Mdm2 protein. This Figure was prepared using MOLMOL. Taken from [170].

Mdm2/MdmX RING Heterodimer

The recent publication of an Mdm2/MdmX RING domain heterodimer X-ray crystal structure [288] [Figure 34] has lead to a better understanding of how Mdm2 acts as an E3 ubiquitin ligase, and lead to the discovery of a proposed mode of action hypothesis for the 5-deazaflavin analogues.

The Mdm2/MdmX RING domain heterodimer X-ray crystal structure [288] shows the structural basis of the functional importance of the C-terminus of the Mdm2 RING domain, which has been reported earlier [289]. The last 7 amino acid residues of the C-terminus tail of Mdm2 RING domain are required for Mdm2 E3 ubiquitin ligase activity and dimerisation [289]. Phenylalanine at 490 position of Mdm2 is shown to be an important residue for Mdm2 E3 ubiquitin ligase activity and dimerisation [289]. The C-terminus tail of Mdm2

can be seen to insert into a hydrophobic groove or cleft of MdmX, and vice versa [Figure 34]. The Mdm2/MdmX heterodimer forms for the ubiquitinylation of MdmX and is also involved in p53 ubiquitinylation [290] as monomeric forms of Mdm2 are relatively ineffective E3 ubiquitin ligases [289-291].

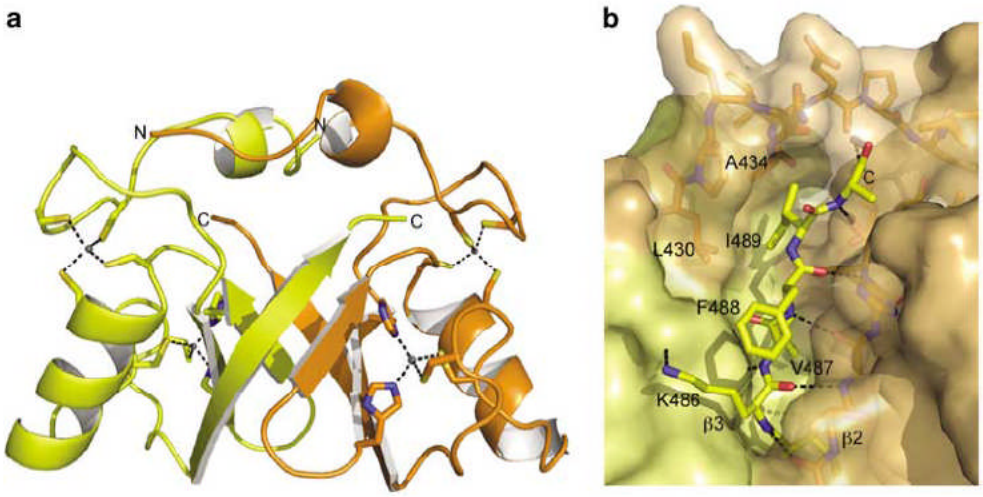


Figure 34. Structure of the Mdm2/MdmX RING domain heterodimer. (a) Cartoon diagram of the Mdm2/MdmX RING domain heterodimer structure. Mdm2 RING is shown in orange and MdmX RING in yellow, with the zinc ions and coordinating residues shown as spheres and sticks, respectively. (b) Details of the heterodimer interface between the C terminus of MdmX RING (yellow sticks), with b2 and the N terminus (top loop) of Mdm2 RING (orange surface). Hydrogen bonds are shown as lines and the key residues that make cross dimer contacts are indicated. Taken from [289].

Mdm2 Homodimer

Mdm2 also forms a homodimer for auto-ubiquitinylation and is also involved in p53 ubiquitinylation [290] as monomeric forms of Mdm2 are relatively ineffective E3 ubiquitin ligases [289-291]. The Mdm2 homodimer [Figure 35] is similar to the structure of Mdm2/MdmX RING domain heterodimer [Figure 32] with the C-terminus tail important for E3 ubiquitn ligase activity and dimerisation inserted into a hydrophobic groove or cleft of the other Mdm2 subunit and vice-versa.

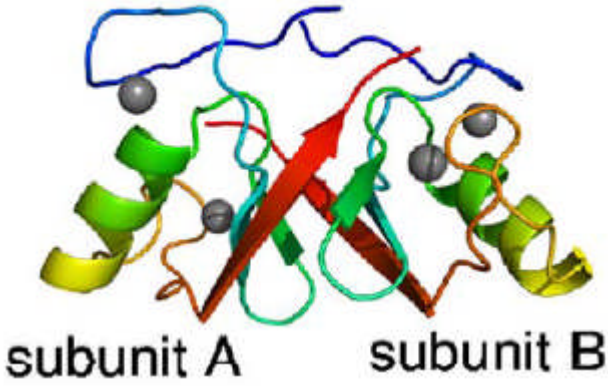


Figure 35. The Mdm2 homodimer. Both Mdm2 subunits labelled A and B and the zinc atoms in grey. The Mdm2 RING domain C-terminus in red fits into a hydrophobic groove/cleft of the other Mdm2 subunit and visa versa similar to the structure of Mdm2/MdmX RING domain heterodimer [Figure 34]. Taken from [170].

Mdm2 Monomer

Since Mdm2 can also function as a monomer as an E3 ubiquitin ligase, one can imagine from the structure that the C terminus tail important for E3 ubiquitin ligase activity can be inserted intramolecularly into its own hydrophobic groove/cleft [Figure 36], as proposed by Poyurovsky et al. [289], forming a *cis* C terminus RING interaction.

Mmd2 Oligomer

Mdm2 oligomers form by the RING domain C-terminus tail, important for E3 ubiquitin ligase activity and dimerisation, inserting into a hydrophobic groove or cleft of another Mdm2 subunit and so on [Figure 36] [289], forming a *trans* C terminus RING interaction. Results by Poyurovsky et al. [289] show that Mdm2 RING domain can induce ubiquitinylation by providing a scaffold for the reaction by forming supramolecular oligomers [Figure 36]. Mdm2 oligomer structure may provide a binding surface for multiple E2s allowing ubiquitin chain elongation required for ubiquitinylation [289].

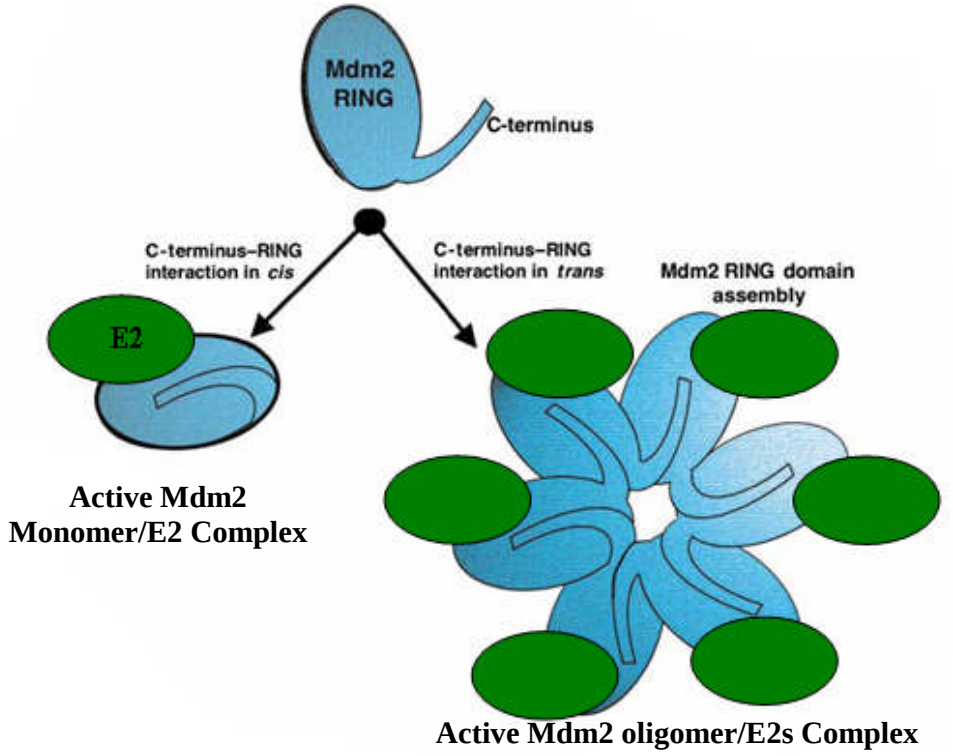


Figure 36. Schematic representation of the RING domain C-terminus of Mdm2 for the monomer and oligomer formation and role in ubiquitinylation. Blue oval represents Mdm2 with the tail respenting the RING domain C-terminus while the green oval represents E2. The extreme C-terminus of Mdm2 is able to form either intramolecular interactions forming a stable monomer that can interact with the required E2 or similar interactions with another Mdm2 RING domain which can lead to oligomer formation. Adapted from [289].

Hypothesis

A possible mode of action of the 5-deazaflavin analogues as inhibitors of Mdm2 E3 ubiquitin ligase activity could be to block this C terminus tail hydrophobic groove/cleft interaction therefore, preventing the formation of the Mdm2/MdmX heterodimer, the Mdm2 homodimer, the E3 ubiquitin ligase active Mdm2 monomer and the Mdm2 oligomer. This seems reasonable since we already know that the 5-deazaflavin compounds work at the level of the RING domain [237] and this might explain why Mdm2 auto-ubiquitinylation is also inhibited by these compounds.

Using the coordinates from the Mdm2/MdmX RING domain heterodimer X-ray crystal structure [288] with computer modelling, HLI98C, **11**, one of the

previously identified hit compounds [237] was docked without defining a binding site. The results were quite surprising insofar as they were all plausible and highly scoring poses placed the ligand exactly in the hydrophobic groove/cleft where the C-terminus tail in the complex structure is [Figure 37].

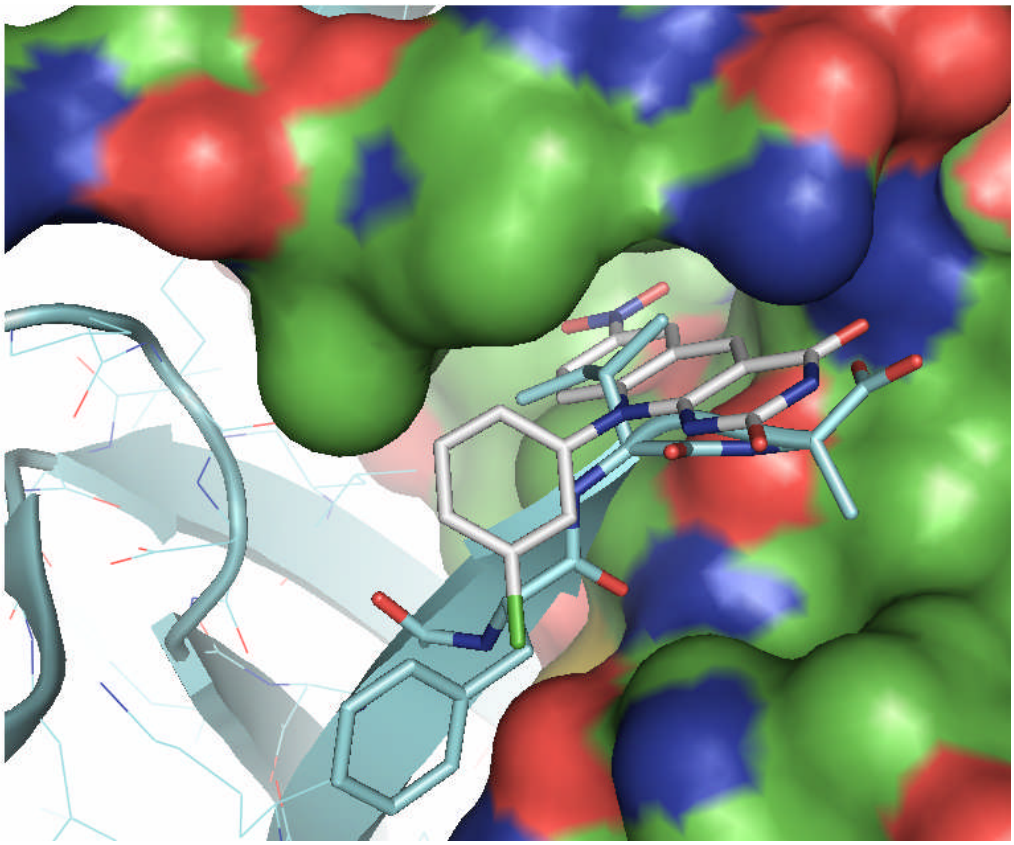


Figure 37. The computer modelling top pose of HLI98C, 11 (grey CPK sticks), superimposed onto the C-terminus tail (cyan CPK sticks and cartoon) of Mdm2 in the hydrophobic groove/cleft of MdmX (green CPK surface). Computer modelling kindly proformed by Prof Fischer of the University of Nottingham, whom provided the diagram.

This is of course not conclusive but probably suggestive enough to test experimentally. This could be done by seeing if the 5-deazaflavin compounds are active as inhibitors of Mdm2 E3 ubiquitin ligase activity also block Mdm2 homodimerisation, Mdm2/MdmX heterodimerisation and Mdm2 oligomerisation; then one could be fairly certain that blocking of the C-terminus tail hydrophobic groove/cleft interaction is occurring. A preliminary biological test could be to test the active 5-deazaflavin compounds to see if they inhibit MdmX ubiquitinylation as well as p53 ubiquitinylation by Mdm2

and Mdm2 auto-ubiquitinylation. Alternatively, collaboration with protein chemists to determine the X-ray crystal structure of 5-deazaflavin in the C-terminus tail hydrophobic groove/cleft interaction of the Mdm2/MdmX heterodimer, Mdm2 homodimer, Mdm2 monomer or Mdm2 oligomer could be carried out to prove this active site hypothesis.

When/If the active site hypothesis of the C-terminus tail hydrophobic groove/cleft interaction inhibition by the 5-deazaflavin compounds to act as inhibitors of Mdm2 E3 ubiquitin ligase activity is proven, this would lead to the use of computer modelling in the drug discovery process. Computer aided design of the 5-deazaflavin template could then be used to produce more potent inhibitors of Mdm2 E3 ubiquitin ligase activity based on the 5-deazaflavin pharmacophore. More likely, computer modelling could be used to find new different classes of Mdm2 E3 ubiquitin ligase inhibitors.

Conclusion

To conclude I have successfully continued the research into finding a small molecule inhibitor of Mdm2 E3 ubiquitin ligase activity to reactivate p53 based on the 10-phenyl-5-deazaflavin template to be used as a potential therapy for cancers that retain wild type p53.

Over 150 compounds have been synthesised with 90 being novel final compounds based on the 10-phenyl-5-deazaflavin template which were biologically tested as potential inhibitors of Mdm2 E3 ubiquitin ligase activity to reactivate p53. A novel synthesis method of 5-deazaflavin using the required 6-anilinouracil and 2-*p*-toluenesulfonyloxybenzaldehyde was devised in the process. A new quantitative biological assay was developed by scientists based at the Beatson Institute for 10-phenyl-5-deazaflavin compounds, showing excellent activity as inhibitors of Mdm2 E3 ubiquitin ligase activity on the previous qualitative or pre-screen biological assay.

The work has established a clear and logical structure-activity relationship between inhibitor activity and structure. An electron-withdrawing hydrophobic substituent at the nine position is a prerequisite for activity, as shown by the inactivity or poor activity of all 5-deazaflavins analogues tested without an electron-withdrawing hydrophobic substituent at the nine position. The hydrophobic nature of the substituent is to be confirmed after the biological results have been obtained for the 9-cyano-5-deazaflavin analogues synthesised, **131** & **134**. The best substituent at the nine position of 5-deazaflavin for potency as inhibitors of Mdm2 E3 ubiquitin ligase activity is the trifluoromethyl group. The N_{10} phenyl is required for Mdm2 inhibitor

activity as shown by the inactivity of N_{10} hydrogen, methyl and benzyl 5-deazaflavin analogues, **90-95**. Substitution on the N_{10} phenyl improves activity possibly due to increased hydrophilicity of compounds with the *meta* position best for inhibitor activity. The best substituent at the N_{10} phenyl *meta* position of 5-deazaflavin for potency as inhibitors of Mdm2 E3 ubiquitin ligase activity is the chloro group. Further SAR will be deduced about the N^3 position of the 5-deazaflavin pharmacophore once the biological results have been obtained for 10-(4-chlorophenyl)-9-trifluoromethyl-3-methyl-5-deazaflavin, **122**, and 10-(4-chlorophenyl)-9-trifluoromethyl-3-ethyl-5-deazaflavin, **123**.

Hit optimization has been successful with the new hit compound, 10-(3-chlorophenyl)-9-trifluoromethyl-5-deazaflavin, **113**, being thirty times more active than the previous identified hit compound 10-(4-chlorophenyl)-7-nitro-5-deazaflavin, **25** [237].

Using the X-ray crystal structure of the Mdm2 MdmX heterodimer [Figure 34] [288], an improved understanding of how Mdm2 acts as an E3 ubiquitin ligase has been described and used to propose an mode of action hypothesis and mode of action of the 5-deazaflavin based inhibitors of Mdm2 E3 ubiquitin ligase activity. Once/If proven this hypothesis could, using computer modelling, lead to further development of 5-deazaflavins analogues as Mdm2 inhibitors and possibly the discovery of new classes of inhibitors.

My PhD work has proven the principle that small molecules can inhibit E3 ubiquitin ligases. I believe that this work has provided the foundation for additional studies by other scientists with continued external funding in a new and developing field of medicinal chemistry.

Future Work

Assay Calibration

To further the research into inhibitors of Mdm2 E3 ubiquitin ligase activity, the acylimidazolone compound, **4**, previously shown to selectively inhibit Mdm2 E3 ubiquitin ligase activity, but not Mdm2 auto-ubiquitinylation, with a published IC_{50} value [292] should be synthesised [Figure 38] [293-297] (work carried out but not completed or discussed) and tested. The acylimidazolone compound, **4**, would then confirm and calibrate the newly developed quantitative biological assay used to obtain IC_{50} data for p53 ubiquitinylation by Mdm2 for the 5-deazaflavin analogues synthesised.

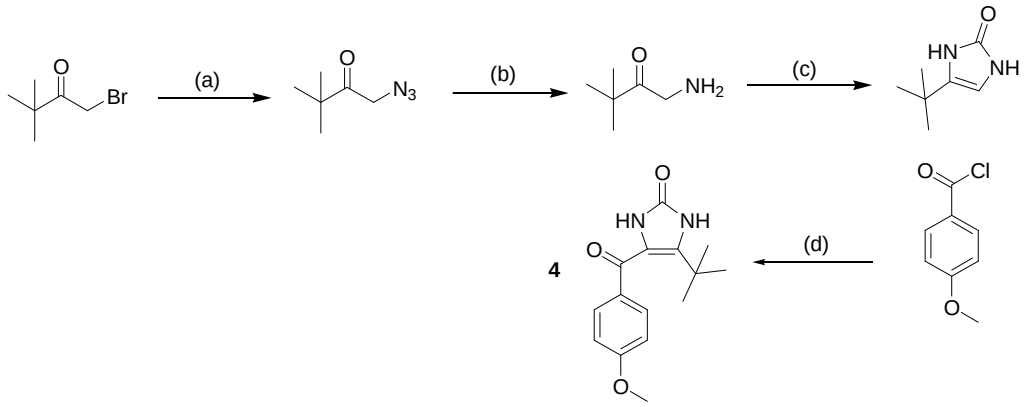


Figure 38. The synthetic pathway to produce the acylimidazolone compound, 4. (a) NaN_3, acetone, stirring (b) H_2, Pd/C, conc HCl and ethanol (c) KOCN (aq) and Δ (d) AlCl, nitrobenzene and Δ [293-297].

Future Medicinal Chemistry Work

Topliss Tree

Using the Topliss decision tree [298], more 5-deazaflavin analogues could be synthesised, further probing the N_{10} phenyl substituent and substituent pattern. In particular the *meta*-trifluoromethyl, *meta*-bromo, *para*-trifluoromethyl and *para*-bromo substituents of the N_{10} phenyl-9-trifluoromethyl-5-deazaflavin

analogues should be synthesised. The Topliss decision tree is an operational scheme or flow chart to aid analogue substituent design [298].

Third Position (N^3) of 5-Deazaflavin

The biological results for 10-(4-chlorophenyl)-9-trifluoromethyl-3-methyl-5-deazaflavin, **122**, and 10-(4-chlorophenyl)-9-trifluoromethyl-3-ethyl-5-deazaflavin, **123**, will prove if the N^3 position of 5-deazaflavin is required for inhibitor activity of Mdm2 E3 ubiquitin ligase ability. If the N^3 position of 5-deazaflavin is not required for inhibitor activity, 5-deazaflavin analogues with acidic solubilising groups (such as carboxylmethyl) and basic solubilising groups (such as morpholine) at the N^3 position should be synthesised (similar to the other N^3 5-deazaflavin analogues) to improve the poor solubility of the 5-deazaflavin compounds.

Five Position of 5-Deazaflavin

The role of substitution at the five position [R_1 -Figure 9] of 5-deazaflavin for activity as an inhibitor of Mdm2 E3 ubiquitin ligase activity requires further investigation. As HLI373, **15** [242, 243], has a 5-dimethylaminopropylamino side chain but lacks the N_{10} phenyl and an electron-withdrawing hydrophobic substituent at the nine position, which from my work is a prerequisite for activity as an inhibitor of Mdm2. HLI373, **15**, has been shown to increase Mdm2 and p53 protein levels, causing apoptosis in a variety of different cancer cell lines [242, 243]. Also the acridine derivatives, **9**, which are structurally similar to 5-deazaflavins with substituents at the five position have been shown to block p53 ubiquitinylation in an unknown manner [233]. Therefore 5-deazaflavin analogues with the dimethylaminopropylamino,

diethylaminopropylamino, morpholinepropylamino and piperidinepropylamino groups at the five position with *m*-chloro on the N_{10} phenyl and the 9-trifluoromethyl should be synthesised [250, 299] and tested.

Proving the Mode of Action Hypothesis

A lot of further work to be undertaken is to prove the hypothesis for Mdm2 mode of action as an E3 ubiquitin ligase and the proposed mode of action hypothesis of the C-terminus tail hydrophobic groove/cleft interaction of Mdm2 inhibition by the 5-deazaflavin compounds. As from the speculative computer modelling it is probably suggestive enough to test this theory experimentally. A preliminary biological test that could be carried out is to test the active 5-deazaflavin compounds ability to inhibit MdmX ubiquitinylation. This experiment would be similar to the biological tests already performed for p53 ubiquitinylation by Mdm2 and Mdm2 auto-ubiquitinylation. Another more conclusive test of mode of action hypothesis would be to see if the 5-deazaflavin active compounds block Mdm2 homodimerisation, Mdm2/MdmX heterodimerisation and Mdm2 oligomerisation; then one could be fairly certain that blocking of the C-terminus tail hydrophobic groove/cleft interaction of Mdm2 and Mdm2/MdmX is occurring. Another potential test to confirm the mode of action hypothesis would be to carry out binding studies of the active 5-deazaflavin compounds with the hydrophobic groove/cleft of Mdm2 which is currently being researched by the biological collaborators. Alternatively, through collaboration with protein chemists the X-ray crystal structure of 5-deazaflavin in the C-terminus tail hydrophobic groove/cleft interaction of the Mdm2/MdmX heterodimer, Mdm2 homodimer, Mdm2 monomer and Mdm2 oligomer could be carried out to prove this active site hypothesis.

If/When the active site hypothesis of 5-deazaflavin inhibition of Mdm2 E3 ubiquitin ligase activity is proven this would lead to the use of computer modelling in the drug discovery process. Therefore computer aided medicinal chemistry design could be carried out on the 5-deazaflavin template drug design or more likely computer modelling will be used to find different classes of Mdm2 E3 ubquitin ligase inhibitors

Experimental

General Information

Chemicals and solvents were purchased from standard suppliers and used without further purification. Merck Kieselgel 60, 230-400 mesh, for flash column chromatography was supplied by Merck KgaA (Darmstadt, Germany) and deuterated solvents were purchased from Goss International Limited (England) and Sigma-Aldrich Company Limited (England).

Reactions were monitored by thin layer chromatography on commercially available pre-coated aluminium backed plates (Merck Kieselgel 60 F^{254}). Visualisation was by examination under UV light (254 and 366 nm). Organic solvents were evaporated under reduced pressure at ≤ 40 °C (water bath temperature). Flash chromatography was performed either using a classical glass column or a Biotage Argonant Flash Master II or a Biotage Flash Master Personal. Purification using preparative layer chromatography was carried out using Fluka silica gel 60 PF^{254} containing gypsum (200 mm x 200 mm x 1 mm).

Melting points were recorded on a Gallenkamp Melting Point apparatus for **51**, **68**, **69**, **87**, **92**, **93**, **101**, **113**, **114**, **104**, **119**, **121-124, 126**, **132, 135**, **136** and **138-145**. All other compound melting points were obtained using a Bibby Stuart Scientific Melting Point Apparatus SMP3. Literature melting points obtained from research papers found using chemical structure searching computer software, such as Sci-Finder Scholar. Where no literature melting points are supplied, no compound data or melting point information was available for that compound synthesised. ^{1}H NMR spectra were recorded on a

Bruker-AV 400 at 400.13 MHz. ^{13}C NMR spectra were recorded at 75 MHz. Chemical shifts (δ) are recorded in ppm with reference to the chemical shift of the deuterated solvent or an internal TMS standard. Coupling constants (J) were recorded in Hz and the significant multiplicities described by singlet (s), broad singlet (br s), doublet (d), triplet (t), quadruplet (q), multiplet (m), doublet of doublets (dd) and doublet of doublets of doublets (ddd). ^{13}C NMR spectra peaks are described as quaternary (Cq), tertiary (CH), secondary (CH_2) and primary (CH_3) identified by DEPT 135 and DEPT 90 NMR spectra. Mass spectra (TOF $ES^{+/-}$) were recorded on a Waters 2795 separation module/micromass LCT platform and calibrated to within +/- 10 ppm with theoretical mass values provided for comparison. FT-IR spectra were recorded as KBr discs in the range of 4000 – 500 cm^{-1}. FT-IR spectra for compounds **40**, **41**, **44**, **48**, **64-66**, **68**, **69**, **135** and **136**, were obtained using an Avatar 360 Nicolet FT-IR spectrophotometer. All other compound FT-IR spectra were obtained using a Thermo Scientific Nicoler IR200 FT-IR spectrophotometer.

Waters 2525 Binary Gradient Module was used for analytical HPLC with a Waters 2487 Dual λ Absorbance UV Detector. Analytical HPLC was used to confirm purity of greater than 95%, by chromatogram integration at 254 nm wavelength, of final compounds using, System A and System B. All retention times (t_R) are quoted in minutes with percentage purity.

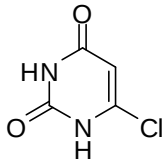
System A: Phenomenex Onyx Monolithic reverse phase C18 column (100 x 3.0 mm) with a flow rate of 3.00 mL/min and UV detection at 254 nm using 70% water and 30% acetonitrile over 10 min.

System B: Phenomenex Kromasil reverse phase C18 column (250 x 4.6 mm) with a flow rate of 1.00 mL/min and UV detection at 254 nm using 50% water and 50% methanol over 35 min.

Synthesis of 6-Chlorouracil, 68 [262, 266, 267, 269]

2, 4, 6-Trichloropyrimidine (25 g, 15.67 mL; 0.14 mol) was added to a warm (temperature greater than 25 ^{O}C) solution of sodium hydroxide (21.86 g; 0.56 mol) in water (150 mL) under stirring and refluxed for 1 h. The hot suspension of crystalline material was acidified with conc hydrochloric acid to pH 1. The resulting mixture was cooled and the precipitate was collected by filtration. The solid was purified by recrystallization from water to yield the pure 6-chlorouracil, **68**.

6-Chlorouracil, 68, (19.06 g, 0.13 mol, 95% yield) as a white solid with a m.pt. of 295-298 ^{O}C (dec) (lit 300 ^{O}C dec) [262]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 5.75 (1H, s, C5-H), 11.31 (1H, br s, N1-H), 12.08 (1H, br s, N2-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 100.32 (CH), 145.19 (Cq), 150.78 (Cq), 163.15 (Cq); HRMS (ESI^{+}): m/z $[M + H]^{+}$ calcd for $C_4H_4ClN_2O_2^{+}$ 146.9956, found 146.9964; IR (KBr): 3095 (NH), 1728 (C=O), 1655 (C=O) cm^{-1}.



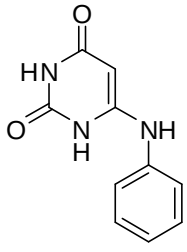
68

Synthesis of 6-(Phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 69 [259, 260, 263, 265, 300]

Method A

6-Chlorouracil, **68** (0.32 g; 2.19 mmol), was added to aniline (6.13 g, 6.00 mL; 65.84 mmol) and placed into a microwave reactor and irradiated for 20 min at 160 ^{O}C. The reaction mixture was cooled and diluted with diethyl ether which caused separation of crystals which were filtered, washed with water and recrystallised from ethanol to yield the pure 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **69**.

6-(Phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 69, (0.25 g, 1.25 mmol, 57% yield) as an off white solid with a m.pt. of 324-326 ^{O}C (dec) (lit 325-327 ^{O}C dec) [259]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 4.68 (1H, s, C5-H), 7.12-7.42 (5H, m, Ph-H), 8.26 (1H, s, N3-H), 10.18 (1H, s, N1-H), 10.47 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): 76.25 (CH), 123.19 (CH), 125.16 (CH), 129.89 (CH) 138.36 (Cq), 151.32 (Cq), 152.68 (Cq), 164.84 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{10}H_{10}N_3O_2^+$ 204.0768, found 204.0784; IR (KBr): 3200 (NH), 3053 (NH), 1753 (C=O), 1604 (C=C) cm^{-1}.



69

Method B

6-Chlorouracil, **68** (0.50 g; 3.41 mmol), was added to aniline (9.53 g, 9.32 mL; 102.36 mmol) and refluxed for 30 min. The reaction mixture was cooled and diluted with diethyl ether which caused separation of crystals which were

filtered, washed with water and recrystallised from ethanol to yield the pure 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **69**.

6-(Phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 69, (0.36 g, 1.79 mmol 53% yield) as an off white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 4.68 (1H, s, C5-H), 7.16-7.40 (5H, m, Ph-H), 8.27 (1H, s, NH), 10.20 (1H, s, N1-H), 10.48 (1H, s, N3-H); HRMS (ESI^{+}): m/z $[M + H]^{+}$ calcd for $C_{10}H_{10}N_3O_2^{+}$ 204.0768, found 204.0785.

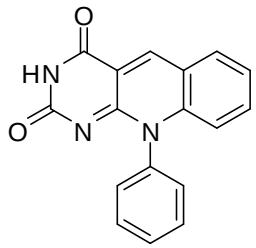
Synthesis of 10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 64, using 2-Halobenzaldehyde [241, 261, 264, 265, 268, 270]

Method A

A mixture of 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **69** (69.75 mg; 0.34 mmol), and 2-chlorobenzaldehyde (58.09 mg, 46.54μL; 0.41 mmol), in DMF (3 mL) was heated to 160 ^{O}C for 30 min in the microwave reactor. Concentration of the solution under reduced pressure and dry column flash chromatography using 95% dichloromethane and 5% methanol was carried out to yield the pure 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, **64**.

10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 64, (90.3 mg, 0.31 mmol, 90% yield) as a yellow solid with a m.pt. of >350 ^{O}C (lit >330 ^{O}C) [264]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.71 (1H, d, *J*= 8.5 Hz, C9-H), 7.43 (2H, d, *J*= 7.1 Hz, Ph2-H), 7.51 (1H, t, *J*= 8.5 Hz, C8-H), 7.61-7.77 (4H, m, C7-H & Ph-H), 8.24 (1H, d, *J*= 8.5 Hz, C6-H), 9.14 (1H, s, C5-H), 11.10 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 112.31 (Cq), 115.68 (Cq), 118.08 (CH), 121.12 (Cq), 125.08 (CH), 128.02 (CH), 129.90 (CH), 130.68 (CH), 131.10 (CH), 135.27 (CH), 136.98 (Cq), 142.40(Cq), 143.27

(CH), 156.21 (Cq), 161.51 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{12}N_3O_2^+$ 290.0924, found 290.0922; IR (KBr): 3419 (NH), 1699 (C=O), 1667 (C=O), 1607 (C=C) cm^{-1}; Anal. HPLC t_R 0.91 min (99.5% pure - System A), 3.55 min (100.0% pure - System B).



64

Method B

A mixture of 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **69** (69.75 mg; 0.34 mmol), and 2-fluorobenzaldehyde (50.88 mg, 43.74 μL; 0.41 mmol), in DMF (3 mL) was heated to 160 ^{O}C for 30 min in the microwave reactor. Concentration of the solution under reduced pressure and dry column flash chromatography using 95% dichloromethane and 5% methanol was carried out to yield the pure 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, **64**.

10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 64, (80.5 mg, 0.27 mmol, 81% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.71 (1H, d, *J*= 8.5 Hz, C9-H), 7.43 (2H, d, *J*= 7.1 Hz, Ph2-H), 7.50 (1H, t, *J*= 8.5 Hz, C8-H), 7.60-7.76 (4H, m, C7-H & Ph-H), 8.23 (1H, d, *J*= 8.5 Hz, C6-H), 9.12 (1H, s, C5-H), 11.05 (1H, s, N3-H); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{12}N_3O_2^+$ 290.0924, found 290.0944.

Method C

A mixture of 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **69** (69.75 mg; 0.34 mmol), and 2-chlorobenzaldehyde (58.09 mg, 46.54 μL; 0.41 mmol), in

DMF (3 mL) was refluxed for 4 h. Concentration of the solution under reduced pressure and dry column flash chromatography using 95% dichloromethane and 5% methanol was carried out to yield the pure 10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, **64** .

10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 64, (84.2 mg, 0.29 mmol, 84% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.71 (1H, d, *J*= 8.5 Hz, C9-H), 7.43 (2H, d, *J*= 7.1 Hz, Ph2-H), 7.51 (1H, t, *J*= 8.5 Hz, C8-H), 7.60-7.76 (4H, m, C7-H & Ph-H), 8.23 (1H, d, *J*= 8.5 Hz, C6-H), 9.12 (1H, s, C5-H), 11.05 (1H, s, N3-H); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{12}N_3O_2^+$ 290.0924, found 290.0922.

Method D

A mixture of 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **69** (69.75 mg; 0.34 mmol), and 2-fluorobenzaldehyde (50.88 mg, 43.74 μL; 0.41 mmol), in DMF (3 mL) was refluxed for 4 h. Concentration of the solution under reduced pressure and dry column flash chromatography using 95% dichloromethane and 5% methanol was carried out to yield the pure 10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, **64**.

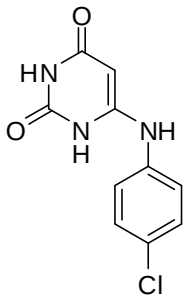
10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 64, (66.8 mg, 0.23 mmol, 67% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.71 (1H, d, *J*= 8.5 Hz, C9-H), 7.43 (2H, d, *J*= 8.1 Hz, Ph2-H), 7.50 (1H, t, *J*= 8.5 Hz, C8-H), 7.60-7.76 (4H, m, C7-H & Ph-H), 8.23 (1H, d, *J*= 8.5 Hz, C6-H), 9.12 (1H, s, C5-H), 11.06 (1H, s, N3-H); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{12}N_3O_2^+$ 290.0924, found 290.0896.

Synthesis of 6-(4-Chlorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 135

[259, 260, 263, 265, 300]

6-Chlorouracil, **68** (1.23 g; 8.41 mmol), was added to 4-chloroaniline (3.21 g; 25.23 mmol) and heated for 30 min at 180 ^{O}C. The reaction mixture was cooled and diluted with diethyl ether which caused separation of crystals which were filtered, washed with water and recrystallised from ethanol to yield the pure 6-(4-chlorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **135**.

6-(4-Chlorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 135, (1.05 g, 4.41 mmol, 53% yield) as an off white solid with a m.pt. of 345-347 ^{O}C (dec) (lit 344-346 ^{O}C dec) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 4.70 (1H, s, C5-H), 7.21 (2H, d, AA'BB' system, *J*= 8.8 Hz, Ph2-H), 7.42 (2H, d, AA'BB' system, *J*= 8.8 Hz, Ph3-H), 8.41 (1H, s, NH), 10.30 (1H, s, N1-H), 10.51 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 77.03 (CH), 124.61 (CH), 128.79 (Cq), 129.77 (CH), 137.56 (Cq), 151.34 (Cq), 152.40 (Cq), 164.86 (Cq); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{10}H_9ClN_3O_2^+$ 238.0378, found 238.0350; IR (KBr): 3196 (NH), 3064 (NH), 1752 (CO), 1608 (C=C) cm^{-1}.



135

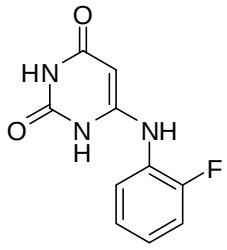
Synthesis of 6-(2-Fluorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 136

[259, 260, 263, 265, 300]

6-Chlorouracil, **68** (0.66 g; 4.52 mmol), was added to 2-fluoroaniline (3.01 g, 2.61 mL; 25.23 mmol) and heated for 30 min at 180 ^{O}C. The reaction mixture

was cooled and diluted with diethyl ether which caused separation of crystals which were filtered, washed with water and recrystallised from ethanol to yield the pure 6-(2-Fluorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **136**.

6-(2-Fluorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 136, (0.51 g, 2.31 mmol, 51% yield) as an off white solid with a m.pt. of 316-318 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 4.45 (1H, s, C5-H), 7.20-7.43 (4H, m, Ph-H), 8.19 (1H, s, NH), 10.32 (1H, s, N1-H), 10.52 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 76.52 (CH), 116.83 (CH, d, *J*= 19.65 Hz), 125.54 (CH, d, *J*= 3.65 Hz), 125.69 (Cq, d, *J*= 11.91 Hz), 126.84 (CH), 127.69 (CH, d, *J*= 7.45 Hz) 151.14 (Cq), 152.81 (Cq), 156.32 (Cq, d, *J*= 245.41 Hz), 164.73 (Cq); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{10}H_9FN_3O_2^+$ 222.0673, found 222.0682; IR (KBr): 3204 (NH), 3055 (NH), 1733 (C=O), 1629 (C=C) cm^{-1}.



136

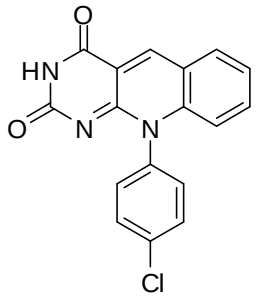
<u>Synthesis of 10-(4-Chlorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 66</u> [241, 261, 264, 265, 268, 270]

Method A

A mixture of 6-(4-chlorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **135** (73.40 mg; 0.31 mmol), and 2-fluorobenzaldehyde (46.84 mg, 39.05 µL; 0.37 mmol), in DMF (3 mL), was heated to 160 ^{O}C for 30 min in the microwave reactor. Concentration of the solution under reduced pressure and dry column flash chromatography using 95% dichloromethane and 5% methanol was

carried out to yield the pure 10-(4-chlorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, **66**.

10-(4-Chlorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 66, (55.90 mg, 0.17 mmol, 56% yield) as a yellow solid with a m.pt. of >350 ^{O}C (lit >300 ^{O}C) [264]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.79 (1H, d, *J*= 8.8 Hz, C9-H), 7.44-7.55 (3H, m), 7.69-7.82 (3H, m), 8.23 (1H, d, *J*= 8.8 Hz, C6-H), 9.13 (1H, s, C5-H), 11.01 (1H, br s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.08 (Cq), 117.56 (CH), 121.47 (Cq), 124.95 (CH), 130.81 (CH), 130.98 (CH), 131.89 (CH), 134.51 (Cq), 135.61 (CH), 136.98 (Cq), 142.05 (Cq), 143.00 (CH), 156.82 (Cq), 159.20 (Cq), 162.34 (Cq); HRMS (ESI^{+}): m/z $[M + H]^{+}$ calcd for $C_{17}H_{11}ClN_3O_2^{+}$ 324.0534, found 324.0551; IR (KBr): 3421 (NH), 1701 (C=O), 1665 (C=O), 1609 (C=C) cm^{-1}; Anal. HPLC t_R 1.69 min (100.0% pure - System A), 4.59 min (98.5% pure - System B).



66

Method B

A mixture of 6-(4-chlorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **135** (73.40 mg; 0.31 mmol), and 2-chlorobenzaldehyde (52.10 mg, 41.75μL; 0.37 mmol), in DMF (3 mL) was heated to 160 ^{O}C for 30 min in the microwave reactor. Concentration of the solution under reduced pressure and dry column flash chromatography using 95% dichloromethane and 5% methanol was

carried out to yield the pure 10-(4-chlorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, **66**.

10-(4-Chlorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 66, (45.5 mg, 0.14 mmol, 46% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.79 (1H, d, *J*= 8.8 Hz, C9-H), 7.47-7.54 (3H, m), 7.74-7.79 (3H, m), 8.24 (1H, d, *J*= 8.8 Hz, C6-H), 9.13 (1H, s, C5-H), 11.12 (1H, s, N3-H); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{11}ClN_3O_2^+$ 324.0534, found 324.0528.

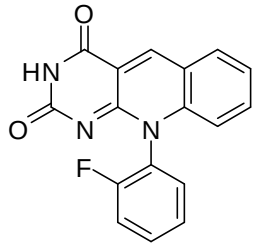
Synthesis of 10-(2-Fluorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 65 [241, 261, 264, 265, 268, 270]

Method A

A mixture of 6-(2-fluorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **136** (71.99 mg; 0.32 mmol), and 2-fluorobenzaldehyde (48.50 mg, 41.17 μL; 0.39 mmol), in DMF (3 mL) was heated to 160 ^{O}C for 30 min in the microwave reactor. Concentration of the solution under reduced pressure and dry column flash chromatography using 95% dichloromethane and 5% methanol was carried out to yield the pure 10-(2-fluorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, **65**.

10-(2-Fluorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 65, (69.9mg, 0.22mmol, 70% yield) as a yellow solid with a m.pt. of 308-310 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.82 (1H, d, *J*= 8.4 Hz, C9-H), 7.50-7.65 (4H, m), 7.69-781 (2H, m), 8.25 (1H, d, *J*= 8.4 Hz, C6-H), 9.16 (1H, s, C5-H), 11.16 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 115.92 (Cq), 116.74 (CH), 117.67 (CH, d, *J*= 19.20 Hz), 121.42 (Cq), 124.98 (Cq, d,

J= 13.25 Hz), 125.33 (CH), 126.62 (CH, d, *J*= 3.75 Hz), 131.03 (CH), 132.21 (CH), 132.57 (CH, d, *J*= 10.05 Hz), 136.11 (CH), 141.38 (Cq), 143.49 (CH), 157.47 (Cq, d, *J*= 250.00 Hz), 156.83 (Cq), 158.90 (Cq), 162.16 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{11}FN_3O_2^+$ 308.0830, found 308.0846; IR (KBr): 3444 (NH), 1705 (C=O), 1654 (C=O), 1611 (C=C) cm^{-1}; Anal. HPLC t_R 1.11 min (98.6% pure - System A), 3.72 min (97.6% pure - System B).



65

Method B

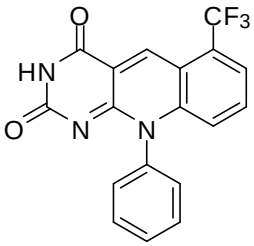
A mixture of 6-(2-fluorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **136** (71.99 mg; 0.32 mmol), and 2-chlorobenzaldehyde (48.50 mg, 41.17μL; 0.39 mmol), in DMF (3 mL) was heated to 160 ^{O}C for 30 min in the microwave reactor. Concentration of the solution under reduced pressure and dry column flash chromatography using 95% dichloromethane and 5% methanol was carried out to yield the pure 10-(2-fluorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, **65**.

10-(2-Fluorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 65, (91.0 mg, 0.29 mmol, 91% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.83 (1H, d, *J*= 8.4 Hz, C9-H), 7.50-7.66 (4H, m), 7.70-7.83 (2H, m), 8.27 (1H, d, *J*= 8.4 Hz, C6-H), 9.17 (1H, s, C5-H), 11.19 (1H, s, N3-H); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{11}FN_3O_2^+$ 308.0830, found 308.0842.

General Procedure to Synthesise 10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione Analogues using 2-Halobenzaldehyde [241, 261, 264, 265, 268, 270]

To 1 equivalent of the desired 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione analogue in DMF was added 1.2 equivalents of the desired 2-halobenzaldehyde (chloro or fluoro). The mixture was heated at 160 ^{O}C for 30 min in the microwave reactor or refluxed for 4 h. Concentration of the solution under reduced pressure and separation by dry column flash chromatography using 95% dichloromethane and 5% methanol to yield the pure desired 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogue.

[a]10-Phenyl-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 40, (30.2 mg, 0.08 mmol, 30% yield) as a yellow solid with a m.pt. of 342-344 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.06 (1H, d, *J*= 8.0 Hz, C9-H), 7.47 (2H, d, *J*= 7.4 Hz, Ph2-H), 7.64-7.76 (3H, m, Ph-H), 7.88 (1H, t, *J*= 8.0 Hz, C8-H), 7.96 (1H, d, *J*= 8.0 Hz, C7-H), 8.87 (1H, s, C5-H), 11.31 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.91 (Cq), 118.12 (Cq), 122.86 (CH), 123.14 (CH), 123.20 (Cq),), 128.78 (CH), 130.13 (CH), 130.89 (CH), 134.57 (CH), 135.55 (CH), 137.94 (Cq), 143.48 (Cq), 156.78 (Cq), 158.78 (Cq), 162.03 (Cq); HRMS (ESI^{+}): m/z [M + H]$^{+}$ calcd for $C_{18}H_{11}F_3N_3O_2^{+}$ 358.0798, found 358.0833; IR (KBr): 3441 (NH), 1713 (C=O), 1679 (C=O), 1627 (C=C) cm^{-1}; Anal. HPLC t_R 2.30 min (97.4% pure - System A), 5.45 min (100.0% pure - System B).



40

[a] microwaved for 1 h at 160 ^{O}C.

10-Phenyl-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 40, (14.5 mg, 0.04 mmol, 15% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.05 (1H, d, *J*= 8.0 Hz, C9-H), 7.46 (2H, d, *J*= 7.68 Hz, Ph2-H), 7.63-7.74 (3H, m, Ph-H), 7.87 (1H, t, *J*= 8.0 Hz, C8-H), 7.96 (1H, d, *J*= 8.0 Hz, C7-H), 8.86 (1H, s, C5), 11.32 (1H, s, N3-H); HRMS (ESI^{+}): m/z $[M + H]^{+}$ calcd for $C_{18}H_{11}F_3N_3O_2^{+}$ 358.0798, found 358.0798.

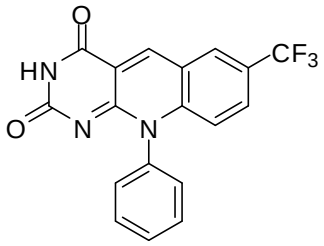
[b]**10-Phenyl-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 40,** (14.5 mg, 0.04 mmol, 15% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.05 (1H, d, *J*= 8.0 Hz, C9-H), 7.46 (2H, d, *J*= 8.00 Hz, Ph2-H), 7.63-7.74 (3H, m, Ph-H), 7.87 (1H, t, *J*= 8.0 Hz, C8-H), 7.96 (1H, d, *J*= 8.0 Hz, C7-H), 8.86 (1H, s, C5-H), 11.32 (1H, s, N3-H); HRMS (ESI^{+}): m/z $[M + H]^{+}$ calcd for $C_{18}H_{11}F_3N_3O_2^{+}$ 358.0798, found 358.0814.

[b] microwaved for 20 min at 160 ^{O}C.

[c]**10-Phenyl-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 40,** (12.7 mg, 0.03 mmol, 13% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.06 (1H, d, *J*= 8.0 Hz, C9-H), 7.46 (2H, d, *J*= 7.01 Hz, Ph2-H), 7.63-7.74 (3H, m, Ph-H), 7.87 (1H, t, *J*= 8.0 Hz, C8-H), 7.96 (1H, d, *J*= 8.0 Hz, C7-H), 8.86 (1H, s, C5-H), 11.32 (1H, s, N3-H); HRMS (ESI^{+}): m/z $[M + H]^{+}$ calcd for $C_{18}H_{11}F_3N_3O_2^{+}$ 358.0798, found 358.0800.

[c] microwaved for 10 min at 160 ^{O}C.

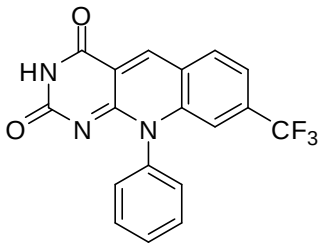
10-Phenyl-7-trifluoromethlypyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 41, (87.2 mg, 0.24 mmol, 87% yield) as a yellow solid with a m.pt. of 304-306 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.87 (1H, d, *J*= 9.3 Hz, C9-H), 7.46 (2H, d, *J*= 7.1 Hz, Ph2-H), 7.63-7.74 (3H, m, Ph-H), 8.01 (1H, d, *J*= 9.3 Hz, C8-H), 8.73 (1H, s, C6-H), 9.23 (1H, s, C5-H), 11.22 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 117.57 (Cq), 118.88 (CH), 121.04 (Cq), 124.99 (Cq), 125.58 (Cq), 128.82 (CH), 129.34 (CH), 130.07 (CH), 130.84 (CH), 137.82 (CH), 142.48 (CH), 144.24 (Cq), 156.80 (Cq), 159.72 (Cq), 162.05 (Cq); HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for $C_{18}H_{11}F_3N_3O_2^+$ 358.0798, found 358.0791; IR (KBr): 3419 (NH), 1706 (CO), 1666 (CO), 1619 (C=C) cm^{-1}; Anal. HPLC t_R 3.36 min (100.0% pure - System A), 6.30 min (99.6% pure - System B).



41

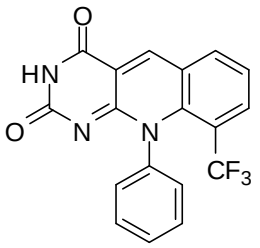
10-Phenyl-8-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 42, (66.7 mg, 0.18 mmol, 67% yield) as a yellow solid with a m.pt. of 347-348 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.82 (1H, s, C9-H), 7.49 (1H, d, *J*= 8.3 Hz, C7-H), 7.65-7.76 (3H, m, Ph-H), 7.84 (2H, d, *J*= 8.6 Hz, Ph2-H), 8.48 (1H, d, *J*= 8.3 Hz, C6-H), 9.22 (1H, s, C5-H), 11.26 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 113.91 (CH), 118.46 (Cq), 120.58 (CH), 123.81 (Cq), 128.85 (CH), 130.27 (CH), 130.95 (CH), 133.52 (CH), 133.72 (Cq), 137.54 (Cq), 141.83 (CH), 142.01 (Cq), 156.76 (Cq), 159.50

(Cq), 162.02 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{11}F_3N_3O_2^+$ 358.0798, found 358.0818; IR (KBr): 3417 (NH), 1706 (C=O), 1666 (C=O), 1616 (C=C) cm^{-1}; Anal. HPLC t_R 3.15 min (96.2% pure - System A), 6.02 min (97.8% pure - System B).



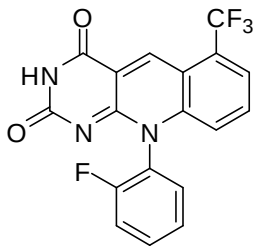
42

10-Phenyl-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 43, (23.2 mg, 0.06 mmol, 23% yield) as a yellow solid with a m.pt. of 312-314 ^{O}C; ^1H NMR (400 MHz, DMSO-d_6): δ/ppm 7.30-7.36 (2H, m, Ph2-H), 7.45-7.51 (3H, m, Ph-H), 7.66 (1H, t, *J*= 7.7 Hz, C7-H), 8.18 (1H, dd, ^4J= 1.4 Hz, ^3J= 7.7 Hz, C8-H), 8.48 (1H, dd, ^4J= 1.4 Hz, ^3J= 7.7 Hz, C6-H), 9.11 (1H, s, C5-H), 11.23 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.79 (Cq), 120.91 (CH), 124.26 (Cq), 124.94 (CH), 128.44 (CH), 129.58 (CH), 129.90 (CH), 131.20 (CH), 136.79 (Cq), 139.46 (Cq), 139.67 (Cq), 143.32 (CH), 156.80 (Cq), 161.18 (Cq), 162.07 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{11}F_3N_3O_2^+$ 358.0798, found 358.0765; IR (KBr): 3424 (NH), 1716 (C=O), 1659 (C=O), 1618 (C=C) cm^{-1}; Anal. HPLC t_R 2.12 min (100.0% pure - System A), 5.20 min (100.0% pure - System B).



43

[a]**10-(2-Fluorophenyl)-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 44,** (26.00 mg, 0.69 mmol, 26% yield) as a yellow solid with a m.pt. of 299-300 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.20 (1H, d, *J*= 8.6 Hz, C9-H), 7.52-7.69 (3H, m, Ph-H), 7.72-7.81 (1H, m, Ph-H), 7.93 (1H, t, *J*= 8.6 Hz, C8-H), 8.00 (1H, d, *J*= 8.6 Hz, C7-H), 8.87 (1H, s, C5-H), 11.37 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.86 (Cq), 117.82 (CH, d, *J*= 19.42 Hz), 118.02 (Cq), 122.10 (CH), 123.63 (CH), 124.59 (Cq), 124.72 (Cq), 126.82 (CH, d, *J*= 3.64 Hz), 130.88 (CH), 132.94 (CH, d, *J*= 7.93 Hz), 135.29 (CH), 136.02 (CH), 147.52 (Cq), 156.60 (Cq), 157.59 (Cq, d, *J*= 248.65 Hz), 158.43 (Cq), 161.73 (Cq); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{18}H_{10}F_4N_3O_2^+$ 376.0704, found 376.0685; IR (KBr): 3433 (NH), 1720 (C=O), 1660 (C=O), 1625 (C=C); Anal. HPLC t_R 2.55 min (100.0% pure - System A), 5.87 min (99.5% pure - System B).



44

[a] microwaved for 1 h at 160 ^{O}C.

10-(2-Fluorophenyl)-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 44, (51.8 mg, 0.13 mmol, 10% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.19 (1H, d, *J*= 8.6 Hz, C9-H), 7.52-7.68 (3H, m, Ph-H), 7.72-7.79 (1H, m, Ph-H), 7.93 (1H, t, *J*= 8.6 Hz, C8-H), 8.00 (1H, d, *J*= 8.6 Hz, C6-H), 8.86 (1H, s, C5-H), 11.40 (1H, s, N3-H); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{18}H_{10}F_4N_3O_2^+$ 376.0704, found 376.0725.

[b]**10-(2-Fluorophenyl)-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 44,** (10.0 mg, 0.02 mmol, 10% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.20 (1H, d, *J*= 8.6 Hz, C9-H), 7.53-7.68 (3H, m, Ph-H), 7.72-7.81 (1H, m, Ph-H), 7.93 (1H, t, *J*= 8.6 Hz, C8-H), 8.01 (1H, d, *J*= 8.6 Hz, C7-H), 8.86 (1H,s, C5-H), 11.40 (1H, s, N3-H); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{18}H_{10}F_4N_3O_2^+$ 376.0704, found 376.0721.

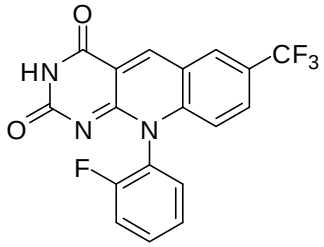
[b] microwaved for 20 min at 160 ^{O}C.

[c]**10-(2-Fluorophenyl)-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 44,** (17.8 mg, 0.04 mmol, 18% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.20 (1H, d, *J*= 8.6 Hz, C9-H), 7.53-7.68 (3H, m, Ph-H), 7.72-7.81 (1H, m, Ph-H), 7.93 (1H, t, *J*= 8.6 Hz, C8-H), 8.01 (1H, d, *J*= 8.6 Hz, C7-H), 8.86 (1H,s, C5-H), 11.40 (1H, s, N3-H); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{18}H_{10}F_4N_3O_2^+$ 376.0704, found 376.0718.

[c] microwaved for 10 min at 160 ^{O}C.

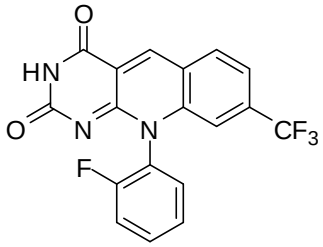
10-(2-Fluorophenyl)-7-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 45, (63.10 mg, 0.16 mmol, 63% yield) as a yellow sold with a m.pt. of 308-309 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.99 (1H, d, *J*= 8.9 Hz, C9-H), 7.52-7.67 (3H, m, Ph-H), 7.71-7.78 (1H, m, Ph-H), 8.05 (1H, dd, ^4J= 1.6 Hz, ^3J= 8.9 Hz, C8-H), 8.75 (1H, d, ^4J= 1.6 Hz, C6-H), 9.24 (1H, s, C5-H), 11.32 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 117.39 (Cq), 117.78 (CH, d, *J*= 18.91 Hz), 118.11 (CH), 121.11 (CH), 124.36 (Cq, d, *J*= 13.59 Hz), 125.45 (Cq), 126.75 (CH, d, *J*= 3.14 Hz), 129.71 (CH), 130.91 (CH), 131.46 (Cq), 132.87 (CH, d, *J*= 7.74 Hz), 142.98 (CH), 143.37 (Cq), 156.73 (Cq), 157.48 (Cq, d, *J*= 250.50 Hz), 159.38 (Cq), 161.82 (Cq); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{18}H_{10}F_4N_3O_2^+$ 376.0704, found

376.0727; IR (KBr): 3415 (NH), 1712 (C=O), 1655 (C=O), 1617 (C=C) cm^{-1}; Anal. HPLC t_R 3.47 min (98.2% pure - System A), 6.74 min (96.8% pure - System B).



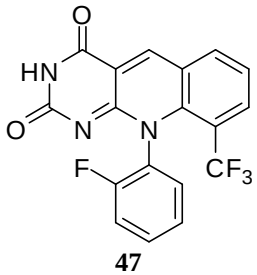
45

10-(2-Fluorophenyl)-8-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 46, (39.10 mg, 0.10 mmol, 39% yield) as a yellow solid with a m.pt. of 315-318 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.90 (1H, s, C9-H), 7.56 (1H, t, *J*= 7.6 Hz, Ph5-H), 7.62-7.69 (2H, m, Ph-H), 7.73-7.80 (1H, m, Ph-H), 7.89 (1H, d, *J*= 8.4 Hz, C7-H), 8.51 (1H, d, *J*= 8.4 Hz, C6-H), 9.23 (1H, s, C5-H), 11.34 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 112.97 (CH, d, *J*= 4.77 Hz), 117.88 (CH, d, *J*= 18.40 Hz), 118.28 (Cq), 121.10 (CH, d, *J*= 3.62 Hz), 123.86 (Cq), 124.29 (Cq, d, *J*= 13.29 Hz), 126.81 (CH, d, *J*= 3.52 Hz), 130.94 (CH), 133.06 (CH, d, *J*= 8.09 Hz), 133.93 (CH), 134.27 (Cq), 141.12 (Cq), 142.34 (CH), 156.63 (Cq), 157.58 (Cq, d, *J*= 251.92 Hz), 159.17 (Cq), 161.77 (Cq HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{10}F_4N_3O_2^+$ 376.0704, found 376.0705; IR (KBr): 3423 (NH), 1708 (C=O), 1672 (C=O), 1622 (C=C) cm^{-1}; Anal. HPLC t_R 3.59 min (97.3% pure - System A), 6.42 min (100.0% pure - System B).



46

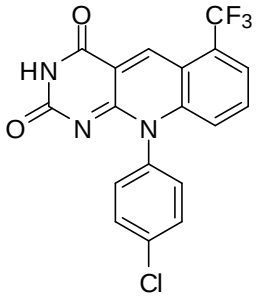
10-(2-Fluorophenyl)-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 47, (65.70 mg, 0.17 mmol, 66% yield) as a yellow solid with a m.pt. of 292-293 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.25-7.31 (2H, m, Ph-H), 7.42 (1H, t, *J*= 9.7 Hz, Ph5-H), 7.55-7.63 (1H, m, Ph-H), 7.69 (1H, t, *J*= 7.9 Hz, C7-H), 8.23 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 7.9 Hz, C8-H), 8.52 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 7.9 Hz, C6-H), 9.15 (1H, s, C5-H), 11.32 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 115.80 (CH, d, *J*= 20.14 Hz), 116.05 (CH), 123.89 (Cq), 124.64 (Cq), 126.75 (CH, d, *J*= 3.64 Hz), 129.70 (CH), 130.95 (CH), 131.14 (Cq), 131.99 (CH, d, *J*= 7.64 Hz), 135.36 (Cq), 136.91 (Cq), 139.57 (Cq), 143.28 (CH), 156.26 (Cq), 158.77 (Cq, d, *J*= 251.75 Hz), 159.99 (Cq), 161.45 (Cq); HRMS (ESI^{+}): m/z [M + H]$^{+}$ calcd for $C_{18}H_{10}F_4N_3O_2^{+}$ 376.0704, found 376.0729; IR (KBr): 3418 (NH), 1703 (C=O), 1679 (C=O), 1631 (C=C) cm^{-1}; Anal. HPLC t_R 2.52 min (98.0% pure - System A), 5.62 min (97.5% pure - System B).



47

[d]10-(4-Chlorophenyl)-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 48, (11.10 mg, 28.33 μmol, 11% yield) as a yellow solid with a m.pt. of 304-306 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.16 (1H,

d, *J*= 8.0 Hz, C9-H), 7.52 (2H, d, AA'BB' system, *J*= 8.8 Hz, Ph2-H), 7.80 (2H, d, AA'BB' system, *J*= 8.8 Hz, Ph3-H), 7.88 (1H, t, *J*= 8.0 Hz, C8-H), 7.97 (1H, d, *J*= 8.0 Hz, C7-H), 8.86 (1H, s, C5-H), 11.33 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.89 (Cq), 118.04 (Cq), 122.94 (CH), 123.27 (CH), 127.48 (Cq), 130.86 (CH), 130.99 (CH), 134.66 (CH), 134.83 (Cq), 135.64 (CH), 136.70 (Cq), 143.28 (Cq), 156.53 (Cq), 158.80 (Cq), 161.88 (Cq); HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for $C_{18}H_{10}ClF_3N_3O_2^+$ 392.0408, found 392.0439; IR (KBr): 3410 (NH), 1716 (C=O), 1670 (C=O), 1624 (C=C) cm^{-1}; Anal. HPLC t_R 3.98 min (100.0% pure - System A), 7.47 min (96.2% pure - System B).



48

[d] microwaved for 6 h at 160 ^{O}C.

[e]10-(4-Chlorophenyl)-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 48, (11.7 mg, 29.86 μmol, 12% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.16 (1H, d, *J*= 8.0 Hz, C9-H), 7.52 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph2-H), 7.80 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph3-H), 7.88 (1H, t, *J*= 8.0 Hz, C8-H), 7.84 (1H, d, *J*= 7.0 Hz, C7-H), 8.86 (1H, s, C5-H), 11.33 (1H, s, N3-H); HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for $C_{18}H_{10}ClF_3N_3O_2^+$ 392.0408, found 392.0405.

[e] microwaved for 2 h at 160 ^{O}C.

10-(4-Chlorophenyl)-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 48, (18.1 mg, 0.04 mmol, 18% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.15 (1H, d, *J*= 8.0 Hz, C9-H), 7.51 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph2-H), 7.79 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph3-H), 7.87 (1H, t, *J*= 8.0 Hz, C8-H), 7.96 (1H, d, *J*= 7.0 Hz, C7-H), 8.85 (1H, s, C5-H), 11.35 (1H, s, N3-H); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{18}H_{10}ClF_3N_3O_2^+$ 392.0408, found 392.0423.

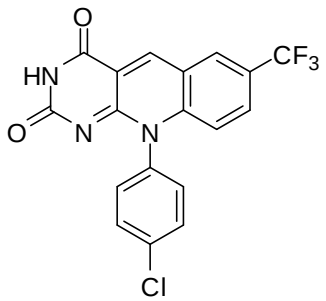
[b]10-(4-Chlorophenyl)-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 48, (40.3 mg, 0.10 mmol, 40% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.15 (1H, d, *J*= 8.0 Hz, C9-H), 7.51 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph2-H), 7.79 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph3-H), 7.87 (1H, t, *J*= 8.0 Hz, C8-H), 7.96 (1H, d, *J*= 8.0 Hz, C7-H), 8.85 (1H, s, C5-H), 11.35 (1H, s, N3-H); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{18}H_{10}ClF_3N_3O_2^+$ 392.0408, found 392.0429.

[b] microwaved for 20 min at 160 ^{O}C.

[c]10-(4-Chlorophenyl)-6-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 48, (40.3 mg, 0.10 mmol, 40% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.16 (1H, d, *J*= 8.0 Hz, C9-H), 7.52 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph2-H), 7.80 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph3-H), 7.88 (1H, t, *J*= 8.0 Hz, C8-H), 7.97 (1H, d, *J*= 8.0 Hz, C7-H), 8.86 (1H, s, C5-H), 11.35 (1H, s, N3-H); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{18}H_{10}ClF_3N_3O_2^+$ 392.0408, found 392.0440.

[c] microwaved for 10 min at 160 ^{O}C.

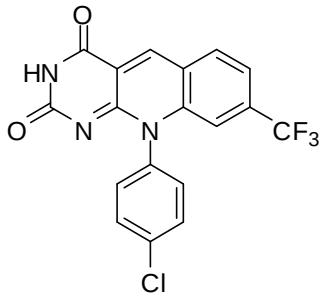
10-(4-Chlorophenyl)-7-trifluoromethlypyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 49, (52.3 mg, 0.13 mmol, 53% yield) as a yellow solid with a m.pt. of 342-343 ^{O}C (lit 342-343 ^{O}C) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.96 (1H, d, *J*= 9.4 Hz, C9-H), 7.50-7.54 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph2-H), 7.77-7.82 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph3-H), 7.99 (1H, d, *J*= 9.4 Hz, C8-H), 8.73 (1H, s, C6-H), 9.22 (1H, s, C5-H), 11.24 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 117.55 (CH), 118.92 (CH), 121.09 (Cq), 124.94 (Cq), 125.56 (Cq), 129.38 (CH), 130.90 (CH), 130.96 (CH), 134.82 (Cq), 136.68 (Cq), 142.58 (CH), 144.13 (Cq), 156.73 (Cq), 159.78 (Cq), 162.01 (Cq); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{18}H_{10}ClF_3N_3O_2^+$ 392.0408, found 392.0416; IR (KBr): 3418 (NH), 1702 (C=O), 1685 (C=O), 1621 (C=C) cm^{-1}; Anal. HPLC t_R 7.15 min (97.5% pure - System A), 9.10 min (100.0% pure - System B).



49

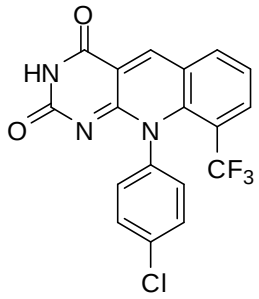
10-(4-Chlorophenyl)-8-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 50, (70.80 mg, 0.18 mmol, 51% yield) as a yellow solid with a m.pt. of 348-349 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.89 (1H, s, C9-H), 7.54 (2H, d, AA'BB' system, *J*= 8.7 Hz, Ph2-H), 7.81(2H, d, AA'BB' system, *J*= 8.7 Hz, Ph3-H), 7.86 (1H, d, *J*= 8.2 Hz, C7-H), 8.49 (1H, d, *J*= 8.2 Hz, C6-H), 9.22 (1H, s, C5-H), 11.28 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 118.40 (CH), 120.69 (CH), 120.72 (CH), 122.36

(Cq), 123.96 (Cq), 130.94 (CH), 131.06 (CH), 133.59 (CH), 134.92 (Cq), 136.31 (Cq), 141.79 (Cq), 141.94 (CH), 156.67 (Cq), 159.58 (Cq), 161.97 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{10}ClF_3N_3O_2^+$ 392.0408, found 392.0374; IR (KBr): 3430 (NH), 1716 (C=O), 1683 (C=O), 1650 (C=C) cm^{-1}; Anal. HPLC t_R 6.18 min (100.0% pure - System A), 8.60 min (97.4% pure - System B).



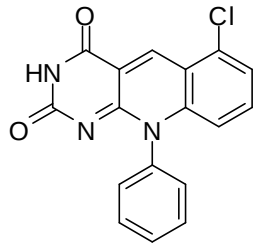
50

10-(4-Chlorophenyl)-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 51, (28.3 mg, 0.07 mmol, 28% yield) as a yellow solid with a m.pt. of 372-375 ^{O}C; ^1H NMR (400 MHz, DMSO-d_6): δ/ppm 7.39 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph2-H), 7.56 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph3-H), 7.67 (1H, t, *J*= 7.7 Hz, C7-H), 8.20 (1H, dd, ^4J= 1.3 Hz, ^3J= 7.7 Hz, C8-H), 8.49 (1H, dd, ^4J= 1.3 Hz, ^3J= 7.7 Hz, C6-H), 9.11 (1H, s, C5-H), 11.25 (1H, s, N3-H); ^{13}C NMR (75MHz, DMSO-d_6): δ/ppm 116.76 (Cq), 118.70 (Cq), 124.38 (Cq), 124.98 (CH), 128.57 (CH), 133.16 (CH), 134.23 (Cq), 135.60 (CH), 137.03 (CH), 139.24 (Cq), 140.15 (Cq), 143.47 (CH), 156.69 (Cq), 161.14 (Cq), 161.97 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{10}ClF_3N_3O_2^+$ 392.0408, found 392.0416; IR (KBr): 3483 (NH), 1715 (C=O), 1659 (C=O), 1618 (C=C) cm^{-1}; Anal. HPLC t_R 4.37 min (100.0% pure - System A), 7.17 min (98.2% pure - System B).



51

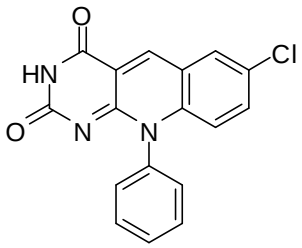
10-Phenyl-6-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 28, (19.7 mg, 0.06 mmol, 20% yield) as a yellow solid with a m.pt. of 339-341 ^{O}C (lit 338-340 ^{O}C dec) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.69 (1H, dd, ^{4}J= 1.2 Hz, ^{3}J= 7.8 Hz, C9-H), 7.43 (2H, d, *J*= 7.3 Hz, Ph2-H), 7.62-7.74 (5H, m, C8-H & C7-H & Ph-H), 9.03 (1H, s, C5-H), 11.25 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 117.23 (Cq), 117.27 (CH), 118.98 (Cq), 125.35 (CH), 128.82 (CH), 130.03 (CH), 130.79 (CH), 134.24 (Cq), 135.64 (CH), 137.02 (CH), 138.05 (Cq), 143.66 (Cq), 156.77 (Cq), 159.03 (Cq), 162.07 (Cq); HRMS (ESI^{+}): m/z [M + H]$^{+}$ calcd for $C_{17}H_{11}ClN_3O_2^{+}$ 324.0534, found 324.0518; IR (KBr): 3419 (NH), 1707 (C=O), 1674 (C=O), 1613 (C=C) cm^{-1}; Anal. HPLC t_R 1.49 min (98.4% pure - System A), 4.52 min (96.3% pure - ystem B).



28

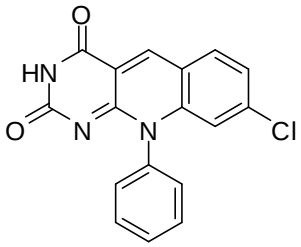
10-Phenyl-7-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 29, (65 mg, 0.20 mmol, 65% yield) as a yellow solid with a m.pt. of 295-296^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.70 (1H, d, *J*= 9.1 Hz, C9-H), 7.43 (2H, d, *J*= 7.3 Hz, Ph2-H), 7.61-7.72 (3H, m, Ph-H), 7.75 (1H, dd, ^{4}J= 2.5 Hz,

^{3}J= 9.1 Hz, C8-H), 8.37 (1H, d, ^{4}J= 2.5 Hz, C6-H), 9.09 (1H, s, C5-H), 11.16 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 117.24 (Cq), 119.64 (CH), 122.47 (Cq), 128.80 (CH), 128.87 (Cq), 129.97 (CH), 130.23 (CH), 130.79 (CH), 135.03 (CH), 137.92 (Cq), 141.05 (Cq), 141.72 (CH), 156.82 (Cq), 159.08 (Cq), 162.17 (Cq); HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for $C_{17}H_{11}ClN_3O_2^+$ 324.0534, found 358.0531; IR (KBr): 3420 (NH), 1702 (C=O), 1663 (C=O), 1614 (C=C) cm^{-1}; Anal. HPLC t_R 1.85 min (97.5% pure - System A), 4.97 min (System B).



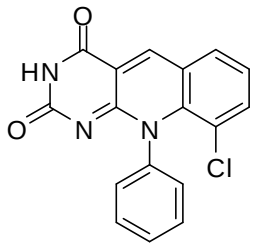
29

10-Phenyl-8-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 30, (35.5 mg, 0.10 mmol, 36% yield) as a yellow solid with a m.pt. of >350 ^{O}C (lit >360 ^{O}C) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.57 (1H, s, C9-H), 7.45 (2H, d, *J*= 7.1 Hz, Ph2-H), 7.58 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 8.6 Hz, C7-H), 7.63-7.74 (3H, m, Ph-H), 8.27 (1H, d, *J*= 8.6 Hz, C6-H), 9.13 (1H, s, C5-H), 11.16 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.43 (Cq), 116.68 (CH), 120.30 (Cq), 125.21 (CH), 128.81 (CH), 130.13 (CH), 130.89 (Cq), 133.71 (CH), 137.67 (Cq), 139.84 (Cq), 142.20 (CH), 142.98 (Cq), 156.82 (Cq), 159.33 (Cq), 162.20 (Cq); HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for $C_{17}H_{11}ClN_3O_2^+$ 324.0534, found 324.0565; IR (KBr): 3429 (NH), 1702 (C=O), 1663 (C=O), 1608 (C=C) cm^{-1}; Anal. HPLC t_R 1.68 min (96.2% pure - System A), 4.82 min (97.5% pure - System B).



30

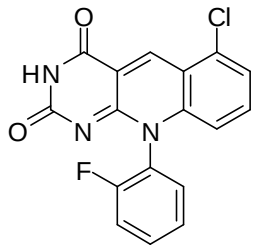
10-Phenyl-9-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 31, (38.8 mg, 0.12 mmol, 39% yield) as a yellow solid with a m.pt. >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.37 (2H, m, Ph2-H), 7.45-7.54 (3H, m, Ph-H), 7.48 (1H, t, *J*= 7.8 Hz, C7-H), 7.83 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.8 Hz, C8-H), 8.23 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.8 Hz, C6-H), 9.10 (1H, s, C5-H), 11.18 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.42 (Cq), 121.22 (Cq), 124.57 (Cq), 125.70 (CH), 129.10 (CH), 129.45 (CH), 130.33 (CH), 132.34 (CH), 137.67 (Cq), 139.26 (CH), 139.62 (Cq), 143.26 (CH), 156.75 (Cq), 160.75 (Cq), 162.08 (Cq); HRMS (ESI^{+}): m/z [M + H]$^{+}$ calcd for $C_{17}H_{11}ClN_3O_2^{+}$ 324.0534, found 324.0513; IR (KBr): 3441 (NH), 1717 (C=O), 1659 (C=O), 1615 (C=C) cm^{-1}; Anal. HPLC t_R 1.53 min (97.2% pure - System A), 4.59 min (100.0% pure - System B).



31

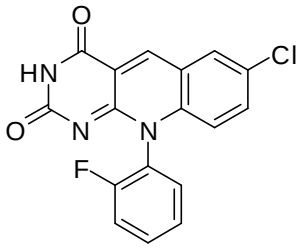
10-(2-Fluorophenyl)-6-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 32, (28.0 mg, 0.08 mmol, 28% yield) as a yellow solid with a m.pt. of 322-323 ^{O}C (lit 323-324 ^{O}C) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.82 (1H, d, *J*= 8.4 Hz, C9-H), 7.51-7.66 (3H, m), 7.70-7.79 (3H, m), 9.04 (1H, s, C5-H), 11.34 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.44 (CH),

117.05 (Cq), 117.75 (CH, d, *J*= 18.66 Hz), 118.98 (Cq), 124.84 (Cq, d, *J*= 12.92 Hz), 125.85 (CH), 126.71 (CH, d, *J*= 3.63 Hz), 130.90 (CH), 132.81 (CH, d, *J*= 7.51 Hz), 134.61 (Cq), 136.31 (CH), 137.61 (CH), 142.77 (Cq), 156.65 (Cq), 157.44 (Cq, d, *J*= 249.89 Hz), 158.74 (Cq), 161.81 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{10}ClFN_3O_2^+$ 342.0440, found 342.0414; IR (KBr): 3464 (NH), 1710 (C=O), 1678 (C=O), 1620 (C=C) cm^{-1}; Anal. HPLC t_R 1.70 min (100.0% pure - System A), 4.89 min (96.8% pure - System B).



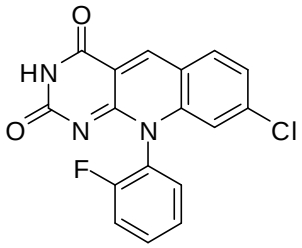
32

10-(2-Fluorophenyl)-7-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 33, (79.1 mg, 0.23 mmol, 79% yield) as a yellow solid with a m.pt. of 329-330 ^{O}C; ^1H NMR (400 MHz, DMSO-d_6): δ/ppm 6.84 (1H, d, *J*= 9.1 Hz, C9-H), 7.51-7.66 (3H, m, Ph-H), 7.71-7.77 (1H, m, Ph-H), 7.80 (1H, dd, ^3J= 2.5 Hz, ^4J= 9.1 Hz, C8-H), 8.41 (1H, d, ^4J= 2.5 Hz, C6-H), 9.12 (1H, s, C5-H), 11.26 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 117.05 (CH), 117.75 (CH, d, *J*= 19.07 Hz), 118.85 (CH), 122.46 (Cq), 124.72 (Cq, d, *J*= 13.64 Hz), 126.68 (CH), 129.30 (Cq), 130.67 (CH), 130.93 (CH), 132.75 (CH, d, *J*= 7.47 Hz), 135.54 (CH), 140.08 (Cq), 142.25 (CH), 157.72 (Cq), 157.57 (Cq, d, *J*= 251.58 Hz), 158.79 (Cq), 161.92 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{10}ClFN_3O_2^+$ 342.0440, found 342.0399; IR (KBr): 3427 (NH), 1707 (C=O), 1652 (C=O), 1615 (C=C) cm^{-1}; Anal. HPLC t_R 2.05 min (100.0% pure - System A), 5.34 min (98.6% pure - System B).



33

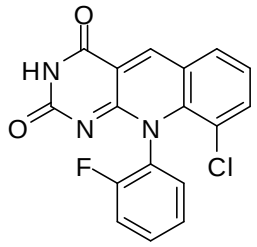
10-(2-Fluorophenyl)-8-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 34, (48.3 mg, 0.14 mmol, 48% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.73 (1H, s, C9-H), 7.55 (1H, t, *J*= 7.4 Hz, Ph5-H), 7.61-7.67 (3H, m, C7-H & Ph-H), 7.72-7.79 (1H, m, Ph-H), 8.31 (1H, d, *J*= 8.5 Hz, C6-H), 9.17 (1H, s, C5-H), 11.25 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 115.90 (CH), 116.21 (Cq), 117.86 (CH, d, *J*= 19.01 Hz), 120.29 (Cq), 124.43 (Cq, d, *J*= 13.26 Hz), 125.74 (CH), 126.75 (CH), 130.88 (CH), 132.90 (CH, d, *J*= 8.01 Hz), 134.07 (CH), 140.49 (Cq), 142.11 (Cq), 142.78 (CH), 156.75 (Cq), 157.32 (Cq, d, *J*= 250.76 Hz), 159.04 (Cq), 161.96 (Cq); HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for $C_{17}H_{10}ClFN_3O_2^+$ 342.0440, found 342.0467; IR (KBr): 3432 (NH), 1700 (C=O), 1668 (C=O), 1608 (C=C) cm^{-1}; Anal. HPLC t_R 1.93 min (98.4% pure - System A), 5.10 min (97.3% pure - System B).



34

10-(2-Fluorophenyl)-8-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 35, (12.4 mg, 0.03 mmol, 12% yield) as a yellow solid with a m.pt. of 298-300 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.34 (1H, t, *J*= 7.55 Hz, Ph5-H), 7.41-7.48 (2H, m, Ph-H), 7.52 (1H, t, *J*= 8.0 Hz, C7-H), 7.58-7.65 (1H, m, Ph-

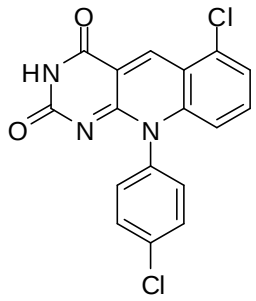
H), 7.88 (1H, dd, ^{4}J= 1.2 Hz, ^{3}J= 8.0 Hz, C8-H), 8.26 (1H, dd, ^{4}J= 1.2 Hz, ^{3}J= 8.0 Hz, C6-H), 9.12 (1H, s, C5-H), 11.27 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.27 (CH, d, *J*= 19.36 Hz), 116.31 (Cq), 120.66 (Cq), 124.56 (Cq), 125.32 (CH, d, *J*= 2.72 Hz), 126.07 (CH), 127.30 (Cq, d, *J*= 13.69 Hz), 131.24 (CH), 132.23 (CH, d, *J*= 7.90 Hz), 132.61 (CH), 137.19 (Cq), 139.20 (CH), 143.65 (CH), 156.65 (Cq), 158.98 (Cq, d, *J*= 248.75 Hz), 160.30 (Cq), 161.86 (Cq); HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for $C_{17}H_{10}ClFN_3O_2^+$ 342.0440, found 342.0446; IR (KBr): 3423(NH), 1709 (C=O), 1678 (C=O), 1619 (C=C) cm^{-1}; Anal. HPLC t_R 1.80 min (96.8% pure - System A), 4.95 min (99.1% pure - System B).



35

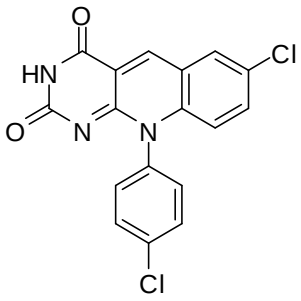
10-(4-Chlorophenyl)-6-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 36, (37.6 mg, 0.10 mmol, 38% yield) as a yellow solid with a m.pt. of 331-332^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): 6.77 (1H, dd, ^{4}J= 2.3 Hz, ^{3}J= 7.1 Hz, C9-H), 7.48 (2H, d, AA'BB' system, *J*= 8.7 Hz, Ph2-H), 7.68-7.71 (2H, m, C7-H & C8-H), 7.78 (2H, d, AA'BB' system, *J*= 8.7 Hz, Ph3-H), 9.01 (1H, s, C5-H), 11.27 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 117.22 (Cq), 117.30 (CH), 118.97 (Cq), 125.42 (CH), 130.86 (CH), 130.90 (CH), 134.25 (Cq), 134.72 (Cq), 135.78 (CH), 136.85 (Cq), 137.08 (CH), 143.50 (Cq), 156.69 (Cq), 159.08 (Cq), 162.04 (Cq); HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for $C_{17}H_{10}Cl_2N_3O_2^+$ 358.0145, found 358.0155; IR (KBr): 3422 (NH), 1703 (C=O),

1672 (C=O), 1607 (C=C) cm^{-1}; Anal. HPLC t_R 2.50 min (99.6% pure - System A), 6.05 min (100.0% pure - System B).



36

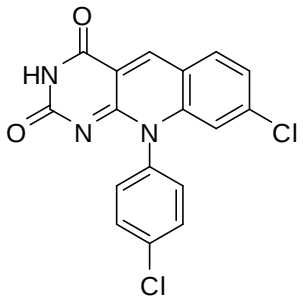
10-(4-Chlorophenyl)-7-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 37, (47.3 mg, 0.13 mmol, 47% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.80 (1H, d, *J*= 9.0 Hz, C9-H), 7.47-7.53 (2H, d, AA'BB' system, *J*= 8.4 Hz, Ph2-H), 7.72-7.79 (3H, m, C8-H & Ph3-H), 8.38 (1H, d, ^{4}J= 2.1 Hz, C6-H), 9.09 (1H, s, C5-H), 11.19 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 117.21 (Cq), 119.64 (CH), 122.48 (Cq), 128.97 (Cq), 130.28 (CH), 130.86 (CH), 130.90 (CH), 134.67 (Cq), 135.04 (CH), 136.72 (Cq), 140.80 (Cq), 141.79 (CH), 156.67 (Cq), 159.11 (Cq), 162.01 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{10}Cl_2N_3O_2^+$ 358.0145, found 358.0152; IR (KBr): 3406 (NH), 1704 (C=O), 1665 (C=O), 1615 (C=C) cm^{-1}; Anal. HPLC t_R 3.42 min (100.0% pure - System A), 6.90 min (98.6% pure - System B).



37

10-(4-Chlorophenyl)-8-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 38, (22.7 mg, 0.06 mmol, 23% yield) as a yellow solid with a m.pt. of >350 ^{O}C

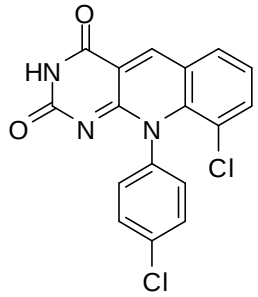
(lit >300 ^{O}C) [264]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.70 (1H, d, ^{4}J= 1.7 Hz, C9-H), 7.51 (2H, d, AA'BB' system, J= 8.6 Hz, Ph2-H), 7.60 (1H, dd, ^{4}J= 1.7 Hz, ^{3}J= 8.6 Hz, C7-H), 7.78 (2H, d, AA'BB' system, J= 8.6 Hz, Ph3-H), 8.28 (1H, d, J= 8.6 Hz, C6-H), 9.13 (1H, s, C5-H), 11.61 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.39 (Cq), 116.65 (CH), 120.32 (Cq), 125.37 (CH), 130.90 (CH), 131.02 (CH), 133.74 (CH), 134.78 (Cq), 136.44 (Cq), 140.05 (Cq), 142.29 (CH), 142.81 (Cq), 156.74 (Cq), 159.45 (Cq), 162.16 (Cq); HRMS (ESI^{+}): m/z [M + H]$^{+}$ calcd for $C_{17}H_{10}Cl_2N_3O_2^{+}$ 358.0145, found 358.0146; IR (KBr): 3432 (NH), 1701 (C=O), 1664 (C=O), 1609 (C=C) cm^{-1}; Anal. HPLC t_R 3.54 min (100.0% pure - System A), 6.82 min (100.0% pure - System B).



38

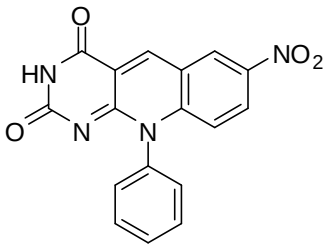
10-(4-Chlorophenyl)-9-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 39, (14.6 mg, 0.04 mmol, 15% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.43-7.52 (3H, m, C7-H & Ph2-H), 7.60 (2H, d, AA'BB' system, J= 8.8 Hz, Ph3-H), 7.85 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.9 Hz, C8-H), 8.28 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.9 Hz, C6-H), 9.09 (1H, s, C5-H), 11.22 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.48 (Cq), 121.00 (Cq), 124.59 (Cq), 125.77 (CH), 129.18 (CH), 132.22 (CH), 132.45 (CH), 134.12 (Cq), 137.53 (Cq), 138.57 (Cq), 139.25 (CH), 143.33 (CH), 156.63 (Cq), 160.81 (Cq), 162.04 (Cq); HRMS (ESI^{+}): m/z [M + H]$^{+}$

calcd for $C_{17}H_{10}Cl_2N_3O_2^+$ 358.0145, found 358.0156; IR (KBr): 3415 (NH), 1715 (C=O), 1655 (C=O), 1613 (C=C) cm^{-1}; Anal. HPLC t_R 3.10 min (100.0% pure - System A), 6.34 min (97.2% pure - System B).



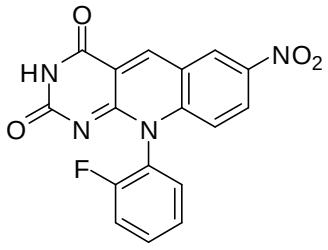
39

10-Phenyl-7-nitropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 17, (66.1 mg, 0.19 mmol, 66% yield) as a yellow solid with a m.pt. of >350 ^{O}C (lit >360 ^{O}C) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.76 (1H, d, *J*= 9.4 Hz, C9-H), 7.36 (2H, d, *J*= 8.0 Hz, Ph2-H), 7.53-7.65 (3H, m, Ph-H), 8.34 (1H, dd, ^{4}J= 2.6 Hz, ^{3}J= 9.4 Hz, C8-H), 9.13 (1H, d, ^{4}J= 2.6 Hz, C6-H), 9.20 (1H, s, C5-H), 11.19 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 118.05 (CH), 119.00 (Cq), 120.92 (Cq), 127.68 (CH), 128.75 (CH), 128.85 (CH), 130.19 (CH), 130.92 (CH), 137.75 (Cq), 142.62 (CH), 143.37 (Cq), 145.60 (Cq), 156.78 (Cq), 159.98 (Cq), 161.90 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{11}N_4O_4^+$ 335.0775, found 335.0776; IR (KBr): 3447 (NH), 1720 (C=O), 1659 (C=O), 1613 (C=C), 1521 (NO_2), 1357 (NO_2) cm^{-1}; Anal. HPLC t_R 0.92 min (99.1% pure - System A), 4.37 min (98.6% pure - System B).



17

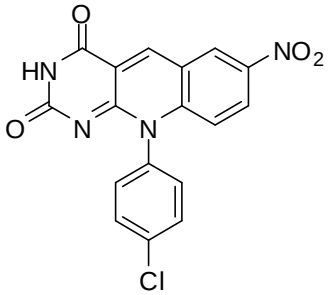
10-(2-Fluorophenyl)-7-nitropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 21, (88.9 mg, 0.25 mmol, 89% yield) as a yellow solid with a m.pt. of 326-327 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.98 (1H, d, *J*= 9.4 Hz, C9-H), 7.53-7.69 (3H, m, Ph-H), 7.73-7.80 (1H, m, Ph-H), 8.48 (1H, dd, ^{4}J= 2.3 Hz, ^{3}J= 9.4 Hz, C8-H), 9.25 (1H, d, ^{4}J= 2.3 Hz, C6-H), 9.33 (1H, s, C5-H), 11.38 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 117.82 (CH, d, *J*= 19.02 Hz), 117.86 (Cq), 118.26 (CH), 120.99 (Cq), 124.54 (Cq, d, *J*= 13.34 Hz), 126.81 (CH, d, *J*= 3.56 Hz), 127.98 (CH), 129.44 (CH), 130.85 (CH), 132.99 (CH, d, *J*= 7.85 Hz), 143.07 (CH), 143.79 (Cq), 144.72 (Cq), 156.65 (Cq), 157.56 (Cq, d, *J*= 242.62 Hz), 159.58 (Cq), 161.65 (Cq); HRMS (ESI^{+}): m/z $[M + H]^{+}$ calcd for $C_{17}H_{10}FN_4O_4^{+}$ 353.0681, found 353.0671; IR (KBr): 3413 (NH), 1711 (C=O), 1685 (C=O), 1616 (C=C), 1488 (NO_2), 1309 (NO_2) cm^{-1}; Anal. HPLC t_R 1.49 min (100.0% pure - System A), 4.60 min (97.2% pure - System B).



21

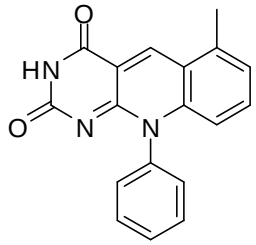
10-(4-Chlorophenyl)-7-nitropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 25, (49.8 mg, 0.13 mmol, 50% yield) as a yellow solid with a m.pt. of 343-344 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.95 (1H, d, *J*= 9.5 Hz, C9-H), 7.52 (2H, d, AA'BB' system, *J*= 8.8 Hz, Ph2-H), 7.80 (2H, d, AA'BB' system, *J*= 8.8 Hz, Ph3-H), 8.42 (1H, dd, ^{4}J= 2.3 Hz, ^{3}J= 9.5 Hz, C8-H), 9.22 (1H, d, ^{4}J= 2.3 Hz, C6-H), 9.30 (1H, s, C5-H), 11.31 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 118.00 (Cq), 119.02 (CH), 120.96 (Cq), 127.66

(CH), 128.86 (CH), 130.82 (CH), 131.02 (CH), 134.88 (Cq), 136.56 (Cq), 142.69 (CH), 143.42 (Cq), 145.46 (Cq), 156.70 (Cq), 160.02 (Cq), 161.85 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{10}ClN_4O_4^+$ 369.0385, found 369.0403; IR (KBr): 3423 (NH), 1709 (C=O), 1665 (C=O), 1614 (C=C), 1543 (NO_2), 1335 (NO_2) cm^{-1}; Anal. HPLC t_R 0.90 min (97.3% pure - System A), 5.80 min (100.0% pure - System B).



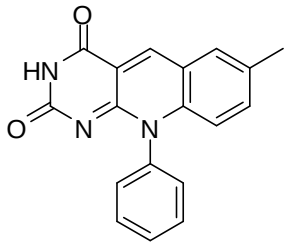
25

10-Phenyl-6-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 52, (382.9 mg, 1.26 mmol, 48% yield) as a yellow solid with a m.pt. of 342-344 ^{O}C (dec); ^1H NMR (400 MHz, DMSO-d_6): δ/ppm 2.75 (3H, s, Me), 6.55 (1H, d, *J*= 8.8 Hz, C9-H), 7.33-7.43 (3H, m, C7-H & Ph2-H), 7.57-7.72 (4H, m, C8-H & Ph-H), 9.02 (1H, s, C5-H), 11.09 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 18.15 (CH), 114.85 (Cq), 115.51 (CH), 119.88 (Cq), 125.61 (CH), 128.41 (CH), 129.34 (CH), 130.24 (CH), 134.87 (CH), 137.96 (Cq), 138.45 (CH), 139.15 (Cq), 142.42 (Cq), 156.42 (Cq), 158.30 (Cq), 162.06 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{14}N_3O_2^+$ 304.1081, found 304.1085; IR (KBr): 3371 (NH), 1700 (C=O), 1651 (C=O), 1598 (C=C) cm^{-1}; Anal. HPLC t_R 1.18 min (98.8% pure - System A), 4.03 min (97.7% pure - System B).



52

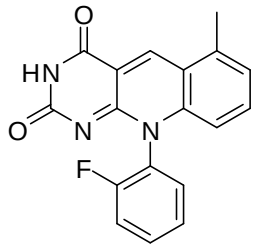
10-Phenyl-7-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 53, (54.8 mg, 0.18 mmol, 55% yield) as a yellow solid with a m.pt. of 342-344 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.40 (3H, s, Me), 6.62 (1H, d, *J*= 8.8 Hz, C9-H), 7.41 (2H, d, *J*= 7.7 Hz, Ph2-H), 7.57 (1H, dd, ^{4}J= 1.74 Hz, ^{3}J= 8.8 Hz, C8-H), 7.60-7.72 (3H, m, Ph-H), 8.00 (1H, s, C6-H), 9.02 (1H, s, C5-H). 11.04 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 20.64 (CH_3), 115.99 (Cq), 117.50 (CH), 121.41 (Cq), 128.84 (CH), 129.77 (CH), 130.66 (CH), 130.84 (CH), 134.41 (Cq), 136.95 (CH), 138.18 (Cq), 140.46 (Cq), 142.51 (CH), 158.74 (Cq), 158.74 (Cq), 162.41 (Cq); HRMS (ESI^{+}): m/z [M + H]$^{+}$ calcd for $C_{18}H_{14}N_3O_2^{+}$ 304.1081, found 304.0836; IR (KBr): 3437 (NH), 1703 (C=O), 1649 (C=O), 1609 (C=C) cm^{-1}; Anal. HPLC t_R 1.37 min (96.2% pure - System A), 4.24 min (96.8% pure - System B).



53

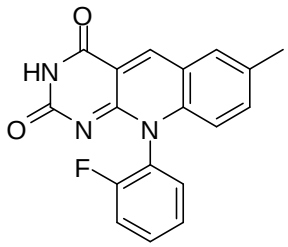
10-(2-Fluorophenyl)-6-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 56, (129.6 mg, 0.40 mmol, 16% yield) as a yellow solid with a m.pt. of 309-310 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): 2.76 (3H, s, Me), 6.66 (1H, d, *J*= 8.0 Hz, C9-H), 7.40 (1H, d, *J*= 8.0 Hz, C7-H), 7.49-7.76 (5H, m, C8-H & Ph-H), 9.05 (1H, s, C5-H), 11.19 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6):

δ/ppm 19.04 (CH_3), 115.01 (CH), 115.06 (Cq), 117.66 (CH, d, *J*= 19.01 Hz), 120.31 (Cq), 125.26 (Cq, d, *J*= 13.60 Hz), 126.11 (CH, d, *J*= 3.51 Hz), 126.53 (CH), 130.98 (CH), 132.54 (CH, d, *J*= 8.10 Hz), 135.95 (CH), 139.63 (CH), 140.17 (Cq), 142.02 (Cq), 156.81 (Cq), 157.43 (Cq, d, *J*= 247.76 Hz), 158.55 (Cq), 162.24 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{13}FN_3O_2^+$ 322.0986, found 322.0978; IR (KBr): 3467 (NH), 1702 (C=O), 1653 (C=O), 1597 (C=C) cm^{-1}; Anal. HPLC t_R 1.34 min (100.0% pure - System A), 4.28 min (99.0% pure - System B).



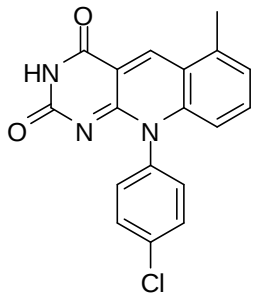
56

10-(2-Fluorophenyl)-7-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 57, (67.9 mg, 0.21 mmol, 68% yield) as a yellow solid with a m.pt. of 317-319 ^{O}C; ^1H NMR (400 MHz, DMSO-d_6): δ/ppm 2.42 (3H, s, Me), 6.74 (1H, d, *J*= 8.7 Hz, C9-H), 7.49-7.65 (4H, m, C8-H & Ph-H), 7.69-7.76 (1H, m, Ph-H), 8.04 (1H, s, C6-H), 9.07 (1H, s, C5-H), 11.14 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 20.63 (CH_3), 115.86 (Cq), 116.65 (CH), 117.65 (CH, d, *J*= 18.79 Hz), 121.42 (Cq), 125.06 (Cq, d, *J*= 13.71 Hz), 126.56 (CH, d, *J*= 3.05 Hz), 130.98 (CH), 131.26 (CH), 132.50 (CH, d, *J*= 6.38 Hz), 134.95 (Cq), 137.50 (CH), 139.62 (Cq), 143.12 (CH), 156.77 (Cq), 157.61 (Cq, d, *J*= 248.01 Hz), 158.59 (Cq), 162.17 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{13}FN_3O_2^+$ 322.0986, found 322.0960; IR (KBr): 3423 (NH), 1705 (C=O), 1657 (C=O), 1609 (C=C) cm^{-1}; Anal. HPLC t_R 1.53 min (98.1% pure - System A), 4.49 min (100.0% pure - System B).



57

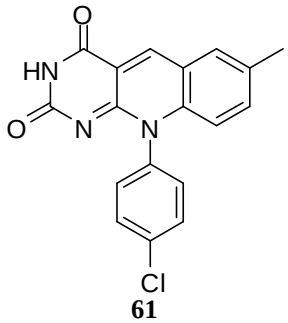
10-(4-Chlorophenyl)-6-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 60, (301.8 mg, 0.89 mmol, 30% yield) as a yellow solid with a m.pt. of 339-341 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.75 (3H, s, Me), 6.62 (1H, d, *J*= 7.9 Hz, C9-H), 7.37 (1H, d, *J*= 7.9 Hz, C7-H), 7.47 (2H, d, AA'BB' system, *J*= 8.7 Hz, Ph2-H), 7.62 (1H, t, *J*= 7.9 Hz, C8-H), 7.76 (2H, d, AA'BB' system, *J*= 8.7 Hz, Ph3-H), 9.02 (1H, s, C5-H), 11.11 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 19.01 (CH_3), 115.30 (Cq), 115.93 (CH), 120.33 (Cq), 126.15 (CH), 130.79 (CH), 130.93 (CH), 134.47 (Cq), 135.45 (CH), 137.23 (Cq), 139.01 (CH), 139.68 (Cq), 142.68 (Cq), 156.79 (Cq), 158.82 (Cq), 162.46 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{13}ClN_3O_2^+$ 338.0691, found 338.0702; IR (KBr): 3424 (NH), 1701 (C=O), 1674 (C=O), 1599 (C=C) cm^{-1}; Anal. HPLC t_R 2.00 min (97.2% pure - System A), 5.37 min (98.2% pure - System B).



60

10-(4-Chlorophenyl)-7-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 61, (47.6 mg, 0.14 mmol, 48% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.49 (3H, s, Me), 6.70 (1H,

d, *J*= 8.8 Hz, C9-H), 7.47 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph2-H), 7.58 (1H, dd, ^{4}J= 1.9 Hz, ^{3}J= 8.8 Hz, C8-H), 7.76 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph3-H), 8.01 (1H, s, C6-H), 9.03 (1H, s, C5-H), 11.07 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 20.68 (CH_3), 116.01 (Cq), 117.48 (CH), 121.44 (Cq), 130.23 (Cq), 130.76 (CH), 130.93 (CH), 134.46 (Cq), 134.49 (Cq), 137.02 (CH), 137.04 (CH), 140.28 (Cq), 142.61 (CH), 156.74 (Cq), 158.85 (Cq), 162.36 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{13}ClN_3O_2^+$ 338.0691, found 338.0670; IR (KBr): 3434 (NH), 1701 (C=O), 1668 (C=O), 1608 (C=C) cm^{-1}; Anal. HPLC t_R 2.50 min (97.4% pure - System A), 5.74 min (99.1% pure - System B).

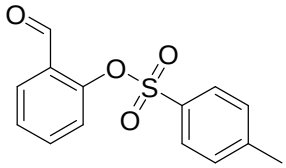


61

Synthesis of 2-*p*-Toluenesulfonyloxybenzaldehyde, 78 [301-303]

A mixture of salicylaldehyde (0.88 g, 0.76mL; 7.23 mmol,) and sodium carbonate (3.06 g; 28.95 mmol), in acetone (20 mL) was stirred at room temperature for 30 min under nitrogen atmosphere. Tosyl chloride (2.75 g; 14.47 mmol) dissolved in acetone (25 mL) was added to the reaction mixture and refluxed for 5 h. Concentration of the solution under reduced pressure and dry column flash chromatography using 66.6% petroleum ether and 33.3% diethyl ether was carried out to yield the pure 2-*p*-toluenesulfonyloxybenzaldehyde, **78**.

2-*p*-Toluenesulfonyloxybenzaldehyde, 78 (1.96 g, 7.09 mmol, 98% yield) as a white solid with a m.pt. of 66-68 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.42 (3H, s, Me), 7.18 (1H, dd, ^{4}J= 0.7 Hz, ^{3}J= 7.8 Hz, C6-H), 7.48 (2H, d, AA'BB' system, J= 8.0 Hz, Ph3-H), 7.52 (1H, ddd, ^{4}J= 1.2 Hz, ^{3}J= 7.8 Hz, C5-H), 7.71-7.75 (3H, m, C4-H & Ph2-H), 7.81 (1H, dd, ^{4}J= 1.2 Hz, ^{3}J= 7.6 Hz, C3-H), 9.94 (1H, s, COH); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.66 (CH_3), 124.14 (CH), 128.62 (CH), 128.88 (CH), 129.18 (CH), 129.44 (Cq), 130.84 (Cq), 130.90 (CH), 136.42 (CH), 147.00 (Cq), 150.51 (Cq), 187.92 (CH).



78

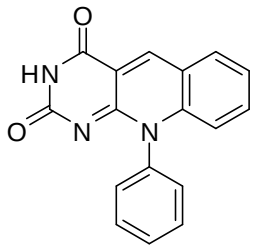
Synthesis of 10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 64, using 2-*p*-Toluenesulfonyloxybenzaldehyde, 75 [241, 261, 264, 265, 268, 270]

Method A

A mixture of 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **69** (70.2 mg; 0.34 mmol), and 2-*p*-toluenesulfonyloxybenzaldehyde, **78** (114.64 mg; 0.41 mmol), in DMF (3 mL) was heated to 160 ^{O}C for 30 min in the microwave reactor. Concentration of the solution under reduced pressure and dry column flash chromatography using 95% dichloromethane and 5% methanol was carried out to yield the pure 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, **64**.

10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 64, (58.7 mg, 0.20 mmol, 59% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.71 (1H, d, J= 8.5 Hz, C9-H), 7.43 (2H, d, J= 7.1 Hz, Ph2-H), 7.50 (1H, t, J=

8.5 Hz, C8-H), 7.60-7.78 (4H, m, C7-H & Ph-H), 8.23 (1H, d, *J*= 8.5 Hz, C6-H), 9.12 (1H, s, C5-H), 11.06 (1H, s, N3-H); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{12}N_3O_2^+$ 290.0924, found 290.0931.



64

Method B

A mixture of 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **69** (70.2 mg; 0.34 mmol), and 2-p-toluenesulfonyloxybenzaldehyde, **78** (114.64 mg; 0.41 mmol), in DMF (3 mL) was heated to 160 ^{O}C for 1 h in the microwave reactor. Concentration of the solution under reduced pressure and dry column flash chromatography using 95% dichloromethane and 5% Methanol was carried out to yield the pure 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, **64**.

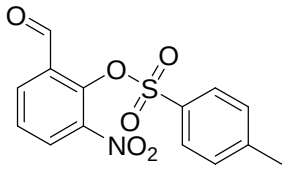
10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 64, (38.8 mg, 0.13 mmol, 39% yield) as a yellow solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.71 (1H, d, *J*= 8.5 Hz, C9-H), 7.43 (2H, d, *J*= 7.1 Hz, Ph2-H), 7.50 (1H, t, *J*= 8.5 Hz, C8-H), 7.60-7.76 (4H, m, C7-H & Ph-H), 8.23 (1H, d, *J*= 8.5 Hz, C6-H), 9.12 (1H, s, C5-H), 11.06 (1H, s, N3-H); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{12}N_3O_2^+$ 290.0924, found 290.0922.

General Procedure to Synthesise 2-*p*-Toluenesulfonyloxybenzaldehyde Analogues [301-303]

A mixture of 1 equivalent of the desired salicyladehyde and 4 equivalents of sodium carbonate, in acetone was stirred at room temperature for 30 min under

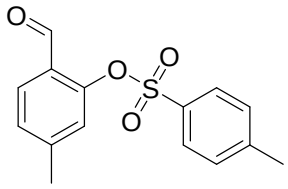
nitrogen atmosphere. 2 Equivalents of tosyl chloride dissolved in acetone was added to the reaction mixture and refluxed for 5 h. Concentration of the solution under reduced pressure and dry column flash chromatography using 66.6% petroleum ether and 33.3% diethyl ether was carried out to yield the pure desired 2-*p*-toluenesulfonyloxybenzaldehyde analogue.

2-*p*-Toluenesulfonyloxy-3-nitrobenzaldehyde, 79 (435.9 mg, 1.35 mmol, 54% yield) as a white solid with a m.pt. of 133-134 ^{O}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 2.50 (3H, s, Me), 7.40 (2H, d, AA'BB' system, *J*= 8.3 Hz, Ph3-H), 7.58 (1H, t, *J*= 8.0 Hz, C5-H), 7.78 (2H, d, AA'BB' system, *J*= 8.3 Hz, Ph2-H), 8.15-8.22 (2H, m, C6-H & C4-H), 10.10 (1H, s COH); ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 21.90 (CH_3), 127.86 (CH), 128.98 (CH), 130.33 (Cq), 130.45 (CH), 130.72 (CH), 132.38 (Cq), 133.03 (CH), 142.72 (Cq), 144.41 (Cq), 147.35 (Cq), 185.63 (CH).



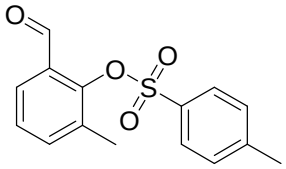
79

2-*p*-Toluenesulfonyloxy-4-methylbenzaldehyde, 80 (630.2 mg, 2.17 mmol, 59% yield) as a white solid with a m.pt. of 93-94 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.36 (3H, s, Me), 2.42 (3H, s, Me), 7.06 (1H, s, C3-H), 7.33 (1H, d, *J*= 7.8 Hz, C5-H), 7.48 (2H, d, AA'BB' system, *J*= 8.2 Hz, Ph3-H), 7.70 (1H, d, *J*= 7.8 Hz, C6-H), 7.76 (2H, d, AA'BB' system, *J*= 8.2 Hz, Ph2-H), 9.83 (1H, s, COH); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.65 (CH_3), 21.66 (CH_3), 124.44 (CH), 126.96 (Cq), 128.86 (CH), 128.96 (CH), 129.31 (CH), 130.85 (CH), 130.90 (Cq), 146.95 (Cq), 147.74 (Cq), 150.51 (Cq), 187.45 (CH).



80

2-*p*-Toluenesulfonyloxy-3-methylbenzaldehyde, 81 (1.04 g, 3.55 mmol, 98% yield) as a white solid with a m.pt. of 68-69 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.03 (3H, s, Me), 2.45 (3H, s, Me), 7.44 (1H, t, *J*= 7.63 Hz, C5-H), 7.52 (2H, d, AA'BB' system, *J*= 8.2 Hz, Ph3-H), 7.62-7.70 (2H, m, C4-H & C6-H), 7.79 (2H, d, AA'BB' system, *J*= 8.2 Hz, Ph2-H), 9.88 (1H, s, COH); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 16.11 (CH_3), 21.69 (CH_3), 126.63 (CH), 128.22 (CH), 128.78 (CH), 130.57 (Cq), 131.06 (CH), 131.46 (Cq), 133.56 (Cq), 138.13 (CH), 147.15 (Cq), 148.93 (Cq), 188.12 (CH).

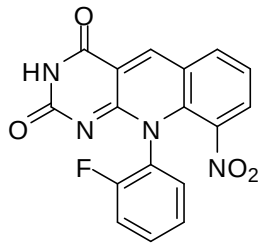


81

General Procedure to Synthesise 10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione Analogues using 2-*p*-Toluenesulfonyloxybenzaldehyde [241, 261, 264, 265, 268, 270]

To 1 equivalent of the desired 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione analogue in DMF was added 1.2 equivalents of the desired 2-p-toluenesulfonyloxybenzaldehyde. The mixture was heated at 160 ^{O}C for 30 min in the microwave reactor or refluxed for 4 h. Concentration of the solution under reduced pressure and separation by dry column flash chromatography using 95% dichloromethane and 5% methanol to yield the pure desired 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogue .

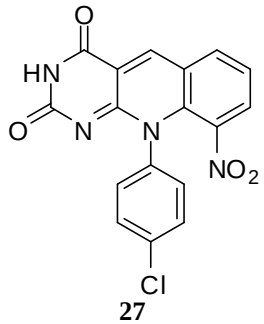
10-(2-Fluorophenyl)-9-nitropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 23, (23.0 mg, 0.06 mmol, 23% yield) as a yellow solid with a m.pt. of 305-307 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.25 (1H, t, *J*= 7.5 Hz, Ph5-H), 7.35-7.44 (2H, m, Ph-H), 7.56-7.74 (2H, m, C7-H & Ph-H), 8.17 (1H, d, *J*= 7.3 Hz, C8-H), 8.50 (1H, d, *J*= 7.3 Hz, C6-H), 9.19 (1H, s, C5-H), 11.36 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.72 (CH, d, *J*= 19.55 Hz), 117.13 (Cq), 124.05 (Cq), 125.11 (CH), 125.21 (Cq), 125.34 (Cq), 125.36 (CH), 130.03 (CH), 132.60 (CH), 133.12 (CH, d, *J*= 7.97 Hz), 136.17 (CH), 140.85 (Cq), 142.95 (CH), 156.53 (Cq), 158.52 (Cq, d, *J*= 252.37 Hz), 159.51 (Cq), 161.71 (Cq); HRMS (ESI^{+}): m/z $[M + H]^{+}$ calcd for $C_{17}H_{10}FN_4O_4^{+}$ 353.0681, found 353.0676; IR (KBr): 3425 (NH), 1712 (C=O), 1679 (C=O), 1619 (C=C), 1487 (NO_2), 1328 (NO_2) cm^{-1}; Anal. HPLC t_R 1.10 min (100.0% pure - System A), 3.98 min (97.6% pure - System B).



23

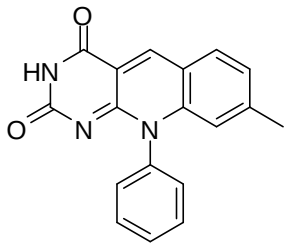
10-(4-Chlorophenyl)-9-nitropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 27 (23.8 mg, 0.06 mmol, 24% yield) as a yellow solid with a m.pt. of 336-338 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.41 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph2-H), 7.55 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph3-H), 7.64 (1H, t, *J*= 7.8 Hz, C7-H), 8.13 (1H, d, *J*= 7.8 Hz, C8-H), 8.48 (1H, d, *J*= 7.8 Hz, C6-H), 9.17 (1H, s, C5-H), 11.30 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 117.45 (Cq), 123.98 (Cq), 124.87 (CH), 129.21 (CH), 131.40 (CH), 132.03 (CH), 132.52 (Cq), 134,63 (Cq), 135.89 (CH), 136.24 (Cq), 141.09

(Cq), 142.62 (CH), 156.53 (Cq), 160.37 (Cq), 161.86 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{17}H_{10}ClN_4O_4^+$369.0385, found 369.0419; IR (KBr): 3422 (NH), 1706 (C=O), 1672 (C=O), 1620 (C=C), 1427 (NO_2), 1352 (NO_2) cm^{-1}; Anal. HPLC t_R 1.63 min (98.4% pure - System A), 4.75 min (100.0% pure - System B).



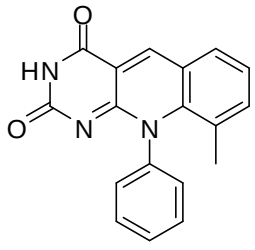
27

10-Phenyl-8-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 54, (64.7 mg, 0.21 mmol, 65% yield) as a yellow solid with a m.pt. of 299-301 ^{O}C; ^1H NMR (400 MHz, DMSO-d_6): δ/ppm 2.32 (3H, s, Me), 6.48 (1H, s, C9-H), 7.35 (1H, d, *J*= 8.1 Hz, C7-H), 7.41 (2H, d, *J*= 7.2 Hz, Ph2-H), 7.59-7.72 (3H, m, Ph-H), 8.12 (1H, d, *J*= 8.1 Hz, C6-H), 9.07 (1H, s, C5-H), 11.03 (1H, s, N3-H); ^{13}C NMR (75 MHz, , DMSO-d_6): δ/ppm 22.66 (CH_3), 115.06 (Cq), 117.05 (CH), 119.58 (Cq), 126.56 (CH), 128.88 (CH), 129.81 (CH), 130.71 (CH), 131.73 (CH), 138.10 (Cq), 142.45 (Cq), 142.67 (CH), 146.79 (Cq), 156.91 (Cq), 159,19 (Cq), 162.49 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{14}N_3O_2^+$ 304.1081, found 304.1021; IR (KBr): 3433 (NH), 1709 (C=O), 1672 (C=O), 1608 (C=C) cm^{-1}; Anal. HPLC t_R 1.20 min (100.0% pure - System A), 4.02 min (97.8% pure - System B).



54

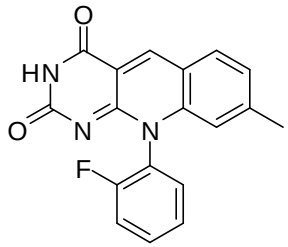
10-Phenyl-9-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 55, (37.8 mg, 0.12 mmol, 38% yield) as a yellow solid with a m.pt. of 337-338 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 1.59 (3H, s, Me), 7.39-7.47 (3H, m, C8-H & Ph2-H), 7.53-7.61 (4H, m, C7-H & Ph-H), 8.10 (1H, d, *J*= 7.9 Hz, C6-H), 9.07 (1H, s, C5-H), 11.07 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 22.34 (CH_3), 115.23 (Cq), 123.04 (Cq), 125.08 (CH), 127.13 (Cq), 129.24 (CH), 129.53 (CH), 130.64 (CH), 131.31 (CH), 140.55 (CH), 140.77 (Cq), 140.95 (Cq), 144.01 (CH), 156.84 (Cq), 160.62 (Cq), 162.35 (Cq); HRMS (ESI$^+$): m/z $[M + H]^+$ calcd for $C_{18}H_{14}N_3O_2^+$ 304.1081, found 304.1097; IR (KBr): 3441 (NH), 1703 (C=O), 1665 (C=O), 1619 (C=C) cm^{-1}; Anal. HPLC t_R 1.28 min (97.2% pure - System A), 14.40 min (97.9% pure - System B).



55

10-(2-Fluorophenyl)-8-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 58, (16.3 mg, 0.05 mmol, 16% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.37 (3H, s, Me), 6.60 (1H, s, C9-H), 7.39 (1H, d, *J*= 80 Hz, C7-H), 7,49-7.65 (3H, m, Ph-H), 7.69-7.77 (1H, m, Ph-H), 8.15 (1H, d, *J*= 8.0 Hz, C6-H), 9.10 (1H, s, C5-H), 11.10 (1H,

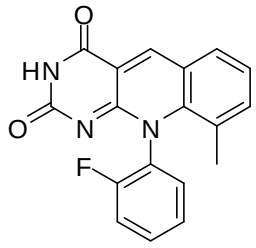
s, N3-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 114.17 (CH, d, *J*= 4.32 Hz), 118.07 (CH, d, *J*= 19.46 Hz), 119.12 (Cq), 122.01 (CH, d, *J*= 4.32 Hz), 123.48 (Cq), 124.73 (Cq, d, *J*= 12.96 Hz), 127.18 (CH, d, *J*= 4.62 Hz), 130.38 (CH), 133.26 (CH, d, *J*= 8.89 Hz), 134.23 (CH), 134.67 (Cq), 140.81 (Cq), 143.37 (CH), 156.84 (Cq), 158.78 (Cq, d, *J*= 263.14 Hz), 159.64 (Cq), 162.24 (Cq); HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{18}H_{13}FN_3O_2^+$ 322.0986, found 322.0995; IR (KBr): 3442 (NH), 1707 (C=O), 1673 (C=O), 1608 (C=C) cm^{-1}; Anal. HPLC t_R 1.34 min (100.0% pure - System A), 5.09 min (98.8% pure - System B).



58

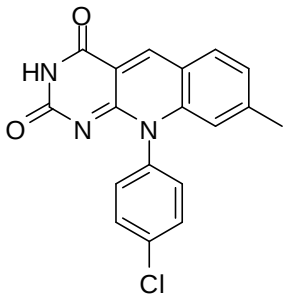
10-(2-Fluorophenyl)-9-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 59, (53.1 mg, 0.16 mmol, 53% yield) as a yellow solid with a m.pt. of 287-290 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 1.68 (3H, s, Me), 7.36-7.54 (4H, m, C8-H & Ph-H), 7.62-7.70 (2H, m, C7-H & Ph-H), 8.13 (1H, d, *J*= 7.4 Hz, C6-H), 9.10 (1H, s, C5-H), 11.16 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.37 (CH_3), 115.11 (Cq), 116.63 (CH, d, *J*= 19.5 6Hz), 123.03 (Cq), 125.40 (Cq), 125.50 (CH, d, *J*= 3.19 Hz), 126.28 (Cq), 128.52 (Cq, d, *J*= 13.26 Hz) 131.63 (CH), 131.84 (CH), 132.38 (CH, d, *J*= 8.08 Hz), 140.35 (Cq), 140.74 (CH), 144.51 (Cq), 156.81 (Cq), 158.84 (Cq, d, *J*= 249.14 Hz), 160.25 (Cq), 162.16 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{13}FN_3O_2^+$ 322.0986, found 322.0959; IR (KBr): 3442 (NH), 1712 (C=O), 1657 (C=O),

1619 (C=C) cm^{-1}; Anal. HPLC t_R 1.40 min (97.4% pure - System A), 15.64 min (96.8% pure - System B).



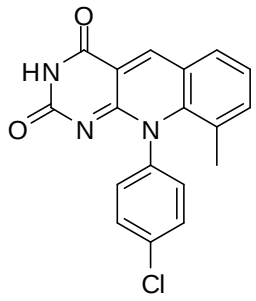
59

10-(4-Chlorophenyl)-8-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 62, (24.3 mg, 0.08 mmol, 24% yield) as a yellow solid with a m.pt. of 346-347 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.36 (3H, s, Me), 6.57 (1H, s, C9-H), 7.36 (1H, d, *J*= 8.1 Hz, C7-H), 7.47 (2H, d, AA'BB' system, *J*= 8.5 Hz, Ph2-H), 7.76 (2H, d, AA'BB' system, *J*= 8.5 Hz, Ph3-H), 8.12 (1H, d, *J*= 8.1 Hz, C6-H), 9.08 (1H, s, C5-H), 11.04 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 22.58 (CH_3), 115.06 (Cq), 117.06 (CH), 119.64 (Cq), 126.66 (CH), 130.84 (CH), 130.99 (CH), 131.79 (CH), 134.46 (Cq), 136.95 (Cq), 142.29 (CH), 142.81 (Cq), 147.11 (Cq), 156.80 (Cq), 159.33 (Cq), 162.47 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{13}ClN_3O_2^+$ 338.0691, found 338.0718; IR (KBr): 3441 (NH), 1694 (C=O), 1663 (C=O), 1602 (C=C) cm^{-1}; Anal. HPLC t_R 2.07 min (98.9% pure - System A), 8.40 min (99.8% pure - System B).



62

10-(4-Chlorophenyl)-9-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 63, (26.4 mg, 0.07 mmol, 26% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 1.67 (3H, s, Me), 7.42 (1H, t, *J*= 7.6 Hz, C7-H), 7.51 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph2-H), 7.59-7.66 (3H, m, C8-H & Ph3-H), 8.11 (1H, d, *J*= 7.6 Hz, C6-H), 9.07 (1H, s, C5-H), 11.09 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 22.81 (CH_3), 115.25 (Cq), 123.04 (Cq), 125.18 (CH), 126.82 (Cq), 129.29 (CH), 131.38 (CH), 132.52 (CH), 134.11 (Cq), 139.81 (Cq), 140.56 (Cq), 140.60 (CH), 144.15 (CH), 156.75 (Cq), 160.68 (Cq), 162.26 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{13}ClN_3O_2^+$ 338.0691, found 338.0664; IR (KBr): 3428 (NH), 1715 (C=O), 1656 (C=O), 1617 (C=C) cm^{-1}; Anal. HPLC t_R 2.12 min (100.0% pure - System A), 2.70 min (98.4% pure - System B).

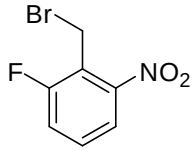


63

Synthesis of 2-Fluoro-6-nitrobenzyl Bromide, 83 [277, 279]

Benzoyl peroxide (2.10 mg; 0.09 mmol) was added to a mixture of 2-fluoro-6-nitrotoluene (2.42 g; 1.90 mL; 15.64 mmol,) and N-bromosuccinimide (2.53 g; 14.22 mmol), in carbon tetrachloride (30 mL) and refluxed for 72 h. The precipitate formed was hot filtered, collected and washed with hot carbon tetrachloride (10 mL) and dry column flash chromatography using 90% petroleum ether and 10% diethyl ether to yield the pure 2-fluoro-6-nitrobenzyl bromide, **83**.

2-Fluoro-6-nitrobenzyl bromide, 83, (1.68 g, 7.17 mmol, 51% yield) as a white solid with a m.pt. of 55-56 ^{O}C; ^{1}H NMR (400MHz, $CDCl_3$): δ/ppm 4.82 (2H, s, CH_2), 7.40 (1H, t, *J*= 8.6 Hz, C4-H), 7.49 (1H, m, C3-H), 7.89 (1H, d, *J*= 8.6 Hz, C5-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 19.21 (CH_2), 120.96 (Cq, d, *J*= 23.40 Hz), 121.20 (CH, d, *J*= 2.85 Hz), 121.74 (CH, d, *J*= 17.44 Hz), 130.16 (CH, d, *J*= 9.53 Hz), 148.78 (Cq), 160.87 (Cq, d, *J*= 253.61 Hz).

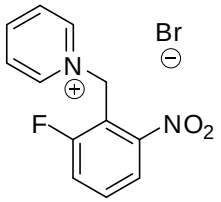


83

Synthesis of 2-Fluoro-6-nitrobenzyl Pyridinium Bromide, 84 [277, 279]

2-Fluoro-6-nitrobenzyl bromide, **83** (1.40 g; 5.98 mmol), and pyridine (0.71 g, 0.70 mL; 8.97 mmol) was refluxed in ethanol (40 mL) for 3.5 h. The reaction mixture was cooled in the fridge for 5 min. The precipitate formed was collected by suction filtration, washed with ethanol and dry column flash chromatography was carried out, using 80% dichloromethane and 20% methanol, to yield the pure 2-fluoro-6-nitrobenzyl pyridium bromide, **84**.

2-Fluoro-6-nitrobenzyl pyridinium bromide, 84, (1.68 g, 7.17 mmol, 91% yield) as a white solid with a m.pt. of 59-60 ^{O}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 6.7 (2H, s, CH_2), 7.86-7.93 (2H, m), 8.11-8.21 (3H, m), 8.67 (1H, t, *J*= 7.6 Hz, C4-H), 9.10 (2H, d, *J*= 5.7 Hz, Py2-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 54.28 (CH_2, d, *J*= 4.43 Hz), 115.57 (Cq, d, *J*= 17.72 Hz), 122.45 (CH, d, *J*= 3.06 Hz), 122.83 (CH, d, *J*= 23.56 Hz), 128.69 (CH), 133.72 (CH, d, *J*= 10.32 Hz), 145.33 (CH), 146.82 (CH), 149.99 (Cq, d, *J*= 4.05 Hz), 162.05 (Cq, d, *J*= 253.66 Hz); m/z $[M + H]^+$ calcd for $C_{12}H_{10}FN_2O_2^+$ 233.0721, found 233.0705.

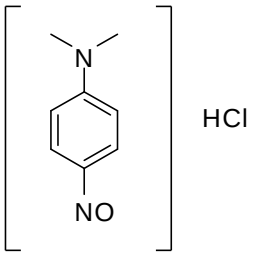


84

Synthesis of *p*-Nitrosodimethylaniline Hydrochloride, 85 [278]

To a mixture of N,N-dimethylamine (1.94 g, 2.03mL; 16.07 mmol) in conc hydrochloric acid (30 mL), at 0-5 ^{O}C, was added sodium nitrite (1.16 g; 16.87 mmol) in water (50 mL) drop wise over 1 h. The reaction mixture was allowed to rise to room temperature and stirred for a further 1 h with the resulting yellow solid collected by suction filtration and washed with water (25 mL) to yield the slightly impure *p*-Nitrosodimethylaniline hydrochloride, **85**.

***p*-Nitrosodimethylaniline hydrochloride, 85,** (2.21 g, 11.84 mmol, 74% yield) as a yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 3.52 (6H, s, Me), 7.11 (2H, d, AA'BB' system, *J*= 9.7 Hz, C2-H), 7.67 (2H, d, AA'BB' system, *J*= 9.7 Hz, C3-H); m/z $[M + H]^+$ calcd for $C_8H_{11}N_2O^+$ 151.0866, found 151.0858.



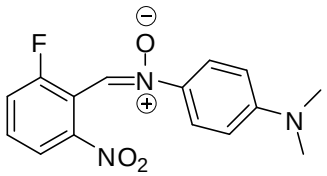
85

Synthesis of N-(*p*-Dimethylaminobenzyl)-α-(6-fluoro-*o*-nitrophenyl) Nitrone, 87 [277, 279]

p-Nitrosodimethylaniline hydrochloride, **85** (1.50 g; 4.79 mmol), was added to 2-fluoro-6-nitrobenzyl pyridinium bromide, **84** (1.07 g; 5.74 mmol), in ethanol (70 mL) and cooled to 0-5 ^{O}C. Sodium hydroxide (0.40 g; 9.82 mmol) in

ethanol (70mL) was added dropwise over 30 min with the temperature maintained at 5-10 ^{O}C under stirring. The reaction mixture was stirred for a further 3 h at room temperature with the yellow precipitate formed filtered and washed with ethanol to yield the pure N-(*p*-dimethylaminobenzyl)-α-(6-fluoro-o-nitrophenyl) nitrone, **87**.

N-(*p*-Dimethylaminobenzyl)-*α*-(6-fluoro-*o*-nitrophenyl) nitrone, 87, (1.06 g, 3.49 mmol, 73% yield) as a yellow solid with a m.pt. of 147-148 ^{O}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 3.02 (6H, s, Me), 6.67 (2H, d, AA'BB' system, *J*= 9.2 Hz, Ph3-H), 7.40 (1H, t, *J*= 8.4 Hz, C4-H), 7.48-7.55 (1H, m, C3-H), 7.69 (2H, d, AA'BB' system, *J*= 9.2 Hz, Ph2-H), 7.84 (1H, d, *J*= 8.4 Hz, C5-H), 8.15 (1H, s, CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 40.33 (CH_3), 111.18 (CH), 114.50 (Cq, d, *J*= 16.92 Hz), 120.05 (CH, d, *J*= 22.08 Hz), 120.32 (CH, d, *J*= 2.93 Hz), 120.91 (CH), 122.59 (CH), 130.43 (CH, d, *J*= 9.44 Hz), 136.84 (Cq), 149.05 (Cq), 151.87 (Cq), 160.62 (Cq, d, *J*= 254.19 Hz); m/z $[M + H]^+$ calcd for $C_{15}H_{15}FN_3O_3^+$ 304.1092, found 304.1087.

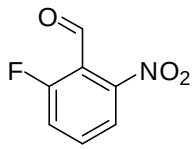


87

<u>Synthesis of 2-Fluoro-6-nitrobenzaldehyde, 82</u> <u>[277, 279]</u>

N-(*p*-Dimethylaminobenzyl)-α-(6-fluoro-o-nitrophenyl) nitrone, **87** (1.00 g; 3.29 mmol), was stirred in 3 molar concentration of sulfuric acid (80 mL) at room temperature. After 30 min dichloromethane (50 mL) was added and liquid extraction carried out. The organic layer was treated with magnesium sulfate, filtered and dried to yield the pure 2-fluoro-6-nitrobenzaldehyde, **82**.

2-Fluoro-6-nitrobenzaldehyde, 82, (0.47 g, 2.79 mmol, 86% yield) as a white solid with a m.pt. of 62-63 ^{O}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 7.50 (1H, t, *J*= 8.5 Hz, C4-H), 7.66-7.73 (1H, m, C3-H), 7.88 (1H, d, *J*= 8.5 Hz, C5-H), 10.31 (1H, s, COH); ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 120.05 (CH, d, *J*= 3.61 Hz), 121.29 (CH, d, *J*= 16.22 Hz), 122.11 (Cq), 122.33 (Cq), 133.59 (CH, d, *J*= 9.17 Hz), 160.80 (Cq, d, *J*= 260.25 Hz), 184.52 (CH); IR (KBr): 1704 (C=O), 1517 (NO_2), 1310 (NO_2) cm^{-1}.



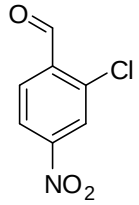
82

Synthesis of 2-Chloro-4-nitrobenzaldehyde, 88 [280, 281]

Under a nitrogen atmosphere and dry conditions di-isobutylaluminum hydride (5.10 mL, 1 molar solution in toluene) in dry toluene (50 mL) was added slowly drop wise to methyl-2-chloro-4-nitrobenzoate (1.00 g; 4.63 mmol) in dry toluene (100 mL) at a temperature range of -70 to -78 ^{O}C. After the reaction mixture was stirred for a further 2 h at -70 to -78 ^{O}C, saturated ammonium chloride solution (50mL) and dichloromethane (50ml) was added and liquid extraction carried out with the organic dichloromethane layer treated with magnesium sulfate and filtered. Concentration of the solution under reduced pressure and separation by flash chromatography using a 66.6% hexane and 33.3% ethyl acetate was performed to yield the pure 2-chloro-4-nitrobenzaldehyde, **88**.

2-Chloro-4-nitrobenzaldehyde, 88, (142.7 mg, 0.76 mmol, 17% yield) as a white solid with a m.pt. of 57-60 ^{O}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 8.08 (1H, d, *J*= 8.5 Hz, C6-H), 8.30 (1H, dd, ^{4}J= 2.2 Hz, ^{3}J= 8.5 Hz, C5-H), 8.43

(1H, d, ^{4}J= 2.2 Hz, C3-H), 10.36 (1H, s, COH); ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 123.19 (CH), 126.20 (CH), 131.44 (CH), 136.43 (Cq), 136.80 (Cq), 151.11 (Cq), 189.38 (CH); IR (KBr): 1719 (C=O), 1528 (NO_2), 1336 (NO_2) cm^{-1}.



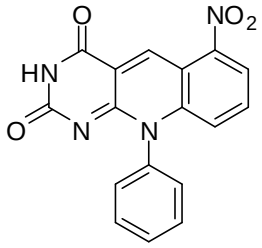
88

General Procedure to Synthesise Nitro Analogues of 10-Phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione [241, 261, 264, 265, 268, 270]

To 1 equivalent of the desired 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione analogue in DMF was added 1.2 equivalents of the desired 2-halobenzaldehyde (chloro or fluoro). The mixture was heated at 160 ^{O}C for 30 min in the microwave reactor or refluxed for 4 h. Concentration of the solution under reduced pressure and separation by dry column flash chromatography using 95% dichloromethane and 5% methanol to yield the pure desired 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogue.

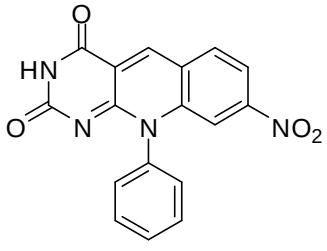
10-Phenyl-6-nitropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 16, (66.50 mg, 0.18 mmol, 67% yield) as a yellow solid with a m.pt. of 348-349 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.08 (1H, d, *J*= 8.3 Hz, C9-H), 7.46 (2H, d, *J*= 7.0 Hz, Ph2-H), 7.63-7.75 (3H, m, Ph-H), 7.87 (1H, t, *J*= 8.3 Hz, C8-H) 8.18 (1H, d, *J*= 8.3 Hz, C7-H), 9.20 (1H, s, C5-H), 11.30 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 118.35 (Cq), 121.55 (CH), 123.27 (CH), 128.80 (CH), 130.01 (Cq), 130.18 (CH), 130.92 (CH), 134.36 (CH),

136.16 (CH), 137.89 (Cq), 140.29 (Cq), 142.95 (Cq), 156.74 (Cq), 159.96 (Cq), 161.87 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{11}N_4O_4^+$ 335.0775, found 335.0783; IR (KBr): 3434 (NH), 1707 (C=O), 1677 (C=O), 1615 (C=C), 1551 (NO_2), 1296 (NO_2) cm^{-1}; Anal. HPLC t_R 2.43 min (98.3% pure - System A), 7.55 min (97.9% pure - System B).



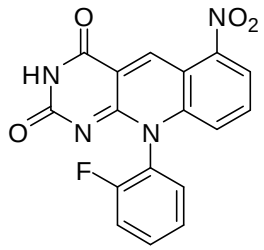
16

10-Phenyl-8-nitropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 18, (42.6 mg, 0.12 mmol, 71% yield) as a yellow solid with a m.pt. of 342-343 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.37 (1H, d, ^{4}J= 1.9 Hz, C9-H), 7.49 (2H, d, *J*= 7.0 Hz, Ph-H), 7.66-7.78 (3H, m, Ph-H), 8.23 (1H, dd, ^{4}J= 1.9 Hz, ^{3}J= 8.6 Hz, C7-H), 8.49 (1H, d, *J*= 8.6 Hz, C6-H), 9.21 (1H, s, C5-H), 11.27 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 112.36 (CH), 118.55 (CH), 119.12 (Cq), 125.20 (Cq), 128.89 (CH), 130.35 (CH), 130.97 (CH), 133.64 (CH), 137.45 (Cq), 141.45 (CH), 142.11 (Cq), 150.45 (Cq), 156.66 (Cq), 159.61 (Cq), 161.88; m/z $[M + H]^+$ calcd for $C_{17}H_{11}N_4O_4^+$ 335.0775, found 335.0764; IR (KBr): 3430 (NH), 1703 (C=O), 1667 (C=O), 1607 (C=C), 1539 (NO_2), 1317 (NO_2) cm^{-1}; Anal. HPLC t_R 1.28 min (99.8% pure - System A), 10.47 min (100.0% pure - System B).



18

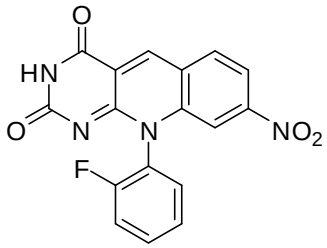
10-(2-Fluorophenyl)-6-nitropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 20, (135.3 mg, 0.38 mmol, 10% yield) as a yellow solid with a m.pt. of 306-308 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm 7.22 (1H, d, *J*= 8.2 Hz, C9-H), 7.52-7.68 (3H, m, Ph-H), 7.72-7.80 (1H, m, Ph-H), 7.92 (1H, t, *J*= 8.2 Hz, C8-H), 8.22 (1H, d, *J*= 8.2 Hz, C7-H), 9.23 (1H, s, C5-H), 11.40 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 114.01 (Cq), 117.85 (CH, d, *J*= 19.35 Hz), 118.09 (Cq), 122.05 (CH), 122.53 (CH), 124.67 (Cq, d, *J*= 13.64 Hz), 126.83 (CH, d, *J*= 3.46 Hz), 130.91 (CH), 132.99 (CH, d, *J*= 8.01 Hz), 135.05 (CH), 136.91 (CH), 142.02 (Cq), 148.21 (Cq), 156.55 (Cq), 157.49 (Cq, d, *J*= 250.29 Hz), 158.38 (Cq), 161.63 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{10}FN_4O_4^+$ 353.0681, found 353.0694; IR (KBr): 3442 (NH), 1707 (C=O), 1678 (C=O), 1620 (C=C), 1518 (NO_2), 1289 (NO_2) cm^{-1}; Anal. HPLC t_R 1.20 min (97.8% pure - System A), 9.28 min (98.7% pure - System B).



20

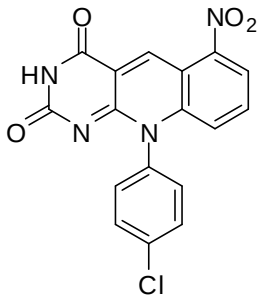
10-(2-Fluorophenyl)-8-nitropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 22, (23.6 mg, 0.06 mmol, 37% yield) as a yellow solid with a m.pt. of 308-310 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.43 (1H, s, C9-H), 7.56-7.72 (3H, m, Ph-H), 7.75-7.83 (1H, m, Ph-H), 8.27 (1H, dd, ^{4}*J*= 2.0 Hz, ^{3}*J*= 8.6 Hz, C7-H), 8.52 (1H, d, *J*= 8.6 Hz, C6-H), 9.24 (1H, s, C5-H), 11.36 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 111.51 (CH), 117.96 (CH, d, *J*= 17.58 Hz), 118.91 (Cq), 119.14 (CH), 124.23 (Cq, d, *J*= 13.18 Hz), 125.25 (Cq), 126.86 (CH, d, *J*= 2.93 Hz), 130.96 (CH), 133.17 (CH, d, *J*= 7.91 Hz), 134.05

(CH), 141.20 (Cq), 141.78 (CH), 150.89 (Cq), 156.56 (Cq), 157.61 (Cq, d, *J*= 250.48 Hz), 159.26 (Cq), 161.64 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{10}FN_4O_4^+$ 353.0681, found 353.0678; IR (KBr): 3420 (NH), 1711 (C=O), 1670 (C=O), 1628 (C=C), 1535 (NO_2), 1342 (NO_2) cm^{-1}; Anal. HPLC t_R 1.37 min (100.0% pure - System A), 4.47 min (98.7% pure - System B).



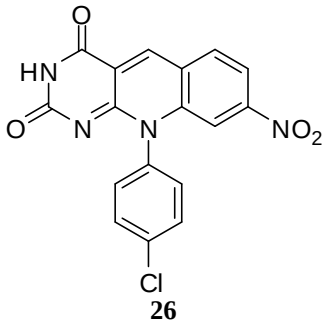
22

10-(4-Chlorophenyl)-6-nitropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 24, (188.9 mg, 0.51 mmol, 20% yield) as a yellow solid with a m.pt. of 258-260 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.18 (1H, d, *J*= 8.2 Hz, C9-H), 7.51 (2H, d, AA'BB' system, *J*= 8.5 Hz, Ph2-H), 7.79 (2H, d, AA'BB' system, *J*= 8.5 Hz, Ph3-H), 7.87 (1H, t, *J*= 8.2 Hz, C8-H), 8.19 (1H, d, *J*= 8.2 Hz, C7-H), 9.19 (1H, s, C5-H), 11.32 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 113.86 (Cq), 118.27 (Cq), 121.64 (CH), 123.37 (CH), 130.87 (CH), 131.04 (CH), 134.43 (CH), 134.89 (Cq), 136.32 (CH), 136.68 (Cq), 142.77 (Cq), 148.05 (Cq), 156.58 (Cq), 158.77 (Cq), 161.82 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{10}ClN_4O_4^+$ 369.0385, found 369.0394; IR (KBr): 3400 (NH), 1715 (C=O), 1649 (C=O), 1614 (C=C), 1531 (NO_2), 1347 (NO_2) cm^{-1}; Anal. HPLC t_R 1.75 min (98.7% pure - System A), 5.22 min (99.0% pure - System B).



24

10-(4-Chlorophenyl)-8-nitropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 26, (33.9 mg, 0.09 mmol, 51% yield) as a yellow solid with a m.pt. of 328-329 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.40 (1H, d, ^{4}J= 1.9 Hz, C9-H), 7.55 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph2-H), 7.83 (2H, d, AA'BB' system, *J*=8.6 Hz, Ph3-H), 8.25 (1H, dd, ^{4}J= 1.9 Hz, ^{3}J= 8.6 Hz, C7-H), 8.49 (1H, d, *J*= 8.6 Hz, C6-H), 9.22 (1H, s, C5-H), 11.31 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 112.21 (CH), 118.66 (CH), 119.08 (Cq), 125.24 (Cq), 130.99 (CH), 131.09 (CH), 133.72 (CH), 135.00 (Cq), 136.22 (Cq), 141.36 (CH), 141.89 (Cq), 150.55 (Cq), 156.56 (Cq), 159.69 (Cq), 161.82 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{10}ClN_4O_4^+$ 369.0385, found 369.0348; IR (KBr): 3429 (NH), 1711 (C=O), 1654 (C=O), 1610 (C=C), 1521 (NO_2), 1310 (NO_2) cm^{-1}; Anal. HPLC t_R 2.13 min (97.4% pure - System A), 5.42 min (100.0% pure - System B).



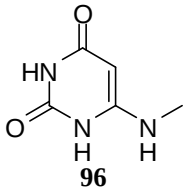
26

<u>Synthesis of 6-(Methylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 96</u>

Methylamine (3.00 mL of 40% aqueous solution) was added to 6-chlorouracil, **68** (500.00 mg; 3.41 mmol), and was heated at 50 ^{O}C for 1 h in a microwave

reactor. Acetone (5 mL) was added to the reaction mixture with the white precipitate formed, collected by suction filtration without further purification to yield the impure desired 6-(methylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **96**.

6-(Methylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 96, (0.35 g, 2.48 mmol, 73% yield) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ/ppm 2.62 (3H, s, Me), 3.35 (1H, broad s, NH), 4.32 (1H, s, C5-H), 6.44 (1H, s, N1-H), 9.22 (1H, broad s, N3-H); m/z $[M + H]^+$ calcd for $C_5H_8N_3O_2^+$ 142.0611, found 142.0597.



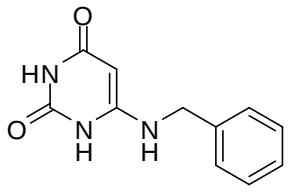
96

Synthesis of 6-(Benzylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 97 [259, 260, 263, 265, 300, 304]

Benzylamine (3.69 g, 3.77 mL; 34.52 mmol) was added to 6-chlorouracil, **68** (337.28 mg; 2.30 mmol), and was heated at 130 ^{O}C for 15 min in a microwave reactor. The reaction mixture was cooled to room temperature and diluted with diethyl ether (10 mL) resulting in the formation of a white precipitate. The white precipitate was collected by suction filtration, washed with water and recrystallised from ethanol to yield the pure desired 6-(benzylamino)pyrimidine-2,4(1*H*,3*H*)-dione, **97**.

6-(Benzylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 97, (324.5 mg, 1.49 mmol, 65% yield) as a pale yellow solid with a m.pt. of 313-314 ^{O}C (lit 313-314 ^{O}C) [304]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 4.26 (2H, d, *J*= 5.8 Hz, CH_2), 4.38 (1H, s, C5-H), 6.62 (1H, t, *J*= 5.8 Hz, NH), 7.24-7.42 (5H, m, Ph-H), 9.77 (1H, s, N1-H), 10.17 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm

45.40 (CH_2), 73.91 (CH), 127.62 (CH), 127.68 (CH), 128.98 (CH), 138.42 (Cq), 151.29 (Cq), 154.48 (Cq), 164.62 (Cq); m/z $[M + H]^+$ calcd for $C_{11}H_{12}N_3O_2^+$ 218.0924, found 218.0927; IR (KBr): 3259 (NH), 3085 (NH), 1728 (CO), 1658 (C=C) cm^{-1}.



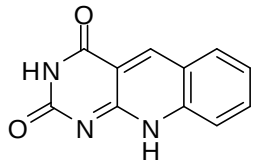
97

General Procedure to Synthesise 10-Substituted-pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione Analogues [241, 261, 264, 265, 268, 270]

To 1 equivalent of the desired 6-(Substituted-amino)pyrimidine-2,4(1*H*,3*H*)-dione analogue in DMF was added 1.2 equivalents of the desired 2-halobenzaldeyhyde (chloro or fluoro). The mixture was heated at 160 ^{O}C for 30 min in the microwave reactor or refluxed for 4 h. Concentration of the solution under reduced pressure and separation by dry column flash chromatography using 95% and dichloromethane 5% methanol to yield the pure desired 10-substituted-pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogues .

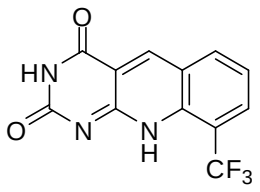
Pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 90, (51.10 mg, 0.23 mmol, 26% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.51-7.57 (1H, m, C9-H), 7.82-7.89 (2H, m, C7-H & C8-H), 8.15 (1H, d, *J*= 8.2 Hz, C6-H), 9.01 (1H, s, C5-H), 11.51 (1H, s, N3-H), 11.71 (1H, s, N10-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 111.52 (Cq), 125.10 (Cq), 125.65 (CH), 127.22 (CH), 130.33 (CH), 133.57 (CH), 139.50 (CH), 149.91 (Cq), 150.57 (Cq), 151.11 (Cq), 162.64 (Cq); m/z $[M + H]^+$ calcd for $C_{11}H_8N_3O_2^+$ 214.0611, found 214.0617; IR (KBr): 3409 (NH), 3177 (NH),

1710 (C=O), 1622 (C=O), 1583 (C=C) cm^{-1}; Anal. HPLC t_R 0.72 min (100.0% pure - System A), 3.05 min (98.9% pure - System B).



90

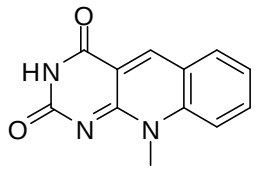
9-Trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 91, (73.8 mg, 0.26 mmol, 74% yield) as a yellow solid; with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.69 (1H, t, *J*= 7.6 Hz, C7-H), 8.25 (1H, d, *J*= 7.6 Hz, C8-H), 8.45 (1H, d, *J*= 7.6 Hz, C6-H), 9.14 (1H, s, C5-H), 11.64 (1H, s, N3-H), 11.80 (1H, s, N10-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 112.57 (Cq), 124.32 (CH), 125.65 (Cq), 131.76 (CH), 131.86 (Cq), 135.30 (CH), 139.94 (CH), 146.13 (Cq), 151.02 (Cq), 151.11 (Cq), 162.36 (Cq); m/z $[M + H]^+$ calcd for $C_{12}H_7F_3N_3O_2^+$ 282.0485, found 282.0505; IR (KBr): 3429 (NH), 3071 (NH), 1711 (C=O), 1654 (C=O), 1610 (C=C) cm^{-1}; Anal. HPLC t_R 1.59 min (99.2% pure - System A), 6.74 min (100.0% pure - System B).



91

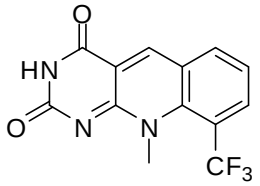
10-Methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 92, (95.0 mg, 2.39 mmol, 48% yield) as a yellow solid with a m.pt. of 360-363 ^{O}C (lit >359 ^{O}C) [254]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 4.03 (3H, s, Me), 7.53 (1H, t, *J*= 7.5 Hz, C8-H), 7.89-7.97 (2H, m, C7-H & C9-H), 8.16 (1H, d, *J*= 7.5 Hz, C6-H), 8.98 (1H, s, C5-H), 11.07 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 32.69 (CH_3), 115.59 (Cq), 117.18 (CH), 121.34 (Cq), 124.82 (CH), 132.05 (CH), 135.77 (CH), 141.24 (Cq), 141.94 (CH), 156.90 (Cq), 157.98

(Cq) 162.54 (Cq); m/z $[M + H]^+$ calcd for $C_{12}H_{10}N_3O_2^+$ 228.0768, found 228.0771; IR (KBr): 3473 (NH), 1714 (C=O), 1624 (C=O), 1607 (C=C) cm^{-1}; Anal. HPLC t_R 0.60 min (100.0% pure - System A), 4.05 min (97.8% pure - System B).



92

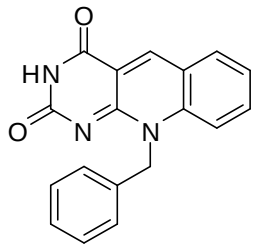
***10-Methyl-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 93,** (3.39 mg, 0.01 mmol, 3% yield) as a yellow solid with a m.pt. of 318-320 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 3.85 (3H, s, Me), 7.67 (1H, t, *J*= 7.8 Hz, C7-H), 8.35 (1H, d, *J*= 7.8 Hz, C8-H), 8.44 (1H, d, *J*= 7.8 Hz, C6-H), 9.00 (1H, s, C5-H), 11.59 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 45.11 (CH_3), 116.56 (Cq), 124.10 (Cq), 124.45 (CH), 132.47 (Cq), 135.92 (CH), 137.03 (CH), 140.62 (Cq), 142.01 (CH), 157.01 (Cq), 160.74 (Cq), 162.19 (Cq); m/z $[M + H]^+$ calcd for $C_{13}H_9F_3N_3O_2^+$ 296.0641, found 296.0643; IR (KBr): 3438 (NH), 1713 (C=O), 1664 (C=O), 1618 (C=C) cm^{-1}; Anal. HPLC t_R 0.79 min (99.4% pure - System A), 7.95 min (99.6% pure - System B).



93

* To fully purify **93**, preparative HPLC was carried out using a Waters 2525 Binary Gradient Module with a Waters 2487 Dual λ Absorbance Detector as the UV FT-IR detector. The system used was a Phenomenex Kromasil reverse phase C18 column (250 x 21.2mm) with a flow rate of 14.10 mL/min and UV detection at 254 nm using linear gradient of 70% water and 30% acetonitrile over 10 min.

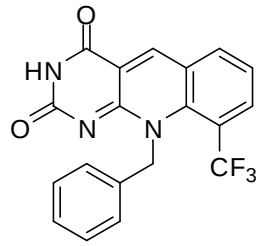
10-Benzylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 94, (71.4 mg, 0.23 mmol, 71% yield) as a yellow solid with a m.pt. of 346-347 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.00 (2H, s, CH_2), 7.21-7.34 (5H, m, Ph-H), 7.49 (1H, t, *J*= 7.9 Hz, C7-H), 7.68 (1H, d, *J*= 7.9 Hz, C9-H), 7.82 (1H, ddd, ^{4}J= 1.5 Hz, ^{3}J= 7.9 Hz, C8-H), 8.20 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.9 Hz, C6-H), 9.07 (1H, s, C5-H), 11.15 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 47.72 (CH_2), 115.87 (Cq), 117.40 (CH), 121.74 (Cq), 124.91 (CH), 126.93 (CH), 127.74 (CH), 129.15 (CH), 132.32 (CH), 135.72 (CH), 136.02 (Cq), 140.46 (Cq), 142.51 (CH), 157.07 (Cq), 158.38 (Cq), 162.56 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{14}N_3O_2^+$ 304.1081, found 304.1065; IR (KBr): 3447 (NH), 1702 (C=O), 1652 (C=O), 1608 (C=C) cm^{-1}; Anal. HPLC t_R 1.17 min (100.0% pure - System A), 12.82 min (99.2% pure - System B).



94

10-Benzyl-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 95, (11.5 mg, 0.03 mmol, 12% yield) as a yellow solid with a m.pt. of 235-237 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 5.87 (2H, s, CH_2), 6.81 (2H, d, *J*= 6.7 Hz, Ph2-H), 7.13-7.21 (3H, m, Ph-H), 7.68 (1H, t, *J*= 7.8 Hz, C7-H), 8.31 (1H, dd,^{4} *J*= 1.3 Hz, ^{3}J= 7.8 Hz, C8-H), 8.47 (1H, d, *J*= 7.8 Hz, C6-H), 9.02 (1H, s, C5-H), 11.26 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 54.41 (CH_2), 117.14 (Cq), 118.17 (Cq), 124.54 (Cq), 125.08 (CH), 126.70 (CH), 127.62 (CH), 128.86 (CH), 135.99 (CH), 136.69 (Cq), 137.34 (CH), 139.88 (Cq), 142.47 (CH), 156.73 (Cq), 160.83 (Cq), 162.02 (Cq); m/z [M +

H]$^+$ calcd for $C_{19}H_{13}F_3N_3O_2^+$ 372.0954, found 372.0973; IR (KBr): 3416 (NH), 1706 (C=O), 1664 (C=O), 1615 (C=C) cm^{-1}; Anal. HPLC t_R 2.34 min (97.5% pure - System A), 8.22 min (96.8% pure - System B).



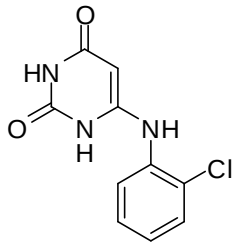
95

General Procedure to Synthesise 6-(2 or 3 or 4-Substituted-phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione Analogues [259, 260, 263, 265, 300]

To 1 equivalent of 6-chlorouracil, **68**, was added 6 equivalents of the desired aniline and refluxed for 1.5 to 3 h. The reaction mixture was cooled and diluted with diethyl ether which caused separation of crystals which were filtered, washed with water and recrystallised from ethanol to yield the 6-(2 or 3 or 4-substituted-phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione analogue.

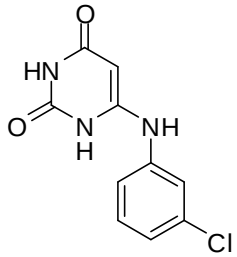
6-(2-Chlorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 137, (0.69g, 2.89 mmol, 69% yield) as a off white solid with a m.pt. of 322-324 ^{O}C (lit 321-323 ^{O}C) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 4.41 (1H, s, C5-H), 7.27 (1H, ddd, ^4J= 1.5 Hz, ^3J= 7.7 Hz, Ph5-H), 7.39 (1H, ddd, ^4J= 1.5 Hz, ^3J= 7.7 Hz, Ph4-H), 7.45 (1H, dd, ^4J= 1.5, ^{3}J= 7.7 Hz, Ph6-H), 7.57 (1H, dd, ^4J= 1.5 Hz, ^3J= 7.7 Hz, Ph3-H), 8.06 (1H, s, NH), 10.33 (1H, s, N1-H), 10.50 (1H, s, N3-H), ^{13}C NMR (75M Hz, DMSO-d_6): δ/ppm 76.62 (CH), 127.24 (CH), 127.82 (CH), 128.67 (CH), 128.87 (Cq), 130.63 (CH), 134.94 (Cq), 151.14 (Cq), 152.60 (Cq), 164.67 (Cq); m/z [M + H]$^+$ calcd for $C_{10}H_9ClN_3O_2^+$

238.0378, found 238.0374, IR (KBr): 3200 (NH), 3048 (NH), 1728 (CO), 1628 (C=C) cm^{-1}.



137

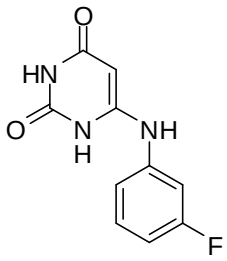
6-(3-Chlorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 138, (467.2 mg, 1.96 mmol, 47% yield) as a off white solid with a m.pt. of 327-328 ^{O}C (lit 328-329 ^{O}C) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 4.75 (1H, s, C5-H), 7.14-7.27 (3H, m, Ph-H), 7.38 (1H, t, *J*= 7.8 Hz, Ph5-H), 8.46 (1H, s, NH), 10.35 (1H, s, N1-H), 10.53 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 77.76 (CH), 121.22 (CH), 122.19 (CH), 124.50 (CH), 131.45 (CH), 134.02 (Cq), 140.36 (Cq), 151.35 (Cq), 152.15 (Cq), 164.83 (Cq); m/z $[M + H]^+$ calcd for $C_{10}H_9ClN_3O_2^+$ 238.0378, found 238.0370; IR (KBr): 3157 (NH), 3044 (NH), 1734 (C=O), 1622 (C=C) cm^{-1}.



138

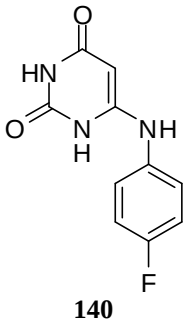
6-(3-Fluorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 139, (1.19 g, 5.40 mmol, 60% yield) as a off white solid with a m.pt. of 320-322 ^{O}C (lit 321-322 ^{O}C) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 4.80 (1H, s, C5-H), 6.92-7.06 (3H, m, Ph-H), 7.36-7.43 (1H, m, Ph-H), 8.47 (1H, s, NH), 10.30 (1H, s, N1-H), 10.53 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 77.74 (CH), 109.38 (CH, d, *J*= 24.31 Hz), 111.29 (CH, d, *J*= 20.98 Hz), 118.44 (CH,

d, *J*= 2.90 Hz), 131.48 (CH, d, *J*= 9.51 Hz), 140.60 (Cq, d, *J*= 10.96 Hz), 151.32 (Cq), 152.05 (Cq), 163.02 (Cq, d, *J*= 244.91 Hz), 164.85 (Cq); m/z [M + H]$^{+}$ calcd for $C_{10}H_9FN_3O_2^{+}$ 222.0673, found 222.0652; IR (KBr): 3248 (NH), 3042 (NH), 1752 (CO), 1616 (C=C) cm^{-1}.



139

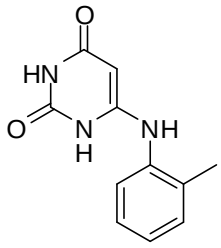
6-(4-Fluorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 140, (819.9 mg, 3.70 mmol, 82% yield) as a pale blue solid with a m.pt. of 338-340 ^{O}C (lit 339-340 ^{O}C) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 4.54 (1H, s, C5-H), 7.18-7.27 (4H, m, Ph-H), 8.18 (1H, s, NH), 10.22 (1H, s, N1-H), 10.43 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_{63}): δ/ppm 76.05 (CH), 116.57 (CH, d, *J*= 22.42 Hz), 126.02 (CH, d, *J*= 8.13 Hz), 134.57 (Cq, d, *J*= 2.90 Hz), 151.34 (Cq), 153.22 (Cq), 159.79 (Cq, d, *J*= 242.76 Hz), 164.77 (Cq); m/z [M + H]$^{+}$ calcd for $C_{10}H_9FN_3O_2^{+}$ 222.0673, found 222.0658; IR (KBr): 3196 (NH), 3090 (NH), 1752 (CO), 1611 (C=C) cm^{-1}.



140

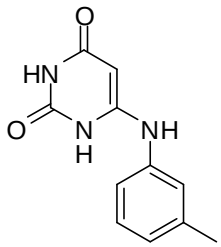
6-(2-Methylphenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 141, (557.5 mg, 2.56 mmol, 56% yield) as a off white solid with a m.pt. of 331-332 ^{O}C (lit 332-333 ^{O}C) [305]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.19 (3H, s, Me), 4.15

(1H, s, C5-H), 7,17-7.33 (4H, m, Ph-H), 7.78 (1H, s, NH), 10.18 (1H, s, N1-H), 10.35 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 17.79 (CH_3), 75.20 (CH), 126.65 (CH), 127.05 (CH), 127.31 (CH), 131.44 (CH), 134.19 (Cq), 136.12 (Cq), 151.27 (Cq), 153.53 (Cq), 164.70 (Cq); m/z $[M + H]^+$ calcd for $C_{11}H_{12}N_3O_2^+$ 218.0924, found 218.0916; IR (KBr): 3175 (NH), 3038 (NH), 1735 (C=O), 1631 (C=C) cm^{-1}.



141

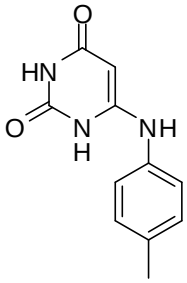
6-(3-Methylphenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 142, (707.4mg, 3.25mmol, 71% yield) as an off white solid with a m.pt. of 303-304 ^{O}C (lit 302-303 ^{O}C) [305]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.30 (3H, s, Me), 4.68 (1H, s, C5-H), 6.95-7.02 (3H, m, Ph-H), 7.26 (1H, t, *J*= 7.5 Hz, Ph5-H), 8.16 (1H, s, NH), 10.10 (1H, s, N1-H), 10.43 (1H, s, N3-H); ^{13}C NMR (75M Hz, DMSO-d_6): δ/ppm 17.78 (CH_3), 75.21 (CH), 126.66 (CH), 127.07 (CH), 127.32 (CH), 131.45 (CH), 134.21 (Cq), 136.12 (Cq), 151.29 (Cq), 153.54 (Cq), 164.70 (Cq); m/z $[M + H]^+$ calcd for $C_{11}H_{12}N_3O_2^+$ 218.0924, found 218.0923; IR (KBr): 3211 (NH), 3053 (NH), 1734 (C=O), 1625 (C=C) cm^{-1}.



142

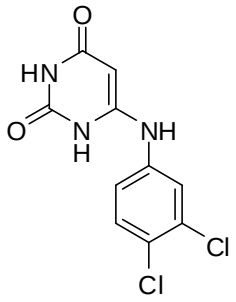
6-(4-Methylphenylamino)pyrimidine-2,4(1*H*,3*H*)-dione 143, (2.52 g, 11.61 mmol, 84% yield) as a off white solid with a m.pt. of 322-324 ^{O}C (lit 321-323

^{O}C) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.28 (3H, s, Me), 4.59 (1H, s, C5-H), 7.08 (2H, d, AA'BB' system, *J*= 8.1 Hz, Ph3-H), 7.18 (2H, d, AA'BB' system, *J*= 8.1 Hz, Ph2-H), 8.10 (1H, s, NH), 10.09 (1H, s, N1-H), 10.41 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 20.92 (CH_3), 75.80 (CH), 123.58 (CH), 130.30 (CH), 134.63 (Cq), 135.58 (Cq), 151.30 (Cq), 152.99 (Cq), 164.81 (Cq); m/z $[M + H]^+$ calcd for $C_{11}H_{12}N_3O_2^+$ 218.0924, found 218.0006, IR (KBr): 3197 (NH), 3030 (NH), 1759 (CO), 1596 (C=C) cm^{-1}.



143

6-(3,4-dichlorophenylamino)pyrimidine-2,4(1*H*,3*H*)-dione, 144, (88.3 mg, 0.32 mmol, 9% yield) as a pale blue solid with a m.pt. of 333-335 ^{O}C (lit 334-335 ^{O}C) [306]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 4.78 (1H, s, C5-H), 7.19 (1H, dd, ^{4}J= 2.5 Hz, ^{3}J= 8.6 Hz, Ph6-H), 7.44 (1H, d, ^{4}J= 2.5 Hz, Ph1-H), 7.59 (1H, d, *J*= 8.6 Hz, Ph5-H), 8.57 (1H, s, NH), 10.43 (1H, s, N1-H), 10.55 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 78.40 (CH), 122.71 (CH), 123.97 (CH), 126.32 (Cq), 131.61(CH), 132.01 (Cq), 139.17 (Cq), 151.36 (Cq), 151.95 (Cq), 164.81 (Cq); m/z $[M + H]^+$ calcd for $C_{10}H_8Cl_2N_3O_2^+$ 271.9988, found 271.9983; IR (KBr): 3154 (NH), 3030 (NH), 1753 (C=O), 1652 (C=C) cm^{-1}.



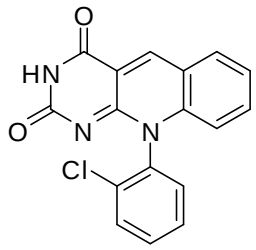
144

General Procedure to Synthesise 10-(2 or 3 or 4-Substituted-phenyl)-9-sbstituted-pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione Analogues [241, 261, 264, 265, 268, 270]

To 1 equivalents of the desired 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione analogue in DMF was added 1.2 equivalents of the desired 2-halobenzaldehyde (chloro or fluoro). The mixture was heated at 160 ^{O}C for 30 min in the microwave reactor or refluxed for 4 h. Concentration of the solution under reduced pressure and separation by dry column flash chromatography using 95% dichloromethane and 5% methanol to yield the pure desired 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogue.

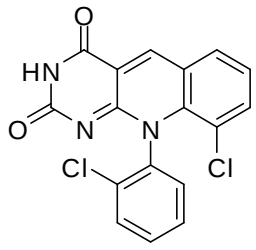
10-(2-Chlorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 98, (70.2 mg, 0.21 mmol, 70% yield) as a yellow solid with a m.pt. of 347-348 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.69 (1H, d, *J*= 8.0 Hz, C9-H), 7.54 (1H, t, *J*= 8.0 Hz, C7-H), 7.62-7.72 (3H, m, Ph-H), 7.71 (1H, ddd, ^{4}J= 1.4 Hz, ^{3}J= 8.0 Hz, C8-H), 7.83-7.87 (1H, m, Ph-H), 8.26 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 8.0 Hz, C6-H), 9.17 (1H, s, C5-H), 11.16 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 115.87 (Cq), 116.68 (CH), 121.40 (Cq), 125.31 (CH), 129.77 (CH), 131.06 (CH), 131.27 (CH), 131.61 (Cq), 131.96 (CH), 132.21 (CH), 135.18 (Cq), 136.11 (CH), 141.07 (Cq), 143.54 (CH), 156.90 (Cq), 158.59 (Cq), 162.21 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{11}ClN_3O_2^+$ 324.0534,

found 324.0547; IR (KBr): 3421 (NH), 1702 (C=O), 1676 (C=O), 1614 (C=C) cm^{-1}; Anal. HPLC t_R 1.17 min (98.6% pure - System A), 11.80 min (97.2% pure - System B).



98

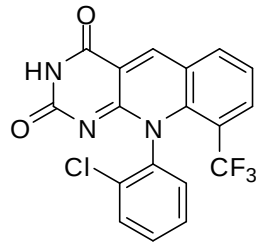
10-(2-Chlorophenyl)-9-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 105, (16.1 mg, 0.04 mmol, 16% yield) as a yellow solid with a m.pt. of 312-313 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.49-7.54 (2H, m), 7.55-7.62 (2H, m), 7.65-7.69 (1H, m), 7.89 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 7.9 Hz, C8-H), 8.27 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 7.9 Hz, C6-H), 9.15 (1H, s, C5-H), 11.28 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.24 (Cq), 120.37 (Cq), 124.35 (Cq), 126.01 (CH), 128.37 (CH), 129.89 (CH), 131.66 (CH), 132.06 (CH), 132.83 (CH), 133.50 (Cq), 136.71 (Cq), 137.01 (Cq), 139.44 (CH), 143.83 (CH), 156.60 (Cq), 160.02 (Cq), 161.82 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{10}Cl_2N_3O_2^+$ 358.0145, found 358.0148; IR (KBr): 3434 (NH), 1702 (C=O), 1675 (C=O), 1618 (C=C) cm^{-1}; Anal. HPLC t_R 2.13 min (100.0% pure - System A), 26.75 min (99.2% pure - System B).



105

10-(2-Chlorophenyl)-9-trifloromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 112, (10.7 mg, 0.02 mmol, 11% yield) as a yellow solid

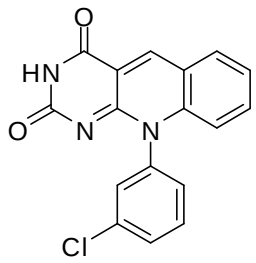
with a m.pt. of 324-325 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.50-7.64 (3H, m), 7.66-7.73 (2H, m), 8.26 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.8 Hz, C8-H), 8.56 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.8 Hz, C6-H), 9.16 (1H, s, C5-H), 11.32 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.71 (Cq), 124.08 (Cq), 125.05 (CH), 127.36 (CH), 130.31 (CH), 131.86 9 (CH), 132.52 (Cq), 134.75 (CH), 135.94 (CH), 136.01 (Cq), 136.88 (Cq), 137.86 (CH), 139.92 (Cq), 143.80 (CH), 156.52 (Cq), 160.74 (Cq), 161.78 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{10}ClF_3N_3O_2^+$ 392.0408, found 392.0449; IR (KBr): 3421 (NH), 1703 (C=O), 1679 (C=O), 1623 (C=C) cm^{-1}; Anal. HPLC t_R 2.88 min (97.8% pure - System A), 34.63 min (98.0% pure - System B).



112

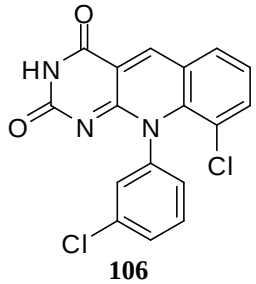
10-(3-Chlorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 99, (54.6 mg, 0.16 mmol, 55% yield) as a yellow solid with a m.pt. of 346-347 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.77 (1H, d, *J*= 7.9 Hz, C9-H), 7.43-7.47 (1H, m, Ph-H), 7.51 (1H, t, *J*= 7.9 Hz, C7-H), 7.66 (1H, s, Ph2-H), 7.71-7.78 (3H, m, C8-H & Ph-H), 8.23 (1H, d, *J*= 7.9 Hz, C6-H), 9.12 (1H, s, C5-H), 11.10 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.03 (Cq), 117.50 (CH), 121.43 (Cq), 124.99 (CH), 127.94 (CH), 129.13 (CH), 130.04 (CH), 131.89 (CH), 132.33 (CH), 134.74 (Cq), 135.67 (CH), 139.38 (Cq), 141.93 (Cq), 143.08 (CH), 156.83 (Cq), 159.13 (Cq), 162.30 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{11}ClN_3O_2^+$ 324.0534, found 324.0511; IR (KBr): 3422 (NH),

1703 (C=O), 1659 (C=O), 1614 (C=C) cm^{-1}; Anal. HPLC t_R 1.35 min (98.9% pure - System A), 8.02 min (97.5% pure - System B).



99

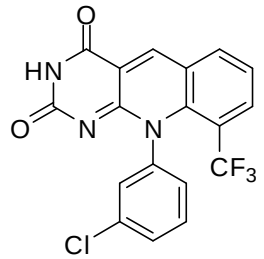
10-(3-Chlorophenyl)-9-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 106, (29.3 mg, 0.08 mmol, 30% yield) as a yellow solid, m.pt. >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.41-7.45 (1H, m, Ph-H), 7.49 (1H, t, *J*= 7.6 Hz, Ph5-H), 7.56 (1H, t, *J*= 8.1 Hz, C7-H), 7.60-7.64 (2H, m, Ph-H), 7.86 (1H, dd, ^{4}J= 1.2 Hz, ^{3}J= 8.1 Hz, C8-H), 8.25 (1H, dd, ^{4}J= 1.2 Hz, ^{3}J= 8.1 Hz, C6-H), 9.10 (1H, s, C5-H), 11.21 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.39 (Cq), 120.84 (Cq), 124.56 (Cq), 125.82 (CH), 129.40 (CH), 129.60 (CH), 130.35 (CH), 130.62 (CH), 132.46 (CH), 133.31 (Cq), 137.39 (Cq), 139.32 (CH), 140.86 (Cq), 143.46 (CH), 156.62 (Cq), 160.75 (Cq), 161.96 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{10}Cl_2N_3O_2^+$ 358.0145, found 358.0151; IR (KBr): 3416 (NH), 1715 (C=O), 1655 (C=O), 1613 (C=C) cm^{-1}; Anal. HPLC t_R 2.48 min (100.0% pure - System A), 8.60 min (98.3% pure - System B).



106

10-(3-Chlorophenyl)-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 113, (54.6 mg, 0.16 mmol, 55% yield) as a yellow solid

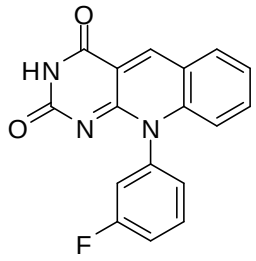
with a m.pt. of 342-343 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.37 (1H, d, *J*= 7.7 Hz, Ph-H), 7.48-7.62 (3H, m, Ph-H), 7.68 (1H, t, *J*= 7.5 Hz, C7-H), 8.21 (1H, d, *J*= 7.5 Hz, C8-H), 8.49 (1H, d, *J*= 7.5 Hz, C6-H), 9.11 (1H, s, C5-H), 11.28 (1H, s, N3-H), ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.76 (Cq), 124.45 (Cq), 125.03 (CH), 129.72 (CH), 130.04 (CH), 130.44 (CH), 131.19 (CH), 132.58 (Cq), 135.61 (CH), 135.66 (Cq), 137.06 (CH), 140.04 (Cq), 141.39 (Cq), 143.56 (CH), 156.70 (Cq), 161.16 (Cq), 161.95 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{10}ClF_3N_3O_2^+$ 392.0408, found 392.0404; IR (KBr): 3421 (NH), 1706 (C=O), 1672 (C=O), 1623 (C=C) cm^{-1}; Anal. HPLC t_R 3.54 min (97.8% pure - System A), 8.69 min (98.9% pure - System B).



113

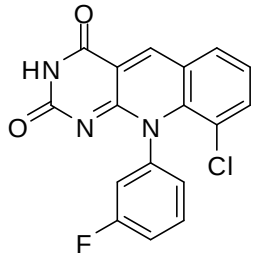
10-(3-Fluorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 100, (20.2 mg, 0.06 mmol, 20% yield) as a yellow solid with a m.pt. of 348-350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.78 (1H, d, *J*= 8.3 Hz, C9-H), 7.29-7.33 (1H, m), 7.43-7.54 (3H, m), 7.71-7.78 (2H, m), 8.23 (1H, dd, ^{4}*J*= 1.3 Hz, ^{3}*J*= 8.3 Hz, C6-H), 9.13 (1H, s, C5-H), 11.09 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.05 (Cq), 166.66 (CH, d, *J*= 24.05 Hz), 117.01 (CH, d, *J*= 20.76 Hz), 117.48 (CH), 121.43 (Cq), 124.97 (CH), 125.34 (CH, d, *J*= 2.95 Hz), 131.87 (CH), 132.39 (CH, d, *J*= 9.06 Hz), 135.65 (CH), 139.46 (Cq, d, *J*= 10.25 Hz), 141.95 (Cq), 143.04 (CH), 156.81 (Cq), 159.10 (Cq), 162.29 (Cq), 163.40 (Cq, d, *J*= 244.81 Hz); m/z $[M + H]^+$ calcd for $C_{17}H_{17}FN_3O_2^+$ 308.0830, found 308.0850; IR (KBr): 3501 (NH), 1696 (C=O),

1608 (C=O), 1561 (C=C) cm^{-1}; Anal. HPLC t_R 0.99 min (98.3% pure - System A), 9.22 min (100.0% pure - System B).



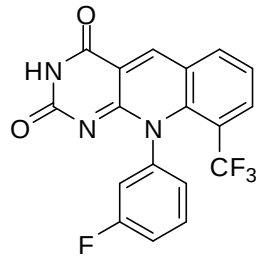
100

10-(3-Fluorophenyl)-9-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 107, (71.0 mg, 0.20 mmol, 71% yield) as a yellow solid with a m.pt. of >365 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.28 (1H, d, *J*= 7.8 Hz, Ph6-H), 7.38-7.45 (2H, m, Ph-H), 7.49 (1H, t, *J*= 7.8 Hz, C7-H), 7.53-7.60 (1H, m, Ph-H), 7.86 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 7.8 Hz, C8-H), 8.25 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 7.8 Hz, C6-H), 9.10 (1H, s, C5-H), 11.21 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.38 (Cq), 116.59 (CH, d, J= 21.21 Hz), 118.11 (CH, d, *J*= 23.80 Hz), 120.94 (Cq), 124.55 (Cq), 125.82 (CH), 126.86 (CH, d, *J*= 2.93 Hz), 130.58 (CH, d, *J*= 8.79 Hz), 132.45 (CH), 137.43 (Cq), 139.33 (CH), 140.88 (Cq, d, *J*= 10.92 Hz), 143.42 (CH), 156.64 (Cq), 160.73 (Cq), 161.96 (Cq), 162.35 (Cq, d, *J*= 243.72 Hz); m/z $[M + H]^+$ calcd for $C_{17}H_{10}ClFN_3O_2^+$ 342.0440, found 342.0453; IR (KBr): 3417 (NH), 1717 (C=O), 1658 (C=O), 1614 (C=C) cm^{-1}; Anal. HPLC t_R 1.63 min (97.0% pure - System A), 19.62 min (98.1% pure - System B).



107

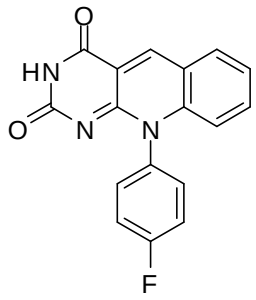
10-(3-Fluorophenyl)-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 114, (64.09 mg, 0.17 mmol, 64% yield) as a yellow solid with a m.pt. of 326-328 ^{O}C (lit 327-329 ^{O}C) [241]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.18 (1H, d, *J*= 7.9 Hz, Ph-H), 7.31-7.41 (2H, m, C7-H & Ph-H), 7.49-7.55 (1H, m, Ph-H), 7.65-7.75 (1H, m, Ph-H), 8.20 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 8.0 Hz, C8-H), 8.49 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 8.0 Hz, C6-H), 9.11 (1H, s, C5-H), 11.27 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.71 (CH, d, *J*= 21.10 Hz), 116.72 (Cq), 119.07 (CH, d, *J*= 24.58 Hz), 124.38 (Cq), 125.04 (Cq), 127.58 (Cq), 130.04 (CH, d, *J*= 8.77 Hz), 131.70 (CH), 132.17 (CH), 135.64 (CH), 137.01 (CH), 140.11 (Cq), 143.54 (CH), 156.72 (Cq), 161.11 (Cq), 161.59 (Cq, d, *J*= 243.57 Hz), 161.98 (Cq); m/z [M + H]$^{+}$ calcd for $C_{18}H_{10}F_4N_3O_2^{+}$ 376.0704, found 376.0739; IR (KBr): 3422 (NH), 1716 (C=O), 1659 (C=O), 1619 (C=C) cm^{-1}; Anal. HPLC t_R 2.23 min (98.7% pure - System A), 2.68 min (99.3% pure - System B).



114

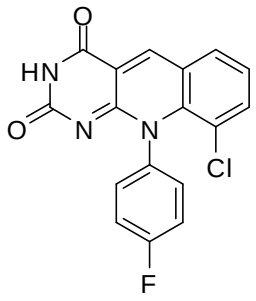
10-(4-Fluorophenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 101, (45.8 mg, 0.14 mmol, 46% yield) as a yellow solid with a m.pt. of 344-346 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 6.77 (1H, d, *J*= 8.2 Hz, C9-H), 7.46-7.57 (5H, m, C7-H & Ph-H), 7.74 (1H, ddd, ^{4}J= 1.3 Hz, ^{3}J= 8.2 Hz, C8-H), 8.23 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 8.2 Hz, C6-H), 9.12 (1H, s, C5-H), 10.86 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.09 (Cq), 117.55 (CH, d, *J*= 4.51 Hz), 117.75 (CH), 121.47 (Cq), 124.90 (CH), 131.21 (CH, d,

J= 8.47 Hz), 131.86 (CH), 134.28 (Cq, d, *J*= 2.94 Hz), 135.58 (CH), 142.35 (Cq), 142.97 (CH), 156.85 (Cq), 159.33 (Cq), 162.36 (Cq), 162.85 (Cq, d, *J*= 247.81 Hz); m/z $[M + H]^+$ calcd for $C_{17}H_{17}FN_3O_2^+$ 308.0830, found 308.0826; IR (KBr): 3417 (NH), 1715 (C=O), 1655 (C=O), 1614 (C=C) cm^{-1}; Anal. HPLC t_R 0.99 min (99.6% pure - System A), 9.15 min (100.0% pure - System B).



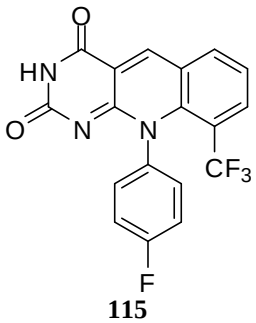
101

10-(4-Fluorophenyl)-9-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 108, (37.08 mg, 0.10 mmol, 37% yield) as a yellow solid with a m.pt. of >360 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.33-7.40 (2H, m, Ph2-H), 7.43-7.52 (3H, m, C7-H & Ph3-H), 7.85 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.9 Hz, C8-H), 8.24 (1H, dd, ^{4}J= 1.40 Hz, ^{3}J= 7.9 Hz, C6-H), 9.09 (1H, s, C5-H), 11.18 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 115.98 (CH, d, *J*= 22.72 Hz), 116.42 (Cq), 121.10 (Cq), 124.58 (Cq), 125.74 (CH), 132.37 (CH, d, *J*= 4.06 Hz), 132.43 (CH), 135.80 (Cq, d, *J*= 3.27 Hz), 137.68 (Cq), 139.28 (CH), 143.32 (CH), 156.70 (Cq), 160.94 (Cq), 161.70 (Cq, d, *J*= 244.07 Hz), 162.05 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{10}ClFN_3O_2^+$ 342.0440, found 342.0465; IR (KBr): 3416 (NH), 1716 (C=O), 1657 (C=O), 1614 (C=C) cm^{-1}; Anal. HPLC t_R 1.63 min (97.4% pure - System A), 20.08 min (98.7% pure - System B).



108

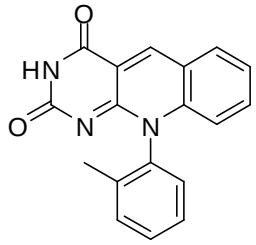
10-(4-Fluorophenyl)-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 115, (45.50 mg, 0.12 mmol, 46% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.29-7.36 (2H, m, Ph2-H), 7.38-7.45 (2H, m, Ph3-H), 7.67 (1H, t, *J*= 7.4 Hz, C7-H), 8.20 (1H, d, *J*= 7.4 Hz, C8-H), 8.49 (1H, d, *J*= 7.4 Hz, C6-H), 9.11 (1H, s, C5-H), 11.24 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 115.41 (CH, d, *J*= 23.01 Hz), 116.76 (Cq), 118.60 (Cq), 118.91 (Cq), 124.34 (Cq), 124.88 (CH), 133.52 (CH, d, *J*= 9.33 Hz), 135.57 (CH), 136.97 (CH), 140.44 (Cq), 143.42 (CH), 156.75 (Cq), 162.00 (Cq), 161.31 (Cq), 162.27 (Cq, d, *J*= 247.93 Hz); m/z $[M + H]^+$ calcd for $C_{18}H_{10}F_4N_3O_2^+$ 376.0704, found 376.0719; IR (KBr): 3420 (NH), 1716 (C=O), 1660 (C=O), 1620 (C=C) cm^{-1}; Anal. HPLC t_R 2.25 min (100.0% pure - System A), 27.65 min (100.0% pure - System B).



115

10-(2-Methylphenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 102, (45.6 mg, 0.15 mmol, 46% yield) as a yellow solid with a m.pt. of 294-296 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.42 (3H, s, Me), 6.73 (1H, d, *J*= 8.0 Hz, C9-H), 7.19-7.25 (2H, m, Ph-H), 7.44 (1H, m, Ph-H), 7.49 (1H, t, *J*= 7.4

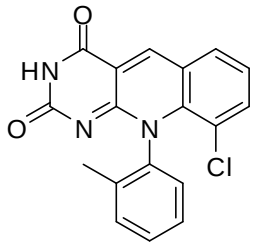
Hz, Ph-H), 7.57 (1H, t, *J*= 8.0 Hz, C7-H), 7.73 (1H, ddd, ^{4}J= 1.4 Hz, ^{3}J= 8.0 Hz, C8-H), 8.21 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 8.0 Hz, C6-H), 9.11 (1H, s, C5-H), 11.06 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.33 (CH_3), 116.05 (Cq), 117.66 (CH), 121.39 (Cq), 124.85 (CH), 125.80 (CH), 129.06 (CH), 130.45 (CH), 130.48 (CH), 131.78 (CH), 135.48 (CH), 138.06 (Cq), 140.39 (Cq), 142.22 (Cq), 142.83 (CH), 156.91 (Cq), 159.05 (Cq), 162,37 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{14}N_3O_2^+$ 304.1081, found 304.1063; IR (KBr): 3425 (NH), 1703 (C=O), 1654 (C=O), 1613 (C=C) cm^{-1}; Anal. HPLC t_R 1.27 min (100.0% pure - System A), 14.65 min (98.9% pure - System B).



102

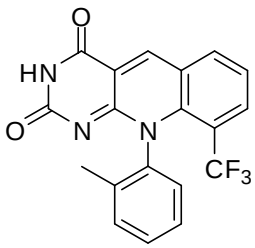
10-(2-Methylphenyl)-9-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 109, (56.5 mg, 0.16 mmol, 57% yield) as a yellow solid with a m.pt. of 292-293 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.36 (3H, s, Me), 7.18-7.24 (2H, m, Ph-H), 7.31-7.36 (1H, m, Ph-H), 7.38-7.43 (1H, m, Ph-H), 7.47 (1H, t, *J*= 7.7 Hz, C7-H), 7.83 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.7 Hz, C8-H), 8.23 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.7 Hz, C6-H), 9.08 (1H, s, C5-H), 11.16 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.30 (CH_3), 116.41 (Cq), 121.29 (Cq), 124.56 (Cq), 125.69 (CH), 127.65 (CH), 128.86 (CH), 130.08 (CH), 130.45 (CH), 132.32 (CH), 137.66 (Cq), 138.52 (Cq), 139.26 (CH), 139.48 (Cq), 143.21 (CH), 156.74 (Cq), 160.73 (Cq), 162.04 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{13}ClN_3O_2^+$ 338.0691, found 338.0718; IR (KBr): 3441 (NH), 1715

(C=O), 1656 (C=O), 1613 (C=C) cm^{-1}; Anal. HPLC t_R 2.20 min (97.6% pure - System A), 32.08 min (96.5% pure - System B).



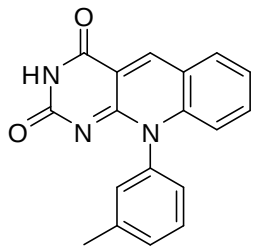
109

10-(2-Methylphenyl)-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 116, (24.0 mg, 0.6 mmol, 24% yield) as a yellow solid with a m.pt. of 347-348 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 1.89 (3H, s, Me), 7.18 (1H, d, *J*= 7.6 Hz, Ph3-H), 7.28 (1H, ddd, ^{4}J= 1.5 Hz, ^{3}J= 7.6 Hz, Ph4-H), 7.33-7.43 (2H, m, Ph-H), 7.67 (1H, t, *J*= 7.7 Hz, C7-H), 8.22 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 7.7 Hz, C8-H), 8.51 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 7.7 Hz, C6-H), 9.12 (1H, s, C5-H), 11.23 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 18.32 (CH_3), 116.89 (Cq), 117.99 (Cq), 118.62 (Cq), 124.19 (CH), 124.73 (Cq), 126.33 (CH), 129.92 (CH), 130.82 (CH), 131.19 (CH), 137.45 (CH), 137.78 (CH), 139.51 (Cq), 140.36 (Cq), 143.20 (CH), 156.86 (Cq), 160.61 (Cq), 162.10 (Cq); m/z $[M + H]^+$ calcd for $C_{19}H_{13}F_3N_3O_2^+$ 372.0954, found 372.0938; IR (KBr): 3421 (NH), 1703 (C=O), 1677 (C=O), 1623 (C=C) cm^{-1}; Anal. HPLC t_R 2.68 min (98.5% pure - System A), 6.22 min (97.5% pure - System B).



116

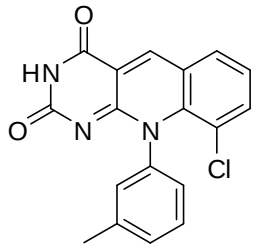
10-(3-Methylphenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 103, (20.8 mg, 0.06 mmol, 21% yield) as a yellow solid with a m.pt. of 328-329 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 1.89 (3H, s, Me), 6.64 (1H, d, *J*= 7.7 Hz, C9-H), 7.34-7.38 (1H, m, Ph-H), 7.47-7.58 (4H, m, C7-H & Ph-H), 7.74 (1H, ddd, ^{4}J= 1.4 Hz, ^{3}J= 7.7 Hz, C8-H), 8.25 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.7 Hz, C6-H), 9.14 (1H, s, C5-H), 11.08 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 17.28 (CH_3), 116.19 (Cq), 116.88 (CH), 121.54 (Cq), 125.08 (CH), 128.38 (CH), 128.70 (CH), 130.09 (CH), 131.98 (CH), 132.04 (CH),135.55 (Cq), 135.84 (CH), 137.01 (Cq), 141.30 (Cq), 142.94 (CH), 157.02 (Cq), 158.45 (Cq), 162.42 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{14}N_3O_2^+$ 304.1081, found 304.1095; IR (KBr): 3413 (NH), 1706 (C=O), 1655 (C=O), 1607 (C=C) cm^{-1}; Anal. HPLC t_R 1.12 min (97.8% pure - System A), 11.44 min (98.0% pure - System B).



103

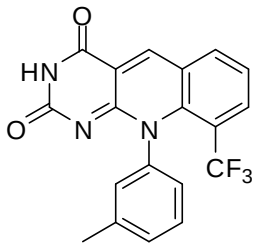
10-(3-Methylphenyl)-9-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 110, (16.9 mg, 0.05 mmol, 17% yield) as a yellow solid with a m.pt. of 342-343 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.04 (3H, s, Me), 7.19 (1H, dd, *J*= 1.0 Hz, *J*= 7.6 Hz, Ph4-H), 7.31 (1H, t, *J*= 7.6 Hz, Ph5-H), 7.36-7.45 (2H, m, Ph-H), 7.49 (1H, t, *J*= 7.8 Hz, C7-H), 7.84 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.8 Hz, C8-H), 8.25 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.8 Hz, C6-H), 9.11 (1H, s, C5-H), 11.18 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 18.10 (CH_3), 116.53 (Cq), 120.90 (Cq), 124.58 (Cq), 125.74 (CH), 127.07 (CH), 129.38

(CH), 129.83 (CH), 130.65 (CH), 132.53 (CH), 137.26 (Cq), 137.61 (Cq), 139.02 (Cq), 139.23 (CH), 143.23 (CH), 156.87 (Cq), 159.97 (Cq), 162.10 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{13}ClN_3O_2^+$ 338.0691, found 338.0653; IR (KBr): 3417 (NH), 1712 (C=O), 1671 (C=O), 1619 (C=C) cm^{-1}; Anal. HPLC t_R 1.97 min (96.8% pure - System A), 24.47 min (97.2% pure - System B).



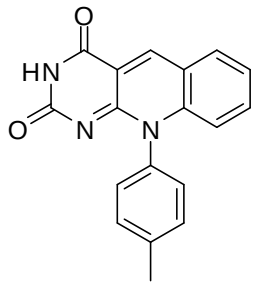
110

10-(3-Methylphenyl)-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 117, (38.6 mg, 0.10 mmol, 39% yield) as a yellow solid with a m.pt. of 296-298 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.31 (3H, s, Me), 7.08 (1H, s, Ph2-H), 7.18 (1H, d, *J*= 7.6 Hz, Ph4-H), 7.29 (1H, d, *J*= 7.6 Hz, Ph6-H), 7.36 (1H, t, *J*= 7.6 Hz, Ph5-H), 7.66 (1H, t, *J*= 7.6 Hz, C7-H), 8.18 (1H, dd, ^4J= 1.5 Hz, ^3J= 7.6 Hz, C8-H), 8.47 (1H, dd, ^4J= 1.5 Hz, ^3J= 7.6 Hz, C6-H), 9.10 (1H, s, C5-H), 11.22 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.78 (CH_3), 116.71 (Cq), 118.95 (Cq), 119.27 (Cq), 124.28 (Cq), 124.82 (CH), 128.12 (CH), 128.66 (CH), 130.10 (CH), 131.11 (CH), 135.51 (CH), 136.79 (CH), 137.96 (Cq), 140.49 (Cq), 143.29 (CH), 156.85 (Cq), 161.23 (Cq), 162.05 (Cq); m/z $[M + H]^+$ calcd for $C_{19}H_{13}F_3N_3O_2^+$ 372.0954, found 372.0954; IR (KBr): 3474 (NH), 1716 (C=O), 1657 (C=O), 1619 (C=C) cm^{-1}; Anal. HPLC t_R 3.00 min (100.0% pure - System A), 4.64 min (99.7% pure - System B).



117

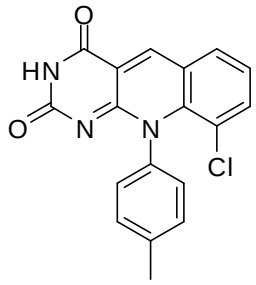
10-(4-Methylphenyl)pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 104, (48.8 mg, 0.16 mmol, 49% yield) as a yellow solid with a m.pt. of 315-316 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.46 (3H, s, Me), 6.76 (1H, d, *J*= 8.2 Hz, C9-H), 7.29 (2H, d, AA'BB' system, *J*= 8.3 Hz, Ph3-H), 7.46-7.53 (3H, m, C7-H & Ph2-H), 7.73 (1H, ddd, ^{4}J= 1.4 Hz, ^{3}J= 8.2 Hz, C8-H), 8.22 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 8.2 Hz, C6-H), 9.11 (1H, s, C5-H), 11.04 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.30 (CH_3), 116.07 (Cq), 117.67 (CH), 121.41 (Cq), 124.86 (CH), 128.58 (CH), 131.16 (CH), 131.82 (CH), 135.43 (CH), 135.53 (Cq), 139.39 (Cq), 142.41 (Cq), 142.78 (CH), 156.87 (Cq), 159.19 (Cq), 162.43 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{14}N_3O_2^+$ 304.1081, found 304.1082; IR (KBr): 3437 (NH), 1698 (C=O), 1671 (C=O), 1611 (C=C) cm^{-1}; Anal. HPLC t_R 1.34 min (98.6% pure - System A), 16.20 min (100.0% pure - System B).



104

10-(4-Methylphenyl)-9-chloropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 111, (19.0 mg, 0.05 mmol, 19% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.42 (3H, s, Me), 7.25 (2H, d,

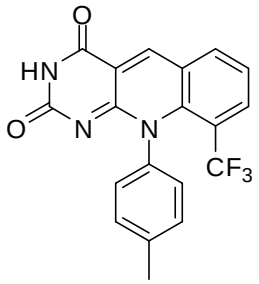
AA'BB' system, *J*= 8.2 Hz, Ph3-H), 7.31 (2H, d, AA'BB' system, *J*= 8.2 Hz, Ph2-H), 7.47 (1H, t, *J*= 7.7 Hz, C7-H), 7.82 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.7 Hz, C8-H), 8.22 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.7 Hz, C6-H), 9.07 (1H, s, C5-H), 11.15 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.38 (CH_3), 116.39 (Cq), 121.40 (Cq), 124.57 (Cq), 125.63 (CH), 129.58 (CH), 130.09 (CH), 132.31 (CH), 137.05 (Cq), 137.83 (Cq), 138.88 (Cq), 139.22 (CH), 143.17 (CH), 156.76 (Cq), 160.88 (Cq), 162.16 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{13}ClN_3O_2^+$ 338.0691, found 338.0701; IR (KBr): 3418 (NH), 1717 (C=O), 1660 (C=O), 1615 (C=C) cm^{-1}; Anal. HPLC t_R 2.38 min (97.6% pure - System A), 5.90 min (100.0% pure - System B).



111

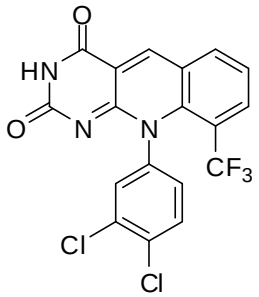
10-(4-Methylphenyl)-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 118, (52.1 mg, 0.14 mmol, 52% yield) as a yellow solid with a m.pt. of 344-346 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.38 (3H, s, Me), 7.19 (2H, d, AA'BB' system, *J*= 8.4 Hz, Ph3-H), 7.27 (2H, d, AA'BB' system, *J*= 8.4 Hz, Ph2-H), 7.65 (1H, t, *J*= 7.9 Hz, C7-H), 8.17 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 7.9 Hz, C8-H), 8.47 (1H, dd, ^{4}J= 1.3 Hz, ^{3}J= 7.9 Hz, C6-H), 9.09 (1H, s, C5-H), 11.21 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.27 (CH_3), 116.72 (Cq), 118.94 (Cq), 124.30 (Cq), 124.80 (CH), 128.94 (CH), 130.92 (CH), 135.52 (CH), 136.79 (CH), 138.18 (Cq), 139.12 (Cq), 140.56 (Cq), 143.28 (CH), 156.80 (Cq), 161.29 (Cq), 162.07 (Cq); m/z $[M + H]^+$ calcd for $C_{19}H_{13}F_3N_3O_2^+$ 372.0954, found 372.0957; IR (KBr): 3438

(NH), 1706 (C=O), 1681 (C=O), 1622 (C=C) cm^{-1}; Anal. HPLC t_R 3.13 min (100.0% pure - System A), 8.57 min (99.7% pure - System B).



118

10-(3,4-Dichlorophenyl)-9-trifluoromethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 119, (62.3 mg, 0.14 mmol, 62% yield) as a yellow solid with a m.pt. of 320-322 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.46 (1H, dd, ^{4}J= 1.1 Hz, ^{3}J= 8.6 Hz, Ph6-H), 7.69 (1H, t, J= 7.8 Hz, C7-H), 7.73 (1H, d, ^{4}J= 1.1 Hz, Ph2-H), 7.81 (1H, d, J= 8.6 Hz, Ph5-H), 8.23 (1H, dd, ^{4}J= 1.6 Hz, ^{3}J= 7.8 Hz, C8-H), 8.50 (1H, dd, ^{4}J= 1.6 Hz, ^{3}J= 7.8 Hz, C6-H), 9.12 (1H, s, C5-H), 11.30 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.71 (Cq), 118.33 (Cq), 121.67 (Cq), 124.46 (Cq), 125.13 (CH), 130.37 (CH), 130.94 (Cq), 132.28 (CH), 132.58 (Cq), 133.14 (CH), 135.71 (CH), 137.30 (CH), 139.84 (Cq), 143.71 (CH), 156.61 (Cq), 161.14 (Cq), 161.88 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_9Cl_2F_3N_3O_2^+$ 426.0018, found 426.0034; IR (KBr): 3486 (NH), 1705 (C=O), 1655 (C=O), 1609 (C=C) cm^{-1}; Anal. HPLC t_R 6.40 min (99.4% pure - System A), 6.35 min (98.7% pure - System B).

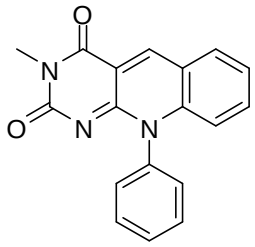


119

General Synthesis of 10-Phenyl-3-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione Analogues

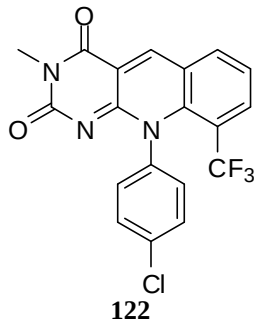
To 1 equivalent of the desired 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogue in methanol (20 mL) was added 2.05 equivalents of sodium methoxide. After stirring for 30 min, 4 equivalents of methyl iodide was added and refluxed for 24 h. Concentration of the solution under reduced pressure and separation by preparative thin layer chromatography using 98% dichloromethane and 2% methanol to yield the pure desired 10-phenyl-3-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogue.

10-Phenyl-3-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 120, (17.4 mg, 0.05 mmol, 33% yield) as a yellow solid with a m.pt. of >350 ^{O}C (lit >360 ^{O}C) [271]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 3.23 (3H, s, Me), 6.74 (1H, d, *J*= 8.2 Hz, C9-H), 7.40-7.78 (4H, m, C7-H & Ph-H), 7.62-7.73 (2H, m, Ph2-H), 7.75 (ddd, ^{4}J= 1.5 Hz, ^{3}J= 8.2 Hz, C8-H), 8.28 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 8.2 Hz, C6-H), 9.19 (1H, s, C5-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 28.01 (CH_3), 115.60 (CH), 117.48 (Cq), 121.61 (Cq), 124.99 (CH), 128.81 (CH), 129.94 (CH), 130.82 (CH), 131.88 (CH), 135.58 (CH), 137.83 (Cq), 142.09 (Cq), 143.47 (CH), 156.30 (Cq), 157.62 (Cq), 161.94 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{14}N_3O_2^+$ 304.1081, found 304.1066; IR (KBr): 1637 (C=O), 1616 (C=O), 1566 (C=C) cm^{-1}; Anal. HPLC t_R 1.40 min (97.9% pure - System A), 14.05 min (100.0% pure - System B).



120

10-(4-Chlorophenyl)-9-trifluoromethyl-3-methylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 122, (6.8 mg, 0.01 mmol, 22% yield) as a yellow solid with a m.pt. of >360 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 5.75 (3H, s, Me), 7.39 (2H, d, AA'BB' system, *J*= 8.7 Hz, Ph2-H), 7.58 (2H, d, AA'BB' system, *J*= 8.7 Hz, Ph3-H), 7.70 (1H, t, *J*= 7.8 Hz, C7-H), 8.23 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.8 Hz, C8-H), 8.54 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.8 Hz, C6-H), 9.18 (1H, s, C5-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.08 (CH_3), 115.84 (Cq), 118.34 (Cq), 123.98 (Cq), 125.12 (CH), 128.68 (CH), 133.86 (CH), 134.57 (Cq), 135.21 (CH), 137.77 (CH), 139.85 (Cq), 140.45 (Cq), 144.17 (CH), 157.04 (Cq), 161.84 (Cq), 162.27 (Cq); m/z $[M + H]^+$ calcd for $C_{19}H_{12}ClF_3N_3O_2^+$ 406.0565, found 406.0571; IR (KBr): 1700 (C=O), 1681 (C=O), 1620 (C=C) cm^{-1}; Anal. HPLC t_R 6.43 min (99.1% pure - System A), 5.12 min (96.8% pure - System B).



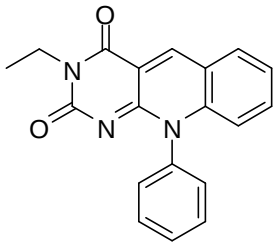
122

General Synthesis of 10-Phenyl-3-ethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione Analogues

To 1 equivalent of the desired 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogues in methanol (20 mL) was added 2.05 equivalents of sodium ethoxide. After stirring for 30 min, 4 equivalents of ethyl iodide were added and refluxed for 24 h. Concentration of the solution under reduced pressure and separation by preparative thin layer chromatography using 98%

dichloromethane and 2% methanol to yield the pure desired 3-ethyl-10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogue.

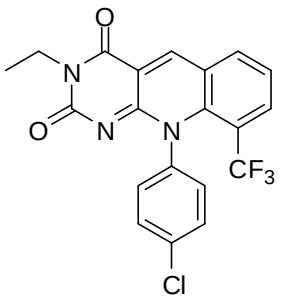
10-Phenyl-3-ethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 121, (10.0 mg, 0.03 mmol, 18% yield) as a yellow solid with a m.pt. >360 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 1.11 (3H, t, *J*= 7.0 Hz, Me), 3.89 (2H, q, *J*= 7.0 Hz, CH_2), 6.73 (1H, d, *J*= 8.0 Hz, C9-H), 7.43 (2H, d, *J*= 7.4 Hz, Ph2-H), 7.51 (1H, t, *J*= 8.0 Hz, C7-H), 7.61-7.80 (4H, m, C8-H & Ph-H), 8.27 (1H, dd, ^{4}J= 1.2 Hz, ^{3}J= 8.0 Hz, C6-H), 9.18 (1H, s, C5-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 8.40 (CH_3), 13.48 (CH_2), 115.83 (CH), 118.05 (Cq), 122.16 (Cq), 125.57 (CH), 127.93 (CH), 130.09 (CH), 131.13 (CH), 131.77 (CH), 135.51 (CH), 138.03 (Cq), 142.25 (Cq), 143.67 (CH), 155.20 (Cq), 158.32 (Cq), 162.93 (Cq); m/z $[M + H]^+$ calcd for $C_{19}H_{16}N_3O_2^+$ 318.1237, found 318.1251; IR (KBr): 1692 (C=O), 1638 (C=O), 1623 (C=C) cm^{-1}; Anal. HPLC t_R 1.99 min (100.0% pure - System A), 22.15 min (100.0% pure - System B).



121

10-(4-Chlorophenyl)-9-trifluoromethyl-3-ethylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 123, (9.3 mg, 0.02 mmol, 43% yield) as a yellow solid with a m.pt. of 278-280 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 1.10 (3H, t, *J*= 7.9 Hz, Me), 3.87 (2H, q, *J*= 7.9 Hz, CH_2), 7.39 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph2-H), 7.57 (2H, d, AA'BB' system, *J*= 8.6 Hz, Ph3-H), 7.69 (1H, t, *J*= 7.8 Hz, C7-H), 8.22 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.8 Hz, C8-H), 8.53 (1H, dd, ^{4}J= 1.4 Hz, ^{3}J= 7.8 Hz, C6-H), 9.17 (1H, s, C5-H); ^{13}C NMR (75

MHz, DMSO-d_6): δ/ppm 9.31 (CH_3), 14.88 (CH_2), 115.88 (Cq), 117.50 (Cq), 124.28 (Cq), 125.11 (CH), 128.52 (CH), 133.32 (CH), 134.67 (Cq), 136.16 (CH), 137.61 (CH), 139.47 (Cq), 140.94 (Cq), 144.28 (CH), 157.10 (Cq), 161.74 (Cq), 162.16 (Cq); m/z $[M + H]^+$ calcd for $C_{20}H_{14}ClF_3N_3O_2^+$ 420.0721, found 420.0692; IR (KBr): 1646 (C=O), 1611 (C=O), 1538 (C=C) cm^{-1}; Anal. HPLC t_R 0.75 min (98.3% pure - System A), 19.62 min (99.4% pure - System B).



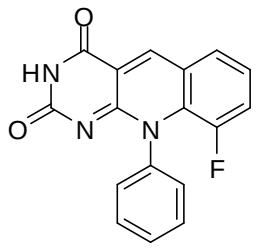
123

General Procedure to Synthesise 10-Phenyl-9-Substituted-pyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione Analogues [241, 261, 264, 265, 268, 270]

To 1 equivalent of the desired 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione analogue in DMF was added 1.2 equivalents of the desired 2-halobenzaldehyde (chloro or fluoro). The mixture was heated at 160 ^{O}C for 30 min in the microwave reactor or refluxed for 4 h. Concentration of the solution under reduced pressure and separation by dry column flash chromatography using 95% dichloromethane and 5% methanol to yield the pure desired 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogue.

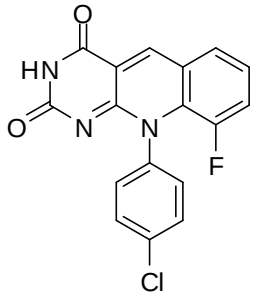
10-Phenyl-9-fluoropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 124, (29.3 mg, 0.17 mmol, 29% yield) as a yellow solid with a m.pt. of 260-262 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.43-7.57 (6H, m, C8-H & Ph-H), 7.60 (1H, ddd, ^4J= 1.5 Hz, ^3J= 6.9 Hz, C7-H), 8.07 (1H, d, J= 6.9 Hz, C6-H),

9.12 (1H, s, C5-H), 11.14 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.78 (Cq), 122.61 (CH, d, *J*= 21.93 Hz), 123.84 (Cq), 125.42 (CH, d, *J*= 8.01 Hz), 128.22 (CH, d, *J*= 4.70 Hz), 128.41 (CH, d, *J*= 3.77 Hz), 129.14 (CH), 129.41 (CH), 130.32 (Cq, d, *J*= 3.79 Hz), 140.98 (Cq, d, *J*= 3.88 Hz), 142.83 (CH, d, *J*= 2.21 Hz), 150.08 (Cq, d, *J*= 250.69 Hz), 156.69 (Cq), 159.98 (Cq), 162.10 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{11}FN_3O_2^+$ 308.0830, found 308.0813; IR (KBr): 3410 (NH), 1703 (C=O), 1667 (C=O), 1613 (C=C) cm^{-1}; Anal. HPLC t_R 1.02 min (99.5% pure - System A), 9.57 min (97.6% pure - System B).



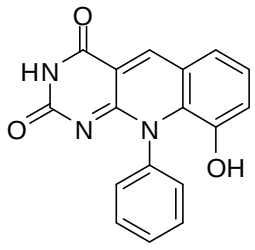
124

10-(4-Chlorophenyl)-9-fluoropyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 126, (20.6 mg, 0.06 mmol, 21% yield) as a yellow solid with a m.pt. of 268-269 ^{O}C (dec); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.46-7.52 (3H, m, C7-H & Ph2-H), 7.59-7.66 (3H, m, C8 & Ph3-H), 8.07 (1H, d, *J*= 7.8 Hz, C6-H), 9.12 (1H, s, C5-H), 11.16 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 116.76 (Cq), 122.65 (CH, d, *J*= 21.88 Hz), 123.84 (Cq), 125.49 (CH, d, *J*= 8.02 Hz), 128.48 (CH, d, *J*= 3.47 Hz), 129.50 (CH), 130.15 (Cq, d, *J*= 4.53 Hz), 130.25 (CH, d, *J*= 4.47 Hz), 133.82 (Cq), 139.84 (Cq, d, *J*= 3.79 Hz), 142.97 (CH), 150.02 (Cq, d, *J*= 251.49 Hz), 156.64 (Cq), 160.05 (Cq), 162.08 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{10}ClFN_3O_2^+$ 342.0440, found 342.0435; IR (KBr): 3431 (NH), 1716 (C=O), 1657 (C=O), 1614 (C=C) cm^{-1}; Anal. HPLC t_R 1.82 min (99.8% pure - System A), 19.97 min (98.2% pure - System B).



126

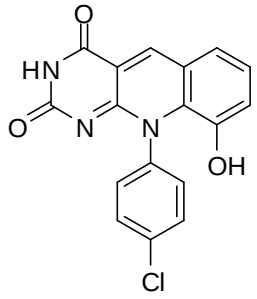
10-Phenyl-9-hydroxypyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 128, (44.6 mg, 0.14 mmol, 45% yield) as a yellow solid with a m.pt. of 207-208 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.09 (1H, dd, ^{4}J= 1.2 Hz, ^{3}J= 7.8 Hz, C8-H), 7.28-7.34 (3H, m), 7.41-7.48 (3H, m), 7.65 (1H, dd, ^{4}J= 1.2 Hz, ^{3}J= 7.8 Hz, C6-H), 9.00 (1H, s, C5-H), 10.00 (1H, s, OH), 10.98 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 115.68 (Cq), 121.79 (CH), 122.63 (CH), 123.67 (Cq), 125.70 (CH), 128.06 (CH), 128.35 (CH), 128.70 (CH), 130.24 (Cq), 142.38 (Cq), 143.58 (Cq), 147.07 (Cq), 156.78 (Cq), 159.96 (Cq), 162.42 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{12}N_3O_3^+$ 306.0873, found 306.0844; IR (KBr): 3475 (NH), 1695 (C=O), 1650 (C=O), 1602 (C=C) cm^{-1}; Anal. HPLC t_R 0.63 min (100.0% pure - System A), 6.18 min (97.4% pure - System B).



128

10-(4-Chlorophenyl)-9-hydroxypyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 130, (17.50 mg, 0.05 mmol, 9% yield) as a yellow solid with a m.pt. of 316-318 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.08 (1H, d, *J*= 7.6 Hz, C8-H), 7.31 (1H, t, *J*= 7.6 Hz, C7-H), 7.37 (2H, d, AA'BB' system, *J*= 8.2 Hz, Ph2-H), 7.50 (2H, d, AA'BB' system, *J*= 8.2 Hz, Ph3-H), 7.76 (1H, d, *J*= 7.6

Hz, C6-H), 8.99 (1H, s, C5-H), 10.16 (1H, s, OH), 11.00 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 115.68 (Cq), 121.84 (CH), 122.70 (CH), 123.69 (Cq), 125.79 (CH), 128.34 (CH), 130.05 (Cq), 130.64 (CH), 132.74 (Cq), 141.26 (Cq), 143.71 (CH), 146.95 (Cq), 156.70 (Cq), 159.95 (Cq), 162.34 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{11}ClN_3O_3^+$ 340.0740, found 340.0713; IR (KBr): 3434 (NH), 1702 (C=O), 1655 (C=O), 1603 (C=C) cm^{-1}; Anal. HPLC t_R 0.80 min (99.8% pure - System A), 10.85 min (100.0% pure - System B).



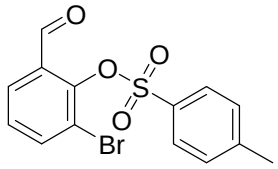
130

Synthesis of 2-*p*-Toluenesulfonyloxy-3-bromobenzaldehyde, 145 [301-303]

A mixture of 2-hydroxy-3-bromobenzaldehyde (565.81 mg, 0.76 mL; 2.81 mmol) and sodium carbonate (2.31 g; 21.85 mmol) in acetone (20 mL) was stirred at room temperature for 30 min under a nitrogen atmosphere. Tosyl chloride (1.07 g; 5.62 mmol) dissolved in acetone (25 mL) was added to the reaction mixture and refluxed for 5 h. Concentration of the solution under reduced pressure and dry column flash chromatography using 66.6% petroleum ether 33.3% and diethyl ether was carried out to yield the pure 2-*p*-toluenesulfonyloxy-3-bromobenzaldehyde, **145**.

2-*p*-Toluenesulfonyloxy-3-bromobenzaldehyde, 145 (0.82 g, 2.32 mmol, 82% yield) as a white solid with a m.pt. of 79-91 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.46 (3H, s, Me), 7.44-7.58 (3H, m), 7.77-7.88 (3H, m),

8.05 (1H, d, *J*= 7.3 Hz, C4-H), 9.86 (1H, s, COH); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.74 (CH_3), 118.58 (Cq), 128.40 (CH), 129.17 (CH), 129.94 (CH), 131.02 (CH), 131.28 (Cq), 132.40 (Cq), 139.98 (CH), 147.44 (Cq), 147.65 (Cq), 187.53 (CH).



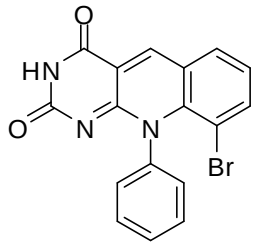
145

General Procedure to Synthesise 10-Phenyl-9-bromopyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione Analogues using 2-p-Toluenesulfonyloxy-3-bromobenzaldehyde, 145 [241, 261, 264, 265, 268, 270]

To 1 equivalent of the desired 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione analogue in DMF was added 1.2 equivalents of the 2-*p*-toluenesulfonyloxy-3-bromobenzaldehyde, **145**. The mixture was heated at 160 ^{O}C for 30 min in the microwave reactor or refluxed for 4 h. Concentration of the solution under reduced pressure and separation by dry column flash chromatography using 95% dichloromethane and 5% methanol to yield the pure desired 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogue.

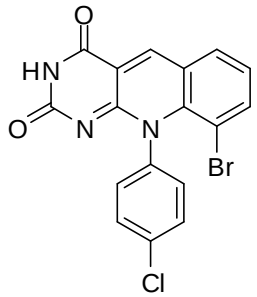
10-Phenyl-9-bromopyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 125, (29.3 mg, 0.07 mmol, 29% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.37-7.42 (3H, m), 7.51-7.55 (3H, m), 8.06 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.8 Hz, C8-H), 8.27 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.8 Hz, C6-H), 9.06 (1H, s, C5-H), 11.16 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 109.28 (Cq), 116.31 (Cq), 124.76 (Cq), 126.06 (CH), 129.11 (CH), 129.63 (CH), 131.02 (Cq), 131.15 (CH), 132.87 (CH), 138.75 (CH), 138.88 (Cq), 143.19 (CH), 156.73 (Cq), 160.73 (Cq), 162.06 (Cq); m/z

$[M + H]^+$ calcd for $C_{17}H_{11}BrN_3O_2^+$ 368.0029, found 367.9998; IR (KBr): 3413 (NH), 1717 (C=O), 1658 (C=O), 1614 (C=C) cm^{-1}; Anal. HPLC t_R 1.55 min (98.5% pure - System A), 21.07 min (99.2% pure - System B).



125

10-(4-Chlorophenyl)-9-bromopyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 127, (36.4 mg, 0.09 mmol, 36% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.40 (1H, t, *J*= 7.7 Hz, C7-H), 7.44 (2H, d, AA'BB' system, *J*= 8.7 Hz, Ph2-H), 7.61 (2H, d, AA'BB' system, *J*= 8.7 Hz, Ph3-H), 8.08 (1H, dd, ^4J= 1.5 Hz, ^3J= 7.7 Hz, C8-H), 8.27 (1H, dd, ^4J= 1.5 Hz, ^3J= 7.7 Hz, C6-H), 9.06 (1H, s, C5-H), 11.19 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 109.12 (Cq), 116.30 (Cq), 124.76 (Cq), 126.15 (CH), 129.18 (CH), 132.95 (CH), 132.96 (CH), 134.33 (Cq), 137.72 (Cq), 138.72 (Cq), 143.19 (CH), 143.34 (CH), 156.68 (Cq), 160.84 (Cq), 162.04 (Cq); m/z $[M + H]^+$ calcd for $C_{17}H_{10}BrClN_3O_2^+$ 401.9639, found 401.9613; IR (KBr): 3439 (NH), 1714 (C=O), 1655 (C=O), 1610 (C=C) cm^{-1}; Anal. HPLC t_R 2.73 min (97.6% pure - System A), 4.17 min (100.0% pure - System B).

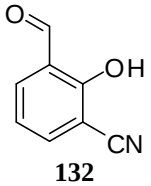


127

Synthesis of 3-Cyano-2-hydroxybenzaldehyde, 132 [286, 287]

To a warm solution of sodium hydroxide (40.29 g; 1007.38 mmol) in water (250 mL) was added 2-hydroxybenzonitrile (15.00 g; 125.92 mmol) under mechanical stirring. After the addition the temperature was raised to 70 ^{O}C and chloroform (30.06 g, 20.31mL; 251.81 mmol) was added slowly drop wise over 1 h. The reaction mixture temperature was kept at 70 ^{O}C with mechanical stirring for a further 3 h. After heating, the reaction mixture was cooled to room temperature and acidified by sulfuric acid to pH 1. Diethyl ether (300 mL) was added and liquid extraction carried out with the diethyl ether layer treated with magnesium sulfate and filtered. Concentration of the solution under reduced pressure and separation by dry column flash chromatography using 95% dichloromethane and 5% methanol was carried out to yield the pure 3-cyano-2-hydroxy-benzaldehyde, **132**.

3-Cyano-2-hydroxybenzaldehyde, 132 (0.47 g, 3.22 mmol, 3% yield) as a white solid with a m.pt. of 114-115 ^{O}C (lit 113-114 ^{O}C) [287]; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.20 (1H, t, *J*= 7.8 Hz, C5-H), 8.01-8.06 (2H, m), 10.14 (1H, s, COH), 11.73 (1H, br s, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 102.25 (Cq), 116.02 (Cq), 121.07 (CH), 123.31 (Cq), 137.26 (CH), 140.36 (CH), 162.04 (Cq), 194.25 (CH); IR (KBr): 3411 (OH), 2235 (CN), 1668 (C=O)cm^{-1}.

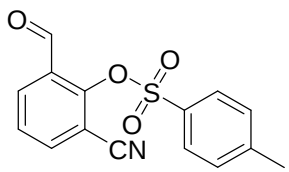


132

Synthesis of 2-*p*-Toluenesulfonyloxy-3-cyanobenzaldehyde, 146 [301-303]

A mixture of 3-Cyano-2-hydroxybenzaldehyde, **132** (250.00 mg; 1.69 mmol), and sodium carbonate (720.45 mg; 6.79 mmol) in acetone (50 mL) was stirred at room temperature for 30 min under nitrogen atmosphere. Tosyl Chloride (647.86 mg; 3.39 mmol) dissolved in acetone (25 mL) was added to the reaction mixture and refluxed overnight. Concentration of the solution under reduced pressure and dry column flash chromatography using 66.6% petroleum ether and 33.3% diethyl ether was carried out to yield the pure 2-*p*-toluenesulfonyloxy-3-cyanobenzaldehyde, **146**.

2-*p*-Toluenesulfonyloxy-3-cyanobenzaldehyde, 146 (481.2 mg, 1.59 mmol, 94% yield) as a white solid with a m.pt. of 84-86 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 2.46 (3H, s, Me), 7.55 (2H, d, AA'BB' system, *J*= 8.5 Hz, Ph3-H), 7.75 (1H, t, *J*= 7.6 Hz, C5-H), 7.82 (2H, d, AA'BB' system, *J*= 8.5 Hz, Ph2-H), 8.15 (1H, dd, *J*=1.76 Hz, *J*= 7.6 Hz, C6-H), 8.28 (1H, dd, *J*= 1.76 Hz, *J*= 7.6 Hz, C4-H), 9.84 (1H, s, COH); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 21.76 (CH_3), 109.73 (Cq), 114.46 (Cq), 129.27 (CH), 129.55 (CH), 129.95 (Cq), 131.22 (Cq), 131.25 (CH), 133.78 (CH), 140.01 (CH), 148.06 (Cq), 150.68 (Cq), 186.55 (CH).

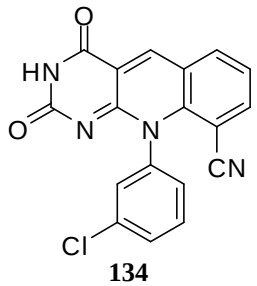


146

General Procedure to Synthesise 10-Phenyl-9-cyanopyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione Analogues using 2-*p*-Toluenesulfonyloxy-3-cyanobenzaldehyde, 146 [241, 261, 264, 265, 268, 270]

To 1 equivalent of the desired 6-(phenylamino)pyrimidine-2,4(1*H*,3*H*)-dione analogue in DMF was added 1.2 equivalents of 2-*p*-toluenesulfonyloxy-3-cyanobenzaldehyde, **146**. The mixture was heated at 160 ^{O}C for 30 min in the microwave reactor or refluxed for 4 h. Concentration of the solution under reduced pressure and separation by flash chromatography using 95% dichloromethane 5% methanol to yield the pure desired 10-phenylpyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione analogue .

10-(3-Chlorophenyl)-9-cyanopyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 134, (96.0 mg, 0.27 mmol, 64% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.53 (1H, d, *J*= 8.1 Hz, Ph6-H), 7.61-7.66 (2H, m), 7.72-7.76 (2H, m), 8.23 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.8 Hz, C8-H), 8.55 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.8 Hz, C6-H), 9.16 (1H, s, C5-H), 11.28 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 101.06 (Cq), 114.89 (Cq), 117.10 (Cq), 122.87 (Cq), 124.90 (CH), 129.74 (CH), 130.76 (CH), 131.44 (CH), 131.78 (CH), 134.32 (Cq), 137.80 (CH), 139.07 (Cq), 141.37 (Cq), 142.98 (CH), 143.66 (CH), 156.55 (Cq), 160.12 (Cq), 161.90 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{10}ClN_4O_2^+$ 349.0687, found 349.0627; IR (KBr): 3420 (NH), 2213 (CN), 1703 (C=O), 1664 (C=O), 1619 (C=C) cm^{-1}; Anal. HPLC t_R 0.90 min (99.5% pure - System A), 6.78 min (98.7% pure - System B).



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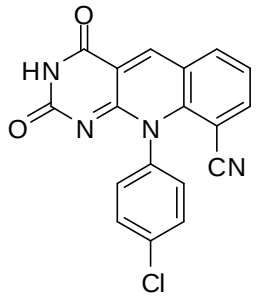
10-(4-Chlorophenyl)-9-cyanopyrimido[4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione, 131, (37.2 mg, 0.10 mmol, 25% yield) as a yellow solid with a m.pt. of >350 ^{O}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.58 (2H, d, AA'BB' system, *J*= 8.7 Hz, Ph2-H), 7.62 (1H, t, *J*= 7.7 Hz, C7-H), 7.68 (2H, d, AA'BB' system, *J*= 8.7 Hz, Ph3-H), 8.23 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.7 Hz, C8-H), 8.54 (1H, dd, ^{4}J= 1.5 Hz, ^{3}J= 7.7 Hz, C6-H), 9.15 (1H, s, C5-H), 11.26 (1H, s, N3-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ/ppm 101.15 (Cq), 114.87 (Cq), 117.12 (Cq), 122.88 (Cq), 124.85 (CH), 130.23 (CH), 132.75 (CH), 135.99 (Cq), 136.81 (Cq), 137.69 (CH), 141.47 (Cq), 142.95 (CH), 143.56 (CH), 156.56 (Cq), 160.23 (Cq), 161.88 (Cq); m/z $[M + H]^+$ calcd for $C_{18}H_{10}ClN_4O_2^+$ 349.0687, found 349.0627; IR (KBr): 3416 (NH), 2198 (CN), 1704 (C=O), 1666 (C=O), 1621 (C=C) cm^{-1}; Anal. HPLC t_R 0.92 min (100.0% pure - System A), 4.92 min (100.0% pure - System B).



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Appendix

Analytical HPLC Retention Times

Waters 2525 Binary Gradient Module was used for analytical HPLC with a Waters 2487 Dual λ Absorbance UV Detector. Analytical HPLC was used to confirm purity of greater than 95%, by chromatogram intergration at 254 nm wavelength, of final compounds using System A and System B. All retention times are quoted in minutes.

System A: Phenomenex Onyx Monolithic reverse phase C18 column (100 x 3.0mm) with a flow rate of 3.00 mL/min and UV detection at 254 nm using 70% water and 30% acetonitrile over 10 minutes.

System B: Phenomenex Kromasil reverse phase C18 column (250 x 4.6 mm) with a flow rate of 1.00 mL/min and UV detection at 254 nm using 50% water and 50% methanol over 35 min.

Compound Number	System A		System B	
	Retention Time (min)	Purity (%)	Retention Time (min)	Purity (%)
64	0.91	99.5	3.55	100
66	1.69	100.0	4.59	98.5
65	1.11	98.6	3.72	97.6
40	2.30	97.4	5.45	100.0
41	3.36	100.0	6.30	99.6
42	3.15	96.2	6.02	97.8
43	2.12	100.0	5.20	100.0
44	2.55	100.0	5.87	99.8
45	3.47	98.2	6.74	96.8
46	3.59	97.3	6.42	100.0
47	2.52	98.0	5.62	97.5
48	3.98	100.0	7.47	96.2
49	7.15	97.5	9.10	100.0
50	6.18	100.0	8.60	97.4
51	4.37	100.0	7.17	98.2

Compound Number	System A		System B	
	Retention Time (min)	Purity (%)	Retention Time (min)	Purity (%)
28	1.49	98.4	4.52	96.3
29	1.85	97.5	4.97	98.4
30	1.68	96.2	4.82	97.5
31	1.53	97.2	4.59	100.0
32	1.70	100.0	4.89	96.8
33	2.05	100.0	5.34	98.6
34	1.93	98.4	5.10	97.3
35	1.80	96.8	4.95	99.1
36	2.50	99.6	6.05	100.0
37	3.42	100.0	6.90	98.6
38	3.54	100.0	6.82	100.0
39	3.10	100.0	6.34	97.2
17	0.92	99.1	4.37	98.6
21	1.49	100.0	4.60	97.2
25	0.90	97.3	5.80	100.0
52	1.18	98.8	4.03	97.7
53	1.37	96.2	4.24	96.8
56	1.34	100.0	4.28	99.0
57	1.53	98.1	4.49	100.0
60	2.00	97.2	5.37	98.2
61	2.50	97.4	5.74	99.1
23	1.10	100.0	3.98	97.6
27	1.63	98.4	4.75	100.0
54	1.20	100.0	4.02	97.8
55	1.28	97.2	14.40	97.9
58	1.34	100.0	5.09	98.8
59	1.40	97.4	15.64	96.8
62	2.07	98.9	8.40	99.8
63	2.12	100.0	2.70	98.4
16	2.43	98.3	7.55	97.9
18	1.28	99.8	10.47	100.0
20	1.20	97.8	9.28	98.7
22	1.37	100.0	4.47	98.7
24	1.75	98.7	5.22	99.0
26	2.13	97.4	5.42	100.0
90	0.72	100.0	3.05	98.9
91	1.59	99.2	6.74	100.0
92	0.60	100.0	4.05	97.8
93	0.79	99.4	7.95	99.6
94	1.17	100.0	12.82	99.2
95	2.34	97.5	8.22	96.8
98	1.17	98.6	11.80	97.2
105	2.13	100.0	26.75	99.2
112	2.88	97.8	34.63	98.0
99	1.35	98.9	8.02	97.5

Compound Number	System A		System B	
	Retention Time (min)	Purity (%)	Retention Time (min)	Purity (%)
106	2.48	100.0	8.60	98.3
113	3.54	97.8	8.69	98.9
100	0.99	98.3	9.22	100.0
107	1.63	97.0	19.62	98.1
114	2.23	98.7	2.68	99.3
101	0.99	99.6	9.15	100.0
108	1.63	97.4	20.08	98.7
115	2.25	100.0	27.65	100.0
102	1.27	100.0	14.65	98.9
109	2.20	97.6	32.08	96.5
116	2.68	98.5	6.22	97.5
103	1.12	97.8	11.44	98.0
110	1.97	96.8	24.47	97.2
117	3.00	100.0	4.64	99.7
104	1.34	98.6	16.20	100.0
111	2.38	97.6	5.90	100.0
118	3.13	100.0	8.57	99.7
119	6.40	99.4	6.35	98.7
120	1.40	97.9	14.05	100.0
122	6.43	99.1	5.12	96.8
121	1.99	100.0	22.15	100.0
123	0.75	98.3	19.62	99.4
124	1.02	99.5	9.57	97.6
126	1.82	99.8	19.97	98.2
128	0.63	100.0	6.18	97.4
130	0.80	99.8	10.85	100.0
125	1.55	98.5	21.07	99.2
127	2.73	97.6	4.17	100.0
134	0.90	99.5	6.78	98.7
131	0.92	100.0	4.92	100.0

Codes

Overleaf is a table of codes used throughout my work.

The thesis number is the number given to the compound as the compound appears in this PhD thesis. The compound number is the unique number for that chemical. The reaction number relates to a specific reaction or batch which can be found in my lab book by the lab book page. The data number connects the hardcopy print out of characterisation data with the location in the data folder.

I have included this information so that in the future anyone can find the compounds, look at the characterisation data or repeat the reaction by looking in my lab book.

All compounds synthesised are stored in sample vials labelled with compound number and reaction number in boxes labelled Michael Dickens in the Centre for Biomolecular Sciences C floor cold room. The lab books and data folders have been handed to my supervisor, Prof Fischer for safe keeping.

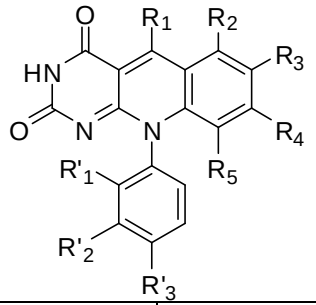
Thesis Number	Compound Number	Reaction Number	Data Number	Lab Book Page
68	MPD001	RMPD004	1	7
69	MPD002 - Method A	RMPD006	3	12
	MPD002 - Method B	RMPD008	5	15
64	MPD003 - Method A	RMPD018	10	31
	MPD003 - Method B	RMPD049	30	80
	MPD003 - Method C	RMPD014	8	25
	MPD003 - Method D	RMPD013	7	23
135	MPD004	RMPD019	11	33
136	MPD005	RMPD028	13	54
66	MPD006 - Method A	RMPD030	14	60
	MPD006 - Method B	RMPD051	31	82
65	MPD007 - Method A	RMPD031	15	61
	MPD007 - Method B	RMPD050	29	81
40	MPD009 - 1 H	RMPD037	18	68
	MPD009 - 30 Min	RMPD061	41	93
	MPD009 - 20 Min	RMPD062	42	94
	MPD009 - 10 Min	RMPD063	43	95
41	MPD012	RMPD040	22	71
42	MPD017	RMPD045	27	76
43	MPD019	RMPD122	84	156
44	MPD011 - 1 H	RMPD038	20	69
	MPD011 - 30 Min	RMPD055	35	86
	MPD011 - 20 Min	RMPD056	36	87
	MPD011 - 10 Min	RMPD057	37	88
45	MPD013	RMPD116	81	149
46	MPD018	RMPD046	34	77
47	MPD020	RMPD115	80	148
48	MPD010 - 6 H	RMPD039	21	70
	MPD010 - 2 H	RMPD035	19	66
	MPD010 - 30 Min	RMPD058	38	89
	MPD010 - 20 Min	RMPD059	39	90
	MPD010 - 10 Min	RMPD060	40	91
49	MPD014	RMPD042	24	73
50	MPD036	RMPD093	65	128
51	MPD037	RMPD081	60	113
28	MPD033	RMPD077	61	109
29	MPD024	RMPD068	48	100
30	MPD027	RMPD101	72	135
31	MPD030	RMPD104	74	138
32	MPD034	RMPD078	62	110
33	MPD025	RMPD099	70	133
34	MPD028	RMPD072	55	104
35	MPD031	RMPD106	75	139
36	MPD035	RMPD110	76	143
37	MPD026	RMPD100	71	134
38	MPD029	RMPD073	56	105
39	MPD032	RMPD076	58	108

Thesis Number	Compound Number	Reaction Number	Data Number	Lab Book Page
17	MPD021	RMPD064	49	97
21	MPD022	RMPD095	66	129
25	MPD023	RMPD067	47	99
52	MPD015	RMPD137	94	179
53	MPD038	RMPD112	77	145
56	MPD016	RMPD138	95	180
57	MPD039	RMPD113	78	146
60	MPD042	RMPD139	96	181
61	MPD040	RMPD114	79	147
78	MPD041	RMPD120	83	153
64	MPD003 - OTs Method A MPD003 - OTs Method B	RMPD131 RMPD132	89 93	173 174
79	MPD045	RMPD200	131	200
80	MPD044	RMPD166	110	212
81	MPD043	RMPD141	104	184
23	MPD053	RMPD150	111	194
27	MPD054	RMPD151	112	195
54	MPD049	RMPD146	113	190
55	MPD046	RMPD143	101	187
58	MPD050	RMPD172	115	218
59	MPD047	RMPD144	102	188
62	MPD051	RMPD148	114	192
63	MPD048	RMPD145	103	189
83	MPD058	RMPD181	124	227
84	MPD059	RMPD212	142	276
85	MPD062	RMPD183	128	231
87	MPD060	RMPD184	129	233
82	MPD061	RMPD185	130	235
88	MPD064	RMPD223	137	291
16	MPD055	RMPD206	133	263
18	MPD067	RMPD219	138	286
20	MPD056	RMPD187	148	237
22	MPD068	RMPD220	139	287
24	MPD057	RMPD188	146	238
26	MPD069	RMPD221	140	288
96	MPD074	RMPD248	152	322
97	MPD157	RMPD439	229	537
90	MPD076	RMPD242	149	315
91	MPD077	RMPD233	147	306
92	MPD078	RMPD234	154	307
93	MPD079	RMPD235	153	308
94	MPD160	RMPD444	237	542
95	MPD161	RMPD454	241	553
137	MPD119	RMPD393	191	491

Thesis Number	Compound Number	Reaction Number	Data Number	Lab Book Page
138	MPD120	RMPD394	192	492
139	MPD121	RMPD395	197	493
140	MPD122	RMPD396	193	492
141	MPD123	RMPD440	230	538
142	MPD124	RMPD441	231	539
143	MPD125	RMPD399	196	497
144	MPD154	RMPD433	232	531
98	MPD126	RMPD403	204	501
105	MPD127	RMPD404	199	526
112	MPD128	RMPD405	203	503
99	MPD129	RMPD406	205	504
106	MPD130	RMPD407	206	505
113	MPD131	RMPD408	202	506
100	MPD132	RMPD409	207	507
107	MPD133	RMPD410	208	508
114	MPD134	RMPD411	209	509
101	MPD135	RMPD412	210	510
108	MPD136	RMPD413	211	511
115	MPD137	RMPD414	212	512
102	MPD138	RMPD415	213	513
109	MPD139	RMPD416	215	514
116	MPD143_2	RMPD446	238	544
103	MPD141	RMPD418	217	516
110	MPD142	RMPD419	218	517
117	MPD140	RMPD417	216	515
104	MPD144	RMPD421	220	521
111	MPD145	RMPD422	221	520
118	MPD146	RMPD423	222	521
119	MPD162	RMPD448	234	546
120	MPD083	RMPD243	155	316
122	MPD165	RMPD456	257	555
121	MPD087	RMPD252	158	326
123	MPD166	RMPD457	261	556
124	MPD147	RMPD424	201	522
126	MPD148	RMPD425	223	523
128	MPD149	RMPD426	224	524
130	MPD150	RMPD451	240	549
145	MPD155	RMPD434	228	532
125	MPD158	RMPD442	236	540
127	MPD159	RMPD443	235	541
132	MPD176	RMPD582	303	690
146	MPD198	RMPD584	305	692
134	MPD199	RMPD585	306	693
131	MPD200	RMPD586	307	694

Biological Results

10-(3 or 4-Substituted-Phenyl)-(2, 3, 4 or 5)-Substituted-5-Deazaflavin Analogues

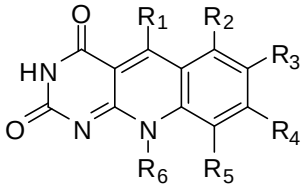


Thesis No	R_2	R_3	R_4	R_5	R'_1	R'_2	R'_3	Pre-Screen	IC_{50} (μM)
64	H	H	H	H	H	H	H	Inactive	ND
66							Cl	Inactive	ND
65					F			Inactive	ND
40	CF_3							Inactive	ND
41		CF_3						Inactive	ND
42			CF_3					Inactive	ND
43				CF_3				Active	27.8
44	CF_3				F			Inactive	ND
45		CF_3			F			Inactive	ND
46			CF_3		F			100-250μM	ND
47				CF_3	F			Active	18.7
48	CF_3						Cl	100-250μM	ND

Thesis No	R_2	R_3	R_4	R_5	R'_1	R'_2	R'_3	Pre-Screen	IC_{50} (μM)
49		CF_3					Cl	100-250μM	ND
50			CF_3				Cl	50-250μM	ND
51				CF_3			Cl	Active	8.0
28	Cl							Inactive	ND
29		Cl						Inactive	ND
30			Cl					Inactive	ND
31				Cl				100-250μM	ND
32	Cl				F			Inactive	ND
33		Cl			F			250μM	ND
34			Cl		F			Inactive	ND
35				Cl	F			Inactive	ND
36	Cl						Cl	100-250μM	ND
37		Cl					Cl	Inactive	ND
38			Cl				Cl	Inactive	ND
39				Cl			Cl	Active	11.9
16	NO_2							Inactive	ND
17		NO_2						Inactive	ND
18			NO_2					Inactive	ND
20	NO_2				F			Inactive	ND
21		NO_2			F			Inactive	ND
22			NO_2		F			Inactive	ND
23				NO_2	F			Active	ND
24	NO_2						Cl	Inactive	ND
25		NO_2					Cl	Active	75.0
26			NO_2				Cl	Inactive	ND
27				NO_2			Cl	Active	ND

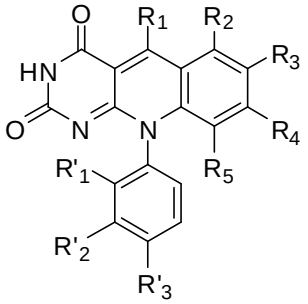
Thesis No	R_2	R_3	R_4	R_5	R'_1	R'_2	R'_3	Pre-Screen	IC_{50} (μM)
52	Me							Inactive	ND
53		Me						Inactive	ND
54			Me					Inactive	ND
55				Me				Inactive	ND
56	Me				F			Inactive	ND
57		Me			F			Inactive	ND
58			Me		F			Inactive	ND
59				Me	F			Inactive	ND
60	Me						Cl	Inactive	ND
61		Me					Cl	Inactive	ND
62			Me				Cl	100-250μM	ND
63				Me			Cl	Inactive	ND

10-Substituted-5-Deazaflavin Analogues



Thesis No	R_5	R_6	Pre-Screen	IC_{50} (μM)
90	H	H	Inactive	ND
91	CF_3	H	Inactive	ND
92		Me	Inactive	ND
93	CF_3	Me	Inactive	ND
94		Bn	Inactive	ND
95	CF_3	Bn	Active	129.2

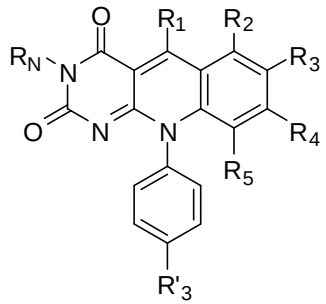
10-(2, 3 or 4-Substituted-Phenyl)-9-Substituted-5-Deazaflavin Analogues



Thesis No	R_5	R'_1	R'_2	R'_3	Pre-Screen	IC_{50} (μM)
98		Cl			Inactive	ND
99			Cl		Inactive	ND
114	CF_3	Cl			Inactive	ND
113	CF_3		Cl		Active	2.5
105	Cl	Cl			Inactive	ND
106	Cl		Cl		Active	>100
100			F		Inactive	ND
101				F	Inactive	ND
114	CF_3		F		Active	13
115	CF_3			F	Active	14
107	Cl		F		Active	>250
108	Cl			F	Inactive	ND
102		Me			Inactive	ND
103			Me		Inactive	ND

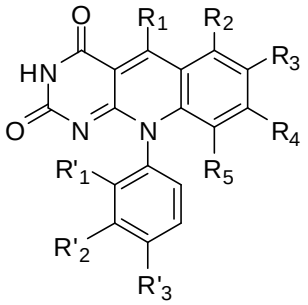
Thesis No	R_5	R'_1	R'_2	R'_3	Pre-Screen	IC_{50} (μM)
104				Me	Inactive	ND
116	CF_3	Me			Inactive	ND
117	CF_3		Me		Active	4.5
118	CF_3			Me	Active	95
109	Cl	Me			Inactive	ND
11	Cl		Me		Inactive	ND
111	Cl			Me	Inactive	ND
119	CF_3		Cl	Cl	Active	10.15
147		F		Cl	Inactive	ND
148	Cl	F		Cl	Inactive	ND

10-Phenyl-3-Substituted-5-Deazaflavin Analogues



Thesis No	R_N	R_5	R'_3	Pre-Screen	IC_{50} (μM)
120	Me			Inactive	ND
121	Et			Inactive	ND
122	Me	CF_3	Cl	ND*	ND*
123	Et	CF_3	Cl	ND*	ND*

10-(3 or 4-Substituted-Phenyl)-9-Substituted-5-Deazaflavin Analogues



Thesis No	R_5	R'_2	R'_3	Pre-Screen	IC_{50} (μM)
128	OH			Inactive	ND
130	OH		Cl	Inactive	ND
125	Br			Active	>100
127	Br		Cl	ND*	ND*
124	F			Inactive	ND
126	F		Cl	Inactive	ND
134	CN	Cl		ND*	ND*
131	CN		Cl	ND*	ND*

* This compound was the previously identified lead compound. ND = Not Determined. ND* = Not Determined with result waiting for testing. Empty squares represent hydrogen atoms.

IC_{50} In Vitro Assay Data 1

Below is the *in vitro* assay data provided for inhibition of Mdm2 E3 ubiquitin ligase activity for compounds **39** (MPD032), **43** (MPD019), **47** (MPD020) & **51** (MPD037).

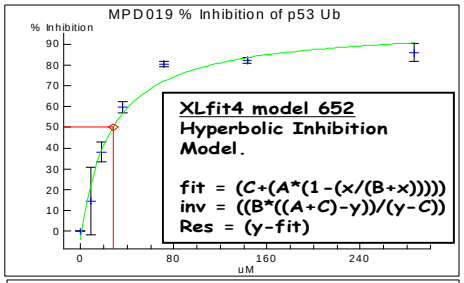
Inhibition of Ub of p53 (in-vitro assay spiked with fluoroescent-Ub)

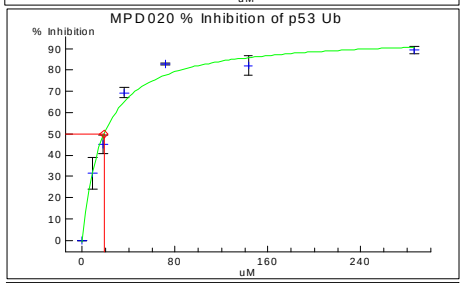
MPD019								
uM	**0.0**	**9.0**	**18.0**	**36.0**	**71.4**	**143.0**	**286.0**	
expt 2	0.0	3.2	41.5	57.8	79.4	81.2	82.8	
expt 4	0.0	25.9	34.7	61.5	81.2	83.2	88.8	IC50
average	0.0	14.5	38.1	59.6	80.3	82.2	85.8	27.8
STDEV	0.0	16.0	4.8	2.6	1.3	1.4	4.3	

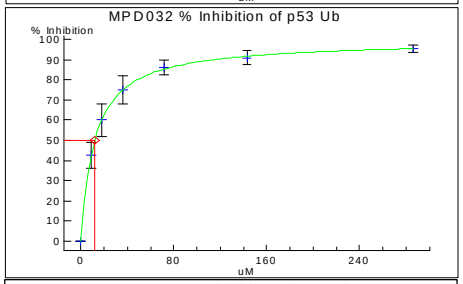
MPD020								
uM	**0.0**	**9.0**	**18.0**	**36.0**	**71.4**	**143.0**	**286.0**	
expt 2	0.0	26.1	42.0	71.1	82.6	78.9	90.5	
expt 3	0.0	36.6	48.2	67.6	83.2	85.4	88.1	
average	0.0	31.4	45.1	69.4	82.9	82.1	89.3	18.7
STDEV	0.0	7.4	4.4	2.5	0.4	4.6	1.7	

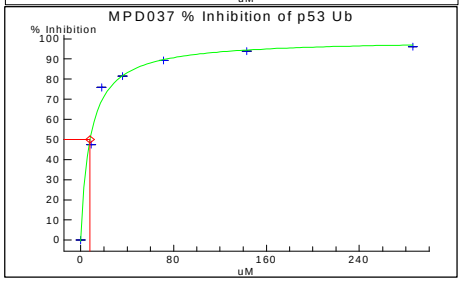
MPD032								
uM	**0.0**	**9.0**	**18.0**	**36.0**	**71.4**	**143.0**	**286.0**	
ex 2	0.0	40.7	47.9	65.6	82.5	86.4	93.4	
ex 3	0.0	44.3	63.3	74.1	85.2	90.1	95.6	
ex 4	0.0	50.6	63.9	82.2	86.2	93.7	94.4	
ex 5	0.0	35.0	64.9	78.2	91.1	93.5	97.7	
average	0.0	42.6	60.0	75.0	86.3	90.9	95.3	11.9
STDEV	0.0	6.5	8.1	7.1	3.6	3.4	1.8	

MPD037								
uM	**0.0**	**9.0**	**18.0**	**36.0**	**71.4**	**143.0**	**286.0**	
expt 2	0.0	34.3	70.5	75.6	85.6	91.5	96.9	
expt 3	0.0	46.0	79.0	79.0	90.9	92.7	95.9	
expt 4	0.0	50.7	73.6	83.9	89.8	96.2	97.4	
expt 5	0.0	59.0	80.3	87.2	90.6	95.3	94.3	
average	0.0	47.5	75.8	81.4	89.2	93.9	96.1	8.0
STDEV	0.0	10.3	4.6	5.2	2.4	2.2	1.4	







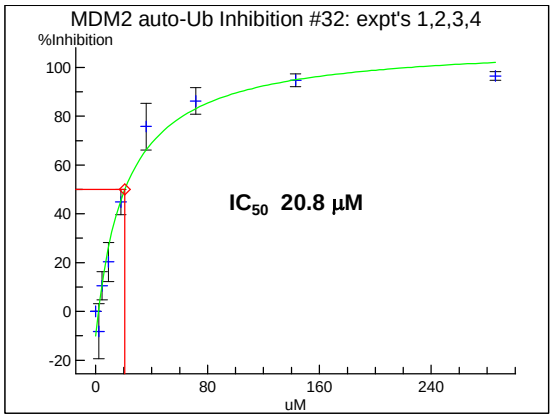


Mdm2 Auto-ubiquitinylation IC_{50} In Vitro Assay Data

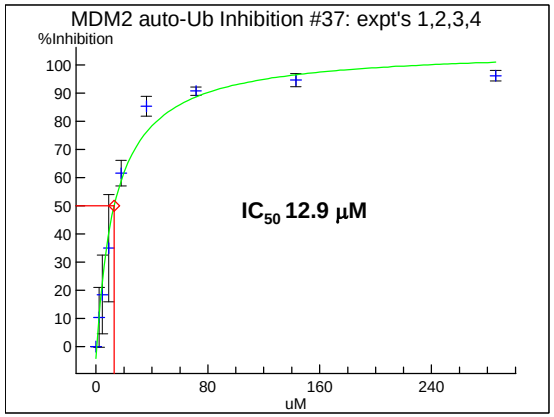
Below is the *in vitro* assay data provided for inhibition of Mdm2 auto-ubiquitinylation for compounds **39** (MPD032) & **51** (MPD037).

Inhibition of mdm2 auto-

	MPD032	0.0	2.2	4.5	9.0	18.0	36.0	71.4	143.0	286.0	µM
expt 1	%Inhibition	0.0	-6.4	16.3	25.9	38.2	65.6	78.4	91.7	93.9	
expt 2	”	0.0	0.0	2.8	28.2	46.4	87.6	90.8	96.6	96.5	
expt 3	”	0.0	-24.6	13.0	11.6	50.6	71.0	87.1	97.2	97.8	
expt 4	”	0.0	-1.7	9.9	15.6	44.0	78.7	88.7	93.5	97.6	
	average	0.0	-8.2	10.5	20.3	44.8	75.7	86.2	94.8	96.4	
	STDEV	0.0	11.3	5.7	8.0	5.2	9.5	5.4	2.6	1.8	



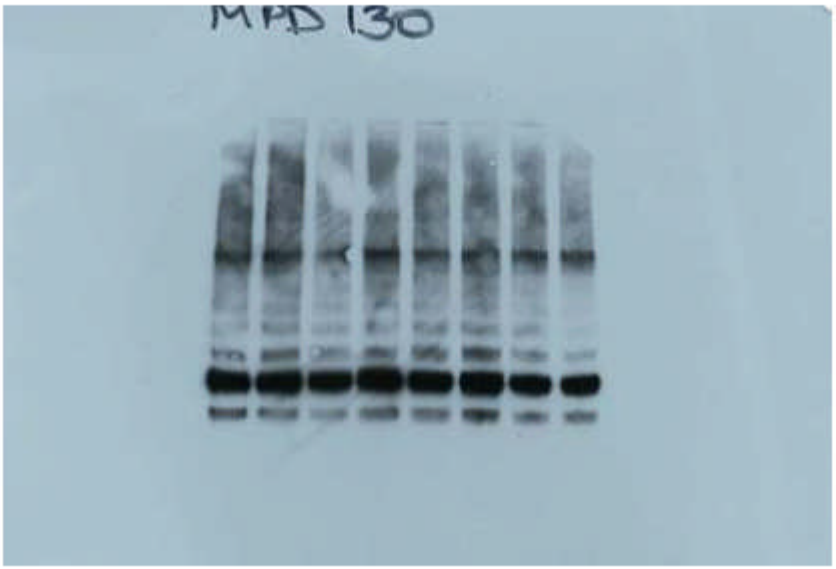
	MPD037	0.0	2.2	4.5	9.0	18.0	36.0	71.4	143.0	286.0	µM
expt 1	%Inhibition	0.0	-0.5	18.4	23.6	62.6	80.3	88.6	91.1	93.4	
expt 2	”	0.0	5.5	8.1	18.2	55.0	86.9	91.2	96.2	97.4	
expt 3	”	0.0	24.2	38.2	60.9	63.6	85.9	91.9	95.5	97.0	
expt 4	”	0.0	12.3	9.0	37.2	65.2	88.4	91.3	95.7	97.0	
	average	0.0	10.4	18.4	35.0	61.6	85.4	90.8	94.6	96.2	
	STDEV	0.0	10.6	14.0	19.0	4.5	3.5	1.5	2.3	1.9	

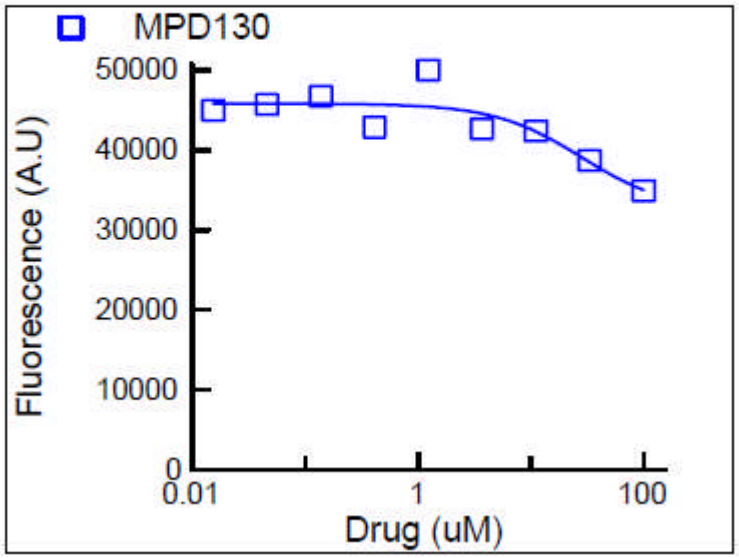


IC_{50} In Vitro Assay Data 2

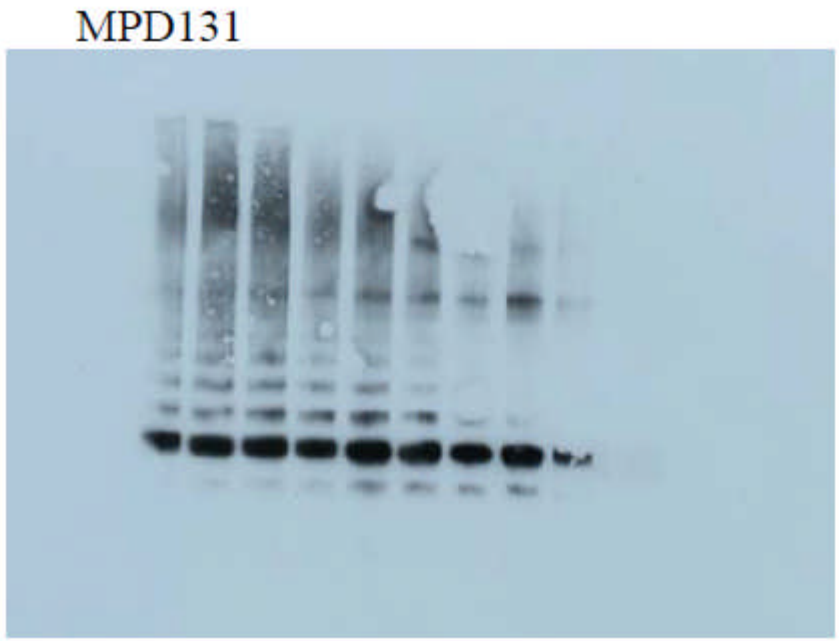
Below is the *in vitro* assay data provided for inhibition of Mdm2 E3 ubiquitin ligase activity for compounds **106** (MPD130), **113** (MPD131), **107** (MPD133), **114** (MPD134), **115** (MPD137), **117** (MPD140), **116** (MPD143) & **118** (MPD146).

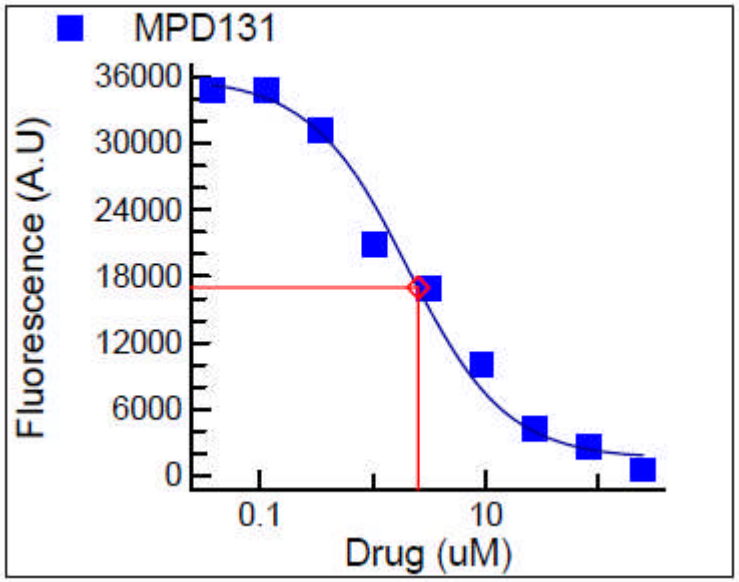
MPD 130



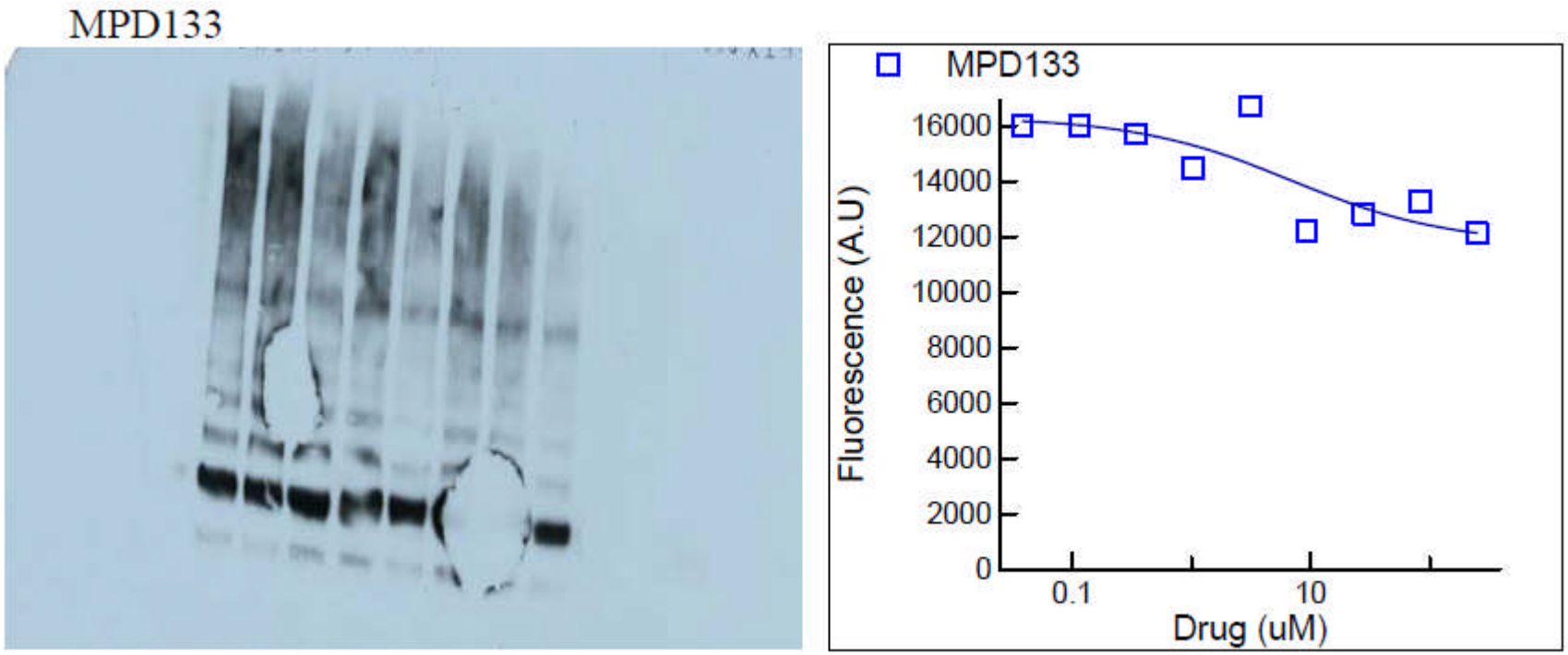


106 (MPD130) IC_{50} > 100 µM



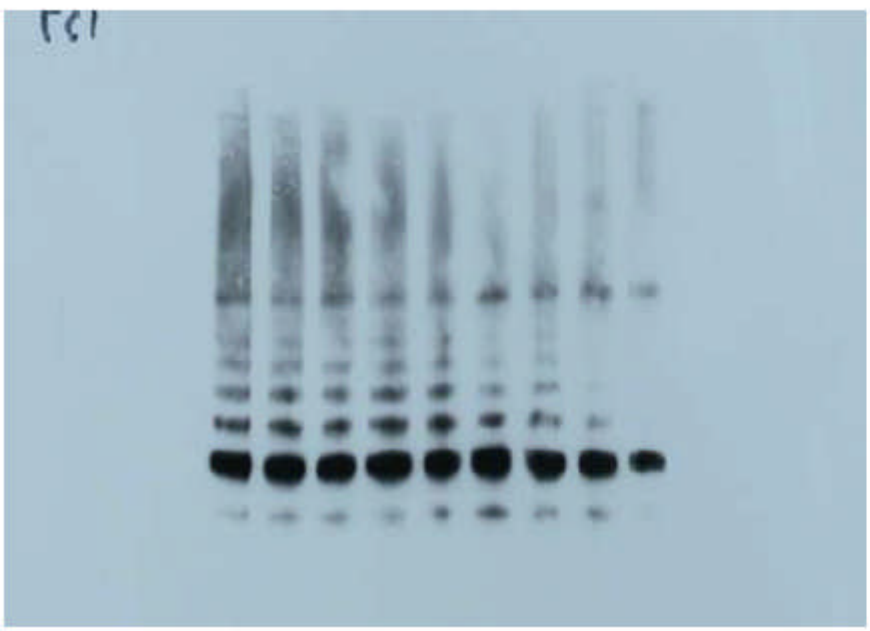


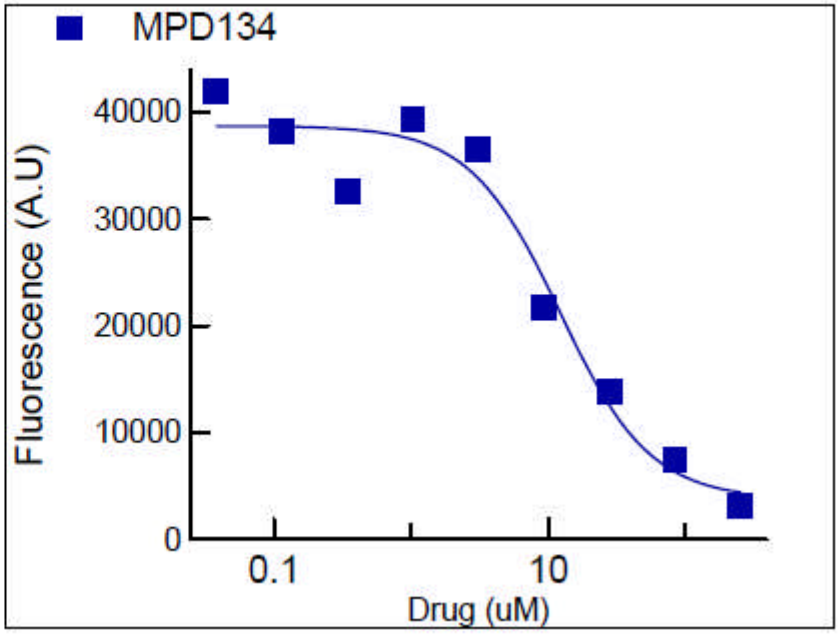
113 (MPD131) IC_{50} = 2.5 µM



107 (MPD133) IC_{50} > 250 µM

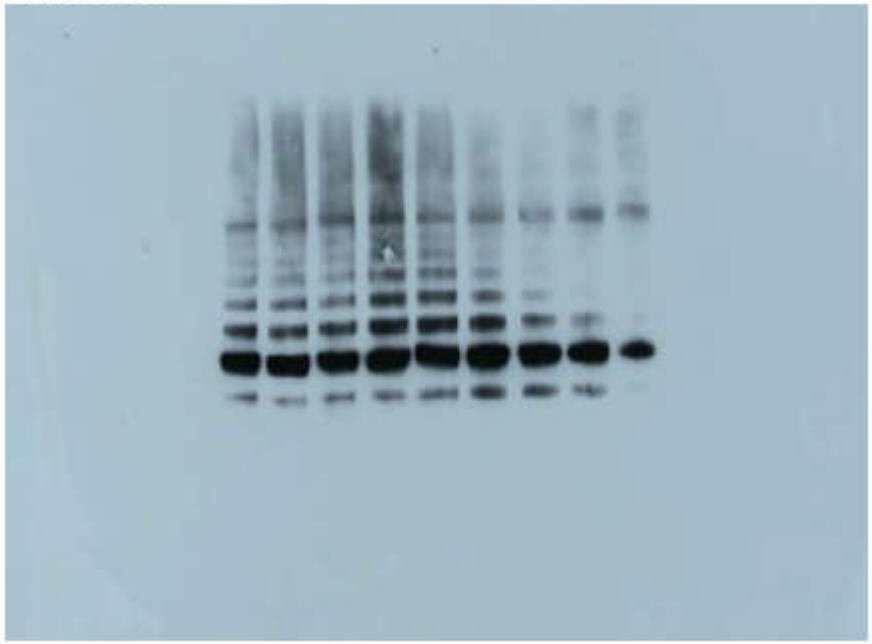
MPD134

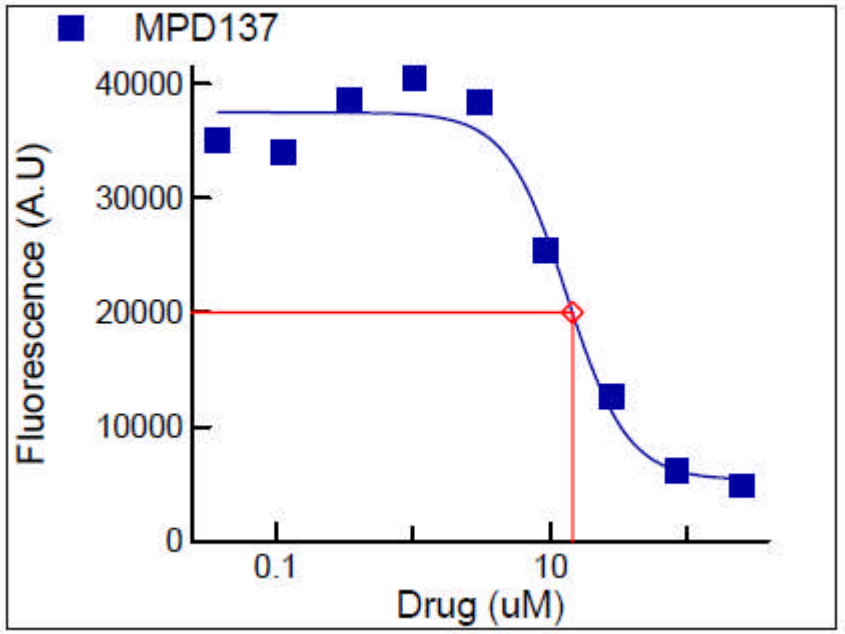




114 (MPD134) IC_{50} = 13 µM

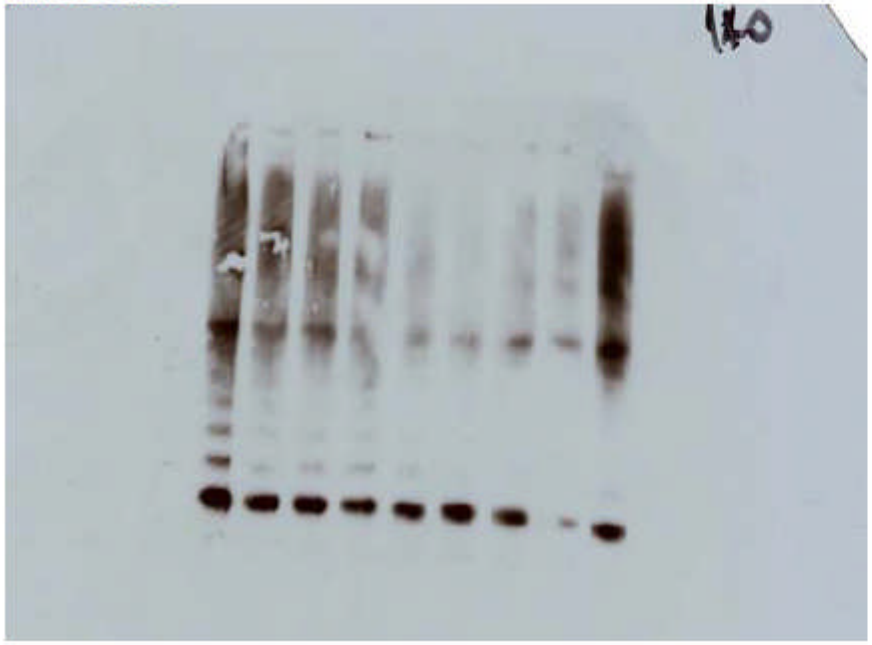
MPD137

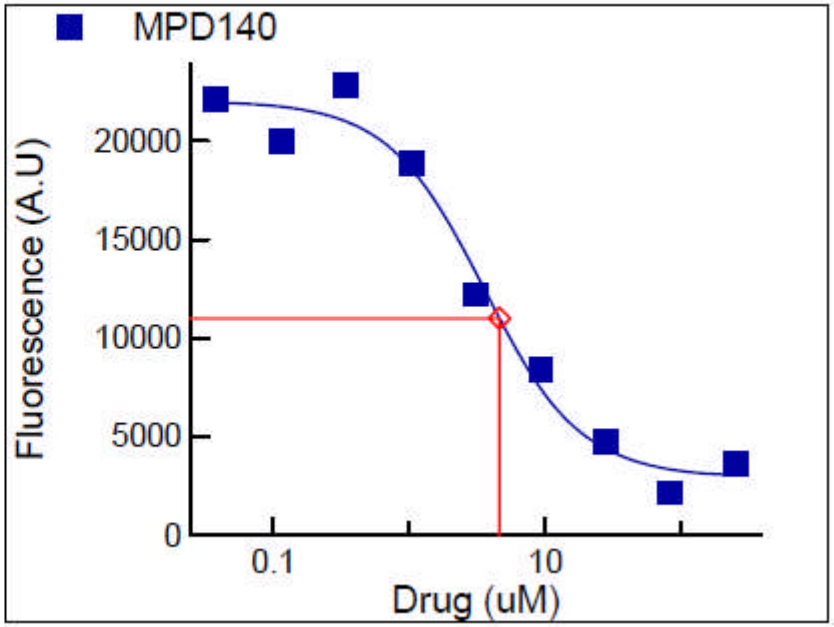




115 (MPD137) IC_{50} = 14 µM

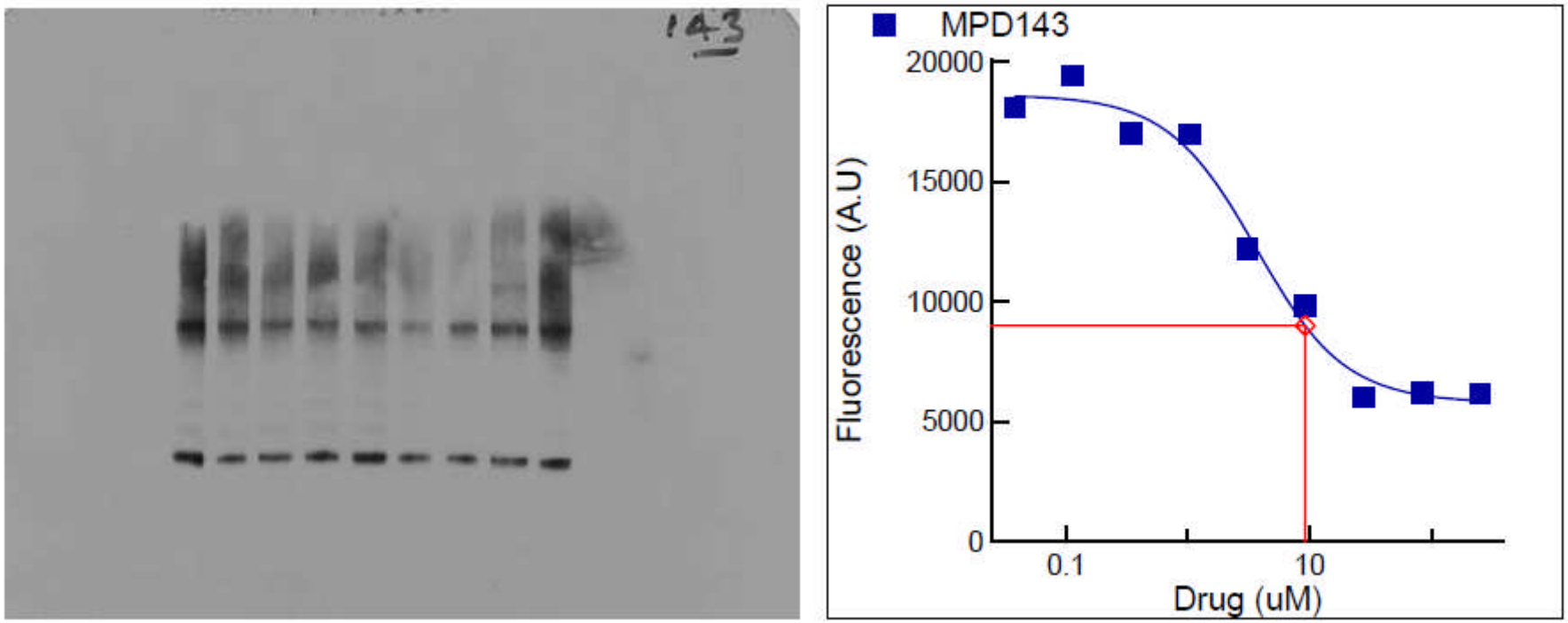
MPD140



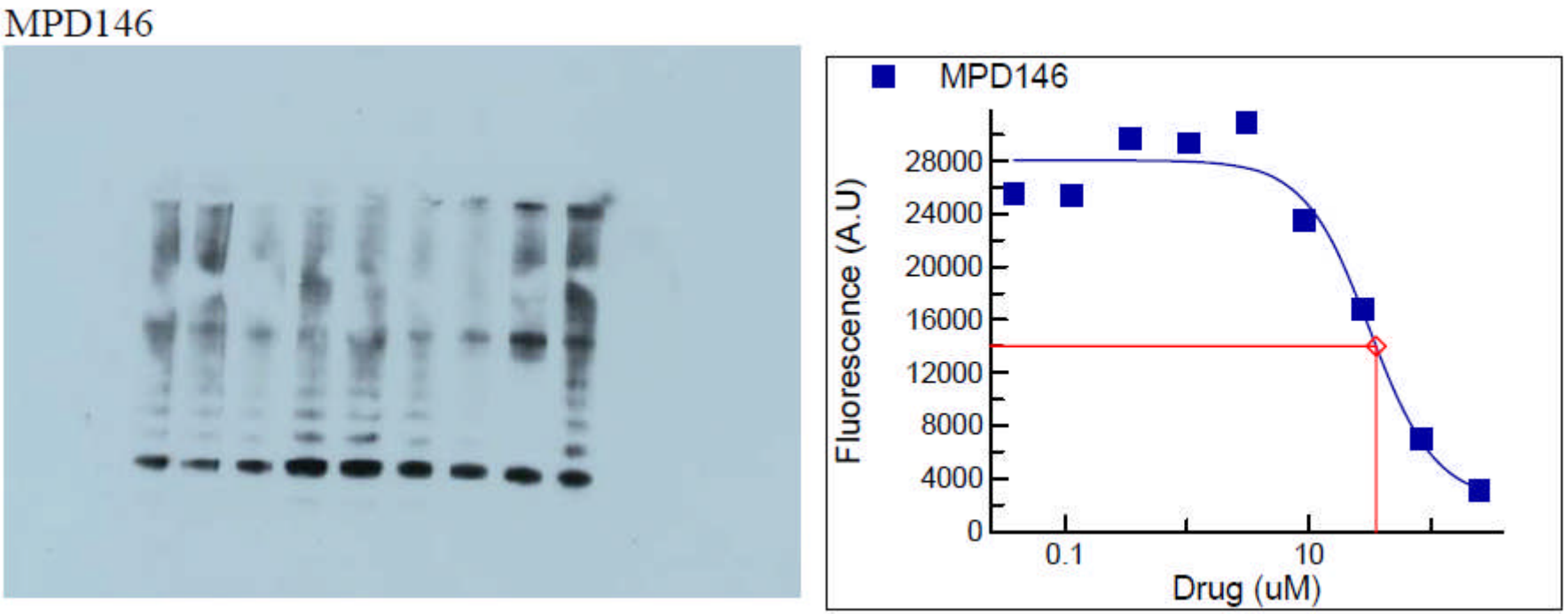


117 (MPD140) IC_{50} = 4.5 µM

MPD143



116 (MPD143) IC_{50} = 9.5 µM



118 (MPD146) IC_{50} = 10.11 µM