New synthetic routes to nitrogen heterocycles: natural products and novel drug scaffolds

Loughborough University

New Synthetic Routes to Nitrogen Heterocycles: Natural Products and Novel Drug Scaffolds

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A Doctoral Thesis

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Last but not the least, I would like to thank my family for the constant support during the course of my studies.

Abbreviations used in the text:

Bu	Butyl
BuLi	*n*-Butyllithium
cm^{-1}	Wave number
δ	Chemical shift
C	Degrees celsius
DCM	Dichloromethane
EtCOCl	Propionylchloride
GC	Gas chromatography
h	hours
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
IR	Infrared
M	Molarity
Me	Methyl
MeCN	Acetonitrile
mL	milliliter
Min.	Minute
mmol	millimole
NMR	Nuclear Magnetic Resonance
OTf	Trifluoromethanesulfonate
Pd/C	Palladium on carbon
Ph	Phenyl
PMB	4-Methoxybenzyl ether
ppm	Parts per million
n-Pr	*n*-Propyl
i-Pr	*iso*-Propyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV	Ultraviolet

Abstract

This thesis is divided into three main sections. The first chapter contains a brief review of nitrogen heterocyclic chemistry. The second chapter reports the results and their discussion of new heterocyclic chemistry, and the experimental details are provided in the fourth chapter.

The first part of chapter two contains the results and discussion related to the synthesis of the revised structure of the natural product acremolin. The second part of chapter two describes the synthesis of *s*-triazine derivatives starting from cyanuric fluoride or chloride., while the third part of this chapter reports advances in the synthesis of an EGFR inhibitor.

An initial attempt to prepare the revised structure of acremolin, compound **52**, was undertaken. During this time, the first total synthesis of the desired structure was reported, so then work continued with an alternative synthetic route. The imidazopurine core was disconnected into two separate imidazole precursors **61** and **62**. These two compounds were both successfully synthesized and isolated, but the yields were very poor, and the coupling reaction between these two precursors could not be achieved. We also prepared two analogues of the revised structure of acremolin, **68** and **69** as potential biologically active compounds. Three analogues of the imidazole precursors **70**-**72** were also prepared in order to allow synthesise of further analogues in the future.

In research on biologically active triazines, three mono-aryl, two di-aryl and one tri-aryl substituted *s*-triazines, compounds of interest as potential anticancer agents, were successfully synthesised. In addition, a number of commercially available primary (e.g. cyclopentylamine) and secondary (e.g. morpholine) amines were also attached to the *s*-triazines, giving six mono-amino, six mono-aryl-mono-amino, two di-amino and two mono-aryl-di-amino *s*-triazines. A number of these compounds are currently undergoing screening for biological activity.

In a further development of nitrogen heterocyclic chemistry, three *N*-2,3,5,6-tetra-4-pyridinamine analogues **96**, **103** and **104** as potential kinase inhibitors were prepared by S_NAr reactions of perfluoroarenes. However, the desired substitution reactions at position-2 proved difficult and were unsuccessful. The amine moieties were then modified

with either 4-toluenesulfonyl or propionic anhydride, giving more acidic secondary amides **105**, **106**, **108** and **109**. These modified compounds were only able to replace the fluoride at position-4 of the fluoropyridine ring in base catalysed S_NAr reactions.

Content

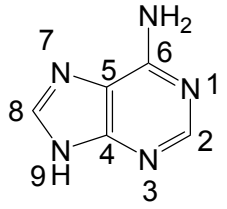
Chapter 1: Introduction

Section 1: Introduction to Biologically Important Nitrogen Hetereocycles

1.1 Brief overview of deoxyribonucleic acid (DNA)

Deoxyribonucleic acid (DNA) is a polymer molecule, often consisting of many thousands or millions of monomer building blocks. The monomer units of DNA are nucleotides, and the polymer is known as a "polynucleotide." Each nucleotide consists of a 5-carbon sugar (deoxyribose), a nitrogen containing heterocyclic (base) attached to the sugar, and a phosphate group. There are four different types of nucleotides, adenine (A), guanine (G), cytosine (C) and thymine (T) found in DNA, differing only in the nitrogenous base.

Adenine **1** and guanine **2** are purines (**Figure 1-1**). Purines are the larger of the two types of bases found in DNA and consist of an imidazole ring fused to a pyrimidine ring. The 9 atoms that make up the fused rings (5 carbon, 4 nitrogen) are numbered 1-9. All ring atoms lie in the same plane.



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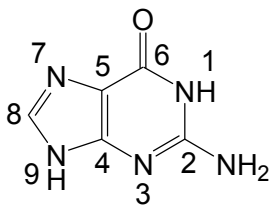
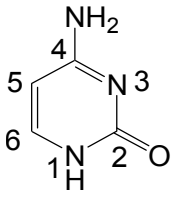


2

Figure 1-1. Adenine **1** and guanine **2**.

Cytosine **3** and thymine **4** are pyrimidines (**Figure 1-2**). The 6 atoms (4 carbon, 2 nitrogen) are numbered 1-6. Like purines, all pyrimidine ring atoms lie in the same plane.



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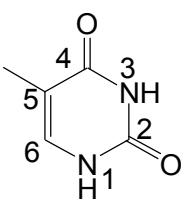
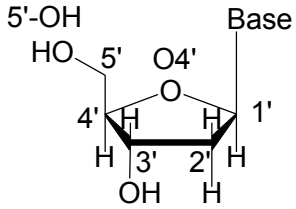


4

Figure 1-2. Cytosine **3** and thymine **4**.

The deoxyribose sugar **5** of the DNA backbone has 5 carbons and 3 oxygens (**Figure 1-3**). The carbon atoms are numbered 1', 2', 3', 4' and 5' to distinguish from the numbering of the atoms of the purine and pyrmidine rings. The hydroxyl groups on the 5'- and 3'-carbons link to the phosphate groups to form the DNA backbone. Deoxyribose lacks a hydroxyl group at the 2'-position when compared to ribose, the sugar component of RNA.



5

Figure 1-3. The deoxyribose sugar **5** in DNA

DNA is a normally double stranded macromolecule. Two polynucleotide chains, held together by weak thermodynamic forces (hydrogen bonding), form a DNA molecule (**Figure 1-4**).

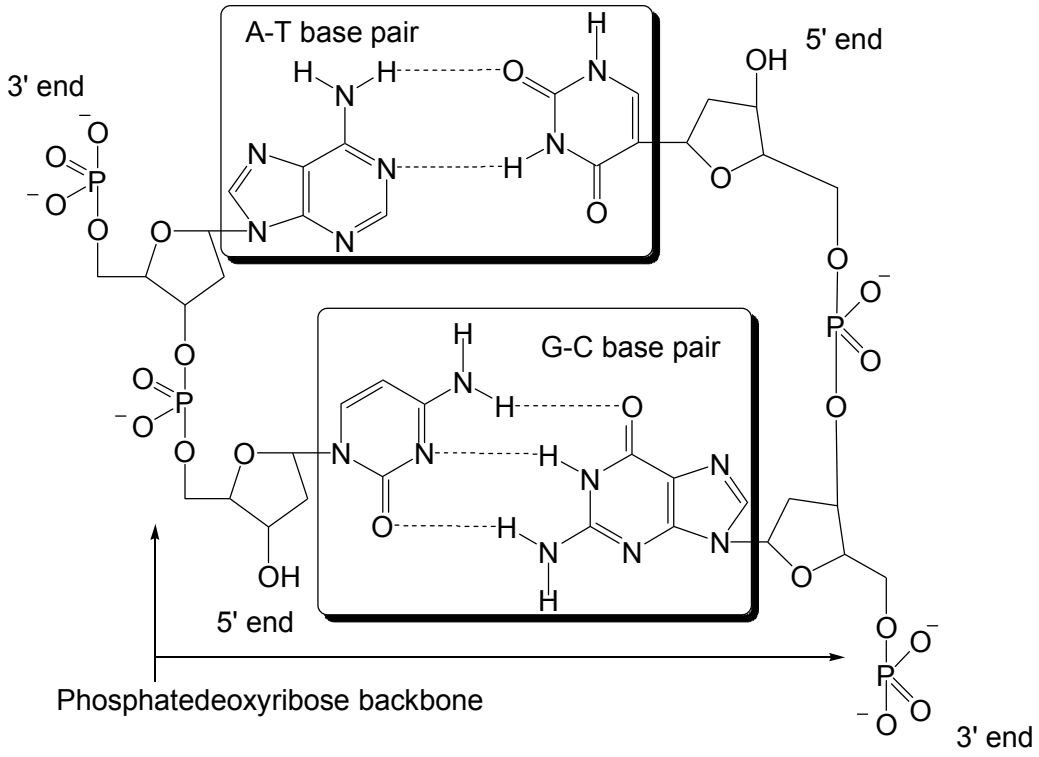


Figure 1-4. Two polynucleotide chains of DNA.

Within the DNA double helix, A forms 2 hydrogen bonds with T on the opposite strand, and G forms 3 hyrdorgen bonds with C on the opposite strand. dA-dT and dG-dC base pairs

are the same length, and occupy the same space within a DNA double helix. Therefore the DNA molecule has a uniform diameter.[1]

1.2 Historical overview of purines

The purine structure **6** is a common and important nitrogen-containing heterocycle in nature.[2] Purine bases, such as adenine **1** and guanine **2**, their corresponding nucleosides, adenosine **7** and guanosine **8** and their metabolic products, the nucleoside inosine and the bases hypoxanthine **9** and xanthine **10**, as well as purine nucleotides, such as adenosine 5' triphosphate (ATP), adenosine 5' diphosphate (ADP), adenosine 5' monophoshate (AMP), guanosine 5' triphosphate (GTP), guanosine 5' diphosphate (GDP) and guanosine 5' monophosphate (GMP), are ubiquitous molecules found within the cells of animals and plants (e.g. anchovies, brains, kidneys, liver, sardines, sweetbreads).[3]

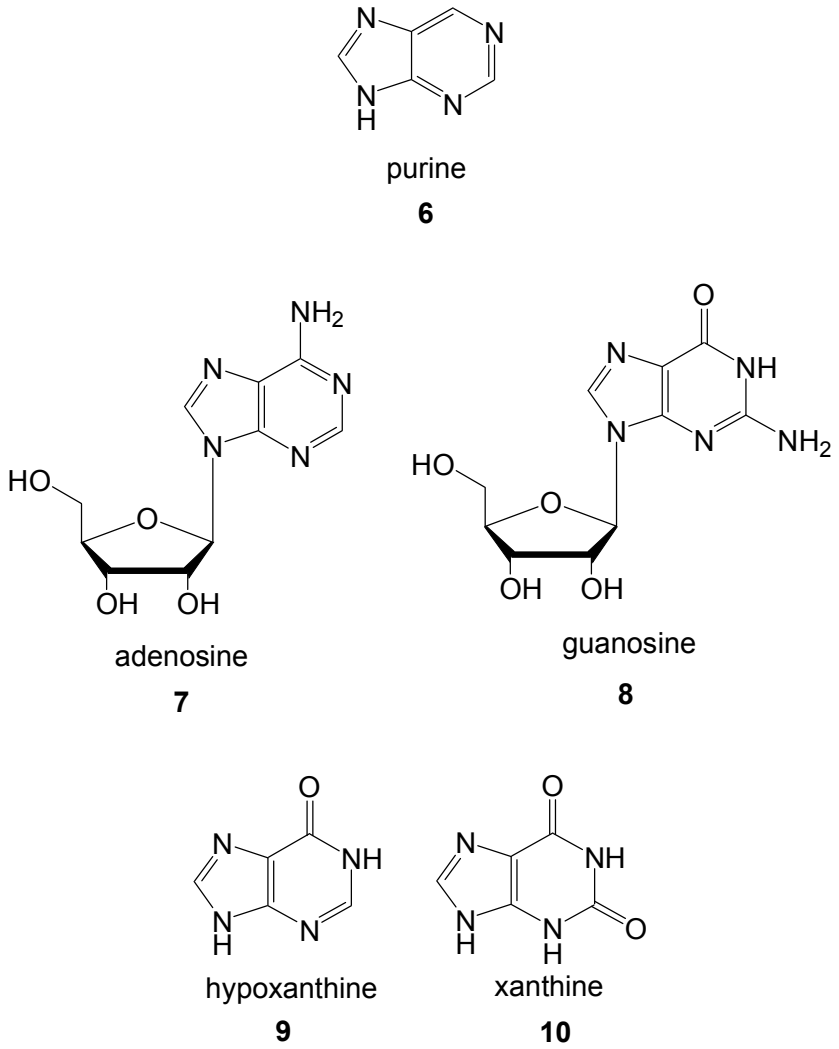


Figure 1-5. Purine-base structures in nature.

Purine bases and their pyrimidine counterparts are the building blocks of the nucleic acids that form deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). ATP is considered the universal cellular energy currency, it and other purine nucleotides and nucleosides are involved in biochemical pathways and energy transfer within the cell. Cyclic nucleotides such as adenosine 3',5'-cylic monophosphate (cAMP) and guanosine 3',5'-cylic monophosphate (cGMP) act as important intracellular second messenger molecules during signal transduction.

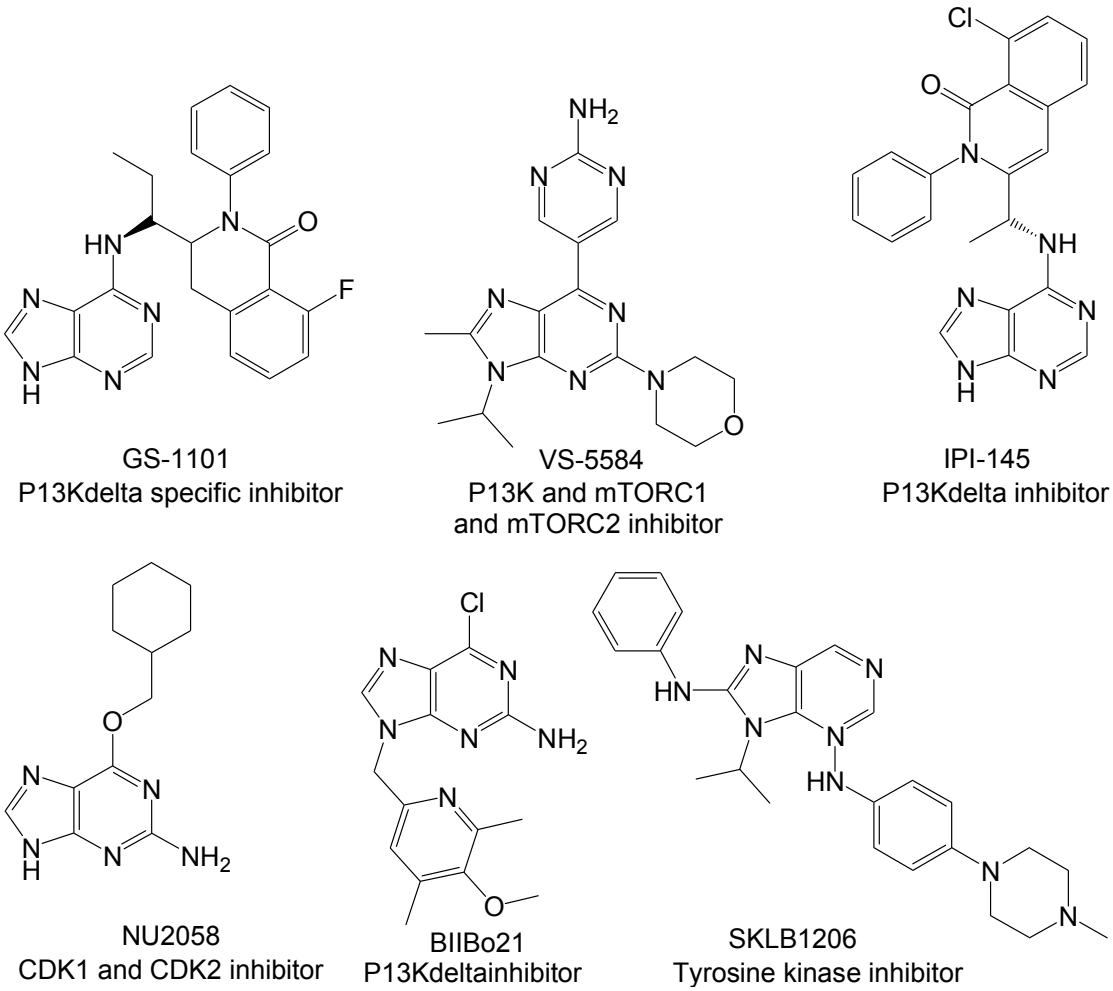
Although purines have mainly been traditionally viewed as having only intracellular roles, about 90 years ago Drury and Szent-Gyorgyi (1929) discovered that extracellular adenosine is a hormone released by the heart during ischemia. Over the last 30 years, however, the roles of adenosine and ATP as neurotransmitters and neuromodulators in the central, peripheral and enteric nervous systems have been elucidated.[4] Specific receptors for ATP[5] and adenosine[6] have now been identified. Outside the nervous system, adenosine and ATP were found to act not only as neurotransmitters, but also as hormones with 'trophic' roles. Trophic substances affect the structure, development, and integrity of a target cell or tissue over a longer time course than the milliseconds to seconds involved in neurotransmission. Trophic effects include the plastic changes involved in memory and learning, sprouting of nerve processes, neuroprotection against noxious stimuli and even regulation of cell number through stimulation of apoptosis (programmed cell death).

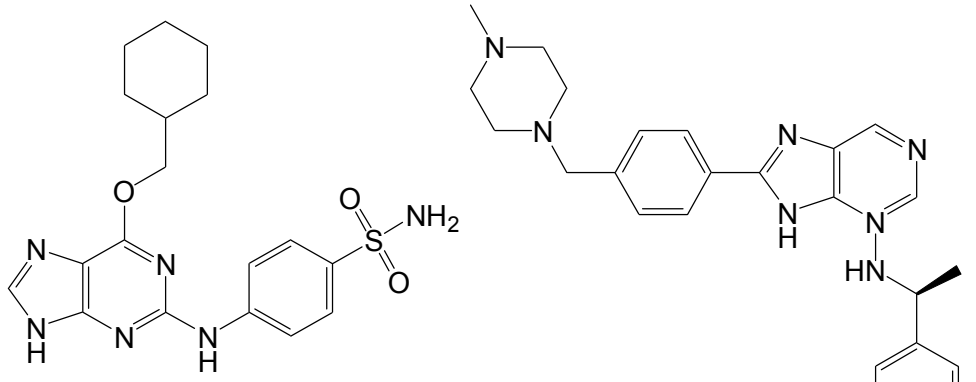
Moreover, such trophic signaling by extracellular purines is not limited to mammals; rather it is phylogenetically ancient and widespread in the plant and animal kingdoms.[7] For example, external GTP alters both the motility and membrane depolarization in the ciliated protist, *Paramecium tetrauelia*.[8]

In animals, signaling by extracellular purines has also been implicated in the control of a wide variety of physiological processes. Extracellular purinergic receptors have been located on numerous animal cell types[9] and their activation affects, for example, sperm motility, fertilization, embryo- genesis and organogenesis.[10] In mammalian cells, purines regulate vascular smooth muscle tone, cardiac muscle, platelet aggregation, gastrointestinal mobility, respiratory and renal function.[11]

1.3 A brief introduction of purine based compounds

Purine is a privileged heterocyclic nucleus which exists in the chemical architecture of various bioactive compounds.[12] The purine nucleus has appeared as an important pharmacophore interacting with the synthesis, functions of nucleic acids and enzymes and is one of the most widely used heterocycles in the development of protein kinase inhibitors. The introduction of substituents on the 2, 6 and 9 positions of the purine ring is anticipated to yield compounds with enhanced binding affinity and selectivity toward kinases.[13] Purine based protein kinase inhibitors in pre-clinical/clinical trials are shown in **Figure 1-6**.



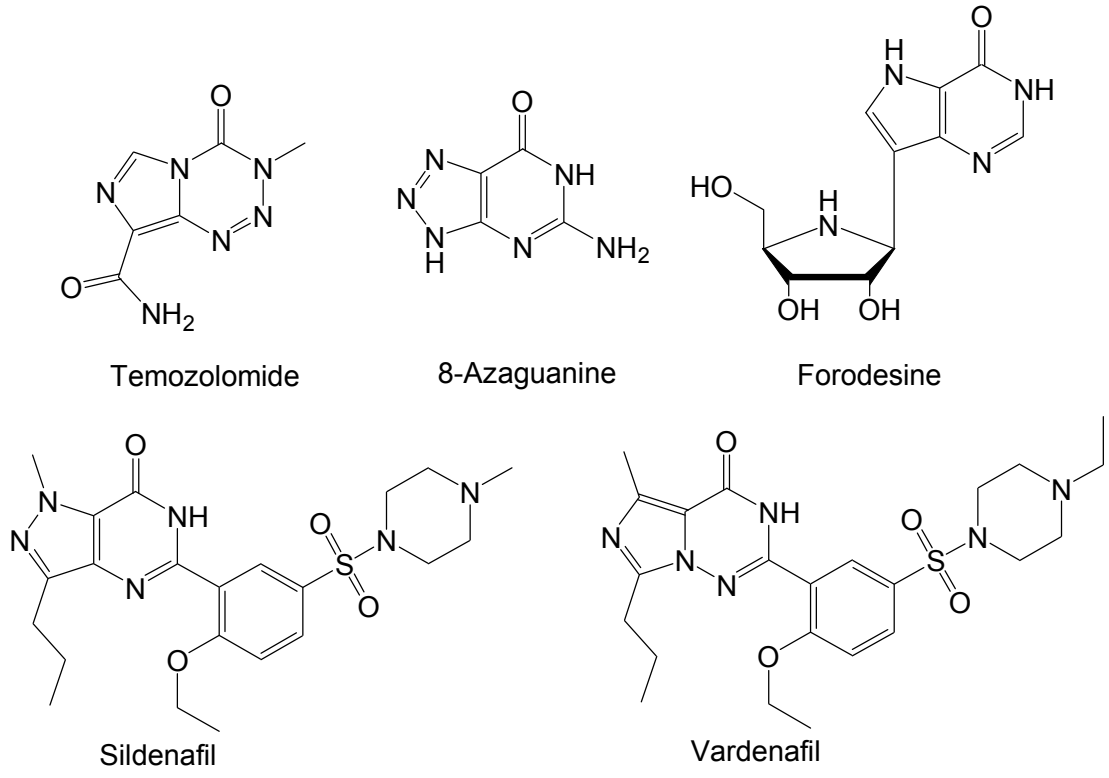


NU6102
CDK1 and CDK2 inhibitor

AEE788
Bispecific tyrosine kinase inhibitor

Figure 1-6. Purine based protein kinase inhibitors in pre-clinical/clinical trials.

A number of purine isostere core based drugs are also available in the market (**Figure 1-7**), including temozolomide used for the chemotherapeutic treatment of brain cancer[14]; 8-azaguanine[15] and forodesine[16] used for the treatment of leukemia; sildenafil and vardenafil,[17] PDE5 inhibitors, are used for the treatment of erectile dysfunction.



Temozolomide

8-Azaguanine

Forodesine

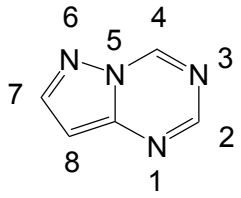
Sildenafil

Vardenafil

Figure 1-7. Therapeutic agents based on isosteric purine skeletons.

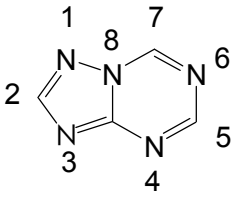
There are extensive and ongoing research and development activities around purine isosteres and one of the promising directions has a focus on using 1,3,5-triazine-based

isosteres of purine, pyrazolo[1,5-a][1,3,5]triazines (5-aza-9-deazapurines **11**) and 1,2,4-triazolo[1,5-a][1,3,5]triazines (5-azapurines **12**)(**Figure 1-8**).[18,19] This group of azolo[1,3,5]triazine systems has a nitrogen atom in the position 5 of the purine ring and therefore can be generally categorized as 5-aza-isosteres of purine.



5-aza-9-deazapurine
pyrazolo[1,5-*a*][1,3,5]-triazine

11

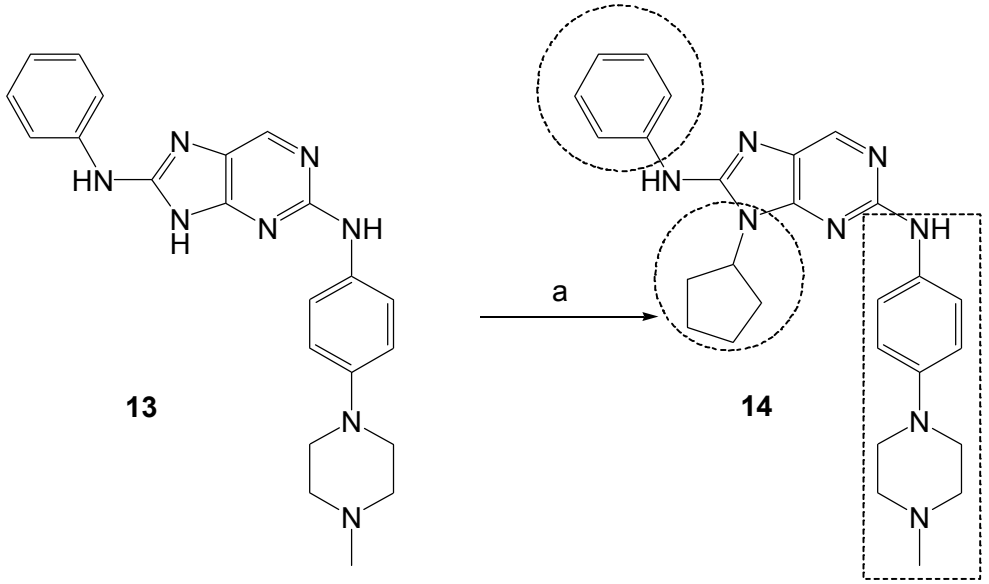


5-azapurine
1,2,4-triazolo[1,5-*a*][1,3,5]-triazine

12

Figure 1-8. 1,3,5-Triazine-based purine-like scaffolds

Yang and co-workers performed a virtual screening followed by kinase inhibitory assays against a library of known kinase inhibitors. Among the tested compounds, *N*-(4-(4-methylpiperazin-1-yl)phenyl)-*N*-phenyl-9*H*-purine-2,8-diamine **13** exhibited ability to inhibit both EGFR-activating and resistance mutations. After the structure was modified at the *N*-9 position of the purine, and with the phenyl ring of the 2-anilino group and the phenyl ring of the 8-anilino group, a series of novel 2,8-dianilinopurine derivatives were synthesized and tested for bioactivity. As a result, compound **14** generated the most anti-tumor activity both *in-vitro* and *in-vivo*.[20]

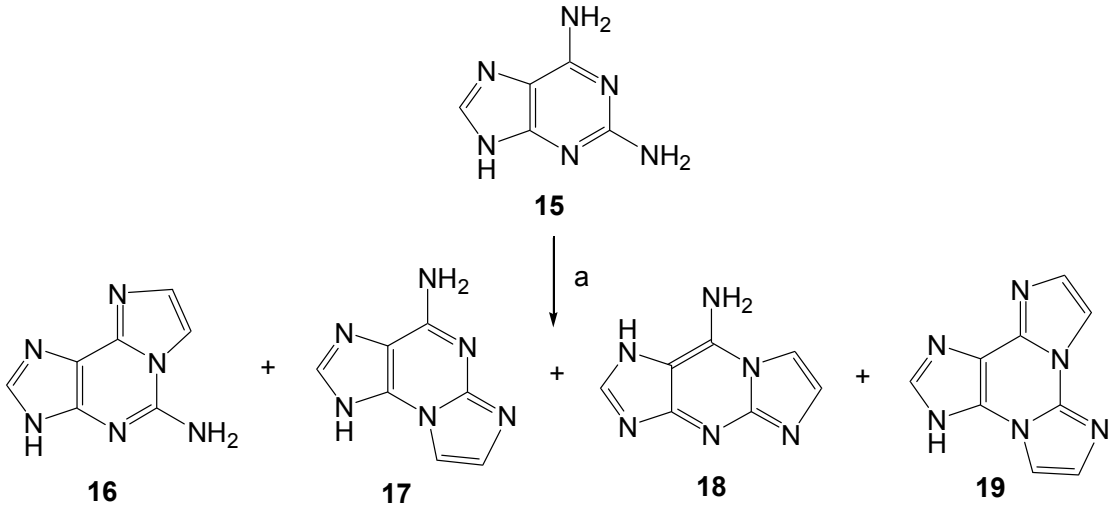


13 **14**

Figure 1-9. 2,8-diamino purine analogs as reversible tyrosine kinase inhibitors. Reagents and conditions: (a) MeOH, 10% Pd/C, H_2, 60 ˚C, 5 h, 48%.

~~Virta *et al.* synthesized four derivatives of a nucleoside base analogue, 2,6-diaminopurine, with an additional five-membered ring fused to the base moiety (**Scheme 1-1**).[21]~~

The exocyclic DNA adducts with an additional five-membered ring fused to the base moiety can be used as models to investigate what may be happening detrimentally on the bimolecular scale.[21] Virta *et al.* synthesized four derivatives of a nucleoside base analogue, 2,6-diaminopurine, with an additional five-membered ring fused to the base moiety (**Scheme 1-1**).[22]



Scheme 1-1. Reagents and conditions: (a) chloroacetaldehyde ($ClCH_2CHO$), water/DMF, pH 4–5, 80 ˚C, 24 h.

Cyclic nucleosides play significant role for antiviral reagents, and they usually exist in biological metabolites[23] and synthetic intermediates.[24] Meng and co-workers synthesized multi-fused rings via intramolecular double C-H activation in purines and benzimidazole structures. (**Scheme 1-10**).[25] A palladium catalyst was employed under acidic conditions and double C-H cyclization was proceeded.

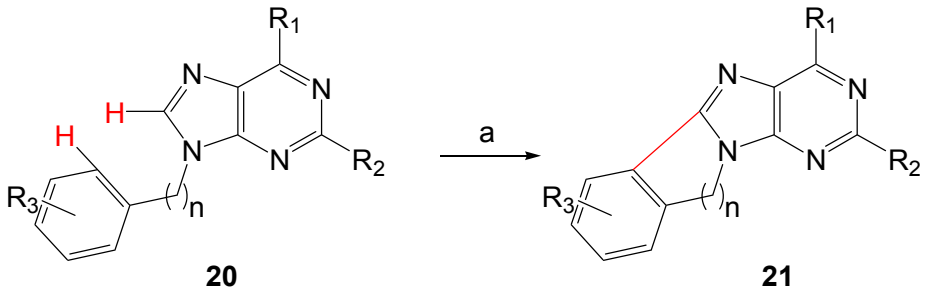


Figure 1-10. Strategies for the synthesis of *N*-fused heterocycles. Where n=1,2,3. Reagents and conditions: (a) $Pd(OAc)_2$ as catalyst, silver acetate, acetic acid, 110 ˚C, 36 h.

1.4 Background to acremolin

Marine microorganisms, particularly actinomycete bacteria and fungi, are an important source of antibacterial, anti-algal and anti-larval compounds, which can potentially be used for biotechnological applications. Hundreds of novel compounds are isolated from these organisms annually.

Shin and co-workers isolated a strain of *Acremonium strictum* which was obtained from an unidentified Choristida sponge collected from Korean waters.[26] A novel modified base acremolin, was isolated as a white amorphous solid after continuous work with this strain using diverse culture media which exhibited weak cytotoxicity against A549 cell line.

After analysis of the white amorphous solid with HR-FAB-MS the molecular formula was identified as $C_{11}H_{13}N_5O$. In addition, IR, ^1H NMR and ^{13}C NMR spectroscopic experiments were performed, and the structure of acremolin was firstly proposed as **22**. Shin and co-workers emphasized the acremolin was first example of a 1*H*-azirine metabolite, which was unprecedented among natural products.

1*H*-azirines and 2*H*-azirines are heterocyles have different number of electrons in the π system as shown in **Figure 1-11**. 2*H*-azirines has been found in a few natural products.[27] In contrast, only 5 examples of short-lived antiaromatic heterocycles, 1*H*-azirines, have been photochemically generated and identified by IR spectroscopy at very low temperatures.[28]

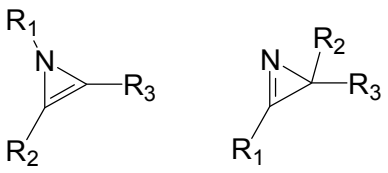


Figure 1-11. 1*H*-azirines (left) and 2*H*-azirines (right)

Klaus raised doubts about the structure **22** due to the acremolin being isolated at room temperature, which is contradictory to all previous experimental and theoretical results.[29]

Then he omitted the 1*H*-azirine moiety and introduced a second fused five-membered cyclic system. After comparing the ^{1}H and ^{13}C NMR data with a published compound 1*H*-imidazo[2,1-b]-purine-4(5*H*)-one (**Figure 1-12**)[30], the structure was reoriented to an isomeric ring system for acremolin, namely a substituted N^2,3-ethenoguanine, which is consistent with all spectroscopic data **23** (**Figure 1-13**).

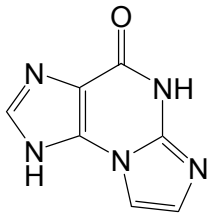
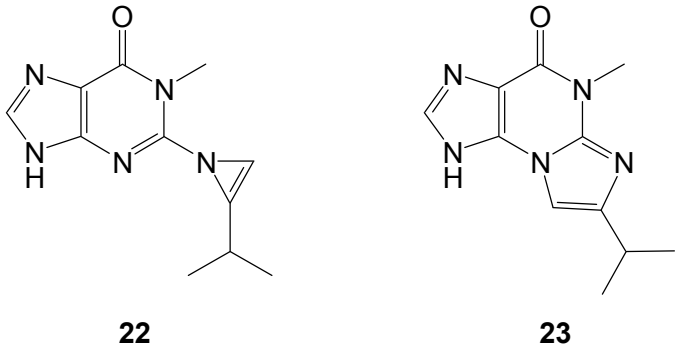


Figure 1-12. 1*H*-imidazo[2,1-b]-purine-4(5*H*)-one



22 **23**

Figure 1-13. Acremolin **22** and the revised structure **23**

1*H*-azirines still only exist as short-lived intermediates and cannot be isolated from natural products at room temperatures. In this thesis, we aim to synthesis the revised structure of acremolin **23** and compare the spectroscope data with the published by Shin and co-workers.

Section 2: Introduction to *s*-triazine and its derivatives

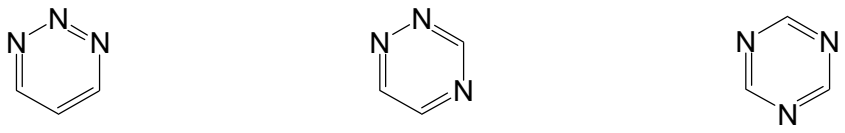
1.5 Introduction to the chemistry of *s*-triazine and its derivatives

This research involves synthesis of new drug candidates based on triazines and the chemistry of this ring and current drugs containing a triazine ring are reviewed here.

The design, synthesis and evaluation of molecules with potential human therapeutic values, remains one of the main objectives of organic and medicinal chemistry. During the past decades, combinatorial chemistry has provided access to chemical libraries based on privileged heterocyclic motifs with utility in medicinal chemistry.[31] Synthesis of nitrogen containing heterocyclic compounds has been attracting increasing interest because of their utility for various biological receptors with a high degree of binding affinity.

Triazines are six-membered aromatic heterocycles comprised of three carbon and three nitrogen atoms. The three isomers shown below in **Figure 1-13**

are 1,2,3-triazine **24**, 1,2,4-triazine **25**, and 1,3,5-triazine **26**.



24 **25** **26**

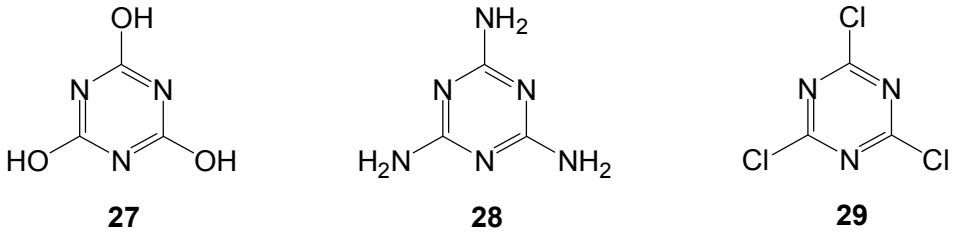
Figure 1-13. Triazine isomers

The 1,3,5-triazines are among the oldest recognized organic compounds known and were discovered in 1776.[32] They have a symmetrical ring consisting of alternating carbon and nitrogen atoms joined by alternating single and double bonds. Compounds of this type are often referred to as *s*-triazines, the ring is numbered in the same manner as a benzene ring starting with a nitrogen atom as number one. 1,3,5-Triazine was unknowingly first synthesized by Nef in 1895 by treating hydrogen cyanide with ethanol in an ether solution saturated with hydrogen chloride. The resulting salt was then treated with base and distilled to give 1,3,5-triazine in a low yield of 10%. Nef incorrectly identified the product as a dimeric species, but Grundmann and Kreutzberger proved the compound to be a trimer of hydrogen cyanide, *s*-triazine in 1954.[33]

Triazine is thermally stable, and will decomposes to form hydrogen cyanide when heated to above 600 °C. The triazine ring is very resistant to electrophilic substitution. However, it

may easily undergo ring cleavage with nucleophiles and is fairly sensitive to hydrolysis by water and other hydroxyl-compounds to a lesser degree. A series of heterocyclic or related compounds can be synthesized from 1,3,5-triazine by introducing bifunctional amines or related compounds.

The most commonly used triazine derivatives are shown below in **Figure 1-14**, cyanuric acid **27**, melamine **28** and cyanuric chloride **29**.

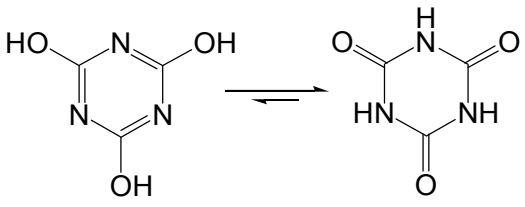


27 **28** **29**

Figure 1-14. Common triazine derivatives **27**-**29**.

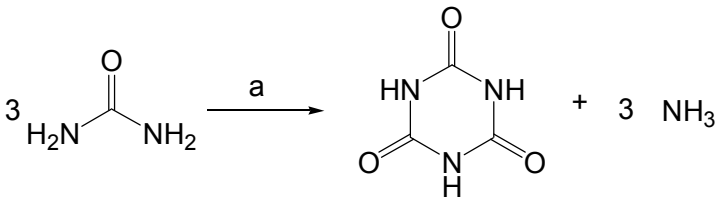
Triazines were first synthesized as the compound known as cyanuric acid **27**. In 1776, Scheele pyrolysed uric acid and obtained cyanuric acid. In 1820, Serullas repeated the work of Scheele to produce cyanuric acid from cyanogen in water. In 1830, the two products were discovered to be the same, and the structure was elucidated by Liebig and Wohler.[34]

Cyanuric acid **27** is thermally unstable. It decomposes to from toxic cyanic acid at high temperature. Cyanuric acid exists preferentially as the oxo tautomer **27b** rather than the hydroxy form **27a** (**Figure 1-15**). Cyanuric acid is industrially prepared from the pyrolysis of urea(**Scheme 1-2**),[34] and it has many commercial applications such as stabilizers of swimming pool disinfectants, household bleach, industrial cleaners, dishwasher detergents and general sanitizers. Naturally occurring cyanuric acid has been found in soil humus.



27a **27b**

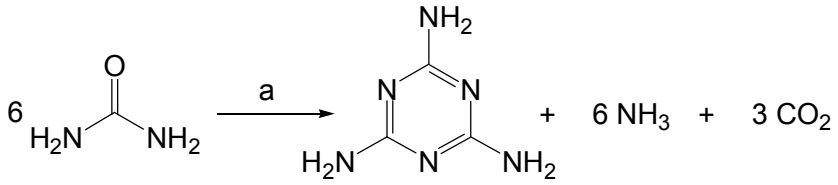
Figure 1-15. Tautomerism of cyanuric acid



27b

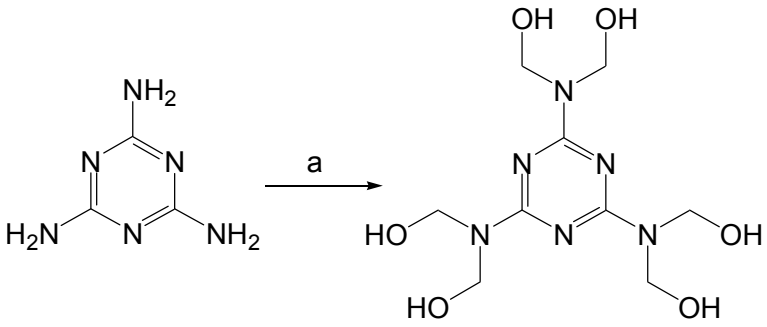
Scheme 1-2. Industrial synthesis of cyanuric acid. Condition: (a) 200-300 °C.

Melamine 28 was first prepared in 1834 by Liebig by fusing potassium thiocyanate with ammonium chloride and then work-up with base.[35] In recent days, melamine is industrially prepared from urea (**Scheme 1-3**).[34] Melamine has been found to naturally occur in nature in meteorites.[32] Melamine can be used to produce resins by reacting with formaldehyde (**Scheme 1-4**).[36]



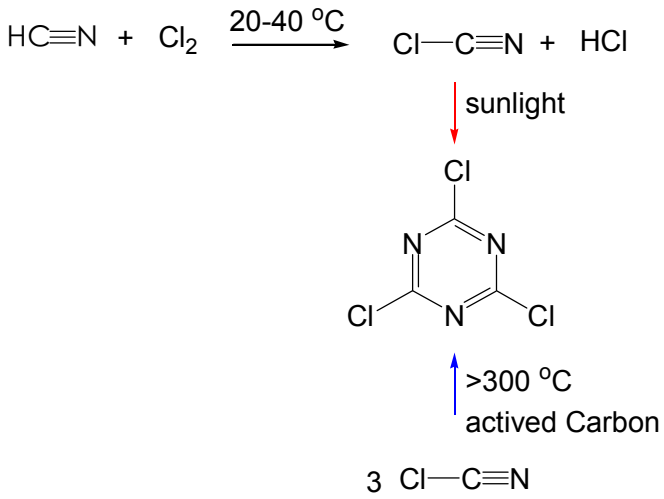
28

Scheme 1-3. Industrial synthesis of melamine **28**. Condition: (a) 390-410 °C.



Scheme 1-4. Resin preparation from melamine. Reagent: (a) 6 equivalents formaldehyde.

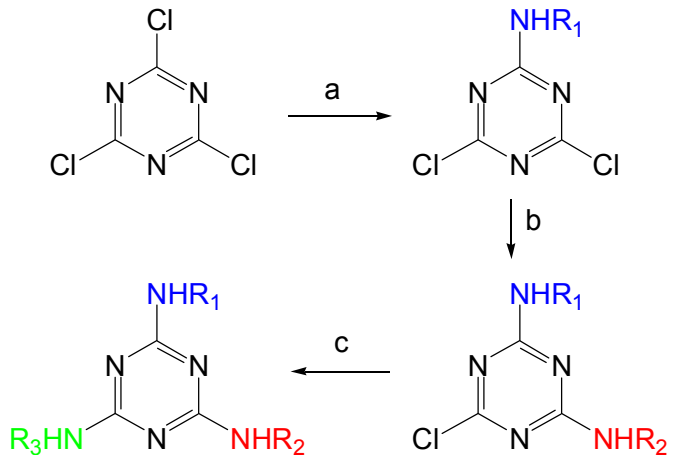
Cyanuric chloride, trichlorotriazine **29**, was first synthesized in 1827 by Serullas, who converted cyanogen chloride to cyanuric chloride using sunlight. The structure was thought to be the trimer of cyanogen chloride for many years, and then corrected by Liebig who prepared the compound by passing chlorine over dry potassium thiocyanate.[32] Cyanuric chloride is produced by the trimerization of chlorinated hydrocyanic acid nowadays (**Scheme 1-5**).[37]



Scheme 1-5. Serullas procedure (top) and industrial synthesis of cyanuric chloride **29** (bottom)

Cyanuric chloride has the far more applications than any other triazine derivatives. The halogenated analogues can react easily with nucleophiles, and they are widely used in S_NAr reactions to prepare derivatives of the triazine ring. The fundamental building block makes It more important than the cyanuric acid **27** when synthesizing the *s*-triazine series. It may be used as an analogous acid chloride equivalent in many organic transformations, including chlorination, dehydration, and coupling reactions.[38-41]

Cyanuric chloride has temperature-dependent differential reactivity for subsequent transformations of chlorides with nucleophiles during S_NAr reactions. The incorporation of amino nucleophiles was selected as an example and the process is illustrated in **Scheme 1-6**.



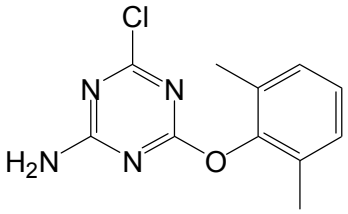
Scheme 1-6. Different reactivity of cyanuric chloride. Reagents and conditions: (a) R_1NH_2, 0 °C; (b) R_2NH_2, 25 °C; (c) R_3NH_2, 70 °C.

As a general rule, which varies from the reactivity of the nucleophile chosen, the first substitution occurs at low temperatures, 0 °C, the second substitution at around room temperature, and the third substitution at elevated temperatures, 70–100 °C[42-45]. This property allows substitution of three different nucleophiles onto the same triazine core, and numerous possible triazine derivatives can be provided.

1.6 Brief introduction to the biological and pharmaceutical applications of *s*-triazine scaffold based derivatives

s-Triazine is a versatile nuclei in various biologically active molecules. The triazine ring will imparts different activity after being substituted of various groups, which provides the basis of designing biologically relevant molecules with widespread applications in drug development.

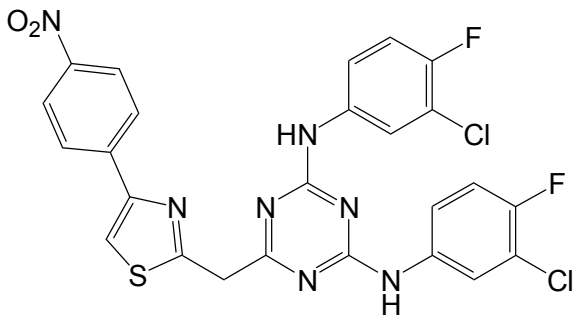
Seo and co-workers[46] described a novel phenoxyl-triazine **30**, which can reach the hydrophobic region of Hsp90 by van der Waals interaction with antiproliferative activity against gefitinib-resistant H1975 cells.



30

Figure 1-16. Hsp90 inhibitor **30**

Gahtori and co-workers[47] have synthesized a series of triazine substituted aromatic and heterocyclic amines as potential antibacterial agents. After structure-activity relationship studies, the presence of a nitro group such as in **31** was found to present the most effective antibacterial activity.

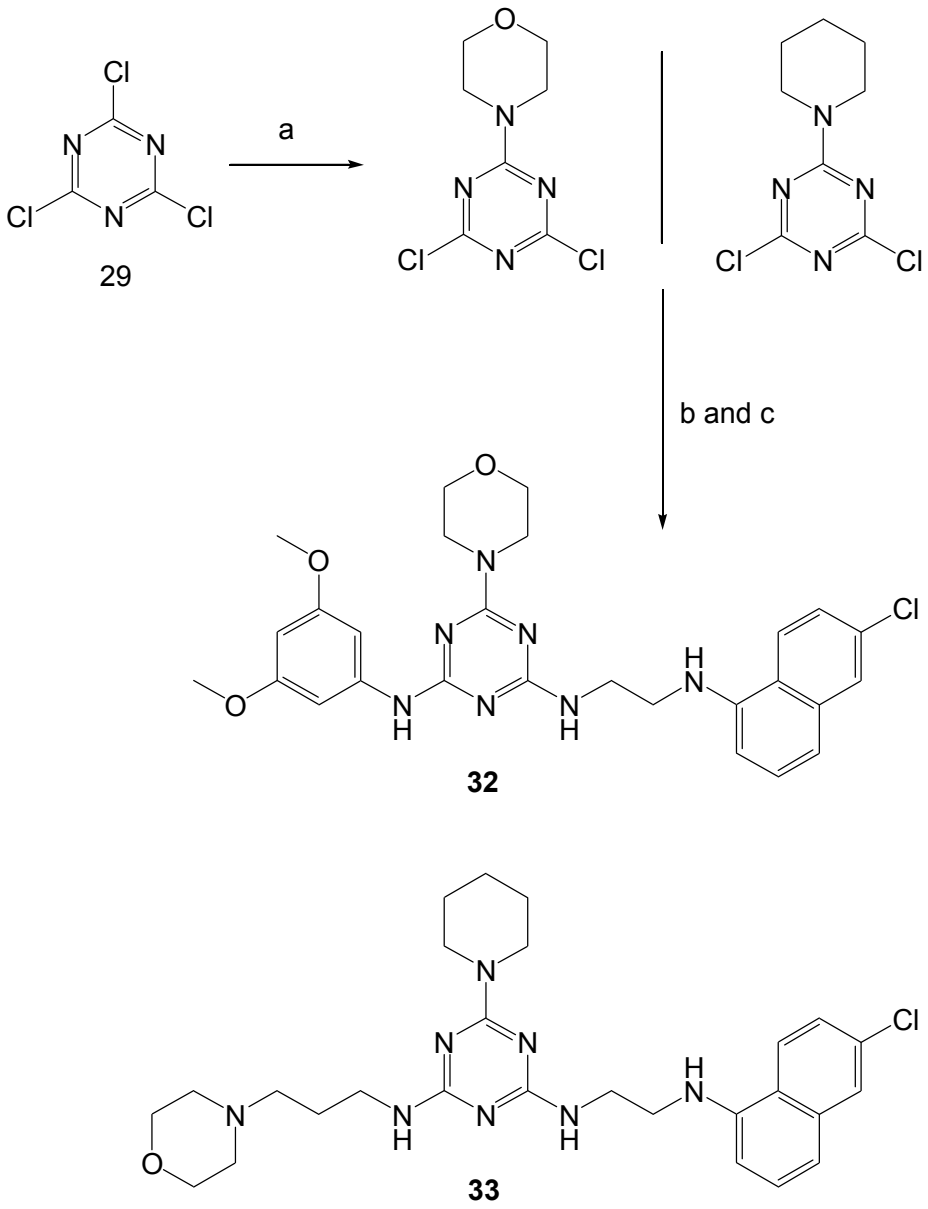


31

Figure 1-17. Phenylthiazoleetriazine hybrid **31**.

Rawat and co-workers[48] described the quinoline moiety substituted with triazine functionalities that can decrease drug resistance while retaining antimalarial activity. Sunduru and co-workers synthesized a serious triazines bearing a quinoline moiety, and **32** and **33** has been found to be the most active against a chloroquinoline sensitive strain. [49]

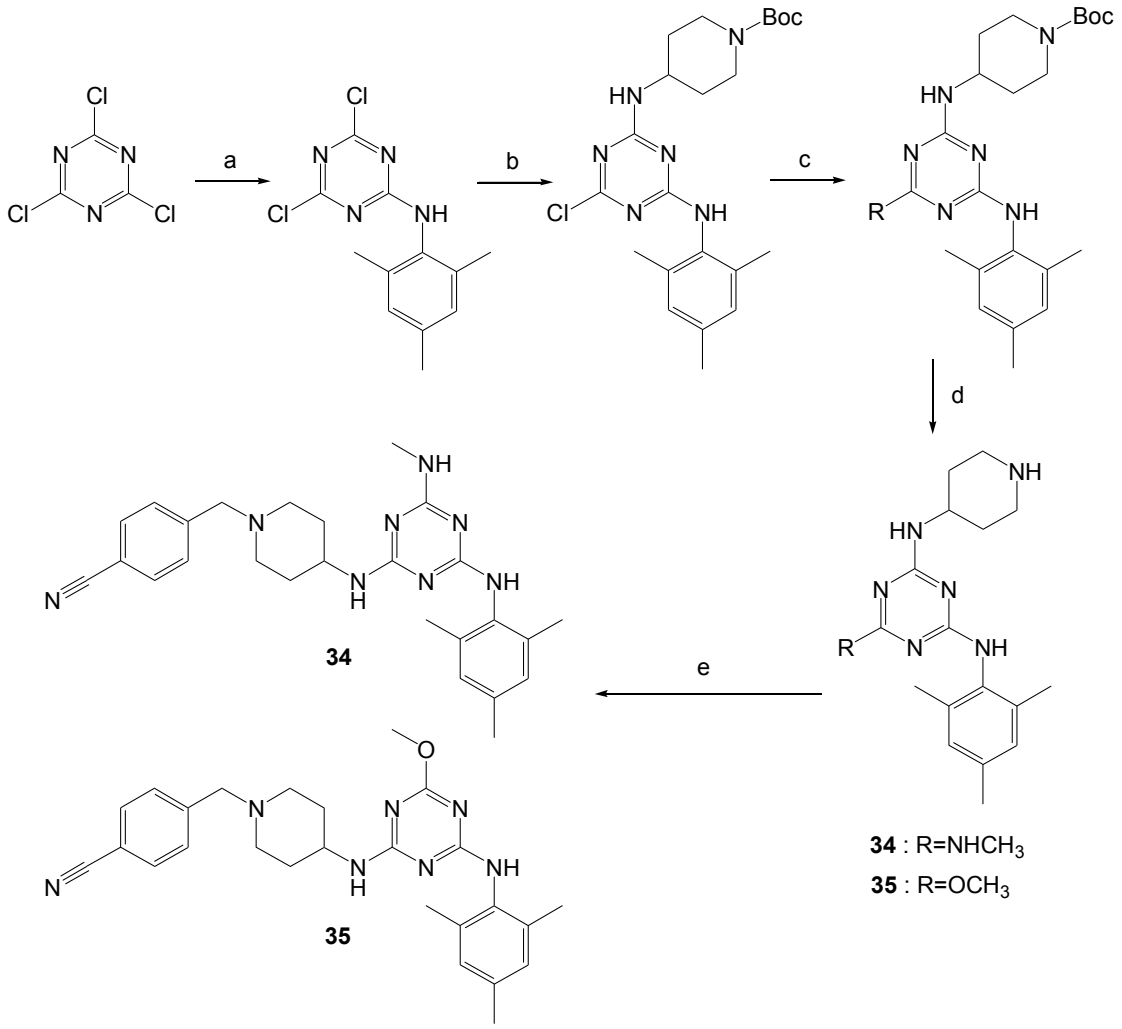
The synthesis of monosubstituted *s*-triazines were accomplished by nucleophilic substitution. Then they were subsequently reacted with N^1-(7-Chloroquinolin-4-yl)ethane-1,2-diamine. The targeted compounds were obtained after the third chloride atom was substituted with further nucleophilic amines.



Scheme 1-7. Quinoline substituted triazine derivatives **32** (above) and **33** (bottom). Reagents and conditions: (a) piperidine/morpholine, K_2CO_3, 0 °C to r.t., anhydrous THF, 1h, 84% and 82%, respectively; (b) N^1-(7-Chloroquinolin-4-yl)ethane-1,2-diamine, K_2CO_3, anhydrous THF, r.t., 4 h; (c) 3-Aminopropylmorpholine/*N*,*N*-Dimethylethylenediamine, K_2CO_3, 80 °C, 16 h, 71% and 74%, respectively.

HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTI) with high antiviral potency, specificity and low cytotoxicity have become an indispensable component in highly active antiretroviral therapy (HAART) regimen.[50] Liu and co-workers[51] have synthesized a novel series of diarylpyrimidines (DAPY) analogs bearing a piperidine triazine moiety. Among them, compounds **34** and **35** are the most potent derivatives which exhibited highest inhibitory activity against HIV-1.

The mono-substituted **s**-triazine was produced starting with cyanuric chloride and 2,4,6-trimethylaniline at 0 °C in the presence of K_2CO_3. The second chloride was then replaced with another primary amine 4-amino-1-Boc-piperidine. Then the third chlorine atom of the product was transformed to methylamino/oxyl group under the conditions of NH_2CH_3 (aq)/ $NaHCO_3$/THF or Na/CH_3OH, respectively. The protecting group was then removed in the presence of trifluoroacetic acid (TFA) at room temperature. The benzyl substituent was then introduced to the substituted the secondary amide.



Scheme 1-8. DAPY derivatives. Reagents and conditions: (a) K_2CO_3, dimethylaniline, THF, 0 °C, 3 h, 91%; (b) $NaHCO_3$ (5% aq), 4-amino-1-Boc-piperidine, THF, 30 °C, 8 h, 92%; (c) $NaHCO_3$ or Na/CH_3OH; (d) TFA, CH_2Cl_2; (e) 4-cyanobenzyl chloride, K_2CO_3, anhydrous DMF, r.t., overnight, 68% and 61%.

Variously substituted *s*-triazine drugs are widely used in the clinic, and some of them are listed in **Table 1-1**.

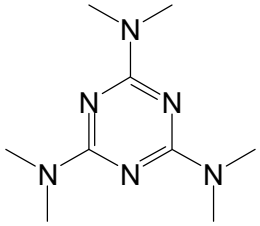
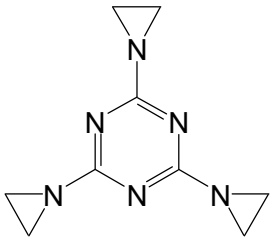
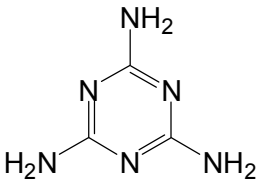
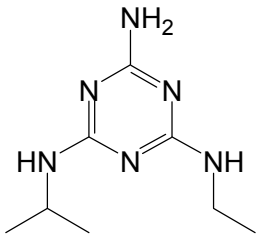
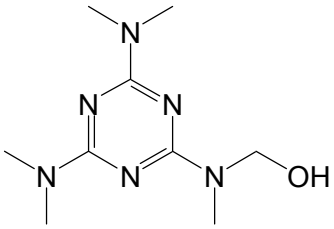
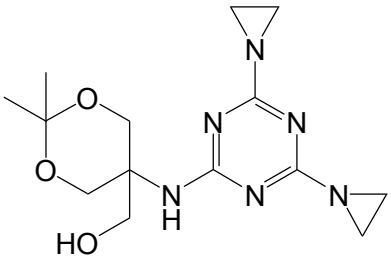
Drug name	Structure	Uses
Altretamine		Refractory ovarian cancer
Triethylenemelamine		Antineophlastic
Melamine		African trypanosomiasis
Atrazine		Itching caused by allergies
HMPMM		Anti-tumor
Dioxadet		Anti-tumor

Table 1-1. Commercial drugs containing triazine nucleus

In this thesis, we aim to develop some triazine-based anti-cancer agents. To start with commercial available cyanuric fluoride/chloride, moieties such as benzyl (e.g. 2,4-dimethoxyphenyl) or amine (e.g. piperazine) will be attached and some mono-, di- or tri-substituted triazines may be formed based on the procedures mentioned above.

Section 3: Introduction to the background of Epidermal Growth Factor Receptor (EGFR) and EGFR inhibitors

1.7 Introduction of Non-Small Cell Lung Cancer (NSCLC)

Between 85% to 90% of lung cancers are identified as non-small cell lung cancer (NSCLC). There are three main subtypes of NSCLC (squamous cell carcinoma, adenocarcinoma and large cell carcinoma). The cells in these subtypes differ in size, shape, and chemical make-up when looked at under a microscope, but they are grouped together because the approach to treatment and prognosis are often very similar. NSCLC is a very aggressive form of neoplasm, more responsible for cancer deaths each year than colon, breast, pancreas and prostate cancers combined together.[52]

1.8 Introduction to EGFR and EGFR inhibitors

Cancer cells are progressive with a gradual transition from normal cells to benign tumors to malignant tumors. They have abnormal membranes and cytoskeletal proteins, and grow and divide at an uncontrolled rapid rate. Over the last 20 years, research efforts has aimed to clarify, and to specifically interfere with the essential alterations in cell physiology that characterize malignancy. Recent research efforts have been focused on developing tumor-specific therapies associated with chemotherapy, which will give theoretically minimum debilitating nonspecific toxicities. By focusing on key molecules integral for cellular function, replication, or tumorigenesis, such specific therapies may exert cytostatic or cytotoxic effects on tumor cells.

The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein that constitutes one of four members of the erbB family of tyrosine kinase receptors. Binding of EGFR to its natural ligands leads to autophosphorylation of receptor tyrosine kinase and subsequent activation of signal transduction pathways that are involved in regulating cellular division, differentiation, and survival. Although present in normal cells, EGFR is overexpressed in a number of tumor cell lines and has been associated with poor prognosis and decreased survival. EGFR activation also plays a role in resistance to chemotherapy and radiation treatment in tumor cells.

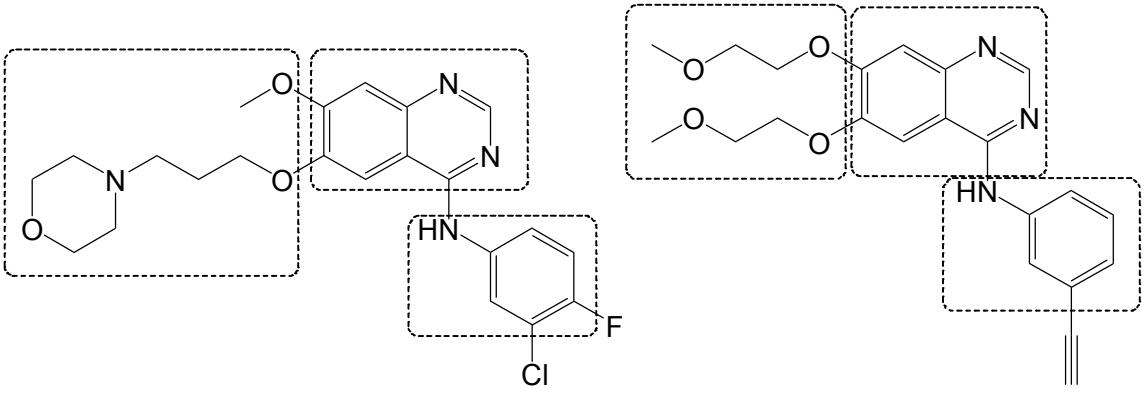
Over the last two decades, a lot of work has been directed at developing anticancer agents that can block with EGFR activity. The most common pharmacologic approaches to inhibiting EGFR have been developed are monoclonal antibodies and low molecular

weight inhibitors. Monoclonal antibodies block ligand binding to the extracellular domain, whereas the small-molecule inhibitors exert their effects on the intracellular part of the receptor to stop tyrosine kinase phosphorylation and further activation of signal transduction pathways. A number of EGFR inhibitors have been developed that can arrest tumor growth and, in some cases, cause tumor regression. When used together with cytotoxic treatments, chemotherapy, or radiation therapy, EGFR inhibitors have been able to enhance their anticancer activity.[53]

In NSCLC, studies have shown that the EGFR is overexpressed in up to 80% of cases, depending on histology.[54] Data from many studies indicate that, on average, 84% of tumors with squamous cell histology will be positive for EGFR.[55] The mean percentages of large cell and adenocarcinomas reported to be positive for EGFR are 68% and 65%, respectively. Overexpression of EGFR in NSCLC is correlated with a high metastatic rate, poor tumor differentiation, and a high rate of tumor growth.[56] Median survival also decreases as the degree of EGFR expression increases, although this conclusion is not supported by all studies.[57,58]

Several approaches have been developed to target the EGFR to interfere with EGFR-mediated cellular effects. The two most intensively studies so far consist of monoclonal antibodies acting directly against the extracellular receptor domain and small-molecule drug compounds that interfere with EGFR tyrosine kinase activity inside the cell. The differing mechanism of action and toxicity profiles of these agents compared with those of traditional cytotoxic compounds, radiation and chemotherapy, provide an ideal rationale for evaluating them in combination with cytotoxic approaches to attempt to achieve additive or even synergistic anticancer activity.

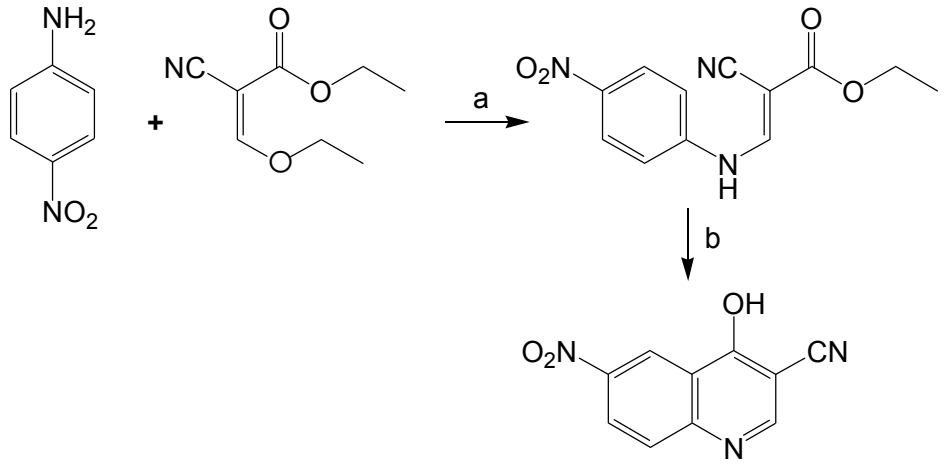
Two orally active low-molecular-weight tyrosine kinase inhibitors (TKIs), ZD1839 (Gefitinib, Iressa) and OSI-774 (Erlotinib, Tarceva) (**Figure 1-18**), have produced specific, reversible inhibition of EGFR tyrosine kinase.[59,60] Gefitinib and erlotinib have induced clinical responses in NSCLC with activating mutations within the EGFR kinase domain.[61-63]



Gefitinib Erlotinib

Figure 1-18. Chemical structures of Getinib and Erlotinib, both of them contain a central quinazoline, with aniline and solubilising groups.

Clinically, the efficacy of these TKIs is often of limited duration because of the emergence of drug resistance conferred by a second mutation: substitution of threonine 790 with methionine (T790M). Threonine 790 is the residue in EGFR located at the entrance to a hydrophobic pocket in the back of the ATP binding cleft, which makes it an important determinant of inhibitor specificity in protein kinases. Yun and co-workers indicated that the introduction of the T790M mutation increased the ATP affinity of the oncogenic activating mutant. The increased ATP affinity is the primary mechanism by which the T790M mutation confers drug resistance.[64-66] The T790M mutation accounts for about half of all resistance to gefitinib and erlotinib.[67] To overcome the T790M mutation related resistance, irreversible ATP-competitive EGFR inhibitors bearing a Michael addition receptor moiety have been developed. For example, Wissner and co-workers prepared 6-amino-4-anilinoquinoline-3-carbonitriles **36** as shown in **Scheme 1-9**.[68] However, these irreversible inhibitors present limited clinical efficacy which is caused by their high toxicities and decreased binding velocity to the mutant kinase.

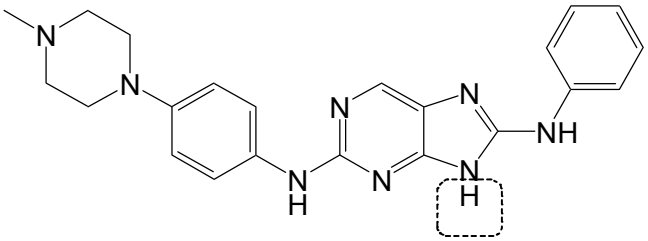


36

Scheme 1-9. Synthesis of 6-Nitro–4-anilinoquinoline-3-carbonitrile building block **36** by Wissner *et al*. Reagents and conditions: (a) Toluene, reflux; (b) Dowtherm, 258 °C.

In recent days, it has been highly desirable to develop novel reversible EGFR inhibitors which can inhibit the drug resistant T790M bearing mutants and remain potent in other activating mutants.[69-72]

Yang and co-workers indicated that a 2,8-dianilinopurine analogue, N^2-(4-(4-methylpiperazin -1-yl)phenyl)-N^8-phenyl-*9H*-purine-2,8-diamine **37** (**Figure 1-19**), exhibited an ability to inhibit both EGFR-activating and resistance mutations.[20] However, the potency against the T790M EGFR mutation of this compound was relatively poor, which made it incapable of overcoming the enhanced ATP binding found in T790M EGFR.

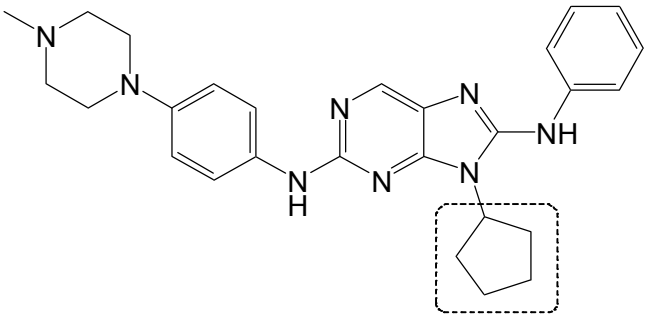


37

Figure 1-19. Structure of inhibitor **37**

Yang and co-workers then successfully optimized **38** from **37** in good yields. The aniline groups at C-2 and C-8 were retained and a cyclopentyl group was attached at N-9 of the purine scaffold. This allowed compound **38** to exhibit significant in vitro antitumor potency against the NSCLC cell lines HCC827 and H1975, which harbor EGFR-activating and drug resistance mutations, respectively. In further tests, compound **38** was indicated as a highly

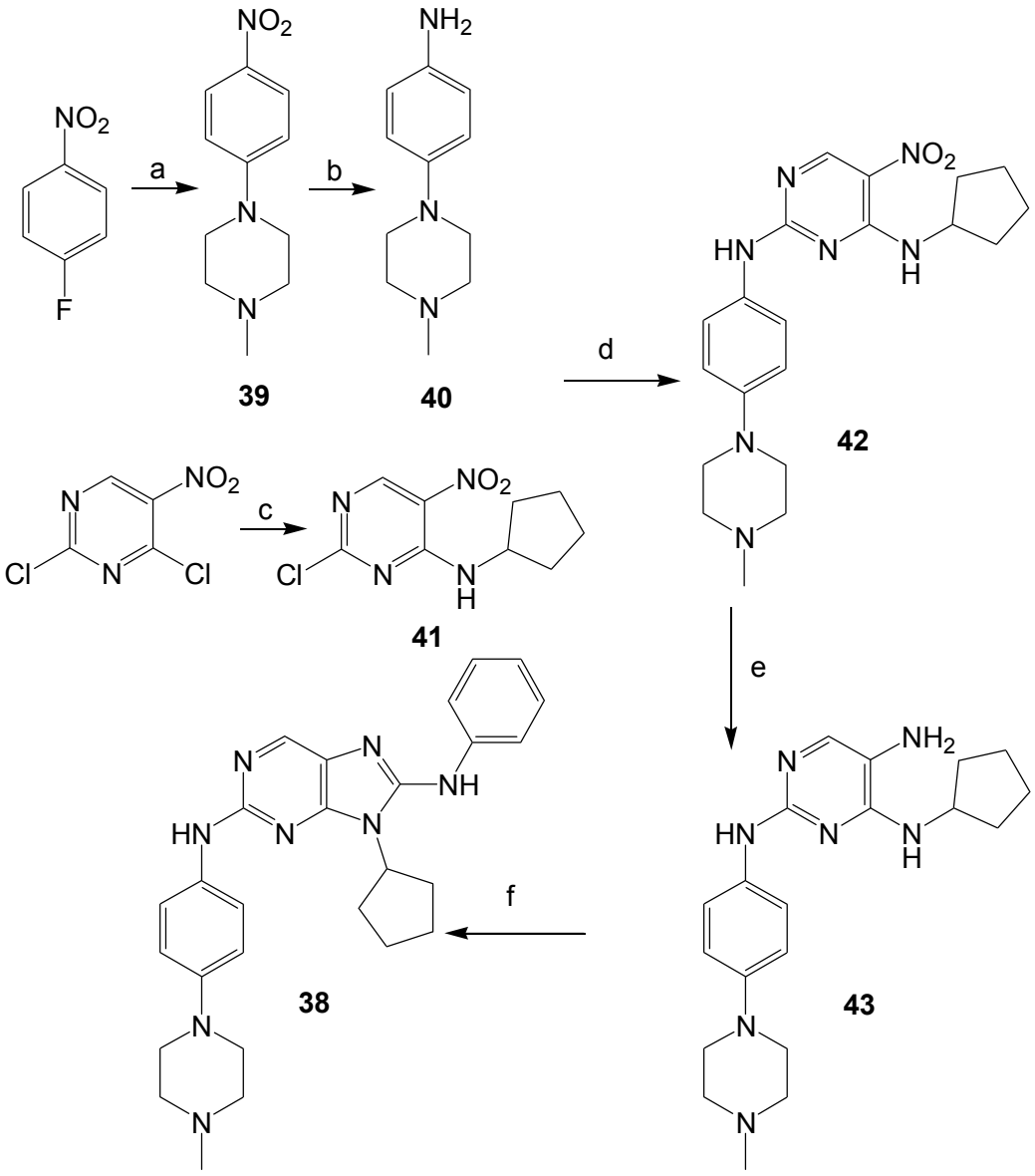
potent kinase inhibitor against both EGFR-activating and resistance mutations in vivo anti-NSCLC studies.



38

Figure 1-20. Structure of optimized purine inhibitor **38**

The synthetic route to the 2,8-dianilinopurine derivative **38** is shown in **Scheme 1-10**.



Scheme 1-10. Reagents and conditions: (a) 1-methylpiperazine, K_2CO_3, DMSO, r.t., 6 h, 95%; (b) H_2, 10% Pd/C, EtOH, r.t., 9 h, 90%; (c) CH_2Cl_2, DIEA, cyclopentylamine, -60 °C to room temperature, 60-75%; (d) n-butanol, 90 °C, 5 h, 76%; (e) H_2, 10% Pd/C, MeOH, 50 °C, 6 h, 85%; (f) CH_2Cl_2, DIEA, EDIC, isothiocyanatobenzene, reflux, 12 h, 35–60%.

The preparation of the first main aniline intermediate **40** began with commercially available 4-fluoronitrobenzene which was reacted with 1-methylpiperazine in the presence of K_2CO_3 in DMSO to produce the intermediate **39**. Catalytic hydrogenation of the nitro group in **39** with palladium on carbon (Pd/C) provided the desired aniline **40** in excellent yield. The second main intermediate **41** is synthesized with commercially available 2,4-dichloro-5-nitropyrimidine with cyclopentylamine. Nucleophilic aromatic substitution of the 2-chloro-

position in **41** with **40** in n-butanol at 90 °C yielded compound **42**. Subsequent hydrogenation of the nitro group in **42** using Pd/C as a catalyst provided a good yield of the desired intermediate **43**, which was treated with isothiocyanotobenzene to produce the final 2,8-dianilinopurine derivative **38**.

Continued research into new methods for heterocyclic synthesis thus remains important for medicinal chemistry and the development of new drugs to combat cancer and other life threatening diseases. In this thesis, we therefore aim to synthesis a new target scaffold around the kinase inhibitor **38**. The aniline and piperazine groups would be remained in our structure, and the preparation would based on the procedure mentioned above.

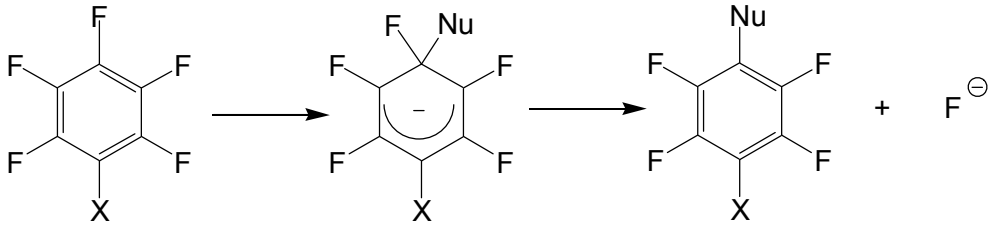
Section 4: Fluorinated aromatic compounds

1.9 Orientation of substitution reactions in polyfluorinated compounds

As a key part of the strategy proposed for the new targets in this work involves stepwise S_NAr reactions of per-fluorinated arenes such as pentafluoropyridine, the chemistry of these highly fluorinated arenes is now discussed.

Similar to electrophilic substitution in benzene derivatives C_6H_5X, the nucleophilic reaction of perfluorobenzene derivatives, C_6F_5X, with nucleophiles can give three sites of attack, ortho, meta and para relative to the group X. ^{19}F NMR spectroscopy has be used used since the very early work to assist in the structure determination of such compounds[73].

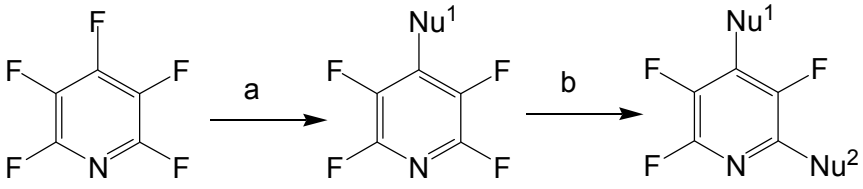
The variation in the proportions of ortho, meta and para substitution of fluorine by nucleophiles in C_6F_5X compounds is very uneven and can be profoundly influenced by the nature of the solvent (X could either be electron donating or withdrawing) (**Scheme 1-11**). [74] Meta substitution is the least common site of attack and requires vigorous conditions. The major substitution product from the vast number of C_6F_5X compounds which have been reacted with nucleophiles is the para isomer.



Meisenheimer intermediate

Scheme 1-11. Para substitution in C_6F_5X compounds *via* Meisenheimer intermediate

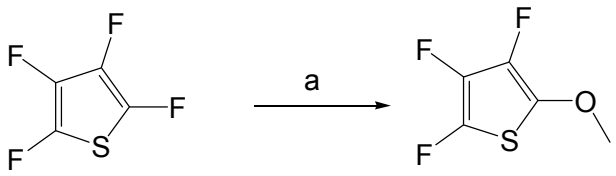
Nucleophilic substitution of fluorine in pentafluoropyridine, the first heterocyclic compound to be prepared,[75] occurs initially almost exclusively at the 4-position, due to stabilization of the charge on the tetrahedral intermediate by nitrogen.[76]



Scheme 1-12. Nucleophilic substitution of hexafluorobenzene and pentafluoropyridine.

Conditions: (a)Nucleophile 1; (b)Nucleophile 2.

Orientation effects also occur in polyfluorinated systems containing five-membered rings with six π electrons. For example, tetrafluorothiophene undergoes substitution at the 2-position under the conditions of NaOMe/MeOH at room temperature (**Scheme 1-13**).[77]



Scheme 1-13. Substitution of the 2-fluorine. Reagents and conditions: $NaOCH_3$/MeOH, 60 ˚C.

1.10 Brief introduction to ^{19}F NMR spectroscopy

As ^{19}F NMR spectroscopy is less widely known and used than ^{1}H or ^{13} C NMR, it is introduced briefly here.

As the new target compounds and the synthetic intermediates leading to them, contain fluorine which is an NMR active nucleus, the background to ^{19}F NMR spectroscopy is reviewed here as this is a powerful analytical method that was used to assist in structure determination in the research reported in this thesis.

Current synthetic methods make possible the production of fluorine-containing analogs of amino acids, nucleosides, lipids and sugars, as well as a wide variety of molecules that are important drugs and agricultural chemicals. Fluorine 19, at 100% natural abundance, is a spin 1/2 nucleus. The technology for obtaining fluorine NMR spectra is virtually the same as that involved in acquiring proton NMR data. However, because a fluorine nucleus in a molecule is on average surrounded by nine electrons, rather than a single electron as is the case with hydrogen, the range of fluorine chemical shifts and the sensitivity of fluorine chemical shifts to the details of the local environment are much higher for fluorine than hydrogen. Signals are thus usually well spaced and give valuable structural information about the compounds under investigation.

The reference signals used for proton and carbon-13 NMR spectroscopy are those from tetramethylsilane (TMS). Fluorine chemical shifts span a very wide range and there is no

single fluorine-containing compound that is experimentally convenient for use as a universal reference compound.[78]

A fluorine reference is usually a compound that is chemically similar to the one under examination. The typical chemical shift values are shown in **Table 1-2**.

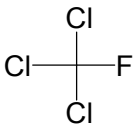
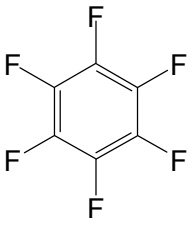
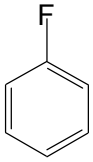
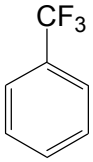
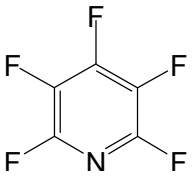
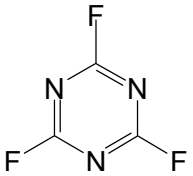
Compound	Formular	Structure	Chemical shifts versus $CFCl_3$ / (p.p.m.)
Trichloro-fluoromethane	$CFCl_3$		0.00
Hexafluorobenzene	C_6F_6		-164.90
Monofluorobenzene	C_6H_5F		-113.15
Trifluoro-toluene	$C_7H_5F_3$		-63.72
Pentafluoropyridine[70]	C_5F_5N		-88.5 -132.5 -160.5
Trifluoro-triazine[71]	$C_3F_3N_3$		-31.3

Table 1-2. Typical ^{19}F NMR chemical shifts. Negative (-) values correspond to upfield shifts, higher-shielding, or lower frequency.

Fluorine will also exhibit strong coupling to protons or carbon atoms in a molecule which can give useful information about structure and orientation of substituents. A disadvantage

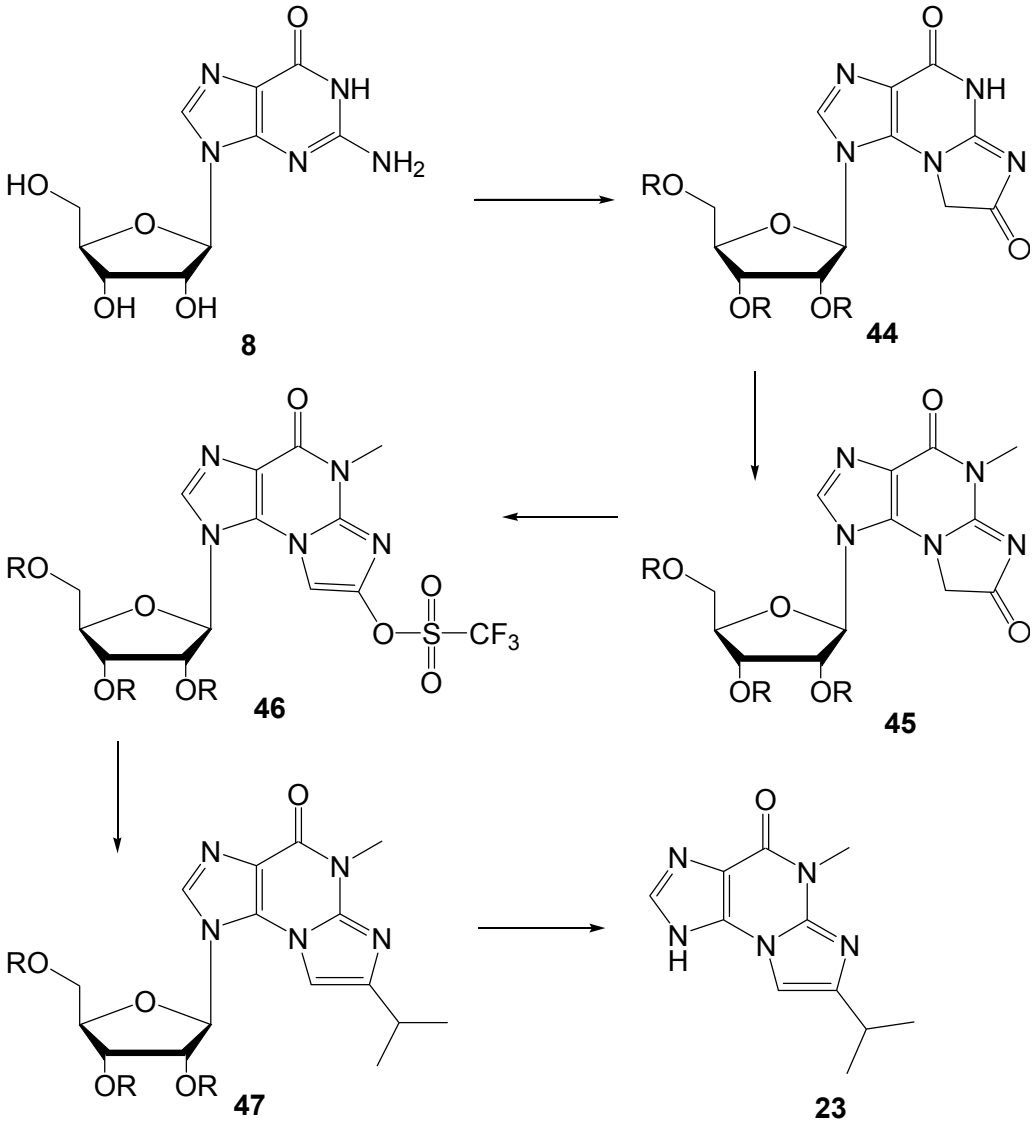
however is the reduced intensity of C-F signals, particularly weak aromatic signals, thus requiring long acquisition times to detect all signals.

^{19}F NMR spectroscopic results will be discussed throughout the thesis, as it proved to be an essential analytical tool in structure determination.

Chapter 2: Results and Discussion

Section 1:

One of our aims was to synthesise the revised structure of acremolin **23** and compare its spectroscopic data with that of the natural product isolated by Shin[26] to confirm if the proposed revised structure was correct. The revised structure of acremolin was first planned to be synthesized in five steps from guanosine, and the proposed synthetic route is shown below (**Scheme 2-1**).

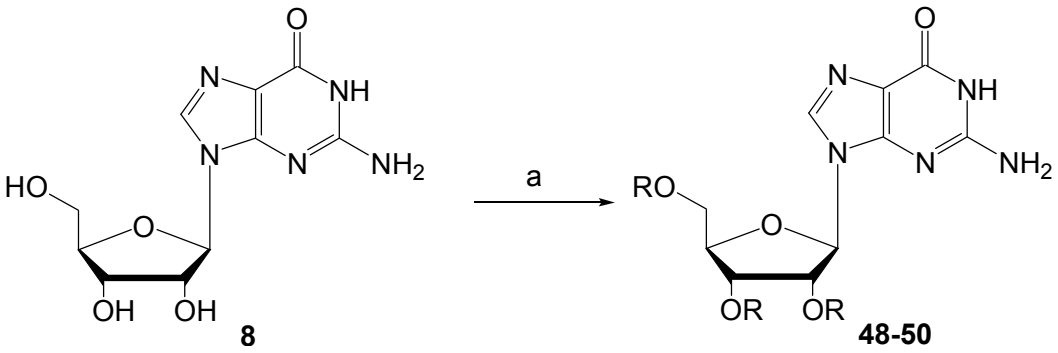


Scheme 2-1. Proposed synthesis of the revised structure of acremolin **23** from guanosine.

Guanosine **8** was selected as the starting material as the imidazole nitrogen is already blocked with the ribose ring, which allows methylation to occur on the pyrimidine ring directly. The second fused imidazole **44** will be formed by reacting **8** with chloroacetyl chloride after the solubility modification on the ribose ring. In the last two steps, **45** will be treated with trifluoromethanesulfonic anhydride to provide triflate derivative **46** before the introduction of the isopropyl group by palladium catalysed coupling to give the target product **23**.

2.1 Solubility modification of guanosine

As guanosine **8** is largely insoluble in general organic solvents, three reagents, acetic anhydride, propionic anhydride and octanoyl chloride were employed to modify the hydroxyl groups on the ribose ring (**Scheme 2-2**) yielding the products **48**-**50**. The reactions were run with the following conditions (**Table 2-1**).



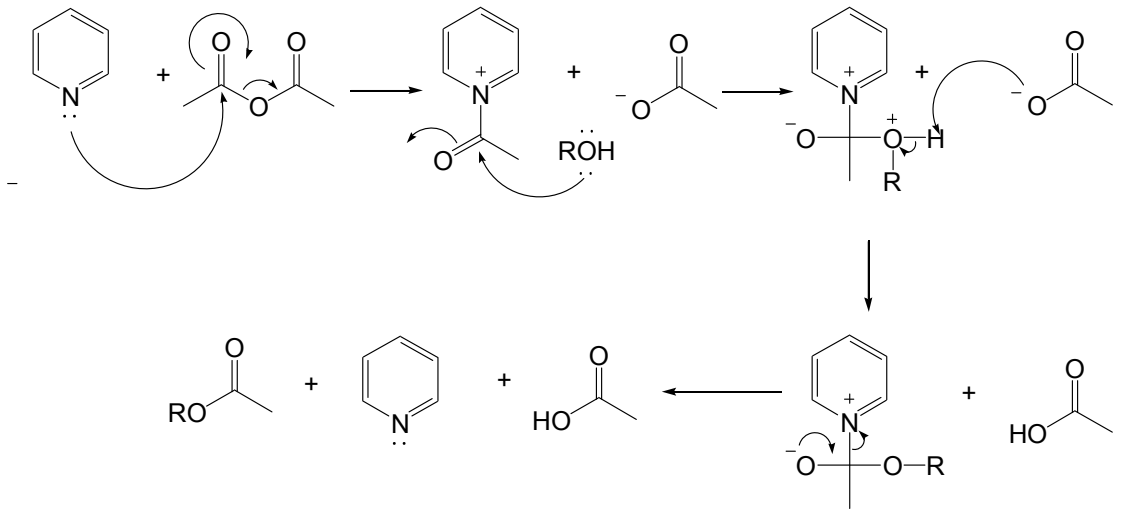
Scheme 2-2. Solubility modification of guanosine. Reagents and conditions: (a) pyridine, DMF, 100 ˚C, 2 h.

Entry	Reactant	Product	R	Yield
1	Acetic anhydride	**48**	Acetyl	75
2	Propionic anhydride	**49**	Propionyl	70
3	Octanoyl chloride	**50**	Octanoyl	—

Table 2-1. Compounds obtained

In the case of esterification with acetic anhydride the currently accepted mechanism involves three steps. First, pyridine and acetic anhydride form in an equilibrium reaction a labile ion pair between the acetate and the acetyl-pyridinium ion. In the second step the

alcohol attacks the acetyl group to form the ester. In this step the acetate counterion removes the proton from the alcohol while the alcohol forms a covalent bond with the acetyl group. The bond from the acetyl group to the catalyst is cleaved to generate the catalyst and the ester. The acetic acid formed will then protonate the pyridine. In the last step of the catalytic cycle the auxiliary base deprotonates the protonated pyridine, reforming the catalyst[81] (**Scheme 2-3**).



Scheme 2-3.

It has been reported that using pyridine and DMF as a mixed solvent could increase the yield of the product. The quaternary ammonium salt formed by pyridine and acetic anhydride could play a significant role as an acylating agent. The large polarity of DMF generates good solubility of the starting materials. The yield reached the peak when the amount of acetic anhydride increased till $n_{\text{guanosine: acetic anhydride}}$ reached 1: 10, and then changed very slightly.[82]

In entry 1 and 2, reactions proceeded smoothly without any difficulties in good yields (75% and 70, respectively). The pure products were obtained as white fine powders after purification via column chromatography.

In ^{1}H NMR spectroscopy, signals representing the acetyl/propionyl showed the right ratio to that of the hydrogen atoms and the diastereotopic protons attached to the ribose ring. As a result, compound **48** could dissolved in organic solvents like dichloromethane, while compound **49** could only be dissolved in some more polar organic solvents like pyridine.

In entry 3, the starting material spot presented after four hours stirring by tracking by the

TLC, and the reaction mixture stayed very turbid, and could not be filtered or dissolved in neither water nor ethyl acetate. The reason was not clear, and the reaction was discontinued.

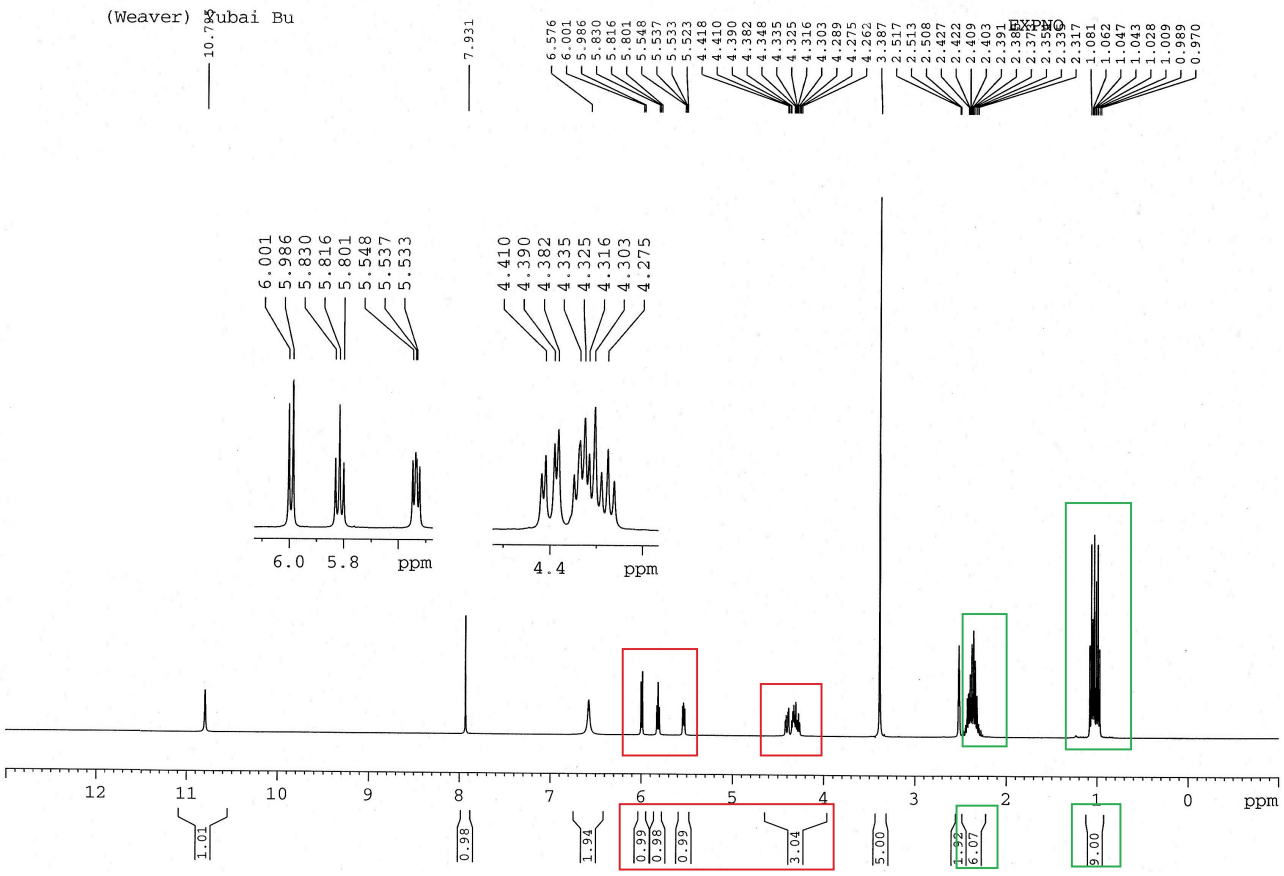


Figure 2-1. ^{1}H NMR spectrum of 2',3',5'-tripropanoate-guanosine **49**, the ribose hydrogen (red grid) and the propionyl hydrogen (green grid) in DMSO-d_6.

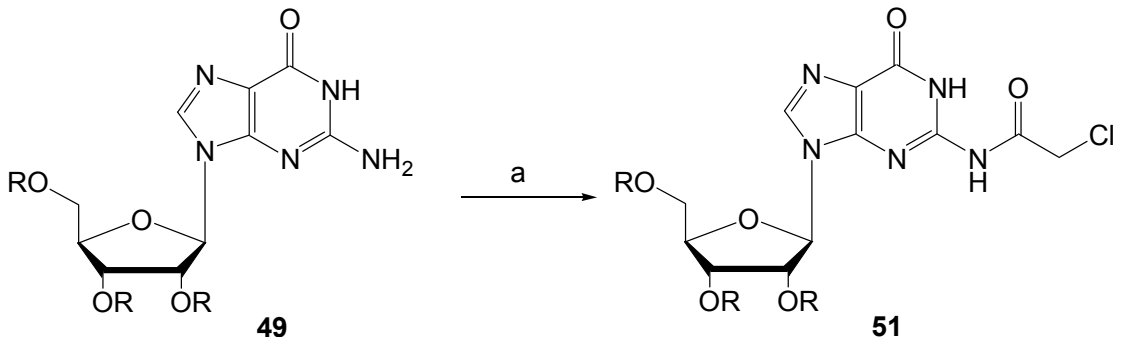
2.2 synthesis of 2',3',5'-tripropanoate-*N*-(2-chloroacetyl)-guanosine 51

In the next step we aimed to form **51** which involved treatment of **48** and **49** with chloroacetyl chloride (**Scheme 2-5**).

Compound **48** and chloroacetyl chloride were stirred in pyridine at 0 ˚C. The reaction mixture formed a brown gel after a very short period. This could be due to the by-product formed by reaction of the chloroacetyl chloride and pyridine. As a result, the reactant needed to have better nucleophilic solubility in a less polar solvent.

The second approach was started with 2',3',5'-tripropanoate-guanosine **49**, chloroacetyl

chloride and *N*-ethyldiisopropylamine formed 2',3',5'-tripropanoate-*N*-(2-chloroacetyl)-guanosine **51** in good yield.



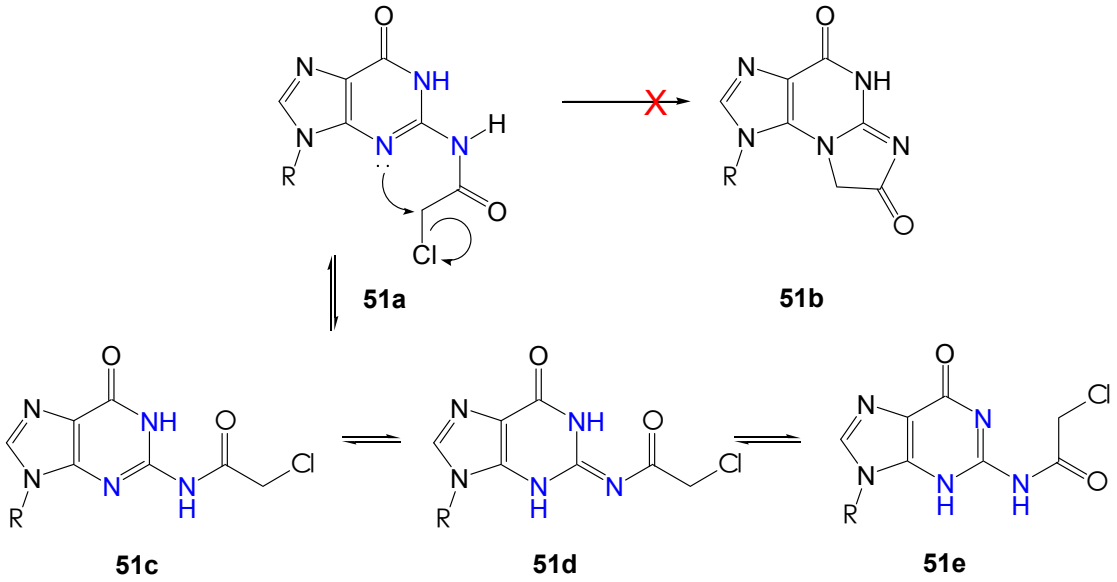
49 **51**

R = propionyl

Scheme 2-5. Synthesis of 2',3',5'-tripropanoate-*N*-(2-chloroacetyl)-guanosine **51**. Reagents and conditions: (a) chloroacetyl chloride, *N*-ethyldiisopropylamine, anhydrous THF, 65 ˚C, 2 h, 73%.

The reaction was firstly run with triethylamine, 80% of the starting material was recovered even though the chloroacetyl chloride was added in excess and the reaction time was increased to18 hours. The could be due to the high reactively of chloroacetyl chloride may have reacted with triethylamine. The reaction finished in 2 hours after a more hindered base *N*-ethyldiisopropylamine was used in excess.

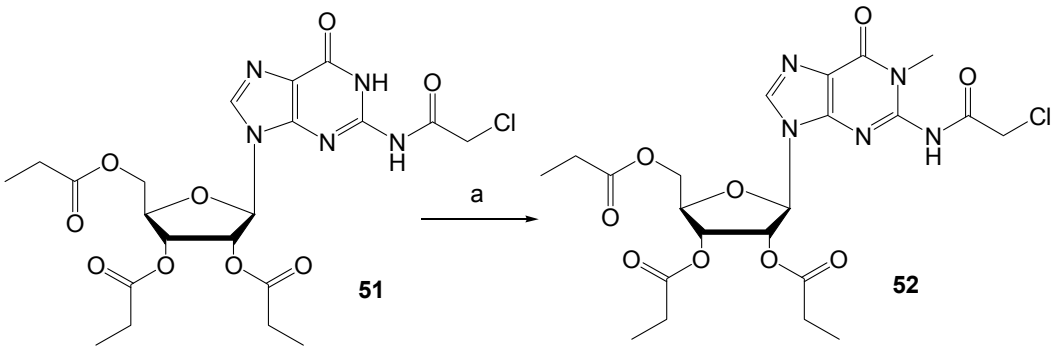
In the high resolution mass spectrum, the signal representing $C_{21}H_{27}{}^{35}ClN_5O_9{}^+$ was observed, which indicated that the chloroacetamide **51a** had formed but did not spontaneously ring close to the tricyclic imidazo-purine **51b**. This was also proved by the presence of two broad singlet signals at δ= 11.92 and 10.38 which represented on NH groups. In addition, a new singlet representing the new introduced CH_2 group (δ=4.36 p.p.m.) in ^1H NMR spectrum. The delocalized electrons between three nitrogen atoms give rise to a tautomer/rotamer mixture of **51c**, **51d** and **51e**, which may also inhibit the cyclization activation.



Scheme 2-6. Cyclization of **51a**.

2.3 Synthesis of 2',3',5'-tripropanoate-*N*-(2-chloroacetyl)-1-methyl-guanosine 52

The next step was to methylate the N-1 of the purine ring in **51** with iodomethane to give 2',3',5'-tripropanoyl-*N*-(2-chloroacetyl)-1-methyl-guanosine **52** in fair yield (**Scheme 2-7**).



Scheme 2-7. Synthesis of 2',3',5'-tripropanoate-*N*-(2-chloroacetyl)-1-methyl-guanosine **52**. Reagents and conditions: (a) iodomethane, anhydrous THF, 40 ˚C, 8 h, 46%.

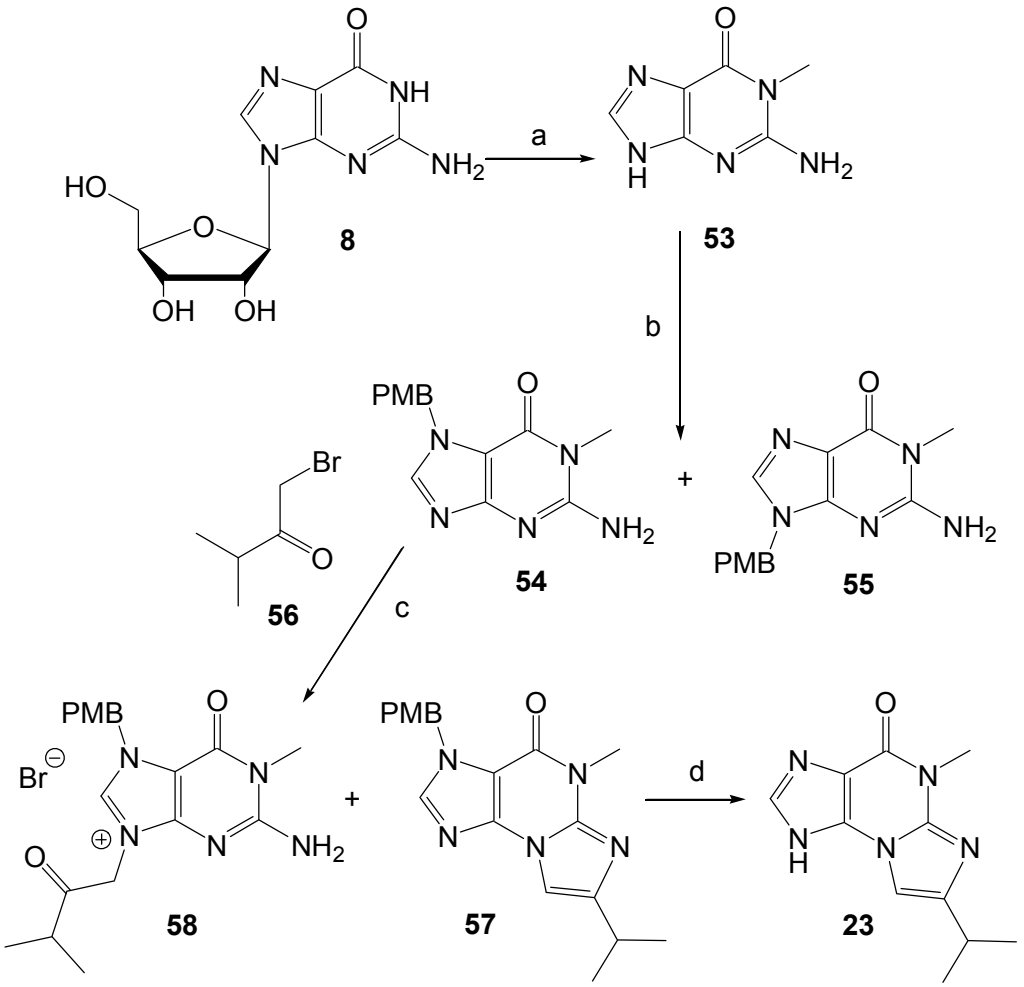
This proceeded smoothly without any difficulties and resulted in fair yields. This pure product was obtained as yellow solid after the purification via column chromatography. In ^{1}H NMR spectroscopy, a new signal at δ=3.28 p.p.m. represents the N_1-CH_3. This was also proved by ^{13}C spectroscopy (a new signal at δ=32.98) and HRMS spectroscopy ($[M+H]^+$ signal matching the expected composition).

Conclusion

Guanosine was successfully modified with acetic anhydride and propionic anhydride, and 2',3',5'-tripropanoate-guanosine **49** was then used as the starting material in the next step due to its good solubility. Chloroacetyl chloride was successfully attached to NH_2 group on C-2. However, the compound **51** failed to spontaneously ring close. The methylation reaction proceeded smoothly at N-1 position, and a precursor to the revised structure of acremolin was successfully formed.

2.4 First total synthesis of acremolin

After we had started our investigation, the first synthesis of the revised structure of the heterocyclic marine natural product, acremolin, was reported (**Scheme 2-8**).[83]



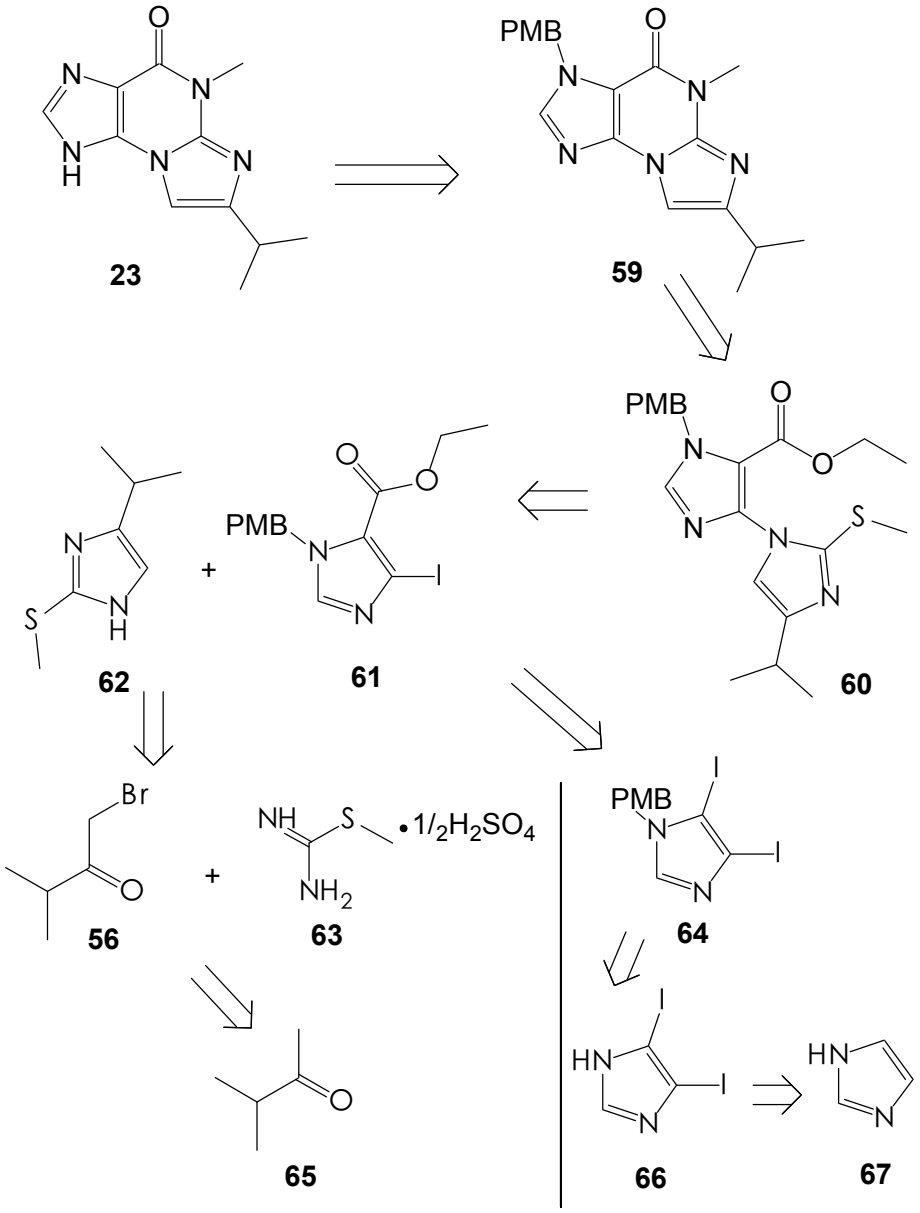
Scheme 2-8. Lawrence procedure.

Lawrence and co-workers chose to prepare 1-methylguanine **53** by methylation of guanosine, and followed by hydrolysis of the nucleoside. Protection of **53** gave a mixture of N^7- and N^9-PMB derivatives **54, 55** (~1.6:1, 56%) from which **54** could be isolated by fractional crystallization (EtOH). Reaction of **54** with bromoketone **56** returned the N^2,3-ethenoguanine derivative **57** (27%) as a single isomer, along with the guaninium salt **58** (54%) resulting from monoalkylation of the imidazole ring. The PMB group was removed under the conditions reported by Fujii and co-workers (90% aq H_2SO_4, toluene, 40 ˚C),[84] and smoothly delivered samples of **23** of high purity, albeit in low yield (39%).

2.5 Synthesis of the revised structure of acremolin from imidazole

Despite this, we wanted to finish our synthesis (and adapt it to form analogues which might have useful biological activity).

A alternative synthetic plan had also been considered, which involved coupling two imidazole precursors to build up the imidazopurine core. As this route differed from Lawrence's method we decided to investigate this approach which is outlined in **Scheme 2-9**.



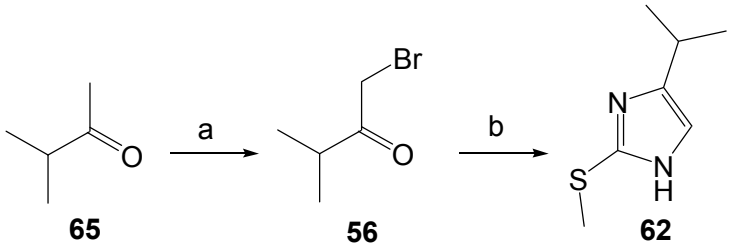
Scheme 2-9.

The second part of the research began by firstly aiming towards the formation of two fragments: 4-isopropyl-2-methylthio-1*H*-imidazole **62** and 4-iodo-1-[(4-methoxyphenyl)

methyl]-1*H*-imidazole-5-carboxylic acid **61**. Compound **62** was proposed to be synthesized via a cyclization reaction between a bromoketone **56** and a thiouronium salt **63**, while compound **61** was proposed to arise after sequential substitution reactions starting with imidazole. Two precursors would be coupled together, and the cyclization reaction in next step would employ methyl amine as the reagent to close the purine ring.

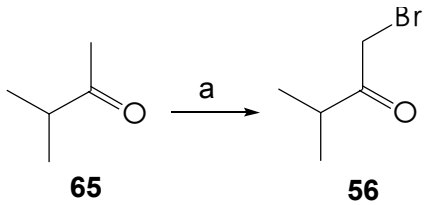
2.6 Synthesis of 2-methylthio-4-isopropyl-1*H*-imidazole 62

We obtained 4-isopropyl-2-methylthio-1*H*-imidazole **62** successfully by a 2-step reaction **Scheme 2-10**.



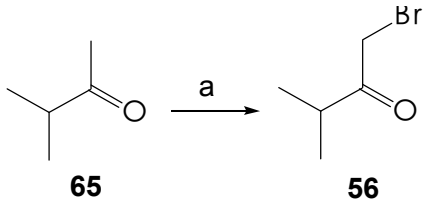
Scheme 2-10. Synthesis of 2-methylthio-4-isopropyl-1*H*-imidazole **62**. Reagents and conditions: (a) bromine, methanol, 0 ˚C, 2 h, 95%; (b) *S*-methylisothiourea hemisulfate salt, sodium hydride, DMF, room temperature, 8 h, 16%.

Dickschat and co-workers had synthesized 2-bromo-3-methyl-2-butanone **56** at low temperature, -78 ˚C yielding the product in 79% (**Scheme 2-11**)[85].



Scheme 2-11. Dickschat procedure. Reagents and conditions: (a) bromine, pentane, -78 ˚C, 79%.

We produced the compound **56** with a different solvent at a higher temperature and obtained the same product in excellent yield (95%).

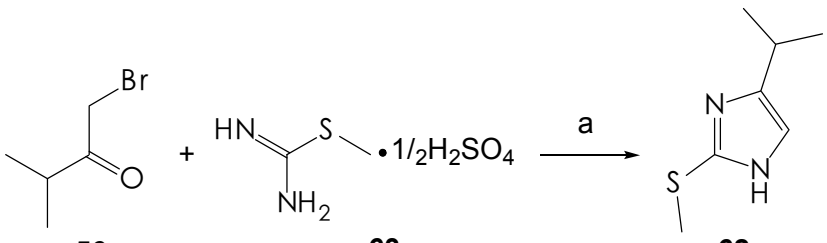


65 **56**

Scheme 2-12. Synthesis of **56**. Reagents and conditions: (a) bromine, methanol, 0 ˚C, 2 h, 95%.

This proceeded smoothly without any difficulties and resulted in excellent yields. GC-MS confirmed the expected composition, C_5H_9BrO by the presence of signals at 163.9 and 165.9 m/z representing the two isotopes of bromine. In addition, the product caused eye irritation characteristic of α-halo-carbonyl compounds. This stage was repeated on a 20 mmol scale. In addition, this stage was also tried on a 40 mmol scale. However, the yield of the predicated mono-substituted product was lowered, as the di- or tri-brominated by-products were formed in a capricious reaction.

The second step was to condense the bromoketone **56** with a thiouronium salt **63** to form the desired imidazole **62** (**Scheme 2-13**). This worked but led to the product in a very disappointing 16% yield. The reactions were run with the following conditions (**Table 2-2**).



56 **63** **62**

Scheme 2-13. Synthesis of **62**. Reagents and conditions: NaH, DMF, room temperature, 8 h, 16%.

Entry	Solvent	Base	Reaction temperature	Reaction time/(hours)	Yield/ (%)
1	Ethanol and water	Triethylamine	Reflux	4	—
2	Ethanol and water	Sodium hydroxide	Reflux	4	—
3	Sodium ethoxide (neat)		Reflux	8	—
4	DMF	Sodium hydride	room temperature	8	16

Table 2-2.

As the S-methylisothiouea hemisufate salt could only be dissolved in water, ethanol and water were used as a mixed solvent in entry 1 and 2. In addition, sodium ethoxide was selected as both the base in ethanol as solvent in entry 3. Unfortunately, all three reactions formed complex product mixture and none of the fractions obtained after chromatography proved to be the expected product after analysis by ^{1}H NMR and MS spectroscopy.

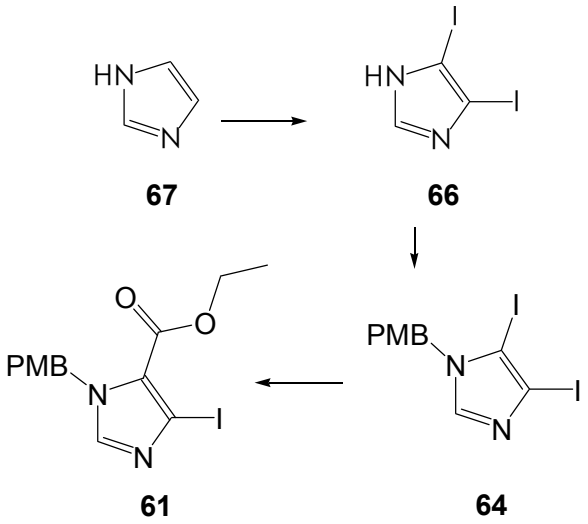
At last, the desired product was isolated after column chromatography in entry 4.

Conclusion

The synthesis of the first imidazole precursor, 4-isopropyl-2-methylthio-1*H*-imidazole **62**, proceeded smoothly via two steps reactions, and then the bromoketone **56** was prepared in excellent yield. After trials with different conditions of bases, solvents and temperature, the desired product was formed successfully iwith the use of sodium hydride in DMF.

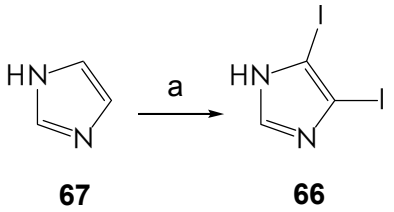
2.7 Synthesis of 4-iodo-5-carboxylic-1-[(4-methoxyphenyl)methyl]-1*H*-imidazole 61

The desired compound **61** was obtained after three consecutive substitution reactions (**Scheme 2-14**).



Scheme 2-14. Synthesis of **61**.

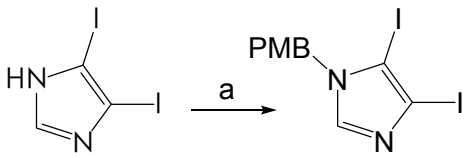
The first stage was to introduce iodine into positions **4** and **5** ~~replace the 4,5-hydrogens~~ at imidazole **67** by iodine and gave **66** (**Scheme 2-15**) in 75% yield.[77]



Scheme 2-15. Synthesis of 4,5-diiodo-1*H*-imidazole **66**.[86] Reagents and conditions: Iodine, NaOH, hexane, deionized water, room temperature, 8 h, 75%.

This proceeded smoothly without any difficulties and resulted in good yields. The reaction mixture was stirred in a basic environment to allow deprotonation of imidazole. The reaction was quenched with 1M HCl until the pH reached 7, the crude product was precipitated. After recrystallization with dilute ethanol, the product **66** was formed as a fine white powder. The melting point (175 ˚C) confirmed this by comparison with the literature value (180 ˚C)[86].

The second stage involved alkylation to form the 4,5-diiodo-1-(4-methoxybenzyl)-1*H*-imidazole and gave **64** in 91% yield.

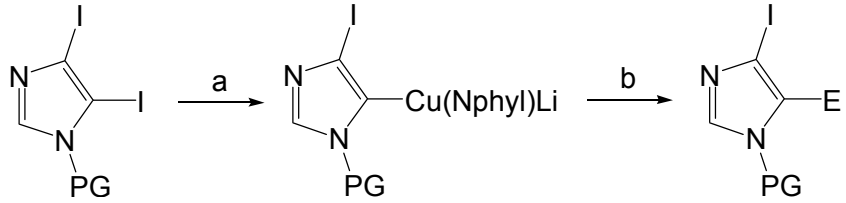


66 **64**

Scheme 2-16. Synthesis of PMB-protected imidazole **61**. Reagents and conditions: PMB-Cl, sodium hydride, anhydrous THF, 55 ˚C, 12 h, 91%.

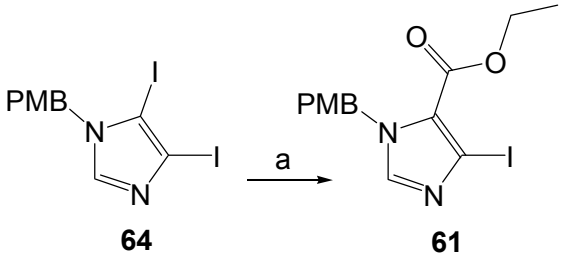
This proceeded smoothly without any difficulties and resulted in excellent yields. The reaction mixture was stirred at 0 ˚C for 1 hour to allow the deprotonation of 4,5-diiodo-imidazole **66**. This pure product gave the same melting point (157 ˚C) compared with the literature[78].

Knochel and co-workers reported a iodine-copper exchange reaction (**Scheme 2-17**). The introduced $(Nphyl)_2CuLi_2$ presented precomplexation to the protecting group (CH_2OEt or Ts) favoring the iodine copper exchange in the ortho-position. Various electrophiles (e.g. EtCOCl) provided the corresponding products in good yields.[87]



Scheme 2-17. Knochel's iodine-copper exchange reaction.

For replacing the iodo-group with the desired ester moiety, the ~~stronger base~~ BuLi was employed to effect lithium halogen exchange (**Scheme 2-18**).



64 **61**

Scheme 2-18. Synthesis of **64**. Reagents and conditions: (a) nitrogen protection i) n-BuLi, anhydrous THF, ii) ethyl chloroformate, r.t., 8 h, 60%.

The desired product **64** was produced smoothly without any difficulties and resulted in fair

yields. This pure product was obtained as yellow oil after the purification via column chromatography. In ^{1}H NMR spectroscopy, new signals at δ=4.36 and 1.32 p.p.m. represent the newly introduced ethyl group. This was also proved by HRMS spectroscopy ($[M+H]^{+}$ signal matching the desired product).

Conclusion

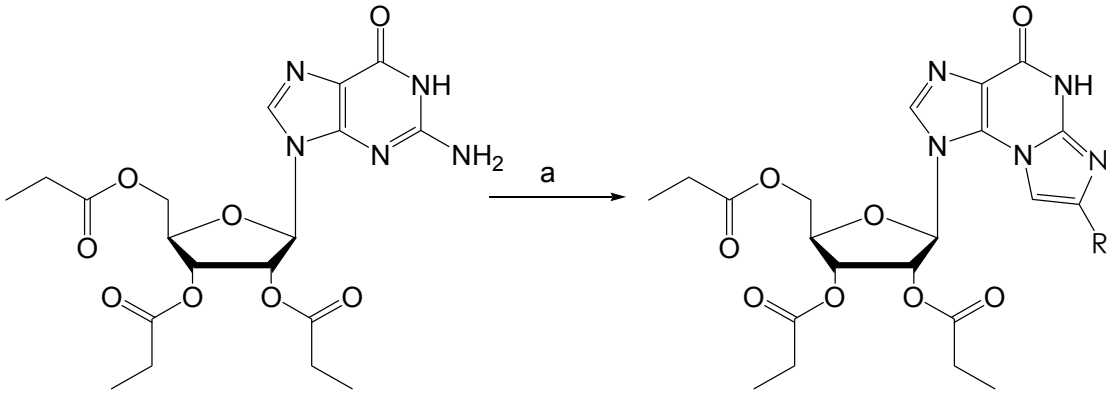
The second precursor, ethyl 4-iodo-1-[(4-methoxyphenyl)methyl]-1*H*-imidazole-5-carboxylate **61**, was synthesised successfully via a three step reaction. The 4,5-position hydrogens were firstly substituted by iodine. And then the amine group was protected with PMB group to give the desired product **64** in excellent yield. One of the iodine atoms was replaced by the carboxylate group using lithium halogen exchange, and which was identified by ^{1}H, ^{13}C NMR spectroscopy and its high resolution mass spectrum.

Although compound **61** was successfully prepared, due to lack of time, and the low yield of the other imidazole component **62** we were not able to investigate the coupling of the two imidazole fragments to compete the core of the acromelin molecule, and further work would be required to complete the natural product target by this approach.

2.8 Other attempts to prepare guanosine and imidazole derivatives

Despite the bioactivity of acremolin being still not totally investigated, we still wanted to synthesis some of derivatives of it. Thus, a number of precursors were prepared based on our products formed above.

We synthesized two analogues 7-(propan-2-yl)-1-(2,3,5-tri-*O*-propanoylpentofuranosyl)-1*H*-imidazo[2,1-*b*]purin-4(5*H*)-one **68** and 7-phenyl-1-(2,3,5-tri-*O*-propanoylpentofuranosyl)-1*H*-imidazo[2,1-*b*]purin-4(5*H*)-one **69** based on Lawrence's last procedure.



49 **68,69**

68 R=propan-2-yl

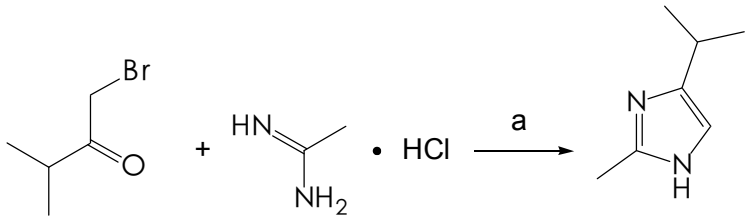
69 R=phenyl

Scheme 2-19. Synthesis of analogues **68** and **69**. Reagents and conditions: (a) 1-bromo-3-methyl-2-butanone in **68** or 2-bromo-1-phenylethanone in **69**, sodium hydride, DMF, 80 ˚C, 12 h, 20% and 12%, respectively.

Both analogues **68** and **69** were isolated in poor yields after the purification via column chromatography. In ^{1}H NMR spectroscopy of compound **68**, most signals belonging to the compound **49** remained the same, the broad singlet represented the NH_2 at C-2 disappeared. In addition, a new singlet signal at δ=7.38 p.p.m., which belongs to the new ring formed, reached the agreement with that reported in the literature.[83]

However, the ^{13}C NMR spectroscopy for both compounds **68** and **69** were very poor, as most signals were very weak due to the aromatic carbon skeleton. We also confirmed the products in the HRMS by the matching theoretical $[M+H]^+$ signal at 512.5022 and 552.5664, respectively.

The imidazole analogues, **70** (**Scheme 2-20**), **71** and **72** were prepared following the same procedure of the synthesis of imidazole **62**. It was to condense the bromoketones with acetamidine groups to formed imidazoles (**Table 2-3**).



56 **70**

Scheme 2-20. Synthesis of **70**. Reagents and conditions: sodium hydride, DMF, r.t., 8 h, 35%.

Sodium hydride was chosen as the base and DMF was used as the very polar solvent. The solution was stirred at room temperature ~~for~~ overnight. The complex product was purified by a column chromatography and the desired products were collected.

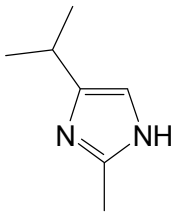
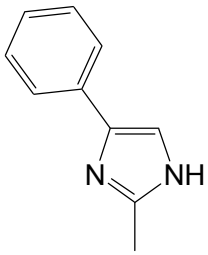
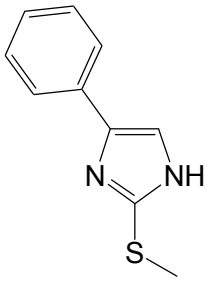
Compound	Structure	Starting material	Reagent	Yield/(%)
70		1-bromo-3-methyl-2ketone	acetamidine	35
71		2-bromo-1-phenyl-ethanone	acetamidine	28
72		1-bromo-3-methyl-2ketone	S-methylisothioure a hemisulfate salt	18

Table 2-3.

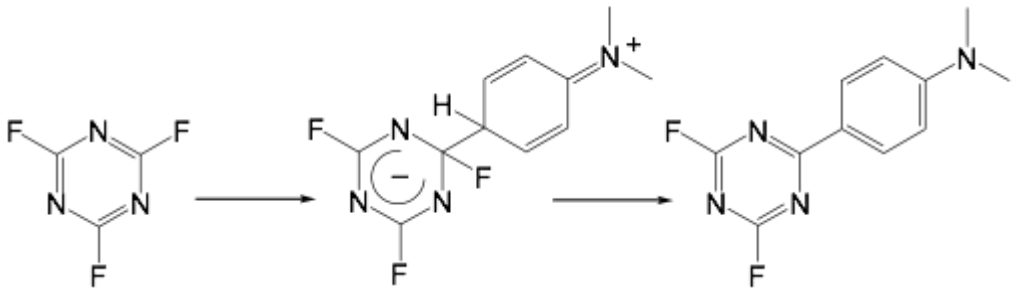
Similar to compound **62**, 4-isopropyl-2-methyl-1*H*-imidazole **70** was isolated as a pale yellow oil. The other two 4-phenyl-substitued imidazoles **71** and **72** were formed as yellow

solids, and the melting points (158 ˚C and 134 ˚C, respectively) were close to those reported before (159 ˚C and 137 ˚C, respectively).[89,90] In ^{1}H spectra of both compounds, signals were presented in correct ratios at the expected chemical shifts.

Section 2:

2.9 Synthesis of 2,4-difluoro-6-monosubstituted *s*-triazines

A new highly-fluorinated class of compounds class was firstly described by Chambers in 1992[91] including the 2,4,6-trifluorotriazine. Aromatic amines were found to act as carbon nucleophiles in reactions with the highly electron deficient fluorinated triazine as shown in **Scheme 2-21**.

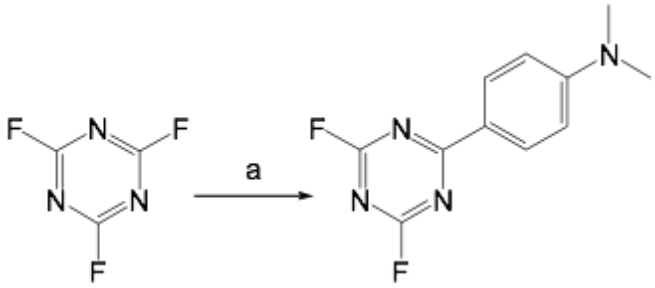


73 **74**

Scheme 2-21. Chambers procedure[91]

As part of our research to develop new triazine derivatives we repeated the reaction of **73** with *N*,*N*-dimethylaniline. We obtained **74** (**Scheme 2-22**) successfully in a yield of 33% (Chamber's yield 28%).

The first fluoride was to be substituted with an electron-donating dimethylaminophenyl moiety as this was known to react with the need for activation. The following substitution reaction should then proceed smoothly, and the conditions would be more easily controlled.



73 **74**

Scheme 2-22. Addition of *N*,*N*-dimethylaniline to cyanuric fluoride **73**. Reagent and conditions: (a) *N*,*N*-dimethylaniline, acetonitrile, reflux, overnight.

Addition of *N*,*N*-dimethylaniline to a solution of cyanuric fluoride in acetonitrile gave an immediate blue coloration, but on heating under reflux the color changed to dark red. The

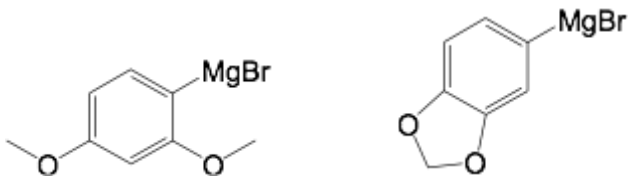
blue color was presumably due to a charge-transfer interaction.

The amount of cyanuric fluoride was added slightly in excess as it could easily be removed as the product was formed as a solid. After overnight reaction, the unwanted by-products including 2-fluoro-4,6-di-(*N*,*N*-dimethylamino-benzen-4-yl)-*s*-triazine and 2,4,6-tri-(*N*,*N*-dimethylamino-benzen-4-yl)-*s*-triazine were not isolated ~~formed~~. The melting point of **74** (235 ˚C) was very close to the compound in literature (234 ˚C). A single peak was shown in ^{19}F NMR spectrum also demonstrated that the two fluoric groups were mirror imaged. The yield was a slightly higher than that shown in literature (28 %).[91]

2.10 Synthesis of 2-fluoro-4,6-disubstituted *s*-triazines

Compounds consisting of di-aryl substituted *s*-triazines were of interest as potential anti-cancer drugs (unpublished results from our collaborators), and the synthesized heterocycles are undergoing testing for biological activity with collaborators at De Montfort University.

Two Grignard reagents (2,4-dimethoxyphenyl)-magnesium bromide **75** and (1,3-benzodioxole)-magnesium bromide **76** were prepared in dried flasks as THF solutions (**Figure 2-2**) as these oxygenated arenes had shown good activity in related compounds prepared by collaborators.

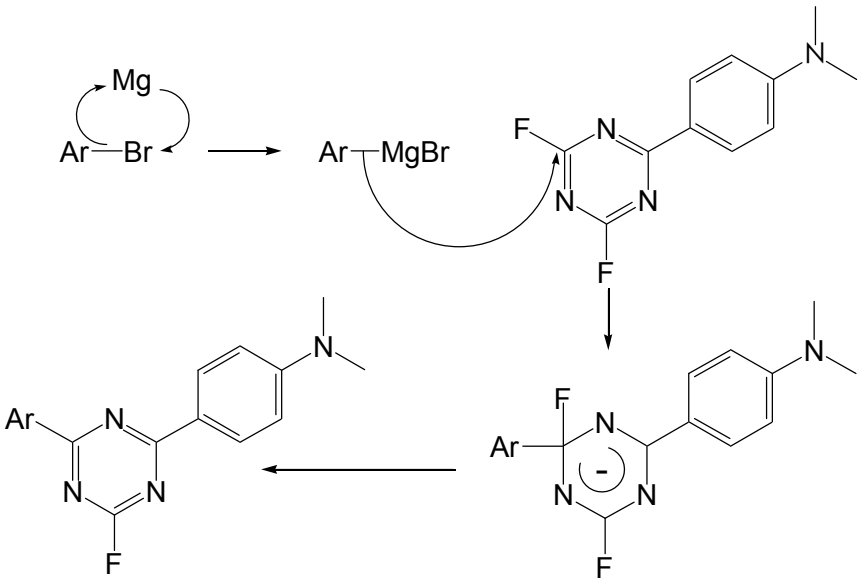


75 **76**

Figure 2-2. Grignard reagents **75** and **76**

The aromatic bromide was dissolved in anhydrous THF and added dropwise to magnesium powder under a nitrogen atmosphere. After the mixture was heated under reflux for 2 hours, the magnesium powder was consumed. The system was allowed to cool to room temperature. The grey colored slurry are then used directly in the required experiments with the fluorinated triazine 74.

We successfully obtained a 2,4-diaryl substituted *s*-triazine compound, 2-fluoro-4-(*N*,*N*-dimethylaminophenyl)-6-(2,4-dimethoxyphenyl)-*s*-triazine **77** starting with the **74** either using Grignard reagent (**Scheme 2-23**) or lithiated arene (**Scheme 2-24, Table 2-4**). The lithium could have a stronger electron-withdrawing inductive effect than magnesium, thus both reactions should proceed smoothly.

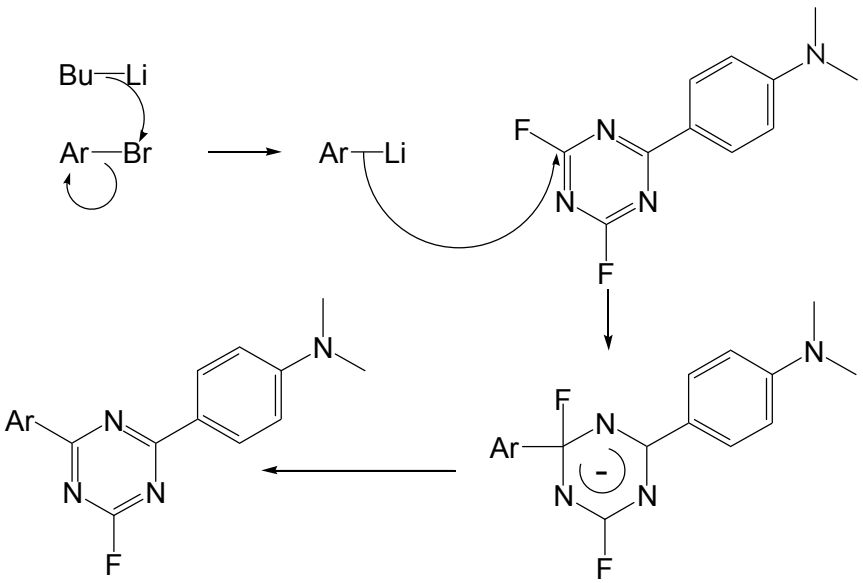


77, 78

77 Ar: 2,4-dimethoxyphenyl

78 Ar: 1,3-benzodioxole-5-yl

Scheme 2-23. Substitution reaction with Grignard reagent. Reagents and conditions: ArBr, Grignard reagent, anhydrous THF, 0˚C to r.t., overnight



77, 78

Scheme 2-24. Substitution reaction with organolithium reagent. Reagents and conditions: ArBr, *n*-BuLi, anhydrous THF, -78˚C to r.t., overnight.

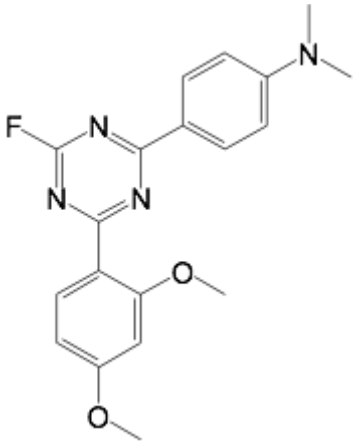
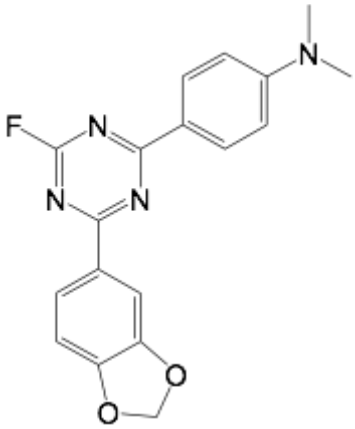
Product	Product structure	Reaction reagent	Yield/(%)
77		Grignard	25
		n-BuLi	22
78		Grignard	Only starting material recycled
		n-BuLi	Only starting material recycled

Table 2-4. The 2,4-diaryl substituted *s*-triazine **77** and **78**.

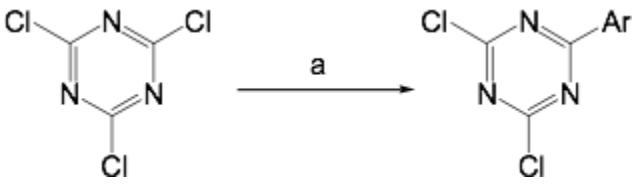
One of the remaining fluorine atoms in **74** was then replaced by 2,4-dimethoxyphenyl Grignard reagent at room temperature to afford **77** by nucleophilic substitution (**scheme 2-22**). The Grignard reagent was added dropwise to keep compound **74** in excess to prevent over addition and formation of the unwanted triaryl compound. A single peak shown in the ^{19}F NMR spectrum occurred at -3 ppm chemical shift, which confirmed to the second substituted group. Although the first substitution of F in 1,3,5-trifluorotriazine occurs with a neutral nucleophile, replacement of the second of third fluorine requires an anionic organometallic reagent.

The failed reaction of **78** may be due to the lower reactivity of the 1,3-benzodioxole-5-yl group, but there is no obvious reason for the lack of reaction.

2.11 Synthesis of 2,4-chloro-6-monoaryl *s*-triazines

Cyanuric chloride **29**, a cheaper and more readily available reagent than the corresponding fluride, was used as starting material in the next steps, and it would be less reactive than the fluoride one, which means the conditions would be more easily controlled.

The first aim of this part was to synthesis the monoaryl-substituted triazines. We obtained two 2,4-dichloro-6-monoaryl-*s*-triazines, **79** and **80**, using nucleophilic substitution via a Grignard reagent or lithiation of a bromoarene with *n*-butyllithium as reagent (**Scheme 2-25**).



29 **79** Ar: 2,4-dimethoxyphenyl

80 Ar: 1,3-benzodioxole-5-yl

Scheme 2-25. Reagents and conditions: (a) Grignard reagent, anhydrous THF, 0˚C to r.t., overnight; (b) ArBr, *n*-BuLi, anhydrous THF, -78˚C to r.t., overnight.

The Grignard reagent was added dropwise to the solution of cyanuric chloride in anhydrous THF at 0 ˚C. The monoaryl-*s*-triazines were purified using column chromatography, where unreacted cyanuric chloride eluted at the very beginning owing to its non-polar structure. As the cyanuric chloride was kept in excess and the temperature was controlled, the unwanted di-substitued by-product was avoided.

From TLC analysis, **79** showed Rf=0.5 (eluent petroleum ether: ethyl acetate=4: 1), while **80** was less polar and exhibited an Rf=0.8 (eluent petroleum ether: ethyl acetate=8: 1).

After comparing the ^{1}H NMR spectrum with the starting material, we confirmed the presence of more deshielded peaks in the aromatic region. Mass spectrometry also revealed the theoretical $[M+H]^{+}$ isotopic signature with strong peaks at 286.0140 and 269.9827 for the $^{35}Cl_2$ isomers of compounds **79** and **80**, respectively.

The products **79** and **80** were also obtained when the Grignard reagent was replaced by the aryllithium reagent. The *n*-BuLi reagent was introduced to the aromatic bromide at

-78˚C in anhydrous THF under nitrogen atmosphere to effect bromine-lithium exchange, and then stirred at room temperature for 30 minutes to complete the aryllithium formation. The reaction mixture was cooled to -78˚C again when the solution of cyanuric chloride introduced rapidly.

Two methods used were listed in **Table 2-5**.

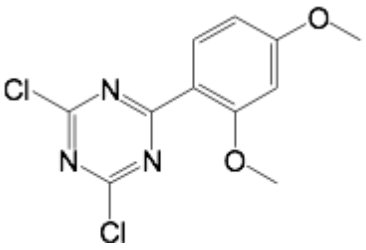
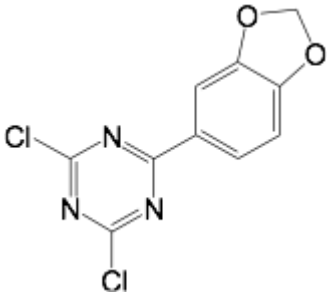
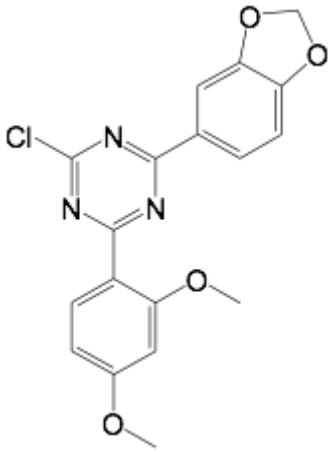
Structure code	Product	Reaction reagent	Yield/(%)
79		Grignard	75
		n-BuLi	73
80		Grignard	70
		n-BuLi	62

Table 2-5.

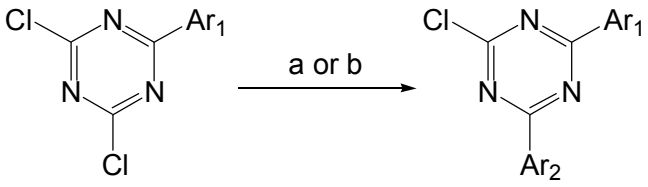
As a conclusion, 2,4-dichloro-6-(2,4-dimethoxyphenyl)-*s*-triazine **79** and 2,4-dichloro-6-(1,3-benzodioxole-5-yl)-*s*-triazine **80** were both successfully prepared via Grignard reagent or using organolithium nucleophile as reagent, with both reactions giving the similar yields. The yields were higher for the reaction of the Grignard and lithium reagents with the trichlorotriazine than the difluoro-monaryl system 74 most likely due to the presence of three halogens.

2.12 Synthesis of 2-chloro-4,6-diaryl *s*-triazines

Our next target was to join 2,4-dimethoxyphenyl and 1,3-benzodioxole-5-yl groups together forming a 2-chloro-4,6-diaryl-*s*-triazine **81** following the procedure discussed in section 2.11 (**Scheme 2-26**).

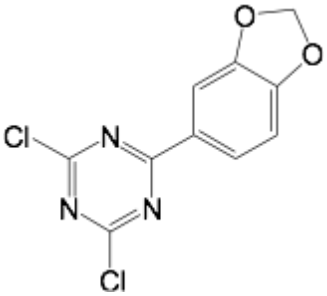
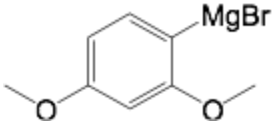


81



Scheme 2-26. Synthesis of 2-chloro-4,6-diaryl-*s*-triazine **81**. Reagents and conditions: (a) Grignard reagent, Ar_2Br, anhydrous THF, 0 ˚C to r.t., overnight; (b) *n*-BuLi, Ar_2Br, (needs Ar_2Br), anhydrous THF, -78˚C to room temperature, overnight.

We attempted to synthesis the target di-aryl-product and our attempts are listed in **Table 2-6**.

Entry	Starting material	Reaction reagent		Yield/ (%)
1			Grignard	Only starting material recycled

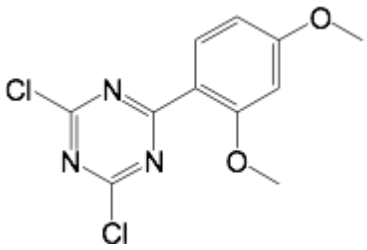
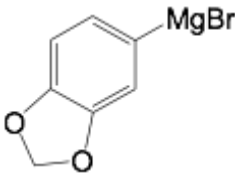
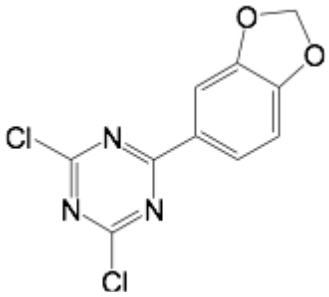
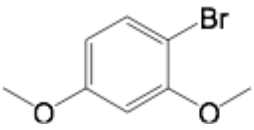
2			Grignard	Only starting material recycled
3			*n*-BuLi	35

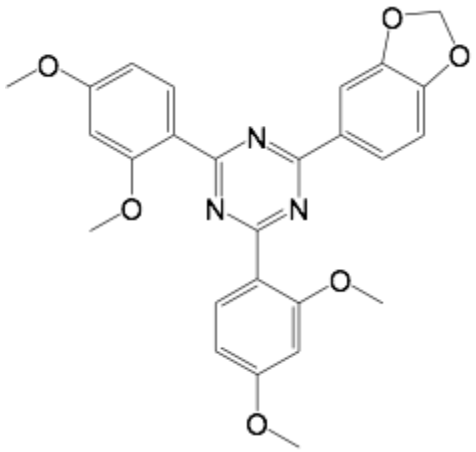
Table 2-6. Synthesis of 2-chloro-4,6-diaryl-*s*-triazine **81**

Our fist attempt was to attach 1,3-benzodioxole-5-yl to the monoaryl compound, 2,4-dichloro-6-(2,4-dimethoxyphenyl)-*s*-triazine **79**, formed in section 2.11 using Grignard reagent. However, no new spots were shown after TLC tracking even when the reaction time was prolonged to 3 days. The lack of reaction was firstly ascribed to the lower activity of 1,3-benzodioxole-5-yl group. However, after the two aromatic group sequence swapped, new product spots were still not observed.

It appeared the dichloro-mono-aryl triazines were considerably less reactive than the corresponding fluorides, or the room temperature could not allow the secondary chloride group substituted by the Grignard reagent. Reactions forming **79**, or **80** gave around 70% yield, whereas **81** was formed in only 35% yield.

Then we decided to use the *lithium* reagent to achieve the nucleophilic substitution. The desirable compound **81** was successfully obtained as a yellow colored oil.

From TLC analysis, compound **81** exhibited higher polarity than compounds **79** and **80**. The ^{1}H and ^{13}C NMR spectra had confirmed the purity of the product. We also confirmed the product by mass spectrometry with a signal matching theoretical $[M+H]^{+}$ value at 372.0735.



82

Additionally, a triary-substitued triazine **82** was obtained with the previous procedure (entry 3) after two equivalence of 2,4-dimethoxyphenyl bromide and Grignard reagent were introduced.

From TLC analysis, compound **82** showed to be even more polar than **81**. The ^{1}H and ^{13}C NMR spectra both confirmed the purity of the product. The signals of 2,4-dimethoxyphenyl group exhibited double intensity than that of 1,3-benzodioxole-5-yl group. We also confirmed the product by the matching theoretical $[M+H]^{+}$ signal at 474.1660 in the high resolution mass spectrum.

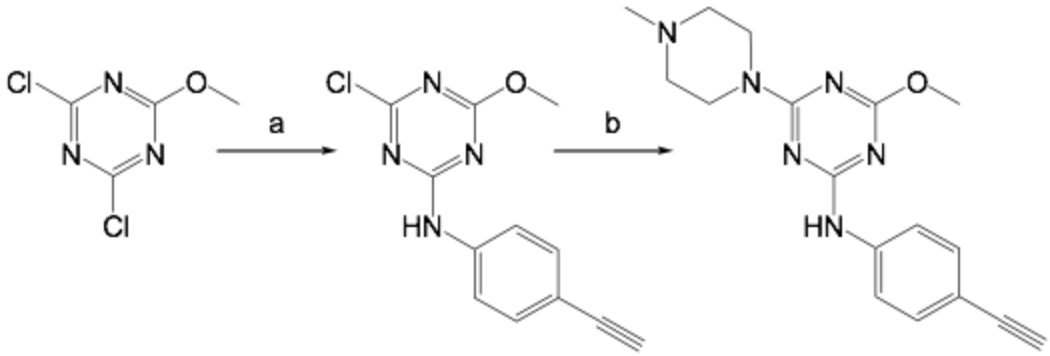
Conclusion

As a conclusion, the first chloride atom on triazines **29** can be replaced smoothly using either Grignard or aryllithium reagent. The yields found were similar for the lithium and Grignard reagents, and there was little to choose between the methods, although the lithium arenes were easier to generate from a practical point of view, and represent the preferred methodology.

2.13 Synthesis of monoamino-substitued *s*-triazines

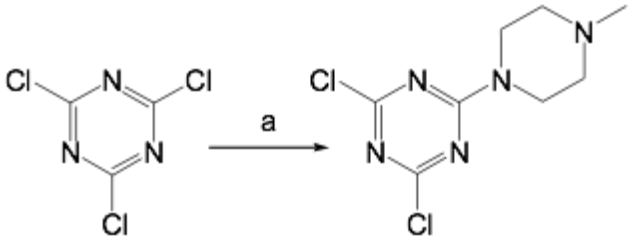
Recent studies have confirmed that several *s*-triazine derivatives bearing morpholine, piperidine and some piperazine moieties are effective against *M. tuberculosis* H37Rv strain.[93]

A series of (piperazinyl/piperidinyl)-*s*-triazine derivatives were prepared by Mewada *et al*., and the general procedure is shown in **Scheme 2-27**.[92]



Scheme 2-27. Mewada procedure. Reagents and conditions: (a) 4-ethynyl-benzenamie, $NaHCO_3$, THF, r.t., 6 h, 85%; (b) 1-methylpiperazine, acetone, 56 ˚C, 4 h, 76%.

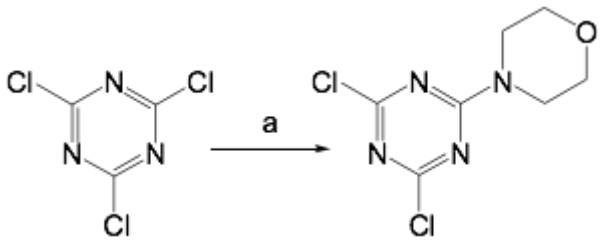
Patel and co-workers had synthesized a series thiazolidin4-one fused *s*-triazines as potential antimicrobial and anticancer agents.[93] 2,4-Dichloro-6-(4-methylpiperzin-1-yl)-*s*-triazine **83** was initially constructed from *N*-methylpiperazine with 2,4,6-trichloro-*s*-triazine as shown in **Scheme 2-28**.



29 **83**

Scheme 2-28. Patel procedure.[93] Reagents and conditions: (a) *N*-methylpiperazine, potassium carbonate, anhydrous acetone, 0 ˚C, 6 h, 89%.

2,4-Dichloro-6-morpholinyl-s-triazine **84** had been had been synthesized by Chuan and co-workers from 2,4,6-trichloro-*s*-triazine and morpholine in the presence of sodium carbonate in water[94].

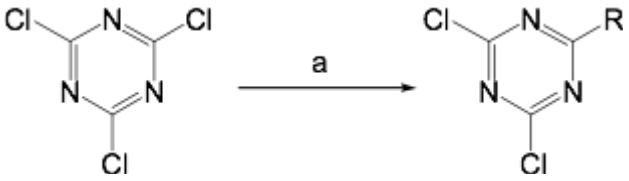


29 **84**

Scheme 2-29. Chuan procedure.[94] Reagents and conditions: (a) Morphline, sodium carbonate, water, 5 ˚C, 3 h, 77%.

The first aim of this part is to syntheses the monoamino-substitued-*s*-triazines using related methods. THF was employed be solvent because acetone can undergo side reactions in the presence of bases.

We obtained a series of compounds of monoamino-substitued-*s*-triazines **83-88** with the general procedure shown in **Scheme 2-30**. The products properties are summarized in **Table 2-5**.



29 **83-87**

Scheme 2-30. Reagents and conditions: (a) amino reagent, THF, potassium carbonate, 0 ˚C, 8 h.

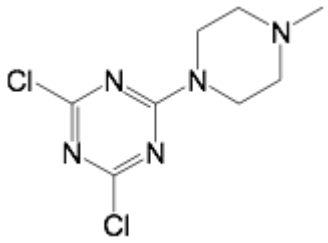
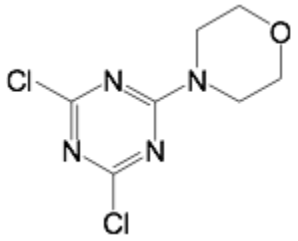
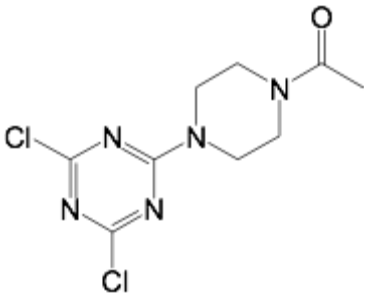
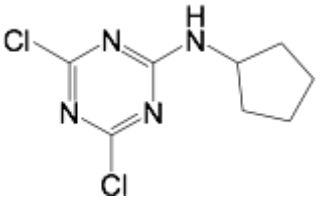
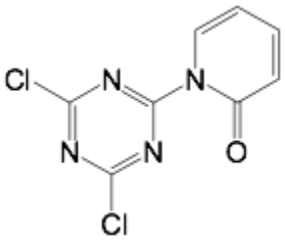
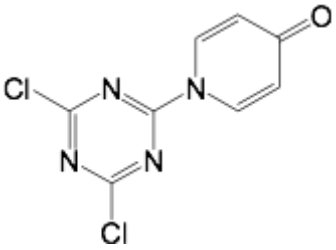
Introduced amine	Product	Product structure	Yield/(%)
1-Methylpiperazine	**83**		62
Morphline	**84**		70
1-Acetyl-piperazine	**85**		65
Cyclopentylamine	**86**		49
2-Pyridone	**87**		54
4-Pyridone	**88**		56

Table 2-7. The monoamino-substitued-*s*-triazines **83-88**.

The reactions of 2,4-dichloro-6-(4-methyl-1-piperazinyl)-*s*-triazine **83** and 2,4-dichloro-6-(4-morpholinyl)-*s*-triazine **84** proceeded smoothly as reported and the products were obtained in good yield of 62% and 70%, respectively. Analytical and spectroscopic data were in

agreement with that reported in literature[93,94].

Another experiment was attempted based on the reaction above. 1-Aceyl-piperazine, was introduced to the cyanuric chloride ring forming 2,4-dichloro-6-(4-acetyl-1-piperazinyl)-*s*-triazine **85** as a colorless oil in good yield. From ^{1}H NMR spectra, peaks due to protons from the piperazine ring were observed in similar region (ca. 2 and 3 ppm) compared to that of compound **83**. A single peak at δ=2.00 p.p.m. corresponding to the methyl group was observed as well.

Two more secondary amino derivatives, 2-pyridone and 4-pyridone, were also employed. As the pyridone anions were highly nucleophilic and could attached easily, TLC analysis showed two spots even when the reactions were run at 0 ˚C. For both reactions, the desired product gave different polarities with the by-product, and they were separated via column chromatography. Through analysis of the mass spectra, the signals of the desired products **87** and **88** were observed, and the signal of the by-product was also detected as the di-substitued compounds were also presented ($n_{mono:di}$=5: 1). Through analysis of the ^{1}H NMR spectra, the products retained the same peak distribution as with the starting material (triplet, doublet, doublet and triplet in sequence from 2-pyridone one, and doublet and doublet in sequence from 4-pyridone one), but shifted to lower field region.

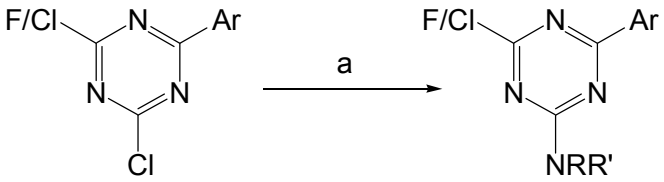
A commercial available primary amine, cyclopentylamine, was introduced to the triazine ring. The desire product was easily obtained as a colorless oil after aqueous work-up. IR spectroscopy was used, and one strong signal in presented NH stretch at ν 3335 cm^{-1} region characterized that the primary amine group of starting material was no longer present. From the ^{1}H NMR spectrum, signals due to protons from the cyclopentyl ring were more deshielded than that of the starting material.

Conclusion

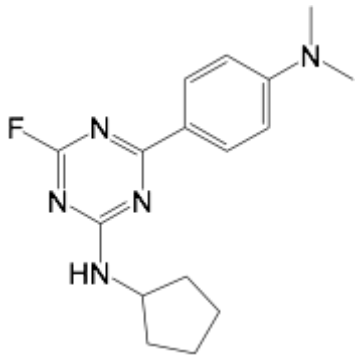
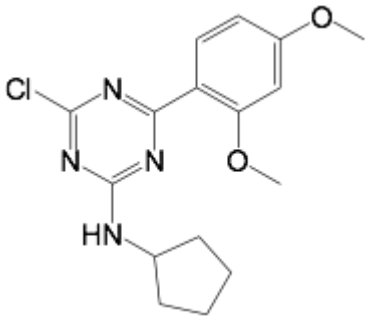
Cyanuric chloride **29** was treated with six commercially available amines. Based on procedures in literature,[93, 94] six monoamino-substituted *s*-triazine intermediates **83**-**88** were successfully prepared. The products were sent to collaborators at De Montfort University for further bioactivity test.

2.14 Synthesis of 2-aryl-4-amino-disubstitued *s*-triazines

We also attempted to synthesis the 2-aryl-4-amino-disubstitued *s*-triazines **89**-**94** starting from the 2,4-dichloro-6-monoaryl-*s*-triazines **74**, **77** and **78** synthesized previously (**Table 2-8**).following the general procedure (**Scheme 2-31**).



Scheme 2-31. Synthesis of the 2,4-dichloro-6-monoaryl-*s*-triazines. Reagent and conditions: (a) amine, THF, potassium carbonate, r.t., 8 h.

Starting material	Introduced amine	Product	Product Structure	Yield/ (%)
74	Cyclopentylamine	**89**		50
79	Cyclopentylamine	**90**		45

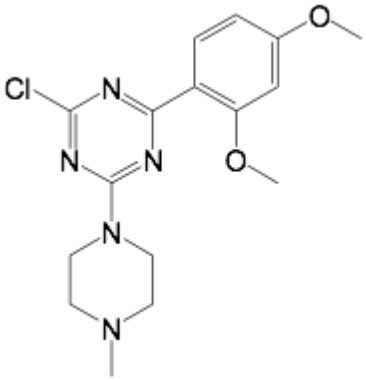
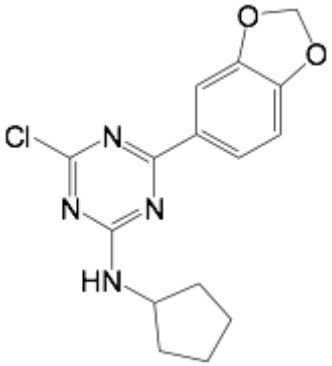
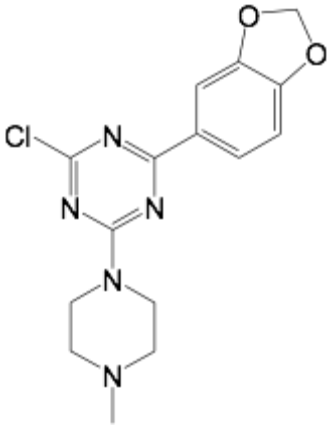
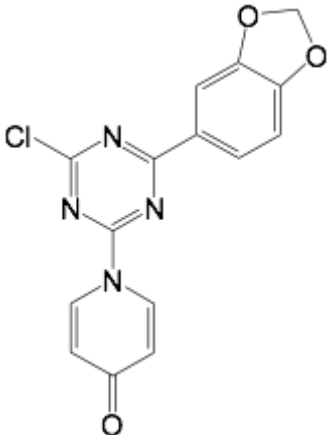
79	1-methylpiperazine	**91**		40
80	Cyclopentylamine	**92**		41
80	1-methylpiperazine	**93**		43
80	2-pyridone	**94**		40

Table 2-8. Synthesis of the 2-aryl-4-amino-disubstitued *s*-triazines **89**-**94**.

2,4-difluoro-6-(*N*,*N*-dimethylaminophenyl)-s-triazine **74** was treated with cyclopentylamine in the presence of triethylamine at room temperature. The reaction was run for 8 hours and the desired product was formed as a pale yellow colored oil. The ^{1}H and ^{13}C NMR proved this product to be pure. In IR spectroscopy, a single peak at n 3330 cm^{-1} representing the NH stretch also proved that the amine attached successfully and the secondary amine was formed.

The predicted of bioactivity score for **89** was analysed via http://www.molinspiration.com/, and showed it has potential uses as a GPCR ligand, kinase inhibitor or enzyme inhibitor.

As the fluoride compound **74** was very reactive, and the temperature or the reaction time may not controlled very well, a tri-substituted by-product was formed, which gave double intensity signals for the amine group in both ^{1}H and ^{13}C NMR spectra than that of **89**.

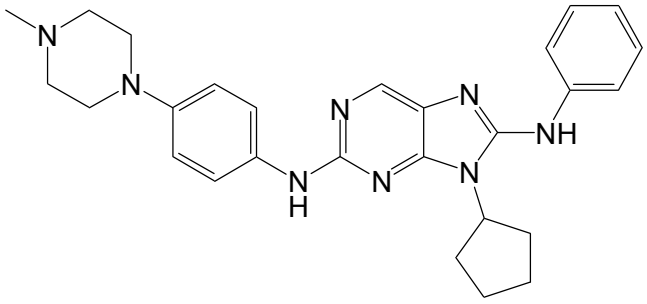
We successfully synthesized and isolated the 2-aryl-4-amino-disubstitued *s*-triazines **90**-**94** in fair yields. The products were confirmed to be pure using ^{1}H NMR spectroscopy and MS spectrometry experiments.

Conclusion

Three mono-aryl substituted *s*-triazines **74**, **79** and **80** were treated with commercially available amines, and a series of 2-aryl-4-amino-disubstitued *s*-triazines **89**-**94** were formed successfully. In future, the reaction temperature and time may need to be controlled more accurately to optimize the yields and reduce by-product formation.

Section 3:

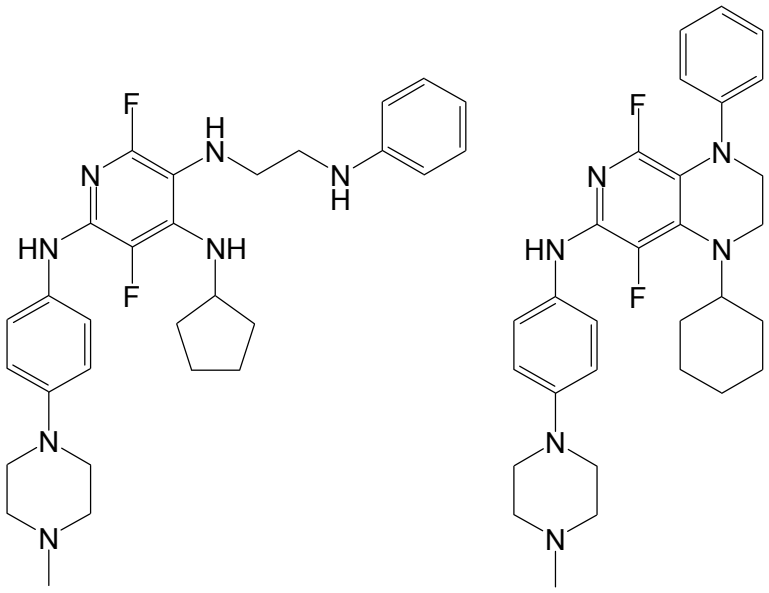
A series of new target scaffolds were designed around the kinase inhibitor **38.** Similar to the structure of ErbB family tyrosine kinase inhibitors in **Figure 1-19**, compound **38** consisted of an aniline and piperazine solubilizing groups, but the quinazoline core is replaced by a purine scaffold. It exhibits antitumor potency against the NSCLC cell line and drug resistance mutations. We wanted to make simpler analogues in fewer steps using our approach with S_NAr reactions.[96]



38

Figure 2-3. Structure of optimized inhibitor **38**.

To simplify the synthesis and improve conformational mobility, the imidazo ring of the purine core has been removed and a series of flexible amine substituents incorporated generating **95** and **100** as targets in this research.



95 **100**

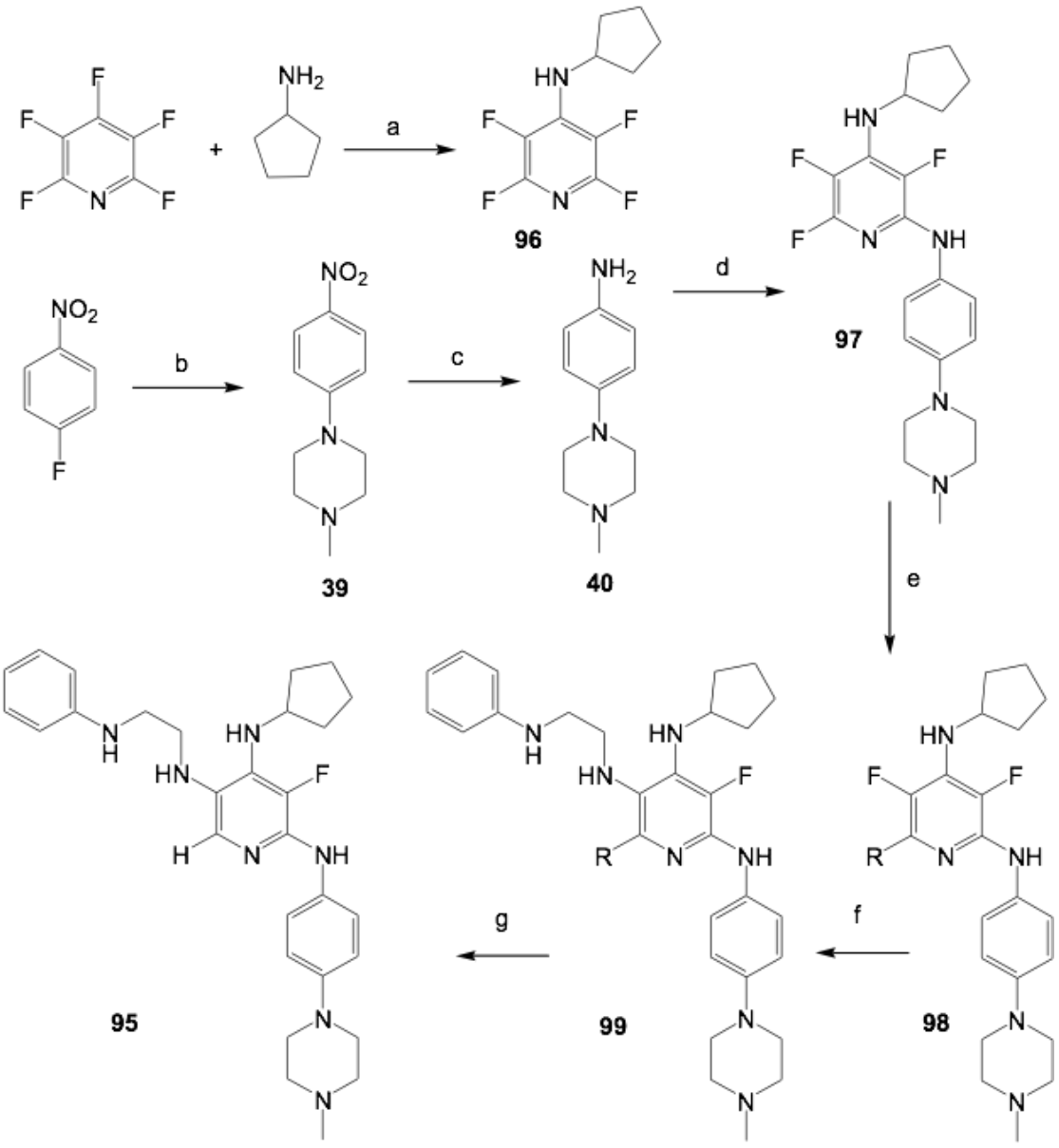
Figure 2-4. Structure of **95** and **100**.

The piperizinylphenylamino group is retained at the position corresponding to C-2 of the original purine ring. The amino substituents should be amenable to insertion by stepwise

S_NAr reaction of an polyfluoropyridine core and will also to increase the number of sp^3 centers and the number of rotatable bonds both desirable in drug candidates.[95]

2.15 Synthesis of 9-Cyclopentyl-*N*^{2}-4(4-methylpiperazin-1-yl)phenyl)-*N*^{8}-phenyl-9*H*-2,8-diamine 95

The first aim of this discussion is concerned with the synthesis of perfluoroarene **38.** Pentafluoropyridine, as the structure core, was proposed to be substituted at the 4-, 2-, 6- and 5-positions sequentially (**Scheme 2-32**).

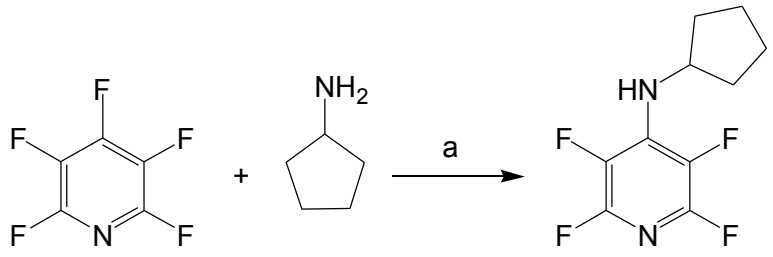


Scheme 2-32. Purposed synthetic route of perfluoroarene **95**. Reagents: (a) Et_3N; (b) 1-methylpiperazine, K_2CO_3; (c) H_2, Pd/C, EtOH (d) base; (e) protecting group; (f) *N*^{1}-cyclopentyl-*N*^{2}-phenyl-1,2-enhaediamine; (g) protecting group removal.

Starting with commercially available pentafluoropyridine, cyclopentylamine would be attached at the *para*-position of the pyridine ring to provide *N*-cyclopentyl-2,3,5,6-tetrafluoro-4-pyridinamine **96**. 4-(4-Methylpiperazin-1-yl)-phenylamine **40** was then be added, with nucleophilc substitution expected to occur at the next most reactive site, the ortho position, forming the intermediate **97**. In order to attach the N^1-phenyl-1,2-ethanediamide group on meta position, a protecting group for the ortho position was considered necessary in procedure. This approach would generate a new set of test compounds which would be screened for anti-cancer activity, and their properties compared to the original inhibitor compound **38**.

2.16 Synthesis of *N*-cyclopentyl-2,3,5,6-tetrafluoro-4-pyridinamine 96

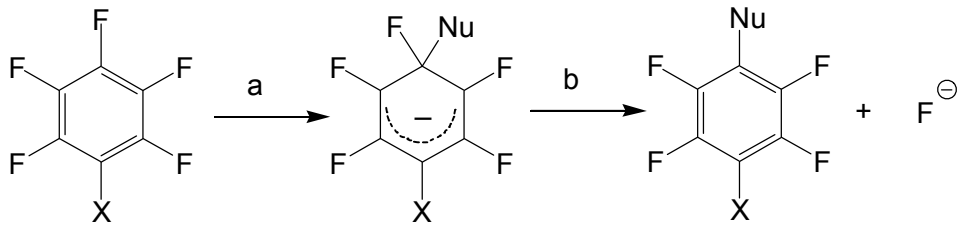
Our first target was the *N*-cyclopentyl-2,3,5,6-tetrafluoro-4-pyridinamine **96** (**Scheme 2-33**).



96

Scheme 2-33. Synthesis of compound **96**. Reagents and conditions: (a) Et_3N, CH_3CN, 0˚C, 8 h, 80%.

Polyfluorinated aromatic systems have an interesting 'mirror-image' relationship with the corresponding hydrocarbon compounds, in that the former generally react with nucleophiles, *via* Meisenheimer complexes[67] that undergo electrophilic substitution, as shown in **Figure 2-5**.



Meisenheimer intermediate

Figure 2-5. 'Mirror-image' chemistry, X=H, CN, OCH_3, etc.

Nucleophilic attack occurs predominantly *para*- to the substituent X in pentafluorobenzene derivatives, regardless of whether the substituent is electron-withdrawing, hydrogen, or electron-donating[76].

We obtained compound **96** following this observation. The reaction proceeded smoothly and pure product was obtained as a pale yellow liquid. On comparing with the ^1H spectrum of cyclopentylamine, the product signals have become deshielded resulting in a downfield shift of 0.8 p.p.m.. In the ^{13}C NMR spectrum, the symmetry of the molecule was apparent. Two very strong signals at δ = 34.7 and 23.7 p.p.m. belong to the two pairs methylene

groups of the cyclopentyl moiety. In the aromatic region, a double triplet peak at 144.3 p.p.m. and a double doublet signal at 130.9 p.p.m. belong to the two pairs of carbons bonded to fluorine, which give $^{1}J_{C\text{-}F}$ coupling constants of 236 and 244 Hz, respectively (**Figure 2–6**).

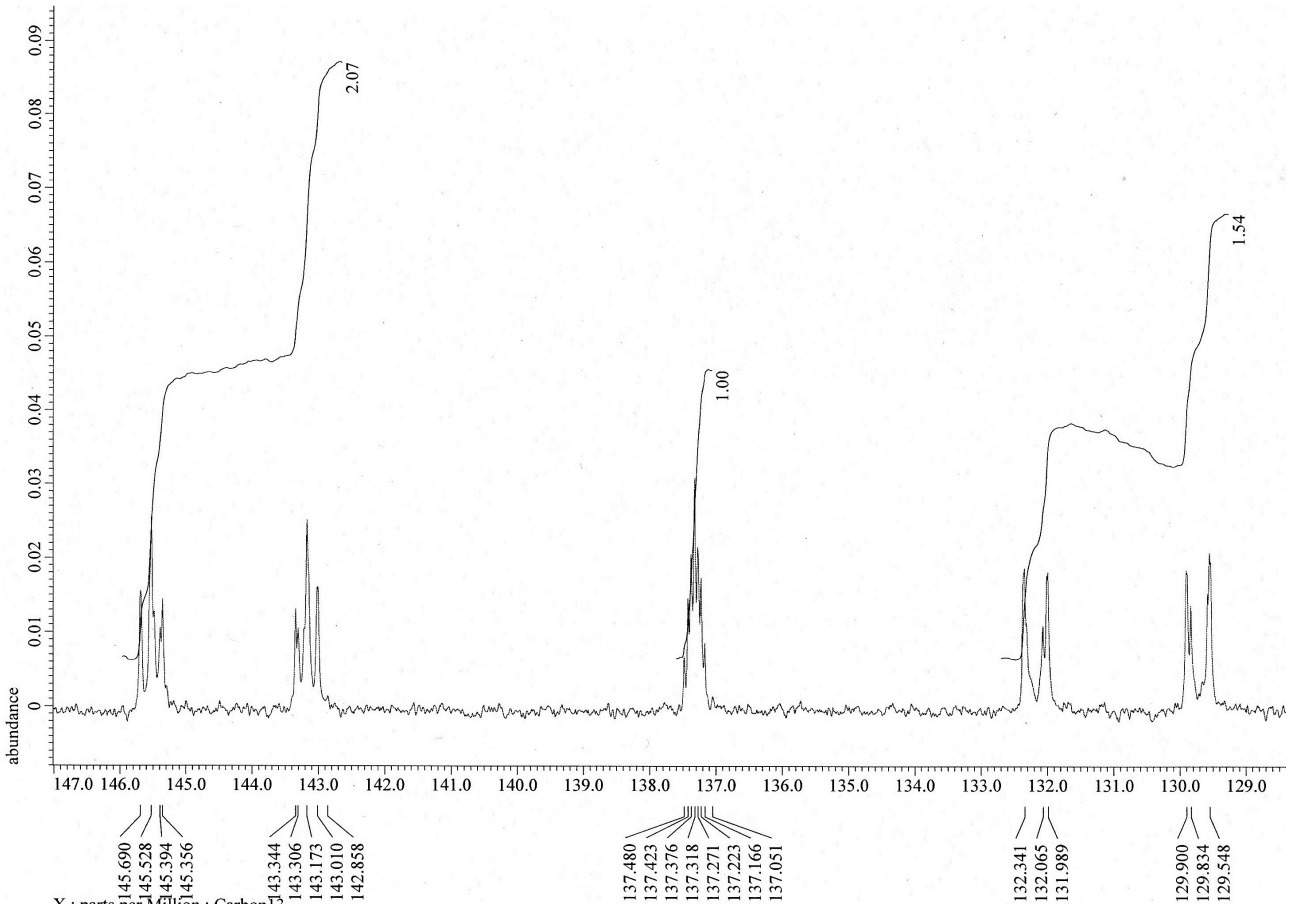


Figure 2-6. C-F coupling in compound **96**: partial ^{13}C NMR spectrum ($CDCl_3$)

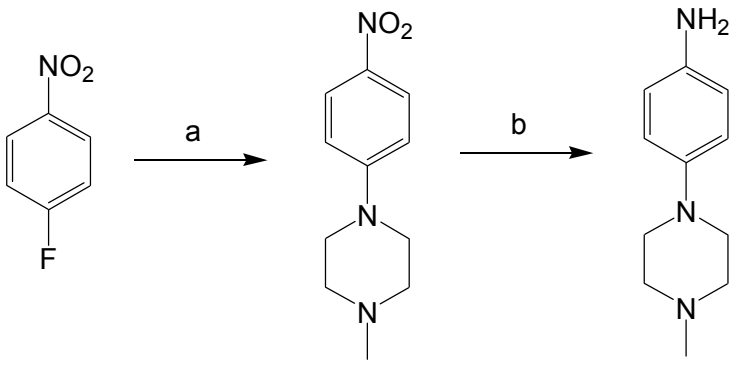
In ^{19}F NMR spectroscopy confirmed the structure by the presence of multiplet peaks at ranges -2.5 and -67.5 ppm representing the two mirror-imaged pairs of fluorine groups from the fluoropyridine ring respectively.

2.17 Synthesis of 1-(4-aminophenyl)-4-methylpiperazine 40

The 1-(4-aminophenyl)-4-methylpiperazine **40** was prepared according to a literature procedures (**Scheme 2-34**)[20].

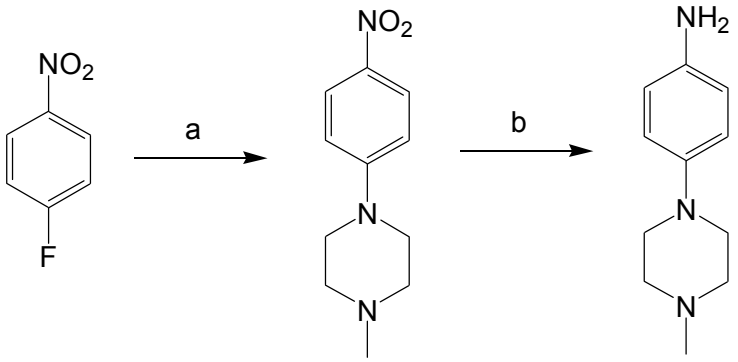
4-Fluoronitrobenzene was treated with methylpiperazine in the presence of potassium carbonate to produce the intermediate 1-methyl-4-(4-nitrophenyl)piperazine in good yield.

The nitro group was catalytically hydrogenated over palladium on carbon (Pd/C) to provided the desired aniline in excellent yield.



Scheme 2-34. Synthesis of 1-methyl-4-(4-nitrophenyl)piperazine **39** and 4-(4-methylpiperazin- 1-yl)phenylamine **40**. Reagents and conditions: (a) 1-methylpiperazine, K_2CO_3, DMSO, r.t., 6 h, 95%; (b) H_2, 10% Pd/C, EtOH, r.t., 9 h, 90%.

We obtained 1-(4-aminophenyl)-4-methylpiperazine **40** successfully by a 2-step reaction (**Scheme 2-35**) with modified conditions to the literature method.



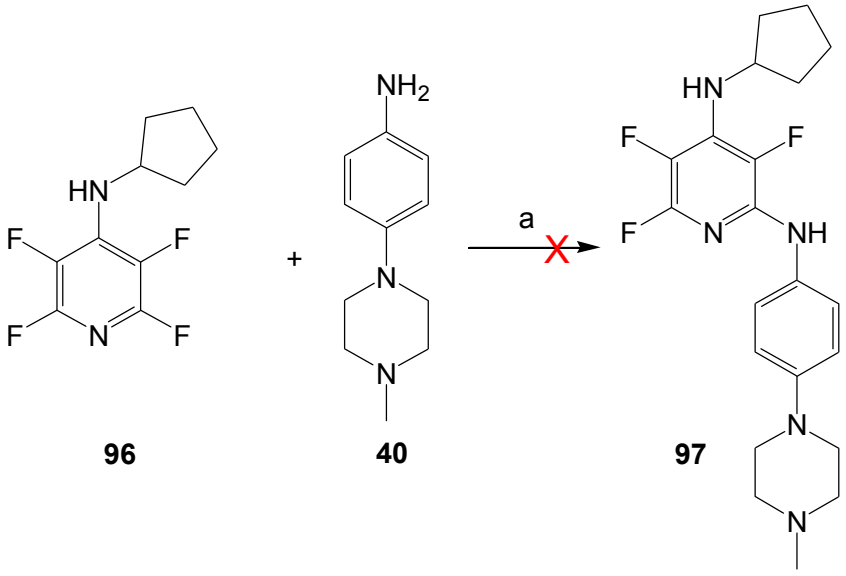
39 **40**

Scheme 2-35. Synthesis of 1-methyl-4-(4-nitrophenyl)-piperazine **39** and 1-(4-amino-phenyl)-4-methylpiperazine **40**. Reagents and conditions: (a) 1-methylpiperazine, Et_3N, CH_3CN, 82 ˚C, 8 h, 90%; (b) H_2, 10% Pd/C, 1 atmosphere, HPLC grade MeOH, 25 ˚C, 10 h, 99%.

The signals in the ^1H and ^{13}C NMR spectra of compound **40** matched the reference data. Compound **39** was yielded as a yellow colored solid, which was soluble in water. Thus the volume of the aqueous phase was minimized in extraction step. Compound **40** was yielded initially as a colorless solid but presented a problem with stability, as the material would

turn black after only 10 minutes in contact with air. It was thought that aerial oxidation of the electron rich arene was occurring leading to decomposition.

Then we aimed to introduce 1-(4-aminophenyl)-4-methylpiperazine **40** at the ortho-position on the pyridine ring in *N*-cyclopentyl-2,3,5,6-tetrafluoro-4-pyridinamine **96**.



Scheme 2-36. Synthesis of scaffold **97**.

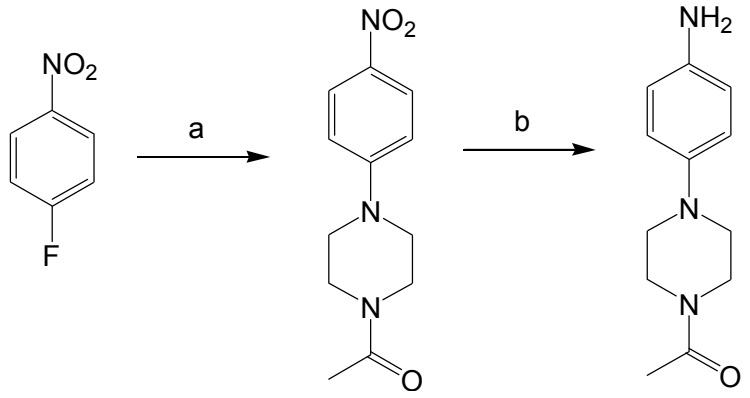
As the substitution reaction at the para-position fluoride had proceeded smoothly at 0 ˚C and gave a good yield, the reaction was firstly run at room temperature for 8 hours. After analysis with TLC plate, however no new spot presented. After aqueous work-up and analysis by ^{1}H NMR spectroscopy, the signals were found to be the same as those of the starting material.

The reaction was then run with a stronger base (sodium hydride) at higher reaction temperature (82 ˚C). Gas evolution occurred at the beginning of the reaction, which indicated that compound **40** was deprotonated with sodium hydride. Unfortunately, the substitution reaction still did not occur.

The crude product presented the same 1H NMR spectrum as the starting material **40**.

2.18 Synthesis of 4-(4-acetylpiperazin-1-yl)-aniline 101[86]

A scaffold of 1-(4-aminophenyl)-4-methylpiperazine **40** was also synthesized by replacing the piperazine methyl group by an acetyl group. The 4-(4-acetylpiperazin-1-yl)-aniline **101** was successfully obtained by a 2-step reaction between fluoronitrobenzene and *N*-acetylpiperazine (**Scheme 2-37**).

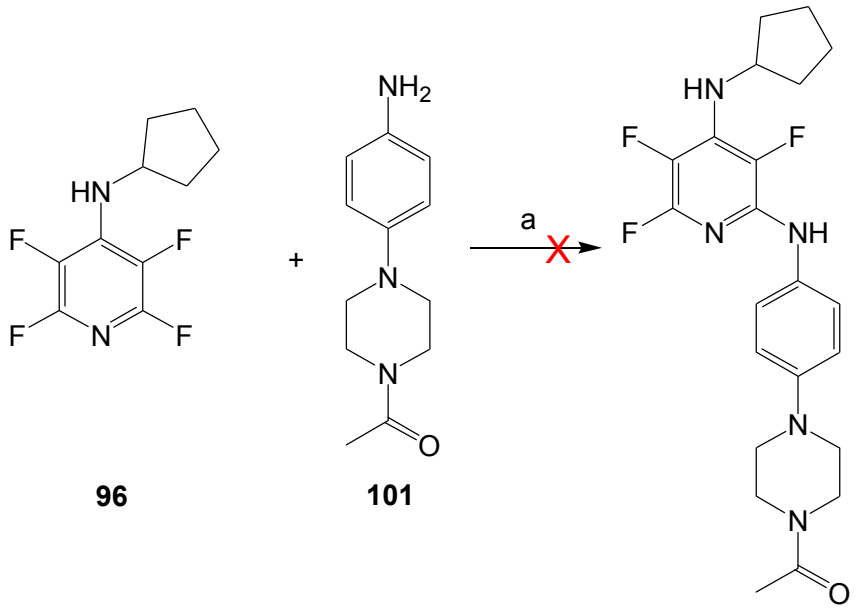


102 **101**

Scheme 2-37. Synthesis of 4-(4-acetylpiperazin-1-yl)-aniline **101**. Reagents and conditions: (a) Et_3N, CH_3CN, 82 ˚C, 8 h, 95%; (b) H_2, 1 atmosphere, HPLC grade methanol, 25 ˚C, 10 h, 99%.

The ^1H and ^{13}C NMR spectra both indicated the product to be pure. The spectra gave similar signals to that of compound **40** and a new signal representing the methyl group.

Following the procedure discussed in section 2.17, the reaction was allowed to run with sodium hydride at a higher reaction temperature (120 ˚C) after the solvent was changed to DMF.



Scheme 2-38. Synthesis of *N*-cyclopentyl-2-[4-(4-acetylpiperazin-1-yl)-anilino]-3,5,6-trifluoro-4-pyridinamine.

Unfortunately again, there was no new product formed, and substitution at the 2-position, normally straightforward, appeared compromised by the electron donating amine substituent at C-4.

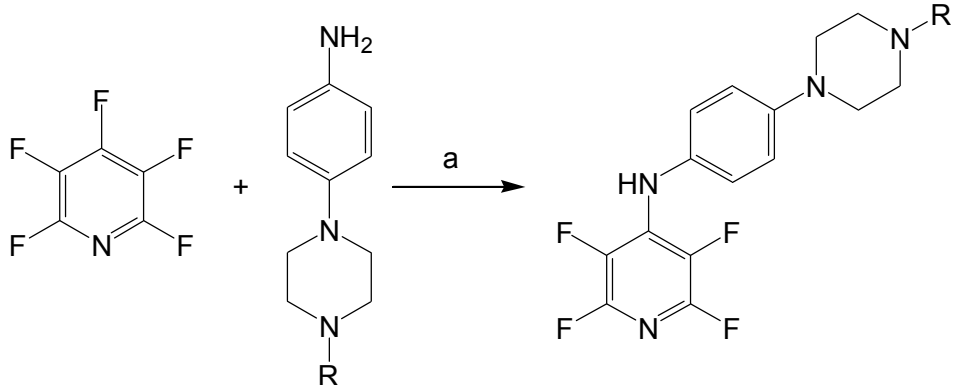
Conclusion

An advanced intermediate, *N*-cyclopentyl-2,3,5,6-tetrafluoro-4-pyridinamine **96**, and two 4-(4-piperazin-1-yl)-anilines **40** and **101** were produced smoothly in good yields. Although the solvent conditions and base were investigated extensively, the desired 2-position substitution reaction proved difficult and the synthesis of the final target compound could not be completed.

2.19 Synthesis of 2,3,5,6-tetrafluoro-N-4-substitued pyridianamines 103 and 104

As mentioned in the section of orientation reactions in polyfluorinated compounds, nucleophilic substitution of fluorine in pentafluoropyridine occurs initially almost exclusively at the para-position. Afterwards at the ortho- and meta-position in turn.

It was expected that cyclepentylamine would attack at the ortho-position more easier than two piperizinylphenylamines **40** and **101** prepared above. We aimed to make analogues of compound **96** , which have the piperzinylphenylamino groups substituted at para-position. We thus obtained 2,3,5,6-tetrafluoro-*N*-4-(4-methyl-1-piperazinyl)phenyl-4-pyridinamine **103** and 2,3,5,6-tetrafluoro-*N*-4-(4-acetyl-1-piperazinyl)phenyl-4-pyridinamine **104** according to the procedure mentioned in section 2.16 (**Scheme 2-39**).



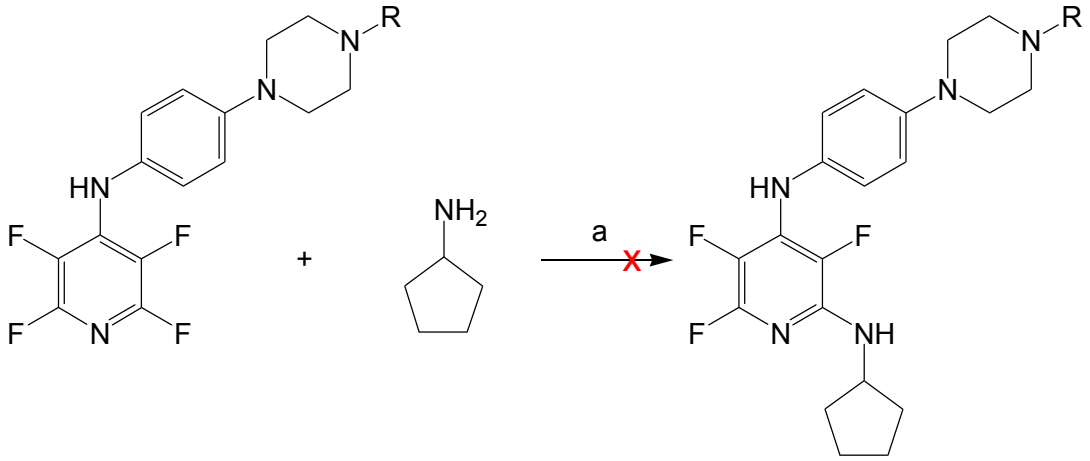
103: R=CH_3
104: R=$COCH_3$

Scheme 2-39. Synthesis of **103** and **104**. Reagents and conditions: (a) Et_3N, CH_3CN, 0 ˚C, 8 h, 70% and 73%, respectively.

The reactions were both proceeded smoothly and pure products were obtained as fine white colored powder. The melting points were both increased by 70 ˚C compared to starting material; **103** (168 ˚C) and **104** (194 ˚C) with **40** (94 ˚C) and **96** (130 ˚C).

The ^{1}H and ^{13}C NMR spectra indicated the products to be pure. ^{19}F NMR spectroscopy confirmed this by the presence of multiplet peaks representing of two mirror-imaged pairs of fluorine groups from the fluoropyridine ring respectively.

We aimed to attach the cyclopentylamine to the ortho-position (C-2) of the pyridine ring in **103** and **104** prepared previously (**Scheme 2-40**). The reactions were run with the following conditions (**Table 2-9**).



103: R=CH_3
104: R=$COCH_3$

Scheme 2-40.

Entry	Solvent	Base	Reaction temperature/(˚C)	Reaction time/(hours)
1	Acetonitrile	Triethylamine	23	8
2	Acetonitrile	Sodium amide	40	72
3	Cyclopentylamine	Sodium hydride	40-100	24
4	TFE/TFA	—	100	48

Table 2-9.

We were unsuccessful in our attempts even though the base was changed in sequence, the reaction temperature was increased while the reaction time was prolonged. After analysis by TLC, no new spot presented. After aqueous work-up and analysis by ^{1}H NMR spectroscopy, the signals were found to be the same as those of the starting material.

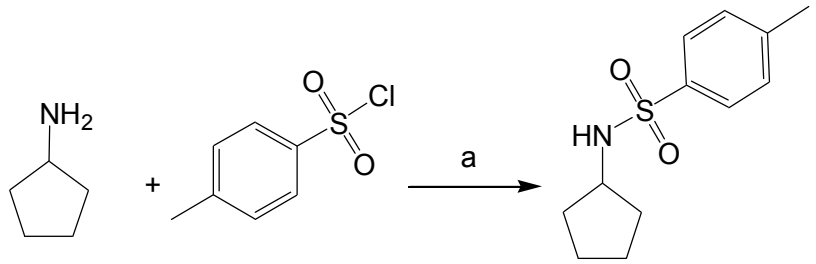
Conclusion

Two 4-(4-piperazin-1-yl)-anilines **40** and **101** were attached to position-4 giving the analogues **103** and **104** both in good yields. Cyclopentylamine with less steric bulk was attempted to be attached at position-2, but the reactions were still unsuccessful.

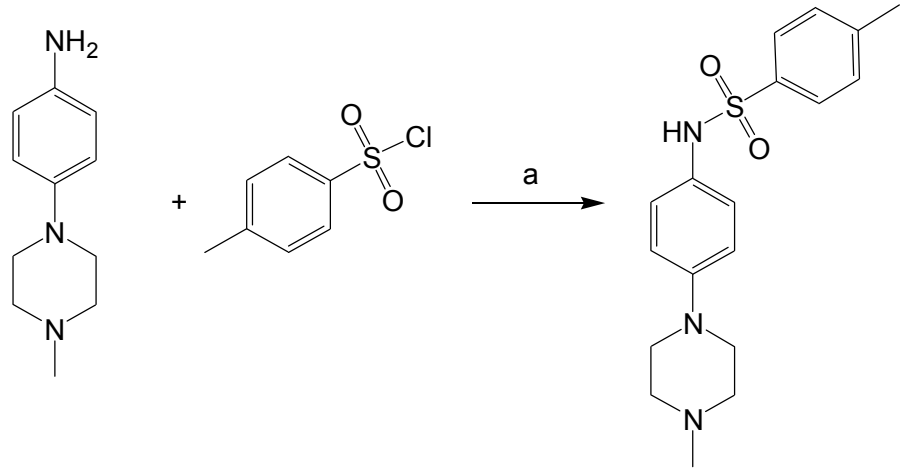
It was supposed that the secondary amine group in compounds **96**, **103** and **104** had blocked the next steps reactions. Thus, we decided to modify the primary amines with the 4-toluenesulfonyl group to improve acidity and reactivity.

2.20 Synthesis of 4-toluenesulfonyl protected secondary amines 105 and 106

The primary amines cyclopentylamine and **40** were treated with 4-toluenesulfonyl chloride, and gave the expected products **105** and **106.** The reactions proceeded successfully and the products could easily isolated after aqueous workup (**Scheme 2-41**).



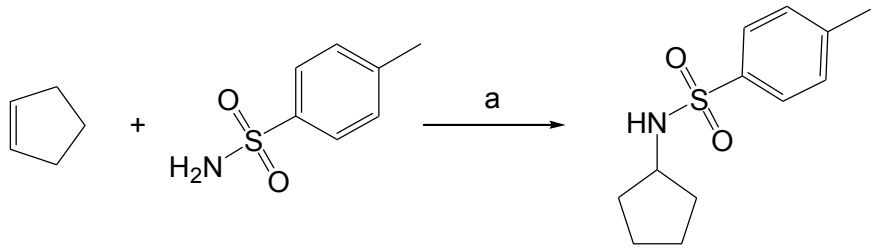
105



106

Scheme 2-41. Synthesis of **105** (above) and **106** (bottom). Reagents and conditions: (a) pyridine, DCM, 0 ˚C, 8 h, 72% and 68, respectively.

N-cyclopentyl-4-methyl-benzenesulfonamide **105** was prepared by Pei and co-workers by another route (**Scheme 2-42**).[97]



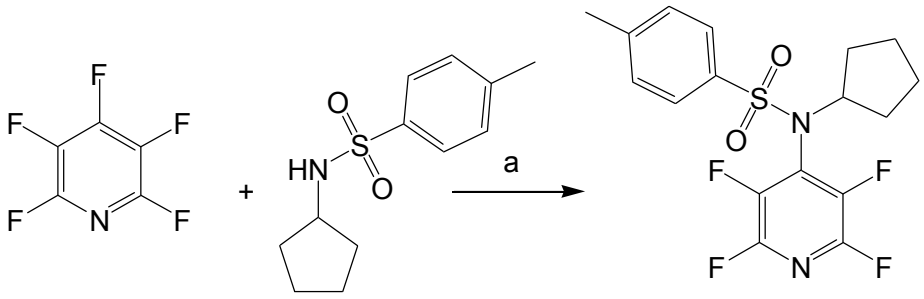
Scheme 2-42. Pei procedure. Reagents and conditions: (a) HOTf-SiO2, H2O, toluene, 20 h, 85 ˚C, 84%.

The melting point and ^{1}H NMR spectroscopy of our product **105** were in agreement with that reported in the literature.

For compound **106**, ^{1}H NMR spectroscopy, ~~the~~ doublet peaks at 7.57, 7.18, 6.94 and 6.74 ppm corresponding to the four pair aromatic protons were observed. Two singlet peaks at 2.43 and 2.35 ppm corresponding to the protons of the methyl groups at both ends of the structure respectively.

2.21 Synthesis of *N*-cyclopentyl-4-methyl-*N*-(2,3,5,6-tetrafluoropyridin-4-yl) benzene-1-sulfonamide 107

According to the procedure mentioned in scheme 2.16, the secondary amine we made above, *N*-cyclopentyl-4-methyl-benzenesulfonamide **105**, was treated with pentafluoro-pyridine in the presence of sodium hydride, and gave the expected product in **107** good yield (**Scheme 2-43**).



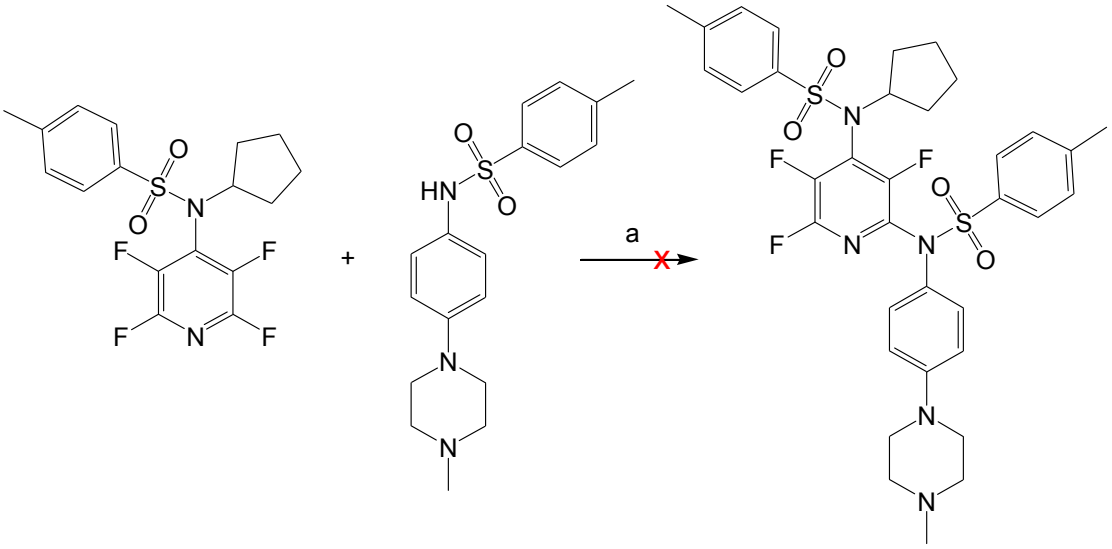
105 **107**

Scheme 2-43. Synthesis of **107**. Reagents and conditions: (a) NaH, CH_3CN, 0 ˚C, 8 h, 80%.

The fluoropyridine ring was attached successfully, which was identified by the ^{19}F NMR spectroscopy by the presence of multiplet peaks at -5.80 and -57.3 ppm representing of two mirror-imaged pairs of fluorine groups.

With the compounds **106** and **107** modified with 4-toluenesulfonyl group, the ortho position substitution reaction was attempted with the reaction conditions below (**Scheme 2-44**). However, the reaction was unsuccessful.

After analysis by TLC, no new spot again was observed. After aqueous work-up and analysis by ^{1}H NMR spectroscopy, the signals were found to be the same as those of the starting material.

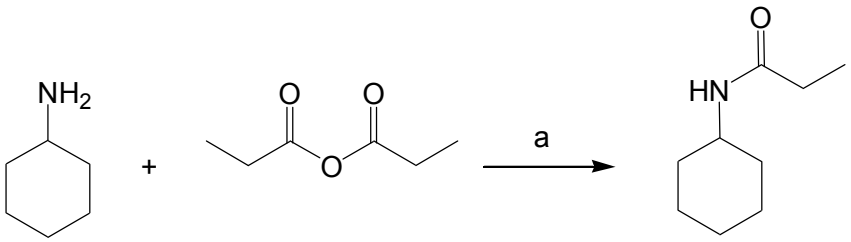


Scheme 2-44. Synthesis of 3,5,6-trifluoro-2,4-disubsttitued-pyridinamine. Reagent and condition: (a) Sodium hydride, DMF, 100 ˚C, 24 h.

Instead of 4-toluenesulfonyl chloride, in next multistep reaction sequence, an acid anhydride, propionic anhydride, was employed as a common acyl source because of its ready availability and stability.

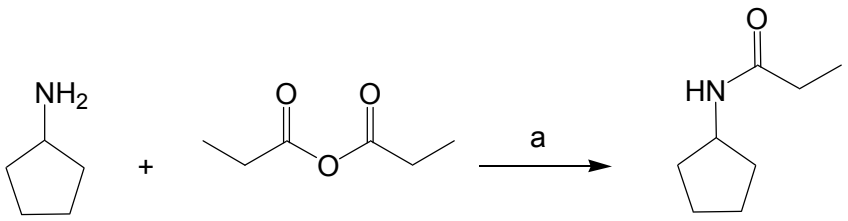
2.22 Synthesis of *N*-cyclopentyl-propanamide 108 and *N*-[4-(4-methyl-1-piperazinyl) phenyl]-propanamide 109

Andrea and co-workers described a mild and convenient procedure for the *N*-acylation of different amines with anhydrides in ethyl acetate at room temperature[98]. The products were formed cleanly and do not require chromatographic purification.

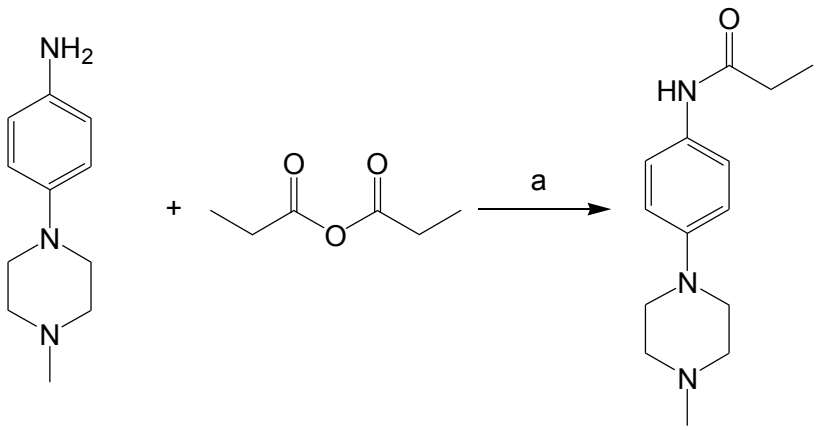


Scheme 2-45. Acylation of amines with anhydrides[98]. Reagents and conditions: (a) N_2 protection, ethyl acetate, potassium carbonate, room temperature, 6 h, 98%.

Following the relative procedure, we modified our two moieties with propionic anhydride and obtained the desired product *N*-cyclopentyl-propanamide **108** and *N*-[4-(4-methyl-1-piperazinyl)phenyl]-propanamide **109** both in excellent yield (**Scheme 2-46**).



108



109

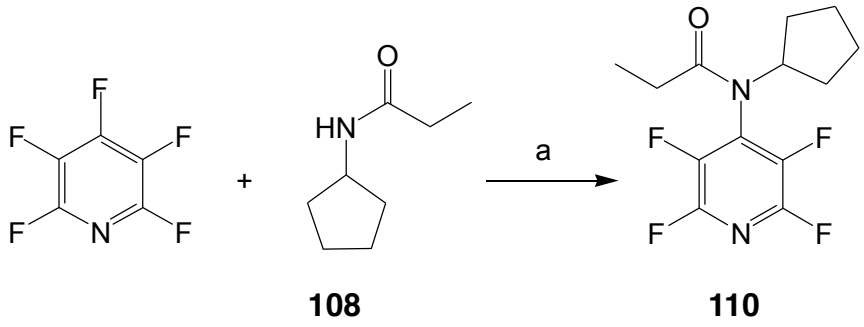
Scheme 2-46. Synthesis of *N*-cyclopentyl-propanamide **108** and *N*-[4-(4-methyl-1-piperazinyl)phenyl]-propanamide **109**. Reagents and conditions: (a) N_2 protection, potassium carbonate, pyridine, dichloromethane, room temperature, 85% and 90%, respectively.

Cyclopentylamine was treated with propionic anhydride in the presence of potassium carbonate under nitrogen protection, and after aqueous workup the expected product **108** was easily isolated in excellent yield. The reaction was exothermic, thus it was started at 0 ˚C and then warmed to room temperature. In IR spectroscopy, a single signal at 3325 was observed.

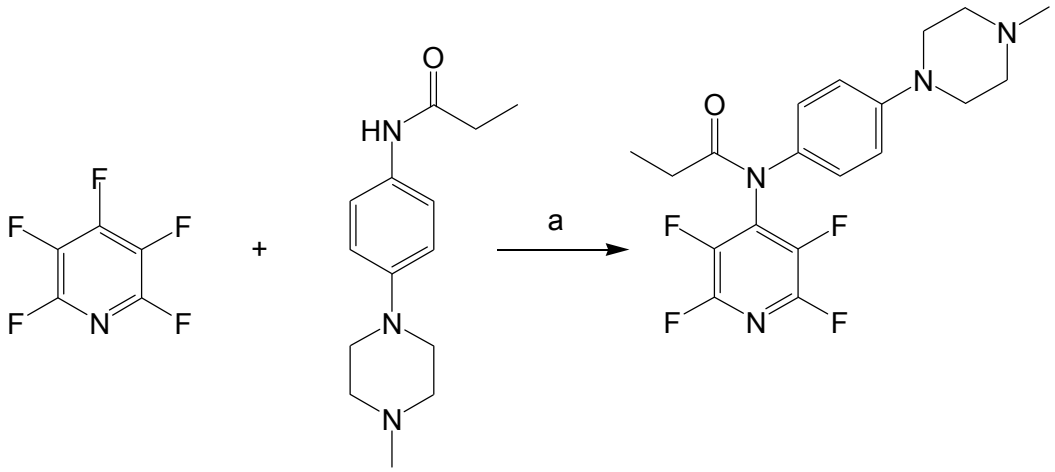
1-(4-Aminophenyl)-4-methylpiperazine **40** was treated with propionic anhydride in the presence of potassium carbonate under nitrogen protection, and after aqueous workup the expected product **109** was easily isolated in excellent yield. In ^{1}H NMR spectroscopy, this was confirmed by the presence of new quartet peaks at 2.35 ppm and triplet peaks at 1.23 ppm corresponding to the protons of the ethyl groups of the propionic group attached. The product was far more stable than the starting material, and did not turning into black color anymore.

2.23 Synthesis of analogues 110 and 111

The acylated products **110** and **111** were then attached to pentafluoropyridine ring following the procedure discussed previously (**Scheme 2-47**).



108 **110**

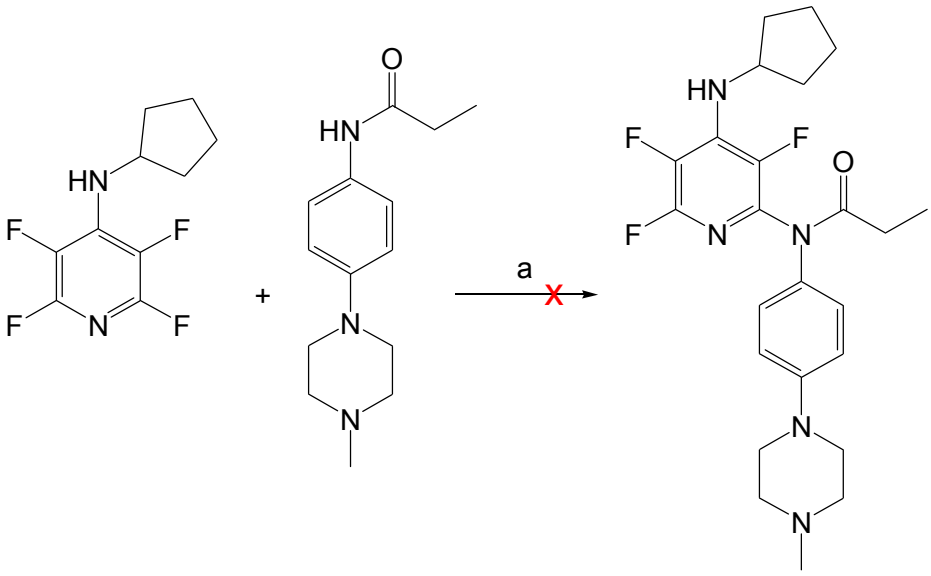


109 **111**

Scheme 2-47. Synthesis of **110** and **111**. Reagents and conditions: (a) NaH, CH_3CN, 0˚C, 8 h, 75% and 70%, respectively.

Compounds **110** and **111** were both produced smoothly without any problems like the para-substituted pentafluoropyridine **96** synthesized previously. The melting point of both products were increased as **40** and **103**. In ^{19}F spectroscopy, two multiplet peaks observed, (-63.86/-7.85 p.p.m. and -80.36/-43.26 for **110** and **111**, respectively)

The ortho-substitution reaction was then tried with the modified products synthesized above (**Table 2-10**) following the conditions tried previously (**Scheme 2-48**).



Scheme 2-48. Synthesis of 2,4-disubstituted-pentafluoropyridine (entry 1).

Entry	Substrate	Reactant	Product yield
1	**96**	**109**	Only starting material recovered
2	**111**	Cyclopentylamine	Only starting material recovered
3	**110**	**109**	Only starting material recovered
4	**111**	**108**	Only starting material recovered

Table 2-10.

~~The reactions were not started~~ Unfortunately, no expected product isolated in any reactions, neither the substrates and the reactants were modified with the propionic anhydride.

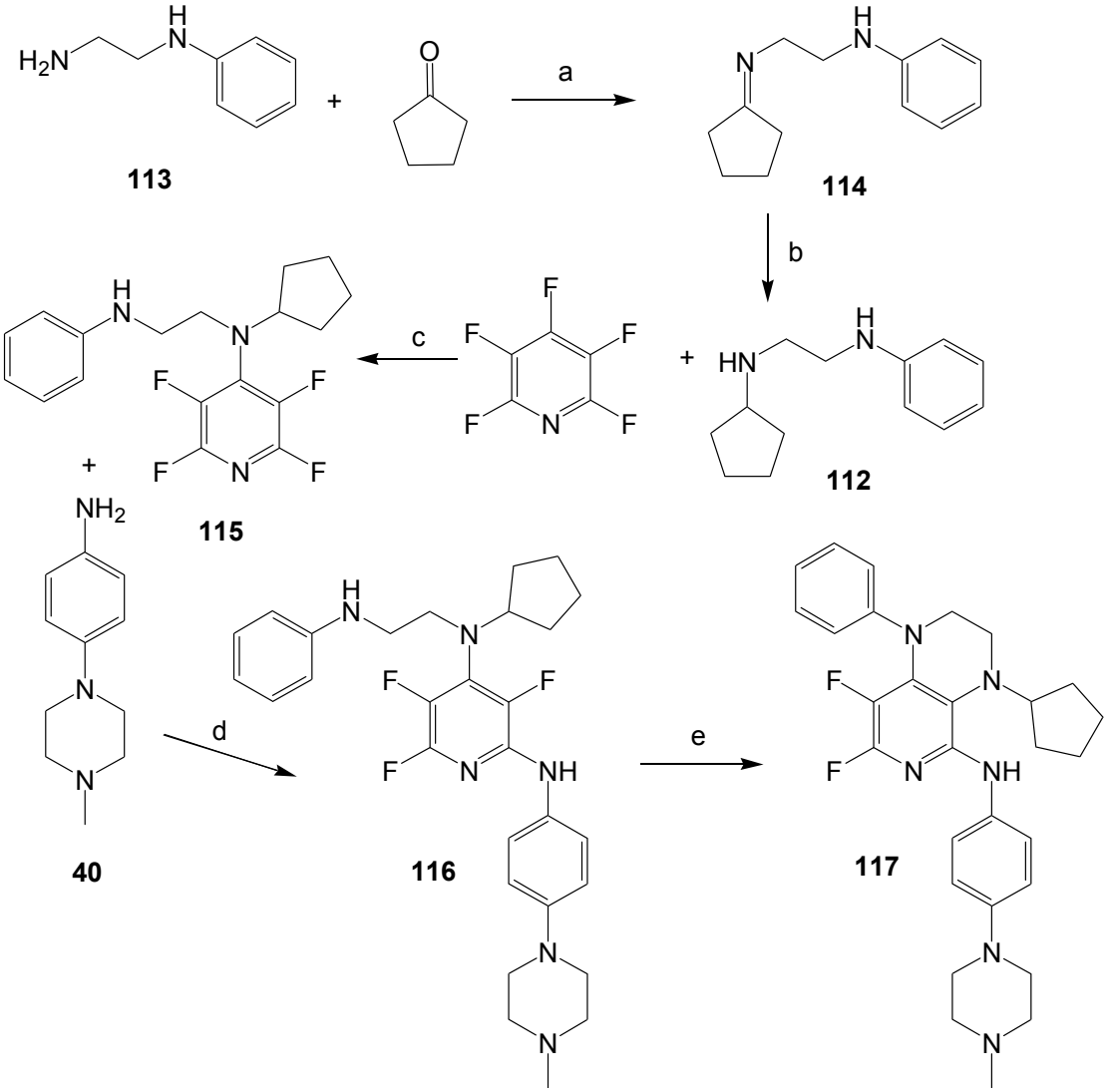
Conclusion

The amine and aniline groups were protected with 4-toluenesulfonyl or propionic anhydride, giving more acidic compounds **105**, **106**, **108** and **109**, and these were attached at position-4 forming **107**, **110** and **111** without any problem. However, these

compounds were still not able to substitute the fluorine at position-2 in attempted base catalysed reactions.

2.24 Synthesis of perfluoroarene 100

The second target of this discussion was aimed to synthesis of perfluoroarene **100** (**Scheme 2-49**).



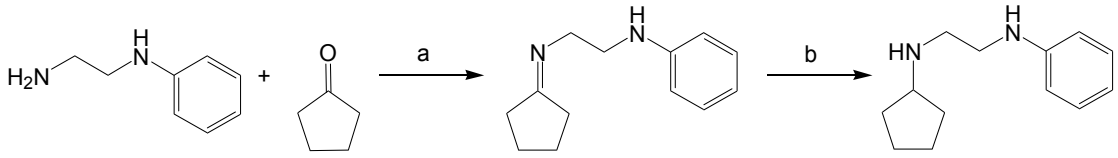
Scheme 2-49. Proposed synthesis route to perfluoroarene **100.** Reagents ~~and conditions~~: (a) ~~dichloromethane,~~ $MgSO_4$; (b) ~~dichloromethane,~~ sodium borohydride; (c) ~~acetonitrile,~~ triethylamine.

The synthesis of perfluoroarene **100** would require making the diamine, *N*[1]-cyclopentyl-*N*[2]-

phenyl-1,2-ehanediamine **112** by reductive amination of cyclopentanone with the phenylethylene diamine (**Scheme 2-48**) and reacting that with pentafluoropyridine first (to add at the para-position), and then adding the piperazinyl aniline **40** in sequence (so it adds at the ortho-position of the pyridine ring.

2.25 Synthesis of *N*¹-cyclopentyl-*N*²-phenyl-1,2-ehanediamine 112

*N*¹-cyclopentyl-*N*²-phenyl-1,2-ehanediamine **26** was successfully synthesized by a 2-step reaction (**Scheme 2-50**).



113 **114** **112**

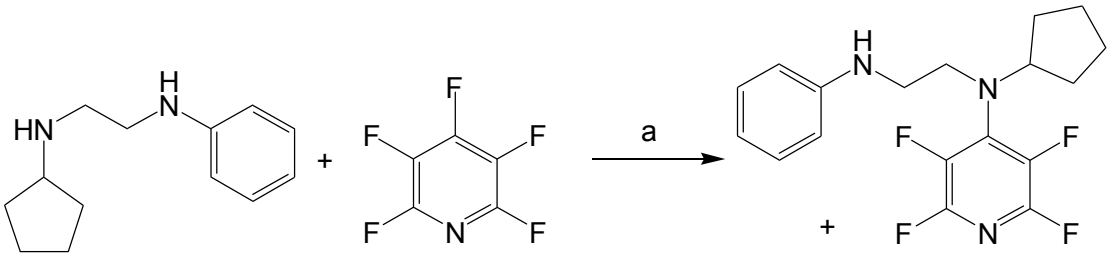
Scheme 2-50. Synthesis of *N*¹-cyclopentyl-*N*²-phenyl-1,2-ehanediamine **112**. Reagents and conditions: (a) cyclopentanone, $MgSO_4$, DCM, r.t., 8 h, 70%; (b) $NaBH_4$, EtOH, room temperature, 8 h, 76%.

N-Pheylethylenediamine **113** was treated with cyclopentanone in the presence of magnesium sulphate, and gave the expected imine **114** in good yield. The magnesium sulphate was added to avoid the reversion, and the filtration was conducted under gravity in order that the water formed could be kept in the dessicant residue. On comparing the IR spectrum with that of the starting material, two NH absorptions at 3291 cm^{-1} were seen to have disappeared. In the ^1H NMR spectrum, signals representing protons on the benzyl ring and cyclopentyl rings were observed in the correct ratio.

As the intermediate **114** was unstable and recent to the amine by hydrolysis, it was reduced with sodium borohydride, and gave the expected product **112** in good yield. In the ^1H spectrum, a new peak at δ 3.08 was observed, which represents the -CH in cyclopentyl ring indicating that the double bond was reduced successfully.

2.26 Synthesis of N^1-cyclopentyl-N^2-phenyl-N^1-(2,3,5,6-tetrafluoropyridin-4-yl)ethane -1,2-diamine 115

The aniline nitrogen of the phenylethylene diamine should be much less nucleophilic than the alkyl amine group so it was hoped that alternatively substituted by-products would not be formed.

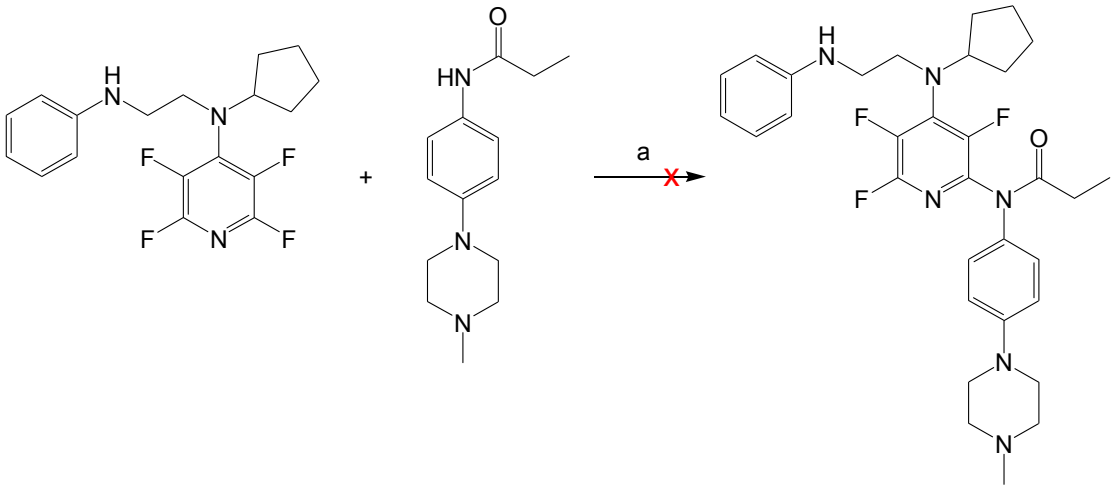


112 **115**

Scheme 2-51. Synthesis of **115**. Reagents and conditions: (a) NaH, CH_3CN, 0 ˚C, 8 h, 70%.

N^1-cyclopentyl-N^2-phenyl-1,2-ehanediamine **112** was treated with pentafluoropyridine in the presence of sodium hydride, and gave the expected product **115** in good yield. In the ^1H spectrum, signals for the protons at two CH_2 group and the cyclopentyl ring moved the chemical shift downfield by 0.4 p.p.m. as the secondary amine was transformed to the tertiary amine and they were more deshielded than the starting material.

Compound **115** was then used as the substrate in next step to form the 2,4-disubsttitued-3,5,6-trifluoropyridinamine (**Scheme 2-52**).



Scheme 2-52. Synthesis of 2,4-disubsttitued-3,5,6-trifluoropyridinamine

Unfortunately, the reactions were not started neither. After tracking by TLC, the product gave similar polarity with the starting material. In the ^{1}H spectrum, signals representing the piperazine were not observed.

Conclusion

In this section, we aimed to prepare the perfluoroarene **95** as an alternate scaffold of the kinase inhibitor **38**. The amine moiety **112** was successfully isolated. Then it replaced the position-4 fluorine atom on pentafluoropyridine smoothly to give compound **115** in good yield. Unfortunately, the desired substitution reactions at position-2 proved difficult and were still unsuccessful.

Chapter 3: Conclusion

In section 1, we hoped to initially prepare the revised structure of the natural product acremolin, compound **52**. During this time, the first total synthesis of the desired structure was reported, so then we continued our synthesis with a alternative synthetic route. The imidazopurine core was disconnected into two separate imidazole precursors **61** and **62**. These two compounds were both successfully synthesized and isolated, but the yields were very poor. The coupling reaction between these two precursors could not be achieved, but may run in future research. We also prepared two analogues of the revised structure of acremolin, **68** and **69** as potential biologically active compounds. Three analogues of the imidazole precursors **70**-**72** were also prepared in order to synthesis further analogues in the future.

In section 2, we developed three mono-aryl, two di-aryl and one tri-aryl substituted *s*-triazines, compounds of interest as potential anticancer agents. In addition, a number of commercially available primary (e.g. cyclopentylamine) and secondary (e.g. morpholine) amines were also attached to the *s*-triazines, giving six mono-amino, six mono-aryl-mono-amino, two di-amino and two mono-aryl-di-amino *s*-triazines. A number of these compounds are currently undergoing screening for biological activity.

In section 3, we prepared three *N*-2,3,5,6-tetra-4-pyridinamine analogues **96**, **103** and **104** as potential kinase inhibitors. However, the desired substitution reactions at position-2 proved difficult and were still unsuccessful. The amine moieties were then modified with either 4-toluenesulfonyl or propionic anhydride, giving the more acidic secondary amides **105**, **106**, **108** and **109**. Unfortunately, these modified compounds were only able to replace the fluoride at position-4 of the fluoropyridine ring in base catalysed S_NAr reactions.

Chapter 4: Experimental

Section 1: General experimental

Anhydrous conditions were obtained using oven/flame dried glassware purged with nitrogen prior to the addition of chemical reagents. A nitrogen atmosphere was maintained throughout reactions where necessary through the use of a nitrogen balloon.

Commercially available solvents were used and not subjected to further purification, except THF which was distilled as needed from sodium and benzophenone. DMSO-d_6 was purchased dry from commercial suppliers. Petroleum ether refers to the fractions with a boiling point between 40-60°C. Sodium hydride refers to a 60% dispersion of NaH in mineral oil.

NMR spectra were recorded (^{1}H, ^{13}C and ^{19}F) using a Bruker or Jeol 400MHz NMR machines. Chemical shifts are reported in ppm ~~for both forms of spectra~~. ^{1}H spectra were recorded at 400MHz, ^{13}C spectra were recorded at 100MHz and ^{19}F spectra were recorded at 376MHz. DEPT analysis was used to assign environment (CH, CH_2, CH_3) to each carbon atom in the ^{13}C spectra. The solvent system used for NMR measurements is specified in brackets.

A Thermofisher executive (orbi) resolution mass spectrometer was used to obtain high resolution mass spectra, with ESI as the ionization source, or these were recored at the UK National Mass Spectometry Facility in Swansea. The solvent used for all samples was methanol.

Analysis by GCMS utilized a Fisons GC 8000 series (AS 800), using a 15 m x 0.25 mm DB-5 column and an electron-impact low resolution mass spectrometer.

IR spectra were obtained on FTIR-8400s.

TLC analysis was carried out on Merck TLC silica gel 60 F_{254} aluminum backed plates and were visualized by vanillin stain or under UV light at 254nm using a UVP chromato-vue cabinet model CC-60. Column chromatography used Apollo Scientific ZEOprep 60 silica with a particle size of 40-63 microns.

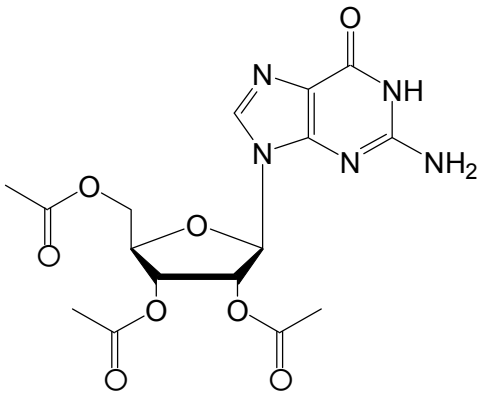
The eluent and concentration used is specified in brackets.

Melting points were recorded using an Electrothermal-IA 9100 melting point instrument and are uncorrected.

Concentration of the Grignard reagents and n-BuLi were measured by diphenylacetic acid titration.

Section 2: experimental for purine analogues

Synthesis of 2',3',5'-acetyl-guanosine[73]



48

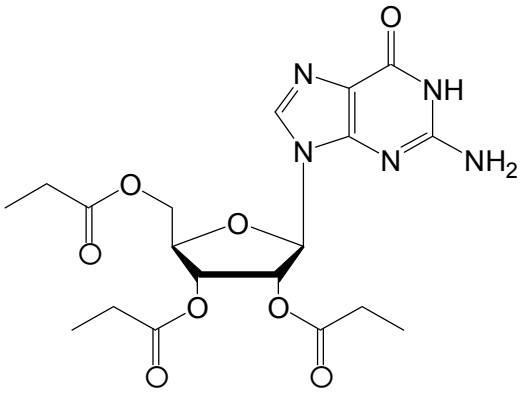
A mixture of guanosine **8** (2.83 g, 0.01 mol), pyridine (0.25 mL, 0.03 mol) and DMF (0.25 mL) was stirred in acetic anhydride (10 mL, 0.10 mol) at 100 ˚C for 2 hours. The reaction mixture was quenched with deionized water (70 mL). Then it was cooled to room temperature and extracted with dichloromethane (3 x 30 mL). The combined organic phases were washed with saturated sodium hydrogen carbonate solvent (3 x 20 mL) and brine, dried over magnesium sulfate. After filtration and purification via column chromatography (elution with methanol: ethyl acetate=1: 10), the title compound was yielded as a white fine powder (3.07 g, 0.0075 mmol, 75%, m.p. 223 ˚C, literature[73] 225 ˚C).

δ_H (400 MHz, DMSO-d_6) 10.72 (s, br, 1H, NH), 7.93 (s, 1H, NCHN), 6.53 (s, br, 2H, NH_2), 5.98 (d, J = 6 Hz, 1H, CH-N in ribose), 5.80 (t, J = 6 Hz, 1H, CH-O in ribose), 5.49 (t, J = 6 Hz, 1H, CH-O in ribose), 4.41-4.37 (m, 1H, CH-C in ribose), 4.34-4.29 (m, 2H, OCH_2), 2.04-2.02 (s, 9H, CH_3)

δ_C (100 MHz, DMSO-d_6) 170.41, 172.63, 172.49, 156.82, 154.25, 151.46, 135.97, 117.12, 84.68, 79.79, 72.41, 70.74, 63.02 (CH_2), 20.91, 20.68, 20.55.

HRMS *m/z* 410.1315. ($C_{16}H_{20}N_5O_8^+$ requires 410.1312).

Synthesis of 2',3',5'-tripropanoate-guanosine



49

A mixture of guanosine **8** (2.83 g, 0.01 mol), pyridine (0.25 mL, 0.03 mol) and DMF (0.25 mL) was stirred in propionic anhydride (12.5 mL, 0.10 mol) at 100 ˚C for 2 hours. The reaction mixture was quenched with deionized water (70 mL). Then it was cooled to room temperature and extracted with dichloromethane (3 x 30 mL). The combined organic phases were washed with saturated sodium hydrogen carbonate solvent (3 x 20 mL) and brine, dried over magnesium sulfate. After filtration and purification via column chromatography (elution with methanol: ethyl acetate=1: 10), the title compound was yielded as a white fine powder (3.16 g, 0.007 mmol, 70%, m.p. 218 ˚C).

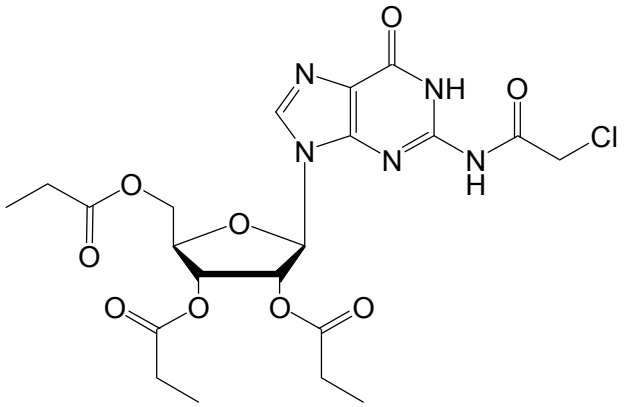
ν_{max} (KBr,cm^{-1}) 3433, 3310, 3187, 2739, 2292, 1751, 1697, 1607, 1535, 1481, 1381.

δ_H (400 MHz, DMSO-d_6) 10.78 (s, br, 1H, NH), 7.93 (s, 1H, NCHN), 6.58 (s, br, 2H, NH_2), 5.99 (d, J = 6 Hz, 1H, CH-N in ribose), 5.82 (t, J = 6 Hz, 1H, CH-O in ribose), 5.54 (t, J = 6 Hz, 1H, CH-O in ribose), 4.41-4.38 (m, 1H, CH-C in ribose), 4.38-4.34 (m, 2H, OCH_2), 2.43-2.32 (m, 6H, CH_2), 1.08-0.97 (m, 9H, CH_3).

δ_C (100 MHz, DMSO-d_6) 173.34, 172.63, 172.49, 156.62, 153.85, 151.06, 135.57, 116.79, 84.52, 79.59, 72.06, 70.24, 63.02 (CH_2), 26.60 (CH_2), 26.54 (CH_2), 26.36 (CH_2), 8.84, 8.82, 8.71.

HRMS *m/z* 452.1787. ($C_{19}H_{26}N_5O_8^+$ requires 452.1798).

Synthesis of 2',3',5'-tripropanoate-*N*-(2-chloroacetyl)-guanosine



51

A mixture of 2',3',5'-tripropanoate-guanosine **49** (0.45 g, 1 mmol) and *N*-ethyldiisopropylamine (0.34 mL, 2 mmol) was stirred in anhydrous THF (10 mL) for 0.5 hours. Chloroacetyl chloride (0.16 mL, 2 mmol) was then added dropwise and the reaction mixture was stirred at 65 ˚C for a further 2 hours. The reaction mixture was cool to room temperature and treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with methanol: ethyl acetate=1: 10), the title compound was yielded as yellow oil (0.38 g, 0.68 mmol, 68%).

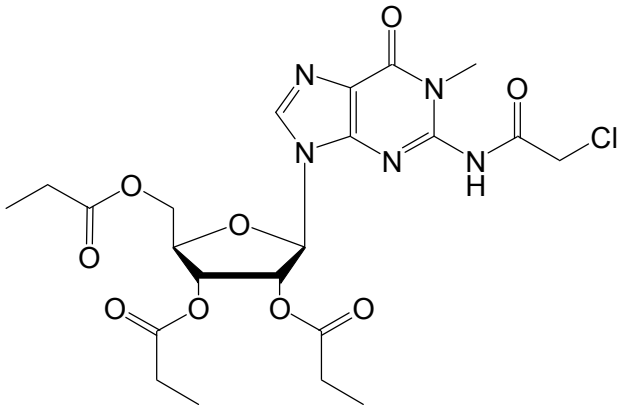
ν_{max} (KBr,cm^{-1}) 3394, 3202, 2947, 1743, 1589, 1373, 1180, 1087, 902, 786, 640;

δ_H (400 MHz, DMSO-d_6) 11.92 (s, br, 1H, NH), 10.38 (s, br, 1H, NH), 7.83 (s, 1H, NCHN), 6.09 (d, J = 6 Hz, 1H, CH-N in ribose), 5.84 (t, J = 6 Hz, 1H, CH-O in ribose), 5.51 (t, J = 6 Hz, 1H, CH-O in ribose), 4.42-4.29 (m, 3H, OCH_2 and -CH next to it), 4.36 (s, 2H, CH_2-Cl), 2.44-2.32 (m, 6H, CH_2), 1.09-1.00 (m, 9H, CH_3).

δ_C (100 MHz, DMSO-d_6) 173.34, 172.63, 172.46, 168.94, 154.71, 148.34, 147.44, 138.06, 120.71, 84.70, 79.88, 72.19, 70.17, 62.98 (CH_2), 43.04 (CH_2), 26.60 (CH_2), 26.54 (CH_2), 26.33 (CH_2), 8.84, 8.82, 8.69.

HRMS *m/z* 528.1491. ($C_{21}H_{27}{}^{35}ClN_5O_9{}^+$ requires 528.1495).

Synthesis of 2'3'5'-tripropanoate-*N*-(2-chloroacetyl)-1-methyl-guanosine



52

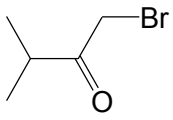
A mixture of 2',3',5'-tripropanoate-*N*-(2-chloroacetyl)-guanosine **51** (put compound numbers into experimental) (1.06 g, 2 mmol) and iodomethane (0.24 mL, 4 mmol) was stirred in anhydrous THF (10 mL) at 40 ˚C for 4 hours. The reaction mixture was cool to room temperature and treated with deionized water (20 mL) and extracted with dichloromethane (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with methanol: ethyl acetate=1: 10), the title compound was yielded as yellow solid (0.51 g, 0.92 mmol, 46%, m.p. 190 ˚C).

δ_H (400 MHz, DMSO-d_6) 10.27 (s, br, 1H, NH), 7.89 (s, 1H, NCHN), 5.98 (d, J = 6 Hz, 1H, CHN in ribose), 5.91 (t, J = 6 Hz, 1H, CHO in ribose), 5.67 (t, J = 6 Hz, 1H, CHO in ribose), ~~4.68,~~ 4.48-4.42 (m, 3H, OCH_2 and CH in ribose), 3.30 (s, 2H, CH_2Cl), 3.28 (s, 3H, NCH_3), 2.46-2.37 (m, 6H, CH_2), 1.21-1.14 (m, 9H, CH_3).

δ_C (100 MHz, DMSO-d_6) 173.33, 172.62, 172.51, 168.94, 154.72, 148.33, 147.44, 138.08, 120.72, 84.72, 79.88, 72.19, 70.17, 63.00 (CH_2), 43.04 (CH_2), 32.98, 26.60 (CH_2), 26.53 (CH_2), 26.32 (CH_2), 8.84, 8.82, 8.71.

HRMS *m/z* 542.1643. ($C_{22}H_{29}{}^{35}ClN_5O_9^+$ requires 542.1648).

Synthesis of 1-bromo-3-methyl-2-butanone[76]



56

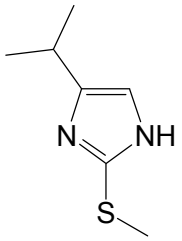
To a solution of 3-methyl-2-butanone (2.12 mL, 20 mmol) in ice-cold methanol (10 mL) was added bromine (1.00 mL, 20 mmol) dropwise. The reaction mixture was stirred at 0 ˚C for 2 hours, and then it was quenched with a solution of sodium hydrogen carbonate (1 g) and sodium hydrogen sulfite (1 g) in deionized water (30 mL). After extraction with diethyl ether (3 x 20 mL) the organic layer were combined, washed with brine and dried over magnesium sulfate. After filtration and the solvent was evaporated the title compound was yielded as pale yellow liquid (3.14 g, 19 mmol, 95%).

δ_H (400 MHz, $CDCl_3$) 4.00 (s, 2H, CH_2Br), 2.98 (septet, J = 7 Hz, 1H, CH), 1.16 (d, J = 7 Hz, 6H, CH_3).

δ_C (100 MHz, $CDCl_3$) 205.80 (CO), 38.36, 33.71 (1C, CH_2), 18.49 (2C, CH_3).

GCMS *m/z* 163.9. ($C_5H_9{}^{79}BrO$ requires 163.9).

Synthesis of 4-isopropyl-2-methylthio-1*H*-imidazole



62

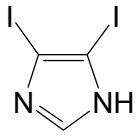
To a solution of S-methylisothiourea hemisulfate salt (1.39 g, 10 mmol) and sodium hydride (1.20 g, 30 mmol) in DMF (3 mL) was added a diluted solution of 1-bromo-3-methyl-2-ketone **56** (1.65 g, 10 mmol) in DMF (1 mL) dropwise. The reaction mixture was stirred at room temperature for 8 hours. Then it was quenched with ethanol (2 mL), and neutralized with 1M HCl till the pH reached slightly higher than 7. After extration with deionized water (20 mL) and diethyl ether (3 x 20 mL), the organic layer were combined, washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=1: 5) the title compound was yielded as yellow oil (0.24 g, 0.72 mmol, 16%).

δ_H (400 MHz, $CDCl_3$) 9.21 (s, br, 1H, NH), 6.30 (s, 1H, NCH), 2.83 (septet, J = 6 Hz, 1H, CH), 1.32 (s, 3H, SCH_3), 1.20 (d, J = 6 Hz, 6H, CH_3).

δ_C (100 MHz, $CDCl_3$) 159.36, 145.81, 118.77, 26.17, 23.34, 21.34 (2C, CH_3).

HRMS *m/z* 157.2552. ($C_7H_{13}N_2{}^{32}S^+$ requires 157.2561).

Synthesis of 4,5-diiodo-1*H*-imidazole[77]



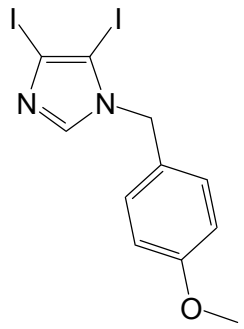
66

To a solution of imidazole (3.40 g, 50 mmol) and sodium hydroxide (8.00 g, 200 mmol) in deionized water (30 mL) was added a solution of iodine (12.69 g, 50 mmol) in hexane (30 mL) dropwise. The reaction was stirred at room temperature for 8 hours. Then it was quenched with 1M HCl till the pH reached slightly higher than 7. After filtration and recrystallized with diluted ethanol the title compound was yielded as white fine powder (12.00 g, 37.50 mmol, 75%, m.p. 175 ˚C, literature[77] 180 ˚C).

δ_H (400 MHz, DMSO-d_6) 12.94 (s, br, 1H, NH), 7.79 (s, 1H, NCHN).

HRMS *m/z* 320.8370. ($C_3H_3N_2{}^{127}I_2{}^+$ requires 320.8380).

Synthesis of 4,5-diiodo-1-(4-methoxybenzyl)-1*H*-imidazole[78]



61

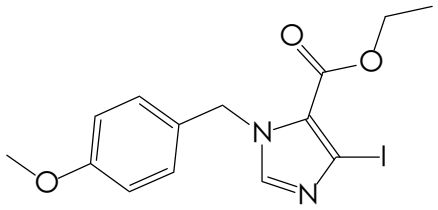
A mixture of 4,5-diiodo-imidazole **66** (3.20 g, 10 mmol) and sodium hydride (0.40 g, 10 mmol) were stirred in anhydrous THF (10 mL) at 0 ˚C for 1 hour. PMB-Cl (1.57 g, 10 mmol) was then added dropwise wth vigorous string. The reaction mixture was stirred at 55 ˚C for 12 hours, and then it was cooled to room temperature and quenched with ethanol (2 mL). After extration with deionized water (20 mL) and dichloromethane (3 x 20 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=20: 1) the title compound was yielded as white solid (4 g, 9.01 mmol, 91%, m.p. 157 ˚C, literature[78] 160 ˚C).

ν_{max} (KBr, cm^{-1}) 3099, 2939, 2832, 1651, 1517, 1481, 1170.

δ_H (400 MHz, DMSO-d_6) 7.55 (s, 1H, NCHN), 7.27 (d, J = 9 Hz, 2H, phenyl), 6.95 (d, J = 9 Hz, 2H, phenyl), 5.06 (s, 2H, CH_2), 3.79 (s, 3H, CH_3).

HRMS *m/z* 440.8954. ($C_{11}H_{11}N_2O^{127}I_2^+$ requires 440.8956).

Synthesis of ethyl 5-iodo-1-[(4-methyoxyphenyl)methyl]-1*H*-imidazole-4-carboxylate



64

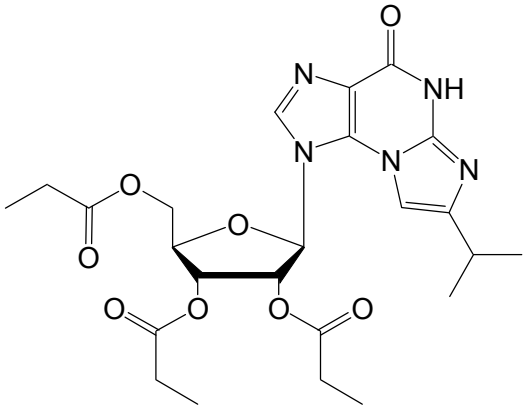
To a dried flask a solution of 4,5-diiodo-1-(4-methoxybenzyl)-1*H*-imidazole **61** (0.88 g, 2 mmol) in anhydrous THF (10 mL) was added a solution of Bu-Li (2.5 M, 0.5 mL, 5 mmol) diluted with anhydrous THF (2 mL) dropwise under nitrogen protection at -78 ˚C. The reaction mixture was warmed to room temperature and stirred for 30 minutes. After cooled to -78 ˚C, a solution of ethyl chloroformate (0.17 mL, 2 mmol) in anhydrous THF (10 mL) was added fast under nitrogen protection. The reaction mixture was warmed to room temperature and left overnight. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum: ethyl acetate=10: 1), the title compound was yielded as pale yellow oil (0.62 g, 1.20 mmol, 60%).

δ_H (400 MHz, DMSO-d_6) 7.70 (s, 1H), 7.32 (d, J = 9 Hz, 2H, phenyl), 6.85 (d, J = 9 Hz, 2H, phenyl), 5.43 (s, 2H, CH_2), 4.36 (quartet, J=7 Hz, 2H, CH_2), 3.77 (s, 3H, OCH_3), 1.32 (t, J = 7 Hz, 3H, CH_3).

δ_C (100 MHz, DMSO-d_6) 159.9, 158.33, 150.9, 150.2, 136.44, 129.73 (2C, phenyl), 113.5 (2C, phenyl), 105.3, 62.41 (CH_2), 58.64 (CH_2), 52.16, 17.3.

HRMS m/z 387.1999. ($C_{14}H_{16}{}^{127}IN_2O_3^+$ requires 387.1996).

Synthesis of 7-(propan-2-yl)-1-(2,3,5-tri-*O*-propanoylpentofuranosyl)-1*H*-imidazo[2,1-*b*]purin-4(5*H*)-one



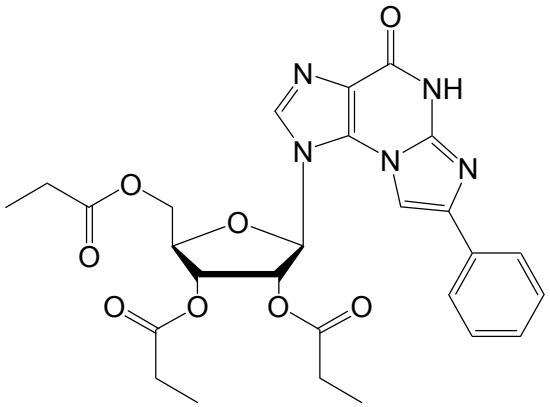
68

A mixture of 2',3',5'-tripropanoate-guanosine **49** (4.51 g, 1 mmol) and 1-bromo-3-methyl-butanone (0.16 g, 1 mmol) was stirred in the presence of sodium hydride (0.08 g, 2 mmol) in DMF (4 mL) at 80 ˚C for 12 hours. After the solvent evaporated, the mixture was diluted with deionized water (20 mL) and extracted with dichloromethane (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and purification via column chromatography (elution with methanol: ethyl acetate=1: 10), the title compound was yielded as pale yellow liquid (0.12 g, 0.2 mmol, 20%).

δ_H (400 MHz, $CDCl_3$) 12.15 (s, br, 1H, NH), 7.99 (s, 1H, NCHN), 7.68 (s, 1H, NCHC), 6.22 (d, J = 6 Hz, 1H, CHN in ribose), 6.01 (t, J = 6 Hz, 1H, CHO in ribose), 5.69 (t, J = 6 Hz, 1H, CHO in ribose), 4.43-4.32 (m, 3H, OCH_2 and CH in ribose), 2.78 (septet, J = 6 Hz, 1H), 2.37-2.28 (m, 6H, $COCH_2$), 1.25 (d, J = 6 Hz, 6H, CH_3), 1.13-1.01 (m, 9H, CH_3).

HRMS *m/z* 512.5022. ($C_{24}H_{26}N_5O_8^+$ requires 512.5014).

Synthesis of 7-phenyl-1-(2,3,5-tri-*O*-propanoylpentofuranosyl)-1*H*-imidazo[2,1-*b*]purin-4(5*H*)-one



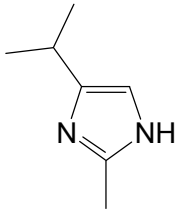
69

A mixture of 2',3',5'-tripropanoate-guanosine **49** (4.51 g, 1 mmol) and 2-bromo-1-phenyl-ethanone (0.20 g, 1 mmol) was stirred in the presence of sodium hydride (0.08 g, 2 mmol) in DMF (4 mL) at 80 ˚C for 12 hours. After the solvent evaporated, the mixture was diluted with deionized water (20 mL) and extracted with dichloromethane (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and purification via column chromatography (elution with methanol: ethyl acetate=1:10), the title compound was yielded as pale yellow liquid (0.07 g, 0.12 mmol, 12%).

δ_H (400 MHz, $CDCl_3$) 12.26 (s, br, 1H, NH), 8.01 (s, 1H, NCHN), 7.73 (d, J = 6.4 Hz, 2H), 7.71 (s, 1H, NCHC), 7.48 (t, J = 8 Hz, 1H), 7.42 (t, J = 5.6 Hz, 2H), 6.23 (d, J = 6 Hz, 1H, CHN in ribose), 5.99 (t, J = 6 Hz, 1H, CHO in ribose), 5.68 (t, J = 6 Hz, 1H, CHO in ribose), 4.46-4.36 (m, 3H, OCH_2 and CH in ribose), 2.39-2.28 (m, 6H, $COCH_2$), 1.13-1.01 (m, 9H, CH_3).

HRMS *m/z* 552.5664. ($C_{27}H_{30}N_5O_8^+$ requires 552.5661).

Synthesis of 4-isopropyl-2-methyl-1*H*-imidazole



70

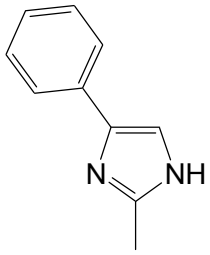
To a solution of acetamidine hydrochloride (0.95 g, 10 mmol) and sodium hydride (0.80 g, 20 mmol) in DMF (4 mL) was added 1-bromo-3-methyl-2-ketone **56** (1.65 g, 10 mmol) dropwise. The reaction mixture was stirred at room temperature for 8 hours. Then it was quenched with ethanol (2 mL), and neutralized with 1M HCl till the pH reached slightly higher than 7. After extration with deionized water (20 mL) and dichloromethane (3 x 20 mL), the organic layer were combined, washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=1: 5) the title compound was yielded as yellow oil (0.44 g, 3.50 mmol, 35%).

δ_H (400 MHz, DMSO-d_6) 7.90 (s, br, 1H, NH), 6.55 (s, 1H, CH), 2.67 (septet, J = 7 Hz, 1H, CH), 2.03 (s, 3H, CH_3), 1.02 (d, J = 7 Hz, 6H, CH_3).

δ_C (100 MHz, $CDCl_3$) 162.83, 145.32, 114.71, 27.47, 22.29 (2C), 17.35.

HRMS *m/z* 111.1889. ($C_7H_{13}N_2^+$ requires 111.1882).

Synthesis of 2-methyl-4-phenyl-1*H*-imidazole[79]



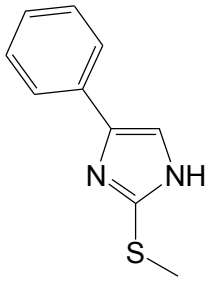
71

To a solution of acetamidine hydrochloride (0.95 g, 10 mmol) and sodium hydride (0.80 g, 20 mmol) in DMF (4 mL) was added 2-bromo-1-phenyl-ethanone (2 g, 10 mmol) dropwise. The reaction mixture was stirred at room temperature for 8 hours. Then it was quenched with ethanol (2 mL), and neutralized with 1M HCl till the pH reached slightly higher than 7. After extration with deionized water (20 mL) and dichloromethane (3 x 20 mL), the organic layer were combined, washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=1: 5) the title compound was yielded as yellow solid (0.44 g, 2.78 mmol, 28%, m.p. 158 ˚C, literature[79] 159 ˚C).

δ_H (400 MHz, DMSO-d_6) 9.53 (s, br, 1H, NH), 7.78 (d, J = 8 Hz, 2H, phenyl), 7.35-7.25 (m, 3H, phenyl), 6.55 (s, 1H, CH), 2.03 (s, 3H, CH_3),

HRMS *m/z* 159.2070. ($C_{10}H_{11}N_2^+$ requires 159.2072).

Synthesis of 2-methylthio-4phenyl-1*H*-imidazole[80]



72

To a solution of S-methylisothiourea hemisulfate salt (1.39 g, 10 mmol) and sodium hydride (0.80 g, 20 mmol) in DMF (4 mL) was added 1-bromo-3-methyl-butanone (1.60 g, 10 mmol) dropwise. The reaction mixture was stirred at room temperature for 8 hours. Then it was quenched with ethanol (2 mL), and neutralized with 1M HCl till the pH reached slightly higher than 7. After extraction with deionized water (20 mL) and dichloromethane (3 x 20 mL), the organic layer were combined, washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=1: 5) the title compound was yielded as yellow solid (0.34 g, 1.78 mmol, 18%, m.p. 134 ˚C, literature[80] 137 ˚C).

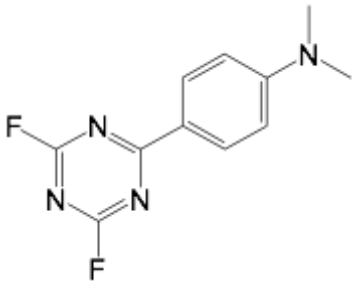
ν_{max} (KBr, cm^{-1}) 3020.

δ_H (400 MHz, DMSO-d_6) 7.80 (d, J = 8 Hz, 2H, phenyl), 7.33 (t, J = 8 Hz, 1H), 7.26 (t, J = 8 Hz, 2H, phenyl), 6.43 (s, 1H, CH), 2.64 (s, 3H, SCH_3).

HRMS *m/z* 191.2734. ($C_{10}H_{11}N_2{}^{32}S^+$ requires 191.2727).

Section 3: experimental for triazine analogues

Synthesis of 2,4-difluoro-6-(*N,N*-dimethylaminophenyl)-*s*-triazine[81]



74

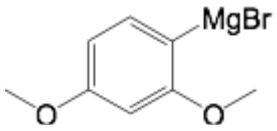
A mixture of cyanuric fluoride **73** (1.02 g, 7.5 mmol) and *N,N*-dimethylaniline (0.88 g, 7 mmol) was refluxed overnight in acetonitrile (5 mL). On cooling a red solid precipitated which was collected by filtration, washed with deionized water and recrystallized from acetonitrile to yield the title compound (0.54 g, 2.3 mmol, 33%, m.p. 235 ˚C, literature[81] 237 ˚C).

δ_H (400 MHz, $CDCl_3$) 8.34 (dt, J = 9, 2 Hz, 2H, phenyl), 6.70 (dt, J = 9, 2 Hz, 2H, phenyl), 3.12 (s, 6H, CH_3).

δ_F (376 MHz, CDCl3) -124.77 (s, 2F).

Preparation of Grignard reagents

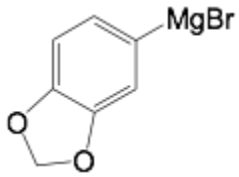
Preparation of (2,4-dimethoxyphenyl)-magnesium bromide



75

To a dried flask containing a suspension of magnesium powder (0.25 g, 20 mmol) in anhydrous THF (10 mL) was added 1-bromo-2,4-dimethoxyl-benzene (1.45 mL, 10 mmol) in anhydrous THF (10 mL) dropwise under nitrogen protection. The mixture was heated under reflux for 2 hours and then cooled to room temperature, and used directly in the required experiment.

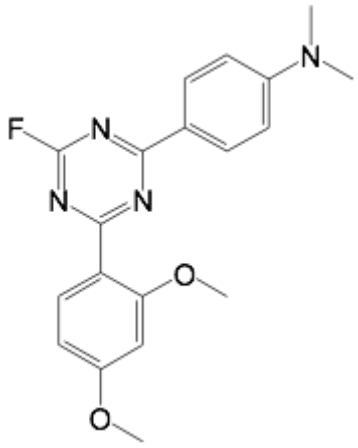
Preparation of (1,3-benzodioxole)-magnesium bromide



76

To a dried flask containing a suspension of magnesium powder (0.25 g, 20 mmol) in anhydrous THF (10 mL) was added 5-bromo-1,3-benzodioxole (1.17 mL, 10 mmol) in anhydrous THF (10 mL) dropwise under nitrogen protection. The mixture was heated under reflux for 2 hours and then cooled to room temperature, and used directly in the required experiment.

Synthesis of 2-fluoro-4-(*N*,*N*-dimethylaminophenyl)-6-(2,4-dimethoxyphenyl)-*s*-triazine



77

To a solution of 2,4-difluoro-6-(*N*,*N*-dimethylaminophenyl)-*s*-triazine **74** (0.54 g, 2.3 mmol) in anhydrous THF (10 mL) was added a solution of (2,4-dimethoxyphenyl)-magnesium bromide **75** in THF (2M, 1 mL, 2 mmol) diluted with anhydrous THF (5 mL) dropwise at 0 ˚C. The reaction was warmed to room temperature and left overnight, and then diluted with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=60: 1), the title compound was yielded as pale yellow oil (0.15 g, 0.5 mmol, 25%).

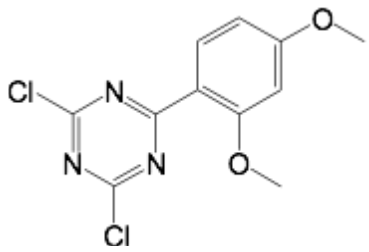
δ_H (400 MHz, $CDCl_3$) 8.44 (d, J = 8 Hz, 2H, phenyl), 8.27 (d, J = 8 Hz, 1H, phenyl), 6.73 (d, J = 9 Hz, 2H, phenyl), 6.62 (dd, J = 9, 2 Hz, 1H, phenyl), 6.56 (d, J = 2 Hz, 1H, phenyl), 3.95 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.09 (s, 6H, CH_3).

δ_C (100 MHz, $CDCl_3$) 175.64 (d, J = 66 Hz, CF coupling, $1C_{tri}$-C_{phenyl}), 171.42 (d, J = 245 Hz, CF coupling, $1C_{tri}$-F), 165.42 (d, J = 66 Hz, CF coupling, $1C_{tri}$-C_{phenyl}), 163.45, 159.06, 154.64, 152.21, 132.5, 131.01 (2C, phenyl), 122.21, 121.25, 111.15 (2C, phenyl), 105.31, 55.88, 55.64, 40.19 (2C, CH_3).

δ_F (376 MHz, $CDCl_3$) -121.76 (s, 1F)

HRMS *m/z* 303.3822. ($C_{19}H_{19}FN_4^+$ requires 303.3818).

Synthesis of 2,4-dichloro-6-(2,4-dimethoxyphenyl)-*s*-triazine



79

Method 1:

To a solution of cyanuric chloride **29** (3.84 g, 20 mmol) in anhydrous THF (20 mL) was added (2,4-dimethoxyphenyl)-magnesium bromide **75** in THF (2M, 10 mL, 20 mmol) dropwise at 0 °C under nitrogen protection. The mixture was warmed to room temperature and left overnight. The reaction was cooled to 0 °C and quenched by addition of saturated NH_4Cl (10 mL), and then extracted with ethyl acetate (20 mL x 3). The organic layer was washed with brine (20 mL) and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=30: 1), the title compound was yielded as white solid (4.29 g, 15 mmol, 75%, m.p. 125 ˚C).

δ_H (400 MHz, $CDCl_3$) 8.22 (d, J = 9 Hz, 1H, phenyl), 6.64 (dd, J = 9, 2 Hz, 1H, phenyl), 6.56 (d, J = 2 Hz, 1H, phenyl), 3.97 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3).

MS *m/z* 286.0140. ($C_{11}H_{10}{}^{35}Cl_2N_3O_2^+$ requires 286.0145).

Method 2:

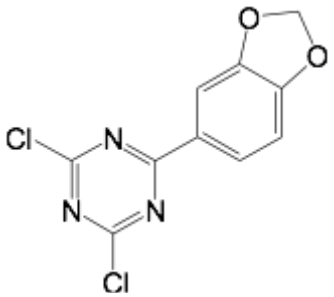
To a dried flask containing a solution of 1-bromo-2,4-dimethoxyl-benzene **75** (2M, 1.45 mL, 10 mmol) in anhydrous THF (10 mL) was added a solution of Bu-Li (2.5 M, 4.4 mL, 11 mmol) diluted with anhydrous THF (2 mL) dropwise under nitrogen protection at -78 ˚C.

The reaction mixture was warmed to room temperature and stirred for 30 minutes. After cooled to -78 ˚C, a solution of cyanuric chloride **29** (1.92 g, 10 mmol) in anhydrous THF (10 mL) was added fast under nitrogen protection. The reaction mixture was warmed to room temperature and left overnight. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and

purification via column chromatography (elution with petroleum: ethyl acetate=10: 1), the title compound was yielded as white solid (4.17 g, 7.3 mmol, 73%).

The product was identified to be the same by comparison (^{1}H NMR) with the compound prepared above.

Synthesis of 2,4-dichloro-6-(1,3-benzodioxole-5-yl)-*s*-triazine



80

Method 1:

To a solution of cyanuric chloride **29** (3.84 g, 20 mmol) in anhydrous THF (20 mL) was added (1,3-benzodioxol-5-yl)-magnesium bromide **76** (2M, 10 mL, 20 mmol) dropwise at 0 °C under nitrogen protection. The mixture was warmed to room temperature and left overnight. The reaction was cooled to 0 °C and quenched by addition of saturated NH_4Cl (10 mL), then extracted with ethyl acetate (20 mL x 3). The organic layer was washed with brine (20 mL) and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=60: 1), the title compound was yielded as white solid (3.78 g, 14 mmol, 70%, m.p. 138 ˚C).

δ_H (400 MHz, $CDCl_3$) 8.17 (dd, J = 8, 2 Hz, 1H, Ar-H), 7.90 (d, J= 2 Hz, 1H, phenyl), 6.91 (d, J = 8 Hz, 1H, phenyl), 6.09 (s, 2H, OCH_2O).

δ_C (100 MHz, $CDCl_3$) 172.12 (2C, C_{tri}-Cl), 165.49 (1C, C_{tri}-C_{phenyl}), 151.58, 147.98, 129.28, 124.49, 108.63, 108.26, 101.77 (CH_2).

HRMS *m/z* 269.9827. ($C_{10}H_6{}^{35}Cl_2N_3O_2{}^+$ requires 269.9832).

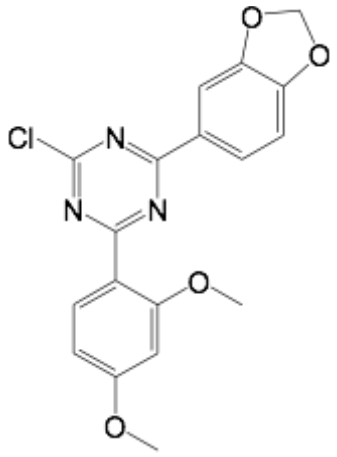
Method 2:

To a dried flask containing a solution of 5-bromo-1,3-benzodioxole **76** (2M, 1.17 mL, 10 mmol) in anhydrous THF (10 mL) was added a solution of Bu-Li (2.5 M, 4.4 mL, 11 mmol) diluted with anhydrous THF (2 mL) dropwise under nitrogen protection at -78 ˚C. The reaction mixture was warmed to room temperature and stirred for 30 minutes. After cooled to -78 ˚C, a solution of cyanuric chloride (1.92 g, 10 mmol) in anhydrous THF (10 mL) was added fast under nitrogen protection. The reaction mixture was warmed to room

temperature and left overnight. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum: ethyl acetate=10: 1), the title compound was yielded as white solid (3.34 g, 6.2 mmol, 62%).

The product was identified to be the same by comparison (^{1}H NMR) with the compound prepared above.

Synthesis of 2-chloro-4-(1,3-benzodioxole-5-yl)-6-(2,4-dimethoxyphenyl)-*s*-triazine



81

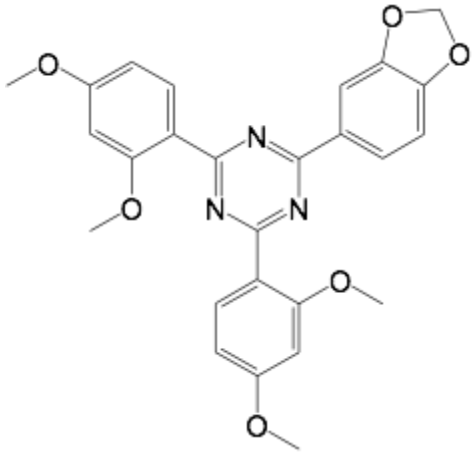
To a dried flask a solution of ~~5-bromo-1,3-benzodioxole (1.17 mL, 10 mmol)~~ 1-bromo-2,4-dimethoxyl-benzene (1.45 mL, 10 mmol) in anhydrous THF was added Bu-Li (2.5 M, 4.4 mL, 11 mmol) in anhydrous THF (10 mL) dropwise under nitrogen protection at -78 ˚C. The reaction mixture was warmed to room temperature and stirred for 30 minutes. After cooled to -78 ˚C, a solution of ~~2,4-dichloro-6-(2,4-dimethoxyphen-1-yl)-*s*-triazine **79** (2.85 g, 10 mmol)~~ 2,4-dichloro-6-(1,3-benzodioxole-5-yl)-*s*-triazine **80** (2.70 g, 10 mmol) in anhydrous THF (10 mL) was added dropwise under nitrogen protection. The reaction mixture was warmed to room temperature and left overnight. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum: ethyl acetate=10: 1), the title compound was yielded as yellow oil (1.30 g, 3.5 mmol, 35%).

δ_H (400 MHz, $CDCl_3$) 8.22 (dd, J = 8, 2 Hz, 1H, phenyl), 8.20 (d, J = 8 Hz, 1H, phenyl), 8.00 (d, J = 2 Hz, 1H, phenyl), 6.92 (d, J = 8 Hz, 1H, phenyl), 6.61 (dd, J = 8, 2 Hz, 1H, phenyl), 6.56 (d, J = 2 Hz, 1H, phenyl), 6.06 (s, 2H, OCH_2O), 3.95 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3).

δ_C (100 MHz, $CDCl_3$) 173.47, 172.16, 171.17, 164.66, 161.59, 152.23, 148.23, 134.58, 129.09, 125.40, 117.29, 109.12, 108.51, 105.45, 101.95 (CH_2), 99.48, 56.25 (-OCH_3), 55.64 (-OCH_3).

HRMS *m/z* 372.0735. ($C_{10}H_6{}^{35}Cl_2N_3O_2^+$ requires 372.0746).

Synthesis of 2-(1,3-benzodioxole-5-yl)-4,6-di-(2,4-dimethoxyphenyl)-*s*-triazine



82

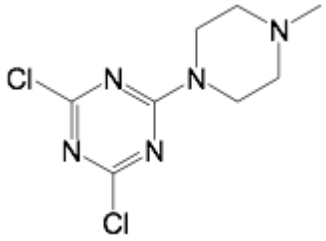
To a dried flask a solution of 1-bromo-2,4-dimethoxyl-benzene (3.19 mL, 22 mmol) in anhydrous THF was added Bu-Li (2.5 M, 9.68 mL, 22 mmol) in anhydrous THF (10 mL) dropwise under nitrogen protection at -78 ˚C. The reaction mixture was warmed to room temperature and stirred for 30 minutes. After cooled to -78 ˚C, a solution of 2,4-dichloro-6-(1,3-benzodioxole-5-yl)-*s*-triazine **80** (2.70 g, 10 mmol) in anhydrous THF (10 mL) was added dropwise under nitrogen protection. The reaction mixture was warmed to 50 ˚C and left overnight. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum: ethyl acetate=10: 1), the title compound was yielded as yellow oil (1.41 g, 3 mmol, 30%).

δ_H (400 MHz, $CDCl_3$) 7.59 (dd, J = 8, 2 Hz, 1H, phenyl), 7.43 (d, J = 2 Hz, 1H, phenyl), 7.38 (d, J = 8 Hz, 2H, phenyl), 6.78 (d, J = 8 Hz, 1H, phenyl), 6.46 (dd, J = 8, 2 Hz, 2H, phenyl), 6.35 (d, J = 2 Hz, 2H, phenyl), 6.00 (s, 2H, OCH_2O), 3.80 (s, 6H, CH_3), 3.45 (s, 6H, CH_3).

δ_C (100 MHz, $CDCl_3$) 176.35 (2C, C_{tri}-C_{phenyl}), 166.25, 163.26 (2C, phenyl), 159.05 (2C, phenyl), 151.92, 147.79, 132.36 (2C, phenyl), 126.73, 125.59, 119.94 (2C, phenyl), 108.98, 107.96, 104.73 (2C, phenyl), 101.95 (CH_2), 98.70 (2C, phenyl), 55.57 (2C, OCH_3), 55.39 (2C, OCH_3).

HRMS *m/z* 474.1654. ($C_{26}H_{24}N_3O_6^+$ requires 474.1660).

Synthesis of 2,4-dichloro-6-(4-methyl-1-piperazinyl)-*s*-triazine[83]



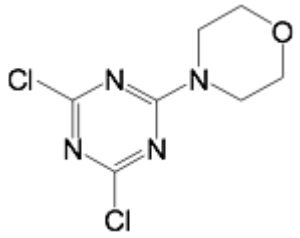
83

A mixture of cyanuric chloride **29** (1.84 g, 10 mmol) and 1-methyl-piperazine (1.10 mL, 10 mmol) was stirred stirred in the presence of potassium carbonate (1.38 g, 10 mmol) in THF (10 mL) at room temperature for 8 hours. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=20: 1), the title compound was yielded colorless oil (1.54 g, 6.20 mmol, 62%).

δ_H (400 MHz, $CDCl_3$) 3.98-3.93 (m, 4H, piperazine), 2.23-2.19 (m, 4H, piperazine), 2.03 (s, 3H, CH_3).

HRMS *m/z* 249.2111. ($C_8H_{12}{}^{35}Cl_2N_5{}^+$ requires 249.2105).

Synthesis of 2,4-dichloro-6-(4-morpholinyl)-*s*-triazine[84]



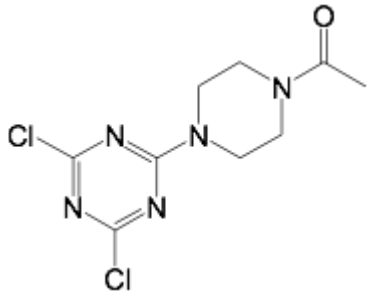
84

A mixture of cyanuric chloride **29** (1.84 g, 10 mmol) and morpholine (0.86 mL, 10 mmol) was stirred stirred in the presence of potassium carbonate (1.38 g, 10 mmol) in THF (10 mL) at room temperature for 8 hours. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration, evaporation and purification via column chromatography (elution with petroleum ether: ethyl acetate=10: 1), the title compound was yielded colorless oil (1.64 g, 7 mmol, 70%, m.p. 155 °C, literature[84] 156 °C).

δ_H (400 MHz, $CDCl_3$) 3.87-3.86 (m, 4H, morpholine), 3.73-3.72 (m, 4H, morpholine).

HRMS *m/z* 236.0677. ($C_7H_9{}^{35}Cl_2N_4O^+$ requires 236.0685).

Synthesis of 2,4-dichloro-6-(4-acetyl-1-piperazinyl)-*s*-triazine



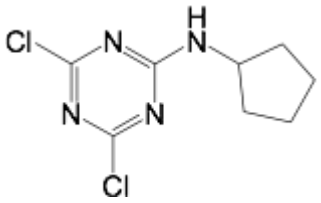
85

A mixture of cyanuric chloride **29** (1.84 g, 10 mmol) and 1-acetyl-piperazine (1.25 mL, 10 mmol) was stirred stirred in the presence of potassium carbonate (1.38 g, 10 mmol) in THF (10 mL) at room temperature for 8 hours. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration, evaporation and purification via column chromatography (elution with petroleum ether: ethyl acetate=20: 1), the title compound was yielded as a colorless oil (1.79 g, 6.49 mmol, 65%).

δ_H (400 MHz, DMSO-d_6) 3.77-3.67 (m, 4H, piperazine), 3.52-3.45 (m, 4H, piperazine), 2.00 (s, 3H, CH_3).

HRMS *m/z* 276.0431. ($C_9H_{12}{}^{35}Cl_2N_5O^+$ requires 276.0424).

Synthesis of 2, 4-dichloro-6-(*N*-cyclopentylamino)-*s*-triazine



86

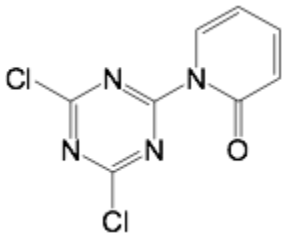
A mixture of cyanuric chloride **29** (0.92 g, 5 mmol) and cyclopentylamine (0.2 mL, 5 mmol) was stirred in the presence of potassium carbonate (0.64 g, 5 mmol) in THF (10 mL) at 0˚C for 5 hours. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether), the title compound was yielded as colorless oil (1.10 g, 4.9 mmol, 49%).

ν_{max} (KBr, cm^{-1}) 3335 (N-H), 2218 (C=N), 1221 (C-N in 2˚ amine), 780 (C-Cl).

δ_H (400 MHz, $CDCl_3$) 5.73 (br, s, 1H, NH), 4.34 (apparent sextet, J = 7 Hz, 1H, CH in amine), 2.09-2.04 (m, 2H, CH_2 in cyclopentyl), 1.72-1.62 (m, 4H, CH_2 in cyclopentyl), 1.51-1.44 (m, 2H, CH_2 in cyclopentyl).

HRMS *m/z* 233.0358. ($C_8H_{11}{}^{35}Cl_2N_4{}^+$ requires 233.0355).

Synthesis of 2,4-dichloro-6-(2-oxo-1(4H)-pyridinyl)-*s*-triazine

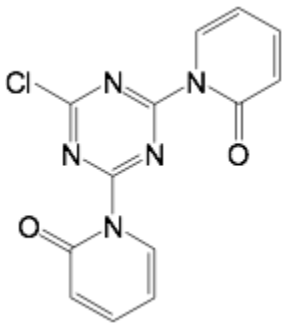


87

A mixture of cyanuric chloride **29** (1.84 g, 10 mmol) and 2-pyridone (0.95 g, 10 mmol) was stirred in the presence of triethylamine (1.39 mL, 10 mmol) in THF (10 mL) at 0 °C for 5 hours. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=20: 1), the title compound was yielded as colorless oil (1.32 g, 5.41 mmol, 54%).

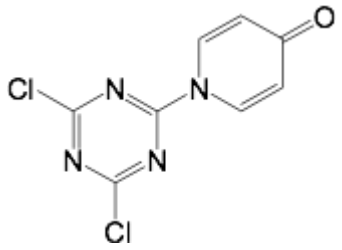
δ_H (400 MHz, $CDCl_3$) 8.38 (d, J = 7 Hz, 1H), 7.86 (t, J = 7 Hz, 1H), 7.31 (t, J = 7 Hz, 1H), 7.11 (d, J = 7 Hz, 1H).
HRMS *m/z* 244.9819. ($C_8H_5{}^{35}Cl_2N_4O^+$ requires 244.9805).

Another by-product, 2-chloro-4,6-di(2-oxo-1(4H)-pyridinyl)-*s*-triazine, was also identified as colorless oil (0.30 g, 1.00 mmol, 10%).



δ_H (400 MHz, $CDCl_3$) 8.35 (d, J = 7 Hz, 2H), 7.77 (t, J = 7 Hz, 2H), 7.20 (t, J = 7 Hz, 2H), 7.05 (d, J = 7 Hz, 2H).
HRMS *m/z* 302.0545. ($C_{13}H_9{}^{35}ClN_5O_2^+$ requires 302.0439).

Synthesis of 2,4-dichloro-6-(4-oxo-1(4H)-pyridinyl)-*s*-triazine



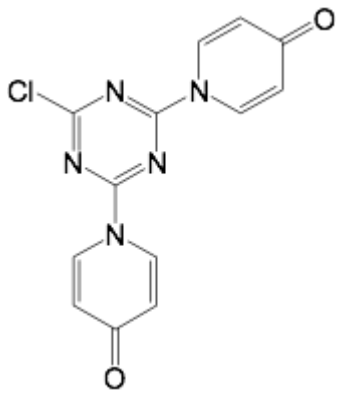
88

A mixture of cyanuric chloride **29** (1.84 g, 10 mmol) and 4-pyridone (0.95 g, 10 mmol) was stirred in the presence of triethylamine (1.39 mL, 10 mmol) in THF (10 mL) at 0 °C for 5 hours. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=1: 1), the title compound was yielded as colorless oil (1.37 g, 5.61 mmol, 56%).

δ_H (400 MHz, $CDCl_3$) 8.27 (d, J = 8 Hz, 2H), 7.31 (d, J = 8 Hz, 2H).

HRMS *m/z* 244.5811. ($C_8H_5{}^{35}Cl_2N_4O^+$ requires 244.9805).

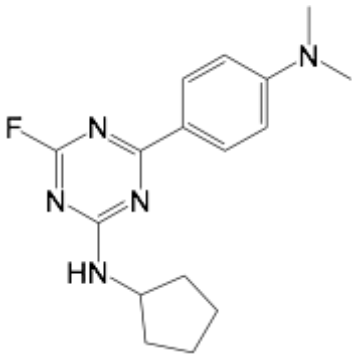
Another by-product, 2-chloro-4,6-di(4-oxo-1(4H)-pyridinyl)-*s*-triazine, was also identified as colorless oil (0.35 g, 1.18 mmol, 12%).



δ_H (400 MHz, $CDCl_3$) 8.51 (d, J = 8 Hz, 4H), 7.24 (d, J = 8 Hz, 4H).

HRMS *m/z* 302.0394. ($C_{13}H_9{}^{35}ClN_5O_2^+$ requires 302.0439).

Synthesis of 2-fluoro-4-(*N*-cyclopentylamino)-6-(*N*,*N*-dimethylaminophenyl)-*s*-triazine



89

A mixture of 2,4-difluoro-6-(2,4-dimethoxyphenyl)-*s*-triazine **74** (1.17 g, 5 mmol) and cyclopentylamine (0.20 mL, 5 mmol) was stirred in the presence of triethylamine (0.70 mL, 5 mmol) overnight in anhydrous THF (10 mL) at room tempreature. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=60: 1), the title compound was yielded as a pale yellow colored oil (0.75 g, 2.5 mmol, 50%).

ν_{max} (KBr, cm^{-1}) 3330 (N-H), 2215 (C=N), 1330 (C-F), 1214 (C-N in 2˚ amine).

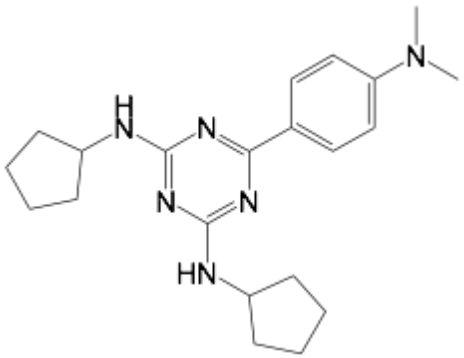
δ_H (400 MHz, $CDCl_3$) 8.22 (d, J = 8 Hz, 2H, phenyl), 6.67 (d, J = 8 Hz, 2H, phenyl), 5.62 (br, s, 1H, NH), 4.33 (apparent sextet, J = 6 Hz, 1H, CH in cyclopentyl), 3.06 (s, 6H, CH_3), 2.16-2.02 (m, 2H, CH_2 in cyclopentyl), 1.78-1.70 (m, 2H, CH_2 in cyclopentyl), 1.68-1.60 (m, 2H, CH_2 in cyclopentyl), 1.56-1.44 (m, 2H, CH_2 in cyclopentyl).

δ_C (100 MHz, $CDCl_3$) 174.46 (d, J = 66 Hz, CF coupling, $1C_{tri}$-C_{phenyl}), 170.02 (d, J = 245 Hz, CF coupling, $1C_{tri}$-F), 167.94 (d, J = 66 Hz, CF coupling, $1C_{tri}$-C_{phenyl}), 153.64, 130.91 (2C, phenyl), 122.21, 111.12 (2C, phenyl), 52.78, 40.18 (2C, CH_3), 33.17 (2C, CH_2), 23.81 (2C, CH_2).

δ_F (376 MHz, $CDCl_3$) -122.96 (s, 1F).

HRMS *m/z* 302.1755. ($C_{16}H_{21}FN_5^+$ requires 302.1776).

Another by-product, 2,4-di(*N*-cyclopentylamino)-6-(*N*,*N*-dimethylaminophenyl)-*s*-triazine, was also obtained as a pale yellow colored oil (0.30 g, 0.75 mmol, 15%).

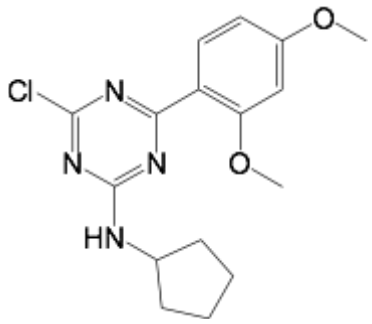


δ_H (400 MHz, $CDCl_3$) 8.21 (d, J = 8 Hz, 2H, phenyl), 6.69 (d, J = 8 Hz, 2H, phenyl), 4.47-4.42 (m, br, 2H, NH), 3.02 (s, 6H, CH_3), 4.35 (apparent sextet, J = 6 Hz, 2H, CH in cyclopentyl), 2.12-1.96 (m, 4H, CH_2 in cyclopentyl), 1.77-1.68 (m, 4H, CH_2 in cyclopentyl), 1.67-1.57 (m, 4H, CH_2 in cyclopentyl), 1.54-1.42 (m, 4H, CH_2 in cyclopentyl).

δ_C (100 MHz, $CDCl_3$) 170.2, 166.5 (2C, C_{tri}-N), 152.77, 129.77 (2C, phenyl), 119.8, 111.27 (2C, phenyl), 52.56 (2C, CH in cyclopentyl), 40.29 (2C, CH_3), 33.41 ($4CH_2$, CH_2 in cyclopentyl), 23.86 ($4CH_2$, CH_2 in cyclopentyl).

HRMS *m/z* 403.2617. ($C_{21}H_{31}N_6^+$ requires 403.2610).

Synthesis of 2-chloro-4-(*N*-cyclopentylamino)-6-(2,4-dimethoxyphenyl)-*s*-triazine



90

A mixture of 2,4-dichloro-6-(2, 4-dimethoxyphen-1-yl)-*s*-triazine **79** (2.85 g, 10 mmol) and cyclopentylamine (0.40 mL, 10 mmol) was stirred in the presence of triethylamine (1.53 mL, 11 mmol) overnight in anhydrous THF (10 mL) at room tempreature. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration, evaporation and purification via column chromatography (elution with petroleum ether: ethyl acetate=30: 1), the title compound was yielded as colorless oil (1.50 g, 4.5 mmol, 45%).

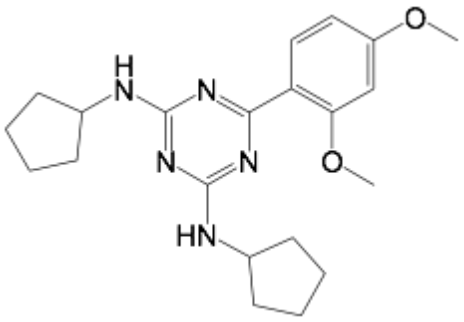
ν_{max} (KBr, cm^{-1}) 3340 (NH), 1235 (CO), 1214 (C-N in 2˚ amine).

δ_H (400 MHz, $CDCl_3$) 7.96 (d, J = 8 Hz, 1H), 6.56 (dd, J = 8, 2 Hz, 1H), 6.54 (d, J = 2 Hz, 1H), 5.64 (s, br, 1H), 4.40 (apparent sextet, J = 6 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 2.12-2.02 (m, 2H), 1.74-1.69 (m, 2H), 1.68-1.62 (m, 2H),1.52-1.42 (m, 2H).

δ_C (100 MHz, $CDCl_3$) 176.41, 171.32, 164.75, 163.16, 158.95, 152.33, 132.76, 119.66, 104.53, 56.21, 55.74. 52.72, 40.16 (2C), 33.21 ($2CH_2$), 23.83 ($2CH_2$).

HRMS *m/z* 335.1258. ($C_{16}H_{20}{}^{35}ClN_4O_2^+$ requires 335.1269).

Another by-product, 2,4-di(*N*-cyclopentylamino)-6-(2,4-dimethoxyphenyl)-*s*-triazine, was also isolated as colorless oil (0.50g, 1.3 mmol, 13%).



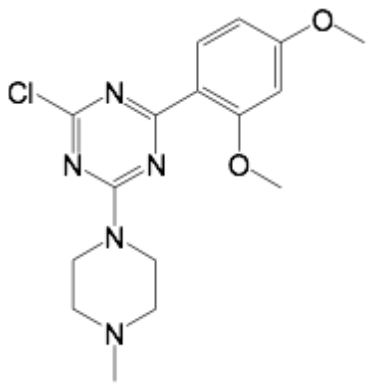
ν_{max} (KBr, cm^{-1}) 3300 (NH), 1220 (C-N in 2˚ amine).

δ_H (400 MHz, $CDCl_3$) 7.96 (d, J = 8 Hz, 1H), 6.54 (dd, J = 8, 2 Hz, 1H), 6.55 (d, J = 2 Hz, 1H), 5.88 (s, br, 2H), 4.55 (apparent sextet, J = 6 Hz, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 2.22-2.12 (m, 4H), 1.83-1.71 (m, 4H), 1.69-1.63 (m, 4H),1.52-1.42 (m, 4H).

δ_C (100 MHz, $CDCl_3$) 174.31, 172.46, 165.11 (2C, C_{tri}-N), 162.88, 158.78, 152.23, 132.32, 119.21, 104.33, 56.25 (1C, OCH_3), 55.64 (1C, OCH_3), 52.51 (2C, CH in cyclopentyl), 33.39 (4C, CH_2 in cyclopentyl), 23.84 (4C, CH_2 in cyclopentyl).

HRMS *m/z* 384.2374. ($C_{21}H_{30}N_5O_2^+$ requires 384.2394).

Synthesis of 2-chloro-4-(4-methyl-1-piperazin-1-yl)-6-(2,4-dimethoxyphen-1-yl)-*s*-triazine



91

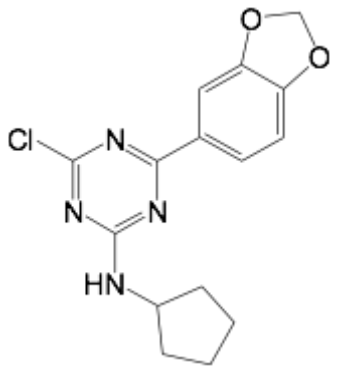
A mixture of 2,4-dichloro-6-(2,4-dimethoxyphen-1-yl)-*s*-triazine **79** (2.85 g, 10 mmol) and 1-methylpiperazine (1.10 mL, 10 mmol) was stirred in the presence of potassium carbonate (1.38 g, 10 mmol) overnight in anhydrous THF (10 mL) at room temperature. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ethyl acetate), the title compound was yielded as colorless oil (1.40 g, 4 mmol, 40 %).

δ_H (400 MHz, $CDCl_3$) 8.10 (d, J = 8 Hz, 1H, phenyl), 6.62 (d, J = 8, 2 Hz, 1H, phenyl), 6.55 (d, J = 2 Hz, 1H, phenyl), 3.95 (s, 3H, CH_3), 3.93 (s, 3H, CH_3), 3.55-3.58 (m, 4H, piperazine), 3.30-3.33 (m, 4H, piperazine), 2.26 (s, 3H, CH_3).

δ_C (100 MHz, $CDCl_3$) 178.34, 174.36, 168.61, 160.32, 156.61, 133.22, 105.33, 102.27, 100.00, 56.22, 55.35, 54.59 (2C, CH_2), 47.31 (2C, CH_2), 46.15.

HRMS *m/z* 349.8142. ($C_{16}H_{20}{}^{35}ClN_5O_2{}^+$ requires 349.8153).

Synthesis of 2-chloro-4-(*N*-cyclopentylamino)-6-(1,3-benzodioxole-5-yl)-*s*-triazine



92

A mixture of 2, 4-dichloro-6-(1,3-benzodioxole-5-yl)-*s*-triazine **80** (0.54 g, 2 mmol) and cyclopentylamine (0.08 mL, 2 mmol) was stirred in the presence of triethylamine (0.28 mL, 2 mmol) overnight in anhydrous THF (10 mL) at room temperature. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=30: 1), the title compound was yielded as colorless oil (0.26 g, 0.82 mmol, 41%).

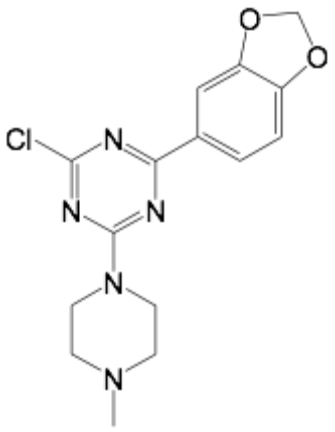
ν_{max} (KBr, cm^{-1}) 3340 (NH), 1350 (C-N in 2˚ amine), 790 (C-Cl).

δ_H (400 MHz, $CDCl_3$) 8.66 (d, J = 8 Hz, 1H, phenyl), 7.87 (dd, J = 8, 2 Hz, 1H, phenyl), 6.90 (d, J = 8 Hz, 1H, phenyl), 6.07 (s, 2H, OCH_2O), 5.61 (br, s, 1H, NH), 4.48 (apparent sextet, J=6 Hz, 1H, CH in cyclopentyl), 2.18-2.07 (m, 2H, CH_2 in cyclopentyl), 1.82-1.67 (m, 4H, CH_2 in cyclopentyl), 1.60-1.48 (m, 2H, CH_2 in cyclopentyl).

δ_C (100 MHz, $CDCl_3$) 172.06, 170.08, 165.71, 151.69, 147.98, 129.28, 124.89, 108.89, 108.19, 101.77 (CH_2), 53.05 (1C, CH in cyclopentyl), 33.11 (2C, CH_2 in cyclopentyl), 23.68 (2C, CH_2 in cyclopentyl).

HRMS *m/z* 319.0945. ($C_{15}H_{16}{}^{35}ClN_4O_2^+$ requires 319.0956).

Synthesis of 2-chloro-4-(4-methyl-1-piperazin-1-yl)-6-(1,3-benzodioxole-5-yl)-*s*-triazine



93

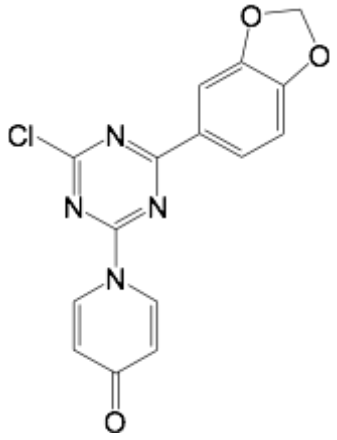
A mixture of 2, 4-dichloro-6-(1,3-benzodioxole-5-yl)-*s*-triazine **80** (2.70 g, 10 mmol) and 1-methylpiperazine (1.10 mL, 10 mmol) was stirred in the presence of potassium carbonate (1.38 g, 10 mmol) overnight in anhydrous THF (10 mL) at room tempreature. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ethyl acetate), the title compound was yielded as colorless oil (1.34 g, 0.43 mmol, 43%).

δ_H (400 MHz, $CDCl_3$) 8.00 (dd, J = 8, 2 Hz, 1H, phenyl), 7.43 (dd, J = 2 Hz, 1H, phenyl), 6.99 (d, J = 8 Hz, 1H, phenyl), 6.07 (s, 2H, OCH_2O), 3.54-3.57 (m, 4H, piperazine), 3.31-3.34 (m, 4H, piperazine), 2.26 (s, 3H, CH_3).

δ_C (100 MHz, $CDCl_3$) 176.33, 171.26, 168.93, 165.09, 150.22, 147.64, 123.31, 108.61, 107.89, 101.44 (1C, OCH_2O), 55.05 (2C, CH_2 in piperazine), 46.37 (2C, CH_2 in piperazine), 43.10 (1C, CH_3)

HRMS *m/z* 302.1170. ($C_{15}H_{17}{}^{35}ClN_5O_2{}^+$ requires 302.1167).

Synthesis of 2-chloro-4-(4-oxo-1(4H)-pyridinyl)-6-(1,3-benzodioxole-5-yl)-*s*-triazine



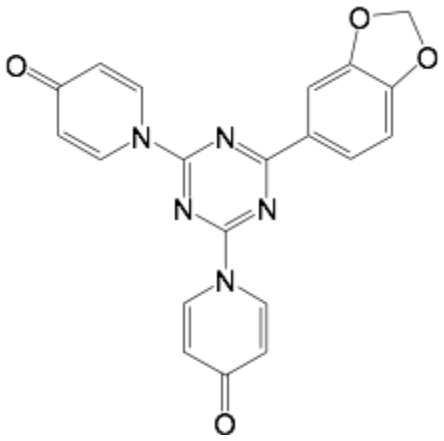
94

A mixture of 2, 4-dichloro-6-(1,3-benzodioxole-5-yl)-*s*-triazine **80** (1.35 g, 5 mmol) and 4-pyridone (0.48 mL, 5 mmol) was stirred in the presence of sodium hydride (0.24 g, 6 mmol) in anhydrous THF (10 mL) at 0 ˚C. The reaction mixture was heat to reflux and left overnight. After the solvent evaporated, the mixture was treated with deionized water (20 mL) and extracted with ethyl acetate (20 x 3 mL). The organic layer was washed with brine and dried over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether: ethyl acetate=30: 1), the title compound was yielded as grey colored solid (0.66 g, 2 mmol, 40%, m.p. 298 ˚C).

δ_H (400 MHz, $CDCl_3$) 8.86 (d, J = 8 Hz, 2H, pyridone), 8.10 (dd, J = 8, 2 Hz, 1H, phenyl), 7.89 (d, J = 2 Hz, 1H, phenyl), 7.07 (d, J = 8 Hz, 1H, phenyl), 6.24 (d, J = 8 Hz, 2H, pyridone), 6.12 (s, 2H, OCH_2O).

HRMS *m/z* 329.0107. ($C_{17}H_{10}{}^{35}ClN_4O_2^+$ requires 329.0436).

Another by-product, 2,4-di(4-oxo-1(4H)-pyridinyl)-6-(1,3-benzodioxole-5-yl)-*s*-triazine, was also identified as grey colored solid (0.68 g, 1.75 mmol, 35%, m.p. 308 ˚C).

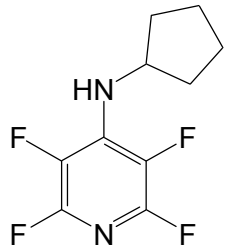


δ_H (400 MHz, $CDCl_3$) 9.22 (d, J = 8 Hz, 4H, pyridone), 8.41 (dd, J = 8, 2 Hz, 1H, phenyl), 8.28 (d, J = 2 Hz, 1H, phenyl), 7.19 (d, J = 8 Hz, 1H, phenyl), 6.38 (d, J = 8 Hz, 4H, pyridone), 6.24 (s, 2H, OCH_2O).

HRMS *m/z* 394.3166. ($C_{20}H_{10}N_5O_4^+$ requires 394.3173).

Section 4: experimental for anticancer compounds

Synthesis of 2,3,5,6-tetrafluoro-*N*-cyclopentyl-4-pyridinamine



96

A mixture containing pentafluoropyridine (0.52 mL, 5 mmol) and cyclopentylamine (0.49 mL, 5 mmol) was stirred in the presence of triethylamine (0.70 mL, 5 mmol) in acetonitrile (5 mL). The reaction mixture was then stirred at 0 ˚C for 8 hours. After the solvent evaporated, the mixture was diluted with deionized water (20 mL) and extracted with ethyl acetate (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether), the title compound was yielded as pale yellow liquid (0.94 g, 4 mmol, 80%).

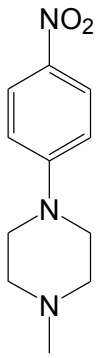
δ_H (400 MHz, $CDCl_3$) 4.44 (br, s, 1H), 4.09 (apparent sextet, J = 6 Hz, 1H), 2.10-2.02 (m, 2H), 1.76-1.62 (m, 4H), 1.56-1.48 (m, 2H).

δ_C (100 MHz, $CDCl_3$) 144.34 (dt, J = 236, 16 Hz, 2C), 137.32 (heptet, J = 6 Hz, 1C), 130.95 (dd, J = 244, 35 Hz, 2C), 56.20, 34.71 ($2CH_2$), 23.74 ($2CH_2$).

δ_F (376 MHz, $CDCl_3$) -67.25 (m, 2F), -2.55 (m, 2F).

HRMS *m/z* 235.0862. ($C_{10}H_{11}F_4N_2^+$ requires 235.0853).

Synthesis of 1-methyl-4-(4-nitrophenyl)-piperazine[20]



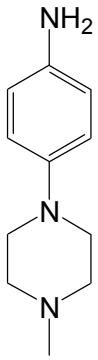
39

A mixture containing 1-methylpiperazine (2.21 mL, 20 mmol) and 1-fluoro-4-nitrobenzene (2.12 mL, 20 mmol) was stirred in the presence of triethylamine (2.80 mL, 20 mmol) overnight in acetonitrile (10 mL) at 82 ˚C. After the solvent evaporated, the mixture was diluted with deionized water (20 mL) and extracted with dichloromethane (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and recrystallization from acetonitrile the title compound was yielded as pale yellow solid (3.98 g, 18 mmol, 90%, m.p. 103 ˚C, literature[20] 104 ˚C).

δ_H (400 MHz, $CDCl_3$) 8.11 (d, J = 9 Hz, 2H), 6.81 (d, J = 9 Hz, 2H), 3.43 (t, J = 5 Hz, 4H), 2.55 (t, J = 5 Hz, 4H), 2.35 (s, 3H).

δ_C (100 MHz, $CDCl_3$) 154.90, 138.55, 126.04 (2C), 112.78 (2C), 54.61 (2C, CH_2), 47.04 (2C, CH_2), 46.14.

Synthesis of 1-(4-aminophenyl)-4-methylpiperazine[20]



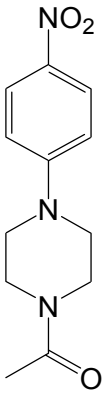
40

1-Methyl-4-(4-nitrophenyl)-piperazine (1.10 g, 5 mmol) in HPLC grade methanol (150 mL) and hydrogen gas were pumped through Pd/C in H cube overnight at 20˚C. After drying and purification via column chromatography (elution with methanol: ethyl acetate=1 : 40), the title compound was yielded as pale yellow solid (0.96 g, 5 mmol, 99%, m.p. 92˚C, literature[20] 94 ˚C)

δ_H (400 MHz, $CDCl_3$) 8.17 (d, J = 9 Hz, 2H), 6.81 (d, J = 9 Hz, 2H), 3.43 (t, J = 5 Hz, 4H), 2.55 (t, J = 5 Hz, 4H), 2.35 (s, 3H). NH_2 not observed in the spectrum.

δ_C (100 MHz, $CDCl_3$) 154.91, 138.55, 126.03 (2C), 112.78 (2C), 54.60 ($2CH_2$), 47.05 (CH_2), 46.14.

Synthesis of 1-acetyl-4-(4-nitrophenyl)-piperazine[86]



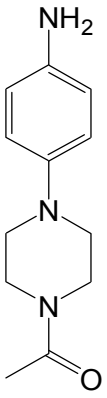
102

A mixture containing 1-acetylpiperazine (1.60 g, 12.5 mmol) and 1-fluoro-4-nitrobenzene (1.06 mL, 10 mmol) was stirred in the presence of triethylamine (1.40 mL, 10 mmol) in acetonitrile (5 mL) at 82˚C for 8 hours. After the solvent was evaporated, the mixture was diluted with deionized water (20 mL) and extracted with dichloromethane (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration, evaporation and recrystallization from acetonitrile the title compound was yielded as pale yellow solid (2.49g, 10 mmol, 99%, m.p. 149 ˚C, literature[86] 150 ˚C).

δ_H (400 MHz, $CDCl_3$) 8.14 (d, J = 9 Hz, 2H), 6.82 (d, J = 9 Hz, 2H), 3.79 (t, J = 5 Hz, 2H), 3.66 (t, J = 5 Hz, 2H), 3.45 (d, J = 5 Hz, 4H), 2.15 (s, 3H).

δ_C (100 MHz, $CDCl_3$) 169.24, 154.47, 139.21, 126.06 (2C), 113.06 (2C), 47.08 (CH_2), 46.96 (CH_2), 45.55 (CH_2), 40.79 (CH_2), 21.43.

Synthesis of 4-(4-acetylpiperazin-1-yl)-aniline[86]



101

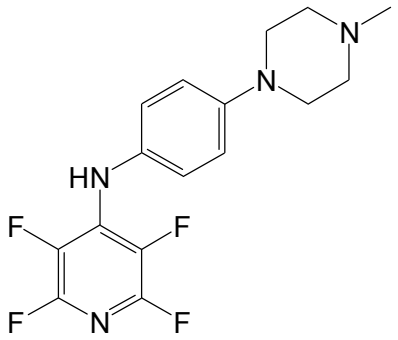
1-Acetyl-4-(4-nitrophenyl)-piperazine (1.25 g, 5 mmol) in HPLC grade methanol (150 mL) and hydrogen gas were pumped through Pd/C in an H cube overnight at 20 ˚C. After evaporation and purification via column chromatography (elution with methanol: ethyl acetate=1: 40), the title compound was yielded as pale yellow solid (1.07 g, 5 mmol, 99%, m.p. 131 ˚C, literature[86] 130 ˚C).

δ_H (400 MHz, DMSO-d_6) 6.62 (d, J = 9 Hz, 2H), 6.45 (d, J = 9 Hz, 2H), 4.59 (s, br, 2H), 3.48 (t, J = 5 Hz, 4H), 2.84 (t, J = 5 Hz, 2H), 2.77 (t, J = 5.2 Hz, 2H), 1.98 (s, 3H).

δ_C (100 MHz, $CDCl_3$) 169.06, 143.96, 141.02, 119.41 (2C), 116.25 (2C), 51.67 (CH_2), 51.26 (CH_2), 46.54 (CH_2), 41.63 (CH_2), 21.46.

HRMS *m/z* 220.1452. ($C_{12}H_{18}N_3O^+$ requires 220.1444).

2,3,5,6-tetrafluoro-*N*-4-(4-methyl-1-piperazinyl)phenyl-4-pyridinamine



103

A mixture containing pentafluoropyridine (0.52 mL, 5 mmol) and 1-(4-aminophenyl)-4-methylpiperazine (0.96 g, 5 mmol) was stirred in the presence of triethylamine (0.70 mL, 5 mmol) in acetonitrile (10 mL). The reaction mixture was then stirred at 0 ˚C for 8 hours. After the solvent evaporated, the mixture was diluted with deionized water (20 mL) and extracted with ethyl acetate (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether), the title compound was yielded as white colored solid (1.19 g, 3.5 mmol, 70%, m.p. 168 ˚C).

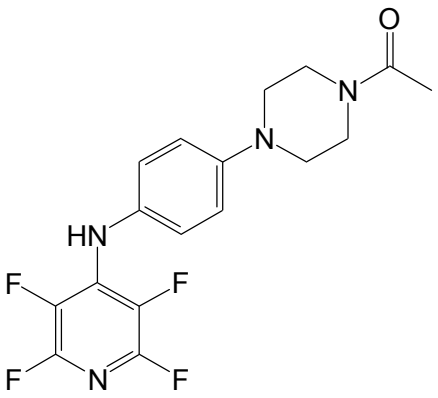
δ_H (400 MHz, $CDCl_3$) 7.05 (d, J = 9 Hz, 2H), 6.88 (d, J = 9 Hz, 2H), 6.25 (s, br, 1H), 3.20 (t, J = 5 Hz, 4H), 2.57 (t, J = 5 Hz, 4H), 2.34 (s, 3H). NH not shown in spectrum.

δ_C (100 MHz, $CDCl_3$) 149.72, 144.33 (d, J = 245 Hz, 2C), 135.42 (m, 1C), 134.2 (d, J = 250 Hz, 2C), 129.86, 124.6 (2C), 116.18 (2C), 55.12 (2C, CH_2), 49.08 (2C, CH_2), 46.23.

δ_F (376 MHz, $CDCl_3$) -159.53 (m, 2F), -94.56 (m, 2F).

HRMS *m/z* 341.1397. ($C_{16}H_{17}F_4N_4^+$ requires 341.1384).

Synthesis of 2,3,5,6-tetrafluoro-*N*-4-(4-acetyl-1-piperazinyl)phenyl-4-pyridinamine



104

A mixture containing pentafluoropyridine (1.04 mL, 10 mmol) and 4-(4-acetylpiperazin-1-yl)-aniline (2.14 g, 10 mmol) was stirred in the presence of triethylamine (1.40 mL, 10 mmol) in acetonitrile (20 mL). The reaction mixture was then stirred at 0˚C for 8 hours. After the solvent evaporated, the mixture was diluted with deionized water (20 mL) and extracted with ethyl acetate (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether), the title compound was yielded as white colored solid (2.76 g, 7.5 mmol, 75%, m.p. 194 ˚C).

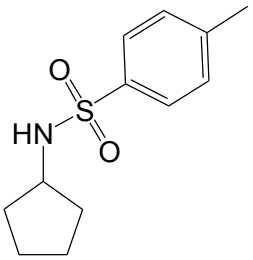
δ_H (400 MHz, $CDCl_3$) 7.07 (d, J = 9 Hz, 2H), 6.89 (d, J = 9 Hz, 2H), 6.24 (s, br, 1H), 3.77 (t, J = 5 Hz, 2H), 3.62 (t, J = 5 Hz, 2H), 3.16 (dt, J = 15, 5 Hz, 4H), 2.14 (s, 3H).

δ_C (100 MHz, $CDCl_3$) 169.14, 149.23, 144.33 (d, J = 240, 2C), 141.33, 135.21 (d, J = 242, 2C), 132.70 (m, 1C), 124.49 (2C), 116.89 (2C), 49.72 (CH_2), 49.39 (CH_2), 46.23 (CH_2), 41.53 (CH_2), 21.45.

δ_F (376 MHz, $CDCl_3$) -68.96 (m, 2F), -4.17 (m, 2F).

HRMS *m/z* 369.1341. ($C_{17}H_{17}F_4N_4O^+$ requires 369.1333).

Synthesis of *N*-cyclopentyl-4-methyl-benzenesulfonamide[87]



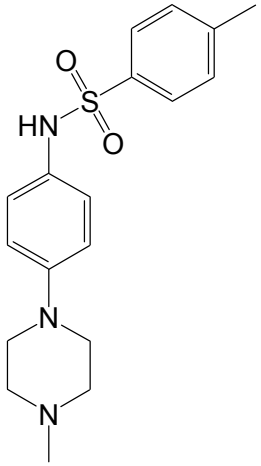
105

p-Toluensulfonyl chloride (3.81 g, 20 mmol) in pyridine (2.5 mL) were added to a diluted solution of cyclopentylamine (1.95 mL, 20 mmol) and potassium carbonate (5.52 g, 40 mmol) in dichloromethane (10 mL) drowsily. The reaction mixture was then stirred at 0˚C for 8 hours. The product was then extracted with dichloromethane (3 x 20mL). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether), the title compound as white fine powder (3.23 g, 13.5 mmol, 67.5%, m.p. 82 ˚C, literature[87] 83 ˚C).

δ_H (400 MHz, $CDCl_3$) 7.76 (d, J = 8 Hz, 2H), 7.27 (d, J = 8 Hz, 2H), 4.97 (s, br, 1H), 3.53 (apparent sextet, J = 6 Hz, 1H), 2.34 (s, 3H), 1.76-1.67 (m, 2H), 1.63-1.53 (m, 2H), 1.49-1.40 (m, 2H), 1.39-1.29 (m, 2H).

HRMS *m/z* 240.1052. ($C_{12}H_{18}NO_2S^+$ requires 240.1053).

Synthesis of 4-methyl-*N*-[4-(4-methylpiperazin-1-yl)phenyl]benzene-1-sulfonamide



106

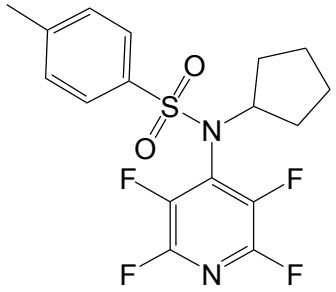
p-Toluensulfonyl chloride (0.95 g, 5 mmol) in pyridine (2.5 mL) were added to a diluted solution of 1-(4-aminophenyl)-4-methylpiperazine (0.96 g, 5 mmol) and potassium carbonate (1.38 g, 10 mmol) in dichloromethane (10 mL) dropwise. The reaction mixture was then stirred at 0 ˚C for 8 hours. The product was then extracted with dichloromethane (3 x 20mL). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether), the title compound was obtained as yellow oil (1.24 g, 3.6 mmol, 72%).

δ_H (400 MHz, $CDCl_3$) 7.57 (d, J = 7 Hz, 2H), 7.18 (d, J = 7 Hz, 2H), 6.94 (d, J = 9 Hz, 2H), 6.74 (d, J = 9 Hz, 2H), 3.19 (t, J = 5 Hz, 4H), 2.69 (t, J = 5 Hz, 4H), 2.43 (s, 3H), 2.35 (s, 3H). NH not observed in the spectrum.

δ_C (100 MHz, $CDCl_3$) 162.45, 143.15, 133.42, 133.25, 129.36 (2C), 127.15(2C), 123.89 (2C), 114.77 (2C), 55.78 ($2CH_2$), 48.08 ($2CH_2$), 46.17, 23.1.

HRMS *m/z* 346.1595. ($C_{18}H_{24}N_3O_2S^+$ requires 346.1584).

Synthesis of *N*-cyclopentyl-4-methyl-*N*-(2,3,5,6-tetrafluoropyridin-4-yl)benzene-1-sulfonamide



107

A mixture containing pentafluoropyridine (1.58 mL, 15 mmol) and *N*-cyclopentyl-4-methyl-benzenesulfonamide (3.23 g, 13.5 mmol) was stirred in the presence of potassium carbonate (2.76 g, 20 mmol) in acetonitrile (20 mL). The reaction mixture was then stirred at 0˚C for 8 hours. After the solvent evaporated, the mixture was diluted with deionized water (20 mL) and extracted with ethyl acetate (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether), the title compound was yielded as pale yellow solid (4.19 g, 11 mmol, 80%).

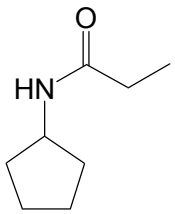
δ_H (400 MHz, $CDCl_3$) 7.74 (d, J = 8 Hz, 2H), 7.29 (d, J = 8 Hz, 2H), 3.57 (quintet, J = 6 Hz, 1H), 2.42 (s, 3H), 1.81-1.72 (m, 2H), 1.65-1.55 (m, 2H), 1.54-1.42 (m, 2H), 1.37-1.28 (m, 2H).

δ_C (100 MHz, $CDCl_3$) 144.32 (d, J = 252 Hz, 2C), 143.37, 137.86, 136.25 (m, 1C), 132.45 (d, J = 255 Hz, 2C), 127.75 (2C), 127.21 (2C), 55.23, 33.59 ($2CH_2$), 23.23 ($2CH_2$), 21.65.

δ_F (376 MHz, $CDCl_3$) -57.3 (m, 2F), -5.80 (m, 2F).

HRMS *m/z* 375.3786. ($C_{17}H_{17}F_4NO_2S^+$ requires 375.3824).

Synthesis of *N*-cyclopentyl-propanamide



108

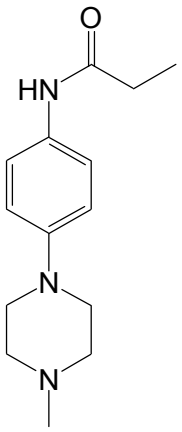
A mixture containing propionic anhydride (10.0 ml, 0.10 mmol) and cyclopentylamine (0.98 mL, 10 mmol) was stirred in the presence of potassium carbonate (2.76 g, 20 mmol) in dichloromethane (5 mL) at 0 ˚C under nitrogen protection. The reaction mixture was stirred for 2 hours. After the solvent evaporated, the mixture was washed with brine and stirred over magnesium sulfate. After filtration, the solvent was evaporated and the title compound was yielded as colorless liquid (1.34 g, 9.5 mmol, 95%).

ν_{max} (KBr, cm^{-1}) 3325 (NH), 1565 (CO).

δ_H (400 MHz, $CDCl_3$) 5.34 (s, br, 1H), 4.18 (heptet, J = 6 Hz, 1H), 2.15 (quartet, J = 7 Hz, 2H), 1.99-1.93 (m, 2H), 1.68-1.53 (m, 4H), 1.36-1.29 (m, 2H), 1.12 (t, J = 7 Hz, 3H).

HRMS *m/z* 142.2154. ($C_8H_{16}NO^+$ requires 142.2182).

Synthesis of *N*-[4-(4-methyl-1-piperazinyl)phenyl]-propanamide



109

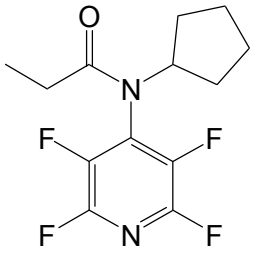
Propionic anhydride (3.88 mL, 30 mmol) in pyridine (0.25 mL) were added to a diluted solution of 1-(4-aminophenyl)-4-methylpiperazine (5.87 g, 31 mmol) and potassium carbonate (8.28 g, 60 mmol) in dichloromethane (20 mL) dropwise under nitrogen protection. The reaction mixture was then stirred at 0 ˚C for 8 hours. The product was then extracted with dichloromethane (3 x 20mL). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration, the solvent was evaporated and the title compound was obtained as pale yellow solid (6.68 g, 27 mmol, 90%, m.p. 218 ˚C).

δ_H (400 MHz, $CDCl_3$) 7.38 (d, J = 8 Hz, 2H), 7.02 (s, br, 1H), 6.88 (d, J = 8 Hz, 2H), 3.18-3.16 (m, 4H), 2.63-2.60 (m, 4H), 2.34 (s, 3H), 2.35 (quartet, J = 7 Hz, 2H), 1.23 (t, J = 7 Hz, 3H).

δ_C (100 MHz, $CDCl_3$) 171.80, 148.21, 130.57, 121.33 (2C), 116.80 (2C), 54.94 ($2CH_2$), 49.44 ($2CH_2$), 45.95, 30.70 (CH_2), 9.87.

HRMS *m/z* 248.1754. ($C_{14}H_{22}N_3O^+$ requires 248.1757).

Synthesis of *N*-cyclopentyl-*N*-(2,3,5,6-tetrafluoropyridin-4-yl)propanamide



110

A mixture containing pentafluoropyridine (1.58 mL, 15 mmol) and *N*-cyclopentyl-propanamide (2.12 g, 15 mmol) were stirred in the presence of sodium hydride (0.60 g, 15 mmol) in dichloromethane (20 mL). The reaction mixture was then stirred at 0 ˚C for 8 hours. After the solvent evaporated, the mixture was diluted with deionized water (20 mL) and extracted with dichloromethane (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether), the title compound was yielded as pale yellow solid (0.94 g, 11.7 mmol, 78%, m.p. 287 ˚C).

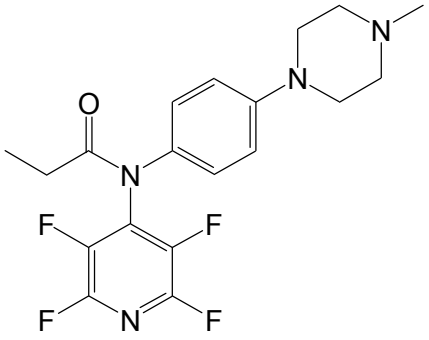
δ_H (400 MHz, $CDCl_3$) 5.35 (br, 1H), 4.20 (apparent sextet, J = 6 Hz, 1H), 2.15 (quartet, J = 7 Hz, 2H), 2.23-1.94 (m, 2H), 1.68-1.56 (m, 4H), 1.37-1.30 (m, 2H), 1.13 (t, J = 7 Hz, 3H).

δ_C (100 MHz, $CDCl_3$) 143.98 (d, J = 248 Hz, 2C), 142.54, 135.44 (m, 1C), 131.55 (d, J = 245 Hz, 2C), 55.15, 33.85 (2C, CH_2), 31.55 (CH_2), 23.15 (2C, CH_2), 9.68.

δ_F (376 MHz, $CDCl_3$) -63.86 (m, 2F), -7.85 (m, 2F).

HRMS *m/z* 291.1138. ($C_{13}H_{15}F_4N_2O^+$ requires 291.1118).

Synthesis of *N*-[4-(4-methylpiperazin-1-yl)phenyl]-*N*-(2,3,5,6-tetrafluoropyridin-4-yl)propanamide



111

A mixture containing pentafluoropyridine (1.58 mL, 15 mmol) and *N*-[4-(4-methyl-1-piperazinyl)phenyl]-propanamide (3.71 g, 15 mmol) were stirred in the presence of triethylamine (2.10 mL, 15 mmol) in acetonitrile (10 mL). The reaction mixture was then stirred at 0˚C for 8 hours. After the solvent evaporated, the mixture was diluted with deionized water (20 mL) and extracted with ethyl acetate (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether), the title compound was yielded as white colored solid (4.46 g, 11.2 mmol, 75%, m.p. 246 ˚C).

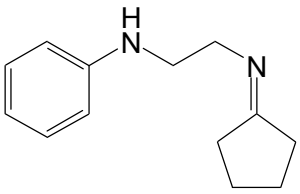
δ_H (400 MHz, $CDCl_3$) 7.37 (d, J = 8 Hz, 2H), 6.88 (d, J = 8 Hz, 2H), 3.16-3.14 (m, 4H), 2.58-2.55 (m, 4H), 2.35 (quintet, J = 7 Hz, 2H), 2.33 (s, 3H), 1.24 (t, J = 7 Hz, 3H).

δ_C (100 MHz, $CDCl_3$) 171.82, 148.11, 144.53 (d, J = 242 Hz, 2C), 137.44 (m, 1C), 130.95 (d, J = 246 Hz, 2C), 130.59, 121.28 (2C), 116.65 (2C), 55.09 (2C, CH_2), 49.54 (2C, CH_2), 46.13, 30.62 (CH_2), 9.57.

δ_F (376 MHz, $CDCl_3$) -80.36 (m, 2F), -43.26 (m, 2F).

HRMS *m/z* 397.3887. ($C_{19}H_{21}F_4N_4O^+$ requires 397.3893).

Synthesis of *N*-[2-(cyclopentylideneamino)ethyl]aniline

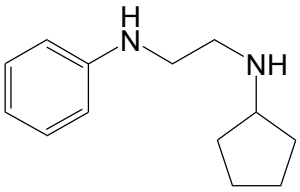


114

N-Pheylethylenediamine (1.31 mL, 10 mmol) and cyclopentanone (0.88 g, 10 mmol) was stirred in dichloromethane (40 mL) in the presence of magnesium sulphate (4.00 g) at room temperature for 8 hours. After filtration, the solvent was evaporated and the title compound was as pale yellow liquid (1.43 g, 7.00 mmol, 70%).

δ_H (400 MHz, $CDCl_3$) 7.19-7.12 (m, 2H), 6.70-6.65 (m, 1H), 6.64-6.61 (m, 2H), 4.10 (s, br, 1H), 3.17 (t, J = 5 Hz, 2H), 2.87 (t, J = 5 Hz, 2H), 1.83-1.70 (m, 4H), 1.63-1.54 (m, 2H), 1.35-1.25 (m, 2H).

Synthesis of N^1-cyclopentyl-N^2-phenyl-1,2-ehanediamine



112

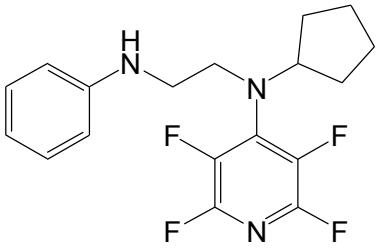
A mixture containing *N*-[2-(cyclopentylideneamino)ethyl]aniline (2.53 g, 12.3 mmol) and sodium borohydride (0.49 g, 13 mmol) was stirred in ethanol (30 mL) at room temperature for 8 hours. The mixture was diluted with deionized water (20 mL) and extracted with ethyl acetate (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration, the solvent was evaporated and the title compound was yielded as pale yellow liquid (1.90 g, 9.40 mmol, 76%).

δ_H (400 MHz, $CDCl_3$) 7.18 (t, J = 8 Hz, 2H), 6.71 (t, J = 8 Hz, 1H), 6.64 (d, J = 8 Hz, 2H), 4.11 (s, br, 1H), 3.21 (t, J = 5 Hz, 2H), 3.08 (apparent sextet, J = 6 Hz, 1H), 2.85 (t, J = 5 Hz, 2H), 1.90-1.81 (m, 2H), 1.75-1.65 (m, 2H), 1.60-1.50 (m, 2H), 1.38-1.28 (m, 2H).

δ_C (100 MHz, $CDCl_3$) 150.45, 132.44 (2C), 118.08, 114.65 (2C), 59.76, 47.55 (CH_2), 45.05 (CH_2), 33.12 (2C, CH_2), 24.22 (2C, CH_2).

HRMS *m/z* 205.1701. ($C_{13}H_{21}N_2^+$ requires 205.1699).

Synthesis of N^1-cyclopentyl-N^2-phenyl-N^1-(2,3,5,6-tetrafluoropyridin-4-yl)ethane-1,2-diamine



115

A mixture containing pentafluoropyridine (0.52 mL, 5 mmol) and N^1-cyclopentyl-N^2-phenyl-1,2-ehanediamine (1.02 g, 5 mmol) was stirred in the presence of sodium hydride (0.60 g, 15 mmol) in dichloromethane (20 mL). The reaction mixture was then stirred at 0˚C for 8 hours. After the solvent evaporated, the mixture was diluted with deionized water (20 mL) and extracted with dichloromethane (20 mL x 3). The organic layer was washed with brine and stirred over magnesium sulfate. After filtration and purification via column chromatography (elution with petroleum ether), the title compound was yielded as white solid (1.27 g, 35 mmol, 70%, m.p. 162 ˚C).

δ_H (400 MHz, $CDCl_3$) 9.48 (s, br, 1H), 7.24-7.18 (m, 2H), 6.79-6.76 (m, 3H), 3.65 (t, J = 6 Hz, 2H), 3.43-3.34 (m, 1H), 3.23 (t, J = 6 Hz, 2H), 2.09-2.00 (m, 2H), 1.94-1.80 (m, 4H), 1.64-1.51 (m, 2H).

δ_C (100 MHz, $CDCl_3$) 151.64, 150.45, 144.12 (d, J = 248 Hz, 2C), 135.43 (m, 1C), 130.45 (d, J = 250 Hz, 2C), 129.27 (2C), 117.94, 116.73 (2C), 59.05, 46.55 (2C, CH_2), 45.44 (2C, CH_2), 35.85 (2C, CH_2), 23.15 (2C, CH_2).

δ_F (376 MHz, $CDCl_3$) -90.67 (m, 2F), -20.45 (m, 2F).

HRMS *m/z* 354.1572. ($C_{18}H_{20}F_4N_3^+$ requires 354.1588).

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