Synthesis of Fluorinated Drug Scaffolds using S_NAr Substitution Reactions

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Contents

Abbreviations

b.p.	Boiling point
d	Doublet splitting pattern
DCM	Dichloromethane
DNA	2-Deoxyribonucleic acid
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
eq.	Equivalent
GC-MS	Gas chromatography–mass spectrometry
HOMO	Highest occupied molecular orbital
HPLC	High-performance liquid chromatography
5-HT_{1D}	5-Hydroxytryptamine receptor 1D
Hz	hertz
IR	Infra-red
J	Coupling constant
MCF-7	Michigan Cancer Foundation-7
m.p.	Melting point
MS	Mass spectrometry
m/z	Mass to charge ratio
NMR	Nuclear magnetic resonance
P	Partition coefficient
ppm	Parts per million
q	Quartet splitting pattern
r.t.	Room temperature
s	Singlet splitting pattern
t	Triplet splitting pattern
THF	Tetrahydrofuran
THP-1	Human leukemia monocytic cell line
TLC	Thin layer chromatography
UV/vis	Ultraviolet–visible

Abstract

Fluorinated arenes are considered valuable in organic chemistry. They display different types of reactivity and physicochemical properties compared to their hydrogen analogues. In this project, our medicinal chemistry programme focused on developing rapidly accessible and modifiable heterocyclic scaffolds. Different classes of fluorinated heteroatom-containing organic compounds including benzothiophenes, (aza)phenoxazines and benzaldehyde phenylhydrazones were synthesised from highly fluorinated aromatic compounds with a diverse range of functional groups appropriate for medicinal chemistry development.

Mechanistic studies for heterocyclic scaffold synthesis were discussed in the project. The mechanisms of the ring-forming reactions were elaborated in detail in each chapter. A range of substituents were introduced flexibly into the aromatic heterocycles, which were designed to meet the requirements for biological screening programmes. New compounds were characterized by ^{1}H, ^{19}F and ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis. The X-ray crystal structures of a fluorinated benzothiophene and two benzopyridooxazine derivatives were obtained confirming the structure and substitution pattern.

From the heterocyclic scaffolds prepared, 6-benzimidazol-1-yl-benzothiophene derivatives (**91**), 3-imidazol-1-yl-pyridobenzoxazine derivatives (**130**) and 4-1-methylpiperazinyl-benzaldehyde phenylhydrazone derivatives (**195**) acted as hit compounds and demonstrated significant trypanocidal activities. SAR studies were employed in structural modifications on these samples to search for the best activities with highest selectivity.

Chapter 1. Introduction

Background

Fluorine is by no means a rare element, being 13th in order of abundance of the elements in the Earth's crust.[1] However, among countless natural products, only a handful of fluorinated organic compounds have been discovered and most of these are metabolites in tropical plants, which shows Nature has a limited ability to generate organofluorine compounds.[2] Since the late 1940s, the number of methods available for incorporating fluorine into organic compounds has increased through numerous chemists' efforts.[3] Organofluorine compounds nowadays are widespread in nearly all parts of our daily life.[4]

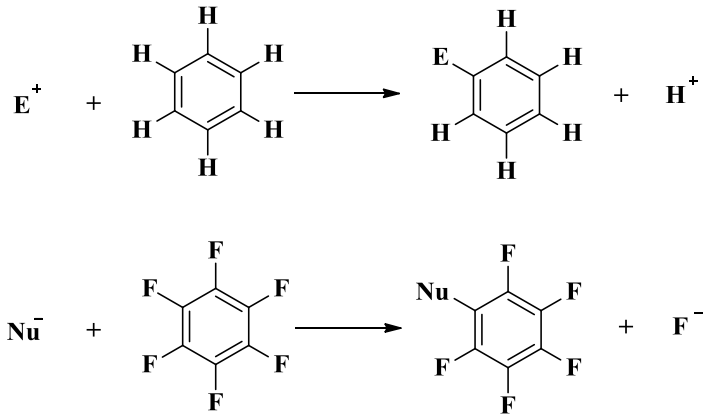
Fluorine is the element of extremes. Fluorine substituents can contribute to a subtle change in molecular conformation or reactivity. Organofluorine compounds often demonstrate unexpected properties and behaviours in comparison with their non-fluorinated parent compounds.[5] In drug design, incorporating fluorine in a molecule can improve metabolic stability due to the significant strength of the carbon-fluorine bond (*ca.*480 kJmol^{-1}).[6] Organofluorine chemistry now is a hot and attractive topic to chemists in a variety of fields.

1.1 Fluorine-Substituent Effects on the Properties of Fluorinated Aromatic Compounds

1.1.1 Electronic effect on fluorinated aromatic compounds

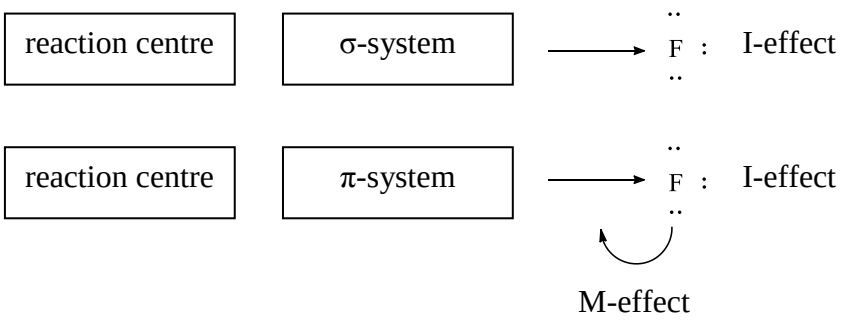
The fluorine atom possesses the strongest attraction to the electron among all elements. Fluorine is recognised as the most electronegative element in the Periodic Table (4.0 on the Pauling Scale),[5] since electronegativity describes the tendency of an atom to attract electrons and sequences the electron-withdrawing ability of an atom.[7] The presence of fluorine in a compound usually contributes to a significant change in the molecule's electron density, and it therefore affects the chemical reactivity of an organic molecule.

In general, fluorine in a perfluoroalkyl or aryl group usually shifts electrons from adjacent atoms to the fluorine atom.[8] The electron density of the molecule is concentrated around the fluorine atom, which makes the neighbouring atom, or atoms, relatively electron-poor. It is caused by this inductive effect. For fluorinated arenes, the strongly electron-withdrawing inductive effect caused by fluorine activates nucleophilic reactions on the reaction centre in fluoroarenes.[9] This is different to the common electrophilic substitution observed on the analogous hydrogen-containing aromatic rings (**Scheme 1**).



Scheme 1: Reaction of electrophilic aromatic substitution and nucleophilic aromatic substitution.[9][10]

On the other hand, for arenes, which consist of a conjugated planar ring system, although introduction of fluorine onto an aromatic compound renders the molecule electron-deficient as the π electron density is withdrawn by the fluorine atoms, the lone pair electrons in a p-orbital on a fluorine substituent also conjugate with the π electron cloud on the aromatic ring, which shifts electron density back into aromatic ring. Thus, there are always both inductively electron-withdrawing and π-electron-donating resonance effects occurring on fluorinated aromatic compounds to consider (**Scheme 2**).[9]



Scheme 2: Transmission of electronic effect to the reaction centre by fluorine.[9]

1.1.2 Acidity of fluorinated arenes

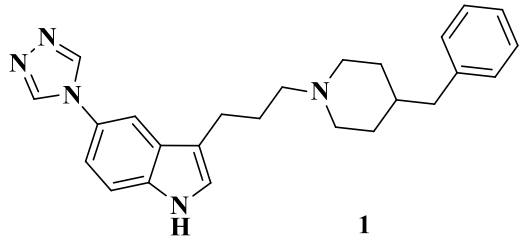
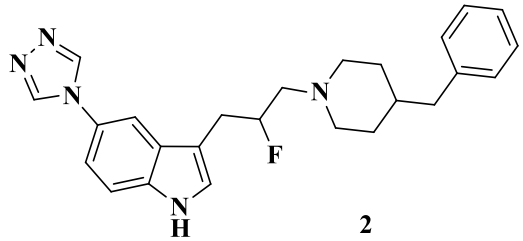
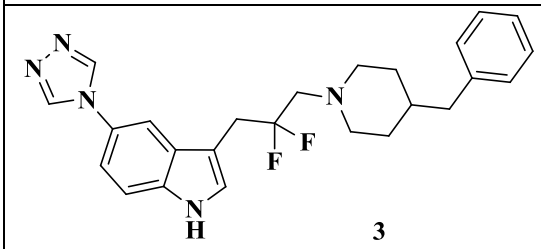
With its strong ability to withdraw electron density, fluorine has very strong effect on the acidity and basicity of adjacent functional groups. It has been reported that the acid strengths of perfluoro derivatives of aromatic and heterocyclic compounds are normally enhanced compared with their parent compounds.[8] For instance, the pK_a value for phenol is 9.8 while the value for its fluorinated analogue compound it is 5.5. Perfluorination of phenol contributes to around a 4.5 pK_a units' reduction compared with its parent compound.[11]

In terms of medicinal chemistry, the perturbation of pK_a usually has a significant impact on the pharmacokinetics properties of a molecule and its binding affinity. In a series of piperidinyl indoles investigated by van Niel *et al.*, [12] it was found that with sequential fluorine incorporation, the pK_a values of the ammonium form of compounds were reduced through decreasing the basicity of the amine. This reduction of basicity, with concomitant weakening of the affinity to the receptor (5-HT_{1D}), had a strong beneficial effect on oral absorption.

The non-fluorinated parent compound **1** is a potent receptor ligand, but has a low bioavailability (**Table 1**). When one fluorine atom was introduced, the mono-fluorinated compound **2** shows a lower pK_a that is still compatible with the requirements for receptor

binding, but now results in a compound of considerably increased bioavailability. Through further introduction of fluorine, the pK_a value for the difluoro compound **3** fell to 6.7. However, this compound is no longer basic enough to achieve high binding affinity for the $5HT_{1D}$ receptor.[13] Although generally beneficial in a series of structurally related compounds, the effect of fluorine substitution on oral bioavailability cannot always be accurately predicted.[14]

Table 1: Basicity and bioavailability in a series of piperidinyl indoles.[12]

Structure	IC_{50}	pK_a	Bioavailability
 **1**	0.3 nM	9.7	poor
 **2**	0.9 nM	8.7	medium
 **3**	78 nM	6.7	-

1.1.3 Lipophilic effect of fluorine-containing groups

Lipophilicity is usually related to the ability of chemical compounds to dissolve in non-polar solvents, and it demonstrates an important chemical character, particularly in medicinal chemistry.[7] For the design of medicines, lipophilicity controls many parameters such as absorption, biological barrier passage and interaction with the macromolecular target.[15]

Generally, the presence of fluorine or a fluorinated group is considered to enhance the lipophilicity of an organic compound.[11] Fluorinated arenes are indeed more lipophilic than their non-fluorinated counterparts as perfluorination, polyfluorination and fluorination adjacent to atoms with π-bonds (except for some α-fluorinated carbonyl compounds) increase the lipophilicity on fluorinated aromatic compounds.[16] The excellent overlap between the fluorine 2s or 2p orbitals with the corresponding orbitals on carbon renders the carbon-fluorine bond highly non-polarisable. The lower polarisability of the C–F bond consequently is regarded to result in increasing lipophilicity on aromatic compounds.[14]

An example showing the increase in the lipophilicity for aromatic compounds is the comparison between fluorobenzene and benzene. Fluorobenzene is known to be more lipophilic than benzene, with the log P value in the octanol/water system for fluorobenzene being 2.46 while that for benzene is 2.29.[17] There is a slight increase in lipophilicity as further fluorine atoms are introduced into a molecule. In a similar manner, 4-trifluoromethylphenol is more lipophilic than its corresponding non-fluorinated compound, 4-methylphenol (**Table 2**).[17]

Table 2: The effect of fluorine substitution on lipophilicity (log PNalk) of arenes.[17]

Compounds	Log P
Fluorobenzene	2.46
Benzene	2.29
4-Trifluoromethylphenol	-0.15
4-Methylphenol	-0.19

However, the introduction of fluorine atoms into an aliphatic molecule sometimes provokes a decrease in the lipophilicity. This was postulated to be due to the strongly electron-withdrawing capabilities of the fluorine.[18] For instance, in aliphatic systems, ethanol (log P = -0.32) is less lipophilic than trifluoroethanol (log P = 0.36). The strong electron-withdrawing effect of trifluoromethyl moiety significantly decreases the basicity of the hydroxyl group. But, this strong inductive effect by the trifluoromethyl group extends up to three CH_2 moieties inserted and there is an obvious difference when the number is beyond four as the inductive effect does not affect the basicity of hydroxyl group (**Table 3**).[11] It is

revealed by the fact that the log P (octanol–water partition) value for 1-pentanol is 1.40, while that for 5,5,5-trifluoropentan-1-ol is 1.15.[18]

Table 3: log P values of straight-chain alkanols.[11]

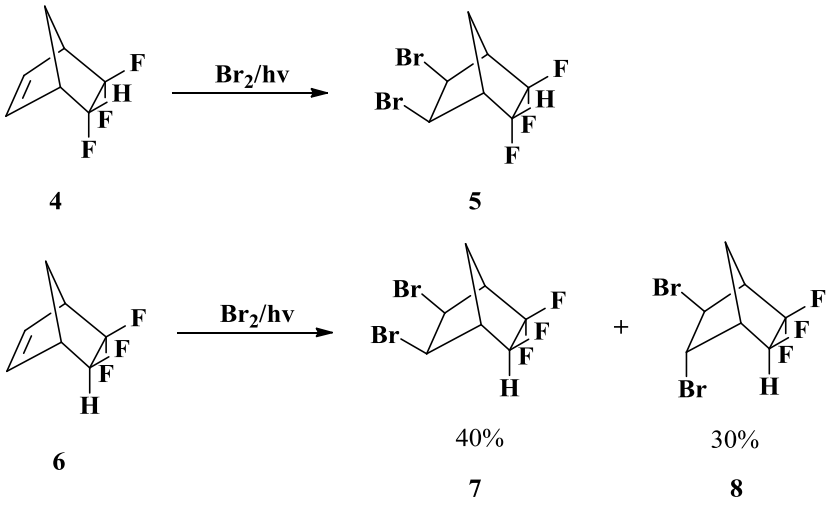
Alcohol	Log P (X=H)	Log P (X=F)
CX_3CH_2OH	-0.32	0.36
$CX_3(CH_2)_2OH$	0.34	0.39
$CX_3(CH_2)_3OH$	0.88	0.90
$CX_3(CH_2)_4OH$	1.40	1.15
$CX_3(CH_2)_5OH$	2.03	1.14

1.1.4 Steric effects of fluorine and fluorine-containing groups

Frequently, fluorine is introduced as an isostere of hydrogen in biomolecules. The size of fluorine also plays an important role in the changes of the molecular conformation besides the electronegativity of fluorine. In terms of size, the van der Waals radius for fluorine is 1.47 Å, which lies between that of hydrogen (1.20 Å) and oxygen (1.57 Å).[16] Despite the slight difference in size, the C-F bond can often replace and mimic the C-H bond with minimal steric consequences.[19]

In practice, fluorine substitution always increases the steric size of alkyl groups. For instance, the steric volume of a trifluoromethyl group is larger than that of a methyl group, and is at least as bulky as an ethyl [20] or isopropyl group.[8] Thus, the effect of fluorine substitution on molecular conformation is quite subtle and sometimes difficult to predict. Methoxybenzenes, for example, usually favour a planar conformation. However, Böhm *et al.*[13] investigated the structure of trifluoromethoxybenzenes searched from the Cambridge Structural Database (CSD) and found the trifluoromethoxyl group lies out of the plane of the phenyl ring. It is due to the steric hindrance effect by the trifluoromethoxyl group.

The nature of the transition state for a chemical or physical process is also affected by steric effects during the reaction. Although the steric size of fluorine is small, it sometimes controls the stereo- and regiochemistries in reactions. Examples of fluorine steric effects can occur in a chemical reaction with two different dynamic processes. *Endo*-trifluoronobornene **4** provides a *cis*-dibromide **5** as the sole product on photolysis with bromine. The first and second bromines approach *endo*-trifluoronorbornene **4** from the less hindered direction, although *trans*-addition of bromine to norbornene is a normal radical reaction pathway. However, in contrast, *exo*-trifluoronorbornene **6** gives a mixture of stereoisomers, the *cis*-dibromide **7** and the *trans*-bromide **8** (**Scheme 3**).[9] The configurations of the final products from the reaction obviously are controlled by the influence of the fluorine.



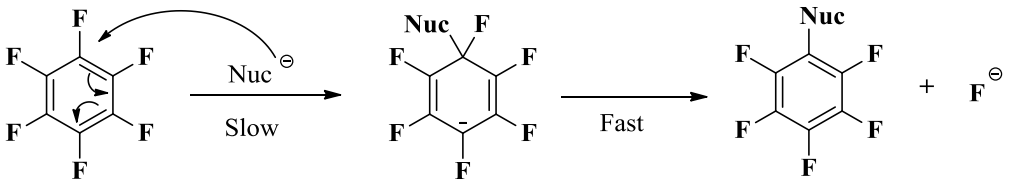
Scheme 3: Different outcome from the photochemical dibromination of trifluoronorbornene.[9]

1.2 Nucleophilic Substitution Reactions

1.2.1 Mechanism of Reactions Induced by Fluorine on Aromatic Rings

Due to its specific electronic characteristics, fluorine allows nucleophilic attack on the aromatic ring, which is hard to effect on hydrogen-based aromatic rings. Thus, fluorinated arenes provide new approaches to the synthesis of novel compounds using nucleophilic substitution.[3]

The mechanisms of the nucleophilic substitution reactions on aromatic compounds are assumed to proceed via a tandem two-step addition-elimination process in the replacement of fluorine from the highly fluorinated aromatic system. Such compounds are firstly susceptible to add nucleophilic reagents on the nucleus of the highly fluorinated aromatic ring and form an intermediate complex on the reaction path. A resonance-stabilized carbanion with a new carbon–nucleophile bond in the intermediate complex called a Meisenheimer complex is formed. Then aromatisation takes place by elimination, as a fluoride ion leaves from the nucleus of the ring in a second step (**Scheme 4**).[21][22]



Scheme 4: Mechanism of the nucleophilc aromatic substitution reaction.[22]

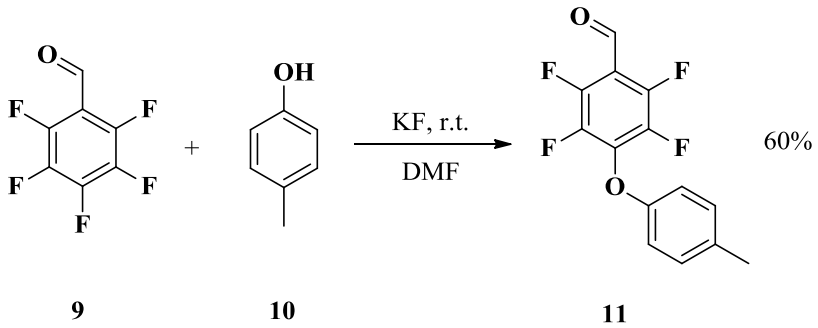
Usually, the formation of the resonance-stabilised Meisenheimer complex is quite slow. This is because the complex usually has a higher energy state than the aromatic reactant. However, the loss of the leaving group is generally very fast, since the complex tends to revert to the aromatic form.[22]

1.2.2 Orientation of Reactions Induced by Fluorine on Perfluorinated Six-Membered Arenes

There is a significant difference in the proportions of *ortho*-, *meta*- and *para*- substitution of fluorine by nucleophiles in the nucleus of polyfluoro-aromatic compounds. Rationalisation of the observed selectivity can be based on several considerations and substitution reactions for fluorinated arenes usually take place in the *para*-position to the first substituent added.[23]

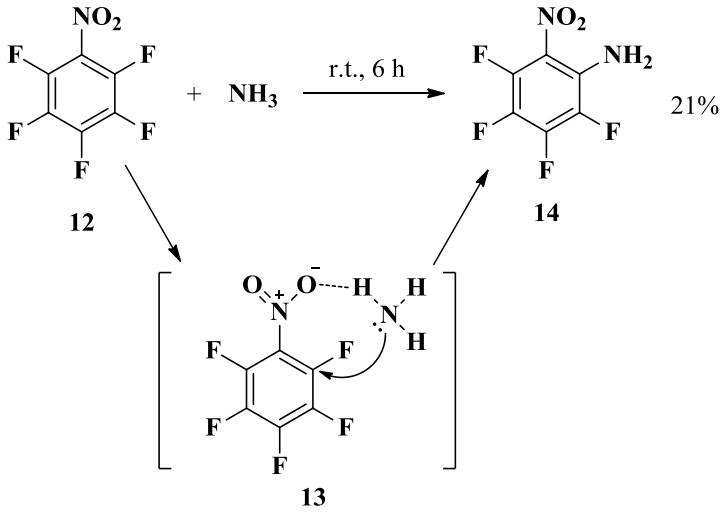
During the process of the nucleophilic aromatic substitution reaction in perfluorinated six-membered aromatic heterocycles, the negative charge of the intermediate adduct is formed and needs to be stabilised. This stabilisation occurs mostly as a result of the combined inductive effects of the fluorine atoms. This inductive effect should cover the concomitant strongly destabilising p–π repulsion of the sp^2-bound fluorine and which is most significant in the position *ortho*- and *para*- to the site of nucleophilic attack. Thus, for a six-membered arene, it is considered that fluorine in the *ortho*-position is most effective for overall stabilisation of the negatively charged intermediate, while *meta*-fluorine is less effective and *para*-fluorine is the least effective.[23] Hence, nucleophilic attack usually takes place at the *para*-position in fluorinated arenes.

A good example illustrates nucleophilic substitution occurring in pentafluorobenzaldehyde (**9**) with 4-methylphenol (**10**) at the *para*-position (**Scheme 5**). 4-Methylphenol acted as nucleophile to attack pentafluorobenzaldehyde at the position *para* to aldehyde group, which provided the sole product **11** with a yield of 60% after crystallisation.[24]



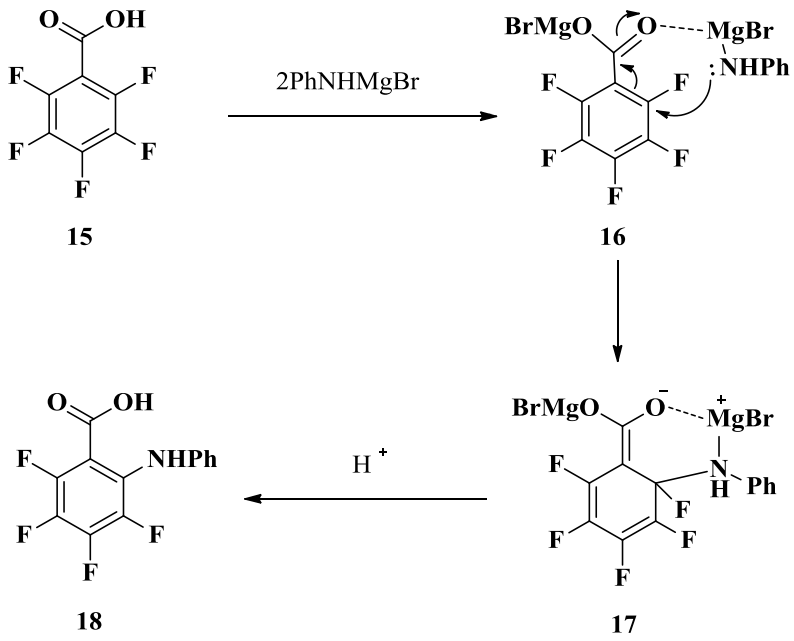
Scheme 5: Reaction of pentafluorobenzaldehdye and 4-methylphenol with *para*-substitution.[24]

Although the major substitution product from the vast number of fluorinated six-membered arenes reacting with nucleophiles is the *para*-isomer irrespective of whether the first substituent is electron-withdrawing or electron-donating, there are some notable exceptions where *ortho*-substitution predominates in the reaction caused by a specific interaction between the substituent in the fluorinated arene and the attacking nucleophile. Brooke *et al.*[25] first reported the predominant *ortho*-regioselectivity in the reaction of pentafluoronitrobenzene (**12**) with ammonia in ether in 1961 (**Scheme 6**). *ortho*-Regioselection is considered to be due to hydrogen bonding between the nucleophile and substituent in a cyclic transition state.



Scheme 6: Reaction of pentafluoronitrobenzene with ammonia.[25]

Many further examples of the enhanced substitution of *ortho*-fluorine in compounds C_6F_5X were subsequently observed.[26] For *ortho*-substitution, it usually requires a specific coordinating substituent in the reactant and the highest yields of *ortho*-isomers obtained are expected in non-polar solvents with metals cations possessing the highest complexing ability when the nucleophile is an anion. An example illustrating a reaction of this type is that of pentafluorobenzoic acid (**15**) with a magnesium amide, which generally undergoes *ortho*-substitution (**Scheme 7**).[27]



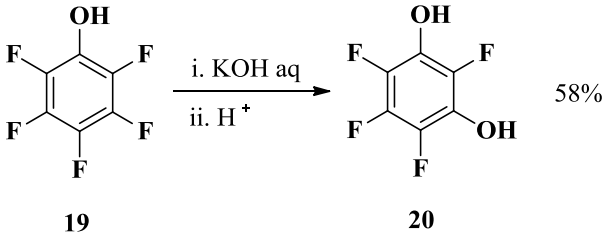
Scheme 7: Reaction of pentafluorobenzoic acid and a magnesium amide via *ortho*-substitution.[27]

From the reaction, a cyclic transition state is formed involving the magnesium cation and the *ortho*-fluorine is substituted by the amide anion. Preservation of the carbonyl-containing group seems to be caused by its low reactivity due to the steric influence of *ortho*-fluorine atoms, and that it will exist as the carboxylate salt in the reaction mixture. In fact, this reaction is considered as the best method of preparation of otherwise difficult to access *o*-alkyl- and *o*-aryltetrafluorobenzoic acids from pentafluorobenzoic acid.[26]

Substitution at the *meta*-Position on fluorinated arenes is the least common but sometimes occurs in a reaction under vigorous conditions with a powerful electron-donating group like

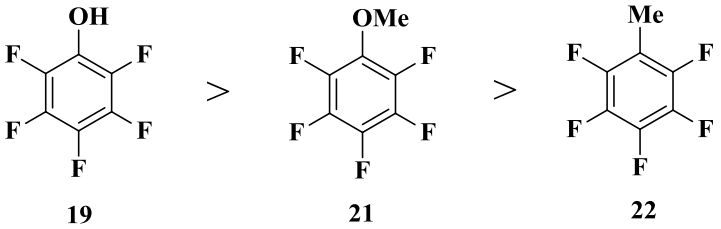
hydroxyl group on the aromatic ring.[28] The strongly electron-donating group provides an electronic effect on the nucleus of the aromatic ring due to conjugation of the π-electrons on the substituent. It was considered as the reason that *meta*-substitution can take place exclusively on the aromatic ring. Thus, certain substituents are usually required with a special interaction to form meta-substituted compounds.

For instance, the reaction of pentafluorophenol (**19**) with potassium hydroxide takes place with *meta*-substitution as shown in **Scheme 8**.[29] The hydroxyl group is an electron-donating group and will be ionised under basic conditions. The oxy-anion substituent on the aromatic ring renders the fluorine substituents at the *meta*-positions less deactivated during the substitution compared with the *ortho*- and *para*- positions. It leads to *meta*-substitution as the predominant process.[27][28]



Scheme 8: Reaction of pentafluorophenol and potassium hydroxide via *meta*-substitution.[29]

Budron *et al.*[28] has investigated nucleophilic substitution reactions of pentafluorotoluene, pentafluoroanisole and pentafluorophenol in order of increasing electron donor capacity of the substituent in the fluorinated arenes with the same nucleophiles (**Scheme 9**).



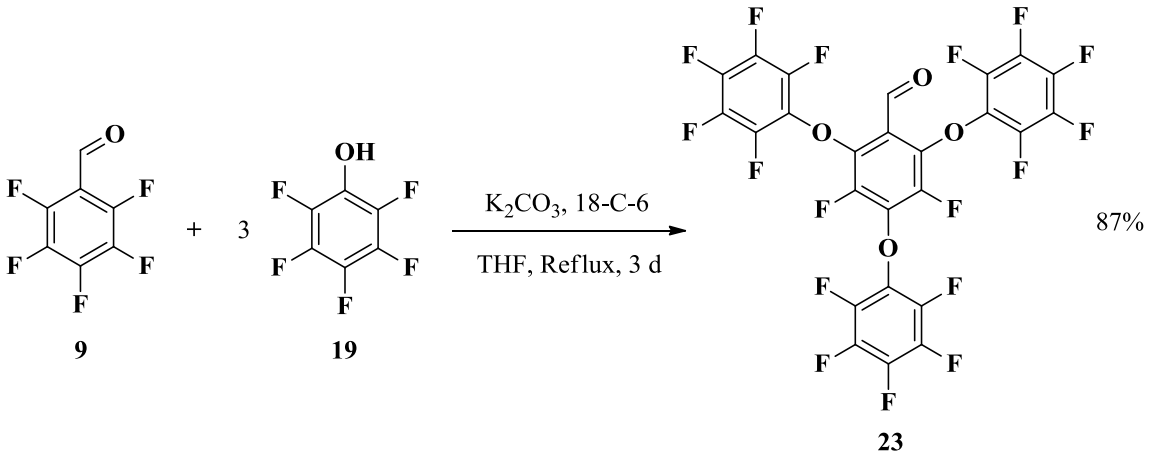
Scheme 9: The increasing propensity to give *meta*-substitution.[28]

From this study, it illustrated that for pentafluorotoluene, the methyl group is not a sufficiently powerful electron donor to overcome the *para*-directing effect of the five fluorine atoms and only *para*-replacement was observed in the reaction, although it does obviously slow the rate of the reaction. Much more strongly electron-donating groups like methoxyl and hydroxyl groups as the substituents overcome the *para*-effect partly or completely on the per-fluorinated arene. Reaction of pentafluoroanisole and sodium methoxide or methyllithium gives a *meta*/*para* replacement ratio of 7:12, while pentafluorophenol provides the *meta*-isomer as the sole product. After investigation, the authors concluded that the much more powerful electron-donating group like O^- group overcomes the *para* effect well, and is the most exclusively *meta*-directing substituent in the polyfluoro-aromatic field.[28]

1.2.3 Poly-substitution on perfluorinated six-membered arenes

For perfluorinated arenes, more than one substitution is usually possible. However, as most groups introduced via nucleophilic substitution are generally electron-donating, the first substituent added to the fluorinated aromatic ring deactivates the ring towards further attack, the next nucleophilic attack is usually more difficult compared with the first.[21]

In most examples of poly-substitution, the nucleophile usually attacks the *ortho*-site after the *para*-position has been substituted, as the *ortho*-position is more susceptible to attack compared with the *meta*-position due to the electronic effect on the perfluorinated arene.[21][23] A good example is the trisubstitution on pentafluorobenzaldehyde illustrated in **Scheme 10**.[30] The structure of trisubstituted derivative of pentafluorobenzaldehyde with pentafluorophenol shows the sequence of the substitutions. Both *ortho*-position substitutions take place after the *para*-position has been attacked. The reaction used 18-crown-6 together with potassium carbonate to generate a more powerful nucleophile as the 18-crown-6 complexes to the K^+ cation which increases the nucleophilicity of the phenoxide. The reaction required heating in refluxing THF for 3 days, which confirmed the second and third nucleophilic attacks are more difficult than the first one even though the aldehyde carbonyl group provides further stabilisation to the Meisenheimer intermediate.



Scheme 10: Reaction of pentafluorobenzaldehyde and pentafluorophenol.[30]

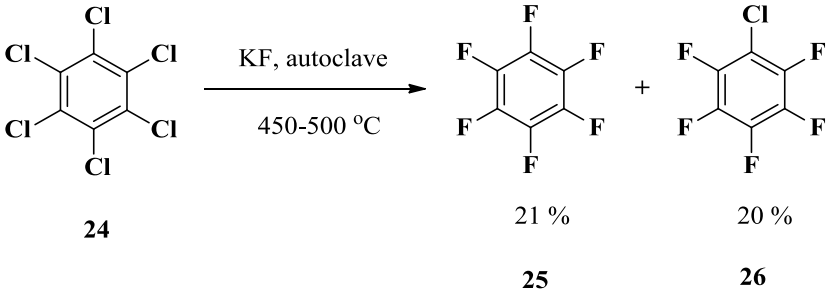
Polysubstitution substitution is very important in organofluorine chemistry, for the introduction of different functional groups into perfluorinated arenes. Especially, polysubstitution provides a large source of heterocyclic scaffolds, and different types of reaction are also possible on the resulting fluorinated hetero-cycles.

1.2.4 Synthesis of perfluorinated arenes

Perfluorination describes the exhaustive replacement of all hydrogen atoms in an organic system by fluorine.[31] It is usually not possible to synthesise perfluoroarenes directly from arenes.

The standard route for the synthesis of perfluorinated heteroaromatic compounds most commonly proceeds via halogen exchange of the corresponding perchlorinated system utilising molten alkali metal-fluoride at very high temperature in an autoclave and in the absence of solvent (**Scheme 11**).[32] This method was first developed by Russian workers (Vorozhtsov *et al.*) in 1963 to synthesise hexafluorobenzene (**25**) as reported by Brooke.[27] The synthetic route is generally via the reaction of potassium fluoride with hexachlorobenzene at around 400-450 °C. The approach provides a good yield compared

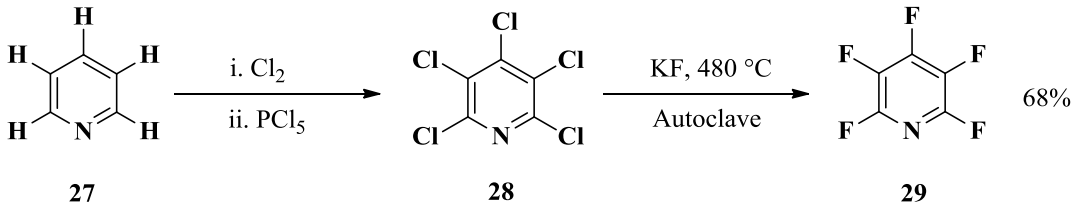
with other methods attempted before, especially for nitrogen-containing perfluoroheteroaromatic systems. [27][32]



24 25 26

Scheme 11: Synthesis of hexafluorobenzene from hexachlorobenzene.[27]

For example, pentafluoropyridine (**29**) is a widely used nitrogen-containing perfluorinated aromatic compound and is readily prepared from pyridine (**27**) using this method. Pentachloropyridine (**28**) is first formed from reacting pyridine with PCl_5 and then gives a high yield of pentafluoropyridine on reaction with potassium fluoride at 480 °C in the absence of solvent using the aforementioned methodology (**Scheme 12**).[32] The temperature of the reaction is suggested to affect the number of halogens exchanged on the aromatic ring, which is the reason why procedures require such a high temperature to ensure complete substitution.



27 28 29

Scheme 12: Synthesis of pentafluoropyridine from pyridine.[32]

1.3 Applications of fluorinated aromatic compounds to pharmaceuticals

Fluorinated arenes currently have widespread use in different fields of industry. The small atomic radius, high electronegativity, and low polarisability of the C-F bond are among the special properties that render fluorine so attractive. These atomic properties translate extensively into equally appealing attributes of fluoroorganic compounds. The introduction of fluorine-containing substituents in structures of known activity has been an important strategy for optimising the properties of pharmaceutical products.[33] Associated with the replacement of a C-H or C-O bond with a C-F bond, some of the properties in biologically active compounds are improved such as higher metabolic stability, increased binding to target molecules, and increased lipophilicity and membrane permeability.[34] Nowadays, about 20-25% of pharmaceuticals used for medical treatment contain a fluorine atom.[33] These statistics make fluorine the "second-favourite heteroatom" after nitrogen in drug design.[34]

1.3.1 Anti-cancer agents containing fluorine

Around the world, tremendous resources are being invested in the prevention, diagnosis, and treatment of cancer. Cancer is currently the second leading cause of death in Europe and North America, thus the discovery and development of anticancer agents has become the key focus of several pharmaceutical companies as well as non-profit government organisations.[35]

1.3.1.1 Chemotherapy and 5-Fluorouracil

Fluorinated anti-cancer agents have been developed as good therapies for targeting cancer. Many fluorinated anti-tumour agents are currently coming onto market. 5-Fluorouracil (**30**), an anticancer drug, was one of the first and is one of the most important examples in

medicinal chemistry.[36] In 1957, Heidelberger *et al.*[36] succeeded in synthesising 5-fluorouracil as an antimetabolite (**Figure 1**). Currently, 5-fluorouracil is used in the treatment of skin cancer and some solid tumours like breast and gastric cancer.[37] It has been listed on the World Health Organisation's List of Essential Medicines.[38]

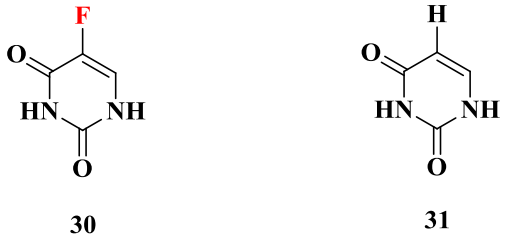
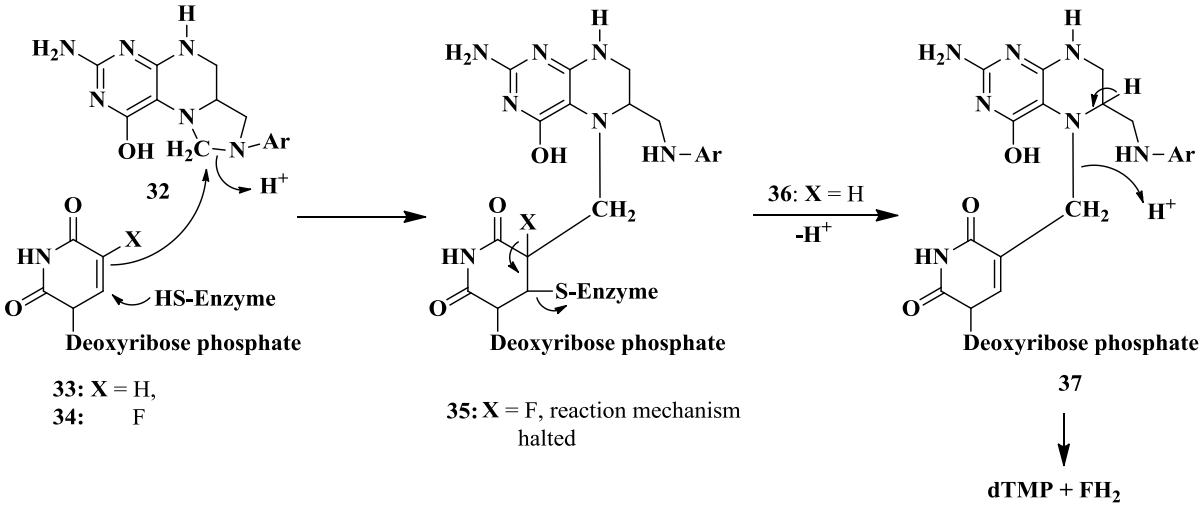


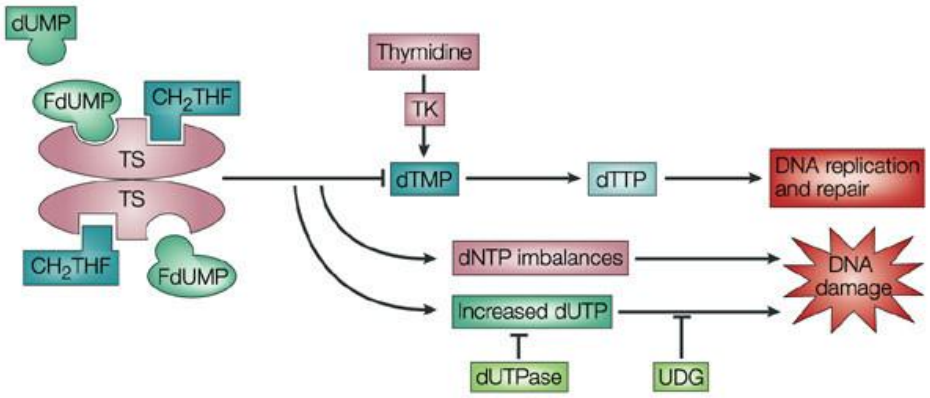
Figure 1: Structures of 5-fluorouracil and uracil.[36]

5-Fluorouracil is a suicide inhibitor which acts through irreversible inhibition of thymidylate synthase (TS) using competitive binding.[39] Thymidylate synthase (TS) is an enzyme which catalyses the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) using 5,10-methylene tetrahydrofolate (CH_2THF) as the methyl donor.[40] The fluorouracil skeleton remains covalently and irreversibly bond to the active site in thymidylate synthase, which finally results in the termination for the synthesis of thymidine (**Scheme 13**).[41]



Scheme 13: Use of 5-fluorouracil as a prodrug for a suicide substrate.[41]

Since dTMP is one of the crucial nucleotides for the early stages of DNA biosynthesis, DNA damage results when the activity of thymidylate synthase is inhibited, and replication and cell division are consequently blocked.[39] Thus, thymidylate synthase inhibitors are important targets for the development of cytotoxic agents. Administration of 5-fluorouracil results in a shortage of dTMP, which leads to cancerous cells suffering apoptosis because of thymineless death (**Scheme 14**).[42]



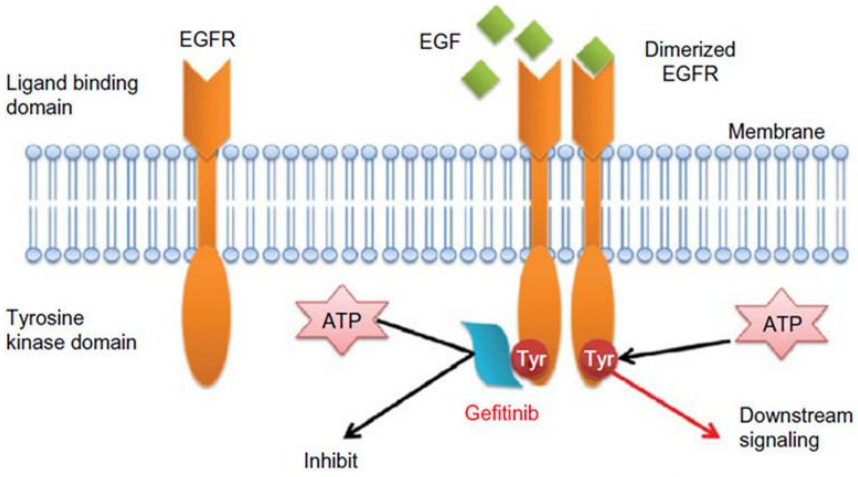
Scheme 14: Mechanism of inhibition of thymidylate synthase (TS) by FdUMP.[42]

1.3.1.2 Protein kinase inhibitors

Chemotherapy such as the administration of 5-fluorouracil currently refers to a major category of cancer treatments that have been used in the last few decades.[43] However, conventional chemotherapy, although directed toward certain macromolecules or enzymes, typically does not discriminate effectively between tumour cells and rapidly dividing normal cells such as those in bone marrow and the gastrointestinal tract (stem cells). It can therefore lead to several serious toxic side effects.[44] Since tumour responses from cytotoxic chemotherapy are usually unpredictable, targeted therapies are of more interest these days. Targeted therapies focus on the interference with molecular targets which have a critical role in tumour growth or progression. There are multiple types of targeted therapies available, but protein kinase inhibitors have become a key focus of development for new therapies.[45]

Protein kinases are enzymes that modify other proteins through supplying phosphate groups chemically (phosphorylation) to serine, threonine or tyrosine residues. The human genome contains about 500 protein kinase genes and up to 30% of all human proteins may be modified by protein kinases.[46] Protein kinases are mediate the majority of cellular pathways, especially those involved in signal transduction during cell and tissue development.[47] Cancerous cells usually develop robust anti-apoptotic signals to avoid stresses and escape the immune system via aberrant signal transduction.[45] In the 1980s, protein tyrosine kinases (PTK) were identified as major players in cancer and nowadays protein kinase inhibitors have been the focus of therapeutic agents for cancer.[45][48] One of the first targeted therapies as a protein kinase inhibitor for cancer treatment was Imatinib (**Figure 2**). During the therapy, only cancerous cells are expected to be killed during the drug's action.[49] Imatinib (**38**) is known as a tyrosine kinase inhibitor with anti-tumour effects in patients with gastrointestinal stromal tumour (GIST) and chronic myelogenous leukemia (CML).[49][50]

Gefitinib (**39**), developed and launched by Astra-Zeneca, is an oral epidermal growth factor receptor (EGFR) inhibitor used for the treatment of certain breast, lung, and other cancers (**Figure 2**).[51] EGFR signal transduction pathways have been implicated in the regulation of various neoplastic processes, including cell-cycle progression, inhibition of apoptosis, tumour-cell motility, invasion, and metastasis. Binding of a specific set of ligands to the receptor promotes EGFR dimerisation and the autophosphorylation of the receptors on tyrosine residues. Several signal transduction pathways downstream of EGFR then become activated, after autophosphorylation upon the receptor (**Scheme 15**).[44] From Wikstrand's reports, it is revealed that continuous activation or amplification and overexpression of EGFR proteins caused by mutations are observed in many human tumours.[52] These mutations eventually result in tumour aggressiveness.



Scheme 15: Mechanism of action of Gefitinib.[53]

Gefitinib interrupts signalling through the EGFR in target cells. It is selectively inhibits EGFR tyrosine kinase by binding to the adenosine triphosphate (ATP)-binding site of the enzyme, which subsequently inhibits the function of the EGFR tyrosine kinase and deactivates the anti-apoptotic signal transduction cascade (**Scheme 15**).[54] Structurally, Gefitinib contains a 3-chloro-4-fluoroaniline moiety linked to a quinazoline core. From X-ray studies, it has been shown that the 3-chloro-4-fluoroaniline moiety fits better than less fluorinated aromatic rings, into the hydrophobic pocket in the back of the ATP binding cleft of EGFR. Especially, from direct binding measurements, Gefitinib binds 20-fold more tightly to the L858R mutant than to the wild-type enzyme.[55] This is difference related to the fluorine substituent in the *para* position on the aniline that extends toward the side chains of Leu-788, Met-766 and Glu-762 and the high affinity between fluorine and the binding site of the enzyme.[44]

Afatinib (**40**) is another irreversible covalent inhibitor of the EGFR family of tyrosine kinases and used as the first-line treatment for patients with different types of metastatic non-small cell lung carcinoma (NSCLC) in many countries.[56] From the molecular structure, Afatinib keeps the quinazoline ring attached to the 3-chloro-4-fluoroaniline substituent as in Gefitinb (**Figure 2**), but bears acrylamide and tetrahydrofuranoxy substituents on the benzene ring of the quinazoline. Afatinib is considered not only active against EGFR mutations targeted by Gefitinib, but also against T790M point mutations in the kinase domain of EGFR which is resistant to these standard therapies.[57]

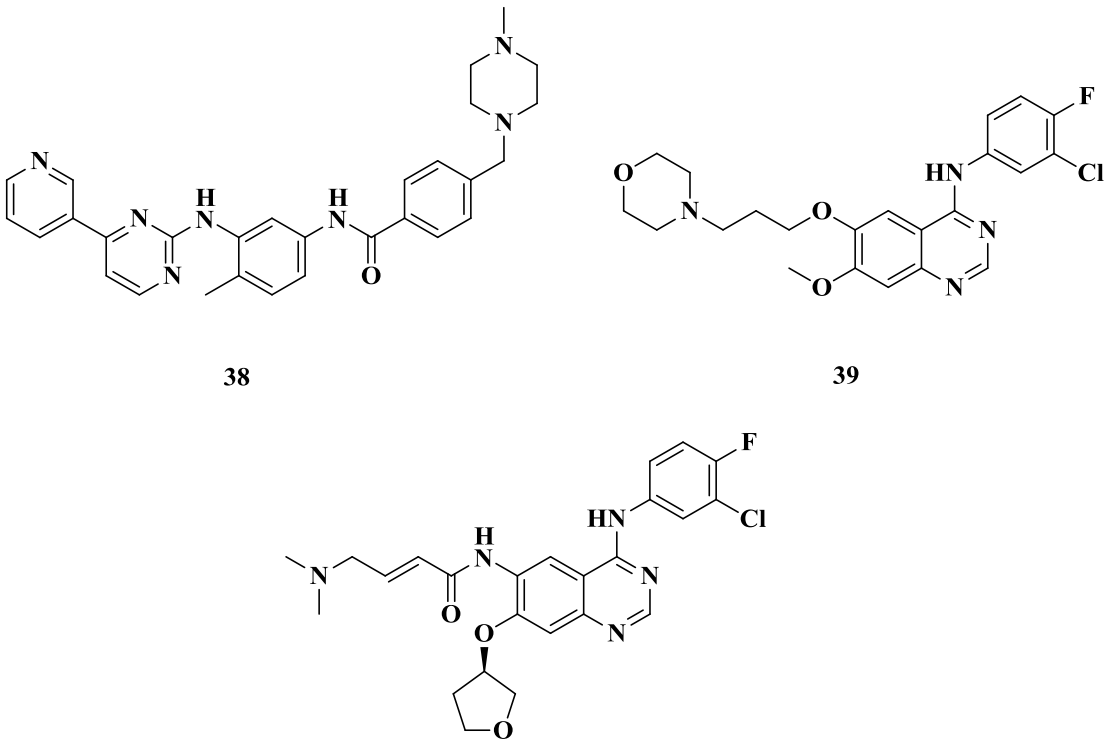


Figure 2: Structures of Imatinib (**38**), Gefitinib (**39**) and Afatinib (**40**).[50][51][56]

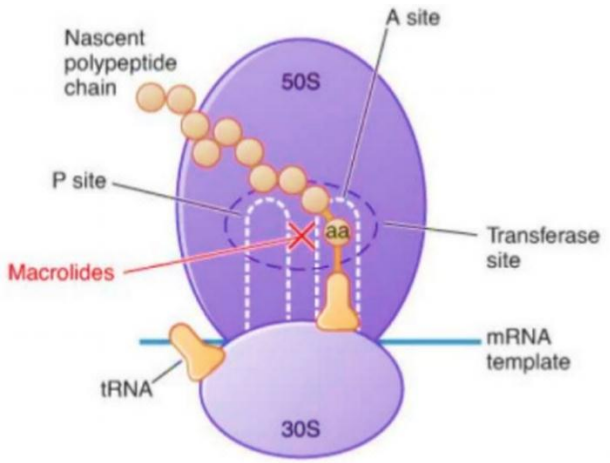
1.3.2 Antibiotic agents

Antibiotics revolutionised medicine in the 20th century and are probably one of the most successful forms of chemotherapy in history.[58] Currently, improvement of antibiotic ability and conquering the drug-resistance are crucial challenges in the development of novel strategies in the search for new antimicrobials.[59]

1.3.2.1 Erythromycin and its fluorinated derivative

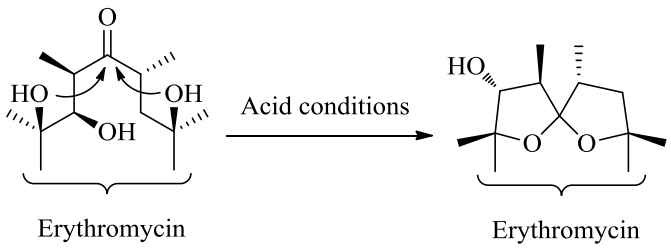
For pharmaceutical products, fluorinated analogues of natural products can modulate or improve their precursors' properties. Erythromycin (**41**) is a macrolide antibiotic which is

safe and effective against a wide range of gram-positive bacteria and is also used to treat an array of infections caused by gram-negative agents including Bronchitis and Legionnaire's disease. It is especially important for the treatment of patients with penicillin allergies.[60] The structure of erythromycin consists of a 14-membered macrocyclic lactone ring with a sugar and an amino sugar attached. Erythromycin acts by binding to 50S subunit of bacterial ribosomes to inhibit translocation (**Scheme 16**).[61]



Scheme 16: Mechanism of macrolides in blocking translation during bacterial protein synthesis.[62]

However, erythromycin unfortunately is unsuitable for the treatment of the *Helicobacter pylori* infection, which causes gastritis and leads to peptic ulcers. This is because Erythromycin is easily decomposed under the acidic conditions of the stomach.[14] The presence of a ketone and two alcohol groups are set up for the acid catalysed intramolecular formation of a ketal, which results in the acid sensitivity of erythromycin (**Scheme 17**).[61]



Scheme 17: Intramolecular ketal formation in erythromycin.[61]

Flurithromycin (**42**), a fluorinated derivative of erythromycin, was developed in 1997 by Pharmacia with the aim of inhibiting the decomposition of erythromycin under acid conditions (**Figure 3**). [14] Flurithromycin has a similar activity spectrum to Erythromycin. Differently, it is more stable than its precursor under low pH conditions. Presence of fluorine at α-position of the carbonyl group decreases electron density around oxygen atom, which makes the carbonyl group hard to be protonated. Additionally, introduction of fluorine into the molecule renders the agent a longer biological half-life, better bioavailability and allows it to reach higher tissue concentrations than Erythromycin *in vivo*, especially for the treatment of *H. pylori* infections.[63]

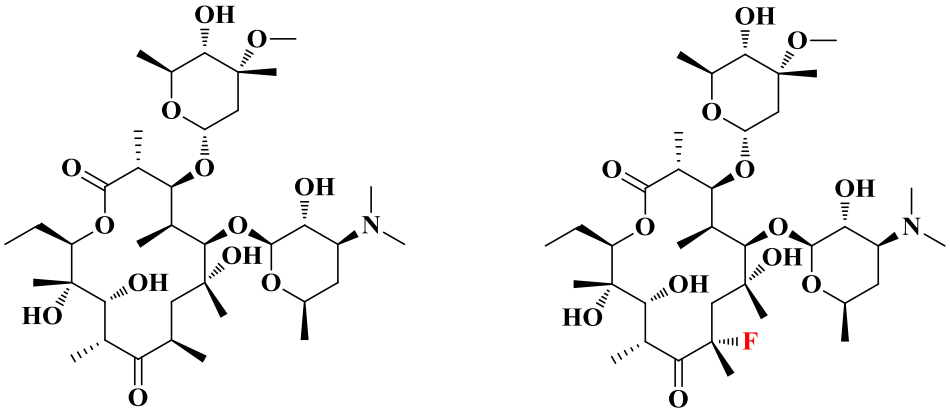


Figure 3: Structures of Erythromycin (**41**) and Flurithromycin (**42**).[14]

1.3.2.2 Fluoroquinolones

The discovery of the fluoroquinolones as antibacterials is another striking example of the strong effect of fluorine atoms on molecular properties. The quinolones are a family of synthetic broad-spectrum antibiotic drugs and usually exert their antibacterial potency by inhibiting topoisomerase IV or DNA gyrase involved in bacterial DNA synthesis.[64] Both of these enzymes are lacking in human cells but essential for bacterial DNA replication, which thereby enable these agents to be both specific and bactericidal.[65] Quinolones originally were derived from quinine and can also act as natural antimicrobials.[66] However, first-

generation quinolones, exemplified by nalidixic acid, were limited by their rather narrow-spectrum antibacterial activities.[13]

Since the first-generation synthetic fluoroquinolone, Norfloxacin, was developed in 1979, further fluoroquinolones have been developed, and currently are the majority of quinolones in clinical use for anti-bacterial agents (**Figure 4**).[64][67] Fluoroquinolones are active against a wide range of gram-negative organisms and several gram-positive aerobes such as *Escherichia coli* and *Salmonella*.[66] Introduction of fluorine in the 6-position led to an improvement of hundreds-fold in the minimum inhibitory concentration (MIC) for common gram-negative bacteria (**Table 4**).[19] It is recognised that fluorination at the 6-position is essential for the fluoroquinolone structure, which is involved in controlling gyrase and bacterial potency.[66]

Table 4: Potency of selected quinolones against Gram-negative bacteria.[66]

	MIC_{90} (mg/L)				
Quinolone	***E. coli***	***Klebsiella* spp.**	***Enterobacter* / *Citrobacter* spp.**	***Serratia* spp.**	***Haemophilus influenzae***
Nalidixic acid	8	16	>64	>64	2
Norfloxacin	0.12	0.5	0.25	2	0.06
Ciprofloxacin	0.03	0.25	0.12	0.5	0.03
Clinafloxacin	0.01	0.03	0.12	0.25	0.01

Domagala *et al.*[68] investigated the role of the fluorine atom in the fluoroquinolone structure in detail. A comparison of several fluoroquinolones and their non-fluorinated parent compounds revealed the 6-fluorine improves both gyrase-complex binding affinity by 2-17 fold and cell penetration by 1-70 fold.[68] This could be because of the increased lipophilicity of the molecule when fluorine is introduced.[69]

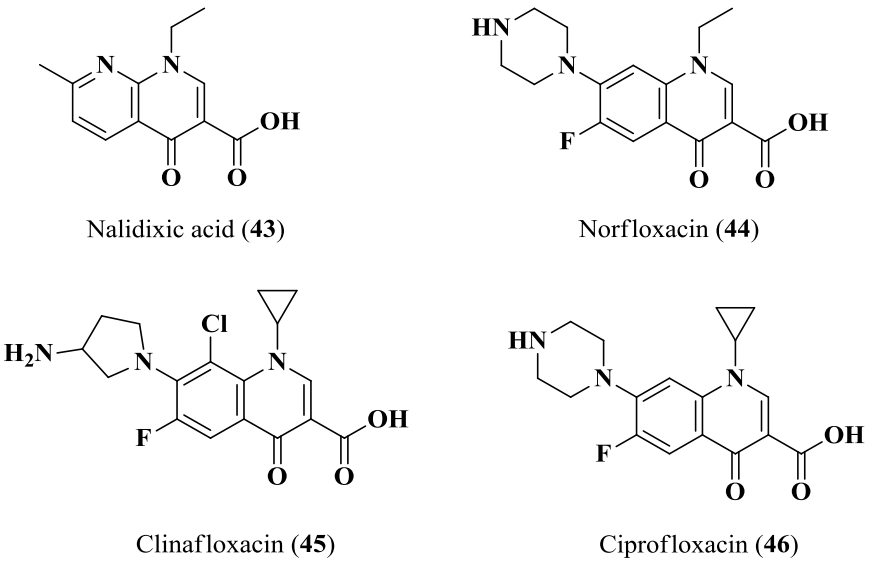


Figure 4: Selected quinolones of historical or commercial significance.[70]

Nowadays, novel fluoroquinolones are being developed to obtain better anti-bacterial activity and to tackle the problem of drug-resistance. JNJ-Q2 (**47**), developed by Furiex Pharmaceuticals (formerly Janssen Pharmaceutica), shows broad-spectrum bactericidal potency against Gram-positive and Gram-negative pathogens (**Figure 5**).[71] It is also found to be active against methicillin-resistant *Staphylococcus aureus* (MRSA) infections.[72] Another new fluoroquinolone antibiotic, Delafloxcain (**48**), has been announced to begin Phase-III clinical trials in community-acquired pneumonia in the USA.[73]

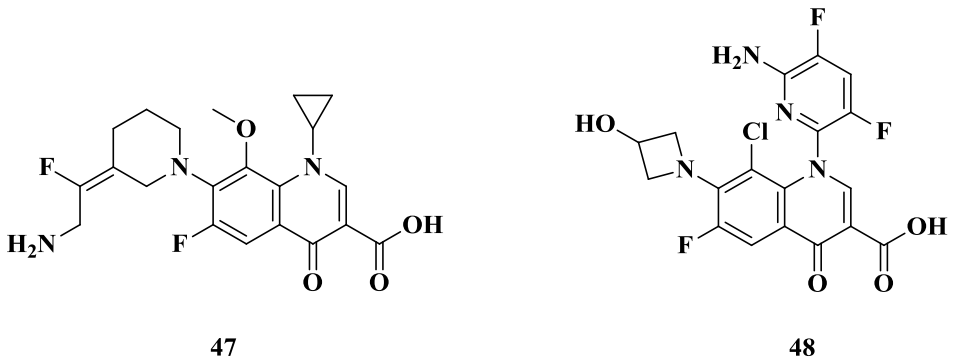


Figure 5: Structures of JNJ-Q2 (**47**) and Delafloxacin (**48**).[71][73]

1.3.3 Anti-protozoal agents

Parasitic infections are still a big problem in tropical and subtropical regions of the world.[74] The Global Burden of Disease Study 2013 studied 240 causes of death world-wide via systematic analysis and reported that parasitic diseases caused more than one million deaths in the year 2013.[75]

1.3.3.1 Antimalarial medication

Malaria continues to be one of the most important infectious diseases in the world and is by far the major parasitic disease killer.[75] In 2013, it was reported there were over 850,000 deaths caused by malaria and *Plasmodium falciparum* is the protozoan overwhelmingly responsible for severe clinical malaria and death.[75][76]

Artemisinin (**49**), isolated from the plant, *Artemisia annua L.*, was found to be a desirable antimalarial, and quickly become the recommended medicine of choice. Artemisinin is a sesquiterpene lactone that contains an endoperoxide bridge, as a part of a 1,2,4-trioxane core (**Figure 6**). This very unusual functional group in medicinal chemistry is an absolute requirement for the molecule's antimalarial activity.[77]

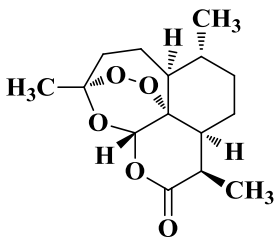


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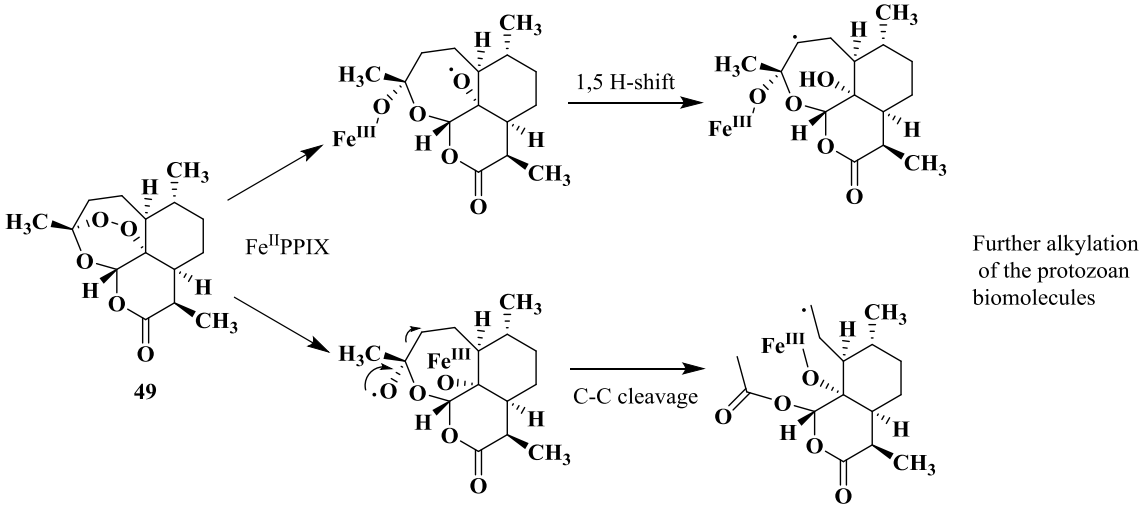
Figure 6: Structure of Artemisinin.[78]

Artemisinin works through a reduction of the endoperoxide group in the presence of ferrous ions in infected haemoglobin and subsequently two possible radical species are formed. Further reactions such as a 1,5-H shift will generate a series of other cytotoxic free radicals which alkylate biomolecules within the parasite and result in cell death (**Scheme 18**).[79]



Scheme 18: Activation of artemisinin by ferrous ions.[79]

Although artemisinin displays its remarkable efficacy, the therapeutic value of artemisinin is still limited by its low solubility in solvents and short half-life in the body, as well as developing resistance.[76][80] The development of chemically and metabolically more stable artemisinins will bring improvement in malaria therapy. Fenozan B07 (**50**) is a novel, second-generation antimalarial 1,2,4-trioxane endoperoxide with potent blood schizontocidal activity against drug-sensitive and drug-resistant rodent malaria parasites. Fenozan B07 like other artemisinins is a potent gametocytocide, but has wider activity towards the malaria parasite life cycle in humans (**Figure 7**).[81] Magueur *et al.* have explored the possibility of adding a C-10-trifluoromethyl group into artemether to improve the hydrolytic stability of the acetal functionality. The C-10 trifluoromethyl-substituted derivative (**51**) impressively was 33 times more stable than artemether in simulated stomach acid and displayed 2-fold higher anti-*Plasmodium berghei in vivo* activity (**Figure 7**).[82]

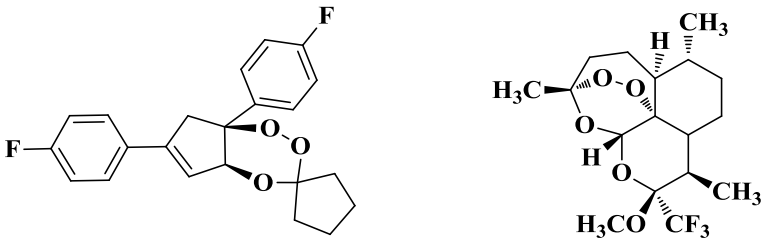


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Figure 7: Structure of Fenozan B07 (**50**) and C-10 trifluoromethyl-substituted artemether (**51**).[81][82]

1.3.3.2 Antitrypanosomal agents

Trypanosomiasis is the name of several diseases in vertebrates caused by parasitic protozoan trypanosomes of the genus *Trypanosoma*. Trypanosomes infect a variety of hosts and cause various diseases, including fatal human diseases like sleeping sickness, caused by *Trypanosoma brucei* (*T. b. gambiense* and *T. b. rhodesiense*), and Chagas disease, caused by *Trypanosoma cruzi*.[83] Both of these diseases cause approximately 18,000 deaths annually in sub-Saharan Africa and Latin America.[75] Current anti-trypanosome agents for sleeping sickness and Chagas disease are most effective early in the course of infection, but they are limited in their application with unsatisfactory toxicities as well as their incapacity to completely eliminate the protozoan from the body, especially in chronically infected patients.

For treatment at the second stage of sleeping sickness (chronic infection), Eflornithine (**52**), a rationally designed ornithine decarboxylase inhibitor with additional difluoromethyl group in comparison to ornithine, appears to be more effective and results in fewer side effects, since it came into medical use in 1990 (**Figure 8**).[84] Eflornithine currently has been a first-line treatment in many regions against *T. b. gambiense*.[85] However, it is expensive and not effective against of *T. b. rhodesiense* due to the parasite's low sensitivity to the drug and the current treatment for second-stage *T. b. rhodesiense* infection is Melarsoprol (**53**) only, even though it causes death in up to 5% of people who take it.[86] This situation highlights the need for the development of safer drugs for treating sleeping sickness caused by *T. b. rhodesiense*.

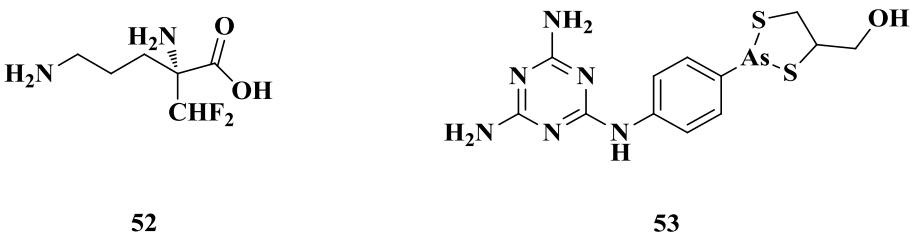

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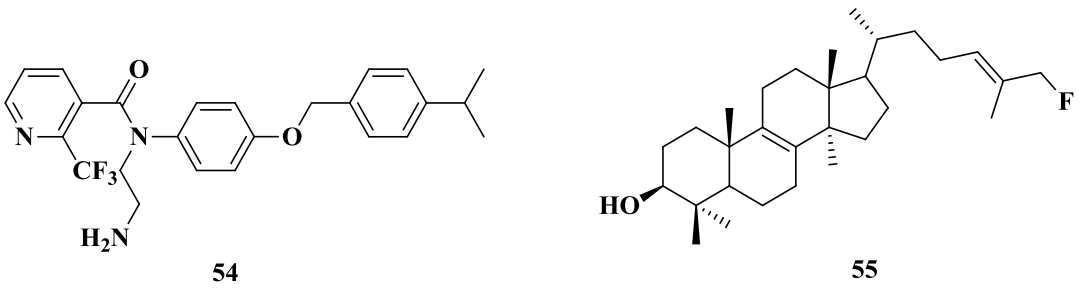
Figure 8: Structures of Eflornithine (**52**) and Melarsoprol (**53**).[85][86]

Many efforts have been made to discover new therapies towards infections caused by *T. b. rhodesiense* and recent literature findings have included the development of *N*-(2-aminoethyl)-*N*-benzyloxyphenyl benzamides (**54**) with a trifluoromethyl group exhibiting significant *in vivo* activity against *T. brucei* parasites as well as a series of fluorinated sterols (**55**), which act as suicide inhibitors to block ergosterol biosynthesis demonstrating activities against *Trypanosoma brucei* (**Figure 9**).[87][88]



54 55

Figure 9: Structures of Potential Anti-*Trypanosoma brucei* agents.[87][88]

In addition, some work has also focused on the treatment of Chagas disease. De Vita *et al.*[89] reported the development of 2-(1*H*-imidazol-1-yl)-1-phenylethanol derivatives (**56**) and found fluorine-substituted compounds exhibited appreciable *in vitro* activity against *T. cruzi* parasites and no toxicity towards mammalian cells. Fluorinated thiosemicarbazones (**57**), investigated by Santos's group, have recently been tested with success against *T. cruzi*. The group concluded the hydrophobicity is an important property for anti-*T. cruzi* activity (**Figure 10**).[90].

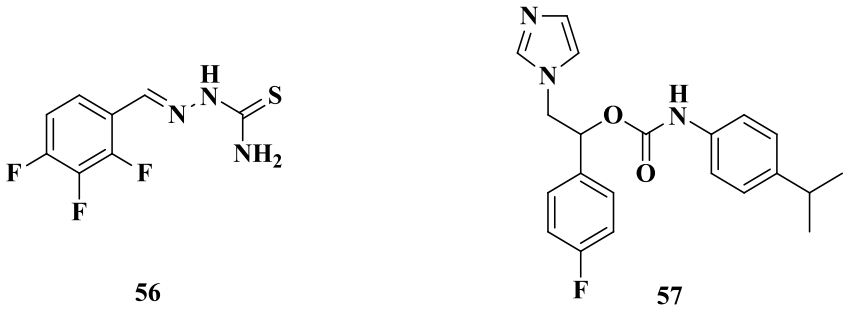


Figure 10: Structures of Potential Anti-*Trypanosome cruzi* agents.[89][90]

Chapter 2: Synthesis of Benzothiophenes and Evaluation of Their Anti-*Trypanosoma brucei. rhodesiense* Activity

2.1 Introduction

Human African Trypanosomiasis, also known as sleeping sickness is caused by infection with *Trypanosoma brucei rhodesiense* (*T.b.r*) or *Trypanosoma brucei gambiense* (*T.b.g*) parasites. There are two stages of sleeping sickness: the haemolymphatic phase and the neurological phase.[91] During the haemolymphatic phase, trypomastigotes circulate within the blood and lymphatic system. If not treated properly effectively, the neurological phase ensues as parasites penetrate the blood brain barrier thus infecting the central nervous system from which patient recovery is unlikely.[92] Generally, the symptoms of the disease show up as poor coordination, changes of behaviour and sensory disturbances. The disease is therefore given its name by the important feature of disordering the sleep cycle.[91]

The current treatment for *T.b.r* infection is out-dated and potentially harmful consisting of Suramin for the haemolymphatic phase (**Figure 11**) and arsenic-based melarsoprol for the neurological phase. These treatments require complicated dosing methods and assocaited side-effects such as reactive encephalopathy have proven fatal in about 9% of patients.[93] Clinical advancements in antitrypanosomal drug development have been limited and increasing resistance urgently requires the development of new drugs to replace existing therapies.

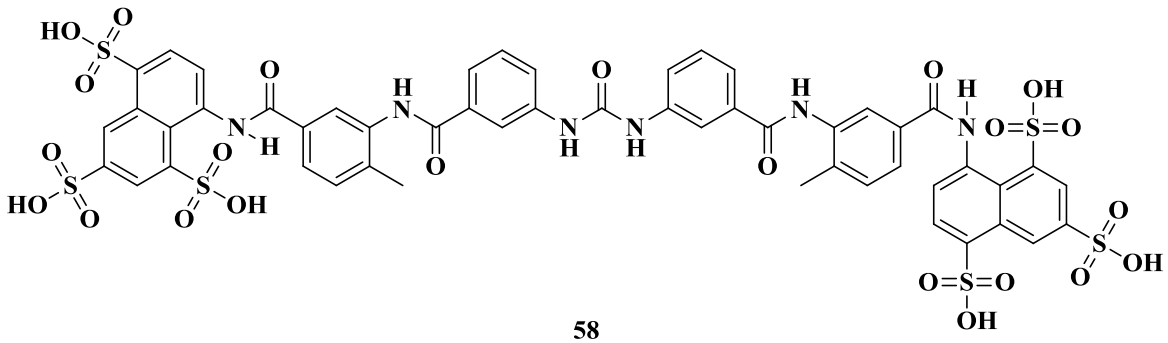


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Figure 11: Structure of Suramin.

Benzothiophene is an important class of heterocycle, consisting of benzene fused to a thiophene ring. It is widespread in pharmaceuticals like leukotriene synthesis inhibitors

(Zileuton), and antifungals (Sertaconazole).[94][95] Different substitution patterns around the benzothiophene scaffold provide good opportunities for drug discovery and synthesis of bioactive structures.[96]

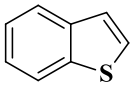

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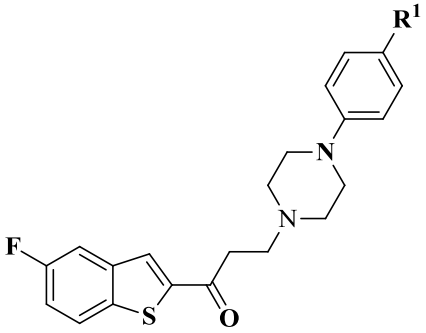
Figure 12: Structure of Benzothiophene.

There have been many efforts on drug discovery using benzothiophene as a scaffold against neglected tropical diseases. Moreno-Viguri *et al.* for example, reported preparation of a series of arylaminoketone derivatives and some containing a benzothiophene sub-structure were tested with success against *T .cruzi* (**Figure 13**).[97] They concluded after *in vitro* and *in vivo* experiments that both compounds demonstrated improved trypanocidal properties and higher activity for treating Chagas disease during acute phase. They also suggested that the *T. cruzi* Fe-SOD Enzyme is a potential target for the candidates.



60: R^1 = **F**,
61: R^1 = CF_3

Figure 13: Structures of benzothiophene based arylaminoketone derivatives.[97]

Two 3-nitrotriazole-based benzothiophene-amides, investigated by Papadopoulou's group, were reported to be effective against both *T. b. rhodesiense* and *T. cruzi* in their study (**Figure 14**). [98] They also found that Type I nitroreductase played an important role in the

activation of benzothiopheneamides as parasites which overexpresses this enzyme (in the presence of tetracycline) were more susceptible to these test candidates.

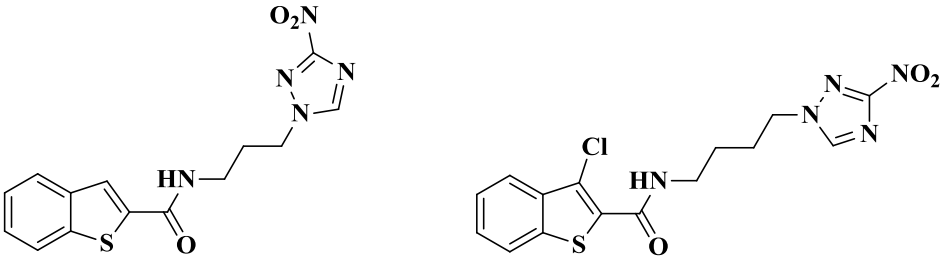
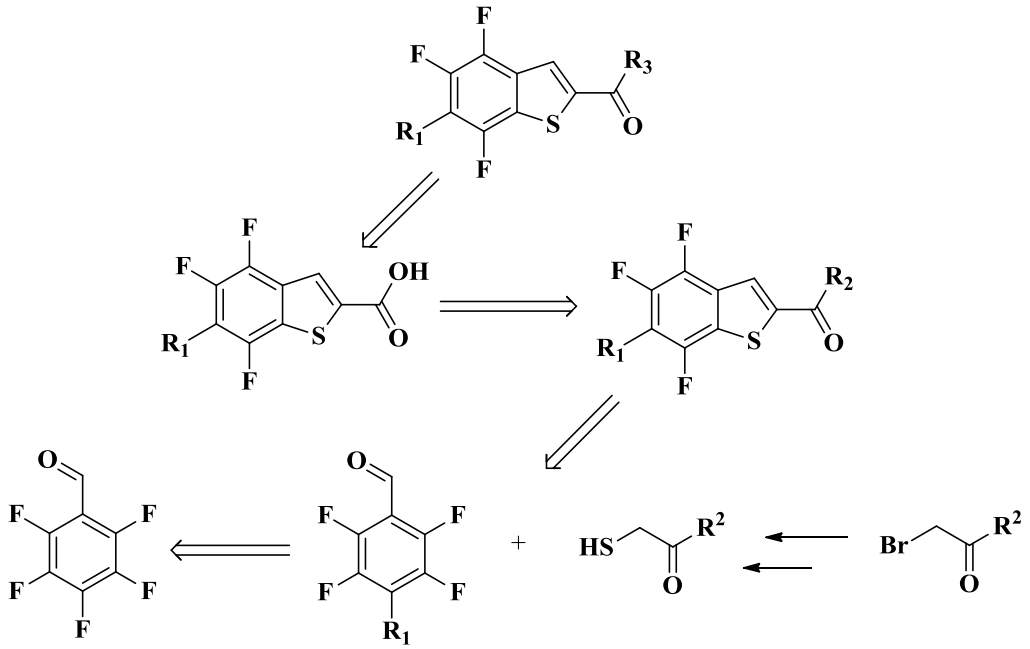


Figure 14: Structures of 3-nitrotriazole-based benzothiopheneamides.[98]

Many researches have illustrated that the benzothiophene structure is a promising and privileged scaffold for discovering potential anti-protozoal agents. It is therefore worthwhile to study the preparation of benzothiophene structures from fluorinated building blocks and to test such fluorinated benzothiophenes for anti-*T.brucei* activity in our research.

2.2 Aims

The investigations described in this chapter were aimed at preparing diversely substituted fluorinated benzothiophene scaffolds using nucleophilic aromatic substitution reactions (S_NAr) of perfluorinated building blocks (**Scheme 19**). Thematic of our medicinal chemistry programme is also to develop rapidly accessible fluorinated heterocyclic scaffolds with potential for anti-*Trypanosoma brucei rhodesiense* activity.



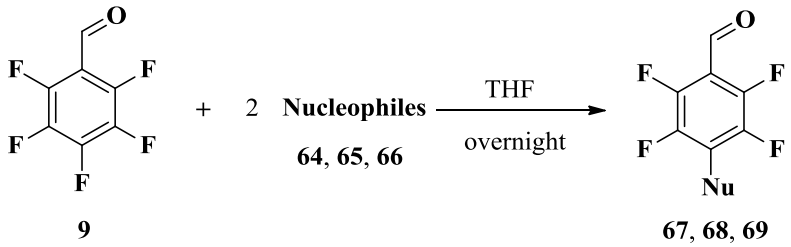
Scheme 19: Retrosynthesis of the target fully substituted core.

2.3 Results and Discussion

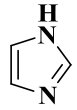
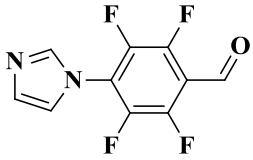
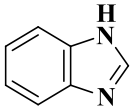
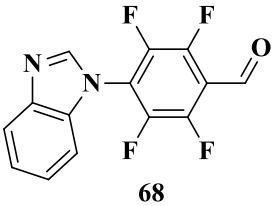
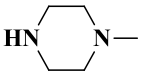
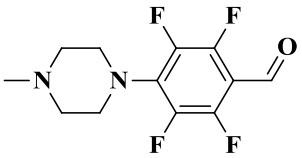
2.3.1 *para*-Substitution on pentafluorobenzaldehyde

Research began by first aiming towards *para*-substitution of pentafluorobenzaldehyde with a range of nitrogen nucleophiles. Pentafluorobenzaldehyde was reacted with three nitrogen-atom containing nucleophiles, namely imidazole, benzimidazole and 1-methylpiperazine. Two molar equivalents of each nucleophile were reacted with pentafluorobenzaldehyde separately at room temperature using THF as solvent (**Table 5**). The method was based on that reported by Fujii's group in 1989.[99]

Table 5: Reaction of pentafluorobenzaldehyde and imidazole or benzimidazole.



9 67, 68, 69

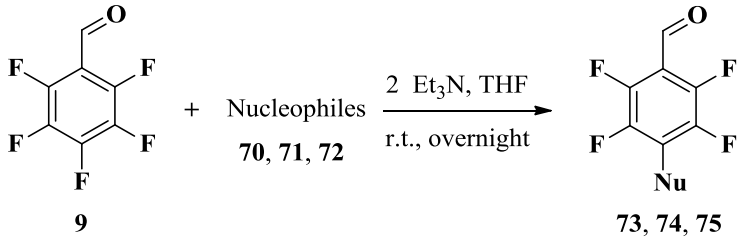
Nucleophiles	Product	Yield
 64	 67	68%
 65	 68	62%
 66	 69	80%

Reaction of pentafluorobenzaldehyde with imidazole mainly provided the known imidazolyl **67** as a red oil, in 68% yield, slightly less than the 84% reported.[99] Substitution with 1-methylpiperazine under the same conditions provided the piperazinyl **69** in good yield (80%). Similarly, reaction of pentafluorobenzaldehyde and benzimidazole was conducted under similar experimental conditions as imidazolyl **67**. A small modification of the reaction time for the synthesis of benzimidazolyl **68** was made, and the reaction duration for benzimidazole was increased to 10 hours longer than imidazole for the reaction to go to completion. In the experiment, two molar equivalents of the nucleophile were used so one could function as an acid scavenger to neutralise the HF generated in the reaction.

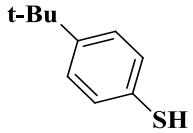
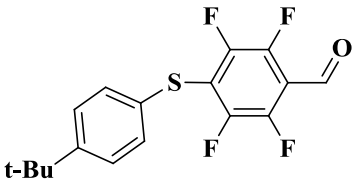
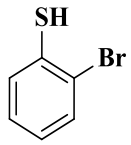
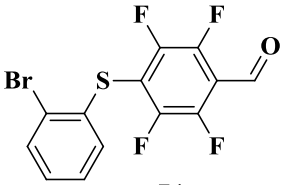
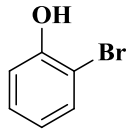
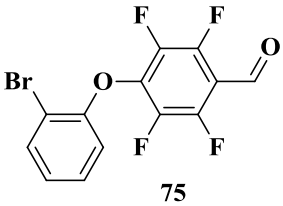
Pentafluorobenzaldehyde was then reacted with selected sulfur- and oxygen-atom containing nucleophiles, including 2-bromothiophenol and 2-bromophenol, to effect substitution at the

para-position. The 2-bromo analogues (**74** and **75**) have potential for further ring forming reaction by activation at the bromine centre by butyllithium exchange and cyclisation onto the meta-position.[100] The *t*-butyl analogue **73** was easy to handle and a less unpleasant thiol. The approach started by studying the synthetic route outlined in **Table 6** using pentafluorobenzaldehyde and the nucleophiles in the presence of two molar equivalent of triethylamine in dry THF at room temperature.

Table 6: Reaction of pentafluorobenzaldehyde and selected nucleophiles.



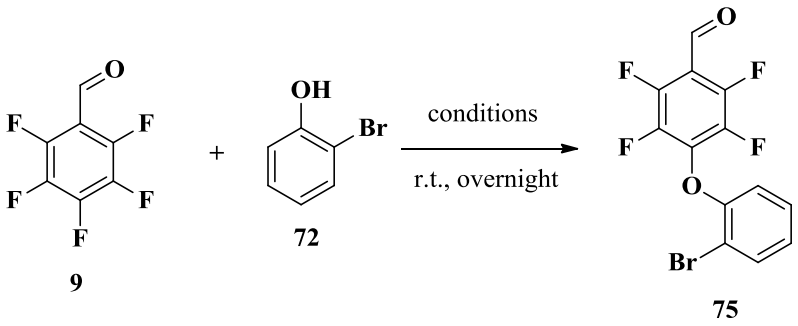
9 73, 74, 75

Nucleophiles	Product	Yield
 70	 73	73%
 71	 74	62%
 72	 75	35%

Three 4-substituted tetrafluorobenzaldehydes (**73**, **74** and **75**) were prepared from the reaction. However, the yield of the diaryl ether **75** was too low to scale up to the large quantities required for further synthesis, and so required reaction optimisation. After a number of investigations of different reaction conditions, the best results were obtained by performing

the reaction without solvent in the presence of five equivalents of triethylamine as base (**Table 7**).

Table 7: The yield of diaryl ether **75** with different amounts of triethylamine.



Solvent	Amount of Et_3N	Yield
THF	2 eq.	35%
THF	4 eq.	45%
THF	5 eq.	47%
None	5 eq.	62%

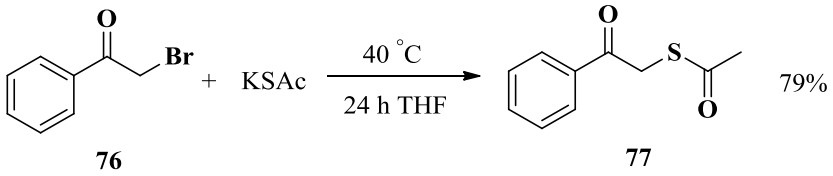
The structures of the six 4-substituted tetrafluorobenzaldehydes (**67**, **68**, **69**, **73**, **74** and **75**) obtained were confirmed by NMR spectroscopy. Singlet signals standing for aldehyde mostly appeared around 10.30 ppm in the ^{1}H NMR spectra. Also, there were two peaks found in the ^{19}F NMR spectra, which indicated the AA'BB' pattern of the tetrafluorobenzaldehyde system.

2.3.2 Preparation of α-mercaptocarbonyl compounds

Continuing our interest in preparing condensed sulfur-containing heterocycles from perfluoroarene precursors, alkanethiols bearing an α-activating group were required before the study. Methyl mercaptoacetate was firstly selected as the next reactant and it was available commercially. 2-Mercapto-1-phenylethanone was chosen as another reactant, which allows synthesis of compounds containing a phenyl ketone substituent instead of an ester as formed with methyl mercaptoacetate. Both were needed to construct the benzothiophene

derivatives via an S_NAr reaction followed by ring-closure reactions with carbonyl group of the range of 4-substituted pentafluorobenzaldehydes.[92]

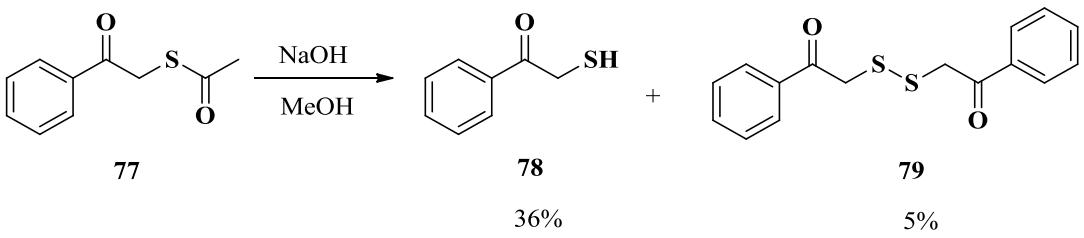
The preparation of 2-mercapto-1-phenylethanone followed the method described by Dehmel *et al.* who reported the synthesis of 2-mercapto-1-(4-methoxyphenyl)ethanone.[101] The reaction was conducted at 40 °C at first with potassium thioacetate and 2-bromoacetophenone **76** as starting materials in THF (**Scheme 20**). Thioacetate was expected to effect an S_N2 reaction on the highly reactive α-bromoketone through the more nucleophilic sulfur atom.



Scheme 20: Reaction of potassium thioacetate and 2-bromoacetophenone.

After 24 hours, there was only one product observable from the reaction via TLC. The known thioester **77** was isolated in 79% yield as a light yellow liquid after purification with silica column chromatography.[102]

The next step was to remove the acetyl group from the thioester **77** to form the target thiol **78**. The reaction was conducted at room temperature for 16 hours using methanol as solvent. Sodium hydroxide was employed as a base (**Scheme 21**).



Scheme 21: Synthesis of thiol **78** from known thioester **77**.

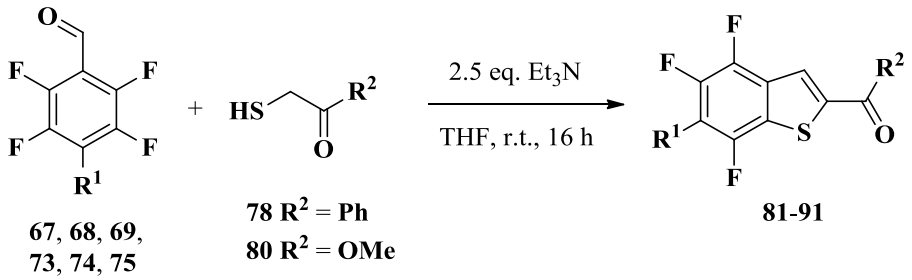
Thiol **78** was isolated as colourless liquid in 36% yield after purification via column chromatography. Another component, disulfide **79**, was obtained as a colourless liquid in 5%. It was suggested that thiol **78** was oxidised to form the corresponding disulfide **79**. Thiol **78** was not stable, and can form disulfide **79** spontaneously in air unless stored under nitrogen at low temperature.

2.3.3 Synthesis of poly-fluorobenzo[*b*]thiophene derivatives

The α-Mercaptocarbonyl compounds prepared were next used to effect a nucleophilic substitution reaction *ortho*- to the aldehyde group in the fluorobenzaldehydes already prepared. It was thought that subsequent condensation of the active methylene group with the adjacent aldehyde would form a fused thiophene ring, since thieno[2,3-*c*]pyridines have been prepared by a related method involving cyclisation onto a nitrile.[103]

Substituted benzaldehyde derivatives (**67**-**69** and **73**-**75**) were reacted with the two different α-mercaptocarbonyl compounds, **78** and **80**. The reactions were conducted at room temperature in THF with 2.5 molar equivalents of triethylamine for 16 hours. Various polyfluorobenzo[*b*]thiophene derivatives were synthesised as summarised in **Table 8**.

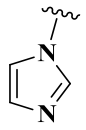
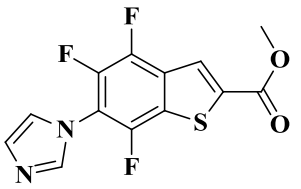
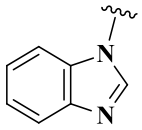
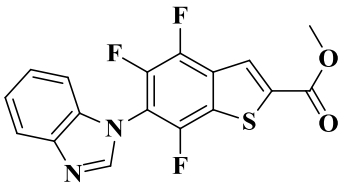
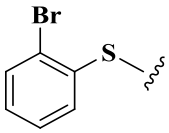
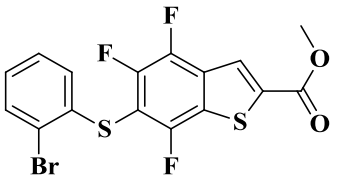
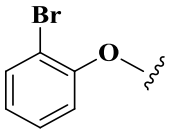
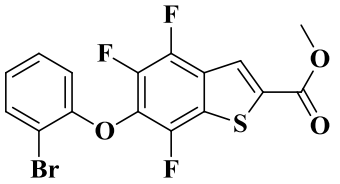
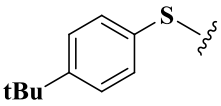
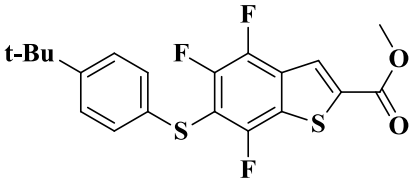
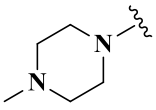
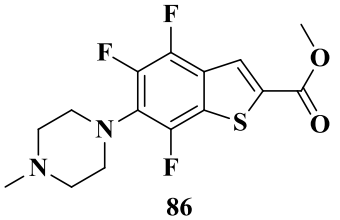
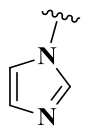
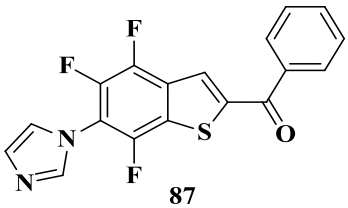
Table 8: Different substituents on the poly-fluorobenzo[*b*]thiophene derivatives.

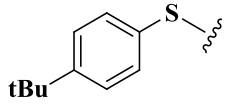
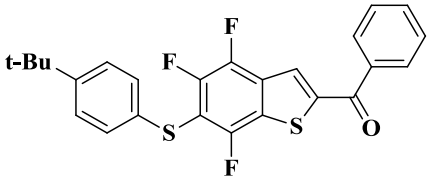
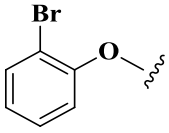
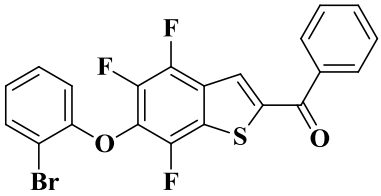
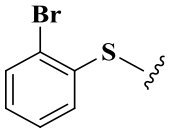
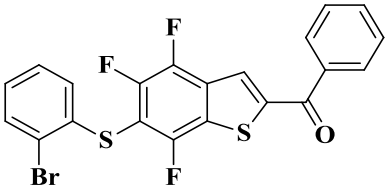
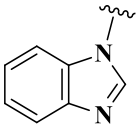
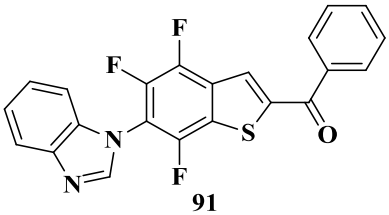


67, **68**, **69**, **73**, **74**, **75**

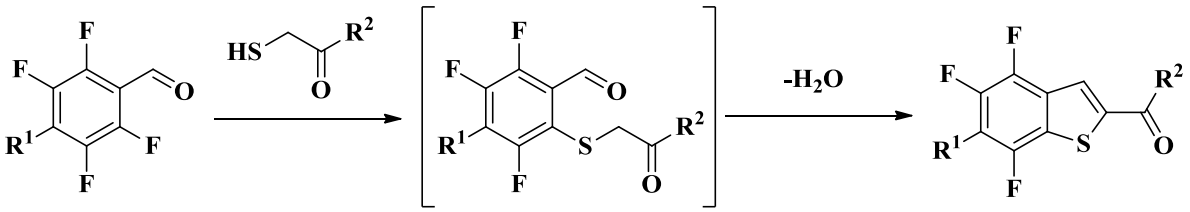
78 R^2 = **Ph**
80 R^2 = **OMe**

81-91

R^1	R^2	Product	Yield
	-OMe	 **81**	60%
	-OMe	 **82**	57%
	-OMe	 **83**	66%
	-OMe	 **84**	78%
	-OMe	 **85**	76%
	-OMe	 **86**	51%
	Phenyl-	 **87**	49%

R^1	R^2	Product	Yield
	Phenyl-	 **88**	49%
	Phenyl-	 **89**	48%
	Phenyl-	 **90**	46%
	Phenyl-	 **91**	51%

The method led to the successful synthesis of poly-fluorobenzo[*b*]thiophene derivatives (**81-91**) in reasonable yields. From a mechanistic perspective, the synthesis was thought to involve S_NAr reaction at position *ortho* to the aldehyde, followed by aldol-type cyclisation, and an intermediate enolate was formed under base catalysis. Addition to the aldehyde group then occurs followed by loss of water (**Scheme 22**).



Scheme 22: Synthesis route for the synthesis of benzothiophene derivatives.

All of the benzothiophene derivatives were identified using NMR spectroscopy and mass spectrometry. For example, in the ^{1}H NMR spectrum of benzimidazolyl benzothiophene **91**, the signal representing the aldehyde group is not present, and a doublet at 8.03 ppm (1H, d, *J* = 3.2 Hz), in contrast, was observed which indicated the proton in the thiophene ring. Three signals were detected in the ^{19}F NMR spectrum, which suggested one fluorine atom in the precursor had undergone substitution.

Benzimidazolyl benzothiophene **91** was further characterised by X-ray crystallography (**Figure 15**), and the structure (and subsequent structures) were determined by Dr Mark Elsegood in the department. Two torsion centres were found in the molecule. The twist angle between the benzimidazole ring and the benzothiophene ring is 54.29°, while the angle between the benzothiophene and benzene rings is 54.57°.

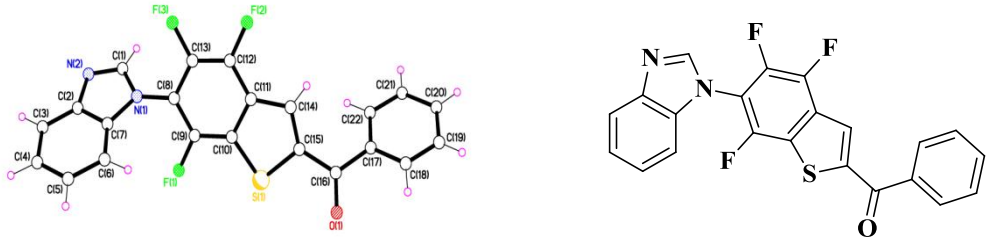
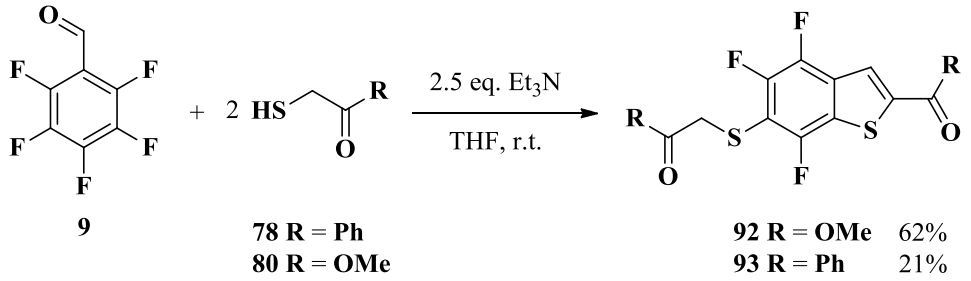


Figure 15: Crystal structure of benzimidazolyl benzothiophene **91**.

With the successful synthesis of benzothiophene derivatives from 4-substituted pentafluoro-benzaldehydes, it appeared attractive to prepare this class of compounds using the benzaldehyde directly. To this end, pentafluorobenzaldehyde **9** was treated with two equivalents of each thiol, **78** and **80**, in the presence of two and half equivalents of base to form the benzothiophene in a one-pot procedure without isolating the intermediate 4-sulfanyl benzaldehydes (**Scheme 23**).



Scheme 23: Reaction of two molar equivalents of thiols and pentafluorobenzaldehyde.

Benzothiophene **92** and **93** were prepared successfully from this reaction and were isolated in yields of 62% and 21%, respectively. Disulfide **79** was also observed from the reaction by NMR spectroscopy, which accounted for the reduction in yield of compound **93**. For both benzothiophene **92** and **93**, a singlet representing the methylene group (3.65 ppm and 4.36 ppm) and doublet (8.09 ppm and 7.93 ppm) on the thiophene ring were found in the ^{1}H NMR spectra, respectively. The presence of three signals in the ^{19}F NMR spectra also supported the formation of benzothiophenes. Since benzothiophene scaffolds were prepared from this approach, it was suggested that the thiols most likely added consecutively to the 4- and 2-positions of the aldehyde starting material. Subsequently, the intramolecular reaction of the 2-substituent occurred with the adjacent aldehyde group to form the benzothiophene scaffold.

2.3.4 Biological testing against *T. b. rhodesiense* with benzothiophenes

The benzothiophene derivatives prepared were evaluated by our collaborators for possible anti-*Trypanosoma brucei* activity and screened for cytotoxicity against MCF_7 cells using a cell-based assay (**Table 9**). Anti-trypanosomal activity assays (carried out by Vanessa Yardley and Hollie Burrell-Saward at the London School of Hygiene and Tropical Medicine), were performed in 96-well microtiter plates and *T. b. rhodesiense* STIB 900 was used *in vitro* screening. Each well containing 100 µl of parasite culture (1 x 10^3 bloodstream forms) was treated with serial drug dilutions at 37 °C for 72 hours in 5% CO_2. Alamar Blue was added into each well after the incubation period and the plates were read under a fluorimeter, and IC_{50} values were determined. Cytotoxicity studies were conducted by Vladimir Krystof at Palacky University, CZ.

Table 9: Anti-*Trypanosoma b. rhodesiense* and cytotoxicity (MCF_7 cell line) screening for benzothiophenes **81-93**.

Compound	*T. b. rhodesiense* IC_{50} [µM]	MCF_7 IC_{50} [µM]
81	129.9	>100
82	0.6	>12
83	11.9	>25
84	30.0	>25
92	>10.0	>25
85	67.2	>50
86	-	-
87	33.0	>60
88	31.4	>25
89	15.9	>25
90	9.8	>25
91	0.5	>25
93	16.0	>50
Melarsoprol	0.05	-
Roscovitine		11

Among the benzothiophene derivatives, **83**, synthesised bearing a 6-(2-bromophenylsufanyl) group demonstrated moderate trypanocidal activity with an IC_{50} value of 11.9 µM, while **84** containing a 6-(2-bromophenoxy) group gave an IC_{50} value of 30 µM. 6-Imidazolyl-substituted analogue, **81**, did not show anti-*T. b. rhodesiense* activity, nor did the 6-(4-*tert*-butylphenylsulfanyl) derivative **85**. However, the 6-benzimidazolyl analogue **82** interestingly demonstrated respectable anti-*T. b. rhodesiense* activity with an IC_{50} value of 0.60 µM, which is the best results of the benzothiophene-2-carboxylate derivatives that were screened.

In the ketone series, **91**, the 2-benzoylbenzothiophene with a bromophenyl sulfanyl substituent showed comparable antiparasitic and cytotoxic activity to its ester analogue **83** with an IC_{50} value of 9.8 µM. Similarly, the imidazolyl-substituted compound **87** showed an increase in trypanocidal activity with an IC_{50} value of 33.0 µM in relation to its carboxylate analogue **81**. Likewise for the *t*-butyl pair **88**/**85** the ketone, **88** showed two-fold better

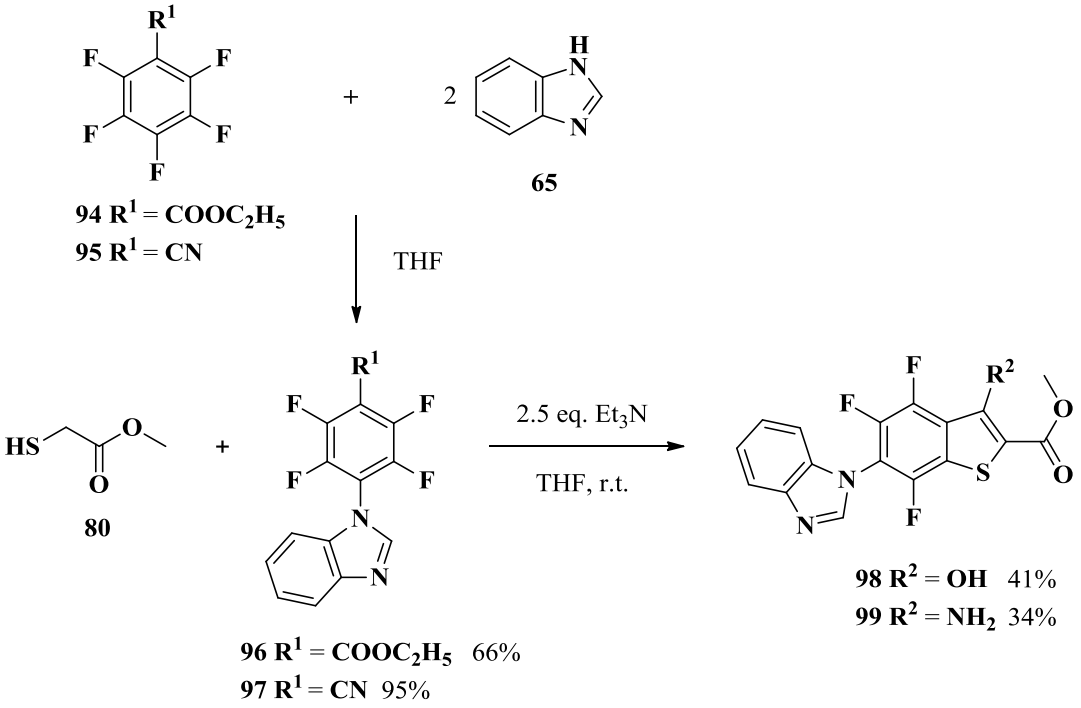
antiparasitic activity than the corresponding ester, while for compounds **93**/**92** comparable trypanocidal activity was observed in the region of 10 µM. Significantly, the benzimidazole-containing compound **91** showed a trypanocidal IC_{50} value of 0.53 µM, comparable to its 2-carboxylate analogue **82**.

The ketone series demonstrated remarkably higher anti-parasitic activity than the 2-carboxylate analogues evaluated. It revealed that a phenyl ketone group increased the activity compared to the methyl ester substituent. In terms of functionality on the fluorobenzene ring, the benzimidazole-containing compounds demonstrated the greatest anti-parasitic activity, and these were classified as hit compounds. Further investigations were then undertaken on the benzimidazole-substituted compounds to enhance their anti-*Trypanosoma brucei* activity by making modifications to the thiophene ring.

2.3.5 Formation of benzimidazole-substituted benzothiophenes from different fluorinated arenes

Modification firstly aimed towards substitution on the benzothiophene at the 3-position with different functional groups, in order to investigate the effect of hydrogen-bond donor substituent at C-3 position for its trypanocidal activity. The reaction was therefore developed starting from different fluorinated arenes containing ester or nitrile groups which have the potential to undergo condensation reactions similar to the tetrafluorobenzaldehyde derivatives. These starting materials were expected to lead to benzothiophenes by a similar mechanism.

Benzimidazole was therefore reacted separately with pentafluorobenzonitrile and ethyl pentafluorobenzoate. The method for the reaction was the same as that used for the synthesis of aldehyde **68**. Following this procedure, ester **96** and nitrile **97** were prepared successfully, and formed as white solids in yields of 66% and 95%, respectively. From the ^{19}F NMR spectra, both showed the same AA'BB' pattern as was observed for aldehyde **68**. In addition, the structure of products confirmed by mass spectrometry as $[M+H^+]$ found at m/z 339.0749 for ester **96** and m/z 292.0492 for nitrile **97**.



Scheme 24: Reaction of benzimidazole with pentafluorobenzonitrile **95** and ethyl pentafluorobenzoate **94**.

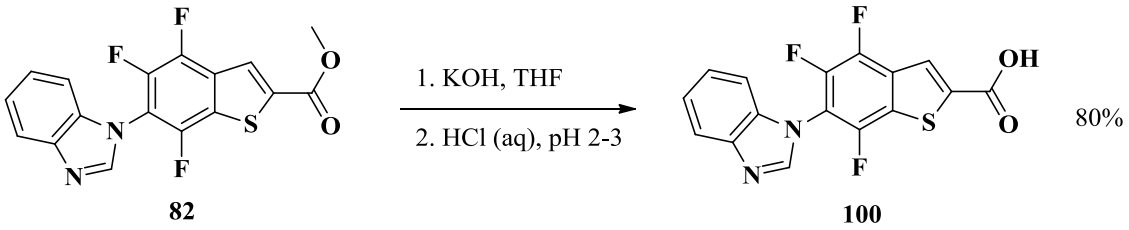
Although the ketone series showed a lower activity than the 2-carboxylate series, methyl mercaptoacetate is available commercially. This contributed to the easier preparation of the 2-carboxylate series. Therefore, ester **96** and nitrile **97** were reacted with methyl mercaptoacetate in the next step using the same method as **82** (**Scheme 24**). After the reactions, alcohol **98** and amine **99** were obtained in yields of 41% and 34%, respectively. Both compounds represent good candidates to investigate the effect of introducting hydrogen bond donors on the biological activities.

2.3.6 Modification on the ester group of methyl 6-(1*H*-benzo[*d*]imidazol-1-yl)-4,5,7-trifluorobenzo[*b*]thiophene-2-carboxylate

As phenyl ketone **91** showed slightly greater anti-parasitic activity than ester **82**, other modifications were proposed to the ester group in ester 68 in an effort to generate analogues with enhanced activity.

2.3.6.1 Hydrolysis of methyl 6-(1H-benzo[d]imidazol-1-yl)-4,5,7-trifluoro-benzo[b]thiophene-2-carboxylate

The investigation began with hydrolysis of ester **82**. The approach followed the method released by Hendrix *et al.* who reported the synthesis of benzo[*b*]thiophene-2-carboxylic acid.[104] Thus, ester **82** was treated with KOH in THF at room temperature for 6 hours and subsequently acidified with hydrochloric acid to pH 2-3 (**Scheme 25**).



Scheme 25: Hydrolysis of ester **82** using KOH.

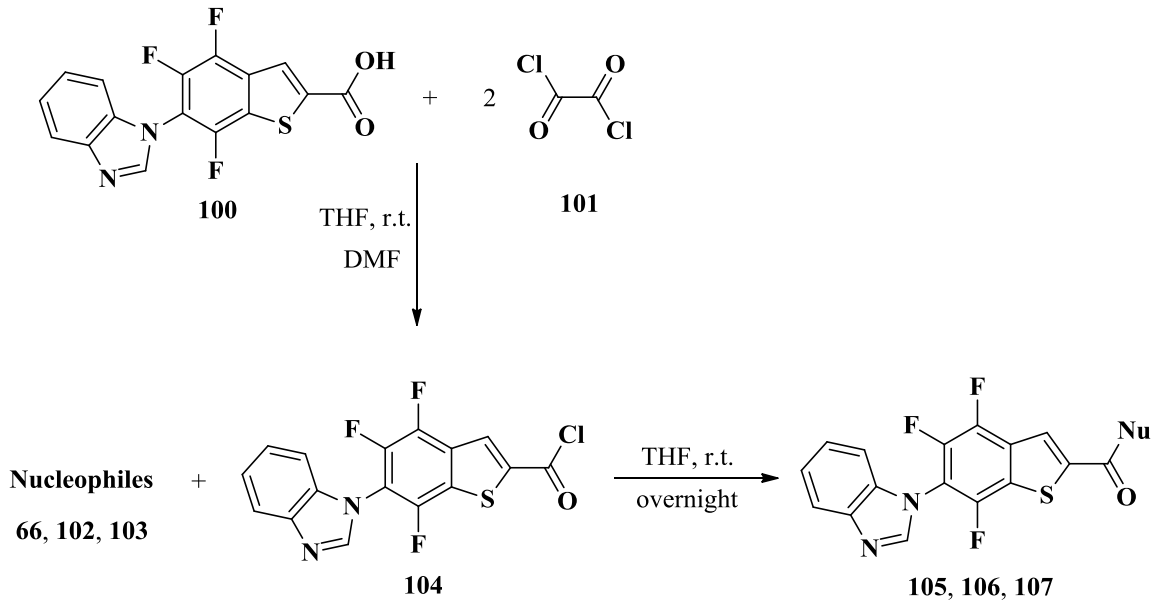
A white solid shown to be the carboxylic acid **100** was isolated from the reaction in a yield of 80% without purification. Both the absence of the methoxy group signal and the presence of a carboxylic acid group signal at 14.34 (1H, br) ppm in the ^{1}H HMR spectrum confirmed the successful saponification of ester **82**. The carboxyl group on acid **100** was an attractive site for further substitution.

2.3.6.2 Substitutions on 6-(1H-benzo[d]imidazol-1-yl)-4,5,7-trifluorobenzo-[b]thiophene-2-carboxylic acid

To activate the carboxylic acid towards different substitutions, acid **100** was firstly converted to the corresponding acyl chloride. The attempt began with the reaction of acid **100** and oxalyl chloride as reported by Courtney *et al.*[105] Excess oxalyl chloride was used as the reagent in the reaction which was conducted at ambient temperature (**Scheme 26**). Several

drops of DMF were added to the reaction as catalyst. DMF is easily converted into an imidoyl chloride with oxalyl chloride, which is the active chlorinating agent.

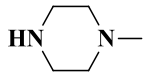
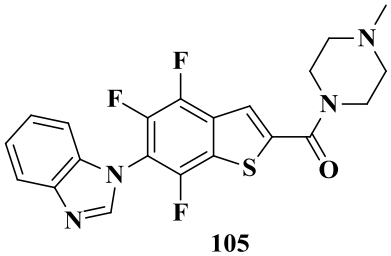
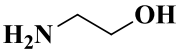
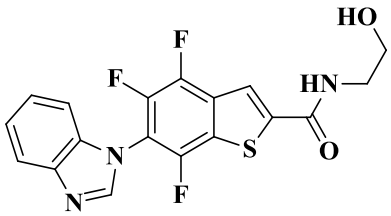
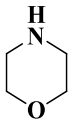
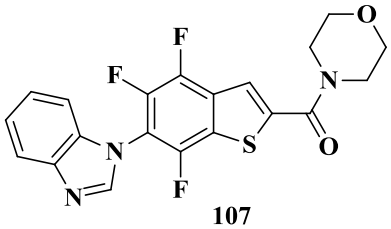
The reaction was stopped after 16 hours as starting material acid **100** disappeared from TLC. The solution was concentrated and a white solid was obtained. Since the acyl chloride was reactive and unstable, the residue was utilised directly for further substitution without purification.



Scheme 26: Substitutions on acid **100**.

Selected amines were reacted with the acyl chloride **104** in the THF without catalyst. The reactions were conducted at room temperature overnight (**Scheme 26**).

Table 10: Substitutions on acyl chloride **104**.

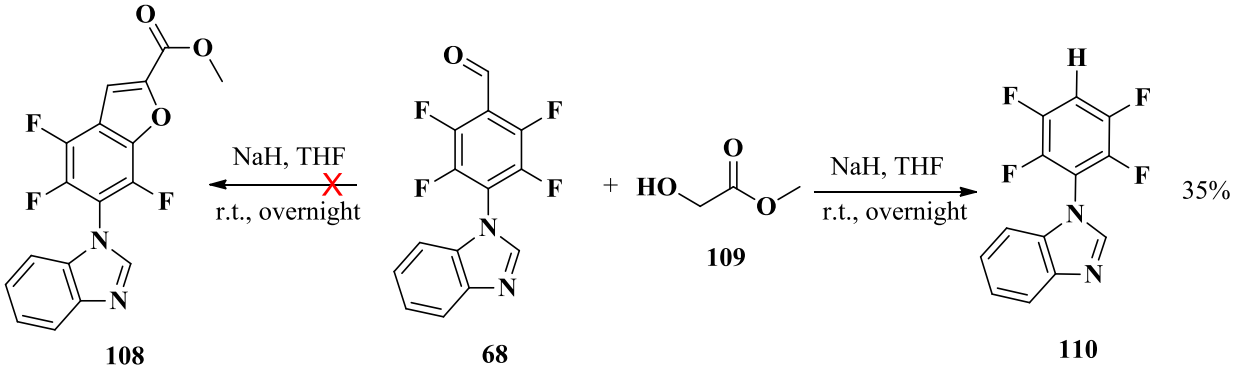
Nucleophiles	Product	Yield
 **66**	 **105**	59%
 **102**	 **106**	55%
 **103**	 **107**	56%

Amides substituted **105**, **106** and **107** were prepared successfully in a moderate yield from these reactions (**Table 10**). Signals indicative of the methylpiperazine, ethanolamine and morpholine moieties were observed in the ^{1}H NMR spectra and mass spectrometry also confirmed the structure of the products as [M+H^{+}] peaks were observed at m/z 431.1151 for **105**, m/z 392.0676 for **106**, m/z 418.0831 for **107**, respectively.

2.3.7 Attempted reaction to form partially fluorinated benzofuran

To extend the scope of fused benzene ring preparation, further study was attempted to form fluorinated benzofuran following the same method used to synthesise partially fluorinated benzothiophene derivatives.

Benzimidazole **68** and methyl glycolate were firstly employed in an attempt to form analogous benzofurans. Two molar equivalents of sodium hydride were used which were expected to both deprotonate the methyl glycolate and base-catalyse the ring-closure process. The whole reaction was run at room temperature with THF as solvent. The synthesis strategy is illustrated in the **Scheme 27**.



Scheme 27. Reaction of benzimidazole **68** and methyl glycolate.

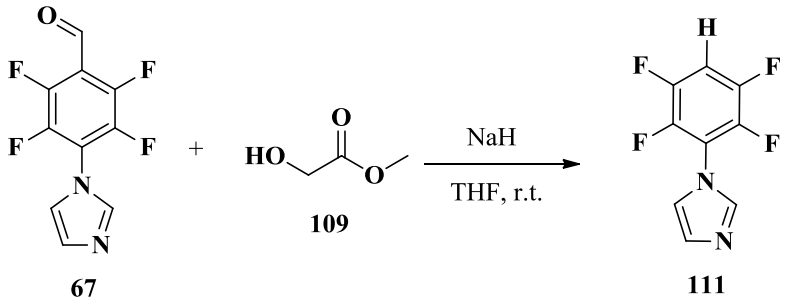
After leaving the reaction mixture stirring overnight, a white solid was obtained. However, there was no evidence of any cyclisation taking place, which was confirmed with GC-MS and NMR spectroscopy. Two peaks found in the ^{19}F NMR spectrum still indicated an AA'BB' pattern in the tetrafluorobenzene system and GC-MS gave a peak at m/z 266.1 suggesting the corresponding molecular formula as $C_{13}H_6F_4N_2$. Both NMR spectroscopy and GC-MS suggested that the product was the tetrafluorophenyl benzimidazole **110**. It appeared that the aldehyde group had been lost and replaced by a hydrogen atom and no evidence for the expected formate by-product was obtained.

To investigate this unexpected reaction, imidazolyl substituted aldehyde **67** was exposed to the same reaction conditions. Considering sodium hydride is a strong base (pK_a = 23), triethylamine (pK_a = 8) was also investigated in a second reaction to screen the influence of base. From TLC monitoring, the reaction with sodium hydride showed one main spot, while the other reaction with triethylamine was not completed as starting materials still remained in the reaction mixture (**Table 11**).

Table 11. Different conditions for the reaction of aldehyde **67** and methyl glycolate.

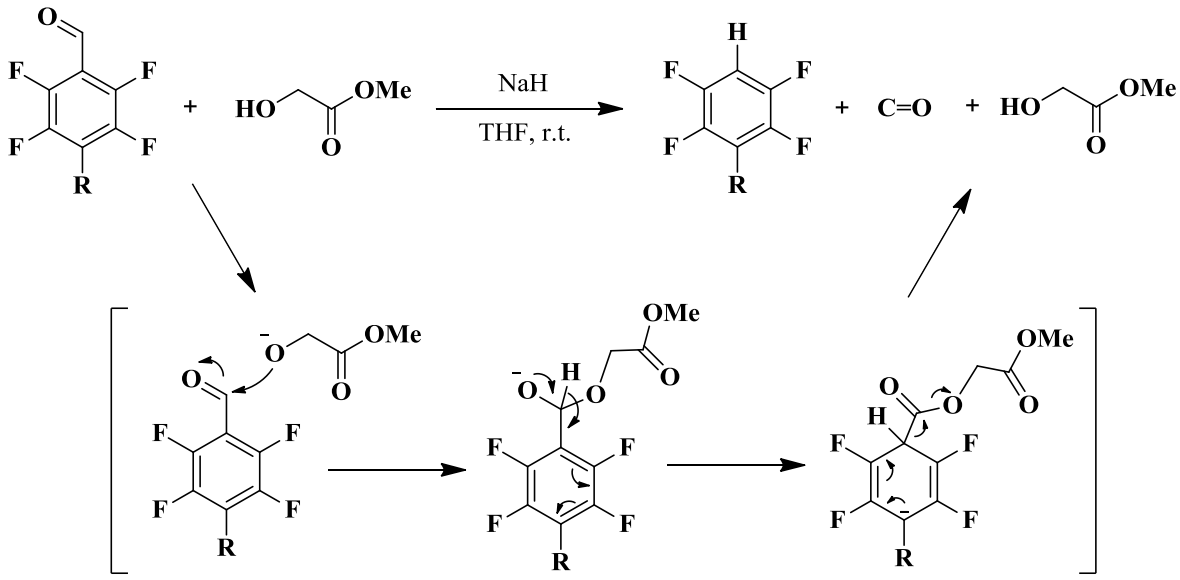
Entry	Condition	Yield
1	2 eq. NaH in THF, r.t.	58%
2	2 eq. Et_3N in THF, r.t.	24% and starting materials

Both reactions provided the same compound as a yellow solid. The product **111** isolated showed again a similar situation had occurred and that the aldehyde group had been cleaved, as confirmed by LC-MS and NMR spectroscopy (**Scheme 28**).



Scheme 28. Reaction of imidazole **67** and methyl glycolate.

The reactions described above, showed that it was difficult to form the analogous benzofurans by the S_NAr-condensation strategy that had been successful for accessing benzothiophenes; the reaction instead led to substitution of the aldehyde group for a hydrogen atom. A possible explanation is that the partially cationic carbonyl carbon of the aldehyde was the more favourable reaction site towards the harder alkoxide nucleophile compared with the 2-position in the aromatic ring which appears to be the preferred site of attack by the softer sulfur-based nucleophile.



Scheme 29: Proposed mechanism for the synthesis of compound 4-sbustituted tetrafluorobenzene.

A possible mechanism (**Scheme 29**) could involve hydride ion transfer from the derived hemi-acetal anion formed after alkoxide addition to the aldehyde group, to the aromatic ring at the *ipso* site. Rearomatisation could then occur by loss carbon monoxide and methyl glycolate anion, finally producing the 4-substituted tetrafluorobenzene. Further work on the selectivity using other types of nucleophile would be of interest to study competition between attack at the aldehyde or the C-2 position.

2.4 Conclusions

The work outlined in this chapter has shown the development of a benzo[*b*]thiophene scaffold from pentafluorobenzaldehyde and a number of nucleophiles, using nucleophilic aromatic substitution as a key step. The benzo[*b*]thiophene derivatives prepared were tested against *Trypanosoma brucei rhodesiense* parasites and the benzimidazole-substituted benzo[*b*]thio-phenes demonstrated appreciable anti-*T. b. rhodesiense* activity.

4-Substituted-tetrafluorobenzaldehydes (**67**-**69** and **73**-**75**) were firstly synthesised from pentafluorobenzaldehyde with a range of nucleophiles including two diazoles, a piperazine, a phenol and two thiols. The reactions provided good yields of product from 62 to 80%.

Treatment of the aldehydes with α-mercaptocarbonyl compounds (**78** and **80**) in the presence of weak base at room temperature led to substitution and condensation of the active methylene group with the adjacent aldehyde to form a fused thiophene ring. A variety of benzo[*b*]thiophene derivatives were prepared using the same reaction conditions in poor to good yield (22-78%). Two benzimidazole-substituted benzo[*b*]thiophenes, **91** (IC_{50} = 0.53 μM) and **82** (IC_{50} = 0.60 μM), showed moderate inhibition against *Trypanosoma brucei rhodesiense* parasites after biological screening. X-ray diffraction analysis confirmed the structure of ester **91** as well.

Based on the structure-activity relationship study, another six benzimidazole-substituted benzo[*b*]thiophene derivatives were prepared. Alcohol **98** and amine **99** were obtained in yields of 41% and 34% respectively through the use of pentafluorobenzoate and pentafluorobenzonitrile as starting materials for the first step. Acid **100** was also prepared by hydrolysis of ester **82** allowing the synthesis of piperazinyl **105**, ethanolamine **106**, morpholinyl **107** amides.

Formation of a benzofuran unfortunately failed using the same conditions as were used in the preparation of benzothiophene ring. The aldehyde group was surprisingly cleaved from the 4-substituted tetrafluorobenzaldehydes and eventually tetrafluorobenzenes **110** and **111** were obtained.

Chapter 3: Preparation of (Aza-) Phenoxazines and Assessment of Their Anti-*Trypanosoma cruzi* Activity

3.1 Introduction

Chagas disease affects about 6 to 7 million people worldwide, mostly in Latin America, and is spreading also in Europe and North America. It is caused by the protozoan parasite *Trypanosoma cruzi*.[106] Chagas disease presents itself in two phases: an acute phase and a chronic phase. During the acute phase, *Trypanosoma cruzi* infection is curable if treatment is initiated soon after infection when a high number of parasites circulate in the blood. However, persons with long-standing *T. cruzi* infection sometimes develop serious gastrointestinal and cardiac problems. This is because the parasites are hidden usually in the heart and digestive muscles, which typically lead to cardiac disorders and enlargement of the oesophagus or colon.[106][107]

Currently, nifurtimox and benznidazole are the only drugs approved for the treatment of Chagas disease (**Figure 16**). Although they work well in the acute phase of the disease, clinical efficacy in patients with the chronic illness is limited.[108][109] In spite of the social and economic importance of Chagas disease, efforts directed toward the discovery of new drugs against the disease remain underdeveloped. Therefore, there is an urgent need for the discovery of new therapeutics displaying anti-*Trypanosomal cruzi* activities.

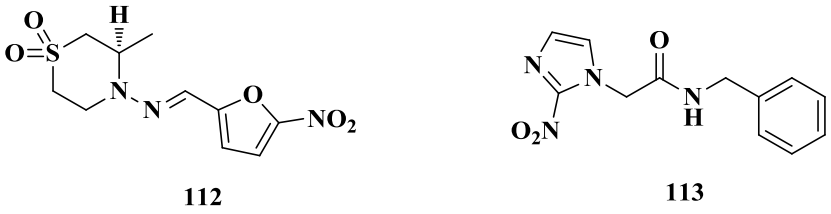
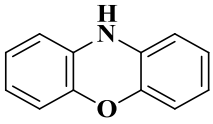


Figure 16: Structure of nifurtimox and benznidazole.[110]

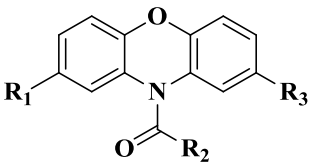
Phenoxazine derivatives consist of two benzene rings fused to a central oxazine structure (**Figure 17**). They are an important class of heterocycles and occur as the central core of some natural products like litmus and dactinomycin.[111][112] Phenoxazine-based analogues also span a wide spectrum of pharmaceutical properties, such as anti-tumour,[113] anti-inflammatory,[114] and anti-neurodegenerative activities.[115]



114

Figure 17: Structure of phenoxazine.

There have also been some significant efforts in early stage drug discovery based on phenoxazine derivatives as antitrypanosomal agents. Marcu *et al.* [116] reported they developed a groups of phenoxazine-derived chloroacetamides which showed inhibitory effect on *Leishmania major* growth. They also found Trypanothione Reductase is a target of chloroacetamides with tricyclic systems. Trypanothione Reductase is common to all parasites of the Trypanosomatidae family such as *T. cruzi* and *L. donovani*.[116] Their study provided opportunities to discover further potential antitrypanosomal agents based on phenoxazine scaffold.

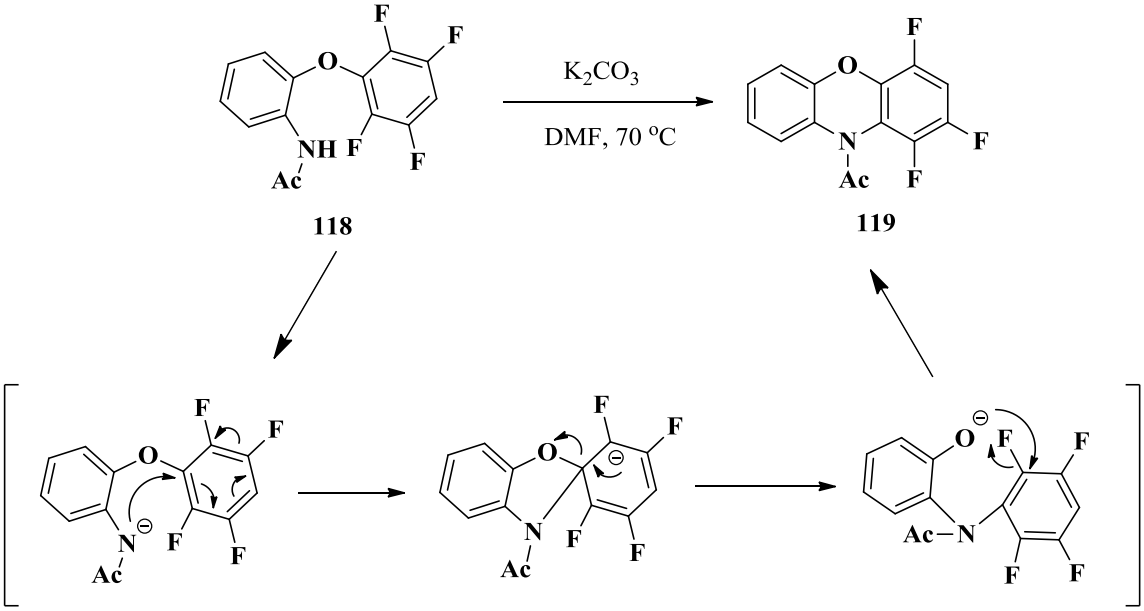


115 R = Cl,
116 R = H,
117 R = CF_3

Figure 18: Structure of phenoxazine-derived chloroacetamides.

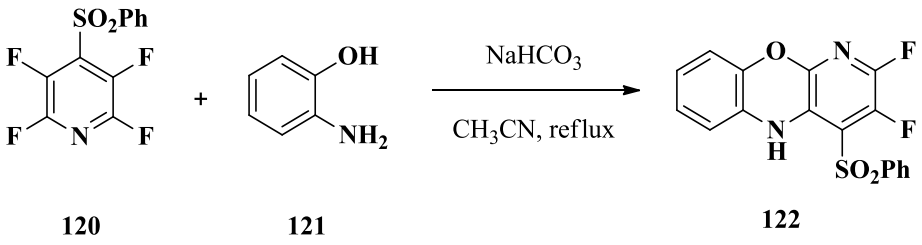
Several scientific publications have reported the formation of phenoxazine rings via nucleophilic substitution reactions of highly fluorinated compounds. In 1986 Kolchina *et al.*[117] first described the formation of the ether **118** by S_NAr substitution of pentafluoro-benzene and its rearrangement and cyclisation to form a phenoxazine ring (**Scheme 30**). The Smiles rearrangement is the intramolecular nucleophilic aromatic substitution reaction incorporating a heteroatom as the nucleophilic component.[118] Kolchina investigated the mechanism of the Smiles rearrangement occurring during the reaction on highly fluorinated arenes in the presence of base.[119] A temperature of over 70 ^oC is needed to activate the

migrating aromatic ring, and the presence of four electron-withdrawing fluorine atoms simultaneously provides activation.[117]



Scheme 30: Substitution and rearrangement of pentafluoropyridine by Kolchina.[117]

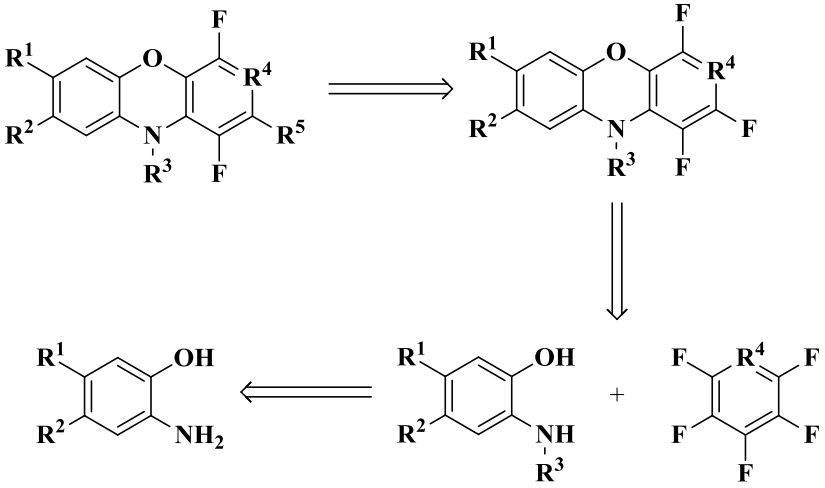
Sandford and co-workers [120] recently also reported a method to prepare pyrido[2,3-*b*] [1,4]benzoxazine system. In Sandford's work, formation of the phenoxazine began with the *para*-phenylsulfonyl-substituted pyridine **120** and ring closure across the *ortho*- and *meta*-positions occurred to form pyridobenzoxazine **122 (Scheme 31)**. They also unambiguously confirmed the structure by X-ray crystallography.



Scheme 31: Preparation of pyrido[2,3-*b*][1,4]benzoxazine system.[120]

3.2 Aims

The aim of the research outlined in this chapter was to synthesise an (aza-)phenoxazine pharmacophore starting from polyfluoro-aromatic compounds (**Scheme 32**). An exploratory study to understand possible anti-*Trypanosoma cruzi* activity was then to be conducted, with the hope of discovering structure-activity relationships based on a series of (aza-)phenoxazine scaffold structures.

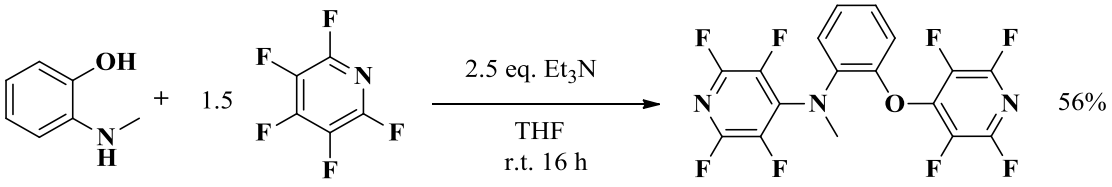


Scheme 32: Proposed synthesis routes to substituted-(aza-)phenoxazines.

3.3 Results and Discussion

3.3.1 Formation of Azaphenoxazine Scaffold

Our research started by studying the synthetic route outlined in **Scheme 33** using 2-(methylamino)phenol and pentafluoropyridine in the presence of triethylamine in dry THF at room temperature. An excess of pentafluoropyridine was used, and was expected to help drive the reaction forward smoothly to completion.



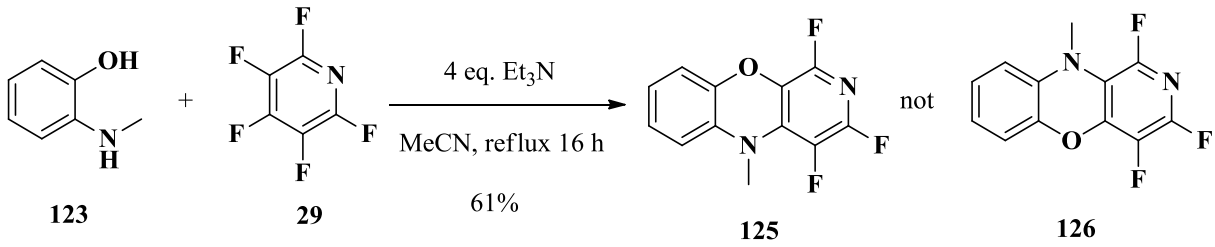
123 29 124

Scheme 33: Reaction of 2-(methylamino) phenol and pentafluoropyridine.

Surprisingly, however, di-pyridine **124** was obtained from the reaction in a yield of 56%. Four peaks were observed in the ^{19}F NMR spectrum as two AA'BB' patterns for each tetrafluoropyridine system, instead of the three peaks expected had cyclisation occurred to form the fluorinated azaphenoxazine. In addition, GC-MS gave a signal at m/z 421.1, which also matched the molecular weight of di-pyridine **124**.

The formation of di-pyridine **124** appears counter-intuitive in that addition of a second pyridine is faster than intramolecular ring closure. Assuming the more nucleophilic phenoxide adds first, the lone pair on the nitrogen must then be inclined to attack another molecule intermolecularly at the *para*-position, rather than at the *meta*-position in the same molecule during an intramolecular process. This suggests the electrophilicity of the *para*-carbon is the dominant controlling factor.

Modification of this reaction appeared attractive to explore a possible preparation of fused oxazine compounds. Pentafluoropyridine was therefore reacted with the same 2-(methylamino)phenol in a 1:1 ratio in the presence of four equivalents of triethylamine under reflux in acetonitrile. The strategy is illustrated in **Scheme 34**.



Scheme 34: Formation of a fluorinated benzo[*b*]pyrido[4,3-*e*][1,4]oxazine using 2-(methylamino)phenol and pentafluoropyridine.

Benzopyridooxazine was successfully isolated from the reaction and obtained as a white solid in good yield (61%). Hydrogen and fluorine atoms were easily assigned by ^{1}H- and ^{19}F NMR spectroscopy. Three peaks were observed in the ^{19}F NMR spectra, which suggested there were two substitutions occurring in the pentafluoropyridine. However, no obvious signal was obtained regarding the relative positions of the nitrogen and oxygen atoms as two nucleophilic substitutions had occurred. From the structure of 2-methylaminophenol, the hydroxyl group should be deprotonated in the presence of triethylamine and it was expected the negatively charged phenoxide would attack first at the 4-position of pentafluoropyridine.[121] The product was further characterised by X-ray crystallography. However, the X-ray crystal structure analysis confirmed the product to be 1,3,4-trifluoro-5-methyl-5*H*-benzo[*b*]pyrido[4,3-*e*][1,4]oxazine **125** in which the methylamino group was located *para* to the pyridine nitrogen (**Figure 19**).

Figure 19: Crystal structure of benzo[*b*]pyrido[4,3-*e*][1,4]oxazine **125**.

From the results of the X-ray diffraction study, four molecules were observed in the asymmetric unit (**Figure 20**). All of the molecules are slightly folded about the N-O crease with different angles, as $O_{(1)}/N_{(1)}$= 13.12(3)°, $O_{(1A)}/N_{(1A)}$= 13.31(4)°, $O_{(1B)}/N_{(1B)}$= 12.84(3)°, $O_{(1C)}/N_{(1C)}$= 9.42(3)° respectively. The crease was suggested to be caused by the flexible

single C-O or C-N bonds. In addition, there are obvious weak (Ar)C–H···F interactions between molecules as the distance between of $F_{(3)}$-$H_{(6A)}$ is 2.51 *Å*.

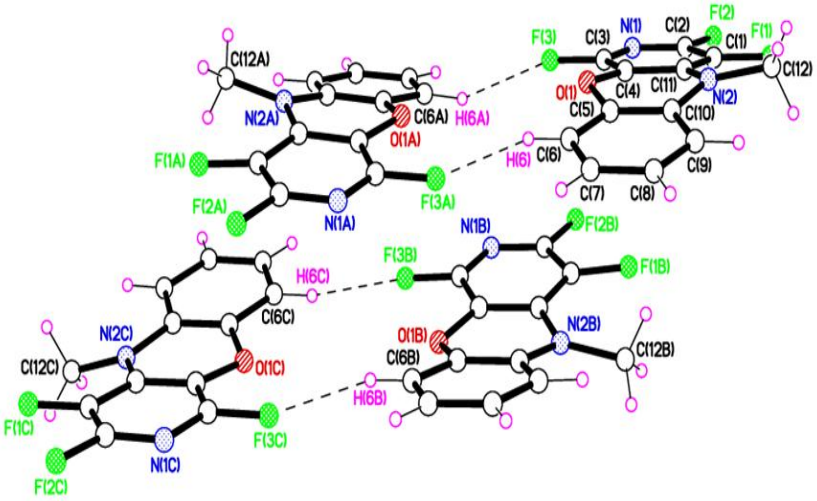


Figure 20: Four molecules in the asymmetric unit.

Formation of the benzo[*b*]pyrido[4,3-*e*][1,4]oxazine **125** is ascribed to initial attack by the phenoxide ion, followed by rearrangement in agreement with previous work by Kolchina's group. They investigated the reaction of 2-aminophenol with fluoroarenes and determined that a smiles rearrangement can occur and is controlled by temperature: the rearrangement is hard to take place in the presence of base at 20 ^{o}C or below, but at higher temperature the rearrangement proceeds easily.[119]

Benzopyridooxazine **125** was also characterised by ultraviolet–visible spectroscopy. In the observed spectrum, oxazine **125** showed a maximum UV absorption λ_{max} at 333 nm with shoulders at 312 nm and 366 nm (**Figure 21**). The preparation of the fused polycyclic oxazine **125** by flow chemistry was also studied, and the reaction monitored by UV-Vis spectroscopy in collaboration with the Christie group in the department. The formation of benzopyridooxazine **125** was successfully monitored in a bespoke microfluidic device constructed by additive manufacturing which incorporated in-line HPLC and UV-Vis analysis.[122]

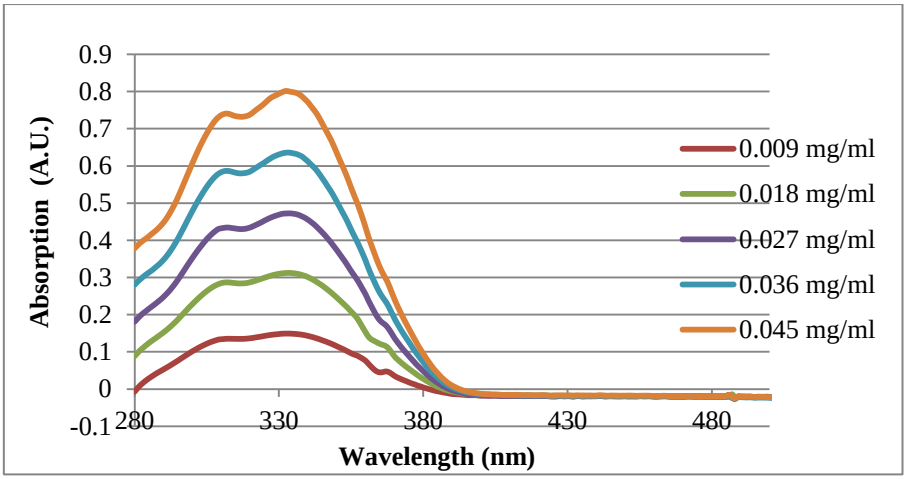
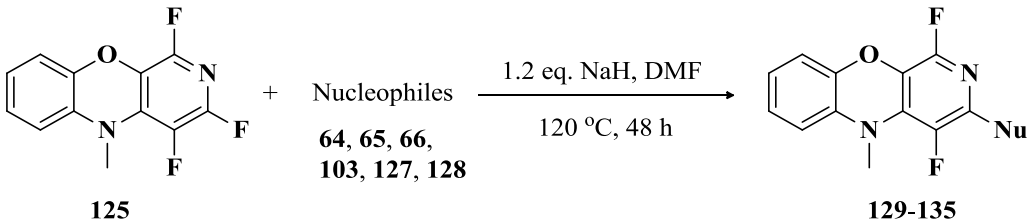


Figure 21: UV-Vis spectrum for oxazine **125**.

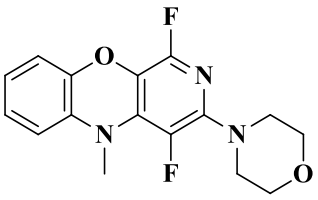
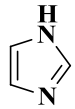
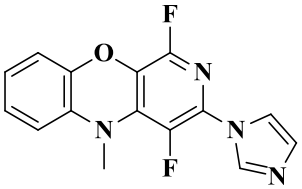
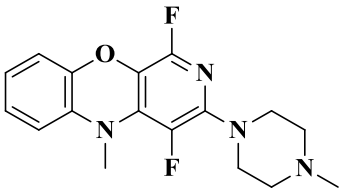
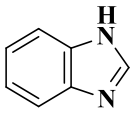
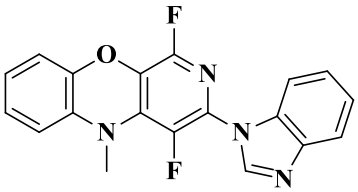
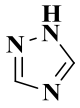
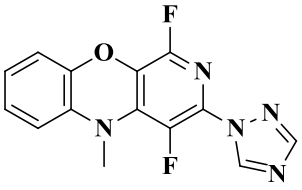
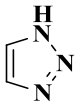
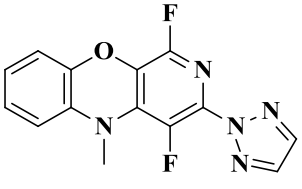
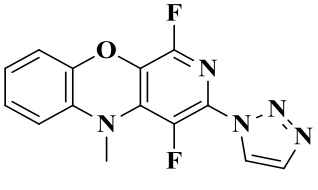
3.3.2 Substitutions on benzo[*b*]pyrido[4,3-*e*][1,4]oxazine system

To develop a set of compounds for biological screening based on the structure prepared above, six amine and aza-heterocyclic nucleophiles were investigated as reaction partners for oxazine **125** for further S_NAr substitution of fluorine. A screen of reaction conditions showed that use of NaH as base at 120 ^oC in DMF for 48 hours was the best method for the reaction (**Table 12**). Lower temperatures or shorter reaction times led to incomplete reaction as observed by TLC monitoring.

Table 12: Substitution reaction of oxazine 44a with different nucleophiles.



125 **129-135**

Nucleophiles	Product	Yield
 103	 129	59%
 64	 130	62%
 66	 131	58%
 65	 132	52%
 127	 133	50%
 128	 134	25%
	 135	24%

Seven 3-substituted benzo[*b*]pyrido[4,3-*e*][1,4]oxazines (**129**-**135**) were obtained successfully from the reactions with six nucleophiles. The compounds were formed in moderate to good yield and characterised by appearance of proton peaks due to the substituent moieties from the ^{1}H NMR spectra, as well as the loss of one fluorine signal from the ^{19}F NMR spectra.

To determine the position of substitution on the oxazines, the solid-state structure of imidazolyl **130** was characterised via X-ray diffraction. From the X-ray crystal structure analysis, it confirmed the imidazolyl **130** to be 1,4-difluoro-3-(1*H*-imidazol-1-yl)-5-methyl-5*H*-benzo[*b*] pyrido[4,3-*e*][1,4]oxazine which shows the imidazole moiety attacked C-3, the position opposite to the oxazine oxygen (**Figure 22**). The fold angle was 7.94° along the vector of benzo[*b*]pyrido[4,3-*e*][1,4]oxazine (oxazine ring), while twist angle between the imidazole ring and benzopyridooxazine was found to be 29.51°.

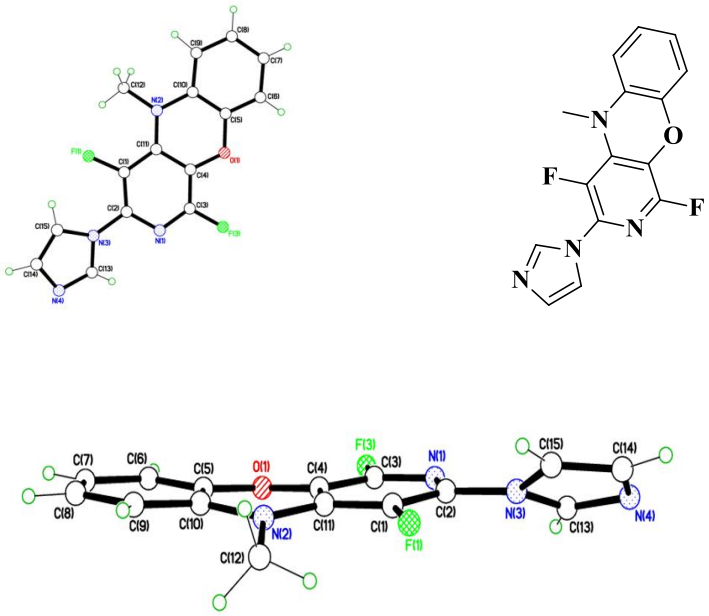
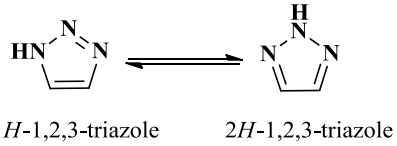


Figure 22: Crystal structure of imidazolyl benzopyrooxazine **130**.

Interestingly, triazoles **134** and **135** were prepared from the reaction of oxazine **125** and 1,2,3-triazole. Due to the annular tautomerism of 1,2,3-triazole, two tautomers, 2*H*-1,2,3-triazole and 1*H*-1,2,3-triazole can exist (**Scheme 35**), and could react with oxazine **125** through N-2 or N-1 affording the different isomers.[123] The difference between triazoles **134**

and **135** was identified from the ^{1}H NMR spectra. Two different NMR signals at 8.14 (1H, s) and 7.81 (1H, s) indicated the 1*H*-1,2,3-triazol-1-yl moiety, while the single peak found at 7.89 (2H, s) represents the equivalents protons in the 2*H*-1,2,3-triazol-2-yl substituted compound.



1*H*-1,2,3-triazole 2*H*-1,2,3-triazole

Scheme 35: Tautomerism of 1,2,3-triazole.[124]

3.3.3 Biological testing against Trypanosoma *cruzi* with benzopyrido-oxazines

The analogues prepared were then evaluated for their anti-*Trypanosoma cruzi* activity and toxicity testing using a cell-based assay by collaborators at the Laboratório de Protozoologia in Brazil (**Table 13**). Intracellular amastigotes of *T. cruzi* (Tulahuen strain) were used for anti-*Trypanosoma cruzi* activity screening and THP-1 cell line was employed for cytotoxicity assay. Two of the analogues, imidazolyl **130** and piperidinyl **131**, show much stronger inhibition of *T. cruzi* growth *in vitro* than does the reference compound benznidazol. The imidazolyl **130** displayed better activity and selectivity than the piperidinyl **131**. The novel **130** thus represents a new hit compound in the design of inhibitors of *Trypanosoma cruzi* and has potential for the treatment of Chagas disease.

Table 13: Anti-*Trypanosoma cruzi* activity data of benzopyridooxazines **129-135**.

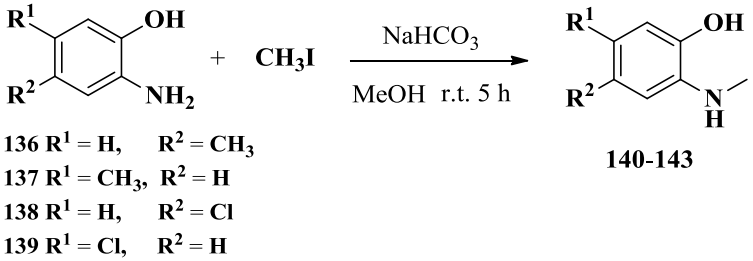
Compound	*T. cruzi* IC_{50} [µM]	THP-1 CC_{50} [µM]	Selectivity
129	-	-	-
130	5.76 (±1.38)	102.4 (±33.46)	17.77
131	6.35 (±2.12)	25.28 (±4.49)	3.98
132	-	-	-
133	-	-	-
134	-	-	-
135	-	-	-
Benznidazol 20 µM	10.18 (±0.30)	>500	>49.11

3.3.4 Synthesis of *N*-substituted 2-aminophenol derivatives

Due to the promising activity shown by the imidazolyl benzopyrooxazine **130**, it was decided to synthesis further benzopyridooxazine analogues substituted with an imidazole ring at the C-3 position. Further development of imidazolyl **130** was therefore investigated to generate compounds with different substitution patterns on the (aza-)phenoxazine ring. Several *N*-substituted 2-aminophenol derivatives were thus employed as precursors for the further formation of benzopyridooxazines and phenoxazines as the approach allowed for the introduction of substituent diversity. The study of the imidazolyl **130** derivatives also aimed to discover improved biological activity and lower toxicity based on the introduction or modification of various functional groups.

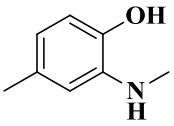
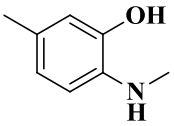
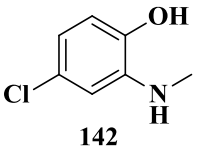
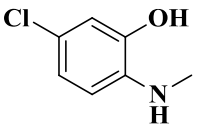
The investigation began with the methylation of 2-aminophenol derivatives on the amino group based on conditions reported by Matralis *et al.*[125] 2-Aminophenol derivatives were treated with iodomethane in the presence of sodium bicarbonate. Methanol was used as solvent instead of the DMF reported to improve the solubility of sodium bicarbonate (**Table 14**). Synthesis of *N*-substituted 2-aminophenol derivatives was to form imidazolyl (aza-) phenoxazine derivatives and to study the effect of electron-donating or electron-withdrawing substituent at different position on the phenoxazine system for its trypanocidal activity.

Table 14: Preparation of *N*-substituted 2-aminophenol derivatives.



136 $R^1 = H$, $R^2 = CH_3$
137 $R^1 = CH_3$, $R^2 = H$
138 $R^1 = H$, $R^2 = Cl$
139 $R^1 = Cl$, $R^2 = H$

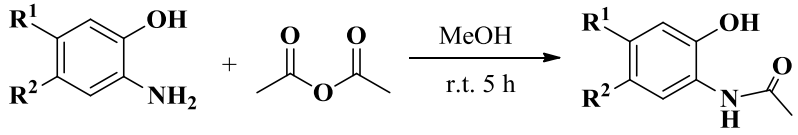
140-143

R^1	R^2	Product	Yield
H	CH_3	 **140**	61%
CH_3	H	 **141**	26%
H	Cl	 **142**	60%
Cl	H	 **143**	32%

Methylation of the 2-aminophenol derivatives was achieved and four analogues were prepared using methyl- and chloride-substituted aminophenols. The yields of the two 4-substituted-2-(methylamino)phenols **140** and **142** from the reactions were reproducibly around 60%, while another two 5-substituted-2-(methylamino)phenols **141** and **143**, were obtained in poor yield between 26-32%. It is not clear if there is any reason for the lower yield with the 5-substituted compounds.

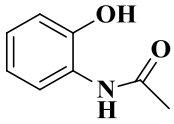
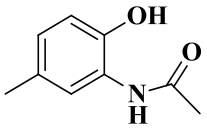
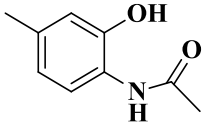
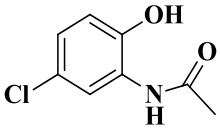
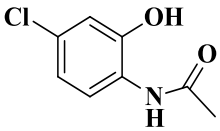
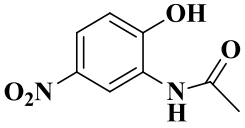
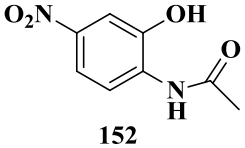
Another group of precursors based on *N*-acetylated 2-aminophenols were also prepared. *N*-substituted (hydroxyphenyl)acetamides were synthesised according to the method reported by Hartmann *et al.*[126] The reactions were conducted between 2-aminophenol derivatives and acetic anhydride in methanol (**Table 15**).

Table 15: Preparation of *N*-(substituted hydroxyphenyl)acetamides.



136 $R^1 = H$, $R^2 = CH_3$
137 $R^1 = CH_3$, $R^2 = H$
138 $R^1 = H$, $R^2 = Cl$
139 $R^1 = Cl$, $R^2 = H$
144 $R^1 = H$, $R^2 = NO_2$
145 $R^1 = NO_2$, $R^2 = H$

146-152

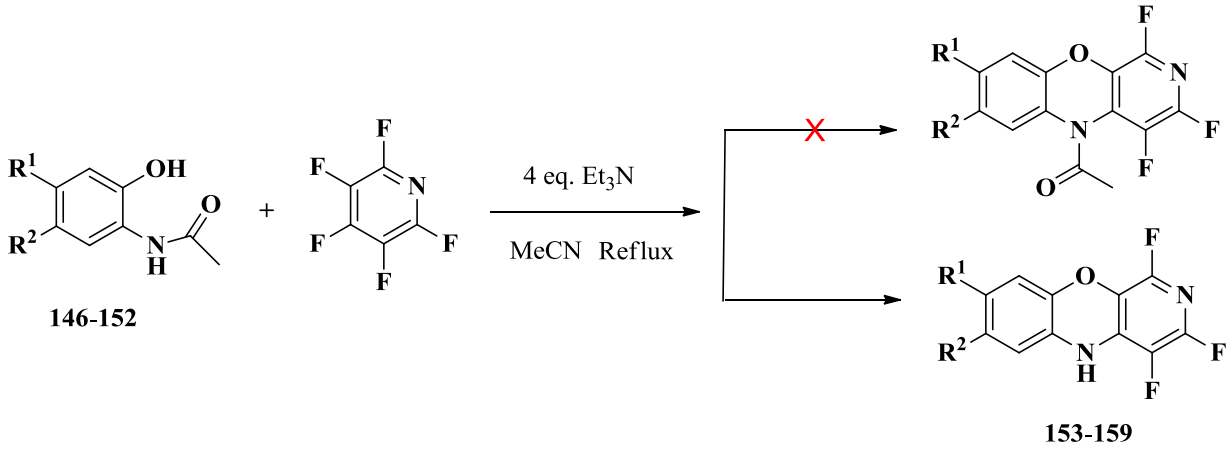
R^1	R^2	Product	Yield
H	H	 **146**	91%
H	CH_3	 **147**	93%
CH_3	H	 **148**	82%
H	Cl	 **149**	87%
Cl	H	 **150**	80%
H	NO_2	 **151**	98%
NO_2	H	 **152**	90%

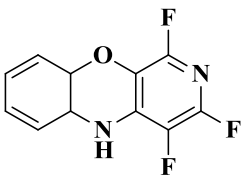
The analogues (**146-152**) were obtained in high yield without the need for further purification. Their structures were confirmed by the absence of one proton signal from the starting amino compound and the presence of the acetyl methyl signal around 2.06 ppm in the ^{1}H NMR spectra. Also the C=O signals were observed around 170 ppm in the ^{13}C NMR spectra.

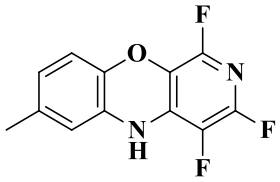
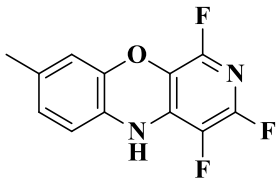
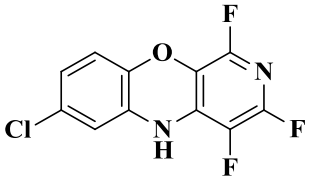
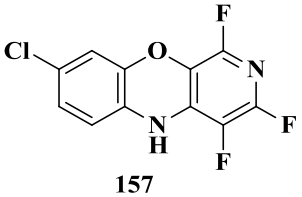
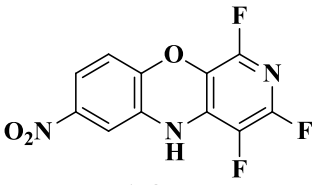
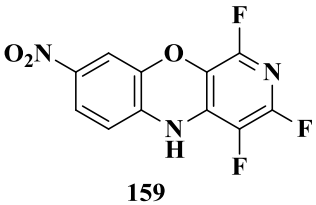
3.3.5 Formation of 3-substituted benzopyridooxazine derivatives

With the two different sets of *N*-substituted 2-aminophenol derivatives in hand, experiments were next investigated to form the corresponding benzopyridooxazines. The *N*-(substituted hydroxyphenyl)acetamides (**146-152**) were first treated with pentafluoropyridine following the methods used successfully to prepare oxazine **125**. The reaction method is outlined in **Table 16**.

Table 16: Formation of benzopyridooxazine using (2-hydroxyphenyl)acetamides and pentafluoropyridine.



R^1	R^2	Product	Yield
H	H	 **153**	60%

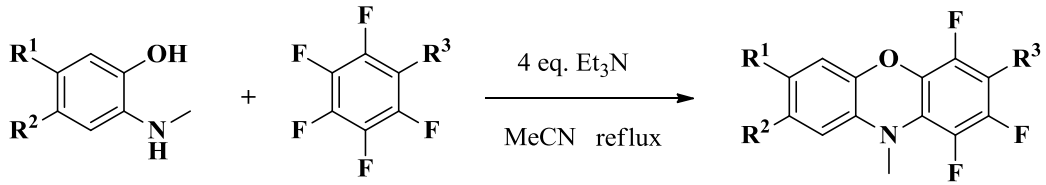
R^1	R^2	Product	Yield
H	CH_3	 **154**	34%
CH_3	H	 **155**	30%
H	Cl	 **156**	56%
Cl	H	 **157**	45%
H	NO_2	 **158**	51%
NO_2	H	 **159**	48%

Heating the acetamides with pentafluoropyridine in the presence of triethylamine led to formation of 1,3,4-trifluoro-5*H*-benzo[*b*]pyrido[4,3-*e*][1,4]oxazines. This was shown by the presence of three signals in the ^{19}F NMR spectra similar to those in benzopyridooxazine **125**, which suggested the same scaffold structure. However, the expected target compounds were unfortunately not obtained, and in all cases the acetyl group was lost as the signal for the methyl group at approximately 2.10 ppm were not present in the ^{1}H NMR spectra. In contrast,

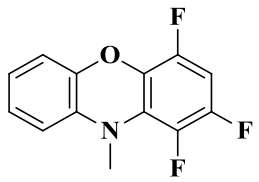
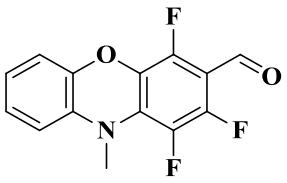
a singlet peak near 10.00 ppm was observed indicating the presence of a N-H in the molecule. The reason that acyl group was cleaved in the reaction is possibly due to the presence of triethylamine at high temperature.[127] Although the products were not prepared as expected, they still represented good scaffold structures for further synthetic modification.

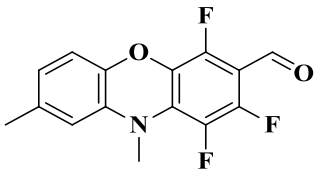
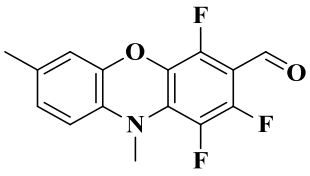
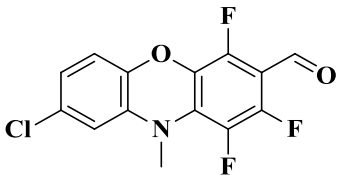
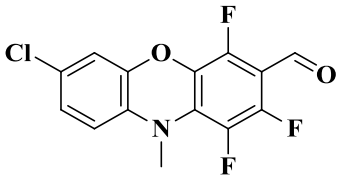
The group of N-methyl-substituted-2-aminophenol derivatives (**140**-**143**) were also reacted with pentafluorobenzene and pentafluorobenzaldehyde using the same conditions for the formation of fused oxazine **125**. This set of reactions involving different functional groups on both the *N*-substituted-2-aminophenol derivative and the fluorinated compounds, was expected to provide further examples for screening and modification. The synthetic route is illustrated in **Table 17**. Pentafluorobenzaldehyde was also chosen as a reactive electron-deficient arene in which the aldehyde group would act as a useful site for further functional group interconversion.

Table 17: Reaction of *N*-substituted-2-aminophenols and highly fluorinated arenes.



140-143 **9 R^3 = CHO** **160 R^3 = H** **161-166**

R^1	R^2	R^3	Product	Yield
H	H	H	 **161**	45%
H	H	CHO	 **162**	63%

R^1	R^2	R^3	Product	Yield
H	CH_3	CHO	 **163**	74%
CH_3	H	CHO	 **164**	50%
H	Cl	CHO	 **165**	59%
Cl	H	CHO	 **166**	59%

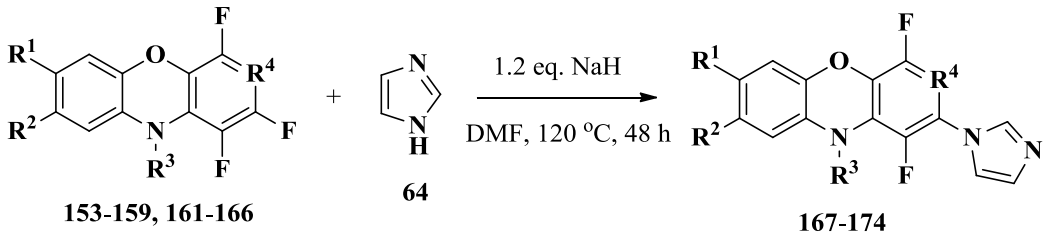
Six substituted benzopyridooxazines (**161-166**) were prepared in moderate to good yield from 45% to 74%. Three fluorine signals were observed in the ^{19}F NMR spectra, which suggested two substitutions took place on the starting materials. All of the products exhibited analytical and spectroscopic data fully in accord with their structures.

3.3.6 Synthesis of further derivatives of the imidazolyl benzopyridooxazine

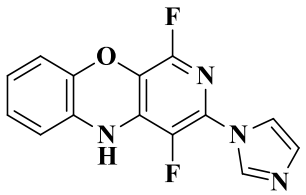
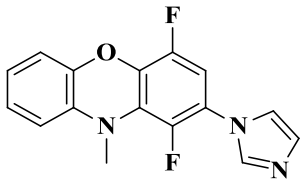
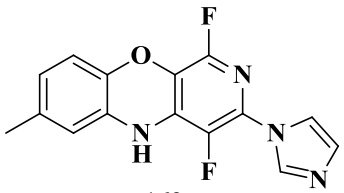
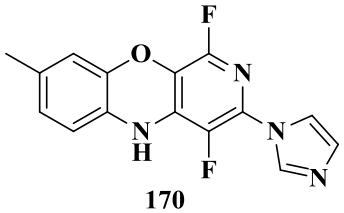
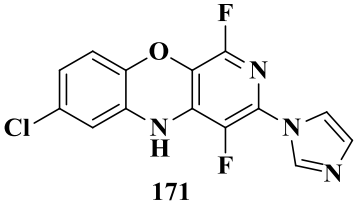
Further reactions introduced the imidazole moiety to each of the thirteen new benzopyidooxazine **125** analogues prepared through S_NAr substitution as in the preparation of imidazolyl **130**. Imidazolyl benzopyidooxazine **130** was used as hit compound in this study as the imidazolyl **130** displayed better activity and lower toxicity than the piperidinyl

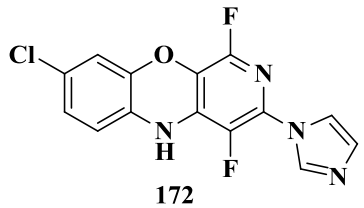
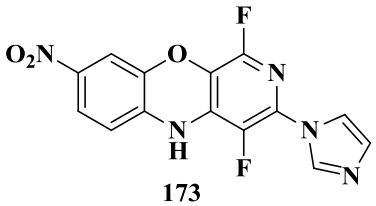
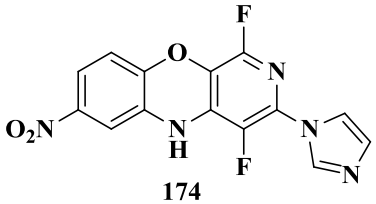
derivative **131**. The synthetic route employed sodium hydride as base in presence of DMF as solvent at 120 ^{o}C (**Table 18**).

Table 18: Synthesis of further derivatives of imidazolyl benzopyridooxazine **130**.



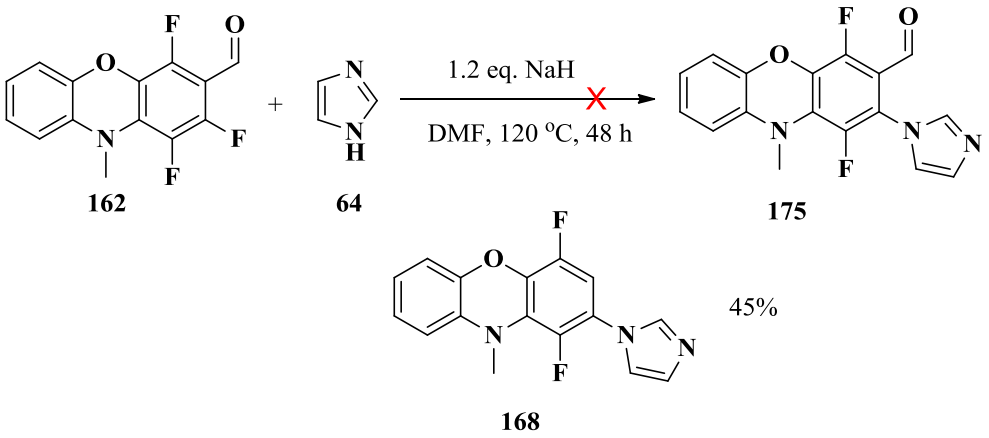
153-159, 161-166 + 64 → 167-174

R_1	R_2	R_3	R_4	Product	Yield
H	H	H	N	 **167**	62%
H	H	CH_3	C-H	 **168**	45%
H	CH_3	H	N	 **169**	55%
CH_3	H	H	N	 **170**	50%
H	Cl	H	N	 **171**	66%

R_1	R_2	R_3	R_4	Product	Yield
Cl	H	H	N	 **172**	49%
H	NO_2	H	N	 **173**	54%
NO_2	H	H	N	 **174**	47%

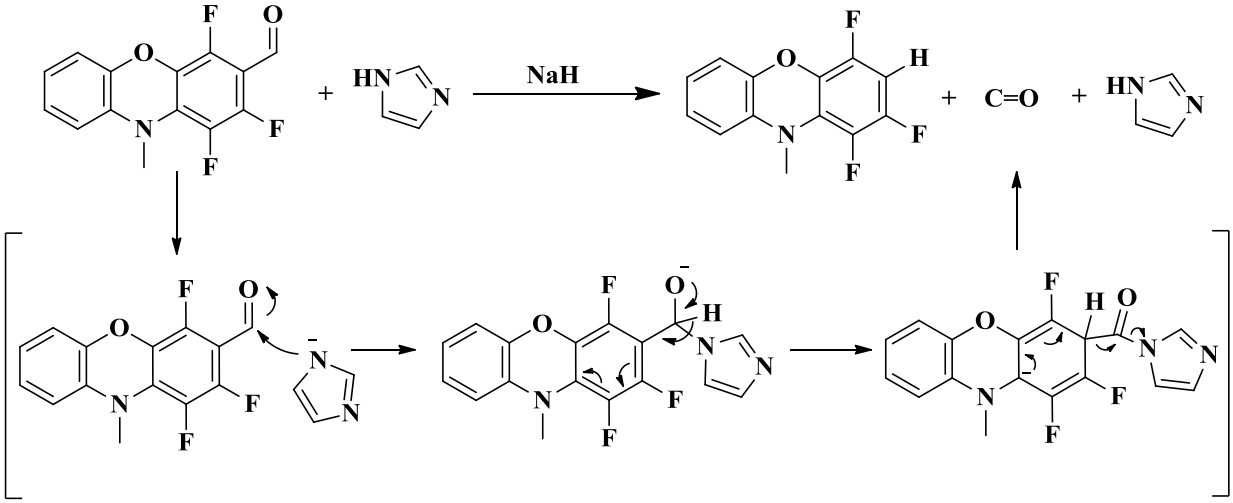
Eight derivatives (**167**-**174**) were obtained successfully from the reactions in moderate yield from 45% to 66%. The imidazolyl moiety signal was observed clearly in the ^{1}H NMR spectra and two doublet peaks were found in the ^{19}F NMR spectra as well. The NMR spectroscopy thus confirmed the occurrence of substitution in the benzopyridooxazine systems.

Aldehyde **162** and its analogues were also treated with the aforementioned conditions. Unfortunately, the expected aldehyde product was not obtained from the reaction (**Scheme 36**). Instead, compound **168** was identified as the final product by NMR spectroscopy, when aldehyde **162** was reacted with imidazole. It revealed that the aldehyde group was surprisingly cleaved from the scaffold structure during the reaction.



Scheme 36: Reaction of imidazole and aldehyde **162** using sodium hydride.

This reaction showed a similar result to the attempted formation of partially fluorinated benzofuran in Chapter 2. Imidazole was deprotonated by sodium hydride and presumably acted as a hard nucleophile, which preferred to react with the partially cationic carbonyl carbon of the aldehyde. Hydride transfer from the alkoxide with aldehyde adduct to C-4 of the fluoroarene ring was possibly involved in the reaction (**Scheme 37**).

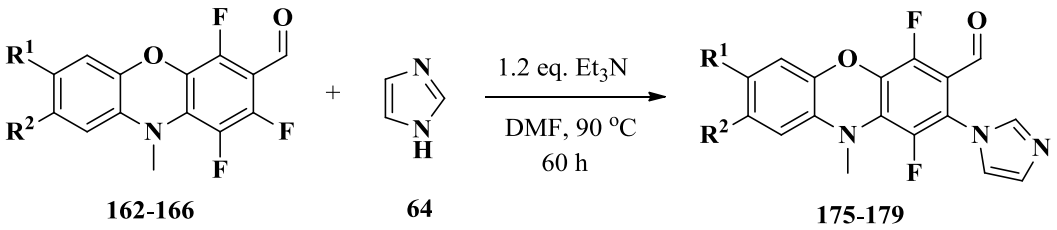


Scheme 37: Proposed mechanism for the synthesis of compound **186** from **162**.

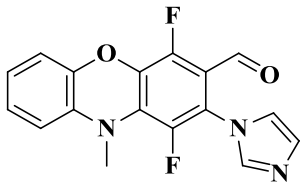
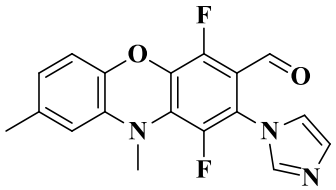
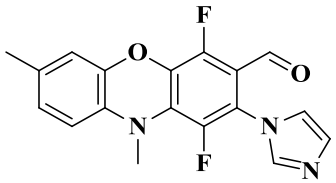
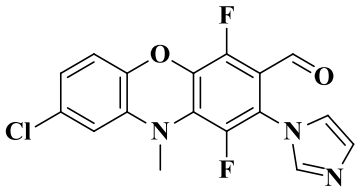
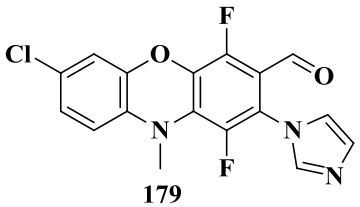
Hence, the reaction conditions were modified to discover the possibility to prepare the desired aldehyde products. Sodium hydride was replaced by the less basic triethylamine and the reaction temperature was also decreased from 120 °C to 90 °C as the boiling point of

triethylamine was 89 °C. After 60 hours heating, traces of starting materials were observed by TLC monitoring, which indicated the reaction was nearly complete. The approach is illustrated in the **Table 19**.

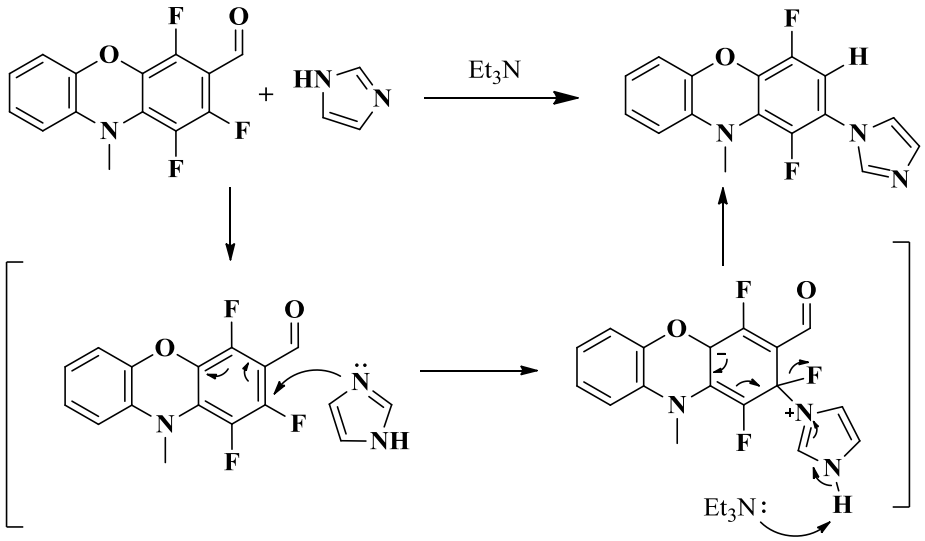
Table 19: Synthesis of 2-imidazolyl phenoxazine-3-carboxaldehydes.



162-166 64 175-179

R^1	R^2	Product	Yield
H	H	 175	54%
H	CH_3	 176	54%
CH_3	H	 177	53%
H	Cl	 178	49%
Cl	H	 179	43%

Under these modified conditions, aldehyde **175** and its derivatives were successfully prepared in a yield between 43-54%. A singlet around 9.59 ppm in the ^{1}H NMR spectra indicated the aldehyde group was retained in the phenoxazine scaffold. There was an obvious difference in reaction outcome depending on whether triethylamine or sodium hydride, when they were used as base in the reaction. Triethylamine was not strong enough to deprotonate imidazole to form a hard nucleophile, and the reaction then preferred to take place substitution of fluorine at 2-position (**Scheme 38**).



Scheme 38: Proposed mechanism for the synthesis of aldehyde **175** and its derivatives.

3.3.7 SAR study on the trypanocidal activity of further derivatives of the imidazolyl benzopyridooxazine

Imidazolyl benzopyridooxazine **130** and its analogues were then screened *in vitro* against *T. cruzi* (Tulahuen strain) and their cytotoxicity against THP-1 cell line was also evaluated in order to calculate the selectivity index of the compounds (**Table 20**).

Table 20: Anti-*Trypanosoma cruzi* activity data of imidazolyl benzopyridooxazine **130** and its derivatives.

Compounds	T. *cruzi* IC_{50} [µM]	THP-1 CC_{50} [µM]	Selectivity
130	5.76 (±1.38)	102.4 (±33.46)	17.77
167	37.46 (±2.54)	>500	>13.35
168	12.70 (±0.67)	>500	>39.37
175	2.62 (±0.27)	>500	>190.83
169	-	-	-
170	-	-	-
171	-	-	-
172	-	-	-
173	-	-	-
174	-	-	-
176	1.93 (±0.26)	>500	>259.06
177	-	>500	
178	1.51(±0.21)	124.2 (±7.90)	82.25
179	33.60 (±3.47)	>500	14.88
Benznidazol 20 µM	10.18 (±0.30)	>500	>49.11

The introduction of an aldehyde group or a hydrogen atom into the 3-position of the phenoxazine ring affected the activity significantly. Aldehyde **175** exhibited increased anti-*T.cruzi* activity by 2-fold compared with its precursor **130** whereas compound **168** containing a CH instead of a nitrogen atom at the 3-position led to decreased activity by 2-fold. Although the aldehyde group is an undesirable functional group because of off-target toxicity in medicinal chemistry, it indicated that introduction of a similar electron-withdrawing group to C-3 might increase the effect of *T. cruzi* growth inhibition. The aldehyde can also be converted readily into other functionality to allow further screening and structure activity studies to be undertaken.

Interestingly, among all of these candidates, the most active was 8-substituted aldehyde analogues (**178** and **176**), which had 7-/5-fold better anti-*T. cruzi* activity, as well as 2-/5-fold higher selectivity in comparison to the reference drug benznidazol, respectively. Comparing

178 and **176**, the chloro-substituted **178** displayed a slight increase in potency over the methyl-substituted derivative **176**. On the other hand, 7-substituted aldehyde analogues were a different story. The methyl-substituted **177** unfortunately showed no activity, while the chloride-substituted analogue **179** was 13-fold less active than its precursor **175**. These results suggested 7-substitution on aldehyde **175** analogues had a negative impact on activity.

Methylation at *N*-10 also affected the activity for phenoxazine derivatives significantly as compound **167** showed a 6-fold decrease in the anti-*T. cruzi* effect compared to compound **130**. Also the combination of 7-/8-substitution and replacement of the methyl group from *N*-10 atom by H atom resulted in a loss of activity for compound **169**-**174**. This revealed that loss of the methyl group attached to *N*-10 is not tolerated and leads to a loss of activity.

3.4 Conclusion

The work outlined in this chapter has shown the development of (aza-)phenoxazine scaffold structures from highly fluorinated arene precursors with a number of bis-nucleophilic aminophenol derivatives, using nucleophilic aromatic substitution (S_NAr). The fluorinated heterocycles prepared were tested against *Trypanosoma cruzi* parasites and imidazole-substituted derivatives demonstrated appreciable anti-*T. cruzi* activity.

Two different sets of *N*-substituted 2-aminophenol precursors were synthesised from 2-aminophenols with iodomethane and acetic anhydride separately. *N*-Methyl-substituted 2-aminophenols (**140**-**143**) were isolated in poor to moderate yields (26-61%), while *N*-(substituted hydroxyphenyl)acetamides (**146**-**152**) were geneated with good yields (80-98%).

A variety of fused oxazines (**125**, **153**-**159**, **161**-**166**) were synthesised successfully by a nucleophilic aromatic substitution ring-closing process involving Smiles rearrangement of the initial adduct formed by reaction with the *N*-substituted 2-aminophenols. The cyclisation products were obtained in a low to good yield (30-74%). X-ray diffraction analysis confirmed

the structure of benzopyridooxazine **125** and indicated rearrangement of the initially formed diaryl ether had occurred.

Different substituents were further introduced into the benzopyridooxazine ring at the 3-position via substitution in the presence of sodium hydride at 120 ^{o}C. Impressively, imidazole-substituted benzopyridooxazine **130** (IC_{50} = 5.76 µM) and methylpiperazine substituted benzopyridooxazine **131** (IC_{50} = 6.35 µM) demonstrated good inhibition against *Trypanosoma cruzi* parasites. A variety of imidazole-substituted benzopyridooxazines and phenoxazines (**167**-**179**) were obtained in moderate yield (43-66%). Among these imidazole-substituted compounds, **176** (IC_{50} = 1.93 µM) and **178** (IC_{50} = 1.51 µM) showed 5-/7-fold better anti-*Trypanosoma cruzi* activities than the current drug benznidazol as reference.

Chapter 4: Formation of 4-Substituted Tetrafluorobenzaldehyde Hydrazones and Their Anti-*Trypanosoma. brucei. rhodesiense* Activity

4.1 Introduction

Hydrazones and their derivatives play a significant role in organic chemistry. The functional diversity of the azomethine group provides a range of different chemistries and applications (**Figure 23**).[128] From the triatomic structure C=N–N, there is a nucleophilic imine, an amino-type (more reactive) nitrogen, and an imine carbon that has both electrophilic and nucleophilic character. In addition, an acidic N–H proton sometimes provides opportunities for H-bonding. All of these features give the hydrazone group its wide range of chemical properties. Also the intrinsic nature of the C=N double bond renders the molecule switchable resulting in configurational isomerism.

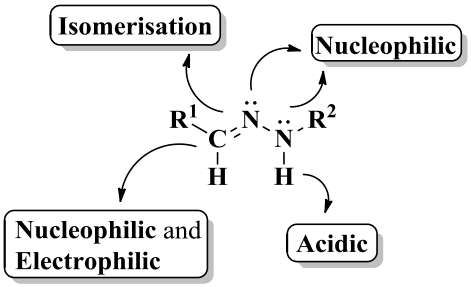
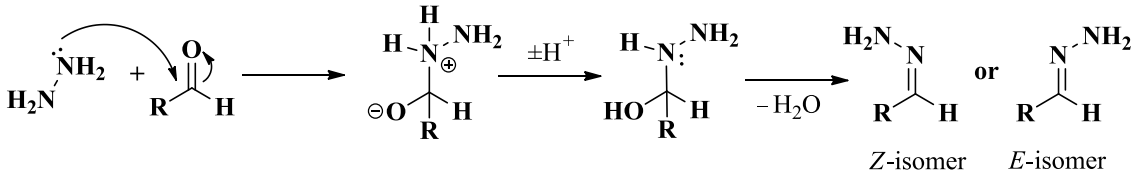


Figure 23. The structural and functional diversity of the hydrazone group.[128]

Hydrazones are easily formed by the replacement of oxygen in ketones or aldehydes with the hydrazine functional group (**Scheme 39**). The reaction usually occurs without catalysis. Hydrazines are classed as α-nucleophiles and have high reactivity due to their elevated HOMO level.



Scheme 39. Mechanism of hydrazone formations.

In recent years, hydrazones have attracted significant attention because of their proposed use in interesting biological and medicinal applications. Compounds containing the hydrazone structure are of importance for drug design and discovery.[129] The synthesis of various benzaldehyde phenylhydrazones has been reported in the literature recently and the compounds found to be active as antimicrobials (**Figure 24**).[130][131][132][133] These researches display good examples for the rational drug design as anti-trypanosomal candidate using the scaffold of benzaldehyde phenylhydrazone.

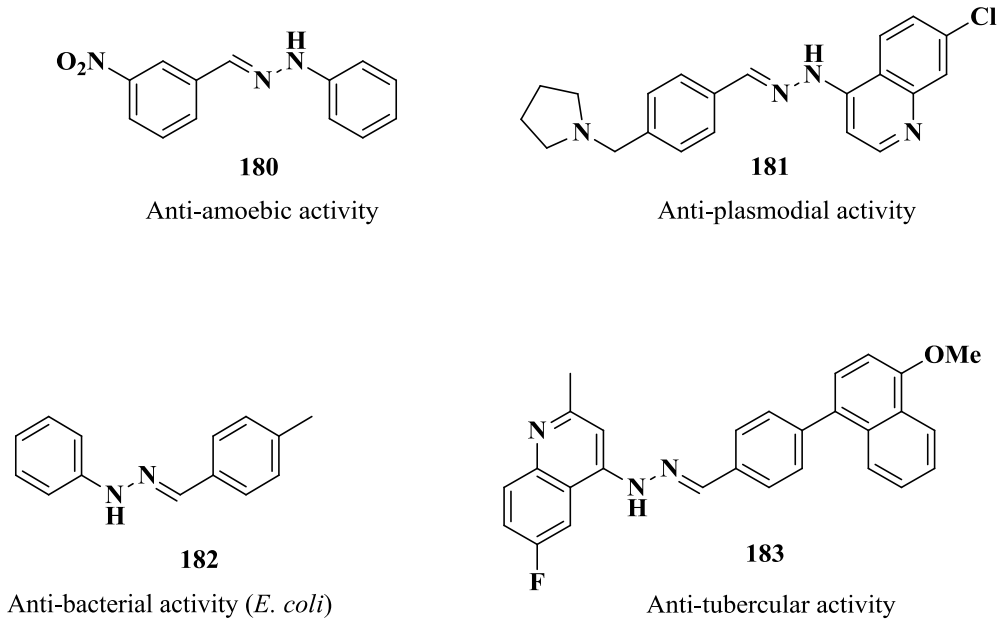
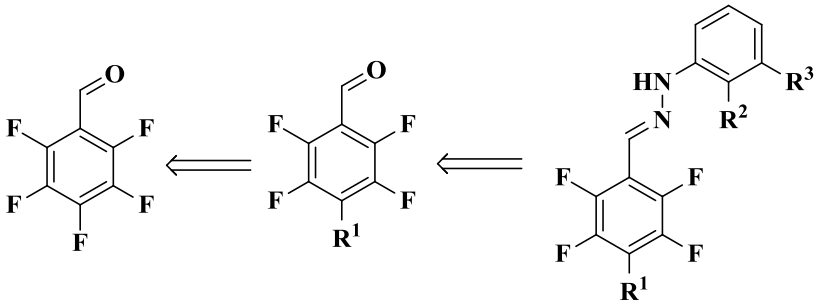


Figure 24. Structures of biologically active hydrazones.

4.2 Aims

The aim of the project focused in this chapter was to further study the scope of the fluorinated benzaldehyde derivatives in hand to prepare benzaldehyde phenylhydrazones (**Scheme 40**) and test these for biological activity as well as exploring the formation of other possible scaffold structures such as benzisoxazoles and quinazolinones by ring-closing reactions of the *ortho*-fluoroaldehyde functionality.



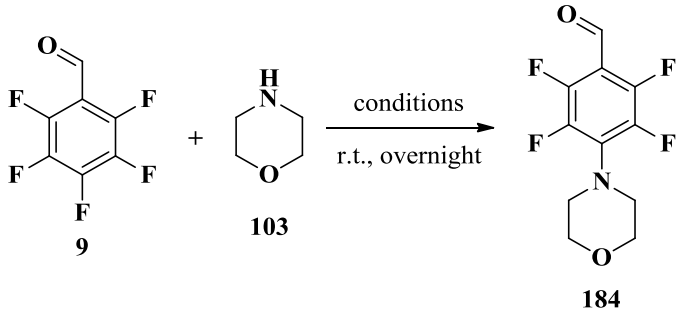
Scheme 40: Retrosynthesis of the target fully substituted core.

4.3 Results and Discussion

4.3.1 *para*-Substitution on pentafluorobenzaldehyde

Following the previous study in Chapter 2, pentafluorobenzaldehyde was reacted with two equivalents of morpholine in THF at room temperature, effecting nucleophilic substitution at the 4-position. The morpholine ring is a widespread structural motif in drug discovery and some compounds containing a morpholine moiety displayed good anti-*T.brucei* activity.[134] Thus, morpholine was employed here with rational design. The yield of aldehyde **184** however was quite low at 36% and starting material was mostly recovered. To improve the yield of the product, the conditions were optimised (**Table 21**).

Table 21: Various reaction conditions tested to synthesise aldehyde **184**.



9 **103** **184**

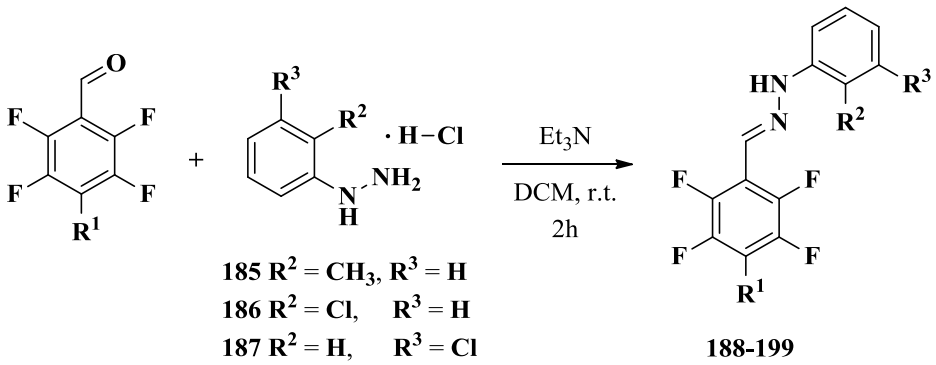
Entry	Conditions	Yield
1	2 eq. morpholine, THF	36%
2	1 eq. morpholine and 2 eq. Et_3N, DMF	44%
3	2 eq. morpholine, DMF	71%

After screening a number of different conditions, DMF was found to be the best solvent with two equivalents of morpholine employed in the reaction (one functions as a base to neutralise the HF generated). Under these conditions, the yield of compound **184** was improved to 71%, which represents a good yield for the substitution at room temperature.

4.3.2 Synthesis of fluorinated aldehyde phenylhydrazones

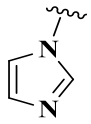
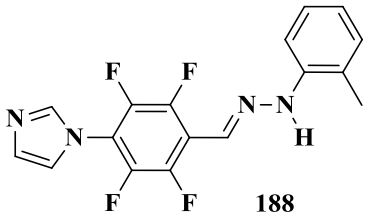
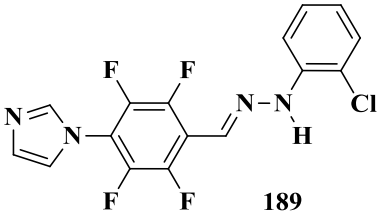
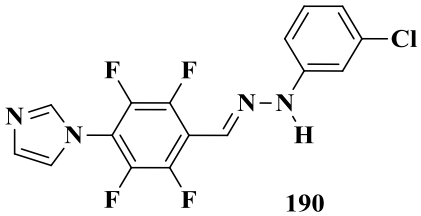
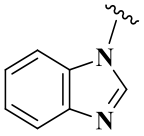
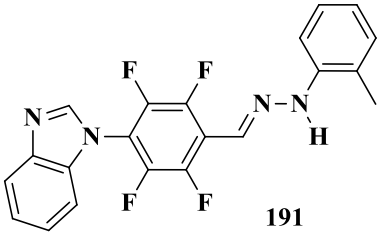
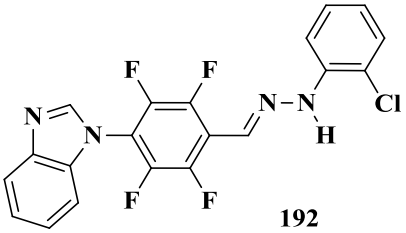
Four 4-substituted compounds (**67**, **68**, **69** and **184**) obtained with different function groups were selected in the study as all possess an aldehyde group. It was of interest to convert these compounds to hydrazone structures. Different hydrazine salts were utilized in the reactions and it was planned to prepare a set of hydrazone compounds for biological testing. Hydrazines are generally stored as stable crystalline salts, which are easy to handle. However, the free base must be generated in the reaction mixture to allow the hydrazine to function as a nucleophile. One equivalent of triethylamine was thus required to generate the free base from the hydrazine hydrochloride salt. The reactions were conducted at room temperature in dichloromethane for 2 hours and a sole product was observed from TLC analysis. The synthetic route is shown in **Table 22**.

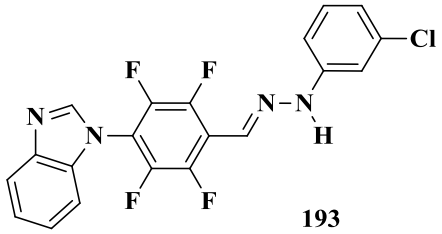
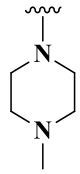
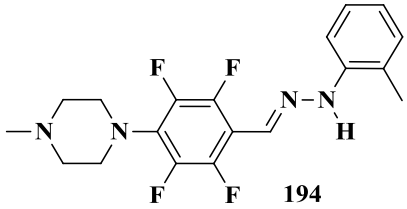
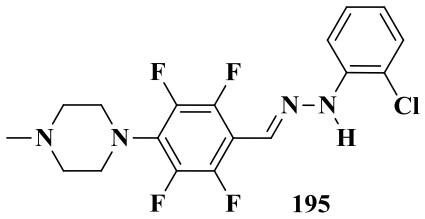
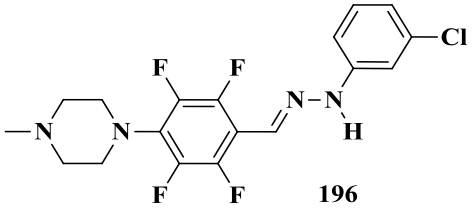
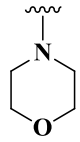
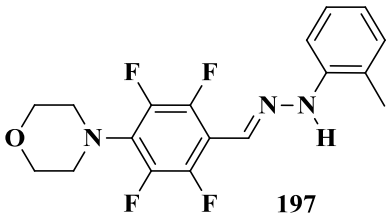
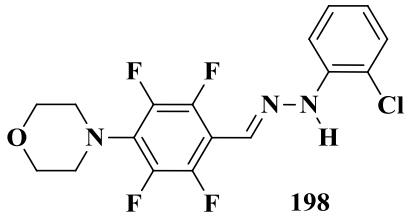
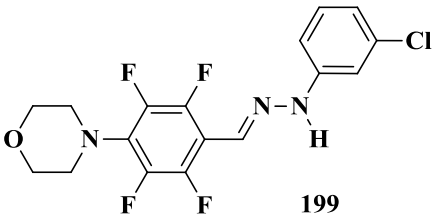
Table 22: Four fluorinated compounds reacted with different hydrazine salts.



185 $R^2 = CH_3$, $R^3 = H$
186 $R^2 = Cl$, $R^3 = H$
187 $R^2 = H$, $R^3 = Cl$

188-199

R^1	R^2	R^3	Product	Yield
	CH_3-	H-	 **188**	77%
	Cl-	H-	 **189**	75%
	H-	Cl-	 **190**	80%
	CH_3-	H-	 **191**	81%
	Cl-	H-	 **192**	85%

R^1	R^2	R^3	Product	Yield
	H-	Cl-	 193	79%
	CH_3-	H-	 194	63%
	Cl-	H-	 195	67%
	H-	Cl-	 196	69%
	CH_3-	H-	 197	76%
	Cl-	H-	 198	77%
	H-	Cl-	 199	74%

A dozen phenylhydrazone derivatives were obtained in good to excellent yields and were generally easy to purify by silica column chromatography. However, the piperazinyl-substituted hydrazone **194** could not be isolated pure when it was subjected to silica column chromatography. The mixture formed suggested the compound was easily decomposed by silica, but it was eventually purified by recrystallisation in moderate yield. Piperazinyl substituted analogues **195** and **196** were separated via by column chromatography on silica gel, although they also did not provide as a good yield as other substituted hydrazones. Possibly the more polar tertiary amine group in the piperazine ring is binding strongly to silica surface. Piperazinyl **195** and **196** tend to remain in the flash column and be difficult to be washed out.

In this part of the research, it was attractive to investigate hydrazine salts with either an electron-withdrawing or electron-donating group at the same position or different positions on the benzene ring. Different hydrazines were employed in the study to discover the effect when different groups were added to the phenol ring to modify electronic or steric properties. A weakly electron-donating group (CH_3-) and a weakly electron-withdrawing group (Cl-) were employed in the study. From the reactions above, there was no obvious difference in the yields when methyl group or choride atom was introduced into the benzene ring. Also for all the cases, only one product was obtained from the reaction and it indicated one isomer was formed. Thus, the stereo-structure of the aldehyde phenylhydrazones was of interest.

From the ^{1}H NMR spectra, proton resonance representing the NH group appeared around 10.0 ppm for all of the phenylhydrazones. There is no obvious evidence to recognize the configuration as *E*-isomer or *Z*-isomer from the proton NMR spectra. Further X-ray diffraction analysis identified the *E*-isomer by confirming the morpholinyl **198** to be (*E*)-4-(4-((2-(2-chlorophenyl)hydrazono)methyl)-2,3,5,6-tetrafluoro-phenyl)morpholine (**Figure 25**).

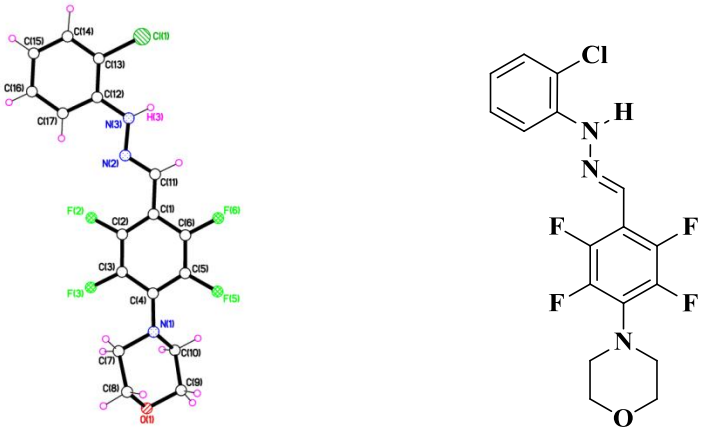


Figure 25: Crystal structure of morpholinyl **198**.

From the results of the X-ray diffraction study, morpholinyl **198** was the asymmetric unit. The molecule interestingly is detected to be curved as banana-shaped rather than twisted with a dihedral angle between rings C(1) > C(6) and C(12) > C17) = 10.40(3)°. A strong H-bond N(3)–H(3)···O(1') gives rise to zig-zag chains parallel to weaker C–H···F/Cl/O interactions, which give an overall 3D network of weak and strong H-bonds (**Figure 26**).

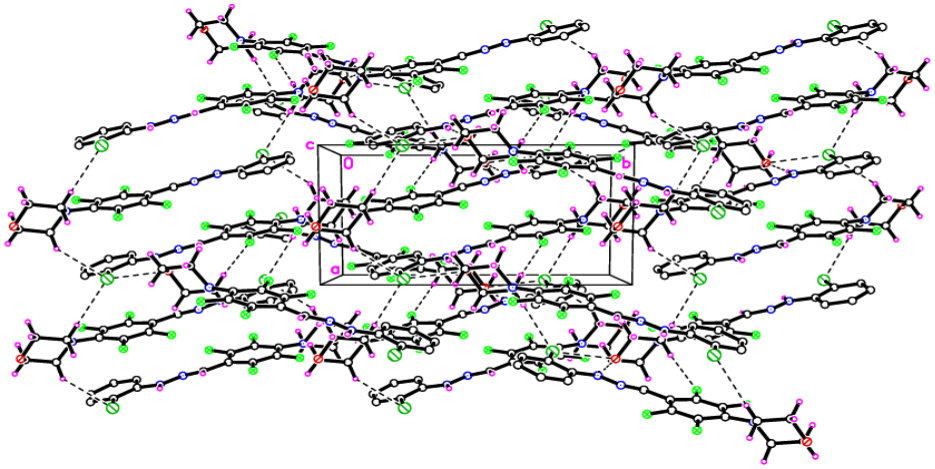


Figure 26: Three molecules in the asymmetric unit.

4.3.3 Biological testing of benzaldehyde phenylhydrazones against *T. b. rhodesiense*

As benzaldehyde phenylhydrazones have previously demonstrated good antimicrobial activity,[130][131][132][133] the derivatives **188**-**199** prepared were also evaluated for anti-*Trypanosoma brucei* activity and toxicity *in vitro* using a cell-based assay carried out by our collaborating partner (**Table 23**). *T. b. rhodesiense* (STIB 900) were used for anti-*Trypanosoma brucei* activity screening and L6 rat skeletal muscle myoblasts were employed for cytotoxicity assay.

Table 23: Evaluation of benzaldehyde phenylhydrazones against *T. b. rhodesiense*.

Compound	*T. b. rhodesiense* IC_{50} [µM]	Cytotox L6 IC_{50} [µM]	Selectivity
188	24.92	206.12	8.27
189	112.48	299.15	2.66
190	9.84	136.82	13.90
191	15.12	43.96	2.91
192	16.89	57.55	3.41
193	14.51	30.34	2.09
194	4.85	8.27	1.70
195	1.63	15.40	9.42
196	4.30	19.31	4.49
197	12.64	96.56	7.64
198	33.84	131.24	3.88
199	14.33	109.47	7.64
Melarsoprol	0.01		
Podophyllotoxin		0.02	

Most candidates showed activity against *T. b. rhodesiense*, which indicated that benzaldehyde phenylhydrazone is good scaffold structure for synthesisins anti-*Trypanosoma brucei* agents. Compound **190** with an imidazolyl group displayed moderate trypanocidal activity with an IC_{50} value of 9.84 µM. However, its imidazolyl analogues, 2-methyl-substituted **188** and 2-

chloro-substituted **189**, in contrast, displayed a decrease in potency by 3-fold and 11-fold, respectively. Benzimidazolyl analogues also showed moderate anti-*Trypanosoma brucei* activity. Surprisingly, there is no obvious difference in activity among the three analogues (**191**, **192** and **193**), which suggests alteration of the functional groups and positions on the benzene ring did not have an obvious effect on their trypanocidal activities for benzimidazolyl analogues. Morpholinyl analogues also demonstrated similar anti-*Trypanosoma brucei* activities to the benzimidazolyl analogues, although the 2-chloro-substituted analogue **198** was 3-fold less active than the other two morpholinyl analogues **197** and **199**.

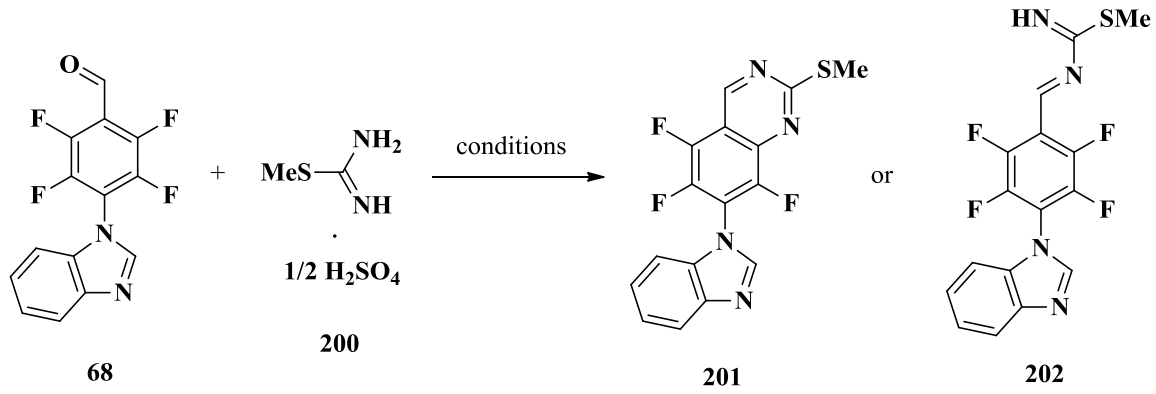
Interestingly, compound **195**, prepared bearing a 1-methyl-piperazinyl moiety demonstrated respectable trypanocidal activity with an IC_{50} value of 1.63 µM and moderate selectivity after cytotoxicity assay. The other two 1-methyl-piperazinyl analogues, **194** and **196** also showed good activity against *T. b. rhodesiense*. It is worth mentioning that compound **194** gave IC_{50} value of 8.27 µM against the normal cell line, indicating its moderate cytotoxicity.

4.3.4 Formation of partially fluorinated quinazoline

Quinazolines are noteworthy in medicinal chemistry, because of their wide spectrum of biological properties.[135] There are several approved drugs containing the quinazoline structure on the market such as gefitinib and prazosin.[51][135] To further study the scope of the fluorinated benzaldehyde derivatives prepared, quinazoline scaffolds were next considered possible targets.

Preparation was attempted from aldehyde **68** as the starting material. The reaction was investigated between aldehyde **68** and *S*-methylisothiouronium sulfate. It was hoped that the urea group in *S*-methylisothiouronium sulfate would both condense with the carbonyl group and effect S_NAr reaction on the neighbouring carbon. The proposed synthetic route is illustrated in (**Table 24**).

Table 24: Reaction of compound **68** and *S*-methylisothiouronium sulfate.



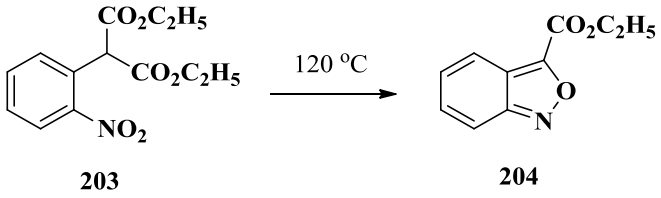
Entry	Solvent	Conditions	Results
1	EtOH	NaH, reflux, 24 h	11% compound **201** and other impurities
2	DMF	NaH, 80 °C , 24 h	13% compound **201** and other impurities
3	DMF	K_2CO_3, 80 °C, 24 h	14% uncyclised compound **202** and other impurities

From the **Table 24**, Entry 1 and 2 showed **201** was obtained from the reaction, though the yield was quite low (about 13%). There were three signals observed in the ^{19}F NMR spectrum and the product was also supported by the presence of an [M+H] ion at m/z 347.0556 in the mass spectrum. Several components were also separated from the reaction mixture, but they showed a complicated mixture by ^{1}H NMR spectroscopy. Sodium hydride was necessary for the approach, as potassium carbonate was not strong enough to form the quinazoline ring as shown in Entry 3. Otherwise, there was no obvious influence of the solvent from aprotic solvent-DMF to protic solvent EtOH from Entry 1 and Entry 2.

4.3.5 Attempted reaction to prepare partially fluorinated benzisoxazole derivatives

The success of ring-forming reactions using substituted fluorinated benzaldehydes prompted us to investigate analogous reactions using fluoronitrobenzene derivatives in which the nitro

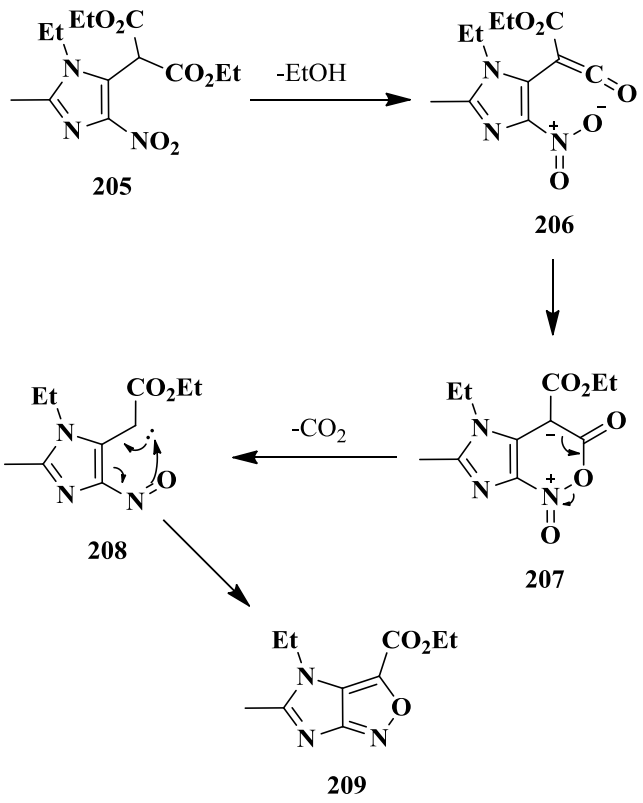
group could be used to form novel ring structures. The benzisoxazole scaffold was next attempted from the fluorinated arene. The first successful synthesis of a benzisoxazole using an *ortho*-nitro group reaction was reported by Grob *et al.* in 1961.[136] They prepared benzisoxazole **204** formed by thermolysis of 2-(2-nitrophenyl) malonate **203** derivative (**Scheme 41**).



203 **204**

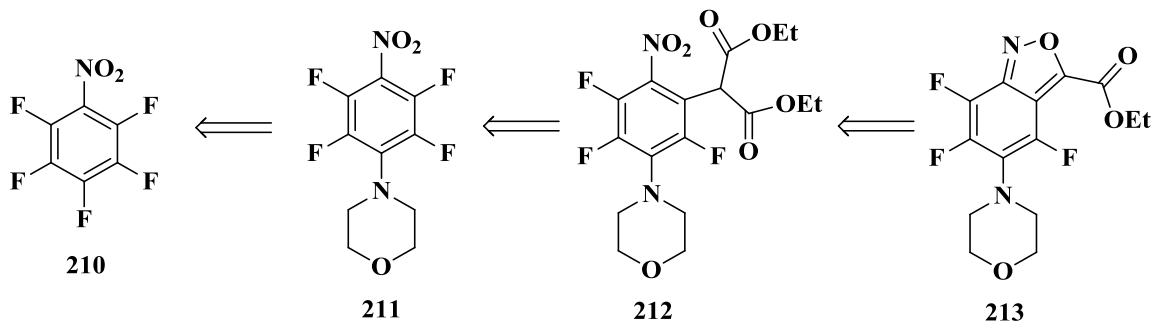
Scheme 41: Synthesis of benzisoxazole by Grob.[136]

In 1999, Tennant *et al.* extended this reaction to heterocyclic systems. He also provided the proposed mechanism for the transformation on their own study for preparing imidazo[4,5-*c*]isoxazole ring system. A reactive ketene intermediate was thought to be generated from elimination of ethanol and underwent cyclisation by attack of the adjacent nitro group to form an intermediate oxoimidazo[4,5-*c*]-1,2-oxazin-*N*-oxide. After loss of CO_2 from the intermediate, a nitroso carbene was generated which underwent final electrocyclic ring closure to form the isoxazole ring (**Scheme 42**). [137]



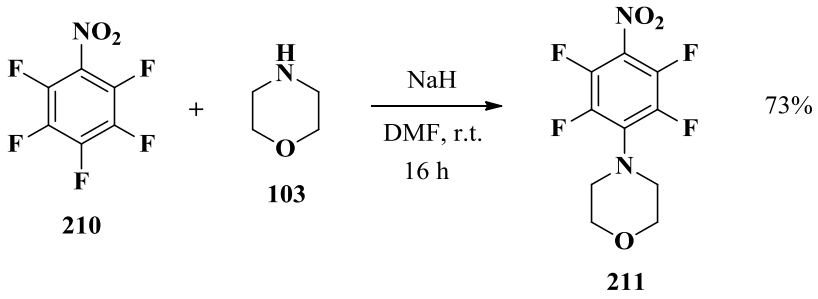
Scheme 42: Proposed mechanism for the transformation in Tennant's work.[137]

To form a benzisoxazole scaffold from pentafluoronitrobenzene, a suitable nucleophile is required adjacent to the nitro group, which can enable the cyclisation reaction. Since a substituent first needed to be introduced at C-4 to direct the key nucleophile to C-2 adjacent to the nitro group, a suitable 4-substituted nitrobenzene needed to be prepared first. The method for the synthesis was envisaged as below (**Scheme 43**).



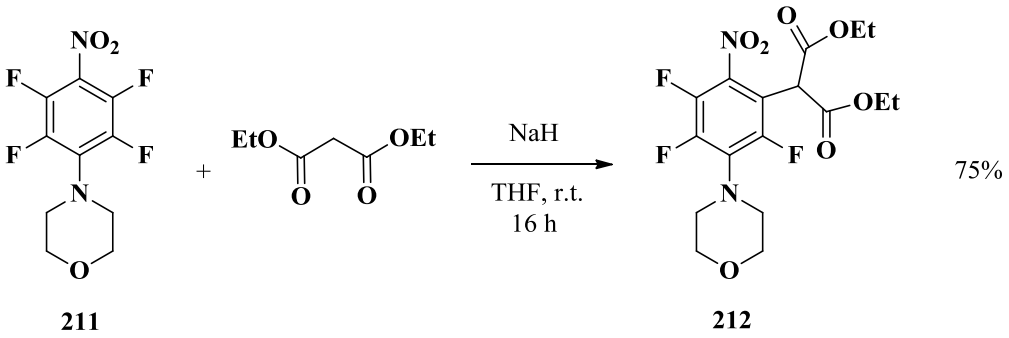
Scheme 43: Proposed synthesis routes to benzisoxazole.

Two equivalents of morpholine were employed as a nucleophile with pentafluoronitrobenzene to prepare a 4-amino substituted tetrafluoronitrobenzene. The reaction was conducted at room temperature in the presence of sodium hydride (**Scheme 44**). The 4-substituted compound **211** was obtained as a yellow solid from the reaction mixture and was isolated in a yield of 73%.



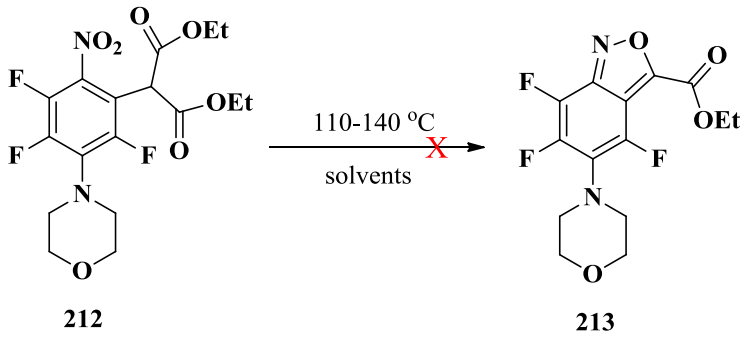
Scheme 44: Reaction of pentafluoronitrobenzene and benzimidazole.

The enolate of diethyl malonate was then used as the next nucleophile and was designed to attack the *ortho*-site of the benzene ring in compound **211** in the next step. Two equivalents of sodium hydride were utilised as base to generate the enolate and to deprotonate the product which was expected to be more acidic than the starting malonate. The reaction was conducted at room temperature in THF overnight (**Scheme 45**). Compound **212** was obtained after silica column chromatography in a good yield of 75%.



Scheme 45: Reaction of compound **211** and diethyl malonate.

Thermolysis of compound **212** was conducted next to investigate the conversion to the novel fluorinated benzisoxazole. Related compounds most likely react by loss of ethanol and CO_2 leading to formation of the isoxazole ring. The compounds was heated under reflux in toluene for 48 hours following the method reported by Tennant *et al*. (**Scheme 46**).[137] However, there was no new component formed during the reaction as shown by TLC analysis.



Scheme 46: Proposed thermolysis of **212** to prepare benzisoxazole **213**.

More forcing reactions were then attempted with different solvents including *o*-xylene and DMF, expected to increase the temperature of the reaction. However, there was no effect on the outcome of the reaction. The results from these attempts suggested that the fluoronitroaryl malonate is resistant to thermal ring closure although it is not obvious what effect fluorine substitution is having on the process.

4.4 Conclusions

The work outlined in this chapter has shown the developments of fluoroarene-derived scaffold structures from substituted benzaldehyde derivatives. A number of compounds were prepared from the reactions with different conditions.

Quinazoline **201** was obtained successfully from benzimidazole **68**, despite in a poor yield of 13%.

Synthesis of a fluorinated benzisoxazole was also attempted from morpholine-substituted benzaldehyde **211**. But the thermal ring closure was not observed and there was no effect on the outcome of the reaction after many forcing reactions.

A dozen aldehyde phenylhydrazone derivatives (**188**-**199**) were obtained easily from the reaction of four tetrafluorobenzaldehydes (**67**, **68**, **69** and **184**) with different hydrazines (**185**-**187**). Among these aldehyde phenylhydrazones, **195** with a 1-methyl-piperazinyl moiety demonstrated significant trypanocidal activity with an IC_{50} value of 1.63 µM.

Chapter 5: Overall Conclusions

The work described in this thesis has demonstrated the formation of three scaffold structures and their respectable trypanocidal activities with specific substituents. These scaffold structures were readily synthesised through nucleophilic aromatic substitution from highly fluorinated arenes as starting materials.

Nucleophilic substitution of a diverse range of nucleophiles with pentafluorobenzoaldehyde gave highly fluorinated aromatic compounds. Subsequent reactions of the compounds with thiols provided a series of poly-fluorobenzo[*b*]thiophene derivatives with various amine and heterocyclic substituents. 6-Benzimidazol-1-ylbenzothiophene derivatives (**91** and **82**) demonstrated significant antitrypanosomal activities (IC_{50} = 0.53 and 0.60 µM) against T. *b. rhodesiense* and no toxicity towards MCF_7 cell line. Further modifications on 6-benzimidazol-1-ylbenzothiophene derivatives were designed and synthesised to find compounds with better activities and selectivity.

An (aza-)phenoxazine scaffold was successfully prepared from the reaction of fluoroarenes with *ortho*-amino phenols through substitution, rearrangement and cyclisation. Further substitution at the *ortho*-position to electron-withdrawing group with different nucleophiles gave a series of (aza-)phenoxazine derivatives and 3-imidazol-1-yl-pyridobenzoxazine derivatives (**130**) which showed good activity (IC_{50} = 5.76 µM) against *T. cruzi* as well as high selectivity over THP-1 cell line. SAR studies on these samples were employed to identify important functional groups and found aldehyde derivatives **176** and **178** demonstrating improved activity (IC_{50} = 1.93 and 1.51 µM).

A fluorinated quinazoline scaffold was also successfully synthesised from the fluorobenzaldehydes, but attempts to prepare benzisoxazole structures from *ortho*-substituted fluoronitrobenzenes failed after several attempts.

A dozen novel fluorinated aldehyde phenylhydrazones were obtained from the tetrafluorobenzaldehydes on reaction with different hydrazines. 1-Methyl-piperazinyl derivatives (**195** and **194**) demonstrated great anti-*T. b. rhodesiense* activities (IC_{50} = 1.63 and 4.30 µM) and moderate selectivity from rat L6 cell.

Chapter 6: Future work

The strategy to the synthesis of fused heterocyclic compounds was successful and represents an interesting approach to heterocyclic synthesis using perfluorinated arenes as building blocks. For further work, the research could still focus on the preparation of more various novel scaffold structures such as phenothiazines and quinolines. More privileged structures could also be taken into account for judicious structural modifications. Another potential area of the research is to synthesise natural product-containing heterocycles from highly fluorinated aromatic compounds. Substitutions on the scaffold structure could also be a strategy to prepare natural products like prenylterphenyllins.

Chapter 7: Experimental

General

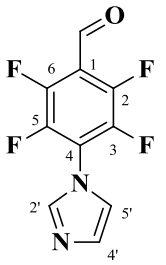
Reagents and solvents were used as received from commercial suppliers apart from THF which was distilled from sodium / benzophenone ketyl radical under a nitrogen atmosphere. All reactions were run under anhydrous conditions in oven-dried glassware (or flame-dried under nitrogen) unless otherwise stated. Organic extracts were dried over magnesium sulfate. Sodium hydride was dispersed in mineral oil as 60% w/w. 40-60 Micron silica gel was employed in flash column chromatography. The yields reported in this thesis are isolated yields. Aluminium backed silica gel was used for TLC analysis and plates were viewed under UV radiation at 254 nm wavelength.

NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 at 400 MHz (^{1}H NMR), 376 MHz (^{19}F NMR), or 100 MHz (^{13}C NMR) on Bruker Advance 400 or Joel JNM-ECS400 instruments. Chemical shifts are given in parts per million (p.p.m) and coupling constants, *J*, were recorded in hertz (Hz). TMS (tetramethylsilane) is used as the internal reference for ^{1}H NMR and ^{19}F NMR chemical shifts are referenced to hexafluorobenzene, ($\delta_F = 0$).

Mass spectra were recorded on a ThermoFisher Exactive (orbitrap) instrument with an ion max source and ESI probe fitted with an Advion Triversa Nanomate to obtain high resolution mass spectra, or were recorded at the EPSRC National Mass Spectrometry Facility in Swansea.

IR spectra were recorded using a PerkinElmer Spectrum 65 FT-IR Spectrometer using KBr discs and FT-IR 8400S with GS10800-X Quest ATR diamond accessory. Elemental analysis was determined using a CE-440 Elemental Analyzer. Melting points were determined using an Electrothermal-IA 9100 instrument.

2,3,5,6-Tetrafluoro-4-(1*H*-imidazol-1-yl)benzaldehyde (67)



A solution of pentafluorobenzoaldehyde (0.39 g, 2.00 mmol) in THF (3 ml) was added to a solution of imidazole (0.27 g, 4.00 mmol) in THF (3 ml). The solution was stirred at room temperature for 16 hours. The reaction mixture was poured into deionised water (10 ml) and was extracted with ethyl acetate (15 ml x 3). The extracts were combined, washed with brine (approx. 15 ml), and dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was purified by chromatography over silica gel (elution with light petroleum/ ethyl acetate = 1.1:1). The appropriate fractions were combined and evaporated under reduced pressure to give the known compound **67** (0.33 g, 68%) as a red oil.[99]

NMR δ_H (400 MHz, $CDCl_3$), 10.31 (1H, s, CHO), 7.88 (1H, s, H-2'), 7.32 (1H, s, H-4'/H-5'), 7.29 (1H, s, H-4'/H-5').

NMR δ_F (376 MHz, $CDCl_3$), 19.4-19.2 (2F, m, F-3, F-5), 14.9-15.0 (2F, m, F-2, F-6).

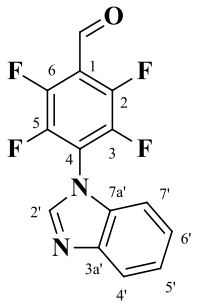
NMR δ_c (100 MHz, $CDCl_3$), 181.5 (CHO), 147.4 (ddd, *J* = 260, 11, 4 Hz, C-2, C-6), 140.7 (ddd, *J* = 255, 12, 5 Hz, C-3, C-5), 137.5 (C-2'), 130.5 (C-4'), 122.0 (t, *J* = 12 Hz, C-4), 119.7 (C-5'), 113.6 (t, *J* = 10 Hz, C-1).

Data were in agreement with those reported in the literature.

IR, ν_{max} /cm^{-1} 1712 (CHO).

MS, m/z found 245.0332, $C_{10}H_5F_4N_2O$, ($M+H^+$) requires 245.0333.

4-(1*H*-Benzimidazol-1-yl)-2,3,5,6-tetrafluorobenzaldehyde (68)



A solution of pentafluorobenzoaldehyde (0.39 g, 2.00 mmol) in THF (3 ml) was added a solution of benzimidazole (0.47 g, 4.00 mmol) in THF (3 ml). The solution was stirred at room temperature for 26 hours. The reaction mixture was poured into deionised water (10 ml) and was extracted with ethyl acetate (15 ml x 3). The extracts were combined, washed with brine (approx. 15 ml) and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was purified by chromatography over silica gel (elution with light petroleum/ ethyl acetate = 8: 5). The combined elution fractions were evaporated under reduced pressure to give compound **68** (0.37 g, 62%) as a yellow solid, m.p. 160-162 °C.

NMR δ_H (400 MHz, $CDCl_3$), 10.42 (1H, s, CHO), 8.09 (1H, t, *J* = 1.8 Hz, H-2'), 7.96-7.92 (1H, m, H-7'), 7.47-7.42 (2H, m, H-5', H-6'), 7.33-7.29 (1H, m, H-4').

NMR δ_F (376 MHz, $CDCl_3$), 19.5-19.4 (2F, m, F-3, F-5), 18.2-18.1 (2F, m, F-2, F-6).

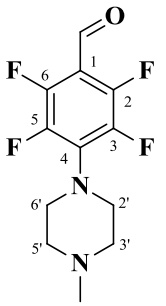
NMR δ_c (100 MHz, $CDCl_3$), 181.6 (CHO), 147.3 (ddd, *J* = 260, 12, 5 Hz, C-2, C-6), 143.2, 142.0 (ddd, *J* = 250, 14, 6 Hz, C-3, C-5), 141.9 (C-2'), 132.7, 125.0 (C-5'/C-6'), 124.1(C-5'/C-6'), 121.0, 120.8 (d, *J* = 10 Hz, C-4), 114.6 (d, *J* = 10 Hz, C-1), 110.7 (t, *J* = 3 Hz).

IR, ν_{max} /cm^{-1} 1705 (CHO).

MS, m/z found 295.0488, $C_{14}H_7F_4N_2O$, ($M+H^+$) requires 295.0489.

Elemental analysis, $C_{14}H_6F_4N_2O$ requires: C, 57.15; H, 2.06; N, 9.52; found: C, 57.19; H, 2.16; N, 9.37.

2,3,5,6-Tetrafluoro-4-(4-methylpiperazin-1-yl)benzaldehyde (69)



A solution of pentafluorobenzoaldehyde (1.96 g, 10.00 mmol) in THF (10 ml) was added a solution of 1-methylpiperazine (2.00 g, 20.00 mmol) in DMF (10 ml). The solution was stirred at room temperature for 26 hours. The reaction mixture was poured into deionised water (30 ml) and was extracted with ethyl acetate (30 ml x 3). The extracts were combined, washed with brine (approx. 30 ml) and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was purified by chromatography over silica gel (elution with light petroleum/ ethyl acetate = 1: 20). The combined elution fractions were evaporated under reduced pressure to give compound **69** (2.21 g, 80%) as a yellow solid. m.p. 65-67 °C.

NMR δ_H (400 MHz, $CDCl_3$), 10.09 (1H, d, *J*= 0.8 Hz, CHO), 3.42 (4H, d, *J*= 4 Hz, H-2', H-6'), 2.50 (4H, t, *J*=4.4 Hz, H-3', H-5'), 2.30 (3H, s, CH_3)

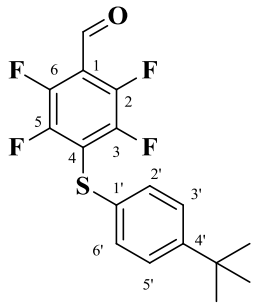
NMR δ_F (376 MHz, $CDCl_3$), 15.2 (2F, dd, *J*=20, 9 Hz, F-3, F-5), 9.5 (2F, dd, *J*=18, 7 Hz, F-2, F-6).

NMR δ_C (100 MHz, $CDCl_3$), 182.1 (C=O), 148.1 (dd, *J*=256, 14 Hz, C-2, C-6/C-3, C-5), 140.1 (dd, *J* =245, 14 Hz, C-2, C-6/C-3, C-5), 135.9 (C-4), 106.7 (t, *J*=10 Hz, C-1), 55.2 (C-2"), 50.6 (t, *J*= 5 Hz, C-1"), 46.2 (C-3").

IR, ν_{max} /cm^{-1} 1682 (CHO), 1628 and 1558 (benzene).

MS, m/z found 277.0953, $C_{12}H_{13}F_4N_2O$, ($M+H^+$) requires 277.0959.

4-[(4-*tert*-Butylphenyl)sulfanyl]-2,3,5,6-tetrafluorobenzaldehyde (73)



Pentafluorobenzaldehyde (0.39 g, 2.00 mmol) was added to a solution of Et_3N (0.40 g, 4.00 mmol) in THF (5 ml). 4-*tert*-Butylbenzenethiol (0.33 g, 2.00 mmol) was added into the reaction dropwise with ice cooling. The mixture was stirred at room temperature for 16 hours. The reaction mixture was poured into deionised water (10 ml) and was extracted with ethyl acetate (15 ml x 3). The combined extracts were washed sequentially with 0.2 M HCl (aq.) solution (10 ml) and deionised water (approx. 15 ml) and dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was purified by silica column chromatography (elution with light petroleum/ ethyl acetate = 50:1) to give sulfide **73** (0.50 g, 73%) as a yellow solid, m.p. 42-44 °C.

NMR δ_H (400 MHz, $CDCl_3$), 10.30 (1H, s, CHO), 7.44-7.36 (4H, m, H-2', H-3', H-5', H-6'), 1.34 (9H, s, CH_3)

NMR δ_F (376 MHz, $CDCl_3$), 29.2 (2F, *J* = 23, 11 Hz, F-3, F-5), 16.9 (2F, *J* = 23, 11 Hz, F-2, F-6).

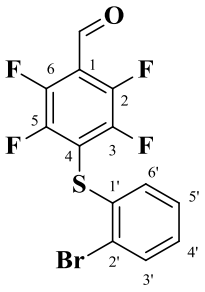
NMR δ_c (100 MHz, $CDCl_3$), 182.3 (CHO), 152.6 (C-4'), 146.6 (dd, *J* = 260, 16 Hz, C-2, C-6), 146.1 (dd, *J* = 260, 10 Hz, C-3, C-5), 132.3 (C-2', C-6'), 127.2 (C-1'), 126.7 (C-3', C-5'), 123.4 (t, *J* = 18 Hz, C-4), 114.4 (t, *J* = 10 Hz, C-1), 34.7 (*C*-$(CH_3)_3$), 31.2(CH_3).

IR, ν_{max} /cm^{-1} 1705 (CHO).

MS, m/z found 343.0778, $C_{17}H_{15}F_4OS$, $(M+H^+)$ requires 343.0774.

Elemental analysis, $C_{17}H_{14}F_4OS$ requires: C, 59.64; H, 4.12; found: C, 59.61; H, 4.06.

4-[(2-Bromophenyl)sulfanyl]-2,3,5,6-tetrafluorobenzaldehyde (74)



Following the general method outlined for sulfide **73**, pentafluorobenzaldehyde (0.39 g, 2.00 mmol) was reacted with 2-bromothiophenol (0.38 g, 2.00 mmol) in THF (2 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 30:1) afforded compound **74** (0.45 g, 62%) as a white solid. m.p 88-90 °C.

NMR δ_H (400 MHz, $CDCl_3$), 10.33 (1H, s, CHO), 7.65 (1H, dd, *J* = 8.0, 1.2 Hz, H-3'), 7.38 (1H, d, *J* = 7.6 Hz, H-6'), 7.30 (1H, td, *J* = 7.6, 1.2 Hz, H-5'), 7.23 (1H, td, *J* = 7.6, 1.6 Hz, H-4').

NMR δ_F (376 MHz, $CDCl_3$), 29.7-29.6 (2F, m, F-3, F-5), 17.4-17.3 (2F, m, F-2, F-6).

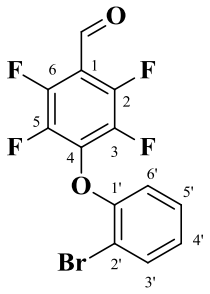
NMR δ_c (100 MHz, $CDCl_3$), 182.2 (CHO), 146.4 (dd, *J* = 260, 13 Hz, C-2, C-6), 146.1 (dd, *J* = 260, 11 Hz, C-3/C-5), 133.8 (C-3'), 132.7 (C-6'), 132.0 (C-1'), 130.1 (C-4'), 128.3 (C-5'), 125.9 (C-2'), 125.5 (t, *J* = 18 Hz, C-4), 114.7 (t, *J* = 11 Hz, C-1).

IR, ν_{max} /cm^{-1} 1705 (CHO).

MS, m/z found 364.9251, $C_{13}H_6{}^{79}BrF_4OS$, $(M+H^+)$ requires 364.9253.

Elemental analysis, $C_{13}H_5BrF_4OS$ requires: C, 42.76; H, 1.38; found: C, 42.73; H, 1.35

4-(2-Bromophenoxy)-2,3,5,6-tetrafluorobenzaldehyde (75)



Pentafluorobenzaldehyde (0.39 g, 2.00 mmol) was added to 1.4 ml Et_3N (1.00 g, 10.00 mmol). 2-Bromophenol (0.35 g, 2.00 mmol) was added to the reaction dropwise with ice cooling. The mixture was stirred at room temperature for 16 hours. The reaction mixture was poured into deionised water (10 ml) and was extracted with ethyl acetate (15 ml x 3). The combined extracts were washed with 0.2 M (aq.) HCl solution (30 ml) and deionised water (approx. 15 ml) successively and dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was purified by using silica column chromatography (elution with light petroleum/ ethyl acetate = 30:1) to afford compound **75** (0.42 g, 62%) as a white solid, m.p. 48-50 °C.

NMR δ_H (400 MHz, $CDCl_3$), 10.31 (1H, s, CHO), 7.67 (1H, dd, *J* = 8.0, 1.6 Hz, H-3'), 7.31 (1H, td, *J* = 8.0, 1.6 Hz, H-5'), 7.11 (1H, td, *J* = 8.0, 1.2 Hz, H-4'), 6.94 (1H, d, *J* = 8.0 Hz, H-6').

NMR δ_F (376 MHz, $CDCl_3$), 17.1-17.0 (2F, m, F-3, F-5), 8.4-8.3 (2F, m, F-4, F-6).

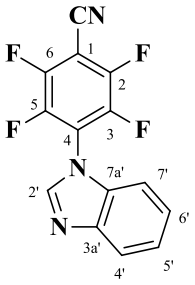
NMR δ_c (100 MHz, $CDCl_3$), 182.0 (CHO), 153.0 (C-1'), 147.6 (ddd, *J* = 260, 17, 6 Hz, C-2, C-6), 140.6 (dd, *J* = 250, 12 Hz, C-3, C-5), 134.2 (C-3'), 128.8 (C-5'), 126.3 (C-4'), 125.9 (C-4), 117.3 (C-6'), 112.6 (C-2'), 111.1 (t, *J* = 10 Hz, C-1).

IR, ν_{max} /cm^{-1} 1705 (CHO).

MS, m/z found 348.9478, $C_{13}H_6{}^{79}Br\ F_4O_2$, $(M+H^+)$ requires 348.9482.

Elemental analysis, $C_{13}H_5BrF_4O_2$ requires: C, 44.85; H, 1.16; found: C, 44.73; H, 1.16.

4-(1H-Benzo[*d*]imidazol-1-yl)-2,3,5,6-tetrafluorobenzonitrile (97)



Following the general method outlined for compound **68**, pentafluorobenzonitrile (0.19 g, 1.00 mmol) was reacted with benzimidazole (0.24 g, 2.00 mmol) in THF (2 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 8:5) afforded compound **97** (0.28 g, 95%) as a white solid. m.p. 180-182 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.06 (1H, s, H-2'), 7.93-7.89 (1H, m, H-7'), 7.46-7.39 (2H, m, H-5', H-6'), 7.28-7.23 (1H, m, H-4').

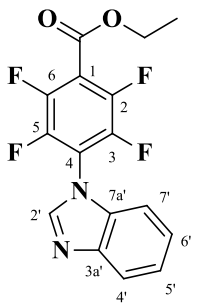
NMR δ_F (376 MHz, $CDCl_3$), 32.6-32.4 (2F, m, F-3, F-5), 20.3-20.1 (2F, m, F-2, F-6).

NMR δ_c (100 MHz, $CDCl_3$), 148.1 (ddt, J = 264, 13, 4 Hz, C-2, C-6), 142.1 (dd, J = 257, 13 Hz, C-3, C-5), 143.1, 141.6 (C-2'), 132.6, 125.3, 124.4, 121.4 (t, J = 13 Hz, C-4), 121.3, 110.5, 106.7, 94.4 (t, J = 17 Hz, C-1).

IR, ν_{max} /cm^{-1} 2245 (CN), 1651 and 1495 (benzene ring).

MS, m/z found 292.0492, $C_{14}H_6N_3F_4$, ($M+H^+$) requires 292.0492.

Ethyl 4-(1*H*-benzo[*d*]imidazol-1-yl)-2,3,5,6-tetrafluorobenzoate (96)



Following the general method outlined for compound **68**, pentafluorobenzoate (0.24 g, 1.00 mmol) was reacted with benzimidazole (0.24 g, 2.00 mmol) in THF (1.5 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 8:5) afforded compound **96** (0.22 g, 66%) as a white solid, m.p. 90-92 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.07 (1H, s, H-2'), 7.92-7.90 (1H, m, H-7'), 7.43-7.38 (2H, m, H-5', H-6'), 7.27-7.25 (1H, m, H-4'), 4.50 (2H, q, *J* = 7.6 Hz, CH_2), 1.44 (3H, t, *J* = 7.2 Hz, CH_3).

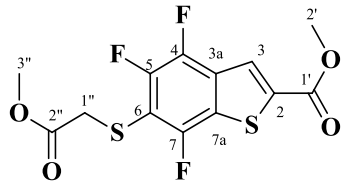
NMR δ_F (376 MHz, $CDCl_3$), 24.9-24.7 (2F, m, F-3, F-5), 17.7-17.5 (2F, m, F-2, F-6).

NMR δ_c (100 MHz, $CDCl_3$), 158.8 (C=O), 145.3 (dd, *J* = 257, 13 Hz, C-2, C-6), 142.9, 142.3 (dd, *J* = 256, 14 Hz, C-3, C-5), 142.0 (C-2'), 133.0, 124.9, 124.0, 120.9, 118.0 (t, *J* = 16 Hz, C-4), 113.4 (t, *J* = 16Hz, C-1), 110.6, 63.4 (CH_2), 14.2 (d, *J* = 10 Hz, CH_3).

IR, ν_{max} /cm^{-1} 1728 (C=O), 1651 and 1489 (benzene ring), 1273 (C-O).

MS, m/z found 339.0749, $C_{16}H_{11}F_4N_2O_2$, ($M+H^+$) requires 339.0751.

Methyl-4,5,7-trifluoro-6-[(2-methoxy-2-oxoethyl)sulfanyl]-1-benzo[*b*]thiophene-2-carboxylate (92)



Methyl mercaptoacetate (0.42 g, 4.00 mmol) was added dropwise to a solution of pentafluorobenzaldehyde (0.39 g, 2.00 mmol) and Et_3N (0.51 g, 5.00 mmol) in THF (5 ml) with ice cooling. The mixture was stirred at room temperature for 16 hours. The reaction mixture was poured into deionised water (15 ml) and was extracted with ethyl acetate (15 ml x 3). The combined extracts were washed with 0.2 M HCl (aq.) solution (20 ml) and deionised water (approx. 20 ml) successively and dried over sodium sulfate. The solution was concentrated under reduced pressure. Compound **92** was isolated (0.44 g, 62%) after purification by silica column chromatography (elution with light petroleum/ethyl acetate = 20:1) as a white solid, m.p. 118-120 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.09 (1H, d, *J* = 3.2 Hz, H-3), 3.98 (3H, s, H-2'), 3.69 (3H, s, H-3"), 3.65 (2H, s, H-1").

NMR δ_F (376 MHz, $CDCl_3$), 52.4 (1F, dd, *J* = 18, 3 Hz), 28.5 (1F, d, *J* = 21 Hz), 18.3 (1F, dd, *J* = 21, 18 Hz).

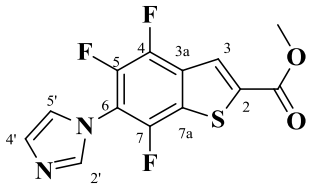
NMR δ_c (100 MHz, $CDCl_3$), 169.2 (C-2"), 161.9 (C-1'), 153.1 (d, *J* = 246 Hz, C-7), 147.3 (ddd, *J* = 242, 15, 4 Hz, C-4/ C-5), 142.4 (ddd, *J* = 253, 14, 4 Hz, C-4/C-5), 137.6 (C-2), 130.4 (ddd, *J* = 15, 7, 4 Hz, C-3a/ C-7a), 125.6-125.2 (m, C-3), 125.5 (d, *J* = 7 Hz, C-3a/ C-7a), 110.1 (t, *J* = 22 Hz, C-6), 53.1 (C-3"), 52.8 (C-2'), 36.1 (C-1").

IR, ν_{max} /cm^{-1} 1728 (C=O), 1273 (C-O).

MS, m/z found 350.9969, $C_{13}H_{10}F_3O_4S_2$, ($M+H^+$) requires 350.9967.

Elemental analysis, $C_{13}H_9F_3O_4S_2$ requires: C, 44.57; H, 2.59; found: C, 44.40; H, 2.53.

Methyl-4,5,7-trifluoro-6-(1*H*-imidazol-1-yl)benzo[*b*]thiophene-2-carboxylate (81)



Methyl mercaptoacetate (0.21 g, 2.00 mmol) was added dropwise to a solution of aldehyde **67** (0.49 g, 2.00 mmol) and Et_3N (0.51 g, 5.00 mmol) in THF (5 ml) with ice cooling. The mixture was stirred at room temperature for 16 hours. The reaction mixture was poured into deionised water (15 ml) and was extracted with ethyl acetate (15 ml x 3). The combined extracts were washed with 0.2 M HCl (aq.) solution (20 ml) and deionised water (approx. 20 ml) successively and dried over sodium sulfate. Compound **81** (0.44 g, 70%) was purified by silica column chromatography (elution with light petroleum/ethyl acetate = 5:3) as a white solid, m.p. 146-148 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.17 (1H, d, *J* = 3.2 Hz, H-3), 7.81 (1H, s, H-2'), 7.29 (1H, s, H-5'), 7.28 (1H, s, H-4'), 3.99 (3H, s, CH_3).

NMR δ_F (376 MHz, $CDCl_3$), 36.2 (1F, d, *J* = 19 Hz), 19.8 (1F, t, *J* = 19 Hz), 14.6 (1F, d, *J* = 20 Hz).

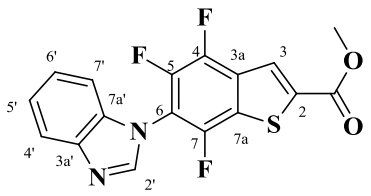
NMR δ_C (100 MHz, $CDCl_3$), 161.5 (C=O), 146.3 (ddd, *J* = 248, 4, 3 Hz, C-7), 143.5 (ddd, *J* = 251, 13, 4 Hz, C-4/C-5), 141.8 (ddd, *J* = 245, 15, 3 Hz, C-4/C-5), 137.9 (C-2), 130.0 (C-4'), 128.6 (ddd, *J* = 18, 6, 3 Hz, C-3a/ C-7a), 125.6 (ddd, *J* = 21, 6, 3 Hz, C-3a/ C-7a), 125.1 (d, *J* = 6 Hz, C-3), 120.4 (C-5'), 114.6 (t, *J* = 16 Hz, C-6), 53.2 (CH_3).

IR, ν_{max} /cm^{-1} 1728 (C=O), 1265 (C-O).

MS, m/z found 313.0264, $C_{13}H_7F_3N_2OS$, ($M+H^+$) requires 313.0253.

Elemental analysis, $C_{13}H_7F_3N_2O_2S$ requires: C, 50.00; H, 2.26; N, 8.97; found: C, 49.91; H, 2.19; N, 8.88.

Methyl-6-(1*H*-benzo[*d*]imidazol-1-yl)-4,5,7-trifluorobenzo[*b*]thiophene-2-carboxylate (82)



Following the general method outlined for compound **81**, aldehyde **68** (0.59 g, 2.00 mmol) was reacted with methyl mecaptoacetate (0.21 g, 2.00 mmol) in THF (4 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 3:1) afforded compound **82** (0.41 g, 57%) as a white solid. m.p. 178-179 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.26 (1H, d, *J* = 3.2 Hz, H-3), 8.10 (1H, s, H-2”), 7.92 (1H, d, *J* = 7.6 Hz, H-7”), 7.42-7.37 (2H, m, H-5”, H-6”), 7.30 (1H, d, *J* = 8.0 Hz, H-4”), 4.02 (3H, s, CH_3).

NMR δ_F (376 MHz, $CDCl_3$), 38.9 (1F, d, *J* = 19 Hz), 20.2 (1F, t, *J* = 19 Hz), 16.8 (1F, d, *J* = 19 Hz).

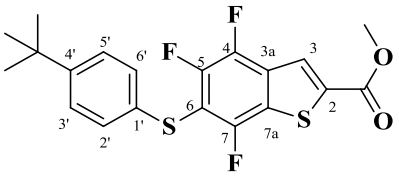
NMR δ_c (100 MHz, $CDCl_3$), 161.5 (C=O), 147.6 (dt, *J* = 251, 4 Hz C-7), 143.1, 142.8 (C-2’), 142.7 (dt, *J* = 249, 5 Hz, C-4/C-5) , 142.5 (dt, *J* = 251, 5 Hz, C-4/C-5), 138.2 (C-2), 133.8, 129.5 (ddd, *J* = 19, 7, 2 Hz, C-3a/ C-7a), 125.8 (ddd, *J* = 17, 5, 2 Hz, C-3a/ C-7a), 125.3 (d, *J* = 5 Hz, C-3), 124.4, 123.5, 120.8, 112.9 (t, *J* = 6 Hz, C-6), 110.4, 53.2 (CH_3).

IR, ν_{max} /cm^{-1} 1720 (C=O), 1249 (C-O).

MS, m/z found 363.0402, $C_{17}H_9F_3N_2O_2S$, ($M+H^+$) requires 363.0410.

Elemental analysis, $C_{17}H_9F_3N_2O_2S$ requires: C, 56.51; H, 2.21; N, 7.75; found: C, 56.36; H, 2.56; N, 7.62.

Methyl 6-((4-(tert-butyl)phenyl)thio)-4,5,7-trifluorobenzo[*b*]thiophene-2-carboxylate (85)



Following the general method outlined for compound **81**, aldehyde **73** (0.68 g, 2.00 mmol) was reacted with methyl mecaptoacetate (0.21 g, 2.00 mmol) in THF (4 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 50:1) afforded compound **85** (0.62 g, 76%) as a light yellow solid. m.p. 127-129 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.15 (1H, d, *J* = 3.2 Hz, H-3), 7.37-7.31 (4H, m, H-2', H-3', H-5', H-6'), 4.00 (3H, s, OCH_3), 1.31 (9H, s, CH_3).

NMR δ_F (376 MHz, $CDCl_3$), 52.6 (1F, d, *J* = 17 Hz), 29.3 (1F, d, *J* = 23 Hz), 18.2 (1F, t, *J* = 20 Hz).

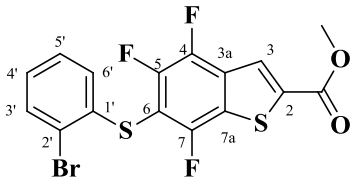
NMR δ_c (100 MHz, $CDCl_3$), 161.9 (C=O), 152.9 (dt, *J* = 248, 5Hz, C-7), 151.1 (C-4'), 147.0 (ddd, *J* = 245, 13, 4 Hz, C-4/ C-5), 142.5 (ddd, *J* = 253, 18, 4 Hz, C-4/C-5), 137.4 (C-2), 130.5 (C-2', C-6'), 130.2 (ddd, *J* = 22, 9, 4 Hz, C-3a/C-7a), 130.0 (C-1'), 126.4 (C-3', C-5'), 125.3 (d, *J* = 5 Hz, C-3a/C-7a), 125.3 (C-3), 111.8 (t, *J* = 22 Hz, C-6), 53.0 (OCH_3), 34.6 (*C*$(CH_3)_3$), 31.2 (CH_3).

IR, ν_{max} /cm^{-1} 1712 (C=O), 1249 (C-O).

MS, m/z found 411.0696, $C_{20}H_{18}F_3O_2S_2$, ($M+H^+$) requires 411.0695.

Elemental analysis, $C_{20}H_{17}F_3O_2S_2$ requires: C, 58.52; H, 4.17; found: C, 58.40; H, 4.12.

Methyl 6-((2-bromophenyl)thio)-4,5,7-trifluorobenzo[*b*]thiophene-2-carboxylate (83)



Following the general method outlined for compound **81**, aldehyde **74** (0.73 g, 2.00 mmol) was reacted with methyl mecaptoacetate (0.21 g, 2.00 mmol) in THF (4 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 30:1) afforded compound **83** (0.57 g, 66%) as a white solid. m.p. 143-145 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.20 (1H, d, *J* = 3.2 Hz, H-3), 7.60 (1H, dd, *J* = 8.0, 1.2 Hz, H-3'), 7.20 (1H, td, *J* = 7.2, 1.2 Hz, H-5'), 7.10 (1H, td, *J* = 7.2, 1.2 Hz, H-4'), 6.94 (1H, d, *J* = 7.6 Hz, H-6'), 4.02 (1H, s, CH_3)

NMR δ_F (376 MHz, $CDCl_3$), 53.7 (1F, dd, *J* = 17, 3 Hz), 29.7 (1F, d, *J* = 21 Hz), 18.9 (1F, t, *J* = 20 Hz).

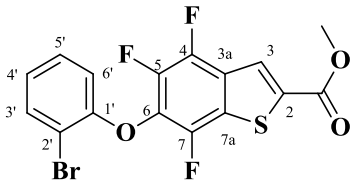
NMR δ_C (100 MHz, $CDCl_3$), 161.9 (C=O), 153.2 (C-7, d, *J* = 248 Hz), 147.0 (ddd, *J* = 250, 16, 5 Hz, C-4/C-5), 142.6 (ddd, *J* = 245, 21, 10 Hz, C-4/C-5), 138.0 (C-2), 135.4 (C-1'), 133.4 (C-3'), 130.5 (dd, *J* = 17, 7 Hz, C-3a/C-7a), 128.9 (C-6'), 128.0 (C-4'), 128.0 (d, *J* = 7 Hz), 125.7, 125.4 (d, *J* = 5 Hz, C-3), 122.7 (C-2'), 109.2 (t, *J* = 21 Hz, C-6), 53.2 (CH_3).

IR, ν_{max} /cm^{-1} 1720 (C=O), 1265 (C-O).

MS, m/z found 432.9129, $C_{16}H_9{}^{79}BrF_3O_2S_2$, ($M+H^+$) requires 432.9174.

Elemental analysis, $C_{16}H_8BrF_3O_2S_2$ requires: C, 44.35; H, 1.86; found: C, 44.18; H, 1.74.

Methyl 6-(2-bromophenoxy)-4,5,7-trifluorobenzo[*b*]thiophene-2-carboxylate (84)



Following the general method outlined for compound **81**, aldehyde **75** (0.70 g, 2.00 mmol) was reacted with methyl mecaptoacetate (0.21 g, 2.00 mmol) in THF (4 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 30:1) afforded compound **84** (0.65 g, 78%) as a white solid. m.p. 126-128 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.19 (1H, d, *J* = 3.2 Hz, H-3), 7.67 (1H, dd, *J* = 8.0, 1.6 Hz, H-3'), 7.24 (1H, td, *J* = 7.6 Hz, 1.6 Hz, H-5'), 7.03 (1H, td, *J* = 7.6, 1.6 Hz, H-4'), 6.78 (1H, dd, *J* = 7.6, 0.8 Hz, H-6'), 4.01 (3H, s, CH_3)

NMR δ_F (376 MHz, $CDCl_3$), 29.9 (1F, dd, *J* = 17, 3 Hz), 19.4 (1F, dd, *J* = 19, 17 Hz), 10.3 (1F, d, *J* = 19 Hz).

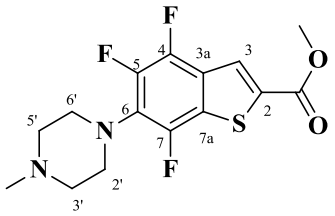
NMR δ_C (100 MHz, $CDCl_3$), 161.9 (C=O), 154.1 (C-1'), 146.0 (dt, *J* = 248, 4 Hz, C-7), 142.8 (ddd, *J* = 254, 12, 4 Hz, C-4/C-5), 141.8 (ddd, *J* = 248, 13, 3 Hz, C-4/C-5), 136.3 (C-2), 134.1 (C-3'), 131.5 (t, *J* = 14 Hz, C-6), 128.7 (C-5'), 125.9 (dd, *J* = 18, 4 Hz, C-3a/C-7a), 125.6 (dd, *J* = 19, 4 Hz, C-3a/C-7a), 125.3 (d, *J* = 6 Hz, C-3), 125.0 (C-4'), 115.5 (C-6'), 111.9 (C-2'), 53.1 (CH_3).

IR, ν_{max} /cm^{-1} 1720 (C=O), 1257 (C-O).

MS, m/z found 416.9400, $C_{16}H_9{}^{79}BrF_3O_3S$, ($M+H^+$) requires 416.9402.

Elemental analysis, $C_{16}H_8BrF_3O_3S$ requires: C, 46.06; H, 1.93; found: C, 46.07; H, 1.93.

Methyl 4,5,7-trifluoro-6-(4-methylpiperazin-1-yl)benzo[*b*]thiophene-2-carboxylate (86)



Following the general method outlined for compound **81**, aldehyde **69** (0.28 g, 1.00 mmol) was reacted with methyl mecaptoacetate (0.11 g, 1.00 mmol) in THF (2 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 3:1) afforded compound **86** (0.19 g, 51%) as a yellow solid. m.p. 88-90 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.04 (1H, d, *J* = 3.6 Hz, H-3), 3.93 (3H, s, OCH_3), 3.37 (4H, s, H-2', H-6'), 2.59 (4H, s, H-3', H-5'), 2.38 (3H, s, CH_3).

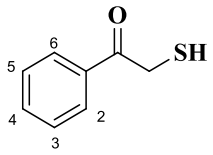
NMR δ_F (376 MHz, $CDCl_3$), 34.2 (1F, d, *J* = 16 Hz), 17.0 (1F, t, *J* = 18 Hz), 14.6 (1F, d, *J* = 19 Hz).

NMR δ_C (100 MHz, $CDCl_3$), 162.4 (C=O), 147.0 (d, *J* = 243 Hz, C-7), 144.1 (dd, *J* = 244, 19 Hz, C-4/C-5), 143.0 (dd, *J* = 247, 14 Hz, C-4/C-5), 134.0 (C-2), 128.5 (t, *J* = 12 Hz, C-3a/C-7a), 126.1 (dd, *J* = 20, 4 Hz, C-3a/C-7a), 125.6 (d, *J* = 6 Hz, C-3), 123.7 (dd, *J* =17, 4 Hz, C-6), 55.6 (C-3', C-5'), 52.9 (OCH_3), 51.0 (C-2', C-6'), 46.3 (CH_3).

IR, ν_{max} /cm^{-1} 1712 (C=O), 1627 and 1519 (benzene ring), 1234 (C-O).

MS, m/z found 345.0881, $C_{15}H_{16}F_3N_2O_2S$, ($M+H^+$) requires 345.0879.

2-Mercapto-1-phenylethanone (78)

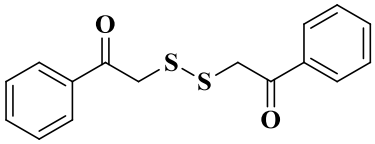


2-Bromoacetophenone (0.40 g, 2.00 mmol) was added to a solution of potassium thioacetate (0.23 g, 2.00 mmol) in THF (5 ml). The reaction mixture was stirred at 40 °C for 24 hours. The mixture was poured into deionised water (10 ml) and was extracted with ethyl acetate (15 ml x 3). The combined extracts were dried over sodium sulphate and concentrated under reduced pressure. The residue (0.30 g, 1.96 mmol) was then treated with 1 M NaOH (aq.) (2 ml) in methanol (4 ml). The reaction mixture was stirred at room temperature for 14 hours. The mixture was poured into deionised water (10 ml) and neutralised with 1 M HCl (aq.) (2 ml). The neutralised mixture was extracted with ethyl acetate (15 ml x 3). The combined extracts were dried with sodium sulfate and concentrated under reduced pressure. The residue was purified by using silica column (elution with light petroleum/ ethyl acetate = 2:1) to give the known thiol **78** (0.11 g, 37%) as a light yellow liquid.

NMR δ_H (400 MHz, $CDCl_3$), 8.01-7.98 (2H, m, H-2, H-6), 7.63 (1H, tt, *J* = 7.6, 1.2 Hz, H-4), 7.52 (2H, t, *J* = 8 Hz, H-3, H-5), 4.00 (2H, d, *J* = 7.6 Hz, CH_2), 2.16 (1H, t, *J* = 7.6 Hz, SH). The data was identical to the literature values.[138]

MS, m/z found 153.0363, C_8H_9OS, ($M+H^+$) requires 153.0369.

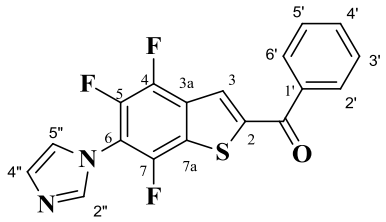
2,2'-Disulfanediylbis(1-phenylethan-1-one) (79)



Further elution afforded the known disulfide **79** as a colourless liquid in 5% (0.01 g) yield.

NMR δ_H (400 MHz, $CDCl_3$), 7.92 (4H, d, *J* = 8.0 Hz), 7. 58 (2H, t, *J* = 8.0 Hz), 7.45 (4H, t, *J* = 8.0 Hz), 4.18 (4H, s, CH_2). Data was identical with the literature values.[138]

Phenyl(4,5,7-trifluoro-6-(1*H*-imidazol-1-yl)benzo[*b*]thiophen-2-yl)methanone (87)



Following the general method outlined for compound **81**, aldehyde **67** (0.49 g, 2.00 mmol) was reacted with thiol **78** (0.30 g, 2.00 mmol) in THF (4 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 3:1) afforded compound **87** (0.35 g, 49%) as a cream solid. m.p. 132-134 °C.

NMR δ_H (400 MHz, $CDCl_3$), 7.98 (1H, d, *J* = 2.8 Hz, H-3), 7.92 (2H, d, *J* = 8.0 Hz, H-2', H-6'), 7.86 (1H, s, H-2"), 7.67 (1H, t, *J* = 7.6 Hz, H-4'), 7.57 (2H, t, *J* = 7.6 Hz, H-3', H-5'), 7.28 (2H, d, *J* = 12.4 Hz, H-4", H-5").

NMR δ_F (376 MHz, $CDCl_3$), 36.6 (1F, d, *J* = 19 Hz), 20.2 (1F, t, *J* = 17 Hz), 14.7 (1F, d, *J* = 17 Hz).

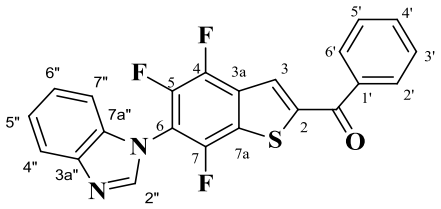
NMR δ_C (100 MHz, $CDCl_3$), 188.1 (C=O), 147.6 (C-2), 146.5 (d, *J* = 255 Hz, C-7), 143.1 (ddd, *J* = 255, 16, 5 Hz, C-4/ C-5), 141.8 (dd, *J* = 250, 14 Hz, C-4/C-5), 138.0 (C-2"), 136.6 (C-1'), 133.6, 129.9 (C-4"), 129.4 (C-2', C-6'), 129.2 (dd, *J* = 19, 7 Hz, C-3a/C-7a), 129.0 (C-3', C-5'), 126.2 (dd, *J* = 19, 7 Hz, C-3a/C-7a), 125.6 (d, *J* = 5 Hz, C-3), 120.5 (C-5"), 114.8 (t, *J* = 15 Hz, C-6).

IR, ν_{max} /cm^{-1} 1643 (C=O).

MS, m/z found 359.0454, $C_{18}H_{10}F_3N_2OS$, $(M+H^+)$ requires 359.0460.

Elemental analysis, $C_{18}H_9F_3N_2OS$ requires: C, 60.33; H, 2.53; N, 7.82; found: C, 60.08; H, 2.60; N, 7.68.

(6-(1H-Benzo[*d*]imidazol-1-yl)-4,5,7-trifluorobenzo[*b*]thiophen-2-yl)(phenyl)methanone (91)



Following the general method outlined for compound **81**, aldehyde **68** (0.59 g, 2.00 mmol) was reacted with thiol **78** (0.30 g, 2.00 mmol) in THF (4 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 3:1) afforded compound **91** (0.42 g, 51%) as an orange solid. m.p. 140-142 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.09 (1H, s, H-2”), 8.04 (1H, d, *J* = 3.2 Hz, H-3), 7.96-7.90 (3H, m, H-7”, H-2’, H-6’) 7.70 (1H, t, *J* = 7.2 Hz, H-4’), 7.59 (2H, t, *J* = 7.6 Hz, H-3’, H-5’), 7.41-7.34 (2H, m, H-5”,H-6”), 7.30 (1H, d, *J* = 5.6 Hz, H-4”).

NMR δ_F (376 MHz, $CDCl_3$), 39.34 (1F, d, *J* = 17 Hz), 20.6 (1F, t, *J* = 20 Hz), 16.9 (1F, d, *J* = 20 Hz).

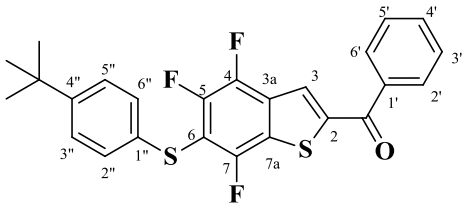
NMR δ_c (100 MHz, $CDCl_3$), 188.2 (C=O), 147.8 (d, *J* = 251 Hz, C-7), 147.7 (C-2), 143.2, 143.1 (ddd, *J* = 254, 13, 4 Hz, C-4/ C-5), 142.8 (C-2”), 142.7 (dd, *J* = 249, 14 Hz, C-4/ C-5), 136.6 (C-1’), 133.8, 133.6 (C-4’), 129.9 (dd, *J* = 17, 4 Hz, C-3a/C-7a), 129.5 (C-2’, C-6’), 129.0 (C-3’, C-5’), 126.2 (ddd, *J* = 18, 4, 3 Hz, C-3a/C-7a), 125.7 (d, *J* = 6 Hz, C-3), 124.6 (C-5”/C-6”), 123.6 (C-5”/C-6”), 120.9 (C-7”), 113.3 (t, *J* = 16 Hz, C-6), 110.5 (C-4”).

IR, ν_{max} /cm^{-1} 1643 (C=O).

MS, m/z found 409.0608, $C_{22}H_{12}F_3N_2OS$, ($M+H^+$) requires 409.0617.

Element analysis, $C_{22}H_{11}F_3N_2OS$: C, 64.70; H, 2.69; N, 6.86; Found: C, 64.47; H, 2.70; N, 6.96.

(6-((4-(*tert*-Butyl)phenyl)thio)-4,5,7-trifluorobenzo[*b*]thiophen-2-yl)(phenyl)methanone (88)



Following the general method outlined for compound **81**, aldehyde **73** (0.68 g, 2.00 mmol) was reacted with thiol **78** (0.30 g, 2.00 mmol) in THF (4 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 50:1) afforded compound **88** (0.45 g, 49%) as a red oil.

NMR δ_H (400 MHz, $CDCl_3$), 7.91 (2H, d, *J* = 2.0 Hz, H-2', H-6'), 7.90 (1H, s, H-3), 7.66 (1H, t, *J* = 7.6 Hz, H-4'). 7.55 (2H, t, *J* = 7.6 Hz, H-3', H-5'), 7.31 (4H, AA'BB', m, H-2", H-3", H-5", H-6"), 1.25 (9H, s, CH_3).

NMR δ_F (376 MHz, $CDCl_3$), 53.0 (1F, d, *J* = 17 Hz), 29.4 (1F, d, *J* = 23 Hz), 18.3 (1F, t, *J* = 21 Hz).

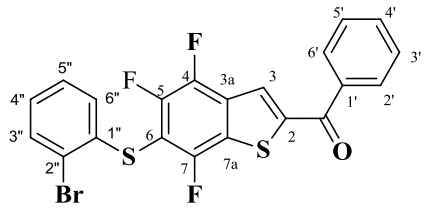
NMR δ_C (100 MHz, $CDCl_3$), 188.6 (C=O), 152.2 (C-7), 151.2, 147.0 (d, *J* = 250 Hz, C-4/C-5), 146.9 (C-2), 142.7 (d, *J* = 250 Hz, C-4/C-5), 136.9 (C-1'), 133.4 (C-4'), 130.7 (C-2", C-6"), 130.5 (C-3a/C-7a), 130.0, 129.4 (C-2', C-6'), 128.9 (C-3', C-5'), 126.7 (C-3a/C-7a), 126.5 (C-3", C-5"), 126.1 (d, *J* = 9 Hz, C-3), 112.3 (t, *J* = 19 Hz, C-6), 34.6 ($C(CH_3)_3$), 29.8 (CH_3).

IR, ν_{max} /cm^{-1} 1646 (C=O).

MS, m/z found 457.0895, $C_{25}H_{20}F_3OS_2$, ($M+H^+$) requires 457.0902.

Elemental analysis, $C_{25}H_{19}F_3OS_2$ requires: C, 65.77; H, 4.19; found: C, 65.73; H, 4.46.

(6-((2-Bromophenyl)thio)-4,5,7-trifluorobenzo[*b*]thiophen-2-yl)(phenyl)methanone (90)



Following the general method outlined for compound **81**, aldehyde **74** (0.73 g, 2.00 mmol) was reacted with thiol **78** (0.30 g, 2.00 mmol) in THF (4 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 30:1) afforded compound **90** (0.46 g, 46%) as a brown solid. m.p. 118-120 °C.

NMR δ_H (400 MHz, $CDCl_3$), 7.96 (1H, d, *J* = 3.2 Hz, H-3), 7.92 (2H, d, *J* = 7.2 Hz, H-2', H-6'), 7.68 (1H, t, *J* = 8.0 Hz, H-4'). 7.58 (1H, d, *J* = 1.6 Hz, H-3"), 7.56 (2H, t, *J* = 7.6 Hz, H-3', H-5'), 7.17 (1H, t, *J* = 7.2 Hz, H-5"), 7.08 (1H, t, *J* = 7.2 Hz, H-4"), 6.94 (1H, d, *J* = 7.6 Hz, H-6").

NMR δ_F (376 MHz, $CDCl_3$), 54.0 (1F, d, *J* = 23 Hz), 29.8 (1F, d, *J* = 17 Hz), 19.1 (1F, t, *J* = 17 Hz).

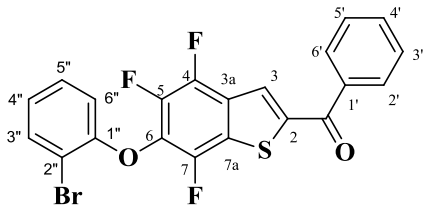
NMR δ_C (100 MHz, $CDCl_3$), 188.5 (C=O), 153.2 (d, *J* = 249 Hz, C-7), 147.4 (C-2), 146.9 (dd, *J* = 249, 17 Hz, C-4/C-5), 142.9 (dd, *J* = 256, 12 Hz, C-4/C-5), 136.7 (C-1'), 135.3, 133.5 (C-4'), 133.4 (C-3"), 131.2 (ddd, *J* = 18, 8, 4 Hz, C-3a/C-7a), 129.4 (C-2', C-6'), 129.1 (C-6"), 129.0 (C-3', C-5'), 128.2 (C-4"), 128.1 (C-5"), 126.0 (C-3), 125.9 (C-3a/C-7a), 122.9 (C-2"), 109.8 (t, *J* = 22 Hz, C-6).

IR, ν_{max} /cm^{-1} 1635 (C=O).

MS, m/z found 478.9370, $C_{21}H_{11}{}^{79}BrF_3OS_2$, ($M+H^+$) requires 478.9381.

Elemental analysis, $C_{21}H_{10}BrF_3OS_2$ requires: C, 52.62; H, 2.10; found: C, 52.45; H, 1.92.

(6-(2-Bromophenoxy)-4,5,7-trifluorobenzo[*b*]thiophen-2-yl)(phenyl)methanone (89)



Following the general method outlined for compound **81**, aldehyde **75** (0.70 g, 2.00 mmol) was reacted with thiol **78** (0.30 g, 2.00 mmol) in THF (4 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 30:1) afforded compound **89** (0.44 g, 48%) as a dark yellow solid. m.p. 110-112 °C.

NMR δ_H (400 MHz, $CDCl_3$), 7.83 (1H, s, H-3), 7.80 (2H, d, *J* = 7.6 Hz, H-2', H-6'), 7.58-7.52 (2H, m, H-4', H-3"), 7.45 (2H, t, *J* = 7.6 Hz, H-3', H-5'), 7.10 (1H, t, *J* = 7.6 Hz, H-5"), 6.90 (1H, t, *J* = 7.2 Hz, H-4"), 6.65 (1H, d, *J* = 8.4 Hz, H-6").

NMR δ_F (376 MHz, $CDCl_3$), 30.3 (1F, d, *J* = 15 Hz), 19.6 (1F, t, *J* = 20 Hz), 10.5 (1F, d, *J* = 20 Hz).

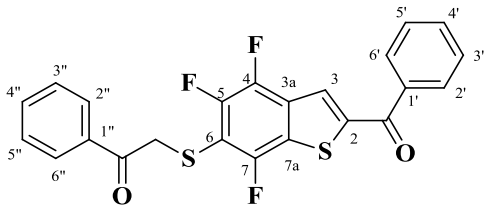
NMR δ_C (100 MHz, $CDCl_3$), 188.4 (C=O), 154.1 (C-1"), 146.1 (dd, *J* = 249, 4 Hz, C-7), 146.0 (C-2), 143.2 (ddd, *J* = 251, 14, 3 Hz, C-4/C-5), 142.1 (ddd, *J* = 251, 14, 4 Hz, C-4/C-5), 136.9, 134.1 (C-3"), 133.3 (C-4'), 131.9 (t, *J* = 14 Hz, C-6), 129.4 (C-2', C-6'), 128.9 (C-3', C-5'), 128.7 (C-5"), 126.4 (dd , *J* = 17, 4 Hz, C-3a/C-7a), 126.3-125.8 (m, C-3, C-3a/C-7a), 125.1 (C-4"), 115.6 (C-6"), 112.0 (C-2").

IR, νmax /cm^{-1} 1643 (C=O).

MS, m/z found 462.9601, $C_{21}H_{11}{}^{79}BrF_3O_2S$, ($M+H^+$) requires 462.9610.

Elemental analysis, $C_{21}H_{10}BrF_3O_2S$ requires: C, 54.44; H, 2.18; found: C, 54.37; H, 2.04.

2-((2-Benzoyl-4,5,7-trifluorobenzo[*b*]thiophen-6-yl)thio)-1-phenylethanone (93)



Following the general method outlined for compound **92**, pentafluorobenzaldehyde (0.39 g, 2.00 mmol) was reacted with thiol **78** (0.61 g, 4.00 mmol) in THF (6 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **93** (0.19 g, 21%) as an orange solid. m.p. 138-139 °C.

NMR δ_H (400 MHz, $CDCl_3$), 7.93-7.89 (5H, m, H-3, H-2', H-6', H-2", H-6"), 7.67 (1H, t, *J* = 7.2 Hz, H-4'), 7.61-7.53 (3H, m, H-3', H-5', H-4"), 7.46 (2H, t, *J* = 7.8 Hz, H-5", H-3"), 4.36 (2H, s, CH_2).

NMR δ_F (376 MHz, $CDCl_3$), 53.0 (1F, d, *J* = 20 Hz), 28.8 (1F, d, *J* = 20 Hz), 18.2 (1F, t, *J* = 19 Hz, H-5).

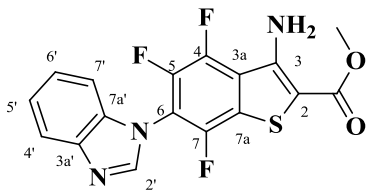
NMR δ_C (100 MHz, $CDCl_3$), 193.2 (C=O), 188.6 (C=O), 153.1 (d, *J* = 246 Hz, C-7), 147.3 (ddd, *J* = 240, 16, 4 Hz, C-4/C-5), 146.9 (C-2), 142.5 (ddd, *J* = 252, 18, 4 Hz, C-4/C-5), 136.8 (C-1'), 135.1 (C-1"), 133.9 (C-4"), 133.4 (C-4'), 130.7 (C-3a/C-7a), 129.4 (C-2', C-6'), 128.9 (C-3', C-5'), 128.8 (C-5", C-3"), 128.6 (C-6", C-2"), 126.0 (d, *J* = 6 Hz, C-3), 125.7 (dd, *J* = 23, 6 Hz, C-3a/C-7a), 110.7 (t, *J* = 22 Hz, C-6), 40.6 (CH_2).

IR, ν_{max} /cm^{-1} 1666 (C=O), 1635 (C=O).

MS, m/z found 443.0374, $C_{23}H_{14}F_3O_2S_2$, ($M+H^+$) requires 443.0382.

Elemental analysis, $C_{23}H_{13}F_3O_2S_2.0.5H_2O$ requires: C, 61.19; H, 3.12; found: C, 61.50; H, 2.83.

Methyl 3-amino-6-(1*H*-benzo[*d*]imidazol-1-yl)-4,5,7-trifluorobenzo[*b*]thiophene-2-carboxylate (99)



Following the general method outlined for compound **82**, nitrile **97** (0.29 g, 1.00 mmol) was reacted with methyl mercaptoacetate (0.11 g, 1.00 mmol) in THF (4 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 3:1) afforded compound **99** (0.13 g, 34%) as a yellow solid. m.p. 216-218 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.09 (1H, s, H-2'), 7.92 (1H, s, H-7'), 7.39-7.34 (2H, m, H-5', H-6'), 7.28 (1H, s, H-4'), 6.32 (2H, s, NH_2), 3.91 (3H, s, CH_3).

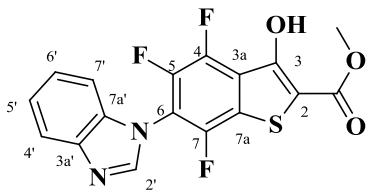
NMR δ_F (376 MHz, $CDCl_3$), 37.9 (1F, d, *J* = 17 Hz), 15.0 (1F, d, *J* = 19 Hz), 16.8 (1F, t, *J* = 19 Hz, F-5).

NMR δ_c (100 MHz, $CDCl_3$), 164.8 (C=O), 147.9 (d, *J* = 251 Hz, C-7), 147.1, 144.0 (dd, *J* = 255, 13 Hz, C-4/C-5), 143.1 (C-2'), 142.0 (dd, *J* = 252, 13 Hz, C-4/C-5), 133.7 (d, *J* = 13 Hz, C-3a/C-7a), 124.6 (C-7'), 123.9 (d, *J* = 15 Hz, C-3a/C-7a), 123.6 (C-5'), 121.5 (d, *J* = 12, 7 Hz), 120.9 (C-6'), 113.5 (t, *J* = 16 Hz, C-6), 110.6 (C-4'), 100.7, 52.1 (CH_3).

IR, ν_{max} /cm^{-1} 3456 (NH), 1655 (C=O), 1607 and 1512 (benzene ring), 1273 (C-O).

MS, m/z found 378.0521, $C_{17}H_{11}F_3N_3O_2S$, ($M+H^+$) requires 378.0519.

Methyl 6-(1*H*-benzo[*d*]imidazol-1-yl)-4,5,7-trifluoro-3-hydroxybenzo[*b*]thiophene-2-carboxylate (98)



Following the general method outlined for compound **82**, ester **96** (0.34 g, 1.00 mmol) was reacted with methyl mercaptoacetate (0.11 g, 1.00 mmol) in THF (4 ml) at room temperature for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 3:1) afforded compound **98** (0.16 g, 41%) as a yellow solid. m.p. 238-240 °C.

NMR δ_H (400 MHz, $CDCl_3$), 10.30 (1H, br, OH), 8.12 (1H, s, H-2'), 7.95 (1H, d, *J* = 7.2 Hz, H-7'), 7.45-7.39 (2H, m, H-5', H-6'), 7.32 (1H, s, H-4'), 4.05 (3H, s, CH_3).

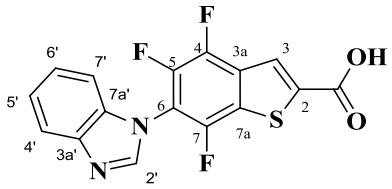
NMR δ_F (376 MHz, $CDCl_3$), 36.7 (1F, d, *J* = 17 Hz), 18.1 (1F, *J* =19 Hz), 16.8 (1F, t, *J* = 19 Hz, F-5).

NMR δ_c (100 MHz, $CDCl_3$), 166.5 (C=O), 157.8, 148.8, 146.3, 144.6, 142.7, 124.7, 123.7, 122.5, 120.8, 110.6, 105.1, 52.9 (CH_3).

IR, ν_{max} /cm^{-1} 1697 (C=O), 1612 and 1512 (benzene ring).

MS, m/z found 379.0361, $C_{17}H_{10}F_3N_2O_3S$, ($M+H^+$) requires 379.0359.

6-(1*H*-Benzo[*d*]imidazol-1-yl)-4,5,7-trifluorobenzo[*b*]thiophene-2-carboxylic acid (100)



A solution of compound **82** (0.36 g, 1.00 mmol) in THF (3 ml) was added a solution of potassium hydroxide (0.14 g, 2.50 mmol) in deionised water (2 ml). The solution was stirred at room temperature for 6 hours. The reaction mixture then was acidified by 1 M HCl (aq.) to pH 2-3. The residues was filtered and washed by deionised water (approx. 10 ml). Pure acid **100** (0.28 g, 80%) was prepared as a white solid, m.p. 295-297 °C.

NMR δ_H (400 MHz, DMSO-d_6), 14.34 (1H, br, OH), 8.57 (1H, s, H-2'), 8.30 (1H, d, *J* = 3.2 Hz, H-3), 7.82-7.78 (1H, m, H-7'), 7.48-7.45 (1H, m, H-4'), 7.35-7.30 (2H, m, H-5', H-6').

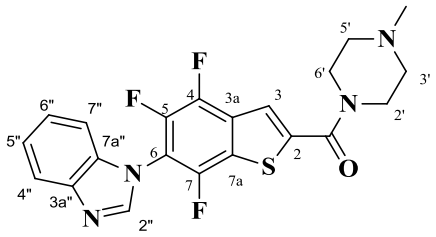
NMR δ_F (376 MHz, DMSO-d_6), 37.6 (1F, d, *J* = 17 Hz), 19.1 (1F, dd, *J* = 21, 17 Hz), 15.6 (1F, d, *J* = 21 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 162.6 (C=O), 148.2 (d, *J* = 251 Hz, C-7), 144.6 (C-2'), 143.2 (dd, *J* = 259, 15 Hz, C-4/C-5), 143.2, 142.5 (dd, *J* = 254, 13 Hz, C-4/C-5), 140.5, 134.3, 129.8 (d, *J* = 12 Hz, C-3a/C-7a), 125.3 (C-3), 125.2 (t, *J* = 20 Hz, C-3a/C-7a), 124.6 (C-6'), 123.6 (C-5'), 120.5 (C-7'), 113.2 (t, *J* = 16 Hz, C-6), 111.4 (C-4').

IR, ν_{max} /cm^{-1} 2365 (b), 1735 (C=O).

MS, m/z found 349.0253, $C_{16}H_8F_3N_2O_2S$, ($M+H^+$) requires 349.0253.

(6-(1*H*-Benzo[*d*]imidazol-1-yl)-4,5,7-trifluorobenzo[*b*]thiophen-2-yl)(4-methylpiperazin-1-yl)methanone (105)



Acid **100** (0.17 g, 0.50 mmol) was suspended in dry THF (1 ml), and oxalyl chloride (0.13 g, 1.00 mmol) was added along with one drop of DMF as a catalyst. The reaction mixture was stirred at room temperature for 16 hours. The residue then was concentrated under reduced pressure and was added into a solution of 1-methylpiperazine (0.05 g, 1.00 mmol) in THF (3 ml). The reaction mixture was stirred at room temperature for 16 hours. The reaction mixture was poured into deionised water (5 ml) and was extracted with ethyl acetate (10 ml x 3). The combined extracts were washed with deionised water (approx. 10 ml) and dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was purified by using silica column chromatography (elution with light petroleum/ ethyl acetate = 2:1) to give compound **105** (0.13 g, 59%) as a white solid, m.p. 193-195 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.06 (1H, s, H-2”), 7.92-7.89 (1H, m, H-7”), 7.66 (1H, d, *J* = 3.2 Hz, H-3), 7.41-7.33 (2H, m, H-6”, H-5”), 7.26-7.23 (1H, m, H-4”), 3.81 (4H, s, H-2’, H-6’), 2.52 (4H, H-3’, H-5’), 2.36 (3H, s, CH_3).

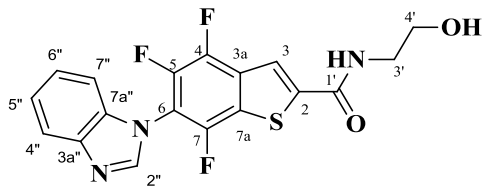
NMR δ_F (376 MHz, $CDCl_3$), 38.6 (1F, d, *J* = 17 Hz), 18.8 (1F, dd, *J* = 20, 17 Hz, F-5), 16.6 (1F, d, *J* = 20 Hz).

NMR δ_c (100 MHz, $CDCl_3$), 161.6 (C=O), 147.7 (d, *J* = 252 Hz, C-7), 143.2, 142.9 (C-2”), 142.8 (dd, *J* = 237, 13 Hz, C-4/ C-5), 142.2 (dd, *J* = 237, 12 Hz, C-4/C-5), 141.9, 133.9, 129.6 (d, *J* = 22 Hz, C-3a/C-7a), 124.5 (C-6”), 124.0 (d, *J* = 24 Hz, C-3a/C-7a), 123.5 (C-5”), 120.9 (C-7”), 119.9 (d, *J* = 5 Hz, C-3), 111.9 (t, *J* = 15 Hz, C-6), 110.4 (C-4”), 54.9 (C-3’, C-5’), 45.8 (C-2’, C-6’), 45.9 (CH_3).

IR, ν_{max} /cm^{-1} 1627 (C=O), 1512 and 1489 (benzene ring).

MS, m/z found 431.1151, $C_{21}H_{18}F_3N_4OS$, ($M+H^+$) requires 431.1148.

6-(1*H*-Benzo[*d*]imidazol-1-yl)-4,5,7-trifluoro-N-(2-hydroxyethyl)benzo[*b*]thiophene-2-carboxamide (106)



Following the general method outlined for compound **105**, acid **100** (0.35 g, 1.00 mmol) was reacted with ethanolamine (0.06 g, 1.00 mmol) in THF (2 ml) for 16 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **106** (0.21 g, 55%) as a light yellow solid. m.p. 83-85 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.06 (1H, s, H-2”), 7.94 (1H, d, *J* = 2.8 Hz, H-3), 7.90 (1H, d, *J* = 6.4, 2.4 Hz, H-7”), 7.40-7.33 (2H, m, H-5”, H-6”), 7.28-7.23 (2H, H-4”), 6.97 (1H, t, *J* = 5.6 Hz, NH), 3.91 (2H, t, *J* = 4.8 Hz, H-4’), 3.69 (2H, dt, *J* = 4.4, 5.6 Hz, H-3’), 2.63 (1H, br, OH).

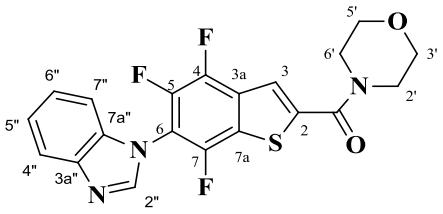
NMR δ_F (376 MHz, $CDCl_3$), 38.8 (1F, d, *J* = 17.7 Hz), 19.3 (1F, dd, *J* = 20, 18 Hz), 16.7 (1F, dd, *J* = 20, 2 Hz).

NMR δ_C (100 MHz, $CDCl_3$), 161.1 (C=O), 147.7 (dt, *J* = 251, 3 Hz, C-7), 144.1 (C-2), 143.0, 142.8 (C-2”), 142.7 (ddd, *J* = 230, 16, 3 Hz, C-4/C-5), 142.3 (ddd, *J*= 243, 14, 5 Hz, C-4/C-5), 133.8, 130.0 (dd, *J* = 16, 10 Hz, C-3a/C-7a), 124.7 (dd, *J* =19, 3 Hz, C-3a/C-4a), 124.5 (C-5”/C-6”), 123.5 (C-5”/C-6”), 120.7 (C-7”), 119.3 (d, *J* = 5 Hz, C-3), 112.2 (t, *J* = 16 Hz, C-6), 110.4 (C-4”), 61.5 (C-4’), 42.7 (C-3’).

IR, ν_{max} /cm^{-1} 3278 (b), 3086 (b), 1635 (C=O), 1550 and 1504 (benzene ring).

MS, m/z found 392.0676, $C_{18}H_{13}F_3N_3O_2S$, ($M+H^+$) requires 392.0675.

(6-(1*H*-Benzo[*d*]imidazol-1-yl)-4,5,7-trifluorobenzo[*b*]thiophen-2-yl)(morpholino) methanone (107)



Following the general method outlined for compound **105**, acid **100** (0.35 g, 1.00 mmol) was reacted with morpholine (0.09 g, 1.00 mmol) in THF (2 ml). Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **107** (0.23 g, 56%) as a white solid. m.p. 201-203 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.08 (1H, s, H-2”), 7.90 (1H, dd, *J* = 7.2, 2.0 Hz, H-3), 7.67 (1H, d, *J* = 3.2 Hz, H-7”), 7.40-7.33 (2H, m, H-5”, H-6”), 7.26-7.23 (1H, H-4”), 3.79 (8H, s, H-2’, H-3’, H-5’, H-6’).

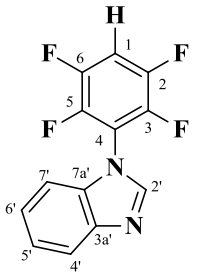
NMR δ_F (376 MHz, $CDCl_3$), 38.7 (1F, dd, *J* = 18, 2 Hz), 18.9 (1F, dd, *J* = 20, 18 Hz), 16.8 (1F, t, *J* = 20, 2 Hz).

NMR δ_C (100 MHz, $CDCl_3$), 161.7 (C=O), 147.7 (dt, *J* = 201, 3 Hz, C-7), 143.2, 142.8 (dd, *J* = 201, 2 Hz, C-4/C-5), 142.8, 142.3 (dd, *J* = 203, 3 Hz, C-4/C-5), 141.5, 133, 129.5 (dd, *J* = 16, 4 Hz, C-3a/C-7a), 124.4, 124.0 (dd, *J* = 15, 3 Hz, C-3a/C-7a), 123.4, 120.9, 120.0 (dd, *J* = 4 Hz, C-3), 112.0 (t, *J* = 13 Hz, C-6), 110.3, 66.8 (C-2’, C-3’, C-5’, C-6’).

IR, ν_{max} /cm^{-1} 2846 (C-H), 1620 (C=O), 1509 and 1481 (benzene ring), 1257 (C-O).

MS, m/z found 418.0831, $C_{20}H_{15}F_3N_3O_2S$, ($M+H^+$) requires 418.0832.

1-(2,3,5,6-Tetrafluorophenyl)-1H-benzo[*d*]imidazole (110)



A solution of compound **68** (0.59 g, 2.00 mmol) in THF (4 ml) was added to NaH (0.20 g, 4.00 mmol). Methyl glycolate (0.18 g, 2.00 mmol) was then added into the reaction dropwise and the solution was stirred at room temperature for 16 hours. The reaction mixture was poured into deionised water (10 ml) and was extracted with ethyl acetate (15 ml x 3). The extracts were combined and washed with brine (approx. 10 ml), and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was subjected to a silica column chromatography (elution with light petroleum/ ethyl acetate = 2:1). The combined elution solutions were evaporated to give the compound **110** (0.19 g, 35%) as a white solid. m.p. 77-79 °C

NMR δ_H (400 MHz, $CDCl_3$), 8.03 (1H, s, H-2'), 7.91-9.88 (1H, m, H-7'), 7.41-7.32 (2H, m, H-5', H-6'), 7.30-7.23 (2H, m, H-4', H-1).

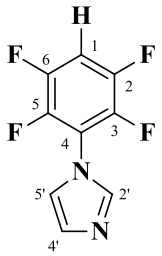
NMR δ_F (376 MHz, $CDCl_3$), 25.7-25.6 (2F, m, F-3, F-5), 16.5-16.4 (2F, m, F-2, F-6).

NMR δ_C (100 MHz, $CDCl_3$), 146.5 (dtd, *J* = 248, 12.4, 3.8 Hz, C-2, C-6), 143.1, 142.4 (C-2'), 142.3 (dd, *J* = 253, 13.3 Hz, C-3, C-5), 133.4, 124.6 (C-6'), 123.7 (C-5'), 120.9 (C-7'), 116.3 (t, *J* = 13.4 Hz, C-4), 110.5 (C-4'), 106.5 (t, *J* = 22 Hz, C-1)

IR, ν_{max} /cm^{-1} 3039 (Ar-H), 1612 and 1504 (benzene).

MS, m/z found 267.0534, $C_{13}H_7F_4N_2$, ($M+H^+$) requires 267.0540.

1-(2,3,5,6-Tetrafluorophenyl)-1*H*-imidazole (111)



Following the general method outlined for compound **110**, aldehyde **67** (0.49 g, 2.00 mmol) was reacted with methyl glycolate (0.18 g, 2.00 mmol) in THF (4 ml). Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2.5:1) afforded compound **111** (0.25 g, 58%) as a light yellow solid. m.p. 93-95 °C.

NMR δ_H (400 MHz, $CDCl_3$), 7.76 (1H, s, H-2'), 7.25-7.11 (3H, m, H-1, H-4', H-5')

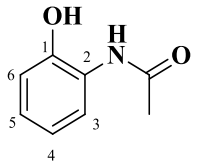
NMR δ_F (376 MHz, $CDCl_3$), 25.7-25.6 (2F, m, F-3, F-5), 16.5-16.4 (2F, m, F-2, F-6).

NMR δ_C (100 MHz, $CDCl_3$), 146.3 (dtd, *J* = 244, 12.4, 3.8 Hz, C-2, C-6), 141.6 (dd, *J* = 250, 19.1 Hz, C-3, C-5), 137.7 (C-2'), 130.2 (C-5'), 120.1 (C-4'), 117.9 (t, *J* = 13.3 Hz, C-4), 105.4 (t, *J* = 21.9 Hz, C-1)

IR, ν_{max} /cm^{-1} 3009 (Ar-H), 1651 and 1504 (benzene).

MS, m/z found 217.0379, $C_9H_5F_4N_2$, ($M+H^+$) requires 217.0383.

2-Acetamidophenol (146)



2-Aminophenol (0.16 g, 1.50 mmol) was added to a solution of acetic anhydride (0.15 g, 1.50 mmol) in methanol (2 ml). The reaction mixture was stirred at room temperature for 5 hours. The mixture was poured into deionised water (10 ml) and extracted with ethyl acetate (15 ml x 3). The combined extracts were dried over sodium sulfate. The solution was concentrated under reduced pressure and the products were purified by recrystallisation (hexane and dichloromethane) and known compound **146** (0.21 g, 91%) was obtained as a white solid. m.p. 206-208 °C. (Literature m.p. 208-210 °C)[139]

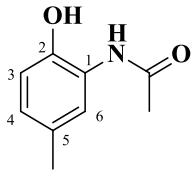
NMR δ_H (400 MHz, DMSO-d_6), 9.70 (1H, s), 9.27 (1H, s), 7.63 (1H, dd, *J* = 8.0, 1.2 Hz, H-3), 6.88 (1H, td, *J* = 7.6, 1.2 Hz, H-5), 6.80 (1H, dd, *J* = 7.6, 1.2 Hz, H-6), 6.71 (1H, td, *J* = 8.0, 1.6 Hz, H-4), 2.05 (3H, s, CH_3).

NMR δ_c (100 MHz, DMSO-d_6), 169.5 (C=O), 148.4, 126.9, 125.1 (C-5), 122.9 (C-3), 119.5 (C-4), 116.4 (C-6), 24.1 (CH_3).

IR, ν_{max} /cm^{-1} 3402 (NH), 3032 (OH), 1651 (C=O), 1589 and 1527 (benzene).

MS, m/z found 152.0709, $C_8H_{10}NO_2$, ($M+H^+$) requires 152.0706.

***N*-(2-Hydroxy-5-methylphenyl)acetamide (147)**



Following the general method outlined for compound **146**, 2-amino-4-methylphenol (0.18 g, 1.50 mmol) and acetic anhydride (0.15 g, 1.50 mmol) was reacted in methanol (4 ml) at room temperature for 5 hours. Work-up as previously described and purification by recrystallisation (hexane and dichloromethane) afforded known compound **147** (0.23 g, 93%) as a white solid. m.p. 158-160 °C. (Literature m.p. 156-157 °C)[140]

NMR δ_H (400 MHz, DMSO-d_6), 9.44 (1H, s), 9.23 (1H, s), 7.44 (1H, s, H-6), 6.69 (2H, s, H-4, H-3), 2.13 (3H, s, CH_3), 2.04 (3H, s, Ar-CH_3).

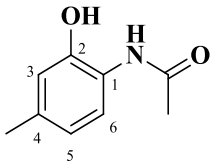
NMR δ_c (100 MHz, DMSO-d_6), 169.5 (C=O), 146.1, 127.9, 126.6, 125.5 (C-3/C-4), 123.3 (C-6), 116.3 (C-3/C-4), 24.1 (Ar-CH_3), 20.9 (CH_3).

Data were in agreement with those reported in the literature.[140]

IR, ν_{max} /cm^{-1} 3263 (NH), 3094 (OH), 1628 (C=O), 1589 and 1550 (benzene).

MS, m/z found 166.0865, $C_9H_{12}NO_2$, ($M+H^+$) requires 166.0863.

***N*-(2-Hydroxy-4-methylphenyl)acetamide (148)**



Following the general method outlined for compound **146**, 2-amino-5-methylphenol (0.18 g, 1.50 mmol) and acetic anhydride (0.15 g, 1.50 mmol) was reacted in methanol (4 ml) at room temperature for 5 hours. Work-up as previously described and purification by recrystallisation (hexane and dichloromethane) afforded known compound **148** (0.20 g, 82%) as a white solid. m.p. 168-170 °C. (Literature m.p. 169-170 °C)[141]

NMR δ_H (400 MHz, DMSO-d_6), 9.59 (1H, s), 9.26 (1H, s), 7.43 (1H, d, *J* = 8 Hz, H-6), 6.62 (1H, s, H-3), 6.52 (1H, d, *J* = 8 Hz, H-5), 2.14 (3H, s, CH_3), 2.02 (3H, s, Ar-CH_3).

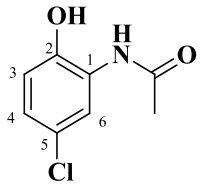
NMR δ_c (100 MHz, DMSO-d_6), 169.4 (C=O), 148.4, 134.5, 124.3, 122.9 (C-6), 120.0 (C-5), 117.1 (C-3), 24.0 (Ar-CH_3), 21.1 (CH_3).

Data were in agreement with those reported in the literature.[141]

IR, ν_{max} /cm^{-1} 3263 (NH), 3070 (OH), 1635 (C=O), 1589 and 1543 (benzene).

MS, m/z found 166.0865, $C_{10}H_5F_4N_2O$, ($M+H^+$) requires 166.0863.

***N*-(2-Hydroxy-5-chlorophenyl)acetamide (149)**



Following the general method outlined for compound **146**, 2-amino-4-chlorophenol (0.22 g, 1.50 mmol) and acetic anhydride (0.15 g, 1.50 mmol) was reacted in methanol (4 ml) at room temperature for 5 hours. Work-up as previously described and purification by recrystallisation (hexane and dichloromethane) afforded known compound **149** (0.24 g, 87%) as a grey solid. m.p. 182-184 °C. (Literature m.p. 183-184 °C)[142]

NMR δ_H (400 MHz, DMSO-d_6), 10.09 (1H, s), 9.24 (1H, s), 7.91 (1H, d, *J* = 2.4 Hz, H-6), 6.89 (1H, dd, *J* = 8.4, 2.4 Hz, H-4), 6.80 (1H, dd, *J* = 8.8 Hz, H-3), 2.06 (3H, s, CH_3).

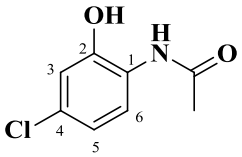
NMR δ_c (100 MHz, DMSO-d_6), 169.6 (C=O), 146.7, 128.3, 123.9 (C-4), 122.6, 121.5 (C-6), 116.8 (C-3), 24.3 (CH_3).

Data were in agreement with those reported in the literature.[142]

IR, ν_{max} /cm^{-1} 3387 (NH), 3032 (OH), 1659 (C=O), 1589 and 1535 (benzene).

MS, m/z found 186.0320, $C_8H_9{}^{35}ClNO_2$, ($M+H^+$) requires 186.0316.

***N*-(2-Hydroxy-4-chlorophenyl)acetamide (150)**



Following the general method outlined for compound **146**, 2-amino-5-chlorophenol (0.22 g, 1.50 mmol) and acetic anhydride (0.15 g, 1.50 mmol) was dissolved in methanol (4 ml) at room temperature for 5 hours. Work-up as previously described and purification by recrystallisation (hexane and dichloromethane) afforded known compound **150** (0.22 g, 80%) as a black solid. m.p. 190-192 °C. (Literature m.p. 186 °C)[143]

NMR δ_H (400 MHz, DMSO-d_6), 10.27 (1H, s), 9.24 (1H, s), 7.73 (1H, d, *J* = 8.8 Hz, H-6), 6.82 (1H, d, *J* = 1.6 Hz, H-3), 6.75 (1H, dd, *J* = 8.4, 2.0 Hz, H-5), 2.04 (3H, s, CH3).

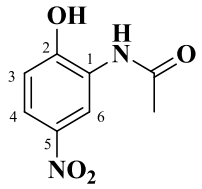
NMR δc (100 MHz, DMSO-d_6), 169.4 (C=O), 149.3, 128.1, 126.2, 123.8 (C-6), 119.1 (C-5), 115.7 (C-3), 24.1 (CH_3).

Data were in agreement with those reported in the literature.[143]

IR, ν_{max} /cm^{-1} 3232 (NH), 3047 (OH), 1623 (C=O), 1581 and 1527 (benzene).

MS, m/z found 186.0320, $C_8H_9{}^{35}ClNO_2$, ($M+H^+$) requires 186.0316.

***N*-(2-Hydroxy-5-nitrophenyl)acetamide (151)**



Following the general method outlined for compound **146**, 2-amino-4-nitrophenol (0.23 g, 1.50 mmol) and acetic anhydride (0.15 g, 1.50 mmol) was dissolved in methanol (4 ml) at room temperature for 5 hours. Work-up as previously described and purification by recrystallisation (hexane and dichloromethane) afforded known compound **151** (0.29 g, 98%) as a yellow solid. m.p. 274-276°C.

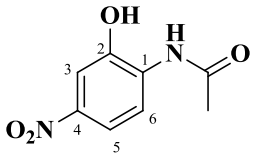
NMR δ_H (400 MHz, DMSO-d_6), 11.6 (1H, s), 9.47 (1H, s), 8.94 (1H, d, *J* = 2.4 Hz, H-6), 7.89 (1H, dd, *J* = 9.2, 2.8 Hz, H-4), 7.02 (1H, d, *J* = 9.2 Hz, H-3), 2.15 (3H, s, CH_3).

NMR δ_c (100 MHz, DMSO-d_6), 169.8 (C=O), 154.2, 139.6, 127.4, 120.9 (C-4), 117.0 (C-6), 115.1 (C-3), 24.3 (CH_3).

IR, ν_{max} /cm^{-1} 3379 (NH), 1659 (C=O), 1589 and 1497 (benzene), 1527 and 1335 (NO_2).

MS, m/z found 197.0560, $C_8H_9N_2O_4$, ($M+H^+$) requires 197.0557.

***N*-(2-Hydroxy-4-nitrophenyl)acetamide (152)**



Following the general method outlined for compound **146**, 2-amino-5-nitrophenol (0.23 g, 1.50 mmol) and acetic anhydride (0.15 g, 1.50 mmol) was dissolved in methanol (4 ml) at room temperature for 5 hours. Work-up as previously described and purification by recrystallisation (hexane and dichloromethane) afforded known compound **152** (0.27 g, 90%) as a white solid. m.p. 261-263 °C. (Literature m.p. 264 °C)[144]

NMR δ_H (400 MHz, DMSO-d_6), 10.95 (1H, s), 9.48 (1H, s), 8.23 (1H, dd, *J* = 9.2, 1.2 Hz, H-6), 7.65 (1H, dd, *J* = 9.2, 2.0 Hz, H-5), 7.62 (1H, d, *J* = 1.6 Hz, H-3), 2.12 (3H, s, CH_3).

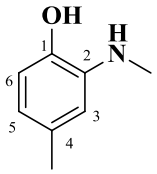
NMR δ_c (100 MHz, DMSO-d_6), 170.1 (C=O), 147.3, 142.8, 134.1, 120.2 (C-6), 115.7 (C-5), 109.6 (C-3), 24.6 (CH_3).

Data were in agreement with those reported in the literature.[144]

IR, ν_{max} /cm^{-1} 3371 (NH), 1666 (C=O), 1589 and 1419 (benzene), 1512 and 1327 (NO_2).

MS, m/z found 197.0560, $C_8H_9N_2O_4$, ($M+H^+$) requires 197.0557.

4-Methyl-2-(methylamino)phenol (140)



Iodomethane (0.24 g, 1.70 mmol) was added to a solution of 2-amino-4-methylphenol (0.18 g, 1.50 mmol) in DMF (2 ml). Sodium bicarbonate (0.13 g, 1.60 mmol) was added. The reaction mixture was stirred at room temperature for 5 hours. The mixture was poured into 10 ml deionised water and extracted with ethyl acetate (15 ml x 3). The extracts were combined and washed with brine (approx. 10 ml), and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (elution with light petroleum/ ethyl acetate = 5:1). The combined elution solutions were evaporated to give compound **140** (0.12 g, 61%) as a pale pink solid. m.p. 103-105 °C.

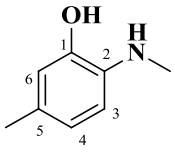
NMR δ_H (400 MHz, DMSO-d_6), 8.82 (1H, s), 6.46 (1H, d, *J* = 7.6 Hz, H-6), 6.19 (1H, s, H-3), 6.26 (1H, dd, *J* = 7.6, 1.2 Hz, H-5), 4.59 (1H, d, *J* = 4.4 Hz), 2.64 (3H, d, *J* = 4.8 Hz, HN-CH_3), 2.11 (3H, s, Ar-CH_3).

NMR δ_c (100 MHz, DMSO-d_6), 142.3, 139.0, 128.6, 116.0 (C-5), 113.4 (C-6), 110.5 (C-3), 30.5 (HN-CH_3), 21.5 (Ar-CH_3).

IR, ν_{max} /cm^{-1} 3325 (NH), 1597 and 1473 (benzene).

MS, m/z found 138.0916, $C_8H_{12}NO$, ($M+H^+$) requires 138.0913.

5-Methyl-2-(methylamino)phenol (141)



Following the general method outlined for compound **140**, 2-amino-5-methylphenol (0.18 g, 1.50 mmol) and iodomethane (0.24 g, 1.70 mmol) was dissolved in methanol (4 ml) with sodium bicarbonate (0.13 g, 1.60 mmol) at room temperature for 5 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 5:1) afforded compound **141** (0.05 g, 26%) as a yellow solid. m.p. 159-161 °C.

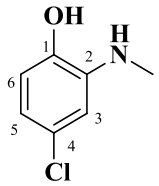
NMR δ_H (400 MHz, DMSO-d_6), 9.00 (1H, s), 6.42 (1H, s, H-6), 6.40 (1H, s, H-3), 6.26 (1H, d, *J* = 7.2 Hz, H-4), 4.46 (1H, s), 2.62 (3H, s, HN-CH_3), 2.06 (3H, s, Ar-CH_3).

NMR δ_c (100 MHz, DMSO-d_6), 144.5, 136.9, 124.5, 120.3 (C-3), 114.7 (C-6), 109.6 (C-4), 30.7 (HN-CH_3), 20.9 (Ar-CH_3).

IR, ν_{max} /cm^{-1} 3317 (NH), 1589 and 1527 (benzene).

MS, m/z found 138.0915, $C_8H_{12}NO$, ($M+H^+$) requires 138.0913.

4-Chloro-2-(methylamino)phenol (142)



Following the general method outlined for compound **140**, 2-amino-4-chlorophenol (0.22 g, 1.50 mmol) and iodomethane (0.24 g, 1.70 mmol) was dissolved in methanol (4 ml) with sodium bicarbonate (0.13 g, 1.60 mmol) at room temperature for 5 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 5:1) afforded compound **142** (0.14 g, 60%) as a black oil.

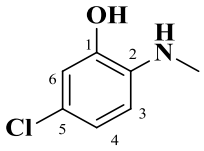
NMR δ_H (400 MHz, DMSO-d_6), 9.44 (1H, s), 6.54 (1H, d, *J* = 8.0 Hz, H-6), 6.34 (1H, dd, *J* = 8.0, 2.4 Hz, H-5), 6.30 (1H, d, *J* = 2.4 Hz, H-3), 5.08 (1H, s), 2.63 (3H, s, HN-CH_3).

NMR δ_c (100 MHz, DMSO-d_6), 143.4, 140.6, 124.0, 114.6 (C-5), 114.1 (C-6), 108.7 (C-3), 30.1 (HN-CH_3).

IR, ν_{max} /cm^{-1} 3170 (b), 1604 and 1512 (benzene).

MS, m/z found 158.0369, $C_7H_9{}^{35}ClNO$, ($M+H^+$) requires 158.0367.

5-Chloro-2-(methylamino)phenol (143)



Following the general method outlined for compound **140**, 2-amino-5-chlorophenol (0.22 g, 1.50 mmol) and iodomethane (0.24 g, 1.70 mmol) was dissolved in methanol (4 ml) with sodium bicarbonate (0.13 g, 1.60 mmol) at room temperature for 5 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 5:1) afforded compound **143** (0.08 g, 32%) as a black solid. m.p. 110-112 °C.

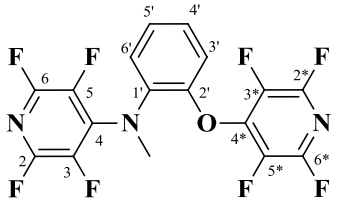
NMR δ_H (400 MHz, DMSO-d_6), 9.67 (1H, s), 6.63 (1H, dd, *J* = 8.0, 2.4 Hz, H-4), 6.59 (1H, d, *J* = 2.4, 2.4 Hz, H-6), 6.33 (1H, d, *J* = 8.0 Hz, H-3), 4.87 (1H, s), 2.63 (3H, s, HN-CH_3).

NMR δ_c (100 MHz, DMSO-d_6), 145.4, 138.4, 119.5 (C-4), 118.7, 113.3 (C-6), 109.9 (C-3), 30.1 (HN-CH_3).

IR, ν_{max} /cm^{-1} 3317 (NH), 1589 and 1473 (benzene).

MS, m/z found 158.0370, $C_7H_9{}^{35}ClNO$, ($M+H^+$) requires 158.0367.

2,3,5,6-Tetrafluoro-*N*-methyl-*N*-(2-((perfluoropyridin-4-yl)oxy)phenyl)pyridin-4-amine (124)



A solution of pentafluoropyridine (0.68 g, 4.00 mmol) in THF (1 ml) was added a solution of 2-methylaminophenol (0.25 g, 2.00 mmol) in THF (1 ml). Triethylamine (0.70 ml, 5.00 mmol) was added into the reaction. The solution was stirred at room temperature for 16 hours. The reaction mixture was poured into deionised water (10 ml) and was extracted with ethyl acetate (15 ml x 3). The extracts were combined and washed with brine (approx. 10 ml), and then dried with sodium sulfate. The solution was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (elution with light petroleum/ ethyl acetate = 20:1). The combined elution solutions were evaporated under reduced pressure to give compound **124** (0.47 g, 56%) as a white solid. m.p. 74-76 °C.

NMR δ_H (400 MHz, $CDCl_3$), 7.35 (1H, dd, *J* = 8.0, 2.0 Hz, H-3'), 7.28-7.20 (2H, m, H-4', H-5'), 6.89 (1H, d, *J* = 7.6 Hz, H-6'), 3.56 (3H, t, *J* = 2.0 Hz, CH_3).

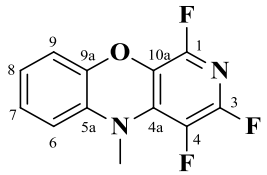
NMR δ_F (376 MHz, $CDCl_3$), 74.0-73.8 (2F, m), 69.4-69.2 (2F, m), 8.9-8.7 (2F, m), 6.8-6.6 (2F, m).

NMR δ_C (100 MHz, $CDCl_3$), 149.8, 144.6 (dt, *J* = 243, 16 Hz), 144.1 (dt, *J* = 243, 15 Hz), 143.6-143.5 (m), 138.6-138.3 (m), 137.3-136.7 (m), 136.5, 134.6-133.9 (m), 127.3, 126.2, 126.1, 116.7, 42.3 (t, *J* = 5 Hz, CH_3).

IR, ν_{max}/cm^{-1} 1635 and 1466 (benzene ring).

MS, m/z found 422.0530, $C_{17}H_8F_8N_3O$, $(M+H^+)$ requires 422.0545.

1,3,4-Trifluoro-5-methyl-5*H*-benzo[*b*]pyrido[3,4-*b*][1,4]oxazine (125)



A solution of pentafluoropyridine (0.85 g, 5.00 mmol) in MeCN (4 ml) was added to a solution of 2-methylaminophenol (0.62 g, 5.00 mmol) in MeCN (4 ml). Triethylamine (2.80 ml, 20.00 mmol) was then added. The solution was heated at reflux for 16 hours. The reaction mixture was poured into deionised water (25 ml) and was extracted with ethyl acetate (25 ml x 3). The extracts were combined and washed with brine (approx. 20 ml), and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (elution with light petroleum/ ethyl acetate = 20:1). The combined elution solutions were evaporated under reduced pressure to give compound **125** (0.77 g, 61%) as a white solid. m.p. 150-152 °C.

NMR δ_H (400 MHz, $CDCl_3$), 6.94 (1H, td, *J* = 8.0, 1.6 Hz, C-7), 6.85 (1H, td, *J* = 8.0, 1.6 Hz, C-8), 6.77 (1H, dd, *J* = 8.0, 1.6 Hz, C-9), 6.67 (1H, dd, *J* = 7.6, 1.2 Hz, C-6), 3.37 (3H, d, *J* = 4.4 Hz).

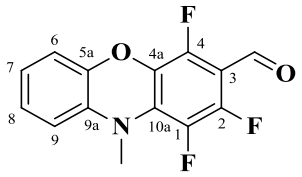
NMR δ_F (376 MHz, $CDCl_3$), 68.8 (1F, dd, *J* = 22, 15 Hz), 66.7 (1F, dd, *J* = 22, 15 Hz), -0.2 (1F, tq, *J* = 22, 5 Hz).

NMR δ_C (100 MHz, $CDCl_3$), 145.6(dt, *J* = 235, 17 Hz), 145.3, 142.9 (dd, *J* = 235, 15 Hz), 136.5 (dt, *J* = 6, 2 Hz, C-4a/C-10a), 132.4, 130.6 (dd, *J* = 247, 6 Hz), 126.39 (dd, *J* = 28, 5 Hz, C-4a/C-10a), 124.9 (C-7), 124.1 (C-8), 116.2 (C-9), 113.5 (C-6), 35.6 (d, *J* = 12.4 Hz, CH_3).

IR, ν_{max}/cm^{-1} 1643 and 1496 (benzene ring)

MS, m/z found 253.0581, $C_{12}H_8F_3N_2O$, $(M+H^+)$ requires 253.0594.

1,2,4-Trifluoro-10-methyl-10*H*-phenoxazine-3-carbaldehyde (162)



Following the general method outlined for compound **125**, pentafluorobenzaldehyde (0.29 g, 1.50 mmol) and 2-methylaminophenol (0.18 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was heated at reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **162** (0.26 g, 63%) as a yellow solid. m.p. 145-147 °C.

NMR δ_H (400 MHz, $CDCl_3$), 10.04 (1H, d, *J* = 1.2 Hz, CHO), 6.94 (1H, td, *J* = 8.0, 1.2 Hz, H-8), 6.86 (1H, td, *J* = 8.0, 1.2 Hz, H-7), 6.79 (1H, dd, *J* = 6.6, 1.2 Hz, H-6), 6.68 (1H, dd, *J* = 8.0, 1.2 Hz, H-9), 3.39 (3H, d, *J* = 5.6 Hz, CH_3).

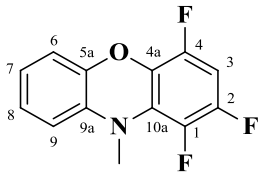
NMR δ_F (376 MHz, $CDCl_3$), 15.3 (1F, dd, *J* = 9, 4 Hz), 15.2 (1F, dd, *J* = 19, 4 Hz), 2.4-2.2 (1F, m).

NMR δ_c (100 MHz, $CDCl_3$), 182.2 (t, *J* = 3 Hz, CHO), 149.0 (ddd, *J* = 257, 14, 8 Hz), 145.8 (ddd, *J* = 255, 7, 2 Hz), 145.2, 135.3 (ddd, *J* = 241, 16, 3 Hz), 132.9, 132.4-132.2 (m, C-4a/C-10a), 131.3 (dt, *J* = 14, 3 Hz, C-4a/C-10a), 124.9 (C-8), 124.1 (C-7), 116.0 (C-6), 113.8 (C-9), 107.2 (t, *J* = 10 Hz, C-3), 36.7 (d, *J* = 12 Hz, CH_3)

IR, ν_{max} /cm^{-1} 1681 (CHO), 1643 and 1473(benzene ring).

MS, m/z found 280.0577, $C_{14}H_9F_3NO_2$, ($M+H^+$) requires 280.0580.

1,2,4-Trifluoro-10-methyl-10*H*-phenoxazine (161)



Following the general method outlined for compound **125**, pentafluorobenzene (0.25 g, 1.50 mmol) and 2-methylaminophenol (0.18 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was heated at reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **161** (0.14 g, 45%) as a white solid. m.p. 118-120 °C.

NMR δ_H (400 MHz, $CDCl_3$), 6.95-6.89 (1H, m, H-8), 6.82-6.77 (2H, m, H-7, H-6), 6.64 (1H, td, *J* = 8.4 Hz, H-9), 6.45-6.38 (1H, m, H-3), 3.35 (3H, d, *J* = 4.8 Hz, CH_3).

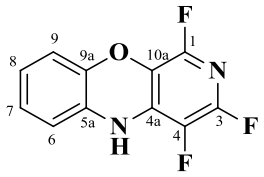
NMR δ_F (376 MHz, $CDCl_3$), 21.9 (1F, t, *J* = 10 Hz), 20.0 (1F, dd, *J* = 21, 10 Hz), 2.9-2.8 (1F, m).

NMR δ_c (100 MHz, $CDCl_3$), 147.0 (dt, *J* = 194, 11 Hz), 145.9, 145.2 (ddd, *J* = 194, 10, 2 Hz), 136.5 (ddd, *J* = 191, 13, 3 Hz), 134.8, 132.0 (dt, *J* = 12, 3 Hz, C-4a/C-10a), 126.9-126.7 (m, C-4a/C-10a), 124.5 (C-8), 122.6 (C-7), 115.7 (C-6), 113.8 (C-9), 97.0 (dd, *J* = 19, 18 Hz, C-3), 37.4 (d, *J* = 9 Hz, CH_3)

IR, ν_{max} /cm^{-1} 1651 and 1481 (benzene ring).

MS, m/z found 252.0585, $C_{10}H_5F_4N_2O$, ($M+H^+$) requires 252.0631.

1,3,4-Trifluoro-5*H*-benzo[*b*]pyrido[3,4-*b*][1,4]oxazine (153)



Following the general method outlined for compound **125**, pentafluoropyridine (0.25 g, 1.50 mmol) and 2-acetamidophenol (0.23 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was heated at reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **153** (0.22 g, 60%) as a white solid. m.p. 225-227 °C.

NMR δ_H (400 MHz, $CDCl_3$), 6.87-6.76 (3H, m, H-7, H-8, H-9), 6.53 (1H, dd, *J* = 6.8, 1.6 Hz, H-6), 5.88 (1H, s, NH).

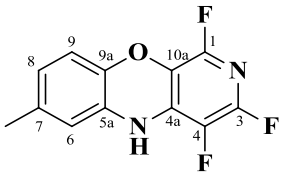
NMR δ_F (376 MHz, $CDCl_3$), 68.8-68.4 (2F, m, F-1, F-3), -5.7 (1F, t, *J* = 23 Hz, F-4).

NMR δ_c (100 MHz, $CDCl_3$), 143.3 (ddd, *J* = 182, 12, 10 Hz), 142.6, 142.5 (ddd, *J* = 189, 11, 5 Hz), 133.7 (dt, *J* = 11, 4 Hz, C-4a/C-10a), 129.4 (ddd, *J* = 198, 26, 5 Hz), 126.6, 124.9 (C-7), 124.4 (C-8), 123.8 (dd, *J* = 24, 5 Hz, C-4a/C-10a), 116.8 (C-9), 114.8 (C-6).

IR, ν_{max} /cm^{-1} 3348 (NH), 1651 and 1535 (benzene ring).

MS, m/z found 239.0426, $C_{11}H_6F_3N_2O$, ($M+H^+$) requires 239.0427.

1,3,4-Trifluoro-7-methyl-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (154)



Following the general method outlined for compound **125**, pentafluoropyridine (0.25 g, 1.50 mmol) and *N*-(2-hydroxy-5-methylphenyl)acetamide (0.25 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was heated at reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **154** (0.12 g, 34%) as a white solid. m.p. 208-210 °C.

NMR δ_H (400 MHz, DMSO-d_6), 9.62 (1H, NH), 6.59 (1H, d, *J* = 7.6 Hz, H-6), 6.52(1H, s, H-9), 6.50 (1H, s, H-8), 2.07 (3H, s, CH_3).

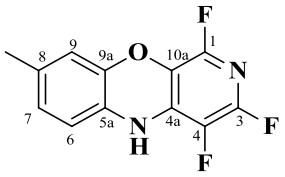
NMR δ_F (376 MHz, DMSO-d_6), 66.0 (1F, dd, *J* = 23, 14 Hz), 65.5 (1F, dd, *J* = 21, 14 Hz), -3.6 (1F, t, *J* = 23 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 143.4 (ddd, *J* = 230, 23, 6 Hz), 142.2 (dd, *J* = 230, 15 Hz), 140.3, 135.6-135.3 (m, C-4a/C-10a), 134.7, 129.6 (ddd, *J* = 245, 32, 4 Hz), 127.7, 124.1 (C-9), 123.7 (d, *J* = 5 Hz, C-4a/C-10a), 116.4 (C-8), 116.1 (C-6), 20.8 (CH_3).

IR, ν_{max} /cm^{-1} 3410 (NH), 1651 and 1512 (benzene ring).

MS, m/z found 253.0588, $C_{12}H_8F_3N_2O$, ($M+H^+$) requires 253.0583.

1,3,4-Trifluoro-8-methyl-5H-pyrido[3,4-*b*][1,4]benzoxazine (155)



Following the general method outlined for compound **125**, pentafluoropyridine (0.25 g, 1.50 mmol) and *N*-(2-hydroxy-4-methylphenyl)acetamide (0.25 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was under reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **155** (0.10 g, 30%) as a white solid. m.p. 218-220 °C.

NMR δ_H (400 MHz, DMSO-d_6), 9.58 (1H, NH), 6.64-6.56 (2H, m, H-6, H-7), 6.54(1H, s, H-9), 2.06 (3H, s, CH_3).

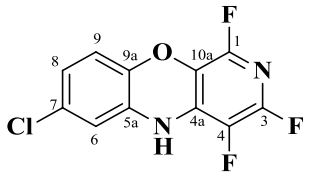
NMR δ_F (376 MHz, DMSO-d_6), 65.7 (1F, dd, *J* = 24, 15 Hz), 65.2 (1F, dd, *J* = 22, 14 Hz), -4.2 (1F, t, *J* = 23 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 143.4 (ddd, *J* = 183, 13, 10 Hz), 142.2 (dd, *J* = 185, 12 Hz), 142.1, 135.5 (dt, *J* = 11, 4 Hz, C-4a/ C-10a), 133.5, 129.4 (ddd, *J* = 196, 25, 4 Hz), 125.5 (C-6), 125.4, 123.6 (dd, *J* = 23, 4 Hz, C-4a/ C-10a), 116.8 (C-9), 115.6 (C-7), 22.6 (CH_3).

IR, ν_{max} /cm^{-1} 3263 (NH), 1651 and 1474 (benzene ring).

MS, m/z found 253.0588, $C_{12}H_8F_3N_2O$, ($M+H^+$) requires 253.0583.

7-Chloro-1,3,4-trifluoro-5H-pyrido[3,4-*b*][1,4]benzoxazine (156)



Following the general method outlined for compound **125**, pentafluoropyridine (0.25 g, 1.50 mmol) and *N*-(2-hydroxy-5-chlorophenyl)acetamide (0.28 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was under reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **156** (0.23 g, 56%) as a cream solid. m.p. 257-259 °C.

NMR δ_H (400 MHz, DMSO-d_6), 9.82 (1H, NH), 6.75-6.69 (2H, m, H-9, H-8), 6.66(1H, d, *J* = 1.2 Hz, H-6).

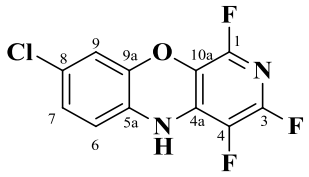
NMR δ_F (376 MHz, DMSO-d_6), 66.5 (1F, dd, *J* = 23, 14 Hz), 65.7 (1F, dd, *J* = 23, 14 Hz), -3.4 (1F, t, *J* = 23 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 143.5 (ddd, *J* = 231, 16, 13 Hz), 142.1 (ddd, *J* = 231, 14, 2 Hz), 141.4, 134.7 (dt, *J* = 13, 6 Hz, C-4a/C-10a), 129.7, 129.6 (ddd, *J* = 230, 32, 5 Hz), 128.8, 123.7 (dd, *J* = 30, 5 Hz, C-4a/C-10a), 123.2 (C-9), 117.7 (C-8), 115.3 (C-6).

IR, ν_{max} /cm^{-1} 3402 (NH), 1651 and 1527 (benzene ring).

MS, m/z found 273.0041, $C_{11}H_5{}^{35}ClF_3N_2O$, ($M+H^+$) requires 273.0037.

8-Chloro-1,3,4-trifluoro-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (157)



Following the general method outlined for compound **125**, pentafluoropyridine (0.25 g, 1.50 mmol) and *N*-(2-hydroxy-4-chlorophenyl)acetamide (0.28 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was under reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **157** (0.19 g, 45%) as a cream solid. m.p. 194-196 °C.

NMR δ_H (400 MHz, DMSO-d_6), 9.81 (1H, NH), 6.88-6.83 (2H, m, H-6, H-7), 6.67(1H, d, *J* = 8.8 Hz, H-9).

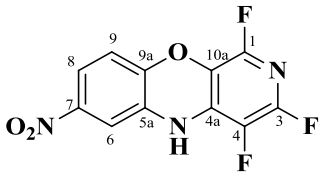
NMR δ_F (376 MHz, DMSO-d_6), 66.6 (1F, dd, *J* = 23, 14 Hz), 65.8 (1F, dd, *J* = 23, 14 Hz), -3.6 (1F, t, *J* = 23 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 143.7 (ddd, *J* = 231, 16, 13 Hz), 143.2, 142.2 (dd, *J* = 232, 16 Hz), 135.2 (dt, *J* = 13, 6 Hz, C-4a/C-10a), 129.6 (ddd, *J* = 246, 32, 4 Hz), 127.5, 127.0, 125.2 (C-6), 123.5 (dd, *J* = 30, 5 Hz, C-4a/C-10a),116.8 (C-9), 116.5 (C-7).

IR, ν_{max} /cm^{-1} 3255 (NH), 1651 and 1527 (benzene ring).

MS, m/z found 273.0041, $C_{11}H_5{}^{35}ClF_3N_2O$, ($M+H^+$) requires 273.0037.

1,3,4-Trifluoro-7-nitro-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (158)



Following the general method outlined for compound **125**, pentafluoropyridine (0.25 g, 1.50 mmol) and *N*-(2-hydroxy-5-nitrophenyl)acetamide (0.29 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was under reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **158** (0.22 g, 51%) as an orange solid. m.p. 267-269 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.11 (1H, NH), 7.57 (1H, dd, *J* = 8.8, 2.8 Hz, H-8), 7.41 (1H, d, *J* = 2.4 Hz, H-6), 6.89 (1H, d, *J* = 8.8 Hz, H-9).

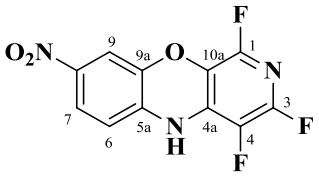
NMR δ_F (376 MHz, DMSO-d_6), 67.6 (1F, dd, *J* = 23, 14 Hz), 66.3 (1F, dd, *J* = 23, 14 Hz), -3.2 (1F, t, *J* = 23 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 147.9, 144.5, 144.0 (ddd, *J* = 232, 16, 13 Hz), 142.2 (dd, *J* = 231, 17 Hz), 134.5 (dt, *J* = 13, 6 Hz, C-4a/C-10a), 129.7 (ddd, *J* = 247, 32, 5 Hz), 129.5, 123.4 (dd, *J* = 30, 5 Hz, C-4a/C-10a), 120.1 (C-8), 116.8 (C-9), 110.2(C-6).

IR, ν_{max} /cm^{-1} 3309 (NH), 1659 and 1489 (benzene ring), 1612 and 1396 (NO_2).

MS, m/z found 284.0283, $C_{11}H_5F_3N_3O_3$, ($M+H^+$) requires 284.0278.

1,3,4-Trifluoro-8-nitro-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (159)



Following the general method outlined for compound **125**, pentafluoropyridine (0.25 g, 1.50 mmol) and *N*-(2-hydroxy-4-nitrophenyl)acetamide (0.29 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was under reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **159** (0.20 g, 48%) as a yellow solid. m.p. 211-213 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.48 (1H, NH), 7.76 (1H, dd, *J* = 8.8, 2.0 Hz, H-7), 7.47 (1H, d, *J* = 2.0 Hz, H-9), 6.81 (1H, d, *J* = 9.2 Hz, H-6).

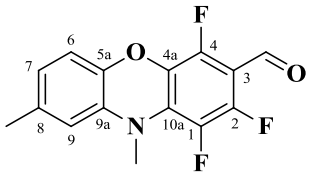
NMR δ_F (376 MHz, DMSO-d_6), 67.4 (1F, dd, *J* = 23, 14 Hz), 66.3 (1F, dd, *J* = 23, 14 Hz), -2.1 (1F, t, *J* = 23 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 143.6 (ddd, *J* = 232, 16, 14 Hz), 142.7, 142.5, 142.0 (dd, *J* = 232, 16 Hz), 135.5, 134.0 (dt, *J* = 13, 6 Hz, C-4a/C-10a), 130.0 (ddd, *J* = 243, 32, 5 Hz), 123.8 (dd, *J* = 30, 5 Hz, C-4a/C-10a), 122.5 (C-7), 115.4 (C-6), 111.6 (C-9).

IR, ν_{max} /cm^{-1} 3279 (NH), 1658 and 1512 (benzene ring), 1589 and 1334 (NO_2).

MS, m/z found 284.0282, $C_{11}H_5F_3N_3O_3$, ($M+H^+$) requires 284.0278.

1,2,4-Trifluoro-8,10-dimethyl-10*H*-phenoxazine-3-carbaldehyde (163)



Following the general method outlined for compound **125**, pentafluorobenzaldehyde (0.29 g, 1.50 mmol) and 4-methyl-2-(methylamino)phenol (0.21 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was under reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **163** (0.31 g, 74%) as a yellow solid. m.p. 141-143 °C.

NMR δ_H (400 MHz, $CDCl_3$), 10.09 (1H, d, *J* = 0.8 Hz, CHO), 6.74-6.68 (2H, m, H-6, H-9), 6.53 (1H, dd, *J* = 1.2 Hz, H-7), 3.43 (3H, d, *J* = 4.0 Hz, N-CH_3), 2.28 (3H, s, CH_3).

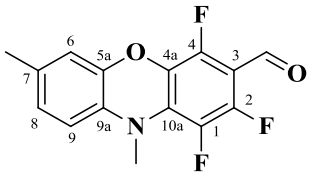
NMR δ_F (376 MHz, $CDCl_3$), 15.1 (1F, dd, *J* = 8, 4 Hz), 14.8 (1F, dd, *J* = 15, 4 Hz), 2.2-2.1 (1F, m).

NMR δ_c (100 MHz, $CDCl_3$), 177.4 (CHO), 144.1 (ddd, *J* = 205, 11, 6 Hz), 141.0 (ddd, *J* = 203, 6, 2 Hz), 140.0 (q, *J* = 3 Hz), 130.7 (ddd, *J* = 193, 13, 2 Hz), 129.9, 127.9, 127.7-127.5 (m, C-4a/C-10a), 126.7 (dt, *J* = 12, 2 Hz, C-4a/C-10a), 119.4 (C-6), 110.9 (C-9), 109.8 (C-7), 102.5 (t, *J* = 8 Hz, C-3), 32.0 (d, *J* = 9 Hz, N-CH_3), 16.3 (CH_3).

IR, ν_{max} /cm^{-1} 1689 (CHO), 1643 and 1473 (benzene ring).

MS, m/z found 294.0738, $C_{15}H_{11}F_3NO_2$, ($M+H^+$) requires 294.0736.

1,2,4-Trifluoro-7,10-dimethyl-10*H*-phenoxazine-3-carbaldehyde (164)



Following the general method outlined for compound **125**, pentafluorobenzaldehyde (0.29 g, 1.50 mmol) and 5-methyl-2-(methylamino)phenol (0.21 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was under reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **164** (0.21 g, 50%) as a yellow solid. m.p. 158-160 °C.

NMR δ_H (400 MHz, $CDCl_3$), 10.08 (1H, d, *J* = 0.4 Hz, CHO), 6.77 (1H, dd, *J* = 6.4, 0.8 Hz, H-8), 6.68 (1H, d, *J* = 1.2 Hz, H-6), 6.61 (1H, d, *J* = 6.4 Hz, H-9), 3.42 (3H, d, *J* = 4.0 Hz, N-CH_3), 2.25 (3H, s, CH_3).

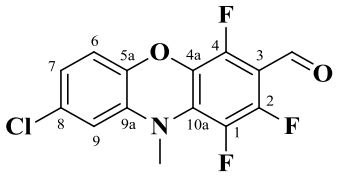
NMR δ_F (376 MHz, $CDCl_3$), 15.1 (1F, dd, *J* = 8, 4 Hz), 14.9 (1F, dd, *J* = 15, 4 Hz), 2.0-1.8 (1F, m).

NMR δ_c (100 MHz, $CDCl_3$), 182.1 (CHO), 149.0 (ddd, *J* = 257, 14, 8 Hz), 145.8 (ddd, *J* = 256, 7, 3 Hz), 144.9, 135.3 (ddd, *J* = 241, 16, 3 Hz), 134.2, 132.6-132.4 (m, C-4a/C-10a), 131.2 (dt, *J* = 14, 4 Hz, C-4a/C-10a), 130.3, 125.0 (C-8), 116.7 (C-6), 113.5 (C-9), 107.0 (t, *J* = 10 Hz, C-3), 36.7 (d, *J* = 12 Hz, N-CH_3), 20.5 (Ar-CH_3).

IR, ν_{max} /cm^{-1} 1689 (CHO), 1643 and 1473(benzene ring).

MS, m/z found 294.0738, $C_{15}H_{11}F_3NO_2$, ($M+H^+$) requires 294.0736.

8-Chloro-1,2,4-trifluoro-10-methyl-10*H*-phenoxazine-3-carbaldehyde (165)



Following the general method outlined for compound **125**, pentafluorobenzaldehyde (0.29 g, 1.50 mmol) and 4-chloro-2-(methylamino)phenol (0.24 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was under reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **165** (0.27 g, 59%) as a yellow solid. m.p. 187-189 °C.

NMR δ_H (400 MHz, $CDCl_3$), 10.12 (1H, d, *J* = 0.8 Hz, CHO), 6.87 (1H, dd, *J* = 6.8, 1.6 Hz, H-7), 6.78 (1H, d, *J* = 6.8 Hz, H-6), 6.70 (1H, d, *J* = 2.0 Hz, H-9), 3.43 (3H, d, *J* = 4.0 Hz, N-CH_3).

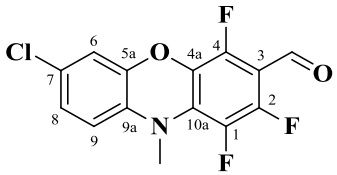
NMR δ_F (376 MHz, $CDCl_3$), 15.8 (1F, dd, *J* = 8, 4 Hz), 15.7 (1F, dd, *J* = 15, 4 Hz), 3.0-2.9 (1F, m).

NMR δ_c (100 MHz, $CDCl_3$), 182.1 (q, *J* = 3 Hz, CHO), 149.0 (ddd, *J* = 258, 14, 7 Hz), 145.8 (ddd, *J* = 256, 7, 2 Hz), 143.8, 135.6 (ddd, *J* = 243, 16, 3 Hz), 134.2, 131.4 (m, C-4a/C-10a), 131.2 (dt, *J* = 3 Hz, C-4a/C-10a), 130.0, 123.5, 116.8, 114.1, 107.9 (t, *J* = 10 Hz, C-3), 36.9 (d, *J* = 12 Hz, CH_3)

IR, ν_{max} /cm^{-1} 1681 (CHO), 1643 and 1465 (benzene ring).

MS, m/z found 314.0191, $C_{14}H_8{}^{35}ClF_3N\,O_2$, ($M+H^+$) requires 314.0190.

7-Chloro-1,2,4-trifluoro-10-methyl-10*H*-phenoxazine-3-carbaldehyde (166)



Following the general method outlined for compound **125**, pentafluorobenzaldehyde (0.29 g, 1.50 mmol) and 5-chloro-2-(methylamino)phenol (0.24 g, 1.50 mmol) was dissolved in MeCN (4 ml). To this mixture, triethylamine (0.84 ml, 6.00 mmol) was added as base and the reaction was under reflux overnight. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 20:1) afforded compound **166** (0.27 g, 59%) as a yellow solid. m.p. 160-162 °C.

NMR δ_H (400 MHz, $CDCl_3$), 10.11 (1H, d, *J* = 0.8 Hz, CHO), 6.96 (1H, dd, *J* = 6.8, 1.6 Hz, H-8), 6.87 (1H, d, *J* = 1.6 Hz, H-6), 6.63 (1H, d, *J* = 6.8 Hz, H-9), 3.43 (3H, d, *J* = 3.6 Hz, N-CH_3).

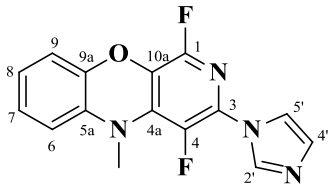
NMR δ_F (376 MHz, $CDCl_3$), 15.9-15.8 (2F, m, F-4, F-2), 2.8-2.7 (1F, m, F-1).

NMR δ_c (100 MHz, $CDCl_3$), 182.1 (dd, *J* = 7, 4 Hz, CHO), 149.1 (ddd, *J* = 258, 14, 8 Hz), 145.9 (dd, *J* = 254, 5 Hz), 145.6, 135.4 (dd, *J* = 239, 16 Hz), 131.8, 131.8-131.7 (m, C-4a/C-10a), 130.9-130.8 (m, C-4a/C-10a), 128.8, 124.6 (C-8), 116.6 (C-6), 114.4 (C-9), 107.6 (t, *J* = 10 Hz, C-3), 36.9 (d, *J* = 12 Hz, CH_3).

IR, ν_{max} /cm^{-1} 1689 (CHO), 1635 and 1473 (benzene ring).

MS, m/z found 314.0195, $C_{14}H_8{}^{35}ClF_3NO_2$, ($M+H^+$) requires 314.0190.

1,4-Difluoro-3-(1*H*-imidazol-1-yl)-5-methyl-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (130)



A solution of compound **125** (0.25 g, 1.00 mmol) in DMF (1 ml) was added a solution of imidazole (0.07 g, 1.00 mmol) in DMF (1 ml) with sodium hydride (0.08 g, 2.00 mmol). The solution was stirred at 120 °C for 48 hours. The reaction mixture was poured into deionised water (10 ml) and was extracted with ethyl acetate (15 ml x 3). The extracts were combined and washed with brine (approx. 10 ml), and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was purified by using column chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1). The combined elution solutions were evaporated under reduced pressure to give compound **130** (0.18 g, 62%) as a white solid. m.p. 156-158 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.18 (1H, s, H-2'), 7.58 (1H, s, H-5'), 7.19 (1H, s, H-4'), 6.99 (1H, td, *J* = 7.6, 1.6 Hz, C-7), 6.90 (1H, td, *J* = 7.6, 1.6 Hz, C-8), 6.83 (1H, dd, *J* = 8.0, 1.2 Hz, C-9), 6.73 (1H, dd, *J* = 8.0, 1.2 Hz, C-6), 3.46 (3H, d, *J* = 5.2 Hz, CH_3).

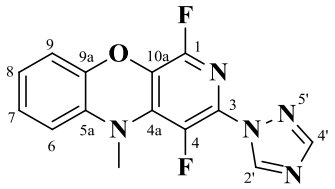
NMR δ_F (376 MHz, $CDCl_3$), 70.6 (1F, d, *J* = 23 Hz), 12.1-11.9 (1F, m).

NMR δ_C (100 MHz, $CDCl_3$), 145.5, 145.4 (d, *J* = 232 Hz, C-4/C-1), 136.3-136.1 (m, C-2', C-3), 135.7 (dd, *J* = 249, 3 Hz, C-4/C-1), 132.9, 130.2 (dd, *J* = 16, 14 Hz), 129.6 (d, *J* = 2 Hz), 127.7 (d, *J* = 31 Hz), 125.1 (C-7), 124.2 (C-8), 117.7 (C-5'), 116.2 (C-9), 114.0 (C-6), 36.4 (d, *J* = 12 Hz).

IR, ν_{max} /cm^{-1} 1589 and 1481 (benzene ring).

MS, m/z found 301.0881, $C_{10}H_5F_4N_2O$, $(M+H^+)$ requires 301.0895.

1,4-Difluoro-5-methyl-3-(1*H*-1,2,4-triazol-1-yl)-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (133)



Following the general method outlined for compound **130**, compound **125** (0.25 g, 1.00 mmol) and 1,2,4-triazole (0.07 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **133** (0.14 g, 50%) as a light yellow solid. m.p. 231-233 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.70 (1H, s, H-2'), 8.12 (1H, s, H-4'), 6.97 (1H, td, *J* = 8.0, 1.6 Hz, H-7), 6.87 (1H, td, *J* = 8.0, 1.2 Hz, H-8), 6.80 (1H, dd, *J* = 7.6, 1.6 Hz, H-9), 6.70 (1H, dd, *J* = 7.6, 1.2 Hz, H-6), 3.43 (d, *J* = 5.2 Hz, CH_3).

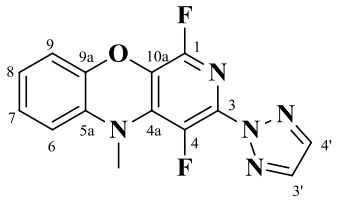
NMR δ_F (376 MHz, $CDCl_3$), 70.6 (1F, d, *J* = 23 Hz), 14.1 (1F, dq, *J* = 23, 5 Hz).

NMR δ_C (100 MHz, $CDCl_3$), 152.9 (C-4'), 145.5, 145.4 (d, *J* = 235 Hz, C-4/C-1), 143.6 (C-2'), 136.7 (t, *J* = 7 Hz), 136.4 (dd, *J* = 254, 4 Hz, C-4/C-1), 132.8, 129.7 (t, *J* = 16 Hz), 129.4 (d, *J* = 32 Hz), 125.4 (C-7), 124.4 (C-8), 116.4 (C-9), 114.1 (C-6), 36.4 (d, *J* = 12 Hz, CH_3).

IR, ν_{max} /cm^{-1} 1635 and 1481 (benzene ring).

MS, m/z found 302.0845, $C_{14}H_{10}F_2N_5O$, ($M+H^+$) requires 302.0848.

1,4-Difluoro-5-methyl-3-(2*H*-1,2,3-triazol-2-yl)-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (134)



Following the general method outlined for compound **130**, compound **125** (0.25 g, 1.00 mmol) and 1,2,3-triazole (0.07 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2.00 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **134** (0.08 g, 25%) as a white solid. m.p. 224-226 °C.

NMR δ_H (400 MHz, $CDCl_3$), 7.89 (2H, s, H-3', H-4'), 6.96 (1H, td, *J* = 8.0, 1.6 Hz, H-7), 6.87 (1H, td, *J* = 8.0, 1.2 Hz, H-8), 6.80 (1H, dd, *J* = 7.6, 1.6 Hz, H-9), 6.69 (1H, dd, *J* = 8.0, 1.2 Hz, H-6), 3.43 (3H, d, *J* = 5.2 Hz, CH_3).

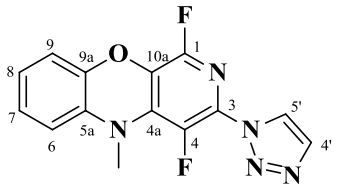
NMR δ_F (376 MHz, $CDCl_3$), 70.9 (1F, d, *J* = 23 Hz), 14.5 (1F, dq, *J* = 23, 5 Hz).

NMR δ_C (100 MHz, $CDCl_3$), 145.6, 145.5 (d, *J* = 235 Hz, C-4/C-1), 137.5 (d, *J* = 254, 4 Hz, C-4/C-1), 136.5-136.3 (C-3', C-4'), 133.0, 131.9 (dd, *J* = 15, 12 Hz), 129.8 (d, *J* = 30 Hz), 125.3 (C-7), 124.3 (C-8), 116.4 (C-9), 114.0 (C-6), 36.4 (d, *J* = 12 Hz, CH_3).

IR, ν_{max} /cm^{-1} 1635 and 1481 (benzene ring).

MS, m/z found 302.0845, $C_{14}H_{10}F_2N_5O$, ($M+H^+$) requires 302.0848.

1,4-Difluoro-5-methyl-3-(1*H*-1,2,3-triazol-1-yl)-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (135)



Another component obtained during the synthesis of compound **134**. The yield of compound **135** was 24% (0.08 g) as a yellow solid. m.p. 199-201 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.14 (1H, s, H-5'), 7.81 (1H, s, H-4'), 6.97 (t, *J* = 7.6 Hz, H-7), 6.88 (1H, t, *J* = 8.0 Hz, H-8), 6.80 (1H, d, *J* = 8.4 Hz, H-9), 6.71 (1H, d, *J* = 8.4 Hz, H-6), 3.44 (1H, d, *J* = 4.8 Hz, CH_3).

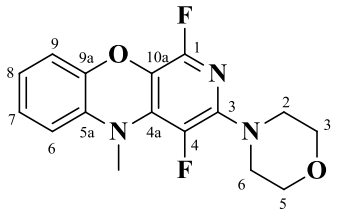
NMR δ_F (376 MHz, $CDCl_3$), 70.3 (1F, d, *J* = 22 Hz), 15.4 (1F, dq, *J* = 23, 5 Hz).

NMR δ_C (100 MHz, $CDCl_3$), 145.5, 145.4 (d, *J* = 235 Hz, C-4/C-1), 137.3 (dd, *J* = 257, 3 Hz, C-4/C-1), 136.8 (t, *J* = 8 Hz), 133.4 (C-4'), 132.9, 129.8 (d, *J* = 12 Hz), 129.5 (d, *J* = 12 Hz), 125.4 (C-7), 124.4 (C-8), 123.6 (C-5'), 116.4 (C-9), 114.1 (C-6), 36.4 (d, *J* = 12 Hz, CH_3).

IR, ν_{max} /cm^{-1} 1635 and 1481 (benzene ring).

MS, m/z found 302.0845, $C_{14}H_{10}F_2N_5O$, ($M+H^+$) requires 302.0848.

1,4-Difluoro-5-methyl-3-morpholino-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (129)



Following the general method outlined for compound **130**, compound **125** (0.25 g, 1.00 mmol) and morpholine (0.09 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **129** (0.18 g, 59%) as a white solid. m.p. 131-133 °C.

NMR δ_H (400 MHz, $CDCl_3$), 6.95 (1H, td, *J* = 7.6, 2.0 Hz, H-7), 6.86 (1H, td, *J* = 7.6, 1.6 Hz, H-8), 6.81 (1H, dd, *J* = 7.6, 1.6 Hz, H-9), 6.70 (1H, dd, *J* = 8.0, 1.2 Hz, H-6), 3.82 (4H, t, *J* = 4.4 Hz, H-3, H-5), 3.39 (3H, d, *J* = 5.2 Hz, CH_3), 3.30 (4H, t, *J* = 4.4 Hz, H-2, H-6).

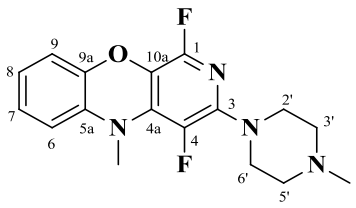
NMR δ_F (376 MHz, $CDCl_3$), 67.3 (1F, d, *J* = 24 Hz), 11.5 (1F, dq, *J* = 24, 5 Hz).

NMR δ_C (100 MHz, $CDCl_3$), 145.9, 144.7 (dd, *J* = 227, 2 Hz, C-1/ C-4), 143.4 (dd, *J* = 16, 11 Hz), 135.3 (dd, *J* = 245, 5 Hz, C-1/C-4), 134.9-134.6 (m), 133.6, 124.3 (C-7), 123.2 (C-8), 122.8, 116.0 (C-9), 113.7 (C-6), 66.7 (C-3, C-5), 48.5 (d, *J*= 5 Hz, C-2, C-6), 36.6 (d, *J* = 12 Hz, CH_3).

IR, ν_{max}/cm^{-1} 2824 (C-H), 1635 and 1581 (benzene ring).

MS, m/z found 320.1190, $C_{16}H_{16}F_2N_3O_2$, ($M+H^+$) requires 320.1205.

1,4-Difluoro-5-methyl-3-(4-methylpiperazin-1-yl)-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (131)



Following the general method outlined for compound **130**, compound **125** (0.25 g, 1.00 mmol) and 1-methylpiperazine (0.10 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2.00 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **131** (0.19 g, 58%) as a white solid. m.p. 131-133 °C.

NMR δ_H (400 MHz, $CDCl_3$), 6.94 (1H, td, *J* = 8.0, 2.0 Hz, H-7), 6.85-6.80 (2H, m, H-8, H-9), 6.69 (1H, dd, *J* = 8.0, 1.2 Hz, H-6), 3.39 (4H, d, *J* = 5.2 Hz, CH_3), 3.36 (3H, t, *J* = 4.4 Hz, H-2', H-6'), 2.55 (4H, t, *J* = 4.8 Hz, H-3', H-5'), 2.36 (3H, s, piperazine- CH_3).

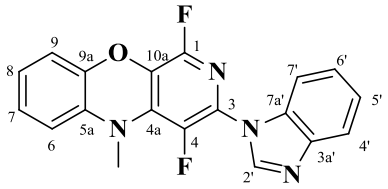
NMR δ_F (376 MHz, $CDCl_3$), 67.3 (1F, d, *J* = 24 Hz), 12.0 (1F, dq, *J* = 24, 5 Hz).

NMR δ_C (100 MHz, $CDCl_3$), 146.0, 144.7 (d, *J* = 225 Hz, C-4/C-1), 143.5 (dd, *J* = 15, 10 Hz), 135.2 (dd, *J* = 244, 5 Hz, C-4/C-1), 134.6 (dd, *J* = 15, 6 Hz), 133.7, 124.2 (C-7), 123.1 (C-8), 122.6 (d, *J* = 32 Hz), 115.9 (C-9), 113.7 (C-6), 54.9 (C-3', C-5'), 47.8 (d, *J* = 5 Hz, C-2', C-6'), 46.1 (piperazine-CH_3), 36.6 (d, *J* = 12 Hz, CH_3).

IR, ν_{max}/cm^{-1} 2939 (C-H), 1643 and 1450 (benzene ring).

MS, m/z found 333.1503, $C_{17}H_{19}F_2N_4O$, ($M+H^+$) requires 333.1521.

3-(1*H*-Benzo[*d*]imidazol-1-yl)-1,4-difluoro-5-methyl-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (132)



Following the general method outlined for compound **130**, compound **125** (0.25 g, 1.00 mmol) and benzimidazole (0.12 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2.00 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **132** (0.18 g, 52%) as a white solid. m.p. 178-180 °C.

NMR δ_H (400 MHz, $CDCl_3$), 8.33 (1H, d, *J* = 2.4 Hz, H-2'), 7.90-7.81 (2H, m, H-7', H-4'), 7.43-7.35 (2H, m, H-6', H-5'), 7.02 (1H, td, *J* = 7.6, 1.6 Hz, H-7), 6.93 (1H, td, *J* = 7.6, 1.6 Hz, H-8), 6.87 (1H, dd, *J* = 7.6, 1.6 Hz, H-9), 6.76 (1H, dd, *J* = 8.0, 1.6 Hz, H-6), 3.49 (3H, d, *J* = 4.8 Hz, CH_3).

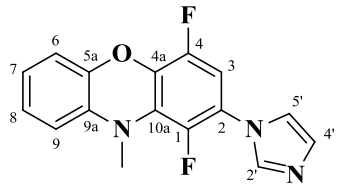
NMR δ_F (376 MHz, $CDCl_3$), 70.9 (1F, d, *J* = 23 Hz), 14.1-14.0 (1F, m).

NMR δ_C (100 MHz, $CDCl_3$), 145.8 (d, *J* = 234 Hz, C-4/C-1), 145.6, 143.4, 141.7 (d, *J* = 10 Hz, C-2'), 137.0 (d, *J* = 247 Hz, C-4/C-1), 136.3 (t, *J* = 7 Hz), 132.9, 132.6, 130.5 (t, *J* = 16 Hz), 128.4 (d, *J* = 32 Hz, C-3), 125.2 (C-7), 124.3 (C-6', C-8), 123.5 (C-5'), 120.5 (C-7'), 116.4 (C-9), 114.0 (C-6), 112.6 (C-4'), 36.4 (d, *J* = 12 Hz, CH_3).

IR, ν_{max}/cm^{-1} 1636 and 1481 (benzene ring).

MS, m/z found 351.1035, $C_{19}H_{13}F_2N_4O$, $(M+H^+)$ requires 351.1052.

1,4-Difluoro-2-(1*H*-imidazol-1-yl)-10-methyl-10*H*-phenoxazine (168)



Following the general method outlined for compound **130**, compound **161** (0.25 g, 1.00 mmol) and imidazole (0.07 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **168** (0.14 g, 45%) as a white solid. m.p. 118-120 °C.

NMR δ_H (400 MHz, $CDCl_3$), 7.69 (1H, s, H-2'), 7.18 (1H, s, H-5'), 7.13 (1H, s, H-4'), 6.98-6.92 (1H, m, H-8), 6.85-6.80 (2H, m, H-7, H-6), 6.67 (1H,d, *J* = 8.4 Hz, H-9), 6.59 (1H, dd, *J* = 10.0, 6.4 Hz, H-3), 3.36 (3H, d, *J* = 5.6 Hz, CH_3).

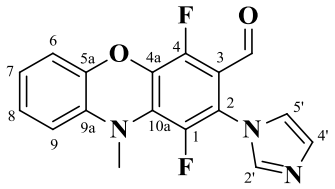
NMR δ_F (376 MHz, $CDCl_3$), 22.5 (1F, dd, *J* = 12, 10 Hz), 17.4-17.2 (1F, m).

NMR δ_c (100 MHz, $CDCl_3$), 146.2 (dd, *J* = 243, 3 Hz, C-1/C-4), 145.8, 140.7 (d, *J* = 244 H, C-1/C-4), 137.23 (C-2'), 136.0 (dd, *J* = 14, 5 Hz), 135.0, 129.8 (C-5'), 127.1 (dd, *J* = 10, 4 Hz), 125.0 (C-8), 122.9 (C-7), 121.4 (t, *J* = 11 Hz), 119.9 (C-4'), 115.9 (C-6), 114.4 (C-9), 104.9 (d, *J* = 23 Hz, C-3), 38.1 (d, *J* = 10 Hz, CH_3).

IR, ν_{max} /cm^{-1} 1635 and 1481 (benzene ring).

MS, m/z found 300.0946, $C_{16}H_{12}F_2N_3O$, ($M+H^+$) requires 300.0943.

1,4-Difluoro-2-(1*H*-imidazol-1-yl)-10-methyl-10*H*-phenoxazine-3-carbaldehyde (175)



A solution of compound **162** (0.28 g, 1.00 mmol) in DMF (1 ml) was added a solution of imidazole (0.07 g, 1.00 mmol) in DMF (1 ml) with triethylamine (0.35 ml, 2.50 mmol). The solution was heated at 90 °C for 60 hours. The reaction mixture was poured into deionised water (10 ml) and was extracted with ethyl acetate (15 ml x 3). The extracts were combined and washed with brine (approx. 15 ml), and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (elution with light petroleum/ ethyl acetate = 2:1). The combined elution solutions were evaporated under reduced pressure to give compound **175** (0.18 g, 54%) as a yellow solid. m.p. 196-198 °C.

NMR δ_H (400 MHz, $CDCl_3$), 9.58 (1H, s, CHO), 7.54 (1H, s, H-2'), 7.24 (1H, s, H-5'), 7.01 (1H, s, H-4'), 6.97 (1H, td, *J* = 7.6, 1.6 Hz, H-8), 6.89 (1H, td, *J* = 7.6, 1.6 Hz, H-7), 6.83 (1H, dd, *J* = 7.6, 1.6 Hz, H-6), 6.69 (1H, dd, *J* = 7.6, 1.6 Hz, H-9), 3.38 (3H, d, *J* = 5.2 Hz, CH_3).

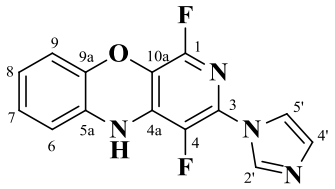
NMR δ_F (376 MHz, $CDCl_3$), 19.0 (1F, m), 16.9 (1F, *J* = 11 Hz).

NMR δ_c (100 MHz, $CDCl_3$), 183.3 (CHO), 147.2 (d, *J* = 258 Hz, C-4/C-1), 145.2, 141.5 (d, *J* = 242 Hz, C-4/C-1), 138.5 (C-2'), 136.3 (dd, *J* = 14, 5 Hz), 133.3, 131.7 (dd, *J* = 10, 4 Hz), 130.0 (C-5'), 125.4 (C-8), 124.3 (C-7), 123.0 (dd, *J* = 15, 5 Hz), 121.5 (C-4'), 116.2(C-6), 114.2 (C-9), 113.8 (d, *J* = 8 Hz, C-3), 37.1 (d, *J* = 12 Hz, CH_3).

IR, ν_{max} /cm^{-1} 1672 (CHO), 1628 and 1481 (benzene ring).

MS, m/z found 328.0894, $C_{17}H_{12}F_2N_3O_2$, ($M+H^+$) requires 328.0892.

1,4-Difluoro-3-(1*H*-imidazol-1-yl)-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (167)



Following the general method outlined for compound **130**, compound **153** (0.24 g, 1.00 mmol) and imidazole (0.07 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2.00 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **167** (0.18 g, 62%) as a white solid. m.p. 282-284 °C.

NMR δ_H (400 MHz, DMSO-d_6), 9.59 (1H, s, NH), 8.11 (1H, s, H-2'), 7.57 (1H, s, H-5'), 7.07 (1H, s, H-4'), 6.84-6.79 (1H, m, H-7), 6.74-6.71 (3H, m).

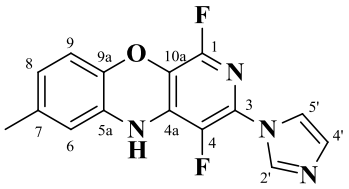
NMR δ_F (376 MHz, DMSO-d_6), 68.4 (1F, d, *J* = 23 Hz), 8.6 (1F, d, *J* = 23 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 144.7 (d, *J* = 182 Hz, C-4/C-1), 142.6, 136.4 (d, *J* = 6 Hz, C-2'), 135.1 (dd, *J* = 199, 3 Hz, C-4/C-1), 134.6 (dd, *J* = 13, 6 Hz), 129.8 (C-4'), 128.9 (dd, *J* = 13, 8 Hz), 128.5, 125.6(C-7), 124.6 (dd, *J* = 25, 2 Hz), 123.9 (C-8), 118.4 (d, *J* = 3 Hz, C-5'), 116.3 (C-6/C-9), 116.0 (C-6/C-9).

IR, ν_{max} /cm^{-1} 3171 (NH), 1589 and 1473 (benzene ring).

MS, m/z found 287.0736, $C_{14}H_9F_2N_4O$, ($M+H^+$) requires 287.0739.

1,4-Difluoro-3-(1*H*-imidazol-1-yl)-7-methyl-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (169)



Following the general method outlined for compound **130**, compound **154** (0.25 g, 1.00 mmol) and imidazole (0.07 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2.00 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **169** (0.17 g, 55%) as a white solid. m.p. 296-298 °C.

NMR δ_H (400 MHz, DMSO-d_6), 9.58 (1H, s, NH), 8.16 (1H, s, H-2'), 7.62 (1H, d, *J* = 1.2 Hz, H-5'), 7.126 (1H, t, *J* = 1.2 Hz, H-4'), 6.66 (1H, d, *J* = 8.0 Hz, H-8), 6.58 (1H, s, H-6), 6.56 (1H, s, H-9), 2.13 (3H, s, CH_3).

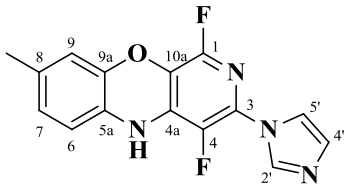
NMR δ_F (376 MHz, DMSO-d_6), 68.4 (1F, d, *J* = 23 Hz), 8.7 (1F, d, *J* = 23 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 144.8 (d, *J* = 227 Hz, C-4/C-1), 140.4, 136.5 (d, *J* = 7 Hz, C-2'), 135.2 (dd, *J* = 248, 4 Hz, C-4/C-1), 134.8, 134.6 (dd, *J* = 16, 7 Hz), 129.8 (C-4'), 128.8 (dd, *J* = 17, 10 Hz), 128.1, 124.5(dd, *J* = 31, 2 Hz), 124.0 (C-9), 118.4 (d, *J* = 4 Hz, C-5'), 116.5 (C-6), 116.1 (C-8), 20.8 (CH_3).

IR, ν_{max} /cm^{-1} 1590 and 1481 (benzene ring).

MS, m/z found 301.0900, $C_{15}H_{11}F_2N_4O$, ($M+H^+$) requires 301.0895.

1,4-Difluoro-3-(1*H*-imidazol-1-yl)-8-methyl-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (170)



Following the general method outlined for compound **130**, compound **155** (0.25 g, 1.00 mmol) and imidazole (0.07 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2.00 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **170** (0.15 g, 50%) as a light yellow solid. m.p. 267-269 °C.

NMR δ_H (400 MHz, DMSO-d_6), 9.50 (1H, s, NH), 8.10 (1H, s, H-2'), 7.56 (1H, d, *J* = 1.2 Hz, H-5'), 7.07 (1H, d, *J* = 1.2 Hz, H-4'), 6.61 (2H, s, H-6, H-9), 6.56 (1H, s, H-7), 2.07 (3H, s, CH_3).

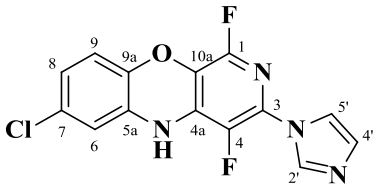
NMR δ_F (376 MHz, DMSO-d_6), 68.4 (1F, d, *J* = 23 Hz), 8.4 (1F, d, *J* = 23 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 144.9 (d, *J* = 227 Hz, C-4/C-1), 142.3, 136.5 (d, *J* = 8 Hz, C-2'), 135.1 (dd, *J* = 248, 3 Hz, C-4/C-1), 134.9-134.8 (m), 133.5, 129.8 (C-4'), 128.8 (dd, *J* = 28, 11 Hz), 125.8, 125.7 (C-6), 124.5 (dd, *J* = 31, 14 Hz), 118.5 (d, *J* = 5 Hz, C-5'), 117.0 (C-7), 115.8 (C-9), 20.7 (CH_3).

IR, ν_{max} /cm^{-1} 3224 (NH), 1597 and 1481 (benzene ring).

MS, m/z found 301.0901, $C_{15}H_{11}F_2N_4O$, ($M+H^+$) requires 301.0895.

7-Chloro-1,4-difluoro-3-(1*H*-imidazol-1-yl)-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (171)



Following the general method outlined for compound **130**, compound **156** (0.27 g, 1.00 mmol) and imidazole (0.07 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2.00 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **171** (0.21 g, 66%) as a white solid. m.p. 262-264 °C.

NMR δ_H (400 MHz, DMSO-d_6), 9.73 (1H, s, NH), 8.12 (1H, s, H-2'), 7.58 (1H, d, *J* = 1.2 Hz, H-5'), 7.08 (1H, s, H-4'), 6.74 (3H, dd, *J* = 12.4, 1.2 Hz, H-9, H-6, H-8).

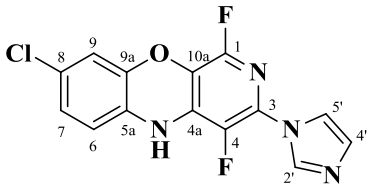
NMR δ_F (376 MHz, DMSO-d_6), 68.9 (1F, d, *J* = 24 Hz), 9.0 (1F, d, *J* = 24 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 144.7 (d, *J* = 228 Hz, C-4/C-1), 141.7, 136.5 (d, *J* = 8 Hz, C-2'), 134.1-133.9 (m), 130.2, 129.9 (C-4'), 129.3-129.1 (m), 128.9, 124.6 (d, *J* = 30 Hz), 123.2 (C-9), 118.4 (d, *J* = 5 Hz, C-5'), 117.8 (C-6), 115.5 (C-8).

IR, ν_{max} /cm^{-1} 3155 (NH), 1643 and 1543 (benzene ring).

MS, m/z found 321.0353, $C_{14}H_8{}^{35}ClF_2N_4O$, ($M+H^+$) requires 321.0349.

8-Chloro-1,4-difluoro-3-(1*H*-imidazol-1-yl)-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (172)



Following the general method outlined for compound **130**, compound **156** (0.27 g, 1.00 mmol) and imidazole (0.07 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2.00 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **172** (0.16 g, 49%) as a light yellow solid. m.p. 293-295 °C.

NMR δ_H (400 MHz, DMSO-d_6), 9.74 (1H, s, NH), 8.12 (1H, s, H-2'), 7.57 (1H, d, *J* = 1.2 Hz, H-5'), 7.08 (1H, H-4'), 6.89-6.87 (2H, m, H-6, H-9), 6.70 (1H, *J* = 6.8 Hz, 2.0 Hz, H-7).

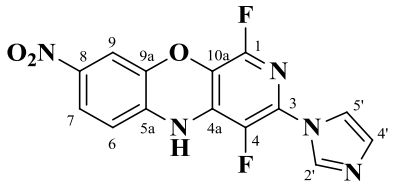
NMR δ_F (376 MHz, DMSO-d_6), 68.9 (1F, d, *J* = 23 Hz), 8.8 (1F, d, *J* = 23 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 144.7 (d. *J* = 228 Hz, C-4/C-1), 143.3, 136.5 (d, *J* = 8 Hz, C-2'), 135.2 (d, *J* = 249 Hz, C-4/C-1), 134.4 (dd, *J* = 16, 7 Hz), 129.9 (C-4'), 129.2 (dd, *J* = 16, 11 Hz), 127.9, 126.9, 125.3 (C-6), 124.2 (d, *J* = 30 Hz, C-3), 118.4 (d, *J* = 4 Hz, C-5'), 116.9 (C-7), 116.6 (C-9).

IR, ν_{max} /cm^{-1} 3155 (NH), 1643 and 1481 (benzene ring).

MS, m/z found 321.0354, $C_{14}H_8{}^{35}ClF_2N_4O$, ($M+H^+$) requires 321.0349.

1,4-Difluoro-3-(1*H*-imidazol-1-yl)-8-nitro-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (173)



Following the general method outlined for compound **130**, compound **159** (0.28 g, 1.00 mmol) and imidazole (0.07 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2.00 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **173** (0.18 g, 54%) as an orange solid. m.p. 275-277 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.40 (1H, s, NH), 8.15 (1H, s, H-2'), 7.77 (1H, dd, *J* = 8.4, 2.4 Hz, H-7), 7.60 (1H, s, H-5'), 7.50 (1H, d, *J* = 2.4 Hz, H-9), 7.09 (1H, s, H-4'), 6.84 (1H, d, *J* = 8.8 Hz, H-6).

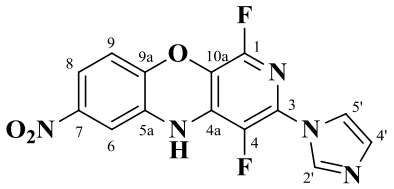
NMR δ_F (376 MHz, DMSO-d_6), 69.4-69.2 (1F, m), 10.1 (1F, d, *J* = 23 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 144.6 (d, *J* = 184 Hz, C-4/C-1), 142.7 , 142.5, 136.5 (d, *J* = 7 Hz, C-2'), 136.0, 135.3 (dd, *J* = Hz, C-4/C-1), 133.2 (dd, *J* = 12, 5 Hz), 130.0 (C-4'), 129.5 (dd, *J* = 14, 8 Hz), 124.5 (d, *J* = 27 Hz), 122.5 (C-7), 118.3 (d, *J* = 4 Hz, C-5'), 115.5 (C-6), 111.6 (C-9).

IR, ν_{max} /cm^{-1} 3155 (NH), 1643 and 1481 (benzene ring), 1581 and 1327 (NO_2).

MS, m/z found 332.0592, $C_{14}H_8F_2N_5O_3$, ($M+H^+$) requires 332.0590.

1,4-Difluoro-3-(1*H*-imidazol-1-yl)-7-nitro-5*H*-pyrido[3,4-*b*][1,4]benzoxazine (174)



Following the general method outlined for compound **130**, compound **158** (0.28 g, 1.00 mmol) and imidazole (0.07 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, sodium hydride (0.08 g, 2.00 mmol) was added as base and the reaction was heated at 120 ^{o}C for 48 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **174** (0.16 g, 47%) as a white solid. m.p. >330 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.07 (1H, s, NH), 8.20 (1H, s), 7.68-7.64 (2H, m), 7.55 (1H, d, *J* = 2.4 Hz), 7.15-7.14 (1H, m), 7.00 (1H, d, *J* = 7.2 Hz).

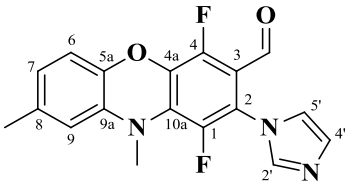
NMR δ_F (376 MHz, DMSO-d_6), 69.0 (1F, *J* = 9 Hz), 9.0 (1F, d, *J* = 21 Hz).

NMR δ_c (100 MHz, DMSO-d_6), 148.2, 144.7, 144.6 (d, *J* = 182 Hz, C-4/C-1), 136.5 (d, *J* = 6 Hz), 135.2 (dd, *J* = 200, 3 Hz, C-4/C-1), 130.4 (dd, *J* = 3, 2 Hz), 129.9, 129.7 (dd, *J* = 24, 8 Hz), 124.2 (dd, *J* = 25, 2 Hz), 119.9, 118.4 (d, *J* = 3 Hz), 116.8, 110.4, 100.0.

IR, ν_{max} /cm^{-1} 3155 (NH), 1651 and 1481 (benzene ring), 1535 and 1342 (NO_2).

MS, m/z found 332.0592, $C_{14}H_8F_2N_5O_3$, ($M+H^+$) requires 332.0590.

1,4-Difluoro-2-(1*H*-imidazol-1-yl)-8,10-dimethyl-10*H*-phenoxazine-3-carbaldehyde (176)



Following the general method outlined for compound **175**, compound **163** (0.37 g, 1.30 mmol) and imidazole (0.09 g, 1.30 mmol) was dissolved in DMF (2 ml). To this mixture, triethylamine (0.35 ml, 2.50 mmol) was added as base and the reaction was heated at 90 ^{o}C for 60 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **176** (0.24 g, 54%) as a yellow solid. m.p. 184-186 °C.

NMR δ_H (400 MHz, $CDCl_3$), 9.59 (1H, s, CHO), 7.54 (1H, s, H-2'), 7.24 (1H, s, H-5'), 7.01 (1H, s, H-4'), 6.74-6.66 (2H, m, H-6, H-7), 6.50 (1H, s, H-9), 3.37 (3H, d, *J* = 5.6 Hz, N-CH_3), 2.25 (3H, s, Ar-CH_3).

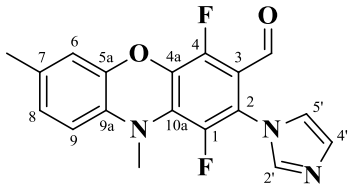
NMR δ_F (376 MHz, $CDCl_3$), 19.0 (1F, m), 16.8 (1F, *J* = 11 Hz).

NMR δ_c (100 MHz, $CDCl_3$), 183.4 (CHO), 147.2 (d, *J* = 256 Hz, C-4/C-1), 143.0, 141.5 (d, *J* = 243 Hz, C-4/C-1), 138.5 (C-2'), 136.5 (dd, *J* = 14, 5 Hz), 135.1, 132.9, 131.9 (dd, *J* = 11, 5 Hz), 130.1 (C-5'), 124.4 (C-7), 122.8 (dd, *J* = 14, 5 Hz), 121.5 (C-4'), 115.9 (C-6), 115.0 (C-9), 113.8 (d, *J* = 9 Hz), 37.2 (d, *J* = 12 Hz, N-CH_3), 21.3 (Ar-CH_3).

IR, ν_{max} /cm^{-1} 1689 (CHO), 1635 and 1489 (benzene ring).

MS, m/z found 342.1053, $C_{18}H_{14}F_2N_3O_2$, ($M+H^+$) requires 342.1049.

1,4-Difluoro-2-(1*H*-imidazol-1-yl)-7,10-dimethyl-10*H*-phenoxazine-3-carbaldehyde (177)



Following the general method outlined for compound **175**, compound **164** (0.34 g, 1.20 mmol) and imidazole (0.08 g, 1.20 mmol) was dissolved in DMF (2 ml). To this mixture, triethylamine (0.35 ml, 2.50 mmol) was added as base and the reaction was heated at 90 ^{o}C for 60 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **177** (0.22 g, 53%) as a brown solid. m.p. 211-213 °C.

NMR δ_H (400 MHz, $CDCl_3$), 9.56 (1H, s, CHO), 7.54 (1H, s, H-2'), 7.23 (1H, s, H-5'), 7.01 (1H, s, H-4'), 6.75 (1H, dd, *J* = 7.6, 0.8 Hz, H-8), 6.67 (1H, d, *J* = 1.6 Hz, H-6), 6.57 (1H, d, *J* = 8.4 Hz, H-9), 3.36 (3H, d, *J* = 5.2 Hz, N-CH_3), 2.22 (3H, s, Ar-CH_3).

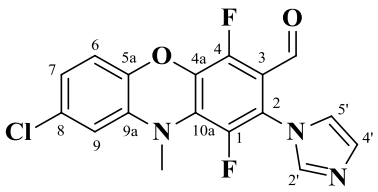
NMR δ_F (376 MHz, $CDCl_3$), 18.9 (1F, m), 16.8 (1F, *J* = 11 Hz).

NMR δ_c (100 MHz, $CDCl_3$), 183.3 (CHO), 147.2 (d, *J* = 257 Hz, C-4/C-1), 144.9, 141.4 (d, *J* = 245 Hz, C-4/C-1), 138.5 (C-2'), 136.1(dd, *J* = 14, 6 Hz), 134.5, 131.9 (dd, *J* = 11, 5 Hz), 130.6, 130.0 (C-5'), 125.5 (C-8), 123.0 (dd, *J* = 15, 6 Hz), 121.5 (C-4'), 116.9 (C-6), 114.0 (C-9), 113.5 (d, *J* = 8 Hz), 37.2 (d, *J* = 12 Hz, N-CH_3), 20.6 (Ar-CH_3).

IR, ν_{max} /cm^{-1} 1682 (CHO), 1635 and 1489 (benzene ring).

MS, m/z found 342.1054, $C_{18}H_{14}F_2N_3O_2$, ($M+H^+$) requires 342.1049.

8-Chloro-1,4-difluoro-2-(1*H*-imidazol-1-yl)-10-methyl-10*H*-phenoxazine-3-carbaldehyde (178)



Following the general method outlined for compound **175**, compound **165** (0.30 g, 1.00 mmol) and imidazole (0.07 g, 1.00 mmol) was dissolved in DMF (2 ml). To this mixture, triethylamine (0.35 ml, 2.50 mmol) was added as base and the reaction was heated at 90 ^{o}C for 60 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **178** (0.18 g, 49%) as a yellow solid. m.p. 181-183 °C.

NMR δ_H (400 MHz, $CDCl_3$), 9.60 (1H, s, CHO), 7.55 (1H, s, H-2'), 7.24 (1H, s, H-5'), 7.01 (1H, d, *J* = 0.8 Hz, H-4'), 6.85 (1H, dd, *J* = 8.8, 2.4 Hz, H-7), 6.77 (1H, d, *J* = 8.4 Hz, H-6), 6.66 (1H, d, *J* = 2.0 Hz, H-9), 3.37 (3H, d, *J* = 6.0 Hz, N-CH_3).

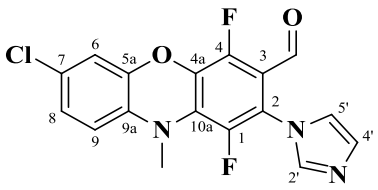
NMR δ_F (376 MHz, $CDCl_3$), 19.7 (1F, m), 17.4 (1F, *J* = 11 Hz).

NMR δ_c (100 MHz, $CDCl_3$), 183.2 (CHO), 147.2 (d, *J* = 257 Hz, C-4/C-1), 143.8, 141.6 (d, *J* = 245 Hz, C-4/C-1), 138.5 (C-2'), 136.2 (dd, *J* = 14, 5 Hz), 134.5, 130.9 (dd, *J* = 11, 5 Hz), 130.5, 130.1 (C-5'), 123.7 (C-7), 123.2 (dd, *J* = 14, 6 Hz), 121.5 (C-4'), 117.0 (C-6), 114.5 (C-9), 114.4 (d, *J* = 9 Hz), 37.3 (d, *J* = 12 Hz, N-CH_3),

IR, ν_{max} /cm^{-1} 1689 (CHO), 1635 and 1481 (benzene ring).

MS, m/z found 362.0507, $C_{17}H_{11}{}^{35}ClF_2N_3O_2$, ($M+H^+$) requires 362.0502.

7-Chloro-1,4-difluoro-2-(1*H*-imidazol-1-yl)-10-methyl-10*H*-phenoxazine-3-carbaldehyde (179)



Following the general method outlined for compound **175**, compound **166** (0.36 g, 1.20 mmol) and imidazole (0.08 g, 1.20 mmol) was dissolved in DMF (2 ml). To this mixture, triethylamine (0.35 ml, 2.50 mmol) was added as base and the reaction was heated at 90 ^{o}C for 60 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **179** (0.19 g, 43%) as a brown solid. m.p. 201-203 °C.

NMR δ_H (400 MHz, $CDCl_3$), 9.59 (1H, s, CHO), 7.54 (1H, s, H-2'), 7.24 (1H, s, H-5'), 7.01 (1H, d, *J* = 1.2 Hz, H-4'), 6.94 (1H, dd, *J* = 8.8, 2.4 Hz, H-8), 6.86 (1H, d, *J* = 2.4 Hz, H-6), 6.60 (1H, d, *J* = 8.8 Hz, H-9), 3.37 (3H, d, *J* = 5.2 Hz, N-CH_3).

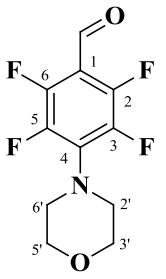
NMR δ_F (376 MHz, $CDCl_3$), 19.5 (1F, m), 17.5 (1F, *J* = 11 Hz).

NMR δ_c (100 MHz, $CDCl_3$), 183.2 (CHO), 147.2 (d, *J* = 258 Hz, C-4/C-1), 145.6, 141.5 (d, *J* = 247 Hz, C-4/C-1), 138.5 (C-2'), 135.8 (dd, *J* = 13, 4 Hz), 132.2, 131.2 (dd, *J* = 10, 5 Hz), 130.2 (C-5'), 129.1, 125.1 (C-8), 123.3 (dd, *J* = 15, 5 Hz), 121.5 (C-4'), 116.8 (C-6), 114.8 (C-9), 114.1 (d, *J* = 9 Hz), 37.3 (d, *J* = 12 Hz, N-CH_3).

IR, ν_{max} /cm^{-1} 1682 (CHO), 1627 and 1481 (benzene ring).

MS, m/z found 362.0509, $C_{17}H_{11}{}^{35}ClF_2N_3O_2$, ($M+H^+$) requires 362.0502.

2,3,5,6-Tetrafluoro-4-(morpholin-4-yl)benzaldehyde (184)



A solution of pentafluorobenzoaldehyde (1.96 g, 10.00 mmol) in DMF (5 ml) was added to a solution of morpholine (1.74 g, 20.00 mmol) in DMF (5 ml). The resulting solution was stirred at room temperature for 16 hours. The reaction mixture was poured into deionised water (20 ml) and was extracted with ethyl acetate (30 ml x 3). The extracts were combined and washed with brine (approx. 20 ml), and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was subjected to a silica column (elution with light petroleum/ ethyl acetate = 10:1). The combined elution fractions were evaporated to give aldehyde **184** (2.33 g, 88%) as a light yellow solid. m.p. 87-89 °C

NMR δ_H (400 MHz, $CDCl_3$), 10.13 (1H, s, CHO), 3.42 (4H, t, *J* = 4.4 Hz, H-3', H-5'), 3.41 (4H, t, *J* = 4.4 Hz, H-2', H-6').

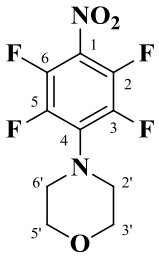
NMR δ_F (376 MHz, $CDCl_3$), 15.5 (2F, dd, *J* = 20, 9 Hz, F-3, F-5), 9.6 (2F, dd, *J* = 19, 7 Hz, F-2, F-6).

NMR δ_C (100 MHz, $CDCl_3$), 182.2 (C=O), 148.1 (ddt, *J* = 255, 13, 4 Hz, C-2, C-6/C-3, C-5), 140.3 (d, *J* = 245, 13 Hz, C-2, C-6/C-3, C-5), 135.5 (d, *J* = 10 Hz, C-4), 107.3 (t, *J* = 10 Hz, C-1), 67.1 (C-3', C-5'), 51.0 (t, *J*= 5 Hz, C-2', C-6').

IR, ν_{max} /cm^{-1} 1689 (CHO), 1628 and 1566 (benzene).

MS, m/z found 264.0636, $C_{11}H_{10}F_4NO_2$, ($M+H^+$) requires 264.0642.

4-(2,3,5,6-Tetrafluoro-4-nitrophenyl)morpholine (211)



A solution of pentafluoronitrobenzene (0.21 g, 1.00 mmol) in DMF (2 ml) was added to NaH (0.08 g, 2.00 mmol). Morpholine (0.09 g, 1.00 mmol) was then added and the solution was stirred at room temperature for 16 hours. The reaction mixture was poured into deionised water (5 ml) and was extracted with ethyl acetate (15 ml x 3). The extracts were combined and washed with brine (approx. 10 ml), and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was subjected to a silica column (elution with light petroleum/ ethyl acetate = 20:1). The combined elution solutions were evaporated under reduced pressure to give compound **211** (0.20 g, 73%) as a yellow solid. m.p. 85-87 °C.

NMR δ_H (400 MHz, $CDCl_3$), 3.81 (4H, t, *J* = 4.4 Hz, H-3', H-5'), 3.41 (4H, dt, *J* = 7.6, 2.4 Hz, H-2', H-6').

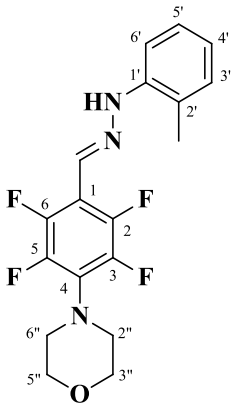
NMR δ_F (376 MHz, $CDCl_3$), 15.2-15.0 (2F, m, F-3, F-5), 11.9-11.7 (2F, m, F-2, F-6).

NMR δ_C (100 MHz, $CDCl_3$), 142.4 (ddt, *J* = 157, 11, 4 Hz, C-2, C-6/C-3, C-5), 140.3 (ddd, *J* = 147, 11, 5 Hz, C-2, C-6/C-3, C-5), 134.1 (tt, *J* = 8, 2 Hz, C-4), 123.1-122.9 (m, C-1), 67.0 (C-3', C-5'), 51.0 (t, *J* = 3 Hz, C-2', C-6').

IR, ν_{max} /cm^{-1} 1627 and 1527 (benzene), 1527 and 1327 (NO_2).

MS, m/z found 281.0550, $C_{10}H_9F_4N_2O_3$, ($M+H^+$) requires 281.0544.

4-(2,3,5,6-Tetrafluoro-4-((*E*)-(2-(2-methylphenyl)hydrazinylidene)methyl)phenyl)-morpholine (197)



A solution of aldehyde **184** (0.26 g, 1.00 mmol) in DCM (1 ml) was added to a solution of *o*-tolylhydrazine hydrochloride (0.16 g, 1.00 mmol) in DCM (1 ml). The solution was stirred at room temperature for 2 hours. The reaction mixture was poured into deionised water (5 ml) and was extracted with ethyl acetate (15 ml x 3). The extracts were combined and washed with brine (approx. 10 ml), and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was subjected to a silica column (elution with light petroleum/ ethyl acetate = 10:1). The combined elution solutions were evaporated to give the compound **197** (0.28 g, 76%) as orange solid. m.p. 157-159 °C

NMR δ_H (400 MHz, DMSO-d_6), 9.95 (1H, s, NH), 8.13 (1H, s, CHN), 7.28 (1H, d, *J* = 7.6 Hz, H-3'), 7.06 (1H, t, *J* = 7.6 Hz, H-4'), 7.02 (1H, d, *J* = 7.6 Hz, H-6'), 6.69 (1H, t, *J* = 7.6 Hz, H-5'), 3.65 (4H, s, H-3", H-5"), 3.18 (4H, s, H-2", H-6"), 2.18 (3H, s, CH_3).

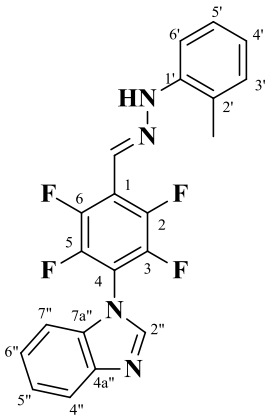
NMR δ_F (376 MHz, DMSO-d_6), 15.8 (2F, dd, *J* = 20, 7 Hz, F-3, F-5), 9.7 (2F, dd, *J* = 20, 7 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 144.5 (dt, *J* = 247, 12 Hz, C-2, C-6/C-3, C-5), 142.9, 142.5 (dd, *J* = 237, 16 Hz, C-2, C-6/C-3, C-5), 130.9 (C-6'), 128.5 (t, *J* = 11 Hz, C-4), 127.3 (C-4'), 126.5 (CHN), 121.4, 120.1 (C-5'), 112.6 (C-3'), 109.1 (t, *J* = 12 Hz, C-1), 66.9 (d, *J* = 15 Hz, C-3", C-5"), 51.4 (C-2", C-6"), 17.9 (CH_3).

IR, ν_{max} /cm^{-1} 3271 (NH), 1650 (C=N), 1589 and 1540 (benzene).

MS, m/z found 368.1374, $C_{18}H_{18}F_4N_3O$, ($M+H^+$) requires 368.1381.

1-(2,3,5,6-Tetrafluoro-4-((*E*)-(2-(2-methylphenyl)hydrazinylidene)methyl)phenyl)-1*H*-benzimidazole (191)



Following the general method outlined for compound **197**, aldehyde **68** (0.29 g, 1.00 mmol) was reacted with *o*-tolylhydrazine hydrochloride (0.16 g, 1.00 mmol) in DCM (2 ml) at room temperature for 2 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 3:1) afforded compound **191** (0.32 g, 81%) as a yellow solid. m.p. 169-171 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.36 (1H, s, NH), 8.52 (1H, s,H-2”), 8.30 (1H, s, CHN), 7.80-7.77 (1H, m, H-7”), 7.48 (1H, d, *J* = 6.8 Hz, H-4”), 7.35-7.32 (3H, m, H-3’, H-6”, H-5”), 7.11 (1H, t, *J* = 7.6 Hz, H-4’), 7.07 (1H, d, *J* = 7.6 Hz, H-6’), 6.77 (1H, t, *J* = 7.6 Hz, H-5’), 2.24 (3H, s, CH_3).

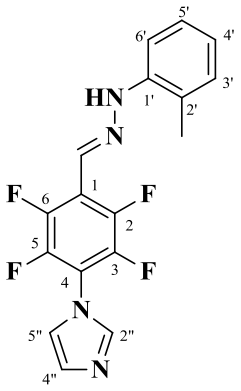
NMR δ_F (376 MHz, DMSO-d_6), 17.8 (2F, dd, *J* = 21, 9 Hz, F-3, F-5), 13.6 (2F, dd, *J* = 22, 9 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 144.3 (C-2”), 144.1 (dd, *J* = 254, 13 Hz, C-2, C-6/C-3, C-5), 143.2, 142.8, 143.0 (dd, *J* = 248, 15 Hz, C-2, C-6/C-3, C-5), 133.9, 131.1 (C-6’), 127.4 (C-4’), 125.4 (CHN), 124.7 (C-6”), 123.7 (C-5”), 121.9, 120.9 (C-5’), 120.5 (C-7”), 116.5 (t, *J* = 11 Hz, C-4), 113.0 (t, *J* = 14 Hz, C-1), 112.9 (C-3’), 111.4 (C-4”), 17.9 (CH_3).

IR, ν_{max} /cm^{-1} 3279 (NH), 1599 and 1512 (benzene).

MS, m/z found 399.1219, $C_{21}H_{15}F_4N_4$, ($M+H^+$) requires 399.1227.

1-(2,3,5,6-Tetrafluoro-4-((*E*)-(2-(2-methylphenyl)hydrazinylidene)methyl)phenyl)-1*H*-imidazole (188)



Following the general method outlined for compound **197**, aldehyde **67** (0.24 g, 1.00 mmol) was reacted with *o*-tolylhydrazine hydrochloride (0.16 g, 1.00 mmol) in DCM (2 ml) at room temperature for 2 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2.25:1) afforded compound **188** (0.27 g, 77%) as a yellow solid. m.p. 223-225 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.29 (1H, s, NH), 8.24 (1H, s, CHN), 8.00 (1H, s, H-2”), 7.53 (1H, s, H-5”), 7.33 (1H, d, *J* = 8.0 Hz, H-3’), 7.17 (1H, s, H-4”), 7.11 (1H, t, *J* = 7.2 Hz, H-4’), 7.06 (1H, d, *J* = 7.6 Hz, H-6’), 6.76 (1H, t, *J* = 7.6 Hz, H-5’), 2.22 (3H, s, CH_3).

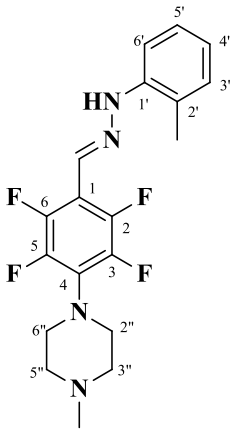
NMR δ_F (376 MHz, DMSO-d_6), 17.4 (2F, dd, *J* = 21, 9 Hz, F-3, F-5), 11.6 (2F, dd, *J* = 21, 9 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 143.9 (dd, *J* = 248, 8 Hz, C-2, C-6/C-3, C-5), 142.5, 142.1 (dd, *J* = 245, 15 Hz, C-2, C-6/C-3, C-5), 138.8 (C-2”), 131.1 (C-6’), 129.9 (C-4”), 127.4 (C-4’), 125.4 (CHN), 121.9, 121.5 (C-5”), 120.9 (C-5’), 115.6 (t, *J* = 11 Hz, C-4), 115.0 (t, *J* = 12 Hz, C-1), 113.0 (C-3’), 17.9 (CH_3).

IR, ν_{max} /cm^{-1} 3248 (NH), 1589 and 1520 (benzene).

MS, m/z found 349.1062, $C_{17}H_{13}F_4N_4$, ($M+H^+$) requires 349.1071.

1-Methyl-4-(2,3,5,6-tetrafluoro-4-((*E*)-(2-(2-methylphenyl)hydrazinylidene)methyl)-phenyl)piperazine (194)



A solution of compound **69** (0.28 g, 1.00 mmol) in DCM (1 ml) was added a solution of *o*-tolylhydrazine hydrochloride (0.16 g, 1.00 mmol) in DCM (1 ml). The solution was stirred at room temperature for 2 hours. The reaction mixture was poured into deionised water (5 ml) and was extracted with ethyl acetate (20 ml x 3). The extracts were combined and washed with brine (approx. 15 ml), and then dried over sodium sulfate. The solution was concentrated under reduced pressure to give the residue. It was washed with solution of hexane and dichloromethane three times and pure compound **194** (0.36 g, 63%) was collected as a yellow solid. m.p.132-134 °C.

NMR δ_H (400 MHz, DMSO-d_6), 9.97 (1H, s, NH), 8.14 (1H, s, CHN), 7.28 (1H, d, *J* = 7.2 Hz, H-3'), 7.07 (1H, t, *J* = 7.2 Hz, H-4'), 7.02 (1H, d, *J* = 7.2 Hz, H-6'), 6.70 (1H, t, *J* = 7.2 Hz, H-5'), 3.23 (4H, s, H-2", H-6"), 2.51 (4H, s, H-3", H-5"), 2.26 (3H, s, N- CH_3), 2.18 (3H, s, Ar-CH_3).

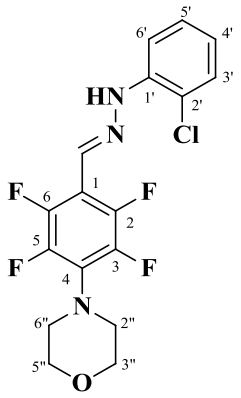
NMR δ_F (376 MHz, DMSO-d_6), 15.7 (2F, dd, *J* = 20, 8 Hz, F-3, F-5), 9.8 (2F, dd, *J* = 21, 7 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 144.5 (dd, *J* = 238, 14 Hz, C-2, C-6/C-3, C-5), 142.9, 142.4 (dd, *J* = 240, 11 Hz, C-2, C-6/C-3, C-5), 131.0 (C-6'), 128.6 (t, *J* = 11 Hz, C-4), 127.3 (C-4'), 126.6 (CHN), 121.4, 120.1 (C-5'), 112.6 (C-3'), 108.9 (t, *J* = 11 Hz, C-1), 55.2 (C-3", C-5"), 50.5 (C-2", C-6"), 45.9 (N-CH_3), 17.9 (Ar-CH_3).

IR, ν_{max} /cm^{-1} 3217 (NH), 1643 (C=N), 1589 and 1500 (benzene).

MS, m/z found 381.1689, $C_{19}H_{21}F_4N_4$, ($M+H^+$) requires 381.1697.

4-(4-((*E*)-(2-(2-Chlorophenyl)hydrazinylidene)methyl)-2,3,5,6-tetrafluorophenyl)-morpholine (198)



Following the general method outlined for compound **197**, aldehyde **184** (0.26 g, 1.00 mmol) was reacted with 2-chlorophenylhydrazine hydrochloride (0.18 g, 1.00 mmol) in DCM (2 ml) at room temperature for 2 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 10:1) afforded compound **198** (0.30 g, 77%) as a white solid. m.p. 199-201 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.30 (1H, s, NH), 8.30 (1H, s, CHN), 7.39 (1H, dd, *J* = 8.0, 1.2 Hz, H-3'), 7.29 (1H, dd, *J* = 8.0, 1.2 Hz, H-6'), 7.21 (1H, td, *J* = 8.0, 0.8 Hz, H-4'), 6.78 (1H, td, *J* = 8.0, 1.2 Hz, H-5'), 3.65 (4H, t, *J* = 4.4 Hz, H-3", H-5"), 3.19 (4H, s, H-2", H-6").

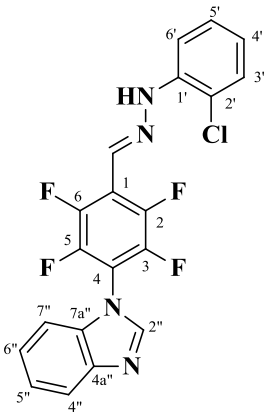
NMR δ_F (376 MHz, DMSO-d_6), 16.3 (2F, dd, *J* = 20, 8 Hz, F-3, F-5), 9.8 (2F, dd, *J* = 21, 8 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 144.8 (dt, *J* = 247, 13 Hz, C-2, C-6/C-3, C-5), 141.2, 142.3 (dd, *J* = 248, 12 Hz, C-2, C-6/C-3, C-5), 129.9 (C-6'), 129.2 (CHN), 129.1 (t, *J* = 11 Hz, C-4), 128.6 (C-4'), 120.9 (C-5'), 116.9, 114.5 (C-3'), 108.4 (t, *J* = 12 Hz, C-1), 67.0 (C-3", C-5"), 51.3 (C-2", C-6").

IR, ν_{max} /cm^{-1} 3271 (NH), 1643 (C=N), 1589 and 1481 (benzene).

MS, m/z found 388.0827, $C_{17}H_{15}{}^{35}ClF_4N_3O$, ($M+H^+$) requires 388.0834.

1-(4-((*E*)-(2-(2-Chlorophenyl)hydrazinylidene)methyl)-2,3,5,6-tetrafluorophenyl)-1*H*-benzimidazole (192)



Following the general method outlined for compound **197**, aldehyde **68** (0.29 g, 1.00 mmol) was reacted with 2-chlorophenylhydrazine hydrochloride (0.18 g, 1.00 mmol) in DCM (2 ml) at room temperature for 2 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 2:1) afforded compound **192** (0.36 g, 85%) as a yellow solid. m.p. 197-199 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.68 (1H, s, NH), 8.53 (1H, s, H-2”), 8.49 (1H, s, CHN), 7.80-7.77 (1H, m, H-7”), 7.50-7.47 (2H, m, H-4”, H-3’), 7.37-7.31 (3H, m, H-5”, H-6”, H-6’), 7.27 (1H, t, *J* = 8.0 Hz, H-4’), 6.86 (1H, td, *J* = 8.0, 1.2 Hz, H-5’).

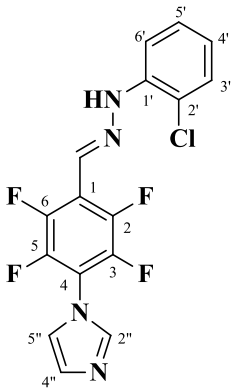
NMR δ_F (376 MHz, DMSO-d_6), 18.4 (2F, dd, *J* = 20, 9 Hz, F-3, F-5), 13.9 (2F, dd, *J* = 21, 10 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 144.4 (m, C-2, C-6/C-3, C-5), 144.3 (C-2”), 143.2, 143.0 (dd, *J* = 248, 14 Hz, C-2, C-6/C-3, C-5), 140.9, 133.9, 130.1 (C-6’), 128.8 (C-4’), 128.2 (CHN), 124.7 (C-6”), 123.8 (C-5”), 121.7 (C-5’), 120.6 (C-7”), 117.4, 116.0 (t, *J* = 11 Hz, C-4), 114.9 (C-3’), 113.8 (t, *J* = 14 Hz, C-1), 111.5 (C-4”).

IR, ν_{max} /cm^{-1} 3317 (NH), 1597 and 1512 (benzene).

MS, m/z found 419.0676, $C_{20}H_{12}{}^{35}ClF_4N_4$, ($M+H^+$) requires 419.0681.

1-(4-((*E*)-(2-(2-Chlorophenyl)hydrazinylidene)methyl)-2,3,5,6-tetrafluorophenyl)-1*H*-imidazole (189)



Following the general method outlined for compound **197**, aldehyde **67** (0.24 g, 1.00 mmol) was reacted with 2-chlorophenylhydrazine hydrochloride (0.18 g, 1.00 mmol) in DCM (2 ml) at room temperature for 2 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 1:1) afforded compound **189** (0.28 g, 75%) as a yellow solid. m.p. 242-244 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.63 (1H, s, NH), 8.42 (1H, s, H-2"), 8.02 (1H, s, CHN), 7.54 (1H, s, H-5"), 7.45 (1H, d, *J* = 8.4 Hz, C-3'), 7.34 (1H, d, *J* = 7.2 Hz, C-6'), 7.26 (1H, t, *J* = 8.0 Hz, C-4'), 7.17 (1H, s, H-4"), 6.85 (1H, td, *J* = 7.2, 1.2 Hz, C-5').

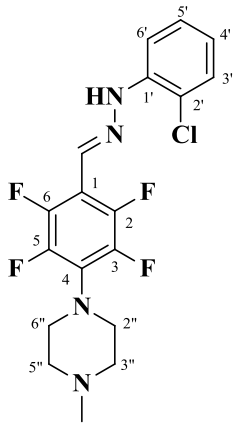
NMR δ_F (376 MHz, DMSO-d_6), 17.9 (2F, dd, *J* = 22, 9 Hz, F-3, F-5), 11.8 (2F, dd, *J* = 22, 9 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 144.3 (m, C-2, C-6/C-3, C-5), 142.0 (m, C-2, C-6/C-3, C-5), 140.9, 138.8(C-2"), 130.1 (C-6'), 129.9 (C-4"), 128.8 (C-4'), 128.1 (CHN), 121.7 (C-5'), 121.5(C-5"), 117.3, 115.7 (t, *J* = 11 Hz, C-4), 115.1 (C-1), 114.8 (C-3').

IR, ν_{max} /cm^{-1} 3237 (NH), 1597 and 1512 (benzene).

MS, m/z found 369.0523, $C_{16}H_{10}{}^{35}ClF_4N_4$, ($M+H^+$) requires 369.0525.

1-(4-((*E*)-(2-(2-Chlorophenyl)hydrazinylidene)methyl)-2,3,5,6-tetrafluorophenyl)-4-methylpiperazine (195)



Following the general method outlined for compound **197**, compound **69** (0.28 g, 1.00 mmol) was reacted with 2-chlorophenylhydrazine hydrochloride (0.18 g, 1.00 mmol) in DCM (2 ml) at room temperature for 2 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 1:20) afforded compound **195** (0.27 g, 67%) as a white solid. m.p. 155-157 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.26 (1H, s, NH), 8.28 (1H, s, CHN), 7.38 (1H, dd, *J* = 7.6, 1.2 Hz, H-3'), 7.27 (1H, dd, *J* = 7.6, 0.8 Hz, H-6'), 7.20 (1H, t, *J* = 7.2 Hz, H-4'), 6.77 (1H, td, *J* = 7.6, 1.2 Hz, H-5'), 3.17 (4H, s, H-2", H-6"), 2.36 (4H, s, H-3", H-5"), 2.16 (3H, s, CH_3).

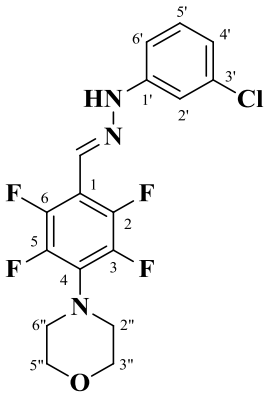
NMR δ_F (376 MHz, DMSO-d_6), 16.2 (2F, dd, *J* = 20, 8 Hz, F-3, F-5), 9.7 (2F, dd, *J* = 20, 8 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 144.8 (dd, *J* = 235, 12 Hz, C-2, C-6/C-3, C-5), 142.3 (dd, *J* = 257, 13 Hz, C-2, C-6/C-3, C-5), 141.3, 129.9 (C-6'), 129.4 (t, *J* = 11 Hz, C-4), 129.3 (CHN), 128.5 (C-4'), 120.8 (C-5'), 116.9, 114.5 (C-3'), 108.0 (t, *J* = 12 Hz, C-1), 55.5 (C-3", C-5"), 50.8 (C-2", C-6"), 46.4 (CH_3).

IR, ν_{max} /cm^{-1} 3333 (NH), 1643 (C=N), 1589 and 1500 (benzene).

MS, m/z found 398.0905, $C_{18}H_{15}{}^{35}ClF_4N_4$, ($M+H^+$) requires 398.0916.

4-(4-((*E*)-(2-(3-Chlorophenyl)hydrazinylidene)methyl)-2,3,5,6-tetrafluorophenyl)-morpholine (199)



Following the general method outlined for compound **197**, compound **184** (0.26 g, 1.00 mmol) was reacted with 3-chlorophenylhydrazine hydrochloride (0.18 g, 1.00 mmol) in DCM (2 ml) at room temperature for 2 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 10:1) afforded compound **199** (0.29 g, 74%) as an orange solid. m.p. 187-189 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.85 (1H, s, NH), 7.80 (1H, s, CHN), 7.20 (1H, t, *J* = 8.0 Hz, H-5'), 6.98 (1H, s, H-2'), 6.86 (1H, d, *J* = 7.6 Hz, H-6'), 6.76 (1H, dd, *J* = 8.0, 1.2 Hz, H-4'), 3.65 (4H, t, *J* = 4.4 Hz, H-3", H-5"), 3.18 (4H, s, H-2", H-6").

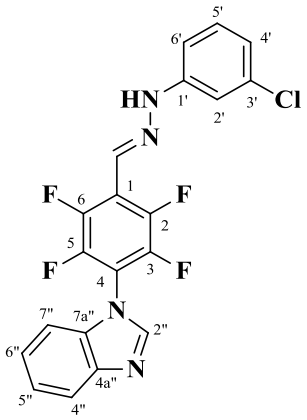
NMR δ_F (376 MHz, DMSO-d_6), 16.0 (2F, dd, *J* = 22, 7 Hz, F-3, F-5), 9.8 (2F, dd, *J* = 20, 6 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 146.4, 144.5 (m, C-2, C-6/C-3, C-5), 142.2 (dd, *J* = 225, 12 Hz, C-2, C-6/C-3, C-5), 134.4, 131.4 (C-5'), 128.9 (t, *J* = 11 Hz, C-4), 126.5 (CHN), 119.5 (C-4'), 111.9 (C-2'), 111.4 (C-6'), 108.4 (t, *J* = 12 Hz, C-1), 67.0 (C-3", C-5"), 51.3 (C-2", C-6").

IR, ν_{max} /cm^{-1} 3248 (NH), 1589 and 1535 (benzene).

MS, m/z found 388.0831, $C_{17}H_{15}{}^{35}ClF_4N_3O$, ($M+H^+$) requires 388.0834.

1-(4-((*E*)-(2-(3-Chlorophenyl)hydrazinylidene)methyl)-2,3,5,6-tetrafluorophenyl)-1*H*-benzimidazole (193)



Following the general method outlined for compound **197**, compound **68** (0.29 g, 1.00 mmol) was reacted with 3-chlorophenylhydrazine hydrochloride (0.18 g, 1.00 mmol) in DCM (2 ml) at room temperature for 2 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 3:1) afforded compound **193** (0.29 g, 79%) as a yellow solid. m.p. 235-237°C.

NMR δ_H (400 MHz, DMSO-d_6), 11.23 (1H, s, NH), 8.52 (1H, s, H-2”), 7.96 (1H, s, CHN), 7.80-7.76 (1H, m, H-7”), 7.47 (1H, d, *J* = 7.2 Hz, H-4”), 7.37-7.30 (2H, m, H-5”, H-6”), 7.25 (1H, t, *J* = 8.0 Hz, H-5’), 7.08 (1H, s, H-2’), 6.96 (1H, d, *J* = 8.4 Hz, H-6’), 6.84 (1H, dd, *J* = 8.4, 0.8 Hz, H-4’).

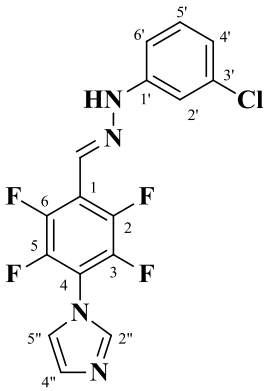
NMR δ_F (376 MHz, DMSO-d_6), 18.2 (2F, dd, *J* = 22, 9 Hz, F-3, F-5), 13.8 (2F, dd, *J* = 20, 9 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 146.0, 144.2 (dd, *J* = 243, 15 Hz, C-2, C-6/C-3, C-5), 144.3 (C-2”), 143.2, 142.9 (dd, *J* = 248, 15 Hz, C-2, C-6/C-3, C-5), 134.5, 133.9, 131.5 (C-5’), 125.5 (CHN), 124.7 (C-6”), 123.7 (C-5”), 120.6 (C-7”), 120.3 (C-4’), 116.0 (t, *J* = 11 Hz, C-4), 113.5 (t, *J* = 14 Hz, C-1), 112.3 (C-2’), 111.8 (C-6’), 111.5 (C-4”).

IR, ν_{max} /cm^{-1} 3217 (NH), 1597 and 1512 (benzene).

MS, m/z found 419.0677, $C_{20}H_{12}{}^{35}ClF_4N_4$, ($M+H^+$) requires 419.0681.

1-(4-((*E*)-(2-(3-Chlorophenyl)hydrazinylidene)methyl)-2,3,5,6-tetrafluorophenyl)-1*H*-imidazole (190)



Following the general method outlined for compound **197**, compound **67** (0.24 g, 1.00 mmol) was reacted with 3-chlorophenylhydrazine hydrochloride (0.18 g, 1.00 mmol) in DCM (2 ml) at room temperature for 2 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 1.1:1) afforded compound **190** (0.30 g, 80%) as a yellow solid. m.p. 245-247 °C.

NMR δ_H (400 MHz, DMSO-d_6), 11.17 (1H, s, NH), 8.01 (1H, s, H-2"), 7.90 (1H, s, CHN), 7.53 (1H, s, H-5"), 7.24 (1H, t, *J* = 8.0 Hz, H-5'), 7.17 (1H, s, H-4"), 7.04 (1H, s, H-2'), 6.93 (1H, d, *J* = 8.4 Hz, H-6'), 6.83 (1H, dd, *J* = 8.0, 1.2 Hz, H-4').

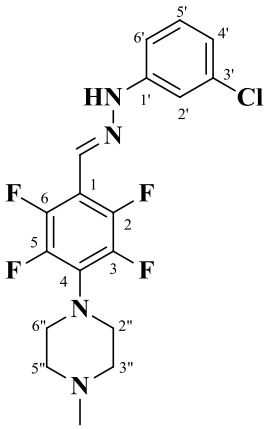
NMR δ_F (376 MHz, DMSO-d_6), 17.6 (2F, dd, *J* = 22, 9 Hz, F-3, F-5), 11.8 (2F, dd, *J* = 22, 9 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 146.0, 144.1 (d, *J* = 256 Hz, C-2, C-6/C-3, C-5), 142.0 (dd, *J* = 247, 15 Hz, C-2, C-6/C-3, C-5), 138.8 (C-2"), 134.5 (C-3'), 131.5 (C-5'), 129.9 (C-4"), 125.5 (CHN), 121.5 (C-5"), 120.3 (C-4'), 115.5 (t, *J* = 12 Hz, C-4), 115.1 (t, *J* = 11 Hz, C-1), 112.3 (C-2'), 111.7 (C-6').

IR, ν_{max} /cm^{-1} 3227 (NH), 1597 and 1528 (benzene).

MS, m/z found 369.0526, $C_{16}H_{10}{}^{35}ClF_4N_4$, ($M+H^+$) requires 369.0525.

1-(4-((*E*)-(2-(3-Chlorophenyl)hydrazinylidene)methyl)-2,3,5,6-tetrafluorophenyl)-4-methylpiperazine (196)



Following the general method outlined for compound **197**, compound **69** (0.28 g, 1.00 mmol) was reacted with 3-chlorophenylhydrazine hydrochloride (0.18 g, 1.00 mmol) in DCM (2 ml) at room temperature for 2 hours. Work-up as previously described and purification by chromatography on silica gel (elution with petrol ether / ethyl acetate = 1:20) afforded compound **196** (0.27 g, 69%) as a white solid. m.p. 155-157 °C.

NMR δ_H (400 MHz, DMSO-d_6), 10.84 (1H, s, NH), 7.80 (1H, s, CHN), 7.20 (1H, t, *J* = 8.4 Hz, H-5'), 6.98 (1H, t, *J* = 1.6 Hz, H-2'), 6.86 (1H, dd, *J* = 8.0, 1.2 Hz, H-6'), 6.76 (1H, dd, *J* = 8.0, 1.2 Hz, H-4'), 3.10 (4H, s, H-2", H-6"), 2.38 (4H, s, H-2", H-6"), 2.17 (3H, s, CH_3).

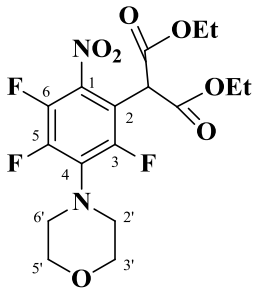
NMR δ_F (376 MHz, DMSO-d_6), 15.9 (2F, dd, *J* = 20, 7Hz, F-3, F-5), 9.7 (2F, dd, *J* = 20, 7 Hz, F-2, F-6).

NMR δ_C (100 MHz, DMSO-d_6), 146.5, 144.7 (dd, *J* = 248, 14 Hz, C-2, C-6/C-3, C-5), 142.3 (dd, *J* = 242, 15 Hz, C-2, C-6/C-3, C-5), 134.4, 131.4 (C-5'), 129.3 (t, *J* = 11 Hz, C-4), 126.7 (CHN), 119.5 (C-4'), 111.9 (C-2'), 111.4 (C-6'), 108.0 (t, *J* = 12 Hz, C-1), 55.5 (C-3", C-5"), 50.9 (C-2", C-6"), 46.4 (CH_3).

IR, ν_{max} /cm^{-1} 3356 (NH), 1643 (C=N), 1589 and 1473 (benzene).

MS, m/z found 398.0911, $C_{18}H_{15}{}^{35}ClF_4N_4$, ($M+H^+$) requires 398.0916.

Diethyl 2-(2,4,5-trifluoro-3-morpholino-6-nitrophenyl)malonate (212)



A solution of compound **211** (0.14 g, 0.50 mmol) in THF (1 ml) was added to NaH (0.04 g, 2.00 mmol). Diethyl malonate (0.08 g, 0.50 mmol) was then added and the solution was stirred at room temperature for 16 hours. The reaction mixture was poured into deionised water (10 ml) and was extracted with ethyl acetate (10 ml x 3). The extracts were combined and washed with brine (approx. 10 ml), and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was subjected to a silica column (elution with light petroleum/ ethyl acetate = 2:1). The combined elution solutions were evaporated under reduced pressure to give the compound **212** (0.16 g, 75%) as a red oil.

NMR δ_H (400 MHz, $CDCl_3$), 4.87 (1H, s, CH), 4.26 (4H, q, *J* = 7.6 Hz, CH_2), 3.79 (4H, t, *J* = 4.4 Hz, H-3', H-5'), 3.35 (4H, s, H-2', H-6'), 1.28 (6H, t, *J* = 7.2 Hz, CH_3).

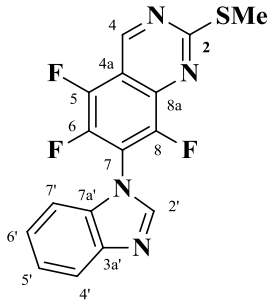
NMR δ_F (376 MHz, $CDCl_3$), 39.4 (1F, t, *J* = 9 Hz), 19.6 (1F, dd, *J* = 21, 9 Hz), 15.6 (1F, dd, *J* = 21, 9 Hz).

NMR δ_C (100 MHz, $CDCl_3$), 165.7 (C=O), 150.1 (dd, *J* = 195, 2 Hz), 144.6 (ddd, *J* = 200, 11, 7 Hz), 142.8 (ddd, *J* = 207, 13, 3 Hz), 133.1 (dd, *J* = 12, 7 Hz), 132.3 (td, *J* = 5, 3 Hz, C-4), 113.6 (dd, *J* = 16, 3 Hz, C-1), 67.1 (C-3', C-5'), 62.6 (CH_2), 50.9 (t, *J* = 3 Hz, C-2', C-6'), 49.2 (CH), 14.0 (CH_3).

IR, ν_{max} /cm^{-1} 1743 (C=O), 1612 and 1527 (benzene), 1527 and 1342 (NO_2).

MS, m/z found 421.1227, $C_{17}H_{20}F_3N_2O_7$, ($M+H^+$) requires 421.1217.

7-(1'*H*-Benzo[*d*]imidazol-1'-yl)-5,6,8-trifluoro-2-(methylsulfanyl)quinazoline (201)



A mixture of compound **68** (0.44 g, 1.50 mmol), S-methylisothiouronium sulfate (0.21 g, 1.50 mmol) and sodium hydride (0.18 g, 4.50 mmol) was added into a 25 ml round-bottom flask with DMF (4 ml). The reaction was conducted at 80 °C for 24 hours. The reaction mixture was poured into deionised water (10 ml) and was extracted with ethyl acetate (15 ml x 3). The extracts were combined and washed with brine (approx. 15 ml), and then dried over sodium sulfate. The solution was concentrated under reduced pressure and the residue was separated by using column chromatography on silica gel (elution with light petroleum/ ethyl acetate = 2:1). The combined elution solutions were evaporated under reduced pressure to give the compound **201** (0.06 g, 13%) as an orange solid. m.p. 162-164 °C

NMR δ_H (400 MHz, $CDCl_3$), 7.97 (1H, s, H-2'), 7.88 (1H, dd, *J* = 7.2, 1.6 Hz, H-7'), 7.37-7.29 (2H, m, H-5', H-6'), 7.23-7.16 (1H, m, H-4), 7.11 (1H, d, *J* = 7.2 Hz, H-4'), 2.14(3H, s, CH_3).

NMR δ_F (376 MHz, $CDCl_3$), 57.1-56.9 (1F, m), 31.2 (1F, ddd, *J* = 22, 10, 5 Hz), 16.9-16.8 (1F, m).

NMR δ_C (100 MHz, $CDCl_3$), 171.2 (C-2), 158.4 (dd, *J* = 249, 11 Hz), 150.2 (dt, *J* = 253, 13 Hz), 144.0 (ddd, *J* = 253, 13, 5 Hz), 143.0, 142.9, 134.1, 127.4 (d, *J* = 10 Hz, C-4a/C-8a), 124.3 (C-5'), 123.5 (d, *J* = 98 Hz, C-7), 123.3 (C-6'), 120.9 (d, *J* = 41 Hz, C-4a/C-8a), 120.8 (C-7'), 110.1 (C-4'), 106.9 (dd, *J* = 30, 20 Hz, C-4), 14.2 (CH_3).

IR, ν_{max}/cm^{-1} 1635 and 1581 (benzene ring).

MS, m/z found 347.0556, $C_{16}H_{10}F_3N_4S$, ($M+H^+$) requires 347.0573.

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