Design, synthesis and pharmacological evaluation of efficacy selective β_2 adrenoceptor agonists

Elliott Lumb BSc MSc

Thesis submitted to the University of Nottingham

for the degree of Doctor of Philosophy

September 2018

Abstract

Asthma and chronic obstructive pulmonary disease both involve constriction or obstruction of the airways and are causes of morbidity and mortality worldwide. To treat these conditions, patients are often prescribed β_2 adrenoceptor agonists (β_2AR). Activation of the β_2AR leads to smooth muscle relaxation within the respiratory tract, and therefore increased airflow throughout the lungs. The caveat of current approved drugs, is that due to structural similarities with the β_2AR endogenous agonist adrenaline, these compounds can also activate the β_1AR. Activation of the β_1AR, increases heart rate and force of contraction. This becomes problematic when patients also suffer from heart disease (40% of COPD patients). There is therefore, a requirement for β_2AR agonists with efficacy selectivity to the β_2AR.

This project aims to develop an efficacy selective β_2AR agonist by linking a β_2AR agonist pharmacophore, to a selective β_1AR antagonist pharmacophore, to form a bivalent compound that will exhibit agonism at the β_2AR, but antagonism at the β_1AR.

Analogues of the naturally occurring β_2AR agonist, S1319, and the highly selective β_1AR antagonist CGP 20712A were synthesised and pharmacologically evaluated (performed by the author and Prof. Jillian Baker). Structure-activity relationship studies were performed in an attempt to identify moieties that were β_2AR efficacy selective and β_1AR affinity selective.

A β_2AR agonist group was attached to several β_1AR antagonist groups to form a number of bivalent compounds which underwent pharmacological evaluation (performed by Prof Jillian Baker). The results of these studies facilitated the rational design of the efficacy selective β_2AR agonist **5.61** which is a partial β_2AR agonist–β_1AR antagonist.

Acknowledgements

Firstly, I would like to thank my supervisors Michael Stocks, Jillian Baker and Peter Scammells for their knowledge and guidance throughout my PhD.

I would like to acknowledge all of those within CBS and MIPS that gave me their time and patience to help me over the last four years. In particular I would like to thank Nick Kindon, Shailesh Mistry and Sarah Cully for their support, and to Aimie Garces and Max Ziemann for the occasional distraction.

This work could not have been done without the staff at the University of Nottingham and Monash University. I am especially thankful to Jane Doughty, Lee Hibbert and Richard Proudman for their technical support and Rob Manners for his help.

I would like to thank Mum, Dad and the rest of my family for their unwavering support and encouragement. This thesis would not have been possible if it was not for them.

Finally, I would like to thank all of my friends who have endured and helped me through the more difficult times of my PhD, and for generally making my time outside of the lab fun. I would particularly like to mention Sean, Phil, Jamie, Laurence, Sarah, Rachel, Lisa, Joe, Isabelle, Cara, Cameron and Sophie.

This work was funded by the University of Nottingham

Abbreviations

β_1AR	β_1-adrenoreceptor
β_2AR	β_2-adrenoreceptor
β_3AR	β_3-adrenoreceptor
ATP	adenosine triphosphate
Ar	aromatic ring
Bn	benzyl
cAMP	cyclic adenosine monophosphate
CC	column chromatography
CHO	Chinese hamster ovary
cLogP	partition coefficient between n-octanol and water
COPD	chronic obstructive pulmonary disease
COSY	correlation spectroscopy
CPM	counts per minute
CRE	cAMP response element
CREB	cAMP response element-binding protein
d	doublet
DCM	dichloromethane
dd	doublet of doublets
DIPEA	*N,N*-Diisopropylethylamine
DMAP	*N,N*-dimethylaminopyridine
DMF	*N,N*-Dimethylformamide

DMSO	dimethyl sulfoxide
dt	doublet of triplets
EC_{50}	concentration required to generate half maximal response
ES	electrospray
GPCR	G protein-coupled receptor
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IA	intrinsic activity
Ic_{50}	molar concentration that inhibits 50% of the maximal response
K_d	dissociation constant
LC-MS	liquid chromatography mass spectrometry
m	multiplet
m	*meta*
m/z	observed ion
M_3	M_3 muscarinic acetylcholine receptor
m-CPBA	*meta*-Chloroperoxybenzoic acid
MeCN	acetonitrile
mw	microwave
NMR	nuclear magnetic resonance
o	*ortho*
p	*para*
Pd/C	palladium on carbon

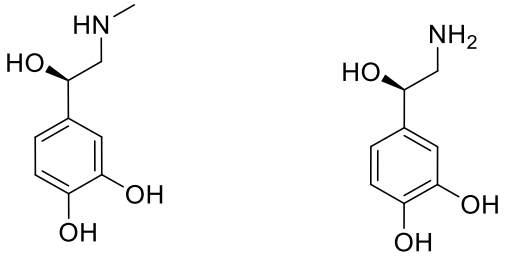
PDE4	Phosphodiesterase type 4
PKA	protein kinase A
*p*NPP	*p*-Nitrophenol phosphate
ppm	parts per million
R_f	retention factor
RP	reverse phase
rt	room temperature
R_t	retention time
s	singlet
SAR	structure-activity relationships
S_NAr	nucleophilic aromatic substitution
SPAP	secreted placental alkaline phosphatase
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TM	transmembrane domain
TOF	time of flight
uLABA	ultra-long β_2AR Agonist

Contents

1. Introduction

1.1 β-Adrenoceptors

The β-adrenoceptors (βAR) are class A, G protein-coupled receptors (GPCRs), which are characterised by a conserved core structure that consists of 7-transmembrane α-helices.[1] The βARs are subdivided into β_1, β_2 and β_3 which are activated by the endogenous hormones, adrenaline (**1.1**) and noradrenaline (**1.2**). The receptors are widely distributed around the body and cause a range of physiological effects.



Adrenaline **1.1**

Noradrenaline **1.2**

Fig 1.1 βAR endogenous hormones

β_1ARs are found predominantly within cardiac tissue, where activation causes an increase in both heart rate and the force of contraction.[2] β_2ARs are also found within cardiac tissue, eliciting the same effect as β_1ARs, but are found in significantly lower numbers (cardiac tissue is comprised of 20-25% β_2ARs).[1, 3] The β_2ARs are primarily distributed within the respiratory tract, where activation induces smooth muscle relaxation and as a result, an opening of the airways. Finally, β_3ARs are localised mainly within adipose tissue and have been shown to play a role within the control of lipid metabolism in rats.[4-5]

1.1.1 β-Adrenoceptor structure

GPCRs are the largest known gene superfamily in the human genome. Each of these receptors are made up of a single polypeptide chain that contains seven hydrophobic transmembrane α-helices.[6] However, each GPCR has a different peptide chain length and sequence, as well as a varying N-terminus function.[7] There are three extracellular and three intracellular loops, as well as an extracellular N-terminus and intracellular C-terminus, all of which have variation between each GPCR.[8-9]

While each of the three βARs do have conserved regions in their amino acid chains, there are also many differences. The β_1 and β_2AR are 48.9% homologous, whereas β_3 is 50.7 and 45.5% homologous to β_1 and β_2 respectively.[10] Furthermore, the amino acids chain length differs between each receptor. The β_1, β_2 and β_3AR contain 477, 413 and 408 amino acids, respectively.[11]

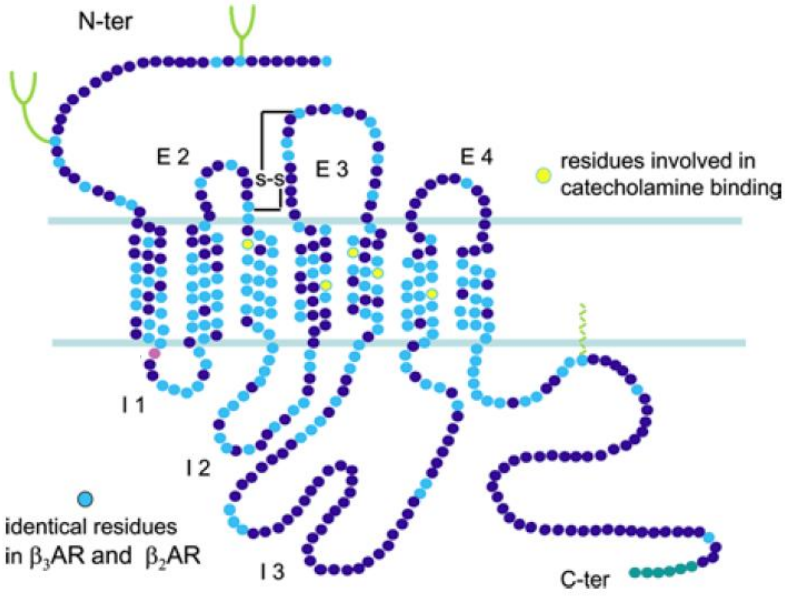


Fig 1.2: Diagram of β-adrenoceptor embedded within cell membrane, showing homology between β_2 and β_3AR.[12]

1.1.2 Receptor activation

The β-adrenoceptors are activated by the catecholamine endogenous hormones, adrenaline (**1.1**) and noradrenaline (**1.2**). Upon binding, the hormones stabilise the activated state of the receptor. This allows the receptor to couple with the intracellular G_s protein.[13] Following this, the G protein α subunit binds to adenylate cyclase which increases the conversion of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP) within the cell. This in turn can activate proteins such as protein kinase A (PKA) which either up or down regulates various signalling cascades (Fig 1.2).[14-15]

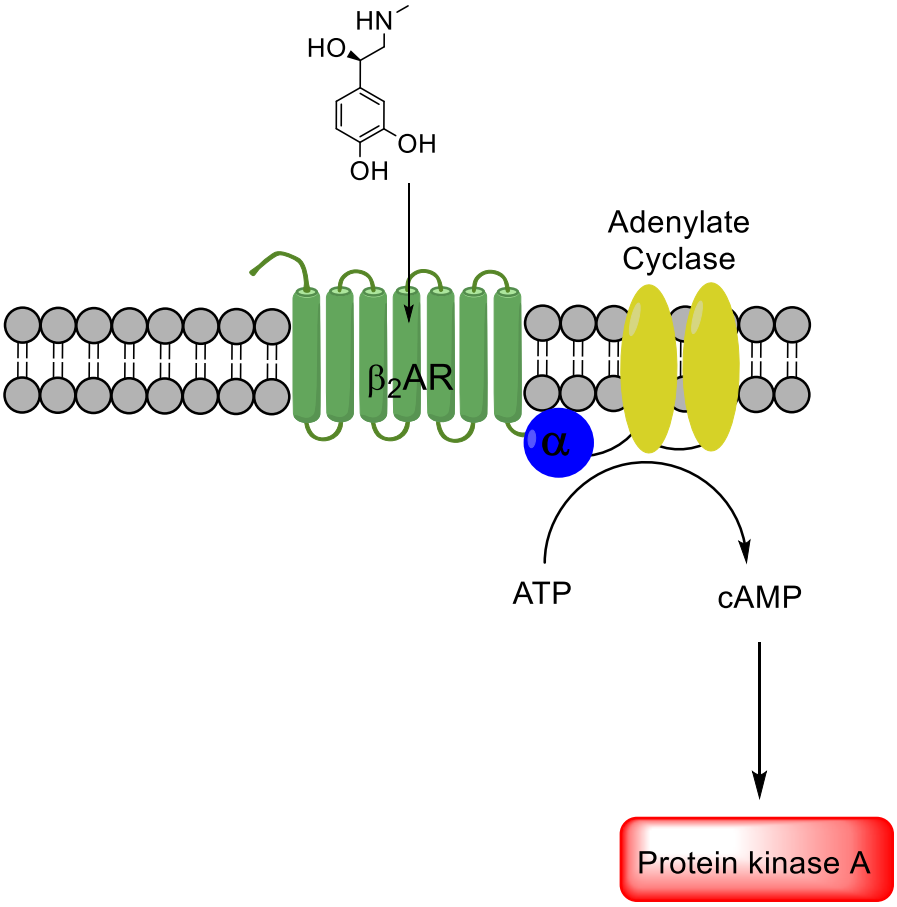


Fig 1.3: Binding of adrenaline **1** causes a conformational change of β_2AR. As a result, the G protein α subunit binds to adenylate cyclase, which catalyses the conversion of ATP into cAMP. The increase of cAMP within the cell activates PKA, which it activates or inhibits numerous signalling pathways.[14]

Mutagenesis studies have identified a number of residues that are essential for catecholamine activation of the βARs. Within the β_2AR there are three serine residues involved in the catecholamine activation of the receptors.[4] The hydroxyl side chains of serine 203, 204 and 207, each on TM5, can form hydrogen bonds with the *meta*- (ser-203 and -204) and *para*-hydroxyl groups (ser-207) of the catechol.[16-17] The interaction of the catechol with these serine residues is essential for catecholamine βAR activation. Additionally, it has been shown that the carboxylate group of aspartic acid 113, located on TM3, interacts with the amine of a catecholamine ligand, this has also been to shown to be important for receptor activation.[6] It is also postulated that asparagine 293 of TM6 could be involved with the stereospecifity of ligand binding (Fig 1.3).[18]

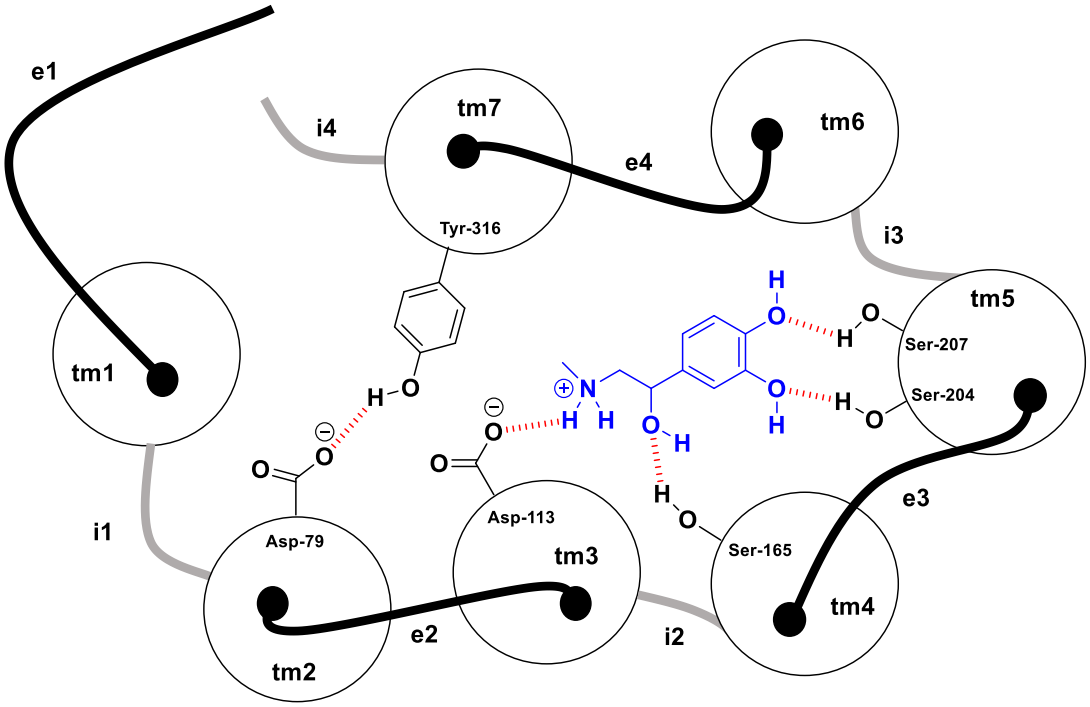


Fig 1.4: adapted schematic of proposed interactions between catecholamine, adrenaline **1.1** and β_2AR.[6]

1.2 β_2AR agonists

1.2.1 Therapeutic relevance

Activation of the β_2AR increases airflow throughout the lungs by relaxing smooth muscle in the respiratory tract. β_2AR agonists can therefore be used to treat conditions in which constriction or obstruction of the airways reduces airflow thus causing breathing difficulty.[19] These types of condition include asthma and chronic obstructive pulmonary disease (COPD).

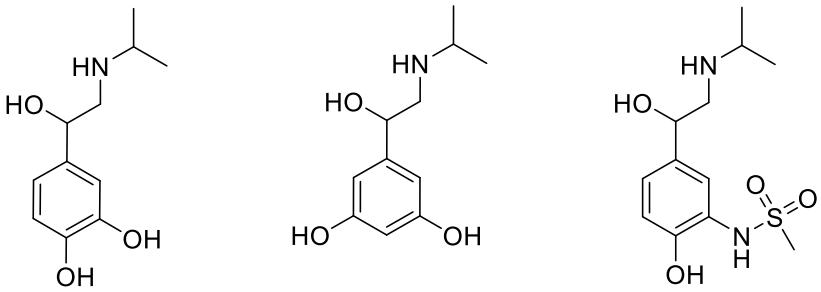
Asthma is an allergic disease that affects 30 million people within Europe.[20] It is triggered by allergens or irritants which cause smooth muscle contraction, airway inflammation and increased mucous production.[21] The constriction of the airways caused by asthma is reversible, but patients can suffer from wheezing, chest tightness and coughing in varying degrees of intensity. In severe cases, asthma can be fatal, and is reported to cause 1000-1500 deaths per year in the UK.[22]

Conversely, the effects of COPD are irreversible and constant and the disease is a major cause of morbidity and mortality throughout the world. COPD is characterised by long term breathing difficulties and poor airflow throughout the lungs and it includes chronic bronchitis and emphysema. In the Western World the disease is most commonly caused by long-term tobacco smoking and effects an estimated 1.2 million people, in the UK alone. [23-24] In developing countries, it is also attributed to the inhalation of smoke from the burning of biomass.[25] Due to a progressive reduction in airflow throughout the lungs, patients afflicted with COPD frequently experience dyspnoea, and the disease can ultimately result in death.[23]

1.2.2 First generation β_2AR agonists

During the early 20th century, it was reported that adrenaline was effective at easing the symptoms of asthma.[26] However, adrenaline (**1.1**) itself was less than an ideal drug; it has to be administered intravenously and causes tachycardia and increases blood pressure, due to activation of β_1ARs. Furthermore, adrenaline is readily metabolised by the enzyme catechol-*o*-methyltransferase, resulting in a very short half-life and accordingly, a short duration of action.[27]

The first step forward in the treatment of asthma with a β_2AR agonist was the discovery of isoprenaline (**1.3**). Isoprenaline exhibits a similar effect in bronchodilation as adrenaline, without increasing blood pressure.[28] However, the widespread use of isoprenaline as a treatment for asthma was hindered by its short half-life; like adrenaline it has the readily metabolised catechol moiety. Additionally, though it has no pressor effect, isoprenaline, increases heart rate.[29]



Isoprenaline **1.3** | Orciprenaline **1.4** | Soterenol **1.5**

Fig 1.5: first generation β_2AR agonists.

Following the discovery of isoprenaline, a number of analogues were synthesised, in an attempt to increase β_2AR selectivity, and increase half-life. These compounds included, orciprenaline (**1.4**) which has a resorcinol moiety rather than a catechol, and the sulfonamide containing soterenol (**1.5**). While each of these were β_2AR agonists with increased metabolic stability, they lacked the required β_2AR selectivity.[30-31]

The first compounds developed that had metabolic stability against catechol-*o*-methyltransferase, and had levels of β_2AR selectivity were salbutamol (**1.6** (pEC_{50}= 6.7, intrinsic activity (IA) = 0.8 (percentage response compared to full agonist isoprenaline), clogP = 0.88)) and terbutaline (**1.7** (pEC_{50}= 5.5, IA = 0.7, clogP = 1.35)).[32-33] The saligenin moiety of salbutamol and resorcinol of tertbutaline satisfy the same pharmacophore as the catechol of adrenaline. However, as with the aforementioned orciprenaline and soterenol, they are not metabolised by the catechol-*o*-methyltransferase, and so have a significantly longer half-lives than adrenaline or isoprenaline.[34] The two compounds each exhibit a duration of action between 3-6 hours, whereas isoprenaline is active for 1-2 hours.[35] Although salbutamol (**1.6**) and tertbutaline (**1.7**) may only have modest β_2AR selectivity (30- and 63-fold, respectively), they were found to have less tachycardia effects than earlier drugs.[35] This is not only due to their levels of selectivity, but because they are inhaled (as are all β_2AR agonists that followed). This maximises the amount of the drug reaching the bronchus, while reducing the amount of drug entering systemic circulation and activating β_1AR on cardiac tissue, causing undesirable side-effects.[36]

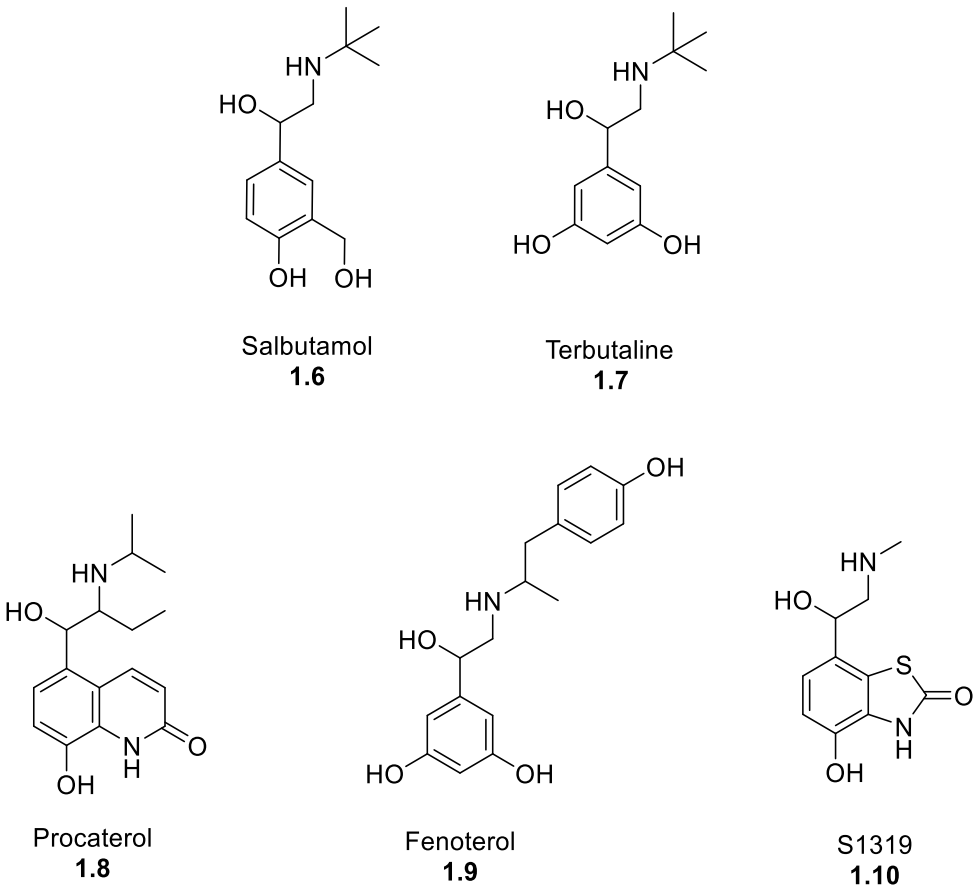


Fig 1.6: first generation βAR agonists

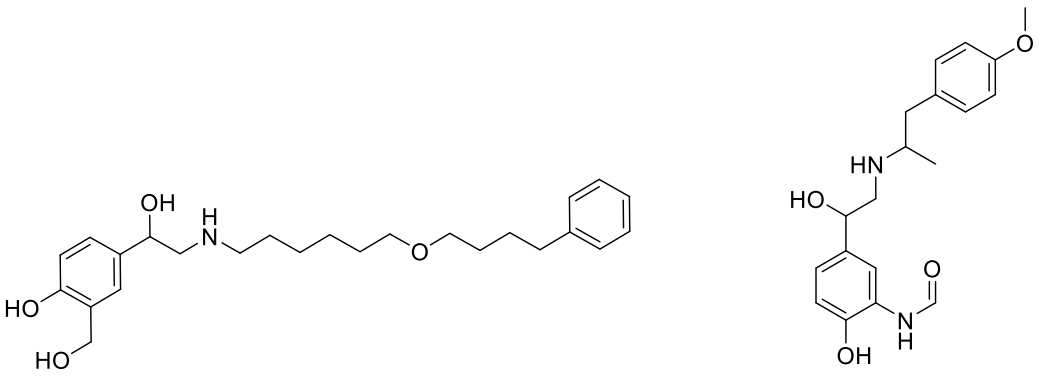
Following the discovery of salbutamol and terbutaline, a number of compounds were developed which included procaterol (**1.8**) and fenoterol (**1.9**). Many of these compounds have increased metabolic stability when compared to isoprenaline and have similar levels of efficacy and β_2AR selectivity compared to salbutamol. However, many of these did not have any additional clinical benefit and so are not widely used.[37-38] Fenoterol was licenced for use in New Zealand, however, its use was associated with an increased risk of death from severe asthma and was therefore withdrawn from the market.[39]

30 years after the discovery of salbutamol, S1319 (**1.10**) was isolated from the marine sponge, *Dysidea sp*. The compound is analogous to adrenaline and the benzothiazolone structure can act as a mimetic of the catechol moiety. It is reported to have similar levels of β_2AR selectivity to salbutamol but, to be significantly more active.[40]

1.2.3 Second generation β_2AR agonists: long acting

Salbutamol **1.6** is still widely used clinically as a relief medication for asthma and COPD, however, as its duration of action is 3-6 hours, it requires multiple dosage per day. The caveat of this being that patients with more severe conditions would have to regularly wake up to take the medication, thus reducing quality of life.[41] Much of the research following the discovery of the first generation β_2AR agonists was therefore concerned with developing compounds that could maintain an effective concentration at the receptor site for significantly longer than 6 hours.

The two main second generation β_2AR agonists are salmeterol (**1.11**) and formoterol (**1.12**), each of which are long acting, as their bronchodilating effect lasts approximately 12 hours.[42] As a result, these compounds require twice daily dosage, alleviating the need for patients to wake up during the night to take the medication.



Salmeterol
1.11

Formoterol
1.12

Fig 1.7: second generation βAR agonists

Although the two drugs have a similar duration of action, their pharmacological profiles differ in a number of ways. Formoterol (pEC_{50}= 9.3, IA = 1, clogP = 2.06) is a highly efficacious, fast acting β_2AR agonist, and is therefore ideal for use as a reliever medication, when fast onset of action is required.[43] Conversely, salmeterol (pEC_{50}= 9.1, IA = 0.6 clogP = 4.15) is a partial agonist, with a slow onset of action.[32, 44] The physical properties of the two drugs also differ, while formoterol is highly water soluble and moderately lipophilic, salmeterol has low water solubility and high

lipophilicity. As with all other β_2AR agonists, they each have a moiety that acts as a catechol mimetic and have a structurally disparate amino group, which is where the differences in the two compounds arises from.[45]

There are a number of theories to explain the long duration of action of salmeterol and formoterol. The most widely accepted hypothesis is the diffusion micro kinetic theory (Fig 1.4).[46-47] The theory explains that compounds with moderate to high levels of lipophilicity such as formoterol and salmeterol can partition into the lipid bilayer. They are then released over a period of time, resulting in a consistent effective concentration of agonist at the receptor, for an extended duration.[48] Salbutamol and terbutaline are both hydrophilic and so are favoured in the extracellular aqueous environment. Consequently, they are cleared from the tissue at a faster rate.[44]

The differences in the onset of action of salmeterol and formoterol can also be explained through the diffusion micro kinetic theory. Salmeterol is highly lipophilic and is therefore rapidly partitioned into the lipid bilayer (within 1 minute) and so there is no response until it diffuses back out of the bilayer (in approximately 25 minutes).[48] Formoterol, however, is moderately lipophilic and hydrophilic, it is therefore thermodynamically favourable for it to exist in both the lipid bilayer and surrounding aqueous environment. Therefore, immediately after administering the drug through inhalation, there is a sufficiently high concentration of the drug in the extracellular environment to bind to, and activate the receptor.[44]

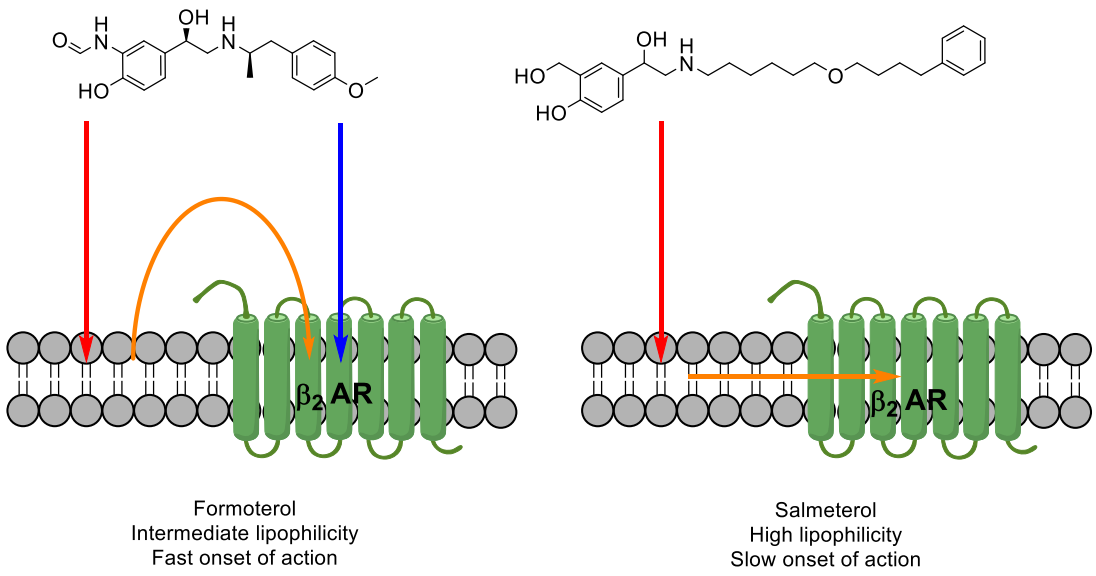


Fig 1.8: Schematic of diffusion micro kinetic theory for formoterol and salmeterol. The red arrow represents membrane partitioning of the compounds, since both are lipophilic, both diffuse into the membrane. The blue arrow, shown only for formoterol indicates immediate membrane activation, this can occur because unlike salmeterol, the compound is thermodynamically stable in both aqueous and lipid environments. The orange arrow shows the movement of drugs from the membrane into the β_2AR. Formoterol is proposed to be released slowly into the aqueous environment, whereas salmeterol moves laterally through the membrane into the β_2AR[48]

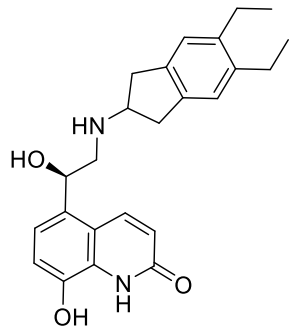
Due to the physical differences between formoterol and salmeterol, it is postulated that the two compounds could have completely different reasons for their long duration of action. An additional theory is that salmeterol, with its long aliphatic group, is able to bind to an exosite within the β_2AR that is distal from the orthosteric binding site. It is hypothesised that salmeterol would be anchored to the exosite, which allows for persistent activation of the receptor.[49]

The two second generation compounds also exhibit high selectivity towards the β_2AR. Formoterol is reported to have 331-fold higher affinity to β_2AR than β_1, while salmeterol has a 3388-fold higher affinity towards the β_2AR.[50] Each of these are significantly more selective towards β_2 than the first-generation compounds. However, the selectivity is based on affinity, which is how well a compound can bind to a receptor and does not take into account whether the receptors is activated. Although both compounds are β_2AR selective, they have are able to bind to and activate the β_1AR.[51] The unprecedented level of selectivity exhibited by salmeterol has been studied by point mutations of the β_2AR. From this, it was found that

introducing mutations to lysine-305 on extracellular loop 3 and histidine-296 in transmembrane 6 had the greatest single effect at reducing salmeterol's affinity. By combining these two mutations, salmeterol's affinity was reduced 275-fold, which is only a 4-fold higher than salmeterol's β_1AR affinity. This suggests that lysine-305 and histidine-296 are important salmeterol's high level of selectivity towards the β_2AR.[52]

1.2.4 Third generation β_2AR agonists: ultra-long acting agonists

Since their approval, salmeterol (**1.11**) and formoterol (**1.12**) have widely been used to treat severe asthma and COPD. However, over the past 20 years, many pharmaceutical companies have endeavoured to develop a β_2AR agonists with longer half-lives. Novartis were the first to obtain approval for a compound in this class, indacaterol (**1.13**), which is the first ultra-long acting β_2AR agonist (uLABA). Indacaterol provides bronchodilation for a full 24 hours, and so unlike previous compounds, only requires once-a-day dosing.[53]



Indacaterol
1.13

Fig 1.9: third generation ultra-long acting βAR agonist

Novartis set out to develop an agonist that could provide once daily treatment and have a fast onset of action. Although there are many hypotheses on how the long duration of action is obtained, their strategy involved using lipophilicity as the basis of the drug design, which appears to be a factor in the duration of action in the second generation compounds.[53] A possible problem that could have arisen is that a compound that is very lipophilic, such as salmeterol, could have a slow onset of

action. However, an additional argument is that salmeterol's slow onset of action may be due to partial receptor agonism, and therefore the requirement to occupy more receptors than the high intrinsic efficacy agonists, such as formoterol.[54]

Indacaterol (pEC_{50}= 7.9, IA = 0.8, clogP = 4.25) has a quinolinone core that acts as a catechol mimetic; this has been previously used in past drugs, such as procaterol. It is a partial agonist at the β_2AR, but has a higher intrinsic efficacy than both salmeterol and salbutamol.[32] Additionally, as desired, indacaterol has a fast onset of action, causing bronchodilation within 5 minutes of inhilation.[55] Indacaterol does not exhibit the same levels of selectivity towards the β_2AR as the second generation compounds; it is approximately 10-fold more selective towards β_2AR than β_1AR.[56] While indacaterol may provide instant and lasting relief for patients suffering from COPD, its lack of selectivity may present problems for patients who also have heart conditions. Upon reaching cardiac tissue, though systemic circulation, indacaterol may induce sustained activation of the β_1AR and therefore prolonged tachycardia.

As with salmeterol and formoterol, there is much debate on how indacaterol could provide the extended duration of action. It has been suggested that the structure of procaterol, which shares the same quinolone core as indacaterol, allows it to bind tightly to the β_2AR, resulting in a long duration of binding, and therefore action.[57] However, contra to this, it has also been demonstrated that slow receptor dissociation is not responsible for the long duration of action of the β_2AR agonists and it is more likely due to lipophicility.[58] The diffusion micro kinetic theory is therefore again favoured as an explanation. Though indacaterol has high lipophilicity, a possible explanation for it having a significantly faster onset of action, is that at physiological pH (indacaterol parent pK_a: NH = 9.71, ArOH = 8.51) it is predominantly zwitterionic (54.1%), whereas salmeterol (parent pK_a: NH = 9.45, ArOH = 9.96) is almost entirely cationic (95.6%) and these different species will interact with tissue differently.[59-60]

Finally, it has been reported that that dual therapy of indacaterol and a long acting anticholinergic bronchodilator could be particularly beneficial for patients suffering

from severe COPD. Accordingly, Novartis have recently launched Ultibro Breezhaler, a combination of indacaterol and the long acting anticholinergic bronchodilator, glycopyrronium bromide.[61]

Another uLABA is olodaterol (**1.14** (pEC_{50}= 9.9, IA = 0.9, clogP = 1.93)) which contains a hydroxy benzoxazinone core as the catechol mimetic; this moiety has not been used in previous β_2AR agonists. The compound is reported to have rapid onset of action and a duration of action of at least 24 hours. It also has a 256-fold higher affinity for the β_2 than β_1AR and presents partial agonism (though higher IA than indacaterol).[62] Although reported to have similar levels of clinical efficacy to indacaterol it is only approved for monotherapy in Russia and Canada.[63] However in both Europe and the USA, it is approved for use in combination with tiotropium, a muscarinic M_3 receptor antagonist, to treat severe COPD.[64]

Abediterol (**1.15** (clogP = 4.07)), an uLABA developed by AstraZeneca, is currently in phase II clinical trials. It is reported to provide 24 hour bronchodilation, fast onset of action, and have a superior or similar selectivity to the β_2AR when compared to other long and ultra-long acting β_2AR agonists.[65-66]

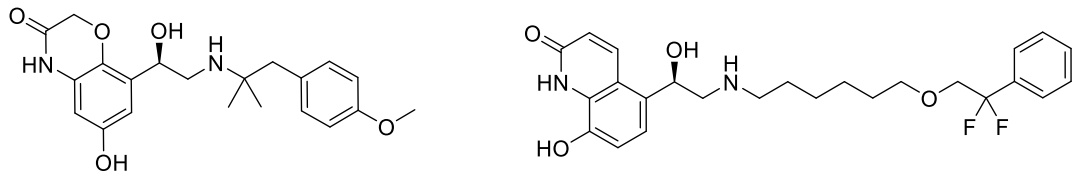
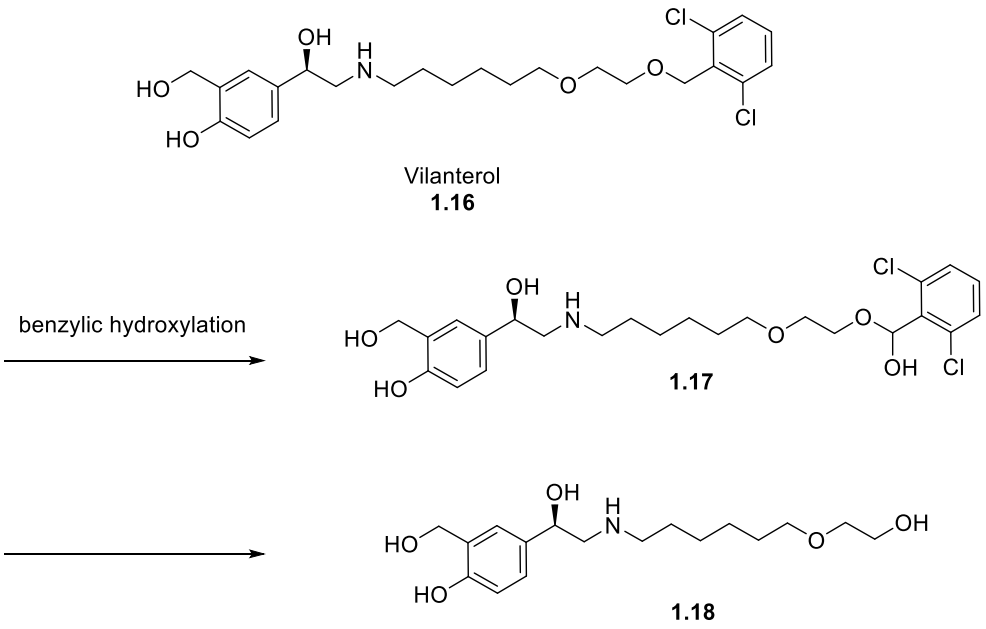


Fig 1.10: third generation ultra-long acting βAR agonists

The uLABA Vilanterol (**1.16** (pEC_{50}= 9.4, IA = 0.7, clogP = 4.14)), was recently approved by the FDA in combination with fluticasone furoate, a corticosteroid. Vilanterol was developed to improve the properties of salmeterol (**1.11**), and as a result has 24 hour duration of action and a faster onset of action.[67] Additionally, the design of vilanterol incorporates an antedrug approach. This is achieved by incorporating structural features that make the compound sensitive to metabolism upon exposure to

systemic circulation. It was hypothesised that by replacing a methylene, within the salmeterol chain, with an oxygen atom, and assuming the same route of metabolism by benzylic hydroxylation occurred, then an unstable hemiacetal (**1.17**) would form and cleave to the alcohol (**1.18**). The alcohol may be less potent than the initial compound, therefore the drug in systemic circulation may not activate cardiac β_1AR receptors, and so the tachycardia related side effects would not occur.[68]



Scheme 1.1: Proposed metabolic inactivation of Vilanterol **1.16**. Benzylic hydroxylation enzyme hydroxylates **1.17** to form unstable hemiacetal **1.18** which cleaves to form less potent β_2AR agonist.[68]

1.3 Dual pharmacology compounds

As previously discussed, a number of β_2AR agonists are given as combination therapy such as Anoro Ellipta, a combination of the vilanterol and umeclidinium and Ultibro Breezhaler, a combination of indacaterol and glycopyrronium bromide. Each of these examples are combinations of a β_2AR agonist and a M_3 receptor antagonist. The rationale for combining these two classes of drug is that for patients with severe COPD, a β_2AR agonist monotherapy is not sufficient.[69] Activation of the M_3 receptor induces bronchoconstriction, and so inhibition of this receptor can have an additive,

or synergistic effect when given with β_2AR agonists, to provide more effective bronchodilation.[70]

A relatively new approach is to develop compounds with dual pharmacology. These are often bivalent compounds with two pharmacophores, that differ in molecular targets, separated by a linker.[71] This approach can provide clinical efficacy similar to that of administering separate compounds, but removes the challenges of co-formulation. Additionally, it may provide simpler pharmacokinetics, and allows for triple therapy in a single inhalation device, where a bivalent compound can be co-formulated with another drug such as a corticosteroid that are also used to treat COPD.[72]

GlaxoSmithKline have developed the first-in-class dual M_3 muscarinic antagonist and β_2AR agonist (MABA), batefenterol (**1.19**). Initial studies involved linking a biphenyl carbamate, which was known to be a potent M_3 antagonist and β_2AR agonist head groups (the pharmacophore than binds to the orthosteric site of the receptor) *via* a range of linkers. A number of alkyl linker lengths were synthesised to probe for a lipophilic pocket that the second pharmacophore could bind to. The two compounds, linked by an alkyl chain with 9 carbon atoms, gave an early lead compound which led to the development of THRX-109156 (**1.20**) and then THRX-494732 (**1.21**), which had the same linker length but additional functionality.[72] However, although each of these compounds had high affinity at each receptor and were potent at the β_2AR, they failed to provide bronchodilation for 24 hours.

It was identified that a high molecular weight and a high total polar surface could achieve longer duration of action of inhaled compounds.[73] The subsequent study therefore focused mainly on the aromatization and substitution of the central cyclohexyl ring of THRX-494732. This led to the discovery of batefenterol (**1.19**). Batefenterol has a 24 hour duration of action, while having comparable affinity (M_3 log K_d = -8.1) to tiotropium, an M_3 antagonist, and similar β_2AR potency (pEC_{50} = 8.0) to indacaterol. Batefenterol is currently in phase II clinical trials.[74]

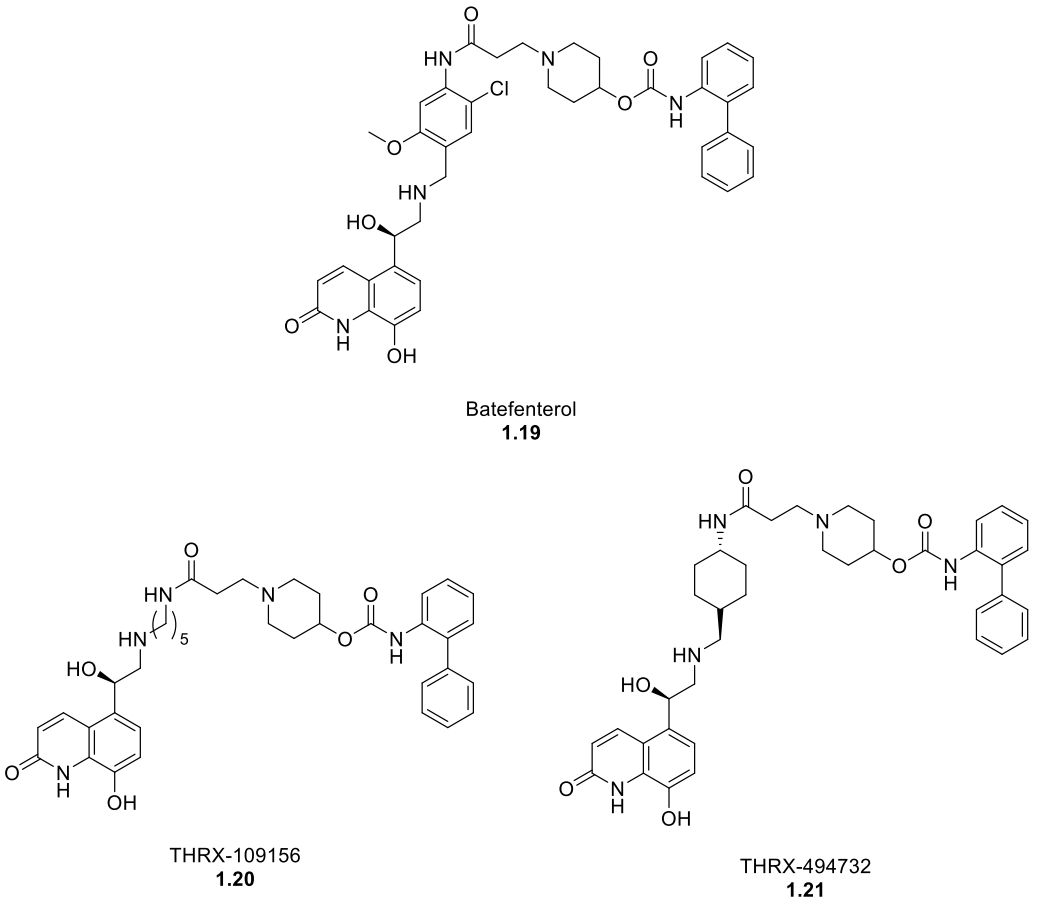


Fig 1.11: dual pharmacology βAR agonists

Pfizer have also attempted to develop a MABA (**1.22**). This was achieved by combining their long-acting muscarinic antagonist, Tolterodine, with a range of β_2AR agonist head groups. From this study a compound was developed that is highly potent at the β_2AR, has high affinity at the M_3 receptor and has a long off rate, providing a long duration of action. Furthermore due to the compounds poor membrane permeability, and high metabolic instability, it is predicted to have a low risk of systemic exposure.[75]

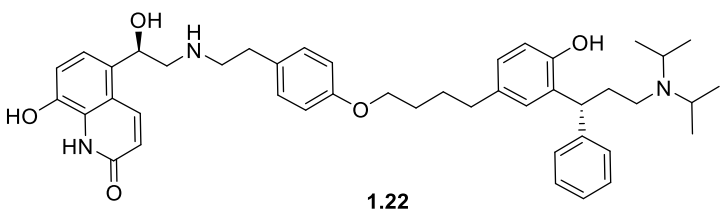
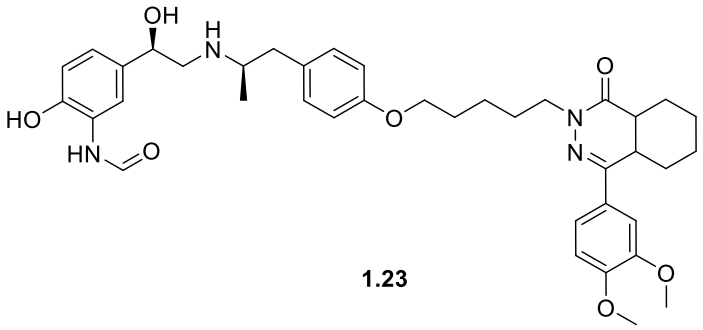


Fig 1.12: dual pharmacology MABA developed by Pfizer

Phosphodiesterase type 4 (PDE4) inhibitors have also been reported to be effective anti-inflammatory agents and implicated in bronchdilation.[76] It has therefore been investigated whether linking formoterol (**1.12**) and a PDE4 antagonist could give a high efficacy bronchodilator. Li *et al*. developed compound (**1.23**) which has been shown to have similar potency (pEC_{50} = 9.0) to formoterol, and higher affinity than the PDE4 antagonist, rolipram. However, the study into whether the two pharmacophores of this compound will have an additive or synergistic effect on bronchodilation, is ongoing.[77]



1.23

Fig 1.13: dual pharmacology βAR agonist-PDE4 inhibitor

1.4 Problems with β_2AR agonists

It is clear from the literature, that the majority of the research carried out over the last 40 years has mainly been concerned with developing compounds with increased duration of action. This has been necessary, to meet a clinical need to improve patient's quality of life and to improve compliance. However, a focus has not been placed on developing compounds that have efficacy selectivity to the β_2AR over the β_1AR, in order to remove cardiac side effects. In some cases, such as vilanterol (**1.16**), an antedrug design was implemented, in order to reduce systemic circulation, but whether this approach works as effectively as suggested, remains hypothetical.

The two most widely prescribed long acting β_2AR agonists, formoterol (**1.12**) and salmeterol (**1.11**), do exhibit very high selectivity (331- and 3388-fold, respectively).

However, though they may have high selectivity, it is reported that each compound has a similar level of intrinsic efficacy at the β_1 and β_2AR; this is also the case for many other marketed β_2AR agonists.[51] The high intrinsic efficacy at both the β_1 and β_2AR likely arises from each of the compounds being based on the endogenous agonist for both receptors, adrenaline (**1.1**). Each compound is made up of a head group containing a catechol mimetic, and an amino alcohol moiety, as seen in adrenaline. The caveat of this being, that when in systemic circulation, though compounds have much higher affinity for the β_2AR, they will be able to activate β_1ARs on cardiac tissue.[78] An increase in heart rate is generally not too problematic for otherwise healthy patients. However, 40% of patients who suffer from COPD, also have heart disease. This becomes problematic, as βAR agonists can aggravate pre-existing cardiac arrhythmia.[79-80]

1.5 β_1AR antagonists

Like the β_2AR, the β_1AR is activated by the endogenous hormones adrenaline and noradrenaline. Whereas the therapeutic modulation of the β_2AR is concerned with the receptors activation and subsequent bronchodilation, the therapeutic benefit of targeting β_1ARs is derived from their inhibition. The β_1ARs are primarily localised on cardiac tissue where they make up 75-80% of the total adrenoceptors in this area.[81] The activation of the receptor, increases both heart rate and the force of contraction. β-blockers, a class of drug used to inhibit β_1ARs, are therefore indicated with the treatment of hypertension and heart disease.[82] Within the β_2AR (which is largely homologous to the β_1AR within the catechol binding region), β-blockers bind to the same orthosteric site as the previously discussed agonists, forming a hydrogen bond with Asp-113 and in some cases Ser-204, but not Ser-207. This difference is predicted to lead to a more flexible coupling of TM-3 and TM-5, which stabilises a receptor configuration that is inactive.[83]

1.5.1 First generation β_1AR antagonists

As with β_2AR agonists, there have been a number of generations of β_1AR antagonists. The first are the non-selective β-blockers, as they have little or no difference in affinity between the β_1 and β_2AR. The first compound reported in this class was dichloroisoprenaline (**1.24**), which has two chlorine atoms, replacing the catechol of isoprenaline (**1.3**). The compound was synthesised while making analogues of isoprenaline, in an attempt to make a compound that is more metabolically stable. However, this was not the case. Instead it was found to be a low efficacy agonist that could antagonise the bronchodilating effect of adrenaline. Though not a pure antagonist, due to its low levels of agonism, it has no clinical benefit itself, but it became the prototype for the β_1AR antagonists that followed.[27] The first of which was pronethalol (**1.25**), a naphthalene containing derivative of isoprenaline. This compound could inhibit the effects of adrenaline, while having no intrinsic efficacy and did not affect α receptors. However, due to causing thymic tumours in mice, it never had any widespread clinical use. Instead, an analogue, propranolol (**1.26** (β_1 log K_D = -8.2), was found to have a similar pharmacological profile, but was safer.[84] In the clinic, propranolol was found to successfully reduce the frequency of angina attacks, and later was found to be useful in the treatment of hypertension and arrhythmias.[85]

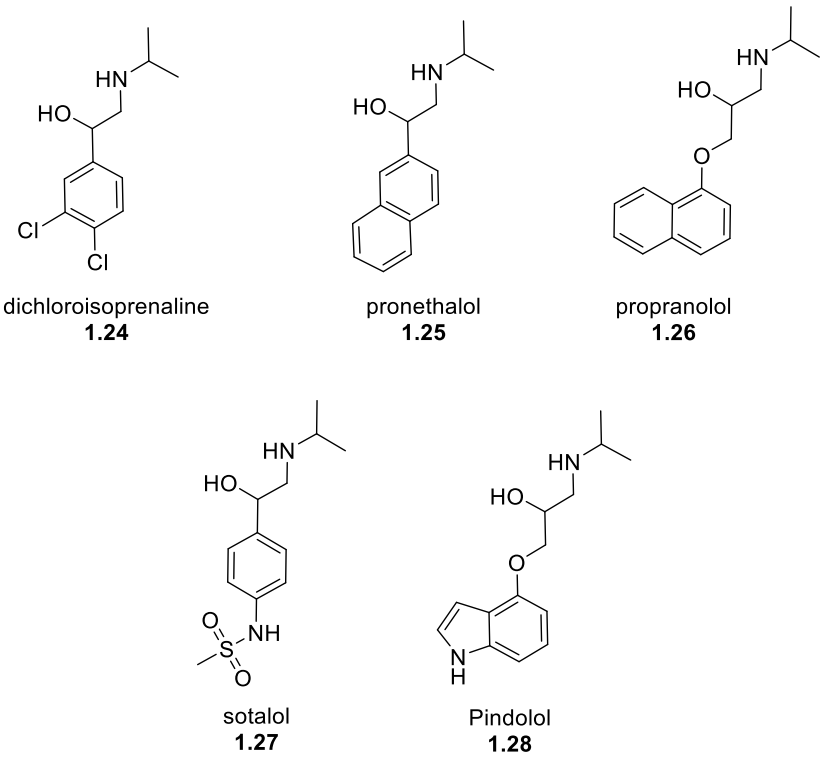


Fig 1.14: First generation β_1AR antagonists

A number of similar compounds followed the discovery of propranolol, including sotalol (**1.27** (β_1 log K_d = -5.8)) and pindolol (**1.28** (β_1 log K_d = -8.6).[50-51] Each of these had varying pharmacological properties. Pindolol, for example, has a higher affinity for the βARs than propranolol, but causes intrinsic sympathomimetic activity – it partially activates the βARs in the absence of the endogenous hormones. This is due to the nitrogen atom of the indole core being able to interact with serine-204, which is partly responsible for βAR activation, therefore being a partial agonist.[86] However, it has been shown that partial agonists offer no clinical benefit compared to the use of full antagonists and could be detrimental.[87] consequently, of the 1st generation non-selective β-blockers, propranolol remains one of the most widely used in the treatment of a number of heart conditions.

1.5.2 Second generation β_1AR antagonists

As stated above, many patients suffering from conditions such as heart disease are also diagnosed with COPD. This makes treatment with first generation β-blockers

difficult, they have no selectivity for β_1 over the β_2AR, so they can inhibit β_2ARs in the lungs and induce bronchospasm.[88] The second generation of β-blockers therefore have higher affinity to the β_1AR and these drugs include metoprolol (**1.29** (β_1 log K_d = -7.3)), atenolol (**1.30** (β_1 log K_d = -6.7)) and bisoprolol (**1.31** (β_1 log K_d = -7.8)). Each of these compounds was based on practolol (**1.32** (β_1 log K_d = -6.1)), which was the first compound to exhibit levels of β_1AR selectivity. The drug itself was withdrawn due to side effects caused by an immune reaction to the drug.[89] Each drug in this class, unlike the first generation compounds, has a substitution, *para*- to the phenyl ether. This was found to be important for β_1AR selectivity.[90]

However, this class of compounds is not absolutely selective to the β_1AR, with the relative affinities being 2.3-, 4.7- and 13.5-fold greater for β_1 than β_2, for metoprolol, atenolol and bisprolol, respectively.[50] Due to relatively low levels of selectivity, bronchospasm side effects can still be observed, particularly at higher doses.[91]

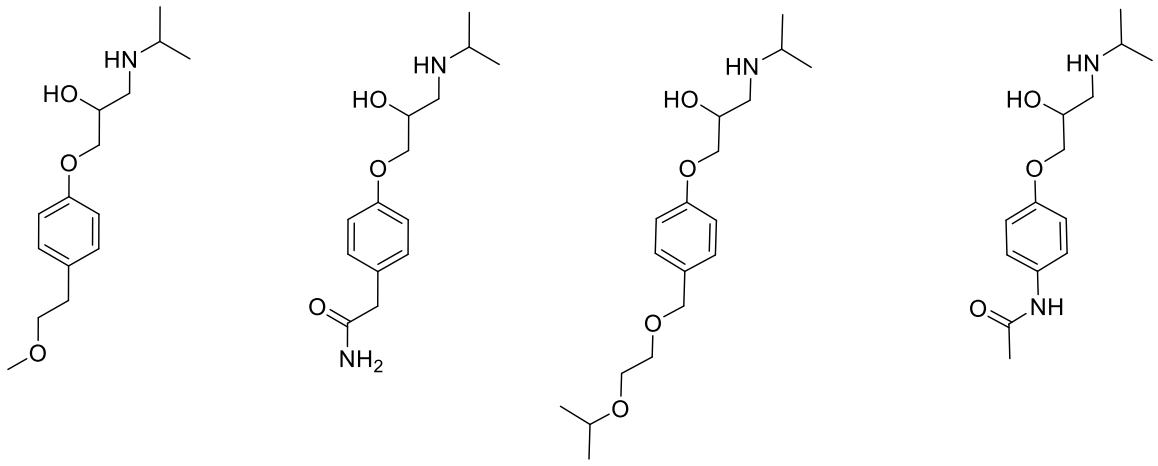
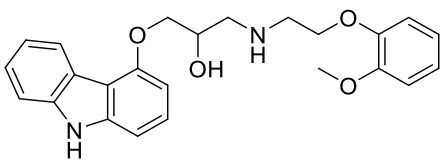


Fig 1.15: second generation β_1AR antagonists

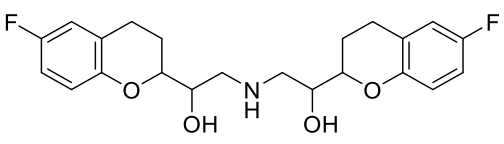
1.5.3 Third generation β_1AR antagonists

The development of third generation β-blockers focused on obtaining vasodilation, in addition to inhibiting the β_1AR. This was achieved in a number of compounds, although the proposed mechanism of how vasodilation is attained, differs between each compound.[92] The need to induce vasodilation in this class of drugs is that it reduces cardiac work and myocardial oxygen demand. This is beneficial for patients suffering from hypertension, an affliction that when treated with β-blocker mono-therapy, only gives 30-60% of patients adequate blood pressure control.[93-94]

Two examples of this class of drug are carvedilol (**1.33** (β_1 log K_D = -8.8)) and nebivolol (**1.34** (β_1 log K_D = -9.1)).[50-51] Carvedilol is a non-selective β-blocker, as it inhibits both β_1 and β_2ARs, as well as the α_1AR. Its affinity for α_1 is how vasodilation is attained and consequently, it has been reported that carvedilol can be effective at treating patients with hypertension.[95] Nevibolol has a different pharmacological profile to carvedilol, it is reported to be the most selective marketed β-blocker, having a 321-fold higher affinity for the β_1AR than β_2AR.[96] furthermore, nevibolol is unique in its mechanism of how it mediates vasodilation. It stimulates endothelial nitric oxide synthase and as a result increases nitric oxide, which can inhibit smooth muscle contraction.[97] Nebivolol is reported to be as effective as other β-blockers at reducing blood pressure and has shown clinical efficacy for treating hypertension. Preliminary trials have also reported that it reduces rates of mortality in heart failure.[98-99]



carvedilol
1.33

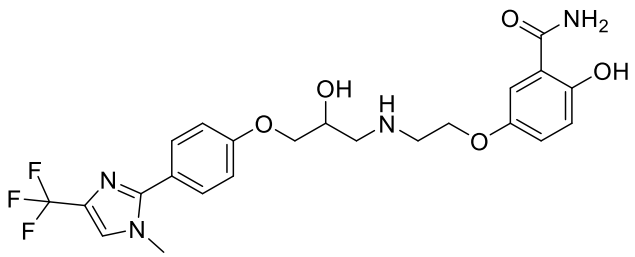


nebivolol
1.34

Fig 1.16: third generation β_1AR antagonists

1.5.4 CGP 20712A

CGP 20712A (**1.35** (β_1 log K_D = -8.8)) has higher affinity for the β_1AR than propranolol and is the most selective of the reported β_1AR antagonists, having a 501-fold greater affinity for the β_1AR than the β_2AR.[50] Though the compound is not used clinically, it has been used extensively in the research of the βARs.[100] CGP 20712A was discovered by Ciba-Geigy in 1985, but despite having unprecedented selective towards the β_1AR, no study has been conducted into the SAR of the compound.[101] Similarly to second generation β-blockers, CGP 20712A has a substitution *para*- to the phenyl ether, which is known to be important for β_1AR selectivity.[90] However, the trifluoromethyl imidazole moiety is not seen in any other compounds of this class. Furthermore, second generation compounds tended to have relatively small isopropyl or *tert*-butyl amino groups, whereas CGP 20712A has a substituted phenyl group attached to the amine by an ethyl linker. Due to modifications on both sides of the compound, in comparison to other selective β-blockers, it is unclear what could be causing the high level of selectivity and greater β_1AR affinity.



CGP 20712A
1.35

Fig 1.17: Highly selective β_1AR antagonist CGP 20712A

1.6 Research aim

It is apparent from the literature that the majority of research conducted in the field of β_2AR agonists has mainly been concerned with the development of compounds with increased duration of action. While this met an important clinical need, relatively little has been done to develop efficacy selective β_2AR agonists; a

compound of this nature would provide bronchodilation through β_2AR activation, but not activate β_1ARs which causes undesirable side effects. It was therefore postulated, that combination of a β_2AR agonist pharmacophore with a β_1AR antagonist pharmacophore in a single molecule could result in an efficacy selective β_2AR agonist (Fig 1.18).

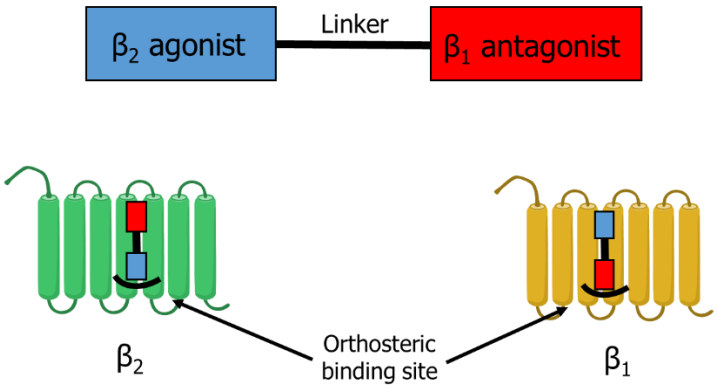


Fig 1.18: schematic of β_2AR agonist-β_1AR antagonist. At the β_2AR, the agonist pharmacophore binds to the orthosteric binding site, eliciting a response. At the β_1AR, the antagonist pharmacophore binds to the orthosteric binding site, inhibiting response.

There is limited understanding into the structure-activity relationship (SAR) of β_2AR agonists in terms of what moieties can give rise to efficacy at the β_2AR but not β_1AR. Therefore, the design and synthesis of a range of analogues based on the β_2AR agonist, S1319 (**1.10**) are performed, followed by an exploration of the SAR of CGP 20712A (**1.35**), a highly selective β_1AR antagonist.

An increased understanding of the SAR of both β_2AR agonists and CGP 20712A could give information on how to incorporate efficacy selectivity into a β_2AR agonist and affinity selectivity into a β_1AR antagonist. This in turn may facilitate the rational design of a bivalent β_2AR agonist–β_1AR antagonist.

2. Pharmacology

To gain initial understanding of the pharmacological profile of compounds it is essential to first carry out *in vitro* pharmacological evaluation, this gives an indication of how they may interact with specific receptors *in vivo*. Within this project, each compound was evaluated by three parameters: the affinity (K_d), which is the concentration required for a ligand to bind to 50% of receptors, the percentage response, which is the percentage maximum effect of a drug when compared to a full agonist, and the potency (EC_{50}) which is the concentration of ligand required to give 50% of the ligand's maximum response.[102]

This data gives insight into the function of each compound at a certain receptor. Assuming a ligand binds to a receptor, the percentage response and potency provide information on whether the compound is an agonist or antagonist. The binding of an agonist stabilises the receptor in such a way that it activates downstream pathways. An agonist can then be subdivided into a full agonist, where a ligand can elicit the maximum response, a partial agonist, which is a ligand that can activate a receptor but not evoke the maximum response or an inverse agonist which exerts the opposite effect of an agonist.[103]

Conversely, an antagonist binds to a receptor, but does not stimulate a response. Furthermore an antagonist can be competitive, where it binds to the orthosteric site and can block the binding of other ligands (such as endogenous agonists), or non-competitive, where it would bind to an allosteric site, altering the conformation of the receptor, so that other ligands cannot bind to the orthosteric site.[104]

2.1 SPAP functional assay

In order to obtain the potency and percentage of isoprenaline response of compounds, a CRE-SPAP (cAMP response element-secreted placental alkaline phosphatase) reporter gene assay was carried out. In this assay, Chinese hamster

ovary (CHO)-K1 cells were transfected with human β_1 or β_2ARs and a CRE-SPAP reporter gene. When an agonist binds to the β_1 or β_2AR, the receptor stabilises a conformation which allows the G Protein α-subunit to bind to adenylate cyclase. This in turn catalyses the conversion of ATP into cAMP.[14] The increase of cAMP activates protein kinase A (PKA) which moves into the nucleus and phosphorylates cAMP response element-binding protein (CREB). The phosphorylated CREB then binds to CRE in the promoter of the gene, thus increasing transcription of SPAP (Fig 2.1). Following SPAP production, the cells are heated, which decomposes all of the enzymes within the cell apart from SPAP, which has a high thermal stability. *p*-Nitrophenol (pNPP) is then added to the samples. SPAP catalyses the hydrolysis of pNPP which causes a colour change which can be measured by colorimetry.[105] From this data, the levels of SPAP production can be deduced, and from this, a ligand's potency and percentage response.

The benefit of using this reporter system is that due to the thermal stability of SPAP, it is easy to measure the levels of secretion. This assay also offers a cheap and safe alternative to radioisotope assays. However, the CRE-SPAP assay is a well coupled system and so a high degree of amplification is observed.[106] Therefore, to further validate any results, a second assay with lower levels of receptor reserve should be perfomed, such as a cAMP assay. Additionally, endogenous activity within the cell can interfere with SPAP production.[107] A countermeasure for this is that when performing the assay, some well are left without the addition of ligand. The response generated by endogenous activity is the 'basel' activity. This level of activity is removed from the observed activity generated by ligands.

The maximum concentration of compound that can be used within this assay is 10 μM, therefore, compounds which are active but do not reach maximal response at this concentration will have a Log EC_{50} value defined as >-5. For these compounds the percentage isoprenaline response will be calculated from the maximum response at 10 μM. Furthermore, both β_1 or β_2AR cell lines were used in this study, these cell lines have different levels of receptor expression and effector coupling efficiencies, therefore direct comparison between the cell lines cannot be done.[51]

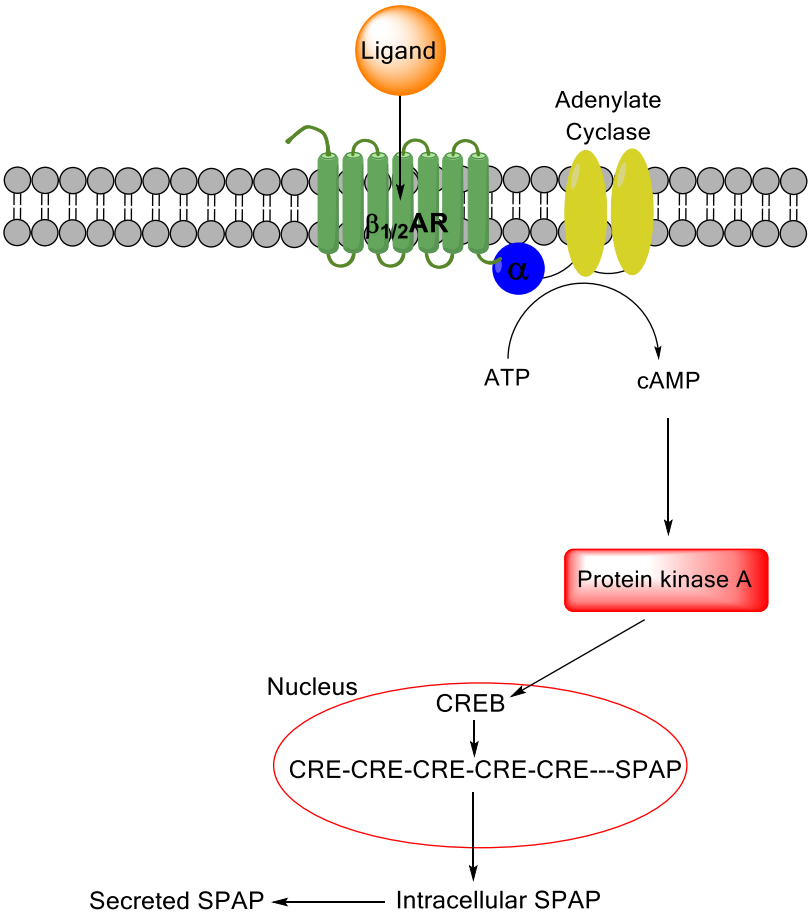


Fig 2.1: the binding of a ligand to the β_1AR and β_2AR causes a conformational change of the receptor which allows the G protein α subunit to bind to adenylate cyclase. This catalyses the conversion of ATP into cAMP and the increase of cAMP activates PKA. Activated PKA then moves into the nucleus and phosphorylates CREB, which binds to CRE in the promoter of reporter gene. As a result the transcription of SPAP is increased.[105]

Before compounds were tested in the assay, the assay first had to be validated. This was done by incubating the cells, transfected with β_1 or β_2ARs, with the β_1AR and β_2AR agonist cimaterol along with the β_2AR selective antagonist, ICI 118551 and β_1AR selective antagonist, CGP 20712A.[50] The results of the validation are presented in table 2.1 and figure 2.2-2.3.

	β_1	n	Literature value	β_2	n	Literature value
Cimaterol Log EC_{50}	-8.78 ± 0.10	12	-8.42 ± 0.08 [51]	9.46 ± 0.14	12	8.94 ± 0.07 [51]
ICI 118551 Log K_d	-7.08 ± 0.21	5	-6.52 ± 0.02 [50]	-9.79 ± 0.10	7	-9.26 ± 0.03 [50]
CGP 20712A Log K_d	-8.97 ± 0.11	4	-8.81± 0.03 [50]	-6.28 ± 0.7	4	-6.11 ± 0.05 [50]

Table 2.1: potency (EC_{50}) of cimaterol and dissociation constant (Log K_d) values of ICI-118551 and CGP-20712A at β_1AR or β_2AR and CRE-SPAP transfected CHO-K1 cells compared against literature values. Values are mean±sem of n separate experiments.

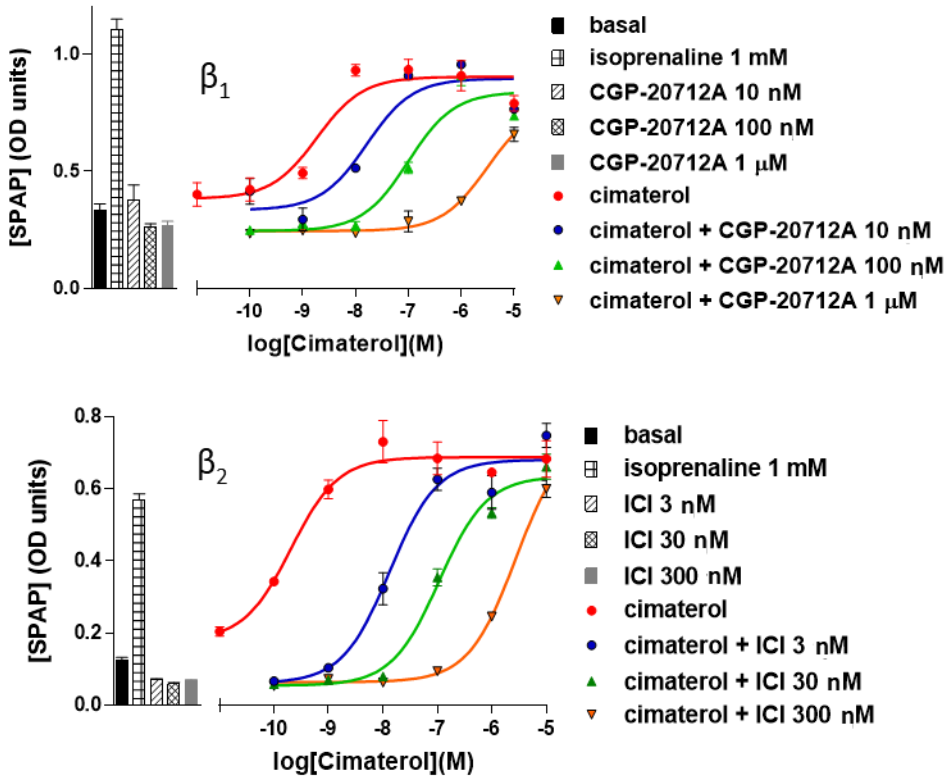


Fig 2.2-2.3: dose-resposnce curve showing activation on β_1AR and β_2AR by the agonist Cimaterol. The response at each receptor is being inhibited by the site-specific antagonists ICI-118551 and CGP-20712A.

The results in table 2.1 shows that the obtained EC_{50} of cimaterol and the K_d values of ICI-118551 and CGP-20712A are close to the literature values. This indicates that the assay is working as expected. In addition to this, the dose-response curves in

Figure 2.2-2.3 illustrate that cimaterol is causing a response in both the β_1AR and β_2AR, and that ICI-118551 and CGP-20712A can inhibit each response. Because ICI-118551 and CGP-20712A are site specific antagonists, inhibition shows that cimaterol is generating a response by activating either the β_1AR and β_2AR, and not at an alternative site in the cells.

2.2 Radioligand binding

To calculate the affinity of ligands, a competition radioligand binding assay was employed. In this assay, receptors are treated with varying concentrations of an unlabelled ligand and then a fixed concentration of a radioactive ligand is added. The assays are then left to reach an equilibrium. If the ligands bind orthosterically with high enough affinity, they can displace the radioligand. Following the incubation, the free radioligands are removed from the samples and the remaining bound radioligand is counted by measuring the ionizing radiation. From these results, the half maximal inhibitory concentration (IC_{50}) can be calculated. With this value, along with the known concentration and K_d of radioligand, the Cheng-Prusoff equation (see Chapter 7) can be applied to calculate the concentration at which the unlabelled ligand is occupying 50% of the receptors.[108] This value is the dissociation constant (K_d), which is the concentration required to bind to half of the receptors and it is how the affinity of a ligand is defined.[109] The highest concentration of compound that can be used in this assay is 100 µM, therefore, when maximum inhibition is not achieved at this concentration, log K_d values will be defined as >-4.

The radioligand binding assay was also validated by testing the known antagonists CGP 20712A and ICI 118551 against the radioligand [^{3}H]-CGP 12177. As shown in table 2.2, the experimental values correspond with literature results, and therefore the assay is functioning as expected.

	Log Kd					
	β_1	n	literature value [85]	β_2	n	literature value [85]
CGP 20712A	-8.09 ± 0.15	3	-8.81 ± 0.03	-5.91 ± 0.37	3	-6.11 ± 0.05
ICI-118551	-6.44 ± 0.05	3	-6.52 ± 0.02	-8.61 ± 0.27	3	-9.26 ± 0.03

Table 2.2: Binding affinities of ICI-118551 and CGP-20712A at the β_1 and β_2 adrenoceptor, compared to literature values. The dissociation constant (log Kd) obtained form ^{3}H-CGP 12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments.

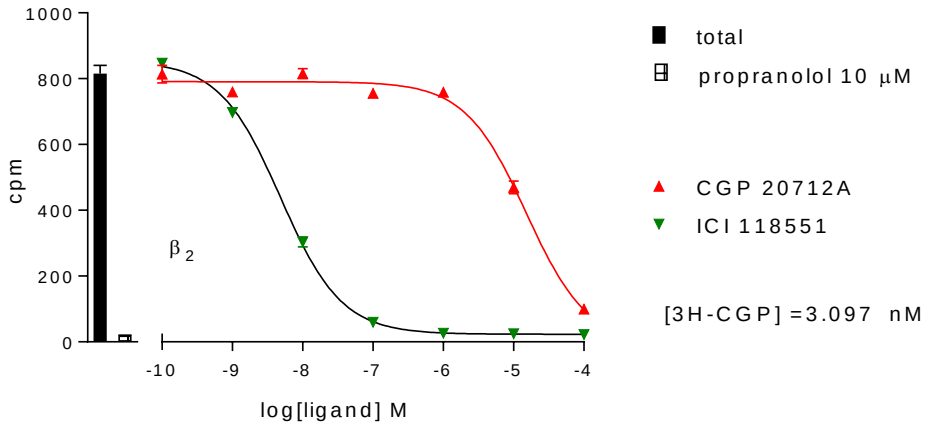


Fig 2.4: radioligand competition binding curve of CGP 20712A and ICI 118551 against the radioligand ^{3}H CGP 12177 at the β_2 adrenoceptor. Ligand concentration *vs* ionizing radiation counts per minute (cpm).

3. Exploration of the SAR of β_2AR agonists

In order to explore the SAR of β_2AR agonists, the design and synthesis of analogues of the β_2AR agonist S1319 (**1.10**) was performed. This compound is reported to be a potent β_2AR agonist and is structurally similar to adrenaline, therefore it will likely occupy the same binding pocket.[40] Additionally, the methyl group on the amine provides a wide scope for derivatization to make analogues. The focus was on making alterations to the amino group, as well as modifications to the benzothiazolone core. The amino group is the main area where alterations to β_2AR agonists are made and has been shown to cause large differences in pharmacological profiles.[110] Although many β_2AR agonists have different core groups, these are typically catechol mimetics, which allows the core structure to interact with Ser-207 and Ser-203/204, as adrenaline does.[111] Alterations to the catechol isostere has not been widely investigated, and therefore modifications were made to the benzothiazolone core structure. These alterations were made in order to explore SAR of β_2AR selectivity in terms of efficacy and affinity.

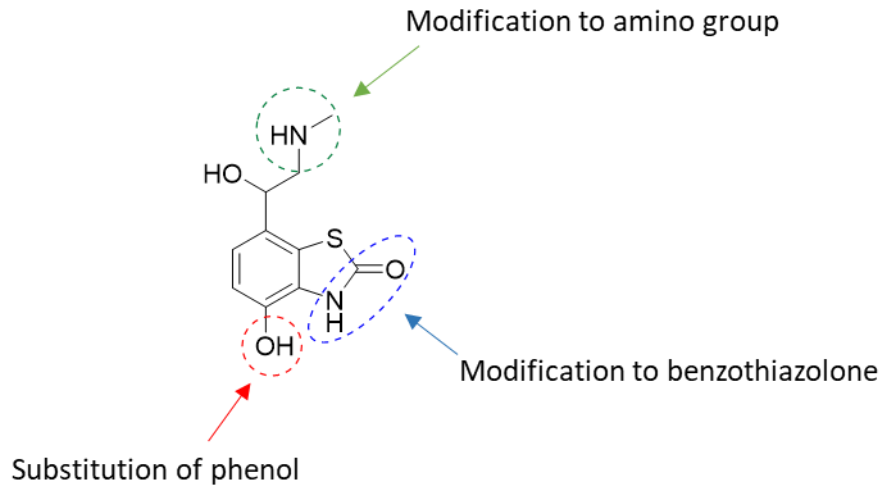
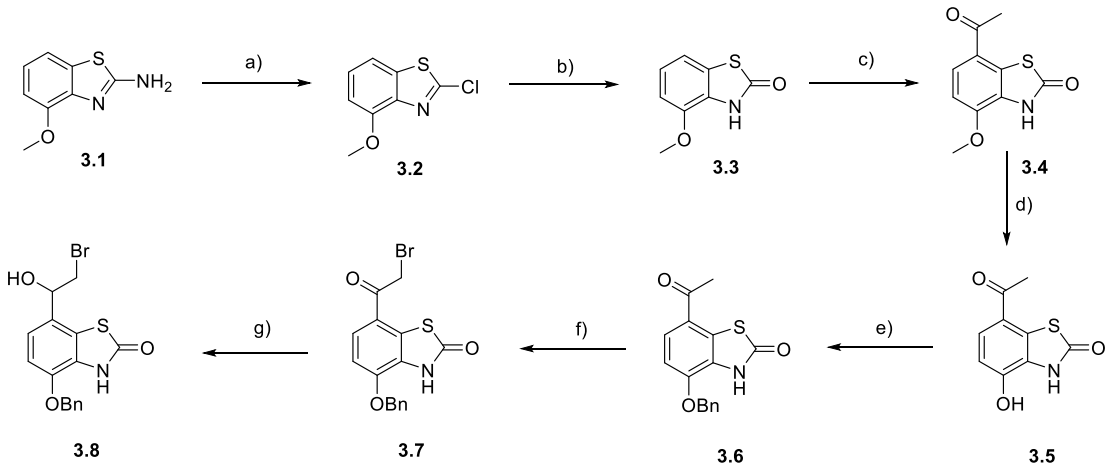


Fig 3.1:areas of investigation around the naturally occurring β_2 adrenoceptor agonist, S1319 (**1.10**)

3.1 Synthesis of S1319 and analogues

3.1.1 Synthesis of S1319 intermediate

To synthesise analogues of S1319 the bromo alcohol intermediate **3.8** was proposed which could be used to make a range of compounds by substituting the bromine with a number of amines. The synthesis of the **3.8** was initially carried out by following a procedure outlined by a group at AstraZeneca (Scheme 3.1).[32]



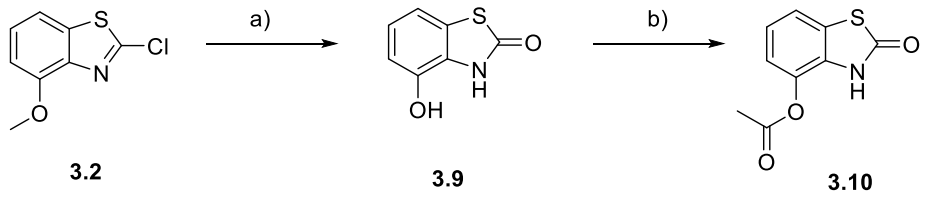
Scheme 3.1: Proposed synthetic route to key intermediate of S1319.

Proposed reagents and conditions: a) sodium nitrate, copper(II) chloride,hydrochloric acid, ethanol, rt. b) Hydrobromic acid (48% in water), reflux. c) aluminium chloride, acetyl chloride, DCM, rt. d) BBr_3, DCM, rt. e) BnBr, K_2CO_3, DMF, 80 °C . f) phenyltrimethylammonium tribromide, THF, 60 °C. g) $NaBH_4$, THF, rt.

The first step in the synthesis of the key intermediate **3.8** was to convert the 2-aminobenzothiazole (**3.1**) to the 2-chlorobenzothiazole (**3.2**) by carrying out a diazotization reaction. A number of literature procedures and optimizations were carried out in this step.[112-113] A mixture of 28% sodium nitrite solution, hydrochloric acid and copper(II) chloride were initially used and variations in reaction times and temperatures were explored. Keeping the reaction cool for the duration and adding the sodium nitrite over 15-60 minutes did not give the desired product. LC-MS indicated there was only starting material and side products. Allowing the reaction to warm to room temperature following addition of sodium nitrite appeared to produce a small amount of compound **3.2**, but with a significant amount of side

products and starting material. Sodium nitrite solution was therefore substituted with *tert*-butyl nitrite and the mixture was heated at 65 ˚C. The addition of *tert*-butyl nitrite over 20 minutes gave the best yield of **3.2** (48%) and minimal formation of side products.

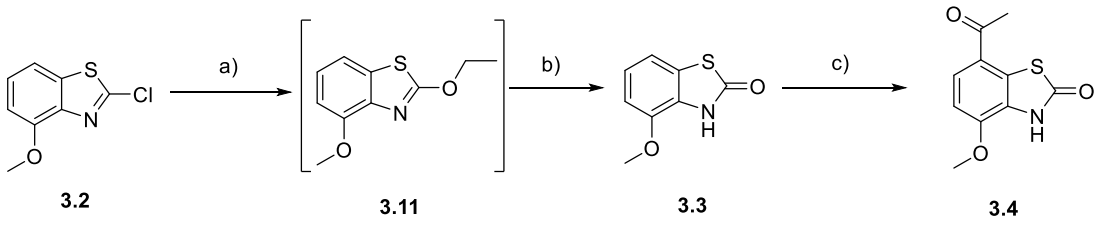
Initially conversion of the 2-chlorobenzothiazole **3.2** into **3.3** was carried out in 48% hydrobromic acid in water.[114] Whilst this successfully formed the benzothiazolone, it also cleaved the methoxy ether. The methyl group was to be removed in a later step so **3.9** was taken forward in the synthesis (scheme 3.2). Unfortunately, the major product of the Friedel-Crafts reaction was the acylated phenol (**3.10**) and increasing the amount of reagents did not result in the required 7-position acylation. The Fries rearrangement, using an acid catalyst such as aluminium chloride was an option, however, this gives a mixture of *ortho*- and *para*- products depending on conditions.[115-116]



Scheme 3.2: Deprotection of methyl ether and subsequent alkylation of phenol from Fridel-Crafts reaction.

Reagents and conditions: a) Hydrobromic acid (48% in water), reflux. b) aluminium chloride, acetyl chloride, DCM, rt.

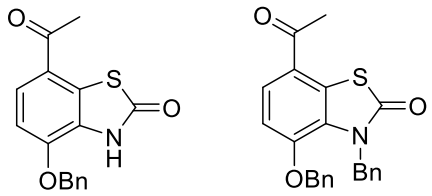
Alternative conditions were utilised which gave **3.3** in good yield. Compound **3.1** was treated with sodium ethoxide to give the 2-ethoxybenzothiazolone intermediate **3.11** which was hydrolysed by heating in 10 M hydrochloric acid to give the benzothiazolone (**3.3**). Subsequent Friedel-Crafts acylation using standard conditions afforded compound **3.4**.[32]



Scheme 3.3: alternative synthetic route to **3.5**

Reagents and conditions: a) sodium ethoxide, ethanol rt. b) conchydrochloric acid, 60 °C, 71% c) aluminium chloride, acetyl chloride, DCM, rt, 65%

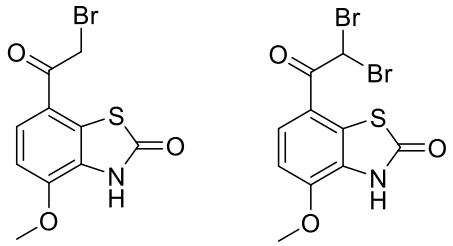
Demethylation of **3.4** was carried out by heating in 48% hydrobromic acid in water to give phenol **3.5**. It was then necessary to reprotect the 4-hydroxy group with a benzyl group which could be removed by hydrogenation in the final step. Initially potassium carbonate was used as the base with 1.5 equivalents of benzyl bromide. However, this resulted in a mixture of **3.6** and **3.12**. This is due to the N-H of the benzothiazolone being moderately acidic (parent pK_a = 11.5), and can therefore be deprotonated by potassium carbonate. A range of conditions were explored including a different base such as triethylamine and varying equivalents of benzyl bromide. The use of trimethylamine and 0.9 equivalents of benzyl bromide alleviated the formation of the di-benzylated product. However, the reaction was very slow and little product was formed after heating overnight. Following the addition of more benzyl bromide and an extra 24 hours of heating, the reaction was worked up. LC-MS and TLC indicated that only a small amount of product had formed and the mixture was primarily unreacted starting material. The reaction was reattempted, however higher yields were not obtained. Protection with a *p*-methoxybenzyl group was also attempted, however this was unsuccessful as none of the desired product was observed. Due to the issues with benzylation, the methyl ether (**3.4**) was carried forward instead as this could also be deprotected later in the synthesis, albeit with harsher conditions.



3.6 **3.12**

Fig 3.2: mono- and *bis*-benzylated benzothiazolone

Bromonation was carried out using the brominating reagent trimethylphenylammonium tribromide. This reaction proceeds by the formation of the reactive enolate form of **3.6**, followed by the electrophilic addition of a bromine. Hydrobromic acid is formed during the reaction which increases the rate at which the enolate is formed, but also results in the formation of the enolate of **3.13** which reacts with a second bromine to form the dibromo product **3.14**.[117] It was not possible to separate these two products. Compound **3.14** could be identified by a singlet peak at 7.92 ppm which corresponds to the α-proton and the ratio of **3.13** to **3.14** were approximately 0.84:0.16. The bromo ketone was then reduced using sodium borohydride, in good yield and following the reduction, the product **3.16** could be separated from the side products of the previous reaction.

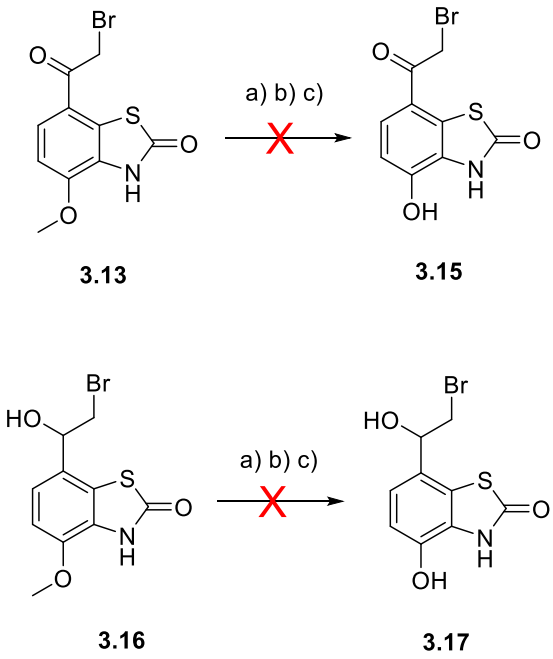


3.13 **3.14**

Fig 3.3: Mono- and *bis*- brominated products of bromination reaction

The deprotection of the methyl ether was attempted on both the bromo ketone **3.13** and bromo alcohol **3.16**. In previous reactions, Hydrobromic acid was used to demethylate the phenol, however the reagent was not used in these reaction due to

the risk of hydrolysis of the bromide. Pyridinium hydrochloride was first used neat, under microwave irradiation, for varying amounts of time and then with the addition of NMP as a solvent. However, from TLC and LC-MS analysis, this produced a number of compounds, none of which were identified as the product. Boron tribromide was also used with both **3.13** and **3.14**. The equivalents of boron tribromide was varied from 4 to 10, but none of these reactions resulted in a significant amount of product by TLC and LC-MS, only starting material and a number of unidentified products.

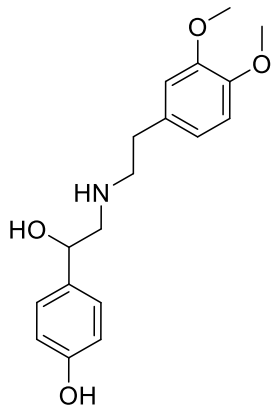


Scheme 3.4: attempted demethylation of **3.11** and **3.13**

Reagents and conditions: a) Hydrobromic acid (48% in water), reflux. b) BBr_3, DCM, rt. c) Py.HCl, NMP, 180 °C.

Due to the issues with being unable to demethylate either **3.13** or **3.16**, the bromo alcohol of **3.16** of was reacted with a number of amines as this would provide analogues with a modified benzothiazolone core and an alternative route could then be taken to synthesise analogues with the phenol. Analogues of S1319 were synthesised with varying amino groups along with a methyl substitution on the phenol. Amino groups were selected for their varying properties. The *tert*-butyl

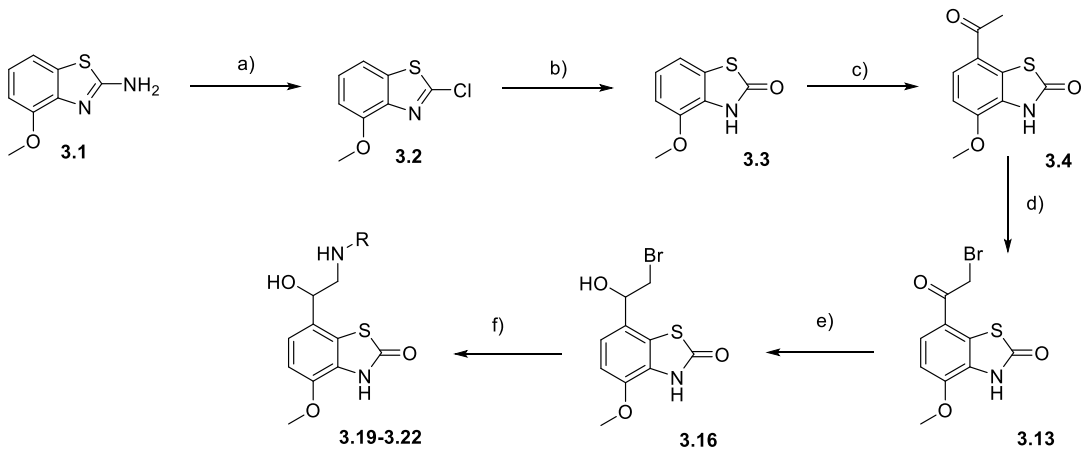
group of **3.19** is known to give high affinity in the βARs and is incorporated in many β_2AR agonists such as salbutamol.[118] The benzylic group of **3.20** was then selected for its ability to form potential π-stacking interactions and the aliphatic chain of **3.21** for its lipophilic properties and similarities to compounds such as salmeterol. Each of these groups with their varying physical properties may therefore be able to interact with different amino acid residues in the βARs. The homovaritylamine moiety of **3.22** is found in the βAR agonist denopamine. Denopamine (**3.18**) is an agonist at both the β_1AR and β_2AR, however it has both affinity and efficacy selectivity towards the β_1AR, it is therefore of interest to see if the amino group is driving this selectivity or if it is due, at least in part, to the headgroup.[51] The nucleophilic substitution of the bromine with each of the amines was carried out under microwave irradiation, giving low yields.



Denopamine

3.18

Fig 3.4: β_1 adrenoceptor selective agonist, denopamine



Scheme 3.5 Synthesis of 4-methoxybenzothiazolone analogues

Reagents and conditions: a) *tert*-butyl nitrite, copper(II) chloride, MeCN, 65 °C, 48%. b) sodium ethoxide, ethanol, 60 °C, hydrochloric acid, 60 °C, 71%. c) Aluminium chloride, acetyl chloride, DCM, rt, 64%. d) phenyltrimethylammonium tribromide, THF, 80 °C, 57%. e) $NaBH_4$, THF, 0 °C, 81%. f) amine, Ethanol, 90 °C, MW, 12 – 31%.

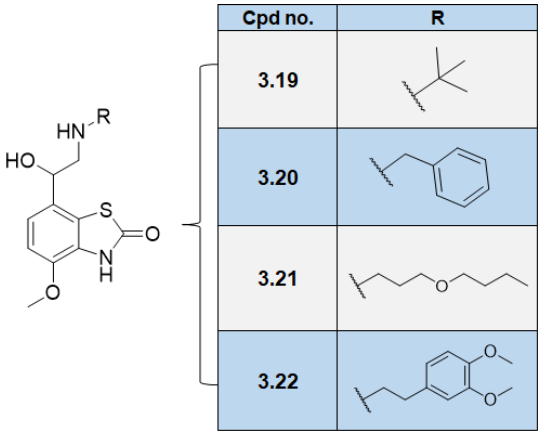


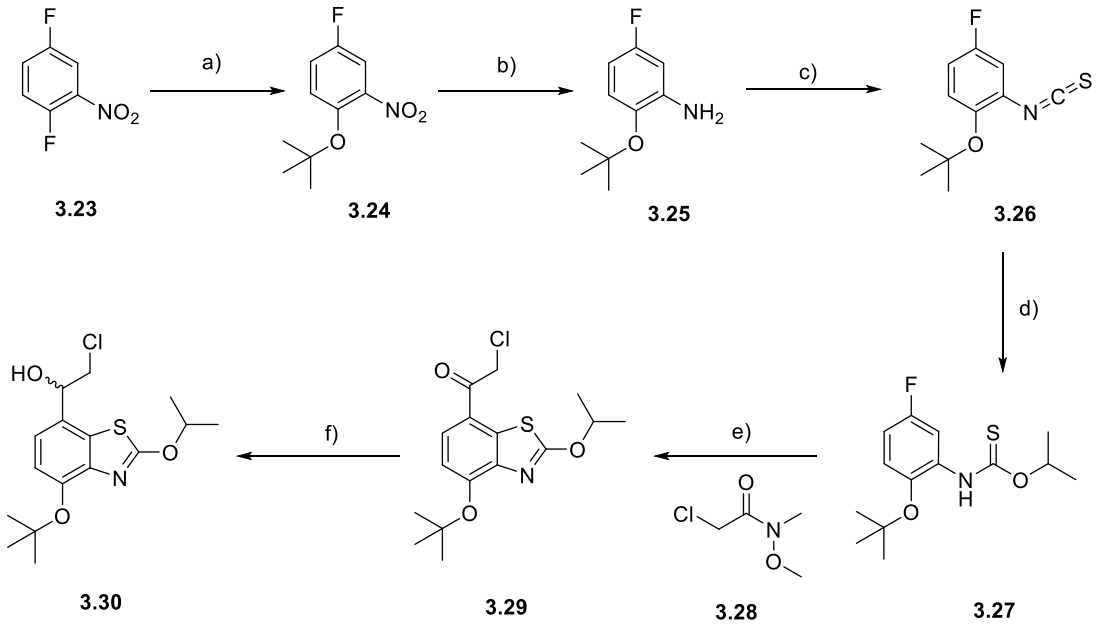
Table 3.1: derivatives of S1319 with *O*-methyl substituted headgroup and various amino groups

3.1.2 Alternative synthesis of S1319 intermediate

Due to the issues with the protection and deprotection steps, an alternative synthetic route to a key intermediate of S1319 was explored. An optimised route, developed by Fairhurst *et al.* was adapted to synthesise the chlorohydrin intermediate **3.30** of S1319.[119] An additional benefit of this synthetic route is that removal of the *tert*-butyl

and isopropyl can be done individually. This leaves the isopropyl group in some of the analogues providing compounds with a modified head group, where the nitrogen of **3.29** may interact differently with serine 203/204, compared to a regular catechol mimetic in the βAR. This interaction is known to be important for catecholamine activation of the receptors (see Chapter 1.1.3).

The first step in the synthesis of the key intermediate **3.30** was to displace the *ortho*-fluorine of **3.23** with potassium *tert*-butoxide by nucleophilic aromatic substitution (S_NAR) to give compound **3.24**. Regioselective substitution of the *ortho*-fluorine was achieved in high yield; this is due to the electron withdrawing effects of the nitro group which directs S_NAr to the 4- and 7-positions of the ring.[120] This was followed by the reduction of the nitro group to an aniline. The reduction was carried out using a palladium on carbon catalyst, under a hydrogen atmosphere, which gives conversion to the aniline in near quantitative yield.



Scheme 3.6: Synthesis of the key intermediate **3.20**

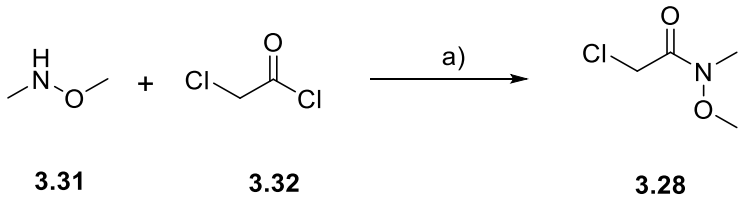
Reagents and conditions: a) potassium *tert*-butoxide, THF, 0 °C – rt, 90%. b) H_2 (balloon), Pt/C, Methanol, rt, 94%. c) carbon disulfide, triethylamine, toluene, ethylchloroformate 0 °C then chloroform, 0 °C – rt, 68%. d) isopropanol, triethylamine, reflux, 89%. e) *tert*-butyllithium, **3.6**, THF, -78 °C - 0 °C, 36%. f) $NaBH_4$, THF, 0 °C – rt, 68%.

The next step in the synthesis was to form the isothiocyanate **3.26** following a procedure by Hodgkins et *al.*[121] The reaction is carried out in two steps, the first of which was to react the aniline **3.25** with carbon disulfide in the presence of triethylamine to form a triethylammonium dithiocarbamate salt. The reaction is carried out in a non-aqueous solvent (toluene) so that the salt can be precipitated out of solution by trituration with petroleum ether. The intermediate salt is then reacted with ethyl chloroformate to afford the desired compound **3.26**. Initially, column chromatography was avoided due to the risk of the isothiocyanate decomposing on the silica gel. ^{1}H NMR indicated that the only impurity was residual ethyl chloroformate, and so the product was at first taken forward without further purification. However, problems arose in sequential steps when a side product formed that was difficult to separate by column chromatography. As a result, during subsequent synthesis of the isothiocyanate **3.26**, column chromatography was used to purify the product, without significant decomposition.

A literature procedure was followed to form the thiocarbamate **3.27**.[122] Isothiocyanate **3.26** was refluxed in isopropanol with trimethylamine. After 24 hours, TLC analysis indicated consumption of starting material, and subsequent work up and purification by column chromatography afforded the product as a yellow solid. ^{1}H NMR indicated that the product was pure, however washing the solid with ice-cold methanol, decolourised it, leaving it as a white solid.

The next reaction, which involves the cyclisation of the thiocarbamate **3.27** to form the benzothiazole **3.29**, is quenched with the Weinreb amide **3.28** which was also synthesised.

To synthesise the Weinreb amide **3.28**, a literature procedure was followed in which Schotten–Baumann type reaction conditions are use.[123] The reaction is carried out in a biphasic system of water and dichloromethane (DCM). This is to prevent the protonation of the amine **3.31** when acid is formed in the reaction. The starting materials, **3.31** and **3.32** stays within the organic phase while the base, which neutralises the acid, remains in the aqueous phase.

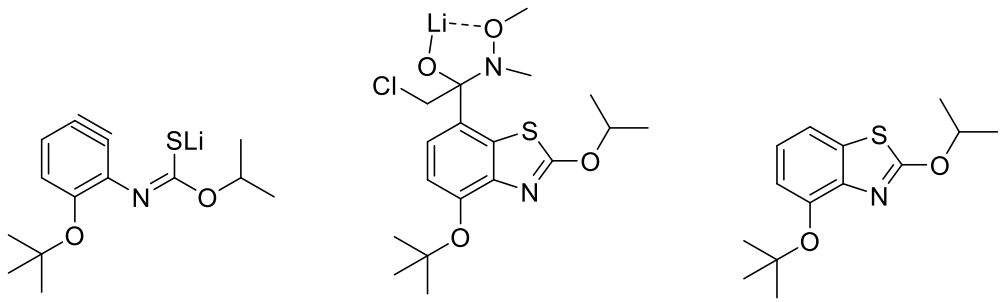


3.31 **3.32** **3.28**

Scheme 3.7. Synthesis of Weinreb amide **3.26**.

Reagents and conditions: i) chloroacetyl chloride, *N,O*-dimethylhydroxylamine hydrochloride, potassium carbonate, DCM, water, 0 °C – rt,, 84%.

The thiocarbamate **3.27** was cyclised to form the benzothiazole **3.28** following a procedure optimised by Fairhurst *et al*.[119] The first step of the reaction involves the addition of 2.8 equivalents of *tert*-butyllithium. The base first deprotonates the thioamide (parent pK_a = 8.1) and then deprotonates the 6-position of the ring to generate the benzyne intermediate **3.33**. The *tert*-butyl group placed on the ring in the first step of the synthesis acts as a steric block that precludes deprotonation at the 3-position, and therefore stops the formation of unwanted side products upon quenching with **3.28**.[119] Following the generation of the benzyne intermediate, the Weinreb amide **3.28** is added which quenches the intermediate lithium anion, to give the product **3.29**. It is important that the Weinreb amide of chloroacetic acid is used in the reaction as it prevents the formation of any unwanted side products resulting from di-addition. Upon acylation of the ring, the tetrahedral intermediate **3.34** is formed which is stabilised by the chelation of the methoxy group with the lithium ion.[124] Due to the high stability of the intermediate, over-addition products are not observed. The tetrahedral intermediate is then decomposed in a mild acid work-up, forming the ketone of the desired product **3.29**.



3.33
Benzyne intermediate

3.34
Tetrahedral intermediate

3.35
Side product

Fig 3.5: benzyne and tetrahedral intermediates formed during benzothiazolone cyclisation reaction and the side product obtained when the benzyne intermediate is quenched with water.

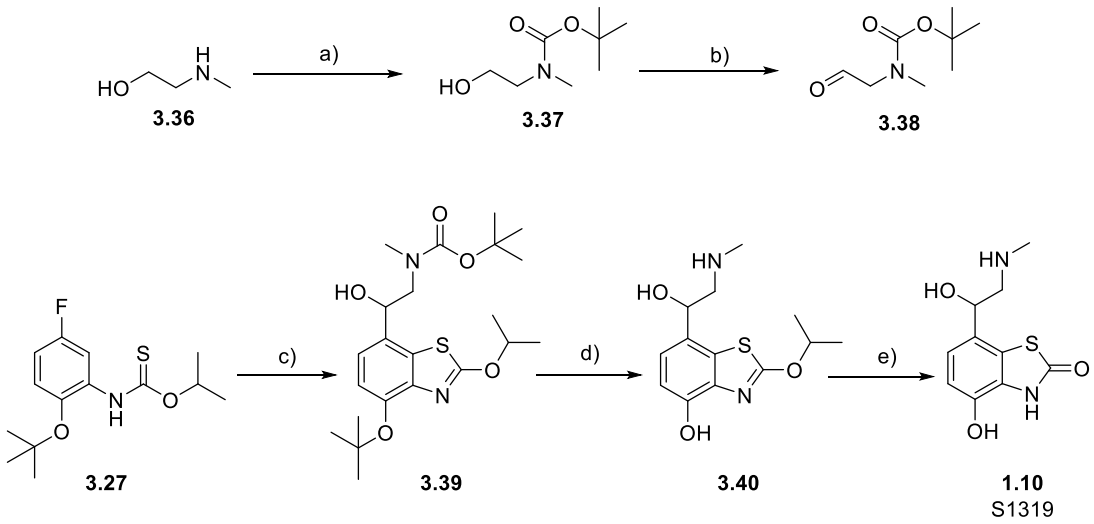
Unfortunately, a relatively low yield of 36% was obtained. This is partly due to the formation of the side product **3.35** which is formed if the benzyne intermediate is quenched with water rather than the Weinreb amide. Although the tetrahydrofuran (THF) was purchased as dry, it was further dried using molecular sieves to make sure as much water is removed as possible in subsequent reactions.[125] The formation of the side product is not only a problem due to a loss of yield but it is also difficult separate from the product **3.29** by column chromatography due to having a similar retention factor (R_f). There is an additional issue with this reaction in that the temperature is altered throughout the reaction, including cooling to -78 °C. As a consequence, the nature of the reaction limits the scale at which the reaction can be carried out. Furthermore, the reaction is quite low yielding and so only a relatively small quantity of the benzothiazole (**3.29**) can be synthesised in one batch.

The final step in synthesis of the key intermediate of S1319 is to reduce the chloro ketone **3.29** to the chlorohydrin **3.30**. Literature procedure was followed, using sodium borohydride as the reducing agent.[126] Initially methanol was used as the solvent, but due to a lack of solubility of **3.29**, reduction did not occur. THF was therefore used as an alternative solvent and the chlorohydrin product was obtained. This reduction forms a chiral centre and therefore two enantiomers were obtained. Resolution of the enantiomers was not attempted at this stage and compounds were ultimately tested as a racemic mixture.

3.1.3 Synthesis of the parent compound S1319

The parent compound S1319 (**1.10**), which was not commercially available was also synthesised to validate the literature results in our own pharmacological assays. The synthesis of S1319 (**1.10**) follows the same route as the key intermediate up to the formation of the thiocarbamate (**3.27**). The cyclisation to form the benzothiazole of **3.39** is also formed *via* the benzyne intermediate **3.33**, but the benzyne is quenched by amino aldehyde **3.38**, rather than the Weinreb amide (**3.28**).

The synthesis of the amino aldehyde (**3.38**) involved the boc-protection of **3.36** and then a Parikh-Doering oxidation, which uses sulfur trioxide pyridine complex and DMSO.[126-127] However ^{1}H NMR indicated that this method failed to oxidise the alcohol. As an alternative, a Swern oxidation was carried out which afforded the oxidised product **3.39** in moderate yield.



Scheme 3.8. Synthesis of the parent compound **1.10**.

Reagents and conditions: a) Di-*tert*-butyl dicarbonate, chloroform, rt, 93%. b) oxalyl chloride, DMSO, triethylamine, DCM, -78 °C - 0 °C, 62%. c) *tert*-butyllithium, **3.35**, THF, -78 °C - 0 °C, 55%. d) TFA, rt, 92% e) formic acid, rt, 36%.

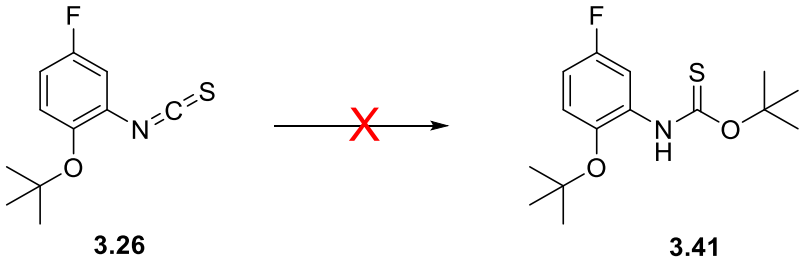
The cyclization of **3.27** into **3.39** uses the same conditions as carried out previously to form the S1319 intermediate **3.30**, However the solvent (THF) was dried over molecular sieves prior to the reaction. As a result, there was no formation of the side

product **3.35** which increased yield compared to the previous analogous reaction and made purification of the product by column chromatography less challenging.

The final steps in the synthetic route to S1319 (**1.10**) were to remove the three protecting groups. The BOCand *tert*-butyl groups could be removed at the same time by stirring **3.39** in trifluoroacetic acid (TFA) which provides deprotection in high yield within 1 hour.[119] Due to the zwitterionic nature of **3.40**, column chromatography was not viable and so C_{18} reverse phase column chromatography was employed to purify the product. As well as the S1319, compound **3.40** was also tested for its biological activity to see if such a modification to the benzothiazolone core would affect activity.

Removal of the isopropyl group was more problematic than the BOCand *tert*-butyl groups. Initially the compound was stirred in TFA at 50 °C but this gave unsatisfactory results.[40] Deprotection was therefore attempted with other acids including hydrochloric and formic acid.[53, 128] The use of formic acid gave the best results, though the reaction takes two days to complete and required reverse phase column chromatography. This gave a moderately low yield of 36%, although this is comparable to the literature yield of 49%.[119]

Due to the problematic nature of removing the isopropyl protecting group and the relative ease of removing a *tert*-butyl group, the replacement of the isopropyl with a *tert*-butyl group was attempted. This was done with the view that both protecting groups could be removed using the same condition. Various reaction conditions were tested to add a *tert*-butoxide into an isothiocyanate. Heating a mixture of **3.26** with potassium *tert*-butoxide with *tert*-butanol as a solvent produced none of the desired product **3.41**. Following this, the same reaction was carried out but at reflux and in the presence of triethylamine. However, this was again unsuccessful. A possible reason for lack of reaction is steric effects, as *tert*-butanol is very bulky. If the reaction is attempted again, a less bulky, high boiling point solvent could be used such as dimethylformamide (DMF).

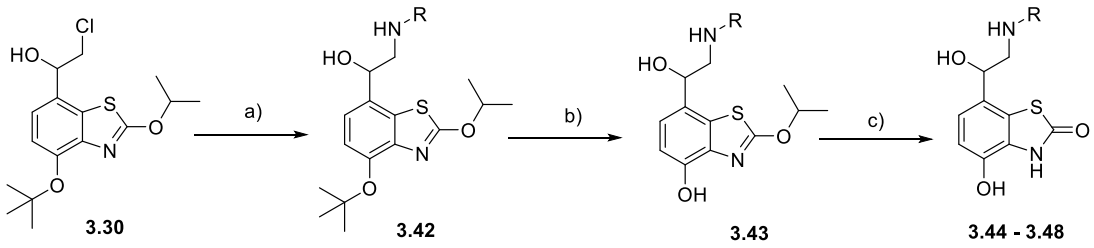


Scheme 3.9. Attempted synthesis of *tert*-butyl thiocarbamate **3.28**.

Reagents and conditions: *tert*-butoxide, *tert*-butanol, TEA, 40 °C

3.1.4 Synthesis of S1319 Analogues

To synthesise the analogues of S1319, a number of amines were used to displace the chlorine of **3.30**. In the literature, the chlorohydrin is first converted to the epoxide, however it should be possible to displace the chlorine directly, which avoids the potential formation of the unwanted benzylic-substituted regioisomer.[53] The amines used to displace the chlorine, were the same derivatives used in previous analogues (see section 3.3.1) for their varying properties.



Scheme 3.10. Synthesis of the S1319 analogues.

Reagents and conditions: a) amine, DMF, 120 °C, 20% / amine, Ethanol, reflux, 50-89%. b) TFA, rt, 33 – 98%. c) formic acid, rt, 26 – 64%.

The first amine used was 3-butoxypropylamine. A range of solvents were tested to carry out the reaction. No reaction occurred when using THF and ethyl acetate so the higher boiling point and more polar DMF was tested at 120 °C. The reaction went to completion, albeit slowly over two days and in low yield. As with the parent compound, protecting groups where removed with TFA and formic acid and the products were purified by HPLC. Due to the low yield obtained from using DMF,

ethanol was used instead for the reaction with other amines. The use of ethanol reduced the reaction time to 16 hours and products were obtained in significantly higher yield.

3.2 Pharmacology of S1319 analogues

CRE-SPAP functional assays and radioligand binding assays were used to determine the pharmacological profiles of each of the compounds at the β_1 and β_2ARs. In this chapter the pharmacological analysis was carried out by the author and Prof. Jillian Baker (Institute of Cell Signalling, University of Nottingham).

3.2.1 Radioligand binding of S1319 analogues

As reported in the literature, the parent compound S1319 (**1.10**) binds to both β_1 and β_2ARs and has selectivity towards the β_2AR.[40] The analogues of **1.10** which share the same benzothiazolone core (**3.45**-**3.48**) also all have the ability to bind to both receptors with varying degrees of affinity. The highest affinity compound is **3.45** which has almost 100-fold higher affinity for the β_2AR than S1319. It is unsurprising however that this compound would have high affinity as it has the *tert*-butyl amino group which is known to bind well within the adrenoceptors.[118] Compounds **3.46**-**3.48** all have very similar affinity to the β_2AR compared to S1319, each of these amino groups therefore appear to have little effect on β_2AR binding.

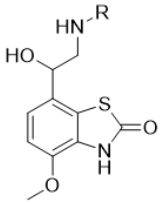
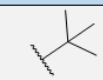
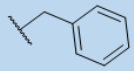
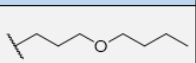
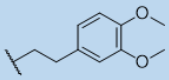
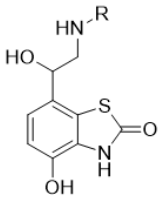
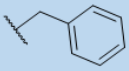
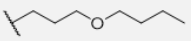
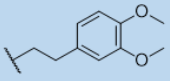
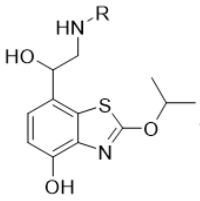
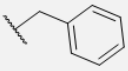
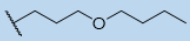
			Log K_d				
	Cpd no.	R	β_1	n	β_2	n	β_2 Fold Selectivity
	3.19		No Binding	4	>-4	4	/
	3.20		No Binding	4	>-4	4	/
	3.21		>-4	4	>-4	4	/
	3.22		>-4	4	>-4	4	/
	3.45		-7.09 ± 0.06	7	-9.25 ± 0.09	7	145
	3.46		-5.73 ± 0.12	6	-7.49 ± 0.10	6	58
	3.47		-6.70 ± 0.06	8	-7.58 ± 0.05	7	7.6
	3.48		-7.50 ± 0.05	5	-7.49 ± 0.10	6	1
	1.10 (S1319)	Me	-5.50 ± 0.06	6	-7.37 ± 0.06	7	74
	3.50		-6.05 ± 0.03	8	-8.00 ± 0.04	8	89
	3.51		-5.06± 0.06	3	-5.95 ± 0.16	7	7.8
	3.52		-5.16 ± 0.13	8	-6.16 ± 0.06	8	10
	3.53	Me	-4.77 ± 0.06	5	-6.33 ± 0.07	8	36

Table 3.2: Binding affinities and β_2AR selectivity of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained from ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem of n separate experiments.

For the compounds **3.45**-**3.48**, there are varying levels of selectivity due to the amino group. The only compounds to increase β_2AR selectivity are the *tert*-butyl compounds (**3.45**, **3.50**), when compared to the parent compound **1.10**. However, the greatest increase is only 2-fold. Each of the other compounds (with the exception of **3.48**) are all β_2AR selective. This suggests that the benzothiazolone headgroup of S1319 is selective to the β_2AR, but an amino group can then tune the level of

selectivity. **3.45** has the same amino group as denopamine, which is slightly selective to the β_1AR. This is the only compound with equivalent levels of affinity for both receptors; this indicates that the amino group does play a role in increasing affinity at the β_1AR as it removes β_2AR selectivity that would be brought about by the headgroup.

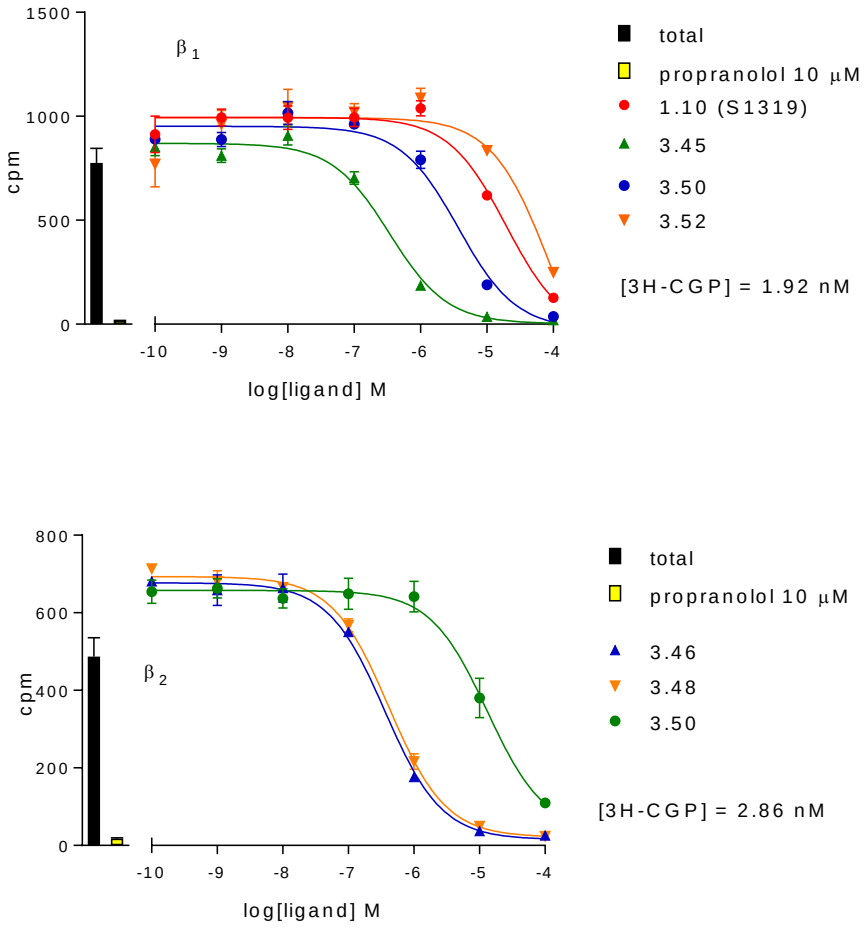


Fig 3.6-3.7: radioligand competition binding curve of compounds against the radioligand ^{3}H CGP 12177 at the β_1 and β_2 adrenoceptors. Ligand concentration *vs* ionizing radiation counts per minute (cpm).

The compounds **3.19**-**3.22**, are each modified with an *O*-methyl group on the headgroup. As a result, all of these compounds have either no affinity, or very low affinity for both receptors, irrespective of the amino group. Alkylation of this phenol removes the compound's ability to form a hydrogen bond with Ser-207.[129] The interaction with this specific amino acid residue is known to be important for catecholamine activation.

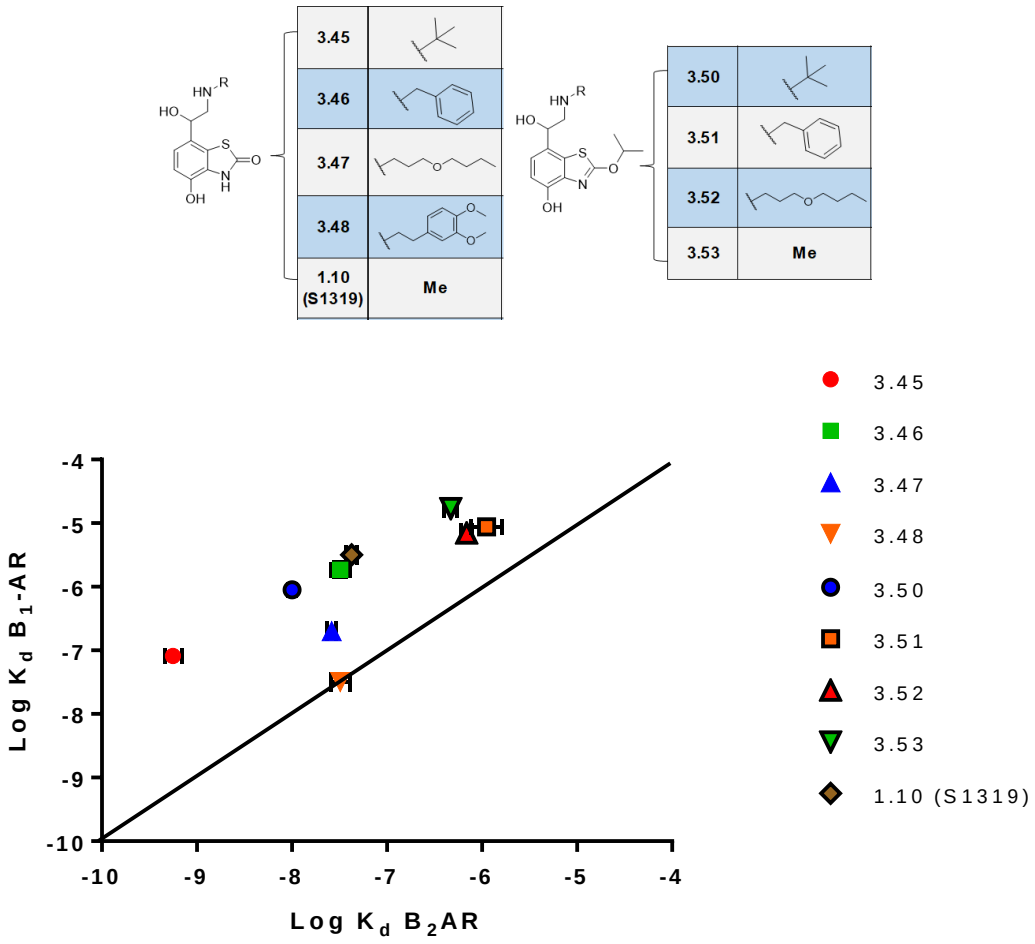


Fig 3.8: graph compares each compounds Log K_d values at the β_1-AR against the Log K_d values at the β_2-AR to identify and selective compounds.

Analogues **3.50**-**3.53** each have an isopropyl group incorporated into their head group. This both removes a proton from the amine which is part of a catechol mimetic and could therefore lead to weaker interactions with Ser-203/204 as it will only be able to act as a hydrogen bond acceptor, rather than both acceptor and donor. Additionally, the isopropyl group adds steric bulk to the compound, which may affect binding. Each of these compounds have reduced affinity at both receptors compared to their amino group analogues (**3.45**-**3.48**), with little change in levels of selectivity. Each compound binds with moderate affinity, so the modification is tolerated by both βARs, but there appears to be no benefit to making this alteration.

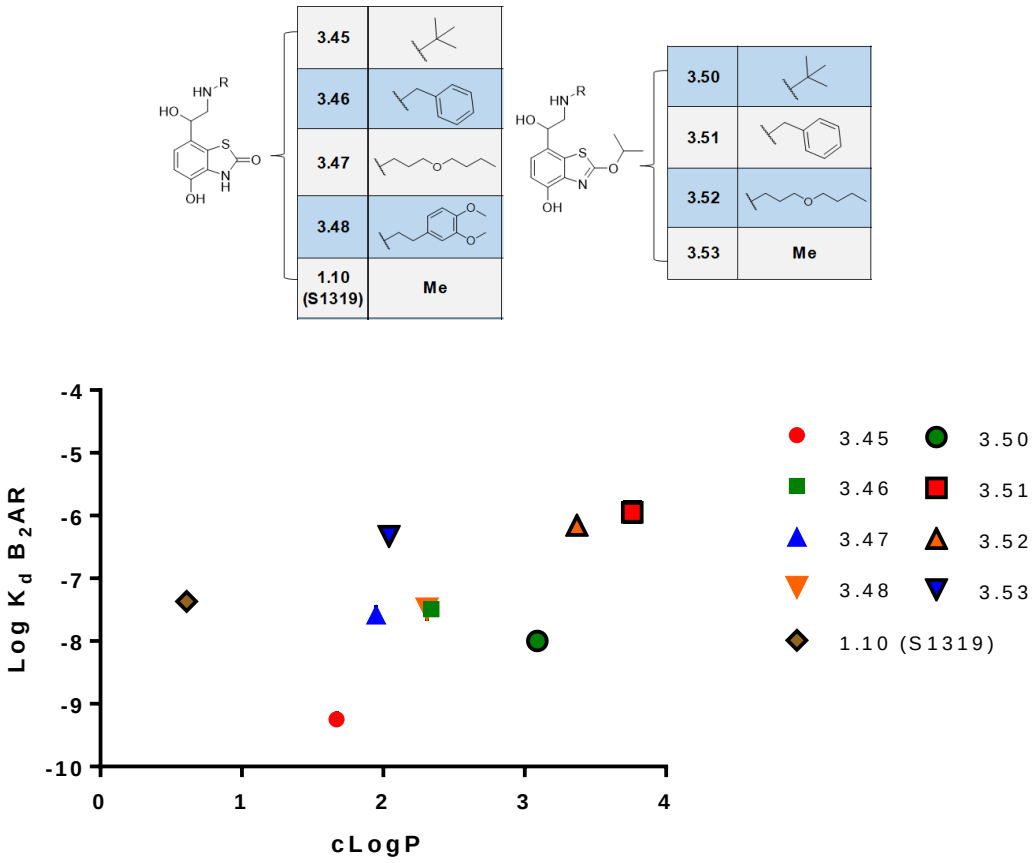


Fig 3.9: the graph compares the Log K_d of each compound at the β_2 adrenoceptor (AR) with the compounds cLogP to see if if there is a correllation between lipophicility and Log K_d.

The affinity of each compound at the β_2AR was also compared to its lipophilicity (cLogP). There appears to be no trend between the lipophilic nature of the compounds and the affinity. Therefore the differences in affinity of this set of compounds can be ascribed to reasons other than increased binding through the hydrophobic effect.

From these results, it would seem that the benzothiazolone headgroup of these compounds has a higher affinity for the β_2 than β_1AR. Of the amino groups studied, despite their varying physical properties, they all have β_2AR selectivity, with the exception of **3.48** (which has equal affinity to both receptors). The only large change observed, in terms of alteration to amino group, is that the *tert*-butyl amino group (**3.45**) significantly increases affinity, compared to the parent compound.

Modifications to the benzothiazolone core offered no benefit to β_2AR selectivity. Affinity to either βAR is almost completely lost when a methyl group is added to the phenol of the head group. The incorporation of an isopropyl group into the headgroup is tolerated by each receptor, but affinity is reduced compared to the parent compounds.

3.2.2 CRE-SPAP functional assay of S1319 analogues

Each of the compounds were tested for their potency (EC_{50}) and percentage response, compared to isoprenaline in both β_1 and β_2ARs. Log EC_{50} values shown as >-4 indicates that compounds can stimulate a response, but the maximum response was generated by concentrations of a compound above 100 μM, which was the highest possible ligand concentration within the CRE-SPAP assay. Additionally, for these compounds, the percentage isoprenaline response value was calculated from the maximum stimulation at 10 μM. The efficacy ratio was also shown, which was calculated by dividing the K_d value by the EC_{50} value for each compound.[51] A higher ratio indicates that less binding was required to reach 50% response of the compound.

Compounds **3.19**-**3.22** which have a methylated phenol have no activity at the β_1AR. At the β_2AR these compounds are observed to be partial agonists, with very low potency, the actual potency is not defined as 50% response was not reached with the concentration of compound that can be used within this assay. This was unsurprising, as these compounds also have very weak affinity at the β_2AR.

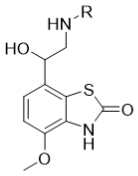
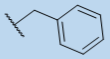
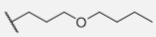
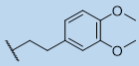
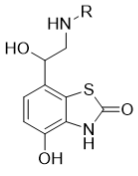
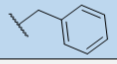
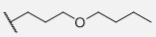
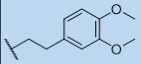
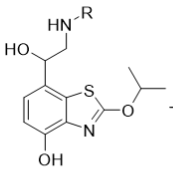
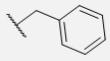
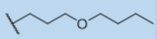
			Log EC_{50}				% isoprenaline	
	Cpd no.	R	β_1	n	β_2	n	β_1	β_2
	3.19		No response	6	>-5	6	No response	62.0 ± 4.2
	3.20		No response	6	>-5	6	No response	89.2 ± 4.9
	3.21		No response	6	>-5	6	No response	29.8 ± 4.9
	3.22		No response	6	>-5	6	No response	22.4 ± 2.4
	3.45		-9.06 ± 0.11	12	-10.02 ± 0.13	12	95.5 ± 3.6	106.6 ± 3.7
	3.46		-7.35 ± 0.18	8	-9.93 ± 0.11	7	96.7 ± 8.1	103.8 ± 4.2
	3.47		-8.16 ± 0.14	13	-9.67 ± 0.11	10	99.0 ± 3.8	101.2 ± 4.8
	3.48		-8.79 ± 0.20	6	-9.50 0.22	7	89.4 ± 2.0	97.6 ± 4.4
	1.10 (S1319)	Me	-6.76 ± 0.14	9	-9.33 ± 0.14	10	93.5 ± 6.8	97.6 ± 3.4
	3.50		-7.88 ± 0.13	12	-8.98 ± 0.10	11	89.3 ± 2.3	98.6 ± 3.6
	3.51		-6.85 ± 0.18	7	-8.53 ± 0.29	8	82.3 ± 7.0	111.2 ± 4.8
	3.52		-6.42 ± 0.10	13	-8.14 ± 0.22	11	92.2 ± 9.7	97.2 ± 3.2
	3.53	Me	-6.09 ± 0.16	11	-8.72 ± 0.14	11	90.6 ± 5.9	94.1 ± 4.0

Table 3.3: log EC_{50} values and % maximum isoprenaline responses of each compound at the β_1 and β_2 adrenoceptor from CRE-SPAP reporter gene assay. Values are mean±sem of n separate experiments.

The parent compound, S1319, was found to be a full agonist at both receptors and is very potent at the β_2AR, having a log EC_{50} value of -9.3. All of the compounds which have the same headgroup as S1319 (**3.45**-**3.48**) are all full agonists at both receptors. The potency of each analogue at the β_2AR remained quite consistent with respect to the parent compound, however, due to differences in affinities, the efficacy ratios do differ, with **3.46** having the highest efficacy ratio at the β_2AR. This means that of this set of compounds, it requires the least receptor occupancy to stimulate 50% response. Compounds **3.47**, **3.48** have a more comparable efficacy ratio compared to the parent compound (**1.10**). The compound with the *tert*-butyl amino group (**3.42**) has the highest potency at the β_2AR within the series, however, it also has the

lowest efficacy ratio, and therefore the high potency is attributed to the high affinity. Conversely, **3.45** has the highest efficacy ratio, at the β_1AR.

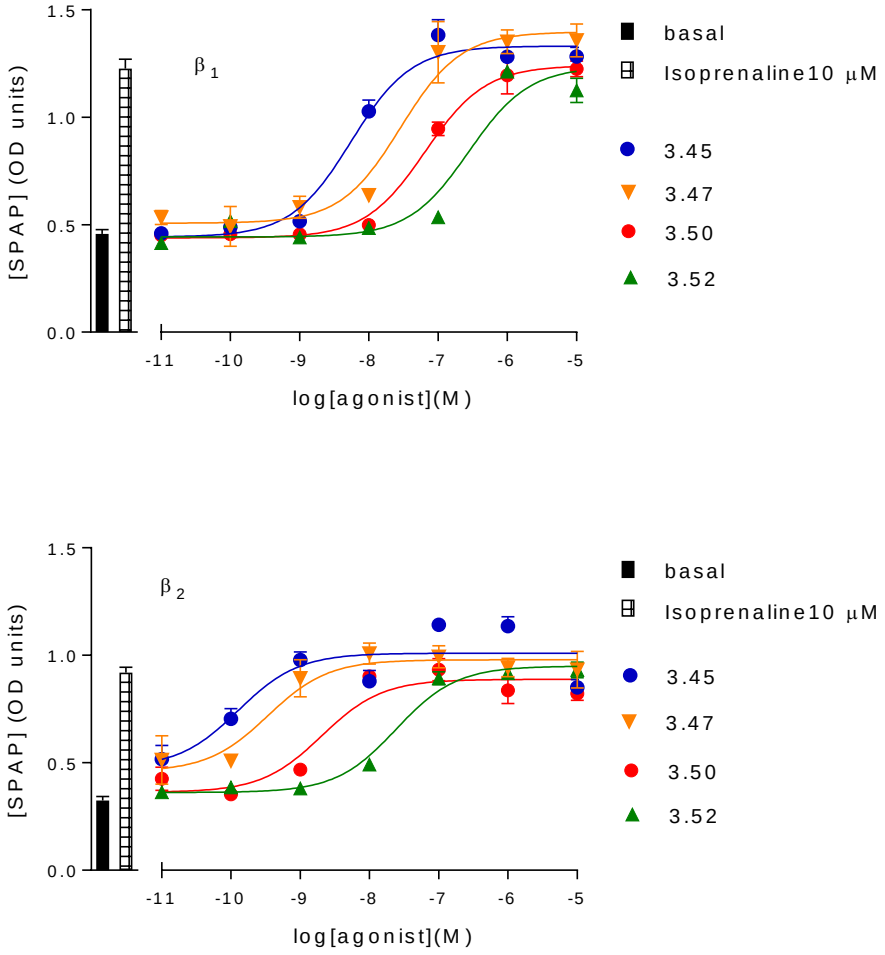


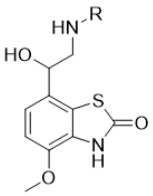
Fig 3.10 – 3.11: dose-response curve showing activation of β_1AR and β_2AR by compounds in table 3.3.

3.3 Summary of β_2AR agonists SAR

The known β_2AR agonist S1319 (**1.10**) was synthesised and evaluated in the CRE-SPAP functional assay and a radioligand binding assay. These studies confirmed the reported pharmacological profile of S1319 that it is a full agonist at the β_1 and β_2ARs, but has affinity selectivity towards the β_2AR.

Analogues of S1319 with structurally diverse amino groups and alterations to the core benzothiazolone were synthesised. An initial synthetic route was performed which led to the formation of a number of analogues. However, this synthetic route posed a number of issues, which meant that the optimization of various steps was required. This was eventually resolved by the application of an entirely different synthetic route. This route led to the synthesis of a number of analogues. However, there was a drawback in that this route gave limited quantiries of final compunds as one of the steps could only be carried out on a limited scale.

Pharmacological analysis of analogues **3.19**-**3.22**, methyl alkylated phenol on the benzothiazolone core had no affinity at either receptor. This was likely due to the methyl ether being unable to form a hydrogen bond with Ser-207, which is important in catechol activation of the βARs.



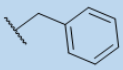
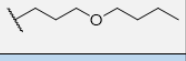
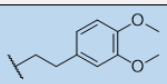
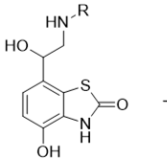
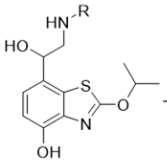
		Log K_d				
Cpd no.	R	β_1	n	β_2	n	β_2 Fold Selectivity
3.19		No Binding	4	>-4	4	/
3.20		No Binding	4	>-4	4	/
3.21		>-4	4	>-4	4	/
3.22		>-4	4	>-4	4	/

Table 3.4: derivatives of S1319 with *O*-methyl substituted headgroup and various amino groups

The compounds with the same benzothiazolone core as the parent compound (**3.45**-**3.48**), were all full agonists and potent at both the β_1 and β_2ARs. All of these compounds with the exception of the *tert*-butyl amino compound (**3.45**) had comparable affinity at the β_2AR to S1319. However, β_1AR affinities were all higher, leading reduced levels of β_2AR selectivity when compared to S1319. Compound **3.45** however, has a significant increase in affinity at both receptors compared to the parent compound and has 140-fold selectivity towards the β_2AR. As these compounds have selectivity towards the β_2AR, this indicates that the

benzothiazolone core has selectivity towards the β_2AR which can be fine-tuned with the amino group. Efficacy can also be altered by the amino group. The *tert*-butyl substituted compound **3.45** was the most potent at the β_2AR, but had the lowest efficacy ratio of the set at the β_2AR. Despite having lower affinity selectivity to the β_2AR, compared to the parent compound, **3.46** had the highest efficacy ratio, therefore requiring the least amount of receptor binding to generate 50% of the maximum response.





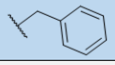
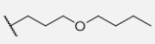
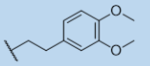
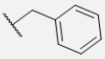
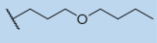
		Log K_d				
Cpd no.	R	β_1	n	β_2	n	β_2 Fold Selectivity
3.45		-7.09 ± 0.06	7	-9.25 ± 0.09	7	145
3.46		-5.73 ± 0.12	6	-7.49 ± 0.10	6	58
3.47		-6.70 ± 0.06	8	-7.58 ± 0.05	7	7.6
3.48		-7.50 ± 0.05	5	-7.49 ± 0.10	6	1
1.10 (S1319)	Me	-5.50 ± 0.06	6	-7.37 ± 0.06	7	74
3.50		-6.05 ± 0.03	8	-8.00 ± 0.04	8	89
3.51		-5.06± 0.06	3	-5.95 ± 0.16	7	7.8
3.52		-5.16 ± 0.13	8	-6.16 ± 0.06	8	10
3.53	Me	-4.77 ± 0.06	5	-6.33 ± 0.07	8	36

Table 3.5: Binding affinities and β_2AR selectivity of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem of n separate experiments.

Compounds that had an extended benzothiazolone core (**3.50**-**3.53**), by incorporation of an isopropyl group were also full, potent agonists at both receptors and followed the same trends seen in the benzothiazolone compounds. However each compound has lower affinity at each receptor when compared to the benzothiazolone analogues, possibly due to a reduction in hydrogen bond formation with Ser203/204, which is implicated in the catecholamine activation of the βARs.[17]

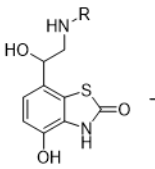
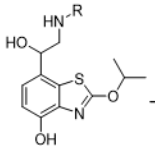
		Efficacy Ratio	
		β_1	β_2
	3.45	93	5.8
	3.46	41	275
	3.47	29	123
	3.48	19	100
	1.10 (S1319)	18	91
	3.50	68	9.5
	3.51	62	380
	3.52	18	95
	3.53	21	245

Table 3.6: efficacy ratio at β_1 and β_2AR of each active compound.

In conclusion, the alteration of the amino group can influence both affinity, and efficacy. The *tert*-butyl group containing compound (**3.45**) has the highest affinity of the set, and is the only compound to have significantly higher affinity at the β_2AR than the parent compound, S1319 (**1.10**). Furthermore, this compound is also the most β_2AR selective compound. However, although having the highest potency, it also has the lowest β_2AR efficacy ratio of the set, so the high potency is driven by high affinity. Conversely, of the set of compounds **3.45** has the highest efficacy ratio at the β_1AR. Therefore **3.45** requires a higher level of β_2AR occupancy than the parent compound to reach half of the maximal response, but at the β_1AR requires less.

Compound **3.46** has similar affinities at the βARs compared to the parent compound and also has a similar level of β_2AR selectivity. However, it has approximately three times higher efficacy ratio at the β_2AR than the parent compound, thus requiring less receptor occupancy to stimulate 50% response. Although there are varying levels of affinity selectivity and different efficacy ratios within the set of compounds, the compounds that bind to both βARs are full agonists at both receptors.

4. SAR of CGP 20712A

CGP 20712A (**1.35**) is a highly selective β_1AR antagonist, with a 501-fold greater affinity for the β_1 than β_2AR.[50] Although the compound was discovered in 1985 by Ciba-Geigy, no structure-activity relationships have been reported.[101] Therefore, it is unknown which moieties of the compound are responsible for the high selectivity towards the β_1AR. An understanding of this may facilitate the rational design of a bivalent compound that contains a β_2AR agonist with a selective β_1AR antagonist.

To investigate the SAR of CGP 20712A, the compound was first split into the headgroup and tailgroup. The headgroup (Blue) is expected to bind to the orthosteric site. This is due to the positioning of the amino-phenoxypropanol moiety which is typical in the headgroups of β_1AR antagonists.[130] The tailgroup (red), is expected to extend away from the orthosteric site. By synthesising analogues of both the headgroup and tailgroup, along with analogues of other βAR antagonists, it could be seen if one side, or both, have preferential binding to the β_1AR. This was followed by further SAR on specific moieties that were thought to provide the β_1AR selectivity.

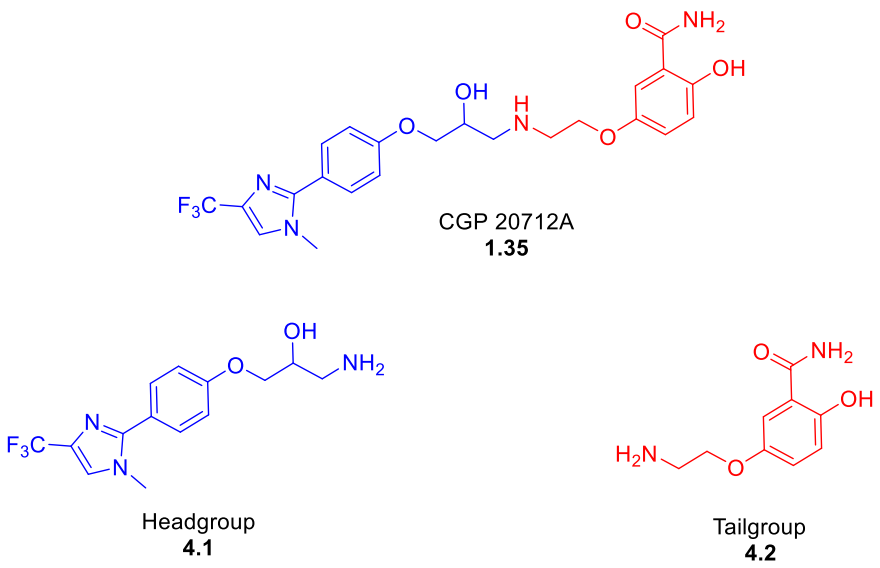
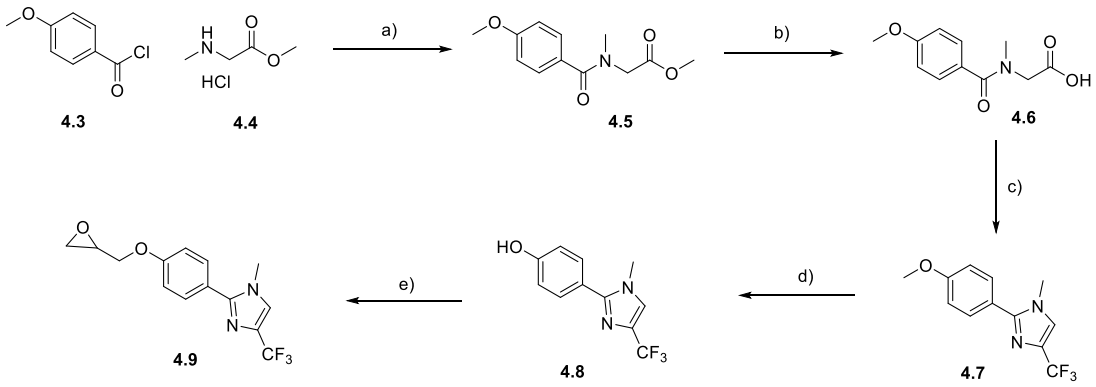


Fig 4.1: highly selective β_1AR antagonist, CGP 20712A divided into headgroup, which binds orthosterically and tailgroup which extends away from the orthosteric site

4.1 CGP 20712A headgroup

4.1.1 Synthesis of CGP 20712A headgroup and analogues

The synthesis of CGP 20712A is reported in the patent literature.[101] However, an alternative synthetic route, adapted from an improved route, developed by Dr Shailesh Mistry (University of Nottingham) was performed.[131]

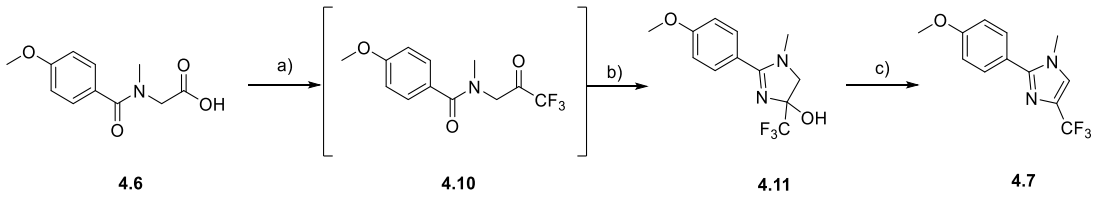


Scheme 4.1. Synthesis of CGP 20712A head group.

Reagents and condition: a) TEA, DCM, rt, 89%. b) $LiOH.H_2O$, THF/H_2O, rt, 72%. c) trifluoroacetic anhydride, DCM, rt. Ammonium acetate, DMF, 70 °C, 26%. d) BBr_3, DCM, -78°C - rt, 86%. e) (±)-epichlorohydrin, Cs_2CO_3, DMF, 50 °C, 59%.

The key intermediate **4.9** was synthesised and then subsequently derivatised by opening the epoxide with an amine. The first step involves the amide formation between the commercially available acid chloride **4.3** and sarcosine methyl ester **4.4**. The reaction gave a high yield, but due to a slight excess of sarcosine methyl ester, a small amount of this starting material remained following work-up. However, the compound was taken forward with the impurity, as this can be removed with ease in the following step. The ester hydrolysis of **4.5** was carried out in standard conditions, using lithium hydroxide in a mixture of THF and water. Following complete conversion of the ester to acid **4.6**, THF was removed *in vacuo* and the basic solution was washed with ethyl acetate. Subsequent acidification of the aqueous medium allows for extraction of the pure product with ethyl acetate.

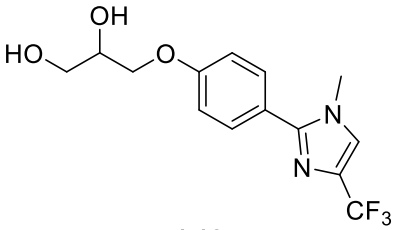
Cyclisation of **4.6** to form imidazole **4.7** was carried out in multiple steps. First a Dakin-West type reaction was carried out with trifluoroacetic anhydride which was expected to form the intermediate **4.10**.[132] The intermediate was not isolated, but redissolved in DMF and heated with ammonium acetate which cyclises **4.10** to form **4.11**. Finally aromatisation of the imidazole can be achieved in two ways. Compound **4.11** can first be isolated and then dissolved in pyridine and reacted with phosphoryl chloride, which gives the product in an overall yield of 27%. Alternatively, following formation of **4.11**, during the workup the product can be washed with 4 M potassium hydroxide. This aromatized the ring and gave 26% yield without the use of hazardous reagents.



Scheme 4.2. Cyclisation to form imidazole.

Reagents and condition: a) trifluoroaceticanhydride, DCM, rt. b) Ammonium acetate, DMF, 70 °C c) phosphoryl chloride, pyridine, 90 °C 27% over 2 steps.

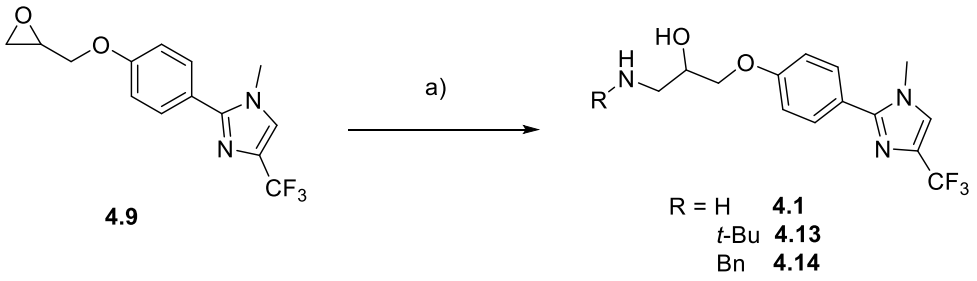
Demethylation of the phenyl ether was carried out using four equivalents of boron tribromide to give phenol **4.8** in high yield. Alkylation of the phenol was first carried out by dissolving **4.8** and sodium hydroxide in epichlorohydrin and heating at 120 °C, under microwave irradiation. Whilst this reaction did form the desired product, it had a modest yield of 48%. It was thought that milder conditions could give a higher yield and so the reaction conditions were altered. Two equivalents of epichlorohydrin, along with potassium carbonate in DMF were heated 80 °C. However, the major product did not have the same mass as expected for **4.9** by LC-MS. Additionally, analysis by ^{1}H NMR indicated that the correct number of peaks were present, however, the expected epoxide CH_2 peaks were shifted downfield by approximately 1.5 ppm, this was indicative of the epoxide product forming and then being hydrolysed to form the diol **4.12**. The reaction temperature was therefore reduced and the use of other bases was also explored. The best conditions were found to be caesium carbonate in DMF at 50 °C which gave the product in an improved, 59% yield.



4.12

Fig 4.2: hydrolysis product of epoxide **4.9**

With the key intermediate synthesised, the epoxide was opened by aminolysis. The amines selected were *tert*-butyl amine, benzylamine and ammonia. *Tert*-butylamine and benzylamine were selected as in Chapter 3 it was shown that the *tert*-butyl group has high affinity towards the βARs and the benzyl group was shown to have moderate affinity. Lastly, an unsubstituted amine was incorporated into **4.9** as without a basic nitrogen substituent, the compounds may still bind to the orthosteric site, but with lower affinity. These three amino groups should have varying βAR binding capability and so any selectivity can be observed to be due to the head group itself, rather than amino group.



4.9

R = H **4.1**
t-Bu **4.13**
Bn **4.14**

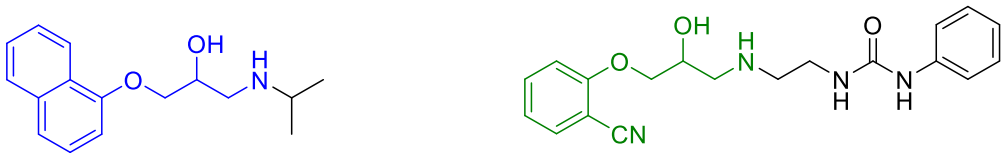
Scheme 4.3.

Reagents and conditions: a) ammonium hydroxide, Methanol, 40 °C, 14% / amines, IPA 50 °C 19-48%

4.1.2 Synthesis of Alternative βAR antagonist headgroups and analogues

Analogues of the headgroups of the βAR antagonists, propranolol and ICI 89406, were also synthesised to compare to the analogues of CGP 20712A. Propranolol has a slightly higher affinity towards the β_2AR, whereas ICI 89406 has selectivity to the β_1AR (69–fold).[50] As with the CGP 20712A headgroup, epoxide intermediates of the

propranolol and ICI 89406 headgroups were synthesised, and then aminolysis of the epoxide was performed to synthesise analogues.

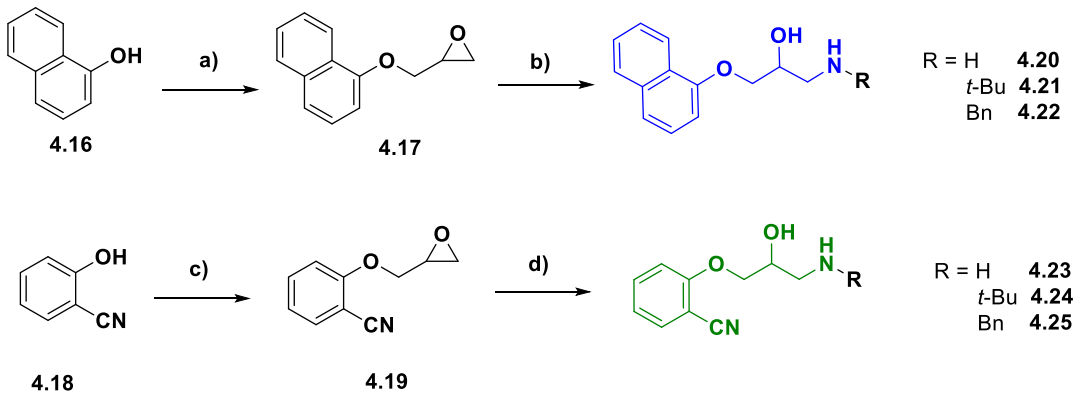


1.26
propanolol

4.15
ICI 89406

Fig 4.3: β-adrenoceptor antagonists, non-selective propranolol and β_2 selective ICI 89406

To synthesise the intermediates, commercially available 1-naphthol (**4.16**) and 2-hydroxybenzonitrile (**4.18**) were reacted with epichlorohydrin to form **4.17** and **4.19** in moderate to good yield (Scheme 4.4). Compound **4.20** was synthesised by reacting epoxide **4.17** with ammonium hydroxide in methanol. Compounds **4.21** and **4.22** were heated in IPA with the intermediate. The same conditions were used to make **4.23**-**4.25**. However, product did not form when **4.19** was reacted with ammonium hydroxide. Changing the ammonia source to 7 M ammonia in methanol gave the product in moderate yield.



Scheme 4.4: synthesis of propranolol and ICI 89406 analogues

Reagents and conditions: a) (±)epichlorohydrin, Cs_2CO_3, DMF, 50°C, 44%. b) ammonium hydroxide, Methanol, 40°C, 15% / amines, IPA 50 °C 26-37% c) (±)epichlorohydrin, Cs_2CO_3, DMF, 50°C, 83%. d) 7 M ammonia in Methanol, 40°C, 41% / amines, IPA 50 °C 18-60%

4.1.3 Pharmacology of headgroup analogues

Radioligand binding assays were used to determine the affinity of each of the compounds at the β_1 and β_2ARs. In this chapter pharmacological analysis was conducted by Prof. Jillian Baker (Institute of Cell Signalling, University of Nottingham).

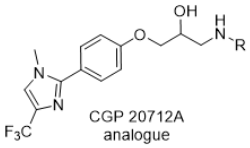
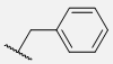
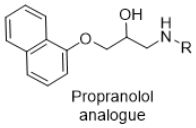
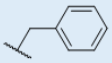
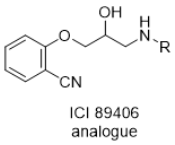
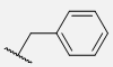
			Log K_d				
	Cpd no.	R	β_1	n	β_2	n	β_2 Selectivity
 CGP 20712A analogue	4.1	H	-5.00 ± 0.05	5	No response	5	/
	4.13		-5.63 ± 0.06	6	>-4	6	/
	4.14		-4.80 ± 0.03	6	>-4	6	/
 Propranolol analogue	4.20	H	-5.47 ± 0.02	6	-6.07 ± 0.04	6	4
	4.21		-7.69 ± 0.03	6	-8.99 ± 0.03	6	20
	4.22		-5.57 ± 0.02	6	-6.67 ± 0.08	6	13
 ICI 89406 analogue	4.23	H	-5.64 ± 0.07	5	-5.66 ± 0.13	5	1
	4.24		-8.21 ± 0.03	6	-8.56 ± 0.04	6	2
	4.25		-5.80 ± 0.02	6	-6.62 ± 0.03	6	6

Table 4.1: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log K_d) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem of n separate experiments.

Of the propranolol analogues (**4.20**–**4.22**), all of them bind to both receptors and have very slight selectivity towards the β_2AR, which is also observed in propranolol.

The addition of the benzyl group on **4.22** only slightly increases affinity compared to the primary amine of **4.20**. As expected, the *tert*-butyl group of **4.21** greatly increases the affinity of the compound at both receptors compared to the primary amine analogue.

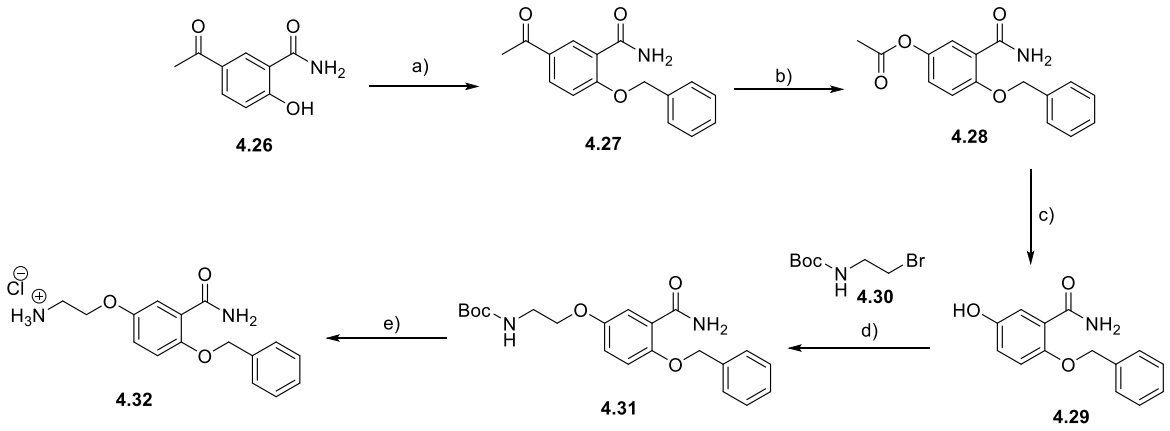
The same trends are seen with the ICI 89406 analogues **4.23**-**4.25**. The primary amine has the lowest affinity at each receptor and the benzyl analogue **4.25** has slightly higher affinity. Similarly, the *tert*-butyl analogue **4.23** has the highest affinity of the set and is approximately 1000-fold higher affinity at the β_2AR than **4.23**. Interestingly, although ICI 89406 is β_1AR selective, each of these analogue compounds have slightly higher affinity towards the β_2AR.[50] This suggested that the tail group of ICI 89406 (right portion of **4.15**), which has been replaced in this set of compounds, is important for the compounds high affinity towards the β_1AR.

The CGP 20712A head group derivatives **4.1**, **4.13**, **4.14** do not follow the same trend as the other two sets of compounds. Each of these compounds binds, although quite weakly, to the β_1AR, but have no measurable affinity to the β_2AR. In the other two series the addition of the *tert*-butyl group increased the affinity approximately 1000-fold, compared to the primary amine, but in this set, the affinity is only increased approximately 3-fold. The relatively weak affinities of these compounds indicates that the CGP 20712A headgroup may have a different binding mode to the other antagonists, or could have less favourable interactions with the orthosteric site. Additionally, due to the lack of measurable affinity at the β_2AR, this suggests that the headgroup is responsible, at least in part, for the higher affinity of CGP 20712A at the β_1 than β_2AR.

4.2 CGP 20712A tailgroup

4.2.1 Synthesis of CGP 20712A tailgroup intermediate

The tail group intermediate **4.32** was synthesised and used to make CGP 20712A and analogues by reacting **4.32** with the antagonist headgroups propranolol and ICI 89406.

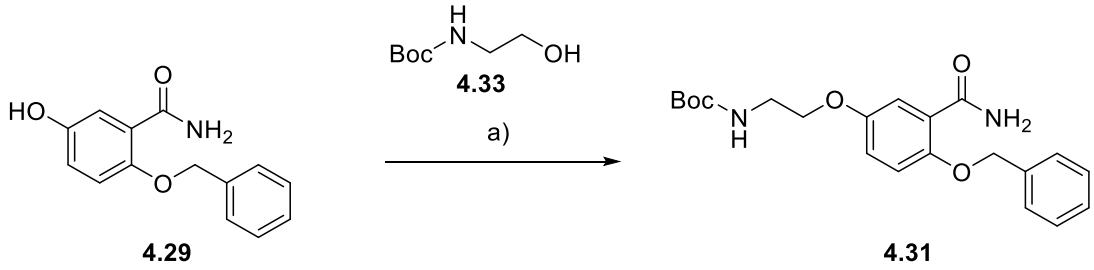


Scheme 4.5: Synthesis of CGP 20712A tail group.

Reagents and conditions: a) BnBr, DIPEA, MeCN, reflux, 98%. b) m-CPBA, DCM, rt, 81%. c) $LiOH.H_2O$, THF/H_2O, rt, 70%. d) Cs_2CO_3, DMF, 60°C, 70%. e) 2 Mhydrochloric acid in diethyl ether, rt, 94%.

The first step in the synthesis of intermediate **4.32** is the benzylation of commercially available **4.26** to protect the phenol in subsequent reactions. This was achieved in near quantitative yield by heating **4.26** with benzyl bromide and DIPEA in acetonitrile. The acetyl **4.27** was then converted to a phenyl ester **4.28** by carrying out a Baeyer-Villiger oxidation, using *m*-CPBA. Subsequent ester hydrolysis with lithium hydroxide in a solvent mixture of 50:50 water and THF gave phenol **4.29.**[132] Alkylation of the phenol **4.29** was initially carried out using a Mitsunobu reaction with DIAD and triphenylphosphine (scheme 4.6). The reaction was successful but gave a moderate yield of 46%. An additional problem was that following purification by column chromatography, not all of the triphenylphosphine oxide by-product could be

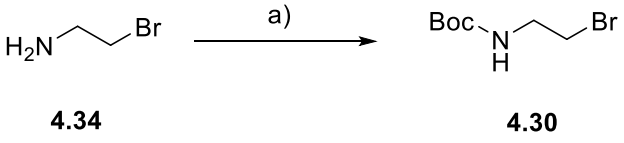
removed from the product. Consequently, alternative alkylation reactions were explored.



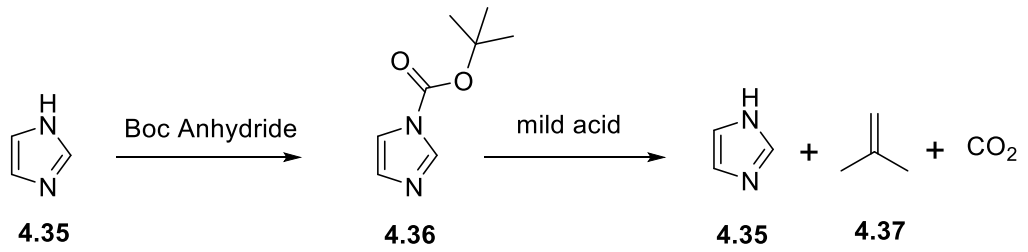
Scheme 4.6. Mitsunobu reaction.

Reagents and conditions: a) DIAD, PPh_3, THF, rt, 46%.

Rather than carrying out a Mitsunobu reaction, the phenol was alkylated with 2-bromoethylamine (**4.34**). This compound required BOCprotection, to avoid polymerisation of the alkylbromide. This was first carried out using one equivalent of BOCanhydride along with DIPEA, however this gave an unsatisfactory yield of 51%. Additional equivalents of BOCanhydride gave higher yield, however, following work-up, ^{1}H NMR indicated that unreacted BOCanhydride remained mixed with the product. The two compounds are difficult to separate by column chromatography, and any remaining anhydride would be reactive in subsequent steps. Therefore the mixture was re-dissolved in DCM and three equivalents of imidazole were added. The mixture was stirred for 30 minutes, which allows the imidazole to react with excess anhydride. The 1-BOCimidazole is liable to hydrolysis under mildly acidic conditions, therefore, the organic solution was washed with dilute acid (0.5 Mhydrochloric acid) which did not deprotect a significant amount of product, but did hydrolyse **4.36** into water soluble products (scheme 4.7).[133] Concentration of the organic solution gave pure product in an improved yield of 72%.



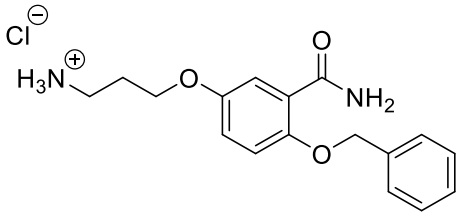
Scheme 4.7: BOCprotection of 2-bromoethylamine
Reagents and conditions: a) BOCanhydride, TEA, imidazole, DCM, rt, 72%.



4.35 **4.36** **4.35** **4.37**

Scheme 4.7: removal of excess BOCanhydride by formation of 1-BOCimidazole and then addition of mild acid.[133]

The BOCprotected amine **4.31** was then used to alkylate the phenol **4.29**. This was preferable to carrying out a Mitsunobu reaction, as no reagents, other than a base is required and so avoids the formation of triphenylphosphine oxide. Initially the reaction was carried using potassium carbonate in DMF at 70 °C, which gave an improved yield of 57%. However, when using the alterative base, caesium carbonate, a yield of 70% was attained. The final step in the synthesis of the intermediate was to deprotect the BOCprotecting group of **4.31**. This was carried out in 2 Mhydrochloric acid in diethyl ether. Although **4.31** is insoluble in diethyl ether, rapid deprotection of the BOCgroup occurred and pure product was obtained in very high yield by filtration. A second tailgroup intermediate, **4.39**, was also synthesised which has a three carbon chain between the oxygen and amine. This was to alter the distance between the head and tailgroup. The same reaction conditions were used as in the synthesis of **4.31**, however a lower yield of 50% was obtained in the alkylation step.

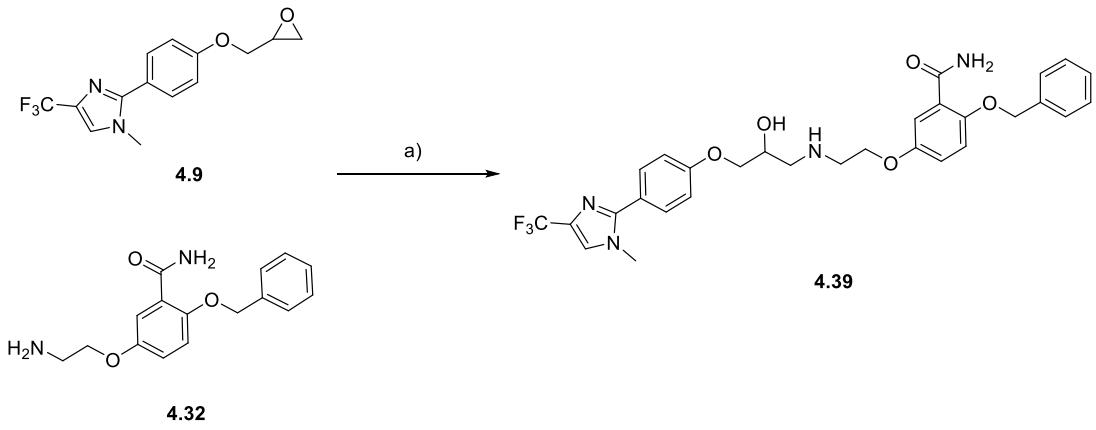


4.38

Fig 4.4: CGP 20712A tailgroup with additional carbon atom in the alkyl chain.

4.2.2 Synthesis of CGP 20712A tailgroup Analogues

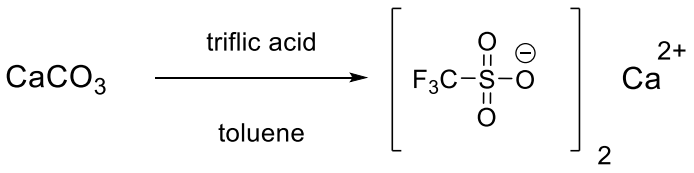
With the intermediate **4.32** prepared, the parent compound CGP 20712A (**1.35**) was synthesised. This was performed by reacting the epoxide **4.9** with the amine **4.32**. Subsequent debenzylation afforded **1.35**.



Scheme 4.8: aminolysis of **4.9** with **4.29**

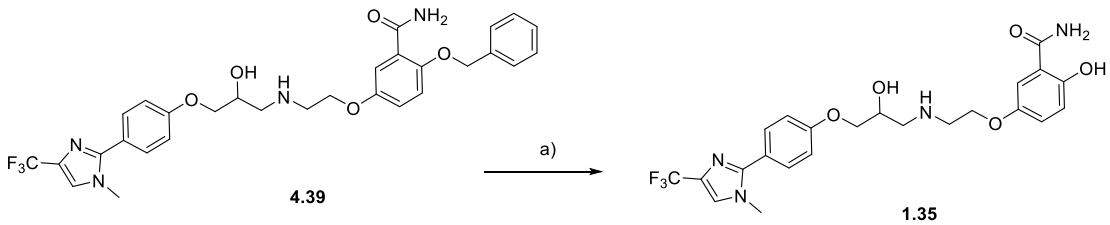
Reagents and conditions: a) calcium triflate, TEA, MeCN, MW 90 °C, 8%.

The first conditions used in the aminolysis of **4.9** with **4.29** was to heat the two compounds in ethanol under microwave irradiation at 90 ˚C, along with trimethylamine. Three equivalents of the amine were used to avoid *bis*-addition of the epoxide onto **4.39**. The starting material was consumed, however a mixture of products formed, none of which could be identified as **4.39** by LC-MS. It is reported that reactions involving the aminolysis of epoxides do not generally produce products cleanly, however, it is also reported that calcium triflate can catalyse the aminolysis of epoxides, giving high yields with equimolar quantities of epoxide and amine.[134] In order to carry out this reaction, the catalyst first had to be prepared. This was done by adding excess triflic acid to a suspension of calcium carbonate in toluene. Filtration and washing of the resulting solid afforded calcium triflate and was used without further purification.



Scheme 4.9: synthesis of calcium triflate

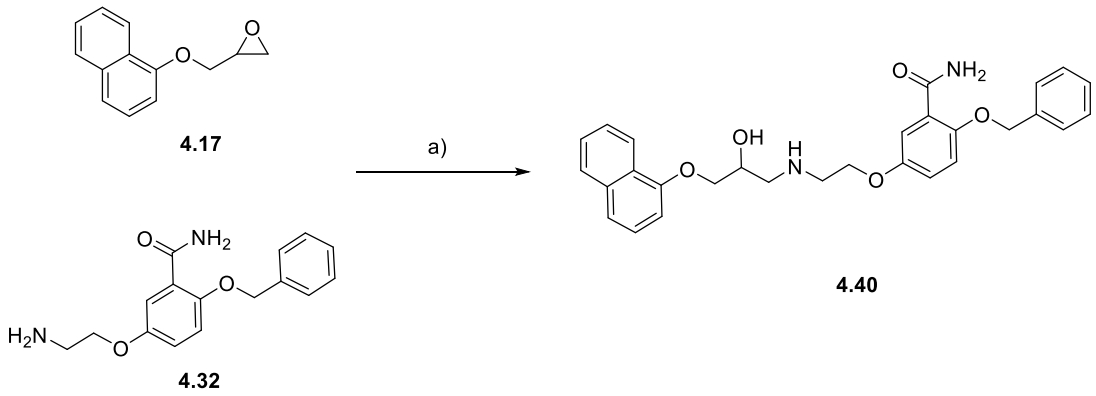
With the catalyst prepared, the literature procedure was carried out, using acetonitrile as the solvent. Starting material was consumed, however following purification, an 8% yield was obtained. The use of ethanol and methanol was also explored, however, there was no improvement in yield. Although low yields were obtained, the products of the three reactions were combined and carried forward to the next step. The benzylated compound **4.39** was also tested in pharmacological assays to see if alkylation of the phenol was tolerated within the βARs. If it was, then it may have been possible to link CGP 20712A to a second pharmacophore element *via* the phenol (See chapter 1.6).



Scheme 4.10: debenzylation of **4.39** to form parent compound CGP 20712A **1.35**
Reagents and conditions: a) Pd/C, Methanol, H_2O, AcOH, H_2, rt, 60%.

To deprotect the benzyl group of **4.39**, the compound was dissolved in a mixture of methanol-water-acetic acid (7:2:1). This solvent system was used as it is reported that the addition of water and acetic acid to methanol increase the reactivity of the hydrogenation.[135] Debenzylation proceeded cleanly, and following column chromatography, **1.35** was obtained in satisfactory yield.

Analogues of CGP 20712A were synthesised with the antagonist head groups of propranolol (**1.26**) and ICI 89406 (**4.15**). As with the synthesis of **4.39**, the epoxide intermediates **4.17** and **4.19** were reacted with the CGP 20712A tail group (**4.32**). Compound **4.17** was initially reacted with the tail group intermediate **4.32** in ethanol at 80 °C. Product was isolated in low yield, however due to a number of side products purifying the product by column chromatography was difficult.

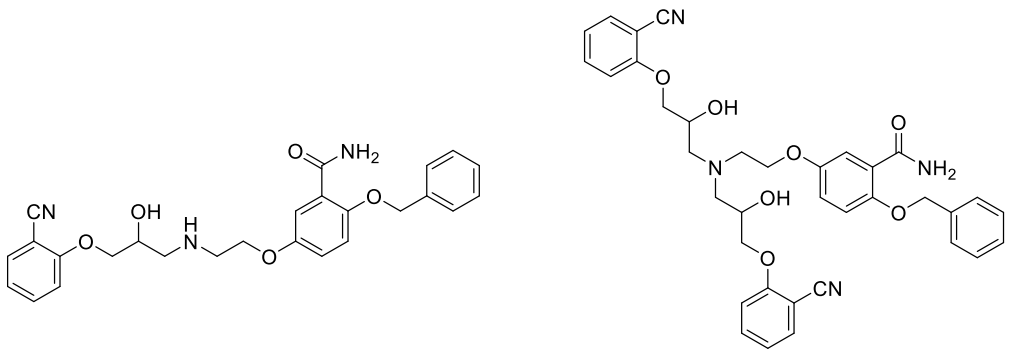


Scheme 4.11: aminolysis of **4.17** with **4.29**

Reagents and conditions: a) DIPEA, Ethanol, 90 °C, 28%.

Low yields were obtained in previous reactions, and so different reaction conditions were explored. Although it was preferable not to use multiple equivalents of the amine **4.32** (due to limited quantities of the compound), three equivalents were reacted with **4.19** in isopropyl alcohol with DIPEA at 70 °C. However, as with methanol and ethanol, **4.32** is insoluble in isopropyl alcohol, and as a result, no reaction occurred. The reaction was therefore repeated in DMF, in which the amine **4.32** is soluble. LC-MS indicated that a small amount of product had formed, however, column chromatography was attempted but the product could not be isolated due to the number of side products with a similar R_f.

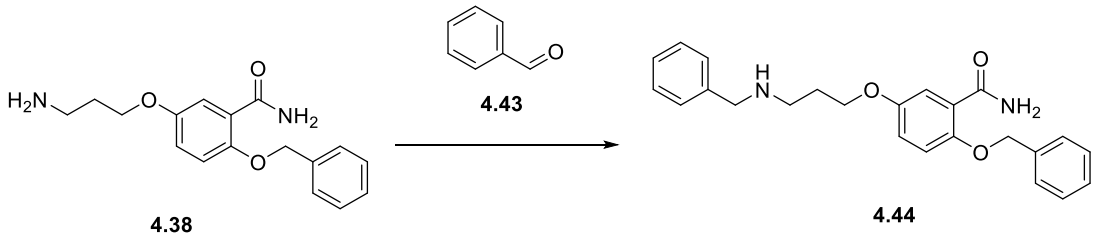
The product could be synthesised, albeit in low yield, but the main issue was that the reaction did not proceed cleanly, making purification difficult. It is reported that the addition of a lithium perchlorate catalyst can increase yields in the aminolysis of epoxides and allows the use equimolar amounts of amine and epoxide. The catalyst is hypothesised to work by the lithium cation coordinating with the epoxide oxygen which makes the epoxide more susceptible to nucleophilic attack.[136] The reaction of the amine **4.32** and epoxide **4.19** using a lithium perchlorate catalyst was attempted in acetonitrile and THF. However, LC-MS analysis indicated that both of these reaction conditions gave predominantly *bis*-substitution of the epoxide onto the amine. Additionally, although a small amount of product formed, it was inseparable from the *bis*-product (**4.42**).



4.41 **4.42**

Fig 4.5: inseparable mono and *bis* products of reaction to form ICI 89406 headgroup – CGP 20712A tailgroup compound

To avoid formation of the *bis*-product, the amine was protected with a benzyl group which could be removed in the final step by hydrogenation. Reductive amination of benzaldehyde (**4.41**) with amine **4.38** was performed, and a number of reducing agents were explored. The alkylation of **4.38** with benzyl bromide was avoided due to the probable di-alkylation.

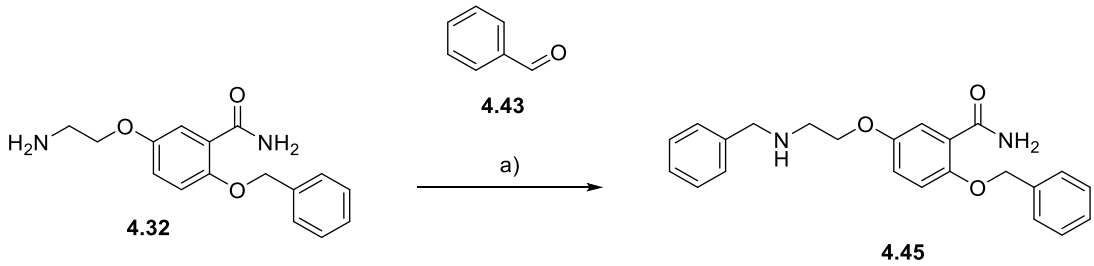


4.38 **4.44**

Scheme 4.12: benzylation of amine by reductive amination
Reagents and conditions: Sodium borohydride, Methanol, rt, 58%.

Sodium triacetoxy borohydride was the first reducing agent to be used. The electron withdrawing nature of the acetoxy groups makes this reagent one of the milder reducing agents, and has been shown to preferentially reduce imines rather than aldehydes.[137] The reduction of the aldehyde would be problematic as it would consume starting material **4.43**, without forming product. However, this procedure did not lead to the production of the desired product (**4.44**). Sodium cyanoborohydride is another mild reducing agent that is widely used in reductive amination reactions, however, this reagent does produce highly toxic cyanide, upon work-up.[138] The use of sodium cyanoborohydride afforded the desired product in a

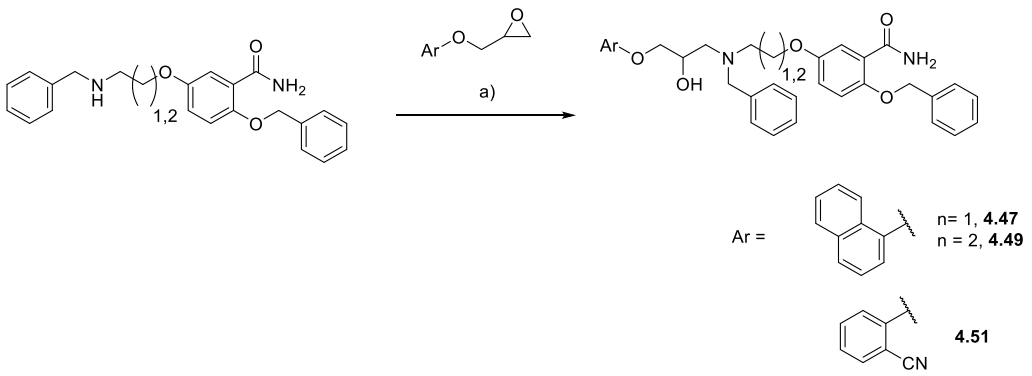
modest yield of 52%. In an attempt to raise the yield of **4.44**, and to avoid the use of highly toxic reagents, the use of sodium borohydride was explored. This was initially avoided due to being a stronger reducing agent (having no electron withdrawing substituents) and could therefore rapidly reduce the aldehyde starting material. In an attempt to avoid this, the amine **4.38** and benzaldehyde were stirred overnight to allow the imine to form. The solution was then cooled and sodium borohydride added. This gave a slightly improved yield of 58%. The use of sodium borohydride as the reducing agent gave the best result as it had the highest yield, safest work-up and simplest purification as the product precipitates out of solution which allows for filtration of the pure product. Sodium borohydride was therefore also used in the reductive amination of **4.32** to **4.45** in which a yield of 64% was obtained.



Scheme 4.13: benzylation of amine by reductive amination

Reagents and conditions: Sodium borohydride, Methanol, rt, 64%.

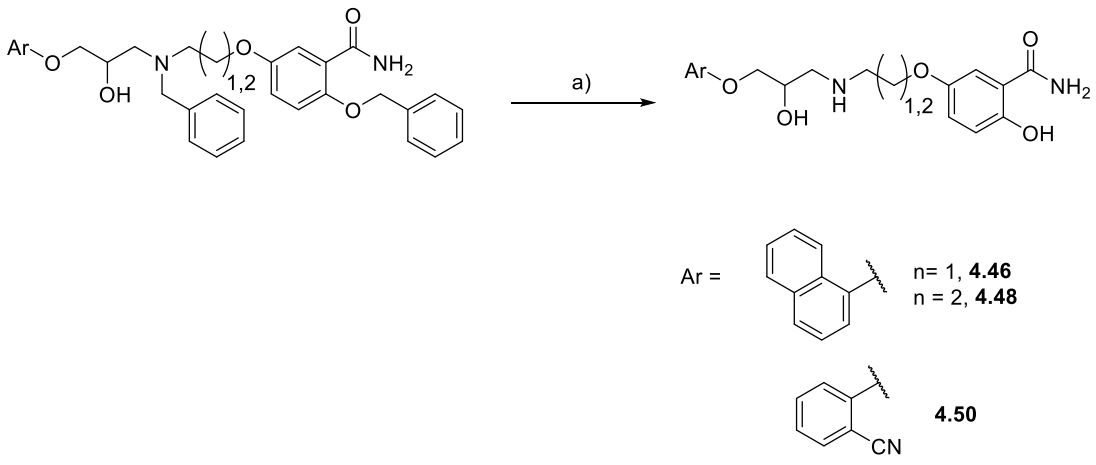
Following benzylation of the tail group intermediates (**4.44** and **4.45**), each of these were reacted with the propranolol and ICI 89406 head group epoxide intermediates. These reactions were carried out in ethanol at 80 °C along with DIPEA. Yields were between 15-27% and each compound was purified by HPLC, as regular phase column chromatography was not sufficient to isolate the products.



Scheme 4.14: aminolysis of benzylated tailgroup intermediate with headgroup intermediates
Reagents and conditions: a) DIPEA, Ethanol, 90 °C, 18-27%.

The benzyl groups were added in an attempt to circumvent the formation of side products and increase yield. However, although the *bis*-product (**4.42**) was unable to form, a number of unidentified side-products did, making purification of the product difficult, and low yields were obtained. This suggests that the attempted aminolysis of an epoxide gives way to a number of side reactions regardless of whether a primary or secondary amine is used as the nuleophile.[139]

The final step in the synthesis of the tail group analogues was to remove the benzyl protecting groups. For compounds with one or two protecting groups the same reaction conditions were used. Each were added to a suspension of palladium on carbon in a mixture of THF-water-acetic acid (7:2:1) under a hydrogen atmosphere. HPLC was used to isolate each product in good yields.

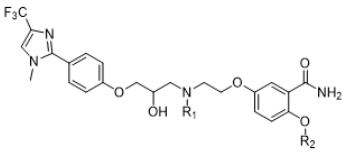


Scheme 4.15: debenzylation of tail group analogues to form
Reagents and conditions: a) Pd/C, THF, H_2O, AcOH, H_2, rt, 60-71%.

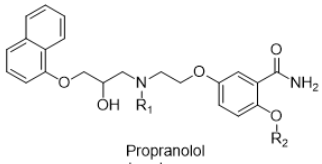
Along with the compounds synthesised by combining the CGP 20712A tail group with alternative headgroups, a number intermediates in the synthesis of **4.46** and **4.48** were tested in a pharmacological assay. These compounds were comprised only of the tailgroup. The purpose of this was to identify if these compounds had any affinity towards either of the βARs without a headgroup. Affinity of these compounds to the βARs could indicate that an earlier hypothesis was wrong, in that the tailgroup does not bind orthosterically.

4.2.3 Pharmacology of tailgroup analogues

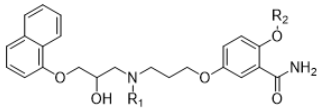
Radioligand binding assays were used to determine the affinity of each of the compounds at the β_1 and β_2ARs. This set of compounds includes the selective β_1AR antagonist CGP 207612A (**1.35**) as well analogues with the same tailgroup as CGP 20712A, but headgroups of different antagonists. Each of these analogues were then further derivatised with benzyl groups on various heteroatoms to see if these alkylations are tolerated.



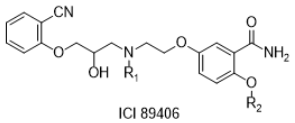
CGP 20712A headgroup



Propranolol headgroup



Propranolol headgroup



ICI 89406 headgroup

			Log K_d				
Cpd no.	R1	R2	β_1	n	β_2	n	Selectivity β1 β2
1.35	H	H	-8.64 ± 0.16	5	-5.60 ± 0.05	5	1096
4.39	H	Bn	-6.02 ± 0.08	5	-5.07 ± 0.08	5	9
4.46	H	H	-9.05 ± 0.12	5	-9.23 ± 0.10	5	2
4.40	H	Bn	-6.63 ± 0.05	5	-8.14 ± 0.05	5	32
4.47	Bn	Bn	-6.80 ± 0.07	5	-7.59 ± 0.05	6	6
4.48	H	H	-8.37 ± 0.14	6	-9.05 ± 0.09	6	5
4.49	Bn	Bn	4.84 ± 0.06	5	5.96 ± 0.09	6	13
4.50	H	H	-8.76 ± 0.16	6	-8.50 ± 0.13	6	2
4.51	Bn	Bn	-4.91 ± 0.07	6	-6.30 ± 0.06	6	25

Table 4.2: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log K_d) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments.

Compound **1.35** (CGP 20712A) has comparable affinities to the literature values.[50] As presented in table 4.2 that **1.35** has a significantly higher affinity towards the β_1AR than β_2AR. **4.39** is analogous to **1.35** but has a benzyl group incorporated onto the phenol of the tailgroup. Interestingly, this causes a significant reduction in affinity

towards the β_1AR, but a relatively small reduction in affinity at the β_2AR. The addition of this group therefore makes **4.39** significantly less β_1AR selective than CGP 20712A.

The headgroup of the non-selective antagonist, propranolol, was attached to the tail group of CGP 20712A to see if the addition of this moiety alone could result in higher affinity towards the β_1AR than β_2AR. Of the compounds (**4.46**, **4.40**, **4.47**), none had any β_1AR selectivity. Each has slightly higher affinity towards the β_2AR, as propranolol does. The same trend can be seen in compounds **4.48**, **4.49**, which have an extra methylene linking the head a group of propranolol and the CGP 20712A tailgroup. Again, each compound has slightly higher affinity towards the β_2AR.

Due to the addition of a methylene, the affinity of **4.48** is slightly lower than that of **4.46** at each receptor, this is possibly due to the tail group moiety becoming further away from favourable interactions with an amino acid residue. Consequently, this interaction will be weaker, or to make the interaction the compound will have to be in a less favourable configuration.

The combination of the ICI 89406 head group and the CGP 20712A tailgroup (**4.50**) has almost equal affinity at both receptors despite ICI 89406 having 69-fold affinity towards the β_1AR. This set of data suggests that the addition of the tail group moiety to any βAR antagonist head group will not make a selective β_1AR antagonist, as the tail group appears to be able to bind to both receptors with approximately equal affinity. Rather, the addition of this group (which can bind well to both receptors) onto a headgroup which binds preferentially towards the β_1AR (such as CGP 20712A, see Chapter 4.1.3) will be selective towards the β_1AR.

As previously mentioned, compound **4.39** has a benzyl group attached to the phenol of the CGP 20712A tail group. This alteration greatly reduces the β_1AR affinity but does not significantly affect β_2AR binding. The same trend can be seen in the other sets of compounds (**4.47**, **4.49**, **4.51**). Addition of a benzyl groups to propranolol and ICI 89406 derivatives **4.46**, **4.48** and **4.50** results in a reduced affinity towards the β_2AR, but in each case, a much greater loss in the affinity at the β_1AR, consequently, each of these compounds are β_2AR selective. This would indicate that the phenol of

the tail group makes an interaction with an amino acid residue, which is either only within the β_1AR, or is more important for receptor stabilisation at the β_1 than β_2AR.

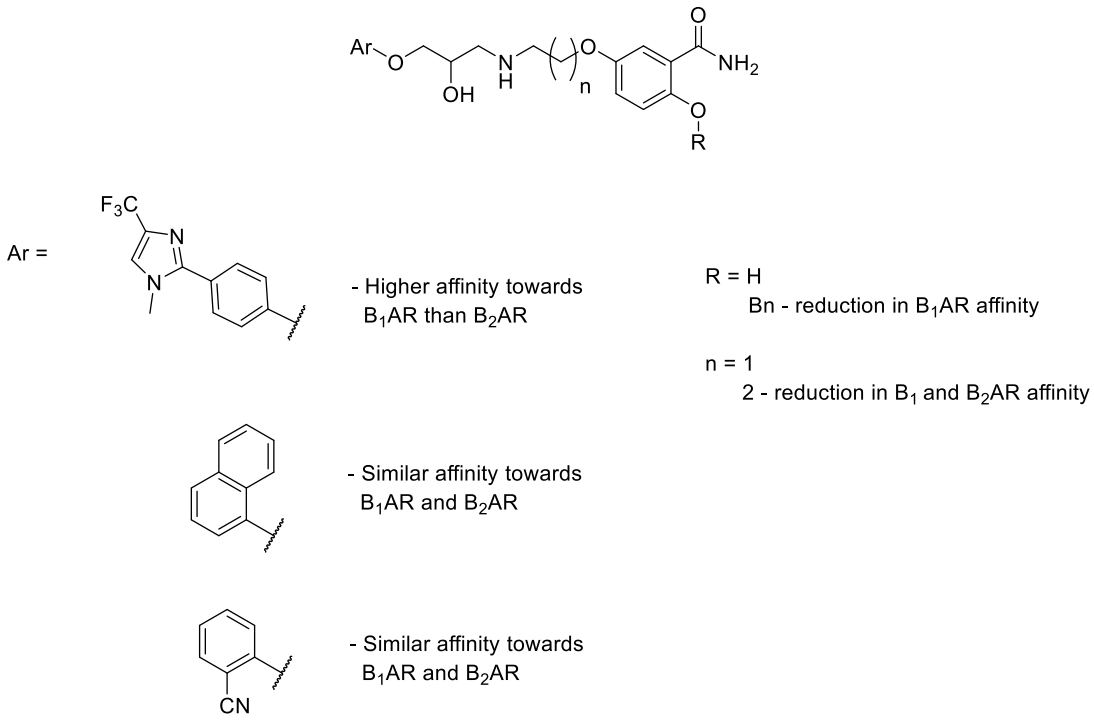
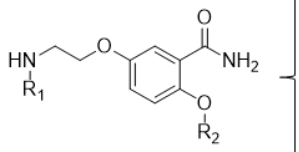
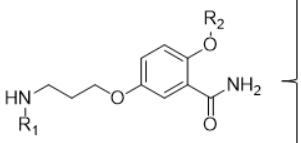


Fig. 4.6: SAR summary of CGP 20712A analogues

The purpose of these studies was to identify if one of the tail or headgroups are responsible for preferential binding of CGP 20712A (**1.35**) to the β_1AR. It appears that the head group is the part of the compound that has higher affinity towards the β_1AR (**4.1**, **4.13** and **4.14**). However the head group has very low affinity (**4.13**), particularly compared to other βAR antagonist headgroups (**4.21**, **4.24**). The tail group of CGP 20712A adds affinity towards both receptors, but with no preference towards either. However, the phenol in the tail group is more important for β_1AR binding than β_2AR (**4.39**). So, because both sides play a role in β_1AR binding, it may be difficult to attach a complete CGP 20712A to a βAR agonist, to form a bivalent compound. The necessity of the phenol for β_1AR binding is particularly problematic as the phenol would be the simplest place to attach a linker to a second pharmacophore. A linker could not be placed on the central amine as alkylation at this position has been

shown to reduces affinity towards both receptors (compounds **4.47 4.49**, **4.51**) and attachment of a linker to the amide would be synthetically, very challenging.





			Log K_d			
Cpd no.	R1	R2	β_1	n	β_2	n
4.52	H	H	No binding	4	No binding	4
3.32	H	Bn	No binding	4	No binding	4
4.45	Bn	Bn	No binding	4	No binding	4
4.53	H	H	No binding	4	No binding	4
4.38	H	Bn	No binding	4	No binding	4

Table 4.3: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments.

The tailgroup moiety and analogues were also tested for affinity at both βARs. This was to investigate if the tail group could bind orthosterically. None of these compounds (Table 4.3) had any affinity towards either receptor which indicates that as postulated, the headgroup of CGP 20712A binds orthosterically and the tail group binds away from this binding site.

These compounds were also tested as antagonists to cimaterol (βAR agonist) in a functional assay. However there was no right shift in the cimaterol response, with a maximum concentration of 10 µM of tailgroup compound. This indicates that these compounds do not bind to the catechol binding site, nor are they allosteric modulators.

4.3 SAR of CGP 20712A headgroup

The headgroup of CGP 20712A has been shown to be selective for the β_1AR, however, it is unknown which specific features of this compound bind preferentially to the β_1AR. Therefore, a set of compounds with subtle variations to the headgroup were synthesised and then tested for their affinity and activity to both βARs to investigate if there are any differences in their pharmacological profiles. The variations focused on the *N*-methyl group, the CF_3 substituent and the size of the aromatic ring (Figure 4.7).

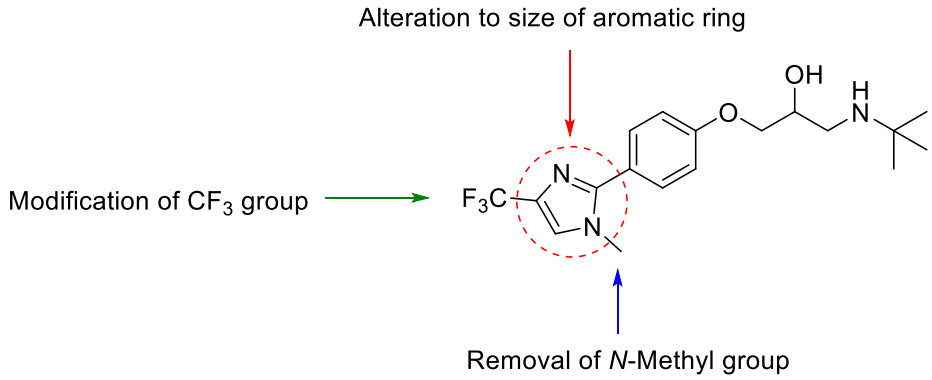
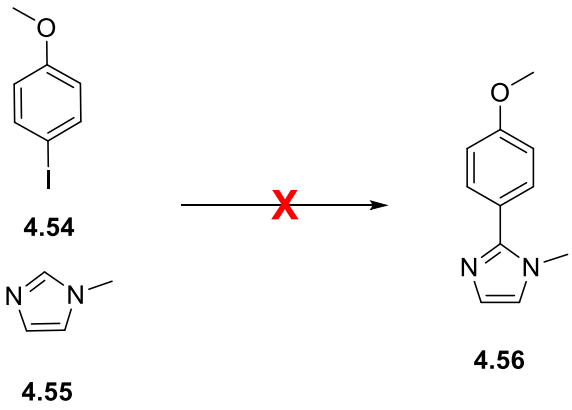


Fig 4.7: areas of investigation around CGP 20712A headgroup.

4.3.1 Synthesis of CF_3 modified analogues

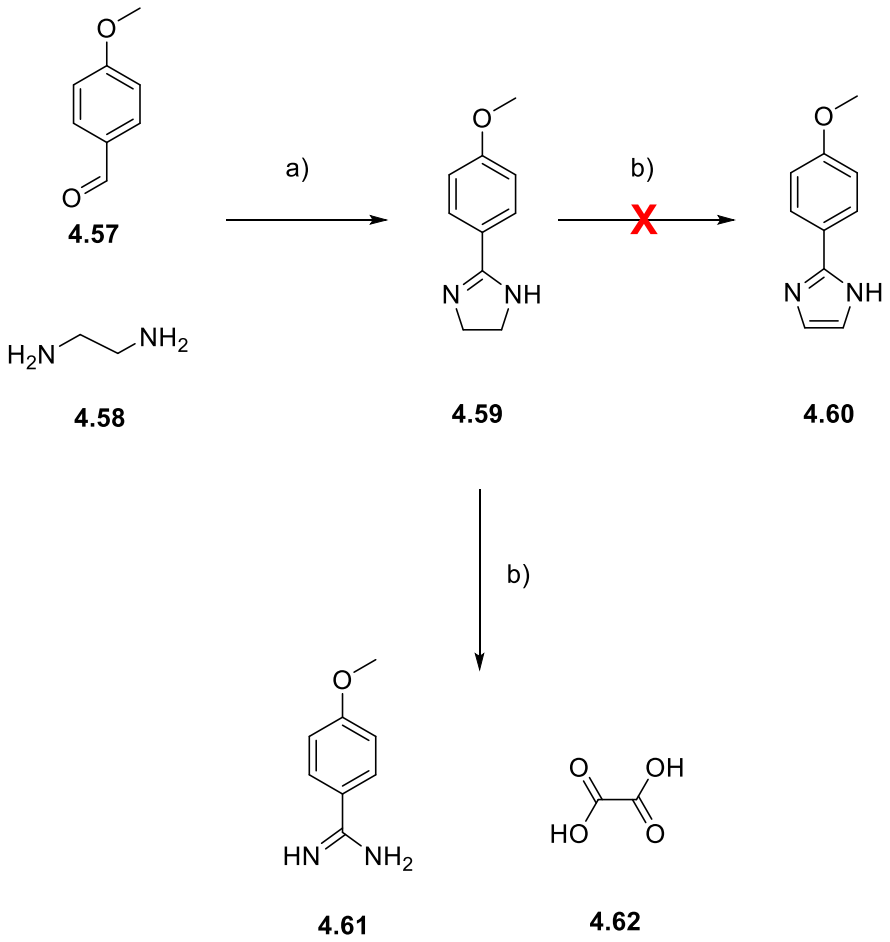
The first analogue that was synthesised was the CGP 20712A headgroup without the CF_3 group. This was attempted by following a literature procedure, which forms an aryl imidazole bond between **4.54** and **4.55** using palladium diacetate and copper iodide (Scheme 4.16).[140] After carrying out the procedure, LC-MS indicated that the reaction was complete as there was only one major product. TLC however indicated, a number of compounds were present within the mixture. Each of these were isolated, but NMR indicated that the majority of the isolated compounds was starting materials. The reaction was repeated, but with no improvements.



Scheme 4.16: attempted palladium mediated coupling of **4.54** and **4.55**.

Reagents and conditions: a) $Pd(OAc)_2$, copper iodide, DMF, 140 °C

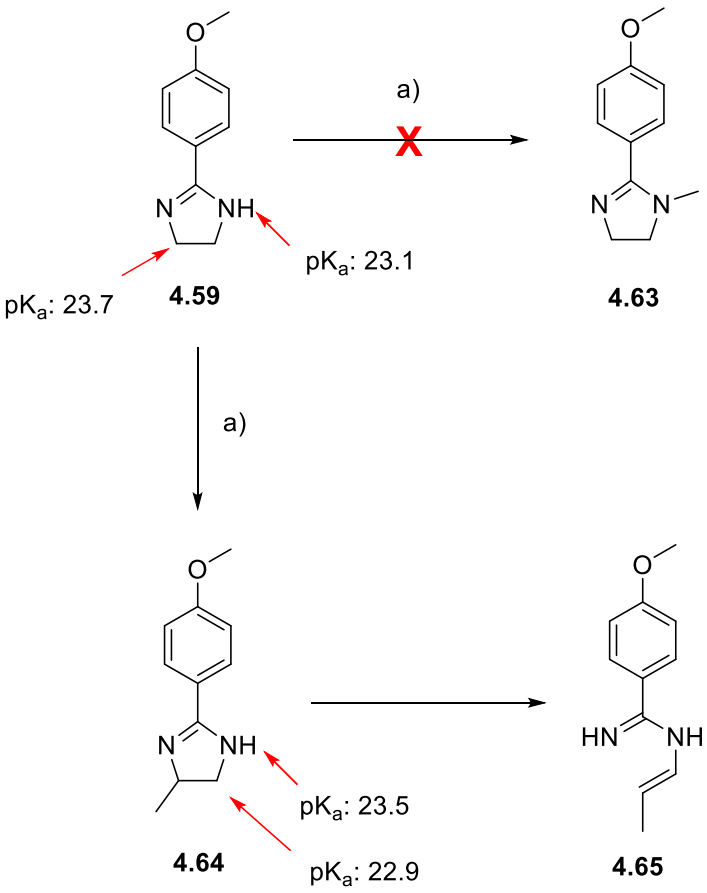
A different approach was taken, by first forming the imidazoline **4.59** and then oxidizing it to the imidazole **4.60** (Scheme 4.17). The imidazoline was formed in modest yield by heating the aldehyde **4.57** and ethylenediamine with potassium carbonate and iodine. To oxidise the imidazole, a literature procedure was followed using potassium permanganate which was adsorbed onto montmorillonite K 10.[141] This reaction was initially carried out overnight, but did not form the desired product as it over oxidised. NMR and LC-MS indicated that over oxidation led to the formation of the two products **4.61** and **4.62**. The reaction was repeated for a shorter period of time, of one hour, however the same product was obtained. The alternative oxidizing agent, manganese(IV) oxide was also used, however, this failed to oxidize the imidazoline.[142]



Scheme 4.17: attempted synthesis of **4.60**

Reagents and conditions: a) potassium carbonate, iodine, *tert*-butanol, 70 °C, 77%. b) $KMnO_4$, MeCN, rt.

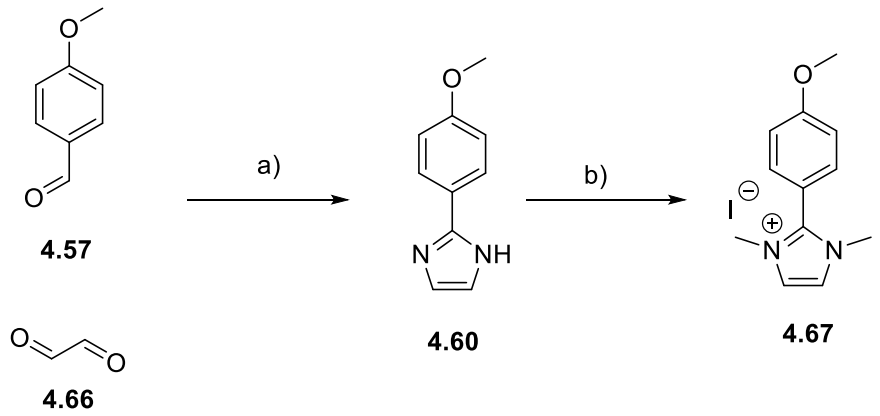
N-Methylation of the imidazoline **4.59** was also attempted to explore whether this compound could be oxidised. Methylation was performed using sodium hydride and methyl iodide, however the expected product (**4.63**) did not form, instead the major product was the ring opened **4.65**. This is because the pK_a of the NH and CH_2 of imidazoline **4.59** are similar and sodium hydride can deprotonate either. This can result in methylation at the 4-position, which reduces the pK_a of the adjacent CH_2 in **4.64**. Deprotonation at this position forms an alkene and opens the ring to form **4.65** (Scheme 4.18).



Scheme 4.18: ring opening of **4.59** to form **4.65**

Reagents and conditions: a) MeI, NaH, DMF, rt.

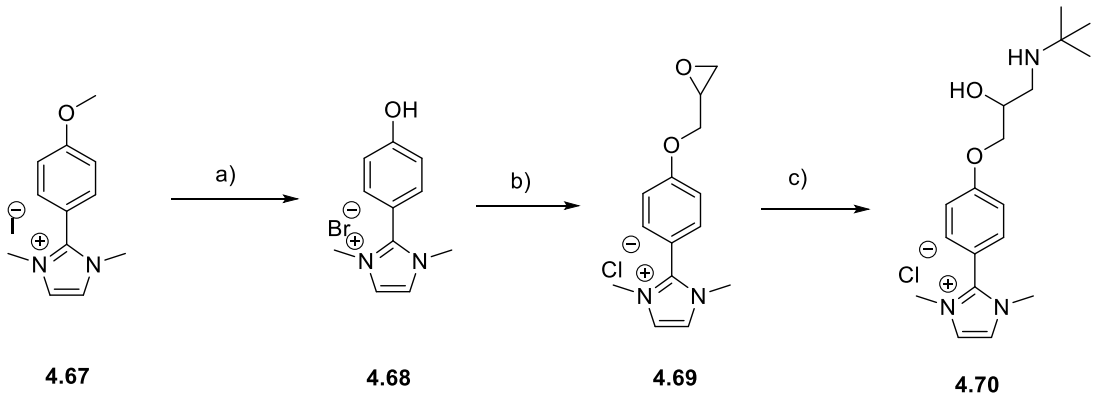
A number of conditions were explored to form the imidazole directly using the aldehyde **4.57** and glyoxal **4.66**. Initially these compounds were dissolved in ammonium hydroxide and stirred overnight.[143] The product formed and was isolated by column chromatography, but in a low yield of 23%. The literature indicated that the use of ammonium acetate as the ammonia source could give higher yields.[144] Initially this reaction was carried out on a 50:50 mixture of water – isopropyl alcohol, however, no reaction occurred. The reaction was repeated but in methanol, which gave the product (**4.60**) in a slightly improved yield of 31% and did not require further purification following work up.



Scheme 4.19: synthesis of **4.67**

Reagents and conditions: a) ammonium acetate, Methanol, rt, 31%. b) MeI, NaH, DMF, 72%.

Methylation of imidazole **4.60** did not give the desired product **4.56**, instead the imidazolium **4.67** was formed. This compound was taken forward to make analogue **4.70**. The phenyl ether was demethylated using boron tribromide; it was postulated that this may also demethylate the quaternized nitrogen, by the same mechanism as methyl phenol demethylation, however this did not occur. The phenol was then alkylated with epichlorohydrin to form **4.69**, and then aminolysis of the epoxide was performed by reacting it with *tert*-butylamine in methanol.

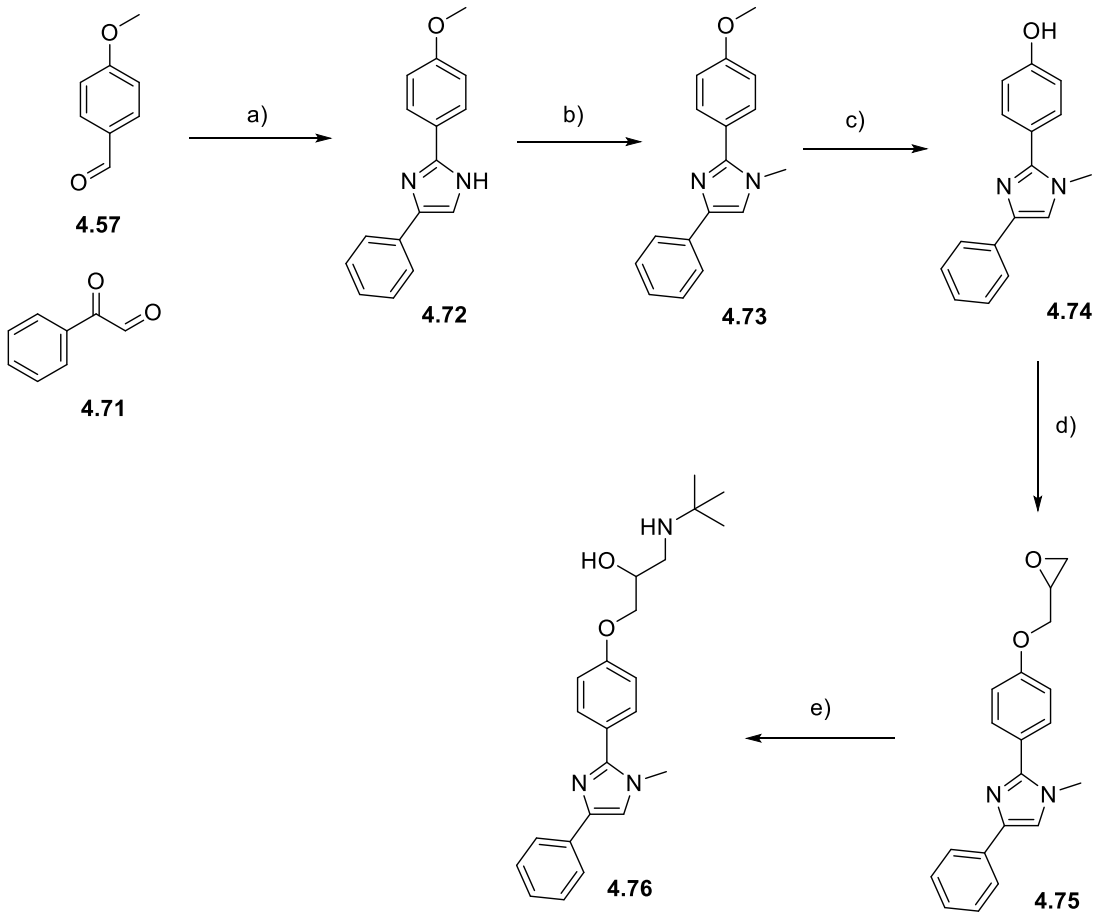


Scheme 4.20: synthesis of **4.70**

Reagents and conditions. a) BBr_3, DCM -78 – 0 °C 98%. b) (±)epichlorohydrin, Cs_2CO_3, 50 °C, 28%.c) *tert*-butylamine, Methanol, 40 °C, 61%.

An analogue where the CF_3 group is replaced by a phenyl ring (**4.76**) was synthesised by a similar synthetic route as **4.70**. However, in the first step phenylglyoxal was used

rather than glyoxal. The imidazole was obtained in satisfactory yield, and subsequent *N*-methylation gave the single *N*-methyl product in good yield. This was followed by demethylation with boron tribromide, alkylation with epichlorohydrin and then aminolysis with *tert*-butylamine.

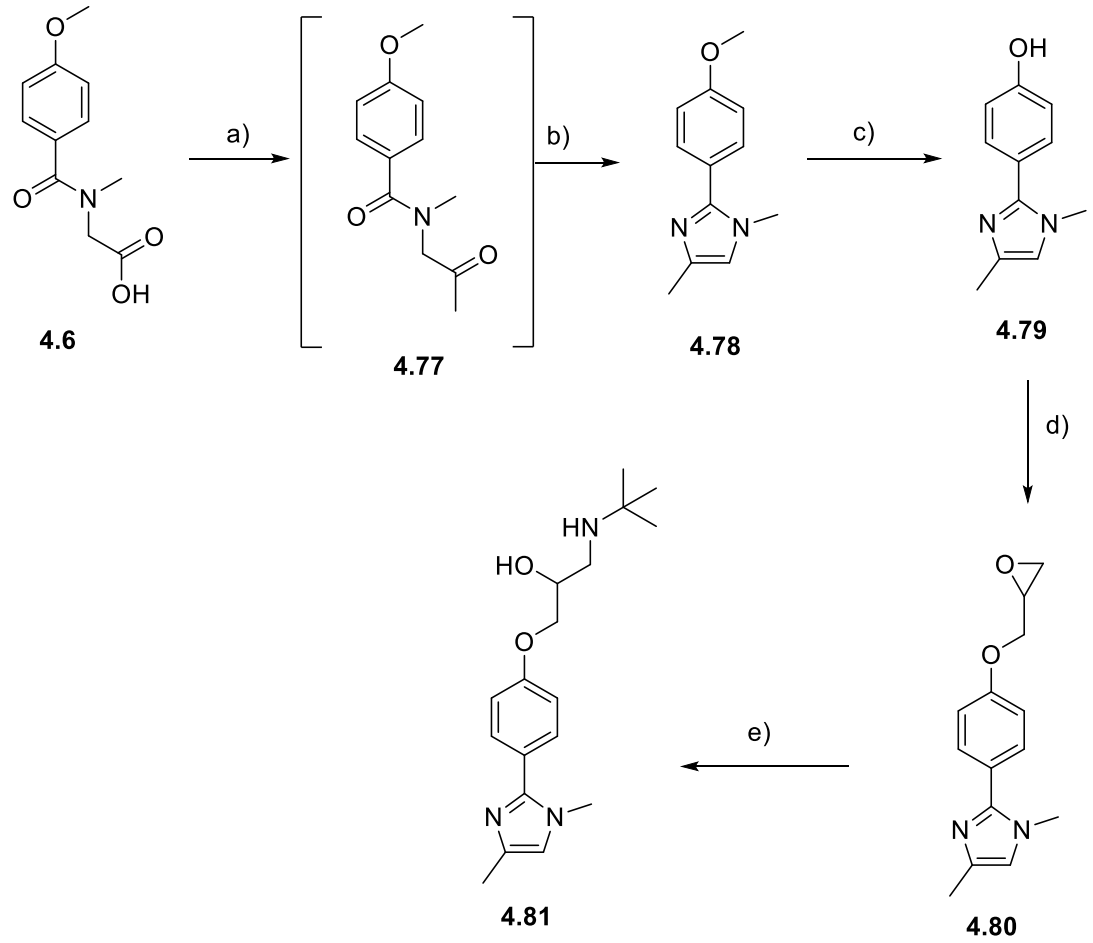


Scheme 4.21: synthesis of **4.76**.

Reagents and conditions: a) ammonium acetate, Methanol, rt, 39%. b) MeI, NaH, DMF, 89% c) BBr_3, DCM -78 – 0 °C 82%. d) (±)epichlorohydrin, Cs_2CO_3, 60 °C, 67%. e) *tert*-butylamine, Methanol, 40 °C, 51%.

A different synthetic route was taken to synthesise the 1,4-dimethyl analogue **4.81**. Compound **4.6** (used in the synthesis of the CGP 20712A headgroup) was dissolved in pyridine, along with acetic anhydride and DMAP. Heating the mixture converted the acid into the intermediate acetyl (**4.77**), which was purified by column chromatography and then redissolved in acetic acid, along with ammonium acetate (Scheme 4.22). The reaction was heated at 100 °C which caused cyclisation to form the imidazole **4.78**, albeit in low yield. Compound **4.78** was then *O*-demethylated,

alkylated with epichlorohydrin, and then **4.81** was formed by opening the epoxide with *tert*-butylamine.



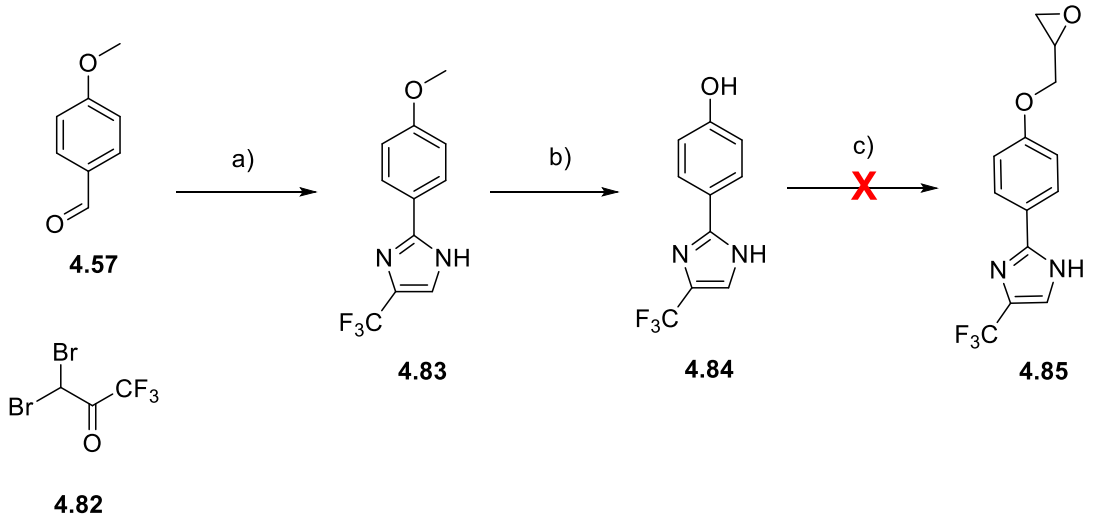
Scheme 4.22: synthesis of **4.81**.

Reagents and conditions: a) DMAP, pyridine, 50 °C. b) ammonium acetate, DMF 70 °C, 14% c) BBr_3, DCM -78 – 0 °C 57%. d) (±)epichlorohydrin, Cs_2CO_3, 60 °C, 67%. e) *tert*-butylamine, Methanol, 40 °C, 55%.

4.3.2 Synthesis of *N*-methyl modified analogues

The synthesis of the *N*-demethylated analogue **4.85** of the CGP 20712A headgroup was carried out by reacting aldehyde **4.57** with **4.82**. This formed the imidazole in modest yield and demethylation of the phenol was then performed using boron tribromide. However, alkylation of the phenol was problematic. Using the same conditions as previously performed, this gave a mixture of many products. LC-MS indicated that the mixture included products that were *O*-alkylated, *N*-alkylated and di-alkylated. This is due to the phenol and imidazole nitrogen both being nucleophilic.

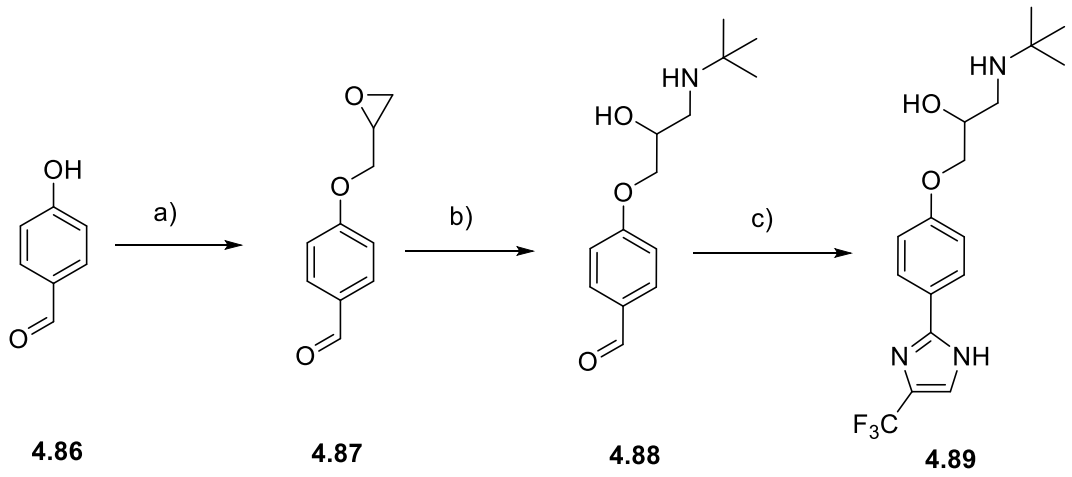
As a consequence an alternative route to **4.85** was explored which involved forming the imidazole in the last step.



Scheme 4.23: attempted synthesis of **4.85**.

Reagents and conditions: sodium acetate, H_2O, 7 N NH_3 in Methanol, 27%. b) BBr_3 DCM DCM -78 – 0 °C 89%. c) (±)epichlorohydrin, Cs_2CO_3, 60 °C.

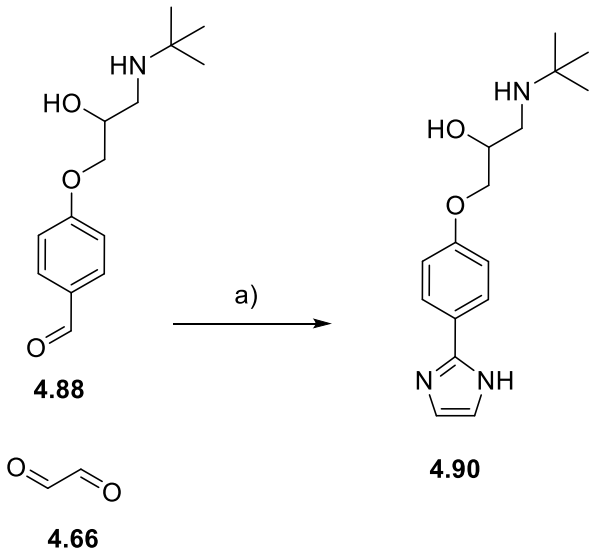
4-Hydroxybenzaldehyde was alkylated with epichlorohydrin and then reacted with *tert*-butylamine to form **4.88**. This was then reacted with **4.82** under the same conditions as previously used to form the CF_3 substituted imidazole. The primary amine analogue of **4.89** was also synthesised, however this was highly insoluble, and therefore could not be tested in the pharmacological assay.



Scheme 4.24: synthesis of **4.89**.

Reagents and conditions: a) (±)epichlorohydrin, Cs_2CO_3, 60 °C, 72%. B) *tert*-butylamine, Methanol 50 °C, 58% c) 4.67, sodium acetate, water, 7M NH_3 in Methanol, 37%.

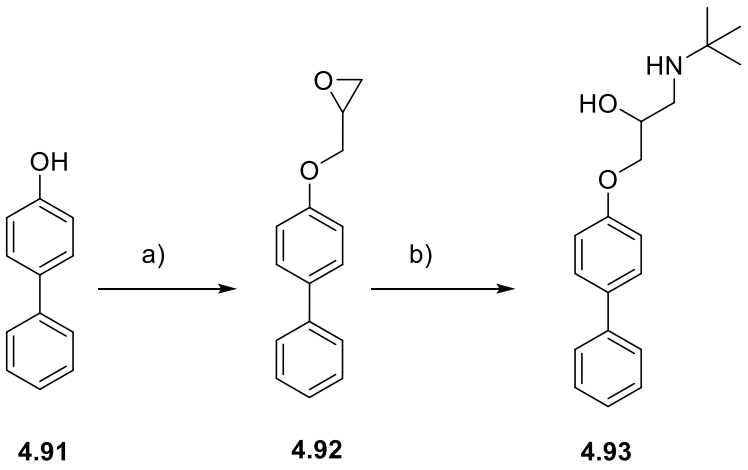
To synthesise **4.90**, compound **4.88** was reacted with glyoxal (Scheme 4.25). This gave a CGP 20712A headgroup analogue without the *N*-methyl or CF_3 groups. Regular phase chromatography was not viable for the purification of this compound, but HPLC successfully isolated **4.90**.



Scheme 4.25: synthesis of **4.90**.
Reagents and conditions: a) ammonium acetate Methanol, rt, 46%.

4.3.3 Synthesis of analogues with modifications to the size of the aromatic ring

The imidazole of the CGP 20712A headgroup was replaced by a phenyl ring, with no substituents to see if there was significant difference in its pharmacological profile compared to the parent headgroup. Little difference in this would indicate that the specifically substituted imidazole ring is not required for the selectivity of CGP 20712A, rather just a flat aromatic ring. To synthesise this analogue, the commercially available 4-phenylphenol was alkylated with epichlorohydrin, using caesium carbonate as the base in DMF. The epoxide was then opened with *tert*-butylamine to form **4.93** (Scheme 4.26).

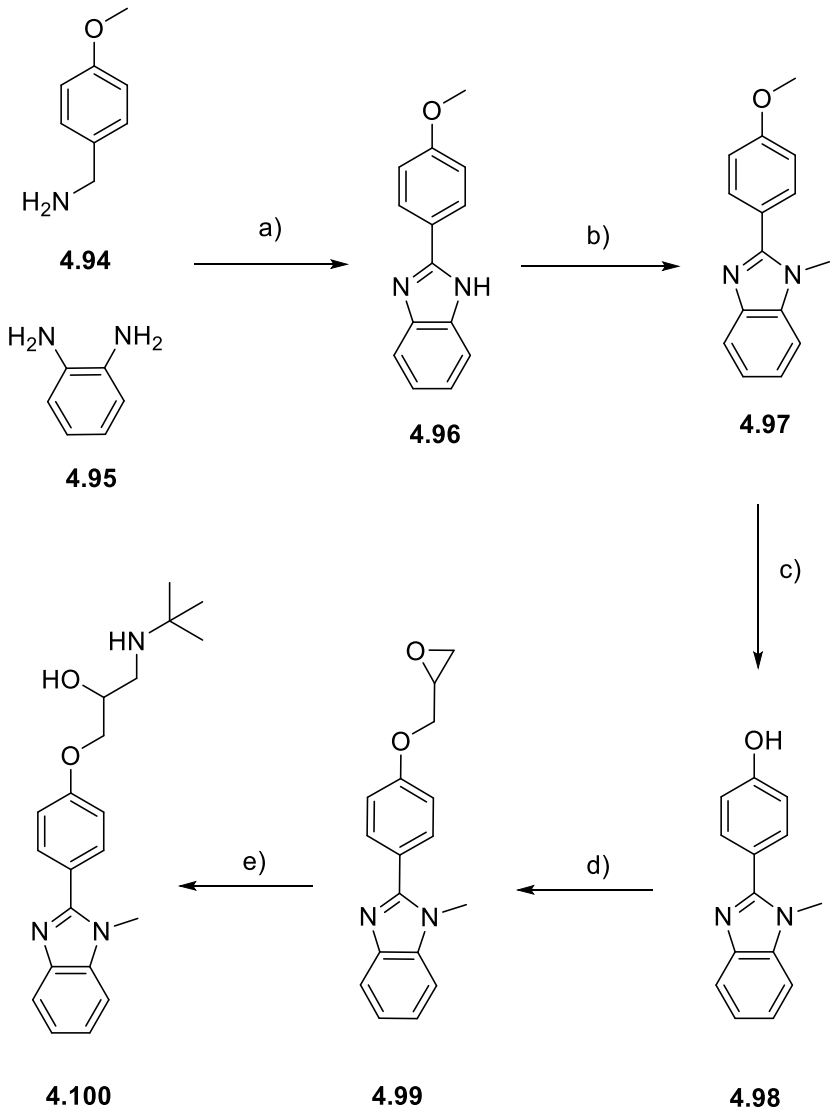


4.91 **4.92** **4.93**

Scheme 4.26: synthesis of **4.91**.

Reagents and conditions: a) (±)epichlorohydrin, Cs_2CO_3, 50 °C, 62%. B) *tert*-butylamine, rt, 63%.

An *N*-methyl benzimidazole analogue was also synthesised, which would have still have the imidazole moiety that is present in CGP 20712A, but with a larger surface, to see if this extended moiety can fit into the same binding region as the parent compound. The benzimidazole moiety is formed by reacting *o*-phenylenediamine, with 4-methoxybenzylamine, at 100 °C in toluene using copper(II) bromide as a catalyst (Scheme 4.27).[145] The subsequent steps follow the same synthetic route as the previous analogues.

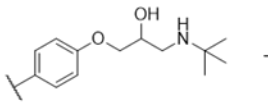


Scheme 4.27: synthesis of **4.100**.

Reagents and conditions: a) copper (ii) bromide, toluene, 100 °C, 17%. b) MeI, NaH, DMF, rt, 63% c) BBr_3, DCM -78 – 0 °C 98%. d) (±) epichlorohydrin, Cs_2CO_3, 50 °C, 64%. e) *tert*-butylamine, Methanol, 40 °C, 39%.

4.3.4 SAR of CGP 20712A headgroup pharmacology

Radioligand binding assays and SPAP functional assays were performed on each of the CGP 20712A headgroup analogues to find the affinities and activities of each of these compounds at the β_1 and β_2ARs. Each of the compounds was compared to **4.13**, to see how subtle changes to the moiety affect the pharmacological profile of the compounds. All pharmacological assays were performed by Prof. Jillian Baker (University of Nottingham).



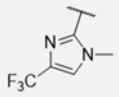
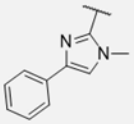
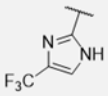
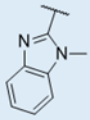
		Log K_d				
Cpd no.	R	β_1	n	β_2	n	β1 Selectivity
4.13		-5.63 ± 0.06	6	>-4	6	/
4.70		No binding	5	No binding	5	/
4.76		-6.59 ± 0.07	5	-5.31 ± 0.09	5	19
4.81		-4.99 ± 0.09	5	>-4	6	/
4.89		-8.00 ± 0.04	5	-7.59 ± 0.06	5	3
4.90		-6.07 ± 0.05	6	>-4	5	/
4.93		-6.46 ± 0.05	5	-5.97 ± 0.06	5	3
4.100		-5.49 ± 0.08	5	>-4	5	/

Table 4.4: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments.

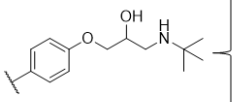
Compound **4.70** has the CF_3 group replaced by a proton and has methyl placed on the nitrogen. This not only removes the proton accepting ability of the nitrogen but also removes an electron withdrawing group from the imidazole. Consequently, this compound is unable to bind to either of the βARs, this could be due to either, or both of the alterations to the imidazole. Compound **4.76** has the CF_3 group replaced by a phenyl group. While this group is electron withdrawing, like CF_3, the phenyl group is much larger. The incorporation of this group increases affinity at both receptors,

compared to the parent compound (**4.13**). The increase in affinity could be due to the compound being more able to bind by the hydrophobic effect, or by making a π-π stacking interaction. The compound is just over 10-fold selective towards the β_1 AR. It is unknown whether this is more or less selective than the parent compound as the precise affinity of the parent compound (**4.13**) at the β_2AR is unknown, therefore the level of selectivity cannot be quantified. In order to better understand how necessary an electron withdrawing group at the 4-position is for binding, compound **4.81** has the CF_3 group replaced by a methyl group, which is slightly smaller and has no electron withdrawing properties.[146] As a result, the affinity is reduced at the β_1AR. Any change at the β_2AR is unknown, again due to limitations of the assay. This set of results has indicated that alkylation of the imidazole 4-position is important for binding, larger groups than CF_3 are tolerated and that groups of an electron withdrawing nature increase affinity.

Compound **4.89** has the largest increase in affinity at both receptors, compared to the parent compound (**4.13**), which is a result of removing the *N*-methyl group. This alteration remioves steric bulk and allows the nitrogen to become proton donating, thus, potentially making an additional interaction with an amino acid residue. This compound also has very low selectivity to the β_1AR. To further investigate the necessity of alkylation at the 4-position, compound **4.90** lacks both the CF_3 and *N*-methyl groups. This compound has higher affinity at the β_1AR, due to the demethylated N, but has approximately 100-fold lower affinity at the β_1AR than **4.89**. This further suggests that substitution at the 4-position is required for higher affinity at this receptor.

Compound **4.93** differs from the parent compound as the imidazole moiety is exchanged for a phenyl group. Although still aromatic, and not significantly larger than the imidazole, this moiety has a significantly different dipole moment as the only substituent is the other phenol group. Therefore, the electron density around this ring will be relatively even. As a result, the affinity at each receptor, compared to **4.13,** is increased and there is less selectivity towards the β_1AR. This demonstrates that a higher electron density around a specific part of the aromatic ring is important for preferential binding towards the β_1AR (in the case of the parent compound,

around the 4-position). Finally compound **4.100** has the parent compound imidazole moiety replaced by a benzimidazole. Though this moiety is larger than the parent compound, affinities are approximately the same, indicating that an increased size of the aromatic ring is tolerated, but offers no benefit.



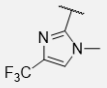
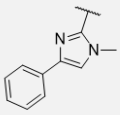
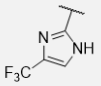
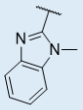
		Log EC_{50}				% Isoprenaline	
Cpd no.	R	β_1	n	β_2	n	β_1	β_2
4.13		No response	7	No response	7	/	/
4.70		No response	5	No response	5	/	/
4.76		No response	5	No response	5	/	/
4.81		No response	5	>-5	5	/	33.0 ± 5.9
4.89		-8.88 ± 0.06	4	-9.01 ± 0.09	4	92.7 ± 12.0	94.3 ± 3.1
4.90		-6.99 ± 0.12	5	-5.91 ± 0.08	5	76.4 ± 4.1	79.4 ± 6.2
4.93		-7.09 ± 0.06	5	-6.34 ± 0.11	4	17.6 ± 0.06	42.8 ± 5.7
4.100		No response	5	>-5	5	/	37.2 ± 6.3

Table 4.5: log EC_{50} values and % maximum isoprenaline responses of each compound at the β_1 and β_2 adrenoceptor from CRE-SPAP reporter gene assay. Values are mean±sem of n separate experiments.

Of the compounds that bind to the βARs, **4.89**, which has higher affinity than the parent compound, is the only one to have no level of activity at either receptor. Interestingly, **4.81** and **4.100** have low levels of β_2AR activity. These results suggest that the alkylation of the 4-position is not only necessary for binding, but the group

should be electron withdrawing (as with **4.13** and **4.76**) to stop the moiety being able to activate the β_2AR, if only at a low level.

The most intriguing result in this set of compounds is that removal of the *N*-methyl (**4.89**) not only greatly increases affinity at each receptor, compared to the parent compound (**4.13**), but it also becomes a full and highly potent agonist at both receptors. It is possible that this is due to the protonated nitrogen being able to interact with one of serine 203, 204 or 207. Interaction with one of these, as shown with catechol containing compounds, stabilises the active conformation of the β_1 and β_2AR. Alternatively, this compound may have a completely different binding mode from the catechol containing compounds, however, proof of either of these hypothesis would require mutagenesis studies. Compound **4.90** provides further evidence to these hypothesis as it is also an agonist at both receptors. The potency of this compound is lower than **4.89** but this is due to the lower affinity of this compound as it lacks alkylation at the 4-position.

As previously discussed, replacement of the imidazole moiety with the phenyl group in **4.78** increases affinity at each receptor. Additionally, this also results in the compound being active at the βARs. This could due to the lack of dipole moment, or it could have a different binding mode to the parent compound.

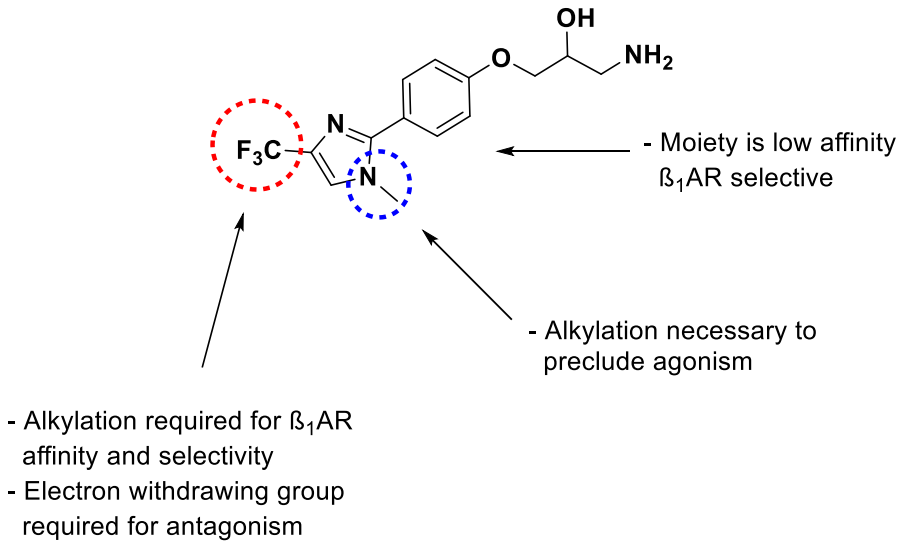


Fig. 4.8: SAR analysis of CGP 20712A headgroup

In conclusion, it appears that to have the best affinity with β_1AR selectivity, 4-position alkylation of the imidazole is required. It is also necessary for both affinity and for antagonism that this group needs to be electron withdrawing. Furthermore,

It has been shown that exchanging the *N*-methly with a protonated amine leads to βAR agonism. This is possibly due to interaction with an amino acid residue such as Ser-207.

4.4 Summary of CGP 20712A SAR

The highly selective β_1AR antagonist, CGP 20712A was divided into a head and tailgroup. Head group analogues were synthesied with various amino groups along with comparable analogues of other βAR antagonists, propranolol and ICI 89406. This study found that the incorporation of a *tert*-butyl group to the headgroups of propranolol and ICI 89406 increased affinity at both receptors 1000-fold compared to a primary amine. However, addition of the same amino group to the CGP 20712A headgroup, resulted in a 3-fold increase at the β_1AR and no measurable increase at the β_2AR compared to the primary amine. With the selected amino groups, the CGP 20712A headgroup was shown to be β_1AR selective.

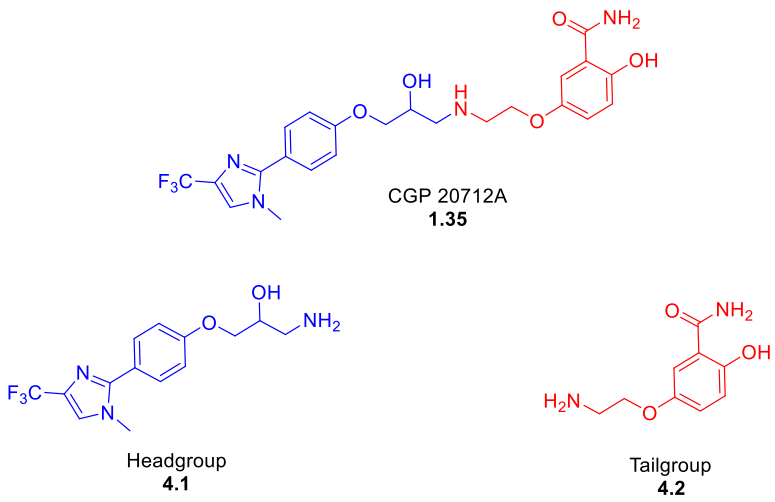


Fig 4.9: highly selective β_1AR antagonist, CGP 20712A divided into headgroup, which binds orthosterically and tailgroup which extends away from the orthosteric site

The tailgroup of CGP20712A was also synthesised and attached to the headgroups of propranolol and ICI 89406. These compounds had high affinity to both receptors, with slight selectivity to the β_2AR. This showed that the addition of this tail group to a nonselective headgroup does not make a β_1AR selective compound, rather it binds well to both receptors. However, it was shown that benzyl alkylation of the tailgroup phenol significantly reduced β_1AR affinity, but had little effect on the β_2AR affinity.

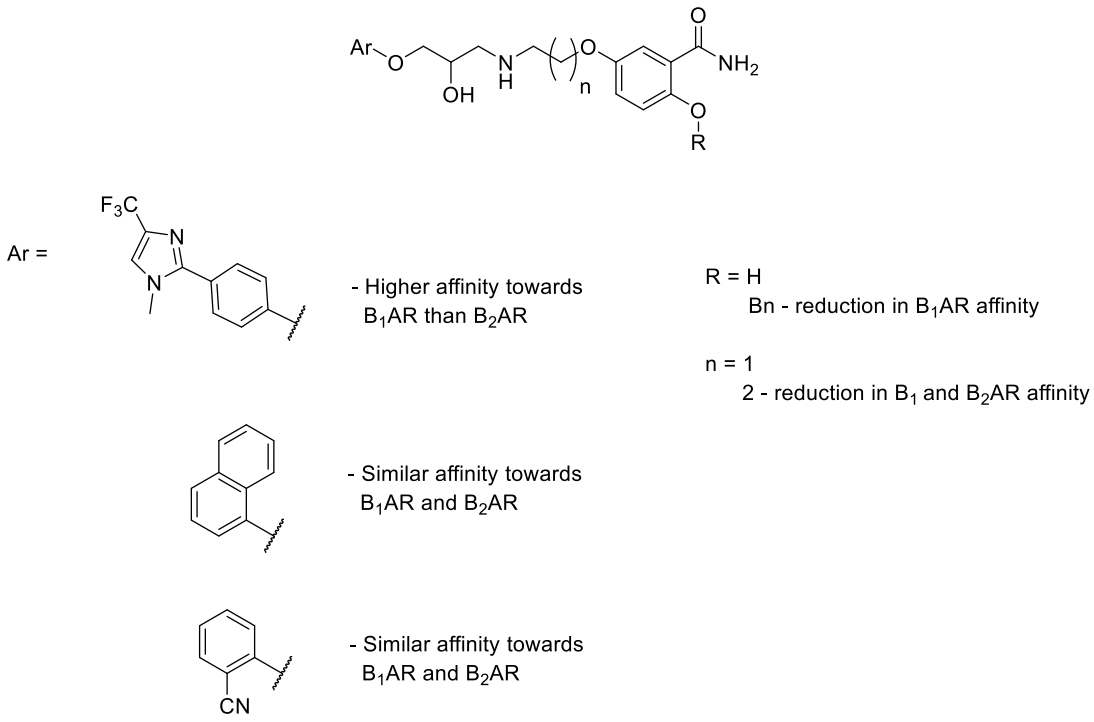


Fig 4.10: SAR summary of CGP 20712A analogues

The studies indicated that the headgroup of CGP 20712A is responsible for the β_1AR selectivity, therefore SAR studies were performed on this moiety. A number of analogues were synthesised with modifications to the imidazole. This study indicated a number of necessary features of the moiety which make it a β_1AR selective antagonist. 4-Position alkylation of the imidazole is necessary to increase affinity at the β_1AR. Furthermore, for the moiety to be antagonist, and increase affinity further, the substituent group is required to be electron withdrawing. Methylation of the nitrogen is also necessary for antagonism. If the nitrogen is protonated, the group becomes a full agonist. This is possibly due to an interaction with Ser-203, -204 or -207.

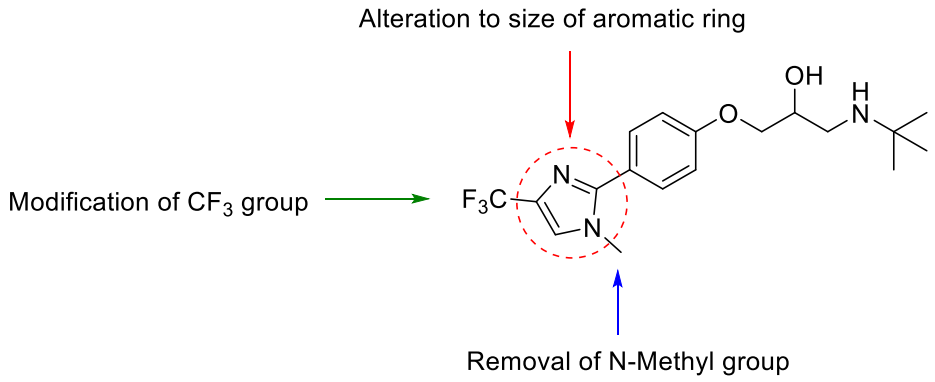


Fig 4.11: areas of investigation around CGP 20712A headgroup.

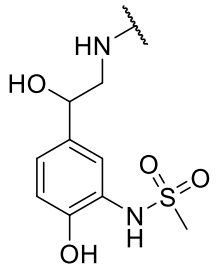
5.1 β_2AR agonist – β_1AR antagonist bivalent compounds

The initial aim was to combine a selective β_1AR antagonist with a βAR agonist (see Chapter 1.6). By looking into the SAR of CGP 20712A it was found that the headgroup of this compound preferentially bound to the β_1AR rather than β_2AR. The headgroup was also found to have low affinity for the β_1AR and therefore in a bivalent compound, may not be able to elicit an antagonist effect at the β_1AR if the agonist side of the compound has higher affinity. However, the tail group (which would, in this case be the agonist moiety in the bivalent compound) can have a great effect on the βAR affinity of the CGP 20712A headgroup, as seen in CGP 20712A itself. This headgroup was therefore attached to a βAR agonist moiety, to make a bivalent compound. A number of other antagonist headgroup compounds were also combined with an agonist for comparison.

5.1 βAR agonist headgroup

5.1.1 Synthesis of βAR agonist headgroup

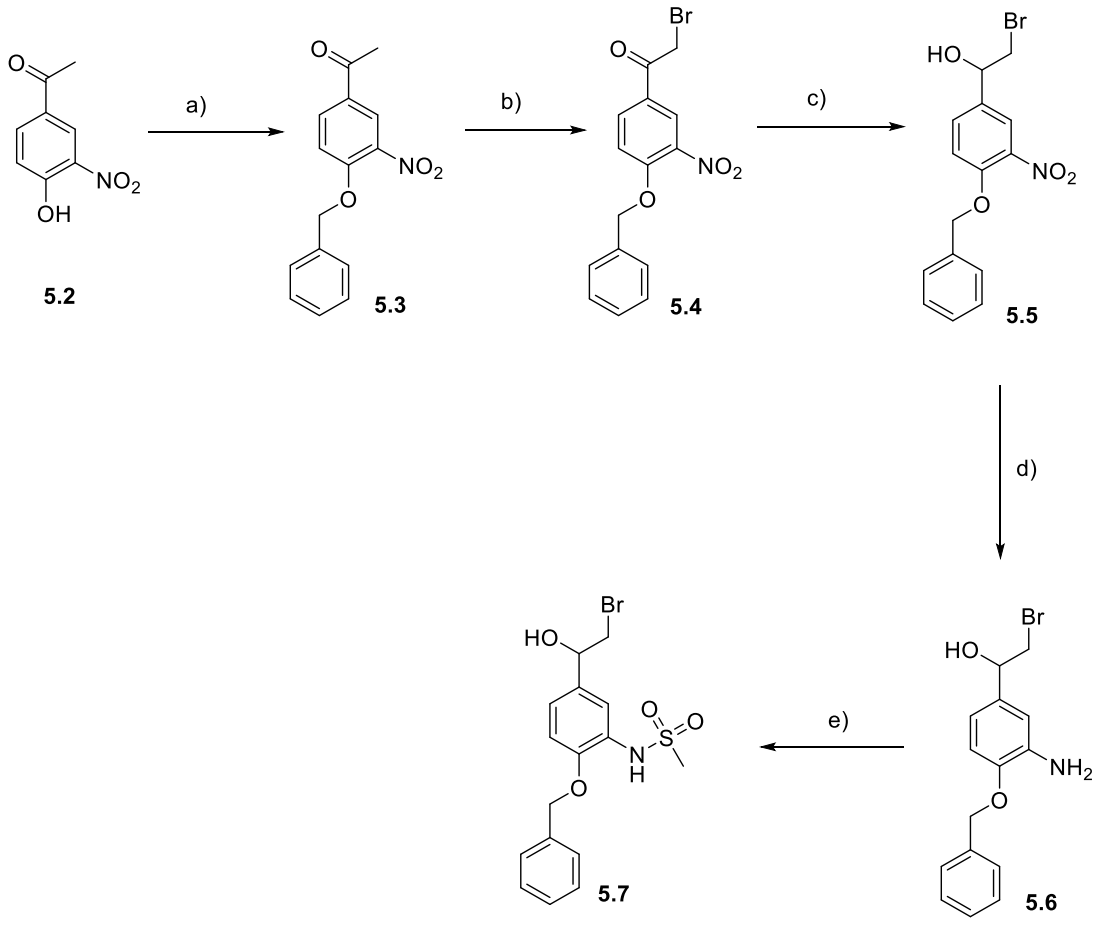
A benzothiazolone βAR agonist headgroup had previously been synthesised (see Chapter 3). However, due to multiple synthetic challenges and low yields which resulted in limited quantities of intermediate compound, an alternative βAR agonist group was selected for the bivalent compounds. The sulfonamide containing compound **5.1**, can act as a catechol mimetic and has the amino alcohol moiety found in adrenaline (**1.1**). This βAR agonist headgroup has been used in β_2AR agonist compounds such as Soterenol (**1.5**).[147]



5.1

Fig 5.1: agonist headgroup for the bivalent compounds

The initial synthetic route to the intermediate **5.7** was based on a synthesis developed by Huang *et al.* (Scheme 5.1).[77] The intermediate compound **5.7** could then be derivatised by nucleophilic substitution of the bromine.



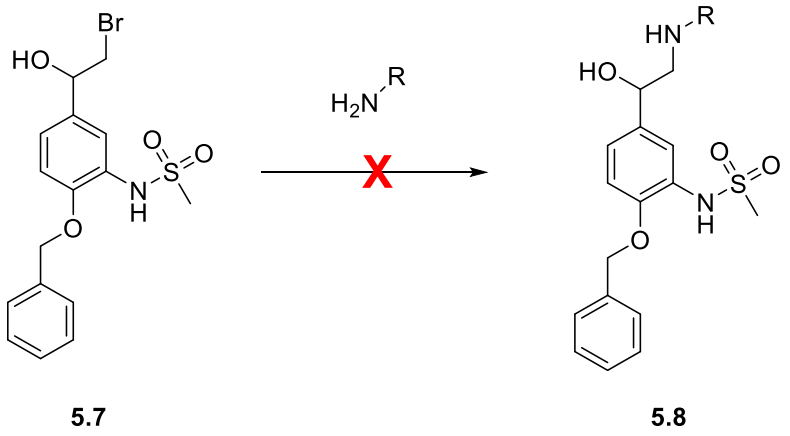
Scheme 5.1: suggested synthesis of **5.7**.

Reagents and conditions: a) BnBr, DIPEA. b) Phenyltrimethylammonium tribromide. c) $NaBH_4$ d) PtO_2, H_2 e) mesyl chloride, pyridine.

The first step in the synthesis of **5.7** is the benzyl protection of the phenol **5.2**, which could be removed in the final step, following substitution on the bromine. Alkylation of the phenol is carried out using benzyl bromide and DIPEA, both of the starting materials are soluble in the solvent (acetonitrile), but after cooling the reaction mixture, the product (**5.3**) precipitated out of solution. Purification is therefore simple as the pure product can be isolated by filtration.

In the subsequent step, the brominating agent phenyltrimethylammonium tribromide was used to form the bromo ketone, as in previous reactions (Chapter 3). This gives an inseparable mixture of the mono and dibromonated products. This was followed by the reduction of the ketone using sodium borohydride. The product of this reaction is difficult to purify due to the mixture left from the previous reaction, but **5.5** can be isolated by column chromatography, in a yield of 37% over the two steps. Reduction of the nitro group of **5.5** cannot be carried out using palladium on carbon, as this would also remove the benzyl protecting group. Therefore, the use of Adams catalyst (platinum(IV) oxide) was explored as this is known reduce nitro groups, but tolerates *O*-benzyl groups.[148] The reaction was carried out multiple times, however, the reaction would not go to completion, and following work up, starting material could be isolated along with the desired product **5.6**. The highest yield form this was 27%. Due to the low yield and incomplete reaction, the use of iron powder with ammonium chloride was also explored. This reaction is carried out under reflux in ethanol, and is significantly harsher than the previous conditions used. Consequently, TLC and LC-MS indicated that a number of compounds formed, none of which could be identified as the product. Therefore, at this point Adam's catalyst was continued to be used. Finally, to form the intermediate **5.7**, the aniline was reacted with methanesulfonyl chloride, with pyridine, which gave product in good yield.

To derivatise the intermediate, amines would be used to displace the bromine. Test reactions with a number of amines, such as *tert*-butylamine and benzylamine were carried out. However, none of these reactions gave significant amount of the desired products. A number of products formed and HPLC had to be used to separate them, however, none of these could be identified as the product.

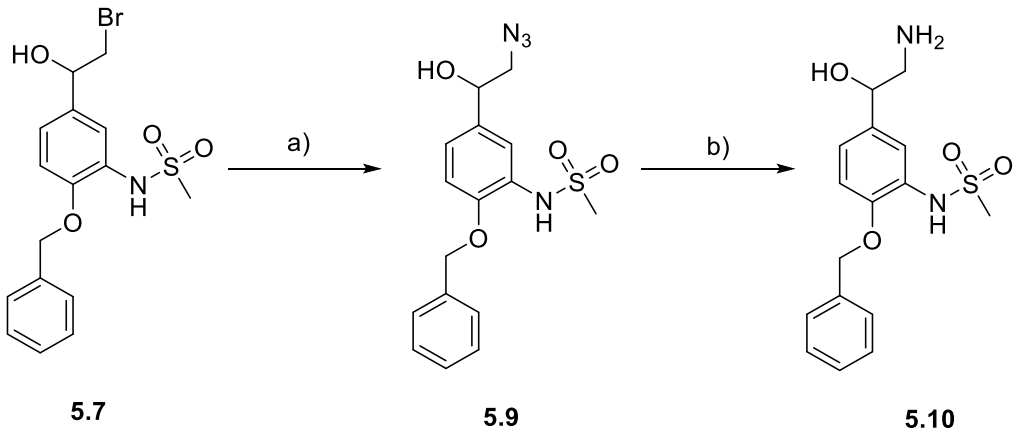


5.7 **5.8**

Scheme 5.2: attempted substitution of bromine with an amine.

Reagents and conditions: a) amine, Ethanol, 80 °C.

As substitution of the bromine with amines was unsuccessful, a different approach was taken. This involved the formation of the amino alcohol **5.10**, which could be used in subsequent reductive amination reactions, or used in the aminolysis of epoxides. The bromine was first displaced by an azide, which is a significantly better nucleophile than the amines that were previously used. The azide was able to displace the bromine without the formation of major side products and a good yield was obtained. Reduction of the azide to the amine could not be carried out with pallidum on carbon and hydrogen as this would remove the benzyl protecting group which was necessary in subsequent steps. A Staudinger reduction was therefore employed (Scheme 5.3). This reaction uses triphenylphosphine to form an intermediate phosphazine with the azido group. N_2 is then lost, and an aqueous work up forms the amine and triphenylphosphine oxide as a by-product. The amine was formed in satisfactory yield, however, the triphenylphosphine oxide by-product could not be completely removed from the desired product by regular phase chromatography, and therefore reverse phase chromatography was also used to isolate the product (**5.10**).

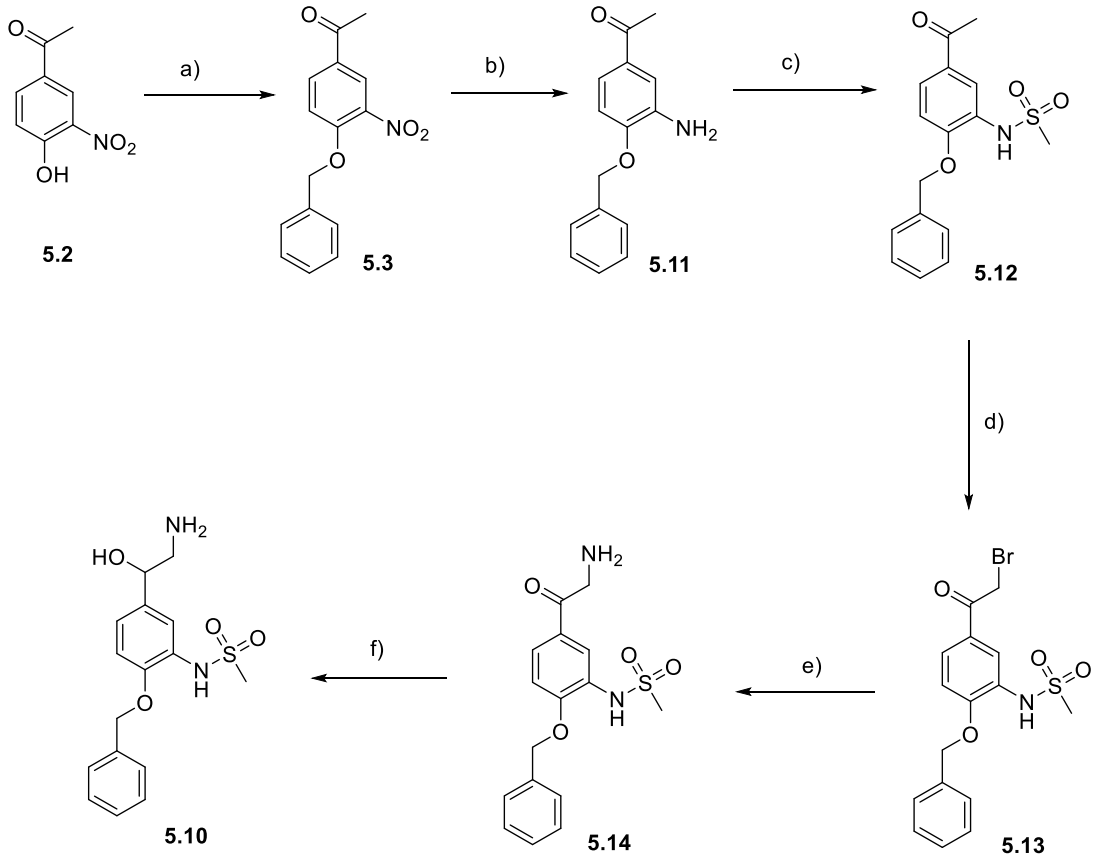


5.7 **5.9** **5.10**

Scheme 5.3: synthesis of **5.10**.
Reagents and conditions: a) Sodium azide, DMF 80 °C, 80%. b) Triphenylphosphine, THF, 38%.

The intermediate compound (**5.10**) was resynthesized, but the synthetic route was revised (scheme 5.4) in an attempt to increase yields and simplify purification in a number of steps. The initial step remained the same as in Scheme 5.1; the phenol was benzyl protected, and a high yield was obtained. Rather than performing the bromination reaction in the next step, this was done later in the synthesis, so that the reactive bromine would not be present in as many reactions, this may reduce the formation of side products. The second step was therefore the reduction of the nitro group **5.3** to the aniline **5.11**. This reaction had initially been performed using Adam's catalyst, however, reactions did not go to completion and low yields were obtained. Consequently, different reducing agents were explored. The use of tin(II) chloride was attempted in which the reaction was heated in ethanol. This gave yields of 32-40%. The solvent was exchanged for methanol, and an increased yield of 57% was obtained. Although this was adequate, the work up and purification of this reaction was laborious. Following the removal of the solvent by reduced pressure, a residue forms which was then extracted with ethyl acetate. However, an emulsion forms between the organic and aqueous layers and traps product within it. This residue can be collected and adsorbed onto silica, which is then washed with chloroform to obtain the product. This is viable on small scale, but less so on the larger scale that was being carried out (>10 g).

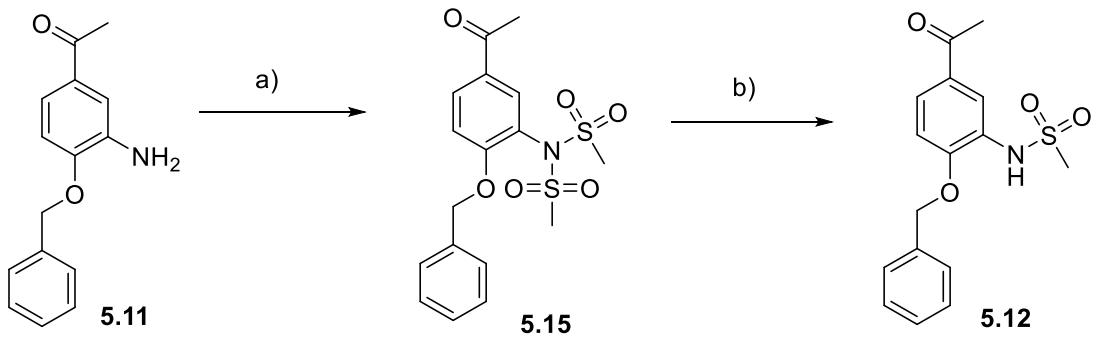
Due to the difficult work-up of the tin(II) chloride reduction, the reduction of the nitro group (**5.3**) was attempted with sodium dithionite and potassium carbonate. However, no reaction occurred. Finally, ammonium chloride and zinc powder were used to reduce the nitro group. This uses mild reaction conditions as it does not require heating. The aniline was found to form within hours, and, to work up the reaction, the zinc was filtered under reduced pressure. The filtrate was then concentrated *in vacuo* and water was added to the residue. This dissolved excess ammonium chloride and pure product precipitated out. A yield of 96% was obtained which was significantly higher than previous reactions.



Scheme 5.4: alternative synthesis of **5.10**.

Reagents and conditions: a) BnBr, DIPEA, MeCN, reflux, 99%. b) ammonium chloride, zinc powder, Methanol, THF, rt, 96%. c) mesyl chloride, pyridine, DCM, rt, 93%. d) Phenyltrimethylammonium tribromide, THF, 65 °C. e) hexamine, $CHCl_3$, rt, Methanol,hydrochloric acid, 60 °C, 51% over 2 steps. f) $NaBH_4$, Methanol, rt, 77%.

The formation of the sulfonamide was carried out using the same conditions as in the previous synthetic route, using mesyl chloride and pyridine to obtain the product in excellent yields of up to 93%. During the workup of this reaction, the volatiles are removed under reduced pressure, however, mesyl chloride is highly toxic. Therefore, during the workup 2 M aqueous KOH was added in an attempt to destroy any excess mesyl chloride. Unfortunately, this resulted in deprotonation of the sulfonamide and the subsequent addition of a second sulphonyl group to form **5.15** (Scheme 5.5). While this is an inefficient way of making the product **5.12**, the second sulfonyl group can be removed by heating **5.15** in 3 M aqueous NaOH. To avoid this step of the reaction, excess reagents were simply removed under reduced pressure and then destroyed once isolated.



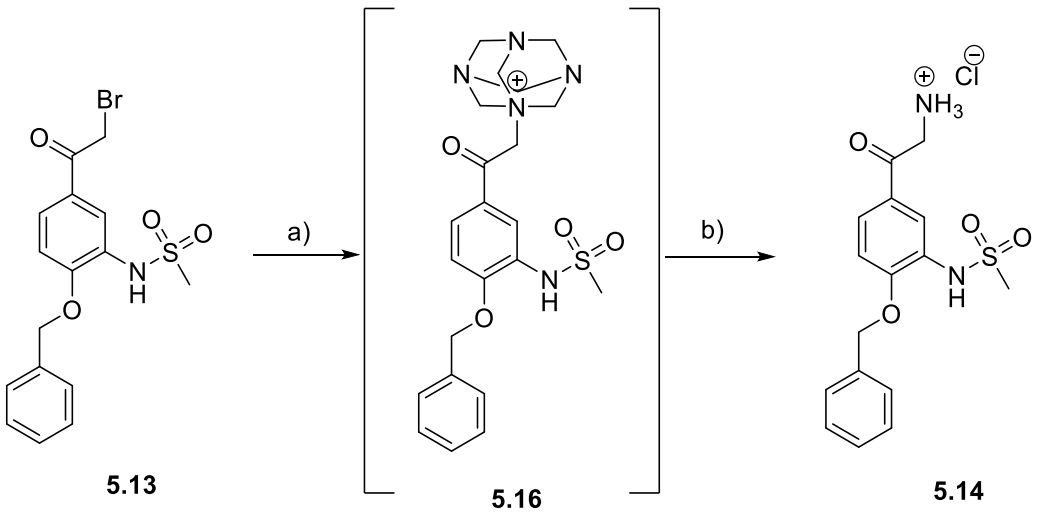
Scheme 5.5. di-addition of sulphonyl groups.

Reagents and conditions: a) mesyl chloride, pyridine, DCM, 2 M KOH, rt. b) 3 M NaOH, H_2O 80 °C, 34%.

In the previous synthetic route, bromination had been carried out using the brominating agent phenyltrimethylammonium tribromide. This gave a mixture of the desired product, dibromide, and starting material in a ratio of 5:2:2. While the dibromide product is not unexpected, due to the product forming a reactive enolate (see chapter 3.1.1), a different brominating agent was investigated to see if the ratios could be improved. The use of copper(II) bromide is reported to give selective bromination in good yields on acetophenones.[149] This is carried out in a 1:1 mixture of ethyl acetate and chloroform at 75 °C. The desired product was formed in this reaction and there was no indication of the dibromide product forming. However, following purification of the product, a low yield of 14% was obtained. Therefore,

although phenyltrimethylammonium tribromide gives an inseparable mixture of compounds at this point in the synthesis, it appeared to be the better option due to the higher yield. The reaction was performed at various temperatures from room temperature to 65 °C, however this made no difference to the ratios obtained.

Previously, to form the amine **5.10** from the bromine **5.7**, the bromine was displaced by azide which was then reduced using the Staudinger reduction. The issue with this approach is that this generates triphenylphosphine oxide which is difficult to completely remove from the product. Additionally, the bromo ketone **5.13** is a mixture of the dibromide product and starting material. Therefore, this may have caused further issues in the purification of the azide. Therefore, a different method, using the Delepine reaction was explored.[150] In this reaction impure **5.13** and hexamine, are dissolved in chloroform (all starting materials are soluble). The mixture was stirred at room temperature and after 10 minutes the quaternary amine intermediate **5.16** began to precipitate out of solution. Full conversion to the intermediate was complete within three hours and the pure intermediate compound can be filtered under vacuum. This removes the dibromide and starting material left over from the previous reaction, which remain dissolved in the filtrate. The solid is then dissolved in a mixture of methanol and concentrated hydrochloric acid. The solution is heated at 65 °C which results in acid hydrolysis of the hexamine moiety to form the amine product **5.14** and formaldehyde (Scheme 5.6). Once complete decomposition to the product occurred, the volatiles were simply removed under reduced pressure and the remaining solid was washed with a small amount of cold methanol to obtain pure product.



5.13 **5.16** **5.14**

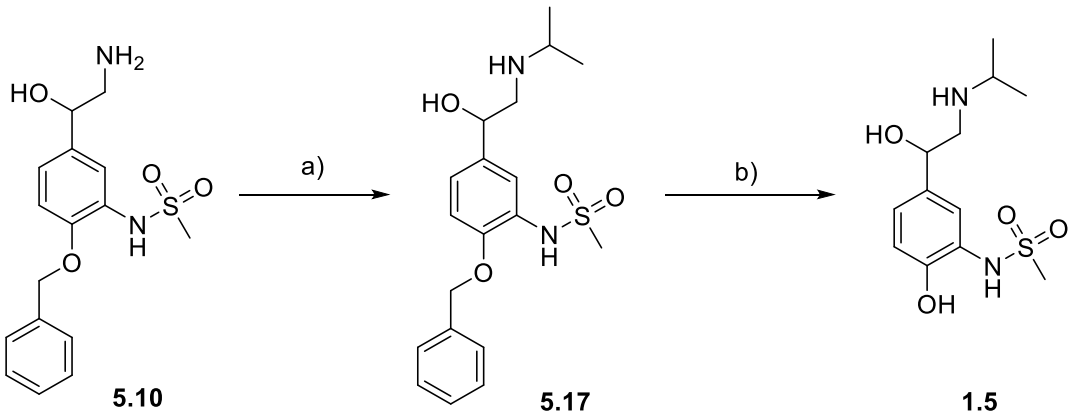
Scheme 5.6: conversion of bromo ketone to aminoketone *via* hexaminium intermediate.
Reagents and conditions: a) hexamine, $CHCl_3$, rt. b) Methanol,hydrochloric acid, 60 °C, 51% over 2 steps.

It was found that the aminoketone **5.14** is unstable when it is not a salt. Addition of a small amount of DIPEA to **5.14** within a solvent causes the solution to turn from clear to pink, and LC-MS indicated that the product degrades into a number of compounds. However, it was unnecessary to desalt **5.14** prior to the subsequent step which was the reduction of the ketone to from the amino alcohol intermediate (**5.10**). The ketone is reduced using the standard reducing agent, sodium borohydride. Full conversion of the ketone occurred, but the workup was found to be problematic. Following the addition of ammonium chloride solution to quench the reaction, the solution was extracted but the product has low solubility in organic solvent, regardless of the pH of the aqueous layer. The use of the polar solvent mixture chloroform-isopropyl alcohol (2:1), resulted in 28% yield. The product would be more water soluble at lower pH due to the salt formation with the amine, therefore in a following attempt, methanol was removed under vacuum and the remaining aqueous solution was basified to pH 10 with sodium hydroxide. Precipitate formed and was filtered. This gave a yield of 45%. The same procedure was carried out a second time, however the pH was adjusted to pH 7, as it was thought that a basic solution could deprotonate the sulfonamide. This again resulted in a precipitate of the pure product, in an increased yield of 57%. Although this yield was satisfactory, TLC and LC-MS indicated that the reaction was proceeding cleanly, and so a higher

yield should have been obtained. The lower yields were thought to be a result of the product retaining some level of water solubility, regardless of the pH. Accordingly, the quench of the reaction and precipitation of the product was carried out using as little aqueous solution as possible. A few drops of concentrated hydrochloric acid were used to quench the reaction, and then left to stir for 30 minutes. The product precipitated out of the methanol solution, of which it is highly insoluble. Following filtration of the product a good yield of 77% was obtained.

5.1.2 Validation of βAR agonist

In order to validate that the sulfonamide containing headgroup **5.1** as a βAR agonist, amino alcohol **5.10** was alkylated with an isopropyl group which is a typical βAR agonist amino group. The isopropyl group was incorporated into **5.10** by a reductive amination using acetone, and sodium borohydride as the reducing agent. Acetone (1.1 equivalents) were used in an attempt to preclude the formation of the tertiary amine product. Consequently, **5.17** formed and was isolated in good yield. The benzyl group of **5.17** was then removed to form the catechol mimetic moiety (Scheme5.6). Debenzylation was performed using palladium on carbon under a hydrogen atmosphere and product **1.5** was isolated in a good yield of 81%. Compound **1.5** was expected to be validated as a βAR agonist in functional CRE-SPAP and radioligand binding assays, as it is one of the first generation agonist, soterenol. The benzylated analogue of this, **5.18**, was also tested in the pharmacological assays to see what effect the benzyl group has on βAR affinity. It was expected that this group should stop the interaction of the phenol with Ser-207 and reduce affinity at both receptors as seen in Chapter 3.3.2, when the phenol was methylated. Pharmacological assasys in this chapter were carried out by Prof. Jillian Baker (University of Nottingham).



Scheme 5.6: synthesis of β_2AR agonist.

Reagents and conditions: a) acetone, $NaBH_4$, Methanol, 61% b) Pd/C, H_2, Methanol, H_2O, AcOH, 81%.

		Log K_d				
	R	β_1	n	β_2	n	β_2 Selectivity
5.17	Bn	-5.54 ± 0.07	5	-5.58 ± 0.14	5	1
1.5	H	-5.48 ± 0.08	5	-6.16 ± 0.02	5	5

Table 5.1: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log K_d) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem of n separate experiments.

		Log EC_{50}				% Isoprenaline	
	R	β_1	n	β_2	n	β_1	β_2
5.17	Bn	No Response	5	-6.47 ± 0.22	3	/	67.3 ± 5.3
1.5	H	-6.26 ± 0.12	4	-7.96 ± 0.09	4	80.1 ± 10.9	97.1 ± 9.2

Table 5.2: log EC_{50} values and % maximum isoprenaline responses of each compound at the β_1 and β_2 adrenoceptor from CRE-SPAP reporter gene assay. Values are mean±sem of n separate experiments.

Compound **1.5** binds to both receptors and is a full agonist at the β_2AR, while being a strong partial agonist at the β_1AR, therefore this group can act as the βAR agonist component of a bivalent compound. Interestingly the addition of the benzyl group to the headgroup did not greatly reduce the affinity of the compound to either βAR. Compared to compound **1.5**, **5.17** only has a slight reduction in affinity towards the β_2AR and maintains the same affinity at the β_1AR. What is more intriguing is that while **5.17** has reduced efficacy at the β_2AR (now a partial agonist, rather than full agonist), efficacy is completely lost at the β_1AR, which makes this compound a partial agonist at the β_2AR but an antagonist at the β_1AR. The aim of this project was to develop a compound that has affinity to both the β_1 and β_2AR while only being active at the β_2AR. Therefore, as **5.17** has this profile it's orthosteric binding moiety was combined with a range of antagonist groups. The headgroup of **1.5** was also used in the bivalent compounds.

5.2 Synthesis of bivalent βAR agonist-antagonist compounds

The β_2AR agonist headgroup group **5.1** was linked to a number of antagonist headgroups such as that of propranolol (**5.18**). Both of these structures contain an amino alcohol moiety, and so rather than link the compounds *via* an alkyl chain, as presented in Chapter 1.3, the two groups were attached directly to form an amino diol moiety, such as in **5.20**.

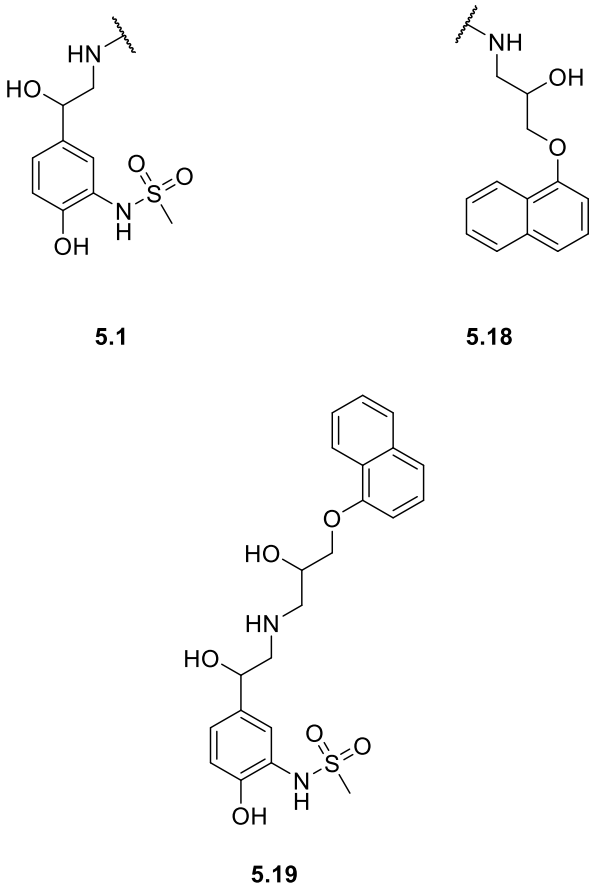
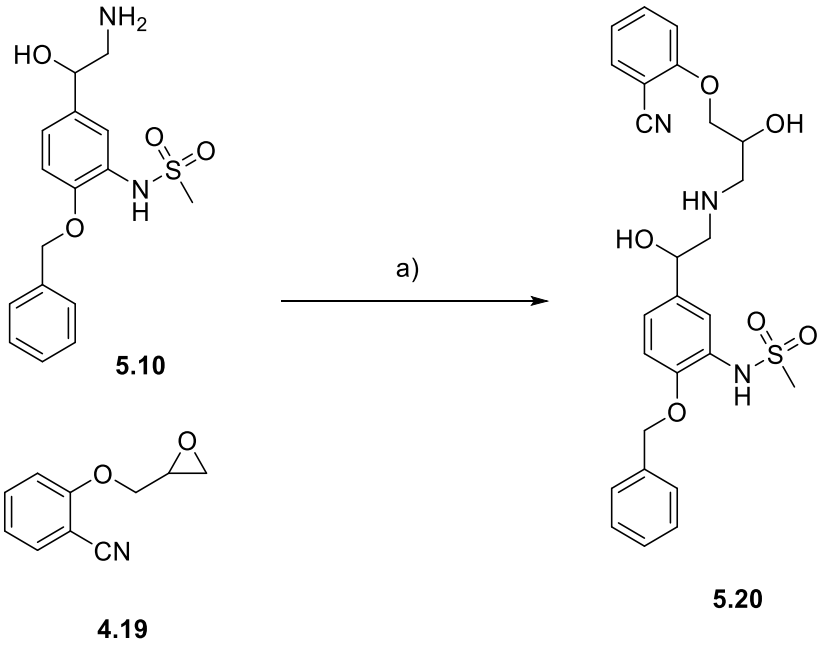


Fig 5.2: agonist headgroup (**5.1**), antagonist headgroup (**5.18**) and bivalent compound (**5.19**)

5.2.1 Aminolysis of antagonist headgroup epoxide with agonist headgroup amine

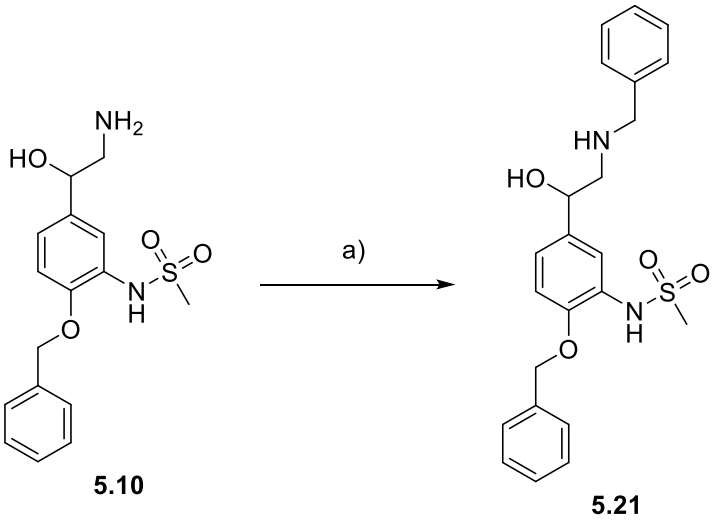
To synthesise these compounds, **5.10** was used to open the epoxide intermediates of the antagonist compounds, which were described in Chapter 4.1.2. In the first instance **5.10** was used to open the epoxide intermediate of ICI 89406 (**4.19**). Compound **5.10** has low solubility in most solvents and therefore the reaction was performed in a number of solvents along with DIPEA as the base to explore conditions that gave the best results. The use of ethyl acetate and isopropyl alcohol gave no product by LC-MS, whereas in DMF a small amount of product formed. The only solvent in which a satisfactory amount of product (**5.20**) formed was in ethanol,

despite the starting material having very low solubility in this solvent. A low yield of 30% was obtained following purification by column chromatography.



Scheme 5.7: synthesis of ICI 89406 bivalent compound.
Reagents and conditions: a) DIPEA, ethanol, 80 °C, 30%

In an attempt to increase the yield and avoid the *bis*-addition of epoxides onto the amine, amino alcohol **5.10** was benzylated to form **5.21**. Benzylation was carried out by reductive amination with benzaldehyde and a number of conditions were explored to find the most ideal procedure.



5.10 **5.21**

Scheme 5.8: synthesis of ICI 89406 bivalent compound.

Reagents and conditions: a) benzaldehyde, $NaBH_4$, Methanol, 22%

Initially, **5.10** was mixed with benzaldehyde and triacetoxy borohydride, as discussed in Chapter 4.2.2, this is one of the milder reducing agents and is more selective to reducing imines than aldehydes. This is important for minimising reduction of the benzaldehyde starting material. This reaction was initially carried out with a small amount of acetic acid in dichloroethane (DCE). However, no reaction occurred. Compound **5.10** was very insoluble in DCE and this could preclude the formation of the imine intermediate that is required to form the product. Compound **5.10** was soluble in DMF, and therefore the reaction was repeated, using DMF as the solvent. Product formed, however, the unwanted *bis*-product (**5.23**) also formed. Column chromatography was carried out to try and separate the two compounds, but due to having a very similar R_f these two compounds were inseparable.

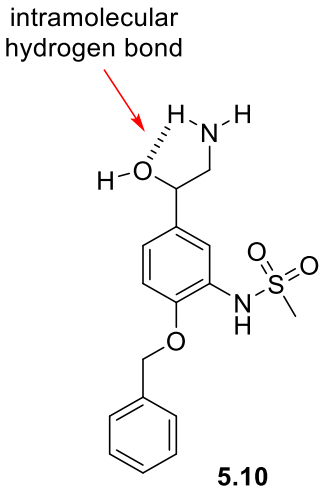
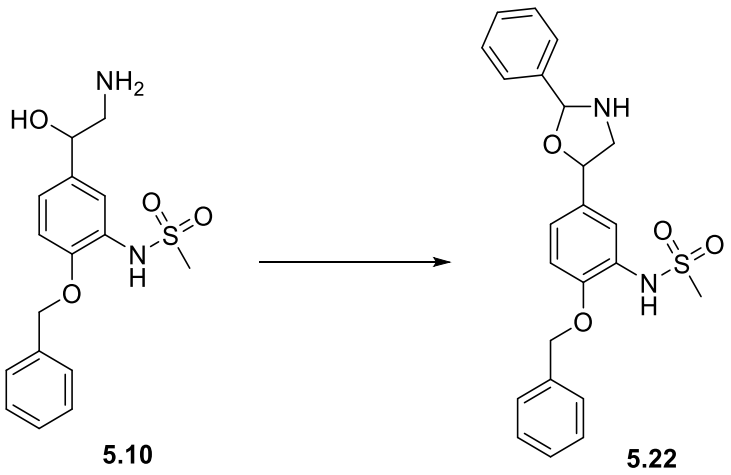


Fig 5.3: Intramolecular hydrogen bond within the amino alcohol moiety

Reductive amination reactions that were conducted in Chapter 4.2.2 were found to give the best yields using sodium borohydride. As this is a stronger reducing agent, the two starting materials were stirred together for 16 hours, to allow the imine to form and then the reducing agent was added. The product formed and was isolated, however, a low yield of 22% was obtained. The low yield could due to a number of factors. As before, the low solubility of the starting material may stop imine formation and sodium borohydride is able to reduce any free benzaldehyde. Alternatively, following imine formation between **5.10** and benzaldehyde, rather than reduction of the imine bond, to form the product, the hydroxyl group may react with the imine to form oxazolidine **5.22**. Although this compound was not isolated, it is possible that during the acidic work up, the compound is hydrolysed to reform the starting material (**5.10**). Additionally, the amino alcohol moiety may able to form an *intra*-molecular 5-membered ring. The additional stability of this moiety, due to the hydrogen bond, may make the formation of the imine less favourable. Finally, to try and increase the yield, sodium cyanoborohydride was used. Again, product formed and could be isolated, in this case a slightly increased yield of 32% was acquired and so in this case appeared to be the best option, although a low yield was still obtained.



Scheme 5.9: formation of oxazolidine

The reductive amination was also carried out on the amino ketone **5.13**, to see if this alleviated solubility issues and the formation of the *bis*-product (**5.23**). However, this reaction did not form any product. Within the reaction the starting material has to be desalted and as previously discussed, this causes the compound to degrade. Due to the low yields when performing reductive aminations, alkylation of the amine using benzyl bromide was attempted. However, as expected, due the increased nucleophilicity of the secondary amine **5.21** compared to the primary amine **5.10** the major product was the *bis*-product, **5.23**.

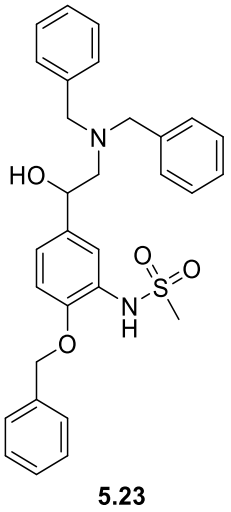
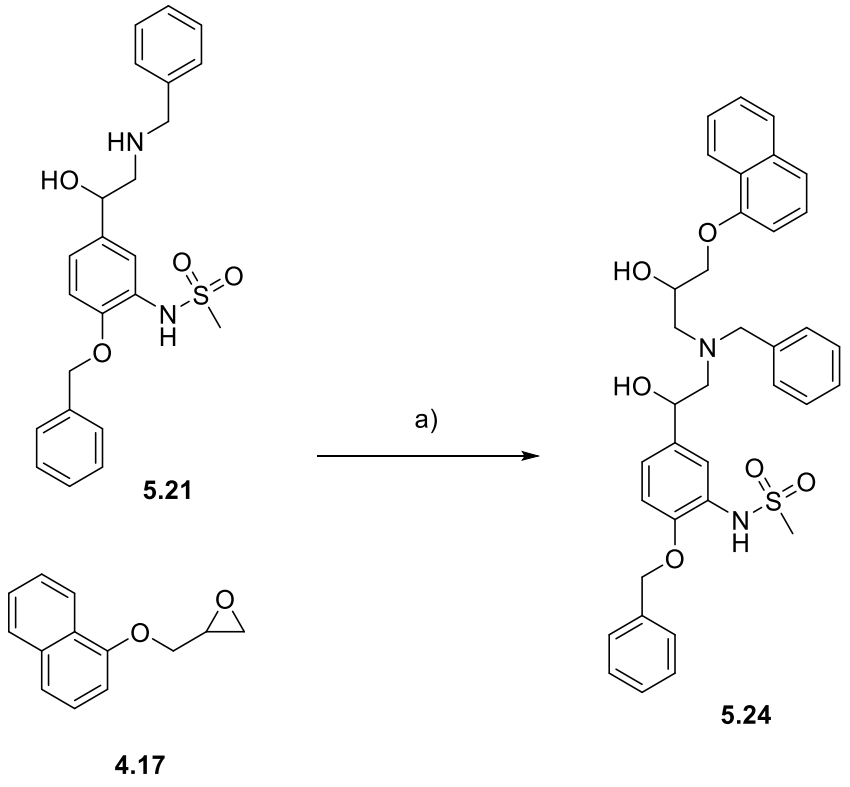


Fig 5.4: *bis*-benzylated product of reaction with benzyl bromide

The benzylated headgroup **5.21** was used to open the epoxide intermediate of propranolol (**4.17**). A 1:1 ratio of epoxide and amine can be used in an aminolysis reaction when the benzylated compound **5.21** was used, whereas multiple equivalents were required when using a primary amine, to prevent di-alkylation of the amine. The use of multiple equivalents was avoided as the headgroup intermediate took many steps to synthesise, and so it was preferable not waste any. The reaction, as in the previous reaction with the primary amine **5.10** and propranolol headgroup (**4.17**) were heated in ethanol, along with DIPEA. Unfortunately, although product formed, both silica chromatography and HPLC were required to isolate the product, and it was obtained in a lower yield than the primary amine (22%).

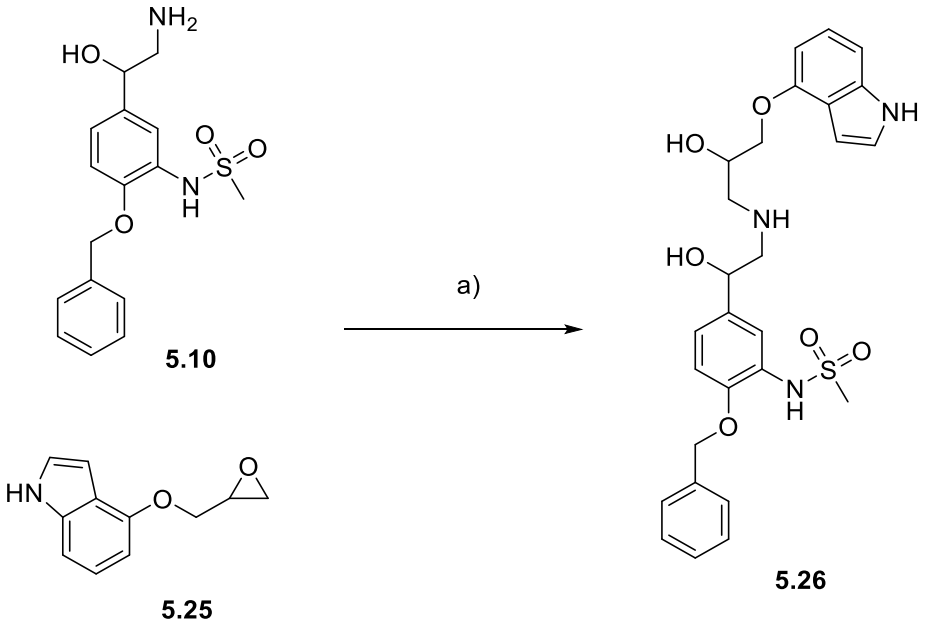


Scheme 5.10 synthesis of dibenzylated propranolol bivalent compound.
Reagents and conditions: a) DIPEA, ethanol, 80 °C, 22%

Due to the low yield of the aminolysis reaction and the low yield when benzylating the amine **5.10**, the opening of the epoxides was continued with the primary amine **5.10**. Although this reaction would require multiple equivalents of the amine, it was

thought that overall this would use less of the amine **5.9** in the one reaction, than when conducting two.

The aminolysis of the pindolol epoxide intermediate (**5.25**) and CGP 20712A intermediate (**4.9**) were performed using two equivalents of **5.10** and otherwise the same conditions as the previous reactions. Both products formed and were purified by column chromatography or HPLC. Yields of these reactions varied between 9 and 49%.

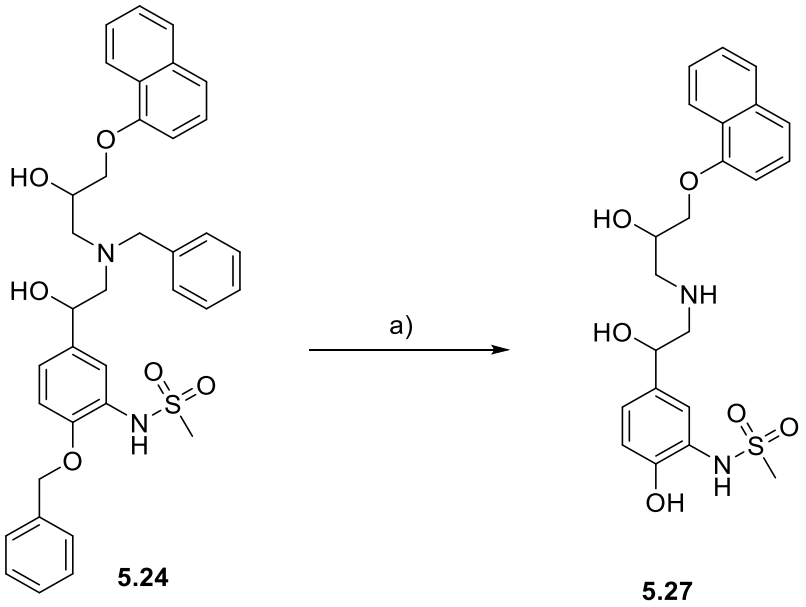


Scheme 5.11 synthesis of benzylated pindolol bivalent compound.
Reagents and conditions: a) DIPEA, ethanol, 80 °C, 13%

5.2.2 Removal of Benzyl Protecting Group

The first debenzylation in this set of compounds was performed on **5.24** which was the only compound within this series that was benzyl protected on both the phenol and amine. This was performed using palladium on carbon under a hydrogen atmosphere. The starting material was soluble in THF and so a solvent mixture of THF, water and acetic acid was used. Following filtration of the palladium and

removal of solvents, NMR indicated that the product was approximately 90% pure. HPLC was therefore used to purify **5.27** which was obtained in a yield of 44%.



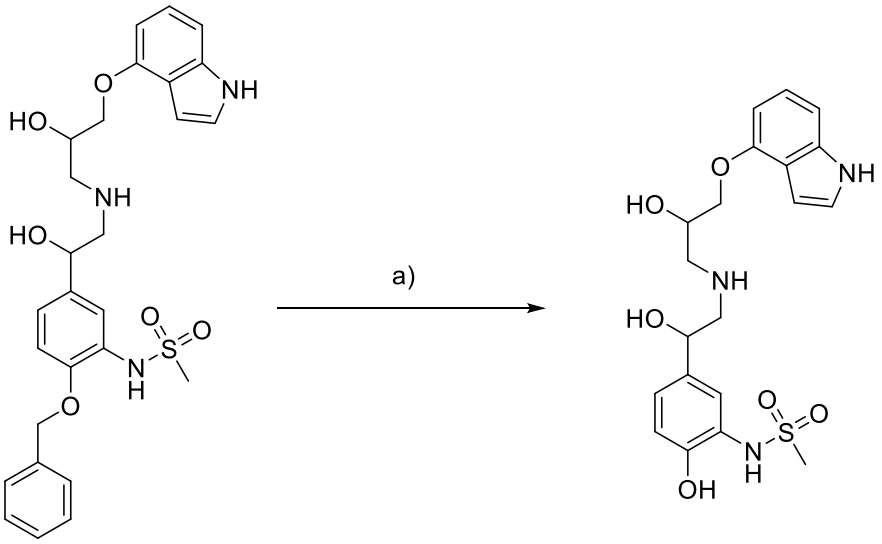
5.24 **5.27**

Scheme 5.12: benzyl deprotection of propranolol bivalent compound.
Reagents and conditions: a) Pd/C, H_2, THF, H_2O, AcOH, 44%.

The other benzylated bivalent compounds **5.20**, **5.26** and **5.30** were all initially deprotected using the same procedure as for **5.24**. For each of these, LC-MS indicated that the starting material had been consumed and that the product had formed cleanly (by the presence of only one peak). However, NMR indicated there was a mixture of compounds in each case. HPLC was performed, which at first, showed one major peak, but alterations to methods showed that the peak could be split into two, indicating the presence of two compounds. Regardless of HPLC method optimization, these two compounds could not be separated. Upon further analysis of the LC-MS spectra, each peak that contained the expected product ion, also contained a second ion that had a mass of 72 higher than the expected mass. Unfortunately in this set of compounds, the unknown compound could not be isolated and characterised (see Chapter 5.4.1).

Due to the formation of the inseparable side product, a different solvent was used for the hydrogenation of the pindolol containing bivalent compound (**5.26**). In this

case, a solvent mixture of ethyl acetate, water and acetic acid was used. This reaction was successful, and none of the unknown side product appeared to form, making purification by HPLC significantly easier than in the previous attempt. The product **5.28** was obtained in a modest yield of 27%.



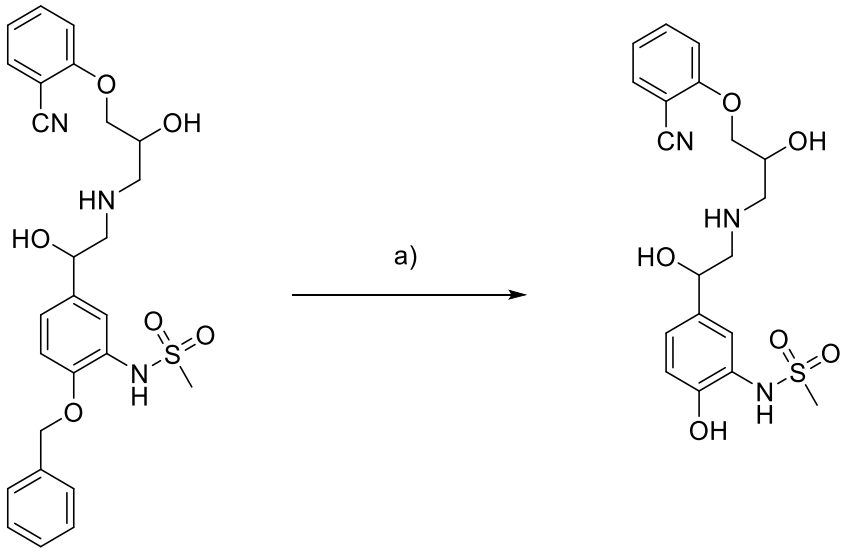
5.26 **5.28**

Scheme 5.13: benzyl deprotection of propranolol bivalent compound.

Reagents and conditions: a) Pd/C, H_2, ethyl acetate, H_2O, AcOH, 44%.

As the previous reaction was successful, the same conditions were used to debenzylate **5.20**. LC-MS indicated that all of the starting material had been consumed and that there was only one major product. However, the mass did not correspond to that of **5.29**. The product was isolated by HPLC, however NMR also indicated that this was not the expected product, due to an extra peak observed at 4.42 ppm. Although the structure of this compound could not be defined, it was hypothesised that under these conditions, the nitrile was reduced to the amine, and then a second, unknown reaction occurred. To avoid the reduction of the nitrile, different hydrogenation conditions were used. A transfer hydrogenation was performed by heating compound **5.20** in methanol along with palladium on carbon and ammonium formate. This reaction appears to be more selective towards debenzylation than reduction of nitriles as none of the side product formed. Silica

chromatography and reverse phase HPLC were used to isolate the product **5.29** in a yield of 15%.

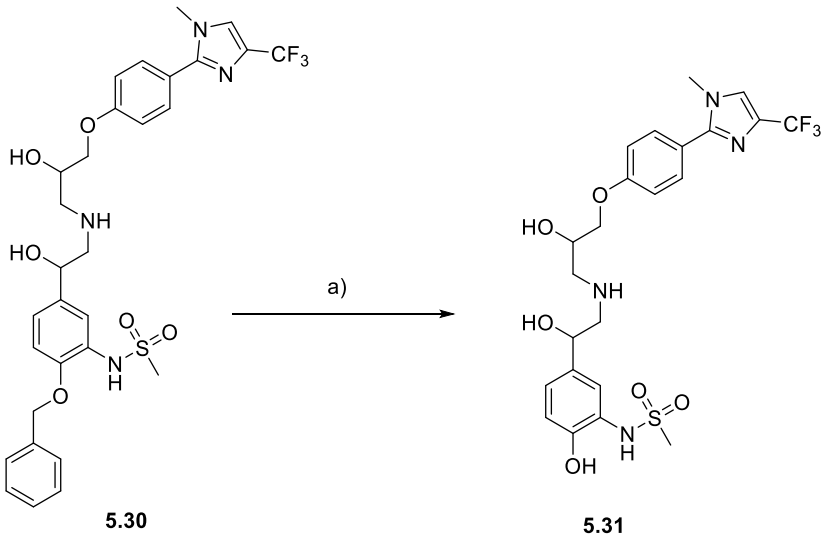


5.20 **5.29**

Scheme 5.14: benzyl deprotection of ICI 89406 bivalent compound.

Reagents and conditions: a) Pd/C, ammonium formate, Methanol, 70 °C 14%.

The debenzylation of the CGP 20712A containing bivalent compound (**5.30**) was also performed using the transfer hydrogenation method. Product formed and was isolated by HPLC. Similarly to **5.28**, a low yield of 14% was obtained. These yields from the transfer hydrogenations are lower than those observed in the hydrogenations using a hydrogen atmosphere. This could be due to the harsher conditions used in the transfer hydrogenation, as the starting material is heated at 70 °C.



Scheme 5.15: benzyl deprotection of CGP 20712A bivalent compound.
Reagents and conditions: a) Pd/C, ammonium formate, Methanol, 70 °C, 14%.

5.2.3 Pharmacology of bivalent βAR agonist-antagonist compounds

Radioligand binding assays and SPAP functional assays were performed on each of the CGP 20712A headgroup analogues to find the affinities and activities of each of these compounds at the β_1 and β_2ARs. The purpose of synthesising and testing these compounds was an attempt to make a bivalent compound which could activate the β_2AR by preferentially binding to the receptor with the agonist side of the compound, while the antagonist pharmacophore of the compound would preferentially bind to the β_1AR and cause antagonism. All pharmacological assays were performed by Prof. Jillian Baker (University of Nottingham).

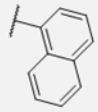
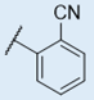
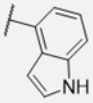
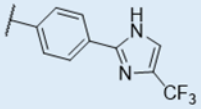
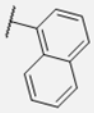
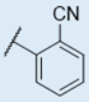
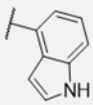
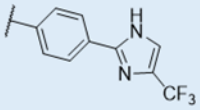
		Log K_d				
Cpd no.	R	β_1	n	β_2	n	β_2 Fold Selectivity
5.24		-5.07 ± 0.03	6	-6.37 ± 0.06	6	20
5.20		-5.40 ± 0.07	4	-6.62 ± 0.07	4	17
5.26		-6.18 ± 0.06	4	-7.74 ± 0.01	4	36
5.30		-4.80 ± 0.03	4	-5.51 ± 0.04	4	5
5.27		-6.66 ± 0.12	5	-7.14 ± 0.09	5	3
5.29		-5.43 ± 0.06	4	-6.02 ± 0.08	4	4
5.28		-6.21 ± 0.03	5	-6.91 ± 0.05	5	5
5.31		IC50 > -4	5	-5.72 ± 0.03	5	/

Table 5.3: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments.

The benzylated phenol compounds **5.24**, **5.20**, **5.26** and **5.30** were tested along with the phenol bivalent compounds, as the addition of the benzyl group had been shown to maintain affinity at both receptors, but only activity at the β_2AR. With the exception of **5.24** which is also *N*-benzylated (and has reduced affinity because of this). The pairs of benzylated and non-benzylated compounds have approximately the same levels of affinity towards each receptor. The addition of this benzyl group

therefore makes little difference to affinity. However, it would be expected that these compounds bind differently than the phenol analogues. The phenol can have proton donating and accepting interaction with Ser-207 whereas the benzyl not only acts as a steric block, but the benzylic oxygen can only be a proton acceptor with Ser-207. None of the compounds have high levels of selectivity towards either receptor. All of them bind preferentially to the β_2AR, but, none of these compounds have significantly higher affinity at the β_2AR. Although, the precise level of selectivity of **5.31** is unknown due to the limitations of the assay. However, for these compounds to be considered as prescribed drugs, they should have similar, or higher affinity towards the β_1AR (if they are antagonists) so that they will bind preferentially to the β_1AR, in areas such as cardiac tissue.

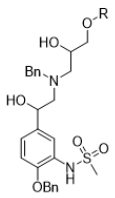
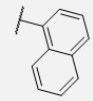
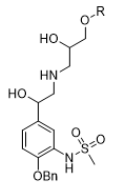
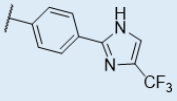
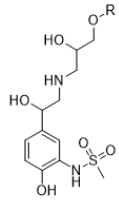
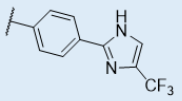
			Log EC_{50}				% Isoprenaline	
	Cpd no.	R	β_1	n	β_2	n	β_1	β_2
	5.24		No response	5	No response	4	/	/
	5.20		>-5	4	-6.99 ± 0.15	4	23.0 ± 2.0	20.4 ± 1.7
	5.26		>-5	3	-7.93 ± 0.19	4	35.7 ± 2.0	20.7 ± 3.5
	5.30		No response	4	>-5	4	/	39.2 ± 5.6
	5.27		>-5	10	-6.96 ± 0.18	6	59.8 ± 4.4	91.6 ± 6.6
	5.29		>-5	4	-6.92 ± 0.22	3	62.9 ± 7.6	90.8 ± 5.1
	5.28		-6.33 ± 0.18	5	-7.42 ± 0.05	3	32.6 ± 5.1	82.5 ± 6.1
	5.31		>-5	5	-6.59 ± 0.14	5	80.3 ± 6.3	89.7 ± 5.8

Table 5.4: log EC_{50} values and % maximum isoprenaline responses of each compound at the β_1 and β_2 adrenoceptor from CRE-SPAP reporter gene assay. Values are mean±sem of n separate experiments.

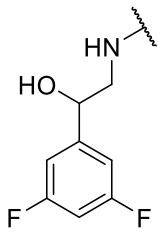
Although the addition of a benzyl group to the nitrogen and phenol (**5.24**) only affects the affinity approximately 10-fold, compared to the debenzylated compound (**5.27**), the activity at both receptors is completely removed. This is possibly due to a reduced interaction with Asp-113 as the nitrogen is less basic. Alternatively the additional benzyl group could form a π-π interaction with Tyr-316 which would alter the conformation of the receptor (see Chapter 1, Fig 1.4). Of the compounds with a benzylated phenol, the ICI 89406 (**5.20**) and pindolol (**5.26**) containing compounds have low levels of activity at both receptors, but have high potency at the β_2AR. It is unknown however, which pharmacophore binds preferentially to which receptor as the headgroups of pindolol and ICI 89406 have been shown to be able to have low levels of efficacy at each receptor.[51] The CGP 20712A headgroup containing compound (**5.30**) only has efficacy at the β_2AR, however this is weak agonism, and has no measurable affinity at the β_1AR. The efficacy at the β_2AR would therefore need to be increased and β_1AR affinity increased to be a useful β_2AR agonist–β_1AR antagonist compound.

The debenzylated bivalent compounds (**5.27**, **5.29**, **5.28** and **5.31**), each have activity at both receptors. At the β_2AR the percentage isoprenaline maximum response is similar for all of the compounds, all of them being full agonists. At the β_1AR there is a greater difference in the percentage isoprenaline maximum response values. While **5.27**, **5.29** and **5.31** are all partial agonists at the β_1AR, **5.28** is a weak agonist. Therefore, although all of these compounds have similar affinity at each receptor and have varying efficacies at the β_1AR while all having full agonism at the β_2AR, they are not β_2AR agonists–β_1AR antagonists as levels of agonism are observed at the β_1AR

5.3 Bivalent compounds with an alternative headgroup

Following the synthesis and pharmacological evaluation of the bivalent compounds with the agonist headgroup **5.1**, a second set were synthesised with an alternative

headgroup. The 3, 5-di-fluoro headgroup **5.32** was expected to be a partial agonist, in keeping with the 3, 5-di-chloro group.[151] The bivalent compounds in Chapter 4 contained an agonist group that was a full agonist at both receptors. This resulted in the bivalent compounds being full agonists at the β_2AR but had varying levels of activity at the β_1AR. Therefore, by incorporating a partial rather than full agonist into a bivalent compound, activity may be tuned in such a way that the compounds would remain active at the β_2AR but not the β_1AR.



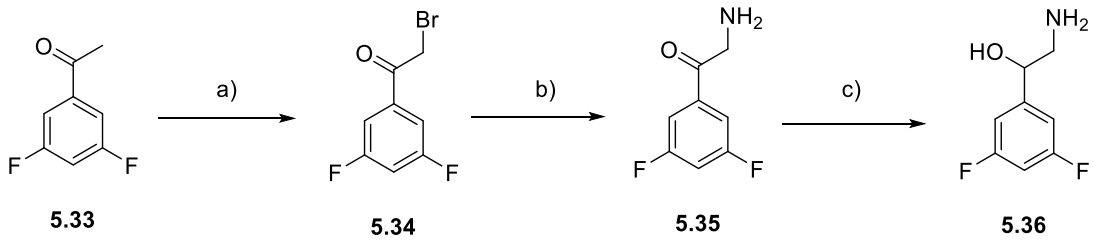
5.32

Fig: 5.5: alternative difluoro β_2AR agonist group

5.3.1 Synthesis of Bivalent compounds with an alternative headgroup

The synthesis of **5.36** follows a similar synthetic route to the sulfonamide intermediate **5.10**. The commercially available **5.33** was brominated, as in previous synthesis, with phenyltrimethylammonium tribromide. As before, this gives an inseparable mixture of product, starting material and dibromo product. However, this is not an issue as in the next step the Delepine reaction was conducted, in which any impurities are removed following the formation of an insoluble intermediate. The amine **5.35** was formed in a good yield of 75% over the two steps (Scheme 5.16).[150] Reduction of the ketone was then performed using sodium borohydride as the reducing agent. A low yield of 21% was obtained. The amino alcohol had high water solubility and could not be extracted in significant quantities with an organic solvent from the aqueous solution. In the synthesis of **5.10** this problem was solved by

developing conditions that allowed the product to precipitate out of solution, however precipitation of **5.36** did not occur.

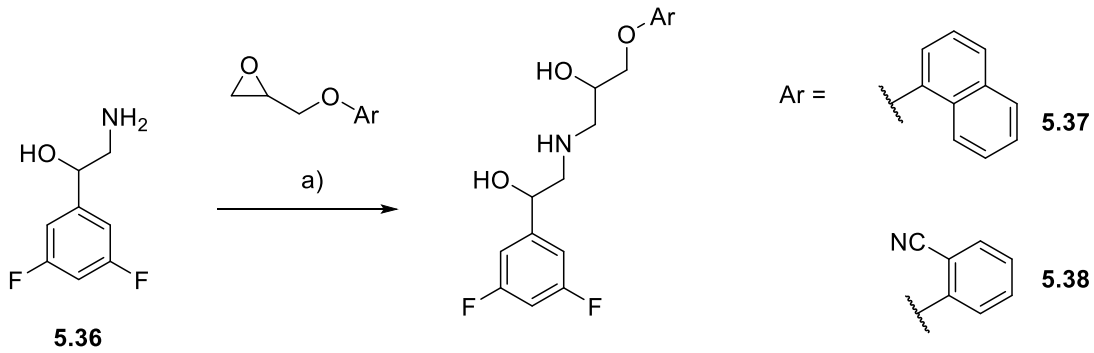


5.33 **5.34** **5.35** **5.36**

Scheme 5.16: synthesis of difluoro agonist headgroup.

Reagents and conditions: a) phenyltrimethylammonium tribromide, THF, 65 °C. b) hexamine, $CHCl_3$, rt, Methanol,hydrochloric acid, 60 °C, 70% over 2 steps. c) $NaBH_4$, Methanol, rt, 21%.

Following the synthesis of intermediate **5.36**, the compound was reacted with the epoxide intermediates of propranolol (**4.17**) and ICI 89406 (**4.19**). These moieties were selected as they are antagonists, with varying pharmacological profils as discussed in previous chapters. The reaction, as with similar previous ones, was carried out with DIPEA in ethanol at 80 °C. Column chromatography was performed to isolate the products, and each were obtained in yields of 22-32%.



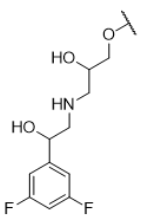
5.36 **5.37** **5.38**

Scheme 5.17: synthesis of bivalent compounds

Reagents and conditions: a) DIPEA, ethanol, 80 °C, 22 – 32%

5.3.2 Pharmacology of bivalent compounds with alternative headgroup

Radioligand binding assays and SPAP functional assays were performed on each of the CGP 20712A headgroup analogues to find the affinities and activities of each of these compounds at the β_1 and β_2ARs. The purpose of synthesising and testing these compounds was an attempt to make a bivalent compound with an alternative, potentially less βAR active agonist group. This may lead to compounds that activate the β_2AR by preferentially binding to the receptor with the agonist side of the compound, while the antagonist part of the compound would preferentially bind to the β_1AR and cause antagonism. All pharmacological assays were performed by Prof. Jillian Baker (University of Nottingham).



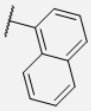
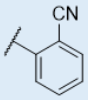
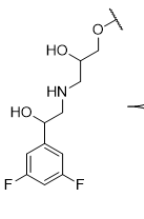
		Log K_d				
Cpd no.	R	β_1	n	β_2	n	β_2 Selectivity
5.37		-6.96 ± 0.04	5	-8.05 ± 0.05	5	12
5.38		-6.70 ± 0.04	5	-7.15 ± 0.02	5	3

Table 5.5: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments.



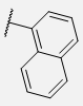
		Log EC_{50}				% Isoprenaline	
Cpd no.	R	β_1	n	β_2	n	β_1	β_2
5.37		No response	4	No response	4	/	/
5.38		-7.19 ± 0.05	4	-7.39 ± 0.06	4	63.6 ± 3.9	40.4 ± 4.0

Table 5.6: log EC_{50} values and % maximum isoprenaline responses of each compound at the β_1 and β_2 adrenoceptor from CRE-SPAP reporter gene assay. Values are mean±sem of n separate experiments.

Both of the compounds with the alternative difluoro agonist head group have affinity to both receptors, each having slight selectivity towards the β_2AR. However, as with the other bivalent compounds, it is unclear which pharmacophore will be preferentially binding to each of the receptors. Although the affinity at each receptor meets the aims of what these compounds are intended to do, the functional activity does not. The propranolol containing compound **5.37** has no activity at either receptor. Therefore, if the difluoro moiety is a partial agonist, this data would indicate that the affinity of the propranolol side of the compound (which is an antagonist) has higher affinity at both receptors and so there is no response. Compound **5.83** has pharmacological properties unlike any of the other bivalent compounds as is has significantly higher β_1AR potency than the other bivalent compounds, whereas the β_2AR potency is in line with the other set of bivalent compounds. Additionally, the β_1AR percentage response is higher than most other compounds, but β_2AR percentage response is lower. Due to the undesirable properties of each of these compounds the difluoro headgroup was not further explored.

5.4 Adrenoceptor agonist with CGP 20712A tailgroup

It has been shown throughout chapter 5 that a benzyl group attached to the phenol of the adrenoceptor agonist headgroup (**5.1**) does not greatly affect the affinity of the compounds at either the β_1 or β_2AR. However, these compounds lose a significant amount of efficacy at the β_1AR compared to the parent compound, while in some cases retaining partial agonism at the β_2AR. Furthermore, it was shown in Chapter 4 that the tail group of CGP 20712A binds well to both the β_1 and β_2AR, when attached to an orthosteric binding headgroup. Additionally, alkylation of the tailgroup phenol with a benzyl group has little effect on β_2AR affinity, but greatly reduces β_1AR affinity. Therefore, **5.39** was synthesised which combines the adrenoceptor agonist **5.1** with the CGP 20712A tailgroup. A series of analogues with benzyl groups added to either, or both head and tailgroup phenols were also synthesised. This was to investigate

whether the affinity and efficacy of the compounds could be tuned, to develop a β_2AR agonist–β_1AR antagonist.

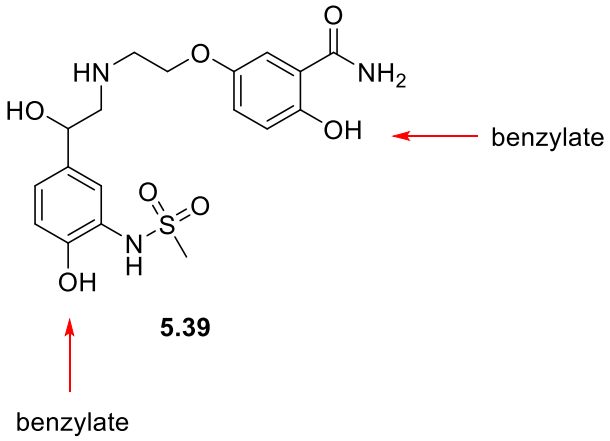
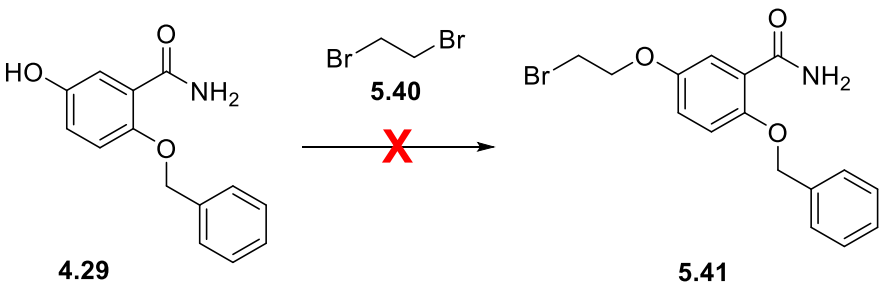


Fig 5.6: adrenoceptor agonist with CGP 20712A tailgroup and positions of benzylation

5.4.1 Synthesis of adrenoceptor agonist with CGP 20712A tailgroup

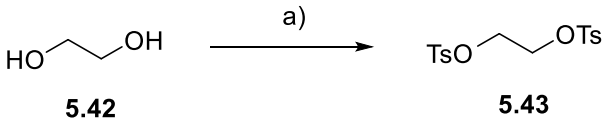
The first step in the synthesis of each of these compounds was to alkylate **4.29** with an ethyl group which had a leaving group on the opposite side of the chain, which could be substituted for the β_2AR agonist headgroup. This was, at first, attempted using dibromoethane. The reaction was carried out in DMF, using various bases at 80 °C. None of these reactions gave any indication by LC-MS or TLC that product formed.



Scheme 5.18: attempted alkylation of phenol
Reagents and conditions: DMF, Cs_2CO_3, 80 °C.

Due to the difficulties in forming **5.41**, a different approach was taken, which first required the tosylation of ethylene glycol. Two equivalents of tosyl chloride were mixed with ethylene glycol along with TEA in DCM (Scheme 5.19). The product in this

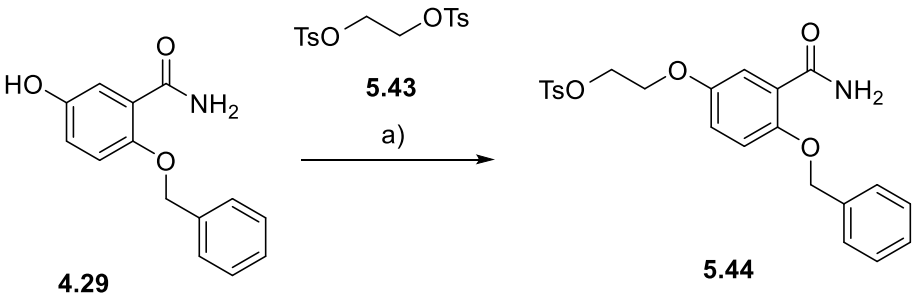
case formed, although not cleanly. This at first appeared to be problematic as the products were highly insoluble and therefore column chromatography was not viable. However, after testing a range of solvents, recrystallization of the tosylated product (**5.43**) could be carried out in ethanol to obtain pure product.



5.42 **5.43**

Scheme 5.19: tosylation of ethylene glycol
Reagents and conditions: tosyl chloride, TEA, DCM rt, 40%.

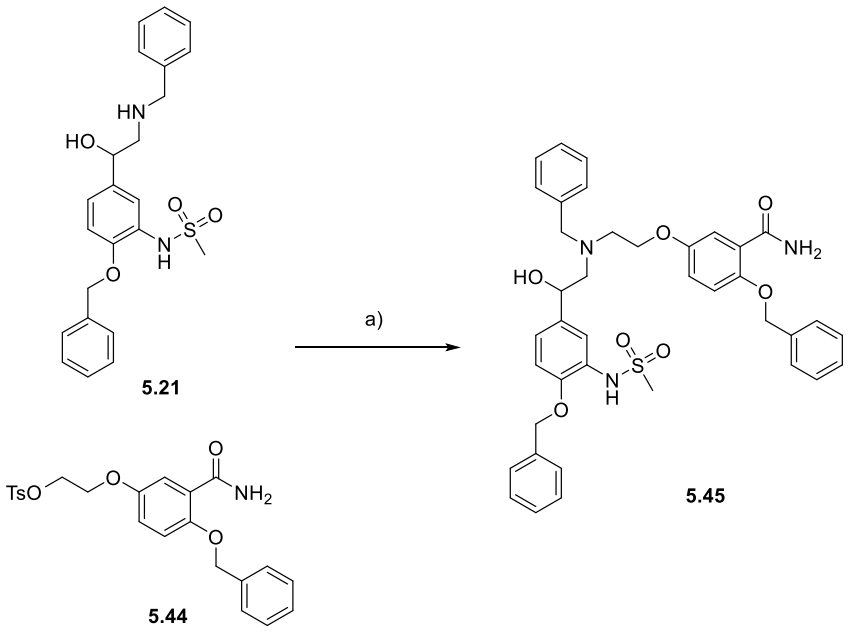
4.29 was then alkylated with **5.43** (Scheme 5.20). This reaction was carried out in DMF at 40 °C, first with potassium carbonate which gave product in a yield of 21%, and then with caesium carbonate which gave an improved yield of 52%.



4.29 **5.44**

Scheme 5.20: alkylation of phenol
Reagents and conditions: Cs_2CO_3, DMF, rt, 52%.

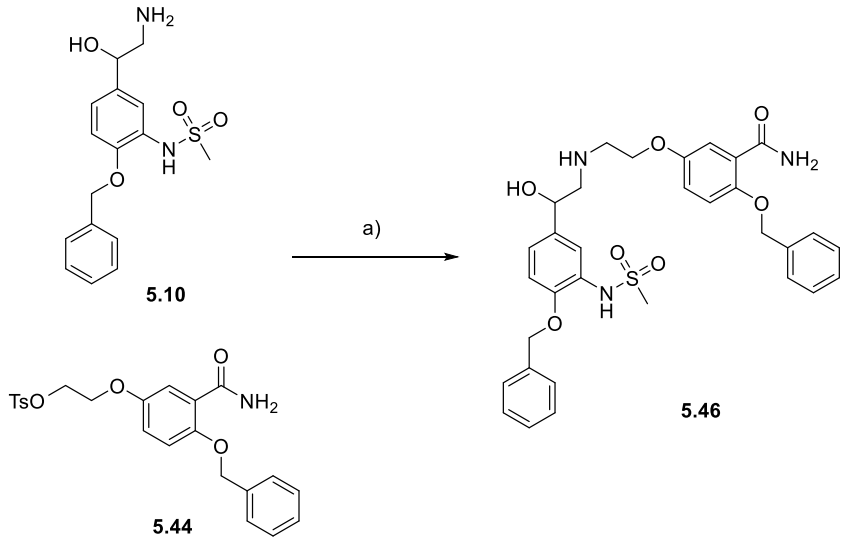
The first compound of this series to be synthesised was **5.45** which was formed by reacting the *N*-benzylated β_2AR agonist headgroup **5.21** with **5.44**. This was performed by mixing the two compounds in DMF along with potassium carbonate at 40 °C overnight. LC-MS indicated that the product (**5.45**) had formed and HPLC was carried out to isolate the product, which was obtained in a yield of 15%.



Scheme 5.21: Synthesis of tri-benzylated analogue

Reagents and conditions: K_2CO_3, DMF, 40 °C, 15%.

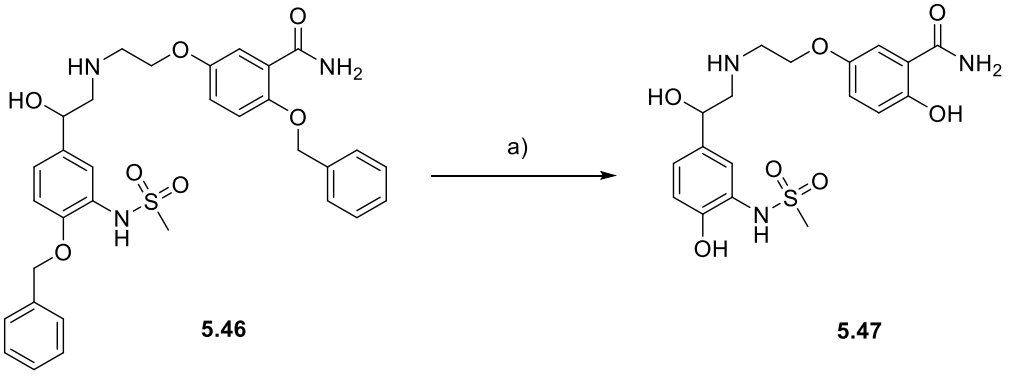
To form compound **5.46** that has benzyl groups on each phenol but not on the nitrogen as in **5.45**, the same reaction as above was performed but using primary amine **5.10** rather than the secondary amine **5.21.** The product was again isolated by HPLC and a yield of 24% was obtained.



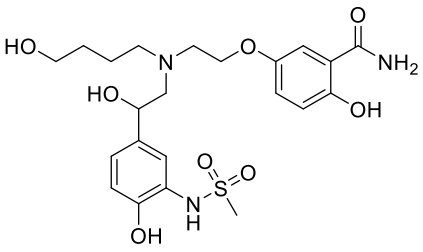
Scheme 5.22: Synthesis of di-benzylated analogue

Reagents and conditions: K_2CO_3, DMF, 40 °C, 24%.

The hydrogenation of **5.46** was then performed to debenzylate both groups. The product (**5.47**) was expected to have the highest affinity and efficacy of the series, but would not be expected to be selective for either receptor. The hydrogenation was carried out using palladium on carbon under a hydrogen atmosphere. A mixture of THF, water and acetic acid was used as the solvent. After stirring overnight, two major products formed, one of which had the expected mass of **5.47** and another that, like the bivalent compounds in Chapter 5.2.2, had a mass of 72 over the expected mass. However, unlike in Chapter 5.2.2, the two products could be separated by column chromatography. The side product was analysed by NMR. The ^{1}H NMR of the side product was almost identical to that of **5.47** but had an additional eight protons in the alkyl region. Due to this and the additional mass of 72, it was hypothesised that during the reaction, the amine of **5.47** (or the amine of the bivalent compounds) attacks THF, which causes it to ring open and results in the alkylation of the amine, giving compound **5.48**.



5.46 **5.47**

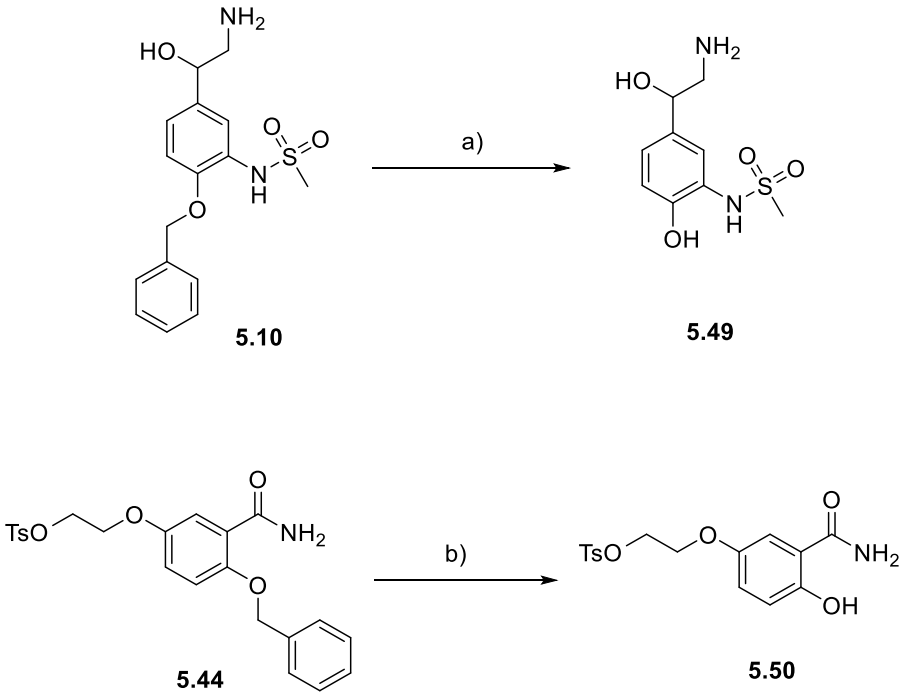


5.48

Scheme 5.23: debenzylation of di-benzylated analogue and *N*-alkylated sideproduct

Reagents and conditions: a) Pd/C, H_2, THF, H_2O, AcOH, 52%.

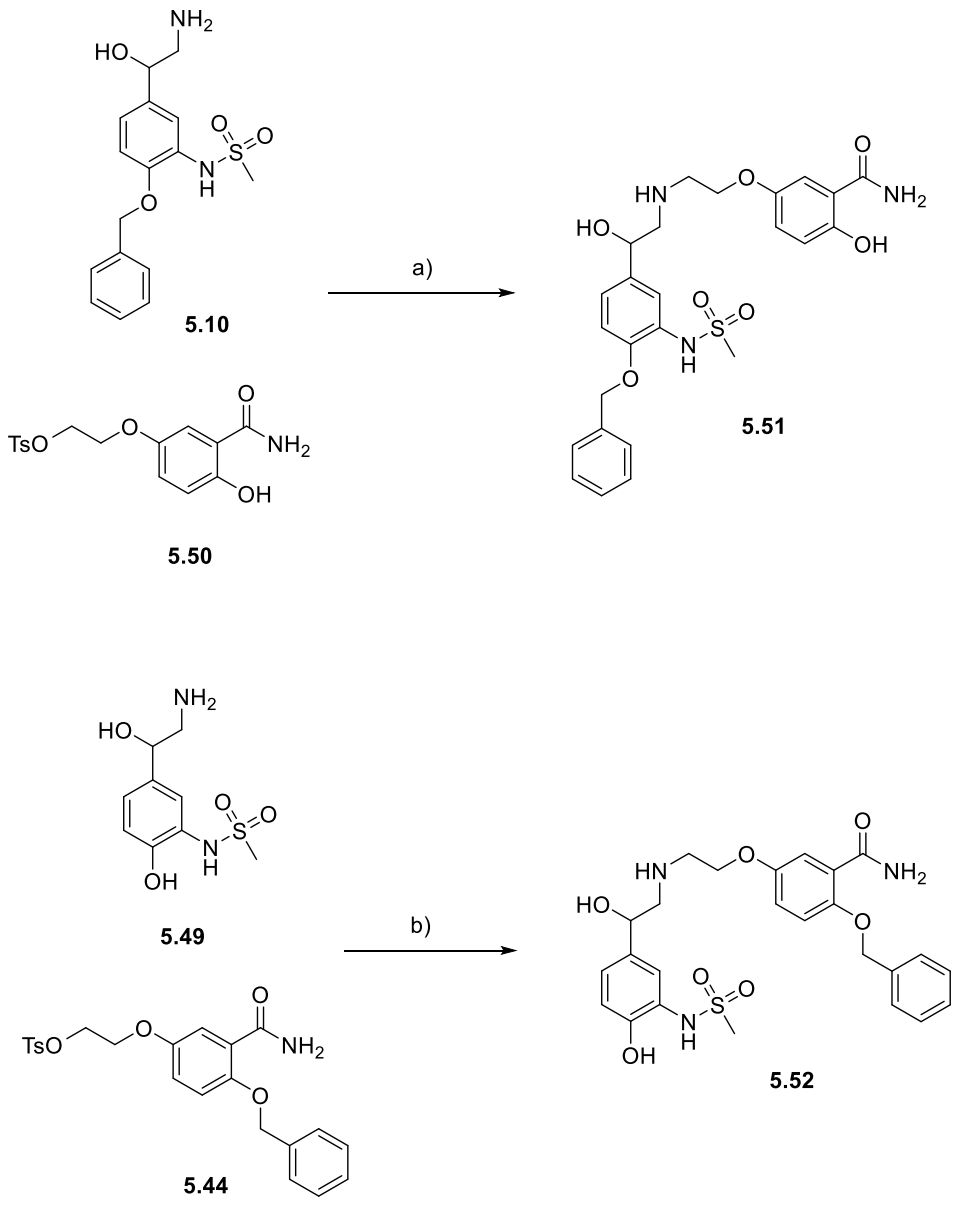
To synthesise the final two compounds, each of which have one benzyl group, on either phenol, either the agonist headgroup (**5.10**) or the tosylated tail group (**5.44**) were debenzylated (Scheme 5.24). As before, these reactions were carried out using palladium on carbon under a hydrogen atmosphere. Both of these products were obtained in excellent yields of over 90%.



Scheme 5.24: debenzylation of head and tailgroups

Reagents and conditions: a) Pd/C, H_2, Methanol, H_2O, AcOH, 98%. b) Pd/C, H_2, EA, H_2O, AcOH, 95%.

Finally, **5.10** and **5.49** were reacted with **5.50** and **5.44**, respectively (Scheme 5.25). These reactions were both carried out in DMF along with DIPEA. Though temperatures varied, in both cases only a small amount of product formed, as well as many side products. It was therefore required to purify each of these by HPLC.



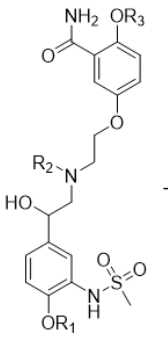
Scheme 5.25: Synthesis of mono-benzylated analogues

Reagents and conditions: a) DIPEA, DMF, 60 °C, 13%. b) DIPEA, DMF, 100 °C, 10%.

5.4.2 Pharmacology of adrenoceptor agonist with CGP 20712A tailgroup compounds

Radioligand binding assays and SPAP functional assays were performed on each of the CGP 20712A headgroup analogues to find the affinities and activities of each of these compounds at the β_1 and β_2ARs. The purpose of synthesising and testing these compounds was to investigate if affinity and activity at the βARs could be tuned by

alkylation of the phenols on either side of the compound. This may then lead to a compound with comparable affinity at each receptor, but with activity, only at the β_2AR. All pharmacological assays were performed by Prof. Jillian Baker (University of Nottingham).

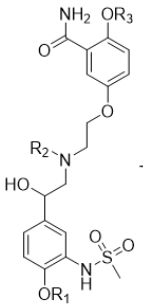


				Log K_d				
Cpd no.	R1	R2	R3	β_1	n	β_2	n	Selectivity β_1 β_2
5.45	Bn	Bn	Bn	>-4	4	>-4	4	/
5.46	Bn	H	Bn	-5.19 ± 0.08	4	-6.32 ± 0.05	4	13
5.47	H	H	H	-7.05 ± 0.27	9	-7.29 ± 0.26	9	2
5.51	Bn	H	H	-6.95 ± 0.24	6	-6.55 ± 0.25	6	3
5.52	H	H	Bn	-5.32 ± 0.06	5	-6.77 ± 0.19	5	28

Table 5.7: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log K_d) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments.

Of this set of compounds, **5.45** has the lowest affinity at each receptor, this is unsurprising as alkylation of the amine in a bivalent compound (**5.24**) also had greatly reduced affinity compared to other compounds. An increase in affinity is seen in the secondary amine analogue **5.46**. With a benzyl group on both the head and tailgroup of the compound, there is just over 10-fold selectivity towards the β_2AR. As expected, **5.47**, which is not alkylated on either phenol has the highest affinity towards each receptor of this series, and there is very little difference in the levels of affinity towards the two receptors. The addition of a benzyl group to the headgroup of the compound **5.51** leads to a slight reduction in β_2AR affinity but no difference at the β_1AR. This corresponds with earlier results in which agonist headgroups were benzylated. Finally, benzylation of the tail group (**5.52**), as performed with CGP

20712A tail group analogues (Chapter 4), gave a significant loss in β_1AR affinity, but a relatively small reduction in β_2AR affinity. Again, this is akin to previous results.



Cpd no.	R1	R2	R3	Log EC_{50} β_1	n	Log EC_{50} β_2	n	% Isoprenaline β_1	% Isoprenaline β_2
5.45	Bn	Bn	Bn	No response	4	No response	4	/	/
5.46	Bn	H	Bn	No response	4	-6.71 ± 0.07	3	/	47.9 ± 4.4
5.47	H	H	H	-7.03 ± 0.19	10	-7.95 ± 0.30	9	93.7 ± 6.2	106.4 ± 5.4
5.51	Bn	H	H	No response	8	-6.62 ± 0.19	7	/	46.6 ± 4.2
5.52	H	H	Bn	>-5	4	-7.25 ± 0.26	4	89.0 ± 14.9	95.2 ± 5.4

Table 5.8: log EC_{50} values and % maximum isoprenaline responses of each compound at the β_1 and β_2 adrenoceptor from CRE-SPAP reporter gene assay. Values are mean±sem of n separate experiments.

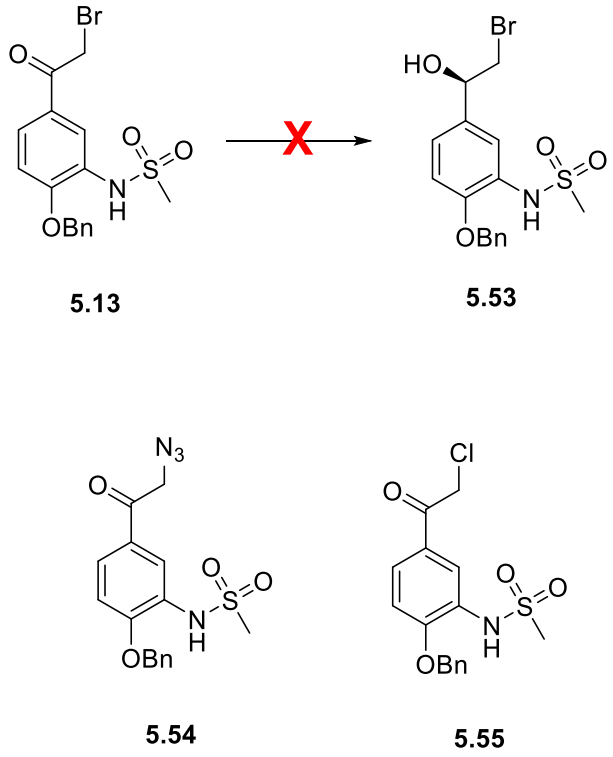
Along with having the lowest affinity of this set of compounds, the *N*-benzylated **5.45** also has no activity at either receptor, possibly due to the *N*-benzyl group altering the interaction with Asp-113. Compound **5.47** is both the highest affinity and most active compound of the series; it is a potent, full agonist at both receptors. In compound **5.52**, the benzyl group is placed on the tail group of the compound which leads to a lower β_1 than β_2AR affinity, consequently, the compound has high potency at the β_2AR but low potency at the β_1AR. These differences are due to affinity as the efficacy ratio is 0.1 and 3 at the β_1 and β_2AR respectively. However, the compound is still a full agonist at each receptor. The two most interesting compounds in the series are **5.46** and **5.51** which are antagonists at the β_1AR but partial agonists at the β_2AR. The lack of activity is likely due to benzylation of the headgroup phenol, which has previously been shown to remove β_1AR activity. While both of these compounds have very similar EC_{50} and percentage maximum isoprenaline response, **5.51** has

higher affinity at both receptors, and has slightly higher affinity at the β_1AR, this compound therefore satisfies the aim of the project.

5.4.3 Asymmetric synthesis of compound 5.44

Compound **5.51** has the pharmacological profile that was desired in the aim of the project. Although this compound is not the combination of an agonist and antagonist group, it does have partial agonism at the β_2AR but is an antagonist at the β_1AR. Compound **5.51** is however, a racemic mixture. Many marketed β_2AR agonists, such as formoterol and salbutamol, are given therapeutically as racemic mixtures.[152-153] However, endogenous adrenaline has the *R* configuration, therefore it may be expected that the *R* and *S* enantiomers of **5.51** have different pharmacological profiles.[154-155] In order to obtain the two separate enantiomers, an alternative, asymmetric synthesis was performed.

The synthesis of these two enantiomers follows the same synthetic route as in scheme 5.4, up to the bromo ketone compound **5.13**. The synthesis then differed by performing an asymmetric Corey-Bakshi-Shibata (CBS) reduction of the ketone. This was first performed on bromo ketone **5.13**. This reaction requires the CBS catalyst and a source of borane (in this case, borane•THF). The reaction is carried out in unstabilised THF. However, this first attempt was unsuccessful as LC-MS and TLC analysis showed that only starting materials were present. Different compounds were also used in this reaction including azido ketone **5.54** and chloro ketone **5.55**. Compound **5.13** was converted to the azido ketone (**5.54**), using sodium azide. Chloro ketone **5.48** was also synthesised from **5.13** using lithium chloride and silver carbonate. However, again, neither of these reactions yielded any product.



Scheme 5.26: attempted asymmetric reduction of bromo ketone. Azido ketone and chloro ketone which were used in attempted asymmetric reduction reactions.

Reagents and conditions: a) *S*-CBS, borane•THF, THF, rt.

Due to none of these compounds reacting, the source of borane was changed. Borane *N,N*-diethylaniline complex was used while the other conditions remained consistent. The reaction was first carried out on the bromo ketone **5.13**, which successfully gave product **5.53**. A possible reason that this reaction worked, whereas previous attempts did not is that borane•THF contained butylated hydroxytoluene as a stabiliser within the THF. This compound can inhibit oxidation reactions occurring, therefore the reaction failed. This compound was then treated with hexamine, in an attempt to form an amine. However, hexamine was unable to displace the bromine, therefore no product formed. The azido ketone compound **5.54** was again formed by displacing the bromine of **5.13** with sodium azide, this was achieved in an excellent yield of 95% and required no further purification, following work up. The CBS reduction, using borane *N,N*-diethylaniline complex along with the *S*-CBS catalyst was performed. The *S*-CBS catalyst gives the *R*-enantiomer of the product. This is due to the intermediate favouring the larger substituent in the equatorial position to minimise steric interactions (shown in figure 5.6).[156] To

determine which substituent is larger, the A-value was used, which defines the most stable orientation of an atom in a molecule and can represent steric bulk (larger groups have higher value).[157] The phenyl group is the larger substituent as it has an A-value of 3.0, whereas azide has a value of 0.6.[158]

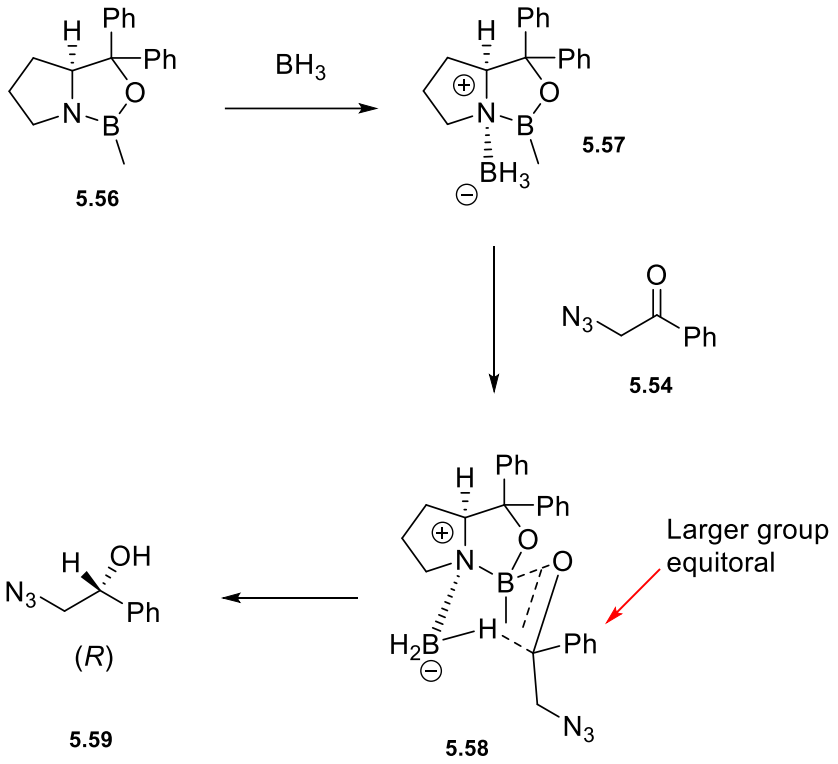
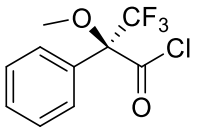


Fig 5.6: schematic example of *S*-CBS reduction of azido ketone

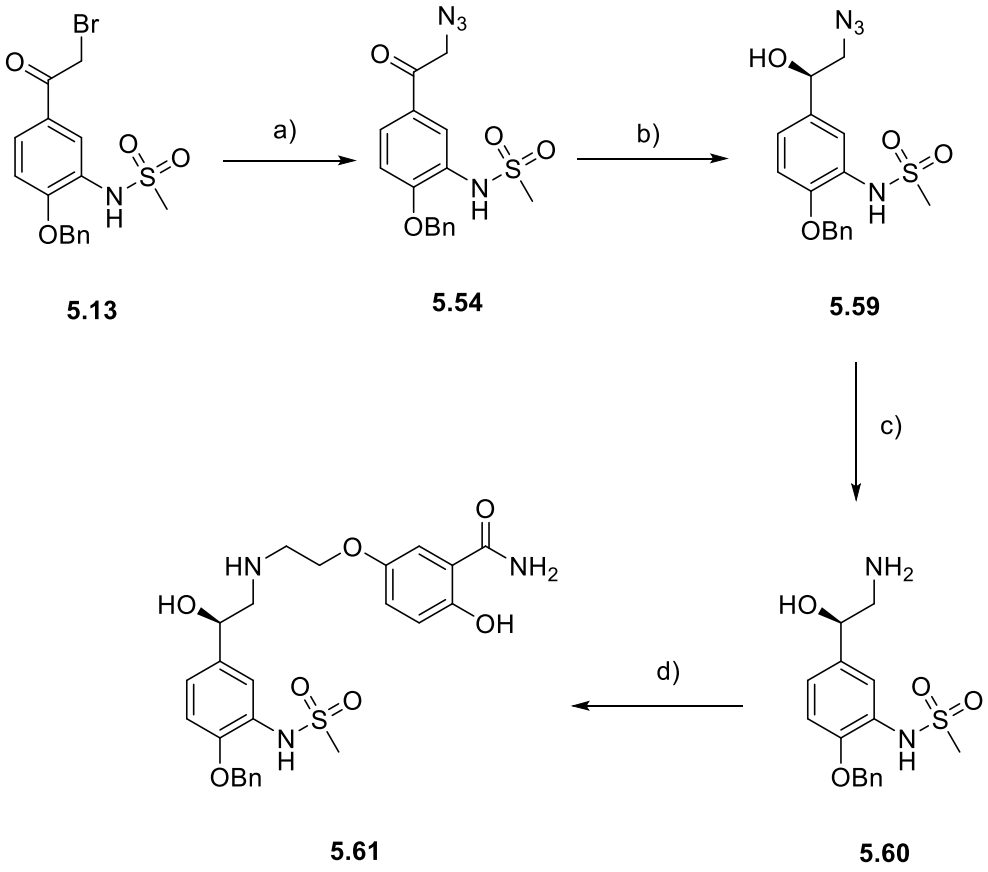
Following the asymmetric reduction of the azido ketone (**5.54**) into the azido alcohol (**5.59**), the enantiomeric excess (ee) was calculated. This was performed by mixing **5.59** with the Mosher acid chloride (**5.60**) and DMAP. This formed a pair of diastereoisomers, the ratio of which could be determined by fluorine NMR, and from this the ee was derived.[159] This indicated that 48% ee of the *R* enantiomer was obtained. Further validation of this was attempted using chiral reverse phase chromatography. Three separate chiral columns were used (two cellulose, one amylose) however, none of these were able to separate the enantiomers. Consequently, the enantiomeric excess could not be validated.



5.60

Fig 5.7: (*S*)-Mosher acid chloride

Reduction of the azide to form **5.59** was initially performed using the Staudinger reduction. LC-MS indicated that product had formed, but also a number of other side products. Consequently, this made isolation of the product very difficult, additionally, as discussed earlier, this reaction produces triphenylphosphine oxide as a by-product which can be difficult to completely remove. Different approaches were therefore explored. The use of palladium on carbon along with hydrogen could not be employed, as this would remove the benzyl group. Therefore, reduction using zinc and ammonium chloride was attempted as this was very effective and mild way of reducing nitro groups in an earlier synthesis. The reaction proceeded cleanly and pure product was afforded without the need for purification following the work up. The amine **5.60** was then reacted with the tosylated tailgroup **5.50** using the same conditions as in analogous racemic reaction (scheme 5.25). As before this reaction produced a number of products and only a small amount of desired product, however, **5.61** was successfully isolated using HPLC. The synthesis was then repeated using the *R*-CBS catalyst to form the *S* enantiomer of **5.61**.



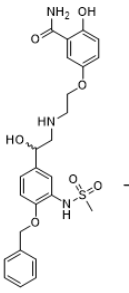
5.13 **5.54** **5.59** **5.60** **5.61**

Synthesis of the *R*-enantiomer of compound **5.51**.

Reagents and conditions: a) sodium azide, DMF, rt, 76%. b) *s*-CBS catalyst, *N,N*-diethylaniline complex, THF, rt, 85% (48% ee*). c) ammonium chloride, zinc powder, Methanol, THF, rt, 38%. d) **5.43**, DIPEA, DMF, 80 °C mw, 14%. * Requires further validation.

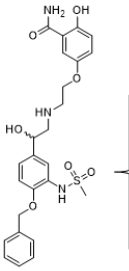
5.4.4 Pharmacology of *R* and *S* enantiomers

Radioligand binding assays and SPAP functional assays were performed on each of the CGP 20712A headgroup analogues to find the affinities and activities of each of these compounds at the β_1 and β_2ARs. As the endogenous agonist, adrenaline, is the *R* enantiomer and that a difference has been reported in the effects of *R*/*S* enantiomers of β_2AR agonists, it would be expected that the two enantiomers would have slightly different effects.[160-161] All pharmacological assays were performed by Prof. Jillian Baker (University of Nottingham).



		Log K_d					
Cpd no.	Configuration	β_1	n	β_2	n	β_1 Selectivity	ee (%)
5.51	*R/S*	-6.95 ± 0.24	6	-6.55 ± 0.25	6	3	0
5.61	*R*	-6.23 ± 0.13	4	-5.93 ± 0.04	4	2	48*
5.62	*S*	-7.08 ± 0.12	5	-6.73 ± 0.09	5	2	/

Table 5.9: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments. * Requires further validation.



		Log EC_{50}				% Isoprenaline		
Cpd no.	Configuration	β_1	n	β_2	n	β_1	β_2	ee (%)
5.51	*R/S*	No response	8	-6.62 ± 0.19	7	/	46.6 ± 4.2	0
5.61	*R*	-7.67 ± 0.38	5	-6.74 ± 0.21	5	11.0 ± 4.1	48.5 ± 2.6	48*
5.62	*S*	-7.46 ± 0.14	5	-7.04 ± 0.13	5	15.2 ± 3.2	44.6 ± 2.3	/

Table 5.10: log EC_{50} values and % maximum isoprenaline responses of each compound at the β_1 and β_2 adrenoceptor from CRE-SPAP reporter gene assay. Values are mean±sem of n separate experiments. * Requires further validation.

The *R* and *S* enantiomers are shown to have slightly different pharmacological properties. The *S* enantiomer (**5.62**) has a higher affinity than the *R* enantiomer (**5.61**) and as should be expected, the racemic mixture has affinities in between the two enantiomers. An unexpected result is that while the racemic mixture has no β_1AR activity, both enantiomers have low levels of β_1AR activity, whereas the mixture has none. The activity of the *R* enantiomer (**5.61**) is however, very close to the limitations of the assay (10% percentage maximum isoprenaline response) and has a large error, whereas compound **5.62** certainly has low level β_1AR activity. Since **5.61** is a partial agonist at the β_2AR, and has negligible activity at the β_1AR. This compound meets the aims of the project. However, it would be preferable to further develop this

compound to have increased levels of activity at the β_2AR without altering the β_1AR activity.

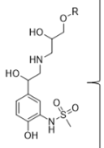
5.5 Summary of β_2AR agonist – β_1AR antagonist bivalent compounds

The β_2AR agonists group (**5.1**) was synthesised and pharmacological analysis showed that it was a non-selective β_2AR agonist. However, it was also found that benzyl alkylation of the phenol (**5.17**) did not greatly affect β_1AR or β_2AR affinity, but did remove β_1AR activity completely while only reducing β_2AR activity to partial agonism.

A range of antagonist head groups were linked the β_2AR agonist groups. The majority of these bivalent compounds bind to both β_1 and β_2ARs, each having slightly higher affinity towards the β_2AR. The exception being CGP 20712A headgroup containing compound (**5.31**) which has no measurable affinity at the β_1AR. As with the benzylated agonist headgroup (**5.17**), alkylation of the phenol of the bivalent compound has little effect on the affinity at each receptor. The *O*-benzylated compounds that are linked to the antagonist groups of ICI 89406 and pindolol (**5.20**, **5.26**) are weak agonists at both receptors. However, at the β_2AR, EC_{50} values are maintained, compared to the agonist parent agonist (**1.5**). Conversely at the β_1AR, EC_{50} values are significantly reduced.

The only compound in this set that is an agonist at the β_2AR and antagonist at the β_1AR is the *O*-benzylated agonist linked to CGP 20712A headgroup (**5.30**). However this compound has low potency, and has quite weak affinity, both of which would have to be increased for this compound to be useful as a β_2AR agonist–β_1AR antagonist. All of debenzylated bivalent compounds have activity at both receptors. However, in each case all of the compounds are full agonists at the β_2AR whereas at the β_1AR they vary between weak and partial agonists. Furthermore, the potencies of compounds **5.27**, **5.29** and **5.28** were comparable to the parent agonist (**1.5**), whereas at the β_1AR, the EC_{50} values are significantly less than **1.5**. However, all of

these compounds present some level of activity at the β_1AR and therefore are not β_2AR agonist–β_1AR antagonists.



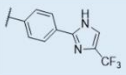
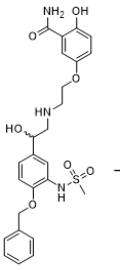
		Log K_d					Log EC_{50}				% Isoprenaline	
Cpd no.	R	β_1	n	β_2	n	β_2 Selectivity	β_1	n	β_2	n	β_1	β_2
5.27		-6.66 ± 0.12	5	-7.14 ± 0.09	5	3	>-5	10	-6.96 ± 0.18	6	59.8 ± 4.4	91.6 ± 6.6
5.29		-5.43 ± 0.06	4	-6.02 ± 0.08	4	4	>-5	4	-6.92 ± 0.22	3	62.9 ± 7.6	90.8 ± 5.1
5.28		-6.21 ± 0.03	5	-6.91 ± 0.05	5	5	-6.33 ± 0.18	5	-7.42 ± 0.05	3	32.6 ± 5.1	82.5 ± 6.1
5.31		IC50 > -4	5	-5.72 ± 0.03	5	/	>-5	5	-6.59 ± 0.14	5	80.3 ± 6.3	89.7 ± 5.8

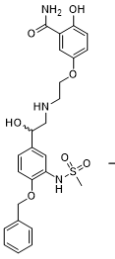
Table 5.11: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments.

It was found that *O*-benzylation of the agonist headgroup resulted in a removal of β_1AR activity, while affinity at each receptor was maintained. Additionally, it had previously been shown that the CGP 20712A tailgroup binds well to both βARs. The agonist group (**5.1**) and CGP 20712A tailgroup were therefore combined and alkylations were made to the phenol groups. This resulted in the synthesis of **5.51**, which has β_2AR partial agonism while being a β_1AR antagonist. As this compound was a racemic mixture, an asymmetric synthesis was performed. The *R* and S enantiomers were synthesised separately (further ee determination is required). Pharmacological characterisation found that the *R* enantiomer had negligible activity at the β_1AR, therefore a partial β_2AR agonist–β_1AR antagonist (**5.61**) was discovered.



		Log K_d					
Cpd no.	Configuration	β_1	n	β_2	n	β_1 Selectivity	ee (%)
5.51	*R/S*	-6.95 ± 0.24	6	-6.55 ± 0.25	6	3	0
5.61	*R*	-6.23 ± 0.13	4	-5.93 ± 0.04	4	2	48*
5.62	*S*	-7.08 ± 0.12	5	-6.73 ± 0.09	5	2	/

Table 5.12: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments. * Requires further validation.



		Log EC_{50}				% Isoprenaline		
Cpd no.	Configuration	β_1	n	β_2	n	β_1	β_2	ee (%)
5.51	*R/S*	No response	8	-6.62 ± 0.19	7	/	46.6 ± 4.2	0
5.61	*R*	-7.67 ± 0.38	5	-6.74 ± 0.21	5	11.0 ± 4.1	48.5 ± 2.6	48*
5.62	*S*	-7.46 ± 0.14	5	-7.04 ± 0.13	5	15.2 ± 3.2	44.6 ± 2.3	/

Table 5.13: log EC_{50} values and % maximum isoprenaline responses of each compound at the β_1 and β_2 adrenoceptor from CRE-SPAP reporter gene assay. Values are mean±sem of n separate experiments. * Requires further validation.

6. Conclusion and future work

6.1 General conclusion

The naturally occurring β_2AR agonist S1319 (**1.10**) was synthesised along with analogues to investigate the structural effects on βAR selectivity in terms of both affinity and efficacy. The synthetic route to form a key intermediate compound had multiple synthetic challenges and low yields in a number of steps, this led to the use of an alternative synthesis. This route was successful and lead to the synthesis of a number of S1319 analogues which were evaluated in functional and binding pharmacological assays.

Analogues of the highly selective β_1AR antagonist CGP 20712A were synthesised. These compounds were used to investigate the SAR of the parent compound in order to define which moieties were accountable for the preferential binding to the β_1AR. The aim was to use this information to find a highly selective β_1AR antagonist pharmacophore which could be linked to a β_2AR agonist group. A selective moiety was discovered, but it had low affinity at the β_1AR.

A range of β_1AR antagonist pharmacophores, including that of CGP 20712A, were linked to a β_2AR agonist group to form a number of bivalent compounds. Each of these compounds had similar affinity at each receptor and had comparable EC_{50} values to the parent βAR agonist compound (**1.5**) at the β_2AR, but significantly reduced EC_{50} values at the β_1AR, which fits the required pharmacological profile. However, the compounds had activity at both receptors, therefore did not fit the specified profile of a β_2AR agonist–β_1AR antagonist.

Using findings from both the SAR of CGP 20712A, and the sets of bivalent compounds, it was possible to rationally design an efficacy selective β_2AR partial agonist, **5.61**.

6.1.1 Exploration of β_2AR agonists SAR conclusions

The known β_2AR agonist S1319 (**1.10**) was synthesised and pharmacological evaluation was perfomed by the author and Prof. Jillian Baker (University of Nottingham). These studies confirmed that S1319 has affinity selectivity towards the β_2AR, but it is a full agonist at both receptors. Analogues were then synthesised using a range of structurally diverse amino groups, along with alterations to the core benzothiazolone, which is a catechol mimetic. An initial synthetic route was performed which led to the formation of a number of analogues. However, this route presented a number of synthetic issues. Consequently, in an effort to simplify the synthesis of a key intermediate compound and to allow the formation of different analogues, an alternative synthetic route was applied. This route led to the synthesis of an intermediate compound that was derivatised to form a number of analogues. However, due to the nature of one of the steps in the synthesis, it could only be carried out on a limited scale, which led to limited quantities of intermediate compound.

Pharmacological analysis of analogues **3.19**-**3.22**, which had a range of amino groups and a methyl alkylated phenol on the benzothiazolone core had no affinity at either receptor. This was likely due to the removal of the hydrogen bond between the phenol and Ser-207 which is important in catechol activation of the βARs. Methyl alkylation at this position is therefore not tolerated.

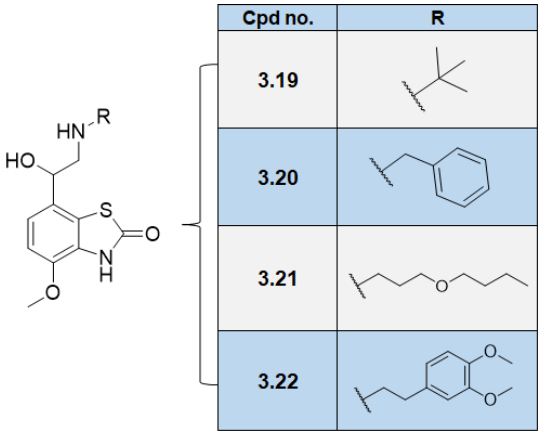
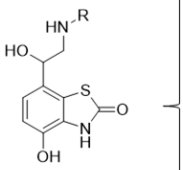
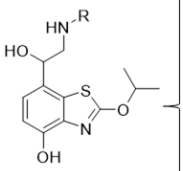


Table 6.1: derivatives of S1319 with *O*-methyl substituted headgroup and various amino groups

The compounds with the same benzothiazolone core as the parent compound (**3.45**-**3.48**), were all full agonists and potent at both the β_1 and β_2ARs. All of these compounds with the exception of the *tert*-butyl amino compound (**3.45**) had comparable affinity at the β_2AR to the parent compound. Variation was found in the β_1AR affinities which were all higher, leading to lower levels of β_2AR selectivity (though all had some level of β_2AR selectivity). Compound **3.45**, however, has a significant increase in affinity at both receptors compared to the parent compound and has 140-fold selectivity towards the β_2AR. Since all of these compounds have some level of selectivity towards the β_2AR, this indicates that the benzothiazolone core has some level of selectivity towards the β_2AR but this can be fine-tuned with the choice of amino group. Efficacy can also be altered by the choice of amino group, although the benzyl substituted compound **3.46** was the most potent at the β_2AR, it had the lowest efficacy ratio of the set at the β_2AR. Despite having lower affinity selectivity to the β_2AR, compared to the parent compound, **3.46** had the highest efficacy ratio, therefore requiring the least amount of receptor binding to generate 50% of the maximum response.





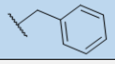
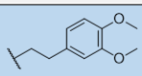
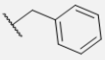
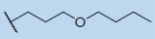
		Log K_d				
Cpd no.	R	β_1	n	β_2	n	β_2 Fold Selectivity
3.45		-7.09 ± 0.06	7	-9.25 ± 0.09	7	145
3.46		-5.73 ± 0.12	6	-7.49 ± 0.10	6	58
3.47		-6.70 ± 0.06	8	-7.58 ± 0.05	7	7.6
3.48		-7.50 ± 0.05	5	-7.49 ± 0.10	6	1
1.10 (S1319)	Me	-5.50 ± 0.06	6	-7.37 ± 0.06	7	74
3.50		-6.05 ± 0.03	8	-8.00 ± 0.04	8	89
3.51		-5.06± 0.06	3	-5.95 ± 0.16	7	7.8
3.52		-5.16 ± 0.13	8	-6.16 ± 0.06	8	10
3.53	Me	-4.77 ± 0.06	5	-6.33 ± 0.07	8	36

Table 6.2: Binding affinities and β_2AR selectivity of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem of n separate experiments.

Compounds that had an extended benzothiazolone core (**3.50**-**3.53**), by incorporation of an isopropyl group were also full, potent agonists at both receptors and followed the same trends seen in the benzothiazolone compounds. The extension of the core group is tolerated within both receptors, however each compound has lower affinity at each receptor when compared to the benzothiazolone analogues, possibly due to weaker interactions with Ser-203/204.

6.1.2 SAR of CGP 20712A conclusions

The highly selective β_1AR antagonist, CGP 20712A was first divided into a head and tailgroup. Analogues of each were synthesised to investigate which moieties of the antagonist bind preferentially to the β_1AR. The head group, with various amino groups were synthesised, along with analogues of the headgroups of other βAR antagonists, propranolol and ICI 89406, for comparison. This study found that the addition of a *tert*-butyl group (known to bind well the βARs) to the headgroups of propranolol and ICI 89406 increased affinity at both receptors 1000-fold compared

to a primary amine. However, addition of the same amino group to the CGP 20712A headgroup, resulted in a 3-fold increase at the β_1AR and no measurable increase at the β_2AR compared to the primary amine. The headgroup was shown to have very low affinity at the β_2AR and only modest affinity at the β_1AR. However, despite low affinity, each of the CGP 20712A headgroup analogues was shown to have β_1AR selectivity.

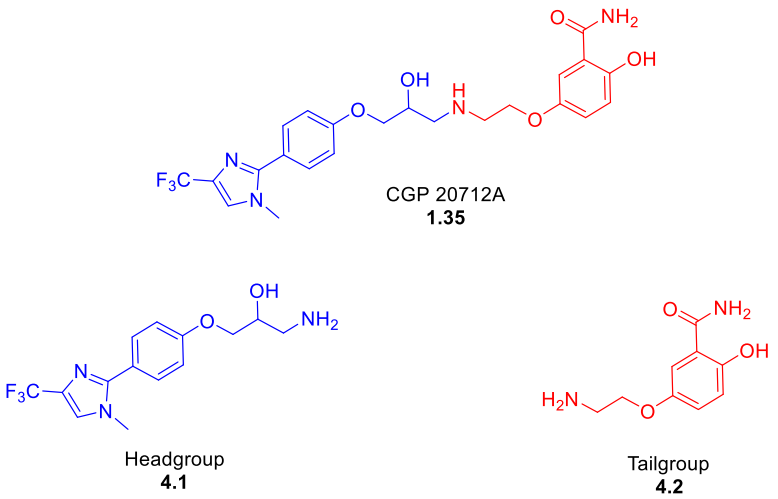


Fig 6.1: highly selective β_1AR antagonist, CGP 20712A divided into headgroup, which binds orthosterically and tailgroup which extends away from the orthosteric site

The tailgroup of CGP20712A was also synthesised and attached to the headgroups of propranolol and ICI 89406. These compounds had high affinity to both receptors, with slight selectivity to the β_2AR. This shows that the addition of this tail group to a nonselective headgroup does not make a β_1AR selective compound, rather it binds well to both receptors. However, it was shown that alkylation of the tailgroup phenol significantly reduced β_1AR affinity, but had less of an effect at the β_2AR.

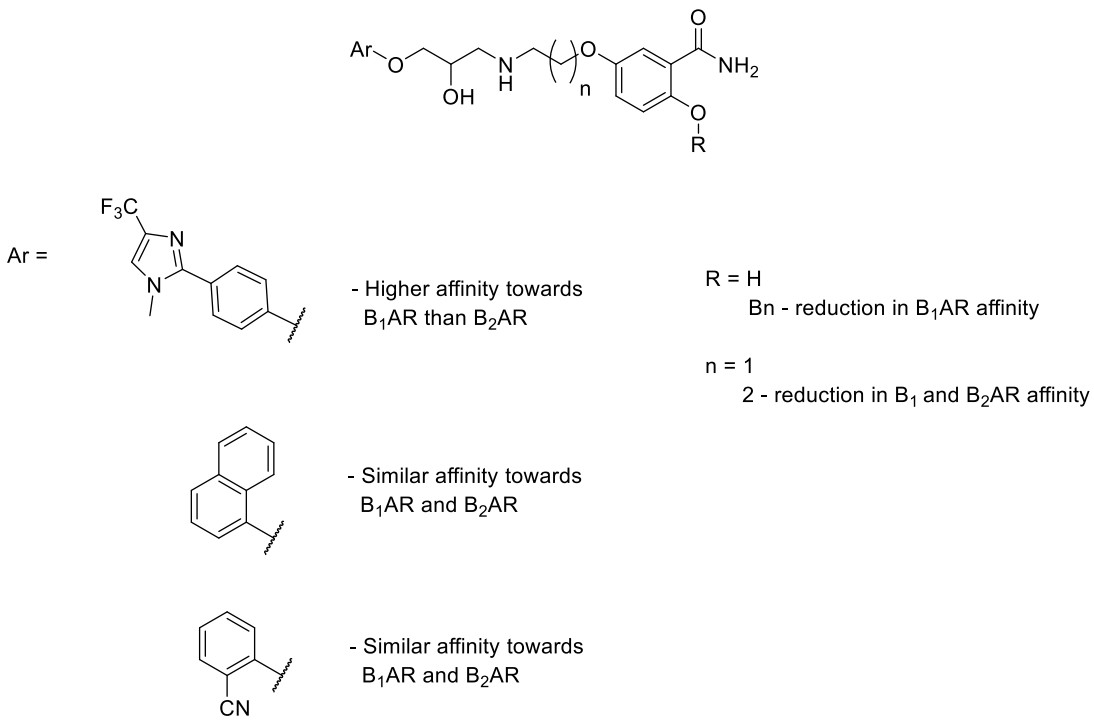


Fig. 6.2: SAR summary of CGP 20712A analogues

As it appeared that the headgroup of CGP 20712A is responsible for the β_1AR selectivity, further SAR studies were performed on this moiety. A number of analogues were synthesised with modifications to the imidazole. This study indicated a number of necessary features of the moiety which make it a β_1AR selective antagonist. To increase affinity at the β_1AR it is necessary for the 4-position of the imidazole to be alkylated, furthermore for the group to be antagonist, and increase affinity further, the group is required to be electron withdrawing. Methylation of the nitrogen is also required as if it is protonated, the group becomes a full agonist. This is possibly due to an interaction with Ser-203, -204 or -207.

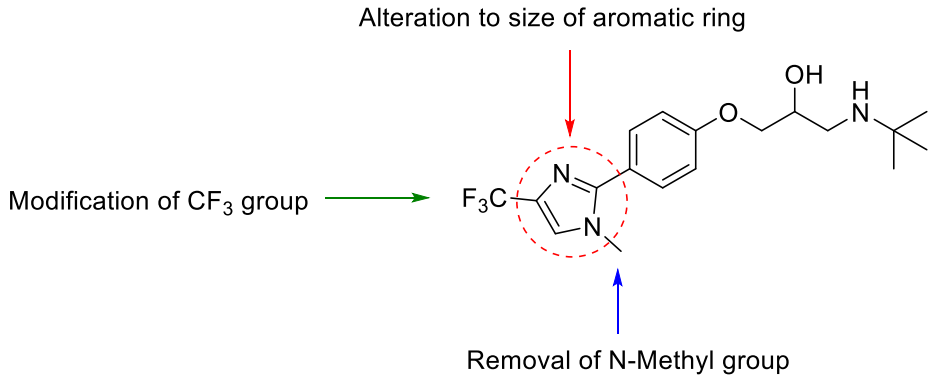


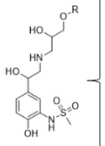
Fig 6.3: areas of investigation around CGP 20712A headgroup.

6.1.3 β_2AR agonist – β_1AR antagonist bivalent compounds conclusions

A β_2AR agonists group (**5.1**) was synthesised, which contained a sulfonamide mimetic of a catechol. A synthetic route to synthesising this compound was based on literature procedure but then further optimised to ease purification and increase yields. Pharmacological analysis of this compound showed that it was a non-selective β_2AR agonist. Furthermore, it was also found that the alkylation of the phenol with a benzyl group (**5.17**) did not greatly affect β_1AR or β_2AR affinity, however it did remove β_1AR activity completely, while reducing activity at the β_2AR to partial agonism.

A range of antagonist head groups were linked the β_2AR agonist groups. This was carried out by aminolysis of antagonist group epoxides with the amine of the agonist group. Low yields were obtained and efforts were made to increase this, however this was unsuccessful, consequently the low yields were accepted and HPLC was used to isolate the products. The majority of the bivalent compounds bind to both receptors, each having slightly higher affinity towards the β_2AR. The exception being the agonist linked group to CGP 20712A headgroup (**5.31**) which has no measurable affinity at the β_1AR due to limitations of the assay, therefore the actual level of selectivity could not be defined. As with the benzylated agonist headgroup (**5.17**), alkylation of the phenol has little effect on the affinity at each receptor. The *O*-benzylated compounds that are linked to the antagonist groups of ICI 89406 and pindolol (**5.20**, **5.26**) are weak agonists at both receptors, but at the β_2AR, EC_{50} values are maintained, compared to the agonist parent agonist (**1.5**). Conversely at the

β_1AR, EC_{50} values are significantly reduced. The only compound in this set that is an agonist at the β_2AR and antagonist at the β_1AR is the *O*-benzylated agonist linked to CGP 20712A headgroup (**5.30**), however this compound has low potency, and has quite weak affinity, both of which would have to be increased for this compound to be useful as a β_2AR agonist–β_1AR antagonist. It is however unclear which side of the compound is preferentially binding to each receptor. Mutagenesis studies would be required to explore this. Of the debenzylated bivalent compounds, all of these compounds have activity at both receptors. However, in each case all of the compounds are full agonists at the β_2AR whereas at the β_1AR they vary between weak and partial agonists. Furthermore, the potencies of compounds **5.27**, **5.29** and **5.28** were comparable to the parent agonist (**1.5**), whereas at the β_1AR, the EC_{50} values are significantly less than **1.5**. However, all of these compounds present some level of activity at the β_1AR and therefore are not β_2AR agonist–β_1AR antagonists.



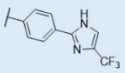
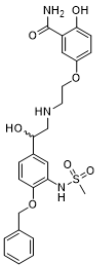
		Log K_d					Log EC_{50}				% Isoprenaline	
Cpd no.	R	β_1	n	β_2	n	β_2 Selectivity	β_1	n	β_2	n	β_1	β_2
5.27		-6.66 ± 0.12	5	-7.14 ± 0.09	5	3	>-5	10	-6.96 ± 0.18	6	59.8 ± 4.4	91.6 ± 6.6
5.29		-5.43 ± 0.06	4	-6.02 ± 0.08	4	4	>-5	4	-6.92 ± 0.22	3	62.9 ± 7.6	90.8 ± 5.1
5.28		-6.21 ± 0.03	5	-6.91 ± 0.05	5	5	-6.33 ± 0.18	5	-7.42 ± 0.05	3	32.6 ± 5.1	82.5 ± 6.1
5.31		IC50 > -4	5	-5.72 ± 0.03	5	/	>-5	5	-6.59 ± 0.14	5	80.3 ± 6.3	89.7 ± 5.8

Table 6.3: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments.

6.1.4 β_2AR agonist with CGP 20712A tailgroup conclusions

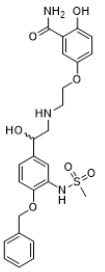
Although the set of bivalent compounds did not provide a β_2AR agonist–β_1AR antagonist with satisfactory affinity and activity, it was shown in Chapter 5 that

benzylation of the agonist group phenol resulted in a removal of β_1AR activity, while affinity at each receptor was maintained. It had also been shown in Chapter 4 that the CGP 20712A tailgroup binds well to both βARs and the affinity at the β_1AR can be fine-tuned by alkylation of the phenol. The agonist group (**5.1**) and CGP 20712A tailgroup were therefore combined and alkylations were made to the phenol groups. This resulted in the synthesis of **5.51**, which has β_2AR partial agonism while being a β_1AR antagonist. As this compound was a racemic mixture, an asymmetric synthesis was performed. The *R* and S enantiomers were synthesised separately, however, further ee determination is required. Pharmacological characterisation of the two enantiomers found that the *R* enantiomer had negligible activity at the β_1AR, while the *S* enantiomer has a low level of activity at the β_1AR. A partial β_2AR agonist–β_1AR antagonist **5.61** was therefore discovered.



		Log K_d					
Cpd no.	Configuration	β_1	n	β_2	n	β_1 Selectivity	ee (%)
5.51	*R/S*	-6.95 ± 0.24	6	-6.55 ± 0.25	6	3	0
5.61	*R*	-6.23 ± 0.13	4	-5.93 ± 0.04	4	2	48*
5.62	*S*	-7.08 ± 0.12	5	-6.73 ± 0.09	5	2	/

Table 6.4: Binding affinities of each compound at the β_1 and β_2 adrenoceptor are shown by the dissociation constant (log Kd) obtained form ^{3}H-CGP12177 radioligand competition binding assay. Values are mean±sem and n of separate experiments. * Requires further validation.



		Log EC_{50}				% Isoprenaline		
Cpd no.	Configuration	β_1	n	β_2	n	β_1	β_2	ee (%)
5.51	*R/S*	No response	8	-6.62 ± 0.19	7	/	46.6 ± 4.2	0
5.61	*R*	-7.67 ± 0.38	5	-6.74 ± 0.21	5	11.0 ± 4.1	48.5 ± 2.6	48*
5.62	*S*	-7.46 ± 0.14	5	-7.04 ± 0.13	5	15.2 ± 3.2	44.6 ± 2.3	/

Table 6.5: log EC_{50} values and % maximum isoprenaline responses of each compound at the β_1 and β_2 adrenoceptor from CRE-SPAP reporter gene assay. Values are mean±sem of n separate experiments. * Requires further validation.

6.2 Future work

The most interesting result of this study was that alkylation of the β_2AR agonist group with a benzyl group removed all activity at the β_1AR while not affecting affinity. A number of studies could be carried out to further explore this. It would be beneficial to discover a moiety that, like the benzyl group, removed β_1AR activity, but did not reduce β_2AR activity.

It is currently unclear if the benzylated compounds are interacting with the catechol binding pocket as the addition of the benzyl group would be expected to remove the phenol interaction with Ser-207. SAR of the benzyl group should be performed by synthesising and then pharmacologically evaluating analogues with altered aromatic groups. A number of avenues could be taken to explore SAR of the benzyl group, an understanding of which could lead to a full β_2AR agonist – β_1AR antagonist. Substitutions could be made to the benzyl group. For example, an electron withdrawing group such as CF_3 (**6.1**), could be placed on the benzyl group at various positions. This would alter the distribution of electron density around the ring and potentially alter interactions within the binding pocket. Alternatively, groups with the ability to form hydrogen bonds could also be placed on the ring (**6.2**) to see if any additional interactions with amino acid residues could be formed.

As shown in **6.3** the benzyl group could be exchanged for a different aromatic group, such as pyridizine, which has a much larger dipole than a benzyl group and could therefore interact differently with the binding pocket. This would not be limited to groups with different dipoles, but also aromatic groups of different sizes and with the ability to form hydrogen bonds. As it is unknown if the binding of **5.17** to the β_2AR is the same as catechol mimetics, or whether a different binding pocket opens, the distance between the phenyl ring and the core structure could therefore be altered as in compound **6.4**. As the phenyl group would go deeper into a binding pocket, this may alter affinity at each receptor. It is also unknown as to whether the sulfonamide group is necessary for **5.18** to activate the β_2AR. If binding differs from that of

catechol binding compounds, then the interaction of the sulfonamide with Ser-203/204 may not occur and therefore be unnecessary.

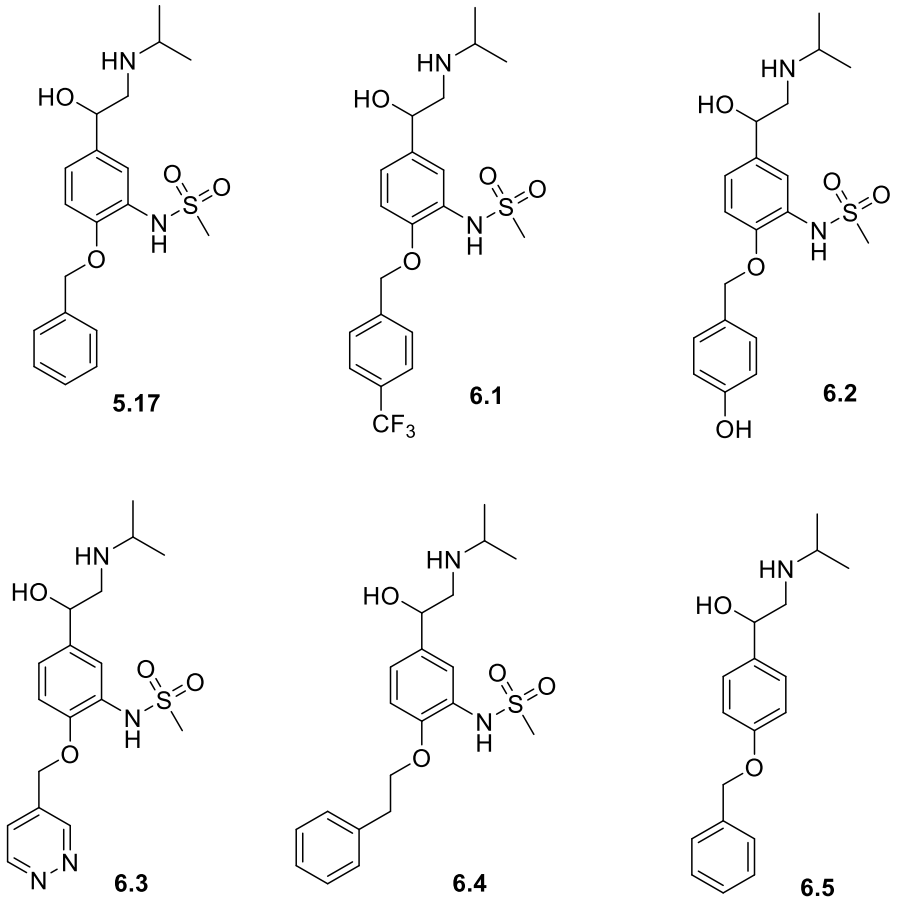


Fig 6.1: proposed compounds with alterations to the benzyl group and catechol mimetic

It is currently unknown whether the pharmacological properties caused by the addition of the benzyl group are specific to the sulfonamide catechol mimetic, or whether this can be applied to other catechol mimetics. In Chapter 3 a benzothiazolone, catechol mimetic was used rather than a sulfonamide. This βAR group provided high affinity, full β_2AR agonists with very high potency. If the addition of a benzyl group to this moiety results in the same reduction of β_1AR activity, then compounds such as **6.6** and **6.7** could have higher β_2AR potency and activity than the sulfonamide analogues.

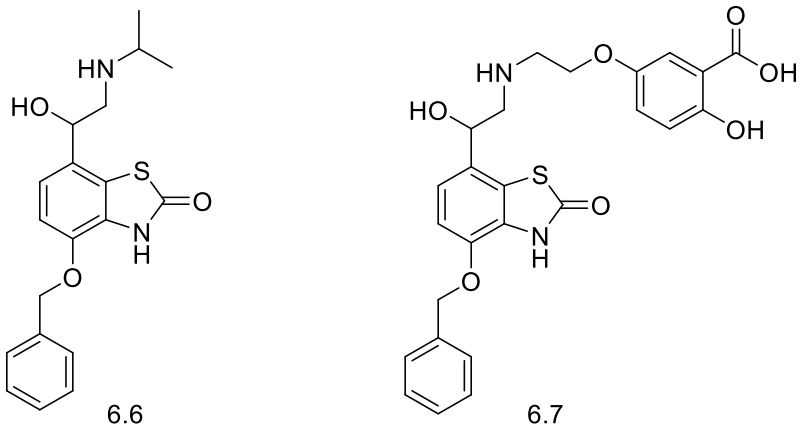
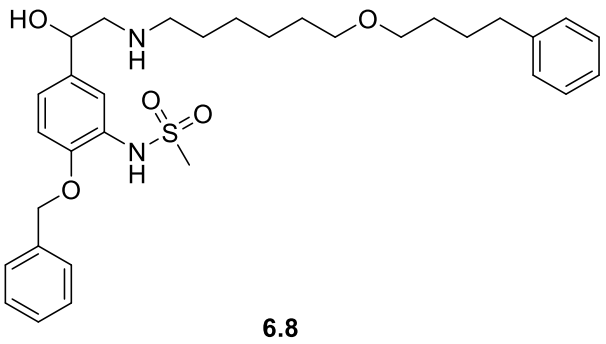


Fig 6.2: benzothiazolone headgroup compounds with benzylated phenol

Following an SAR study of the benzyl group, and altering the catechol mimetic moiety, alternative amino groups could be used. Salmeterol for example, has a very high affinity and potency at the β_2AR which is, in part, due to the amino group.[161-162] Amino groups such as this one, and those of other high affinity β_2AR agonists could be incorporated into the headgroup of **5.18**, such as **6.8**, which may form a high affinity and highly potent β_2AR partial agonist which has no activity at the β_1AR.



6.8

Fig 6.3: benzylated sulfonamide headgroup with salmeterol amino group

If the aforementioned compounds have the required pharmacological profile, while having higher affinity and activity than compound **5.61** in *in vitro* assays, the next step would be to perform *in vivo* studies to obtain the pharmacokinetics and pharmacodynamics of the compounds. Modification of to these compounds to comply with Lipinski's guidelines will not be required as they would be inhaled rather

than given orally.[163] Overall, the future aim is to develop a compound that has high affinity to both the β_1AR and the β_2AR while exhibiting full agonism at the β_2AR but an inhibitory effect at the β_1AR. Additionally, appropriate pharmacokinetics and pharmacodynamics will be required. This type of drug would be an invaluable treatment for patients that suffer from asthma or COPD who cannot currently use standard treatments due to additional heart related conditions.[80] Consequently for these patients, quality of life could be substantially increased.

7. Experimental

7.1 Chemistry

All chemical reagents were purchased from commercial suppliers and used without further purification. Compounds were characterised by ^{1}H NMR, ^{13}C NMR and LC-MS or HRMS. For previously published compounds a reference has been provided.

Unless otherwise stated, reactions were performed at ambient temperature. Following organic extraction of an aqueous work-up, the organic phase was dried over $MgSO_4$ or Na_2SO_4 and then filtered, prior to rotary evaporation.

^{1}H and ^{13}C NMR spectra were recorded at 400.13 MHz and 101.62 MHz respectively in deuterated solvents on a Brüker Avance 400 MHz Ultrashield Plus spectrometer. Chemical shifts are referenced to residual solvent peaks and are quoted in ppm. Coupling constants (*J*) are reported to the nearest 0.1 Hz. Assignment of spectra was based on expected chemical shifts and coupling constants.

LC-MS was recorded on an Agilent 1200 Series coupled to the 6120 quadrupole mass spectrometer or a Shimadzu UFLCXR HPLC system combined with an Applied Biosystems MDS SCIEX API2000 electrospray ionization mass spectrometer (ESI-MS) with a Gemini 3 µm C18 110 Å, LC Column 50 x 2 mm. A solvent system of an increasing gradient of acetonitrile (5 to 95%) in water, each containing 0.1% formic acid. A flow rate of 0.5 mL/min was used. UV detection was at 220 and 254 nm. m/z values are given in Daltons to one decimal place. HRMS analyses were recorded using an Agilent 6224 TOF LC-MS coupled to an Agilent 1290 Infinity, or a Bruker microTOF II mass spectrometer, using electrospray ionization (ESI-TOF).

Analytical reverse-phase HPLC was performed on a Waters HPLC system using a Phenomenex Luna C8 100 Å column (150 × 4.6 mm, 5 µm). A solvent system of 0.1% TFA/H_2O and 0.1% TFA/MeCN was used with a gradient of (method a) 5-95% MeCN over 5 minutes or (method b) 30-95% MeCN over 5 minutes, with a flow rate of 1.0 mL/min and monitored at 214 and/ or 254 nm using a Waters 996 Photodiode Array

detector. Unless otherwise stated, all final compounds were of >95% purity, by LC-MS or analytical HPLC at the quoted wavelength.

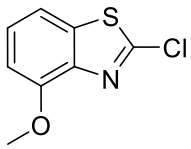
Thin layer chromatography (TLC) was carried out on Merck TLC Silica gel 60 Å F_{254} plates. Flash column chromatography was performed using Davisil silica gel 40–63 micron or Merck Geduran silica gel 60 Å (230–240 µm). All Retention factors (R_f) are given to two decimal places along with the solvent system. Reversed phase column chromatography was performed using a Puriflash C_{18}-Reversed phase 50 µM 20 g flash column. Preperative RP-HPLC was performed on a Waters 2767 sample manager, coupled to a Waters 2525 binary gradient module and a Waters 2457 dual wavelength absorbance detector. The column used was a Phenomenex Gemini 5 µm NX C18 110Å, 150x21 mm.

Partition coefficient (cLogP) values were calculated using MarvinSketch 18.4.

7.1.1 Synthesis

All known compounds and synthetic routes in section 7.1.1 are referenced.

2-Chloro-4-methoxybenzothiazole (3.2) [164]



4-Methoxybenzothiazol-2-amine (10 g, 55.5 mmol) was dissolved in acetonitrile (80 mL) along with copper(II) chloride (11.19 g, 83.2 mmol) and *tert*-butyl nitrite (8.58 g, 83.2 mmol). The mixture was heated to 65 °C for 2 hours and then diluted with 1Nhydrochloric acid and extracted with ethyl acetate. The combined organic layers were then washed with water and then brine. The Solution was dried over magnesium sulphate and concentrated under reduced pressure. Column chromatography (hexanes–ethyl acetate, 80:20) was used to isolate **26** (5.31 g, 48%).

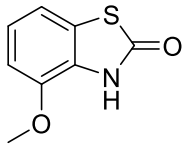
R_f: 0.33 (hexanes–ethyl acetate, 80:20).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (dd, *J* = 8.2, 1.0 Hz, ArH, 1H), 7.44 (t, *J* = 8.1 Hz, ArH, 1H), 7.09 (dd, *J* = 8.2, 0.9 Hz, ArH, 1H), 3.94 (s, CH_3, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 152.4, 150.3, 140.3, 137.2, 127.1, 113.8, 108.3, 55.9.

LC-MS: Calculated for C_8H_7ClNOS $[M+H]^+$ 199.9. found: 199.9

4-Methoxybenzothiazol-2(3*H*)-one (3.3) [32]



3.2 (8.87 g, 44.4 mmol) was dissolved in 21% sodium ethoxide solution (50 mL) and stirred at 60 °C for 24 hours. Ethanol was then removed under reduced pressure and this was followed by the addition of 37%hydrochloric acid solution (50 mL). The mixture was stirred for 2 hours at 60 °C and then diluted with water. The mixture was extracted with ethyl acetate (×2), the organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was filtered through a pad of silica (hexanes – ethyl acetate, 60:40) to afford the title compound (7.06 g, 71%).

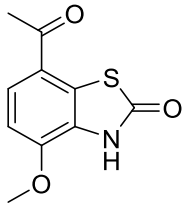
R_f: 0.41 (hexanes–ethyl acetate, 60:40).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.84 (s, NH, 1H), 7.17 – 7.05 (m, ArH, 2H), 6.96 (dd, *J* = 8.0, 1.3 Hz, ArH, 1H), 3.86 (s, CH_3, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.8, 144.8, 125.4, 123.5, 123.0, 114.6, 108.6, 55.8.

LC-MS: Calculated for $C_8H_8NO_2S$ $[M+H]^+$ 182.0. found: 182.0

7-Acetyl-4-methoxybenzothiazol-2(3*H*)-one (3.4) [32]



3.3 (4 g, 22.1 mmol) was dissolved in anhydrous DCM (100 mL) and the solution was cooled to 5 °C. Aluminium chloride (7.36 g, 55.2 mmol) was added and then acetyl chloride (3.81 g, 48.6 mmol) was added dropwise over 20 minutes. The reaction mixture was then stirred for 4 hours at room temperature. The reaction was quenched by carefully pouring onto a mixture of 2Nhydrochloric acid and ice. This was followed by the addition of DCM and a saturated solution of potassium sodium tartrate. The mixture was stirred at ambient temperature overnight and then the mixture was extracted with DCM (× 3), washed with brine and the combined organic extracts were dried over magnesium sulfate. The yellow solid was purified by column chromatography (petroleum ether (40-60) – ethyl acetate, 60:40) to afford the title compound as a white solid (3.18 g, 65%).

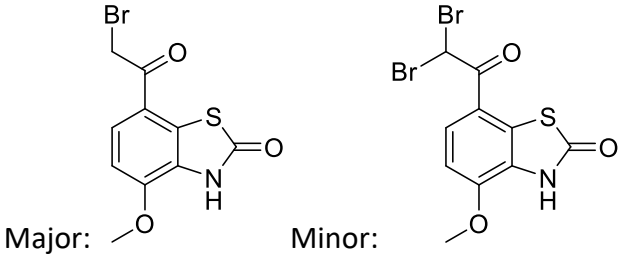
R_f: 0.17 (petroleum ether (40-60) – ethyl acetate, 60:40).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (s, NH, 1H), 7.92 (d, *J* = 8.7 Hz, ArH, 1H), 7.11 (d, *J* = 8.7 Hz, ArH, 1H), 3.97 (s, O-CH_3, 3H), 2.59 (s, Ac, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 196.1, 172.3, 148.3, 126.7, 126.6, 123.5, 123.1, 108.1, 56.4, 25.8.

LC-MS: Calculated for $C_1H_{10}NO_3S$ $[M+H]^+$ 224.0. found: 224.0

7-(2-Bromoacetyl)-4-methoxybenzothiazol-2(3*H*)-one (3.13) [32]



3.4 (7.5 g, 33.6 mmol) was dissolved in THF (100 mL) under N_2 and phenyl trimethyl ammonium tribromide (13.26 g, 35.3 mmol). The mixture was heated at 80 °C and after 4 hours TLC indicated that the reaction was complete. The mixture was allowed to cool to room temperature and then water was added. The precipitate was filtered under vacuum to give the title compound as a white solid. Further purification was not required (5.8 g, 57% including dibromiode side product).

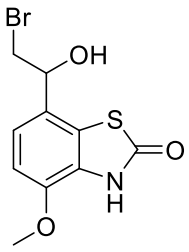
R_f: 0.51 (ethyl acetate)

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.08 (s, NH, 1H), 7.98 (d, *J* = 8.8 Hz, ArH, 1H), 7.15 (d, *J* = 8.8 Hz, ArH, 1H), 4.91 (s, CH_2, 2H), 4.00 (s, CH_3, 3H)

^{13}C NMR (101 MHz, DMSO-d_6) δ 189.8, 171.8, 148.8, 127.1, 126.7, 124.6, 120.1, 108.2, 56.5, 32.5

LC-MS: Calculated for $C_{10}H_9BrNO_3S$ $[M+H]^+$ 301.9. found: 302.2

7-(2-Bromo-1-hydroxyethyl)-4-methoxybenzo[d]thiazol-2(3*H*)-one (3.16)



3.13 (0.26 g, 0.86 mmol) was dissolved in THF (4 mL) and cooled to 0 °C. Sodium borohydride (0.036 g, 0.94 mmol) was added portion wise to the cooled solution. The mixture was stirred for 30 minutes at 0 °C then allowed to warm to room temperature and stir for a further 1 hour. The solution was cooled in an ice bath and

then acidified with 1Nhydrochloric acid. The solution was allowed to stir for 10 minutes and then the solvent was removed *in vacuo* to give the crude product as a yellow solid. The crude product was filtered through a pad of silica, eluting with a mobile phase of EtOAc to give **30** as a white solid (0.21 g, 81%).

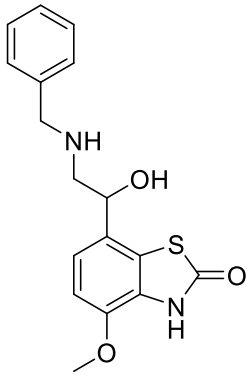
R_f: 0.48 (DCM – Methanol 96:4)

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.77 (s, NH, 1H), 7.09 (d, *J* = 8.6 Hz, ArH, 1H), 7.03 – 6.95 (d, *J* = 8.6 Hz, ArH, 1H), 6.17 (d, *J* = 4.2 Hz, OH, 1H), 4.81 (dt, *J* = 7.1, 4.5 Hz, CH, 1H), 3.85 (s, CH_3, 3H), 3.66 – 3.51 (m, CH_2 2H)

^{13}C NMR (101 MHz, DMSO-d_6) δ 170.6, 144.1, 128.3, 125.8, 121.8, 121.0, 108.2, 71.4, 55.9, 38.3

LC-MS: Calculated for $C_{10}H_{11}BrNO_3S$ $[M+H]^+$ 303.0. found: 304.0

7-(2-(Benzylamino)-1-hydroxyethyl)-4-methoxybenzo[d]thiazol-2(3*H*)-one (3.20)



3.16 (0.037 g, 0.12 mmol) was dissolved in Ethanol (0.4 mL) along with benzylamine (0.028 g, 0.26 mmol). The mixture was heated at 90 °C under microwave irradiation for 1 hour. Water was added and the product was extracted with EtOAc (× 3), washed with brine and then dried over $MgSO_4$. The Solvent was evaporated *in vacuo* to give the crude product as an orange solid. The product was purified by column chromatography (petroleum ether (40-60) – ethyl acetate – triethylamine, 40:60:1 → 30:70:1) to give the title compound as a white solid (0.011 g, 27.8 mmol).

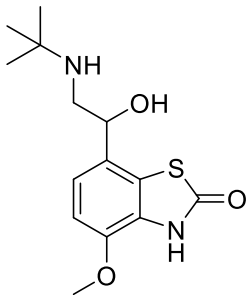
R_f: 0.18 (petroleum ether (40-60) – ethyl acetate – triethylamine, 30:70:1)

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.11 (s, NH, 1H), 7.40 – 7.19 (m, ArH, 5H), 7.03 – 6.89 (m, ArH, 2H), 5.00 (s, CH, 1H), 3.85 (s, CH_3, 3H), 3.74 (t, *J* = 6.7 Hz, $BnCH_2$, 1H), 3.61 (d, *J* = 13.4 Hz, Bn-CH_2, 1H), 3.37 – 3.28 (m, N-CH_2, 2H)

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.7, 143.9, 140.1, 128.1, 128.0, 126.9, 126.6, 126.3, 122.3, 121.1, 108.4, 64.2, 63.2, 55.9, 50.8

LC-MS: Calculated for $C_{17}H_{19}N_2O_3S$ $[M+H]^+$ 331.1. found: 331.2. R_t: 1.96 min (254 nm)

7-(2-(*tert*-Butylamino)-1-hydroxyethyl)-4-methoxybenzothiazol-2(3*H*)-one (3.19)



3.16 (0.04 g, 0.13 mmol) and *tert*-butylamine (0.04 g, 0.53 mmol) were dissolved in ethanol (1.5 mL) and heated at 50 °C under microwave irradiation for 30 minutes. The solvent was removed *in vacuo* and column chromatography was carried out to purify the product, eluting with DCM – methanol (100:0 → 96:4). The title compound was obtained as a light yellow solid (0.01g, 26%).

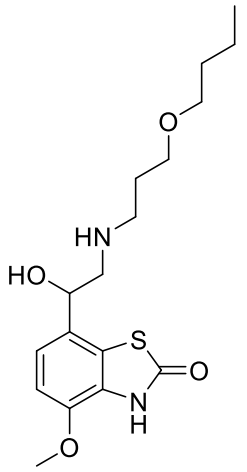
R_f: 0.28 (DCM – methanol 96:4).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.52 (s, NH, 1H), 6.96 (d, *J* = 8.6 Hz, ArH, 1H), 6.86 (d, *J* = 8.4 Hz, ArH, 1H), 5.02 (s, CH, 1H), 3.82 (s, CH_3, 3H), 3.17 (s, CH_2, 2H), 0.95 (s, *t*-Bu, 9H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 172.1, 143.3, 130.8, 126.2, 121.4, 120.9, 108.3, 64.7, 58.1, 55.8, 50.8, 28.7

LC-MS: Calculated for $C_{14}H_3ON_2O_3S$ $[M+H]^+$ 297.2. found: 297.1. R_t: 0.97 min (254 nm)

7-(2-((3-Butoxypropyl)amino)-1-hydroxyethyl)-4-methoxybenzothiazol-2(3*H*)-one (3.21)



3.16 (0.05 g, 0.16 mmol) and 3-hydroxypropylamine (0.065 g, 0.49 mmol) were dissolved in in ethanol (1.5 mL) and heated at 50 °C under microwave irradiation for 1 hour. The solvent was removed *in vacuo* to give a brown oil. Column chromatography was performed to purify the product, eluting with DCM – methanol (100:0 → 95:5) to give the title compound was as a white solid (0.007g, 12%).

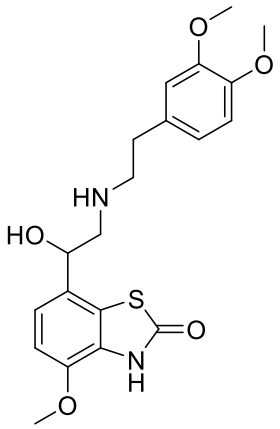
R_f: 0.36 (DCM – methanol 95:5).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.58 (s, NH, 1H), 6.97 (d, *J* = 8.3 Hz, ArH, 1H), 6.88 (d, *J* = 8.5 Hz, ArH, 1H), 4.98 (t, *J* = 5.6 Hz, CH, 1H), 3.83 (s, O-CH_3, 3H) 3.68 (dd, *J* = 8.9, 4.6 Hz, N-CH_2, 2H), 3.42 - 3.25 (m, $\underline{CH_2}$-O-$\underline{CH_2}$, 4H), 2.37 (qt, *J* = 11.4, 6.7 Hz, N-CH_2, 2H), 1.65 (q, *J* = 6.5 Hz, NH-CH_2-$\underline{CH_2}$, 2H), 1.40 (q, *J* = 6.7 Hz, CH_3-CH_2,$\underline{CH_2}$, 2H), 1.24 (sxt, *J* = 7.4 Hz, CH_3-$\underline{CH_2}$, 2H), 0.82 (t, *J* = 7.3 Hz, CH_3, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.8, 143.8, 127.4, 126.2, 122.1, 121.0, 108.3, 69.6, 68.3, 64.4, 64.3, 55.8, 44.7, 31.3, 29.5, 18.8, 13.7

LC-MS: Calculated for $C_{17}H_{27}N_2O_4S$ $[M+H]^+$ 355.2. found: 355.2. R_t: 2.02 min (254 nm)

7-(2-((3,4-Dimethoxyphenethyl)amino)-1-hydroxyethyl)-4-methoxybenzothiazol-2(3*H*)-one (3.22)



3.16 (0.04 g, 0.12 mmol) and 3,4-dimethoxyphenethylamine (0.066 g, 0.36 mmol) were dissolved in ethanol (1.5 mL) and heated at 90 °C under microwave irradiation for 1 hour. The solvent was removed *in vacuo* to give a brown residue. Column chromatography was carried out to purify the product, eluting with DCM – methanol (100:0 → 96:4) to give the title compound was as a white solid (0.015g, 31%).

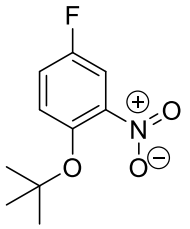
R_f: 0.36 (DCM – methanol 96:4)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.97 (d, *J* = 8.4 Hz, ArH, 1H), 6.92 – 6.73 (m, ArH, 3H), 6.67 (dd, *J* = 8.1, 2.0 Hz, ArH, 1H), 5.00 (t, *J* = 5.4 Hz, CH, 1H), 3.83 (s, CH_3, 3H), 3.71 (s, Ph-O-CH_3, 3H). 3.69 (s, Ph-O-CH_3, 3H), 3.41-3.28 (m, BnCH_2, 2H), 2.75 – 2.63 (m, N-CH_2, 2H), 2.63 – 2.51 (m, N-CH_2, 2H)

^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.8, 148.6, 147.0, 143.8, 132.5, 127.3, 126.2, 122.0, 121.1, 120.3, 112.4, 111.9, 108.3, 64.3, 63.7, 55.8, 55.4, 55.3, 48.9, 35.7

LC-MS: Calculated for $C_{20}H_{25}N_2O_5S$ $[M+H]^+$ 405.1. found: 405.1. R_t: 1.95 min (254 nm)

1-(*tert*-Butoxy)-4-fluoro-2-nitrobenzene (3.24) [119]



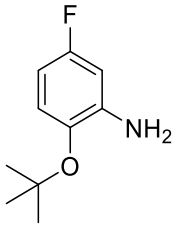
2,5-Difluoronitrobenzene (15.0 g, 94 mmol) was dissolved in anhydrous THF (190 mL) under N_2 and was cooled to 0 °C. Potassium *tert*-butoxide (15.9 g, 141 mmol) was added portion-wise to the cooled solution. The mixture was allowed to warm to room temperature over 2 hours and then left to stir at ambient temperature for 24 hours. The mixture was diluted with DCM and washed with saturated aqueous ammonium chloride. The aqueous phase was back extracted with DCM and the organic layers were combined and dried over $MgSO_4$, concentrated *in vacuo* and then filtered over a pad of silica to give **3.2** as a brown oil (17.96 g, 90%).

R_f: 0.69 (toluene)

^{1}H NMR: (400 MHz, Chloroform-d) δ 7.46 – 7.39 (m, ArH, 1H), 7.25 – 7.12 (m, ArH, C4H, 2H), 1.35 (s, *t*-Bu, 9H)

^{13}C NMR: (101 MHz, Chloroform-d) δ 158.1, 155.6, 145.4, 126.3, 125.7 (C-F), 125.5 (C-F), 119.9, 112.1, 110.7 (C-F), 110.5 (C-F), 83.0, 28.6

2-(*tert*-Butoxy)-5-fluoroaniline (3.25) [119]



3.24 (13.5 g, 63.2 mmol) was added to platinum on carbon (1.0 g) in Methanol (120 mL) and was stirred under 1 atmosphere of H_2 for 18 hours. The catalyst was removed by filtration and the solvent was removed under reduced pressure to afford the title compound as a brown oil (10.9 g, 94%).

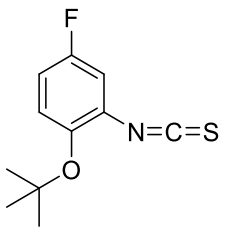
R_f: 0.31 (toluene)

^{1}H NMR: (400 MHz, Chloroform-d) δ 6.85 (dd, *J* = 8.8, 5.6 Hz, ArH, 1H), 6.42 (dd, *J* = 10.0, 3.0 Hz, Ar-C3H, 1H), 6.32 (td, *J* = 8.6, 3.0 Hz, ArH, 1H), 3.90 (br, s, NH_2 2H), 1.37 (s, *t*-Bu, 9H)

^{13}C NMR: (101 MHz, Chloroform-d) δ 158.2, 142.1, 138.9, 123.6, 104.5 (C-F), 104.3 (C-F), 104.2, 103.8 (C-F), 103.6 (C-F), 102.7, 80.0, 28.9

LC-MS: Calculated for $C_{10}H_{15}FNO$ $[M+H]^+$ 184.1. found: 184.2

1-(*tert*-Butoxy)-4-fluoro-2-isothiocyanatobenzene (3.26) [119]



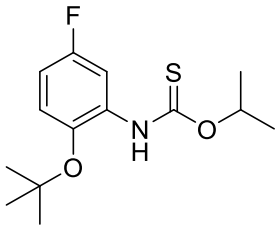
3.25 (0.78 g, 4.26 mmol) was dissolved in the minimum amount of toluene and treated with carbon disulfide (0.32 g, 4.26 mmol). The mixture was cooled to 0 °C and triethylamine (0.43 g, 4.26 mmol) was added. The mixture was allowed to warm to room temperature and was stirred for 24 hours. After stirring the solvent was removed under reduced pressure and the resulting oil was treated with petroleum ether which caused precipitation of the triethylammonium dithiocarbamate salt. The salt was filtered, washed with petroleum ether and allowed to air dry for 10 minutes. The solid was dissolved in chloroform (5 mL) along with triethylamine (0.59 mL, 4.26 mmol). The reaction was cooled to 0 °C and ethyl chloroformate (0.41 mL, 4.26 mmol) was added dropwise over 15 minutes with stirring. The resulting solution was stirred for 10 minutes at 0 °C and then allowed to warm to room temperature over 1 hour. The solution was washed with 3Mhydrochloric acid and water (× 2). The organic layer was dried over sodium sulfate and the solvent was removed under reduced to pressure to afford **3.4** as a yellow oil (0.65 g, 68%).

R_f: 0.49 (Hexane)

^{1}H NMR: (400 MHz, Chloroform-d) δ 7.04 (dd, J = 9.0, 5.3 Hz, ArH, 1H), 6.94 – 6.78 (m, ArH, 2H), 1.42 (s, *t*-Bu, 9H)

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 159.1, 149.1, 140.2, 127.3, 124.51, 114.2, 112.5, 81.8, 28.0. C-F not observed.

o-Isopropyl (2-(*tert*-butoxy)-5-fluorophenyl)carbamothioate (3.27) [119]



3.26 (6.5 g, 28.9 mmol) was dissolved in 2-propanol (22.1 mL, 289 mmol) along with triethylamine (2.92 g, 28.9 mmol), the solution was refluxed for 24 hours under N_2. The solution was concentrated under reduced pressure and the resulting crude product was purified by column chromatography (hexane–ethyl acetate, 9:1). A yellow solid was obtained that was subsequently washed with ice-cold methanol to afford the title compound as an off white solid (6.99 g, 89%).

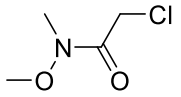
R_f: 0.34 (hexane–ethyl acetate, 9:1).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 8.68 (br, s, NH, 1H), 7.46 (br, s, ArH, 1H), 6.98 (dd, *J* = 8.9, 5.4 Hz, ArH, 1H), 6.71 (ddd, *J* = 8.9, 7.9, 3.1 Hz, ArH, 1H), 5.66 (sept, *J* = 6.3 Hz, *i*-Pr, 1H), 1.44 (d, *J* = 5.2 Hz, *i*-Pr, 6H), 1.38 (s, *t*-Bu, 9H).

^{13}C NMR: (101 MHz, Chloroform-d) δ 187.2 159.4, 140.3 132.9, 123.2, 114.3 (C-F), 114.1 (C-F), 112.5 (C-F), 112.2 (C-F), 110.3, 108.6, 81.3, 77.2, 28.7, 21.7.

LC-MS: Calculated for $C_{14}H_{21}FNO_2S$ $[M+H]^+$ 286.2. found: 286.2

2-Chloro-*N*-methoxy-*N*-methylacetamide (3.28) [123]

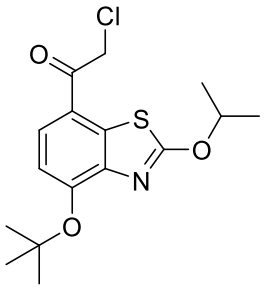


Chloroacetyl chloride (9.04 g, 80 mmol) was dissolved in DCM (50 mL). To this solution, a solution of N,O-Dimethylhydroxylamine hydrochloride (6.54 g, 67 mmol) in water (50 mL) was added. The biphasic solution was cooled to 0 °C and potassium carbonate (11.06 g, 0.08 mmol) was slowly added. The biphasic solution was allowed to warm to room temperature and stirred for 12 hours. The solution was extracted with DCM and the organic phase was washed with saturated sodium bicarbonate and brine. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give **3.6** as a clear yellow oil (7.72 g, 84%).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 4.24 (s, Cl-CH_2 2H), 3.75 (s, O-CH_3 3H), 3.23 (s, N-CH_3, 3H).

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 167.6, 61.8, 40.9, 32.7.

1-(4-(*tert*-Butoxy)-2-isopropoxybenzothiazol-7-yl)-2-chloroethan-1-one (3.29) [165]



3.27 (2.5 g, 8.76 mmol) was dissolved in anhydrous THF (60 mL) and cooled to -78 °C (CO_2/acetone bath) and *tert*-butyllithium (8.0 mL, 24.5 mmol) was added dropwise to the stirring solution. The mixture was warmed to -10 °C over 90 minutes and then re-cooled to -78 °C, this was followed by the addition of **3.28** (1.56 g, 11.4 mmol) dissolved in THF (1.7mL). The reaction mixture was warmed to 0 °C over 60 minutes and then quenched with saturated ammonium chloride (40 mL). The mixture was extracted with ethyl acetate (× 3) and the combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by

flash column chromatography (DCM) to give **3.7** as a white crystalline solid (1.08 g, 36%).

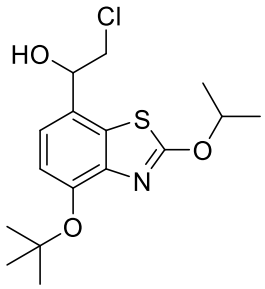
R_f: 0.50 (DCM)

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.82 – 7.74 (m, ArH, 1H), 7.13 (dd, *J* = 8.5, 3.5 Hz, ArH, 1H), 5.48 (sept, *J* = 6.2 Hz, *i*-Pr, 1H), 4.81 (s, Cl-CH_2, 2H), 1.52 (s, *t*-Bu, 9H), 1.49 (d, *J* = 6.3 Hz, *i*-Pr, 6H)

^{13}C NMR: (101 MHz, Chloroform-d) δ 189.0, 175.4, 153.2, 146.2, 134.4, 125.7, 122.9, 120.1, 82.5, 76.2, 45.2, 29.3, 22.0

LC-MS: Calculated for $C_{16}H_{21}ClNO_3S$ $[M+H]^+$ 342.1. found: 342.1

1-(4-(*tert*-Butoxy)-2-isopropoxybenzothiazol-7-yl)-2-chloroethan-1-ol (3.30) [165]



3.29 (1.08 g, 3.16 mmol) was dissolved in THF (10 mL) and $NaBH_4$ (0.12 g, 3.16 mmol) was added portion wise at 0 °C. The mixture was stirred at 0 °C for 30 minutes and then at room temperature for a further 30 minutes. The reaction was cooled to 0 °C and then acidified with ammonium chloride, this was followed by the removal of THF *in vacuo*. The solution was extracted with DCM (× 3) and the combined organic layers were washed with saturated sodium chloride (× 3) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (hexane–diethyl ether, 80:20) to afford the title compound as a brown crystalline solid (0.74 g, 68%).

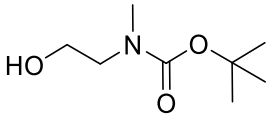
R_f: 0.19 (hexane–diethyl ether, 80:20).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.05 (d, *J* = 8.2 Hz, ArH, 1H), 6.96 (d, *J* = 8.2 Hz, ArH, 1H), 5.45 (sept, *J* = 6.2 Hz, *i*-Pr, 1H), 5.02 – 4.94 (m, HC-OH, 1H), 3.84 – 3.69 (m, Cl-CH_2, 2H), 1.45 (d, 6.4 Hz, *i*-Pr 6H), 1.41 (s, *t*-CH_3, 9H).

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 172.0, 147.5, 146.4, 130.3, 128.3, 121.9, 121.1, 81.0, 76.0, 73.9, 49.3, 29.1, 21.9.

LC-MS: Calculated for $C_{16}H_{23}ClNO_3S$ $[M+H]^+$ 344.1. found: 344.2

***tert*-Butyl (2-hydroxyethyl)(methyl)carbamate (3.37) [166]**



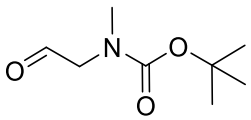
Di-*tert*-butyl dicarbonate (5.89 g, 27.0 mmol) was dissolved in chloroform (10 mL) and added dropwise to a solution of 2-(methylamino)ethanol (2 g, 27.0 mmol) in chloroform (20 mL) at approximately 5 °C. The mixture was stirred at room temperature for 18 hours and then concentrated *in vacuo*. The residue was purified by column chromatography (ethyl acetate) to give the product as a clear colourless oil (4.41 g, 93%).

R_f: 0.48 (ethyl acetate).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 3.72 (t, *J* = 5.4 Hz, HO-CH_2 2H), 3.37 (t, *J* = 5.4 Hz, N-CH_2, 2H), 2.90 (s, N-CH_3, 3H), 2.56 (s, OH, 1H), 1.44 (s, *t*-CH_3, 9H)

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 156.0, 80.0, 61.6, 51.5, 35.6, 28.5.

***tert*-Butyl methyl(2-oxoethyl)carbamate (3.38) [167]**



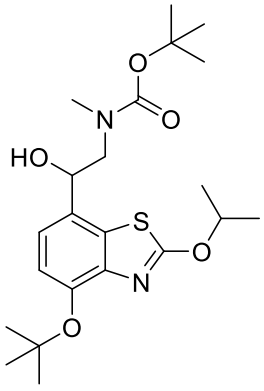
Under anhydrous conditions oxalyl chloride (1.25 g, 9.84 mmol) was dissolved in DCM (25 mL) and cooled to -78 °C. To the solution, dry DMSO (1.46 mL, 20.54 mmol) was

added dropwise and then allowed to stir for 15 minutes. The alcohol **3.37** (1.5 g, 8.56 mmol) was dissolved in DCM (4.3 mL) and added dropwise to the mixture. The solution was stirred for a further 15 minutes and then triethylamine (4.33 g, 42.8 mmol) was added dropwise. The reaction mixture was stirred for 1 hour at -78 °C and then allowed to warm to room temperature over 1 hour. The reaction mixture was diluted with DCM and quenched by the addition of water. The aqueous layer was acidified with aqueous 2Nhydrochloric acid and then extracted with DCM (× 2). The combined organic layers were washed with 1%hydrochloric acid in brine and then 5% sodium bicarbonate solution. The aqueous layer was extracted with DCM. The combined organic layers were washed with brine and then dried over magnesium sulfate and then concentrated under reduced pressure to afford **3.17** as a clear yellow oil (0.93 g, 62%).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 9.59 (s, O=CH, 1H), major rotomers: 3.89 (s, N-CH_2, 2H), 2.95 (s, N-CH_3, 3H), 1.41 (s, *t*-Bu, 9H), minor rotomers: 4.00 (s, N-CH_2, 2H), 2.92 (s, N-CH_3, 3H), 1.46 (s, *t*-Bu, 9H).

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 198.7, 36.0, 28.4, major rotomer: 155.4, 80.8, 59.3 minor rotomers: 156.2, 80.6, 58.9.

tert-Butyl(2-(4-(_tert_-butoxy)-2-isopropoxybenzothiazol-7-yl)-2hydroxyethyl)(methyl)carbamate (3.39) [119]



A solution of **3.27** (1.30 g, 4.6 mmol) in anhydrous THF (8 mL) and cooled to -78 °C (CO_2/acetone bath) and *tert*-butyllithium (1.22 mL, 1.96 mmol) was added dropwise

to the stirring solution. The mixture was warmed to -10 °C over 90 minutes and then re-cooled to -78 °C, this was followed by the addition of the carbamate **3.38** (0.145 g, 0.84 mmol) dissolved in THF (0.5 mL). The reaction mixture was warmed to 0 °C over 60 minutes and then quenched with saturated ammonium chloride (6 mL). The mixture was extracted with ethyl acetate (× 3) and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (Hexanes–ethyl acetate, 90:10 → 75:25) to give **3.18** as a clear yellow oil (0.169 g, 55%).

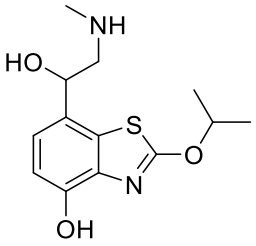
R_f: 0.20 (Hexanes–ethyl acetate, 80:20).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.05 (s, ArH, 1H), 6.98 (d, *J* = 7.8 Hz, ArH, 1H), 5.45 (p, *J* = 6.2 Hz, ^{i}Pr-CH, 1H), 5.01 (s, HO-CH, 1H), 3.61 – 3.54 (m, N-CH_2, 2H), 2.63 (s, N-CH_3, 3H), 1.46 (s, t-Bu-CH_3, 9H), 1.44 (d, *J* = 6, 2 × *i*-Pr, 6H), 1.40 (s, *t*-Bu, 9H).

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 172.1, 146.8, 146.2, 131.1, 130.0, 122.0, 120.6, 80.7, 77.4, 76.0, 74.0, 56.2, 36.0, 29.1, 28.6, 22.0.

LC-MS: Calculated for $C_{22}H_{35}N_2O_5S$ $[M+H]^+$ 438.2. found: 440.2

7-(1-Hydroxy-2-(methylamino)ethyl)-2-isopropoxybenzothiazol-4-ol (3.40) [119]



3.39 (0.039 g, 0.088 mmol) was dissolved in TFA (0.3 mL) and stirred for 1 hour at room temperature. Methanol was added and then blown down with N_2, this was repeated until all TFA was removed. A yellow oil was obtained which was purified with C_{18} reverse phase column chromatography (H_2O–Methanol–TFA, 100:0:0.01 → 0:100:0.01). The title compound was afforded as a clear colourless oil (0.023 g, 92%).

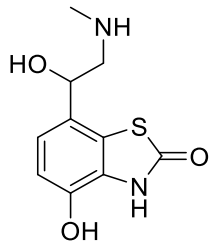
^{1}H NMR: (400 MHz, DMSO-d_6) δ 9.80 (s, ArOH, 1H), 7.01 (d, *J* = 8.2 Hz, ArH, 1H), 6.82 (d, *J* = 8.2 Hz, ArH, 1H), 6.55 (m, HC-OH, 1H), 5.36 (sept, *J* = 6.2 Hz, *i*-Pr-CH, 1H), 4.96

(dt, *J* = 8.1, 3.9 Hz, HO-CH, 1H), 4.11 (s, NH, 1H), 3.06 (s, N-CH_2, 2H), 2.59 (s, N-CH_3, 3H), 1.42 (d, *J* = 6.2 Hz, *i*-Pr, 6H).

^{13}C NMR: (101 MHz, DMSO) δ 170.8, 149.0, 138.5, 128.7, 125.0, 121.7, 111.7, 75.5, 67.7, 53.0, 32.8, 21.6.

LC-MS: Calculated for $C_{13}H_{19}N_2O_3S$ $[M+H]^+$ 283.1 found 283.2. R_t: 1.09 min (254 nm)

4-Hydroxy-7-(1-hydroxy-2-(methylamino)ethyl)benzothiazol-2(3*H*)-one (1.10) [119]



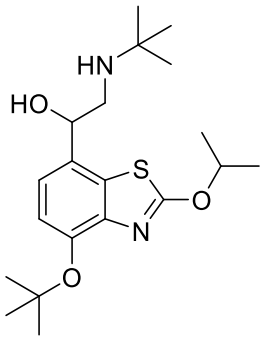
3.40 (0.05 g, 0.118 mmol) was dissolved in formic acid (0.5 mL) and left to stir at ambient temperature for 48 hours. The crude product was purified using C_{18} reverse phase column chromatography (H_2O–Methanol–TFA, 100:0:0.01 → 0:100:0.01) to give the product as a clear colourless oil (0.010 g, 36%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 11.68 (s, NH, 1H), 10.23 (s, ArOH, 1H), 6.92 (d, *J* = 8.3 Hz, ArH, 1H), 6.78 (d, *J* = 8.3 Hz, ArH, 1H), 6.44 (m, HO-CH, 1H), 4.89 – 4.81 (m, HO-CH, 1H), 3.06 – 2.96 (m, N-CH_2, 2H), 2.60 (s, N-CH_3, 3H)

^{13}C NMR: (101 MHz, DMSO-d_6) δ 170.2, 142.4, 140.9, 125.5, 125.4, 121.2, 120.5, 112.1, 67.6, 48.5, 32.8

LC-MS: Calculated for $C_{10}H_{13}N_2O_3S$ $[M+H]^+$ 241.1. found: 241.1. R_t: 0.31 min (254 nm)

1-(4-(*tert*-Butoxy)-2-isopropoxybenzothiazol-7-yl)-2-(*tert*-butylamino)ethan-1-ol (7.1)



3.30 (0.08 g, 0.23mmol) was dissolved in *tert*-butylamine (1 mL) and stirred at 35 °C for 3 days. Excess *tert*-butylamine was removed by repeatedly adding methanol and blowing down with nitrogen. The product was purified by column chromatography (DCM–Methanol–ammonia, 99:1:1→98:2:1) to give title compound as a clear colourless oil (0.078 g, 89%).

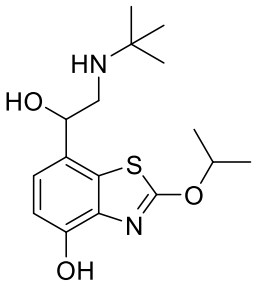
R_f: 0.11(DCM–Methanol–ammonia, 98:2:1).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.03 (d, *J* = 8.2 Hz, ArH, 1H), 6.96 (d, *J* = 8.2 Hz, ArH, 1H), 5.46 (sept, *J* = 6.2 Hz, *I*-Pr, 1H), 4.68 (dd, *J* = 9.4, 3.7 Hz, HO-CH, 1H), 2.95 (dd, *J* = 12.1, 3.7 Hz, N-CH_2, 1H), 2.73 (dd, *J* = 12.1, 9.5 Hz, N-CH_2, 1H), 1.50 – 1.39 (m, 2 × *I*-Pr, 6H), 1.41 (s, *t*-Bu, 9H), 1.14 (s, *t*-Bu, 9H)

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 172.2, 146.8, 146.3, 131.2, 129.9, 121.8, 120.6, 80.6, 75.6, 71.9, 51.0, 48.1, 29.2, 29.1, 22.0

LC-MS: Calculated for $C_{20}H_{33}N_2O_3S$ $[M+H]^+$ 381.2. found: 381.2

7-(2-(*tert*-Butylamino)-1-hydroxyethyl)-2-isopropoxybenzothiazol-4-ol (3.50)



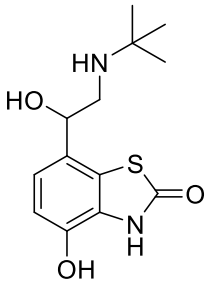
7.1 (0.038 g, 0.10 mmol) was dissolved in TFA (0.3 mL) and stirred for 1 hour at room temperature. Methanol was added and then blown with N_2, this was repeated until all TFA was removed. The crude white solid was purified by C_{18} reverse phase column chromatography (H_2O–Methanol–TFA, 100:0:0.01 → 0:100:0.01) to give the title compound as a white solid (0.016 g, 49%).

^{1}H NMR: (400 MHz, Methanol-d_4) δ 7.13 – 7.06 (m, ArH, 1H), 6.83 (d, *J* = 8.2 Hz, ArH, 1H), 5.44 (hept, *J* = 6.2 Hz, ^{i}Pr-CH, 1H), 5.01 (dd, *J* = 8.6, 5.1 Hz, HO-CH, 1H), 3.21 – 3.06 (m, N-CH_2, 2H), 1.59 – 1.42 (m, *I*-Pr, 6H), 1.38 (s, *t*-Bu, 9H)

^{13}C NMR (101 MHz, Methanol-d_4) δ 173.6, 150.4, 140.3, 130.3, 126.5, 123.0, 112.7 77.26, 70.36, 58.25, 47.8, 25.7, 22.1

LC-MS: Calculated for $C_{16}H_{25}N_2O_3S$ $[M+H]^+$ 325.2. found: 325.3. R_t: 2.00 min (254 nm)

7-(2-(*tert*-Butylamino)-1-hydroxyethyl)-4-hydroxybenzothiazol-2(3*H*)-one (3.45)



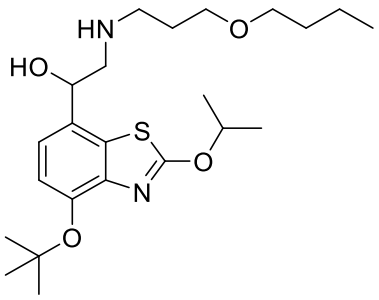
3.50 (0.015 g, 0.046 mmol) was dissolved in formic acid (0.15 mL) and left to stir at ambient temperature for 48 hours. The crude white solid was purified using C_{18} reverse phase column chromatography (H_2O–Methanol–TFA, 100:0:0.01 → 0:100:0.01) to give the title compound as a white solid (0.004 g, 64%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.95 (d, *J* = 8.3 Hz, ArH, 1H), 6.75 (d, *J* = 8.3 Hz, ArH, 1H), 4.78 (dd, *J* = 10.0, 3.2 Hz, HO-CH, 1H), 2.95 – 2.78 (m, N-CH_2, 2H), 1.22 (s, *t*-Bu, 9H), exchangeable protons not observed.

^{13}C NMR: (101 MHz, DMSO-d_6) δ 170.3, 142.4, 125.5, 121.2, 120.6, 112.2, 111.6, 68.9, 46.7, 25.7

LC-MS: Calculated for $C_{13}H_{19}N_2O_3S$: *m/z* 283.1 $[M+H]^+$; found 283.2. R_t: 0.46 min (254 nm)

1-(4-(*tert*-Butoxy)-2-isopropoxybenzothiazol-7-yl)-2-((3-butoxypropyl)amino)ethan-1-ol (7.2)



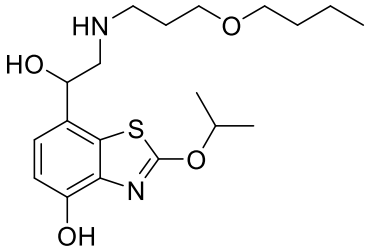
3.30 (0.08 g, 0.233 mmol) was dissolved in ethanol (1 mL) along with 3-butoxypropylamine (0. 1.38 g, 0.93 mmol) and heated at 70 °C for 90 minutes. Saturated sodium bicarbonate (1 mL) was added and the solution was extracted with ethyl acetate (× 3), the combined organic layers were washed with brine, dried over magnesium sulfate, and then concentrated under reduced pressure. The crude yellow oil was purified by C_{18} reverse phase column chromatography (H_2O–Methanol–TFA, 100:0:0.01 → 0:100:0.01) to give the title compound as a clear colourless oil (0.05 g, 56%).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.04 – 6.93 (m, ArH, 2H), 5.44 (hept, *J* = 6.3 Hz, i-Pr, 1H), 5.34 – 5.29 (m, HO-CH, 1H), 3.73 – 3.54 (m, 2 × CH_2 4H), 3.51 – 3.43 (m, N-CH_2, 2H), 3.26 – 3.14 (m, CH_2, 2H), 1.54 – 1.41 (m, *i*-Pr, 3 × CH_2 12H), 1.40 (s, *t*-Bu, 9H), 0.91 (dt, *J* = 9.9, 7.3 Hz, CH_2-CH_3, 3H).

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 172.4, 147.4, 146.6, 129.9, 128.4, 122.0, 120.7, 81.00, 76.0, 72.0, 70.0, 68.4, 53.1, 48.2, 31.6, 29.2, 25.5, 22.0, 19.4, 14.0.

LC-MS: Calculated for $C_{23}H_{39}N_2O_4S$ $[M+H]^+$ 439.3. found: 440.3.

7-(2-((3-Butoxypropyl)amino)-1-hydroxyethyl)-2-isopropoxybenzothiazol-4-ol (3.52)



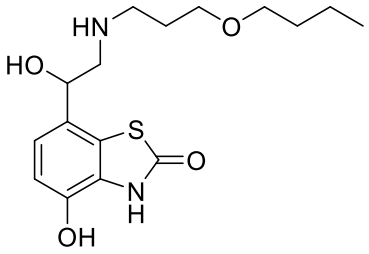
7.2 (0.05 g, 0.11 mmol) was dissolved in TFA (0.5 mL) and stirred for 1 hour at room temperature. Methanol was added and then blown with N_2, this was repeated until all TFA was removed. The crude clear colourless oil was purified by C_{18} reverse phase column chromatography (H_2O–Methanol–TFA, 100:0:0.01 → 0:100:0.01) to give the title compound as a clear colourless oil (0.014 g, 33%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 9.80 (s, OH, 1H), 7.01 (d, *J* = 8.3 Hz, ArH, 1H), 6.81 (d, *J* = 8.2 Hz, ArH, 1H), 6.55 (s, HC-OH, 1H), 5.35 (p, *J* = 6.2 Hz, ^{i}Pr-CH, 1H), 4.96 (d, *J* = 9.4 Hz, HO-CH, 1H), 3.45 – 3.31 (m, N-CH_2, 2H), 3.35 – 3.22 (m, CH_2, 2H), 3.01 (d, *J* = 7.3 Hz, CH_2, 2H), 1.86 (tt, *J* = 14.1, 6.9 Hz, CH_2 2H), 1.53 – 1.36 (m, *i*-Pr, 2 × CH_2 10H), 1.35 – 1.25 (m, CH_2 2H), 0.91 – 0.77 (m, CH_2-CH_3, 3H)

^{13}C NMR: (101 MHz, DMSO-d6) δ 170.83 150.0, 138.6, 128.8, 125.5, 121.8, 111.7, 75.6, 69.8, 67.9, 67.2, 51.6, 45.2, 31.2, 25.2, 21.6, 18.9, 13.8

LC-MS: Calculated for $C_{19}H_{31}N_2O_4S$: *m/z* 383.2 $[M+H]^+$; found 383.0 R_t: 2.20 min (254 nm)

7-(2-((3-Butoxypropyl)amino)-1-hydroxyethyl)-4-hydroxybenzothiazol-2(3*H*)-one (3.47)



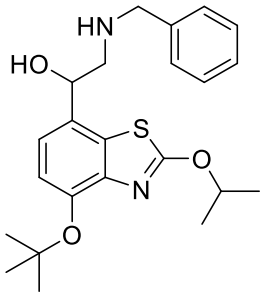
3.52 (0.007 g, 0.018 mmol) was dissolved in formic acid (0.1 mL) and left to stir at ambient temperature for 48 hours. The crude product was purified using C_{18} reverse phase column chromatography (H_2O–Methanol–TFA, 100:0:0.01 → 0:100:0.01) to give the title compound as a clear colourless oil (0.004 g, 64%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 11.67 (s, NH, 1H), 10.19 (s, OH, 1H), 6.93 (d, *J* = 8.3 Hz, ArH, 1H), 6.76 (d, *J* = 8.3 Hz, ArH, 1H), 6.43 (m, HC-OH, 1H), 4.89 – 4.81 (m, HO-CH, 1H), 3.41 (t, *J* = 5.9 Hz, N-CH_2, 2H), 3.02 (dd, *J* = 13.8, 7.5 Hz, 2 × CH_2, 4H), 1.85 (tt, *J* = 13.6, 7.1 Hz, CH_2, 2H), 1.53 – 1.12 (m, 3 × CH_2, 6H), 0.92 – 0.78 (m, CH_2-CH_3, 3H).

^{13}C NMR (101 MHz, DMSO-d6) δ 170.3, 142.4, 125.5, 125.5, 121.2, 120.7, 112.2, 69.8, 67.7, 67.2, 51.6, 45.1, 31.2, 25.7, 18.9, 13.8

LC-MS: Calculated for $C_{16}H_{25}N_2O_4S$: *m/z* 341.2 $[M+H]^+$; found 341.3. R_t: 1.91 min (254 nm)

2-(Benzylamino)-1-(4-(*tert*-butoxy)-2-isopropoxybenzothiazol-7-yl)ethan-1-ol (7.3)



3.30 (0.08 g, 0.23 mmol) was dissolved in ethanol (1 mL) and benzylamine (0.054 g, 0.51 mmol) was added. The mixture was refluxed for 16 hours and then allowed to

cool. Saturated sodium bicarbonate (1 mL) was added and the solution was extracted with ethyl acetate (× 3), the combined organic layers were washed with brine, dried over magnesium sulfate, and then concentrated under reduced pressure. The crude yellow oil was purified with column chromatography (DCM–Methanol–ammonia, 99.5:0.5:0.5→96:4:0.5) to give **3.26** as a clear yellow oil (0.048 g, 50%).

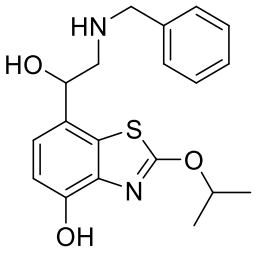
R_f: 0.48 (DCM–Methanol–ammonia, 99:1:0.5).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 – 7.30 (m, BnH, 5H), 7.05 – 6.92 (m, ArH, 2H), 5.44 (h, *J* = 6.2 Hz, *i*-Pr, 1H), 4.82 (dd, *J* = 9.1, 3.6 Hz, HO-CH, 1H), 3.92 – 3.79 (m, N-CH_2, 2H), 3.10 – 2.83 (m, Bn-CH_2, 2H) , 1.52 – 1.39 (m, *i*-Pr, 6H), 1.40 (s, *t*-Bu, 9H), exchangeable protons not observed.

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 172.2, 146.9, 146.3, 139.3, 130.8, 130.0, 128.7, 128.4, 127.5, 121.9, 120.6, 80.7, 75.7, 71.3, 54.2, 53.4, 29.2, 22.0

LC-MS: Calculated for $C_{23}H_{31}N_2O_3S$ $[M+H]^+$ 415.2 found: 415.1.

7-(2-(Benzylamino)-1-hydroxyethyl)-2-isopropoxybenzothiazol-4-ol (3.51)



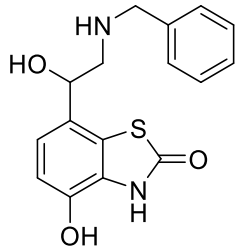
7.3 (0.048 g, 0.116 mmol) was dissolved in TFA (0.4 mL) and stirred for 1 hour at room temperature. Methanol was added and then blown with N_2, this was repeated until all TFA was removed. The title compound was afforded as a white solid (0.040 g, 98%).

^{1}H NMR: (400 MHz, Methanol-d_4) δ 7.54 – 7.42 (m, BnH, 5H), 7.03 (d, *J* = 8.2 Hz, ArH, 1H), 6.80 (d, *J* = 8.2 Hz, ArH, 1H), 5.42 (sept, *J* = 6.2 Hz, *i*-Pr, 1H), 5.07 (dd, *J* = 9.4, 4.3 Hz, HO-CH, 1H), 4.27 (s, BnCH_2, 2H), 3.24 – 3.07 (m, N-CH_2, 2H), 1.45 (d, *J* = 6.2 Hz, *i*-pr, 6H).

^{13}C NMR: (101 MHz, Methanol-d_4) δ 173.7, 150.4, 140.3, 132.3, 131.1, 130.8, 130.3, 130.2, 126.2, 123.0, 112.7, 77.3, 69.7, 52.5, 52.1, 22.1.

LC-MS: Calculated for $C_{19}H_{23}N_2O_3S$: *m/z* 359.1[M+H]$^+$; found 359.2. R_t: 2.09 min (254 nm)

7-(2-(Benzylamino)-1-hydroxyethyl)-4-hydroxybenzothiazol-2(3*H*)-one (3.46)



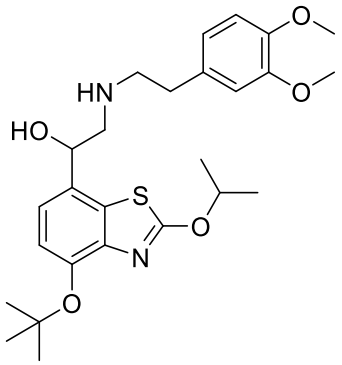
3.51 (0.02 g, 0.056 mmol) was dissolved in formic acid (0.2 mL) and left to stir at ambient temperature for 48 hours. The crude product was purified using C_{18} reverse phase column chromatography (H_2O–Methanol–TFA, 90:10:0.01 → 0:100:0.01) to give the title compound as a white solid (0.004 g, 26%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 11.66 (s, NH, 1H), 10.20 (s, OH, 1H), 7.47 (m, BnH, 5H), 6.89 (d, *J* = 8.3 Hz, ArH, 1H), 6.75 (d, *J* = 8.3 Hz, ArH, 1H), 6.43 (s, HC-OH, 1H), 4.88 (s, HO-CH, 1H), 4.19 (d, *J* = 3.0 Hz, N-CH_2, 2H), 2.97 (d, *J* = 6.3 Hz, BnCH_2, 2H).

^{13}C NMR: (101 MHz, DMSO-d_6) δ 170.2, 142.5, 141.1, 130.1, 129.0, 128.7, 125.5, 125.2, 121.2, 120.6, 112.1, 67.7, 50.9, 50.1.

LC-MS: Calculated for $C_{16}H_{17}N_2O_3S$: 317.1 [M+H]$^+$; found 317.0. R_t: 1.71 min (254 nm)

1-(4-(*tert*-Butoxy)-2-isopropoxybenzothiazol-7-yl)-2-((3,4-dimethoxyphenethyl)amino)ethan-1-ol (7.4)



3.8 (0.05 g, 0.145 mmol) was dissolved in ethanol (0.5 mL) and 2-(3,4-dimethoxyphenyl)ethan-1-amine (0.053 g, 0.290 mmol) was added. The mixture was refluxed for 16 hours and then allowed to cool. Saturated sodium bicarbonate (0.5 mL) was added and the solution was extracted with ethyl acetate (× 3), the combined organic layers were washed with brine, dried over magnesium sulfate, and then concentrated under reduced pressure. The crude yellow oil was purified with column chromatography (DCM–Methanol–ammonia, 99:1:0.5→98:2:0.5) to give **3.29** as a clear colourless oil (0.036 g, 51%).

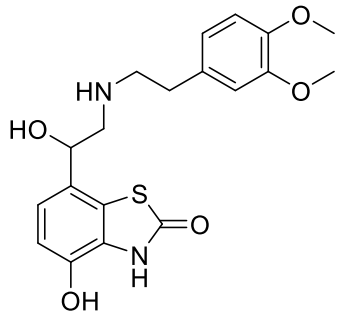
R_f: 0.48 (DCM–Methanol–ammonia, 96:4:0.5)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.04 – 6.89 (m, ArH, 2H), 6.83 – 6.69 (m, ArH, 3H), 5.45 (p, *J* = 6.2 Hz, *i*-Pr, 1H), 4.79 (dd, *J* = 9.3, 3.6 Hz, HO-CH, 1H), 3.86 (d, *J* = 6.4 Hz, 2 × O-CH_3, 6H), 3.02 – 2.68 (m, 3 × CH_2, 6H), 1.49 – 1.35 (m, 2 × ^{i}Pr-CH_3, 6H), 1.39 (s, *t*-Bu, 9H)

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 172.1, 149.2, 147.7, 146.9, 146.3, 132.1, 130.9, 129.9, 121.8, 120.7, 120.5, 112.2, 111.5, 80.6, 77.4, 75.7, 71.2, 56.0, 54.6, 50.7, 35.9, 29.1, 22.0

LC-MS: Calculated for $C_{26}H_{39}N_2O_5S$ $[M+H]^+$ 489.2. found 489.1

7-(2-((3,4-Dimethoxyphenethyl)amino)-1-hydroxyethyl)-4-hydroxybenzothiazol-2(3H)-one (3.48)



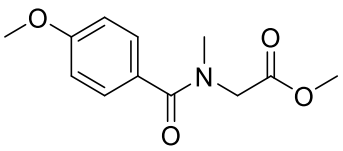
7.4 (0.016 g, 0.032 mmol) was dissolved in formic acid (0.15 mL) and left to stir at ambient temperature for 48 hours. The crude product was purified using C_{18} reverse phase column chromatography (H_2O–Methanol–TFA, 100:0:0.01 → 60:40:0.01) to give the title compound as a white solid (0.006 g, 47%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 11.67 (s, NH, 1H), 10.20 (s, OH, 1H), 7.00 – 6.86 (m, ArH, 3H), 6.80 – 6.71 (m, ArH, 2H), 6.46 (s, HC-OH, 1H), 4.88 (s, HO-CH, 1H), 3.73 (d, *J* = 10.6 Hz, 2 × O-CH_3, 6H), 3.17 (s, N-CH_2, 2H), 3.06 (s, N-CH_2, 2H), 2.96 – 2.78 (m, Ar-CH_2, 2H), exchangeable NH not observed .

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 170.3, 147.7, 142.4, 139.0, 130.9, 129.4, 126.1, 125.5, 122.8, 121.2, 120.6, 112.5, 111.5, 67.8, 55.5, 48.2, 37.4, 31.0

LC-MS: Calculated for $C_{19}H_{23}N_2O_3S$ $[M+H]^+$ 391.1. found: 391.2. R_t: 1.84 min (254 nm)

Methyl *N*-(4-methoxybenzoyl)-*N*-methylglycinate (4.5) [168]



Sarcosine methyl ester hydrochloride (5 g, 35.8 mmol) was dispersed in DCM (50 mL) and TEA (6.9 g, 68.2 mmol) was added. The mixture was cooled in an ice bath and then 4-methoxybenzoyl chloride (5.82 g, 34.1 mmol) was added. The mixture was stirred overnight at room temperature. Water was added and the mixture was

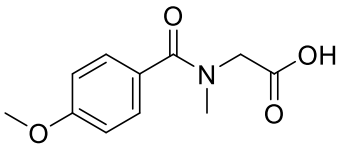
extracted with DCM (×2). The organic layers were combined, washed with 1Mhydrochloric acid solution, saturated aq. Sodium hydrogen carbonate and then water. The organic layer was then dried over magnesium sulfate and concentrated *in vacuo* to give a yellow oil. diethyl ether was added and heated which caused yellow solid to crash out. The solid was filtered and the filtrate concentrated *in vacuo* to give the product as yellow oil (7.25 g, 89% including small amount of starting material).

^{1}H NMR: (400 mhz, $CDCl_3$) δ 7.53 – 7.28 (m, ArH, 2H), 6.92 – 6.74 (t, *J* = 10.5 Hz, ArH, 2H), 4.30 – 3.96 (m, N-$\underline{CH_2}$, 2H), 3.80 (s, Ph-O-$\underline{CH_3}$, 3H), 3.75 (s, COO$\underline{CH_3}$, 3H), 3.07 (s, N-CH_3, 3H)

^{13}C NMR: (101 MHz, $CDCl_3$) δ 171.9, 169.8, 160.9, 132.8, 129.4, 113.9, 55.4, 52.2, 49.3, 31.0.

LC-MS: calculated for $C_{12}H_{16}NO_4$ $[M+H]^+$ 238.1. found: 237.9

***N*-(4-Methoxybenzoyl)-N-methylglycine (4.6)** [169]



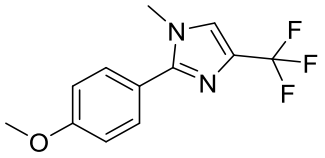
4.5 (7.25 g, 30.6 mmol) was dissolved in THF (25 mL) and water (25 mL), lithium hydroxide monohydrate (1.92 g, 45.8 mmol) was then added. The solution was stirred for 16 hours at ambient temperature. THF was removed under reduced pressure, ethyl acetate was added to the aqueous solution followed by excess aq. 2Mhydrochloric acid. The aqueous solution was then extracted with EA. The organic layer was then dried over magnesium sulfate and concentrated *in vacuo* to give the product as a yellow solid (4.9 g, 72%).

^{1}H NMR: (400 mhz, DMSO) δ 7.46 – 7.21 (m, ArH, 2H), 6.99 (dd, *J* = 8.7, 4.8 Hz, ArH, 2H), 4.16 – 3.93 (m, N-$\underline{CH_2}$, 2H), 3.77 (s, Ph-O-$\underline{CH_3}$, 3H), 2.98 (s, N-CH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 170.5, 167.0, 162.5, 131.3, 129.1, 113.5, 55.4, 48.9, 38.8.

LC-MS: calculated for $C_{11}H_{14}NO_4$ $[M+H]^+$ 224.1. found: 224.0

2-(4-Methoxyphenyl)-1-methyl-4-(trifluoromethyl)-1*H*-imidazole (4.7) [101]



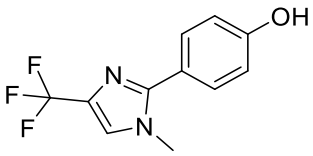
4.6 (4.9 g, 22.0 mmol) was dissolved in DCM (60 mL) under nitrogen and was cooled to 0 °C. Trifluoroacetic anhydride (13.83 g, 65.9 mmol) was added and the mixture was stirred overnight at room temperature. Volatiles were removed *in vacuo* to give a brown oil. The residue was redissolved in DMF (60 mL) under nitrogen and was cooled to 0 °C. Ammonium acetate (2.54 g, 32.9 mmol) was added and the mixture was heated at 70 °C for 4 hours. DMF was removed under reduced pressure, water was added and the aqueous solution was extracted with ethyl acetate (×3). The organic layers were combined and washed with an aq. solution of 1M potassium hydroxide and then water, dried over magnesium sulfate and concentrated *in vacuo* to give a yellow solid. diethyl ether was added and heated, solid crashed out and was filtered. The filtrate was concentrated in vacuo to give an orange oil which was purified by CC, eluting with PE-EA (80:20→50:50). The product was isolated as a pale yellow oil (1.49 g, 26%).

^{1}H NMR: (400 mhz, $CDCl_3$) δ 7.50 – 7.44 (m, ArH, 2H), 7.19 (s, imid-H, 1H), 6.93 – 6.87 (m, ArH, 2H), 3.77 (s, O-CH_3, 3H), 3.64 (s, N-CH_3, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 160.63, 149.15, 132.2, 130.4, 130.0, 129.2 (q, CF_3) 121.5, 114.1, 113.8, 55.4, 34.8.

LC-MS: calculated for $C_{12}H_{12}F_3N_2O$ $[M+H]^+$ 257.1. found: 256.9

4-(1-Methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl)phenol (4.8) [101]



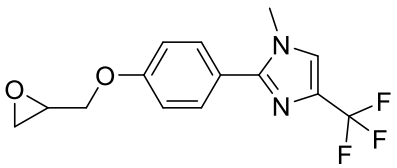
4.7 (0.75 g, 2.93 mmol) was dissolved in dry DCM (15 mL) under nitrogen. The solution was cooled to -78 °C in a dry ice/acetone bath. Boron tribromide 1M in hexane (11.71 mL, 11.7 mmol) was added dropwise and then the mixture was allowed to room temperature. After 1.5 hours of stirring, the reaction had complete and the solution was carefully poured into methanol. The solution was concentrated under reduced pressure to give a dark brown solid. A small amount of ethyl acetate was added followed by excess PE. The brown crystalline product was then filtered under vacuum (0.61 g, 86%).

^{1}H NMR: (400 mhz, DMSO) δ 8.03 (s, imid-H, 1H), 7.59 – 7.51 (m, ArH, 2H), 6.95 – 6.89 (m, ArH, 2H), 3.76 (s, N-CH_3, 3H), exchangeable OH not observed.

^{13}C NMR: (101 MHz, DMSO) δ 159.4, 148.8, 130.9, 125.4, 124.1, 120.7, 118.7, 115.9, 35.5. CF_3 not observed.

LC-MS: calculated for $C_{11}H_{10}F_3N_2O$ $[M+H]^+$ 243.1. found: 242.9

1-Methyl-2-(4-(oxiran-2-ylmethoxy)phenyl)-4-(trifluoromethyl)-1*H*-imidazole (4.9) [101]



4.8 (0.4 g, 1.64 mmol) was dissolved in DMF (12 mL) and caesium carbonate (1.07 g, 3.29 mmol) was added followed by the addition of (±)epichlorohydrin (0.22 g, 1.18 mmol). The mixture was stirred at 50 °C overnight and then DMF was removed *in vacuo* and the residue was redissolved in EA. The organic solution was washed with water (×4), dried over magnesium sulfate and then concentrated under reduced

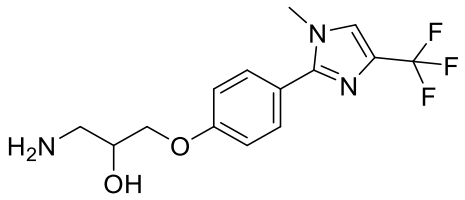
pressure to give a yellow oil. The oil was dissolved in DCM and filtered through a pad of silica with a mobile phase of DCM. The product was isolated as a white oil (0.29 g, 59%).

^{1}H NMR: (400 mhz, $CDCl_3$) δ 7.61 – 7.51 (m, ArH, 2H), 7.31 – 7.26 (m, imid-H 1H), 7.05 – 6.97 (m, ArH, 2H), 4.34 – 4.22 (m, O-$\underline{CH_2}$ 1H), 3.99 (dd, *J* = 11.0, 5.8 Hz, O-$\underline{CH_2}$ 1H), 3.74 (s, N-CH_3, 3H), 3.44 – 3.33 (m, epox-CH_1, 1H), 2.99 – 2.89 (m, epox-CH_2 , 1H), 2.78 (dd, *J* = 4.9, 2.6 Hz, epox-CH_2, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 159.4, 148.7, 130.4, 123.1, 122.2, 121.4, 120.4, 114.7, 68.8, 50.0, 44.6, 34.7. CF_3 not observed.

LC-MS: calculated for $C_{14}H_{14}F_3N_2O_2$ $[M+H]^+$ 299.1. found: 298.9

1-Amino-3-(4-(1-methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl)phenoxy)propan-2-ol (4.1) [101]



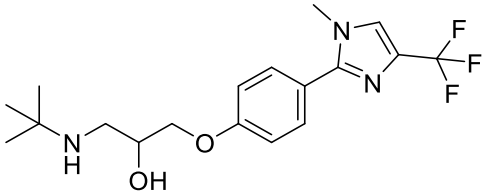
4.9 (0.15 g, 0.5 mmol) was dissolved in ammonium hydroxide solution (0.5 mL) and acetonitrile (0.5 mL). The solution was stirred at room temperature overnight. Volatiles were removed under reduced pressure and the residue was purified by column chromatography, eluting with DCM-Methanol-TEA (90:10:0.1). The product was isolated as a light tallow oil (0.011 g, 14%).

R_f: 0.26 (DCM-Methanol-TEA (90:10:0.1))

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.89 (s, imidazole ArH, 1H), 7.68 – 7.60 (m, ArH, 2H), 7.10 – 7.03 (m, ArH, 2H), 4.07 – 3.94 (m, O-$\underline{CH_2}$-CH, 2H), 3.92 – 3.84 (m, O-CH_2-$\underline{CH}$, 1H), 3.55 (s, NCH_3, 3H), 2.85 (dd, *J* = 12.8, 4.3 Hz, NH_2-$\underline{CH_2}$, 1H), 2.71 (dd, *J* = 12.8, 7.3 Hz, NH_2-$\underline{CH_2}$, 1H), exchangeable protons not observed.

^{13}C NMR: (101 MHz, DMSO) δ 159.7, 148.5, 130.5, 129.8, 123.8, 122.2, 115.6, 115.0, 70.6, 68.9, 43.9, 35.2. CF_3 not observed

1-(*tert*-Butylamino)-3-(4-(1-methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl)phenoxy)propan-2-ol (4.13)



4.9 (0.03 g, 0.10 mmol) was added to *tert*-butylamine (1 mL) and stirred for 16 hours at room temperature and then volatiles were removed *in vacuo.* COLUMN CHROMATOGRAPHY was carried out eluting with DCM-Methanol-TEA (95:5:0.1) to isolate the product as a white solid (0.07 g, 19%).

R_f: 0.32 (DCM-Methanol-TEA (95:5:0.1)).

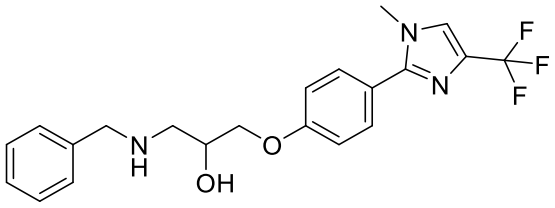
^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.58 – 7.51 (m, ArH, 2H), 7.28 (s, imid-H, 1H), 7.04 – 6.96 (m, ArH, 2H), 4.08 – 3.96 (m, O$\underline{CH_2CH}$, 3H), 3.73 (s, NCH_3, 3H), 2.96 – 2.86 (m, NCH_2, 1H), 2.79 – 2.66 (m, NCH_2, 1H), 1.16 (s, *t*-Bu, 9H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 158.6, 147.9, 138.8 129.9, 129.3, 121.0, 120.4, 113.6, 69.5, 67.1, 56.0, 43.6, 33.7, 27.8. CF_3 not observed

HRMS: calculated for $C_{18}H_{25}F_3N_3O_2$ $[M+H]^+$ 372.1893. found: 372.1901.

Analytical HPLC: R_t 3.02 min (method b, 254 nm)

1-(Benzylamino)-3-(4-(1-methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl)phenoxy)propan-2-ol (4.14)



4.9 (0.04 g, 0.134 mmol) and benzylamine (0.057 g, 0.536 mmol) were dissolved in IPA (1 mL) and heated under microwave irradiation at 50 °C for 30 minutes. Volatiles were removed under reduced pressure then COLUMN CHROMATOGRAPHY was carried out eluting with DCM-Methanol-TEA (98:2:0.1 → 95:5:0.1) to afford the product as an orange oil (0.026 g, 48%).

R_f: 0.37 (DCM-Methanol-TEA (95:5:0.1)).

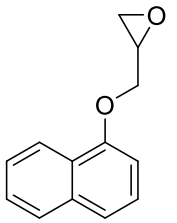
^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.8 Hz, ArH, 2H), 7.40 – 7.26 (m, ArH/imid-H, 6H), 6.96 (d, *J* = 8.8 Hz, ArH, 2H), 4.15 (dt, *J* = 8.7, 5.0 Hz, OHCH, 1H), 4.04 – 3.96 (m, OCH_2, 2H), 3.91 (q, *J* = 13.2 Hz, $BnCH_2$ 2H), 3.72 (s, NCH_3, 3H), 2.96 (dd, *J* = 12.2, 3.7 Hz, NCH_2, 1H), 2.85 (dd, *J* = 12.2, 8.2 Hz, NCH_2, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 159.6, 149.0, 138.1, 131.5, 130.5, 128.8, 128.6, 127.8, 123.2, 122.2, 121.6, 114.8, 70.4, 67.8, 53.5, 50.9, 34.9. CF_3 not observed

HRMS: calculated for $C_{21}H_{23}F_3N_3O_2$ $[M+H]^+$ 203.5905. found: 203.5906.

Analytical HPLC: R_t 2.98 min (method b, 254 nm)

2-((Naphthalen-1-yloxy)methyl)oxirane (4.17) [170]



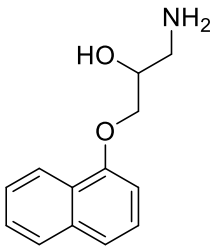
1-Naphthol (1 g, 6.94 mmol) was dissolved in DMF (40 mL) along with caesium carbonate (4.52 g, 13.88 mmol) and (±)-epichlorohydrin (0.77 g, 8.33 mmol). The mixture was stirred at 60 °C overnight. DMF was removed under reduced pressure then water was added to the residue. The aqueous solution was extracted with ethyl acetate (×3), dried over magnesium sulfate, concentrated *in vacuo*. The product was then purified by COLUMN CHROMATOGRAPHY eluting with PE-EA (9:1) to obtain the product as a white solid (0.61 g, 44%).

^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.39 – 8.32 (m, ArH, 1H), 7.88 – 7.80 (m, ArH, 1H), 7.56 – 7.46 (m, ArH, 3H), 7.42 – 7.36 (m, ArH, 1H), 6.85 – 6.79 (m, ArH, 1H), 4.39 (dd, *J* = 11.0, 3.0 Hz, O-CH_2, 1H), 4.13 (dd, *J* = 11.0, 5.6 Hz, O-CH_2, 1H), 3.49 (ddt, *J* = 5.7, 4.1, 2.9 Hz, epoxCH_1, 1H), 2.96 (dd, *J* = 4.9, 4.2 Hz, epoxCH_2 1H), 2.85 (dd, *J* = 4.9, 2.7 Hz, epox CH_2, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 154.2, 134.5, 127.3, 126.5, 126.5, 125.7, 125.3, 122.0, 120.9, 105.1, 68.9, 50.2, 44.8.

LC-MS: calculated for $C_{13}H_{13}O_2$ $[M+H]^+$ 201.1. found: 200.9

1-Amino-3-(naphthalen-1-yloxy)propan-2-ol (4.20) [171]



2-((naphthalen-1-yloxy)methyl)oxirane (0.03 g, 0.15 mmol) was dissolved in a mixture of methanol (1mL) and ammonium hydroxide solution (1mL). The solution was stirred for 16 hours at ambient temperature and then methanol was removed *in vacuo*. Precipitate formed and was filtered under vacuum. The product was collected as a white solid (0.05g, 15%).

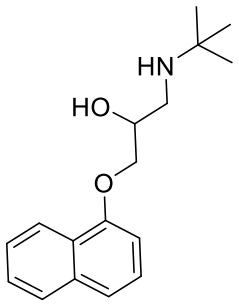
^{1}H NMR: (400 mhz, $CDCl_3$) δ 8.27 – 8.20 (m, ArH, 1H), 7.86 – 7.76 (m, ArH, 1H), 7.54 – 7.42 (m, ArH, 3H), 7.41 – 7.33 (m, ArH, 1H), 6.87 – 6.78 (m, ArH, 1H), 4.24 – 4.08 (m, O$\underline{CH_2CH}$, 3H), 3.09 (dd, *J* = 12.9, 3.9 Hz, NCH_2, 1H), 2.98 (dd, *J* = 12.9, 6.7 Hz, NCH_2, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 154.2, 134.5, 127.5, 126.4, 125.8, 125.5, 125.3, 121.7, 120.7, 104.9, 70.5, 70.2, 44.2.

LC-MS: calculated for $C_{13}H_{16}NO_2$ $[M+H]^+$ 218.1. found: 218.0.

Analytical HPLC: R_t 4.32 min (method b, 254 nm, 90%)

1-(*tert*-Butylamino)-3-(naphthalen-1-yloxy)propan-2-ol (4.21) [172]



2-((naphthalen-1-yloxy)methyl)oxirane (0.05 g, 0.25 mmol) was added to *tert*-butylamine (1mL) and stirred over night at ambient temperature and then volatiles were removed *in vacuo.* COLUMN CHROMATOGRAPHY was carried out eluting with DCM-Methanol-TEA (95:5:0.1) to isolate the product as a yellow amorphous solid (0.18 g, 26%).

R_f: 0.28 (95:5:0.1).

^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.16 (ddd, *J* = 7.1, 3.2, 1.4 Hz, ArH, 1H), 7.72 – 7.62 (m, ArH, 1H), 7.40 – 7.29 (m, ArH, 2H), 7.23 (s, ArH, 1H), 7.22 – 7.17 (m, ArH, 1H), 6.59 (t, *J* = 5.4 Hz, ArH, 1H), 4.60 (ddd, *J* = 9.8, 6.7, 2.4 Hz, OHCH, 1H), 4.11 (td, *J* = 9.6, 4.6 Hz, OCH_2, 1H), 4.05 – 3.97 (m, OCH_2, 1H), 3.30 (dd, *J* = 12.1, 2.4 Hz, NCH_2, 1H), 3.08 (dt, *J* = 9.7, 8.0 Hz, NCH_2, 1H), 1.37 (s, tBu, 9H).

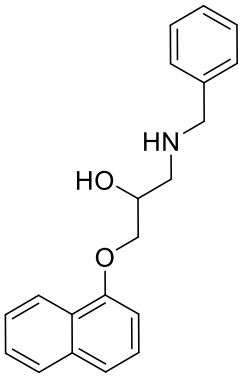
^{13}C NMR: (101 MHz, $CDCl_3$) δ 154.0, 134.5, 127.5, 126.5, 125.8, 125.5, 125.4, 121.9, 120.8, 105.0, 69.9, 66.1, 57.0, 45.6, 26.1.

HRMS: calculated for $C_{17}H_{24}NO_2$ $[M+H]^+$ 274.1802. found: 274.1805.

LC-MS: R_t: 2.08 min (254 nm).

Analytical HPLC: R_t 3.18 min (method b, 254 nm)

1-(Benzylamino)-3-(naphthalen-1-yloxy)propan-2-ol (4.22) [173]



2-((naphthalen-1-yloxy)methyl)oxirane (0.1 g, 0.5 mmol) and benzylamine (0.21 g, 2 mmol) were dissolved in IPA (2 mL) and heated at 50 °C overnight. Volatiles were removed under reduced pressure. The residue was heated in a small amount of DE, which caused white solid to crash out. The solid was filtered and collected to afford the product as a white solid (0.057 g, 37%).

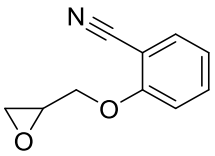
^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.2 Hz, ArH, 1H), 7.77 (d, *J* = 7.5 Hz, ArH, 1H), 7.54 – 7.50 (m, ArH, 2H), 7.49 – 7.28 (m, ArH, 8H), 6.69 (d, *J* = 7.3 Hz, OH, 1H), 4.55 (dd, *J* = 8.5, 3.7 Hz, OHCH, 1H), 4.20 – 4.13 (m, $BnCH_2$, 2H), 4.11 (dd, *J* = 9.5, 4.6 Hz, OCH_2, 1H), 4.02 (dd, *J* = 9.5, 5.8 Hz, OCH_2, 1H), 3.20 (dd, *J* = 12.5, 3.1 Hz, NCH_2 1H), 3.12 (dd, *J* = 12.4, 9.4 Hz, NCH_2, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 153.9, 134.5, 129.9, 129.2, 129.1, 127.6, 126.6, 125.8, 125.5, 125.4, 121.9, 121.0, 114.5, 105.0, 69.8, 66.5, 52.2, 50.1.

HRMS: calculated for $C_{20}H_{22}NO_2$ $[M+H]^+$ 308.1645. found: 308.1649.

LC-MS: R_t: 3.0 min (254 nm).

2-(Oxiran-2-ylmethoxy)benzonitrile (4.19) [174]



2-hydroxybenzonitrile (0.5 g, 4.20 mmol) was dissolved in DMF (20 mL) along with caesium carbonate (2.05 g, 6.30 mmol) and (±)-epichlorohydrin (0.58 g, 6.30 mmol). The mixture was stirred at 50 °C overnight. DMF was removed under reduced pressure then water was added to the residue. The aqueous solution was extracted with ethyl acetate (×3), washed with brine, dried over magnesium sulfate, concentrated *in vacuo*. The product was then purified by COLUMN CHROMATOGRAPHY eluting with PS-EA (75:25→50:50) to isolate the product as a white solid (0.61 g, 83%).

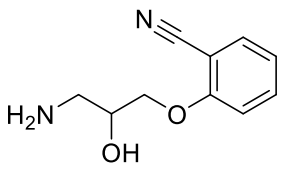
Rf: 0.31 (PS-EA (75:25))

^{1}H NMR: (400 mhz, $CDCl_3$) δ 7.62 – 7.49 (m, ArH, 2H), 7.07 – 6.97 (m, ArH, 2H), 4.37 (dd, *J* = 11.4, 3.0 Hz, O-CH_2, 1H), 4.13 (dd, *J* = 11.4, 5.2 Hz, O-CH_2, 1H), 3.40 (ddt, *J* = 5.2, 4.1, 2.8 Hz, epoxCH_1, 1H), 2.96 – 2.92 (m, epoxCH_2, 1H), 2.85 (dd, *J* = 4.8, 2.6 Hz, epoxCH_2, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 159.7, 135.1, 133.7, 121.4, 116.3, 113.2, 100.5, 69.7, 49.3, 43.6.

LC-MS: calculated for $C_{10}H_{10}NO_2$ $[2M+H]^+$ 350.2. found: 350.9

2-(3-amino-2-hydroxypropoxy)benzonitrile



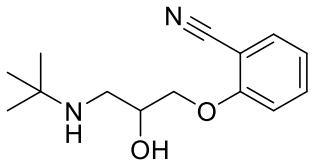
4.19 (0.2 g, 1.14 mmol) was dissolved in 7N ammonia in methanol (5 mL). The solution was stirred for 16 hours at 40 °C and the volatiles were removed *in vacuo*.

The residue was purified by COLUMN CHROMATOGRAPHY eluting with DCM – Methanol (99:1 → 85:15) to obtain the product as a white solid (0.09 g, 41%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 (dd, *J* = 7.5, 1.7 Hz, ArH, 1H), 7.68 (ddd, *J* = 8.9, 7.5, 1.7 Hz, ArH, 1H), 7.28 (d, *J* = 8.5 Hz, ArH 1H), 7.12 (t, *J* = 7.5 Hz, ArH, 1H), 5.90 (s, OH 1H), 4.23 – 4.10 (m, O-CH_2-CH, 3H), 3.12 – 2.99 (m, N-CH_2 1H), 2.98 – 2.82 (m, N-CH_2, 1H).

^{13}C NMR (101 MHz, DMSO) δ 160.3, 135.5, 134.1, 121.8, 116.8, 113.6, 101.1, 70.7, 65.8, 41.9.

2-(3-(*tert*-Butylamino)-2-hydroxypropoxy)benzonitrile (4.24) [175]



2-(oxiran-2-ylmethoxy)benzonitrile (0.1 g, 0.57 mmol) and tert-butylamine (0.25 g, 3.42 mmol) were dissolved in IPA (2 mL) and heated at 50 °C overnight. Volatiles were removed under reduced pressure. COLUMN CHROMATOGRAPHY was carried out eluting with DCM-Methanol-TEA (95:5:0.1) to afford the product as a clear oil (0.092 g, 60%).

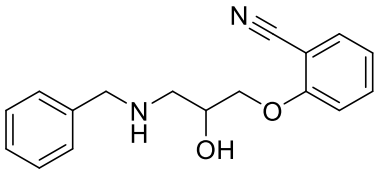
^{1}H NMR: (400 mhz, $CDCl_3$) δ 7.58 – 7.49 (m, 2H), 7.05 – 6.98 (m, 2H), 4.12 (d, *J* = 4.8 Hz, 2H), 4.04 (m, H), 2.97 (dd, *J* = 12.1, 4.4 Hz, 1H), 2.82 (dd, *J* = 12.1, 6.9 Hz, 1H), 1.16 (s, 9H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 160.6, 134.5, 133.8, 121.2, 116.5, 112.6, 102.3, 71.7, 67.7, 51.2, 44.4, 28.9.

HRMS: calculated for $C_{14}H_{21}N_2O_2$ $[M+H]^+$ 249.1602. found: 249.1598.

Analytical HPLC: R_t 2.62 min (method b, 254 nm)

2-(3-(Benzylamino)-2-hydroxypropoxy)benzonitrile (4.25)



2-(oxiran-2-ylmethoxy)benzonitrile (0.65 g, 3.71 mmol) and benzylamine (1.59 g, 14.8 mmol) were dissolved in IPA (15 mL) and heated at 50 °C overnight. Volatiles were removed under reduced pressure. Water was added and the aqueous solution was extracted with ethyl acetate (×3), washed with water, brine and dried over magnesium sulfate. COLUMN CHROMATOGRAPHY was carried out eluting with DCM-Methanol-TEA (96:4:0.1) to afford the product as a light yellow solid (0.19 g, 18%).

R_f: 0.34 (DCM-Methanol-TEA (96:4:0.1)).

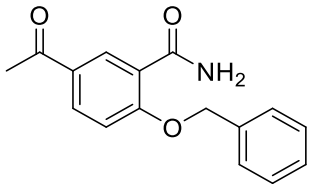
^{1}H NMR: (400 mhz, DMSO) δ 7.71 (dd, *J* = 7.7, 1.7 Hz, ArH, 1H), 7.68 – 7.60 (m, ArH, 1H), 7.34 – 7.18 (m, ArH, 6H), 7.08 (td, *J* = 7.6, 0.8 Hz, ArH, 1H), 5.06 (s, OH, 1H), 4.15 (dd, *J* = 10.1, 4.6 Hz, O-CH_2, 1H), 4.07 (dd, *J* = 10.0, 5.8 Hz, O-CH_2, 1H), 3.95 (m, CH, 1H), 3.77 – 3.67 (m, BnCH_2, 2H), 2.69 (dd, *J* = 11.9, 5.3 Hz, N-CH_2, 1H), 2.62 (dd, *J* = 11.9, 6.4 Hz, N-CH_2, 1H).

^{13}C NMR: (101 MHz, DMSO) δ 160.3, 140.8, 135.0, 133.6, 128.0, 127.8, 126.5, 120.9, 116.4, 113.1, 100.6, 71.5, 67.9, 53.0, 51.5.

HRMS: calculated for $C_{17}H_{19}N_2O_2$ $[M+H]^+$ 283.1441. found: 283.1443.

Analytical HPLC: R_t: 2.85 min (method b, 254 nm).

5-Acetyl-2-(benzyloxy)benzamide (4.27) [176]



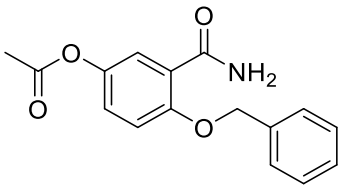
5-acetylsalicylamide (12 g, 67.0 mmol) was dissolved in MeCN (50 mL) along with DIPEA (13.0 g, 100.5 mmol) and benzyl bromide (17.2 g, 100.5 mmol). The mixture was heated at reflux for 4 hours and then MeCN was evaporated *in vacuo,* water was added and precipitate formed which was filtered under vacuum. The product was obtained as a light pink solid (11.75 g, 98%).

^{1}H NMR: (400 mhz, DMSO) δ 8.33 (d, *J* = 2.4 Hz, ArH, 1H), 8.04 (dd, *J* = 8.7, 2.4 Hz, ArH, 1H), 7.67 (s, NH_2, 2H), 7.55 – 7.48 (m, ArH, 2H), 7.46 – 7.27 (m, ArH, 4H), 5.35 (s, $BnCH_2$, 2H), 2.54 (s, $COCH_3$, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 196.1, 165.9, 159.5, 136.0, 132.4, 130.9, 129.6, 128.5, 128.1, 127.7, 123.8, 113.2, 70.2, 26.4.

LC-MS: calculated for $C_{16}H_{16}NO_3$ $[M+H]^+$ 270.1. found: 269.9

4-(Benzyloxy)-3-carbamoylphenyl acetate (4.28)



4.27 (7 g, 26 mmol) was dispersed in chloroform (200 mL) and m-CPBA (73%, 18.5 g, 78 mmol) was added. The mixture was stirred at room temperature for 3 days and then additional m-CPBA (6.15 g, 26 mmol) was added and stirred for a further 16 hours. The mixture was diluted with DCM and then washed with saturated aq. sodium hydrogen carbonate (×2) and then water. The organic layer was dried over

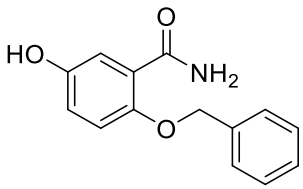
magnesium sulfate and concentrated *in vacuo* to afford the product as an off white solid (6.02 g, 81%).

^{1}H NMR: (400 mhz, $CDCl_3$) δ 7.95 (s, ArH, 1H), 7.73 (s, NH_2, 1H), 7.46 – 7.35 (m, ArH/NH_2, 5H), 7.21 (dd, *J* = 8.9, 3.0 Hz, ArH, 1H), 7.12 (d, *J* = 8.9 Hz, ArH, 1H), 7.07 (d, *J* = 8.9 Hz, ArH, 1H), 5.18 (s, $BnCH_2$, 2H), 2.29 (s, $COOCH_3$, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 170.1, 165.8, 154.8, 144.7, 135.4, 129.1, 129.0, 128.0, 126.5, 125.6, 122.3, 113.7, 72.0, 21.1.

LC-MS: calculated for $C_{16}H_{16}NO_4$ $[M+H]^+$ 286.1. found: 285.9

2-(Benzyloxy)-5-hydroxybenzamide (4.29)



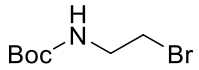
30 (9.18 g, 32.2 mmol) and lithium hydroxide monohydrate (2.03 g, 48.3 mmol) were dissolved in THF (50 mL) and water (50 mL). The mixture was stirred for 16 hours under nitrogen and then THF was removed under reduced pressure. The solution was basified with aq. 2M KOH and then washed with EA. The aqueous layer was then acidified with aq. 2Mhydrochloric acid and extracted with ethyl acetate (×3). The combined organic layers were washed with water then dried over magnesium sulfate. The solution was concentrated in vacuo to give a brown solid. Petroleum ether was added and the precipitate was filtered under vacuum and washed with diethyl ether to afford pure product as a light brown solid (5.48 g, 70%).

^{1}H NMR: (400 mhz, DMSO) δ 9.22 (s, OH, 1H), 7.62 (s, NH_2, 1H), 7.49 (s, NH_2, 1H), 7.47 (d, *J* = 7.2 Hz, ArH, 2H), 7.42 – 7.31 (m, ArH, 3H), 7.25 (d, *J* = 3.1 Hz, ArH, 1H), 7.04 (d, *J* = 8.9 Hz, ArH, 1H), 6.83 (dd, *J* = 8.8, 3.2 Hz, ArH, 1H), 5.14 (s, $BnCH_2$, 2H).

^{13}C NMR: (101 MHz, DMSO) δ 166.1, 151.1, 149.1, 136.8, 128.5, 128.0, 127.8, 123.8, 118.6, 116.7, 115.1, 70.7.

LC-MS: calculated for $C_{14}H_{14}NO_3$ $[M+H]^+$ 244.1. found: 243.9

***tert*-butyl (2-bromoethyl)carbamate (4.30)**

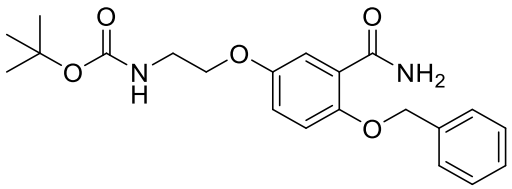


2-bromoethylamine hydrobromide (5 g, 24.4 mmol) was suspended in DCM (60 mL) and BOCanhydride (6.18 g, 22.0 mmol) was added. The solution was cooled to 0 °C and DIPEA (3.15 g, 24.4 mmol) was added. The mixture was stirred at room temperature for 16 hours. Water was added, and then extracted with DCM. The extract was washed with water, dried over magnesium sulfate and then concentrated under reduced pressure. DCM (40 mL) was added along with imidazole (4.03 g, 59.3 mmol) and the solution was stirred for 30 minutes. The solution was washed with 0.5 M aq.hydrochloric acid (× 3) and the organic layer was concentrated under reduced pressure to give the pure product as a clear oil (4.8 g, 72%)

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 5.09 (s, NH, 1H), 3.46 (q, *J* = 6.0 Hz, N-CH_2, 2H), 3.39 (d, *J* = 5.8 Hz, Br-CH_2, 2H), 1.38 (s, *t*-Bu, 9H).

^{13}C NMR: (101 MHz, Chloroform-*d*) δ 155.6, 77.3, 39.81, 30.88, 27.85.

***tert*-Butyl (2-(4-(benzyloxy)-3-carbamoylphenoxy)ethyl)carbamate (4.31)**



31 (1g, 4.11 mmol) was dissolved in DMF (40 mL) along with caesium carbonate (4.02 g, 12.3 mmol) and then tert-butyl (2-bromoethyl)carbamate (1.38 g, 6.17 mmol) was added. The mixture was stirred at 60 °C for 2 days and then DMF was removed under reduced pressure. Water was added to the residue and then the aqueous solution was extracted with DCM (×3), dried over magnesium sulfate and concentrated *in*

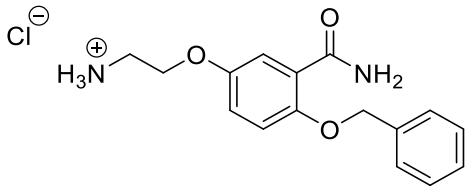
vacuo. The product was then purified by CC, eluting with $CHCl_3$-Methanol (100:0→95:5) to afford the product as a white solid (1.11g, 70%).

^{1}H NMR: (400 mhz, $CDCl_3$) δ 7.82 (s, NH_2, 1H), 7.76 (s, NH_2, 1H), 7.46 – 7.33 (m, 5H), 7.05 – 6.96 (m, ArH, 2H), 5.80 (s, ArH, 1H), 5.14 (s, $BnCH_2$, 2H), 4.99 (s, NH, 1H), 4.03 (t, *J* = 5.2 Hz, O-CH_2, 2H), 3.51 (d, *J* = 5.2 Hz, N-CH_2, 2H), 1.45 (s, tBu, 9H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 166.5, 153.1, 151.7, 135.7, 129.1, 128.8, 128.2, 127.9, 125.3, 120.2, 117.3, 114.5, 72.1, 40.2, 28.5, 28.0, 21.5.

LC-MS: calculated for $C_{21}H_{27}N_2O_5$ $[M+H]^+$ 387.2. found: 386.9

5-(2-Aminoethoxy)-2-(benzyloxy)benzamide (4.32)



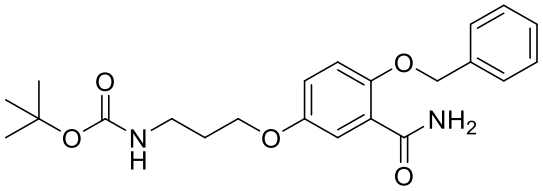
33 (1.1 g, 2.85 mmol) was dispersed in 2Mhydrochloric acid in diethyl ether (20 mL) and stirred at room temperature overnight. Precipitate was filtered under vacuum to afford the pure product (0.77 g, 94%).

^{1}H NMR: (400 mhz, DMSO) δ 7.67 (s, NH_2, 1H), 7.61 (s, NH_2, 1H), 7.51 – 7.45 (m, ArH, 2H), 7.45 – 7.30 (m, ArH, 4H), 7.18 (d, *J* = 9.1 Hz, ArH, 1H), 7.12 – 7.07 (m, ArH, 1H), 5.22 (s, $BnCH_2$, 2H), 4.14 (t, *J* = 5.1 Hz, OCH_2, 2H), 3.18 (dd, *J* = 10.5, 5.4 Hz, NCH_2, 2H).

^{13}C NMR: (101 MHz, DMSO) δ 165.9, 151.7, 150.7, 136.6, 128.6, 128.1, 127.8, 123.9, 118.7, 116.6, 115.0, 70.6, 64.9, 38.3.

LC-MS: calculated for $C_{16}H_{19}N_2O_3$ $[M+H]^+$ 387.1. found: 287.0

***tert*-Butyl (3-(4-(benzyloxy)-3-carbamoylphenoxy)propyl)carbamate (7.5)**



4.29 (0.8 g, 3.29 mmol) was dissolved in DMF (20 mL) along with caesium carbonate (3.21 g, 9.87 mmol) and then *tert*-butyl (3-bromopropyl)carbamate (1.18 g, 4.94 mmol) was added. The mixture was stirred at 60 °C for 16 hours and then DMF was removed under reduced pressure. Water was added to the residue and then the aqueous solution was extracted with DCM (×3), dried over magnesium sulfate and concentrated *in vacuo.* The product was then purified by CC, eluting with DCM-Methanol (100:0→95:5) to afford the product as a white solid (0.65 g, 50%).

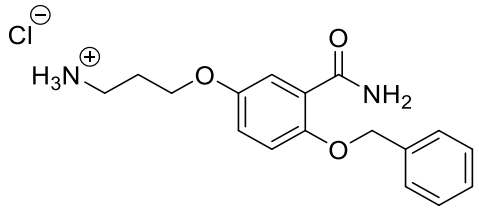
R_f: 0.36 (DCM-Methanol (95:5)).

^{1}H NMR: (400 mhz, DMSO) δ 7.65 (s, NH_2, 1H), 7.55 (d, *J* = 13.6 Hz, ArH, 1H), 7.51 – 7.44 (m, NH_2/ArH, 2H), 7.44 – 7.30 (m, ArH, 3H), 7.14 (d, *J* = 9.1 Hz, ArH, 1H), 7.01 (dd, *J* = 9.1, 3.3 Hz, ArH, 1H), 6.89 (t, *J* = 5.1 Hz, ArH, 1H), 5.19 (s, $BnCH_2$, 2H), 3.93 (t, *J* = 6.3 Hz, O-CH_2, 2H), 3.06 (dd, *J* = 12.7, 6.3 Hz, $CH_2\underline{CH_2}CH_2$, 2H), 1.86 – 1.73 (m, NCH_2, 2H), 1.37 (s, tBu, 9H).

^{13}C NMR: (101 MHz, DMSO) δ 165.9, 155.6, 152.4, 150.2, 136.7, 128.5, 128.0, 127.7, 123.9, 118.4, 115.7, 115.0, 77.4, 70.6, 65.7, 36.8, 29.2, 28.2.

LC-MS: calculated for $C_{22}H_{29}N_2O_5$ $[M+H]^+$ 401.2. found: 401.2

5-(3-Aminopropoxy)-2-(benzyloxy)benzamide (4.38)



7.5 (0.65 g, 1.62 mmol) was suspended in 2Mhydrochloric acid in diethyl ether (12 mL) and stirred at room temperature for 16 hours. Precipitate was filtered under vacuum and washed with diethyl ether to afford the pure product as a white solid (0.54 g, 99%).

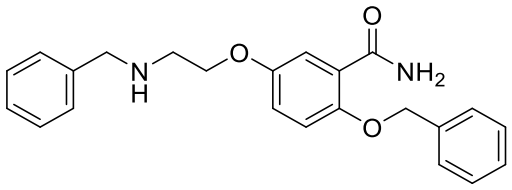
^{1}H NMR: (400 MHz, DMSO) δ 7.98 (s, NH_3, 3H), 7.66 (s, NH_2, 1H), 7.59 (s, NH_2, 1H), 7.48 (d, *J* = 7.6 Hz, ArH, 2H), 7.44 – 7.29 (m, ArH. 4H), 7.16 (d, *J* = 9.1 Hz, ArH, 1H), 7.04 (dd, *J* = 9.0, 3.2 Hz, ArH, 1H), 5.21 (s, $BnCH_2$, 2H), 4.03 (t, *J* = 6.1 Hz, O-CH_2, 2H), 2.94 (dd, *J* = 12.7, 6.4 Hz, $CH_2\underline{CH_2}CH_2$, 2H), 2.08 – 1.93 (m, NCH_2, 2H).

^{13}C NMR: (101 MHz, DMSO) δ 165.9, 152.1, 150.4, 136.6, 128.5, 128.0, 127.7, 123.8, 118.5, 115.9, 115.0, 70.5, 65.1, 36.2, 26.8.

LC-MS: calculated for $C_{17}H_{21}N_2O_3$ $[M+H]^+$ 301.1. found: 301.0

Analytical HPLC: R_t 2.82 min (method b, 254 nm, 91%)

5-(2-(Benzylamino)ethoxy)-2-(benzyloxy)benzamide (4.45)



4.32 (0.5 g, 1.55 mmol) was added to methanol (10 mL) along with TEA (0.16 g, 1.55 mmol) followed by the addition of benzaldehyde (0.16 g, 1.55 mmol). The mixture was stirred at room temperature overnight and then cooled to 0-5 °C. Sodium borohydride (0.18 g, 4.66 mmol) was added portion wise and the cooled solution was stirred for 30 minutes and then allowed to warm to room temperature. After stirring for a further 30 minutes aq. saturated ammonium chloride was added. White precipitate formed which was filtered under vacuum and washed with diethyl ether to afford the product as a white solid (0.37 g, 64%).

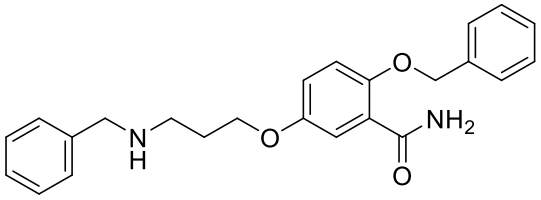
^{1}H NMR: (400 mhz, DMSO) δ 7.68 (s, NH_2, 1H), 7.65-7.55 (m, ArH/NH_2, 2H), 7.50 – 7.31 (m, ArH, 10H), 7.19 (d, *J* = 9.0 Hz, ArH, 1H), 7.10 (dd, *J* = 9.0, 3.2 Hz, ArH, 1H), 5.22 (s, $BnCH_2$, 2H), 4.28 (t, *J* = 5.1 Hz, OCH_2, 2H), 4.21 (s, $BnCH_2$, 2H), 3.26 (t, *J* = 5.1 Hz, NCH_2, 2H), 3.17 (s, NH, 1H).

^{13}C NMR: (101 MHz, DMSO) δ 166.3, 152.0, 151.1, 137.1, 132.5, 130.6, 129.3, 129.0, 129.0, 128.5, 128.2, 124.3, 119.0, 116.9, 115.5, 71.0, 64.3, 50.6, 45.8.

HRMS: calculated for $C_{23}H_{25}N_2O_3$ $[M+H]^+$ 377.1860. found: 377.1861

Analytical HPLC: R_t 3.16 min (method b, 254 nm)

5-(3-(benzylamino)propoxy)-2-(benzyloxy)benzamide (4.44)



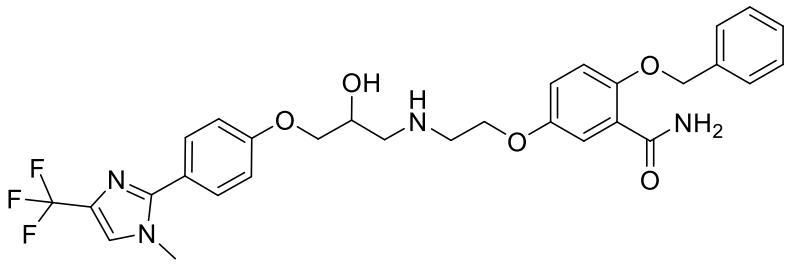
4.38 (0.10 g, 0.30 mmol) was added to methanol (2 mL) along with TEA (0.03 g, 0.30 mmol) followed by the addition of benzaldehyde (0.032g, 0.30 mmol). The mixture was stirred at room temperature for 2 hours and then cooled to 0-5 °C. Sodium borohydride (0.034 g, 0.89 mmol) was added portion wise and the cooled solution was stirred for 30 minutes and then allowed to warm to room temperature. After stirring for a further 30 minutes aq. saturated ammonium chloride was added. Methanol was removed under reduced pressure and white precipitate formed which was filtered under vacuum and washed with diethyl ether to afford the product as a white solid (0.068 g, 58%).

^{1}H NMR: (400 mhz, DMSO-d_6) δ 7.69 – 7.64 (m, NH_2, 1H), 7.59 (s, NH_2, 1H), 7.50 (td, *J* = 8.0, 1.5 Hz, ArH, 4H), 7.44 – 7.32 (m, ArH, 7H), 7.16 (d, *J* = 9.1 Hz, ArH, 1H), 7.02 (dd, *J* = 9.0, 3.3 Hz, ArH, 1H), 5.21 (s, O-CH_2-Bn, 2H), 4.09 – 3.99 (m, N-CH_2-Bn, Bn-O-CH_2, 4H), 2.97 (t, *J* = 7.4 Hz, N-CH_2, 2H), 2.06 (p, *J* = 6.4 Hz, CH_2-CH_2-CH_2, 2H).

^{13}C NMR: (101 MHz, DMSO) δ 166.4, 164.5, 152.6, 150.8, 137.1, 129.9, 129.0, 129.0, 128.8, 128.5, 128.2, 126.4, 124.3, 118.9, 116.3, 115.5, 71.0, 65.9, 51.2, 44.7, 26.8 (2 aromatic C not observed).

LC-MS: calculated for $C_{30}H_{32}F_3N_4O_5$ $[M+H]^+$ 391.2. found: 390.9

2-(Benzyloxy)-5-(2-((2-hydroxy-3-(4-(1-methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl)phenoxy)propyl)amino)ethoxy)benzamide (4.39)



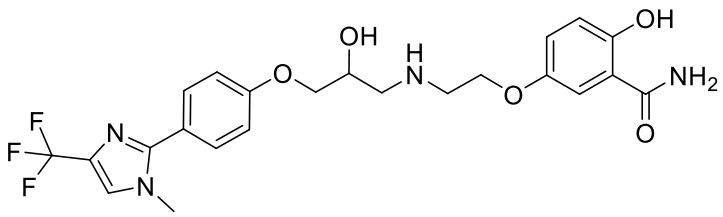
4.32 (0.02g, 0.067 mmol) and **4.9** (0.02 g, 0.067 mmol) were dissolved in MeCN (1 mL) along with TEA (0.02 g, 0.20 mmol) and calcium triflate (0.01 g, 0.034 mmol). The mixture was heated at 90 °C for 1 hour under microwave irradiation. Volatiles were removed *in vacuo* and the residue was purified by column chromatography, eluting with DCM-Methanol-TEA (90:10:0.1). The Product was isolated as a white solid (0.003 g, 8%).

R_f: 0.37 (DCM-Methanol-TEA (90:10:0.1))

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.90 (d, *J* = 5.3 Hz, imidazole ArH, 1H), 7.71 – 7.56 (m, ArH, 4H), 7.50 (d, *J* = 8.2 Hz, ArH, 2H), 7.45 – 7.32 (m, ArH, 4H), 7.16 (d, *J* = 9.2 Hz, ArH, 1H), 7.11 – 7.03 (m, ArH, 3H), 5.22 (s, BnCH_2, 2H), 4.11 – 3.88 (m, O-$\underline{CH_2}$-$\underline{CH}$, O-$\underline{CH_2}$-CH_2, 5H), 3.77 (s, N-CH_3, 3H), 3.05 (t, *J* = 5.6 Hz, O-CH_2-$\underline{CH_2}$, 1H), 2.93 (t, *J* = 5.6 Hz, O-CH_2-$\underline{CH_2}$, 1H), 2.84 – 2.65 (m, N-$\underline{CH_2}$-CH, 2H).

LC-MS: calculated for $C_{30}H_{32}F_3N_4O_5$ $[M+H]^+$ 585.2. found: 585.2 R_t: 2.33 min (254 nm)

2-Hydroxy-5-(2-((2-hydroxy-3-(4-(1-methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl)phenoxy)propyl)amino)ethoxy)benzamide (1.35) [101]



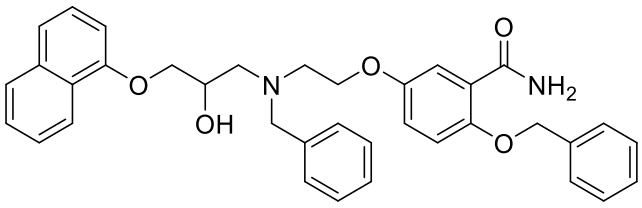
4.39 (0.008 g, 0.014 mmol) was dissolved in a mixture of Methanol (0.7 mL), water (0.2 mL) and acetic acid (0.1 mL). Pd/C (0.001 g) was added and the mixture was stirred under a hydrogen atmosphere overnight. Pd/C was filtered and then volatiles were removed *in vacuo*. The residue was purified by column chromatography, eluting with DCM-Methanol-TEA (90:10:0.1). The product was isolated as a white solid (0.004 g, 60%).

R_f: 0.20 (DCM-Methanol-TEA (90:10:0.1))

^{1}H NMR: (400 MHz, DMSO-d_6) δ 12.51 (s, OH, 1H), 8.47 (s, NH_2, 1H), 7.96 (s, NH_2, imidazole ArH, 2H), 7.76 – 7.70 (m, ArH, 2H), 7.56 (d, *J* = 3.0 Hz, ArH, 1H), 7.18 – 7.13 (m, ArH, 3H), 6.93 (d, *J* = 9.0 Hz, ArH, 1H), 4.35 – 4.24 (m, O-$\underline{CH_2}$-$\underline{CH}$, 3H), 4.13 (d, *J* = 5.1 Hz, O-$\underline{CH_2}$-CH_2, 2H), 3.82 (s, N-CH_3, 3H), 2.38 – 2.33 (m, O-CH_2-$\underline{CH_2}$, 1H), 2.28 – 2.20 (m, O-CH_2-$\underline{CH_2}$, 1H), 2.09 – 2.03 (m, N-$\underline{CH_2}$-CH, 2H).

LC-MS: calculated for $C_{23}H_{26}F_3N_4O_5$ $[M+H]^+$ 495.2. found: 495.2. R_t: 2.13 min (254 nm, 90%).

5-(2-(Benzyl(2-hydroxy-3-(naphthalen-1-yloxy)propyl)amino)ethoxy)-2-(benzyloxy)benzamide (4.47)



4.16 (0.05 g, 0.14 mmol) was dissolved in ethanol (2 mL) along with DIPEA (0.018 g, 0.14 mmol) and **4.45** (0.027 g, 0.14 mmol). The mixture was heated at 80 °C overnight and then volatiles were removed under reduced pressure to give an orange oil. COLUMN CHROMATOGRAPHY was carried out eluting with DCM-Methanol-TEA (99:1:0.1 → 95:5:0.1) and then HPLC, eluting with MeCN:H_2O:TFA (30:70:0.01 → 100:0:0.01 over 10 minutes, eluted at 7.1 min). The product was obtained as a white solid (0.14 g, 18%).

R_f: 0.45 (DCM-Methanol-TEA 97.5:2.5:0.1).

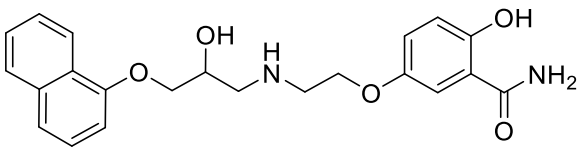
^{1}H NMR: (400 mhz, $CDCl_3$) δ 8.10 (s, ArH, 1H), 7.91 (d, *J* = 8.4 Hz, ArH, 1H), 7.78 (dd, *J* = 8.6, 5.6 Hz, ArH, 2H), 7.54 (t, *J* = 6.9 Hz, ArH, 2H), 7.52 – 7.30 (m, ArH, 11H), 7.18 (dd, *J* = 8.6, 5.6 Hz, ArH, 1H), 7.10 (dd, *J* = 9.0, 2.9 Hz, ArH, 1H), 6.99 (d, *J* = 9.0 Hz, ArH, 1H), 6.77 (d, *J* = 7.5 Hz, OH, 1H), 5.14 (s, $BnCH_2$, 2H), 4.64 (d, *J* = 13.1 Hz, O$\underline{CH_2}$. 2H), 4.48 (d, *J* = 12.4 Hz, OCH_2/O$\underline{CH}$ 3H), 4.28 (d, *J* = 5.6 Hz, NCH_2, 1H), 4.09 – 4.01 (m, NCH_2, 1H), 3.79 – 3.64 (m, $BnCH_2$/NCH_2, 3H), 3.46 (dd, *J* = 20.7, 10.1 Hz, NCH_2, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 153.4, 152.8, 151.5, 135.1, 134.4, 131.4, 130.5, 129.6, 129.1, 129.0, 128.9, 128.2, 128.1, 127.9, 127.6, 126.5, 125.8, 125.3, 125.1, 121.4, 121.1, 114.6, 105.8, 104.9, 72.0, 71.3, 67.8, 64.7, 63.4, 54.3, 45.6 (1 C not observed $CONH_2$).

HRMS: calculated for $C_{36}H_{37}N_2O_5$ $[M+H]^+$ 577.2697. found: 577.2705.

Analytical HPLC: R_t 3.81 min (method b, 254 nm, 90%)

2-Hydroxy-5-(2-((2-hydroxy-3-(naphthalen-1-yloxy)propyl)amino)ethoxy)benzamide (4.46)



4.47 (0.018 g, 0.037 mmol) was dissolved in a mixture of THF (1.4 mL), water (0.4 mL) and acetic acid (0.2 mL). Pd/C (0.005 g) was added and the mixture was stirred under a hydrogen atmosphere overnight. Pd/C was filtered and then volatiles were removed *in vacuo*. The product was purified by HPLC, eluting with MeCN:H_2O:TFA (5:95:0.01 → 100:0:0.01 over 10 minutes, eluted at 5.8 minutes). The product was obtained as a white solid (0.10 g, 71%).

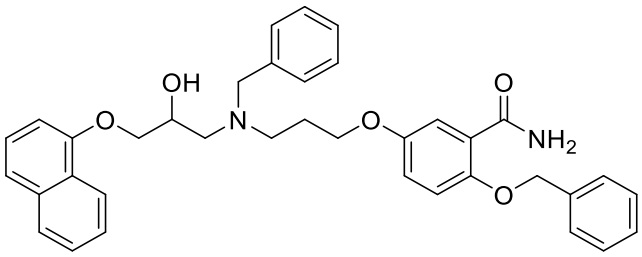
^{1}H NMR: (400 mhz, Methanol-d_4) δ 8.15 – 8.11 (m, ArH, 1H), 7.70 (d, *J* = 8.1 Hz, ArH, 1H), 7.40 – 7.33 (m, ArH, 3H), 7.33 – 7.25 (m, ArH, 2H), 7.00 (dd, *J* = 9.0, 3.1 Hz, ArH, 1H), 6.84 (dd, *J* = 7.6, 1.0 Hz, ArH, 1H), 6.76 (d, *J* = 9.0 Hz, ArH, 1H), 4.43 – 4.33 (m, O-CH_2-CH, 1H), 4.23 – 4.06 (m, O-CH_2-CH, O-CH_2-CH_2, 4H), 3.52 – 3.41 (m, CH_2-N-CH_2, 3H), 3.30 (dd, *J* = 12.8, 9.8 Hz, CH_2-N-CH_2, 1H).

^{13}C NMR: (101 MHz, MeOD) δ 171.1, 154.6, 153.9, 150.4, 134.6, 127.1, 126.1, 125.5, 125.3, 124.9, 121.9, 121.2, 120.5, 118.0, 115.5, 113.0, 104.7, 69.6, 65.2, 63.6, 50.0, 46.7.

HRMS: calculated for $C_{22}H_{25}N_2O_5$ $[M+H]^+$ 397.1758. found: 397.1758.

Analytical HPLC: R_t 3.78 min (method a, 254 nm)

5-(3-(Benzyl(2-hydroxy-3-(naphthalen-1-yloxy)propyl)amino)propoxy)-2-(benzyloxy)benzamide (4.49)



4.16 (0.021 g, 0.103 mmol) was dissolved in ethanol (2 mL) along with DIPEA (0.013 g, 0.103 mmol) and 5-(3-(benzylamino)propoxy)-2-(benzyloxy)benzamide (0.04 g, 0.103 mmol). The mixture was heated at 80 °C for 3 days and then volatiles were removed under reduced pressure to give an orange oil. COLUMN CHROMATOGRAPHY was carried out eluting with DCM-Methanol-TEA (95:5:0.1) and this was followed by a second COLUMN CHROMATOGRAPHY eluting with DCM-Methanol-TEA (99:1:0.1 → 97.5:2.5:0.1). The product was isolated as a yellow solid (0.016 g, 27%).

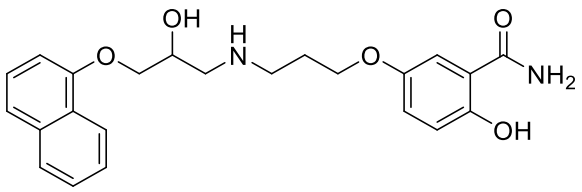
^{1}H NMR: (400 mhz, DMSO) δ 8.12 (d, *J* = 8.1 Hz, ArH, 1H), 7.83 (d, *J* = 8.0 Hz, ArH, 1H), 7.63 (s, NH_2, 1H), 7.56 (s, NH_2, 1H), 7.50-7.27 (m, ArH, 11H), 7.16 (m, ArH, 3H), 7.03 (d, *J* = 9.1 Hz, ArH, 1H), 6.86 – 6.79 (m, ArH, 2H), 5.15 (s, $BnCH_2$, 2H), 4.98 (d, *J* = 4.7 Hz, OCH, 1H), 4.09 (d, *J* = 7.0 Hz, OCH_2, 2H), 3.99 (dd, *J* = 10.5, 6.4 Hz, OCH_2 1H), 3.93 – 3.79 (m, $BnCH_2$, 2H), 3.73 (d, *J* = 13.8 Hz, OCH_2, 1H), 3.59 (d, *J* = 13.8 Hz, NCH_2, 1H), 2.77 (dd, *J* = 13.1, 6.3 Hz, NCH_2, 1H), 2.66 (dd, *J* = 12.7, 6.6 Hz, NCH_2, 1H), 2.58 (dd, *J* = 12.9, 5.9 Hz, NCH_2, 1H), 1.86 (dd, *J* = 18.6, 12.3 Hz, $CH_2\underline{CH_2}CH_2$, 2H).

^{13}C NMR: (101 MHz, DMSO) δ 166.0, 154.1, 152.4, 150.1, 139.4, 136.7, 134.0, 128.6, 128.5, 128.0, 128.0, 127.7, 127.3, 126.6, 126.3, 126.2, 125.0, 125.0, 123.8, 121.8, 119.7, 118.1, 115.8, 114.9, 104.8, 70.6, 70.6, 67.3, 65.9, 58.9, 56.6, 50.6, 26.5.

HRMS: calculated for $C_{37}H_{39}N_2O_5$ $[M+H]^+$ 591.2853. found: 591.2861.

Analytical HPLC: R_t 3.90 min (method b, 254 nm)

2-Hydroxy-5-(3-((2-hydroxy-3-(naphthalen-1-yloxy)propyl)amino)propoxy)benzamide (4.48)



4.49 (0.011 g, 0.019 mmol) was dissolved in a mixture of THF (0.7 mL), water (0.2 mL) and acetic acid (0.1 mL). Pd/C (0.02 g) was added and the mixture was stirred under a hydrogen atmosphere overnight. Pd/C was filtered and then volatiles were removed *in vacuo*. The residue was then purified by HPLC eluting with MeCN:H_2O:TFA (30:70:0.01 → 100:0:0.01 over 10 minutes, eluted at 5.9 minutes) and the product was obtained as a clear oil (0.006 g, 75%).

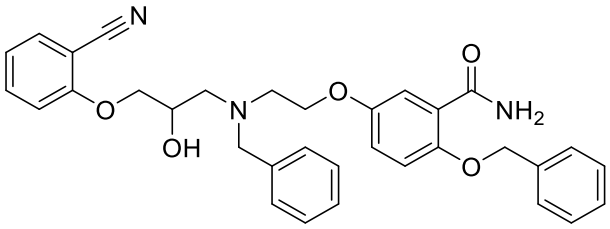
^{1}H NMR: (400 mhz, MeOD) δ 8.23 – 8.13 (m, ArH, 1H), 7.75 – 7.68 (m, ArH, 1H), 7.44 – 7.24 (m, ArH, 5H), 6.97 (dd, *J* = 9.0, 3.1 Hz, ArH, 1H), 6.84 (d, *J* = 7.2 Hz, ArH, 1H), 6.74 (d, *J* = 9.0 Hz, ArH, 1H), 4.48 (s, O-CH_2, 2H), 4.39 – 4.31 (m, CH, 1H), 4.20 – 4.07 (m, O-$\underline{CH_2}$-CH_2, 2H), 4.03 (t, *J* = 5.7 Hz, O-CH_2-CH_2-$\underline{CH_2}$, 2H), 3.37 (dd, *J* = 12.7, 3.1 Hz, N-CH_2, 1H), 3.29 – 3.24 (m, N-CH_2, 1H), 2.18 – 2.09 (m, O-CH_2-$\underline{CH_2}$-CH_2, 2H), exchangable protons not observed.

^{13}C NMR: (101 MHz, MeOD) δ 172.7, 155.7, 155.3, 152.3, 136.0, 128.5, 127.5, 126.9, 126.8, 126.3, 123.1, 122.7, 121.9, 119.3, 116.7, 114.2, 106.1, 71.1, 67.4, 66.8, 51.6, 47.3, 27.0.

HRMS: calculated for $C_{23}H_{27}N_2O_5$ $[M+H]^+$ 411.1914. found: 411.1920.

Analytical HPLC: R_t 3.07 min (method b, 254 nm)

5-(2-(Benzyl(3-(2-cyanophenoxy)-2-hydroxypropyl)amino)ethoxy)-2-(benzyloxy)benzamide (4.51)



4.18 (0.03 g, 0.167 mmol) was dissolved in ethanol (2 mL) along with DIPEA (0.022 g, 0.167 mmol) and **4.45** (0.06 g, 0.167 mmol). The mixture was heated at 80 °C for 3 days and then additional **4.45** (0.015 g, 0.084 mmol) and DIPEA (0.011 g, 0.084 mmol) were added and after an additional 16 hours of stirring, volatiles were removed under reduced pressure. COLUMN CHROMATOGRAPHY was carried out eluting with DCM-Methanol-TEA (99:1:0.1 → 95:5:0.1) and then the column was flushed with methanol-acetone to obtain a clear oil. HPLC was then carried out, eluting with MeCN:H_2O:TFA (30:70:0.01 → 100:0:0.01 over 10 minutes, eluted at 6.2 minutes). The product was obtained as a clear oil (0.14 g, 15%).

R_f: 0.47 (DCM-Methanol-TEA 99:1:0.1)

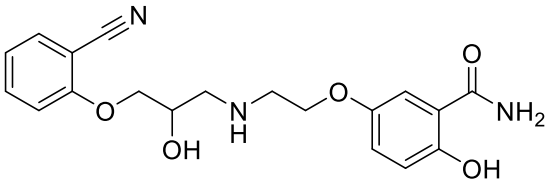
^{1}H NMR: (400 mhz, $CDCl_3$) δ 7.82 (s, NH_2, 1H), 7.76 (s, NH_2, 1H), 7.56 – 7.31 (m, ArH, 13H), 7.07 (dd, *J* = 9.0, 2.5 Hz, ArH, 1H), 7.02 (t, *J* = 7.6 Hz, ArH, 2H), 6.94 (d, *J* = 8.4 Hz, ArH, 1H), 5.75 (s, OH, 1H), 5.14 (s, $BnCH_2$, 2H), 4.23 (s, $BnCH_2$, 2H), 4.17 – 4.05 (m, O-CH_2-CH, O-CH_2, 4H), 3.98 (dd, *J* = 9.3, 6.8 Hz, O-CH_2, 1H), 3.35 – 3.05 (m, CH_2-N-CH_2, 4H)

^{13}C NMR: (101 MHz, $CDCl_3$) δ 158.9, 151.2, 150.9, 150.0, 134.6, 133.4, 133.3, 132.5, 129.4, 129.3, 128.0, 127.7, 126.8, 123.6, 120.3, 119.4, 116.1, 116.0, 113.5, 111.5, 101.0, 76.2, 70.9, 69.5, 59.0, 56.4, 52.5, 47.2 (1 C not observed, $CONH_2$).

HRMS: calculated for $C_{33}H_{34}N_3O_5$ $[M+H]^+$ 552.2493. found: 552.2498.

Analytical HPLC: R_t 3.56 min (method b, 254 nm)

5-(2-((3-(2-Cyanophenoxy)-2-hydroxypropyl)amino)ethoxy)-2-hydroxybenzamide (4.50)



4.51 (0.010 g, 0.018 mmol) was dissolved in a mixture of THF (0.7 mL), water (0.2 mL) and acetic acid (0.1 mL). Pd/C (0.02 g) was added and the mixture was stirred under a hydrogen atmosphere overnight. Pd/C was filtered and then volatiles were removed in vacuo. The residue was then purified by HPLC eluting with MeCN:H_2O:TFA (30:70:0.01 → 100:0:0.01 over 10 minutes, eluted at 4.4 minutes) and the product was obtained as a clear oil (0.005 g, 71%).

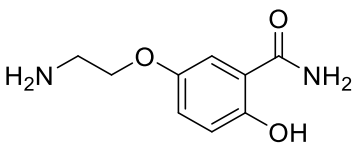
^{1}H NMR: (400 mhz, MeOD) δ 7.58 – 7.52 (m, ArH, 2H), 7.35 (d, *J* = 3.0 Hz, ArH, 1H), 7.11 (d, *J* = 8.7 Hz, ArH, 1H), 7.05 – 7.00 (m, ArH, 2H), 6.77 (d, *J* = 9.0 Hz, ArH, 1H), 4.32 – 4.25 (m, CH, 1H), 4.20 (t, *J* = 4.9 Hz, O-$\underline{CH_2}$-CH, 2H), 4.15 (dd, *J* = 9.9, 4.5 Hz, O-CH_2, 1H), 4.08 (dd, *J* = 9.9, 5.6 Hz, O-CH_2, 1H), 3.49 – 3.43 (m, N-CH_2, 2H), 3.40 – 3.35 (m, N-CH_2, 1H), 3.29 – 3.24 (m, N-CH_2, 1H).

^{13}C NMR: (101 MHz, MeOD) δ 173.1, 162.0, 160.1, 150.4, 134.7, 133.3, 121.9, 121.4, 118.0, 115.4, 113.0, 112.5, 101.4, 70.4, 64.8, 63.6, 49.8, 48.3.

HRMS: calculated for $C_{19}H_{22}N_3O_5$ $[M+H]^+$ 372.1554. found: 372.1558.

Analytical HPLC: R_t 3.38 min (method b, 254 nm)

5-(2-Aminoethoxy)-2-hydroxybenzamide (4.52) [177]



4.32 (0.03 g, 0.093 mmol) was dissolved in a mixture of THF (0.7 mL), water (0.2 mL) and acetic acid (0.1 mL). Pd/C (0.005 g) was added and the mixture was stirred under

a hydrogen atmosphere overnight. Pd/C was filtered and then volatiles were removed in vacuo to afford the product as a white solid (0.016 g, 86%).

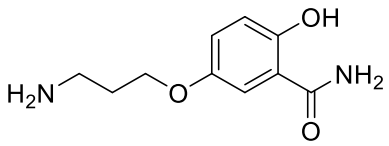
^{1}H NMR: (400 mhz, DMSO) δ 7.52 (d, *J* = 3.0 Hz, ArH, 1H), 7.08 (dd, *J* = 9.0, 3.0 Hz, ArH, 1H), 6.85 (d, *J* = 9.0 Hz, ArH, 1H), 4.14 (t, *J* = 5.1 Hz, O-CH_2, 2H), 3.23 – 3.13 (m, N-CH_2, 2H).

^{13}C NMR: (101 MHz, DMSO) δ 171.3, 155.4, 149.8, 122.6, 118.3, 114.3, 112.5, 65.1, 38.3.

HRMS: calculated for $C_9H_{13}N_2O_3$ $[M+H]^+$ 197.0921. found: 197.0922.

Analytical HPLC: R_t 2.30 min (method b, 254 nm)

5-(3-Aminopropoxy)-2-hydroxybenzamide (4.53)



4.38 (0.03 g, 0.09 mmol) was dissolved in a mixture of THF (0.7 mL), water (0.2 mL) and acetic acid (0.1 mL). Pd/C (0.005 g) was added and the mixture was stirred under a hydrogen atmosphere overnight. Pd/C was filtered and then volatiles were removed in vacuo to afford the product as a white solid (0.015 g, 81%).

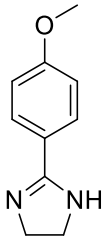
^{1}H NMR: (400 mhz, DMSO) δ 8.41 (s, NH_2, 1H), 7.85 (s, NH_2, 1H), 7.48 (d, *J* = 2.9 Hz, ArH, 1H), 7.05 (dd, *J* = 9.0, 3.0 Hz, ArH, 1H), 6.82 (d, *J* = 9.0 Hz, ArH, 1H), 4.02 (t, *J* = 6.1 Hz, O-CH_2, 2H), 2.93 (t, *J* = 7.4 Hz, N-CH_2, 2H), 2.06 – 1.93 (m, $CH_2\underline{CH_2}CH_2$, 2H)

^{13}C NMR: (101 MHz, DMSO) δ 171.5, 155.1, 150.2, 121.9, 118.2, 114.3, 112.7, 65.4, 36.3, 26.9

HRMS: calculated for $C_{10}H_{15}N_2O_3$ $[M+H]^+$ 211.1077. found: 211.1078.

Analytical HPLC: R_t 2.32 min (method b, 254 nm)

2-(4-methoxyphenyl)-4,5-dihydro-1*H*-imidazole (4.59)

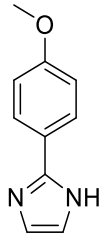


4-methoxyanisaldehyde (1.0 g, 7.34 mmol) was dissolved in *tert*-butanol (20 mL) along wiith ethylene diamine (0.49 g, 8.08 mmol). The mixture was stirred at 70 °C for 30 minutes, then potassium carbonate (4.06 g, 22.4 mmol) and iodine (2.33 g, 9.18 mmol) were added. The mixture was stirred overnight and then *tert*-butanol was removed under reduced pressure. The residue was re-dissolved in ethyl acetate and washed with water. The oranic layer was then washed with sodium thiosulphate solution and then again with water. The organic later was dried over magnesium sulpate and then concentrated under reduced pressure to give the product as a light brown solid (1.0 g, 77%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.81 – 7.73 (m, ArH, 2H), 7.01 – 6.92 (m, ArH 6H), 6.75 (s, NH, 1H), 3.79 (s, CH_2 2H), 3.57 (s, CH_2 2H).

^{13}C NMR: (101 MHz, DMSO-d_6) δ 163.6, 161.1, 129.1, 123.6, 113.9, 55.6, 49.2.

2-(4-Methoxyphenyl)-1*H*-imidazole (4.60) [178]



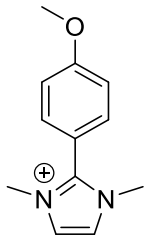
4-methoxyanisaldehyde (2 g, 14.7 mmol) was dissolved in Methanol (50 mL) along with ammonium chloride (5.67 g, 73.5 mmol). Glyoxal (40 wt. % in H2O, 2.13 g, 14.7 mmol) was added dropwise and the solution was stirred at rt for 16h. Volatiles were removed and water was added to the residue and then the solution was extracted

with EA. The organic layers were dried, concentrated *in vacuo* to give the product as an off white solid (0.79 g, 31%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.89 – 7.82 (m, ArH, 2H), 7.06 (s, imid H, 2H), 7.03 – 6.96 (m, ArH 2H), 3.79 (s, CH_3, 3H).

LC-MS: calculated for $C_{10}H_{11}N_2O$ $[M+H]^+$ 175.1. found: 175.2

2-(4-Methoxyphenyl)-1,3-dimethyl-1*H*-imidazol-3-ium (4.67)



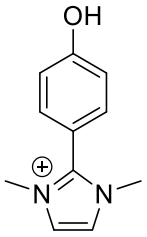
4.60 (0.48 g, 2.76 mmol) was dissolved in DMF (10 mL) and NaH (60% dispersion in mineral oil, 0.2 g, 8.28 mmol) was added. The mixture was stirred at rt for 5 minutes and then MeI (0.78 g, 5.51 mmol) was added. The mixture was stirred at rt overnight and then volatiles were removed *in vacuo.* Toluene was added and the precipitate was filtered, washed with toluene and chloroform. The filtrate was concentrated under reduced pressure to give the product as a yellow solid (0.40 g, 72%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.85 (s, imid H, 2H), 7.74 – 7.67 (m, ArH, 2H), 7.26 – 7.21 (m, ArH 2H), 3.88 (s, OCH_3, 3H), 3.68 (s, 2×N-CH_3, 6H).

^{13}C NMR: (101 MHz, DMSO) δ 161.9, 144.2, 132.3, 122.9, 114.8, 112.7, 55.6, 35.6.

LC-MS: calculated for $C_{12}H_{16}N_2O$ $[M+H]^+$ 203.1. found: 203.2

2-(4-Hydroxyphenyl)-1,3-dimethyl-1*H*-imidazol-3-ium (4.68)



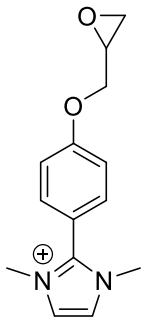
4.67 (1 g, 4.92 mmol) was dissolved in dry DCM (20 mL) and cooled to -78 °C in an acetone/ice bath. BBr_3 (1M in DCM, 19.7 mL, 19.7 mmol) was added dropwise. The solution was allowed to warm to room temperature and stir for 3h. The reaction mixture was poured onto methanol and then concentrated under reduced pressure, diethyl ether was added to the residue and precipitate was filtered to give the product as a brown solid (0.28 g, 98%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.85 (s, imid H, 2H), 7.60 – 7.54 (m, ArH, 2H), 7.07 – 7.00 (m, ArH, 2H), 3.68 (s, 2×N-CH_3, 6H).

^{13}C NMR: (101 MHz, DMSO) δ 160.7, 144.6, 132.2, 122.8, 116.1, 110.9, 36.1.

LC-MS: calculated for $C_{11}H_{14}N_2O$ $[M+H]^+$ 189.1. found: 189.2

1,3-Dimethyl-2-(4-(oxiran-2-ylmethoxy)phenyl)-1H-imidazol-3-ium (4.69)



4.68 (0.64 g, 3.38 mmol) was dissolved in DMF (10 mL) along with caesium carbonate (2.20 g, 6.76 mmol) and then (±)epichlorohydrin (0.63 g, 6.76 mmol). The mixture was stirred at 60 °C for 4h. Volatiles were removed *in vacuo* and the residue was

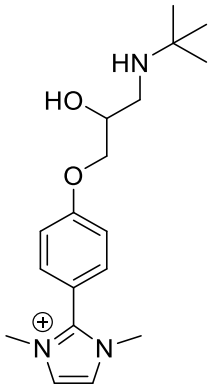
purified by CC, eluting with DCM–Methanol (100:0 →85:15) to give the product as a white solid (0.23 g, 28%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 6.86 (s, imid H, 2H), 6.83 – 6.76 (m, ArH, 2H), 6.52 – 6.44 (m, ArH, 2H), 3.69 (dd, *J* = 11.5, 2.4 Hz, O-CH_2, 1H), 3.19 (dd, *J* = 11.5, 6.3 Hz, O-CH_2, 1H), 2.94 (s, 2×N-CH_3, 6H), 2.58 (tt, *J* = 6.5, 2.8 Hz, epox CH 1H), 2.10 (t, *J* = 4.6 Hz, epox CH_2, 1H), 1.98 (dd, *J* = 5.0, 2.6 Hz, epox CH_2, 1H).

^{13}C NMR: (101 MHz, DMSO) δ 161.1, 144.5, 131.0, 122.0, 114.7, 112.1, 68.5, 48.8, 42.6, 34.1.

LC-MS: calculated for $C_{14}H_{18}N_2O_2$ $[M+H]^+$ 245.1. found: 245.2

2-(4-(3-(*tert*-Butylamino)-2-hydroxypropoxy)phenyl)-1,3-dimethyl-1*H*-imidazol-3-ium (4.70)



4.69 (0.05 g, 0.20 mmol) was dissolved in *tert*-butylamine (1 mL) and Methanol (1 mL) and stirred 40 °C 16 hours. Volatiles were removed *in vacuo* to give the product as a white solid (0.39 g, 61%).

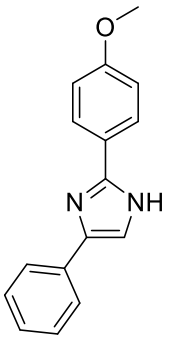
^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.85 (s, imidazole ArH, 2H), 7.72 – 7.65 (m, ArH, 2H), 7.29 – 7.21 (m, ArH, 2H), 4.13 (dd, *J* = 9.9, 4.4 Hz, O-$\underline{CH_2}$-CH, 1H), 4.02 (dd, *J* = 9.9, 6.0 Hz, O-$\underline{CH_2}$-CH, 1H), 3.89 – 3.79 (m, O-CH_2-$\underline{CH}$, 1H), 3.70 (s, N-CH_3 ×2, 6H) 2.70 – 2.56 (m, N-CH_2, 2H), 1.05 (s, *t*Bu, 9H).

^{13}C NMR: (101 MHz, DMSO) δ 162.0, 144.8, 132.7, 123.4, 115.8, 113.1, 71.6, 69.3, 50.2, 45.5, 36.1, 29.2.

HRMS: calculated for $C_{18}H_{29}N_3O_2$ $[M+H]^+$ 318.2176. found: 318.2174

LC-MS: R_t 0.38 min (254 nm)

2-(4-Methoxyphenyl)-4-phenyl-1*H*-imidazole (4.72) [179]



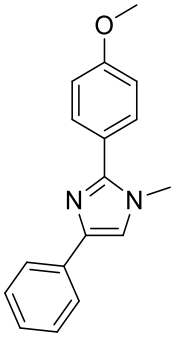
4-anisaldehyde (5.08 g, 37.3 mmol) was dissolved in Methanol (100 mL) along with ammonium acetate (14.37 g, 186.4 mmol). Phenylglyoxal hydrate (5 g, 37.3 mmol) was added and the mixture was stirred at rt overnight. Volatiles were removed *in vacuo*, water was added to the residue and then extracted with EA. The organic layer was washed with water, brine and then dried over magnesium sulfate. The solution was concentrated under reduced pressure to give the product as a yellow solid (3.8 g, 39%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 8.12 (d, *J* = 8.6 Hz, ArH, 2H), 8.02 (s, ArH, 1H), 7.91 (d, *J* = 7.7 Hz, ArH, 2H), 7.47 (t, *J* = 7.6 Hz, ArH, 2H), 7.38 (t, *J* = 7.4 Hz, ArH, 1H), 7.12 (d, *J* = 8.5 Hz, ArH, 2H), 3.81 (s, CH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 162.22, 145.2, 134.34, 129.4, 129.3, 129.2, 128.0, 126.0, 116.5, 116.5, 115.1, 56.0.

LC-MS: calculated for $C_{16}H_{15}N_2O$ $[M+H]^+$ 251.1. found: 251.1

2-(4-Methoxyphenyl)-1-methyl-4-phenyl-1*H*-imidazole (4.73)



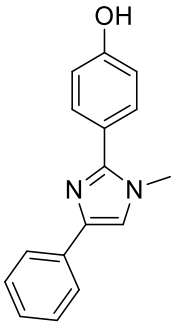
4.72 (1.5 g, 6.0 mmol) was dissolved in DMF (10 mL) and NaH (60% dispersion in mineral oil, 0.72 g, 18.0 mmol) was added. The mixture was stirred at rt for 5 minutes and then MeI (2.13 g, 15.0 mmol) was added. The mixture was stirred at rt overnight and then volatiles were removed *in vacuo.* Water was added and the precipitate was filtered and washed with water to give the product as a pale yellow solid (1.40 g, 89%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.81 – 7.74 (m, ArH, 2H), 7.71 – 7.63 (m, ArH, 3H), 7.35 (t, *J* = 7.7 Hz, ArH, 2H), 7.24 – 7.16 (m, ArH, 1H), 7.10 – 7.02 (m, ArH, 2H), 3.82 (s, O-CH_3 3H), 3.73 (s, N-CH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 159.8, 147.4, 139.5, 134.9, 130.2, 128.9, 126.6, 124.6, 123.3, 119.7, 114.3, 55.7, 34.9.

LC-MS: calculated for $C_{17}H_{17}N_2O$ $[M+H]^+$ 265.1. found: 265.2

4-(1-Methyl-4-phenyl-1*H*-imidazol-2-yl)phenol (4.74)

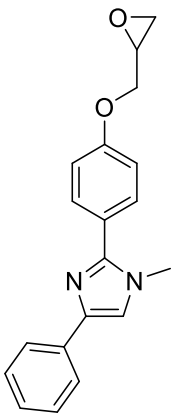


4.73 (0.8 g, 3.03 mmol) was dissolved in dry DCM (20 mL) and cooled to -78 °C in an acetone/ice bath. BBr_3 (1M in DCM, 12.1 mL, 12.1 mmol) was added dropwise. The solution was allowed to warm to room temperature and stir for 3h. The reaction mixture was poured onto methanol and then concentrated under reduced pressure, the residue was filtered through a pad of celite, eluting with DCM, to give the product as a brown solid, carried forward without further purification (0.62 g, 82%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.89 – 7.83 (m, imid-H 1H), 7.80 – 7.66 (m, ArH, 3H), 7.64 – 7.41 (m, ArH, 4H), 7.12 – 7.03 (m, ArH, 2H), 3.88 (s, CH_3, 3H).

LC-MS: calculated for $C_{16}H_{15}N_2O$ $[M+H]^+$ 251.1. found: 251.3

1-Methyl-2-(4-(oxiran-2-ylmethoxy)phenyl)-4-phenyl-1*H*-imidazole (4.75)



4.74 (0.28 g, 1.20 mmol) was dissolved in DMF (10 mL) along with caesium carbonate (0.73 g, 2.24 mmol) and then (±)epichlorohydrin (0.21 g, 2.24 mmol). The mixture

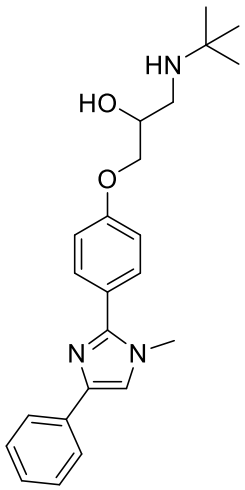
was stirred at 50 °C for 3h. Volatiles were removed *in vacuo* and then water was added, the precipitate was filtered to give pure product (0.25 g, 67%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.78 (d, *J* = 7.7 Hz, imidazole ArH, 1H), 7.74 – 7.59 (m, ArH, 4H), 7.43 – 7.32 (m, ArH, 2H), 7.26 – 7.17 (m, ArH, 1H), 7.16 – 7.06 (m, ArH, 2H), 4.46 – 4.38 (m, O-$\underline{CH_2}$-CH, 1H), 3.93 (dt, *J* = 11.7, 6.5 Hz, O-$\underline{CH_2}$-CH, 1H), 3.79 (d, *J* = 24.5 Hz, N-CH_3, 3H), 3.42 – 3.35 (m, O-CH_2-$\underline{CH}$, 1H), 2.88 (t, *J* = 4.7 Hz, epox-CH_2, 1H), 2.75 (dd, *J* = 5.1, 2.7 Hz, epox-CH_2, 1H).

^{13}C NMR: (101 MHz, DMSO) δ 159.2, 158.8, 134.9, 130.2, 130.0, 128.9, 126.6, 124.6, 119.7, 115.2, 114.9, 69.5, 50.1, 44.2, 34.9.

LC-MS: calculated for $C_{19}H_{19}N_2O_2$ $[M+H]^+$ 307.4. found: 307.2

1-(*tert*-Butylamino)-3-(4-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)phenoxy)propan-2-ol (4.76)



4.75 (0.05 g, 0.20 mmol) was dissolved in *tert*-butylamine (1 mL) and Methanol (1 mL) and stirred 40 °C 3 days. Volatiles were removed *in vacuo* and the product was purified by HPLC, eluting with MeCN:H_2O:TFA (5:95:0.01 → 20:80:0.01 over 8 minutes, eluted at 7.7 minutes). (0.31 g, 51%).

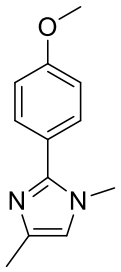
^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.79 – 7.70 (m, ArH, 4H), 7.48 – 7.37 (m, ArH, 3H), 7.24 (s, ArH, 1H), 7.04 – 6.96 (m, ArH, 2H), 3.81 (s, O-$\underline{CH_2}$-CH 3H), 3.51 (s, N-CH_3, 3H), 3.20 (d, *J* = 12.0 Hz, N-CH_2, 1H), 2.90 – 2.77 (m, N-CH_2, 1H), 1.30 (s, *t*-Bu, 9H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 162.1, 160.9, 145.8, 131.4, 129.4, 129.1, 125.9, 118.2, 115.0, 69.5, 65.1, 56.9, 45.7, 35.5, 25.4 (2 aromatic carbon not observed).

HRMS: calculated for $C_{23}H_{30}N_3O_2$ $[M+H]^+$ 380.2333. found: 380.2344

LC-MS: R_t: 1.80 min (254 nm)

2-(4-Methoxyphenyl)-1,4-dimethyl-1*H*-imidazole (4.78) [180]



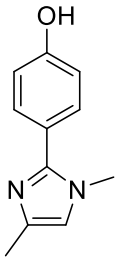
4.6 (2 g, 8.97 mmol) was dissolved in pyridine (20 mL) along with acetic anhydride and DMAP (0.11 g, 0.90 mmol). The mixture was stirred at 50 °C overnight, then volatiles were removed *in vacuo* and intermediate was isolated by column chromatography eluting with DCM-Methanol (95:5) to give an orange oil. The intermediate (0.5 g, 2.3 mmol) was dissolved in acetic acid (20 mL) along with ammonium acetate (0.17 g, 23 mmol) and heated at 100 °C overnight. The mixture was concentrated under reduced pressure and then purified by column chromatography eluting with DCM-Methanol (95:5) to give pure product (0.26 g, 14%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.62 – 7.53 (m, ArH, 2H), 7.08 – 6.98 (m, ArH, 2H), 6.92 (s, imidazole ArH, 1H), 3.80 (s, O-CH_3, 3H), 3.64 (s, N-CH_3, 3H), 2.11 (s, CH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 159.7, 145.8, 135.2, 130.0, 128.8, 119.8, 114.3, 55.6, 34.5, 13.6.

LC-MS: calculated for $C_{12}H_{15}N_2O$ $[M+H]^+$ 203.1. found: 203.3

4-(1,4-Dimethyl-1*H*-imidazol-2-yl)phenol (4.79)

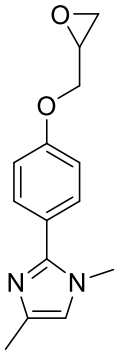


4.78 (0.18 g, 0.89 mmol) was dissolved in dry DCM (6 mL) and cooled to -78 °C in an acetone/ice bath. BBr_3 (1M in DCM, 3.56mL, 3.56 mmol) was added dropwise. The solution was allowed to warm to room temperature and stirred for 3 hours. The reaction mixture was poured onto methanol and then concentrated under reduced pressure. Water was added and the solution was extracted with EA. The organic layer was concentrated *in vacuo* to give pure product (0.98 g, 57%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.64 – 7.57 (m, ArH, 2H), 7.47 (s, imidazole ArH, 1H), 7.04 – 6.99 (m, ArH, 2H), 3.88 (s, N-CH_3, 3H), 2.30 (s, CH_3, 3H).

LC-MS: calculated for $C_{11}H_{13}N_2O$ $[M+H]^+$ 189.2. found: 189.2

1,4-Dimethyl-2-(4-(oxiran-2-ylmethoxy)phenyl)-1*H*-imidazole (4.80)



4.79 (0.10g, 0.52 mmol) was dissolved in DMF (5 mL) along with caesium carbonate (0.34 g, 1.04 mmol) and then (±)epichlorohydrin (0.34 g, 1.04 mmol). The mixture was stirred at 50 °C overnight. Water was added and then extracted with EA. The organic layer was concentrated *in vacuo* to give and orange oil which was purified by

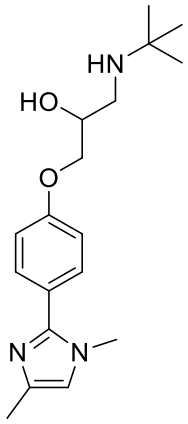
column chromatography eluting with DCM-METHANOL (95-5) to give the product as a clear oil (0.25 g, 67%).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.59 – 7.53 (m, ArH, 2H), 7.03 – 6.94 (m, ArH, 2H), 6.66 (s, imidazole ArH, 1H), 4.27 (dd, *J* = 11.0, 3.1 Hz, O-CH_2, 1H), 3.99 (dd, *J* = 11.0, 5.7 Hz, O-CH_2,1H), 3.38 (ddt, *J* = 5.7, 4.1, 2.7 Hz, epox-CH, 1H), 2.92 (t, *J* = 4.5 Hz, epox-CH_2, 1H), 2.78 (dd, *J* = 4.9, 2.7 Hz, epox-CH_2 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 162.6, 158.9, 134.5, 130.2, 118.7, 114.7, 72.5, 69.2, 50.5, 45.2, 34.7.

LC-MS: calculated for $C_{14}H_{17}N_2O_2$ $[M+H]^+$ 245.1. found: 245.3

1-(*tert*-Butylamino)-3-(4-(1,4-dimethyl-1*H*-imidazol-2-yl)phenoxy)propan-2-ol (4.81)



4.80 (0.03 g, 0.12 mmol) was dissolved in *tert*-butylamine (1mL) and Methanol (1 mL) and stirred at 40 °C for 3 days. Volatiles were removed *in vacuo* and the product was purified by HPLC, eluting with MeCN:H_2O:TFA (15:85:0.01 → 95:5:0.01 over 11 minutes, eluted at 8.0 minutes) to isolate the product (0.021 g, 55%).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.62 – 7.56 (m, ArH, 2H), 7.03 – 6.96 (m, ArH, 2H), 6.86 (s, imidazole ArH, 1H), 4.11 – 4.01 (m, CH, 1H), 3.86 (d, *J* = 5.6 Hz, O-CH_2,

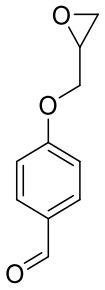
2H), 3.80 (s, N-CH_3, 3H), 3.23 (t, *J* = 10.6 Hz, N-CH_2, 1H), 2.98 (t, *J* = 10.6 Hz, N-CH_2, 1H), 2.37 (s, CH_3, 3H), 1.42 (s, *t*-Bu 9H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 161.7, 160.9, 144.3, 130.9, 130.4, 119.6, 115.4, 69.6, 65.2, 57.1, 45.3, 35.2, 25.6, 9.7.

HRMS: calculated for $C_{18}H_{28}N_3O_2$ $[M+H]^+$ 318.2176. found: 318.2182.

LC-MS: R_t: 0.57 min (254 nm)

4-(Oxiran-2-ylmethoxy)benzaldehyde (4.87) [181]



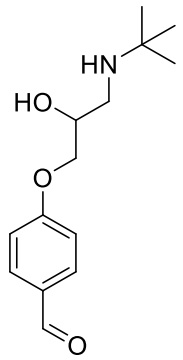
4-hydroxybenzaldehyde (5 g, 41.0 mmol) was dissolved in DMF (50 mL) along with K_2CO_3 (11.32 g, 81.9 mmol) and (±)epichlorohydrin (7.58 g, 81.9 mmol). The mixture was stirred at 80 °C overnight and then volatiles were removed *in vacuo.* Water was added to the residue and the mixture was extracted with EA. The organic layers were combined and washed with water, then dried and concentrated under reduced pressure. The product was purified by CC, eluting with DCM (5.26 g, 72%).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 9.91 (s, aldehyde, 1H), 7.90 – 7.82 (m, ArH, 2H), 7.08 – 7.01 (m, ArH, 2H), 4.36 (dd, *J* = 11.1, 2.9 Hz, O-$\underline{CH_2}$, 1H), 4.03 (dd, *J* = 11.1, 5.9 Hz, O-$\underline{CH_2}$, 1H), 3.40 (ddt, *J* = 5.9, 4.1, 2.8 Hz, epox CH_1 1H), 2.95 (dd, *J* = 4.9, 4.1 Hz, epox CH_2, 1H), 2.80 (dd, *J* = 4.9, 2.8 Hz, epox CH_2, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 190.7, 163.3, 132.0, 129.2, 114.9, 69.0, 49.8, 44.5.

LC-MS: calculated for $C_{10}H_{11}O_3$ $[M+H]^+$ 179.1. found: 179.2

4-(3-(*tert*-Butylamino)-2-hydroxypropoxy)benzaldehyde (4.88)



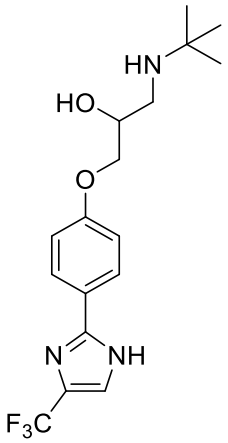
4.87 (1.0 g, 5.6 mmol) was dissolved in *tert*-butylamine (10 mL) and heated at 50 °C overnight. Volatiles were removed *in vacuo.* The resulting solid was washed with Methanol and DE, to give the pure product as a white solid (1.16 g, 58%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 8.24 (s, aldehyde, 1H), 7.72 – 7.63 (m, ArH, 2H), 7.02 – 6.94 (m, ArH, 2H), 4.02 (dd, *J* = 9.8, 4.5 Hz, O-$\underline{CH_2}$, 1H), 3.91 (dd, *J* = 9.8, 6.1 Hz, O-$\underline{CH_2}$, 1H), 3.80 (q, *J* = 5.8 Hz, CH_2-$\underline{CH}$OH, 1H), 2.67 – 2.53 (m, NH-$\underline{CH_2}$, 2H), 1.22 (s, *t*-Bu, 9H).

^{13}C NMR: (101 MHz, DMSO) δ 160.9, 154.7, 130.0, 129.6, 114.9, 71.3, 69.4, 57.0, 45.6, 30.1 (1 C not observed, HC=O).

LC-MS: calculated for $C_{14}H_{22}NO_3$ $[M+H]^+$ 252.2. found: 252.4

1-(*tert*-Butylamino)-3-(4-(4-(trifluoromethyl)-1*H*-imidazol-2-yl)phenoxy)propan-2-ol (4.89)



1,1-dibromo-3,3,3-trifluoroacetate (0.43 g, 1.59 mmol) and sodium acetate (0.26 g, 3.19 mmol) were dissolved in water (5 mL) and heated at reflux for 30 minutes. The solution was cooled and added to **4.88** (0.4 g, 1.159 mmol), dissolved in 7N ammonia in methanol (10 mL). The mixture was stirred at rt for 3 days. Volatiles were removed *in vacuo* and MeCN was added to the residue. The suspension was heated and solid crashed out which was filtered. The filtrate was concentrated under reduced pressure to give an orange oil which was purified by COLUMN CHROMATOGRAPHY eluting with DCM–Methanol (100:0→80:20) to give a yellow solid product (0.21 g, 37%).

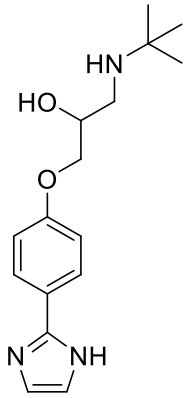
^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.97 – 7.90 (m, ArH, 2H), 7.86 (s, imidazole ArH, 1H), 7.12 – 7.05 (m, ArH 2H), 4.23 – 4.16 (m, CH_2-CHOH, 1H), 4.08 (d, *J* = 5.2 Hz, O-CH_2, 2H), 3.24 – 3.10 (m, NH-CH_2, 1H), 3.02 – 2.90 (m, NH-CH_2, 1H), 1.32 (s, *t*-Bu, 9H).

^{13}C NMR: (101 MHz, DMSO) δ 160.2, 148.6, 128.2, 124.3, 122.8, 119.5, 116.2, 70.6, 66.4, 57.8, 52.2, 25.9. CF_3 not observed

HRMS: calculated for $C_{17}H_{23}F_3N_3O_2$ $[M+H]^+$ 358.1737. found: 358.1749.

LC-MS: R_t: 2.05 min (254 nm)

1-(4-(1*H*-Imidazol-2-yl)phenoxy)-3-(tert-butylamino)propan-2-ol (4.90) [182]



4.88 (0.4 g, 1.59 mmol) was dissolved in Methanol (20 mL) along with ammonium acetate (0.61 g, 7.95 mmol). Glyoxal (40% in water) was added and the solution was stirred at room temperature overnight. Volatiles were removed and the product was purified by HPLC, eluting with MeCN:H_2O:TFA (5:95:0.01 → 19:81:0.01 over 6 minutes then 19:81:0.01 → 95:5:0.01 over 4 minutes, eluted at 8.1 minutes) to obtain the product as a white solid (0.21 g, 46%).

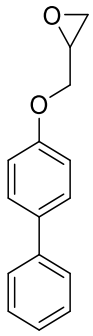
^{1}H NMR: (400 MHz, Methanol-d_4) δ 7.95 – 7.90 (m, ArH, 2H), 7.57 (s, imidazole ArH, 2H), 7.28 – 7.21 (m, ArH, 2H), 4.34 – 4.25 (m, CH, 1H), 4.18 (d, *J* = 5.1 Hz, O-CH_2, 2H), 3.36 – 3.29 (m, N-CH_2, 1H), 3.21 – 3.09 (m, N-CH_2, 1H), 1.44 (s, *t*-Bu, 9H).

^{13}C NMR: (101 MHz, MeOD) δ 161.7, 144.8, 128.5, 119.3, 115.6, 115.5, 69.9, 65.7, 56.9, 44.0, 24.3.

HRMS: calculated for $C_{16}H_{24}N_3O_2$ $[M+H]^+$ 290.1863. found: 290.1878

LC-MS: R_t 0.47 min (254 nm)

2-(([1,1'-Biphenyl]-4-yloxy)methyl)oxirane (4.92) [183]

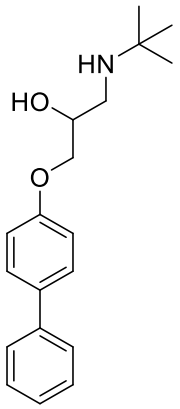


4-phenylphenol (2 g, 11.75 mmol) was dissolved in DMF (30 mL) along with epichlorohydrin (1.38 mL, 17.63 mmol) and caesium carbonate (7.66 g, 23.5 mmol). The mixture was heated at 50 °C for 16 hours. Volatiles were removed *in vacuo* and then water was added. The precipitate was filtered and washed with water. The product was isolated as a white solid by CC, eluting with PE–EA (80:20) (1.66 g, 62%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.64 – 7.56 (m, ArH, 4H), 7.47 – 7.37 (m, ArH, 2H), 7.35 – 7.27 (m, ArH, 1H), 7.08 – 7.02 (m, ArH, 2H), 4.37 (dd, *J* = 11.4, 2.7 Hz, O-CH_2, 1H), 3.88 (dd, *J* = 11.4, 6.5 Hz, O-CH_2, 1H), 3.35 (ddt, *J* = 6.8, 4.2, 2.7 Hz, epoxide CH_1, 1H), 2.86 (dd, *J* = 5.1, 4.2 Hz, epoxide CH_2, 1H), 2.73 (dd, *J* = 5.1, 2.7 Hz, epoxide CH_2, 1H).

^{13}C NMR: (101 MHz, DMSO-d_6) δ 157.8, 139.7, 132.8, 128.8, 127.7, 126.7, 126.1, 114.9, 69.0, 49.1, 43.7.

1-([1,1'-Biphenyl]-4-yloxy)-3-(tert-butylamino)propan-2-ol (4.93) [184]



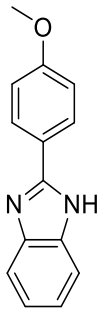
4.92 (0.2 g, 0.88 mmol) was dissolved in Methanol (3 mL) and *tert*-butylamine (3 mL). The mixture was heated at 40 °C overnight. Volatiles were removed *in vacuo* and then 1Mhydrochloric acid in diethyl ether (2 mL) was added along with diethyl ether (2 mL), the white precipitate was filtered, to give the product as a white solid (0.17 g, 63%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.65 – 7.58 (m, ArH, 4H), 7.43 (t, *J* = 7.6 Hz, ArH, 2H), 7.31 (t, *J* = 7.4 Hz, ArH, 1H), 7.05 (d, *J* = 8.6 Hz, ArH, 2H), 4.10 – 3.97 (m, O-$\underline{CH_2}$,$\underline{CH}$-OH 3H), 3.00 (d, *J* = 10.8 Hz,NH-$\underline{CH_2}$ 1H), 2.83 (dd, *J* = 12.1, 8.2 Hz, NH-$\underline{CH_2}$, 1H), 1.24 (s, *t*-Bu, 9H).

^{13}C NMR: (101 MHz, DMSO-d_6) δ 158.0, 139.7, 132.7, 128.8, 127.7, 126.7, 126.1, 115.0, 70.1, 66.5, 44.4, 40.7, 26.0.

LC-MS: calculated for $C_{19}H_{26}NO_2$ $[M+H]^+$ 300.2. found: 300.2. R_t 2.25 min (254 nm)

2-(4-Methoxyphenyl)-1*H*-benzimidazole (4.96)



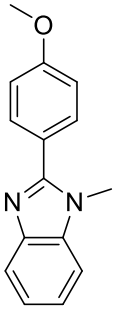
o-Phenyldiamine (2.0 g, 18.5 mmol) and 4-methoxybenzylamine (3.8 g, 27.7 mmol) were dissolved in toluene (100 mL) and $CuBr_2$ (0.041 g, 0.19 mmol) was added. The mixture was stirred at 100 °C for 16 h and then the mixture was filtered through a pad of silica. The filtrate was concentrated *in vacuo* then purified by COLUMN CHROMATOGRAPHY eluting with DCM–Methanol (100:0→95:5) to give a brown solid product (0.7 g, 17%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 8.15 – 8.07 (m, ArH, 2H), 7.55 (s br, ArH, 2H), 7.20 – 7.13 (m, ArH, 2H), 7.14 – 7.08 (m, ArH, 2H), 3.84 (s, CH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 160.5, 151.3, 134.9, 128.0, 123.2, 117.2, 114.5, 114.3, 55.3.

LC-MS: calculated for $C_{14}H_{13}N_2O$ $[M+H]^+$ 225.1. found: 225.0

2-(4-Methoxyphenyl)-1-methyl-1*H*-benzimidazole (4.97)



4.96 (0.46 g, 2.05 mmol) was dissolved in DMF (5 mL) and NaH (60% dispersion in mineral oil) (0.25 g, 6.15 mmol) was added. The mixture was stirred at rt for 5 minutes

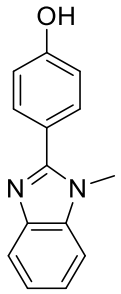
and then MeI (0.35 g, 2.46 mmol) was added. The mixture was stirred at rt overnight and then volatiles were removed *in vacuo.* The resulting solid was suspended in water and filtered under vacuum to give pure product (0.3 g, 63%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.84 – 7.77 (m, ArH, 2H), 7.68 – 7.64 (m, ArH, 1H), 7.61 – 7.56 (m, ArH, 1H), 7.31 – 7.20 (m, ArH, 2H), 7.16 – 7.10 (m, ArH 2H), 3.87 (s, CH_3, 3H) 3.86 (s, CH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 160.2, 153.0, 142.4, 136.5, 130.7, 122.4, 122.0, 121.7, 118.7, 114.1, 110.3, 55.3, 31.6.

LC-MS: calculated for $C_{15}H_{15}N_2O_2[M+H]^+$ 239.1. found: 239.2

4-(1-Methyl-1*H*-benzimidazol-2-yl)phenol (4.98)



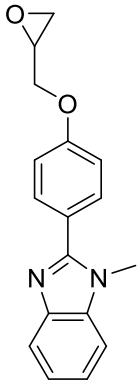
4.97 (0.3 g, 1.26 mmol) was dissolved in dry DCM (5 mL) and cooled to -78 °C in an acetone/ice bath. BBr_3 (1M in DCM, 5mL, 5 mmol) was added dropwise. The solution was allowed to warm to room temperature and stir for 16h. The reaction mixture was poured onto methanol and then concentrated under reduced pressure. The product was purified by CC, eluting with DCM–Methanol (95:5) to give the product as a beige solid (0.28 g, 98%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 10.53 (s, OH, 1H), 7.96 (dd, *J* = 6.9, 2.3 Hz, ArH, 1H), 7.83 – 7.76 (m, ArH, 3H), 7.62 – 7.52 (m, ArH, 2H), 7.11 – 7.05 (m, ArH, 2H), 4.00 (s, CH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 161.1, 150.9, 133.7, 132.1, 125.5, 125.0, 116.1, 114.6, 112.6, 32.5.

LC-MS: calculated for $C_{14}H_{13}N_2O$ $[M+H]^+$ 225.1. found: 225.2

1-Methyl-2-(4-(oxiran-2-ylmethoxy)phenyl)-1*H*-benzimidazole (4.99)



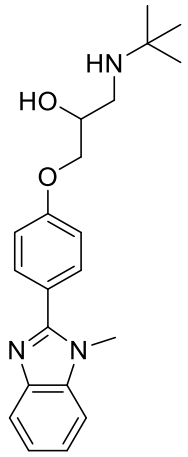
4.98 (0.33 g, 1.47 mmol) was dissolved in DMF (10 mL) along with caesium carbonate (0.96 g, 2.95 mmol) and then (±)epichlorohydrin (0.27 g, 2.95 mmol). The mixture was stirred at 50 °C overnight. Volatiles were removed *in vacuo* and the residue was purified by CC, eluting with DCM–Methanol (100:0 →98:2) to give the product as a viscous yellow oil (0.26 g, 64%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.83 – 7.79 (m, ArH, 2H), 7.66 (d, ArH, 1H), 7.59 (d, *J* = 7.5, 1.6 Hz, 1H), 7.26 (dtd, *J* = 18.6, 7.2, 1.3 Hz, ArH, 3H), 7.18 – 7.15 (m, ArH, 2H), 4.46 (dd, *J* = 11.4, 2.6 Hz, O-CH_2, 1H), 3.95 (dd, *J* = 11.4, 6.6 Hz, O-CH_2, 1H), 3.87 (s, CH_3, 3H), 3.42 – 3.36 (m, epox CH, 1H), 2.89 (t, *J* = 4.7 Hz, epox CH_2, 1H), 2.76 (dd, *J* = 5.1, 2.6 Hz, epox CH_2, 1H).

^{13}C NMR: (101 MHz, DMSO) δ 159.2, 152.9, 142.4, 136.5, 130.7, 122.7, 122.0, 121.7, 118.7, 114.6, 110.3, 69.1, 50.6, 49.6, 43.7, 31.6.

LC-MS: calculated for $C_{19}H_{19}N_2O_2$ $[M+H]^+$ 281.1. found: 281.2

1-(*tert*-Butylamino)-3-(4-(1-methyl-1*H*-benzo[d]imidazol-2-yl)phenoxy)propan-2-ol (4.100)



4.99 (0.065 g, 0.23 mmol) was dissolved in Methanol (2 mL) and *tert*-butylamine (2 mL). The mixture was heated at 40 °C overnight. Volatiles were removed under reduced pressure and the product was purified by HPLC, eluting with MeCN:H_2O:TFA (5:95:0.01 → 30:70:0.01 over 8 minutes then 30:70:0.01 → 95:5:0.01 over 4 minutes, eluted at 7.9 minutes) to obtain the product as a white solid (0.032 g, 39%).

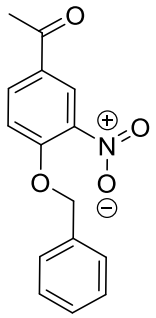
^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.80 – 7.69 (m, ArH, 3H), 7.62 – 7.44 (m, ArH, 3H), 7.05 (d, *J* = 8.4 Hz, ArH, 2H), 4.37 (s, CH, 1H), 4.07 – 3.98 (m, O-CH_2, N-CH_3, 5H), 3.31-3.22 (m, N-CH_2, 1H), 3.13-3.02 (m, N-CH_2, 1H), 1.44 (s, *t*-Bu, 9H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 161.9, 150.1, 133.0, 131.8, 131.1, 126.6, 126.1, 115.7, 115.1, 114.2, 111.5, 69.8, 65.5, 57.3, 50.8, 32.6, 25.6.

HRMS: calculated for $C_{21}H_{28}N_3O_2$ $[M+H]^+$ 354.2176. found: 354.2190

LC-MS: R_t 1.03 min (254 nm, 91%)

1-(4-(Benzyloxy)-3-nitrophenyl)ethan-1-one (5.3) [76]



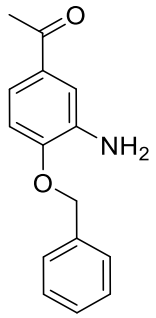
4-hydroxy-3-nitroacetophenone (20 g, 110.4 mmol) was dissolved in MeCN (200 mL) and DIPEA (15.0 g, 115.9 mmol) was added. Benzyl bromide was added over 5 minutes and then the mixture was heated to reflux over 3 hours. The mixture was cooled and then water was added, precipitate started to form and the mixture was stirred for 30 minutes. The precipitate was filtered under vacuum and washed with water to give the product was a white solid (27.8 g, 93%).

^{1}H NMR: (400 mhz, DMSO) δ 8.42 (d, *J* = 1.6 Hz, ArH, 1H), 8.21 (dd, *J* = 8.8, 1.6 Hz, ArH, 1H), 7.57 (d, *J* = 8.8 Hz, ArH, 1H), 7.45-7.27 (m, ArH, 5H), 5.42 (s, $BnCH_2$, 2H), 2.58 (s, $COCH_3$, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 195.3, 154.0, 139.4, 135.4, 134.0, 129.3, 128.5, 128.2, 127.4, 125.0, 115.4, 70.8, 26.5.

LC-MS: calculated for $C_{15}H_{14}NO_4$ $[M+H]^+$ 272.1. found: 272.2

1-(3-Amino-4-(benzyloxy)phenyl)ethan-1-one (5.4) [185]



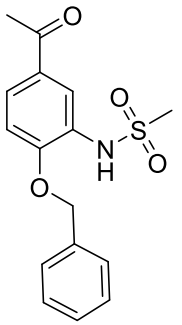
5.3 (8 g, 29.5 mmol) was dissolved in Methanol (100 mL) and THF (100 mL). Ammonium chloride (15.8 g, 295 mmol) and zinc powder (19.3 g, 295 mmol) were added and the mixture was stirred at room temperature for 16 hours. Zinc was removed by filtration under vacuum and the filtrate was concentrated to give a yellow solid. The solid was washed with water, then redissolved in THF. The Solution was filtered and the filtrate was concentrated *in vacuo* to give the pure product as a yellow solid (6.82 g, 96%).

^{1}H NMR: (400 mhz, DMSO) δ 8.00 (d, *J* = 2.1 Hz, ArH, 1H), 7.92 (dd, *J* = 8.6, 2.1 Hz, ArH, 1H), 7.62 – 7.56 (m, ArH, 2H), 7.44 – 7.29 (m, ArH, 4H), 5.35 (s, $BnCH_2$, 2H), 2.52 (s, $COCH_3$, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 195.8, 176.3, 154.3, 135.9, 129.9, 128.4, 128.1, 127.7, 123.3, 122.5, 112.9, 70.2, 26.3.

LC-MS: calculated for $C_{15}H_{16}NO_2$ $[M+H]^+$ 242.1. found: 242.0

***N*-(5-Acetyl-2-(benzyloxy)phenyl)methanesulfonamide (5.11) [186]**



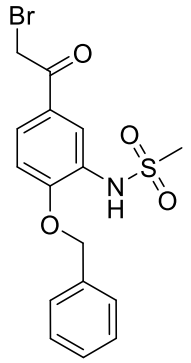
5.3 (5 g, 20.7 mmol) was dissolved in DCM (60 mL) and pyridine was added (3.28 g, 41.5 mmol). The solution was cooled to 5 °C and then methane sulfonyl chloride (4.75 g, 41.5 mmol) dissolved in DCM (4mL) was added dropwise. The solution was stirred at 5 °C for 30 minutes and then allowed to warm to room temperature and stirred for a further 16 hours. Aq. 2Mhydrochloric acid was added (50 mL) to the solution and then extracted with DCM (×3). The organic layers were combined and washed with water then brine, dried over magnesium sulfate and concentrated *in vacuo.* diethyl ether was added to the residue and heated, solid crashed out and was filtered under vacuum to afford the product as a green solid (4.55 g, 69%).

^{1}H NMR: (400 mhz, DMSO) δ 9.20 (s, NH, 1H), 7.87 – 7.80 (m, ArH, 2H), 7.59 – 7.52 (m, ArH, 2H), 7.45 – 7.30 (m, ArH, 3H), 7.28 – 7.21 (m, ArH, 1H), 5.29 (s, $BnCH_2$, 2H), 2.93 (s, SCH_3, 3H), 2.51 (s, $COCH_3$, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 196.0, 155.5, 136.1, 129.8, 128.4, 128.0, 127.9, 127.8, 126.1, 125.7, 112.6, 70.0, 40.4, 26.3.

LC-MS: calculated for $C_{15}H_{17}NO_4S$ $[M+H]^+$ 319.4. found: 319.8

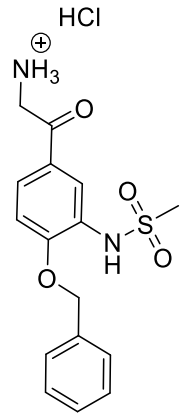
***N*-(2-(Benzyloxy)-5-(2-bromoacetyl)phenyl)methanesulfonamide (5.13)** [185]



5.12 (3.98 g, 12.5 mmol) was dissolved in THF (80 mL) and phenyltrimethylammonium tribromide (5.15 g, 13.7 mmol) was added. The mixture was stirred at 65 °C for 2 hours and then THF was removed under reduced pressure. Water was added and the aqueous solution was extracted with ethyl acetate (×3), washed with water, brine and dried over magnesium sulfate. The volatiles were removed in vacuo to give the product as a light brown solid. The product was carried forward without purification (7.37 g).

LC-MS: calculated for $C_{16}H_{17}BrNO_4S$ $[M+H]^+$ 397.0. found: 397.7

***N*-(2-(Benzyloxy)-5-glycylphenyl)methanesulfonamide hydrochloride (5.14)**



5.13 (3.53 g, 8.86 mmol) was dissolved in $CHCl_3$ (200 mL), hexamine (1.37 g, 14.0 mmol) was added and the solution was stirred at ambient temperature for 4 hours. Precipitate formed and was filtered; the precipitate was then redissolved in

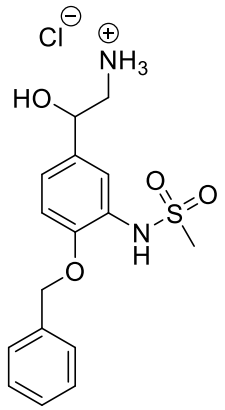
methanol (50 mL) and conc.hydrochloric acid (25 mL) and stirred overnight at 60 °C. Volatiles were removed in vacuo, to afford pink solid, a small amount of diethyl ether was added and the solid was filtered under vacuum and collected to afford the product as a pink solid (1.35 g, 30% over 2 steps).

^{1}H NMR: (400 mhz, DMSO) δ 9.32 (s, NH, 1H), 8.27 (s, NH_3, 3H), 7.89 (dt, *J* = 3.5, 2.1 Hz, ArH, 2H), 7.58 – 7.53 (m, ArH, 2H), 7.45 – 7.29 (m, ArH. 4H), 5.33 (s, $BnCH_2$, 2H), 4.52 (s, $COCH_2$, 2H), 2.96 (s, SCH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 191.0, 156.4, 136.0, 128.4, 128.1, 127.8, 126.5, 126.5, 125.5, 121.2, 112.9, 70.2, 44.5, 40.6.

LC-MS: calculated for $C_{16}H_{20}ClN_2O_4S$ $[M+H]^+$ 334.2. found: 334.9

_N_-(5-(2-Amino-1-hydroxyethyl)-2-(benzyloxy)phenyl)methanesulfonamide (5.10) [187]



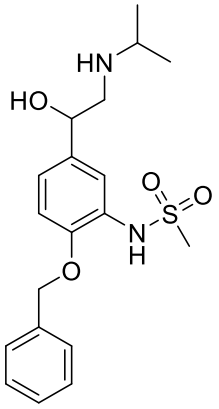
5.14 (2.18 g, 5.89 mmol) was dissolved in methanol (80 mL) and the solution was cooled to 0-5 °C followed by the portion-wise addition of sodium borohydride (0.89 g, 23.56 mmol). The cooled mixture was stirred for 30 minutes then allowed to warm to ambient temperature at which point it was stirred for a further hour. The reaction was quenched by the addition of aq. 1Mhydrochloric acid and then methanol was removed under reduced pressure. The aqueous solution was basified to pH 10 with aq. 1M NaOH and precipitate formed. The solid was filtered to afford the pure product as a white solid (0.90 g, 45%).

^{1}H NMR: (400 mhz, DMSO) δ 8.21 (s, NH_3, 3H), 7.54 (d, *J* = 7.1 Hz, ArH, 2H), 7.40 (dd, *J* = 10.1, 4.6 Hz, ArH, 2H), 7.36 – 7.27 (m, ArH, 2H), 7.16 (dt, *J* = 20.0, 5.3 Hz, ArH, 2H), 6.03 (d, *J* = 3.8 Hz, CHOH, 1H), 5.18 (s, BnCH_2, 2H), 4.75 (d, *J* = 9.6 Hz, CHOH, 1H), 2.99 – 2.91 (m, NCH_2, 1H), 2.92 (s, SCH_3, 3H), 2.78 (dd, *J* = 12.6, 9.9 Hz, NCH_2, 1H).

^{13}C NMR: (101 MHz, DMSO) δ 151.0, 136.7, 134.2, 128.3, 127.8, 127.6, 125.8, 124.1, 123.8, 112.9, 69.7, 68.5, 45.6, 40.2.

LC-MS: calculated for $C_{16}H_{21}N_2O_4S$ $[M+H]^+$ 336.1. found: 336.9

***N*-(2-(Benzyloxy)-5-(1-hydroxy-2-(isopropylamino)ethyl)phenyl)methanesulfonamide (5.17)**



5.10 (0.1 g, 0.30 mmol) and acetone (0.019 mL, 0.33 mmol) were dissolved in Methanol (2 mL) and left to stir overnight at rt. $NaBH_4$ (0.034 g 0.89 mmol) was added and the mixture was stirred for a further 30 minutes. 4M aq.hydrochloric acid was added and then volatiles were removed *in vacuo.* The product was purified by COLUMN CHROMATOGRAPHY eluting with DCM–Methanol (90:10) to give the product as a white solid (0.068 g, 61%).

Rf: 0.28 (with DCM–Methanol (90:10)).

^{1}H NMR: (500 MHz, DMSO-d_6) δ 7.59 – 7.51 (m, ArH, 2H), 7.44 – 7.38 (m, ArH, 2H), 7.38 – 7.30 (m, ArH, 2H), 7.21 (dd, *J* = 8.5, 2.2 Hz, ArH, 1H), 7.14 (d, *J* = 8.5 Hz, ArH, 1H), 6.11 (s, OH, 1H), 5.20 (s, BnCH_2, 2H), 4.84 (d, *J* = 10.1 Hz, BnCH 1H), 3.33 – 3.25

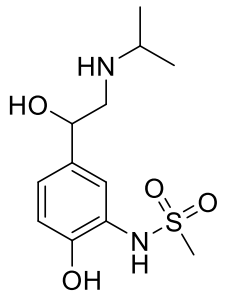
(m, iPr-CH, 1H (partially underneath water peak)), 3.04 (d, *J* = 11.8 Hz, NH-$\underline{CH_2}$, 1H), 2.93 (s, NH-$\underline{CH_2}$, S$\underline{CH_3}$, 4H), 1.23 (dd, *J* = 10.5, 6.5 Hz, *i*Pr, 6H).

^{13}C NMR: (126 MHz, DMSO) δ 151.6, 137.2, 134.7, 128.8, 128.3, 128.1, 126.3, 124.6, 124.4, 113.4, 70.2, 68.5, 51.1, 50.2, 19.4, 18.6.

HRMS: calculated for $C_{19}H_{27}N_2O_4S$ $[M+H]^+$ 379.1686. found: 379.1695

LC-MS: R_t 2.06 min (254 nm)

***N*-(2-Hydroxy-5-(1-hydroxy-2-(isopropylamino)ethyl)phenyl)methanesulfonamide (1.5)**



5.17 (0.05 g, 0.13 mmol) was dissolved in Methanol (0.7 mL), water (0.2 mL) and acetic acid (0.1 mL). Pd/C (0.01 g) was added and the mixture was stirred under a hydrogen atmosphere for 4h. Pd/C was removed by filtration and the filtrate was concentrated under reduced pressure. The clear brown oil was then purified by COLUMN CHROMATOGRAPHY eluting with DCM–Methanol (100:0→80:20) to give the product as a white solid (0.03 g, 81%).

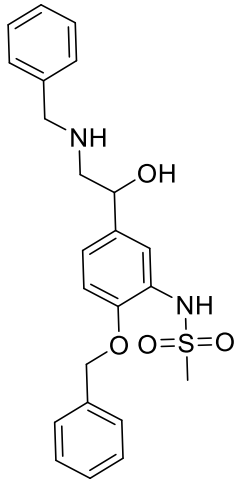
^{1}H NMR: (500 MHz, DMSO-d_6) δ 7.24 (d, *J* = 2.2 Hz, ArH, 1H), 7.07 (dd, *J* = 8.2, 2.2 Hz, ArH, 1H), 6.92 (d, *J* = 8.2 Hz, ArH, 1H), 4.80 (dd, *J* = 10.2, 2.9 Hz, Bn$\underline{CH,}$ 1H), 3.34 – 3.26 (m, iPr-CH, 1H (underneath water peak, identified by COSY 2D NMR)), 3.01 – 2.83 (m, NH-$\underline{CH_2}$, S$\underline{CH_3}$, 5H), 1.22 (dd, *J* = 10.0, 6.4 Hz, *i*Pr, 6H).

^{13}C NMR: (126 MHz, DMSO) δ 150.9, 133.2, 124.8, 124.6, 124.5, 116.1, 68.7, 51.4, 50.0, 45.9, 19.5.

HRMS: calculated for $C_{12}H_{21}N_2O_4S$ $[M+H]^+$ 289.1217. found: 289.1226

LC-MS: R_t 0.35 min (254 nm)

N-(5-(2-(Benzylamino)-1-hydroxyethyl)-2-(benzyloxy)phenyl)methanesulfonamide (5.21) [187]



5.10 (0.45 g, 1.21 mmol) was dissolved in methanol (20 mL) along with TEA (0.073 g, 1.21 mmol) and benzaldehyde (0.13 g, 1.21 mmol). The mixture was stirred at room temperature overnight and then cooled to 0-5 °C followed by the portion-wise addition of sodium borohydride (0.89 g, 23.6 mmol). The cooled mixture was stirred for 30 minutes then allowed to warm to ambient temperature at which it was stirred for a further 30 minutes. The reaction was quenched by the addition of aq. saturated ammonium chloride and then methanol was removed in vacuo. The aqueous solution was extracted with ethyl acetate (×3), the organic layers were combined, washed with water, brine, dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by CC, eluting with DCM-Methanol-TEA (99:1:0.1 → 97.5:2.5:0.1) to afford the product as a clear oil (0.11 g, 22%).

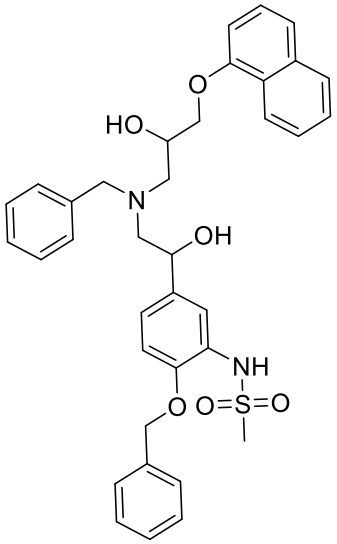
R_f: 0.47 (DCM-Methanol-TEA (97.5:2.5:0.1)).

^{1}H NMR: (400 mhz, $CDCl_3$) δ 7.50 (d, *J* = 2.0 Hz, ArH, 1H), 7.44 – 7.27 (m, ArH, 10H), 7.14 (dd, *J* = 8.5, 1.9 Hz, ArH, 1H), 6.96 (d, *J* = 8.5 Hz, OH, 1H), 5.09 (s, $BnCH_2$, 2H), 4.70 (dd, *J* = 9.1, 3.4 Hz, CH, 1H), 3.88 – 3.78 (m, $BnCH_2$, 2H), 2.94 – 2.87 (m, SO_2CH_3, N-CH_2, 4H), 2.72 (dd, *J* = 12.2, 9.1 Hz, N-CH_2, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 148.1, 139.3, 136.1, 135.8, 128.9, 128.7, 128.6, 128.3, 127.9, 127.4, 126.3, 123.0, 118.9, 112.3, 71.2, 71.1, 56.3, 53.4, 39.3.

HRMS: calculated for $C_{23}H_{27}N_2O_4S$ $[M+H]^+$ 427.1686. found: 427.1689.

N-(5-(2-(Benzyl(2-hydroxy-3-(naphthalen-1-yloxy)propyl)amino)-1-hydroxyethyl)-2-(benzyloxy)phenyl)methanesulfonamide (5.24)



5.21 (0.05 g, 0.12 mmol) was dissolved in ethanol (1 mL) along with DIPEA (0.015 g, 0.14 mmol) and **4.17** (0.023 g, 0.12 mmol). The mixture was stirred at 80 °C overnight and then volatiles were removed under reduced pressure. The residue was then purified by CC, eluting with DCM-Methanol-TEA (97.5:2.5:0.1) and then HPLC eluting with MeCN:H_2O:TFA (30:70:0.01 → 100:0:0.01 over 10 minutes, eluted at 7.4 minutes) to isolate the product as a yellow oil (16 mg, 22%).

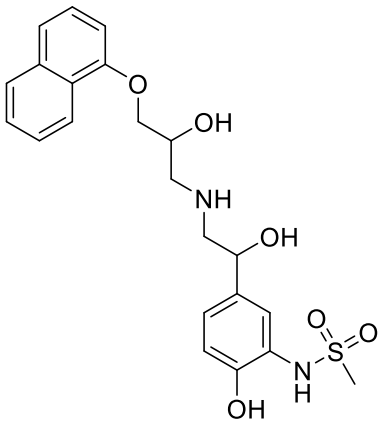
R_f: 0.33 (DCM-Methanol-TEA (97.5:2.5:0.1)).

^{1}H NMR: (400 mhz, $CDCl_3$) δ 7.96 (dd, *J* = 8.2, 1.5 Hz, ArH, 1H), 7.73 (d, *J* = 8.0 Hz, ArH, 1H), 7.48 – 7.25 (m, ArH, 14H), 7.10 – 7.03 (m, ArH, 1H), 6.89 (dd, *J* = 8.5, 1.6 Hz, ArH, 1H), 6.78 – 6.69 (m, ArH, 2H), 5.12 (s, OH, 1H), 5.01 (d, *J* = 2.7 Hz, $BnCH_2O$ 2H), 4.58 (dd, *J* = 36.1, 22.9 Hz, O-CH_2, 2H), 4.39 (t, *J* = 12.5 Hz, CH, 1H), 4.22 (td, *J* = 10.2, 4.6 Hz, N-CH_2, 1H), 4.07 – 3.99 (m, N-CH_2, 1H), 3.59 – 3.27 (m, $BnCH_2N$, N-CH_2, 4H), 2.85 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 153.6, 148.4, 135.6, 134.6, 131.6, 131.6, 130.7, 129.8, 129.0, 128.9, 127.9, 127.8, 126.7, 126.5, 125.9, 125.5, 125.3, 123.0, 121.6, 121.5, 121.3, 118.2, 112.7, 105.1, 77.8, 71.2, 69.6, 69.5, 57.6, 41.5, 39.7.

HRMS: calculated for $C_{36}H_{39}N_2O_6S$ $[M+H]^+$ 627.2523. found: 627.2529.

N-(2-Hydroxy-5-(1-hydroxy-2-((2-hydroxy-3-(naphthalen-1-yloxy)propyl)amino)ethyl)phenyl)methanesulfonamide (5.27)



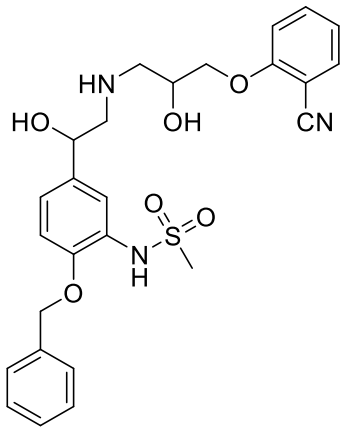
5.24 (0.012 g, 0.019 mmol) was dissolved in a mixture of THF (0.7 mL), water (0.2 mL) and acetic acid (0.1 mL). Pd/C (0.005 g) was added and the mixture was stirred under a hydrogen atmosphere overnight. Pd/C was filtered and then volatiles were removed *in vacuo*. HPLC was then carried out eluting with MeCN:H_2O:TFA (30:70:0.01 → 100:0:0.01 over 10 minutes, eluted at 4.8 minutes) to afford the product as a clear oil (0.004g 44%).

^{1}H NMR: (400 mhz, MeOD) δ 8.33 – 8.25 (m, ArH, 1H), 7.81 (dd, *J* = 6.3, 3.1 Hz, ArH, 1H), 7.52 – 7.43 (m, ArH, 4H), 7.41 – 7.36 (m, ArH, 1H), 7.19 – 7.15 (m, ArH, 1H), 6.93 (t, *J* = 7.9 Hz, ArH, 2H), 4.99 – 4.94 (m, BnCH, 1H), 4.48 (td, *J* = 9.6, 4.5 Hz,CH, 1H), 4.29 – 4.16 (m, O-CH_2, 2H), 3.55 – 3.44 (m, N-CH_2, 2H), 3.42 – 3.35 (m, N-CH_2, 1H), 3.27 – 3.20 (m, N-CH_2, 1H), 2.94 (s, SO_2CH_3, 3H).

HRMS: calculated for $C_{22}H_{27}N_2O_6S$ $[M+H]^+$ 447.1584. found: 447.1589.

Analytical HPLC: R_t 2.94 min (method b, 254 nm)

***N*-(2-(Benzyloxy)-5-(2-((3-(2-cyanophenoxy)-2-hydroxypropyl)amino)-1-hydroxyethyl)phenyl)methanesulfonamide (5.20)**



5.10 (0.43 g, 1.14 mmol) was dissolved in ethanol (15 mL) along with **4.19** (0.10 g, 0.57 mmol) and DIPEA (0.15 g, 1.14 mmol). The mixture was stirred at 80 °C for 2 days and then volatiles were removed under reduced pressure. The product was purified by COLUMN CHROMATOGRAPHY eluting with DCM–Methanol (100:0→95:5). Product was isolated as a white solid (0.11 g, 37%).

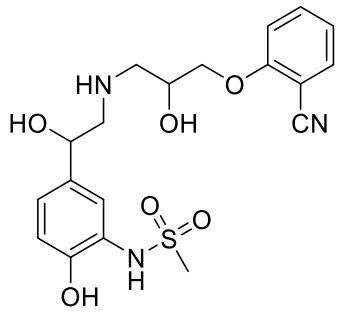
^{1}H NMR: (400 MHz, Methanol-d_4) δ 7.65 (dd, *J* = 6.8, 1.8 Hz, ArH, 2H), 7.51 (dd, *J* = 6.8, 1.8 Hz, ArH, 3H), 7.44 – 7.32 (m, ArH, 3H), 7.27 – 7.19 (m, ArH, 2H), 7.15 – 7.08 (m, ArH, 2H), 5.23 (s, Bn$\underline{CH_2}$, 2H), 4.84 (m, Bn$\underline{CH}$, 1H), 4.30 – 4.12 (m, O-$\underline{CH_2}$-$\underline{CH}$OH, 3H), 3.27 – 2.95 (m, $\underline{CH_2}$-NH-$\underline{CH_2}$, 4H), 2.90 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, MeOD) δ 160.3, 150.7, 136.6, 135.0, 134.6, 133.3, 128.2, 127.8, 127.5, 126.1, 123.9, 122.7, 121.1, 116.0, 112.6, 112.6, 101.3, 70.9, 70.0, 69.6, 66.7, 55.3, 50.7, 38.6.

LC-MS: calculated for $C_{26}H_{30}N_3O_6S$ $[M+H]^+$ 512.2. found: 512.2. R_t: 2.21 min (254 nm)

Analytical HPLC: R_t 3.17 min (method b, 254 nm, 90%)

***N*-(5-(2-((3-(2-Cyanophenoxy)-2-hydroxypropyl)amino)-1-hydroxyethyl)-2-hydroxyphenyl)methanesulfonamide (5.29)**



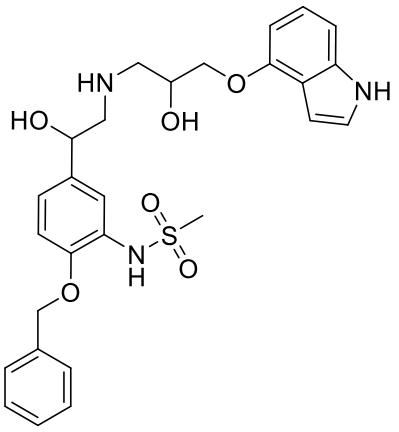
5.20 (0.05 g, 0.10 mmol) was dissolved in Methanol (3 mL) and water (0.5 mL). Ammonium formate (0.06 g, 0.98 mmol) and Pd/C (0.01 g) were added. The mixture was heated ay 70 °C for 5h. Pd/C was filtered under vacuum, the filtrate was concentrated *in vacuo* and then purified by CC, eluting with DCM–Methanol (100:0→80:20), followed by HPLC eluting with water-MeCN and 0.01% TFA (5:95:0.01 → 95:5:0.01 over 14 minutes, eluted at 7.1 minutes). White solid product (0.006 g, 12%).

^{1}H NMR: (400 MHz, Methanol-d_4) δ 7.69 – 7.65 (m, ArH, 2H), 7.45 (d, *J* = 2.1 Hz, ArH, 1H), 7.26 – 7.11 (m, ArH, 3H), 6.94 (d, *J* = 8.3 Hz, ArH, 1H), 4.96 (ddd, *J* = 10.2, 5.1, 3.4 Hz, BnCH, 1H), 4.39 (m, CH_2-CHOH-CH_2 1H), 4.30 – 4.14 (m, O-CH_2 2H), 3.47 – 3.44 (m, NH-CH_2, 1H), 3.37-3.26 (m, NH-CH_2, 2H) 3.22 – 3.18 (m, NH-CH_2, 1H), 2.96 (s, SCH_3, 3H).

^{13}C NMR: (101 MHz, MeOD) δ 160.1, 150.3, 134.7, 133.3, 132.2, 124.7, 124.1, 123.1, 121.4, 115.9, 115.2, 112.6, 101.4, 70.6, 68.1, 64.7, 53.8, 49.6, 38.2.

LC-MS: calculated for $C_{19}H_{24}N_3O_6S$ $[M+H]^+$ 422.1. found: 422. R_t: 2.17 min (254 nm)

***N*-(5-(2-((3-((1*H*-Indol-4-yl)oxy)-2-hydroxypropyl)amino)-1-hydroxyethyl)-2-(benzyloxy)phenyl)methanesulfonamide (5.26)**



5.10 (0.25 g, 0.74 mmol) was dissolved in ethanol (10 mL) along with **5.25** (0.070 g, 0.37 mmol) and DIPEA (0.096 g, 0.74 mmol). The mixture was stirred at 80 °C overnight and then volatiles were removed under reduced pressure. The product was purified by COLUMN CHROMATOGRAPHY eluting with DCM–Methanol (100:0→85:15). Product was isolated as a white solid (0.026 g, 13%).

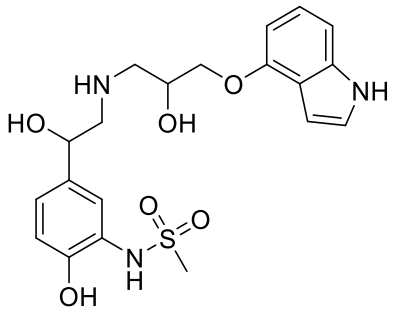
^{1}H NMR: (400 mhz, Methanol-d_4) δ 7.36 (m, ArH, 3H), 7.27 (m, ArH, 2H), 7.24 – 7.20 (m, ArH, 1H), 7.11 (dt, *J* = 8.5, 1.8 Hz, ArH, 1H), 7.01 (d, *J* = 3.2 Hz, ArH, 1H), 6.98 (d, *J* = 8.6 Hz, ArH, 1H), 6.94 – 6.87 (m, ArH, 2H), 6.45 (dt, *J* = 3.2, 0.9 Hz, ArH, 1H), 6.39 (dd, *J* = 6.7, 1.7 Hz, ArH, 1H), 5.07 (s, Bn-CH_2, 2H), 4.80 – 4.77 (m, BnCH, 1H), 4.16 (m, CH_2-CHOH-CH_2, 1H), 4.05-3.94 (m, O-CH_2-CH, 2H), 3.12 (dd, *J* = 12.7, 3.7 Hz, CH_2-NH-CH_2, 1H), 3.03 – 2.91 (m, CH_2-NH-CH_2, 3H), 2.75 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, MeOD) δ 151.9, 150.7, 137.8, 136.6, 134.7, 128.3, 127.8, 127.5, 126.1, 124.0, 122.8, 122.7, 121.5, 118.7, 112.7, 104.9, 99.8, 98.2, 70.3, 70.0, 69.6, 66.8, 55.0, 51.0, 38.6.

LC-MS: calculated for $C_{27}H_{32}N_3O_6S$ $[M+H]^+$ 526.2. found: 526.2

Analytical HPLC: R_t 3.82 min (method a, 254 nm)

***N*-(5-(2-((3-((1*H*-Indol-4-yl)oxy)-2-hydroxypropyl)amino)-1-hydroxyethyl)-2-hydroxyphenyl)methanesulfonamide (5.28)**



5.26 (0.018 g, 0.034 mmol) was dissolved in ethyl acetate (1.4 mL), water (0.4 mL) and acetic acid (0.2 mL). Pd/C (0.002 g) was added and the mixture was stirred under a hydrogen atmosphere at rt overnight. Pd/C was filtered off and volatiles removed *in vacuo*. The residue was purified by COLUMN CHROMATOGRAPHY eluting with DCM–Methanol (100:0→80:20). The product was isolated as a white solid (0.004 g, 27%).

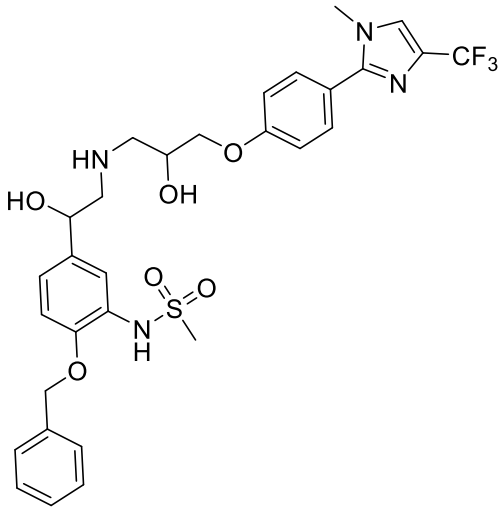
^{1}H NMR: (400 MHz, Methanol-d_4) δ 7.43 (d, *J* = 2.2 Hz, ArH, 1H), 7.15 (m, ArH, 2H), 7.03 (m, ArH, 2H), 6.91 (d, *J* = 8.3 Hz, ArH, 1H), 6.57 (d, *J* = 3.1 Hz, ArH, 1H), 6.53 (dd, *J* = 6.6, 1.8 Hz, ArH, 1H), 4.91 – 4.88 (m, BnCH, 1H (partially under water peak)), 4.33 (q, *J* = 5.2 Hz, CH_2-$\underline{CH}$OH-CH_2, 1H), 4.23 – 4.09 (m, O-$\underline{CH_2}$-CHOH, 2H), 3.29 (m, CH_2-NH-$\underline{CH_2}$, 1H), 3.20 – 3.05 (m, $\underline{CH_2}$-NH-$\underline{CH_2}$, 3H), 2.94 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, MeOD) δ 161.4, 159.3, 146.8, 144.5, 133.6, 133.5, 133.4, 132.9, 131.2, 127.8, 124.7, 114.3, 109.3, 107.9, 79.8, 77.5, 77.1, 61.7, 61.4, 38.5.

HRMS: calculated for $C_{20}H_{26}N_3O_6S$ $[M+H]^+$ 436.1537. found: 436.1547

LC-MS: R_t 1.06 min (254 nm)

***N*-(2-(Benzyloxy)-5-(1-hydroxy-2-((2-hydroxy-3-(4-(1-methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl)phenoxy)propyl)amino)ethyl)phenyl)methanesulfonamide (5.30)**



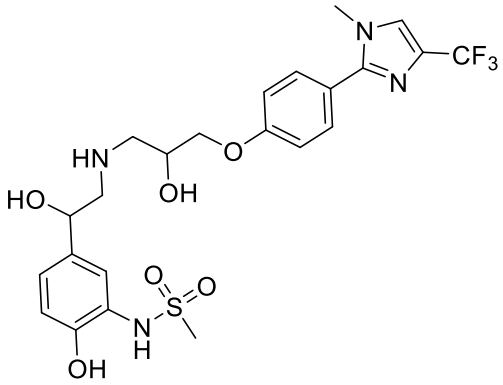
5.10 (0.5 g, 1.34 mmol) was dissolved in ethanol (10 mL) along with **4.9** (0.20 g, 0.67 mmol) and DIPEA (0.17 g, 1.34 mmol). The mixture was stirred at 80 °C for 16h and then volatiles were removed under reduced pressure. The product was purified by COLUMN CHROMATOGRAPHY eluting with DCM–Methanol (100:0→85:15). Product was isolated as a clear yellow oil (0.22 g, 49%).

^{1}H NMR: (400 mhz, Methanol-d_4) δ 7.55 (d, *J* = 1.5 Hz, ArH, 1H), 7.50 – 7.45 (m, ArH, 2H), 7.39-7.35 (m, ArH, 2H), 7.30 – 7.20 (m, ArH, 4H), 7.11 (dd, *J* = 8.5, 2.2 Hz, ArH, 1H), 7.03 – 6.97 (m, 3H), 5.10 (s, Bn$\underline{CH_2}$, 2H), 4.73-4.68 (s, Bn$\underline{CH}$, 1H), 4.08 (d, *J* = 8.6 Hz, O-CH_2-$\underline{CH}$OH, 1H), 3.99 – 3.94 (m, O-$\underline{CH_2}$-CHOH, 2H), 3.65 (s, N-$\underline{CH_3}$, 3H), 2.99 – 2.80 (m, $\underline{CH_2}$-NH-$\underline{CH_2}$, 4H), 2.78 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 159.5, 151.5, 148.5, 137.2, 135.1, 132.7, 130.5, 128.8, 128.3, 128.1, 126.2, 124.5, 123.9, 122.4, 117.7, 115.1, 113.4, 70.6, 70.2, 53.5, 53.5, 49.0, 41.8, 35.2, 21.5 (1 aromatic carbon not observed). CF_3 not observed.

LC-MS: calculated for $C_{30}H_{34}F_3N_4O_6S$ $[M+H]^+$ 635.2. found: 635.2. R_t: 1.97 min (254 nm)

***N*-(2-Hydroxy-5-(1-hydroxy-2-((2-hydroxy-3-(4-(1-methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl)phenoxy)propyl)amino)ethyl)phenyl)methanesulfonamide (5.31)**



5.30 (0.075 g, 0.12 mmol) was dissolved in Methanol (3 mL) and water (0.5 mL). Ammonium formate (0.075 g, 1.18 mmol) and Pd/C (0.01 g) were added. The mixture was heated ay 70 °C for 4h. Pd/C was filtered off and the filtrate was concentrated under reduced pressure. The residue was purified by HPLC eluting with water-MeCN and 0.01% TFA (5:95:0.01 → 95:5:0.01 over 14 minutes, eluted at 8.3 minutes), to give white solid product (0.009 g, 14%).

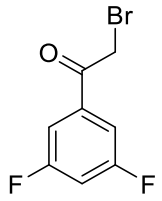
^{1}H NMR: (400 MHz, Methanol-d_4) δ 7.68 (s, ArH, 1H), 7.60 (d, *J* = 8.3 Hz, ArH, 2H), 7.44 (d, *J* = 2.2 Hz, ArH, 1H), 7.18-7.12 (m, ArH, 3H), 6.92 (d, *J* = 8.3 Hz, ArH, 1H), 4.97-4.92 (m, Bn<u>CH</u>, 1H), 4.39-4.29 (m, O-CH_2-<u>CH</u>OH, 1H), 4.18-4.08 (m, O-<u>CH_2</u>-CHOH, 2H), 3.76 (s, N-<u>CH_3</u>, 3H), 3.42 (ddd, *J* = 12.9, 9.6, 3.2 Hz, CH_2-NH-<u>CH_2</u> 1H), 3.30 – 3.16 (m, <u>CH_2</u>-NH-<u>CH_2</u> 3H), 2.94 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, MeOD) δ 159.7, 150.3, 149.2, 132.2, 130.3, 130.0 (q, CF_3), 129.8, 129.4, 129.0, 124.7, 124.1, 123.1, 122.5, 121.5, 120.45, 115.2, 114.5, 70.0, 68.0, 65.0, 53.8, 49.8, 38.2, 33.8.

HRMS: calculated for $C_{23}H_{28}F_3N_4O_6S$ $[M+H]^+$ 545.1680. found: 545.1680

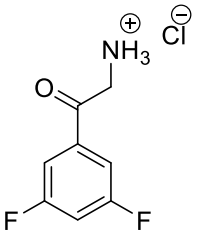
LC-MS: R_t 2.03 min (254 nm)

2-Bromo-1-(3,5-difluorophenyl)ethan-1-one (5.34)



1-(3,5-difluorophenyl)ethan-1-one (1.0 g, 6.40 mmol) was dissolved in THF (50 mL) and phenyltrimethylammonium tribromide (2.65 g, 7.05 mmol) was added. The mixture was heated at 50 °C overnight. Precipitate was filtered and filtrate was concentrated *in vacuo* to give an impure oil which was carried forward without purification.

2-Amino-1-(3,5-difluorophenyl)ethan-1-one (5.35)



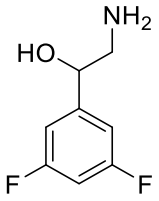
5.34 (1.35 g, 5.77 mmol) was dissolved in $CHCl_3$ (50 mL), hexamine (0.89 g, 6.35 mmol) was added and the solution was stirred at ambient temperature for 3 hours. Precipitate formed and was filtered; the precipitate was then redissolved in methanol (40 mL) and conc.hydrochloric acid (20 mL) and stirred overnight at 60 °C. Volatiles were removed *in vacuo*, to afford a white solid, a small amount of diethyl ether was added and the solid was filtered under vacuum and collected to afford the product as a white solid (0.83 g, 70% over 2 steps).

^{1}H NMR: (400 mhz, DMSO-d_6) δ 8.58 (s, NH_3, 3H), 7.82 – 7.65 (m, ArH, 3H), 4.60 (q, J = 5.5 Hz, CH_2, 2H).

^{13}C NMR: (101 MHz, DMSO) δ 191.7, 164.1, 161.6, 139.3, 137.1, 111.9, 110.2, 45.5. CF_3 not observed.

LC-MS: calculated for $C_8H_8F_2NO$ $[M+H]^+$ 172.0. found: 172.0

2-Amino-1-(3,5-difluorophenyl)ethan-1-ol (5.36)

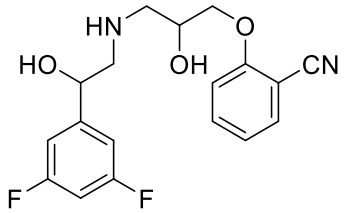


5.35 (0.83 g, 4.0 mmol) was dissolved in Methanol (10 mL) and cooled to 0 °C. Sodium borohydride (0.55 g, 14.5 mmol) was added portion wise to the cooled solution. The mixture was stirred for 30 minutes at 0 °C then allowed to warm to room temperature and stir for a further 30 minutes. The solution was cooled in an ice bath and then acidified with 1Nhydrochloric acid. The solution was allowed to stir for 10 minutes and then the solvent was removed *in vacuo* to give a crude solid. Water was added and the mixture was extracted with EA. The organic later was concentrated under reduced pressure to give the pure product as a white solid (0.15 g, 21%).

^{1}H NMR: (400 mhz, Chloroform-*d*) δ 6.79 (s, ArH, 2H), 6.62 (s, ArH, 1H), 4.53 (s, CH, 1H), 2.93 (s, CH_2, 1H), 2.68 (s, CH_2, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 171.1, 146.8, 132.1, 108.7, 72.8, 48.7. CF_3 not observed.

2-(3-((2-(3,5-Difluorophenyl)-2-hydroxyethyl)amino)-2-hydroxypropyl)benzonitrile (5.38)



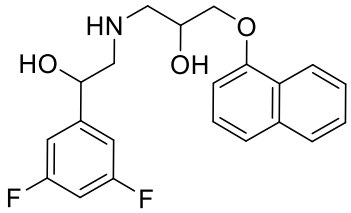
5.36 (0.05 g, 0.29 mmol) and **4.19** (0.025 g, 0.14 mmol) were dissolved in Ethanol (2 mL) along with DIPEA (0.028 g, 0.216 mmol) and heated at 80 °C overnight. Volatiles were removed *in vacuo* and the residue was purified by column chromatography, eluting with DCM-Methanol-TEA (99:1:0.1→85:15:0.1) to afford the product as a white solid (0.011 g, 22%).

^{1}H NMR: (400 mhz, Methanol-d_4) δ 7.68 – 7.60 (m, ArH, 2H), 7.24 – 7.17 (m, ArH, 1H), 7.10 (td, *J* = 7.6, 0.9 Hz, ArH, 1H), 7.06 – 6.97 (m, ArH, 2H), 6.83 (tt, *J* = 9.1, 2.4 Hz, ArH, 1H), 4.85 – 4.79 (m, Ar-CH, 1H), 4.20 – 4.10 (m, O-CH_2-CH, 3H), 3.03 – 2.75 (m, CH_2-NH-CH_2, 4H).

^{13}C NMR: (101 MHz, MeOD) δ 164.2, 164.1, 161.8, 160.5, 148.2, 134.5, 133.3, 120.9, 116.0, 112.5, 108.3 (q C-F), 102.0 (t, C-F), 101.3, 71.1, 70.7, 67.8, 56.2, 51.1.

LC-MS: calculated for $C_{18}H_{19}F_2N_2O_3$ $[M+H]^+$ 349.1. found: 348.9. R_t 4.60 min (254 nm)

1-((2-(3,5-Difluorophenyl)-2-hydroxyethyl)amino)-3-(naphthalen-1-yloxy)propan-2-ol (5.37)



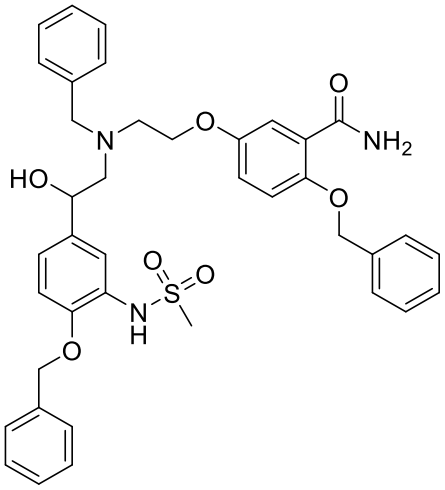
5.36 (0.041 g, 0.24 mmol) and **4.17** (0.024 g, 0.18 mmol) were dissolved in Ethanol (2 mL) along with DIPEA (0.028 g, 0.216 mmol) and heated at 80 °C overnight. Volatiles were removed *in vacuo* and the residue was purified by column chromatography, eluting with DCM-Methanol-TEA (99:1:0.1→80:20:0.1) to afford the product as a white solid (0.014 g, 32%).

^{1}H NMR: (400 mhz, Methanol-d_4) δ 8.35 – 8.26 (m, ArH, 1H), 7.84 – 7.77 (m, ArH, 1H), 7.53 – 7.33 (m, ArH, 4H), 7.06 – 6.97 (m, ArH, 2H), 6.92 (dt, *J* = 7.5, 1.0 Hz, ArH, 1H), 6.82 (tt, *J* = 9.1, 2.4 Hz, ArH, 1H), 4.87 – 4.80 (m, Ar-CH, 1H), 4.31 – 4.22 (m, O-CH_2-CH, 1H), 4.18 (dd, *J* = 5.8, 1.6 Hz, O-CH_2 2H), 3.08 – 2.75 (m, CH_2-NH-CH_2, 4H).

^{13}C NMR: (101 MHz, MeOD) δ 164.4, 164.2, 161.8, 161.7, 154.4, 148.3, 134.6, 127.0, 125.9, 125.5, 124.7, 121.6, 120.0, 108.4 (q C-F), 104.5, 101.8 (t, C-F), 70.5, 68.7, 68.3, 56.2, 51.6.

LC-MS: calculated for $C_{21}H_{22}F_2NO_3$ $[M+H]^+$ 373.2. found: 373.8. R_t 5.0 min (254 nm)

5-(2-(Benzyl(2-(4-(benzyloxy)-3-(methylsulfonamido)phenyl)-2-hydroxyethyl)amino)ethoxy)-2-(benzyloxy)benzamide (5.45)



5.21 (0.017 g, 0.038 mmol) and **5.44** (0.02 g, 0.047 mmol) were dissolved in DMF (1 mL) along with potassium carbonate (0.01 g, 0.070 mmol) and heated at 40 °C overnight. Water was added and the mixture was extracted with EA. The organic layer was concentrated *in vacuo* and the resulting residue was purified by HPLC eluting with water-MeCN and 0.01% TFA (30:70:0.01 → 95:5:0.01 over 10 minutes, eluted at 7.1 minutes), to give white solid product (0.004 g, 15%).

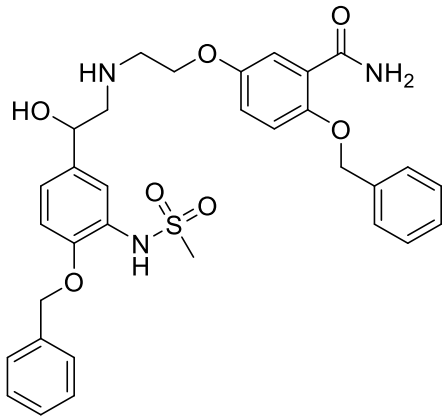
^{1}H NMR: (400 mhz, Methanol-d_4) δ 7.39 – 7.21 (m, ArH, NH_2, 20H), 7.10 – 7.02 (m, ArH, 2H), 6.87 (dd, *J* = 9.0, 3.2 Hz, ArH, 1H), 5.11 (s, Bn-CH_2-O, 2H), 5.02 (s, Bn-CH_2-O, 2H), 4.85 – 4.78 (m, CH, 1H), 4.12 (s, Bn-CH_2-N, 2H), 3.98 – 3.80 (m, CH_2-CH_2, 4H), 3.05 – 2.89 (m, N-CH_2, 2H), 2.81 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, MeOD) δ 167.9, 155.4, 152.5, 151.4, 136.3, 136.2, 133.6, 131.0, 130.9, 129.6, 129.3, 128.9, 128.4, 128.3, 128.1, 128.0, 127.7, 127.6, 127.5, 127.2, 122.1, 118.9, 116.9, 114.7, 113.2, 71.3, 70.3, 67.8, 67.2, 53.0, 50.8, 48.9, 38.6.

HRMS: calculated for $C_{39}H_{42}N_3O_7S$ $[M+H]^+$ 696.2738. found: 696.2747

LC-MS: R_t 3.06 min (254 nm)

2-(Benzyloxy)-5-(2-((2-(4-(benzyloxy)-3-(methylsulfonamido)phenyl)-2-hydroxyethyl)amino)ethoxy)benzamide (5.46)



5.10 (0.05 g, 0.15 mmol) and **5.44** (0.033 g, 0.074 mmol) were dissolved in DMF (1 mL) along with DIPEA (0.014 g, 0.11 mmol) and heated at 40 °C overnight. The solution was concentrated *in vacuo* and the resulting residue was purified by HPLC eluting with water-MeCN and 0.01% TFA (30:70:0.01 → 95:5:0.01 over 10 minutes, eluted at 5.6 minutes), to give white solid product (0.009 g, 24%).

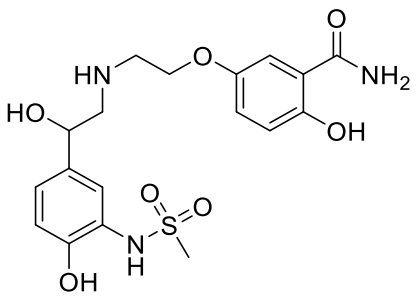
^{1}H NMR: (400 mhz, Methanol-d_4) δ 7.66 (d, *J* = 3.1 Hz, ArH, 1H), 7.53 – 7.47 (m, ArH, 5H), 7.45 – 7.32 (m, ArH, 6H), 7.29 – 7.14 (m, ArH, 4H), 5.27 (s, Bn-CH_2, 2H), 5.25 (s, Bn-CH_2, 2H), 4.98 (dd, *J* = 10.2, 3.3 Hz, CH, 1H), 4.35 – 4.29 (m, O-CH_2, 2H), 3.55 (t, *J* = 5.0 Hz, N-CH_2, 2H), 3.32 – 3.29 (m, N-$\underline{CH_2}$-CH, 1H), 3.26 – 3.18 (m, N-$\underline{CH_2}$-CH, 1H), 2.91 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, MeOD) δ 167.8, 152.1, 152.0, 151.0, 136.5, 136.2, 133.6, 128.4, 128.3, 128.1, 127.9, 127.6, 127.5, 126.3, 123.9, 122.8, 122.2, 119.6, 116.4, 114.9, 112.8, 71.3, 70.3, 67.9, 63.5, 53.6, 46.5, 38.6.

HRMS: calculated for $C_{32}H_{36}N_3O_7S$ $[M+H]^+$ 606.2268. found: 606.2277

Analytical HPLC: R_t 3.30 min (method b, 254 nm)

2-Hydroxy-5-(2-((2-hydroxy-2-(4-hydroxy-3-(methylsulfonamido)phenyl)ethyl)amino)ethoxy)benzamide (5.47)



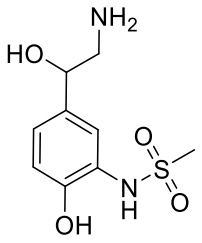
5.46 (0.011 g, 0.018 mmol) was dissolved in THF (1.4 mL), water (0.4 mL) and acetic acid (0.2 mL). Pd/C (0.002 g) was added and the mixture was stirred under a hydrogen atmosphere at rt overnight. Pd/C was filtered off and volatiles removed *in vacuo*. The residue was purified by COLUMN CHROMATOGRAPHY eluting with DCM–Methanol (100:0→70:30). The product was isolated as a white solid (0.004 g, 52%).

^{1}H NMR: (400 mhz, Methanol-d_4) δ 7.30 (d, *J* = 2.6 Hz, ArH, 2H), 7.00 (ddd, *J* = 18.2, 8.6, 2.6 Hz, ArH, 2H), 6.77 (dd, *J* = 13.9, 8.6 Hz, ArH, 2H), 4.05 (t, *J* = 5.1 Hz, O-CH_2, 2H), 3.12 (t, *J* = 5.1 Hz, O-CH_2-$\underline{CH_2}$, 2H), 2.91 (d, *J* = 6.6 Hz, N-CH_2 2H), 2.83 (s, SO_2CH_3 3H). BnCH not observed, peak undernateth water peak (COSY 2D NMR).

HRMS: calculated for $C_{18}H_{24}N_3O_7S$ $[M+H]^+$ 426.1329. found: 426.1343

Analytical HPLC: R_t 3.04 min (method a, 254 nm)

***N*-(5-(2-Amino-1-hydroxyethyl)-2-hydroxyphenyl)methanesulfonamide (5.49)** [188]



5.10 (1.0 g, 2.69 mmol) was dissolved in Methanol (7 mL), water (0.2 mL) and acetic acid (1 mL). Pd/C (0.1 g) was added and the mixture was stirred under a hydrogen

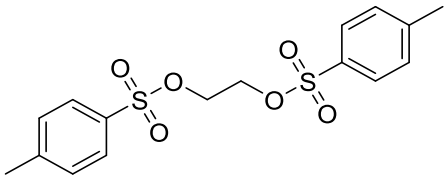
atmosphere at rt overnight. Pd/C was filtered and volatiles removed *in vacuo* to give a white solid product (0.65 g, 98%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.21 (d, *J* = 2.1 Hz, ArH, 1H), 7.05 (dd, *J* = 8.3, 2.1 Hz, ArH, 1H), 6.95 (d, *J* = 8.3 Hz, ArH, 1H), 4.71 (dd, *J* = 9.7, 3.1 Hz, BnCH, 1H), 2.94 (s, SO_2CH_3, 3H), 2.93-2.88 (m, NH-CH_2, 1H) 2.76 (dd, *J* = 12.7, 9.7 Hz, NH-CH_2, 1H).

^{13}C NMR: (101 MHz, DMSO) δ 150.8, 133.1, 124.8, 124.5, 124.3, 116.2, 69.2, 49.0, 46.4.

LC-MS: calculated for $C_9H_{15}N_2O_4S$ $[M+H]^+$ 247.1. found: 247.3

Ethane-1,2-diyl bis(4-methylbenzenesulfonate) (5.43)



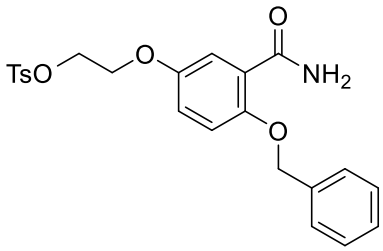
Ethylene glycol (2 g, 32.2 mmol) and tosyl chloride (12.3 g, 64.4 mmol) were dissolved in DCM (50 mL) and cooled to 0 °C, TEA (6.52 g, 64.4 mmol) was added dropwise and the mixture was stirred at room temperature overnight. Volatiles were removed *in vacuo* and the solid was recrystallized from ethanol to give pure product (4.8 g, 40%)

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.72 (m, ArH, 4H), 7.47 (d, *J* = 8.1 Hz, ArH, 4H), 4.19 (s, CH_2 ×2, 4H), 2.43 (s, CH_3 ×2, 6H).

^{13}C NMR: (101 MHz, DMSO) δ 145.6, 132.3, 130.6, 128.0, 68.3, 21.5.

LC-MS: calculated for $C_{16}H_{19}O_6S_2$ $[M+H]^+$ 371.0. found: 370.7

2-(4-(Benzyloxy)-3-carbamoylphenoxy)ethyl 4-methylbenzenesulfonate (5.44)



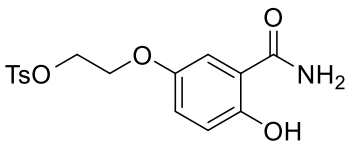
5.43 (2.8 g, 7.56 mmol) and **4.29** (0.92 g, 3.78 mmol) were dissolved in DMF (30 mL) along with caesium carbonate and stirred at ambient temperature for 16 hours. DMF was removed under reduced pressure. Water was added to the residue and extracted with EA. The organic layer was concentrated *in vacuo* and the resulting solid was purified by column chromatography eluting with DCM–Methanol (100:0→95:5) to afford the pure product as a white solid (0.85 g, 52%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.84 – 7.76 (m, ArH, 2H), 7.62 (d, *J* = 23.3 Hz, NH_2, 2H), 7.51 – 7.45 (m, ArH, 4H), 7.44 – 7.31 (m, ArH, 3H), 7.24 (d, *J* = 3.3 Hz, ArH, 1H), 7.13 (d, *J* = 9.0 Hz, ArH, 1H), 6.95 (dd, *J* = 9.0, 3.3 Hz, ArH, 1H), 5.20 (s, $BnCH_2$, 2H), 4.36 – 4.27 (m, O-CH_2, 2H), 4.16 – 4.07 (m, TsO-CH_2, 2H), 2.42 (s, CH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 166.3, 152.0, 151.0, 145.5, 137.1, 132.7, 130.6, 129.0, 128.5, 128.2, 128.1, 124.5, 118.8, 116.5, 115.4, 71.0, 69.6, 66.3, 21.5.

LC-MS: calculated for $C_{23}H_{24}NO_6S$ $[M+H]^+$ 442.1. found: 442.3

2-(3-Carbamoyl-4-hydroxyphenoxy)ethyl 4-methylbenzenesulfonate (5.50)



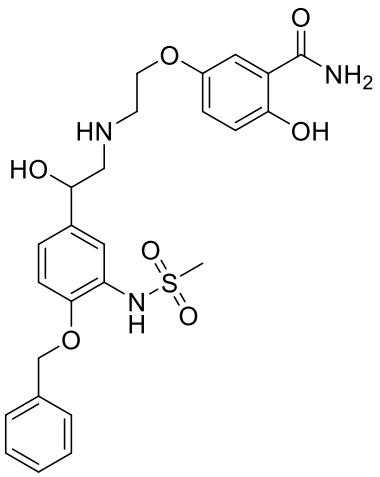
5.44 (0.2 g, 0.45 mmol) was dissolved in ethyl acetate (5 mL). Pd/C (0.1 g) was added and the mixture was stirred under a hydrogen atmosphere at rt overnight. Pd/C was filtered and volatiles removed *in vacuo* to give a white solid product (0.15 g, 95%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 8.34 (s, $CONH_2$, 1H), 7.86 (s, $CONH_2$, 1H), 7.79 (d, *J* = 8.2 Hz, TsArH, 2H), 7.46 (dd, *J* = 8.5, 2.4 Hz, TsArH, 2H), 7.35 (d, *J* = 3.0 Hz, ArH, 1H), 6.93 (dd, *J* = 9.0, 3.0 Hz, ArH, 1H), 6.78 (d, *J* = 9.0 Hz, ArH, 1H), 4.34 – 4.26 (m, CH_2, 2H), 4.14 – 4.05 (m, CH_2, 2H), 2.42 (s, CH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 172.1, 155.8, 150.0, 145.4, 132.6, 130.6, 128.1, 122.3, 118.6, 114.5, 113.0, 69.5, 66.4, 21.5.

LC-MS: calculated for $C_{16}H_{18}NO_6S$ $[M+H]^+$ 352.1. found: 352.2

5-(2-((2-(4-(Benzyloxy)-3-(methylsulfonamido)phenyl)-2-hydroxyethyl)amino)ethoxy)-2-hydroxybenzamide (5.51)



5.50 (0.05 g, 0.15 mmol) and **5.10** (0.10 g, 0.30 mmol) were dissolved in DMF (4mL) along with DIPEA (0.058 g, 0.45 mmol). The mixture was heated ay 100 °C under microwave irradiation for 30 minutes. The mixture was concentrated under reduced pressure and the residue was purified by HPLC eluting with water-MeCN and 0.01% TFA (5:95:0.01 → 95:5:0.01 over 12 minutes, eluted at 8.0 minutes), White solid product (0.010 g, 13%).

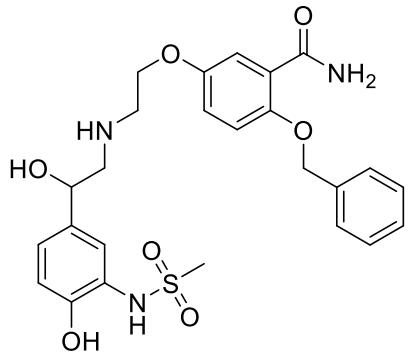
^{1}H NMR: (400 MHz, Methanol-d_4) δ 7.53 – 7.42 (m, ArH, 4H), 7.42 – 7.29 (m, ArH, 3H), 7.25 (dd, *J* = 8.5, 2.1 Hz, ArH, 1H), 7.18 – 7.08 (m, ArH, 2H), 6.89 (d, *J* = 9.0 Hz, ArH, 1H), 5.22 (s, $BnCH_2$, 2H), 4.96 (dd, *J* = 10.2, 3.2 Hz, bnCH, 1H), 4.28 (t, *J* = 5.0 Hz, O-CH_2, 2H), 3.52 (t, *J* = 5.0 Hz, NH-$\underline{CH_2}$-CH_2, 2H), 3.36-3.28 (m, NH-$\underline{CH_2}$, 1H), 3.25-3.17 (m, NH-$\underline{CH_2}$, 1H), 2.89 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, MeOD) δ 172.5, 156.0, 152.4, 151.8, 137.9, 135.0, 129.7, 129.3, 128.9, 127.7, 125.3, 124.2, 123.3, 119.4, 116.8, 114.4, 114.2, 71.7, 69.4, 65.1, 55.0, 47.9, 40.0.

HRMS: calculated for $C_{25}H_{30}N_3O_7S$ $[M+H]^+$ 516.1799. found: 516.1818

LC-MS: R_t: 2.12 min (254 nm)

2-(Benzyloxy)-5-(2-((2-hydroxy-2-(4-hydroxy-3-(methylsulfonamido)phenyl)ethyl)amino)ethoxy)benzamide (5.52)



5.44 (0.1 g, 0.23 mmol) and **5.49** (0.11 g, 0.45 mmol) were dissolved in DMF (3mL) along with DIPEA (0.058 g, 0.45 mmol). The mixture was heated ay 100 °C under microwave irradiation for 30 minutes. The mixture was concentrated under reduced pressure and the residue was purified by HPLC eluting with water-MeCN and 0.01% TFA, (5:95:0.01 → 95:5:0.01 over 13 minutes, eluted at 9.0 minutes), White solid product (0.011 g, 10%).

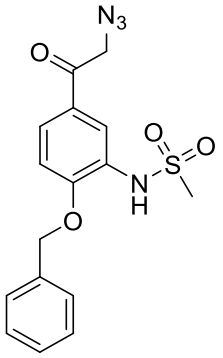
^{1}H NMR: (400 MHz, Methanol-d_4) δ 7.64 (d, *J* = 3.0 Hz, ArH, 1H), 7.51 – 7.45 (m, ArH, 2H), 7.44 – 7.32 (m, ArH, 4H), 7.24 – 7.12 (m, ArH, 3H), 6.92 (d, *J* = 8.3 Hz, ArH, 1H), 5.25 (s, $BnCH_2$, 2H), 4.93 (dd, *J* = 10.2, 3.3 Hz, BnCH, 1H), 4.30 (t, $O\text{-}CH_2$, *J* = 5.0 Hz, 2H), 3.53 (t, *J* = 5.0 Hz, NH-$\underline{CH_2}$-CH_2, 2H), 3.32 – 3.28 (m, NH-$\underline{CH_2}$, 1H), 3.24 – 3.17 (m, NH-$\underline{CH_2}$, 1H), 2.94 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, MeOD) δ 169.0, 153.2, 151.5, 148.7, 137.4, 133.3, 129.6, 129.2, 128.8, 125.9, 125.3, 124.3, 123.4, 120.8, 117.6, 116.4, 116.1, 72.5, 69.3, 64.7, 54.9, 47.7, 39.4.

HRMS: calculated for $C_{25}H_{30}N_3O_7S$ $[M+H]^+$ 516.1799. found: 516.1816

LC-MS: R_t: 2.09 min (254 nm)

N-(5-(2-Azidoacetyl)-2-(benzyloxy)phenyl)methanesulfonamide (5.54) [187]



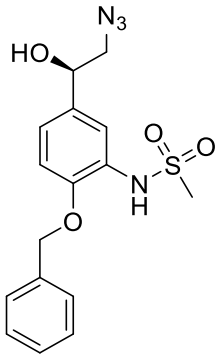
5.13 (0.5 g, 1.26 mmol) was dissolved in DMF (5 mL) along with sodium azide (0.09 g, 1.38 mmol). The mixture was stirred at ambient temperatire for 16 hours. Water was added and the mixture was extracted with EA. The organic layer was washed with water, then concentrated in vacuo to give pure product (0.34 g, 76%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.85 – 7.78 (m, ArH, 2H), 7.59 – 7.53 (m, ArH, 2H), 7.46 – 7.32 (m, ArH, 3H), 7.28 (d, *J* = 8.6 Hz, ArH, 1H), 5.31 (s, Bn-CH_2, 2H), 4.83 (s, N_3-CH_2, 2H), 2.96 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, DMSO) δ 193.1, 156.5, 136.5, 128.9, 128.5, 128.3, 128.1, 127.6, 126.9, 125.7, 113.3, 70.6, 54.8, 41.0.

LC-MS: calculated for $C_{16}H_{18}N_4O_4S$ $[M+H]^+$ 361.1. found: 361.3

(*R*)-*N*-(5-(2-Azido-1-hydroxyethyl)-2-(benzyloxy)phenyl)methanesulfonamide (5.59) [188]



(*S*)-CBS catalyst (0.046 g, 0.17 mmol) was dissolved in unstabilised THF (5 mL) along with borane-*N,N*-diethylaniline at 0 °C. The mixture was stirred for 10 minutes and then **5.54** (0.2 g, 0.55 mmol), dissolved in THF (2 mL) was added). The mixture was stirred at room temperature overnight. Water was added and stirred for 10 minutes followed by the removal of volatiles *in vacuo*. Column chromatography was used to isolate the product, eluting with DCM–Methanol (100:0→90:10) to afford the pure product (0.17 g, 85%).

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.56 (d, *J* = 2.1 Hz, ArH, 1H), 7.47 – 7.35 (m, ArH, 5H), 7.19 (dd, *J* = 8.4, 2.2 Hz, ArH, 1H), 7.03 (d, *J* = 8.4 Hz, ArH, 1H), 6.84 (s, OH, 1H), 5.14 (s, $BnCH_2$, 2H), 4.89 – 4.82 (m, CH, 1H), 3.50 – 3.46 (m, N_3-CH_2, 2H), 2.95 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 148.4, 135.5, 134.1, 128.9, 128.7, 127.8, 126.5, 123.0, 118.7, 112.4, 72.7, 71.2, 57.9, 39.3.

ee determination: (*S*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.002 g, 0.008 mmol) was added to **5.59** (0.002 g, 005 mmol) along with DMAP (0.001 g, 0.005 mmol) in an NMR tube with DMSO (0.75 mL).

The above procedure was repeated with N-(5-(2-azido-1-hydroxyethyl)-2-(benzyloxy)phenyl)methanesulfonamide (racemic mixture):

Both compounds, along with (*S*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride were analysed by ^{19}F NMR (all in deuterated DMSO).

(*R*)-*N*-(5-(2-Azido-1-hydroxyethyl)-2-(benzyloxy)phenyl)methanesulfonamide:

^{19}F NMR (376 MHz, DMSO-d_6) δ -69.2 (s, inergration = 1), -70.5 (s, residual mosher acid chloride), -71.3 (s, integration = 0.35).

N-(5-(2-azido-1-hydroxyethyl)-2-(benzyloxy)phenyl)methanesulfonamide (racemic mixture):

^{19}F NMR (376 MHz, DMSO-d_6) δ -69.2 (s, inergration = 1), -70.5 (s, residual mosher acid chloride), -71.3 (s, integration = 1).

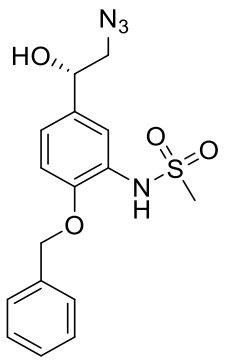
(*S*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride:

^{19}F NMR (376 MHz, DMSO-d_6) δ -70.5 (s, mosher acid chloride).

The integration of the two compounds in the racemic mixture have an integration of 1:1 therefore 0% ee. The integration of the two compounds in the (*R*)-enantiomer is 1:0.35. Further determination is required.

ee: 48% (further determination required)

(*S*)-*N*-(5-(2-Azido-1-hydroxyethyl)-2-(benzyloxy)phenyl)methanesulfonamide (7.6)[188]



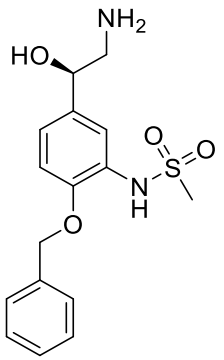
R-CBS catalyst (0.074 g, 0.27 mmol) was dissolved in unstabilised THF (5 mL) along with borane-*N,N*-diethylaniline (0.29 g, 1.78 mmol) at 0 °C. The mixture was stirred for 10 minutes and then **5.54** (0.32 g, 0.89 mmol), dissolved in THF (3 mL) was added. The mixture was stirred at room temperature for 3 hours. Water was added and stirred for 10 minutes followed by the removal of volatiles *in vacuo*. Water was added and the mixture was extracted with EA. The organic layer was washed with 2M aq.hydrochloric acid and then water. The organic layer was concentrated under reduced pressure to afford the pure product (0.31 g, 96%). ee not determined for (*S*)-enantiomer.

^{1}H NMR: (400 MHz, Chloroform-*d*) δ 7.56 (d, *J* = 2.1 Hz, ArH, 1H), 7.46 – 7.37 (m, 5H), 7.19 (dd, *J* = 8.4, 2.1 Hz, ArH, 1H), 7.03 (d, *J* = 8.4 Hz, ArH, 1H), 6.84 (s, OH, 1H), 5.14 (s, Bn-CH_2, 2H), 4.86 (dd, *J* = 6.9, 5.0 Hz, CH, 1H), 3.54 – 3.42 (m, N_3-CH_2, 2H), 2.95 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 148.4, 135.5, 134.1, 128.9, 128.7, 127.8, 126.5, 123.0, 118.7, 112.4, 72.7, 71.2, 57.9, 39.3.

ee Determination was not performed on the *(s)*- enantiomer.

(*R*)-*N*-(5-(2-Amino-1-hydroxyethyl)-2-(benzyloxy)phenyl)methanesulfonamide (5.60) [187]



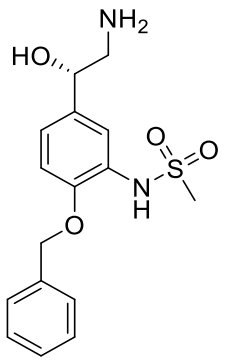
5.59 (0.02 g, 0.055 mmol) was dissolved in a mixture of Methanol (2 mL) and THF (2 mL). Ammonium chloride (0.29 g, 0.55 mmol) and zinc powder (0.36 g, 0.55 mmol) were added and the mixture was stirred at ambient temperature for 16 $_2$h. Zinc power was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography, eluting with DCM-Methanol (100:0→80:20) to afford the product (0.052 g, 38%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.57 – 7.51 (m, ArH, 2H), 7.43 – 7.28 (m, ArH, 4H), 7.19 – 7.09 (m, ArH, 2H), 5.18 (s, Bn-CH_2, 2H), 4.64 (dd, *J* = 9.2, 3.4 Hz, CH, 1H), 2.95 – 2.83 (m, SO_2CH_3, N-CH_2, 4H), 2.77 – 2.66 (m, N-CH_2, 1H).

^{13}C NMR: (101 MHz, DMSO) δ 151.4, 137.2, 135.5, 128.8, 128.3, 128.1, 126.2, 124.5, 124.3, 113.3, 70.5, 70.2, 49.0, 40.7.

LC-MS: calculated for $C_{16}H_{22}N_2O_4S$ $[M+H]^+$ 337.1. found: 337.2

(*S*)-*N*-(5-(2-amino-1-hydroxyethyl)-2-(benzyloxy)phenyl)methanesulfonamide (7.7) [187]



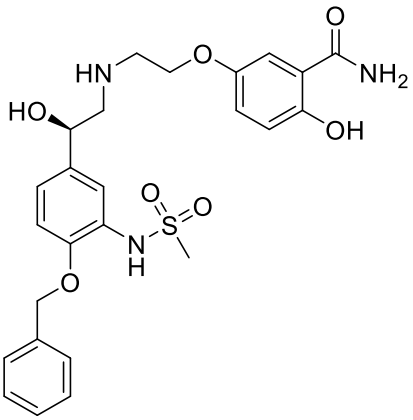
7.6 (0.03 g, 0.083 mmol) was dissolved in a mixture of Methanol (5 mL) and THF (5 mL). Ammonium chloride (0.44 g, 0.83 mmol) and zinc powder (0.52 g, 0.83 mmol) were added and the mixture was stirred at ambient temperature for 16h. Zinc power was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography, eluting with DCM-Methanol (100:0→80:20) to afford the product (0.12 g, 44%).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.56 – 7.51 (m, ArH, 2H), 7.40 (t, *J* = 7.4 Hz, ArH, 2H), 7.37 – 7.31 (m, ArH, 1H), 7.29 (d, *J* = 2.0 Hz, ArH, 1H), 7.18 – 7.09 (m, ArH, 2H), 5.18 (s, Bn-CH_2, 2H), 4.66 (dd, *J* = 9.5, 3.4 Hz, CH, 1H), 2.95 – 2.84 (m, SO_2CH_3, N-CH_2, 4H), 2.77 – 2.65 (m, N-CH_2, 1H).

^{13}C NMR: (101 MHz, DMSO) δ 151.4, 137.2, 135.5, 128.8, 128.3, 128.1, 126.2, 124.5, 124.3, 113.3, 70.5, 70.2, 49.0, 40.7.

LC-MS: calculated for $C_{16}H_{22}N_2O_4S$ $[M+H]^+$ 337.1. found: 337.2

(*R*)-5-(2-((2-(4-(Benzyloxy)-3-(methylsulfonamido)phenyl)-2-hydroxyethyl)amino)ethoxy)-2-hydroxybenzamide (5.61)



5.50 (0.027 g, 0.077 mmol) and **5.60** (0.052 g, 0.15 mmol) were dissolved in DMF (1 mL) along with DIPEA (0.030 g, 0.23 mmol). The mixture was heated ay 80 °C under microwave irradiation for 30 minutes. The mixture was concentrated under reduced pressure and the residue was purified by HPLC eluting with water-MeCN and 0.01% TFA, (5:95:0.01 → 95:5:0.01 over 13 minutes, eluted at 8.3 minutes), White solid product (0.006 g, 14%).

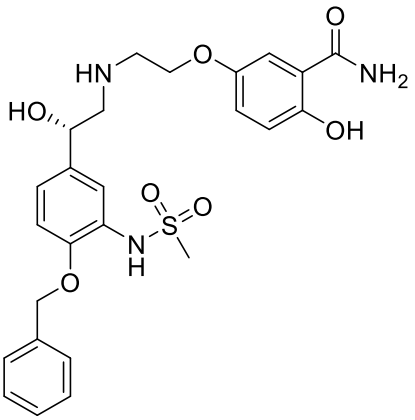
^{1}H NMR: (400 MHz, Methanol-d_4) δ 7.54 – 7.45 (m, ArH, 3H), 7.44 – 7.31 (m, ArH, 4H), 7.27 (dd, *J* = 8.5, 2.2 Hz, ArH, 1H), 7.18 – 7.10 (m, ArH, 2H), 6.90 (d, *J* = 9.0 Hz, ArH, 1H), 5.24 (s, Bn-CH_2, 2H), 5.03 – 4.95 (m, CH, 1H), 4.30 (t, *J* = 5.0 Hz, O-CH_2, 2H), 3.53 (t, *J* = 5.0 Hz, O-CH_2-$\underline{CH_2}$, 2H), 3.32 – 3.28 (m, N-CH_2, 1H), 3.26 – 3.14 (m, N-CH_2, 1H), 2.90 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, MeOD) δ 171.2, 154.7, 150.9, 150.5, 136.5, 133.8, 128.3, 127.8, 127.5, 126.3, 123.9, 122.8, 121.9, 118.0, 115.4, 113.0, 112.7, 70.3, 68.3, 64.1, 53.9, 46.7, 38.6.

HRMS: calculated for $C_{25}H_{30}N_3O_7S$ $[M+H]^+$ 516.1799. found: 516.1815

LC-MS: R_t: 2.15 min (254 nm)

(*R*)-5-(2-((2-(4-(Benzyloxy)-3-(methylsulfonamido)phenyl)-2-hydroxyethyl)amino)ethoxy)-2-hydroxybenzamide (5.62)



5.50 (0.060 g, 0.17 mmol) and **7.7** (0.12 g, 0.34 mmol) were dissolved in DMF (2 mL) along with DIPEA (0.066 g, 0.51 mmol). The mixture was heated ay 80 °C under microwave irradiation for 30 minutes. The mixture was concentrated under reduced pressure and the residue was purified by eluting with water-MeCN and 0.01% TFA, with a gradient of 5→95% MeCN. White solid product (0.01 g, 11%).

^{1}H NMR: (400 MHz, Methanol-d_4) δ 7.53 – 7.47 (m, ArH, 3H), 7.47 – 7.32 (m, ArH, 4H), 7.26 (dd, *J* = 8.5, 2.2 Hz, ArH, 1H), 7.17 – 7.10 (m, ArH, 2H), 6.90 (d, *J* = 9.0 Hz, ArH, 1H), 5.24 (s, Bn-CH_2, 2H), 4.96 (dd, *J* = 10.0, 3.2 Hz, CH, 1H), 4.28 (t, *J* = 4.9 Hz, O-CH_2, 2H), 3.49 (t, *J* = 4.9 Hz, O-CH_2-$\underline{CH_2}$, 2H), 3.30 – 3.24 (m, N-CH_2, 1H), 3.23 – 3.12 (m, N-CH_2, 1H), 2.90 (s, SO_2CH_3, 3H).

^{13}C NMR: (101 MHz, MeOD) δ 171.2, 154.7, 150.9, 150.5, 136.5, 133.8, 128.3, 127.8, 127.5, 126.3, 123.9, 122.8, 121.9, 118.0, 115.4, 113.0, 112.7, 70.3, 68.3, 64.1, 53.9, 46.7, 38.6.

HRMS: calculated for $C_{25}H_{30}N_3O_7S$ $[M+H]^+$ 516.1799. found: 516.1821

LC-MS: R_t: 2.14 min (254 nm)

7.2 Pharmacology

Pharmacology in Chapter 3 was carried out by the author and Prof. Jillian Baker (University of Nottingham). Pharmacology in subsequent chapters was performed by Prof Jillian Baker.

7.2.1 Whole cell culture

Prof Jillian Baker performed the transfection, dilution cloning and isolation of a stable clone. All reagents used were obtained from Sigma chemicals and all plates were obtained from Corning Costar. CHO-K1-β_1 and CHO-K1-β_2 cells were used throughout this study.

All cell lines were cultured in Dulbecco's modified Eagle's medium/nutrient mix F12 (DMEM/F12) supplemented with 2 mM L-glutamine and 10% heat-inactivated foetal calf serum (FCS). CHO cells were incubated at 37 °C, 5% CO2. All cell culture procedures were performed in a class II laminar flow hood using sterile techniques.

7.2.2 Passaging of cells

All CHO cell lines were passaged once they had grown to confluence in a T75 flask. Media was aspirated and the cells were washed with warm Dulbecco's phosphate buffered saline (PBS) (5-10 mL). PBS was removed and cells were then incubated with trypsin/EDTA (1 mL) for 2-3minutes or until cells were loosened from the bottom of the flask. Once detached, the trypsin/EDTA was neutralised with media (10 mL) and the resulting suspension was spun at 1000 rpm for five minutes. The media was removed and then the pellet was re-suspended in media (1 mL); this step involved rapid agitation to ensure a uniform suspension was produced. The 1 mL suspension was then added to media (9 mL) and the resulting suspension was dispensed as appropriate into 75 mL flasks. All CHO cell lines were generally split 1:10.

7.2.3 Seeding into cell plates

All CHO cells from confluent T75 flasks were removed from the flask and centrifuged as described above. Media was removed from the pelleted cells, followed by the addition of 8 mL of media. 1 mL of the suspended cells was then added to 9 mL of media and of the 10 mL, 100 µL was dispensed into each well of a 96-well plate. One T75 flask was used to set up a maximum of eight 96-well plates.

7.2.4 Freezing and thawing cells

Cells from confluent T75 flasks were removed from the flask and centrifuged as described previously. One pellet from a T75 flask was carefully re-suspended in the freezing medium (2mL, 10% DMSO in FCS). 1 mL of cell suspension was transferred to each cryovails. Cryovials were placed in a Mr Frosty freezing container which was placed immediately in a -80 °C freezer.

Cells were thawed by removing one cyrovial from the freezer and warmed to 37 °C in a 37 °C bath. The contents of the vial were suspended into media (10 mL) and the resulting suspension was spun at 1000 rpm for five minutes. Supernatant was removed and the pellet was resuspended in media (1 mL) and transferred to a 75 mL flask containing 20 mL of media. After 24 hours, media was removed and replaced with fresh media.

7.2.5 SPAP assay

Cells were grown to confluence in 96-well plates. Media was removed and 100 µL of DMEM/F12 containing 2 mM L-glutamine (serum-free media) was added 24 hours prior to the experiment. On the day of the experiment, media was replaced with fresh serum-free media (90 µL). Diluted compounds (in 10 µL media) were then added to the plates in triplicates and incubated for 5 hours at 37°C in a humidified atmosphere of 5% CO2/95% air. Media was then removed and replaced with 40 µL of fresh serum-free media and incubated for a further one hour. Plates were then placed in an oven at 65 °C and left for 30 minutes. Plates were allowed to cool and then p-Nitrophenol phosphate in diethanolamine buffer (100 µL) was added to each

sample and incubated at 37°C in air for 15 minutes. The plates were then read at 405 nm using a Dynatech Laboratories MRX plate reader and the data was converted to optical density (OD) units.

7.2.6 [^{3}H]-CGP 12177 whole-cell binding

Cells were seeded into 96-well, clear bottomed white-sided plates, 24 hours prior to the assay. On the day of experimentation all media was aspirated and different concentrations of the ligands, diluted in serum free media (100 µl/well), were added in triplicates. The total and non-specific binding was determined by adding serum free media for total binding and 10 µM of propranolol for non-specific binding. A fixed concentration of [^{3}H]-CGP 12177 in serum free media (100 µl/well) was then added to each well. The cells were incubated for two hours at 37 °C, 5% CO_2. Cells were washed twice with cold PBS (200 µl/well), and Microscint 20 (100 µl/well) was added. A white sticker was added to the base of the plate; the plates were sealed and left overnight. Finally, the plates were counted on a Topcount.

7.2.7 Data analysis

All data are represented as mean ± standard error. The n in the text refers to the number of separate experiments (a separate experiment requires cells plated from a separate flask and separate drug dilution used throughout the experiment). A maximal isoprenaline concentration (10 µL) was included in two triplicates in each CRE-SPAP plate so agonist responses could be expressed as a percentage of isoprenaline maximum. Percentage of isoprenaline maximum was calculated by:

$$\% \text{ isoprenaline} = ((\text{max ligand response} - \text{basal}) / (\text{max isoprenaline response} - \text{basal})) \times 100$$

Sigmoidal agonist concentration response curves (in the presence and absence of antagonists) were fitted to the following equation through computer-assisted nonlinear regression using the program GraphPad Prism 6.

$$\text{Response} = (E_{max} \text{ X } [A]) / (EC_{50} + [A])$$

Where E_{max} is the maximal response, [A] is the agonist concentration and EC_{50} is the concentration of agonist that produces 50% of maximal response.

Antagonist dissociation constants were assessed at fixed antagonist concentrations (assuming competitive antagonism) by observing the shift in the agonist concentration-response curve using the equation:

$$DR = 1 + [A]/K_d$$

Where DR is the ratio of the concentrations of the agonist required to produce an identical response in the presence and absence of antagonist, [A] is the concentration of antagonist, and K_D is the antagonist dissociation constant.

From the IC_{50} value and the known concentration of radioligand [^{3}H]-CGP 12177, a K_d (concentration required to bind to half of the receptors) value was calculated using the equation:

$$K_d = IC_{50} / (1+[^3\text{H-CGP 12177}] / K_d\ ^3\text{H-CGP 12177})$$

Where IC_{50} is the concentration of the ligand where binding of the radioligand is reduced by half. To determine the concentration of the radioligand, 50 µl was added to scintilation vials along with scintilation fluid (10 mL) in a triplicate. Each vial was counted on a scintillation counter which gave dpm values. dpm was then converted to to the concentration of the radioligand. To obtain the K_d of the radioligand, a saturation binding experiment as performed. From this data, the non-specific binding was subtracted from the total binding, to give specific binding. This gives the maximim specific binding (Bmax) and the K_d, which is the radioligand concentration required to achieve half-maximum binding.

8. References

1. Madamanchi, A., β-Adrenergic receptor signaling in cardiac function and heart failure. *McGill Journal of Medicine : MJM* **2007,** *10* (2), 99-104.
2. Xiao, R. P.; Lakatta, E. G., Beta 1-adrenoceptor stimulation and beta 2-adrenoceptor stimulation differ in their effects on contraction, cytosolic Ca2+, and Ca2+ current in single rat ventricular cells. *Circulation Research* **1993,** *73* (2), 286.
3. Barnes, P. J., Distribution of Receptor Targets in the Lung. *Proceedings of the American Thoracic Society* **2004,** *1* (4), 345-351.
4. Krief, S.; Lönnqvist, F.; Raimbault, S.; Baude, B.; Van Spronsen, A.; Arner, P.; Strosberg, A. D.; Ricquier, D.; Emorine, L. J., Tissue distribution of beta 3-adrenergic receptor mRNA in man. *The Journal of Clinical Investigation* **1993,** *91* (1), 344-349.
5. Arch, J. R. S., The discovery of drugs for obesity, the metabolic effects of leptin and variable receptor pharmacology: perspectives from β3-adrenoceptor agonists. *Naunyn-Schmiedeberg's Archives of Pharmacology* **2008,** *378* (2), 225.
6. Strosberg, A. D., Structure, function, and regulation of adrenergic receptors. *Protein Science : A Publication of the Protein Society* **1993,** *2* (8), 1198-1209.
7. Katritch, V.; Cherezov, V.; Stevens, R. C., Diversity and Modularity of G Protein-Coupled Receptor Structures. *Trends in Pharmacological Sciences* **2012,** *33* (1), 17-27.
8. Gether, U., Uncovering Molecular Mechanisms Involved in Activation of G Protein-Coupled Receptors. *Endocrine Reviews* **2000,** *21* (1), 90-113.
9. Bockaert, J.; Pin, J. P., Molecular tinkering of G protein-coupled receptors: an evolutionary success. *The EMBO Journal* **1999,** *18* (7), 1723-1729.
10. Emorine, L. J.; Marullo, S.; Briend-Sutren, M. M.; Patey, G.; Tate, K.; Delavier-Klutchko, C.; Strosberg, A. D., Molecular characterization of the human beta 3-adrenergic receptor. *Science* **1989,** *245* (4922), 1118.
11. Strosberg, A. D., Structure and function of the β3-adrenergic receptor. *Annual Review of Pharmacology and Toxicology* **1997,** *37* (1), 421-450.
12. Strosberg, A. D.; Nahmias, C., G-protein-coupled receptor signalling through protein networks. *Biochemical Society Transactions* **2007,** *35* (1), 23.
13. Yeagle, P. L.; Albert, A. D., G-protein coupled receptor structure. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2007,** *1768* (4), 808-824.
14. Billington, C. K.; Ojo, O. O.; Penn, R. B.; Ito, S., cAMP regulation of airway smooth muscle function. *Pulmonary Pharmacology & Therapeutics* **2013,** *26* (1), 112-120.
15. Rasmussen, S. G. F.; DeVree, B. T.; Zou, Y.; Kruse, A. C.; Chung, K. Y.; Kobilka, T. S.; Thian, F. S.; Chae, P. S.; Pardon, E.; Calinski, D.; Mathiesen, J. M.; Shah, S. T. A.; Lyons, J. A.; Caffrey, M.; Gellman, S. H.; Steyaert, J.; Skiniotis, G.; Weis, W. I.; Sunahara, R. K.; Kobilka, B. K., Crystal structure of the β2 adrenergic receptor–Gs protein complex. *Nature* **2011,** *477*, 549.
16. Strader, C. D.; Candelore, M. R.; Hill, W. S.; Sigal, I. S.; Dixon, R. A., Identification of two serine residues involved in agonist activation of the beta-adrenergic receptor. *Journal of Biological Chemistry* **1989,** *264* (23), 13572-13578.
17. Liapakis, G.; Ballesteros, J. A.; Papachristou, S.; Chan, W. C.; Chen, X.; Javitch, J. A., The Forgotten Serine: A critical role for ser-2035.42 in ligand binding to and activation of the β2-adrenergic receptor. *Journal of Biological Chemistry* **2000,** *275* (48), 37779-37788.
18. Wieland, K.; Zuurmond, H. M.; Krasel, C.; Ijzerman, A. P.; Lohse, M. J., Involvement of Asn-293 in stereospecific agonist recognition and in activation of the beta 2-adrenergic receptor. *Proceedings of the National Academy of Sciences of the United States of America* **1996,** *93* (17), 9276-9281.

19. Keatings, V. M.; Collins, P. D.; Scott, D. M.; Barnes, P. J., Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *American Journal of Respiratory and Critical Care Medicine* **1996,** *153* (2), 530-534.
20. Price, D.; Fletcher, M.; van der Molen, T., Asthma control and management in 8,000 European patients: the REcognise Asthma and LInk to Symptoms and Experience (REALISE) survey. *Npj Primary Care Respiratory Medicine* **2014,** *24*, 14009.
21. Erle, D. J.; Sheppard, D., The cell biology of asthma. *The Journal of Cell Biology* **2014,** *205* (5), 621.
22. Mukherjee, M.; Stoddart, A.; Gupta, R. P.; Nwaru, B. I.; Farr, A.; Heaven, M.; Fitzsimmons, D.; Bandyopadhyay, A.; Aftab, C.; Simpson, C. R.; Lyons, R. A.; Fischbacher, C.; Dibben, C.; Shields, M. D.; Phillips, C. J.; Strachan, D. P.; Davies, G. A.; McKinstry, B.; Sheikh, A., The epidemiology, healthcare and societal burden and costs of asthma in the UK and its member nations: analyses of standalone and linked national databases. *BMC Medicine* **2016,** *14* (1), 113.
23. Celli, B. R., Predictors of mortality in COPD. *Respiratory Medicine* **2010,** *104* (6), 773-779.
24. Louie, S.; Zeki, A. A.; Schivo, M.; Chan, A. L.; Yoneda, K. Y.; Avdalovic, M.; Morrissey, B. M.; Albertson, T. E., The asthma–chronic obstructive pulmonary disease overlap syndrome: pharmacotherapeutic considerations. *Expert Review of Clinical Pharmacology* **2013,** *6* (2), 197-219.
25. Adeloye, D.; Chua, S.; Lee, C.; Basquill, C.; Papana, A.; Theodoratou, E.; Nair, H.; Gasevic, D.; Sridhar, D.; Campbell, H.; Chan, K. Y.; Sheikh, A.; Rudan, I.; Global Health Epidemiology Reference, G., Global and regional estimates of COPD prevalence: Systematic review and meta–analysis. *Journal of Global Health* **2015,** *5* (2), 020415.
26. Barger, G.; Dale, H. H., Chemical structure and sympathomimetic action of amines. *The Journal of Physiology* **1910,** *41* (1-2), 19-59.
27. Waldeck, B., Beta-adrenoceptor agonists and asthma--100 years of development. *Eur J Pharmacol* **2002,** *445* (1-2), 1-12.
28. Konzett, H., On the discovery of isoprenaline. *Trends in Pharmacological Sciences* **1981,** *2*, 47-49.
29. Cazzola, M.; Page, C. P.; Calzetta, L.; Matera, M. G., Pharmacology and Therapeutics of Bronchodilators. *Pharmacological Reviews* **2012,** *64* (3), 450.
30. Shanks, R. G.; Brick, I.; Hutchison, K.; Roddie, I. C., Stimulation of adrenergic beta-receptors by orciprenaline. *British Medical Journal* **1967,** *1* (5540), 610-612.
31. Farmer, J. B.; Levy, G. P.; Marshall, R. J., A comparison of the β-adrenoceptor stimulant properties of salbutamol, orciprenaline and soterenol with those of isoprenaline. *Journal of Pharmacy and Pharmacology* **1970,** *22* (12), 945-947.
32. Stocks, M. J.; Alcaraz, L.; Bailey, A.; Bonnert, R.; Cadogan, E.; Christie, J.; Connolly, S.; Cook, A.; Fisher, A.; Flaherty, A.; Hill, S.; Humphries, A.; Ingall, A.; Jordan, S.; Lawson, M.; Mullen, A.; Nicholls, D.; Paine, S.; Pairaudeau, G.; St-Gallay, S.; Young, A., Design driven HtL: The discovery and synthesis of new high efficacy β2-agonists. *Bioorganic & Medicinal Chemistry Letters* **2011,** *21* (13), 4027-4031.
33. Jack, D., The 1990 Lilly Prize Lecture. A way of looking at agonism and antagonism: lessons from salbutamol, salmeterol and other beta- adrenoceptor agonists. *British Journal of Clinical Pharmacology* **1991,** *31* (5), 501-514.
34. Cullum, V. A.; Farmer, J. B.; Jack, D.; Levy, G. P., Salbutamol: A new, selective β-adrenoceptive receptor stimulant. *British Journal of Pharmacology* **1969,** *35* (1), 141-151.
35. Warrell, D. A.; Robertson, D. G.; Howes, J. N.; Conolly, M. E.; Paterson, J. W.; Beilin, L. J.; Dollery, C. T., Comparison of Cardiorespiratory Effects of Isoprenaline and Salbutamol in Patients with Bronchial Asthma. *British Medical Journal* **1970,** *1* (5688), 65-70.

36. Kelly, H. W.; Murphy, S., Beta-Adrenergic Agonists for Acute, Severe Asthma. *Annals of Pharmacotherapy* **1992,** *26* (1), 81-91.
37. Crowe, M. J.; Counihan, H. E.; O'Malley, K., A comparative study of a new selective beta 2-adrenoceptor agonist, procaterol and salbutamol in asthma. *British Journal of Clinical Pharmacology* **1985,** *19* (6), 787-791.
38. Hockley, B.; Johnson, N. M., Fenoterol versus salbutamol nebulization in asthma. *Postgraduate Medical Journal* **1983,** *59* (694), 504-505.
39. Crane, J.; Flatt, A.; Jackson, R.; Ball, M.; Pearce, N.; Burgess, C.; Kwong, T.; Beasley, R., Prescribed fenoterol and death from asthma in new zealand, 1981-83; case-control study. *The Lancet* **1989,** *333* (8644), 917-922.
40. Suzuki, H.; Shindo, K.; Ueno, A.; Miura, T.; Takei, M.; Sakakibara, M.; Fukamachi, H.; Tanaka, J.; Higa, T., S1319: A novel β2-adrenoceptor agonist from a marine sponge Dysidea sp. *Bioorganic & Medicinal Chemistry Letters* **1999,** *9* (10), 1361-1364.
41. Juniper, E. F.; Johnston, P. R.; Borkhoff, C. M.; Guyatt, G. H.; Boulet, L. P.; Haukioja, A., Quality of life in asthma clinical trials: comparison of salmeterol and salbutamol. *American Journal of Respiratory and Critical Care Medicine* **1995,** *151* (1), 66-70.
42. Palmqvist, M.; Persson, G.; Lazer, L.; Rosenborg, J.; Larsson, P.; Lotvall, J., Inhaled dry-powder formoterol and salmeterol in asthmatic patients: onset of action, duration of effect and potency. *European Respiratory Journal* **1997,** *10* (11), 2484.
43. Wegener, T.; Hedenström, H.; Melander, B., Rapid Onset of Action of Inhaled Formoterol in Asthmatic Patients. *CHEST* **1992,** *102* (2), 535-538.
44. Lotvall, J., Pharmacological similarities and differences between beta2-agonists. *Respir Med* **2001,** *95 Suppl B*, S7-11.
45. Santus, P.; Radovanovic, D.; Paggiaro, P.; Papi, A.; Sanduzzi, A.; Scichilone, N.; Braido, F., Why use long acting bronchodilators in chronic obstructive lung diseases? An extensive review on formoterol and salmeterol. *European Journal of Internal Medicine* **2015,** *26* (6), 379-384.
46. Anderson, G. P., Formoterol: Pharmacology, molecular basis of agonism, and mechanism of long duration of a highly potent and selective β2-adrenoceptor agonist bronchodilator. *Life Sciences* **1993,** *52* (26), 2145-2160.
47. Szczuka, A.; Wennerberg, M.; Packeu, A.; Vauquelin, G., Molecular mechanisms for the persistent bronchodilatory effect of the β(2)-adrenoceptor agonist salmeterol. *British Journal of Pharmacology* **2009,** *158* (1), 183-194.
48. Anderson, G. P.; Linden, A.; Rabe, K. F., Why are long-acting beta-adrenoceptor agonists long-acting? *European Respiratory Journal* **1994,** *7* (3), 569.
49. Green, S. A.; Spasoff, A. P.; Coleman, R. A.; Johnson, M.; Liggett, S. B., Sustained Activation of a G Protein-coupled Receptor via "Anchored" Agonist Binding: Molecular localization of the salmeterol exosite within the β2-adrenergic receptor. *Journal of Biological Chemistry* **1996,** *271* (39), 24029-24035.
50. Baker, J. G., The selectivity of β-adrenoceptor antagonists at the human β1, β2 and β3 adrenoceptors. *British Journal of Pharmacology* **2005,** *144* (3), 317-322.
51. Baker, J. G., The selectivity of β-adrenoceptor agonists at human β(1)-, β(2)- and β(3)-adrenoceptors. *British Journal of Pharmacology* **2010,** *160* (5), 1048-1061.
52. Baker, J. G.; Proudman, R. G.; Hill, S. J., Salmeterol's extreme beta2 selectivity is due to residues in both extracellular loops and transmembrane domains. *Molecular pharmacology* **2015,** *87* (1), 103-20.
53. Baur, F.; Beattie, D.; Beer, D.; Bentley, D.; Bradley, M.; Bruce, I.; Charlton, S. J.; Cuenoud, B.; Ernst, R.; Fairhurst, R. A.; Faller, B.; Farr, D.; Keller, T.; Fozard, J. R.; Fullerton, J.; Garman, S.; Hatto, J.; Hayden, C.; He, H.; Howes, C.; Janus, D.; Jiang, Z.; Lewis, C.; Loeuillet-Ritzler, F.; Moser, H.; Reilly, J.; Steward, A.; Sykes, D.; Tedaldi, L.; Trifilieff, A.; Tweed, M.; Watson, S.; Wissler, E.; Wyss, D., The Identification of Indacaterol as an

Ultralong-Acting Inhaled β2-Adrenoceptor Agonist. *Journal of Medicinal Chemistry* **2010,** *53* (9), 3675-3684.
54. Rosethorne, E. M.; Turner, R. J.; Fairhurst, R. A.; Charlton, S. J., Efficacy is a contributing factor to the clinical onset of bronchodilation of inhaled β2-adrenoceptor agonists. *Naunyn-Schmiedeberg's Archives of Pharmacology* **2010,** *382* (3), 255-263.
55. Moen, M. D., Indacaterol: in chronic obstructive pulmonary disease. *Drugs* **2010,** *70* (17), 2269-2280.
56. Battram, C.; Charlton, S. J.; Cuenoud, B.; Dowling, M. R.; Fairhurst, R. A.; Farr, D.; Fozard, J. R.; Leighton-Davies, J. R.; Lewis, C. A.; McEvoy, L.; Turner, R. J.; Trifilieff, A., In vitro and in vivo pharmacological characterization of 5-[(R)-2-(5,6-diethyl-indan-2-ylamino)-1-hydroxy-ethyl]-8-hydroxy-1H-quinolin-2-o ne (indacaterol), a novel inhaled beta(2) adrenoceptor agonist with a 24-h duration of action. *The Journal of pharmacology and experimental therapeutics* **2006,** *317* (2), 762-70.
57. Voss, H.-P.; Donnell, D.; Bast, A., Atypical molecular pharmacology of a new long-acting β2-adrenoceptor agonist, TA 2005. *European Journal of Pharmacology: Molecular Pharmacology* **1992,** *227* (4), 403-409.
58. Sykes David, A.; Charlton Steven, J., Slow receptor dissociation is not a key factor in the duration of action of inhaled long-acting β2-adrenoceptor agonists. *British Journal of Pharmacology* **2011,** *165* (8), 2672-2683.
59. Lombardi, D.; Cuenoud, B.; Krämer, S. D., Lipid membrane interactions of indacaterol and salmeterol: Do they influence their pharmacological properties? *European Journal of Pharmaceutical Sciences* **2009,** *38* (5), 533-547.
60. Cazzola, M.; Bardaro, F.; Stirpe, E., The role of indacaterol for chronic obstructive pulmonary disease (COPD). *Journal of Thoracic Disease* **2013,** *5* (4), 559-566.
61. Montuschi, P.; Ciabattoni, G., Bronchodilating Drugs for Chronic Obstructive Pulmonary Disease: Current Status and Future Trends. *Journal of Medicinal Chemistry* **2015,** *58* (10), 4131-4164.
62. Bouyssou, T.; Hoenke, C.; Rudolf, K.; Lustenberger, P.; Pestel, S.; Sieger, P.; Lotz, R.; Heine, C.; Büttner, F. H.; Schnapp, A.; Konetzki, I., Discovery of olodaterol, a novel inhaled β2-adrenoceptor agonist with a 24h bronchodilatory efficacy. *Bioorganic & Medicinal Chemistry Letters* **2010,** *20* (4), 1410-1414.
63. Gibb, A.; Yang, L. P. H., Olodaterol: First Global Approval. *Drugs* **2013,** *73* (16), 1841-1846.
64. Ferguson, G. T.; Karpel, J.; Bennett, N.; Clerisme-Beaty, E.; Grönke, L.; Voß, F.; Buhl, R., Effect of tiotropium and olodaterol on symptoms and patient-reported outcomes in patients with COPD: results from four randomised, double-blind studies. *npj Primary Care Respiratory Medicine* **2017,** *27* (1), 7.
65. Beier, J.; Pujol, H.; Seoane, B.; Jimenez, E.; Astbury, C.; Massana, E.; Ruiz, S.; de Miquel, G., Abediterol, a novel long-acting β2-agonist: bronchodilation, safety, tolerability and pharmacokinetic results from a single-dose, dose-ranging, active-comparator study in patients with COPD. *BMC Pulmonary Medicine* **2016,** *16* (1), 102.
66. Beier, J.; Fuhr, R.; Massana, E.; Jiménez, E.; Seoane, B.; de Miquel, G.; Ruiz, S., Abediterol (LAS100977), a novel long-acting β2-agonist: Efficacy, safety and tolerability in persistent asthma. *Respiratory Medicine* **2014,** *108* (10), 1424-1429.
67. Slack, R. J.; Barrett, V. J.; Morrison, V. S.; Sturton, R. G.; Emmons, A. J.; Ford, A. J.; Knowles, R. G., In vitro pharmacological characterization of vilanterol, a novel long-acting beta2-adrenoceptor agonist with 24-hour duration of action. *The Journal of pharmacology and experimental therapeutics* **2013,** *344* (1), 218-30.
68. Procopiou, P. A.; Barrett, V. J.; Bevan, N. J.; Biggadike, K.; Box, P. C.; Butchers, P. R.; Coe, D. M.; Conroy, R.; Emmons, A.; Ford, A. J.; Holmes, D. S.; Horsley, H.; Kerr, F.; Li-Kwai-Cheung, A.-M.; Looker, B. E.; Mann, I. S.; McLay, I. M.; Morrison, V. S.; Mutch, P. J.; Smith,

C. E.; Tomlin, P., Synthesis and Structure−Activity Relationships of Long-acting β2 Adrenergic Receptor Agonists Incorporating Metabolic Inactivation: An Antedrug Approach. *Journal of Medicinal Chemistry* **2010,** *53* (11), 4522-4530.
69. Cazzola, M.; Molimard, M., The scientific rationale for combining long-acting β2-agonists and muscarinic antagonists in COPD. *Pulmonary Pharmacology & Therapeutics* **2010,** *23* (4), 257-267.
70. Cazzola, M.; Matera, M. G., The effective treatment of COPD: Anticholinergics and what else? *Drug Discovery Today: Therapeutic Strategies* **2006,** *3* (3), 277-286.
71. Karellas, P.; McNaughton, M.; Baker, S. P.; Scammells, P. J., Synthesis of Bivalent β2-Adrenergic and Adenosine A1 Receptor Ligands. *Journal of Medicinal Chemistry* **2008,** *51* (19), 6128-6137.
72. Hughes, A. D.; Chin, K. H.; Dunham, S. L.; Jasper, J. R.; King, K. E.; Lee, T. W.; Mammen, M.; Martin, J.; Steinfeld, T., Discovery of muscarinic acetylcholine receptor antagonist and beta 2 adrenoceptor agonist (MABA) dual pharmacology molecules. *Bioorganic & Medicinal Chemistry Letters* **2011,** *21* (5), 1354-1358.
73. Ritchie, T. J.; Luscombe, C. N.; Macdonald, S. J. F., Analysis of the Calculated Physicochemical Properties of Respiratory Drugs: Can We Design for Inhaled Drugs Yet? *Journal of Chemical Information and Modeling* **2009,** *49* (4), 1025-1032.
74. Ambery, C. L.; Wielders, P.; Ludwig-Sengpiel, A.; Chan, R.; Riley, J. H., Population Pharmacokinetics and Pharmacodynamics of GSK961081 (Batefenterol), a Muscarinic Antagonist and β(2)-Agonist, in Moderate-to-Severe COPD Patients: Substudy of a Randomized Trial. *Drugs in R&D* **2015,** *15* (3), 281-291.
75. Jones, L. H.; Baldock, H.; Bunnage, M. E.; Burrows, J.; Clarke, N.; Coghlan, M.; Entwistle, D.; Fairman, D.; Feeder, N.; Fulton, C.; Hilton, L.; James, K.; Jones, R. M.; Kenyon, A. S.; Marshall, S.; Newman, S. D.; Osborne, R.; Patel, S.; Selby, M. D.; Stuart, E. F.; Trevethick, M. A.; Wright, K. N.; Price, D. A., Inhalation by design: Dual pharmacology β-2 agonists/M3 antagonists for the treatment of COPD. *Bioorganic & Medicinal Chemistry Letters* **2011,** *21* (9), 2759-2763.
76. Shan, W.-J.; Huang, L.; Zhou, Q.; Jiang, H.-L.; Luo, Z.-H.; Lai, K.-f.; Li, X.-S., Dual β2-adrenoceptor agonists-PDE4 inhibitors for the treatment of asthma and COPD. *Bioorganic & Medicinal Chemistry Letters* **2012,** *22* (4), 1523-1526.
77. Huang, L.; Shan, W.; Zhou, Q.; Xie, J.; Lai, K.; Li, X., Design, synthesis and evaluation of dual pharmacology β2-adrenoceptor agonists and PDE4 inhibitors. *Bioorganic & Medicinal Chemistry Letters* **2014,** *24* (1), 249-253.
78. Salpeter, S. R.; Ormiston, T. M.; Salpeter, E. E., Cardiovascular Effects of β-Agonists in Patients With Asthma and COPD: A Meta-Analysis. *Chest* **2004,** *125* (6), 2309-2321.
79. Cazzola, M.; Imperatore, F.; Salzillo, A.; Di Perna, F.; Calderaro, F.; Imperatore, A.; Matera, M. G., Cardiac Effects of Formoterol and Salmeterol in Patients Suffering from COPD with Preexisting Cardiac Arrhythmias and Hypoxemia. *Chest* **1998,** *114* (2), 411-415.
80. Baker, J. G.; Wilcox, R. G., beta-Blockers, heart disease and COPD: current controversies and uncertainties. *Thorax* **2017,** *72* (3), 271-276.
81. Mason, R. P.; Giles, T. D.; Sowers, J. R., Evolving Mechanisms of Action of Beta Blockers: Focus on Nebivolol. *Journal of Cardiovascular Pharmacology* **2009,** *54* (2), 123-128.
82. Frishman William, H., A Historical Perspective on the Development of β-Adrenergic Blockers. *The Journal of Clinical Hypertension* **2007,** *9* (s4), 19-27.
83. Freddolino, P. L.; Kalani, M. Y.; Vaidehi, N.; Floriano, W. B.; Hall, S. E.; Trabanino, R. J.; Kam, V. W.; Goddard, W. A., 3rd, Predicted 3D structure for the human beta 2 adrenergic receptor and its binding site for agonists and antagonists. *Proc Natl Acad Sci U S A* **2004,** *101* (9), 2736-41.

84. Stapleton, M. P., Sir James Black and propranolol. The role of the basic sciences in the history of cardiovascular pharmacology. *Texas Heart Institute Journal* **1997,** *24* (4), 336-342.
85. Alderman, E. L.; Davies, R. O.; Crowley, J. J.; Lopes, M. G.; Brooker, J. Z.; Friedman, J. P.; Graham, A. F.; Matlof, H. J.; Harrison, D. C., Dose response effectiveness of propranolol for the treatment of angina pectoris. *Circulation* **1975,** *51* (6), 964.
86. Clark, B. J.; Menninger, K.; Bertholet, A., Pindolol--the pharmacology of a partial agonist. *British Journal of Clinical Pharmacology* **1982,** *13* (Suppl 2), 149S-158S.
87. Fitzgerald, J. D., Do partial agonist beta-blockers have improved clinical utility? *Cardiovascular Drugs and Therapy* **1993,** *7* (3), 303-310.
88. Albouaini, K.; Andron, M.; Alahmar, A.; Egred, M., Beta-blockers use in patients with chronic obstructive pulmonary disease and concomitant cardiovascular conditions. *International Journal of Chronic Obstructive Pulmonary Disease* **2007,** *2* (4), 535-540.
89. Garylarde, P. M.; Sarkany, I., Letter: Side effects of practolol. *British Medical Journal* **1975,** *3* (5980), 435.
90. Smith, L. H.; Crowther, A. F.; Howe, R., .beta.-Adrenergic blocking agents. 10. (3-Amino-2-hydroxypropoxy)anilides. *Journal of Medicinal Chemistry* **1971,** *14* (6), 511-513.
91. Weber, M. A., The Role of the New β-Blockers in Treating Cardiovascular Disease. *American Journal of Hypertension* **2005,** *18* (S6), 169S-176S.
92. Bristow Michael, R.; Roden Robert, L.; Lowes Brian, D.; Gilbert Edward, M.; Eichhorn Eric, J., The role of third-generation beta-blocking agents in chronic heart failure. *Clinical Cardiology* **2009,** *21* (S1), I3-I13.
93. Pedersen, M. E.; Cockcroft, J. R., The vasodilatory beta-blockers. *Current Hypertension Reports* **2007,** *9* (4), 269-277.
94. Shin, J.; Johnson, J. A., Pharmacogenetics of β-Blockers. *Pharmacotherapy* **2007,** *27* (6), 874-887.
95. Leonetti, G.; Egan, C. G., Use of carvedilol in hypertension: an update. *Vascular Health and Risk Management* **2012,** *8*, 307-322.
96. Gupta, S.; Wright Harold, M., Nebivolol: A Highly Selective β1-Adrenergic Receptor Blocker That Causes Vasodilation by Increasing Nitric Oxide. *Cardiovascular Therapeutics* **2008,** *26* (3), 189-202.
97. Ritter, J. M., Nebivolol: Endothelium-Mediated Vasodilating Effect. *Journal of Cardiovascular Pharmacology* **2001,** *38*, S13-S16.
98. Fongemie, J.; Felix-Getzik, E., A Review of Nebivolol Pharmacology and Clinical Evidence. *Drugs* **2015,** *75* (12), 1349-1371.
99. Cheng, J. W. M., Nebivolol: A third-generation β-blocker for hypertension. *Clinical Therapeutics* **2009,** *31* (3), 447-462.
100. Dooley, D. J.; Bittiger, H.; Reymann, N. C., CGP 20712 A: a useful tool for quantitating β1- and β2-adrenoceptors. *Eur J Pharmacol* **1986,** *130* (1), 137-139.
101. Fuhrer, W.; Certain antihypertensive 1-[2-[3-carbamoyl-4-hydroxy phenoxy]alkylene amino]-heterocyclic phenoxy-propanol derivatives. **1985**.
102. Hulme Edward, C.; Trevethick Mike, A., Ligand binding assays at equilibrium: validation and interpretation. *British Journal of Pharmacology* **2010,** *161* (6), 1219-1237.
103. Zhu, B. T., Mechanistic explanation for the unique pharmacologic properties of receptor partial agonists. *Biomedicine & Pharmacotherapy* **2005,** *59* (3), 76-89.
104. Lambert, D. G., Drugs and receptors. *Continuing Education in Anaesthesia Critical Care & Pain* **2004,** *4* (6), 181-184.
105. Hill, S. J.; Baker, J. G.; Rees, S., Reporter-gene systems for the study of G-protein-coupled receptors. *Current Opinion in Pharmacology* **2001,** *1* (5), 526-532.

106. Stoddart, L. A.; Vernall, A. J.; Briddon, S. J.; Kellam, B.; Hill, S. J., Direct visualisation of internalization of the adenosine A3 receptor and localization with arrestin3 using a fluorescent agonist. *Neuropharmacology* **2015,** *98*, 68-77.
107. Naylor, L. H., Reporter gene technology: the future looks bright. *Biochemical Pharmacology* **1999,** *58* (5), 749-757.
108. Cheng, H. C., The power issue: determination of KB or Ki from IC50: A closer look at the Cheng–Prusoff equation, the Schild plot and related power equations. *Journal of Pharmacological and Toxicological Methods* **2001,** *46* (2), 61-71.
109. Flanagan, C. A., Chapter 10 - GPCR-radioligand binding assays. In *Methods in Cell Biology*, K. Shukla, A., Ed. Academic Press: 2016; Vol. 132, pp 191-215.
110. Sears, M. R.; Lötvall, J., Past, present and future—β2-adrenoceptor agonists in asthma management. *Respiratory Medicine* **2005,** *99* (2), 152-170.
111. Schmidt, D.; Gunera, J.; Baker, J. G.; Kolb, P., Similarity- and Substructure-Based Development of β2-Adrenergic Receptor Ligands Based on Unusual Scaffolds. *ACS Medicinal Chemistry Letters* **2017,** *8* (5), 481-485.
112. Roberts, C.; Zhang, Y.; Beaumier, F.; Lepissier, L.; Marineau, J. J.; Rahl, P. B.; Sprott, K.; Ciblat, S.; Sow, B.; Larouche-Gauthier, R.; Berstler, L. Preparation of substituted benzothiazoles for the modulation of Myc activity. WO2016197078A1, 2016.
113. Dupont-Passelaigue, E.; Le Roy, I.; Pignier, C. Preparation of N-[(4-substituted piperazin-1-yl)carbonyl]-heteroaryl sulfonamides and analogs as Kv potassium channel blockers. WO2012069503A1, 2012.
114. Okita, T.; Otsuka, N. Preparation of 1,3-benzothiazol-2(3H)-one derivatives, β2 receptor agonists containing them, their use as therapeutic drugs for COPD, and their glucuronides. JP2005187357A, 2005.
115. Commarieu, A.; Hoelderich, W.; Laffitte, J. A.; Dupont, M.-P., Fries rearrangement in methane sulfonic acid, an environmental friendly acid. *Journal of Molecular Catalysis A: Chemical* **2002,** *182-183*, 137-141.
116. Bagno, A.; Kantlehner, W.; Kress, R.; Saielli, G.; Stoyanov, E., Fries Rearrangement of Aryl Formates: A Mechanistic Study by Means of 1H, 2H, and 11B NMR Spectroscopy and DFT Calculations. *The Journal of Organic Chemistry* **2006,** *71* (25), 9331-9340.
117. Becker, R.; van den Broek, S. A. M. W.; Nieuwland, P. J.; Koch, K.; Rutjes, F. P. J. T., Optimisation and Scale-up of α-Bromination of Acetophenone in a Continuous Flow Microreactor. *Journal of Flow Chemistry* **2012,** *2* (3), 87-91.
118. Kaumann, A. J.; Morris, T. H.; Birnbaumer, L., A comparison of the influence of N-isopropyl and N-tert butyl substituents on the affinity of ligands for sinoatrial β-adrenoceptors in rat atria and β-adrenoceptors coupled to the adenylyl cyclase in kitten ventricle. *Naunyn-Schmiedeberg's Archives of Pharmacology* **1979,** *307* (1), 1-8.
119. Fairhurst, R. A.; Janus, D.; Lawrence, A., Synthesis of the Marine Sponge Derived β2-Adrenoceptor Agonist S1319. *Organic Letters* **2005,** *7* (21), 4697-4700.
120. Woiwode, T. F.; Rose, C.; Wandless, T. J., A Simple and Efficient Method for the Preparation of Hindered Alkyl−Aryl Ethers. *The Journal of Organic Chemistry* **1998,** *63* (25), 9594-9596.
121. Hodgkins, J. E.; Reeves, W. P., The Modified Kaluza Synthesis. III. The Synthesis of Some Aromatic Isothiocyanates. *The Journal of Organic Chemistry* **1964,** *29* (10), 3098-3099.
122. Stanetty, P.; Krumpak, B., Novel Synthesis of Benzothiazole Derivatives via Directed Lithiation and Aryne-Mediated Cyclization Followed by Quenching with Electrophiles. *The Journal of Organic Chemistry* **1996,** *61* (15), 5130-5133.
123. Shen, Z.; Dornan, P. K.; Khan, H. A.; Woo, T. K.; Dong, V. M., Mechanistic Insights into the Rhodium-Catalyzed Intramolecular Ketone Hydroacylation. *Journal of the American Chemical Society* **2009,** *131* (3), 1077-1091.

124. Pace, V.; Castoldi, L.; Alcantara, A. R.; Holzer, W., Highly efficient and environmentally benign preparation of Weinreb amides in the biphasic system 2-MeTHF/water. *RSC Advances* **2013,** *3* (26), 10158-10162.
125. Williams, D. B. G.; Lawton, M., Drying of Organic Solvents: Quantitative Evaluation of the Efficiency of Several Desiccants. *The Journal of Organic Chemistry* **2010,** *75* (24), 8351-8354.
126. Kato, S.; Harada, H.; Morie, T., Efficient synthesis of (6R)-6-amino-1-methyl-4-(3-methylbenzyl)hexahydro-1H-1,4-diazepine from methyl (2R)- and (2S)-1-benzyloxycarbonylaziridine-2-carboxylates. *Journal of the Chemical Society, Perkin Transactions 1* **1997,** (21), 3219-3226.
127. Parikh, J. R.; Doering, W. v. E., Sulfur trioxide in the oxidation of alcohols by dimethyl sulfoxide. *Journal of the American Chemical Society* **1967,** *89* (21), 5505-5507.
128. Nawrat, C. C.; Moody, C. J., Total Synthesis of Mevashuntin. *Organic Letters* **2012,** *14* (6), 1484-1487.
129. Kikkawa, H.; Kurose, H.; Isogaya, M.; Sato, Y.; Nagao, T., Differential contribution of two serine residues of wild type and constitutively active β2-adrenoceptors to the interaction with β2-selective agonists. *British Journal of Pharmacology* **2009,** *121* (6), 1059-1064.
130. Agustian, J.; Kamaruddin, A. H.; Bhatia, S., Single enantiomeric β-blockers—The existing technologies. *Process Biochemistry* **2010,** *45* (10), 1587-1604.
131. BAKER, J. G.; Fischer, P. M.; Fromont, C.; Gardiner, S. M.; Hill, S. J.; Jadhav, G.; Kellam, B.; Mistry, S.; WOOLARD, J. Novel ether linked compounds and improved treatments for cardiac and cardiovascular disease. 2012.
132. Tran, K.-V.; Bickar, D., Dakin−West Synthesis of β-Aryl Ketones. *The Journal of Organic Chemistry* **2006,** *71* (17), 6640-6643.
133. Basel, Y.; Hassner, A., Imidazole and Trifluoroethanol as Efficient and Mild Reagents for Destruction of Excess Di-tert-butyl Dicarbonate [(BOC)2O]. *Synthesis* **2001,** *2001* (04), 0550-0552.
134. Cepanec, I.; Litvić, M.; Mikuldaš, H.; Bartolinčić, A.; Vinković, V., Calcium trifluoromethanesulfonate-catalysed aminolysis of epoxides. *Tetrahedron* **2003,** *59* (14), 2435-2439.
135. Takagi, H.; Isoda, T.; Kusakabe, K.; Morooka, S., Effects of Solvents on the Hydrogenation of Mono-Aromatic Compounds Using Noble-Metal Catalysts. *Energy & Fuels* **1999,** *13* (6), 1191-1196.
136. Azizi, N.; Saidi, M. R., Solid lithium perchlorate as a powerful catalyst for the synthesis of β-aminoalcohols under solvent-free conditions. *Canadian Journal of Chemistry* **2005,** *83* (5), 505-507.
137. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D., Reductive Amination of Aldehydes and Ketones with Sodium Triacetoxyborohydride. Studies on Direct and Indirect Reductive Amination Procedures1. *The Journal of Organic Chemistry* **1996,** *61* (11), 3849-3862.
138. Lane, C. F., Sodium Cyanoborohydride - A Highly Selective Reducing Agent for Organic Functional Groups. *Synthesis* **1975,** *1975* (03), 135-146.
139. Pujala, B.; Rana, S.; Chakraborti, A. K., Zinc Tetrafluoroborate Hydrate as a Mild Catalyst for Epoxide Ring Opening with Amines: Scope and Limitations of Metal Tetrafluoroborates and Applications in the Synthesis of Antihypertensive Drugs (RS)/(R)/(S)-Metoprolols. *The Journal of Organic Chemistry* **2011,** *76* (21), 8768-8780.
140. Fabio, B.; Silvia, C.; Renzo, R., Palladium- and Copper-Mediated Direct C-2 Arylation of Azoles — Including Free (NH)-Imidazole, -Benzimidazole and -Indole — Under Base-Free and Ligandless Conditions. *European Journal of Organic Chemistry* **2006,** *2006* (6), 1379-1382.

141. Mohammadpoor-Baltork, I.; Abdollahi-Alibeik, M., Mild, efficient, and chemoselective dehydrogenation of 2-imidazolines, bis-imidazolines, and N-substituted-2-imidazolines with potassium permanganate supported on montmorillonite K-10. *Canadian Journal of Chemistry* **2005,** *83* (2), 110-114.
142. Wiglenda, T.; Gust, R., Structure−Activity Relationship Study To Understand the Estrogen Receptor-Dependent Gene Activation of Aryl- and Alkyl-Substituted 1H-Imidazoles. *Journal of Medicinal Chemistry* **2007,** *50* (7), 1475-1484.
143. Chen, J.; Wang, Z.; Li, C.-M.; Lu, Y.; Vaddady, P. K.; Meibohm, B.; Dalton, J. T.; Miller, D. D.; Li, W., Discovery of Novel 2-Aryl-4-benzoyl-imidazoles Targeting the Colchicines Binding Site in Tubulin As Potential Anticancer Agents. *Journal of Medicinal Chemistry* **2010,** *53* (20), 7414-7427.
144. Bowman, R. K.; Brown, A. D.; Cobb, J. H.; Eaddy, J. F.; Hatcher, M. A.; Leivers, M. R.; Miller, J. F.; Mitchell, M. B.; Patterson, D. E.; Toczko, M. A.; Xie, S., Synthesis of HCV Replicase Inhibitors: Base-Catalyzed Synthesis of Protected α-Hydrazino Esters and Selective Aerobic Oxidation with Catalytic Pt/Bi/C for Synthesis of Imidazole-4,5-dicarbaldehyde. *The Journal of Organic Chemistry* **2013,** *78* (23), 11680-11690.
145. Xiao, T.; Xiong, S.; Xie, Y.; Dong, X.; Zhou, L., Copper-catalyzed synthesis of benzazoles via aerobic oxidative condensation of o-amino/mercaptan/hydroxyanilines with benzylamines. *RSC Advances* **2013,** *3* (36), 15592-15595.
146. Belot, V.; Farran, D.; Jean, M.; Albalat, M.; Vanthuyne, N.; Roussel, C., Steric Scale of Common Substituents from Rotational Barriers of N-(o-Substituted aryl)thiazoline-2-thione Atropisomers. *The Journal of Organic Chemistry* **2017,** *82* (19), 10188-10200.
147. Cho, Y. W.; Aviado, D. M.; Lish, P. M., Efficacy of a new bronchodilator, soterenol, on experimental locked-lung syndrome in dogs. *Journal of Allergy and Clinical Immunology* **1968,** *42* (1), 36-48.
148. Avery, M. A.; Verlander, M. S.; Goodman, M., Synthesis of 6-aminoisoproterenol. *The Journal of Organic Chemistry* **1980,** *45* (14), 2750-2753.
149. King, L. C.; Ostrum, G. K., Selective Bromination with Copper(II) Bromide1. *The Journal of Organic Chemistry* **1964,** *29* (12), 3459-3461.
150. Blažzević, N.; Kolbah, D.; Belin, B.; Šunjić, V.; Kajfež, F., Hexamethylenetetramine, A Versatile Reagent in Organic Synthesis. *Synthesis* **1979,** *1979* (03), 161-176.
151. Donaldson, K. S., B. Uehling, D. Therapeutic biaryl derivatives. 26/06/2001, 2001.
152. Kharidia, J.; Fogarty, C. M.; LaForce, C. F.; Maier, G.; Hsu, R.; Dunnington, K. M.; Curry, L.; Baumgartner, R. A.; Hanrahan, J. P., A pharmacokinetic/pharmacodynamic study comparing arformoterol tartrate inhalation solution and racemic formoterol dry powder inhaler in subjects with chronic obstructive pulmonary disease. *Pulmonary Pharmacology & Therapeutics* **2008,** *21* (4), 657-662.
153. Westerhof, F. J.; Zuidhof, A. B.; Kok, L.; Meurs, H.; Zaagsma, J., Effects of salbutamol and enantiomers on allergen-induced asthmatic reactions and airway hyperreactivity. *European Respiratory Journal* **2005,** *25* (5), 864.
154. Huber, T.; Menon, S.; Sakmar, T. P., Structural Basis for Ligand Binding and Specificity in Adrenergic Receptors: Implications for GPCR-Targeted Drug Discovery. *Biochemistry* **2008,** *47* (42), 11013-11023.
155. Boulton, D. W.; Fawcett, J. P., Beta2-agonist eutomers: a rational option for the treatment of asthma? *American journal of respiratory medicine : drugs, devices, and other interventions* **2002,** *1* (5), 305-11.
156. Quallich, G. J.; Blake, J. F.; Woodall, T. M., A combined synthetic and ab initio study of chiral oxazaborolidines structure and enantioselectivity relationships. *Journal of the American Chemical Society* **1994,** *116* (19), 8516-8525.
157. Allinger, N. L., Table of Conformational Energies—1967. In *Topics in Stereochemistry*, Allinger, N. L., Ed.

158. Schneider, H. J.; Hoppen, V., Carbon-13 nuclear magnetic resonance substituent-induced shieldings and conformational equilibriums in cyclohexanes. *The Journal of Organic Chemistry* **1978,** *43* (20), 3866-3873.
159. Fossey, J. S.; Anslyn, E. V.; Brittain, W. D. G.; Bull, S. D.; Chapin, B. M.; Le Duff, C. S.; James, T. D.; Lees, G.; Lim, S.; Lloyd, J. A. C.; Manville, C. V.; Payne, D. T.; Roper, K. A., Rapid Determination of Enantiomeric Excess via NMR Spectroscopy: A Research-Informed Experiment. *Journal of Chemical Education* **2017,** *94* (1), 79-84.
160. Nelson, H. S.; Bensch, G.; Pleskow, W. W.; DiSantostefano, R.; DeGraw, S.; Reasner, D. S.; Rollins, T. E.; Rubin, P. D., Improved bronchodilation with levalbuterol compared with racemic albuterol in patients with asthma. *Journal of Allergy and Clinical Immunology* **1998,** *102* (6), 943-952.
161. Zhang, X.-Y.; Zhu, F.-X.; Olszewski, M. A.; Robinson, N. E., Effects of enantiomers of β2-agonists on ACh release and smooth muscle contraction in the trachea. *American Journal of Physiology-Lung Cellular and Molecular Physiology* **1998,** *274* (1), L32-L38.
162. Johnson, M.; Butchers, P. R.; Coleman, R. A.; Nials, A. T.; Strong, P.; Summer, M. J.; Vardey, C. J.; Whelan, C. J., The pharmacology of salmeterol. *Life Sciences* **1993,** *52* (26), 2131-2143.
163. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings1PII of original article: S0169-409X(96)00423-1. The article was originally published in Advanced Drug Delivery Reviews 23 (1997) 3–25.1. *Advanced Drug Delivery Reviews* **2001,** *46* (1), 3-26.
164. Sharpe, C. J.; Palmer, P. J.; Evans, D. E.; Brown, G. R.; King, G.; Shadbolt, R. S.; Trigg, R. B.; Ward, R. J.; Ashford, A.; Ross, J. W., Basic ethers of 2-anilinobenzothiazoles and 2-anilinobenzoxazoles as potential antidepressants. *Journal of Medicinal Chemistry* **1972,** *15* (5), 523-529.
165. Beattie, D.; Bradley, M.; Brearley, A.; Charlton, S. J.; Cuenoud, B. M.; Fairhurst, R. A.; Gedeck, P.; Gosling, M.; Janus, D.; Jones, D.; Lewis, C.; McCarthy, C.; Oakman, H.; Stringer, R.; Taylor, R. J.; Tuffnell, A., A physical properties based approach for the exploration of a 4-hydroxybenzothiazolone series of β2-adrenoceptor agonists as inhaled long-acting bronchodilators. *Bioorganic & Medicinal Chemistry Letters* **2010,** *20* (17), 5302-5307.
166. Basel, Y.; Hassner, A., Di-tert-butyl Dicarbonate and 4-(Dimethylamino)pyridine Revisited. Their Reactions with Amines and Alcohols1. *The Journal of Organic Chemistry* **2000,** *65* (20), 6368-6380.
167. Herkommer, D.; Dreisigacker, S.; Sergeev, G.; Sasse, F.; Gohlke, H.; Menche, D., Design, Synthesis, and Biological Evaluation of Simplified Side Chain Hybrids of the Potent Actin Binding Polyketides Rhizopodin and Bistramide. *ChemMedChem* **2015,** *10* (3), 470-489.
168. Ghosh, S. C.; Ngiam, J. S. Y.; Seayad, A. M.; Tuan, D. T.; Johannes, C. W.; Chen, A., Tandem oxidative amidation of benzyl alcohols with amine hydrochloride salts catalysed by iron nitrate. *Tetrahedron Letters* **2013,** *54* (36), 4922-4925.
169. DeRuiter, J.; Davis, R. A.; Wandrekar, V. G.; Mayfield, C. A., Relative structure-inhibition analyses of the N-benzoyl and N-(phenylsulfonyl) amino acid aldose reductase inhibitors. *Journal of Medicinal Chemistry* **1991,** *34* (7), 2120-2126.
170. Pchelka, B. K.; Loupy, A.; Petit, A., Improvement and simplification of synthesis of 3-aryloxy-1,2-epoxypropanes using solvent-free conditions and microwave irradiations. Relation with medium effects and reaction mechanism. *Tetrahedron* **2006,** *62* (47), 10968-10979.

171. Kamal, A.; Khanna, G. B. R.; Krishnaji, T.; Tekumalla, V.; Ramu, R., New chemoenzymatic pathway for β-adrenergic blocking agents. *Tetrahedron: Asymmetry* **2005,** *16* (8), 1485-1494.
172. Pchelka, B.; Plenkiewicz, J., MICROWAVE-PROMOTED SYNTHESIS OF 1-ARYLOXY-3-ALKYLAMINO-2-PROPANOLS. *Organic Preparations and Procedures International* **1998,** *30* (1), 87-90.
173. Tacon, C.; Guantai, E. M.; Smith, P. J.; Chibale, K., Synthesis, biological evaluation and mechanistic studies of totarol amino alcohol derivatives as potential antimalarial agents. *Bioorganic & Medicinal Chemistry* **2012,** *20* (2), 893-902.
174. Kopka, K.; Wagner, S.; Riemann, B.; Law, M. P.; Puke, C.; Luthra, S. K.; Pike, V. W.; Wichter, T.; Schmitz, W.; Schober, O.; Schäfers, M., Design of new β1-selective adrenoceptor ligands as potential radioligands for in vivo imaging. *Bioorganic & Medicinal Chemistry* **2003,** *11* (16), 3513-3527.
175. Sayyed, I. A.; Thakur, V. V.; Nikalje, M. D.; Dewkar, G. K.; Kotkar, S. P.; Sudalai, A., Asymmetric synthesis of aryloxypropanolamines via OsO4-catalyzed asymmetric dihydroxylation. *Tetrahedron* **2005,** *61* (11), 2831-2838.
176. Gold, E. H.; Chang, W.; Cohen, M.; Baum, T.; Ehrreich, S.; Johnson, G.; Prioli, N.; Sybertz, E. J., Synthesis and comparison of some cardiovascular properties of the stereoisomers of labetalol. *Journal of Medicinal Chemistry* **1982,** *25* (11), 1363-1370.
177. Fuhrer, W.; Ostermayer, F.; Zimmermann, M.; Meier, M.; Mueller, H., .beta.-Adrenergic blocking agents: substituted phenylalkanolamines. Effect of side-chain length on .beta.-blocking potency in vitro. *Journal of Medicinal Chemistry* **1984,** *27* (7), 831-836.
178. Kargar, H., Rapid and efficient biomimetic oxidation of 2-imidazolines to their corresponding imidazoles with NaIO4 catalyzed by Mn(salophen)Cl. *Inorganic Chemistry Communications* **2011,** *14* (6), 863-866.
179. Lee, E.; Ryu, T.; Shin, E.; Son, J.-Y.; Choi, W.; Lee, P. H., Synthesis of 2-Bromoimidazoles from Alkynes, N-Sulfonylazides, and Bromocyanides. *Organic Letters* **2015,** *17* (10), 2470-2473.
180. Hong, L.; Shao, Y.; Zhang, L.; Zhou, X., Ln[N(SiMe3)2]3-Catalyzed Cross-Diinsertion of CN/CC into an NH Bond: Facile Synthesis of 1,2,4-Trisubstituted Imidazoles from Propargylamines and Nitriles. *Chemistry – A European Journal* **2014,** *20* (28), 8551-8555.
181. Yang, J.; Liu, X.; Huang, C.; Zhou, C.; Li, Y.; Zhu, D., Construction and Photophysical Properties of Organic–Inorganic Nanonetworks Based on Oligo(phenylenevinylene) and Functionalized Gold Nanoparticles. *ChemPhysChem* **2010,** *11* (3), 659-664.
182. Baldwin, J. J.; Engelhardt, E. L.; Hirschmann, R.; Lundell, G. F.; Ponticello, G. S.; Ludden, C. T.; Sweet, C. S.; Scriabine, A.; Share, N. N.; Hall, R., .beta.-Adrenergic blocking agents with acute antihypertensive activity. *Journal of Medicinal Chemistry* **1979,** *22* (6), 687-694.
183. Schmitt, M. L.; Hauser, A.-T.; Carlino, L.; Pippel, M.; Schulz-Fincke, J.; Metzger, E.; Willmann, D.; Yiu, T.; Barton, M.; Schüle, R.; Sippl, W.; Jung, M., Nonpeptidic Propargylamines as Inhibitors of Lysine Specific Demethylase 1 (LSD1) with Cellular Activity. *Journal of Medicinal Chemistry* **2013,** *56* (18), 7334-7342.
184. Cai, Y.; Lian, M.; Li, Z.; Meng, Q., Enantioselective α-hydroxylation of β-keto esters catalyzed by chiral S-timolol derivatives. *Tetrahedron* **2012,** *68* (38), 7973-7977.
185. de Koning, P. D.; Gladwell, I. R.; Moses, I. B.; Panesar, M. S.; Pettman, A. J.; Thomson, N. M., Development of an Enabling Route to PF-00610355: A Novel Inhaled β2-Adrenoreceptor Agonist. *Organic Process Research & Development* **2011,** *15* (6), 1247-1255.
186. Washburn, W. N.; Sher, P. M.; Poss, K. M.; Girotra, R. N.; McCann, P. J.; Gavai, A. V.; Mikkilineni, A. B.; Mathur, A.; Cheng, P.; Dejneka, T. C.; Sun, C. Q.; Wang, T. C.; Harper, T. W.; Russell, A. D.; Slusarchyk, D. A.; Skwish, S.; Allen, G. T.; Hillyer, D. E.; Frohlich, B. H.;

Abboa-Offei, B. E.; Cap, M.; Waldron, T. L.; George, R. J.; Tesfamariam, B.; Ciosek, C. P.; Ryono, D.; Young, D. A.; Dickinson, K. E.; Seymour, A. A.; Arbeeny, C. M.; Gregg, R. E., Beta 3 agonists. Part 1: evolution from inception to BMS-194449. *Bioorganic & Medicinal Chemistry Letters* **2001,** *11* (23), 3035-3039.
187. Matsubara, K. Method for the preparation of tricyclic amino alcohol derivatives through azides. **2002**.
188. Hu, B.; Ellingboe, J.; Han, S.; Largis, E.; Lim, K.; Malamas, M.; Mulvey, R.; Niu, C.; Oliphant, A.; Pelletier, J.; Singanallore, T.; Sum, F.-W.; Tillett, J.; Wong, V., Novel (4-Piperidin-1-yl)-phenyl Sulfonamides as Potent and Selective Human β3 Agonists. *Bioorganic & Medicinal Chemistry* **2001,** *9* (8), 2045-2059.