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Synthesis of potential inhibitors of estrone sulfatase in the treatment of hormone-dependent breast cancer

A THESIS SUBMITTED IN ACCORDANCE WITH THE CONDITIONS GOVERNING CANDIDATES FOR THE DEGREE OF DOCTOR OF PHILOSOPHIAE

BY

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Rest in peace knowing all is and will be fine with those left behind.

ABSTRACT

Estrone sulfatase (E1STS) belongs to a family of enzymes, namely the steroid sulfatases, which catalyse the conversion of the biologically inactive compound to the more potent biologically active steroid. In particular, E1STS catalyses the conversion of estrone sulfate to estrone (E1) and is therefore a pivotal enzyme in the progression of hormone-dependent breast cancer in postmenopausal women. The use of aromatase (AR) inhibitors, such as anastrozole, has led to the reduction of plasma levels of E1 by as much as 98%, however, it has been suggested that the high levels of E1 found within breast cancer cells are due to the activity of E1STS and is therefore a non-AR route which is not affected through the use of AR inhibitors.

A number of compounds, both steroidal and non-steroidal, have been synthesised and subsequently evaluated as inhibitors of E1STS, however, from the ranges of compounds evaluated, 667-COUMATE remains the only compound to enter Phase I clinical trials. Within our own group, we have previously synthesised a number of compounds based on the 4-sulfamoylated derivatives of a series of alkyl 4-hydroxybenzoic acid esters, indeed, the cyclo-octyl derivative was found to be more potent than 667-COUMATE. However, these compounds are known to be unstable in the plasma due to the presence (and action) of esterases. As such, we considered the synthesis of 4-sulfonated derivatives of both the mono- and di-substitiuted *N*-alkyl-4-hydroxybenzamide. In the synthesis of the target compounds, we utilised a reaction scheme which involved the initial synthesis of the mono- and di-substituted *N*-alkyl-4-hydroxybenzamide followed by the conversion of the 4-hydroxy moiety to the sulfamate, methansulfonate or trifluoromethansulfonate derivatives. However, a number of difficulties in the synthesis and purification of the *N*-alkyl-4-hydroxybenzamide from 4-hydroxybenzoic acid led us to consider the use of a protecting group for the 4-hydroxy moiety. As such, we considered (and utilised) the initial synthesis of 4-acetoxybenzoic acid followed by the synthesis of *N*-alkyl-4-hydroxybenzamide via the appropriate acyl chloride. The conversion of the 4-hydroxy moiety to the sulfonate derivatives involved the reaction between *N*-alkyl-4-hydroxybenzamide and the appropriate sulfonyl chloride. In general, the reactions proceeded in moderate to good yield with a few problems, namely the purification of the target compounds. Although the sulfonated products are currently undergoing biochemical evaluation, initial pK_a (a physicochemical factor which has been shown to play an important role in determining biochemical activity) studies suggest that the benzamide-based compounds are potentially weak inhibitors of E1STS.

In an effort to produce novel inhibitors of E1STS, the synthesis of non-phenolic based inhibitors was also considered within the current study. In particular, synthesis of sulfamoylated derivatives of alkyl and benzyl alcohols was undertaken involving a reaction between the appropriate alcohol and aminosulfonyl chloride. In general, the reactions proceeded in low to good yield with the main problem once again being the purification of the product. These compounds are also currently undergoing biochemical evaluation, however, from molecular modelling studies undertaken within the group, they would initially appear to be weak inhibitors of E1STS. Within this range, however, the α-substituted halogen containing compounds (as these possess the potential to stabilise the alkoxide ion being formed as a result of the hydrolysis of the sulfamate moiety) may be the more potent inhibitors in comparison to the non-halogenated compounds.

Abbreviations

δ_C	^{13}CNMR
δ_H	^{1}HNMR
λ	wavelength
ν	wave number
μM	micro molar
17β-HSD	17β-hydroxysteroid dehydrogenase
AR	aromatase
ASA	aryl sulfatase A
ASB	aryl sulfatase B
ASCl	amino sulfonyl chloride
br.s	broad singlet
br.d	broad doublet
CDK	cyclin dependent kinase
CKI	CDK inhibitor
d	doublet
DCM	dichloromethane
dd	doublet of doublets
DHEAS	dehydroepiandrosterone sulfate
DHEA-STS	dehydroepiandrosterone sulfatase
DMA	*N,N*-dimethylacetamide
DMF	*N,N*-dimethylformamide
DNA	deoxyribonucleic acid
E1	estrone
E1S	estrone sulfate
E2	estradiol
ER	estrogen receptors
E1STS	estrone sulfatase
et al.	and others
FGly	formyl glycine
g	grams
GCMS	gas chromatography-mass spectrometry

h	hour(s)
HRMS	high resolution mass spectra
HRT	hormone replacement therapy
Hz	hertz
IC_{50}	compound concentration that gives 50% inhibition
K_2CO_3	potassium carbonate
K_i	inhibition constant
$K_{i,app}$	apparent inhibition constant
K_m	Michaelis constant
LRMS	low resolution mass spectra
MHL	Madurahydroxylactone
M	molar (mol/dm^3)
m.p.	melting point
mg	milligram
MHz	mega hertz
min	minute(s)
ml	millilitre
mmol	millimoles
MS	mass spectrometry
MSCl	methane sulfonyl chloride
$NaCO_3$	sodium carbonate
NADPH	nicotinamide adenine dinucleotide phosphate
NaH	sodium hydride
nM	nanomolar
pK_a	acid dissociation constant
q	quartet
R_f	retention factor
s	singlet
sex	sextuplets
SHBG	sex hormone binding globulin
STS	Steroid sulfatase
t	triplets
tt	triplet of triplets
td	triplet of doublets

T	testosterone
TEA	triethylamine
TFMSCl	trifluoromethane sulfonyl chloride
TLC	thin layer chromatography
t_R	retention time
v/v	volume per volume
yr	year(s)

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CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

1.1 Cancer

Cancer describes the cellular disease state in which the normal reproductive function of cells has been compromised, leading to abnormal and/or uncontrolled cell division. Cancer can be categorised by the tissue of origin, as such, there are over 100 forms of the disease, having a number of subtypes depending on the affected organ and course of disease progression. Due to the variety of the forms the disease takes, there is no general aetiology of cancer, and so treatment is determined on an individual basis, according to the tissue in which the cancer manifests (Jefford and Irminger-Finger, 2006).

1.2 Cellular basis of cancer

The behaviour and characteristics of cancerous cells differ from that of surrounding healthy cells, usually progressing sequentially through the main disease markers at varying rates. These markers include abnormal cell proliferation, loss of tissue specific character, invasiveness of adjacent tissue and finally metastasis, with the infiltration of body cavaties and fluid transport systems i.e. blood circulatory and lymph systems. An understanding of the nature of normal and abnormal cell behaviour can give an indication of how best to approach the research into drug development for cancer.

1.2.1 The cell cycle

When behaving normally, cells follow the cell cycle, a repeating pattern of well regulated events required to produce new cells (Figure 1); in general, this occurs to replace damaged or dead cells in response to various growth-stimulating factors (Reynolds and Schecker, 1995). The speed at which the cells go through the cell cycle differs from tissue to tissue, depending on the cell function e.g. the turn around for the cycle will be more rapid in the cells of the stomach lining and skin, than those of the muscle.

The cycle is considered to be composed of four major steps; the cycle begins with the preparation of the DNA for replication, in G_1 phase. At this point, there is a pause in the process, a restriction point, where feedback controls are able to

halt the duplication of DNA if damage is detected. The cycle continues with stimulation provided by growth factors going on to the S and G_2 phases where the main processes associated with mitosis occur. G_0 represents a rest phase where the activities of replication do not take place (Hartwell and Weinert, 1989). As cells differentiate they may leave the cycle entirely thereby resulting in specialised cells that have no capacity to replicate.

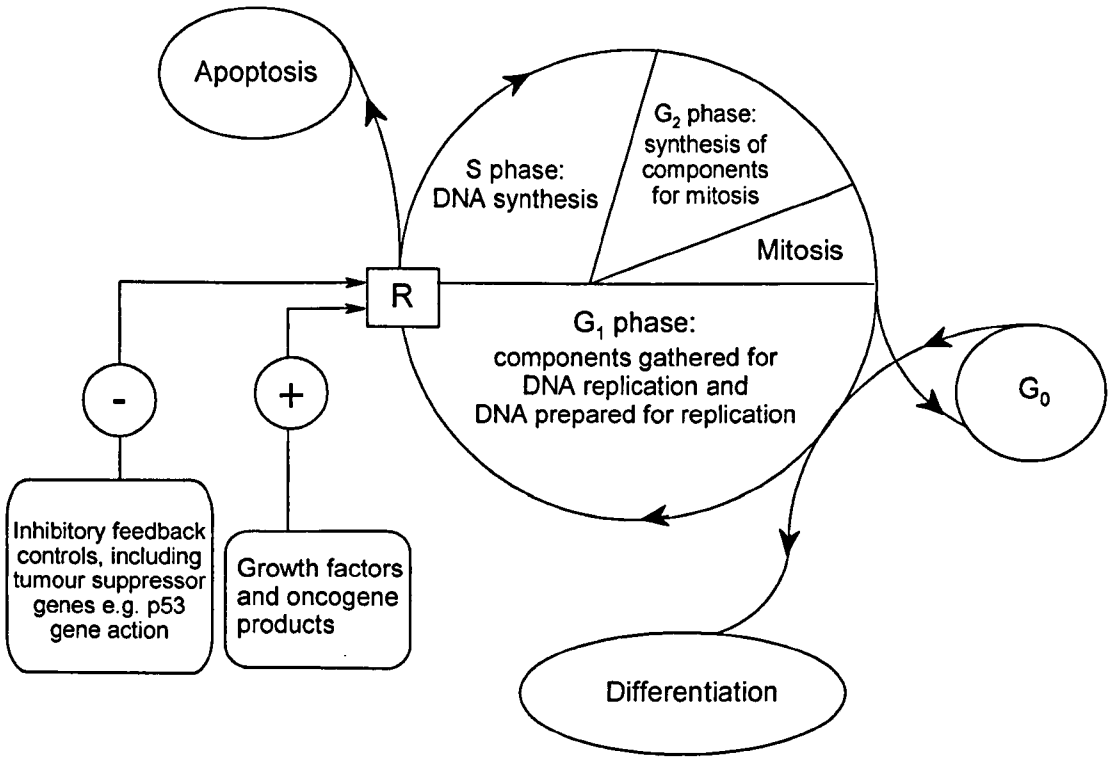


Figure 1: Cell cycle, adapted from Rang *et al.* (1998).

1.2.2 Cell cycle controls

The control of the cell cycle is highly regulated by a number of interconnecting protein-based mechanisms. The main family of proteins that control the progression of the cell cycle are the cyclins, in particular, the cyclin dependent kinases (CDKs), the cyclin dependent kinase inhibitors (CKIs) and the tumour suppressor gene product, p53 (Gali-Muhtasib and Bakkar, 2002; Golias *et al.*, 2005). Gene alterations in neoplasia generally affect three pathways: the cell cycle, apoptosis and differentiation of the cell. As such, changes in one of the three pathways can have a major impact on the normal functioning of another and therefore lead to the formation of tumours (Corn and El-Deiry, 2002).

1.2.3 The p53 protein

The tumour suppressor gene product, the p53 protein, is essential to cell replication. In normal cell replication, p53 prevents uncontrolled proliferation by an arrest in the G1 phase of the cell cycle and apoptosis (Kristensen and Børresen-Dale, 2000); this may be in response to genetoxic damage via radiation or chemical mutagenesis (Bitton *et al.*, 2005). This is achieved mainly through its ability to act as a transcription factor, binding to specific sites of the p53-responsive genes (Meek, 2004), allowing for DNA repair ahead of replication and cell division.

In studies it has been noted that mutations in the p53 gene have been found in approximately 50% of all human cancers (Soussi and Béroud, 2001), with p53 signaling dysfunctions found in over 80% of all cancers (Donehower, 2006). Cells that express mutant p53 do not arrest at the restriction point and are likely to proceed into S phase or G_2/M phases with DNA damage. The loss of this gene's function can lead to compound errors, progressing to tumescent cell growth.

1.3 Breast cancer aetiology and pathogenisis

The aetiology of hormone-dependent breast cancer development has yet to be fully elucidated. Many epidemiological studies have been undertaken to rationalise the initiation and progression of the disease, however, the results of many have been vague at best and contradictiory at worst.

Discussed here are some of the proposed factors which may be involved in the aetiology of breast cancer, however, it is not fully understood whether it is just one or a combination of many factors that bring about the onset of breast cancer (Table 1).

Whilst breast cancer is predominantly a female disease, male breast cancer has been shown to be an equally aggressive disease although the difference in incidence between males and females differs by over 100 fold (Adami *et al.*, 1995). Incidence of breast cancer in different populations has also been shown

to be more environmental than genetic. For instance, Japan has a lower rate of breast cancer than that observed in North America, however, migrants from Japan within America have been found to possess similar rates of incidence of the disease as those of the native population within the second and third generations.

Factor	**High risk**	**Low risk**
Gender	Female	Male
Birth country	N. America, N. Europe	Asia, Africa
Age of menarche/ menopause	<12/ >55yr	>14/ <45yr
Age of 1st full term pregnancy	>30	<20
Age	>45	<25
Relatives diagnosed at early age	Yes	No
History of breast cancer	Yes	No
History of other hormone-dependent cancer (endometrial or ovarian)	Yes	No
BRCA1/2	Yes	No
Familial history of disease	Yes	No
Oral contraception/ HRT	Yes	No
Weight	Obese	Under weight

Table 1: Summary of established breast cancer risk factors (Hulka and Moorman, 2001; Key *et al.*, 2001).

The length of time between the onset of puberty and the age at which menopause is reached (the 'estrogen window hypothesis') also appears to have a profound effect on the risk of breast cancer. This observation was supported by the decrease in breast cancer incidence discovered in patients who had undergone bilateral oopherectomy before menopause, particularly if relatively early in life (Key *et al.*, 2001).

Early age of first full-term pregnancy has also been shown to confer some protection from the onset of breast cancer. The risk appears to decrease with each successive child, especially if the first born was before the age of 20 years.

The decrease in risk is hypothesised to be due to the interruption of the menstrual cycle, breaking up the amount of exposure the hormone-dependent cells have to hormonal stimuli (i.e. narrowing of the 'estrogen window') (Travis and Key, 2003).

Incidence of the disease appears to start to increase in females between 25 and 30 years of age, rising dramatically after the age of 40 (Table 1; Hulka and Moorman, 2001). As such, breast cancer remains the leading cause of morbidity due to cancer for females between 20 and 59 years of age, and the second and third cause of cancer death in women aged between 60 and 79 and over 80 years of age, respectively (Sakorafas *et al.*, 2002). The age at which the disease is diagnosed has also been observed to be a major factor in survivability of the disease; in general, the onset of breast cancer in premenopausal women often results in tumour cells having a far more rapid and aggressive disease profile than if the disease was diagnosed in post-menopuasal women.

Predisposition has also been observed in women who have been shown to express the BRCA1 and BRCA2 genes. The BRCA genes are responsible for the encoding of proteins that are involved in DNA repair and cell cycle control usually in G_1 and early S phase (Vogelstein and Kinzler, 2004). Furthermore, the inherited risk factors for the familial risk for breast cancer is then increased, with the gene being found in 80-90% of cases where a familial link was postulated to be the cause of breast cancer (Kristensen and Børresen-Dale, 2000). The presence of this gene has been found to induce around 45% of hormone-dependent breast cancer tumours and is particularly prevalent in women suffering from early onset breast cancer (Wooster *et al.*, 2003).

The use of exogenous sources of estrogen, such as oral contraceptives and hormone replacement therapy (HRT), has also been shown to increase the risk of breast cancer development. With contraception, the short term risks are relatively low, especially in younger females, however, the risk of the disease increases with chronic use (five or more years continuous treatment). The same pattern was also observed with HRT, that is, long term use resulted in up to 35% increased risk of development of breast cancer. The cessation of exposure to the

external estrogens would appear to lower the risk, returning to 'normal' levels over an extended period of time (Travis and Key, 2003).

1.4 Estrogens and breast cancer

From the preceeding discussion, it is clear that the aetiology of breast cancer still remains unclear, however, a strong correlation appears to exist between exposure to estrogen and the risk of breast cancer initiation and progression. In general, factors affecting the length of exposure to estrogens seems to be of particular importance, with prolonged contact without cessation increasing the risk of development of hormonal dependent breast cancer (Key *et al.*, 2001; Thijssen, 2004).

A direct link between estrogens and breast cancer was shown by Beatson in 1896. Furthermore, approximately two-thirds of breast cancers occur after the menopause in women, and studies have shown that tumours have elevated levels of estrogens compared to free estrogen levels observed in plasma. This led to the conclusion that breast cancer cells possess the requisite enzymes to synthesise and interconvert estrogens locally (Nakata *et al.*, 2003).

The biosythesis of steroids plays a pivotal role in the bioregulation of a great number of systems essential to maintaining the normal functioning of the body. The majority of sex steroid production occurs in the adrenal cortex, ovaries and the placenta. Biosynthesis of steroids involves the initial synthesis of cholesterol, which in turn is synthesised within the body from acetate using acetyl coenzyme A derived from dietary intake. Cholesterol is then converted to the sex steroids (androgens and estrogens) via a series of steroid precursors in the steroidal cascade, with various enzymes catalysing the biosynthetic steps (Figure 2). The major biosynthetic step in the synthesis of estrogens is therefore the aromatisation of the A-ring of androstenedione by aromatase (AR) leading to estrone (E1) while subsequent enzymes convert E1 to the most potent estrogen, namely estradiol (E2).

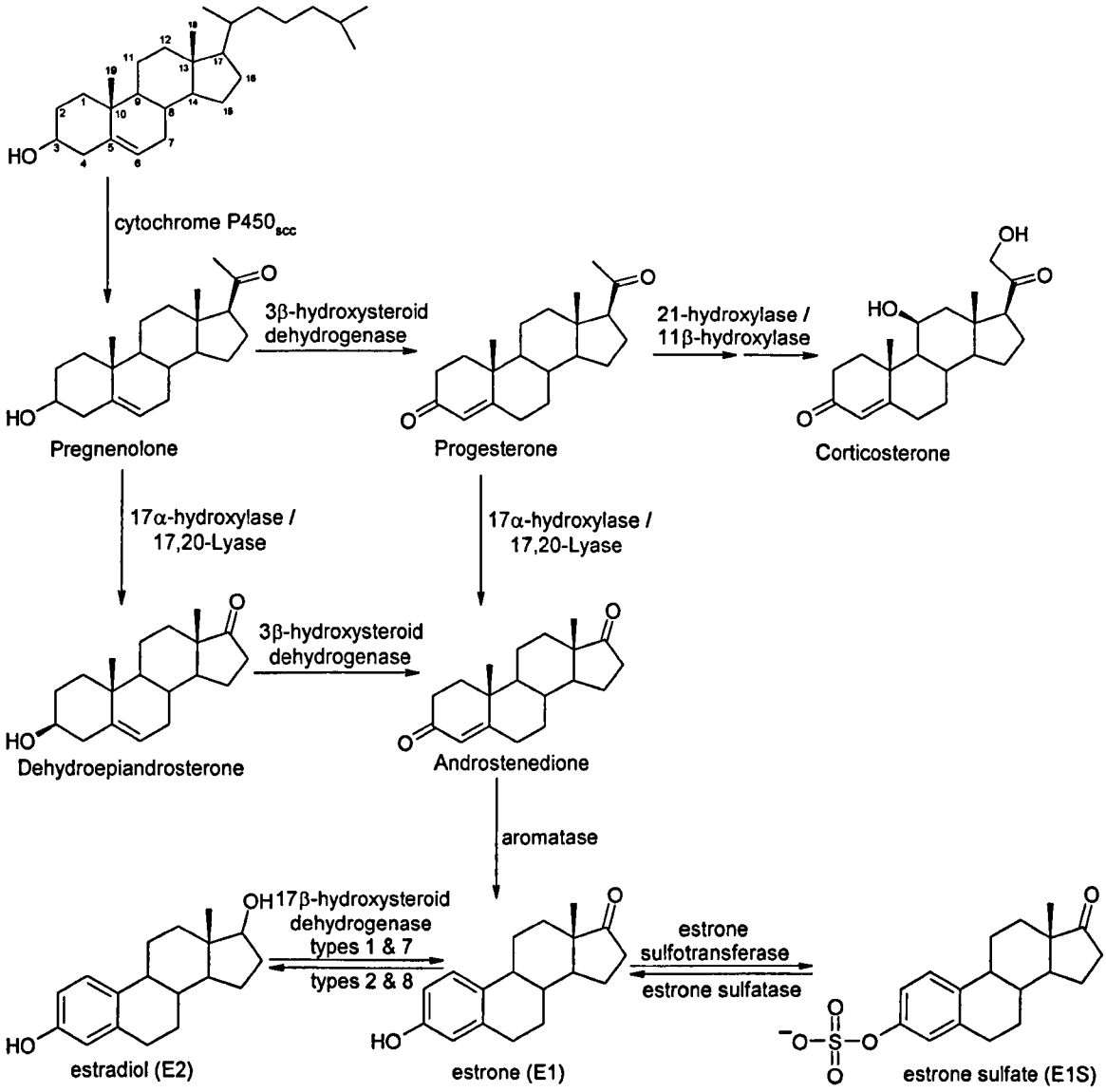


Figure 2: Enzymes involved in the regulation of steroid biosynthesis.

The majority of estrogens produced are transported within the plasma mainly bound to sex hormone binding globulin (SHBG) or albumin. However, once the estrogen molecules enter hormone-dependent cells they interact with estrogen receptors (ER) to illicit an estrogenic response (Figure 3).

That is, estrogens (e.g. E1 or E2) enter the target cell via diffusion across the cellular membrane, where they bind to an ER to form an estrogen-ER complex. This complex is activated by transcription coregulators, dimerises, and then interacts with the appropriate portions of DNA (DNA estrogen-response elements), leading to cell proliferation (Girault *et al.*, 2006). Of the estrogens, E2 has been shown to be the stronger ligand and hence its potent estrogenic

response, having an approximate 10-fold positive effect on the proliferation of breast cancer cells over E1.

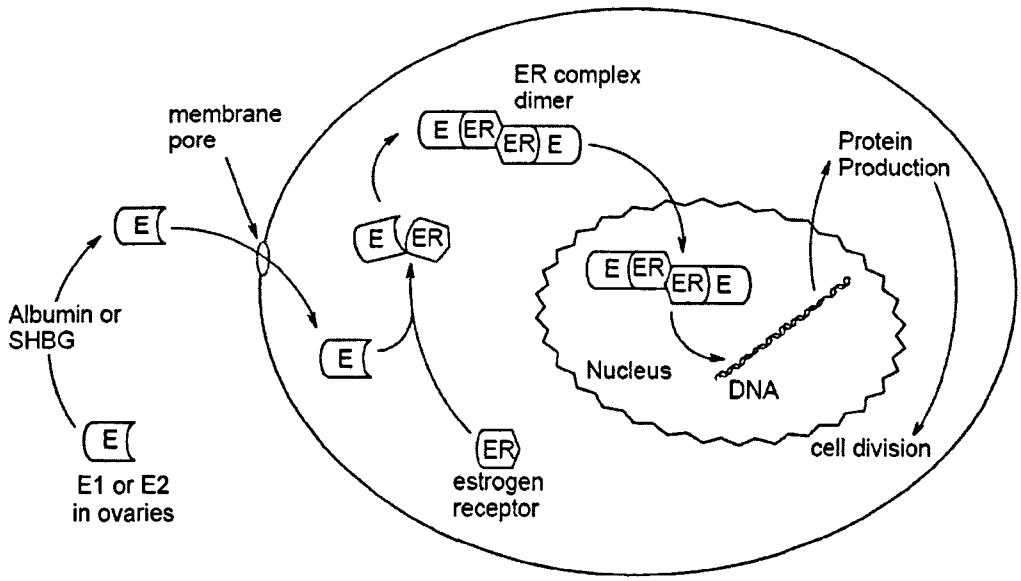


Figure 3: Estrogen-ER interaction within a hormone-dependent cell (adapted from Girault *et al.*, 2006).

From the consideration of the role of the steroidal cascade in the biosynthesis of the estrogens and the aetiology of breast cancer, we can conclude that factors which result in the lowering of the estrogen levels within tumours would lead to cell death, as such, the enzymes involved in estrogen biosynthesis are important targets in the fight against hormone-dependent breast cancer. Some of these enzymes will now be discussed.

1.5 17β-Hydroxysteroid dehydrogenase (17β-HSD)

17β-Hydroxysteroid dehydrogenase (17β-HSD) is a family of enzymes responsible for the biosynthesis of the active form of various sex hormones, in particular, E2 (Figure 4) and testosterone (T). 17β-HSD enters the steroidogenic pathway at the final step of the biosynthesis of E2, converting the C17=O group to a C17-β hydroxyl moiety - an important group for potent biological activity (Luu-The, 2001).

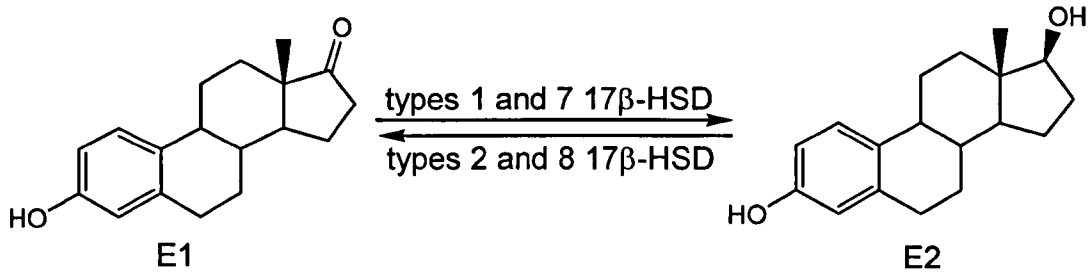


Figure 4: Interconversion of E1 and E2 by 17β-HSD.

There are currently around 14 isozymes of 17β-HSD characterised, at least 10 of which are found in humans (Lukacik *et al.,* 2006; Thijssen, 2004). Despite the common features of the natural substrates, the isozymes have very low homology, with the reaction catalysed also being different, e.g. types 1, 3, 5 and 7 catalysing reductive reactions, while types 2, 4 and 8 catalyse oxidative reactions (Mindnich *et al.*, 2004; Adamski and Jakob, 2001). However, due to the number of isozymes, the potential for the development of a single drug agent is unclear (Coldham and James, 1990).

1.6 Aromatase (AR)

AR is a membrane-bound cytochrome P-450 enzyme that catalyses the conversion of C_{19} androgens to C_{18} estrogens via the aromatisation of the steroid A ring (Figure 2). AR is a monooxygenase comprised of two proteins: the aromatase cytochrome P-450 and the reduced nicotinamide adenine dinucleotide diphosphate (NADPH)-cytochrome P-450 reductase (Perez *et al.*, 1992). The instability of the enzyme when removed from the membrane has prevented the crystal structure of AR from being determined.

AR is found predominantly in the ovaries in premenopausal women, as well as in smaller quantities in the peripheral tissues (e.g. adipose and muscular tissue) in postmenopausal women (Santen and Harvey, 1999). AR achieves the biosynthesis of E1 by the oxidation of androstenedione in three sequential steps, each requiring oxygen and NADPH (Figure 5) (Watanabe and Ishimura, 1989). E2 can also be synthesised via this route through the use of T as the natural substrate, although the binding affinity of T to AR is lower than androstenedione.

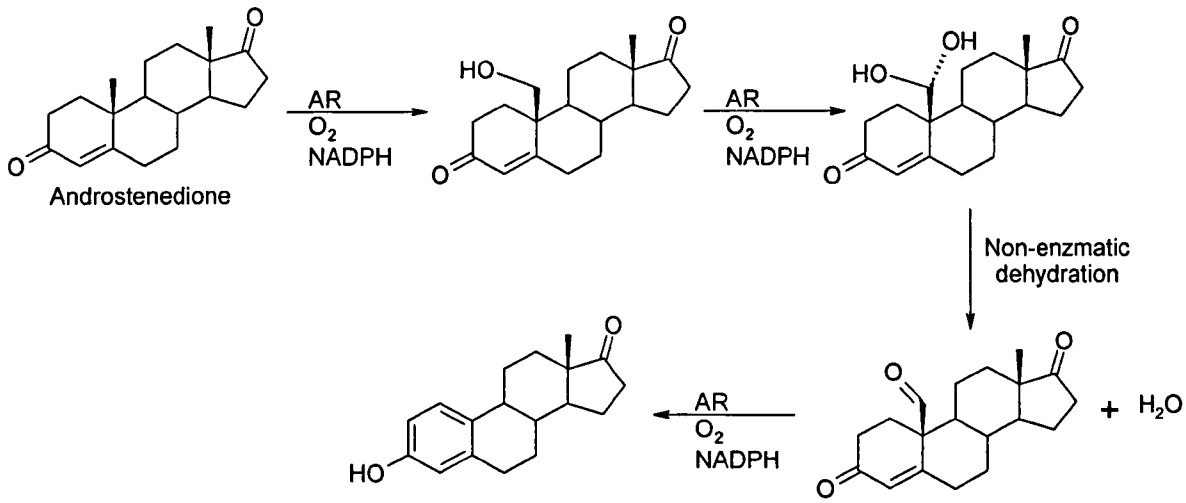


Figure 5: Conversion of androstenedione to E1 via the action of AR.

Due to the position of AR within the steroidal cascade, AR has been the focus of extensive research for sometime and has resulted in the synthesis of numerous potent inhibitors with greatly reduced clinical side effects; it is the last enzyme within the steroidal cascade, as such, inhibition of this enzyme would result in fewer side-effects since its inhibition would not affect the biosynthesis of alternative steroids.

As such, steroidal inhibitors such as formestane (Lentaron®) and exemestane (Aromasin®), and non-steroidal inhibitors such as aminoglutethimide, letrozole (Femara), vorozole (Rivizor) and anastrozole (Arimidex) (Figure 6) have been found to possess highly potent inhibitory activity against AR with minimal side-effects (Lake and Hudis, 2002; Hill and Moore, 2002).

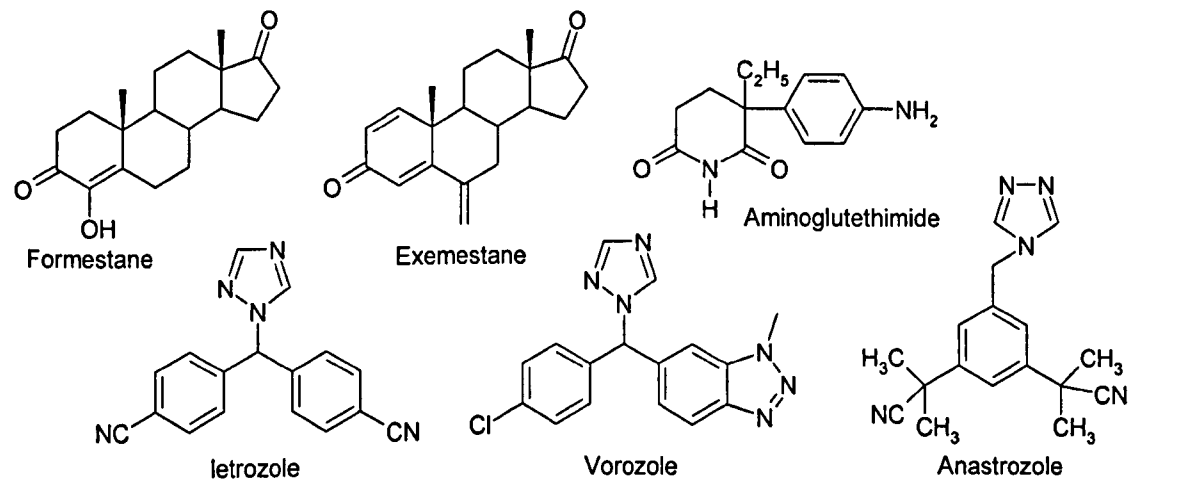


Figure 6: steroidal and non-steroidal inhibitors of AR.

It has also been proposed that using a combination of compounds which are able to selectively inhibit or have dual inhibitory activity against 17β-HSD (types 1 and 7) as well as estrone sulfatase (E1STS) and/ or AR, would lead to significantly more effective estrogen ablation than the inhibition of one ezyme alone. And so combinatorial drug regimen are frequenly considered in the treatment of hormone dependent breast cancer (Lukacik *et al.,* 2006).

1.7 Estrone sulfatase (E1STS)

Estrone sulfate (E1S) accumulates in the adipose tissue throughout a female's adult life. E1 is converted to the sulfoconjugated form by the action of the enzyme estrone sulfotransferase (Figure 7).

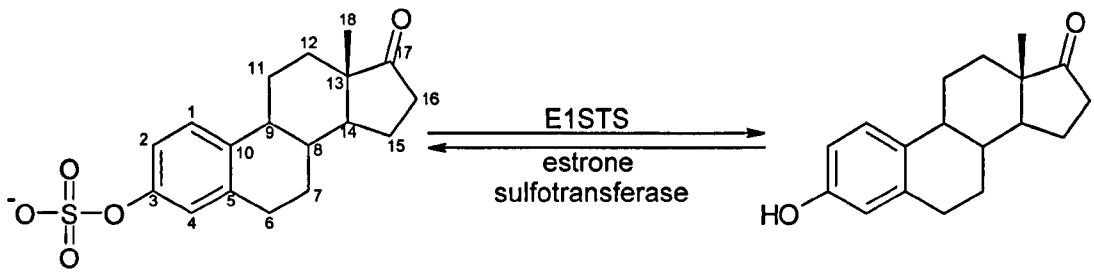


Figure 7: Interconversion between E1S and E1.

E1S is found in most bodily tissue, predominantly in estrogen-dependent cells, such as mammary and uterine endometrial cells (Pasqualini *et al.*, 1989). In pre-menopausal women, the sulfatase pathway functions at a minimum rate, as the vast majority of E1 is synthesised via the AR pathway, much of which is converted to the conjugated sulfate by estrone sulfotransferase (Hobkirk, 1993). E1S is also readily taken up by the adipose tissue to form a large reservoir that can be converted back to E1 (Santner *et al.,* 1984).

This stored source of estrogens is then converted back into the active form by the action of E1STS (Figure 7); being a non-AR route, it is not blocked by the conventional AR inhibitors and provides an alternative route to the estrogens thereby sustaining tumour growth. The sulfatase route is therefore thought to contribute to the majority of stimulation observed in breast tumours, and is estimated to account for 10 times the estrogen content of tumours than the amount produced by the AR pathway (Reed *et al.*, 1996).

E1STS is a microsomal enzyme thought to be one of a family of aryl sulfatase enzymes which exists in a glycosolated form, identifiable due to its ability to bind to concanavalin-A-sephorose (Purohit *et al.*, 1998a). Unstable outside of the membrane, the isolation of pure enzyme has as yet not been possible. However, the family of enzymes displays such a significant degree of homology allowing active site models to be determined from the crystal structure of bacterial derived aryl sulfatase A (ASA) and aryl sulfatase B (ASB) (Smith *et al.*, 2001). The active site, in particular the substrate binding pocket, was shown to be positive in nature with a modification of a conserved glycine residue – the nature of which will be further discussed with respect to the mechanism of action of this enzyme.

1.7.1 Steroid sulfatase (STS)

There has been some discussion over the identities of the sulfatase enzymes. It is believed that E1STS and dehydroepiandrosterone sulfatase (DHEA-STS), which converts dehydroepiandrosterone sulfate (DHEAS) to dehydroepiandrosterone (DHEA), are the same enzyme. Compounds designed to inhibit E1STS have been found to also inhibit DHEA-STS, thereby adding support to the 'one enzyme hypothesis' (Pasqualini and Chetrite 2005).

Another pathway which is also catalysed by a sulfatase based enzyme and which has also been shown to stimulate breast tumours is the conversion of androstenediol sulfate to androstenediol. The latter steroid is an androgen, however, it has been shown to stimulate the growth of breast tumour cells. The action of androstenediol in promoting tumour cells appears to be through the binding of this steroid with ER present in the cytoplasm of hormone-dependent cells, as such, they behave in a similar manner to the estrogens although to a much lesser extent than E1 or E2. The majority of androstenediol is converted to DHEA and subsequently to DHEAS in premenopausal women and is then stored in a similar manner to E1S (Adams *et al.*, 1981).

The non-estrogenic stimulation of breast cancer tumours may give an insight into why AR inhibitors alone have not led to a decrease in tumour growth. This highlights the value of the design and synthesis of compounds that can inhibit

both estradiol and androstenediol synthesis, through the inhibition of the STS family of enzymes, thus reducing the levels of hormones that can stimulate tumours.

1.7.2 Mechanism of E1STS enzyme action

As previously mentioned, E1STS converts the stored E1S into the more potent estrogen E1. The mechanism by which this occurs is not fully understood, however, a number of hypotheses have been proposed. Figure 8 shows the first hypothesis of mechanistic elucidation as proposed by Woo *et al.* (1996a). The authors proposed several potential routes, all involving the initial interaction of the substrate with the active site via hydrogen bond formation between the aryl oxygen atom and a suitable functionality within the active site pocket.

In route A the direct dissociation of the sulfate moiety from the steroid backbone is represented. This occurs due to the intitial hydrogen bond formation that weakens the sulfur-oxygen bond. This is followed by the simultaneous release of sulfur trioxide and water, prior to the evolution of E1. Route B depicts the nucleophilic attack of the sulfur by water, possibly bound to the active site. This may occur via a concerted step resulting in the release of E1.

The authors also suggest route C which involves a nucleophilic attack by an amino acid residue within the active site that leads to a sulfoenzyme intermediate and the liberation of E1, whilst the hydrolysis of this reconstitutes the residues in the active site (Figure 8).

Following an extensive series of studies into a number of physicochemical factors and their role in determining the overall inhibitory activity of sulfamate based inhibitors, pK_a was found to be a major physicochemical factor (Ahmed *et al.*, 2002a). As result of these studies, an alternative mechanism was put forward by Ahmed *et al.* (2002b) based on the studies of Waldow *et al.* (1999) which rationalised the pK_a requirement of the parent phenols and the biochemical activity of sulfamate derivatives.

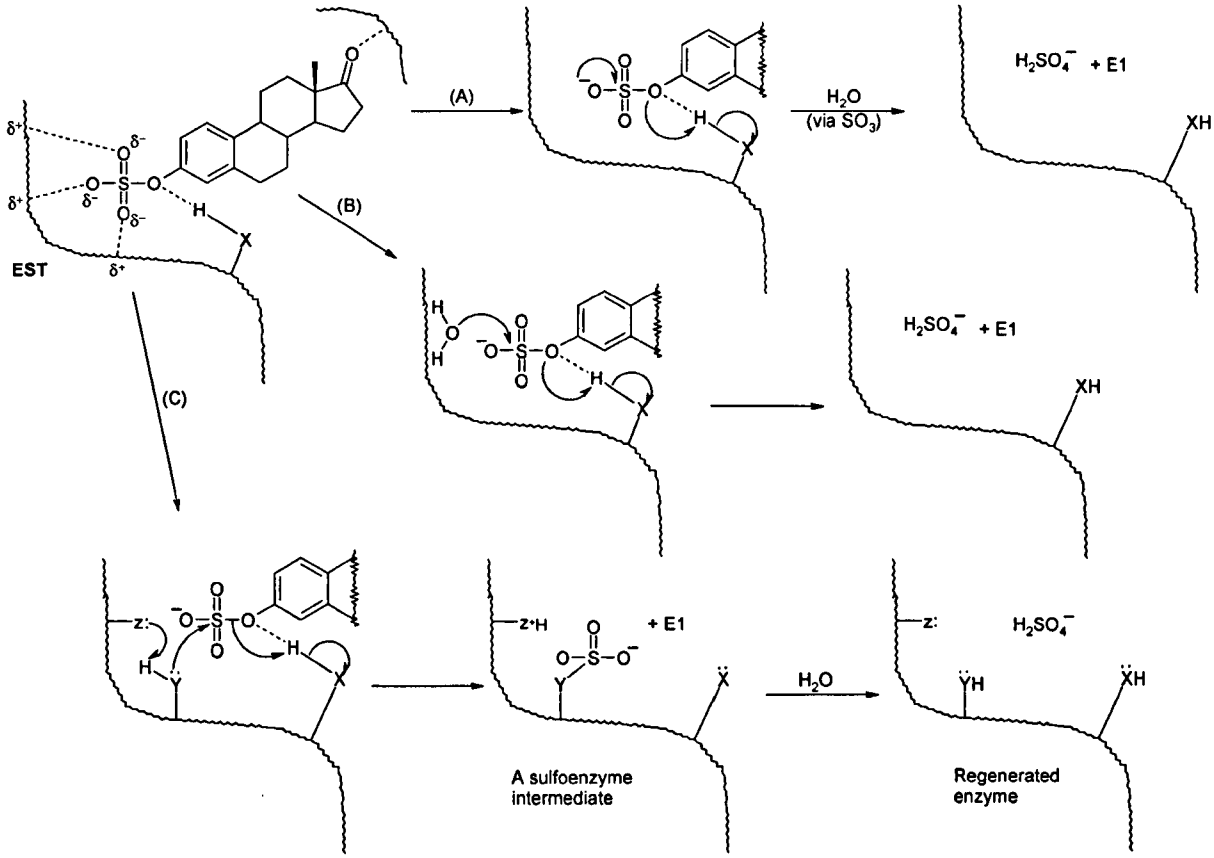


Figure 8: Mechanisms of E1S hydrolysis by E1STS via three potential routes (adapted from Woo *et al.*, 1996a).

In the mechanism proposed by Ahmed *et al.* (2002b), the initial attack is of the substrate's sulfonate group by the gem-diol moiety within the active site resulting in the cleavage of the S-OR bond and resulting in the loss of the hydrophobic steroid backbone from the active site; giving rise to formylglycine (FGly) and sulfonic acid. The sulfonic acid then leaves the active site and the carbonyl group is hydrolysed to reconstitute the gem-diol (Figure 9; Ahmed *et al.*, 2002c).

Although the exact mechanism has yet to be elucidated, the latter mechanism by Ahmed *et al.* (2002b) has been utilised in the rationalisation of the irreversible inhibitory activity observed within the sulfamate based inhibitors as well as reversible inhibitors (Ahmed *et al.*, 2002c; Ahmed *et al.*, 2002d).

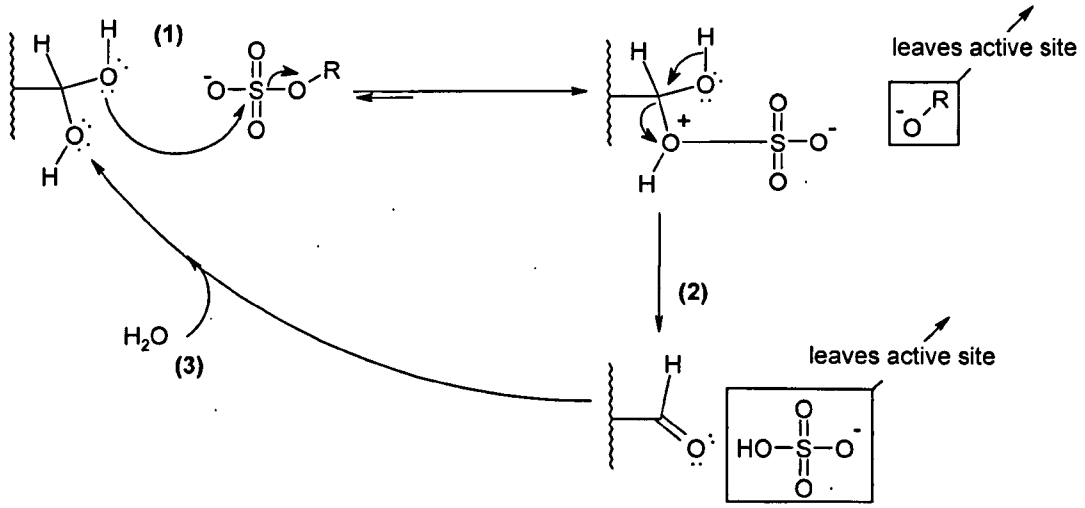


Figure 9: Mechanism of action for E1STS; **(1)** gem-diol in active site of E1STS, **(2)** hydrolysis to formyl glycine (FGly) and sulfonic acid, **(3)** reformation of the gem-diol moiety.

1.8 Steroidal inhibitors of E1STS

Among the first steroidal inhibitors discovered as an E1STS inhibitor was androstandiol sulfate (Figure 10) which was found to possess a K_i value of 2.0μM when evaluated against placental microsomal enzyme (Nguyen *et al.*, 1993).

Another compound which was found to possess weak inhibitory activity against E1STS was 17β-hydroxy-2,4,17α-pregnadien-20-yno[2,3-D]isoxazole (danazol), a 17α-ethinyl testosterone derivative used in the treatment of endometriosis (Carlstrom *et al.*, 1984a). Danazol inhibited E1STS in placental microsomes by 60% (inhibitor concentration 10μM) (Selcer *et al.*, 1996). Moderate potency was also displayed when evaluated against intact MCF-7 cancer cell lines, and was found to possess 62% inhibition at 10μM inhibitor concentration with the substrate concentration at physiological levels i.e. 2nM (Purohit *et al.*, 1992).
The observation of inhibitory activity possessed by androstandiol sulfate, led workers to consider the replacement of the sulfate group of E1S in an attempt to synthesise compounds that would compete with the natural substrate for the active site. The interest in steroidal analogues led to the E1 derivative estrone-3-*O*-methyl-phosphonothionate (E1-MTP, Figure 10). This was the first E1STS

inhibitor to be specifically designed and synthesised as an E1STS inhibitor, possessing a K_i value of 37.5μM (Duncan *et al.*, 1993) and an IC_{50} value of 90nM in intact MCF-7 breast cancer cell lines (Purohit *et al.*, 1995). This compound had already been shown to be able to mimic the substrate (Cox *et al.*, 1979) and was found to be some 14 times more potent than danazol.

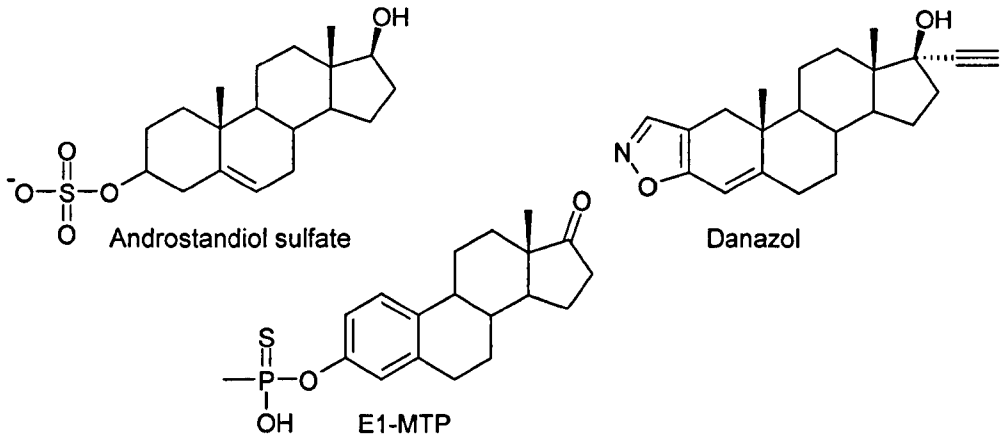
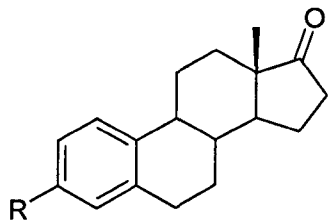


Figure 10: Initial steroidal inhibitors of E1STS.

The detailed study of the inhibitory activity of E1-MTP showed that this compound inhibited E1STS in a reversible manner, more specifically it was found to be a competitive and time-dependent inhibitor. Furthermore, due to the stability of E1-MTP to *in vivo* metabolism, it became the early template in design of potential novel inhibitors of E1STS.

This strategy of generating the lead compounds to inhibit E1STS led to many weak reversible inhibitors. The most notable of these being E1 heptyl alkyl sulfonate (**3**) and E1 phosphonate (**6**), which were found to possess 91% and 80% inhibition (at 10μM) of E1STS respectively (Table 2) (Anderson *et al.*, 1997; Woo *et al.*, 1997).



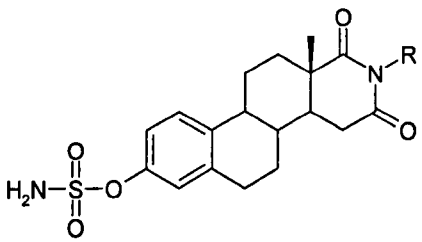
	R	% Activity
-	$-OSO_2NH_2$ (EMATE)	99% 0.1μM [1,4]
1	$-OSO_2NHCH_3$	80% 0.1μM[1,4]
2	$-OSO_2N(CH_3)_2$	50% 0.1μM[1,4]
3	$-OSO_2C_6H_4CH_3$	30% 0.1μM[1,3]
4	$-OSO_2CH_3$	28% 10μM[1,3]
5	$-OSO_2C_4H_9$	17% 10μM [1,3]
6	$-OPO_2H-$	80% 10μM [1,6]
7	$-OPO_2CH_3^-$	41% 10μM [1,6]
8	$-NHSO_2NH_2$	53% 50μM [2,5]
9	$-SHSO_2NH_2$	12% 50μM [2,5]

Table 2: E1 derived E1STS inihibitors ([1]intact MCF-7 cells, [2]Placental microsomes; [3]Howarth *et al.,* 1997; [4]Woo *et al.*, 1997; [5]Woo *et al.*, 1996a; [6]Anderson *et al.*, 1997).

Further derivatisation of the sulfonate moiety led workers to the sulfamate functionality and the subsequent discovery of estrone-3-*O*-sulfamate (EMATE) which was found to possess irreversible inhibitory actvity and high potency against E1STS (99% at 0.1μM), however, EMATE was also found to be highly estrogenic. The discovery of estrogenicity within EMATE led to the design and synthesis of a series of compounds, all possessing the 3-*O*-sulfamoylation of the estrone backbone as the basic pharmacophore (Woo *et al.*, 1996a).

One particular set of derivatives included a series of D-ring derivatives of EMATE (Table 3). In general, the compounds synthesised were found to possess weaker biological activity than EMATE. However, two compounds were found to possess good inhibitory activity, compound **11** was found to be only marginally less potent than EMATE possessing an IC_{50} value of 12nM (in comparison, EMATE was found to possess an IC_{50} value of 8nM under similar conditions). Compound **12** (STX213), however, was found to be an extremely potent inhibitor possessing an IC_{50} value of 1nM when evaluated using placental microsomal assay. As a result of its potent inhibitory acvtivity combined with its increased stability *in vivo* and lack of estrogenicity, STX213 has recently entered Phase I clinical trails (Foster *et al.*, 2006). Potent inhibitory activity was also

observed in the benzyl compound (**15**) (possessing an IC_{50} value of 3nM) whilst the (3-pyridyl) methyl derivative (**17**) (possessing an IC_{50} value of 1nM) was found to be equipotent to compound **12** and therefore some 8 times more potent than EMATE.



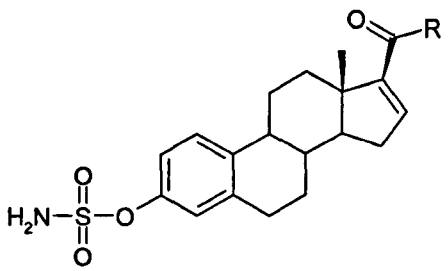
	R	IC_{50} (nM)
-	EMATE	8
10	H	20
11	Methyl	12
12	*n*-Propyl	1
13	*n*-Pentyl	150
14	Cyclopropylmethyl	74
15	Benzyl	3
16	$-CH=CH_2$	80
17	(3-Pyridyl)methyl	1
18	4-*t*-Butylbenzyl	>100

Table 3: D-ring derivatives of EMATE evaluated against placental microsome (Fischer *et al.*, 2003; Sterix Ltd. 2002c).

Consideration of the structure-activity relationship of the D-ring derivatised compounds (Table 3) shows that some large groups [e.g. (3-pyridyl)methyl] are well tollerated whilst more flexible functionalities (e.g. pentyl chain) are not. This increase in biological activity with the increase in the overall size of the R group is surprising since the large groups would be expected to undergo steric hindrance. This obserservation may indicate potential interaction between the inhibitor and the active site which counteracts the effects of increased steric volume of the molecule (Fischer *et al.*, 2003).

Other C17 derivatives of EMATE have also been synthesised and subsequently evaluated against E1STS. For example, in one series of compounds, the C17 carbonyl group was replaced with a carboxylate functionality (Tables 4a and 4b). In general, a number of the synthesised compounds were found to possess greater potency than EMATE, e.g. within the range of compounds from **19** to **28**, all of these compounds are observed to be more potent than EMATE, with

compound **19** being the most potent and was found to possess an IC_{50} value of 1nM. However, the most potent inhibitor within the full range of compounds is **38** which was found to possess an IC_{50} value of 0.45nM. A number of compounds were also found to be weaker inhibitors of E1STS in comparison to EMATE, e.g. compound **36** and **37** were found to possess IC_{50} values of 26nM and 50.0nM respectively.

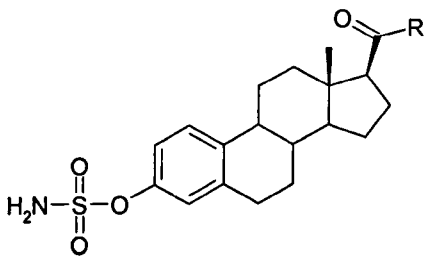


	R	IC_{50} (nM)
19	NH-Cyclopentyl	1.0[2]
20	NH-$(CH_2)_2OCH_3$	2.8[2]
21	NH-*i*-C_3H_7	3.2[1]
22	$N(C_2H_5)_2$	3.6[1]
23	1-Prrolidinyl	4.4[2]
24	N-*i*-$(C_3H_7)_2$	4.8[1]
25	NH-*n*-C_3H_7	5.0[1]
26	NH-Allyl	5.6[2]
27	NH-NH-($_2$-Pyridinyl)	6.0[2]
28	NH-(4-COOHbenzyl)	6.0[2]
29	NH-Pyridazinyl	9.4[2]
30	NH-$(CH_2)_4OH$	9.8[2]
31	NH-$(CH_2)_5COOCH_3$	10.0[2]
32	NH-Ph	10.0[2]
33	OC_2H_5	12.0[1]
34	NH-CH_3	12.0[1]
35	OC_3H7	13.0[1]
36	NH-$C(CH_3)_2CH_2OH$	26.0[2]
37	OH	50.0[1]

Table 4a: C-17-modifications of E1 derived sulfamate E1STS inhibitors evaluated against purified E1STS ([1]Duquesne Univ. of the Holy Ghost, 1999; [2]Duquesne Univ. of the Holy Ghost & Kyowa Hakko Kogyo Co. Ltd., 2000; [3]Kyowa Hakko Kogyo Co. Ltd., 2001).

Rationalisation of the structure activity relationship suggested that the more potent compounds possess increased hydrophobicity, but with little or no increase in the overall size of functionality about the C-17 area of the D-ring.

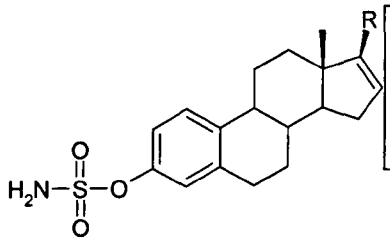
That the rationale may have some validity is illustrated by the activity of compounds such as **19** (Table 4a) and **38** (Table 4b), which displayed IC_{50} values of 1nM and 0.45nM respectively (Duquesne Univ. of the Holy Ghost & Kyowa Hakko Kogyo Co. Ltd., 1999 and 2000).



	R	IC_{50} (nM)
38	$NH-(CH_2)_6CH_3$	0.45[1]
39	1-Pyrrolidinyl	6.0[2]
40	$NH-(CH_2)_2OCH_3$	15.5[2]
41	NH-*i*-C_3H_7	17.0[3]
42	NH-*n*-C_3H_7	19.0[3]

Table 4b: C-17-modifications of E1 derived sulfamate E1STS inhibitors evaluated against purified E1STS ([1]Duquesne Univ. of the Holy Ghost, 1999; [2]Duquesne Univ. of the Holy Ghost & Kyowa Hakko Kogyo Co. Ltd., 2000; [3]Kyowa Hakko Kogyo Co. Ltd., 2001).

Further modifications of the D-ring of EMATE led other workers within the field to the synthesis of some highly potent inhibitors of E1STS (Table 5a and 5b). For example, compound **43** was found to be an extremely potent inhibitor possessing an IC_{50} value of 0.02nM in comparison to EMATE which, under similar conditions, was found to possess an IC_{50} value of 8nM, as such, it was found to be some 400 times more potent than EMATE. Other highly potent inhibitors of E1STS was also obtained as a result of the reduction of the C=C within the D-ring of compounds such as **43**. That is, reduction of the D-ring in **43** led to the synthesis of **46**, a weaker inhibitor than **43**, however, the derivatisation the substituent attached to the C17 carbon of the setroid back bone resulted in a range of compounds which were found to possess potent inhibitory activity and equipotent to **43**. For example, the ethyl, *n*-propyl and (*E*)-propylidene derivatives (compound **47**, **48** and **50**) were all found to be equipotent to **43**. A major difference was however discovered in the latter series of compounds, that is, whilst **43** was found to be estrogenic, compounds **48** and **49** (which were 1.7 and 4.8 times weaker than **43** respectively) were found to lack estrogenic properties.

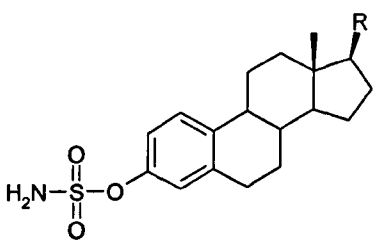


	R	IC_{50} (nM)	Estrogenicity
43	Acetyl	0.02[1]	Y
44	3-Pyridyl	12.0[2]	N/A
45	$C(CH_3)_2OH$	13.0[2]	N/A

Table 5a: C-17 modifications of E1 derivatives evaluated against purified E1STS ([1]Sri Int., 1999; [2]Kyowa Hakko Kogyo Co. Ltd., 2001).

The structure activity relationship determination suggests that there is an apparent strong correlation between alky chain length and size at the C-17 position of the steroid backbone in the determination of overall inhibitory activity. However, the presence or lack of the C17 double bond appears to play an important role in not only determining the overall inhibitory activity, but also the estrogenic property of the inhibitor.

Further consideration of the D-ring reduced series of compounds suggests that the alkyl chain length has some effect on the estrogenic property of the compounds. That is, consideration of compound **47** in comparison to **48** or **49** would appear to suggest that increase in steric bulk about the C17 area of the steroid backbone results in a lowering of the estrogenic characteristic of the inhibitors of E1STS.

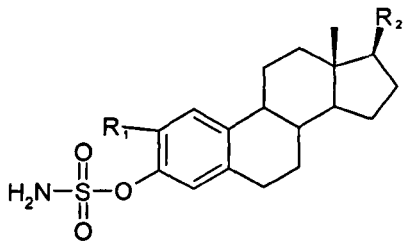


	R	IC_{50} (nM)	Estrogenicity
46	Acetyl	2.0[1]	N
47	Ethyl	0.020[1]	Y
48	*n*-Propyl	0.034[1]	N
49	*n*-Butyl	0.096[1]	N
50	(*E*)-Propylidene	0.027[1]	Y

Table 5b: C17 derivatives of EMATE evaluated against purified E1STS ([1]Sri Int., 1999; [2]Kyowa Hakko Kogyo Co. Ltd., 2001).

Derivatisation of the C17 position in combination with derivatisation of the A-ring of the steroid backbone was also undertaken by several workers within the field. However, in general, the modifications did not improve on the activity observed

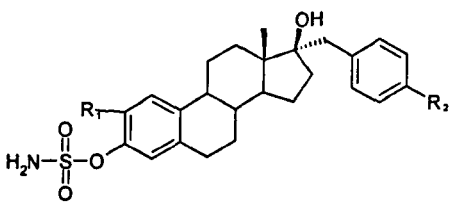
in other steroid derivatives, with the exception of compound **51** which was found to possess an IC_{50} value of 0.3nM (Table 6a).



	R_1	R_2	IC_{50} (nM)
51	CN	OAc	0.3[1]
52	OCH_3	C_2H_5	1.5[1]
53	OCH_3	OSO_2NH_2	32[2,4]
54	C_2H_5	OSO_2NH_2	33[2,4]
55	OC_2H_5	OSO_2NH_2	100[2,4]

Table 6a: A-ring and C-17 modifications of E1 derivatives evaluated against purified E1STS ([1]Sri Int., 1999; [2]Sterix Ltd., 2002a; [2]Sterix Ltd., 2002b; [4]Sterix Ltd., 2002d).

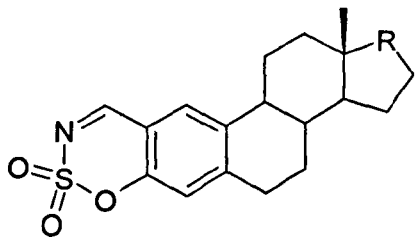
The addition of large bulky groups (such as a substituted phenyl ring) about the C17 position of the steroid backbone in an effort to increase the hydrophobicity of the overall inhibitor only resulted in decreasing the overall inhibitory activity with the most potent of these being compound **56**, which was found to possess an IC_{50} value of 40nM (Sri Int., 1999).



	R_1	R_2	IC_{50} (nM)
56	SCH_3	H	44[2,3]
57	SCH_3	t-C_4H_9	80[2,3]
58	OCH_3	H	430[2,4]
59	OCH_3	t-C_4H_9	4300[2,4]
-	EMATE		18[2]

Table 6b: A-ring and C-17 modifications of E1 derived evaluated against purified E1STS ([1]Sri Int., 1999; [2]Sterix Ltd., 2002a; [2]Sterix Ltd., 2002b; [4]Sterix Ltd., 2002d).

One series of compounds which contained a major modification about the A ring of the steroid backbone was the oxathiazine based compounds (Table 7). In general, these compounds were found to be weaker inhibitors of E1STS in comparison to EMATE (result from previous assay determination), however, further derivatisation of both the ring-A and ring-D of the steroid backbone led to a range of irreversible inhibitors that were found to possess greatly reduced estrogenic character compared to EMATE.



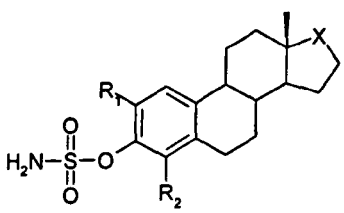
	R	IC_{50} (nM)
60	C=O	9
61	C-OAc	12
62	C-OH	20
63	C=CH-CH_3 (Z)	63
64	C=CHCH_2CH_3 (Z)	58
65	C=CHCH_2CH_3 (E)	<1000
66	C=C=CH_2	74
67	C-CH_3	45
68	C-CH_2CH_3	50
69	C-$(CH_2)_2CH_3$	120
70	C=CH-CO_2Et	36
71	C=C$(CN)_2$	22
72	C-OCH_3	35
73	C-αCl	62
-	EMATE	8

Table 7: Inhibition data for steroidal oxathiazine derivatives evaluated against MCF-7 (adapted from Peters *et al.*, 2003).

The most potent compound found within this range was found to be compound **60** which was a direct derivative of EMATE and which was found to possess an IC_{50} value of 9nM in intact MCF-7 cells, as such, it was equipotent to EMATE. A range of derivatives were then synthesised by the investigators which involved the derivatisation of the D-ring and which led to the next most potent inhibitor, namely compound **61** (which was found to possess an IC_{50} value of 12nM). Compound **61** contained an acetate functionality at the C17 position and was found to be 1.3 times weaker than compound **60.** An increase in the overall size and volume occupied by the C17 substituent resulted in a further decrease in inhibitory activity, with compound **65** ([17(20)*E*]-propylideneestra-1,3,5(10)-trien-[3,2,e]-1',2',3'-oxathiazine-2',2'-dioxide) proving to be an extremly weak inhibitor of E1STS (possessing an IC_{50} greater than 500nM) in comparison to EMATE. It is interesting to note that the *E*-isomer was found to possess an IC_{50} value of 58nM, clearly the bulky nature of the *Z*-isomer resulted in increased steric hindrance which led to a greatly reduced binding ability.

A series of compounds containing substituents on the steroid A-ring have also been studied as inhibitors of E1STS, many of which showed comparable inhibitory activity with EMATE (result from previous assay determination) - a series of halogenated derivatives were considered as well as a number of compounds containing electron-withdrawing groups. For example, compound **75** contains a nitro group at the C2 position of the backbone and was found to possess an IC_{50} value of 0.07μM against E1STS in intact MCF-7 cells at 0.01μM (Woo *et al.*, 1996). In comparison, EMATE was found to possess an IC_{50} value of 0.07μM.

Consideration of the halogen-based derivatives (substituted at the C-2 position) shows that both the addition of groups able to act as electron-withdrawing groups attached to the sulfamate containing A-ring may play an important role in determining biological activity, with 2-chloro-EMATE (**79**) being the most potent of the halogen range, possessing an IC_{50} value of 0.8nM against E1STS from human placental microsomes (Sterix Ltd., 2001).



	X	R_1	R_2	Activity
74	CH_2	H	H	97%[2,3,5]
75	C=O	NO_2	H	0.07μM[1,6]
76	C=O	H	NO_2	0.8nM[1,6]
77	(E)C=NOH	H	H	>99%[2,4,10]
78	C=O	F	H	5.6nM[1,9]
79	C=O	Cl	H	0.8nM[1,9]
80	C=O	Br	H	1.7nM[1,9]
81	C=O	I	H	6.1nM[1,9]
82	C=O	CH_2OCH_3	H	2nM[2,7]
83	C=O	OCH_3	H	30nM[1,6]
84	CH(β-OSO_2NH_2)	OCH_3	H	39nM[1,8]
-	EMATE			8nM

Table 8: Examples of EMATE derived irreversible E1STS inhibitors ([1]Placental E1STS, [2]intact MCF-7 cells; inhibitory concentration [3]0.01μM and [4]0.1μM; [5]Woo *et al.*, 1996; [6]Purohit *et al.*, 1998b; [7]Sri Int., 1999; [8]Poirier and Boivin, 1998; [9]Sterix Ltd., 2001; [10]Hejaz *et al.*, 1999).

This indicated that the electron withdrawing capacity of the substituents on the A-ring may play an important factor in determining overall inhibitory activity an important factor within the sulfamate based inhibitors was supported by the synthesis and subsequent evaluation of 2-ethoxy-EMATE (**83**), and which was found to be a weak inhibitor compared to EMATE (result from previous assay determination) itself, with an IC_{50} of 30nM (Purohit *et al.*, 1998b) and the 2-propanoyl equivalent having an IC_{50} of 2nM (Sri Int., 1999). This led to the synthesis of the 2- and 4-nitro substituted derivative of EMATE, both of which showed considerable activity against E1STS. 2-Nitro-EMATE (**75**) possessed an IC_{50} value of 0.07μM when evaluated against placental E1STS, and 4-nitro-EMATE (**76**) was found to possess an IC_{50} value of 0.8nM, and therefore was more potent than the 2-chloro-EMATE derivative.

Whilst sulfamoylated derivatives of estrone remain the major target for most workers within the field, a small number of workers have considered the use of non-sulfamated derivatives and which has resulted in the synthesis of a small range of potent and irreversible inhibitors of E1STS. Compound **89** (the 3-formyl derivative of E1) (Figure 11) was found to be the most potent of this range and was found to possess an IC_{50} of 0.42μM - in comparison, EMATE was found to possess an IC_{50} value of 0.056μM under the same conditions. Alternative compounds have also been considered although these compounds have been shown to possess poor inhibition, e.g. compounds **85** to **88** were all found to possess IC_{50} values >50μM (Schreiner and Billich, 2004).

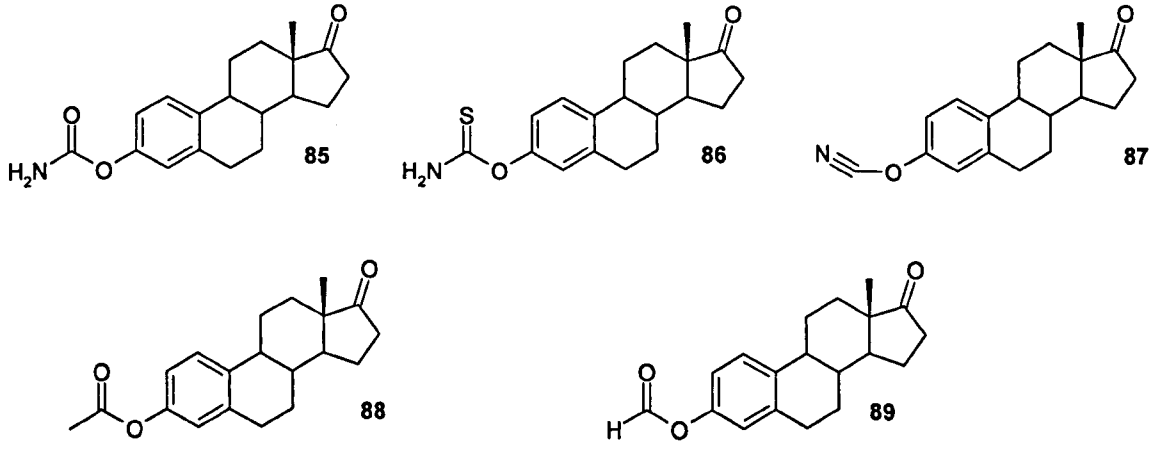


Figure 11: Non sulfamoyl based, irreversible inhibitor of E1STS.

1.9 Non-steroidal inhibitors of E1STS

As stated previously, the most potent steroidal E1STS inhibitors *todate* were found to be EMATE, STX213 (**12**), 4-nitro-EMATE (**76**) and 2-chloro-EMATE (**79**), however, these inhibitors proved to be highly estrogenic, as well as undergoing metabolism to the estrone derivative. As such, non-steroidal compounds were also investigated and this has resulted in numerous potent inhibitors with varying structural features.

The first class of non-steroidal E1STS inhibitors were based on 2-(hydroxyphenyl) indole sulfate. Used to directly mimic the relationship between E1STS and the sulfated A-ring of E1S, this range of compounds produced some potent inhibitors, the most active of these being 3-methyl-1-pentafluorophenylmethyl-6-sulfooxy-2-(4-sulfooxyphenyl)-4-trifluoromethyl indole (**90**) (Figure 12), which was found to possess an IC_{50} value of 80μM against partially purified enzyme extracted from calf uterus (Birnbock and von Angerer, 1990).

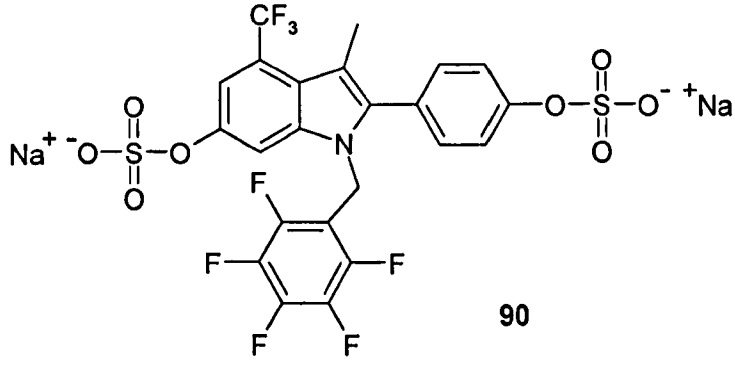
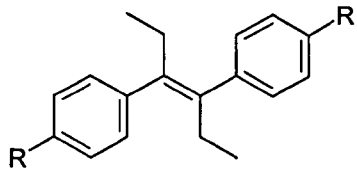


Figure 12: 2-(Hydroxyphenyl) indole sulfate (**90**) as an E1S mimic, first non-steroidal E1STS inhibitor.

Among the first non-steroidal inhibitors of E1STS to be synthesised were compounds that mimicked the steroidal AD-rings (Table 10). Compound **91** was shown to be a potent inhibitor of E1STS possessing an IC_{50} value of 10nM against MCF-7 cells. Although possessing potent inhibitory activity, the compound was limited as a potential drug candidate since its congener, trans-1,2-diphenylethylene [or (*E*)-stilbene] and its derivatives, are known to be highly estrogenic (Chen *et al.*, 1996). The biochemical evaluation of the non-sulfamated derivative (**92**) further supported the importance of the use of the sulfamate

moiety in the inhibition of E1STS and was found to be significantly weaker than **91** and was found to be a reversible inhibitor possessing an IC_{50} value of 10μM.



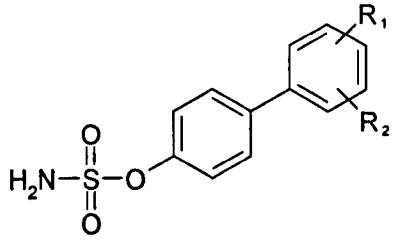
	R	IC_{50}
91	OSO_2NH_2	10nM
92	$SO_2N(CH_3)_2$	10μM

Table 10: Examples of AD-ring mimics as inhibitors evaluated against intact MCF-7 cells (Reed *et al.*, 1996).

The use of the biphenyl backbone was also investigated as a potential mimic of the steroid AC-ring (Table 11). A range of compounds were synthesised where the biphenyl ring moiety was substituted with numerous varying functionalities, for example, cyano groups were utilised across both ring systems (compound **93**) and which was found to possess 94% inhibition against E1STS at 3nM (Teikoku Hormone MFG Co. Ltd., 2001). A series of 4-alky-ester derivatives were also investigated (compounds **93** to **96**, Table 11) which proved to be relatively weak inhibitors of E1STS when compared to EMATE. Indeed, the parent unsubstituted biphenyl sulfamate (**97**) demonstrated extremely poor inhibitory activity and was found to be 10 to 20 time less potent than the corresponding 4-alky ester derivatives. Consideration of the structure-activity relationship determination suggested that within the latter series of compounds, the carbonyl moiety may undergo hydrogen bonding interaction with the active site, in particular, the C=O group may mimic the steroid D-ring carbonyl interaction between the steroid backbone and the active site of E1STS and that this interaction may be in some part responsible for the potency observed within compounds.

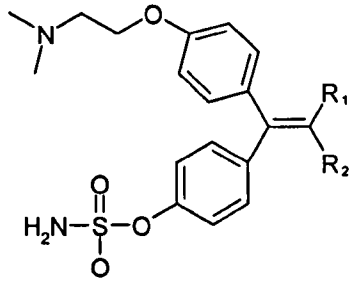
Sulfamate derivatives of hydroxytamoxifen have also been investigated. Using E1STS obtained from rat liver microsomal preparations, two compounds: the (*E*)-isomer (**98**) and; (*Z*)-isomer (**99**) of hydroxytamoxifen were evaluated and were found to possess apparent K_i values of 35.9μM and 500μM respectively (Table 12). More importantly, the compounds were found to be reversible inhibitors and are therefore the only sulfamate based compounds which do not possess irreversible inhibitory activity against E1STS. From the consideration of the

structure-activity relationship determination of the series of compounds based on hydroxytamoxifen, it was proposed that the orientation of the phenyl ring system clearly affected the ability of the compound to bind to the activie site, that is, steric hindrance in the case of compound **99** was the major factor which led to the poor inhibitory activity (Chu *et al.*, 1999).



	R_1	R_2	IC_{50} (μM)
-	EMATE		0.1
93	CN	H	6.7
94	4-COOMe	H	5.2
95	4-COOEt	H	4.2
96	4-COOPr	H	3.5
97	H	H	76

Table 11: Biphenyl based inhibitors of E1STS (Ahmed *et al.*, 2002a).

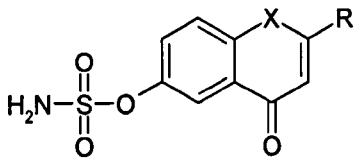


	R_1	R_2	$K_i$app
98	Ph	C_2H_5	35.9μM
99	C_2H_5	Ph	>500μM

Table 12: Hydroxytamoxifen sulfamate E1STS inhibitors (Rat liver microsomes, Chu *et al.*, 1999).

Sulfamate-based chromenone derivatives have been investigated (Nussbaumer *et al.*, 2002) and were found to be potent inhibitors of E1STS and the consideration of the structure-activity relationship determination of the chromenone derivatives gave some interesting insights that proved to be useful in the design of further novel inhibitors of E1STS. In the range of compounds synthesised it was observed that an increase in alkyl chain length led to an increase in inhibitory activity, e.g. consideration of the *n*-propyl (**100**) (posessing an IC_{50} value of 722nM) and *n*-nonyl (**101**) (posessing an IC_{50} value of 403nM) clearly suggests that an increase in the hydrophobicity of the molecule results in an increase in inhibitory activity. Derivatisation of compound **101** involving the introduction of branching of the alkyl chain gave compound **102** which was found

to possess an IC_{50} of 78nM and which therefore supports the contribution of hydrophobicity in determining the overall inhibitory activity. That steric hindrance is also a factor (previously observed in different ranges of inhibitors) was further supported by the synthesis and evaluation of the derivative of compound **100** containing a *t*-butyl function, namely compound **103** which was found to possess an IC_{50} value of 22nM. Further derivatisation of the chromenone backbone led to 1-adamantyl thiochromenone (**106**), a highly potent inhibitor of E1STS possessing an IC_{50} value of 0.34nM, and therefore being 170 times more potent than EMATE (Nussbaumer *et al.*, 2002).



	X	R	IC_{50} (nM)
-	EMATE	-	56
100	O	*n*-Propyl	722
101	O	*n*-Nonyl	403
102	O	1,1-Dimethylnonyl	78
103	O	*t*-Butyl	22
104	O	4-Pentybicyclo[2.2.2]-oct-1-yl	11
105	O	1-Adamantyl	5.6
106	S	1-Adamantyl	0.34

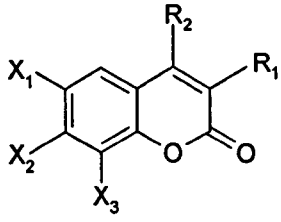
Table 13: Chromenone base inhibitors of E1STS (Nussbaumer *et al.*, 2002).

As a result of the potent inhibitory activity observed within the series of chromenone-based compounds, the authors evaluated the estrogenicity of these inhibitors, in particular, compounds **101**, **103** and **106**. While compound **103** was found to stimulate estrogen-dependent MCF-7 cells, compound **106** was shown to possess highly potent estrogenic effect, stimulating MCF-7 cells by 99% at 100nM (Nussbaumer *et al.*, 2003b). Compound **101**, however, did not exhibit any estrogenic activity at concentrations upto 1μM, far above the IC_{50} of 89pM against MCF-7 cells; in comparison, EMATE was found to possess an IC_{50} value of 21pM (Billich *et al.*, 2000).

A large number of AB-ring mimics have been investigated, including the coumarin-based sulfamate inhibitors which were found to inhibit E1STS in a similar fashion to EMATE (Table 13), that is this range of compounds were found to possess irreversible inhibition in a time- and concentration-dependent manner,

however, unlike EMATE, these compounds lacked the potent estrogenic properties.

A large number of different derivatives of the coumarin backbone were synthesised and many of the compounds were found to be highly potent inhibitors of E1STS (Table 14), for example, 4-methylcoumarin-7-O-sulfamate (COUMATE, **108**) and **113**. The latter compound was more potent than EMATE with an IC_{50} value of 380nM, thereby inhibiting E1STS by 96.9% when evaluated against placental microsomes, leading to a range of compounds in which modification of this area was the focus, particularly a range of tricyclic coumarin sulfamates (Woo *et al.*, 1996b).

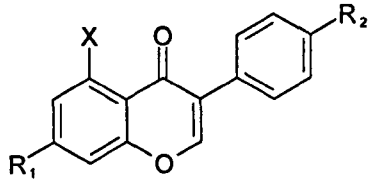


	R_1	R_2	X_1	X_2	X_3	% Inhibition
107	H	H	H	H_2NSO_2O-	H	93.0
108	H	CH_3	H	H_2NSO_2O-	H	78.2
109	H	CF_3	H	H_2NSO_2O-	H	79.4
110	CH_3	CH_3	H	H_2NSO_2O-	CH_3	17.4
111	H	CH_3	H_2NSO_2O-	H	H	<10
112	H	H	OCH_3	H_2NSO_2O-	H	<10
113	CH_3	CH_3	H	H_2NSO_2O-	H	96.9
114	H	CH_3	H_2NSO_2O-	OH	H	<10
115	H	CH_3	H_2NSO_2O-	H_2NSO_2O-	H	<10
EMATE						99.0

Table 14: Coumarin based sulfamates E1STS inhibitors (10μM) evaluated against placental microsomes (Woo *et al.*, 1996b).

Compounds mimicking the AB ring system and based on the naturally derived flavanoid backbone have also been investigated, in particular, the sulfate derivatives , daidzein 4'-*O*-sulfate (**119**) and daidzein 4',7-di-*O*-sulfate (**120**) have also been investigated (IC_{50} values of 6μM and 1.5μM respectively). These proved to be relatively potent, competitive inhibitors of E1STS (Wong and Keung, 1997).

The isoflavone sulfamate related compounds (**116** to **120**, Table 15) were also investigated as alternative AB ring system mimics and were found to be potent and irreversible inhibitors of E1STS, whilst the bis-sulfamates were found to be more potent than the mono-sulfamates; unfortunately both sets of compounds proved to be significantly less potent *in vivo* than EMATE, the mono- and bis-sulfamates showing 62% and 81% inhibitory activity (for compounds **119** and **120** respectively) in intact MCF-7 cells at 1μM. These flavonoid derivatives, although shown to have some inhibitory activity, their metabolite (**118**) exhibited estrogenic properties that would make them unlikely candidates as lead compounds in drug design.



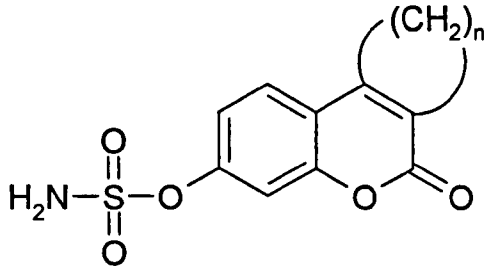
	X	R_1	R_2	Activity
116	H	OSO_3^-	OSO_3^-	1μM[1,3]
117	H	OH	OSO_3^-	5.9μM[1,3]
118	H	OH	OH	-[3]
119	OH	OSO_2NH_2	H	83%[2,4]
120	OH	OSO_2NH_2	OSO_2NH_2	90%[2,4]

Table 15: Natural flavonoid based inhibitors of E1STS ([1]K_i in μM, [2]percentage inhibition in intact MCF-7 at 1μM, [3]Wong and Keung, 1997; [4]Purohit *et al.*, 1999).

As can be observed, within the flavonoid range of compounds, a third phenyl ring system is present, and in an effort to produce ABC ring system mimics, the derivatisation of the coumarin backbone was undertaken so as to produce compounds which contained the additional ring. Biochemical evaluation of these compounds showed that these tricyclic compounds possessed significantly increased inhibitory activity when compared to the parent AB ring mimics, such as COUMATE. Indeed, the results of this study showed the tricyclic compounds to possess over 100 times more potent inhibitory activity than COUMATE, and 3 to 20 times more potent inhibitory activity than EMATE, for example, 667-COUMATE (**123**) and 6610-COUMATE (**126**) were found to possess IC_{50} values of 8nM and 1nM respectively (Table 16).

The tricyclic sulfamate-based compounds were all found to inhibit E1STS in an irreversible manner, and more importantly, exhibited none of the estrogenic properties of EMATE. The increased potency compared to COUMATE is thought

to be due the aliphatic cyclo-alkyl ring that enables the compound to interact with E1STS far more readily (Malini *et al.*, 2000). 667-COUMATE (**123**), although not the most potent of the coumarin based compounds synthesised, recently entered Phase 1 clinical trials and has been shown found to be effective *in vivo* (Stanway *et al.*, 2006).

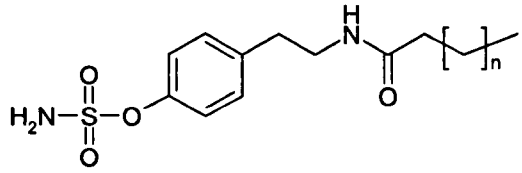


	n	IC_{50} (nM)
121	3	200
122	4	70
123	5	8
124	6	30
125	7	2.4
126	8	1
127	9	13
128	10	60
129	11	75
130	13	370
EMATE	-	8

Table 16: E1STS tricyclic coumarin base sulfamates evaluated against placental microsomes (Malini *et al.*, 2000).

Among the first A-ring inhibitors to be synthesised were the derivatives of 4-*O*-sulfamoyl-*N*-alkanoyl tyramines (Table 17). The most potent of the series was compound **138** (4-*O*-sulfamoyl-*N*-tetradecanoyl tyramine, DU-14) which was found to possess an IC_{50} value of 56nM.

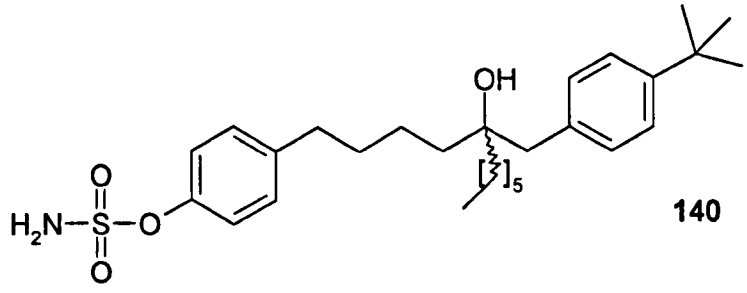
The activity decreased considerably for compound **139** (IC_{50} value 158nM) despite being one carbon longer, presumably due to steric hindrance between the alkanoyl group and the hydrophobic group at the active site. These compounds were found to lack estrogenic property when evaluated against estrogen-dependent MCF-7 cells (Li *et al.*, 1996).



	n	IC_{50} (nM)
131	4	14300
132	5	1880
133	6	600
134	7	253
135	8	180
136	9	74
137	10	61
138	11	56
139	12	158

Table 17: First mono-aryl tyramine based E1STS inhibitors (Li *et al.,* 1996).

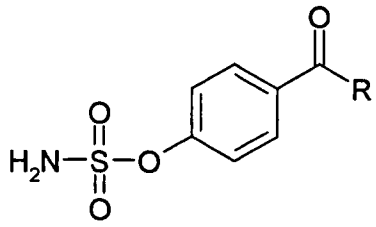
Compound **140** (Figure 14) was based on monoaryl tyramine compounds and was found to possess an IC_{50} value of 0.4nM in homogenates of HEK-293 cells transfected with STS (Ciobanu *et al.*, 2002). Compound **140** was therefore found to be more potent than EMATE and **138** (IC_{50} values of 0.9nM and 1.6nM respectivley under similar conditions). Changes in the aliphatic chain length did not improve the potency of this compound, the activity decreasing markedly with any alteration.



140

Figure 14: Potent inhibitor of E1STS (Ciobanu *et al.*, 2002).

Other mono-aryl sulfamates have been considered (Tables 18 and 19). Assay of the phenyl ketone sulfamate-based compounds showed that they were potent inhibitors of E1STS, with the most potent of the range, compound **148**, possessing an IC_{50} of 3.4μM (Ahmed *et al.*, 2002a).



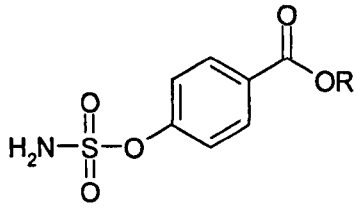
	R	IC_{50} (μM)
141	H	254
142	CH_3	302
143	C_2H_5	116.4
144	C_3H_7	39.8
145	C_4H_9	20.9
146	C_6H_{13}	5.0
147	C_7H_{15}	5.6
148	C_8H_{17}	3.4
149	C_9H_{19}	13
EMATE		0.5

Table 18: Sulfamated phenyl ketone inhibitors of E1STS (placental microsome assay; Ahmed *et al.*, 2002a).

This led to the synthesis of a series of phenyl ester sulfamates being synthesised (Table 19); the initial study containing a range of straight chain esters (**150** to **159**), resulted in inhibitors that were comparable to the phenyl ketones, most notably compounds **155**, **156** and **159**, which possessed IC_{50} values of 3.8μM, 3.4μM and 5.0μM respectively. Derivatisation of the straight alkyl chain moiety led to the developemnt of compound **163** (possessing an IC_{50} value of 0.17μM) which was found to be more potent than 667-COUMATE (possessing an IC_{50} value of 0.21μM).

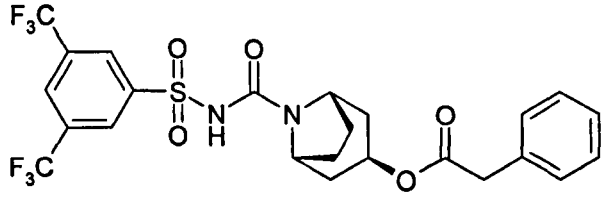
1.10 Non-sulfamate inhibitor of E1STS

With the importance of the sulfonates established, in particular, the aminosulfonates, some research groups experimented with differing types of sulfonates. In a series of nortropinyl-arylsulfonylurea derivatives, the most potent was shown to be compound **164** (Figure 13), which possessed an IC_{50} of 84nM. Being only slightly less potent than EMATE, this possessed an IC_{50} of 56nM in the same assay. Compound **164** is also one of the most potent reversible inhibitors of E1STS synthesised *todate* (Nussbaumer *et al.*, 2003a).



	R	Activity IC_{50} (μM)
150	CH_3	31.6
151	C_2H_5	31.6
152	C_3H_7	13.2
153	C_4H_9	10.5
154	C_5H_{11}	5.9
155	C_6H_{13}	3.8
156	C_7H_{15}	3.4
157	C_8H_{17}	5.0
158	C_9H_{19}	4.8
159	$C_{10}H_{21}$	22.4
160	*c*-C_5H_9	9.3
161	*c*-C_6H_{11}	1.7
162	*c*-C_7H_{13}	0.5
163	*c*-C_8H_{15}	0.17
-	EMATE	0.5
-	COUMATE	13.8
-	667-COUMATE	0.21

Table 19: sulfamated phenyl ester inhibitors of E1STS evaluated against placental microsome (Patel *et al.*, 2004).



164

Figure 13: Potent reversible nonsteroidal inhibitor of E1STS (Nussbaumer *et al.*, 2003a).

The natural product of soil bacterium *Nonomuria rubra*, madurahydroxylactone (MHL) was found to have moderate activity against E1STS (Figure 14).

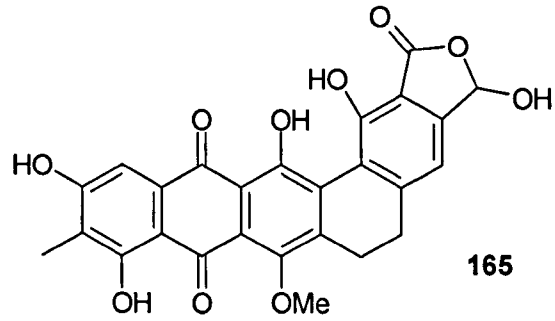
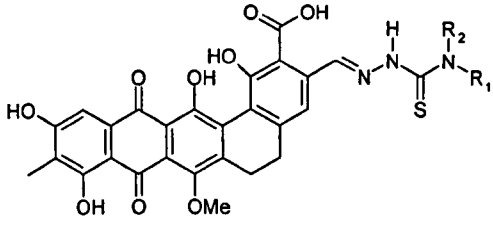


Figure 14: natural product MHL inhibits E1STS.

The discovery of the inhibitory activity displayed by MHL led to the synthesis of a series of thiosemicarbazones (Table 20).



	R_1	R_2	IC_{50} (μM)
166	-	-	41
167	H	*c*-C_3H_5	22
168	H	*c*-C_5H_9	3.2
169	H	*c*-C_6H_{11}	0.46
170	H	*c*-C_7H_{13}	1.8
171	H	*c*-C_8H_{17}	15
172	H	Ph	4.8
173	H	*c*-C_6H_{10}-4-Me	4.0
174	CH_3	*c*-C_6H_{11}	1.4
EMATE	-	-	0.08
COUMATE	-	-	2.6

Table 20: Thiosemicarbazones derivatives as potent E1STS inhibitors (adapted froom Jütten, 2002).

The most potent compound found in this range was **169**, which was found to possess an IC_{50} value of 0.46μM (under similar conditions EMATE was found to possess an IC_{50} value of 0.08μM). Consideration of the biological activity observed within compounds **167-171** show how the increase in alkyl chain length (and therefore hydrophobicity) led to an increase in inhibitory activity up to the cylcohexyl moiety, with rapid decline in potency observed thereafter. The nature of substrate binding was investigated with the synthesis of compound **172** showing an increase in potency with the aromatic R_2 group possessing an IC_{50} value of 4.8μM, being 10 times less potent than **169**.

1.11 Mechanism of irreversible inhibition of E1STS

As previously mentioned, the mechanism for the inhibition of E1STS has yet to be fully elucidated. This is mainly due to the difficulty in studying the active site, since obtaining a crystal structure of this membrane-bound enzyme has proved to be difficult. However, crystal structures of bacteria-derived free sulfatases have led to some more detailed structure of the active site of E1STS, which has aided the further understanding of the mechanism of action of E1STS. For example, the differences in the resting forms of the formyl glycine (FGly) observed in the crystal structures of ASA and ASB (Bond *et al.*, 1997) has led to two different mechanisms of action of E1STS.

One suggested mechanism involves attack by the oxygen atoms of the *gem*-diol on the sulfur of the sulfate moiety within the substrate, thereby resulting in the loss of RO^- ion and the sulfated form of the enzyme. The reformation of the free FGly through the expulsion of the sulfate moiety is then followed by the hydrolysis of the aldehyde regenerating the gem-diol (Figure 15a).

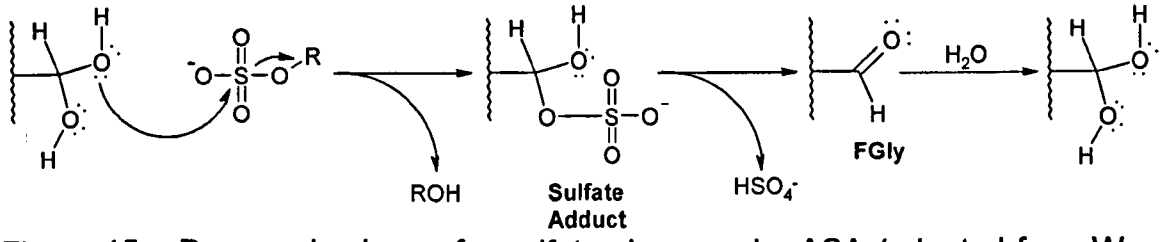


Figure 15a: Proposed scheme for sulfate cleavage by ASA (adapted from Woo *et al.*, 2000).

In the reaction catalysed by ASB, the resting state is thought to be the FGly and not the gem-diol, as such, the nucleophilic oxygen of the sulfate moiety attacks the aldehidic carbonyl of the FGly (Figure 15b). The resultant sulfate ester is hydrolysed, resulting in the formation of the sulfate adduct and the alcoholic substrate.

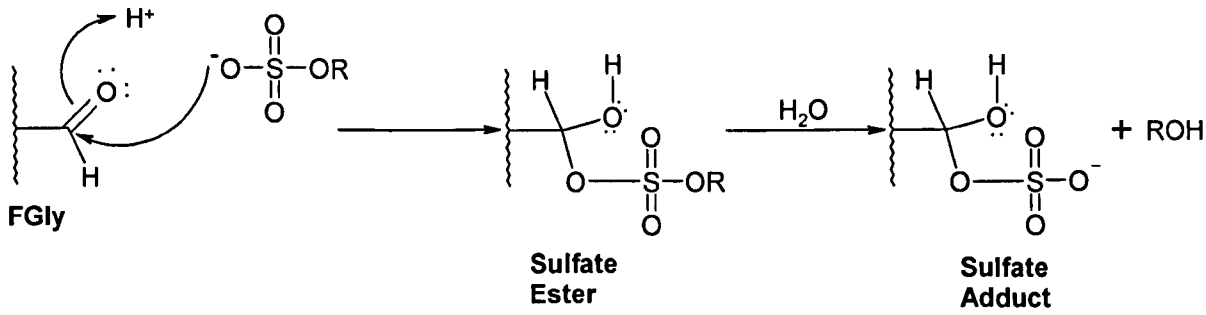


Figure 15b: Proposed scheme for sulfate cleavage by ASB (adapted from Woo *et al.*, 2000).

From the consideration of the mechanism of action postulated, mechanisms have been proposed to rationalise the inhibitory activity of the aryl sulfamates, such as EMATE and 667-COUMATE, and based on the inhibitory activity of these sulfamate-based compounds, Woo *et al.* (2000) postulated one possible mechanism (Figure 16). In general, the mechanism proposes that the formyl group of E1STS is subjected to nucleophilic attack by the lone pair of electrons on the nitrogen atom of the sulfamate moiety. The result of this is a hemiaminal intermediate (I) (Figure 16) which then undergoes hydrolysis to yield the alcohol (e.g 667-coumarin, in the case of attack by 667-COUMATE) and the sulfamoylated enzyme (II) (Figure 16).

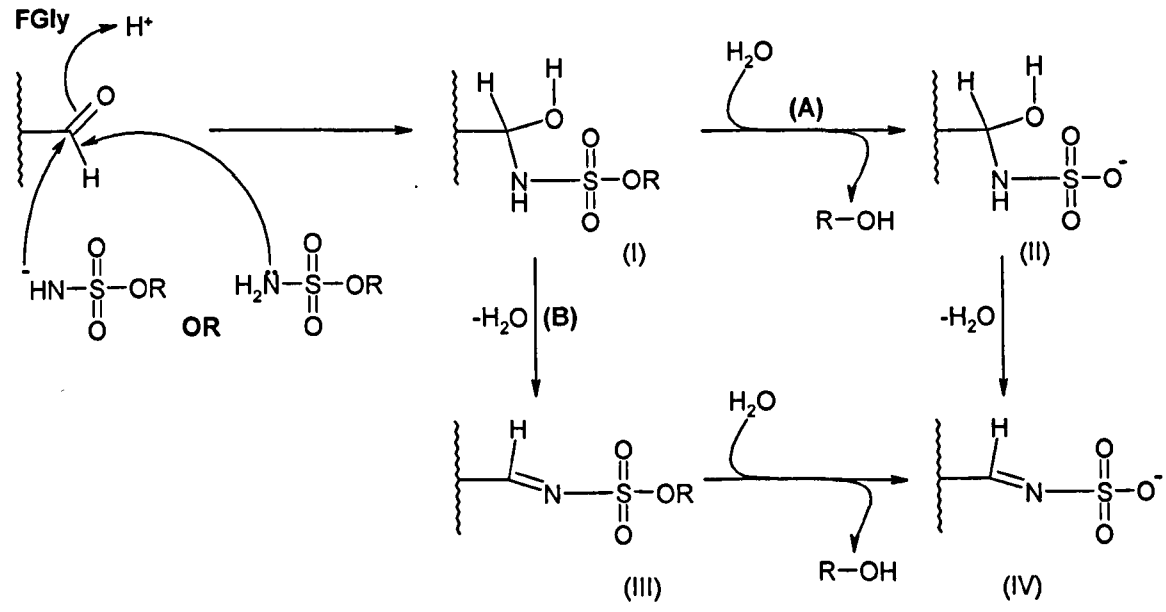


Figure 16: Proposed mechanism of E1STS by aryl sulfamate based compounds (modified from Woo *et al.*, 2000).

It is proposed by the authors that a dehydration step occurs (Step A) which results in the formation of a sulfamoylated imine moiety within the enzyme active site, thereby irreversibly inhibiting the enzyme at the end of path A (Figure 16). Alternatively (in pathway B) the dehydration of the hemiaminal intermediate (I) and subsequent hydrolysis of the resultant ester (III) results in the overall irreversible inhibition of E1STS.

Although the above mechanisms descibed help rationalise the inhibitory activity observed in a number of aryl sulfamate-based inhibitors of E1STS, crucially, it does not help rationalise the lack of inhibition observed within a range of sulfamates. For example, a range of alkyl sulfamate-based compounds have previously been synthesised and evaluated against E1STS and were found to be non-inhibitors; the compounds were evaluated at a concentration of 1mM and no significant levels of inhibition were observed (Ahmed *et al.*, 2000b). The mechanism postulated above would suggest that the alkyl sulfamates would be expected to show some levels of inhibition.

Furthermore, in their extensive studies on the 4-hydroxybenzoic acid esters and 4-hydroxyphenyl ketones, it proposed that the major determining factor in determining overall inhibitory activity observed within aryl and alkyl sulfamate-based compounds were pK_a and logP (Ahmed *et al.*, 2000a; Ahmed *et al.*, 2002c). It was further suggested that the involvment of pK_a was related to the stability of the alkoxide or the phenoxide ion resulting from the initial cleavage of the RO-sulfamate bond and which is therefore an important step in the inhibition process (Figure 17). This was therefore a direct contradiction to the mechanism proposed by Woo *et al.* (2000), in which the cleavage of the RO-sulfamate bond does not occur until imine production.

In the mechanism proposed by Ahmed *et al.* (2002c) the substrate alcohol is released from the active site prior to attack of the FGly carbonyl (Figure 17). In this mechanism, attack is initiated by the action of one of oxygen atoms within the *gem*-diol moiety of the hydrolysed FGly. As such, this aids the rationalisation of the dependence of the pK_a of the parent alochol (and therefore RO^-) of the inhibitor showing good correlation with the inhibitory activity observed within the

sulfamte derivatives of a wide range of inhibitors as well as non-inhibitors. The resulting hemiaminal then undergoes conversion to the FGly which allows the NH_2 moiety with sulfamic acid to undertake attack of the FGly C=O bond, eventually resulting in the formation of the imine functionality within the active site.

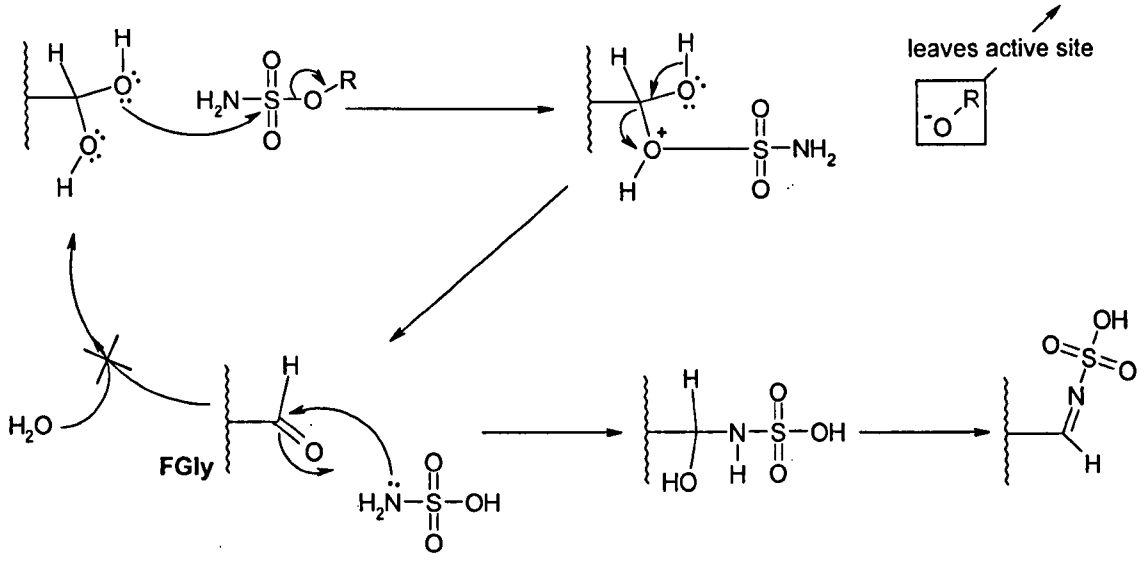


Figure 17: Mechanism for the irreversible inhibition of E1STS (Ahmed *et al.*, 2002b).

1.12 Basis of current investigation

The use of hormone therapy, in particular estrogen ablation therapy, has been the major focus for the treatment of hormone-dependent breast cancer. The current direction attempts to decrease the concentration of circulating estrogen by interrupting the stages of the biosynthetic pathways that lead to the formation of E1 and E2 within the steroidal cascade. E1STS is the enzyme responsible for the conversion of E1S, the stored form of the estrogen, to its non-conjugated and active form, E1. The inhibition of this enzyme has been shown to lead to a marked decrease in the circulating E1 in the body of postmenopausal women; this would therefore lead to the loss of stimulus for estrogen-dependent breast cancer cells.

The potency of sulfamate compounds, in particular, the irreversible nature of their inhibition, led to a line of research in which the aminosulfonyl group became the focal point for potent compounds (e.g. 667-COUMATE). Previously, a series of highly potent inhibitors of E1STS have been synthesised based on the sulfamated derivatives of esters of 4-hydroxybenzoic acid. However, esters are known to be metabolically unstable due to the esterases present within plasma. The synthesis of compounds which would result in metabolically stable compounds whilst possessing the sulfamate moiety for potent and irreversible inhibition of E1STS would allow us the opporunity to improve on the biological activity previously observed in compound **163** (page 36, Table 19) and which was found to be more potent than 667-COUMATE. As such, the synthesis of amide derivatives (Figure 20) would allow us to circumvent the metabolism of the ester functionality by the esterases.

In previous studies, the synthesis of alkyl sulfamates has been attempted where it was shown that alkyl sulfamates containing electron-withdrawing groups α to the carbon attached to the OH group in the parent alkyl alcohol (and subsequently derivatised to the sulfamate moiety) results in an overall increase in inhibitory activity. In an effort to further investigate this hypothesis, it is proposed to undertake the synthesis of sulfamate derivatives of substituted benzyl alcohol and substituted alkyl alcohols (Figure 20). Through the use of

electron-withdrawing groups, we would be able (after biochemical evaluation) to correlate IC_{50} with pK_a of these and the benzamide based compounds.

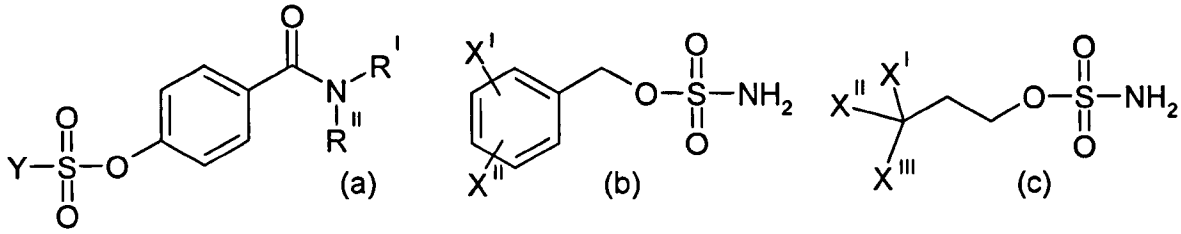


Figure 20: Compounds to be investigated (a) *N*-alkyl benzamides, (b) benzyl alcohol and (c) substituted ethanols (R^{I}= Alkyl groups; R^{II}= H, alkyl groups; Y= Amino, methane and trifluoromethane moieties; $X^{I, II \& III}$= F, Cl, Br or NO_2).

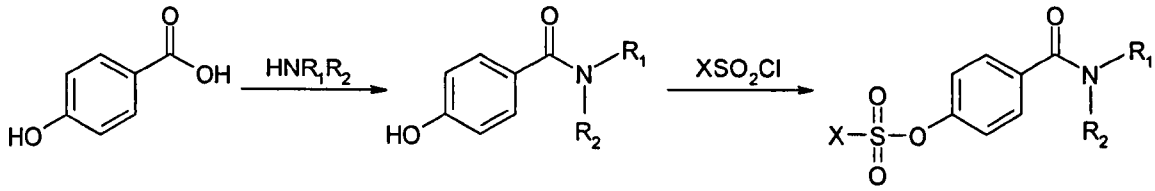
CHAPTER 2

SYNTHESIS OF SULFONATED DERIVATIVES OF *N*-ALKYL DERIVATIVES OF 4-HYDROXY BENZAMIDE

2.0 SYNTHESIS OF SULFONATED DERIVATIVES OF 4-HYDROXY BENZAMIDE

2.1 Discussion

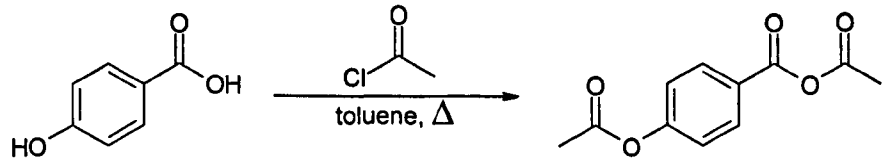
The synthesis of the *N*-alkyl benzamides was achieved following the following general scheme depicted in Scheme 1.



Scheme 1: General reaction scheme for the synthesis of *N*-alkyl benzamide sulfonates (R_1= alkyl groups; R_2= H or alkyl groups; X= NH_2, CH_3 or CF_3).

The first step in the synthesis of the target compounds involved the synthesis of a range of the *N*-alkyl derivatives of 4-hydroxybenzamide followed by the sulfonation of the 4-hydroxy moiety to give a range of *N*-alkyl derivatives as the target compound.

The synthesis of amides from carboxylic acids may be undertaken via several different routes; however, due to the presence of the 4-OH moiety within the starting material, we considered the initial protection of the OH through the formation of an ester functionality followed by the synthesis of the desired amide functionality (Scheme 2).

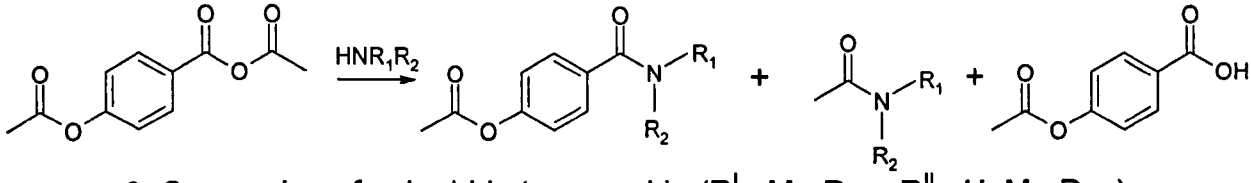


Scheme 2: Ester and anhydride formation around 4-hydroxy benzoic acid.

The formation of an ester functionality may be brought about by the reaction of an alcohol with a carboxylic acid, however, the reaction is reversible and an equilibrium is reached. In this type of reaction an acid catalyst is often used to facilitate the reaction- the acid is hypothesised to be involved in the protonation of the carboxyl oxygen, thereby activating the carbonyl carbon toward nucleophilic attack by the oxygen atom of the alcohol. Furthermore, the equilibrium may be 'forced' to the products through the use of excess alcohol or

through the removal of water from the system (e.g. via the use of Dean-Stark distillation apparatus) (Furness *et al.*, 1996). In the synthesis of the amide, the carboxylic acid moiety can now be activated by the conversion to an acyl chloride or an anhydride (and as a result of the protection of the 4-OH moiety no side reactions would therefore be expected) allowing the activated acyl moiety to react with the appropriated amine to yield the corresponding amide

We hypothesised that the two steps outlined above could be combined into a 'one-pot' synthesis, whereby the protection of the phenolic OH moiety could be achieved whilst simultaneously activating the carboxylic moiety. That is, we considered the reaction between 4-hydoxybenzoic acid and acety chloride, the resulting anhydride (where the 4-OH is also converted to the ester functionality) can undergo reaction with an appropriate amine to give the target amide (Scheme 3).

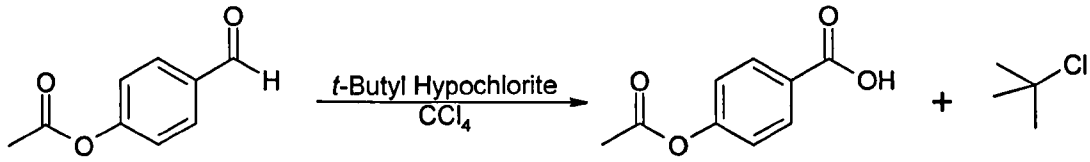


Scheme 3: Conversion of anhydride to an amide (R^{I}= Me-Dec; R^{II}= H, Me-Dec).

Unfortunately, whilst the 'one-pot' approach gave the desired compound the yield was found to be relatively low (≤36%). We investigated the reason for the low yield and discovered that this was mainly due to the synthesis of two major impurities, namely the appropriate derivative of acetylamide and 4-acetoxy benzoic acid. In an effort to impove the yield (since the 4-hydroxybenzamide is the major starting material in the synthesis of a number of sulfonate based target compounds), we re-considered the 'one-pot' synthetic approach, in particular, we considered the sythesis of the amide from initial synthesis of 4-acetoxy benzoic acid.

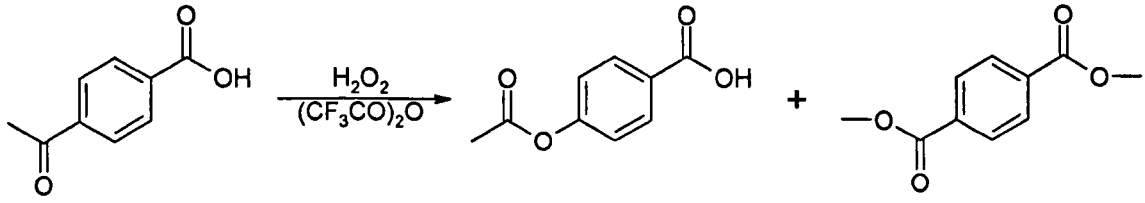
In 1951, Ginburg reported the sythesis of 4-acetoxy benzoic acid in high yield (91%) which involved the reaction between 4-acetoxy benzaldehyde and *t*-butyl hypochlorite in carbon tetrachloride. The reaction is hypothesised to proceed through the rapid production of 4-acetoxy benzoyl chloride; prolonged heating of the mixture would lead to the production of the aromatic acid and *t*-butyl chloride,

which is distilled off continually to give 4-acetoxy benzoic acid in high yield (Scheme 4).

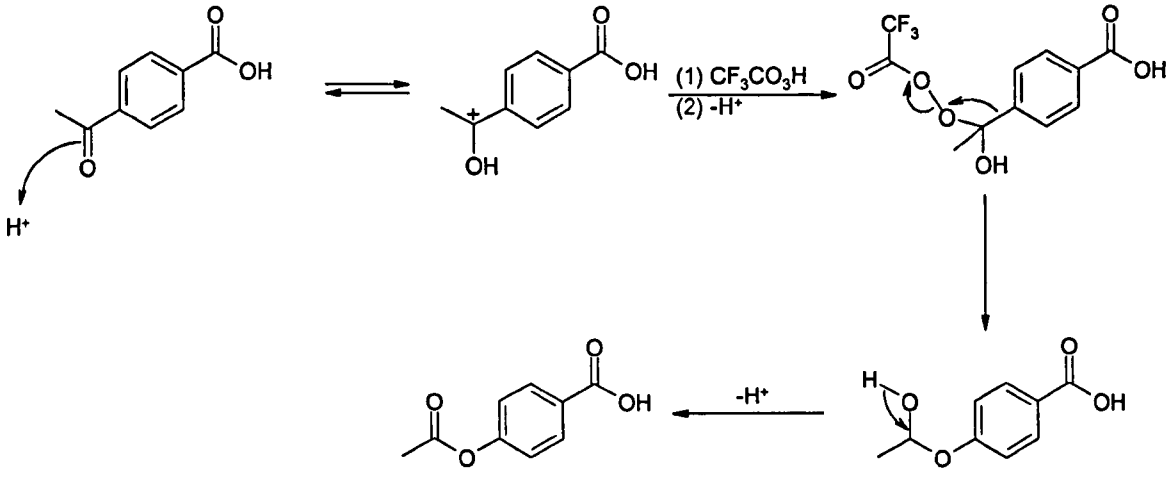


Scheme 4: Synthesis of 4-acetoxy benzoic acid.

An alternative route was explored by Smissman *et al.* (1968) who used the Baeyer-Villiger reaction. This involves the oxidation of 4-acetyl benzoate to produce 4-acetoxy benzoic acid. This was achieved by the reaction of hydrogen peroxide and trifluoroacetic anhydride which is hypothesised to generate a peroxy acid that gave the product in high yield (92%) with dimethyl terephthalate as the major impurity (Scheme 5a & b) (Sykes, 1986).



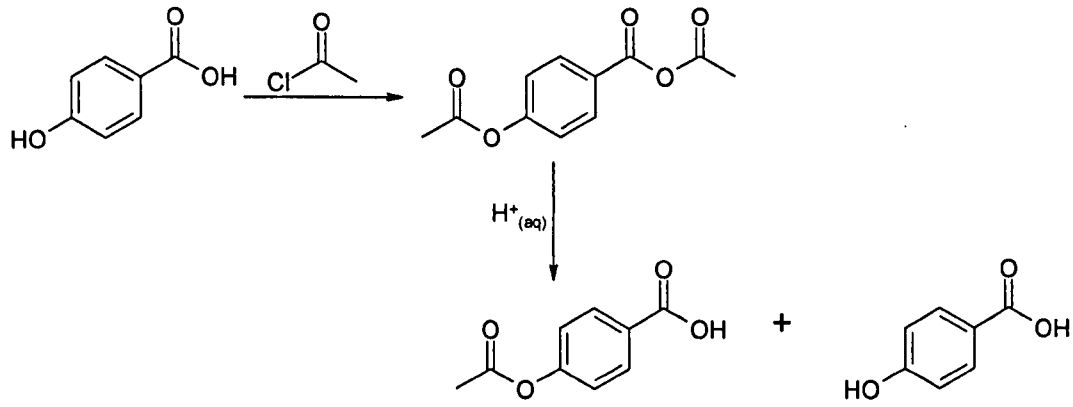
Scheme 5a: Baeyer-Villiger scheme.



Scheme 5b: Baeyer-Villiger reaction mechanism of 4-acetylbenzoate.

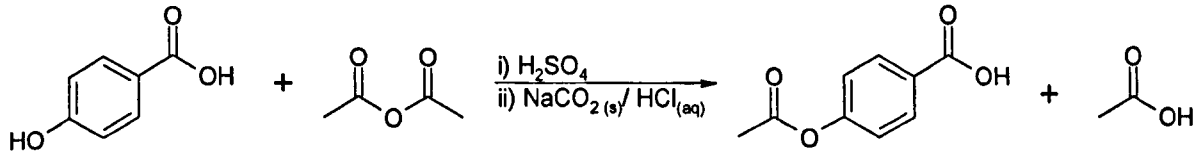
In the current study, we considered the reaction between 4-hydroxy benzoic acid and acetyl chloride (outlined in Scheme 2). However, in a modification of Scheme 6, the hydrolysis of the anhydride back to the carboxylic acid using dilute aqueous HCl was considered. The reaction proceeded without any major problems, however, the yield for this step was found to be low (≤10%) and on analysis of the crude product, we observed the synthesis of a large quantity of 4-

hydroxy benzoic acid (Scheme 6). The use of excess quantities of acetyl chloride did not improve the yield, as such, we hypothesised that the product, whilst being produced in the reaction, was potentially undergoing acid hydrolysis (in particularly the ester moiety) due to the HCl, therefore resulting in the synthesis of 4-hydroxy benzoic acid. In an effort to reduce the acid hydrolysis of the ester, we attempted the work up with varying concentrations of HCl, however, the yield could not be improved (Jiang *et al.*, 1984).



Scheme 6: 4-acetoxybenzoic acid synthesis using acetyl chloride and acid hydrolysis.

A review of the literature suggested the use of base rather than acid in the hydrolysis of the anhydride. For example, in the sythesis of acetylsalicylic acid, sodium carbonate ($NaCO_3$) was used together with dilute solution of HCl which resulted in the sythesis of the desired compound (Zubrick, 1997). On carrying out the work up with the $NaCO_3$ in the place HCl, 4-acetoxybenzoic acid was obtained in increase yield (80%) and without any problems (Scheme 5).

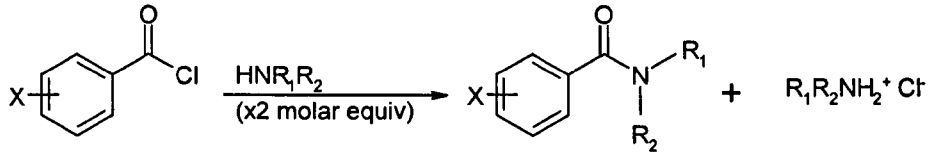


Scheme 7: 4-Acetoxy benzoic acid formation using acetic anhydride.

2.1.1 Synthesis of benzamide derivatives

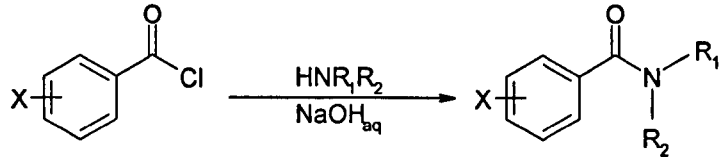
Alkyl benzamides may be formed via various different routes. One of the highest yielding reactions involves the reaction of a benzoyl chloride with the appropriate amine (primary or secondary), however, the difficulty with this route involves the evolution of HCl. To counteract this acid production, the amine is often used in excess, such that the excess would therefore be expected to give an amide salt

and has been shown to produce the amide in high yield and purity (Scheme 8; Clayden *et al.*, 2001).



Scheme 8: Benzoylation of amines (R_1= Alkyl groups; R_2= H, alkyl groups).

Other methods include those based on the Schotten-Baumann method of amination, where the amine is added in molar equivalent quantities together with a base [e.g. aqueous sodium hydroxide (NaOH)] to remove HCl produced within the reaction mixture (Scheme 9).



Scheme 9: Schotten-Baumann method of amination (R_1= Alkyl groups; R_2= H, alkyl groups).

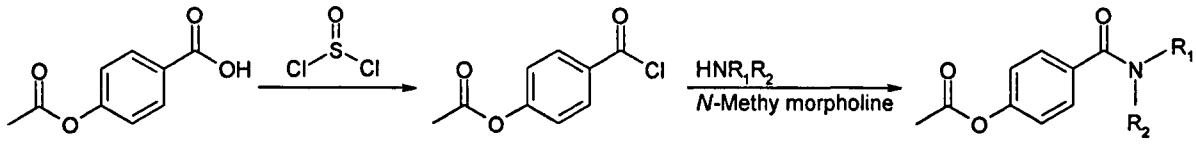
In certain cases the reaction may be carried out directly in the aqueous solution as it is considered if the acid chloride is used in excess the rate of attack of the acid chloride will be slower than the rate of reaction of the acid and the amine (Harwood *et al.*, 2001).

In another example, a two-phase system was utilised, in which the organic components of the reaction were dissolved in a suitable organic solvent, for example dichloromethane. Aqueous base is added to the system, however, since the base remains within the aqueous layer, it was found to be unable to react with the acid chloride, whilst the HCl was found to be partitioned out of the organic and into the aqueous layer (Harwood *et al.*, 2001).

In the synthesis of the amides, we initially considered the direct reaction between the amine (in excess) and 4-acetoxybenzoyl chloride (Scheme 8) – the latter having been synthesised by the reaction of 4-acetoxy benzoic acid with excess thionyl chloride in anhydrous toluene. The synthesis of the dialkyl benzamides proceeded without any major problems following this method. However, the

synthesis of the short chain mono-alkyl containing amides (particularly the methyl and ethyl) proved to be extremely troublesome, the yields being relatively low (ranging from 15% for compound **208** to 20% for compound **212**).

A variation on the Schotten-Baumann method of amination was discovered which involved the use of an organic base (in particular, *N*-methyl morpholine) (Vale, 1957). The major advantage reported with this method involves the precipitation of a salt (presumed to be *N*-methyl morpholine hydrochloride) which results from the reaction of the HCl formed during the reaction. The target amide was then recovered by filtration and removal of the solvent.



Scheme 10: Amide formation of the carboxylic acid (R_1= Alkyl groups; R_2= H, alkyl groups).

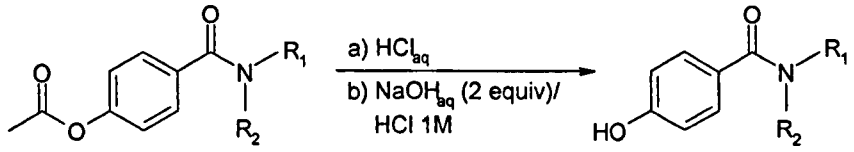
The use of *N*-methyl morpholine resulted in the synthesis of the larger alkyl chain containing compounds in good yield (in excess of 90% at this stage) and without any major problems.

As previously mentioned, the use of HCl in the hydrolysis of the anhydride also resulted in the hydrolysis of the ester moiety. Acid hydrolysis is presumed to involve the initial protonation of the carbonyl oxygen followed by the subsequent attack of the carbonyl carbon by an oxygen atom on the water molecule. The reaction is driven by the presence of excess water, forcing the equilibrium steps toward the acid rather than back toward ester reformation.
Initially the hydrolysis was attempted using dilute HCl solution (1.0M) and gentle heating. The reaction did indeed result in the hydrolysis of the ester moiety, however, the major product was found to be 4-hydroxybenzoic acid, that is, the acid had also resulted in the hydrolysis of the amide moiety. The use of weaker acid and lack of heat did not yield the target compound in sufficient quantity (the highest yield obtained was 20%).

Esters may also be hydrolysed under basic conditions. The hydroxide ion directly attacks the carbonyl carbon to give the alcohol and the carboxylic acid - the use

of excess base results in the formation of the salt of the carboxylic acid, thereby resulting in complete hydrolysis.

In the synthesis of *N*-alkyl 4-hydroxybenzamide, a modification of Vale (1957) was usitilised. In the modified method, the amide was dissolved in 2 equivalents of aqueous solution of NaOH followed by the acidification (Scheme 11). The use of the modified method gave the required amides in good yield (ranging from 71% for compound **180** to 93% for compound **192**). However, in the synthesis of the mono-alkyl benzamide, major problems were encountered.



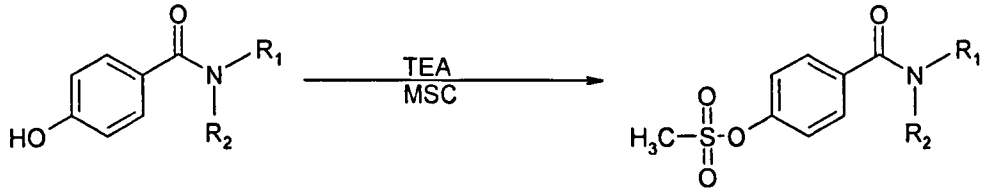
Scheme 11: Hydrolysis of phenolic ester (R_1= Alkyl groups; R_2= H, alkyl groups).

In the initial attempts to synthesise the mono-methyl and ethyl benzamide derivatives the yields were low (≤20%). Analysis of the crude product did not indicate any difference from the other alky benzamides synthesised. An extraction of the aqueous solution used in the base hydrolysis of 4-acetoxy benzamide revealed that the hydrolysed forms of the methyl and ethyl benzamides were dissolved. This proved difficult to recover form the aqueous layer, with only small amounts of it dissolving in organic solvents used to extract. We also discovered that the compounds were found to undergo hydrolysis of the amide functionality, resulting in poor yield. To add to the problem of hydrolysis, the purification of the shorter chain length compounds also proved to be difficult, with multiple chromatography columns being required to be run to produce pure compound thereby further reducing the overall yield (9%). This was overcome to a certain extent by acidifying to pH5 instead of pH2 as in the usual base hydrolysis work-up, however, this did not solve the yield problem entirely, but gave adequate quantities for the use in subsequent reactions.

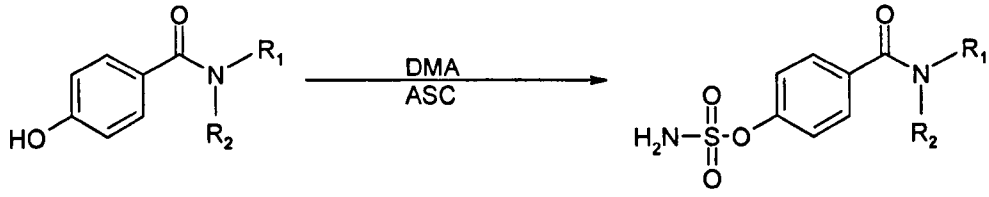
2.1.2 Synthesis of 4-sulfonated derivatives of 4-hydroxybenzamide

In the synthesis of 4-sulfonate derivatives of 4-hydroxybenzamide, the reaction outlined in Scheme 1 (step 2) was undertaken. In general, the reactions involved reacting derivatives of *N*-alkyl 4-hydroxybenzamide with the appropriate sulfonyl

chloride [in the presence of the weak base such as triethylamine (TEA) for the methanesulfonate and trifluoromethane sulfonate (Scheme 12a) – no base was used in the case of aminosulfonyl chloride] under anhydrous conditions to give the corresponding sulfonated derivatives. The reactions were found to progress in relatively good yield and, in general, without too many problems. For example, in the synthesis of the methanesulfonate derivatives, the reaction between methanesulfonyl chloride and the appropriate *N*-alkyl 4-hydroxybenzamide led to the synthesis of the target compounds in good yield, ranging from 30% for compound **194** and 73% for compound **182**.



Scheme 12a: Synthesis of methane sulfonate and trifluoromethane sulfonate derivatives of 4-hydroxy alkyl benzamide (R_1= Alkyl groups; R_2= H, alkyl groups).

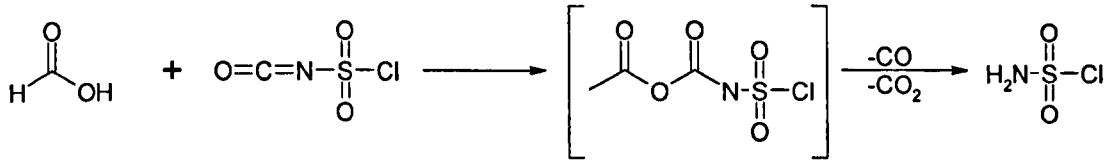


Scheme 12b: Synthesis of sulfamate derivatives of 4-hydroxy alkyl benzamide (R_1= Alkyl groups; R_2= H, alkyl groups).

However, in the synthesis of the short chain mono-*N*-alkyl benzamide derivatives, in particular, the methyl (**209**) and ethyl (**213**) derivatives, we discovered that a number of by-products were observed both on thin layer chromatography (TLC) as well as gas chromatography-mass spectrometry (GC-MS) and the purification of the pure product proved to be difficult.

We hypothesised that the synthesis of the mono-methyl and ethyl benzamide derivatives faired poorly due to the labile proton of the nitrogen, which could act as another reaction centre for the sulfonyl chloride to react at. This led to low yields in these compounds that was difficult to overcome. Despite this the reactions gave enough products for analysis and any possible testing that might be done in the future.

In the synthesis of the amino sulfonate derivatives, the reaction between 4-hydroxyphenyl ketones and aminosulfonyl chloride led to a number of problems which have previously been well documented. In summary, this reaction has been reported in the literature to be extremly troublesome and led to poor yields. The main problem with this reaction has been mainly due to the extremely poor stability of both the aminosulfonyl chloride and the target compound. That is, aminosulfonyl chloride is not readily available commercially, due to its rapid degradation (Woo *et al.*, 1996a) and was therefore synthesised *in situ* prior to immediate use (Scheme 13).

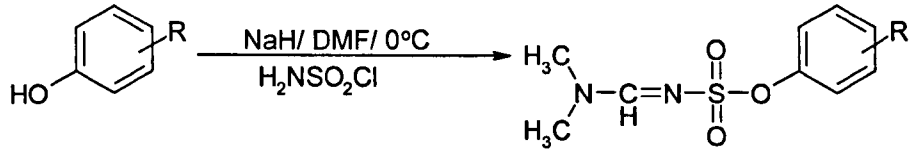


Scheme 13: Amino sulfonyl chloride synthesis.

The reaction leading to the formation of the aminosulfonyl chloride is believed to involve an initial nucleophilic attack (by the methanoic acid) on the chlorosulfonyl isocyanate, resulting in an intermediate which undergoes decarbonylation and decarboxylation to give aminosulfonyl chloride. In the initial report on the synthesis of aminosulfonyl chloride (Appel and Berger, 1958), the authors extracted the product into benzene, filtered, and removed the solvent under vacuum without the use of heat. In the present study, benzene was replaced with anhydrous toluene, however, this resulted in a problem involving the removal of the solvent. That is, toluene could not be readily removed at room temperature and any heating resulted in the degradation of the product; as a consequence, the product was not purified and was left in solution in toluene, after decanting.

As previously mentioned, the synthesis of sulfamate-based compounds as potential inhibitors of E1STS has been extensively studied within our group and the initial synthesis of the sulfamate-based compounds was previously attempted using sodium hydride (NaH) as a base for the reaction (James, 2000; Patel, 2004), in a similar manner to Woo *et al.* (1996a). However, it was discovered that an undesired dimethylformamide (DMF) adduct was formed (Scheme 14). Schwarz *et al.* (1996) in their report proposed that the DMF adduct

was formed through the attack on the carbonyl carbon atom of DMF by the lone pair of electrons on the nitrogen atom of the sulfamate moiety, followed by the dehydration of the product during the work up to give the imine type product (Scheme 13) (Woo *et al.*, 1998).



Scheme 14: Formation of the DMF adduct.

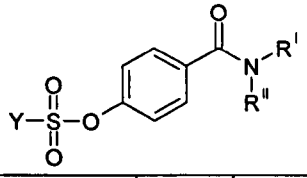
Okada *et al.* (2000) discovered a successful method of sulfamoylation which avoided the use of a base and thereby avoiding the production of the DMF adduct. In their study, the authors used different bases with various solvents in an attempt to increase the yield, however, they concluded that the use of dimethylacetamide (DMA) as the solvent in the absence of a base was sufficient to give the target compound in relatively high yield. Furthermore, the authors found that the solvent used for the reaction had a profound effect on the rate of sulfamoylation.

In our hands, no problems were encountered using DMA and as such, all of the sulfamoylation reactions of the various *N*-alkyl 4-hydroxybenzamide derivatives were undertaken using the above method and the reactions proceeded smoothly however in poor to moderate yields ranging from 12% (**193**) to 45% (**205**).

As before, the synthesis of the sulfamate derivatives of mono-alkyl benzamide based compounds resulted in numerous problems. The main issue with these compounds was the number of side-reactions taking place, making them very difficult to purify. The identities of these products indicate the involvement of the nitrogen proton in the reaction; or the nitrogen itself, activated by the presence of the labile proton, resulting in unwanted side reactions. However, those compounds which were purified (as indicated by elemental analysis) were found to give unusual spectral data, in particular, the ^{1}H NMR proved to be difficult to obtain although the ^{13}C NMR did not give any unusual patterns (for the same sample with the ^{13}C NMR being undertaken immediately after the ^{1}H NMR experiment), as such, the majority of the mono-alkyl benzamide sulfamate-based

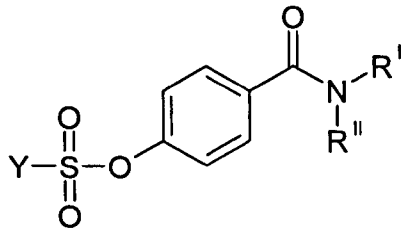
compounds have been reported with limited spectral data, i.e. the ^1H NMR spectra is missing for a number of compounds.

The target compounds synthesised in this study are summarised in the following tables.



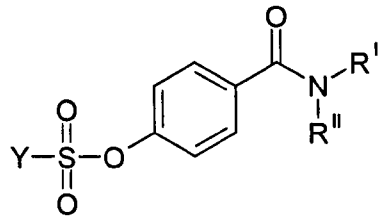
R'	R''	Y	Compound No	page
CH_3	CH_3	NH_2	**178**	60
CH_2CH_3	CH_2CH_3	NH_2	**182**	62
$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	NH_2	**186**	65
$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	NH_2	**190**	68
$(CH_2)_4CH_3$	$(CH_2)_4CH_3$	NH_2	**194**	70
$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	NH_2	**198**	73
$(CH_2)_7CH_3$	$(CH_2)_7CH_3$	NH_2	**202**	76
$(CH_2)_9CH_3$	$(CH_2)_9CH_3$	NH_2	**206**	78
CH_3	CH_3	CH_3	**179**	60
CH_2CH_3	CH_2CH_3	CH_3	**183**	63
$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	CH_3	**187**	66
$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	CH_3	**191**	68
$(CH_2)_4CH_3$	$(CH_2)_4CH_3$	CH_3	**195**	71
$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	CH_3	**199**	74
$(CH_2)_7CH_3$	$(CH_2)_7CH_3$	CH_3	**203**	76
$(CH_2)_9CH_3$	$(CH_2)_9CH_3$	CH_3	**207**	79
CH_3	CH_3	CF_3	**180**	61
CH_2CH_3	CH_2CH_3	CF_3	**184**	64
$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	CF_3	**188**	66
$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	CF_3	**192**	69
$(CH_2)_4CH_3$	$(CH_2)_4CH_3$	CF_3	**196**	72
$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	CF_3	**200**	74
$(CH_2)_7CH_3$	$(CH_2)_7CH_3$	CF_3	**204**	77
$(CH_2)_9CH_3$	$(CH_2)_9CH_3$	CF_3	**208**	79

Table 20a: Sulfonate derivatives of 4-hydroxybenzamide



R'	R''	Y	Compound No	page
CH_2CH_3	H	NH_2	**213**	82
$(CH_2)_2CH_3$	H	NH_2	**217**	85
$(CH_2)_3CH_3$	H	NH_2	**221**	87
$(CH_2)_4CH_3$	H	NH_2	**225**	90
$(CH_2)_5CH_3$	H	NH_2	**229**	92
$(CH_2)_6CH_3$	H	NH_2	**233**	95
$(CH_2)_7CH_3$	H	NH_2	**237**	97
$(CH_2)_8CH_3$	H	NH_2	**241**	100
$(CH_2)_9CH_3$	H	NH_2	**245**	102
CH_3	H	CH_3	**210**	81
CH_2CH_3	H	CH_3	**214**	83
$(CH_2)_2CH_3$	H	CH_3	**218**	85
$(CH_2)_3CH_3$	H	CH_3	**222**	88
$(CH_2)_4CH_3$	H	CH_3	**226**	90
$(CH_2)_5CH_3$	H	CH_3	**230**	93
$(CH_2)_6CH_3$	H	CH_3	**234**	95
$(CH_2)_7CH_3$	H	CH_3	**238**	98
$(CH_2)_8CH_3$	H	CH_3	**242**	100
$(CH_2)_9CH_3$	H	CH_3	**246**	103
CH_3	H	CF_3	**211**	81
CH_2CH_3	H	CF_3	**215**	84
$(CH_2)_2CH_3$	H	CF_3	**219**	86
$(CH_2)_3CH_3$	H	CF_3	**223**	88
$(CH_2)_4CH_3$	H	CF_3	**227**	91
$(CH_2)_5CH_3$	H	CF_3	**231**	93
$(CH_2)_6CH_3$	H	CF_3	**235**	96
$(CH_2)_7CH_3$	H	CF_3	**239**	98
$(CH_2)_8CH_3$	H	CF_3	**243**	101
$(CH_2)_9CH_3$	H	CF_3	**247**	103

Table 20b: Sulfonate derivatives of 4-hydroxybenzamide



R^I	R^{II}	Y	Compound No	page
c-C_3H_5	H	NH_2	**249**	105
c-C_5H_9	H	NH_2	**253**	107
c-C_6H_{11}	H	NH_2	**257**	110
c-C_7H_{13}	H	NH_2	**261**	112
c-C_8H_{15}	H	NH_2	**265**	114
c-C_3H_5	H	CH_3	**250**	105
c-C_5H_9	H	CH_3	**254**	108
c-C_6H_{11}	H	CH_3	**258**	110
c-C_7H_{13}	H	CH_3	**262**	112
c-C_8H_{15}	H	CH_3	**266**	115
c-C_3H_5	H	CF_3	**251**	106
c-C_5H_9	H	CF_3	**254**	108
c-C_6H_{11}	H	CF_3	**259**	111
c-C_7H_{13}	H	CF_3	**263**	113
c-C_8H_{15}	H	CF_3	**267**	115

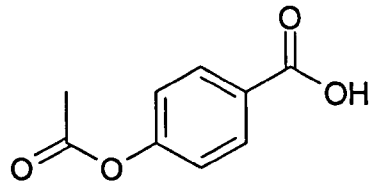
Table 20c: Sulfonate derivatives of 4-hydroxybenzamide

2.2 Materials and method

Chemicals were purchased from Sigma-Aldrich Company Ltd. (The Old Brickyard, Gillingham, UK), Lancaster Synthesis Ltd. (Newgate, Lancashire, UK) and Avocado Research Chemicals (Shore Road, Lancashire, UK). Structure elucidation was verified by ^{1}H and ^{13}CNMR (Jeol Eclispe+ 400 MHz and 100 MHz respectively). Infrared spectrometry was carried out on a Perkin-Elmer Fourier Transform-Paragon 1000 infrared spectrometer. Gas chromatography-mass spectra were obtained using Hewlett 5890 Packard series II GCMS machines. Low resolution mass spectra obtained using Varian 1200L Quadrupole MS. Melting points are uncorrected and were carried out on a Stuart Scinetific SMP3 melting point apparatus or Gallenkamp melting point instrument. High resolution mass spectroscopy was carried out at Chemistry Department Mass Spectrometry Sevice (King's College London, London, UK). Elemental analysis was carried out by the CHN microanalysis service (London School of Pharmacy, London, UK) using a Bruker Apex III System.

2.3 Synthesis of sulfonated derivatives of 4-hydroxy benzamide

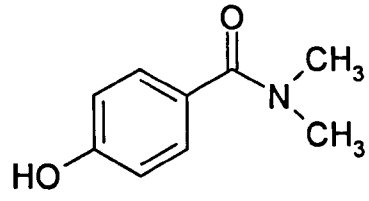
Synthesis of 4-acetoxy benzoic acid (**175**)



4-Hydroxy benzoic acid (9.70g, 70.23mmol) was dispersed in acetic anhydride (13ml, 137.53mmol), concentrated sulfuric acid (5 drops) was added in a dropwise manner and the mixture refluxed for 40min. The mixture was allowed to cool and water (250ml) added and the mixture left to stir. The mixture was filtered and washed with water (3x25ml). The solid was then added to saturated sodium bicarbonate solution ($NaHCO_3$, 125ml) and the resulting solution was filtered and poured added to ice cold aqueous HCl solution. The resulting solid was filtered, washed with cold water and dried under suction. The solid was purified via column chromatography (DEE, 100%) to give the product **175** as a white solid [10.06g, 79.51%; m.p. 189.6-190.4°C, lit. m.p. 191-192.5°C (Van Etten *et al.*, 1967); R_f: 0.329 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3080.00 (OH), 2986.66 (PhH), 2673.56 (CH) 1754.07 (RC=O), 1681.75 (HO-C=O); $\delta_H(CDCl_3)$: 8.13 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.19 (2H, d, J=8.97Hz, Ph<u>H</u>), 2.13 (3H, s, OCC<u>H</u>$_3$); $\delta_C(CDCl_3)$: 171.28, 168.96 (C=O), 155.67, 131.97, 126.92, 121.86 (ArC), 21.27 (CH_3); GC: t_R 7.80min; LRMS (EI): m/z 180 (M^+, 13%), 138 [M^+- OC_2H_2, 100%].

Synthesis of 4-hydroxy-*N,N*-dimethyl-benzamide (**176**)

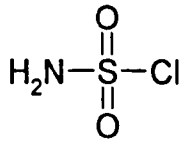


4-Acetoxy benzoic acid (2.01g, 11.14mmol) was added to a solution of thionyl chloride (1.99ml, 27.28mmol) in anhydrous toluene and the mixture left to reflux for 4hr. The solvent and excess thionyl chloride was removed under vacuum and the resultant oil was dissolved in a solution of *N*-methylmorpholine (1.25ml,

11.37mmol) in anhydrous toluene (50ml). Dimethyl amine [5.88ml (2M in THF), 11.76mmol] was added to the solution in a dropwise manner and the solution left to stir for 72hr. The reaction mixture was filtered and the solvent removed under vacuum to give a brown solid. Sodium hydroxide (NaOH) (14.48ml, 1.24M) was added to the solid over a period of five minutes and the resulting mixture left to stir for 1hr. The resulting solution was then acidified to pH2 with aqueous HCl (2M) and filtered. The product was purified using flash chromatography (DEE, 100%) to give **176** as a white solid [(1.14g, 62.07%; m.p. 159.1-160.6°C, lit. m.p. 162-163°C (Baker and Wallace, 1972); R_f 0.09 (DEE, 100%)].

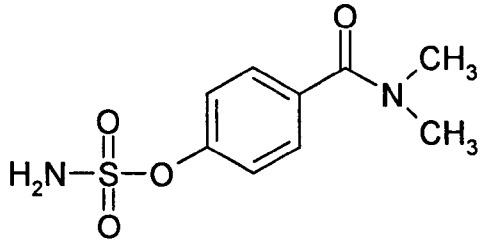
$\nu_{(max)}$(Film)cm^{-1}: 3151.59 (PhOH), 2935.37 (CH), 1608.01 (C=O); δ_H(d_6-Acetone): 8.78 (1H, s, PhO<u>H</u>), 7.23 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.76 (2H, d, J=8.79Hz, Ph<u>H</u>), 2.91 [6H, br.s, N(C<u>H</u>$_3$)$_2$]; δ_C(d_6-Acetone): 171.47 (C=O), 159.42, 130.10, 128.56, 115.49 (ArC); GC: t_R 8.95 min; LRMS (EI): m/z 165 (M^+, 17%), 121 [M^+- $N(CH_3)_2$, 100%].

Synthesis of amino sulfonyl chloride (**177**)



Methanoic acid (1.00ml, 26.5mmol) was added cautiously to chlorosulfonyl isocynate (2.30ml, 26.4mmol), whilst stirring at 0°C. Upon the evolution of carbon monoxide and carbon dioxide, a white precipitate was formed. Anhydrous toluene (60ml) was added to dissolve the precipitate and was left to stir for 1hr. The resultant solution was added to the next step of the reaction without purification.

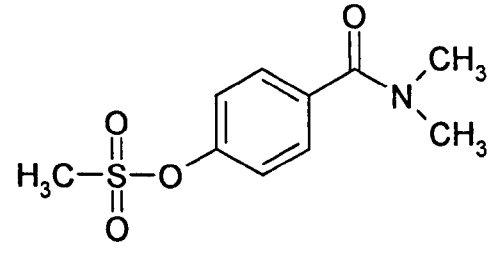
Synthesis of 4-[(dimethylamino)carbonyl]phenyl sulfamate (**178**)



Tp a solution of 4-hydroxy-*N,N*-dimethyl-benzamide (0.60g, 3.61mmol) in *N,N*-dimethyl acetamide (DMA, 2ml), amino sulfonyl chloride (20ml, ~8.8mmol) was added, and the mixture stirred for 3hr at 0°C. The reaction was quenched with saturated sodium chloride solution (NaCl, 50ml), extracted into ethyl acetate (2x50ml) and washed with water (3x30ml). The organic layer was dried over anhydrous magnesium sulfate ($MgSO_4$), filtered and the solvent removed under vacuum to give a white solid. The crude product was purified via column chromatography (DEE, 100%) to give the purified product **178** as a white solid [0.33g, 38%; mp: 142.7-144.4°C; R_f: 0.077 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3334.64 (NH_2), 3195.57 (PhH), 3070.11 (CH), 1606.15 (C=O); δ_H(d_6-Acetone): 7.40 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.27 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.14 (2H, s, SN<u>H</u>$_2$), 2.90 [6H, s, N(C<u>H</u>$_3$)$_2$]; δ_C(d_6-Acetone): 170.36 (C=O), 151.87, 136.38, 129.51, 123.02 (ArC), 35.12 (NCH_3); HRMS (EI): Found m/z 267.04085 (M^++Na), $C_9H_{12}O_4N_2SNa$, Calculated m/z 267.04100.

Synthesis of 4-[(dimethylamino)carbonyl]phenyl methanesulfonate (**179**)

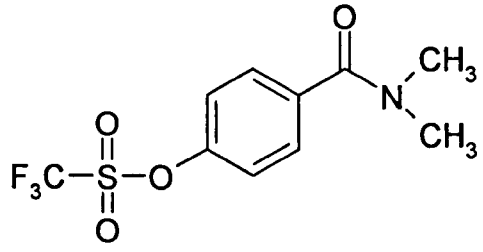


To a solution of 4-hydroxy-*N,N*-dimethyl-benzamide (0.51g, 3.09mmol) in dichloromethane (DCM), triethyl amine (TEA) (0.50ml, 3.59mmol) was added and the solution left to stir for 10min. Methane sulfonyl chloride (0.30ml, 3.86mmol) was then added the solution was refluxed for 3hr. After cooling, the resulting solution was poured onto ice and extracted into ethyl acetate (3x25ml).

The combined organic layer was then washed with saturated sodium carbonate (Na_2CO_3, 2x30ml) and then water (2x30ml) before being dried over anhydrous $MgSO_4$. The solvent was removed under vacuum to give a pale yellow solid. The crude product was purified via column chromatography [ethyl acetate (80%): DEE (20%)] to give the purified product **179** as a white solid [0.36g, 47.62%; mp: 99.0-100.2°C; R_f: 0.78 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3043.87 (PhH), 3023.19 (CH), 2941.0 (NCH_3), 1622.85 (C=O); δ_H(d_6-Acetone): 7.43 (2H, d, J=8.79, Ph<u>H</u>), 7.26 (2H, d, J=8.79Hz, PhH), 3.10 (3H, s, SC<u>H</u>$_3$), 3.05 [3H, s, N(C<u>H</u>$_3$)CH_3], 2.92 [3H, s, N(CH_3)C<u>H</u>$_3$]; δ_C (d_6-Acetone): 170.08 (C=O), 150.92, 137.01, 129.84, 122.89 (ArC), 37.65 (SCH_3), 35.34 (NCH_3); GC: t_R 11.14min; LRMS (EI): m/z 243 (M^+, 13%), 121 [M^+-$C_3H_{10}NSO_2$, 100%]; HRMS (EI): Found m/z 266.04575 (M^++Na) $C_{10}H_{13}O_4NSNa_1$, Calculated m/z 266.04575.

Synthesis of 4-[(dimethylamino)carbonyl]phenyl trifluoromethanesulfonate (**180**)

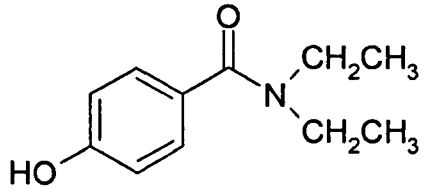


Compound **180** was synthesised using the same method as **179** with 4-hydroxy-*N,N*-dimethyl-benzamide (0.39g, 2.34mmol) in TEA (0.50ml, 3.59mmol) using trifluoromethane sulfonyl chloride (0.50ml, 4.73mmol) instead of methane sulfonyl chloride. The reaction was left to reflux for 72hr to give the crude product as a brown solid. The crude product was purified via column chromatography [ethyl acetate (80%): DEE (20%)] to give **180** as an off white solid [0.36g, 52.17%; mp: 49.4-50.7°C; R_f: 0.24 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3489.51 (PhH), 2934.20 (CH) 1637.37 (C=O); δ_H($CDCl_3$): 7.46 (2H, d, J=8.42Hz, Ph<u>H</u>), 7.26 (2H, d, J=8.42Hz, Ph<u>H</u>), 3.05 (3H, s, NCH_3), 2.92 (3H, s, NCH_3); δ_C($CDCl_3$): 169.77 (C=O), 150.03, 136.72, 129.36, 121.62 (ArC), 39.60, 35.51 (CH_3); GC: t_R 8.18min; LRMS (EI): m/z 297 (M^+, 29%), 253 [M^+-

$N(CH_3)_2$, 100%]; HRMS (EI): Found m/z 320.01707 (M^++Na) $C_{10}H_{10}O_4F_3NSNa_1$, Calculated m/z 320.01748; Elemental analysis: Found C40.50%, H3.40%, N4.81% $C_{10}H_{10}O_4F_3NS$, Calculated C40.41%, H3.39%, N4.71%.

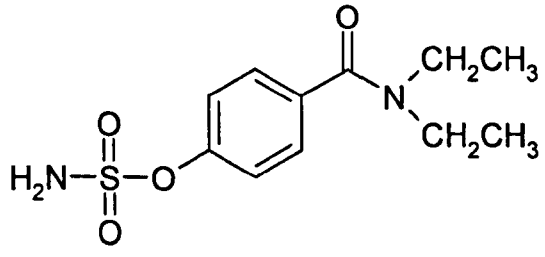
Synthesis of 4-hydroxy-*N,N*-diethyl-benzamide (**181**)



Compound **181** was synthesised via the same method as compound **176**, using 4-acetoxy benzoic acid (5.30g, 29.44mmol), thionyl chloride (2.10ml, 28.79mmol), *N*-methyl morpholine (3.20ml, 29.11mmol) and diethyl amine (4.59ml, 44.18mmol). Hydrolysis was achieved with NaOH (2.49g, 1.24M), and acidified with HCl (1M) to give the crude product as a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **181** as a white solid [4.05g, 71.24%; mp: 122.2-122.8°C; R_f: 0.17 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3418.89 (PhOH), 2984.5 (CH), 2801.58 (NCH_3), 1605.52 (C=O); δ_H (d_6-Acetone): 8.75 (1H, s, PhO<u>H</u>), 7.17 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.75 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.31 [4H, d, J=7.14Hz, $N(C\underline{H}_2CH_3)_2$], 1.05 [6H, t, J=7.14Hz, $N(CH_2C\underline{H}_3)_2$]; δ_C(d_6-Acetone): 171.45 (C=O), 159.14, 129.51, 129.14, 115.64 (ArC), 13.85 (CH_3); GC: t_R 9.64min; LRMS (EI): m/z 193 (M^+, 13%), 121 [M^+-$N(C_2H_5)_2$, 100%].

Synthesis of 4-[(diethylamino)carbonyl]phenyl sulfamate (**182**)

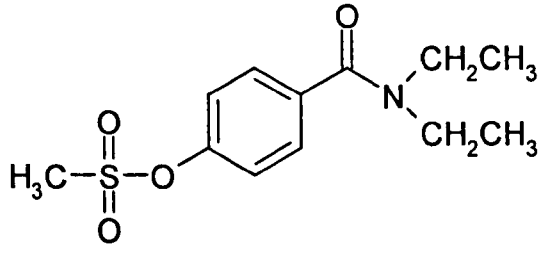


Compound **182** was synthesised via the same method as **178** using 4-hydroxy-*N,N*-ethyl-benzamide (0.82g, 4.26mmol). The crude product was

purified via column chromatography (DEE, 100%) to give **182** as an off-white solid [0.29g, 24.65%; mp: 136.5-137.9°C; R_f: 0.17 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3187.71 (NH_2), 3064.84 (PhH), 2976.83 (CH), 1607.73 (C=O); δ_H(d_6-Acetone): 7.53 (2H, d, J=8.79Hz, Ph$\underline{H}$), 7.44 (2H, d, J=8.79Hz, PhH), 7.31 (2H, s, SN$\underline{H}_2$), 3.19 [4H, m, N(C$\underline{H}_2$CH$_3$)$_2$], 1.23 [6H, m, N(CH$_2$C$\underline{H}_3$)$_2$]; δ_C(d_6-Acetone): 169.49 (C=O), 151.91, 136.42, 127.98, 122.37 (ArC); LRMS (EI): m/z 272 (M^+, 8%), 121 [M^+- $C_4H_{12}N_2SO_2$, 100%].

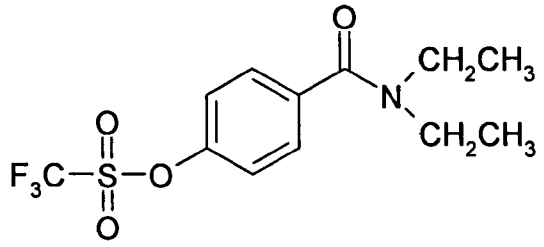
Synthesis of 4-[(diethylamino)carbonyl]phenyl methanesulfonate (**183**)



Compound **183** was synthesised via the same method as **179** using 4-hydroxy-*N,N*-ethyl benzamide (0.71g, 3.67mmol), TEA (0.60ml, 4.30mmol) and methane sulfonyl chloride (0.30ml, 3.86mmol). The crude product was purified via column chromatography (DEE, 100%) to give **183** as a white solid [0.73g, 73.64%; mp: 88.1-90.2°C; R_f: 0.18 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3020.61 (PhH), 2998.68 (CH), 2973.81 (NCH_3), 1624.43 (C=O); δ_H(d_6-Acetone): 7.41 (2H, d, J=8.42, Ph$\underline{H}$), 7.32 (2H, d, J=8.42Hz, Ph$\underline{H}$), 3.27 [7H, m, SC$\underline{H}_3$, N(C$\underline{H}_2$CH$_3$)$_2$], 2.91 [6H, m, (CH$_2$C$\underline{H}_3$)$_2$]; δ_C(d_6-Acetone): 169.20 (C=O), 149.94, 137.06, 128.34, 122.29 (ArC), 36.91 (SCH_3), 28.91 (CH_2), 13.64 (CH_3); GC: t_R 11.75min; LRMS (EI): m/z 271 (M^+, 17%), 199 [M^+- N(C_2H_5)$_2$, 100%].

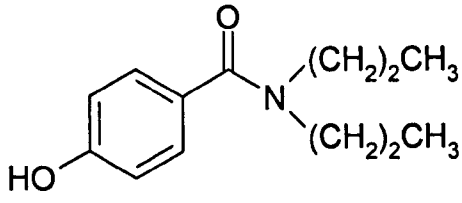
Synthesis of 4-[(diethylamino)carbonyl]phenyl trifluoromethanesulfonate (**184**)



Compound **184** was synthesised using the same method **179** as using 4-hydroxy-*N,N*-diethyl-benzamide (0.45g, 2.35mmol), TEA (0.40ml, 2.87mmol) and trifluoromethane sulfonyl chloride (0.30ml, 2.82mmol). The crude product was purified via column chromatography [ethyl acetate (80%): DEE (20%)] to give **184** as pale yellow oil [0.52g, 68.03%; R_f: 0.51 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3064.13 (PhH), 2978.08 (CH), 2879.32 (NCH), 1636.07 (C=O); δ_H(d_6-Acetone): 7.69 (2H, d, J=8.79Hz, PhH), 7.62 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.45 [4H, m, N(C<u>H</u>$_2$CH$_3$)$_2$], 1.24 [3H, m, N(CH$_2$C<u>H</u>$_3$)$_2$]; δ_C (d_6-Acetone): 169.14 (C=O), 149.64, 138.77, 129.01, 121.63 (ArC), 50.53, 46.19 (NCH$_2$), 21.79, 20.62 (CH$_2$), 10.83, 10.43 (CH$_3$); GC: t_R 10.15min; LRMS (EI): m/z 325 (M^+, 17%), 253 [M^+-N(C$_2$H$_5$)$_2$, 100%].

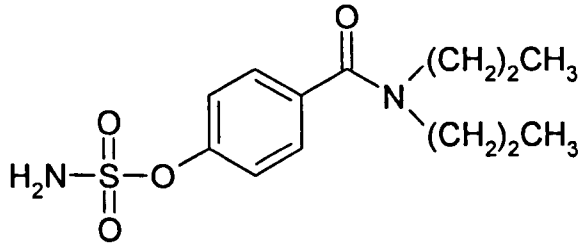
Synthesis of 4-hydroxy-*N,N*-dipropyl-benzamide (**185**)



Compound **185** was synthesised via the same method as compound **176** using 4-acetoxy benzoic acid (2.73g, 15.15mmol), thionyl chloride (1.65ml, 22.62mmol), *N*-methyl morpholine (2.50ml, 22.74mmol) and dipropyl amine (3.20ml, 23.34). Hydrolysis was achieved with NaOH (1.23g, 1.28M), and acidified with HCl (1M) to give the crude product as a light brown semi-solid. The crude product was purified via column chromatography (DEE, 100%) to give **185** as an off-white solid [2.71g, 81.12%; mp: 92.2-93.9°C; R_f: 0.25 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3173.49 (PhOH), 2964.06 (PhH), 2934.26 (CH), 2874.88 (NCH_3), 1605.42 (C=O); δ_H(d_6-Acetone): 8.81 (1H, s, PhO<u>H</u>), 7.27 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.87 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.24 [4H, br.s, N(C<u>H</u>$_2$CH$_2$CH$_3$)$_2$], 1.50 (4H, m, N(CH$_2$C<u>H</u>$_2$CH$_3$)$_2$], 0.73 [6H, m, N(CH$_2$CH$_2$C<u>H</u>$_3$)$_2$]; δ_C(d_6-Acetone): 171.34 (C=O), 158.42, 129.09, 128.70, 115.03 (ArC), 21.40 (CH_2), 11.51, 10.84 (CH_3); GC: t_R 10.45min; LRMS (EI): m/z 221 (M^+, 8%), 121 [M^+- N(C_3H_7)$_2$, 100%].

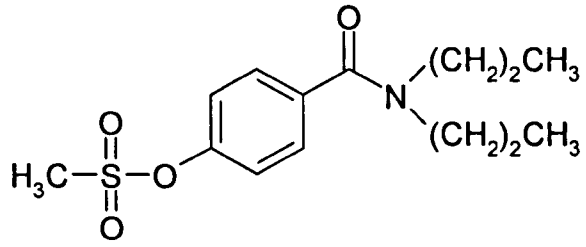
Synthesis of 4-[(dipropylamino)carbonyl]phenyl sulfamate (**186**)



Compound **186** was synthesised via the same method as **178** using 4-hydroxy-*N,N*-dipropyl-benzamide (0.54g, 2.44mmol). The crude product was purified via column chromatography (DEE 100%) to give **186** as a white solid [0.14g, 19%; mp: 107.2-109.1°C; R_f: 0.37 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3199.75 (NH_2), 3078.49 (PhH), 2935.15 (CH), 2872.84 (N-CH), 1608.85 (C=O); δ_H(d_6-Acetone): 7.51 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.44 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.32 (2H, s, SN<u>H</u>$_2$), 3.40 [4H,m, N(C<u>H</u>$_2$CH$_2$CH$_3$)$_2$], 1.68 [4H, m, N(CH$_2$C<u>H</u>$_2$CH$_3$)$_2$], 0.90 [6H, m, N(CH$_2$CH$_2$C<u>H</u>$_3$)$_2$]; δ_c(d_6-Acetone): 170.08 (C=O), 150.89, 136.44, 128.16, 122.36 (ArC), 21.81 (CH_2), 10.87 (CH_3); LRMS (EI): m/z 249 (M^+, 14%), 121 (M^+ - $C_6H_{16}N_2SO_2$, 100%).

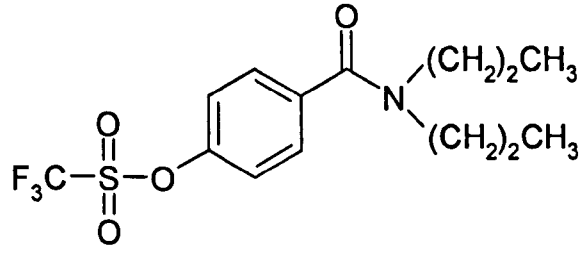
Synthesis of 4-[(dipropylamino)carbonyl]phenyl methanesulfonate (**187**)



Compound **187** was synthesised via the same method as **179** using 4-hydroxy-*N,N*-dipropyl-benzamide (0.87, 3.93mmol), TEA (0.60ml, 4.30mmol) and methane sulfonyl chloride (0.40ml, 5.15mmol). The crude product was purified via column chromatography (DEE, 100%) to give **187** as pale yellow solid [0.30g, 25.55%; mp: 50.60-51.10°C; R_f: 0.29 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 2964.42CH (PhH), 2934.55 (CH), 2875.11 (NCH_3), 1629.18 (C=O); δ_H(d_6-Acetone): 7.39 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.32 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.23 [7H, m, SC<u>H</u>$_3$, N(C<u>H</u>$_2$CH$_2$CH$_3$)$_2$], 1.52 [4H, m, N(CH$_2$C<u>H</u>$_2$CH$_3$)$_2$], 0.73 [6H, m, N(CH$_2$CH$_2$C<u>H</u>$_3$)$_2$]; δ_C(d_6-Acetone): 169.72 (C=O), 149.89, 137.15, 128.49, 122.25 (ArC), 36.88 (SCH_3), 29.26 (CH_2), 10.84 (CH_3); GC: t_R 12.76min; LRMS (EI): m/z 298 (M^+-1, 13%), 199 [M^+- NH(C_3H_7)$_2$, 100%].

Synthesis of 4-[(dipropylamino)carbonyl]phenyl trifluoromethanesulfonate (**188**)

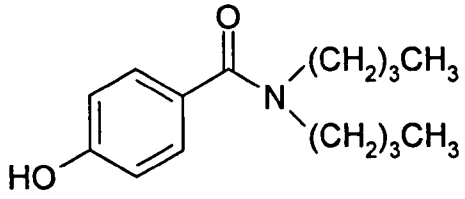


Compound **188** was synthesised using the same method **179** as using 4-hydroxy-*N,N*-dipropyl-benzamide (0.86g, 3.91mmol), TEA (0.70ml, 5.02mmol) and trifluoromethane sulfonyl chloride (0.55ml, 5.17mmol). The crude product was purified via column chromatography [ethyl acetate (80%): DEE (20%)] to give **188** as a pale yellow oil [0.10g, 7.53%; R_f: 0.73 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3064.12 (PhH), 2967.42 (CH), 2877.56 (NCH), 1636.87 (C=O); δ_H(d_6-Acetone): 7.58 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.53 (2H, d, J=8.97Hz, Ph<u>H</u>), 3.31

[4H, m, $N(C\underline{H}_2CH_2CH_3)_2$], 1.52 [4H, m, $N(CH_2C\underline{H}_2CH_3)_2$], 0.81 [6H, m, $N(CH_2CH_2C\underline{H}_3)_2$]; δ_C(d_6-Acetone): 169.14 (C=O), 149.64, 138.77, 129.01, 121.63 (ArC), 50.53, 46.19 (NCH_2), 21.79, 20.62 (CH_2), 10.83, 10.43 (CH_3); GC: t_R 11.66min; LRMS (EI): m/z 353 (M^+, 7%), 121 [M^+- $C_7H_{15}F_3NSO_2$, 100%]; Elemental analysis: Found C47.46%, H5.23%, N4.05% $C_{14}H_{18}O_4F_3NS$, Calculated C47.59%, H5.13%, N3.96%.

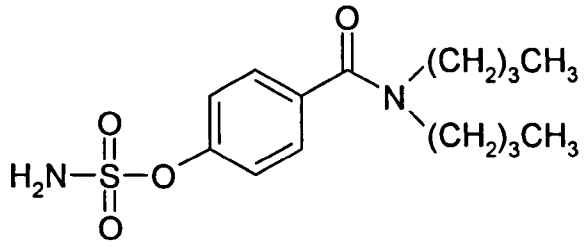
Synthesis of 4-hydroxy-*N,N*-dibutyl-benzamide (**189**)



Compound **189** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (2.73g, 15.15mmol) thionyl chloride (1.65ml, 22.62mmol), *N*-methyl morpholine (2.50ml, 22.74mmol) and dibutyl amine (3.90ml, 22.96mmol). Hydrolysis was achieved with NaOH (1.23g, 1.28M), and acidified with HCl (1M) to give the crude product as a brown semi-solid. The crude product was purified via column chromatography (DEE, 100%) to give **189** as an off-white solid [1.83g, 48.55%; mp: 86.8-88.3°C; R_f: 0.31 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3173.23 (PhOH), 2958.45 (PhH), 2932.16 (CH), 2872.43 (NCH_3), 1605.21 (C=O); δ_H(d_6-Acetone): 8.69 (1H, s, Ph$O\underline{H}$), 7.16 (2H, d, J=8.79Hz, Ph$\underline{H}$), 6.76 (2H, d, J=8.79Hz, Ph$\underline{H}$), 3.27 [4H, m, $N(C\underline{H}_2)_2$], 1.45 (4H, m, $N(CH_2C\underline{H}_2)_2$], 1.17 [4H, m, $(C\underline{H}_2CH_3)_2$], 0.78 [6H, m, $(C\underline{H}_3)_2$]; δ_C(d_6-Acetone): 171.05 (C=O), 158.29, 128.94, 128.56, 114.87 (ArC), 19.88 (CH_2), 13.28 (CH_3); GC: t_R 11.47min; LRMS (EI): m/z 249 (M^+, 13%), 121 [M^+- $N(C_4H_9)_2$, 100%].

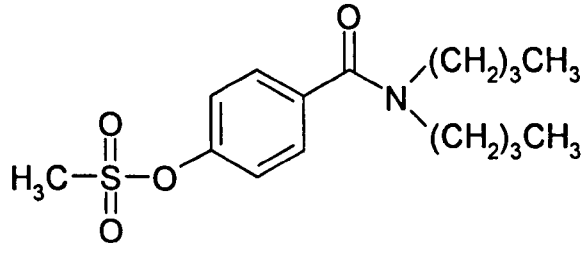
Synthesis of 4-[(dibutylamino)carbonyl]phenyl sulfamate (**190**)



Compound **190** was synthesised via the same method as **178** using 4-hydroxy-*N,N*-dibutyl-benzamide (0.45g, 1.80mmol). The crude product was purified via column chromatography (DEE, 100%) to give **190** as a white solid [0.22, 38.13%; mp: 144.4-145.6°C; R_f: 0.46 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3305.53 (NH_2), 3071.67 (PhH), 2933.26 (CH), 2872.84 (NCH), 1608.86 (C=O); δ_H(d_6-Acetone): 7.44 (2H, d, J=8.79Hz, Ph$\underline{H}$), 7.37 (2H, d, J=8.79Hz, Ph$\underline{H}$), 7.27 (2H, s, SN$\underline{H}_2$), 3.37 [4H,m, N(C$\underline{H}_2$)$_2$], 1.58 [4H, m, N($CH_2C\underline{H}_2$)$_2$], 1.25 [4H, m, ($C\underline{H}_2CH_3$)$_2$], 0.87 [6H, m, ($CH_2C\underline{H}_3$)$_2$]; δ_c(d_6-Acetone): 169.85 (C=O), 150.84, 136.52, 128.16, 122.32 (ArC), 30.71, 19.74 (CH_2), 13.26 (CH_3); LRMS (EI): m/z 327 (M^+-1, 2%), 121 [M^+- $C_8H_{18}N_2SO_2$, 100%]; HRMS (EI): Found m/z 351.13590 (M^++Na) $C_{15}H_{24}O_4N_2SNa$, Calculated m/z 351.134899.

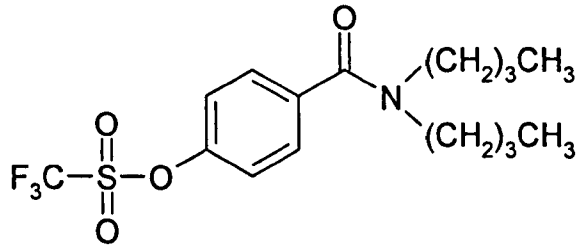
Synthesis of 4-[(dibutylamino)carbonyl]phenyl methanesulfonate (**191**)



Compound **191** was synthesised via the same method as **179** using 4-hydroxy-*N,N*-dibutyl-benzamide (1.17g, 4.70mmol), TEA (0.75ml, 5.38mmol) and methane sulfonyl chloride (0.45ml, 5.79mmol). The crude product was purified via column chromatography (DEE, 100%) to give **191** as an off white solid [0.95g, 61.56%; mp: 42.2-43.4°C; R_f: 0.39 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 2958.80 (PhH), 2932.88 (CH), 2872.66 (NCH_3), 1629.44 (C=O); δ_H(d_6-Acetone): 7.40 (2H, d, J=8.79Hz, Ph$\underline{H}$), 7.32 (2H, d, J=8.79Hz, Ph$\underline{H}$) 3.26 [7H, m, SC$\underline{H}_3$, N(C$\underline{H}_2$)$_2$], 1.49 [4H, m, N($CH_2C\underline{H}_2$)$_2$], 1.13 [4H, m, ($C\underline{H}_2CH_3$)$_2$], 0.77 [6H, m, ($CH_2C\underline{H}_3$)$_2$]; δ_C(d_6-Acetone): 170.33 (C=O), 149.54, 136.59, 128.53, 122.18 (ArC), 44.69 (CH_2), 37.57 (SCH_3), 30.86, 19.93 (CH_2), 14.00 (CH_3); GC: t_R 15.18min; LRMS (EI): m/z 327 (M^+, 4%), 199 (M^+ - N(C_4H_9)$_2$, 100%); Elemental analysis: Found C58.71%, H7.68%, N4.35% $C_{16}H_{25}O_4NS$, Calculated C58.39%, H7.70%, N4.28%.

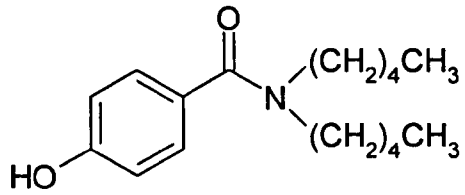
Synthesis of 4-[(dibutylamino)carbonyl]phenyl trifluoromethanesulfonate (**192**)



Compound **192** was synthesised using the same method as **179** using 4-hydroxy-*N,N*-dibutyl-benzamide (0.79g, 3.17mmol), TEA (0.60ml, 4.30mmol) and trifluoromethane sulfonyl chloride (0.45ml, 4,23mmol). The crude product was purified via column chromatography [ethyl acetate (80%): DEE (20%)] to give **192** as a pale yellow oil [0.68g, 56.48%; R_f: 0.79 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3064.84 (PhH), 2961.53 (CH), 2875.02 (NCH) 1636.75 (C=O); δ_H($CDCl_3$): 7.42 (2H, d, J=8.79Hz, Ph$\underline{H}$), 7.28 (2H, d, J=8.79Hz, Ph$\underline{H}$), 3.46 [2H, t, J=7.14Hz, N(C$\underline{H}_2$CH$_2$)], 3.13 (2H, t, J=6.96Hz, NCH$_2$$\underline{C}H_2$), 1.38 [8H, m, ((C$_2\underline{H}_4$)CH$_3$)$_2$], 0.95 (3H, t, J=6.96Hz, CH$_2$C$\underline{H}_3$), 0.75 (3H, t, J=6.96Hz, CH$_2$C$\underline{H}_3$); δ_C($CDCl_3$): 169.76 (C=O), 149.72, 137.76, 128.73, 121.38 (ArC), 48.87, 44.75 (NCH_2), 30.83, 29.66, 20.36, 19.74 (CH_2), 13.95, 13.56 (CH_3); GC: t_R 14.79min; LRMS (EI): m/z 381 (M^+, 2%), 253 [M^+- N(C_4H_9)$_2$, 100%]; Elemental analysis: Found C50.42%, H5.79%, N3.67% $C_{16}H_{22}O_4F_3NS$, Calculated C50.39%, H5.81%, N3.67%.

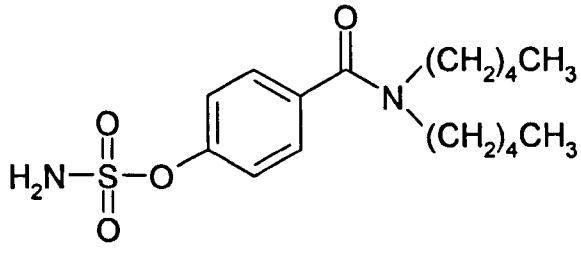
Synthesis of 4-hydroxy-*N,N*-dipentyl-benzamide (**193**)



Compound **193** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (2.56g, 14.21mmol), thionyl chloride (1.35ml, 18.51mmol), *N*-methyl morpholine (2.00ml, 18.19mmol) and dipentyl amine (3.50ml, 17.07mmol). Hydrolysis was achieved with NaOH (1.14g, 1.24M), and acidified with HCl (1M) to give the crude product as a brown semi-solid. The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **193** as an off-white solid [3.66g, 93.02%; mp: 95.6-97.1°C; R_f: 0.37 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3181.25 (PhOH), 2956.60 (PhH), 2931.01 (CH), 2870.83 (NCH_3), 1605.43 (C=O); δ_H(d_6-Acetone): 8.79 (1H, s, PhO<u>H</u>), 7.23 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.82 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.37 [4H, s, $N(C\underline{H}_2)_2$], 1.60 [4H, s, $N(CH_2C\underline{H}_2)_2$], 1.30 [8H, s, $(C_2\underline{H}_4)_2$] 0.86 [6H, m, $(C\underline{H}_3)_2$]; δ_C($CDCl_3$): 171.39 (C=O), 160.37, 158.54, 128.53, 114.95 (ArC), 28.91, 22.24 (CH_2), 13.54 (CH_3); GC: t_R 12.73min; LRMS (EI): m/z 277 (M^+, 6%), 121 [M^+ - $N(C_5H_{11})_2$, 100%].

Synthesis of 4-[(dipentylamino)carbonyl]phenyl sulfamate (**194**)

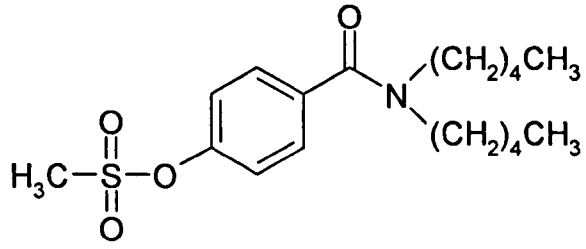


Compound **194** was synthesised via the same method as **178** using 4-hydroxy-*N,N*-dipentyl-benzamide (0.74g, 2.69mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **194** as a white solid [0.11g, 11.87%; mp: 115.5-116.0°C; R_f: 0.58 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3197.30 (NH_2), 3072.54 (PhH), 2931.95 (CH), 2871.21 (NCH), 1609.93 (C=O); δ_H(d_6-Acetone): 7.51 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.44 (2H, d,

J=8.79Hz, Ph$\underline{H}$), 7.31 (2H, s, SN$\underline{H}_2$), 3.45 [4H, m, N(C$\underline{H}_2$)$_2$], 1.78 [4H, m, N(CH$_2$C$\underline{H}_2$)$_2$], 1.37 [8H, m, (C$_2\underline{H}_4$)$_2$], 0.96 [6H, m, (CH$_2$C$\underline{H}_3$)$_2$]; δ_c(d$_6$-Acetone): 169.93 (C=O), 150.87, 136.47, 128.16, 122.34 (ArC), 48.79, 22.35, 22.03 (CH$_2$), 13.51 (CH$_3$); LRMS (EI): m/z 355 (M^+-1, 3%), 121 [M^+ - $C_{10}H_{22}N_2SO_2$, 100%].

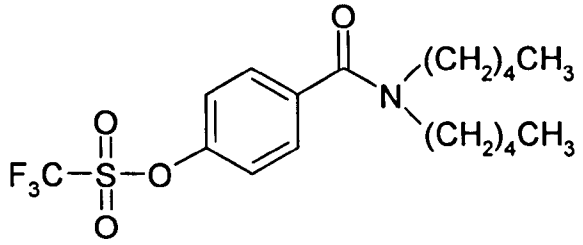
Synthesis of 4-[(dipentylamino)carbonyl]phenyl methanesulfonate (**195**)



Compound **195** was synthesised via the same method as **179** using 4-hydroxy-*N,N*-dipentyl-benzamide (0.22g, 0.80mmol), TEA (0.20ml, 1.43mmol) and methane sulfonyl chloride (0.10ml, 1.29mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **195** as an off white solid [0.086g, 30.36%; mp: 41.0-41.5°C; R_f: 0.49 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 2930.96 (CH), 2871.17 (NCH$_3$), 1633.58 (C=O); δ_H(d$_6$-Acetone): 7.40 (2H, d, J=8.79Hz, Ph$\underline{H}$), 7.32 (2H, d, J=8.79Hz, Ph$\underline{H}$), 3.22 [7H, m, SC$\underline{H}_3$, N(C$\underline{H}_2$)$_2$], 1.51 [4H, m, N(CH$_2$C$\underline{H}_2$)$_2$], 1.26 [4H, m, (C$\underline{H}_2$CH$_2$CH$_3$)$_2$], 1.05 [4H, m (C$\underline{H}_2$CH$_3$)$_2$], 0.71 [6H, m, (CH$_2$C$\underline{H}_3$)$_2$]; δ_C(d$_6$-Acetone): 169.57 (C=O), 149.89, 137.16, 128.48, 122.24 (ArC), 48.72, 44.45 (CH$_2$), 36.85 (SCH$_3$), 27.17, 22.35 (CH$_2$), 13.43 (CH$_3$); GC: t_R 12.76min; LRMS (EI): m/z 298 (M^+, 5%), 199 [M^+-N(C$_5$H$_{11}$)$_2$, 100%]; Elemental analysis: Found: C60.78%, H8.28%, N3.95%, $C_{18}H_{29}NO_4S$ Calculated: C60.82%, H8.22%, N3.94%.

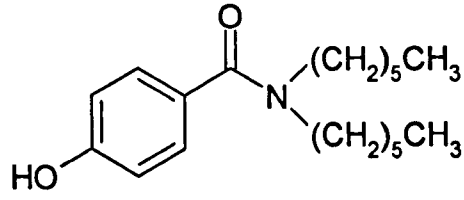
Synthesis of 4-[(dipentylamino)carbonyl]phenyl trifluoromethanesulfonate (**196**)



Compound **196** was synthesised using the same method **179** as using 4-hydroxy-*N,N*-dipentyl-benzamide (0.71g, 2.58mmol), TEA (0.60ml, 4.30ml) and trifluoromethane sulfonyl chloride (0.50ml, 4.70mmol). The crude product was purified via column chromatography [ethyl acetate (80%): DEE (20%)] to give **196** as a pale yellow oil [0.57g, 49.98%; R_f: 0.88 (DEE, 100%)].

$\nu_{(max)}$(Film) cm^{-1}: 2932.80 (CH), 2862.46 (NCH_3) 1638.10 (C=O); δ_H(d_6-Acetone): 7.68 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.62 (2H, d, J=8.97Hz, Ph<u>H</u>), 3.34 [4H, m, $N(C\underline{H}_2)_2$], 1.60 [4H, m, $N(CH_2C\underline{H}_2)_2$], 1.44 [4H, m, $(C\underline{H}_2CH_2CH_3)_2$], 1.21 [4H, m, $(C\underline{H}_2CH_3)_2$], 0.85 [6H, m, $(CH_2C\underline{H}_3)_2$]; δ_C(d_6-Acetone): 169.00 (C=O), 149.65, 138.79, 129.00, 121.63 (ArC) 48.73, 44.51, 27.13, 22.36 (CH_2) 13.32 (CH_3); GC: t_R 16.26min; LRMS (EI): m/z 409 (M^+, 6%), 253 (M^+-$N(C_5H_{11})_2$, 100%); Elemental analysis: Found C52.80%, H6.45%, N3.43% $C_{18}H_{26}O_4F_3NS$, Calculated C52.80%, H6.40%, N3.67%.

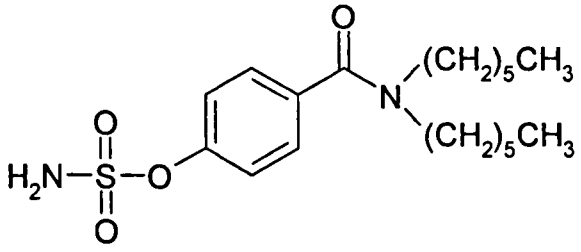
Synthesis of 4-hydroxy-*N,N*-dihexyl-benzamide (**197**)



Compound **197** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (2.51g, 13.91mmol), thionyl chloride (1.4ml, 18.51mmol), *N*-methyl morpholine (2.0ml, 18.19mmol) and dihexyl amine (4.7ml, 17.98mmol). Hydrolysis was achieved with NaOH (1.11g, 1.21M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified via column chromatography [DEE (60%): petroleum spirit 40-60°C (40%)] to give **197** as an off-white solid [3.81g, 89.81%; mp: 76.6-77.5°C; R_f: 0.40 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3217.69 (PhOH), 2955.36 (PhH), 2929.05 (CH), 2857.73 (NCH_3), 1605.44 (C=O); δ_H(d_6-Acetone): 8.83 (1H, s, PhO<u>H</u>), 7.27 (2H, d, J=8.60Hz, Ph<u>H</u>), 6.86 (2H, d, J=8.60Hz, Ph<u>H</u>), 3.39 [4H, m, N(C<u>H</u>$_2$)$_2$], 1.61 [4H, m, N(CH_2C<u>H</u>$_2$)$_2$], 1.29 [12H, m, (C_3<u>H</u>$_6$)$_2$], 0.86 [6H, m, (C<u>H</u>$_3$)$_2$]; δ_C(d_6-Acetone): 171.81 (C=O), 159.04, 129.63, 129.29, 115.61 (ArC), 32.15, 27.15, 23.19 (CH_2) 14.24 (CH_3); GC: t_R 14.47min; LRMS (EI): m/z 305 (M^+, 6%), 121 [M^+- N(C_6H_{13})$_2$, 100%].

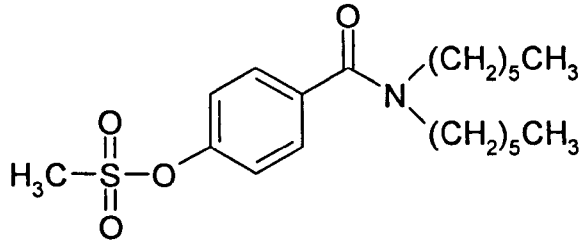
Synthesis of 4-[(dihexylamino)carbonyl]phenyl sulfamate (**198**)



Compound **198** was synthesised via the same method as **178** using 4-hydroxy-*N,N*-dihexyl-benzamide (0.29g, 0.96mmol). The crude product was purified via column chromatography [DEE (60%): petroleum spirit 40-60°C (40%)] to give **198** as a white solid [0.17g, 45.01%; mp: 45.4-46.5°C; R_f: 0.62 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3331.4 (NH), 2956.94 (PhH), 1609.58 (C=O); δ_H(d_6-Acetone): 7.52 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.45 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.31 (2H, s, SN<u>H</u>$_2$), 3.44 [4H,m, N(C<u>H</u>$_2$)$_2$], 1.69 [4H, m, N(CH_2C<u>H</u>$_2$)$_2$], 1.31 [12H, m, (C_6<u>H</u>$_{12}$)$_2$], 0.94 [6H, m, (CH_2C<u>H</u>$_3$)$_2$]; δ_c(d_6-Acetone): 169.84 (C=O), 150.84, 136.54, 128.15, 122.33 (ArC), 29.24, 22.42 (CH_2), 13.47 (CH_3); HRMS (EI): Found m/z 385.21515 (M^++Na) $C_{19}H_{32}N_2O_4SNa$, Calculated 385.21315.

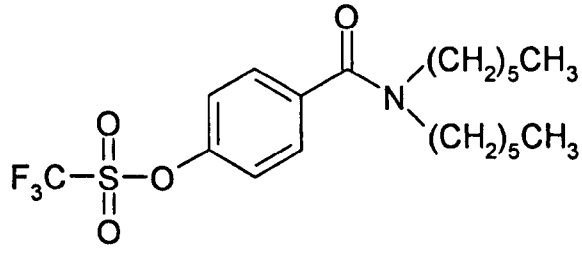
Synthesis of 4-[(dihexylamino)carbonyl]phenyl methanesulfonate (**199**)



Compound **199** was synthesised via the same method as **178** using 4-hydroxy-*N,N*-dihexyl-benzamide (0.49g, 1.62mmol), TEA (0.25ml, 1.79mmol) and methane sulfonyl chloride (0.15ml, 1.93mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give **199** as a pale yellow oil [0.34, 55.24%; R_f: 0.53 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 2955.52 (PhH), 2929.65 (CH), 2858.08 (NCH_3), 1633.23 (C=O); δ_H(d_6-Acetone): 7.39 (2H, d, J=8.97Hz, Ph<u>H</u>) 7.32 (2H, d, J=8.97Hz, Ph<u>H</u>) 3.22 [7H, m, SC<u>H</u>$_3$, N(C<u>H</u>$_2$)$_2$], 1.13 [12H, m (C_3<u>H</u>$_6$)$_2$], 0.75 [6H, m, (C<u>H</u>$_3$)$_2$]; δ_C(d_6-Acetone): 169.57 (C=O), 149.89, 137.15, 128.49, 122.24 (ArC), 36.88 (SCH_3), 26.64, 26.11, 22.41 (CH_2), 13.51 (CH_3); GC: t_R 19.71min; LRMS (EI): m/z 382 (M^+, 4%), 199 [M^+- $N(C_6H_{13})_2$, 100%]; Elemental analysis: Found: C62.85% H8.38% N3.65% $C_{20}H_{33}NO_4S$ Calculated: C62.63% H8.67% N3.65.

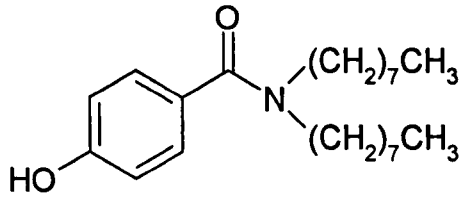
Synthesis of 4-[(dihexylamino)carbonyl]phenyl trifluoromethanesulfonate (**200**)



Compound **200** was synthesised using the same method **179** as using 4-hydroxy-*N,N*-dihexyl-benzamide (1.07, 3.31mmol), TEA (0.60ml, 4.30mmol) and trifluoromethane sulfonyl chloride (0.5ml, 4.70mmol). The crude product was purified via column chromatography [ethyl acetate (80%): DEE (20%)] to give **200** as a pale yellow oil [0.84g, 57.89%; R_f: 0.91 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3058.02 (PhH), 2931.28 (CH), 2859.53 (NCH), 1638.33 (C=O); δ_H(d_6-Acetone): 7.67 (2H, d, J=8.79Hz, Ph$\underline{H}$), 7.26 (2H, d, J=8.79Hz, Ph$\underline{H}$), 3.34 [4H, m, N(C$\underline{H}_2$)$_2$], 1.59 [4H, m, N(CH$_2$C$\underline{H}_2$)$_2$], 1.15 [12H, m, (C$_3\underline{H}_6$)$_2$], 0.85 [6H, m, (CH$_2$C$\underline{H}_3$)$_2$]; δ_C(d_6-Acetone): 168.98 (C=O), 149.65, 138.79, 129.00, 121.62 (ArC), 48.78, 44.53, 31.57, 31.09, 27.39, 26.59, 26.05, 22.46, 22.30 (CH$_2$), 13.49, 13.38 (CH$_3$); GC: t_R 16.26min; LRMS (EI): m/z 437 (M^+, 4%), 253 [M^+-N(C$_6$H$_{13}$)$_2$, 100%]; HRMS (EI): Found m/z 460.17475 (M^++Na) $C_{20}H_{30}NO_4F_3SNa$, Calculated m/z 460.17400.

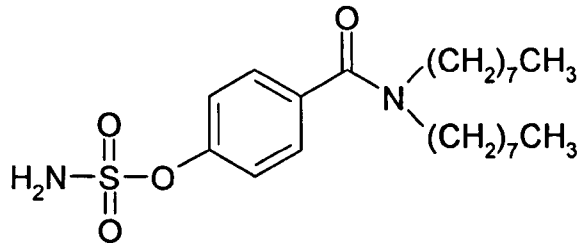
Synthesis of 4-hydroxy-*N,N*-dioctyl-benzamide (**201**)



Compound **201** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (2.73g, 15.15mmol), thionyl chloride (1.65ml, 22.62mmol), *N*-methyl morpholine (2.50ml, 22.74mmol) and dioctyl amine (7.00ml, 23.16mmol). Hydrolysis was achieved with NaOH (1.23g, 1.28M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **201** as a waxy white solid [1.94g, 23.79%; mp: 132.1-133.9°C; R_f: 0.44 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3375.90 (PhOH), 2925.43 (CH), 2854.22 (NCH), 1610.37 (C=O); δ_H(d_6-Acetone): 8.90 (1H, s, PhO$\underline{H}$), 7.26 (2H, d, J=8.60Hz, Ph$\underline{H}$), 6.88 (2H, d, J=8.60Hz, Ph$\underline{H}$), 3.41 [4H, m, N(C$\underline{H}_2$)$_2$], 1.63 [4H, m, N(CH$_2$C$\underline{H}_2$)$_2$], 1.29 [20H, m, ((C$_5\underline{H}_{10}$)CH$_3$)$_2$], 0.90 [6H, t, J=7.14Hz, (C$\underline{H}_3$)$_2$]; δ_C(d_6-Acetone): 171.15 (C=O), 158.39, 128.79, 128.55, 114.89 (ArC), 31.77, 29.19, 26.76, 22.52 (CH$_2$) 13.60 (CH$_3$); GC: t_R 21.01min; LRMS (EI): m/z 360 (M^+, 1%), 121 [M^+-N(C$_8$H$_{17}$)$_2$, 100%].

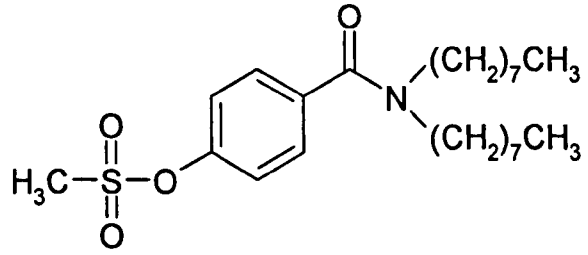
Synthesis of 4-[(dioctylamino)carbonyl]phenyl sulfamate (**202**)



202 was synthesised via the same method as **176** using 4-hydroxy-*N,N*-dioctyl-benzamide (0.55g, 1.51mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **202** as a white solid [0.28g, 42.44%; mp: 145.6-147.2°C; R_f: 0.66 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3415.33 (NH_2), 3079.33 (PhH), 2923.59 (CH), 2850.05 (NCH), 1634.91 (C=O); δ_C(d_6-Acetone): 165.56 (C=O), 152.63, 133.63, 128.82, 122.09 (ArC), 39.72, 31.77, 29.95, 28.99, 26.89, 22.48 (CH_2), 13.56 (CH_3). LRMS (EI): m/z 440 (M^+, 17%), 121 [M^+- $C_{16}H_{35}N_2SO_2$, 100%].

Synthesis of 4-[(dioctylamino)carbonyl]phenyl methanesulfonate (**203**)

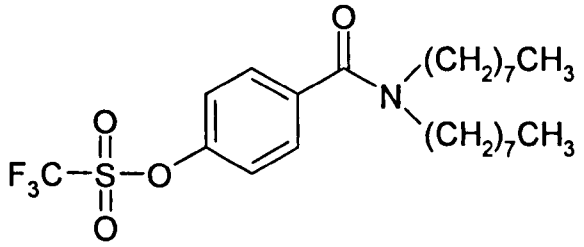


Compound **203** was synthesised via the same method as **179** using 4-hydroxy-*N,N*-dioctyl-benzamide (0.89g, 2.47mmol), TEA (0.40ml, 2.87mmol) and methane sulfonyl chloride (0.25ml, 3.22mmol,). The crude product was purified via column chromatography [DEE (60%): petroleum spirit 40-60°C (40%)] to give **203** as a white solid [0.67g, 61.97%; mp: 146.3-147.1°C; R_f: 0.59 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 2927.05 (CH), 2852.64 (NCH_3), 1636.39 (C=O); δ_H(d_6-Acetone): 7.40 (2H, d, J=8.79Hz, Ph$\underline{H}$), 7.32 (2H, d, J=8.79Hz, Ph$\underline{H}$), 3.22 [7H, m, SC$\underline{H}_3$, N(C$\underline{H}_2$)$_2$], 1.51 [4H, m, N(CH_2C$\underline{H}_2$)$_2$], 1.20 [20H, m, (($C_5\underline{H}_{10}$)CH_3)$_2$], 0.78 [6H, m, (CH_2C$\underline{H}_3$)$_2$]; δ_C(d_6-Acetone): 169.59 (C=O), 149.91, 137.12, 128.50, 122.23

(ArC), 48.77, 44.51 (CH_2), 36.88 (SCH_3), 31.74, 27.47, 26.95, 26.40, 22.50 (CH_2), 13.58 (CH_3); GC: t_R 33.72min; LRMS (EI): m/z 439 (M^+, 2%), 199 [M^+-$N(C_8H_{17})_2$, 100%].

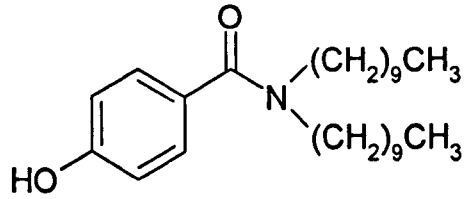
Synthesis of 4-[(dioctylamino)carbonyl]phenyl trifluoromethanesulfonate (**204**)



Compound **204** was synthesised using the same method **179** as using 4-hydroxy-*N,N*-dioctyl-benzamide (0.78g, 2.17mmol), TEA (0.4ml, 2.97mmol) and trifluoromethane sulfonyl chloride (0.3ml, 2.82mmol). The crude product was purified via column chromatography [ethyl acetate (80%): DEE (20%)] to give **204** as a waxy pale yellow solid [0.75g, 70.26%; m.p. 46.7-47.9°C; R_f: 0.93 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3078.49 (PhH), 2930.28 (CH), 2858.19 (NCH), 1641.99 (C=O); δ_H(d_6-Acetone): 7.60 (2H, d, J= 8.97Hz, Ph$\underline{H}$), 7.54 (2H, d, J= 8.97Hz, Ph$\underline{H}$), 3.48 [2H, t, J=7.32Hz, N($C\underline{H}_2$)CH_2], 3.23 [2H, t, J=7.32Hz, N(CH_2)$C\underline{H}_2$], 1.61 [2H, m, N($CH_2C\underline{H}_2$)$_2$], 1.25 [20H, m, (($C_5C\underline{H}_{10}$)CH_3)$_2$], 0.84 [6H, t, J=7.14Hz, ($CH_2C\underline{H}_3$)$_2$]; δ_C($CDCl_3$): 168.98 (C=O), 149.65, 138.79, 129.01, 121.61 (ArC) 48.78 (NCH_2), 44.58, 31.74, 27.45, 26.93, 26.38, 22.48 (CH_2), 13.53 (CH_3); GC: t_R 15.93min; LRMS (EI): m/z 493 (M^+, 1%), 253 [M^+ - $NH(C_8H_{17})_2$, 100%].

Synthesis of 4-hydroxy-*N,N*-didecyl-benzamide (**205**)

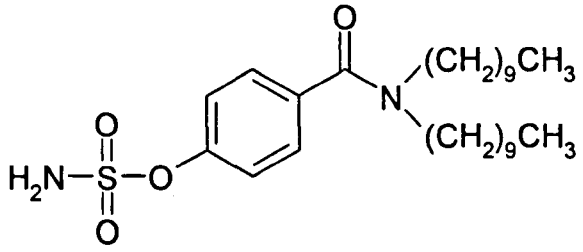


Compound **205** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (3.23g, 17.90mmol), thionyl chloride (1.35ml, 18.51mmol), *N*-methyl morpholine (2.00ml, 18.19mmol) and didecyl amine (5.33g, 17.92mmol).

Hydrolysis was achieved with NaOH (1.33g, 1.23M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified via column chromatography [DEE (60%): petroleum spirit 40-60°C (40%)] to give **205** as a waxy white solid [6.02g, 80.60%; mp: 48.0-49.9°C; R_f: 0.49 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3406.02 (PhOH), 2924.04 (CH), 2853.54 (NCH_3), 1606.46 (C=O); δ_H(d_6-Acetone): 8.86 (1H, s, PhO$\underline{H}$), 7.23 (2H, d, J=8.42Hz, Ph$\underline{H}$), 6.84 (2H, d, J=8.42Hz, Ph$\underline{H}$), 3.37 [4H, m, N($C\underline{H}_2)_2$], 1.63 [4H, m, N($CH_2C\underline{H}_2)_2$],1.26 [28H, m, (($C_7\underline{H}_{14}$)$CH_3)_2$], 0.87 [6H, t, J=6.96Hz, ($C\underline{H}_3)_2$]; δ_C(d_6-Acetone): 171.15 (C=O), 158.39, 128.80, 128.56, 114.90 (ArC), 31.86, 29.51, 26.75, 22.56 (CH_2) 13.62 (CH_3);

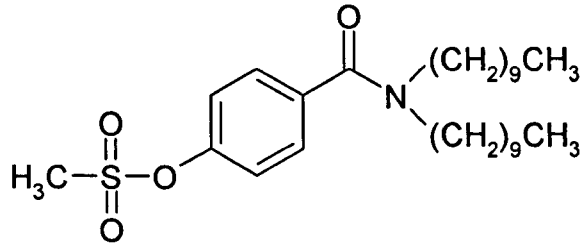
Synthesis of 4-[(didecylamino)carbonyl]phenyl sulfamate (**206**)



Compound **206** was synthesised via the same method as **178** using 4-hydroxy-*N,N*-didecyl-benzamide (0.55g, 1.33mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **206** as a white solid [0.30g, 45.46%; mp: 59.1-60.4°C; R_f: 0.71 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3199.55 (NH_2), 3075.85 (PhH), 2925.77 (CH), 2854.52 (NCH), 1611.14 (C=O); δ_H(d_6-Acetone): 7.43 (2H, d, J=8.97Hz, Ph$\underline{H}$), 7.36 (2H, d, J=8.97Hz, Ph$\underline{H}$), 7.23 (2H, s, SN$\underline{H}_2$), 3.36 [4H, m, N($C\underline{H}_2)_2$], 1.61 [4H, m, N($CH_2C\underline{H}_2)_2$], 1.27 [28H, m, (($C_7\underline{H}_{14}$)$CH_3)_2$], 0.88 [6H, t, J=6.96Hz, ($CH_2C\underline{H}_3)_2$]; δ_C(d_6-Acetone): 169.90 (C=O), 150.88, 136.43, 128.15, 122.35 (ArC), 48.84, 44.53, 31.84, 29.50, 29.27, 26.96, 22.55 (CH_2), 13.61 (CH_3); HRMS (EI): Found m/z 519.32270 (M^++Na) $C_{20}H_{30}N_2O_4SNa$, Calculated m/z 519.32188.

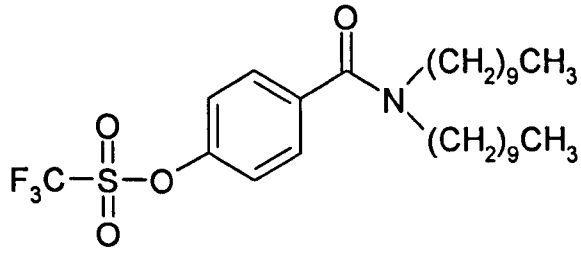
Synthesis of 4-[(didecylamino)carbonyl]phenyl methanesulfonate (**207**)



Compound **207** was synthesised via the same method as **179** using 4-hydroxy-*N,N*-didecyl-benzamide (1.00g, 2.39mmol), TEA (0.4ml, 2.87mmol) and methane sulfonyl chloride (0.25ml, 3.22mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **207** as a transparent pale yellow coloured oil [0.73ml, 61.62%; R_f: 0.67 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 2926.02 (CH), 2854.34 (NCH_3), 1635.79 (C=O); δ_H(d_6-Acetone): 7.57 (2H, d, J=8.79Hz, Ph$\underline{H}$), 7.49 (2H, d, J=8.79Hz, Ph$\underline{H}$) 3.39 [7H, m, SC$\underline{H}_3$, N(C$\underline{H}_2$)$_2$], 1.71 [4H, m, ($CH_2C\underline{H}_2$)$_2$], 1.39 [28H, m (($C_7\underline{H}_{14}$)CH_3)$_2$], 0.96 [6H, br.t, 6.96Hz, ($CH_2C\underline{H}_3$)$_2$]; δ_C(d_6-Acetone): 169.60 (C=O), 149.91, 137.06, 128.50, 122.23 (ArC), 48.79, 44.56 (CH_2), 36.89 (SCH_3), 31.84, 29.47, 29.27, 27.51, 26.95, 22.55 (CH_2), 13.61 (CH_3); Elemental analysis: Found C67.91%, H9.95%, N2.76% $C_{28}H_{49}NO_4S$, Calculated C67.84%, H9.96%, N2.83%.

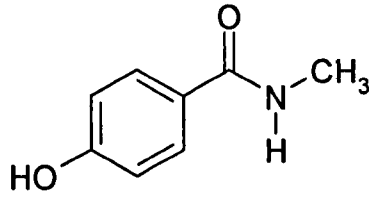
Synthesis of 4-[(didecylamino)carbonyl]phenyl trifluoromethanesulfonate (**208**)



Compound **208** was synthesised using the same method as **179** using 4-hydroxy-*N,N*-didecyl-benzamide (0.39g, 0.94mmol), TEA (0.20ml, 1.43mmol) and trifluoromethane sulfonyl chloride (0.15ml, 1.41mmol). The crude product was purified via column chromatography [DEE (50%): hexane (50%)] to give **208** as a pale yellow solid [0.24g, 32.91%; mp: 42.5-43.2°C; R_f: 0.98 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 2955.63 (PhH), 2926.80 (CH), 2855.50 (NCH), 1639.22 (C=O); δ_H($CDCl_3$): 7.43 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.29 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.45 [2H, t, J=7.32Hz, N(C<u>H</u>$_2$)$_2$], 3.12 [2H, t, J=7.32Hz, N(C<u>H</u>$_2$)$_2$], 1.25 [32H, m, ((C$_8$C<u>H</u>$_{16}$)CH$_3$)$_2$], 0.84 [6H, t, J=7.14Hz, (CH$_2$C<u>H</u>$_3$)$_2$]; δ_C($CDCl_3$): 169.74 (C=O), 149.73, 137.79, 128.74, 121.58 (ArC) 49.15 (NCH$_2$), 45.05, 31.95, 29.65, 29.50, 29.37, 29.13, 37.55, 22.74 (CH$_2$), 14.17 (CH$_3$); Elemental analysis: Found C61.12%, H8.27%, N2.56% $C_{28}H_{46}NO_4F_3S$, Calculated C61.18%, H8.43%, N2.55%.

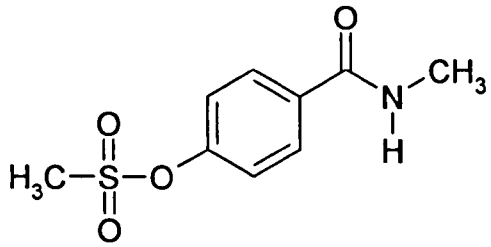
Synthesis of 4-hydroxy-*N*-methyl-benzamide (**209**)



Compound **209** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (3.85g, 19.44mmol) and methyl amine [10.85ml (2M in THF), 21.70mmol]. The crude product was purified using column chromatography (DEE 100%) to give **209** as a white solid (0.32g, 11.07%; R_f: 0.089 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3361.63 (PhOH), 2920.84 (PhH), 1634.17 (C=O); δ_H(d_6-Acetone): 9.11 (1H, s, PhO<u>H</u>), 7.86 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.68 (1H, br.s, N<u>H</u>), 6.96 (2H, d, J=8.79Hz, Ph<u>H</u>), 2.95 (3H, br.d, J=4.76Hz, NHC<u>H</u>$_3$); δ_C(d_6-Acetone): 167.72 (C=O), 160.93, 129.74, 127.04, 115.68 (ArC), 26.60 (CH$_3$); GC: t_R 8.03min; LRMS (EI): m/z 151 (M^+, 28%), 121 [M^+ - NH(CH$_3$), 100%].

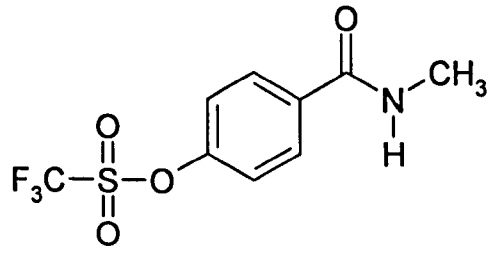
Synthesis of 4-[(methylamino)carbonyl]phenyl methanesulfonate (**210**)



Compound **210** was synthesised via the same method as **179** using 4-hydroxy-*N*-methyl-benzamide (0.14g, 0.92mmol), TEA (0.17ml, 1.43mmol) and methane sulfonyl chloride (0.10ml, 1.29mmol). The crude product was purified via column chromatography (DEE 100%) to give **210** as a white solid (0.10g, 48.03%; mp: 175.1-175.9°C; R_f: 0.048 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3316.77 (NH), 2978.50 (PhH), 2943.78 (CH), 2879.07 (NCH), 1635.08 (C=O); δ_H(d_4-Methanol): 7.62 (2H, d, J=8.97Hz, Ph<u>H</u>) 7.20 (2H, d, J=8.97Hz, Ph<u>H</u>), 3.05 (3H, s, SC<u>H</u>$_3$), 2.71 (3H, s, NC<u>H</u>$_3$); δ_C(d_4-Methanol): 168.06 (C=O), 151.80, 133.31, 128.87, 121.99 (ArC), 36.48 (SCH_3), 25.63 (NCH_3); GC: t_R 11.72min; LRMS (EI): m/z 229 (M^+, 40%), 199 [M^+ - NH(CH_3), 100%].

Synthesis of 4-[(methylamino)carbonyl]phenyl trifluoromethanesulfonate (**211**)

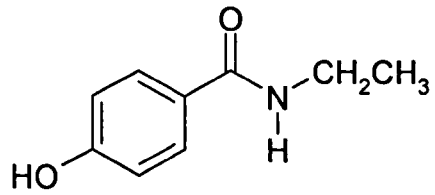


Compound **211** was synthesised using the same method **179** as using 4-hydroxy-*N*-methyl-benzamide (0.12g, 0.79mmol), TEA (0.15ml, 1.07mmol) and trifluoromethane sulfonyl chloride (0.15ml, 1.41mmol). The crude product was purified via column chromatography (DEE, 100%) to give **211** as a pale yellow solid (0.13g, 62.07%; mp: 55.6-56.4°C; R_f: 0.077 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3356.97 (CH), 1641.53 (C=O); δ_H(d_6-Acetone): 8.06 (2H, d, J=8.97Hz, Ph<u>H</u>) 7.88 (1H, br. s, N<u>H</u>), 7.56 (2H, d, J=8.79Hz, Ph-<u>H</u>), 2.91 (2H, d,

J=4.76Hz, NHC$\underline{H}_3$); δ_C(d_6-Acetone): 165.40 (C=O), 151.26, 135.71, 129.61, 121.53 (ArC), 25.97 (NCH_3); GC: t_R 7.40min; LRMS (EI): m/z 283 (M^++1, 40%), 253 [M^+ - $NH(CH_3)$, 100%].

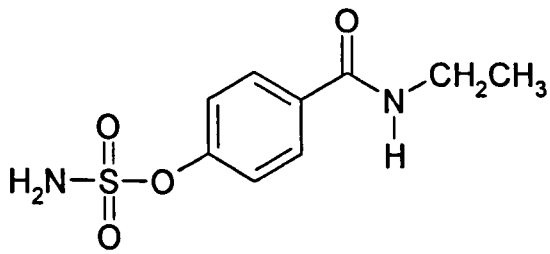
Synthesis of Synthesis of 4-hydroxy-*N*-ethyl-benzamide (**212**)



Compound **212** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (3.41g, 18.92mmol), thionyl chloride (1.40ml, 19.19mmol), *N*-methyl morpholine (2.08ml, 18.92mmol) and ethyl amine [9.50ml (2M in THF)], 19.00mmol]. Hydrolysis was achieved with NaOH (1.50g, 1.25M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **212** as a white solid (1.38g, 44.19%; mp: 98.3-101.8°C; R_f: 0.16 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3362.94 (NH), 2921.29 (PhH), 2849.65 (NCH), 1633.99 (C=O); δ_H(d_6-Acetone): 8.92 (1H, s, PhOH), 7.80 (2H, d, J=8.97Hz, PhH), 7.59 (1H, br.s, N$\underline{H}$), 6.89 (2H, d, J=8.97Hz, PhH), 3.40 (2H, q, J=7.14Hz, NHC$\underline{H}_2$$CH_3$), 1.17 (3H, t, J=7.14Hz, CH_2C$\underline{H}_3$); δ_C(d_6-Acetone): 166.79 (C=O), 160.80, 129.77, 127.35, 115.60 (ArC), 35.02 (CH_2), 15.28 (CH_3); GC: t_R 9.58min; LRMS (EI): m/z 165 (M^+, 44%), 121 [M^+ - $NH_2(C_3H_7)$, 100%].

Synthesis of 4-[(ethylamino)carbonyl]phenyl sulfamate (**213**)

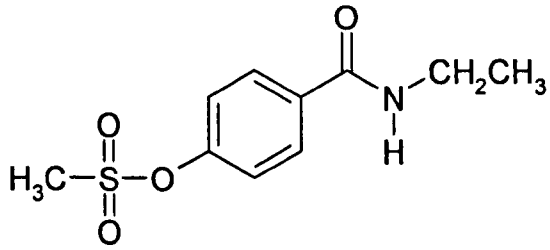


Compound **213** was synthesised via the same method as **178** using 4-hydroxy-*N*-ethyl-benzamide (0.22g, 1.35mmol). The crude product was purified via column chromatography [ethyl acetate (70%): hexane (30%)] to give **213** as

an off-white solid (0.025g, 7.27%; mp: 165.3-166.5°C; R_f: 0.25 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3330.97 (NH), 3197.38 (NH), 3073.78 (PhH), 2978.80 (CH), 2938.75 (NCH), 1628.35 (C=O); δ_C(d_6-Acetone): 165.35 (C=O) 152.63, 133.64, 128.78, 122.05 (ArC) 34.47 (NCH_2), 14.33 (CH_3); LRMS (EI): m/z 244 (M^+, 17%), 121 [M^+- $C_2H_7N_2SO_2$, 100%].

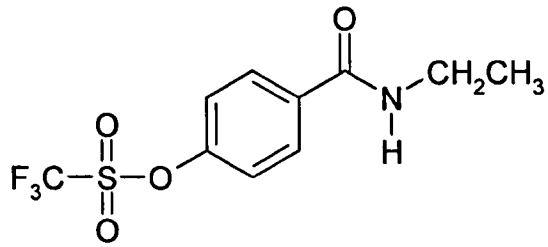
Synthesis of 4-[(ethylamino)carbonyl]phenyl methanesulfonate (**214**)



Compound **214** was synthesised via the same method as **179** using 4-hydroxy-*N*-ethyl-benzamide (0.19g, 1.15mmol), TEA (0.20ml, 1.43mmol) and methane sulfonyl chloride (0.16ml, 1.50mmol). The crude product was purified via column chromatography (DEE, 100%) to give **214** as a white solid (0.15g, 53.44%; 148.0-149.1°C; R_f: 0.095 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3316.77 (NH), 2978.50 (PhH), 2943.78 (CH), 2879.07 (NCH), 1635.08 (C=O); δ_H(d_6-Acetone): 7.91 (2H, d, J=8.97Hz, Ph$\underline{H}$) 7.85 (1H, br. s, N$\underline{H}$) 7.42 (2H, d, J=8.97Hz, Ph$\underline{H}$) 3.42 (2H, m, NHC$\underline{H}_2$) 3.32 (3H, s, SC$\underline{H}_3$), 1.19 (3H, t, J=7.32Hz, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 160.20 (C=O), 151.20, 134.07, 128.95, 122.19 (ArC), 37.82 (SCH_3), 35.19 (CH_2), 14.94 (CH_3); GC: t_R 14.15min; LRMS (EI): m/z 243 (M^+, 44%), 199 [M^+- $NH(C_2H_5)$, 100%].

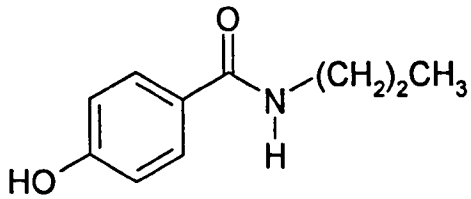
Synthesis of 4-[(ethylamino)carbonyl]phenyl trifluoromethanesulfonate (**215**)



Compound **215** was synthesised using the same method as **179** using 4-hydroxy-*N*-ethyl-benzamide (0.20g, 1.19mmol), TEA (0.25ml, 1.79mmol) and trifluoromethane sulfonyl chloride (0.15ml, 1.69mmol). The crude product was purified via column chromatography [ethyl acetate (80%): DEE (20%)] to give **215** as an off white solid (0.063g, 21.95%; 73.1-73.9°C; R_f: 0.18 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3337.01 (NH), 2989.10 (CH), 1643.76 (C=O); δ_H(d_6-Acetone): 8.15 (2H, d, J=8.97Hz, Ph<u>H</u>) 8.01 (1H, br.s, NH), 7.62 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.51 (2H, m, NHC<u>H</u>$_2$), 1.27 (3H, t, J=7.14Hz, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 164.77 (C=O), 151.25, 135.89, 129.65, 121.48 (ArC), 34.60 (NCH_2), 14.23 (CH_3).

Synthesis of 4-hydroxy-*N*-propyl-benzamide (**216**)

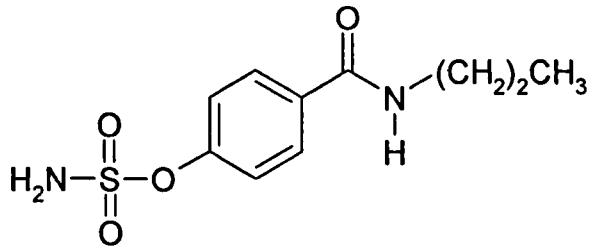


Compound **216** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (2.24, 12.44mmol), thionyl chloride (1.00ml, 13.71mmol), *N*-methyl morpholine (1.45ml, 13.19mmol) and propyl amine (1.08ml, 13.14mmol). Hydrolysis was achieved with NaOH (1.40g, 1.27M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **216** as a white solid (0.96g, 42.98%; mp: 97.3-98.6°C; R_f: 0.21 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3293.00 (NH), 2964.64 (PhH), 2934.56 (CH), 2874.98 (NCH), 1629.92 (C=O); δ_H(d_6-Acetone): 9.16 (1H, s, PhO<u>H</u>), 7.82 (2H, d, J=8.79Hz,

PhH), 7.72 (1H, br.s, NH), 6.89 (2H, d, J=8.79Hz, PhH), 3.34 (2H, m, $NHCH_2$), 1.96 (2H, sex, J=7.51Hz, $CH_2CH_2CH_3$), 0.83 (3H, t, J=7.51Hz, CH_2CH_3); δ_C(d_6-Acetone): 167.34 (C=O) 161.01, 129.82, 127.03 ,115.66 (ArC) 42.10, 23.59 (CH_2) 11.71 (CH_3); GC: t_R 9.96min; LRMS (EI): m/z 179 (M^+, 14%), 121 [M^+-$NH_2(C_3H_7)$].

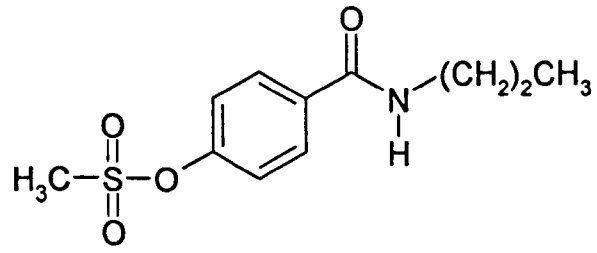
Synthesis of 4-[(propylamino)carbonyl]phenyl sulfamate (**217**)



Compound **217** was synthesised via the same method as **178** using 4-hydroxy-*N*-propyl-benzamide (0.34g, 1.92mmol). The crude product was purified via column chromatography [ethyl acetate (70%): hexane (30%)] to **217** as an off-white solid (0.12g, 24.24%; mp: 150.0-151.0°C; R_f: 0.30 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3315.46 (NH), 2966.57 (PhH), 2939.04 (CH), 1637.24 (C=O); δ_C(d_6-Acetone): 165.60 (C=O) 152.63, 133.62, 128.81, 122.09 (ArC) 41.46 (NCH_2), 22.78 (CH_2) 13.57 (CH3); HRMS (EI): Found m/z 281.05848 (M^++Na) $C_{10}H_{14}O_4N_2SNa$, Calculated m/z 281.05665.

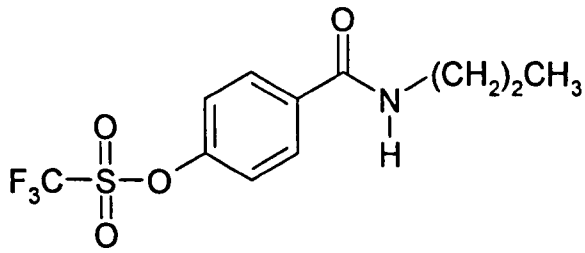
Synthesis of 4-[(propylamino)carbonyl]phenyl methanesulfonate (**218**)



Compound **218** was synthesised via the same method as **179** using 4-hydroxy-*N*-propyl-benzamide (1.19g, 6.65mmol), TEA (1.3ml, 9.33mmol) and methane sulfonyl chloride (0.70ml, 9.01mmol). The crude product was purified via column chromatography (DEE, 100%) to give **218** as a white solid (0.31g, 16.94%; mp: 121.3-122.8°C; R_f: 0.14 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3305.80 (NH), 2960.59 (PhH), 2872.60 (NCH), 1633.49 (C=O); δ_H(d_6-Acetone): 7.89 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.76 (1H, br.s, N<u>H</u>), 7.32 (2H, d, J=8.97Hz, Ph<u>H</u>), 3.26 (2H, m, NHC<u>H</u>$_2$), 3.23 (3H, s, SC<u>H</u>$_3$), 1.51 (2H, m, C<u>H</u>$_2$), 0.84 (3H, t, J=7.32Hz, CH$_2$C<u>H</u>$_3$); δ_C(d_6-Acetone): 165.35 (C=O), 151.58, 134.33, 129.12, 122.08 (ArC), 41.46 (CH$_2$), 37.00 (S-CH$_3$), 22.73 (CH$_2$), 10.94 (CH$_3$); GC: t_R 15.05min; LRMS (EI): m/z 258 (M$^+$, 4%), 199 [M$^+$ - NH(C$_3$H$_7$)].

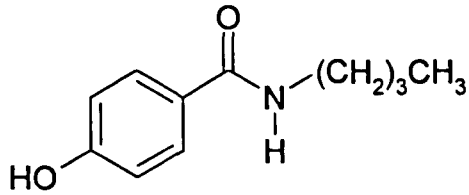
Synthesis of 4-[(propylamino)carbonyl]phenyl trifluoromethanesulfonate (**219**)



Compound **219** was synthesised using the same method as **179** using 4-hydroxy-*N*-propyl-benzamide (0.73g, 4.09mmol), TEA (0.80ml, 5.74mmol) and trifluoromethane sulfonyl chloride (0.60ml, 5.64mmol). The crude product was purified via column chromatography [ethyl acetate (80%): DEE (20%)] to give **219** as a (0.83g, 65.36mmol; mp: 114.7-115.5°C; R_f: 0.14 [DEE (90%): hexane (10%)].

$\nu_{(max)}$(Film)cm^{-1}: 3296.62 (NH), 2977.55 (PhH), 2935.53 (CH), 2877.73 (NCH), 1608.10 (C=O); δ_H(d_6-Acetone): 8.10 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.98 (1H, br. s, NH), 7.59 (2H, d, J=8.97Hz, Ph<u>H</u>), 3.41 (2H, m, NHC<u>H</u>$_2$), 1.66 (2H, sex, J=7.32, CH$_2$C<u>H</u>$_2$CH$_3$) 0.98 (3H, t, J=7.32Hz, CH$_2$C<u>H</u>$_3$); δ_C(d_6-Acetone): 164.98 (C=O), 151.24, 135.93, 129.68, 121.48 (ArC), 41.54 (NCH$_2$), 22.66 (CH$_2$), 10.92 (CH$_3$); GC: t_R 8.79min; LRMS (EI): m/z 311 (M$^+$, 19%), 253 [M$^+$ - NH(C$_3$H$_7$)].

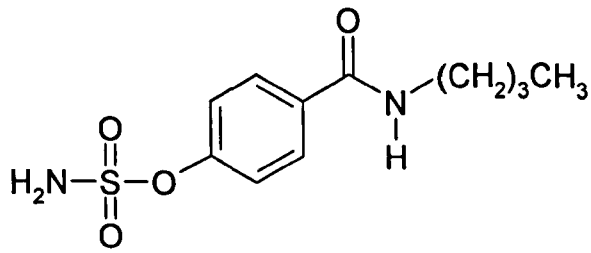
Synthesis of 4-hydroxy-*N*-butyl-benzamide (**220**)



Compound **220** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (2.22g, 12.34mmol), thionyl chloride (1.00ml, 13.71mmol), *N*-methyl morpholine (1.4ml, 12.73mmol) and butyl amine (1.4ml, 13.15mmol). Hydrolysis was achieved with NaOH (1.33g, 1.23M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **220** as a white solid (1.99g, 83.48%; mp: 108.2-110.5°C; R_f: 0.27 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3300.82 (NH), 3185.58 (PhOH), 2959.00 (PhH), 2932.55 (CH), 2872.56 (NCH), 1608.01 (C=O); δ_H(d_6-Acetone): 9.11 (1H, s, PhO<u>H</u>) 7.81 (2H, d, J=8.97Hz, Ph<u>H</u>) 7.68 (1H, br.s, N<u>H</u>) 6.89 (2H, d, J=8.97Hz, Ph<u>H</u>) 3.39 (2H, m, NHC<u>H</u>$_2$), 1.56 (2H, m, C<u>H</u>$_2$CH$_3$), 0.93 (3H, t, J=7.32Hz, C<u>H</u>$_3$); δ_C(d_6-Acetone): 164.35 (C=O), 158.14, 126.99, 124.37 ,112.84 (ArC) 37.19, 29.80, 17.94 (CH_2), 11.27 (CH_3); GC: t_R 10.64min; LRMS (EI): m/z 193 (M^+, 13%), 121 ([M^+-$NH_2(C_4H_9)$], 100%).

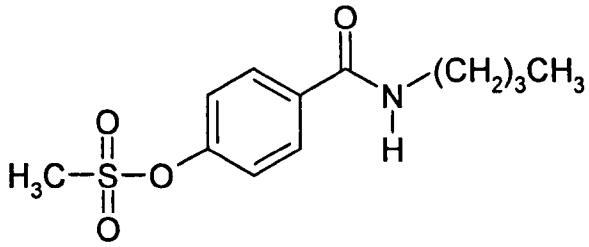
Synthesis of 4-[(butylamino)carbonyl]phenyl sulfamate (**221**)



Compound **221** was synthesised via the same method as **178** using 4-hydroxy-*N*-butyl-benzamide (0.298, 1.54mmol). The crude product was purified via column chromatography [ethyl acetate (70%): hexane (30%)] to give **221** as an off-white solid (0.16g, 37.99%; mp: 134.8-135.6°C; R_f: 0.33 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3413.82 (NH), 3298.17 (NH amd), 2954.29 (PhH), 2859.56 (NCH), 1632.30 (C=O); δ_C(d_6-Acetone): 165.64 (C=O), 152.65, 133.58, 128.83, 122.10 (ArC), 39.41 (NCH_2), 31.67, 19.98 (CH_2), 13.32 (CH_3); HRMS (EI): Found 295.07310 m/z (M^++Na) $C_{11}H_{16}N_2O_4SNa$, Calculated m/z 295.07230.

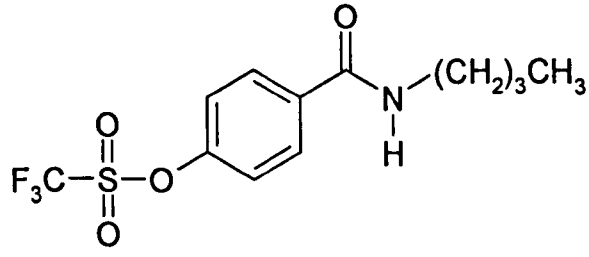
Synthesis of 4-[(butylamino)carbonyl]phenyl methanesulfonate (**222**)



Compound **222** was synthesised via the same method as **179** using 4-hydroxy-*N*-butyl-benzamide (0.57g, 2.94mmol), TEA (0.80ml, 5.74mmol) and methane sulfonyl chloride (0.60ml, 7.75mmol). The crude product was purified via column chromatography (DEE, 100%) to give **222** as a white solid (0.31g, 38.30%; mp: 128.5-130.4°C; R_f: 0.17 [DEE (90%): hexane (10%)].

$\nu_{(max)}$(Film)cm^{-1}: 3329.77 (NH), 2951.94 (PhH), 2871.73 (NCH), 1633.01 (C=O); δ_H(d_6-Acetone): 7.88 (2H, d, J=8.97Hz, Ph<u>H</u>) 7.74 (1H, br.s, N<u>H</u>), 7.32 (2H, d, J=8.97Hz, Ph<u>H</u>), 3.30 (2H, m, NHC<u>H</u>$_2$) 3.23 (3H, s, SCH_3) 1.49 (2H, m, $CH_2C\underline{H}_2CH_2$), 1.29 (2H, $CH_2C\underline{H}_2CH_3$), 0.80 (3H, t, J=7.32Hz, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 165.33 (C=O), 151.57, 134.33, 129.12, 122.08 (ArC), 39.39 (CH_2), 37.00 (SCH_3), 31.68, 19.98 (CH_2), 13.30 (CH_3); GC: t_R 15.97min; LRMS (EI): m/z 272 (M^+, 13%), 199 [M^+- $NH_2(C_4H_9)$, 100%].

Synthesis of 4-[(butylamino)carbonyl]phenyl trifluoromethanesulfonate (**223**)

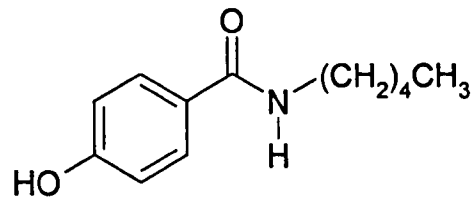


Compound **223** was synthesised using the same method as **179** using 4-hydroxy-*N*-butyl-benzamide (0.70g, 3.64mmol), TEA (0.70ml, 5.02mmol) and

trifluoromethane sulfonyl chloride (0.50ml, 4.70mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **223** as an off white solid (0.33g, 27.59%; mp: 57.3-58.1°C; R_f: 0.64 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3308.34 (NH), 2962.03 (PhH), 2935.07 (CH), 2874.51 (NCH), 1643.38 (C=O); δ_H(d_6-Acetone): 8.15 (2H, d, J=8.97Hz, Ph$\underline{H}$), 8.01 (1H, br.s, N$\underline{H}$), 7.63 (2H, d, J=8.97Hz, Ph$\underline{H}$), 3.49 (2H, m, NHC$\underline{H}_2$), 1.67 (2H, m, $CH_2C\underline{H}_2CH_2$), 1.48 (2H, m, $CH_2C\underline{H}_2CH_3$), 1.00 (3H, t, J=7.32Hz, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 164.92 (C=O), 151.24, 135.95, 129.67, 121.47 (ArC), 39.47 (NCH_2), 31.61, 19.95 (CH_2), 13.26 (CH_3); GC: t_R 11.73min; LRMS (EI): m/z 325 (M^+, 5%), 253 [M^+-NH(C_4H_9), 100%]; HRMS (EI): Found m/z 348.04860 (M^++Na) $C_{12}H_{16}O_4NSNa$, Calculated m/z 348.04878.

Synthesis of 4-hydroxy-*N*-pentyl-benzamide (**224**)

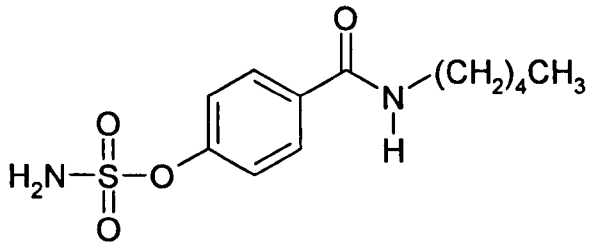


Compound **224** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (3.87g, 21.48mmol), thionyl chloride (1.70ml, 23.31mmol), *N*-methyl morpholine (2.50ml, 22.74mmol) and pentyl amine (2.70ml, 23.12mmol). Hydrolysis was achieved with NaOH (1.95g, 1.22M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **224** as a white solid (3.34g, 75.12%; mp: 107.4-109.3°C; R_f: 0.32 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3313.00 (NH), 3168.51 (PhOH), 2957.44 (PhH), 2931.62 (CH), 2871.10 (NCH), 1607.86 (C=O); δ_H(d_6-Acetone): 9.10 (1H, s, PhO$\underline{H}$), 7.87 (2H, d, J=8.97Hz, Ph$\underline{H}$), 7.71 (1H, br.s, N$\underline{H}$), 6.95 (2H, d, J=8.97Hz, Ph$\underline{H}$), 3.44 (2H, m, NHC$\underline{H}_2$), 1.42 (4H, m, $C_2\underline{H}_4CH_3$), 0.95 (3H, m, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 166.35 (C=O), 160.17, 129.07, 126.48 ,114.90 (ArC), 39.55, 29.45, 29.16, 22.32

(CH_2), 13.54 (CH_3); GC: t_R 13.76min; LRMS (EI): m/z 207 (M^+, 12%), 121 [M^+ - $NH(C_5H_{11})$, 100%].

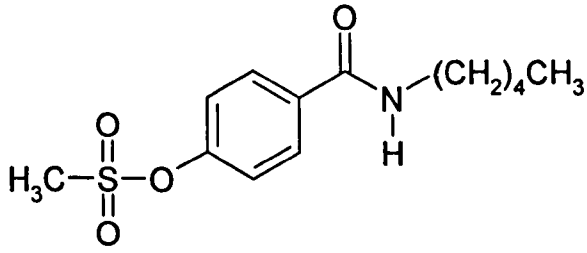
Synthesis of 4-[(pentylamino)carbonyl]phenyl sulfamate (**225**)



Compound **225** was synthesised via the same method as **178** using 4-hydroxy-*N*-pentyl-benzamide (0.48g, 2.30mmol). The crude product was purified via column chromatography [ethyl acetate (70%): hexane (30%)] to give **225** as an off-white solid (0.34g, 51.03%; mp: 130.3-132.4°C; R_f: 0.35 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3406.86 (NH), 3288.76 (NH amd), 2950.45 (PhH), 2923.16 (CH), 2853.81 (NCH), 1633.10 (C=O); HRMS (EI): Found m/z 309.08877 (M^++Na) $C_{12}H_{20}O_4N_2SNa$, Calculated m/z 309.08795.

Synthesis of 4-[(pentylamino)carbonyl]phenyl methanesulfonate (**226**)

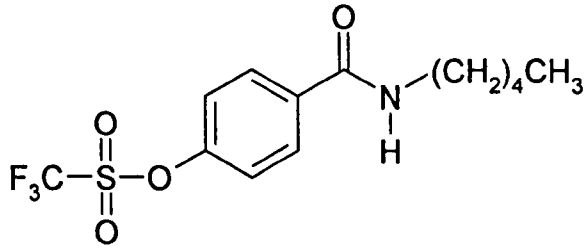


Compound **226** was synthesised via the same method as **179** using 4-hydroxy-*N*-pentyl-benzamide (0.73g, 3.53mmol), TEA (0.40ml, 2.87mmol) and methane sulfonyl chloride (0.30ml, 3.86mmol). The crude product was purified via column chromatography [DEE (70%): hexane (30%)] to give **226** as a white solid (0.50g, 49%; mp: 129.9-131.3°C; R_f: 0.20 [DEE (90%): hexane (10%)].

$\nu_{(max)}$(Film)cm^{-1}: 3310.68 (NH), 2953.45 (PhH), 2925.95 (CH), 2858.21 (NCH), 1635.84 (C=O); δ_H(d_6-Acetone): 7.89 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.74 (1H, br.s,

N$\underline{H}$), 7.32 (2H, d, J=8.97Hz, Ph$\underline{H}$), 3.30 (2H, q, J=5.86Hz, NHC$\underline{H}_2$CH$_2$), 3.23 (3H, s, SC$\underline{H}_3$), 1.96 (2H, quin, J=2.20Hz, CH$_2$C$\underline{H}_2$CH$_2$), 1.51 (2H, quin, J=7.14Hz, CH$_2$C$\underline{H}_2$CH$_2$), 1.26 (2H, m, C$\underline{H}_2$CH$_3$), 0.80 (3H, t, J=7.14Hz, CH$_2$C$\underline{H}_3$); δ_C(d$_6$-Acetone): 165.30 (C=O), 151.58, 134.33, 129.12, 122.08 (ArC), 39.68 (CH$_2$), 37.00 (SCH$_3$), 29.10, 28.86, 22.28 (CH$_2$), 13.30 (CH$_3$); GC: t_R 16.80min; LRMS (EI): m/z 286 (M$^+$, 2%), 199 [M$^+$ - NH(C$_5$H$_{11}$), 100%].

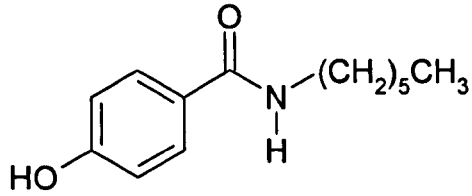
Synthesis of 4-[(pentylamino)carbonyl]phenyl trifluoromethanesulfonate (**227**)



Compound **227** was synthesised using the same method as **179** using 4-hydroxy-*N*-pentyl-benzamide (0.45g, 2.16mmol), TEA (0.40ml, 2.87mmol) and trifluoromethane sulfonyl chloride (0.20ml, 1.88mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **227** as a white solid (0.13g, 17.71%; mp: 80.1-82.5°C; R_f: 0.72 [DEE (90%): hexane (10%)].

$\nu_{(max)}$(Film)cm^{-1}: 3319.44 (NH), 2958.20 (PhH), 2930.44 (CH), 2859.68 (NCH), 1642.37 (C=O); δ_H(d$_6$-Acetone): 8.15 (2H, d, J=8.97Hz, Ph$\underline{H}$), 8.01 (1H, br.s, N$\underline{H}$), 7.63 (2H, d, J=8.97Hz, Ph$\underline{H}$), 3.48 (2H, m, NHC$\underline{H}_2$), 1.70 (2H, m, CH$_2$C$\underline{H}_2$CH$_2$), 1.43 (4H, m, C$\underline{H}_2$C$\underline{H}_2$CH$_3$), 0.97 (3H, t, J=7.14Hz, CH$_2$C$\underline{H}_3$); δ_C(d$_6$-Acetone): 164.94 (C=O), 151.24, 135.93, 129.68, 121.48 (ArC), 39.75 (NCH$_2$), 29.19, 22.08, 22.26 (CH$_2$), 13.48 (CH$_3$); GC: t_R 10.76min; LRMS (EI): m/z 339 (M$^+$, 6%), 253 [M$^+$-NH(C$_5$H$_{11}$), 100%].

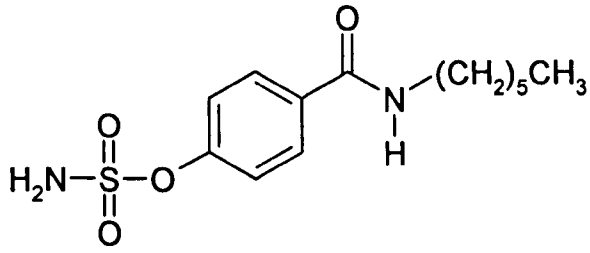
Synthesis of 4-hydroxy-*N*-hexyl-benzamide (**228**)



Compound **228** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (3.17g, 17.57mmol), thionyl chloride (1.80ml, 24.68mmol), *N*-methyl morpholine (2.55ml, 23.19mmol) and hexyl amine (3.10ml, 23.47mmol). Hydrolysis was achieved with NaOH (1.59g, 1.24M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **228** as a white solid (3.45g, 88.84%; mp: 88.5-87.3°C; R_f: 0.36 [DEE (90%): hexane (10%)].

$\nu_{(max)}$(Film)cm^{-1}: 3313.00 (NH), 3168.51 (PhOH), 2957.44 (PhH), 2931.62 (CH), 2871.10 (NCH), 1607.86 (C=O); δ_H(d_6-Acetone): 8.83 (1H, s, O<u>H</u>), 7.27 (2H, d, J=8.60Hz, Ph<u>H</u>), 6.86 (2H, d, J=8.60Hz, Ph<u>H</u>), 3.39 [4H, br. s, N(C<u>H</u>$_2$)$_2$], 1.61 [4H, br. s, N(CH_2C<u>H</u>$_2$)$_2$], 1.29 [12H, br. s, (C_3<u>H</u>$_6$)$_2$], 0.86 [6H, m, (C<u>H</u>$_3$)$_2$]; δ_C(d_6-Acetone): 171.81 (C=O), 159.04, 129.63, 129.29, 115.61 (ArC), 32.15, 27.15, 23.19 (CH_2), 14.24 (CH_3); GC: t_R 13.76min; LRMS (EI): m/z 221 (M^+, 13%), 121 [M^+-NH(C_6H_{13}), 100%].

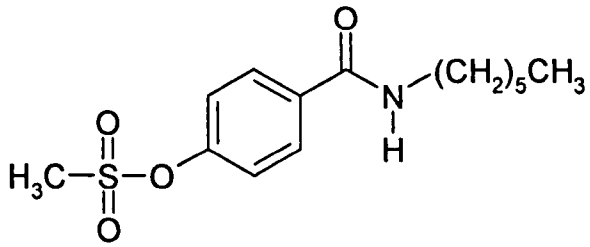
Synthesis of 4-[(hexylamino)carbonyl]phenyl sulfamate (**229**)



Compound **229** was synthesised via the same method as **178** using 4-hydroxy-*N*-hexyl-benzamide (0.23g, 0.97mmol). The crude product was purified via column chromatography [ethyl acetate (70%): hexane (30%)] to give **229** as an off-white solid (0.099g, 34.12%; mp: 130.7-132.9°C; R_f: 0.36 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3405.96 (NH), 3282.30 (NH amd), 2954.59 (PhH), 2915.93 (CH), 2849.78 (NCH), 1629.64 (C=O); δ_C(d_6-Acetone): 167.77 (C=O) 153.08, 132.75, 128.63, 121.89 (ArC) 39.85, 31.39, 29.14, 26.48, 22.36 (CH_2), 13.12 (CH_3); LRMS (EI): m/z 300 (M^+, 14%), 121 [M^+- $NH_2(C_6H_{13})$].

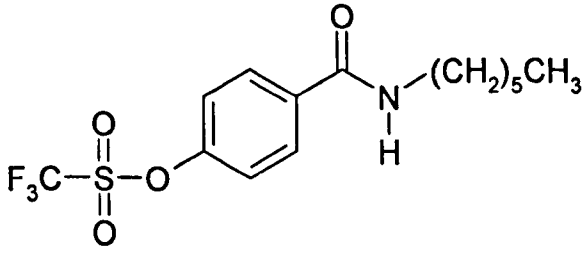
Synthesis of 4-[(hexylamino)carbonyl]phenyl methanesulfonate (**230**)



Compound **230** was synthesised via the same method as **179** using 4-hydroxy-*N*-hexyl-benzamide (0.51g, 2.32mmol), TEA (0.40ml, 2.87mmol) and methane sulfonyl chloride (0.30ml, 3.86mmol). The crude product was purified via column chromatography [DEE (70%): hexane (30%)] to give **230** as a white solid (0.47g, 67.98%; mp: 129.5-130.2°C; R_f: 0.21 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3337.20 (NH), 2954.00 (PhH), 2936.54 (CH), 2815.67 (NCH), 1633.72 (C=O); δ_H(d_6-Acetone): 7.98 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.86 (1H, br.s, N<u>H</u>), 7.42 (2H, d, J=8.97Hz, Ph<u>H</u>), 3.39 (2H, m, NHC<u>H</u>$_2$), 3.32 (3H, s, SC<u>H</u>$_3$), 1.60 (2H, m, $CH_2C\underline{H}_2CH_2$), 1.34 (6H, m, $C_3\underline{H}_6CH_3$), 0.89 (3H, t, J=6.96Hz, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 165.33 (C=O), 151.88, 134.33, 129.13, 122.08 (ArC), 39.37 (CH_2), 37.01 (SCH_3), 31.51, 29.53, 26.61, 22.48 (CH_2), 13.52 (CH_3); GC: t_R 17.64min; LRMS (EI): m/z 300 (M^+, 2%), 199 [M^+ - $NH(C_6H_{13})$, 100%].

Synthesis of 4-[(hexylamino)carbonyl]phenyl trifluoromethanesulfonate (**231**)

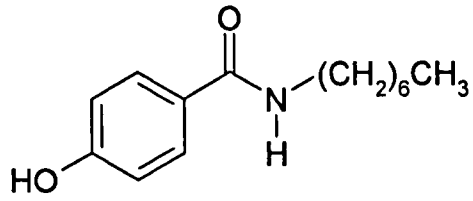


Compound **231** was synthesised using the same method as **179** using 4-hydroxy-*N*-hexyl-benzamide (1.41g, 6.36mmol), TEA (1.20ml, 8.61mmol) and

trifluoromethane methane sulfonyl chloride (0.90ml, 8.47mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%)] to give **231** as a (1.36g, 60.36%; mp: 57.5-58.3°C; R_f: 0.74 [DEE (90%): hexane (10%)]).

δ_H(d_6-Acetone): 8.06 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.92 (1H, br. s, N<u>H</u>), 7.55 (2H, d, J=8.97Hz, Ph<u>H</u>), 3.39 (2H, m, NHC<u>H</u>$_2$), 1.60 (2H, m, $CH_2C\underline{H}_2CH_2$) 1.33 (6H, m, C_3H_6), 0.97 (3H, t, J=7.14Hz, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 164.92 (C=O), 151.24, 135.96, 129.68, 121.46 (ArC), 39.81 (NCH_2), 31.49, 29.47, 26.85, 22.45 (CH_2), 13.48 (CH_3); GC: t_R 11.63min; LRMS (EI): m/z 353 (M^+, 5%), 253 [M^+-$NH(C_6H_{13})$].

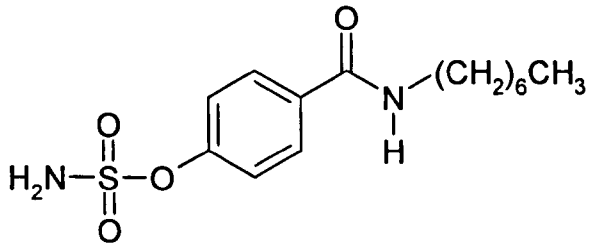
Synthesis of 4-hydroxy-*N*-heptyl-benzamide (**232**)



Compound **232** was synthesised via the same method as **176** using 4-acetoxy benzoic acid (3.44g, 19.08mmol), thionyl chloride (1.50ml, 20.56mmol), *N*-methyl morpholine (2.5ml, 22.74mmol) and heptyl amine (3.4ml, 22.72). Hydrolysis was achieved with NaOH (1.68g, 1.24M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **232** as a white solid (4.09g, 91.17%; mp: 85.3-86.9°C; R_f: 0.38 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3313.80 (NH), 3169.15 (PhOH), 2955.43 (PhH), 2955.43 (CH), 2856.28 (NCH), 1630.28 (C=O); δ_H(d_6-Acetone): 8.99 (1H, s, PhO<u>H</u>), 7.86 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.64 (1H, s, N<u>H</u>), 6.94 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.34 (2H, m, NHC<u>H</u>$_2$), 1.39 (8H, m, $C_2\underline{H}_8CH_3$), 0.95 (3H, t, J=6.96Hz, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 166.18 (C=O), 160.06, 129.03, 126.63, 114.85 (ArC) 39.57 (NCH_2), 31.77, 29.76, 26.93, 22.48 (CH_2), 13.54 (CH_3); GC: t_R 13.64min; LRMS (EI): m/z 235 (M^+, 11%), 121 [M^+- $NH(C_7H_{15})$, 100%].

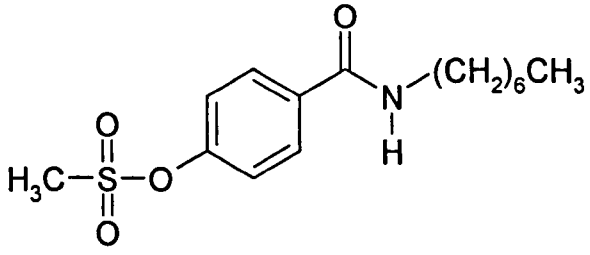
Synthesis of 4-[(heptylamino)carbonyl]phenyl sulfamate (**233**)



Compound **233** was synthesised via the same method as **178** using 4-hydroxy-*N*-heptyl-benzamide (0.95g, 4.07mmol). The crude product was purified via column chromatography [ethyl acetate (60%): DEE (40%)] to give **233** as an off-white solid (1.04g, 81.82%; mp: 143.4-144.8°C; R_f: 0.35 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film) cm^{-1}: 3414.38 (NH), 3294.17 (NH amd), 2923.20 (PhH), 2849.31 (CH), 2849.31 (NCH), 1635.86 (C=O); δ_C(d_6-Acetone): 165.43 (C=O), 152.59, 133.72, 128.79, 122.08 (ArC), 39.67, 31.77, 26.92, 22.50 (CH_2), 13.57 (CH_3).

Synthesis of 4-[(heptylamino)carbonyl]phenyl methanesulfonate (**234**)

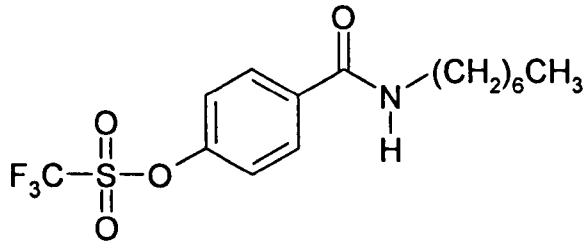


Compound **234** was synthesised via the same method as **179** using 4-hydroxy-*N*-heptyl-benzamide (0.77g, 3.29mmol), TEA (0.50ml, 3.59mmol) and methane sulfonyl chloride (0.30ml, 3.86mmol). The crude product was purified via column chromatography [DEE (70%): hexane (30%)] to give **234** as a white solid (0.89g, 86.02%; mp: 130.5-131.8°C; R_f: 0.21 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3305.89 (NH), 2953.35 (PhH), 2920.52 (CH), 2848.39 (NCH), 1633.27 (C=O); δ_H(d_6-Acetone): 7.90 (2H, d, J=8.97Hz, Ph$\underline{H}$), 7.42 (2H, d, J=8.97Hz, Ph$\underline{H}$), 3.39 (2H, t, J=7.14, NHC$\underline{H}_2$), 3.33 (3H, s, SC$\underline{H}_3$), 1.61 (2H, m, NHCH$_2$C$\underline{H}_2$), 1.31 (8H, C$_4\underline{H}_8$CH$_3$), 0.87 (3H, t, J=7.14Hz, CH$_2$C$\underline{H}_3$); δ_C(d_6-Acetone): 165.24 (C=O), 151.58, 134.30, 129.12, 122.08 (ArC), 39.59 (CH_2), 37.00 (SCH_3),

31.76, 29.56, 28.98, 26.89, 22.48 (CH_2), 13.54 (CH_3); GC: t_R 18.47min; LRMS (EI): m/z 314 (M^+, 3%), 199 [M^+ - $NH(C_7H_{15})$, 100%].

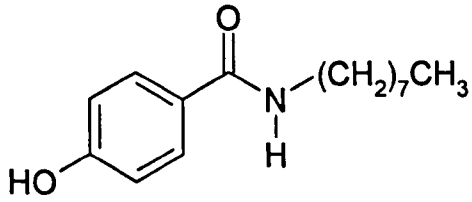
Synthesis of 4-[(heptylamino)carbonyl]phenyl trifluoromethanesulfonate (**235**)



Compound **235** was synthesised using the same method as **179** using 4-hydroxy-*N*-heptyl-benzamide (0.53, 2.26mmol), TEA (0.50ml, 3.59mmol) and trifluoromethane methane sulfonyl chloride (0.40ml, 3.76mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **235** as a (0.63g, 71.58%; mp: 56.7-57.1°C; R_f: 0.77 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3344.59 (NH), 2957.44 (PhH), 2921.98 (CH), 2851.96 (NCH), 1634.90 (C=O); δ_H(d_6-Acetone): 8.15 (2H, d, J=8.97Hz, PhH), 8.01 (1H, br.s, NH), 7.62 (2H, d, J=8.97Hz, PhH), 3.48 (2H, m, NHC$\underline{H}_2$), 1.69 (2H, m, $CH_2C\underline{H}_2CH_2$), 1.41 (8H, m, $C_4\underline{H}_8$), 0.95 (3H, m, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 164.93 (C=O), 151.24, 135.93, 129.68, 121.47 (ArC), 39.81 (NCH_2), 31.74, 29.50, 28.97, 26.87, 22.47 (CH_2), 13.52 (CH_3); GC: t_R 12.67min; LRMS (EI): m/z 367 (M^+, 6%), 253 (M^+ - NC_7H_{16}, 100%).

Synthesis of 4-hydroxy-*N*-octyl-benzamide (**236**)

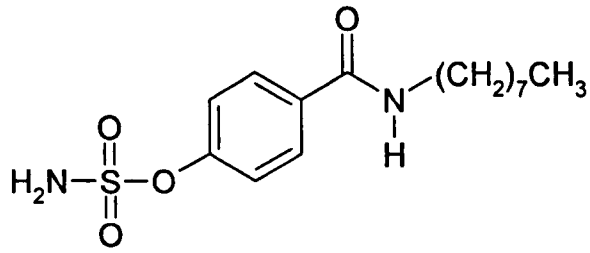


Compound **236** was synthesised via the same method as **176**, using 4-acetoxy benzoic acid (2.30g, 12.75mmol), thionyl chloride (1.00ml, 13.71mmol), *N*-methyl morpholine (1.50ml, 13.64mmol) and octyl amine (2.10ml, 12.67). Hydrolysis was achieved with NaOH (1.02g, 1.24M), and acidified with

HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography [DEE (70%): hexane (30%)] to give **236** as a white solid (3.00g, 94.29%; mp: 69.1-69.8°C; R_f: 0.43 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3313.82 (NH), 3160.40 (PhOH), 2954.51 (PhH), 2926.04 (CH), 2854.74 (NCH), 1629.44 (C=O); δ_H(d_6-Acetone): 9.01 (1H, s, PhO<u>H</u>) 7.81 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.63 (1H, m, N<u>H</u>), 6.88 (2H, d, J=8.79Hz, Ph<u>H</u>) 3.38 (2H, m, NHC<u>H</u>$_2$), 1.59 (10H, m, C_5<u>H</u>$_{10}CH_3$), 0.89 (3H, t, J=6.96Hz, CH_2C<u>H</u>$_3$); δ_C(d_6-Acetone): 166.28 (C=O) 160.11, 129.05, 126.52, 114.86 (ArC) 39.57, 31.78, 29.76, 29.31, 28.86, 26.97, 22.51 (CH_2) 13.56 (CH_3); GC: t_R 16.48min; LRMS (EI): m/z 249 (M^+, 13%), 121 [M^+- NH(C_8H_{17}), 100%].

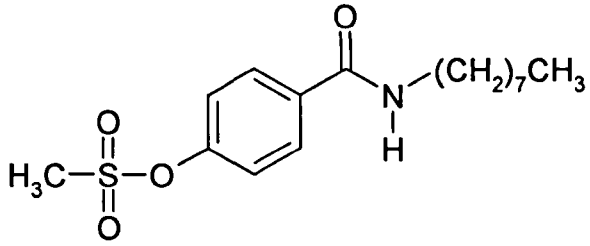
Synthesis of 4-[(octylamino)carbonyl]phenyl sulfamate (**237**)



Compound **237** was synthesised via the same method as **178** using 4-hydroxy-*N*-octyl-benzamide (0.28g, 1.13mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%)] to give **237** as an off-white solid (0.15g, 41.21%; mp: 143.2-145.1°C; R_f: 0.36 [DEE (90%): hexane (10%)].

$\nu_{(max)}$(Film)cm^{-1}: 3300.83 (NH), 2953.90 (PhH), 2919.02 (CH), 2848.30 (NCH), 1632.60 (C=O); δ_C(d_6-Acetone): 165.66 (C=O) 152.66, 133.57, 128.84, 122.09 (ArC) 31.77, 29.57, 28.99, 26.90, 22.48 (CH_2) 13.57 (CH_3); LRMS (EI): m/z 249 (M^+, 8%), 121 (M^+ - $C_8H_{18}N_2SO_2$, 100%).

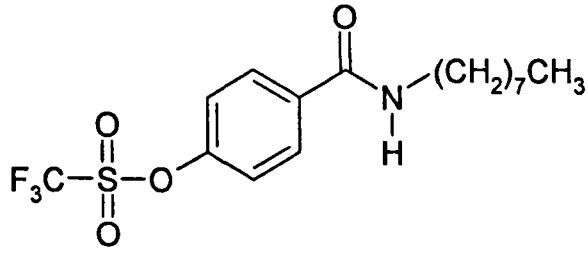
Synthesis of 4-[(octylamino)carbonyl]phenyl methanesulfonate (**238**)



Compound **238** was synthesised via the same method as **179** using 4-hydroxy-*N*-octyl-benzamide (1.09g, 4.38mmol), TEA (0.75ml, 5.38mmol) and methane sulfonyl chloride (0.5ml, 6.43mmol). The crude product was purified via column chromatography [DEE (50%): hexane (50%)] to give **238** as a white solid (0.56g, 39.30%; mp: 132.6-133.8°C; R_f: 0.24 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3305.89 (NH), 2953.35 (PhH), 2920.52 (CH), 2848.39 (NCH), 1633.27 (C=O); δ_H(d_6-Acetone): 7.98 (2H, d, J=8.97Hz, Ph$\underline{H}$), 7.85 (1H, s, N$\underline{H}$), 7.42 (2H, d, J=8.97Hz, Ph$\underline{H}$), 3.38 (2H, m, NHC$\underline{H}_2$), 3.33 (3H, s, SC$\underline{H}_3$), 1.61 (2H, m, $CH_2C\underline{H}_2CH_2$), 1.32 (10H, $C_5\underline{H}_{10}$), 0.87 (3H, t, J=6.96Hz, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 165.30 (C=O), 151.58, 134.33, 129.12, 122.08 (ArC), 39.72, 39.59 (CH_2), 37.00 (S-CH_3), 31.77, 29.56, 28.85, 26.93, 22.51 (CH_2), 13.55 (CH_3); GC: t_R 19.49min; LRMS (EI): m/z 328 (M^+, 3%), 199 (M^+ - NC_8H_{18}, 100%).

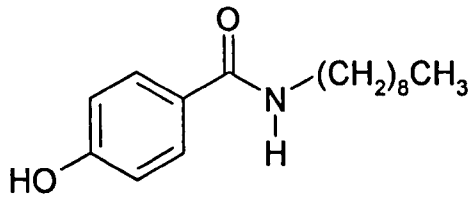
Synthesis of 4-[(octylamino)carbonyl]phenyl trifluoromethanesulfonate (**239**)



Compound **239** was synthesised using the same method as **179** using 4-hydroxy-*N*-octyl-benzamide (0.35g, 1.42mmol), TEA (0.30ml, 2.15mmol) and trifluoromethane sulfonyl chloride (0.2ml, 1.88mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **239** as a (0.35g, 64.38%; mp: 52.5-53.4°C; R_f: 0.78 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3307.50 (NH), 2956.98 (PhH), 2928.01 (CH), 2856.61 (NCH), 1643.68 (C=O); δ_H(d_6-Acetone): 8.15 (2H, d, J=8.97Hz, Ph<u>H</u>) 8.02 (1H, br.s, N<u>H</u>), 7.63 (2H, d, J=8.97Hz, Ph<u>H</u>), 3.48 (2H, m, NHC<u>H</u>$_2$), 1.69 (2H, m, $CH_2C\underline{H}_2CH_2$), 1.40 (10H, m, $C_5\underline{H}_{10}$), 0.94 (3H, m, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 164.90 (C=O), 151.24, 135.93, 129.68, 121.49 (ArC), 39.80 (NCH_2), 31.77, 29.50, 29.26, 29.21, 26.92, 22.51 (CH_2), 13.54 (CH_3); GC: t_R 13.48min; LRMS (EI): m/z 381 (M^+, 14%), 253 [M^+- $NH(C_8H_{17})$].

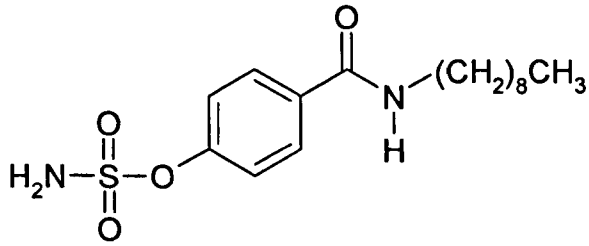
Synthesis of 4-hydroxy-*N*-nonyl-benzamide (**240**)



Compound **240** was synthesised via the same method as compound **176** using 4-acetoxy benzoic acid (2.59g, 14.37mmol), thionyl chloride (1.20ml, 16.45mmol), *N*-methyl morpholine (1.60ml, 14.55mmol) and nonyl amine (2.70ml, 14.74mmol). Hydrolysis was achieved with NaOH (1.23g, 1.28M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **240** as a white solid (3.52g, 93.26%; mp: 64.5-65.3°C; R_f: 0.47 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3307.04 (NH), 3154.04 (PhOH), 2954.84 (PhH), 2935.70 (CH), 2854.60 (NCH), 1633.82 (C=O); δ_H(d_6-Acetone): 9.02 (1H, s, PhO<u>H</u>), 7.81 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.64 (1H, m, N<u>H</u>), 6.89 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.38 (2H, m, NHC<u>H</u>$_2$), 1.60 (2H, m, $CH_2C\underline{H}_2CH_2$), 1.32 (12H, m, $C_6\underline{H}_{12}$), 0.89 (3H, t, J=6.96Hz, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 166.30 (C=O), 160.13, 129.06, 126.50, 114.87 (ArC), 39.57, 31.82, 29.75, 29.52, 29.35, 29.06, 26.96, 22.53 (CH_2), 13.57 (CH_3); GC: t_R: 17.24min; LRMS (EI): m/z 263 (M+, 13%), 121 [M^+ - $NH(C_9H_{19})$, 100%].

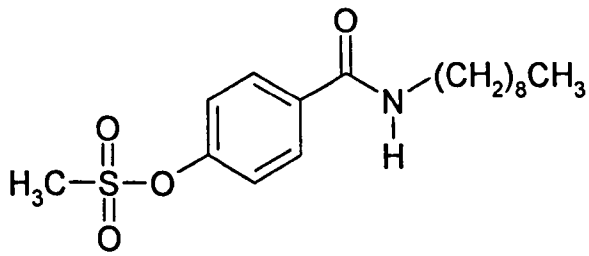
Synthesis of 4-[(nonylamino)carbonyl]phenyl sulfamate (**241**)



Compound **241** was synthesised via the same method as **178** using 4-hydroxy-*N*-nonyl-benzamide (0.35, 1.34mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%)] to give **241** as an off-white solid (0.12g, 25.54%; mp: 132.3-134.2°C; R_f: 0.38 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3415.88 (NH), 3294.79 (NH amd), 2919.56 (PhH), 2219.59 (CH), 2848.47 (NCH), 1634.39 (C=O); δ_C(d_6-Acetone): 165.49 (C=O), 152.62, 133.67, 128.08, 122.08 (ArC) 39.41, 39.57, 31.81, 29.59, 29.51, 29.32, 26.93, 22.52 (CH_2), 13.56 (CH_3); HRMS (EI): Found m/z 365.15050 (M^++Na) $C_{16}H_{26}O_4N_2SNa$, Calculated m/z 365.15055.

Synthesis of 4-[(nonylamino)carbonyl]phenyl methanesulfonate (**242**)

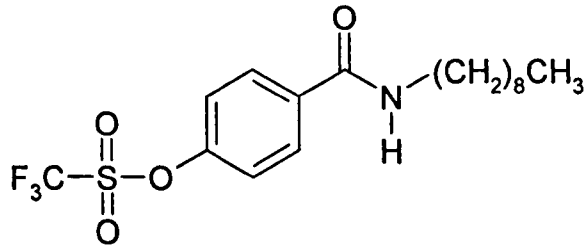


Compound **242** was synthesised via the same method as **179** using 4-hydroxy-*N*-nonyl-benzamide (0.91g, 3.49mmol), TEA (0.60ml, 4.30mmol) and methane sulfonyl chloride (0.40ml, 5.15mmol). The crude product was purified via column chromatography [DEE (50%): hexane (50%)] to give **242** as a white solid (1.06g, 89.98%; mp: 43.8-45.3°C; R_f: 0.24 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3305.89 (NH), 2953.35 (PhH), 2920.52 (CH), 2848.39 (NCH), 1633.27 (C=O); δ_H(d_6-Acetone): 7.99 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.87 (1H, s, N<u>H</u>), 7.41 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.40 (2H, m, NHC<u>H</u>$_2$), 3.32 (3H, s, SC<u>H</u>$_3$), 1.61

(2H, m, $NHCH_2C\underline{H}_2$), 1.33 (12H, m, $C_6\underline{H}_{12}CH_3$), 0.87 (3H, t, J=6.96Hz, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 165.33 (C=O), 151.58, 134.32, 129.13, 122.08 (ArC), 39.73, 39.60 (CH_2), 37.00 (SCH_3), 31.81, 29.56, 29.50, 29.31, 26.93, 22.52 (CH_2), 13.56 (CH_3); GC: t_R: 20.46min; LRMS (EI): m/z 314 (M+, 3%), 199 (M^+-NC_9H_{20}, 100%).

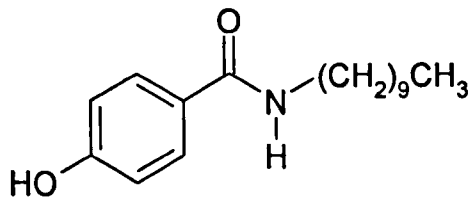
Synthesis of 4-[(nonylamino)carbonyl]phenyl trifluoromethanesulfonate (**243**)



Compound **243** was synthesised using the same method **179** as using 4-hydroxy-*N*-nonyl-benzamide (0.45g, 1.70mmol), TEA (0.40ml, 2.87mmol) and trifluoromethane sulfonyl chloride (0.20ml, 1.88mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **243** as a (0.54g, 80.96%; mp: 53.6-54.5°C; R_f: 0.82 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3345.93 (NH), 2956.08 (PhH), 2920.45 (CH), 2849.22 (NCH), 1643.68 (C=O); δ_H(d_6-Acetone): 8.15 (2H, d, J=8.97Hz, Ph$\underline{H}$), 8.01 (1H, br.s, N$\underline{H}$), 7.62 (2H, d, J=8.97Hz, Ph$\underline{H}$), 3.48 (2H, m, $NHC\underline{H}_2$), 1.69 (2H, m, $NHCH_2C\underline{H}_2$), 1.40 (12H, m, $C_6\underline{H}_{12}CH_3$), 0.93 (3H, m, $CH_2C\underline{H}_3$); δ_C(d_6-Acetone): 164.89 (C=O), 151.24, 135.94, 129.68, 121.48 (ArC), 39.80 (NCH_2), 31.81, 29.49, 29.30, 26.90, 22.51 (CH_2), 13.54 (CH_3); GC: t_R: 4.37min; LRMS (EI): m/z 395 (M^+, 13%), 253 (M^+ - NC_9H_{20}, 100%).

Synthesis of 4-hydroxy-*N*-decyl-benzamide (**244**)

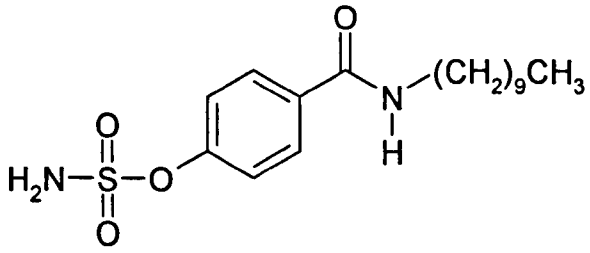


Compound **244** was synthesised via the same method as **176** using 4-acetoxy benzoic acid (3.42g, 19.00mmol), thionyl chloride (1.50ml,

20.56mmol), *N*-methyl morpholine (2.50ml, 22.74mmol) and decyl amine (4.20ml, 20.95mmol). Hydrolysis was achieved with NaOH (0.85g, 1.24M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **244** as a white solid (2.40g, 50.75%; mp: 61.2-62.9°C; R_f: 0.49 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3299.66 (NH), 3114.19 (PhOH), 2954.93 (PhH), 2925.60 (CH), 2854.39 (NCH), 1635.27 (C=O); δ_H(d_6-Acetone): 8.90 (1H, s, PhO<u>H</u>), 7.78 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.56 (1H, br.s, N<u>H</u>), 6.86 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.35 (2H, m, NHC<u>H</u>$_2$), 1.58 (2H, m, NCH$_2$C<u>H</u>$_2$CH$_2$), 1.28 (14H, m, C$_7$<u>H</u>$_{14}$), 0.87 (3H, t, J=6.96Hz, CH$_2$C<u>H</u>$_3$); δ_C(d_6-Acetone): 166.05 (C=O), 160.01, 129.00, 126.67, 114.81 (ArC), 39.49, 39.00, 31.82, 29.76, 29.67, 26.96, 26.87, 22.51, 22.10 (CH$_2$), 13.54 (CH$_3$); GC: t_R: 16.04min; LRMS (EI): m/z 277 (M^+, 12%), 199 (M^+-$NC_{10}H_{21}$, 100%).

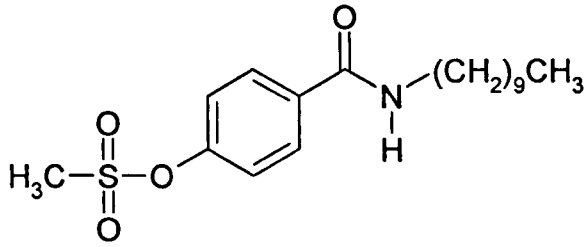
Synthesis of 4-[(decylamino)carbonyl]phenyl sulfamate (**245**)



Compound **245** was synthesised via the same method as **178** using 4-hydroxy-*N*-decyl-benzamide (0.54g, 1.94mmol). The crude product was purified via column chromatography [Ethyl acetate (60%): DEE (40%)] to give **245** as an off-white solid (0.13g, 18.73%; mp: 137.9-139.5°C; R_f: 0.39 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3333.86 (NH), 2954.34 (PhH), 2918.00 (CH), 2848.86 (NCH), 1629.49 (C=O); HRMS (EI): Found m/z 378.17123 (M^++Na) $C_{17}H_{28}N_2O_4SNa$, Calculated m/z 378.17095.

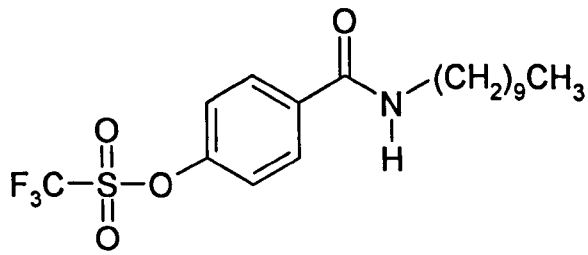
Synthesis of 4-[(decylamino)carbonyl]phenyl methanesulfonate (**246**)



Compound **246** was synthesised via the same method as **179** using 4-hydroxy-*N*-decyl-benzamide (0.36g, 1.29mmol), TEA (0.20ml, 1.43mmol) and methane sulfonyl chloride (0.15ml, 1.93mmol). The crude product was purified via column chromatography [DEE (50%): hexane (50%)] to give **246** as a white solid (0.35g, 76.11%; mp: 137.9-139.5°C; R_f: 0.089 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3343.65 (NH), 2937.70 (PhH), 2918.09 (CH), 2846.78 (NCH), 1633.34 (C=O); δ_H($CDCl_3$): 7.80 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.33 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.06 (1H, s, N<u>H</u>), 3.42 (2H, m, NHC<u>H</u>$_2$), 3.15 (3H, s, SC<u>H</u>$_3$), 1.59 (2H, m, NHCH$_2$C<u>H</u>$_2$), 1.29 (14H, m, C$_7$<u>H</u>$_{14}$CH$_3$), 0.86 (3H, t, J=6.96Hz, CH$_2$C<u>H</u>$_3$); δ_C(d_6-Acetone): 165.24 (C=O), 151.58, 134.28, 129.13, 122.08 (ArC), 39.59, 38.86 (CH_2), 37.00 (S-CH_3), 31.82, 31.55, 29.69, 29.24, 26.23, 22.52 22.20 (CH_2), 13.56 (CH_3); GC: t_R: 21.75min; LRMS (EI): m/z 356 (M+, 2%), 199 (M^+ - $NC_{10}H_{23}$, 100%).

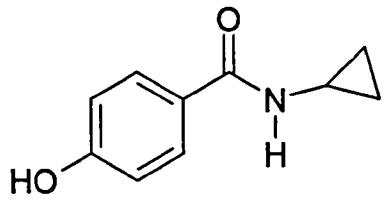
Synthesis of 4-[(decylamino)carbonyl]phenyl trifluoromethanesulfonate (**247**)



Compound **247** was synthesised using the same method as **179** using 4-hydroxy-*N*-decyl-benzamide (0.53g, 1.91mmol), TEA (0.40ml, 2.87mmol) and trifluoromethane sulfonyl chloride (0.20ml, 1.88mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **247** as a (0.44g, 56.68%; mp: 65.9-67.2°C; R_f: 0.85 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3347.05 (NH), 2956.47 (PhH), 2919.94 (CH), 2849.65 (NCH), 1630.96 (C=O); δ_H(d_6-Acetone): 8.15 (2H, d, J=8.97Hz, Ph<u>H</u>), 8.02 (1H, br.s, N<u>H</u>), 7.63 (2H, d, J=8.97Hz, Ph<u>H</u>), 3.48 (2H, m, NHC<u>H</u>$_2$), 1.69 (2H, m, NHCH$_2$C<u>H</u>$_2$), 1.40 (14H, m, C$_7$<u>H</u>$_{14}$CH$_3$), 0.93 (3H, m, CH$_2$C<u>H</u>$_3$); δ_C(d_6-Acetone): 164.89 (C=O), 151.24, 135.94, 129.67, 121.48 (ArC), 39.80 (NCH$_2$), 31.82, 29.53, 29.50, 26.30, 26.91, 22.52 (CH$_2$), 13.54 (CH$_3$); LRMS (EI): m/z 409 (M$^+$, 6%), 253 (M$^+$ - NC$_{10}$H$_{22}$, 100%).

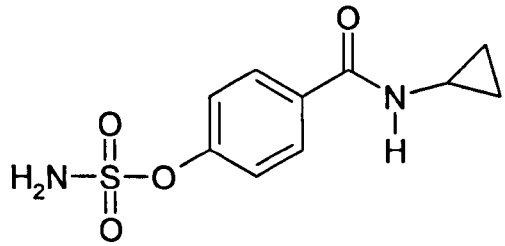
Synthesis of 4-hydroxy-*N*-cyclopropyl benzamide (**248**)



Compound **248** was synthesised via the same method as **176** using 4-acetoxy benzoic acid (2.29, 12.68mmol), thionyl chloride (2.00ml, 27.42mmol), *N*-methyl morpholine (1.50ml, 13.64mmol) and cyclopropyl amine (1.00ml, 14.26mmol). Hydrolysis was achieved with NaOH (1.10g, 1.30M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **248** as a white solid [1.14g, 50.96%; mp: 160.2-160.7°C; R_f: 0.16 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3251.73 (NH), 3092.85 (PhOH), 3009.77 (CH), 1607.60 (C=O); δ_H(d_6-Acetone): 9.14 (1H, s, PhO<u>H</u>), 7.84 (2H, d, J=8.79Hz, PhH), 7.72 (1H, br.s, N<u>H</u>), 6.94 (2H, d, J=8.97Hz, Ph<u>H</u>), 3.12 (1H, s, C<u>H</u>), 0.74 (4H, m, C$_2$<u>H</u>$_4$); δ_C(d_6-Acetone): 167.74 (C=O), 160.31, 129.00, 126.11, 114.88 (ArC), 22.98 (CH), 5.67 (CH$_2$); GC: t_R 9.78min; LRMS (EI): m/z 177 (M$^+$, 11%), 121 (M$^+$ - HNC$_3$H$_5$, 100%)

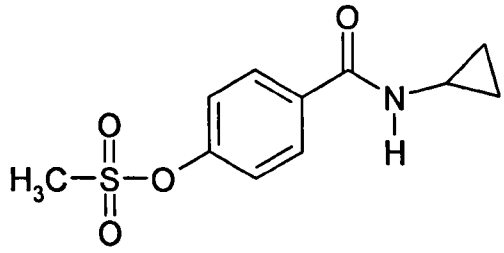
Synthesis of 4-[(cyclopropylamino)carbonyl]phenyl sulfamate (**249**)



Compound **249** was synthesised via the same method as **178** using 4-hydroxy-*N*-cyclopropy-benzamide (0.22g, 1.22mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%)] to give **249** as a white solid (0.14g, 45.46%; 165.2-166.4°C; R_f: 0.13 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3285.01 (NH), 3058.50 (CH), 3006.41 (NCH), 1624.74 (C=O); δ_C(d_6-Acetone): 166.76 (C=O), 152.69, 133.35, 128.82, 122.04 (ArC), 23.08 (CH), 5.64 (CH_2); HRMS (EI): Found m/z 279.041046 $C_{10}H_{12}O_4N_2SNa$, Calculated m/z 279.040999;

Synthesis of 4-[(cyclopropylamino)carbonyl]phenyl methanesulfonate (**250**)

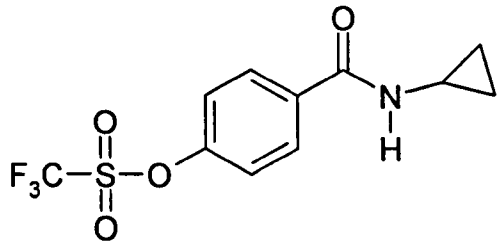


Compound **250** was synthesised via the same method as **179** using 4-hydroxy-*N*-cyclopropy-benzamide (0.21g, 1.19mmol), TEA (0.15ml, 1.04mmol) and methane sulfonyl chloride (0.16ml, 2.06mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%)] to give **250** as a white solid [0.29g, 96.46%; mp: 188.3-189.9°C; R_f: 0.12 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3284.98 (NH), 2964.64 (PhH), 3044.72 (CH), 2942.23 (NCH), 1626.58 (C=O); δ_H(d_4-Methanol): 7.78 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.29 (2H, d, J=8.60Hz, Ph<u>H</u>), 3.15 (3H, s, SC<u>H</u>$_3$), 2.74 (1H, m, NC<u>H</u>), 0.70 (2H, m, C<u>H</u>$_2$), 0.53(2H, m, C<u>H</u>$_2$); δ_C(d_4-Methanol): 151.85, 133.22, 128.97, 121.93 (ArC), 36.45

(SCH_3), 22.73(CH), 5.19 (CH_2); GC: t_R 11.90min; LRMS (EI): m/z 255 (M^+, 15%), 199 (M^+ - HNC_3H_6, 100%).

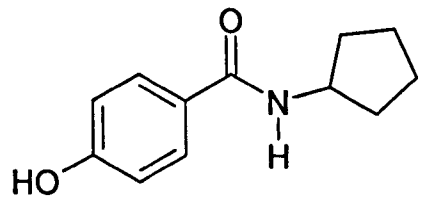
Synthesis of 4-[(cyclopropylamino)carbonyl]phenyl trifluoromethanesulfonate (**251**)



Compound **251** was synthesised via the same method as **179** using 4-hydroxy-*N*-cyclopropy-benzamide (0.29g, 1.04mmol), TEA (0.40ml, 2.87mmol) and trifluoromethane sulfonyl chloride (0.20ml, 1.88mmol). The crude product was purified via column chromatography [DEE (80%): hexane (20%)] to give **251** as a white solid [0.28g, 87.29%; mp: 95.5-96.4°C; R_f: 0.48 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3305.42 (NH), 3079.44 (PhH), 3013.03 (CH), 1637.74 (C=O); δ_H(d_6-Acetone): 7.77 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.24 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.44 (1H, s, N<u>H</u>), 2.81 (1H, m, NC<u>H</u>), 0.80 (2H, m, C<u>H</u>$_2$), 0.56 (2H, m, C<u>H</u>$_2$); δ_C($CDCl_3$): 167.32 (C=O), 151.44, 134.76, 129.26, 121.64 (ArC), 23.39 (CH), 56.83 (CH_2); GC: t_R 11.25min; LRMS (EI): m/z 309 (M^+, 13%), 253 (M^+- HNC_3H_5, 100%).

Synthesis of 4-hydroxy-*N*-cyclopentyl benzamide (**252**)

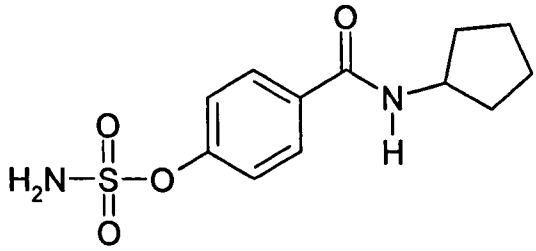


Compound **252** was synthesised via the same method as **176** using 4-acetoxy benzoic acid (2.49g, 13.84mmol), thionyl chloride (1.65ml, 22.62mmol), *N*-methyl morpholine (1.60ml, 14.55mmol) and cyclopentyl amine (1.50ml, 15.18mmol). Hydrolysis was achieved with NaOH (1.11g, 1.26M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was

purified using column chromatography (DEE, 100%) to give **252** as a white solid [2.58g, 90.88%; mp: 214.3-214.9°C; R_f: 0.30 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3276.72 (NH), 3142.16 (PhOH), 2961.89 (CH), 2868.68 (NCH), 1602.60 (C=O); δ_H(d_6-Acetone): 8.95 (1H, s, PhO<u>H</u>), 7.84 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.44 (1H, br.s, N<u>H</u>), 6.92 (2H, d, J=8.97Hz, Ph<u>H</u>), 4.42 (1H, s, C<u>H</u>), 2.05 (2H, m, C<u>H</u>$_2$), 1.80 (2H, m, C<u>H</u>$_2$), 1.65 (6H, m, C_2<u>H</u>$_4$); δ_C(d_6-Acetone): 165.92 (C=O), 159.98, 129.10, 126.70, 114.74 (ArC), 51.39 (CH), 32.59, 23.72 (CH_2); GC: t_R 14.14min; LRMS (EI): m/z 205 (M^+, 15%), 121 (M^+- NC_5H_{10}, 100%).

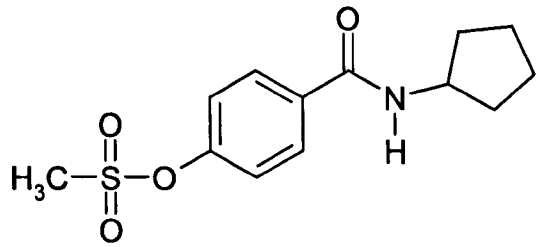
Synthesis of 4-[(cyclopentylamino)carbonyl]phenyl sulfamate (**253**)



Compound **253** was synthesised via the same method as **178** using 4-hydroxy-*N*-cyclopenty-benzamide (0.23g, 1.11mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%)] to give **253** as a white solid [0.27g, 84.11%; mp: 167.3-168.9°C; R_f: 0.24 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3176.07 (NH), 2955.02 (CH), 2866.45 (NCH), 1630.55 (C=O); δ_C(d_4-Methanol): 167.69 (C=O), 153.02, 132.87, 128.73, 121.79 (ArC), 51.82 (CH), 32.10, 23.64 (CH_2); HRMS (EI): Found m/z 307.07245 $C_{12}H_{16}O_4N_2SNa$ Calculated m/z 307.07230.

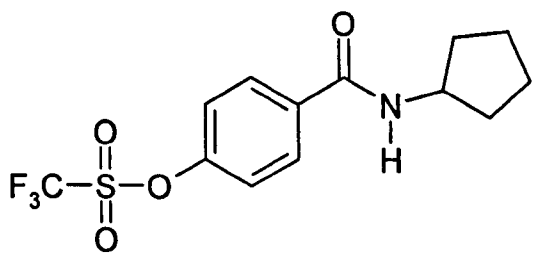
Synthesis of 4-[(cyclopentylamino)carbonyl]phenyl methanesulfonate (**254**)



Compound **254** was synthesised via the same method as **179** using 4-hydroxy-*N*-cyclopenty-benzamide (0.28g, 1.34mmol), TEA (0.25ml, 1.79mmol) and methane sulfonyl chloride (0.15ml, 1.93mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%)] to give **254** as a white solid [0.33g, 87.71%; mp: 157.5-158.6°C; R_f: 0.24 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3284.08 (NH), 3067.70 (PhH), 3033.97 (CH), 2962.97 (NCH), 1630.62 (C=O); δ_H(d_6-Acetone): 7.78 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.31 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.10 (1H, m, N<u>H</u>), 4.36 (1H, m, NC<u>H</u>), 3.14 (3H, s, SC<u>H</u>$_3$), 2.10 (2H, m, C<u>H</u>$_2$), 1.66 (4H, m, C$_2$<u>H</u>$_4$), 1.47 (2H, m, C<u>H</u>$_2$); δ_C(d_6-Acetone): 165.94 (C=O), 151.14, 134.24, 128.96, 122.17 (ArC), 51.97 (CH), 37.79 (SCH_3), 33.29, 23.89 (CH_2).

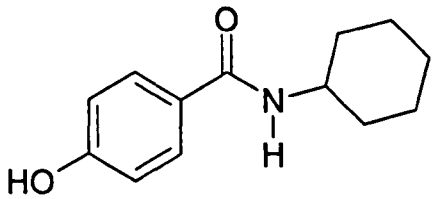
Synthesis of 4-[(cyclopentylamino)carbonyl]phenyl trifluoromethanesulfonate (**255**)



Compound **255** was synthesised via the same method as **179** using 4-hydroxy-*N*-cyclopenty-benzamide (0.37g, 1.82mmol), TEA (0.40ml, 2.87mmol) and trifluoromethane sulfonyl chloride (0.25ml, 2.35mmol). The crude product was purified via column chromatography [DEE (80%): hexane (20%)] to give **255** as a white solid [0.53g, 86.07%; mp: 116.1-117.0°C; R_f: 0.78 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3307.08 (NH), 2962.55 (CH), 2871.75 (NCH), 1634.63 (C=O); δ_H(d_6-Acetone): 7.77 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.26 (2H, d, J=8.97Hz, Ph<u>H</u>), 6.05 (1H, br.d J=6.41Hz, N<u>H</u>), 4.31 (1H, sex, J=7.14Hz, C<u>H</u>), 2.02 (2H, m, C<u>H</u>$_2$), 1.52 (6H, m, C_3<u>H</u>$_6$); δ_C($CDCl_3$): 165.53 (C=O), 151.31, 135.31, 129.21, 121.63 (ArC), 52.06 (CH), 33.27, 23.88 (CH_2); GC: t_R 13.22min; LRMS: m/z 337 (M^+, 7%), 270 (M^+- HNC_5H_9 , 100%).

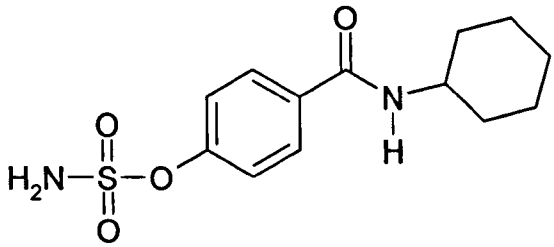
Synthesis of 4-hydroxy-*N*-cyclohexyl benzamide (**256**)



Compound **256** was synthesised via the same method as **176** using 4-acetoxy benzoic acid (2.74g, 15.23mmol), thionyl chloride (1.30ml, 17.82mmol), *N*-methyl morpholine (2.00ml, 18.19mmol) and cyclohexyl amine (2.00ml, 17.50mmol). Hydrolysis was achieved with NaOH (1.22g, 1.24M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **256** as a white solid [1.52g, 45.65%; mp: 265.2-266.7°C; R_f: 0.36 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3315.12 (NH), 2928.40 (CH), 2852.49 (NCH), 1606.95 (C=O); δ_H(d_4-Methanol): 7.58 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.76 (2H, d, J=8.79Hz, Ph<u>H</u>), 3.72 (1H, m, N<u>H</u>), 1.68 (5H, m, C<u>H</u>C_2<u>H</u>$_4$), 1.25 (6H, m, C_3<u>H</u>$_6$); δ_C(d_4-Methanol): 167.99 (C=O), 160.56, 129.10, 125.51, 114.63 (ArC), 49.09 (CH), 32.52, 25.36, 25.16 (CH_2); GC: t_R 15.03min; LRMS (EI): m/z 219 (M^+, 13%), 121 (M^+ - HNC_6H_{11}, 100%).

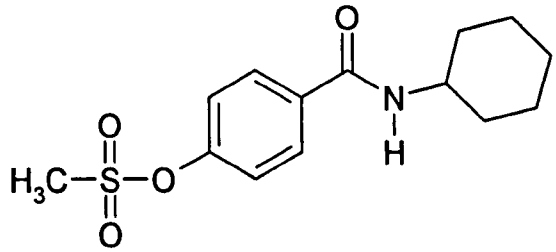
Synthesis of 4-[(cyclohexylamino)carbonyl]phenyl sulfamate (**257**)



Compound **257** was synthesised via the same method as **178** using 4-hydroxy-*N*-cyclohexy-benzamide (0.28g, 1.29mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%)] to give **257** as a white solid [0.26g, 67.12%; mp: 164.5-165.1°C; R_f: 0.31 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3308.38 (NH), 2928.71 (CH), 2853.01 (NCH), 1630.11 (C=O); δ_C(d_4-Methanol): 167.07 (C=O), 153.03, 132.95, 128.70, 121.79 (ArC), 49.37 (CH), 32.10, 27.81, 24.25 (CH_2); HRMS (EI): Found m/z 321.08832, $C_{13}H_{18}O_4N_2SNa$, Calculated 321.08795.

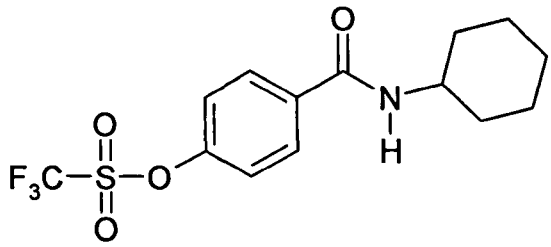
Synthesis of 4-[(cyclohexylamino)carbonyl]phenyl methanesulfonate (**258**)



Compound **258** was synthesised via the same method as **179** using 4-hydroxy-*N*-cyclohexy-benzamide (0.33g, 1.48mmol), TEA (1.25ml, 1.79mmol) and methane sulfonyl chloride (0.14ml, 1.80mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%) to give **258** as a white solid [0.34g, 76.28%; mp: 182.4-183.2°C; R_f: 0.29 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3319.67 (NH), 3038.60 (PhH), 2938.01 (CH), 2850.16 (NCH), 1632.67 (C=O); δ_H(d_6-Acetone): 7.74 (2H, d, J=8.60Hz, Ph<u>H</u>), 7.26 (2H, d, J=8.60Hz, Ph<u>H</u>), 5.97 (1H, s, N<u>H</u>), 3.89 (1H, m, NC<u>H</u>), 3.10 (3H, s, SCH_3), 2.10 (2H, m, C<u>H_2</u>); δ_C(d_6-Acetone): 165.41 (C=O), 151.13, 134.39, 128.97, 122.17 (ArC), 49.02 (CH), 37.79 (SCH_3), 33.25, 22.60, 24.99 (CH_2).

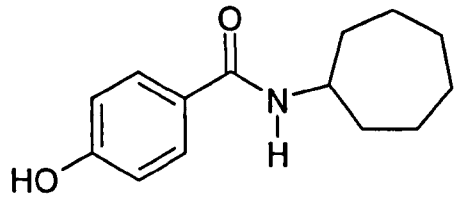
Synthesis of 4-[(cyclohexylamino)carbonyl]phenyl trifluoromethanesulfonate (**259**)



Compound **259** was synthesised via the same method as **179** using 4-hydroxy-*N*-cyclohexy-benzamide (0.30g, 1.38mmol), TEA (0.30ml, 2.15mmol) and trifluoromethane sulfonyl chloride (0.20, 1.88mmol). The crude product was purified via column chromatography [DEE (80%): hexane (20%)] to give **259** as a white solid [0.42g, 86.50%; mp: 132.8-133.6°C; R_f: 0.85 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3318.06 (NH), 2919.65 (CH), 2855.45 (NCH) 1633.10 (C=O); δ_H(d_6-Acetone): 7.86 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.35 (2H, d, J=8.97Hz, Ph<u>H</u>), 6.04 (1H, d, J=7.69Hz, N<u>H</u>), 4.16 (1H, m, NC<u>H</u>), 2.08(2H, m, C<u>H</u>$_2$), 1.62 (10H, m, C_5<u>H</u>$_{10}CH_3$); δ_C($CDCl_3$): 164.72 (C=O), 151.34, 135.51, 129.17, 121.65 (ArC), 51.33 (CH), 35.23, 28.08, 24.20 (CH_2); LRMS (EI): m/z 351 (M^+, 13%), 270 (M^+-C_6H_9, 100%).

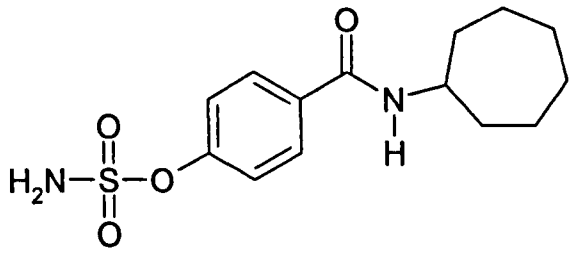
Synthesis of 4-hydroxy-*N*-cycloheptyl benzamide (**260**)



Compound **260** was synthesised via the same method as **176** using 4-acetoxy benzoic acid (2.29g, 12.68mmol), thionyl chloride (2.00ml, 27.42mmol), *N*-methyl morpholine (1.40ml, 12.73mmol) and cycloheptyl amine (1.70ml, 13.34mmol). Hydrolysis was achieved with NaOH (1.10g, 1.31M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **260** as a white solid [2.72g, 91.97%; mp: 245.3-246.2°C; R_f: 0.35 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3335.71 (NH), 2926.69 (CH), 2856.39 (NCH), 1626.87 (C=O); δ_H(d_6-Methanol): 7.58 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.69 (2H, d, J=8.60Hz, Ph<u>H</u>), 3.91 (1H, m, N<u>H</u>), 1.86 (2H, m, C<u>H</u>$_2$), 1.53 (10H, m, C_5<u>H</u>$_{10}$); δ_C(d_4-Methanol): 167.65 (C=O), 160.52, 128.91 125.59, 114.63 (ArC), 51.29 (CH), 34.57, 27.81, 24.25 (CH_2); GC: t_R 12.64min; LRMS (EI): m/z 233 (M^+, 14%), 121 (M^+ - HNC_7H_{13}, 100%).

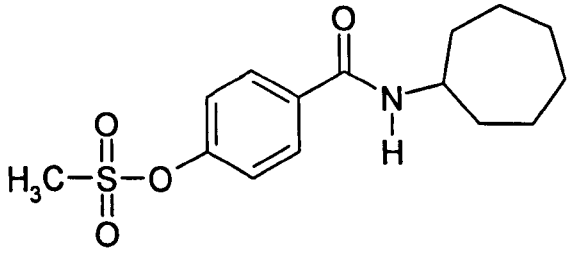
Synthesis of 4-[(cycloheptylamino)carbonyl]phenyl sulfamate (**261**)



Compound **261** was synthesised via the same method as **178** using 4-hydroxy-*N*-cyclohepty-benzamide (0.26g, 1.11mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%)] to give **261** as a white solid [0.093g, 26.69%; mp: 136.3-137.5°C; R_f: 0.35 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3021.95 (NH), 2899.63 (CH), 2844.19 (NCH), 1629.55 (C=O); δ_C(d_4-methanol): 167.66 (C=O), 160.50, 128.91, 125.59, 121.39 (ArC), 51.29 (CH), 34.57, 27.81, 24.25 (CH_2).

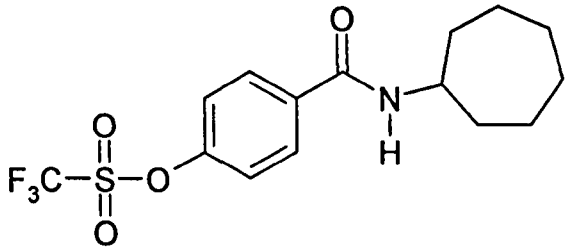
Synthesis of 4-[(cycloheptylamino)carbonyl]phenyl methanesulfonate (**262**)



Compound **262** was synthesised via the same method as **179** using 4-hydroxy-*N*-cyclohepty-benzamide (0.38g, 1.65mmol), TEA (0.30ml, 2.15mmol) and methane sulfonyl chloride (0.15ml, 1.93mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%) to give **262** as a white solid [0.38g, 73.96%; mp: 175.2-175.9°C; R_f: 0.31 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3325.49 (NH), 3035.58 (PhH), 2933.19 (CH), 2856.15 (NCH), 1630.23 (C=O); δ_H(d_6-Acetone): 7.82 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.35 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.10 (1H, m, N<u>H</u>), 4.15 (1H, m, NC<u>H</u>), 3.18 (3H, s, SC<u>H</u>$_3$), 2.04 (2H, m, C<u>H</u>$_2$), 1.60 (10H, m, C$_5$<u>H</u>$_{10}$); δ_C(d_6-Acetone): 165.14 (C=O), 151.13, 134.43, 128.90, 122.18 (ArC), 49.02 (CH), 37.79 (SCH_3), 33.22, 28.10, 24.21 (CH_2).

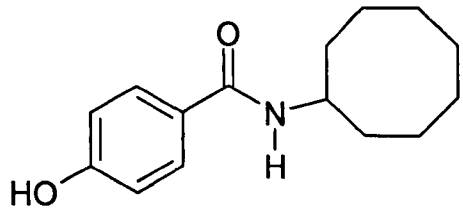
Synthesis of 4-[(cycloheptylamino)carbonyl]phenyl trifluoromethanesulfonate (**263**)



Compound **263** was synthesised via the same method as **179** using 4-hydroxy-*N*-cyclohepty-benzamide (0.36g, 1.53mmol), TEA (0.35ml, 2.51mmol) and trifluoromethane sulfonyl chloride (0.20ml, 1.88mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **263** as a white solid [0.47g, 83.04%; mp: 116.9-117.8°C; R_f: 0.89 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3352.11 (NH), 2919.00 (CH), 2855.57 (NCH), 1633.90 (C=O); δ_H(d_6-Acetone): 7.86 (2H, d, J=8.97Hz, Ph<u>H</u>), 7.35 (2H, d, J=8.97Hz, Ph<u>H</u>), 6.04 (1H, d, J=7.69Hz, N<u>H</u>), 4.16 (1H, m, NC<u>H</u>), 2.08(2H, m, C<u>H</u>$_2$), 1.62 (10H, m, C$_5$<u>H</u>$_{10}$); δ_C($CDCl_3$): 164.72 (C=O), 151.34, 135.51, 129.17, 121.65 (ArC), 51.33 (CH), 35.23, 28.08, 24.20 (CH_2); GC: t_R 12.64min; LRMS (EI): m/z 367 (M^+, 3%), 270 (M^+ - C_7H_{13}, 100%)

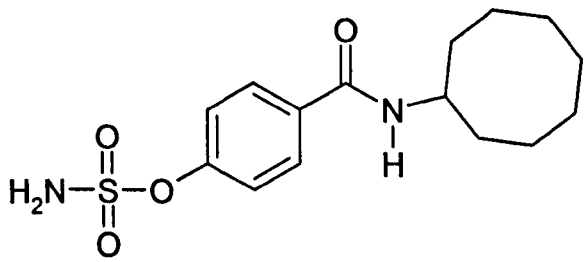
Synthesis of 4-hydroxy-*N*-cyclooctyl benzamide (**264**)



Compound **264** was synthesised via the same method as **176** using 4-acetoxy benzoic acid (2.69g, 14.94mmol), thionyl chloride (1.65ml, 22.62mmol), *N*-methyl morpholine (1.70ml, 24.58mmol) and cyclooctyl amine (2.50ml, 17.92mmol). Hydrolysis was achieved with NaOH (1.10g, 1.15M), and acidified with HCl (1M) to give a brown semi-solid. The crude product was purified using column chromatography (DEE, 100%) to give **264** as a white solid [2.61g, 70.82%; mp: 250.4-252.1°C; R_f: 0.37 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3125.08 (PhOH), 2919.43 (CH), 2850.85 (NCH), 1606.67 (C=O); δ_H(d_4-Methanol): 7.57 (2H, d, J=8.79Hz, Ph$\underline{H}$), 6.70 (2H, d, J=8.79Hz, Ph$\underline{H}$), 4.80 (1H, m, N$\underline{H}$), 1.86 (2H, m, C$\underline{H}_2$), 1.60 (12H, m, $C_6\underline{H}_{12}$); δ_C(d_4-Methanol): 167.64 (C=O), 160.51, 128.91 125.60, 114.63 (ArC), 45.95 (CH), 32.20, 26.81, 25.53, 23.91 (CH_2); GC: t_R 15.48min; LRMS (EI): m/z 247 (M^+, 14%), 121 (M^+ - HNC_7H_{13}, 100%).

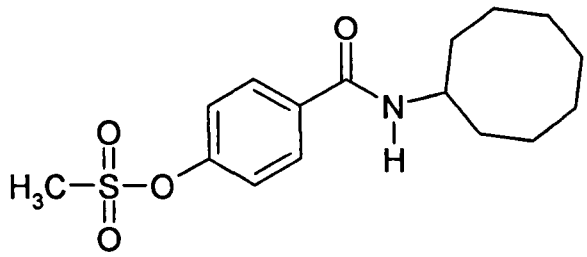
Synthesis of 4-[(cyclooctylamino)carbonyl]phenyl sulfamate (**265**)



Compound **265** was synthesised via the same method as **178** using 4-hydroxy-*N*-cyclooctyl-benzamide (0.35g, 1.40mmol). The crude product was purified via column chromatography [ethyl acetate (60%): hexane (40%)] to give **265** as a white solid [0.045g, 9.93%; R_f: 0.36 (DEE, 100%)].

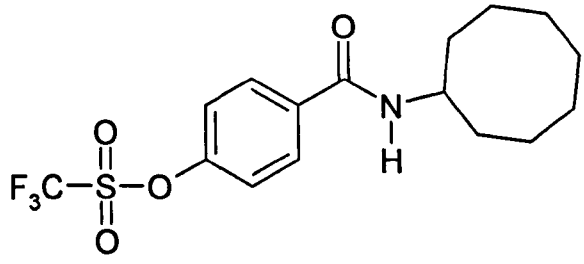
$\nu_{(max)}$(Film)cm^{-1}: 3118.04 (NH), 2914.24 (CH), 2848.80 (NCH), 1630.02 (C=O); δ_C(d_4-Methanol): 166.70 (C=O), 153.00, 133.06, 128.72, 121.79 (ArC), 60.27 (CH), 50.26, 32.10, 26.81, 25.53, 23.90, 19.61, 13.19 (CH_2).

Synthesis of 4-[(cyclooctylamino)carbonyl]phenyl methanesulfonate (**266**)



Compound **266** was synthesised via the same method as **179** using 4-hydroxy-*N*-cyclooctyl-benzamide (0.40g, 1.60mmol), TEA (0.30ml, 2.73mmol) and methane sulfonyl chloride (0.12g, 1.54mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **266** as a white solid [0.31g, 61.15%; mp: 141.9-143.1°C; R_f: 0.32 (DEE, 100%)].
$\nu_{(max)}$(Film)cm^{-1}: 3317.18 (NH), 3029.63 (PhH), 2910.03 (CH), 2843.60 (NCH), 1628.48 (C=O); δ_H(d_6-Acetone): 7.78 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.31 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.08 (1H, d, J=7.69Hz, N<u>H</u>), 4.16 (1H, m, NC<u>H</u>), 3.14 (3H, s, SC<u>H</u>$_3$), 1.59 (14H, m, C_7<u>H</u>$_{14}$); δ_C(d_6-Acetone): 165.16 (C=O), 151.13, 134.43, 128.94, 122.18 (ArC), 50.20 (CH), 37.32 (SCH_3), 33.52, 27.22, 25.53 23.81 (CH_2).

Synthesis of 4-[(cyclooctylamino)carbonyl]phenyl trifluoromethanesulfonate (**267**)



Compound **267** was synthesised via the same method as **179** using 4-hydroxy-*N*-cyclooctyl-benzamide (0.35g, 1.44mmol), TEA (0.30ml, 2.15mmol) and trifluoromethane sulfonyl chloride (0.20ml, 1.88mmol). The crude product was purified via column chromatography [DEE (60%): hexane (40%)] to give **267** as a white solid [0.38g, 71.02%; mp: 68.6-69.9°C; R_f: 0.93 (DEE, 100%)].

$\nu_{(max)}$(Film)cm^{-1}: 3292.69 (NH), 2919.33 (CH), 2856.39 (NCH) 1632.68 (C=O); δ_H(d_6-Acetone): 7.78 (2H, d, J=8.79hz, Ph<u>H</u>), 7.31 (2H, d, J=8.79Hz, Ph<u>H</u>), 6.08 (1H, d, J=7.69Hz, N<u>H</u>), 4.16 (1H, m, NC<u>H</u>), 3.14 (3H, s, SC<u>H</u>$_3$), 1.59 (14H, m, C_7<u>H</u>$_{14}$); δ_C(d_6-Acetone): 165.16 (C=O), 151.13, 134.43, 128.94, 122.18 (ArC), 50.20 (CH), 37.32 (SCH_3), 33.52, 27.22, 25.53 23.81 (CH_2); GC: t_R 12.75min; LRMS (EI): m/z 379 (M^+, 12%), 253 (M^+ - HNC_8H_{15}, 100%)

CHAPTER 3

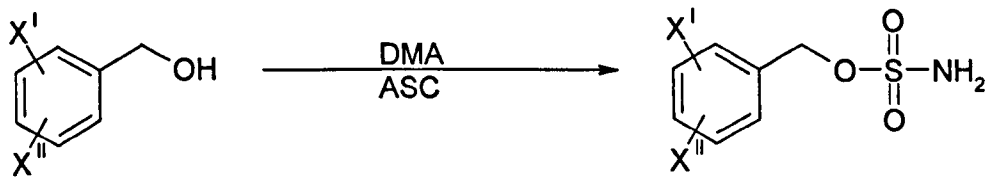
SYNTHESIS OF SULFAMATE DERIVATIVES OF ALKYL AND BENZYL ALCOHOLS

3.0 SYNTHESIS OF SULFAMATE DERIVATIVES OF ALKYL AND BENZYL ALCOHOLS

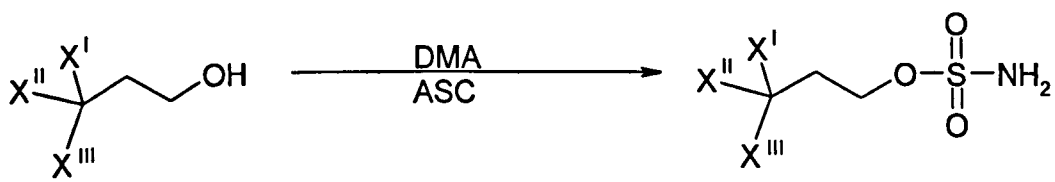
3.1 Discussion

As previously mentioned, in an effort to synthesise metabolically stable sulfamate-based inhibitors of E1STS, we considered the synthesis of a series of alkyl sulfamates containing an electron-withdrawing functionality so as to allow the active site of E1STS to undertake a nucleophilic substitution type reaction, thereby releasing the alkoxide moiety whilst maintaining the sulfamate moiety within the active site as proposed in the mechanism of Ahmed *et al.* (2002a). It should be noted that non-sulfamate derivatives have previously been reported (Ahmed *et al.*, 2002c) where it was observed that the use of methane sulfonate based compounds of alkyl alcohols resulted in extremely poor inhibitory activity, as such, these derivatives were excluded from this study and only the sulfamate derivatives were designed as potential inhibitors.

In the synthesis of the target compounds, we considered the reaction between amino sulfonyl chloride and the appropriate alcohol (Scheme 15), both benzyl alcohol and alkyl alcohol in a suitable aprotic solvent.



Scheme 15a. Synthesis of alkyl sulfamate-based compounds as potential inhibitors of E1STS (R^{I}= Alkyl groups; R^{II}= H alkyl groups)



Scheme 15b. Synthesis of alkyl sulfamate-based compounds as potential inhibitors of E1STS ($X^{I, II \& III}$= F, Cl, Br or NO_2).

In general, the reactions proceeded in good yield and without any major problems and gave the target compounds in low to moderate yield [ranging from 4% (for compound **278**) to 72% (for compound **293**)]. However, the low yield obtained for compound **278** was not due to the stability of the compound nor the lack of reaction. The main problem in attaining good to high yield was found to be due to the purification of the target compounds using column chromatography. That is, due to the close similarity between the product and the starting material, the sulfamate derivatives were found to be difficult to separate from the crude mixture and although a solvent system was found which allowed sufficient separation between the product and the starting alcohol, the problems with the separation resulted in the product being obtained in poor yield.

In the synthesis of the alcohols, the alkyl alcohols were commercially available as were a number of derivatives of benzyl alcohol. A small number of compounds (e.g. 2, 3-dichlorobenzyl alcohol compound **281**) were not, however, commercially available and in these cases it was necessary to synthesise the appropriate benzyl alcohol derivatives. This was undertaken involving the reduction of the appropriate benzyladehyde using sodium borohydride as the reducing agent. The reactioms proceeded in good yield (for example, 72% yield was obtained for the reduction of 2, 3- dichlorobenzaldehyde to 2, 3-dichlorobenzyl alcohol) and without any major problems and the compounds were purified using column chromatography.

The target compounds in this study are listed in the following tables:

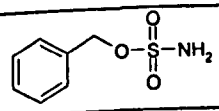
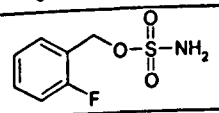
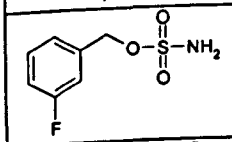
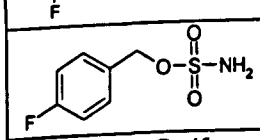
Name	Compound Number	Page
	**268**	122
	**269**	122
	**270**	123
	**271**	123

Table 21a: Sulfamate derivatives of substituted benzyl and ethyl alcohols

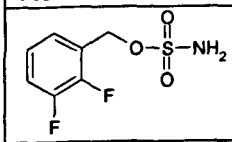
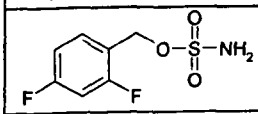
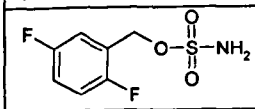
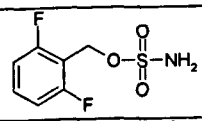
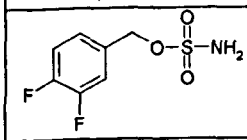
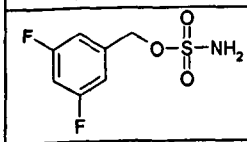
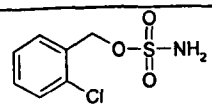
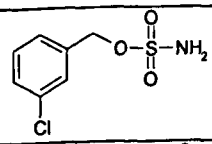
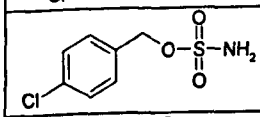
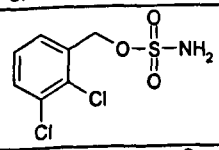
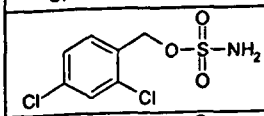
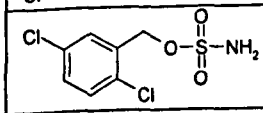
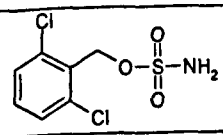
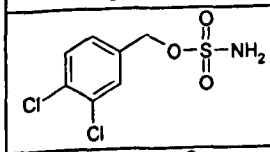
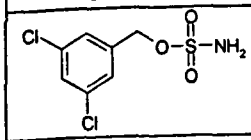
Name	Compound Number	Page
	**272**	124
	**273**	124
	**274**	125
	**275**	125
	**276**	126
	**277**	126
	**278**	127
	**279**	127
	**280**	128
	**282**	129
	**283**	129
	**284**	130
	**285**	130
	**286**	131
	**287**	131

Table 21b: Sulfamate derivatives of substituted benzyl and ethyl alcohols

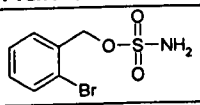
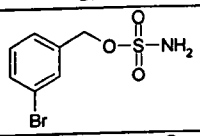
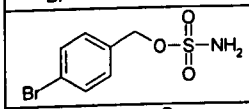
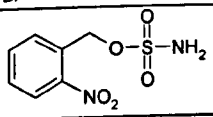
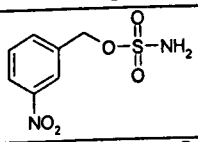
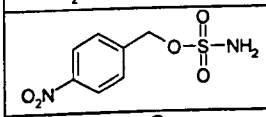
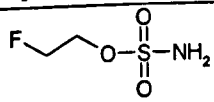
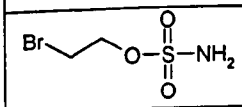
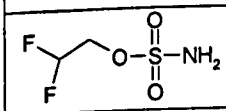
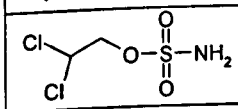
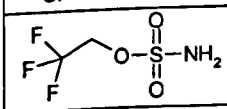
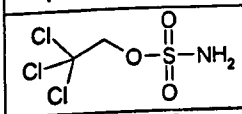
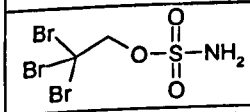
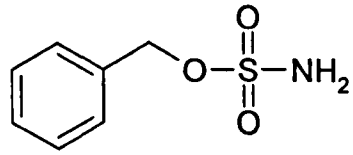
Name	Compound Number	Page
	**288**	132
	**289**	132
	**290**	133
	**291**	133
	**292**	134
	**293**	134
	**294**	135
	**295**	135
	**296**	136
	**297**	136
	**298**	137
	**299**	137
	**300**	138

Table 21c: Sulfamate derivatives of substituted benzyl and ethyl alcohols

3.2 Synthesis of sulfamate derivatives of substituted benzyl alcohol

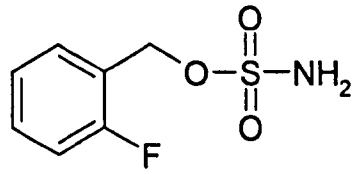
Synthesis of benzyl sulfamate (**268**)



Compound **268** was synthesised via the same method as **178** using Benzyl alcohol (0.73g, 6.73mmol). The crude product was purified via column chromatography [DEE (60%): petroleum spirit 40-60°C (40%)] to give **268** as a white solid (0.27g, 21.67%; mp: 78.9-80.3; R_f: 0.85 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3364.52 (NH_2), 3277.11 (PhH), 1339.04 (S=O); δ_H (d_6-Acetone): 7.40 (5H, m, Ph<u>H</u>), 6.80 (2H, s, N<u>H</u>$_2$), 5.15 (2H, s, C<u>H</u>$_2$); δ_C (d_6-Acetone): 135.60 (C-O), 129.42, 129.34, 129.20, 128.55 (PhH) 71.80 (CH_2); LRMS (EI): m/z 187 (M^+, 17%), 91 (M^+- H_2O_3SN, 100%).

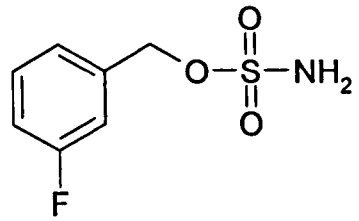
Synthesis of 2-fluorobenzyl sulfamate (**269**)



Compound **269** was synthesised via the same method as **178** using 2-fluorobenzyl alcohol (1.10g, 8.74mmol). The crude product was purified via column chromatography [DEE (50%): hexane (50%)] to give **269** as a white solid (0.69g, 13.47%; mp: 93.8-94.3°C; R_f: 0.45 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3367.6 (NH_2), 3267.8 (PhH), 1191.0 (S=O); δ_H($CDCl_3$): 7.25 (4H, m, Ph<u>H</u>), 5.32 (2H, s, C<u>H</u>$_2$), 4.85 (2H, s, N<u>H</u>$_2$); δ_C(d_6-Acetone): 161.06 (d, $^1J_{CF}$=247.52Hz, ArC, <u>C</u>F), 131.22 (d, $^3J_{CF}$=8.46Hz, ArC, FCCH<u>C</u>H), 124.61 (d, $^4J_{CF}$=3.07Hz, ArC, FCCHCHC<u>H</u>), 122.08 (d, $^6J_{CF}$=14.61Hz, ArC, FC<u>C</u>CH$_2$), 66.12 (d, $^7J_{CF}$=4.61Hz, CH_2); Elemental analysis: Found C41.06%, H3.82%, N6.73%, $C_7H_8FNO_3S$, Calculated C40.97%, H3.93%, N6.83%.

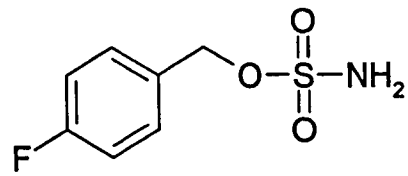
Synthesis of 3-fluorobenzyl sulfamate (**270**)



Compound **270** was synthesised via the same method as **178**, using 3-fluorobenzyl alcohol (1.18g, 9.36mmol). The crude product was purified via column chromatography [DEE (50%): hexane (50%)] to give **270** as a white solid (0.69g, 35.94%; mp: 99.1-100.4°C; R_f: 0.43 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3352.7 (NH_2), 3274.0 (PhH), 1354.9 (S=O); δ_H($CDCl_3$): 7.19 (3H, m, Ph$\underline{H}$), 5.09 (2H, s, C$\underline{H}_2$), 4.77 (2H, s, N$\underline{H}_2$); δ_C(d_6-Acetone): 162.72 (d, $^1J_{CF}$=245.99Hz, ArC, $\underline{C}$F), 138.72 (d, $^5J_{CF}$=6.62Hz, ArC, $\underline{C}CH_2$), 129.99 (d, $^3J_{CF}$=8.46Hz, ArC, FCCH$\underline{C}$H), 123.21 (d, $^4J_{CF}$=2.31Hz, ArC, FCCHCH$\underline{C}$H), 129.99 (d, $^3J_{CF}$=8.46Hz, ArC, FCCH$\underline{C}$H), 114.90 (d, $^2J_{CF}$=22.21Hz, ArC, FC$\underline{C}$H), 65.83 (CH_2).

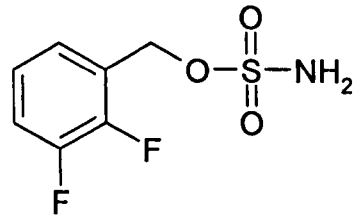
Synthesis of 4-fluorobenzyl sulfamate (**271**)



Compound **271** was synthesised via the same method as **178**, using 4-fluorobenzyl alcohol (1.30g, 10.33mmol). The crude product was purified via column chromatography [DEE (50%): hexane (50%)] to give **271** as a white solid (0.49g, 23.16%; mp: 94.8-96.1°C; R_f: 0.33 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3414.16 (NH_2), 3268.19 (PhH), 1227.58 (S=O); δ_H($CDCl_3$): 7.32 (2H, m, Ph$\underline{H}$), 7.02 (2H, m, Ph$\underline{H}$), 5.04 (2H, s, C$\underline{H}_2$), 4.93 (2H, s, N$\underline{H}_2$); δ_C(d_6-Acetone): 162.57 (d, $^1J_{CF}$=246.75Hz, ArC, $\underline{C}$F), 132.01 (d, $^4J_{CF}$=3.07Hz, ArC, $\underline{C}CH_2$), 130.08 (d, $^3J_{CF}$=8,46Hz, ArC, C$\underline{H}CCH_2$), 115.40 (d, $^2J_{CF}$=21.52Hz, ArC, FC$\underline{C}$H), 66.12 (CH_2).

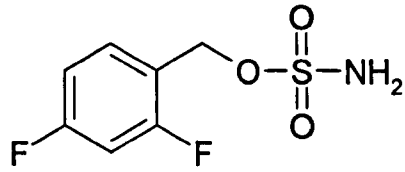
Synthesis of 2, 3-difluorobenzyl sulfamate (**272**)



Compound **272** was synthesised via the same method as **178**, using 2,3-difluorobenzyl alcohol (0.97g, 6.70mmol). The crude product was purified via column chromatography [DEE (50%): hexane (50%)] to give **272** as a white solid (0.11g, 7.43%; mp: 88.6-90.1°C; R_f: 0.40 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3361.36 (NH_2), 3263.53 (PhH), 1349.36 (S=O); δ_H($CDCl_3$): 7.12 (3H, m, Ph<u>H</u>), 5.22 (2H, s, C<u>H</u>$_2$), 4.83 (2H, br.s, N<u>H</u>$_2$); δ_C(d_6-Acetone): 125.58 (d, $^5J_{CF}$=5.38Hz, ArC, <u>C</u>HCCH$_2$), 124.52 (dd, $^4J_{CFa}$=6.92Hz, $^4J_{CFb}$=4.61Hz, ArC, CH<u>C</u>HCH), 123.15 (d, $^3J_{CF}$=11.53Hz, ArC, CFCF<u>C</u>H), 118.55 (d, $^6J_{CF}$=16.91Hz, ArC, <u>C</u>CH$_2$), 65.73 (d, $^7J_{CF}$=3.84Hz, <u>C</u>H$_2$).

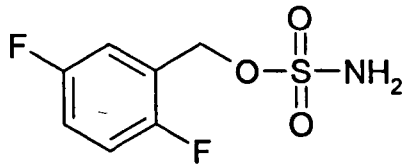
Synthesis of 2, 4-difluorobenzyl sulfamate (**273**)



Compound **273** was synthesised via the same method as **178** using 2,4-difluorobenzyl alcohol (0.94g, 6.52mmol). The crude product was purified via column chromatography [DEE (50%): hexane (50%)] to give **273** a white solid (0.28g, 19.23%; mp: 133.5-134.3°C; R_f: 0.46 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3356.6 (NH_2), 3260.0 (Ar), 1160.4 (S=O), δ_H(d_6-Acetone): 7.53 (1H, m, Ph<u>H</u>), 7.09 (2H, m, Ph<u>H</u>), 6.89 (2H, s, N<u>H</u>$_2$), 5.26 (2H, m, C<u>H</u>$_2$); δ_C(d_6-Acetone): 161.84 (dd, $^1J_{CFa}$=243.68Hz, $^1J_{CFb}$=6.92Hz, ArC, <u>C</u>F), 132.32 (t, $^6J_{CF}$=9.99Hz, ArC, <u>C</u>CH$_2$), 111.64 (dd, $^4J_{CFa}$=25.37Hz, $^4J_{CFb}$=6.15Hz, ArC, CF<u>C</u>HCH), 110.85 (t, $^2J_{CF}$=19.22, ArC, CF<u>C</u>HCF); Elemantal analysis: Found C37.71%, H3.25%, N6.28%, $C_7H_8FNO_3S$, Calculated: C37.67%, H3.16%, N6.28%, LRMS (EI): m/z 223 (M^+, 3%), 127 (M^+- H_2O_3SN, 100%).

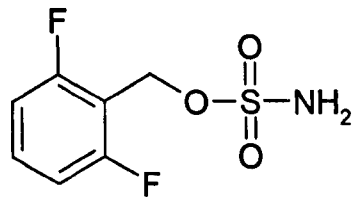
Synthesis of 2,5-difluorobenzyl sulfamate (**274**)



Compound **274** was synthesised via the same method as **178** using 2,5-difluorobenzyl alcohol (1.00g, 6.96mmol). The crude product was purified via column chromatography [DEE (50%): hexane (50%)] to give **274** a white solid (0.47g, 30.32%; mp: 101.1-102.8°C; R_f: 0.50 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3382.87 (NH_2), 3280.44 (PhH), 1168.47 (S=O); δ_H(d_6-Acetone): 7.34 (3H, m, Ph<u>H</u>), 7.00 (2H, s, N<u>H</u>$_2$), 5.30 (2H, br.s, C<u>H</u>$_2$); δ_C(d_6-Acetone): 158.63 (dd, $^1J_{CFa}$=243.68Hz, $^1J_{CFb}$=2.31Hz, ArC, <u>C</u>FC), 156.89 (dd, $^4J_{CFa}$=245.22Hz, $^4J_{CFb}$=2.31Hz, ArC, CH<u>C</u>FCH), 124.04 (dd, $^2J_{CFa}$=16.91Hz, $^2J_{CFb}$=8.46Hz, ArC, CF<u>C</u>H), 117.35 (d, $^5J_{CF}$=8.46Hz, ArC, <u>C</u>FCH), 117.11 (m, ArC, <u>C</u>HC), 116.88 (m, ArC, CF<u>C</u>HCH) ; LRMS (EI): m/z 223 (M^+, 7%), 91 (M^+-H_2O_3SN, 100%).

Synthesis of 2, 6-difluorobenzyl sulfamate (**275**)

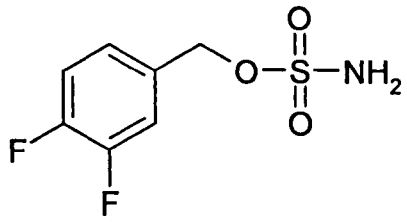


Compound **275** was synthesised via the same method as **178**, using 2,6-difluorobenzyl alcohol (1.00g, 6.92mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give **275** a white solid (0.14g, 8.80%; mp: 87.3-88.5°C; R_f: 0.44 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3358.47 (NH_2), 3260.55 (PhH), 1166.10 (S=O); δ_H($CDCl_3$): 7.41 (1H, m, Ph<u>H</u>), 6.87 (2H, m, Ph<u>H</u>), 5.21 (2H, s, C<u>H</u>$_2$), 4.83 (2H, br.s, N<u>H</u>$_2$); δ_C(d_6-Acetone): 111.82 (dd, $^2J_{CFa}$=21.52Hz, $^2J_{CFb}$=3.84Hz, ArC, CF<u>C</u>HCH), 104.30 (t, $^3J_{CF}$=25.37Hz, ArC, CF<u>C</u>), 65.93 (d, $^5J_{CF}$=3.07, CH_2); Elemental analysis: Found

C37.46%, H3.17%, N6.26%, $C_7H_8FNO_3S$, Calculated: C37.67%, H3.16%, N6.28%.

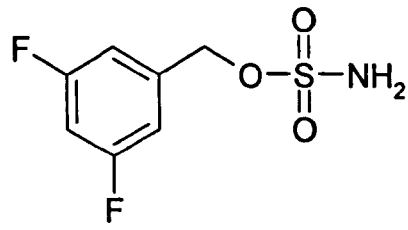
Synthesis of 3,4-difluorobenzyl sulfamate (**276**)



Compound **276** was synthesised via the same method as **178** using 3,4-difluorobenzyl alcohol (1.04g, 7.23mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give **276** a white solid (0.31g, 19.12%; mp: 70.6-71.5°C; R_f: 0.38 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3374.23 (NH_2), 3286.07 (PhH), 1289.30 (S=O); δ_H($CDCl_3$): 7.18 (3H, m, Ph<u>H</u>), 5.13 (2H, s, C<u>H</u>$_2$), 5.01 (2H, br.s, N<u>H</u>$_2$); δ_C(d_6-Acetone): 150.24 (dd, $^1J_{CFa}$=245.99Hz, $^1J_{CFb}$=12.30Hz, ArC,CCH<u>C</u>F), 150.04 (dd, $^2J_{CFa}$=244.45Hz, $^2J_{CFb}$=10.76Hz, ArC, CF<u>C</u>FCH), 132.74 (dd, $^4J_{CFa}$=5.38Hz, $^4J_{CFb}$=3.84Hz, ArC, CF<u>C</u>HCH), 125.25 (dd, $^4J_{CFa}$=6.92Hz, $^4J_{CFb}$=3.07Hz, ArC, <u>C</u>HCCH$_2$); LRMS (EI): m/z 223 (M^+, 13%), 91 (M^+- H_2O_3SN, 100%).

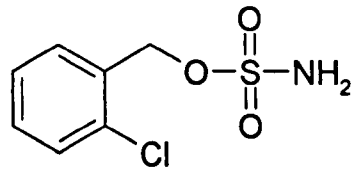
Synthesis of 3, 5-difluorobenzyl sulfamate (**277**)



Compound **277** was synthesised via the same method as **178** using 3,5-difluorobenzyl alcohol (1.03g, 7.13mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give **277** a white solid (0.84g, 52.75%; mp: 102.8-104.2°C; R_f: 0.48 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3369.12 (NH_2), 3266.29 (PhH), 1355.39 (S=O); δ_H($CDCl_3$):7.91 (1H, d, J=8.86Hz Ph<u>H</u>), 6.81 (2H, m, Ph<u>H</u>), 5.10(2H, s, C<u>H</u>$_2$), 4.77 (2H, br.s, N<u>H</u>$_2$); δ_C(d_6-Acetone): 150.24 (dd, $^1J_{CFa}$=247.52Hz, $^1J_{CFb}$=13.84Hz, ArC, <u>C</u>F), 139.67 (t, $^4J_{CFa}$=8.46Hz, ArC, <u>C</u>H), 110.85 (dd, $^3J_{CFa}$=19.99Hz, $^3J_{CFb}$=6.92Hz, ArC, C<u>H</u>CCH$_2$), 103.59 (t, $^2J_{CF}$=24.60Hz, ArC, CF<u>C</u>HCF), 69.92 (CH_2)

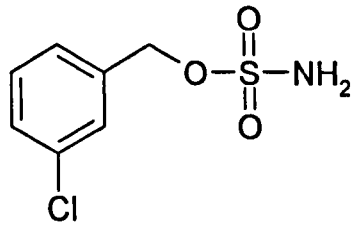
Synthesis of 2-chlorobenzyl sulfamate (**278**)



Compound **278** was synthesised via the same method as **178** using 2-chlorobenzyl alcohol (1.02g, 7.18mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to to give **278** a white solid (0.062g, 3.88%; mp: 106.5-107.5°C; R_f: 0.45 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3347.6 (NH_2), 3259.0 (PhH), 3112.8 (CH_2), 1181.6 (S=O), δ_H($CDCl_3$): 7.29 (4H, m, Ph<u>H</u>), 5.31 (2H, s, C<u>H</u>$_2$), 4.77 (2H, br.s, N<u>H</u>$_2$); δ_C(d_6-Acetone): 134.11, 133.38, 131.28, 131.19, 130.32, 128.18 (Ar), 68.86 (CH_2); Elemental analysis: Found: C37.92%, H3.63%, N6.29%, $C_7H_8ClNO_3S$, Calculated: C37.93%, H3.64%, N6.32%.

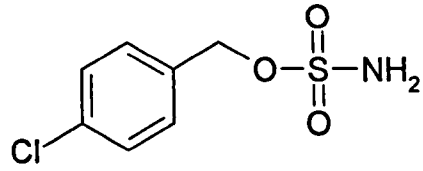
Synthesis of 3- chlorobenzyl sulfamate (**279**)



Compound **279** was synthesised via the same method as **178** using 3-chlorobenzyl alcohol (1.16g, 8.15mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give **279** a white solid (0.58g, 32.28%; mp: 82.9-83.9°C; R_f: 0.40 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3371.20 (NH_2), 3283.40 (PhH), 1138.80 (S=O); δ_H($CDCl_3$): 7.36 (1H, m, Ph<u>H</u>), 7.10 (2H, m, Ph<u>H</u>), 5.18 (2H, s, C<u>H</u>$_2$), 4.87 (2H, s, N<u>H</u>$_2$); δ_C($CDCl_3$):130.43, 124.01, 116.30, 116.10, 115.52, 115.30 (Ar), 71.71 (CH_2); LRMS (EI): m/z 223 (M^++1, 1%), 109 (M^+- H_2O_2SNCl, 100%).

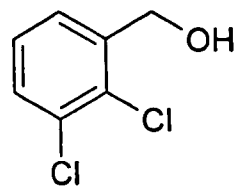
Synthesis of 4- chlorobenzyl sulfamate (**280**)



Compound **280** was synthesised via the same method as **178** using 4-chlorobenzyl alcohol (1.07g, 7.50mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give **280** a white solid (0.54g, 32.29%; mp: 105.5-106.3°C; R_f: 0.46 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3371.0 (NH_2), 3274.8 (CH_2), 1173.7 (S=O); δ_H($CDCl_3$): 7.32 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.28 (2H, d, J=8.79Hz, Ph<u>H</u>), 5.11 (2H, s, C<u>H</u>$_2$), 4.62 (2H, s, N<u>H</u>$_2$); δ_C(d_6-Acetone): 134.76, 134.62, 130.85, 129.39 (Ar), 70.86 (CH_2); Found: C38.13%, H3.56%, N6.28%, $C_7H_8ClNO_3S$, Calculated: C37.93%, H3.64%, N6.32%.

Synthesis of 2, 3-dichlorobenzyl Alcohol (**281**)

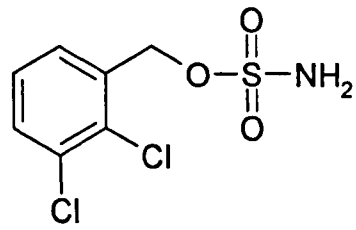


2, 3-Dichlorobenzaldehyde (3.21g, 18.36mmol) was dissolved in anhydrous THF and sodium borohydride (1.84g, 22.17mmol) was added to the mixture. This mixture was allowed to stir for 30hr at rt. The reaction mixture was poured onto ice and was acidified to pH1. The compound was extracted into DCM and the solvent removed under reduced pressure to give the crude product. The crude product was purified via column chromatography [DEE (70%): petroleum spirit

40-60°C (30%)] to give **281** as a white solid (2.35g, 72.15%; mp: 189.6-190.4°C; R_f: 0.72 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3320.00 (OH), 3220.50 (PhH) 1179.54 (S=O); δ_H(d_6-acetone): 7.61 (1H, m, Ph<u>H</u>), 7.47 (1H, m, Ph<u>H</u>), 7.36 (1H, t, J=7.68Hz, Ph<u>H</u>), 4.73 (2H, d, J=5.31Hz, C<u>H</u>$_2$), 4.62 (1H, t, J=5.31Hz; C<u>H</u>$_2$); δ_C(d_6-Acetone): 142.49, 131.99, 129.34, 128.66, 127,84, 126.29 (ArC), 61.48 (CH_2). GC: t_R 4.76min; LRMS (EI): m/z 180 (M^+, 8%), 141 (M^+ - ^{37}Cl- 2)

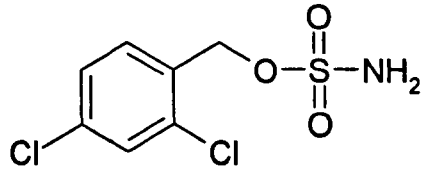
Synthesis of 2, 3- dichlorobenzyl sulfamate (**282**)



282 was synthesised via the same method as **178** using **281** (0.92g, 5.21mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to the purified product **282** give a white solid (0.36g, 26.71%; mp: 91.4-92.4°C; R_f: 0.56 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3210.93 (PhH), 1179.26 (S=O); δ_H($CDCl_3$): 7.44 (2H, m, Ph<u>H</u>), 7.24 (1H, m, Ph<u>H</u>), 5.31 (2H, s, C<u>H</u>$_2$), 4.88 (2H, br.s, N<u>H</u>$_2$); δ_C($CDCl_3$): 133.60, 131.88, 131.08, 128.13, 127.62 (Ar), 69.77 (CH_2).

Synthesis of 2, 4- dichlorobenzyl sulfamate (**283**)

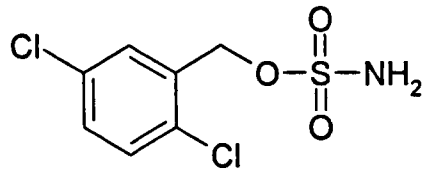


Compound **283** was synthesised via the same method as **178** using 2,4-dichlorobenzyl alcohol (1.07g, 6.03mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give

283 a white solid (0.40g, 25.67%; mp: 124.1-125.5°C; R_f: 0.48 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3348.26 (NH_2), 3255.68 (PhH), 1162.89 (S=O); δ_H(d_6-acetone): 7.58 (1H, d, J=8.4Hz, Ph<u>H</u>), 7.53 (1H, d, 2.2Hz, Ph<u>H</u>), 7.42 (1H, dd, J_{ab}=8.4Hz, J_{ax}=2.2Hz, Ph<u>H</u>), 6.9 (2H, s, N<u>H</u>$_2$), 5.2 (2H, s, C<u>H</u>$_2$); δ_C(d_6-Acetone): 135.63, 134.99, 132.41, 129.88, 128.35 (Ar), 68.18 (CH_2); Found: C32.87%, H2.76%, N5.52%, $C_7H_7Cl_2NO_3S$ Calculated: C32.83%, H2.75%, N5.47%.

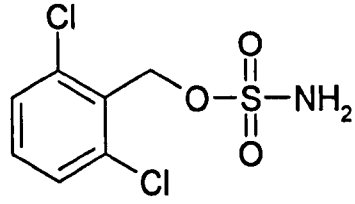
Synthesis of 2, 5- dichlorobenzyl sulfamate (**284**)



Compound **284** was synthesised via the same method as **178** using 2,5-dichlorobenzyl alcohol (0.79g, 4.45mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give **284** as a white solid (0.33g, 28.54%; mp: 98.1-99.9°C; R_f: 0.53 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3411.0 (NH_2), 3298.8 (PhH), 1165.80 (S=O); δ_H(d_6-Acetone): 7.68 (1H, d, J=2.56Hz, Ph<u>H</u>), 7.59 (1H, d, J=8.60Hz, Ph<u>H</u>), 7.53 (1H, dd, J_{ab}=8.60Hz, J_{ax}=2.56Hz, Ph<u>H</u>), 7.06 (2H, br.s, N<u>H</u>$_2$), 5.33 (2H, s, C<u>H</u>$_2$); δ_H(d_6-Acetone): 134.83, 132.68, 131.57, 131.08, 130.11, 129.75 (ArC), 67.29 (CH_2); LRMS (EI): m/z 256 (M^+, 17%), 141 (M^+- H_2O_2SNCl, 100%).

Synthesis of 2, 6- dichlorobenzyl sulfamate (**285**)

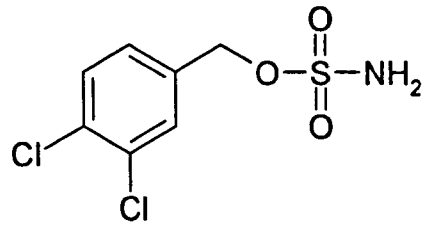


Compound **285** was synthesised via the same method as **178** using 2,6-dichlorobenzyl alcohol (0.99g, 5.61mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give

285 a white solid (0.41g, 28.08%; mp: 172.3-174.9°C; R_f: 0.44 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3362.3 (NH2), 3277.1 (PhH), 1182.5 (S=O); δ_H(d_6-Acetone): 7.54 (3H, m, Ph<u>H</u>), 7.01 (2H, br.s, N<u>H</u>$_2$), 5.52 (2H, s, C<u>H</u>$_2$); δ_C(d_6-Acetone): 137.62, 132.59, 130.65, 129.48 (Ar), 66.33 (CH_2); Elemental analysis: Found: C33.03%, H2.83% N5.41%, $C_7H_7Cl_2NO_3S$ Calculated: C32.83%, H2.75%, N5.47%.

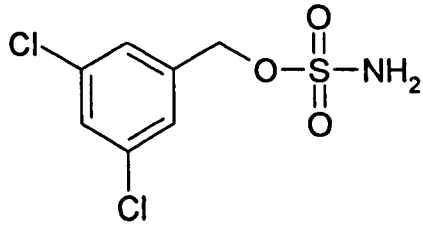
Synthesis of 3, 4- dichlorobenzyl sulfamate (**286**)



Compound **286** was synthesised via the same method as **178** using 3,4-dichlorobenzyl alcohol (1.08g, 6.11mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give **286** as a white solid (0.49g, 31.33%; mp: 106.6-107.2°C; R_f: 0.40 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3384.70 (NH_2), 3287.80 (PhH), 1185.20 (S=O); δ_H($CDCl_3$): 7.47 (2H, m, Ph<u>H</u>), 7.24 (1H, m, Ph<u>H</u>), 5.13 (2H, s, C<u>H</u>$_2$), 4.78 (2H, br.s, N<u>H</u>$_2$); δ_C($CDCl_3$): 130.86, 130.38, 127.63 (Ar), 70.91 (CH_2); LRMS (EI): m/z 256 (M^+, 17%), 159 (M^+- H_2O_3SN, 100%).

Synthesis of 3, 5-dichlorobenzyl sulfamate (**287**)

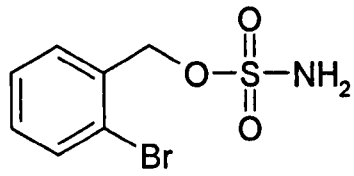


Compound **287** was synthesised via the same method as **178** using 3,5-dichlorobenzyl alcohol (1.03g, 5.84mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give

287 as a white solid (0.19, 12.77%; mp: 91.3-92.1°C; R_f: 0.42 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3430.80 (NH_2), 3370.20 (PhH), 1353.40 (S=O); δ_H(d_6-Acetone): 7.39 (1H, m, Ph<u>H</u>), 7.32 (2H, m, Ph<u>H</u>), 5.10 (2H, br.s, N<u>H</u>$_2$), 4.97 (2H, s, C<u>H</u>$_2$); δ_C(d_6-Acetone): 142.09, 134.67,128.24, 127.38, 126.56, 126.01 (ArC), 68.99 (CH_2); LRMS (EI): m/z 256 (M^+, 7%), 159 (M^+- H_2O_2SN, 100%).

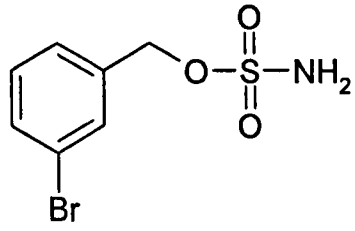
Synthesis of 2-bromobenzyl sulfamate (**288**)



Compound **288** was synthesised via the same method as **178** using 2-bromobenzyl alcohol (1.07g, 5.74mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give **288** as a white solid (0.80g, 52.33%; mp: 122.7-123.3°C; R_f: 0.47 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3347.1 (NH_2), 3256.7 (PhH), 3116.2 (CH_2), 1183.3 (S=O); δ_H(d_6-Acetone): 7.59 (4H, m, Ph<u>H</u>), 7.00 (2H, br.s, N<u>H</u>$_2$), 5.32 (2H, s, C<u>H</u>$_2$); δ_C(d_6-Acetone): 134.88, 133.54, 131.27, 131.12, 128.69, 123.64 (Ar), 70.97 (CH_2); Elemental analysis: Found: C31.72% H3.04% N5.19%; $C_7H_7BrNO_3S$, Calculated: C31.59% H3.03% N5.26%.

Synthesis of 3- bromobenzyl sulfamate (**289**)

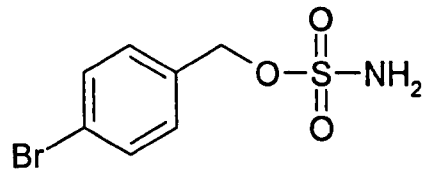


Compound **289** was synthesised via the same method as **178** using 3-bromobenzyl alcohol (1.36g, 7.27mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give

289 as a white solid (0.90g, 46.51%; mp: 106.9-107.7°C; R_f: 0.43 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3351.50 (NH_2), 3273.50 (PhH), 1354.90 (S=O); δ_H($CDCl_3$): 7.52 (1H, m, Ph<u>H</u>), 7.25 (2H, m, Ph<u>H</u>), 5.15 (2H, s, C<u>H</u>$_2$), 4.80 (2H, br.s, N<u>H</u>$_2$); δ_C($CDCl_3$): 135.56, 132.33, 131.47, 130.38, 127.03, 122.79 (Ar), 71.57 (CH_2); LRMS (EI): m/z 267 (M^+, 17%), 169 (M^+- H_2O_3SN, 100%).

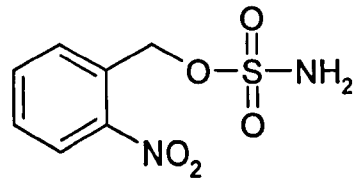
Synthesis of 4- bromobenzyl sulfamate (**290**)



Compound **290** was synthesised via the same method as **178** using 4-bromobenzyl alcohol (1.03g, 5.52mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give **290** as a white solid (0.48g, 32.46%; mp: 227.3-229.5°C; R_f: 0.39 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3365.2 (NH_2), 3283.0 (PhH); δ_H($CDCl_3$): 7.56 (2H, d, J=8.6Hz, Ph<u>H</u>), 7.39 (2H, d, J=8.6Hz, Ph<u>H</u>), 6.83 (2H, s, C<u>H</u>$_2$), 5.14 (2H, s, N<u>H</u>$_2$), δ_C(d_6-Acetone): 135.15, 132.42, 131.14, 122.94 (Ar), 70.87 (CH_2), Found: C31.79%, H3.04%, N5.26%, $C_7H_8BrNO_3S$, Calculated: C31.59%, H3.03%, N5.26%.

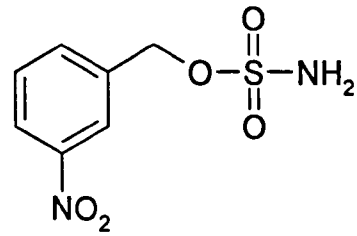
Synthesis of 2-nitrobenzyl sulfamate (**291**)



Compound **291** was synthesised via the same method as **178** using 2-nitrobenzyl alcohol (1.20g, 7.87mmol). The crude product was purified via column chromatography [DEE (50%): petroleum spirit 40-60°C (50%)] to give **291** as a white solid (0.51g, 27.72%; mp: 98.9-100.7°C; R_f: 0.18 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3348.6 (NH_2), 3252.5 (PhH), 1514.8, 1345.6 (NO_2), 1163.5 (S=O); δ_H(d_6-Acetone): 8.10 (1H, d, J=8Hz, Ph<u>H</u>), 7.75 (2H, m, Ph<u>H</u>), 7.60 (1H, m, Ph<u>H</u>), 7.07 (2H, s, N<u>H</u>$_2$), 5.55 (2H, s, C<u>H</u>$_2$); δ_C(d_6-Acetone): 148.10, 134.93, 131.89, 130.11, 129.75, 125.66 (Ar), 68.11 (CH_2), Found: C36.25%, H3.52%, N12.08%, $C_7H_8N_2O_5S$ required: C36.21%, H3.47%, N12.06%.

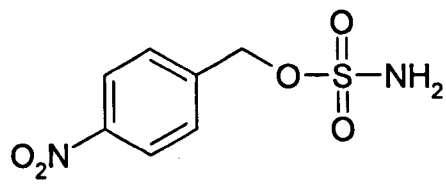
Synthesis of 3- nitrobenzyl sulfamate (**292**)



Compound **292** was synthesised via the same method as **178** using 3-nitrobenzyl alcohol (1.59g, 10.40mmol). The crude product was purified via column chromatography [DEE (60%)/: petroleum spirit 40-60°C (40%)] to give **292** as white solid (0.66g, 27.24%; 104.7-105.9°C; R_f: 0.45 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3350.6 (NH_2), 3274.0 (PhH), 1355.1 (NO_2), 1185.1 (S=O); δ_H(d_6-Acetone): 7.41 (4H, m, Ph<u>H</u>), 6.78 (2H, br.s, N<u>H</u>$_2$), 5.00 (2H, s, C<u>H</u>$_2$); δ_C($CDCl_3$): 135.57, 132.31, 131.46, 130.38, 127.01, 122.78 (Ar), 71.55 (CH_2); LRMS (EI): m/z 232 (M^+, 17%), 152 (M^+- H_2O_2SN, 100%).

Synthesis of 4-nitrobenzyl sulfamate (**293**)

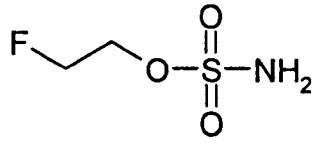


Compound **293** was synthesised via the same method as **178** using 4-nitrobenzyl alcohol (1.07g, 6.99mmol). The crude product was purified via column chromatography [DEE: petroleum spirit 40-60°C (50% v/v)] to give **293** as a white solid (1.17g, 72.29%; mp: 89.4-89.9°C; R_f: 0.15 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3348.6 (NH_2), 3252.5 (PhH), 1514.8, 1345.6 (NO_2), 1163.5 (S=O), δ_H(d_6-Acetone): 8.20 (2H, d, J=8.79Hz, Ph<u>H</u>), 7.52 (2H, d, J=8.79Hz, Ph<u>H</u>), 5.23 (2H, s, C<u>H</u>$_2$), 4.74 (2H, s, N<u>H</u>$_2$); δ_C(d_6-Acetone): 148.84, 143.33, 129.62, 124.41 (ArC), 70.18 (CH_2); Found: C36.40%, H3.46%, N12.01%, $C_7H_8N_2O_5S_1$ Calculated: C36.21%, H3.47%, N12.06%.

3.3 Synthesis of Amino sulfonates of substituted ethyl alcohol

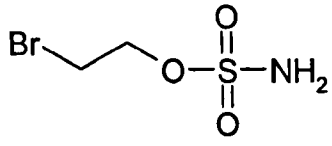
Synthesis of 2-fluoroethyl sulfamate (**294**)



Compound **294** was synthesised via the same method as **178** using 2-fluoroethanol (0.67g, 10.49mmol). The crude product was purified via column chromatography [DEE (40%): petroleum spirit 40-60°C (60%)] to give **294** as a white solid (032g, 21.53%; mp: 55.4-56.7°C; R_f: 0.17 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3396.48 (NH), 3301.12 (CH), 1171.83 (S=O); δ_H(d_6-Acetone): 6.67 (2H, br.s, N<u>H</u>$_2$), 4.66 (1H, m, FHC<u>H</u>), 4.54 (1H, m, F<u>H</u>CH), 4.37 [1H, m, O(HC<u>H</u>)], 4.24 [1H, m, O(<u>H</u>CH)], δ_C(d_6-Acetone): 82.18, 80.50, 68.74, 68.54 (CH_2); LRMS (EI): m/z 144 (M^+, 17%), 80 (M^+- H_2O_1SN, 100%).

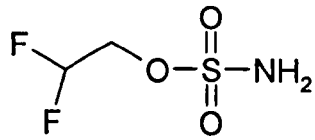
Synthesis of 2-bromoethyl sulfamate (**295**)



Compound **295** was synthesised via the same method as **178** using 2-bromoethanol (1.80g, 14.42mmol). The crude product was purified via column chromatography [DEE (40%): petroleum spirit 40-60°C (60%)] to give **295** as a brown solid (0.38g, 10.74%; mp: 40.4-42.2°C; R_f: 0.51 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3361.98 (NH), 3278.30 (CH), 1167.52 (S=O); δ_H(d_6-Acetone): 4.24 (2H, t, J=5.86Hz, $BrCH_2$), 3.70 (2H, t, J=5.86Hz, OCH_2); δ_C(d_6-Acetone): 68.96 ($BrCH_2$), 29.02 (CH_2); LRMS (EI): m/z 204 (M^+, 6%), 106 (M^+- H_2O_3SN, 100%).

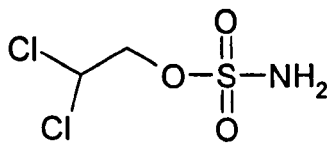
Synthesis of 2, 2-difluoroethyl sulfamate (**296**)



Compound **296** was synthesised via the same method as **178** using 2,2-difluoroethanol (0.52g, 6.31mmol). The crude product was purified via column chromatography [DEE (40%): petroleum spirit 40-60°C (60%)] to give **296** as a white solid (0.38g, 37.77%; mp: 48.4-49.5°C; R_f: 0.52 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3399.75 (NH), 3296.11 CH), 1087.30 (S=O); δ_H(d_6-Acetone): 6.89 (2H, br.s, N$\underline{H}_2$), 6.14 (1H, tt, J=3.48Hz, $F_2C\underline{H}$), 4.29 (2H, td, J=3.48Hz, OCH_2); δ_C(d_6-Acetone): 115.46, 113.08, 110.69 (CH), 66.94, 66.66, 66.38 (CH_2); LRMS (EI): m/z 162 (M^+, 3%), 80 (M^+- H_2O_2SN, 100%).

Synthesis of 2, 2-dichloroethyl sulfamate (**297**)

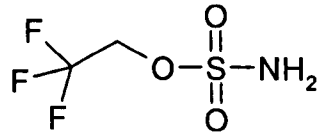


Compound **297** was synthesised via the same method as **178** using 2, 2-dichloroethanol (1.04g, 9.06mmol). The crude product was purified via column chromatography [DEE (40%): petroleum spirit 40-60°C (60%)] to give **297** a waxy white solid (0.93g, 53.01%; mp: 42.1-42.5°C; R_f: 0.62 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3377.94 (NH), 3279.72 (CH), 1175.47 (S=O); δ_H(d_6-Acetone): 7.11 (2H, br.s, N$\underline{H}_2$), 6.36 (1H, t, J=5.49Hz, C$\underline{H}$), 4.56 (2H, d, J=5.49Hz, C$\underline{H}_2$);

δ_C(d_6-Acetone): 73.19 (CH), 69.37 (CH_2); LRMS (EI): m/z 194 (M^+, 17%), 79 (M^+-CH_2O_3SN, 100%).

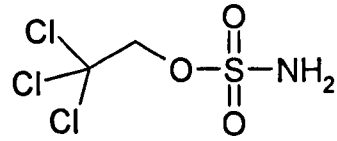
Synthesis of 2, 2, 2-trifluoroethyl sulfamate (**298**)



298 was synthesised via the same method as **178** using 2, 2, 2-trifluoroethanol (1.82g, 18.15mmol). The crude product was purified via column chromatography [DEE (40%): petroleum spirit 40-60°C (60%)] to give **298** as a white solid (0.59g, 18.25%; mp: 69.2-71.7°C; R_f: 0.67 [DEE (90%): hexane (10%)]).

$\nu_{(max)}$(Film)cm^{-1}: 3386.61 (NH), 3284.88 (CH), 1167.19 (S=O); δ_H($CDCl_3$); 5.04 (2H, br.s, N$\underline{H}_2$), 4.87 (2H, s, C$\underline{H}_2$); δ_C($CDCl_3$) 81.27 (CF_3), 32.41 (CH_2); LRMS (EI): m/z 180 (M^+, 40%), 64 (M^+- CH_5O_3SN, 100%).

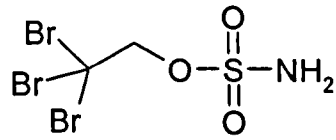
Synthesis of 2, 2, 2-trichloroethyl sulfamate (**299**)



Compound **299** was synthesised via the same method as **178** using 2, 2, 2-trichloroethanol (1.72g, 11.48mmol). The crude product was purified via column chromatography [DEE (40%): petroleum spirit 40-60°C (60%)] to give **299** a white solid [0.81g, 30.82%; mp: 62.4-63.2°C, lit. m.p. 46.5-49.2°C Ahmed *et al.* (2002c) ; R_f: 0.60 [DEE (90%): hexane (10%)].

$\nu_{(max)}$(Film)cm^{-1}: 3385.18 (NH), 3291.64 (CH), 1176.21 (S=O); δ_H($CDCl_3$): 5.42 (2H, br.s, N$\underline{H}_2$), 4.67 (2H, s, C$\underline{H}_2$); δ_C($CDCl_3$): 93.07 (CCl_3), 78.62 (CH_2); LRMS (EI): m/z 230 (M^+, 17%), 79 (M^+- CH_5O_3SN, 100%).

Synthesis of 2, 2, 2-tribromoethyl sulfamate (**300**)



Compound **300** was synthesised via the same method as **178** using 2, 2, 2-tribromoethanol (1.40g, 4.94mmol). The crude product was purified via column chromatography [DEE (40%): petroleum spirit 40-60°C (60%)] to give **300** as a pale brown solid (1.13g, 63.28%; mp: 125.127.2°C; R_f: 0.58 [DEE (90%): hexane (10%)].

$\nu_{(max)}$(Film)cm^{-1}: 3384.20 (NH), 3290.94 (CH), 1172.89 (S=O); δ_H(Acetone): 7.03 (2H, br.s, N$\underline{H}_2$), 4.72 (2H, s, C$\underline{H}_2$); δ_C(Acetone): 80.65 (CBr_3), 33.55 (CH_2).

CHAPTER 4

DISCUSSION

4.0 DISCUSSION

4.1 Synthesis of sulfonated derivatives of 4-hydroxy benzamide

As previously mentioned, the synthesis of the sulfonated derivatives of 4-hydroxy benzamide was undertaken in an effort to produce potential inhibitors of E1STS. Previous studies have shown that of the three sulfonate derivatives, the sulfamate-based compounds would be expected to result in potent irreversible inhibitors whilst the methanesulfonate- and trifluoro-based compounds would be expected to possess moderate, but more importantly, reversible inhibition (due to the lack of the NH_2 group, which therefore would not result in the production of the imine moiety within the active site).

The sulfonated derivatives of 4-hydroxy benzamide are currently undergoing biochemical evaluation using microsomal enzymes obtained from rat liver, as such, no initial screening data was available to produce a structure activity relationship or to consider the inhibitory activity for the synthesised compounds in comparison to the previously reported compounds, in particular, those based on the 4-sulfamoylated derivatives of alkyl esters of 4-hydroxybenzoic acid and which have previously shown extremely potent inhibitory activity.

However, as previously reported by Ahmed *et al.* (2002a), pK_a has been postulated to be an important factor in determining the overall inhibitory activity of the sulfamate derivatives, as such, an indication of the pK_a of the parent 4-hydroxy benzamide would allow us to hypothesise the potential inhibitory activity of the sulfamate derivatives - the other sulfonate derivatives (namely, methane sulfonate and trifluoromethane sulfonate) derivatives have previously been shown to inhibit E1STS in a reversible manner (James, 2000) and no correlation has yet been reported between reversible inhibition of E1STS and pK_a (or indeed any other physicochemical factor other than logP).

In an attempt to postulate the potential inhibitory activity possessed by the sulfamate derivatives of the 4-hydroxy benzamide-based compounds, the pK_a of the parent phenolic derivative was undertaken. This involved the disolving of the parent phenolic compound (between 2 and 4mg) in borax buffer (100ml)

following which the UV spectra was recorded between a wavelength of 200-450nm. The absorbance of the main peak was adjusted to approximately 1 absorbance unit by the addition of either the buffer solution or the phenolic compound. After the removal of any undissolved particulates, 20ml of the original solution was pipetted into three 25ml volumetric flasks, and made up to 25ml with either 2M HCl (Aa), borax buffer pH9.0 (A) or 2m NaOH (Ab). The UV spectrum of each solution was obtained and the absorbances at the wavelength corresponding to the 2M NaOH solution maxima was recorded. The pK_a was then determined using the following equation (Harwood and Moody, 2001):-

$$\textbf{Mole fraction of dissociated phenol= X} = \frac{[ArO^-]}{[ArO^-] + [ArOH]}$$

$$= \frac{(A - Aa)}{(Ab - Aa)}$$

$$pK_a = pH + \log[(1-X)/X]$$

The pK_a is hypothesised to be related to the stability of the phenoxide ion produced as a result of the cleavage of the S-OR bond within the sulfamate-based inhibitors. In an effort to correlate pK_a values with the structure, the partial charge on the phenolic oxygen was calculated - the structures were constructed within CaChe (version 7.5; Fujitsu Ltd) and minimised using MM3 parameters followed by optimisation (and therefore partial charge calculation) using PM5 parameters. For comparison, the pK_a values for the parent phenolic-based compounds (from methyl to propyl) of the previously reported sulfamate derivatives of 4-hydroxy benzoic acid esters were also determined (Table 21).

Compound	pK_a	Partial charge on phenolic O^-	Calculated logP
150	8.28	-0.606	0.981
151	8.22	-0.607	1.323
152	8.03	-0.607	1.792
176	10.10	-0.624	0.577
181	9.45	-0.649	1.262
185	9.15	-0.647	2.200
189	10.03	-0.647	2.992

Table 21: Comparison of the pK_a values of the compounds synthesised within the current study with the pK_a values of the sulfamate derivatives of 4-hydroxybenzoic acid esters.

From the initial consideration of the pK_a values, we observe that the benzamide-based compounds possess higher pK_a values than the similar ester-based compounds. As such, the above values would appear to suggest that (based on the pK_a values determined) the sulfamate derivatives of 4-hydroxy benzamide would be expected to possess weaker inhibitory activity against E1STS than the corresponding ester-based compounds. That is, in their initial work, Ahmed *et al.* (2002a) suggested that the optimum pK_a value for potent E1STS inhibition is approximately 8.02. However, the above benzamide compounds would appear to possess much greater pK_a values (average of 9.68) and would therefore be extremely weak acids, therefore they would be expected to show weak inhibitory activity. It should be noted that phenol possesses a pK_a value of 9.98, with the above four 4-hydroxy benzamide compounds showing an average pK_a value of 9.68; the inhibitory activity of the benzamide-based compounds would therefore be expected to be similar to that of phenol. Indeed, in a previous study, phenyl sulfamate was found to possess an IC_{50} value greater than 10mM, whilst the 4-fluorophenyl sulfamate derivative (which possessed a pK_a value of 9.16) was found to possess an IC_{50} value of 2089μM (Ahmed *et al.*, 2001). We therefore postulate that the benzamide based compounds would be expected to be extremely weak inhibitors of E1STS in comparison to the sulfamate derivatives of 4-hydroxy benzoic acid esters.

That the above hypothesis may indeed be correct can be further supported by the observation that the sulfamate derivatives of 4-hydroxy benzamide synthesised within the current study were chemically more stable than the sulfamate derivatives of 4-hydroxy benzoic acid esters. That is, it was discovered by James (2000) that the ester-based sulfamate derivatives were found to undergo hydrolysis with standing, even when refrigerated, indeed a range of sulfamate derivatives of 4-hydroxy benzoic acid esters were found to undergo hydrolysis soon after workup. The sulfamate derivatives of 4-hydroxybenazmide were, however, found to be extremely stable with long term exposure to the atmosphere.

In a study, Ahmed *et al.* (2002d) suggested that the calculated logP value of the parent phenolic compound also appeared to be an important factor (assuming

optimum pK_a had been achieved) in determining the overall inhibitory activity against E1STS [logP was calculated using Project Leader version 7.5, Fujitsu Ltd; using the atomic typing scheme of Ghose *et al.* (1998) – it should be noted *that the parameters for the aminosulfonate* moiety is not available, as such, the logP value for the parent phenolic *derivative was calculated within Project* Leader]. The most potent compound reported within the sulfamate derivatives of 4-hydroxy benzoic acid ester-based inhibitors is the cyclooctyl 4-aminosulfonyl benzoate (**163**), and which was found to possess a calculated logP value of 3.8. From the initial consideration of the values obtained for the 4-hydroxy benzamide-based compounds, it would appear that the di-pentyl derivative would be a close mimic to the calculated logP value of **163**, however, the high pK_a value would result in poor inhibitory activity.

4.2 Synthesis of sulfamate derivatives of alkyl and benzyl alcohols

The synthesis of a range of benzyl and alkyl sulfamates were undertaken following the observation that halogenated derivatives of acetic acid possessed increased acidity. Since the strength of the S-OR bond has been postulated to be a major factor in determining the overall inhibitory activity of the sulfamated derivatives of benzyl and alkyl alcohol, we postulated that the derivatisation of the benzyl and alkyl backbone would allow us to synthesise potentially good inhibitors of E1STS. The compounds synthesised are currently undergoing biochemical evaluation, however, we can conclude that these compounds would be expected to possess poor inhibitory activity due to the low calculated logP value – as mentioned above, the optimum logP observed within the sulfamate derivatives of 4-hydroxy benzoic acid esters was found to be approximately 3.8, however, within the alkyl sulfamate-based compound, the logP values are much lower than 3.8 (Table 22). It should be emphasised that the logP values used are the values for the non-sulfamated derivatives since the appropriate parameters are not available for the sulfamate moiety, as such, the proceeding discussion relates to the non-sulfamated compound, however, the trend in logP would be expected to be the same since the only additional moiety (the sulfamate group) is present in the final compound – any use of the sulfamated derivative within Project Leader to calculate logP values result in an error.

Compound number	Calculated logP value
268	0.561
280	1.079
290	1.353
293	0.514
295	-0.449
297	-0.274
294	-1.056
296	-0.960
298	-0.241
299	0.318

Table 22: Calculated logP of the parent alcohol derivatives for a small range of benzyl and alkyl sulfamates.

Indeed, the only compound which approaches the logP of compound **163** is compound **280** (4-chlorobenzyl sulfamate), however, from the consideration of compounds within the 4-hydroxyphenyl ketone-based compounds, a logP of approximately 1.1 was observed within compound **142** (which was found to possess an IC_{50} value of 302μM). As such, these compounds possess the potential to be moderate inhibitors of E1STS. It should be noted that whilst the logP value may be appropriate for moderate inhibition, the stability of the S-O-Benzyl bond would result in poor inhibitory activity. In an attempt to determine the stability of the S-O bond, we undertook a molecular modelling study to calculate the charge differences resulting from the various substituents, however, this proved to be disappointing with similar charges being observed on the O of the S-OR bond - Figures 21a and 21b shows the charge differences between compounds **268** and **293** (charge difference of 0.013 being observed).

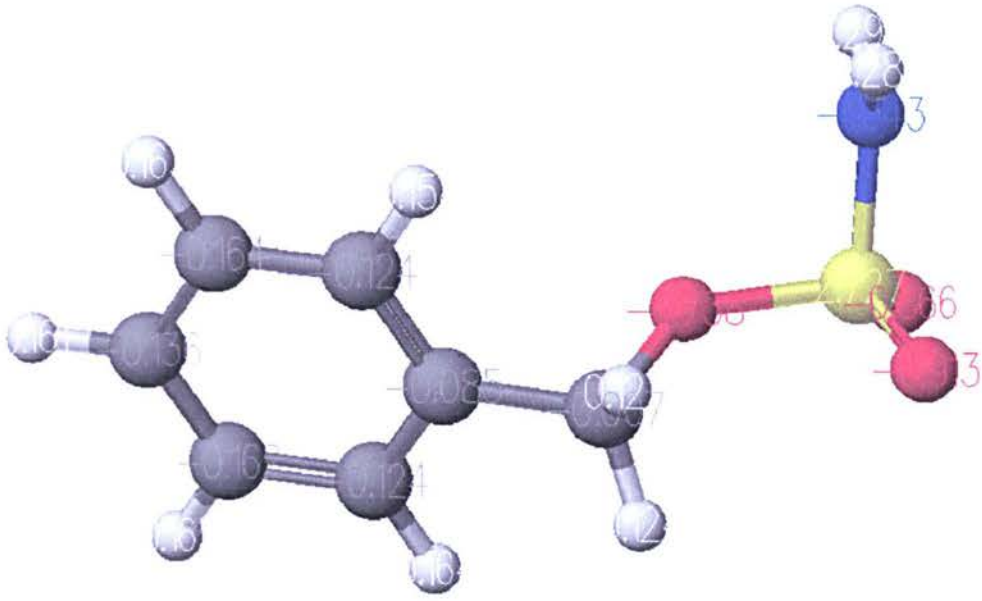


Figure 21a: Partial charge calculated within compound **268**.

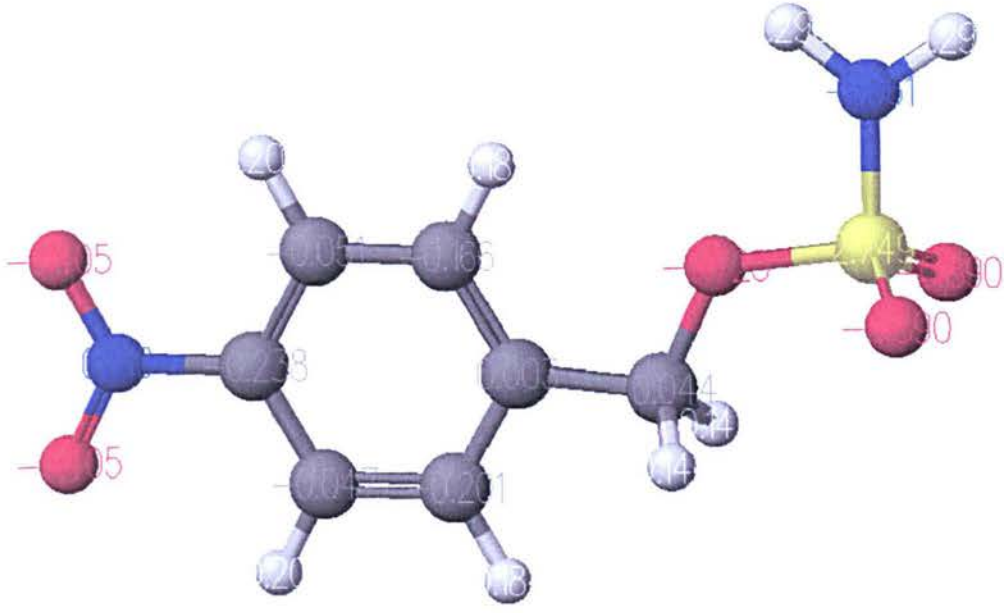


Figure 21b: Partial charge calculated within compound **293**.

Within the alkyl sulfamate based compounds there are no compounds which possess logP values close to 1.0, as such, these compounds would be expected to be extremely poor inhibitors – the biological activity would be further reduced by the greater stability of the S-O-Alkyl bond within this range of compounds as can be observed by the partial charge calculations being undertaken on compounds **296** and **298** (Figures 22a and 22b).

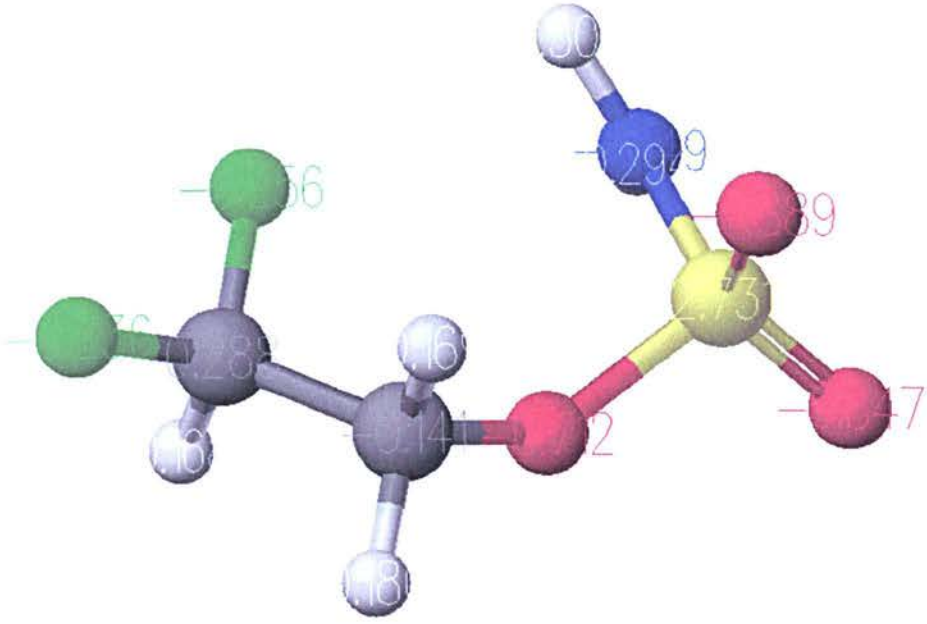


Figure 22a: Partial charge calculated within compound **296**.

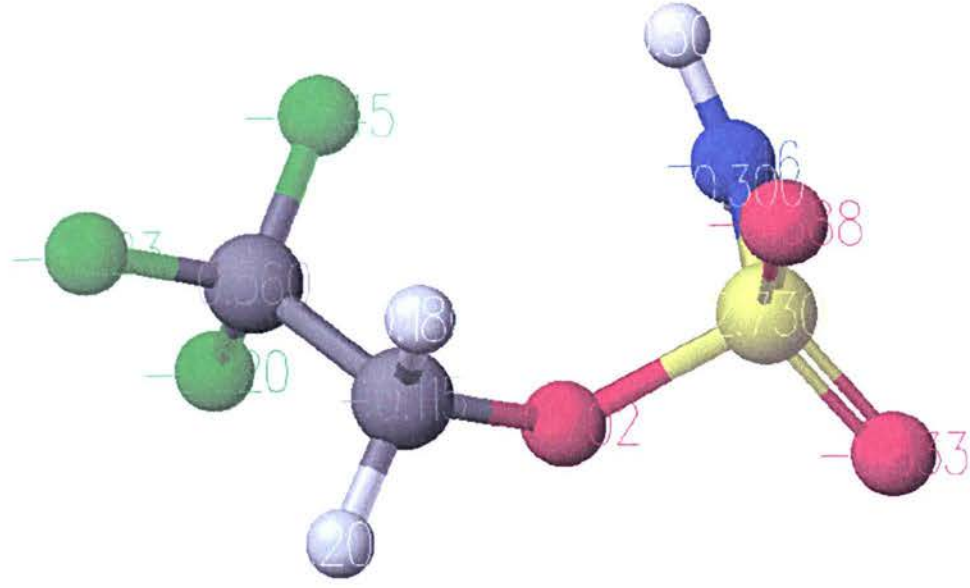


Figure 22b: Partial charge calculated within compound **298**.

In summary, the two ranges of compounds would be expected to be extremely weak inhibitors of E1STS due to the stability of the S-OR bonds and the low logP values observed within the two ranges of compounds.

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