KINGSTON UNIVERSITY

SYNTHESIS, BIOCHEMICAL EVALUATION AND PHYSICOCHEMICAL PROPERTY DETERMINATION OF A RANGE OF POTENTIAL ESTRONE SULFATASE INHIBITORS IN THE TREATMENT OF HORMONE-DEPENDENT BREAST CANCER

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

BY

Timothy Cartledge

FACULTY OF SCIENCE
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Abstract

The inhibition of enzymes within the steroidal cascade has been shown to lead to a reduction in tumour mass as a result of a reduction in the levels of steroids present both within the plasma and within tumour cells. For example, in postmenopausal women, the use of enzyme inhibitors has led to the treatment of hormone-dependent breast cancer. Enzymes such as aromatase, 17β-hydroxysteroid dehydrogenase [types 1 (17β-HSD1) and 3 (17β-HSD3)] and estrone sulfatase (ES) are some of the enzymes involved in the biosynthesis of steroids and have therefore become biochemical targets in the design and synthesis of novel drugs against this disease. Inhibitors of ES have thus far been investigated involving the use of the sulfamate moiety as the inhibiting moiety. Within the current study, the synthesis and biochemical evaluation of a number of compounds (both steroidal and non-steroidal) with varying structural features has been undertaken, in particular, the synthesis of sulfonate (as opposed to sulfamate) derivatives has been investigated.

The results show that the sulfonate (methane sulfonate and trifluoromethanesulfonate) derivatives of 4-hydroxyphenyl ketone-based compounds were found to possess weak inhibitory activity against ES (from rat liver microsomes at a final inhibitor concentration of 100μM) in comparison to the two standard compounds used within the study, namely EMATE and COUMATE. For example, within the methanesulfonate derivatives of 4-hydroxyphenyl ketone based compounds, the most potent compounds were: methanesulfonic acid 4-nonyl-phenyl ester (**304**) (which was found to possess ~36% inhibitory activity against ES); methanesulfonic acid 4-decyl-phenyl ester (**305**) (which was found to possess ~38% inhibitory activity against ES); methanesulfonic acid 4-cyclobutane carbonyl phenyl ester (**307**) (which was found to possess ~38% inhibitory activity against ES) and methanesulfonic acid 4-cyclopentane carbonyl phenyl ester (**308**) (which was found to possess ~38% inhibitory activity against ES). The trifluoromethanesulfonate derivatives of 4-hydroxyphenyl ketones were found to be extremely weak inhibitors of ES and were weak inhibitors in comparison to both standard compounds as well as the methanesulfonate derivatives of the 4-hydroxyphenyl ketone-based compound, indeed, the most potent compound was trifluromethanesulfonic acid 4-cyclobutane carbonyl phenyl ester (**321**) which was found to possess ~29% inhibitory activity against ES. Within the estrone (E1)- and estradiol (E2)- based compounds, a number of different sulfonate derivatives were evaluated (but not synthesised) within this study, and a small number of these compounds was found to possess good inhibitory activity (at [I]=100μM), however, in general, these compounds were also weak inhibitors in comparison to EMATE and COUMATE. Some of the more potent inhibitors within this range of compounds include: methanesulfonic acid E1 (**369**) (which was found to possess ~47% inhibitory activity against ES); trifluoro-methanesulfonic acid E1 (**370**) (which was found to possess ~58% inhibitory activity against ES) and; di-methanesulfonic acid E2 (**371**) (which was found to possess ~41% inhibitory activity against ES). Within the range of E1- and E2-based compounds, it was observed that the inhibitory activity decreased with increasing size of the substituent on the sulfonate moiety, the introduction of a biphenyl ring on the sulfonate moiety resulted in a marked decrease in inhibitory activity. A range of thiosemicarbazone-based inhibitors were also evaluated and were shown to possess moderate inhibitory activity, however, they were weak inhibitors in comparison to the two standard compounds used. For example, compound **401** was found to possess ~73% inhibitory activity against ES, whilst compound **381** was found to possess ~70% inhibitory activity against ES - a major problem with these compounds is that they were found to rapidly degrade when dissolved in solution (such as ethanol or DMSO). In conclusion, the compounds synthesised proved to have weak levels of inhibitory activity, however, they have provided some insight into the design of novel inhibitors of ES.

Contents

LIST OF ABBREVIATIONS

Numbers in bold are the compound numbers

1-10

4-Methylcoumarin-7-*O*-sulfamate	COUMATE
5α-reductase	5AR
17α-hydroxylase/17,20-lyase	$P450_{17\alpha}$
17β-hydroxysteroid dehydrogenase	17β-HSD

A

Acid disassociation constant	pK_a
Androstenediol	Adiol
Androstendione sulphate	Adiol-S
Aromatase	AR

B

Broad singlet	bs

C

D

Dehydroepiandrosterone	DHEA
Dehydroepiandrosterone sulphate	DHEA-S
Dichloromethane	DCM
Diethyl ether	DEE
Dimethyl sulfoxide	DMSO
Doublet	d
Doublet of doublets	dd

E

Electron ionisation	EI

Electron spray	ES
Estradiol	E2
Estrogen	E
Estrogen receptor	ER
Estrone	E1
Estrone sulfatase	ES
Estrone sulfate	E1S
Estrone-3-*O*-sulfamate	EMATE
Estrone-3-*O*-methyl-phosphonothionate	E1-MTP

F

G

Gas chromatography	GC

H

High resolution mass spectrometry	HRMS
Human breast adrenocarcinoma cell lines	MCF-7
Hydrochloric acid	HCl
Hydroxysteroid dehydrogenase	HSD

I

Infrared	IR
Inhibitory concentration at 50%	IC_{50}

J

K

L

Low resolution mass spectrometry	LRMS

M

Magnesium sulphate	$MgSO_4$
Michaelis menton constant	K_m

N

Nuclear magnetic resonance	NMR

O

P

Partition coefficient	Log P
Petroleum spirit	Pet spirit
Potassium chloride	KCl

Q

Quartet	q

R

Retention time	t_R

S

Sex hormone binding globulin	SHBG
Sextet	sex
Singlet	s
Sodium bicarbonate	$NaHCO_3$
Sodium hydroxide	NaOH
Structure activity-relationship	SAR

T

Tetrahydronaphthol	THC
Thin layer chromatography	TLC
Triethylamine	TEA

Triplet	t
U	
Ultra violet	UV
V	
Velocity	V
W	
X	
Y	
Z	

Chapter 1: Introduction

1.0 INTRODUCTION

1.1 Breast cancer

Since 1975 there has been a steady increase in breast cancer cases, and in 2008 it is estimated that breast cancer will be the most commonly occurring cancer in women in the USA, with an estimated occurrence of 26% of all cancers. This is approximately twice the estimated occurrence of the second largest occurring cancer (lung and bronchus), which accounts for 14% of all cancer cases. Furthermore, breast cancer has the second highest mortality rate of cancers in women with 15% of all cancer-caused deaths (Jemal et al, 2008).

During the past 20 years, research has been focused on the early detection and standardised treatment of breast cancer, however, it is suggested that since breast cancer was responsible for 192,200 new cancer cases in 2001, this approach may not be effective (Greenlee et al, 2001).

The aetiology of breast cancer still remains unclear, with conflicting evidence for the matrices of the risk factors for pre- and post-menopausal women. However, there are a number of known risk factors, and estrogens have been implicated as being a major factor (Table 1.1).

1.2 Estrogens and breast cancer

The relationship between the ovaries and breast cancer was first recorded by Beatson (1896) when he observed the healing of locally recurrent breast cancer in a woman who had undergone bilateral oophorectomy. Furthermore, it has been shown that breast cancer growth is promoted and stimulated by estrogenic hormones (James et al, 1980; Lippman et al, 1988). It has also been found that estrogen levels are higher in breast cancer tissue than any other tissue within the female body; the long-term exposure to this family of compounds therefore appears to increase the risk of developing the disease, thus establishing a further

link between estrogens and breast cancer (Pasqualini et al, 1995). More specifically, estradiol (E2) has been reported to be the main initiator and promoter of estrogen-dependent tissue growth (Pasqualini et al, 1989).

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	>30yr	<20yr
Age	>45yr	<25yr
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/ Hormone replacement therapy (HRT)	Yes	No
Weight	Obese	Underweight

Table 1.1: Summary of established breast cancer risk factors (Sakorafas et al, 2002).

1.3 Estrogen stimulation

Estrogen (synthesised in the ovaries and adrenals in pre-menopausal women) is transported around the body complexed with albumin or sex hormone binding globulin (SHBG). Once within the breast tissue, the estrogen enters the cell via passive diffusion. Within the cell cytoplasm, the estrogen forms a complex with an estrogen receptor (ER), before undergoing dimerisation with another estrogen-ER complex. The dimer subsequently enters the nucleus and interacts with the DNA at the estrogen responsive elements, inducing transcription followed by protein synthesis and subsequent cell division (Figure 1.1).

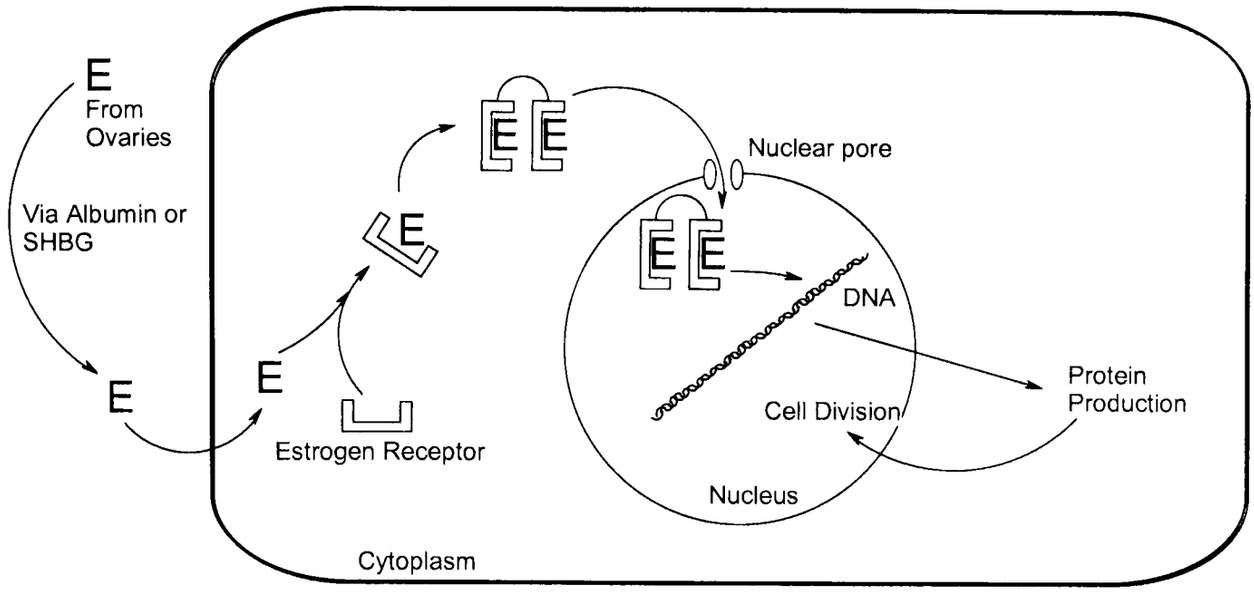


Figure 1.1: The action of estrogen stimulation (E= estrogen).

1.4 Androgens and breast cancer

It has been shown that ER can also be stimulated by the androgens, more specifically androstenediol (Adiol) (Adams et al, 1981; Poulin et al, 1986). Adiol has a much lower affinity for ER than E2, however, it is present in the plasma at a much higher concentration, approximately 100 times that of E2, as such, the weaker interaction between Adiol and ER, which results in a weak stimulation, is overcome by the high concentration of the androgen (Bonney et al, 1983).

1.5 Biosynthetic pathway of estrogens

In the biosynthesis of estrogens, three main enzyme complexes are involved: aromatase (AR) which catalyses the conversion of C_{19} androgens to C_{18} estrogens involving aromatisation of the steroid A ring (Dowsett et al, 1989; Reed et al, 1990); 17β-hydroxysteroid dehydrogenase (17β-HSD) which catalyses the conversion of estrone (E1) to E2 (McNeill et al, 1986; Luu-The et al, 1989); and estrogen sulfatase (ES), which converts the stored steroidal sulfate [estrone sulfate (E1S)] back to the more potent steroid form (Figure 1.2).

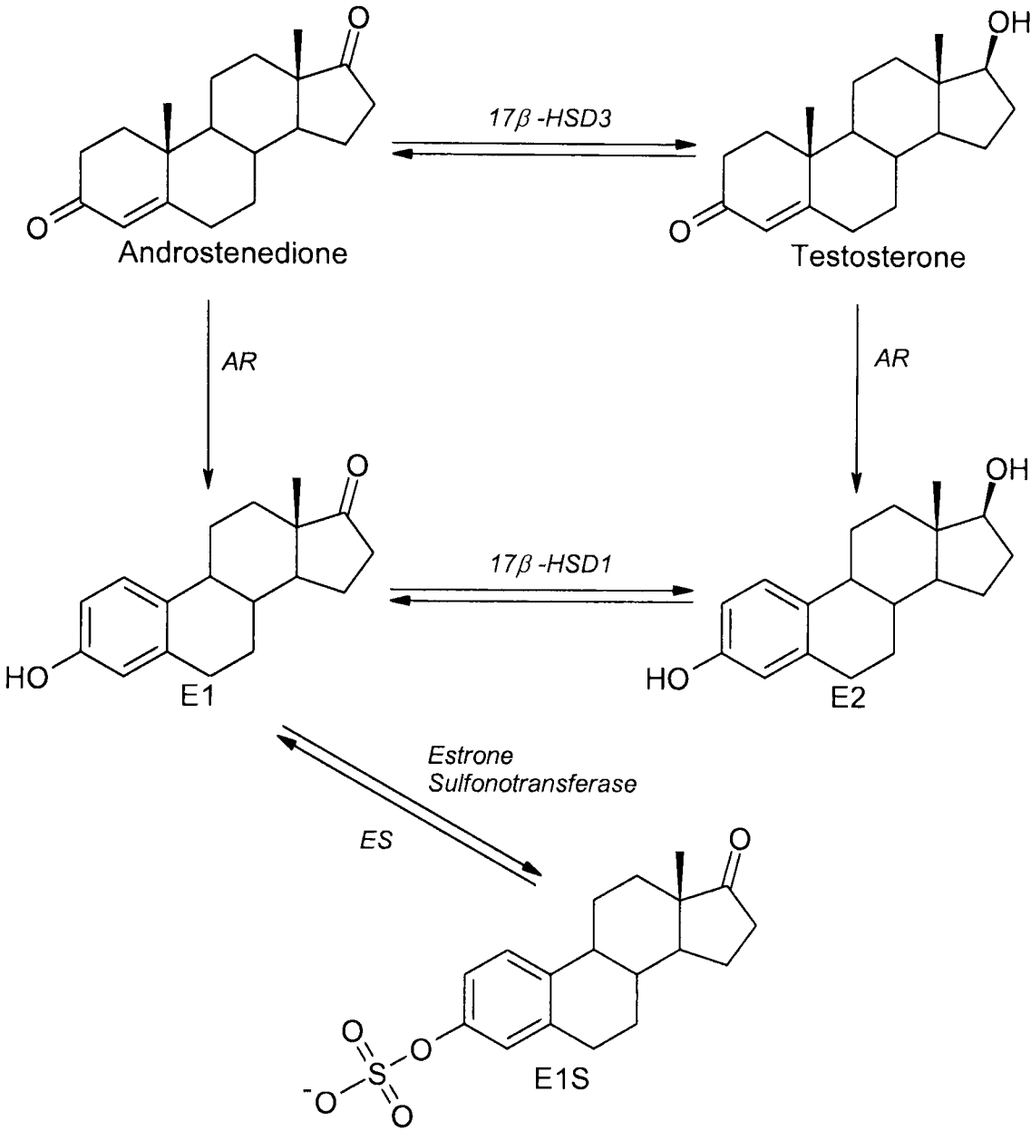


Figure 1.2: Biosynthesis of the estrogens where 17β-HSD1 is 17β-HSD type 1 and 17β-HSD3 is 17β-HSD type 3 (Rang et al, 1999).

1.6 Biosynthetic pathway to androgen biosynthesis

Over 90% of Adiol in post-menopausal women originates from dehydroepiandrosterone-3-sulfate (DHEA-S) which is either converted to Adiol sulfate (Adiol-S) with subsequent enzymatic hydrolysis to Adiol, or from dehydroepiandrosterone (DHEA) via DHEA sulfatase with subsequent enzymatic reduction to Adiol (Poortman et al, 1980) (Figure 1.3).

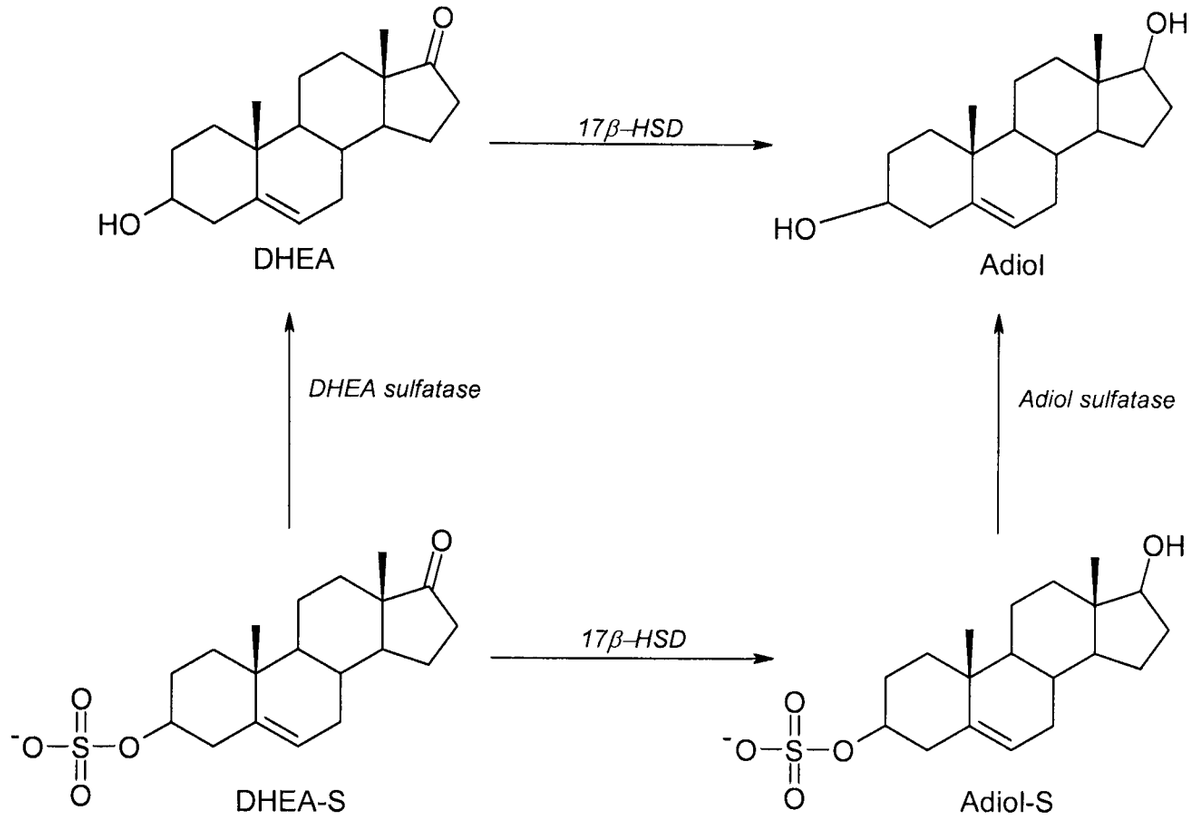


Figure 1.3: Biosynthetic pathways to Adiol (adapted from Poortman et al, 1980).

1.7 Sulfatase family of enzymes

Sulfatases have been postulated to be a family of enzymes that catalyse the hydrolytic conversion of the sulfate derivative to the corresponding non-sulfate-based alcohol (e.g. E1) and are widely found within the human body (Roy, 1971). The current view suggests, however, that a single sulfatase enzyme is responsible for the cleavage of all 3-*O*-sulfates (Dibbelt and Kuss, 1991). This hypothesis is further supported by recent work which shows that compounds synthesised to inhibit ES also inhibit DHEA-sulfatase (Pasqualini and Chetrite, 2005).

1.8 ES

In pre-menopausal women, estrogens are biosynthesised from androgen precursers via the AR pathway (Figure 1.2) within the ovaries and to a lesser extent in the liver, adrenal glands and breast, and any excess estrogen is stored in adipose tissue in the sulfated form as E1S (Santner et al, 1984). In post-menopausal women, the enzymatic pathway for the biosynthesis of the estrogens

from androgens is no longer active, as such, the majority of the estrogen is obtained from the stored (sulfated) form via the action of ES. It is estimated that up to 10 times as much estrogen in breast tumour tissue is derived from the ES pathway as opposed to the catalytic activity of AR. As such, the ES pathway is thought to be the major contributor to active estrogen synthesis, and therefore is responsible for the long term exposure to the estrogens and therefore to the stimulation of breast tumours (Santner et al, 1984; Reed et al, 1996).

The mechanism for the removal of the sulfate group by ES was proposed by our own group (Ahmed et al, 2002a) (Figure 1.4). The mechanism involves an inital attack of the sulfate group by the lone pair of electrons on the *gem*-diol moiety of a formyl-glycine residue, in particular, the sulfur atom of the substrate, resulting in the hydrolysis of the steroid sulfate; the steroid backbone then leaves the active site as the phenolic ion. The sulfate group is then lost from the active site, resulting in the formation of the aldehydic moiety, which is subsequently hydrolysed on the addition of water to the *gem*-diol moiety which is stabilised by a magnesium ion and a calcium ion within the enzyme active site (Figure 1.4) (Ahmed et al, 2002a).

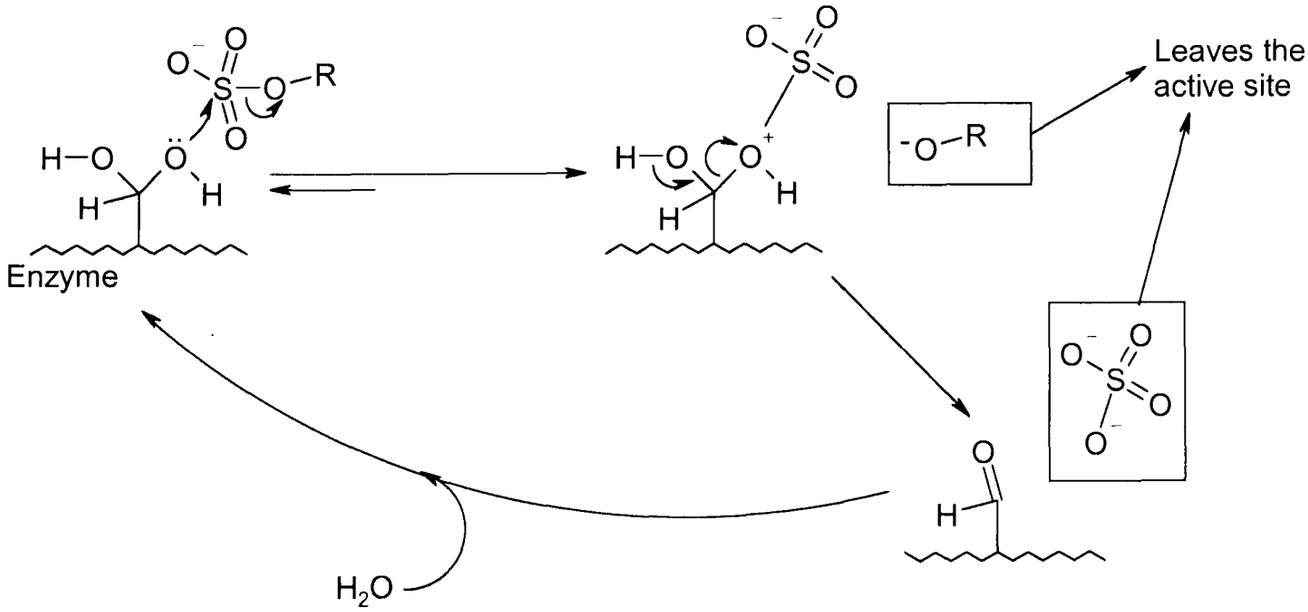


Figure 1.4: Mechanism of desulfatation (adapted from Ahmed et al, 2002a).

1.9 Treatments of breast cancer

There are a number of treatments for breast cancer that do not target enzymes in the steroidal cascade, these are namely surgery, radiotherapy and chemotherapy. Surgery is used in the removal of a portion of the breast, or the removal of the full breast and lymph nodes. Radiotheraphy involves teletherapy, where a focused beam of radiation is used to kill tumour cells or brachytherapy, where a radioactive pellet is inserted into the tumour. Chemotherapy uses cytotoxic chemicals to destroy the cancerous cells.

1.10 Endocrine therapy in breast cancer

Two thirds of post-menopausal breast cancer patients have hormone-dependent tumours, and as such, these would be the most responsive to endocrine treatment. The main marker of hormone-dependent breast cancers is the presence of ER: the higher the occurrence of ER, the greater the possibility of the cancerous tissue responding to endocrine therapy (Ali and Coombes, 2000).

Endocrine therapy is the first-line form of treatment for hormone-dependent breast cancer, involving the blocking of estrogenic stimulation (De Jong and Blijham, 1999), of which there are two main methods used: either blocking the interaction of the estrogen at the target cell (via anti-estrogens) or; the inhibition of the synthesis of the estrogens (Santen et al, 1999).

1.11 Anti-estrogens

The most successful treatment of breast cancer *todate* is tamoxifen (Figure 1.5), which is an ER antagonist, binding to the ER in the cytoplasm of estrogen-dependent cells (Buckley, 1997). This prevents estrogen molecules from binding to the receptor, thus preventing estrogen stimulation and tumour growth. Clinical trials have indicated that tamoxifen is an effective palliative therapy for 50% of patients with hormone-dependent breast cancer (Ravdin et al, 1992). However,

serious side-effects have been shown to occur during treatment with tamoxifen, including: an increased possibility of endometrial cancer in some patients due to increased stimulation of endometrial tissue, and, development of resistance to tamoxifen, resulting in ineffective treatment and therefore leading to disease progression (Clarke and Lippman, 1992).

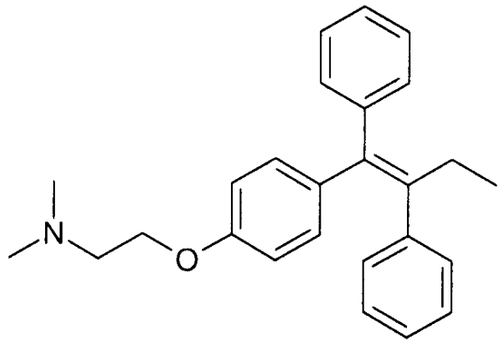
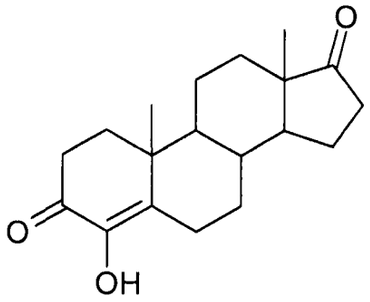


Figure 1.5: Structure of tamoxifen.

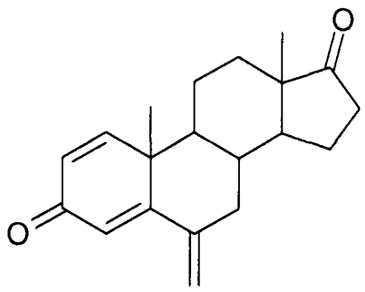
1.12 AR inhibitors

Inhibition of AR would reduce the synthesis and therefore plasma levels of E1 and E2 without inhibiting other enzymes involved in the steroidal cascade, in particular, the synthesis of adrenal corticoids (Brodie et al, 1999). However, negative feedback from this inhibition has been shown to result in changes or effects upon other biosynthetic pathways in pre-menopausal women, stimulating the production of estrogens from other pathways.

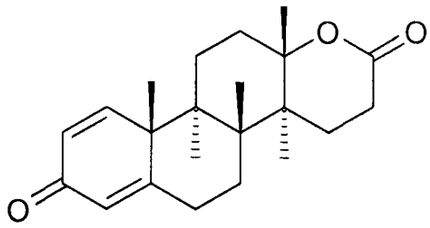
There are two types of inhibitors of AR: steroidal and non-steroidal. These types can be further subdivided into reversible, irriversible and: type I, which compete with the natural substrate for binding to the active site (Figure 1.6) (Santen et al, 1999); and type II, which bind to the P-450 haem moiety in the enzyme complex (Figure 1.7) (Brodie and Njar, 1996).



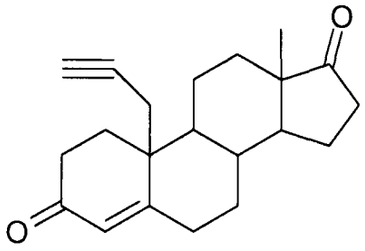
Formestane



Exemestane



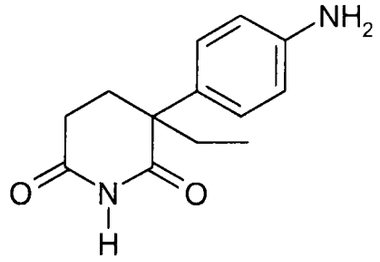
Testolactone



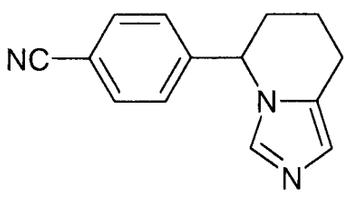
10-(2-Propynyl)estr-4-ene-3,17-dione (PED)

Figure 1.6: Examples of steroidal type I aromatase inhibitors.

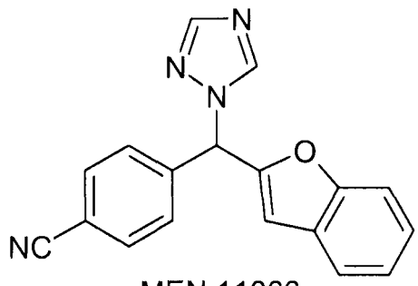
Synthetic analogues of adrostenedione and testosterone e.g. formestane, exemestane, testolactone and 10-(2-propynyl)estr-4-ene-3,17-dione (Figure 1.6), have been developed as inhibitors, of which formestane was the first selective AR inhibitor to be used in the clinic in the treatment of estrogen-dependent breast cancer (Brodie et al, 1999).



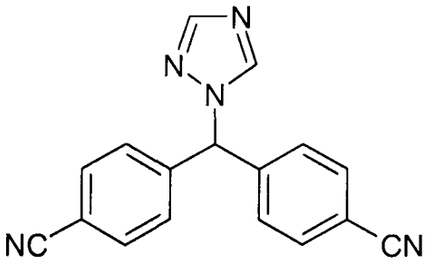
Aminoglutethimide



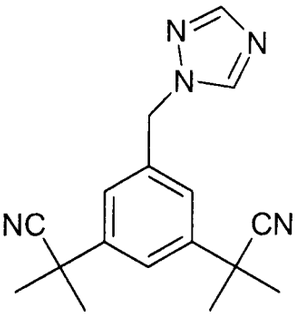
Fadrozole



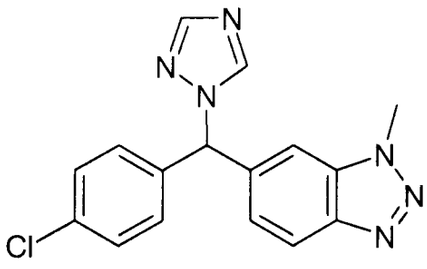
MEN 11066



Letrozole



Anastrozole



Vorozole

Figure 1.7: Examples of non-steroidal type II aromatase inhibitors.

Non-steroidal inhibitors have also been designed and subsequently synthesised (Figure 1.7); they bind reversibly with the Fe within the cytochrome P-450 haem of the enzyme, thus blocking the substrate from binding to the active site, interfering with steroidal hydroxylation and thereby preventing the aromatisation of the steroidal A ring within the substrate (Brodie and Njar, 1996; Brodie, 2002).

The first non-steroidal AR inhibitor to enter the clinic was aminoglutethimide (Figure 1.7). Originally produced as an anticonvulsant, the drug was discovered to inhibit the synthesis of adrenal steroids and displayed therapeutic properties towards hormone–dependent breast cancer in pre-menopausal women (Santen et al, 1974). The drug, however, does possess serious side-effects including ataxia, dizzyness, the possibility of adrenal insufficiencies and other symptoms associated with a lack of specificity (Brodie, 2002).

Other inhibitors have been synthesised and biologically evaluated against AR, for example, fadrozole, vorozole, letrozole and anastrozole (Figure 1.7). The current most potent AR inhibitor available is letrozole. However, MEN 11066 has been shown to display slightly less potency than letrozole but more specificity and is currently undergoing clinical trials (Muratori et al, 2003). It has also been shown that anastrozole has significantly better therapeutic properties than tamoxifen in the treatment of estrogen-dependent breast cancer, with less side-effects (Hayward and Dixon, 2003).

1.13 ES inhibitors

The targeting of ES as a potential treatment of hormone-dependent breast cancer is still in its early stages, with no compounds having entered the market that target ES, and only a limited number of compounds having entered clinical trials. There is, however, a great deal of interest in this field and a number of steroidal and non-steroidal compounds are currently (or have been) under investigation.

Since ES is believed to be the same enzyme as DHEA-sulfatase, any inhibition of ES would not only reduce the plasma and tissue concentration of E2 but also

Adiol, a secondary stimulant of hormone-dependent breast cancer (as discussed above).

1.14 Steroidal inhibitors of ES

The first steroidal inhibitor to show inhibitory activity against ES was danazol, a synthetic isoxazolic derivative of 17α-ethynyltestosterone (Figure 1.8) (Carlstrom, 1984a; Carlstrom, 1984b), which was found to possess ~60% inhibition at an inhibitor concentration of 10μM (Selcer et al, 1996), with previous data displaying inhibition in MCF-7 cell lines by 38% at 10μM (Purohit et al, 1992).

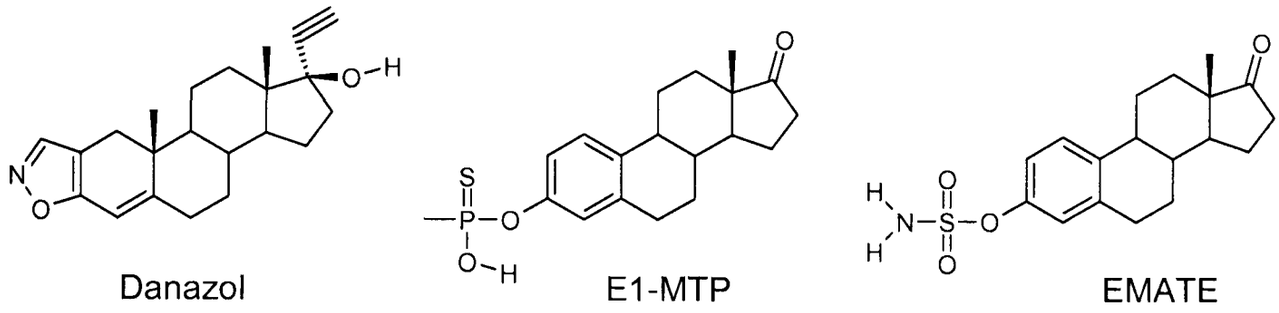
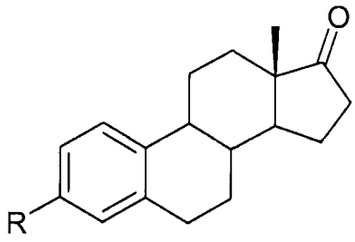


Figure 1.8: Steroidal inhibitors of ES.

Compounds were synthesised specifically to fit known data, and the idea that natural steroid sulfates could inhibit ES activity (Reed et al, 1994); the first was E1-3-*O*-methyl-phosphonothionate (E1-MTP) (Figure 1.8). E1-MTP was found to be 14 times more potent than danazol, and was also found to undergo reversible inhibition of ES, and when tested against MCF7 cell lines displayed competitive inhibition as the cells returned to their natural equilibrium after exhaustion of E1-MTP. Structure activity-relationship (SAR) studies were undertaken, which revealed structural characteristic requirements for an ES inhibitor (Duncan et al, 1993; Purohit et al, 1995). Results from the SAR study led to a series of reversible inhibitors and the discovery of the first irriversible inhibitor of ES, estrone-3-*O*-sulfamate (EMATE) (Figure 1.8). EMATE displayed inhibition in a time- and dose-dependent manner, and biochemical evaluation showed it to be a highly potent inhibitor, displaying upto 95% inhibition at 2nM (Howarth et al, 1994; Purohit et al, 1995). However, there are significant problems with EMATE, making it far from ideal: it is highly unstable and it is itself estrogenic, since after inhibition

of ES, the inhibitor undergoes de-sulfatation, resulting in the formation of E1 and subsequently E2, which is the main stimulant of estrogen-sensitive tissue. As a result, derivitisation of EMATE was undertaken so as to design and synthesise compounds with lowered estrogenicity and greater clinical stability (Tables 1.2 and 1.3).

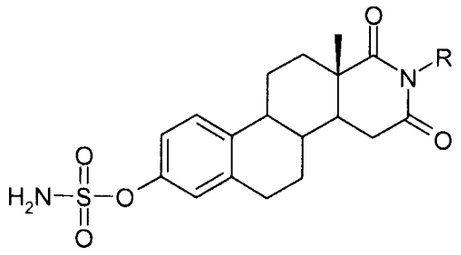


Compound	R	% Activity at concentration
EMATE	$-OSO_2NH_2$	99% 0.1μM [1,4]
1	$-OSO_2NHCH_3$	80% 0.1μM[1,4]
2	$-OSO_2N(CH_3)_2$	50% 0.1μM[1,4]
3	$-OSO_2C_6H_4CH_3$	30% 0.1μM[1,3]
4	$-OSO_2CH_3$	28% 10μM[1,3]
5	$-OSO_2C_4H_9$	17% 10μM [1,3]
6	$-OPO_2H-$	80% 10μM [1,6]
7	$-OPO_2CH_3^-$	41% 10μM [1,6]
8	$-NHSO_2NH_2$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

Table 1.2: E1 derived ES inihibitors ([1]intact MCF7 cells, [2]placental microsomes, [3] [Howarth et al, 1997], [4] [Woo et al, 1997], [5] [Woo et al, 1996a], [6] [Anderson et al, 1997]).

The ranges of EMATE derivatives were in general, found to possess weaker biological activity than EMATE, with only four compounds possessing equipotent inhibitory activity (Table 1.3). Compounds **11** and **12** (STX-213) were found to possess IC_{50} values of 12nM and 1nM respectively (in comparison, EMATE was found to possess an IC_{50} value of 8nM under similar conditions), as such **12** was found to be 8 times more potent than EMATE. As a result of its potency, lack of estrogenicity and improved stability (with repect to EMATE), **12** recently entered

Phase I clinical trails (Foster et al, 2006). Potent inhibitory activity was also observed in the benzyl compound (**15**) (which was found to possess an IC_{50} value of 3nM) whilst the (3-pyridyl) methyl derivative (**17**) (IC_{50}=1nM) was found to be equipotent to **12**.

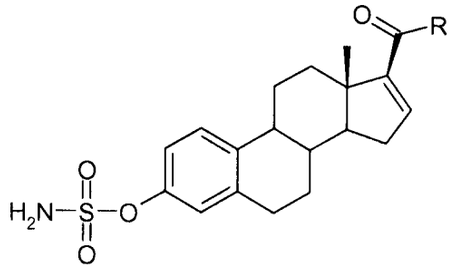


Compound	R	IC_{50} (nM)
EMATE	-	8
10	H	20
11	Methyl	12
12	*n*-Propyl	1
13	*n*-Pentyl	150
14	Cyclopropylmethyl	74
15	Benzyl	3
16	$-CH=CH_2$	80
17	(3-Pyridyl)methyl	1
18	4-*t*-Butylbenzyl	>100

Table 1.3: D-ring derivatives of EMATE evaluated against placental microsomes (Fischer et al, 2003; Potter and Reed, 2002c).

Further derivatisations of EMATE have also been undertaken and the compounds evaluated against ES, for example, the replacement of the C(17) keto group (Tables 1.4 to 1.7) (Li and Selcer, 1999; Tanabe et al., 1999; Li et al, 2000; Kyowa Hakko Kogyo Co. Ltd., 2001;). In compounds **19** to **42**, the C(17) keto group was replaced with a carbonyl functionality, for example, compound **33** replaced the keto with an ethyl ester. Of this range, only compounds **19** to **28** were found to be more potent than EMATE, with compound **19** displaying an IC_{50} value of 1nM and compound **38** being the most potent within the range, possessing an IC_{50}

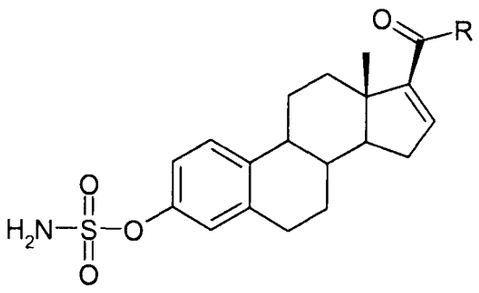
value of 0.45nM. In general, the remaining compounds were found to be weaker inhibitors of ES in comparison to EMATE (e.g. compounds **36** and **37** were found to possess IC_{50} values of 26nM and 50nM respectively and were therefore 3 and 6 times less potent than EMATE).



Compound	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-Pyrolidinyl	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]

Table 1.4: C(17) derivatisation of E1 derived sulfamate ES inhibitors evaluated against purified ES ([1] Li and Selcer, 1999; [2].Li et al, 2000).

Further derivatisation of the D-ring oxygen within EMATE resulted in a range of highly potent inhibitors of ES (Tables 1.6 and 1.7) (Kyowa Hakko Kogyo Co. Ltd., 2001; Tanabe et al, 1999). Within this range, compound **43** was found to be 400 times more potent than EMATE, possessing an IC_{50} value of 0.02nM (EMATE in comparison was found to possess an IC_{50} value of 8nM under similar conditions).



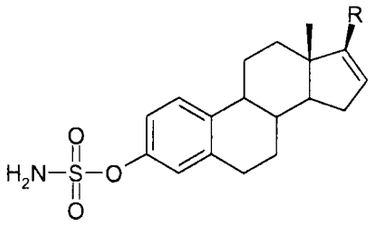
Compound	R	IC_{50} (nM)
27	NH-NH-($_2$-Pyridinyl)	6.0[2]
28	NH-(4-COOHbenzyl)	6.0[2]
29	NH-Pyridazinyl	9.4[2]
30	NH-$(CH_2)_4OH$	9.8[2]
31	NH-$(CH_2)_5COOCH_3$	10.0[2]
32	NH-Ph	10.0[2]
33	OC_2H_5	12.0[1]
34	NH-CH_3	12.0[1]
35	OC_3H_7	13.0[1]
36	NH-$C(CH_3)_2CH_2OH$	26.0[2]
37	OH	50.0[1]
38	NH-$(CH_2)_6CH_3$	0.45[1]
39	1-Pyrolidinyl	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 1.5: C(17) derivatisation of E1 derived sulfamate ES inhibitors evaluated against purified ES ([1] Li and Selcer, 1999; [2].Li et al, 2000; [3] Kyowa Hakko Kogyo Co. Ltd., 2001).

Further derivatisations included the reduction of the C=C within the D-ring of compounds such as **43**, thereby resulting in compound **46**. However, **46** was found to be a weaker inhibitor than **43**, although the derivatisation of the C(17) substituent led to a range of compounds which were found to possess potent inhibitory activity and were 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 discovered in the latter series of compounds, 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.

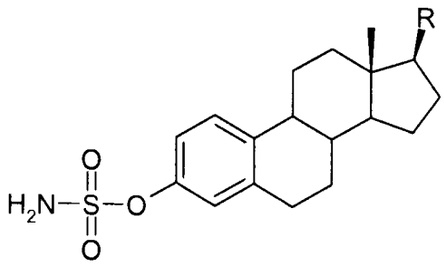
The SAR of compounds **27-42** suggests that there is an apparent strong correlation between alkyl chain length and size at the C(17) position of the steroid backbone in the determination of overall inhibitory activity. However, the presence or lack of the C(16) double bond appears to play an important role in not only determining the overall inhibitory activity, but also the estrogenic property possessed by the inhibitor (Tanabe et al, 1999).



Compound	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

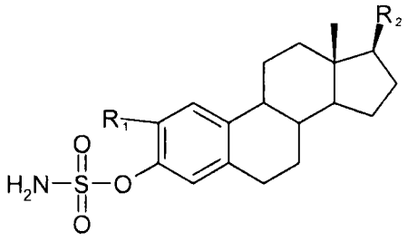
Table 1.6: C-(17) modifications of E1 derivatives evaluated against purified ES ([1] Tanabe et al, 1999; [2] Kyowa Hakko Kogyo Co. Ltd., 2001).

Derivatisation of both the C(17) position and the A-ring was also investigated by several workers (Tables 1.8 and 1.9). In general, the modifications did not improve on the inhibitory activity observed (compared to EMATE), however, compound **51** was found to possess an IC_{50} value of 0.3nM, making it more potent than EMATE (Table 1.8). Furthermore, it was shown that the addition of large bulky groups about the C(17) position of the steroid backbone resulted in a reduction of the overall inhibitory activity, evident from the reduced inhibitory activity of the benzyl derivatives, the most potent compound within the series being **56** (Table 1.9), which was found to possess an IC_{50} value of 44nM (Tanabe et al, 1999).



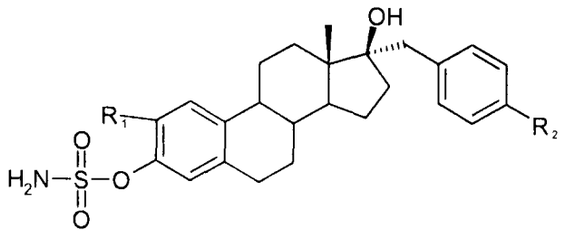
Compound	R	IC_{50} (nM)	Estrogenicity
46	Acetyl	2.0	N
47	Ethyl	0.020	Y
48	*n*-Propyl	0.034	N
49	*n*-Butyl	0.096	N
50	(*E*)-Propylidene	0.027	Y

Table 1.7: C17 derivatives of EMATE evaluated against purified E1S (Kyowa Hakko Kogyo Co. Ltd., 2001).



Compound	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[2,3,4]
54	C_2H_5	OSO_2NH_2	33[2,3,4]
55	OC_2H_5	OSO_2NH_2	100[2,3,4]

Table 1.8: A-ring and C-(17) modifications of E1 derivatives evaluated against purified ES ([1] Tanabe et al, 1999; [2] Potter and Reed, 2002a; [3] Potter and Reed, 2002b; [4] Potter and Reed, 2002d).

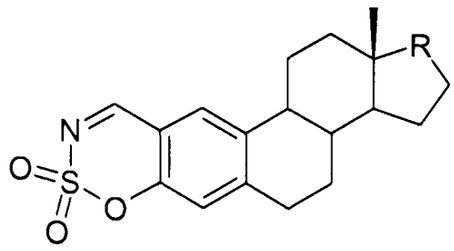


Compound	R_1	R_2	IC_{50} (nM)
56	SCH_3	H	44[1,2]
57	SCH_3 OCH_3	*t*-C_4H_9	80[1,2]
58	OCH_3	H	430[1,3]
59	H	*t*-C_4H_9	4300[1,3]
EMATE	-	-	18[1]

Table 1.9: A-ring and C-(17) modifications of E1 derived evaluated against purified ES ([1] Potter and Reed, 2002a; [2] Potter and Reed, 2002b; [3] Potter and Reed, 2002d).

One series of compounds which involved major derivatisation of the steroid backbone was the oxathiazine-based compounds (Table 1.10) (Peters et al, 2003). These compounds were, in general, found to be weaker inhibitors of ES in comparison to EMATE, apart from compound **60** which was found to be equipotent to EMATE. However, continued derivatisation of both ring-A and ring-D of the steroid backbone led to a range of irreversible inhibitors that were found to possess greatly reduced estrogenicity in comparison to EMATE (Peters et al, 2003).

The most potent compound found within the oxathiazine based range (Table 1.10) was compound **60**, which was found to possess an IC_{50} value of 9nM in intact MCF-7 cells and was therefore equipotent to EMATE (which was found to possess an IC_{50} of 8nM under similar conditions) (Peters et al, 2003). A range of derivatives was then investigated involving the derivatisation of the D-ring, which led to compound **61** (IC_{50} value of 12nM) which contained an acetate functionality at the C(17) position but was found to be 1.3 times weaker than compound **60**.

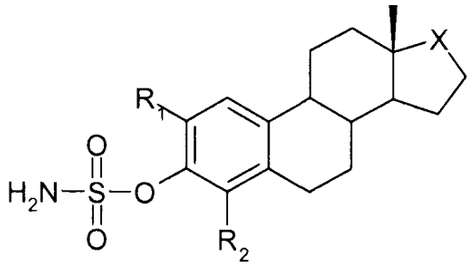


Compound	R	IC_{50} (nM)
60	C=O	9
61	C-OAc	12
62	C-OH	20
63	C=CH-CH_3 (Z)	63
64	C=CHCH_2CH_3 (Z)	58
65	C=CHCH_2CH_3 (E)	<1000
66	C=C=CH_2	74
67	C-CH_3	45
68	C-CH_2CH_3	50
69	C-$(CH_2)_2CH_3$	120
70	C=CH-CO_2Et	36
71	C=C$(CN)_2$	22
72	C-OCH_3	35
73	C-αCl	62
EMATE		8

Table 1.10: Inhibition data for steroidal oxathiazine derivatives evaluated against MCF-7 (Peters et al, 2003).

Further investigations showed that an increase in the overall size and volume of the C(17) substituent resulted in a decrease in inhibitory activity, as such compound **65** proved to be an extremely weak inhibitor of ES (possessing an IC_{50} greater than 500nM) in comparison to EMATE. It is interesting to note that the *Z*-isomer of **65** (i.e. compound **64**) was found to possess an IC_{50} value of 58nM; it was proposed that the bulky nature of the *E*-isomer resulted in increased steric hindrance, resulting in weaker binding of the inhibitor to the ES active site (Peters et al, 2003).

Another series of compounds containing substituents on the steroid A-ring has been studied as inhibitors of ES, many of which showed comparable inhibitory activity to EMATE. In particular, a series of halogenated derivatives were considered, as well as a number of compounds containing electron-withdrawing groups (Table 1.11). For example, compound **75** contains a 2-nitro group and was found to possess an IC_{50} value of 0.07μM against ES in intact MCF-7 cells (Woo et al, 1996a), (under similar conditions, EMATE was found to possess an IC_{50} value of 8nM).



Compound	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	OCH_2CH_3	H	2nM[2,7]
83	C=O	OCH_3	H	30nM[1,6]
84	CH(β-SO_2NH_2)	OCH_3	H	39nM[1,8]
EMATE	-	-	-	8nM[11]

Table 1.11: Examples of EMATE derived irreversible ES inhibitors [[1]Placental ES, [2]intact MCF-7 cells, inhibitory concentration [3]0.01μM and [4]0.1μM, [5] (Woo et al, 1996a), [6] (Purohit et al, 1998b), [7] (Kyowa Hakko Kogyo Co. Ltd., 2001), [8] (Poirier and Boivin, 1998), [9] (Reed et al, 2001), [10] (Hejaz et al, 1999), [11] (Potter and Reed, 2002a)].

This led to the synthesis of 4-nitro-EMATE (**76**) which was found to possess an IC_{50} value of 0.8nM, and was therefore considerably more potent than **75** and EMATE. Among the halogenated derivatives, 2-chloro-EMATE (**79**) was found to be the most potent, possessing an IC_{50} value of 0.8nM against ES from human placental microsomes (Reed et al, 2001). The synthesis and subsequent evaluation of other substituted (non-halogenated) compounds resulted in compounds which were found to be weak inhibitors in comparison to EMATE, e.g. 2-methoxy-EMATE (**83**) was found to possess an IC_{50} of 30nM (Purohit et al, 1998b) whereas the 2-ethoxy derivative (**82**) was found to possess an IC_{50} of 2nM (Tanabe et al, 1999).

Whilst sulfamoylated derivatives of E1 remain the major target for most workers within the field, a small number of workers have considered the use of non-sulfamated derivatives and this has resulted in the synthesis of a small range of potent and irreversible inhibitors of ES. Compound **89** (the 3-formyl derivative of E1) (Figure 1.9) was found to be the most potent of this range, with an IC_{50} of 0.42μM - in comparison, EMATE was found to possess an IC_{50} value of 0.056μM under the same conditions. The others in the study have been shown to possess poor inhibition, e.g. compounds **85** to **88** were all found to possess IC_{50} values greater than 50μM (Schreiner and Billich, 2004).

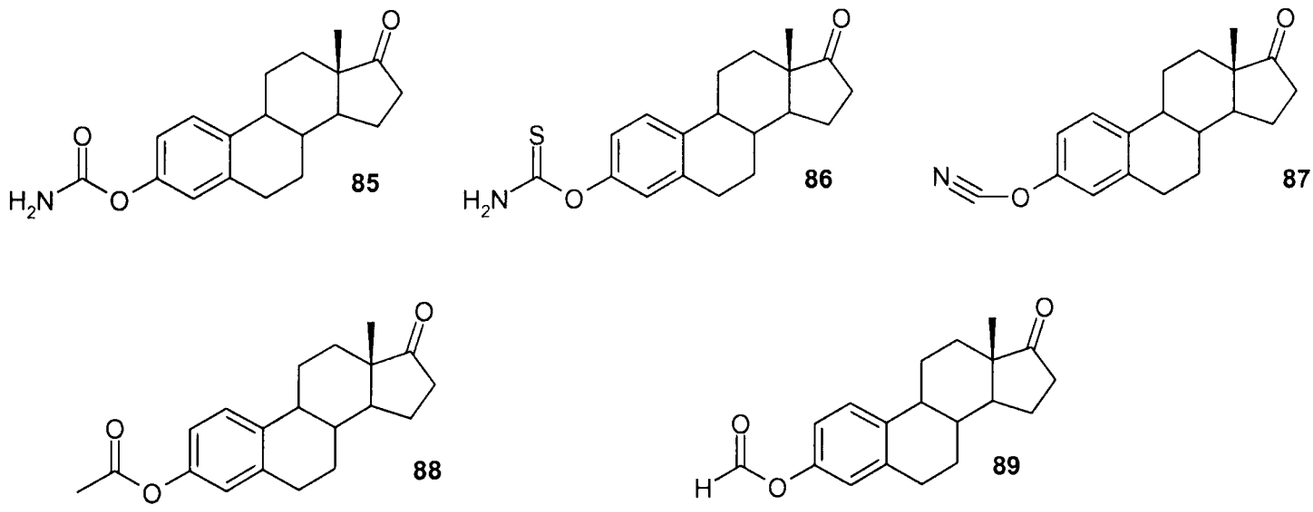


Figure 1.9: Non-sulfamoyl based irreversible inhibitor of ES.

1.15.1 Non-steroidal inhibitors

In an effort to overcome the primary major drawback in steroidal ES inhibitors, i.e. possession of estrogenic activity, the synthesis of non-steroidal, and thus non-estrogenic inhibitors, has been a major area of focus.

A substituted indole backbone was present in the first non-steroidal inhibitors synthesised, and of these, the most potent was found to be 3-methyl-1-pentafluorophenylmethyl-6-sulfooxy-2-(4-sulfooxyphenyl)-4-trifluoromethyl indole (compound **90**) (Figure 1.10) which was found to be a reversible competitive inhibitor, possessing an IC_{50} of 80μM, using partially purified enzyme from calf uterus (Brinbock and Von Angerer, 1990).

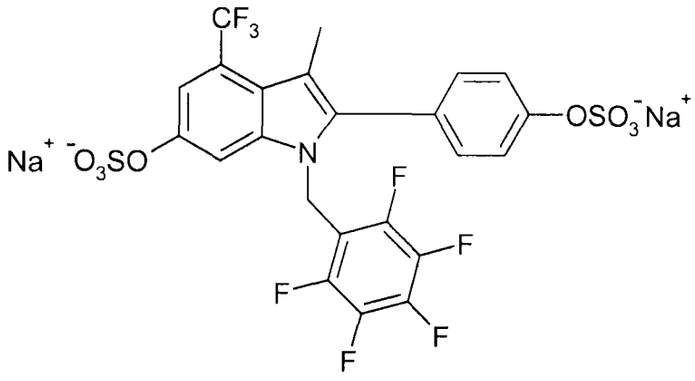


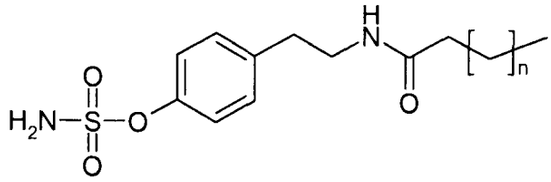
Figure 1.10. 3-Methyl-1-pentafluorophenylmethyl-6-sulfooxy-2-(4-sulfooxyphenyl)-4-trifluoromethylindole (**90**).

The development of the steroidal inhibitors demonstrated that in mimicking the steroidal backbone of E1S, the inhibitors possessed some estrogenic activity, therefore development of the non-steroidal inhibitors focused upon partially mimicking sections of the steroidal backbone. These will be discussed below.

1.15.2 A ring mimics

A series of A ring mimics based around 4-*O*-sulfamoyl-*N*-alkanoyl tyramine (Table 1.12) was studied (Li et al, 1996). It was hypothesised that the phenyl group would mimic the A-ring of the steroid backbone whilst the alkyl group would match the hydrophobic requirements of the steroidal substrate; as such, the extension of

the alkonyl chain resulted in an increase in potency. The most potent compound of the series was compound **98**, which was found to possess an IC_{50} value of 56nM, whilst the least potent was compound **91**, which was found to possess an IC_{50} value of 14.3μM.



Compound	n	IC_{50} (nM)
91	4	14300
92	5	1880
93	6	600
94	7	253
95	8	180
96	9	74
97	10	61
98	11	56
99	12	158

Table 1.12: First mono-aryl tyramine based ES inhibitors (Li et al, 1996).

In a more recent study by Ciobanu et al (2002), a tyramine-based compound (compound **100**) (Figure 1.11) was found to be more potent than EMATE, possessing an IC_{50} value of 0.4nM in homogenates of HEK-293 cells transfected with ES (EMATE possessed an IC_{50} values of 0.9nM under similar conditions) (Ciobanu et al, 2002). Changes in the aliphatic chain length did not improve the potency of this compound, the activity decreasing with any alteration.

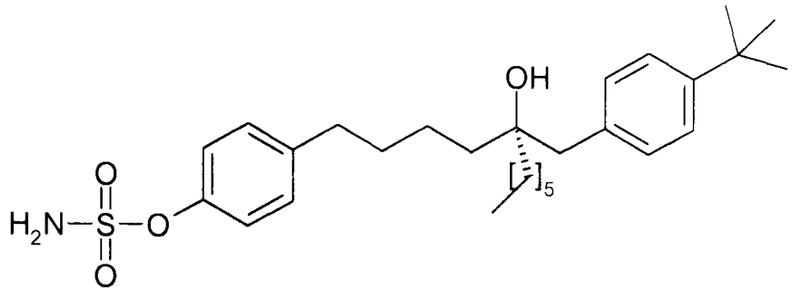


Figure 1.11: Potent inhibitor of ES (**100**) (Ciobanu et al, 2002).

In 1998, Woo et al published a proposed pharmacophore based on the observed SAR of the previous compounds, the main feature being the aminosulfonate group attached to a substituted phenyl ring, the substituents upon the ring being employed to promote activity (Figure 1.12) (Woo et al, 1998).

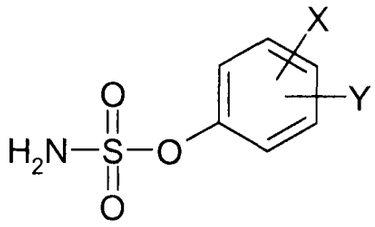
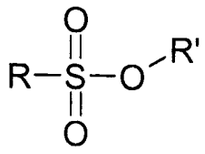


Figure 1.12: Proposed pharmacophore by Woo et al (1998), where X= H or substituent, Y= additional functionality including fused or adjacent/remote ring structures.

A series of substituted aminosulfonated phenols and aminosulfonated straight chain alkyl alcohols were synthesised within our own group to determine the SAR of the compounds and then subsequently determine the mechanism of inhibition. The aminosulfonated straight chain compounds (Table 1.13) showed interesting inhibitiory activity. For example, the unsubstituted aminosulfonated straight chain derivatives (**104-106**) were observed to lack any inhibitory activity (tested at 10mM); the methane sulfonated derivatives, however, displayed some inhibition (compound **107**, 28% inhibition at 1mM). Further investigation showed that the inclusion of halogens on the alkyl chain increased potency considerably with **101** showing 60% inhibition at 1mM (Ahmed et al, 1999b; Ahmed et al, 2000; Ahmed et al, 2002a; Ahmed et al 2002c)

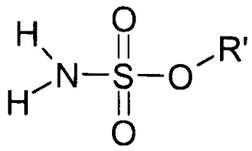


Compound	R	R'	Percentage inhibition
101	NH_2	$Cl_3C\text{-}CH_2$	60.0[2]
102	NH_2	$Cl_2HC\text{-}CH_2$	30.0[2]
103	NH_2	$ClH_2C\text{-}CH_2$	15.0[2]
104	NH_2	$CH_3\text{-}(CH_2)_5\text{-}CH_2\text{-}$	0[1]
105	NH_2	$CH_3\text{-}(CH_2)_6\text{-}CH_2\text{-}$	0[1]
106	NH_2	$CH_3\text{-}(CH_2)_7\text{-}CH_2\text{-}$	0[1]
107	CH_3	$CH_3\text{-}(CH_2)_7\text{-}CH_2\text{-}$	28.0[2]
108	CH_3	$CH_3\text{-}(CH_2)_{10}\text{-}CH_2\text{-}$	17.0[2]
COUMATE	NH_2	4-methyl coumarin	99.5[2]
EMATE	NH_2	Estrone	99.8[2]

Table 1.13: Sulfamated straight chain alcohol inhibitors of ES using placental microsome assay, [1] at inhibitor concentration of 10mM, [2] at inhibitor concentration 1mM (Ahmed et al, 2002a).

The aminosulfonated phenyl derivatives (Table 1.14) showed varied biological activity and were all less potent than EMATE and COUMATE (see section 1.15.3), but were found to possess a good trend in SAR data. The trend showed that there was a relationship between biological activity and pK_a, for example compound **111** with a pK_a of 10.0 was found to possess an IC_{50} of 2089μM however compound **123** with a pK_a of 8.28 was found to possess an IC_{50} of 120μM. An optimum pK_a value for increased activity was discovered around 8.3.

The results from the phenolic and straight chain compounds (Tables 1.13 and 1.14) led to the development of a revised pharmacophore (Figure 1.13).



Compound	R'	Percentage inhibition	IC_{50} (μM)	pK_a
109	Phenyl	29.7	>10000	-
110	4-Methylphenyl	27.4	>10000	10.2
111	3-Methylphenyl	39.5	2089±50	10.0
112	4-Fluorophenyl	37.0	537±21.2	9.8
113	3-Fluorophenyl	79.6	2089±50.0	9.16
114	4-Chlorophenyl	37.6	1585±66.1	9.5
115	3-Chlorophenyl	62.0	537±21.2	9.0
116	4-Bromophenyl	58.8	912±12.4	9.29
117	3-Bromophenyl	75.1	257±6.3	8.95
118	4-Iodophenyl	66.0	560±16.2	-
119	3-Iodophenyl	89.4	120±1.2	-
120	4-Cyanophenol	74.4	300±3.3	8.02
121	3-Cyanophenol	84.3	191±4.3	8.54
122	4-Nitrophenol	82.5	330±10.3	7.15
123	3-Nitrophenol	90.4	120±3.9	8.28
COUMATE	4-methyl coumarin	99.5	12±0.16	-
EMATE	Estrone	99.8	0.5±0.01	

Table 1.14: Sulfamated substituted phenolic inhibitors of ES using placental microsome assay, at inhibitor concentration 1mM (Owen et al, 2002e)

As a result of the revised pharmacophore, a number of A ring mimics were synthesised and evaluated, including a number of sulfamated phenyl esters (Tables 1.15 and 1.16). The compounds were found to be potent inhibitors of ES and were pivotal in aiding the understanding of the mechanism of ES and the role of the physicochemical factors involved in the inhibition of ES (Ahmed et al, 2001a; Ahmed et al, 2001c; Ahmed et al 2002c; Owen et al, 2003).

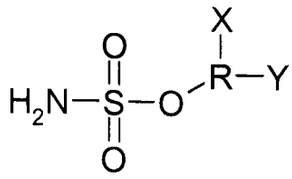
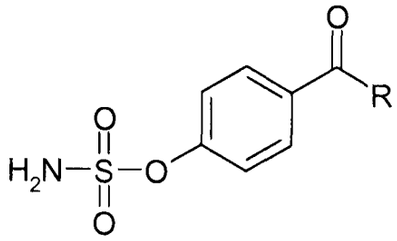


Figure 1.13: New pharmacophore for the inhibition of ES by Ahmed et al (2002b) (R=aliphatic or aromatic carbon backbone, X= electron withdrawing groups, Y=additional functionality including fused or adjacent/remote ring structures).

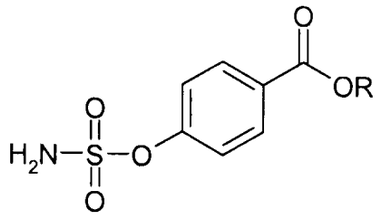


Compound	R	IC_{50} (μM)
124	H	254
125	CH_3	302
126	C_2H_5	116.4
127	C_3H_7	39.8
128	C_4H_9	20.9
129	C_6H_{13}	5.0
130	C_7H_{15}	5.6
131	C_8H_{17}	3.4
132	C_9H_{19}	13
EMATE		0.5

Table 1.15: Sulfamated phenyl ketone inhibitors of ES using placental microsome tissue assay (Ahmed et al, 2000).

Consideration of the compounds based on the 4-*O*-sulfamoylated derivatives of 4-hydroxyphenyl ketones (compounds **124** to **132**) resulted in the discovery of compound **131**, which was found to possess an IC_{50} of 3.4μM, as such, it was found to be more potent than COUMATE but less potent than EMATE; all compounds under study were found to be irriversible inhibitors, inhibiting ES in a

time- and concentration-dependent manner due to the aminosulfonyl moiety (Patel et al, 2003).



Compound number	R	Activity IC_{50} (μM)
133	CH_3	31.6
134	C_2H_5	31.6
135	C_3H_7	13.2
136	C_4H_9	10.5
137	C_5H_{11}	5.9
138	C_6H_{13}	3.8
139	C_7H_{15}	3.4
140	C_8H_{17}	5.0
141	C_9H_{19}	4.8
142	$C_{10}H_{21}$	22.4
143	*c*-C_5H_9	9.3
144	*c*-C_6H_{11}	1.7
145	*c*-C_7H_{13}	0.5
146	*c*-C_8H_{15}	0.17
-	EMATE	0.5
-	COUMATE	13.8
-	667-COUMATE	0.21

Table 1.16: Sulfamated phenyl ester inhibitors of E1S evaluated against placental microsome (Patel et al, 2003).

Ester-based compounds were also synthesised (Table 1.16), in particular, the *n*-alkyl chain containing esters (**133** to **142**), resulted in inhibitors that were comparable in activity to the phenyl ketone-based compounds, most notably

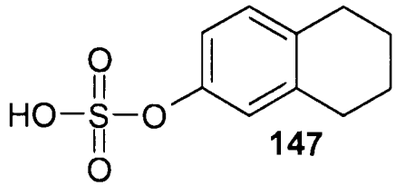
compounds **138**, **139** and **142**, which possessed IC_{50} values of 3.8μM, 3.4μM and 5.0μM respectively (Patel 2003a, Patel et al, 2003; Patel et al, 2004). Derivatisation of the straight alkyl chain moiety led to the development of compound **146** (possessing an IC_{50} value of 0.17μM) which was found to be more potent than 667-COUMATE (see section1.15.4) (possessing an IC_{50} value of 0.21μM) (Patel et al, 2003).

Non-steroidal formate derivatives were investigated following on from the discovery of the steroidal formate inhibitors. These were tested against ES in comparison to the sulfamated derivative of the same parent compounds. It was observed that the formate derivative of phenol was inactive up to a concentration of 30μM, in comparison to the sulfamated derivative which displayed inhibition of IC_{50}>10μM, thus showing that the formate was a less potent inhibitor than the sulfamate (Schreiner, 2004).

1.15.3 AB ring mimics

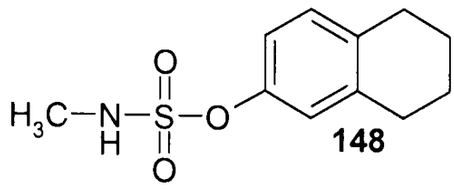
Other workers investigated mimicking the A and B rings of E1S, and as such a series of tetrahydronaphthol (THN) sulfamate derivatives was the first to be synthesised (**147-152**, Figure 1.14). Modifications were undertaken upon the ring structure, incorporating functional groups into the ring structure and substitutions upon the ring with varied activity (**153-148**, Figure 1.15). The most potent compound was **153**, which was found to be a weak irreversible inhibitor with an IC_{50} of 1mM in MCF-7 cell lines (Reed and Potter, 1998).

A number of coumarin-based sulfamate derivatives was synthesised (compounds **159-165**, Table 1.17). These displayed non-estrogenic activity and were found to inhibit ES in a time- and concentration-dependent manner, with compounds **162**, **164** and **165** showing 99% inhibition at 10μM (Woo et al, 2000; Woo et al, 1998).



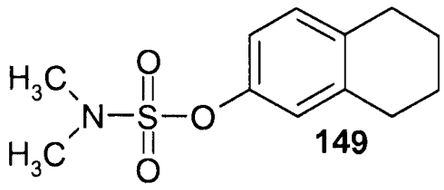
147

IC_{50} 1μM[a], 100μM 12.5%[2]



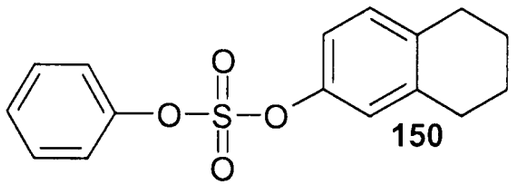
148

IC_{50} 25μM[1]



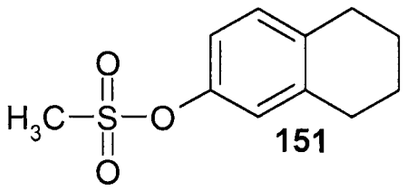
149

IC_{50} 750μM[1]



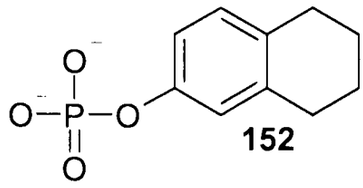
150

10μM 0%[1]



151

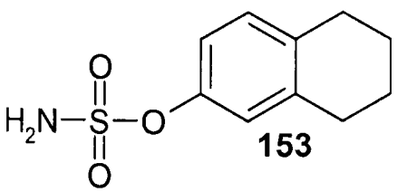
10μM 0%[1]



152

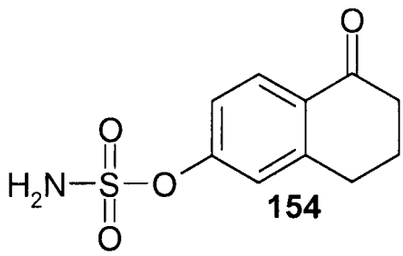
K_i 360μM (pH 7.0)[2]

Figure 1.14: THN sulfamate derivatives and activity ([1]intact MCF7 breast cancer cell line assay; [2]human placental microsome assay) (Reed and Potter, 1998).



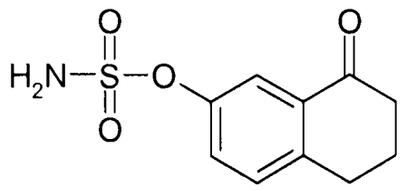
153

10μM 19.9%



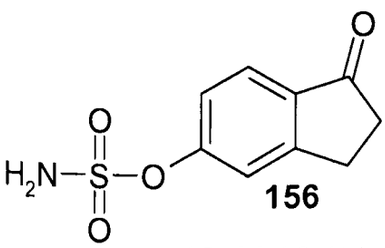
154

10μM 16.1%



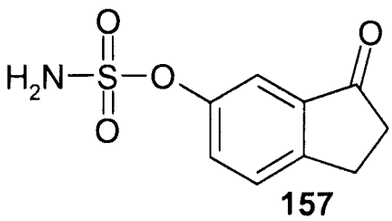
155

10μM 12.3%



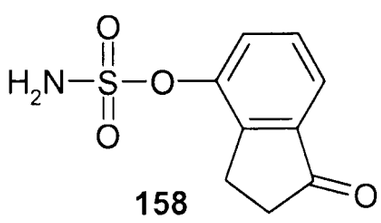
156

10μM 16.1%



157

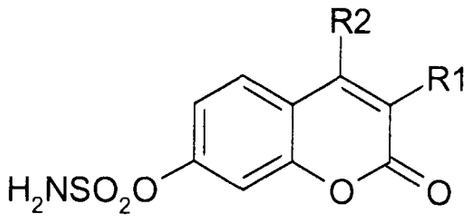
10μM <10%



158

10μM <10%

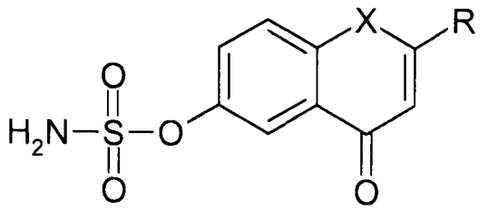
Figure 1.15: Modified THN sulfamate derivatives and their activity (human placental microsome assay) (Reed and Potter, 1998).



			% Inhibition of ES in placental microsomes	
Compound	R1	R2	At 1μM	At 10μM
COUMATE (**159**)	H	CH_3	63	93
160	H	H	-	78
161	CH_3	CH_3	88	97
162	H	CH_2CH_3	88	>99
163	H	$CH_2(CH_3)CH_3$	94	96
164	CH_2CH_3	CH_3	96	>99
165	$CH_2(CH_3)CH_3$	CH_3	97	>99

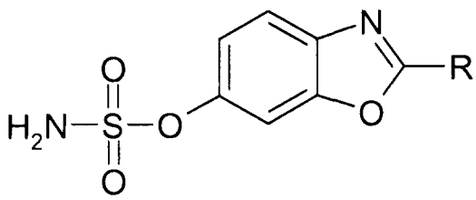
Table 1.17: COUMATE and COUMATE derivatives and their respective activity (adapted from Woo et al, 2000; Woo et al, 1998).

Other compounds were synthesised by other workers using the coumarin-based compounds as lead compounds (Tables 1.18 and 1.19). Some of these compounds were found to possess good inhibitory activity, in particular, the 1-adamantyl derivatives of COUMATE (**171** and **172**), which diplayed IC_{50} values of 5.6 and 0.34nm respectively. The oxazolidine derivatives (Table 1.19) were found to be much poorer inhibitors than COUMATE, with the 1-adamantyl aminosulfamated oxazolidine derivative (**173**) being 500 times less potent than the corresponding COUMATE derivative (IC_{50}=2800nM)



Compound	X	R	Activity IC_{50} (nM)
166	O	*n*-Propyl	722
167	O	*n*-Nonyl	403
168	O	1,1-Dimethylnonyl	78
169	O	*t*-Butyl	22
170	O	4-Pentylbicyclo-[2.2.2]-oct-1-yl	11
171	O	1-Adamantyl	5.6
172	S	1-Adamantyl	0.34

Table 1.18: Modified aminosulfonated isocoumarin derivatives (Novartis, 2002a; Nussbaumer et al, 2002a).



Compound	R	Activity IC_{50} (nM)
173	1-Adamantyl	2800
174	(1-Adamantyl)methyl	1792
175	(2-Adamantylidene)methyl	196
176	Cyclohexylidenemethyl	319

Table 1.19: Modified aminosulfonated oxazolidine derivatives (Novartis, 2002b; Schreiner et al, 2003).

6-Adamantan-2-ylidene-hydroxy-benzoxazole (**177**, Figure 1.16) was derivatised as a formate, and evaluated against ES in comparison to the sulfamated derivative of the same molecule. It was observed that the formate derivative

possessed an IC_{50} value of 1.5μM in comparison to the sulfamated derivative of **177** (IC_{50}=0.26μM, thus displaying that the formate derivative was less potent in comparison to the sulfamate (Schreiner, 2004).

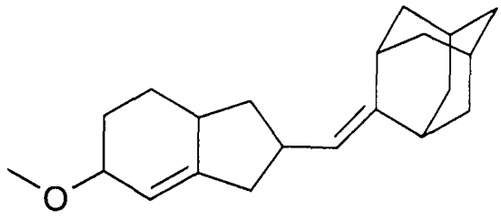
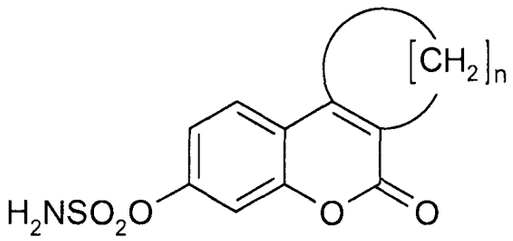


Figure 1.16: 6-adamantan-2-ylidene-hydroxy-benzoxazole (**177**).

1.15.4 ABC ring mimics

Following on from the derivatives of COUMATE, a series of tricyclic compounds was synthesised, which resulted in a series of highly potent non-steroidal inhibitors of ES. Ring formation across C(3)-C(4) of the coumarin backbone led to a series of compounds, of which 667-COUMATE (**180**) possessed the most potent inhibitory activity (IC_{50}=8nM, in placental microsomes) (**178-181**, Table 1.20), as such, it was found to be more potent than EMATE (IC_{50}= 25nM). 667-COUMATE also entered phase 1 clinical trials (Woo et al, 2000).

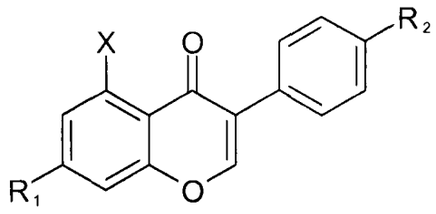


		% Inhibition of ES in placental microsomes	
Compound	n	At 0.1μM	At 1μM
178	5	37	91
179	6	63	93
667-COUMATE (180)	7	91	>99
181	8	89	>99

Table 1.20: Tricyclic COUMATE derivatives and their respective inhibitory activity (adapted from Woo et al, 2000).

1.15.5 ABD ring mimics

A range of isoflavone sulfamate-based compounds was investigated as potential mimics of the steroidal ABD rings (**182** to **186**) (Table 1.21). They were found to be potent and irreversible inhibitors of ES, with the monosulfamate compound (**185**) showing 83% inhibitory activity in intact MCF-7 cells at 1μM whilst the bis-sulfamate derivative (**186**) was found to be the most potent of the range, showing 90% inhibitory activity under the same conditions; they were however less potent than EMATE. The flavonoid derivatives, in particular, their metabolite (**184**), were found to possess potent estrogenic property. The sulfate derivatives, daidzein 4',7-di-*O*-sulfate (**182**) and daidzein 4'-*O*-sulfate (**183**), were found to possess IC_{50} values of 6μM and 1.5μM respectively, and were found to be potent competitive inhibitors of ES, with K_i values of 1μM and 5.91μM respectively (Wong and Keung, 1997).



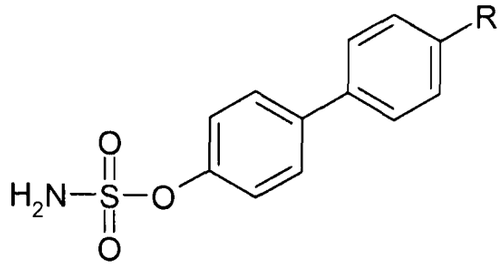
Compound	X	R_1	R_2	Activity
182	H	OSO_3^-	OSO_3^-	1μM[1]
183	H	OH	OSO_3^-	5.9μM[1]
184	H	OH	OH	0[2]
185	OH	OSO_2NH_2	H	83%[2]
186	OH	OSO_2NH_2	OSO_2NH_2	90%[2]

Table 1.21: Natural flavonoid-based inhibitors of ES ([1]K_i in μM), [2]percentage inhibition in intact MCF7 cells at 1μM (Wong and Keung, 1997).

1.15.6 AC ring mimics

Using molecular modelling data and SAR studies, a series of AC ring mimics was synthesised and biochemically evaluated (Table 1.22) (Ahmed et al, 2002a;

Ahmed et al, 2002c; Ahmed et al, 2002d). In particular, a series of compounds based on the biphenyl backbone [that had previously been acknowledged as a potential steroid mimic (Abell and Henderson, 1995)] was considered.



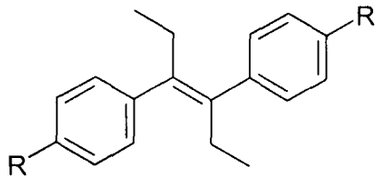
Compound	R	IC_{50} (μM)
EMATE	-	0.1
187	4-CN	6.7
188	4-COOMe	5.2
189	4-COOEt	4.2
190	4-COOPr	3.5
191	H	76

Table 1.22: Biphenyl-based inhibitors of ES (Ahmed et al, 2002a; Ahmed et al, 2002c, Ahmed et al, 2002d).

The biphenyl moiety was derivatised with various functional groups, for example, a cyano group was added at the 4- position (compound **187**), which was found to possess 94% inhibition against ES at 3nM (Koizumi, 2001). The unsubstituted biphenyl sulfamate (**191**) was found to possess an IC_{50} value of 76μM (EMATE displayed an IC_{50} value of 0.1μM under similar conditions), and was the weakest of the range. A series of 4-alky-ester derivatives was also investigated (**188** to **190**, Table 1.22) and although they displayed potentcy 10 to 20 times more potent than the parent biphenyl, they proved to be weak inhibitors of ES when compared to EMATE. Consideration of the SAR suggested that the carbonyl moiety on the 4-alkyl-ester derivatives may undergo hydrogen bonding with the active site, in particular, the C=O group may mimic estogen's D-ring carbonyl interaction with the ES active site, and that this interaction may be in some part responsible for the potency observed within these compounds (Ahmed et al, 2002d).

1.15.7 AD ring mimics

AD ring mimics were among the first non-steroidal inhibitors to be synthesised, specifically a range based on stilbene (Table 1.23). Of the range, stilbene bis-sulfate (compound **192**) was found to display potent inhibition against ES (IC_{50}=10nM, intact MCF-7 cell line assay) (Reed et al, 1996). This was expected since the compounds are based upon stilbene, a known estrogen mimic, but as a result, their use is limited as the trans-1,2-diphenylethylene [or (*E*)-stilbene] (and its derivatives) are known to be highly estrogenic (Chen et al, 1996).



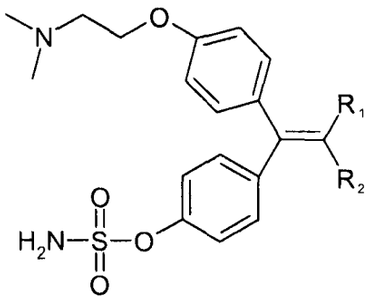
Compound	R	IC_{50}
192	OSO_2NH_2	10nM
193	$OSO_2N(CH_3)_2$	10μM

Table 1.23: Examples of AD-ring mimics as inhibitors evaluated against intact MCF-7 cells (Reed et al, 1996).

Derivatisation of the sulfamate group, more specifically, replacing the sulfamide nitrogen hydrogens by methyl groups (compound **193**) decreased inhibitory activity. The compound was found to be significantly less potent than **192** (IC_{50}=10μM, intact MCF7 breast cancer cell line assay) and was also found to be a reversible inhibitor, thus demonstrating the importance of the sulfamate moiety (Reed et al, 1996).

Hydroxytamoxifen sulfamate derivatives were also investigated (Table 1.24); two compounds: the (*E*)-isomer (**194**) and (*Z*)-isomer (**195**) of hydroxytamoxifen were evaluated against ES from rat liver microsomes and were found to possess apparent K_i values of 35.9μM and 500μM (Chu et al, 1999). Interestingly, both **194** and **195** was found to be reversible inhibitors, indeed, these two compounds are the only sulfamate-based compounds *todate* that do not possess irreversible

inhibition against ES. After investigation of the SAR of the hydroxytamoxifen derivatives, it was postulated that the orientation of the phenyl ring system clearly affected the ability of the compound to bind to the active site. It was also proposed that in the case of compound **195**, the poor inhibitory activity was due to steric hindrance (Chu et al, 1999).

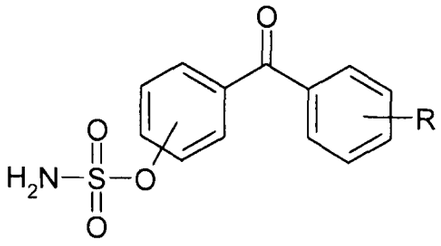


	R_1	R_2	$K_i$app
194	Ph	C_2H_5	35.9μM
195	C_2H_5	Ph	>500μM

Table 1.24: Hydroxytamoxifen sulfamate ES inhibitors [rat liver microsomes (Chu et al, 1999)].

1.15.8 Miscellaneous non-steroidal inhibitors

Nussbaumer et al (2002b) investigated the benzophenone moiety as a ligand backbone, resulting from this, a series of potent benzophenone disulfamates were evaluated (Table 1.25). Compounds **196-204** displayed very similar potency to each other indicating that the presence of the side groups at the 3 and 4 positions provides no realistic benefit, but further side chains in these positions could promote activity. The bis-sulfamates (compounds **205-207**) showed a significant improvement in biological activity, particularly with the substitution in the 4 position (compound **207**), being only 3.5 times less potent than EMATE (Nussbaumer et al, 2002b)



	Position of		IC_{50} (μM)
	$-OSO_2NH_2$	R	
EMATE	-	-	0.056
196	4	H	5.1
197	3	H	5.7
198	4	3-OMe	5.2
199	4	2-OMe	4.8
200	4	2-OH	4.6
201	3	3-OMe	7.1
202	3	3-OH	6.9
203	3	4-OMe	6.9
204	3	4-OH	5.0
205	3	$3-OSO_2NH_2$	3.2
206	3	$4-OSO_2NH_2$	0.78
207	4	$4-OSO_2NH_2$	0.19

Table 1.25: ES inhibitors based on benzophenone sulfamate [recombinant human ES in comparison to EMATE (Nussbaumer et al, 2002b)].

During high-throughput screening of a library of compounds against ES, Nussbaumer et al (2003) discovered a novel reversible inhibitor that inhibited ES in a non-time-dependent manner (**208**). In an effort to rationalise the SAR of these compounds, **208** was derivatised (Figure 1.18 and Table 1.26). It was observed that the presence of an electronegative group on the benzylsulfonyl moiety increased biological activity, and that the size and position of this substituent also influenced activity, demonstrating a possible active site interaction. **219** (IC_{50}=1.89μM) was found to be the most potent compound in the study, but was still 60 times weaker than EMATE.

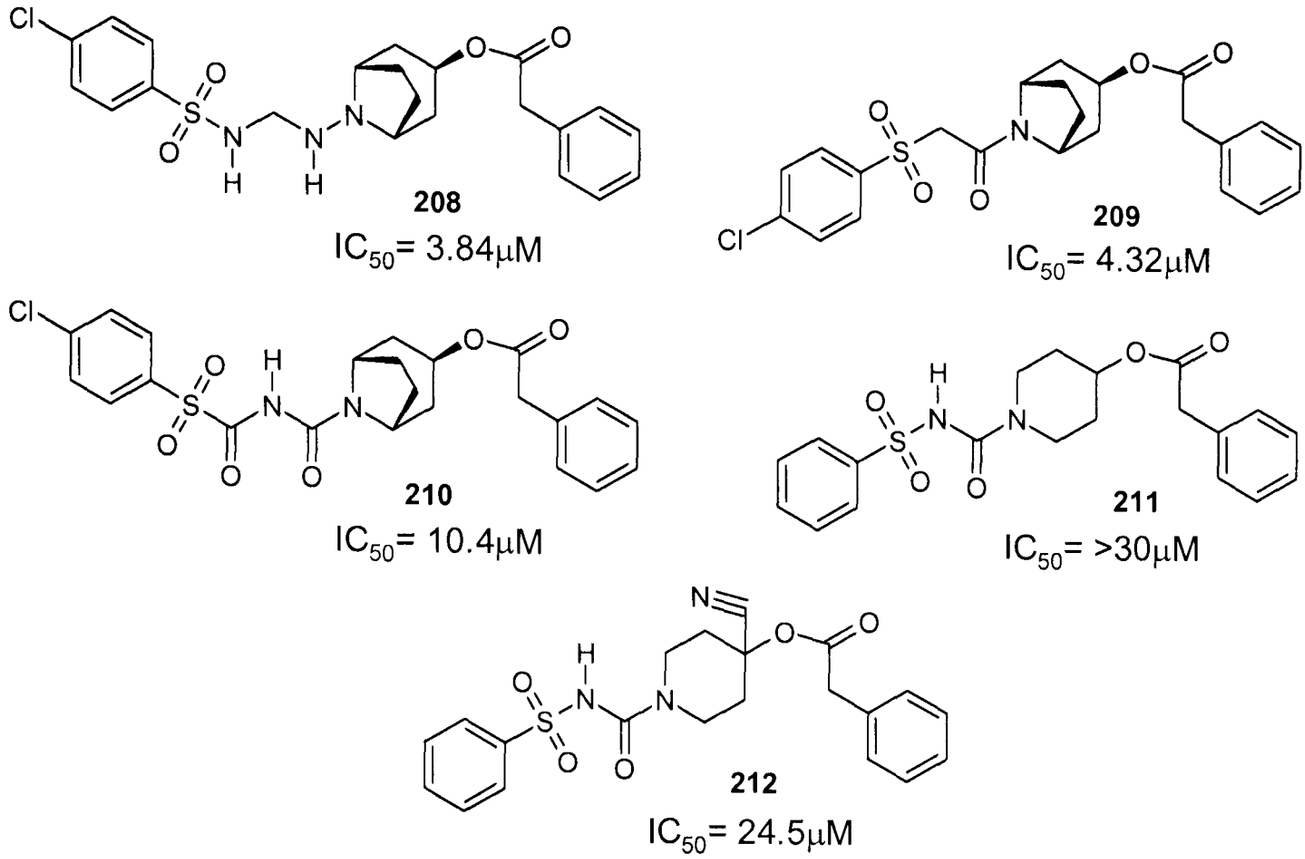
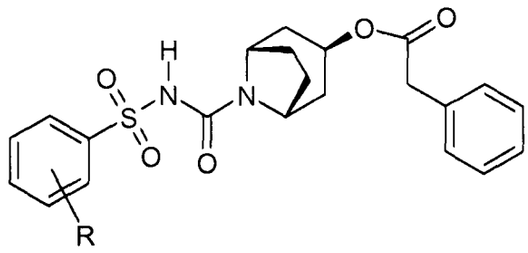


Figure 1.18: Novel nortropinyl-arylsulfonylureas, reversible inhibitors of ES (Nussbaumer et al, 2003).



	R=	IC_{50} (μM)
213	4-Cl	6.72
214	4-F	39.2
215	4-Br	6.15
216	4-H	>30
217	4-Me	37.1
218	4-CF_3	7.47
219	3,5-diCF_3	1.89

Table 1.26: Novel nortropinyl-arylsulfonylureas, reversible inhibitors of ES (Nussbaumer et al, 2003).

1.16 Mechanism of ES Inhibition

Woo et al (2000) suggested the first mechanism for the inhibition of ES by a sulfamate-based compound (Figure 1.19).

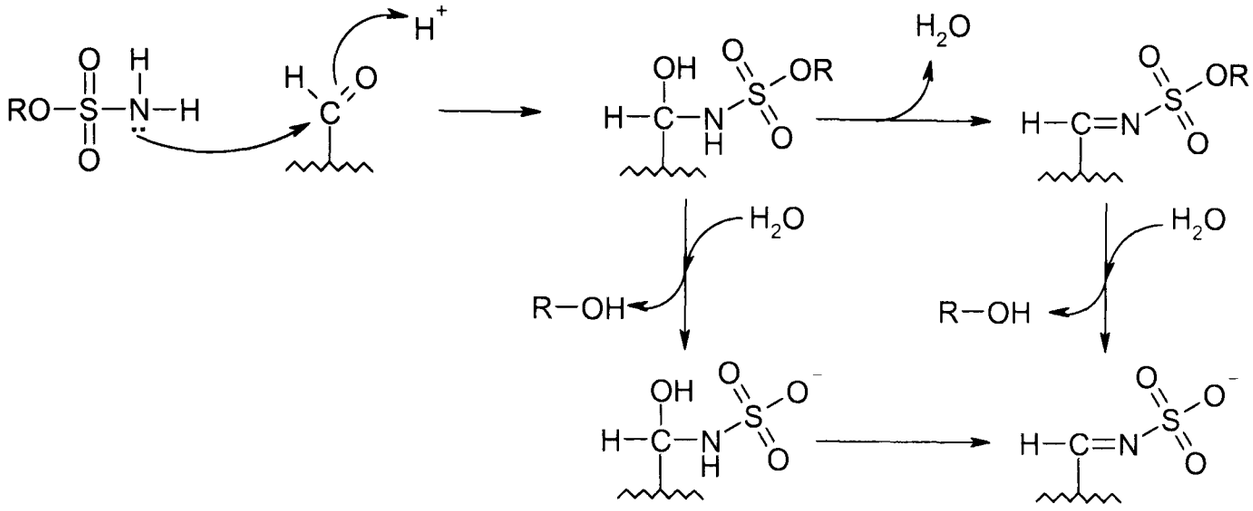


Figure 1.19: Proposed mechanism of ES inhibition (Woo et al, 2000).

However the straight chain sulfamate compounds [**109-123** (Ahmed et al, 2002e)] showed this mechanism to be incorrect. If the mechanism proposed by Woo et al (2000) was correct, then compounds **109-123** would have shown significant biological inhibition, as they possess the sulfamate moiety which would attack the aldehydic group within the acitve site, thus inhibiting ES; instead, the compounds were seen to be very poor inhibitors indeed.

Researching into a number of physicochemical properties, in particular pK_a and SAR data, led Ahmed et al (2001b, 2002a, 2002e) to the current, recognised mechanism for sulfamate inhibition of ES (Figure 1.20).

The initial step is believed to involve the cleavage of the S-OR bond via the nucleophilic attack of the sulfur in the aminosulfonate group by a lone pair of electrons from the *gem*-diol. This is the pivotal step as the attack enables the formation of the RO^- ion and more importantly, the formation of an aldehydic moiety within the active site. The sulfamic acid subsequently attacks the aldehydic group via the NH_2 moiety, resulting in the irreversible inhibition of the enzyme via the formation of an imine functionality.

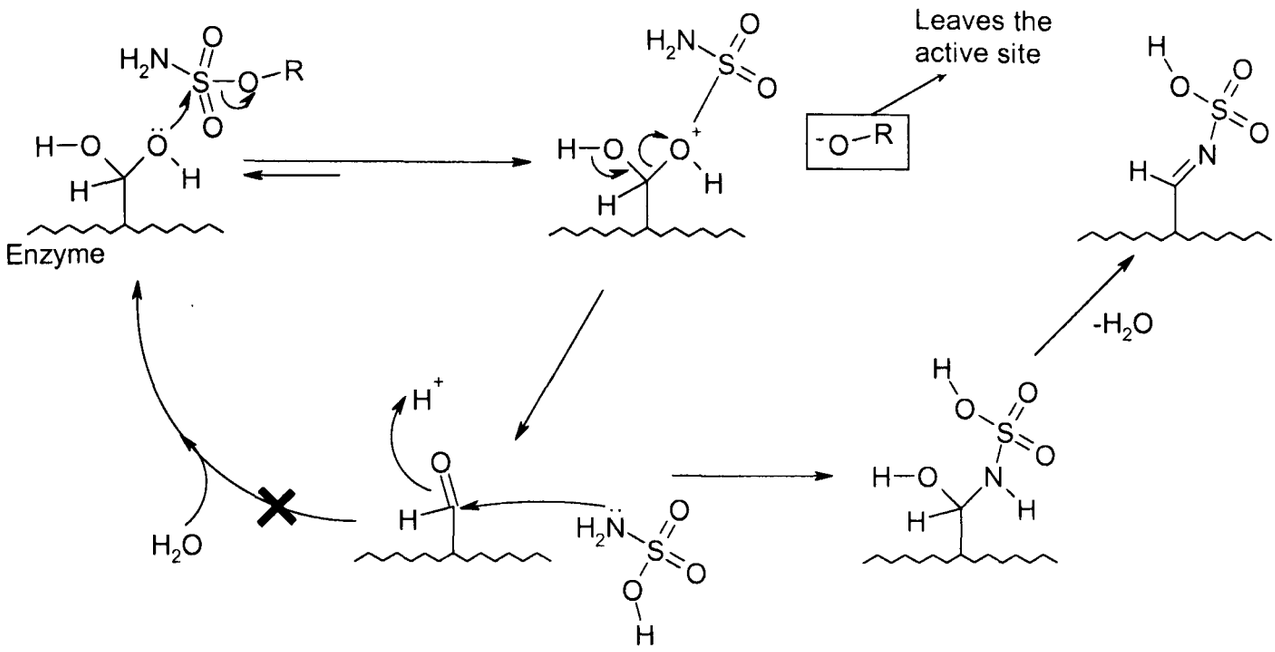


Figure 1.20: Mechanism of ES inhibition (adapted from Ahmed et al, 2002a).

1.17 Basis of present investigation

The use of estrogen ablation therapy has been the major focus for the treatment of estrogen-dependent breast cancer. The current treatments attempt to decrease the production of estrogen within tumour cells by interrupting the stages of the biosynthetic pathways that lead to the formation of E1 and E2 within the steroidal cascade, as such, a number of aromatase inhibitors have recently entered the clinic. ES is the enzyme responsible for the conversion of E1S, the stored form of the estrogen, to its non-conjugated and active form, E1 and which can therefore be metabolised to the more potent estrogen, namely E2. The inhibition of ES has been shown to lead to a marked decrease in the levels of circulating E1 in the body of postmenopausal women and therefore to the loss of stimulus for estrogen-dependent breast cancer cells in postmenopausal women.

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 ES has been synthesised based on the sulfamated derivatives of 4-hydroxyphenyl ketone based compounds. However, the sulfamate derivatives of 4-hydroxybenzoic acid, in particular, the ester (both alkyl and cycloalkyl) based compounds were shown to possess significant levels of inhibition against ES, indeed compound **146** (4-sulfamoyloxy-benzoic acid cyclooctyl ester) was found to be slightly more potent than 667-COUMATE. In previous studies, it has been discovered that a decrease in the pK_a of the parent phenolic compound has resulted in an increase in the inhibitory activity of the sulfamate derivatives. In an effort to investigate the potential use of alternative sulfonate groups in the inhibition of ES, we considered the use of previously reported physicochemical factors in the design and synthesis of novel inhibitors of ES. As such, the initial aim of the study involves the use of alternative sulfonate functionalities (e.g. methanesulfonate and trifluoromethane sulfonate moieties) so as to lead to the synthesis of potential inhibitors of ES. That is, we proposed to use the previously reported increase in stability of the phenoxide ion (and which led to an increase in the inhibitory activity of the sulfamate based compounds) to

increase the potency of methanesulfonate based compounds, through the use of factors such as pK_a (involving the incorporation of bromine atoms into the phenyl ring system), as such, the derivatisation of 4-hydroxyphenyl carbonyl containing compounds is a major target within the current project (Figures 1.21 and 1.22). Furthermore, so as to correlate the biological activity of the synthesised compounds with the acidity of the phenolic OH moiety, the pK_a of the parent phenolic compounds will be undertaken and the compounds evaluated for inhibitory activity against rat liver microsomes.

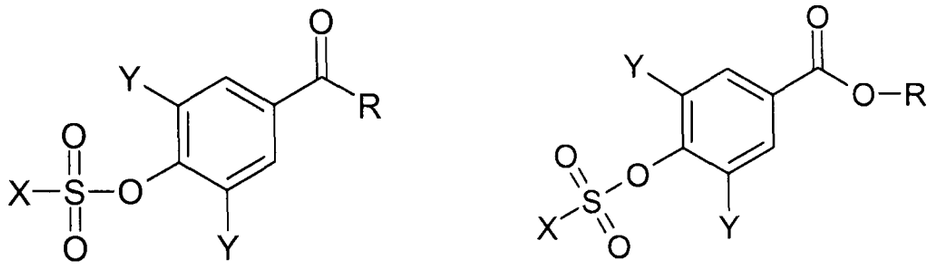


Figure 1.21: Proposed inhibitors (R= alkyl or aryl moiety; X= CH_3 or CF_3; Y= H or Br)

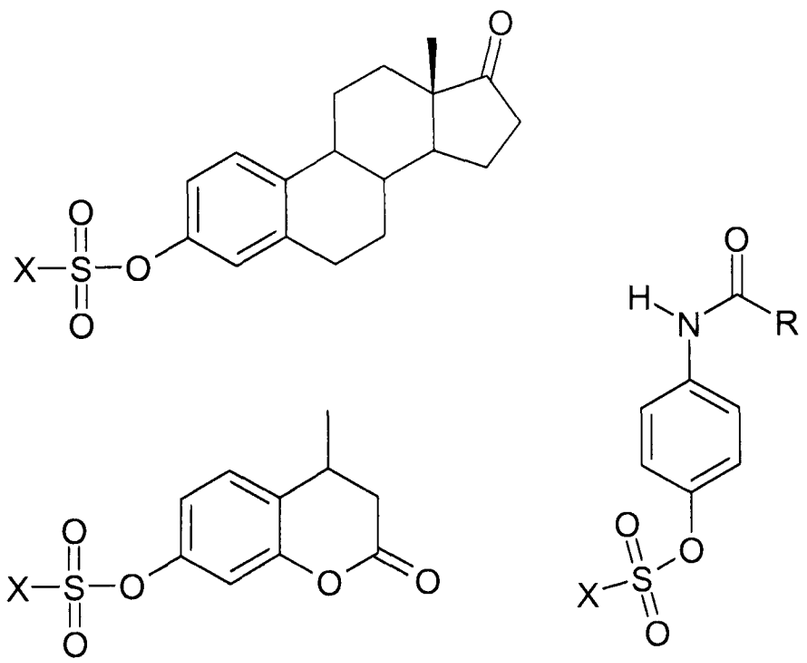


Figure 1.21: Proposed inhibitors (R= alkyl or aryl moiety; X= CH_3, CF_3, NH_2, $N(CH_3)_2$)

Chapter 2: Synthesis of hydroxybenzoic acid esters and derivatives

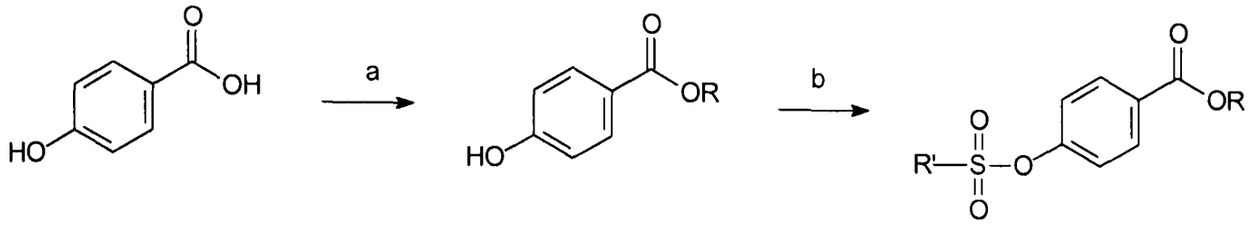
2.0 Synthesis of hydroxybenzoic acid esters and derivatives

2.1 Discussion

As previously mentioned, the sulfamate moiety appears to possess potent irreversible inhibitory activity when attached to an aromatic ring system. More specifically, James (2000) and Patel (2003a) have shown that 4-sulfamoylated derivatives of benzoic acid possess potent inhibitory activity against ES. Furthermore, it was suggested that increasing the stability of the phenoxide ion may potentially lead to potent inhibitors of ES. Indeed, Ahmed et al (2001a) have shown that the use of electron-withdrawing groups (such as nitro, cyano and bromine functionalities) resulted in an increase in the potency of inhibitors based on the benzoic acid backbone. However, highly electron-withdrawing groups which were able to stabilise the phenoxide ion (e.g. nitro moiety) resulted in non-enzymatic hydrolysis of the sulfamate moiety, thereby leading to a slight decrease in potency when compared to the brominated derivatives due to the degradation of the inhibitor. Whilst the sulfamate based compounds have been extensively considered within the literature, the use of alternative sulfonate functionalities has been ignored since previously these have been shown to possess weak inhibitory activity against ES. In an attempt to improve the inhibitory activity of these alternative sulfonate containing compounds, we considered the previous reports regarding the structure-activity relationship (SAR) determination of ES inhibitors and concluded that the use of RO^- (where R=phenyl moiety within the phenoxide ion) stabilising groups may lead to an increase in inhibitory activity in compounds containing non-sulfamated groups. We therefore considered the synthesis of methanesulfonate derivatives of 4-hydroxybenzoic acid – it should be noted that the sulfamate derivatives have been reported previously as potent inhibitors of ES and were therefore not repeated within the current study.

In the synthesis of the non-brominated derivatives of the 4-methanesulfonates of 4-hydroxybenzoic acid, the reactions outlined in Scheme 2.1 were undertaken and were found to progress in moderate to excellent yield [ranging from ~82% for

compound **260** (propyl 4-methanesulfonylbenzoate) to ~55% for compound **266** (nonyl 4-methanesulfonylbenzoate)] and without any major problems.



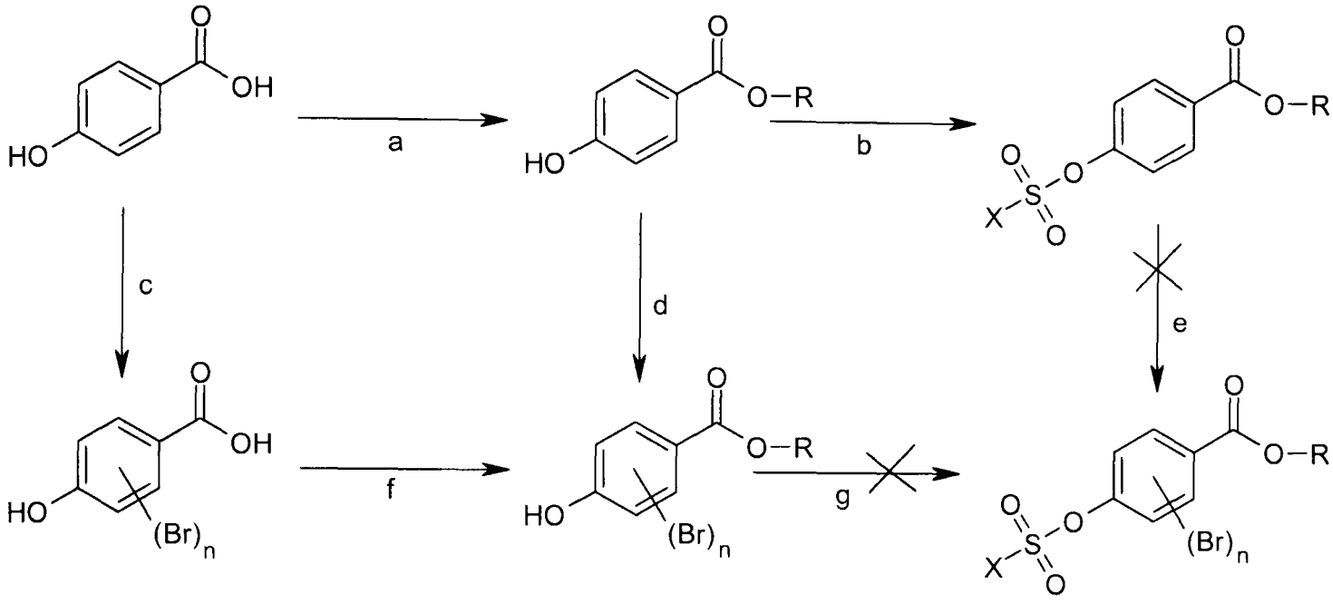
Scheme 2.1: Synthesis of sulfonate derivatives of esters of 4-hydroxybenzoic acid (where a=ROH/H^+/Δ; b=sulfonyl chloride/DCM; R= alkyl and cycloalkyl moiety; R'=CH_3).

In an effort to observe any increase in inhibitory activity with increased stability of the RO^- ion, we considered the synthesis of mono- and dibrominated derivatives of 4-hydroxybenzoic acid (followed by the subsequent conversion to the methanesulfonate derivative) - as proposed in the mechanism of inhibition of ES, the increased stability of the phenoxide ion would be expected to decrease the stability of the S-OR bond and thereby result in increased inhibitory activity (Ahmed et al, 2002a).

In the synthesis of the sulfonate derivatives of esters of 3-bromo- and 3,5-dibromo-4-hydroxybenzoic acid, three routes exist (Scheme 2.2). That is: the first route involves the synthesis of the initial ester derivative of 4-hydroxybenzoic acid followed by the bromination of the phenyl ring prior to sulfamoylation (steps a, d and g; Scheme 2.2); the second route, however, involves the initial bromination of 4-hydroxybenzoic acid followed by the esterification of the carboxylic acid moiety which is then followed by the conversion of the 4-hydroxy moiety to the sulfonate derivative (steps c, f and g; Scheme 2.2), and; the final potential route involves the initial synthesis of the ester followed by the derivatisation of the 4-hydroxy moiety through the sulfonation reaction prior to the bromination step (steps a, b and e; Scheme 2.2).

In the synthesis of the target 3-monobrominated compounds (**234** to **243**), we considered the literature methods for the bromination of 4-hydroxybenzoic acid derivatives. In general, the bromination step was undertaken using the ester

derivative as opposed to the carboxylic acid derivative (Cavill and Vincent, 1945). For example, Samson and Santos (1934) undertook the initial synthesis of methyl 3-bromo-4-hydroxybenzoate followed by the bromination of the methyl ester using an excess of bromine water, however, a high proportion of the dibrominated product was formed as a result of this route.

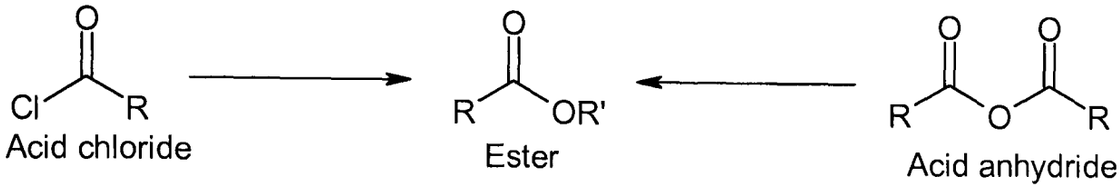


Scheme 2.2: Synthesis of the sulfonate derivatives of esters of 4-hydroxybenzoic acid (a and f=ROH/H^+/Δ; b and g=sulfonyl chloride/DCM/TEA; c, d and e=Br_2/CH_3COOH; n=0, 1 or 2; R= alkyl and cycloalkyl moiety; X=NH_2, CH_3 or CF_3).

An alternative method of brominating the esters of 4-hydroxybenzoic acid involved the reaction between the appropriate ester and *N*-bromosuccinimide as the brominating reagent (Oberhauser, 1997). Using this method, yields of 93% to 97% were obtained of the target 3-bromo derivatives, however, as with the method of Sampson and Santos (1934), the dibrominated derivative was found to be the major by-product. As a result of the reported problems highlighted within the literature, we concluded that the esterification of the purchased 3-bromo-4-hydroxybenzoic acid was the better route to the target methanesulfonated derivatives.

A relatively rapid route to the synthesis of esters involves the reaction between the alcohols and an acid chloride or an acid anhydride (Scheme 2.3), the former being

highly reactive and therefore would be expected to give greater yield in less time in comparison to the alternative methods of esterification. However, due to the presence of the phenolic OH moiety, the use of acid chlorides or indeed anhydrides would lead to increased by-products, that is the more reactive carboxylic acid derivatives would be expected to react with the phenolic OH moiety leading to the synthesis of potential 'diesters' which would remove the phenolic moiety thereby preventing the synthesis of the target 4-methanesulfonate derivative.



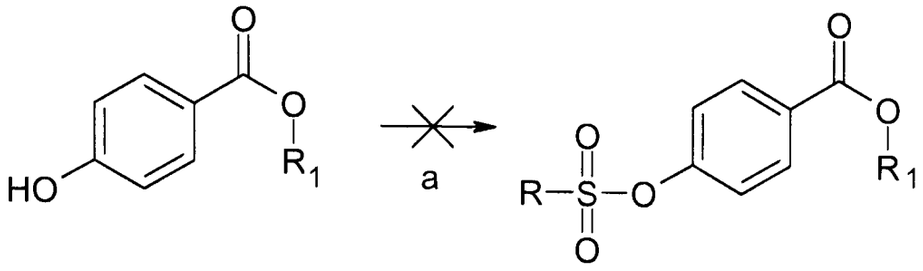
Scheme 2.3: Formation of an ester involving the use of acid chloride or acid anhydride.

Esters may also be formed by the direct reaction of a carboxylic acid with an alcohol (this reaction is often acid-catalysed and is reversible). The acid catalyst is postulated to protonate the carbonyl oxygen of the acid thereby allowing the carbonyl carbon atom to be more readily attacked by the nucleophilic oxygen of the alcohol; water is eliminated, yielding the appropriate ester. The equilibrium which exists may be altered in favour of the products by the use of either excess alcohol or the removal of one of the products. The removal of the water produced as a result of the esterification reaction may be achieved by using anhydrous toluene as a reaction solvent (Carey, 2000), as such, the water produced in the reaction is removed as an azeotropic mixture through the use of a Dean-Stark apparatus (Furness et al, 1996). In the synthesis of the target esters, we concluded that the use of the direct esterification would be the better route, as such, the acid catalysed esterification (with a large excess of the appropriate alcohol) afforded us the range of esters from the methyl to the pentyl derivatives of 3-bromo-4-hydroxybenzoic acid in moderate to good yield [ranging from ~30% yield for compound **238** (pentyl 3-bromo-4-hydroxybenzoate) to ~60% yield for compound **236** (propyl 3-bromo-4-hydroxybenzoate)].

Whilst the reactions were, in general, without major problems, the parent carboxylic acid (3-bromo-4-hydroxybenzoic acid) was found to contain a small quantity of the di-brominated derivative as an impurity and was present in approximately 2% quantity, as such, the impurity was significant enough to be detected in the NMR spectra and TLC, as such, the esters were required to be purified by column chromatography to remove the dibrominated derivative.

Problems were encountered with the synthesis of the hexyl to decyl derivatives. That is, in the synthesis of the methyl to the pentyl esters, an excess of alcohol was used in relation to the parent carboxylic acid, which was later removed under vacuum to give the ester in a good yield. However, the higher molecular weight alcohols (from hexanol to decanol) could not be removed easily under vacuum, and therefore the ratio of the appropriate alcohol to the acid was lowered, with the final compounds being purified by column chromatography (allowing the loss of the excess alcohol). For these reactions, the Dean-Stark method as well as an increased reaction time (between 48h to 96h) were used and allowed the target estrs to be obtained in poor to good yields for the hexyl to the decyl esters of 3-bromo-4-hydroxybenzoic acid following purification by column chromatography [ranging from ~13% yield for compound **242** (nonyl 3-bromo-4-hydroxybenzoate) to ~69% yield for compound **239** (hexyl 3-bromo-4-hydroxybenzoate)]. Similar problems were encountered in the synthesis of the cycloalkyl-based ester derivatives resulting in poor yields (in most cases less than 5%).

As previously mentioned, the target compounds were the sulfonate derivatives of the esters of 4-hydroxybenzoic acid, as such, sulfamate and methanesulfonate derivatives were the major target compounds. The synthesis of the sulfamated compounds involved the method previously described by Ahmed et al (2004a) in the synthesis of the sulfamated derivatives of 4-hydroxybenzoic acid esters. That is, the various esters of 4-hydroxybenzoic acid were stirred in the presence of an *in situ* preparation of aminosulfonyl chloride using dimethyl acetamide (DMA) as the reaction solvent (Scheme 2.4).



Scheme 2.4: Synthesis of sulfonated derivatives of the esters of 4-hydroxybenzoic acid (a=DMA/NH_2SO_2Cl; R_1=alkyl and cycloalkyl moiety; R=NH_2).

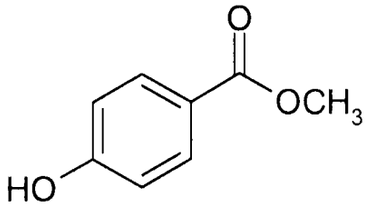
However, in the synthesis of the compounds based on the brominated derivatives of 4-hydroxybenzoic acid, it was found that the aminosulfonate products were difficult to isolate due mainly to the lack of stability of the product. Indeed it was discovered that the products underwent rapid hydrolysis to give back the brominated 4-hydroxybenzoic acid ester. Furthermore, when we considered the synthesis of the methanesulfonate derivatives, we discovered that the brominated derivatives of the esters of 4-hydroxybenzoic acid were easily hydrolysed back to the parent hydroxybenzoic acid ester. The lack of stability of the products made it very difficult to isolate any product, for full spectral analysis. Indeed, James (2000) had previously reported that phenolic based inhibitors containing electron-withdrawing groups were able to undergo non-enzymatic hydrolysis. As such, we propose that the mono- and dibrominated derivatives undergo self-hydrolysis, and therefore, the reaction scheme was abandoned for the bromo derivatives of the sulfamate- and methanesulfonate-based compounds of 3-bromo- and 3,5-dibromo-derivatives of 4-hydroxybenzoic acid. Only the methanesulfonate containing target compounds of the non-brominated derivatives of 4-hydroxybenzoic acid have been reported together with the 3-bromo- and 3,5-brominated intermediates of 4-hydroxybenzoic acid.

2.2 Materials and methods

Chemicals were purchased from Sigma-Aldrich Company Ltd (Poole, Dorset, England), and checked for purity by ^{1}H and ^{13}C NMR (JEOL 400MHz and 100MHz respectively) using either $CDCl_3$, d_6 acetone or d_6 methanol as the solvent unless otherwise stated. Infrared spectrometry was obtained on a Perkin-Elmer Fourier transform-Paragon 1000 IR spectrometer. Gas chromatography-mass spectrometry was undertaken on a Hewlett 5890 Packard series II GCMS at a flow rate of 0.58mL/min, and a temperature range increasing from 120-270°C at the rate of 10°C/min. Melting points are uncorrected and were obtained on a Buchi 512 or a Gallenkamp Instrument. Elemental analysis was carried out by the CHN microanalysis service (London School of Pharmacy, London, UK). HRMS was carried out by Kings College Mass spectrometry service.

2.3 Synthesis of the esters of 4-hydroxybenzoic acid

Methyl-4-hydroxybenzoate (220):

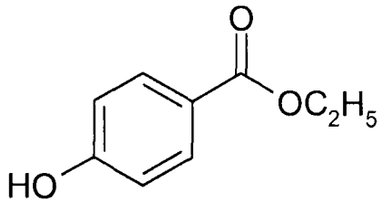


4-Hydroxybenzoic acid (2.31g, 16.71mmol) was dissolved in methanol (50mL, 1.23mol) and left to stir for 30min. Concentrated sulfuric acid (10M, 0.1mL) was cautiously added and the solution refluxed for 18h. After cooling, the solvent was removed under vacuum and the resulting oil was neutralised with saturated sodium bicarbonate ($NaHCO_3$) solution (50mL), extracted into dichloromethane (DCM) (40mL) and the organic layer was washed with water (3 x 50mL). The DCM layer was dried over anhydrous magnesium sulfate ($MgSO_4$), filtered, and the solvent removed under vacuum to yield an off-white solid which was purified via column chromatography to give **220** as an off-white solid (2.00g, 78.8% yield); m.p.=130.4-132.2°C [lit. m.p.=125-126°C (Graham and Lewis, 1978)]; R_f=0.62

[diethyl ether (DEE)/petroleum spirit (pet spirit) 40-60°C (50/50)]; GC: t_R=6.30min; LRMS (EI): 152 (M^+, 35%), 121 (M^+-CH_3O, 100%), 93 (M^+-$C_2H_3O_2$, 21%).

$\nu_{(max)}$ (Film) cm^{-1}: 3257.9 (Ph-OH), 1686.6 (C=O), 1608.7 (Ar C=C); δ_H d_6 acetone: 9.08 (1H, s, O<u>H</u>), 7.79 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.83 (2H, dd, J=8.9Hz, Ph<u>H</u>), 3.72 (3H, s, O<u>C</u>H_3); δ_C d_6 acetone: 166.21 (<u>C</u>O), 161.79 (<u>C</u>O, Ar), 131.62 (<u>C</u>H, Ar), 121.74 (<u>C</u>H, Ar), 115.23 (<u>C</u>, Ar), 51.09 (O<u>C</u>H_3).

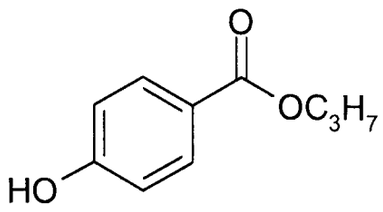
Ethyl-4-hydroxybenzoate (221):



Compound **221** was synthesised following the same procedure as for compound **220** except that 4-hydroxybenzoic acid (2.36g, 17.11mmol) was dissolved in ethanol (50mL, 1.37mol). An off-white solid was obtained which was purified via column chromatography to give **221** as an off-white coloured solid (1.92g, 84.4% yield); m.p.=120.8-122.4°C [lit. m.p.=115°C (Andrade et al, 1964)]; R_f=0.65 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.01min; LRMS (EI): 166 (M^+, 23%), 138 (M^+-C_2H_4, 22%), 121 (M^+-C_2H_5O, 100%), 93 (M^+-$C_3H_5O_2$, 14%).

$\nu_{(max)}$ (Film) cm^{-1}: 3215.9 (Ph-OH), 1672.4 (C=O), 1608.4 (Ar C=C); δ_H d_6 acetone: 9.06 (1H, s, O<u>H</u>), 7.80 (2H, dd, J=9.0Hz, Ph<u>H</u>), 6.82 (2H, dd, J=9.0Hz, Ph<u>H</u>), 4.19 (2H, q, J=7.1Hz, OC<u>H</u>$_2$), 1.23 (3H, t, J=7.1Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 165.72 (<u>C</u>O), 161.73 (<u>C</u>O, Ar), 131.57 (<u>C</u>H, Ar), 121.99 (<u>C</u>H, Ar), 115.19 (<u>C</u>, Ar), 60.09 (O<u>C</u>H_2), 13.86 (<u>C</u>H_3).

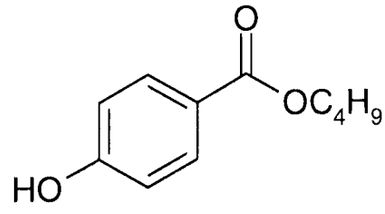
Propyl-4-hydroxybenzoate (222):



Compound **222** was synthesised following the same procedure as for compound **220** except that 4-hydroxybenzoic acid (2.57g, 18.66mmol) was dissolved in propanol (50mL, 1.04mol). An off-white solid was obtained which was purified via column chromatography to give **222** as an off-white coloured solid (2.43g, 72.3% yield); m.p.=98.2-99.7°C [lit. m.p.=88.0-90.0°C (Owen et al, 2003)]; R_f=0.66 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.58min; LRMS (EI): 180 (M^+, 8%), 138 (M^+-C_3H_6, 77%), 121 (M^+-C_3H_7O, 100%), 93 (M^+-$C_4H_7O_2$, 12%); Elemental analysis: found C 66.68%, H 6.75%; $C_{10}H_{12}O_3$ requires C 66.65%, H 6.71%.

$\nu_{(max)}$ (Film) cm^{-1}: 3277.1 (Ph-OH), 1676.7 (C=O), 1606.7 (Ar C=C); δ_H d_6 acetone: 9.08 (1H, s, O<u>H</u>), 7.81 (2H, dd, J=9.0Hz, Ph<u>H</u>), 6.82 (2H, dd, J=9.0Hz, Ph<u>H</u>), 4.10 (2H, t, J=6.6Hz, OC<u>H</u>$_2$), 1.96 (2H, m, C<u>H</u>$_2$), 0.91 (3H, t, J=7.4Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 165.75 (<u>C</u>O), 161.73 (<u>C</u>O, Ar), 131.57 (<u>C</u>H, Ar), 121.96 (<u>C</u>H, Ar), 115.19 (<u>C</u>, Ar), 65.66 (O<u>C</u>H$_2$), 22.06 (<u>C</u>H$_2$), 9.97 (<u>C</u>H$_3$).

Butyl-4-hydroxybenzoate (223):

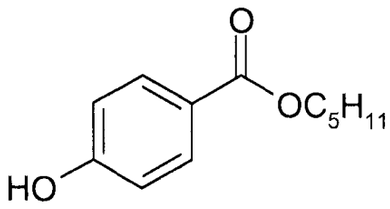


Compound **223** was synthesised following the same procedure as for compound **220** except that 4-hydroxybenzoic acid (2.13g, 15.46mmol) was dissolved in butanol (50mL, 0.83mol). An off-white solid was obtained which was purified via column chromatography to give **223** as an off-white coloured solid (1.83g, 61.0% yield); m.p.=71.0-72.3°C [lit. m.p.=64-66°C (Owen et al, 2003)]; R_f=0.67 [DEE/pet

spirit 40-60°C (50/50)]; GC: t_R=8.25min; LRMS (EI): 194 (M^+, 6%), 138 (M^+-C_4H_8, 91%), 121 (M^+-C_4H_9O, 100%), 93 (M^+-$C_5H_9O_2$, 13%).

$\nu_{(max)}$ (Film) cm^{-1}: 3383.8 (Ph-OH), 1683.4 (C=O), 1607.9 (Ar C=C); δ_H d_6 acetone: 9.06 (1H, s, O<u>H</u>), 7.79 (2H, dd, J=8.8Hz, Ph<u>H</u>), 6.82 (2H, dd, J=8.8Hz, Ph<u>H</u>), 4.15 (2H, t, J=6.6Hz, OC<u>H</u>$_2$), 1.63 (2H, m, C<u>H</u>$_2$), 1.38 (2H, m, C<u>H</u>$_2$), 0.87 (3H, t, J=7.3Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 165.73 (<u>C</u>O), 161.72 (<u>C</u>O, Ar), 131.57 (<u>C</u>H, Ar), 121.98 (<u>C</u>H, Ar), 115.18 (<u>C</u>, Ar), 63.89 (O<u>C</u>H$_2$), 30.83 (<u>C</u>H$_2$), 19.13 (<u>C</u>H$_2$), 13.22 (<u>C</u>H$_3$).

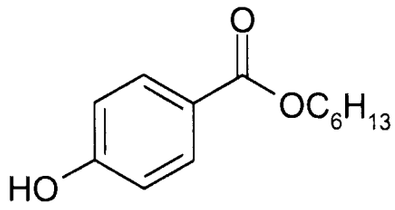
Pentyl-4-hydroxybenzoate (224):



Compound **224** was synthesised following the same procedure as for compound **220** except that 4-hydroxybenzoic acid (2.59g, 18.81mmol) was dissolved in pentanol (50mL, 0.71mol). A pale yellow solid was obtained which was purified via column chromatography to give **224** as an off-white coloured solid (2.44g, 62.4% yield); m.p.=42.6-43.9°C [lit. m.p.=54°C (Andrade et al, 1964)]; R_f=0.68 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.83min; LRMS (EI): 208 (M^+, 3%), 138 (M^+-C_5H_{10}, 100%), 121 (M^+-$C_5H_{11}O$, 94%), 93 (M^+-$C_6H_{11}O_2$, 13%); Elemental analysis: found C 54.53%, H 6.34%; $C_{12}H_{16}O_3$ requires C 54.53%, H 6.34%.

$\nu_{(max)}$ (Film) cm^{-1}: 3358.5 (Ph-OH), 1684.7 (C=O), 1608.5 (Ar C=C); δ_H d_6 acetone: 9.08 (1H, s, O<u>H</u>), 7.81 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.30 (2H, dd, J=8.9Hz, Ph<u>H</u>), 4.15 (2H, t, J=7.0Hz, OC<u>H</u>$_2$), 1.64 (2H, m, C<u>H</u>$_2$), 1.31 (4H, m, C<u>H</u>$_2$), 0.82 (3H, t, J=7.1Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 166.55 (<u>C</u>O), 162.59 (<u>C</u>O, Ar), 132.42 (<u>C</u>H, Ar), 122.84 (<u>C</u>H, Ar), 116.05 (<u>C</u>, Ar), 65.05 (<u>C</u>H$_2$), 65.05 (<u>C</u>H$_2$), 29.32 (<u>C</u>H$_2$), 29.01 (<u>C</u>H$_2$), 23.09 (<u>C</u>H$_2$), 14.33 (<u>C</u>H$_3$).

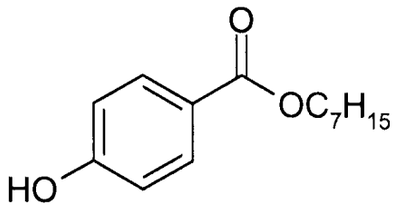
Hexyl-4-hydroxybenzoate (225):



4-Hydroxybenzoic acid (2.32g, 16.79mmol) was dissolved in toluene (75mL) with hexanol (3.0mL, 23.89mmol), and stirred for 30min. Concentrated sulfuric acid (10M, 0.1mL) was cautiously added and refluxed using a Dean-Stark apparatus for 90h. After cooling, the organic phase was neutralised with saturated $NaHCO_3$, extracted into ethyl acetate (3 x 50mL) and washed with water (2 x 40mL). The solvent was dried over $MgSO_4$, filtered, and the solvent removed under vacuum to yield an off-white coloured solid which was purified via column chromatography to give **225** as an off-white coloured solid (2.47g, 66.3% yield); m.p.=45.2-46.6°C [lit. m.p.=42-44°C (Cavill and Vincent, 1947)]; R_f=0.69 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.47min; LRMS (EI): 222 (M^+, 10%), 138 (M^+-C_6H_{12}, 100%), 121 (M^+-$C_6H_{13}O$, 74%), 93 (M^+-$C_7H_{13}O_2$, 3%).

$\nu_{(max)}$ (Film) cm^{-1}: 3352.2 (Ph-OH), 1675.9 (C=O), 1606.6 (Ar C=C); δ_H d_6 acetone: 9.14 (1H, s, O<u>H</u>), 7.89 (2H, dd, J=8.8Hz, Ph<u>H</u>), 6.92 (2H, dd, J=8.8Hz, Ph<u>H</u>), 4.24 (2H, t, J=6.9Hz, OC<u>H</u>$_2$), 1.73 (2H, m, C<u>H</u>$_2$), 1.38 (6H, m, C<u>H</u>$_2$), 0.89 (3H, t, J=7.1Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 166.59 (<u>C</u>O), 162.59 (<u>C</u>O, Ar), 132.41 (<u>C</u>H, Ar), 122.82 (<u>C</u>H, Ar), 116.06 (<u>C</u>, Ar), 65.06 (O<u>C</u>H$_2$), 32.28 (<u>C</u>H$_2$), 29.56 (<u>C</u>H$_2$), 26.51 (<u>C</u>H$_2$), 23.29 (<u>C</u>H$_2$), 14.34 (<u>C</u>H$_3$).

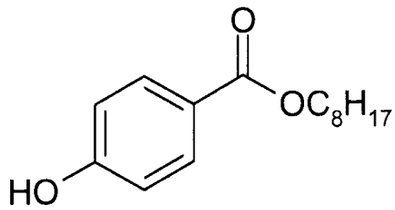
Heptyl-4-hydroxybenzoate (226):



Compound **226** was synthesised following the same procedure as for compound **225** except that 4-hydroxybenzoic acid (2.81g, 20.34mmol) was dissolved in toluene (75mL) and heptanol (2.9mL, 20.51mmol). An off-white solid was obtained which was purified via column chromatography to give **226** as an off-white coloured solid (2.89g, 60.2% yield); m.p.=49.3-50.7°C [lit. m.p.=47.0-48.5°C (Neubert et al, 1991)]; R_f=0.72 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.99min; LRMS (EI): 236 (M^+, 2%), 138 (M^+-C_7H_{14}, 100%), 121 (M^+-$C_7H_{15}O$, 67%), 93 (M^+-$C_8H_{15}O_2$, 10%).

$\nu_{(max)}$ (Film) cm^{-1}: 3346.5 (Ph-OH), 1684.4 (C=O), 1608.7 (Ar C=C); δ_H d_6 acetone: 9.09 (1H, s, O<u>H</u>), 7.80 (2H, dd, J=8.8Hz, Ph<u>H</u>), 6.83 (2H, dd, J=8.8Hz, Ph<u>H</u>), 4.15 (2H, t, J=6.6Hz, OC<u>H</u>$_2$), 1.65 (2H, m, C<u>H</u>$_2$), 1.27 (8H, m, C<u>H</u>$_2$), 0.78 (3H, t, J=6.9Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 165.76 (<u>C</u>O), 161.76 (<u>C</u>O, Ar), 131.57 (<u>C</u>H, Ar), 121.95 (<u>C</u>H, Ar), 115.20 (<u>C</u>, Ar), 64.23 (O<u>C</u>H$_2$), 31.71 (<u>C</u>H$_2$), 28.93 (<u>C</u>H$_2$), 28.76 (<u>C</u>H$_2$), 25.98 (<u>C</u>H$_2$), 22.47 (<u>C</u>H$_2$), 13.55 (<u>C</u>H$_3$).

Octyl-4-hydroxybenzoate (227):

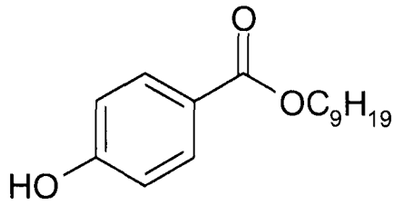


Compound **227** was synthesised following the same procedure as for compound **225** except that 4-hydroxybenzoic acid (2.74g, 19.83mmol) was dissolved in toluene (75mL) and octanol (2.9mL, 18.34mmol). An off-white solid was obtained which was purified via column chromatography to give **227** as an off-white coloured solid (2.65g, 57.8% yield); m.p.=46.9-48.6°C [lit. m.p.=51°C (Andrade et

al, 1964)]; R_f=0.77 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=10.57min; LRMS (EI): 250 (M^+, 2%), 138 (M^+-C_8H_{16}, 100%), 121 (M^+-$C_8H_{17}O$, 56%), 93 (M^+-$C_9H_{17}O_2$, 8%).

$\nu_{(max)}$ (Film) cm^{-1}: 3351.9 (Ph-OH), 1682.7 (C=O), 1608.5 (Ar C=C); δ_H d_6 acetone: 9.08 (1H, s, O<u>H</u>), 7.79 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.83 (2H, dd, J=8.9Hz, Ph<u>H</u>), 4.15 (2H, t, J=6.6Hz, OC<u>H</u>$_2$), 1.64 (2H, m, C<u>H</u>$_2$), 1.27 (10H, m, C<u>H</u>$_2$), 0.78 (3H, t, J=6.9Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 165.74 (<u>C</u>O), 161.73 (<u>C</u>O, Ar), 131.57 (<u>C</u>H, Ar), 121.57 (<u>C</u>H, Ar), 115.18 (<u>C</u>, Ar), 64.21 (O<u>C</u>H$_2$), 31.74 (<u>C</u>H$_2$), 29.20 (<u>C</u>H$_2$), 28.74 (<u>C</u>H$_2$), 28.16 (<u>C</u>H$_2$), 25.99 (<u>C</u>H$_2$), 22.50 (<u>C</u>H$_2$), 13.54 (<u>C</u>H$_3$).

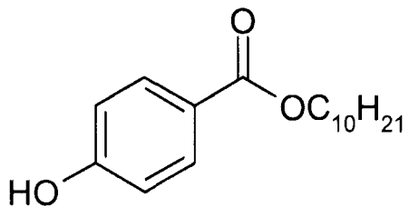
Nonyl-4-hydroxybenzoate (228):



Compound **228** was synthesised following the same procedure as for compound **225** except that 4-hydroxybenzoic acid (2.47g, 17.88mmol) was dissolved in toluene (75mL) and nonanol (3.2mL, 18.34mmol). An off-white solid was obtained which was purified via column chromatography to give **228** as an off-white coloured solid (2.59g, 54.9% yield); m.p.=42.5-44.0°C [lit. m.p.=43.5-44.0°C (Neubert et al, 1991)]; R_f=0.79 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=11.13min; LRMS (EI): 264 (M^+, 1%), 138 (M^+-C_9H_{18}, 100%), 121 (M^+-$C_9H_{19}O$, 50%), 93 (M^+-$C_{10}H_{19}O_2$, 7%).

$\nu_{(max)}$ (Film) cm^{-1}: 3375.7 (Ph-OH), 1677.1 (C=O), 1605.1 (Ar C=C); δ_H d_6 acetone: 9.13 (1H, s, O<u>H</u>), 7.89 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.91 (2H, dd, J=8.9Hz, Ph<u>H</u>), 4.23 (2H, t, J=6.9Hz, OC<u>H</u>$_2$), 1.74 (2H, quin J=6.9Hz, C<u>H</u>$_2$), 1.36 (12H, m, C<u>H</u>$_2$), 0.87 (3H, t, J=6.9Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 166.59 (<u>C</u>O), 162.59 (<u>C</u>O, Ar), 131.57 (<u>C</u>H, Ar), 122.82 (<u>C</u>H, Ar), 116.05 (<u>C</u>, Ar), 65.07 (O<u>C</u>H$_2$), 32.65 (<u>C</u>H$_2$), 30.29 (<u>C</u>H$_2$), 30.08 (<u>C</u>H$_2$), 30.04 (<u>C</u>H$_2$), 28.76 (<u>C</u>H$_2$), 26.84 (<u>C</u>H$_2$), 23.38 (<u>C</u>H$_2$), 14.42 (<u>C</u>H$_3$).

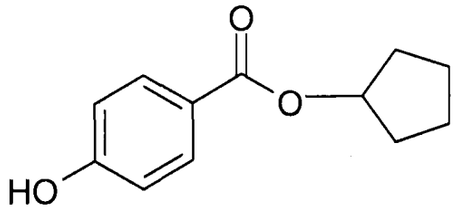
Decyl-4-hydroxybenzoate (229):



Compound **229** was synthesised following the same procedure as for compound **225** except 4-hydroxybenzoic acid (2.03g, 14.69mmol) was dissolved in toluene (50mL) and decanol (2.8ml, 14.73mmol). The crude oil was purified via column chromatography to give **229** as a white solid (1.06g, 25.9% yield); m.p.=32.1-34.0°C; R_f 0.81 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=19.19min; LRMS (EI): 278 (M^+, 1%), 138 (M^+-$C_{10}H_{20}$, 100%), 121 (M^+-$C_{10}H_{21}O$, 52%), 93 (M^+-$C_{11}H_{21}O_2$, 6%).

$\nu_{(max.)}$ (Film) cm^{-1}: 3354.7 (Ph-OH), 1683.7 (C=O); δ_H $CDCl_3$: 7.94 (2H, dd, J=8.9Hz, PhH), 6.93 (2H, dd, J=8.9Hz, PhH), 6.37 (1H, bs, OH), 4.30 (2H, t, J=6.9Hz, OCH_2), 1.76 (2H, quin, CH_2), 1.36 (14H, m, CH_2), 0.88 (3H, t, J=6.9Hz, CH_3); δ_c $CDCl_3$: 167.53 (CO), 160.7 (CO, Ar), 131.9 (CH, Ar), 122.2 (CH, Ar), 115.3 (C, Ar), 65.3 (OCH_2), 31.9 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 28.7 (CH_2), 26.0 (CH_2), 22.7 (CH_2), 14.1 (CH_3).

Cyclopentyl 4-hydroxy benzoate (230):

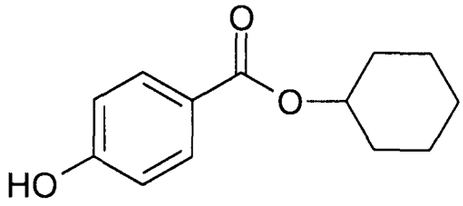


Compound **230** was synthesised following the same procedure as for compound **225**, except that 4-hydroxybenzoic acid (2.04g, 14.84mmol) was dissolved in toluene (75mL) and cyclopentanol (15mL, 0.16mol). A yellow solid was obtained which was purified by column chromatography to give **230** as a yellow solid (0.83g, 27.1% yield); m.p.=119.9-121.8°C; R_f=0.40 [DEE/pet spirit 40-60°C

(50/50)]; GC: t_R=9.90min; LRMS (EI): 206 (M^+, 1%), 138 (M^+-C_5H_8, 100%), 121 (M^+-C_5H_9O, 50%), 93 (M^+-$C_6H_9O_2$, 7%).

$\nu_{(max)}$ (Film) cm^{-1}: 3331.0 (Ph-OH), 1678.3 (C=O); δ_H $CDCl_3$: 7.93 (2H, dd, J=8.9Hz, PhH), 6.93 (2H, dd, J=8.9Hz, PhH), 5.46 (1H, m, OCH), 1.86 (8H, m, CH_2); δ_C $CDCl_3$: 167.71 (CO), 160.13 (CO, Ar), 131.84 (CH, Ar), 123.00 (CH, Ar), 115.18 (C, Ar), 32.81 (CH_2), 23.83 (CH_2).

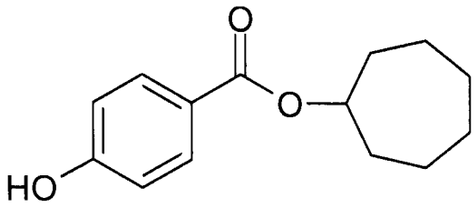
Cyclohexyl 4-hydroxy benzoate (231):



Compound **231** was synthesised following the same procedure as for compound **225**, except that 4-hydroxybenzoic acid (2.01g, 14.55mmol) was dissolved in toluene (70mL) and cyclohexanol (15mL, 0.14mol). A brown solid was obtained, which was purified by column chromatography to give **231** as an light brown solid (0.1g, 3.1% yield); m.p.=106.3-107.8°C [lit. m.p.=120-121°C (De Fazi and Berti, 1951)]; R_f=0.50 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=10.41min; LRMS (EI): 220 (M^+, 3%), 138 (M^+-C_6H_{10}, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3341.2 (Ph-OH), 1677.1 (C=O); δ_H $CDCl_3$: 8.0 (2H, dd, J=8.9Hz, PhH), 6.97 (2H, dd, J=8.9Hz, PhH), 5.01 (1H, m, OCH), 1.65 (10H, m, CH_2); δ_C $CDCl_3$: 166.58 (CO), 160.35 (CO, Ar), 131.90 (CH, Ar), 122.89 (CH, Ar), 115.24 (C, Ar), 31.65 (CH_2), 25.45 (CH_2), 23.63 (CH_2).

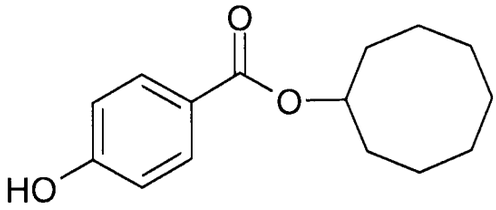
Cycloheptyl 4-hydroxy benzoate (232):



Compound **232** was synthesised following the same procedure as for compound **225**, except that 4-hydroxybenzoic acid (1.99g, 14.40mmol) was dissolved in toluene (75mL) and cycloheptanol (15mL, 0.12mol). A brown solid was obtained, which was purified by column chromatography to give **232** as a light brown solid (0.47g, 13.9% yield); m.p.=98.7-100.2°C; R_f=0.50 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=11.37min; LRMS (EI): 234 (M^+, 2%), 121 (M^+-$C_7H_{13}O$, 100%).

$\nu_{(max)}$ (Film)cm^{-1}: 3342.1 (Ph-OH), 2858.9 (CH), 1677.3 (C=O); δ_H $CDCl_3$: 7.93 (2H, dd, J=8.8Hz, PhH), 6.96 (2H, dd, J=8.8Hz, PhH), 5.62 (1H, s, OH), 5.24 (1H, m, OCH), 1.84 (12H, m, CH_2); δ_C $CDCl_3$: 159.73 (CO), 131.84 (CH, Ar), 115.10 (C, Ar), 33.68 (CH_2), 28.33 (CH_2), 22.93 (CH_2).

Cyclooctyl 4-hydroxy benzoate (233):

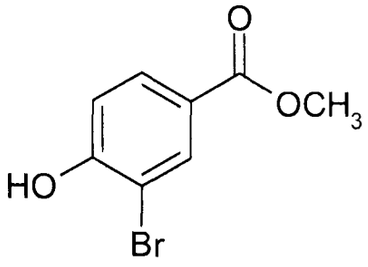


Compound **233** was synthesised following the same procedure as for compound **225**, except that 4-hydroxybenzoic acid (2.04g, 14.77mmol) was dissolved in toluene (75mL) and cyclooctanol (15mL, 0.22mol). A yellow oil was obtained which was purified by column chromatography to give **233** as a yellow oil (1.10g, 30.0% yield). R_f=0.50 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=11.64min; LRMS (EI): 248 (M^+), 121 (M^+-$C_8H_{15}O$, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3334.4 (Ph-OH), 2924.2 (CH), 1676.9 (C=O); δ_H $CDCl_3$: 7.97 (2H, dd, J=7.9Hz, PhH), 6.84 (2H, dd, J=7.9Hz, PhH), 5.47 (1H, s, OH), 5.22 (1H, m, OCH), 1.63 (14H, m, CH_2); δ_C $CDCl_3$: 131.49 (CH, Ar), 121.80 (CH, Ar), 31.44 (CH_2), 27.12 (CH_2), 25.32 (CH_2), 22.87 (CH_2).

2.4 Synthesis of the esters of 3-bromo-4-hydroxybenzoic acid

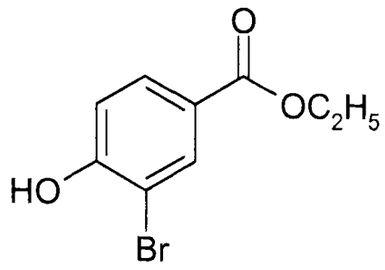
Methyl 3-bromo-4-hydroxybenzoate (234):



3-Bromo-4-hydroxybenzoic acid (1.47g, 6.77mmol) was dissolved in methanol (30mL, 1.18mol) and left to stir for 30min. Concentrated sulfuric acid (10M, 0.1mL) was cautiously added and the solution refluxed for 18h. After cooling, the solvent was removed under vacuum and the resulting oil was neutralised with saturated $NaHCO_3$ solution (50mL) and extracted into DCM (40mL). The organic layer was washed with water (3 x 50mL) and then drying over anhydrous $MgSO_4$, filtered, and the solvent removed under vacuum to yield an off-white solid which was purified via column chromatography to give **234** as an off-white solid (0.70g, 44.7% yield); m.p.=106.3-107.8°C [lit. m.p.=107-108°C (Cavill and Vincent, 1945)]; R_f=0.29 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=6.94min; LRMS (EI): 232 (M^+, 38%), 230 (M^+, 42%), 201 (M^+-CH_3O, 97%), 199 (M^+-CH_3O, 100%), 173 (M^+-$C_2H_3O_2$ 10%), 171 (M^+-$C_2H_3O_2$, 10%), 92 (M^+-$C_2O_2BrH_3$, 19%).

$\nu_{(max)}$ (Film) cm^{-1}: 3321.8 (Ph-OH), 1696.1 (C=O), 1601.3 (Ar C=C); δ_H $CDCl_3$: 8.18 (1H, dd, J=2.0Hz, PhH), 7.91 (1H, dd, J=2.0Hz, J=8.6Hz, PhH), 7.04 (1H, dd, J=8.6Hz, PhH), 6.11 (1H, s, OH), 3.89 (3H, s, OCH_3); δ_C $CDCl_3$: 165.65 (CO), 156.22 (CO, Ar), 133.96 (CBr, Ar), 130.99 (C, Ar), 123.96 (C, Ar), 115.78 (CH, Ar), 110.05 (CH, Ar), 52.21 (OCH_3).

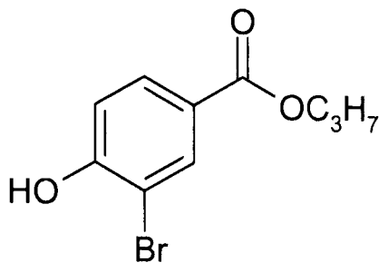
Ethyl 3-bromo-4-hydroxybenzoate (235):



Compound **235** was synthesised following the same procedure as for compound **234** except that 3-bromo-4-hydroxybenzoic acid (1.50g, 6.90mmol) was dissolved in ethanol (30mL, 0.82mol). Removal of the solvent gave an off-white solid was obtained which was purified via column chromatography to give **235** as an off-white coloured solid (0.98g, 57.9% yield); m.p.=100.4-101.3°C [lit. m.p.=103°C (Meyer, 1901)]; R_f=0.33 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=7.50min; LRMS (EI): 246 (M^+, 26%), 244 (M^+, 27%), 218 (M^+-C_2H_2, 32%), 216 (M^+-C_2H_4, 33%), 201 (M^+-C_2H_5O, 100%), 199 (M^+-C_2H_3O, 98%), 92 (M^+-$C_3H_4O_2Br$, 21%); Elemental analysis: found C 44.17%, H 3.75%; $C_9H_9BrO_3$ requires C 44.11%, H 3.70%.

$\nu_{(max)}$ (Film) cm^{-1}: 3234.0 (Ph-OH), 1676.1 (C=O), 1603.6 (Ar C=C); δ_H $CDCl_3$: 8.17 (1H, d, J=2.0Hz, PhH), 7.91 (1H, d, J=2.0Hz, J=8.6Hz, PhH), 7.03 (1H, d, J=8.4Hz, PhH), 5.89 (1H, s, OH), 4.34 (2H, t, J=7.1Hz, OCH_2), 1.36 (3H, t, J=7.1Hz, CH_3); δ_C $CDCl_3$: 165.15 (CO), 156.10 (CO, Ar), 133.87 (CBr, Ar), 130.97 (C, Ar), 124.36 (C, Ar), 115.72 (CH, Ar), 110.02 (CH, Ar), 65.13 (OCH_2), 14.30 (CH_3).

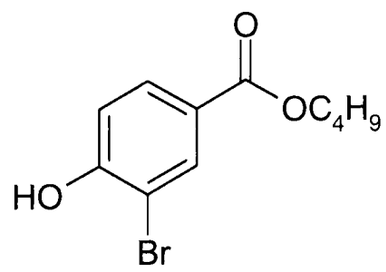
Propyl 3-bromo-4-hydroxybenzoate (236):



Compound **236** was synthesised following the same procedure as for compound **234** except that 3-bromo-4-hydroxybenzoic acid (1.51g, 6.96mmol) was dissolved in propanol (30mL, 0.62mol). Removal of the solvent gave a pale yellow solid which was purified via column chromatography to give **236** as an off-white coloured solid (1.09g, 60.3% yield); m.p.=74.8-76.8°C [lit. m.p.=88-89°C (Hirai, 1957)]; R_f=0.35 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=8.14min; LRMS (EI): 260 (M^+, 14%), 258 (M^+, 13%), 218 (M^+-C_3H_7, 96%), 216 (M^+-C_3H_4, 97%), 201 (M^+-C_3H_6O, 99%), 199 (M^+-C_3H_5O, 100%), 92 (M^+-$C_4H_6O_2Br$, 26%).

$\nu_{(max)}$ (Film) cm^{-1}: 3337.3 (Ph-OH), 1686.2 (C=O), 1601.0 (Ar C=C); δ_H $CDCl_3$: 8.18 (1H, d, J=2.0Hz, PhH), 7.92 (1H, dd, J=2.0Hz, J=8.4Hz, PhH), 7.04 (1H, d, J=8.4Hz, PhH), 5.93 (1H, s, OH), 4.25 (2H, t, J=7.1Hz, OCH_2), 1.77 (2H, m, J=7.5Hz, CH_2), 1.00 (3H, t, J=7.5Hz, CH_3); δ_C $CDCl_3$: 165.15 (CO), 156.04 (CO, Ar), 133.82 (CBr, Ar), 130.99 (C, Ar), 124.46 (C, Ar), 115.73 (CH, Ar), 110.05 (CH, Ar), 66.70 (OCH_2), 22.09 (CH_2), 10.49 (CH_3).

Butyl 3-bromo-4-hydroxybenzoate (237):

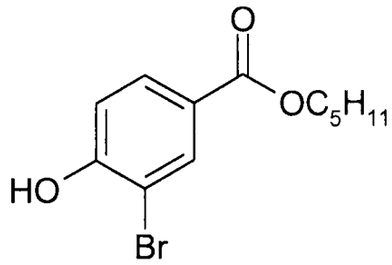


Compound **237** was synthesised following the same procedure as for compound **234** except that 3-bromo-4-hydroxybenzoic acid (1.49g, 6.87mmol) was dissolved

in butanol (70mL, 1.17mol) and left to stir for 30min. The solvent was removed under vacuum to yield a light brown coloured solid which was purified via column chromatography to give **237** as an off-white coloured solid (0.66g, 35.2% yield); m.p.=86.6-87.1°C [lit. m.p.=83-84°C (Cavill and Vincent, 1945)]; R_f=0.37 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=8.98min; LRMS (EI): 274 (M^+, 10%), 272 (M^+, 10%), 218 (M^+-C_4H_6, 100%), 216 (M^+-C_4H_8, 96%), 201 (M^+-C_4H_7O, 64%), 199 (M^+-C_4H_9O, 64%), 173 (M^+-$C_5H_9O_2$, 5%), 171 (M^+-$C_5H_9O_2$, 5%), 92 (M^+-$C_5H_8O_2Br$, 9%); Elemental analysis: found C 48.37%, H 4.92%; $C_{11}H_{13}BrO_3$ requires C 48.37%, H 4.80%.

$\nu_{(max)}$ (Film) cm^{-1}: 3326.0 (Ph-OH), 1686.2 (C=O), 1600.7 (Ar C=C); δ_H $CDCl_3$: 8.17 (1H, d, J=2.0Hz, PhH), 7.91 (1H, dd, J=2.0Hz, J=8.4Hz, PhH), 7.042 (1H, d, J=8.4Hz, PhH), 5.88 (1H, s, OH), 4.29 (2H, t, J=6.8Hz, OCH_2), 1.73 (2H, m, J=7.3Hz, CH_2), 1.46 (2H, m, J=7.3Hz, CH_2), 0.96 (3H, t, J=7.3Hz, CH_3); δ_C $CDCl_3$: 165.12 (CO), 156.01 (CO, Ar), 133.80 (CBr, Ar), 131.00 (C, Ar), 124.51 (C, Ar), 115.72 (CH, Ar), 110.05 (CH, Ar), 65.00 (OCH_2), 30.76 (CH_2), 19.24 (CH_2), 13.74 (CH_3).

Pentyl 3-bromo-4-hydroxybenzoate (238):

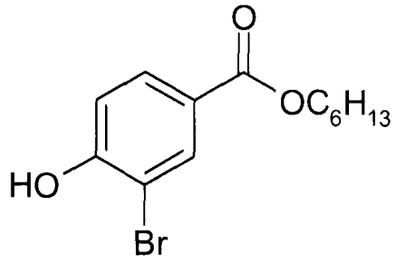


Compound **238** was synthesised following the same procedure as for compound **234** except that 3-bromo-4-hydroxybenzoic acid (1.50g, 6.86mmol) was dissolved in pentanol (30mL, 0.42mol). The solvent was removed under vacuum to yield a light brown coloured solid which was purified via column chromatography to give **238** as a pale cream coloured solid (0.58g, 29.6% yield); m.p.=68.9-69.5°C [lit. m.p.=64-66°C (Hirai, 1957)]; R_f=0.29 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=9.42min; LRMS (EI): 288 (M^+, 5%), 286 (M^+, 4%), 218 (M^+-C_5H_{11}, 98%), 216

(M^+-C_5H_8, 100%), 201 (M^+-$C_5H_{10}O$, 61%), 199 (M^+-C_5H_9O, 60%), 173 (M^+-$O_2C_6H_{10}$, 7%), 171 (M^+-$C_6H_9O_2$, 6%), 92 (M^+-$C_6H_{10}BrO_2$, 15%); Elemental analysis: found C 50.24%, H 5.23%; $C_{12}H_{15}BrO_3$ requires C 50.19%, H 5.27%.

$\nu_{(max)}$ (Film) cm^{-1}: 3337.1 (Ph-OH), 1690.4 (C=O), 1601.8 (Ar C=C); δ_H $CDCl_3$: 8.11 (1H, d, J=2.0Hz, PhH), 7.85 (1H, dd, J=2.0Hz, J=8.6Hz, PhH), 6.98 (1H, d, J=8.4Hz, PhH), 5.84 (1H, s, OH), 4.21 (2H, t, J=6.8Hz, OCH_2), 1.68 (2H, m, J=6.9Hz, CH_2), 1.32 (4H, m, CH_2), 0.85 (3H, t, J=6.9Hz, CH_3); δ_C $CDCl_3$: 165.13 (CO), 155.99 (CO, Ar), 133.80 (CBr, Ar), 130.98 (C, Ar), 124.47 (C, Ar), 115.71 (CH, Ar), 110.04 (CH, Ar), 65.30 (OCH_2), 28.40 (CH_2), 28.14 (CH_2), 22.34 (CH_2), 13.95 (CH_3).

Hexyl 3-bromo-4-hydroxybenzoate (239):

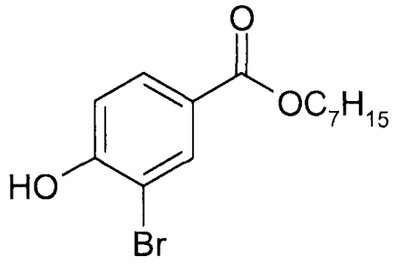


3-Bromo-4-hydroxybenzoic acid (1.48g, 6.85mmol) was dissolved in toluene (75mL) and hexanol (2.5mL, 19.9mmol), and stirred for 30min. Concentrated sulfuric acid (10M, 0.1mL) was cautiously added and refluxed using a Dean-Stark apparatus for 96h. After cooling, the organic phase was neutralised with saturated $NaHCO_3$, extracted into ethyl acetate (3 x 50mL) and washed with water (2 x 40mL). The solvent was dried over anhydrous $MgSO_4$, filtered, and the solvent removed under vacuum to yield an off-white coloured solid which was purified via column chromatography to give **239** as a pale cream coloured solid (1.42g, 68.6% yield); m.p.=59.4-60.7°C [lit. m.p=60-62°C (Hirai, 1957)]; R_f=0.31 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=9.78min; LRMS (EI): 302 (M^+, 1%), 300 (M^+, 1%), 218 (M^+-C_6H_{10}, 89%), 216 (M^+-C_6H_{11}, 100%), 201 (M^+-$C_6H_{11}O$, 64%), 199 (M^+-$C_6H_{13}O$, 61%), 173 (M^+-$C_7H_{11}O_2$, 9%), 171 (M^+-$C_7H_{11}O_2$, 8%), 92 (M^+-$C_7H_{12}O_2Br$, 47%);

Elemental analysis: found C 51.94%, H 5.65%; $C_{13}H_{17}BrO_3$ requires C 51.84%, H 5.69%.

$\nu_{(max)}$ (Film) cm^{-1}: 3349.3 (Ph-OH), 1683.5 (C=O), 1601.3 (Ar C=C); δ_H $CDCl_3$: 8.10 (1H, d, J=2.0Hz, PhH), 7.85 (1H, dd, J=2.0Hz, J=8.6Hz, PhH), 6.98 (1H, d, J=8.4Hz, PhH), 5.92 (1H, s, OH), 4.21 (2H, t, J=6.8Hz, OCH_2), 1.68 (2H, m, J=6.9Hz, CH_2), 1.31 (6H, m, CH_2), 0.83 (3H, t, J=6.9Hz, CH_3); δ_C $CDCl_3$: 165.17 (CO), 156.05 (CO, Ar), 133.83 (CBr, Ar), 130.96 (C, Ar), 124.42 (C, Ar), 115.72 (CH, Ar), 110.04 (CH, Ar), 65.32 (OCH_2), 31.43 (CH_2), 28.64 (CH_2), 25.65 (CH_2), 22.52 (CH_2), 13.98 (CH_3).

Heptyl 3-bromo-4-hydroxybenzoate (240):

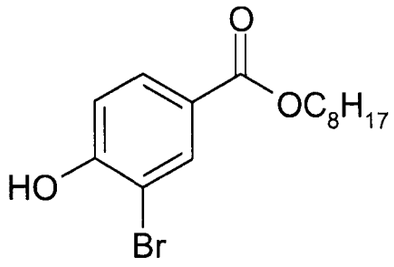


Compound **240** was synthesised following the same procedure as for compound **239** except that 3-Bromo-4-hydroxybenzoic acid (1.53g, 7.05mmol) was dissolved in toluene (50mL) and heptan-1-ol (1.0mL, 7.07mmol). To give a yellow solid which was purified via column chromatography to give **240** as an off-white coloured solid (0.47g, 21.2% yield); m.p.=57.0-58.1°C [lit. m.p.=58-59°C (Hirai, 1957)]; R_f=0.40 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=10.56min; LRMS (EI): 316 (M^+, 4%), 314 (M^+, 4%), 218 (M^+-C_7H_{12}, 100%), 216 (M^+-C_7H_{15}, 99%), 201 (M^+-$C_7H_{13}O$, 43%), 199 (M^+-$C_7H_{15}O$, 42%), 173 (M^+-$C_8H_{13}O_2$, 4%), 171 (M^+-$C_8H_{15}O_2$, 4%), 92 (M^+-$C_8H_{14}O_2Br$, 8%); Elemental analysis: found C 53.17%, H 6.12%; $C_{14}H_{19}BrO_3$ requires C 53.35%, H 6.08%.

$\nu_{(max)}$ (Film) cm^{-1}: 3340.3 (Ph-OH), 1686.2 (C=O), 1602.9 (Ar C=C); δ_H $CDCl_3$: 8.15 (1H, d, J=2.0Hz, PhH), 7.90 (1H, dd, J=2.0HZ J=8.6Hz, PhH), 7.02 (1H, d, J=8.6Hz, PhH), 6.02 (1H, s, OH), 4.26 (2H, t, J=6.8Hz, OCH_2), 1.72 (2H, m,

J=6.8Hz, C$\underline{H}_2$), 1.33 (8H, m, C$\underline{H}_2$), 0.86 (3H, t, J=6.8Hz, C$\underline{H}_3$); δ_C $CDCl_3$: 165.14 (C$\underline{O}$), 156.05 (C$\underline{O}$, Ar), 133.81 (C$\underline{Br}$, Ar), 130.97 (C$\underline{}$, Ar), 124.46 (C$\underline{}$, Ar), 115.70 (C$\underline{H}$, Ar), 110.03 (C$\underline{H}$, Ar), 65.32 (OC$\underline{H}_2$), 31.69 (C$\underline{H}_2$), 28.92 (C$\underline{H}_2$), 28.71 (C$\underline{H}_2$), 25.95 (C$\underline{H}_2$), 22.56 (C$\underline{H}_2$), 14.03 (C$\underline{H}_3$).

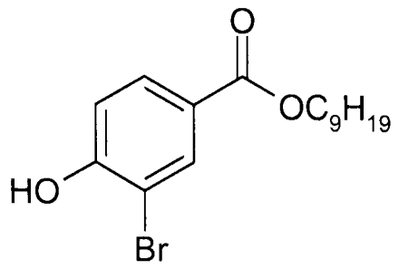
Octyl 3-bromo-4-hydroxybenzoate (241):



Compound **241** was synthesised following the same procedure as for compound **239** except that 3-bromo-4-hydroxybenzoic acid (1.50g, 6.87mmol) was dissolved in toluene (50mL) and octanol (10.0mL, 63.6mmol). A yellow coloured solid was obtained which was purified via column chromatography to give **241** as an off-white coloured solid (1.58g, 67.2% yield); m.p.=41.8-43.7°C [lit. m.p.=45-47°C (Hirai, 1957)]; R_f=0.42 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=11.14min; LRMS (EI): 330 (M^+, 1%), 328 (M^+, 1%), 218 (M^+-C_8H_{14}, 100%), 216 (M^+-C_8H_{16}, 98%), 201 (M^+-$C_8H_{15}O$, 41%), 199 (M^+-$C_8H_{17}O$, 43%), 173 (M^+-$C_9H_{15}O_2$, 4%), 171 (M^+-$C_9H_{15}O_2$, 4%), 92 (M^+-$C_9H_{16}O_2Br$, 14%); Elemental analysis: found C 54.81%, H 6.46%; $C_{15}H_{21}BrO_3$ requires C 54.72%; H 6.43%.

$\nu_{(max)}$ (Film) cm^{-1}: 3336.7 (Ph-OH), 1693.2 (C=O), 1602.6 (Ar C=C); δ_H $CDCl_3$: 8.16 (1H, d, J=2.0Hz, Ph$\underline{H}$), 7.95 (1H, dd, J=2.0HZ J=8.4Hz, Ph$\underline{H}$), 7.03 (1H, d, J=8.4Hz, Ph$\underline{H}$), 6.11 (1H, s, O$\underline{H}$), 4.26 (2H, t, J=6.8Hz, OC$\underline{H}_2$), 1.73 (2H, m, J=6.8Hz, C$\underline{H}_2$), 1.35 (10H, m, C$\underline{H}_2$), 0.87 (3H, t, J=6.8Hz, C$\underline{H}_3$); δ_C $CDCl_3$: 165.25 (C$\underline{O}$), 156.13 (C$\underline{O}$, Ar), 133.86 (C$\underline{Br}$, Ar), 130.94 (C$\underline{}$, Ar), 124.32 (C$\underline{}$, Ar), 115.72 (C$\underline{H}$, Ar), 110.01 (C$\underline{H}$, Ar), 65.36 (OC$\underline{H}_2$), 31.76 (C$\underline{H}_2$), 29.20 (C$\underline{H}_2$), 29.15 (C$\underline{H}_2$), 28.65 (C$\underline{H}_2$), 25.98 (C$\underline{H}_2$), 22.61 (C$\underline{H}_2$), 14.07 (C$\underline{H}_3$).

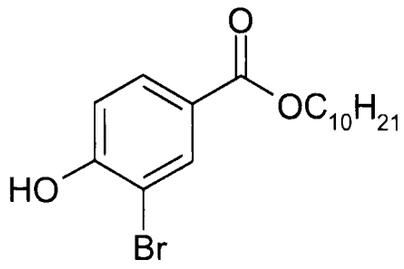
Nonyl 3-bromo-4-hydroxybenzoate (242):



Compound **242** was synthesised following the same procedure as for compound **239** except that 3-bromo-4-hydroxybenzoic acid (1.49g, 6.86mmol) was dissolved in toluene (50mL) and nonan-1-ol (1.5mL, 8.6mmol). A light yellow coloured solid was obtained which was purified via column chromatography to give **242** as an off-white coloured solid (0.29g, 12.5% yield); m.p.=48.2-49.3°C; R_f=0.44 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=11.54min; LRMS (EI): 344 (M^+, 1%), 342 (M^+, 1%), 218 (M^+-C_9H_{16}, 98%), 216 (M^+-C_9H_{18}, 100%), 201 (M^+-$C_9H_{17}O$, 38%), 199 (M^+-$C_9H_{19}O$, 40%), 173 (M^+-$C_{10}H_{17}O_2$, 4%), 171 (M^+-$C_{10}H_{17}O_2$, 4%), 92 (M^+-$C_{10}H_{18}O_2Br$, 10%).

$\nu_{(max)}$ (Film) cm^{-1}: 3322.5 (Ph-OH), 1682.6 (C=O), 1601.0 (Ar C=C); δ_H $CDCl_3$: 8.18 (1H, d, J=2.0Hz, PhH), 7.92 (1H, dd, J=2.0HZ J=8.6Hz, PhH), 7.05 (1H, d, J=8.4Hz, PhH), 6.16 (1H, s, OH), 4.28 (2H, t, J=6.8Hz, OCH_2), 1.75 (2H, m, J=6.8Hz, CH_2), 1.40 (12H, m, CH_2), 0.88 (3H, t, J=6.6Hz, CH_3); δ_C $CDCl_3$: 165.28 (CO), 156.18 (CO, Ar), 133.90 (CBr, Ar), 130.94 (C, Ar), 124.32 (C, Ar), 115.74 (CH, Ar), 110.02 (CH, Ar), 65.37 (OCH_2), 31.86 (CH_2), 29.49 (CH_2), 29.27 (CH_2), 29.25 (CH_2), 28.66 (CH_2), 25.98 (CH_2), 22.64 (CH_2), 14.08 (CH_3).

Decyl 3-bromo-4-hydroxybenzoate (243):

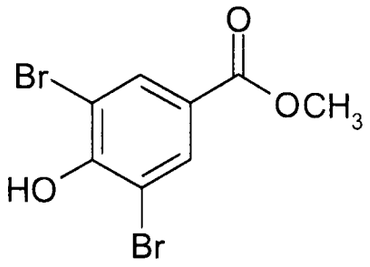


Compound **243** was synthesised following the same procedure as for compound **239** except that 3-bromo-4-hydroxybenzoic acid (0.99g, 4.58mmol) was dissolved in toluene (50mL) and decan-1-ol (1.6mL, 8.4mmol). A brown coloured solid was obtained which was purified via column chromatography to give **243** as an off-white coloured solid (0.26g, 15.6% yield); m.p.=53.6-54.9°C; R_f=0.36 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=12.25min; LRMS (EI): 358 (M^+, 1%), 356 (M^+, 1%), 218 (M^+-$C_{10}H_{18}$, 99%), 216 (M^+-$C_{10}H_{20}$, 100%), 201 (M^+-$OC_{10}H_{19}$, 39%), 199 (M^+-$C_{10}H_{21}O$, 42%), 173 (M^+-$C_{11}H_{21}O_2$, 4%), 171 (M^+-$C_{11}H_{19}O_2$, 4%), 92 (M^+-$C_{11}H_{21}O_2Br$, 12%).

$\nu_{(max)}$ (Film) cm^{-1}: 3407.5 (Ph-OH), 1693.8 (C=O), 1600.0 (Ar C=C); δ_H $CDCl_3$: 8.16 (1H, d, J=2.0Hz, PhH), 7.90 (1H, dd, J=2.0HZ J=8.6Hz, PhH), 7.02 (1H, d, J=8.6Hz, PhH), 5.96 (1H, s, OH), 4.26 (2H, t, J=6.8Hz, OCH_2), 1.73 (2H, m, J=6.9Hz, CH_2), 1.37 (14H, m, CH_2), 0.86 (3H, t, J=6.9Hz, CH_3); δ_C $CDCl_3$: 165.16 (CO), 156.08 (CO, Ar), 133.85 (CBr, Ar), 130.97 (C, Ar), 124.49 (C, Ar), 115.74 (C, Ar), 110.05 (C, Ar), 65.33 (OCH_2), 31.87 (CH_2), 29.52 (CH_2), 29.50 (CH_2), 29.27 (CH_2), 29.26 (CH_2), 26.26 (CH_2), 26.00 (CH_2), 22.65 (CH_2), 14.07 (CH_3).

2.5 Synthesis of the esters of 3,5-dibromo-4-hydroxybenzoic acid

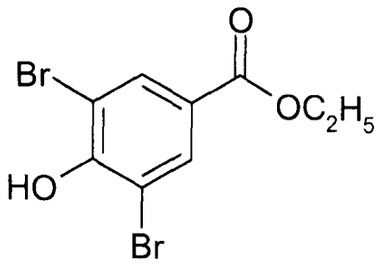
Methyl 3,5-dibromo-4-hydroxybenzoate (244):



3,5-Dibromo-4-hydroxy-benzoic acid (1.02g, 3.45mmol) was dissolved in toluene and methanol (10mL, 0.39mol) and left to stir for 30min. Concentrated sulfuric acid (10M, 0.1mL) was cautiously added and the solution refluxed, for 6h. After cooling, the solvent was removed under vacuum and the resulting oil was neutralised with saturated $NaHCO_3$ solution (50mL), extracted into DCM (40mL) and washed with water (3 x 50mL). The DCM layer was dried over anhydrous $MgSO_4$, filtered, and solvent removed under vacuum to yield an off-white solid which was purified via column chromatography to give **244** as an off-white crystalline solid (0.88g, 82.2% yield); m.p.=124.2-125.8°C [lit. m.p.=104.3-106.0°C (Patel, 2003b)]; R_f=0.45 [DEE/pet spirit 40-60°C (40/60)]; GC: t_R=8.95min; LRMS (EI): 310 (M^+, 45%), 279 (M^+-OCH_3, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3420.0 (Ph-OH), 1693.6 (C=O); δ_H d_6 Methanol: 8.00 (2H, s, PhH), 4.96 (3H, s, OCH_3); δ_C d_6 Methanol: 164.40 (CO), 155.10 (CO, Ar), 133.10 (CBr, Ar), 123.05 (CH, Ar), 110.90 (C, Ar), 51.72 (OCH_3).

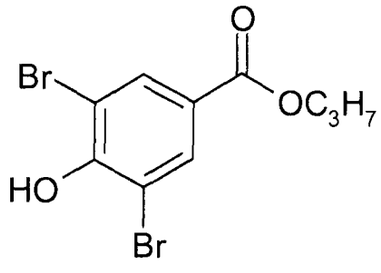
Ethyl 3,5-dibromo-4-hydroxybenzoate (245):



Compound **245** was synthesised following the same procedure as for compound **244**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.00g, 3.39mmol), and ethanol (10mL, 0.27mol) was used in place of methanol. The solvent was removed under vacuum to give **245** as an off-white crystalline solid (0.59g, 53.6% yield); m.p.=101.7-102.3°C [lit. m.p.=104.1-105.6°C (Patel, 2003b)]; R_f=0.53 [DEE/pet spirit 40-60°C (30/70)]; GC: t_R=9.38min; LRMS (EI): 324 (M^+, 35%), 279 (M^+-OC_2H_5, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3343.9 (Ph-OH), 2990.1 (CH), 1700.9 (C=O); δ_H $CDCl_3$: 8.14 (2H, s, PhH), 6.29 (1H, s, OH), 4.36 (2H, q, J=8.0Hz, OCH_2), 1.38 (3H, t, J=7.0Hz, CH_3); δ_C $CDCl_3$: 164.08 (CO), 153.05 (CO, Ar), 133.57 (CBr, Ar), 125.10 (CH, Ar), 109.63 (C, Ar), 61.54 (OCH_2), 14.27 (CH_3).

Propyl 3,5-dibromo-4-hydroxybenzoate (246):

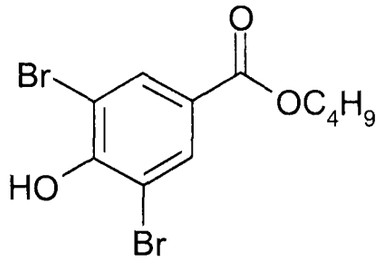


3,5-Dibromo-4-hydroxy-benzoic acid (1.00g, 3.38mmol), propan-1-ol (10mL, 0.20mol) and concentrated sulphuric acid (1mL, 10mol) were dissolved in toluene (25mL), and the mixture was refluxed for 6h. After cooling to room temperature, the mixture was neutralised with saturated $NaHCO_3$ solution and the resulting mixture was allowed to stand for 15min, poured into ice and then extracted into DCM (2 x 25mL). The organic phase was dried over anhydrous $MgSO_4$, the

mixture filtered and removal of the solvent under vacuum gave **246** as an off-white crystalline solid (0.61g, 53.5% yield); m.p.=109.8-110.5°C [lit. m.p.=107.2-108.8°C (Patel, 2003b)]; R_f=0.51 [DEE/pet spirit 40-60°C (40/60)]; GC: t_R=9.91min; LRMS (EI): 338 (M^+, 16%), 296 (M^+-C_3H_6, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3306.0 (Ph-OH), 2966.4 (CH), 1699.0 (C=O), 1588.0 (Ar C=C); δ_H $CDCl_3$: 8.14 (2H, s, PhH), 6.25 (1H, s, OH), 4.24 (2H, t, J=7.0Hz, OCH_2), 1.77 (2H, t, J=7.0Hz, CH_2), 1.01 (3H, t, J=7.0Hz, CH_3); δ_C $CDCl_3$: 164.01 (CO), 153.03 (CO, Ar), 133.56 (CBr, Ar), 125.10 (CH, Ar), 109.63 (C, Ar), 67.10 (OCH_2), 22.03 (CH_2), 10.47 (CH_3).

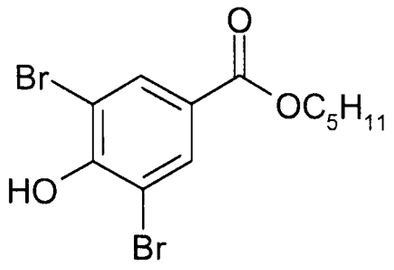
Butyl 3,5-dibromo-4-hydroxybenzoate (247):



Compound **247** was synthesised following the same procedure as for compound **246**, except that 3,5-dibromo-4-hydroxy-benzoic acid (0.98g, 3.31mmol), and butan-1-ol (10mL, 0.17mol) was used in place of propan-1-ol. Removal of the solvent under vacuum gave **247** as an off-white crystalline solid (0.87g, 74.4% yield); m.p.=89.1-91.5°C [lit. m.p.=90.2-91.0°C (Patel, 2003b)]; R_f=0.69 [DEE/pet spirit 40-60°C (40/60)]; GC: t_R=10.48min; LRMS (EI): 352 (M^+, 9%), 296 (M^+-C_4H_8, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3309.6 (Ph-OH), 1701.4 (C=O); δ_H $CDCl_3$: 8.13 (2H, s, PhH), 6.27 (1H, s, OH), 4.28 (2H, t, J=7.0Hz, OCH_2), 1.72 (2H, m, CH_2), 1.43 (2H, m, CH_2), 0.97 (3H, t, J=7.0Hz, CH_3); δ_C $CDCl_3$: 164.04 (CO), 153.03 (CO, Ar), 133.55 (CBr, Ar), 125.11 (CH, Ar), 109.63 (C, Ar), 65.42 (OCH_2), 30.68 (CH_2), 19.19 (CH_2), 13.72 (CH_3).

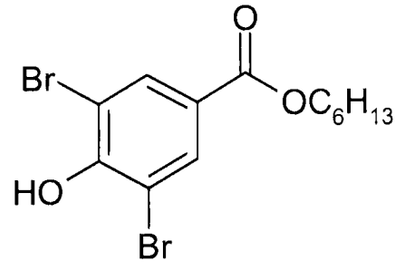
Pentyl 3,5-dibromo-4-hydroxybenzoate (248):



Compound **248** was synthesised following the same procedure as for compound **246**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.01g, 3.41mmol), and pentan-1-ol (10mL, 0.14mol) was used in place of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave **248** as a crude oil. Column chromatography of the crude oil gave **248** as a pale yellow crystalline solid (0.82g, 65.6% yield); m.p.=61.4-62.1°C; R_f=0.57 [DEE/pet spirit 40-60°C (20:80)]; GC: t_R=11.07min; LRMS (EI): 366 (M^+, 10%), 296 (M^+-C_5H_{10}, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3377.4 (Ph-OH), 1704.4 (C=O); δ_H $CDCl_3$: 8.13 (2H, s, PhH), 6.30 (1H, s, OH), 4.27 (2H, t, J=7.0Hz, OCH_2), 1.76 (2H, m, CH_2), 1.35 (4H, m, CH_2), 0.92 (3H, t, J=7.0Hz, CH_3); δ_C $CDCl_3$: 164.04 (CO), 153.03 (CO, Ar), 133.56 (CBr, Ar), 125.14 (CH, Ar), 109.63 (C, Ar), 65.71 (OCH_2), 28.34 (CH_2), 28.08 (CH_2), 22.32 (CH_2), 13.95 (CH_3).

Hexyl 3,5-dibromo-4-hydroxybenzoate (249):

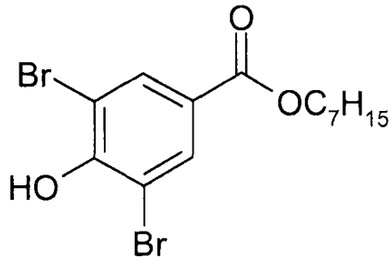


Compound **249** was synthesised following the same procedure as for compound **246**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.03g, 3.48mmol), and hexan-1-ol (10mL, 0.12mol) was used in place of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave a crude oil. Column chromatography of the crude oil gave **249** as a yellow crystalline solid (0.93g, 70.5% yield);

m.p.=59.1-59.6°C; R_f=0.46 [DEE/pet spirit 40-60°C (10/90)]; GC: t_R=11.66min; LRMS (EI): 380 (M^+, 9%), 296 (M^+-C_6H_{12}, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3374.2 (Ph-OH), 1703.0 (C=O); δ_H $CDCl_3$: 8.14 (2H, s, PhH), 6.33 (1H, s, OH), 4.28 (2H, t, J=7.0Hz, OCH_2), 1.80 (2H, m, CH_2), 1.36 (6H, m, CH_2), 0.90 (3H, t, J=7.0Hz, CH_3); δ_C $CDCl_3$: 164.05 (CO), 153.05 (CO, Ar), 133.55 (CBr, Ar), 125.11 (CH, Ar), 109.64 (C, Ar), 65.73 (OCH_2), 31.42 (CH_2), 28.39 (CH_2), 25.61 (CH_2), 22.51 (CH_2), 13.99 (CH_3).

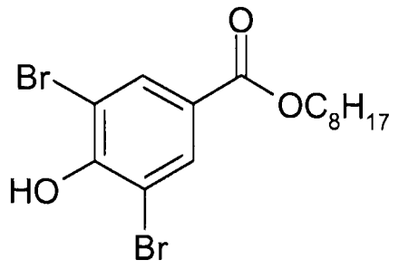
Heptyl 3,5-dibromo-4-hydroxybenzoate (250):



Compound **250** was synthesised following the same procedure as for compound **246**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.00g, 3.38mmol), and heptan-1-ol (10mL, 0.10mol) was used in place of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave a crude oil. Column chromatography of the crude oil gave **250** as an off-white crystalline solid (0.82g, 61.7% yield); m.p.=68.5-69.2°C; R_f=0.54 [DEE/pet spirit 40-60°C (30/70)]; GC: t_R=12.40min; LRMS (EI): 394 (M^+, 3%), 296 (M^+-C_7H_{14}, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3386.0 (Ph-OH), 1702.2 (C=O); δ_H $CDCl_3$: 8.14 (2H, s, PhH), 6.28 (1H, s, OH), 4.30 (2H, t, J=7.0Hz, OCH_2), 1.80 (2H, m, CH_2), 1.35 (8H, m, CH_2), 0.89 (3H, t, J=7.0Hz, CH_3); δ_C $CDCl_3$: 164.04 (CO), 153.03 (CO, Ar), 133.56 (CBr, Ar), 125.14 (CH, Ar), 109.64 (C, Ar), 65.74 (OCH_2), 31.68 (CH_2), 28.91 (CH_2), 28.63 (CH_2), 25.91 (CH_2), 22.58 (CH_2), 14.05 (CH_3).

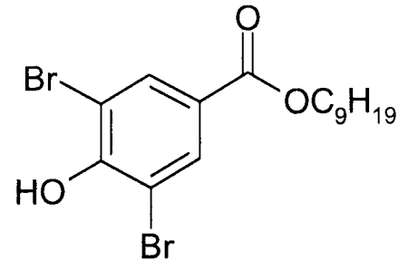
Octyl 3,5-dibromo-4-hydroxybenzoate (251):



Compound **251** was synthesised following the same procedure as for compound **246**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.00g, 3.38mmol), and octan-1-ol (10mL, 93.18mmol) was used in place of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Column chromatography of the crude oil gave **251** as a light yellow crystalline solid (0.89g, 64.5% yield); m.p.=65.1-66.2°C; R_f=0.50 [DEE/pet spirit 40-60°C (20/80)]; GC: t_R=13.12min; LCMS: 408 (M^+, 36%), 296 (M^+-C_8H_{16}, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3382.5 (Ph-OH), 1702.6 (C=O); δ_H $CDCl_3$: 8.14 (2H, s, PhH), 6.28 (1H, s, OH), 4.30 (2H, t, J=7.0Hz, OCH_2), 1.75 (2H, m, CH_2), 1.35 (10H, m, CH_2), 0.88 (3H, t, J=7.0Hz, CH_3); δ_C $CDCl_3$: 164.05 (CO), 153.04 (CO, Ar), 133.56 (CBr, Ar), 125.14 (CH, Ar) 109.64 (C, Ar), 65.74 (OCH_2), 31.77 (CH_2), 29.19 (CH_2), 28.15 (CH_2), 28.62 (CH_2), 25.95 (CH_2), 22.62 (CH_2), 14.08 (CH_3).

Nonyl 3,5-dibromo-4-hydroxybenzoate (252):

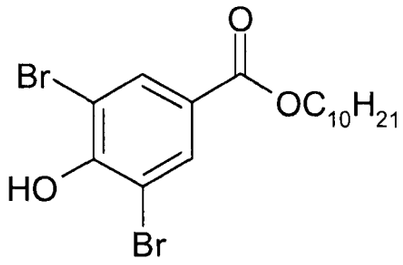


Compound **252** was synthesised following the same procedure as for compound **246**, except that 3,5-dibromo-4-hydroxy-benzoic acid (0.96g, 3.24mmol), and nonan-1-ol (10mL, 83.82mmol) was used in place of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Column chromatography of the crude oil gave **252** as an off-white crystalline solid (0.72g,

52.6% yield); m.p.=60.7-61.2°C; R_f=0.57 [DEE/pet spirit 40-60°C (10/90)]; GCMS: t_R=14.13min; LRMS (EI): 422 (M^+, 4%), 296 (M^+-C_9H_{18}, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3382.5 (Ph-OH), 1702.8 (C=O); δ_H $CDCl_3$: 8.14 (2H, s, PhH), 6.27 (1H, s, OH), 4.30 (2H, t, J=7.0Hz, OCH_2), 1.67-1.79 (2H, m, CH_2), 1.32 (12H, m, CH_2), 0.87 (3H, t, J=7.0Hz, CH_3); δ_C $CDCl_3$: 164.04 (CO), 153.03 (CO, Ar), 133.56 (CBr, Ar), 125.14 (CH, Ar), 109.63 (C, Ar), 65.74 (OCH_2), 31.83 (CH_2), 29.43 (CH_2), 29.24 (CH_2), 28.63 (CH_2), 25.94 (CH_2), 22.65 (CH_2), 14.09 (CH_3).

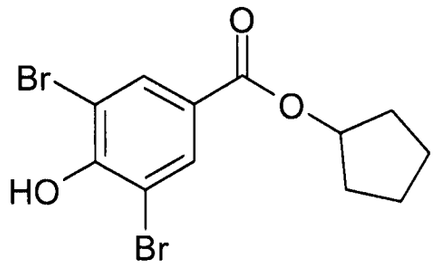
Decyl 3,5-dibromo-4-hydroxybenzoate (253):



Compound **253** was synthesised following the same procedure as for compound **246**, except that 3,5-dibromo-4-hydroxy-benzoic acid (0.95g, 3.21mmol), and decan-1-ol (10mL, 76.21mmol) was used in place of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Column chromatography of the crude oil gave **253** as an off-white crystalline solid (0.72g, 51.8% yield); m.p.=64.8-65.9°C; R_f=0.52 [DEE/pet spirit 40-60°C (10/90)]; GC: t_R=15.12min; LRMS (EI): 434 (M^+, 3%), 296 (M^+-$C_{10}H_{18}$, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3345.4 (Ph-OH), 1700.8 (C=O); δ_H $CDCl_3$: 8.13 (2H, s, PhH), 6.29 (1H, s, OH), 4.27 (2H, t, J=7.0Hz, OCH_2), 1.73 (2H, m, CH_2), 1.32 (14H, m, CH_2), 0.87 (3H, t, J=7.0Hz, CH_3); δ_C $CDCl_3$: 164.05 (CO), 153.04 (CO, Ar), 133.56 (CBr, Ar), 125.14 (CH, Ar), 109.64 (C, Ar), 65.74 (OCH_2), 31.87 (CH_2), 29.51 (CH_2), 29.28 (CH_2), 29.24 (CH_2), 25.94 (CH_2), 22.66 (CH_2), 14.10 (CH_3).

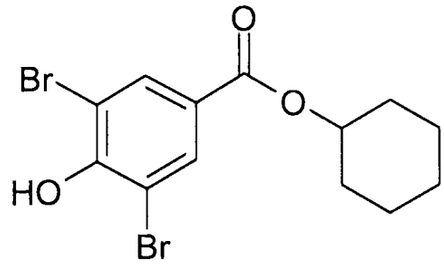
Cyclopentyl 3,5-dibromo-4-hydroxybenzoate (254):



Compound **254** was synthesised following the same procedure as for compound **246**, except that 3,5-dibromo-4-hydroxy-benzoic acid (0.94g, 3.18mmol), and cyclopentanol (10mL, 0.12mol) was used in place of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Column chromatography of the crude oil gave **254** as pale brown crystalline solid (0.68g, 58.6% yield); m.p.=145.7-146.2°C; R_f=0.70 [DEE/pet spirit 40-60°C (20/80)]; GC: LRMS (EI): t_R=11.52min; 364 (M^+, 5%), 279 (M^+-OC_5H_9, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3335.7 (Ph-OH), 1701.9 (C=O); δ_H $CDCl_3$: 8.10 (2H, s, PhH), 6.25 (1H, s, OH), 5.34 (1H, m, OCH), 1.97 (8H, m, CH_2); δ_C $CDCl_3$: 163.73 (CO), 152.93 (CO, Ar), 133.49 (CBr, Ar), 125.51 (CH, Ar), 109.58 (C, Ar), 77.32 (OCH), 32.73 (CH_2), 23.79 (CH_2).

Cyclohexyl 3,5-dibromo-4-hydroxybenzoate (255):

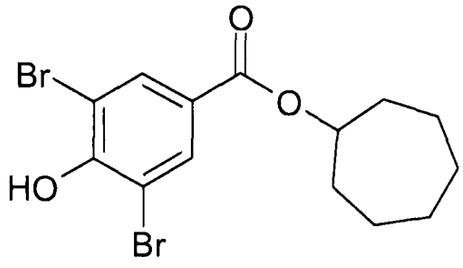


Compound **255** was synthesised following the same procedure as for compound **246**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.00g, 3.38mmol), and cyclohexanol (10mL, 0.10mol) was used in place of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Column chromatography of the crude oil gave **255** as a pale brown crystalline solid (0.68g,

53.1% yield); m.p.=126.3-126.8°C; R_f=0.39 [DEE/pet spirit 40-60°C (20/80)]; GC: t_R=12.35min; LRMS (EI): 378 (M^+, 5%), 82 (M^+-$C_7H_4Br_2O_3$, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3367.8 (Ph-OH), 1700.0 (C=O); δ_H $CDCl_3$: 8.07 (2H, s, PhH), 6.25 (1H, s, OH), 4.94 (1H, m, OCH), 1.50 (10H, m, CH_2); δ_C $CDCl_3$: 162.14 (CO), 151.72 (CO, Ar), 132.30 (CBr, Ar), 124.34 (CH, Ar), 108.36 (C, Ar), 72.73 (OCH), 30.38 (CH_2), 24.11 (CH_2), 22.50 (CH_2).

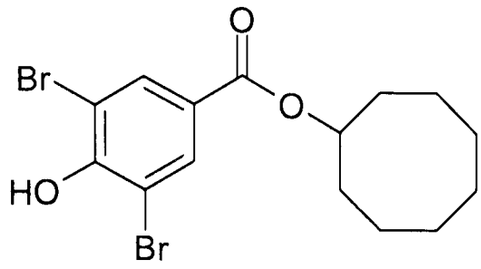
Cycloheptyl 3,5-dibromo-4-hydroxybenzoate (256):



Compound **256** was synthesised following the same procedure as for compound **246** except that 3,5-dibromo-4-hydroxy-benzoic acid (1.06g, 3.58mmol), and cycloheptanol (10mL, 0.09mol) was used in place of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Column chromatography of the crude oil gave **256** as an off-white crystalline solid (0.05g, 3.6% yield); m.p.=120.2-121.4°C; R_f=0.36 [DEE/pet spirit 40-60°C (20/80)]; GC: t_R=13.69 min; LRMS (EI): 392 (M^+, 2%), 96 (M^+-$C_7H_4O_3Br_2$, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3390.0 (Ph-OH), 1699.5 (C=O), 1601.0 (Ar C=C); δ_H $CDCl_3$: 8.06 (2H, s, PhH), 6.25 (1H, s, OH), 5.18 (1H, m, OCH), 1.72 (12H, m, CH_2); δ_C $CDCl_3$: 163.10 (CO), 153.00 (CO, Ar), 133.51 (CBr, Ar), 125.91 (CH, Ar), 109.57 (C, Ar), 33.80 (CH_2), 28.27 (CH_2), 22.85 (CH_2);

Cyclooctyl 3,5-dibromo-4-hydroxybenzoate (257):

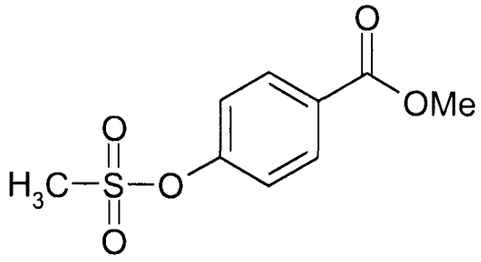


Compound **257** was synthesised following the same procedure as for compound **246**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.02g, 3.45mmol), and cyclooctanol (10mL, 0.08mol) was used in place of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Column chromatography of the crude oil gave **257** as an off-white crystalline solid (0.62g, 44.3% yield); m.p.=153.6-154.2°C; R_f=0.62 [DEE/pet spirit 40-60°C (20/80)]; GC: t_R=15.25min; LCMS: 406 (M^+, 7%), 82 (M^+-$C_{15}H_{17}O_3Br$, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3374.9 (Ph-OH), 1697.1 (C=O); δ_H $CDCl_3$: 8.05 (2H, s, PhH), 6.19 (1H, s, OH), 5.08 (1H, m, CH), 1.47 (14H, m, CH_2); δ_C $CDCl_3$: 163.10 (CO), 152.01 (CO, Ar), 133.51 (CBr, Ar), 125.90 (CH, Ar), 109.57 (C, Ar), 31.50 (CH_2), 27.09 (CH_2), 25.34 (CH_2), 22.91 (CH_2).

2.6 Synthesis of the methane sulfonic acid esters of 4-hydroxybenzoic acid

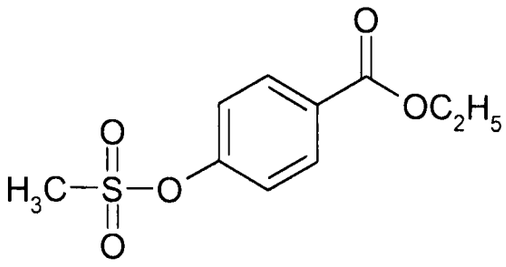
Methyl 4-methanesulfonylbenzoate (258):



Compound **220** (0.98g, 6.42mmol) was dissolved in anhydrous DCM (75mL) with triethyl amine (TEA) (2.0mL, 14.34mmol), and stirred for 10min. Methanesulfonyl chloride (1.5mL, 19.29mmol) was added and the solution refluxed for 2h. The reaction was poured onto ice (100mL), the organic layer was separated and washed with water (3 x 50mL), saturated $NaHCO_3$ solution (2 x 50mL) prior to and water (3 x 50mL). The solvent was dried over anhydrous $MgSO_4$, filtered, and the solvent removed under vacuum to yield an off-white coloured solid which was purified via column chromatography to give **258** as an off white coloured solid (0.94g, 63.6% yield); m.p.=86.1-87.8°C [lit. m.p=89-90°C (Percec et al, 1995)]; R_f=0.39 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.86min; LRMS (EI): 230 (M^+, 53%), 199 (M^+-CH_3O, 35%), 152 (M^+-CH_2SO_2, 74%), 121 (M^+-$C_2H_5SO_3$, 100%), 92 (M^+-$C_3H_6SO_4$, 13%).

$\nu_{(max)}$ (Film) cm^{-1}: 1719.7 (C=O), 1600.6 (Ar C=C), 1356.20 (SO_2R_2); δ_H d_6 acetone: 8.02 (2H, dd, J=8.9Hz, PhH), 7.39 (1H, dd, J=8.9Hz, PhH), 3.91 (3H, s, OCH_3), 3.27 (3H, s, CH_3); δ_C d_6 acetone: 165.42 (CO), 153.11 (CO, Ar), 131.45 (CH, Ar), 129.21 (C, Ar), 122.40 (CH, Ar), 51.80 (OCH_3), 37.19 (CH_3SO_3).

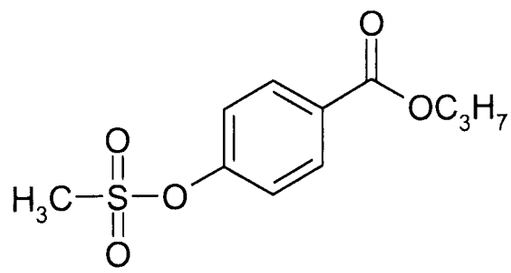
Ethyl 4-methanesulfonylbenzoate (259):



Compound **259** was synthesised following the same procedure as for compound **258** except that compound **221** (1.27g, 7.65mmol) was stirred in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol) for 30min prior to the addition of methanesulfonyl chloride (1.5mL, 19.29mmol). The reaction mixture was refluxed for 4h. The solvent was removed under vacuum which gave an off-white solid which was purified via column chromatography to give **259** as an off-white coloured solid (1.36g, 72.8% yield); m.p.=45.8-47.2°C; R_f=0.44 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.00min; LRMS (EI): 244 (M^+, 27%), 199 (M^+-C_2H_5O, 74%), 138 (M^+-$C_3H_6SO_2$, 58%), 121 (M^+-$C_3H_7SO_3$, 100%), 92 (M^+-$C_4H_8SO_4$, 19%).

$\nu_{(max)}$ (Film) cm^{-1}: 1715.9 (C=O), 1602.0 (Ar C=C), 1372.20 (SO_2R_2); δ_H $CDCl_3$: 8.04 (2H, dd, J=8.9Hz, PhH), 7.29 (2H, dd, J=8.9Hz, PhH), 4.31 (2H, q, J=7.1Hz, OCH_2), 3.12 (3H, s, SCH_3), 1.32 (3H, t, J=7.1Hz, CH_3); δ_C $CDCl_3$: 165.46 (CO), 152.45 (CO, Ar), 131.74 (CH, Ar), 129.68 (C, Ar), 121.93 (CH, Ar), 61.45 (OCH_2), 37.91 (CH_3SO_3), 14.38 (CH_3).

Propyl 4-methanesulfonylbenzoate (260):

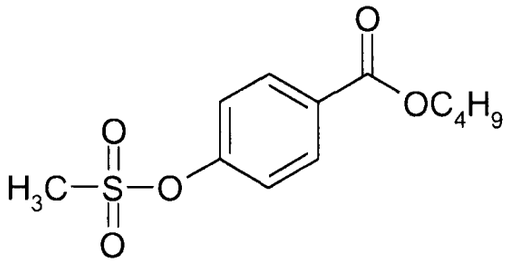


Compound **260** was synthesised following the same procedure as for compound **258** except that compound **222** (0.52g, 2.88mmol) was stirred in anhydrous DCM

(50mL) with TEA (1.2mL, 8.60mmol) 30min for prior to the addition of methanesulfonyl chloride (0.7mL, 9.04mmol). The reaction mixture was subsequently refluxed for 4h. The solvent was removed under vacuum which gave an off-white solid which was purified via column chromatography to give **260** as an off-white solid (0.61g, 82.1% yield); m.p.=53.1-54.2°C; R_f=0.49 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.61min; LRMS (EI): 258 (M^+, 33%), 216 (M^+-C_3H_6, 65%), 199 (M^+-C_3H_7O, 65%), 138 (M^+-$C_4H_8SO_2$, 100%), 121 (M^+-$C_4H_9SO_3$, 76%), 92 (M^+-$C_5H_{10}SO_4$, 14%).

$\nu_{(max)}$ (Film) cm^{-1}: 1709.6 (C=O), 1601.0 (Ar C=C), 1359.9 (SO_2R_2); δ_H $CDCl_3$: 8.03 (2H, dd, J=8.9Hz, PhH), 7.39 (2H, dd, J=8.9Hz, PhH), 4.18 (2H, t, J=6.6Hz, OCH_2), 3.26 (3H, s, SCH_3), 1.68 (2H, m, CH_2), 0.92 (3H, t, J=7.3Hz, CH_3); δ_C $CDCl_3$: 165.94 (CO), 153.07 (CO, Ar), 131.43 (CH, Ar), 129.50 (C, Ar), 122.42 (CH, Ar), 66.53 (OCH_2), 37.15 (CH_3SO_3), 21.94 (CH_2), 9.93 (CH_3).

Butyl 4-methanesulfonylbenzoate (261):

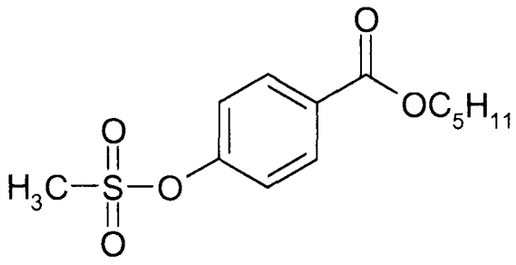


Compound **261** was synthesised following the same procedure as for compound **258** except that compound **223** (0.55g, 2.84mmol) was stirred in anhydrous DCM (75mL) with TEA (1.2mL, 8.60mmol) for 30min prior to the addition of methanesulfonyl chloride (0.7mL, 9.04mmol). The reaction mixture was subsequently refluxed for 4h. The solvent was removed under vacuum which gave an off-white solid which was purified via column chromatography to give **261** as an off-white coloured solid (0.58g, 75.1% yield); m.p.=46.7-48.3°C; R_f=0.51 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=14.46; LRMS (EI): 272 (M^+, 2%), 216 (M^+-C_4H_8, 65%), 199 (M^+-C_4H_9O, 55%), 138 (M^+-$C_5H_{11}SO_2$, 100%), 121 (M^+-

$C_5H_{10}SO_3$, 71%); Elemental analysis: found C 53.12%, H 5.91%; $C_7H_8SO_3$ requires C 52.93%, H 5.92%.

$\nu_{(max)}$ (Film) cm^{-1}: 1715.1 (C=O), 1602.9 (Ar C=C), 1368.8 (SO_2R_2); δ_H d_6 acetone: 8.16 (2H, dd, J=8.9Hz, PhH), 7.53 (2H, dd, J=8.9Hz, PhH), 4.36 (2H, t, J=6.6Hz, OCH_2), 3.41 (3H, s, SCH_3), 1.79 (2H, m, CH_2), 1.53 (2H, m, CH_2), 1.01 (3H, t, J=7.3Hz, CH_3); δ_C d_6 acetone: 164.98 (CO), 153.07 (CO, Ar), 131.49 (CH, Ar), 129.50 (C, Ar), 122.42 (CH, Ar), 64.79 (OCH_2), 37.15 (CH_3SO_3), 30.67 (CH_2), 19.08 (CH_2), 13.22 (CH_3).

Pentyl 4-methanesulfonylbenzoate (262):

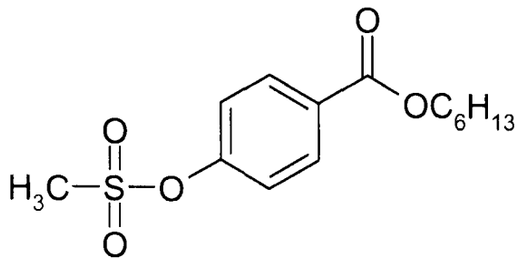


Compound **262** was synthesised following the same procedure as for compound **258** except that compound **224** (0.53g, 2.57mmol) was stirred in anhydrous DCM (75mL) with TEA (1.2mL, 8.60mmol) for 30min prior to the addition of methanesulfonyl chloride (0.7mL, 9.04mmol). The reaction mixture was subsequently refluxed for 4h. The solvent was removed under vacuum which gave an off-white solid which was purified via column chromatography to give **262** as an off-white coloured solid (0.48g, 65.3% yield); m.p.=65.7-67.4°C; R_f=0.54 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=10.74min; LRMS (EI): 286 (M^+, 1%), 216 (M^+-C_5H_{10}, 46%), 199 (M^+-$C_5H_{11}O$, 46%), 138 (M^+-$C_6H_{12}SO_2$, 100%), 121 (M^+-$C_6H_{13}SO_3$, 69%), 92 (M^+-$C_7H_{14}SO_4$, 20%); Elemental analysis: found C 54.39%, H 6.34%; $C_7H_8SO_3$ requires C 54.53%, H 6.34%.

$\nu_{(max)}$ (Film) cm^{-1}: 1702.4 (C=O), 1601.3 (Ar C=C), 1372.20 (SO_2R_2); δ_H d_6 acetone: 8.17 (2H, dd, J=8.9Hz, PhH), 7.53 (2H, dd, J=8.9Hz, PhH), 4.36 (2H, t, J=6.6Hz, OCH_2), 3.40 (3H, s, SCH_3), 1.82 (2H, m, CH_2), 1.46 (4H, m, CH_2), 0.97

(3H, t, J=7.1Hz, C$\underline{H}_3$); δ_C d_6 acetone: 164.98 ($\underline{C}O$), 153.08 ($\underline{C}O$, Ar), 131.42 ($\underline{C}H$, Ar), 129.52 ($\underline{C}$, Ar), 122.41 ($\underline{C}H$, Ar), 65.07 (O$\underline{C}H_2$), 37.15 ($\underline{C}H_3SO_3$), 28.09 ($\underline{C}H_2$), 28.09 ($\underline{C}H_2$), 22.21 ($\underline{C}H_2$), 13.45 ($\underline{C}H_3$).

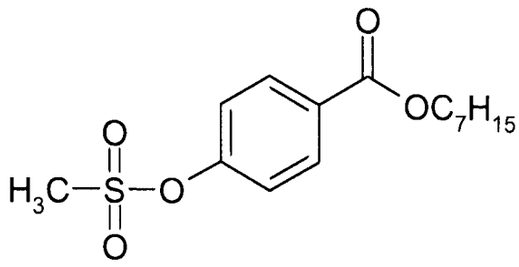
Hexyl 4-methanesulfonylbenzoate (263):



Compound **263** was synthesised following the same procedure as for compound **258** except that compound **225** (1.76g, 7.94mmol) was stirred in anhydrous DCM (75mL) with TEA (1.2mL, 8.60mmol) for 30min prior to the addition of methanesulfonyl chloride (0.7mL, 9.04mmol). The reaction mixture was subsequently refluxed for 4h. The solvent was removed under vacuum which gave a brown solid which was purified via column chromatography to give **263** as an off-white coloured solid (1.56g, 65.5% yield); m.p.=49.4-48.6°C; R_f=0.55 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=11.36min; LRMS (EI): 300 (M^+, 1%), 216 (M^+-C_6H_{12}, 78%), 199 (M^+-$C_6H_{13}O$, 57%), 138 (M^+-$C_7H_{14}SO_2$, 100%), 121 (M^+-$C_7H_{15}SO_3$, 68%), 92 (M^+-$C_8H_{16}SO_4$, 16%).

$\nu_{(max)}$ (Film) cm^{-1}: 1659.8 (C=O), 1604.9 (Ar C=C), 1361.9 (SO_2R_2); δ_H d_6 acetone: 8.12 (2H, dd, J=8.6Hz, Ph$\underline{H}$), 7.48 (2H, dd, J=8.6Hz, Ph$\underline{H}$), 4.31 (2H, t, J=7.1Hz, O$\underline{CH}_2$), 3.36 (3H, s, S$\underline{CH}_3$), 1.76 (2H, m, $\underline{CH}_2$), 1.36 (6H, m, $\underline{CH}_2$), 0.89 (3H, t, J=7.1Hz, C$\underline{H}_3$); δ_C d_6 acetone: 165.72 ($\underline{C}O$), 153.79 ($\underline{C}O$, Ar), 132.18 ($\underline{C}H$, Ar), 130.24 ($\underline{C}$, Ar), 123.14 ($\underline{C}H$, Ar), 65.86 (O$\underline{C}H_2$), 37.93 ($\underline{C}H_3SO_3$), 32.16 ($\underline{C}H_2$), 29.33 ($\underline{C}H_2$), 26.37 ($\underline{C}H_2$), 23.19 ($\underline{C}H_2$), 14.32 ($\underline{C}H_3$).

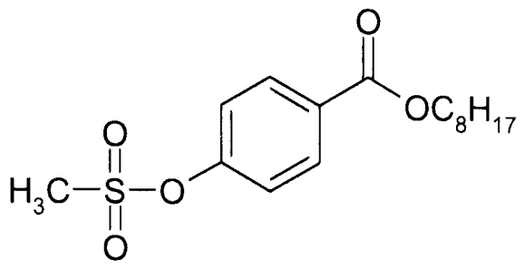
Heptyl 4-methanesulfonylbenzoate (264):



Compound **264** was synthesised following the same procedure as for compound **258** except that compound **226** (0.52g, 2.18mmol) was stirred in anhydrous DCM (75mL) with TEA (1.2mL, 8.60mmol) for 30min prior to the addition of methanesulfonyl chloride (0.7mL, 9.04mmol). The reaction mixture was subsequently refluxed for 4h. The solvent was removed under vacuum which gave a brown coloured solid which was purified via column chromatography to give **264** as a pale brown coloured solid (0.47g, yield 68.7%); m.p.=43.0-44.2°C; R_f=0.58 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=11.98min; LRMS (EI): 314 (M^+, 1%), 216 (M^+-C_7H_{14}, 97%), 199 (M^+-$C_7H_{15}O$, 57%), 138 (M^+-$C_8H_{16}SO_2$, 100%), 121 (M^+-$C_8H_{17}SO_3$, 66%), 92 (M^+-$C_9H_{18}SO_4$, 15%); Elemental analysis: found C 53.39%, H 7.05%; $C_7H_8SO_3$ requires C 57.30%, H 7.05%.

$\nu_{(max)}$ (Film) cm^{-1}: 1711.5 (C=O), 1600.7 (Ar C=C), 1371.2 (SO_2R_2); δ_H d_6 acetone: 8.17 (2H, dd, J=8.9Hz, PhH), 7.53 (2H, dd, J=8.9Hz, PhH), 4.36 (2H, t, J=6.6Hz, OCH_2), 3.40 (3H, s, SCH_3), 1.81 (2H, m, CH_2), 1.40 (8H, m, CH_2), 0.93 (3H, t, J=6.9Hz, CH_3); δ_C d_6 acetone: 164.98 (CO), 153.08 (CO, Ar), 131.41 (CH, Ar), 129.52 (C, Ar), 122.41 (CH, Ar), 65.09 (OCH_2), 37.15 (CH_3SO_3), 31.68 (CH_2), 25.88 (CH_2), 22.44 (CH_2), 13.51 (CH_3).

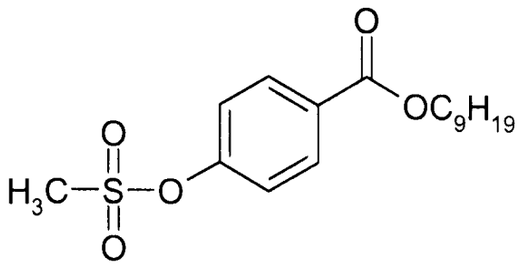
Octyl 4-methanesulfonylbenzoate (265):



Compound **265** was synthesised following the same procedure as for compound **258** except that compound **227** (0.50g, 2.00mmol) was stirred in anhydrous DCM (75mL) with TEA (1.2mL, 8.60mmol) for 30min prior to the addition of methanesulfonyl chloride (0.7mL, 9.04mmol). The reaction mixture was subsequently refluxed for 4h. The solvent was removed under vacuum which gave a brown coloured solid which was purified via column chromatography to give **265** as a pale brown coloured solid (0.42g, yield 64.0%); m.p.=51.8-53.0°C; R_f=0.60 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=12.68min; LRMS (EI): 328 (M^+, 1%), 216 (M^+-C_8H_{16}, 100%), 199 (M^+-$C_8H_{17}O$, 53%), 138 (M^+-$C_9H_{18}SO_2$, 95%), 121 (M^+-$C_9H_{19}SO_3$, 14%).

$\nu_{(max)}$ (Film) cm^{-1}: 1710.8 (C=O), 1600.8 (Ar C=C), 1370.9 (SO_2R_2); δ_H $CDCl_3$: 8.09 (2H, dd, J=8.9Hz, PhH), 7.33 (2H, dd, J=8.9Hz, PhH), 4.30 (2H, t, J=6.7Hz, OCH_2), 3.16 (3H, s, SCH_3), 1.74 (2H, q, J=6.9Hz, CH_2), 1.32 (10H, m, CH_2), 0.86 (3H, t, J=6.9Hz, CH_3); δ_C $CDCl_3$: 165.52 (CO), 152.44 (CO, Ar), 131.74 (CH, Ar), 129.72 (C, Ar), 121.94 (CH, Ar), 65.63 (OCH_2), 37.89 (CH_3SO_3), 31.87 (CH_2), 29.30 (CH_2), 29.26 (CH_2), 28.75 (CH_2), 26.09 (CH_2), 22.72 (CH_2), 14.17 (CH_3).

Nonyl 4-methanesulfonylbenzoate (266):

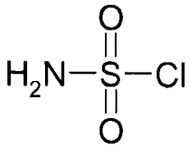


Compound **266** was synthesised following the same procedure as for compound **258** except that compound **228** (1.19g, 4.50mmol) was stirred in anhydrous DCM (75mL) with TEA (1.2mL, 8.60mmol) for 30min prior to the addition of methanesulfonyl chloride (0.7mL, 9.04mmol). The reaction mixture was subsequently refluxed for 4h. The solvent was removed under vacuum which gave an off-white crystalline solid which was purified via column chromatography to give **266** as an off-white crystalline solid (0.84g, yield 54.5%); m.p.=47.0-48.3°C; R_f=0.62 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=13.52min; LRMS (EI): 342 (M^+, 1%), 216 (M^+-C_9H_{18}, 100%), 199 (M^+-$C_9H_{18}O$, 49%), 138 (M^+-$C_{10}H_{20}SO_2$, 76%), 121 (M^+-$C_{10}H_{20}SO_3$, 47%).

$\nu_{(max)}$ (Film) cm^{-1}: 1711.3 (C=O), 1600.5 (Ar C=C), 1370.8 (SO_2R_2); δ_H d_6 acetone: 8.12 (2H, dd, J=8.6Hz, PhH), 7.53 (2H, dd, J=8.6Hz, PhH), 4.32 (2H, t, J=7.1Hz, OCH_2); 3.36 (3H, s, SCH_3), 1.77 (2H, m, CH_2), 1.37 (12H, m, CH_2), 0.87 (3H, t, J=6.8Hz, CH_3); δ_C d_6 acetone: 165.78 (CO), 153.88 (CO, Ar), 132.25 (CH, Ar), 130.33 (C, Ar), 123.21 (CH, Ar), 65.93 (OCH_2), 37.99 (CH_3SO_3), 32.64 (CH_2), 31.87 (CH_2), 30.08 (CH_2), 30.05 (CH_2), 29.46 (CH_2), 26.77 (CH_2), 23.38 (CH_2), 14.45 (CH_3).

2.7 Attempted synthesis of the 3-bromo-4-sulfamoyloxy-benzoic acid esters

Aminosulfonyl chloride (267):



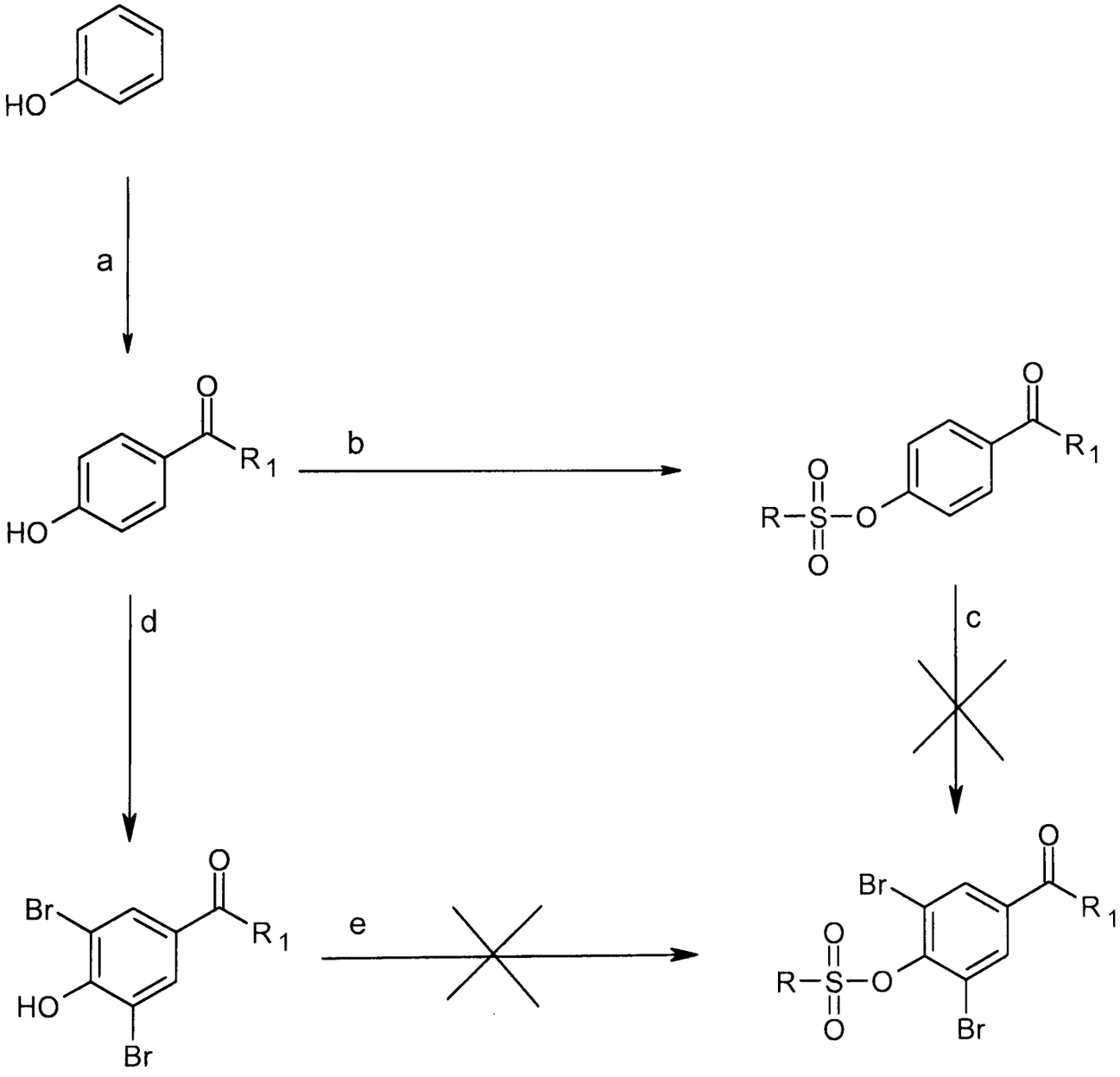
Formic acid (1.0mL, 26.5mmol) was added in a dropwise manner to a stirred solution of chlorosulfonyl isocyanate (2.3mL, 26.4mmol) at 0°C. The solution was left to stir for 30min. After the evolution of gases had ceased and an off-white precipitate had formed, anhydrous toluene (50mL) was added and the solution was left to stir for a further 1h. The resulting solution was decanted before being used in the aminosulfonation steps without further purification.

Chapter 3: Synthesis of the 4-hydroxy phenyl ketones and derivatives

3.0 Synthesis of the 4-hydroxy phenyl ketones and derivatives

3.1 Discussion

The synthesis of sulfonated derivatives of 4-hydroxyphenyl ketones was undertaken using the reactions outline in Scheme 3.1.



Scheme 3.1. Synthesis of derivatives of 4-hydroxyphenyl ketones (where a=$AlCl_3$/phenol/DCM; b and e=RSO_2Cl/DCM/TEA/Δ; c and d=Br_2/CH_3COOH).

The first step involves Friedel-Crafts acylation; it has been suggested that aluminium chloride reacts with the acid chloride to form the electrophilic acylium ion (Fessenden et al, 1998). The phenol ring then undergoes electrophilic substitution to give the required aryl ketone - an excess of aluminuium chloride is required so as to form a complex involving the aryl carbonyl group in the product. Treatment with water during workup liberates the required ketone (Sykes, 1988). The acylium ion is resonance stabilised, as such, rearrangement is not observed (as with alkylation where the formation of a stable carbocation leads to rearrangement resulting in numerous by-products depending upon the alkyl halide). As such, the formation of an acylium ion is crucial to the progress of the reaction.

The acylation reaction proceeded smoothly and gave the desired compounds in good yield, however under the Friedel-Crafts reaction conditions, the ortho substituted product was observed in small quantities but was removed via column chromatography. With regards to potential impurities, such as disubstituted compounds, these were not observed, potentially due firstly to the electron-withdrawing ability of the carbonyl group (which therefore deactivates the aromatic ring system, decreasing the potential of disubstitution, or indeed any other polysubstitution products); secondly, the phenolic OH moiety is also believed to undergo interaction with the $AlCl_3$, thereby producing a bulky complex which prevents ortho substitution(s) (to the OH group) as a result of steric hindrance.

The acylation step in Scheme 3.1 was found to proceed without any major problems and the phenyl ketone products were obtained (after column chromatography) in moderate yields {ranging from ~43% for compound **277** [1-(4-Hydroxy-phenyl)-dodecan-1-one] to ~74% for compound **268** [1-(4-Hydroxy-phenyl)-ethanone]}.

The second step in the synthesis of the target compounds involves either the bromination of the 4-hydroxyphenyl ketones (steps d and e; Scheme 3.1) or the sulfonation of the 4-hydroxy moiety (steps b and c; Scheme 3.1). In the synthesis of the target compounds, we attempted the initial synthesis of the sulfonated derivative of 4-hydroxyphenyl ketone (step b; Scheme 3.1; compounds **296** to

324) followed by the bromination of the sulfonated derivative (step c; Scheme 3.1). The reaction to produce the methanesulfonate and trifluoromethanesulfonate derivatives of 4-hydroxyphenyl ketones proceeded in good to excellent yield [ranging from ~35% for compound **311** (trifluromethanesulfonic acid 4-acetyl-phenyl ester) to ~87% for compound **304** (methanesulfonic acid 4-nonyl-phenyl ester)] without any major problems.

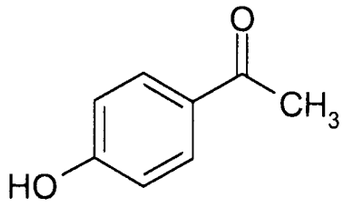
In the synthesis of the brominated derivatives, however, we discovered that the synthesis of the brominated derivatives of methanesulfonate- and trifluoromethanesulfonate-based compounds proved to be extremely difficult. Indeed, prolonged reaction time (upto a maximum of two weeks) did not yield any target compound across the full range. The lack of any product is postulated to be due to the bulky nature of the methanesulfonate moiety resulting in steric hindrance. As such, we abandoned this route in preference to steps d and e within Scheme 3.1. It should be noted that a previous study by Patel (2003b) to synthesise the aminosulfonate derivatives also proved to be difficult and the attempted bromination of the sulfamate derivatives of 4-hydroxyphenyl ketone-based compounds resulted in hydrolysis of the aminosulfonate moiety, as such, the synthesis of the aminosulfonate derivatives was not attempted.

As previously shown in section 2.1, bromination of the phenyl ring system occurs via electrophilic aromatic substitution. The π electrons from the aromatic C=C bond act as a nucleophile attacking bromine. In the synthesis of the target compounds, we undertook the initial bromination, which resulted in the target compounds (step d; Scheme 3.1; compounds **283** to **295**) in good yield [ranging from ~53% for compound **288** (3,5-dibromo-4-hydroxyheptanophenone) to ~73% for compound **284** (3,5-dibromo-4-hydroxypropiophenone)] and without any major problems. It should be noted that no monobrominated by-product was observed, indeed, the bromine was added in excess so as to reduce any possibility of the production of the monobrominated by-product.

Step e (Scheme 3.1) involves the reaction between the appropriate sulfonyl chloride and the 4-hydroxyphenyl ketone-based compound. However, attempts to synthesise the target compounds failed and no target compound was produced.

3.2 Synthesisof the 4-hydroxy phenyl ketones

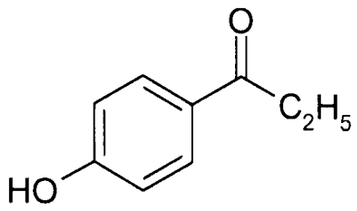
1-(4-Hydroxy-phenyl)-ethanone (268):



Aluminium trichloride (4.54g, 34.00mmol) was added to a solution of phenol (1.53g, 16.30mmol) in anhydrous DCM (15mL). The slurry was left to stir for 1h before acetyl chloride (1.30mL, 18.20mmol) was added in a dropwise manner. The solution was left to stir for a further 14h. The reaction was quenched using an ice-cold solution of aqueous hydrochloric acid (HCl) (1M, 30mL) and extracted into DEE (2 x 50mL). The combined organic layer was extracted into sodium hydroxide (NaOH) (2M, 2 x 50mL) and then acidified to pH 2 using aqueous HCl (1M, 40mL). The product was extracted into DEE (2 x 50mL), the organic layer washed with water (2 x 50mL) and dried over anhydrous $MgSO_4$. The solvent was removed under vacuum to give a brown solid. Column chromatography of the crude solid gave **268** as a white solid (1.63g, 73.5% yield); m.p.=109.4-110.3°C [lit. m.p.=110.2-110.4°C (Buehler et al, 1937)]; R_f=0.35 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=5.60min; LRMS (EI): 136 (M^+, 41%), 121 (M^+-CH_3, 100%), 93 (M^+-C_2H_3O, 28%); Elemental analysis: found C 70.42%, H 5.88%; $C_8H_8O_2$ requires C 70.58%, H 5.92%; HRMS (ES): found 137.05971 $C_8H_9O_2$ requires 137.15586.

$\nu_{(max)}$ (Film) cm^{-1}: 3315.6 (Ph-OH), 1661.6 (C=O), 1605.3 (Ar C=C), δ_H d_6 acetone: 9.20 (1H, s, O<u>H</u>), 7.89 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.91 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.48 (3H, s, C<u>H</u>$_3$); δ_C d_6 acetone: 196.36 (<u>C</u>O), 162.63 (<u>C</u>O), 131.57 (<u>C</u>H, Ar), 130.51 (<u>C</u>H, Ar), 115.97 (C, Ar), 26.34 (<u>C</u>H$_3$).

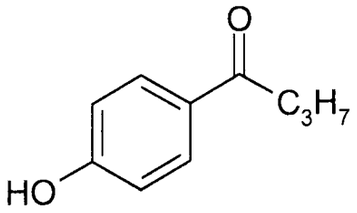
1-(4-Hydroxy-phenyl)-propan-1-one (269):



Compound **269** was synthesised following the same procedure as for compound **268** except that phenol (1.56g, 16.62mmol) was stirred with aluminium trichloride (4.54g, 34.01mmol) in anhydrous DCM (15mL) prior to the addition of propanoyl chloride (1.5mL, 17.19mmol). The crude solid was purified by column chromatography to give **269** as a white solid (1.87g, 75.0% yield); m.p.=151.1-152.5°C [lit. m.p.=152-153°C (Aulin-Erdtman and Sanden, 1968)]; R_f=0.40 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=6.13min; LRMS (EI): 150 (M^+, 12%), 121 (M^+-C_2H_5, 100%), 93 (M^+-C_3H_5O, 26%); Elemental analysis: found C 71.84%, H 6.71%; $C_9H_{10}O_2$ requires C 71.98%, H 6.71%; HRMS (ES): found 151.07536 $C_9H_{11}O_2$ requires 151.18244.

$\nu_{(max)}$ (Film) cm^{-1}: 3169.3 (CH), 1650.0 (C=O), 1604.7 (Ar C=C), δ_H d_6 acetone: 9.16 (1H, s, O<u>H</u>), 7.90 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.92 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.93 (2H, q, J=7.3Hz, C<u>H</u>$_2$), 1.11 (3H, t, J=7.3Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 199.01 (<u>C</u>O), 162.49 (<u>C</u>O), 131.20 (<u>C</u>H, Ar), 130.20 (<u>C</u>H, Ar), 115.99 (<u>C</u>, Ar), 31.63 (<u>C</u>H$_2$), 8.72 (<u>C</u>H$_3$).

1-(4-Hydroxy-phenyl)-butan-1-one (270):

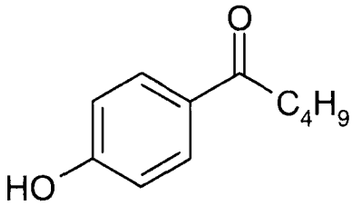


Compound **270** was synthesised following the same procedure as for compound **268** except that phenol (1.54g, 16.41mmol) was stirred with aluminium trichloride (4.57g, 34.23mmol) in anhydrous DCM (15mL) prior to the addition of butyryl

chloride (1.80mL, 17.21mmol). The crude solid was purified by column chromatography to give **270** as a light brown solid (1.54g, 57.2% yield); m.p.=92.9-93.6°C [lit. m.p.=93-94°C (Krausz and Martin, 1965)]; R_f=0.55 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=6.57min; LRMS (EI): 164 (M^+, 15%), 149 (M^+-CH_3, 1%), 136 (M^+-C_2H_4, 12%), 121 (M^+-C_3H_7, 100%), 93 (M^+-C_4H_7O, 14%); Elemental analysis: found C 73.07%, H 7.29%; $C_{10}H_{12}O_2$ requires C 73.15%, H 7.37%; HRMS (ES): found 165.09101 $C_{10}H_{13}O_2$ requires 165.20902.

$\nu_{(max)}$ (Film) cm^{-1}: 3361.4 (Ph-OH), 1658.3 (C=O), 1602.2 (Ar C=C); δ_H d_6 acetone: 7.90 (2H, dd, J=8.9Hz, PhH), 6.92 (2H, dd, J=8.9Hz, PhH), 2.89 (2H, t, J=7.3Hz, CH_2), 1.67 (2H, sex, J=7.3Hz, CH_2), 0.95 (3H, t, J=7.3Hz, CH_3); δ_C d_6 acetone: 198.47 (CO), 162.36 (CO), 131.08 (CH, Ar), 130.13 (CH, Ar), 115.79 (C, Ar), 40.21 (CH_2), 18.38 (CH_2), 13.97 (CH_3).

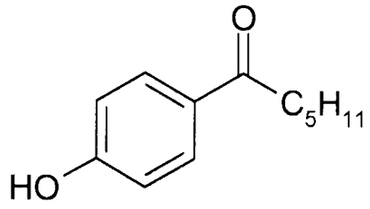
1-(4-Hydroxy-phenyl)-pentan-1-one (271):



Compound **271** was synthesised following the same procedure as for compound **268** except that phenol (1.50g, 15.99mmol) was stirred with aluminium trichloride (4.63g, 34.68mmol) in anhydrous DCM (15mL) prior to the addition of valeryl chloride (2.1mL, 17.60mmol). The crude solid was purified by column chromatography to give **271** as a white solid (1.60g, 56.2% yield); m.p.=63.9-64.9°C [lit. m.p.=62-63°C (Coulthard et al, 1930)]; R_f=0.57 [DEE/pet spirit 40-60°C (50/50)]: GC: t_R=7.04min; LRMS (EI): 178 (M^+, 5%), 149 (M^+-C_2H_5, 4%), 136 (M^+-C_3H_6, 46%), 121 (M^+-C_4H_9, 100%), 93 (M^+-C_5H_9O, 11%); Elemental analysis: found C 73.98%, H 7.90%; $C_{11}H_{14}O_2$ requires C 74.13%, H 7.92%; HRMS (ES): found 179.10666 $C_{11}H_{15}O_2$ requires 179.23560.

$\nu_{(max)}$(Film) cm^{-1}: 3295.7 (Ph-OH), 1656.4 (C=O), 1601.2 (Ar C=C); δ_H d_6 acetone: 9.17 (1H, s, O<u>H</u>), 7.91 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.92 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.92 (2H, t, J=7.3Hz, C<u>H</u>$_2$), 1.64 (2H, m, C<u>H</u>$_2$), 1.38 (2H, m, C<u>H</u>$_2$), 0.91 (3H, t, J=7.3Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 198.66 (<u>C</u>O), 162.51 (<u>C</u>O), 131.30 (<u>C</u>H, Ar), 130.41 (<u>C</u>H, Ar), 116.01 (<u>C</u>, Ar), 38.25 (<u>C</u>H$_2$), 27.48 (<u>C</u>H$_2$), 23.16 (<u>C</u>H$_2$), 14.32 (<u>C</u>H$_3$).

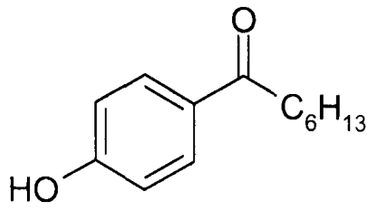
1-(4-Hydroxy-phenyl)-hexan-1-one (272):



Compound **272** was synthesised following the same procedure as for compound **268** except that phenol (1.56g, 16.62mmol) was stirred with aluminium trichloride (4.54g, 34.01mmol) in anhydrous DCM (15mL) prior to the addition of hexanoyl chloride (2.50mL, 17.66mmol). The crude solid was purified by column chromatography to give **272** as an off-white solid (1.71g, 55.3% yield); m.p.=62.6-64.5°C [lit. m.p.=63-64°C (Coulthard et al, 1930)]; R_f=0.58 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.48min; LRMS (EI): 192 (M^+, 3%), 149 (M^+-C_3H_7, 6%), 136 (M^+-C_4H_8, 56%), 121 (M^+-C_5H_{11}, 100%), 93 (M^+-$C_6H_{11}O$, 13%); Elemental analysis: found C 74.99%, H 8.40%; $C_{12}H_{16}O_2$ requires C 74.97%, H 8.39%; HRMS (ES): found 193.1223 $C_{12}H_{17}O_2$ requires 193.26218.

$\nu_{(max)}$ (Film) cm^{-1}: 3296.0 (Ph-OH), 1656.3 (C=O), 1601.9 (Ar C=C); δ_H d_6 acetone: 9.15 (1H, s, O<u>H</u>), 7.90 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.90 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.92 (2H, t, J=7.3Hz, C<u>H</u>$_2$), 1.67 (2H, m, C<u>H</u>$_2$), 1.34 (4H, m, C<u>H</u>$_2$), 0.89 (3H, t, C<u>H</u>$_3$); δ_C d_6 acetone: 198.44 (<u>C</u>O), 162.29 (<u>C</u>O), 131.10 (<u>C</u>H, Ar), 130.24 (<u>C</u>H, Ar), 115.79 (<u>C</u>, Ar), 38.28 (<u>C</u>H$_2$), 32.14 (<u>C</u>H$_2$), 24.83 (<u>C</u>H$_2$), 23.10 (<u>C</u>H$_2$), 14.13 (<u>C</u>H$_3$).

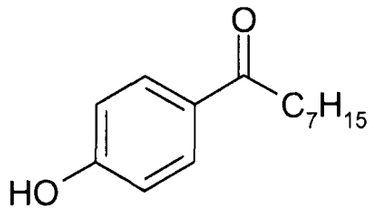
1-(4-Hydroxy-phenyl)-heptan-1-one (273):



Compound **273** was synthesised following the same procedure as for compound **268** except that phenol (1.54g, 16.63mmol) was stirred with aluminium trichloride (4.54g, 34.01mmol) in anhydrous DCM (15mL) prior to the addition of heptanoyl chloride (2.70mL, 17.45mmol). The crude solid was purified by column chromatography to give **273** as an off-white solid (1.94g, 57.6% yield); m.p.=93.0-94.1°C [lit. m.p.=93-94°C (Coulthard et al, 1930)]; R_f=0.62 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.91min; LRMS (EI): 206 (M^+, 4%), 149 (M^+-C_4H_9, 9%), 136 (M^+-C_5H_{10}, 74%), 121 (M^+-C_6H_{13}, 100%), 93 (M^+-$C_7H_{13}O$, 11%); Elemental analysis: found C 75.84%, H 8.78%; $C_{13}H_{18}O_2$ requires C 75.69%, H 8.80%; HRMS (ES): found 207.1380 $C_{13}H_{19}O_2$ requires 207.28876.

$\nu_{(max)}$ (Film) cm^{-1}: 3316.0 (Ph-OH), 1661.2 (C=O), 1600.6 (Ar C=C); δ_H d_6 acetone: 9.15 (1H, s, O<u>H</u>), 7.90 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.91 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.92 (2H, t, J=7.3Hz, C<u>H</u>$_2$), 1.66 (2H, m, C<u>H</u>$_2$), 1.32 (6H, m, C<u>H</u>$_2$), 0.88 (3H, t, C<u>H</u>$_3$); δ_C d_6 acetone: 198.65 (<u>C</u>O), 162.49 (<u>C</u>O), 131.30 (<u>C</u>H, Ar), 130.43 (<u>C</u>H, Ar), 115.99 (<u>C</u>, Ar), 38.53 (<u>C</u>H$_2$), 32.54 (<u>C</u>H$_2$), 25.29 (<u>C</u>H$_2$), 23.30 (<u>C</u>H$_2$), 14.38 (<u>C</u>H$_3$).

1-(4-Hydroxy-phenyl)-octan-1-one (274):

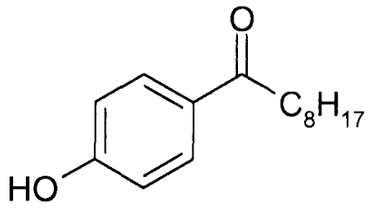


Compound **274** was synthesised following the same procedure as for compound **268** except that phenol (1.55g, 16.52mmol) was stirred with aluminium trichloride (4.62g, 34.60mmol) in anhydrous DCM (15mL) prior to the addition of octanoyl

chloride (3.00mL, 17.54mmol). The crude solid was purified by column chromatography to give **274** as a white solid (2.17g, 59.7% yield); m.p.=63.2-63.7°C [lit. m.p.=62.5-63.5°C (Ralston et al, 1940)]; R_f=0.63 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.29min; LRMS (EI): 220 (M^+, 7%), 149 (M^+-C_5H_{11}, 7%), 136 (M^+-C_6H_{12}, 67%), 121 (M^+-C_7H_{15}, 100%), 93 (M^+-$C_8H_{15}O$, 10%); HRMS (ES): found 221.1536 $C_{14}H_{21}O_2$ requires 221.31534.

$\nu_{(max)}$ (Film) cm^{-1}: 3311.8 (Ph-OH), 1660.3 (C=O), 1600.5 (Ar C=C); δ_H d_6 acetone: 9.15 (1H, s, O<u>H</u>), 7.90 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.91 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.92 (2H, t, J=7.3Hz, C<u>H</u>$_2$), 1.67 (2H, m, C<u>H</u>$_2$), 1.32 (8H, m, C<u>H</u>$_2$), 0.88 (3H, t, J=6.9Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 198.65 (<u>C</u>O), 162.49 (<u>C</u>O), 131.30 (<u>C</u>H, Ar), 130.45 (<u>C</u>H, Ar), 115.99 (<u>C</u>, Ar), 38.54 (<u>C</u>H$_2$), 32.58 (<u>C</u>H$_2$), 25.35 (<u>C</u>H$_2$), 23.35 (<u>C</u>H$_2$), 14.40 (<u>C</u>H$_3$).

1-(4-Hydroxy-phenyl)-nonan-1-one (275):

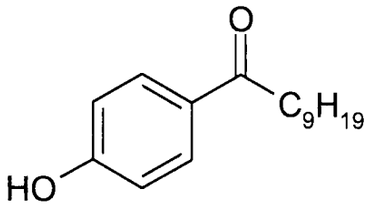


Compound **275** was synthesised following the same procedure as for compound **268** except that phenol (1.52g, 16.25mmol) was stirred with aluminium trichloride (4.61g, 34.53mmol) in anhydrous DCM (15mL) prior to the addition of nonanoyl chloride (3.2mL, 17.77mmol). The crude solid was purified by column chromatography to give **275** as a cream solid (2.64g, 69.4% yield); m.p.=56.1-57.2°C [lit. m.p.=55.5-56.5°C (Kolobielski et al, 1968)]; R_f=0.64 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.68min; LRMS (EI): 234 (M^+, 2%), 149 (M^+-C_6H_{13}, 10%), 136 (M^+-C_7H_{14}, 90%), 121 (M^+-C_8H_{17}, 100%), 93 (M^+-$C_9H_{17}O$, 12%); HRMS (ES): found 235.1693 $C_{15}H_{23}O_2$ requires 235.34192.

$\nu_{(max)}$ (Film) cm^{-1}: 3294.7 (Ph-OH), 1655.3 (C=O), 1601.2 (Ar C=C); δ_H d_6 acetone: 9.15 (1H, s, O<u>H</u>), 7.91 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.92 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.92 (2H, t, J=7.3Hz, C<u>H</u>$_2$), 1.66 (2H, m, C<u>H</u>$_2$), 1.31 (10H, m, C<u>H</u>$_2$), 0.87 (3H, t,

J=6.9Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 198.67 (<u>C</u>O), 162.50 (<u>C</u>O), 131.29 (<u>C</u>H, Ar), 130.41 (<u>C</u>H, Ar), 116.00 (<u>C</u>, Ar), 38.53 (<u>C</u>H$_2$), 32.64 (<u>C</u>H$_2$), 25.34 (<u>C</u>H$_2$), 23.36 (<u>C</u>H$_2$), 14.41 (<u>C</u>H$_3$).

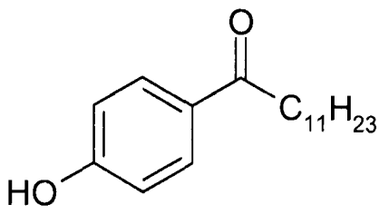
1-(4-Hydroxy-phenyl)-decan-1-one (276):



Compound **276** was synthesised following the same procedure as for compound **268** except that phenol (1.53g, 16.32mmol) was stirred with aluminium trichloride (4.56g, 34.15mmol) in anhydrous DCM (15mL) prior to the addition of decanoyl chloride (3.6mL, 17.34mmol). The crude solid was purified by column chromatography to give **276** as a white solid (2.96g, 73.1% yield); m.p.=55.9-56.5°C [lit. m.p.=64-65°C (Woodcock et al, 1955)]; R_f=0.66 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.07min; LRMS (EI): 248 (M^+, 6%), 149 (M^+-C_7H_{15}, 14%), 136 (M^+-C_8H_{16}, 90%), 121 (M^+-C_9H_{19}, 100%), 93 (M^+-$C_{10}H_{19}O$, 11%); Elemental analysis: found C 77.41%, H 9.61%; $C_{16}H_{24}O_2$ requires C 77.38%, H 9.74%; HRMS (ES): found 249.18491 $C_{16}H_{25}O_2$ requires 249.36850.

$\nu_{(max)}$ (Film) cm^{-1}: 3287.3 (Ph-OH), 1656.4 (C=O), 1604.3 (Ar C=C); δ_H d_6 acetone: 9.16 (1H, s, O<u>H</u>), 7.91 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.92 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.92 (2H, t, J=7.3Hz, C<u>H</u>$_2$), 1.67 (2H, m, C<u>H</u>$_2$), 1.32 (12H, m, C<u>H</u>$_2$), 0.87 (3H, t, J=6.9Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 198.66 (<u>C</u>O), 162.50 (<u>C</u>O), 131.30 (<u>C</u>H, Ar), 130.44 (<u>C</u>H, Ar), 116.00 (<u>C</u>, Ar), 38.54 (<u>C</u>H$_2$), 32.68 (<u>C</u>H$_2$), 25.35 (<u>C</u>H$_2$), 23.39 (<u>C</u>H$_2$), 14.42 (<u>C</u>H$_3$).

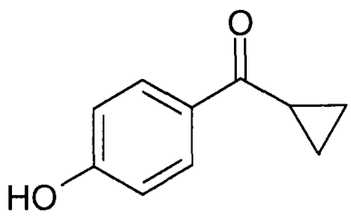
1-(4-Hydroxy-phenyl)-dodecan-1-one (277):



Compound **277** was synthesised following the same procedure as for compound **268** except that phenol (1.54g, 16.43mmol) was stirred with aluminium trichloride (4.53g, 33.93mmol) in anhydrous DCM (15mL) prior to the addition of dodecanoyl chloride (4.2mL, 17.68mmol). The crude solid was purified by column chromatography to give **277** as a cream solid (1.95g, 43.0% yield); m.p.=60.9-62.8°C [lit. m.p.=70-71°C (Ralston and Bauer, 1940)]; R_f=0.72 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.93min; LRMS (EI): 276 (M^+, 7%), 149 (M^+-C_9H_{19}, 11%), 136 (M^+-$C_{10}H_{20}$, 100%), 121 (M^+-$C_{11}H_{23}$, 80%), 93 (M^+-$C_{12}H_{23}O$, 8%); HRMS (ES): found 277.21621 $C_{18}H_{29}O_2$ requires 277.42206.

$\nu_{(max)}$ (Film) cm^{-1}: 3390.0 (Ph-OH), 1678.3 (C=O), 1605.3 (Ar C=C); δ_H d_6 acetone: 9.16 (1H, s, O<u>H</u>), 7.90 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.92 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.92 (2H, t, J=7.3Hz, C<u>H</u>$_2$), 1.67 (2H, m, C<u>H</u>$_2$), 1.33 (16H, m, C<u>H</u>$_2$), 0.87 (3H, t, J=6.9Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 198.65 (<u>C</u>O), 162.49 (<u>C</u>O), 131.30 (<u>C</u>H, Ar), 130.45 (<u>C</u>H, Ar), 115.99 (<u>C</u>, Ar), 38.54 (<u>C</u>H$_2$), 32.70 (<u>C</u>H$_2$), 25.35 (<u>C</u>H$_2$), 23.39 (<u>C</u>H$_2$), 14.42 (<u>C</u>H$_3$).

Cyclopropyl-(4-Hydroxy-phenyl)-methanone (278):

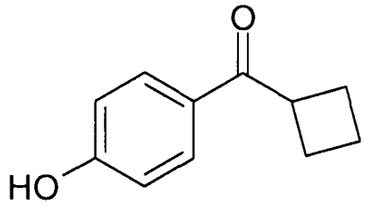


Compound **278** was synthesised following the same procedure as for compound **268** except that phenol (1.57g, 16.71mmol) was stirred with aluminium trichloride (4.51g, 33.78mmol) in anhydrous DCM (15mL) prior to the addition of

cyclopropane carbonyl chloride (1.60mL, 17.61mmol). The crude solid was purified by column chromatography to give **278** as a cream solid (2.09g, 77.2% yield); m.p.=106.8-108.6°C [lit. m.p.=105-107°C (Magano et al, 2006)]; R_f=0.33 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=6.84min; LRMS (EI): 162 (M^+, 28%), 133 (M^+-C_2H_5, 3%), 121 (M^+-C_3H_5, 100%), 93 (M^+-C_4H_5O, 17%); HRMS (ES): found 163.07536 $C_{10}H_{11}O_2$ requires 163.19314.

$\nu_{(max)}$ (Film) cm^{-1}: 3264.7 (Ph-OH), 1643.1 (C=O), 1601.6 (Ar C=C); δ_H $CDCl_3$: 7.95 (2H, dd, J=8.9Hz, PhH), 6.91 (2H, dd, J=8.9Hz, PhH), 2.65 (1H, m, CH), 1.23 (2H, m, CH_2), 1.04 (2H, m, CH_2); δ_C $CDCl_3$: 201.24 (CO), 161.27 (CO), 130.90 (CH, Ar), 130.33 (CH, Ar), 115.62 (C, Ar), 17.07 (CH), 12.02 (CH_2).

Cyclobutyl-(4-Hydroxy-phenyl)-methanone (279):

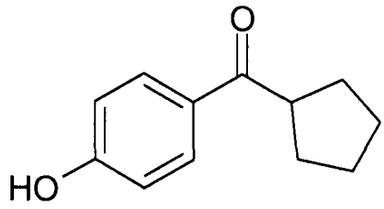


Compound **279** was synthesised following the same procedure as for compound **268** except that phenol (1.55g, 16.54mmol) was stirred with aluminium trichloride (4.58g, 34.31mmol) in anhydrous DCM (15mL) prior to the addition of cyclobutane carbonyl chloride (2.0mL, 16.88mmol). The crude solid was purified by column chromatography to give **279** as a cream solid (2.48g, 85.2% yield); m.p.=95.5-96.9°C [lit. m.p.=102.4-105.4°C (Patel, 2003b)]; R_f=0.45 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.35min; LRMS (EI): 176 (M^+, 4%), 148 (M^+-C_2H_4, 1%), 121 (M^+-C_4H_7, 100%), 93 (M^+-C_5H_7O, 6%); Elemental analysis: found C 74.93%, H 7.01%; $C_{11}H_{12}O_2$ requires C 74.98%, H 6.86%; HRMS (ES): found 177.09101 $C_{11}H_{13}O_2$ requires 177.21972.

$\nu_{(max)}$ (Film) cm^{-1}: 3279.6 (Ph-OH), 1651.2 (C=O), 1602.2 (Ar C=C); δ_H d_6 acetone: 9.20 (1H, s, OH), 7.84 (2H, dd, J=8.9Hz, PhH), 6.90 (2H, dd, J=8.9Hz, PhH), 4.03 (1H, m, CH), 2.28 (4H, m, CH_2), 1.82 (2H, m, CH_2); δ_C d_6 acetone: 199.15 (CO),

162.54 (CO), 131.55 (CH, Ar), 128.73 (CH, Ar), 116.09 (C, Ar), 42.45 (CH), 25.69 (CH_2), 18.68 (CH_2).

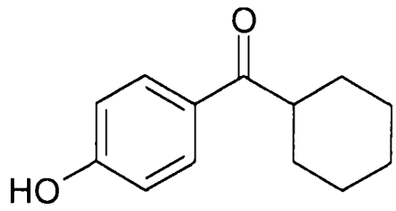
Cyclopentyl-(4-Hydroxy-phenyl)-methanone (280):



Compound **280** was synthesised following the same procedure as for compound **268** except that phenol (1.54g, 16.42mmol) was stirred with aluminium trichloride (4.63g, 34.68mmol) in anhydrous DCM (15mL) prior to the addition of cyclopentane carbonyl chloride (2.1mL, 17.91mmol). The crude solid was purified by column chromatography to give **280** as a light brown solid (2.26g, 72.4% yield); m.p.=105.6-107.1°C [lit. m.p.=111-113.2°C (Patel, 2003b)]; R_f=0.50 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.73min; LRMS (EI): 190 (M^+, 12%), 149 (M^+-C_3H_5, 5%), 121 (M^+-C_5H_9, 100%), 93 (M^+-C_6H_9O, 12%); HRMS (ES): found 191.10666 $C_{12}H_{15}O_2$ requires 191.24630.

$\nu_{(max)}$ (Film) cm^{-1}: 3267.8 (Ph-OH), 1651.7 (C=O), 1601.4 (Ar C=C); δ_H d_6 acetone: 9.20 (1H, s, OH), 7.93 (2H, dd, J=8.9Hz, PhH), 6.93 (2H, dd, J=8.9Hz, PhH), 3.75 (1H, m, CH), 1.84 (4H, m, CH_2), 1.64 (4H, m, CH_2); δ_C d_6 acetone: 200.72 (CO), 162.27 (CO), 131.53 (CH, Ar), 129.80 (CH, Ar), 115.83 (C, Ar), 46.20 (CH), 26.77 (CH_2).

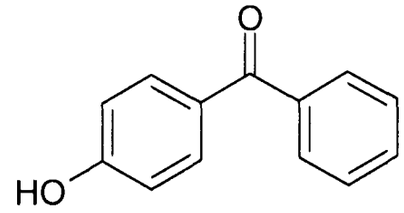
Cyclohexyl-(4-Hydroxy-phenyl)-methanone (281):



Compound **281** was synthesised following the same procedure as for compound **268** except that phenol (1.52g, 16.20mmol) was stirred with aluminium trichloride (4.59g, 34.38mmol) in anhydrous DCM (15mL) prior to the addition of cyclohexane carbonyl chloride (2.4mL, 17.86mmol). The crude solid was purified by column chromatography to give **281** as a light brown solid (2.43g, 73.5% yield); m.p.=104.7-106.6°C [lit. m.p.=110-111.2°C (Patel, 2003b)]; R_f=0.53 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.20min; LRMS (EI): 204 (M^+, 18%), 149 (M^+-C_4H_7, 5%), 121 (M^+-C_6H_{11}, 100%), 93 (M^+-$C_7H_{11}O$, 8%); HRMS (ES): found 205.12231 $C_{13}H_{17}O_2$ requires 205.27288.

$\nu_{(max)}$ (Film) cm^{-1}: 3291.3 (Ph-OH), 1650.9 (C=O), 1601.6 (Ar C=C); δ_H d_6 acetone: 9.28 (1H, s, O<u>H</u>), 7.80 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.83 (2H, dd, J=8.9Hz, Ph<u>H</u>), 3.22 (1H, m, C<u>H</u>), 1.71 (4H, m, C<u>H</u>$_2$), 1.33 (4H, m, C<u>H</u>$_2$), 1.16 (2H, m, C<u>H</u>$_2$); δ_C d_6 acetone: 201.08 (<u>C</u>O), 161.70 (<u>C</u>O), 130.69 (<u>C</u>H, Ar), 128.44 (<u>C</u>H, Ar), 115.27 (<u>C</u>, Ar), 44.64 (<u>C</u>H), 26.01 (<u>C</u>H$_2$), 25.63 (<u>C</u>H$_2$), 25.29 (<u>C</u>H$_2$).

Cyclobenzyl-(4-Hydroxy-phenyl)-methanone (282):



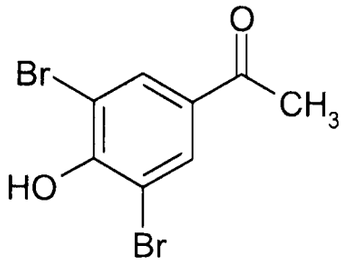
Compound **282** was synthesised following the same procedure as for compound **268** except that phenol (1.54g, 16.42mmol) was stirred with aluminium trichloride (4.70g, 35.20mmol) in anhydrous DCM (15mL) prior to the addition of benzoyl chloride (2.4mL, 20.67mmol). The crude solid was purified by column

chromatography to give **282** as a cream solid (2.67g, 82.1% yield); m.p.=138.9-140.2°C [lit. m.p.=135°C (Blakey et al, 1927)]; R_f=0.38 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.31min; LRMS (EI): 198 (M^+, 67%), 141 (M^+-C_4H_9, 2%), 121 (M^+-C_6H_5, 100%), 93 (M^+-C_7H_5O, 11%); HRMS (ES): found 199.0754 $C_{13}H_{11}O_2$ requires 199.22524.

$\nu_{(max)}$ (Film) cm^{-1}: 3322.6 (Ph-OH), 3069.8 (Ar-C), 1643.2 (C=O), 1601.2 (Ar C=C); δ_H d_6 acetone: 9.19 (1H, s, O<u>H</u>), 7.64 (4H, m, Ph<u>H</u>), 7.52 (1H, m, Ph<u>H</u>), 7.44 (2H, m, Ph<u>H</u>), 6.88 (2H, dd, J=8.9Hz, Ph<u>H</u>); δ_C d_6 acetone: 194.38 (<u>C</u>O), 161.72 (<u>C</u>O), 138.72 (<u>C</u>, Ar), 132.60 (<u>C</u>H, Ar), 131.72 (<u>C</u>H, Ar), 129.39 (<u>C</u>H, Ar), 129.23 (<u>C</u>H, Ar), 128.30 (<u>C</u>H, Ar), 115.16 (<u>C</u>, Ar).

3.3 Synthesis of the 3,5-dibromo-4-hydroxy phenyl ketones

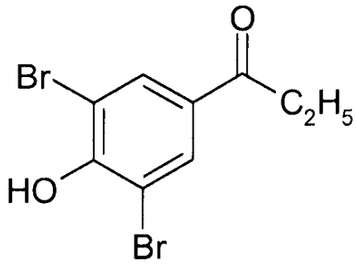
3,5-Dibromo-4-hydroxyacetophenone (283):



Bromine water was added to a solution of **268** (1.05g, 7.71mmol) in glacial acetic acid (15mL) and the mixture left to stir for 3h. The mixture was extracted into DEE (2 x 50mL), washed with water (2 x 50mL) and dried over anhydrous $MgSO_4$. The solvent was removed under vacuum to give **283** as an off-white solid (1.47g, 64.4% yield); m.p.=189-191°C [lit. m.p.=187°C (Krausz and Martin, 1965)]; R_f=0.60 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=7.39min; LRMS (EI): 294 (M^+, 38%), 279 (M^+-CH_3, 100%), 251 (M^+-C_2H_3O, 11%), 170 (M^+-C_2H_4BrO, 12%); HRMS (ES): found 292.8807 $C_8H_7Br_2O_2$ requires 294.94798.

$\nu_{(max)}$ (Film) cm^{-1}: 3224.3 (Ph-OH), 1663.4 (C=O), 1581.5 (Ar C=C); δ_H d_6 acetone: 8.13 (2H, s, Ar-H), 2.56 (3H, s, CH_3); δ_C d_6 acetone: 194.40 (CO), 154.89 (CO), 133.35 (CBr, Ar), 132.37 (CH, Ar), 110.08 (C, Ar), 26.16 (CH_3).

3,5-Dibromo-4-hydroxypropiophenone (284):

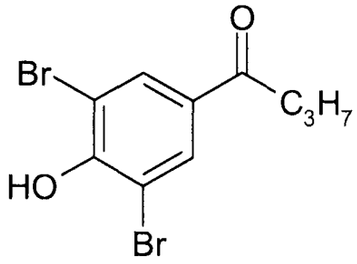


Compound **284** was synthesised following the same procedure as for compound **283** except that **269** (1.12g, 6.83mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column

chromatography to give **284** as a pale yellow solid (1.54g, 72.7% yield); m.p.=111.8-113.9°C [lit. m.p.=115°C (Krausz and Martin, 1965)]; R_f=0.68 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=7.80min; LRMS (EI): 308 (M^+, 25%), 279 (M^+-C_2H_5, 100%), 251 (M^+-C_3H_5O, 9%), 172 (M^+-C_3H_4BrO, 13%); HRMS (ES): found 308.8949 $C_9H_9Br_2O_2$ requires 308.97456.

$\nu_{(max)}$ (Film) cm^{-1}: 3334.8 (Ph-OH), 1675.7 (C=O), 1582.6 (Ar C=C); δ_H d_6 acetone: 9.45 (1H, bs, O<u>H</u>), 8.13 (2H, s, Ph<u>H</u>), 3.02 (2H, q, J=7.1Hz, C<u>H</u>$_2$), 1.13 (3H, t, J=7.1Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 197.32 (<u>C</u>O), 155.36 (<u>C</u>O), 133.25 (<u>C</u>H, Ar), 132.25 (<u>C</u>H, Ar), 111.57 (<u>C</u>Br, Ar), 31.86 (<u>C</u>H_2), 8.36 (<u>C</u>H_3).

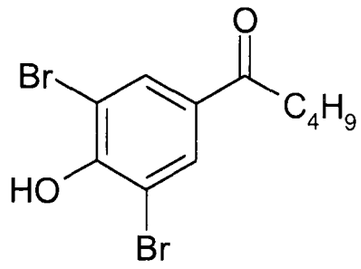
3,5-Dibromo-4-hydroxybutyrophenone (285):



Compound **285** was synthesised following the same procedure as for compound **283** except that **270** (1.19g, 7.26mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column chromatography to give **285** as an off-white solid (1.21g, 55.6% yield); m.p.=106.7-108.9°C [lit. m.p.=117°C (Buu-Hoi et al, 1954)]; R_f=0.72 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=8.12min; LRMS (EI): 322 (M^+, 11%), 279 (M^+-C_3H_7, 100%), 251 (M^+-C_4H_7O, 10%), 172 (M^+-C_4H_6BrO, 11%); HRMS (ES): found 320.9120 $C_{10}H_{11}Br_2O_2$ requires 323.00114.

$\nu_{(max)}$ (Film) cm^{-1}: 3310.0 (Ph-OH), 1672.5 (C=O), 1581.2 (Ar C=C); δ_H d_6 acetone: 9.31 (1H, s, O<u>H</u>), 8.14 (2H, s, Ph<u>H</u>), 2.99 (2H, t, J=7.1Hz, C<u>H</u>$_2$), 1.67 (2H, m, C<u>H</u>$_2$), 0.95 (3H, t, J=7.3Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 196.61 (<u>C</u>O), 155.10 (<u>C</u>O), 133.05 (<u>C</u>H, Ar), 132.19 (<u>C</u>H, Ar), 111.19 (<u>C</u>Br, Ar), 40.19 (<u>C</u>H_2), 17.95 (<u>C</u>H_2), 13.71 (<u>C</u>H_3).

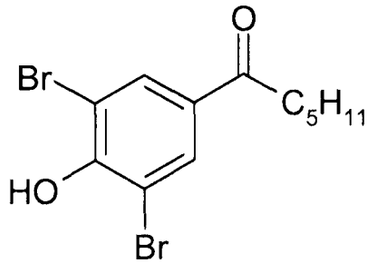
3,5-Dibromo-4-hydroxyvalerophenone (286):



Compound **286** was synthesised following the same procedure as for compound **283** except that **271** (1.02g, 5.71mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column chromatography to give **286** as an off-white solid (1.13g, 58.5% yield); m.p.=68.6-69.4°C [lit. m.p.=75°C (Buu-Hoi et al, 1954)]; R_f=0.78 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=8.52min; LRMS (EI): 336 (M^+, 7%), 279 (M^+-C_4H_9, 100%), 251 (M^+-C_5H_9O, 11%), 172 (M^+-C_5H_8BrO, 13%); HRMS (ES): found 334.9277 $C_{11}H_{13}Br_2O_2$ requires 337.02772.

$\nu_{(max)}$ (Film) cm^{-1}: 3409.7 (Ph-OH), 1674.2 (C=O), 1583.8 (Ar C=C); δ_H $CDCl_3$: 8.04 (2H, s, PhH), 6.33 (1H, s, OH), 2.85 (2H, t, J=7.3Hz, CH_2), 1.66 (2H, m, CH_2), 1.36 (2H, m, CH_2), 0.92 (3H, t, J=7.3Hz, CH_3); δ_C $CDCl_3$: 196.96 (CO), 153.31 (CO), 132.53 (CH, Ar), 132.02 (CH, Ar), 110.29 (CBr, Ar), 38.18 (CH_2), 26.50 (CH_2), 22.58 (CH_2), 14.11 (CH_3).

3,5-Dibromo-4-hydroxyhexanophenone (287):

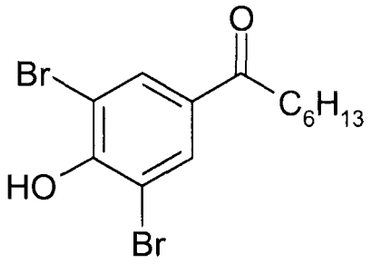


Compound **287** was synthesised following the same procedure as for compound **283** except that **272** (1.07g, 5.50mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column chromatography to give **287** as an off-white solid (1.17g, 60.4% yield); m.p.=70.7-

72.4°C [lit. m.p.=68°C (Buu-Hoi et al, 1954)]; R_f=0.80 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=8.92min; LRMS (EI): 350 (M^+, 5%), 294 (M^+-C_4H_8, 100%), 279 (M^+-C_5H_{11}, 95%), 251 (M^+-$C_6H_{11}O$, 9%), 172 (M^+-$C_6H_{10}BrO$, 14%); HRMS (ES): found 350.9418 $C_{12}H_{15}Br_2O_2$ requires 351.0543.

$\nu_{(max)}$ (Film) cm^{-1}: 3367.3 (Ph-OH), 1677.6 (C=O), 1580.6 (Ar C=C); δ_H $CDCl_3$: 8.04 (2H, s, PhH), 6.36 (1H, s, OH), 2.84 (2H, t, J=7.3Hz, CH_2), 1.68 (2H, m, CH_2), 1.32 (4H, m, CH_2), 0.88 (3H, t, J=6.9Hz, CH_3); δ_C $CDCl_3$: 196.88 (CO), 153.21 (CO), 132.42 (CH, Ar), 131.89 (C, Ar), 110.18 (CBr, Ar), 38.31 (CH_2), 31.50 (CH_2), 24.39 (CH_2), 22.57 (CH_2), 14.03 (CH_3).

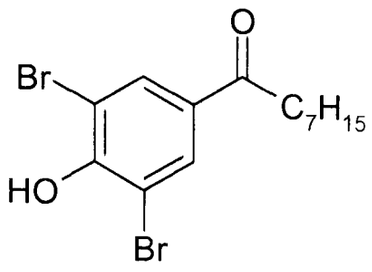
3,5-Dibromo-4-hydroxyheptanophenone (288):



Compound **288** was synthesised following the same procedure as for compound **283** except that **273** (1.26g, 6.12mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column chromatography to give **288** as a cream solid (1.11g, 52.9% yield); m.p.=55.3-56.9°C [lit. m.p.=71°C (Buu-Hoi et al, 1954)]; R_f=0.84 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=9.32min; LRMS (EI): 364 (M^+, 3%), 294 (M^+-C_5H_{10}, 100%), 279 (M^+-C_6H_{13}, 78%), 251 (M^+-$C_7H_{13}O$, 8%), 172 (M^+-$C_7H_{12}BrO$, 13%); HRMS (ES): found 364.9575 $C_{13}H_{17}Br_2O_2$ requires 365.08088.

$\nu_{(max)}$ (Film) cm^{-1}: 3386.5 (Ph-OH), 1738.3 (C=O), 1580.6 (Ar C=C); δ_H $CDCl_3$: 8.04 (2H, s, PhH), 2.84 (2H, t, J=7.3Hz, CH_2), 1.67 (2H, m, CH_2), 1.28 (6H, m, CH_2), 0.86 (3H, t, J=6.7Hz, CH_3); δ_C $CDCl_3$: 197.04 (CO), 153.35 (CO), 132.52 (CH, Ar), 131.96 (C, Ar), 110.30 (CBr, Ar), 38.44 (CH_2), 31.78 (CH_2), 29.09 (CH_2), 24.36 (CH_2), 22.68 (CH_2), 14.21 (CH_3).

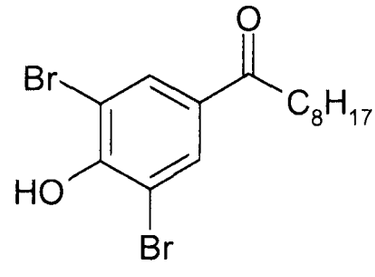
3,5-Dibromo-4-hydroxyoctanophenone (289):



Compound **289** was synthesised following the same procedure as for compound **283** except that **274** (1.29g, 5.50mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column chromatography to give **289** as yellow oil (1.15g, 55.0% yield); R_f=0.88 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=9.76min; LRMS (EI): 378 (M^+, 4%), 294 (M^+-C_6H_{12}, 100%), 279 (M^+-C_7H_{15}, 68%), 251 (M^+-$C_8H_{15}O$, 7%), 172 (M^+-$C_8H_{14}BrO$, 11%); HRMS (ES): found 378.9731 $C_{14}H_{19}Br_2O_2$ requires 379.10746.

$\nu_{(max)}$ (Film) cm^{-1}: 3357.3 (Ph-OH), 1678.3 (C=O), 1580.6 (Ar C=C); δ_H d_6 acetone: 8.12 (2H, s, PhH), 1.64 (2H, m, CH_2), 1.33 (10H, m, CH_2), 0.86 (3H, t, J=6.9Hz, CH_3); δ_C d_6 acetone: 196.73 (CO), 154.91 (CO), 132.96 (CH, Ar), 132.15 (C, Ar), 110.98 (CBr, Ar), 32.15 (CH_2), 24.40 (CH_2), 22.94 (CH_2), 20.13 (CH_2), 14.02 (CH_3).

3,5-Dibromo-4-hydroxynonanophenone (290):

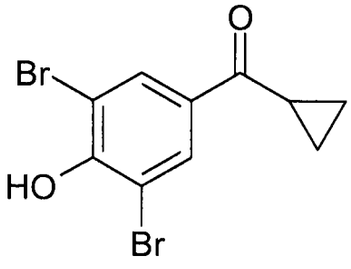


Compound **290** was synthesised following the same procedure as for compound **283** except that **275** (1.38g, 6.31mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column chromatography to give **290** as clear oil (1.24g, 53.2% yield); R_f=0.92 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=10.25min; LRMS (EI): 392 (M^+, 5%), 294 (M^+-

C_7H_{14}, 100%), 279 (M^+-C_8H_{17}, 55%), 251 (M^+-$C_9H_{17}O$, 5%), 172 (M^+-$C_9H_{16}BrO$, 10%); HRMS (ES): found 392.9888 $C_{15}H_{21}Br_2O_2$ requires 393.13404.

$\nu_{(max)}$ (Film) cm^{-1}: 3350.8 (Ph-OH), 1677.9 (C=O), 1580.7 (Ar C=C); δ_H d_6 acetone: 8.13 (2H, s, PhH), 1.66 (2H, m, CH_2), 1.31 (12H, m, CH_2), 0.86 (3H, t, J=6.9Hz, CH_3); δ_C d_6 acetone: 196.86 (CO), 155.01 (CO), 133.09 (CH, Ar), 132.30 (C, Ar), 111.09 (CBr, Ar), 32.36 (CH_2), 24.53 (CH_2), 23.10 (CH_2), 14.16 (CH_3).

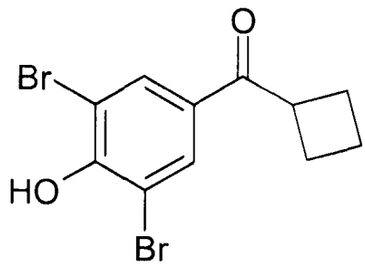
Cyclopropyl (3,5-dibromo-4-hydroxyphenyl)methanone (291):



Compound **291** was synthesised following the same procedure as for compound **283** except that **278** (1.05g, 6.46mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column chromatography to give **291** as a cream solid (1.46g, 70.6% yield); m.p.=114.9-116.8°C; R_f=0.72 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=8.46min; LRMS (EI): 320 (M^+, 31%), 279 (M^+-C_3H_5, 100%), 251 (M^+-C_4H_5O, 4%), 172 (M^+-C_4H_4BrO, 9%); HRMS (ES): found 320.8949 $C_{10}H_9Br_2O_2$ requires 320.98526.

$\nu_{(max)}$ (Film) cm^{-1}: 3364.5 (Ph-OH), 1658.6 (C=O), 1581.0 (Ar C=C); δ_H $CDCl_3$: 8.11 (2H, s, PhH), 6.43 (1H, bs, OH), 2.50 (1H, m, CH), 1.22 (2H, m, CH_2), 1.04 (2H, m, CH_2); δ_C $CDCl_3$: 197.09 (CO), 153.26 (CO), 132.78 (CH, Ar), 132.51 (C, Ar), 110.26 (CBr, Ar), 17.11 (CH), 12.23 (CH_2).

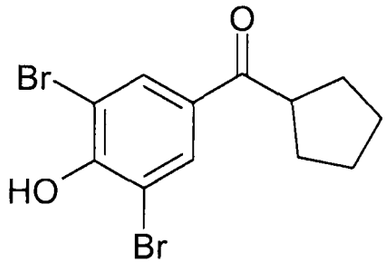
Cyclobutyl (3,5-dibromo-4-hydroxyphenyl)methanone (292):



Compound **292** was synthesised following the same procedure as for compound **283** except that **279** (1.12g, 6.35mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column chromatography to give **292** as a yellow solid (1.31g, 61.8% yield); m.p.=103.8-105.2°C; R_f=0.76 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=8.84min; LRMS (EI): 334 (M^+, 8%), 279 (M^+-C_4H_7, 100%), 251 (M^+-C_5H_7O, 6%), 172 (M^+-C_5H_6BrO, 10%); HRMS (ES): found 334.9105 $C_{11}H_{11}Br_2O_2$ requires 335.01184.

$\nu_{(max)}$ (Film) cm^{-1}: 3377.3 (Ph-OH), 1671.2 (C=O), 1579.9 (Ar C=C); δ_H $CDCl_3$: 7.95 (2H, s, PhH), 6.44 (1H, bs, OH), 3.85 (1H, m, CH), 2.27 (4H, m, CH_2), 2.06 (1H, m, CH), 2.04 (1H, m, CH); δ_C $CDCl_3$: 197.65 (CO), 153.31 (CO), 132.74 (CH, Ar), 130.53 (C, Ar), 110.33 (CBr, Ar), 42.00 (CH), 25.27 (CH_2), 18.31 (CH_2).

Cyclopentyl (3,5-dibromo-4-hydroxyphenyl)methanone (293):

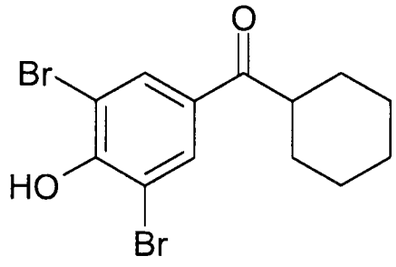


Compound **293** was synthesised following the same procedure as for compound **283** except that **280** (1.27g, 6.68mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column chromatography to give **293** as yellow solid (1.38g, 59.3% yield); m.p.=90.7-92.9°C; R_f=0.78 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=9.28min; LRMS (EI): 348

(M^+, 11%), 279 (M^+-C_5H_9, 100%), 251 (M^+-C_6H_9O, 7%), 172 (M^+-C_6H_8BrO, 9%); HRMS (ES): found 346.9277 $C_{12}H_{13}Br_2O_2$ requires 349.03842.

$\nu_{(max)}$ (Film) cm^{-1}: 3382.4 (Ph-OH), 1738.6 (C=O), 1579.9 (Ar C=C); δ_H $CDCl_3$: 8.05 (2H, s, PhH), 3.53 (1H, m, CH), 1.87 (4H, m, CH_2), 1.69 (4H, m, CH_2); δ_C $CDCl_3$: 199.40 (CO), 153.21 (CO), 132.90 (CH, Ar), 131.80 (C, Ar), 110.28 (CBr, Ar), 46.24 (CH), 30.18 (CH_2), 26.45 (CH_2).

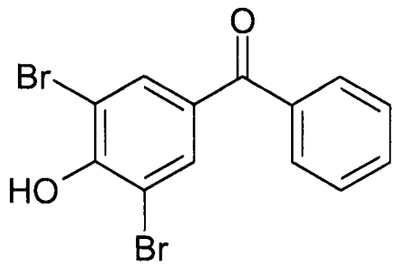
Cyclohexyl (3,5-dibromo-4-hydroxyphenyl)methanone (294):



Compound **294** was synthesised following the same procedure as for compound **283** except that **281** (1.33g, 6.53mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column chromatography to give **294** as a clear oil (1.53g, 64.7% yield); R_f=0.80 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=9.82min; LRMS (EI): 362 (M^+, 14%), 279 (M^+-C_6H_{11}, 100%), 251 (M^+-$C_7H_{11}O$, 5%), 172 (M^+-$C_7H_{10}BrO$, 8%); HRMS (ES): found 360.9433 $C_{13}H_{15}Br_2O_2$ requires 363.0650.

$\nu_{(max)}$ (Film) cm^{-1}: 3380.7 (Ph-OH), 1738.7 (C=O), 1579.0 (Ar C=C); δ_H $CDCl_3$: 8.02 (2H, s, PhH), 6.39 (1H, bs, OH), 3.09 (1H, m, CH), 1.81 (4H, m, CH_2), 1.37 (6H, m, CH_2); δ_C $CDCl_3$: 200.28 (CO), 153.21 (CO), 132.72 (CH, Ar), 131.26 (C, Ar), 110.36 (CBr, Ar), 45.52 (CH), 29.56 (CH_2), 26.02 (CH_2), 25.91 (CH_2).

3,5-dibromo-4-hydroxyphenyl (phenyl) methanone (295):

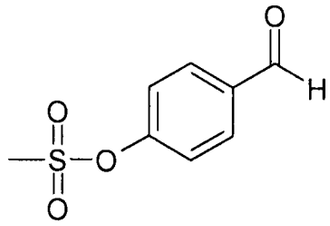


Compound **295** was synthesised following the same procedure as for compound **283** except that **282** (1.25g, 6.31mmol) was dissolved in glacial acetic acid (15mL) prior to the addition of bromine water. The crude solid was purified by column chromatography to give **295** as clear oil (1.45g, 64.5% yield); R_f=0.74 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=9.81min; LRMS (*m/z*): 356 (M^+, 33%), 279 (M^+-C_6H_5, 46%), 105 (M^+-$C_6H_3OBr_2$, 100%), 77 (M^+-$C_7H_3Br_2O_2$, 44%); HRMS (ES): found 356.8949 $C_{13}H_9Br_2O_2$ requires 357.01736.

$\nu_{(max)}$ (Film) cm^{-1}: 3380.4 (Ph-OH), 1650.1 (C=O), 1578.6 (Ar C=C); δ_H $CDCl_3$: 7.29 (2H, m, PhH), 7.06 (2H, m, PhH), 6.94 (1H, m, PhH), 6.81 (2H, m, PhH), 5.72 (1H, bs, OH); δ_C $CDCl_3$: 193.20 (CO), 153.06 (CO), 137.05 (CH, Ar), 134.45 (CH, Ar), 132.93 (CH, Ar), 131.98 (CH, Ar), 129.93 (C, Ar), 128.75 (C, Ar), 110.08 (CBr, Ar).

3.4 Synthesis of the methanesulfonic acid esters of 4-hydroxy phenyl ketone based compounds

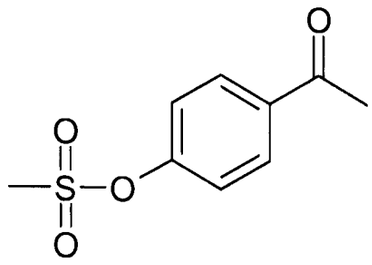
Methanesulfonic acid 4-formyl-phenyl ester (296):



4-hydroxybenzaldehyde (1.03g, 8.44mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min. Methanesulfonyl chloride (1.0mL, 12.86mmol) was added and refluxed for 2h. The reaction was poured onto ice (100mL). The organic layer separated, washed with water (3 x 50mL), washed with a saturated solution of $NaHCO_3$ (2 x 50mL) and washed with water (3 x 50mL). The solvent was dried over anhydrous $MgSO_4$, filtered, and the solvent removed under vacuum to yield a light brown coloured solid which was purified via recrystalisation in DEE to give **296** as a pale brown solid (1.14g, 67.5% yield); m.p.=64.1-65.3°C; [lit. m.p.=64-65°C (Looker and Hayes, 1957)]; R_f=0.25 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=6.74min; LRMS (EI): 200 (M^+, 86%), 135 (M^+-CH_5SO, 17%), 121 (M^+-CH_3SO_2, 100%), 93 (M^+-C_7H_7O, 12%), 79 (M^+-$C_7H_5O_2$, 28%); HRMS (ES): found 201.02161 $C_8H_9O_4S$ requires 201.2198.

$\nu_{(max)}$ (Film) cm^{-1}: 1697.3 (C=O), 1598.4 (Ar C=C); δ_H d_6 acetone: 10.08 (1H, s, C<u>H</u>), 8.06 (2H, dd, J=8.9Hz, Ph<u>H</u>), 7.58 (2H, dd, J=8.9Hz, Ph<u>H</u>), 3.39 (3H, s, SC<u>H</u>$_3$); δ_C d_6 acetone: 190.99 (<u>C</u>O), 153.82 (<u>C</u>O), 135.48 (<u>C</u>H, Ar), 131.47 (<u>C</u>H, Ar), 122.99 (<u>C</u>, Ar), 37.33 (<u>C</u>H_3SO_3).

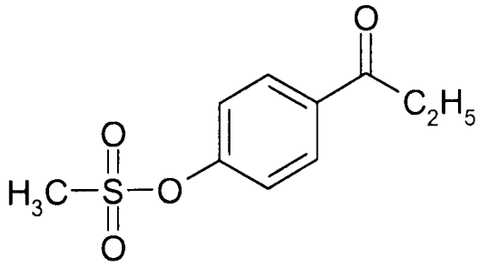
Methanesulfonic acid 4-acetyl-phenyl ester (297):



Compound **297** was synthesised following the same procedure as for compound **296** except that compound **268** (1.04g, 7.64mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **297** as a pale brown solid (1.19g, 72.7% yield); m.p.=69.7-71.2°C [lit. m.p.=70-71°C (Kametani et al, 1964)]; R_f=0.20 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.23min; LRMS (EI): 214 (M^+, 23%), 199 (M^+-CH_3, 67%), 121 (M^+-$C_2H_5SO_2$, 100%), 92 (M^+-$C_3H_6SO_3$, 13%); HRMS (ES): found 215.03726 $C_9H_{11}O_4S$ requires 215.2463.

$\nu_{(max)}$ (Film) cm^{-1}: 1685.9 (C=O), 1596.5 (Ar C=C); δ_H d_6 acetone: 8.11 (2H, dd, J=8.9Hz, PhH), 7.48 (2H, dd, J=8.9Hz, PhH), 3.36 (3H, s, SCH_3), 2.61 (3H, s, CH_3); δ_C d_6 acetone: 196.71 (CO), 153.57 (CO), 136.75 (CH, Ar), 131.01 (CH, Ar), 123.01 (C, Ar), 37.84 (CH_3SO_3), 26.63 (CH_3).

Methanesulfonic acid 4-propyl-phenyl ester (298):

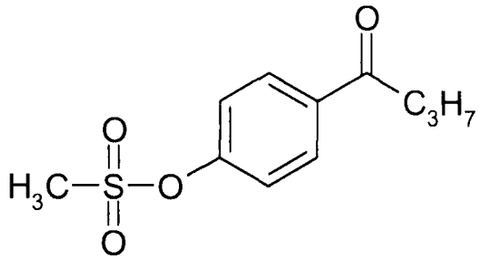


Compound **298** was synthesised following the same procedure as for compound **296** except that compound **269** (1.11g, 7.40mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the

addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **298** as a pale brown solid (1.23g, 72.9% yield); m.p.=76.5-77.4°C; R_f=0.31 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.66min; LRMS (EI): 228 (M^+, 8%), 199 (M^+-C_2H_5, 100%), 149 (M^+-CH_3SO_2, 2%), 121 (M^+-$C_3H_7SO_2$, 96%), 92 (M^+-$C_4H_8SO_3$, 12%); HRMS (ES): found 251.03485 $C_{10}H_{12}O_4SNa$ requires 251.25518.

$\nu_{(max)}$ (Film) cm^{-1}: 1688.6 (C=O), 1598.6 (Ar C=C); δ_H d_6 acetone: 8.10 (2H, dd, J=8.9Hz, PhH), 7.48 (2H, dd, J=8.9Hz, PhH), 3.36 (3H, s, SCH_3), 3.09 (2H, q, J=7.1Hz, CH_2), 1.15 (3H, t, J=7.1Hz, CH_3); δ_C d_6 acetone: 199.53 (CO), 153.67 (CO), 136.78 (CH, Ar), 130.86 (CH, Ar), 123.22 (C, Ar), 38.03 (CH_3SO_3), 32.29 (CH_2), 8.37 (CH_3).

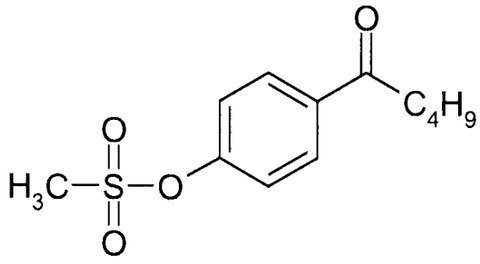
Methanesulfonic acid 4-butyl-phenyl ester (299):



Compound **299** was synthesised following the same procedure as for compound **296** except that compound **270** (1.10g, 6.71mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **299** as a pale brown solid (0.99g, 60.9% yield); m.p.=63.5-64.7°C; R_f=0.36 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.99min; LRMS (EI): 242 (M^+, 1%), 199 (M^+-C_3H_7, 100%), 163 (M^+-CH_3SO_2, 18%), 121 (M^+-$C_4H_9SO_2$, 95%), 92 (M^+-$C_5H_{10}SO_3$, 12%); Elemental analysis: found C 54.80%, H 5.85%; $C_{11}H_{14}O_4S$ requires C 54.53%, H 5.82%.

$\nu_{(max)}$ (Film) cm^{-1}: 1683.1 (C=O); δ_H d_6 acetone: 8.10 (2H, dd, J=8.9Hz, PhH), 7.47 (2H, dd, J=8.9Hz, PhH), 3.35 (3H, s, SCH_3), 3.02 (2H, t, J=7.3Hz, CH_2), 1.71 (2H, m, CH_2), 0.96 (3H, t, J=7.3Hz, CH_3); δ_C d_6 acetone: 198.29 (CO), 152.82 (CO), 136.08 (CH, Ar), 130.09 (CH, Ar), 122.38 (C, Ar), 40.09 (CH_2), 37.20 (CH_3SO_3), 17.39 (CH_2), 13.24 (CH_3).

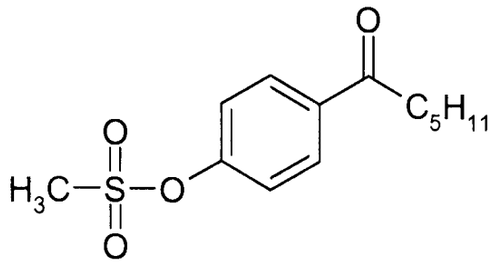
Methanesulfonic acid 4-pentyl-phenyl ester (300):



Compound **300** was synthesised following the same procedure as for compound **296** except that compound **271** (1.08g, 6.06mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **300** as a pale brown solid (1.34g, 86.3% yield); m.p.=62.5-63.9°C; R_f=0.42 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.41min; LRMS (EI): 256 (M^+, 1%), 214 (M^+-C_3H_6, 79%), 199 (M^+-C_4H_9, 90%), 121 (M^+-$C_5H_{11}SO_2$, 100%), 92 (M^+-$C_6H_{12}SO_3$, 12%); Elemental analysis: found C 56.05%, H 6.26%; $C_{12}H_{16}O_4S$ requires C 56.23%, H 6.29%.

$\nu_{(max)}$ (Film) cm^{-1}: 1684.4 (C=O); δ_H d_6 acetone: 8.12 (2H, dd, J=8.9Hz, PhH), 7.48 (2H, dd, J=8.9Hz, PhH), 3.35 (3H, s, SCH_3), 3.05 (2H, t, J=7.3Hz, CH_2), 1.67 (2H, m, CH_2), 1.41 (2H, m, CH_2), 0.92 (3H, t, J=7.3Hz, CH_3); δ_C d_6 acetone: 199.20 (CO), 153.65 (CO), 136.90 (CH, Ar), 130.93 (CH, Ar), 123.20 (C, Ar), 38.78 (CH_2), 38.02 (CH_3SO_3), 27.02 (CH_2), 23.02 (CH_2), 14.27 (CH_3).

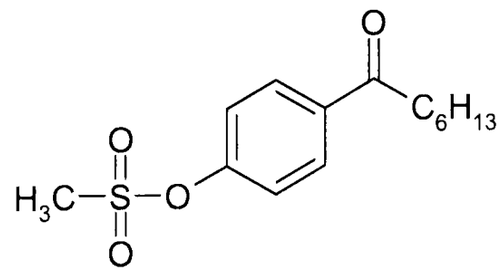
Methanesulfonic acid 4-hexyl-phenyl ester (301):



Compound **301** was synthesised following the same procedure as for compound **296** except that compound **272** (1.04g, 5.41mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **301** as a pale brown solid (0.95g, 64.9% yield); m.p.=79.8-81.1°C; R_f=0.44 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.80min; LRMS (EI): 270 (M^+, 1%), 214 (M^+-C_4H_8, 100%), 199 (M^+-C_5H_{11}, 87%), 121 (M^+-$C_6H_{13}SO_2$, 94%), 92 (M^+-$C_7H_{14}SO_3$, 16%); Elemental analysis: found C 57.68%, H 6.72%; $C_{13}H_{18}O_4S$ requires C 57.76%, H 6.71%.

$\nu_{(max)}$ (Film) cm^{-1}: 1684.4 (C=O); δ_H d_6 acetone: 8.11 (2H, dd, J=8.9Hz, PhH), 7.48 (2H, dd, J=8.9Hz, PhH), 3.36 (3H, s, SCH_3), 3.05 (2H, t, J=7.3Hz, CH_2), 1.70 (2H, m, CH_2), 1.37 (4H, m, CH_2), 0.89 (3H, t, J=7.1Hz, CH_3); δ_C d_6 acetone: 199.24 (CO), 153.71 (CO), 136.81 (CH, Ar), 130.95 (CH, Ar), 123.23 (C, Ar), 39.02 (CH_2), 38.03 (CH_3SO_3), 32.20 (CH_2), 24.61 (CH_2), 23.26 (CH_2), 14.30 (CH_3).

Methanesulfonic acid 4-heptyl-phenyl ester (302):

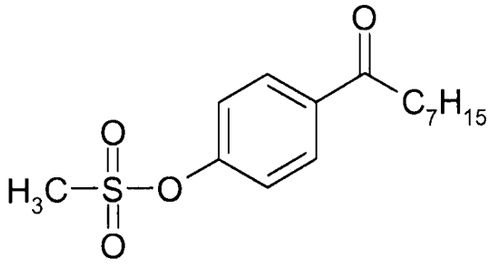


Compound **302** was synthesised following the same procedure as for compound **296** except that compound **273** (0.98g, 4.75mmol) was dissolved in anhydrous

DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **302** as a pale brown solid (0.89g, 65.9% yield); m.p.=79.1-80.2°C; R_f=0.45 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.21min; LRMS (EI): 284 (M^+, 1%), 214 (M^+-C_5H_{10}, 100%), 199 (M^+-C_6H_{13}, 64%), 121 (M^+-$C_7H_{15}SO_2$, 65%), 92 (M^+-$C_8H_{16}SO_3$, 13%); Elemental analysis: found C 59.19%, H 7.10%; $C_{14}H_{20}O_4S$ requires C 59.13%, H 7.09%.

$\nu_{(max)}$ (Film) cm^{-1}: 1684.4 (C=O); δ_H d_6 acetone: 8.12 (2H, dd, J=8.9Hz, PhH), 7.48 (2H, dd, J=8.9Hz, PhH), 3.35 (3H, s, SCH_3), 3.05 (2H, t, J=7.1Hz, CH_2), 1.69 (2H, m, CH_2), 1.33 (6H, m, CH_2), 0.87 (3H, t, J=7.1Hz, CH_3); δ_C d_6 acetone: 199.23 (CO), 153.67 (CO), 136.94 (CH, Ar), 130.95 (CH, Ar), 123.22 (C, Ar), 39.07 (CH_2), 38.03 (CH_3SO_3), 32.49 (CH_2), 24.87 (CH_2), 23.27 (CH_2), 14.37 (CH_3).

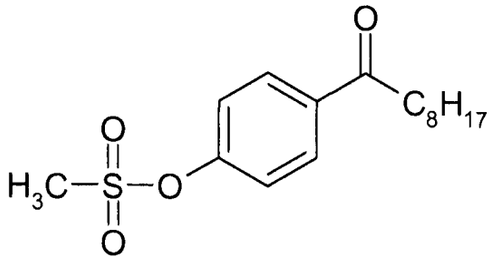
Methanesulfonic acid 4-octyl-phenyl ester (303):



Compound **303** was synthesised following the same procedure as for compound **296** except that compound **274** (1.16g, 5.27mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **303** as a pale brown solid (1.17g, 74.5% yield); m.p.=86.9-88.9°C; R_f=0.47 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.61min; LRMS (EI): 298 (M^+, 1%), 214 (M^+-C_6H_{12}, 100%), 199 (M^+-C_7H_{15}, 61%), 121 (M^+-$C_8H_{17}SO_2$, 66%), 92 (M^+-$C_9H_{18}SO_3$, 13%); HRMS (ES): found 299.1312 $C_{15}H_{23}O_4S$ requires 299.4053.

$\nu_{(max)}$ (Film) cm^{-1}: 1683.4 (C=O); δ_H d_6 acetone: 8.11 (2H, dd, J=8.9Hz, PhH), 7.49 (2H, dd, J=8.9Hz, PhH), 3.36 (3H, s, SCH_3), 3.06 (2H, t, J=7.1Hz, CH_2), 1.70 (2H, m, CH_2), 1.31 (8H, m, CH_2), 0.88 (3H, t, J=7.1Hz, CH_3); δ_C d_6 acetone: 199.20 (CO), 153.64 (CO), 136.90 (CH, Ar), 130.93 (CH, Ar), 123.19 (C, Ar), 39.05 (CH_2), 38.02 (CH_3SO_3), 32.53 (CH_2), 24.90 (CH_2), 23.32 (CH_2), 14.39 (CH_3).

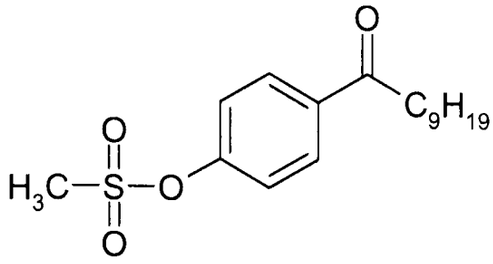
Methanesulfonic acid 4-nonyl-phenyl ester (304):



Compound **304** was synthesised following the same procedure as for compound **296** except that compound **275** (0.95g, 4.06mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **304** as a pale brown solid (1.11g, 87.4% yield); m.p.=89.5-89.9°C; R_f=0.49 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=10.07min; LRMS (EI): 312 (M^+, 1%), 214 (M^+-C_7H_{14}, 100%), 199 (M^+-C_8H_{17}, 51%), 121 (M^+-$C_9H_{19}SO_2$, 50%), 92 (M^+-$C_{10}H_{20}SO_3$, 11%); HRMS (ES): found 313.1468 $C_{16}H_{25}O_4S$ requires 313.4318.

$\nu_{(max)}$ (Film) cm^{-1}: 1684.8 (C=O); δ_H d_6 acetone: 8.12 (2H, dd, J=8.9Hz, PhH), 7.48 (2H, dd, J=8.9Hz, PhH), 3.35 (3H, s, SCH_3), 3.05 (2H, t, J=7.3Hz, CH_2), 1.70 (2H, m, CH_2), 1.31 (10H, m, CH_2), 0.87 (3H, t, J=7.1Hz, CH_3); δ_C d_6 acetone: 199.24 (CO), 153.68 (CO), 136.95 (CH, Ar), 130.96 (CH, Ar), 123.23 (C, Ar), 39.08 (CH_2), 38.03 (CH_3SO_3), 32.64 (CH_2), 24.93 (CH_2), 23.37 (CH_2), 14.42 (CH_3).

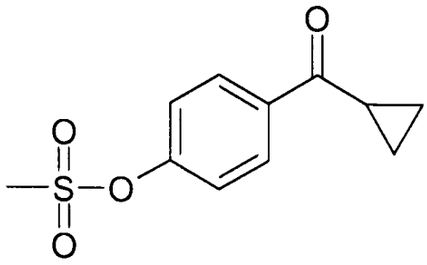
Methanesulfonic acid 4-decyl-phenyl ester (305):



Compound **305** was synthesised following the same procedure as for compound **296** except that compound **276** (1.24g, 5.00mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **305** as a pale brown solid (1.24g, 76.0% yield); m.p.=94.5-95.3°C; R_f=0.51 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=10.56min; LRMS (EI): 326 (M^+, 1%), 214 (M^+-C_8H_{16}, 100%), 199 (M^+-C_9H_{19}, 48%), 121 (M^+-$C_{10}H_{21}SO_2$, 46%), 92 (M^+-$C_{11}H_{22}SO_3$, 8%); Elemental analysis: found C 62.81%, H 8.01%; $C_{17}H_{26}O_4S$ requires C 62.55%, H 8.03%.

$\nu_{(max)}$ (Film) cm^{-1}: 1684.6 (C=O); δ_H d_6 acetone: 8.11 (2H, dd, J=8.9Hz, PhH), 7.48 (2H, dd, J=8.9Hz, PhH), 3.35 (3H, s, SCH_3), 3.05 (2H, t, J=7.1Hz, CH_2), 1.70 (2H, m, CH_2), 1.31 (12H, m, CH_2), 0.87 (3H, t, J=7.1Hz, CH_3); δ_C d_6 acetone: 199.21 (CO), 153.66 (CO), 136.93 (CH, Ar), 130.94 (CH, Ar), 123.21 (C, Ar), 39.07 (CH_2), 38.03 (CH_3SO_3), 32.66 (CH_2), 24.92 (CH_2), 23.37 (CH_2), 14.41 (CH_3).

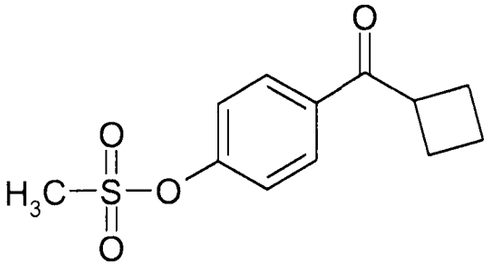
Methanesulfonic acid 4-cyclopropane carbonyl phenyl ester (306):



Compound **306** was synthesised following the same procedure as for compound **296** except that compound **278** (1.13g, 6.97mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **306** as a pale brown solid (1.15g, 68.7% yield); m.p.=68.9-69.4°C; R_f=0.25 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.32min; LRMS (EI): 240 (M^+, 59%), 199 (M^+-C_3H_5, 100%), 161 (M^+-CH_3SO_2, 24%), 121 (M^+-$C_4H_7SO_2$, 64%), 69 (M^+-C_4H_5O, 6%); HRMS (ES): found 241.0529 $C_{11}H_{13}O_4S$ requires 241.2835.

$\nu_{(max)}$ (Film) cm^{-1}: 1668.9 (C=O), 1598.6 (Ar C=C); δ_H $CDCl_3$: 8.04 (2H, dd, J=8.9Hz, PhH), 7.35 (2H, dd, J=8.9Hz, PhH), 3.16 (3H, s, SCH_3), 2.59 (1H, m, CH), 1.21 (2H, m, CH_2), 1.05 (2H, m, CH_2); δ_C $CDCl_3$: 199.37 (CO), 152.33 (CO), 136.96 (CH, Ar), 130.21 (CH, Ar), 122.15 (C, Ar), 37.93 (CH_3SO_3), 17.39 (CH_2), 12.14 (CH_3).

Methanesulfonic acid 4-cyclobutane carbonyl phenyl ester (307):

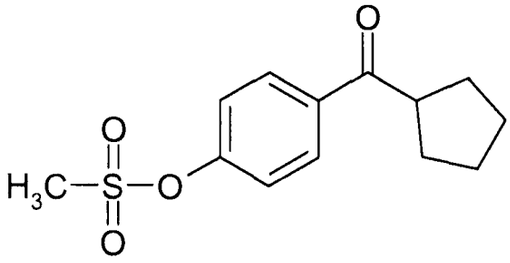


Compound **307** was synthesised following the same procedure as for compound **296** except that compound **279** (1.07g, 6.07mmol) was dissolved in anhydrous

DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **307** as a pale brown solid (1.18g, 76.4% yield); m.p.=82.2-82.9°C; R_f=0.30 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.72min; LRMS (EI): 254 (M^+, 5%), 199 (M^+-C_4H_7, 100%), 121 (M^+-$C_5H_9SO_2$, 64%); HRMS (ES): found 255.0686 $C_{12}H_{15}O_4S$ requires 255.3100.

$\nu_{(max)}$ (Film) cm^{-1}: 1679.0 (C=O), 1597.1 (Ar C=C); δ_H d_6 acetone: 8.04 (2H, dd, J=8.9Hz, PhH), 7.48 (2H, dd, J=8.9Hz, PhH), 4.15 (1H, m, CH), 3.35 (3H, s, SCH_3), 2.31 (4H, m, CH_2), 2.11 (1H, m, CH), 1.86 (1H, m, CH); δ_C d_6 acetone: 199.60 (CO), 153.70 (CO), 135.29 (CH, Ar), 131.23 (CH, Ar), 123.31 (C, Ar), 42.83 (CH), 38.05 (CH_3SO_3), 25.58 (CH_2), 18.62 (CH_2).

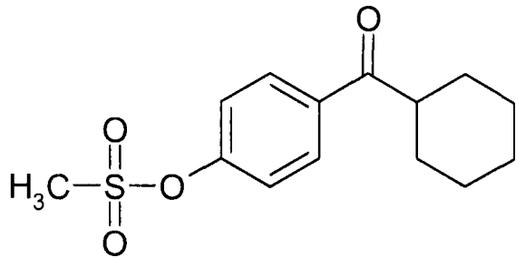
Methanesulfonic acid 4-cyclopentane carbonyl phenyl ester (308):



Compound **308** was synthesised following the same procedure as for compound **296** except that compound **280** (1.04g, 5.47mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **308** as a pale brown solid (1.17g, 79.8% yield); m.p.=69.5-71.4°C; R_f=0.35 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.13min; LRMS (EI): 268 (M^+, 5%), 199 (M^+-C_5H_9, 100%), 189 (M^+-CH_3SO_2, 14%), 121 (M^+-$C_6H_{11}SO_2$, 52%); Elemental analysis: found C 58.24%, H 6.05%; $C_{13}H_{16}O_4S$ requires C 58.19%, H 6.01%; HRMS (ES): found 269.0842 $C_{13}H_{17}O_4S$ requires 269.3365.

$\nu_{(max)}$ (Film) cm^{-1}: 1681.4 (C=O), 1596.7 (Ar C=C); δ_H d_6 acetone: 8.14 (2H, dd, J=8.9Hz, PhH), 7.48 (2H, dd, J=8.9Hz, PhH), 3.83 (1H, m, CH), 3.36 (3H, s, SCH_3), 1.94 (2H, m, CH_2), 1.83 (2H, m, CH_2), 1.66 (4H, m, CH_2); δ_C d_6 acetone: 201.21 (CO), 153.44 (CO), 136.42 (CH, Ar), 131.20 (CH, Ar), 123.03 (C, Ar), 46.80 (CH), 37.84 (CH_3SO_3), 26.74 (CH_2).

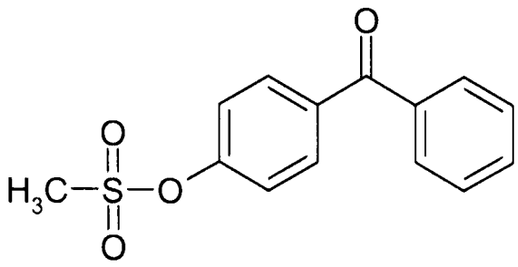
Methanesulfonic acid 4-cyclohexane carbonyl phenyl ester (309):



Compound **309** was synthesised following the same procedure as for compound **296** except that compound **281** (1.06g, 5.19mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **309** as a pale bown solid (1.22g, 83.2% yield); m.p.=95.9-96.9°C; R_f=0.39 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.58min; LRMS (EI): 282 (M^+, 3%), 199 (M^+-C_6H_{11}, 100%), 187 (M^+-CH_3SO_3, 4%), 121 (M^+-$C_7H_{13}SO_2$, 41%); HRMS (ES): found 283.0999 $C_{14}H_{19}O_4S$ requires 283.3630.

$\nu_{(max)}$ (Film) cm^{-1}: 1681.9 (C=O), 1596.9 (Ar C=C); δ_H d_6 acetone: 8.10 (2H, dd, J=8.9Hz, PhH), 7.49 (2H, dd, J=8.9Hz, PhH), 3.41 (1H, m, CH), 3.36 (3H, s, SCH_3), 1.79 (4H, m, CH_2), 1.70 (1H, m, CH), 1.42 (4H, m, CH_2), 1.27 (1H, m, CH); δ_C d_6 acetone: 202.23 (CO), 153.44 (CO), 135.87 (CH, Ar), 131.01 (CH, Ar), 123.11 (C, Ar), 45.81 (CH), 37.82 (CH_3SO_3), 26.56 (CH_2), 26.15 (CH_2).

Methanesulfonic acid 4-benzyl carbonyl phenyl ester (310):

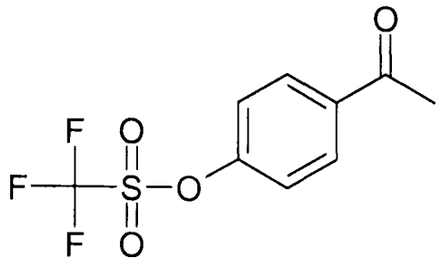


Compound **310** was synthesised following the same procedure as for compound **296** except that compound **282** (1.04g, 5.25mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol), and stirred for 10min prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol). The solvent was removed under vacuum to give a dark brown solid which was purified by column chromatography to give **310** as a pale brown solid (0.90g, 63.9% yield); m.p.=104.2-106.2°C [lit. m.p.=102.5-104.5°C (Copping et al, 1979)]; R_f=0.33 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.66min; LRMS (EI): 276 (M^+, 100%), 199 (M^+-C_6H_5, 98%), 169 (M^+-C_7H_7O, 24%), 121 (M^+-$C_7H_7SO_2$, 69%), 105 (M^+-$C_7H_7SO_3$, 67%); Elemental analysis: found C 60.75%, H 4.39%; $C_{14}H_{12}O_4S$ requires C 60.86%, H 4.38%.

$\nu_{(max)}$ (Film) cm^{-1}: 1659.1 (C=O), 1596.8 (Ar C=C), 1150.8 (S=O); δ_H d_6 acetone: 7.90 (2H, dd, J=8.9Hz, PhH), 7.80 (2H, m, PhH), 7.67 (1H, m, PhH), 7.55 (4H, m, PhH), 3.37 (3H, s, SCH_3); δ_C d_6 acetone: 195.37 (CO), 153.38 (CO), 138.17 (CH, Ar), 137.32 (CH, Ar), 133.59 (CH, Ar), 132.68 (CH, Ar), 130.64 (CH, Ar), 129.42 (C, Ar), 123.11 (C, Ar), 38.02 (CH_3SO_3).

3.5 Synthesis of the trifluoromethanesulfonic acid esters of 4-hydroxy phenyl ketone based compounds

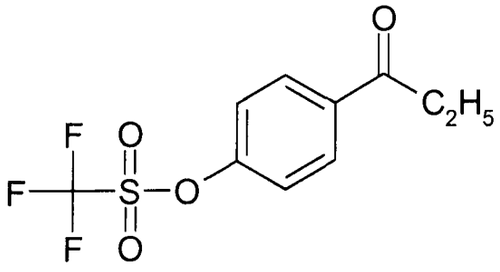
Trifluromethanesulfonic acid 4-acetyl-phenyl ester (311):



Compound **311** was synthesised following the same procedure as for compound **296** except that trifluoromethanesulfonyl chloride (TFMSC) (1.1mL, 10.31mmol) and **268** (1.23g, 9.05mmol) was used in place of methane sulfonyl chloride. The crude solid was purified by column chromatography to give **311** as yellow oil (0.84g, 34.7% yield); R_f=0.67 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=5.02min; LRMS (EI): 268 (M^+, 17%), 253 (M^+-CH_3, 100%), 120 (M^+-$C_2H_3F_3SO_2$, 15%), 95 (M^+-$C_3F_3SO_3$, 29%); HRMS (ES): found 269.0090 $C_9H_8F_3SO_4$ requires 269.2178.

$\nu_{(max)}$ (Film) cm^{-1}: 1693.3 (C=O), 1595.1 (Ar C=C), 1214.7 (S=O); δ_H d_6 acetone: 8.17 (2H, dd, J=8.9Hz, PhH), 7.61 (2H, dd, J=8.9Hz, PhH), 2.69 (3H, s, CH_3); δ_C d_6 acetone: 196.68 (CO), 153.33 (CO), 138.28 (CH, Ar), 131.69 (CH, Ar), 122.70 (C, Ar), 26.90 (CH_3).

Trifluromethansulfonic acid 4-propyl-phenyl ester (312):

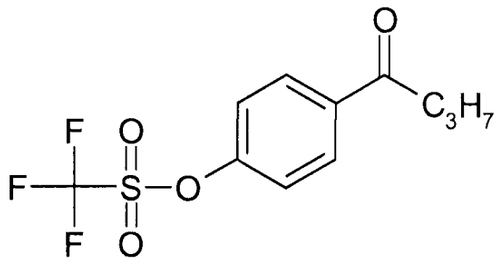


Compound **312** was synthesised following the same procedure as for compound **311** except that **269** (1.14g, 7.58mmol) was used in place of **268**. The crude oil

was purified by column chromatography to give **312** as yellow oil (0.99g, 46.2% yield); R_f=0.80 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=5.56min; LRMS (EI): 282 (M^+, 7%), 253 (M^+-C_2H_5, 100%), 120 (M^+-$C_3H_5F_3SO_2$, 23%), 95 (M^+-$C_4H_2F_3SO_3$, 34%); HRMS (ES): found 283.0246 $C_{10}H_{10}F_3SO_4$ requires 283.2443.

$\nu_{(max)}$ (Film) cm^{-1}: 1695.1 (C=O), 1596.7 (Ar C=C), 1215.9 (S=O); δ_H d_6 acetone: 8.20 (2H, dd, J=8.9Hz, PhH), 7.62 (2H, dd, J=8.9Hz, PhH), 3.13 (2H, q, J=7.1Hz, CH_2), 1.16 (3H, t, J=7.1Hz, CH_3); δ_C d_6 acetone: 199.31 (CO), 153.22 (CO), 138.17 (CH, Ar), 131.35 (CH, Ar), 122.71 (C, Ar), 32.43 (CH_2), 8.26 (CH_3).

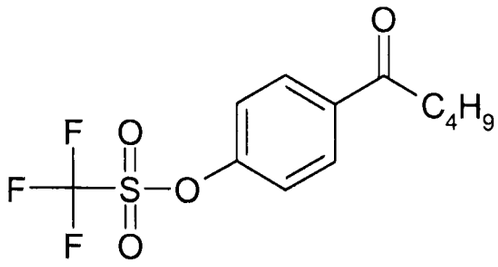
Trifluromethanesulfonic acid 4-butyl-phenyl ester (313):



Compound **313** w was synthesised following the same procedure as for compound **311** except that **270** (1.15g, 6.99mmol) was used in place of **268**. The crude oil was purified by column chromatography to give **313** as colourless oil (1.15g, 55.5% yield); R_f=0.85 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=6.05min; LRMS (EI): 281 (M^+-CH_3, 1%), 268 (M^+-C_2H_5, 15%), 253 (M^+-C_3H_7, 100%), 120 (M^+-$C_4H_7F_3SO_2$, 13%), 95 (M^+-$C_5H_4F_3SO_3$, 15%); HRMS (ES): found 297.0403 $C_{11}H_{12}F_3SO_4$ requires 297.2708.

$\nu_{(max)}$ (Film) cm^{-1}: 1692.6 (C=O), 1594.8 (Ar C=C), 1210.4 (S=O); δ_H d_6 acetone: 8.20 (2H, dd, J=8.9Hz, PhH), 7.61 (2H, dd, J=8.9Hz, PhH), 3.06 (2H, t, J=7.1Hz, CH_2), 1.71 (2H, m, CH_2), 0.97 (3H, t, J=7.1Hz, CH_3); δ_C d_6 acetone: 198.85 (CO), 153.23 (CO), 138.29 (CH, Ar), 131.39 (CH, Ar), 122.71 (C, Ar), 41.03 (CH_2), 18.11 (CH_2), 14.01 (CH_3).

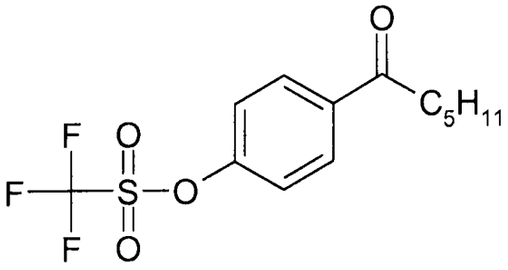
Trifluromethanesulfonic acid 4-pentyl-phenyl ester (314):



Compound **314** was synthesised following the same procedure as for compound **311** except that **271** (1.20g, 6.74mmol) was used in place of **268**. The crude oil was purified by column chromatography to give **314** as colourless oil (1.17g, 56.1% yield); R_f=0.89 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=6.53min; LRMS (EI): 281 (M^+-C_2H_5, 3%), 268 (M^+-C_3H_6, 86%), 253 (M^+-C_4H_9, 100%), 120 (M^+-$C_5H_9F_3SO_2$, 26%), 95 (M^+-$C_6H_6F_3SO_3$, 31%); HRMS (ES): found 311.0559 $C_{12}H_{14}F_3SO_4$ requires 311.2973.

$\nu_{(max)}$ (Film) cm^{-1}: 1694.3 (C=O), 1594.8 (Ar C=C), 1212.5 (S=O); δ_H $CDCl_3$: 8.04 (2H, dd, J=8.9Hz, PhH), 7.35 (2H, dd, J=8.9Hz, PhH), 2.94 (2H, t, J=7.1Hz, CH_2), 1.70 (2H, m, CH_2), 1.38 (2H, m, CH_2), 0.93 (3H, t, J=7.3Hz, CH_3); δ_C $CDCl_3$: 198.67 (CO), 152.37 (CO), 136.94 (CH, Ar), 130.40 (CH, Ar), 121.67 (C, Ar), 38.53 (CH_2), 26.27 (CH_2), 22.47 (CH_2), 13.96 (CH_3).

Trifluromethanesulfonic acid 4-hexyl-phenyl ester (315):

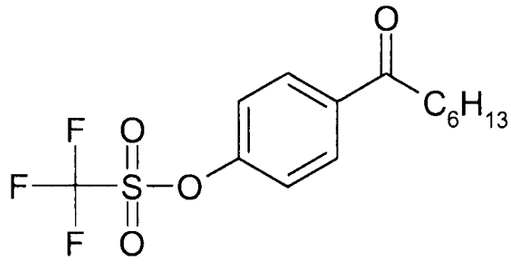


Compound **315** was synthesised following the same procedure as for compound **311** except that **272** (1.08g, 5.63mmol) was used in place of **268**. The crude oil was purified by column chromatography to give **315** as yellow oil (1.38g, 75.5% yield); R_f=0.93 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.00min; LRMS (EI): 324 (M^+, 1%), 268 (M^+-C_4H_8, 100%), 253 (M^+-C_5H_{11}, 88%), 120 (M^+-$C_6H_{11}F_3SO_2$,

18%), 95 (M^+-$C_7H_8F_3SO_3$, 21%); HRMS (ES): found 325.0716 $C_{13}H_{16}F_3SO_4$ requires 325.3238.

$\nu_{(max)}$ (Film) cm^{-1}: 1692.3 (C=O), 1595.1 (Ar C=C), 1214.0 (S=O); δ_H d_6 acetone: 8.19 (2H, dd, J=8.9Hz, PhH), 7.60 (2H, dd, J=8.9Hz, PhH), 3.08 (2H, t, J=7.3Hz, CH_2), 1.70 (2H, m, CH_2), 1.36 (4H, m, CH_2), 0.90 (3H, t, J=7.1Hz, CH_3); δ_C d_6 acetone: 198.99 (CO), 153.23 (CO), 138.31 (CH, Ar), 131.43 (CH, Ar), 122.71 (C, Ar), 39.14 (CH_2), 31.29 (CH_2), 24.48 (CH_2), 23.26 (CH_2), 14.31 (CH_3).

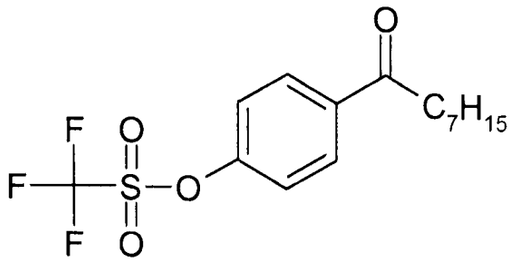
Trifluromethanesulfonic acid 4-heptyl-phenyl ester (316):



Compound **316** was synthesised following the same procedure as for compound **311** except that **273** (0.97g, 4.72mmol) was used in place of **268**. The crude oil was purified by column chromatography to give **316** as colourless oil (1.23g, 77.1% yield); R_f=0.96 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.41min; LRMS (EI): 338 (M^+, 1%), 268 (M^+-C_5H_{10}, 100%), 253 (M^+-C_6H_{13}, 67%), 120 (M^+-$C_7H_{13}F_3SO_2$, 15%), 95 (M^+-$C_8H_{10}F_3SO_3$, 20%); HRMS (ES): found 339.0872 $C_{14}H_{18}F_3SO_4$ requires 339.3503.

$\nu_{(max)}$ (Film) cm^{-1}: 1692.8 (C=O), 1595.2 (Ar C=C), 1215.8 (S=O); δ_H d_6 acetone: 8.21 (2H, dd, J=8.9Hz, PhH), 7.62 (2H, dd, J=8.9Hz, PhH), 3.10 (2H, t, J=7.3Hz, CH_2), 1.74 (2H, m, CH_2), 1.36 (6H, m, CH_2), 0.92 (3H, t, J=7.1Hz, CH_3); δ_C d_6 acetone: 198.96 (CO), 153.07 (CO), 138.26 (CH, Ar), 131.44 (CH, Ar), 122.72 (C, Ar), 39.18 (CH_2), 32.48 (CH_2), 24.75 (CH_2), 23.28 (CH_2), 14.37 (CH_3).

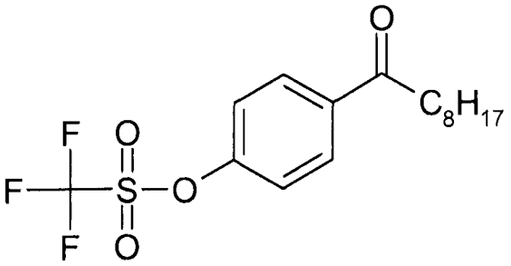
Trifluromethanesulfonic acid 4-octyl-phenyl ester (317):



Compound **317** was synthesised following the same procedure as for compound **311** except that **274** (1.11g, 5.03mmol) was used in place of **268**. The crude oil was purified by column chromatography to give **317** as colourless oil (1.21g, 68.1% yield); R_f=0.90 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.81min; LRMS (EI): 352 (M^+, 1%), 268 (M^+-C_6H_{12}, 100%), 253 (M^+-C_7H_{15}, 59%), 120 (M^+-$C_8H_{15}F_3SO_2$, 15%), 95 (M^+-$C_9H_{12}F_3SO_3$, 15%); HRMS (ES): found 353.1029 $C_{15}H_{20}F_3SO_4$ requires 353.3768.

$\nu_{(max)}$ (Film) cm^{-1}: 1694.5 (C=O), 1594.9 (Ar C=C), 1214.4 (S=O); δ_H $CDCl_3$: 8.04 (2H, dd, J=8.9Hz, PhH), 7.35 (2H, dd, J=8.9Hz, PhH), 2.94 (2H, t, J=7.3Hz, CH_2), 1.71 (2H, m, CH_2), 1.28 (8H, m, CH_2), 0.86 (3H, t, J=7.1Hz, CH_3); δ_C $CDCl_3$: 198.68 (CO), 152.37 (CO), 136.95 (CH, Ar), 130.39 (CH, Ar), 121.67 (C, Ar), 38.82 (CH_2), 31.76 (CH_2), 29.31 (CH_2), 29.18 (CH_2), 24.20 (CH_2), 22.68 (CH_2), 14.13 (CH_3).

Trifluromethanesulfonic acid 4-nonyl-phenyl ester (318):

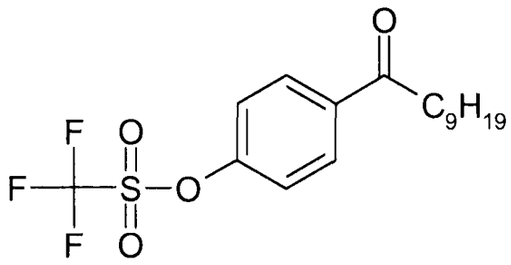


Compound **318** was synthesised following the same procedure as for compound **311** except that **275** (1.10g, 4.68mmol) was used in place of **268**. The crude solid was purified by column chromatography to give **318** as yellow solid (1.15g, 67.3% yield); m.p.=35.9-36.6°C; R_f=0.92 [DEE/pet spirit 40-60°C (50/50)]; GC:

t_R=8.19min; LRMS (EI): 366 (M^+, 1%), 268 (M^+-C_7H_{14}, 100%), 253 (M^+-C_8H_{17}, 61%), 120 (M^+-$C_9H_{17}F_3SO_2$, 15%), 95 (M^+-$C_{10}H_{14}F_3SO_3$, 17%); HRMS (ES): found 367.1185 $C_{16}H_{22}F_3SO_4$ requires 367.4033.

$\nu_{(max)}$ (Film) cm^{-1}: 1693.9 (C=O), 1595.0 (Ar C=C), 1213.2 (S=O); δ_H d_6 acetone: 8.19 (2H, dd, J=8.9Hz, PhH), 7.61 (2H, dd, J=8.9Hz, PhH), 3.08 (2H, t, J=7.1Hz, CH_2), 1.70 (2H, m, CH_2), 1.28 (10H, m, CH_2), 0.87 (3H, t, J=6.9Hz, CH_3); δ_C d_6 acetone: 198.91 (CO), 153.15 (CO), 138.24 (CH, Ar), 131.35 (CH, Ar), 122.63 (C, Ar), 39.11 (CH_2), 32.57 (CH_2), 24.73 (CH_2), 23.30 (CH_2), 14.34 (CH_3).

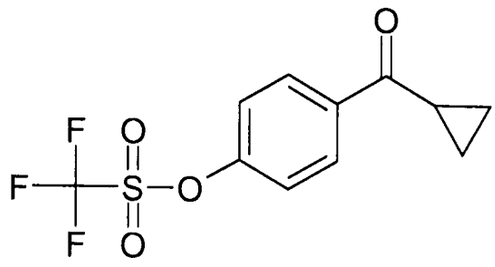
Trifluromethanesulfonic acid 4-decyl-phenyl ester (319):



Compound **319** was synthesised following the same procedure as for compound **311** except that **276** (0.99g, 3.99mmol) was used in place of **268**. The crude solid was purified by column chromatography to give **319** as off-white solid (0.95g, 62.6% yield); m.p.=40.5-42.1°C; R_f=0.94 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.55min; LRMS (EI): 380 (M^+, 1%), 268 (M^+-C_8H_{16}, 100%), 253 (M^+-C_9H_{19}, 43%), 120 (M^+-$C_{10}H_{19}F_3SO_2$, 11%), 95 (M^+-$C_{11}H_{16}F_3SO_3$, 14%); HRMS (ES): found 381.1342 $C_{17}H_{24}F_3SO_4$ requires 381.4298.

$\nu_{(max)}$ (Film) cm^{-1}: 1692.7 (C=O), 1595.3 (Ar C=C), 1214.0 (S=O); δ_H d_6 acetone: 8.21 (2H, dd, J=8.9Hz, PhH), 7.62 (2H, dd, J=8.9Hz, PhH), 3.09 (2H, t, J=7.3Hz, CH_2), 1.71 (2H, m, CH_2), 1.29 (12H, m, CH_2), 0.87 (3H, t, J=6.9Hz, CH_3); δ_C d_6 acetone: 199.01 (CO), 153.22 (CO), 138.31 (CH, Ar), 131.42 (CH, Ar), 122.71 (C, Ar), 39.18 (CH_2), 32.67 (CH_2), 24.80 (CH_2), 23.38 (CH_2), 14.41 (CH_3).

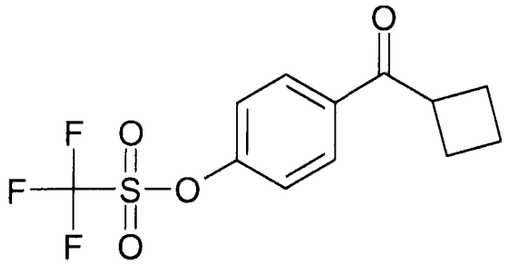
Trifluromethanesulfonic acid 4-cyclopropane carbonyl phenyl ester (320):



Compound **320** was synthesised following the same procedure as for compound **311** except that **278** (0.98g, 6.07mmol) was used in place of **268**. The crude oil was purified by column chromatography to give **320** as colourless oil (0.84g, 46.9% yield); R_f=0.76 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=6.35min; LRMS (EI): 294 (M^+, 40%), 253 (M^+-C_3H_5, 100%), 120 (M^+-$C_4H_5F_3SO_2$, 24%), 95 (M^+-$C_5H_2F_3SO_3$, 38%), 69 (M^+-$C_7H_4F_3SO_3$, 45%); HRMS (ES): found 295.0246 $C_{11}H_{10}F_3SO_4$ requires 295.2550.

$\nu_{(max)}$ (Film) cm^{-1}: 1675.8 (C=O), 1596.1 (Ar C=C), 1217.0 (S=O); δ_H d_6 acetone: 8.26 (2H, dd, J=8.9Hz, PhH), 7.64 (2H, dd, J=8.9Hz, PhH), 2.88 (1H, m, CH), 1.11 (4H, m, CH_2); δ_C d_6 acetone: 199.09 (CO), 153.26 (CO), 138.91 (CH, Ar), 131.39 (CH, Ar), 122.75 (C, Ar), 17.72 (CH), 12.06 (CH_2).

Trifluromethanesulfonic acid 4-cyclobutane carbonyl phenyl ester (321):

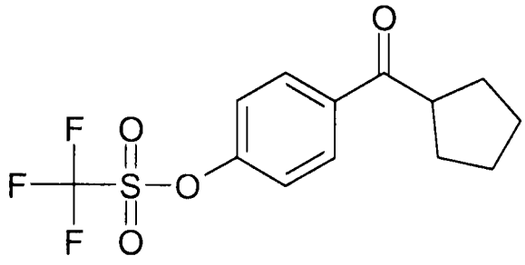


Compound **321** was synthesised following the same procedure as for compound **311** except that **279** (1.08g, 6.12mmol) was used in place of **268**. The crude oil was purified by column chromatography to give **321** as colourless oil (0.96g, 50.8% yield); R_f=0.84 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=6.84min; LRMS (EI): 308 (M^+, 1%), 253 (M^+-C_4H_7, 100%), 120 (M^+-$C_5H_7F_3SO_2$, 11%), 95 (M^+-

$C_6H_4F_3SO_3$, 16%), 69 (M^+-$C_8H_6F_3SO_3$, 6%); HRMS (ES): found 309.0403 $C_{12}H_{12}F_3SO_4$ requires 309.2815.

$\nu_{(max)}$ (Film) cm^{-1}: 1685.5 (C=O), 1595.4 (Ar C=C), 1215.2 (S=O); δ_H d_6 acetone: 8.14 (2H, dd, J=8.9Hz, PhH), 7.61 (2H, dd, J=8.9Hz, PhH), 4.18 (1H, m, CH), 2.23 (4H, m, CH_2), 2.11 (1H, m, CH), 1.86 (1H, m, CH); δ_C d_6 acetone: 199.37 (CO), 153.24 (CO), 136.69 (CH, Ar), 131.72 (CH, Ar), 122.82 (C, Ar), 42.90 (CH), 25.54 (CH), 18.60 (CH_2).

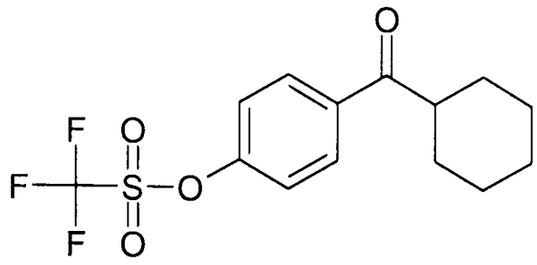
Trifluromethanesulfonic acid 4-cyclopentane carbonyl phenyl ester (322):



Compound **322** was synthesised following the same procedure as for compound **311** except that **280** (0.99g, 5.23mmol) was used in place of **268**. The crude solid was purified by column chromatography to give **322** as yellow solid (1.01g, 60.2% yield); m.p.=46.2-47.9°C; R_f=0.88 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.28min; LRMS (EI): 322 (M^+, 3%), 253 (M^+-C_5H_9, 100%), 120 (M^+-$C_6H_9F_3SO_2$, 19%), 95 (M^+-$C_7H_6F_3SO_3$, 21%), 69 (M^+-$C_9H_8F_3SO_3$, 15%); HRMS (ES): found 323.0559 $C_{13}H_{14}F_3SO_4$ requires 323.3080.

$\nu_{(max)}$ (Film) cm^{-1}: 1686.7 (C=O), 1594.2 (Ar C=C), 1212.4 (S=O); δ_H d_6 acetone: 8.21 (2H, dd, J=8.9Hz, PhH), 7.62 (2H, dd, J=8.9Hz, PhH), 3.85 (1H, m, CH), 1.95 (2H, m, CH_2), 1.84 (2H, m, CH_2), 1.67 (4H, m, CH_2); δ_C d_6 acetone: 200.98 (CO), 152.98 (CO), 137.82 (CH, Ar), 131.68 (CH, Ar), 122.52 (C, Ar), 46.95 (CH), 26.73 (CH_2).

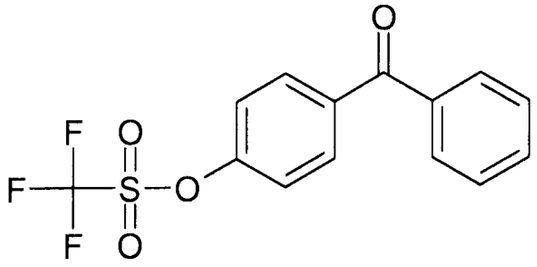
Trifluoromethanesulfonic acid 4-cyclohexane carbonyl phenyl ester (323):



Compound **323** was synthesised following the same procedure as for compound **311** except that **281** (1.06g, 5.18mmol) was used in place of **268**. The crude solid was purified by column chromatography to give **323** as off-white solid (1.13g, 65.1% yield); m.p.=46.5-48.3°C; R_f=0.90 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.68min; LRMS (EI): 336 (M^+, 5%), 253 (M^+-C_6H_{11}, 100%), 120 (M^+-$C_7H_{11}F_3SO_2$, 13%), 95 (M^+-$C_8H_8F_3SO_3$, 15%), 69 (M^+-$C_{10}H_{10}F_3SO_3$, 9%); HRMS (ES): found 337.0716 $C_{14}H_{16}F_3SO_4$ requires 337.3345.

$\nu_{(max)}$ (Film) cm^{-1}: 1687.0 (C=O), 1594.4 (Ar C=C), 1209.4 (S=O); δ_H d_6 acetone: 8.19 (2H, dd, J=8.9Hz, PhH), 7.62 (2H, dd, J=8.9Hz, PhH), 3.42 (1H, m, CH), 1.82 (5H, m, CH_2), 1.40 (5H, m, CH_2); δ_C d_6 acetone: 202.27 (CO), 153.17 (CO), 137.48 (CH, Ar), 131.70 (CH, Ar), 122.80 (C, Ar), 46.13 (CH), 26.73 (CH_2), 26.31 (CH_2).

Trifluoromethanesulfonic acid 4-benzyl carbonyl phenyl ester (324):



Compound **324** was synthesised following the same procedure as for compound **311** except that **282** (1.13g, 5.69mmol) was used in place of **268**. The crude solid was purified by column chromatography to give **324** as yellow solid (1.29g, 68.9% yield); m.p.=40.5-41.9°C [lit. m.p.=41-42°C (Wolfe and Buchwald, 1997)]; R_f=0.95 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.75min; LRMS (EI): 330 (M^+, 41%), 253

(M^+-C_6H_5, 43%), 105 (M^+-$C_7H_4F_3SO_3$, 100%), 77 (M^+-$C_8H_4F_3SO_4$, 24%); HRMS (ES): found 331.0246 $C_{14}H_{10}F_3SO_4$ requires 331.2871.

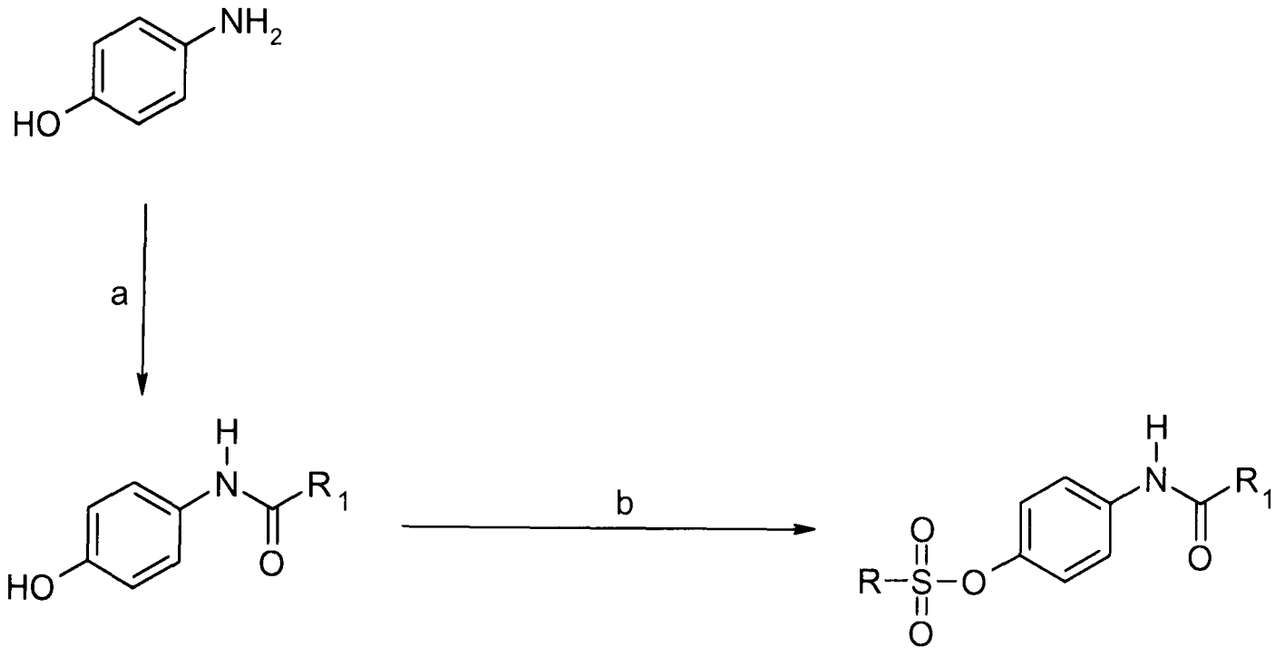
$\nu_{(max)}$ (Film) cm^{-1}: 1665.5 (C=O), 1597.1 (Ar C=C), 1216.6 (S=O); δ_H d_6 acetone: 8.00 (2H, dd, J=8.9Hz, PhH), 7.83 (2H, m, PhH), 7.69 (3H, m, PhH), 6.59 (2H, m, PhH); δ_C d_6 acetone: 195.05 (CO), 152.90 (CO), 138.93 (CH, Ar), 137.85 (CH, Ar), 133.86 (CH, Ar), 133.09 (CH, Ar), 130.74 (C, Ar),129.53 (CH, Ar), 122.62 (C, Ar).

Chapter 4: Synthesis of the N-(4-Hydroxy-phenyl)-alkylamides and derivatives.

4.0 Synthesis of the *N*-(4-hydroxy-phenyl)-alkylamides and derivatives

4.1 Discussion

In the design of potential inhibitors ES, we considered the use of the 4-hydroxyphenylamine backbone, in particular, the derivatisation of the amine moiety to the amide followed by the derivatisation of the 4-hydroxy moiety to the sulfonate derivative (Scheme 4.1).



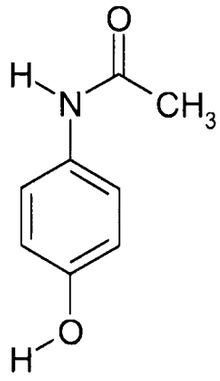
Scheme 4.1: Synthesis of sulfonated derivatives of 4-hydroxyphenylamine [a=$R_1COOCOR_1/H_2O/\Delta$; b=sulfonyl chloride/DCM/TEA; where R=CH_3 or $(CH_3)_2N$; R_1=methyl to nonyl]

In the synthesis of the amide intermediates, we considered the literature method for the preparation of *N*-(4-hydroxy-phenyl)-acetamide (i.e. paracetamol), that is, the carboxylic anhydride is reacted with the 4-hydroxyphenylamine to give the intermediate which is then derivatised involving the reaction with the appropriate sulfonyl chloride. The reactions proceeded without any major problems and the intermediates (compounds **325** to **333**) were produced in good to excellent yield

{ranging from ~57% for compound **330** [*N*-(4-hydroxy-phenyl)-heptyramide] to ~91% for compound **325** [*N*-(4-hydroxy-phenyl)-acetamide]}. In the synthesis of the target sulfonated derivatives, the reactions previously discussed were utilised, as such, the appropriate sulfonyl chloride (methane sulfonyl chloride or *N*,*N*-dimethylaminosulfonyl chloride) was reacted with the 4-hydroxy containing intermediate in the presence of TEA and anhydrous DCM to give the target compounds in good to moderate yield. That is, where methanesulfonyl chloride was utilised, the target compounds (compounds **334** to **342**) were obtained in good to excellent yield {ranging from ~60% for compound **335** [4-(propanoylamino)phenyl methanesulfonate] to ~91% for compound **341** [4-(nonanoylamino)phenyl methanesulfonate]}. With *N*,*N*-dimethylaminosulfonyl chloride, the reactions also proceeded without any major problems and in good yield {ranging from ~46% for compound **343** [4-(acetylamino)phenyl dimethylulfamate] to ~65% for compound **346** [4-(pentanoylamino)phenyl dimethylulfamate]}.

4.2 Synthesis of the N-(4-Hydroxy-phenyl)-alkylamides

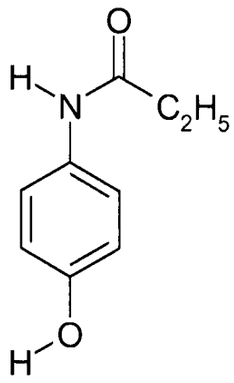
N-(4-Hydroxy-phenyl)-acetamide (325):



4-Aminophenol (5.78g, 52.9mmol) was dissolved in water (100mL) and acetic anhydride (5.0mL, 52.9mmol) was added and the mixture heated for 15min. The mixture was colled and filtered to give an off-white solid which was purified via recrystalisation from water to give **325** as an off-white crystalline solid (7.23g, 90.5% yield); m.p.=172.8-174.6°C [lit. m.p=167-168°C (Srinivas et al, 2003)]; R_f=0.21 (DEE); GC: t_R=8.03min; LRMS (EI): 151 (M^+, 30%), 109 (M^+-C_2H_3O, 100%), 80 (M^+-$C_5H_{10}O$, 15%), 53 (M^+-$C_6H_{10}NO$, 58%); HRMS (ES): found 152.0706 $C_8H_{10}NO_2$ requires 152.17294.

$\nu_{(max)}$ (Film) cm^{-1}: 3321.6 (Ph-OH), 1660.1 (C=O), 1609.7 (Ar C=C); δ_H d_6 acetone: 8.96 (1H, bs, NH), 8.17 (1H, bs, OH), 7.43 (2H, dd, J=8.9Hz, PhH), 6.75 (2H, dd, J=8.9Hz, PhH), 2.02 (3H, s, CH_3); δ_C d_6 acetone: 168.40 (CO), 154.29 (C, Ar), 132.71 (C, Ar), 121.76 (CH, Ar), 115.92 (CH, Ar), 24.08 (CH_3).

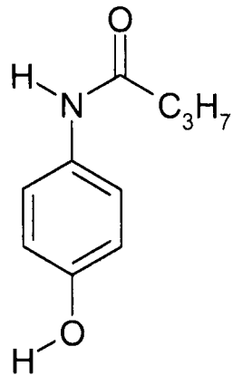
***N*-(4-Hydroxy-phenyl)-propionamide (326):**



Compound **326** was synthesised following the same procedure as for compound **325** except that propionic anhydride (7.0mL, 54.6mmol) was added to 4-aminophenol (5.03g, 47.9mmol). The mixture was allowed to cool to room temperature and filtered to give a crude off-white solid which was purified via recrystallisation from water to give **326** as an off-white crystalline solid (6.53g, 82.5% yield); m.p.=175.4-177.3°C [lit. m.p=172-173°C (Pedrazzoli et al, 1966); R_f=0.49 (DEE); GC: t_R=8.56min; LRMS (EI): 165 (M^+, 24%), 109 (M^+-C_3H_5O, 100%), 80 (M^+-$C_5H_{10}O$, 7%), 53 (M^+-$C_6H_{10}NO$, 5%); Elemental analysis: found C 65.25%, H 6.71%, N 8.39%; $C_9H_{11}NO_2$ requires C 65.44%, H 6.71%, N 8.48%.

$\nu_{(max)}$ (Film) cm^{-1}: 3307.07 (Ph-OH), 1664.7 (C=O), 1603.7 (Ar C=C); δ_H d_6 acetone: 8.84 (1H, bs, NH), 8.11 (1H, bs, OH), 7.45 (2H, dd, J=8.8Hz, PhH), 6.75 (2H, dd, J=8.8Hz, PhH), 2.31 (2H, q, J=7.5Hz, CH_2), 1.12 (3H, t, J=7.5Hz, CH_3); δ_C d_6 acetone: 172.06 (CO), 154.16 (C, Ar), 132.84 (C, Ar), 121.71 (CH, Ar), 115.91 (CH, Ar), 30.60 (CH_2), 10.12 (CH_3).

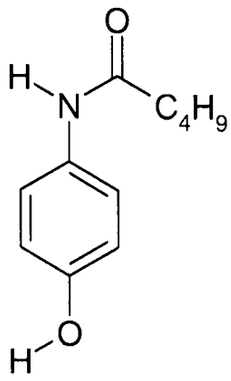
***N*-(4-Hydroxy-phenyl)-butyramide (327):**



Compound **327** was synthesised following the same procedure as for compound **325** except that butionic anhydride (7.0mL, 42.9mmol) was added to 4-aminophenol (4.32g, 39.6mmol). The mixture was allowed to cool to room temperature and filtered to give a pink coloured solid which was purified via recrystallisation from water to give **327** as an off-white crystalline solid (5.91g, 83.4% yield); m.p.=141.6-143.5°C [lit. m.p=138-140°C (Pedrazzoli et al, 1966)]; R_f=0.58 (DEE); GC: t_R=9.05min; LRMS (EI): 179 (M^+, 16%), 109 (M^+-C_4H_7O, 100%), 80 (M^+-$C_5H_{10}O$, 4%), 53 (M^+-C_5H_9NO, 3%); Elemental analysis: found C 66.98%, H 7.32%, N 7.83%; $C_{10}H_{13}NO_2$ requires C 67.02%, H 7.31%, N 7.82%.

$\nu_{(max)}$ (Film) cm^{-1}: 3314.9 (Ph-OH), 1642.7 (C=O), 1606.1 (Ar C=C); δ_H d_6 acetone: 8.84 (1H, bs, NH), 8.15 (1H, bs, OH), 7.46 (2H, dd, J=8.9Hz, PhH), 6.75 (2H, dd, J=8.9Hz, PhH), 2.28 (2H, t, J=7.3Hz, CH_2), 1.66 (2H, m, CH_2), 0.94 (3H, t, J=7.3Hz, CH_3); δ_C d_6 acetone: 171.35 (CO), 154.25 (C, Ar), 132.72 (C, Ar), 121.82 (CH, Ar), 115.91 (CH, Ar), 39.53 (CH_2), 19.76 (CH_2), 14.09 (CH_3).

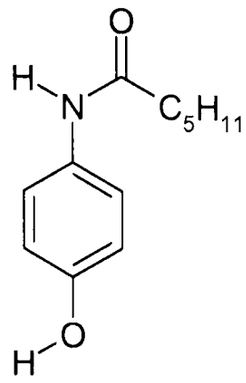
***N*-(4-Hydroxy-phenyl)-pentyramide (328):**



Compound **328** was synthesised following the same procedure as for compound **325** except that pentanoic anhydride (9.5mL, 48.1mmol) was added to 4-aminophenol (5.01g, 46.5mmol). The mixture was allowed to cool to room temperature and filtered to give a pink coloured solid which was purified via recrystallisation from water, and column chromatography to give **328** as an off-white crystalline solid (7.35g, 79.1% yield); m.p.=93.0-94.6°C [lit. m.p=99.5°C (Duffy et al, 2001)]; R_f=0.67 (DEE); GC: t_R=9.66min; LRMS (EI): 193 (M^+, 7%), 151 (M^+-C_3H_6, 2%), 109 (M^+-C_5H_9O, 100%), 81 (M^+-C_6H_4NO, 4%), 57 (M^+-$C_7H_6NO_2$, 3%); Elemental analysis: found C 69.70%, H 7.77%, N 6.60%; $C_{11}H_{15}NO_2$ requires C 68.37%, H 7.82%, N 7.25%.

$\nu_{(max)}$ (Film) cm^{-1}: 3313.1 (Ph-OH), 1648.0 (C=O), 1610.5 (Ar C=C); δ_H d_6 acetone: 8.91 (1H, bs, NH), 8.17 (1H, bs, O<u>H</u>), 7.46 (2H, dd, J=8.9Hz, Ph<u>H</u>), 6.75 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.31 (2H, q, J=7.5Hz, C<u>H</u>$_2$), 1.64 (2H, quint, J=7.5Hz, C<u>H</u>$_2$), 0.90 (3H, t, J=7.5Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 171.56 (<u>C</u>O), 154.28 (<u>C</u>, Ar), 132.69 (<u>C</u>, Ar), 121.85 (<u>C</u>H, Ar), 115.91 (<u>C</u>H, Ar), 37.38 (<u>C</u>H$_2$), 28.58 (<u>C</u>H$_2$), 23.08 (<u>C</u>H$_2$), 14.18 (CH$_3$).

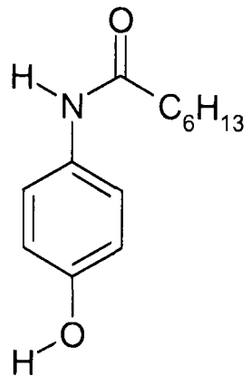
***N*-(4-Hydroxy-phenyl)-hexyramide (329):**



Compound **329** was synthesised following the same procedure as for compound **325** except that hexanoic anhydride (9.5mL, 41.1mmol) was added to 4-aminophenol (4.91g, 45.0mmol). The mixture was allowed to cool to room temperature and filtered to give a pink coloured solid which was purified via recrystallisation from water and subsequent column chromatography to give **329** as an off-white crystalline solid (6.88g, 80.7% yield); m.p.=113.8-115.4°C [lit. m.p=112°C (Fierz-David et al, 1939)]; R_f=0.70 (DEE); GC: t_R=10.18min; LRMS (EI): 207 (M^+, 7%), 151 (M^+-C_4H_8, 2%), 109 (M^+-$C_6H_{11}O$, 100%), 81 (M^+-$C_7H_{12}NO$, 3%), 55 (M^+-$C_8H_{10}NO_2$, 2%); Elemental analysis: found C 68.95%, H 8.28%, N 6.89%; $C_{11}H_{15}NO_2$ requires C 69.54%, H 8.27%, N 6.76%.

$\nu_{(max)}$ (Film) cm^{-1}: 3305.5 (Ph-OH), 1650.7 (C=O), 1610.6 (Ar C=C); δ_H d_6 acetone: 8.98 (1H, bs, NH), 8.23 (1H, bs, OH), 7.46 (2H, dd, J=8.9Hz, PhH), 6.76 (2H, dd, J=8.9Hz, PhH), 2.31 (2H, t, J=7.5Hz, CH_2), 1.66 (2H, quin, J=7.5Hz, CH_2), 1.31 (4H, m, CH_2), 0.88 (3H, t, J=6.9Hz, CH_3); δ_C d_6 acetone: 171.78 (CO), 154.35 (C, Ar), 132.55 (C, Ar), 121.95 (CH, Ar), 115.92 (CH, Ar), 37.62 (CH_2), 32.22 (CH_2), 26.16 (CH_2), 23.14 (CH_2), 14.29 (CH_3).

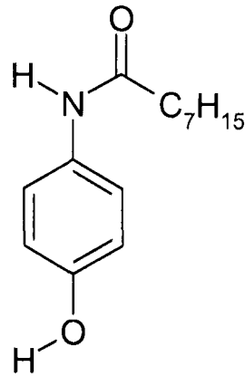
***N*-(4-Hydroxy-phenyl)-heptyramide (330):**



Compound **330** was synthesised following the same procedure as for compound **325** except that heptanoic anhydride (13mL, 49.2mmol) was added to 4-aminophenol (5.54g, 50.7mmol). The mixture was allowed to cool to room temperature and filtered to give a crude off-white solid which was purified via column chromatography to give **330** as an off-white crystalline solid (6.17g, 57.1% yield); m.p.=114.1-115.7°C [lit. m.p=114°C (Fierz-David et al, 1939)]; R_f=0.73 (DEE); GC: t_R=10.87min; LRMS (EI): 221 (M^+, 8%), 151 (M^+-C_5H_{10}, 2%), 109 (M^+-$C_7H_{13}O$, 100%), 81 (M^+-$C_8H_{14}NO$, 3%), 51 (M^+-$C_9H_{12}NO_2$, 2%); HRMS (ES): found 222.1494; $C_{13}H_{20}NO_2$ requires 222.3073.

$\nu_{(max)}$ (Film) cm^{-1}: 3311.7 (Ph-OH), 1651.3 (C=O), 1610.6 (Ar C=C); δ_H d_6 acetone: 8.88 (1H, bs, NH), 8.12 (1H, bs, OH), 7.46 (2H, dd, J=8.9Hz, PhH), 6.75 (2H, dd, J=8.9Hz, PhH), 2.30 (2H, t, J=7.3Hz, CH_2), 1.65 (2H, quin, J=7.3Hz, CH_2), 1.31 (6H, m, CH_2), 0.88 (3H, t, J=6.9Hz, CH_3); δ_C d_6 acetone: 171.49 (CO), 154.24 (C, Ar), 132.77 (C, Ar), 121.80 (CH, Ar), 115.91 (CH, Ar), 37.68 (CH_2), 32.43 (CH_2), 26.42 (CH_2), 23.27 (CH_2), 14.38 (CH_3).

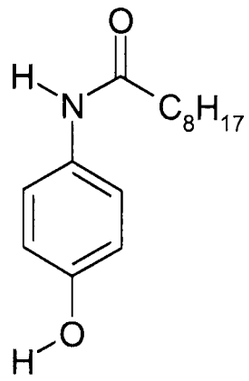
***N*-(4-Hydroxy-phenyl)-octyramide (331):**



Compound **331** was synthesised following the same procedure as for compound **325** except that octanoic anhydride (14.0mL, 47.1mmol) was added to 4-aminophenol (5.23g, 47.9mmol). The mixture was allowed to cool to room temperature and filtered to give a pink coloured solid which was purified via column chromatography to give **331** as an off-white crystalline solid (6.97g, 63.0% yield); m.p.=123.3-124.8°C [lit. m.p=123°C (Fierz-David et al, 1939)]; R_f=0.76 (DEE); GC: t_R=11.46min; LRMS (EI): 235 (M^+, 1%), 151 (M^+-C_6H_{12}, 2%), 109 (M^+-$C_8H_{15}O$, 100%), 81 (M^+-$C_9H_{16}NO$, 4%), 51 (M^+-$C_{10}H_{14}NO_2$, 7%); Elemental analysis: found C 71.46%, H 8.98%, N 5.89%; $C_{11}H_{15}NO_2$ requires C 71.46%, H 8.98%, N 5.89%.

$\nu_{(max)}$ (Film) cm^{-1}: 3308.9 (Ph-OH), 1651.4 (C=O), 1610.6 (Ar C=C); δ_H d_6 acetone: 8.46 (1H, bs, NH), 8.09 (1H, bs, OH), 7.46 (2H, dd, J=8.8Hz, PhH), 6.75 (2H, dd, J=8.8Hz, PhH), 2.30 (2H, t, J=7.5Hz, CH_2), 1.65 (2H, quin, J=7.5Hz, CH_2), 1.29 (8H, m, CH_2), 0.88 (3H, t, J=6.9HZ, CH_3); δ_C d_6 acetone: 171.89 (CO), 154.66 (C, Ar), 133.29 (C, Ar), 122.22 (CH, Ar), 116.37 (CH, Ar), 38.13 (CH_2), 33.02 (CH_2), 30.50 (CH_2), 30.36 (CH_2), 26.92 (CH_2), 23.81 (CH_2), 14.86 (CH_3).

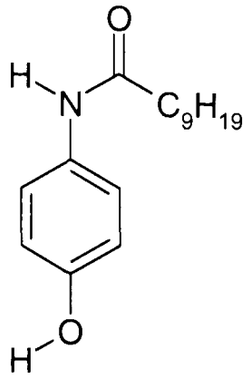
***N*-(4-Hydroxy-phenyl)-nonyramide (332):**



Compound **332** was synthesised following the same procedure as for compound **325** except that nonanoic anhydride (13.0mL, 43.5mmol) was added to 4-aminophenol (4.89g, 44.8mmol). The mixture was allowed to cool to room temperature and filtered to give a pink coloured solid which was purified via column chromatography to give **332** as an off-white crystalline solid (7.36g, 67.8% yield); m.p.=125.5-126.3°C [lit. m.p=124°C (Fierz-David et al, 1939)]; R_f=0.79 (DEE); GC: t_R=11.99min; LRMS (EI): 249 (M^+, 7%), 151 (M^+-C_7H_{14}, 3%), 109 (M^+-$C_9H_{17}O$, 100%); HRMS (ES): found 250.1807 $C_{15}H_{24}NO_2$ requires 250.3611.

$\nu_{(max)}$ (Film) cm^{-1}: 3315.0 (Ph-OH), 1651.4 (C=O), 1611.1 (Ar C=C); δ_H d_6 acetone: 8.82 (1H, bs, NH), 8.08 (1H, bs, OH), 7.36 (2H, dd, J=8.9Hz, PhH), 6.65 (2H, dd, J=8.9Hz, PhH), 2.26 (2H, t, J=7.3Hz, CH_2), 1.56 (2H, quin, J=7.3Hz, CH_2), 1.18 (10H, m, CH_2), 0.78 (3H, t, J=7.1Hz, CH_3); δ_C d_6 acetone: 172.01 (CO), 154.69 (C, Ar), 133.14 (C, Ar), 122.19 (CH, Ar), 116.34 (CH, Ar), 38.10 (CH_2), 33.06 (CH_2), 30.60 (CH_2), 30.51 (CH_2), 30.43 (CH_2), 26.89 (CH_2), 23.77 (CH_2), 14.83 (CH_3).

***N*-(4-Hydroxy-phenyl)-decyramide (333):**

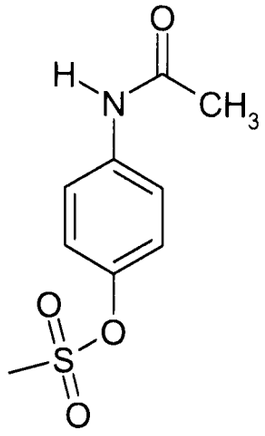


Compound **333** was synthesised following the same procedure as for compound **325** except that decanoic anhydride (13.52g, 41.3mmol) was added to 4-aminophenol (4.57g, 43.6mmol). The mixture was allowed to cool to room temperature and filtered to give a pink coloured solid which was purified via column chromatography to give **333** as an off-white crystalline solid (7.14g, 65.7% yield); m.p.=126.8-128.5°C [lit. m.p=130.5°C (Fierz-David et al, 1939)]; R_f=0.81 (DEE); GC: t_R=12.80min; LRMS (EI): 263 (M^+, 5%), 151 (M^+-C_8H_{16}, 4%), 109 (M^+-$C_{10}H_{19}O$, 100%), 81 (M^+-$C_{10}H_{18}NO$, 2%); Elemental analysis: found C 72.95%, H 9.58%, N 5.34%; $C_{16}H_{26}NO_2$ requires C 72.97%, H 9.57%, N 5.32%; HRMS (ES): found 264.1958; $C_{16}H_{26}NO_2$ requires 264.1958.

$\nu_{(max)}$ (Film) cm^{-1}: 3308.7 (Ph-OH), 1651.4 (C=O), 1610.7 (Ar C=C); δ_H d_6 acetone: 8.88 (1H, bs, NH), 8.13 (1H, bs, OH), 7.46 (2H, dd, J=8.9Hz, PhH), 6.74 (2H, dd, J=8.9Hz, PhH), 2.31 (2H, t, J=7.3Hz, CH_2), 1.66 (2H, quin, J=7.3Hz, CH_2), 1.28 (12H, m, CH_2), 0.87 (3H, t, J=6.9Hz, CH_3); δ_C d_6 acetone: 171.58 (CO), 154.13 (C, Ar), 132.76 (C, Ar), 121.79 (CH, Ar), 115.91 (CH, Ar), 37.68 (CH_2), 32.67 (CH_2), 30.31 (CH_2), 30.24 (CH_2), 30.09 (CH_2), 30.08 (CH_2), 26.47 (CH_2), 23.38 (CH_2), 14.42 (CH_3).

4.3 Synthesis of the 4-(alkylamino)phenyl methanesulfonates

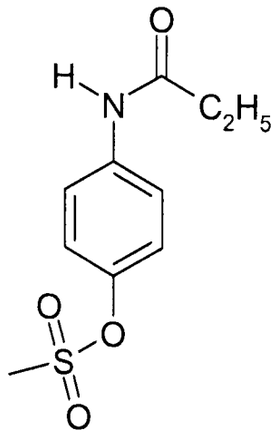
4-(Acetylamino)phenyl methanesulfonate (334):



Compound **325** (0.57g, 3.79mmol) was dissolved in anhydrous DCM (75mL) with TEA (1.0mL, 7.19mmol) and stirred for 10min. Methanesulfonyl chloride (0.7mL, 9.04mmol) was added and the solution refluxed for 8h. After cooling, the reaction mixture was poured onto ice (100mL). The organic layer was washed with water (3 x 50mL), $NaHCO_3$ (2 x 50mL) and water (3 x 50mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **334** as an off-white crystalline solid (0.63g, 72.4% yield); m.p.=172.3-173.5°C [lit. m.p=177°C (Ritter et al, 2004)]; R_f=0.12 (DEE); GC: t_R=10.72min; LRMS (EI): 229 (M^+, 16%), 150 (M^+-CH_3SO_2, 15%), 108 (M^+-$C_3H_5SO_3$, 100%), 53 (M^+-$C_6H_{10}SNO_3$, 3%); HRMS (ES): found 230.0482 $C_9H_{10}NSO_4$ requires 230.25022.

$\nu_{(max)}$ (Film) cm^{-1}: 3298.84 (N-H), 2941.0 (C-H), 1662.1 (C=O), 1605.6 (Ar C=C), 1355.9 (SO_2R_2); δ_H d_6 acetone: 9.35 (1H, bs, NH), 7.72 (2H, dd, J=8.9Hz, PhH), 7.28 (2H, dd, J=8.9Hz, PhH), 3.25 (3H, s, SCH_3), 2.09 (3H, s, CH_3); δ_C d_6 acetone: 170.83 (CO), 145.54 (C, Ar), 139.36 (C, Ar), 123.16 (CH, Ar), 120.77 (CH, Ar), 37.10 (CH_3SO_3), 24.02 (CH_3).

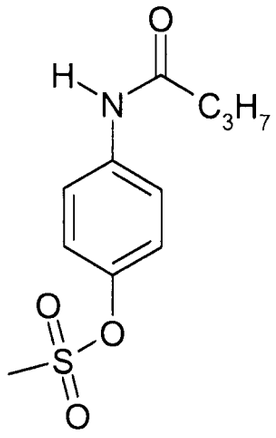
4-(Propanoylamino)phenyl methanesulfonate (335):



Compound **335** was synthesised via the same method as for compound **334** except compound **326** (0.53g, 3.18mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.50mL, 3.60mmol) and stirred for 10min before the addition of methanesulfonyl chloride (0.40mL, 6.46mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **335** as an off-white crystalline solid (0.46g, 60.1% yield); m.p.=144.9-146.2°C; R_f=0.31 (DEE); GC: t_R=10.96min; LRMS (EI): 243 (M^+, 9%), 187 (M^+-C_3H_4O, 2%), 164 (M^+-CH_3O_2S, 5%), 108 (M^+-$C_4H_7O_3S$, 100%), 57 (M^+- $C_7H_8NO_3S$, 32%); HRMS (ES): found 244.0638 $C_{10}H_{14}NO_4S$ requires 244.2879.

$\nu_{(max)}$ (Film) cm^{-1}: 1664.7 (C=O), 1603.7 (Ar C=C), 1356.20 (SO_2R_2); δ_H d_6 acetone: 9.26 (1H, bs, N<u>H</u>), 7.75 (2H, dd, J=9.1Hz, Ph<u>H</u>), 7.27 (2H, dd, J=9.1Hz, Ph<u>H</u>), 3.25 (3H, s, SC<u>H</u>$_3$), 2.38 (2H, q, J=7.5Hz, C<u>H</u>$_2$), 1.14 (3H, t, J=7.5Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 172.77 (<u>C</u>O), 145.73 (<u>C</u>, Ar), 139.59 (<u>C</u>, Ar), 123.40 (<u>C</u>H, Ar), 121.04 (<u>C</u>H, Ar), 37.35 (<u>C</u>H_3SO_3), 30.65 (<u>C</u>H_2), 9.86 (<u>C</u>H_3).

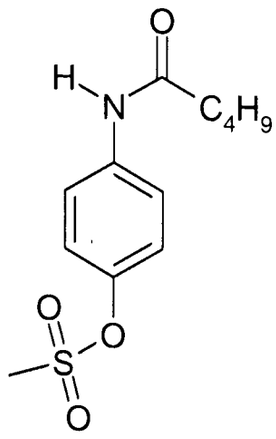
4-(Butanoylamino)phenyl methanesulfonate (336):



Compound **336** was synthesised via the same method as for compound **334** except compound **327** (0.84g, 4.67mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.38mmol) and stirred for 10min before the addition of methanesulfonyl chloride (0.7mL, 9.04mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **336** as an off-white crystalline solid (0.88g, 73.1% yield); m.p.=123.7-124.9°C; R_f=0.48 (DEE); GC: t_R=11.51min; LRMS (EI): 257 (M^+, 16%), 187 (M^+-C_4H_7O, 8%), 108 (M^+-$C_5H_9O_3S$, 100%), 92 (M^+-$C_5H_{11}NO_3S$, 1%), 71 (M^+-$C_7H_8NO_3S$, 1%); HRMS (ES): found 258.0795 $C_{12}H_{16}NO_4S$ requires 258.29918.

$\nu_{(max)}$ (Film) cm^{-1}: 3314.4 (N-H), 1660.2 (C=O), 1603.6 (Ar C=C), 1357.9 (SO_2R_2); δ_H d_6 acetone: 9.28 (1H, bs, NH), 7.75 (2H, dd, J=8.9Hz, PhH), 7.28 (2H, dd, J=8.9Hz, PhH), 3.24 (3H, s, SCH_3), 2.34 (2H, q, J=7.3Hz, CH_2), 1.68 (2H, sex, J=7.3Hz, CH_2), 0.92 (3H, t, J=7.3Hz, CH_3); δ_C d_6 acetone: 172.49 (CO), 146.26 (C, Ar), 140.07 (C, Ar), 123.90 (CH, Ar), 121.55 (CH, Ar), 40.07 (CH_2), 37.83 (CH_3SO_3), 20.06 (CH_2), 14.52 (CH_3).

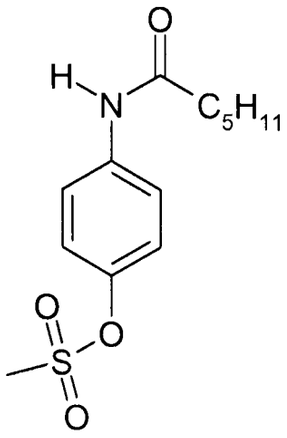
4-(Pentanoylamino)phenyl methanesulfonate (337):



Compound **337** was synthesised via the same method as for compound **334** except compound **328** (0.64g, 3.30mmol) was dissolved in anhydrous DCM (75mL) with TEA (1.0mL, 7.19mmol) and stirred for 10min before the addition of methanesulfonyl chloride (0.7mL, 9.04mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **337** as an off-white crystalline solid (0.56g, 62.1% yield); m.p.=115.7-117.1°C; R_f=0.56 (DEE); GC: t_R=12.17min; LRMS (EI): 271 (M^+, 10%), 187 (M^+-C_5H_9O, 11%), 108 (M^+-$C_6H_{11}O_3S$, 100%), 85 (M^+-$C_7H_8NO_3S$, 15%), 57 (M^+-$C_9H_{12}NO_3S$, 15%).

$\nu_{(max)}$ (Film) cm^{-1}: 3327.7 (N-H), 1660.6 (C=O), 1603.5 (Ar C=C), 1358.8 (SO_2R_2); δ_H d_6 acetone: 9.30 (1H, bs, NH), 7.76 (2H, dd, J=9.1Hz, PhH), 7.27 (2H, dd, J=9.1Hz, PhH), 3.25 (3H, s, SCH_3), 2.37 (2H, q, J=7.5Hz, CH_2), 1.65 (2H, quin, J=7.5Hz, CH_2), 1.38 (2H, quin, J=7.5Hz, CH_2), 0.91 (3H, t, J=7.3Hz, CH_3); δ_C d_6 acetone: 171.32 (CO), 145.51 (C, Ar), 139.43 (C, Ar), 123.14 (CH, Ar), 120.81 (CH, Ar), 37.20 (CH_2), 37.09 (CH_3SO_3), 28.10 (CH_2), 22.78 (CH_2),13.92 (CH_3).

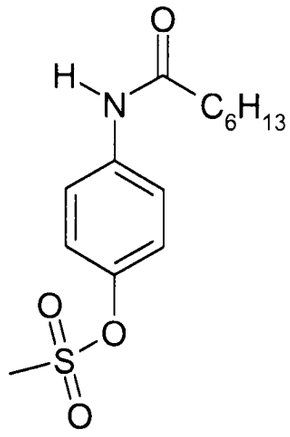
4-(Hexanoylamino)phenyl methanesulfonate (338):



Compound **338** was synthesised via the same method as for compound **334** except compound **329** (0.49g, 2.36mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.50mL, 3.60mmol) and stirred for 10min before the addition of methanesulfonyl chloride (0.40mL, 6.46mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **338** as an off-white crystalline solid (0.54g, 79.8% yield); m.p.=104.3-105.7°C; R_f=0.64 (DEE); GC: t_R=12.86min; LRMS (EI): 285 (M^+, 7%), 229 (M^+-C_4H_8, 2%), 187 (M^+-$C_6H_{10}O$, 12%), 108 (M^+-$C_7H_{13}O_3S$, 100%), 71 (M^+-$C_8H_8NO_4S$, 10%); HRMS (ES): found 286.1108 $C_{13}H_{20}NO_4S$ requires 286.37214.

$\nu_{(max)}$ (Film) cm^{-1}: 3321.8 (N-H), 2931.2 (C-H), 1658.5 (C=O), 1605.3 (Ar C=C), 1358.2 (SO_2R_2); δ_H d_6 acetone: 9.27 (1H, bs, NH), 7.75 (2H, dd, J=8.9Hz, PhH), 7.27 (2H, dd, J=8.9Hz, PhH), 3.25 (3H, s, SCH_3), 2.36 (2H, t, J=7.6HZ, CH_2), 1.67 (2H, quin, J=7.5Hz, CH_2), 1.33 (4H, m, CH_2), 0.89 (3H, t, J=6.9HZ, CH_3); δ_C d_6 acetone: 172.89 (CO), 149.76 (C, Ar), 140.19 (C, Ar), 123.21 (CH, Ar), 120.85 (CH, Ar), 37.49 (CH_2), 37.13 (CH_3SO_3), 31.99 (CH_2), 25.73 (CH_2), 22.96 (CH_2), 14.08 (CH_3).

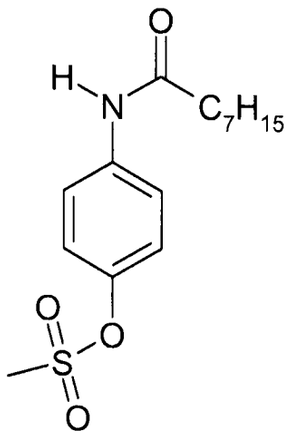
4-(Heptanoylamino)phenyl methanesulfonate (339):



Compound **339** was synthesised via the same method as for compound **334** except compound **330** (0.61g, 2.76mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.50mL, 4.8mmol) and stirred for 10min before the addition of methanesulfonyl chloride (0.40mL, 6.46mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **339** as an off-white crystalline solid (0.68g, 81.9% yield); m.p.=121.4-122.9°C; R_f=0.65 (DEE); GC: t_R=13.78min; LRMS (EI): 299 (M^+, 7%), 229 (M^+-C_5H_{10}, 4%), 187 (M^+-$C_7H_{12}O$, 16%), 108 (M^+-$C_8H_{15}O_3S$, 100%), 85 (M^+- $C_8H_{13}NO_4S$, 5%); HRMS (ES): found 300.1264 $C_{14}H_{22}NO_4S$ requires 300.39902.

$\nu_{(max)}$ (Film) cm^{-1}: 3330.9 (N-H), 1660.1 (C=O), 1603.9 (Ar C=C), 1360.2 (SO_2R_2); δ_H d_6 acetone: 9.27 (1H, bs, N<u>H</u>), 7.75 (2H, dd, J=8.9Hz, Ph<u>H</u>), 7.27 (2H, dd, J=8.9Hz, Ph<u>H</u>), 3.25 (3H, s, SC<u>H</u>$_3$), 2.37 (2H, t, J=7.5Hz, C<u>H</u>$_2$), 1.67 (2H, quin, J=7.5Hz, C<u>H</u>$_2$), 1.32 (6H, m, C<u>H</u>$_2$), 0.88 (3H, t, J=7.0Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 171.92 (<u>C</u>O), 145.51 (<u>C</u>, Ar), 139.41 (<u>C</u>, Ar), 123.16 (<u>C</u>H, Ar), 120.80 (<u>C</u>H, Ar), 37.49 (<u>C</u>H$_2$), 37.09 (<u>C</u>H$_3$SO$_3$), 32.16 (<u>C</u>H$_2$), 29.43 (<u>C</u>H$_2$), 25.95 (<u>C</u>H$_2$), 23.00 (<u>C</u>H$_2$), 14.12 (<u>C</u>H$_3$).

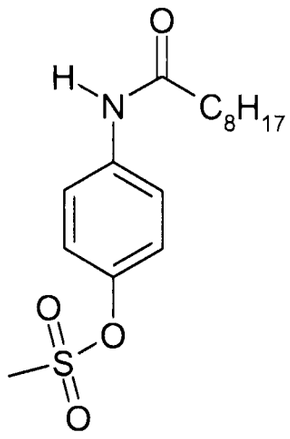
4-(Octanoylamino)phenyl methanesulfonate (340):



Compound **340** was synthesised via the same method as for compound **334** except compound **331** (0.58g, 2.47mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.50mL, 3.60mmol) and stirred for 10min before the addition of methanesulfonyl chloride (0.40mL, 6.46mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **340** as an off-white crystalline solid (0.60g, 77.6% yield); m.p.=129.7-130.9°C; R_f=0.66 (DEE); GC: t_R=14.85min; LRMS (EI): 313 (M^+, 4%), 229 (M^+-C_6H_{12}, 4%), 187 (M^+-$C_8H_{15}O$, 15%), 127 (M^+-$C_8H_{10}O_3S$, 7%), 108 (M^+-$C_9H_{17}O_3S$, 100%), 57 (M^+-$C_{11}H_{15}NO_4S$, 21%); HRMS (ES): found 314.1421 $C_{15}H_{24}NO_4S$ requires 314.3971.

$\nu_{(max)}$ (Film) cm^{-1}: 3323.0 (N-H), 2918.4 (C-H), 1657.8 (C=O), 1605.5 (Ar C=C), 1362.8 (SO_2R_2); δ_H d_6 acetone: 9.27 (1H, bs, NH), 7.75 (2H, dd, J=9.1Hz, PhH), 7.28 (2H, dd, J=9.1Hz, PhH), 3.25 (3H, s, SCH_3), 2.37 (2H, t, J=7.3Hz, CH_2), 1.67 (2H, quin, J=7.3Hz, CH_2), 1.29 (8H, m, CH_3), 0.88 (3H, t, J=6.9Hz, CH_3); δ_C d_6 acetone: 172.18 (CO), 153.74 (C, Ar), 136.89 (C, Ar), 123.41 (CH, Ar), 121.06 (CH, Ar), 37.74 (CH_2), 37.36 (CH_3SO_3), 32.53 (CH_2), 29.97 (CH_2), 26.25 (CH_2), 23.33 (CH_2), 14.38 (CH_3).

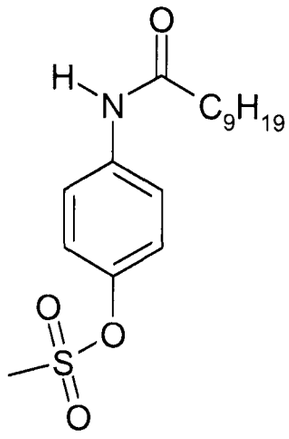
4-(Nonanoylamino)phenyl methanesulfonate (341):



Compound **341** was synthesised via the same method as for compound **334** except compound **332** (0.50g, 2.0mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.50mL, 3.60mmol) and stirred for 10min before the addition of methanesulfonyl chloride (0.40mL, 6.46mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **341** as an off-white crystalline solid (0.59g, 90.5% yield); m.p.=115.4-117.1°C; R_f=0.67 (DEE); GC: t_R=16.12min; LRMS (EI): 327 (M^+, 4%), 229 (M^+-C_7H_{14}, 5%), 187 (M^+-$C_9H_{16}O$, 22%), 108 (M^+-$C_{10}H_{19}O_3S$, 100%), 57 (M^+- $C_{12}H_{16}NO_4S$, 10%).

$\nu_{(max)}$ (Film) cm^{-1}: 3321.9 (N-H), 2930.1 (C-H), 1659.8 (C=O), 1604.9 (Ar C=C), 1361.9 (SO_2R_2); δ_H d_6 acetone: 9.28 (1H, bs, NH), 7.76 (2H, dd, J=9.0Hz, PhH), 7.27 (2H, dd, J=9.0Hz, PhH), 3.24 (3H, s, SCH_3), 2.37 (2H, t, J=7.3Hz, CH_2), 1.67 (2H, quin, J=7.3Hz, CH_2), 1.32 (10H, m, CH_2), 0.87 (3H, t, J=7.0Hz, CH_3); δ_C d_6 acetone: 172.18 (CO), 145.77 (C Ar), 139.70 (C, Ar), 123.55 (CH, Ar), 121.05 (CH, Ar), 37.73 (CH_2), 37.33 (CH_3SO_3), 32.63 (CH_2), 30.16 (CH_2), 30.01 (CH_2), 29.99 (CH_2), 26.24 (CH_2), 23.35 (CH_2), 14.40 (CH_3).

4-(Decanoylamino)phenyl methanesulfonate (342):

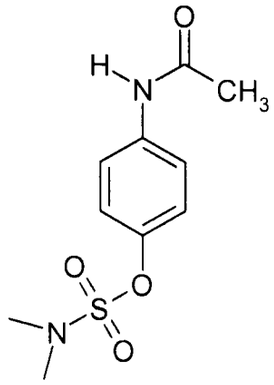


Compound **342** was synthesised via the same method as for compound **334** except compound **333** (0.52g, 1.98mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.50mL, 3.60mmol) and stirred for 10min before the addition of methane sulfonyl chloride (0.40mL, 6.46mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **342** as an off-white crystalline solid (0.44g, 65.2% yield); m.p.=133.8-134.4°C; R_f=0.68 (DEE); GC: t_R=17.69min; LRMS (EI): 341 (M^+, 4%), 229 (M^+-C_8H_{16}, 6%), 187 (M^+-$C_{10}H_{18}O$, 24%), 155 (M^+-$C_8H_{10}O_3S$, 6%), 108 (M^+-$C_{11}H_{21}O_3S$, 100%), 71 (M^+-$C_{12}H_{16}NO_4S$, 6%); HRMS (ES): found 342.1734 $C_{17}H_{28}NO_4S$ requires 342.47966.

$\nu_{(max)}$ (Film) cm^{-1}: 3324.1 (N-H), 2915.6 (C-H), 1659.1 (C=O), 1605.3 (Ar C=C), 1363.9 (SO_2R_2); δ_H d_6 acetone: 9.27 (1H, bs, NH), 7.75 (2H, dd, J=9.1Hz, PhH), 7.27 (2H, dd, J=9.1Hz, PhH), 3.25 (3H, s, SCH_3), 2.37 (2H, t, J=7.3Hz, CH_2), 1.67 (2H, quin, J=7.3Hz, CH_2), 1.32 (12H, m, CH_3), 0.87 (3H, t, J=6.9Hz, CH_3); δ_C d_6 acetone: 173.24 (CO), 142.69 (C, Ar), 134.87 (C, Ar), 123.35 (CH, Ar), 120.99 (CH, Ar), 37.68 (CH_2), 37.29 (CH_3SO_3), 32.58 (CH_2), 30.23 (CH_2), 30.15 (CH_2), 30.01 (CH_2), 29.96 (CH_2), 26.19 (CH_2), 23.33 (CH_2), 14.35 (CH_3).

4.4 Synthesis of the Dimethylsulfamic acid esters 4-alkylamino-phenyl esters

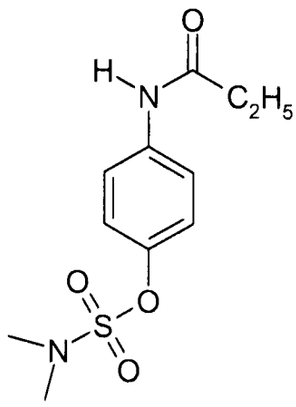
4-(Acetylamino)phenyl dimethylulfamate (343):



Compound **325** (0.73g, 4.88mmol) was dissolved in anhydrous DCM (75mL) with TEA (mL, mmol) and stirred for 10min. *N,N*-dimethyl amino sulfonyl chloride (0.50mL, 4.66mmol) was added and the solution refluxed for 16h. After cooling, the reaction mixture was poured onto ice (100mL). The organic layer was washed with water (3 x 50mL), sodium carbonate solution (2 x 50mL) and water (3 x 50mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **343** as an off-white crystalline solid (0.55g, 46.0% yield) m.p.=66.3-67.9°C; R_f=0.09 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=11.30min; LRMS (EI): 258 (M^+, 11%), 150 (M^+-$C_2H_6NO_2S$, 5%), 108 (M^+-$C_4H_9NO_3S$, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3251.2 (NH), 1658.8 (C=O), 1608.8 (Ar C=C), 1362.6 (SO_2R_2); δ_H $CDCl_3$: 7.80 (1H, bs, N<u>H</u>), 7.56 (2H, dd, J=9.1Hz, Ph<u>H</u>), 7.18 (2H, dd, J=9.1Hz, Ph<u>H</u>), 2.94 (6H, s, NC<u>H</u>$_3$), 2.16 (3H, s, C<u>H</u>$_3$); δ_C $CDCl_3$: 168.89 (<u>C</u>O), 146.14 (<u>C</u>, Ar), 136.93 (<u>C</u>, Ar), 122.50 (<u>C</u>H, Ar), 121.13 (<u>C</u>H, Ar), 46.15 (<u>C</u>H$_3$N), 24.69 (<u>C</u>H$_3$).

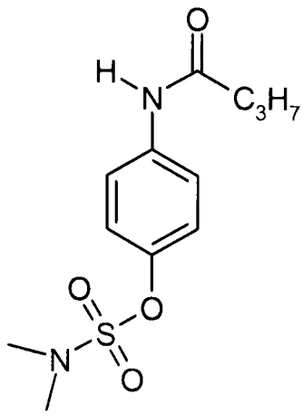
4-(Propanoylamino)phenyl dimethylulfamate (344):



Compound **344** was synthesised via the same method as for compound **343** except compound **326** (0.69g, 4.21mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.5mL, 4.8mmol) and stirred for 10min before the addition of *N,N*-dimethyl amino sulfonyl chloride (0.45mL, 4.19mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **344** as an off-white crystalline solid (0.61g, 53.8% yield); m.p.=85.1-86.4°C; R_f=0.16 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=11.78min; LRMS (EI): 272 (M^+, 100%), 164 (M^+-$C_2H_6NO_2S$, 3%), 108 (M^+-$C_5H_{11}NO_3S$, 5%), 57 (M^+-$C_8H_{11}N_2O_3S$, 30%); HRMS (ES): found M^+ 273.0904 $C_{11}H_{17}N_2O_4S$ requires M^+ 273.33306.

$\nu_{(max)}$ (Film) cm^{-1}: 3306.9 (NH), 1664.8 (C=O), 1606.74 (Ar C=C), 1364.1 (SO_2R_2); δ_H d_6 acetone: 9.27 (1H, bs, N<u>H</u>), 7.75 (2H, dd, J=8.9Hz, Ph<u>H</u>), 7.25 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.94 (6H, s, NC<u>H</u>$_3$), 2.47 (2H, t, J=7.6Hz, CH_2), 1.14 (3H, t, J=7.6Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 173.24 (<u>C</u>O), 146.79 (<u>C</u>, Ar), 139.69 (<u>C</u>, Ar), 123.52 (<u>C</u>H, Ar), 121.25 (<u>C</u>H, Ar), 39.36 (<u>C</u>H_3N), 31.06 (<u>C</u>H_2), 10.29 (<u>C</u>H_3).

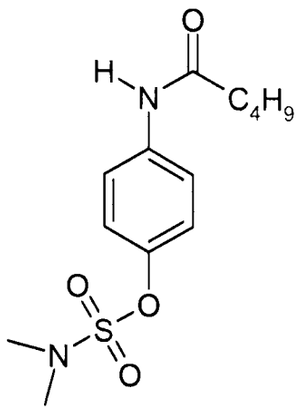
4-(Butanoylamino)phenyl dimethylulfamate (345):



Compound **345** was synthesised via the same method as for compound **343** except compound **327** (0.74g, 4.17mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.5mL, 4.8mmol) and stirred for 10min before the addition of *N,N*-dimethyl amino sulfonyl chloride (0.45mL, 4.19mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **345** as an off-white crystalline solid (0.59g, 49.2% yield); m.p.=106.8-108.3°C; R_f=0.25 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=12.36min; LRMS (EI): 286 (M^+, 18%), 216 (M^+-C_4H_6O, 3%), 179 (M^+-$C_2H_5NO_2S$, 3%), 108 (M^+-$C_6H_{12}NO_3S$, 100%), 71 (M^+-$C_8H_{11}N_2O_3S$, 30%); HRMS (ES): found 287.1066 $C_{12}H_{19}N_2O_4S$ requires 287.3599.

$\nu_{(max)}$ (Film) cm^{-1}: 3307.9 (NH), 1666.9 (C=O), 1608.8 (Ar C=C), 1360.87 (SO_2R_2); δ_H d_6 acetone: 9.25 (1H, bs, N<u>H</u>), 7.74 (2H, dd, J=8.9Hz, Ph<u>H</u>), 7.25 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.95 (6H, s, NC<u>H</u>$_3$), 2.34 (2H, t, J=7.3Hz, C<u>H</u>$_2$), 1.68 (2H, sex, J=7.3Hz, C<u>H</u>$_2$), 0.95 (3H, t, J=7.3Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 171.99 (<u>C</u>O), 146.42 (<u>C</u>, Ar), 139.20 (<u>C</u>, Ar), 123.12 (<u>C</u>H, Ar), 120.95 (<u>C</u>H, Ar), 39.57 (<u>C</u>H$_2$), 38.95 (<u>C</u>H$_3$N), 19.56 (<u>C</u>H$_2$), 14.04 (<u>C</u>H$_3$).

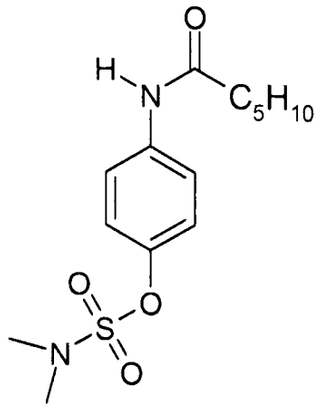
4-(Pentanoylamino)phenyl dimethylulfamate (346):



Compound **346** was synthesised via the same method as for compound **343** except compound **328** (0.75g, 0.40mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.50mL, 4.8mmol) and stirred for 10min before the addition of *N,N*-dimethyl amino sulfonyl chloride (0.40mL, 3.82mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **346** as an off-white crystalline solid (0.75g, 65.4% yield); m.p.=74.3-75.6°C; R_f=0.26 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=13.20min; LRMS (EI): 300 (M^+, 13%), 216 (M^+-C_5H_8O, 5%), 151 (M^+-$C_5H_{12}NO_2S$, 3%), 108 (M^+-$C_7H_{15}NO_3S$, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 3329.0 (NH), 1663.5 (C=O), 1605.8 (Ar C=C), 1362.9 (SO_2R_2); δ_H d_6 acetone: 9.26 (1H, bs, N<u>H</u>), 7.74 (2H, dd, J=9.1Hz, Ph<u>H</u>), 7.25 (2H, dd, J=9.1Hz, Ph<u>H</u>), 2.94 (6H, s, N-C<u>H</u>$_3$), 2.36 (2H, t, J=7.5Hz, C<u>H</u>$_2$), 1.65 (2H, quin, J=7.5Hz, C<u>H</u>$_2$), 1.36 (2H, sex, J=7.5Hz, C<u>H</u>$_2$), 0.91 (3H, t, J=7.5Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 172.19 (<u>C</u>O), 146.41 (<u>C</u>, Ar), 139.20 (<u>C</u>, Ar), 123.10 (<u>C</u>H, Ar), 120.96 (<u>C</u>H, Ar), 38.94 (<u>C</u>H$_3$N), 37.68 (<u>C</u>H$_2$), 32.19 (<u>C</u>H$_2$), 25.93 (<u>C</u>H$_2$), 23.14 (<u>C</u>H$_2$), 14.28 (<u>C</u>H$_3$).

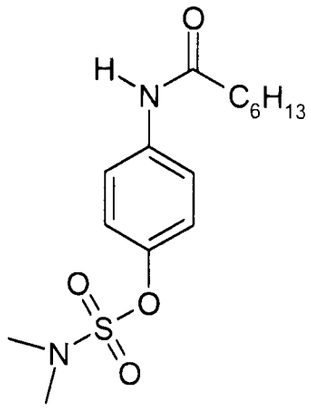
4-(Hexanoylamino)phenyl dimethylulfamate (347):



Compound **347** was synthesised via the same method as for compound **343** except compound **329** (0.68g, 3.28mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.5mL, 4.8mmol) and stirred for 10min before the addition of *N,N*-dimethyl amino sulfonyl chloride (0.35mL, 3.26mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **347** as an off-white crystalline solid (0.64g, 61.9% yield); m.p.=81.5-83.0°C; R_f=0.38 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=14.39min; LRMS (EI): 314 (M^+, 16%), 216 (M^+-$C_6H_{10}O$, 3%), 179 (M^+-$C_2H_6NO_2S$, 3%), 108 (M^+-$C_8H_{17}NO_3S$, 100%); HRMS (ES): found 315.1379 $C_{14}H_{24}N_2O_4S$ requires 315.4137.

$\nu_{(max)}$ (Film) cm^{-1}: 3307.5 (NH), 1665.8 (C=O), 1600.64 (Ar C=C), 1362.3 (SO_2R_2); δ_H d_6 acetone: 9.28 (1H, bs, N<u>H</u>), 7.73 (2H, d, J=8.9Hz, Ph<u>H</u>), 7.25 (2H, d, J=8.9Hz, Ph<u>H</u>), 3.06 (6H, s, NC<u>H</u>$_3$), 2.36 (2H, t, J=7.5Hz, C<u>H</u>$_2$), 1.68 (2H, quin, J=7.3Hz, C<u>H</u>$_2$), 1.32 (4H, m, C<u>H</u>$_2$), 0.88 (3H, t, J=6.9Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 172.19 (<u>C</u>O), 146.41 (<u>C</u>, Ar), 139.20 (<u>C</u>, Ar), 123.10 (<u>C</u>H, Ar), 120.96 (<u>C</u>H, Ar), 38.94 (<u>C</u>H$_3$N), 37.68 (<u>C</u>H$_2$), 32.19 (<u>C</u>H$_2$), 25.93 (<u>C</u>H$_2$), 23.14 (<u>C</u>H$_2$), 14.28 (<u>C</u>H$_3$).

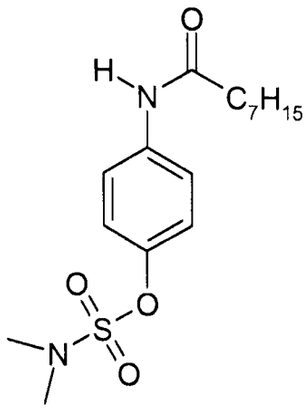
4-(Heptanoylamino)phenyl dimethylulfamate (348):



Compound **348** was synthesised via the same method as for compound **343** except compound **330** (0.76g, 3.44mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.5mL, 4.8mmol) and stirred for 10min before the addition of *N,N*-dimethyl amino sulfonyl chloride (0.35mL, 3.26mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **348** as an off-white crystalline solid (0.61g, 56.6% yield); m.p.=86.9-88.3°C; R_f=0.40 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=15.17min; LRMS (EI): 328 (M^+, 12%), 258 (M^+-C_5H_{10}, 2%), 216 (M^+-$C_7H_{12}O$, 9%), 151 (M^+-$C_6H_{12}NO_3S$, 3%), 108 (M^+-$C_9H_{19}NO_3S$, 100%); Elemental analysis: found C 55.01%, H 7.41%, N 8.33%; $C_{15}H_{25}N_2O_4S$ requires C 54.86%, H 7.37%, N 8.53%; HRMS (ES): found 329.1535 $C_{15}H_{26}N_2O_4S$ requires 329.4406.

$\nu_{(max)}$ (Film) cm^{-1}: 3316.0 (NH), 1658.97 (C=O), 1603.3 (Ar C=C), 1364.2 (SO_2R_2); δ_H d_6 acetone: 9.28 (1H, bs, N<u>H</u>), 7.73 (2H, dd, J=8.9Hz, Ph<u>H</u>), 7.25 (2H, dd, J=8.9Hz, Ph<u>H</u>), 3.06 (6H, s, NC<u>H</u>$_3$), 2.36 (2H, t, J=7.5Hz, C<u>H</u>$_2$), 1.68 (2H, quin, J=7.5Hz, C<u>H</u>$_2$), 1.32 (4H, m, C<u>H</u>$_2$), 0.88 (3H, t, J=6.9Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 172.19 (<u>C</u>O), 146.41 (<u>C</u>, Ar), 139.20 (<u>C</u>, Ar), 123.10 (<u>C</u>H, Ar), 120.96 (<u>C</u>H, Ar), 38.94 (<u>C</u>H$_3$N), 37.68 (<u>C</u>H$_2$), 32.19 (<u>C</u>H$_2$), 25.93 (<u>C</u>H$_2$), 23.14 (<u>C</u>H$_2$), 14.28 (<u>C</u>H$_3$).

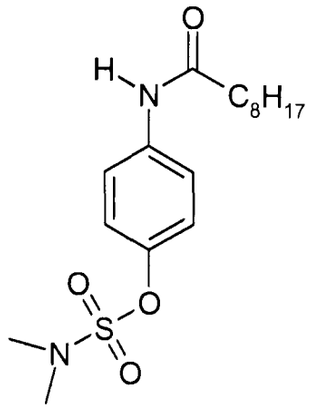
4-(Octanoylamino)phenyl dimethylulfamate (349):



Compound **349** was synthesised via the same method as for compound **343** except compound **331** (0.79g, 3.38mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.5mL, 4.8mmol) and stirred for 10min before the addition of *N,N*-dimethyl amino sulfonyl chloride (0.35mL, 3.26mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **349** as an off-white crystalline solid (0.69g, 62.2% yield); m.p.=61.6-63.5°C; R_f=0.44 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=16.92min; LRMS (EI): 342 (M^+, 12%), 216 (M^+-$C_8H_{14}O$, 12%), 127 (M^+-$C_8H_{11}N_2O_3S$, 12%), 108 (M^+-$C_{10}H_{20}NO_3S$, 100%), 57 (M^+-$C_{12}H_{17}N_2O_4S$, 21%); HRMS (ES): found 343.1692 $C_{16}H_{28}N_2O_4S$ requires 343.4676.

$\nu_{(max)}$ (Film) cm^{-1}: 3309.1 (NH), 1658.97 (C=O), 1656.3 (Ar C=C), 1364.5 (SO_2R_2); δ_H d_6 acetone: 9.31 (1H, bs, N<u>H</u>), 7.73 (2H, dd, J=9.1Hz, Ph<u>H</u>), 7.24 (2H, dd, J=9.1Hz, Ph<u>H</u>), 2.93 (6H, s, NC<u>H</u>$_3$), 2.36 (2H, t, J=7.4Hz, C<u>H</u>$_2$), 1.67 (2H, quin, J=7.3Hz, C<u>H</u>$_2$), 1.30 (8H, m, C<u>H</u>$_2$), 0.86 (3H, t, J=6.9Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 172.18 (<u>C</u>O), 146.35 (<u>C</u>, Ar), 139.13 (<u>C</u>, Ar), 123.04 (<u>C</u>H, Ar), 120.91 (<u>C</u>H, Ar), 38.88 (<u>C</u>H$_3$N), 37.65 (<u>C</u>H$_2$), 32.44 (<u>C</u>H$_2$), 29.89 (<u>C</u>H$_2$), 29.78 (<u>C</u>H$_2$), 26.19 (<u>C</u>H$_2$), 26.19 (<u>C</u>H$_2$), 14.32 (<u>C</u>H$_3$).

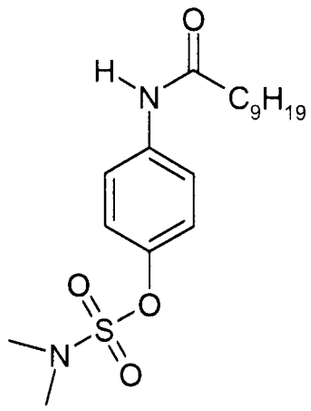
4-(Nonanoylamino)phenyl dimethylulfamate (350):



Compound **350** was synthesised via the same method as for compound **343** except compound **332** (0.74g, 3.00mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.5mL, 4.8mmol) and stirred for 10min before the addition of *N,N*-dimethyl amino sulfonyl chloride (0.30mL, 2.79mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **350** as an off-white crystalline solid (0.74g, 74.5% yield); m.p.=80.1-81.7°C; R_f=0.46 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=18.04min; LRMS (EI): 356 (M^+, 10%), 216 (M^+-$C_9H_{16}O$, 11%), 141 (M^+-$C_8H_{11}N_2O_3S$, 10%), 108 (M^+-$C_{11}H_{22}NO_3S$, 100%), 57 (M^+-$C_{13}H_{19}N_2O_4S$, 14%); HRMS (ES): found 371.2005 $C_{18}H_{32}N_2O_4S$ requires 371.5212.

$\nu_{(max)}$ (Film) cm^{-1}: 3345.4 (NH), 1663.5 (C=O), 1604.7 (Ar C=C), 1363.7 (SO_2R_2); δ_H d_6 acetone: 9.21 (1H, bs, NH), 7.70 (2H, dd, J=9.1Hz, PhH), 7.20 (2H, dd, J=9.1Hz, PhH), 2.91 (6H, s, NCH_3), 2.33 (2H, t, J=7.4, CH_2), 1.63 (2H, quin, J=7.3Hz, CH_2), 1.28 (10H, m, CH_2), 0.86 (3H, t, J=6.9Hz, CH_3); δ_C d_6 acetone: 171.42 (CO), 145.67 (C, Ar), 138.48 (C, Ar), 122.37 (CH, Ar), 120.20 (CH, Ar), 38.20 (CH_3N), 37.99 (CH_2), 31.88 (CH_2), 29.38 (CH_2), 29.27 (CH_2), 29.23 (CH_2), 25.50 (CH_2), 22.60 (CH_2), 13.66 (CH_3).

4-(Decanoylamino)phenyl dimethylulfamate (351):



Compound **351** was synthesised via the same method as for compound **343** except compound **333** (0.81g, 3.08mmol) was dissolved in anhydrous DCM (75mL) with TEA (0.5mL, 4.8mmol) and stirred for 10min before the addition of *N,N*-dimethyl amino sulfonyl chloride (0.30mL, 2.79mmol). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via column chromatography to give **351** as an off-white crystalline solid (0.68g, 66.2% yield); m.p.=78.0-79.8°C; R_f=0.48 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=20.61min; LRMS (EI): 370 (M^+, 12%), 216 (M^+-$C_{10}H_{18}O$, 14%), 155 (M^+-$C_8H_{11}N_2O_3S$, 8%), 108 (M^+-$C_{12}H_{25}NO_3S$, 100%), 71 (M^+-$C_{13}H_{19}N_2O_4S$, 21%).

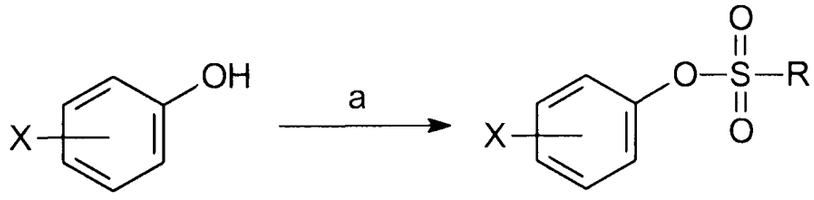
$\nu_{(max)}$ (Film) cm^{-1}: 3337.5 (NH), 1661.8 (C=O), 1604.9 (Ar C=C), 1361.3 (SO_2R_2); δ_H d_6 acetone: 9.23 (1H, bs, N<u>H</u>), 7.74 (2H, dd, J=8.9Hz, Ph<u>H</u>), 7.25 (2H, dd, J=8.9Hz, Ph<u>H</u>), 2.95 (6H, s, NC<u>H</u>$_3$), 2.36 (2H, t, J=7.3Hz, C<u>H</u>$_2$), 1.67 (2H, quint, J=7.3Hz, C<u>H</u>$_2$), 1.33 (12H, m, C<u>H</u>$_2$), (3H, t, J=6.9Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 173.19 (<u>C</u>O), 146.41 (<u>C</u>, Ar), 139.29 (<u>C</u>, Ar), 123.14 (<u>C</u>H, Ar), 120.92 (<u>C</u>H, Ar), 38.96 (<u>C</u>H$_3$N), 37.74 (<u>C</u>H$_2$), 32.65 (<u>C</u>H$_2$), 30.28 (<u>C</u>H$_2$), 30.21 (<u>C</u>H$_2$), 30.08 (<u>C</u>H$_2$), 30.02 (<u>C</u>H$_2$), 26.24 (<u>C</u>H$_2$), 23.37 (<u>C</u>H$_2$), 14.41 (<u>C</u>H$_3$).

Chapter 5: Synthesis of miscellaneous compounds

5.0 Synthesis of miscellaneous compounds

5.1 Discussion

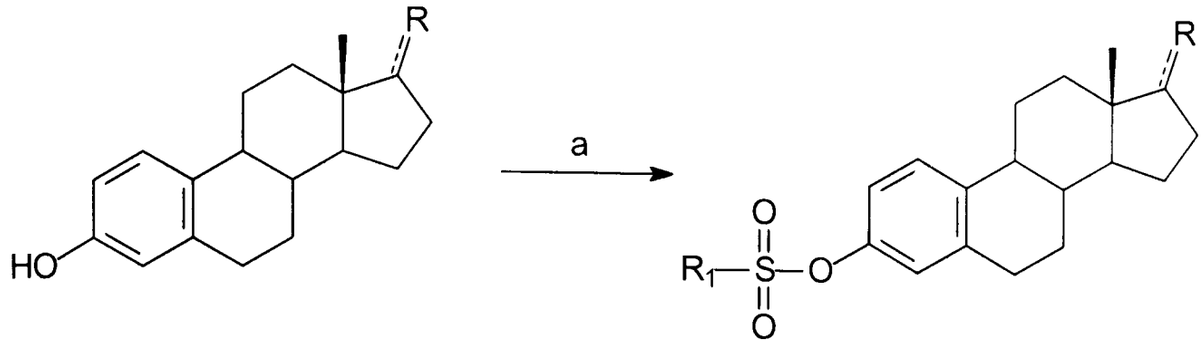
As previously discussed, the initial aim of this project involved the consideration of alternative sulfonate groups in an attempt to discover potential inhibitors of ES. As such, we considered a series of non-steroidal compounds based upon the phenolic backbone, that is, we considered the synthesis of sulfonate derivatives of substituted phenol (e.g. halogen, nitro, cyano containing compounds). The synthesis of these compounds followed the general procedures previously discussed in the synthesis of methanesulfonate-based compounds (e.g. Schemes 2.1 and 2.2), as such, in the synthesis of the methanesulfonate derivatives of phenol, we utilised the reaction outline in Scheme 5.1.



Scheme 5.1 The synthesis of methanesulfonate-based derivatives of substituted phenol (where a=sulfonyl chloride/DCM/Δ)

The reaction outlined above did not prove to be troublesome and gave the target compounds (**352** to **368**) in good to excellent yield (ranging from ~57% for compound **365** (methanesulfonic acid 3,4-dimethyl-phenyl ester) to ~95% for compound **353** (methanesulfonic acid 3-bromo-phenyl ester)].

In the search for alternative inhibitors of ES, we also considered the steroidal backbone in an attempt to discover novel compounds. As such, we considered the synthesis of a series of E1- and E2-based compounds using the reaction outlined below (Scheme 5.2). We also considered the synthesis of the standard compound EMATE which was utilised within the assay in an effort to compare the inhibitory activity of the synthesised compounds in comparison to a compound which has previously been considered as a potential drug substance.



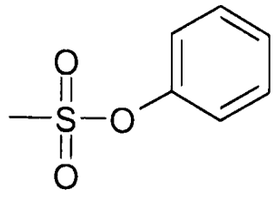
Scheme 5.1 The synthesis of methanesulfonate-based derivatives of substituted phenol (where a=sulfonyl chloride/DCM/Δ; R=O or OSO_2CH_3; R_1=CH_3, CF_3, NH_2)

The reactions proceeded without any major problems to give the target compounds in good to excellent yield [ranging from ~47% yield for **370** (trifluoro-methanesulfonic acid E1) to ~92% yield for **369** (methanesulfonic acid E1)]. We also synthesised a derivative of E2 where both hydroxy groups were derivatised so as to give the di-methanesulfonate derivative of E2 (namely compound **371** which was obtained in ~55% yield and without any major problems).

Finally, a number of coumarin-based compounds were also synthesised including COUMATE and 667-COUMATE, which were used as standard non-steroidal inhibitors (in particular COUMATE) of ES within our assays. The reaction used was similar to that outlined in Scheme 5.1 and gave the compounds in poor to excellent yield [ranging from ~35% yield for 667-COUMATE to ~78% yield for **372** (methanesulfonic acid 4-methyl-2-oxo-2H-chromen-7-yl ester)].

5.2 Synthesis of the methanesulfonic esters of substituted phenols

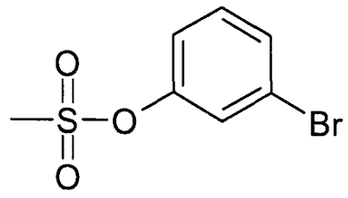
Methanesulfonic acid phenyl ester (352):



Phenol (0.96g, 10.21mmol) was dissolved in anhydrous DCM (75mL) with TEA (2.0mL, 14.34mmol) and stirred for 10min. Methanesulfonyl chloride (1.0mL, 12.86mmol) was added and the solution refluxed for 2h. After cooling, the reaction mixture was poured onto ice (100mL). The organic layer was washed with water (3 x 50mL), $NaHCO_3$ (2 x 50mL) and water (3 x 50mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give an off-white coloured solid which was purified via recyrstalisation from ether to give **352** as an off-white crystalline solid (1.23g, 70.1% yield); m.p.=59.6-60.3°C [lit. m.p.=61-62°C (Modro, 1976)]; R_f=0.31 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=6.27min; LRMS (EI): 172 (M^+, 23%), 109 (M^+-C_5H_3, 1%), 94 (M^+-CH_3O_2S, 100%), 79 (M^+-C_6H_5O, 5%), 65 (M^+-C_6H_8O, 37%); HRMS (ES): found 195.0085010 $C_7H_8O_3SNa$ requires 195.0086359.

$\nu_{(max)}$ (Film) cm^{-1}: 1587.9 (Ar C=C), 1356.2 (SO_2R_2); δ_H d_6 acetone: 7.49 (2H, m, PhH), 7.36 (3H, m, PhH), 3.27 (3H, s, SCH_3); δ_C d_6 acetone: 150.74 (C, Ar), 130.94 (CH, Ar), 128.18 (CH, Ar), 123.14 (CH, Ar), 14.41 (CH_3SO_3).

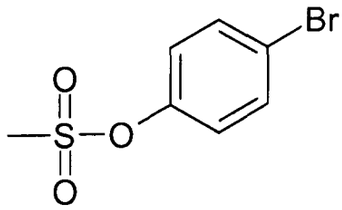
Methanesulfonic acid 3-bromo-phenyl ester (353):



Compound **353** was synthesised following the same procedure as for compound **352** except that 3-bromophenol (1.04g, 6.01mmol) was added to a solution of TEA (1.2mL, 8.6mmol) in anhydrous DCM (75mL), prior to the addition of methanesulfonyl chloride (1.0mL, 12.86mmol), furthermore, the reaction mixture was refluxed for 5h. Removal of the solvent gave a yellow coloured oil which was purified via column chromatography to give **353** as a yellow coloured oil (1.43g, 94.6% yield). R_f=0.53 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.24min; LRMS (EI): 252 (M^+, 1%) 174 (M^+-CH_3O_2S, 16%), 172 (M^+-CH_3O_2S, 18%), 145 (M^+-$C_2H_3O_3S$, 18%), 143 (M^+-$C_2H_3O_3S$, 18%), 92 (M^+-CH_3O_2SBr, 36%), 79 (M^+-$C_2H_4O_2SBr$, 66%), 63 (M^+-$C_2H_3O_3SBr$, 100%); Elemental analysis: found C 33.43%, H 2.81%; $C_7H_8SO_3Br$ requires C 33.48%, H 2.81%.

$\nu_{(max)}$ (Film) cm^{-1}: 1579.2 (Ar C=C), 1371.5 (SO_2R_2); δ_H d_6 acetone: 7.49 (2H, m, PhH), 7.37 (1H, m, PhH), 7.30 (1, m, PhH), 3.26 (3H, s, SCH_3); δ_C d_6 acetone: 150.22 (C, Ar), 131.62 (CBr, Ar), 130.49 (C, Ar), 125.56 (C, Ar), 122.19 (C, Ar), 121.53 (C, Ar), 37.00 (CH_3SO_3).

Methanesulfonic acid 4-bromo-phenyl ester (354):

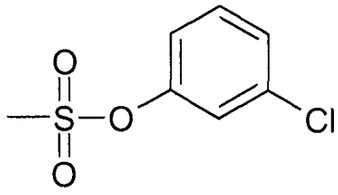


Compound **354** was synthesised following the same procedure as for compound **352** except that 4-bromophenol (1.20g, 6.94mmol) was added to a solution of TEA (2.0mL, 14.34mmol) in anhydrous DCM (75mL), prior to the addition of methane

sulfonyl chloride (0.8mL, 10.29mmol). Removal of the solvent gave a yellow coloured solid which was purified via column chromatography to give **354** as an off-white coloured solid (1.29g, 74.06% yield); m.p.=78.3-80.0°C [lit. m.p=81.9-83.2°C (Baum et al, 1995)]; R_f=0.27 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.34min; LRMS (EI): 252 (M^++2, 2%), 250 (M^+, 2%), 174 (M^+-CH_3O_2S, 47%), 145 (M^+-$C_2H_3O_3S$, 55%), 143 (M^+-$C_2H_3O_3S$, 18%), 117 (M^+-$C_4H_5O_3S$, 15%), 63 (M^+-$C_2H_3O_3SBr$, 100%); Elemental analysis: found C 33.52%, H 2.82%; $C_7H_8BrSO_3$ requires C 33.48%; H 2.81%.

$\nu_{(max)}$ (Film) cm^{-1}: 1367.9 (SO_2R_2); δ_H d_6 acetone: 7.58 (2H, dd, J=8.9Hz, PhH), 7.24 (2H, dd, J=8.9Hz, PhH), 3.26 (3H, s, SCH_3); δ_C d_6 acetone: 148.99 (C, Ar), 133.11 (CBr, Ar), 124.47 (C, Ar), 120.13 (C, Ar), 36.83 (CH_3SO_3).

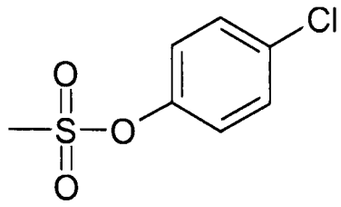
Methanesulfonic acid 3-chloro-phenyl ester (355):



Compound **355** was synthesised following the same procedure as for compound **352** except that 3-chlorophenol (0.99g, 7.70mmol) was added to a solution of TEA (1.2mL, 8.60mmol) in anhydrous DCM (75mL), prior to the addition of methane sulfonyl chloride (0.8mL, 10.29mmol), furthermore, the reaction mixture was refluxed for 4h. Removal of the solvent gave a brown oil which was purified via column chromatography to give **355** as a brown crystalline solid (1.13g, 70.89% yield); m.p.=31.4-32.9°C [lit. m.p=36-36.5°C (Carnahan et al, 1976)], R_f=0.43 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=6.89min; LRMS (EI): 208 (M^+, 9%), 206 (M^+, 24%), 128 (M^+-CH_4O_2S, 100%), 99 (M^+-$C_2H_2O_3S$, 29%).

$\nu_{(max)}$ (Film) cm^{-1}: 1468.1 (Ar C=C), 1365.4 (SO_2R_2); δ_H d_6 acetone: 7.42 (4H, m Ph-H), 3.34 (3H, s, SCH_3); δ_C d_6 acetone: 150.96 (C, Ar), 135.38 (CCl, Ar), 132.11 (C, Ar), 128.34 (CH, Ar), 123.51 (CH, Ar), 121.88 (CH, Ar), 37.83 (CH_3SO_3).

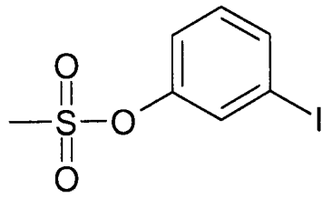
Methanesulfonic acid 4-chloro-phenyl ester (356):



Compound **356** was synthesised following the same procedure as for compound **352** except that 4-chlorophenol (1.01g, 7.86mmol) was added to a solution of TEA (1.2mL, 8.60mmol) in anhydrous DCM (75mL), prior to the addition of methane sulfonyl chloride (0.8mL, 10.29mmol), furthermore, the reaction mixture was refluxed for 4h. Removal of the solvent gave an off-white crystalline solid which was purified via column chromatography [DEE/pet spirit 40-60°C (50/50)] to give **356** as an off-white crystalline solid (1.03g, 63.0% yield); m.p.=65.1-66.4°C [lit. m.p=68-69°C (Carnahan et al, 1976)], R_f=0.45 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=7.71min; LRMS (EI): 208 (M^+, 14%), 206 (M^+, 38%), 128 (M^+-CH_4O_2S, 100%), 99 (M^+-$C_2H_2O_3S$, 49%).

$\nu_{(max)}$ (Film) cm^{-1}: 1369.7 (SO_2R_2); δ_H d_6 acetone: 7.52 (2H, dd, J=9.1Hz, PhH), 7.39 (2H, dd, J=9.1Hz, PhH), 3.32 (3H, s, SCH_3); δ_C d_6 acetone: 149.64 (C, Ar), 133.79 (CCl, Ar), 131.39 (C, Ar), 125.40 (C, Ar), 38.15 (CH_3SO_3).

Methanesulfonic acid 3-Iodo-phenyl ester (357):

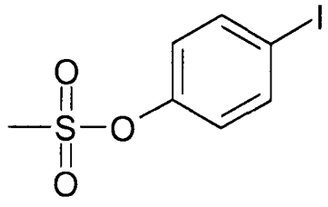


Compound **357** was synthesised following the same procedure as for compound **352** except that 3-Iodophenol (1.00g, 4.54mmol) was added to a solution of TEA (1.0mL, 7.17mmol) in anhydrous DCM (75mL), prior to the addition of methane sulfonyl chloride (0.8mL, 10.29mmol), furthermore, the reaction mixture was subsequently refluxed for 5.5h. Removal of the solvent gave a brown solid which

was purified via column chromatography to give **357** as a pale yellow crystalline solid (1.20g, 88.7% yield); m.p.=60.1-61.5°C; R_f=0.38 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.09min; LRMS (EI): 298 (M^+, 8%), 220 (M^+-CH_3O_2S, 38%), 191 (M^+-$C_2H_3O_2S$, 12%), 92 (M^+-CH_3O_2SI, 100%), 64 (M^+-C_7H_7OI, 64%); Elemental analysis: found C 28.20%, H 2.37%; $C_7H_8SO_3I$ requires C 28.20%; H 2.37%.

$\nu_{(max)}$ (Film) cm^{-1}: 1575.7 (Ar C=C), 1360.3 (SO_2R_2); δ_H d_6 acetone: 7.69 (2H, m, PhH), 7.29 (1H, m, PhH), 7.19 (1H, m, PhH), 3.23 (3H, s, SCH_3); δ_H d_6 acetone: 149.94 (C, Ar), 136.50 (C, Ar), 131.71 (CI), 131.27 (C, Ar), 122.04 (C, Ar), 93.39 (C, Ar), 37.00 (CH_3SO_3).

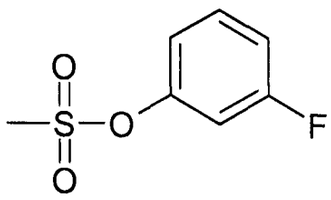
Methanesulfonic acid 4-Iodo-phenyl ester (358):



Compound **358** was synthesised following the same procedure as for compound **352** except that 4-Iodophenol (1.01g, 4.59mmol) was added to a solution of TEA (1.0mL, 7.17mmol) in anhydrous DCM (75mL), prior to the addition of methane sulfonyl chloride (0.50mL, 6.43mmol), furthermore the reaction mixture was subsequently refluxed for 4h. Removal of solvent gave a pale yellow crystalline solid which was purified via column chromatography to give **358** as a pale yellow crystalline solid (0.95g, 69.4% yield); R_f=0.41 [DEE/pet spirit 40-60°C (50/50)] m.p.=103.7-105.2°C; GC: t_R=9.23min; LRMS (EI): 298 (M^+, 4%), 219 (M^+-CH_3O_2S, 25%), 127 (M^+-$C_7H_8SO_3$, 5%), 92 (M^+-CH_3O_2SI, 85%), 64 (M^+-C_7H_7OI, 100%); Elemental analysis: found C 28.31%, H 2.37%; $C_7H_8SO_3I$ requires C 28.20%, H 2.37%.

$\nu_{(max)}$ (Film) cm^{-1}: 1357.8 (SO_2R_2); δ_H d_6 acetone: 7.76 (2H, dd, J=8.9Hz, PhH), 7.09 (2H, dd, J=8.9Hz, PhH), 3.22 (3H, s, SCH_3); δ_C d_6 acetone: 149.78 (C, Ar), 139.22 (CI), 124.64 (C, Ar), 91.28 (C, Ar), 36.86 (CH_3SO_3).

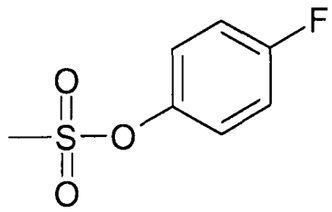
Methanesulfonic acid 3-fluoro-phenyl ester (359):



Compound **359** was synthesised following the same procedure as for compound **352** except that 3-flurophenol (1.09g, 9.73mmol) was added to a solution of TEA (2.0mL, 14.34mmol) in anhydrous DCM (60mL), prior to the addition of methane sulfonyl chloride (0.8mL, 10.29mmol), furthermore, the reaction mixture was refluxed for 7h. Removal of the solvent gave a yellow oil which was purified via column chromatography to give **359** as a yellow oil (1.41g, 76.3% yield); R_f=0.56 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=5.94min; LRMS (EI): 190 (M^+, 1%), 126 (M^+-C_5H_3, 1%), 112 (M^+-CH_3O_2S, 100%), 96 (M^+-CH_2O_3S, 10%), 83 (M^+-$C_2H_2O_3S$, 82%); Elemental analysis: found C 44.01%, H 3.72%; $C_7H_8SO_3F$ requires C 44.21%, H 3.71%.

$\nu_{(max)}$ (Film) cm^{-1}: 1605.4 (Ar C=C), 1357.7 (SO_2R_2); δ_H d_6 acetone: 7.44 (1H, m, PhH), 7.12 (3H, m, PhH), 3.24 (3H, s, SCH_3); δ_C d_6 acetone: 164.12 and 161.66 (CF, Ar), 150.51 and 150,40 (C, Ar), 131.34 and 131.25 (CH,Ar), 118.46 and 118.42 (CH, Ar), 114.39 and 114.17 (CH, Ar), 110.34 and 110.09 (CH, Ar), 36.17 (CH_3SO_3).

Methanesulfonic acid 4-fluoro-phenyl ester (360):

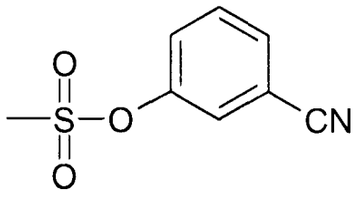


Compound **360** was synthesised following the same procedure as for compound **352** except that 4-flurophenol (0.98g, 8.75mmol) was added to a solution of TEA (2.0mL, 14.34mmol) in anhydrous DCM (50mL), prior to the addition of methane

sulfonyl chloride (1.0mL, 5.89mmol), furthermore, the reaction mixture was refluxed for 6h. Removal of the solvent gave a yellow oil which was purified via column chromatography to give **360** as a yellow oil (1.41g, 85.4% yield); R_f=0.58 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=6.06min; LRMS (EI): 190 (M^+, 1%), 126 (M^+-C_5H_3, 1%), 112 (M^+-CH_3O_2S, 67%), 95 (M^+-CH_2O_3S, 2%), 83 (M^+-$C_2H_2O_3S$, 100%); HRMS (ES): found 212.99850 $C_7H_8SO_3FNa$ requires 212.9992141.

$\nu_{(max)}$ (Film) cm^{-1}: 1599.5 (Ar C=C), 1368.7 (SO_2R_2); δ_H d_6 acetone: 7.32 (2H, m, PhH), 7.17 (2H, m, PhH), 3.20 (3H, s, SCH_3); δ_C d_6 acetone: 159.95 (C, Ar), 145.79 (CF, Ar), 124.34 (CH, Ar), 124.26 (CH, Ar), 116.74 (CH, Ar), 116.50 (CH, Ar), 36.63 (CH_3SO_3).

Methanesulfonic acid 3-cyano-phenyl ester (361):

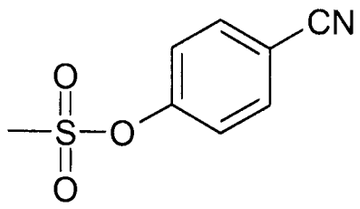


Compound **361** was synthesised following the same procedure as for compound **352** except that 3-cyanophenol (1.05g, 8.82mmol) was stirred in anhydrous DCM (50mL) with TEA (2.0mL, 14.34mmol) 15min prior to the addition of methanesulfonyl chloride (0.8mL, 11.65mmol). The reaction mixture was subsequently refluxed for 5h. The solvent was removed to yield a brown solid which was purified via column chromatography to give a yellow oil (1.21g, 71.4% yield); m.p.=56.9-58.6°C; R_f=0.28 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.43min; LRMS (EI): 197 (M^+, 8%), 119 (M^+-CH_3SO_2, 100%), 90 (M^+-$C_2H_3SNO_2$, 26%), 79 (M^+-C_7H_4N, 55%); Elemental analysis: found C 48.91%, H 3.59%, N 7.14%; $C_7H_8SNO_3$ requires C 48.72%; H 3.58%, N 7.10%.

$\nu_{(max)}$ (Film) cm^{-1}: 2235.6 (C≡N), 1578.4 (Ar C=C), 1357.9 (SO_2R_2); δ_H d_6 acetone: 7.73 (2H, m, PhH), 7.63 (2H, m, PhH), 3.31 (3H, s, SCH_3); δ_C d_6 acetone: 149.78

(C, Ar), 131.52 (C≡N), 131.18 (CH, Ar), 127.57 (CH, Ar), 126.01 (CH, Ar), 117.29 (CH, Ar), 113.87 (CH, Ar), 37.16 (CH_3SO_3).

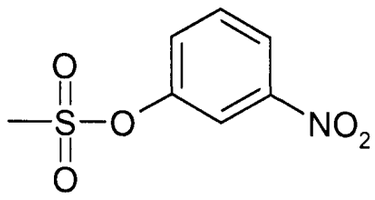
Methanesulfonic acid 4-cyano-phenyl ester (362):



Compound **362** was synthesised following the same procedure as for compound **352** except that 4-cyanophenol (1.00g, 8.40mmol) was stirred in anhydrous DCM (50mL) with TEA (1.2mL, 8.60mmol) 15min prior to the addition of methanesulfonyl chloride (0.8mL, 11.65mmol). The reaction mixture was subsequently refluxed for 5h. The solvent was removed to yield a brown solid which was purified via column chromatography to give **362** as an off-white crystalline solid (1.17g, 70.7% yield); m.p.=85.6-87.2°C [lit. m.p=89-90°C (Percec et al, 1995)], R_f=0.28 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=8.43min; LRMS (EI): 197 (M^+, 2%), 119 (M^+-CH_3SO_2, 100%), 90 (M^+-$C_2H_3SNO_2$, 35%), 79 (M^+-C_7H_4N, 57%); Elemental analysis: found C 48.81%, H 3.58%, N 7.10%; $C_8H_7SNO_3$ requires C 48.72%, H 3.58%, N 7.10%.

$\nu_{(max)}$ (Film) cm^{-1}: 2232.3 (C≡N), 1599.7 (Ar C=C), 1361.8 (SO_2R_2); δ_H d_6 acetone: 7.73 (2H, dd, J=9.1Hz, PhH), 7.63 (2H, dd, J=9.1Hz, PhH), 3.31 (3H, s, SCH_3); δ_C d_6 acetone: 149.78 (C, Ar), 131.52 (C≡N), 131.18 (CH, Ar), 127.57 (CH, Ar), 126.01 (CH, Ar), 117.29 (CH, Ar), 113.87 (CH, Ar), 37.16 (CH_3SO_3).

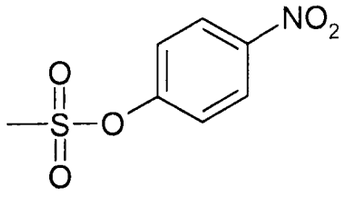
Methanesulfonic acid 3-nitro-phenyl ester (363):



Compound **363** was synthesised following the same procedure as for compound **352** except that 3-nitrophenol (1.02g, 7.30mmol) was stirred in anhydrous DCM (50mL) with TEA (1.2mL, 8.60mmol) 15min prior to the addition of methanesulfonyl chloride (0.8mL, 11.65mmol). The reaction mixture was subsequently refluxed for 5.5h. A pale yellow crystalline solid was produced which was purified via column chromatography to give **363** as a pale yellow crystalline solid (1.35g, 85.2% yield); m.p.=65.3-66.6°C; R_f=0.33 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.09min; LRMS (EI): 217 (M^+, 1%), 139 (M^+-CH_3SO_2, 21%), 92 (M^+-CH_3NSO_4, 13%), 79 (M^+-$C_6H_4NO_2$, 100%).

$\nu_{(max)}$ (Film) cm^{-1}: 1528.7 (NO_2), 1362.7 (NO_2); δ_H d_6 acetone: 8.19 (1H, m, PhH), 8.13 (1H, m, PhH), 7.74 (2H, m, PhH), 3.34 (3H, s, $SC\underline{H}_3$) ; δ_C d_6 acetone: 159.74 ($\underline{C}$, Ar), 149.77 ($\underline{C}$H, Ar), 131.32 ($\underline{C}NO_2$, Ar), 128.98 ($\underline{C}$H, Ar), 122.16 ($\underline{C}$H, Ar), 117.70 ($\underline{C}$H, Ar), 37.16 ($\underline{C}H_3SO_3$).

Methanesulfonic acid 4-nitro-phenyl ester (364):

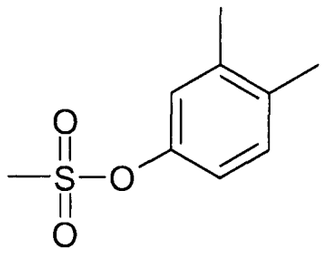


Compound **364** was synthesised following the same procedure as for compound **352** except that 3-nitrophenol (0.99g, 7.13mmol) was stirred in anhydrous DCM (50mL) with TEA (1.2mL, 8.60mmol) 15min prior to the addition of methanesulfonyl chloride (0.8mL, 11.65mmol). The reaction mixture was subsequently refluxed for 5.5h. A pale yellow crystalline solid was produced which was purified via column chromatography to give **364** as a pale yellow crystalline

solid (0.97g, 58.2% yield); m.p.=93.2-95.9°C; R_f=0.34 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=9.31min; LRMS (EI): 217 (M^+, 1%), 139 (M^+-CH_3SO_2, 44%), 92 (M^+-CH_3NSO_4, 35%), 79 (M^+-$C_6H_4NO_2$, 100%); Elemental analysis: found C 38.75%, H 3.25%, N 6.35%: $C_6H_7SNO_5$ requires C 38.71%, H 3.25%, N 6.45%.

$\nu_{(max)}$ (Film) cm^{-1}: 1521.3 (NO_2), 1360.5 (SO_2R_2), 1346.9 (NO_2); δ_H d_6 acetone: 8.28 (2H, d, J=9.4Hz, PhH), 7.56 (2H, d, J=9.4Hz, PhH), 3.34 (3H, s, $SC\underline{H}_3$) ; δ_C d_6 acetone: 154.02 ($\underline{C}$, Ar), 146.53 ($\underline{C}NO_2$, Ar), 125.75 ($\underline{C}H$, Ar), 123.36 ($\underline{C}H$, Ar), 37.44 ($\underline{C}H_3SO_3$).

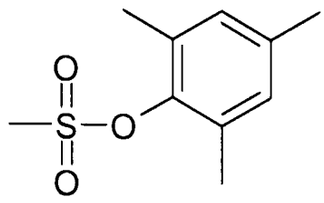
Methanesulfonic acid 3,4-dimethyl-phenyl ester (365):



Compound **365** was synthesised following the same procedure as for compound **352** except that 3,4-dimethylphenol (1.41g, 11.55mmol) was stirred in anhydrous DCM (50mL) with TEA (4mL, 28.66mmol) 15min prior to the addition of methanesulfonyl chloride (2.0mL, 32.03mmol). Removal of the solvent gave a light brown which was purified via column chromatography to give **365** as a light brown crystalline solid (1.32g, 57.1% yield); m.p.=51.8-52.9°C; R_f=0.44 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=10.94min: LRMS (EI): 200 (M^+, 46%), 125 (M^+-CH_3SO_2, 100%), 92 (M^+-$C_3H_8SO_2$, 23%); HRMS (ES): found 223.039936; $C_9H_{12}SO_3Na$ requires 223.24177.

$\nu_{(max)}$ (Film) cm^{-1}: 1604.8 (Ar C=C), 1356.5 (SO_2R_2); δ_H d_6 acetone: 7.03 (3H, m, Ph$\underline{H}$), 3.13 (3H, s, $SC\underline{H}_3$), 2.18 (3H, s, $C\underline{H}_3$), 2.16 (3H, s, $C\underline{H}_3$); δ_C d_6 acetone: 147.90 ($\underline{C}$, Ar), 138.63 ($\underline{C}CH_3$, Ar), 135.76 ($\underline{C}CH_3$, Ar), 122.98 ($\underline{C}$, Ar), 119.28 ($\underline{C}$, Ar), 36.59 ($\underline{C}H_3SO_3$), 18.97 ($\underline{C}H_3$), 18.35 ($\underline{C}H_3$).

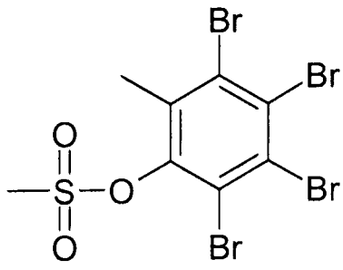
Methanesulfonic acid 2,4,6-trimethyl-phenyl ester (366):



Compound **366** was synthesised following the same procedure as for compound **352** except that 2,4,6-trimethylphenol (0.98g, 7.20mmol) was stirred in anhydrous DCM (50mL) with TEA (2.0mL, 14.33mmol) 15min prior to the addition of methanesulfonyl chloride (1.2mL, 19.21mmol). Removal of the solvent gave a light brown solid was produced which was purified via column chromatography to give a light brown coloured crystalline solid (1.14g, 74.1% yield); m.p.=60.3-61.2°C; R_f=0.53 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=11.08min; LRMS (EI): 214 (M^+, 18%), 135 (M^+-CH_3SO_2, 100%), 92 (M^+-$C_4H_{11}SO_2$, 20%); Elemental analysis: found C 56.05%, H, 6.59%: $C_{10}H_{14}SO_3$ requires C 56.01%, H 6.65%.

$\nu_{(max)}$ (Film) cm^{-1}: 2936.5 (CH_3), 1603.4 (Ar C=C), 1352.1 (SO_2R_2); δ_H d_6 acetone: 6.83 (2H, s, PhH), 3.32 (3H, s, SCH_3), 2.22 (3H, s, CH_3), 2.15 (3H, s, CH_3); δ_C d_6 acetone: 145.05 (C, Ar), 136.31 (CCH_3, Ar), 131.67 (CCH_3, Ar), 129.76 (CH, Ar), 38.60 (CH_3, Ar), 36.59 (CH_3SO_3), 19.83 (CH_3), 16.73 (CH_3).

Methanesulfonic acid 2,3,4,5 tetabromo6 methyl-phenyl ester (367):

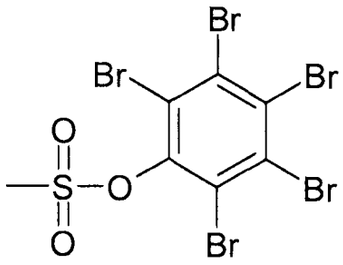


Compound **367** was synthesised following the same procedure as for compound **352** except that 2,3,4,5-tetrabromo-6-methylphenol (1.04g, 2.45mmol) was stirred in anhydrous DCM (50mL) with TEA (1.0mL, 7.16mmol) 15min prior to the addition of methanesulfonyl chloride (1.0mL, 16.01mmol). The reaction mixture was

subsequently refluxed for 24h. An off-white solid was produced which was recrystallised in hexane to give an off-white solid crystalline solid (0.99g, 80.5% yield); m.p.=135.1-136.6°C; R_f=0.52 [DEE/pet spirit 40-60°C (50/50)]; GC: t_R=18.44min: LRMS (EI): 502 (M^+, 30%), 423 (M^+-CH_3SO_2, 100%), 343 (M^+-CH_3SO_2Br, 11%), 263 (M^+-$CH_3SO_2Br_2$, 7%).

$\nu_{(max)}$ (Film) cm^{-1}: 1345.6 (SO_2R_2); δ_H d_6 acetone: 3.32 (3H, s, $SC\underline{H}_3$), 2.51 (3H, s, $C\underline{H}_3$); δ_C d_6 acetone: 145.76 (<u>C</u>, Ar), 136.28 (<u>C</u>Br, Ar), 128.25 (<u>C</u>Br, Ar), 127.51 (<u>C</u>Br, Ar), 126.57 (<u>C</u>Br, Ar), 121.58 (<u>C</u>, Ar), 40.40 ($\underline{C}H_3SO_3$), 21.36 ($\underline{C}H_3$).

Methanesulfonic acid 2,3,4,5,6 pentabromo-phenyl ester (368):

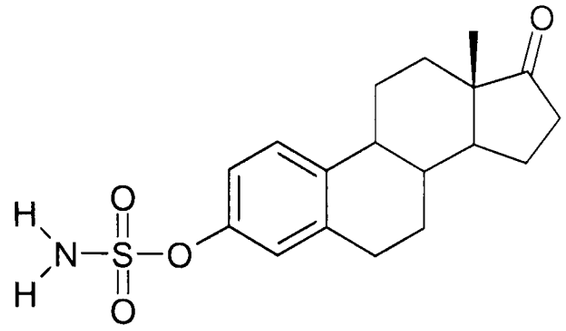


Compound **368** was synthesised following the same procedure as for compound **352** except that 2,3,4,5,6-pentabromophenol (1.29g, 2.64mmol) was stirred in anhydrous DCM (50mL) with TEA (1.0mL, 7.16mmol) 15min prior to the addition of methanesulfonyl chloride (1.0mL, 16.01mmol). The reaction mixture was subsequently refluxed for 24h. An off-white solid was produced which was purified by column chromagraphy to give **368** as an off-white solid crystalline solid (0.93g, 62.2% yield); m.p.=174.3-176.5°C; R_f=0.41 [DEE/pet spirit 40-60°C (50/50)]; t_R=20.99min: 488 (M^+-Br, 100%), 299 (M^+-CH_3SO_2, 100%), 220 (M^+-CH_3SO_2Br, 11%), 141 (M^+-$CH_3SO_2Br_2$, 7%)

$\nu_{(max)}$ (Film) cm^{-1}: 1363.9 (SO_2R_2); δ_H d_6 acetone: 3.32 (3H, s, $SC\underline{H}_3$); δ_C d_6 acetone: 146.38 (<u>C</u>, Ar), 128.93 (<u>C</u>Br, Ar), 127.79 (<u>C</u>Br, Ar), 122.43 (<u>C</u>Br, Ar), 41.80 ($\underline{C}H_3SO_3$).

5.3 Synthesis of miscellaneous compounds

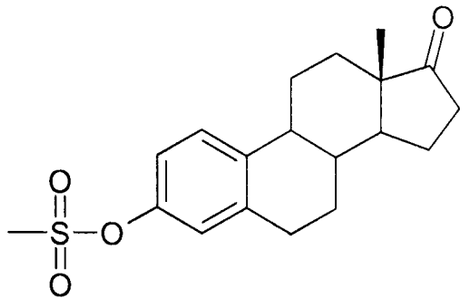
EMATE:



E1 (1.04g, 3.85mmol) was dissolved in anhydrous dimethylacetamide (DMA) (10mL) and left to stir for 30min, prior to the addition of **267**. The mixture was subsequently stirred for 10h, before being quenched with brine and extracted into DEE (3 x 30mL). The organic layer was washed with water (3 x 25mL) and dried over anhydrous $MgSO_4$, the solvent was removed under vacuum to give an off white solid which was purified by column chromatography to give **EMATE** as an off-white solid (0.97g, 72.2% yield); m.p.=194.3-195.6°C [lit. m.p=195-197°C (Woo et al, 1998)]; R_f=0.66 (DEE); LRMS (EI): 349 (M^+, 1%), 270 (M^+-NH_2O_2S, 100%), 185 (M^+-$C_5H_9NO_3S$, 27%), 159 (M^+-$C_7H_{12}NO_3S$, 16%), 79 (M^+-$C_{18}H_{21}O_2$, 4%).

$\nu_{(max)}$ (Film) cm^{-1}: 3283.1 (NH), 1727.1 (C=O), 1379.1 (SO_2R_2); δ_H d_6 acetone: 7.35 (1H, d, J=8.4Hz, PhH), 7.05 (4H, m, NH_2, Ph-H), 2.90 (2H, m, C<u>H</u>$_2$, steroid), 2.42 (3H, m, C<u>H</u>, steroid), 2.09 (3H, m, C<u>H</u>, steroid), 1.86 (1H, m, C<u>H</u>, steroid), 1.56 (6H, m, C<u>H</u>, steroid), 0.91 (3H, m, C<u>H</u>$_3$, steroid); δ_C d_6 acetone: 219.17 (<u>C</u>O), 149.24 (<u>C</u>O, Ar), 139.04 (<u>C</u>, Ar), 138.97 (<u>C</u>, Ar), 127.23 (<u>C</u>H, Ar), 122.82 (<u>C</u>H, Ar), 120.03 (<u>C</u>H, Ar), 50.86 (<u>C</u>H), 48.15 (<u>C</u>), 44.81 (<u>C</u>H), 38.69 (<u>C</u>H), 35.85 (<u>C</u>H$_2$), 32.35 (<u>C</u>H$_2$), 29.84 (<u>C</u>H$_2$), 26.80 (<u>C</u>H$_2$), 26.33 (<u>C</u>H$_2$), 21.92 (<u>C</u>H$_2$), 13.88 (<u>C</u>H$_3$).

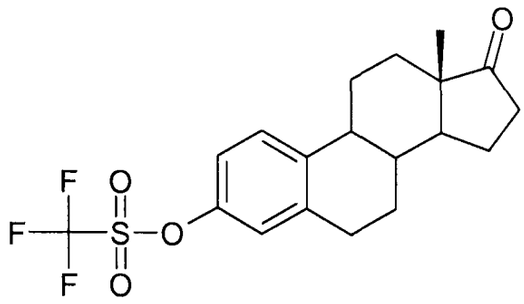
Methanesulfonic acid E1 (369):



Compound **368** was synthesised following the same procedure as for compound **334** except E1 (1.13g, 4.18mmol) was dissolved in anhydrous DCM (75mL) with TEA (1.0mL, 7.16mmol), and stirred for 30min. Methanesulfonyl chloride (1.0mL, 16.01mmol) was added and refluxed for 16h. A pale brown solid was produced, which was purified via flash chromatography to give **368** as a pale brown solid (1.34g, 92.0% yield); m.p.=153.8-155.2°C [lit. m.p=155-157°C (Schwarz et al, 1975)]; R_f=0.52 (DEE); GC: t_R=23.26min; LRMS (EI): 348 (M^+, 100%), 291 (M^+-C_3H_5O, 24%), 251 (M^+-C_6H_9O, 19%), 213 (M^+-C_6H_9O, 25%), 185 (M^+-C_6H_9O, 7%), 133 (M^+-$C_{13}H_{15}O$ 13%), 97 (M^+-$C_{13}H_{15}O_3S$ 21%); Elemental analysis: found C 65.50%, H 6.95%; $C_{19}H_{24}O_4S$ requires C 65.49%, H 6.94%.

$\nu_{(max)}$ (Film) cm^{-1}: 2945.8 (CH), 1727.7 (C=O), 1565.7 (Ar C=C), 1362.5 (SO_2R_2); δ_H $CDCl_3$: 7.32 (1H, d, J=8.6Hz, PhH), 6.99 (2H, m, PhH), 3.11 (3H, s, $OC\underline{H}_3$), 2.92 (2H, m, $C\underline{H}_2$, steroid), 2.16 (6H, m, C<u>H</u>, steroid), 1.51 (7H, m, C<u>H</u>, steroid), 0.89 (3H, m, $C\underline{H}_3$, steroid); δ_C $CDCl_3$: 220.63 (<u>C</u>O), 147.78 (<u>C</u>O, Ar), 140.47 (<u>C</u>, Ar), 139.49 (<u>C</u>, Ar), 127.40 (<u>C</u>H, Ar), 121.44 (<u>C</u>H, Ar), 118.50 (<u>C</u>H, Ar), 50.56 (<u>C</u>H), 48.05 (<u>C</u>), 44.29 (<u>C</u>H), 37.94 ($\underline{C}H_3SO_3$), 36.00 ($\underline{C}H_2$), 31.67 ($\underline{C}H_2$), 29.58 ($\underline{C}H_2$), 26.28 ($\underline{C}H_2$), 25.88 ($\underline{C}H_2$), 25.90 ($\underline{C}H_2$), 21.76 ($\underline{C}H_2$), 13.99 ($\underline{C}H_3$).

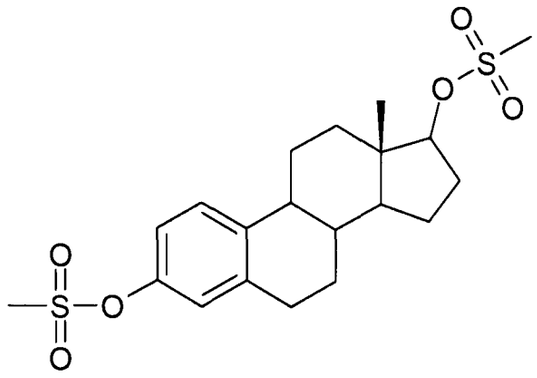
Trifluoro-methanesulfonic acid E1 (370):



Compound **368** was synthesised following the same procedure as for compound **311**, except that E1 (1.24g, 4.59mmol) was dissolved in anhydrous DCM (75mL) with TEA (1.0mL, 7.16mmol), and stirred for 30min. Trifluoromethanesulfonyl chloride (0.7mL, 6.56mmol) was added and refluxed for 16h. An off-white solid was produced, which was purified via column chromatography to give **369** as an off white crystalline solid (0.86g, 46.8% yield); m.p.=83.4-85.0°C [lit. m.p=99-101°C (Horwitz et al, 1986)]; R_f=0.14 (DEE); GC: t_R=18.65min; LRMS (EI): 402 (M^+, 100%), 292 (M^+-$C_7H_{10}O$, 26%), 269 (M^+-CF_3SO_2, 8%), 213 (M^+-$C_4H_3F_3SO_3$, 72%); Elemental analysis: found C 56.98%, H 5.28%; $C_{19}H_{21}F_3O_4S$ requires C 56.71%, H 5.26%.

$\nu_{(max)}$ (Film) cm^{-1}: 1736.3 (C=O), 1608.1 (Ar C=C); δ_H $CDCl_3$: 7.28 (1H, d, J=8.4Hz, PhH), 7.01 (2H, m, PhH), 2.91 (2H, m, C<u>H</u>$_2$, steroid), 2.27 (6H, m, C<u>H</u>, steroid), 1.48 (7H, m, C<u>H</u>, steroid), 0.89 (3H, s, C<u>H</u>$_3$, steroid); δ_C $CDCl_3$: 220.80 (<u>C</u>O), 147.35 (<u>C</u>O, Ar), 139.39 (<u>C</u>, Ar), 139.06 (<u>C</u>, Ar), 127.15 (<u>C</u>H, Ar), 122.22 (<u>C</u>H, Ar), 119.22 (<u>C</u>H, Ar), 50.59 (<u>C</u>H), 48.10 (<u>C</u>), 44.33 (<u>C</u>H), 38.08 (<u>C</u>H), 37.54 (<u>C</u>F_3SO_3), 36.04 (<u>C</u>H_2), 31.71 (<u>C</u>H_2), 29.61 (<u>C</u>H_2), 26.39 (<u>C</u>H_2), 25.90 (<u>C</u>H_2), 21.78 (<u>C</u>H_2), 14.01 (<u>C</u>H_3).

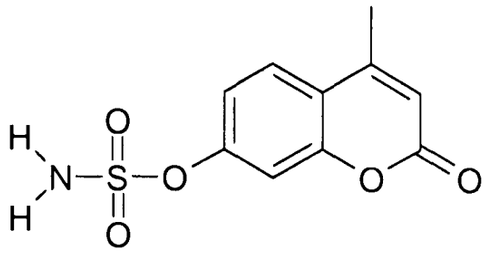
Di-methanesulfonic acid E2 (371):



Compound **370** was synthesised following the same procedure as for compound **334** except E2 (0.96g, 3.52mmol) was dissolved in anhydrous DCM (75mL) with TEA (1.0mL, 7.16mmol), and stirred for 30min. Methanesulfonyl chloride (1.0mL, 16.01mmol) was added and refluxed for 16h. A pale brown solid was produced, which was purified via column chromatography to give **370** as a pale brown solid (0.83g, 55.0% yield); m.p.=174.9-176.2°C; R_f=0.10 (DEE); LRMS (EI): 428 (M^+, 67%), 350 (M^+-CH_3O_2S, 4%), 237 (M^+-$C_8H_{15}O_3S$, 39%), 237 (M^+-$C_{10}H_{17}O_3S$, 23%), 79 (M^+-$C_{19}H_{25}O_4S$, 100%); Elemental analysis: found C 56.07%, H 6.55%; $C_{20}H_{28}O_6S$ requires C 56.05%, H 6.59%.

$\nu_{(max)}$ (Film) cm^{-1}: 2931.8 (CH), 1490.7 (Ar C=C), 1348.4 (SO_2R_2); δ_H $CDCl_3$: 7.27 (1H, m, PhH), 6.98 (2H, m, PhH), 3.10 (3H, s, $SC\underline{H}_3$), 2.99 (3H, s, $SC\underline{H}_3$), 2.86 (2H, m, $C\underline{H}_2$, steroid), 2.26 (3H, m, C$\underline{H}$, steroid), 2.04 (1H, m, C$\underline{H}$, steroid), 1.83 (2H, m, C$\underline{H}$, steroid), 1.37 (4H, m, C$\underline{H}$, steroid), 0.85 (3H, m, $C\underline{H}_3$, steroid); δ_C $CDCl_3$: 147.23 ($\underline{C}$O, Ar), 127.03 ($\underline{C}$H, Ar), 122.04 ($\underline{C}$H, Ar), 119.03 ($\underline{C}$H, Ar), 89.29 ($\underline{C}$O), 49.20 ($\underline{C}$H), 43.94 ($\underline{C}$H), 43.34 ($\underline{C}$H), 38.16 ($\underline{C}H_3SO_3$), 37.39 ($\underline{C}H_3SO_3$), 36.37 ($\underline{C}H_2$), 31.00 ($\underline{C}H_2$), 29.49 ($\underline{C}$H), 28.01 ($\underline{C}$), 26.80 ($\underline{C}H_2$), 25.90 ($\underline{C}H_2$), 23.13 ($\underline{C}H_2$), 11.80 ($\underline{C}H_3$).

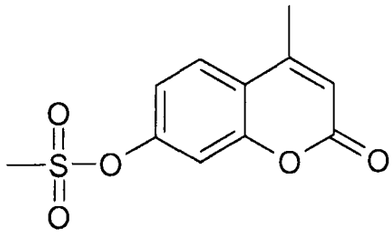
COUMATE



COUMATE was synthesised following the same procedure as for **EMATE**, except that 4-methylumbelliferone (1.01g, 5.73mmol) was dissolved in anhydrous DMA (10mL) and left to stir for 30min, prior to the addition of **267**. Removal of the solvent gave an off white solid which was purified by column chromatography to give **COUMATE** as an off-white solid (0.97g, 72.2% yield); m.p.=163.8-165.2°C [lit. m.p=165-167°C (Woo et al, 1998)]; R_f=0.41 (DEE).

$\nu_{(max)}$ (Film) cm^{-1}: 3315.7 (NH), 1694.1 (C=O), 1608.5 (Ar C=C), 1529.8 (NH), 1378.8 (SO_2R_2); δ_H d_6 acetone: 7.86 (1H, d, J=8.8, PhH), 7.39 (2H, bs, NH_2), 7.29 (2H, m, PhH), 6.33 (1H, d, J=1.3Hz Ph-H), 2.50 (3H, d, J=1.3Hz, $C\underline{H}_3$); δ_C d_6 acetone: 160.23 (O$\underline{C}$O, Ar), 155.09 ($\underline{C}$O, Ar), 153.64 ($\underline{C}$, Ar), 153.29 ($\underline{C}$, Ar), 127.41 ($\underline{C}$H, Ar), 119.37 ($\underline{C}$, Ar), 119.22 ($\underline{C}$H, Ar), 115.29 ($\underline{C}$H, Ar), 111.11 ($\underline{C}$H, Ar), 18.65 ($\underline{C}H_3$).

Methanesulfonic acid 4-methyl-2-oxo-2H-chromen-7-yl ester (372):

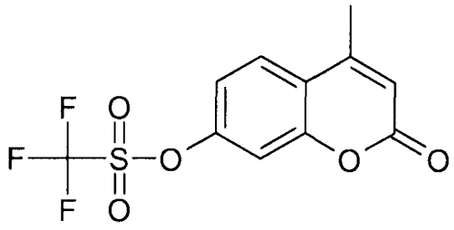


Compound **371** was synthesised following the same procedure as for compound **334** except 4-methylumbelliferone (1.14g, 6.46mmol) was dissolved in anhydrous DCM (75mL) with TEA (1.0mL, 7.16mmol), and stirred for 30min. Methanesulfonyl chloride (1.0mL, 16.01mmol) was added and refluxed for 24h. Removal of the solvent gave an off-white coloured solid which was purified via column

chromatography to give **371** as (1.27g, 77.6% yield); m.p.=148.7-149.8°C [lit. m.p=165°C (Dragota, 1989)]; R_f=0.31 [DEE/pet spirit 40-60°C (70/30)]; GC: t_R=11.84min; LRMS (EI): 254 (M^+, 69%), 176 (M^+-CH_2O_2S, 100%), 147 (M^+-$C_2H_3O_3S$, 93%), 91 (M^+-$C_5H_7O_4S$, 52%).

$\nu_{(max)}$ (Film) cm^{-1}: 1712.1 (C=O), 1609.4 (Ar C=C), 1363.6 (SO_2R_2); δ_H d_6 acetone: 7.80 (1H, m, PhH), 7.26 (2H, m, PhH), 6.26 (1H, d, J=1.3Hz Ph-H), 3.31 (3H, s, SC<u>H</u>$_3$), 3.26 (3H, d, J=1.3Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 159.22 (O<u>C</u>O, Ar), 154.34 (<u>C</u>O, Ar), 152.29 (<u>C</u>, Ar), 151.59 (<u>C</u>, Ar), 126.84 (<u>C</u>H, Ar), 119.11 (<u>C</u>, Ar), 118.35 (<u>C</u>H, Ar), 114.79 (<u>C</u>H, Ar), 110.52 (<u>C</u>H, Ar), 37.21 (<u>C</u>H_3SO_3), 17.79 (<u>C</u>H_3).

Trifluoro-methanesulfonic acid 4-methyl-2-oxo-2H-chromen-7-yl ester (373):

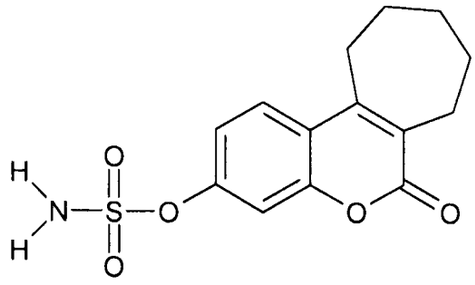


Compound **372** was synthesised following the same procedure as for compound **311**, except that 4-methylumbelliferone (1.08g, 6.12mmol) was dissolved in anhydrous DCM (75mL) with TEA (1.0mL, 7.16mmol), and stirred for 30min. Trifluoromethanesulfonyl chloride (0.7mL, 6.56mmol) was added and refluxed for 18h. An off-white solid was produced, which was purified via column chromatography to give **372** as an off white crystalline solid (0.71g, 37.9% yield); m.p.=88.1-89.9°C [lit. m.p=83-84°C (Kover and Antus, 2005)]; R_f=0.31 [DEE/pet spirit 40-60°C (70/30)], t_R=9.19min: 308 (M^+, 100%), 175 (M^+-CF_3O_2S, 55%), 147 (M^+-$C_3H_4F_3O_2S$, 55%), 119 (M^+-$C_3F_3O_4S$, 16%), 91 (M^+-$C_5H_4F_3O_4S$, 18%); Elemental analysis: found C 42.65%, H 2.28%; $C_{11}H_7SF_3O_5$ requires C 42.86%, H 2.29%.

$\nu_{(max)}$ (Film) cm^{-1}: 1742.8 (C=O), 1608.1 (Ar C=C), 1367.9 (SO_2R_2); δ_H d_6 acetone: 8.00 (1H, m, PhH), 7.49 (2H, m, PhH), 6.43 (1H, d, J=1.3Hz Ph-H), 2.53 (3H, d, J=1.3Hz, C<u>H</u>$_3$); δ_C d_6 acetone: 155.52 (O<u>C</u>O, Ar), 153.68 (<u>C</u>O, Ar), 151.59 (<u>C</u>, Ar),

128.38 (C, Ar), 126.84 (CH, Ar), 118.37 (CH, Ar), 116.52 (CH, Ar), 111.13 (CH, Ar), 46.46 (CF_3SO_3), 18.66 (CH_3).

667-COUMATE:



667-COUMATE was synthesised following the same procedure as for **EMATE**, except that 3-hydroxy-6-oxo-8, 9, 10, 11-tetrahydro-7H-cyclohepta-[c][1]benzopyran (1.01g, 4.41mmol) was dissolved in anhydrous DMA (10mL) and left to stir for 30min, prior to the addition of **267**. Removal of the solvent gave an off white solid which was purified by column chromatography to give **667-COUMATE** as an off-white solid (0.48g, 34.8% yield); m.p.=173.1-174.8°C [lit. m.p=169-171°C (Woo et al, 2001)]; R_f =0.54 [DEE/chloroform (50/50)]; HRMS (ES): found 310.0749; $C_{14}H_{15}NO_5S$ requires 310.0744.

$\nu_{(max)}$ (Film) cm^{-1}: 3273.9 (NH), 1687.7 (C=O), 1380.7 (SO_2R_2); δ_H d_6 acetone: 7.96 (1H, m, PhH), 7.43 (2H, s, N-H_2), 7.37 (2H, m, PhH), 3.08 (2H, m, CH_2), 2.93 (2H, m, CH_2), 1.98 (2H, m, CH_2), 1.75 (2H, m, CH_2), 1.63 (2H, m, CH_2); δ_C d_6 acetone: 161.35 (CO), 153.70 (CO, Ar), 152.51 (C, Ar), 128.94 (C, Ar), 126.42 (CH, Ar), 118.86 (CH, Ar), 110.81 (C, Ar), 32.38 (CH_2), 28.29 (CH_2), 27.07 (CH_2), 26.03 (CH_2), 25.47 (CH_2).

Chapter 6: Determination of pK_a

6.0 Determination of pK_a

6.1 Introduction

pK_a refers to the negative logarithm of the acid dissociation constant (K). Through the change in UV absorption by the phenolic group under acidic, pH 9, and basic conditions, the pK_a can be determined using a photometric technique (Harwood and Moody, 1996). In particular, the formation of the phenoxide ion (Figure 6.1) alters the UV absorbtion, both wavelength and intensity, when compared to the phenolic form. This change in UV absorbtion is caused by the delocalisation of the ion into the aromatic ring.

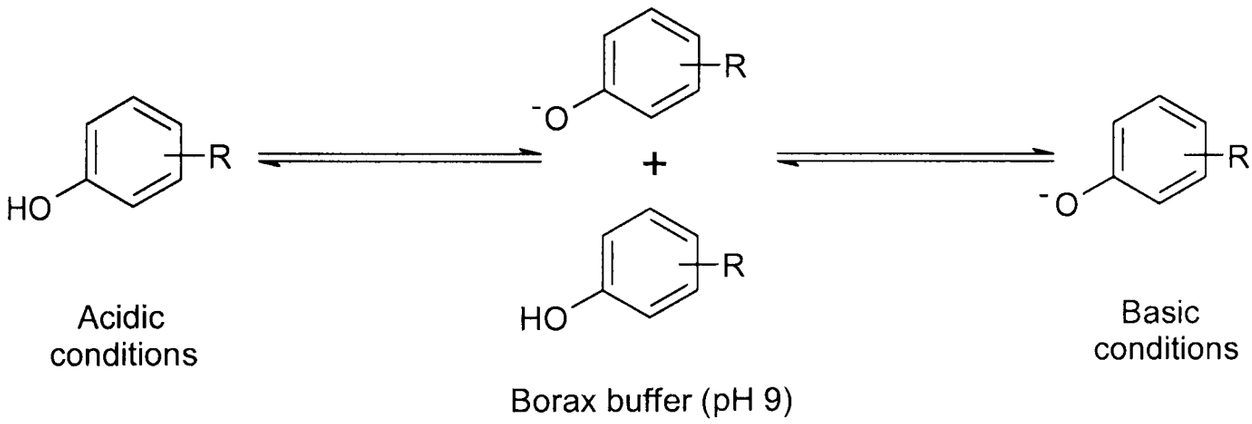


Figure 6.1: The effects of acidic, pH 9, and basic conditions on phenolic based compounds.

It has been proposed that pK_a plays a vital role in the inhibition of ES and is an important physicochemical factor in the inhibition of this enzyme. The hydrolysis of the sulfate group in the proposed mechanism of action of ES results in the formation of a phenolic ion, as such, the optimisation of the pK_a value would then be expected to affect the biological activity of sulfonate based inhibitiors of ES (Figure 6.2) (Ahmed et al, 2001a; Ahmed et al, 2002c; Ahmed et al, 2002e; Owen et al, 2003). In an effort to study the effect of pK_a on the inhibitory activity of other sulfonate based inhibitors (e.g. methanesulfonates), the determination of the pK_a was undertaken using the photometric method.

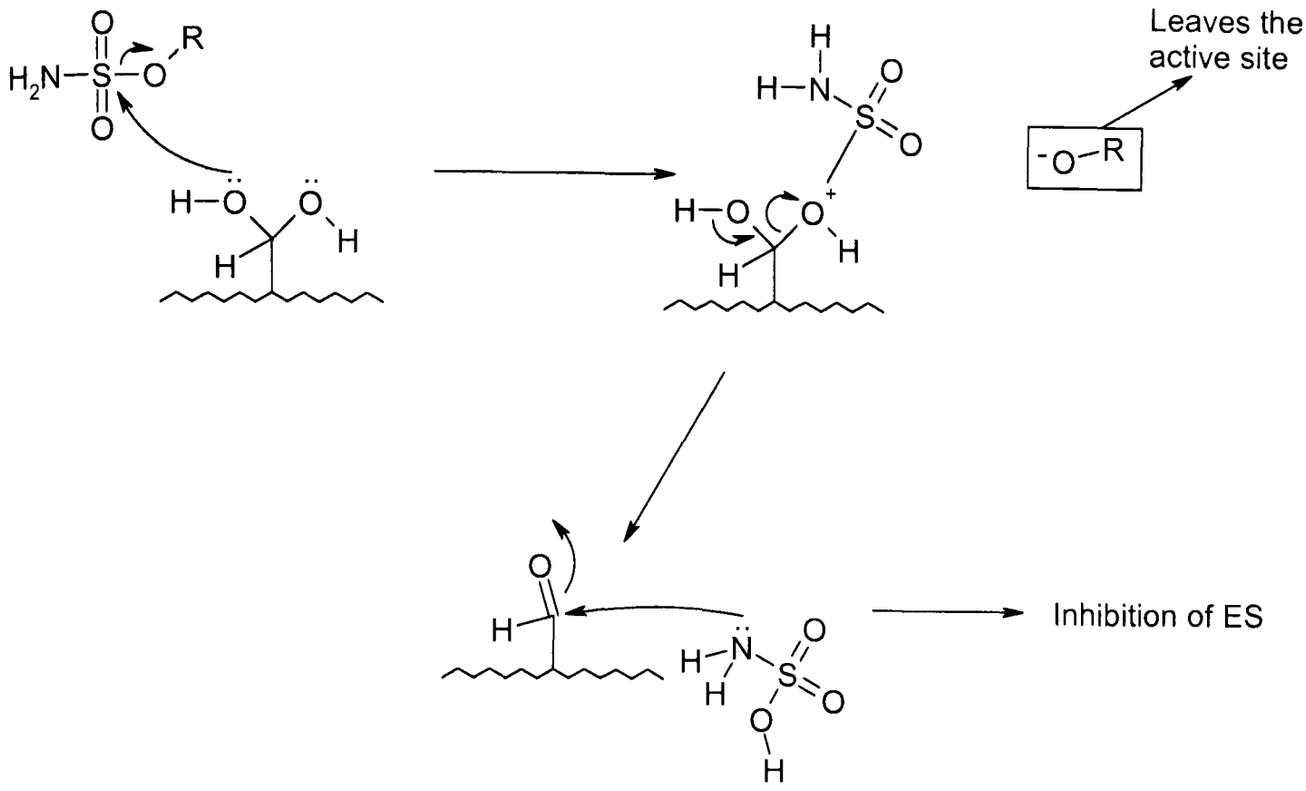


Figure 6.2: The formation of a phenoxide ion in the proposed mechanism of inhibition of ES (Ahmed et al, 2001b; Ahmed at al, 2002e)

6.2 Materials and Methods

The phenolic compounds under investigation were either purchased from Sigma-Aldrich Company Ltd (Poole, Dorset, England), or synthesised in the laboratory. All compounds were checked for purity by ^{1}H and ^{13}C NMR (JEOL 400 MHz and 100 MHz respectively). Ultraviolet spectroscopy was carried out on a CARY 100 Scan UV-visible spectrophotometer.

6.2.1 Solutions

Borax buffer (pH 9.0)

A: Sodium tetraborate decahydrate 9.535g in 1000mL of water

B: HCl 0.1M [HCl (2mL, 37%m/v) made up to 200mL with water].

B was added to A until a pH of 9.0 was reached.

HCl (2M)

HCl (17.2mL, 38%m/v) made up to 100mL with water.

NaOH (2M)

NaOH (8.0g) made up to 100mL with water.

6.2.2 Procedure

The phenolic compound (2-4mg) was added to borax buffer (200mL), and the UV spectrum recorded between 200-450nm. The absorbance of the major peak was then adjusted to approximately 1 absorbance unit either by the addition of more buffer or more of the phenolic compound. After decanting the solution free of any undissolved phenolic compound, 20mL of this stock solution was placed into 3 separate volumetric flasks (25mL) and made up to 25mL with HCl (2M) (Aa, Equation 6.1), borax buffer pH 9.0 (A, Equation 6.1), or NaOH (2M) (Ab, Equation 6.1). The UV spectrum of each solution was recorded and the absorbance of each solution at the wavelength corresponding to the NaOH (2M) (fully dissociated) maxima, was determined. The pK_a was then determined from Equations 6.1 and 6.2.

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

$$X = \frac{A-Aa}{Ab-Aa}$$

Equation 6.1: Determination of the mole fraction of dissociated phenol, where Aa, A and Ab represent the UV absorption in acidic, pH 9.0 and basic conditions respectively.

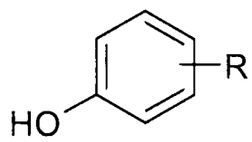
$$pK_a = pH + \log[(1-X)/X]$$

Equation 6.2: Determination of pK_a, where X represents the mole fraction of dissociated phenol and pH refers to the pH 9.0 of the borax buffer.

6.3 Results

6.3.1 Method validation

The results obtained for the substituted phenol based compound are shown in Table 6.1, and their experimentally determined pK_a values are shown in Table 6.2 [for comparison, the literature pK_a values (Lit. pK_a) are also reported].



Compound	λMax (nm)	Aa	A	Ab	X
Phenol	287	0.023	0.273	2.164	0.117
3-Bromophenol	293	0.014	0.867	1.430	0.602
3-Chlorophenol	292	0.149	0.802	1.548	0.466
3-Fluorophenol	267	0.675	0.667	0.652	0.333
3-Nitrophenol	252	0.232	0.709	0.805	0.833
4-Bromophenol	293	0.032	0.373	1.242	0.281
4-Chlorophenol	298	0.028	0.326	0.896	0.343
4-Fluorophenol	264	0.885	0.897	1.199	0.038
4-Nitrophenol	316	3.737	0.527	0.445	0.975

Table 6.1: pK_a values determined for the substituted phenols [where Aa=absorbtion of the 20mL stock solution with HCl (5mL, 2M), A=absorbtion of the 20mL stock solution with 5mL borax buffer and, Ab=absorbtion of the 20mL stock solution with NaOH (5mL, 2M), X=calculated value from Equation 6.1].

Compound	pK_a	Lit. pK_a
Phenol	9.88	9.89
3-Bromophenol	8.82	N/A
3-Chlorophenol	9.06	8.85
3-Fluorophenol	9.30	N/A
3-Nitrophenol	8.30	8.28
4-Bromophenol	9.41	N/A
4-Chlorophenol	9.28	9.18
4-Fluorophenol	10.39	N/A
4-Nitrophenol	7.41	7.15

Table 6.2: pK_a values for the substituted phenols (where N/A = not available) (Lide, 1998)

A plot of experimentally obtained pK_a values versus available Lit. pK_a values (Lide, 1998) shows a good correlation and as such validates the method used in the determination of the pK_a (Figure 6.2).

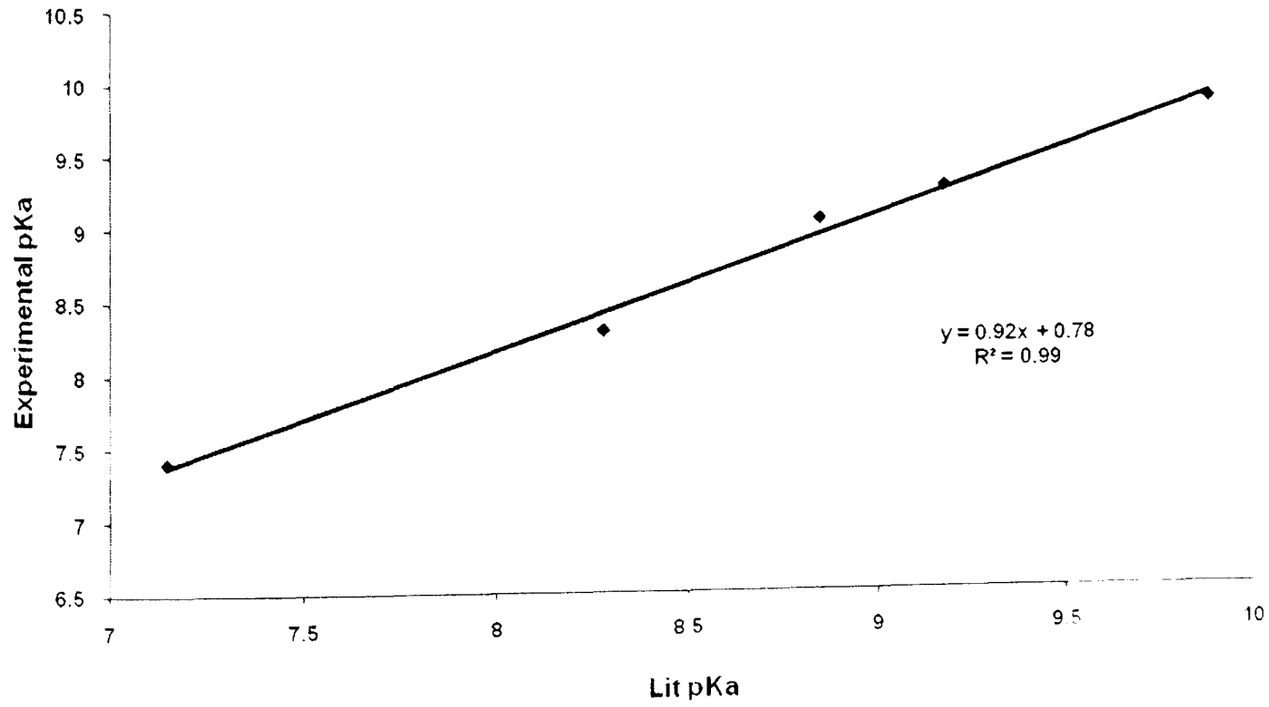
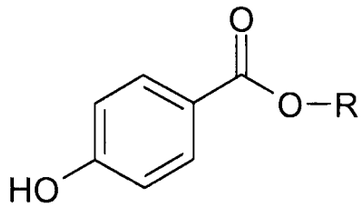


Figure 6.2: Plot of lit. pK_a vs experimentally observed pK_a for a range of substitued phenols.

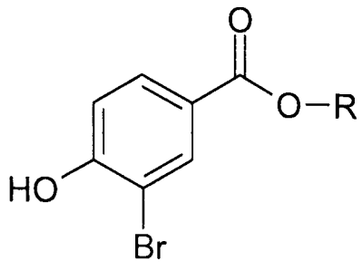
6.3.2 Results

The results of the pK_a determination of a number of the parent hydroxy compounds synthesised within this study are shown in Tables 6.3 to 6.7. A number of the larger chain alkyl containing compounds displayed poor solubility within the buffer, and as such, the experimental pK_a for these compounds could not be easily obtained.



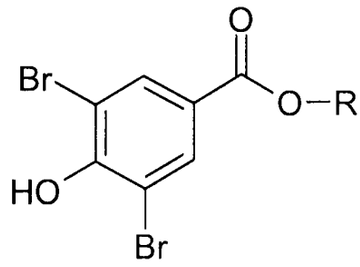
Compound	R	pK_a
220	CH_3	9.15
221	C_2H_5	9.32
222	C_3H_7	9.01
223	C_4H_9	9.12
224	C_5H_{11}	9.27
225	C_6H_{13}	9.08
226	C_7H_{15}	9.21
Average	-	9.16

Table 6.3: Experimentally determined pK_a values for a range of 4-hydroxybenzoates.



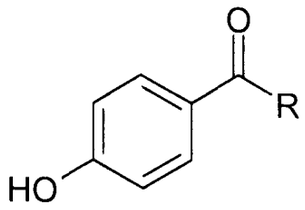
Compound	R	pK_a
234	CH_3	8.45
235	C_2H_5	8.43
236	C_3H_7	8.19
237	C_4H_9	8.82
238	C_5H_{11}	8.07
239	C_6H_{13}	8.44
240	C_7H_{15}	8.37
Average	-	8.39

Table 6.4: Experimentally determined pK_a values for a range of mono-brominated derivatives of alkyl 4-hydroxybenzoates.



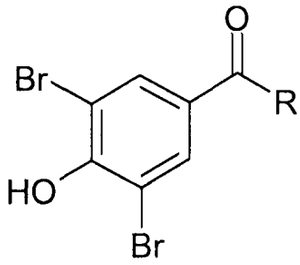
Compound	R	pK_a
244	CH_3	8.08
245	C_2H_5	7.99
246	C_3H_7	8.16
247	C_4H_9	8.21
248	C_5H_{11}	7.98
249	C_6H_{13}	8.39
250	C_7H_{15}	8.30
Average	-	8.15

Table 6.5: Experimentally determined pK_a values for a range of 3,5-dibrominated derivatives of alkyl 4-hydroxybenzoates.



Compound	R	pK_a
268	CH_3	8.06
269	C_2H_5	8.16
270	C_3H_7	8.11
271	C_4H_9	8.30
272	C_5H_{11}	8.13
273	C_6H_{13}	7.82
274	C_7H_{15}	8.55
Average	-	8.16

Table 6.6: Experimentally determined pK_a values for a range of 4-hydroxy phenyl ketone based compounds.



Compound	R	pK_a
283	CH_3	7.48
284	C_2H_5	6.90
285	C_3H_7	6.93
286	C_4H_9	6.13
287	C_5H_{11}	8.64
288	C_6H_{13}	7.86
Average	-	7.32

Table 6.7: Experimentally determined pK_a values for a range of 3,5-dibrominated derivatives of 4-hydroxy phenyl ketone based compounds.

Chapter 7: Biochemical evaluation of compounds

7.0 Biochemical evaluation of compounds

7.1 Introduction

The hydrolysis of the sulfate group in E1S is catalysed by ES. The inhibition and activity of ES can be determined using a radiometric biological assay (Li et al, 1996), which involves the incubation of radiolabelled substrate (namely 6,7-^{3}H E1S) in the presence of ES from rat liver. 6,7-^{3}H E1 formed as a result of the hydrolysis, is then extracted into toluene and the radioactivity determined. The degree of inhibition is then determined by comparing the level of radioactivity detected in the tubes with the potential inhibitors to the control tubes (which lacked both microsomal fraction and inhibitor) and the 100% tubes which lacks inhibitor.

7.2 Materials and methods

Rat livers (Sprague-Dawley - breeders) were supplied by Charles Rivers UK Ltd (Margate, Kent). 6,7-^{3}H E1S, ammonium salt (2120.1GBq/mmol, 37MBq/mL) and the scintillation fluid Optiscint, 'Hi Safe', were purchased from Perkin Elmer (Beaconsfield, Buckinghamshire). All other chemicals and solvents were purchased from Sigma-Aldrich Chemical Co. (Dorset, U.K.). All disposable pipettes, vials and tubes where purchased from Elkay (Coleshill, Birmingham). Radioactivity was measured using a Perkin-Elmer Tri-carb 2900TR scintillation counter.

7.2.1 Buffer solutions

The following buffers and solutions were prepared for use in the assay and microsomal preparation:

Tris HCl buffer 0.1M, pH 7.4, containing 0.154M KCl and 1mM EDTA

Tris(hydroxymethyl)aminomethane (Tris) (24.23g) was dissolved in 500mL of water, KCl (11.49g) and EDTA (0.76g) were then added and dissolved. The pH

was then adjusted to pH 7.2 using 0.2M HCl (~400mL). The solution was then made up to a total volume of 2000mL with distilled water.

Tris HCl buffer 0.1M, pH 7.2

Tris (6.06g) was dissolved in 250mL water, and the pH adjusted to pH 7.3 using 0.2M HCl (~100mL). The solution was then made up to 480mL with distilled water, and the pH checked, and adjusted to 7.2 using 0.2M HCl. The solution was made up to a total volume of 500mL with distilled water.

Tris HCl buffer 50mM, pH 7.2

Aliquots of Tris HCl buffer (0.1M) were diluted by half with water to produce pH 7.2 (50mM) buffer solution (125mL of 0.1M, pH 7.2 Tris HCl buffer made up to 250mL with distilled water).

7.2.2 Microsomal preparation

All procedures were carried out at 4°C. Livers from Sprague-Dawley rats were cleaned of any extraneous connective tissue and fibres, washed with Tris HCl buffer containing KCl and EDTA, blotted dry, then weighed. The livers were chopped roughly with scissors and homogenised in Tris HCl buffer containing KCl and EDTA (1g tissue to 3mL buffer) using an ultra Turrax mincer at maximum speed for 20s, three times, interspersed with 1min cooling periods. The resulting homogenate was then further homogenised using a Potter homogeniser.

The microsomal fraction was obtained by differential centrifugation. That is, the homogenate was centrifuged for 20min at 11,000RPM (9,000-10,000g). The resultant pellet was discarded and the supernatant spun for a further 60min at 40,000RPM (100,000g). The pellet (microsomal fraction) was re-suspended in buffer and centrifuged for 60min at 40,000RPM (10,0000g). The washed pellet was suspended in Tris HCl buffer containing KCl and EDTA (0.1M, pH 7.2, 150ml) using a Potter homogeniser. Aliquots (0.5mL) of suspension were pipetted into capped 1.5mL plastic eppendorf tubes, snap-frozen in liquid nitrogen, and stored at -20°C until required.

7.2.3 Substrate preparation

A stock solution of 6,7-^{3}H E1S was prepared by transferring radiolabelled 6,7-^{3}H E1S (125μl, 5MBq, 0.125nmol) to a vial and removing the ethanol under a stream of nitrogen. Non-radiolabelled E1S in ethanol (5ml, 4.0mM) was added to give a concentration of 4.0mM (1MBq/mL). 25μL of this solution in each assay tube (1mL) gives a final substrate concentration of 100μM, and a maximum tube radioactivity of 0.025MBq.

7.2.4 Protein determination assay

The Folin-Lowry assay was used to determine the protein concentration within the microsomal fraction (Lowry et al, 1951). In the assay the protein concentration was determined colourimetrically at 750nm, via the formation of a chromophoric complex between an alkaline copper-phenol reagent and the protein, more specifically the tyrosine and tryptophan residues of the protein backbone, comparing this reading to that on a standard curve of a series of bovine serum albumin dilutions (Gibson and Skett, 1999).

A series of bovine albumin solutions at ranging concentrations was prepared (in triplicate) with the total volume of each tube being 1mL and concentration of protein between 0-200μg/mL. The liver fraction microsomes were diluted by a factor of 125 (40μl/5mL), and 3 x 1mL aliquots dispensed into tubes and the protein concentration determined alongside the standards.

The alkaline copper-phenol reagent was made by adding a solution of $NaHCO_3$ (2%) in NaOH (0.1M, 200mL) added to copper sulfate (1%, 2mL), and sodium potassium tartrate (2%, 2mL). Aliquots (5mL) were added at 30s intervals to each of the test tubes. After standing for 10min, a 50% diluted solution of Folin-Ciocalteau reagent (0.5mL) was added to each tube. The tubes were immediately vortexed and allowed to stand at room temperature (30min). The optical density (750nm) of each solution was measured against the blank (i.e. distilled water).

The protein concentration was determined from the standard protein calibration curve (Figure 7.1) and was found to be 8.34mg/mL.

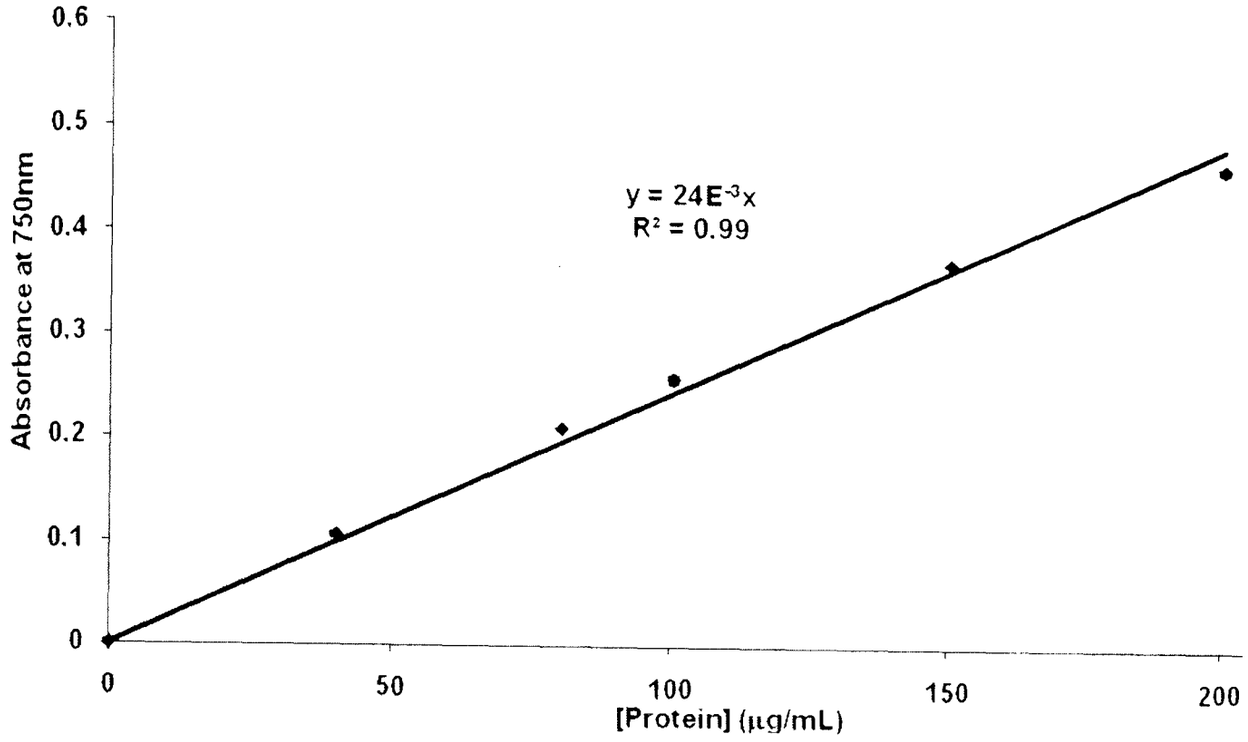


Figure 7.1: Protein determination graph.

7.3 Validation of assay

7.3.1 Determination of non-enzymatic hydrolysis

In order to validate the ES assay it was necessary to determine the quantity of radioactivity present in the toluene layer in the absence of the enzyme compared to when the enzyme was present. This was accomplished by adding prepared substrate E1S (25µL, final assay concentration 100µM, 0.025MBq) in triplicate to the assay tubes, followed by removal of the ethanol under a stream of nitrogen. Tris-HCl buffer (50mM, pH 7.2, 0.975mL) was then added and the assay mixture warmed for 5min at 37°C in a shaking water bath. The microsomes were diluted [1 epindorf (500µL) made upto 4mL with Tris HCl buffer, protein concentration 1.04mg/mL] and the assay was initiated by the addition of the diluted microsomes (100% tubes) or boiled diluted microsomes (blanks) (25µL,final protein

concentration in assay 26μg/mL). After 10min of incubation at 37°C, the assay tubes were quenched by the addition of toluene (4mL) and placed on ice. Each tube was vortexed for 45s and centrifuged (3,000RPM) for 15min. Aliquots (1mL) of each toluene layer were added to Optiscint (5mL) and counted for tritium for 5min per tube.

A negligible amount of radiation (less than 1%) was detected in the blanks compared to the 100% tubes, thereby validating the assay. The blanks, however, were run in each assay to ensure interassay consistency.

7.3.2 Interassay consistency

Interassay variation was determined by measuring the inhibitory effect of the two standards EMATE and COUMATE, as well as the blanks, in each assay undertaken. The variation between assays was less than 5%.

7.4 Determination of assay parameters

A number of preliminary assays were undertaken to determine the parameters and concentrations required for the biological screening.

7.4.1 Time dependency assay

In order to ensure that the assays were performed within the linear phase of the enzymatic reaction, a time dependency assay was undertaken. Radiolabelled substrate was dispensed into tubes in triplicate (4.5μL, 36μM final concentration) and the ethanol removed under a stream of nitrogen. Tris-HCl buffer (50mM, pH 7.2, 475μL), was added to the tubes and warmed (37°C) in a shaking water bath alongside the diluted rat liver microsomes [1 epindorf (500μL) diluted to 4mL with Tris HCl buffer, 1.04mg/mL] for 10min. The diluted microsomes (12.5μL, final assay concentration 26μg/mL) were then added to the assay tubes to initiate the assay. After incubation for 2, 7, 10, 20, 30, 40, 50 and 60min, the tubes were quenched with toluene (4mL) and vortexed for 45s, followed by centrifugation at

3,000RPM for 15min. Aliquots (1mL) of the organic layer from each tube were removed and dispensed into a scintillation vial with Optiscint (5mL), and the vials counted for tritium for 5min. The same assay was also undertaken with an increased substrate concentration (22µL, 184µM final concentration) (Figure 7.2).

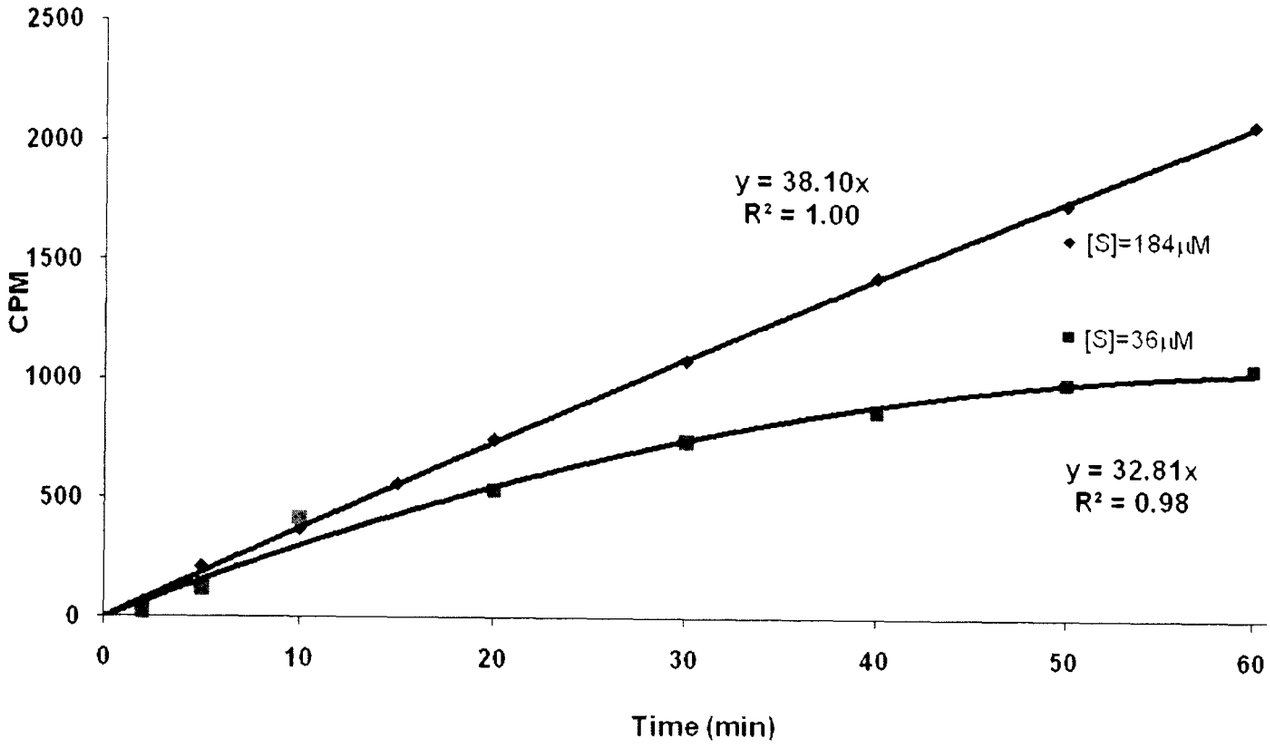


Figure 7.2: Time dependency graph.

7.4.2 Protein dependency

A protein dependency assay was undertaken in order to determine whether the rate of conversion of E1S to E1 during the assay was directly proportional to the protein concentration and in order to determine the optimum protein concentration to use.

The prepared substrate (25µl, final assay concentration 100µM, 0.025MBq) was added to each assay tube. The ethanol was removed under a stream of nitrogen, and Tris-HCl buffer (50mM, pH 7.2, 700-975µL) added. The microsomes were diluted to give final assay concentrations ranging from 0-260µg/mL in a tube volume of 1mL

The assay tubes and microsomes were incubated for 5min at 37°C in a shaking water bath before initiating the assay by adding the appropriate volume of microsomes to each tube (to give a final assay volume of 1mL). After 10min of incubation at 37°C, the assay tubes were quenched by the addition of toluene (4mL) and placed on ice. Each tube was vortexed for 45s, and centrifuged (3,000RPM) for 15min. 1mL aliquots of the toluene layer from each tube were added to Optiscint (5mL) and counted for tritium for 5min.

The graph for the protein dependency (Figure 7.3) shows that there is a linear relationship up to 120μg/mL.

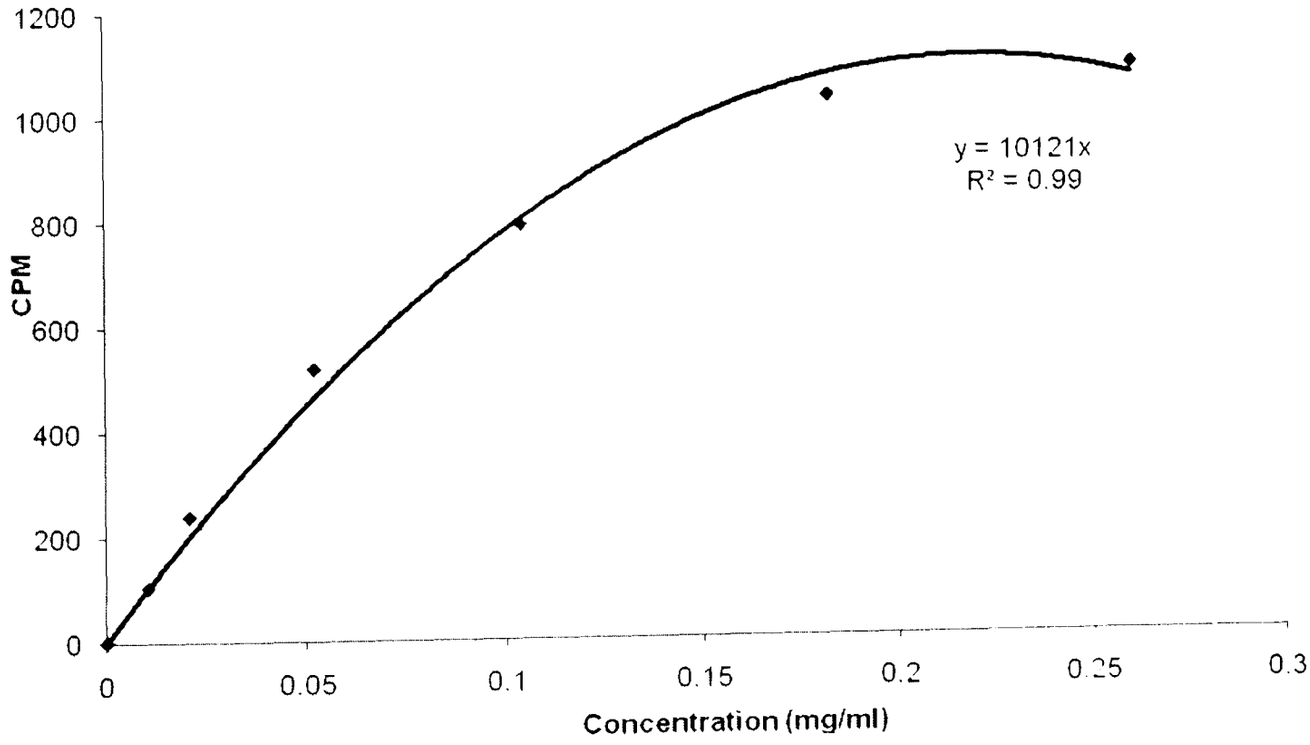


Fig 7.3: Protein dependency graph.

7.4.3 Determination of Michaelis constant, K_m

The final assay volume was 1mL. The prepared radiolabelled substrate was diluted with ethanol (200μL in 4mL, to give a concentration of 200μM, 0.05MBq). Different volume aliquots of the diluted substrate were dispensed in triplicate to the

assay tubes to give final assay concentrations of 0 to 40μM, and the ethanol was evaporated under a stream of nitrogen. Tris-HCl buffer in different volume aliquots (to give a total volume of 1mL, 50mM, pH 7.2) was added, and the tubes incubated (37°C) in a shaking water bath alongside the diluted microsomes [1 epindorf (500μL) diluted to 4mL with Tris HCl buffer, 1.04mg/mL] for 5min. The assay was initiated by the addition of microsomes (25μL, final concentration 26μg/mL). After 13.5min incubation at 37°C, the assay tubes were quenched by the addition of toluene (4mL) and vortexed prior to being placed on ice. Each tube was vortexed for 45s and centrifuged (3,000RPM) for 15min. Aliquots (1mL) of each toluene layer were added to Optiscint (5mL) and counted for tritium for 5min.

For the determination of kinetic parameters, the 100% value of each of the substrate concentrations was required. This was determined by pipetting the substrate in corresponding volume aliquots to those used in the assay directly into scitilation vials, to which toluene (1mL) and Optiscint (5mL) were added and counted via the liquid scintillation counter.

The velocity, V (μM/min/μg), for each substrate concentration, [S]/μM, was calculated using Equation 7.1, where [P] = protein concentration (μg/mL) and CPM = counts per minute.

$$V = \frac{\text{CPM mean} \times [S]\mu M}{\text{Time (min)} \times [P]\mu g/mL \times \text{CPM (100\%)}}.$$

Equation 7.1: Calculation of velocity.

7.4.4 Graphical determination of K_m

Five different general methods were used to determine K_m and V_{max} for E1S (Figures 7.4 to 7.7)

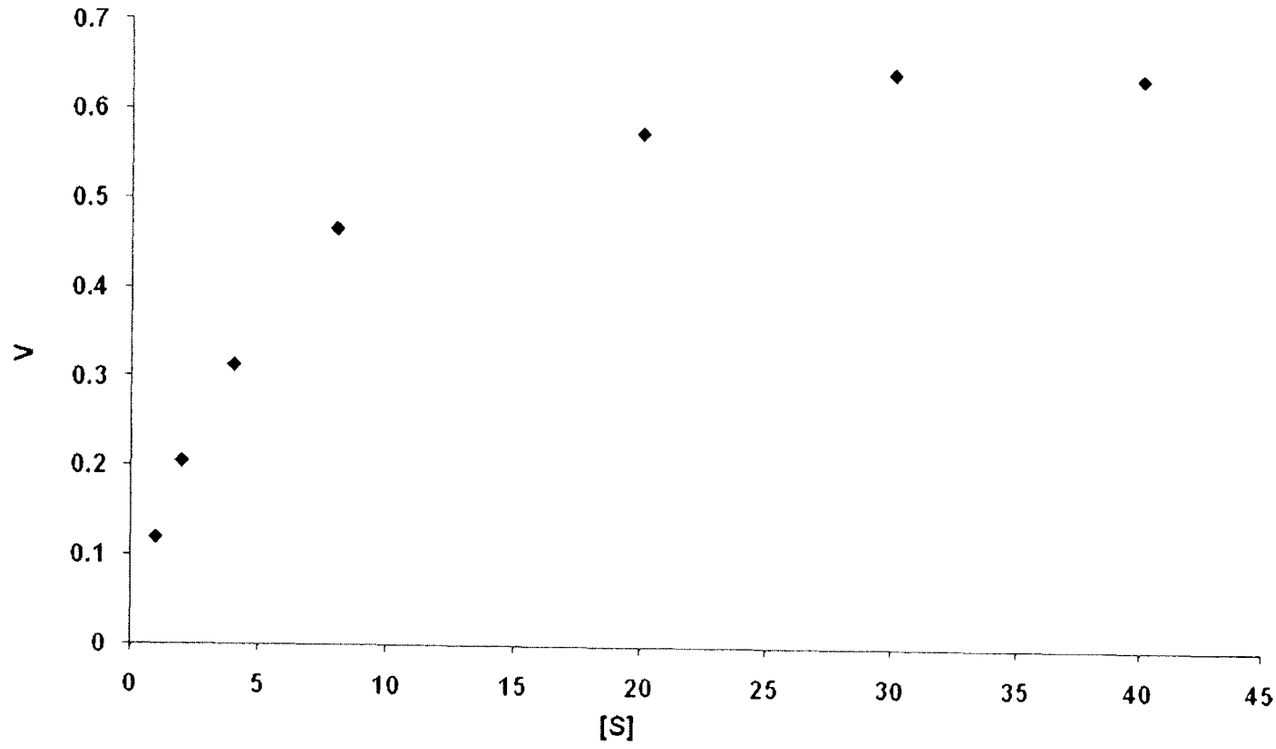


Figure 7.4: Michaelis-Menten plot.

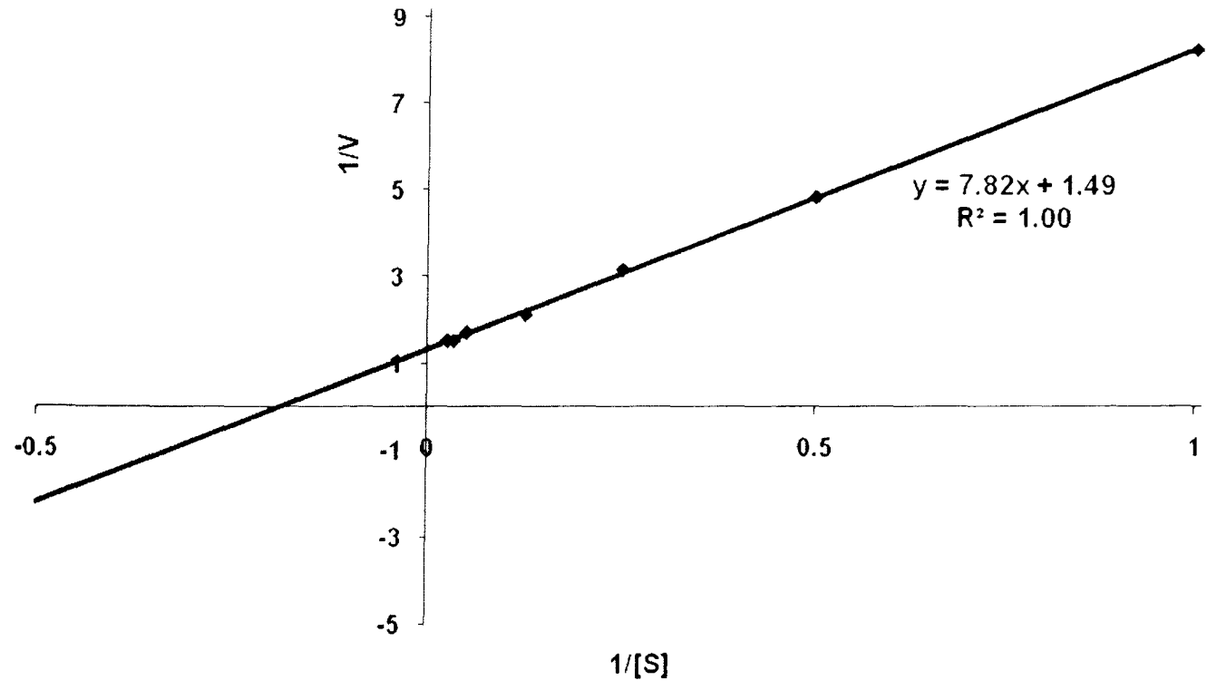


Figure 7.5: Lineweaver-Burke plot.

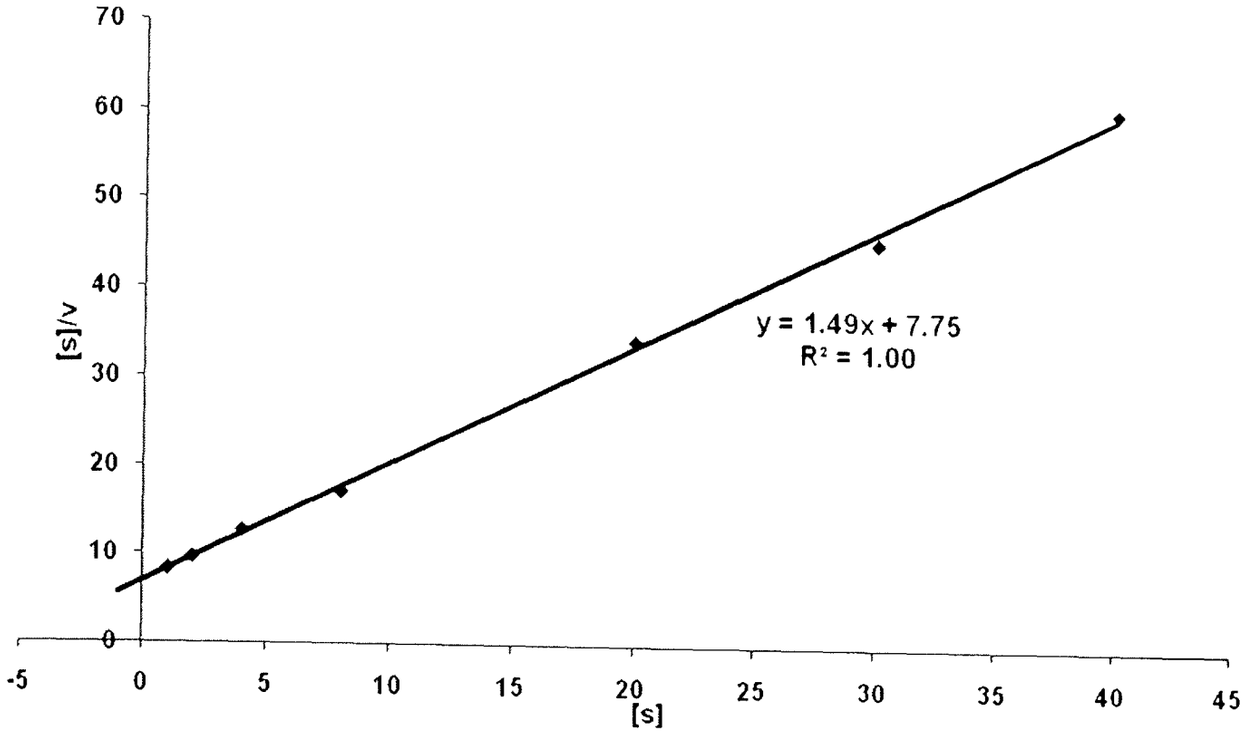


Figure 7.6: Hanes-Woolf plot.

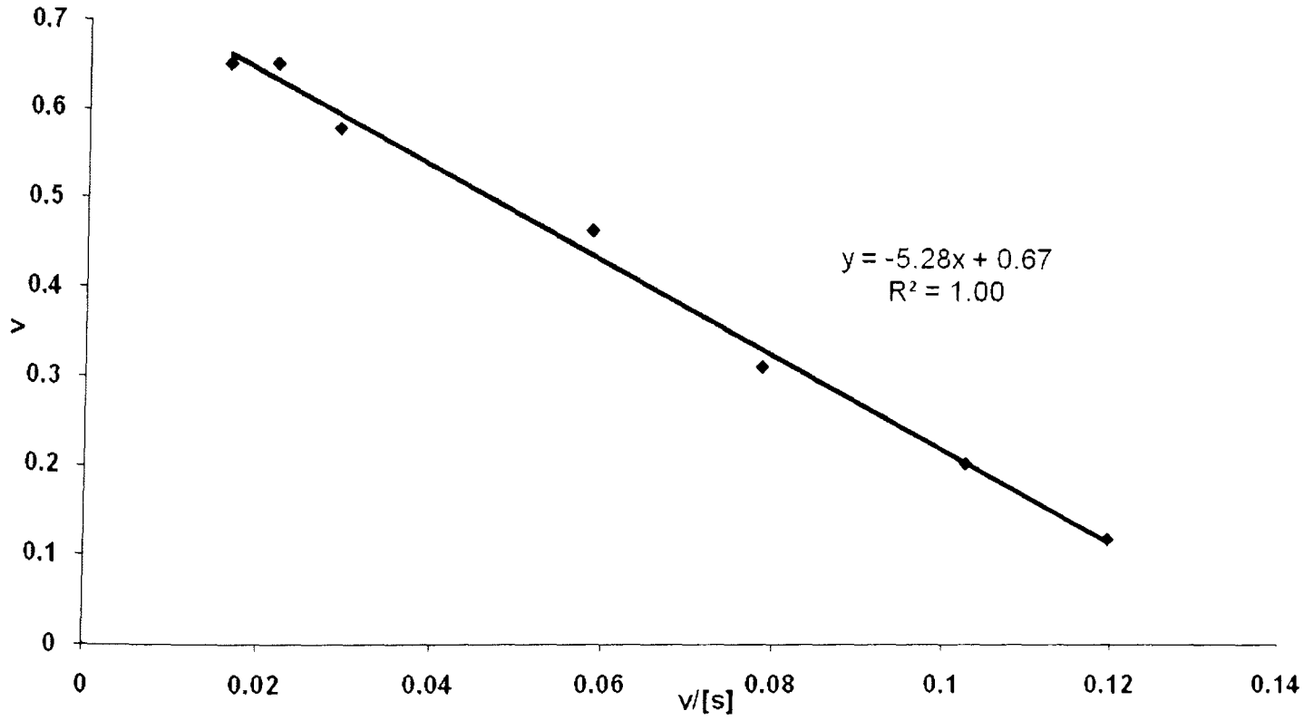


Figure 7.7: Eadie-Hofstee plot.

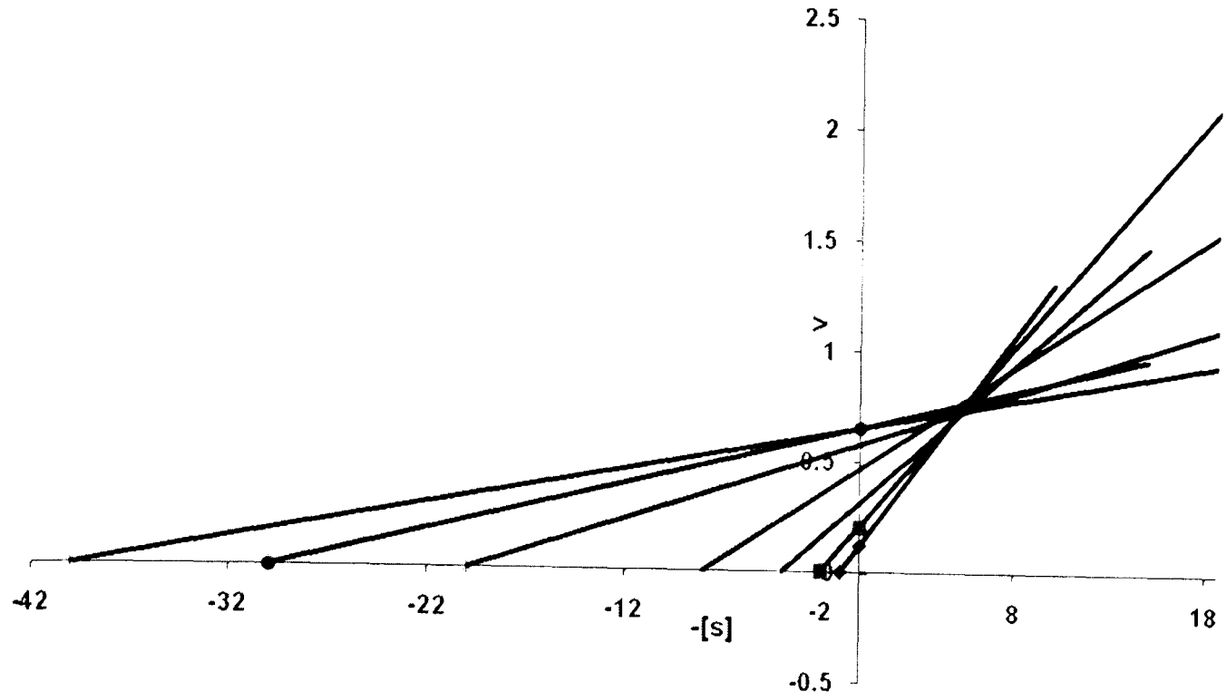


Figure 7.8: Cornish-Bowden plot.

7.4.5 Discussion

The K_m values determined from these graphs and the average are summarised in Table 7.1.

Type of plot used	K_m (μM)
Michaelis-Menten	5.10
Lineweaver-Burke	5.24
Hanes-Woolf	5.20
Eadie-Hofstee	5.28
Cornish-Bowden	5.40
Average	5.20±0.10

Table 7.1: K_m values for E1S against microsomal preparation from rat liver.

The average K_m for E1S from the results obtained from Table 7.1 was found to be 5.20±0.10μM. Interassay consistency gave a mean K_m for E1S against rat liver

microsomal preparation of 5.52±0.44μM. Evans et al (1991) found the K_m of E1S against placental microsomal tissue to be 6.83μM. James (2000), using human placental microsomes found the K_m to be 13.3±2μM, whilst Patel (2003a) under similar conditions, also discovered the IC_{50} to be 13.3±1.6μM. As such, the K_m appears to be in approximate agreement with Evans et al (1991).

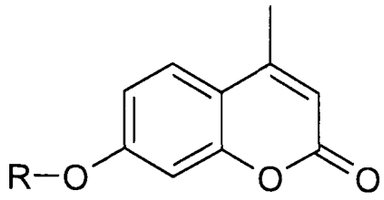
7.5 Initial screening

All assay incubations were carried out in triplicate. The total assay volume was 1mL. The prepared radiolabelled substrate was diluted with buffer to give a solution of 6,7-^{3}H E1S (10μL=50μM/tube; approx 750,000CPM/tube). The diluted substrate 6,7-^{3}H E1S (10μL) was then dispensed into each tube and the ethanol removed under a stream of nitrogen.

The inhibitors (dissolved in DMSO, 20μL, 100μM final concentration) were dispensed into each assay tube. The blank and 100% tubes contained 20μL of DMSO but no inhibitor. Tris-HCl buffer (0.05M, pH 7.2, 0.955mL) was added to each tube. Rat liver microsomes were then diluted with Tris-HCl buffer [1 epindorf (500μL) diluted to 4mL with Tris buffer, 1.04mg/mL]. The microsomes and assay tubes were pre-incubated for 5min at 37°C in a shaking water bath prior to the addition of the microsomes (25μL, final assay concentration 26μg/mL) to the tubes. After 13.5min incubation (at 37°C), the assay was quenched by the addition of toluene (4mL) and the mixture vortexed and placed on ice. Each tube was vortexed for 45s and centrifuged (3,000RPM) for 15min. Aliquots (1mL) of each toluene layer were added to Optiscint (5mL) and counted for tritium for 5min. Control samples with no inhibitor (100% tubes) and no microsomes (blanks) were incubated simultaneously in order to calculate percentage inhibition.

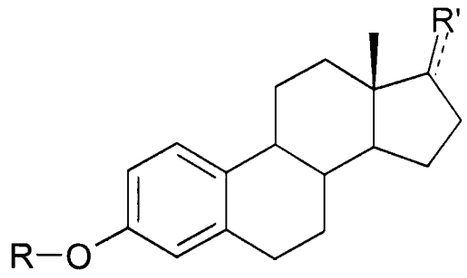
7.5.1 Results

The compounds were screened in triplicate, and each assay repeated such that n=6 (Tables 7.2 to 7.7). Some compounds that were synthesised by other group members were also screened (Tables 7.3, 7.6 and 7.7).



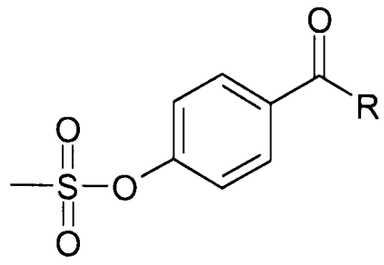
Compound	R	% inhibition (100μM)
COUMATE	NH_2SO_2	97.0±0.4
372	CH_3SO_2	27.0±2.4
373	CF_3SO_2	30.2±5.0

Table 7.2: Inhibitory data for coumarin based compounds against ES.



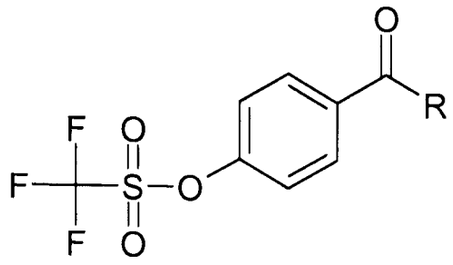
Compound	R	R'	% inhibition ([I]=100μM)
EMATE	NH_2SO_2	=O	98.2±0.0
369	CH_3SO_2	=O	47.2±2.4
370	CF_3SO_2	=O	57.7±0.2
371	CH_3SO_2	CH_3SO_2-O	40.5±1.3
374	$C_6H_5SO_2$	=O	33.3±1.5
375	4-$ClC_6H_4SO_2$	=O	15.0±0.4
376	4-$BrC_6H_4SO_2$	=O	20.8±3.6
377	4-$IC_6H_4SO_2$	=O	14.3±1.4
378	4-$NO_2C_6H_4SO_2$	=O	21.4±10.7
379	4-$CF_3C_6H_4SO_2$	=O	21.3±2.5
380	4-C_6H_5-$C_6H_4SO_2$	=O	5.3±2.0

Table 7.3: Inhibitory data for various sulfonated derivatives of E1 and E2 against ES.



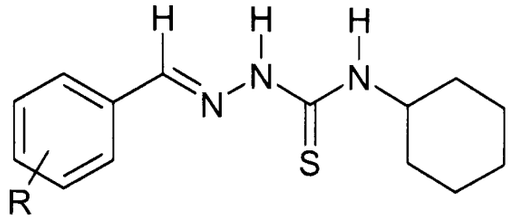
Compound	R	% inhibition ([I]=100μM)
296	H	0
297	CH_3	0
298	C_2H_5	4.6±2.9
299	C_3H_7	9.1±0.5
300	C_4H_9	16.8±3.2
301	C_5H_{11}	9.0±0.4
302	C_6H_{13}	19.9±2.8
303	C_7H_{15}	31.9±5.1
304	C_8H_{17}	36.2±2.1
305	C_9H_{19}	37.2±2.1
306	C_3H_5	13.1±1.1
307	C_4H_7	38.1±1.8
308	C_5H_9	49.0±0.9
309	C_6H_{11}	38.4±1.6
310	C_6H_5	21.3±1.1
COUMATE	-	96.7±0.7
EMATE	-	98.0±0.8

Table 7.4: Inhibitory data for methane sulfonated derivatives of 4-hydroxyphenyl ketone based compounds against ES.



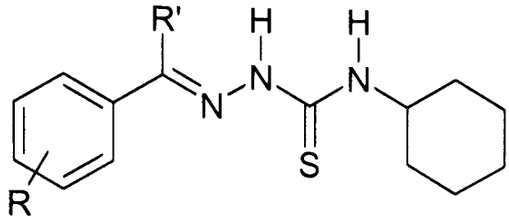
Compound	R	% inhibition ([I]=100μM)
311	CH_3	6.1±0.3
312	C_2H_5	14.0±0.4
313	C_3H_7	8.6±0.8
314	C_4H_9	16.1±0.7
316	C_6H_{13}	13.7±0.7
317	C_7H_{15}	9.4±0.8
318	C_8H_{17}	2.4±0.9
320	C_3H_5	19.1±4.0
321	C_4H_7	29.1±0.8
322	C_5H_9	20.8±1.4
324	C_6H_5	14.4±0.1
COUMATE	-	96.7±0.1
EMATE	-	97.7±0.1

Table 7.5: Inhibitory data for trifluoromethane sulfonated derivatives of 4-hydroxyphenyl ketone based compounds against ES.



Compound	R	% inhibition ([I]=100μM)
381	H	69.6±0.5
382	2-Cl	31.5±0.3
383	2-Br	30.7±0.5
384	2-NO_2	57.3±0.3
385	2-CH_3	ND
386	3-Cl	39.3±0.1
387	3-Br	ND
388	3-NO_2	61.0±0.2
389	3-CH_3	52.5±0.3
390	4-Cl	34.9±0.9
390	4-Br	13.5±0.4
391	4-NO_2	49.6±2.5
392	4-CH_3	43.1±0.4
COUMATE	-	96.8±0.1
EMATE	-	98.0±0.1

Table 7.6: Inhibitory data for thiosemicarbazone based compounds against ES (where ND= not determined).



Compound	R	R'	% inhibition ([I]=100μM)
393	H	CH_3	44.2±0.0
394	H	CH_2CH_3	68.1±0.4
395	3-Br	CH_3	54.2±1.2
396	3-Cl	CH_3	22.4±4.6
397	3-CH_3	CH_3	34.3±1.9
398	4-Br	CH_3	50.4±1.6
399	4-Cl	CH_3	28.0±1.0
400	4-CH_3	CH_3	44.2±2.8
401	2-OH, 5-Br	H	72.9±5.7
402	Ph	H	61.5±1.0
COUMATE	-	-	96.8±0.1
EMATE	-	-	98.0±0.1

Table 7.7: Inhibitory data for thiosemicarbazone based compounds against ES.

Chapter 8: Discussion

8 Discussion

The compounds within the current report were evaluated against ES using EMATE and COUMATE as the two standard compounds and the inhibitory activity outlined in Tables 7.2 to 7.7.

In general, the sulfonate-based compounds have shown extremely disappointing inhibitory activity with the most potent inhibitor within the range of sulfonate-based compounds being compound **370** which was found to possess ~58% inhibitory activity against ES at an inhibitor concentration of 100μM using rat liver microsomes – under similar conditions, both EMATE and COUMATE were found to possess ~99% and ~97% inhibition against ES respectively, as such, no IC_{50} value determinations were undertaken. Detailed consideration of the inhibitory activity for the non-steroidal compounds show that, in general, the methanesulfonate derivatives possess the greater inhibitory activity against ES in comparison to the trifluoromethanesulfonate-based compounds, however, the difference in inhibitory activity is not significant. That is, consideration of the inhibitory activity within the 4-hydroxyphenyl ketone based compounds shows that compound **308** is found to possess ~49% inhibitory activity (at [I]=100μM) against ES whilst compound 322, under similar assay conditions, is found to possess ~21% inhibitory activity against ES.

As previously mentioned, compound **370** was found to possess the most potent inhibitory activity observed within the range of compounds synthesised in the current project, indeed, the E1- and E2-based compounds were found to possess, in general, greater inhibitory activity in comparison to the non-steroidal inhibitors. Furthermore, whilst only initial screening data is considered here, the steroidal compounds appear to suggest that an increase in the bulky nature of the substituent on the sulfonate moiety results in decreased inhibitory activity. For example, the methanesulfonate derivative (compound **369**) is found to possess ~47% inhibitory activity (at [I]=100μM), under similar assay condition, the substitution of the methane moiety with a phenyl ring (compound **374**) results in a decrease in inhibitory activity to ~33% inhibition. The introduction of a biphenyl

ring on the sulfonate moiety (compound **380**) results in a total loss of inhibitory activity, that is **380** is found to possess ~5% inhibitory activity. Substitution of the phenyl ring on compound **374** with halogens as opposed to bulky group such as a phenyl ring system, also results in a decrease in inhibitory activity. That is, substitution of the phenyl ring in compound **374** with a bromine atom results in a decrease in inhibitory activity in comparison to the parent compound **374**, however, in comparison to compound **380**, the inhibitory activity would appear to have been increased.

The thiosemicarbazone-based inhibitors (compounds **381** to **402**, and which were synthesised by another member of the research group) have shown moderate inhibitory activity, however, even these compounds have proved to be weak inhibitors in comparison to the two standard compounds used. For example, compound **401** was found to possess ~73% inhibitory activity against ES at [I]=100μM, in comparison to the two standard compounds, namely, EMATE and COUMATE, which were found to possess ~97% and ~98% respectively under similar assay conditions. Compound 380 was found to be equipotent and was found to possess ~70% inhibitory activity against ES at [I]=100μM. However, a major problem was discovered with these compounds and is the major reason for the lack of IC_{50} values for these compounds, that is, the thiosemicarbazone-based inhibitors were found to rapidly degrade when dissolved in solvents (such as ethanol or DMSO) prior to their addition to the assay mixture. Indeed, attempts to determine the IC_{50} value for these compounds did not prove to be successful and due to both lack of quantity of compound and time, attempts to determine the IC_{50} values were abandoned.

Detailed consideration of the inhibitory activity so as to provide some structure-activity relationship is difficult since these compounds have been shown to be allosteric inhibitors, as such, the binding sites for these compounds is unknown. As such, traditional molecular modelling approaches cannot be used since the binding site(s) of these compounds is not known. Furthermore, the use of superimposing of these compounds (onto the backbone of estrone sulfate) would not yield any useful results since estrone sulfate has not been shown to be an

allosteric inhibitor. As such, there is currently no modelling technique available to consider the inhibitory activity of the thiosemicarbazone-based inhibitors.

Figure 22 shows excellent correlation between the experimentally derived pK_a and those from the literature, indicating the accuracy of the procedure for the determination of the pK_a of phenolic compounds.

It has been reported previously that pK_a plays a major role in ES inhibition (Ahmed et al, 2001). The role of pK_a and the increase in biological activity with the pK_a value has been suggested to be due to the ability of the sulfamated compound to undergo hydrolyse resulting in the formation of the phenoxide ion (Figure 8.1).

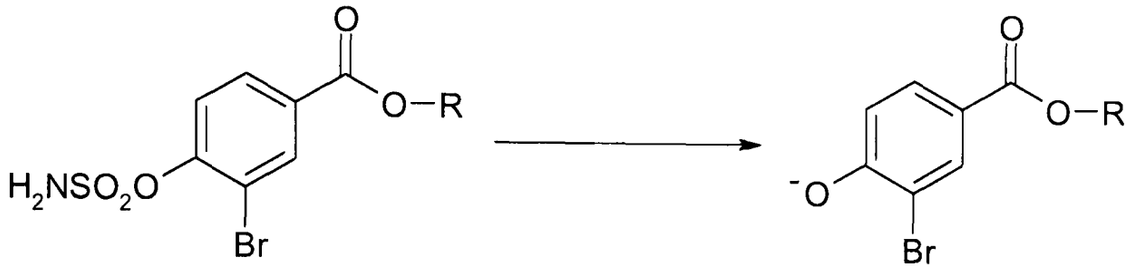


Figure 8.1: Hydrolysis of the sulfamate group to give sulfamic acid and phenoxide ion (R=CH_3 to $C_{10}H_{21}$).

Indeed, when the biological activity of sulfamated compounds against IC50 was considered, it was observed that there was a decrease in IC50, reaching a minimum value at on optimum pK_a of 8.3 – it should be noted that below a pK_a of 8.3, the sulfamate compounds were found to undergo non-enzymatic hydrolysis. Within the current report a similar study was undertaken so as to determine the pK_a of the compounds synthesised within the current study (which are summarised within Tables 6.3 to 6.7). It was observed that the brominated derivatives [as has been previously predicted by James (2000)] that the pK_a decreases with increasing numbers of bromine substituents within the phenyl ring (Tables 6.3, 6.4 ad 6.5). It has been previously proposed that this is due to a weakening of the S-OR bond, a such, we hypothesised that the decrease in the S-OR bond would allow the metanesulfonate- and trifluoromethanesulfonate-based compounds to possess increased inhibitory activity when compared to the non-brominated derivatives. From the consideration of the lack of stability within the methanesulfonate- and trifluoromethanesulfonate-based compounds, the initial

hypothesis would appear to have some validity as these compounds were found to lack chemical stability in comparison to the non-brominated derivatives.

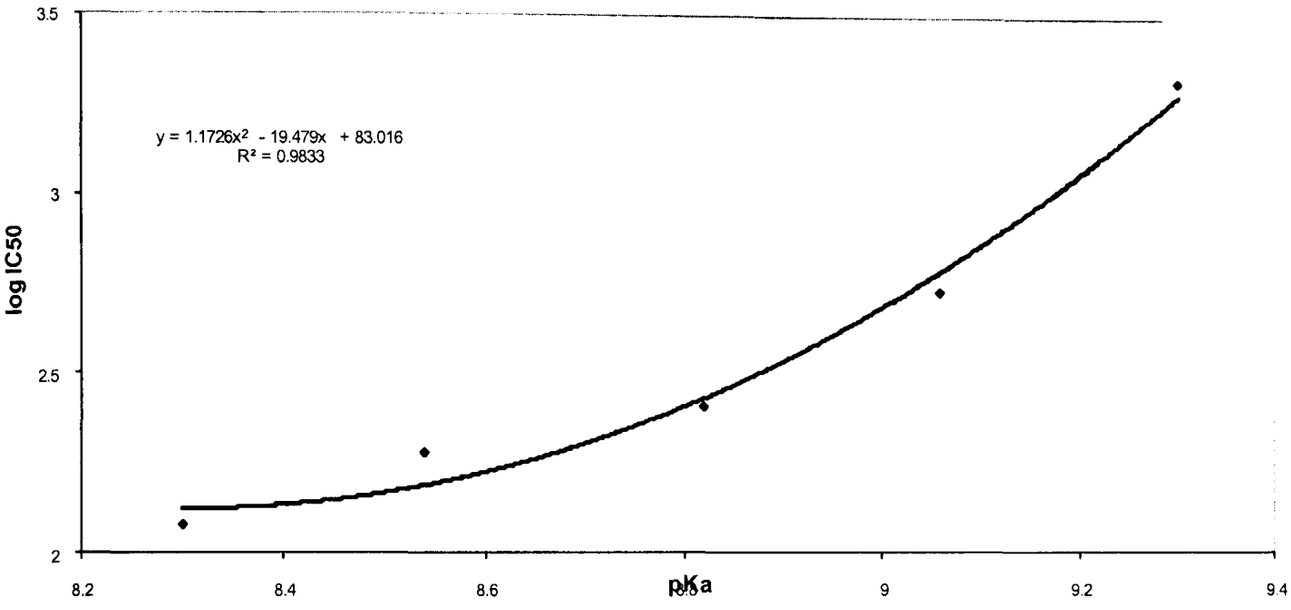


Figure 8.2: Comparison of pK_a with log IC_{50} for meta-substituted phenols.

The 3-bromo-4-hydroxybenzoic esters have an experimentally derived average pK_a of ~8.4, which would therefore appear to be approaching the optimum pK_a for the formation of the phenoxide ion (Figure 8.2). As such, pK_a value would appear to suggest that the 3-bromo-4-sulfamoyloxy-benzoic acid esters would readily undergo non-enzymatic hydrolysis and dissociate back to the parent phenoxide ion – this is indeed what is observed, as such, the current study would appear to suggest that in the design of inhibitors of ES, the pK_a of the phenolic component should not possess a pK_a value close to 8.4. Furthermore, the low pK_a would also appear to have had an effect in the case of the brominated derivatives of 4-hydroxyphenyl ketone-based compounds since these too were found to possess greatly reduced chemical stability when substituted with bromine.

In conclusion, the biochemical evaluation of the synthesised compounds within the current study proved to be disappointing and no significant level of inhibition was

observed. However, the consideration of the inhibitory activity and chemical stability provides us with some initial insight into the potential design of further novel inhibitors of ES, i.e. the acidity of the phenolic component (and therefore the pK_a of the parent phenol) is an important factor in determining both the inhibitory activity of the sulfonated derivatives but also plays an important role in the chemical stability of the target sulfonated compounds.

Chapter 9: References

9 References

-A-

Abell A. D., Henderson B. R. Steroidal and non-steroidal inhibitors of steroid 5-alpha-reductase. Curr. Med. Chem. 1995: 2: 583.

Adams J. B., Barcia M., Rochefort H. Estrogenic effects of physiological concentrations of 5-androstene-3β, 17β-diol and its metabolism in MCF7 human breast cancer cells. Cancer Res. 1981: 41: 4720-4726.

Ahmed S., James K., Sampson L. Derivation of a possible transition-state for the reaction catalysed by the enzyme Estrone Sulfatase (ES). Bioorg. Med. Chem. Lett. 1999a: 9: 1645-1650.

Ahmed S., James K., Sampson L., Mastri C. Structure-activity relationship study of the enzyme estrone sulfatase. Biochem. Biophys. Res. Commun. 1999b: 254: 811-815.

Ahmed S., James K., Patel C. K. First report of the investigation into the importance of pK(a) in the inhibition of estrone sulfatase by sulfamate containing compounds. Biochem. Biophys. Res. Commun. 2000: 272: 583-585.

Ahmed S., James K., Owen C. P., Patel C. K., Patel M. Novel inhibitors of the enzyme estrone sulfatase (ES). Bioorg. Med. Chem. Lett. 2001a: 11: 841-844.

Ahmed S., Owen C. P., James K., Patel C. K., Patel M. Acid dissociation constant, a potential physicochemical factor in the inhibition of the enzyme estrone sulfatase (ES). Bioorg. Med. Chem. Lett. 2001b: 11: 899-902.

Ahmed S., Owen C., James K., Patel C. K., Patel M. Hydrophobicity, a physicochemical factor in the inhibition of the enzyme estrone sulfatase. Bioorg. Med. Chem. Lett. 2001c: 11: 2525-2528.

Ahmed S., James K., Owen C. P., Patel C. K., Sampson L. The mechanism of the irreversible inhibition of estrone sulfatase (ES) through the consideration of a range of methane- and amino-sulfonate-based compounds. Bioorg. Med. Chem. Lett. 2002a: 12: 1279-1282.

Ahmed S., Owen C. P., James K., Sampson L., Patel C. K. Review of estrone sulfatase and its inhibitors – an important new target against hormone dependent breast cancer. Curr. Med. Chem. 2002b: 80: 263-273.

Ahmed S., Owen C. P., James K., Patel C. K. Synthesis and biochemical evaluation of novel and potent inhibitors of the enzyme oestrone sulphatase (ES). J. Steroid Biochem. Mol. Biol. 2002c: 80: 419-427.

Ahmed S., James K., Owen C. P. Design, synthesis and biochemical evaluation of AC ring mimics as novel inhibitors of the enzyme estrone sulfatase (ES). J. Steroid Biochem. Mol. Biol. 2002d: 82: 425-435.

Ahmed S., Owen C. P., James K., Patel C. K. Evidence for the mechanism of the irreversible inhibition of oestrone sulphatase by aminosulphonate based compounds. J. Steroid Biochem. Mol. Biol. 2002e: 80: 429-440.

Ahmed S., Owen C. P., Patel C. K. Inhibition of estrone sulfatase (ES) by alkyl and cycloalkyl ester derivatives of 4-[(aminosulfonyl)oxy] benzoic acid. Bioorg. Med. Chem. Lett. 2004a: 14: 605-609.

Ahmed S., Owen C. P., Patel C. K., Aidoo-Gyamfi K. Structure-Activity Relationship Determination Study of a series of Novel Compounds as Potential Inhibitors of the Enzyme Estrone Sulfatase. Lett. Drug Des. Discov. 2004b: 1: 35-44.

Ali S., Coombes R. C. Estrogen receptor alpha in human breast cancer: Occurance and significance. J Mammary Gland Biol. Neoplasia. 2000: 5(3): 271-281.

Anderson C., Freeman J., Lucas L. H., Farley M., Dalhoumi H., Widlanski T. Estrone sulfatase: Probing structural requirements for substrate and inhibitor recognition. Biochemistry. 1997: 36: 2586-2594.

Andrade G., De Leon R. Jr., Brunch H. Mortaldidad por algunas causas prevenibles. Investigacion interamericana de mortalidad. Salud Publica Mex. 1964: 6: 848-855

Appel A., Berger G. Über das hydrazidosulfamide. Chem. Ber. 1958: 91: 339-1341.

Aulin-Erdtman G., Sanden R. Spectrographic contributions to lignin chemistry IX – Absorption properties of some 4-hydroxyphenyl, guaiacyl, and 4-hydroxy-3.5-dimethoxyphenyl type model compounds for hardwood lignins. Acta Chem. Scand. 1968: 22: 1187.

-B-

Baum J. C., Black B. E., Precedo L., Goehl J. E., Langler R. F. Sulfonyl esters elucidation of the first sequence in the trithioorthoformate reaction. Can. J. Chem. 1995: 73: 44-452.

Beatson G. T. On the treatment of inoperable cases of carcinoma of the mama:suggestions for a new method of treatment with illustrative cases. Lancet 1896: 2: 104-107.

Blakey W., Jones W. I., Scarborough H. A. Substitution products of 4-hydroxybenzophenone and of its methyl ether. J. Chem. Soc. 1927: 2865.

Bonney R. C., Reed M. J., Davidson K., Beranek P. A., James V. H. T. The relationship between 17β-hydroxysteroid dehydrogenase activity and oestrogen concentrations in human breast tumours and in normal breast tissue. Clin. Endocr. 1983: 19: 727-739.

Brinbock H., Von Angerer E. Sulfate derivatives of 2-phenylindols as novel steroid sulfatase inhibitors. Biochem. Pharmacol. 1990: 33: 1709.

Brodie A. M. H., Njar V. C. O. Aromatase inhibitors and breast cancer. Semin. Oncol. 1996: 23: 10-20.

Brodie A. M. H., Lu Q., Long B. Aromatase and its inhibitors. J Steroid Biochem. Mol. Biol. 1999: 69: 205-210.

Brodie A. M. H. Aromatase inhibitors in breast cancer. Trends in endocrinology and metabolism. 2002: 13: 61-65.

Buckley C. H. Tamoxifen-associated postmenopausal endometriosis. Histopathology. 1997: 31: 296-296.

Buehler C. A., Gardner T. S., Clemens M. L. Parachor studies at various temperatures. J Org. Chem. 1937: 2: 137.

Buu-Hoi N. P., Xuong N. D., Lavit D. Halogenated *o*- and *p*-phenolic ketones. J. Chem. Soc. 1954: 1034.

-C-

Carey F. A. Organic Chemistry. McGraw: 2000.

Carlstrom K., Doberl A., Pousette A., Rannevik G., Wilking N. Inhibition of steroid sulfatase by danazol. Acta Obstet. Gynec. Scand. 1984a: 367: 1223-1229.

Carlstrom K. In Recent Results in Cancer Research (Eds: Leclercq G., Toma S., Paridaems R., Heuson J.C.). Springer-Verlag Berlin. 1984b: 145-149.

Carnahan J. C., Closson W. D., Ganson J. R., Juckett D. A., Quaal K.S. Mechanism of cleavage of aryl alkanesulfonates by electron-donors. J. Am. Chem. Soc. 1976: 98: 2526-2531.

Cavill G. W. K., Vincent J. I. Derivatives of 4-hydroxybenzoic acid and its methyl and n-butyl esters. J. Soc. Chem. Ind. London. 1945: 64: 212-214.

Cavill G. W. K., Vincent J. I. Esters of 4-hydroxybenzoic acid and related compounds. Relation between the fungistatic activity and physicochemical properties of the esters. J. Soc. Chem. Ind. London. 1947: 66: 175-182.

Chen C. W., Oberley T. D., Roy D. Inhibition of stilbene estrogen-induced cell proliferation of renal epithelial cells through the modulation of insulin-like growth factor-I receptor expression. Cancer Lett. 1996: 105: 51-59

Chu G. H., Peters A., Selcer K. W., Li P. K. Synthesis and sulfatase inhibitory activities of (E)- and (Z)-4-hydroxytamoxifen sulfamates. Bioorg. Med. Chem. Lett. 1999: 9: 141-144.

Ciobanu L. C., Luu-The V., Poirier D. Nonsteroidal compounds designed to mimic potent steroid sulfatase inhibitors. J. Steroid Biochem. Mol. Biol. 2002: 80: 339-353.

Clarke R., Lippman M. E. Antiestrogens resistance: mechanisms and reversal, in: Teicher B. A. (Ed). Drug resistance in oncology. Marcel Dekker Inc. New York. 1992: 501-536.

Coulthard C. E., Marshall J., Pyman F. L. The variation of phenol coefficients in homologous series of phenols. J. Chem. Soc. 1930: 1-6: 280.

Copping L. G., Kerry J. C., Watkins T. I., Willis R. J., Palmer B. H. Pesticidally active benzophenone hydrazone derivatives, methods for their production and pesticidal compositions and methods. Boots company. EP3913. 1979.

-D-

De Fazi R., Berti G. Anal. Chim.1951: 41: 621-631.

De Jong P. C., Blijham G. H. New aromatase inhibitors for the treatment of advanced breast cancer in postmenopausal women. Netherlands J. Medicine. 1999: 55: 50-58.

Dibbelt L., Kuss E. Human placental steroid-sulfatase solubilised with a cholic-acid derivative: Molecular mass, kinetic properties and susceptibility to glycosidases. Bio. Chem. Hoppe-Seyler. 1991: 372: 173-185.

Dragota I., Ionescu D., Tarnauceanu E., Dobre V., Niculescuduvaz I. Potential anticancer agents synthesis of new methanesulfonate derivatives from benzopyrans and naphtopyrans. Revue Roum. Chem. 1976: 21: 1543-1554.

Dowsett M., Cunniongham D. C., Stein R. C., Evans S., Dehennin L., Hedly A., Coombes R. C. Dose-related endocrine effects and pharmacokinetics of oral and intramuscular 4-hydroxyandrostenedione in postmenopausal breast-cancer. Cancer Res. 1989: 49: 1306-1311.

Duffy J. C., Dearden J. C., Rostron C. Design, synthesis and biological testing of a novel series of anti-inflammatory drugs. J. Pharm. Pharmacol. 2001: 53: 11: 1505-1514.

Duncan L., Purohit A., Howarth N. M., Potter B. V. L., Reed M. J. Inhibition of estrone sulfatase activity by estrone-3-methylthiophosphonate: A potential therapeutic agent on breast cancer. Cancer Res. 1993: 53: 298-303.

-E-

Evans T. R. J., Rowlands M. G., Jarman M., Coombes R. C. Inhibition of estrone sulfatase enzyme in human placental and human breast carcinoma. J. Steroid Biochem. Mol. Biol. 1991: 39: 493-499.

-F-

Fessenden R. J., Fessenden J.S., Logue M. W. Organic Chemistry, Brooks Cole; 6th edition. 1998

Fierz-David H. E., Jadassohn W., Kleemann A. Zum Problem der Anaphylaxie mit chemisch bekannten Substanzen .1939: 22: 1: 3–18.

Fischer D. S., Chander S. K., Woo L. W. L., Fenton J. C., Purohit Q., Reed M. J. Potter B. V. L. Novel D ring modified steroid derivatives as potent, non-estrogenic steroid sulfatase inhibitors with in vivo activity. J. Steroid Biochem. Mol. Biol. 2003: 84: 343-349.

Foster P. A., Newman S. P., Chander S. K., Stengel C., Jhalli R., Woo L. W. L., Potter B. V. L., Reed M. J., Purohit A. In vivo efficacy of STX213, a second generation steroid sulfatase inhibitor, for hormone-dependent breast cancer therapy. Clin. Cancer Res. 2006: 12: 5543-5549.

Furness B. S., Hannaford A. G., Smith P. W., Tatchell A. R. Vogel's textbook of practical organic chemistry. Published by Longman. 1996: Fifth Edition.

-G-

Gibson G. G., Skett P. Introduction to Drug Metabolism. Second Edition. 1994: Published by Stanley Thornes Publishers Ltd.

Graham R., Lewis J. R. Synthesis of 9-Oxoxanthen-2-carboxylic acids. J. Chem. Soc. Perkin Trans. 1. 1978: 8: 876-881.

Greenlee R. T., Hill-Marmon M. B., Murray T, Thun M. Cancer Statistics. CA: A Cancer Journal for Clinicians: 2001: 51: 15-36.

-H-

Harwood L. M., Moody C. J. Experimental organic chemistry principles and practice. 1996: Published by Blackwell science.

Hayward R. L., Dixon J. M. Current limits of knowledge in adjuvant and neoadjuvant endocrine therapy of breast cancer: the need for more clinical research. Surgical Oncology. 2003: 12: 289-304.

Hejaz H. A. M., Purohit A., Mahon M. F., Reed M. J., Potter B. V. L. Recent advances in the development of steroid sulphatase inhibitors. J. Med. Chem. 1999: 42: 3188-3192.

Hirai. Yakugaku Zasshi. 1957: 77: 1285-1286.

Horwitz J. P., Iyer V. K., Vardhan H. B., Corombos J., Brooks S.C. *In-vitro* inhibition of estrogen sulfoconjugation by some 2-substituted and 4-substituted "estra-1,3,5(10)-Trien-17-Beta-OLS1A. J. Med. Chem. 1986: 29: 692-698.

Howarth N. M., Purohit A., Potter B. V. L., Reed M.J. Estrone sulfamates: Potent inhibitors of estrone sulfatase. J. Med. Chem. 1994: 37: 219-221.

Howarth N. M., Purohit A., Reed M. J., Potter B. V. L. Estrone sulfonates as inhibitors of estrone sulfatase. Steroids. 1997: 62: 346-350.

-J-

James K. Synthesis and biochemical evaluation of enzyme inhibitors as potential anti-tumour agents. Thesis. Kingston University. 2000.

James V. H. T., Read M. J. Steroid hormones and human cancer. Prog. Cancer Res. Ther. 1980: 14: 471-487.

Jemal A., Siegel R. S., Ward E., Hao Y., Xu J., Murray T., Thun M. J. Cancer statistics 2008. Can. Cancer J. Clin. 2008: 58: 71-96.

-K-

Kametani T., Umezawa O., Sekine K., Oda T., Ishiguro M., Mizuno D. Studies on anti-cancer agents: XI. Synthesis of methanesulphonates of various carbinols and phenolic compounds and their pharmacological activity. Yakugaku Zasshi. 1964: 84: 237: Chem. Abstr. 1964: 61: 600d.

Koizumi N., Okada M., Iwashita S., Takegawa S., Nakagawa T., Takahashi H. Tomohitoteikoku Hormone MFG Co. Ltd. WO0102349, 2001.

Kolobielski M. Homogeneous non-ionic detergent *p*-n-nonylphenoxydecaethoxyethanol. J. Am. Oil Chem. Soc. 1968: 45: 616.

Kover J., Antus S. Facile deoxygenation of hydroxylated flavonoids by palladium-catalysed reduction of its triflate derivatives. Zeitschrift fur naturforschung section B-A. J. Chem. Sciences. 2005: 60: 792-796.

Krausz F., Martin R. Fries reaction 1–esters of phenols and certain mono-substituted phenols. Bull. Soc. Chim. Fr. 1965: 2: 2192.

Kyowa Hakko Kogyo Co. Ltd., WO0181363, 2001.

-L-

Li P. K., Milano S., Kluth L., Rhodes M. E. Synthesis and sulfatase inhibitory activities of non-steroidal estrone sulfatase inhibitors. J. Steroid. Biochem. Mol. Biol. 1996: 59: 41-48.

Li P. K., Selcer K. W. Steroid sulfatase inhibitors and methods for making and using the same. Duquesne Univ. of the Holy Ghost. WO9903876, 1999.

Li P. K., Akinaga S., Murakata C. Steroid sulfatase inhibitors and methods for making and using the same. Duquesne Univ. of the Holy Ghost & Kyowa Hakko Kogyo Co. Ltd. WO0043408, 2000.

Lide D. R. Handbook of chemistry and physics. Published by CRC press. 1998: 78th Edition.

Lippman M. E., Dickson R. B., Gelman E. P., Rosen N., Knabbe C., Bates S., Bronzert D., Huff K., Kasid A. Growth regulatory and peptide production by human breast carcinoma cells. J. Steroid Biochem. Mol. Biol. 1988: 30: 53-61.

Looker J. H., Heyes C. H. Aroylation by mesyl chloride-carboxylic acid mixtures in pyridine. Synthesis of depside derivatives. J. Am. Chem. Soc. 1957: 79: 745.

Lota R., Olusanjo M., Dhanani S., Owen C., Ahmed S. Synthesis, biochemical evaluation and rationalisation of the inhibitory activity of a range of 4-hydroxyphenyl ketones as potent and specific inhibitors of the type 3 of 17β-hydroxysteroid dehydrogenase (17β-HSD3). J. Steroid Biochem. Mol. Biol. 2008: 111: 128-137.

Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 1951: 265-275.

Luu-The V., Labrie C., Simard J., Luchance Y., Zhao H. F., Conet J., Labrie F. Characterization cDNAs for human oestradiol 17β-dehydrogenase and assignment of the gene to chromosomes 17: Evidence for two mRNA species with distinct 5' termini in human placenta. Mol. Cell. Endocrinol. 1989: 3: 1301-1309.

-M-

Magano J., Chen M. H., Clark J. D., Nussbaumer T. 2-(Diethylamino)ethanethiol, a new reagent for the odourless deprotection of aromatic methyl ethers. J. Org. Chem. 2006: 71: 7103.

McNeill J. M., Reed M. J., Beranek P. A., Bonney R. C., Ghilchick M. W., Robinson D. J., James V. H. T. A comparison of the *in vivo* uptake of ^{3}H-oestradiol by normal breast and breast tumour tissue in postmenopausal women. Int. J. Cancer. 1986: 38: 193-196.

Meyer H. Darstellung der Bromoxybenzoesäuren. Monatatsh. Chem. 1901: 22: 437.

Modro T.A., Pioch J. Substituent effects of phosphorus and arsenic containing groups in aromatic-subsititution .6. orientation in nitration of some anilides and phenyl esters. Can. J. Chem. 1976: 54: 560-565.

Muratori M., Lippi A., Mancina R., Iafrate E. M., Cirillo R., Lopez G., Bigioni M., Maggi M., Criscuoli M., Maggi C. A. Pharmacological profile of MEN 11066, a novel potent and selective aromatase inhibitor. J. Steroid Biochem. Mol. Biol. 2003: 84: 503-512.

-N-

Neubert M.E., Leung K., Jirousek M.R., Ezenyilimba M.C., Sabolkeast S., Ziemnickamerchant B., Sharma R.B. Mol. Crystals Liq. Crystals. 1991: 197: 21-41.

Novartis patent. Chromanone and thiochromanone compounds. AG: WO9952890. 2002a.

Novartis patent. Benzoxa- und benzthiazolylsulfamate sowie ihre verwendung als steroid-sulfataseinibitoren. AG: WO0136398, 2002b.

Nussbaumer P., Lehr P., Billich A. 2-substituted 4-(thio)chromerione 6-O-sulfamates: Potent inhibitors of human steroid sulfatase. J. Med. Chem. 2002a: 45: 4310-4320.

Nussbaumer P., Bilban M., Billich A. 4,4'Benzophenone-*O,O'*-disulfamate: A potent Inhibitor of Steroid Sulfatase. Bioorg. Med. Chem. Lett. 2002b: 12: 2093-2095.

Nussbaumer P., Geyl D., Horvath A., Lehr P., Wolff B., Billich A. Nortropinyl-Arylsulfonylureas as Novel Reversible Inhibitors of Human Steroid Sulfatase. Bioorg. Med. Chem. Lett. 2003: 13: 3673-3677.

-O-

Oberhauser T. A new bromination method for phenols and Anisoles: NBS/HBF_4•Et_2O in CH_3CN. J. Org. Chem. 1997: 62: 4504-4506.

Okada M., Iwashita S., Koizumi N. Efficient general method for sulfamoylation of a hydroxyl group. Tetrahedron Lett. 2000: 41: 7047-7051.

Owen C., James K., Sampson L., Ahmed S. Synthesis and biochemical evaluation of some novel benzoic acid based esters as potential inhibitors of oestrone sulphatase. J. Pharm. Pharmacol. 2003: 55: 85-93.

-P-

Pasqualini J.R., Gelly C., Nguyen B.L., Vella C. Importance of estrogen sulfates in breast cancer. J. Steroid Biochem. Mol. Biol. 1989: 34: 155-163.

Pasqualini J.R., Chetrite G., Nguyen B.L., Maloche C., Delalonde L., Talbi M., Feinsein M.C., Blacker C., Bottella J., Paris J. Estrone sulfate-sulfatase and 17-Beta-hydroxysteroid dehydrogenase-activities – A hypothesis for their role in the evolution of human breast-cancer from hormone-dependence to hormone-independence. J. Steroid Biochem. Mol. Biol. 1995: 53: 407-412.

Pasqualini J.R. Differential effects of progestins on breast tissue enzymes. J. Maturitas. 2003: 46S1: S45-S54.

Pasqualini J.R., Chetrite, G.S. Recent insight on the control of enzymes involved in estrogen formation and transformation in human breast cancer. J. Steroid. Biochem. Mol. Biol. 2005: 93: 221-236.

Patel C. K. Synthesis and biochemical evaluation of inhibitors of estrone sulfatase as potential anti-tumour agents. Thesis. Kingston University. 2003a.

Patel C. K., Owen C. P., Ahmed S. The design, synthesis, and in vitro biochemical evaluation of a series of esters of 4-[(aminosulfonyl)oxy]benzoate as novel and highly potent inhibitors of estrone sulfatase. Biochem. Biophys. Res. Commun. 2003: 307: 778-781.

Patel C. K., Owen C. P., Ahmed S. Inhibition of estrone sulfatase (ES) by alkyl and cycloalkyl ester derivatives of 4-[(aminosulfonyl)oxyl] benzoic acid. Bioorg. Med. Chem. Lett. 2004: 14: 605-609.

Patel M. Synthesis of a series of aminosulfonated compounds as potential inhibitors of estrone sulfatase. Thesis. Kingston University. 2003b.

Pedrazzoli A., Cipelletti G. M. [p-Acetylaminophenol derivatives. 3. 6-Acetamido-3, 4-dihydro-2H-1, 3-benzoxazin-2, 4-diones-3-substituted]. Boll. Chem. Farm. Italian. 1966: 105: 393-399.

Percec V., Bae J. Y., Zhao M. Y., Hill D. H. Aryl Mesylates in metal –catalyzed homocoupling and cross coupling reactions, functional symmetrical biaryls from phenols via nickel-catalyzed homocoupling of their mesylates. J. Org. Chem. 1995: 60: 176-185.

Peters R. H., Chao W. R., Sato B., Shigeno K., Zaveri N. T., Tanabe M. Steroidal oxathiazine inhibitors of estrone sulfatase. Steroids. 2003: 68: 97-110.

Poortman J., Andriesse R., Agema A., Donker G.H., Schwarz F., Thijssen J. H. H., 1980: 219-240. In Adrenal Androgens, Eds: Genazzani A. R., Thijssen J. H. H., Siiteri P. K. New York: Raven Press.

Poirier D., Boivin R. P. 17 α-alkyl- or 17 α-substituted benzyl-17 β-estradiols: A new family of estrone-sulfatase inhibitors. Bioorg. Med. Chem. Lett. 1998: 8: 1891-1896.

Potter B. V. L., Reed M. J. 17-aryl-linker derivatised estrogen 3-sulphamates as inhibitors of steroid sulphatase. Sterix Ltd. WO0216393, 2002a.

Potter B. V. L., Reed M. J. Thioether-sulphamate steroids as steroid sulphatase inhibitors and anti-cancer compounds. Sterix Ltd.: WO0216394, 2002b.

Potter B. V. L., Reed M. J. Steroidal compounds for inhibiting steroid sulphatase. Sterix Ltd.: WO0232409, 2002c.

Potter B. V. L., Reed M. J. Oestrogen-17-sulphamates as inhibitors of steroid sulphatase. Sterix Ltd.: WO0216392, 2002d.

Poulin R., Labrie F. Stimulation of cell-proliferation and estrogenic response by adrenal C-19-δ-5-steroids in the ZR-75-1 human-breast cancer cell-line. Cancer Res. 1986: 46: 4933-4937.

Purohit A., Reed M. J. Oestrogen sulphatase activity in hormone-dependent and hormone-independent breast cancer cell lines: Modulation by steroidal and non-steroidal therapeutic agents. Int. J. Cancer. 1992: 50: 901-905.

Purohit A., Williams G.J., Howarth N. M., Potter B.V.L., Reed M.J. Inactivation of steroid sulfatase by an active site directed inhibitor. Biochemistry. 1995: 34: 11508-11514.

Purohit A., Vernon K. A., Hummelinck A. E. W., Woo L. W. L., Hejaz H. A. M., Potter B. V. L., Reed M. J. The development of a-ring modified analogues of oestrone-3-*O*-sulphamate as potent steroid sulphatase inhibitors with reduced oestrogenicity. J. Steroid Biochem. Mol. Biol. 1998b: 64: 269-275.

-R-

Ralston A. W., Bauer S. T. Relation between chain length and orientation in the acylation of phenol. J. Org. Chem. 1940: 5: 165.

Rang P. H., Dale M. M., Ritter J. M. Pharmacology: Churchill Livingstone: Edinburgh: New York. 1999.

Ravdin P. M., Green S., Dorr T. M., McGuire W. L., Fabian C., Pugh R. P., Carter R. D., Rivkin S. E., Borst J. R., Belt R. J., Metch B., Osborne C. K. Prognostic significance of the progesterone receptor levels in estrogen receptor-positive patients with metastatic breast cancer treated with tamoxifen: results of a prospective Southwest Oncology Group Study. J Clin. Oncol. 1992: 10: 1284-1291.

Reed M. J., Lai L.C., Owen M. A., Singh A., Coldham N. G., Purohit A., Ghlichik M. W., Shaikh N. A., James V. H. T. Effect of treatment with 4-hydroxyandrostenedione on the peripheral conversion of androstenedione to estrone and in vitro tumour aromatase activity in postmenopausal women with breast cancer. Cancer Res. 1990: 50: 193-196.

Reed M. J., Purohit. A. Inhibition of steroid sulfatases. Oxford Uni. Press. 1994: 2: 481-494.

Reed M. J., Purohit A., Woo L. W. L., Potter B. V. L. The development of steroid sulfatase inhibitors. Endocrine-Related Cancer. 1996: 3: 9-23.

Reed M. J., Potter B. V. L. Steroid sulfatase inhibitors. Sterix Ltd WO 98/24802. 1998.

Reed M. J., Potter B. V. L., Hejaz H., Purohit A. Halogenated sulphamate-, phosphonate-, thiophosphonate-, sulphonate- and sulphonamide- compounds as inhibitors of steroid sulphatase. Sterix Ltd.: WO0144268, 2001.

Ritter T., Stanek K.; Larrosa I., Carreira E. M. Mild Cleavage of Aryl Mesylates: Methanesulfonate as Potent Protecting Group for Phenols. Org. Lett. 2004: 6: 1513-1514.

Roy A. B. The hydrolysis of sulphate esters, in: The Enzymes. Boyer P. D. (Ed.), Academic Press: New York.1971: 2-19.

-S-

Sakorafas G. H., Krespis E., Pavlakis G. Risk estimation for breast cancer development: a clinical perspective. Surgical Oncology. 2002: 10: 183-192.

Sam K. M., Boivin R. P., Tremblay M. R., Auger S., Poirier D. C16 and C17 derivatives of estradiol as inhibitors of 17 beta-hydroxysteroid dehydrogenase type 1: chemical synthesis and structure-activity relationships. Drug Des. Discov. 1998: 15: 157-180.

Samson A. M., Santos A. C. The mercuriation of nipasol, a propyl ester of p-hydroxy benzoic acid. Univ. Philippines Sci. Bl. 1934: 4: 149-154.

Santen R. J., Lipton A., Kendall J. Successful medical adrenalectomy with aminoglutethimide: Role of altered drug metabolism. J. Am. Med. Assoc. 1974: 230: 1661-1665.

Santen R. J., Harvey H. A. Use of aromatase inhibitors in breast carcinoma. Endocr.-Relat. Cancer. 1999: 6: 75-92.

Santner S. J., Feil P. D., Santen R. J. In-situ estrogen production via the estrone sulfatase pathway in breast tumours: Relative importance versus the aromatase pathway. J. Clin. Endocrinol. Metab. 1984: 59: 29-33.

Schreiner E. P., Winiski A. P., Wolff B., Billich A. 6-(2-Adamantan-2-ylidene-hydroxybenzoxazole)-*O*-sulfamate: A potent non-steroidal irreversible inhibitor of human steroid sulfatase. Bioorg. Med. Chem. Lett. 2003: 13: 4313-4316.

Schreiner E. P., Billich A. Estrone formate: a novel type of irreversible inhibitor of human steroid sulfatase. Bioorg. Med. Chem. Lett. 2004: 14: 4999-5002.

Schwarz S., Weber G., Schreiber M. Steroids 15. Pharmazie. 1975: 30: 17-21.

Selcer K. W., Jagannathan S., Rhodes M. E., Li P. K. Inhibition of placental estrone sulfatase activity and MCF7 breast cancer cell proliferation by estrone-3-amino derivatives. J. Steroid Biochem. Mol. Biol. 1996: 59: 83-91.

Smith H. J., Nicholls P. J., Simons C., Le Lain R. Inhibitors of steroidogenesis as agents for the treatment of hormone dependent cancers. Exp. Opin. Ther. Patents. 2001: 11: 5.

Srinivas K. V. N. S., Mahender I., Das B. Studies on novel synthetic methodologies, part 21. Efficient and rapid one-pot conversions of aldehydes into nitriles and ketones into amides using silica chloride under microwave irradiation. Chem Lett. 2003: 32: 738-739.

Sykes P. A Guidebook to Mechanisms in Organic Chemistry. Published by Longman Scientific and Technical. 1988: Sixth edition.

-T-

Tanabe M., Peters R. H., Chao W. R., Shigeno K. Estrone sulfamate inhibitors of estrone sulfatase, and associated pharmaceutical compositions and methods of use. Stanford research institute Int. WO9933858, 1999.

Tremblay M. R., Luu-The V., Leblanc G., Noel P., Breton E., Labrie F., Poirier D. Spironolactone-related inhibitors of type II 17β-hydroxysteroid dehydrogenase: chemical synthesis, receptor binding affinities, and proliferative/antiproliferative activities. Bioorg. Med. Chem. 1999: 7: 1013-23.

-V-

Vogel C. L. Hormonal approaches to breast cancer treatment and prevention: An overview. Semin Oncol. 1996: 23: 2-9.

-W-

Wolfe J. P., Buchwald S. L. Palladium-catalyzed amination of aryl triflates. J. Org. Chem. 1997: 62: 1264.

Wong C. K., Keung W. M. Daidzein sulfoconjugates are potent inhibitors of steroid sulfatase. Biochem. Biophys. Res. Commun. 1997: 233: 579-583.

Woo L. W. L., Lightowler M., Purohit A., Reed M. J., Potter B. V. L. Heteroatom-substituted analogues of the active-site directed inhibitor estra-1, 3, 5 (10)-trien-17-one-3-sulphamate inhibit estrone sulphatase by a different mechanism. J. Steroid. Biochem. Mol. Biol. 1996a: 57:79-88.

Woo L. W. L., Purohit A., Reed M.J., Potter B. V. L. Oestrone 3-*O*-(N-acetyl)sulphamate, a potential molecular probe of the active site of oestrone sulphatase. Bioorg. Med. Chem. Lett. 1997: 7: 3075-3080.

Woo L. W. L., Howarth N. M., Purohit A., Hejaz H. A. M., Reed M. J., Potter B. V. L. Steroidal and non-steroidal sulphamates as potent inhibitors of steroid sulphatase J. Med. Chem. 1998: 41: 1068-1083.

Woo L. W. L., Purohit A., Malini B., Reed M. J., Potter B. V. L. Potent active site-directed inhibition of steroid sulphatase by tricyclic coumarin-based sulphamates. Chem. Biol. 2000: 7: 773-791.

Woodcock D. Fungicidal activity and chemical constitution. Part IV. Synthesis of 5-n-alkyl-8-hydroxyquinolines. J. Chem. Soc. 1955: 2: 4391.