SYNTHESIS AND BIOCHEMICAL EVALUATION OF ENZYME INHIBITORS IN THE TREATMENT OF HORMONE-DEPENDENT CANCER.

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

BY

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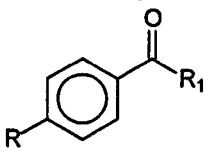
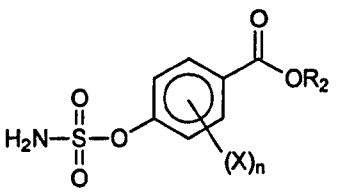
ABSTRACT

The biosynthesis of sex steroids is undertaken by a number of important enzymes such as aromatase, 17β-hydroxysteroid dehydrogenase (types 1 and 3) and estrone sulfatase (ES). Through the inhibition of these enzymes it is possible to reduce the amount of steroids present, which in turn reduces the stimulation of hormone-dependent tissues including breast and prostate cancer cells. Within the current study of my project, we have undertaken the synthesis and limited biochemical evaluation of a number of compounds of varying structural features and which were synthesised as potential enzyme inhibitors in treatment of hormone-dependent cancers. As such, a series of compounds based on the two compounds below were synthesised.



(a)



(b)

Compounds based upon structure (a) were synthesised where R=OH and R_1=alkyl chain or cycloalkyl moiety and evaluated against 17β-HSD1 and 17β-HSD3. The results show that the 4-hydroxyphenyl ketone-based compounds were found to be highly potent against type 3 in comparison to type 17β-HSD1. For example, 1-(4-hydroxy-phenyl)-nonan-1-one (**165**) was found to possess inhibitory activity of 83.53±0.48% (at [I] =100μM) (IC_{50} of 2.86 ± 0.03μM) against 17β-HSD3. Under similar conditions, **165** was found to possess inhibitory activity of 36.32±0.33% (at [I] =100μM) against type1. A range of compounds were also synthesised based on biphenyl ketones where R=phenyl ring and R_1= alkyl chain or cycloalkyl moiety. These compounds were found to be weaker inhibitors of 17β-HSD3 (in comparison to the 4-hydroxyphenyl ketones), however, interestingly they were found to possess greater inhibitory activity against 17β-HSD1. In an effort to determine the selectivity of these compounds against the overall class of HSD enzymes, all inhibitors were evaluated for inhibitory activity against an alternative member of the family of HSD enzymes, namely 3β-hydroxysteroid dehydrogenase (3β-HSD). In general, the synthesised compounds were found to possess weak inhibitory activity against 3β-HSD at inhibitor concentration of 100μM and 500μM. Derivatives of 4-hydroxyphenyl ketones were synthesised (for example, containing sulfamate or methanesulfonate groups substituted at the para position of the phenyl ketones), however, due to the end of the project, they were not evaluated for inhibitory activity against 17β-HSD1 and 3 or against 3β-HSD.

The synthesis of a series of potential inhibitors of ES based on esters of 4-hydroxybenzoic acid [b] were attempted, however, due to severe problems related to the stability of the final sulfamate derivative, the final compounds were not synthesised and only the alkyl and cycloalkyl esters of 4-hydroxybenzoic acid are reported in the current report.

LIST OF ABBREVIATIONS

Acid dissociation constant	pK_a
Aldo-ketoreductase	AKR
Aluminium trichloride	$AlCl_3$
Androgen sensitive	AR^+
Δ5-androstene-3β,17β-diol	Δ5-diol
Androstenedione	AD
4-Androstenedione	Δ^4-DIONE
Aromatase	AR
17beta-hydroxysteroid dehydrogenase	17β-HSD
2-(2-Chlorophenyl)-2-(4- Chlorophenyl)-1,1-dicholoroethane	DDD
Deuterated acetone	d_6-Acetone
Deuterated chloroform	$CDCl_3$
Deuterated methanol	d_6-methanol
Dichloromethane	DCM
Dimethylacetamide	DMA
Dehydroepiandrosterone	DHEA
Dehydroepiandrosterone sulfatase	DHEA-STS
Dehydroepiandrosterone sulphate	DHEAS
Dibenzothiazocine	DBT
5α-dihydrotestosterone	DHT
Dimethylformamide	DMF
Doublet	d
Electron ionisation	EI
Estradiol	E2
Estriol	E3
Estrogen-receptors	ERs
Estrogen-receptor positive/negative	ER+/ER-

Estrone	E1
Estrone sulfatase	ES
Estrone sulfotransferase	EST
Gas Chromatography Mass Spectrometry	GCMS
High resolution mass spectrometry	HRMS
Hours	h
Hydrochloric acid	HCl
17α-Hydroxypregnenolone	17αOH-PREG
17α-Hydroxyprogesterone	17αOH-P
Hydroxysteroid dehydrogenase	HSD
Inhibitor concentration that Gives 50% inhibition	IC_{50}
Michaelis-Menten constant	K_m
Micromolar concentration	µM
Minimolar concentration	mM
Molecular ion	M^+
Inhibition constant	K_i
Literature melting point	lit. m.p.
Low resolution mass spectrometry	LRMS
Luteinising hormone	LH
Magnesium sulfate	$MgSO_4$
Mass spectrometry	MS
Melting point	m.p.
Minutes	min
Multiplet	m
Nanomolar concentration	nm
Nicotinamide adenine (phosphate) Nucleotides	NAD(P)(H)
***N,N*-dimethylacetamide**	DMA
Nuclear magnetic resonance	NMR
Potassium carbonate	K_2CO_3

Pregnenolone	PREG
Progesterone	P
Progesterone receptor	PR
Progesterone receptor positive/negative	PR+/PR-
Quartet	q
Quintet	quin
Refractive f	R_f
Retention time	t_R
Room temperature	RT
Sextet	sex
Short chain dehydrogenase/reductase	SDR
Singlet	s
Sodium carbonate	Na_2CO_3
Sodium hydrogen carbonate	$NaHCO_3$
Sodium hydride	NaH
Sodium hydroxide	NaOH
Structure activity relationship	SAR
Sulfuric acid	H_2SO_4
Triethylamine	TEA
Testosterone	T
Tetrahydrodibenzazocine	THB
Thin layer chromatography	TLC
Triplet	t
Water	H_2O
Wavelength	λ
Wavenumber	ν

AMINO ACID ABBREVIATIONS

Alanine	Ala
Asparagine	Asn
Glutamic acid	Glu

Glycine	Gly
Histidine	His
Isoleucine	Ile
Leucine	Leu
Lysine	Lys
Phenylalanine	Phe
Proline	Pro
Serine	Ser
Threonine	Thr
Tryptophan	Trp
Tyrosine	Tyr
Valine	Val
Methionine	Met

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CHAPTER 1

Introduction

1.0 INTRODUCTION

1.1 Cancer

There are roughly more than 100 different types of cancers present, which could emerge in various ways, and forming many distinct subtypes in each affected organ. Factors that aid the progression of the rate and scope of the tumour include biological, immunological, environmental and genetic factors (Jefford and Irminger-Finger, 2006). It is one of the major causes of death in developed countries; it will affect one in three in the population at some time of their life, and in Europe, there are 2.9 million new cases and 1.7 million deaths from the disease each year (Boyle and Ferlay, 2005). Also as the elderly population increase, the incidence of cancer will continue to increase (Quinn et al, 2003).

There are six main physiological changes that consist of novel capabilities acquired during tumour development. These include self-sufficiency in growth signals, insensitivity to growth inhibitory signals, evasion of programmed cell death (apoptosis), limitless potential to replicate, persistent angiogenesis, and metastasis via tissue invasion (Jefford and Irminger-Finger, 2006).

1.2 Breast cancer

Other than lung and large bowel cancer, breast cancer is the most common type of cancer amongst women, and the number that is diagnosed with breast cancer is about 180,000 each year. In Western countries such as Europe, USA, Canada and South America, 25-30% of the total incidence of cancers in women are represented by breast cancers, and accounts for 15-18% of mortality (Pasqualini, 2004).

Two-thirds of breast cancers are detected in postmenopausal women, and the life-time risk of a woman developing breast cancer is 1 in 8 in the United States, 1 in 12 in Europe and 1 in 80 in Japan (Pasqualini, 2004). Breast

cancer is one of the most prevalent types of cancer, and epidemiological and clinical studies have shown that approximately two-thirds of breast tumours are estrogen-dependent (Henderson and Cannellos, 1980).

1.2.1 Causes of breast cancer

The exact causes of breast cancer are not known, but studies have shown that the risk of breast cancer increases with age. This disease is very uncommon in those under the age of 35, and most breast cancers occur over the age of 50, and the risk is especially high for women over the age of 60. The factors believed to be involved in increasing the chance of developing breast cancer are explained briefly in Table 1a below:

Risk factor	Explanation
Family history	Risk is increased if a family member, for example a mother or sister has had breast cancer.
Diet	Dietary fat increases the risk.
Radiation therapy	X-rays etc are known to increase the risk of breast cancer.
Breast density	Breast cancers occur in dense tissue and not in fatty tissue.
Late childbearing	Risk is increased as longer exposure to estrogens.
Age of menarche	Early menstruation and late meno-pause increases the chance of breast cancer.
Geographical location	Risk is increased for women in developed countries compared to developing countries.
Alcohol	Studies have shown that this increases the risk.

Table 1a: Table showing the risk factors associated with breast cancer.

1.2.2 Breast Cancer and Estrogens

Breast cancer can be divided into two sub-types, namely hormone-dependent and hormone-independent. In the former case, the cancer depends on hormones for growth, i.e. estrogen stimulation (Santen et al, 1990). About 95% of breast cancers are hormone-dependent initially, whether they occur in pre- or postmenopausal women, whereby the more potent estrogen, estradiol (E2) plays a very important role in the development and progression of the cancer (Pasqualini, 2004).

Estrogens are produced by the granulosa cells in the ovary as a result of stimulation by follicle stimulating hormone (FSH) from androgen precursor molecules, which are themselves derived from thecal cells stimulated by luteinising hormone (LH). Estrogens are also produced in moderate quantity by the placenta and in small amounts by the testis in males and by the adrenal cortex in both sexes. Some other tissues, such as the liver, muscle, fat and hair follicles, can also convert steroid precursors into estrogens.

Roles of estrogens include stimulating the maturation in females during puberty, development of the breast and maintaining the normal structure of skin and blood vessels (Green et al, 1993).

In the management of breast cancer, hormone-receptors are widely accepted as prognostic and therapeutic tools. The expression of estrogen-receptors (ERs) is thought to be of great importance due to the fact that majority of all breast cancers are ER-positive (ER+) and progesterone receptor (PR)-positive (PR+), hence making these tumours acquiescent to treatment with adjuvant hormonal therapy. Nearly 33% of breast cancers are hormone-insensitive, whereby they lack both ER and PR. These tumours are more common among younger breast cancer patients. ER- tumours characteristically have a poor prognosis, and they do not respond to conventional hormone therapies (Pommier et al, 2006).

1.2.3 Estrogen Synthesis

The starting substance for steroid biosynthesis is cholesterol (Figure 1.1). The immediate precursors to estrogens are androgenic substances androstenedione or testosterone. There are three main endogenous estrogens in humans namely estrone (E1), estradiol (E2), and estriol (E3). E2 is the principle estrogen secreted by the ovary, and is the most potent.

In post-menopausal women, there is also found to be a high level of circulating estrone sulphate. Estrone sulfate is probably the principle estrogen reservoir, since its levels are known to be 8- to 10-fold higher than those of free estrone. Breast tissue concentrations of estrone sulfatase (the enzyme that converts estrone sulfate to estrone) are 1000-fold higher than those of aromatase (Noel et al, 1981; Samojlik et al, 1982; James et al, 1987; Pasqualini et al, 1986).

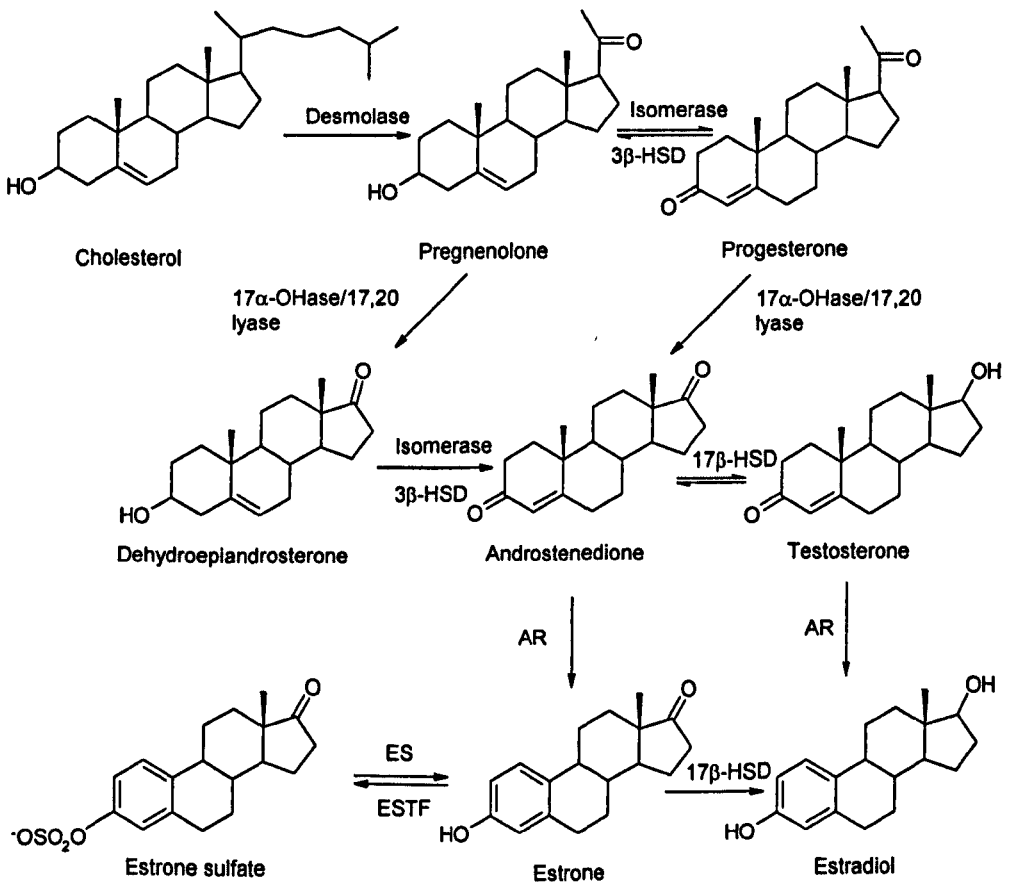


Figure 1.1: Diagram showing steroid biosynthesis (Rang et al, 1998), where HSD=Hydroxysteroid dehydrogenase, AR=Aromatase, OHase=hydroxylase, ES=Estrone sulfatase, ESTF=Estrone sulfotransferase.

1.3 Therapies for Breast Cancer

There have been many therapies developed for the treatment of breast cancer. The type of treatment used depends on the stage of the tumour and these are shown in Table 1b below.

Therapy	Method of treatment
Surgery	Lumpectomy or mastectomy.
Radiotherapy	Use of ionising rays.
Chemotherapy	Use of for example alkylating agents such as cyclophosphamide that interfere with DNA strands and hence stop transcription.
Hormonal therapy	Use of anti-estrogens for example tamoxifen or an aromatase inhibitor such as Letrozole.
Gene therapy	Insertion of normal gene into non-specific location, within the genome to replace the non-functional gene.
Immunotherapy	Use of monoclonal antibodies for example Rituximab, to specifically target cells.

Table 1b: Table showing the types of treatments available for breast cancer.

Hormonal therapy can be used in all stages of hormone-dependent breast cancer (Osborne et al, 1980) and those most currently in use involve either a reduction in levels of stimulating hormones in the body or the prevention of their stimulatory effects.

Hormonal therapy is a vital part of the management of most women with breast cancer regardless of whether it is in the localised (primary) or metastatic (secondary) stages. A number of hormonal agents are clinically in

use, including antiestrogens (e.g. tamoxifen), progestins, androgens, estrogens, luteinising hormone-releasing hormone (LH-RH) analogues and enzyme inhibitors (Ingle et al, 1999). Another method used in an attempt to prevent the stimulation of breast cancer by estrogens is by surgical removal of the ovaries (oophorectomy).

1.3.1 Hormonal therapy via inhibition of estrogens

Blockade of the terminal steps in estrogen biosynthesis is the preferred strategy, preventing the inhibition of production of other important steroids (Santen et al, 1999). There are three main enzyme complexes involved in estrogen synthesis in breast tumours, namely; Aromatase (AR) – which converts androgens to estrogens, 17beta-hydroxysteroid dehydrogenase (17β-HSD) – which transforms E1 into its biologically active form, E2 and finally Estrone sulfatase (ES) – which hydrolyses estrone sulfate to E1 (Figure 1.2). Each of these enzymes has been targeted in an attempt to reduce the levels of stimulating estrogens. At present, estrogen synthesis inhibitors are used as a second-line therapy, but an AR inhibitor, anastrozole, is now thought to be more effective than tamoxifen (the first-line treatment for women with advanced breast cancer) and will replace it soon as first-line therapy.

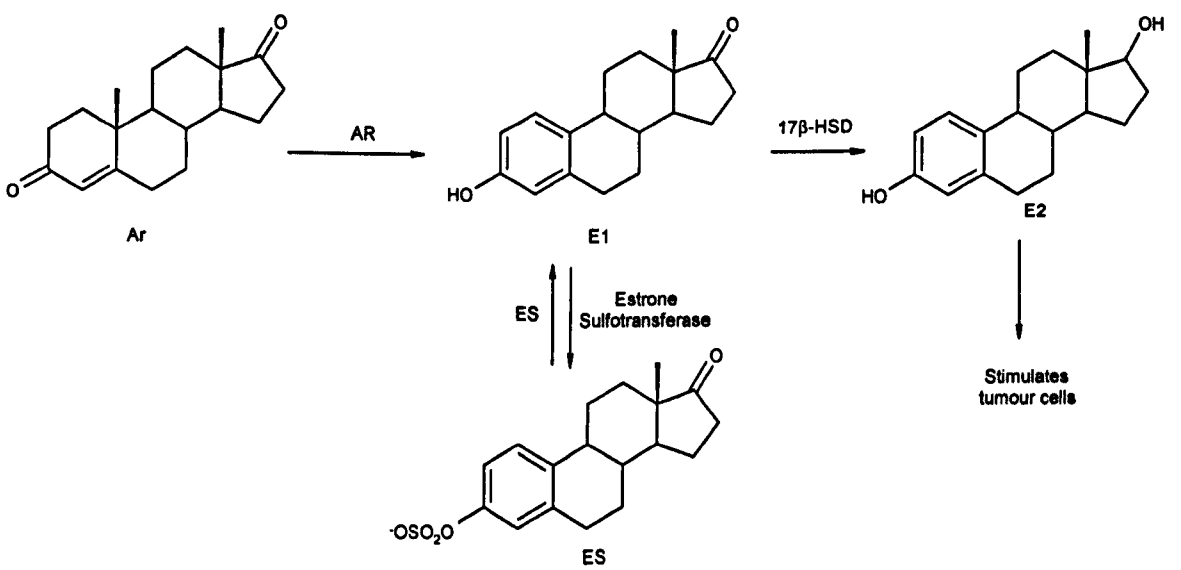


Figure 1.2: Enzymes involved in estrogen synthesis.

1.4 Hydroxysteroid dehydrogenases (HSDs)

HSDs are responsible for the pre-receptor regulation of steroid hormone action (Morris et al., 2003), and they do so by converting steroids at positions 3, 5, 11, 17 and 20 of the steroid backbone.

1.4.1 17β-HSDs

Steroid hormone activation and deactivation is carried out by 17β-HSDs. The family of 17β-HSDs, which by conversion at position 17, modulate biological potency of estrogens and androgens; the keto-forms being inactive, whereas the hydroxy-forms are active and these access the receptors (Mindnich et al, 2004) (Figure 1.3).

There are different isozymes of 17β-HSDs known. They are coded by different amino acid (aa) sequences revealing different subcellular localisations, as well as cofactor and substrate preferences. 17β-HSDs belong to two protein super families; namely the short chain dehydrogenase/reductase (SDR) and aldo-ketoreductase (AKR) (Oppermann et al, 1999; Penning, 2003).

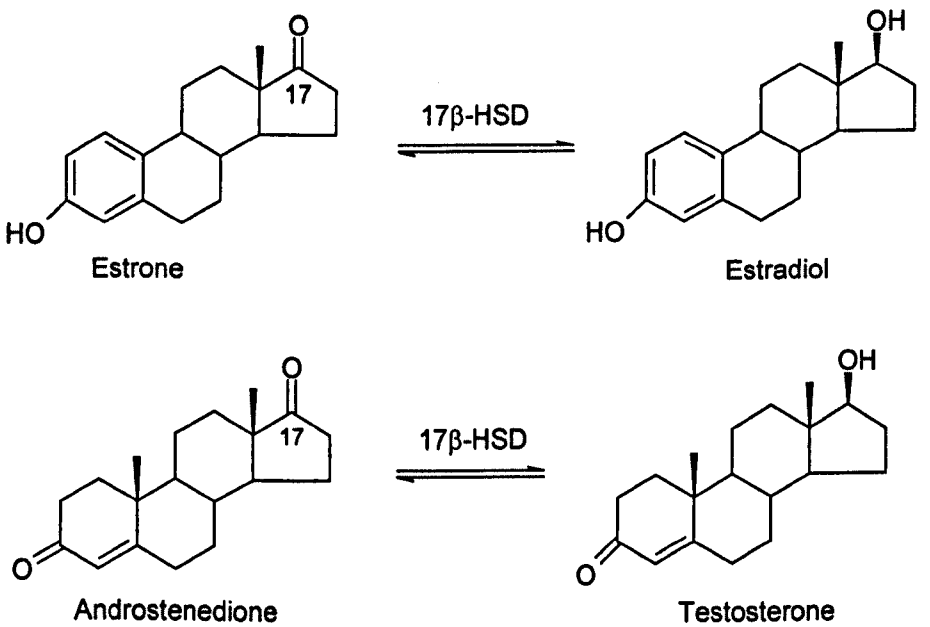


Figure 1.3: The action of 17β-HSD on estrogens and androgens by redox reactions at position 17 (Mindnich et al, 2003).

At present, fourteen forms of 17β-HSDs are known, most of which are members of the SDR protein superfamily. Not all known 17β-HSD forms are present in every species, 12 types are found in humans, 17β-HSD types 6 and 9 being found in rodents so far. One exception is 17β-HSD type 5 that belongs to the AKR-family. These isozymes utilise nicotinamide adenine (phosphate) nucleotides NAD(P)(H) as cofactors for reduction or oxidation reactions of steroids (Adamski et al, 2003).

SDRs are mainly multimeric enzymes, with a monomer mass of about 30kDa, and these transfer the pro-*S*-hydride ion from the cofactor to the steroid, whereas AKRs are monomeric, with a monomer molecular mass of around 37kDa, and these transfer the 4-pro-*R*-hydride ion from the cofactor to the steroid substrate (Mindnich et al, 2004).

It was observed by Ryan and Engel (1953), that E1 and E2 were metabolised by human tissues, and shortly after that, Langer and Engel (1958) described the first partial purification of 17β-HSD from human placenta.

17β-HSD type 1 (17β- HSD1) contributes towards breast cancer and its main involvement is in the role of E2 synthesis via the reduction of E1 and is expressed mainly in the placenta, ovary and breast (Poirier and Tremblay, 1998). It is also expressed in carcinoma *in situ* and invasive ductal carcinoma. In addition, its co-expression was positively correlated with estrogen receptor status in invasive ductal carcinoma cases. These results indicate that breast carcinoma can effectively convert E1 to active E2 and thus exerts estrogenic actions on tumour cells through the estrogen receptor (Gunnarson et al, 2001). 17β-HSD1 also catalyses the conversion of DHEA to Δ5-androstene-3β,17β-diol (Δ5-diol).

17β-HSD2 is mainly expressed in the breast, endometrium, placenta, liver, small intestine, kidney, pancreas and colon (Blomquist et al, 1985[b]) and plays a major role in the inactivation of potent steroid hormones by oxidising E2 and

testosterone (T) to E1 and androstenedione (AD) respectively (Wu et al, 1993). These reactions can occur in virtually all tissues (Peltoketo et al, 1999).

17β-HSD3 and 17β-HSD4 are expressed in the testis and in peripheral tissues respectively (Poirier and Tremblay, 1998). The reduction of AD to T is catalysed by 17β-HSD3, whereas 17β-HSD4 catalyses the conversion of Δ5-diol to DHEA (Adamski et al, 1995, Labrie et al, 2000) and the oxidation (inactivation) of E2.

T formation from AD is catalysed by 17β-HSD5 (Lin et al, 1997; Rheault et al, 1999) and it catalyses the conversion of DHEA to Δ5-diol.

17β-HSD8 catalyses the oxidation of E2 and 17β-HSD2 and 17β-HSD8 might be involved in the catalysis of T oxidation (Andersson and Moghrabi, 1997; Fomitcheva et al, 1998). 5α-Reductase reduces T at position 5 to produce the potent androgen DHT. DHT may undergo reduction at position 3 to form the weak androgen 5α-androstane-3α,17β-diol, and if that is the case, then the reaction might be catalysed by 17β-HSD5, 7 and 10 as well as 3α-hydroxysteroid dehydrogenases (Penning et al, 2001; Törn et al, 2003).

17β-HSD10 also carries out the oxidation of E2 and is found universally (Adamski et al, 1995; Fomitcheva et al, 1998; He et al, 1999; Vihko et al, 2001; Wu et al, 1993). 17β-HSD13 activity has not been demonstrated as yet, and 17β-HSD14 is expressed in the kidney and in the central nervous system and is involved in E2 and T inactivation (Lukacik et al, 2006).

Table 1c shows a summary of some of the isozymes, their function and the disease pathology.

Enzyme type	Human function	Disease or participation in pathology
17β-HSD1	E2 synthesis	Breast and prostate cancer
17β-HSD2	E2 and T inactivation	Endometriosis, colon and prostate cancer
17β-HSD3	T synthesis	Pseudohermaphroditism
17β-HSD4	E2 inactivation and androgen metabolism	D-specific multifunctional protein deficiency
17β-HSD5	T synthesis	Not known
17β-HSD6 (known in rats only)	Not known	Not known
17β-HSD7	E2 synthesis	Not known
17β-HSD8	Estrogen and androgen inactivation	Not known
17β-HSD9	Not known	Not known
17β-HSD10	Estrogen and androgen inactivation	Alzheimer's disease
17β-HSD11	Estrogen and androgen inactivation	Not known
17β-HSD 12	Fatty acid synthesis	Not known
17β-HSD 13	Not known	Not known
17β-HSD 14	E2 and T inactivation	Not known

Table 1c: Table showing the different forms of 17β-HSD (Mindnich et al, 2004).

1.4.2 Mechanism for 17β-HSDs

The chemical mechanism for HSDs was revealed by site-directed mutagenesis, whereby seven active site mutants namely; Y55F, Y55S, H117A, D50N, D50E, K84R and K84M were over expressed and purified to

homogeneity from *E. coli* (Pawlowski and Penning, 1994; Schlegel et al, 1998). All mutants formed the binary complex *E*.NADPH unimpeded (Penning et al, 1999).

The mechanism of action of the 17β-HSD family of enzymes is thought to occur in two ways, and involves either an oxidative or a reductive mechanism. The reductive catalytic mechanism is shown in Figure 1.4.

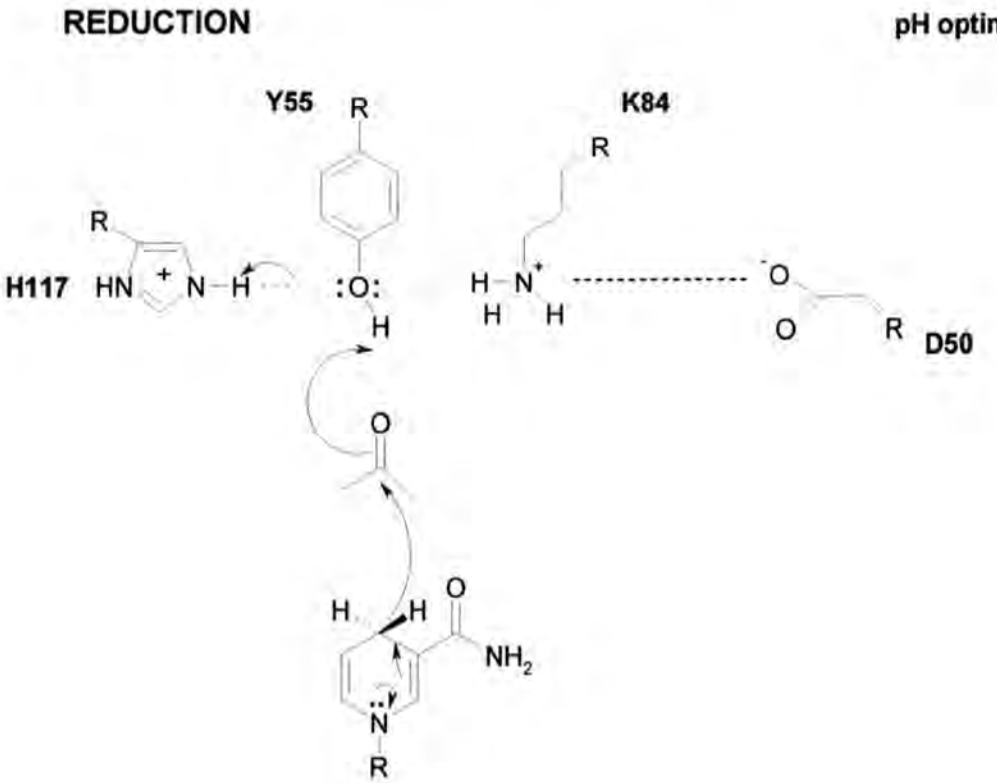


Figure 1.4: Catalytic Mechanism for 17β-HSD via the reductive route (Penning et al, 1999).

In the reductive mechanism, histamine (H117) aids proton donation by tyrosine (Y55). Y55 donates it proton to the acceptor carbonyl group of the steroid. The tyrosyl hydroxyl group of the imidazole ring of H117 undergoes protonation (Penning et al, 1999).

The oxidative catalytic mechanism, on the other hand is shown in Figure 1.5.

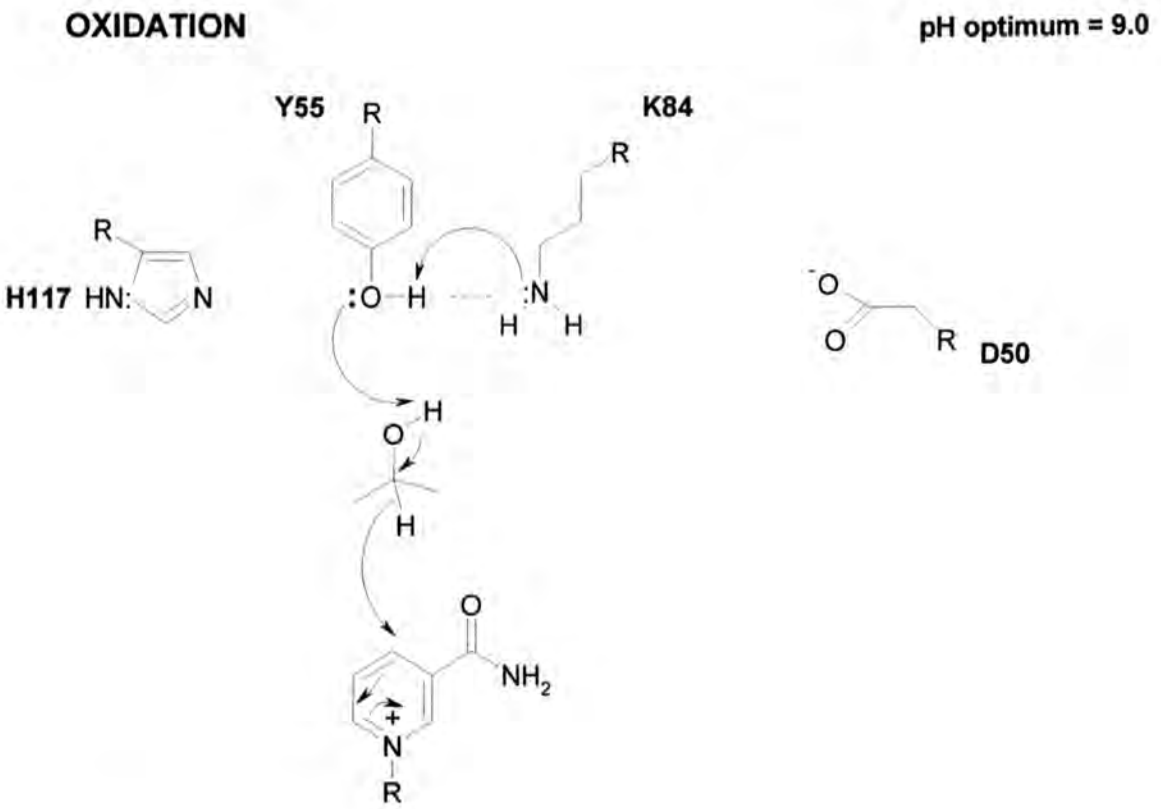


Figure 1.5: The catalytic mechanism of 17β-HSD via the oxidative route. (Penning et al, 1999)

In the oxidation step, the amino group of K84 aids proton removal by Y55, and forms a phenolate anion on Y55. Hydride transfer is facilitated from the steroid alcohol (Penning et al, 1999).

1.4.3 Enzyme structure

17β-HSD1 belongs to the SDR family, and requires a Tyr-X-X-X-Lys motif and a Tyr-Lys-Ser catalytic triad for activity (Ghosh et al, 1995; and Ghosh et al, 1994).

An example of how binding occurs at the catalytic site of the enzyme, the structure of an inhibited ternary complex of 17β-HSD1 with $NADP^+$ and 3-hydroxyestra-1,3,5,7-tetraen-17-one (equilin) is shown in Figure 1.6. Equilin is one of the major components of estrogens used in estrogen replacement therapy, along with estrone and 17α-dihydroequilin. It was observed that the 17β-HSD1-equilin complex crystallised in space group $P2_12_12_1$ with a dimer in the asymmetric unit. A homo-dimer is known to be the functional unit of the enzyme (Lin et al, 1992), and it is this holo-form that represents a true ternary complex of the wild-type enzyme, with the cofactor and a steroidal ligand.

With regards to the ligand-binding interactions, both equilin and $NADP^+$ have well-defined electron density in the A subunit of the dimeric enzyme. The structure of the active-site of the 17β-HSD1-equilin complex for the A subunit is shown below in Figure 1.6.

The equilin molecule forms four hydrogen bond contacts with protein atoms, and the 17-keto oxygen accepts protons from catalytic residues Tyr-155 and Ser-142 (2.7 and 2.8Å, respectively) at the catalytic end of the steroid-binding cleft. The 3-hydroxy group of the ligand forms a bifurcated hydrogen bond to His-221 and Glu-282 (2.9 and 2.9Å, respectively) at the recognition end of the cleft. Therefore with the exception of these two hydrophilic ends, the rest of the steroid-binding cleft is almost exclusively hydrophobic.

The residues Val-143, Met-147, Leu-149, Pro-150, Asn-152, and Tyr-218 compose the protein surface in the vicinity of the β-Face of the nearly coplanar A-B rings of the ligand (Figure 1.7).

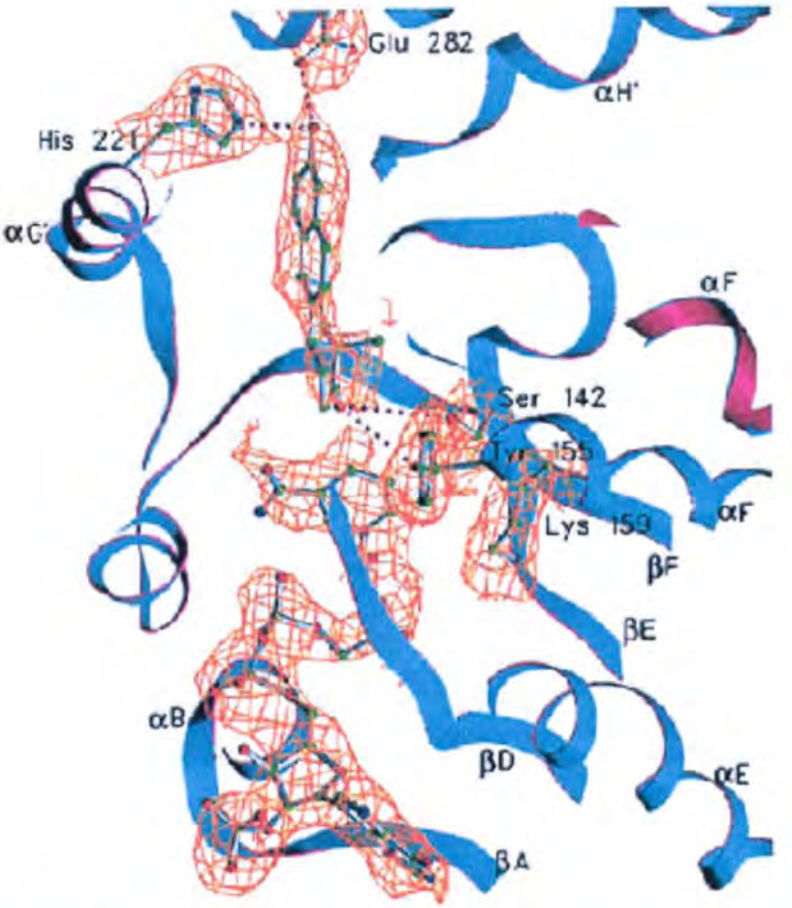


Figure 1.6: Active-site view within the A subunit, $NADP^+$ and equilin, with side chains of amino acid residues Ser-142, Tyr-155, and Lys-159 at the catalytic end and His-221 and Glu-282 at the recognition end of the active-site cleft. Equilin is not included above, and blue ribbon represents the backbone of the A subunit, and the magenta ribbon represents the B subunit (Sawacki et al, 1999).

The residues Val-225 and Pro-187 are within Van der Waals contact distances to the equilin α face, whereas residues Leu-262, Leu-263 and Met-279 are at the floor of the cleft. Figure 1.6 shows the schematic view of the steroid-binding cleft, from a 90° angle, whereas in Figure 1.7, it is perpendicular to the β-face of equilin. The amino acid side chains involved in hydrogen bond formation with the ligand (as well as those side chains lining the cleft) are depicted (Sawacki et al, 1999).

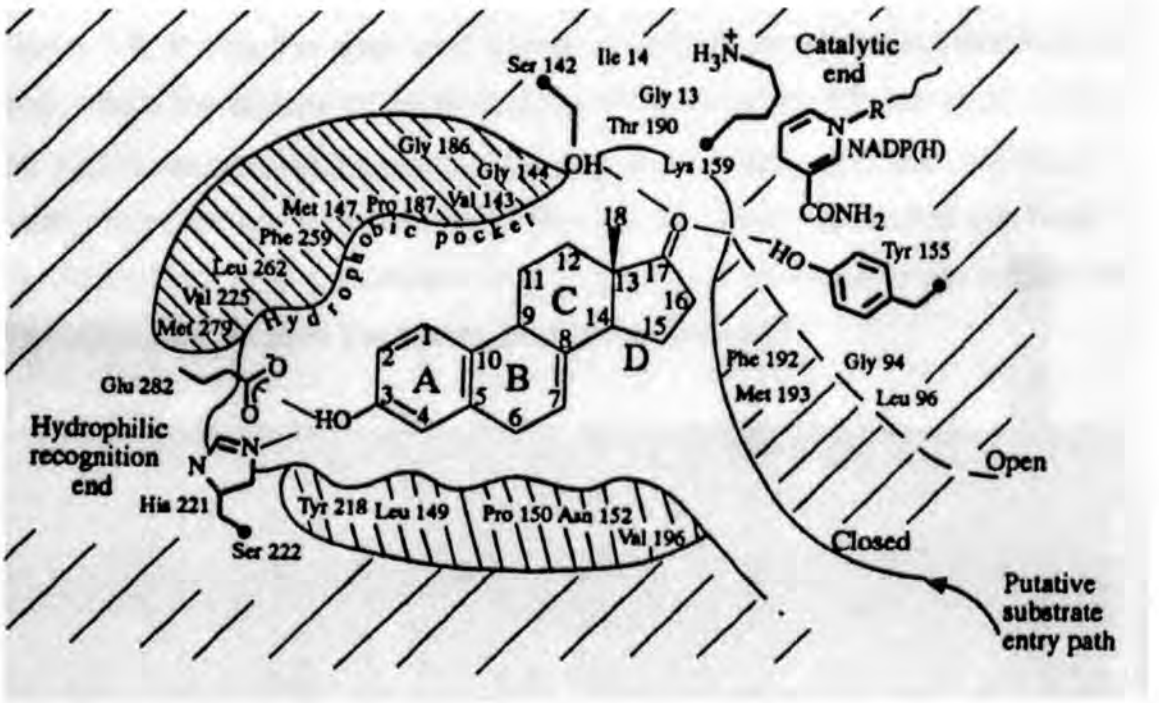


Figure 1.7: Diagram of the ligand-binding pocket, viewed roughly perpendicular to the β-Face of the equilin molecule (Sawacki et al, 1999).

As well as containing the hydrophobic environment of the cleft, Phe-192 and Met-193 from the substrate-entry loop line the entry path, and in the apoenzyme structure, the substrate-entry loop adopts an *open* conformation providing unrestricted access to the active site cleft (Ghosh et al, 1995). The substrate-entry loop forms a *closed* conformation instead, in the 17β-HSD1-equilin complex, whereby the polypeptide chain with residues 186-201 moves towards the catalytic cleft, hence restricting active site access, as shown in Figure 1.7 above. Van der Waals interactions are formed with the ligand molecule, in this *closed* conformation (Sawacki et al, 1999).

For the mechanism of the hydride transfer, the structure-based hypothesis was proposed, as such that an electrophilic attack on the C17-keto oxygen through strong hydrogen-bonding interactions by the hydroxyl groups from Tyr-155, Ser-142, or both, as well as correct orientation and proximity of C4 β-hydride of the nicotinamide at the α-face of estrone, are required for the transition state initiation of the reaction.

Figure 1.8, shows the open and closed conformations of the substrate-entry loop, where the dimeric C^{α} backbones of the apoenzyme (Ghosh et al, 1995), the H221L mutant-NAD complex (Mazza et al, 1998), and the 17β-HSD1-equilin complex are superimposed. The overall tertiary structures are nearly identical except for the substrate-entry loop (shown as thicker cross section of the backbone) between the strand βF and the helix αG".

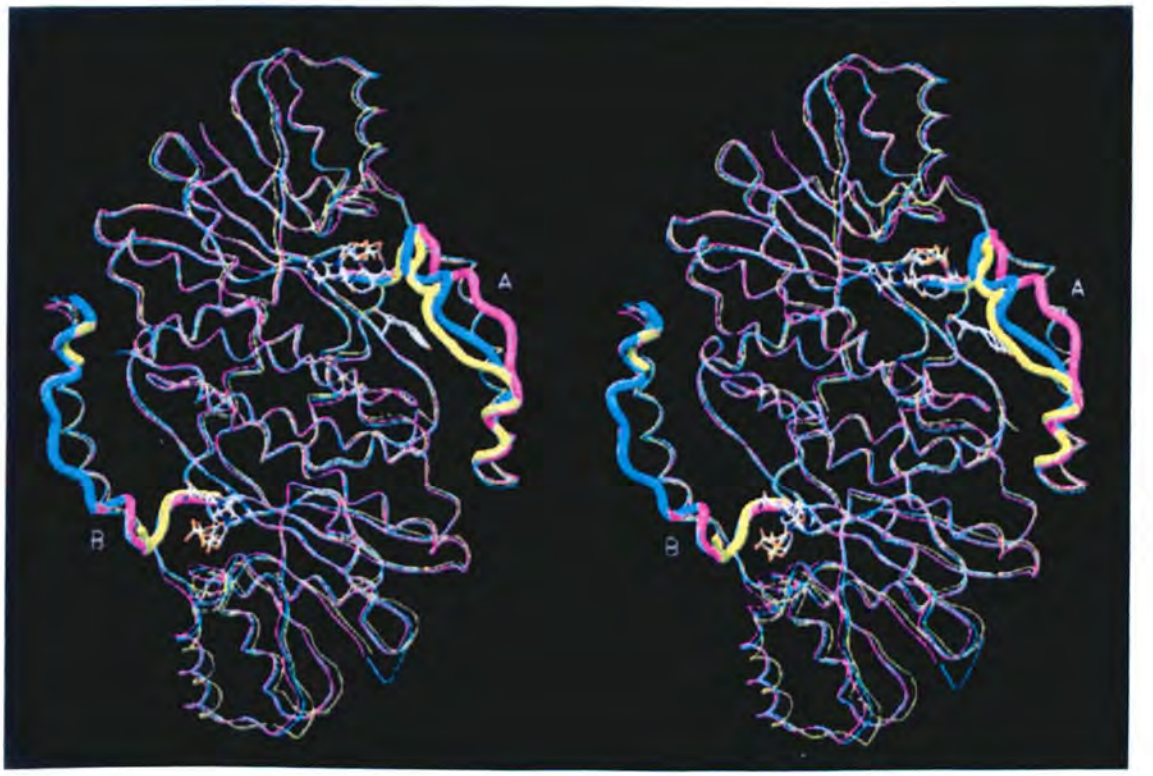


Figure 1.8: Superimposition of the C^{α} backbones of the dimmers of the 17β-HSD1-equilin complex (shown in blue), the apo-17β-HSD1 (shown in magenta) (Ghosh et al, 1995)-E_2-complex (shown in yellow) (Breton et al, 1996) illustrating the differences between the substrate-entry loop structures of the A and B subunits. The A subunit loop of 17β-HSD1-equilin complex is in the *closed* conformation, whereas the B subunit was modelled after the apo form (Sawicki et al, 1999).

The loop in the A subunit containing well defined electron density except for the Lys-195 side chain and packs against both equilin and $NADP^+$ via Phe-192 and Met-193 is shown in Figure 1.9. Both of these line the substrate-entry

path and are involved in Van der Waals interactions with the D ring of the equilin and the nicotinamide head group of $NADP^+$. When the substrate-entry loop is closed, the ligand in the steroid-binding cleft gets trapped and thus occludes the entry path, as shown in Figure 1.7.

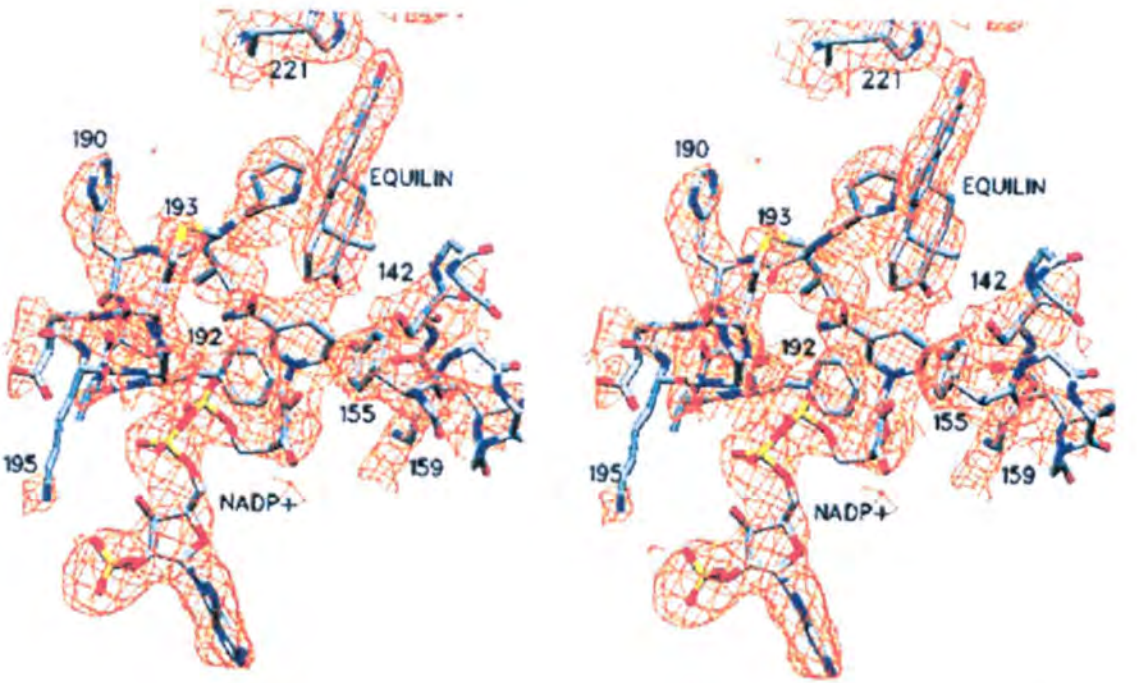


Figure 1.9: Substrate-entry loop structure of the A subunit with $NADP^+$ and equilin. The substrate-entry loops residues are labelled along with the catalytic triad and His-221. The electron density map contoured is shown in orange colour and the densities for all the residues before Ser-142 were removed for clarity (Sawicki et al, 1999).

The location and overall conformation of the cofactor $NADP^+$ are similar to those in crystal structures of other short-chain dehydrogenases/reductases (Duax et al, 1996). The bound $NADP^+$ is present in an extended conformation with the adenine ring in an *anti* conformation and the nicotinamide ring in a *syn* conformation. This conformation is consistent with the 4-*Pro-S* hydride transfer from the B face of the nicotinamide ring.

There are 11 different residues that are involved for the specific contacts between the protein and the other cofactor $NADP^+$. These are Ser-11, Ser-12, Ile-14, Arg-37, Leu-64, Val-66, Asn-90, Gly-92, Tyr-155, Thr-190 and Phe-192 (Figure 1.10). The adenine nucleoside moiety lies in the cleft surrounded by five segments of the polypeptide, βA-to-αB turn (residues 9-12), βB-to-αC turn (residues 36-38), end of βC (residues 64-66), residues 90-93 from βD and val-113 from αE. Stabilisation of the nicotinamide nucleoside moiety is achieved through hydrogen-bonding contacts and hydrophobic interactions (Sawicki et al, 1999).

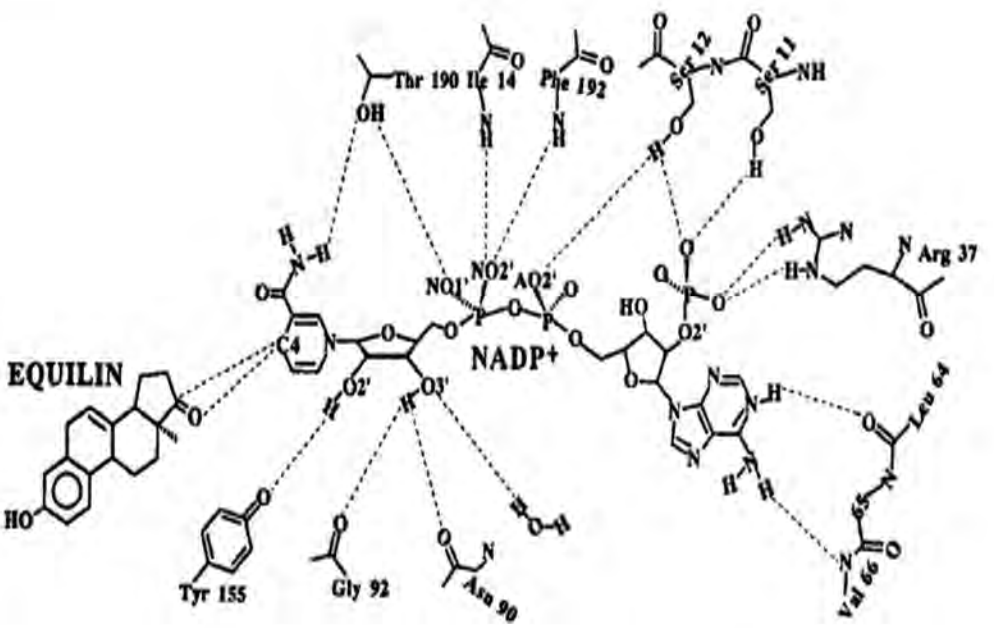


Figure 1.10: Diagram showing $NADP^+$ interactions with protein and equilin atoms, with the residues and atoms that are involved in direct contacts being labelled appropriately (Sawicki et al, 1999).

17β-HSD inhibitors

The synthesis of inhibitors for 17β-HSDs in general is difficult as there are many isozymes present for 17β-HSD, and so cannot be targeted singly for the inhibitors. Hence the isozymes have been looked at and their inhibitors have

been synthesised for each isozyme. The inhibitors for 17β-HSD1 and 3 are described in detail below.

1.5 17β-HSD1 inhibitors

Early work on the inhibitors of 17β-HSD1 was based on E1 or E2 derivatives which were substituted at C-2 or C-4 (or both), at C-3 or altered in number or location of double bonds (substituted at C-1, disubstituted at C-1, and C-2, disubstituted at C-1 and C-4). Also, substitution with an oxygenated B-ring and a substituted C-ring or D-ring was carried out. Jaraback and Sack (1969) investigated other compounds for their inhibitory effect on the reduction of E2 to E1 and most of the compounds which included E2 analogues, C19 and C21 steroid hormones and non-steroidal compounds, were found to inhibit the reduction of E2. From these compounds, 17-desoxy-E2, o,p'-DDD and U-11-100A (shown in Figure 1.11) were found to be the best inhibitors, having inhibition constants (K_i) values of 0.19, 0.22 and 0.61μM, respectively. For equilin, an inhibitor concentration (IC_{50}) of 1.9μM was observed, which was similar to the IC_{50} value of U-11-100A (1.5μM) (Poirier, 2003).

Figure 1.12 shows some inhibitors which include16-difluoro-E1, m,p'-DDD, diethylstilbestrol, and E1 and these inhibited the enzyme with K_i values of 2.5, 2.8, 3.3 and 3.7μM respectively (Poirier, 2003).

The inhibition of 17β-HSD1 from 3-methyl-O-E2 oxidation to 3-methyl-O-E1 (with the K_m value being 2.2μM), by C18 and C19 steroids and by non steroidal alcohols was discovered by Blomquist et al, (1978). The non-steroidal alcohols, which were the simple forms of alcohols, were found to be poor inhibitors, with the exception of phenol, which was a non-competitive inhibitor with a K_i value of 0.7mM. The other alcohols were competitive inhibitors with K_i values of 2, 9, 10 and 37mM, respectively for 1,9-decanediol, cyclohexanol, benzyl alcohol and cyclopentanol (Poirier, 2003).

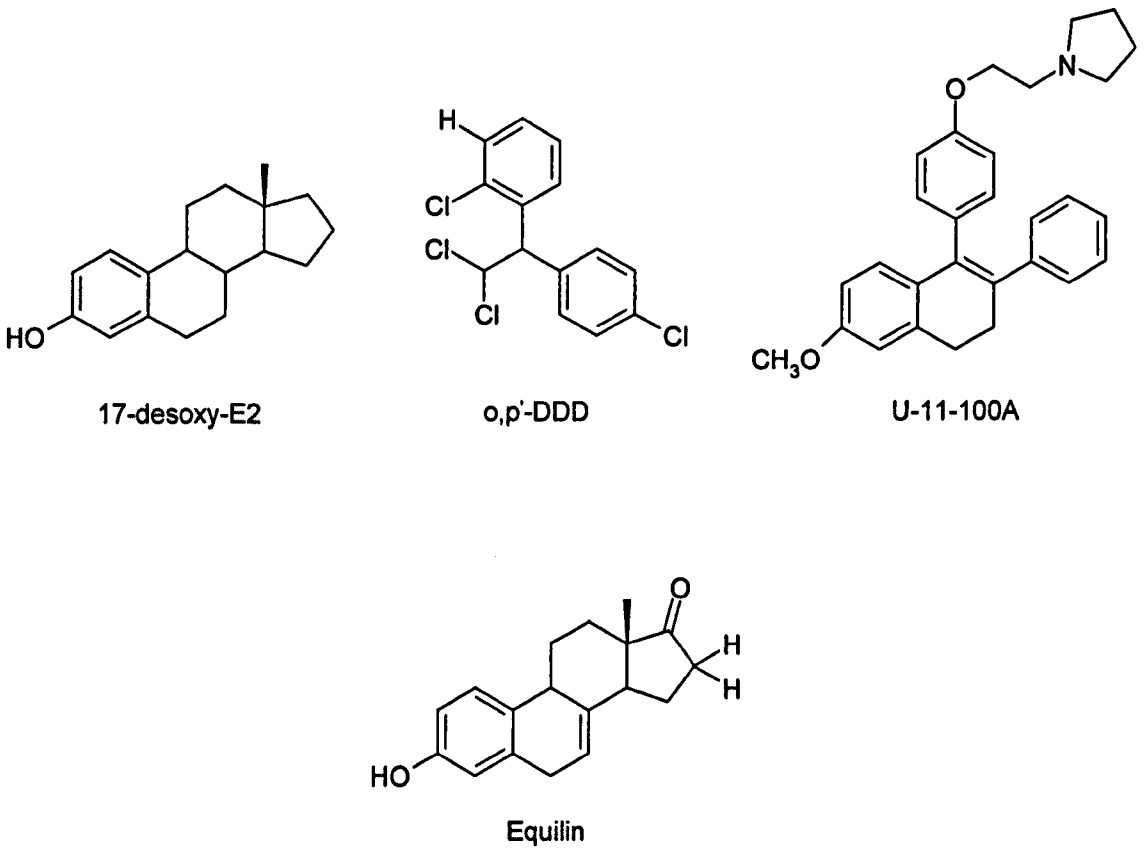
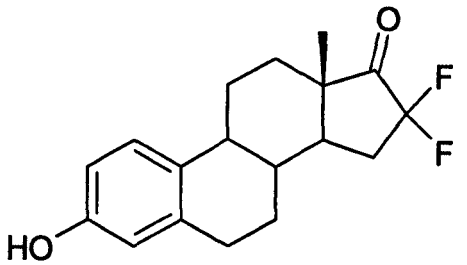
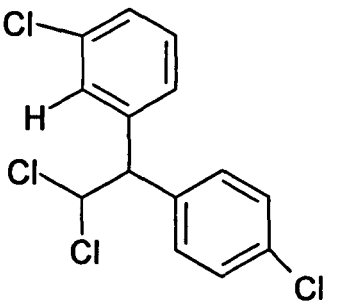


Figure 1.11: Some of the early inhibitors synthesised (Poirier, 2003).

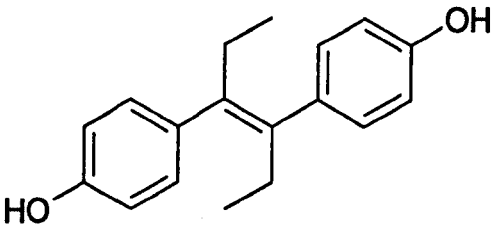
The C19 steroids 3β-hydroxy-5,16-androstadiene, 5-androsten-3β-ol, 5-androstene-3β,16β-diol and 3β-hydroxy-5-androsten-16-one were tested competitively and these inhibited the enzyme moderately with K_i values of 1.8, 6.0, 25.5 and 36.8μM respectively. They showed weak inhibition for the typical steroids T and DHEA with the K_i values being greater than 225μM. The C18 steroids [1,3,5(10)-estratrien-3-ol, 1,3,5(10),16-estratetraen-3-ol, E1, 1,3,5(10)-estratrien-3-16β-diol, 1,3,5(10)-estratrien-3,16β,17β-triol, 1,3,5(10)-estratrien-3,17α-diol] were found to be the best competitive inhibitors with K_i values of 0.04, 0.17, 0.4, 0.8, 1.1, 2.2μM respectively (Figure 1.13). Diethylstilbestrol was found to be a good inhibitor of type 1 with a K_i value of 0.8μM (Poirier, 2003).



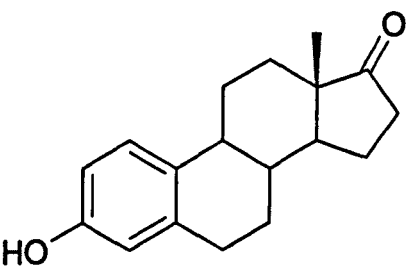
16-difluoro-E1



m,p'-DDD

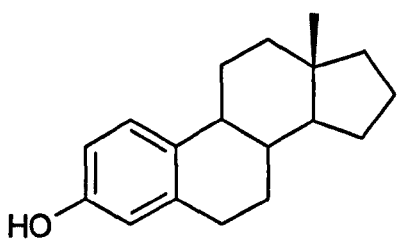


Diethylstilbestrol

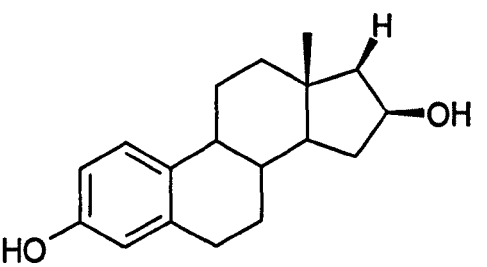


E1

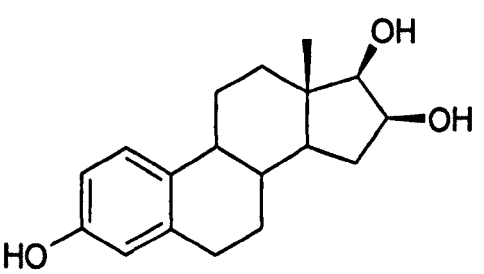
Figure 1.12: Inhibitors of 17β-HSD (Poirier, 2003).



1,3,5(10)-estratrien-3-ol
1,3,5(10),16-estratetraen-3-ol ($\Delta^{16,17}$)



1,3,5(10)-estratrien-3,16β-diol



1,3,5(10)-estratrien-3,16β,17β-triol

Figure 1.13: Examples of the C18 steroid inhibitors (Poirier, 2003).

1.5.1 Affinity labelling agents

Some of the compounds were used to determine information regarding the active site of 17β-HSD1, and these included E1 analogues as well as steroid analogues, as shown in Figures 1.14a and 1.14b. These compounds bear reactive groups which enable them to react with certain amino acid residues of the enzyme, via irreversible covalent bonding. In addition to these steroid analogues (**1-14**), which were known to target the substrate binding domain, analogues of the cofactor or of its adenosine moiety (compounds **15** and **16**) were synthesised to target the cofactor binding domain (Poirier, 2003).

As seen in Figures 1.14a and 1.14b, various reactive groups were used at various positions on the steroidal skeleton, for example, bromo-acetoxy on compounds **6-8** and on compounds **11-14**, as well as iodo (on compound **1**), bromomethyl (on compounds **2** and **3**), bromoacetamido (on compounds **4** and **5**), arylazido-β-alanine (on compounds **9** and **10**), chloroacetyl (on compound **15**), and 5'*p*-fluorosulfonylbenzoyl (on compound **16**).

On the whole, the affinity labelling compounds had a drawback in that they had a low selectivity, however, and this was not seen as a major drawback in terms of the enzyme active site study. Their use in more complex biological systems were prevented however as many of these compounds had shown estrogenic activity. An example was the compound 16-bromoacetoxy-E1, which was hydrolysed to the weak estrogen, E3. Hence these compounds, in general would not have contributed to being good inhibitors for 17β-HSD due to their low selectivity and their estrogenic properties (Poirier, 2003).

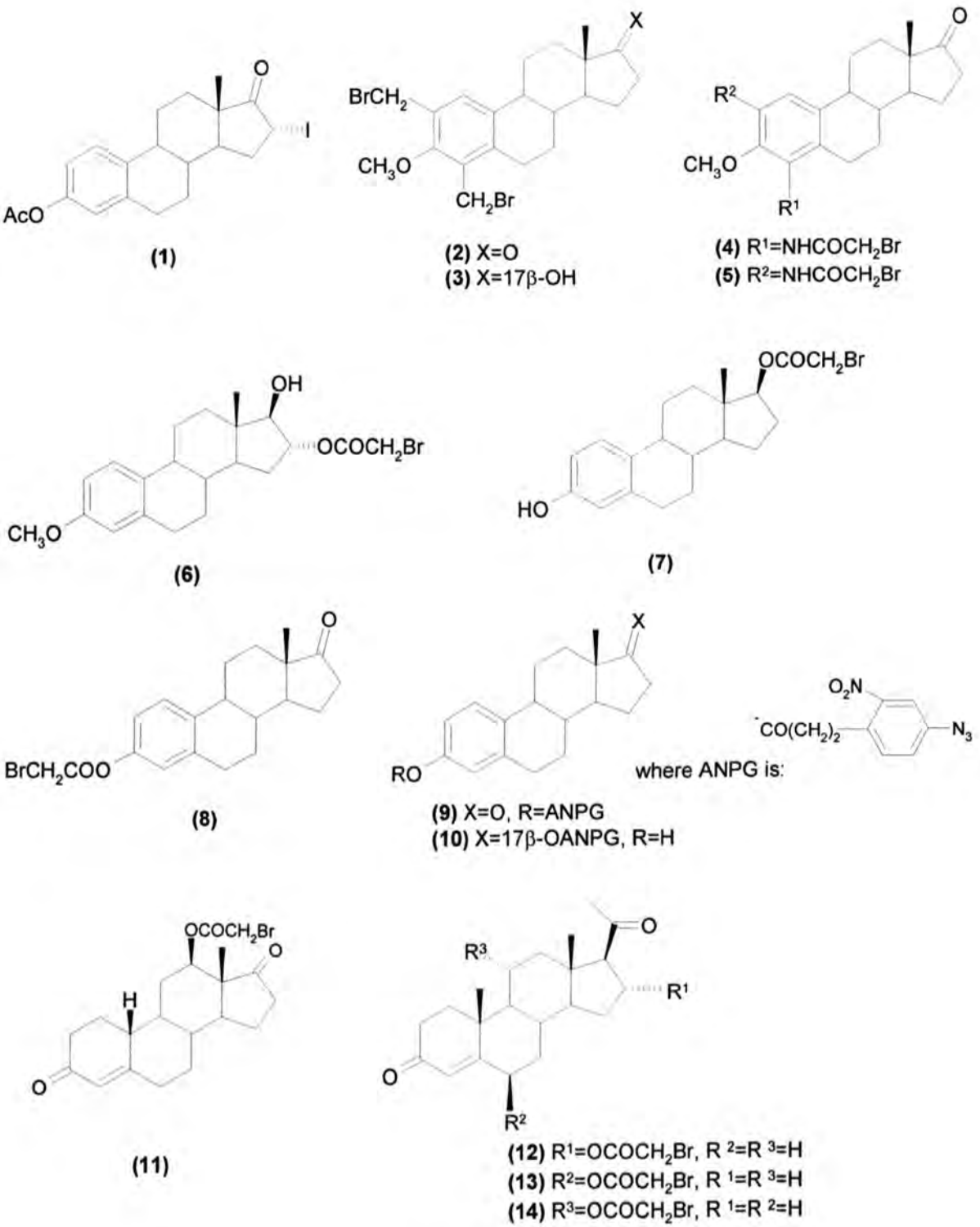
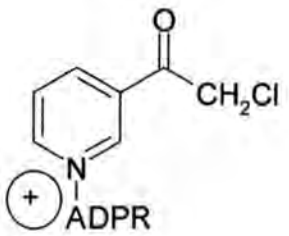
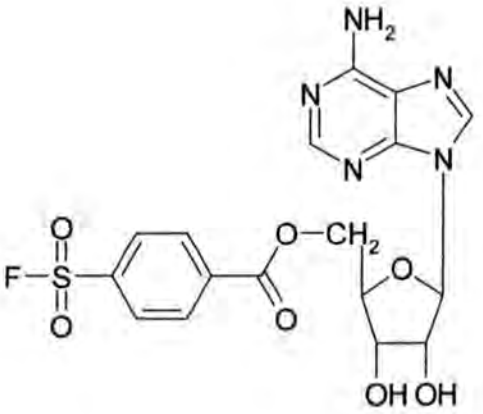


Figure 1.14a: Figure showing a range of steroidal inhibitors.



(15)

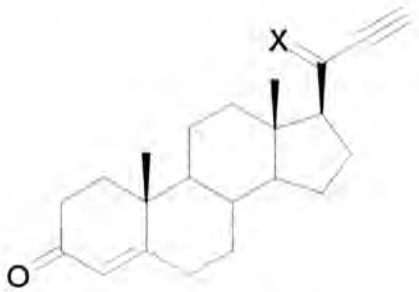


(16)

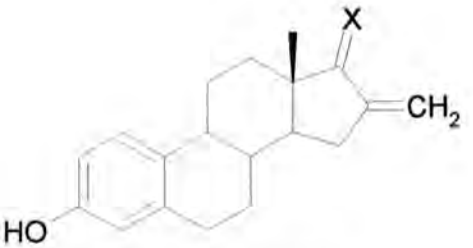
Figure 1.14b: Figure showing natural substrate E1 compounds and steroid analogues as well as cofactor analogues (Poirier, 2003).

1.5.2 Suicide inhibitors

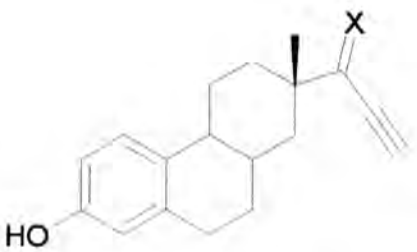
In this case, the compounds were known to have acquired alkylating activity after being transformed by the enzyme, hence gaining a higher potential selectivity. Covey et al (1990) developed inhibitors by introducing a α,β-unsaturated alcohol in position C20, C17 or pseudo C17 of a steroid nucleus, and produced the inhibitors **17**, **19** and **21** shown below in Figure 1.15. Compounds **18**, **20** and **22** were synthesised following the enzymatic oxidation of the allylic alcohols (**17**, **19** and **21**) into α,β-unsaturated ketones (Michael acceptors), and these form a covalent bond, inactivating the enzyme via a nucleophilic 1,4-addition of an amino acid residue (Poirier, 2003). Due to oxidation rate problems, modifications such as an introduction of an electron-donating or electron-withdrawing group were carried out, but this did not yield valuable results. Thereafter, introducing a trifluoromethyl group, which is a strong withdrawing group at the end of the triple bond, as in compound **23**, provided a species reactive enough to inactivate the enzyme without the oxidation step leading to compound **24**.



(17) X=H/OH
(18) X=O



(19) X=H/OH
(20) X=O



(21) X=H/OH
(22) X=O

(23) X=H/OH
(24) X=O

Figure 1.15: Examples of suicide inhibitors (Poirier, 2003).

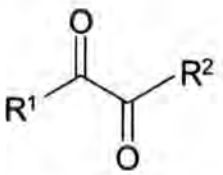
1.5.3 Diketones

Compound **25**, (shown in Figure 1.16), was used by Inano et al, (1983) to examine the functional role of some amino acid residues of 17β-HSD 1. By photooxidising the enzyme with **25**, methionyl and tyrosyl residues were completely destroyed and histidyl, arginyl, threonyl and leucyl residues were partially modified. As well as **25**, compounds **26, 27, 28, 29, 30** and **31** were found to inactivate the enzyme and the $t_{1/2}$ for compounds **25-31** were 2.0, 0.02, 0.19, 0.33, 4.4, 29 and 5.3 h respectively, at a concentration of 40mM and a pH of 8.5.

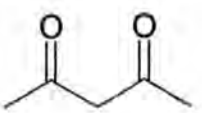
Furthermore, the presence of an arginine in the steroid binding region was suggested due to the addition of a diketone group to a C18-steroid skeleton, as shown for compound **32**, and this inactivated the enzyme in a time-dependent fashion by a modification of arginyl residues (Poirier, 2003). The

concentration at which it was tested was 4mM, and the $t_{1/2}$ for **32** was 0.80h and this compares more advantageously to compound **26**, which inactivated the enzyme at $t_{1/2}$=0.24h, even though it is smaller in size and also a more reactive compound. The steroid inhibitor was further tested and found to inhibit the enzyme competitively, from human placenta against E2 (non-competitively against NAD), in a phosphate buffer of pH 7.2, and this suggested the reversible binding of the compound to the substrate-binding site. Conversely, compound **32** showed an irreversible inactivation at $t_{1/2}$=0.8-1.0h at a concentration of 4mM, and pH 8.5, in a time-dependent manner (Poirier, 2003).

Hence, as with affinity labels, the diketone inhibitors were used to understand the enzyme in more detail rather than using them as therapeutic agents.



(25) $R^1=R^2=CH_3$
(26) $R^1=R^2=CH_2Br$
(27) R^1=Phenyl, $R^2=CH_3$
(28) R^1=Phenyl, R^2=H
(29) $R^1/R^2=(CH_2)_4$
(30) $R^1=R^2$=H



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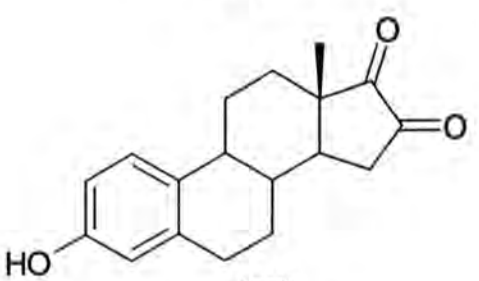


(32)

Figure 1.16: Diketones as inhibitors (Poirier, 2003).

1.5.4 Fatty acids

The effect of fatty acid activity on soluble 17β-HSD1 from human placenta was examined by Blomquist et al (1985[a]) and a few fatty acids namely oleic, arachidonic, linoleic and linolenic acids were tested. These were seen to decrease the enzymatic transformation of E2 into E1 from 100% to 0, 0, 42 and 66% respectively. The fatty acids are shown in Figure 1.17 below.

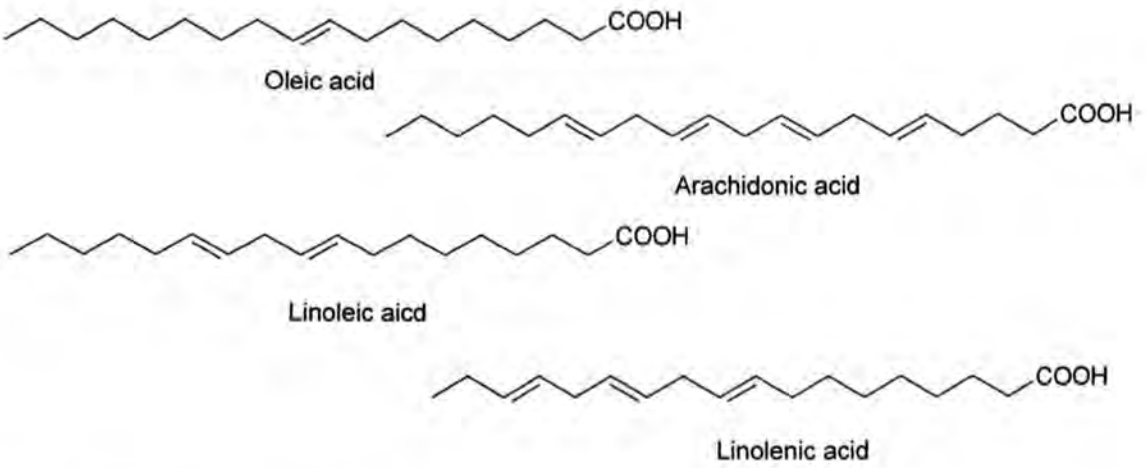
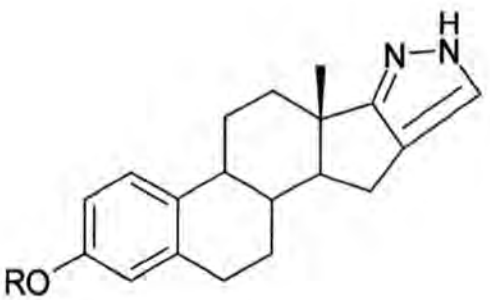


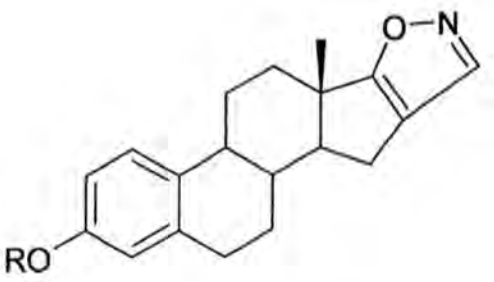
Figure 1.17: Fatty acids used against 17β-HSD1 activity (Poirier, 2003).

The compounds that showed no inhibitory effects were the methyl and ethyl esters of oleic acid, the saturated fatty acid stearic acid, prostaglandin E2 and prostaglandin $F_{2\alpha}$. The cofactors NAD, NADH, NADP and NADPH are known to protect against fatty acid inhibition, whereas steroid substrates do not. Finally, the critical micelle concentration of 50μM is required for the inhibition of the enzyme by oleic acid, and the mechanism of inhibition appears to involve the binding of fatty acid micelles at or near sites capable of binding pyridine nucleotides (Blomquist et al, 1985).

1.5.5 Pyrazoles and isoxazoles



(33) R=H
(34) R=CH_3



(35) R=H
(36) R=CH_3

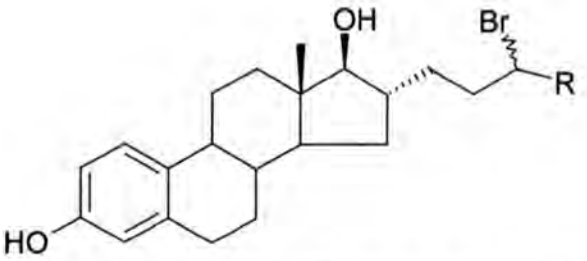
Figure 1.18: Figure showing pyrazoles and isoxazoles as inhibitors (Poirier, 2003).

The compounds **33-36** (Figure 1.18) were shown to inhibit the enzyme competitively for the reaction of E2 to E1 from human placenta. These were E1 derivatives that have isoxazole (**35** and **36)** fused to the 16,17 position on the D-ring (Sweet et al, 1991). The pyrazole based derivative **33** was observed as a better inhibitor than the isoxazole based compound **35**, with compound **33** having an IC_{50} of 4.1μM and compound **35** having a K_i of 69.4μM, and for compounds **34** and **36** the K_i values were 12.8 and 424.5μM respectively and a free CH_3-O group is an important requirement for inhibitory activity. It was concluded overall that compound **33** is a better inhibitor than the natural substrate E1 which had the Ki value of 9.5μM as an inhibitor. The good enzyme affinity can be explained by the fact that there are specific intramolecular hydrogen bonds present between the pyrazole and the phenol groups of the hydroxysteroid and the three specific histidyl residues of the enzyme, hence stabilising the inhibitor-enzyme complex (Poirier, 2003).

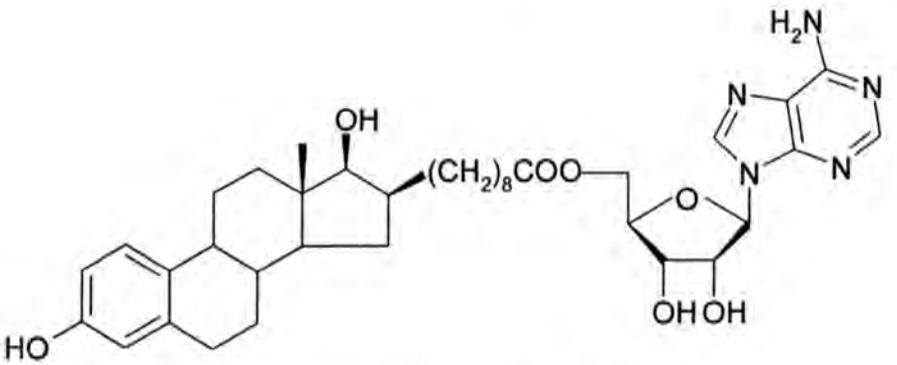
1.5.6 E1 and E2 C-16 derivatives

Inhibitor **37**, shown in Figure 1.19, was developed using classical structure-activity relationship (SAR) studies (Tremblay and Poirier, 1998). Also, cofactor hybrid inhibitors that contained two components, E2 and adenosine, which interacted with two different enzyme-binding sites were looked at (Qiu et al, 2002), one of them being compound **38** shown below in Figure 1.19.

The structures of the three series of compounds (shown in Figure 1.20) were tested against the reductive 17β-HSD1 activity. Table 1d shows the IC_{50} values of the different compounds for each of the series.



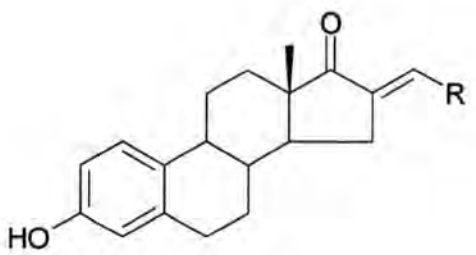
(37) R=H or $(CH_2)_5CONBuMe$



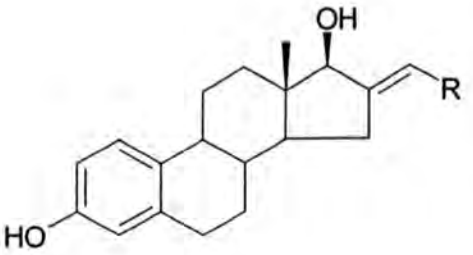
(38) EM-1745

Figure 1.19: Previously designed inhibitors that interacted with different enzyme-binding sites (Poirier et al, 2003).

IC_{50} values for the reference inhibitors EM-1745, E1 and E2 were observed as 0.05, 0.56-0.58 and 7.3μM, respectively (Poirier, 2003). For compound **39**, the R group was seen to decrease the ability of the enone system to inactivate the enzyme. Hence, compounds **39a-i** showed low inhibitory activity compared to the lead compound **39j**. However, in the case of compound **40**, R increases the inhibitory activity if compared with compounds **40a-i** (Thomas et al, 1983).



(39)



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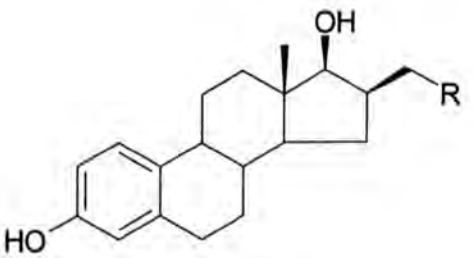


(41)

Figure 1.20: Structures of series of compounds that were tested against 17β-HSD1 (Poirier et al, 2003).

R group	Compounds 39 IC_{50} (μM)	Compounds 40 IC_{50} (μM)	Compounds 41 IC_{50} (μM)
a) Phenyl	10-32	3.4-5.6	0.79-1.0
b) 4-Br-phenyl	>30	-	-
c) 2-pyridyl	>20-40	9.3-12.6	2.4-5.5
d) 3-pyridyl	8.6-18	7.5-16	3.3-4.2
e) 4-NMe_2-Phenyl	>40	7.0-14	4.0-5.7
f) 2-Pyrrole	1.1-3.4	-	-
g) 2-Imidazole	3.8-4.0	-	-
h) Vinyl ($CH=CH_2$)	2.7-5.5	4.5-5.0	1.8-3.5
i) Ethyl (CH_2CH_3)	1.0-1.4	9.0-20	1.5-1.8
j) H	0.26-0.33	14-32	0.72-1.3

Table 1d: IC_{50} values in μM of the series of inhibitors (Poirier et al, 2003).

Series **41** proved even better inhibitors, especially for the phenylmethyl derivative **41a**, which has an IC_{50} of 0.79-1.0μM. For the aryl derivatives, the potency was observed to have increased in the order of series **39<40<41**. This could be explained by the presence of a more flexible 16β-methylene group, and this allows a better positioning of the aryl moiety and in doing so, there are fewer steric interactions with enzyme amino acid residues (Poirier et al, 2003). Inhibitor **41a** was observed to be a more potent inhibitor than EM-251 (R=H, in Figure 14), but weaker than the hybrid inhibitor EM-1745.

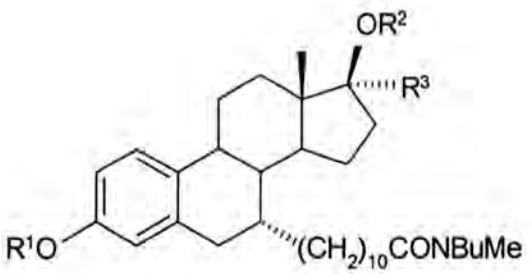
1.5.7 Antiestrogens

The compounds **42-52** (shown in Figure 1.21) had potent inhibitory effects on 17β-HSD activities as well as antiestrogenic activity (Labrie et al, 1992). These are derivatives of E2 and they contain a 7α-alkylamide side chain and a D-ring modification, such as a halogen atom or a double bond. All compounds except **43** showed potency as competitive inhibitors for all the enzyme activities such as in the case of the reaction of E1 to E2, E2 to E1, Δ^4-dione to T and T to Δ^4 –dione in uterine tissue.

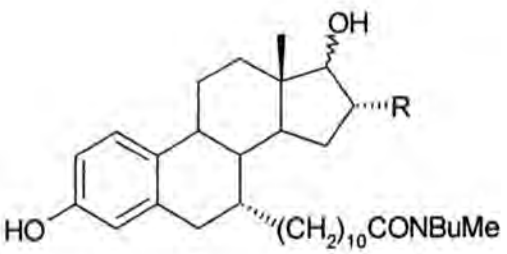
Tamoxifen (**53**), which is nonsteroidal, was compared to EM-139 (**49**), which is steroidal. Compound **53** showed no inhibitory effect if compared to compound **49**, which showed inhibition of E1 to E2 activity by 40 and 70% respectively (Poirier, 2003). However in another model, **53** showed slight inhibition of E1 to E2, as did its metabolites **54** and **55** and the pure steroidal antiestrogen **52**, with IC_{50} values of >1000, 2150, >1000 and 890μM, respectively (Santner and Santen, 1993). These compounds have been shown to have a dual inhibitory action, as such, they block both the estrogen receptor, providing the antiestrogen effect, and estrogen formation providing the inhibitory effect on the enzyme. Hence in this case, dual inhibitors have excellent properties suitable for their use in estrogen-sensitive disease treatment, but their selectivity and their inhibitory potency needs to be improved for 17β-HSD1 (Poirier, 2003).

1.5.8 Progestins

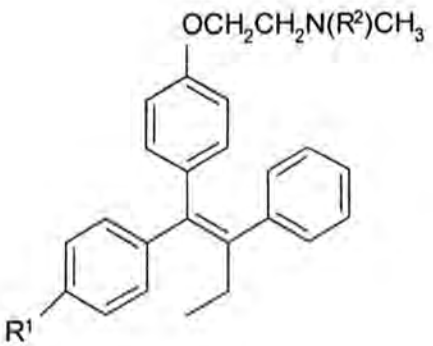
Progestins were investigated for their activity of inhibition on 17β-HSD1 by Chetrite et al (1996, 1997, 1999 and 2001). The compounds that were tested for type 1 activity (Figure 1.22) were tested on hormone-dependent MCF-7 and T-47D human breast cancer cell lines. They were the well-known progestins nomegestrol acetate and medrogestone as well as tibolone (**56**) and its metabolites (**57-59**).



(42) $R^1=R^2=R^3=H$; $\Delta^{13,14}$
(43) $R^1=C_6H_5CO$; $R^2=R^3=H$; $\Delta^{13,14}$
(44) $R^1=R^2=H$; $R^3=C\equiv CH$; $\Delta^{15,16}$
(45) $R^1=C_6H_{11}CO$; $R^2=H$, $R^3=C\equiv CH$; $\Delta^{15,16}$



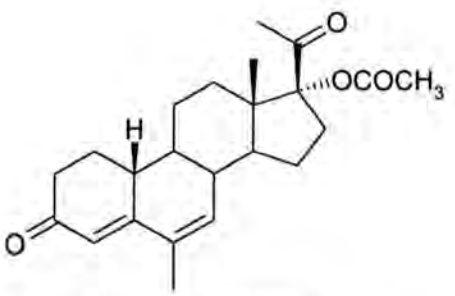
(46) R=F; 17α-OH
(47) R=Cl; 17α-OH
(48) R=F; 17β-OH
(49) R=Cl; 17β-OH
(50) R=Br, 17β-OH
(51) R=I; 17β-OH
(52) R=H; 17β-OH



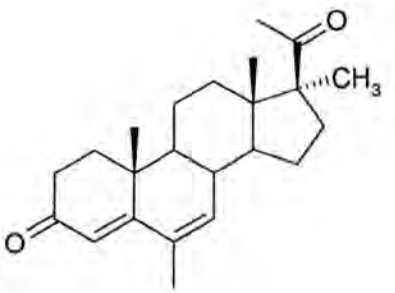
(53) $R^1=H$, $R^2=CH_3$
(54) $R^1=OH$, $R^2=CH_3$
(55) $R^1=R^2=H$

Figure 1.21: Antiestrogens as inhibitors for 17β-HSD (Poirier, 2003).

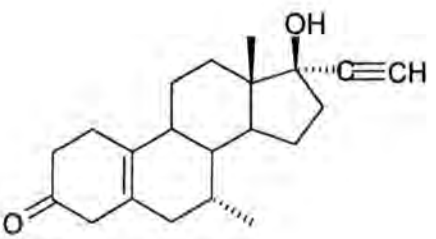
The IC_{50} values of these compounds varied for the MCF-7 cells (0.79 to 22.84μM) and for the T-47D cells (0.45 to 35.25μM), and these antiestrogens have also been observed to inhibit the hydrolysis of sulfates and hence stimulate the formation of sulfates (Poirier et al, 2003). These compounds showed weak inhibitions but can offer a treatment method for estrogen-dependent diseases.



Nomegestrol acetate



Medrogestone



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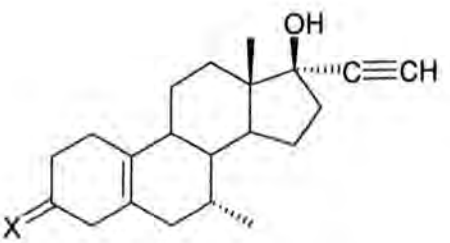
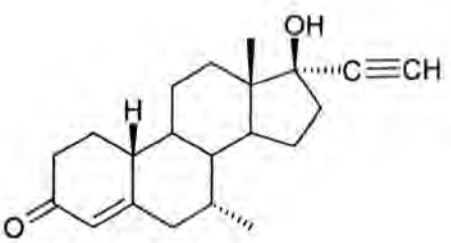


(57) X=3α-OH
(58) X=3β-OH



(59)

Figure 1.22: Progestins as potential inhibitors for 17β-HSD (Poirier, 2003).

1.5.9 Phytoestrogens

These belong to a family of natural products with a variety of biological properties. Phytoestrogens examples include coumarin, coumestrol, flavone,

flavanone, isoflavone and chalcone and these were tested by Makela et al (1995) and they inhibited 17β-HSD1 measuring the conversion of E1 to E2 in MCF-7 and T-47D breast cancer cells using purified human placental cytosolic 17β-HSD. Figure 1.23 shows examples of the phytoestrogens and Table 1e and 1f below shows examples of phytoestrogens and their inhibitory values.

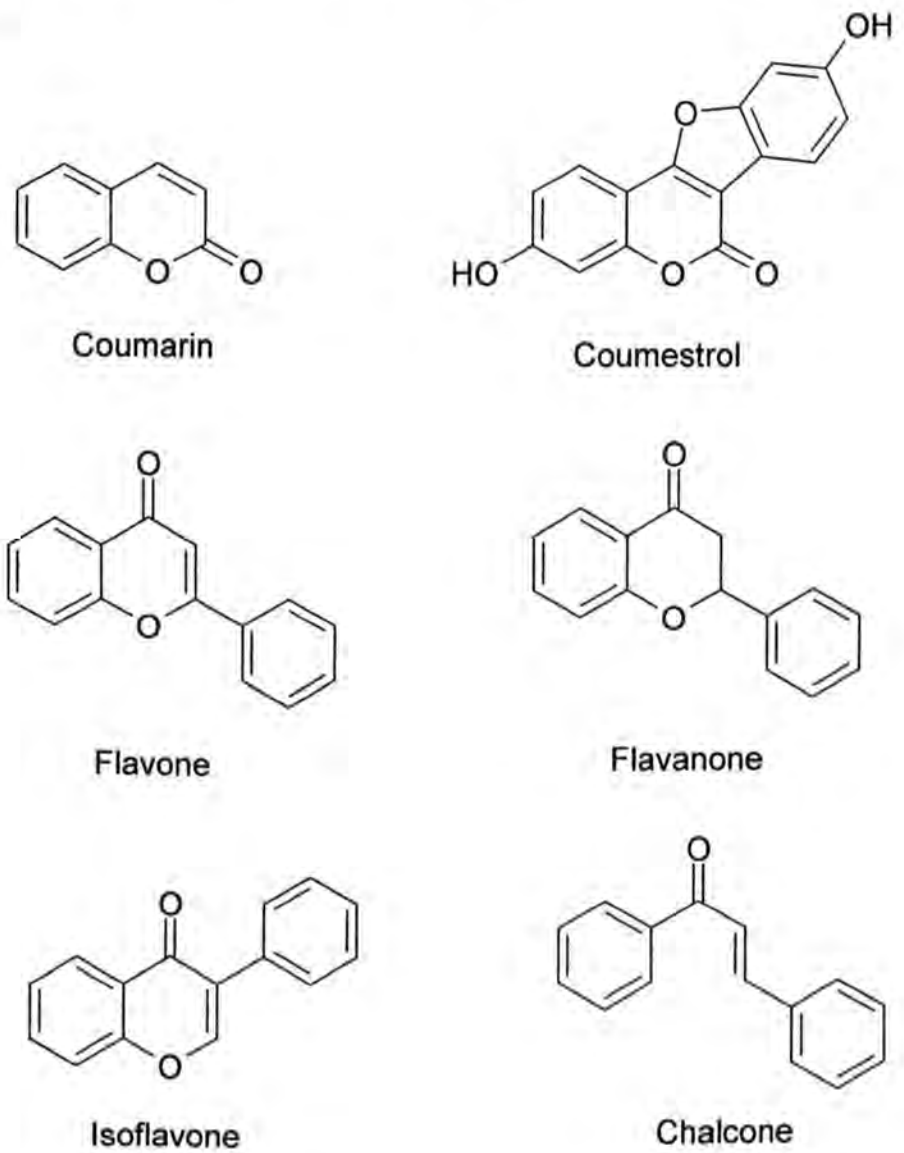


Figure 1.23: Examples of some of the phytoestrogenic scaffolds as inhibitors of 17β-HSD (Poirier, 2003).

Name	% of E1 to E2 conversion (at 1.2 μM)	IC_{50} (μM)
Coumarin	No inhibition observed	Not determined
Coumestrol	18.3	0.2
6α, 11α-reduced form of cholesterol	Not determined	>50
Flavone	No inhibition observed	No inhibition observed
6-hydroxy-flavone	No inhibition observed	Not determined
7-hydroxy-flavone	69.4	0.9
7-methoxy-flavone	Not determined	No inhibition observed
chrysin	No inhibition observed	3.6
5-hydroxy-7-methoxy-flavone	No inhibition observed	Not determined
apigenin	57.7	0.3
5,7-dihydroxy-4'-methoxy-flavone	38.5	Not determined
3,5,7-trihydroxy-flavone	59.1	Not determined
3',4',5,7-tetrahydroxy-flavone	No inhibition observed	0.6
quercetin	No inhibition observed	Not determined
flavanone	No inhibition observed	Not determined
4'-hydroxy-flavanone	No inhibition observed	Not determined
7-hydroxy-flavanone	Not determined	28.0
4',5,7-trihydroxy-flavanone	53.0	15.0
3',4',5,7-tetrahydroxy-flavanone	Not determined	No inhibition observed
3',5,7-trihydroxy-4'-methoxy-flavanone	Not determined	No inhibition observed
4',7-dihydroxy-isoflavone	68.1	10

Table 1e: Examples of phytoestrogens and their inhibition values (Poirier, 2003).

7-hydroxy-4'-methoxy-isoflavone	No inhibition observed	>50
4',5,7-trihydroxy-isoflavone (genistein)	62.8	1.0
5,7-dihydroxy-4'-methoxy-isoflavone (biochanin A)	No inhibition observed	4.9
3',4',7-trihydroxy-isoflavone	Not determined	5.2
5,4'-dihydroxy-7-methoxy-isoflavone	Not determined	>50
2'-hydroxy-chalcone	Not determined	>50
4'-hydroxy-chalcone	Not determined	No inhibition observed
4-hydroxy-chalcone	Not determined	16.0
2',4'-dihydroxy-chalcone	Not determined	34.6
2',4-dihydroxy-chalcone	Not determined	>50
2'-hydroxy-4'-methoxy-chalcone	Not determined	No inhibition observed
2',4',4-tihydroxy-4'-chalcone	Not determined	33.8
2',4',6',4-tetrahydroxy-dihydrochalcone	Not determined	>50
2',4',6',3,4-pentahydroxy-chalcone	Not determined	No inhibition observed
2',4',6',3-tetrahydroxy-4-methoxy-chalcone	Not determined	No inhibition observed

Table 1f: Examples of phytoestrogens and their inhibition values (Poirier, 2003).

Coumestrol was the most potent inhibitor, whereas 4,4'-dihydroxybiphenyl, genistein, β-sitosterol and β-sitostanol were weak inhibitors. The remaining compounds were inactive at the concentration they were tested (1.2μM). At

0.12 and 1.2μM respectively, coumestrol and genistein inhibited the reduction of E1 to E2 in T-47D breast cancer cells. Also compared to many flavanoids and isoflavanoids that were tested and which showed some inhibition against 17β-HSD1, coumestrol was still the most potent inhibitor.

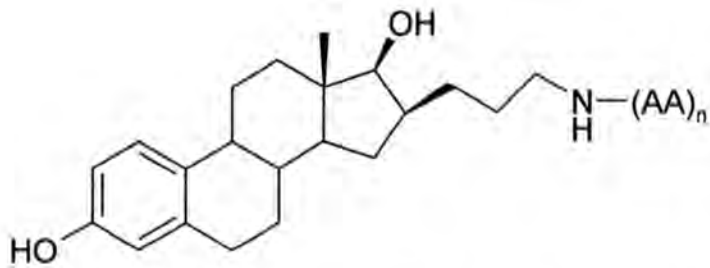
SAR was carried out for 17β-HSD1 and 2 inhibitions. By varying the position and the number of hydroxy groups, it was determined which compound inhibited what isozyme. Chalcones showed low potent activity compared to their flavanoid analogues (Poirier, 2003).

1.5.10 Hybrid inhibitors

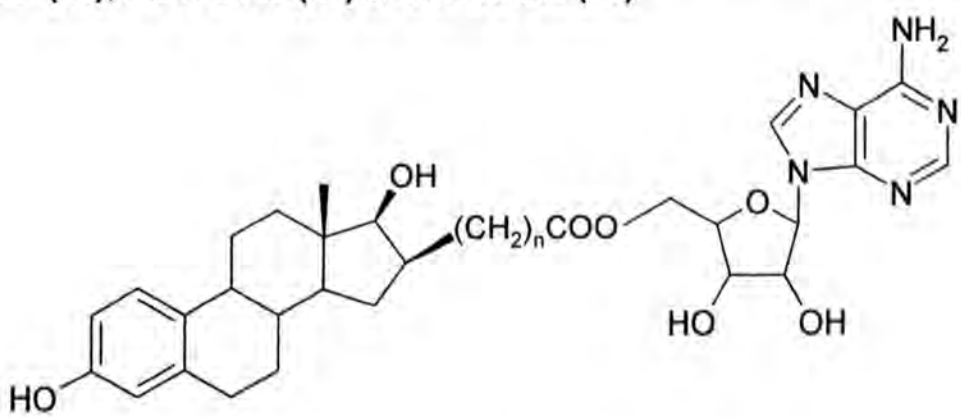
Hybrid inhibitors (Figure 1.24) were investigated by Tremblay et al (2001), using the rationale that the compounds should contain both hydrophilic and hydrophobic components that should interact with both the steroid binding domain and some amino acid residues of the cofactor binding domain of the enzyme. Examples of these inhibitors (**60-68**) included E2 derivatives containing various polar groups such as some amino acids. Once the enzyme screening was carried out, it was observed that none of the inhibitors showed much reductive activity for type 1. Hence a method of increasing interactions with the enzyme was looked at by Poirier et al (2002), as well as investigating the hybrid-molecule types.

Compounds **69-71**, which were E2-adenosine hybrid compounds, were then synthesised to follow the substrate and cofactor sites' (Lin et al, 1983), structure-activity relationships (Tremblay and Poirier, 1998; Poirier et al, 2000) and molecular modelling studies of the enzyme inhibition (Zhu et al, 1993; Ghosh et al, 1995; Azzi et al, 1996). Hence these compounds were designed so at to interact with the two binding domains of the enzyme, one being the adenosine moiety to interact with the cofactor binding domain and the E2 moiety to interact with the substrate binding domain. An alkyl–chain spacer was placed between the E2 and adenosine moieties (Poirier, 2003).

The adenosine-E2 hybrids (**69-71)** inhibited the enzyme reduction of E1 to E2 with IC_{50} values of 93, 52 and 140nM, respectively and the inhibition depended on the spacer length between the two components i.e. where n=7, 8 and 9. Molecular modelling had predicted the optimal spacer length to be 8 methylene groups, and following testing, that was confirmed. With these compounds however, more research needs to be carried out in terms of *in vivo* and *in vitro* testing, as well as to optimise their biological activity (Poirier, 2003).



60-68 $(AA)_n$=Gly **(60)**, Leu **(61)**, Phe **(62)**, Pro **(63)**, Lys **(64)**, Glu **(65)**, Ser **(66)**, Ala-Ala-Ala **(67)** or Glu-Ala-Ala **(68)**



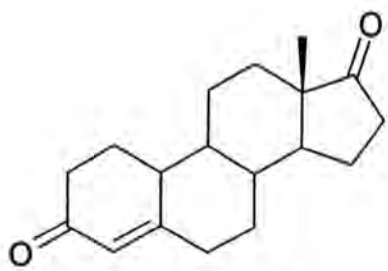
69-71 [n=7 **(69)**, 8 **(70)** or 9 **(71)**]

Figure 1.24: Examples of hybrid inhibitors (Poirier, 2003).

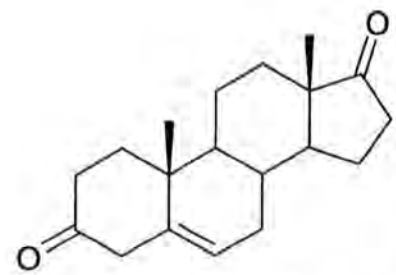
1.6 17β-HSD 3 inhibitors

1.6.1 Early work and Atamestane

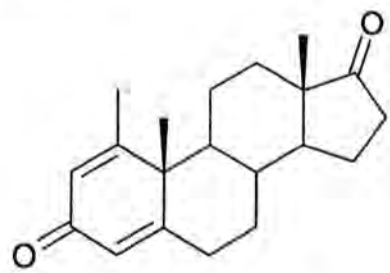
Pittaway (1983) discovered that for the inhibition of 17β-HSD3, a steroid scaffold with a carbonyl at position 17 and a non aromatic A-ring were important factors. From twenty steroids tested, only two compounds namely, 4-estrene-3,17-dione and 5-androstene-3,17-dione (Figure 1.25) showed significant competitive inhibition, for the reduction of Δ^4-dione using NADPH, with K_i values of 2.4 and 6.8μM, respectively. It was further discovered by Lombardo et al (1993) that an inhibitor called Atamestane, which is a known irreversible aromatase inhibitor, was a potent competitive inhibitor of the reduction process, whereby Δ^4-dione is converted to T, in human testes. The K_i value was not reported but the K_m value was found to be 1μM, which was measured for the atamestane reduction to its 17β-OH analogue (Poirier, 2003).



4-estrene-3,17-dione



5-androstene-3,17-dione



Atamestane

Figure 1.25: Examples of early inhibitors (Poirier, 2003).

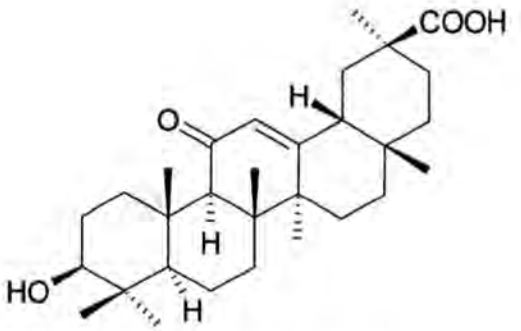
1.6.2 Licorice components

The *in vivo* hydrolysis of the active component of licorice glycyrrhizic acid produces glycyrrhetinic acid (Figure 1.26), which was found to have blocked the formation of T from Δ^4-dione, with an IC_{50} value of 4μM. This was not found to be a selective inhibitor for 17β-HSD3, as it was shown to also inhibit 17,20-lyase activity (converting 17-hydroxyprogesterone into Δ^4-dione) (Armanini et al, 1999).

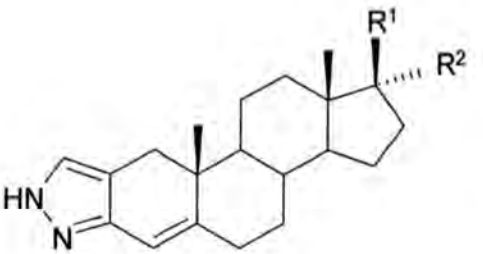
1.6.3 Pyrazoles and isoxazoles

Figure 1.26 shows compounds **72** and **73**, which are 2,3-pyrazoles and compounds **74-77**, which are 3,4-steroidal fused pyrazoles, and which were found to inhibit 17β-HSD3 from the bacterial system, *Pseudomonas testosterone* (Ferrari and Arnold, 1963). Compounds **74-76** were found to be more potent inhibitors for the oxidation of T to Δ^4-dione, with K_i values of 6, 7 and 100nM respectively, than compounds **72** and **73**, which have K_i values of 20 and 15nM respectively.

77 is the isoxazole analogue of **75**, which was found to be a less active inhibitor with a K_i value of 190nM. The pyrazoles are examples of steroidal inhibitors which were found to inhibit the enzyme in a competitive reversible manner, however, the 17β-HSD3 from the bacterial system was found to not be a good model for the inhibitors, therefore, these fused pyrazoles should be tested in another enzyme system (Poirier, 2003).

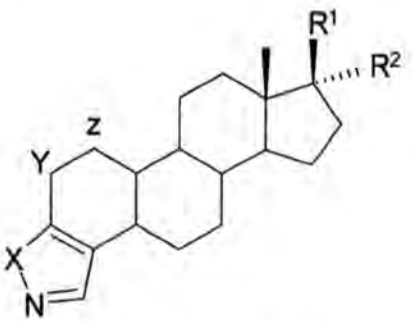


Glycyrrhetinic acid



(72) R^1=OH, R^2=CH_3

(73) R^1=$CH(CH_3)CH_2OH$, R^2=H



(74) R^1=OH, R^2=CH_3, X=NH

(75) R^1=$CH(CH_3)CH_2OH$, R^2=H, X=NH

(76) R^1=$CH(CH_3)CH_2OH$, R^2=H, X=NH, $\overset{1,2}{\triangle}$, Y, Z = $\overset{1,2}{\triangle}$

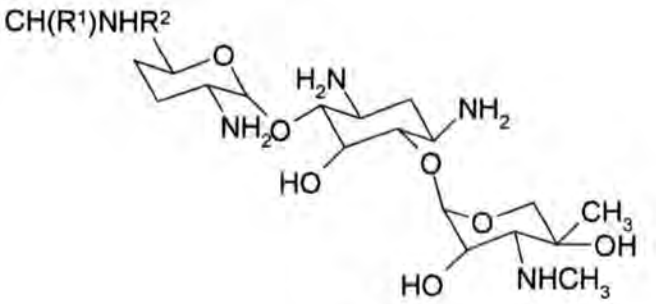
(77) R^1=$CH(CH_3)CH_2OH$, R^2=H, X=O

Figure 1.26: Licorice components and pyrazoles and isoxazoles as examples of inhibitors of 17β-HSD 3 (Poirier, 2003).

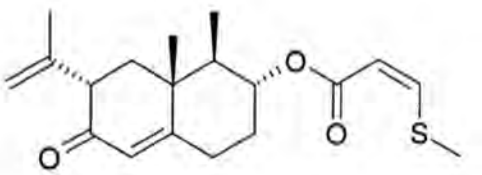
1.6.4 Commercially available drugs

Some commercially available drugs for example, gentamicin and the sesquiterpene S-petasin, were looked at and tested on the enzyme 17β-HSD3 (Figure 1.27). Gentamicin is an aminoglycoside which is used for the treatment of Gram negative bacterial infections and was tested on rat testicular steroidogenesis (Ghosh and Dasgupta, 1999). For type 3 activity, the percentage inhibitions at doses of 80 and 100 mg/kg were 34 and 37% with the compound being a reversible inhibitor. S-petasin, is an anti-inflammatory and analgesic, which was tested *in vivo* and *in vitro*. *In vivo*, S-petasin was injected (1μg/kg), and the level of basal plasma T concentration was found to decrease by 38% in adult male rats after 30 min. *In vitro*, S-

petasin (0-43μM) was incubated with rat testicular interstitial cells in the presence of Δ^4-dione (1nM) and the production of T decreased significantly at S-petasin concentrations of 4.3 and 43μM (Poirier, 2003).



Gentamicin (is a mixture of C1, C2 and C1a forms)
C1 (R^1=R^2=CH_3)
C2 (R^1=CH_3, R^2=H)
C1a (R^1=R^2=H)



S-petasin

Figure 1.27: Examples of commercially available drugs gentamicin and S-petasin as potential 17β-HSD3 inhibitors (Poirier, 2003).

1.6.5 Arochlor-1248

Arochlor-1248 consists of a commercial mixture of poly-(tri-, tetra-, and penta-) chlorinated biphenyls, and the effect of inhibition on rat testicular androgenesis was carried out by Andric et al (2000). The effect of Arochlor-1248 was as such, that *in vivo*, a decrease in levels of serum T and DHT was observed (29-33%). In *in vitro* tests, Arochlor-1248 had an effect on inhibition but only at a micro molar concentration of 10μM and a major effect on inhibition as such, the conversion of progesterone (P) into T and DHT was

inhibited, hence making Arochlor-1248 specific towards the enzyme 17α-hydroxylase/lyase (Poirier, 2003).

1.6.6 Metal salts

Environmental compounds such as metal salts (cationic form), cadmium chloride, and also the presence of excess of NADP during the conversion of T into Δ^4-dione inhibited the activity of 17β-HSD3 (Poirier, 2003).

1.6.7 Phytoestrogens and other nonsteroidal compounds

Nonsteroidal compounds (Figure 1.28) under this category were screened for the conversion of Δ^4-dione in the presence of NADPH using human testicular microsomes as a source of 17β-HSD3, and these included p-benzoquinones, flavones, isoflavones and triphenylethene derivatives (Le Lain et al, 2001). The substrate (Δ^4-dione) concentration was 2μM and the IC_{50} values of the compounds ranged from 2.7 to 100.5μM and the most potent compounds were found to be **79** (2,5-diphenyl-p-benzoquinone at 2.7μM), **78** (phenyl-p-benzoquinone at 5.7μM), **80** (7-hydroxyflavone at 9.0μM), **82** (triphenylethene derivative at 9.1μM), **81** (baicalein at 9.3μM) and **95** (biochanin A at 10.8μM). Comparing these compounds to the K_m values, their potency remained low, and after their SAR studies were carried out, the hydroxyl groups of the flavones were regarded as a factor that might be involved in inhibition. However the phytoestrogens, flavone and isoflavones showed estrogenic activity and/or inhibitory activities for other steroidogenic enzymes.

These compounds were then tested on rat testicular microsomes, where the hormone inhibitory activity was found to be at 0.77μM, however, this enzyme preparation was found to be much less sensitive to enzyme inhibition, hence preventing its use as a more readily available source of the enzyme (Le Lain et al, 2001). Thereafter, six coumarin and eleven triphenylethene derivatives were tested, and 2,5-diphenyl-p-benzoquinone (**79**) was found to be a better inhibitor than the triphenylethene derivatives, and two coumarins namely

umbelliferone (**83**) and 4-methylumbelliferone (**84**) showed better inhibition with IC_{50} values of 1.4 and 0.9μM, respectively (Poirier, 2003).

Compounds **85-92** which are a series of tetralins were patented and tested by Smith et el (2001) and the structure of these tetralins is similar to **95** (biochanin). Compounds **85-87**, upon being tested in human testicular microsomes, at pH 7.5 for the reduction of Δ^4-dione at 2μM to T, gave IC_{50} values of 1.8, 8.3 and 7.0μM respectively. Compounds **85-92** were further tested on rat testes for the reduction of Δ^4-dione at 0.5μM, and the percentages of inhibition at 100μM were from 58% to 79%. Compounds **85** and **87** were found to be less potent on the rat enzyme than on the human enzyme and the dichloro derivative **88** was found to be the most potent inhibitor with an IC_{50} of 32.7μM. Compounds such as **93** that consist of the general structure of novel tetralone or benzopyrane derivatives, are similar to and related to compounds such as **85-90**, and these compounds were used as preventative and/ or curative drugs for various diseases sensitive to male and female hormones. Compound **94** is also an example of a benzofuran derivative that was used as treatment for hormone-sensitive diseases, which inhibited 17β-HSD activity (Nakakoshi et al, patent).

1.6.8 Androsterone derivatives

Poirier et al (1995) investigated the C-19-steroid androsterone (ADT, **96**) as a potential starting nucleus to develop 17β-HSD3 inhibitors. Based on the results that were obtained during the development of 17β-HSD1 inhibitors, derivatives of ADT were synthesised, which were substituted at position 16 with a bromopropyl side chain (Tremblay and Poirier, 1998). These inhibitors however turned out to be weak inhibitors for 17β-HSD3 (Ngatcha et al, 2002). The compounds **97-107** which are shown in Figure 1.29, bear different 3β substituents, and their IC_{50} values obtained were in the range of 57-200nM which was better than ADT (300nM).

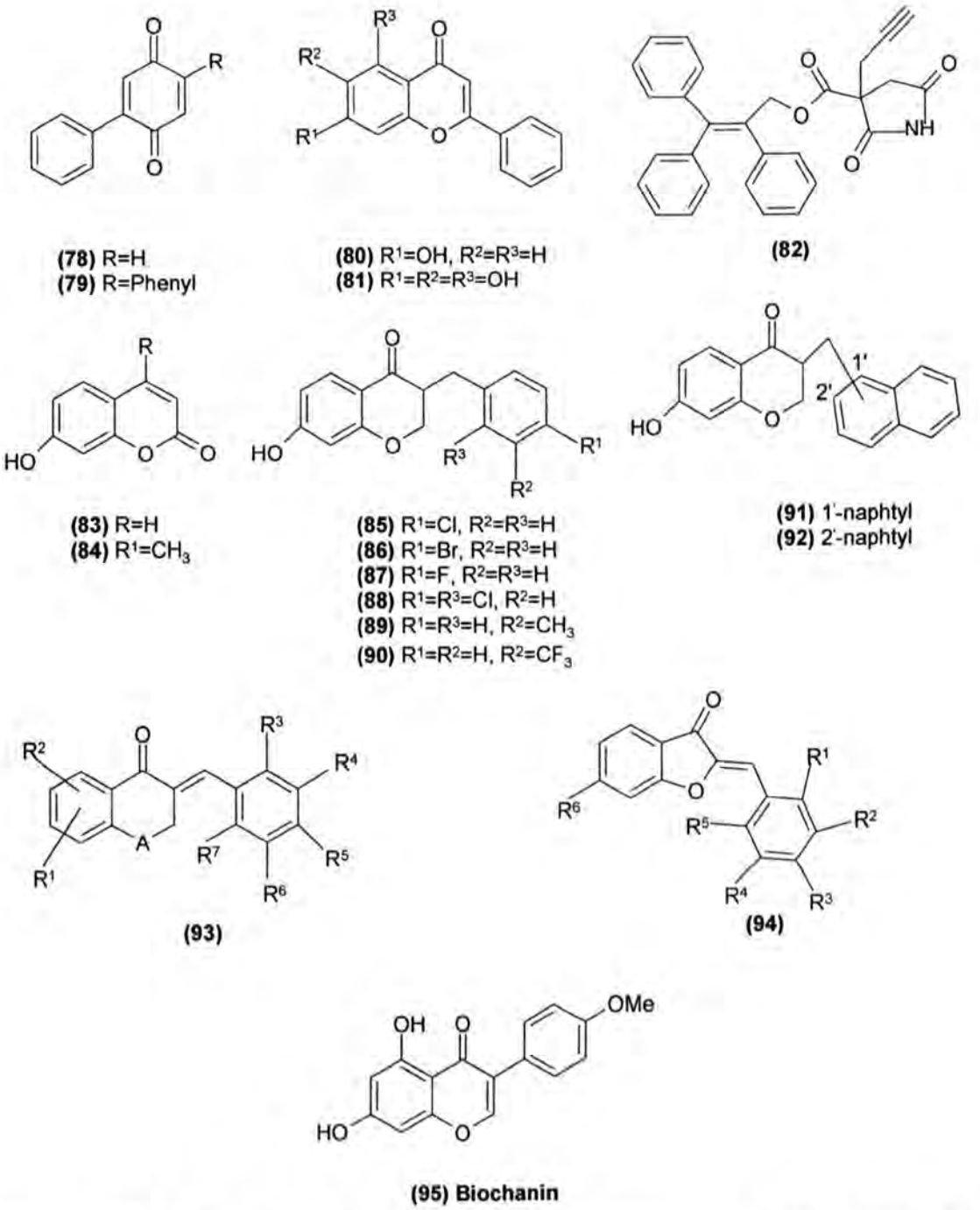


Figure 1.28: Examples of phytoestrogens and other nonsteroidal compounds as inhibitors (Poirier, 2003).

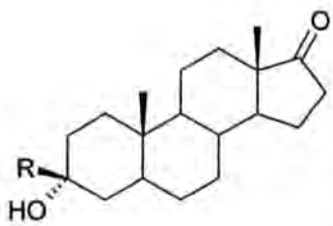
The compounds that were found to be potent in this group were **106** (3β-phenylmethyl-ADT), **104** (3β-cyclohexyethyl-ADT), **98** (3β-propyl-ADT), **99** (3β-*sec*-butyl-ADT), with IC_{50} values of 57, 60, 67 and 73nM respectively (Ngatcha et al, 2000). Furthermore, these compounds showed specific inhibition as such, they did not inhibit any other reductive 17β-HSD types. The disadvantage with using these compounds was that they induced proliferation

of androgen sensitive (AR^+) Shionogi cells, thereby indicating androgenic activity.

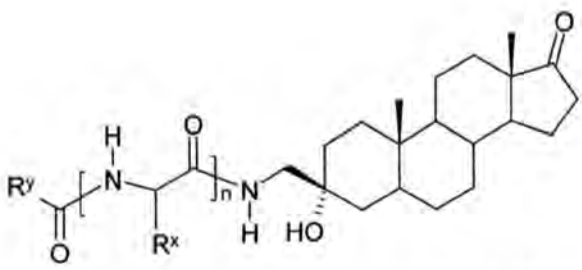
Following this, compounds were synthesised using solid-phase parallel synthesis by Maltais et al (2001), to avoid the problem of androgenic activity as well as to optimise a new class of inhibitors. These compounds consisted of model libraries of 3β-peptido-3α-hydroxy-5α-androstan-17-ones with 1, 2 or 3 molecular diversity levels. The compounds from the libraries that were found to be potent inhibitors were six members of the level 3 library, which bear at least one phenyl group. Compound **108** was found to inhibit the enzyme, with an IC_{50} value of 227nM, and this was twice as potent as the natural substrate Δ^4-dione which was used as an inhibitor itself. To test out its androgenic activity, compound **108** was tested on AR^+ cells and it was found to induce proliferation slightly at 1μM and no proliferative activity at 0.1μM (Poirier, 2003).

A parallel liquid-phase approach was developed whereby the inhibitory activity of the 3β-substituted ADT derivatives was optimised (Maltais and Poirier, 1998; Maltais et al, 2002). Following this, three libraries of 3β-amidomethyl-ADT derivatives (168, 56 and 49 members), as well as two molecular diversity levels on the amide (R^1 and R^2) were synthesised. These compounds were screened and the most potent inhibitors were found to be compounds **109-112** with IC_{50} values of 35-85nM.

Also a 25-membered library, which consisted of 3-carbamate-N-substituted-5α-androstan-17-one was synthesised, which allowed more rigid molecules to be prepared, and which had two levels of molecular diversity within the local area that was occupied by the adamantane group. One of the most potent inhibitors from this particular library was compound **113**, which showed similar potency to compound **111** and showed no androgenic activity. Compounds **96-113** are shown in Figure 1.29 below, and Table 1g shows the IC_{50} values and their androgenic activity values for compounds **109-113**, which were compared to compounds **106** and **96** (Poirier, 2003).

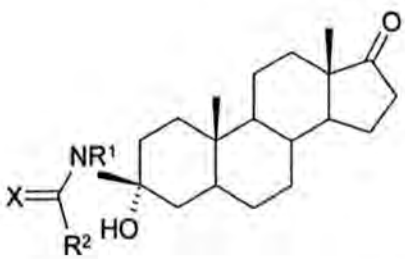


96 (ADT) R=H
97 R=CH_3
98 R=$CH_3(CH_2)_2$
99 R=$CH_3CH_2(CH_3)CH$
100 R=$CH_3(CH_2)_5$
101 R=$CH_3(CH_2)_7$
102 R=Cyclohexyl
103 R=Cyclohexyl-CH_2
104 R=Cyclohexyl-$(CH_2)_2$
105 R=Phenyl
106 R=Phenyl-CH_2
107 R=Phenyl-$(CH_2)_2$

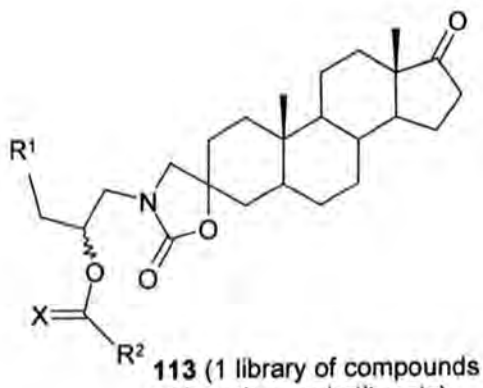


39 compounds with n=0, 1 or 2
R^x=Phenylmethyl, isopropyl or isobutyl
R^y=Phenyl, phenyl propyl or hexyl

108 n=2, R^x=Phenylmethyl
R^y=Hexyl



109-112 (3 libraries of compounds with various substituents)
Table 1g consists of the various R^1, R^2 and X substituents



113 (1 library of compounds with various substituents)
Tables 1g shows the substituents

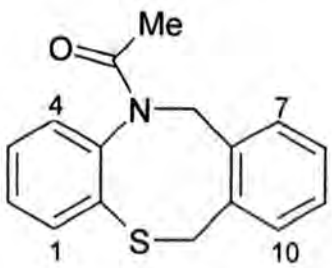
Figure 1.29: Androsterone derivatives as inhibitors for 17β-HSD3 (Poirier, 2003).

1.6.9 Novel series of tetrahydrodibenzazocines

These inhibitors were based on a dibenzazocine core, and they inhibited the enzyme at picomolar to low nanomolar concentrations, in cell-free enzymatic as well as in cell-based transcriptional reporter assays (Fink et al, 2006). The initial focus was based on structures shown below (Figure 1.30) which consisted of a dibenzothiazocine (DBT) and tetrahydrodibenzazocine (THB) series.

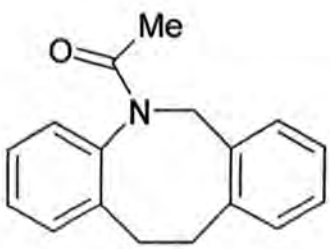
Compound	X	R1	R2	IC_{50} (nM)	Androgenic activity (%) @ 0.1 μM	Androgenic activity (%) @ 1 μM
109	O	Octyl	Cyclopropyl	57	0	0
110	O	Cyclohexylmethyl	Cyclopropyl	85	1	0
111	O	Adamantylmethyl	Propyl	35-57	7	96
112	H_2	Adamantylmethyl	Propyl	80	3	7
113	O	Morpholino	Cyclopentylethyl	74	4	6
106	-	-	-	57-98	27	100
96 (ADT)	-	-	-	182-330	14	2
Δ^4-dione	-	-	-	489-758	Not determined	Not determined

Table 1g: IC_{50} values for inhibitors of 17β-HSD3 and their androgenic activity on Shionogi (AR^+) cells (Poirier, 2003).



Dibenzothiazocine (DBT)

IC_{50}(μM) for enzyme = 0.40 and for cellular = 0.32



Tetrahydrodibenzazocine (THE

IC_{50}(μM) for enzyme = 0.09 and for cellular = 0.22

Figure 1.30: Inhibitors based on tetrahydrodibenzazocines (Fink et al 2006).

After the SARs in the DBT and THB series were carried out, it was found that within the DBT series, the derivatives with the addition of a halogen at the 2-

or 3- position yielded in a 4- to 10- fold improvement in potency in the biochemical as well as cell-based assays. The results are shown in Table 1h below.

Substitution group	IC_{50} (μM) for enzyme assay	IC_{50} (μM) for cellular assay
2-Cl	0.10	0.16
3-Cl	0.03	0.17
2-F	0.09	0.29
2-Br	0.25	0.12

Table 1h: Table showing inhibition values for the halogenated 2- and 3- substitutions (Fink et al, 2006).

The optimum substitution was chlorine, as the activity was found to decrease with both Fluorine and bromine. Alongside these values, the 2,3-dichloro derivative was found to be the most potent but only in the enzymatic assay (IC_{50}=0.01μM) and not in the cell-based assay (IC_{50}=0.13μM). It was also found that by substituting at positions 1- or 4- did not yield in inhibition, and this suggested that the enzyme makes close contacts with these regions of the DBT nucleus.

In case of the THB series, halogen substitution of the A-ring did not improve activity, however bromine substitution on the C-ring at either position 8- or 9- improved both the biochemical and cellular activity. For the 8- substitution, IC_{50} values of the enzymatic and cellular assays were 0.009 and 0.032μM, respectively, and for the 9- substitution being 0.007 and 0.036μM respectively, with an R group on the nitrogen on ring B being a C(O)Me group in both compounds. Furthermore, substitutions at the 8- and 9- positions with groups as a primary amine or a primary carboxamide, resulted in low activity. Data suggested this the region of the molecule (8-) extends into a hydrophobic pocket, and the addition of a phenyl group at position 8- confirmed this

hypothesis, by giving values such as 0.00002μM for the enzyme assay and 0.004μM for the cellular assay (Fink et al, 2006).

Further to the addition of the phenyl group at position 8-, electron-donating groups such as methyl ester groups at the *ortho*-, *meta*- and *para*- positions were added around the aryl ring, and it was observed that the *ortho*- position was preferred with IC_{50} values of 0.002 (enzyme assay) and 0.005μM (cellular-based assay). With the use of electron-withdrawing groups such as the CN group on once again the *ortho*-, *meta*- and *para*- positions, reduced activity was observed. The next step was the use of sulphonamides at the *ortho*- position, which increased the potency by a 100-fold (0.002 and 0.009μM for the enzyme and the cellular-based assay, respectively). A dramatic increase in potency was further observed by the incorporation of a carbonyl group at the *ortho*- position. The methyl ester showed picomolar activity with IC_{50} values of 0.00002 (enzyme assay) and 0.0005μM (cell-based assay). The methyl ketone was found to be equipotent with values of 0.00002 (enzyme assay) and 0.0005μM (cell-based assay).

These results and compounds were found to be potent against this enzyme and hence may provide an alternative approach for the disruption of testosterone biosynthesis by examining its role in both endocrine-sensitive and –resistant prostate tumour models (Fink et al, 2006).

1.7 3β-Hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase (3β-HSD)

3β-HSD is a membrane-bound enzyme that is dependent on NAD^+, and catalyses also the conversion of pregnenolone (PREG) to P, 17α-hydroxypregnenolone (17αOH-PREG) to 17α-hydroxyprogesterone (17αOH-P), Δ^5-diol to T and DHEA to 4-androstenedione (Δ^4-DIONE). The reaction is shown in Figure 1.31. AD is converted to E2 by placental AR and 17β-HSD, which takes part in initiating labour in humans after triggering off a cascade of events (Rainey et al, 2002; Kacsoh, 2000). 3β-HSD's deficiency causes a rare

form of congenital adrenal hyperplasia, which is associated with a male pseudohermaphroditism (Durocher et al, 2005).

Two types of 3β-HSD were isolated from humans (Luu et al, 1989; Rheaume et al, 1991), which share a very high homology (93.4% amino acids identity) and are expressed in a tissue-specific manner (Lachance et al, 1991). Type 1 is expressed in the placenta, skin and mammary glands, whereas type 2 is expressed in the gonads and adrenals (Rheaume et al, 1991). This enzyme is also present in several peripheral tissues such as the liver, adipose tissue, brain, kidney, epididymis, vas deferens, lung, prostate, bone and cardiovascular tissue, where it is involved in the formation of sex steroids (Durocher et al, 2005).

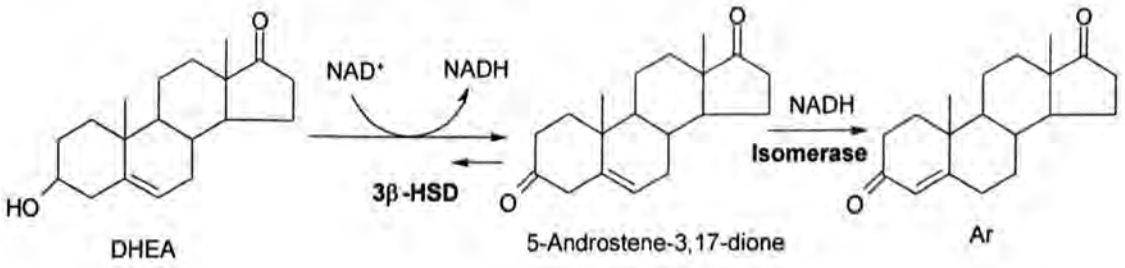


Figure 1.31: 3β-HSD/isomerase catalysing two reactions on a single enzyme protein (Thomas et al, 2004).

1.8 Estrone Sulfatase (ES)

Human ES is a member of a protein family in a group of hydrolytic enzymes, from the human arylsulfatase family, and there are at least six namely A, B, C, D, E and F (Pasqualini, 2004).

ES converts the stored (sulfated) form of the estrogens to the active (non-sulfated) form, thereby allowing the stimulation of estrogen-dependent tumours via a non-aromatase (AR) pathway (which is, therefore, not blocked by AR inhibitors) (Ahmed et al, 2002). E_2 formation in human breast tumours

was assessed quantitatively, and that indicated that the metabolism of EST via the sulfatase pathway produces 100-500 times more E_2 than androgen aromatisation. It was also found that the breast tissue possesses the enzyme EST that is involved in the conversion of estrogens to their sulfates which are biologically inactive (Shields-Botella et al, 2005).

ES is a monomer microsomal enzyme, which is membrane-bound to endoplasmic reticulum, and as mentioned, is responsible for the conversion of estrone sulfate to E1 (Figure 1.32), but it also controls DHEA formation from dehydroepiandrosterone sulphate (DHEAS) (Labrie et al, 2003; Hernandez-Guzman et al, 2001). It is found in the mammary cells, uterine cells (Brinbock, 1990) and in target organs for example, liver, endometrium, ovaries, bone, brain, prostate, white blood cells and adipocytes. It is found predominantly in placenta and in breast cancer tissue (Pasqualini, 2004).

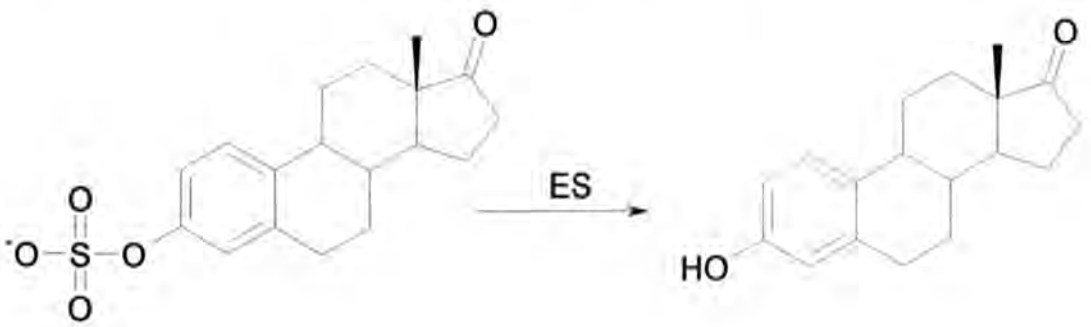


Figure 1.32: Action of the enzyme ES on estrone sulfate (Brinbock, 1990).

Much of the estrone formed from the AR pathway (Figure 1.1) is metabolised to the conjugated sulfate [by EST (Hobkirk et al, 1993)], which can act as a reservoir for the formation of E1, by the enzyme ES.

ES exists in a glycosylated form, which has been demonstrated by its ability to bind to concanavalin-A-sepharose (Purohit et al, 1998). The instability of this enzyme outside the microsome has prevented the pure enzyme from being isolated.

The exact mechanism of desulphonation has yet to be fully determined, however, studies are underway to crystallise the steroidal sulfatase enzyme, enabling a full x-ray crystal structure to be determined (Purohit et al, 1998). There is also controversy as to whether ES and another enzyme, dehydroepiandrosterone sulfatase (DHEA-STS) which converts DHEA-S to DHEA, are the same or different enzymes (Reed et al, 1996). Strong evidence has been shown to suggest that they are in fact the same enzyme (Dibbelt et al, 1986). An ES inhibitor was shown to inhibit the hydrolysis of both estrone sulfate and DHEA-S, suggesting that only one enzyme is responsible for both. Transferring a copy of the placental steroid sulfatase gene (cDNA) into COS-1 cells presented further evidence. The steroid sulfatase enzyme was capable of hydrolysing both sulfates (E1 and DHEA), demonstrating that only one enzyme is responsible for the hydrolysis of both alkyl and aryl steroid sulfates (Purohit et al, 1994).

Androstenediol is thought to be as important in the stimulation of breast cancer cells as (E2), with 90% of androstenediol synthesised in post-menopausal women originating form DHEA-S. This is then converted to DHEA through the action of DHEA-STS, and further metabolised to androstenediol under the action of testicular 17β HSD3 (Andersson et al, 1995). Another route may be the hydrolysis of androstenediol sulfate to androstenediol (Figure 1.33). Androstenediol possesses estrogenic properties and can bind to estrogen receptors, stimulating the growth of breast tumours (Adams et al, 1981).

Tumour stimulation without estrogens may explain why AR inhibitors have failed to give the desired response rates in hormone-dependent breast tumours, emphasising the importance of developing a sulfatase inhibitor which would inhibit both estradiol and androstenediol synthesis, thereby reducing the levels of tumour-stimulating hormones.

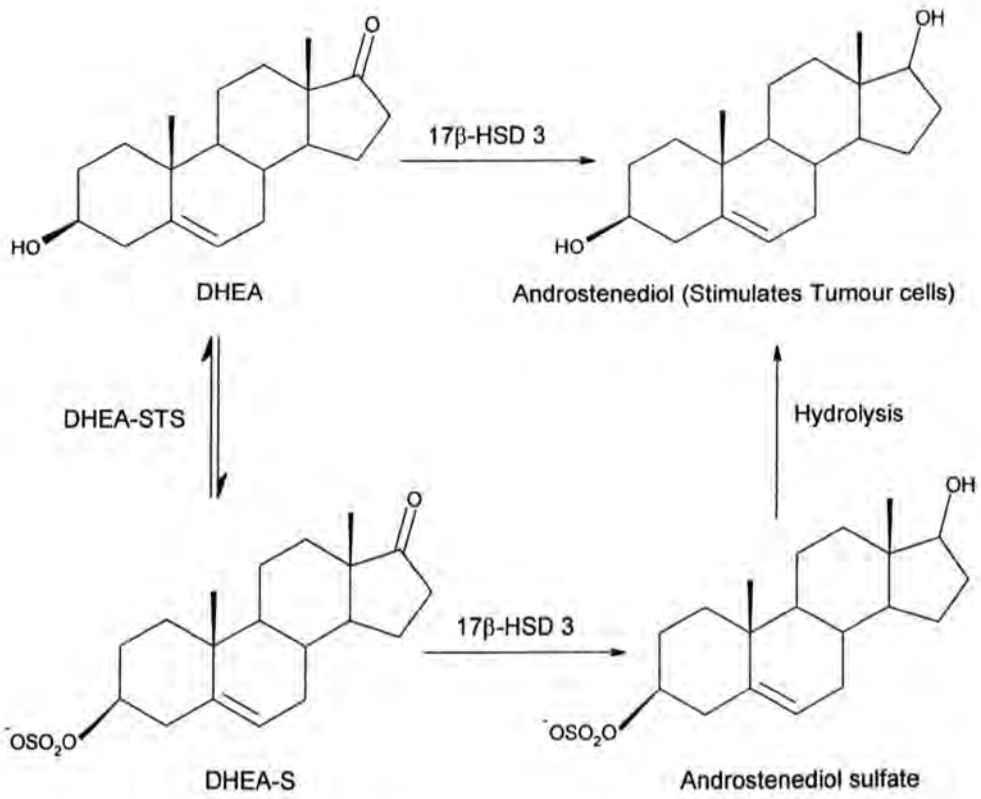


Figure 1.33: Route showing the formation of androstenediol.

1.9 Mechanism of estrone sulfatase

From previous studies, it was found that potent inhibitors of ES contain an aminosulfonate moiety, which is known to inhibit the enzyme irreversibly, whereas compounds containing different substituents have shown to be weak and reversible inhibitors. Not much information is known about the active site of ES, and so to work out the nature of the pharmacophore as well as to rationalise the irreversible activity of the aminosulfonate based compounds, SAR studies were carried out extensively, which would aid further in understanding the mechanism. It was also established that factors such as pK_a of the parent phenol is an important factor in the inhibition of the enzyme, whereby the hydrolysis of the S-OR bond was seen as a very important feature in the inhibition process (Ahmed et al, 2002). This is shown in Figure 1.34.

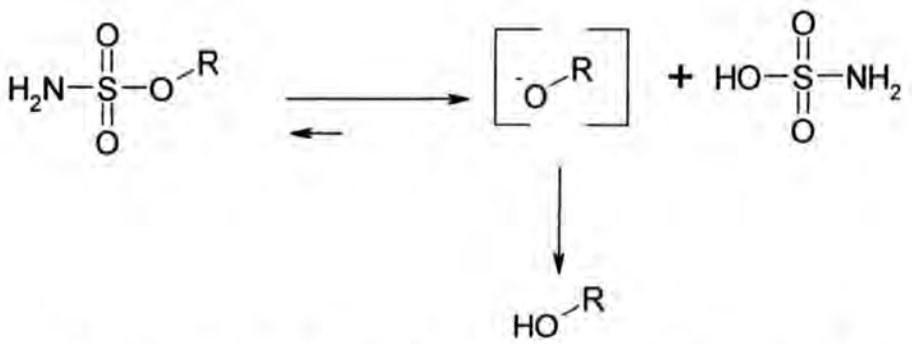


Figure 1.34: Hydrolysis of phenyl aminosulfonate containing compounds (Ahmed et al, 2002).

Hence using synthesised alkyl and phenyl sulfamates, the inhibitory activity was evaluated so as to understand the mechanism in detail. In addition to this, the SAR studies of the phenyl substituted sulfamate based compounds provided a good correlation between pK_a and inhibitory activity, and also confirmed the moiety used, as such, the use of strong electron withdrawing groups for example, NO_2 and CN attached to the phenyl ring resulted in potent inhibitory activity as compared to electron donating groups for example CH_3, which resulted in poor inhibitory activity. Therefore, using this rationale of the stabilisation effect of the electron withdrawing group on the phenoxide ion (Ahmed et al, 2000), it was suggested that the inhibitory activity of the phenolic compounds depended on the sulfamate moiety, which undergoes hydrolysis and hence is important in irreversible inhibition.

Hence the first step of the mechanism, which is the crucial step, involves the cleavage of the S-OR bond, which results in the formation of RO^- and sulfamic acid. Sulfamic acid was found to be involved in the inhibition of ES rather than the alkyl or phenyl sulfamate. Therefore after taking into consideration the proposed mechanism by Ahmed et al, of the desulphatation of estrone sulphate (shown in Figure 1.35), as well as the SAR data for the sulfamate based compounds, a novel mechanism (shown in Figure 1.36) for the irreversible inhibition of ES was proposed (Ahmed et al, 2002).

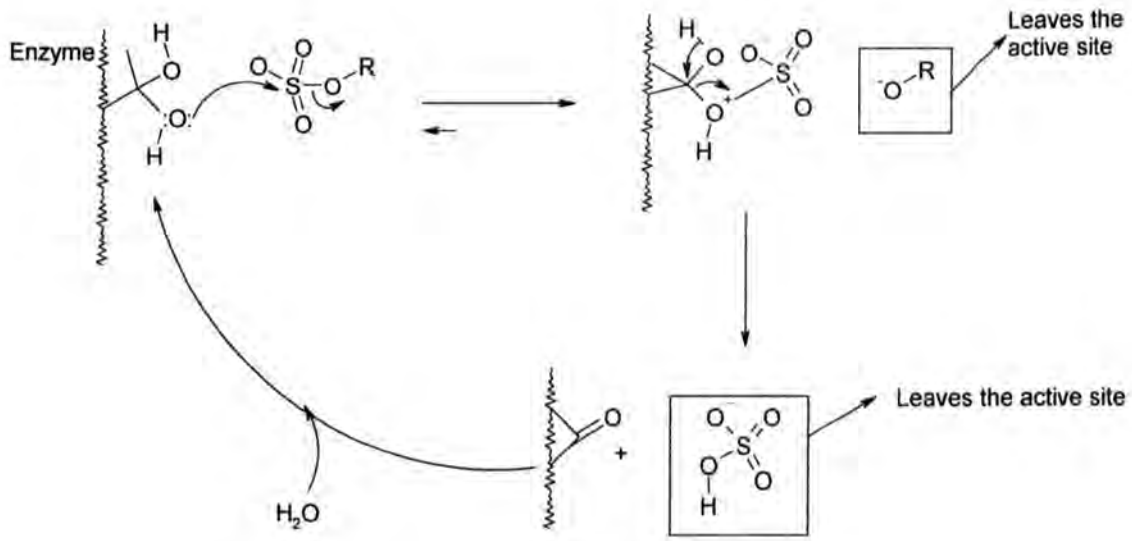


Figure 1.35: Desulphatation reaction catalysed by ES (Ahmed et al, 2002).

As mentioned previously, cleavage of the S-OR bond occurs via the attack by the gem-diol moiety on the sulfamate group. The gem-diol moiety is proposed to exist in the active site of the enzyme [(Waldow et al, 1999), as shown in Figure 1.35)]. This attack allows the formation of an aldehydic moiety, the RO^- ion and sulfamic acid, and an important step in the catalytic and inhibition process, is the formation of the C=O group [(Waldow et al, 1999; Bond et al, 1997), (as shown in Figure 1.35)]. This confirms the fact that without the formation of the aldehydic group from the gem-diol, it is not possible for irreversible inhibition to take place.

The NH_2 group of the sulfamate then attacks the newly formed aldehydic group, which results in the formation of an imine type structure, and that involves the loss of a water molecule, hence resulting in irreversible inhibition of ES (as shown in Figure 1.36). Another fact whereby irreversible inhibition of ES has occurred is due to the formation of the C=N bond, which causes the enzyme to not cycle through the catalytic process (Ahmed et al, 2002).

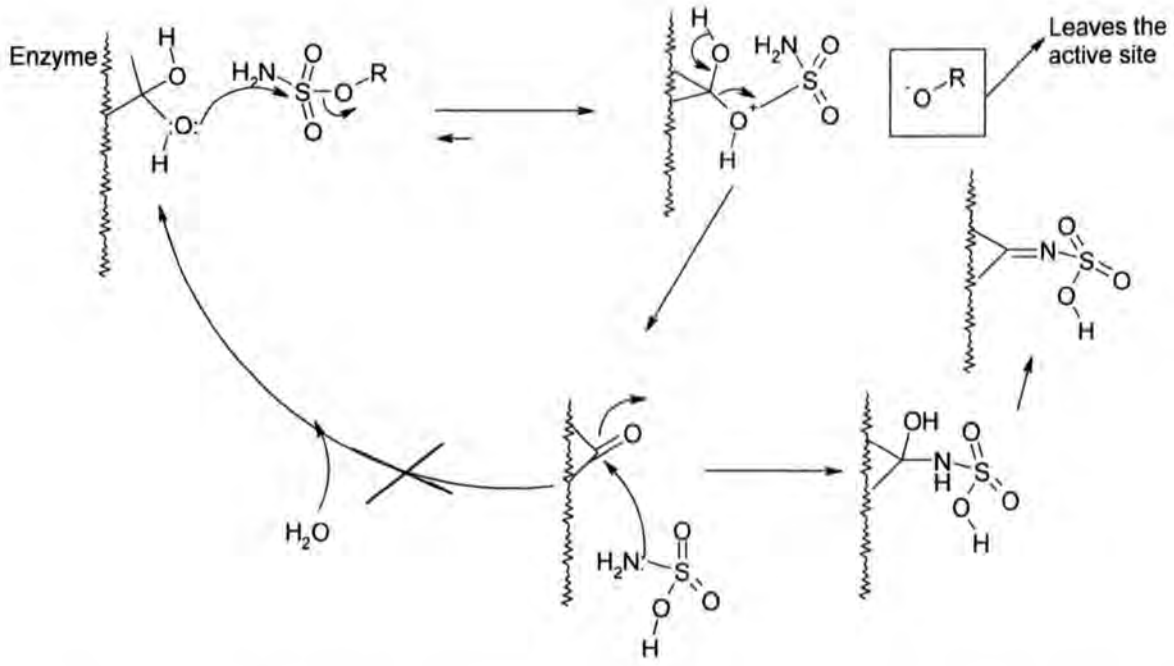


Figure 1.36: Mechanism of irreversible inhibition of ES by sulfamate based compounds and the release of sulfamic acid via the initial cleavage of the S-OR bond (Ahmed et al, 2002).

Hydrophobicity was another factor which was still not fully understood and explained as to how its involvement affects the inhibition, especially the log of the partition coefficient. To investigate this, a series of 4-hydroxycinnamic acid esters were sulfamated and these showed that the hydrophobicity of the carbon backbone of the inhibitors was indeed an important factor in ES inhibition (Ahmed and Patel, 2002). This further led to the proposal that the RO^- was expelled out of the active site by the log*P* of the inhibitors which were involved in destabilising the enzyme-product complex and at the same time, allowing the sulfamic acid to attack the C=O group within the active site, and this led to the conclusion that high inhibitory activity of the sulfamated compounds was due to high hydrophobicity (Ahmed et al, 2002).

With highly hydrophobic compounds, the RO^- is expelled out of the active site due the group becoming unstable, and due to that, the sulfamic acid attacks the C=O group leading to the imine product and hence the irreversible inhibition of ES. This rationale can be used therefore for the desulphatation of estrone sulphate (as shown in Figure 1.35), as such the hydrolysis of estrone

sulphate forms the estrone anion, which in turn causes destabilisation of the enzyme-product complex, and that results in the steroid moiety getting expelled and hence undergoing an irreversible inhibition (Ahmed et al, 2002).

1.10 Estrone sulfatase inhibitors

There are no ES inhibitors currently available on the market as yet, but synthesis of a large number of steroidal and non-steroidal compounds has been carried out which have shown potent inhibitory activity against this enzyme.

1.10.1 Steroidal inhibitors

Danazol (Figure 1.37) was an inhibitor synthesised that was found to possess inhibitory activity (Carlstrom et al, 1984a), and was also found to be a relatively weak competitive inhibitor in mammary cell lines with K_i values of 2.3-8.2μM (Nguyen et al, 1993) and inhibited placental microsomal ES by 60% at 10μM inhibitor concentrations (Selcer et al, 1996) .

E1-MTP (Figure 1.37) was the first steroid-based compound, that was a specific ES inhibitor (Cox et al, 1979), and it acted as a steroid sulfate structural mimic when used for the generation of antibodies which recognised estrone sulfate. It was found to be 14 times more potent than danazol, and is a reversible inhibitor acting in a time-independent and competitive manner, i.e. after the inhibitor had been removed from the assay mixture after a given amount of time, ES activity was restored. It possessed a K_i value of 37.5μM (Duncan et al, 1993) and an IC_{50} value of 90nM in intact MCF-7 breast cancer cell lines (Purohit et al, 1995).

EMATE (estrone-3-O-sulfamate) (Figure 1.37) in intact MCF7 breast cancer cells showed 95% ES inhibition (at 2nM), and an IC_{50} value of 65pM in MCF-7 cells (Purohit et al, 1995; Howarth et al, 1994) and 4nM in placental

microsomes. EMATE, compared to E1-MTP, was found to be a potent irreversible inhibitor (Purohit et al, 1995).

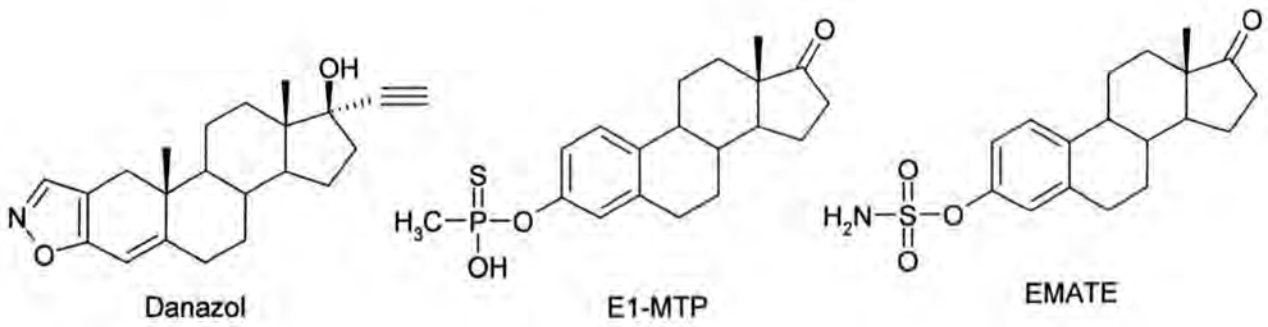


Figure 1.37: Figure showing examples of steroidal inhibitors of ES (Ahmed at al 2002).

1.10.2 Non-steroidal inhibitors

The steroidal inhibitors were found to be potent inhibitors, however some possessed estrogenic activity, therefore non-steroidal inhibitors were investigated and synthesised.

A large number of non-steroidal inhibitors have been synthesised, and considered as mimics of the steroidal backbone, for example, A, AB and AD ring mimics of the steroid backbone.

4-Methylcoumarin-7-O-sulfamate (COUMATE) (Figure 1.38) has been found to be a non-estrogenic active site directed ES inhibitor (Purohit et al, 1996), and possessed an IC_{50} value of 380nM and was found to inhibit ES in a time- and concentration dependent manner (Purohit et al, 1996). A series of tricyclic coumarin sulfamates has also been synthesised, and 667-COUMATE (Figure 1.38) was the most potent inhibitor, and was three times more potent than EMATE and a hundred times more potent than COUMATE in inhibiting ES.

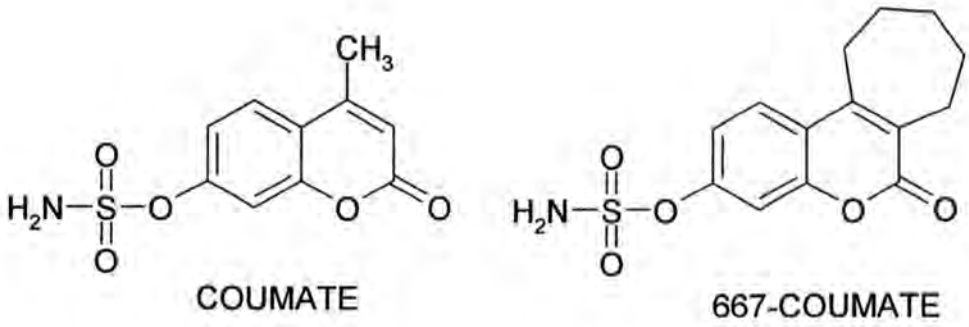
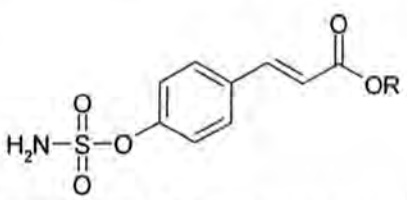


Figure 1.38: Non-steroidal inhibitors (Woo et al, 2000).

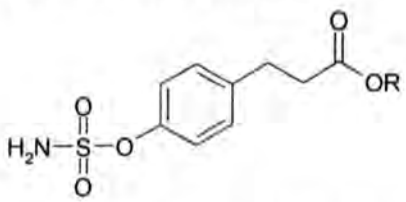
Sulfamated esters of 4-hydroxycinnamic acid and 3-(4-hydroxyphenyl) propanoic acid

A series of sulfamated esters of 4-hydroxycinnamic acid and 3-(4-hydroxyphenyl) propanoic acid (shown in Figure 1.39) were synthesised by Ahmed et al, and these were tested for their mode of action as well as their physicochemical properties via pK_a and LogP studies, and also the SAR was determined. These compounds' activity was compared to the standard compounds EMATE, COUMATE and 667-COUMATE (Ahmed et al, 2004). The compounds and their IC_{50} values are shown below in Table 1i.



Sulfamate esters of 4-hydroxycinnamic acid

114 R=CH_3
115 R=CH_2CH_3
116 R=$(CH_2)_2CH_3$
117 R=$(CH_2)_3CH_3$
118 R=$(CH_2)_4CH_3$
119 R=$(CH_2)_5CH_3$
120 R=$(CH_2)_6CH_3$
121 R=$(CH_2)_7CH_3$
122 R=$(CH_2)_8CH_3$



Sulfamate esters of 3-(4-hydroxy phenyl) propanoic acid

123 R=CH_3
124 R=CH_2CH_3
125 R=$(CH_2)_2CH_3$
126 R=$(CH_2)_3CH_3$
127 R=$(CH_2)_4CH_3$
128 R=$(CH_2)_5CH_3$
129 R=$(CH_2)_6CH_3$
130 R=$(CH_2)_7CH_3$
131 R=$(CH_2)_8CH_3$

Figure 1.39: Sulfamates of 4-hydroxycinnamic acid and 3-(4-hydroxyphenyl) propanoic acid esters (Ahmed et al, 2004).

Compound	$IC_{50}(\mu M)$
114	790±13.4
115	334±1.4
116	274±8.4
117	63.5±2.1
118	992.5±12.0
119	1486±31.1
120	2707±71.4
121	>10,000
122	>10,000
123	3775±96.1
124	3285±67.2
125	3377±46.7
126	5039±24.7
127	8560±12.0
128	12003±31.1
129	>10,000
130	>10,000
131	>10,000
EMATE	0.5±0.04
COUMATE	13.8±0.07
667-COUMATE	0.21±0.01

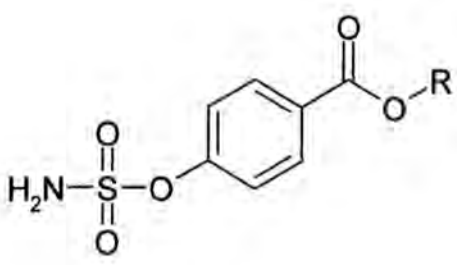
Table 1i: Sulfamated esters of 4-hydroxycinnamic acid and 3-(4-hydroxyphenyl) propanoic acid and their IC_{50} values (Ahmed et al, 2004).

The esters of 4-sulfamoylated cinnamic acid were found to be weak inhibitors compared to the standard compounds EMATE, COUMATE and 667-COUMATE. Compound **117** was found to be the most potent with an IC_{50} value of 63.5±2.1μM, but this in comparison to the three standard compounds, was a weak inhibitor. Furthermore, the ester derivatives of 3-(4-sulfamoylated phenyl) propanoic acid, which were the reduced forms of compounds **114-122**, were synthesised. These compounds were found to be

very weak as inhibitors, compared to the sulfamoylated esters of the 4-hydroxycinnamic acid and the three standard compounds. It was proposed that in the case of the sulfamated esters of 4-hydroxycinnamic acid, the reason behind the reduced inhibitory activity was the fact that there was freedom of rotation of the two C-C bonds, which resulted in the overall conjugation to decrease. In doing so, the stabilisation of the phenoxide ion, which was produced via the sulfamate group hydrolysis, had reduced. Another factor also related to the inhibitory activity was the size of the molecule and in this case, it was the chain length, which when increased caused steric interaction with the active site that corresponded to the C(17) area of the steroid backbone.

Sulfamates of 4-hydroxyphenyl esters

Following the compounds above which were synthesised by the group, the next series of compounds that were synthesised and their activity was tested, were a series of sulfamates of 4-hydroxybenzoic acid esters (Shown in Figure 1.40). The series consisted of straight-chain and cyclic-alkyl esters and some of these were tested by initial screening with IC_{50} values also being obtained (Table 1j) (Ahmed et al, 2003).



132 R=C_5H_{11}
133 R=C_6H_{13}
134 R=C_7H_{15}
135 R=C_8H_{17}
136 R=C_5H_{10} (cyclic)
137 R=C_6H_{12} (cyclic)
138 R=C_7H_{14} (cyclic)
139 R=C_8H_{16} (cyclic)

Figure 1.40: Some of the sulfamates of 4-hydroxybenzoic acid esters (Ahmed et al, 2003).

Compound	$IC_{50}(\mu M)$
132	5.9±0.44
133	3.8±0.16
134	3.4±0.25
135	5.0±0.26
136	9.3±0.07
137	1.7±0.07
138	0.5±0.03
139	0.2±0.01

Table 1j: Table showing the sulfamates of 4-hydroxybenzoic acid esters with their IC_{50} values (Ahmed et al, 2003).

From the synthesis and data obtained, it was found that all compounds above showed good inhibitory activity, and were also more potent than the standard COUMATE (with an IC_{50} value of 12±0.3μM), and the sulfamates of the esters numbered **132-135** were weaker than EMATE (with an IC_{50} of 0.5±0.01μM) and 667-COUMATE (with an IC_{50} value of 0.25±0.01μM). Compounds **136-139** on the other hand, which were the sulfamates of the cyclic esters were found to be equipotent to EMATE and 667-COUMATE, with the cyclooctyl derivative **(139)** being roughly 1.5 times more potent than the latter compound. Finally, these compounds were found to inhibit ES in an irreversible manner and were time-dependent (i.e. could not be dialysed with time) especially compound **139**, which was found to be more potent than 667-COUMATE, which has been in Phase I clinical trials (Ahmed et al, 2003).

AC ring mimics

These compounds were synthesised after the hypothesis that they would inhibit ES by mimicking the steroidal backbone, as such these compounds consisted of sulfamated biphenyls, where the biphenyl moiety would mimic the A and C rings of the steroid backbone and the R group would be involved in hydrogen bonding with any H-bonding group(s) that would be present within

the active site of ES (Ahmed et al, 2002). The structure of the compound is shown in Figure 1.41. The sulfamates that were synthesised were 4'-*O*-sulfamoyl-4-biphenyls and their inhibitory activity and their mode of action was investigated.

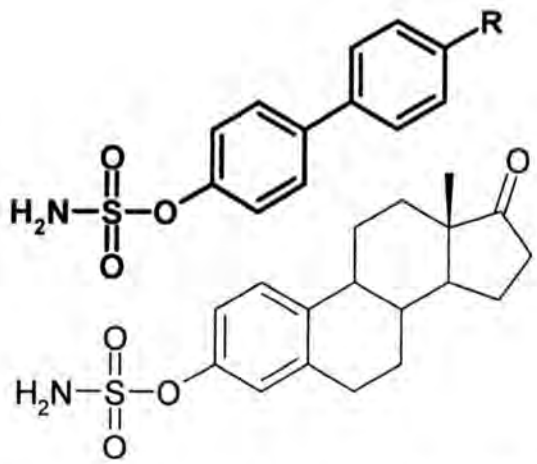


Figure 1.41: Biphenyl sulfamates relationship to EMATE (Ahmed et al, 2002).

The compounds were tested using a literature method (Purohit et al, 1998) and the results are shown below in Table 1k, with the compounds and their corresponding R groups, the compound numbers and their IC_{50} values.

Compound (R)	Compound Number	IC_{50} (μM/tube)
H	**140**	76.1±0.9
CN	**141**	6.7±0.01
COOMe	**142**	5.2±0.07
COOEt	**143**	4.2±0.01
COOPr	**144**	3.5±0.01
COOBu	**145**	5.8±0.02

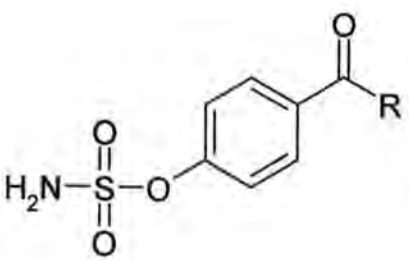
Table 1k: Table showing the inhibition data for the synthesised biphenyl sulfamates (Ahmed et al, 2002).

All the compounds were found to be weak inhibitors in comparison to EMATE (0.5±0.01μM) and 667-COUMATE (0.2±0.01μM). However comparing the compounds to COUMATE (10±0.3μM), all the compounds showed good

inhibitory activity except compound **140**; compound **144** was found to be approximately three times more potent than COUMATE. These compounds were also found to be irreversible inhibitors of ES and were good inhibitors and hence the biphenyl structure is under further investigation to improve potency (Ahmed et al, 2002).

Sulfamates of 4-hydroxyphenyl ketones

Following the synthesis of the sulfamates of the esters, the research group investigated the activity of sulfamates of 4-hydroxyphenyl ketones (Figure 1.42). These compounds were also tested for their mode of action and for their biochemical evaluation, and their IC_{50} values are shown in Table 1l.



146 R=H
147 R=CH_3
148 R=C_2H_5
149 R=C_3H_7
150 R=C_4H_9
151 R=C_6H_{13}
152 R=C_7H_{15}
153 R=C_8H_{17}
154 R=C_9H_{19}
155 R=Ph
156 R=CH=CHPh
157 R=CH_2Ph

Figure 1.42: Sulfamates of 4-hydroxyphenyl ketones (Ahmed et al, 2002).

Compounds **146-157** were found to be potent inhibitors, and in comparison to EMATE ($0.5\pm0.001\mu M$), compound **153** (which was the most potent compound of the group) was about 6.8 times weaker. With regards to COUMATE, most of the compounds were either equipotent or higher in potency than COUMATE, as such, compounds **151**, **152** and **153** were 2.4, 2.1 and 3.5 times more potent respectively and are the known potent compounds to date and which were also found to be irreversible inhibitors (Ahmed et al, 2002).

Compound	IC_{50} (μM) at [S]=50 μM
146	254±10.1
147	302±6.7
148	116.4±4.2
149	39.8±1.4
150	20.9±0.38
151	5.0±0.36
152	5.6±0.19
153	3.4±0.13
154	13±0.05
155	63±1.83
156	263±5.5
157	33±1.1

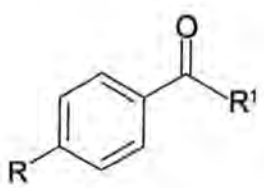
Table 1l: Table showing the inhibition data for the sulfamates of 4-hydroxyphenyl ketones (Ahmed et al, 2002).

1.11 Basis of present investigation

Androgens have been proposed as key endocrine factors for the initiation and progression of hormone-dependent diseases such as prostate and breast cancer. One of the methods of treatment is the deprivation of hormones by the inhibition of enzymes involved in androgen and estrogen biosynthesis, in particular, the 17β-HSD (more specifically isozyme type 3) and ES family of enzymes.

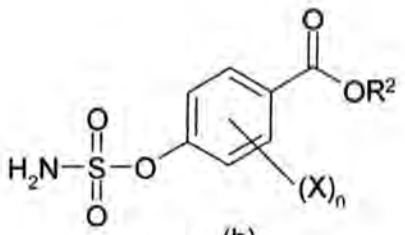
Extensive work has been carried out to develop highly potent and selective inhibitors of 17β-HSD3 and ES. It is thought that the development of inhibitors for these enzymes may lead to an improvement in the treatment for hormone-dependent diseases. Although extensive work has been undertaken in this area, there is a need for further development, since only a few compounds have been shown to be suitable for clinical trials.

The aim of the present investigation involves the synthesis of potential inhibitors of both 17β-HSD3 (Figure 1.43a) and ES (Figure 1.43b) based on the compounds shown in Figure 1.43. The compounds will then undergo biochemical evaluation to determine the potency of these compounds against the two enzymes.



(a)

R=OH, H_2NSO_2O, H_3CSO_2O and Ph
R^1=Alkyl chain from C_1 to C_{12} and cycloalkyl groups



(b)

R^2=Alkyl chain length and cycloalkyl group
$(X)_n$=unsubtituted, 3-Br, and 3,5-Br

Figure 1.43: General structures of potential inhibitors of: the 17β-HSD family of enzymes [compound (a)] and; ES [compound (b)].

CHAPTER 2

Synthesis of 4-hydroxyphenyl ketones and their derivatives

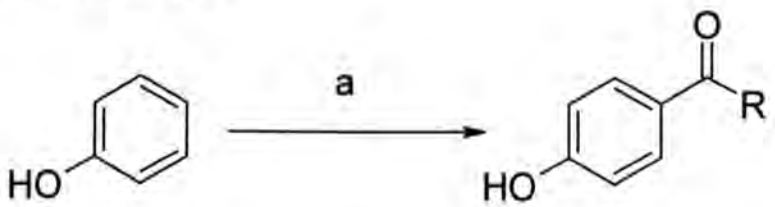
2.1 Discussion

From initial molecular modelling studies, it was suggested that the carbonyl moiety may be a good mimic of the steroid C(17)=O functional group (Owen and Ahmed, 2004). As such, we undertook the synthesis of the 4-hydroxyphenyl ketones and its derivatives according to Scheme 2.1. In an attempt to study the effects of the removal/substitution of the 4-hydroxy moiety, we also undertook the sulfonation of the phenolic moiety as outlined in Scheme 2.1.

The synthesis of the 4-hydroxyphenyl ketones involves acylation of the phenol moiety through the Friedel-Crafts acylation reaction. In this particular reaction, the phenol is allowed to initially react with the excess aluminium chloride ($AlCl_3$). In reactions involving non-hydroxylated phenyl derivatives, the acyl chlorides are often allowed to complex with the $AlCl_3$ prior to the addition of the phenyl compound. It is postulated that as a result of the initial interaction between phenol and $AlCl_3$ leads to the formation of a complex between the phenolic oxygen and the $AlCl_3$. The complex formed is postulated to firstly reduce the possibility of ester formation on the addition of the acid chloride. Secondly, and more importantly, the bulky nature of the complex is thought to sterically hinder the ortho positions of the aromatic ring system. As such, only the para-substituted product would be expected to be produced.

Furthermore, for the Friedel-Crafts acylation reaction to take place, an acylium ion is required to be formed as a result of the reaction between acyl chloride and $AlCl_3$, which then undergoes nucleophilic attack by the phenyl ring, as such, excess $AlCl_3$ is required to be used (Fessenden and Fessenden, 1993). The acylium ion is resonance stabilised, as such, rearrangement of the acylium ion is not observed where as with Friedel-Crafts alkylation (where a carbocation formation is a requirement) rearrangement, so as to produce the more stable carbocation, is observed for alkyl chains containing greater than a C_3 moiety.

The acylation step in Scheme 2.1 was found to proceed, in general, without any major problems and the 4-hydroxyphenyl ketone products (compounds **158** to **172**) were obtained in poor to good yields, ranging from ~14% for the 1-(4-hydroxy-phenyl)-decan-1-one (**166**) to ~53% for 1-(4-hydroxy-phenyl)-propan-1-one (**159**) after purification involving column chromatography or recrystallisation. Furthermore, under the Friedel-Crafts reaction conditions used within the current study, no ortho substitution product was observed. Also, no disubstitution (or indeed any other polysubstitution) products were observed. This is presumably due to the electron withdrawing ability of the carbonyl group thereby leading to the deactivation of the phenyl ring system by the carbonyl group.



R=C_2H_3, **(158)**	R=C_9H_{17}, **(165)**
R=C_3H_5, **(159)**	R=$C_{10}H_{19}$, **(166)**
R=C_4H_7, **(160)**	R=$C_{11}H_{21}$, **(167)**
R=C_5H_9, **(161)**	R=$C_{12}H_{23}$, **(168)**
R=C_6H_{11}, **(162)**	R=C_4H_7 (Cyclic), **(169)**
R=C_7H_{13}, **(163)**	R=C_5H_9 (Cyclic), **(170)**
R=C_8H_{15}, **(164)**	R=C_6H_{11} (Cyclic), **(171)**
	R=C_7H_{13} (Cyclic) , **(172)**

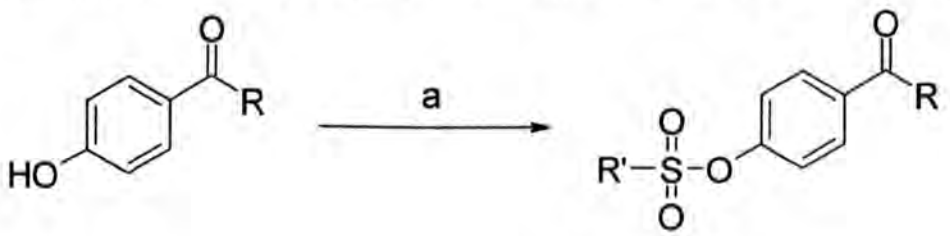
Scheme 2.1: Synthesis of 4-hydroxyphenyl ketones (a=DCM/RCOCl/$AlCl_3$; R=alkyl chain from C_1 to C_{12} and cycloalkyl groups).

The yields for the larger alkyl chain containing compounds were presumed to be due to the extraction procedure where sodium hydroxide is used to generate the sodium salt of the phenol and it is presumed that with the larger alkyl containing compounds, the increased hydrophobicity due to the alkyl chain resulted in a lowering of the amount of compound being extracted into the sodium hydroxide layer, thereby resulting in reduced yield.

With regards to the cyclo derivatives (compounds **169** to **172**), we observe that the yield ranges from ~18% for the cycloheptyl-(4-hydroxyphenyl)-methanone (**172**) to 45% for the cyclopentyl-(4-hydroxyphenyl)-methanone (**170**) and cyclohexyl-(4-hydroxyphenyl)-methanone (**171**). The poor yield obtained for **172**, appears not to be related to the hydrophobicity as the corresponding acyl chloride was required to be synthesised from the carboxylic acid and which presumably did not go to completion or was hydrolysed during the removal of the excess thionyl chloride.

2.1.1 Synthesis of derivatives of 4-hydroxyphenyl ketones

In the synthesis of derivatives of 4-hydroxyphenyl ketones, the reactions outlined in Scheme 2.2 were undertaken to produce the different derivatives.



Where R'=CH3

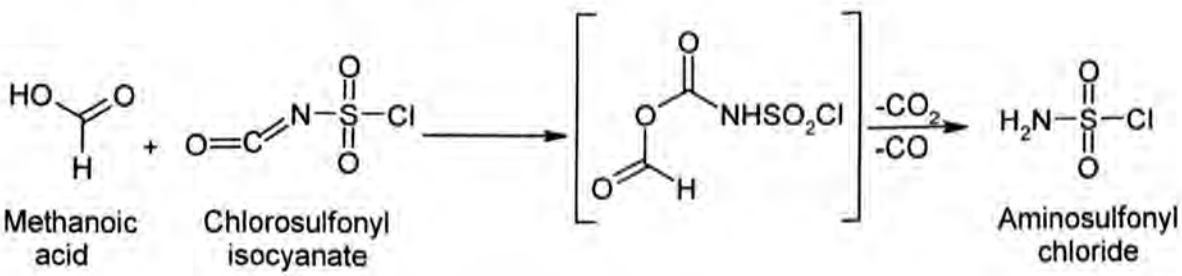
R=Ph, **(173)**
R=H, **(174)**
R=C_2H_3, **(175)**
R=C_3H_5, **(176)**
R=C_4H_7, **(177)**
R=C_5H_9, **(178)**
R=C_6H_{11}, **(179)**
R=C_7H_{13}, **(180)**

R=C_8H_{15}, **(181)**
R=C_9H_{17}, **(182)**
R=$C_{10}H_{19}$, **(183)**
R=$C_{11}H_{21}$, **(184)**
R=C_3H_5 (Cyclic), **(185)**
R=C_4H_7 (Cyclic), **(186)**
R=C_5H_9 (Cyclic), **(187)**
R=C_6H_{11} (Cyclic), **(188)**
R=C_7H_{13} (Cyclic), **(189)**

Scheme 2.2: Synthesis of derivatives of sulfamic acid phenyl esters and sulfamic acid 2,6-dibromophenyl esters (a=toluene/TEA/R'SO_2Cl/Δ; R=alkyl chain from C_1 to C_{12} and cycloalkyl groups; R'=CH_3 and NH_2).

In general, the reactions involved the reaction of the 4-hydroxyphenyl ketone with the appropriate sulfonyl chloride using anhydrous conditions to give the corresponding sulfonated derivatives. The reactions were found to progress well in good yield. For example, in the synthesis of the methanesulfonate derivatives (compounds **173** to **189**), the reaction between methanesulfonyl chloride and the appropriate 4-hydroxyphenyl ketones lead to the synthesis of the target compounds in low to good yields, the low yield ranging from 15%, [for methanesulfonic acid 4-undecanoyl-phenyl ester (**184**)] to an excellent yield of 97% [for methanesulfonic acid 4-octanoyl-phenyl ester and methanesulfonic acid 4-nonanoyl-phenyl ester (**181** and **182**)].

However, in the synthesis of the sulfamate derivatives (compounds **192** to **201**), the reaction between 4-hydroxyphenyl ketones and aminosulfonyl chloride did lead to some problems which have previously been reported (Scheme 2.2). In general, this reaction proved to be troublesome and led to poor yields, however, the poor yield is though to be due to the poor stability of both the aminosulfonyl chloride and the target compound. Moreover, aminosulfonyl chloride is not readily available commercially, due to its rapid degradation (James, 2000); it was therefore synthesised *in situ* prior to immediate use (Scheme 2.3).

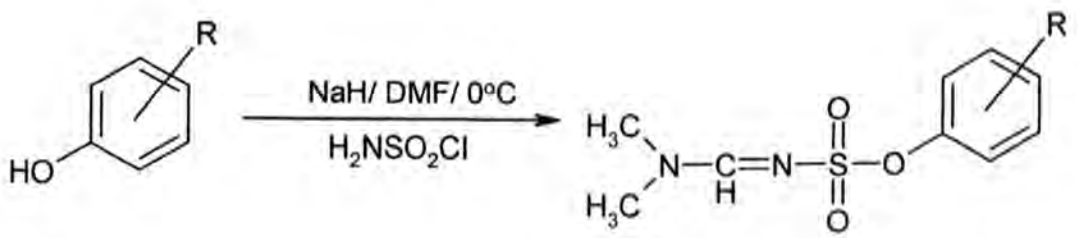


Scheme 2.3. Formation of aminosulfonyl chloride.

The reaction leading to the formation of the aminosulfonyl chloride (Scheme 2.3) is believed to involve an initial nucleophilic attack (by the methanoic acid) on the chlorosulfonyl isocyanate, resulting in an intermediate which undergoes decarbonylation and decarboxylation to give aminosulfonyl chloride. In their synthesis of aminosulfonyl chloride, Appel and Berger

(1958) extracted the product into benzene, filtered, and removed the solvent under vacuum. In the present study, benzene was replaced with anhydrous toluene, resulting in a problem involving the removal of the high boiling point solvent. That is, toluene could not be removed at room temperature and any heating resulted in the degradation of the product, as a consequence, the product was not purified and was left in solution in toluene.

As previously mentioned, the synthesis of aminosulfamate has been extensively studied within our group and the initial synthesis of the sulfamate derivatives of the 4-hydroxyphenyl ketones was initially attempted using sodium hydride (NaH) as a base for the reaction (James, 2000; Patel, 2003), in a similar manner to Woo et al (1996). However, we discovered that an undesired dimethylformamide (DMF) adduct was formed (Scheme 2.4). The so called 'DMF adduct' had previously been reported by Shwarz et al (1996). In their report, the authors proposed that the DMF adduct was formed through the attack on the carbonyl carbon atom of DMF by the lone pair of electrons on the nitrogen atom of the aminosulfonate group, followed by the dehydration of the product during the work up to give the imine type product (Scheme 2.4) (Woo et al, 1998).

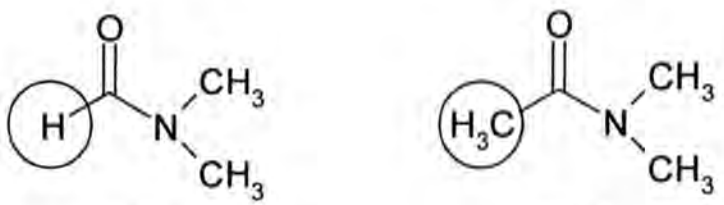


Scheme 2.4. The formation of the DMF adduct.

As a result, alternative methods of sulfamoylation were investigated and it was found by Patel (2003[a]) that by using a weak base such as anhydrous potassium carbonate (K_2CO_3) was used, successful synthesis of the sulfamates was achieved, but in very poor yields (generally less than 10%).

Okada et al (2000) discovered a successful method of sulfamoylation which did not involve the use of any base and as such possessed the possibility of obtaining the target aminosulfonated compound without the production of the DMF adduct. In their study, the authors considered both the change in base and solvent in an attempt to increase the yield, however, they concluded that the use of *N,N*-dimethylacetamide (DMA) as the solvent in the absence of a base was enough to obtain the target compounds in relatively high yield.

More specifically, in an attempt to optimise their results, the authors studied both the amounts of base and aminosulfonyl chloride used in varying quantities. They found that the solvent used for the reaction had a profound effect on the rate of sulfamoylation and in particular, they found that the use of DMA alone resulted in not only a faster reaction but also had minimal by-product formation. Elimination of a base altogether led to the highest yields compared to that of a sulfamoylation reaction with both solvent and base present. The authors also suggested that the formation of the DMF adduct is attributed to a competing reaction between DMF and aminosulfonyl chloride and that since the α-proton in DMF is replaced in DMA with a methyl group (Figure 2.1). DMA therefore possesses a greatly reduced reactivity for the competing reaction, thereby eliminating the competing side reaction and therefore removing any possibility of DMA-adduct formation.

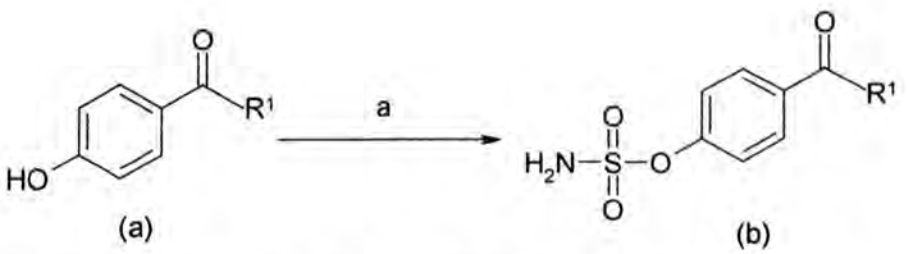


DMF DMA

Figure 2.1: Comparisons between DMF and DMA.

In our hands, no problems were encountered using DMA and as such, all of the sulfamoylation reactions of the 4-hydroxyphenyl ketones were undertaken using the above method (Scheme 2.4) and the reactions proceeded smoothly, however, in poor to moderate yields ranging from 5% [for sulfamic acid 4-

acetyl-phenyl ester (**192**)] to 33% [for sulfamic acid 4-propionyl-phenylester (**193**)].



(a) (b)

R¹=Alkyl chain from C_2 to C_{12} and cycloalkyl groups

$R^1=C_2H_3$, **(192)**
$R^1=C_3H_5$, **(193)**
$R^1=C_4H_7$, **(194)**
$R^1=C_5H_9$, **(195)**
$R^1=C_6H_{11}$, **(196)**

R¹=Alkyl chain length and cycloalkyl groups

$R^1=C_7H_{13}$, **(197)**
$R^1=C_8H_{15}$, **(198)**
$R^1=C_9H_{17}$, **(199)**
$R^1=C_4H_7$ (Cyclic), **(200)**
$R^1=C_5H_9$ (Cyclic), **(201)**

Scheme 2.5: Synthesis of derivatives of sulfamic acid phenyl ketones (a=toluene/NH_2SO_2Cl, R^1=alkyl chain length from C_2 to C_{12} and cycloalkyl groups).

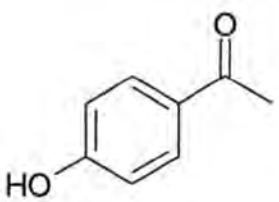
2.2 EXPERIMENTAL

2.3 MATERIALS AND METHOD

Chemicals were purchased from Sigma-Aldrich Company Ltd (Poole, Dorset, England) and from Lancaster (Morecambe, Lancashire, England). Structure elucidation was checked by ^{1}H and ^{13}C NMR (JEOL 400MHz and 100MHz or Brucker 300MHz and 75.5MHz respectively) using either $CDCl_3$, or d_6-acetone as solvent. Infrared spectrometry was carried out on a Perkin-Elmer Fourier Transform-Paragon 1000 infrared spectrometer. Gas chromatography-mass spectrometry was carried out on a Hewlett 5890 Packard series II GC-MS 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 undertaken at the School of Pharmacy, London, and high-resolution mass spectrometry was undertaken at Kings College, London.

2.4 Synthesis of 1-(4-hydroxy-phenyl)-ketones

1-(4-Hydroxy-phenyl)-ethanone (158)

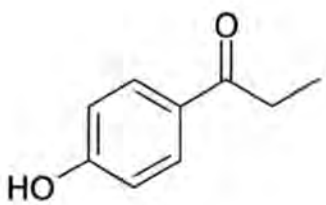


Aluminium chloride (1.50g, 21mmol) was added to a solution of phenol (0.50g, 10.6mmol) in anhydrous dichloromethane (DCM) (10mL). The slurry was left stirring for 30min before acetyl chloride (0.83mL, 11.7mmol) was added in a dropwise manner. The solution was then left to stir for 14h. The reaction was quenched using an ice-cold solution of aqueous hydrochloric acid (HCl) (1M, 30mL) and then extracted into diethyl ether (2 x 50mL). The combined organic layers were extracted into sodium hydroxide solution (NaOH) (2M, 2 x 50mL) and then acidified to pH2 using aqueous HCl (1M). The crude product was

extracted into diethyl ether (2 x 50mL) and the organic layer was washed with H_2O (2 x 50mL) and dried over anhydrous magnesium sulfate ($MgSO_4$). The solution was filtered and the solvent was removed *in vacuo* to give a brown solid. Flash chromatography of the crude solid gave **158** as a white solid (0.68g, 47.00% yield) [m.p. 109.8-110.1°C; R_f 0.26 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 110.2-110.4°C (Buehler et al, 1937)].

$\nu_{(max)}$(Film) cm^{-1}: 3321.93 (OH), 1662.55 (C=O), 1603.51 (Ar C=C); $\delta_H(CDCl_3)$: 7.89 (2H, d, J=8.79Hz, Ph-H), 6.91 (2H, d, J=8.97Hz, Ph-H), 2.56 (3H, s, CH_3); $\delta_C(CDCl_3)$: 198.59 (C=O), 160.74, 131.19, 129.55, 115.51 (ArC), 26.29 (CH_3); GC: t_R 6.06min; LRMS (EI): 136 (M^+, 30%), 121 (M^+-CH_3, 100%); Elemental analysis: Found C, 70.42%; H, 5.88%; $C_8H_8O_2$ requires C, 70.58%; H, 5.92%.

1-(4-Hydroxy-phenyl)-propan-1-one (159)

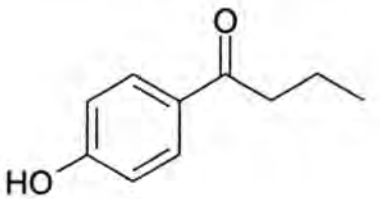


Compound **159** was synthesised in a similar manner to **158** except that propanoyl chloride (1.02mL, 11.7mmol) was used in place of acetyl chloride. Flash chromatography of the crude solid gave **159** as an off- white solid (0.84g, 52.83% yield) [m.p. 158.2-158.6°C; R_f 0.32 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 152-153°C (Aulin-Erdtman and Sanden, 1968)].

$\nu_{(max)}$(Film) cm^{-1}: 3222.83 (OH), 1650.16 (C=O), 1605.78 (Ar C=C); $\delta_H(CDCl_3)$: 7.85 (2H, d, J=8.42Hz, Ph-H), 6.81 (2H, d, J=8.42Hz, Ph-H), 2.89 (2H, q, J_{AB}=7.32Hz, J_{AB}=7.14Hz, O=C-CH_2), 1.15 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.32Hz, CH_2CH_3); $\delta_C(CDCl_3)$: 198.96 (C=O), 152.58, 130.56, 115.26 (ArC), 31.39

($\underline{C}H_2CH_3$), 8.38 ($\underline{C}H_3$); GC: t_R 6.81min; LRMS (EI): 150 (M^+, 9%), 121 (M^+-CH_3CH_2, 100%); Elemental analysis: Found C, 71.84%; H, 6.71%; $C_9H_{10}O_2$ requires C, 71.98%; H, 6.71%.

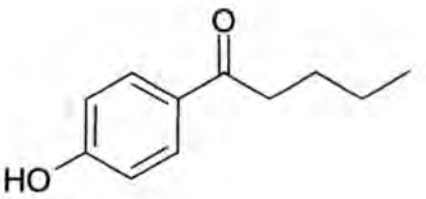
1-(4-Hydroxy-phenyl)-butan-1-one (160)



Compound **160** was synthesised in a similar manner to **158** except that butyryl chloride (1.22mL, 11.7mmol) was used in place of acetyl chloride. Flash chromatography of the crude solid gave **160** as a white solid (0.74g, 42.53% yield) [m.p. 99.6-100.0°C; R_f 0.29 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 93-94°C (Krausz and Martin, 1965)].

$\nu_{(max)}$(Film) cm^{-1}: 3370.69 (OH), 2963.17 (CH), 1660.27 (C=O), 1601.95 (Ar C=C); δ_H($CDCl_3$): 7.89 (2H, d, J=8.79Hz, Ph-$\underline{H}$), 6.90 (2H, d, J=8.70Hz, Ph-$\underline{H}$), 2.89 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-$C\underline{H}_2$), 1.74 (2H, sex, J_{AB}=7.32Hz, J_{AB}=7.51Hz, $C\underline{H}_2CH_3$), 0.98 (3H, t, J_{AB}=7.51Hz, J_{AB}=7.32Hz, $C\underline{H}_3$); δ_C($CDCl_3$): 200.89 ($\underline{C}$=O), 159.98, 130.81, 129.30, 115.43 (Ar$\underline{C}$), 40.25 (O=C-$\underline{C}H_2$), 18.17 ($\underline{C}H_2CH_3$), 13.90 ($\underline{C}H_3$); GC: t_R 7.40min; LRMS (EI): 164 (M^+, 11%), 121 (M^+-C_3H_7, 100%); Elemental analysis: Found C, 73.07%; H, 7.29%; $C_{10}H_{12}O_2$ requires C, 73.15%; H, 7.37%.

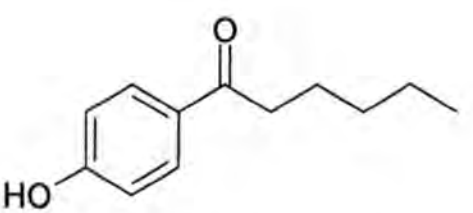
1-(4-Hydroxy-phenyl)-pentan-1-one (161)



Compound **161** was synthesised in a similar manner to **158** except that pentanoyl chloride (1.40mL, 11.7mmol) was used in place of acetyl chloride. Flash chromatography of the crude solid gave **161** as a white solid (0.78g, 41.27% yield) [m.p. 62.5-63.3°C; R_f 0.27 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 62-63°C (Coulthard et al, 1930)].

$\nu_{(max)}$(Film) cm^{-1}: 3341.95 (OH), 2958.91 (CH), 1656.82 (C=O), 1602.03 (Ar C=C), 1581.05; $\delta_H(CDCl_3)$: 7.89 (2H, d, J=8.60Hz, Ph-H), 6.95 (1H, s, OH), 6.90 (2H, d, J=8.60Hz, Ph-H), 2.91 (2H, t, J=7.51Hz, O=C-CH_2), 1.69 (2H, quin, J_{AB}=7.51Hz, J_{AB}=7.69Hz, $CH_2CH_2CH_3$), 1.38 (2H, sex, J_{AB}=7.51Hz, J_{AB}=7.69Hz, J_{AB}=7.32Hz, CH_2CH_3), 0.92 (3H, t, J_{AB}=7.51Hz, J_{AB}=7.32Hz, CH_3); $\delta_C(CDCl_3)$: 200.56 (C=O), 160.65, 130.82, 129.75, 115.43 (ArC), 38.06 (O=C-CH_2), 26.89 ($CH_2CH_2CH_3$), 22.51 (CH_2CH_3), 13.89 (CH_3); GC: t_R 8.09min; LRMS (EI): 178 (M^+, 7%), 121 (M^+-C_4H_9, 100%); Elemental analysis: Found C, 73.98%; H, 7.90%; $C_{11}H_{14}O_2$ requires C, 74.13%; H, 7.92%.

1-(4-Hydroxy-phenyl)-hexan-1-one (162)

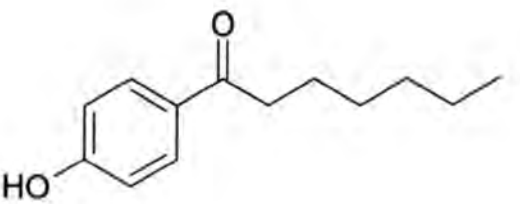


Compound **162** was synthesised in a similar manner to **158** except that hexanoyl chloride (1.66mL, 11.7mmol) was used in place of acetyl chloride.

Flash chromatography of the crude solid gave **162** as a white solid (0.92g, 45.10% yield) [m.p. 64.2-64.6°C; R_f 0.34 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 63-64°C (Coulthard et al, 1930)].

$\nu_{(max)}$(Film) cm^{-1}: 3316.21 (OH), 2956.73 (CH), 1655.38 (C=O), 1602.19 (Ar C=C); $\delta_H(CDCl_3)$: 7.89 (2H, d, J=8.79Hz, Ph -H), 7.20 (1H, s, OH), 6.90 (2H, d, J=8.79Hz, Ph-H), 2.90 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.69Hz, O=C-CH_2), 1.67-1.74 (2H, m, O=C-CH_2CH_2), 1.28-1.38 (4H, m, $(CH_2)_2CH_3$), 0.87 (3H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, CH_3); $\delta_C(CDCl_3)$: 200.63 (C=O), 160.49, 130.83, 129.04, 115.44 (ArC), 38.31 (O=C-CH_2), 31.55 (O=C-CH_2CH_2), 24.50 ($CH_2CH_2CH_3$), 22.46 (CH_2CH_3), 13.91 (CH_3); GC: t_R 8.72min; LRMS (EI): 192 (M^+, 11%), 121 (M^+-C_5H_{11}, 100%); Elemental analysis: Found C, 74.99%; H, 8.40%; $C_{12}H_{16}O_2$ requires C, 74.97%; H, 8.39%.

1-(4-Hydroxy-phenyl)-heptan-1-one (163)

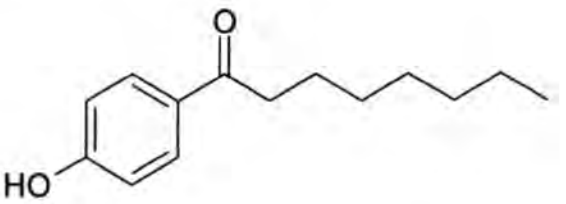


Compound **163** was synthesised in a similar manner to **158** except that heptanoyl chloride (1.81mL, 11.7mmol) was used in place of acetyl chloride. Flash chromatography of the crude solid gave **163** as a white solid (0.98g, 44.75% yield) [m.p. 95.2-95.7°C; R_f 0.30 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 92-94°C (Patent, Chem. Abstract, 1966)].

$\nu_{(max)}$(Film) cm^{-1}: 3315.50 (OH), 2923.40 (CH), 1661.23 (C=O), 1598.27 (Ar C=C); $\delta_H(CDCl_3)$: 7.89 (2H, d, J=8.79Hz, Ph-H), 6.95 (1H, s, OH), 6.89 (2H, d, J=6.77Hz, Ph-H), 2.90 (2H, t, J_{AB}=7.51Hz, J_{AB}=7.32Hz, O=C-CH_2), 1.70 [4H, quin, J_{AB}=7.51Hz, J_{AB}=7.32Hz, $(CH_2)_2$], 1.23-1.38 [4H, m, $(CH_2)_2CH_3$], 0.86 (3H, t, J_{AB}=6.96Hz, J_{AB}=7.14Hz, CH_3); $\delta_C(CDCl_3)$: 200.38 (C=O), 160.63,

130.81, 129.00, 115.43 (Ar<u>C</u>), 38.36 (O=C-<u>C</u>H_2), 31.62 (O=C-CH_2<u>C</u>H_2), 29.07 (<u>C</u>$H_2CH_2CH_2CH_3$), 24.76 (<u>C</u>$H_2CH_2CH_3$), 22.50 (<u>C</u>H_2CH_3), 14.02 (<u>C</u>H_3); GC: t_R 9.33min; LRMS (EI) 206 (M^+, 13%), 121 (M^+-C_6H_{13}, 100%); Elemental analysis: Found C, 75.84%; H, 8.78%; $C_{13}H_{18}O_2$ requires C, 75.69%; H, 8.80%.

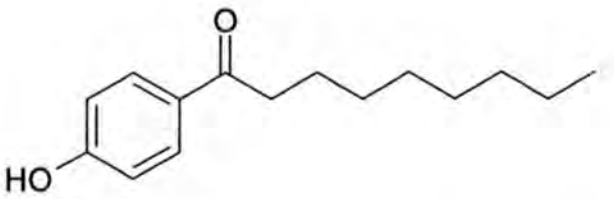
1-(4-Hydroxy-phenyl)-octan-1-one (164)



Compound **164** was synthesised in a similar manner to **158** except that octanoyl chloride (2.00mL, 11.7mmol) was used in place of acetyl chloride. Flash chromatography of the crude solid gave **164** as a white solid (1.63g, 34.83% yield) [m.p. 72.9-73.2°C; R_f 0.29 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 62.5-63.5°C (Ralston and Bauer, 1940)].

$\nu_{(max)}$(Film) cm^{-1}: 3303.50 (OH), 2921.90 (CH), 1654.63 (C=O), 1601.77 (Ar C=C); δ_H($CDCl_3$): 7.79 (2H, d, J=8.97Hz, Ph-<u>H</u>), 7.11 (1H, s, O<u>H</u>), 6.81 (2H, d, J=8.97Hz, Ph-<u>H</u>), 2.81 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.69Hz, O=C-C<u>H</u>$_2$), 1.60 (2H, quin, J_{AB}=7.69Hz, J_{AB}=7.14Hz, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-CH_2C<u>H</u>$_2$), 1.12-1.18 [8H, m, (C<u>H</u>$_2$)$_4$$CH_3$], 0.75 (3H, t, J_{AB}=6.77Hz, J_{AB}=6.96Hz, C<u>H</u>$_3$); δ_C($CDCl_3$): 200.73 (<u>C</u>=O), 160.76, 130.84, 129.64, 115.45 (Ar<u>C</u>), 38.37 (O=C-<u>C</u>H_2), 31.68 (O=C-CH_2<u>C</u>H_2), 29.37 (<u>C</u>H_2), 29.10 [<u>C</u>$H_2$$(CH_2)_2CH_3$], 24.83 (<u>C</u>$H_2CH_2CH_3$), 22.60 (<u>C</u>$H_2CH_3$), 14.06 (<u>C</u>$H_3$): GC: t_R 10.76min; LRMS (EI): 220 (M^+, 3%), 121 (M^+-C_7H_{15}, 100%).

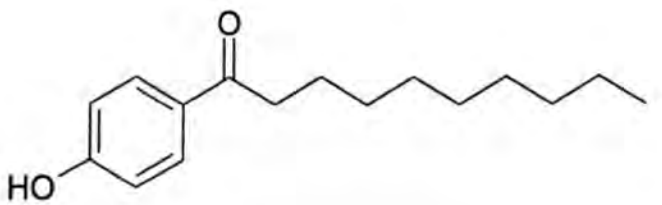
1-(4-Hydroxy-phenyl)-nonan-1-one (165)



Compound **165** was synthesised in a similar manner to **158** except that nonanoyl chloride (2.11mL, 11.7mmol) was used in place of acetyl chloride. Flash chromatography of the crude solid gave **165** as a white solid (0.81g, 32.53% yield) [m.p. 57.2-57.6°C; R_f 0.29 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 55.5-56.5°C (Kolobielski, 1968)].

$\nu_{(max)}$(Film) cm^{-1}: 3370.73 (OH), 2934.33 (CH), 1661.87 (C=O), 1602.50 (Ar C=C); $\delta_H(CDCl_3)$: 7.90 (2H, d, J=8.79Hz, Ph-H), 6.90 (2H, d, J=8.79Hz, Ph-H), 6.76 (1H, s, OH), 2.90 (2H, t, J=7.51Hz, O=C-CH_2), 1.70 (2H, quin, J_{AB}=7.69Hz, J_{AB}=7.23Hz, J_{AB}=7.51Hz, O=C-CH_2CH_2), 1.24-1.36 [10H, m, $(CH_2)_5CH_3$], 0.85 (3H, t, J_{AB}=6.59Hz, J_{AB}=7.14Hz, CH_3); $\delta_C(CDCl_3)$: 200.36 (C=O), 160.73, 130.78, 129.82, 115.38 (ArC), 38.35 (O=C-CH_2), 31.81 (O=C-CH_2CH_2), 29.40 [O=C-$(CH_2)_2CH_2$], 29.14 [$CH_2(CH_2)_2CH_3$], 24.77 ($CH_2CH_2CH_3$), 22.63 (CH_2CH_3), 14.07 (CH_3); GC: t_R 11.44min; LRMS (EI): 234 (M^+, 5%), 121 (M^+-C_8H_{17}, 100%).

1-(4-Hydroxy-phenyl)-decan-1-one (166)

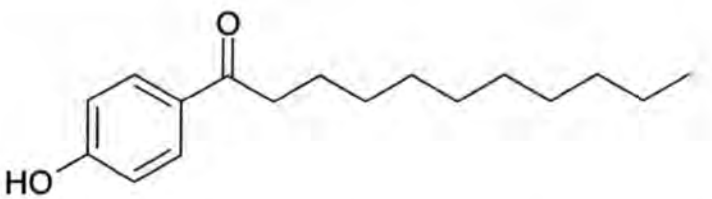


Compound **166** was synthesised in a similar manner to **158** except that decanoyl chloride (2.42mL, 11.7mmol) was used in place of acetyl chloride.

Flash chromatography of the crude solid gave **166** as a white solid (0.36g, 13.64% yield) [m.p. 67.2-67.8°C; R_f 0.31 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 64-65°C (Woodcock, 1955)].

$\nu_{(max)}$(Film) cm^{-1}: 3301.81 (OH), 2921.34 (CH), 1654.70 (C=O), 1601.59 (Ar C=C); $\delta_H(CDCl_3)$: 7.84 (2H, d, J=8.79Hz, Ph-H), 6.85 (2H, d, J=8.79Hz, Ph-H), 6.72 (1H, s, OH), 2.85 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.69Hz, O=C-CH_2), 1.66 (2H, quin, J_{AB}=7.51Hz, J_{AB}=7.32Hz, J_{AB}=7.14Hz, J_{AB}=7.69Hz, O=C-CH_2CH_2), 1.18-1.29 [12H, m, $(CH_2)_6CH_3$], 0.80 (3H, t, J_{AB}=6.77Hz, J_{AB}=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 200.39 (C=O), 160.48, 130.80, 129.85, 115.40 (ArC), 38.36 (O=C-CH_2), 31.85 (O=C-CH_2CH_2), 29.44 (O=C-$CH_2CH_2CH_2$), 29.41 (CH_2), 29.26 [O=C-$(CH_2)_3CH_2$], 24.77 ($CH_2CH_2CH_3$), 22.65 (CH_2CH_3), 14.09 (CH_3); GC: t_R 12.12min; LRMS (EI): 248 (M^+, 5%), 121 (M^+-C_9H_{19}, 100%); Elemental analysis: Found C, 77.41%; H, 9.61%; $C_{16}H_{24}O_2$ requires C, 77.38%; H, 9.74%.

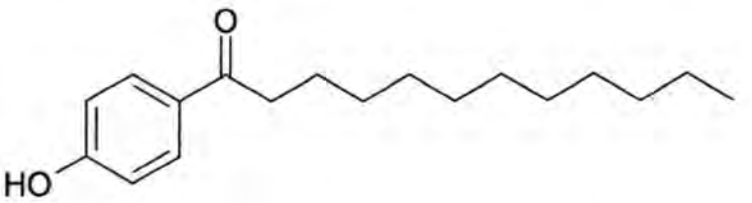
1-(4-Hydroxy-phenyl)-undecan-1-one (167)



Thionyl Chloride (1.17mL) was added dropwise to a solution of undecanoic acid (2.00g) in anhydrous toluene (50mL). The reaction mixture was refluxed for 3h. After that time, the reaction mixture was cooled down to room temperature and toluene washes were carried out until there was no further trace of thionyl chloride. Undecanoyl chloride (2.4g, 11.7mmol) was obtained as a brown oil, which was then used immediately in place of acetyl chloride. Flash chromatography of the crude solid gave **167** as a light brown solid (1.19g, 43.17% yield) [m.p. 53.7-54.2°C; R_f 0.23 diethyl ether/petroleum ether 40-60°C (30:70)].

$\nu_{(max)}$(Film) cm^{-1}: 3257.45 (OH), 2948.80 (CH), 1656.26 (C=O), 1603.38 (Ar C=C); δ_H(d_6-Acetone): 9.32 (1H, s, O<u>H</u>), 7.89 (2H, d, J=8.79Hz, Ph-<u>H</u>), 6.90 (2H, d, J=8.79Hz, Ph-<u>H</u>), 2.98 (2H, s, O=C-C<u>H</u>$_2$), 2.03-2.05 (2H, m, O=C-CH$_2$C<u>H</u>$_2$), 1.64-1.67 [2H, m, O=C-(CH$_2$)$_2$C<u>H</u>$_2$], 1.27-1.39 [12H, m, (C<u>H</u>$_2$)$_6$CH$_3$], 0.86 (3H, t, J_{AB}=6.77Hz, J_{AB}=6.96Hz, C<u>H</u>$_3$); δ_C(d_6-Acetone): 205.55 (<u>C</u>=O), 161.78, 130.44, 129.10, 115.17 (Ar<u>C</u>), 37.68 (O=C-<u>C</u>H$_2$), 29.44 (O=C-CH$_2$<u>C</u>H$_2$), 24.50 (<u>C</u>H$_2$), 22.52 (<u>C</u>H$_2$CH$_2$CH$_3$), 13.56 (<u>C</u>H$_3$); GC: t_R 11.88min; LRMS (EI): 262 (M^+, 5%), 136 (M^+-C_9H_{18}, 100%).

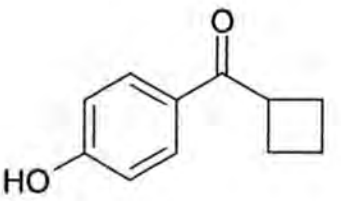
1-(4 -Hydroxy-phenyl)-dodecan-1-one (168)



Compound **168** was synthesised in a similar manner to **158** except that dodecanoyl chloride (2.78mL, 11.7mmol) was used in place of acetyl chloride. Flash chromatography of the crude solid gave **168** as an off-white solid (0.72g, 24.57% yield) [m.p. 69.6-70.4°C; R_f 0.26 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 70-71°C (Ralston et al, 1942)].

$\nu_{(max)}$(Film) cm^{-1}: 3285.15 (OH), 2919.90 (<u>C</u>H), 1655.36 (<u>C</u>=O), 1603.71 (Ar C=C); δ_H($CDCl_3$): 7.80 (2H, d, J=8.97Hz, Ph-<u>H</u>), 6.83 (2H, d, J=8.79Hz, Ph-<u>H</u>), 2.81 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-C<u>H</u>$_2$), 1.58 (2H, quin, J_{AB}=7.32Hz, J_{AB}=7.14Hz, J_{AB}=7.51Hz, O=C-CH$_2$C<u>H</u>$_2$), 1.18-127 (14H, m, (C<u>H</u>$_2$)$_7$CH$_3$), 0.78 (3H, t, J_{AB}=6.77Hz, J_{AB}=6.96Hz, C<u>H</u>$_3$); δ_C($CDCl_3$): 200.10 (<u>C</u>=O), 160.51, 130.76, 129.56, 115.37 (Ar<u>C</u>), 38.35 (O=C-<u>C</u>H$_2$), 31.88 (O=C-CH$_2$<u>C</u>H$_2$), 29.60 [O=C-(CH$_2$)$_2$<u>C</u>H$_2$], 29.44 [O=C-(CH$_2$)$_3$<u>C</u>H$_2$], 24.77 [O=C-(CH$_2$)$_4$<u>C</u>H$_2$], 22.66 (<u>C</u>H$_2$CH$_3$), 14.09 (<u>C</u>H$_3$); GC: t_R 13.70min; LRMS (EI): 276 (M^+, 6%), 136 (M^+-$C_{10}H_{20}$, 100%).

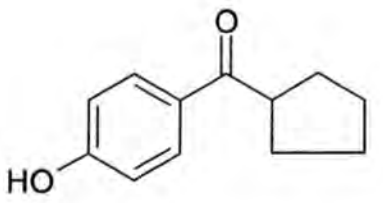
Cyclobutyl-(4-hydroxyphenyl)-methanone (169)



Compound **169** was synthesised in a similar manner to **158** except that cyclobutane carbonyl chloride (1.33mL, 11.7mmol) was used in place of acetyl chloride. Flash chromatography of the crude solid gave **169** as a white solid (0.71g, 37.97% yield) [m.p. 112.9-113.4°C; R_f 0.21 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 102.4-105.4°C (Patel, M., 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3259.00 (OH), 1649.29 (C=O), 1603.43 (Ar C=C); $\delta_H(CDCl_3)$: 7.82 (2H, d, J=8.06Hz, Ph-H), 6.88 (2H, d, J=8.79Hz, Ph-H), 6.56 (1H, s, OH), 3.96 (1H, quin, J_{AB}=8.42Hz, J_{AB}=8.60Hz, O=C-CH, cyclo), 2.39 (2H, quin, J_{AB}=9.34Hz, J_{AB}=10.25Hz, J_{AB}=9.15Hz, J_{AB}=8.97Hz, O=C-CH-CH_2, cyclo), 2.23-2.30 (2H, m, O=C-CH-CH_2CH_2, cyclo), 2.05 [2H, quin, J_{AB}=9.15Hz, J_{AB}=8.42Hz, $(CH_2)_2CH_2$, cyclo]; $\delta_C(CDCl_3)$: 200.40 (C=O), 160.25, 130.95, 128.30, 115.41 (ArC), 42.01 [O=C-CH$(CH_2)_3$, cyclo], 25.26 (CH_2, cyclo), 18.17 (CH_2, cyclo); GC: t_R 8.54min; LRMS (EI): 176 (M^+, 11%), 121 (M^+-C_4H_7, 100%); Elemental analysis: Found C, 74.93%; H, 7.01%; $C_{11}H_{12}O_2$ requires C, 74.98%; H, 6.86%.

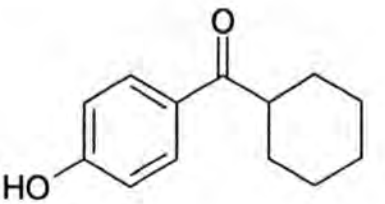
Cyclopentyl-(4-hydroxyphenyl)-methanone (170)



Compound **170** was synthesised in a similar manner to **158** except that cyclopentane carbonyl chloride (1.42mL, 11.7mmol) was used in place of acetyl chloride. Flash chromatography of the crude solid gave **170** as a white solid (0.91g, 45.27% yield) [m.p. 122.7-123.2°C; R_f 0.28 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 111.0-113.2°C (Patel, M., 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3201.76 (OH), 2950.44 (CH), 1640.67 (C=O), 1603.66 (Ar C=C); $\delta_H(CDCl_3)$: 7.90 (2H, d, J=9.70Hz, Ph-H), 6.89 (2H, d, J=8.24Hz, Ph-H), 6.47 (1H, s, OH), 3.66 (1H, quin, J_{AB}=7.87Hz, J_{AB}=8.06Hz, O=C-CHCH$_2$, cyclo), 1.86-1.92 [4H, m, (CH$_2$)$_2$CH$_2$, cyclo], 1.70-1.75 [4H, m, (CH$_2$)$_2$, cyclo]; $\delta_C(CDCl_3)$: 202.32 (C=O), 160.21, 131.08, 129.83, 115.34 (ArC), 46.11 (O=C-CH), 30.22 [(CH$_2$)$_2$, cyclo], 26.31 [(CH$_2$)$_2$, cyclo]; GC: t_R 9.14min; LRMS (EI): 190 (M^+, 14%), 121 (M^+-C_5H_9, 100%).

Cyclohexyl-(4-hydroxyphenyl)-methanone (171)

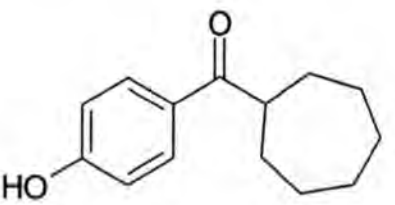


Compound **171** was synthesised in a similar manner to **158** except that cyclohexane carbonyl chloride (1.57mL, 11.7mmol) was used in place of acetyl chloride. Flash chromatography of the crude solid gave **171** as a white

solid (0.97g, 44.81% yield) [m.p. 104.7-105.2°C; R_f 0.25 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 110.0-111.2°C (Patel, M., 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3285.51 (OH), 2931.61 (CH), 1651.24 (C=O), 1601.63 (Ar C=C); δ_H($CDCl_3$): 7.89 (2H, d, J=8.79Hz, Ph-H), 6.91 (2H, d, J=8.97Hz, Ph-H), 3.18-3.24 (1H, m, O=C-CH, cyclo), 1.37 [10H, m, CH-(CH_2)$_5$, cyclo]; δ_C($CDCl_3$): 203.59 (C=O), 160.44, 131.19, 115.45 (ArC), 45.17 (O=C-CH), 29.59 [(CH_2)$_2$, cyclo], 25.90 [(CH_2)$_3$, cyclo]; GC: t_R 9.74min; LRMS (EI): 204 (M^+, 9%), 121 (M^+-C_6H_{11}, 100%).

Cycloheptyl-(4-hydroxyphenyl)-methanone (172)



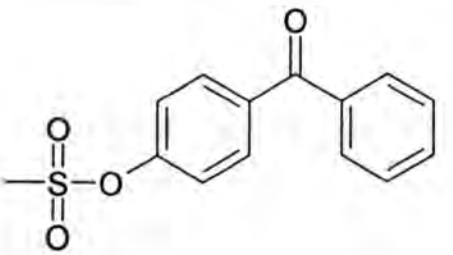
Thionyl Chloride (1.53mL) was added dropwise to a solution of cycloheptanoic acid (2.00g) in anhydrous toluene (50mL). The reaction mixture was refluxed for 3h. After that time, the reaction mixture was cooled down to room temperature and toluene washes were carried out until there was no further trace of thionyl chloride present. Cycloheptanoyl chloride (1.7g, 11.7mmol) was obtained as a dark brown oil, which was then used immediately in place of acetyl chloride. Flash chromatography of the crude solid gave **172** as a white solid (0.41g, 17.67% yield) [m.p. 119.4-119.7°C; R_f 0.41 diethyl ether/petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 3319.02 (OH), 2924.31 (CH), 1649.02 (C=O), 1601.03 (Ar C=C); δ_H($CDCl_3$): 7.83 (2H, d, J=8.79Hz, Ph-H), 6.86 (2H, d, J=8.97Hz, Ph-H), 3.31-3.37 (1H, m, O=C-CH), 1.81-1.88 (2H, m, CHCH_2, cyclo), 1.69-1.77 (2H, m, CH_2, cyclo), 1.51-1.58 [8H, m, (CH_2)$_4$, cyclo]; δ_C($CDCl_3$): 204.32 (C=O),

160.34, 131.14, 129.15, 115.61 (Ar<u>C</u>), 46.47 (O=C-<u>C</u>H, cyclo), 31.21 (O=C-CH<u>C</u>H_2, cyclo), 28.32 (<u>C</u>H_2, cyclo), 26.97 [(<u>C</u>H_2)$_4$, cyclo]; GC: t_R 19.79min; LRMS (EI): 218 (M^+, 3%), 121 (M^+-C_7H_{13}, 100%). Elemental analysis: Found C, 77.08%; H, 8.31%; $C_{14}H_{18}O_2$ requires C, 77.03%; H, 8.31%.

2.5 Synthesis of methanesulfonic acid 4-alkyl-phenyl esters

Methanesulfonic acid 4-benzoyl-phenyl ester (173)

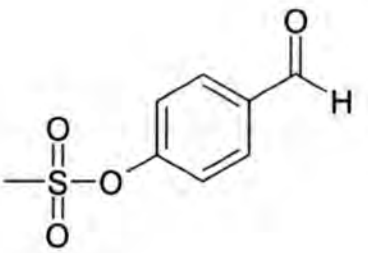


Methane sulfonyl chloride (0.23mL, 0.128mol) was added dropwise to a stirred solution of 4-hydroxybenzophenone (0.53g, 0.106mol), in triethylamine (TEA) (0.39mL, 0.117mol), and DCM (50mL) at room temperature. The resulting mixture was then refluxed for 3h. After cooling to room temperature, it was quenched in ice, washed with H_2O (3x20mL), and cold saturated sodium bicarbonate (Na_2CO_3) (3x20mL). The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave **173** as an off-white solid (0.63g, 85.14% yield) [m.p. 108.3-108.8°C; R_f 0.43 diethyl ether/petroleum ether 40-60°C (70:30); Lit. m.p. 102.5-104.5°C (Patent, Boots Comp, 1979)].

$\nu_{(max)}$(Film) cm^{-1}: 1656.72 (C=O), 1596.44 (Ar C=C), 1370.32, 1150.39 (S=O); δ_H($CDCl_3$): 7.81 (2H, d, J=8.79Hz, Ph-<u>H</u>), 7.72 (2H, d, J=8.42Hz, Ph-<u>H</u>), 7.55 (2H, t, J_{AB}=8.60Hz, J_{AB}=7.51Hz, Ph-<u>H</u>), 7.43 (3H, t, J_{AB}=7.87Hz, J_{AB}=7.32Hz, Ph-<u>H</u>), 3.15 (3H, s, O=S=O-C<u>H</u>$_3$); δ_C($CDCl_3$): 195.12 (<u>C</u>=O), 151.98, 137.12, 136.64, 132.91, 132.14, 130.08, 128.55, 121.98 (Ar<u>C</u>), 38.00 (O=S=O-<u>C</u>H_3);

GC: t_R 12.14min; LRMS (EI): 276 (M^+, 84%), 199 (M^+-C_6H_5, 100%); HRMS (EI): Found 299.0349, $C_{14}H_{12}O_4Na_1S_1$ requires 299.3133.

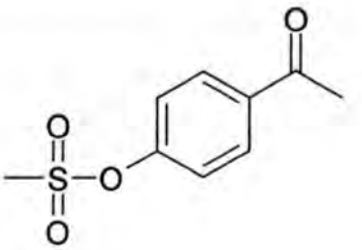
Methanesulfonic acid 4-formyl-phenyl ester (174)



Compound **174** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.38mL, 0.128mol), 4-hydroxybenzaldehyde (0.54g, 0.106mol) and TEA (0.63mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The solution was then filtered and removal of the solvent *in vacuo* gave **174** as a brown solid (0.70g, 79.55% yield) [m.p. 68.2-68.7°C; R_f 0.27 diethyl ether/petroleum ether 40-60°C (70:30); Lit. m.p. 64-65°C (Looker and Hayes, 1957)].

$\nu_{(max)}$(Film) cm^{-1}: 1696.97 (C=O), 1598.16, 1590.08 (Ar C=C), 1363.11, 1146.58 (S=O); δ_H($CDCl_3$): 9.96 (1H, s, O=C-H), 7.90 (2H, d, J=8.79Hz, Ph-H), 7.40 (2H, d, J=8.60Hz, Ph-H), 3.15 (3H, s, O=S=O-CH_3); δ_C($CDCl_3$): 190.61 (C=O), 153.35, 135.15, 131.72, 122.73 (ArC), 38.17 (O=S=O-CH_3); GC: t_R 7.87min; LRMS (EI): 200 (M^+, 77%), 121 (M^+-CH_3SO_2, 100%).

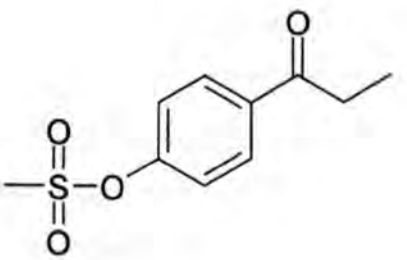
Methanesulfonic acid 4-acetyl-phenyl ester (175)



Compound **175** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.68mL, 0.128mol), compound **158** (1.07g, 0.106mol), and TEA (1.13mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was filtered and removal of the solvent *in vacuo* gave **175** as an off-white solid (1.01g, 70.14% yield) [m.p. 76.1-76.7°C; R_f 0.11 diethyl ether/petroleum ether 40-60°C (50:50); lit. m.p. 72-73°C (James, 2000)].

$\nu_{(max)}$(Film) cm^{-1}: 1682.08 (C=O), 1596.39 (Ar C=C), 1373.01, 1156.31 (S=O); $\delta_H(CDCl_3)$: 7.96 (2H, d, J=8.97Hz, Ph-H), 7.31 (2H, d, J=8.79Hz, Ph-H), 3.13 (3H, s, O=S=O-CH_3), 2.55 (3H, s, O=C-CH_3); $\delta_C(CDCl_3)$: 196.91 (C=O), 152.42, 135.80, 130.80, 122.17 (ArC), 38.01 (O=S=O-CH_3), 26.74 (O=C-CH_3); GC: t_R 8.61min; LRMS (EI): 183 (M^+, 2%), 121 (M^+-$SOCH_2$, 100%).

Methanesulfonic acid 4-propionyl-phenyl ester (176)

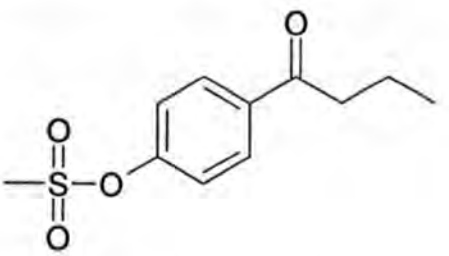


Compound **176** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.62mL, 0.128mol), compound **159** (1.02g, 0.106mol), and

TEA (1.02mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was filtered and removal of the solvent *in vacuo* gave a crude solid. Flash chromatography of the crude solid gave **176** as a white solid (0.45g, 29.03% yield) [m.p. 77.2-77.6°C; R_f 0.17 diethyl ether/petroleum ether 40-60°C (50:50); lit. m.p. 78-79°C (James, 2000)].

$\nu_{(max)}$(Film) cm^{-1}: 1681.55 (C=O), 1597.36 (Ar C=C), 1369.28, 1151.89 (S=O); $\delta_H(CDCl_3)$: 7.97 (2H, d, J=8.97Hz, Ph-H), 7.31 (2H, d, J=8.97Hz, Ph-H), 3.13 (3H, s, O=S=O-CH_3), 2.93 (2H, q, J_{AB}=7.32Hz, J_{AB}=7.14Hz, O=C-CH_2), 1.16 (3H, t, J=7.14Hz, CH_3); $\delta_C(CDCl_3)$: 199.21 (C=O), 152.16, 135.54, 130.17, 122.16 (ArC), 37.96 (O=S=O-CH_3), 32.01 (O=C-CH_2), 8.21 (CH_3); GC: t_R 9.20min; LRMS (EI): 228 (M^+, 9%), 199 (M^+-C_2H_5, 100%). HRMS (EI): Found 251.0349. $C_{10}H_{12}O_4Na_1S_1$ requires 251.2687.

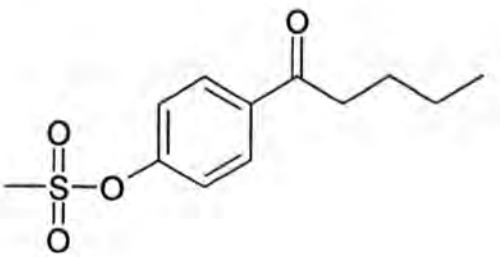
Methanesulfonic acid 4-butyryl-phenyl ester (177)



Compound **177** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.28mL, 0.128mol), compound **160** (0.50g, 0.106mol), and TEA (0.47mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave **177** as an off-white solid (0.70g, 94.59% yield) [m.p. 68.9-69.4°C; R_f 0.31 diethyl ether/petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 1683.20 (C=O), 1597.26 (Ar C=C), 1374.41, 1155.58 (S=O); δ_H($CDCl_3$): 7.96 (2H, d, J=8.97Hz, Ph-H), 7.31 (2H, d, J=8.97Hz, Ph-H), 3.14 (3H, s, O=S=O-CH_3), 2.87 (2H, t, J_{AB}=7.14Hz, J_{AB}=7.32Hz, O=C-CH_2), 1.71 (2H, sex, J_{AB}=7.51Hz, J_{AB}=7.32Hz, CH_2CH_3), 0.94 (3H, t, J_{AB}=7.51Hz, J_{AB}=7.32Hz, CH_3); δ_C($CDCl_3$): 198.95 (C=O), 152.16, 136.06, 130.23, 122.15 (ArC), 40.66 (O=S=O-CH_3), 37.96 (O=C-CH_2), 17.73 (CH_2CH_3), 13.92 (CH_3); GC: t_R 9.68min; LRMS (EI): 242 (M^+, 2%), 199 (M^+-C_3H_7, 100%). HRMS (EI): Found 265.0505, $C_{11}H_{14}O_4Na_1S_1$ requires 265.2958.

Methanesulfonic acid 4-pentanoyl-phenyl ester (178)

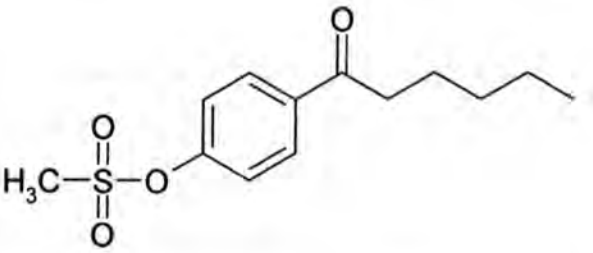


Compound **178** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.26mL, 0.128mol), compound **161** (0.50g, 0.106mol), and TEA (0.43mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave the crude solid. Flash chromatography of the crude solid gave **178** as a white solid (0.62g, 86.11% yield) [m.p. 64.7-65.0°C; R_f 0.32 diethyl ether/petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 1684.19 (C=O), 1335.68, 1155.34 (S=O); δ_H($CDCl_3$): 7.96 (2H, d, J=8.97Hz, Ph-H), 7.31 (2H, d, J=8.97Hz, Ph-H), 3.14 (3H, s, O=S=O-CH_3), 2.89 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-CH_2), 1.65 (2H, quin, J_{AB}=7.51Hz, J_{AB}=7.69Hz, J_{AB}=7.32Hz, O=C-CH_2CH_2), 1.34 (2H, sex, J_{AB}=7.51Hz, J_{AB}=7.32Hz, CH_2CH_3), 0.89 (3H, t, J_{AB}=7.51Hz, J_{AB}=7.32Hz, CH_3); δ_C($CDCl_3$): 198.95 (C=O), 152.16, 135.80, 130.24, 122.15 (ArC), 38.50

(O=S=O-$\underline{C}H_3$), 37.95 (O=C-$\underline{C}H_2$), 26.41 (O=C-$CH_2\underline{C}H_2$), 22.51 ($\underline{C}H_2CH_3$), 14.02 ($\underline{C}H_3$); GC: t_R 10.26min; LRMS (EI): 256 (M^+, 2%), 199 (M^+-C_4H_9, 100%). HRMS (EI): Found 257.0842, $C_{12}H_{16}O_4Na_1S_1$ requires 257.3229.

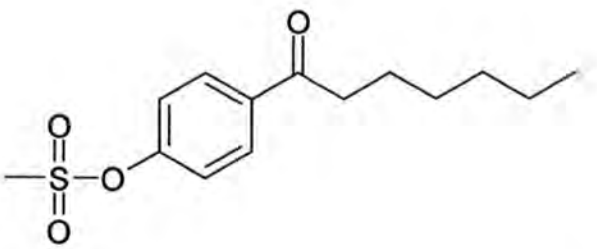
Methanesulfonic acid 4-hexanoyl-phenyl ester (179)



Compound **179** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.24mL, 0.128mol), compound **162** (0.50g, 0.106mol), and TEA (0.40mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave **179** as an off-white solid (0.60g, 85.71% yield) [m.p. 82.0-82.6°C; R_f 0.33 diethyl ether/petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 1683.06 (C=O), 1597.48 (Ar C=C), 1335.60, 1155.17 (S=O); δ_H($CDCl_3$): 8.01 (2H, d, J=8.97Hz, Ph-$\underline{H}$), 7.35 (2H, d, J=8.79Hz, Ph-$\underline{H}$), 3.14 (3H, s, O=S=O-C$\underline{H}_3$), 2.93 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-C$\underline{H}_2$), 1.71 (2H, quin, J_{AB}=7.51Hz, J_{AB}=7.32Hz, O=C-CH_2C$\underline{H}_2$), 1.31-1.37 [4H, m, (C$\underline{H}_2$)$_2$$CH_3$], 0.89 (3H, t, J_{AB}=4.94Hz, J_{AB}=7.14Hz, CH_2C$\underline{H}_3$); δ_C($CDCl_3$): 199.21 ($\underline{C}$=O), 152.42, 138.10, 136.06, 130.24, 122.15 (Ar$\underline{C}$), 38.75 (O=S=O-$\underline{C}H_3$), 37.96 (O=C-$\underline{C}H_2$), 31.55 (O=C-$CH_2\underline{C}H_2$), 24.01 ($\underline{C}H_2CH_2CH_3$), 22.60 ($\underline{C}H_2CH_3$), 14.04 ($\underline{C}H_3$); GC: t_R 20.01min; LRMS (EI): 270 (M^+, 7%), 214 (M^+-C_4H_8, 100%). HRMS (EI): Found 293.0818, $C_{13}H_{18}O_4Na_1S_1$ requires 293.3500.

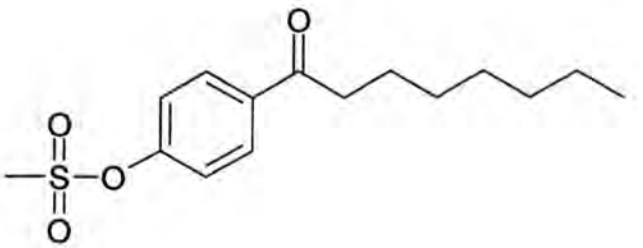
Methanesulfonic acid 4-heptanoyl-phenyl ester (180)



Compound **180** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.23mL, 0.128mol), compound **163** (0.40g, 0.106mol), and TEA (0.37mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave **180** as an off-white solid (0.46g, 83.64% yield) [m.p. 81.3-81.6°C; R_f 0.29 diethyl ether/ petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 1684.17 (C=O), 1597.11 (Ar C=C), 1335.78, 1154.77 (S=O); $\delta_H(CDCl_3)$: 7.96 (2H, d, J=8.97Hz, Ph-H), 7.31 (2H, d, J=8.79Hz, Ph-H), 3.14 (3H, s, O=S=O-CH_3), 2.88 (2H, t, J_{AB}=7.14Hz, J_{AB}=7.69Hz, O=C-CH_2), 1.66 (2H, quin, J_{AB}=7.14Hz, J_{AB}=7.51Hz, J_{AB}=7.69Hz, O=C-CH_2CH_2), 1.21-1.28 [6H, m, $(CH_2)_3CH_3$], 0.82 (3H, t, J=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 198.95 (C=O), 152.16, 135.80, 130.24, 122.15 (ArC), 38.79 (O=S=O-CH_3), 37.96 (O=C-CH_2), 31.72 (O=C-CH_2CH_2), 29.07 ($CH_2CH_2CH_2CH_3$), 24.28 ($CH_2CH_2CH_3$), 22.61 (CH_2CH_3), 14.14 (CH_3); GC: t_R 21.01min; LRMS (EI): 284 (M^+, 8%), 214 (M^+-C_6H_{12}, 100%). HRMS (EI): Found 307.0975, $C_{14}H_{20}O_4Na_1S_1$ requires 307.3771.

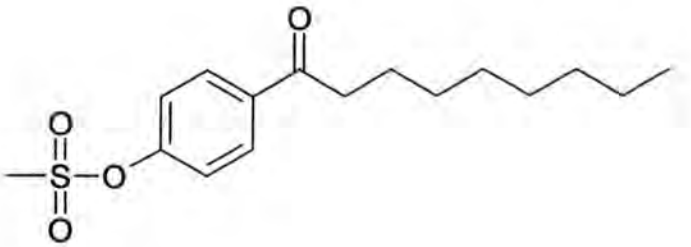
Methanesulfonic acid 4-octanoyl-phenyl ester (181)



Compound **181** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.21mL, 0.128mol), compound **164** (0.50g, 0.106mol), and TEA (0.35mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave **181** as an off-white solid (0.69g, 97.10% yield) [m.p. 95.1-95.6°C; R_f 0.29 diethyl ether/petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 1683.22 (C=O), 1597.31 (Ar C=C), 1335.82, 1153.63 (S=O); $\delta_H(CDCl_3)$: 7.96 (2H, d, J=8.97Hz, Ph-H), 7.31 (2H, d, J=8.97Hz, Ph-H), 3.14 (3H, s, O=S=O-CH_3), 2.88 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-CH_2), 1.66 (2H, quin, J_{AB}=7.51Hz, J_{AB}=7.14Hz, J_{AB}=7.32Hz, O=C-CH_2CH_2), 1.26-1.32 [2H, m, $CH_2(CH_2)_3CH_3$], 1.21-1.24 [6H, m, $(CH_2)_3CH_3$], 0.81 (3H, t, J_{AB}=6.77Hz, J_{AB}=7.14Hz, CH_3); $\delta_C(CDCl_3)$: 198.95 (C=O), 152.42, 136.06, 130.24, 122.15 (ArC), 38.79 (O=S=O-CH_3), 37.95 (O=C-CH_2), 31.78 (O=C-CH_2CH_2), 29.36 [O=C-$(CH_2)_2CH_2$], 29.20 24.33 ($CH_2CH_2CH_3$), 22.71 (CH_2CH_3), 14.18 (CH_3); GC: t_R 21.98min; LRMS (EI): 298 (M^+, 1%), 214 (M^+-C_6H_{12}, 100%); HRMS (EI): Found 321.1131, $C_{15}H_{22}O_4Na_1S_1$ requires 321.4042 .

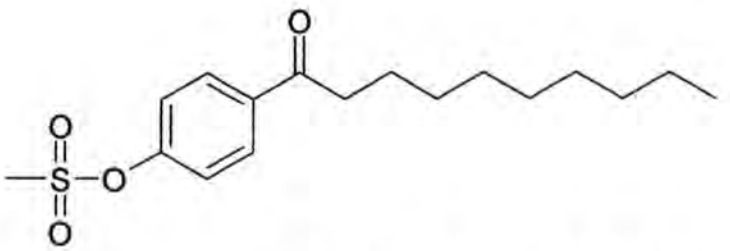
Methanesulfonic acid 4-nonanoyl-phenyl ester (182)



Compound **182** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.20mL, 0.128mol), compound **165** (0.50g, 0.106mol), and TEA (0.33mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave **182** as an off-white solid (0.65g, 97.01% yield) [m.p. 94.8-95.2°C; R_f 0.21 diethyl ether/petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 1684.15 (C=O), 1597.17 (Ar C=C), 1336.45, 1156.36 (S=O); $\delta_H(CDCl_3)$: 8.00 (2H, d, J=8.97Hz, Ph-H), 7.35 (2H, d, J=8.97Hz, Ph-H), 3.17 (3H, s, O=S=O-CH_3), 2.92 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-CH_2), 1.70 (2H, quin, J_{AB}=7.51Hz, J_{AB}=7.14Hz, J_{AB}=7.32Hz, O=C-CH_2CH_2), 1.19-1.28 [2H, m, $CH_2(CH_2)_4CH_3$], 1.30-1.36 [8H, m, $(CH_2)_4CH_3$], 0.85 (3H, t, J=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 198.95 (C=O), 152.16, 136.31, 130.24, 122.15 (ArC), 38.79 (O=S=O-CH_3), 37.95 (O=C-CH_2), 31.91 (O=C-CH_2CH_2), 29.50 (O=C-$(CH_2)_2CH_2$), 29.24 [$CH_2(CH_2)_2CH_3$], 24.33 ($CH_2CH_2CH_3$), 22.74 (CH_2CH_3), 14.19 (CH_3); GC: t_R 12.88min; LRMS (EI): 312 (M^+, 2%), 214 (M^+-C_7H_{14}, 100%); HRMS (EI): Found 335.1288, $C_{16}H_{24}O_4Na_1S_1$ requires 335.4313.

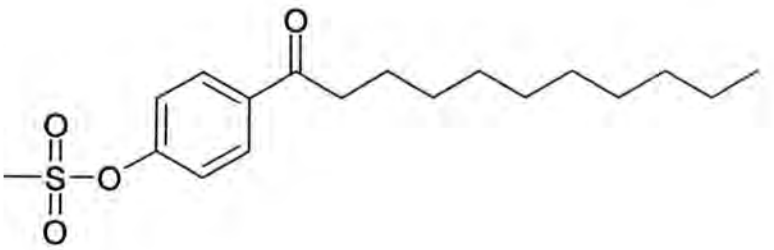
Methanesulfonic acid 4-decanoyl-phenyl ester (183)



Compound **183** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.18mL, 0.128mol), compound **166** (0.52g, 0.106mol), and TEA (0.31mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave a crude solid. Recrystallisation of the crude solid from absolute alcohol gave **183** as a shiny white solid (0.11g, 16.18% yield) [m.p. 100.6-101.0°C; R_f 0.29 diethyl ether/petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 1683.88 (C=O), 1467.22 (Ar C=C), 1336.22, 1155.83 (S=O); $\delta_H(CDCl_3)$: 8.00 (2H, d, J=8.79Hz, Ph-H), 7.35 (2H, d, J=8.79Hz, Ph-H), 3.17 (3H, s, O=S=O-CH_3), 2.92 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-CH_2), 1.70 (2H, quin, J_{AB}=7.32Hz, J_{AB}=6.96Hz, O=C-CH_2CH_2), 1.58 (2H, s, O=C-$(CH_2)_2CH_2$), 1.28-1.35 [2H, m, $CH_2(CH_2)_4CH_3$], 1.17-1.27 [8H, m, $(CH_2)_4$], 0.85 (3H, t, J_{AB}=6.59Hz, J_{AB}=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 199.09 (C=O), 152.16, 136.02, 130.24, 122.14 (ArC), 38.79 (O=S=O-CH_3), 37.95 (O=C-CH_2), 31.95 (O=C-CH_2CH_2), 29.54 (O=C-$(CH_2)_2CH_2$), 29.40 [$CH_2(CH_2)_3CH_3$], 29.36 [$CH_2(CH_2)_2CH_3$], 24.33 ($CH_2CH_2CH_3$), 22.75 (CH_2CH_3), 14.19 (CH_3); GC: t_R 13.70min; LRMS (EI): 326 (M^+, 2%), 214 (M^+-C_8H_{16}, 100%); HRMS (EI): Found 349.1444, $C_{17}H_{26}O_4Na_1S_1$ requires 349.4584.

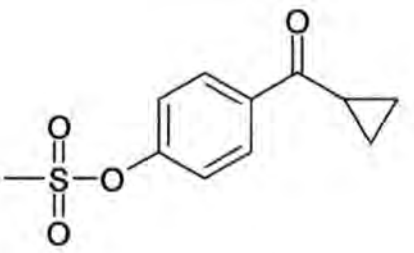
Methanesulfonic acid 4-undecanoyl-phenyl ester (184)



Compound **184** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.18mL, 0.128mol), compound **167** (0.50g, 0.106mol), and TEA (0.29mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave a crude solid. Recrystallisation of the crude solid from absolute alcohol gave **184** as a shiny white solid (0.10g, 15.38% yield) [m.p. 99.2-99.7°C; R_f 0.35 diethyl ether/petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 1683.61 (C=O), 1467.81 (Ar C=C), 1372.70, 1336.37 (Ar C=C), 1155.41 (S=O); $\delta_H(CDCl_3)$: 8.00 (2H, d, J=8.79Hz, Ph-H), 7.35 (2H, d, J=8.79Hz, Ph-H), 3.17 (3H, s, O=S=O-CH_3), 2.92 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-CH_2), 1.70 (2H, quin, J_{AB}=7.51Hz, J_{AB}=7.14Hz, J_{AB}=7.32Hz, O=C-CH_2CH_2), 1.57 [2H, s, O=C-$(CH_2)_2CH_2$], 1.24-1.35 [12H, m, $(CH_2)_6$], 0.85 (3H, t, J_{AB}=6.77Hz, J_{AB}=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 199.07 (C=O), 152.33, 136.03, 130.23, 122.12 (ArC), 38.79 (O=S=O-CH_3), 37.96 (O=C-CH_2), 31.97 (O=C-CH_2CH_2), 29.65 [O=C-$(CH_2)_2CH_2$], 29.57 [$CH_2(CH_2)_4CH_3$], 29.53 [$CH_2(CH_2)_3CH_3$], 29.40 [$CH_2(CH_2)_2CH_3$], 24.33 ($CH_2CH_2CH_3$), 22.75 (CH_2CH_3), 14.19 (CH_3); GC: t_R 14.72min; LRMS (EI): 340 (M^+, 2%), 214 (M^+-C_9H_{18}, 100%).

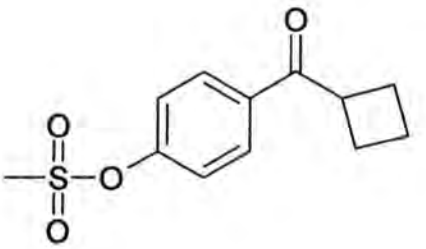
Methanesulfonic acid 4-cyclopropanecarbonyl-phenyl ester (185)



Compound **185** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.29mL, 0.128mol), cyclopropyl-(4-hydroxy-phenyl)-methanone (0.50g, 0.106mol), and TEA (0.47mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave a crude solid. Flash chromatography of the crude solid gave **185** as a white solid (0.14g, 18.92% yield) [m.p. 69.7-70.1°C; R_f 0.44 diethyl ether/petroleum ether 40-60°C (20:80)].

$\nu_{(max)}$(Film) cm^{-1}: 1667.19 (C=O), 1598.15 (Ar C=C), 1372.34, 1154.98 (S=O); $\delta_H(CDCl_3)$: 8.02 (2H, d, J=8.79Hz, Ph-H), 7.33 (2H, d, J=8.97Hz, Ph-H), 3.13 (3H, s, O=S=O-CH_3), 2.54-2.60 (1H, m, O=C-CH), 1.17-1.21 (2H, m, O=C-$CHCH_2$), 0.99-1.03 (2H, m, O=C-$CHCH_2CH_2$); $\delta_C(CDCl_3)$: 199.20 (C=O), 152.14, 136.96, 130.17, 122.08 (ArC), 37.93 (O=S=O-CH_3), 17.39 (O=C-CH), 12.05 (CH_2); GC: t_R 10.22min; LRMS (EI): 240 (M^+, 45%), 199 (M^+-C_3H_5, 100%).

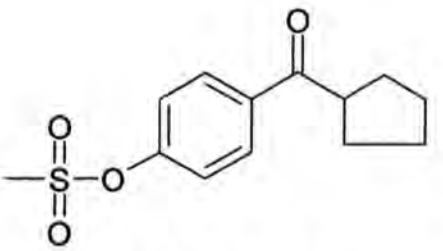
Methanesulfonic acid 4-cyclobutanecarbonyl-phenyl ester (186)



Compound **186** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.27mL, 0.128mol), compound **169** (0.50g, 0.106mol), and TEA (0.45mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave **186** as an off-white solid (0.81g, 91.36% yield) [m.p. 77.4-77.9°C; R_f 0.53 diethyl ether/ petroleum ether 40-60°C (80:20)].

$\nu_{(max)}$(Film) cm^{-1}: 1678.50 (C=O), 1596.80 (Ar C=C), 1368.95, 1151.40 (S=O); $\delta_H(CDCl_3)$: 7.89 (2H, d, J=8.79Hz, Ph-H), 7.29 (2H, d, J=8.79Hz, Ph-H), 3.90 (1H, quin, J_{AB}=7.69Hz, J_{AB}=8.97Hz, J_{AB}=8.06Hz, O=C-CH), 3.12 (3H, s, O=S=O-CH_3), 2.26-2.37 (2H, m, O=C-CHCH_2), 1.97-2.09 (2H, sex, J_{AB}=8.60, J_{AB}=8.97Hz, J_{AB}=8.79Hz, J_{AB}=10.98Hz, O=C-CHCH_2CH_2), 1.82-1.90 (2H, m, CH_2); $\delta_C(CDCl_3)$: 199.46 (C=O), 152.28, 134.58, 130.50, 122.16 (ArC), 42.28, 37.95 (O=S=O-CH_3), 37.43 (O=C-CH), 25.10 (O=C-CHCH_2), 18.22 (CH_2); GC: t_R 10.69min; LRMS (EI): 254 (M^+, 4%), 199 (M^+-C_4H_7, 100%).

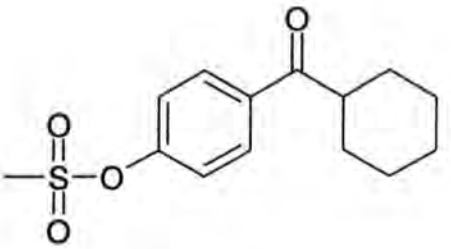
Methanesulfonic acid 4-cyclopentanecarbonyl-phenyl ester (187)



Compound **187** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.24mL, 0.128mol), compound **170** (0.50g, 0.106mol), and TEA (0.40mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave a crude solid. Recrystallisation of the crude solid from absolute alcohol gave **187** as an off-white solid (0.11g, 15.49% yield) [m.p. 77.3-77.8°C; R_f 0.37 diethyl ether/petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 2958.10, 2864.62 (CH_2), 1673.96 (C=O), 1595.38 (Ar C=C), 1368.89, 1150.53 (S=O); δ_H($CDCl_3$): 7.97 (2H, d, J=8.79Hz, Ph-H), 7.31 (2H, d, J=8.79Hz, Ph-H), 3.61 (1H, quin, J_{AB}=8.06Hz, J_{AB}=7.51Hz, J_{AB}=7.69Hz, O=C-CH), 3.12 (3H, s, O=S=O-CH_3), 1.82-1.88 (2H, m, O=C-CHCH_2), 1.57-1.69 (2H, m, O=C-CHCH_2CH_2), 1.53 (2H, s, CH_2); δ_C($CDCl_3$): 201.25 (C=O), 152.42 (C-O), 135.86 130.63, 122.08 (ArC), 46.53 (O=S=O-CH_3), 37.95 (CO-CH), 29.99, 26.38 (CH_2); GC: t_R 11.38min; LRMS (EI): 268 (M^+, 2%), 199 (M^+-C_5H_9, 100%).

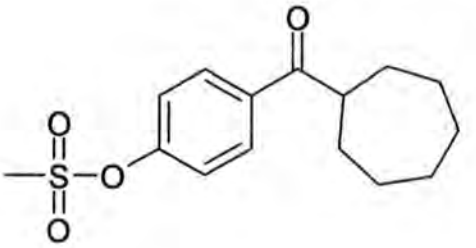
Methanesulfonic acid 4-cyclohexanecarbonyl-phenyl ester (188)



Compound **188** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.24mL, 0.128mol), compound **171** (0.50g, 0.106mol), and TEA (0.40mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave a crude solid. Recrystallisation of the crude solid from absolute alcohol gave **188** as a pale brown solid (0.10g, 13.70% yield) [m.p. 102.5-102.9°C; R_f 0.34 diethyl ether/ petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 2931.87, 2854.07 (CH_2), 1679.54 (C=O), 1595.38 (Ar C=C), 1371.37, 1150.56 (S=O); δ_H($CDCl_3$): 7.98 (2H, d, J=8.79Hz, Ph-H), 7.35 (2H, d, J=8.79Hz, Ph-H), 3.68-3.94 (1H, m , O=C-CH), 3.21 (3H, s, O=S=O-CH_3), 1.59 (2H, s, O=C-CHCH_2), 1.38-1.52 (2H, m, CH_2), 1.20-1.32 (2H, m, CH_2); δ_C($CDCl_3$): 202.02 (C=O), 152.16, 135.29, 130.44, 122.18 (ArC), 45.82, 37.94 (O=S=O-CH_3), 29.40 (O=C-CH), 25.96 (O=C-CHCH_2), 25.85 (CH_2); GC: t_R 21.80min; LRMS (EI): 282 (M^+, 5%), 199 (M^+-C_6H_{11}, 100%).

Methanesulfonic acid 4-cycloheptanecarbonyl-phenyl ester (189)

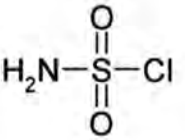


Compound **189** was synthesised in a similar manner to **173** except methane sulfonyl chloride (0.21mL, 0.128mol), compound **172** (0.50g, 0.106mol), and TEA (0.35mL, 0.117mol) were used. The organic phase was washed with H_2O (3x20mL) and dried over anhydrous $MgSO_4$. The mixture was then filtered and removal of the solvent *in vacuo* gave a crude solid. Recrystallisation of the crude solid from absolute alcohol gave **189** as shiny off-white crystals (0.13g, 19.12% yield) [m.p. 87.8-88.3°C; R_f 0.33 diethyl ether/ petroleum ether 40-60°C (50:50)].

$\nu_{(max)}$(Film) cm^{-1}: 2925.96, 2855.61 (CH_2), 1681.05 (C=O), 1596.32 (Ar C=C), 1371.90, 1150.54 (S=O); δ_H($CDCl_3$): 7.93 (2H, d, J=8.97Hz, Ph-H), 7.31 (2H, d, J=8.97Hz, Ph-H), 3.29-3.36 (1H, m, O=C-CH), 3.12 (3H, s, O=S=O-CH_3), 1.69-1.78 (2H, m, O=C-CHCH_2), 1.59-1.67 (2H, m, CH_2), 1.50-1.57 (2H, m, CH_2); δ_C($CDCl_3$): 203.04 (C=O), 151.91, 135.29, 130.48, 122.16 (ArC), 46.81, 37.94 (O=S=O-CH_3), 30.79 (O=C-CH), 28.39 (O=C-CHCH_2), 26.80 (CH_2); GC: t_R 13.24min; LRMS (EI): 296 (M^+, 2%), 199 (M^+-C_7H_{13}, 100%).

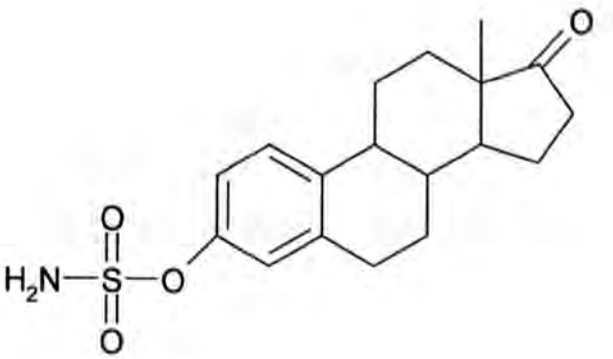
2.6 Synthesis of the sulfamic acid alkylphenyl ester derivatives

Aminosulfonyl chloride (190)



Anhydrous chlorosulfonyl isocyanate (2mL, ~0.23mol) was pipetted in an oven-dried pear-shaped flask under an atmosphere of nitrogen gas at 0°C. Formic acid (0.75mL, 0.02mol) was then carefully added dropwise resulting in the evolution of a gas and the formation of a white precipitate. The mixture was left to stir at 0°C for 30min, after which anhydrous toluene was added, and the mixture was left to stir for a further 1h after which, it was used for the next step below immediately without any purification.

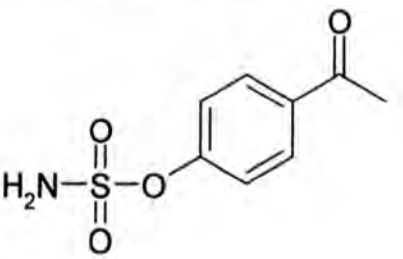
EMATE (191)



A solution of **190** in toluene (20mL, ~0.23mol) was added to a stirred mixture of estrone (0.74g, 0.37mol) and DMA (1mL) at 0°C. The mixture was left stirring at 0°C for 3h, after which it was poured into saturated brine solution (50mL) and extracted into ethyl acetate (2x50mL). The organic phase was washed with brine (2 x 25mL) and then dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give **191** as a red-brown solid (1.39g, 60.00% yield) [m.p. 195.4-197.3°C; R_f 0.30 diethyl ether/petroleum ether 40-60°C (70/30); lit. m.p. 195-197°C (Woo et al, 1998)].

$\nu_{(max)}$(Film) cm^{-1}: 3366.4, 3286.5 (NH_2), 1735.1 (C=O), 1365.2, 1179.8 (S=O); δ_H($CDCl_3$): 7.35 (1H, d, J=8.00Hz, Ph-H), 7.10 (1H, d, J=8.00Hz, Ph-H), 6.50 (1H, d, Ph-H), 5.00 (2H, s, N-H_2), 2.82-2.95 (2H, m, O=C-CH_2), 1.88-2.01 (13H, m, steroid backbone), 0.80 (3H, s, CH_3); δ_C($CDCl_3$): 149.30 (C-O), 139.00, 138.00, 128.20, 122.50, 119.90 (Ar-C), 50.00, 48.90, 43.90, 38.80, 35.11, 31.40, 29.85, 25.90, 25.10, 21.88, 13.90 (steroid backbone).

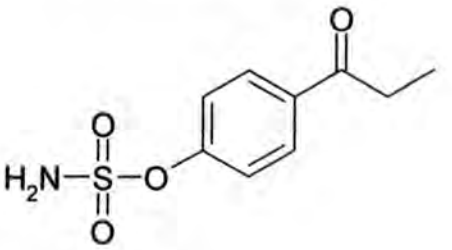
Sulfamic acid 4-acetyl-phenyl ester (192)



Compound **192** was synthesised in a similar manner to **191** except compound **158** (0.51g, 0.36mol) and DMA (1.0mL) were used. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash column chromatography of the crude solid gave **192** as a white solid (0.12g, 4.81% yield) [m.p. 124.4-124.9°C; R_f 0.14 diethyl ether/petroleum ether 40-60°C (50:50); lit. m.p. 123.5-125.5°C (Ahmed et al, 2002)].

$\nu_{(max)}$(Film) cm^{-1}: 3351.20, 3187.64 (NH_2), 11670.97 (C=O), 1595.20 (Ar), 1384.78, 1182.81 (S=O); δ_H(d_6-Acetone): 8.07 (2H, d, J=8.97Hz, Ph-H), 7.44 (2H, d, J=8.97Hz, Ph-H), 7.30 (2H, brs, NH_2), 2.59 (3H, s, CH_3); δ_C(d_6-Acetone): 196.08 (C=O), 154.09, 135.60, 130.08, 121.21 (Ar-C), 25.92 (CH_3).

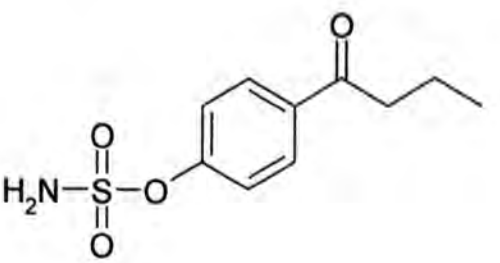
Sulfamic acid 4-propionyl-phenyl ester (193)



Compound **193** was synthesised in a similar manner as **191** except that compound **159** (0.51g, 0.33mol) was used. The mixture was filtered and the solvent was removed *in vacuo* to give a crude oil. Flash column chromatography of the crude oil gave **193** as white crystals (0.26g, 33.33% yield) [m.p. 105.8-106.2°C; R_f 0.21 (diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 106-107°C (Ahmed et al, 2002)].

$\nu_{(max)}$(Film) cm^{-1}: 3380.10, 3190.60, (NH_2), 1681.28 (C=O), 1599.18 (Ar C=C), 1383.63, 1182.26 (S=O); δ_H(d_6-Acetone): 7.98 (2H, d, J=8.97Hz, Ph-H), 7.34 (2H, d, J=8.97Hz, Ph-H), 7.20 (2H, brs, NH_2), 2.97 (2H, q, J=7.14Hz, CH_2CH_3), 1.05 (3H, t, J=7.14Hz, CH_3); δ_C(d_6-Acetone): 198.74 (C=O), 153.96, 135.41, 129.74, 122.22 (Ar-C), 31.40 (CH_2), 7.57 (CH_3).

Sulfamic acid 4-butyryl-phenyl ester (194)

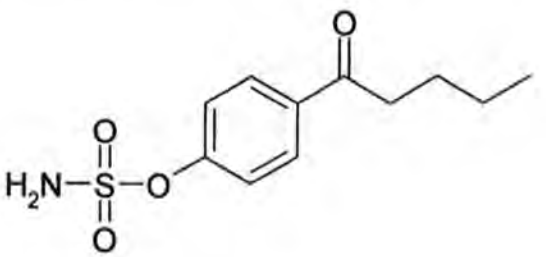


Compound **194** was synthesised in a similar manner as **191** except that compound **160** (0.50g, 0.19mol) was used. The mixture was filtered and the solvent was removed *in vacuo* to give a crude oil. Flash column

chromatography of the crude oil gave **194** as white crystals (0.13g, 17.57% yield) [m.p. 95.1-95.6°C; R_f 0.23 (diethyl ether/petroleum ether 40-60°C (50:50); lit. m.p. 94-97°C (Ahmed et al, 2002)].

$\nu_{(max)}$(Film) cm^{-1}: 3349.29 (NH_2), 1676.95 (C=O), 1597.18 (Ar), 1381.60 (S=O); δ_H(d_6-Acetone): 7.98 (2H, d, J=8.97Hz, Ph-H), 7.34 (2H, d, J=8.97Hz, Ph-H), 7.20 (2H, brs, NH_2), 2.92 (2H, t, J=7.14Hz, CH_2CH_2), 1.62 (2H, sex, J=7.32Hz, CH_2CH_3), 0.88 (3H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, CH_3); δ_C(d_6-Acetone): 198.35 (C=O), 153.97, 135.58, 129.81, 122.24 (Ar-C), 40.06 ($CH_2CH_2CH_3$), 17.45 (CH_2CH_3), 13.19 (CH_3).

Sulfamic acid 4-pentanoyl-phenyl ester (195)

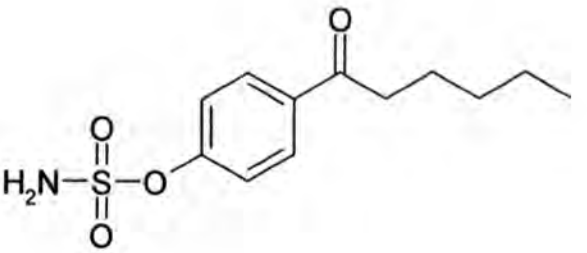


Compound **195** was synthesised in a similar manner as **191** except that compound **161** (0.51g, 0.28mol) was used. The mixture was filtered and the solvent was removed *in vacuo* to give a crude oil. Flash column chromatography of the crude oil gave **195** as a white crystals (0.09g, 12.16% yield) [m.p. 80.5-81.0°C; R_f 0.33 diethyl ether/petroleum ether 40-60°C (50:50); lit. m.p. 80-83°C (Ahmed et al, 2002)].

$\nu_{(max)}$(Film) cm^{-1}: 3363.01 (NH_2), 1678.03 (C=O), 1592.15 (Ar C=C), 1383.89, 1182.43 (S=O); δ_H(d_6-Acetone): 7.99 (2H, d, J=8.97Hz, Ph-H), 7.34 (2H, d, J=8.97Hz, Ph-H), 7.20 (2H, brs, NH_2), 2.95 (2H, t, J_{AB}=7.14Hz, J_{AB}=7.32Hz, O=C-CH_2), 1.52-1.60 (2H, m, $CH_2CH_2CH_3$), 1.19-1.36 (2H, m, CH_2CH_3), 0.84 (3H, t, J=7.32Hz, CH_3); δ_C(d_6-Acetone): 198.45 (C=O), 153.96, 135.57,

129.82, 122.24 (Ar-C), 37.91 [$\underline{C}H_2(CH_2)_2CH_3$], 26.27 ($\underline{C}H_2CH_2CH_3$), 22.18 ($\underline{C}H_2CH_3$), 13.39 ($\underline{C}H_3$).

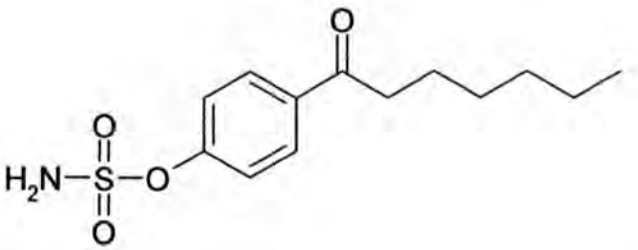
Sulfamic acid 4-hexanoyl-phenyl ester (196)



Compound **196** was synthesised in a similar manner as **191** except that compound **162** (0.52g, 0.27mol) was used. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash column chromatography of the crude solid gave **196** as a white solid (0.12g, 16.44% yield) [m.p. 114.2-114.7°C; R_f 0.38 diethyl ether/petroleum ether 40-60°C (50:50); lit. m.p. 114.9-115.8°C (Patel, M., 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3391.03, 3275.00 (NH_2), 2930.65 (CH), 1681.69 (C=O), 1378.61, 1181.85 (S=O); δ_H(d_6-Acetone): 7.99 (2H, d, J=8.97Hz, Ph-$\underline{H}$), 7.34 (2H, d, J=8.97Hz, Ph-$\underline{H}$), 7.20 (2H, brs, $N\underline{H}_2$), 2.94 (2H, t, J=7.32Hz, O=C-$C\underline{H}_2$), 1.52-1.65 [2H, m, $C\underline{H}_2(CH_2)_2CH_3$], 1.21-1.35 [4H, m, $(C\underline{H}_2)_2CH_3$], 0.65-0.87 (3H, m, $C\underline{H}_3$); δ_C(d_6-Acetone): 198.45 ($\underline{C}$=O), 153.96, 135.57, 129.82, 122.23 (Ar-$\underline{C}$), 38.14 (O=C-$\underline{C}H_2$), 31.34 (O=C-$CH_2\underline{C}H_2$), 23.82 ($\underline{C}H_2CH_2CH_3$), 22.38 ($\underline{C}H_2CH_3$), 13.42 ($\underline{C}H_3$).

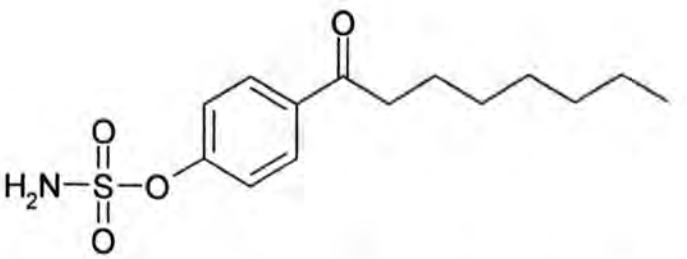
Sulfamic acid 4-heptanoyl-phenyl ester (197)



Compound **197** was synthesised in a similar manner as **191** except that compound **163** (0.50g, 0.24mol) was used. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash column chromatography of the crude solid gave **197** as a white solid (0.14g, 20.29% yield) [m.p. 80.5-81.0°C; R_f 0.38 (diethyl ether/petroleum ether 40-60°C (50:50); lit. m.p. 80-84°C (Ahmed et al, 2002)].

$\nu_{(max)}$(Film) cm^{-1}: 3390.21, 3274.20 (NH_2), 2929.94 (CH), 1682.04 (C=O), 1378.04, 1181.98 (S=O); δ_H(d_6-Acetone): 7.99 (2H, d, J=8.97Hz, Ph-H), 7.34 (2H, d, J=8.97Hz, Ph-H), 7.20 (2H, brs, NH_2), 2.94 (2H, t, J=7.32Hz, O=C-CH_2), 1.52-1.67 [2H, m, $CH_2(CH_2)_3CH_3$], 1.12-1.29 [6H, m, $(CH_2)_3CH_3$], 0.66-0.88 (3H, m, CH_3); δ_C(d_6-Acetone): 198.46 (C=O), 153.97, 135.57, 129.82, 122.23 (Ar-C), 38.19 (O=C-CH_2), 31.62 (O=C-CH_2CH_2), 24.10 ($CH_2CH_2CH_2CH_3$), 22.41 (CH_2CH_3), 13.49 (CH_3).

Sulfamic acid 4-octanoyl-phenyl ester (198)

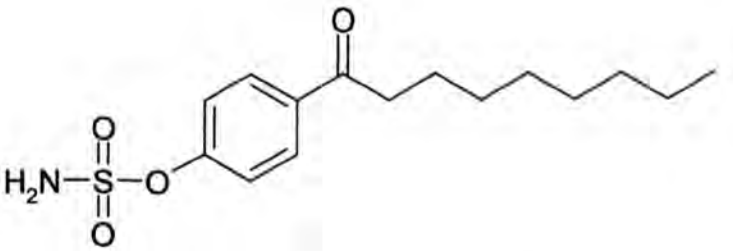


Compound **198** was synthesised in the same way as **191** except that compound **164** (0.55g, 0.25mol) was used. The mixture was filtered and the

solvent was removed *in vacuo* to give a crude solid. Flash column chromatography of the crude solid gave **198** as a white solid (0.16g, 21.33% yield) [m.p. 106.3-106.8°C; R_f 0.11 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 105-107°C (Ahmed et al, 2002)].

$\nu_{(max)}$(Film) cm^{-1}: 3388.93, 3272.56 (NH_2), 2918.44 (CH), 1682.14 (C=O), 1597.84 (Ar C=C), 1377.67, 1181.96 (S=O); δ_H(d_6-Acetone): 7.99 (2H, d, J=8.97Hz, Ph-H), 7.34 (2H, d, J=8.97Hz, Ph-H), 7.20 (2H, brs, NH_2), 2.94 (2H, t, J=7.32Hz, O=C-CH_2), 1.68-1.75 (2H, m, O=C-CH_2CH_2), 1.25-1.37 [8H, m, $(CH_2)_4CH_3$], 0.79 (3H, t, J=7.14Hz, CH_3); δ_C(d_6-Acetone): 198.48 (C=O), 153.96 (Ar-C-O), 135.57, 129.82, 122.23 (Ar-C), 38.19 (O=C-CH_2), 31.69 (O=C-CH_2CH_2), 29.12 [O=C-$(CH_2)_2CH_2$], 29.09 [$CH_2(CH_2)_2CH_3$], 24.15 ($CH_2CH_2CH_3$), 22.47 (CH_2CH_3), 13.51 (CH_3).

Sulfamic acid 4-nonanoyl-phenyl ester (199)

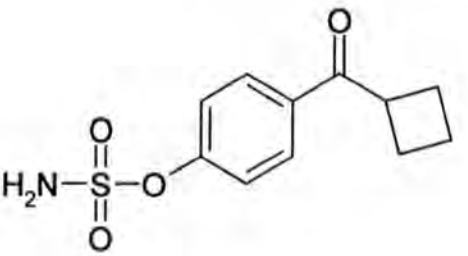


Compound **199** was synthesised in the same way as **191** except that compound **165** (0.52g, 0.22mol) was used. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash column chromatography of the crude solid gave **199** as a white solid (0.10g, 14.29% yield) [m.p. 103.8-104.3°C; R_f 0.56 diethyl ether/petroleum ether 40-60°C (70:30); lit. m.p. 102-104°C (Ahmed et al, 2002)].

$\nu_{(max)}$(Film) cm^{-1}: 3389.49, 3273.87 (NH_2), 2917.25 (CH), 1683.23 (C=O), 1599.09 (Ar), 1378.13, 1182.24 (S=O); δ_H($CDCl_3$): 7.97 (2H, d, J=8.00Hz, Ph-H), 7.29 (2H, d, J=8.00Hz, Ph-H), 7.25 (2H, brs, NH_2), 2.92 (2H, t, J=7.00Hz,

O=C-C$\underline{H}_2$), 2.79 (2H, t, J=7.00Hz, O=C-$CH_2C\underline{H}_2$), 2.16-2.20 [2H, m, O=C-$(CH_2)_2C\underline{H}_2$], 1.75 [8H, t, J=7.00Hz, $(C\underline{H}_2)_4CH_3$], 0.85 (3H, t, J=7Hz, $C\underline{H}_3$); $\delta_C(CDCl_3)$: 189.06 ($\underline{C}$=O), 160.69 (Ar-$\underline{C}$-O), 130.85, 129.73, 115.45, 96.44 (Ar-C), 38.39 (O=C-$\underline{C}H_2$), 31.83 (O=C-$CH_2\underline{C}H_2$), 24.83 ($\underline{C}H_2$), 22.66 ($\underline{C}H_2CH_3$), 14.11 ($\underline{C}H_3$).

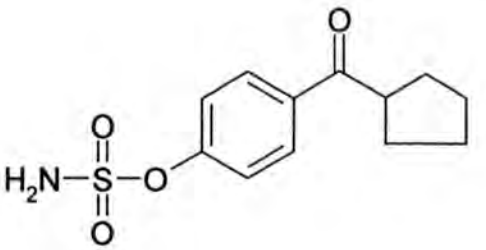
Sulfamic acid 4-cyclobutanecarbonyl-phenyl ester (200)



Compound **200** was synthesised in the same way as **191** except that compound **169** (0.55g, 0.31mol) was used. The mixture was filtered and the solvent was removed *in vacuo* to give a crude oil. Flash column chromatography of the crude oil gave **200** as white crystals (0.26g, 32.50% yield) [m.p. 101.9-102.4°C; R_f 0.52 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 101.2-102.5°C (Patel, M., 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3162.34 (NH_2), 2947.00(CH), 1669.96 (C=O), 1597.61, 1499.81 (Ar), 1385.50, 1181.23 (S=O); $\delta_H(CDCl_3)$: 7.92 (2H, d, J=8.97Hz, Ph-$\underline{H}$), 7.34 (2H, d, J=8.97, Ph-$\underline{H}$), 7.23 (2H, brs, $N\underline{H}_2$), 4.01-4.09 (1H, m, O=C-C$\underline{H}$), 2.74-2.80 [4H, m, $(C\underline{H}_2)_2$, cyclo], 2.19-2.26 (2H, m, J=7Hz, $C\underline{H}_2$, cyclo); $\delta_C(CDCl_3)$: 205.45 ($\underline{C}$=O), 153.96 (Ar-$\underline{C}$-O), 133.52, 130.11, 122.35 (Ar-C), 41.94 (O=C-$\underline{C}$H), 24.74 ($\underline{C}H_2$, cyclo), 17.76 ($\underline{C}H_2$, cyclo).

Sulfamic acid 4-cyclopentanecarbonyl-phenyl ester (201)



Compound **201** was synthesised in the same way as **191** except that compound **170** (0.56g, 0.29mol) was used. The mixture was filtered and the solvent was removed *in vacuo* to give a crude oil. Flash column chromatography of the crude oil gave **201** as a white solid (0.20g, 25.32% yield) [m.p. 103.8-104.3°C; R_f 0.59 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 104.7-106.0°C (Patel, M., 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3266.33, 3102.13 (NH_2), 2955.80 (CH), 1671.23 (C=O), 1596.74, 1499.66 (Ar C=C), 1387.74, 1181.90 (S=O), 870.09; δ_H($CDCl_3$): 8.01 (2H, d, J=8.79Hz, Ph-H), 7.35 (2H, d, J=8.97, Ph-H), 7.23 (2H, brs, NH_2), 3.71-3.79 (1H, m, O=C-CH), 1.95-1.97 [4H, m, 2X $CHCH_2$, cyclo], 1.55-1.59 [4H, m, $CHCH_2$, cyclo]; δ_C($CDCl_3$): 205.43 (C=O), 153.89 (Ar-C-O), 135.24, 130.28, 122.28 (Ar-C), 46.10 (O=C-CH), 28.66 [$(CH_2)_4$, cyclo].

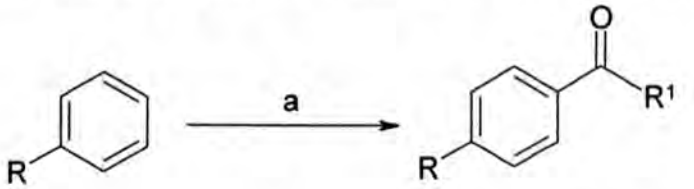
CHAPTER 3

Synthesis of 1-biphenyl-4-yl-ketones and 1-(4-cyclohexyl-phenyl)-ketones

3.0 Synthesis of 1-biphenyl-4-yl ketones and 1-(4-cyclohexyl-phenyl)-ketones

3.1 Discussion

The 1-biphenyl-4-yl ketones and 1-(4-cyclohexyl-phenyl)-ketones were synthesised using the general reaction scheme 3.1 below:



Where R=Ph

$R^1=C_2H_3$, **(202)**
$R^1=C_3H_5$, **(203)**
$R^1=C_4H_7$, **(204)**
$R^1=C_5H_9$, **(205)**
$R^1=C_6H_{11}$, **(206)**
$R^1=C_7H_{13}$, **(207)**
$R^1=C_8H_{15}$, **(208)**
$R^1=C_9H_{17}$, **(209)**
$R^1=C_{10}H_{19}$, **(210)**
$R^1=C_{12}H_{23}$, **(211)**
$R^1=C_4H_7$ (Cyclo), **(212)**
$R^1=C_5H_9$ (Cyclo), **(213)**
$R^1=C_6H_{11}$ (Cyclo), **(214)**

Where R=Cyclohexyl

$R^1=C_2H_3$, **(215)**
$R^1=C_3H_5$, **(216)**
$R^1=C_4H_7$, **(217)**
$R^1=C_5H_9$, **(218)**
$R^1=C_6H_{11}$, **(219)**
$R^1=C_7H_{13}$, **(220)**
$R^1=C_8H_{15}$, **(221)**
$R^1=C_9H_{17}$, **(222)**
$R^1=C_{10}H_{19}$, **(223)**
$R^1=C_{12}H_{23}$, **(224)**
$R^1=C_3H_5$ (Cyclo), **(225)**
$R^1=C_4H_7$ (Cyclo), **(226)**
$R^1=C_5H_9$ (Cyclo), **(227)**

Scheme 3.1 Scheme outlining the synthesis of 1-biphenyl-4-yl ketones (R=Ph) and 1-(4-cyclohexyl-phenyl)-ketones [(R=cyclohexyl) (R^1=straight chain alkyl and cyclo alkyl moiety) (a=DCM/RCOCl/$AlCl_3$; R^1=alkyl chain from C_2 to C_{12} and cycloalkyl groups)].

The reaction involves Friedel-Crafts acylation as utilised in the synthesis of 4-hydroxyphenyl ketones (Chapter 2; Scheme 2.1). In this particular reaction, however, the addition of the starting materials was different to that in the synthesis of the 4-hydroxyphenyl ketones. That is, in the synthesis of the latter series of compounds, the phenol was added to the $AlCl_3$ initially and the

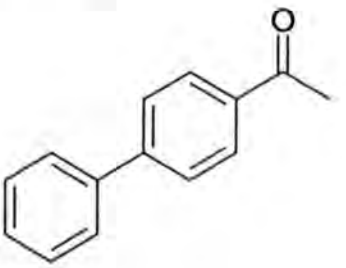
mixture was allowed to stir prior to the addition of the acyl chloride. In the synthesis of the 1-biphenyl-4-yl ketones and 1-(4-cyclohexyl-phenyl)-ketones, however, the lack of a hydroxy group meant that the initial complex formation between $AlCl_3$ and the phenolic oxygen atom was not required. As a result, the acyl chlorides were initially stirred with the $AlCl_3$ prior to the addition of biphenyl (in the synthesis of 1-biphenyl-4-yl ketones) or the 4-cyclohexylbenzene [in the synthesis of 1-(4-cyclohexyl-phenyl)-ketones]. Excess $AlCl_3$ in the reaction is used as it forms a complex with the acyl functionality. The ketone product is also complexed with the $AlCl_3$, however, the desired ketone can be liberated by the treatment of water during the 'work-up' (Fessenden and Fessenden, 1998). The use of the catalyst ensures that the acylation takes place on the carbon atom and not the oxygen atom, resulting in the formation of a carbonyl functionality which leads to the deactivation of the phenyl ring system and which in turn, results in the formation of the mono substituted product only. Furthermore, only the formation of a para substituted product is observed (as shown by the GC-MS and ^{1}H-NMR) in the Friedel-Crafts reaction.

In general, the reactions proceeded in good yield and without major problems. For example, in the synthesis of 1-biphenyl-4-yl ketones, 98% yield was obtained in the synthesis for 1-biphenyl-4-yl-ethanone (**202**) whilst the lowest yield obtained was 35% for 1-biphenyl-4-yl-nonan-1-one (**209**) – it should be noted that most of these compounds were obtained in high purity upon workup. In the synthesis of 1-(4-cyclohexyl-phenyl)-ketones, ~98% yield was obtained in the synthesis for 1-(4-cyclohexyl-phenyl)-hexan-1-one (**219**) whilst the lowest yield obtained was 19% for 1-(4-Cyclohexyl)-phenyl)-dodecan-1-one **(224)** – following column chromatography or recrystallisation. However, in the synthesis of the undecyl and cycloheptyl derivatives of both series of compounds, the appropriate acyl chlorides were not available, as such, the acyl chlorides were synthesised involving the reaction (under anhydrous conditions) between the appropriate carboxylic acid and an excess of thionyl chloride. The attempted synthesis of the undecyl and cycloheptyl derivatives were then carried out in a similar manner to the other ketone derivatives, that is, the appropriate acyl chloride was stirred with the $AlCl_3$ prior to the addition

of biphenyl or 4-cyclohexyl benzene. GC-MS analysis of the reaction mixture (involving the analysis of the MS fragmentation pattern of the peaks within the GC) showed the presence of the product, however, attempted column chromatography and recrystallisation yielded no pure product. Several attempts were made to synthesise the two missing compounds, however, the compounds could not be isolated and the synthesis of the undecyl and cycloheptyl derivatives were abandoned.

3.1.1 Synthesis of 1-biphenyl-4-yl ketones

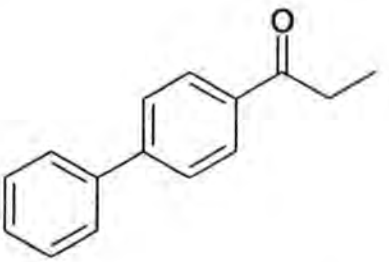
1-Biphenyl-4-yl-ethanone (202)



Acetyl chloride (0.46mL) was added in a dropwise manner to a solution of $AlCl_3$ (0.86g, 21mmol) in anhydrous DCM (100mL). The slurry was left to stir for 30min after which, biphenyl (0.50g, 10.6mol) was added. The reaction mixture was then left to stir for 14h, after which it was quenched with a mixture of ice and water (150mL) and washed with aqueous HCl (0.5M, 2 X 50mL), H_2O (100mL), saturated $NaHCO_3$ solution (50mL) and H_2O (2 X 100mL). The combined organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was then filtered, and the solvent was removed *in vacuo* to give **202** as a pale yellow solid. (0.68g, 97.69% yield) [m.p 131.5-132.9°C; R_f 0.49 diethyl ether/petroleum ether 40-60°C (30:70); lit.m.p. 124.5-125°C (Takebayashi et al, 1970)].

$\nu_{(max)}$(Film) cm^{-1}: 1680.90 (C=O), 1610.00 (Ar C=C), 1358.40 (CH); $\delta_H(CDCl_3)$: 8.04 (2H, d, J=8.60Hz, Ph-H), 7.69 (2H, d, J=8.60Hz, Ph-H), 7.62 (2H, d, J=7.78Hz, Ph-H), 7.47 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.69Hz, Ph-H), 2.64 (3H, s, CH_3); $\delta_C(CDCl_3)$: 198.01 (C=O), 145.65, 139.51, 135.68, 128.94, 128.89, 128.07, 127.25, 127.20 (ArC), 26.65 (CH_3); GC: t_R 8.93min; LRMS (EI): 196 ($M^{+\cdot}$ 56%), 181 (M^{+}-CH_3, 100%); Elemental analysis: Found C, 85.68%; H, 6.08%; $C_{14}H_{12}O$ requires C, 85.68%; H, 6.16%.

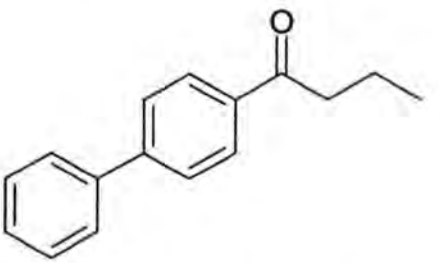
1-Biphenyl-4-yl-propan-1-one (203)



Compound **203** was synthesised in a similar manner to **202** except that propionyl chloride (0.57mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.56g, 10.6mol) was used. The organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give **203** as a pale yellow solid (0.78g, 97.44% yield) [m.p. 114.6-115.2°C; R_f 0.70 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 102°C (Hartung et al, 1935)].

$\nu_{(max)}$(Film) cm^{-1}: 2978.80 (CH), 1685.90 (C=O), 1445.90 (CH); $\delta_H(CDCl_3)$: 8.03 (2H, d, J=8.60Hz, Ph-H), 7.67 (2H, d, J=8.60Hz, Ph-H), 7.62 (2H, d, J=7.51Hz, Ph-H), 7.48 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.69Hz, Ph-H), 3.04 (2H, q, J_{AB}=8.60Hz, J_{AB}=7.32Hz, J_{AB}=5.86Hz, CH_2CH_3), 1.25 (3H, t, J_{AB}=7.51Hz, J_{AB}=7.14Hz, CH_3); $\delta_C(CDCl_3)$: 200.11 (C=O), 145.39, 139.77, 135.68, 128.91, 128.55, 128.14, 127.21, 127.19 (ArC), 31.81 (CH_2CH_3), 8.29 (CH_3); GC: t_R 9.56min; LRMS (EI): 210 (M^+, 19%), 181 (M^+-C_2H_5, 100%); Elemental analysis: Found C, 85.57%; H, 6.76%; $C_{15}H_{14}O$ requires C, 85.68%; H, 6.71%.

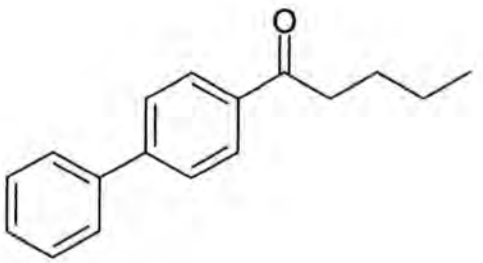
1-Biphenyl-4-yl-butan-1-one (204)



Compound **204** was synthesised in a similar manner to **202** except that butyryl chloride (0.67mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.50g, 10.6mol) was used. The organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent removed *in vacuo* to give **204** as a yellow solid (0.67g, 77.01% yield) [m.p. 104.1-105.2°C; R_f 0.65 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 96-97°C (Cromwell et al, 1953)].

$\nu_{(max)}$(Film) cm^{-1}: 2960.70 (CH), 1678.40 (C=O), 1603.50, 1449.70 (Ar C=C); $\delta_H(CDCl_3)$: 8.04 (2H, d, J=8.79Hz, Ph-H) , 7.68 (2H, d, J=8.60Hz, Ph-H), 7.62 (2H, d, J=7.51Hz, Ph-H), 7.47 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.69Hz, Ph-H), 2.98 (2H, t, J=7.32Hz, O=C-CH_2), 1.81 (2H, sex, J_{AB}=7.32Hz, J_{AB}=7.69Hz, J_{AB}=6.96Hz, J_{AB}=7.51, CH_2CH3), 1.02 (3H, t, J=7.32Hz, CH_3); $\delta_C(CDCl_3)$: 200.11 (C=O), 145.52, 139.92, 135.68, 128.91, 128.62, 128.14, 127.23, 127.21 (ArC), 40.56 (O=C-CH_2), 17.84 (O=C-CH_2CH_2), 13.90 (CH_3); GC: t_R 9.99min; LRMS (EI): 224 (M^+, 15%), 181 (M^+-C_3H_7, 100%); Elemental analysis: Found C, 85.74%; H, 7.24; $C_{16}H_{16}O$ requires C, 85.68%; H, 7.19%.

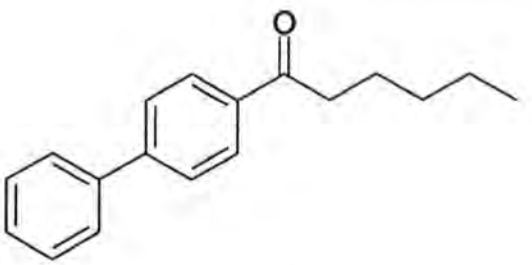
1-Biphenyl-4-yl-pentan-1-one (205)



Compound **205** was synthesised in a similar manner to **202** except that valeryl chloride (0.77mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.55g, 10.6mol) was used. The organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give **205** as a yellow solid (0.74g, 87.06% yield) [m.p. 88.9-89.1°C; R_f 0.62 diethyl ether/petroleum spirit 40-60°C (30:70); lit. m.p. 79.5-80.5 °C (Boots et al, 1976)].

$\nu_{(max)}$(Film) cm^{-1}: 2952.50 (CH), 1675.90 (C=O), 1602.60 (Ar C=C), 1466.00 (CH), 1449.50 (CH); $\delta_H(CDCl_3)$: 8.03 (2H, d, J=8.60Hz, Ph-H), 7.68 (2H, d, J=8.79Hz, Ph-H), 7.62 (2H, d, J=7.60Hz Ph-H), 7.46 (3H, t, J_{AB}=7.14Hz, J_{AB}=8.24Hz, Ph-H), 3.00 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.69Hz, O=C-CH_2), 1.75 (2H, quin, J_{AB}=7.51Hz, J_{AB}=7.69Hz, J_{AB}=7.32Hz, $CH_2CH_2CH_3$), 1.43 (2H, sex, J_{AB}=7.51Hz, J_{AB}=7.41Hz, CH_2CH_3), 0.97 (3H, t, J_{AB}=8.24Hz, J_{AB}=6.77Hz, CH_3); $\delta_C(CDCl_3)$: 200.37 (C=O), 145.58, 139.90, 135.73, 128.92, 128.65, 128.15, 127.24, 127.21 (Ar-C), 38.38 (O=C-CH_2), 26.55 (O=C-CH_2CH_2), 22.50 (CH_2CH_3), 13.95 (CH_3); GC: t_R 10.58min; 238 (M^+, 7%), 181 (M^+-C_4H_9), 100%]; Elemental analysis: Found C, 85.68%; H, 7.72%; $C_{17}H_{18}O$ requires C, 85.67%; H, 7.61%.

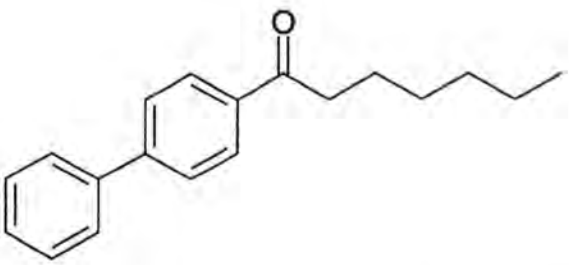
1-Biphenyl-4-yl-hexan-1-one (206)



Compound **206** was synthesised in a similar manner to **202** except that hexanoyl chloride (0.91mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.51g, 10.6mol) was used. The organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash chromatography of the crude solid gave **206** as a white solid (0.50g, 60.24% yield) [m.p. 107.8-108.5°C; R_f 0.80 diethyl ether/petroleum spirit 40-60°C (30:70); lit. m.p. 97°C (Koelbel et al, 1959)].

$\nu_{(max)}$(Film) cm^{-1}: 2927.29, 1677.30 (C=O); $\delta_H(CDCl_3)$: 8.02 (2H, d, J=8.42Hz, Ph-H), 7.67 (2H, d, J=8.60Hz, Ph-H), 7.61 (2H, d, J=7.87Hz, Ph-H), 7.46 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.69Hz, Ph-H), 2.97 (2H, t, J_{AB}=8.24Hz, J_{AB}=7.51Hz, O=C-CH_2), 1.75 (2H, quin, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-CH_2CH_2), 1.59 (2H, s, $CH_2CH_2CH_3$), 1.34-1.39 (2H, m, CH_2CH_3), 0.91 (3H, t, J=8Hz, CH_3); $\delta_C(CDCl_3)$: 200.50 (C=O), 145.50, 139.92, 135.73, 128.91, 128.64, 128.14, 127.23, 127.18 (Ar-C), 38.63 (O=C-CH_2), 31.55 (O=C-CH_2CH_2), 24.14 ($CH_2CH_2CH_3$), 22.54 (CH_2CH_3), 13.95 (CH_3); GC: t_R 11.14min; LRMS (EI): 252 (M^+, 6%), 181 (M^+-C_5H_{11}, 100%); Elemental analysis: Found C, 85.45%; H, 8.04%; $C_{18}H_{20}O$ requires C, 85.67%; H, 7.99%.

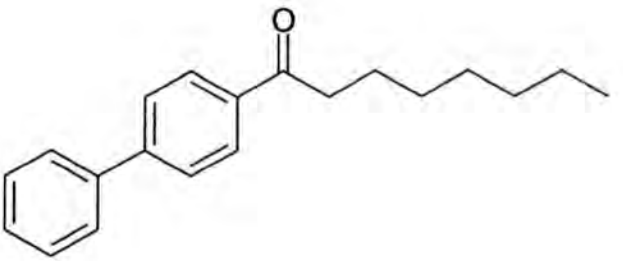
1-Biphenyl-4-yl-heptan-1-one (207)



Compound **207** was synthesised in a similar manner to **202** except that heptanoyl chloride (1.00mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.51g, 10.6mol) was used. The organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash chromatography of the crude solid gave **207** as a white solid (0.64g, 72.73% yield) [m.p. 96.3-96.9°C; R_f 0.77 diethyl ether/petroleum spirit 40-60°C (30:70); lit. m.p. 85.5-86.5°C (Long and Henze, 1941)].

$\nu_{(max)}$(Film) cm^{-1}: 2950.90 (CH aliphatic), 1676.40 (C=O), 1466.00 (CH); $\delta_H(CDCl_3)$: 8.00 (2H, d, J=8.06Hz, Ph-H), 7.66 (2H, d, J=8.06Hz, Ph-H), 7.61 (2H, d, J=7.32Hz, Ph-H), 7.40 (3H, t, J=7.14Hz, J=7.69Hz, Ph-H), 2.92 (2H, t, J_{AB}=7.32, J_{AB}=7.51Hz, O=C-CH_2), 1.68 (2H, quin, J_{AB}=7.14Hz, J_{AB}=7.69Hz, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-CH_2CH_2), 1.56 [2H, quin, J_{AB}=7.51Hz, J_{AB}=7.32Hz, J_{AB}=7.69Hz, $CH_2(CH_2)_2CH_3$], 1.28-1.41 [4H, m, $(CH_2)_2CH_3$], 0.82 (3H, t, J_{AB}= 7.14Hz, J_{AB}=6.96Hz, CH_2CH_3); $\delta_C(CDCl_3)$: 200.37 (C=O), 145.52, 139.92, 135.75, 128.91, 128.64, 128.14, 127.23, 127.21 (Ar-C), 38.67 (O=C-CH_2), 31.66 (O=C-CH_2CH_2), 29.06 [O=C-$(CH_2)_2CH_2$], 24.41 ($CH_2CH_2CH_3$), 22.52 (CH_2CH_3), 14.04 (CH_3); GC: LRMS (EI) t_R 11.81min; 266 (M^+, 17%), 181 (M^+-C_6H_{13}, 100%); Elemental analysis: Found C, 85.57%; H, 8.34%; $C_{19}H_{22}O$ requires C, 85.67%; H, 8.32%.

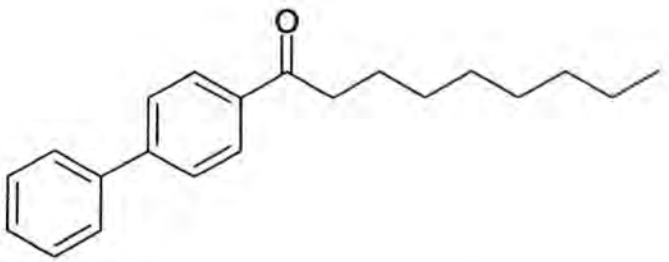
1-Biphenyl-4-yl-octan-1-one (208)



Compound **208** was synthesised in a similar manner to **202** except that octanoyl chloride (1.11mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.51g, 10.6mol) was used. The organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash chromatography of the crude solid gave **208** as a white solid (0.44g, 47.31% yield) [m.p. 102.103.2°C; R_f 0.40, diethyl ether/petroleum spirit 40-60°C (10:90); lit. m.p. 100-100.5°C (Matsuda et al, 1954)].

$\nu_{(max)}$(Film) cm^{-1}: 2928.87, 1677.86 (C=O), 1603.43 (Ar C=C), 1465.38 (CH), $\delta_H(CDCl_3)$: 7.96 (2H, d, J=8.60Hz, Ph-H) , 7.60 (2H, d, J=8.60Hz, Ph-H), 7.55 (2H, d, J=8.24Hz, Ph-H), 7.40 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.69Hz, Ph-H), 2.92 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.69Hz, O=C-CH_2), 1.68 (2H, quin, J_{AB}=7.51Hz, J_{AB}=7.14Hz, J_{AB}=7.32Hz, O=C-CH_2CH_2), 1.21-1.36 [8H, m, $(CH_2)_4CH_3$], 0.81 (3H, t, J_{AB}=4.76Hz, J_{AB}=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 201.00 (C=O), 145.77, 139.89, 135.80, 129.04, 128.77, 128.27, 127.36, 127.31 (Ar-C), 38.80 (O=C-CH_2), 31.84 (O=C-CH_2-CH_2), 29.47 [O=C-$(CH_2)_2CH_2$], 29.28 [$CH_2(CH_2)_2CH_3$], 24.57 ($CH_2CH_2CH_3$), 22.75 (CH_2CH_3), 14.21 (CH_3); GC LRMS (EI) t_R 13.01min; 280 (M^+, 12%), 196 (M^+-C_6H_{12}, 100%); Elemental analysis: Found C, 85.58%; H, 8.57%; $C_{20}H_{24}O$ requires C, 85.67%; H, 8.63%.

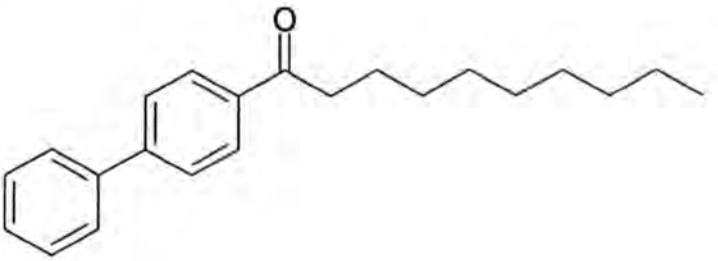
1-Biphenyl-4-yl-nonan-1-one (209)



Compound **209** was synthesised in a similar manner to **202** except that nonanoyl chloride (1.17mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.51g, 10.6mol) was used. The organic layers were collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash chromatography of the crude solid gave **209** as a white solid (0.34g, 35.05% yield) [m.p. 96.7-97.1°C; R_f 0.49, diethyl ether/petroleum spirit 40-60°C (10:90); lit. m.p. 93°C (Ruolene et al, 1984)].

$\nu_{(max)}$(Film) cm^{-1}: 2953.81, 1680.97 (C=O), 1467.13 (CH); δ_H($CDCl_3$): 7.96 (2H, t, J=8.60Hz, Ph-H), 7.60 (2H, d, 8.79Hz, Ph-H), 7.55 (2H, d, 8.24Hz, Ph-H), 7.39 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.69Hz, Ph-H), 2.91 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-CH_2), 1.68 (2H, quin, J_{AB}=7.51Hz, J_{AB}=7.32Hz, O=C-CH_2CH_2), 1.17-1.34 [10H, m, $(CH_2)_5CH_3$], 0.81 (3H, t, J=6.96Hz, CH_3); δ_C($CDCl_3$): 200.23 (C=O), 145.62, 139.89, 135.80, 129.04, 128.77, 128.27, 127.36, 127.30 (Ar-C), 38.80 (O=C-CH_2), 31.96 (O=C-CH_2CH_2), 29.57 [O=C-$(CH_2)_2CH_2$], 29.30 [$CH_2(CH_2)_2CH_3$], 24.57 ($CH_2CH_2CH_3$), 22.77 (CH_2CH_3), 14.23 (CH_3); GC: t_R 13.85min; LRMS (EI): 294 (M^+, 12%), 196 (M^+-C_7H_{14}, 100%); Elemental analysis: Found C, 85.61%; H, 8.94% $C_{18}H_{21}O$ requires C, 85.67%; H, 8.90%.

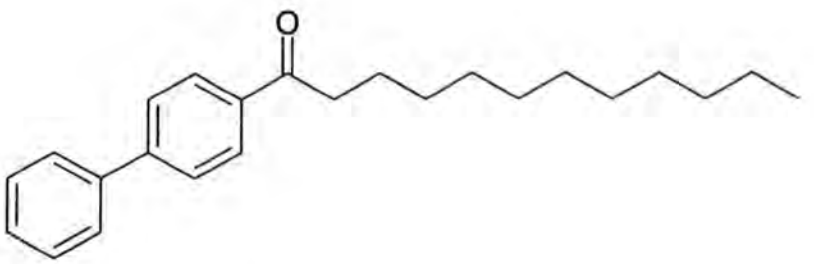
1-Biphenyl-4-yl-decan-1-one (210)



Compound **210** was synthesised in a similar manner to **202** except that decanoyl chloride (1.35mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.51g, 10.6mol) was used. The organic layers were collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash chromatography of the crude solid gave **210** as a white solid (0.60g, 58.82% yield) [m.p. 102.5-102.8°C; R_f 0.52, diethyl ether/petroleum spirit 40-60°C (10:90); lit. m.p. 102-104°C (Thompson et al, 1991)].

$\nu_{(max)}$(Film) cm^{-1}: 2914.18, 2847.90 (CH), 1677.60 (C=O), 1460.78 (CH) $\delta_H(CDCl_3)$: 8.02 (2H, d, J=8.60Hz, Ph-H), 7.66 (2H, d, J=8.60Hz, Ph-H), 7.61 (2H, d, J=8.24Hz, Ph-H), 7.46 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.69Hz, Ph-H), 2.97 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-CH_2), 1.74 (2H, quin, J_{AB}=7.51Hz, J_{AB}=7.32Hz, O=C-CH_2CH_2), 1.18-1.38 [12H, m, $(CH_2)_6CH_3$], 0.87 (3H, t, J_{AB}=6.77Hz, J_{AB}=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 200.35 (C=O), 145.62, 135.86, 133.82, 129.93, 129.04, 128.77, 128.27, 127.36 (Ar-C), 38.81 (O=C-CH_2), 31.99 (O=C-CH_2CH_2), 29.61 [O=C-$(CH_2)_2CH_2$], 29.60 [O=C-$(CH_2)_3CH_2$], 29.52 [$CH_2(CH_2)_3CH_3$], 29.40 [$CH_2(CH_2)_2CH_3$], 24.58 ($CH_2CH_2CH_3$), 22.78 (CH_2CH_3), 14.23 (CH_3); GC: t_R 25.17min; LRMS (EI): 308 (M^+,6%), 196 (M^+-C_8H_{16}, 100%).

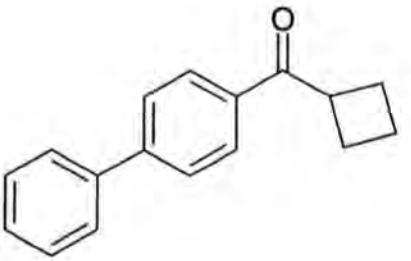
1-Biphenyl-4-yl-dodecan-1-one (211)



Compound **211** was synthesised in a similar manner to **202** except that dodecanoyl chloride (1.54mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.51g, 10.6mol) was used. The organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give the crude solid. Flash chromatography of the crude solid gave **211** as a white solid (0.37g, 41.07% yield) [m.p. 107.1-107.5°C; R_f 0.89 diethyl ether/petroleum spirit 40-60°C (30:70); lit. m.p. 101-102°C (Gilman and Ford, 1939)].

$\nu_{(max)}$(Film) cm^{-1}: 2914.70 (CH), 1678.92 (C=O), 1461.21 (CH). $\delta_H(CDCl_3)$: 8.02 (2H, d, J=8.60Hz Ph-H), 7.67 (2H, d, J=8.42Hz, Ph-H), 7.62 (2H, d, J=8.60Hz, Ph-H), 7.46 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.69Hz, Ph-H), 2.97 (2H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, O=C-CH_2), 1.74 (2H, quin, J_{AB}=7.32Hz, J_{AB}=7.14Hz, J_{AB}=7.69Hz, O=C-CH_2CH_2), 1.60 [2H, s, O=C-$(CH_2)_2CH_2$], 1.19-1.35 [14H, m, $(CH_2)_7CH_3$], 0.87 (3H, t, J_{AB}=6.59Hz, J_{AB}=7.14Hz, CH_3); $\delta_C(CDCl_3)$: 200.39 (C=O), 145.77, 139.89, 135.88, 135.54, 129.04, 128.76, 128.26, 127.35, 127.30 (Ar-C), 38.80 (O=C-CH_2), 32.02 (O=C-CH_2CH_2), 29.74 [O=C-$(CH_2)_2CH_2$], 24.58 [O=C-$(CH_2)_3CH_2$], 22.80 (CH_2CH_3), 14.23 (CH_3); GC: t_R 28.41min; LRMS (EI): 336 (M^+, 5%), 196 (M^+-$C_{10}H_{20}$, 100%).

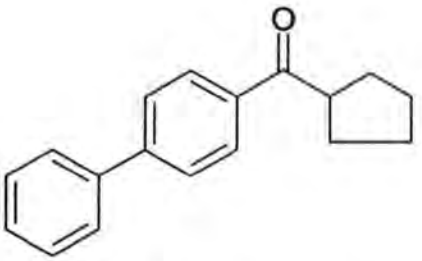
1-Biphenyl-4-yl-cyclobutyl-methanone (212)



Compound **212** was synthesised in a similar manner to **202** except that cyclobutanecarbonyl chloride (0.73mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.54g, 10.6mol) was used. The organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash chromatography of the crude solid gave **212** as an off-white solid (0.71g, 85.54% yield) [m.p. 86.0-86.5°C; R_f 0.65, diethyl ether/petroleum spirit 40-60°C (30:70); lit. m.p. 81-82°C (Morand and Samad, 1977)].

$\nu_{(max)}$(Film) cm^{-1}: 2979.82 (CH), 1672.17 (C=O), 1600.27 (Ar C=C), 1446.21 (CH); $\delta_H(CDCl_3)$: 7.89 (2H, d, J=8.60Hz, Ph-H), 7.59 (2H, d, J=8.79Hz, Ph-H), 7.54 (2H, d, J=6.96Hz, Ph-H), 7.39 (3H, t, J_{AB}=7.14Hz, J_{AB}=8.06Hz, Ph-H), 3.96 (1H, quin, J_{AB}=9.52Hz, J_{AB}=9.34Hz, O=C-CH), 2.33-2.43 (2H, m, O=C-CHCH_2), 2.23-2.29 (2H, m, CH_2, cyclo), 2.04 (2H, sex, J_{AB}=8.97Hz, J_{AB}=8.42Hz, J_{AB}=9.15Hz, J_{AB}=8.60Hz, CH_2, cyclo); $\delta_C(CDCl_3)$: 200.74 (C=O), 145.51, 139.89, 134.27, 128.26, 127.35, 127.33, (Ar-C), 42.35 (O=C-CH), 25.23 (O=C-CHCH_2, cyclo), 18.29 (CH_2, cyclo); GC: t_R 11.37min; LRMS (EI): 236 (M^+, 2%), 181 (M^+-C_4H_7); Elemental analysis: Found C, 86.41%; H, 6.81%; $C_{17}H_{16}O$ requires C, 86.41%; H, 6.82%.

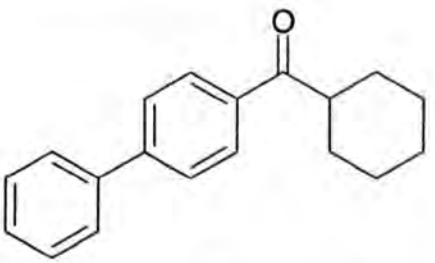
1-Biphenyl-4-yl-cyclopentyl-methanone (213)



Compound **213** was synthesised in a similar manner to **202** except that cyclopentanecarbonyl chloride (0.79mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.50g, 10.6mol) was used. The organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash chromatography of the crude solid gave **213** as a white solid (0.81g, 96.43% yield) [m.p. 61.6-62.5°C; R_f 0.84, diethyl ether/petroleum spirit 40-60°C (20:80); lit. m.p. 61-63°C (Morand, 1964)].

$\nu_{(max)}$(Film) cm^{-1}: 2953.01 (CH), 1603.14 (C=O), 1559.90 (Ar C=C), 1486.30 (CH). $\delta_H(CDCl_3)$: 8.00 (2H, d, J=8.60Hz, Ph-H), 7.61 (2H, d, J=8.60Hz, Ph-H), 7.55 (2H, d, J=8.24Hz, Ph-H), 7.40 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.69Hz, Ph-H), 3.68 (1H, quin, J_{AB}=7.87Hz, J_{AB}=8.06Hz, J_{AB}=7.69Hz, O=C-CH), 1.83-1.93 (4H, m, O=C-CHCH_2, cyclo), 1.64-1.72 (4H, m, CH_2, cyclo); $\delta_C(CDCl_3)$: 202.52 (C=O), 145.48, 140.08, 135.70, 129.17, 129.04, 128.76, 128.24, 127.36 (Ar-C), 46.50 (O=C-CH), 30.12 (O=C-CHCH_2, cyclo), 26.44 (CH_2, cyclo); GC: t_R 12.05 min; LRMS (EI): 250 (M^+, 15%), 181 (M^+-C_5H_9); Elemental analysis: Found C, 86.34%; H, 7.25%; $C_{18}H_{18}O$ requires C, 86.36%; H, 7.25%.

1-Biphenyl-4-yl-cyclohexyl-methanone (214)

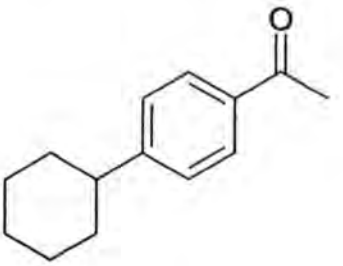


Compound **214** was synthesised in a similar manner to **202** except that cyclohexanecarbonyl chloride (0.87mL, 11.7mmol) was used instead of acetyl chloride and biphenyl (0.51g, 10.6mol) was used. The organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash chromatography of the crude solid gave **214** as an off-white solid (0.52g, 57.14% yield) [m.p. 85.2-85.6°C; R_f 0.92 diethyl ether/petroleum spirit 40-60°C (50:50); lit. m.p. 85-86°C (Morand and Samad, 1977)].

$\nu_{(max)}$(Film) cm^{-1}: 2928.73 (CH), 1676.42 (C=O), 1448.25 (CH); $\delta_H(CDCl_3)$: 8.02 (2H, d, J=8.79Hz, Ph-H), 7.67 (2H, d, J=8.60Hz, Ph-H), 7.62 (2H, d, J=7.51Hz, Ph-H), 7.47 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.69Hz, Ph-H), 3.29 (1H, t, J_{AB}=11.35Hz, J_{AB}=11.17Hz, O=C-CH), 1.81-2.01 (2H, m, O=C-CHCH_2, cyclo), 1.69-1.76 (4H, m, CH_2, cyclo), 1.35-1.57 (4H, m, CH_2, cyclo); $\delta_C(CDCl_3)$: 203.60 (C=O), 145.51, 135.08, 133.61, 129.04, 128.97, 128.25, 127.35 (Ar-C), 51.84 (O=C-CH), 45.78 (O=C-CHCH_2, cyclo), 35.59 (CHCH_2CH_2, cyclo), 29.56 (O=C-CHCH_2, cyclo), 26.08 (CH_2, cyclo), 25.99 (CH_2); GC: t_R 12.87min; LRMS (EI): 264 (M^+, 17%), 181 (M^+-C_6H_{11}, 100%).

3.1.2 Synthesis of 1-(4-cyclohexyl-phenyl)-ketones

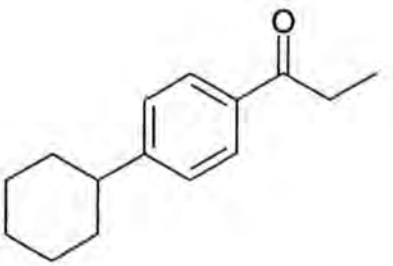
1-(4-Cyclohexyl-phenyl)-ethanone (215)



Acetyl chloride (0.44mL, 21mmol) was added in a drop wise manner to a solution of aluminium chloride (0.86g, 21mmol) in anhydrous DCM (100mL). The slurry was left to stir for 30min after which, cyclohexyl-benzene (0.51g, 10.6mmol) was added. The reaction mixture was then left to stir for 14h. The reaction was quenched using a mixture of ice and water (150mL) and washed with aqueous 0.5M HCl (2 X 50mL), H_2O (1 X 100mL), saturated $NaHCO_3$ solution (1 X 50mL) and H_2O (2 X 100mL). The combined organic layer was collected and dried over anhydrous $MgSO_4$. The mixture was then filtered, and the solvent was removed *in vacuo* to give **215** as a pale yellow solid (0.62g, 96.88% yield) [m.p. 67.6-68.1°C; R_f 0.64 diethyl ether/petroleum ether 40-60°C (30:70); lit.m.p. 67-69°C (Mowry et al, 1946)].

$\nu_{(max)}$(Film) cm^{-1}: 2923.25 (CH), 1670.91 (C=O), 1605.85 (Ar C=C); $\delta_H(CDCl_3)$: 7.88 (2H, d, J=8.42Hz, Ph-H), 7.28 (2H, t, J_{AB}=8.24Hz, J_{AB}=9.34Hz, Ph-H), 2.57 (3H, s, CH_3), 1.84-1.86 (1H, m, CH), 1.34-1.48 [4H, m, $(CH_2)_2$, cyclo], 1.21-1.31 [6H, m, $(CH_2)_3$, cyclo]; $\delta_C(CDCl_3)$: 197.67 (C=O), 153.87, 135.12, 128.64, 127.14 (ArC), 44.77 (CH), 34.20 (CH_2, cyclo), 26.81 (CH_2, cyclo), 26.66 (CH_2), 26.12 (CH_3); GC: t_R 9.39min; LRMS (EI): 202 (M^+, 2%), 91 (M^+-$C_7H_{11}O$, 100%); Elemental analysis: Found C, 83.10%; H, 8.95%; $C_{14}H_{18}O$ requires C, 83.12%; H, 8.97%.

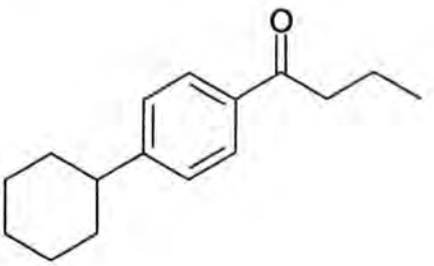
1-(4-Cyclohexyl-phenyl)-propan-1-one (216)



Compound **216** was synthesised in a similar manner to **215** except that propionyl chloride (0.54mL, 11.7mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.53g, 3.30mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give **216** as a pale yellow solid (0.70g, 97.00% yield) [m.p. 45.8-46.2°C; R_f 0.22 petroleum ether 40-60°C (100%); lit.m.p. 46-48°C (Lavagnino and White, 1970)].

$\nu_{(max)}$(Film) cm^{-1}: 2924.78 (CH), 1684.72 (C=O), 1606.37 (Ar C=C); $\delta_H(CDCl_3)$: 7.88 (2H, d, J=8.24Hz, Ph-H), 7.27 (2H, t, J_{AB}=8.42Hz, J_{AB}=6.41Hz, Ph-H), 2.97 (2H, q, J_{AB}=7.32Hz, J_{AB}=7.14Hz, CH_2), 2.52-2.54 (1H, m, CH), 1.79-1.86 [4H, m, $(CH_2)_2$, cyclo], 1.34-1.47 [6H, m, $(CH_2)_3$, cyclo], 1.20 (3H, t, J_{AB}=7.14Hz, J_{AB}=7.87Hz, CH_2CH_3); $\delta_C(CDCl_3)$: 200.69 (C=O), 153.58, 134.87, 128.29, 127.11 (ArC), 44.76 (CH), 34.21 (CH_2, cyclo), 31.76 (O=C-CH_2), 26.81 (CH_2, cyclo), 26.12 (CH_2, cyclo), 8.44 (CH_3); GC: t_R 8.39min; LRMS (EI): 216 (M^+, 2%), 187 (M^+-C_2H_5, 100%); Elemental analysis: Found C, 82.99%; H, 9.33%; $C_{15}H_{20}O$ requires C, 83.29%; H, 9.32%.

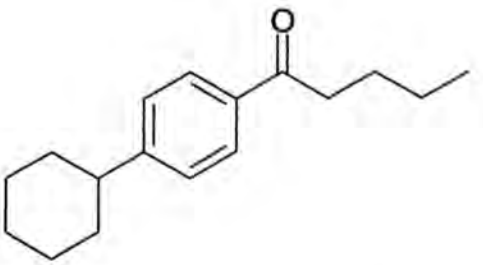
1-(4-Cyclohexyl-phenyl)-butan-1-one (217)



Compound **217** was synthesised in a similar manner to **215** except that butyryl chloride (0.65mL, 11.7mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.51g, 3.18mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give **217** as a pale yellow solid (0.71g, 97.26% yield) [m.p. 39.8-40.8°C; R_f 0.23 petroleum ether 40-60°C (100%); lit.m.p. 41°C (Chang, 1934)].

$\nu_{(max)}$(Film) cm^{-1}: 2926.51 (CH), 1681.85 (C=O), 1605.54 (Ar C=C); $\delta_H(CDCl_3)$: 7.88 (2H, d, J=7.87Hz, Ph-H), 7.27 (2H, t, J_{AB}=8.42Hz, J_{AB}=6.04Hz, Ph-H), 2.91 (1H, t, J_{AB}=7.14Hz, J_{AB}=7.51Hz, CH), 2.45-2.61 (2H, m, O=C-CH_2), 1.71-1.80 [4H, m, $(CH_2)_2$, cyclo], 1.36-1.47 (2H, m, CH_2CH_3), 1.23-1.30 [6H, m, $(CH_2)_3$, cyclo], 0.99 (3H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, CH_3); $\delta_C(CDCl_3)$: 200.27 (C=O), 153.58, 135.06, 128.36, 127.10 (ArC), 44.76 (O=C-CH_2), 40.52 (CH), 34.21 (CH_2, cyclo), 26.82 (CH_2, cyclo), 26.12 (CH_2, cyclo), 17.98 (CH_2CH_3), 14.03 (CH_3); GC: t_R 22.19min; LRMS (EI): 230 (M^+, 5%), 187 (M^+-C_3H_7, 100%); Elemental analysis: Found C, 84.45%; H, 9.60%; $C_{16}H_{22}O$ requires C, 84.43%; H, 9.63%.

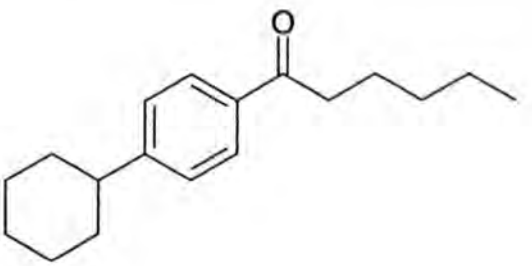
1-(4-Cyclohexyl-phenyl)-pentan-1-one (218)



Compound **218** was synthesised in a similar manner to **215** except that pentanoyl chloride (0.76mL, 11.7mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.50g, 3.12mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give **218** as an off-white solid (0.72g, 94.74% yield) [m.p. 48.5-49.2°C; R_f 0.23 petroleum ether 40-60°C (100%)].

$\nu_{(max)}$(Film) cm^{-1}: 2926.00 (CH), 1683.58 (C=O), 1604.91 (Ar C=C); δ_H($CDCl_3$): 7.90 (2H, d, J=8.24Hz, Ph-H), 7.29 (2H, t, J_{AB}=8.42Hz, J_{AB}=5.31Hz, Ph-H), 2.95 (1H, t, J_{AB}=7.32Hz, J_{AB}=7.69Hz, CH), 2.51-2.58 (2H, m, O=C-CH_2), 1.81-1.94 [4H, m, $(CH_2)_2$, cyclo], 1.68-1.79 [6H, m, $(CH_2)_3$, cyclo], 1.24-1.33 [4H, m, $(CH_2)_2CH_3$], 0.95 (3H, t, J=7.32Hz, CH_3); δ_C($CDCl_3$): 200.43 (C=O), 153.58, 135.04, 128.38, 127.10 (ArC), 44.76 (CH), 38.34 (CH_2, cyclo), 34.21 (CH_2), 26.82 (CH_2, cyclo), 26.70 (CH_2, cyclo), 26.13 (CH_2, cyclo), 22.62 (CH_2CH_3), 14.06 (CH_3); GC: t_R 11.13min; LRMS (EI): 244 (M^+, 1%), 187 (M^+-C_4H_9, 100%); Elemental analysis: Found C, 83.66%; H, 9.81%; $C_{17}H_{24}O$ requires C, 83.55%; H, 9.90%.

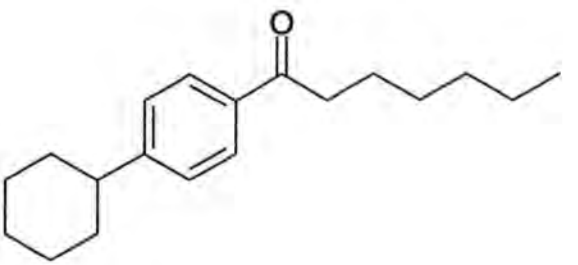
1-(4-Cyclohexyl-phenyl)-hexan-1-one (219)



Compound **219** was synthesised in a similar manner to **215** except that hexanoyl chloride (0.97mL, 11.7mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.50g, 3.12mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give **219** as a yellow solid (0.78g, 97.50% yield) [m.p. 44.9-45.3°C; R_f 0.18 petroleum ether 40-60°C (100%); lit.m.p. 39-40°C (Beger and Thielemann, 1981)].

$\nu_{(max)}$(Film) cm^{-1}: 2926.41 (CH), 1683.73 (C=O), 1605.52 (Ar C=C); $\delta_H(CDCl_3)$: 7.87 (2H, d, J=8.24Hz, Ph-H), 7.27 (2H, t, J_{AB}=8.60Hz, J_{AB}=5.68Hz, Ph-H), 2.92 (1H, t, J=7.51Hz, CH), 2.51-2.59 (2H, m, O=C-CH_2), 1.82-1.94 [4H, m, $(CH_2)_2$, cyclo], 1.67-1.79 [6H, m, $(CH_2)_3$, cyclo], 1.38-1.43 (2H, m, O=C-CH_2CH_2), 1.35 [4H, m, $(CH_2)_2CH_3$], 0.90 (3H, t, J=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 200.43 (C=O), 153.57, 135.05, 128.37, 127.10 (ArC), 44.76 (O=C-CH_2), 38.58 (CH_2, cyclo), 34.21 [$(CH_2)_2$, cyclo], 31.69 (CH_2, cyclo), 29.19 (CH_2, cyclo), 26.82 (CH_2, cyclo), 26.13 (CH_2, cyclo), 24.29 (O=C-CH_2CH_2), 22.64 (CH_2), 14.07 (CH_3); GC: t_R 22.19min; LRMS (EI): 258 (M^+, 8%), 187 (M^+-C_5H_{11}, 100%); Elemental analysis: Found C, 83.80%; H, 10.22%; $C_{18}H_{26}O$ requires C, 83.67%; H, 10.14%).

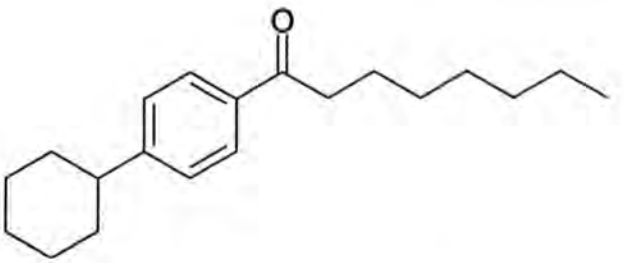
1-(4-Cyclohexyl)-phenyl)-heptan-1-one (220)



Compound **220** was synthesised in a similar manner to **215** except that heptanoyl chloride (3.23mL, 21.73mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.51g, 3.18mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give **220** as an off-white solid (0.80g, 91.95% yield) [m.p. 42.1-42.4°C; R_f 0.28 petroleum ether 40-60°C (100%)].

$\nu_{(max)}$(Film) cm^{-1}: 2925.83 (CH), 1682.81 (C=O), 1605.79 (Ar C=C); $\delta_H(CDCl_3)$: 7.82 (2H, d, J=8.42Hz, Ph-H), 7.21 (2H, d, J=8.06Hz, Ph-H), 2.86 (1H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, CH), 2.44-2.53 (2H, m, O=C-CH_2), 1.73-1.85 (4H, m, CH_2, cyclo), 1.61-1.72 (6H, m, CH_2, cyclo), 1.28-1.36 (2H, m, O=C-CH_2CH_2), 1.20-1.25 [6H, m, $(CH_2)_3CH_3$], 0.82 (3H, t, J=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 200.49 (C=O), 153.18, 135.03, 128.37, 127.10 (ArC), 44.75 (O=C-CH_2), 38.63 (CH_2, cyclo), 34.21 (CH_2, cyclo), 31.78 ($CH_2CH_2CH_3$), 29.19 (CH_2, cyclo), 26.82 (CH_2, cyclo), 26.12 (CH_2, cyclo), 24.56 ($CH_2CH_2CH_3$), 22.64 (CH_2CH_3), 14.16 (CH_3); GC: t_R 11.77min; LRMS (EI): 272 (M^+, 1%), 187 (M^+-C_6H_{13}, 100%).

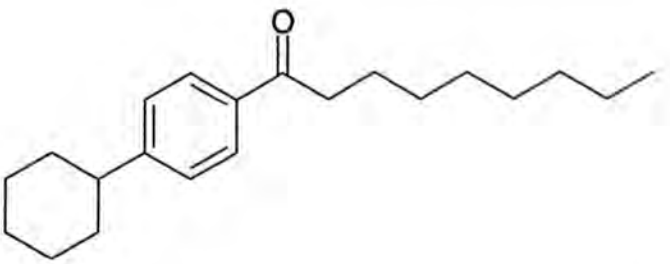
1-(4-Cyclohexyl)-phenyl)-octan-1-one (221)



Compound **221** was synthesised in a similar manner to **215** except that octanoyl chloride (3.58mL, 21mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.86g, 5.38mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give a crude solid. Recrystallisation of the crude solid from methanol gave **221** as a white solid (0.48g, 53.93% yield) [m.p. 52.2-52.6°C; R_f 0.23 petroleum ether 40-60°C (100%)].

$\nu_{(max)}$(Film) cm^{-1}: 2921.51 (CH), 1682.21 (C=O), 1605.49 (Ar C=C); $\delta_H(CDCl_3)$: 7.82 (2H, d, J=8.42Hz, Ph-H), 7.21 (2H, d, J=8.06Hz, Ph-H), 2.86 (1H, t, J_{AB}=7.32Hz, J_{AB}=7.69Hz, CH), 2.45-2.52 (2H, m, O=C-CH_2), 1.73-1.85 [4H, m, $(CH_2)_2$, cyclo], 1.61-1.71 [6H, m, $(CH_2)_3$, cyclo], 1.31-1.38 (2H, m, O=C-CH_2CH_2), 1.24-1.30 [6H, m, $(CH_2)_3CH_3$], 1.18-1.23 (2H, m, CH_2CH_3), 0.82 (3H, t, J=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 200.43 (C=O), 145.09, 135.05, 128.37, (ArC), 44.76 (O=C-CH_2), 38.63 (O=C-CH_2CH_2), 34.21 (CH_2, cyclo), 31.82 (CH_2, cyclo), 29.47 (CH_2, cyclo), 29.25 (CH_2, cyclo), 26.82 (CH_2), 26.13 (CH_2), 24.60 [$(CH_2)_2$], 22.73 (CH_2CH_3), 14.19 (CH_3); GC: t_R 22.43min; LRMS (EI): 286 (M^+, 4%), 187 (M^+-C_7H_{15}, 100%).

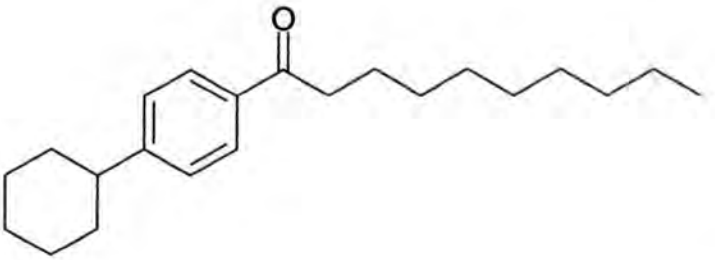
1-(4-Cyclohexyl)-phenyl)-nonan-1-one (222)



Compound **222** was synthesised in a similar manner to **215** except that nonanoyl chloride (1.17mL, 6.49mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.51g, 3.18mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give a crude solid. Recrystallisation of the crude solid from methanol gave **222** as a white solid (0.36g, 40.45% yield) [m.p. 45.8-46.2°C; R_f 0.17 petroleum ether 40-60°C (100%)].

$\nu_{(max)}$(Film) cm^{-1}: 2924.91 (CH), 1682.54 (C=O), 1605.98 (Ar C=C); $\delta_H(CDCl_3)$: 7.87 (2H, d, J=8.42Hz, Ph-H), 7.26 (2H, d, J=8.24Hz, Ph-H), 2.91 (1H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, CH), 2.50-2.57 (2H, m, O=C-CH_2), 1.79-1.91 [4H, m, $(CH_2)_2$, cyclo], 1.66-1.76 [6H, m, $(CH_2)_3$, cyclo], 1.36-1.41 (2H, m, O=C-CH_2CH_2), 1.28-1.36 (2H, m, O=C-$(CH_2)_2CH_2$), 1.19-1.29 [8H, m, $(CH_2)_4CH_3$], 0.86 (3H, t, J_{AB}=6.77Hz, J_{AB}=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 200.43 (C=O), 153.56, 135.05, 128.37, 127.09 (ArC), 44.76 (O=C-CH_2), 38.63 (O=C-CH_2CH_2), 34.69 [$(CH_2)_2$, cyclo], 34.20 (CH_2, cyclo), 31.94 (O=C-$(CH_2)_2CH_2$), 29.55 (CH_2, cyclo), 29.53 (CH_2, cyclo), 29.28 (CH_2, cyclo), 26.82 (CH_2, cyclo), 26.13 (CH_2, cyclo), 24.60 ($CH_2CH_2CH_3$), 22.76 (CH_2CH_3), 14.20 (CH_3); GC: t_R 13.36min; LRMS (EI): 300 (M^+, 2%), 202 (M^+-C_7H_{14}, 100%).

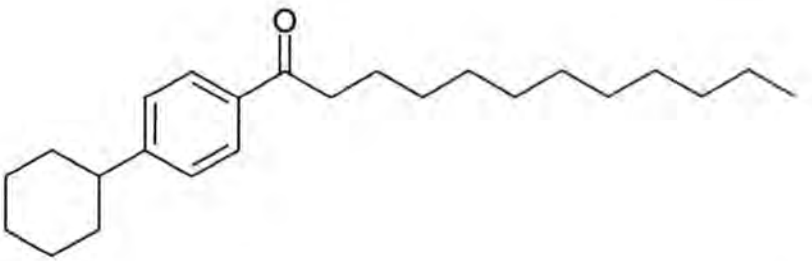
1-(4-Cyclohexyl)-phenyl)-decan-1-one (223)



Compound **223** was synthesised in a similar manner to **215** except that decanoyl chloride (1.29mL, 6.76mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.50g, 3.13mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give a crude solid. Recrystallisation of the crude solid from methanol gave **223** as a pale brown solid (0.42g, 42.86% yield) [m.p. 54.2-54.7°C; R_f 0.15 petroleum ether 40-60°C (100%); lit.m.p. 20°C (Buu-Hoi et al, 1945)].

$\nu_{(max)}$(Film) cm^{-1}: 2920.85 (CH), 1678.76 (C=O), 1605.54 (Ar C=C); $\delta_H(CDCl_3)$: 7.82 (2H, d, J=8.42Hz, Ph-H), 7.21 (2H, d, J=8.24Hz, Ph-H), 2.86 (1H, t, J_{AB}=7.32Hz, J_{AB}=7.51Hz, CH), 2.45-2.52 (2H, m, O=C-CH_2), 1.75-1.84 [4H, m, $(CH_2)_2$, cyclo], 1.61-1.71 [6H, m, $(CH_2)_3$, cyclo], 1.31-135 (2H, m, O=C-CH_2CH_2), 1.24-1.30 (2H, m, O=C-$(CH_2)_2CH_2$), 1.12-1.22 [10H, m, $(CH_2)_5CH_3$], 0.80 (3H, t, J_{AB}=6.77Hz, J_{AB}=6.96Hz, CH_3); $\delta_C(CDCl_3)$: 200.49 (C=O), 153.44, 135.03, 128.37, 127.09 (ArC), 44.75 (O=C-CH_2), 38.63 (O=C-CH_2CH_2), 34.21 (CH_2, cyclo), 31.97 (O=C-$(CH_2)_2CH_2$), 29.60 [$CH_2(CH_2)_4CH_3$], 29.51 (CH_2), 29.57 (CH_2, cyclo), 29.38 (CH_2, cyclo), 26.82 (CH_2, cyclo), 26.13 (CH_2, cyclo), 24.60 ($CH_2CH_2CH_3$), 22.77 (CH_2CH_3), 14.21 (CH_3); GC: t_R 14.30min; LRMS (EI): 314 (M^+, 5%), 202 (M^+-C_8H_{16}, 100%).

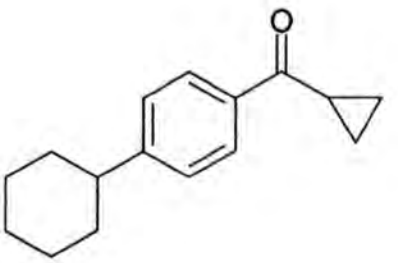
1-(4-Cyclohexyl)-phenyl)-dodecan-1-one (224)



Compound **224** was synthesised in a similar manner to **215** except that dodecanoyl chloride (4.98mL, 22.8mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.86g, 5.38mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give a crude solid. Recrystallisation of the crude solid from methanol gave **224** as a pale yellow solid (0.35g, 19.02% yield) [m.p. 61.8-62.3°C; R_f 0.77 diethyl ether/petroleum ether 40-60°C (10:90); lit.m.p. 54°C (Buu-Hoi et al, 1945)].

$\nu_{(max)}$(Film) cm^{-1}: 2917.67 (CH), 1679.30 (C=O), 1605.20 (Ar C=C); δ_H($CDCl_3$): 7.93 (2H, d, J=8.42Hz, Ph-H), 7.29 (2H, d, J=8.24Hz, Ph-H), 2.61-2.68 (1H, m, CH), 2.52-2.58 (2H, m, O=C-CH_2), 1.82-1.91 [4H, m, $(CH_2)_2$, cyclo], 1.74-1.78 [6H, m, $(CH_2)_3$, cyclo], 1.71-1.74 (2H, m, O=C-CH_2CH_2), 1.34-1.47 [2H, m, O=C-$(CH_2)_2CH_2$], 1.23-1.30 (2H, m, CH_2), 1.18-1.22 [12H, m, $(CH_2)_6CH_3$], 1.00 (3H, t, J=7.14Hz CH_3); δ_C($CDCl_3$): 200.23 (C=O), 153.18, 135.80, 128.31, 127.07 (ArC), 59.61 (O=C-CH_2), 44.76 (O=C-CH_2CH_2), 34.23 (CH_2, cyclo), 26.83 (CH_2, cyclo), 26.14 (CH_2, cyclo), 17.05 (CH_2), 11.50 (CH_3); GC: t_R 16.90min; LRMS (EI): 342 (M^+, 3%), 202 (M^+-$C_{10}H_{20}$, 100%).

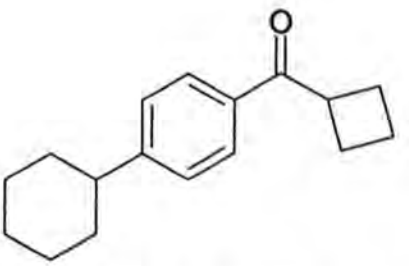
(4-Cyclohexyl-phenyl)-cyclopropyl-methanone (225)



Compound **225** was synthesised in a similar manner to **215** except that cyclopropane carbonyl chloride (0.34mL, 3.76mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.50g, 3.13mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give a crude solid. Recrystallisation of the crude solid from methanol gave **225** as a white solid (0.47g, 66.20% yield) [m.p. 89.0-89.5°C; R_f 0.40 diethyl ether/petroleum ether 40-60°C (10:90)].

$\nu_{(max)}$(Film) cm^{-1}: 2921.37 (CH), 1661.91 (C=O), 1605.39 (Ar C=C); $\delta_H(CDCl_3)$: 7.87 (2H, d, J=8.42Hz, Ph-H), 7.26 (2H, d, J=8.24Hz, Ph-H), 2.91 (1H, t, J_{AB}=7.32Hz, J_{AB}=7.69Hz, Ph-H), 2.50-2.57 (1H, m, O=C-CH), 1.32-1.41 (2H, m, CH_2, cyclo), 0.86 (2H, t, J_{AB}=6.59Hz, J_{AB}=7.14Hz, CH_2, cyclo); $\delta_C(CDCl_3)$: 200.43 (C=O), 135.05, 134.17, 128.38, 127.09 (ArC), 44.76 (Ar-CH), 38.63 (CH_2, cyclo), 34.21 (CH_2, cyclo), 32.00 (CH_2, cyclo), 29.60 (CH_2, cyclo), 26.82 (CH_2, cyclo), 24.61 (CH_2, cyclo), 19.16 (CH_2, cyclo), 11.50 (CH_2); GC: t_R 19.07min; LRMS (EI): 228 (M^+, 33%), 187 (M^+-C_3H_5, 100%).

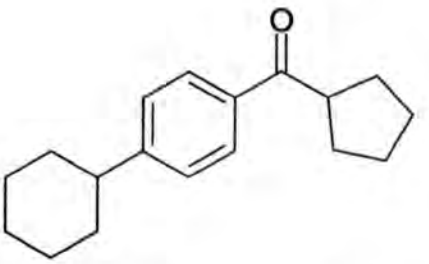
Cyclobutyl-(4-cyclohexyl-phenyl)-methanone (226)



Compound **226** was synthesised in a similar manner to **215** except that cyclobutane carbonyl chloride (2.3mL, 20.10mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.50g, 3.13mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give a crude solid. Recrystallisation of the crude solid from absolute alcohol gave **226** as a crystalline pale brown solid (0.18g, 23.68% yield) [m.p. 59.5-59.8°C; R_f 0.57 diethyl ether/petroleum ether 40-60°C (10:90)].

$\nu_{(max)}$(Film) cm^{-1}: 2926.08 (CH), 1676.21 (C=O), 1605.21 (Ar C=C); $\delta_H(CDCl_3)$: 7.83 (2H, d, J=8.42Hz, Ph-H), 7.27 (2H, d, J=8.60Hz, Ph-H), 3.98 (1H, quin, J_{AB}=8.60Hz, J_{AB}=8.42Hz, Ph-H), 2.51-2.58 (1H, m, O=C-CH), 2.37-2.46 (2H, m, CH_2, cyclo), 2.26-2.30 (2H, m, CH_2, cyclo), 2.02-2.13 (2H, m, CH_2, cyclo), 1.80-1.96 (1H, m, CH, cyclo), 1.64 (2H, s, CH_2, cyclo), 1.35-1.48 (2H, m, CH_2, cyclo), 1.21-1.32 (2H, m, CH_2, cyclo); $\delta_C(CDCl_3)$: 200.76 (C=O), 153.52, 133.57, 128.62, 127.11 (ArC), 44.77 (O=C-CH_2), 42.22 (O=C-CH), 34.20 [$(CH_2)_2$, cyclo], 26.82 (CH_2, cyclo), 26.13 (CH_2, cyclo), 25.21 (CH_2, cyclo), 18.27 (CH_2, cyclo); GC: t_R 10.99min; LRMS (EI): 242 (M^+, 7%), 187 (M^+-C_4H_7, 100%).

(4-Cyclohexyl-phenyl)-cyclopentyl-methanone (227)



Compound **227** was synthesised in a similar manner to **215** except that cyclopentane carbonyl chloride (2.5mL, 20.08mmol) was used instead of acetyl chloride and cyclohexyl-benzene (0.50g, 3.13mmol) was used. The mixture was then filtered, and the solvent was removed *in vacuo* to give a crude solid. Recrystallisation of the crude solid from absolute alcohol gave **227** as a crystalline off-white solid (0.21g, 26.25% yield) [m.p. 75.1-75.6°C; R_f 0.65 diethyl ether/petroleum ether 40-60°C (10:90)].

$\nu_{(max)}$(Film) cm^{-1}: 2925.99 (CH), 1671.28 (C=O), 1604.12 (Ar C=C); $\delta_H(CDCl_3)$: 7.89 (2H, d, J=8.42Hz, Ph-H), 7.27 (2H, d, J=8.60Hz, Ph-H), 3.68 (1H, quin, J=7.87Hz, Ph-H), 2.50-2.57 (1H, m, O=C-CH), 1.82-1.92 (2H, m, CH_2, cyclo), 1.68-1.74 (2H, m, CH_2, cyclo), 1.59-1.66 (2H, m, CH_2, cyclo), 1.21-1.30 (2H, m, CH_2, cyclo); $\delta_C(CDCl_3)$: 199.46 (C=O), 153.38, 134.85, 128.76, 127.05 (ArC), 46.33 (O=C-CH), 44.74 (CH_2, cyclo), 34.21 (CH_2, cyclo), 30.14 (CH_2, cyclo), 26.83 (CH_2, cyclo), 26.41 (CH_2, cyclo), 26.14 (CH_2, cyclo); GC: t_R 11.59min; LRMS (EI): 256 (M^+, 1%), 187 (M^+-C_5H_9, 100%).

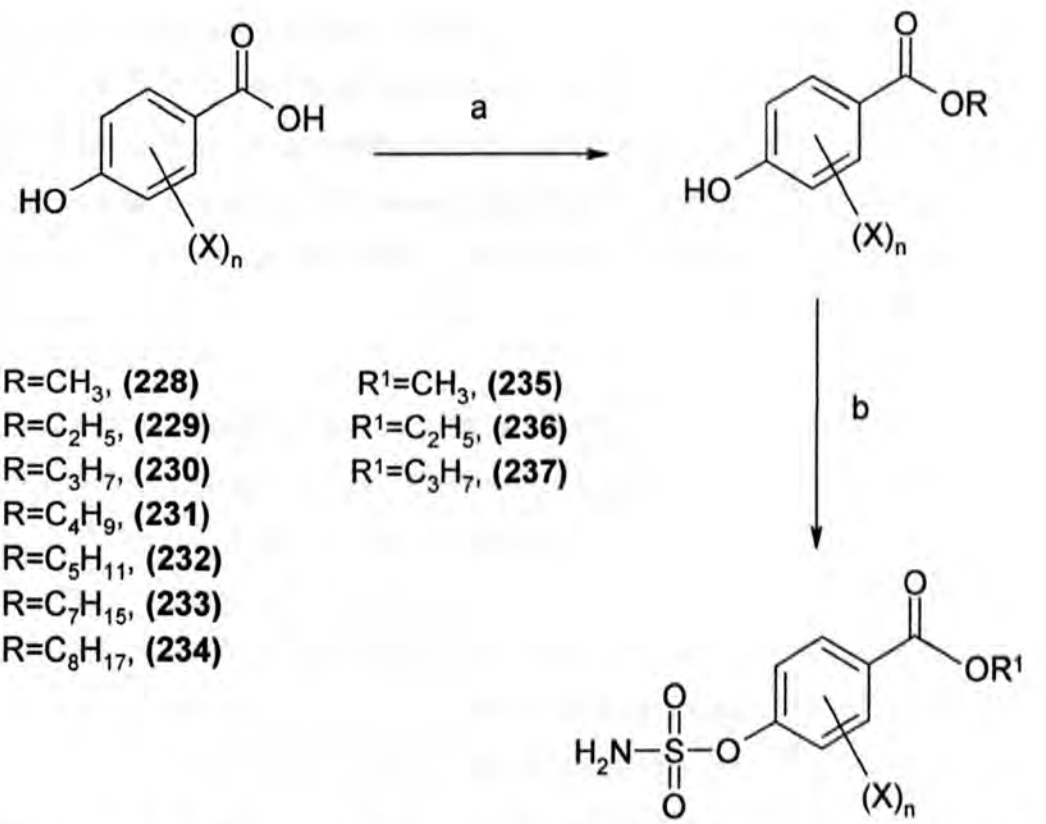
CHAPTER 4

Attempted synthesis of sulfamate derivatives of 4-hydroxybenzoic acid esters

4.0 Attempted synthesis of sulfamate derivatives of 4-hydroxybenzoic acid esters.

4.1 Discussion

As previously mentioned, the sulfamate moiety appears to possess potent inhibitory activity when attached to a phenyl ring system. More specifically, James (2000) has shown that 4-sulfamoylated derivatives of benzoic acid possess potent inhibition of ES. Furthermore, it was suggested that increasing the stability of the phenoxide ion may potentially lead to potent inhibitors of ES. In an attempt to increase the potency of inhibitors based on the benzoic acid backbone, we considered the synthesis of mono- and di-brominated derivatives, as such, in the synthesis of the sulfamate derivatives of the esters of 4-hydroxybenzoic acid, the reactions outlined in Scheme 4.1 were utilised:



R=CH_3, **(228)**
R=C_2H_5, **(229)**
R=C_3H_7, **(230)**
R=C_4H_9, **(231)**
R=C_5H_{11}, **(232)**
R=C_7H_{15}, **(233)**
R=C_8H_{17}, **(234)**

R^1=CH_3, **(235)**
R^1=C_2H_5, **(236)**
R^1=C_3H_7, **(237)**

Scheme 4.1: Synthesis of the sulfamate derivatives of esters of 4-hydroxybenzoic acid (a=ROH/H^+/Δ; b=sulfamoyl chloride/DMA; X=H or Br; n=0, 1 or 2; R= alkyl and cycoalkyl moiety).

The synthesis of the 4-hydroxybenzoate esters involved refluxing 4-hydroxybenzoic acid with the appropriate alcohol (in slight excess) and a few drops of concentrated sulfuric acid as a catalyst. The reaction was refluxed for 6h, and the work up involved addition of 2M NaOH, to neutralise the acidic mixture. In general, the reactions proceeded in good to poor yields ranging from 46% (for compound **228**) to 11% (for compound **234**) - the percentage yield appeared to decrease within increasing alkyl chain length. With the di-brominated esters, the reactions again proceeded in excellent to extremely poor yield ranging from 82% (for compound **243**) to 4% (for compound **255**), however, unlike the non-brominated esters, no clear trend is visible. Finally, with the mono-brominated esters, the reactions again proceeded in good to poor yield ranging from 57% (for compound **259**) to 12% (for compound **264**) – in general, an overall decrease in yield is observed with increasing alkyl chain length.

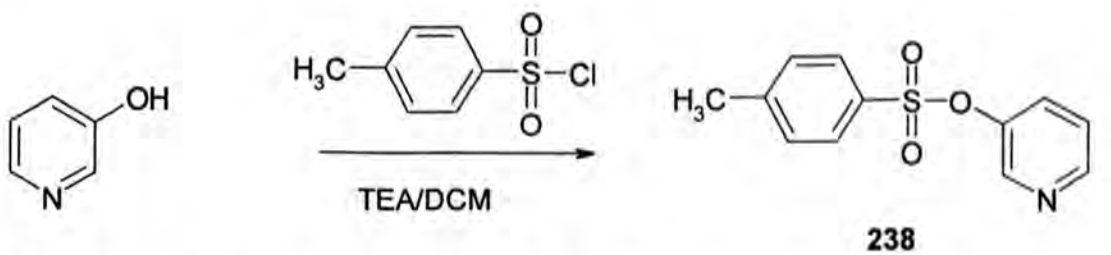
A problem was, however, encountered with the removal of the excess alcohol since the longer alkyl chain containing alcohols were found to possess high boiling points and therefore could not be easily distilled. This resulted in low yielding reactions, for example the 4% yield obtained for compound **255**, was mainly due to the attempts made to purify the compound. The higher yielding products, e.g. compound **243**, were obtained without the use of column chromatography.

The 4-hydroxybenzoate esters were then aminosulfonated using the method previously discussed for the synthesis of the sulfamated derivatives of 4-hydroxy ketones. That is, the various esters of 4-hydroxybenzoic acid were stirred in the presence of freshly prepared aminosulfonyl chloride solution using DMA as the solvent (Chapter 2; Scheme 2.2). However, in the synthesis of the compounds based on 4-hydroxybenzoic acid, it was found that the aminosulfonated products were difficult to isolate due mainly to the lack of stability of the product. Indeed we discovered that the products underwent rapid hydrolysis to give back the 4-hydroxybenzoic acid ester and the aminosulfonated derivative. This proved to be a major problem since any column chromatography was found to enhance hydrolysis. Furthermore, it

was discovered that the compounds could not be stored, even at 0°C. The instability of the products made it impossible to isolate any product, let alone for spectral analysis. In a study by Cartledge (2004), it was discovered that the brominated derivatives did indeed stabilise the phenoxide ion. That is, the pK_a values for a range of substituted and non-substituted derivatives of esters of 4-hydroxy benzoic acid were studied using a UV spectroscopic technique. Cartledge discovered that the mono- and di-brominated compounds were highly acidic in nature when compared to the non-brominated derivatives. James (2000) had previously reported that phenolic based inhibitors containing electron withdrawing groups undergo self-hydrolysis. As such, we propose that the mono- and di-brominated derivatives undergo self-hydrolysis, and therefore, the reaction scheme was abandoned for the bromo derivatives. The sulfamated derivatives of 4-hydroxybenzoic acid also proved to be a problem and only three compounds are reported. It was found that compounds with alkyl chain greater than pentyl underwent hydrolysis whilst the compounds were being purified using column chromatography (the remaining compounds were observed on thin layer chromatography but no GC-MS analysis were undertaken on the sulfamate derivatives due to the instability of the sulfamate group).

4.2 Synthesis of toluene-4-sulfonic acid pyridin-3-yl-ester

The *p*-toluene sulfonation of 3-hydroxypyridine using *p*-toluene sulfonyl chloride was also attempted (Scheme 4.2).

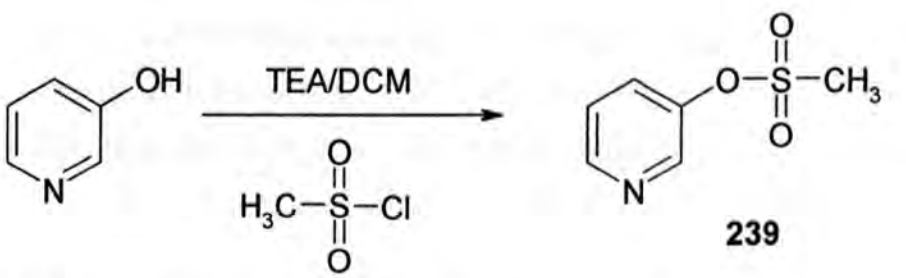


Scheme 4.2: Para-toluene sulfonation of 3-hydroxypyridine

This method was carried out by refluxing a stirring solution the reactants in DCM and TEA for 3h. Compound **238** was obtained in a satisfactory yield (66%).

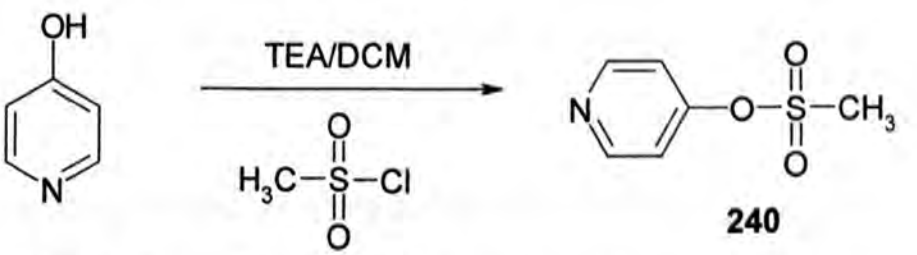
4.3 Methane sulfonation of the 4-hydroxy derivatives of phenyl azole and pyridine

Methane sulfonations of 4-hydroxyphenyl azole (Scheme 4.5) and the 3-substituted and 4-substituted hydroxypyridines were also carried out (Schemes 4.3 and 4.4).



Scheme 4.3: Methanesulfonation of 3-hydroxypyridine

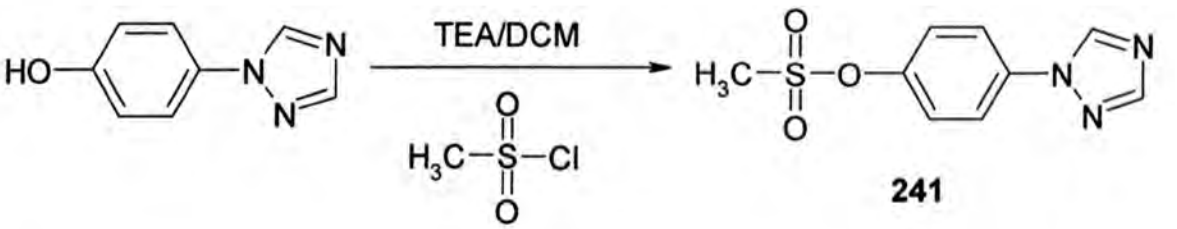
In the case of 3-hydroxy pyridine, this reaction was straight-forward. The two starting materials were commercially available and the reaction was left to reflux overnight, in triethylamine (TEA) and dichloromethane (DCM), as the solvents. An excellent yield for compound **239** was obtained (83%).



Scheme 4.4: Methanesulfonation of 4-hydroxypyridine

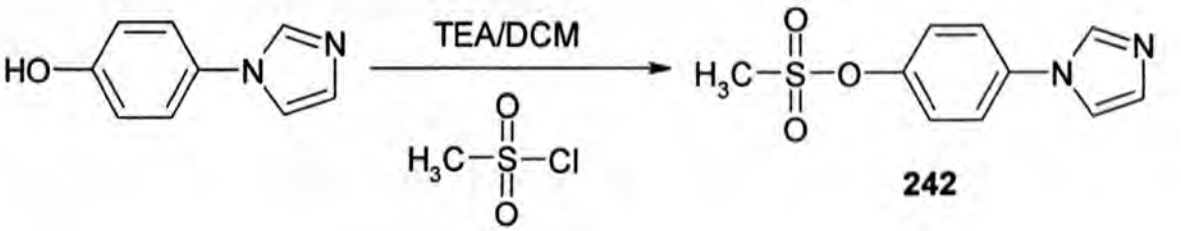
For the reaction with 4-hydroxypyridine, as shown in Scheme 4.4, the starting materials were once again commercially available but this time the reaction

was refluxed for 3h, in TEA and DCM and compound **240** was obtained with a yield of 63%



Scheme 4.5: Methanesulfonation of 4-hydroxyphenyltriazole

In the case of 4-hydroxyphenyl triazole, as shown in Scheme 4.5, the starting materials were commercially available and refluxed for 3h. This compound however, was difficult to work up, as it was forming a sticky residue in the flask. The yield obtained for compound **241** was 49%.



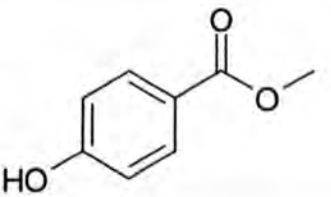
Scheme 4.6: Methanesulfonation of 4-hydroxyphenylimidazole

For the reaction shown in Scheme 4.6, the starting materials were also refluxed for 3h and in this case, the work up was slightly easier compared to the compounds shown in the schemes above. Compound **242** was obtained in an excellent yield of 74%.

In general, the problems encountered with these compounds was the fact that they were found to be problematic as to establish a satisfactory thin-layer chromatography system (TLC) and so the purity and analysis depended on the other available analytical techniques such as NMR and IR.

4.4 Synthesis of the 4-hydroxybenzoic acid alkyl esters

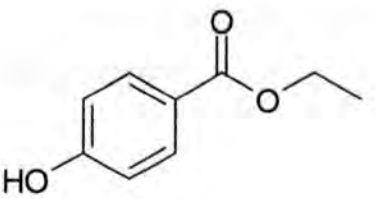
4-Hydroxy-benzoic acid methyl ester (228)



4-Hydroxy-benzoic acid (2.22g, 0.016mol), methanol (10mL) and concentrated sulfuric acid (H_2SO_4) (1mL) were dissolved in toluene (20mL), and the mixture was refluxed for 6h. After cooling to room temperature, the mixture was neutralised with 2M NaOH and the resulting mixture was allowed to stand for 15min, before being poured into a solution of ice/water. A precipitate was observed, which was collected and dried to give **228** as a white solid (1.12g, 45.71% yield) [m.p. 136.4-137.5°C); R_f 0.43 diethyl ether/petroleum ether 40-60°C (40:60); lit.m.p. 131-132°C (Inouye, H. et al, 1979)].

$\nu_{(max)}$(Film) cm^{-1}: 3263.6 (OH), 2360.8 (CH), 1688.2 (C=O), 1609.6, 1586.3, 1438.6 (Ar C=C), 1316.8 (C-H), 1282.7 (C-O); $\delta_H(CDCl_3)$: 8.01 (2H, d, J=9.00Hz, Ph-H), 7.25 (2H, d, J=9.00Hz, Ph-H), 6.9 (1H, s, OH), 3.85 (3H, s, OCH_3), $\delta_C(CDCl_3)$: 166.30 (C=O), 160.00 (Ph-C-O), 132.00, 122.00, 115.20 (Ar-C), 50.50 (OCH_3); GC: t_R 6.46min; LRMS (EI): 152 (M^+, 41%), 121 (M^+-OCH_3, 100%).

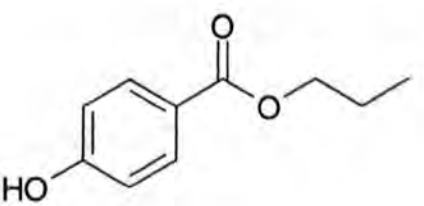
4-Hydroxy-benzoic acid ethyl ester (229)



Compound **229** was synthesised in the same way as **228** except 4-hydroxy-benzoic acid (2.11g, 0.015mol) and ethanol (10mL) was used instead of methanol. The precipitate was collected and dried to give **229** as a white solid (0.88g, 34.65% yield) [m.p. 117.2-117.7°C; R_f 0.23 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 117-118°C (Gutkowska and Biniecki, 1962)].

$\nu_{(max)}$(Film) cm^{-1}: 3221.20 (OH), 2927.90 (CH), 1674.80 (C=O), 1609.60, 1591.50, 1449.70, 1371.50 (Ar C=C), 1287.70 (C-O); $\delta_H(CDCl_3)$: 7.96 (2H, d, J=9.00Hz, Ph-H), 7.25 (2H, d, J=9.00Hz, Ph-H), 6.85 (1H, s, OH), 4.34 (2H, q, J=7.00Hz, OCH_2CH_3), 1.37 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 167.00 (C=O), 160.00 (Ar-C-O), 131.87, 115.12 (Ar-C), 60.78 (OCH_2), 14.34 (CH_3); GC: t_R 6.95 min; LRMS (EI): 166 (M^+, 25%), 121 (M^+-OC_2H_5, 100%).

4-Hydroxy-benzoic acid propyl ester (230)

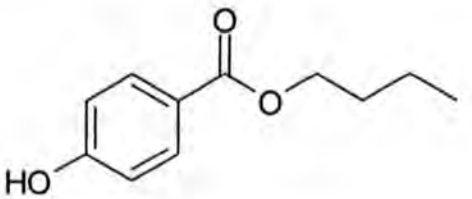


4-Hydroxy-benzoic acid (2.07g, 0.015mol), propan-1-ol (10mL) and concentrated sulfuric acid (H_2SO_4) (1mL) were dissolved in toluene (20mL), and the mixture was refluxed for 6h. After cooling to room temperature, the mixture was neutralised with 2M NaOH and the resulting mixture was allowed to stand for 15min, before being poured into a solution of ice/water and then

extracted into DCM (25mL). The organic phase was dried over anhydrous $MgSO_4$ and the mixture was filtered and removal of the solvent *in vacuo* gave **230** as a white solid (0.46g, 17.04% yield) [m.p. 109.2-109.9°C; R_f 0.47 diethyl ether/petroleum ether 40-60°C (40:60); Lit. m.p. 95-97°C (Blaug and Ahsan, 1961)].

$\nu_{(max)}$(Film) cm^{-1}: 3269.60 (OH), 2980.20 (CH), 1677.00 (C=O), 1606.70, 1588.20, 1439.90 (Ar C=C); $\delta_H(CDCl_3)$: 7.94 (2H, d, J=9.00Hz, Ph-H), 7.30 (2H, d, J=9.00Hz, Ph-H), 6.89 (1H, s, OH), 4.26 (2H, t, J=7.00Hz, OCH_2), 1.75-1.79 (2H, m, CH_2CH_3), 1.02 (3H, t, J=7.00Hz, CH_3), $\delta_C(CDCl_3)$: 167.46 (C=O), 160.61 (Ar-C-O), 131.90, 122.15, 115.31 (Ar-C), 66.68 (OCH_2), 22.04 (CH_2CH_3), 10.47 (CH_3); GC: t_R 7.64 min; 180 (M^+, 13%), 121 (M^+-OC_3H_7, 100%).

4-Hydroxy-benzoic acid butyl ester (231)

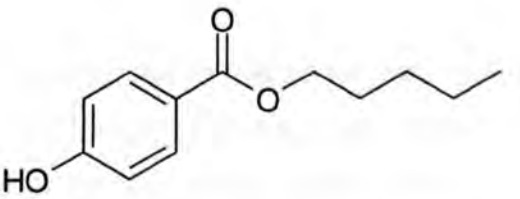


Compound **231** was synthesised in the same way as **230** except 4-hydroxy-benzoic acid (2.02g, 0.015mol), and butanol (10mL) was used instead of propan-1-ol. Removed of the solvent *in vacuo* gave **231** as a white solid (0.43g, 15.14% yield) [m.p. 80.2-81.9°C; R_f 0.51 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 73°C (Blaug and Ahsan, 1961)].

$\nu_{(max)}$(Film) cm^{-1}: 3356.50 (OH), 2960.30 (CH), 1683.70, 1608.60 (Ar C=C), 1444.70 (CH); $\delta_H(CDCl_3)$: 7.95 (2H, d, J=9.00Hz, Ph-H), 7.26 (2H, d, J=9.00Hz, Ph-H), 6.88 (1H, s, OH), 4.28 (2H, t, J=7.00Hz, OCH_2), 1.62-1.81 (2H, m, $CH_2CH_2CH_3$), 1.30-1.58 (2H, m, CH_2CH_3), 0.96 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 167.26 (C=O), 160.40 (Ar-C-O), 131.89, 122.38, 115.27 (Ar-

C), 64.92 (O$\underline{C}H_2$), 30.72 ($\underline{C}H_2CH_2CH_3$), 19.24 ($\underline{C}H_2CH_3$), 13.73 ($\underline{C}H_3$); GC: t_R 8.32 min; LRMS (EI): 194 (M^+, 11%), 138 (M^+-C_4H_8, 100%).

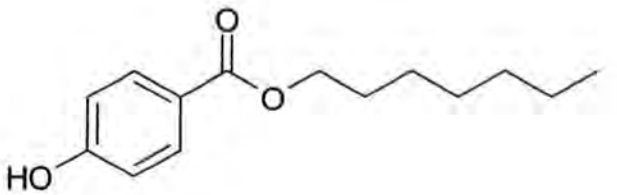
4-Hydroxy-benzoic acid pentyl ester (232)



Compound **232** was synthesised in the same way as **230** except 4-hydroxy-benzoic acid (2.01g, 0.015mol), and pentanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave **232** as a white solid (0.52g, 17.16% yield) [m.p. 53.4-55.1°C; R_f 0.56 diethyl ether/petroleum ether 40-60°C(40:60); lit. m.p. 54°C (De Leon et al, 1964)].

$\nu_{(max)}$(Film) cm^{-1}: 3344.30 (OH), 2958.20 (CH), 1683.80 (C=O), 1592.00, 1514.60, 1445.00 (Ar C=C); δ_H($CDCl_3$): 7.94 (2H, d, J=9.00Hz, Ph-$\underline{H}$), 7.26 (2H, d, J=9.00Hz, Ph-$\underline{H}$), 6.88 (1H, s, O$\underline{H}$), 4.27 (2H, t, J=7.00Hz, OC$\underline{H}_2$), 1.60-1.91 (2H, m, C$\underline{H}_2$), 1.21-1.52 [4H, m, (C$\underline{H}_2$)$_2$CH_3], 0.91 (3H, t, J=7.00Hz, C$\underline{H}_3$); δ_C($CDCl_3$): 167.23 ($\underline{C}$=O), 160.39 (Ar-$\underline{C}$-O), 131.89, 122.41, 115.27 (Ar-$\underline{C}$), 65.20 (O$\underline{C}H_2$), 28.38 (OCH$_2\underline{C}H_2$), 28.16 [O(CH$_2$)$_2\underline{C}H_2$], 22.33 ($\underline{C}H_2CH_3$), 13.96 ($\underline{C}H_3$); GC: t_R 8.97 min; LRMS (EI): 208 (M^+, 8%), 138 (M^+-C_5H_{10}, 100%).

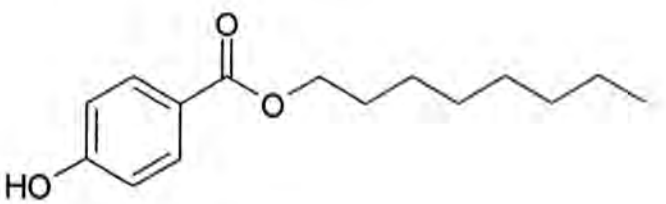
4-Hydroxy-benzoic acid heptyl ester (233)



Compound **233** was synthesised in the same way as **230** except 4-hydroxy-benzoic acid (2.05g, 0.015mol), and heptanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave **233** as a pale yellow solid (0.47g, 13.39% yield) [m.p. 56.8-57.3°C; R_f 0.33 diethyl ether/petroleum ether 40-60°C (30:70); lit.m.p. 55.6-57.9°C (Patel, M., 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3344.20 (OH), 2929.60 (CH), 1683.60 (C=O), 1591.90, 1514.80, 1445.30, 1312.10 (Ar C=C); δ_H($CDCl_3$): 7.94 (2H, d, J=9.00Hz, Ph-H), 6.89 (2H, d, J=9.00Hz, Ph-H), 6.69 (1H, s, OH), 4.29 (2H, t, J=7.00Hz, OCH_2), 1.61-1.92 (2H, m, OCH_2CH_2), 1.61-1.92 [8H, m, $(CH_2)_4CH_3$], 0.88 (3H, t, J=7.00Hz, CH_3); δ_C($CDCl_3$): 167.15 (C=O), 160.30 (Ar-C-O), 131.88, 122.49, 115.25 (Ar-C), 65.19 (OCH_2), 31.70 (OCH_2CH_2), 28.92 [$(CH_2)_2$], 28.69 (CH_2), 25.97 (CH_2), 22.56 (CH_2CH_3), 14.04 (CH_3); GC: t_R 10.11min; LRMS (EI): 236 (M^+, 5%), 138 (M^+-C_7H_{14}, 100%).

4-Hydroxy-benzoic acid octyl ester (234)



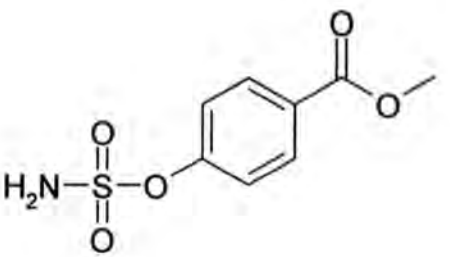
Compound **234** was synthesised in the same way as **230** except 4-hydroxy-benzoic acid (2.00g, 0.014mol), and octanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave **234**

as a yellow solid (0.38g, 10.50% yield) [m.p. 58.9-59.4°C; R_f 0.29 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 51.0-51.6°C (Olivier, 1937)].

$\nu_{(max)}$(Film) cm^{-1}: 3346.30 (OH), 2927.80 (CH), 1683.40 (C=O), 1608.80, 1591.70, 1514.80, 1445.00 (Ar C=C); $\delta_H(CDCl_3)$: 7.95 (2H, d, J=9.00Hz, Ph-H), 6.89 (2H, d, J=9.00Hz, Ph-H), 6.64 (1H, s, OH), 4.29 (2H, t, J=7.00Hz, OCH_2), 1.62-1.91 (2H, m, OCH_2CH_2), 1.13-1.49 [10H, m, $(CH_2)_5CH_3$], 0.87 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 167.14 (C=O), 160.27 (Ar-C-O), 131.89, 122.52, 115.25 (Ar-C), 65.19 (OCH_2), 31.77 (OCH_2CH_2), 29.22 [$CH_2(CH_2)_4CH_3$], 29.17 [$CH_2(CH_2)_3CH_3$], 28.69 (CH_2), 26.02 (CH_2), 22.62 (CH_2CH_3), 14.07 (CH_3); GC: t_R 10.70 min; LRMS (EI): 250 (M^+, 6%), 138 (M^+-C_8H_{16}, 100%).

4.5 Synthesis of the sulfamic acid 4-alkylphenyl esters

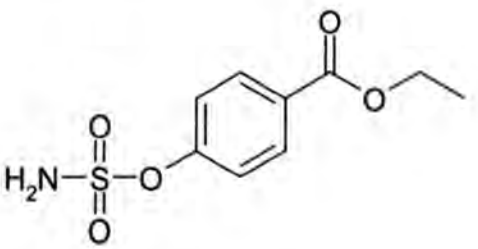
4-Sulfamoyloxy-benzoic acid methyl ester (235)



A solution of **190** in toluene (20mL, ~230 mmol) was added to a stirred mixture of **228** (0.62g, 4.08mmol) in DMA (0.6mL) at 0°C for 3h, after which, it was poured into saturated brine solution (2 x 50mL) and extracted into ethyl acetate (2 x 25mL). The organic phase was washed with brine (2 x 25mL) and dried over anhydrous $MgSO_4$. The mixture was filtered, and removal of the solvent *in vacuo* gave a crude solid. Flash column chromatography of the crude solid gave **235** as a white solid (0.21g, 22.34% yield) [m.p. 177.6-178.7°C; R_f 0.10 diethyl ether/petroleum ether 40-60°C (30:70); lit.m.p. 118-121°C (Patel et al, 2004)].

$\nu_{(max)}$(Film) cm^{-1}: 3424.10 (NH_2), 3204.10, 3424.10 (NH_2), 2810.00 (CH), 1673.80 (C=O), 1603.30, 1506.90, 1433.50 (Ar C=C), 1389.10, 1287.70 (S=O); δ_H(d_6-Acetone): 8.06 (2H, d, J=8.00Hz, Ph-H), 7.27 (2H, d, J=8.00Hz, Ph-H), 7.25 (2H, s, NH_2), 3.88 (3H, s, OCH_3); δ_C(d_6-Acetone): 166.90 (C=O), 156.01 (Ar-C-O), 131.44, 127.60, 122.55 (Ar-C), 52.1 (OCH_3).

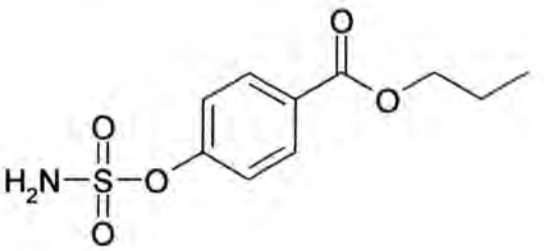
4-Sulfamoyloxy-benzoic acid ethyl ester (236)



Compound **236** was synthesised in the same way as **235** except **229** (0.60g, 3.61mmol) in DMA (0.6mL) was used. Removal of the solvent *in vacuo* gave a crude solid. Flash column chromatography of the crude solid gave **236** as a white solid (0.21g, 23.60% yield) [m.p. 98.6-99.3°C; R_f 0.13 diethyl ether/petroleum ether (40:60); lit. m.p. 83-86°C (Owen et al, 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3318.70, 3100.00 (NH_2), 2985.00 (CH), 1701.80 (C=O), 1601.80, 1501.20, 1453.30 (Ar C=C), 1369.90, 1282.60 (S=O); δ_H(d_6-Acetone): 8.07 (2H, d, J=8.00Hz, Ph-H), 7.45 (2H, d, J=8.00Hz, Ph-H), 7.25 (2H, s, NH_2), 4.30-4.38 (2H, m, CH_2CH_3), 1.32-1.37 (3H, m, CH_3); δ_C(d_6-Acetone): 166.00 (C=O), 132.10 (Ar-C-O), 131.90, 130.00, 123.04 (Ar-C), 61.90 (CH_2CH_3), 14.44 (CH_3).

4-Sulfamoyloxy-benzoic acid propyl ester (237)

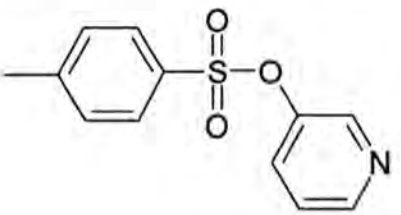


Compound **237** was synthesised in the same way as **235** except **230** (0.56g, 2.89mmol) in DMA (0.6mL) was used. Removal of the solvent *in vacuo* gave a crude solid. Flash column chromatography of the crude solid gave **237** as a white solid (0.10g, 12.66% yield) [m.p. 99.7-101.9°C; R_f 0.15 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 58-60°C (Owen et al, 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3430.10, 3347.10 (NH_2 stretch), 2969.90 (CH aliphatic stretch), 1715.70 (C=O), 1601.90, 1501.90, 1463.40 (Ar), 1388.10, 1277.60 (S=O), 1217.50 (C-O); $\delta_H(CDCl_3)$: 8.02 (2H, d, J=8.00Hz, Ph-H), 7.43 (2H, d, J=8.00Hz, Ph-H), 7.25 (2H, s, NH_2), 4.25 (2H, t, J=7.00Hz, OCH_2), 2.03-2.05 (2H, m, CH_2CH_3), 1.01 (3H, t, J=7.00Hz, CH_3) ; $\delta_C(CDCl_3)$: 166.00 (C=O), 155.10 (Ar-C-O), 132.00, 131.38, 129.90 (Ar-C), 67.00 (OCH_2), 22.50 (CH_2CH_3), 11.02 (CH_3).

4.6 Synthesis of toluene-4-sulfonic acid pyridin-3-yl-ester

Toluene-4-sulfonic acid pyridin-3-yl-ester (238)

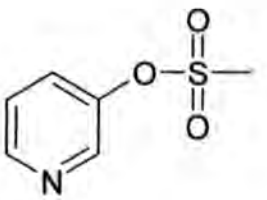


p-toluene sulfonyl chloride (2.4g, 13mmol) was added dropwise to a stirred solution of 3-hydroxypyridine (1g, 105mmol) in TEA (1.63mL, 169mmol), and anhydrous DCM (50mL) at room temperature and refluxed for 3h. After cooling down to room temperature, it was quenched in ice, washed with H_2O (3x20mL) and cold saturated Na_2CO_3 (3x20mL). The organic phase was washed again with H_2O (3x20mL) and then dried over anhydrous $MgSO_4$. The mixture was filtered and the solvent was removed *in vacuo* to give a crude solid. Flash column chromatography of the crude solid gave **238** as a shiny white crystalline solid (1.82g, 66% yield) [m.p. 98.7-99.3°C; R_f 0.11 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 80°C (Cavallito and Haskell, 1944)].

$\nu_{(max)}$(Film) cm^{-1}: 3430.10, 3347.10 (NH), 2969.90 (CH), 1715.70 (C=O), 1601.90, 1501.90, 1463.40 (Ar C=C), 1388.10, 1277.60 (S=O); δ_H(*d*$_6$-Acetone): 8.51 (2H, d, J=8.00Hz, Ar-H), 8.22 (2H, d, J=8.00Hz, Ar-H), 7.75 (2H, d, J=8.00Hz, Ph-H), 7.50 (2H, d, J=8.00Hz, Ph-H), 2.46 (3H, s, CH_3); δ_C(*d*$_6$-Acetone): 149.21, 147.43, 147.20 (Ar-C), 132.67, 131.06, 130.69, 129.32 (Ar-C), 125.32, 20.91; GC: t_R 10.14 min; LRMS (EI): 249 (M^+, 5%), 91 (M^+-$SO_3C_5H_4N$, 100%).

4.7 Synthesis of the methanesulfonic acid pyridin-*N*-yl esters

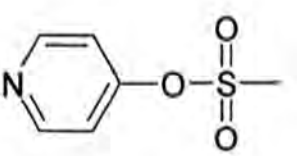
Methanesulfonic acid pyridin-3-yl ester (239)



Compound **239** was synthesized in the same way as **238**, except methane sulfonyl chloride (0.98mL, 128mmol), 3-hydroxypyridine (1.04g, 106mmol) and TEA (1.94mL, 117mmol) were used. The mixture was filtered and removal of the solvent *in vacuo* gave **239** as long crystalline white crystals (1.54g, 83% yield) [m.p. 69.7-72.9°C; R_f 0.39 ether (100%); lit. m.p. 59-60°C (Lyle and Boyle, 1974)].

$\nu_{(max)}$(Film) cm^{-1}: 3030.00 (C-N), 2922.10 (CH), 1597.90 (C=C), 1573.90 (C=N), 1479.70 (CH), 1428.30 (CH), 1377.40 (CH), 1201.60 (C-N), 1366.50, 1164.30 (S=O); $\delta_H(CDCl_3)$: 8.59 (1H, d, J=8.00Hz, C<u>H</u>), 7.70 (1H, d, J=8.00Hz, C<u>H</u>), 7.35-7.44 (1H, m, C<u>H</u>), 3.19 (3H, s, J=8.00Hz, C<u>H</u>$_3$); $\delta_C(CDCl_3)$: 148.05 (<u>C</u>-O), 143.21 (<u>C</u>H), 130.28 (<u>C</u>H), 124.63 (<u>C</u>H), 37.85 (<u>C</u>H$_3$); GC: t_R 5.98 min; LRMS (EI): 173 (M^+, 22%), 95 (M^+-SO_2CH_2, 100%).

Methanesulfonic acid pyridin-4-yl ester (240)

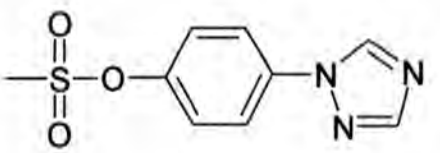


Compound **240** was synthesised in the same way as **238** except methane sulfonyl chloride (0.98mL, 128mmol), 4-hydroxypyridine (1.04g, 106mmol) and TEA (1.94mL, 117mmol) were used. The mixture was filtered and

removal of the solvent *in vacuo* gave **240** as a dark orange solid (1.20g, 63% yield) [m.p. 161.1-161.6°C; R_f 0.10 ether (100%)].

$\nu_{(max)}$(Film) cm^{-1}: 3410.30 (CH), 2360.80 (CH), 1622.70 (C=C), 1498.50 (CH), 1254.20 (C-O), 1197.00 (S=O); δ_H($CDCl_3$): 9.05 (2H, d, J=8.00Hz, Ph-H), 7.04 (2H, s, CH), 4.80 (1H, s, CH_3); δ_C($CDCl_3$): 144.15 (C-O-SO_2CH_3), 141.81 (C-N-CH), 120.72 (ArC), 37.8 (CH_3); GC: t_R 6.06 min; LRMS (EI): 173 (M^+, 90%), 95 (M^+-SO_2CH_2, 100%).

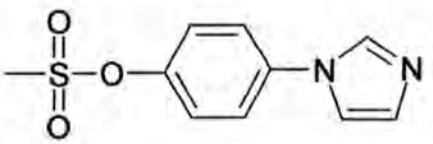
Methanesulfonic acid 4-[1,2,4]-triazol-1-yl-phenyl ester (241)



Compound **241** was synthesised in the same way as **238** except methane sulfonyl chloride (0.58mL, 128mmol), 4-hydroxy phenyl triazole (1.01g, 106mmol) and TEA (1.14mL, 117mmol) were used. The mixture was filtered and removal of the solvent *in vacuo* gave **241** as an off-white solid (0.74g, 49% yield) [m.p. 172.3-173.3°C; R_f 0.17 chloroform (100%)].

$\nu_{(max)}$(Film) cm^{-1}: 3410.00 (CH), 2360.90 (CH), 1517.60 (C=N), 1359.50 (CH), 1277.50 (C-O), 1180.30 (S=O); δ_H(d_6-methanol): 9.35 (1H, s, Ar-H), 8.28 (2H, s, Ph-H), 7.98 (2H, d, J=8.00Hz, Ph-H), 7.48-7.67 (2H, m, Ph-H), 3.49 (3H, s, CH_3); δ_C(d_6-methanol): 154.61 (CH), 144.65 (C-N), 137.10, 125.78 (CH), 123.13 (Ar), 38.8 (CH_3); GC: t_R 10.55 min; LRMS (EI): 239 (M^+, 57%), 160 (M^+-SO_2CH_3, 100%).

Methanesulfonic acid 4-imidazol-1-yl-phenyl ester (242)



Compound **242** was synthesised in the same way as **238** except methane sulfonyl chloride (0.58mL, 128mmol), 4-hydroxyphenyl imidazole (1.00g, 106mmol) and TEA (1.15mL, 117mmol) were used. The mixture was filtered and removal of the solvent *in vacuo* gave **242** as an off-white solid (1.11g, 74.39% yield) [m.p. 97.4-98.6°C; R_f 0.75 methanol (100%)].

$\nu_{(max)}$(Film) cm^{-1}: 3396.30 (C-N), 1515.30, 1364.00, 1156.40 (S=O), 1304.80 (CH); $\delta_H(CDCl_3)$: 8.25 (1H, s, C<u>H</u>, imidazole), 7.76 (2H, d, J=8.00Hz, C<u>H</u>), 7.61 (2H, s, C<u>H</u>-imidazole), 7.53 (2H, d, J=8.00Hz, Ph-<u>H</u>), 3.31 (3H, s, C<u>H</u>$_3$); $\delta_C(CDCl_3)$: 149.15 (Ar-<u>C</u>), 136.34 (Ar-<u>C</u>), 130.06 (Ar-<u>C</u>), 124.65, 123.44, 119.25 (Ar-<u>C</u>), 37.0 (<u>C</u>H$_3$); GC: t_R 11.05 min; LRMS (EI): 238 (M^+, 97%), 159 (M^+-SO_2CH_3, 100%).

4.8 Synthesis of derivatives of 3,5-dibromo-4-hydroxy-benzoic acid alkyl/cycloalkyl esters

3,5-Dibromo-4-hydroxy-benzoic acid methyl ester (243)

3,5-Dibromo-4-hydroxy-benzoic acid (1.02g, 3.45mmol), methanol (10mL) and concentrated H_2SO_4 (1mL) were dissolved in toluene (25mL), and the mixture

was refluxed for 6h. After cooling to room temperature, it was neutralised with 2M NaOH, and the resulting mixture was allowed to stand for 15min, before being poured into a solution of ice/water. A precipitate had formed, which was then filtered and dried to give **243** as a white solid (0.88g, 82.24% yield) [m.p. 124.2-125.8°C; R_f 0.45 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 104.3-106.0°C (Patel, M., 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3420.00 (OH), 1693.60 (C=O), 15300, 1431.50 (Ar C=C), 1272.50 (C-O); δ_H(d_6-Methanol): 8.00 (2H, s, Ph-H), 4.96 (3H, s, OCH_3); δ_C(d_6-Methanol): 164.40 (C=O), 155.10 (Ar-C-O), 133.10, 123.05, 110.90 (Ar-C), 51.72 (OCH_3); GC: t_R 8.95 min; LRMS (EI): 310 (M^+, 45%), 279 (M^+-OCH_3, 100%).

3,5-Dibromo-4-hydroxy-benzoic acid ethyl ester (244)

Compound **244** was synthesised in the same way as compound **243**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.00g, 3.39mmol), and ethanol (10mL) was used instead of methanol. The precipitate was filtered and dried to give **244** as a white solid (0.59g, 54.13% yield) [m.p. 101.7-102.3°C; R_f 0.53 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 104.1-105.6°C (Patel, M., 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3343.90 (OH), 2990.10 (CH), 1700.90 (C=O), 1588.00, 1553.20, 1472.10, 1469.70 (Ar C=C), 1299.30 (C-O); δ_H($CDCl_3$): 8.14 (2H, s, Ph-H), 6.29 (1H, s, OH), 4.36 (2H, q, J=8.00Hz, OCH_2CH_3), 1.38 (3H, J=7.00Hz, CH_3); δ_C($CDCl_3$): 164.08 (C=O), 153.05 (Ar-C-O), 133.57, 125.10,

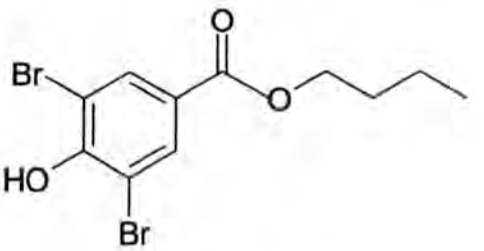
109.63 (Ar-C), 61.54 (OCH_2CH_3), 14.27 (CH_3); GC: t_R 9.38 min; LRMS (EI): 324 (M^+, 35%), 279 (M^+-OC_2H_5, 100%).

3,5-Dibromo-4-hydroxy-benzoic acid propyl ester (245)

3,5-Dibromo-4-hydroxy-benzoic acid (1.00g, 3.38mmol), propan-1-ol (10mL) and concentrated H_2SO_4 (1mL) were dissolved in toluene (25mL), and the mixture was refluxed for 6h. After cooling to room temperature, the mixture was neutralised with 2M NaOH and the resulting mixture was allowed to stand for 15min, before being poured into a solution of ice/water and then extracted into DCM (25mL). The organic phase was dried over anhydrous $MgSO_4$. The mixture was filtered and removal of the solvent *in vacuo* gave **245** as a white solid (0.61g, 53.51% yield) [m.p. 109.8-110.5°C; R_f 0.51 diethyl ether/petroleum ether 40-60°C(40:60); lit. m.p. 107.2-108.8°C (Patel, M., 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3306.00 (OH), 2966.40 (CH), 1699.00 (C=O), 1588.00, 1554.40, 1480.30, 1395.70 (Ar C=C), 1299.30 (C-O); δ_H($CDCl_3$): 8.14 (2H, s, Ph-H), 6.25 (1H, s, OH), 4.24 (2H, t, J=7.00Hz, OCH_2), 1.77 (2H, t, J=7.00Hz, CH_2CH_3), 1.01 (3H, t, J=7.00Hz, CH_3); δ_C($CDCl_3$): 164.01 (C=O), 153.03 (Ar-C-O), 133.56, 125.10, 109.63 (Ar-C), 67.10 ($CH_2CH_2CH_3$), 22.03 (CH_2CH_3), 10.47 (CH_3); GC: t_R 9.91 min; LRMS (EI): 338 (M^+, 16%), 296 (M^+-C_3H_6, 100%).

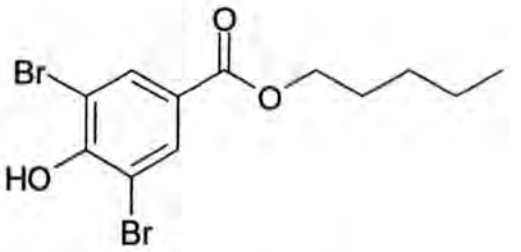
3,5-Dibromo-4-hydroxy-benzoic acid butyl ester (246)



Compound **246** was synthesised in the same way as compound **245**, except that 3,5-dibromo-4-hydroxy-benzoic acid (0.98g, 3.31mmol), and butanol (10mL) was used instead of propan-1-ol. Removal of the solvent *in vacuo* gave **246** as a white solid (0.87g, 74.36% yield) [m.p. 89.1-91.5°C; R_f 0.69 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 90.2-91.0°C (Patel, M., 2003)].

$\nu_{(max)}$(Film) cm^{-1}: 3309.60 (OH), 2958.10 (CH), 1701.40 (C=O), 1588.80, 1555.90, 1480.60, 1393.50 (Ar C=C), 1299.20 (C-O); $\delta_H(CDCl_3)$: 8.13 (2H, s, Ph-H), 6.27 (1H, s, OH), 4.28 (2H, t, J=7.00Hz, OCH_2), 1.65-1.80 (2H, m, $CH_2CH_2CH_3$), 1.38-1.51 (2H, m, CH_2CH_3), 0.97 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 164.04 (C=O), 153.03 (Ar-C-O), 133.55, 125.11, 109.63 (Ar-C), 65.42 (OCH_2), 30.68 ($CH_2CH_2CH_3$), 19.19 (CH_2CH_3), 13.72 (CH_3); GC: t_R 10.48min; LRMS (EI): 352 (M^+, 9%), 296 (M^+-C_4H_8, 100%).

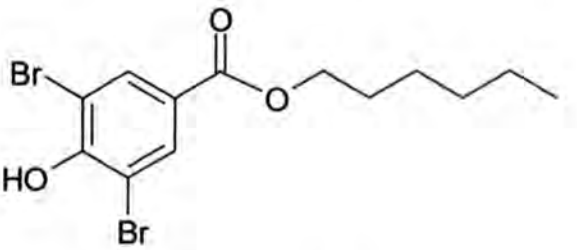
3,5-Dibromo-4-hydroxy-benzoic acid pentyl ester (247)



Compound **247** was synthesised in the same way as compound **245**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.01g, 3.41mmol), and pentanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave a crude oil. Flash column chromatography of the crude oil gave **247** as a pale yellow solid (0.82g, 65.60% yield) [m.p. 61.4-62.1°C; R_f 0.57 diethyl ether/petroleum ether 40-60°C (20:80)].

$\nu_{(max)}$(Film) cm^{-1}: 3377.40 (OH), 2957.90 (CH), 1704.40 (C=O), 1587.90, 1572.10, 1468.00, 1400.30 (Ar C=C), 1295.90 (C-O); $\delta_H(CDCl_3)$: 8.13 (2H, s, Ph-H), 6.30 (1H, s, OH), 4.27 (2H, t, J=7.00Hz, OCH_2), 1.72-180 [2H, m, $CH_2(CH_2)_2CH_3$], 1.22-1.54 [4H, m, $(CH_2)_2CH_3$], 0.92 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 164.04 (C=O), 153.03 (Ar-C-O), 133.56, 125.14, 109.63 (Ar-C), 65.71 (OCH_2), 28.34 [$CH_2(CH_2)_2CH_3$], 28.08 ($CH_2CH_2CH_3$), 22.32 (CH_2CH_3), 13.95 (CH_3); GC: t_R 11.07min; LRMS (EI): 366 (M^+, 10%), 296 (M^+-C_5H_{10}, 100%).

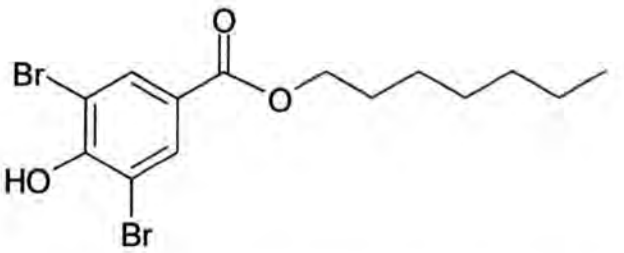
3,5-Dibromo-4-hydroxy-benzoic acid hexyl ester (248)



Compound **248** was synthesised in the same way as compound **245**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.03g, 3.48mmol), and hexanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave a crude oil. Flash column chromatography of the crude oil gave **248** as a yellow solid (0.93g, 70.45% yield) [m.p. 59.1-59.6°C; R_f 0.46 diethyl ether/petroleum ether 40-60°C (10:90)].

$\nu_{(max)}$(Film) cm^{-1}: 3374.20 (OH), 2930.10 (CH), 1703.00 (C=O), 1587.80, 1557.60, 1474.80, 1399.60 (Ar C=C), 1256.20 (C-O); $\delta_H(CDCl_3)$: 8.14 (2H, s, Ph-H), 6.33 (1H, s, OH), 4.28 (2H, t, J=7.00Hz, OCH_2), 1.71-1.90 (2H, m, $CH_2(CH_2)_3CH_3$), 1.22-1.49 [6H, m, $(CH_2)_4CH_3$], 0.90 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 164.05 (C=O), 153.05 (Ar-C-O), 133.55, 125.11, 109.64 (Ar-C), 65.73 [$CH_2(CH_2)_4CH_3$], 31.42 [$CH_2(CH_2)_3CH_3$], 28.39 [$CH_2(CH_2)_2CH_3$], 25.61 ($CH_2CH_2CH_3$), 22.51 (CH_2CH_3), 13.99 (CH_3); GC: t_R 11.66min; LRMS (EI): 380 (M^+, 9%), 296 (M^+-C_6H_{12}, 100%).

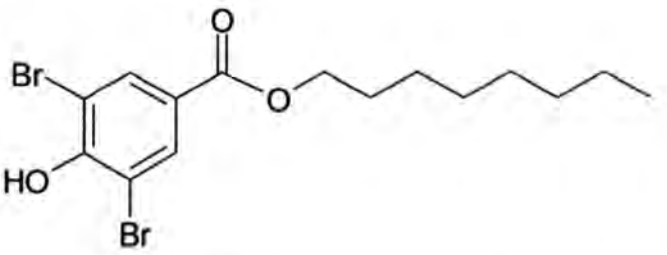
3,5-Dibromo-4-hydroxy-benzoic acid heptyl ester (249)



Compound **249** was synthesised in the same way as compound **245**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.00g, 3.38mmol), and heptanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave a crude oil. Flash column chromatography of the crude oil gave **249** as a pale yellow solid (0.82g, 61.65% yield) [m.p. 68.5-69.2°C; R_f 0.54 diethyl ether/petroleum ether 40-60°C (30:70)].

$\nu_{(max)}$(Film) cm^{-1}: 3386.00 (OH), 2928.20 (CH), 1702.20 (C=O), 1587.80, 1558.90, 1473.60, 1399.40 (Ar C=C), 1257.20 (C-O); $\delta_H(CDCl_3)$: 8.14 (2H, s, Ph-H), 6.28 (1H, s, OH), 4.30 (2H, t, J=7.00Hz, OCH_2), 1.70-1.91 (2H, m, OCH_2CH_2), 1.21-1.49 [8H, m, $(CH_2)_4CH_3$], 0.89 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 164.04 (C=O), 153.03 (Ar-C-O), 133.56, 125.14, 109.64 (Ar-C), 65.74 (OCH_2), 31.68 [$CH_2(CH_2)_2CH_3$], 28.91 (CH_2), 28.63 (CH_2), 25.91 ($CH_2CH_2CH_3$), 22.58 (CH_2CH_3), 14.05 (CH_3); GC: t_R 12.40min; LRMS (EI): 394 (M^+, 3%), 296 (M^+-C_7H_{14}, 100%).

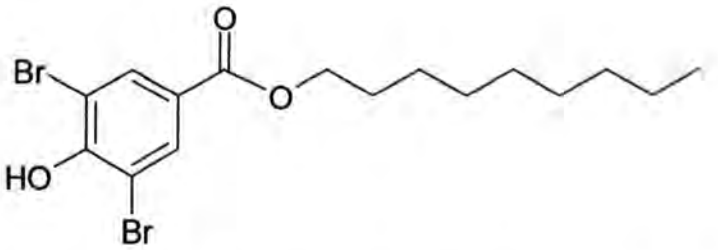
3,5-Dibromo-4-hydroxy-benzoic acid octyl ester (250)



Compound **250** was synthesised in the same way as compound **245**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.00g, 3.38mmol), and octanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Flash column chromatography of the crude oil gave **250** as a white solid (0.89g, 64.49% yield) [m.p. 65.1-66.2°C; R_f 0.50 diethyl ether/petroleum ether 40-60°C (20:80)].

$\nu_{(max)}$(Film) cm^{-1}: 3382.50 (OH), 2925.60 (CH), 1702.60 (C=O), 1587.80, 1558.70, 1472.70, 1399.60 (Ar C=C), 1256.90 (C-O); δ_H($CDCl_3$): 8.14 (2H, s, Ph-H), 6.28 (1H, s, OH), 4.30 (2H, t, J=7.00Hz, OCH_2), 1.69-1.83 (2H, m, OCH_2CH_2), 1.20-1.48 [10H, m, $(CH_2)_5CH_3$], 0.88 (3H, t, J=7.00Hz, CH_3); δ_C ($CDCl_3$): 164.05 (C=O), 153.04 (Ar-C-O), 133.56, 125.14, 109.64 (Ar-C), 65.74 (OCH_2), 31.77 (OCH_2CH_2), 29.19 [$O(CH_2)_2CH_2$], 28.15 (CH_2), 28.62 ($CH_2CH_2CH_2CH_3$), 25.95 ($CH_2CH_2CH_3$), 22.62 (CH_2CH_3), 14.08 (CH_3); GC: t_R 13.12min; LCMS (EI): 408 (M^+, 36%), 296 (M^+-C_8H_{16}, 100%).

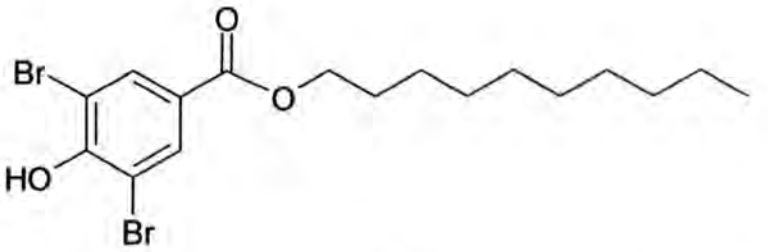
3,5-Dibromo-4-hydroxy-benzoic acid nonyl ester (251)



Compound **251** was synthesised in the same way as compound **245**, except that 3,5-dibromo-4-hydroxy-benzoic acid (0.96g, 3.24mmol), and nonanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Flash column chromatography of the crude oil gave **251** as a white solid (0.72g, 52.55% yield) [m.p. 60.7-61.2°C; R_f 0.57 diethyl ether/petroleum ether 40-60°C (10:90)].

$\nu_{(max)}$(Film) cm^{-1}: 3382.56 (OH), 2924.95 (CH), 1702.84 (C=O), 1467.52, 1399.49, 1295.40 (Ar), 1256.80 (C-O); $\delta_H(CDCl_3)$: 8.14 (2H, s, Ph-H), 6.27 (1H, s, OH), 4.30 (2H, t, J=7.00Hz, OCH_2), 1.67-1.79 (2H, m, OCH_2CH_2), 1.19-1.45 [12H, m, $(CH_2)_6CH_3$], 0.87 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 164.04 (C=O), 153.03 (Ar-C-O), 133.56, 125.14, 109.63 (Ar-C), 65.74 (OCH_2), 31.83 (OCH_2CH_2), 29.43 (CH_2), 29.24 [$CH_2(CH_2)_4CH_3$], 28.63 [$CH_2(CH_2)_3CH_3$], 25.94 ($CH_2CH_2CH_3$), 22.65 (CH_2CH_3), 14.09 (CH_3); GCMS: t_R 14.13min; LRMS (EI): 422 (M^+, 4%), 296 (M^+-C_9H_{18}, 100%).

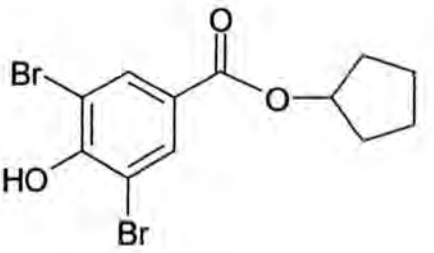
3,5-Dibromo-4-hydroxy-benzoic acid decyl ester (252)



Compound **252** was synthesised in the same way as compound **245**, except that 3,5-dibromo-4-hydroxy-benzoic acid (0.95g, 3.21mmol), and decanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Flash column chromatography of the crude oil gave **252** as a white solid (0.72g, 51.80% yield) [m.p. 64.8-65.9°C; R_f 0.52 diethyl ether/petroleum ether 40-60°C (10:90)].

$\nu_{(max)}$(Film) cm^{-1}: 3345.40 (OH), 2920.00 (CH), 1700.80 (C=O), 1589.50, 1554.20, 1481.30, 1394.60 (Ar C=C), 1264.20 (C-O); δ_H($CDCl_3$): 8.13 (2H, s, Ph-H), 6.29 (1H, s, OH), 4.27 (2H, t, J=7.00Hz, OCH_2), 1.69-1.82 (2H, m, OCH_2CH_2), 1.19-1.47 [14H, m, $CH_2(CH_2)_7CH_3$], 0.87 (3H, t, J=7.00Hz, CH_3); δ_C($CDCl_3$): 164.05 (C=O), 153.04 (Ar-C-O), 133.56, 125.14, 109.64 (Ar-C), 65.74 (OCH_2), 31.87 [$O(CH_2)CH_2$], 29.51 [$CH_2(CH_2)_5CH_3$], 29.28 (CH_2), 29.24 ($CH_2CH_2CH_2CH_3$), 25.94 ($CH_2CH_2CH_3$), 22.66 (CH_2CH_3), 14.10 (CH_3); GC: t_R 15.12min; LRMS (EI): 434 (M^+, 3%), 296 (M^+-$C_{10}H_{18}$, 100%).

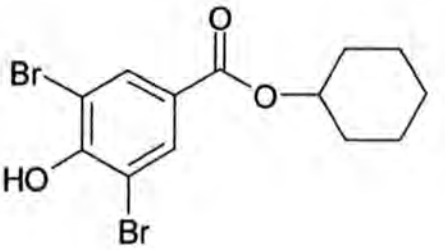
3,5-Dibromo-4-hydroxy-benzoic acid cyclopentyl ester (253)



Compound **253** was synthesised in the same way as compound **245**, except that 3,5-dibromo-4-hydroxy-benzoic acid (0.94g, 3.18mmol), and cyclopentanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Flash column chromatography of the crude oil gave **253** as pale brown crystals (0.68g, 58.62% yield) [m.p. 145.7-146.2°C; R_f 0.70 diethyl ether/petroleum ether 40-60°C (20:80)].

$\nu_{(max)}$(Film) cm^{-1}: 3335.70 (OH), 2966.50 (CH), 1701.90 (C=O), 1588.00, 1553.30, 1478.10, 1366.10 (Ar C=C), 1257.30 (C-O); $\delta_H(CDCl_3)$: 8.10 (2H, s, Ph-H), 6.25 (1H, s, OH), 5.28-5.45 (1H, m, OCH), 1.88-2.07 [8H, m, $(CH_2)_4$, cyclo]; $\delta_C(CDCl_3)$: 163.73 (C=O), 152.93 (Ar-C-O), 133.49, 125.51, 109.58 (Ar-C), 77.32 (OCH), 32.73 ($OCHCH_2$, cyclo), 23.79 (CH_2, cyclo); GC: LRMS (EI): t_R 11.52min; 364 (M^+, 5%), 279 (M^+-OC_5H_9, 100%).

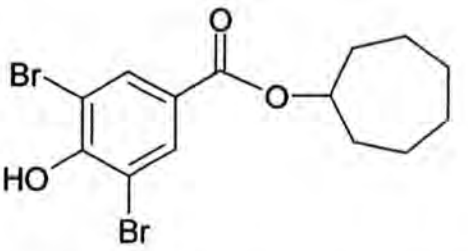
3,5-Dibromo-4-hydroxy-benzoic acid cyclohexyl ester (254)



Compound **254** was synthesised in the same way as compound **245**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.00g, 3.38mmol), and cyclohexanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Flash column chromatography of the crude oil gave **254** as a pale brown solid (0.68g, 53.13% yield) [m.p. 126.3-126.8°C; R_f 0.39 diethyl ether/ petroleum ether 40-60°C (20:80)].

$\nu_{(max)}$(Film) cm^{-1}: 3367.80 (OH), 2934.00 (CH), 1700.00 (C=O), 1587.70, 1478.50, 1450.80, 1399.70 (Ar C=C), 1253.80 (C-O); δ_H($CDCl_3$): 8.07 (2H, s, Ph-H), 6.25 (1H, s, OH), 4.88-4.99 (1H, m, OCH), 1.40-1.58 [10H, m, $(CH_2)_5$, cyclo]; δ_C($CDCl_3$): 162.14 (C=O), 151.72 (Ar-C-O), 132.30, 124.34, 108.36 (Ar-C), 72.73 (OCH), 30.38 ($CHCH_2$), 24.11 (CH_2, cyclo), 22.50 (CH_2, cyclo); GC: t_R 12.35min; LRMS (EI): 378 (M^+, 5%), 82 (M^+-$C_7H_4Br_2O_3$, 100%).

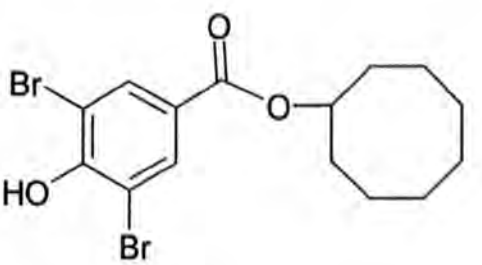
3,5-Dibromo-4-hydroxy-benzoic acid cycloheptyl ester (255)



Compound **255** was synthesised in the same way as compound **245** except that 3,5-dibromo-4-hydroxy-benzoic acid (1.06g, 3.58mmol), and cycloheptanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Flash column chromatography of the crude oil gave **255** as shiny white crystals (0.05g, 3.57% yield) [m.p. 120.2-121.4°C; R_f 0.36 diethyl ether/petroleum ether 40-60°C (20:80)].

$\nu_{(max)}$(Film) cm^{-1}: 3390.00 (OH), 2926.90 (CH), 1699.50 (C=O), 1601.00, 1570.00, 1475.30, 1295.60 (Ar C=C), 1258.50 (C-O); δ_H($CDCl_3$): 8.06 (2H, s, Ph-H), 6.25 (1H, s, OH), 5.11-5.25 (1H, m, OCH), 1.66-1.80 [12H, m, $(CH_2)_6$, cyclo]; δ_C($CDCl_3$): 163.10 (C=O), 153.00 (Ar-C-O), 133.51, 125.91, 109.57 (Ar-C), 33.80 ($OCHCH_2$), 28.27 (CH_2, cyclo), 22.85 (CH_2, cyclo); GC: t_R 13.69 min; LRMS (EI): 392 (M^+, 2%), 96 (M^+-$C_7H_4O_3Br_2$, 100%).

3,5-Dibromo-4-hydroxy-benzoic acid cyclooctyl ester (256)



Compound **256** was synthesised in the same way as compound **245**, except that 3,5-dibromo-4-hydroxy-benzoic acid (1.02g, 3.45mmol), and cyclooctanol

(10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave the crude as an oil. Flash column chromatography of the crude oil gave **256** as shiny white crystals (0.62g, 44.29%) [m.p. 153.6-154.2°C; R_f 0.62 diethyl ether/petroleum ether 40-60°C (20:80)].

$\nu_{(max)}$(Film) cm^{-1}: 3374.90 (OH), 2923.20 (CH), 1697.10 (C=O), 1587.40, 1557.40, 1475.10, 1399.60 (Ar C=C), 1255.70 (C-O); δ_H($CDCl_3$): 8.05 (2H, s, Ph-H), 6.19 (1H, s, OH), 5.01-5.18 (1H, m, OCH), 1.39-1.58 [14H, m, $(CH_2)_7$, cyclo]; δ_C($CDCl_3$): 163.10 (C=O), 152.01 (Ar-C-O), 133.51, 125.90, 109.57 (Ar-C), 31.50 ($OCHCH_2$), 27.09 (CH_2), 25.34 (CH_2, cyclo), 22.91 (CH_2, cyclo); GC: t_R 15.25 min; LCMS (EI): 406 (M^+, 7%), 82 (M^+-$C_{15}H_{17}O_3Br$, 100%).

4.9 Synthesis of 3-Bromo-4-hydroxy-benzoic acid alkyl/cycloalkyl esters

3-Bromo-4-hydroxy-benzoic acid methyl ester (257)

3-Bromo-4-hydroxy-benzoic acid (1.08g, 4.98mmol), methanol (10mL) and concentrated H_2SO_4 (1mL) were dissolved in toluene (25mL), and the mixture was refluxed for 6h. After cooling to room temperature, the mixture was neutralised with 2M NaOH and was allowed to stand for 15min, before being poured into a solution of ice/water. A precipitate was observed, which was then filtered and dried to give **257** as a white solid (0.37g, 32.17% yield) [m.p. 114.1-114.6°C; R_f 0.33 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 107-108°C (Cavill, 1945)].

$\nu_{(max)}$(Film) cm^{-1}: 3347.50 (OH), 1693.30 (C=O), 1601.00 (C=C), 1437.10 (CH), 1290.30 (C-O), 1193.80 (C-C), 1117.50 (C-C); δ_H($CDCl_3$): 8.19 (2H, d, J=9.00Hz, Ph-H), 7.91 (1H, d, J=9.00Hz, Ph-H), 7.05 (1H, d, J=8.00Hz, Ph-H), 6.02 (1H, s, OH), 3.91 (3H, s, OCH_3); δ_C($CDCl_3$): 165.61 (C=O), 156.18 (Ar-C), 133.93, 131.01, 124.01, 115.77 (Ar-C), 110.07 (C-Br), 52.20 (OCH_3); GC: t_R 6.73 min; LRMS (EI): 231 (M^+, 40%), 200 (M^+-OCH_3, 100%).

3-Bromo-4-hydroxy-benzoic acid ethyl ester (258)

Compound **258** was synthesised in a similar manner to **257** except 3-bromo-4-hydroxy-benzoic acid (1.11 g, 5.12mmol), and ethanol (10mL) was used instead of methanol. The precipitate was filtered and dried to give **257** as a white solid (0.67g, 53.60% yield) [m.p. 117.4-117.9°C; R_f 0.35 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 103°C (Meyer, 1901)].

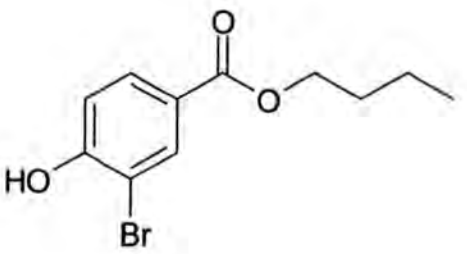
$\nu_{(max)}$(Film) cm^{-1}: 3236.00 (OH), 1676.70 (C=O), 1603.90 (C=C), 1423.50 (CH), 1394.30 (CH), 1369.90 (CH), 1292.50 (C-O), 1145.60 (C-C), 1045.10 (C-C); δ_H($CDCl_3$): 8.18 (2H, d, J=7.00Hz, Ph-H), 7.92 (1H, d, J=7.00Hz, Ph-H), 7.05 (1H, d, J=9.00Hz, Ph-H), 5.98 (1H, s, OH), 4.35 (2H, q, J=7.00Hz, OCH_2CH_3), 1.38 (3H, s, CH_2CH_3); δ_C($CDCl_3$): 164.85 (C=O), 156.06 (Ar-C), 133.85, 130.99, 115.72 (Ar-C), 110.03 (C-Br), 61.13 (OCH_2CH_3), 14.31 (CH_2CH_3); GC: t_R 7.48 min; LRMS (EI): 246 (M^+, 41%), 199 (M^+-OC_2H_7, 100%).

3-Bromo-4-hydroxy-benzoic acid propyl ester (259)

3-Bromo-4-hydroxy-benzoic acid (1.10 g, 5.07mmol), propan-1-ol (10mL) and concentrated H_2SO_4 (1mL) were dissolved in toluene (25mL), and the mixture was refluxed for 6h. After cooling to room temperature, the mixture was neutralised with 2M NaOH. It was then allowed to stand for 15min, before being poured into a solution of ice/water and then extracted into DCM (25mL). The organic phase was dried over anhydrous $MgSO_4$ and the mixture was filtered. Removal of the solvent *in vacuo* gave **259** as a white solid (0.69g, 52.67% yield) [m.p. 87.3-87.8°C; R_f 0.33 diethyl ether/petroleum ether 40-60°C (30:70); lit. m.p. 88-89°C (Hirai, 1957)].

$\nu_{(max)}$(Film) cm^{-1}: 3310.50 (OH), 2969.40 (CH), 1684.20 (C=O), 1601.20 (C=C), 1496.00 1391.50 (Ar C=C), 1286.90 (C-O), 1115.90 (C-C); δ_H($CDCl_3$): 8.17 (1H, d, J=9.00Hz, Ph-H), 7.92 (2H, d, J=9.00Hz, Ph-H), 7.04 (2H, d, J=7.00Hz, Ph-H), 6.0 (1H, s, OH), 4.25 (2H, t, J=7.00Hz, OCH_2), 1.73-1.82 (2H, m, CH_2CH_3), 1.01 (3H, t, J=7.00Hz, CH_2CH_3); δ_C($CDCl_3$): 165.11 (C=O), 155.39 (Ar-C), 133.82, 130.99, 115.72, 109.62 (Ar-C), 66.70 (OCH_2), 22.09 (CH_2CH_3), 10.49 (CH_2CH_3); GC: t_R 7.93 min; LRMS (EI): 258 (M^+, 8%), 199 (M^+-OC_3H_7, 100%).

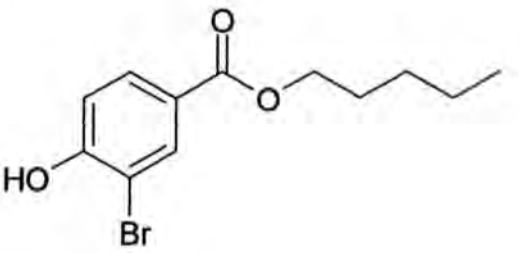
3-Bromo-4-hydroxy-benzoic acid butyl ester (260)



Compound **260** was synthesised in the same way as compound **259**, except that 3-bromo-4-hydroxy-benzoic acid (1.08g, 4.98mmol), and butanol (10mL) was used instead of propan-1-ol. Removal of the solvent *in vacuo* gave **260** as a white solid (0.77g, 56.62% yield) [m.p. 91.2-91.7°C; R_f 0.38 diethyl ether/petroleum ether 40-60°C (40:60); lit.m.p. 83-84°C (Cavill, 1945)].

$\nu_{(max)}$(Film) cm^{-1}: 3329.10 (OH), 2959.10 (CH), 1691.70 (C=O), 1602.10, 1508.20, 1424.10 (Ar), 1288.00 (C-O); $\delta_H(CDCl_3)$: 8.18 (1H, d, J=9.00Hz, Ph-H), 7.92 (1H, d, J=9.00Hz, Ph-H), 7.05 (1H, d, J=9.00Hz, Ph-H), 5.96 (1H, s, OH), 4.30 (2H, t, J=7.00Hz, OCH_2), 1.74 (2H, quin, J_{AB}=7.00Hz, J_{AB}=8.00Hz, $CH_2CH_2CH_3$), 1.46 (2H, sex, J_{AB}=7.00Hz, J_{AB}=8.00Hz, CH_2CH_3), 0.98 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 165.11 (C=O), 156.05 (Ar-C-O), 133.82, 130.98, 115.72 (Ar-C), 65.01 (OCH_2), 30.74 ($CH_2CH_2CH_3$), 19.24 (CH_2CH_3), 13.75 (CH_3); GC: t_R 8.61min; LRMS (EI): 272 (M^+, 8%), 216 (M^+-C_4H_8, 100%).

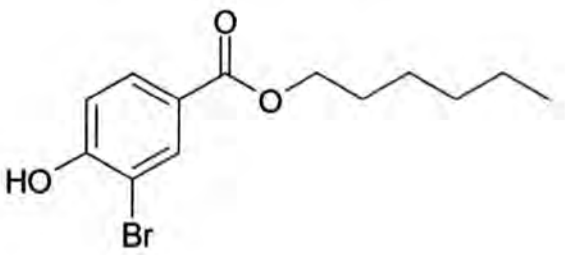
3-Bromo-4-hydroxy-benzoic acid pentyl ester (261)



Compound **261** was synthesised in the same way as compound **259**, except that 3-bromo-4-hydroxy-benzoic acid (1.17g, 5.39mmol), and pentanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave **261** as a white solid (0.63g, 40.65% yield) [m.p. 66.7-67.2°C; R_f 0.29 diethyl ether/petroleum ether 40-60°C (30:70); lit.m.p. 64-66°C (Hirai, 1957)].

$\nu_{(max)}$(Film) cm^{-1}: 3357.80 (OH), 2957.80 (CH), 1686.40 (C=O), 1600.90, 1415.10, 1410.10 (Ar), 1287.30 (C-O); $\delta_H(CDCl_3)$: 8.18 (1H, d, J=9.00Hz, Ph-H), 7.93 (1H, d, J=7.00Hz, Ph-H), 7.05 (1H, d, J=9.00Hz, Ph-H), 6.04 (1H, s, OH), 4.29 (2H, t, J=7.00Hz, OCH_2), 1.72-1.79 (2H, m, OCH_2CH_2), 1.42-1.51 (4H, m, CH_2CH_3), 0.93 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 165.21 (C=O), 156.09 (Ar-C-O), 133.85, 130.96, 124.40, 110.04 (Ar-C), 65.32 (OCH_2), 28.39 (OCH_2CH_2), 28.13 ($CH_2CH_2CH_3$), 22.33 (CH_2CH_3], 13.96 (CH_3); GC: t_R 9.29 min; LRMS (EI): 286 (M^+, 5%), 216 (M^+-C_5H_{10}, 100%).

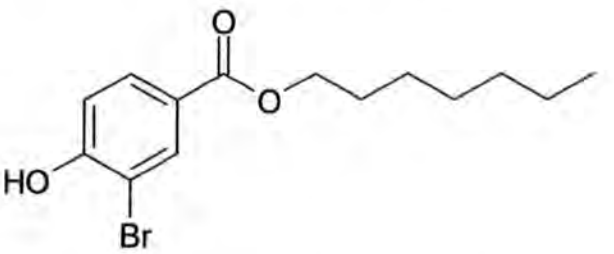
3-Bromo-4-hydroxy-benzoic acid hexyl ester (262)



Compound **262** was synthesised in the same way as compound **259**, except that 3-bromo-4-hydroxy-benzoic acid (1.06g, 4.88mmol), and hexanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave **262** as a pale yellow solid (0.67g, 45.58%) [m.p. 65.7-66.2°C; R_f 0.29 diethyl ether/petroleum ether 40-60°C (30:70); lit.m.p. 60-62°C (Hirai, 1957)].

$\nu_{(max)}$(Film) cm^{-1}: 3348.50 (OH), 2930.50 (CH), 1687.40 (C=O), 1494.60, 1467.50, 1414.20, 1390.00 (Ar), 1287.60 (C-O); $\delta_H(CDCl_3)$: 8.18 (1H, d, J=9.00Hz, Ph-H), 7.92 (1H, d, J=8.00Hz, Ph-H), 7.05 (1H, d, J=9.00Hz, Ph-H), 5.98 (1H, s, OH), 4.28 (2H, t, J=7.00Hz, OCH_2), 1.71-1.78 (2H, m, OCH_2CH_2), 1.29-1.37 [6H, m, $(CH_2)_3CH_3$], 0.91 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 165.10 (C=O), 156.14 (Ar-C-O), 133.83, 130.96, 115.72 (Ar-C), 65.32 (OCH_2), 31.44 (OCH_2CH_2), 28.64 [$(CH_2)_2CH_3$], 25.66 ($CH_2CH_2CH_3$), 22.52 (CH_2CH_3), 13.99 (CH_3); GC: t_R 9.87 min; LRMS (EI): 300 (M^+, 6%), 216 (M^+-C_6H_{12}, 100%).

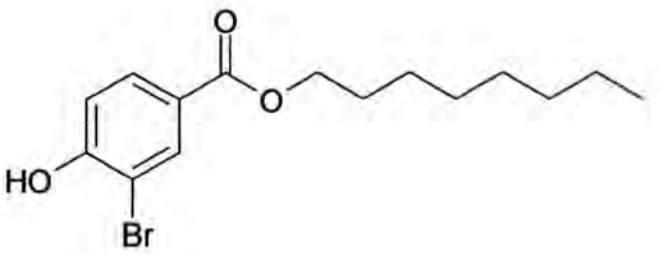
3-Bromo-4-hydroxy-benzoic acid heptyl ester (263)



Compound **263** was synthesised in the same way as compound **259**, except that 3-bromo-4-hydroxy-benzoic acid (1.01g, 4.65mmol), and heptanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave **263** as a pale yellow solid (0.54g, 36.73% yield) [m.p. 65.8-66.3°C; R_f 0.39 diethyl ether/petroleum ether 40-60°C (40:60); lit.m.p. 58-59°C (Hirai, 1957)].

$\nu_{(max)}$(Film) cm^{-1}: 3360.00 (OH), 2928.27 (CH), 1686.86 (C=O), 1495.58, 1413.17 (Ar), 1286.92 (C-O); $\delta_H(CDCl_3)$: 8.17 (1H, d, J=8.00Hz, Ph-H), 7.92 (1H, d, J=7.00Hz, Ph-H), 7.05 (2H, d, J=8.00Hz, Ph-H), 5.98 (1H, s, OH), 4.28 (2H, t, J=7.00Hz, OCH_2), 1.75 (2H, quin, J_{AB}=7.00Hz, J_{AB}=8.00Hz, OCH_2CH_2), 1.25-1.36 [8H, m, $(CH_2)_4CH_3$], 0.89 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 165.35 (C=O), 156.05 (Ar-C-O), 133.83, 124.19, 109.87 (Ar-C), 65.33 (OCH_2), 31.70 (OCH_2CH_2), 28.93 (CH_2), 28.60 (CH_2), 25.96 ($CH_2CH_2CH_3$), 22.58 (CH_2CH_3), 14.05 (CH_3); GC: t_R 10.39 min; LRMS (EI): 314 (M^+, 8%), 216 (M^+-C_7H_{14}, 100%).

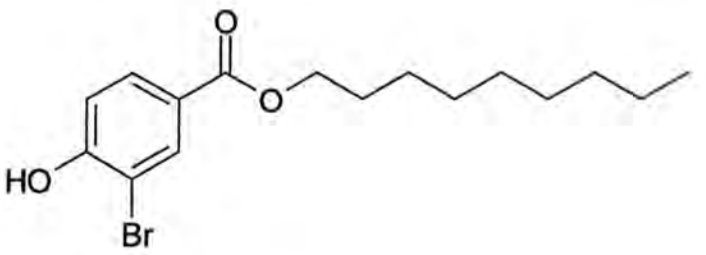
3-Bromo-4-hydroxy-benzoic acid octyl ester (264)



Compound **264** was synthesised in the same way as compound **259**, except that 3-bromo-4-hydroxy-benzoic acid (1.10g, 5.07mmol), and octanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave **264** as a white solid (0.63g, 37.72% yield) [m.p. 41.7-42.2°C; R_f 0.42 diethyl ether/petroleum ether 40-60°C (40:60); lit. m.p. 45-47°C (Hirai, 1957)].

$\nu_{(max)}$(Film) cm^{-1}: 3362.02 (OH), 2926.41 (CH), 1686.54 (C=O), 1600.37, 1412.94 (Ar), 1286.63 (C-O); δ_H($CDCl_3$): 8.18 (1H, d, J=8.00Hz, Ph-H), 7.92 (1H, d, J=7.00Hz, Ph-H), 7.04 (2H, d, J=7.00Hz, Ph-H), 5.98 (1H, s, OH), 4.28 (2H, t, J=7.00Hz, OCH_2), 1.75 (2H, quin, J_{AB}=9.00Hz, J_{AB}=8.00Hz, OCH_2CH_2), 1.25-1.36 [10H, m, $(CH_2)_5CH_3$], 0.88 (3H, t, J=7.00Hz, CH_3); δ_C($CDCl_3$): 165.35 (C=O), 155.89 (Ar-C-O), 133.82, 130.98, 124.19, 115.72, 109.87 (Ar-C), 65.34 (OCH_2), 31.78 (OCH_2CH_2), 28.83 (CH_2), 28.68 (CH_2), 25.96 (CH_2), 22.64 (CH_2CH_3), 14.07 (CH_3); GC: t_R 11.22 min; LRMS (EI): 329 (M^+, 3%), 218 (M^+-C_8H_{15}, 100%).

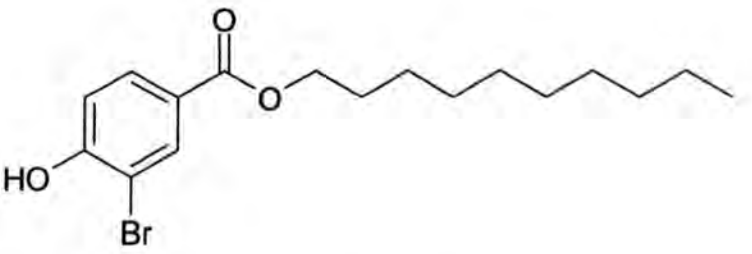
3-Bromo-4-hydroxy-benzoic acid nonyl ester (265)



Compound **265** was synthesised in the same way as compound **259**, except that 3-bromo-4-hydroxy-benzoic acid (1.2g, 5.53mmol), and nonanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave **265** as a white solid (0.23g, 12.11% yield) [m.p. 48.7-49.2°C; R_f 0.29 diethyl ether/petroleum ether 40-60°C (30:70)].

$\nu_{(max)}$(Film) cm^{-1}: 3339.40 (OH), 2924.80 (CH), 1688.30 (C=O), 1600.40 (Ar C=C), 1286.90 (C-O); $\delta_H(CDCl_3)$: 8.18 (1H, d, J=8.00Hz, Ph-H), 7.93 (1H, d, J=8.00Hz, Ph-H), 7.05 (1H, d, J=8.00Hz, Ph-H), 6.03 (1H, s, OH), 4.28 (2H, t, J=7.00Hz, OCH_2), 1.75 (2H, quin, J_{AB}=7.00Hz, J_{AB}=8.00Hz, OCH_2CH_2), 1.21-1.36 [12H, m, $(CH_2)_6CH_3$], 0.88 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 164.01 (C=O), 155.63 (Ar-C-O), 133.90, 131.35, 116.01, 110.13 (Ar-C), 64.87 (OCH_2), 31.83 (OCH_2CH_2), 29.25 [$O(CH_2)_2CH_2$], 22.65 (CH_2CH_3), 14.09 (CH_3); GC: t_R 11.61 min; LRMS (EI): 344 (M^+, 2%), 216 (M^+-C_9H_{20}, 100%).

3-Bromo-4-hydroxy-benzoic acid decyl ester (266)



Compound **266** was synthesised in the same way as compound **259**, except that 3-bromo-4-hydroxy-benzoic acid (1.2g, 5.53mmol), and decanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave **266** as a white solid (0.35g, 17.77% yield) [m.p. 56.0-56.5°C; R_f 0.27 diethyl ether/petroleum ether 40-60°C (30:70)].

$\nu_{(max)}$(Film) cm^{-1}: 3356.55 (OH), 2925.42 (CH), 1686.51 (C=O), 1601.12 (Ar C=C), 1287.29 (C-O); $\delta_H(CDCl_3)$: 8.18 (1H, d, J=9.00Hz, Ph-H), 7.91 (1H, d, J=8.00Hz, Ph-H), 7.05 (1H, d, J=8.00Hz, Ph-H), 6.06 (1H, s, OH), 4.27 (2H, t, J=7.00Hz, OCH_2), 1.70-1.81 (2H, m, OCH_2CH_2), 1.20-1.48 [14H, m, $(CH_2)_7CH_3$], 0.88 (3H, t, J=7.00Hz, CH_3); $\delta_C(CDCl_3)$: 164.01 (C=O), 156.08 (Ar-C-O), 133.85, 130.96, 124.40, 115.72 (Ar-C), 65.34 (OCH_2), 31.87 (OCH_2CH_2), 29.51 [$O(CH_2)_2CH_2$], 29.21 (CH_2), 28.67 (CH_2), 25.98 (CH_2), 22.65 (CH_2CH_3), 14.10 (CH_3); GC: t_R 12.24 min; LRMS (EI): 358 (M^+, 2%), 216 (M^+-$C_{10}H_{22}$, 100%).

3-Bromo-4-hydroxy-benzoic acid cyclopentyl ester (267)

Compound **267** was synthesised in the same way as compound **259**, except that 3-bromo-4-hydroxy-benzoic acid (1.2g, 5.53mmol), and cyclopentanol (10mL) was used instead of propan-1-ol. Removal of the solvent using the Kugerhol apparatus gave **267** as white long crystalline crystals (0.39g, 24.68%) [m.p. 123.9-124.4°C; R_f 0.13 diethyl ether/ petroleum ether 40-60°C (40:60)].

$\nu_{(max)}$(Film) cm^{-1}: 3339.75 (OH), 2964.58 (CH), 1682.19 (C=O), 1601.77 (Ar C=C), 1287.73 (C-O); $\delta_H(CDCl_3)$: 8.12 (1H, d, J=8.00Hz, Ph-H), 7.88 (1H, d, J=8.00Hz, Ph-H), 7.02 (1H, d, J=8.00Hz, Ph-H), 5.33-5.37 (1H, m, OH), 1.88-1.97 (1H, m, OCH), 1.74-1.85 [4H, m, OCH$(CH_2)_2$, cyclo], 1.61-1.68 [4H, m, $(CH_2)_2$, cyclo]; $\delta_C(CDCl_3)$: 165.09 (C=O), 155.37 (Ar-C-O), 133.73, 130.92, 124.69, 115.66 (Ar-C), 110.12 (OCH), 32.75 [$(CH_2)_2$, cyclo], 23.80 [$(CH_2)_2$, cyclo]; GC: t_R 9.58 min; LRMS (EI): 284 (M^+, 3%), 199 (M^+-OC_5H_9, 100%).

CHAPTER 5

Biochemical evaluation of compounds against HSD enzymes

5.1 Biochemical evaluation of compounds against HSD enzymes

A small range of the phenyl ketone based compounds (as potential inhibitors of 17β-HSD isozymes) were evaluated (by Dr. Sachin Dhanani at Kingston University) against rat testicular microsomal enzyme. In particular, the compounds based on 4-hydroxyphenyl ketone (compounds **158** to **172**) and biphenyl ketone (compounds **202** to **214**) were evaluated against type 1 and type 3 isozymes of 17β-HSD. In an effort to determine specificity towards 17β-HSD, the compounds were also evaluated against 3β-HSD. The results are therefore quoted as the mean of triplicate determinations (n=9) (Table 5.1-5.6).

Structure	% Inhibition at 100μM	IC_{50} Value (μM)
Baicalein	38.78 ± 1.36	185.92 ± 12.70
7-Hydroxyflavone	53.93 ± 1.07	66.98 ± 0.95
158	36.59 ± 0.52	1708.92 ± 170.71
159	39.04 ± 0.42	150.56 ± 12.21
160	60.18 ± 0.77	89.51 ± 6.73
161	61.81 ± 0.89	60.52 ± 5.83
162	76.40 ± 0.18	18.02 ± 0.96
163	80.26 ± 0.20	7.84 ± 0.36
164	82.58 ± 0.49	6.52 ± 0.18
165	83.53 ± 0.48	2.86 ± 0.03
166	81.39 ± 0.09	4.97 ± 0.25
168	78.92 ± 0.58	7.55 ± 0.32
169	65.88 ± 0.42	27.15 ± 2.23
170	64.77 ± 0.40	33.19 ± 1.62
171	62.94 ± 0.82	36.16 ± 2.45
172	63.62 ± 0.78	29.66 ± 0.76

Table 5.1 Results from preliminary screening and IC_{50} data of some 4-hydroxyphenyl ketones for type 3 17β-HSD activity.

Structure	% Inhibition at 100μM
Baicalein	49.43 ± 1.16
7-Hydroxyflavone	66.42 ± 2.05
202	0
203	0
204	20.36 ± 1.74
205	8.55 ± 1.00
206	10.22 ± 1.72
207	1.18 ± 0.30
208	0
209	13.29 ± 0.26
210	17.86 ± 1.14
211	16.52 ± 0.91
212	4.30 ± 0.40
213	15.22 ± 1.64
214	23.48 ±0.35

Table 5.2 Results from preliminary screening data of some biphenyl ketones for type 3 17β-HSD activity.

Structure	% Inhibition at 100μM
Baicalein	31.63 ± 4.91
7-Hydroxyflavone	25.63 ± 2.94
158	20.83 ± 0.91
159	17.47 ± 0.91
160	35.11 ± 2.92
161	39.76 ± 1.31
162	45.68 ± 1.34
163	47.58 ± 2.60
164	36.87 ±0.89
165	36.32 ± 0.33
166	28.15 ± 3.46
168	30.61 ± 3.78
169	42.47 ± 2.66
170	43.97 ± 0.78
171	49.54 ± 2.57
172	47.23 ± 2.41

Table 5.3 Results from preliminary screening data of some 4-hydroxyphenyl ketones for type 1 17β-HSD activity.

Structure	% Inhibition at 100μM
Baicalein	31.63 ± 4.91
7-Hydroxyflavone	25.63 ± 2.94
202	31.24 ± 2.79
203	33.50 ± 2.73
204	36.59 ± 0.56
205	45.49 ± 0.53
206	33.55 ± 0.73
207	36.05 ± 0.66
208	34.79 ± 0.25
209	31.26 ± 0.28
210	28.23 ± 1.64
211	29.43 ± 0.37
212	34.47 ± 0.10
213	44.04 ± 1.72
214	48.79 ± 0.22

Table 5.4 Results from preliminary screening data of some biphenyl ketones for type 1 17β-HSD activity.

Structure	% Inhibition
Baicalein	28.26 ± 0.79[a], 24.01 ± 2.44[b]
7-Hydroxyflavone	0[b]
158	4.72 ± 1.10[b]
159	0[b]
160	0[b]
161	0[b]
162	0[b]
163	3.08 ± 1.02[b]
164	5.71 ± 0.30[b]
165	9.02 ± 0.41[b]
166	4.23 ± 0.59[a], 21.08 ± 2.08[b]
168	20.11 ± 0.73[a], 40.68 ± 1.09[b]
169	21.47 ± 1.17[b]
170	33.59 ± 1.03[b]
171	4.31 ± 2.35[a], 34.56 ± 1.26[b]
172	9.83 ± 1.43[a], 47.42 ± 0.47[b]

Table 5.5 Results from preliminary screening data of some 4-hydroxyphenyl ketones for 3β-HSD activity, (where [a][I]=100μM and [b][I]=500μM).

Structure	% Inhibition at 100μM	% Inhibition at 500μM
Baicalein	28.26 ± 0.79	24.01 ± 2.44
7-Hydroxyflavone	0	0
202	5.56 ± 0.08	12.91 ± 0.21
203	10.63 ± 0.70	5.35 ± 0.07
204	9.72 ± 0.03	12.40 ± 0.22
205	25.51 ± 0.08	10.33 ± 0.33
206	4.34 ± 0.95	24.10 ± 0.07
207	7.68 ± 0.78	22.97 ± 0.51
208	0	24.72 ± 0.97
209	0	0
210	0	0
211	4.38 ± 0.63	0
212	0	4.36 ± 0.68
213	19.81 ± 0.76	23.38 ± 0.12
214	21.80 ± 0.26	22.37 ± 1.13

Table 5.6 Results from preliminary screening data of some biphenyl ketones for 3β-HSD activity.

CHAPTER 6

Discussion of biological data

6.1 Discussion of Biological data

As described above, the compounds synthesised within the currently study were evaluated against three HSD enzymes, namely: 17β-HSD3, 17β-HSD1 and 3β-HSD. The latter enzyme was chosen so as to evaluate the potential specificity of the synthesised compounds against the general family of HSD enzymes. In an effort of gain some data as to the level of inhibition observed within the synthesised compounds in comparison to previously reported inhibitors, we considered the flavonoid based compounds. That is, a number of workers within the field have previously undertaken biochemical evaluation of flavonoid-based compounds, in particular, Baicalein and 7-hydroxyflavone, as such, these two compounds were evaluated with our novel inhibitors for comparison.

6.2 Inhibition of 17β-HSD3

The results of the biochemical evaluation of the potential inhibitors of 17β-HSD3 (Tables 5.1 and 5.2) show that the 4-hydroxyphenyl ketone-based compounds are potent inhibitors of 17β-HSD3, in comparison to the two standards used. Detailed consideration of the biochemical data shows that there are two compounds that are potent inhibitors of this isozyme, namely **165** (IC_{50}=2.86μM) and **166** (IC_{50}=4.97μM). As such, **165** is around 65 and 23 times more potent than Baicalein (IC_{50}=185.92μM) and 7-hydroxyflavone (IC_{50}=66.98μM) respectively. A number of other compounds also show good inhibitory activity, for example, compounds **163** (IC_{50}=7.84μM), **164** (IC_{50}=6.52μM) and **168** (IC_{50}=7.55μM) possess good inhibitory activity in comparison to the two standard compounds.

A detailed consideration of the observed inhibitory activity within the 4-hydroxyphenyl ketone based compounds show that there is a good correlation between the potency of the compounds and the alkyl chain length, that is, the inhibitory activity increases with increasing alkyl chain length [and therefore the logarithm of the calculated partition coefficient (logP) (calculated using

Quantum CaChe Project Leader, Fujitsu Ltd)]. Indeed a plot of logP versus IC_{50} supports the initial observation resulting in a very good correlation (R^2=0.95) (Figure 6.1). Consideration of the Figure 6.1 shows that there is an optimum logP of approximately 3.8 and 4.3 and which corresponds approximately to compound **256**.

Molecular modelling of these compounds were undertaken within our group (Lota et al, 2006) which suggested that the 4-hydrophenyl ketone-based inhibitors may possess two possible modes of binding to the 17β-HSD3 active site when superimposed onto the steroid backbone (it should be noted that currently, there are no reported crystal structures of 17β-HSD3, as such, the modelling study was initially undertaken so as to gain valuable information regarding the active site of this isozyme). That is, the inhibitors are presumed to bind to the active site where the carbonyl moiety [as previously suggested by Owen and Ahmed (2004)] within the inhibitor mimics the C(17)=O of the substrate and therefore is presumably reduced by NADPH within the 17β-HSD3 active site. As such, one mode of binding involves the alkyl chain extending towards the area of space normally occupied by the steroid backbone (i.e. rings A, B and C), placing the 4-hydroxyphenyl moiety beyond the (C15) and C(16) position of the steroid backbone (Figure 6.2). The alternative mode of superimpositioning involves the 4-hydroxyphenyl being positioned so that the alkyl chain is extended far beyond the D-ring (Figure 6.3).

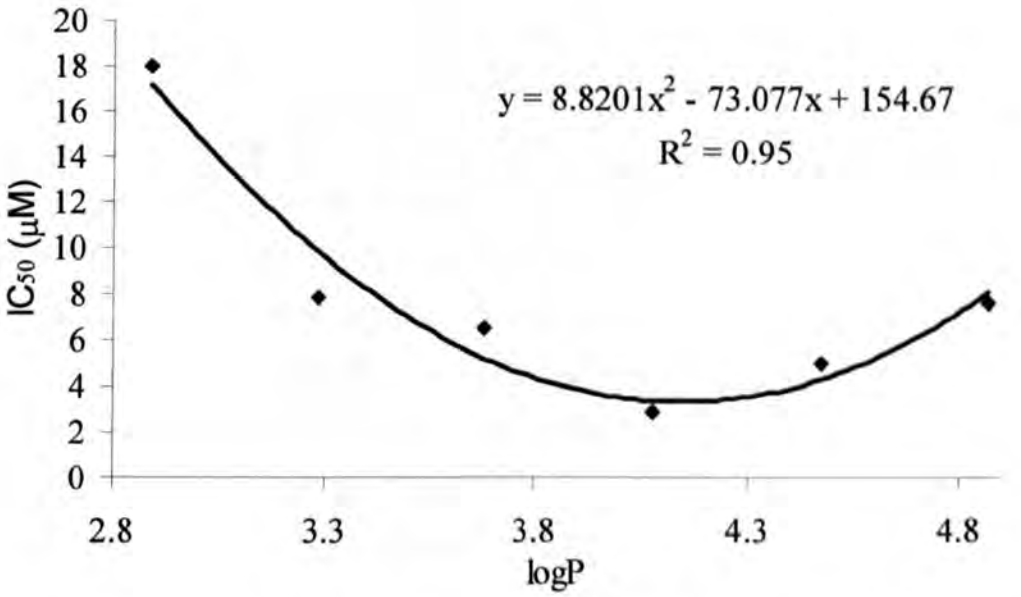


Figure 6.1: Plot of IC_{50} versus calculated logP for a small range of the compounds (from **5** to **10**) synthesised within the current study (Lota et al, 2006).

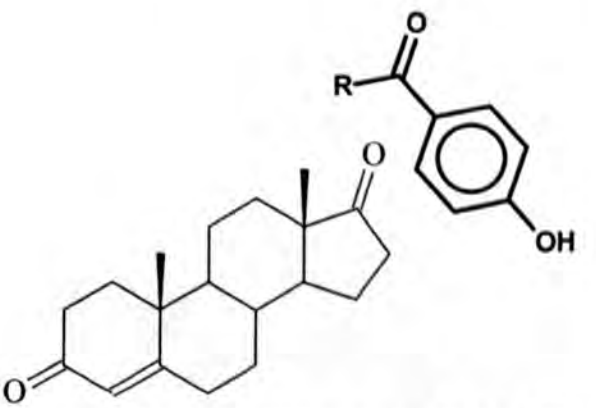


Figure 6.2: Superimposing of novel inhibitor onto the backbone of A

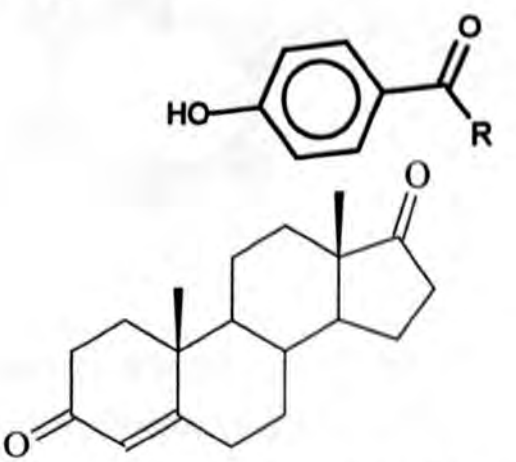


Figure 6.3: Alternative mode of superimposing of novel inhibitor onto the backbone of A

As previously mentioned, Owen and Ahmed (2004) have previously suggested that the area of the enzyme active site corresponding to the D-ring of the natural substrate may be populated with hydrogen donor and bonding groups as well as NADPH co-factor. As such, binding in such a manner where 'large' alkyl chain extends out beyond the D-ring as suggested by Figure 6.3 is highly unlikely due to steric interactions which would therefore lead to reduced inhibitory activity. We therefore suggest that the mode of binding suggested in Figure 6.2 is more preferable and is the mode of binding for the majority of the compounds - the modelling of compounds **165** and **159**, small alkyl chain containing compounds (Figures 6.3 and 6.4) shows the superimpositioning of the inhibitors onto the steroid backbone in such a manner.

Furthermore, from the consideration of the potency of the larger alkyl chain containing compounds, we suggest that the 4-hydroxyphenyl moiety may be involved in hydrogen bonding interactions with the active site about the C(15) and C(16) area of the steroid backbone and which would result in stronger binding and therefore increased inhibitory activity.

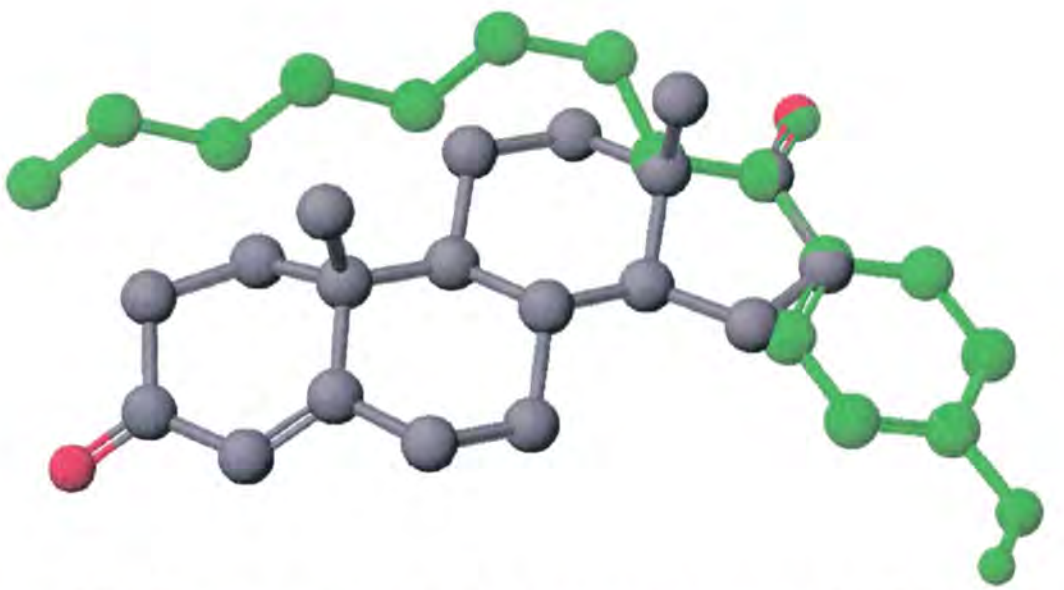


Figure 6.3: Superimposition of a low energy conformer of **165** (in ball and stick representation) onto the backbone of A (Lota et al, 2006).

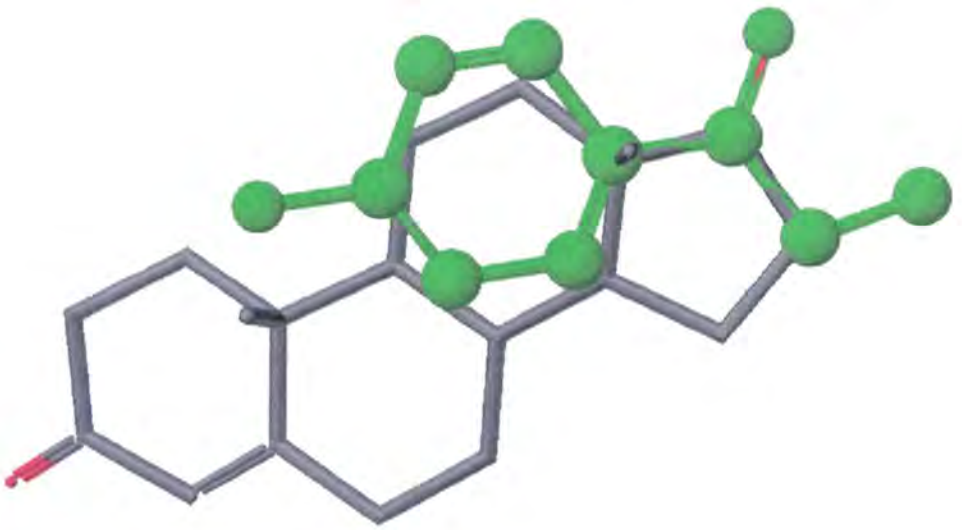


Figure 6.4: Superimposition of **159** (in ball and stick representation) onto the backbone of A (Lota et al, 2006).

That the 4-hydroxyl moiety is able to undergo this favourable hydrogen bonding interaction, resulting in the increased potent inhibitory activity observed within the larger inhibitors of 17β-HSD3, is supported by the observation (Dhanani, 2006) that compounds lacking hydrogen bonding substituents on the phenyl moiety possessed extremely poor inhibitory activity. Furthermore, the poor inhibitory activity observed within the biphenyl based compounds would appear to further support our hypothesis regarding the ability of the larger alkyl chain containing compounds to be involved in hydrogen bonding to groups within the active site of 17β-HSD3.

6.3 Inhibition of 17β-HSD1

The compounds were also evaluated against 17β-HSD1 in an effort to evaluate the selectivity of the 4-hydroxyphenyl ketones range of compounds. From the consideration of the results, we observe that these compounds possess poor inhibitory activity and would appear therefore to be selective inhibitors of the 17β-HSD family of enzymes.

The biphenyl-based compounds, however, possess some potency against this isozyme, as such, this would appear to be initially an interesting observation whereby the loss of a hydrogen bonding group and replacement

with a hydrophobic moiety results in a 'switch' from being 17β-HSD3 inhibitors to 17β-HSD1 inhibitors. As previously mentioned, the structure of 17β-HSD1 has previously been reported and the orientation of groups about the active site has also been proposed (Sawacki et al, 1999). An initial modelling study undertaken by Ahmed (2005) and Olusanjo et al (2007) involved the superimposition of a range of compounds onto the natural substrate bound to active of 17β-HSD1. From his study, Ahmed was therefore able to rationalise the inhibitory activity of a range of the compounds synthesised within the current study.

In general, the 4-hydroxyphenyl ketone based compounds were found to be positioned such that the long alkyl chain containing compounds (e.g. **166**) were found to undergo steric interaction with the co-factor, where as the smaller alkyl chain containing compounds (e.g. **158**) were found to be positioned within the active site such that they are not involved in any steric interaction (Figure 6.5).

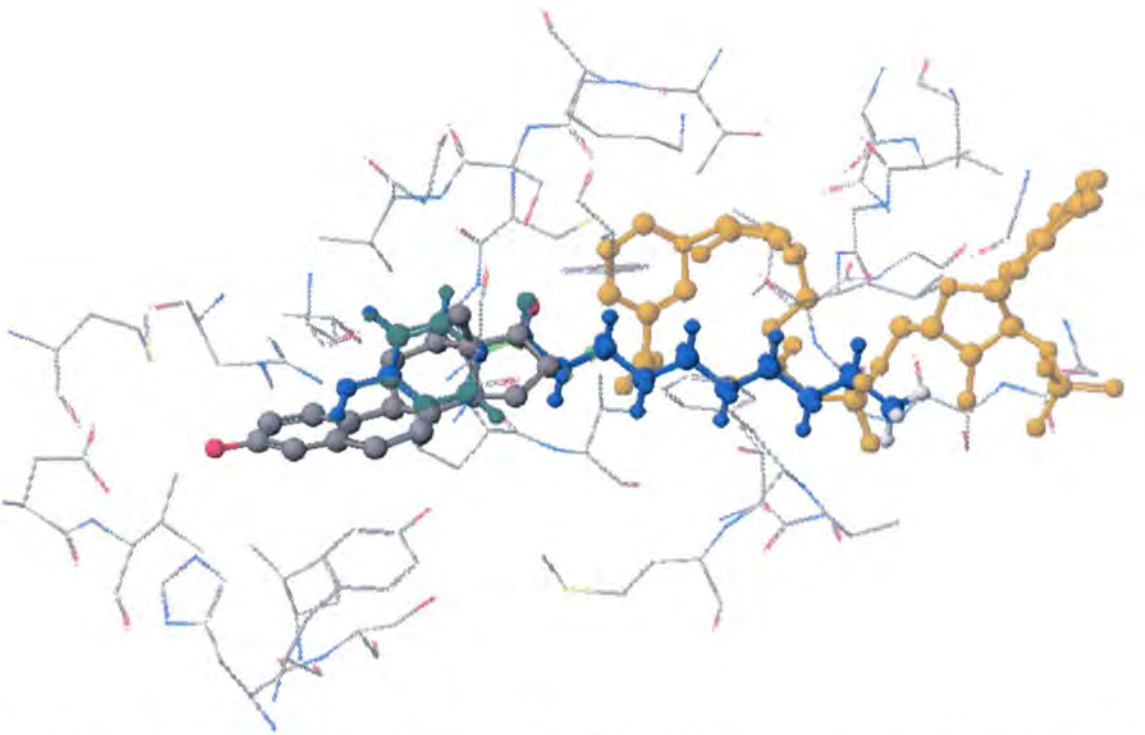


Figure 6.5: Superimposition of compounds **166** (in purple) and **158** (in green) onto the steroid backbone complexed within 17β-HSD1.

As such, the modelling study suggests that in the inhibition of 17β-HSD1, small alkyl chain containing compounds are required so as to be able to fit within the active site. Furthermore, in their study, Olusanjo et al (2007) superimposed compounds **205** and **214** which were found to possess good inhibitory activity against 17β-HSD1 (Figure 6.6).

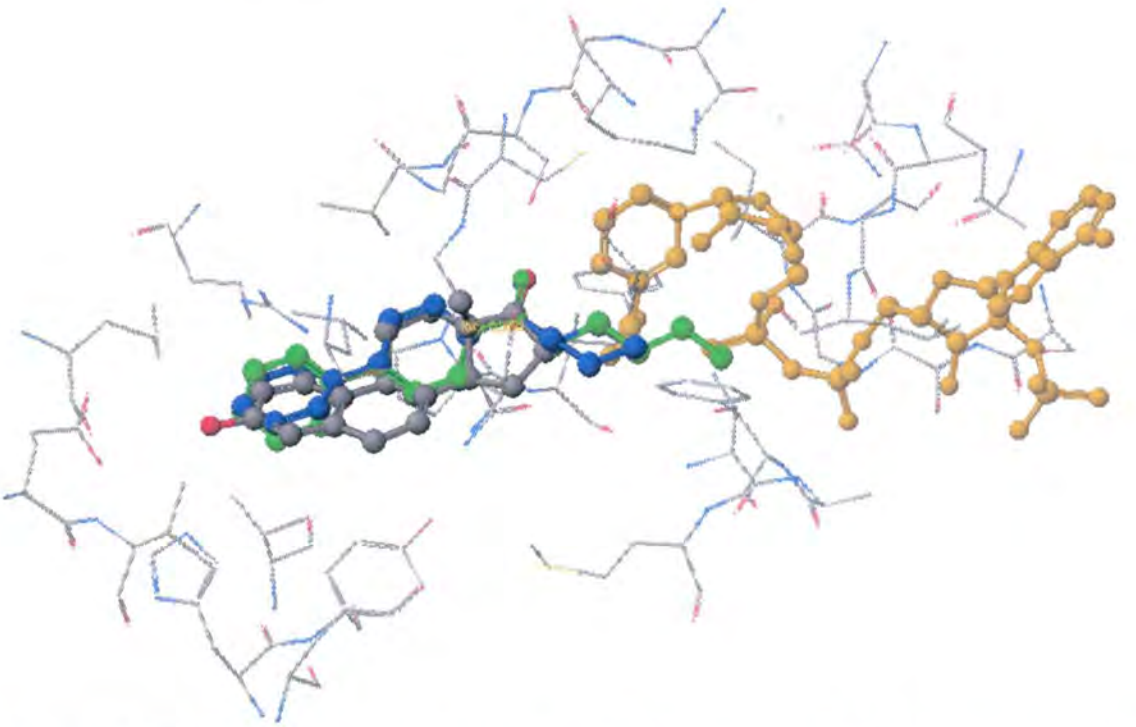


Figure 6.6: To show the superimpositioning of compounds **205** (in green) and **214** (in purple) onto the steroid backbone complexed within 17β-HSD1.

From the superimpositioning study, we observe that (as with 17β-HSD3) the longer alkyl chain containing compound (Figure 6.6; in green) undergoes steric interaction whilst the cyclic based compound (and which therefore possess shorter overall length) is found to possibly undergo less steric interaction due to the smaller overall size of the cyclic moiety. With respect to the increased inhibitory activity observed within these compounds against 17β-HSD1, Olusanjo et al hypothesise that logP may also be a contributory factor in determining the overall inhibitory activity. That is, consideration of the calculated logP values shows that estrone is found to possess a logP value of 4.54 whilst compounds 205 and 214 are found to possess logP values of 4.86 and 4.92 respectively

6.4 Inhibition of 3β-HSD

All the potential 17β-HSD1 and 17β-HSD3 inhibitors were evaluated against 3β-HSD in an effort to determine the general specificity of the synthesised compounds against the overall family of HSD enzymes. From the consideration of the observed inhibitory activity, it would appear that the designed compounds are all, in general, non-inhibitors of 3β-HSD and therefore would appear to be specific inhibitors of 17β-HSD3 (in the case of the 4-hydroxyketone based compounds) or 17β-HSD1 (in the case of the biphenyl ketone based compounds). Due to the weak inhibitory activity of the compounds against 3β-HSD, no molecular modelling study was undertaken.

6.5 Conclusion

In conclusion, the series of potential inhibitors synthesised within the current study have proved to be, in general, specific and potent inhibitors of 17β-HSD1 and 17β-HSD3 and were found to be non-inhibitors of 3β-HSD. As such, these compounds have proved to be good lead compounds in the further design and subsequent synthesis of novel and potent inhibitors of either 17β-HSD1 (in the fight against hormone-dependent breast cancer) or 17β-HSD3 (in the fight against hormone-dependent prostate cancer).

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