# SYNTHESIS AND BIOCHEMICAL EVALUATION OF POTENTIAL INHIBITORS OF 17β-HYDROXYSTEROID DEHYDROGENASE FOR TREATMENT OF HORMONE-DEPENDENT PROSTATE CANCER

**THESIS**

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in

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at

Kingston University

by

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# DECLARATION

This thesis entitled **"Synthesis and biochemical evaluation of potential inhibitors of 17β-hydroxysteroid dehydrogenase for treatment of hormone-dependent prostate cancer"** is based upon work conducted by the author in the School of Pharmacy and Chemistry at Kingston University London. All of the work described herein is original unless otherwise acknowledged in the text or by references.

*Siamak Soltani Khankahdani*

# Dedication

**I would like to dedicate this manuscript to my parents, my wife and my son.**

# ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisors Prof. John E. Brown and Prof. Elizabeth Tyrrell for their help and guidance. Special thanks must be expressed to Prof. Tyrrell for her invaluable help during the writing of this manuscript. I would also like to pay my gratitude and thanks to Miss Ammarra Abdulah, Dr Jean-Marie Peron and Dr Julian Swinden for their kind help and support during my studies. I would like to thank all of my friends and colleagues who morally supported me during my research. I wish all of them the best of luck and happiness in their lives.

# ABSTRACT

It has been shown that the majority of benign prostatic hyperplasia (BPH) and prostate cancers are dependent on androgen production within the body. The biosynthesis of androgens is catalysed by different enzymes however one of the enzymes, 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3), converts the C(17)=O carbonyl moiety of androstenedione ($\Delta^4$-dione) to the corresponding C(17)-OH hydroxyl group of testosterone (T).

It has been hypothesised that inhibition of 17β-HSD3 may cause a decrease in the level of androgens which in turn leads to a reduction in the genesis of androgen-dependent prostatic diseases. The utilisation of enzyme inhibition as a therapeutic agent, in the treatment of breast cancer, has been tested on postmenopausal women by using aromatase inhibitors (e. g; exemestane, anastrazole and latrazole). This approach has proved to be successful and the impact of enzyme inhibition was led to a reduction in cancer growth. This process has now found a clinical application.

From molecular modelling studies it was postulated that any potential inhibitor of 17β-HSD3 should contain a carbonyl moiety, mimicking the C(17)=O of the natural substrate, as well as an aromatic ring adjacent to the carbonyl group. With these criteria in mind results from our laboratories showed that from a library of candidates those based upon 4-hydroxyphenyl ketones showed some potential.

The main focus of this present study was to fine tune the enzyme inhibitor analogues and hence optimise inhibitory activity of 4-hydroxyphenyl ketones. We have successfully synthesised a range of novel derivatives of 4-hydroxyphenyl ketones such as the 4-methanesulfonate and 4-acetate ester derivatives. In general, the reactions have proceeded very well with the yields ranging from 65% to 88% and 91% to 97% respectively.

The results of biochemical evaluation studies suggested that the acetate ester derivatives, in particular compounds (**149**) and (**150**) exhibited good inhibitory activity against 17β-HSD type 3 of about 40% compared to standard inhibitors such as 7-hydroxyflavone and baicalein which resulted in about 13% and 14% inhibitory activity respectively.

In addition a range of non-steroidal B, C, D ring mimics of the natural substrate of 17β-HSD type 3 were synthesised in good yields (65% to 85%). The biochemical evaluation of these compounds also showed good inhibitory activity; in fact compound (**107**) exhibited about 43% inhibition in comparison to the above standards which had inhibition of about 25% and 31% respectively.

In conclusion we have successfully synthesised and biochemically evaluated a number of enzyme inhibitors for the enzyme 17β-HSD type 3. The two types of active inhibitors were structurally dissimilar suggesting that they may have different modes of binding. This outcome requires further investigation in order to establish and identify how this inhibition is taking place.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| 5α-reductase | 5α-R |
| 17β-hydroxysteroid dehydrogenase | 17β-HSD |

**A**

| | |
|---|---|
| Adrenocorticotropic hormone | ACTH |
| Aldo-ketoreductase | AKR |
| Aluminium chloride | $AlCl_3$ |
| Androgen sensitive human prostate andrenocarcinoma cell | LNCaP |
| Androstenedione | $\Delta^4$-dione |
| Androsterone | ADT |
| Angstrom | Å |
| Aspartic acid | Asp |

**B**

| | |
|---|---|
| Benign prostatic hyperplasia | BPH |
| Bovine serum albumin | BSA |

**C**

| | |
|---|---|
| Complimentary dexyribonucleic acid | cDNA |
| Counts per minute | CPM |
| Cyclooxygenase | COX |

**D**

| | |
|---|---|
| Dehydroepiandrosterone | DHEA |
| Deuterated chloroform | $CDCl_3$ |
| Dichloromethane | DCM |
| Dihydrotestosterone | DHT |
| Dimethyl sulfoxide | DMSO |
| Doublet | d |

**E**

| | |
|---|---|
| Electron spray | ES |
| Estradiol | $E_2$ |
| Estrone | $E_1$ |
| Estrogen receptor | ER |

**F**

**G**

| | |
|---|---|
| Gas chromatography mass spectroscopy | GC-MS |
| Gonadotropin releasing hormone | GnRH |

**H**

| | |
|---|---|
| High resolution mass spectroscopy | HRMS |
| Histadine | His |
| Hour | h |

| | |
|---|---|
| Human embryonic kidney cell | HEK-293 |
| Human epithelial carcinoma cell line | A431 |
| Hydrochloric acid | HCl |
| Hydroxysteroid dehydrogenase | HSD |

**I**

| | |
|---|---|
| Inhibitory concentration at 50% | $IC_{50}$ |
| Inhibition constant | $K_i$ |

**J**

**K**

| | |
|---|---|
| Kilo Dalton | KDa |
| Kilo gram | Kg |

**L**

| | |
|---|---|
| Low resolution mass spectroscopy | LRMS |
| Luteinizing hormone | LH |
| Luteinizing hormone releasing hormone | LHRH |
| Lysine | Lys |

**M**

| | |
|---|---|
| Michaelis constant | $K_m$ |
| Microgram | µg |

| | |
|---|---|
| Micromolar concentration | μM |
| Milligram | mg |
| Millimolar concentration | mM |
| Molecular ion | $M^+$ |
| Multiplet | m |

**N**

| | |
|---|---|
| Nanomolar concentration | nM |
| Nicotinamide adenine dinucleotide phosphate | NADPH |
| Non-steroidal anti-inflammatory drug | NSAID |
| Nuclear magnetic resonance | NMR |

**P**

| | |
|---|---|
| Partition coefficient | Log P |
| Polycystic kidney disease | PKD |
| Prostatic specific antigen | PSA |

**Q**

| | |
|---|---|
| Quartet | q |
| Quintet | Quin |

**R**

| | |
|---|---|
| Rat retinol dehydrogenase type 1 | RoDH1 |
| Retention time | $t_R$ |

| | |
|---|---|
| Retinol dehydrogenases | RDHs |
| Retinol short-chain dehydrogenase/reductase | DHRS |

**S**

| | |
|---|---|
| Structure activity relationship | SAR |
| Short-chain reductase | SDR |
| Singlet | s |

**T**

| | |
|---|---|
| Testosterone | T |
| Thin layer chromatography | TLC |
| Triethylamine | TEA |
| Triplet | t |
| Tritium | $^{3}H$ |
| Tyrosine | Tyr |

**U**

| | |
|---|---|
| Ultra violet | UV |

**V**

| | |
|---|---|
| Visible | VIS |

**W**

| | |
|---|---|
| Wavelength | $\lambda$ |

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# CHAPTER 1: INTRODUCTION

# 1. INTRODUCTION

## 1.1. CANCER

Cancer is a term that refers to any disorder of cell growth and uncontrolled proliferation of cell without undergoing the process of cell death (apoptosis). Hence excessive cell proliferation happens which induces the creation of a tumour (Marieb, 2001). The process by which the healthy cell changes into a tumour cell is not known, as yet, although many elements have been proposed such as; chemical damage or certain viral infection. Nevertheless the mutation of genetic material is a common factor among the links which result in the loss of cellular control which then leads to uncontrolled cell proliferation.

Generally tumours can be divided into two groups;

i) Benign ii) Malignant

In both cases excessive cell proliferation occurs which distinguishes them from healthy cells. The benign tumour cells are generally unable to metastesise and as such are harmless to the patient's health. If the benign tumour cells grow too large closed to a vital organ such as brain it can be life threatening. The malignant cells however can be life threatening and also exhibit characteristics such as loss of tissue specificity, invasiveness and metastasis (Rang *et al*, 2003).

### 1.1.1. Excessive cell division

Healthy cells react to stimulatory signals to reproduce and also to inhibitory growth factor which control the state and well being of the cells produced. However a tumour cell, in contrast with healthy cells, can simply react to the stimulatory growth factor and as a result undergo cell division which leads to excess growth (Gupta and Massague, 2006).

### 1.1.2. Loss of tissue specificity

The cells that constitute a tumour change in comparison to the healthy cells from which they originated and result in the loss of appearance and functionality (Rang *et al,* 2003).

### 1.1.3. Invasiveness

As tumour growth progresses the cells (mass) can move within the extracellular matrix into closed tissues and then compete with the healthy cells for nutrients and space (Oppenheimer, 2006).

### 1.1.4. Metastasis

Metastasis is the aptitude of tumour cells to separate themselves from the original site (primary tumour) to a remote site by moving between the lymphatic system and blood stream (secondary tumour) (Chiang and Massague, 2008).

## 1.2. PROSTATE GLAND

The prostate gland is a gland found in men. It is the size of walnut and is situated in the pelvic area below the bladder and in front of rectum. It contains many nerves, smooth muscle, connective tissues and lymphatic vessels. The urethra, the left and right ejaculatory ducts pass through the prostate. The role of the prostate gland is to secrete an alkaline fluid which forms a component of semen and helps the mobility of sperms during ejaculation. The fluid also serves to neutralise the acidity of vaginal fluid (McNeal, 1981).

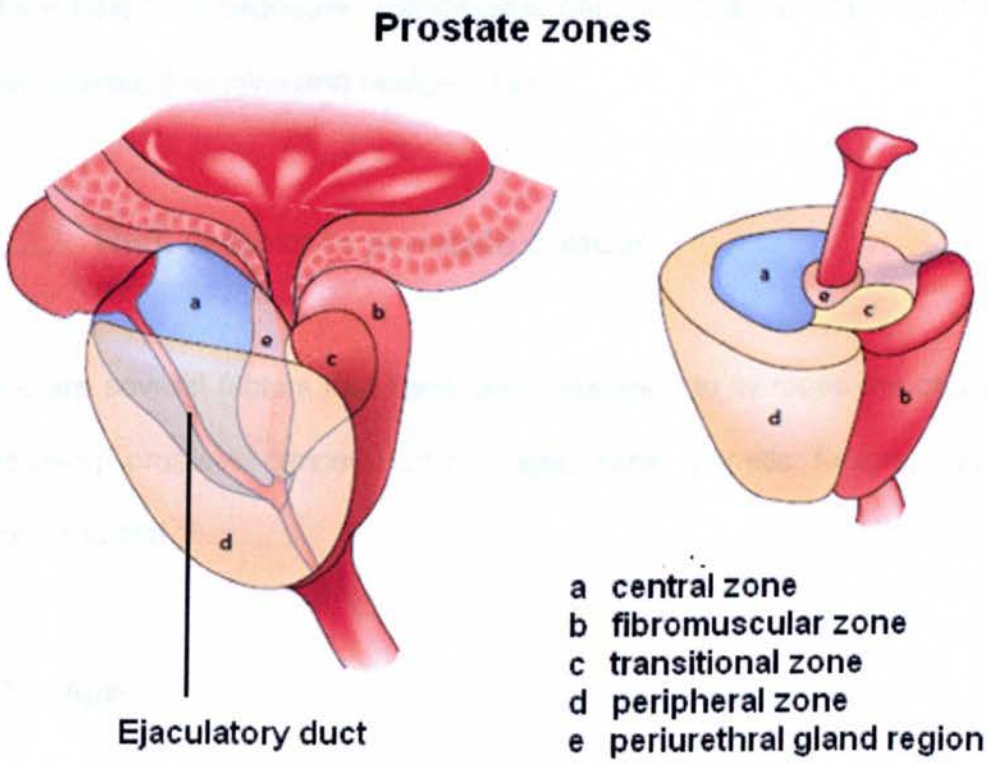



Figure 1.1: Different sections of the prostate gland (adapted from De Marzo *et al*, 2007)

### 1.2.1. Prostate cancer

Prostate cancer is the second largest cause of death from cancer in the United States of America and the third most common type of male cancer death after lung and colorectal cancer (Jemal *et al*, 2009, Ferlay *et al*, 2007). Prostate cancer has been reported to occur mostly in the peripheral zone of the prostate gland (De Marzo *et al*, 2007). Prostate cancer can be divided into two categories hormone-dependent and hormone-independent, however the exact cause of prostate cancer has not been determined but it has been postulated that the long term exposure to androgens can increase the growth and spread of the disease (Huggins and Hodges, 1941).

### 1.2.2. Risk factors in prostate cancer

There are several factors that have been assumed to increase the chances of developing prostate cancer namely; age, race, genetic factors, hormonal factors and diet.

#### 1.2.2.1. Age

Age is a significant contributing factor to prostate cancer because as males pass the age of fifty the prostate gland starts to increase in size. This growth of prostate is largely benign with a low increase in the prostatic specific antigen (PSA) level, as the prostate gland gets larger however this gland becomes predisposed to malignancies or abnormalities (Swallow and Kirby, 2006).

#### 1.2.2.2. **Race and ethnicity**

Studies have shown that prostate cancer varies immensely among race and ethnic groups. For example African Americans have been shown to have higher tendency to develop prostate cancer in comparison to Japanese who are amongst the lowest. It has been hypothesised that these differences could be due to both dietary and lifestyle effects (Jemal *et al*, 2009; Colli and Colli, 2006).

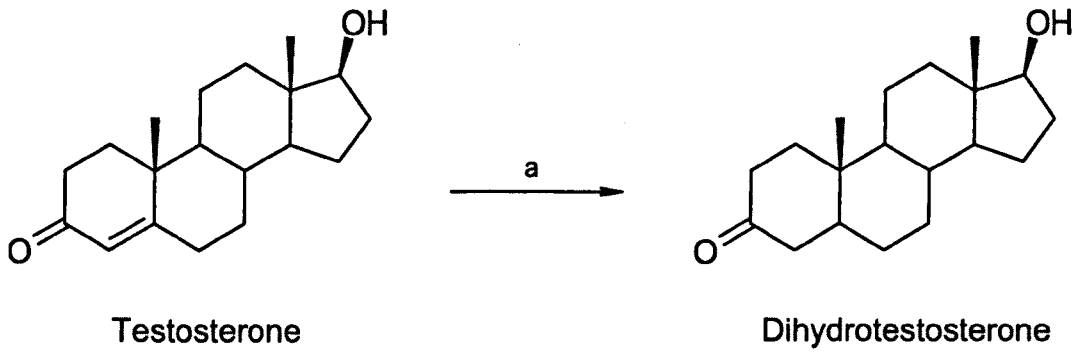
#### 1.2.2.3. **Diet**

Epidemiological studies have shown the importance of dietary influences in prostate cancer. The consumption of fatty acids tends to increase the risk of the disease (Krazeisen *et al*, 2001), however, diets high in selenium, vitamin-E and lycopene have shown to decrease the risk of prostate cancer (Giovannucci *et al*, 2002, Wilkinson and Chodak, 2003).

### 1.2.3. **The role of hormones**

Because prostate cancer is an androgen-dependent illness it needs testosterone for its development and expansion. Testosterone is transformed into dihydrotestosterone (DHT) within the prostatic cells by the action of an enzyme 5α-reductase (5α-R) type 1 and type 2 (Scheme 1.1). Dihydrotestosterone is the most potent androgen that has been generally associated with the commencement and expansion of prostate illness such as

prostate cancer. The production and maintenance of the level of androgens within the body is governed by the action of the hypothalamus gland (Figure 1.2). Although low level of androgens are synthesised in the adrenal glands, the vast proportion of androgens are produced in the Leydig cells of testes (Gregory *et al*, 2001). The role of the hypothalamus is to release a hormone called gonadotropin-releasing hormone (GnRH) which stimulates the anterior pituitary gland to release adrenocorticotropic hormone (ACTH) and luteinizing hormone (LH). While adrenocorticotropic hormone acts on the adrenal gland, situated above kidney, and stimulates the production of androgens, luteinizing hormone attaches to and activates the Leydig cells of the testes to synthesise testosterone (Purves *et al*, 2004).




Scheme 1.1: Conversion of testosterone into dihydrotestosterone within prostatic cell (a= 5α-R)

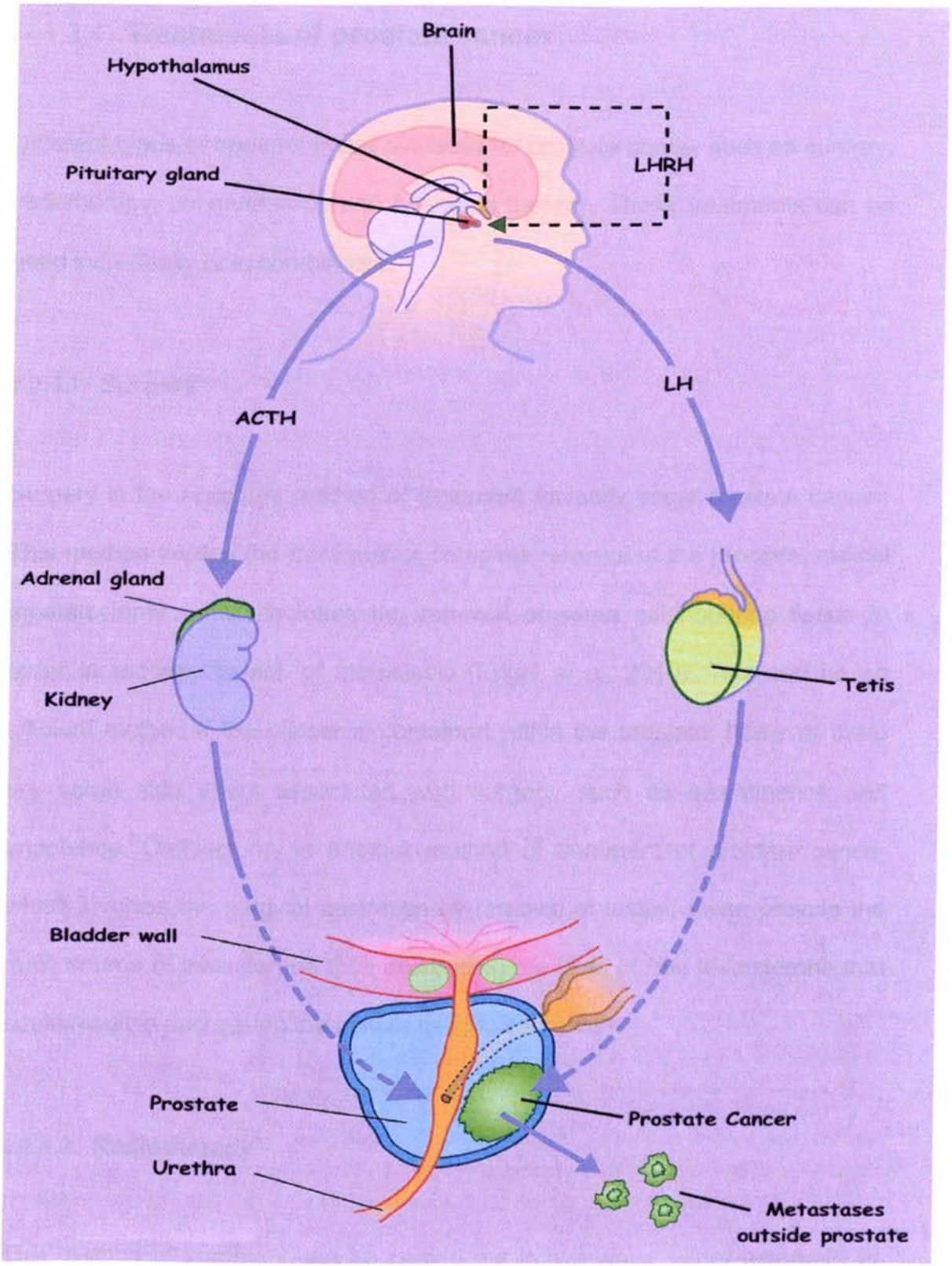



Figure 1.2: The loop involved in the production of testosterone and dihydrotestosterone (adapted from Hall, 2000).

## 1.2.4. Treatments of prostate cancer

Different types of treatments are available for prostate cancer such as surgery, radiotherapy, chemotherapy and hormonal therapy. These treatments can be used individually or in combination.

### 1.2.4.1. Surgery

Surgery is the accepted method of treatment for early stage prostate cancer. This method implies the fractional or complete removal of the prostate, radical prostatectomy, which includes the removal of some neighbouring tissue in order to reduce the risk of metastasis (Engel *et al*, 2010). This can be an efficient method if the cancer is contained within the prostate; however there are some side effect associated with surgery, such as incontinence and impotence. Orchiectomy is another method of treatment of prostate cancer which involves the surgical castration by removal of testes, these provide the main source of testosterone thus decreasing the level of free testosterone that could result in decreasing the cancer cell proliferation.

### 1.2.4.2. Radiotherapy

This method of treatment can be carried out in two ways, either externally or internally. External radiotherapy involves the use of x-rays to eradicate the cancer cell whereas internal radiotherapy involves the use of radioactive seeds containing Iodine$^{125}$ that implanted within the prostate gland (brachytherapy).

The radiation emitted from these seeds slowly damage and eradicate the cancer cells. The side effect associated with this method is that radiation may damage the healthy cells too (Hoskin and Coyle, 2005).

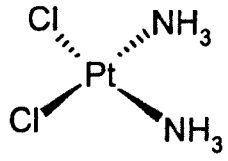
#### 1.2.4.3. **Chemotherapy**

Chemotherapy is sometimes used for the cure of hormone-dependent prostate cancer and more often used in advanced levels of cancer. Chemotherapeutic agents such as cross-linking agents [eg: cisplatin (**1**) or carboplatin (**2**)], plant alkaloids [eg: vinblastine (**3**) or vincristine (**4**)] or anti-metabolites such as purine, pyrimidine analogues [eg: azathiopurine (**5**), mercaptopurine (**6**) or fluorouracil (**7**)] (Figure 1.3) generally encourage apoptosis (by changing the morphology of the cell e.g a change in the cell membrane shape) and cell degradation by destroying cells which divide rapidly. This kind of treatment can be carried out singly or in combination with other treatments such as surgery (Haynes *et al*, 2008).

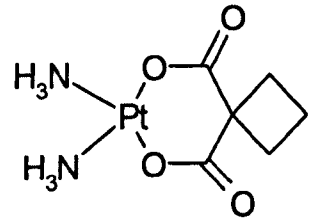
#### 1.2.4.4. **Hormonal therapy**

Hormonal therapy (androgen ablation) is concerned with the interaction on the pathway by which androgens, testosterone and dihydrotestosterone are produced. Therefore it focuses on reducing the production of androgens or blocking androgenic stimulation of the disease by chemicals which eventually, after a while, would slow down or inhibit the growth of prostate cancer. At the present time there is several hormonal therapy methods employed to treat

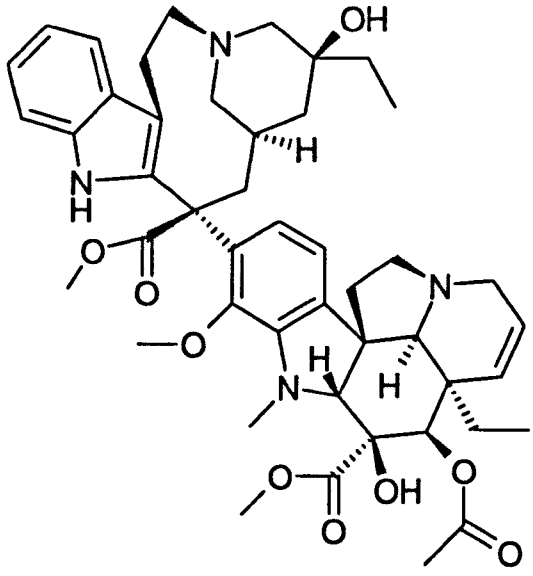
prostate cancer. The most common therapeutic methods in clinical use are orchiectomy, anti-androgens and luteinizing hormone-releasing hormone (LHRH) agonist or antagonist (Isbarn *et al*, 2009).



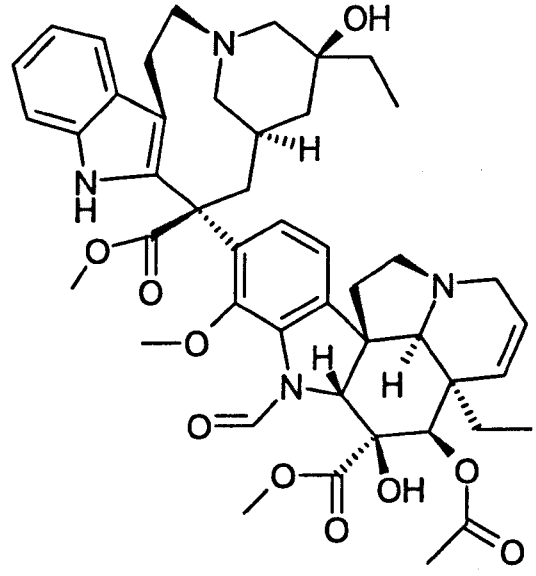

(1) Cisplatin



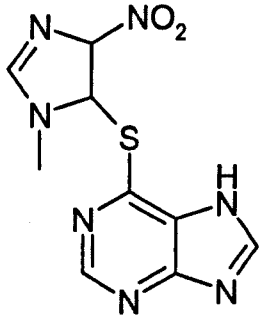

(2) Carboplatin



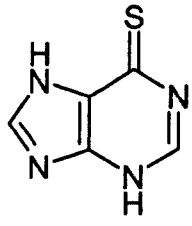

(3) Vinblastine



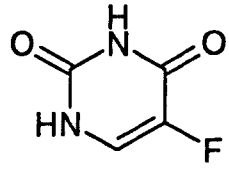

(4) Vincristine




(5) Azathiopurine




(6) Mercaptopurine

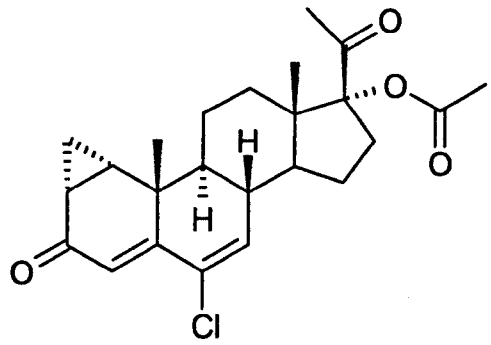



(7) Fluorouracil

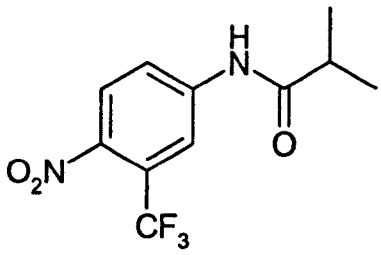
Figure 1.3: Cross-linking, plant alkaloids and anti-metabolite agents used as anti-cancer agent

### 1.2.4.5. **Anti-androgens**

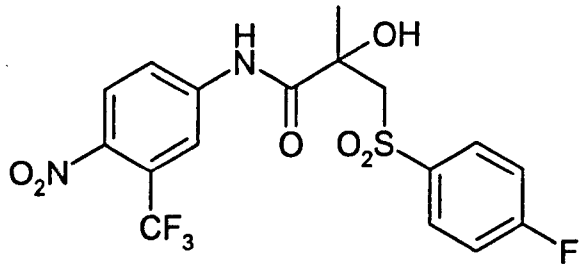
Anti-androgens are drugs that bind competitively to prostatic receptor sites for androgenic hormones (androgen receptor antagonist) and thus halt testosterone and dihydrotestosterone from binding thereby inhibiting the stimulation of the prostate cancer. There are two classes of anti-androgens, steroidal and non steroidal. An example of a steroidal androgen antagonist is cyproterone acetate (**8**) (Figure 1.4) although this drug can interfere with progestin and glucocortinoid receptors. Examples of non-steroidal anti-androgens are flutamide (**9**) and bicalutamide (**10**) (Figure 1.4) (Wakabayashi *et al*, 2005). There are some side effects associated with the use of these drugs such as impotence, loss of libido, vomiting, diarrhoea, skin rashes, liver problems and a reduction in the blood cell count.




(8) Cyproterone acetate



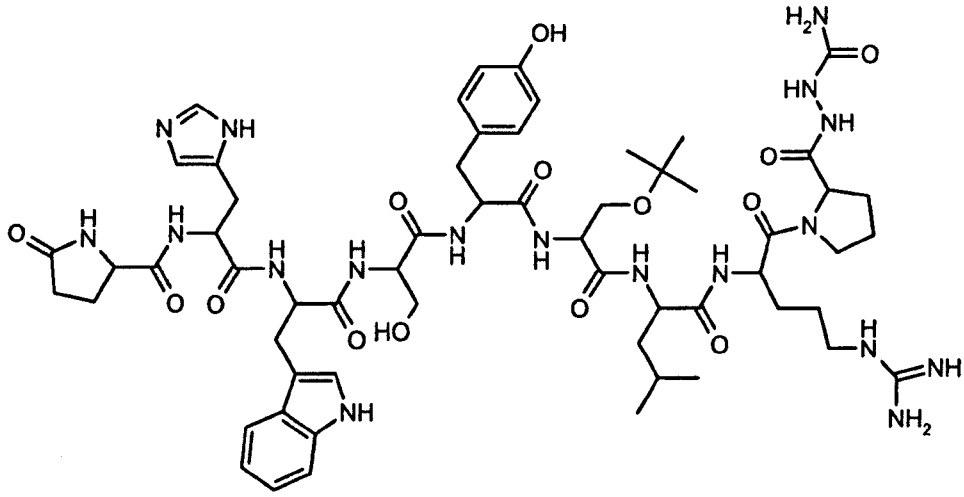

(9) Flutamide



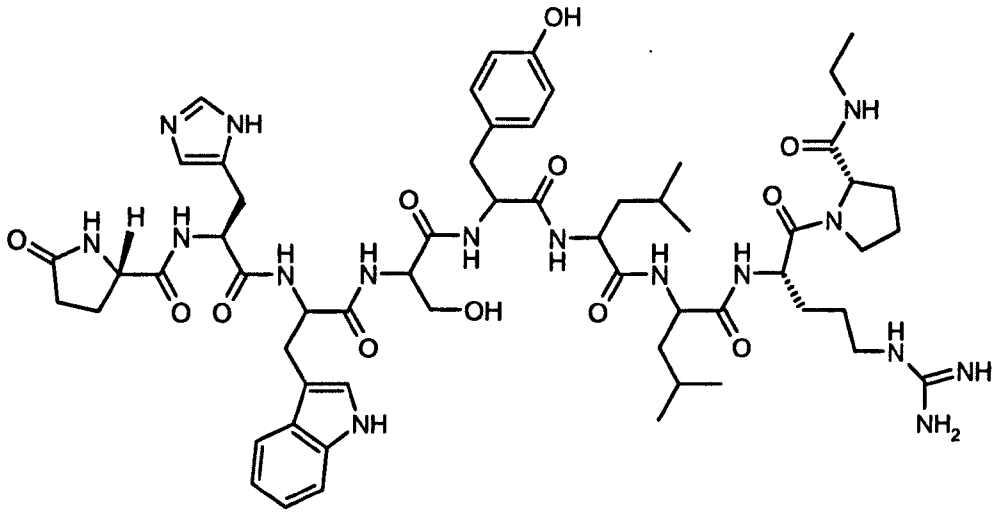

(10) Bicalutamide

Figure 1.4: Steroidal and non-steroidal anti-androgens used for treatment of prostate cancer

The use of luteinizing hormone-releasing hormone analogues is another method of hormonal therapy. These analogues can have agonist or antagonist effects (Isbarn *et al*, 2009). Luteinizing hormone-releasing hormone agonists such as goserelin (**11**) or leuprolide (**12**) (Figure 1.5) act by binding to the luteinizing hormone-releasing hormone (natural) receptor sites and at first increase the production of luteinizing hormone which leads to a increased stimulation of the testis and the production of testosterone. However, the continuous administration of drug overwhelms the body's natural need of testosterone which eventually leads to down-regulation of luteinizing hormone production and stimulation of testis.



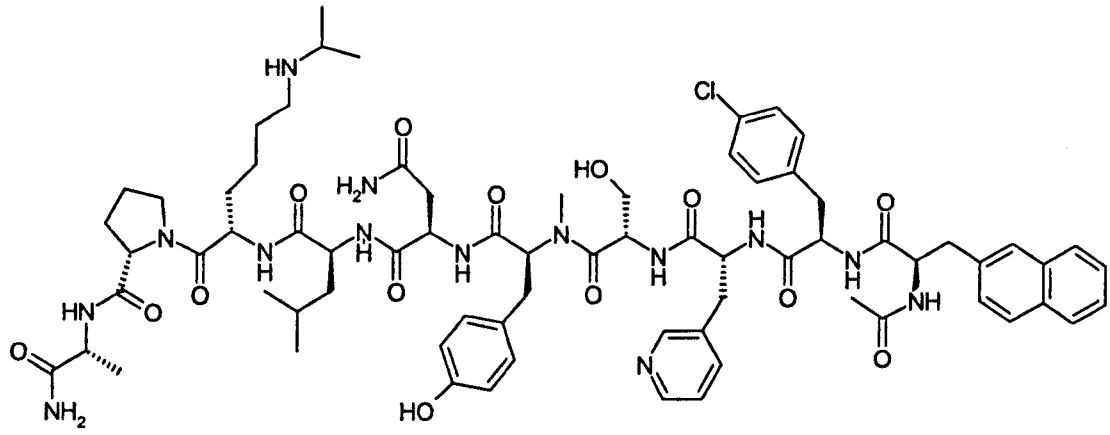
(11) Goserelin



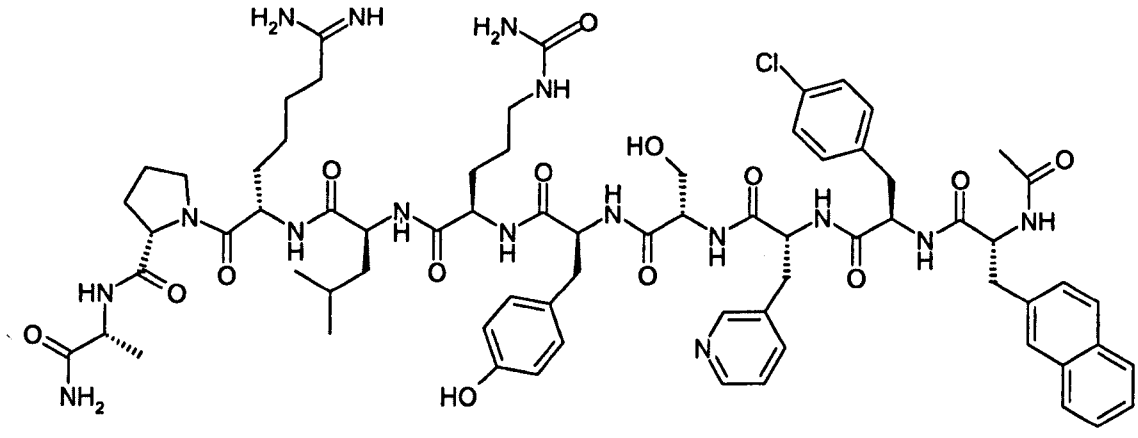
(12) Leuprolide

Figure 1.5: Agonist luteinizing hormone analogues used in the treatment of prostate cancer

Luteinizing hormone releasing hormone antagonists such as abarelix (**13**) and cetrorelix (**14**) (Figure 1.6) operate by binding to luteinizing hormone-releasing hormone receptor sites directly and switching off the stimulation of testis (Debruyne *et al*, 2008).



(13) Abarelix



(14) Cetrorelix

Figure 1.6: Antagonist luteinizing hormone analogues used in the treatment of prostate cancer

## 1.3. HYDROXYSTEROID DEHYDROGENASES

The hydroxysteroid dehydrogenases (HSDs) are a group of enzymes which play an important part in the biosynthesis (activation and inactivation) of most steroid hormones. Within the steroidogenic tissue they catalyse the last stage in androgen, estrogens and progesterone biosynthesis and by doing so, maintaining the intracellular amount of receptor ligands between tissue specific

expressions of special HSDs. These enzymes have been classified according to the reactions accomplished at the hormone structure (Nobel *et al*, 2001). Hydroxysteroid dehydrogenases modify potent steroid hormones into weak metabolites within the peripheral tissues as well as steroid hormone target tissues. As a result they coordinate the amount of hormone that can interact with members of the nuclear receptor super family, eventually balancing gene expression. Hydroxysteroid dehydrogenases catalyse bidirectional reactions and therefore an important challenge is to understand the mechanism by which they can be engaged in both production and inactivation of steroid hormones (Penning, 1997).

The complementary deoxyribonucleic acid (cDNA) cloning of HSD, from human placenta microsomes, reveals that each HSD consists of diverse isoforms that demonstrate tissue specificity in expression; this together with the properties of each isoform (reductase or dehydrogenase) have the ability to designate the role of the enzyme in steroid hormone activity. The improvement in cDNA isolation has indicated that HSDs belong to two protein phylogenies (families) (Penning, 1997):

- The short chain dehydrogenase / reductase (SDR) super family such as 3β-HSD, 11β HSD and 17β-HSD (Krozowski, 1994).

- The aldo keto reductase (AKR) super family such as 3α or 20α HSD (Pawlowski *et al*, 1991 / Miura *et al*, 1994).

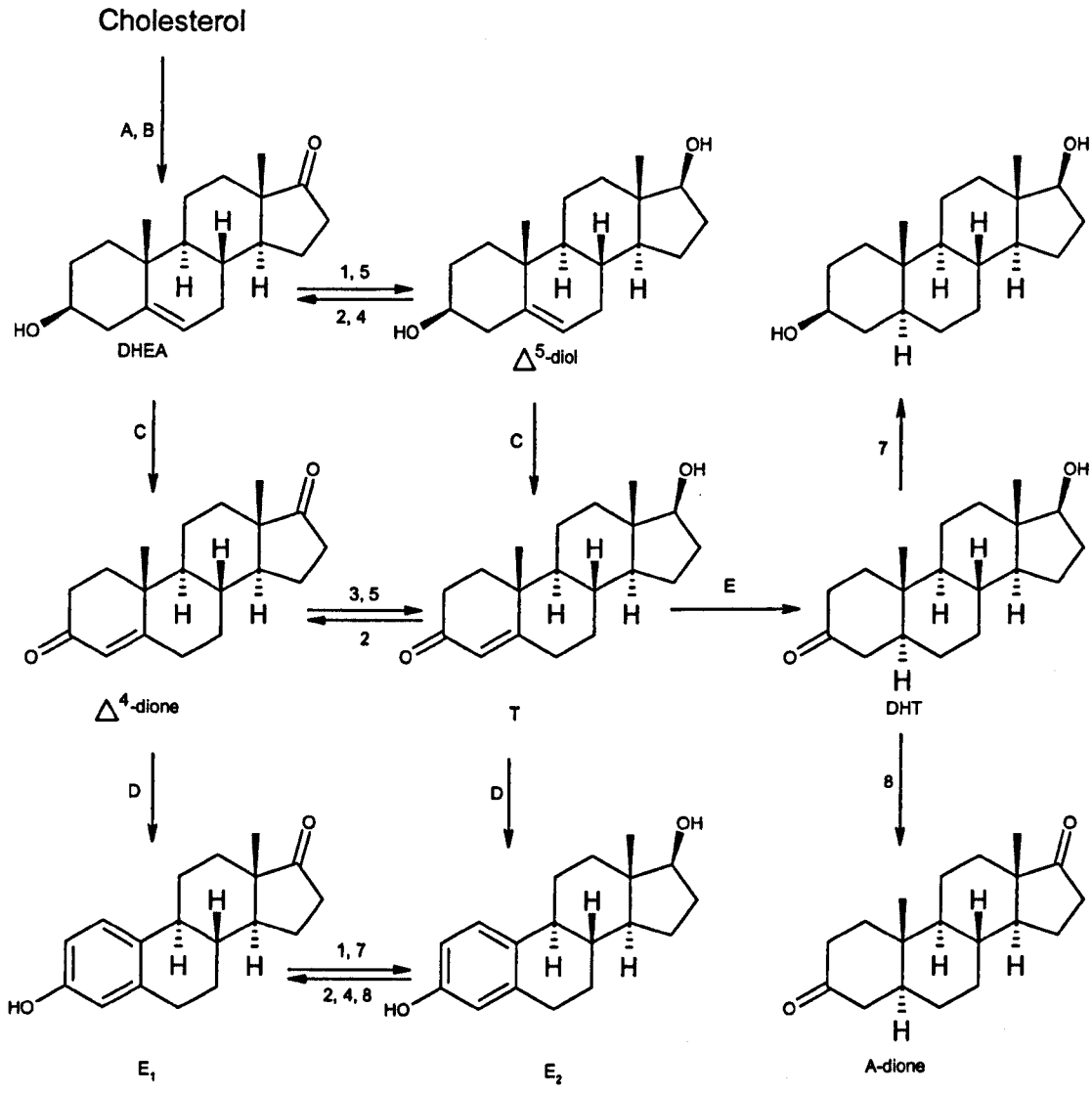
The differences in these two super-families are in their structure and the selective way of hydride transfer, but both exhibit similar chemical mechanisms

in their interaction with tyrosine residue acting as a catalytic base (Shafqat *et al*, 2003).

### 1.3.1. 17β- Hydroxysteroid dehydrogenase (17β-HSD)

The 17β-hydroxysteroid dehydrogenase enzymes play an important part in steroidogenesis. They are involved in the biosynthesis and inter-conversion of steroid hormones and in their regulation. These enzymes have aroused considerable interest due to the fact that they have the ability to accurately regulate hormonal activity and closely maintain cellular responses. As a result they have aroused pharmaceutical interest in the fight against hormone-dependent cancers such as breast or prostate cancer (Prehn *et al*, 2009). The 17β-hydroxysteroid dehydrogenase isoforms catalyse the last stage of androgen and estrogen biosynthesis (Scheme 1.2). In the testicular Leydig cells 17β-HSD transforms androstenedione ($\Delta^4$-dione), an androgen precursor, into testosterone (T) a more potent androgen. In the placenta and ovary, 17β-HSD catalyses the last stage of estrogen biosynthesis in which it transforms the less potent estrogen ($E_1$) into the potent estrogen estradiol ($E_2$) (Scheme 1.2) (Poirier, 2003). When these actions occur in androgen or estrogen target tissues, 17β-HSD isoforms are well placed to regulate ligand possession of each distinctive steroid hormone receptor and therefore could work as a molecular switch. This kind of behaviour of 17β-HSD can arrest the development of hormone-dependent cancers such as prostate and breast cancers. 17β-Hydroxysteroid dehydrogenase uses nicotinamide adenine dinucleotide phosphate (NADPH) or $NADP^+$ as cofactor and their action is

reversible but unidirectional. Every individual type of these enzymes has a selective affinity, path of activity and a particular tissue distribution (Allan *et al*, 2008).




Scheme 1.2: The role of different isoforms of 17β-HSD in human steroidogenesis

The isoforms of 17β-HSD are indicated by numbers and the remaining are: A: P450 side chain cleavage; B: P450 17α-hydroxylase/P450 17,20-lyase; C: 3β-hydroxysteroid dehydrogenase/$\Delta^5$-$\Delta^4$-isomerases; D: aromatase; E: 5α-reductase; DHEA: dehydroepiandrosterone; $\Delta^5$-diol: 5-androstene-3β,17β-diol; 3β-diol: 5α-androstane-3β,17β-diol; $\Delta^4$-dione: 4-androstene-3,17-dione; T: testosterone; DHT: dihydrotestosterone; $E_1$: estrone; $E_2$: estradiol; A-dione: 5α-androstane-3,17-dione (adapted from Poirier, 2003).

#### 1.3.1.1. Mechanism of action of 17β-HSD

In the production of testosterone and estradiol ($E_2$), 17β-HSD types 1, 3, 5, and 7 catalyse the reductive reactions whereas types 2, 4 and 8 catalyse the oxidative reactions (Luu-The, 2001). The mechanisms of both reduction and oxidation are shown in (Figure 1.7) (Penning, 1997).

The enzyme catalysed reduction of androgens or estrogens needs a functional group which must be protonated whereas the enzyme catalysed oxidation requires a functional group which must be deprotonated. Therefore these groups can be classified as general acids or bases. As postulated in (Figure 1.7) by Penning (1997), in the reduction process His117 assists proton donation by Tyr55 to the proton acceptor carbonyl of the steroid (C17) and in the oxidation process Lys84 assists proton removal by Tyr55 by behaving as a general base. Therefore Tyr55 depends upon diverse interactions in the complex to operate as a general base / acid catalyst. During the reduction process the reaction needs a direct hydride relocation from NADPH which is assisted by a proton transfer to a delocalised positive charge on the imidazole ring of His117; in the oxidation process the proton transfer is initiated by Lys84 which forms a phenol anion on Tyr55 to remove a proton from steroid alcohol (C17) and this facilitates the hydride transfer to $NADP^+$.

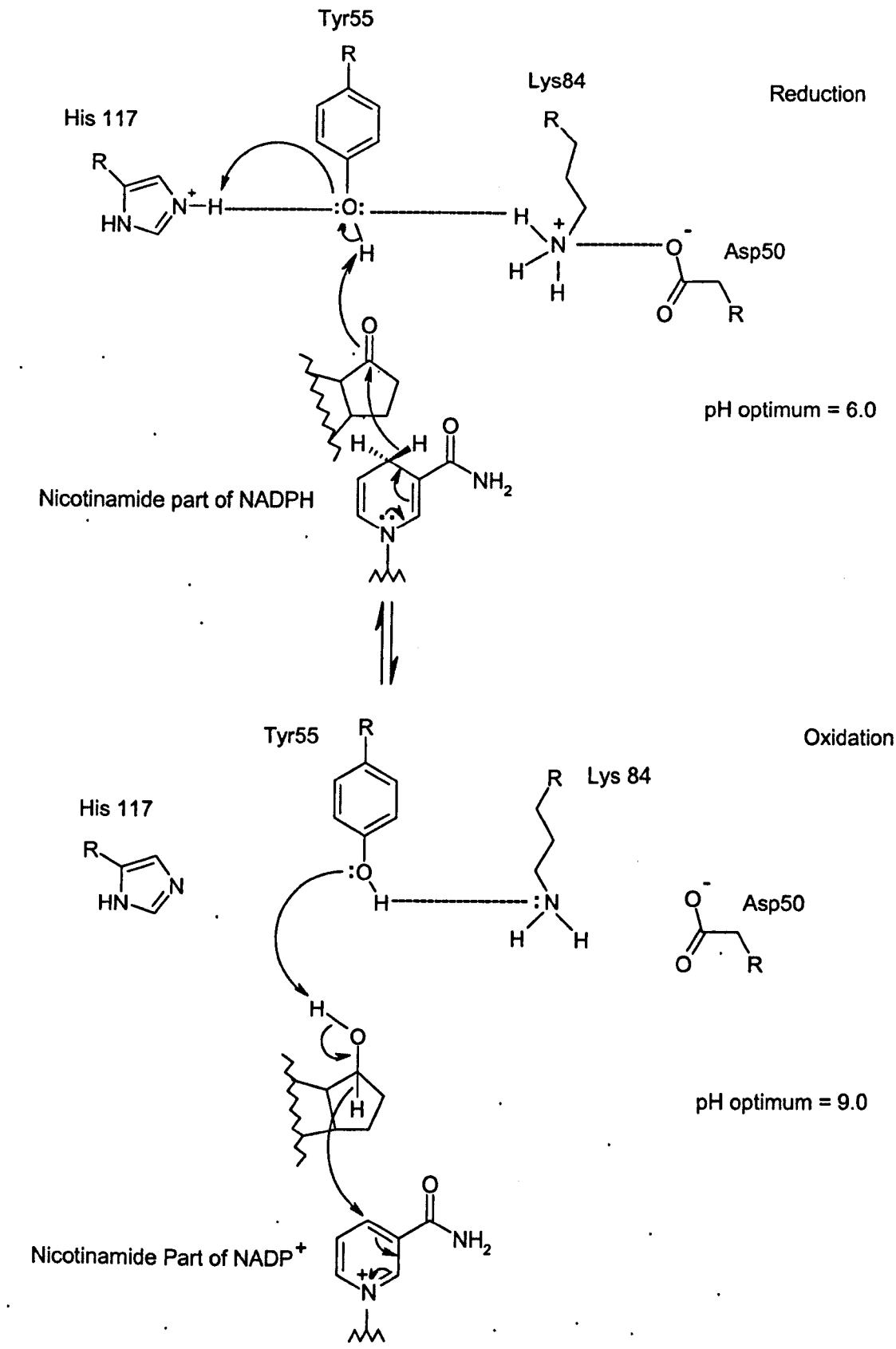



Figure 1.7: Proposed mechanism of action for 17β-HSD (adopted from Penning *et al*, 1997)

#### 1.3.1.2. **The isoforms of 17β-HSD**

To date, 15 isoforms of 17β-HSD have been identified of which 13 are found in humans (Luu-The *et al*, 2008). Apart from 17β-HSD type 5 which is an aldo-keto reductase (AKR), the rest are from the superfamily of short chain dehydrogenase/reductases (SDRs) (Labrie *et al*, 2000). Members of this family catalyse different biochemical reactions and share about 25% of overall sequence identity (Rizner *et al*, 2001). The isoforms of 17β-HSD show some differences in their tissue distribution, catalytic preferences, substrate specificity, sub-cellular localisation and mechanism of regulation. One of the distinctive characteristics of 17β-HSDs is their connection and overlapping substrate specificity with other enzymes; as an example, similar characteristics have been observed between retinol dehydrogenases (RDHs) and rodent 17β-HSD type 5 and 9.

### 1.3.2. **17β-Hydroxysteroid dehydrogenase Type 1**

One of the first 17β-HSDs to be cloned and structurally identified was 17β-HSD type 1 (Duax *et al*, 2000). This enzyme occurs as a soluble homodimer and consists of 327 amino acid sequences with the subunit mass of about 32kDa. 17β-HSD 1 mainly catalyses the reductive transformation of low activity estrone ($E_1$) to the most potent estradiol ($E_2$) (Lukacik *et al*, 2006). 17β-HSD 1 is mainly found in ovaries, breast tissues and placenta and due to its appearance and its adopted reduction reaction pattern, constitutes a leading source of peripheral and gonadol estradiol synthesis (Peltoketo *et al*, 1999).

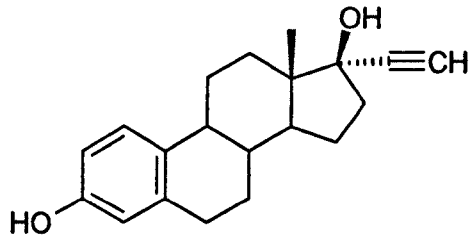
### 1.3.3. 17β-Hydroxysteroid dehydrogenase Type 2

17β-HSD type 2 was initially cloned from human prostate cDNA library and is an important human enzyme which catalyses the conversion of estrogenic and androgenic substrates. This enzyme is not only accountable for oxidation of estradiol ($E_2$) into estrone ($E_1$), but is also responsible for the conversion of testosterone (T) into $\Delta^4$-dione (Scheme 1.2). It is involved in the degradation and control of most potent estrogens and androgens (Poirier, 2003). This enzyme (17β-HSD2) can be found in endometrial tissues such as liver, small intestine, kidney, pancreas, prostate and colon and is particularly highly disclosed in placenta (Peltoketo et al, 1999, Bydal et al, 2004). As 17β-HSD2 is a membrane-bound enzyme whose accurate active site is unknown, but it has been postulated that existence of 387 amino acid residues within the active site would cause enzyme substrate specificity (Ghosh *et al*, 1995).

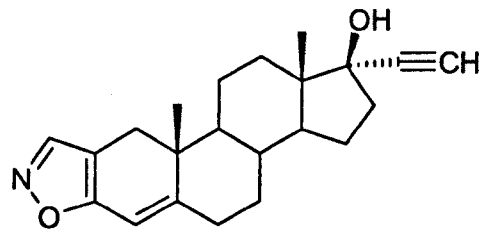
#### 1.3.3.1. Steroidal inhibitors

Blomquist *et al (1984)* published results into the inhibitory activity of some steroid derivatives and non-steroidal agonists and antagonists of the estrogen receptor (ER) against microsomal 17β-HSD 2 of human placenta for oxidation of testosterone to and $\Delta^4$-dione. It was observed that 17α-ethynyl-E2 (**15**) and the synthetic steroid danazol (**16**) (Figure 1.8) both had good binding affinity with enzyme inhibitor constant ($K_i$) values of 0.3μM and 0.6μM respectively in comparison with the natural substrate $E_2$ of 0.8μM. However 17α-ethynyl-E2

was a full agonist of ER while danazol was noticed for its androgenic effect (Blomquist *et al*, 1984).



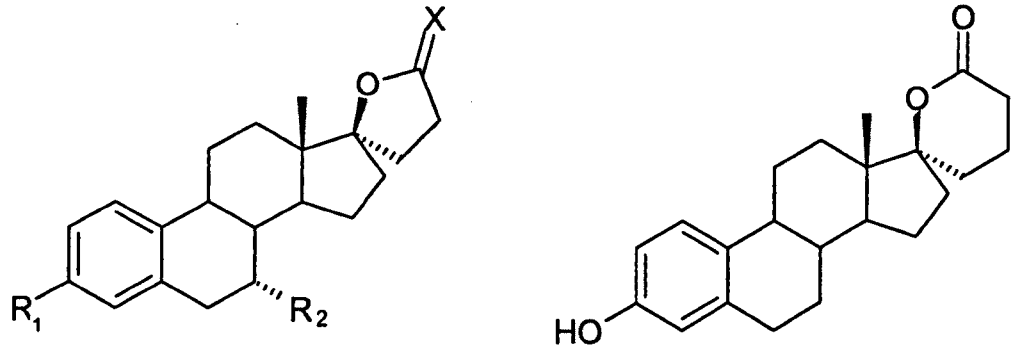

(15) 17α-ethynyl-E2




(16) Danazol

Figure 1.8: Inhibitors of 17β-HSD 2

Blomquist *et al* valuated the effects of the lactone (**17**) (Figure 1.9) incubated in cell culture against cell line A431. These cells were obtained from an epidermoid carcinoma of the vulva. It was observed that (**17**) has a higher affinity toward reduction of $\Delta^4$-dione to testosterone with a $K_i$ value of 1.8μM in comparison to oxidation of testosterone to $\Delta^4$-dione with a $K_i$ value of 2.3μM (Blomquist *et al*, 1997). Sam *et al* tried to remove the estrogenic activity of (**17**) with the synthesis of (**18**) which was not only shown to be a good inhibitor of microsomal 17β-HSD2 with $IC_{50}$ value of 0.35μM which was similar to the lactone (**17**), but also was a selective inhibitor of 17β-HSD2 because of its spiro-γ-lactone moiety. Furthermore, (**18**) exhibited no androgenic or estrogenic behaviour due to the presence of alkylamide side chain at position 7α of the steroidal nucleus (Sam *et al*, 2000).




17 (X=O, $R_1$=OH, $R_2$=H)

18 (X=O, $R_1$=OH, $R_2$=$(CH_2)$10CONBuMe)

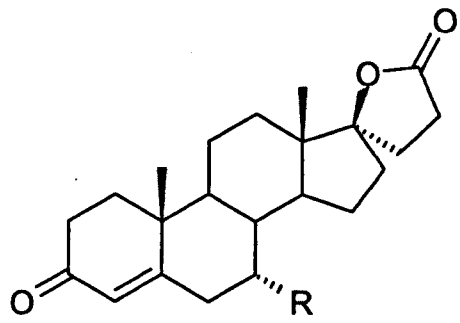
19

Figure 1.9: Lactone derivatives as inhibitors of 17β-HSD2

In order to optimise the activity and minimize the estrogenic effects of (**17**), a new group of C18-steroid lactone derivatives (Figure 1.9) were synthesised and evaluated from which Lactone derivative, (**19**) with a six member δ-lactone was found to possess the highest inhibitory activity with $IC_{50}$ value of 6.0nM and was 150 times more effective than the natural substrate, $\Delta^4$-dione, when used as inhibitor of HEK-293 cells transfected with 17β-HSD2 (Bydal *et al*, 2004).

Sam *et al* (1995) exploited the derivatisation of steroidal spiro-γ-lactone (**17**) and synthesised a range of compounds with the changes in the A and B-rings to evaluate against microsomal 17β-HSD in conversion of $\Delta^4$-dione into testosterone. From their results they noticed that (**20**) was the best inhibitor with 22% inhibition at 1.0μM, nevertheless, it was a sluggish inhibitor. Tremblay *et al* (1999) explored the effect of substitution of the 7α-acetylthio of spironolactone (**20**) with thioalkyl, thioaryl side chain moieties (Figure 1.10) in order to enhance the inhibitory activity of C19-lactones. The results of screening revealed that whereas compounds (**20**, **21** and **25**) were weak inhibitors of 17β-HSD2,

thioalkyl (**22-24**) and thioaryl analogues (**26** and **27**) exhibited good inhibitory activity. In fact thiobenzyl analogue (**27**) was shown to be the most effective inhibitor with the $IC_{50}$ value of 0.42nM.

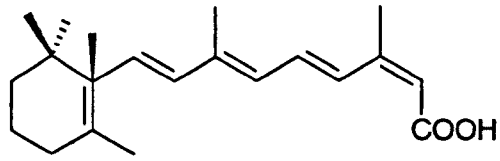



20 (R=$SCOCH_3$)
21 (R=$\Delta^{6-7}$)
22 (R=$SCH_2CH_3$)
23 (R=$S(CH_2)_2CH_3$)
24 (R=$S(CH_2)_5CH_3$)
25 (R=SCOPh)
26 (R=SPh)
27 (R=$SCH_2Ph$)

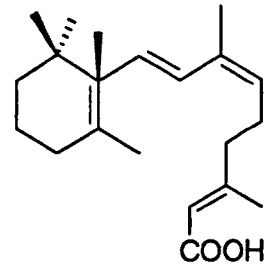
Figure 1.10: C19-steroidal lactone as inhibitors of 17β-HSD2

### 1.3.3.2. **Non-steroidal inhibitors**

In 1994 Murray *et al* examined some retinoids to determine their inhibitory activity against microsomal 17β-HSD2 from rat liver. The results obtained for 13-cis-retinoic acid (**28**) and 9-*cis*-retinoic acid (**29**) (Figure 1.11) show that they possess inhibitory activity with $K_i$ values of 2.4μM and 4.1μM respectively (Murray *et al*, 1994). In 1998 Thiboutot *et al* reported that 13-*cis*-retinoic acid has no inhibition effect on 17β-HSD2 obtained from human sebaceous glands (Thiboutot *et al*, 1998).




(28) 13-*Cis*-retinoic acid




(29) 9-*Cis*-retinoic acid

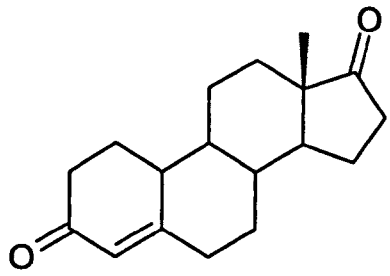
Figure 1.11: Retinoic acids as inhibitors of 17β-HSD2

### 1.3.4. 17β-Hydroxysteroid dehydrogenase Type 3

17β-Hydroxysteroid dehydrogenase 3 (17β-HSD3) was the third enzyme from this family to be identified (Luu-The, 2001). This enzyme is highly expressed in the testes and seminal vesicles. It is also found in a small level in the prostate gland (Vicker *et al*, 2009). 17β-Hydroxysteroid dehydrogenase 3 belongs to the short-chain dehydrogenase/reductase (SDRs) super family (Prehn *et al*, 2009). It consists of 310 amino acids with a mass of about 35KDa. It has been found that 17β-HSD3 deficiency causes male pseudohemaphroditism in youth (Luu-The *et al*, 2008). Despite the crystal structure of the enzyme has not been determined yet it is known that it is bound through the N-terminal transmembrane domain to the endoplasmic reticulum (Lukacik *et al*, 2006). The main function of 17β-HSD3 is the conversion of low activity $\Delta^4$-dione to the more active form testosterone in a NADPH-dependent manner (Laplante and Poirier, 2008). The role of this enzyme in the biosynthesis of testosterone makes it an interesting biochemical target for trestment of hormone-dependent prostate cancer (Vicker *et al*, 2009).

### 1.3.4.1. **Steroidal inhibitors**

The first generation of inhibitors of 17β-HSD 3 was reported by Pittaway in 1983. He suggested that a good inhibitor of 17β-HSD3 should contain a carbonyl at position 17 and a non aromatic A-ring. In this pursuit he synthesised a range of steroidal compounds and tested them against 17β-HSD3 isolated from canine testis. Pittaway found two good inhibitors, among his compounds, 4-estrene-3,17-dione (**31**) and 5-androstene-3,17-dione (**32**) (Figure 1.12) which had the best inhibitory activity with $K_i$ values of 2.4μM and 6.8μM respectively (Pittaway, 1983).

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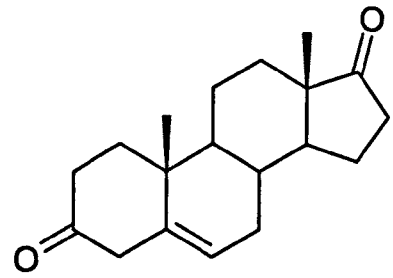


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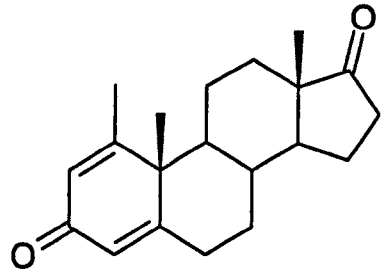


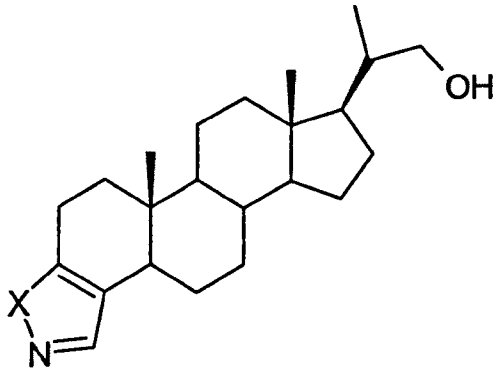

(33) Atamestane

Figure 1.12: Steroidal inhibitors of 17β-HSD3

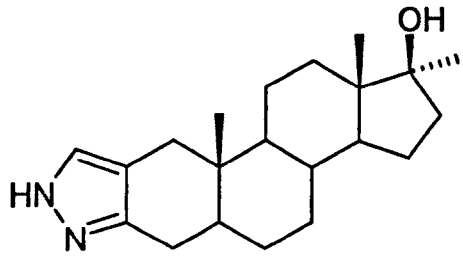
Compound (**31**) was found the most potent and differed from the natural substrate $\Delta^4$-dione by the absence of the C-19 methyl group and compound

(**32**) by change in position of carbon carbon double bond ($\Delta^4$ to $\Delta^5$). The Lombardo group discovered that atamestane (**33**) (Figure 1.12) recognised for its irreversible inhibitory activity against aromatase was a good competitive inhibitor for the reduction of $\Delta^4$-dione to testosterone using NADPH against 17β-HSD3 with the $K_i$ value of 1μM. Atamestane in contrast with natural substrate $\Delta^4$-dione has C1-C2 double bond as well as C1 methyl group (Lombardo *et al*, 1993).

Levy and co-workers examined the effect of C2, C3-pyrazole and C3, C4-pyrazoles on the specificity of inhibition of 17β-HSD3 enzyme isolated from the bacterium *Pseudomonas testosteroni.* They showed that C3, C4-pyrazole compounds (**34-36**) (Figure 1.13) were effective inhibitors with $K_i$ values of 6nM, 7nM and 100nM respectively and C2, C3-pyrazole compounds (**37**) and (**38**) (Figure 1.13) with $K_i$ values of 20nM and 15nM for oxidation of testosterone to $\Delta^4$-dione. In conclusion they showed that the steroidal pyrazoles were effective and selective types of competitive inhibitors of 17β-HSD type 3. In fact these inhibitors didn't show any inhibitory activity toward 5α-reductase from rat prostatic microsomes and 3α-HSD from rat liver and 3α (20β)-HSD from *Streptomyces hydrogenans* at elevated concentration. But knowing the fact that 17β-HSD3, obtained from bacterial source, would have catalysed the oxidation of 3β-hydroxy and also 17β-hydroxy, as a result 17β-HSD3 obtained from *pseudomonas testosteroni* would not be a suitable source of 17β-HSD3 for assay and another source of enzyme should be considered such as rat testes (Levy *et al*, 1987).




34 (X=NH)
35 (X=NH, $\Delta^{1,2}$)
36 (X=O)

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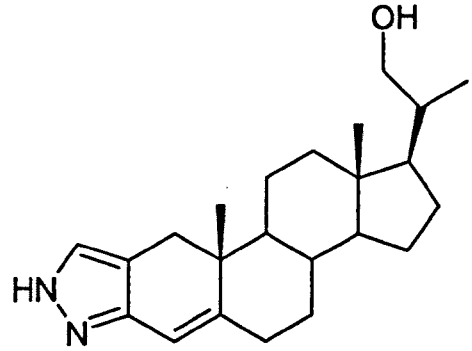


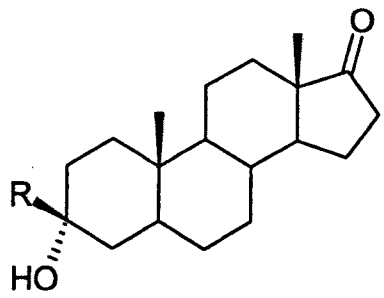

38

Figure 1.13: Fused azole compounds as inhibitors of 17β-HSD3

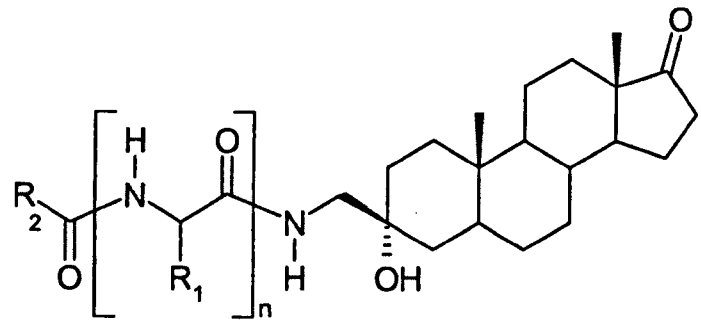
The evaluation and study of about eighty different types of steroids persuaded Poirier *et al* (1995) to consider the C19-steroid androsterone (ADT: **39**) (Figure 1.14) as promising starting material for synthesising new inhibitors of 17β-HSD type 3. As such they synthesised several compounds and their inhibitory activity was determined using the microsomal fraction of HEK-293 cells transfected with 17β-HSD type 3. The first group of inhibitors synthesised were based on substitution of ADT at the 3β position, compounds (**40-50**) (Figure 1.14) with $IC_{50}$ values ranged from 57nM to 200nM. These were much better results in comparison to the ADT with $IC_{50}$ values of 330nM. From this range of compounds 3β-phenylmethyl-ADT (**49**), 3β-cyclohexyethyl-ADT (**47**), 3β-propyl-ADT (**41**) and 3β-sec-butyl-ADT (**42**) had shown the most inhibitory activity with

$IC_{50}$ values of 57nM, 60nM, 67nM and 73nM respectively and because no other kind of interaction or inhibition was noticed would indicate their specific inhibition (Tchédam-Ngatcha *et al*, 2000).

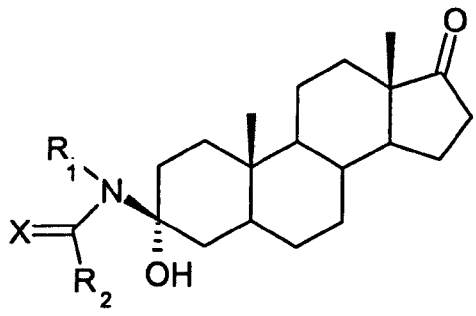
Maltais *et al* tried to optimise and reduce the androgenic effect of ADT. As such they accomplished solid-phase parallel synthesis of 3β-peptido-3α-hydroxy-5α-androstan-17-ones in three levels of molecular distinction. They synthesised and biologically evaluated inhibitory activity of their compounds against 17β-HSD. From the results obtained they noticed that third level compounds which contained at least one phenyl group were the best inhibitors, specially compound (**51**) with an $IC_{50}$ value of 227nM which was even better than the natural substrate $\Delta^4$-dione ($IC_{50}$=489nM) when used alone. They also notice that (**51**) had a minor androgenic activity at 1μM concentration, but none at 0.1μM concentration (Maltais *et al*, 2001). The same group also tried to optimize the potency of 3β-substituted ADT derivatives by parallel liquid-phase synthesis approach (Maltais *et al*, 2002). As such they synthesised several libraries of compounds and examined them. From which four compounds (**52-56**) found to posses good inhibitory activity with $IC_{50}$ values ranged from 35nM to 85nM. Also they synthesised range of compounds with various $R_1$ and $R_2$ groups of 3-carbamate-N-substituted-5α-androtan-17-one in order to displace adamentane group. Biological evaluation against 17β-HSD type 3 of compounds shown that compound (**57**) was the most potent of them all with similar activity to (**55**), but without androgenic activity (Maltis and Poirier, 1998; Maltis *et al*, 2002).



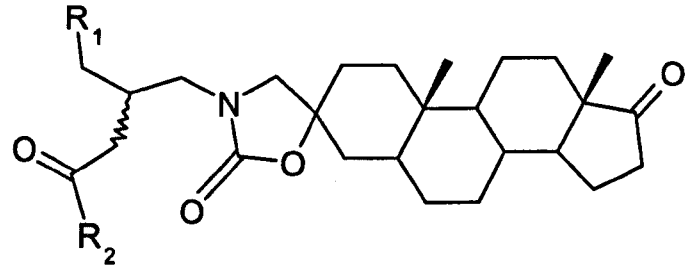

39 (ADT) (R=H)
40 (R=$CH_3$)
41 (R=$CH_3(CH_2)_2$)
42 (R=$CH_3CH_2(CH_3)CH$)
43 (R=$CH_3(CH_2)_5$)
44 (R=$CH_3(CH_2)_7$)
45 (R=Cyclohexyl)
46 (R=Cyclohexyl-$CH_2$)
47 (R=Cyclohexyl-$(CH_2)_2$)
48 (R=Phenyl)
49 (R=Phenyl-$CH_2$)
50 (R=Phenyl-$(CH_2)_2$)




51 (n=2, $R_1$=Phenylmethyl, $R_2$=Hexyl)




52 (X=O, $R_1$=Octyl, $R_2$=Cyclopropyl)
53 (X=O, $R_1$=Octyl, $R_2$=Cyclopropyl)
54 (X=O, $R_1$=Cyclohexylmethyl, $R_2$=Cyclopropyl)
55 (X=O, $R_1$=Adamentylmethyl, $R_2$=Propyl)
56 (X=$H_2$, $R_1$=Adamentylmethyl, $R_2$=Propyl)

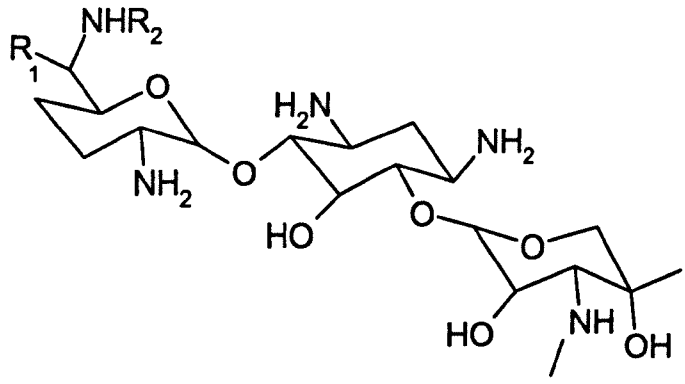



57 ($R_1$=Morpholine, $R_2$=Cyclopentylethyl)

Figure 1.14: ADT derivatives inhibitors of 17β-HSD3

### 1.3.4.2. **Non-steroidal Inhibitors**

Ghosh and Dasgupta investigated the effect of the antibiotic Gentamicin (**59**) (Figure 1.15) on the male rats over a period of seven days with dosage of 40, 60, 80 and 100mg/kg. They found reductions in the activity of 3β-HSD and 17β-HSD3 occurred in a dose dependent manner. In fact gentamicin showed inhibitory of 34% and 37% activity at doses of 80 and 100mg/kg respectively toward 17β-HSD3 (Ghosh and Dasgupta, 1999). However (**59**) was not a good inhibitor as high amount of sample would require to causing a response.



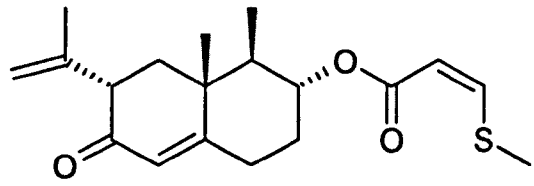

(59)

Figure 1.15: Inhibitor of 17β-HSD3

Gentamicin is the combination of a, b and c forms.
a; ($R_1$=$R_2$=$CH_3$), b; ($R_1$=$CH_3$, $R_2$=H), c; ($R_1$=$R_2$=H)

Lin *et al* examined a well known anti-inflammatory agent sesquiterpene s-petasin (**60**) (Figure 1.16) *in vivo* against the production of testosterone by rat testes and demonstrated that it requires only one dose of s-petasin at 1μg/kg via intravenous injection to bring down plasma level of testosterone by 38% in just half an hour. They also tested this agent *in vitro*. They incubated rat

testicular interstitial cell together with (**60**) in dosages from 0 to 43μM and natural substrate at 1nM concentration. They observed that testosterone production decreased significantly as the concentrations of (**60**) increased from 4.3μM to 43μM. They believed that their findings were due to the lowering of the activities of 17β-HSD (17-ketosteroid reductase) and adenylyl cyclase (Lin *et al*, 2000).




(60) Sesquiterpene S-petasin

Figure 1.16: Inhibitor of 17β-HSD3

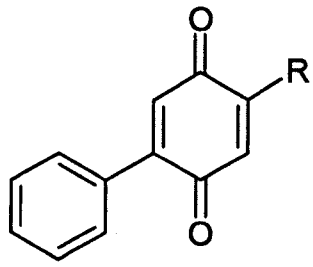
The inhibitory activity of some poly-chlorinated (tri, tetra and penta) biphenyl compounds was investigated *in vivo* and *in vitro* by Andric *et al* against rat testicular androgenesis. *In vivo* they observed that the testosterone and dihydrotestosterone level of serum was reduced by about 29-33% in 24h after a single intraperitoneal injection at about 10μg/kg (Andric *et al*, 2000).

Das and Dasgupta studied the effect of nickel salts as inhibitors of 17β-HSD3. They found that a diet containing nickel sulphate could substantially decrease the action of 3β-HSD and 17β-HSD3 and thus the production of testosterone. They observed that this reduction was higher when a low protein intake diet was applied in comparison with a normal diet (Das and Dasgupta, 2002).

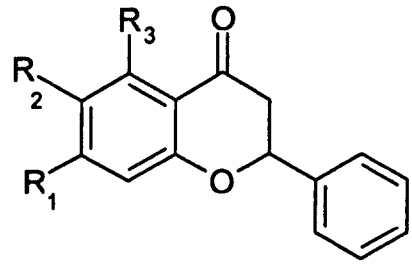
Ghosh *et al* studied the inhibitory activity of cadmium on 17β-HSD3 in the rat toad testis. They noticed that one injection of cadmium chloride (0.5mg/kg)

reduced the level of testosterone and DHT in the serum by 47% after seven days (Ghosh *et al*, 1987). However cadmum chloride was not a good inhibitor due to its toxicity.

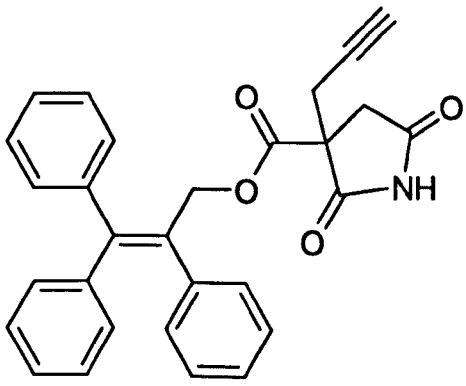
Le Lain *et al* examined a range of nonsteroidal compounds as inhibitors of 17β-HSD3 obtained from human testicular microsomes to inhibit the conversion of $\Delta^4$-dione to testosterone with substrate concentration at 2μM in presence of NADPH. They obtained $IC_{50}$ of samples ranging from 2.7μM to 100.5μM and the most potent inhibitors were, 2, 5-diphenyl-p-benzoquinone (**61**), phenyl-p-benzoquinone (**62**), 7-hydroxy flavone (**63**), baicalein (**64**), triphenylethene derivative (**65**), and biochanin A (**66**) (Figure 1.17) with $IC_{50}$ values of 2.7μM, 5.7μM, 9.0μM, 9.1μM, 9.3μM and 10.8μM respectively (Le Lain *et al*, 2001).



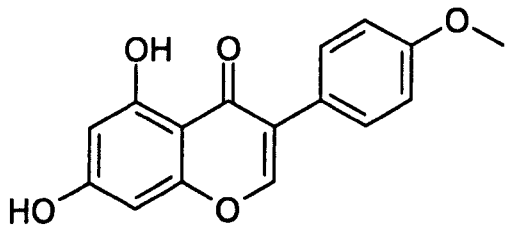

61 (R=Phenyl)
62 (R=H)




63 ($R_1$=OH, $R_2$=$R_3$=H)
64 ($R_1$=$R_2$=$R_3$=OH)




65

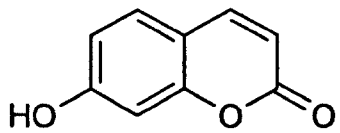



(66) Biochanin A

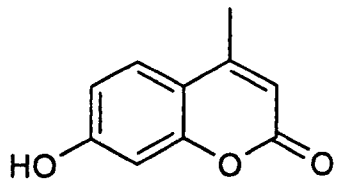
Figure 1.17: Non-steroidal inhibitors of 17β-HSD3

In the following study Le Lain *et al* tested coumarin based compounds umbelliferone (**67**) and 4-methylumbelliferone (**68**) (Figure 1.8) against 17β-HSD3. They observed that these compounds were potent inhibitors with $IC_{50}$ values of 1.4μM and 0.9μM respectively (Le Lain *et al*, 2002).

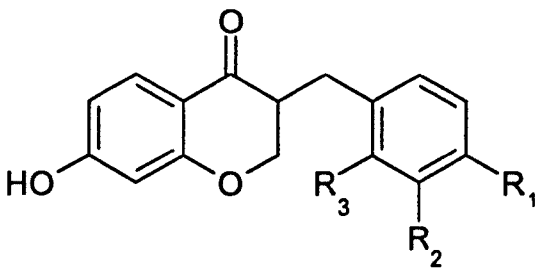
In 2001 Smith *et al* evaluated a series of compounds based on benzyl tetralin using human testes microsomes as a source of 17β-HSD3. The inhibitory activity of samples (**69-71**) (Figure 1.8) was determined at substrate concentrations of 2.0μM and $IC_{50}$ values of 1.8μM, 8.3μM and 7.0μM respectively. These compounds (**69-71**) were also evaluated for their inhibitory effect against rat testes microsomal 17β-HSD for reduction of $\Delta^4$-dione at 100μM concentration. The $IC_{50}$ values obtained ranged from 58% to 79% inhibition which is greater in contrast to the human enzyme. The compound (**72**) was found to be the most active with an $IC_{50}$ value of 32.7μM. However the lack of specificity toward 17β-HSD type 3 made these compounds less suitable as drugs (Smith *et al*, 2001).




67




68




69 ($R_1$=Cl, $R_2$=$R_3$=H)

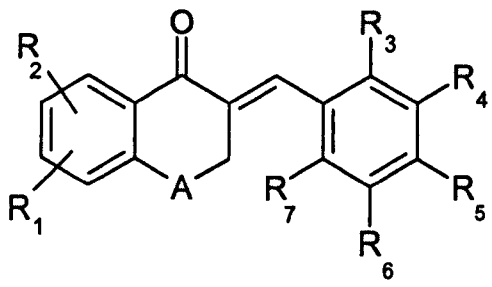
70 ($R_1$=Br, $R_2$=$R_3$=H)

71 ($R_1$=F, $R_2$=$R_3$=H)

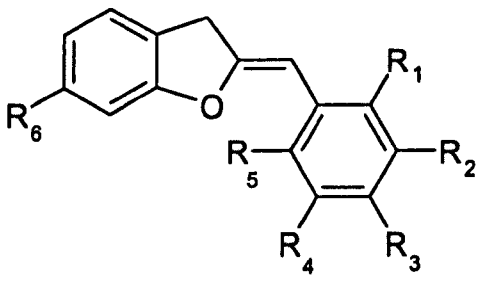
72 ($R_1$=$R_3$=Cl, $R_2$=H)

Figure 1.18: Coumarin and benzyl tetralin derivatives as inhibitors of 17β-HSD3

Novel derivatives of tetralone (**73**) and benzofuranone (**74**) have been shown (Figure 1.19) to exert some inhibitory activity toward 17β-HSD type 3 (Yoshihama *et al*, 1998; Yoshihama *et al*, 1999).



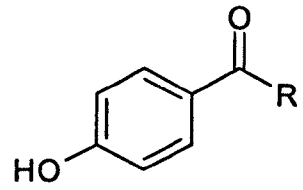

73




74

Figure 1.19: Non-steroidal inhibitors of 17β-HSD3

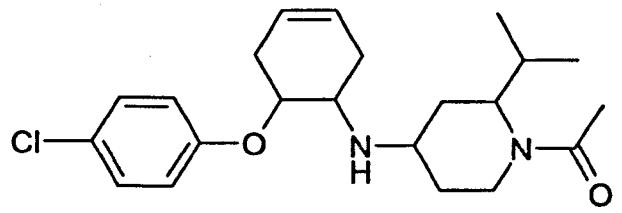
Lota *et al* (2006) were concluded from an extensive molecular modelling study that the carbonyl functional group was a pivotal feature of any potential inhibitor of the 17β-HSD family involved in the reduction of the steroid C(17)=O moiety within the natural substrate and as such they synthesised and biologically evaluated several non-steroidal 4-hydroxyphenyl ketone compounds. They assayed their compounds against rat testes microsomal enzyme with reference to that of baicalein and 7-hydroxy flavone (Lota *et al*, 2006). The results obtained showed that the majority of samples were more effective inhibitors in comparison to the references (baicalein and 7-hydroxy flavone) apart from (**75**) acetophenone. The best inhibitory results were achieved from (**77-81**) (Table 1.1) and it was learnt that (**79**) was the best inhibitor in the range. This study revealed that by addition of an alkyl chain the inhibition was increased due to increased hydrophobicity of the compounds until they reached the optimum partition coefficient (log P) and after that inhibition decreased (Lota *et al*, 2006).



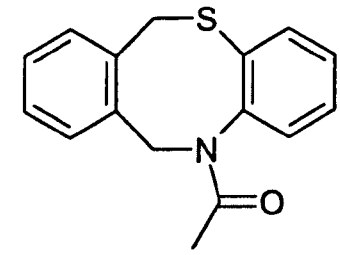

| Compound | R= | % inhibition [I]=100μM | $IC_{50}$ (μM) |
|---|---|---|---|
| 75 | $CH_3$ | 36.6±0.50 | 1708.92±170.71 |
| 76 | $C_4H_9$ | 61.81±0.89 | 60.52±5.82 |
| 77 | $C_6H_{13}$ | 80.26±0.20 | 7.84±0.36 |
| 78 | $C_7H_{15}$ | 82.58±0.49 | 6.52±0.18 |
| 79 | $C_8H_{17}$ | 83.53±0.48 | 2.86±0.03 |
| 80 | $C_9H_{19}$ | 81.39±0.09 | 4.97±0.25 |
| 81 | $C_{11}H_{23}$ | 78.92±0.58 | 7.55±0.32 |
| - | Baicalain | 38.78±1.36 | 185.92±12.70 |
| - | 7-hydroxyflavone | 53.59±0.52 | 66.98±0.95 |

Table 1.1: 4-hydroxyphenyl ketones as potential inhibitors of 17β-HSD 3

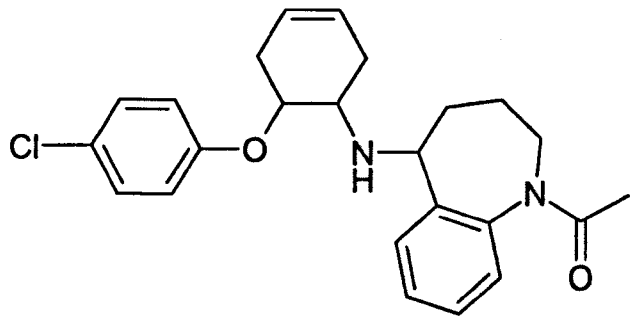
Day *et al* examined about fifty compounds for their inhibitory activity toward 17β-HSD3. From their results they found that STX2171 (**82**) and STX2624 (**83**) (Figure 1.20) had shown $IC_{50}$ values of 208nM and 441nM respectively at about 10μM concentration (Vicker *et al*, 2009). These results were not far from that of dibenzothiazocine (STX2622) (**84**) used as a reference with an $IC_{50}$ value of 201nM. STX2171(**82**) and STX2624 (**83**) were both found to be selective for 17β-HSD3 inhibition with a negligible activity toward 17β-HSD type 2 at 10μM concentration and none toward 17β-HSD type 1 when they were evaluated *in vivo* and *in vitro* adopting radiometric assay with whole prostate cancer cell lines (LNCaP) (Day *et al*, 2009).




(82) STX2171




(84) STX2622




(83) STX2624

Figure 1.20: Inhibitorsof 17β-HSD

### 1.3.5. **17β-Hydroxysteroid dehydrogenase Type 4**

Human 17β-HSD type 4 is a unique multifunctional enzyme as it encodes for peroxisomal 736 amino acid protein. This enzyme has the molecular mass of 80KDa (Labrie *et al*, 2000) comprises three different domains (N-terminal dehydrogenase, hydratase, and sterol carrier like domain) (Lukacik *et al*, 2006) and it is widely distributed around the body including in the liver, heart, prostate, lung, kidney, pancreas, thymus, ovary, intestine, and several human breast cell lines. Its main function is the oxidation of estradiol ($E_2$) to estrone ($E_1$) and metabolising fatty acid β-oxidation (Luu-The *et al*, 2008). The mutation of 17β-HSD4 (Figure 1.21) gene can cause Zellwegar syndrome, distinguished by

signs such as craniofacial dysmorfism, macrocephaly and other syndromes which lead to a short life expectancy after birth (Lukacik *et al*, 2006).

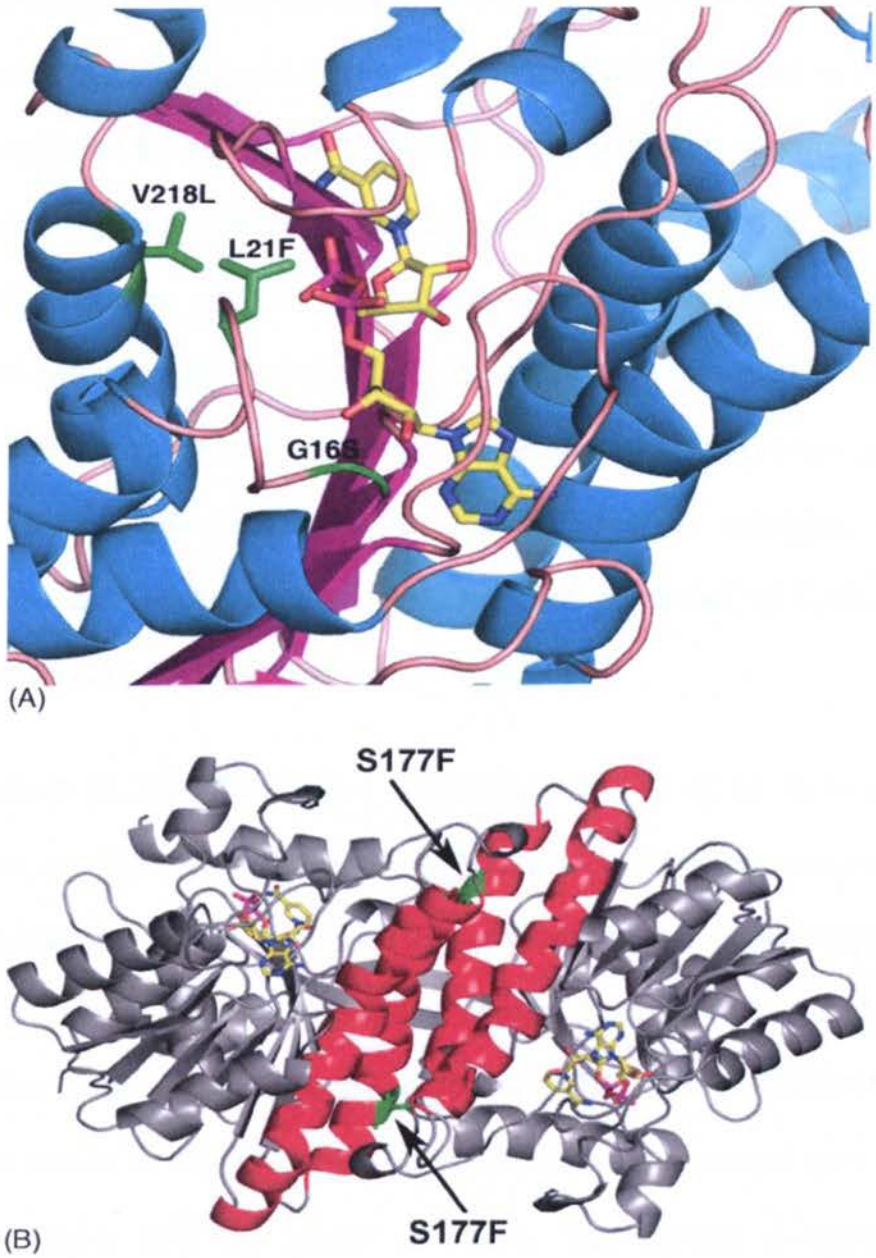



Figure 1.21: Mutation in human 17β-HSD4.

(A) Mutation at three sites (V218L, L21F and G16S) close to $NAD^+$ and cofactor site (B) Mutation to amino acid phenylanaline which could interfere with dimerisation (Lukacik *et al*, 2006).

### 1.3.6. 17β-Hydroxysteroid dehydrogenase Type 5

17β-Hydroxysteroid dehydrogenase type 5 is a special kind of 17β-HSDs due to the fact that this enzyme belongs to the aldo-ketoreductase super family (AKR) in contrast to the rest of 17β-HSDs members which appertain to short-chain dehydrogenase/reductase (SDR). It is mainly found in testes and extragonadal tissues such as prostate and liver and catalyses the NADPH-dependent reduction of $\Delta^4$-dione to testosterone (Luu-The et al, 2001). 17β-HSD5 is a monomeric kind of enzyme with the size of 37KDa. It has α/β barrel structure which binds cofactor in an anti-conformation, catalyses 4-Pro-R-hydride shift and encloses a safeguarded catalytic tetrad of Tyr55, Lys84, Asp50 and His117 mechanism (Penning et al, 2001).

The structure of 17β-HSD 5 was determined by Qiu *et al*, in two ternary complexes as 17β-HSD5/$\Delta^4$-dione/NADP and 17β-HSD5/testosterone/NADP. This enzyme sustains a hydrophobic substrate-binding pocket due to the enclosed protein residues which produce an egg shape cavity at the C-terminal end of the barrel. The $\Delta^4$-dione complex (17β-HSD5/$\Delta^4$-dione/NADP) is positioned inside of the enzyme and is compact and narrow with scalene triangular shape and the length of 450Å. Testosterone complex (17β-HSD5/testosterone/NADP) is open to the surface and substrate (T) is located toward the cofactor-binding site with the larger cavity site compared to $\Delta^4$-dione complex and the length of about 790Å (Figure 1.22) (Qiu *et al*, 2004).

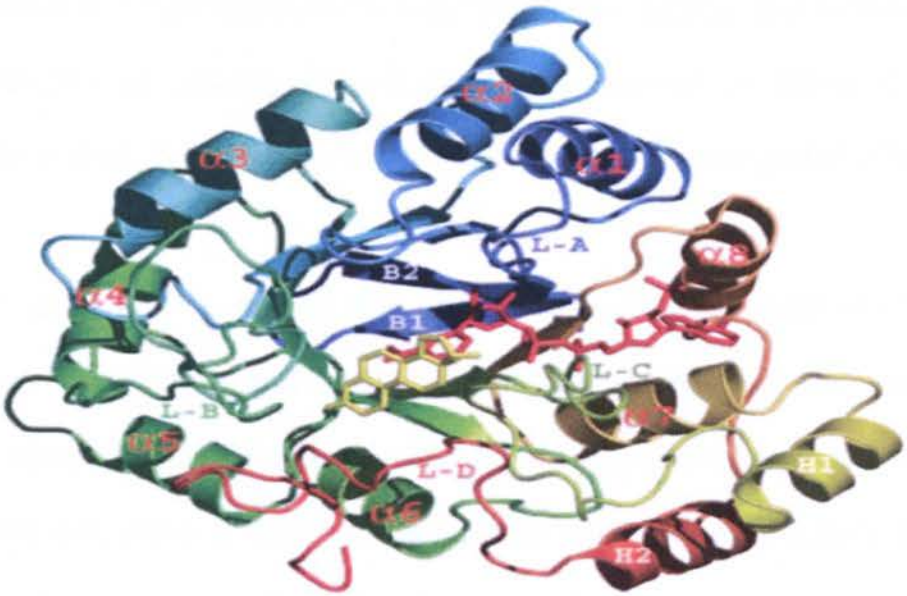



Figure 1.22: Sketch representation of 17β-HSD5/ Testosterone/ NADP complex.

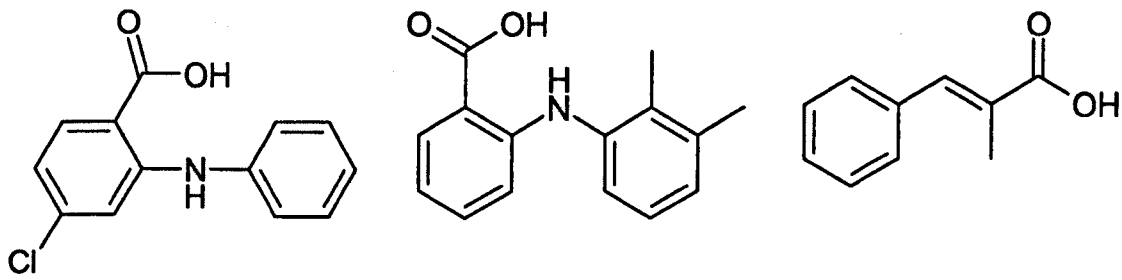
The testosterone is represented with yellow stick and NADP with red stick color. The two β-strands (dark blue) form a β-hairpin turn at the N-terminal of the β—barrel. The α-helix (yellow and brown). The large loops A (blue), B (green), C (light green) and D (red) form the substrate and cofactor binding sites at the C-terminal end of the α/β barrel (adapted from Qiu et al, 2004).

### 1.3.6.1. **Non-steroidal inhibitors**

From structure activity relationship (SAR) studies Penning *et al* (2006) suggested the development of non-steroidal anti-inflammatory drug (NSAID) analogues of N-phenylanthranilic acid. It was hoped that these would not interact with the cyclooxigenase (COX) enzyme and that they would retain their activity and specificity toward AKR1C3 enzyme (AKR1C3 is sometimes referred to as 17β-HSD 5) (Penning *et al*, 2006). They synthesised several compounds and evaluated against AKR1C3 and found that 4-chloro-N-phenylanthranilic acid (**85**) (Figure 1.23) had the highest inhibitory activity with

$IC_{50}$ value of 3.0μM without effecting COX-1 and COX-2, but mefenamic acid (**86**) despite its potency ($IC_{50}$=0.39μM) (Bauman *et al*, 2004) showed no selectivity at all, in fact it inhibited all AKR enzymes (Penning *et al*, 2006).

Brozic *et al* (2006) studied the use of trans-cinnamic acid analogues as inhibitors of AKR1C3 and in this pursuit they synthesised and evaluated a range of compounds. The results obtained using spectrophotometric technique, revealed that some of their compounds had good inhibitory activity in the low micromolar range, in fact the best result was obtained from the α-methylcinnamic acid (**87**) (Figure 1.23) with the $IC_{50}$ value of 6.4μM (Brozic *et al*, 2006).




85 (86) Mefenamic acid 87

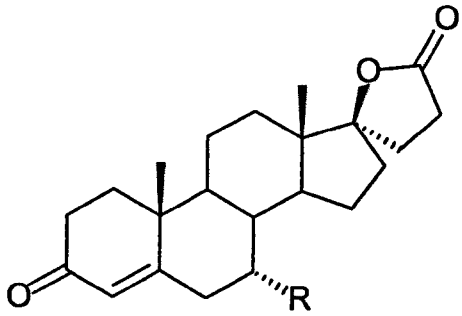
Figure 1.23: Cinnamic acid and *N*-phenylanthranilic acid analogues as inhibitors of AKR1C3

### 1.3.6.2. **Steroidal inhibitors**

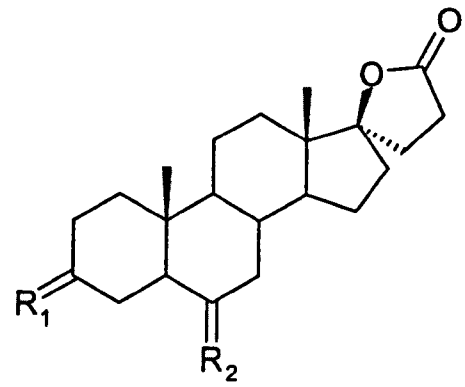
Bydal *et al* evaluated a series of C19-steroids bearing spiro- δ-lactones (**88-97**) (Figure 1.24) for their inhibitory activity against 17β-HSD 5 transfected in HEK-293 cells. The group found mono-spiro-γ-lactones (**88-91**) gave good inhibitory activity of 57%, 61%, 45% and 63% at 3μM concentration (results obtained

using substrate [$^{14}$C] $\Delta^4$-dione and phosphoimager system instrument) respectively whereas the bi-spiro-lactone (**92**) showed poor activity (Bydal *et al*, 2009).

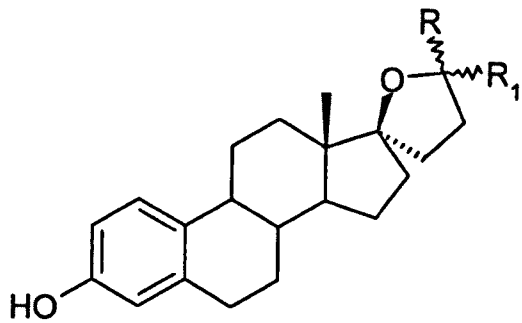
The same group also synthesised and evaluated some C18-steroid lactones based on estradiol ($E_2$) derivatives at 0.3μM and 3μM concentrations. The results showed that compound (**93**) was a more potent inhibitor (47% and 79% inhibition) compared to (7-27% and 29-63% inhibitions) of C19-steroid lactones (**88-92**). In the same studies the effect of a carbonyl group was tested by using *O*-methylated spiro-lactols (**94** and **95**) at 3μM concentration. These compounds shown 61% and 16% inhibitory activity respectively that were less potent compared to (**93**) with 79% inhibitory activity. The effects of increased lactone ring size was test by compound (**96**) (six member ring) and compound (**97**) (seven member ring) at concentrations of 0.3μM and 3μM. In general these compounds have shown good inhibitory activities of 92% and 90% inhibitions at 0.3μM and 95% and 93% inhibitions at 3μM (Bydal *et al*, 2009).



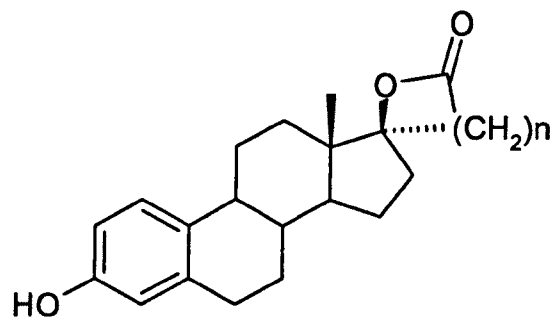

88 (R=$SCOCH_3$)
89 (R=H)




90 ($R_1$=$R_2$=O)
91 ($R_1$=H/$OCH_3$, $R_2$=H)
92 ($R_1$=spiro-γ-butyrolactone, $R_2$=H/H)




93 (R=O)
94 (R&$R_1$=H/$OCH_3$) (less polar)
95 (R&$R_1$=$OCH_3$/H) (more polar)




96 (n=3)
97 (n=4)

Figure 1.24: C18 and C19-steroid lactones as inhibitors of 17β-HSD5

## 1.3.7. 17β-Hydroxysteroid dehydrogenase Type 6

17β-Hydroxysteroid dehydrogenase type 6 has not been found in human (Lukacik *et al*, 2006) however it is found in rodents where it is responsible for the inactivation of androgens (Mindnich *et al*, 2004). 17β-HSD 6 shares about 65% identity with rat's type 1 retinol dehydrogenase (RoDH1) and selectively catalyses the conversion of 3α-diol to androsterone (Labrie *et al*, 2000).

### 1.3.8. 17β-Hydroxysteroid dehydrogenase Type 7

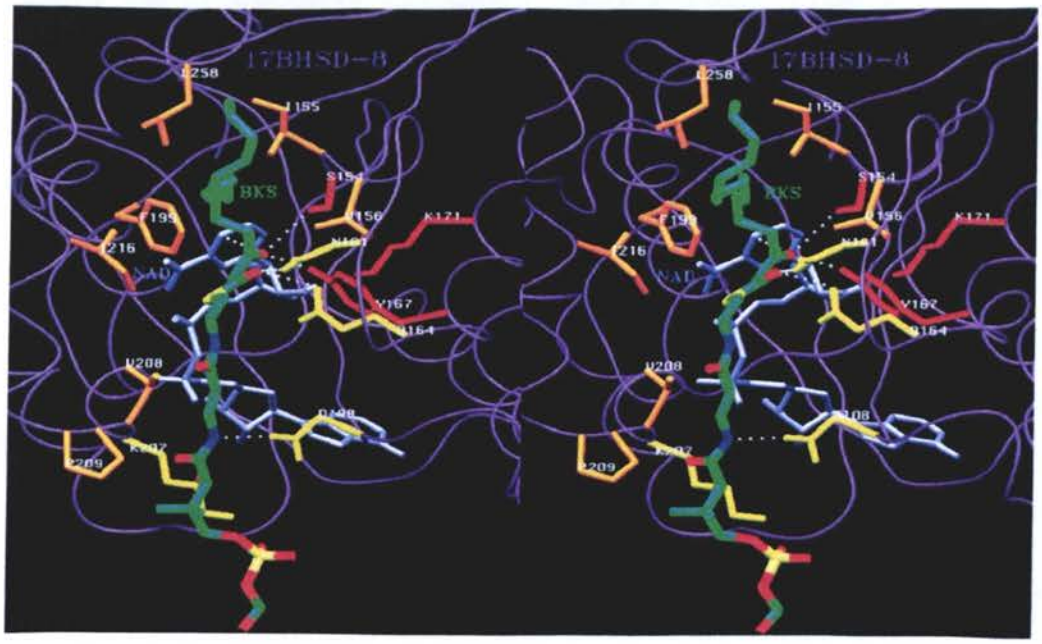
Duan *et al* (1996) initially classified 17β-HSD7 as a prolactin receptor associated protein and was cloned from a corpus luteum cDNA library (Duan *et al*, 1996). The human 17β-HSD 7 was identified by RT-PCR in pregnant uterus and placenta and in the ovaries of non pregnant females and is mapped to chromosome 10p11.2 where some human diseases were mapped for such as prostate cancer. This enzyme shares about 78% amino acid homology with rat (Torn *et al*, 2003), it has the molecular mass of 37KDa or 341 amino acids and can be detected within the breast, ovary, and placenta, testes, prostate and liver. 17β-HSD 7 shares less that 20% identity with other 17β-HSDs (Labrie *et al*, 2000). The high concentration of this enzyme can readily convert inactive estrone ($E_1$) to the most potent estrogen estradiol ($E_2$) in a NADPH dependent manner which is believed to play an important part in estrogen sensitive cells and tissues (Krazeisen *et al*, 1999). 17β-HSD7 is recognised to exhibit a 3-keto reductase activity facilitating the conversion of DHT into 5α-androstane-3β, 17β-diol (Luu-The *et al*, 2008).

### 1.3.9. 17β-Hydroxysteroid dehydrogenase Type 8

17β-hydroxysteroid dehydrogenase type 8 is originally known as the product of Ke6 gene which has been associated with inactive form of polycystic kidney disease (PKD) in mice (Fomitcheva *et al*, 1998). This gene encodes a protein of 274 amino acids (Labrie *et al*, 2000). Additionally it has been postulated that this enzyme is engaged in the regulation of fatty acid metabolism. This enzyme

not only effectively transforms estradiol ($E_2$) to estrone ($E_1$) but also converts testosterone to $\Delta^4$-dione to a lesser extent using $NADP^+$ as cofactor. It is extensively produced in ovary and testis as well as kidney and liver (Pletnev and Duax, 2005). The function of 17β-HSD 8 in human is not clear as yet. The similarities of 17β-HSD 8 with *E. coli* acyl-carrier protein reductase compared to other 17β-HSD (type 4) caused Pletnev and Duax to undertake molecular modelling of enzyme with putative ketoacyl-CoA substrate and estradiol respectively. The results showed that ketoacyl-CoA sat adequately into the substrate-binding pocket compared to the steroid which suggesting the function of enzyme in fatty acid metabolism rather than the steroid metabolism (Figure 1.25) (Moeller and Adamski, 2006).

(A)




(B)

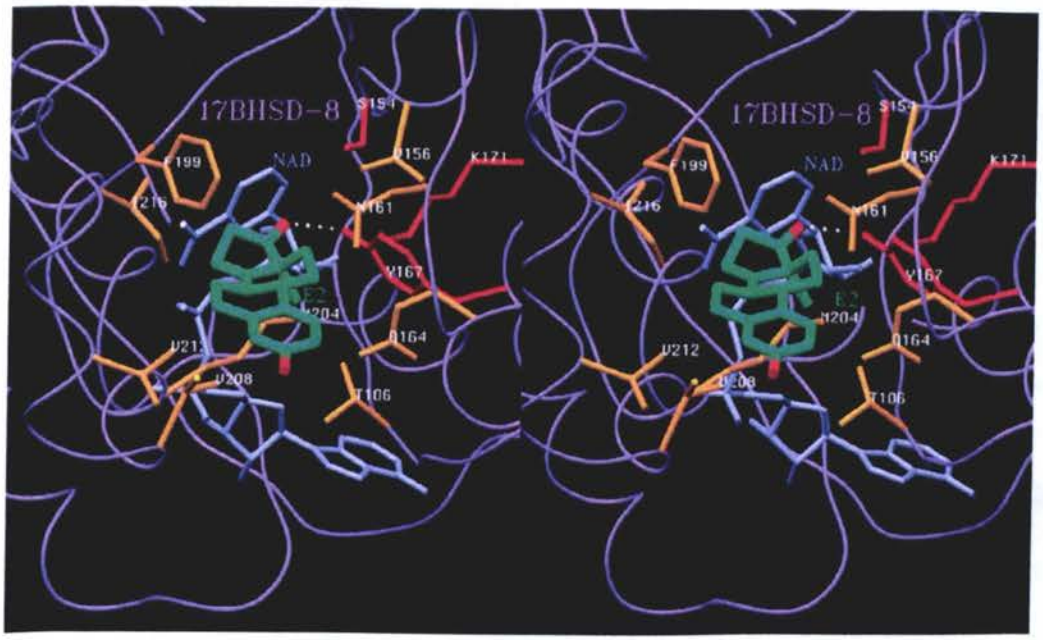



Figure 1.25: Stereoview of binding pocket of 17β-HSD8.

(A) Ketoacyl-CoA, cofactor and 17β-HSD8, (B) estradiol, cofactor and 17β-HSD8 in the ternary complex (adopted from Pletnev & Duax, 2005)

### 1.3.10. 17β-Hydroxysteroid dehydrogenase Type 9

The enzyme 17β-HSD 9 for the first time was identified in mouse (Su *et al*, 1999) and possesses 3α-, 17β- and retinol-dehydrogenase activity (Napoli, 2001). There is no human ortholog known of this enzyme.

### 1.3.11. 17β-Hydroxysteroid dehydrogenase Type 10

17β-Hydroxysteroid dehydrogenase type 10 was primarily described as short chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD). It is a homotetrameric protein with the molecular mass of 108KDa (He *et al*, 1998). This enzyme is confined in mitochondria through a N-terminal non-cleavable targeting signal (He *et al*, 2001; Shafqat *et al*, 2003). 17β- hydroxysteroid dehydrogenase 10 is NAD(H) dependent and is vital for metabolism of branched chain fatty acids, isoleucine and also oxidises 17β-estradiol and converts 5α-androstanediol into 5α-dihydrotestosterone and it might be responsible for the activation of androgens in the prostate (He *et al*, 1998). 17β-HSD 10 is not only expressed in the brain and prostate but its presence is also detected in the liver and heart (He *et al*, 2000; He *et al*, 2001). Moeller and Adamski postulated that this enzyme may have a role in pathogenesis of Alzheimer's disease due to its ability to bind to β-amyloid peptide (Moeller and Adamski, 2006).

### 1.3.12. 17β-Hydroxysteroid dehydrogenase Type 11

It has been discovered that 17β-HSD 11 transforms 5α-reduced steroid 5α-androstanediol into androsterone in an intact cell based assay. 5α-androstanediol steroid possibly have a part in rodents labor (Chai *et al*, 2003). It has been thought that this enzyme is situated through a N-terminal transmembrane helix to estrogen receptor (Lukacik *et al*, 2006). Some data suggests that 17β-HSD11 enzymatic activity might be toward the fatty acid metabolism rather than steroid metabolism (Motojima, 2004). 17β-HSD11 was found to be like human retinal short-chain dehydrogenase/reductase retSDR2. This enzyme alters 5α-androstane-3α, 17β-diol to androsterone and it is highly expressed in various tissues such as pancreas, kidney, liver, lung, adrenal, ovary and heart (Chai *et al*, 2003).

### 1.3.13. 17β-Hydroxysteroid dehydrogenase Type 12

17β-Hydroxysteroid dehydrogenase type 12 was identified as a homolog of yeast microsomal enzyme YBR159w involved in fatty acid expansion. It is located at p11.2 on human chromosome 11 (Moon and Horton, 2003). 17β-HSD 12 has the same genomic structure as 17β-HSD 3 (Luu-The *et al*, 2006) with molecular mass of 33KDa (Sakurai *et al*, 2006). This enzyme shares about 40% sequence identity to that of mouse 17β-HSD (Mindnich *et al*, 2004b). 17β-HSD 12 is highly expressed in heart, skeletal muscle, liver, kidney, adrenal gland, testis, placenta and cerebrum and to a lesser extent is expressed in pancreas, small and large intestine, trachea, lung and thyroid (Sakurai *et al*,

2006). This enzyme efficiently converts estrone ($E_1$) into estradiol ($E_2$) in transfected HEK-293 cells (Luu-The *et al*, 2006).

### 1.3.14. 17β-Hydroxysteroid dehydrogenase Type 13

Liu *et al* (2007) cloned 17β-HSD type 13 from human liver cDNA library (Liu *et al*, 2007). This enzyme has about 65% amino acid sequence similarities to retinal short-chain dehydrogenase/reductase (DHRS8) and is situated on chromosome 4q22.1 (Moeller and Adamski, 2006). 17β-HSD 13 is found in the liver, lung and intestine and it has about 78% homology share with 17β-HSD type 11 (Luu-The *et al*, 2008). It is believed that this enzyme plays a role in retinol metabolism (Moeller and Adamski, 2006).

### 1.3.15. 17β-Hydroxysteroid dehydrogenase Type 14

17β-Hydroxysteroid dehydrogenase type 14 is one of the newly identified 17β-HSD-enzymes and is acknowledged as DHRS10 and retSDR3 too (Jansson, 2009). This enzyme has the same substrate specificity as 17β-HSD 2 and catalyses the oxidation of C19 as well as C18-steroid as substrates (Luu-The *et al*, 2008). 17β-HSD 14 is $NAD^+$ dependent estradiol dehydrogenase which not only has a role in the conversion of estradiol ($E_2$) to estrone ($E_1$), but also may play a part in oxidation of androgens and fatty acids (Jansson, 2008). Structural determination of 17β-HSD 14 revealed that the active site can welcome steroid substrate in a suitable catalytically orientation (Lukacik *et al*, 2006). Even though this enzyme has some identity shared with other members

of the 17β-HSDs family and also its crystal structure is determined but still further investigation are required (Lukacik *et al*, 2006; Jansson, 2008).

### 1.3.16. 17β-Hydroxysteroid dehydrogenase Type 15

17β-Hydroxysteroid dehydrogenase type 15 was lately described by Luu-The *et al* (2008). This enzyme is expressed in the prostate and is capable of converting 5α-dione into dihydrotestosterone (DHT) (Luu-The *et al*, 2008). Currently further investigations are in progress.

## 1.4. AIMS OF CURRENT INVESTIGATION

Androgens have been proposed as the vital endocrine factors implicated in the initiation and the development of hormone-dependent diseases in males such as prostate cancer and benign prostatic hyperplasia (BPH) (Luu-The *et al*, 2008). One approach for treatment of hormone-dependent cancers is concerned with the removal of free sex-hormone testosterone (**99**) in the body by using enzyme inhibitors to influence androgen biosynthesis, in particular the enzymes 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) and type 5 (Figure 1.26).


Figure 1.26: Biosynthesis of testosterone from $\Delta^4$-dione

Researchers have postulated that the development of a 17β-HSD inhibitor could lead to a better and more effective treatment of hormone-dependent prostate cancer and BPH (Poirier, 2003).

As such 17β-hydroxysteroid dehydrogenase types 3 and 5 have attracted much interest as the main biochemical target in the quest to develop new and effective treatments for hormone-dependent prostate cancer and benign prostatic hyperplasia (BPH) (Olusanjo and Ahmed, 2007). Therefore much effort has been put in this field to synthesise highly potent and specific inhibitors

of these enzymes. Despite attempts by other groups there are still no inhibitors available in the market. In fact many inhibitors have been synthesised such as (**31-84**) but so far none have reached clinical trials.

Therefore the aims of this project were to synthesise and biochemically evaluate some non-steroidal inhibitors of the enzyme17β-HSD3. In order to synthesise a novel class of non-steroidal inhibitors with high similarities to the natural substrate $\Delta^4$-dione (**98**) we decided to chemically modify (**98**). The structural modifications that we propose is to retain most of the features of the natural substrate (**98**) but to synthesise compounds in which the A ring present in (**98**) has been opened e.g. in structure (**A**) (Figure 1.27). It is proposed that this modification to the natural substrate might produce an inhibitor which can mimic the natural substrate without exerting any of the unwanted steroidal effects.

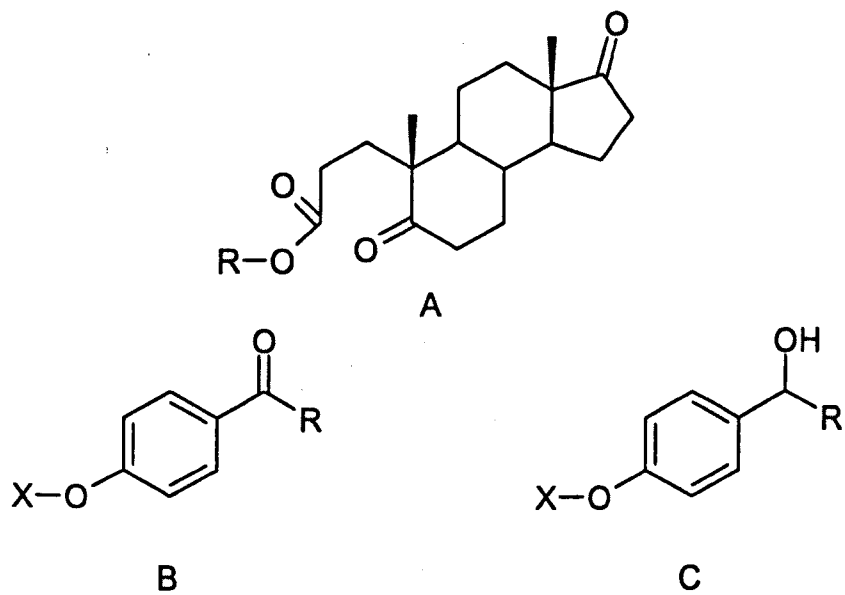
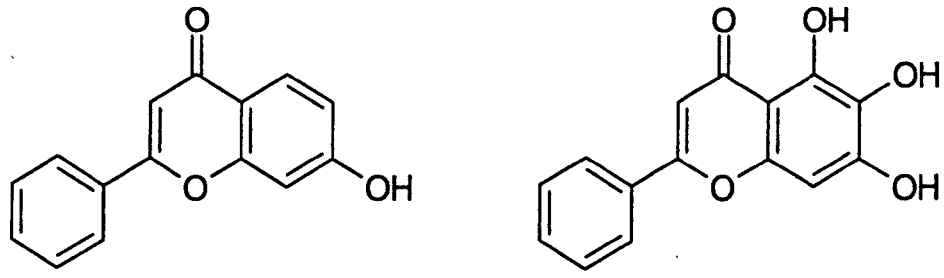



Figure 1.27: General structure of potential inhibitors synthesised in this project (X = H, $CH_3SO_2$, $CH_3CO$; R = alkyl or cycloalkyl groups)

We also plan to synthesise and biochemically evaluate the analogues of

known inhibitors of 17β-HSD 3 namely 7-hydroxy flavone (**63**) and baicalein (**64**) (Figure 1.28) reported by Le Lain *et al* (2001).




**63** **64**

Figure 1.28: Known inhibitors of 17β-HSD3

These compounds will be designed to contain a carbonyl group which may mimic the C(17)=O of the natural substrate (**98**) as well as other pharmacophores such as the aromatic ring. As such a library of 4-hydroxyphenyl ketones was synthesised and biochemically evaluated against the enzyme 17β-HSD 3 within the group (Lota *et al*, 2006). The good inhibitory activities of these compounds led these studies to further investigation on the effects of substitution of the phenolic proton by sulfonation and esterification to observe whether these changes could enhance the inhibitory activity of compounds or reduce the activity. These compounds were based upon the general structure (**B**) (Figure 1.27). When these compounds interact with the target enzyme (17β-HSD3), the carbonyl functional group alpha to the benzene ring would be reduced to a hydroxyl moiety in the same manner as (**98**) is reduced by the enzyme 17β-HSD3 to (**99**). In order to study the effects of reduced forms of inhibitors (products) upon the type 2 17β-HSD which is responsible for the oxidation of (**99**) to (**98**) it was decided to synthesis these set of compounds with the general structure (**C**) (Figure 1.27).

# CHAPTER 2: RESULTS & DISCUSSION

# 2. RESULTS & DISCUSSION

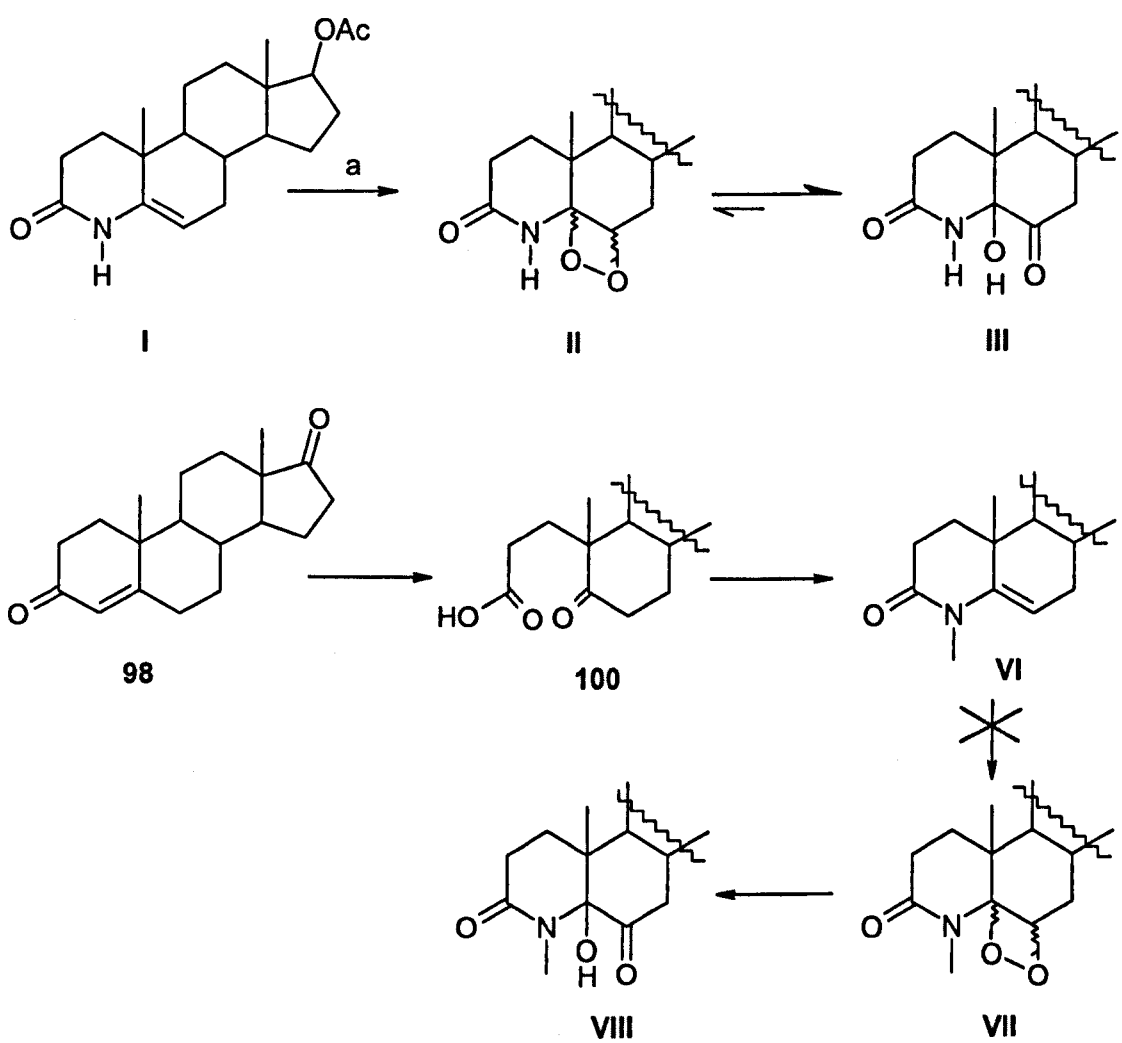
## 2.1. INTRODUCTION

The aims of these studies center around the synthesis of novel inhibitors of 17β-HSD3 as a potential therapeutic treatment for hormone-dependent prostate cancer and benign prostate hyperplasia (BPH). This was undertaken by the chemical synthesis of a number of libraries of chemically related compounds which were biochemically screened for inhibitory activity. The synthesis of these libraries is detailed along with the rationale for their inclusion in a portfolio of derivatives. The results from the various screening activities are then described.

## 2.2. SYNTHESIS OF FIRST LIBRARY BASED UPON DERIVATIVES OF THE NATURAL SUBSTRATE (17B-HSD3)

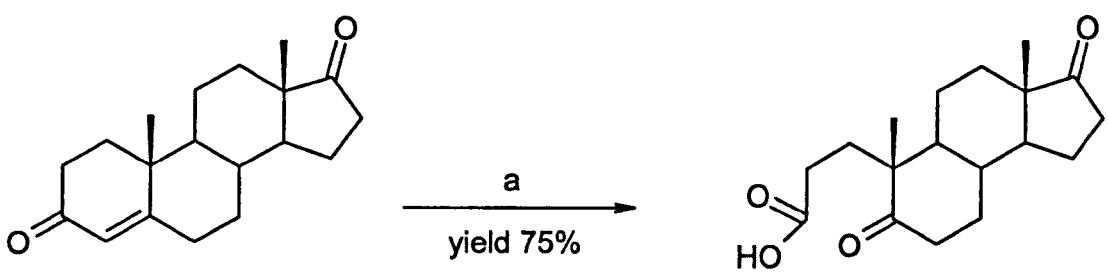
The initial target molecules for use as potential novel inhibitors of the natural substrate of 17β-HSD3 were based upon the structure that resembled the natural substrate itself (**98**) (Figure 1.26). The rationale for this approach was based upon the fact that chemically modified derivatives of (**98**), whilst still retaining some components of the natural substrate and hence structurally analogous to the natural substrate, may nevertheless reduce the androgenic effects of (**98**) and thus enhance the inhibitory activity by binding to the same enzyme active site.

The modification of the A-ring of $\Delta^4$-dione **(98)** has been the subject of previous studies by Piniella *et al* (1987). They observed that the photooxygenation of **(I)** preferentially converted it to ketol **(III)** via the intermediate dioxetane **(II)**. They hypothesised that this phenomenon may be due to the presence of the N-H functional group. In order to confirm their hypothesis they synthesised compound **(VI)** by opening the A-ring of **(98)** and substituting N-H with N-$CH_3$. After subjecting **(VI)** to photooxygenation process, no traces of dioxetane **(VII)** or ketol **(VIII)** were observed (Scheme 2.1).




Scheme 2.1: Photooxygenation of lactom **(I)** and **(VI)**.

In this pursuit we used a modified literature method (Piniella *et al*, 1987) (Von Rudloff solution).The oxidation of $\Delta^4$-dione (**98**) was carried out using a mixture of sodium periodate ($NaIO_4$) and potassium permanganate ($KMnO_4$) under slightly basic conditions. This was prepared by the addition of sodium carbonate to a mixture of tert-butanol and water as reaction solvent. The use of one equivalent of $NaIO_4$ failed to fully convert the starting material. We therefore gradually increased the amount of $NaIO_4$ to seven equivalents and the desired keto acid (**100**) was obtained in good yield of 75% (Scheme 2.2).




**98** **100**

Scheme 2.2: A-ring opening of $\Delta^4$-dione (a=$NaIO_4$, $KMnO_4$, $Na_2CO_3$, t-butanol, $H_2O$)

With the desired ring opened derivative (**100**) synthesised the formation of a series of ester derivatives was undertaken.

Esters can be synthesised in several different ways such as:

(i) direct reaction between alcohols and carboxylic acid

(ii) reaction of alcohol with acid anhydride or

(iii) reaction of alcohol with acyl chloride

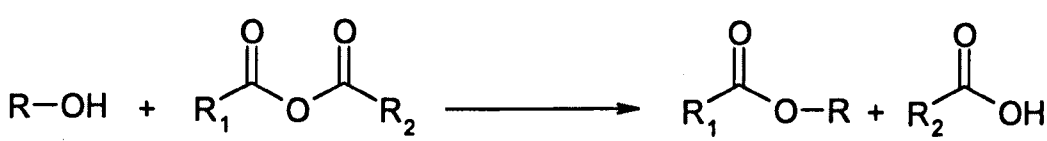
(i) Esterification via acid catalyst (Noland *et al*, 1980))

The direct reaction of a carboxylic acid and an alcohol is a reversible reaction and the best result was obtained *via* addition of a catalytic amount of concentrated acid i.e. sulphuric acid ($H_2SO_4$). During this reaction steric hindrance plays an important role in the rate of production of the ester. In general primary alcohols or carboxylic acids are more reactive than secondary or tertiary reagents during esterifications (Scheme 2.3).


Scheme 2.3: Direct esterification reaction of alcohol and carboxylic acid

(ii) Esterification via acid anhydride (Nishimura and Saneyoshi, 1980)

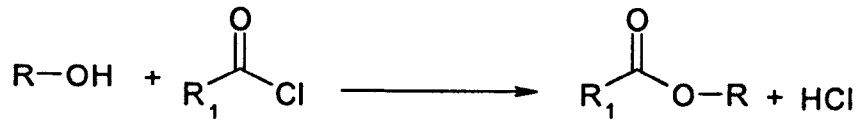
Esters can be also synthesised by the reaction of an alcohol and an acid anhydride using pyridine as a base (Scheme 2.4).




Scheme 2.4: Esterification of alcohol using acid anhydride

(iii) Esterification via acyl chloride (Cuilleron *et al*, 1981)

Acid chlorides readily react with primary alcohols to yield esters in a good yield (Scheme 2.5). The reaction proceeds by a nucleophilic acyl substitution of chloride with R-O$^-$ ion with the production of ester.



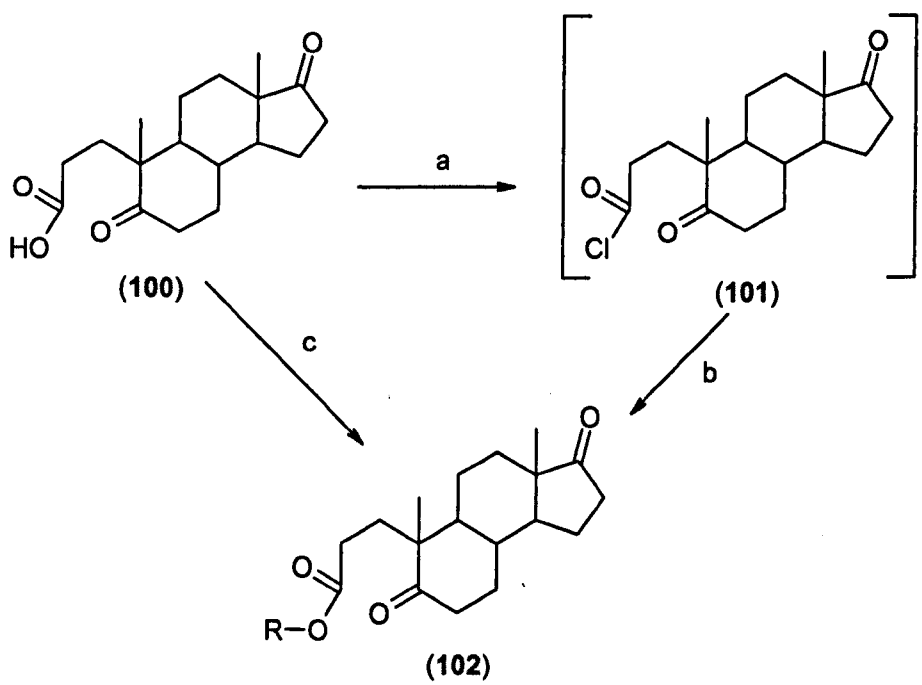

Scheme 2.5: Esterification of alcohol using acyl chloride

The esterification of the keto-acid (**100**) was carried out in one of two ways;

I. For the low carbon chain alcohol i.e. methanol to butan-1-ol, the method of esterification using a catalytic amount of acid was used.

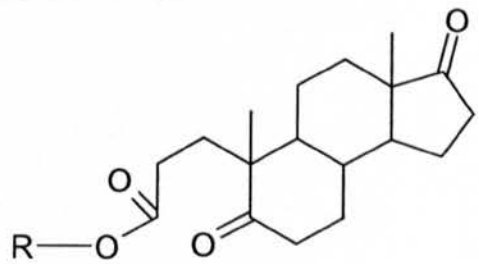
II. For the higher molecular weight alcohols the keto acid (**100**) was first derivatised to an acyl chloride (**101**) (Scheme 2.6) and then reacted with the appropriate alcohol to yield the desired compounds.

As mentioned earlier the rate of reaction can be slow for the bulky alcohol and so the time of reaction was increased to 5h.




Scheme 2.6: Esterification of keto-acid (**100**) (a = $SOCl_2$, Δ; b = alcohol, Δ; c = alcohol, $H^+$, Δ)

In general these reactions all proceeded well and the 26 desired compounds (**103-128**) (Table 2.1) were obtained in a good yield for example compound (**123**) 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cyclopentylmethyl ester was obtained in (79% yield).




| Compounds | R= | % yield | Compounds | R= | % yield |
|---|---|---|---|---|---|
| **100** | H | 75 | **117** | Cyclopentyl | 73 |
| **103** | $CH_3$ | 77 | **118** | Cyclohexyl | 72 |
| **104** | $C_2H_5$ | 78 | **119** | Cyclohepyl | 71 |
| **105** | $C_3H_7$ | 73 | **120** | Cyclo-octyl | 73 |
| **106** | $C_4H_9$ | 70 | | | |
| **107** | $C_5H_{11}$ | 71 | **Cyclic alkane with spacers ($CH_2$)** | | |
| **108** | $C_6H_{13}$ | 75 | **121** | Cyclopropyl | 73 |
| **109** | $C_7H_{15}$ | 76 | **122** | Cyclobutyl | 73 |
| **110** | $C_8H_{17}$ | 73 | **123** | Cyclopentyl | 79 |
| **111** | $C_9H_{19}$ | 71 | **124** | Cyclohexyl | 76 |
| **112** | $C_{10}H_{21}$ | 69 | **125** | Cyclohepyl | 67 |
| **113** | $C_{11}H_{23}$ | 67 | **126** | Cyclo-octyl | 71 |
| **114** | $C_{12}H_{25}$ | 68 | | | |
| **115** | $C_{13}H_{27}$ | 69 | **127** | 2-adamentyl | 70 |
| **116** | Cyclobutyl | 77 | **128** | borneoyl | 69 |

Table 2.1: The synthesis of ester derivatives (**103-128**) of $\Delta^4$-dione (**98**)

All derivatives were extensively characterised using a range of spectroscopic techniques. In the $^1H$ NMR spectra all of the protons were readily detectable however in the $^{13}C$ NMR it was found that some of the peaks overlapped. For example the $^1H$ and $^{13}C$ NMR spectra of compound (**112**) are shown (Figure 2.1 and Figure 2.2). Analysis of the $^{13}C$ NMR spectrum indicates the resonance

for four carbons were overlapping at regions δ29.71 and δ29.48ppm (Figure 2.2). This phenomenon was seen for a number of compounds where overlapping of carbon resonances were seen, the assigned carbon underlined. The full spectral data of this range of compounds can be seen in the experimental section (pp**114-141).**

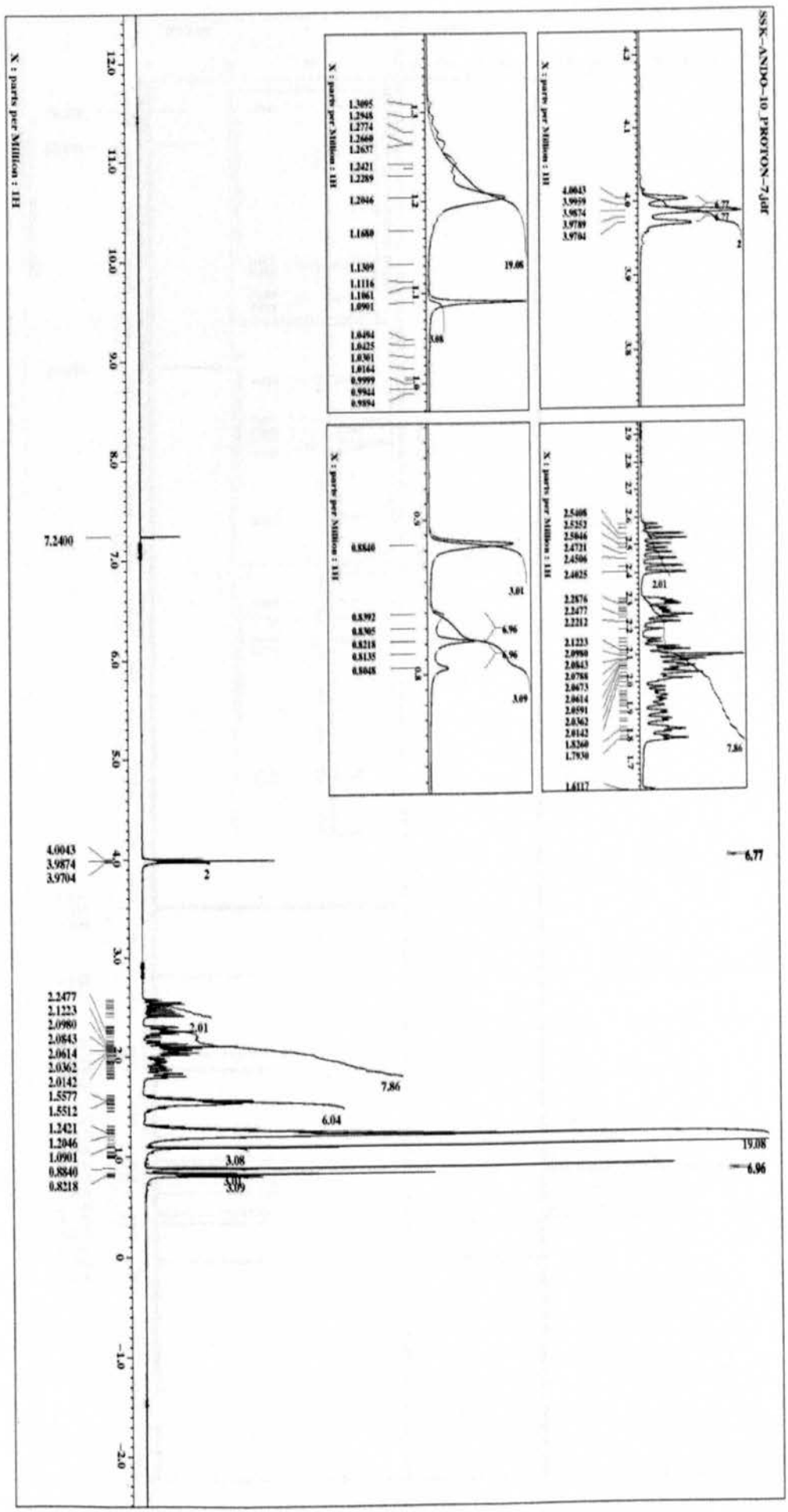



Figure 2.1: $^{1}$H NMR of compound (**112**)

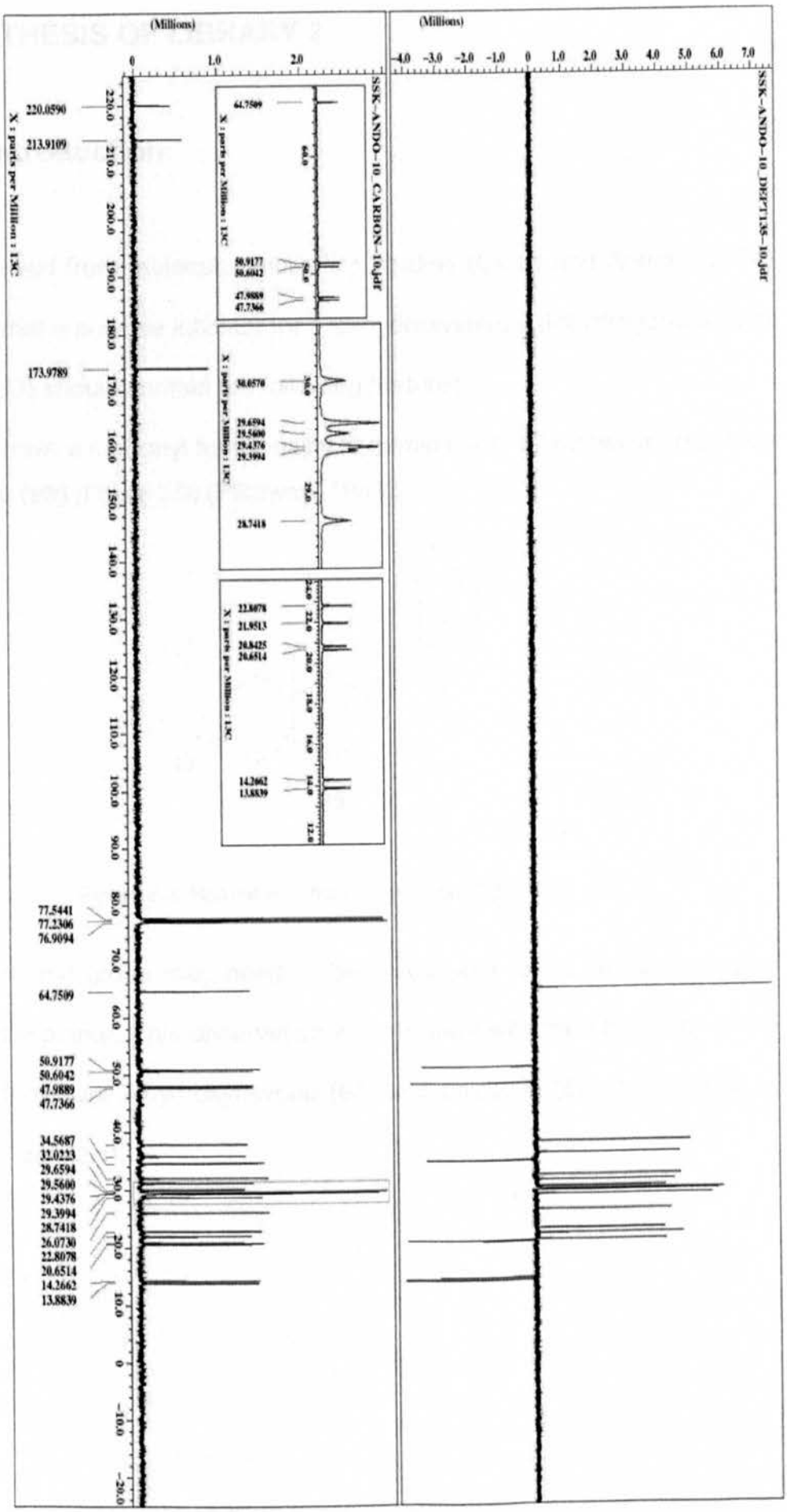


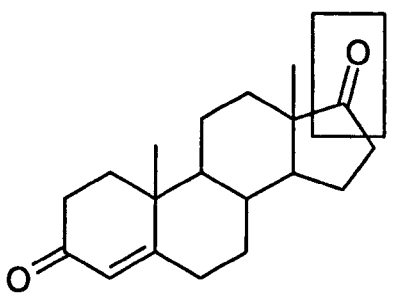

Figure 2.2: $^{13}C$ NMR of compound (**112**)

## 2.3. SYNTHESIS OF LIBRARY 2

### 2.3.1. Introduction

Results derived from molecular modelling studies (Owen and Ahmed, 2004) suggested that a suitable inhibitor for 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) should contain the following features:

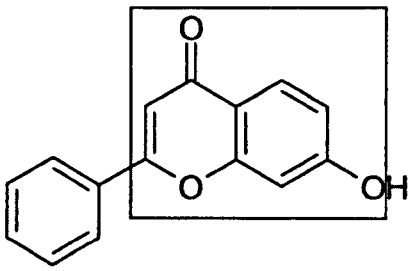
(i) Should have a carbonyl functionality to mimic the C-17 carbonyl present in $\Delta^4$-dione (**98**) (Figure 2.3) (Pittaway, 1983).



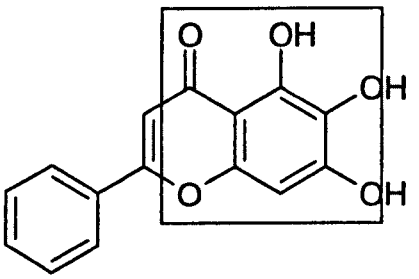

**98**

Figure 2.3: Natural substrate of enzyme 17β-HSD3

(ii) The carbonyl group may need to be conjugated to an aromatic ring ie: should be planar. This observation is consistent with the structures of the known inhibitors 7-hydroxyflavone (**63**) and baicalein (**64**) (Le Lain *et al*, 2001) (Figure 2.4).



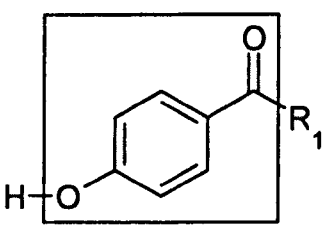

**63**




**64**

Figure 2.4: Known inhibitors of 17β-HSD3 used as standards

It was thus concluded from these modelling studies that an appropriate starting point with which to base the second library of inhibitors might be a compound as relatively simple in structure as 4-hydroxyphenyl ketone (**129**). This was rationalised upon the basis that it contains key pharmacophores present in both (**63**) and (**64**) and is a structure that is consistent with the molecular modelling studies (Figure 2.5).



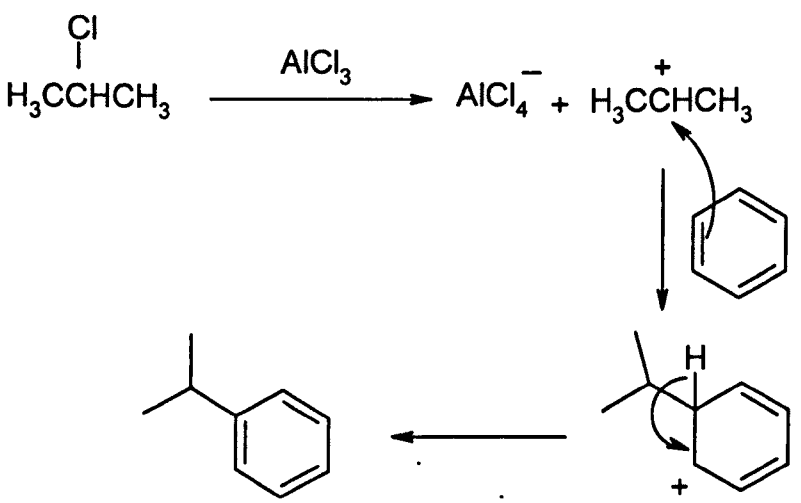

**(129)**

Figure 2.5: General structure of inhibitors

### 2.3.2. Synthesis of novel and potential inhibitors of 17β-HSD3 for biochemical screening

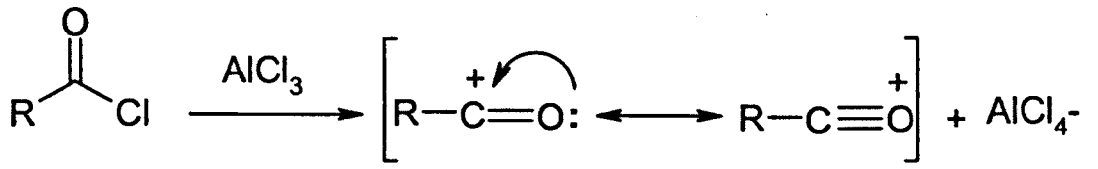
Results previously obtained within this research group indicated that the hydroxyl group was an important feature of a potential inhibitor which would afford an opportunity for hydrogen bonding in the enzyme active site. Therefore

to confirm this hypothesis it was decided to substitute the hydroxyl moiety with the corresponding sulfonyl and ester moieties to see whether the inhibitory activity of compounds would be affected or not. The synthesis of this library of compounds based upon (**129**) was readly accomplished using Friedel-Crafts acylation reactions. The Friedel-Crafts reaction is an electrophilic aromatic substitution reaction in which the electrophilic carbocation is generated by the reaction of an alkyl halide with $AlCl_3$ (Scheme 2.7). The alkylation itself is carried out by the attack of an electron pair from benzene ring to the carbocation to form a carbon - carbon bond. Subsequent loss of a proton from the benzene ring produces the alkylated product.



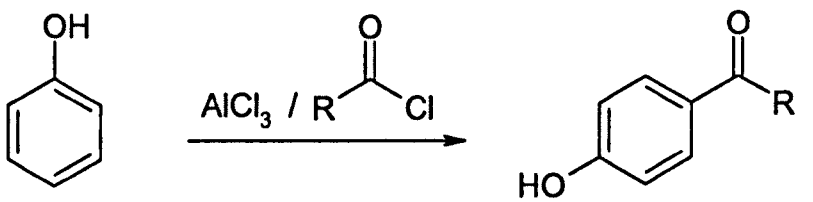

Scheme 2.7: The formation of a carbocation using $AlCl_3$ and electrophilic aromatic substitution of benzene

The Friedel-Crafts acylation reaction is similar to that of alkylation only using acyl halide instead of alkyl halide. The formation of the electrophile is assisted by the presence of $AlCl_3$ and the reactive electrophile is resonance stabilised (Scheme 2.8).




Scheme 2.8: Resonance stabilisation of carbocation.

The Friedel-Crafts acylation of phenol was carried out in the presence of an excess of $AlCl_3$ (Scheme 2.9) using a procedure described previously (Lota *et al*, 2006).




Scheme 2.9: Friedel-Craft acylation of phenol

The role of the Lewis acid ($AlCl_3$) is to form a complex with the R-C(O)-Cl and to generate an acyl cation equivalent. The regiochemistry of the reaction is explained in terms of the ortho-para directing effect of the –OH group which serves to activate the aromatic ring via a resonance effect (Figure 2.6).

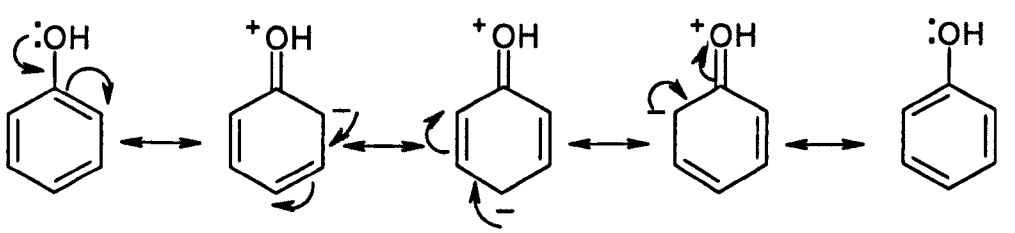
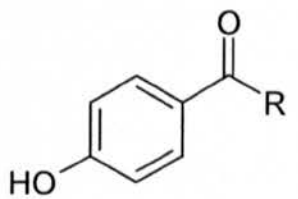



Figure 2.6: Resonace stabilisation of phenol

The formation of a complex between R-OH (Phenol) and $AlCl_3$ sterically discards the *ortho* position thus favouring acylation at the *para* position. In general the acylation reactions were performed without any major difficulties to

afford a small library of the corresponding derivatives (**130-142**) in good to moderate yields. As a general observation the yields of the acylation reactions reflected the length of the hydrocarbon chain thus compound (**131**) 1-(4-hydroxy phenyl) propan-1-one (R=$C_2H_5$) was formed (71% yield) whereas compound (**138**) 1-(4-hydroxy phenyl) decan-1-one (R=$C_9H_{19}$) was formed (34% yield). Once acylated the benzene ring becomes deactivated to further electrophilic aromatic substitution reaction compared to phenol itself and so further acylations were not observed (Table 2.2).




| Compounds | R= | % yield | Compounds | R= | % yield |
|---|---|---|---|---|---|
| **130** | $CH_3$ | 69 | **137** | $C_8H_{17}$ | 40 |
| **131** | $C_2H_5$ | 71 | **138** | $C_9H_{19}$ | 34 |
| **132** | $C_3H_7$ | 57 | **139** | Cyclopropyl | 51 |
| **133** | $C_4H_9$ | 59 | **140** | Cyclobutyl | 38 |
| **134** | $C_5H_{11}$ | 54 | **141** | Cyclopentyl | 39 |
| **135** | $C_6H_{13}$ | 51 | **142** | Cyclohexyl | 34 |
| **136** | $C_7H_{15}$ | 43 | | | |

Table 2.2: Synthesised 4-hydroxyphenyl ketones

The IR spectrum of compound (**132**) (Figure 2.7) shows the presence of an OH stretch at 3559.9 $cm^{-1}$ and a carbonyl peak appeared at 1655.3 $cm^{-1}$. The full spectral data of this range of compounds can be seen in the experimental section (pp**142-154**).

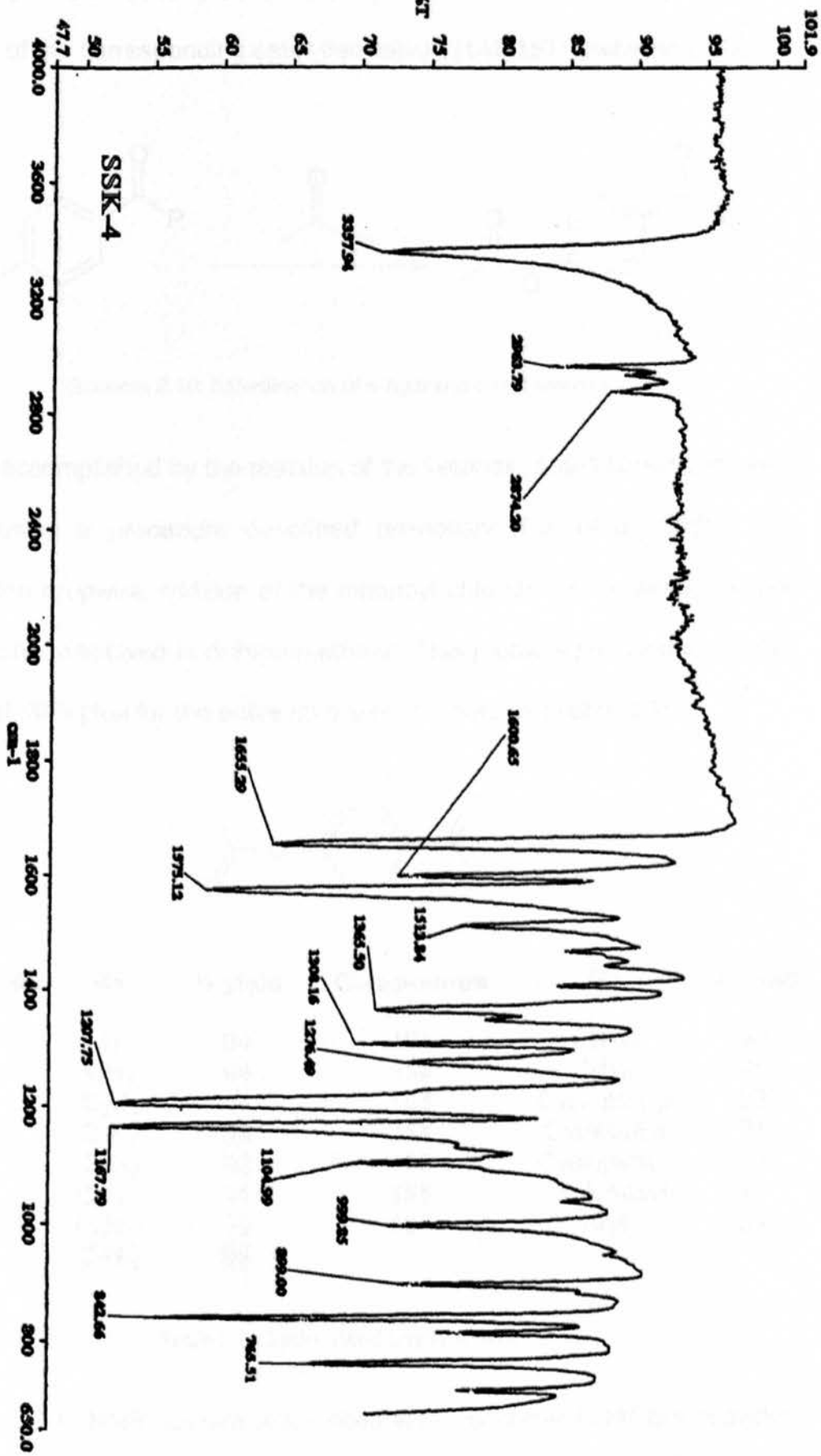
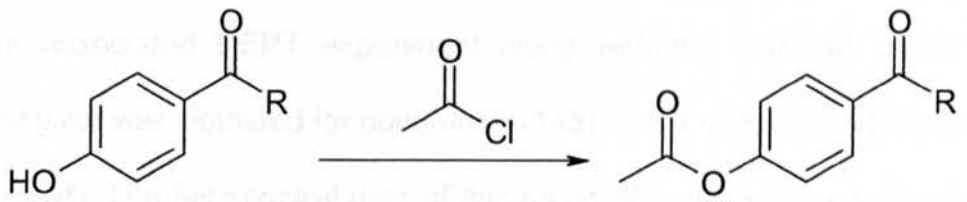


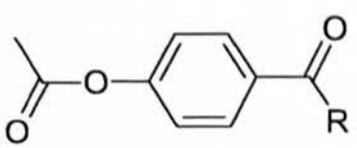

Figure 2.7: The IR spectrum of compound (132)

With the phenolic derivatives (**130-142**) in hand the next stage was the synthesis of the corresponding ester derivatives (**143-157**) (Scheme 2.10).




Scheme 2.10: Esterification of 4-hydroxyphenyl ketones

This was accomplished by the reaction of the ketones (**130-142**) with ethanoyl chloride using a procedure described previously (Dai *et al*, 1995). This involved the dropwise addition of the ethanoyl chloride into a stirred solution of the ketone, dissolved in dichloromethane. This protocol provided the esters in yields of 90% plus for the entire library of compounds (Table 2.3).




| Compounds | R= | % yield | Compounds | R= | % yield |
|---|---|---|---|---|---|
| **143** | H | 94 | **151** | $C_8H_{17}$ | 94 |
| **144** | $CH_3$ | 94 | **152** | $C_9H_{19}$ | 95 |
| **145** | $C_2H_5$ | 96 | **153** | Cyclopropyl | 92 |
| **146** | $C_3H_7$ | 93 | **154** | Cyclobutyl | 91 |
| **147** | $C_4H_9$ | 92 | **155** | Cyclopentyl | 92 |
| **148** | $C_5H_{11}$ | 91 | **156** | Cyclohexyl | 91 |
| **149** | $C_6H_{13}$ | 92 | **157** | Aryl | 94 |
| **150** | $C_7H_{15}$ | 92 | | | |

Table 2.3: Synthesised phenyl esters

The $^1H$ and $^{13}C$ NMR spectra associated with derivative (**150**) are provided (Figure 2.9). In the $^1H$ NMR spectra the characteristic 1,4-disubstitution

aromatic ring pattern is visible. In the corresponding $^{13}C$ NMR spectrum two resonances at δ 199.50ppm and δ 169.10ppm are shown for the two carbonyl groups as well as the two quarternary aromatic carbon atoms which disappear on the associated DEPT experiment along with the carbonyl peaks. The highest yield was obtained for compound (**145**) acetyl 4-propionyl phenyl ester (96% yield). The full spectral data of this range of compounds can be seen in the experimental section (pp**155-169**).

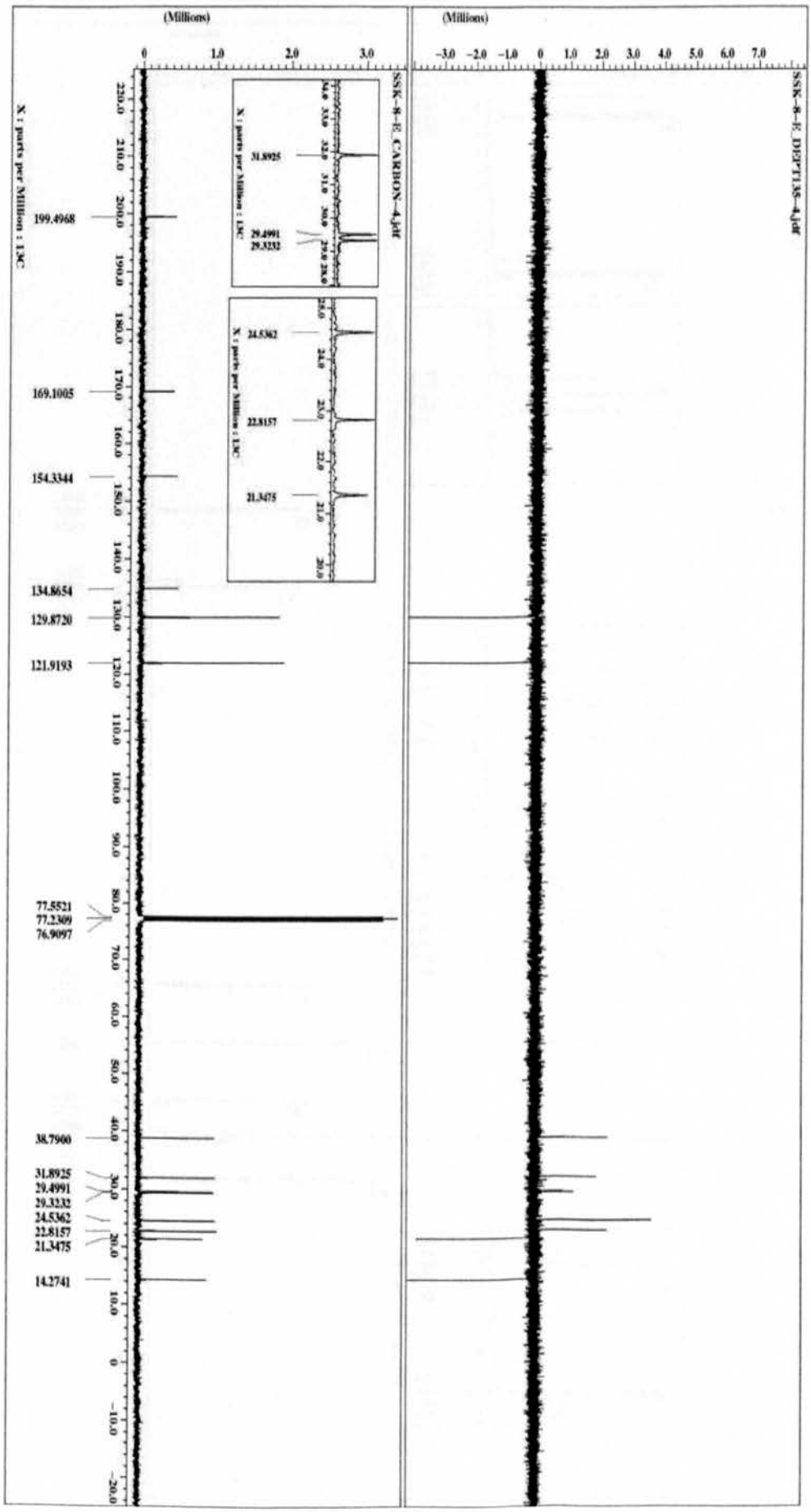



Figure 2.8: $^{13}$C-NMR spectra of compound (**150**)

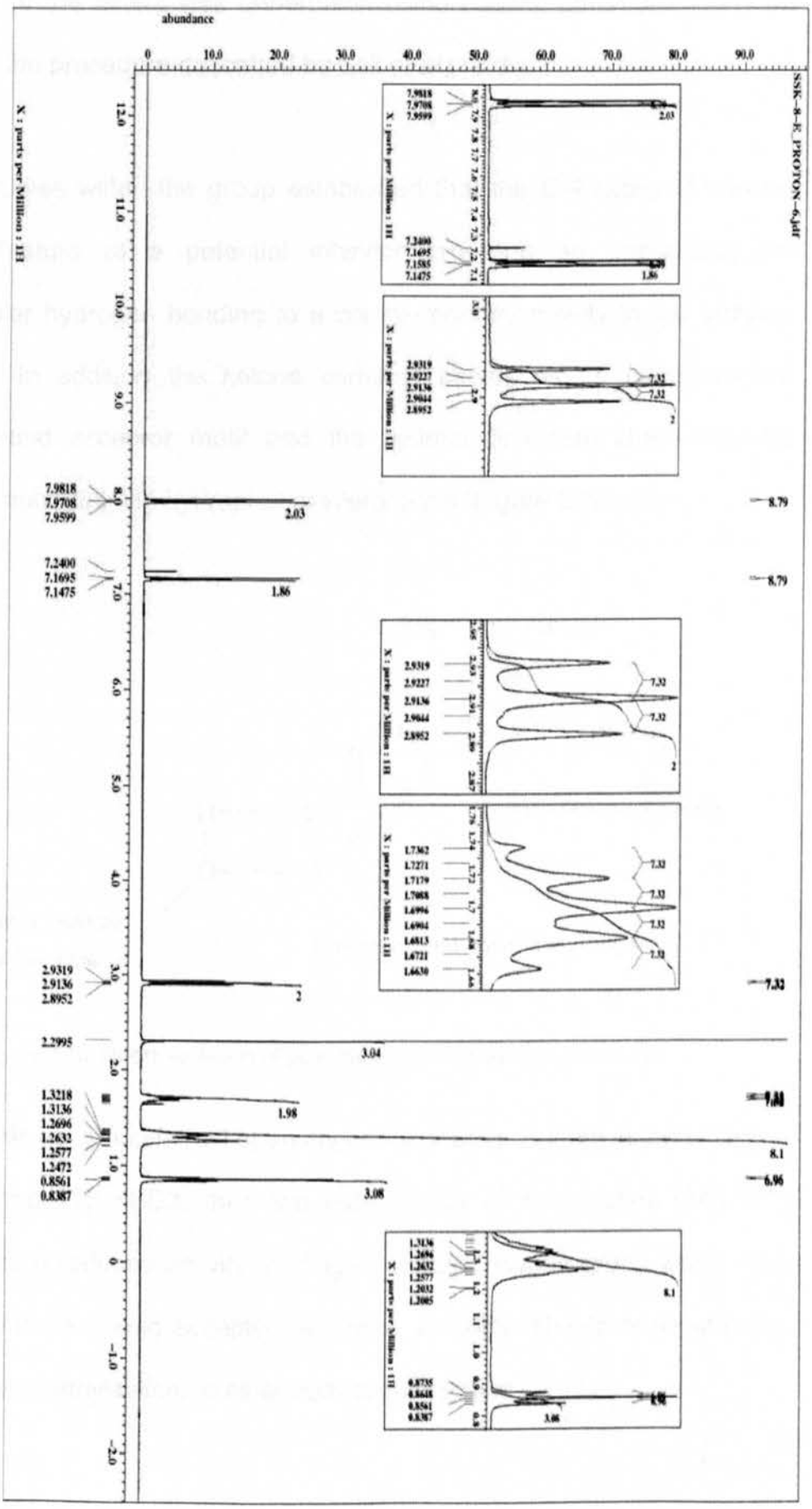



Figure 2.9: $^{1}$H- NMR spectra of compound (**150**)

Purification of the esters was undertaken using column chromatography on silica using the procedure described by Still *et al* (1978).

Previous studies within the group established that the C-4 hydroxyl was an important feature of a potential inhibitor providing an opportunity for intermolecular hydrogen bonding to a complementary moiety in the enzyme active site. In addition the ketone carbonyl serves as an intermolecular hydrogen bond acceptor motif and the hydrocarbon side chain may be involved in stabilising via hydrophobic interactions (Figure 2.10).

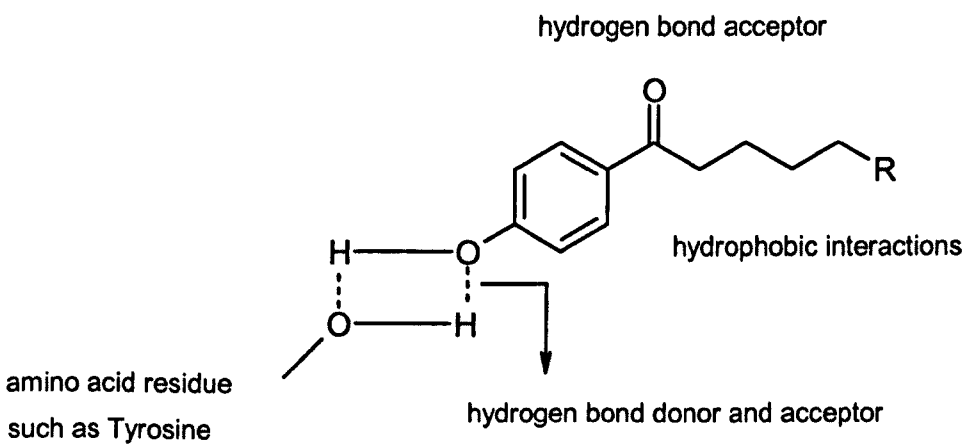
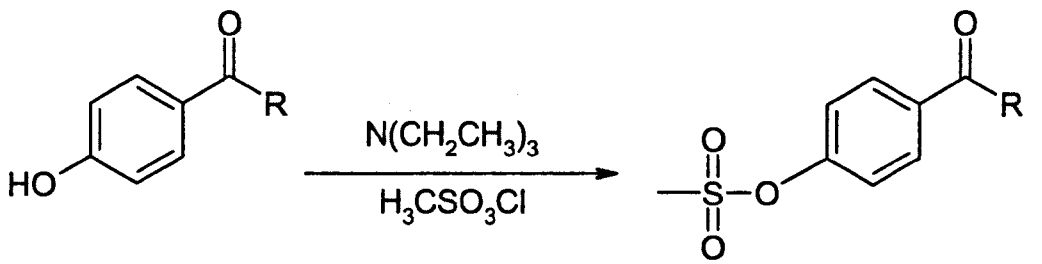



Figure 2.10: Representation of possible mode of binding in active site

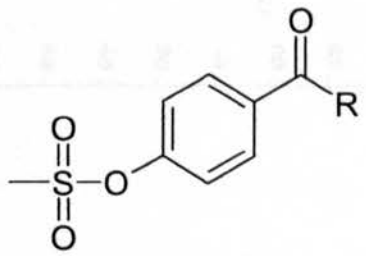
If the C-4 hydroxyl group is part of an important pharmacophore in the inhibition of the enzyme 17β-HSD3, then the ester library of compounds (**143-157**) should exhibit a reduced activity although the ester oxygen group which may still be a hydrogen bond acceptor. A similar outcome should arise with the corresponding methylsulfonate ester derivatives (Scheme 2.11).




Scheme 2.11: Synthesis of methane sulfonate based ketones

## 2.3.3. Synthesis of sulfonate ester derivatives

The sulfonates (**158-172**) (Table 2.4) were obtained using a modified literature method (Andraos *et al*, 1998) in good yields for the entire range. This involved heating the phenoxide anion of the phenols (**130-142**) to reflux temperatures in the presence of an excess of methanesulfonyl chloride. For example compound (**161**) methanesulfonic acid 4-butyryl-phenyl ester was obtained (88% yield). The corresponding IR spectrum for (**161**) (Figure 2.11) showed the presence of carbonyl at 1682.7cm$^{-1}$, the aromatic C=C at 1595.8 cm$^{-1}$ and a strong peak at 1369.8 cm$^{1}$ for the O-$SO_2$-$CH_3$. The full spectral data of this range of compounds can be seen in the experimental section (pp**170-184**)



| Compounds | R= | % yield | Compounds | R= | % yield |
|---|---|---|---|---|---|
| **158** | H | 81 | **166** | $C_8H_{17}$ | 79 |
| **159** | $CH_3$ | 76 | **167** | $C_9H_{19}$ | 85 |
| **160** | $C_2H_5$ | 86 | **168** | Cyclopropyl | 71 |
| **161** | $C_3H_7$ | 88 | **169** | Cyclobutyl | 83 |
| **162** | $C_4H_9$ | 81 | **170** | Cyclopentyl | 74 |
| **163** | $C_5H_{11}$ | 84 | **171** | Cyclohexyl | 85 |
| **164** | $C_6H_{13}$ | 79 | **172** | Aryl | 85 |
| **165** | $C_7H_{15}$ | 78 | | | |

Table 2.4: Synthesised methanesulfonated ketones.

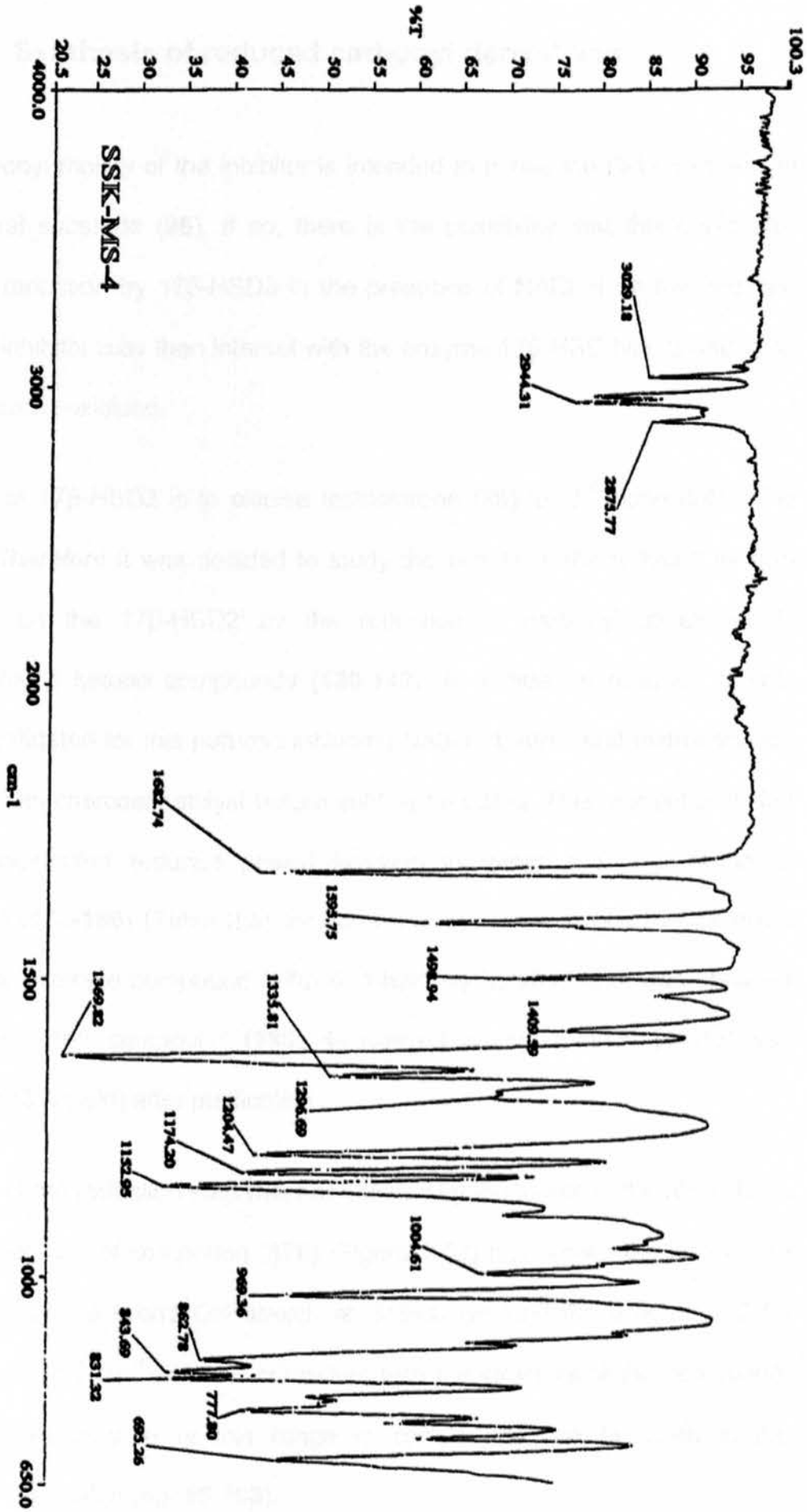


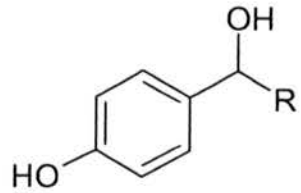

Figure 2.11: The IR spectrum of the compound (**161**)

### 2.3.4. **Synthesis of reduced carbonyl derivatives**

The carbonyl moiety of the inhibitor is intended to mimic the C-17 carbonyl of the natural substrate (**98**). If so, there is the possibility that this could also undergo reduction by 17β-HSD3 in the presence of NADPH. In this reduced form the inhibitor may then interact with the enzyme 17β-HSD type 2 and in so doing become oxidised.

The role of 17β-HSD2 is to oxidise testosterone (**99**) to $\Delta^4$-dione (**98**) using $NADP^+$. Therefore it was decided to study the effects of the reduced form of inhibitors on the 17β-HSD2 by the reduction of carbonyl moiety of 4-hydroxyphenyl ketone compounds (**130-142**). A number of reducing agents were investigated for this purpose including $NaBH_4$, $LiAlH_4$, and hydrogenation on Palladium charcoal catalyst before settling on $LiBH_4$. This reagent provided the corresponding reduced phenyl ketones to afford a further range of molecules (**173-186**) (Table 2.5) for screening purposes in good to excellent yields. For example compound (**176**) 4-(1-hydroxy butyl) phenol was obtained (95% yield) and compound (**186**) 4-(hydroxyl phenyl methyl) phenol was obtained (76% yield) after purification.

The yield of the reduction step was not affected by the length of the alkyl chain. The IR spectrum of compound (**176**) (Figure 2.12) has shown the loss of the carbonyl group, a broad OH stretch at 3334.8 $cm^{-1}$and the aromatic (C=C) stretch at 1614.0 $cm^{-1}$ which is consistent with the structure of the compound. The full spectral data of this range of compounds can be seen in the experimental section (pp**185-198**).




| Compounds | R= | % yield | Compounds | R= | % yield |
|---|---|---|---|---|---|
| **173** | H | 84 | **180** | $C_7H_{15}$ | 80 |
| **174** | $CH_3$ | 86 | **181** | $C_8H_{17}$ | 80 |
| **175** | $C_2H_5$ | 84 | **182** | $C_9H_{19}$ | 80 |
| **176** | $C_3H_7$ | 95 | **183** | Cyclobutyl | 90 |
| **177** | $C_4H_9$ | 85 | **184** | Cyclopentyl | 84 |
| **178** | $C_5H_{11}$ | 83 | **185** | Cyclohexyl | 86 |
| **179** | $C_6H_{13}$ | 86 | **186** | Aryl | 76 |

Table 2.5: Possible inhibitors of 17β-HSD2

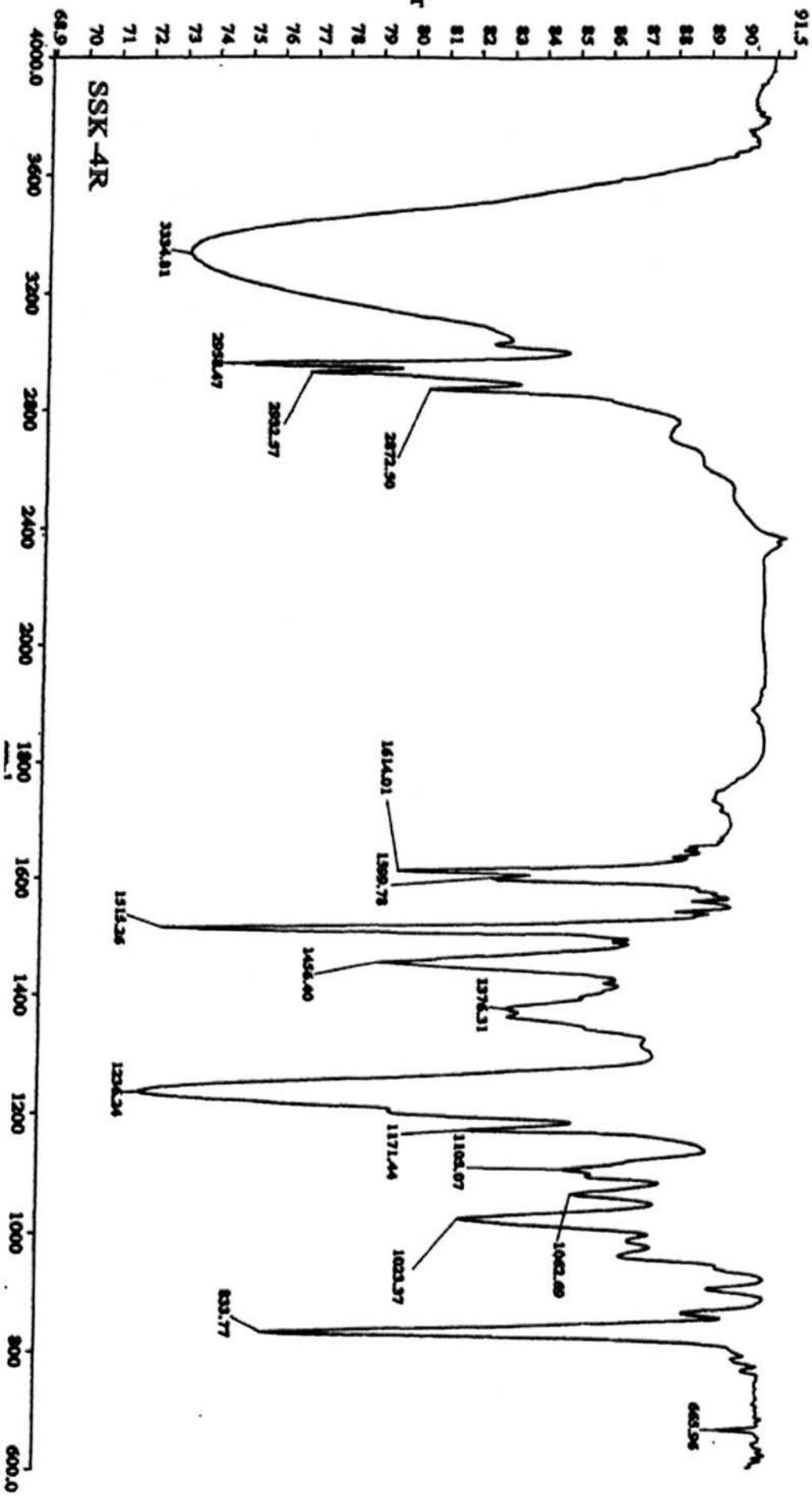
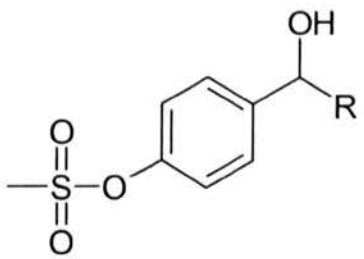



Figure 2.12: The IR spectrum of compound (**176**)

The attempts to reduce the carbonyl functionality of corresponding methanesulfonate derivatives (**158-172**) using $LiBH_4$ led to hydrolysis of the sulfonyl group back to the phenol. Eventually the desired compounds (**187-201**) (Table 2.6) were obtained in good yields using $NaBH_4$ in ethanol. For example compound (**188)** methanesulfonic acid 4-(1-hydroxy ethyl) phenyl ester was obtained (88% yield) and compound (**200**) methanefulfonic acid 4-(cyclohexyl hydroxyl methyl) phenyl ester was obtained (75% yield).




| Compounds | R= | % yield | Compounds | R= | % yield |
|---|---|---|---|---|---|
| **187** | H | 86 | **195** | $C_8H_{17}$ | 80 |
| **188** | $CH_3$ | 88 | **196** | $C_9H_{19}$ | 77 |
| **189** | $C_2H_5$ | 84 | **197** | Cyclopro | 80 |
| **190** | $C_3H_7$ | 88 | **198** | Cyclobut | 77 |
| **191** | $C_4H_9$ | 77 | **199** | Cyclopent | 77 |
| **192** | $C_5H_{11}$ | 80 | **200** | Cyclohex | 75 |
| **193** | $C_6H_{13}$ | 81 | **201** | Aryl | 80 |
| **194** | $C_7H_{15}$ | 77 | | | |

Table 2.6: Possible inhibitors of 17β-HSD2

The IR spectrum of compound (**190**) (Figure 2.13) shows the loss of the carbonyl stretch at 1682.7cm$^{-1}$, the presence of broad OH stretch at 3401.9cm$^{-1}$ the strog peak at 1365.4 cm$^{-1}$ for the O-$SO_2$-$CH_3$ and the aromatic (C=C) stretch at 1602.8 cm$^{-1}$ which is consistent with the structure of the compound. The full spectral data of this range of compounds can be seen in the experimental section (pp**199-213**).

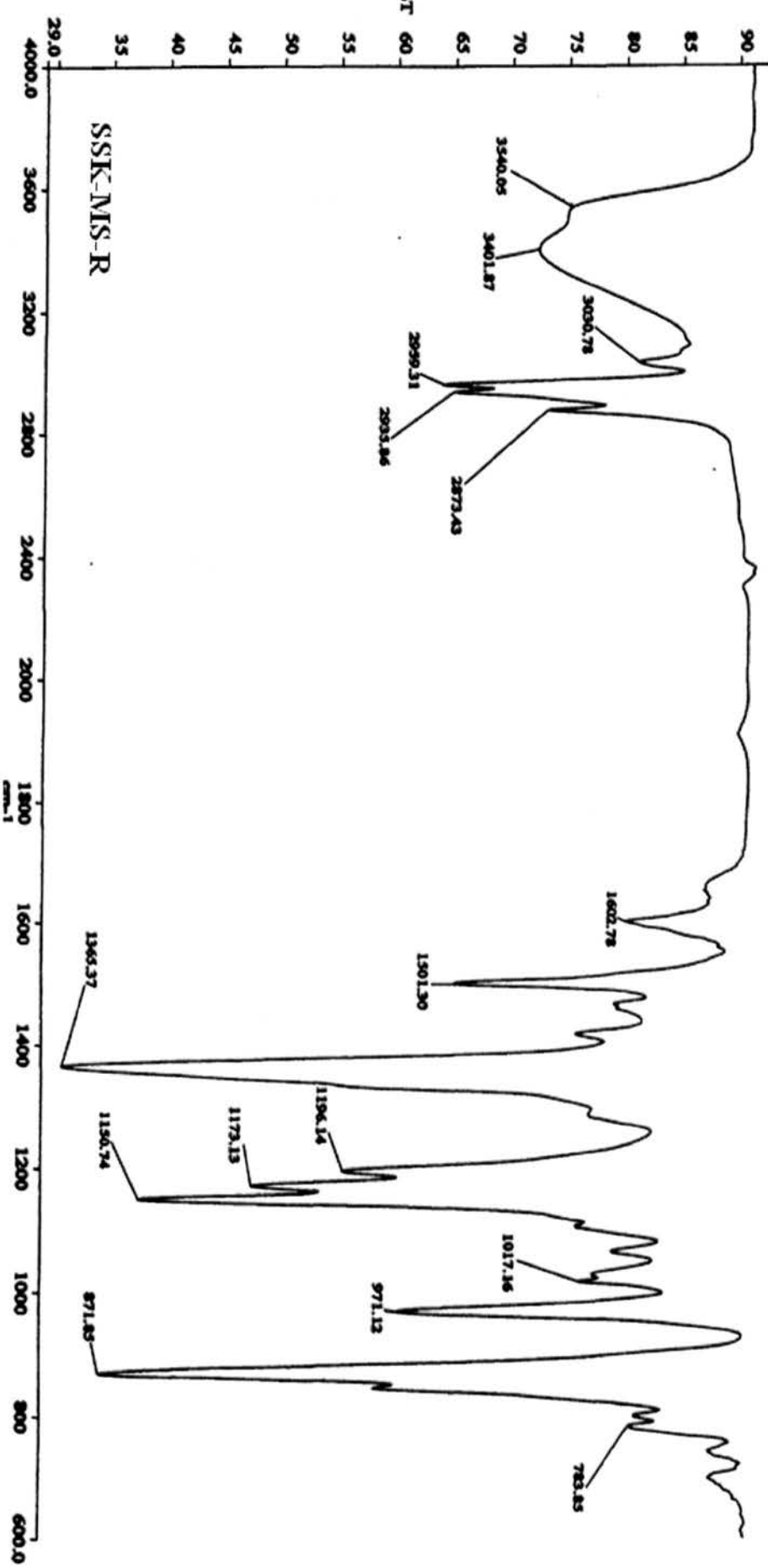



Figure 2.13: The IR spectrum of compound (**190**)

The various classes of synthesised inhibitors for biochemical screening are summarised in Table 2.7.

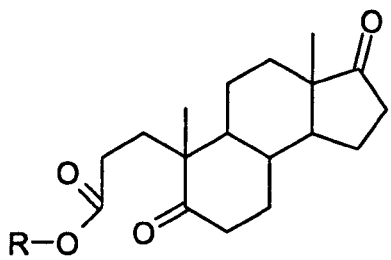
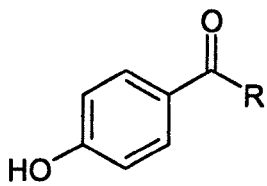
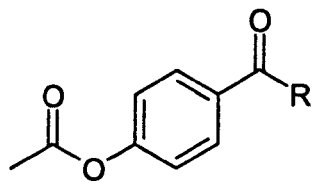
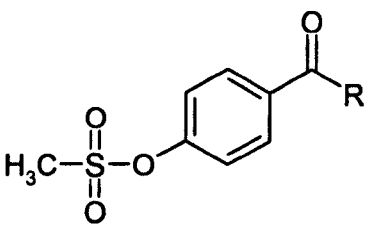
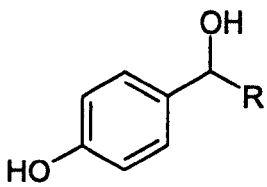
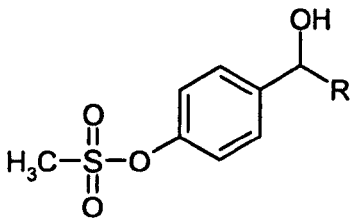
| Set 1 | Set 2 | Set 3 |
|---|---|---|
|  |  |  |
| 26 compounds<br>Compounds (**103-128**) | 13 compounds<br>Compounds (**130-142**) | 15 compounds<br>Compounds (**143-157**) |
| **Set 4** | **Set 5** | **Set 6** |
|  |  |  |
| 15 compounds<br>Compounds (**158-172**) | 14 compounds<br>Compounds (**173-186**) | 15 compounds<br>Compounds (**187-201**) |

Table 2.7: Summary of synthesised inhibitors

Due to limitation in terms of time and laboratory availability only set 1, set 3 and set 4 libraries of derivatives were actually screened for biochemical evaluation against 17β-HSD 3 obtained from rat testes.

# CHAPTER 3: BIOCHEMICAL EVALUATION

# 3. INTRODUCTION

The conversion of low active $\Delta^4$-dione (**98**) into its biologically active form, testosterone (**99**), is catalysed by the enzyme 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) using NADPH as a cofactor. In the current study the inhibitory activity of a number of synthesised compounds was determined against 17β-HSD3 by using a method derived from the literature (Le Lain *et al*, 2001). The assay was carried out by incubation in a shaking water bath at 37°C. The radiolabelled substrate and the synthesised compounds were incubated for 35 minutes along with rat testicular microsome, as a source of enzyme, in the presence of NADPH in a phosphate buffer solution (pH 7.4). The reaction was quenched by the addition of diethyl ether (2mL) and the organic layer was separated. The aquous layer was further extracted by another volume of diethyl ether (2mL) and the organic layers were combined in a new tube before removal of solvent. The residue was reconstituted in acetone (30μL) and vortexed, and spotted on thin layer chromatography (TLC) plate along with the steroid carriers ($\Delta^4$-dione and testosterone). The plates were developed using (dichloromethane / ethyl acetate 70:30 as mobile phase. After development of the TLC plates the steroids were identified under ultra violet (UV) lamp. The silica spots were cut off and placed in scintillation vials. Acetone (1mL) and scintillation fluid (3mL) were added to each vial. Vials were vortexed and placed in a scintillation counter for reading of tritium in each vial.

## 3.1. MATERIALS

D-Glucose-6-phosphate and D-glucose-6-phosphate dehydrogenase (suspention in 3.2M ammonium sulphate solution) were purchased from Roche Diagnostics, Lewes, East Sussex. Radioactive $\Delta^4$-dione (**98**) and testosterone, and β-NADP mono-sodium salt were obtained from Sigma-Aldrich, Poole, Dorset.

The radioactivity of samples was measured by a LKB Wallac 1217 Rackbeta Liquid Scientillation Counter and the scintillation fluid used was Optiscint Hisafe from Perkin-Elmer Life and Analytical Science, Beaconfield, Bucks.

The rat testes (Sprague-Dawley) were purchased from Charles Rivers, Margate, Kent. Testes were homogenised using an ultra-Turrax mincer (Janke and Kunkel, Germany) and further homogenisation was carried out using Teflon in glass homogeniser (Potter-Elvejhem). The separation of the mixture was carried out using a Backmann Coulter ultra-centrifugation. A UNICAM 8700 series ultra violet / visible (UV/VIS) spectrometer was used to read the absorbance of samples at 750 nm.

### 3.1.1. Buffer preparation

Different buffers used in the assay were prepared as follows:

#### 3.1.1.1. Sodium phosphate buffer 50mM pH 7.4

(A) Sodium dihydrogen orthophosphate dihydrate (156.01g/mol, 3.90g, 50mmol) was dissolved in distilled water (500mL).

(B) Disodium hydrogen orthophosphate dihydrate (177.99g/mol, 4.46g, 50mmol) was dissolved in distilled water (500mL).

Solution (A) was added to solution (B) until the pH of 7.4 was achieved.

#### 3.1.1.2. Potassium phosphate buffer 50mM pH 7.4

(A) Potassium dihydrogen orthophosphate (136.09g/mol, 13.61g, 100mmol) was dissolved in distilled water (500mL).

(B) Dipotassium hydrogen orthophosphate (174.18g/mol, 34.84, 200mmol) was dissolved in distilled water (1L).

Solution (A) was added to solution (B) until the pH 7.4 was achieved. The solution (250mL) was further diluted to (50mM) using distilled water (750mL).

#### 3.1.1.3. Sucrose phosphate buffer pH 7.4

Sucrose (342.30g/mol, 21.39g) was dissolved in potassium phosphate buffer (50mM) pH 7.4 (250mL).

#### 3.1.1.4. **Folin-Lowry solutions**

Standard bovine serum albumin (BSA) (200μg/mL, 5mg) was dissolved in distilled water (25mL).

(A) Copper sulphate solution (159.60g/mol, 0.25g) was dissolved in distilled water (25mL).

(B) Potassium sodium tartrate (282.22g/mol, 0.50g) was dissolved in distilled water (25mL).

Sodium hydroxide solution (NaOH) (40.00g/mol, 1.00g, 0.1M) was dissolved in distilled water (250mL).

(C) Sodium carbonate anhydrous (105.99g/mol, 5g) was dissolved in NaOH 0.1M solution (250mL).

Reagent A = 2mL of (A) + 2mL of (B) + 200mL of (C).

Reagent B = Folin-Cicalteau reagent (10mL) was diluted with distilled water (10mL).

### 3.1.2. **Preparation of NADPH-generating system**

Following components were used for the prepation of NADPH-generating system (1mL)

a) NADP-sodium salt (8.6mg)

b) D-glucose-6-phosphate (28.2mg)

c) D-glucose-6-phosphate dehydrogenase (15μL)

d) Sodium phosphate buffer pH 7.4 (50mM, 1000μL)

### 3.1.3. Preparation of testicular microsomes

Sprague-Dawley rat testes were decapsulated and kept in sucrose phosphate buffer solution at (pH 7.4). The first homogenisation of the tissues was carried out using ultra-Turrax mincer while maintaining the temperature at 4°C. Tissues were further homogenised using Potter homogeniser and subsequently centrifuged for 20 min at 4°C and 12,000 rpm (10,000 g). The supernatant was transferred into a new tube and further centrifuged for 1h at 4°C and 35,000 rpm (100,000 g). The supernatant was discarded and the pellets were suspended in sodium phosphate buffer (pH7.4) and homogenised using a potter homogeniser. Aliquots of the suspension (500μL) were transferred into eppendorf tubes and snap frozen in liquid nitrogen and stored at -70°C for future usage.

### 3.1.4. Substrate preparation of [1, 2, 6, 7-$^3$H] $\Delta^4$-dione (100μM)

The substrate required for assay was the combination of the radiolabelled (hot) and non-radiolabelled (cold) $\Delta^4$-dione and was prepared by transferring radiolabelled (tritium, $^3$H) [1, 2, 6, 7-$^3$H] of $\Delta^4$-dione (11.5μM, 20μL) in toluene:ethanol (9:1) into a clean vial solvent was evaporated under a flow of nitrogen. Then non-radiolabelled $\Delta^4$-dione in propane-1, 2-diol (88.5μM, 1mL) was added which resulted to the final concentration of 100μM.

### 3.1.5. Determination of protein content of the rat testicular Microsomes

Folin-Lowry (Lowry *et al*, 1951) method was the method used to determine the protein concentration of the rat testicular microsomes. The assay depends on the presence of phenolic amino acid in the protein. The production of cupric and peptide bond complex (between the alkalin cooper-phenol reagent used and the tyrosine and tryptophan residue of the protein) was measured at λmax of 750 nm. The protein content was determined colorimetrically with reference to bovine serum albumin (BSA) as standard. Standard solutions (0, 40, 80, 120, 160 and 200μg/mL) of bovine serum albumin protein were prepared from stock (section 3.1.1.4) in triplicate with distilled water. The microsomal protein was diluted by the factor of 100 (250μL in 25mL of distilled water) and 1mL in triplicate tested alongside the standards. Aliquots (5mL) of the reagent (A) (section 3.1.1.4) were then added to each test tube and vortexed at 30 seconds intervals. After standing for 10 minutes, reagent (B) (Folin-Cicalteau reagent) (section 3.1.1.4) (500μL) was added to each tube, vortexed and allowed to stand at room temperature for 30 minutes. The optical density (absorbance) of each solution was measured at λmax 750nm against blank (Table 3.1). A standard curve of absorbance against protein concentration was then plotted (Figure 3.1). The protein concentration of the testicular microsome was determined to be 411.67μg/mL.

| Vol. BSA (μL) | Conc. (μg/mL) | OD1 | OD2 | OD3 | Average | Average adjusted |
|---|---|---|---|---|---|---|
| 0 | 0 | 0.026 | 0.025 | 0.026 | 0.026 | 0.000 |
| 200 | 40 | 0.137 | 0.146 | 0.175 | 0.153 | 0.127 |
| 400 | 80 | 0.267 | 0.273 | 0.296 | 0.279 | 0.253 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 600 | 120 | 0.396 | 0.408 | 0.492 | 0.432 | 0.406 |
| 800 | 160 | 0.500 | 0.499 | 0.509 | 0.503 | 0.477 |
| 1000 | 200 | 0.585 | 0.595 | 0.620 | 0.600 | 0.574 |
| Test sample | X | 1.238 | 1.260 | 1.284 | 1.261 | **1.235** |

Table 3.1: Folin Lowry results obtained at 750nm

The average adjusted absorbance of testicular microsome = 1.235

From the graph y = 0.003x where y = 1.235 so

1.235 = 0.003x → x = 1.235 / 0.003 = 411.6666 ≈ 411.67μg/mL

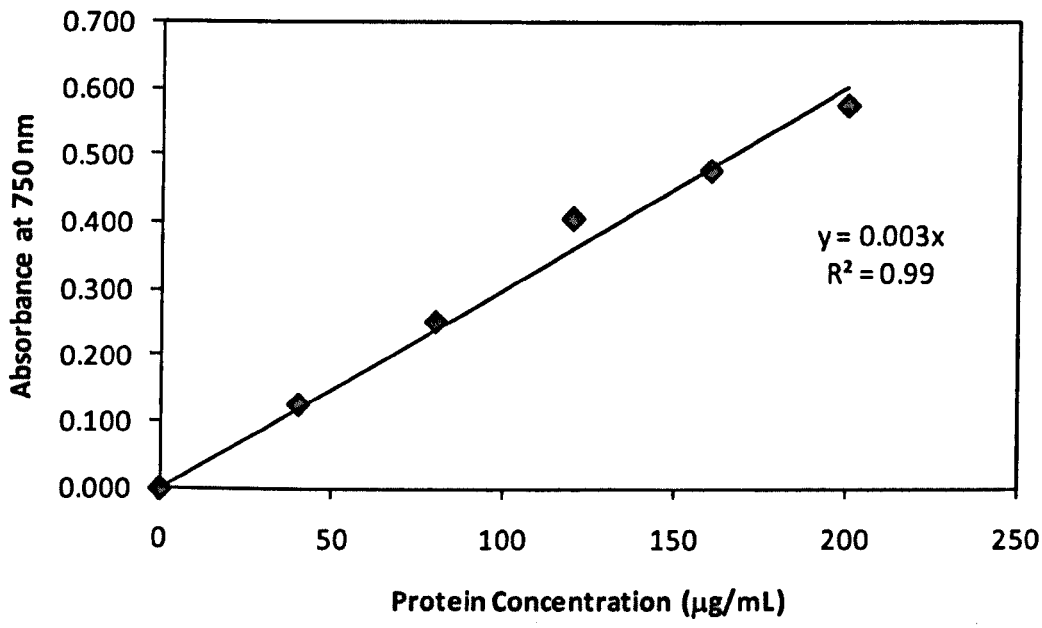



Figure 3.1: Folin-Lowry plot for determination of microsomal protein content

### 3.1.6. **Protein dependency assay**

Different concentration of protein (0.21, 0.41, 0.61, 0.82, 1.03 and 1.24mg/mL) were used in this assay. The substrate $\Delta^4$-dione (Cold and hot) (1μM final concentration, 10μL), NADPH-generating system (50μL) and

sodium phosphate buffer (pH 7.4, made up to 1mL) (Table 3.2) was incubated at 37°C in a shaking water bath. The reaction was initiated by the addition of the rat testicular microsome.

| | Vol. ( μL) | Vol. ( μL) | Vol. ( μL) | Vol. ( μL) | Vol. ( μL) | Vol. ( μL) |
|---|---|---|---|---|---|---|
| Substrate | 10 | 10 | 10 | 10 | 10 | 10 |
| NADPH | 50 | 50 | 50 | 50 | 50 | 50 |
| Enzyme | 5 | 10 | 15 | 20 | 25 | 30 |
| Buffer | 935 | 930 | 925 | 920 | 915 | 910 |
| Tot. Vol. | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |

Table 3.2: Volumes required for protein dependant assay

The assay mixture was incubated at 37°C for 35 minutes. The reaction was quenched by addition of diethyl ether (2mL), vortexed and allowed to stand in ice for 15 minutes. The organic layer was separated into a clean tube and the aqueous layer was re-extracted by adding diethyl ether (2mL). The organic extracts were combined and the sovent was removed under a flow of nitrogen. Acetone (30mL) was then added to each tube, vortexed and samples were spotted onto silica based TLC plates along with carrier steroids ($\Delta^4$-dione and testosterone, 5mg/mL). Plates were developed using a mobile phase consisting of dichloromethane and ethyl acetate (70:30). The steroids spots were identified using an ultra violet lamp. Spots were cut off from the plates and placed into a clean scintillation vial. Acetone (1mL) was then added, and vortexed in order to dissolve steroids from the silica plates and scintillation fluid (3mL) was added and the mixture was again vortexed. The samples were read for tritium ($^3H$) for 3 minutes each and the percentage conversion of $\Delta^4$-dione to testosterone was calculated using the equation 1:

$$\text{percentage conversion} = \frac{\text{CPM (Product)}}{\text{CPM (Product) + CPM (Substrate)}} \times 100$$

Equation 1: Formula for the calculation of percentage conversion of $\Delta^4$-dione into testosterone (where CPM = counts / minute)

The percentage conversion against protein concentration mg/mL (Figure 3.2) was then plotted and the linear relationship was observed up to protein concentration of 0.21mg/mL.

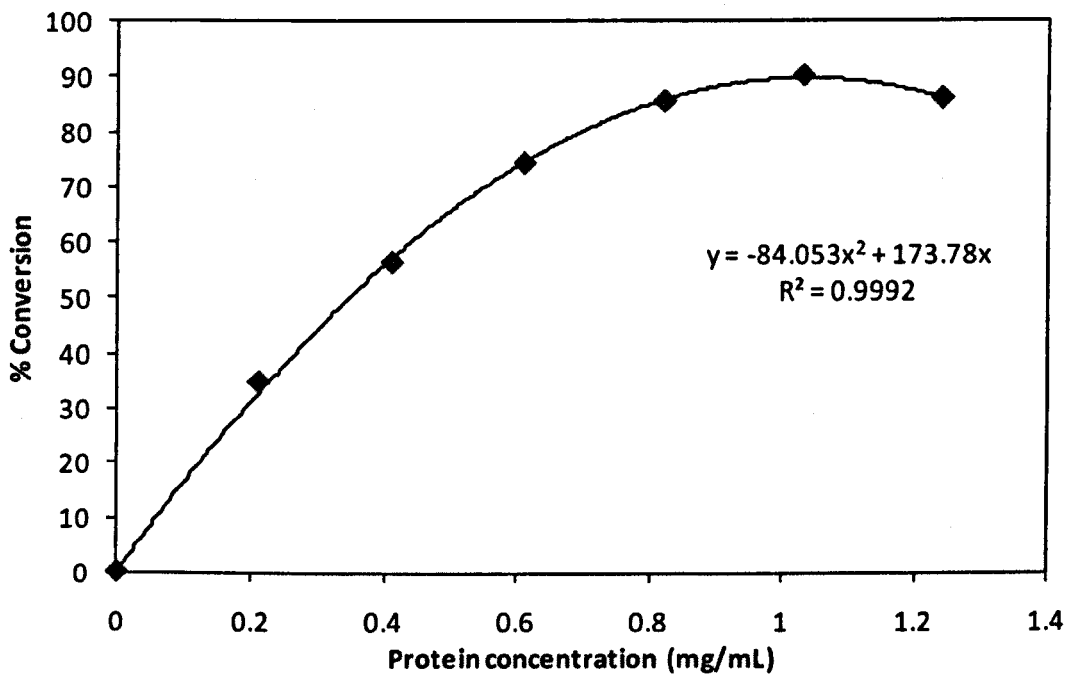



Figure 3.2: Protein dependency assay for the conversion of substrate to product

### 3.1.7. **Time-dependency assay**

Time-dependency assay carried out in order to see whether the assay was within the linear phase of enzymatic reaction.

The assay was carried out in triplicate with the final concentration of prepared radiolabelled $\Delta^4$-dione of 1μM (10μL), NADPH-generating system (50μL) and sodium phosphate buffer pH 7.4 (Table 3.3)

| | Vol.( μL) | Vol.( μL) | Vol.( μL) | Vol.( μL) | Vol.( μL) | Vol.( μL) |
|---|---|---|---|---|---|---|
| Substrate | 10 | 10 | 10 | 10 | 10 | 10 |
| NADPH | 50 | 50 | 50 | 50 | 50 | 50 |
| Buffer | 940 | 935 | 935 | 935 | 935 | 935 |
| Enzyme | 0 | 5 | 5 | 5 | 5 | 5 |
| Tot. Vol. | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| Time / min | 0 | 5 | 10 | 20 | 30 | 45 |

Table 3.3: Volumes required for time dependant assay

The tubes were incubated in a shaking water bath at 37°C for 5 minutes. The assay was initiated by the addition of testicular microsomes (final assay concentration of 0.21mg/mL, 5μL). The addition of testicular microsome was carried out at different intervals of (10, 20, 30, 45, 60 and 90 minutes). The assay tubes were quenched by the addition of diethyl ether (2mL) and vortexed. The assay was completed as pereviouslly described (section 4.1.6) and the percentage conversion for each tube was calculated using equation 1.

The time-dependency curve (percentage conversion against time) was plotted (Figure 3.3) and linear relationship was observed up to 35 minutes.

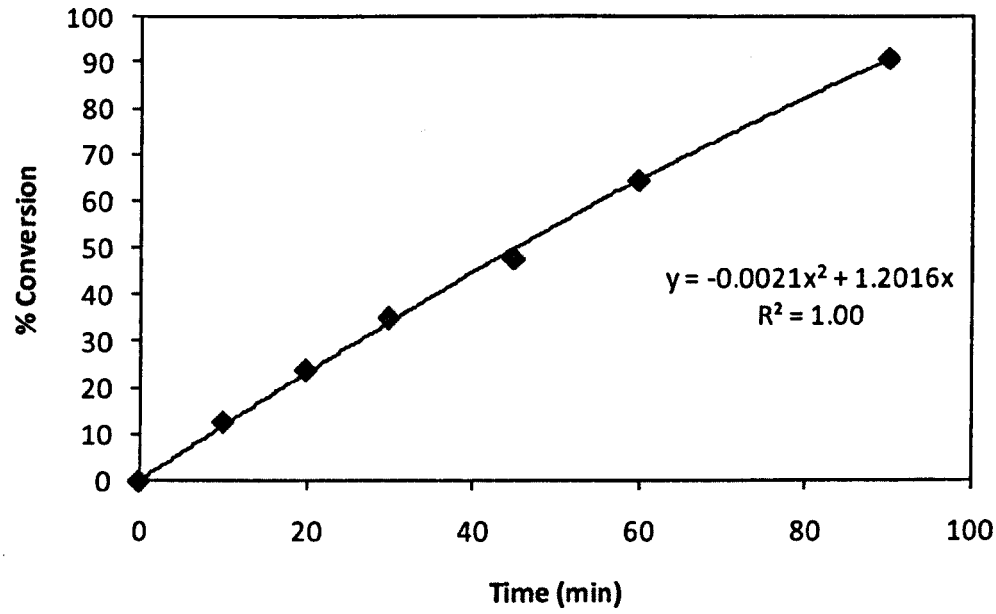



Figure 3.3: Time dependency assay plot

### 3.1.8. Determination of Michaelis constant ($K_m$)

The Michaelis Constant ($K_m$) is a measure of the affinity of the substrate for the enzyme and may be explained as the substrate concentration at which the initial rate is half of the maximum velocity ($V_{max}$) and was obtained. The substrate (100μM) was serially diluted by propane-1,2-diol to give the final concentrations of 0.25, 0.5, 1, 2, 4 and 6μM. The incubation mixtures (1mL) consisting of NADPH-generating system (50μL), substrate and phosphate buffer solution pH 7.4 were incubated at 37°C (Table 3.4). The assay was started by the addition of the testicular microsomes which were defrosted and warmed to 37°C prior to the addition (5μL, 0.21 mg/mL).

| | Vol. / μL | Vol. / μL | Vol. / μL | Vol. / μL | Vol. / μL | Vol. / μL |
|---|---|---|---|---|---|---|
| Substrate | 10 (25μM) | 10 (50μM) | 10 (100μM) | 20 (100μM) | 40 (100μM) | 60 (100μM) |
| NADPH | 50 | 50 | 50 | 50 | 50 | 50 |
| Buffer | 935 | 935 | 935 | 925 | 905 | 885 |
| Enzyme | 5 | 5 | 5 | 5 | 5 | 5 |
| Tot. Vol. | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| Final sub.Conc. | 0.25 μM | 0.5 μM | 1 μM | 2 μM | 4 μM | 6 μM |

Table 3.4: Volumes and concentrations required for determination of Km

The solutions were incubated, in a shaking water bath at 37°C, for 35 minutes. The reaction was quenched by the addition of diethyl ether (2mL) and placed in the ice. The assay was completed as previously described (section 3.1.6)

The velocity for each substrate concentration was calculated using the equation 2 where unit for (V) was μM/min/mg.

$$V = \frac{\text{CPM (testosterone) x substrate conc. [S] (}\mu\text{M)}}{\text{CPM (androstenedione+testosterone) x protein conc. (mg/mL) x time (min)}}$$

Equation 2: Formula for calculation of (V)

### 3.1.9. Graphical determination of Michaelis constant ($K_m$)

$K_m$ was calculated using five different plots (Figure 3.4-Figure 3.8).

Equations required for the calculation of $K_m$ are as below:

Line weaver Burk equation $(1 / V = K_m / V_{max} \times 1 / S + 1 / V_{max})$

Hanes-Woolf equation $(S / V = 1 / V_{max} \times S + K_m / V_{max})$

Eadie-Hofstee equation $(V = -K_m \times V / S + V_{max})$

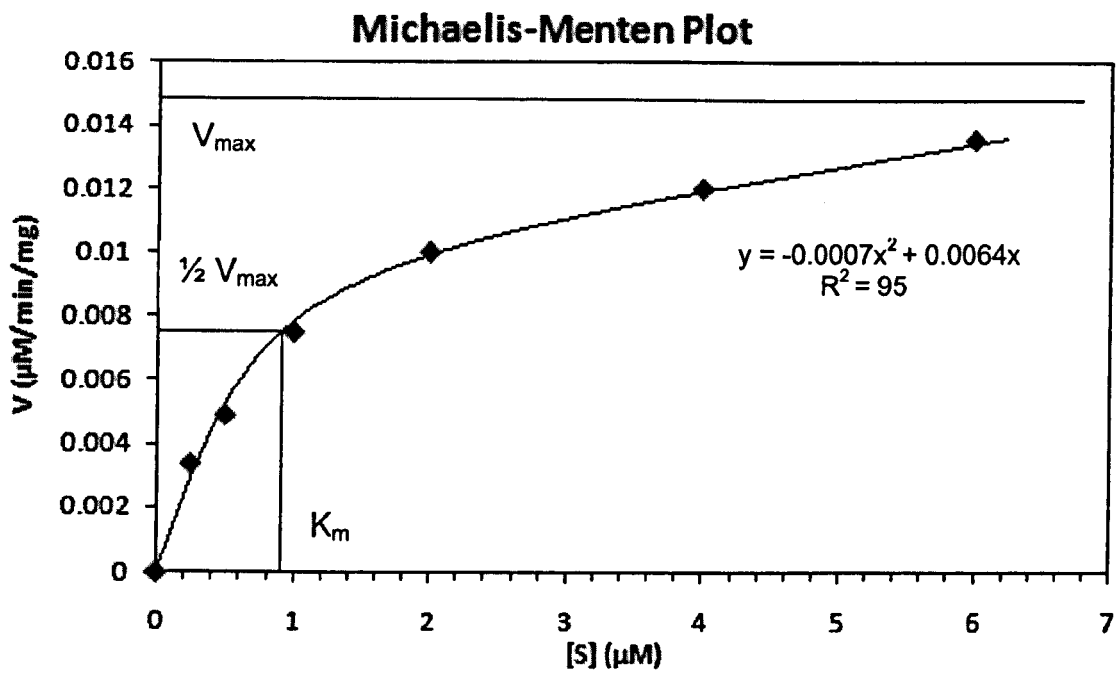



Figure 3.4: Michaelis Menten plot for 17β-HSD3

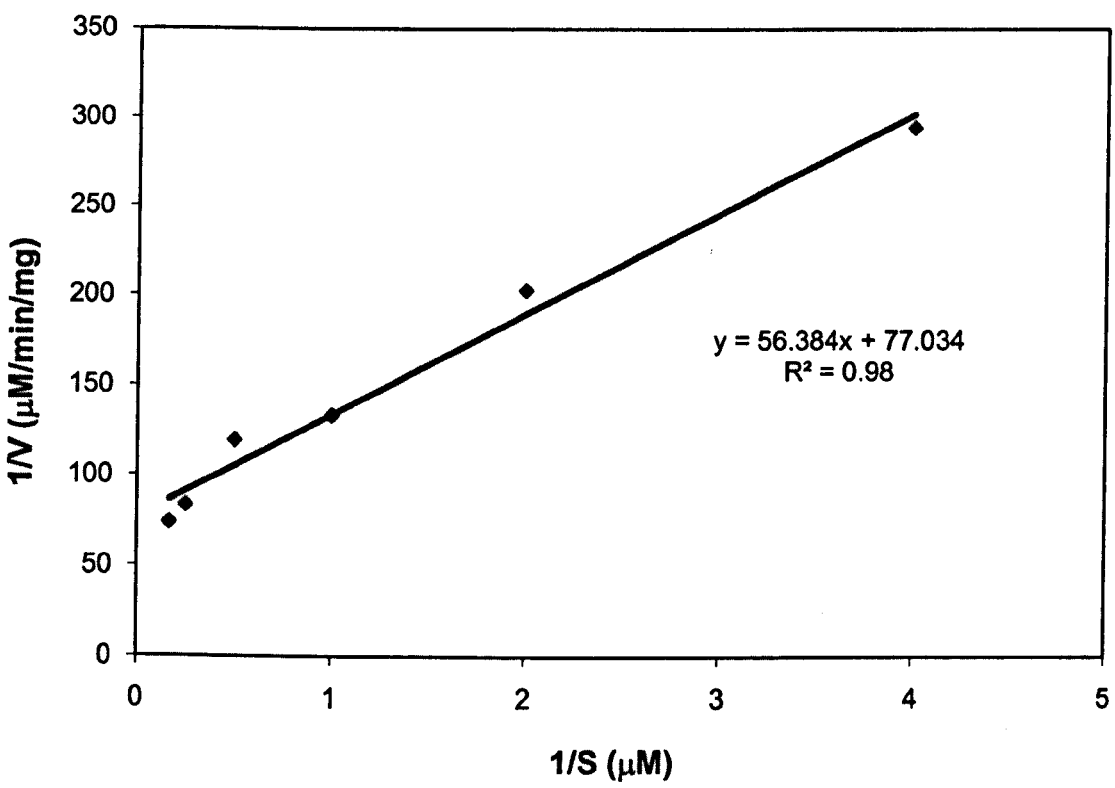



Figure 3.5: Lineweaver-Burk plot

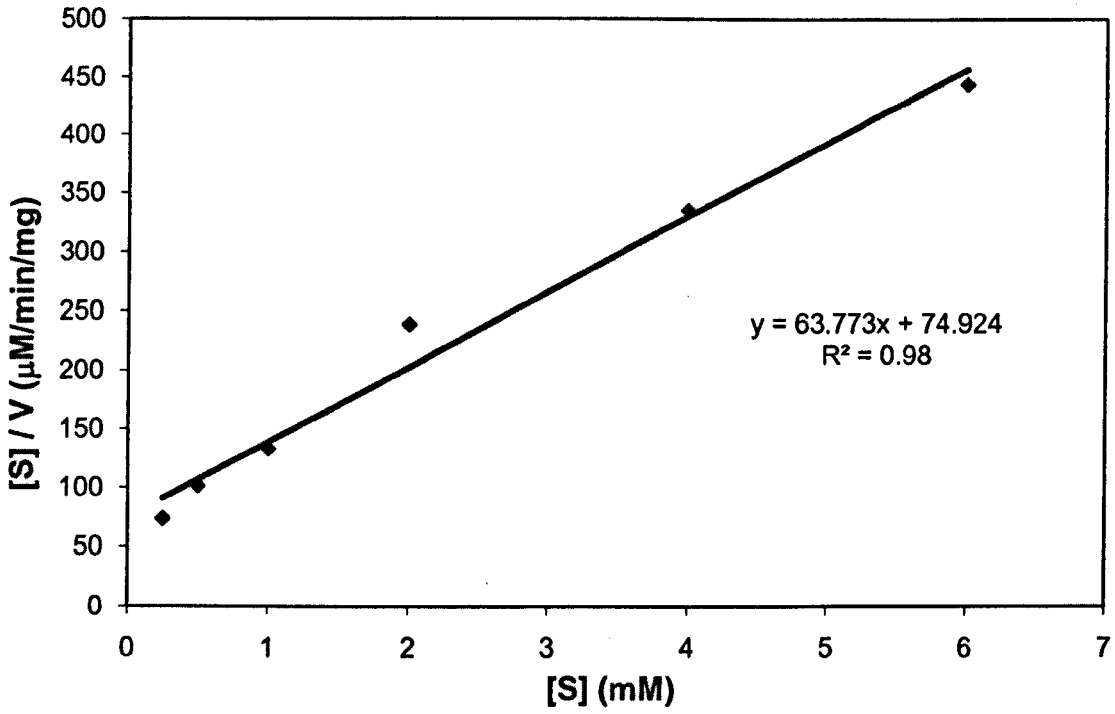



Figure 3.6: Hanes-Woolfe plot

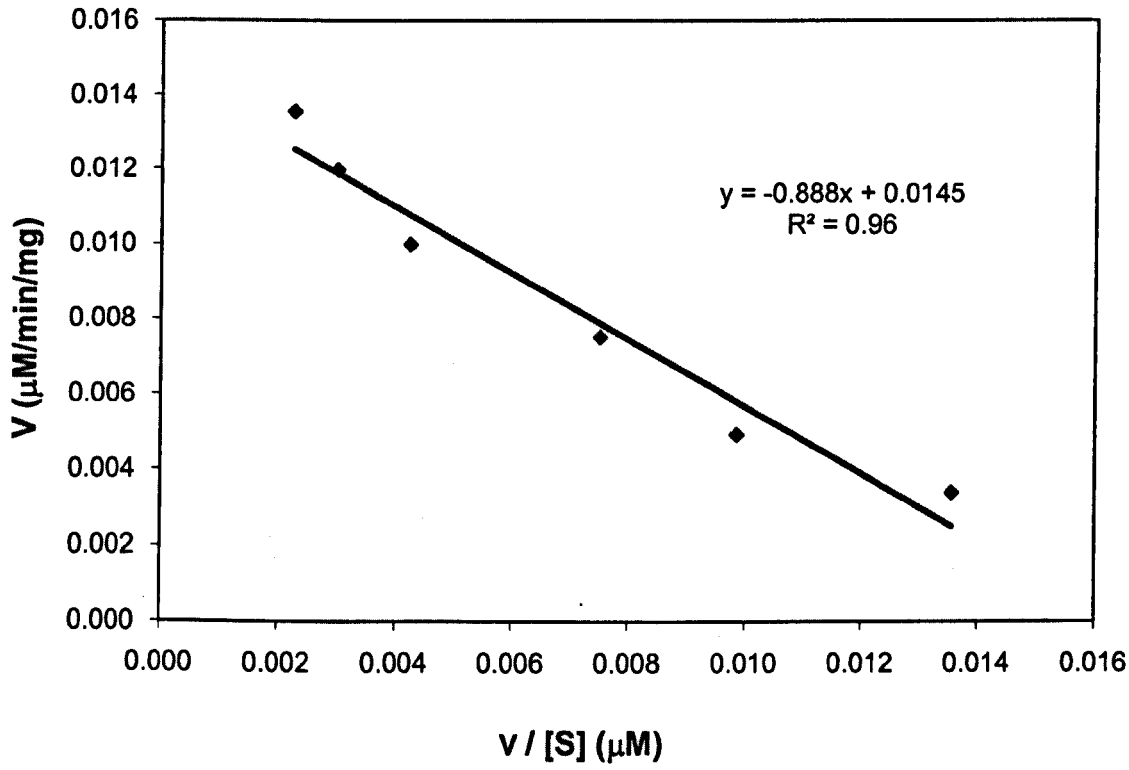



Figure 3.7: Eadie-Hofstee plot

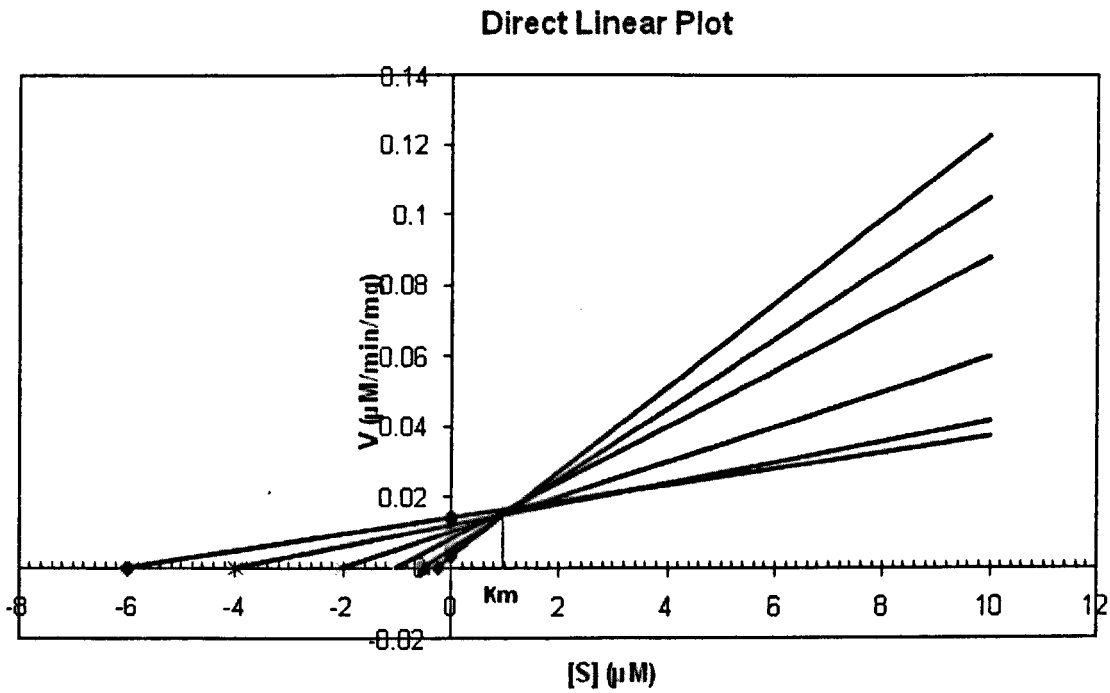



Figure 3.8: Cornish-Bowden plot

The $K_m$ value was determined from the average of five different graphical methods (Table 3.5). The results have been statistically analysed using Q-test method.

| plot | $K_m$ (µM) |
|---|---|
| Michaelis Menten | 0.90 |
| Lineweaver-Burk | 0.73 |
| Hanes-Woolfe | 1.17 |
| Eadie-Hofstee | 0.89 |
| Cornish-Bowden | 0.90 |
| **Average $K_m$** | **0.92** |

Table 3.5: $K_m$ values obtaines for 17β-HSD3

#### 3.1.9.1. **Discussion**

The Michaelis Constant ($K_m$) was determined from five different graphical methods (Figure 3.4-Figure 3.8) and averaged to be 0.92μM for 17β-HSD3 using microsomal fraction from rat testes, whilst Le Lain *et al* (2001) had the $K_m$ value of 0.77μM for the conversion of $\Delta^4$-dione to testosterone.

### 3.1.10. **Preliminary screening of synthesised compounds against 17β-HSD3**

In order to determine the initial inhibitory activity of synthesised compounds against 17β-HSD3, the synthesised compounds and standards (7-hydroxyflavone and baicalein) were dissolved in dimethyl sulfoxide (DMSO) to a required final concentration. The total assay volume was 1000μL and the substrate concentration of about 3 $K_m$ was used with a single inhibitor concentration of (100μM) in triplicate. All the volumes and concentrations were prepared as shown below (Table 3.6). The samples were incubated in a shaking water bath at 37°C for 35 minutes. The reaction mixture was quenched using diethyl ether (2mL). The assay was completed as previously described (section 3.1.6) and the percentage conversion was determined equation 3.

| | Assay mixture |
|---|---|
| **substrate** | 27μL (2.7μM) |
| **Inhibitor** | 20μL (100μM) |
| **NADPH** | 50 μL |
| **Buffer** | 898 μL |
| **Enzyme** | 5μL (0.21μM) |
| **Tot. Vol.** | 1000 μL |

Table 3.6: Volumes and concentrations required for initial screening

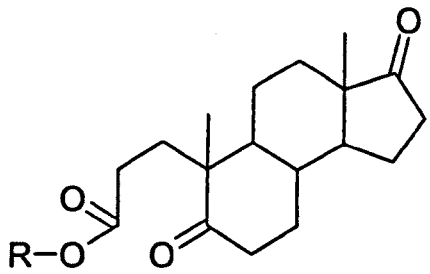
$$\text{Percentage inhibition} = \frac{\text{Avg \% conversion of blank - Avg \% conversion of inhibitor}}{\text{Avg \% conversion of blank}} \times 100$$

Equation 3: Formula for calculation of percentage inhibition of synthesised compounds

### 3.1.10.1. **Results**

The results of initial biochemical evaluation of tested compounds are tabulated in Table 3.7 - Table 3.10 with 7-hydroxyflavone (**63**) and baicalein (**64**) as standards. As the assays were carried out on different days, the percentage inhibitions of standards are quoted for each assay in order to take into account the variability of conducting assays of this nature.

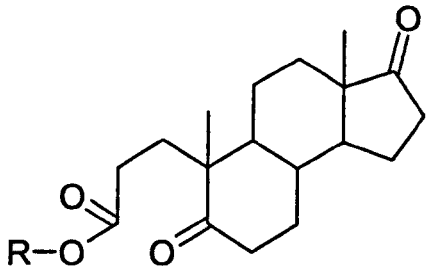
The compounds which are highlighted had the highest inhibitory activity toward 17β-hydroxysteroid dehydrogenase 3 in comparison to standards (**63**) and (**64**).




**(102)**

| Compound | R= | % inhibition of 17β-HSD3 ([I]=100μM) |
|---|---|---|
| **Standard 63** | N/A | 25.01±0.18 |
| **Standard 64** | N/A | 30.73±0.27 |
| **103** | $CH_3$ | 16.82±0.36 |
| **104** | $C_2H_5$ | 15.40±0.28 |
| **105** | $C_3H_7$ | 31.68±0.24 |
| **106** | **$C_4H_9$** | **38.96±0.08** |
| **107** | **$C_5H_{11}$** | **43.18±0.05** |
| **118** | **$C_6H_{13}$** | **38.00±0.09** |
| **109** | $C_7H_{15}$ | 34.66±0.03 |
| **110** | $C_8H_{17}$ | 28.88±0.27 |
| **111** | $C_9H_{19}$ | 16.16±0.36 |
| **112** | $C_{10}H_{21}$ | NQ |
| **113** | $C_{11}H_{23}$ | NQ |
| **114** | $C_{12}H_{25}$ | 21.94±0.62 |
| **115** | $C_{13}H_{27}$ | 25.87±0.18 |

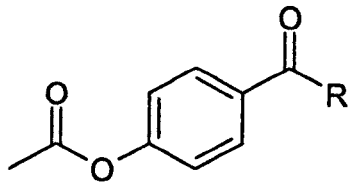
Table 3.7: Initial screening results obtained from set 1 (**103-115**) against 17β-HSD3



**(102)**

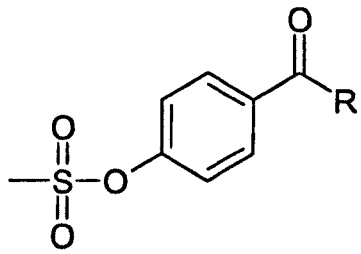
| Compound | R= | % inhibition of 17β-HSD3 ([I]=100μM) |
|---|---|---|
| **Standard 63** | N/A | 28.89±0.45 |
| **Standard 64** | N/A | 35.18±2.08 |
| **116** | $C_4H_7$ | 33.79±0.56 |
| **117** | **$C_5H_9$** | **38.85±0.31** |
| **118** | **$C_6H_{11}$** | **38.09±0.23** |
| **119** | $C_7H_{13}$ | 32.70±2.00 |
| **120** | **$C_8H_{15}$** | **39.76±0.15** |
| **121** | $C_4H_7$ | 35.28±0.62 |
| **122** | $C_5H_9$ | 36.88±0.40 |
| **123** | $C_6H_{11}$ | 36.64±0.30 |
| **124** | $C_7H_{13}$ | 36.52±0.41 |
| **125** | $C_8H_{15}$ | 36.36±0.33 |
| **126** | $C_9H_{17}$ | 35.18±0.54 |
| **127** | $C_{10}H_{15}$ | NQ |
| **128** | $C_{10}H_{17}$ | 28.15±2.15 |

Table 3.8: Initial screening results obtained from set 1 (**116-128**) against 17β-HSD3



| Compound | R= | % inhibition of 17β-HSD3 ([I]=100μM) |
|---|---|---|
| **Standard 63** | N/A | 12.90±0.31 |
| **Standard 64** | N/A | 13.66±0.16 |
| **143** | H | 14.31±0.47 |
| **144** | $CH_3$ | 29.38±0.02 |
| **145** | $C_2H_5$ | 13.00±0.44 |
| **146** | $C_3H_7$ | 17.83±0.18 |
| **147** | $C_4H_9$ | 25.84±0.41 |
| **148** | $C_5H_{11}$ | 36.49±0.34 |
| **149** | **$C_6H_{13}$** | **40.25±0.17** |
| **150** | **$C_7H_{15}$** | **40.51±0.14** |
| **151** | $C_8H_{17}$ | 37.05±0.18 |
| **152** | $C_9H_{19}$ | 16.45±0.02 |
| **153** | Cyclo$C_3H_5$ | 31.60±0.49 |
| **154** | Cyclo$C_4H_7$ | 23.12±0.43 |
| **155** | Cyclo$C_5H_9$ | 32.87±0.50 |
| **156** | **Cyclo$C_6H_{11}$** | **38.22±0.18** |
| **157** | Aryl | 32.85±0.44 |

Table 3.9: Initial screening results obtained from set 3 against 17β-HSD3



| Compound | R= | % inhibition of 17β-HSD3 ([I]=100μM) |
|---|---|---|
| **Standard 63** | N/A | 27.35±0.51 |
| **Standard 64** | N/A | 34.58±0.32 |
| **158** | H | 18.21±0.18 |
| **159** | $CH_3$ | NQ |
| **160** | $C_2H_5$ | 9.20±0.42 |
| **161** | $C_3H_7$ | 16.96±0.22 |
| **162** | $C_4H_9$ | 13.63±0.53 |
| **163** | $C_5H_{11}$ | 15.86±0.56 |
| **164** | $C_6H_{13}$ | 21.52±0.39 |
| **165** | $C_7H_{15}$ | 12.24 ±1.23 |
| **166** | $C_8H_{17}$ | 17.75±0.81 |
| **167** | $C_9H_{19}$ | NQ |
| **168** | Aryl | 17.43±0.28 |
| **169** | Cyclo$C_3H_5$ | 19.07±0.35 |
| **170** | Cyclo$C_4H_7$ | 16.88±0.12 |
| **171** | Cyclo$C_5H_9$ | 17.98±0.26 |
| **172** | Cyclo$C_6H_{11}$ | 20.31±0.33 |

Table 3.10: Initial screening results obtained from set 4 against 17β-HSD3

## 3.2. DISCUSSION

The initial screening of compounds (**103-128**) (set 1 & 2) shows several compounds were equipotent or better inhibitor than the standards. The best inhibitors in the range were compounds (**106-108, 117, 118, 120** and **127**) which provided the greatest inhibition compared to the standards. In fact compound (**107**) with (43%) inhibition was the best inhibitor in this range.

The results of initial screening of compounds (**143-157**) (set 3) showed that all compounds had good inhibitory activity toward 17β-HSD3 except for compound (**145**) which have shown poor inhibitory activity compared to standards. In this set of derivatives, compounds (**149**) and (**150**) were the best inhibitors in the range with (40.3%) and (40.5%) inhibitory activity respectively. These were approximately three times more potent than the standards, 7-hydroxyflavone (**63**) and baicalein (**64**) which give (12.90%) and (13.66%) inhibition respectively. Lota *et al* (2006) had previously reported that 1-(4-hydroxy phenyl) nonan-1-one (**79**) was the best inhibitor in the range they studied, showing about (83.5%) inhibitory activity. This was about 1.5 to 2 times more potent than their standards, (**63**) and (**64**) with (53.6%) and (38.8%) inhibition respectively. Upon consideration of our results obtained, it can be said that our compounds were better inhibitors as compared to those obtained by Lota *et al* (2006).

Molecular modelling of compounds (**79**) and (**150**) *in vacuo* using Sigress Explorer Ultra 7.7.0.47 (Copyright 2000-2007 Fujitsu Ltd. 1999-2000 Oxford Molecular Ltd) showed that compounds adopt a planar global energy

minimum. Superimposition of compounds (Figure 3.9) suggests that there should be enough space within the active site pocket to allow the acetyl group of (**150**) to fit in and there should also be some electron donor groups which can interact with oxygen atom that may stabilise the inhibitor/enzyme complex.

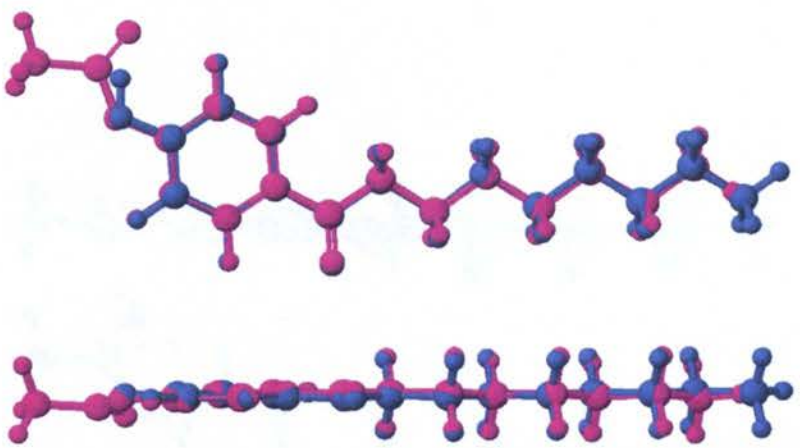


Figure 3.9: Superimpositioning of (**79**) presented in dark blue upon (**150**) presented in purple

Screening of the methanesulfonate-based compounds (**158-172**) (set 4) showed they had low inhibitory activity towards 17β-HSD3 in comparison to 4-hydroxyphenyl ketones. In fact, compound (**164)** the most potent inhibitor in the range, exhibited about (21%) inhibitory activity in contrast to standards, 7-hydroxyflavone (**63**) and baicalein (**64**) which possessed about (27%) and (34%) inhibitory activity respectively. These results are in agreement with the study carried out by Lota *et al* (2006) that showed that removal of the hydroxyl proton of the 4-hydroxyphenyl-based ketones reduced their inhibitory activity against 17β-HSD3. However, this could also be due to the fact that the methanesulfonate moiety may not fit properly within the active site. To

investigate this hypothesis, we modelled compound (**166**) using analagous conditions. The global energy minimum conformer of compound (**166**) was superimposed on that of compound (**150**) (Figure 3.10). It was observed that the methyl group of the methanesulfonate moiety was standing out of plane. This might explain why (**166**) had low inhibitory activity due to a poorer fit into the active site of the enzyme than compound (**150**).

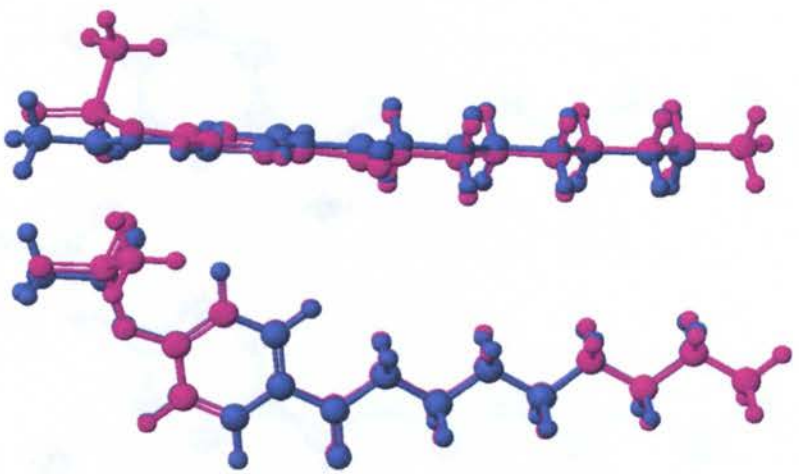


Figure 3.10: Superimpositioning of (**150**) presented in dark blue upon (**166**) presented in purple.

The partial charges of relevant atoms for compounds (**79**), (**150**) and (**166**) was also calculated (Figure 3.11) and it was observed that all the carbonyl oxygens have similar partial charges of about (-0.400). The partial charges of the phenolic oxygen of compound (**79**) and compound (**150**) were similar, -0.350 and -0.327 respectively, but in compound (**166**) (set 4) the phenolic oxygen had a partial charge of -0.671 (Figure 3.11). The sulfonated moiety was highly charged, with the oxygens attached to the sulphur atom having partial charges of about -0.900 whereas the sulphur atom itself had a partial

charge of about +2.738. It was also observed that the methyl group of compound (**150**) had a partial charge of about -0.339 which was about 3 times smaller than that of the methyl group of compound (**166**) with a partial charge of about -1.106. The high polarity of the methane sulfonate based compounds could suggest why this set of compounds was not able to stabilise in a hydrophobic pocket of active site and as such resulting in poorer inhibition.

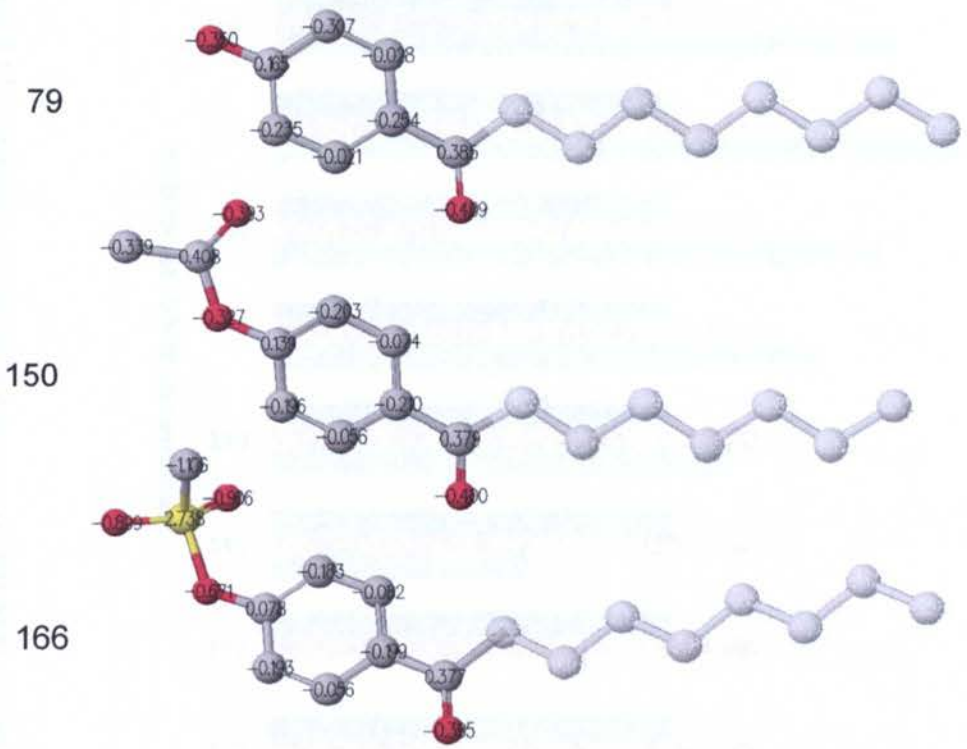



Figure 3.11: Partial electronic charges for compounds (**79**), (**150**) and (**166**).

The inhibitory activities of tested compounds against standards are represented by charts in (Figure 3.12 - Figure 3.15).

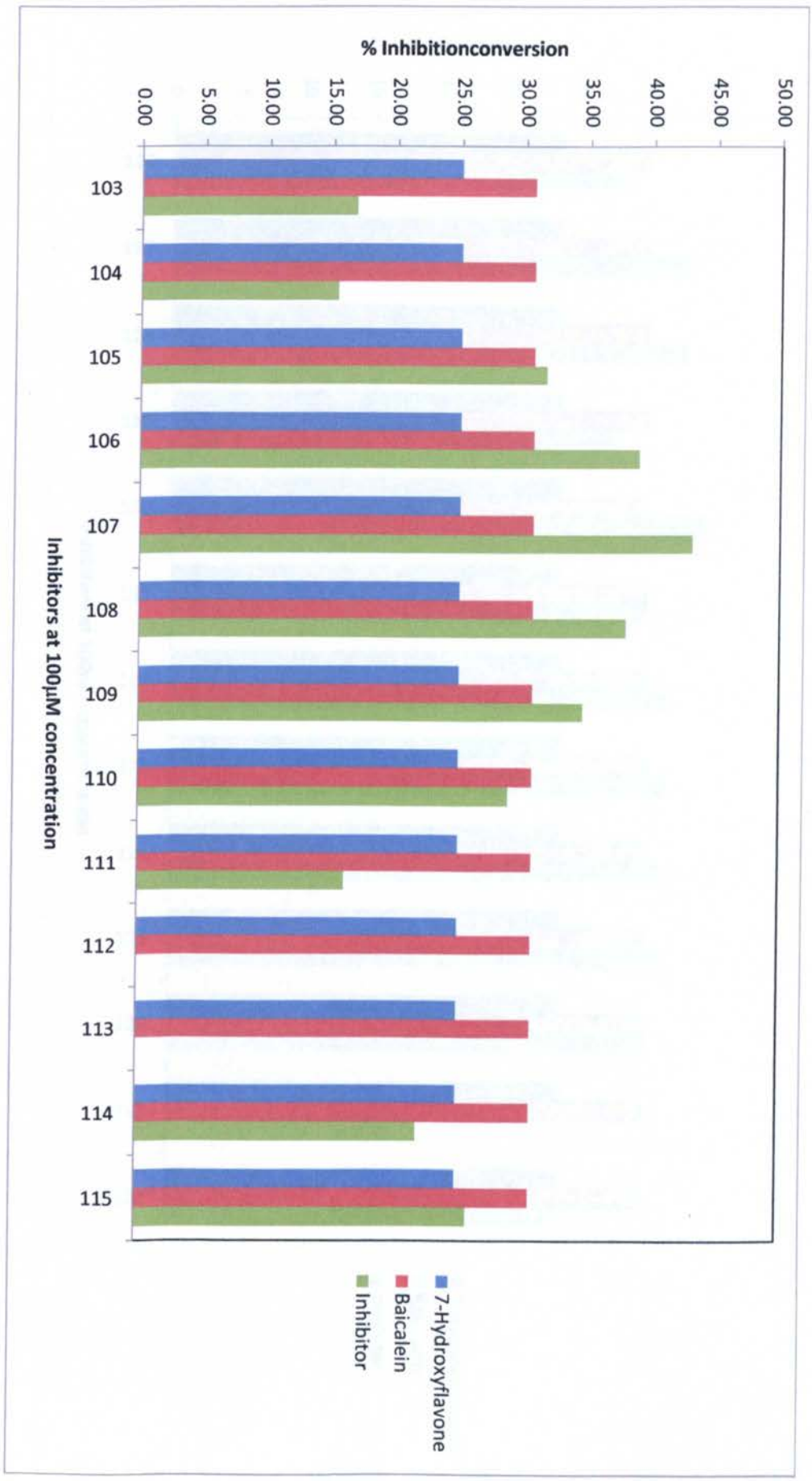



Figure 3.12: Percentage inhibition of compounds (**103-115**) against standards.

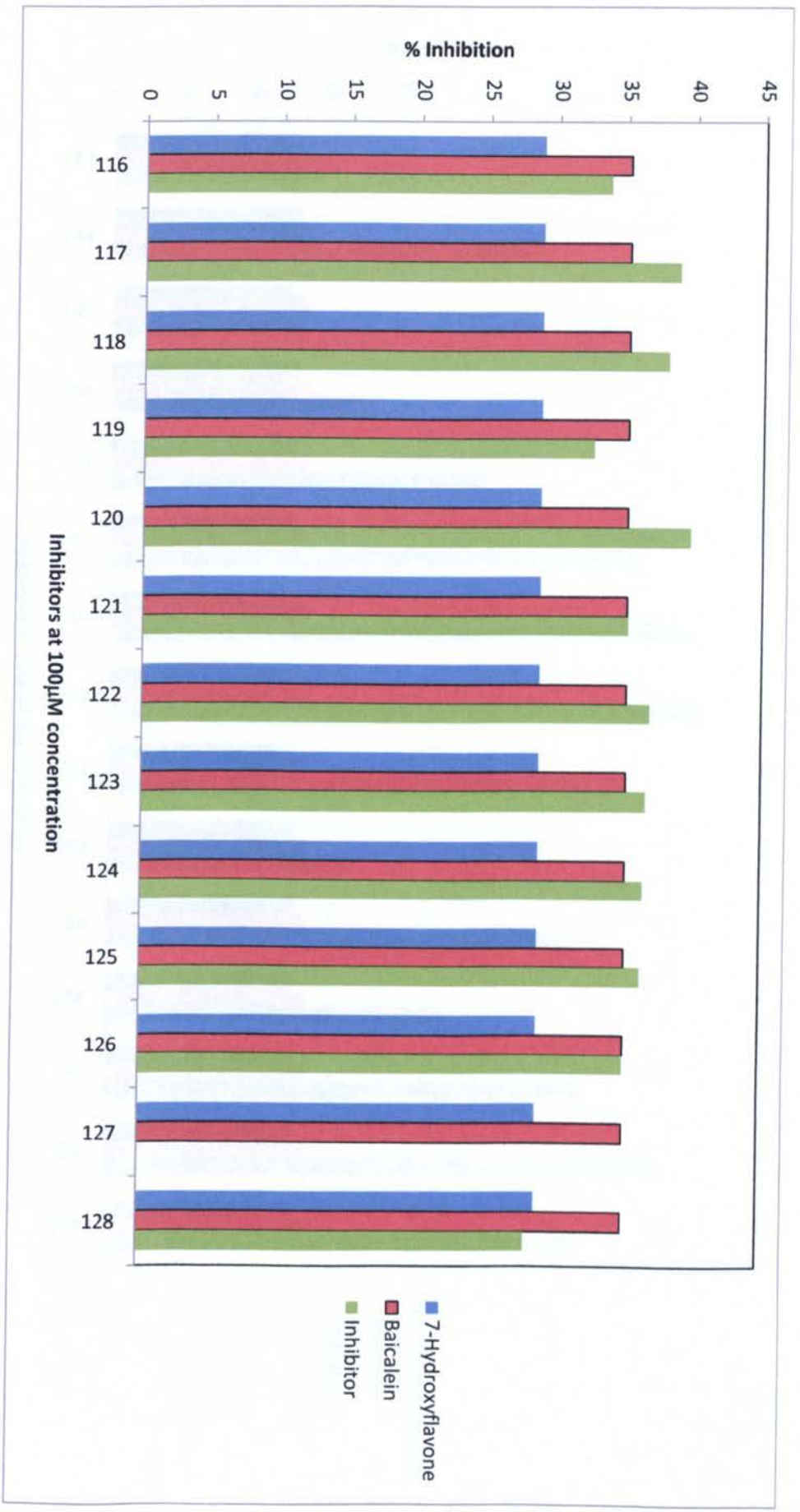



Figure 3.13: Percentage inhibition of compounds (**116-128**) against standar

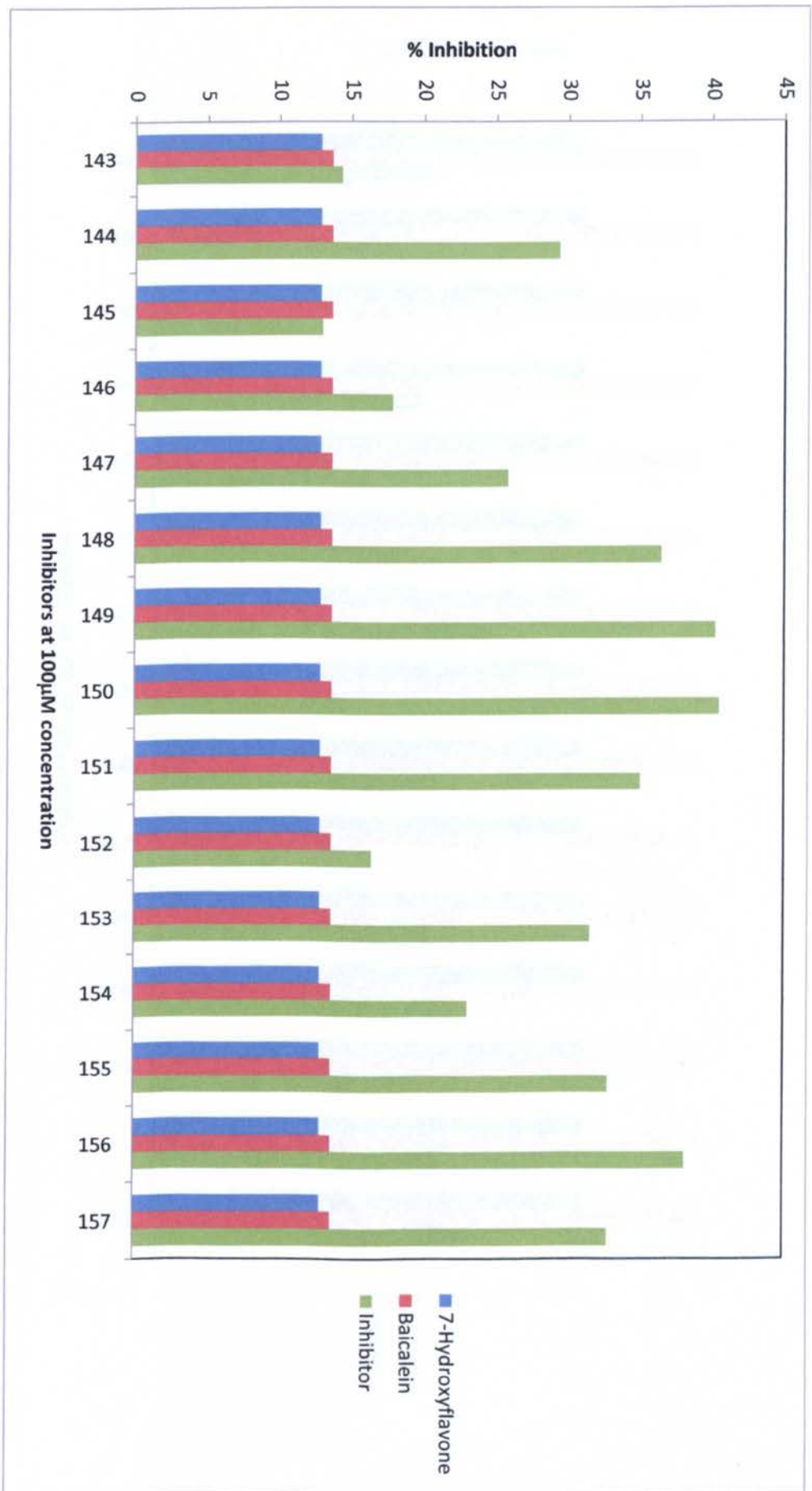



Figure 3.14: Percentage inhibition of compounds (**143-157**) against standards.

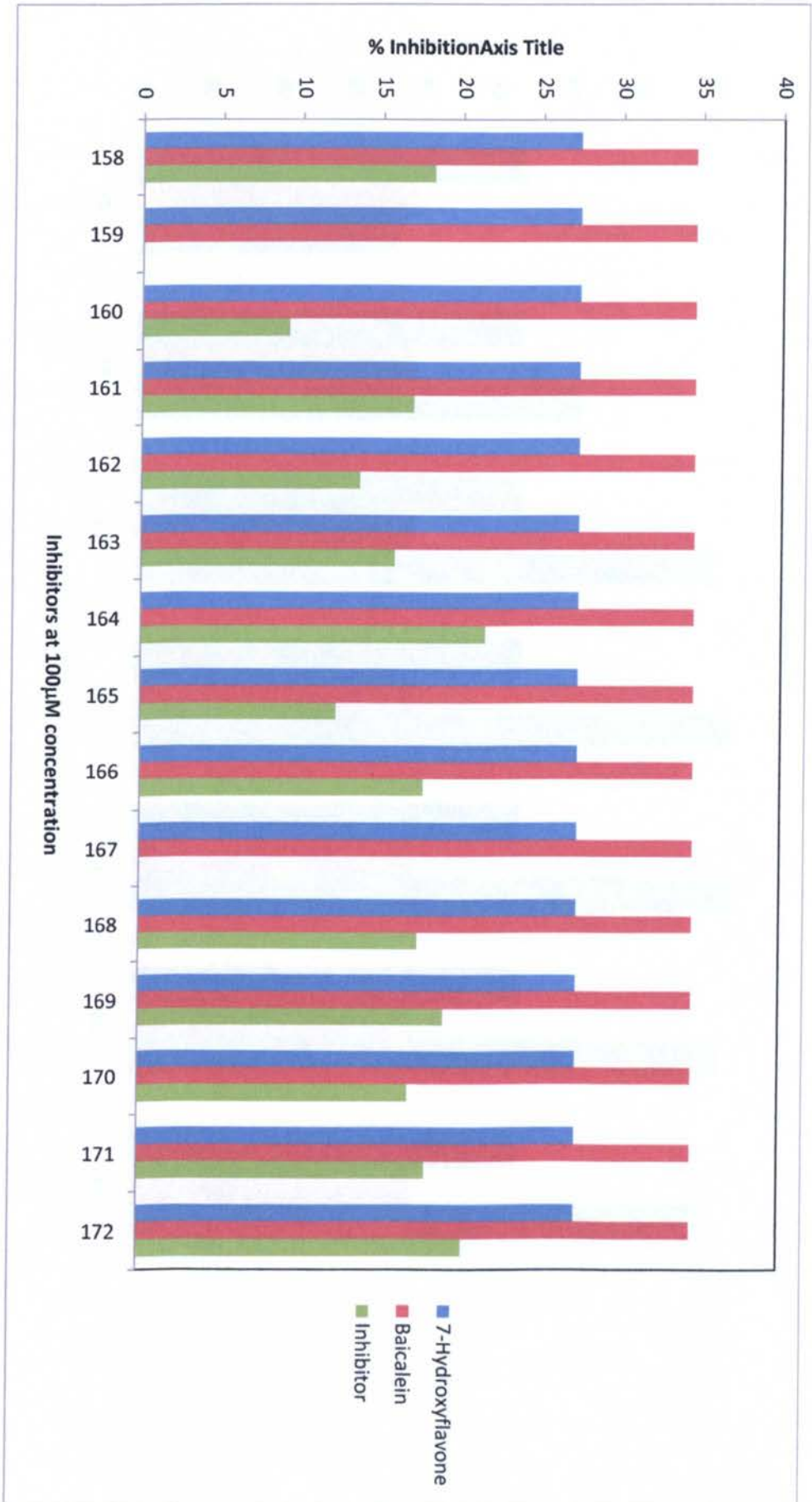



Figure 3.15: Percentage inhibition of compounds (**158-172**) against standards.

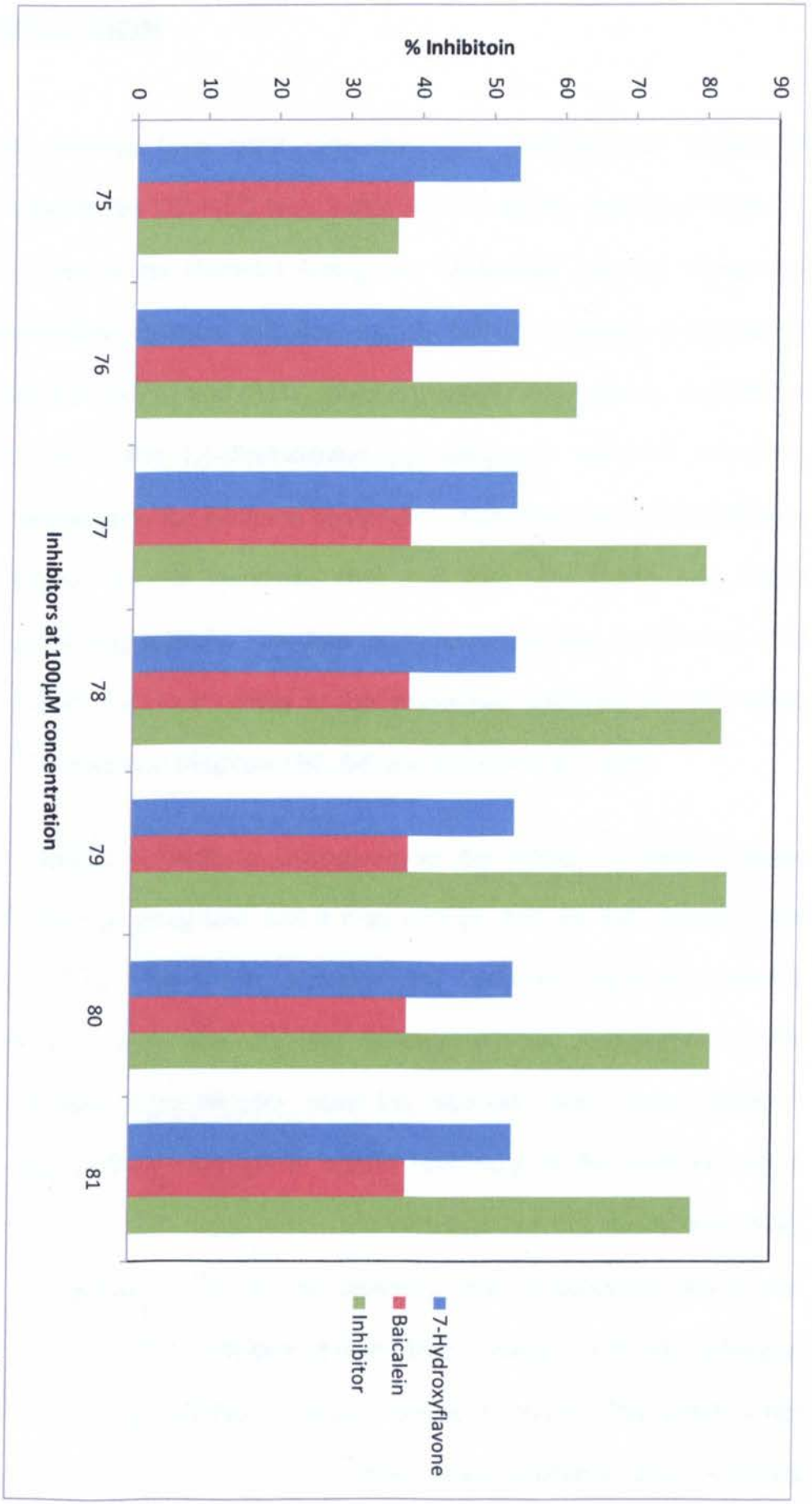


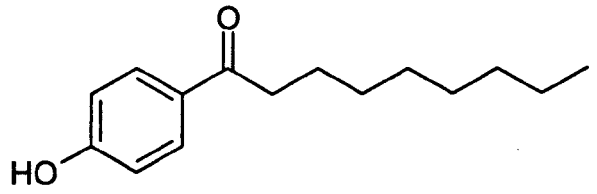

Figure 3.16: Percentage inhibition obtained by Lota *et al* (2006) for compounds (**75-81**) against standards.

## 3.3. CONCLUSION

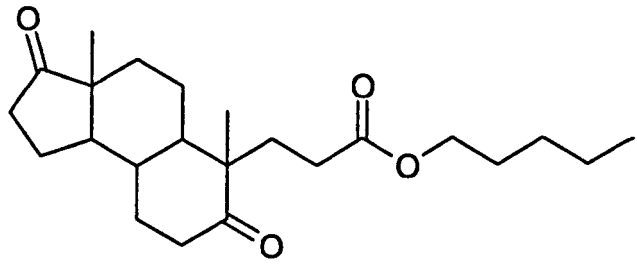
The results obtained from initial screening have identified two classes of potential inhibitors for 17β-HSD type 3 that are structurally unrelated. In set 1, the library in which the steroidal A-ring was oxidatively cleaved, compound (**107**) demonstrated optimum inhibitory activity (43%) compared to standards (**63**) and (**64**) with (25%) and (31%) inhibitory activity respectively. Analysis of the data for set 3, the 1,4-disubstituted aryl analogues exhibited enhanced activity by comparison for instance compound (**150**) showed (41%) inhibitory activity compared to the standards (**63**) and (**64**) with (13%) and (14%) inhibitory activity respectively. The data for compound (**150**), on the face of it, appears to exhibit superior activity to that previously published for (**79**) when the data for the standard inhibitors (**63, 64**) are taken into account.

Overall an attempt to derive a conclusion on the activity of these inhibitor candidates is not an easy task and it may well be that the two different sets work by different mechanisms however the lipophilic character appears important and in both sets provides optimal activity, irrespective of the remaining structure of the inhibitor, when the alkyl side chain approximates to C8-C9 in length. What perhaps is a little less clear is the involvement of hydrogen bonding in the active site. Thus although our two candidates (**107**) and (**150**) can engage in bonding via hydrogen atom acceptance neither can donate in contrast to (**79**). However should (**150**) undergo an *in-situ* hydrolysis the outcome (**150a**) should demonstrate a similar activity to (**79**), which is not observed. Clearly these outcomes provide more questions than answers

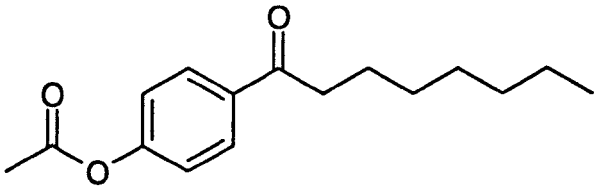
nevertheless novel inhibitors have been identified providing further scope for on-going investigations.



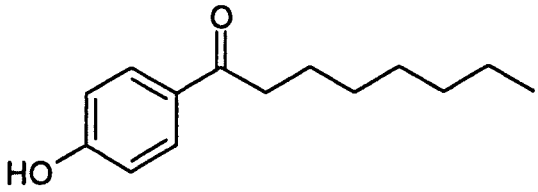

**79**




**107**




**150**




**150a**

Figure 3.17: Inhibitors of 17β-HSD 3

## 3.4. FUTURE WORK

The results have shown several potential and good inhibitors of the target enzyme, 17β-HSD type 3 which could be further investigated.

Therefore the future work should concentrate on:

i. Obtaining the $IC_{50}$ values of the most potent inhibitors. This can be done using the same method as in section 3.1.6 for intial screening. The $IC_{50}$ assay is carried out in 5/7 different concentration e.g; for the $IC_{50}$ of **(150)** with 41% inhibitory activity can be assayed at 30, 60, 90, 120, 150, 180 and 210μM concentrations and from the plot of percentage inhibition against concentrations the $IC_{50}$ value can be obtained.

ii. Biochemically evaluate these compounds against other enzymes such as 17β-HSD type 1, 17β-HSD type 2, 17α-hydroxylase/20-lyaes, 3β-HSD, in order to see the specificity of these inhibitors.

iii. Then carry out tissue culture assay and test the best inhibitors with the lowest $IC_{50}$ in cell culture against testes cell line to see whether the inhibitor can inhibit the enzyme activity in intact cell or not.

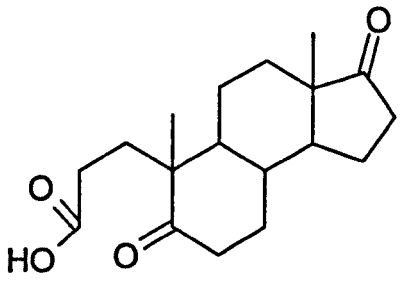
# CHAPTER 4: METHOD AND MATERIALS

# 4. MATERIAL AND METHODS

The reagents used were obtained from either Aldrich Chemical Co. Ltd. (Dorset, UK), Lancaster Synthesis Ltd. (Morecambe, England), Alfa Aesar (Heysham, Lancashire). The NMR spectra were obtained using $^{1}$H-NMR (400MHz) and $^{13}$C-NMR (100MHz) on Jeol Fourier Transform Nuclear Magnetic Resonance spectrometer, using deuterated chloroform ($CDCl_3$) or $d_6$-acetone as solvent unless otherwise stated. Infrared spectrometry was obtained on a Perkins Elmer Fourier transform-paragon 1000 infrared spectrometer using potassium chloride plates (using evaporated drop technique). Melting points are uncorrected and were obtained with a Gallenkamp variable heater instrument. Analytical thin layer chromatography (TLC) was carried out on silica gel on PET polyester and visualised by short – wave ultraviolet radiation and immersion in potassium permanganate ($KMnO_4$). The Gas chromatography-mass spectrometry were obtained with a Hewlett Packard 5890 series II gas chromatograph/Hewlett Packard 5971 series mass selective detector. High-resolution mass spectroscopy of the synthesised compounds was obtained from the mass spectroscopy service, King's College London and Kingston University. Purification via flash column chromatography was performed on silica gel 60, the solvent petroleum spirit (40-60°C) and diethyl ether using the Still *et al* (1978) method.

## 4.1. SYNTHESIS

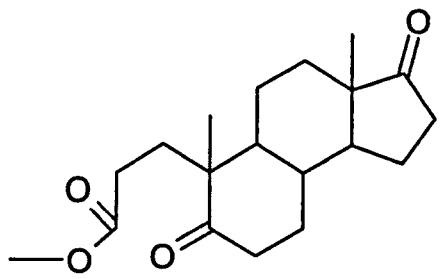
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid (100):**




Androstendione (**98**) (0.5g, 1.7mmol) was dissolved in t-butyl alcohol (120mL) and water (60mL). Sodium carbonate (0.3g, 2.8mmol) was added and the mixture was heated to reflux temperature. Solutions of sodium periodate (2.6g, 12.2mmol) and potassium permanganate (0.03g, 0.2mmol) in water (150mL) was warmed to approximately 70°C and added in a drop-wise manner to the refluxing solution. After the addition, the reaction mixture was heated to reflux for a further 4h and then the solution was cooled and filtered. The t-butyl alcohol was removed in vacuo and the resulting aqueous layer was acidified with concentrated HCl (20.3mL) until a pH of 2 was reached. The acidic layer was extracted with DCM (3x50mL). The organic layers were combined and washed with water (3x50mL) and dried over anhydrous $MgSO_4$ and filtered at the pump. The solvent was removed under vacuum to leave yellow oil. Purification by flash column chromatography gave (**100**) as a pale yellow oil (0.4g, 75% yield); $R_f$ = 0.11, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3114.5 (OH), 1736.1, 1703.1 (C=O); $\delta_H$ ($CDCl_3$): 5.26 (1H, s, **OH**), 2.50 (2H, m, $CO_2HC\mathbf{H}_2$), 2.05 (9H, m, **CH**, $C\mathbf{H}_2$), 1.55 (4H, m, $C\mathbf{H}_2$), 1.24 (4H, m, $C\mathbf{H}_2$), 1.11 (3H, s, $C\mathbf{H}_3$), 0.89 (3H, s, $C\mathbf{H}_3$): $\delta_C$ ($CDCl_3$): 220.45, 214.30, 179.55 (**C**=O), 50.83 (**C**H), 50.59 ($\mathbf{C}H_2$), 48.13 (**C**H), 47.79 ($\mathbf{C}H_2$), 37.85, 35.85 ($\mathbf{C}H_2$), 34.57 (**C**H), 31.09, 30.09, 29.32, 29.22, 21.96, 20.87 ($\mathbf{C}H_2$), 20.54, 13.90 ($\mathbf{C}H_3$); $t_R$=13.3min; LRMS (*M/Z*): 289 ($M^+$-HO, 19%), 288 ($M^+$-$H_2O$, 100%), 260 ($M^+$-$CH_2O_2$, 4%), 233 ($M^+$-$C_3H_5O_2$, 45%); HRMS (ES): Found 306.1819 $C_{18}H_{26}O_4$ required 306.1831.

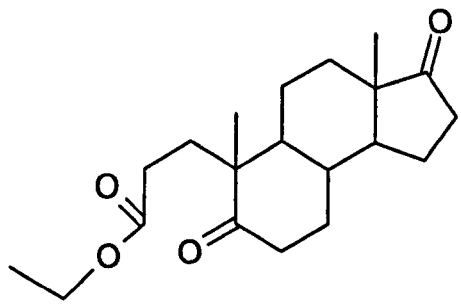
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid methyl ester (103):**



Compound **(100)** (0.5g 16.3mmol) was dissolved in anhydrous toluene (50mL). To this solution methanol (1mL) in excess and concentrated sulphuric acid ($H_2SO_4$) (4 drops) were added and the mixture heated to refluxe for 6h. After cooling, the organic layer was washed with water (3x50mL) and dried over anhydrous $MgSO_4$, filtered at the pump. The solvent was removed under vacuum to produce a brown coloured oil which was purified via flash column chromatography to give **(103)** as a pale yellow oil (0.4g 77% yield); $R_f$ = 0.21, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.9, 1703.0 (C=O), 1170.2 (C-O); $\delta_H$ ($CDCl_3$): 3.59 (3H, s, $CH_3O$), 2.48 (2H, m, $CH_2$), 2.07 (9H, m, $CH_2$, CH), 1.53 (4H, m, $CH_2$), 1.23 (4H, m, $CH_2$), 1.08 (3H, s, $CH_3$), 0.87 (3H, s, $CH_3$); $\delta_C$ ($CDCl_3$): 220.04, 213.88, 174.28 (C=O), 51.71 ($CH_3O$), 50.85 (CH), 50.54 (C), 47.95 (CH), 47.70 (C), 37.82, 35.78 ($CH_2$), 34.52 (CH), 31.11, 30.02, 29.58, 29.20, 21.91, 20.82 ($CH_2$), 20.61, 13.86 ($CH_3$); $t_R$=9.2min; LRMS (*M/Z*): 320 ($M^+$, 3%), 305 ($M^+$-$CH_3$, 13%), 261 ($M^+$-$C_2H_3O_2$, 3%), 234 ($M^+$-$C_4H_6O_2$, 100%), 219 ($M^+$-$C_5H_9O_2$, 52%); HRMS (ES): Found 343.1870 $C_{19}H_{28}O_4Na$ required 343.1885.

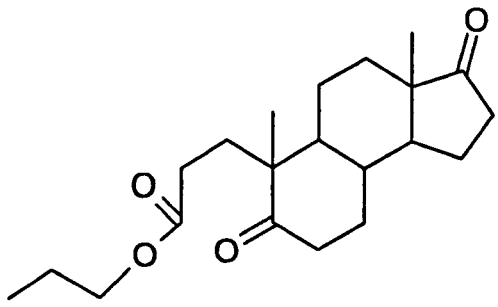
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid ethyl ester (104):**



Compound **(104)** was synthesised in a similar manner to **(103)** except that **(100)** (0.8g, 2.6mmol) was reacted with ethanol (1mL) in excess. The crude product was purified via flash column chromatography to give **(104)** as pale yellow coloured oil (0.7g, 78% yield); $R_f$ = 0.26, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.1, 1703.6 (C=O), 1176.7 (C-O); $\delta_H$ ($CDCl_3$): 4.08 (2H, q, J=7.1Hz, $CH_2O$), 2.50 (2H, m, $CH_2$), 2.10 (9H, m, $CH_2$, CH), 1.56 (4H, m, $CH_2$), 1.25 (7H, m, $CH_2$, $CH_3$), 1.11 (3H, s, $CH_3$), 0.90 (3H, s, $CH_3$); $\delta_C$ ($CDCl_3$): 220.14, 213.96, 173.93 (C=O), 60.54 ($CH_2O$), 50.96 (CH), 50.64 (C), 48.04 (CH), 47.79 (C), 37.90, 35.85 ($CH_2$), 34.61 (CH), 31.20, 30.10, 29.58, 29.50, 22.00, 20.89 ($CH_2$), 20.70, 14.41, 13.94 ($CH_3$); $t_R$=9.4min; LRMS (*M/Z*): 334 ($M^+$, 3%), 319 ($M^+$-$CH_3$, 15%), 289 ($M^+$-$C_2H_5O$, 18%), 234 ($M^+$-$C_5H_8O_2$, 100%), 219 ($M^+$-$C_6H_{11}O_2$, 56%); HRMS (ES): Found 335.2208 $C_{20}H_{31}O_4$ required 335.2222.

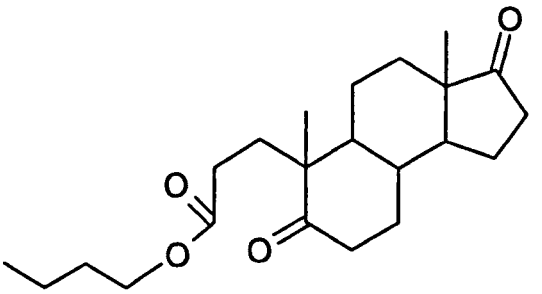
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid propyl ester (105):**



Compound (**105**) was synthesised in a similar manner to (**103**) except that (**100**) (0.6g, 2.0mmol) was reacted with 1-propanol (1mL) in excess. The crude product was purified via flash column chromatography to give (**105**) as pale yellow coloured oil (0.5g, 73% yield); $R_f$ = 0.29, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1736.1, 1702.4 (C=O), 1174.0 (C-O); $\delta_H$ ($CDCl_3$): 3.98 (2H, t, J=6.8$H_Z$, $CH_2O$), 2.50 (2H, m, $CH_2$), 2.10 (9H, m, $CH_2$, CH), 1.55 (6H, m, $CH_2$), 1.25 (4H, m, $CH_2$), 1.10 (3H, s, $CH_3$), 0.90 (3H, s, $CH_3$), 0.89 (3H, t, J=7.3$H_Z$, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 220.12, 213.97, 174.01 (**C**=O), 66.18 (**C**$H_2O$), 50.95 (**C**H), 50.64 (**C**), 48.02 (**C**H), 47.78 (**C**), 37.90, 35.85 (**C**$H_2$), 34.60 (**C**H), 31.18, 30.10, 29.59, 29.46, 22.13, 21.98, 20.88 (**C**$H_2$), 20.70, 13.92, 10.60 (**C**$H_3$); $t_R$=9.8min; LRMS (*M/Z*): 348 ($M^+$, 3%), 333 ($M^+$-$CH_3$, 13%), 289 ($M^+$-$C_3H_7O$, 28%), 234 ($M^+$-$C_6H_{10}O_2$, 100%), 219 ($M^+$-$C_7H_{13}O_2$, 49%); HRMS (ES): found 371.2215 $C_{21}H_{32}O_4Na$ required 371.2198.

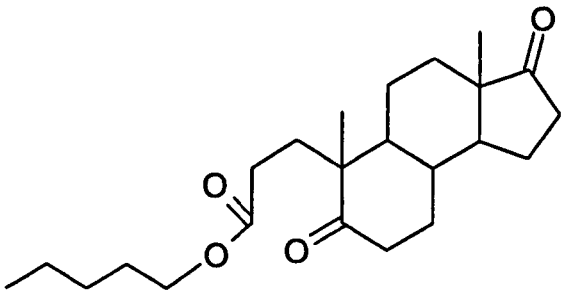
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid butyl ester (106):**



Compound **(106)** was synthesised in a similar manner to **(103)** except that **(100)** (0.6g, 2.0mmol) was reacted with 1-butanol (2mL) in excess. The crude product was purified via flash column chromatography to give **(106)** as pale yellow coloured oil (0.5g, 70% yield); $R_f$ = 0.32, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.2, 1703.9 (C=O), 1173.2 (C-O); $\delta_H$ ($CDCl_3$): 4.01 (2H, t, J=6.8$H_Z$, $CH_2O$), 2.50 (2H, m, $CH_2$), 2.08 (9H, m, $CH_2$, CH), 1.55 (6H, m, $CH_2$), 1.29 (6H, m, $CH_2$), 1.10 (3H, s, $CH_3$), 0.89 (6H, m, $CH_3$); $\delta_C$ ($CDCl_3$): 220.11, 213.95, 174.01 (C=O), 64.46 ($CH_2O$), 50.94 (CH), 50.63 (C), 48.00 (CH), 47.77 (C), 37.89, 35.84 ($CH_2$), 34.59 (CH), 31.17, 30.81, 30.08, 29.58, 29.45, 21.97, 20.87 ($CH_2$), 20.68, 19.31, 13.90, 13.89 ($CH_3$); $t_R$=10.2min; LRMS (*M/Z*): 362 ($M^+$, 3%), 347 ($M^+$-$CH_3$, 11%), 289 ($M^+$-$C_4H_9O$, 21%), 234 ($M^+$-$C_7H_{12}O_2$, 100%), 219 ($M^+$-$C_8H_{15}O_2$, 47%); HRMS (ES): Found 385.2340 $C_{22}H_{34}O_4Na$ required 385.2355.

**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid pentyl ester (107):**

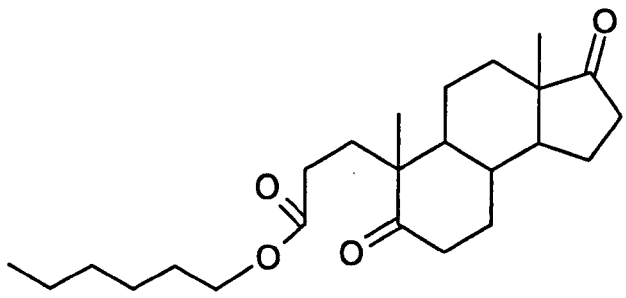


Compound (**100**) (0.5g, 1.6mmol) was dissolved in anhydrous toluene (50mL). Thionyl chloride (0.3mL, 4.1mmol) in excess was added and the solution was heated to reflux for 4h. After this period the solvent was removed in vacuo and anhydrous toluene (50mL) and 1-pentanol (2mL) in excess were added and the mixture was heated to reflux for 5h. After cooling, the organic layer was washed with water (3x50mL), dried over anhydrous $MgSO_4$, filtered at the pump and the solvent was removed under vacuum to produce a brown coloured oil. Purification by flash column chromatography gave (**107**) as a pale yellow oil (0.5g, 71% yield); $R_f$ = 0.34, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.0, 1703.9 (C=O), 1172.2 (C-O); $\delta_H$ ($CDCl_3$): 4.01 (2H, t, J=6.8Hz, $CH_2O$), 2.50 (2H, m, $CH_2$), 2.10 (9H, m, $CH_2$, CH), 1.56 (6H, m, $CH_2$), 1.28 (8H, m, $CH_2$), 1.11 (3H, s, $CH_3$), 0.90 (3H, s, $CH_3$), 0.86 (3H, t, J=6.9Hz, $CH_3CH_2$); $\delta_C$ ($CDCl_3$): 220.13, 213.98, 174.03 (C=O), 64.78 ($CH_2O$), 50.96 (CH), 50.65 (C), 48.04 (CH), 47.79 (C), 37.91, 35.85 ($CH_2$), 34.61 (CH), 31.20, 30.10, 29.59, 29.48, 28.48, 28.26, 22.51, 22.00, 20.89 ($CH_2$), 20.70, 14.15, 13.94 ($CH_3$); $t_R$=10.7min; LRMS (*M/Z*): 376 ($M^+$, 2%), 361 ($M^+$-$CH_3$, 9%), 289

($M^+$-$C_5H_{11}O$, 18%), 234 ($M^+$-$C_8H_{12}O_2$, 100%), 219 ($M^+$-$C_9H_{17}O_2$, 42%); HRMS (ES): Found 399.2512 $C_{23}H_{36}O_4Na$ required 399.2511.

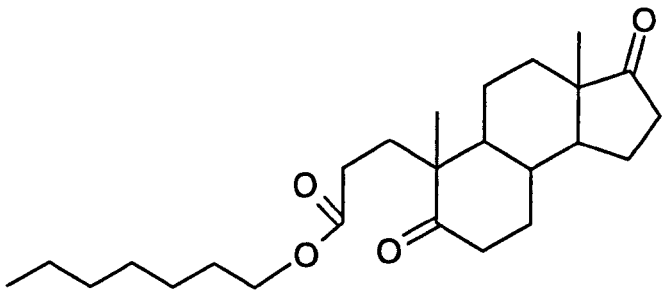
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid hexyl ester (108):**



Compound **(108)** was synthesised in a similar manner to **(107)** except that **(100)** (0.8g, 2.6mmol) was reacted with 1-hexanol (2mL) in excess. The crude product was purified via flash column chromatography to give **(108)** as pale yellow coloured oil (0.6g, 75% yield); $R_f$ = 0.36, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.9, 1704.6 (C=O), 1171.8 (C-O); $\delta_H$ ($CDCl_3$): 3.99 (2H, t, J=6.8Hz, $CH_2O$), 2.49 (2H, m, $CH_2$), 2.08 (9H, m, $CH_2$, CH), 1.54 (6H, m, $CH_2$), 1.25 (10H, m, $CH_2$), 1.09 (3H, s, $CH_3$), 0.89 (3H, s, $CH_3$), 0.83 (3H, t, J=6.9Hz, $CH_3CH_2$); $\delta_C$ ($CDCl_3$): 220.11, 213.96, 174.02 (C=O), 64.78 ($CH_2O$), 50.96 (CH), 50.64 (C), 48.04 (CH), 47.78 (C), 37.90, 35.84 ($CH_2$), 34.61 (CH), 31.60, 31.18, 30.10, 29.59, 29.47, 28.73, 25.77, 22.70, 21.98, 20.88 ($CH_2$), 20.68, 14.17, 13.92 ($CH_3$); $t_R$=11.1min; LRMS (*M/Z*): 390 ($M^+$, 2%), 375 ($M^+$-$CH_3$, 8%), 289 ($M^+$-$C_6H_{13}O$, 14%), 234 ($M^+$-$C_9H_{14}O_2$, 100%), 219 ($M^+$-$C_{10}H_{19}O_2$, 39%); HRMS (ES): Found 413.2655 $C_{24}H_{38}O_4Na$ required 413.2668.

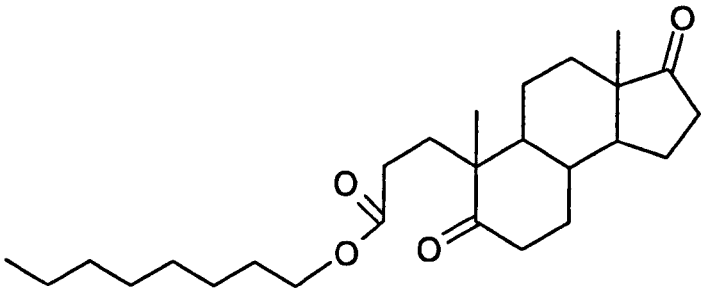
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid heptyl ester (109):**



Compound **(109)** was synthesised in a similar manner to **(107)** except that **(100)** (0.7g, 2.3mmol) was reacted with ethanol (1mL) in excess. The crude product was purified via flash column chromatography to give **(109)** as pale yellow coloured oil (0.7g, 76% yield); $R_f$ = 0.37, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.9, 1704.7 (C=O), 1170.9 (C-O); $\delta_H$ ($CDCl_3$): 4.00 (2H, t, J=6.8Hz, $CH_2O$), 2.51 (2H, m, $CH_2$), 2.09 (9H, $CH_2$, CH), 1.54 6H, m, $CH_2$), 1.25 (12H, m, $CH_2$), 1.10 (3H, s, $CH_3$), 0.90 (3H, s, $CH_3$), 0.84 (3H, t, J=6.8Hz, $CH_3CH_2$); C$\delta_C$ ($CDCl_3$): 220.10, 213.95, 174.01 (**C**=O), 64.78 (**C**$H_2O$), 50.96 (**C**H), 50.64 (**C**), 48.03 (**C**H), 47.78 (**C**), 37.90, 35.84 (**C**$H_2$), 34.61 (**C**H), 31.88, 31.18, 30.10, 29.59, 29.47, 29.09, 28.78, 26.07, 22.74, 21.98, 20.88 (**C**$H_2$), 20.68, 14.24, 13.92 (**C**$H_3$); $t_R$=11.7min; LRMS (*M/Z*): 404 ($M^+$, 2%), 389 ($M^+$-$CH_3$, 8%), 289 ($M^+$-$C_7H_{15}O$, 16%), 234 ($M^+$-$C_{10}H_{16}O_2$, 100%), 219 ($M^+$-$C_{11}H_{21}O_2$, 36%); HRMS (ES): Found 427.2834 $C_{25}H_{40}O_4Na$ required 427.2824.

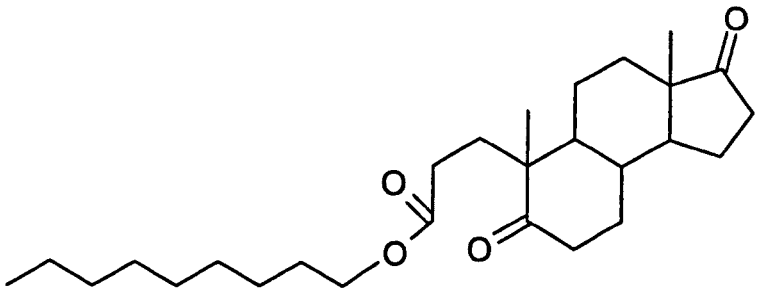
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid octyl ester (110):**



Compound **(110)** was synthesised in a similar manner to **(107)** except that **(100)** (0.6g, 2.0mmol) was reacted with 1-octanol (2mL) in excess. The crude product was purified via flash column chromatography to give **(110)** as pale yellow coloured oil (0.6g, 73% yield); $R_f$ = 0.39, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1738.2, 1704.8 (C=O), 1170.4 (C-O); $\delta_H$ ($CDCl_3$): 4.01 (2H, t, J=6.8$H_Z$, $CH_2O$), 2.21 (2H, m, $CH_2$), 2.08 (8H, m, $CH_2$), 1.58 (6H, m, $CH_2$), 1.26 (15H, m, $CH_2$, CH), 1.11 (3H, s, $CH_3$), 0.90 (3H, s, $CH_3$), 0.84 (3H, t, J=6.9$H_Z$, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 220.13, 213.98, 174.04 (**C**=O), 64.81 (**C**$H_2$O), 50.98 (**C**H), 50.67 (**C**), 48.05 (**C**H), 47.80 (**C**), 37.92, 35.87 (**C**$H_2$), 34.63 (**C**H), 31.97, 31.20, 30.12, 29.61, 29.49, 29.41, 29.37, 28.80, 26.13, 22.83, 22.01, 20.90 (**C**$H_2$), 20.71, 14.30, 13.95 (**C**$H_3$); $t_R$=12.2min; LRMS (*M/Z*): 418 ($M^+$, 3%), 403 ($M^+$-$CH_3$, 11%), 289 ($M^+$-$C_8H_{17}O$, 17%), 234 ($M^+$-$C_{11}H_{18}O_2$, 100%), 219 ($M^+$-$C_{12}H_{23}O_2$, 33%); HRMS (ES): Found 419.3176 $C_{26}H_{43}O_4$ required 419.3161.

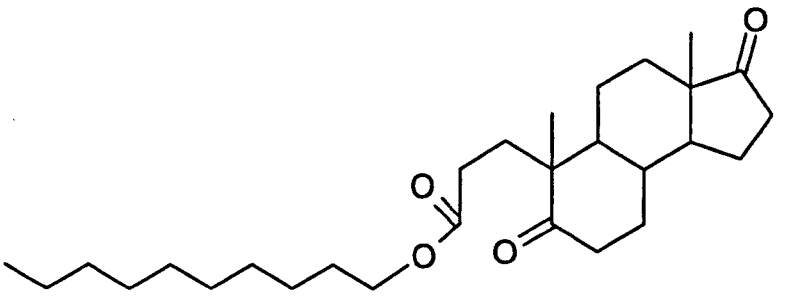
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid nonyl ester (111):**



Compound **(111)** was synthesised in a similar manner to **(107)** except that **(100)** (1.0g, 3.3mmol) was reacted with 1-nonanol (1mL) in excess. The crude product was purified via flash column chromatography to give **(111)** as pale yellow coloured oil (1.0g, 71% yield); $R_f$ = 0.43, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1738.6, 1705.9 (C=O), 1171.0 (C-O); $\delta_H$ ($CDCl_3$): 4.02 (2H, t, J=7.0Hz, $CH_2O$), 2.51 (2H, m, $CH_2$), 2.08 (9H, m, CH, $CH_2$), 1.57 (6H, m, $CH_2$), 1.26 (16H, m, $CH_2$), 1.11 (3H, s, $CH_3$), 0.90 (3H, s, $CH_3$), 0.84 (3H, t, J=7.0Hz, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 220.12, 213.97, 174.03 (C=O), 64.81 ($CH_2O$), 50.98 (CH), 50.66 (C), 48.04 (CH), 47.79 (C), 37.91, 35.86 ($CH_2$), 34.62 (CH), 32.03, 31.20, 30.11, 29.66, 29.60, 29.49, 29.45, 29.42, 28.80, 26.12, 22.5, 22.10, 20.89 ($CH_2$), 20.70, 14.30, 13.94 ($CH_3$); $t_R$=12.5min; LRMS (*M/Z*): 432 ($M^+$, 2%), 417 ($M^+$-$CH_3$, 2%), 289 ($M^+$-$C_9H_{19}O$, 12%), 234 ($M^+$-$C_{11}H_{16}O_2$, 100%), 219 ($M^+$-$C_{12}H_{23}O_2$, 35%); HRMS (ES): Found 455.3145 $C_{27}H_{44}O_4Na$ required 455.3137.

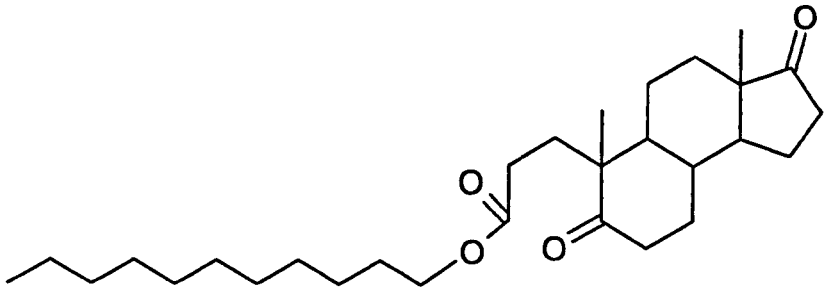
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid decyl ester (112):**



Compound **(112)** was synthesised in a similar manner to **(107)** except that **(100)** (0.7g, 2.3mmol) was reacted with 1-decanol (0.5mL) in excess. The crude product was purified via flash column chromatography to give **(112)** as pale yellow coloured oil (0.7g, 69% yield); $R_f$ = 0.46, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1738.5, 1704.9 (C=O), 1169.8 (C-O); $\delta_H$ ($CDCl_3$): 3.99 (2H, t, J=6.8$H_Z$, $CH_2O$), 2.49 (2H, m, $CH_2$), 2.07 (8H, m, $CH_2$), 1.53 (6H, m, $CH_2$), 1.20 (19H, m, $CH_2$, CH), 1.09 (3H, s, $CH_3$), 0.88 (3H, s, $CH_3$), 0.82 (3h, t, J=6.9$H_Z$, $CH_3$); $\delta_C$ ($CDCl_3$): 220.10, 213.96, 174.02 (**C**=O), 64.80 (**C**$H_2$O), 50.96 (**C**H), 50.65 (**C**), 48.03 (**C**H), 47.78 (**C**), 37.91, 35.85 (**C**$H_2$), 34.61 (**C**H), 32.07, 31.20, 30.11, 29.71, 29.60, 29.48, 29.45, 28.79, 26.12, 22.86, 22.00, 20.89 (**C**$H_2$), 20.70, 14.30, 13.93 (**C**$H_3$); $t_R$=13.2min; LRMS (*M/Z*): 446 ($M^+$, 5%), 431 ($M^+$-$CH_3$, 7%), 289 ($M^+$-$C_{11}H_{21}O$, 15%), 234 ($M^+$-$C_{12}H_{18}O_2$, 100%), 219 ($M^+$-$C_{13}H_{25}O_2$, 42%); HRMS (ES): Found 469.3304 $C_{28}H_{46}O_4Na$ required 469.3294.

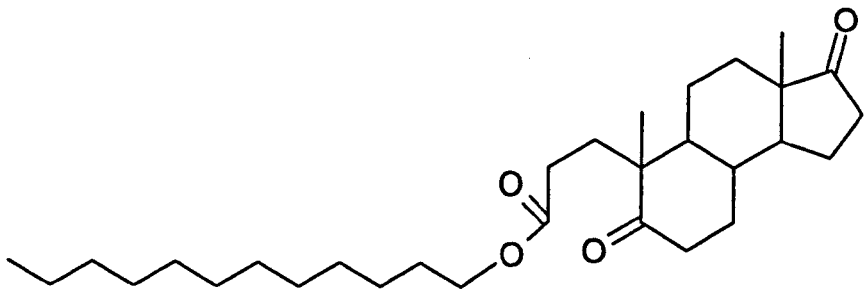
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid undecyl ester (113):**



Compound **(113)** was synthesised in a similar manner to **(107)** except that **(100)** (1.1g, 3.6mmol) was reacted with 1-undecanol (0.8mL) in excess. The crude product was purified via flash column chromatography to give **(113)** as pale yellow coloured oil (1.1g, 67% yield); $R_f$ = 0.47, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1738.5, 1705.0 (C=O), 1169.4 (C-O); $\delta_H$ ($CDCl_3$): 4.01 (2H, t, J=6.7Hz, $CH_2O$), 2.51 (2H, m, $CH_2$), 2.12 (9H, m, $CH_2$, CH), 1.58 (6H, m, $CH_2$), 1.25 (20H, m, $CH_2$), 1.11 (3H, s, $CH_3$), 0.90 (3H, s, $CH_3$), 0.84 (3H, t, J=6.6Hz, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 220.11, 213.96, 174.04 (**C**=O), 64.82 (**C**$H_2$O), 50.98 (**C**H), 50.67 (**C**), 48.06 (**C**H), 47.80 (**C**), 37.92, 35.86 (**C**$H_2$), 34.63 (**C**H), 32.09, 31.21, 30.12, 29.79, 29.77, 29.71, 29.62, 29.52, 29.49, 29.46, 28.80, 26.14, 22.88, 22.01, 20.91 (**C**$H_2$), 20.71, 14.32, 13.95 (**C**$H_3$); $t_R$=14.2min; LRMS (*M/Z*): 460 ($M^+$, 3%), 445 ($M^+$-$CH_3$, 5%), 289 ($M^+$-$C_{12}H_{23}O$, 16%), 234 ($M^+$-$C_{13}H_{20}O_2$, 100%), 219 ($M^+$-$C_{14}H_{27}O_2$, 27%); HRMS (ES): Found 461.3614 $C_{29}H_{49}O_4$ required 461.3631.

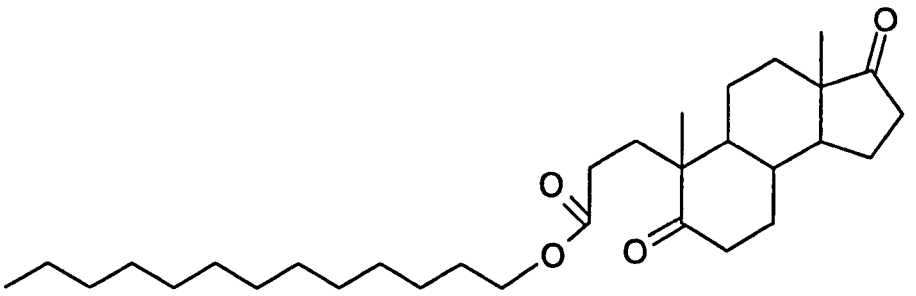
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid dodecyl ester (114):**



Compound **(114)** was synthesised in a similar manner to **(107)** except that **(100)** (1.8g, 5.9mmol) was reacted with 1-dodecanol (1.3mL) in excess. The crude product was purified via flash column chromatography to give **(114)** as pale yellow coloured oil (1.9g, 68% yield); $R_f$ = 0.48, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1738.9, 1705.4 (C=O), 1170.2 (C-O); $\delta_H$ ($CDCl_3$): 4.00 (2H, t, J=6.8$H_Z$, $CH_2O$), 2.51 (2H, m, $CH_2$), 2.08 (9H, m, $CH_2$, CH), 1.56 (6H, m, $CH_2$), 1.23 (22H, $CH_2$, CH), 1.11 (3H, s, $CH_3$), 0.90 (3H, s, $CH_3$), 0.84 (3H, t, J=6.8$H_Z$, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 220.10, 213.95, 174.03 (**C**=O), 64.81 (**C**$H_2$O), 50.97 (**C**H), 50.65 (**C**), 48.04 (**C**H), 47.78 (**C**), 37.91, 35.85 (**C**$H_2$), 34.62 (**C**H), 32.09, 31.20, 30.10, 29.83, 29.81, 29.76, 29.71, 29.60, 29.53, 29.48, 29.45, 28.80, 26.12, 22.87, 22.00, 20.89 (**C**$H_2$), 20.70, 14.31, 13.93 (**C**$H_3$); $t_R$=15.2min; LRMS (*M/Z*): 474 ($M^+$, 3%), 459 ($M^+$-$CH_3$, 5%), 289 ($M^+$-$C_{13}H_{25}O$, 19%), 234 ($M^+$-$C_{14}H_{22}O_2$, 100%), 219 ($M^+$-$C_{15}H_{29}O_2$, 24%); HRMS (ES): Found 475.3780 $C_{30}H_{51}O_4$ required 475.3787.

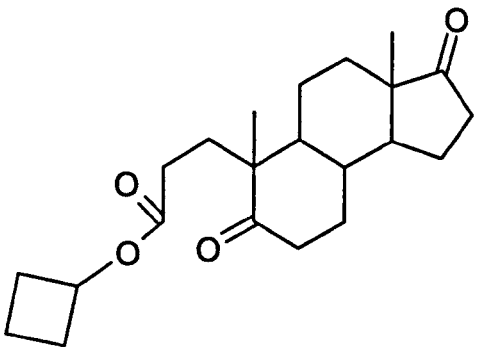
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid tridecyl ester (115):**



Compound **(115)** was synthesised in a similar manner to **(107)** except that **(100)** (0.9g, 2.9mmol) was reacted with 1-tridecanol (0.9g) in excess. The crude product was purified via flash column chromatography to give **(115)** as pale yellow coloured oil (1.0g, 69% yield); $R_f$ = 0.13, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1739.1, 1705.6 (C=O), 1170.6 (C-O); $\delta_H$ ($CDCl_3$): 4.00 (2H, t, J=6.8$H_Z$, $CH_2O$), 2.51 (2H, m, $CH_2$), 2.09 (9H, m, $CH_2$, CH), 1.57 (6H, m, $CH_2$), 1.26 (24H, m, $CH_2$), 1.11 (3H, s, $CH_3$), 0.90 (3H, m, $CH_3$), 0.84 (3H, t, J=6.8$H_Z$, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 220.10, 213.96, 174.02 (C=O), 64.80 ($CH_2O$), 50.96 (CH), 50.65 (C), 48.04 (CH), 47.79 (C) 37.91, 35.85 ($CH_2$), 34.62 (CH), 32.10, 31.20, 30.11, 29.86, 29.83, 29.77, 29.71, 29.60, 29.54, 29.48, 29.46, 28.80, 26.13, 22.88, 22.00, 20.89 ($CH_2$), 20.70, 14.32, 13.94 ($CH_3$); $t_R$=16.3min; LRMS (*M/Z*): 488 ($M^+$, 4%), 473 ($M^+$-$CH_3$, 5%), 289 ($M^+$-$C_{14}H_{27}O$, 21%), 234 ($M^+$-$C_{15}H_{24}O_2$, 100%), 219 ($M^+$-$C_{16}H_{31}O_2$, 23%); HRMS (ES): Found 511.3782 $C_{31}H_{52}O_4Na$ required 511.3763.

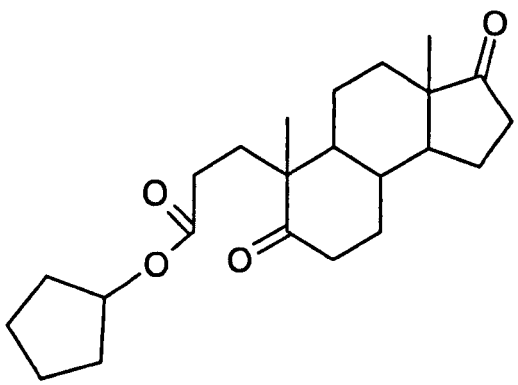
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cyclobutyl ester (116):**



Compound **(116)** was synthesised in a similar manner to **(107)** except that **(100)** (0.8g, 2.6mmol) was reacted with cyclobutanol (0.3mL) in excess. The crude product was purified via flash column chromatography to give **(116)** as pale yellow coloured oil (0.7g, 77% yield); $R_f$=0.37, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1736.4, 1703.0 (C=O), 1170.9 (C-O); $\delta_H$ ($CDCl_3$): 4.92 (1H, quin, J=7.7$H_Z$, 7.5$H_Z$, **CH**O), 2.49 (2H, m, **CH**$_2$), 2.01 (14H, m, **CH**$_2$), 1.54 (5H, m, **CH**$_2$, **CH**), 1.24 (4H, m, **CH**$_2$), 1.09 (3H, s, **CH**$_3$), 0.89 (3H, s, **CH**$_3$); $\delta_C$ ($CDCl_3$): 220.13, 213.96, 173.27 (**C**=O), 68.81 (**C**HO), 50.94 (**C**H), 50.62 (**C**), 48.02 (**C**H), 47.77 (**C**), 37.89, 35.84 (**C**H$_2$), 34.59 (**C**H), 31.17, 30.49, 30.47, 30.08, 29.48, 29.43, 21.97, 20.86 (**C**H$_2$), 20.67, 13.91 (**C**H$_3$), 13.68 (**C**H$_2$); $t_R$=10.6min; LRMS (*M/Z*): 360 ($M^+$, 4%), 289 ($M^+$-$C_4H_7O$, 50%), 260 ($M^+$-$C_5H_8O_2$, 7%), 247 ($M^+$-$C_6H_9O_2$, 5%), 234 ($M^+$-$C_7H_{10}O_2$, 100%); HRMS (ES): Found 361.2391 $C_{22}H_{33}O_4$ required 361.2379.

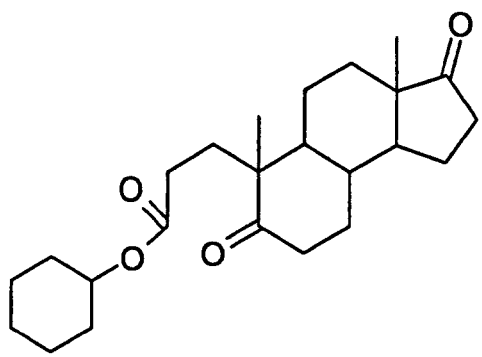
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cyclopentyl ester (117):**



Compound **(117)** was synthesised in a similar manner to **(107)** except that **(100)** (0.9g, 2.9mmol) was reacted with cyclopentanol (0.3mL). The crude product was purified via flash column chromatography to give **(117)** as pale yellow coloured oil (0.8g, 73% yield); $R_f$ = 0.39, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1736.5, 1703.6 (C=O), 1161.1 (C-O); $\delta_H$ ($CDCl_3$): 5.10 (1H, m, **CH**O), 2.50 (2H, m, **CH**$_2$), 2.06 (11H, m, **CH**$_2$, **CH**), 1.58 (10H, m, **CH**$_2$, **CH**), 1.24 (4H, m, **CH**$_2$, **CH**), 1.10 (3H, s, **CH**$_3$), 0.90 (3H, s, **CH**$_3$); $\delta_C$ ($CDCl_3$): 220.02, 214.00, 173.73 (**C**=O), 77.12 (**C**HO), 50.98 (**C**H), 50.66 (**C**), 48.02 (**C**H), 47.79 (**C**), 37.92, 35.86 (**C**H$_2$), 34.61 (**C**H), 32.82, 32.80, 31.20, 30.10, 29.73, 29.55, 23.91, 22.00, 20.87 (**C**H$_2$), 20.71, 13.94 (**C**H$_3$); $t_R$=11.2min; LRMS (*M/Z*): 374 ($M^+$, 4%), 289 ($M^+$-$C_5H_9O$, 54%), 260 ($M^+$-$C_6H_{10}O_2$, 15%), 247 ($M^+$-$C_7H_{11}O_2$, 9%), 234 ($M^+$-$C_8H_{12}O_2$, 100%); HRMS (ES): Found 397.2373 $C_{23}H_{34}O_4Na$ required 397.2355.

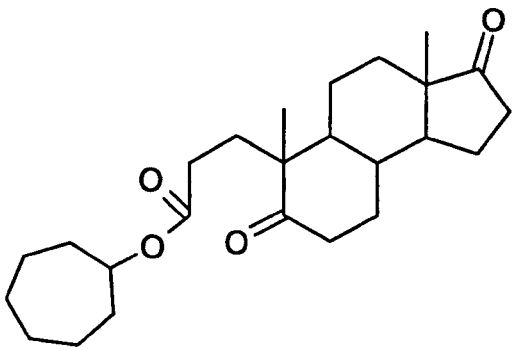
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cyclohexyl ester (118):**



Compound **(118)** was synthesised in a similar manner to **(107)** except that **(100)** (1.1g, 3.6mmol) was reacted with cyclohexanol (0.4mL) in excess. The crude product was purified via flash column chromatography to give **(118)** as pale yellow coloured oil (1.0g, 72% yield); $R_f$ = 0.41, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu$(max) (Film) cm-1: 1736.3, 1703.6 (C=O), 1174.4 (C-O); $\delta_H$ ($CDCl_3$): 4.70 (1H, m, CHO), 2.50 (2H, m, $CH_2$), 2.05 (10H, m, $CH_2$), 1.59 (7H, m, $CH_2$, CH), 1.27 (10H, m, $CH_2$), 1.10 (3H, s, $CH_3$), 0.90 (3H, s, $CH_3$); $\delta_C$ ($CDCl_3$): 220.15, 214.00, 173.35 (C=O), 72.71 (CHO), 50.97 (CH), 50.68 (C), 48.02 (CH), 47.79 (C), 37.91, 35.86 ($CH_2$), 34.61 (CH), 31.82, 31.19, 30.10, 29.81, 29.57, 25.57, 23.94, 22.00, 20.88 ($CH_2$), 20.72, 13.93 ($CH_3$); $t_R$=11.8min; LRMS (*M/Z*): 388 ($M^+$, 2%), 289 ($M^+$-$C_6H_{11}O$, 11%), 260 ($M^+$-$C_7H_{12}O_2$, 15%), 247 ($M^+$-$C_8H_{13}O_2$, 7%), 234 ($M^+$-$C_9H_{14}O_2$, 100%); HRMS (ES): Found 411.2502 $C_{24}H_{36}O_4Na$ required 411.2511.

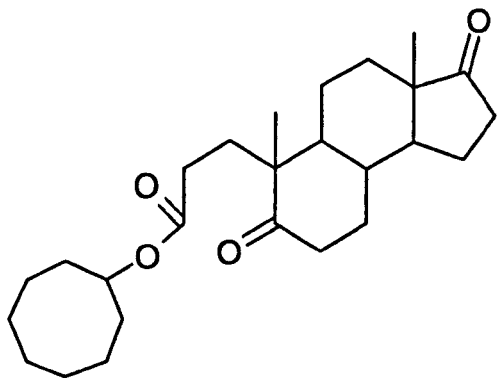
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cycloheptyl ester (119):**



Compound **(119)** was synthesised in a similar manner to **(107)** except that **(100)** (1.4g, 4.6mmol) was reacted with 1-heptanol (0.6mL) in excess. The crude product was purified via flash column chromatography to give **(119)** as pale yellow coloured oil (1.3g, 71% yield); $R_f$ = 0.42, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.1, 1704.2 (C=O), 1165.6 (C-O); 4.87 (1H, m, **CH**O), 2.50 (2H, m, **CH**$_2$), 2.07 (11H, m, **CH**$_2$, **CH**), 1.41 (18H, m, **CH**$_2$), 1.10 (3H, s, **CH**$_3$), 0.90 (3H, s, **CH**$_3$); $\delta_H$ ($CDCl_3$): 220.18, 214.01, 173.28 (**C**=O), 75.21 (**C**HO), 50.96 (**C**H), 50.66 (**C**), 48.00 (**C**H), 47.78 (**C**), 37.90, 35.85 (**C**H$_2$), 34.59 (**C**H), 33.96, 31.17, 30.09, 29.84, 29.57, 28.45, 23.05, 21.98, 20.86 (**C**H$_2$), 20.70, 13.91 (**C**H$_3$); $t_R$=12.7min; LRMS (*M/Z*): 402 ($M^+$, 2%), 289 ($M^+$-$C_7H_{13}O$, 31%), 260 ($M^+$-$C_8H_{14}O_2$, 14%), 247 ($M^+$-$C_9H_{15}O_2$, 7%), 234 ($M^+$-$C_{10}H_{16}O_2$, 100%); HRMS (ES): Found 425.2708 $C_{25}H_{38}O_4Na$ required 425.2668.

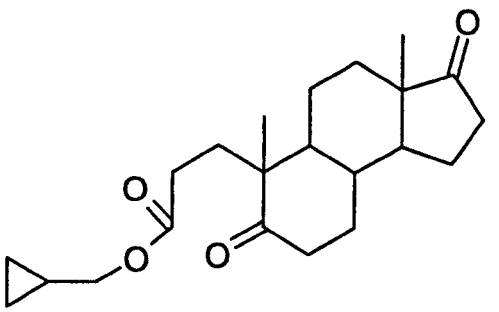
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cyclooctyl ester (120)):**



Compound **(120)** was synthesised in a similar manner to **(107)** except that **(100)** (1.1g, 3.6mmol) was reacted with cyclooctanol (0.5mL) in excess. The crude product was purified via flash column chromatography to give **(120)** as pale yellow coloured oil (1.1g, 73% yield); $R_f$ = 0.43, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1738.0 1704.7 (C=O), 1174.6 (C-O); $\delta_H$ ($CDCl_3$): 4.86 (1H, hept, J=4.0$H_Z$, 4.4$H_Z$, **C**HO), 2.50 (2H, m, **C**$H_2$), 1.79 (27H, m, **C**$H_2$, **C**H), 1.24 (4H, m, **C**$H_2$), 1.09 (3H, s, **C**$H_3$), 0.88 (3H, s, **C**$H_3$); $\delta_C$ ($CDCl_3$): 220.10, 213.94, 173.23 (**C**=O), 75.11 (**C**$H_2$O), 50.92 (**C**H), 50.62 (**C**), 47.96 (**C**H), 47.74 (**C**), 37.86, 35.81 (**C**$H_2$), 34.55 (**C**H), <u>31.64</u>, 31.14, 30.05, 29.81, 29.54, <u>27.20</u>, 25.51, 23.07, 23.06, 21.95, 20.82 (**C**$H_2$), 20.66, 13.88 (**C**$H_3$); $t_R$=23.5min; LRMS (*M/Z*): 416 ($M^+$, 3%), 289 ($M^+$-$C_8H_{15}O$, 38%), 260 ($M^+$-$C_9H_{16}O_2$, 10%), 247 ($M^+$-$C_{10}H_{17}O_2$, 7%), 234 ($M^+$-$C_{11}H_{18}O_2$, 100%); HRMS (ES): Found 439.2836 $C_{26}H_{40}O_4Na$ required 439.2824.

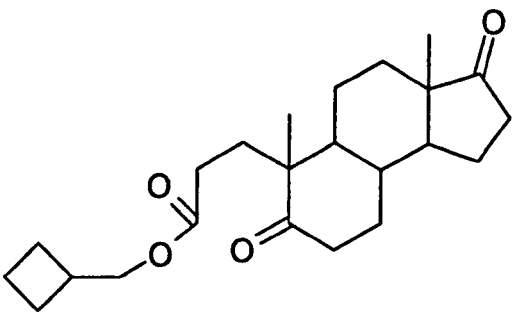
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cycloprpoylmethyl ester (121):**



Compound **(121)** was synthesised in a similar manner to **(107)** except that **(100)** (0.7g, 2.5mmol) was reacted with cyclopropanemethanol (0.4mL) in excess. The crude product was purified via flash column chromatography to give **(121)** as pale yellow coloured oil (0.6g, 73% yield); $R_f$ = 0.38, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1736.4, 1702.7 (C=O), 1164.5 (C-O); $\delta_H$ ($CDCl_3$): 3.85 (2H, d, J=7.3Hz, $CH_2O$), 2.51 (2H, m, $CH_2$), 2.11 (9H, m, $CH_2$, CH), 1.58 (4H, m, $CH_2$), 1.24 (4H, m, $CH_2$), 1.07 (4H, m, $CH_3$, CH), 0.90 (3H, s, $CH_3$), 0.52 (2H, m, $CH_2$), 0.23 (2H, m, $CH_2$); $\delta_C$ ($CDCl_3$): 220.09, 213.93, 173.99 (**C**=O), 69.35 (**C**$H_2$), 50.93 (**C**H), 50.61 (**C**), 47.99 (**C**H), 47.74 (**C**), 37.86, 35.81 (**C**$H_2$), 34.57 (**C**H), 31.16, 30.07, 29.51, 29.43, 21.95, 20.85 (**C**$H_2$), 20.66 (**C**H), 13.90, 9.90 (**C**$H_3$), 3.37 (**C**$H_2$); $t_R$=10.6min; LRMS (*M/Z*): 360 ($M^+$, 10%), 289 ($M^+$-$C_4H_7O$, 14%), 260 ($M^+$-$C_5H_8O_2$,16%), 247 ($M^+$-$C_6H_9O_2$, 6%), 234 ($M^+$-$C_7H_{10}O_2$, 100%); HRMS (ES): Found 383.2202 $C_{22}H_{32}O_4Na$ required 383.2198.

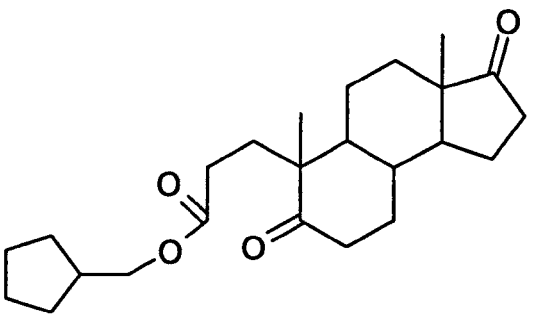
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cyclobutylmethyl ester (122):**



Compound **(122)** was synthesised in a similar manner to **(107)** except that **(100)** (0.9g, 2.9mmol) was reacted with cyclobutanemethanol (0.4mL) in excess. The crude product was purified via flash column chromatography to give **(122)** as pale yellow coloured oil (0.8g, 73% yield); $R_f$ = 0.40, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.2, 1703.8 (C=O), 1170.6 (C-O); $\delta_H$ ($CDCl_3$): 3.98 (2H, d, J=6.8Hz, $CH_2O$), 2.49 (2H, m, $CH_2$), 1.98 (16H, m, $CH_2$, CH), 1.54 (4H, m, $CH_2$), 1.23 (4H, m, $CH_2$), 1.09 (3H, s, $CH_3$), 0.89 (3H, s, $CH_3$); $\delta_C$ ($CDCl_3$): 220.13, 213.97, 174.10 (C=O), 68.42 ($CH_2O$), 50.93 (CH), 50.63 (C), 47.99 (CH), 47.76 (C), 37.88, 35.82 ($CH_2$), 34.58 (CH), 34.18, 31.16, 30.07, 29.58, 29.38, 24.90, 21.97, 20.86 ($CH_2$), 20.67 ($CH_3$), 18.56 ($CH_2$), 13.90 ($CH_3$); $t_R$=12.3min; LRMS (*M/Z*): 374 ($M^+$, 5%), 289 ($M^+$-$C_5H_9O$, 11%), 260 ($M^+$-$C_6H_{10}O_2$, 15%), 247 ($M^+$-$C_7H_{11}O_2$, 5%), 234 ($M^+$-$C_8H_{12}O_2$, 100%); HRMS (ES): Found 397.2354 $C_{23}H_{34}O_4Na$ required 397.2355.

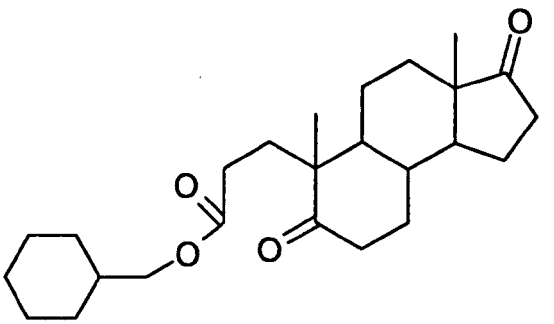
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cyclopentylmethyl ester (123):**



Compound **(123)** was synthesised in a similar manner to **(107)** except that **(100)** (0.9g, 2.9mmol) was reacted with cyclopentanemethanol (0.5mL) in excess. The crude product was purified via flash column chromatography to give **(123)** as pale yellow coloured oil (0.9g, 79% yield); $R_f$ = 0.42, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.5, 1703.9 (C=O), 1168.3 (C-O); $\delta_H$ ($CDCl_3$): 3.89 (2H, d, J=7.1$H_Z$, $CH_2O$), 2.50 (2H, m, $CH_2$), 2.04 (10H, m, $CH_2$, CH),1.54 (10H, $CH_2$, CH), 1.23 (6H, m, $CH_2$), 1.10 (3H, s, $CH_3$), 0.89 (3H, s, $CH_3$); $\delta_C$ ($CDCl_3$): 220.11, 213.95, 174.03 (C=O), 68.53 ($CH_2O$), 50.87 (CH), 50.58 (C), 47.92 (CH), 47.72 (C), 38.60 (CH), 37.83, 35.78 ($CH_2$), 34.52 (CH), 31.11, 30.02, 29.53, 29.46, 29.40, 25.38, 21.92, 20.81 ($CH_2$), 20.64, 13.86 ($CH_3$); $t_R$=11.8min; LRMS (*M/Z*): 388 ($M^+$, 6%), 289 ($M^+$-$C_6H_{11}O$, 18%), 260 ($M^+$-$C_7H_{12}O_2$, 18%), 247 ($M^+$-$C_8H_{13}O_2$, 6%), 234 ($M^+$-$C_9H_{14}O_2$, 100%); HRMS (ES): Found 411.2491 $C_{24}H_{36}O_4Na$ required 411.2511.

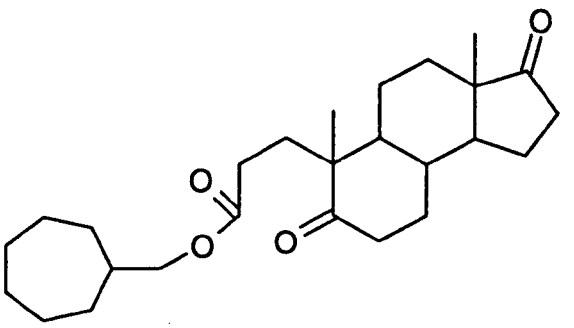
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cyclohexylmethyl ester (124):**



Compound **(124)** was synthesised in a similar manner to **(107)** except that **(100)** (0.8g, 2.6mmol) was reacted with cyclohexanemethanol (0.5mL) in excess. The crude product was purified via flash column chromatography to give **(124)** as pale yellow coloured oil (0.8g, 76% yield); $R_f$ = 0.43, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.8, 1703.9 (C=O), 1171.2 (C-O); $\delta_H$ ($CDCl_3$): 3.82 (2H, d, J=6.8$H_Z$, $CH_2O$), 2.50 (2H, m, $CH_2$), 2.08 (9H, m, $CH_2$, CH), 1.59 (10H, m, $CH_2$), 1.22 (7H, m, $CH_2$, CH), 1.11 (3H, s, $CH_3$), 0.90 (5H, m, $CH_2$, $CH_3$); $\delta_C$ ($CDCl_3$): 220.01, 213.99, 174.06 (C=O), 69.380 ($CH_2O$), 50.96 (CH), 50.66 (C), 48.01 (CH), 47.78 (C), 37.90 ($CH_2$), 37.22 (CH), 35.85 ($CH_2$), 34.61 (CH), 31.18, 30.09, 29.86, 29.58, 29.45, 26.53, 25.84, 21.99, 20.89 ($CH_2$), 20.71, 13.93 ($CH_3$); $t_R$=12.5min; LRMS (*M/Z*): 402 ($M^+$, 2%), 289 ($M^+$-$C_7H_{13}O$, 10%), 260 ($M^+$-$C_8H_{14}O_2$, 13%), 247 ($M^+$-$C_9H_{15}O_2$, 6%), 234 ($M^+$-$C_{10}H_{16}O_2$, 100%); HRMS (ES): Found 425.2655 $C_{25}H_{38}O_4Na$ required 425.2668.

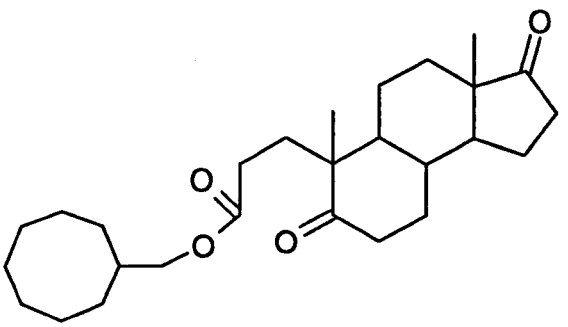
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cycloheptylmethyl ester (125):**



Compound **(125)** was synthesised in a similar manner to **(107)** except that **(100)** (0.9g, 2.9mmol) was reacted with cycloheptanemethanol (0.4mL) in excess. The crude product was purified via flash column chromatography to give **(125)** as pale yellow coloured oil (0.8g, 67% yield); $R_f$ = 0.45, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.7, 1704.2 (C=O), 1169.3 (C-O); $\delta_H$ ($CDCl_3$): 3.81 (2H, d, J=6.8Hz, $CH_2O$), 2.50 (2H, m, $CH_2$), 1.84 (30H, m, $CH_2$, CH), 1.10 (3H, s, $CH_3$), 0.89 (3H, s, $CH_3$); $\delta_C$ ($CDCl_3$): 220.09, 213.97, 174.08 (C=O), 69.75 ($CH_2O$), 50.95 (CH), 50.64 (C), 48.00 (CH), 47.77 (C), 38.65 (CH), 37.90, 35.84 ($CH_2$), 34.59 (CH), 31.17, 31.09, 30.08, 29.57, 29.46, 28.66, 26.41, 21.98, 20.87 ($CH_2$), 20.70, 13.91 ($CH_3$); $t_R$=13.5min; LRMS (*M/Z*): 416 ($M^+$, 3%), 289 ($M^+$-$C_8H_{15}O$, 16%), 260 ($M^+$-$C_9H_{16}O_2$, 15%), 247 ($M^+$-$C_{10}H_{17}O_2$, 7%), 234 ($M^+$-$C_{11}H_{18}O_2$, 100%); HRMS (ES): Found 439.2845 $C_{26}H_{40}O_4Na$ required 439.2824.

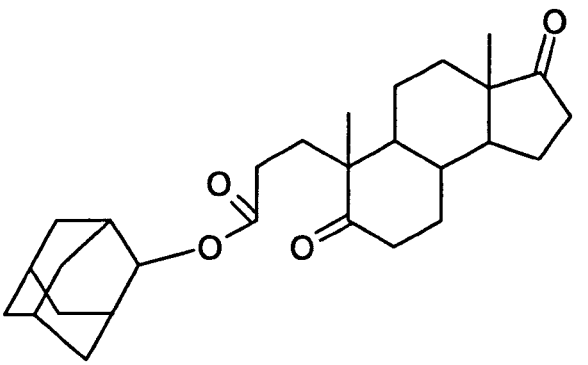
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid cyclooctylmethyl ester (126):**



Compound (**126**) was synthesised in a similar manner to (**107**) except that (**100**) (0.8g, 2.6mmol) was reacted with cyclooctanemethanol (0.5mL) in excess. The crude product was purified via flash column chromatography to give (**126**) as pale yellow coloured oil (0.8g, 71% yield); $R_f$ = 0.47, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.6, 1704.6 (C=O), 1170.0 (C-O); $\delta_H$ ($CDCl_3$): 3.77 (2H, d, J=6.8Hz, $CH_2O$), 2.47 (2H, m, $CH_2$), 2.04 (7H, m, $CH_2$, CH), 1.48 (19H, m, $CH_2$, CH), 1.21 (6H, m, $CH_2$), 1.08 (3H, s, $CH_3$), 0.87 (3H, s, $CH_3$); $\delta_C$ ($CDCl_3$): 220.09, 213.95, 174.02 (C=O), 70.07 ($CH_2O$), 50.86 (CH), 50.57 (C), 47.91 (CH), 47.70 (C), 37.82 ($CH_2$), 36.82 (CH), 35.77 ($CH_2$), 34.51 ($CHCH_2O$), 31.10, 30.00, 29.50, 29.39, 29.28, 26.97, 26.53, 26.65, 25.37, 21.90, 20.80 ($CH_2$), 20.59, 13.84 ($CH_3$); $t_R$=14.7min; LRMS (*M/Z*): 430 ($M^+$, 6%), 289 ($M^+$-$C_9H_{17}O$, 24%), 260 ($M^+$-$C_{10}H_{18}O_2$, 24%), 247 ($M^+$-$C_{11}H_{19}O_2$, 7%), 234 ($M^+$-$C_{12}H_{20}O_2$, 100%); HRMS (ES): Found 453.2982 $C_{27}H_{42}O_4Na$ required 453.2981.

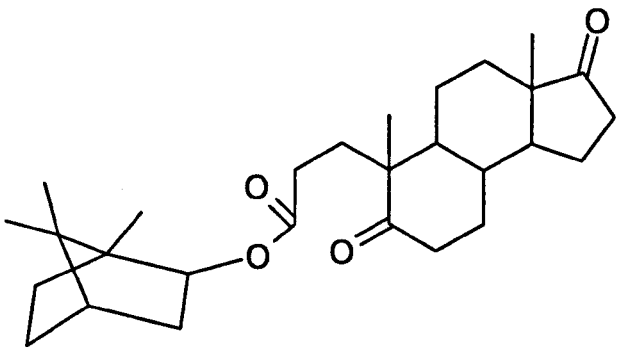
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid adamantan-2-yl ester (127):**



Compound **(127)** was synthesised in a similar manner to **(107)** except that **(100)** (1.1g, 3.6mmol) was reacted with 2-adamantanol (0.6g, 3.9mmol). The crude product was purified via flash column chromatography to give **(127)** as pale yellow coloured oil (1.1g, 70% yield); $R_f$ = 0.39, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1737.0, 1704.3 (C=O), 1172.3 (C-O); $\delta_H$ ($CDCl_3$): 4.87 (1H, s, **CH**CO), 2.51 (2H, m, **CH**$_2$), 2.30 (2H, m, **CH**$_2$), 1.88 (25H, m, **CH**$_2$, **CH**), 1.26 (4H, m, **CH**$_2$), 1.12 (3H, s, **CH**$_3$), 0.90 (3H, s, **CH**$_3$); $\delta_C$ ($CDCl_3$): 220.14, 214.03, 173.31 (**C**=O), 77.05 (**C**HCO), 50.96 (**C**H), 50.71 (**C**), 48.00 (**C**H), 47.79 (**C**), 37.93, 37.57, 36.50, 35.86 (**C**H$_2$), 34.62, 32.01, 32.00 (**C**H), 31.98 (**C**H$_2$), 31.89, 30.10, 29.93, 29.67 (**C**H$_2$), 27.41, 27.17 (**C**H), 22.00, 20.89 (**C**H$_2$), 20.74, 13.93 (**C**H$_3$); $t_R$=12.5min; LRMS (*M/Z*): ($M^+$, 0.4%), 260 ($M^+$-$C_{11}H_{16}O_2$, 2%), 234 ($M^+$-$C_{13}H_{18}O_2$, 24%), 207 ($M^+$-$C_{15}H_{21}O_2$, 10%), 135 ($M^+$-$C_{18}H_{25}O_4$, 100%); HRMS (ES): Found 441.3019 $C_{28}H_{41}O_4$ required 441.3005.

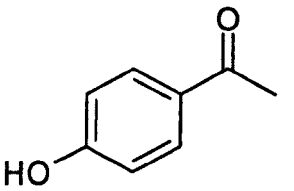
**The synthesis of 3-(3a,6-Dimethyl-3,17-dioxo-dodecahydro-cyclopenta[a]naphthalene-6-yl)-propionic acid 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester (128):**



Compound **(128)** was synthesised in a similar manner to **(107)** except that **(100)** (0.8g, 2.6mmol) was reacted with borneol (0.5g, 3.2mmol). The crude product was purified via flash column chromatography to give **(128)** as pale yellow coloured oil (0.8g, 69% yield); $R_f$ = 0.40, diethyl ether / petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1736.8, 1704.7 (C=O), 177.1 (C-O); $\delta_H$ ($CDCl_3$): 4.80 (1H, d. Q, J=2.0$H_Z$, 1.3$H_Z$, CHO), 2.48 (2H, m, $CH_2$), 2.01 (10H, m, $CH_2$, CH), 1.59 (6H, m, $CH_2$, CH), 1.21 (6H, m $CH_2$, CH), 1.10 (3H, s, $CH_3$), 0.91 (4H, m $CH_3$, CH), 0.85 (3H, s, $CH_3$), 0.82 (3H, s, $CH_3$), 0.77 (3H, s, $CH_3$); $\delta_C$ ($CDCl_3$): 220.09, 214.02, 174.22 (**C**=O), 77.55 (**C**-O), 50.93 (**C**H), 50.67, 48.86 (**C**), 48.00 (**C**H), 47.91, 47.76 (**C**), 45.01 (**C**H), 37.90, 36.93, 35.83 (**C**$H_2$), 34.59 (**C**H), 31.17, 30.09, 29.67, 29.60, 28.17, 27.25, 21.97, 20.86, 20.69 (**C**$H_2$), 19.86, 18.99, 13.90, 13.73 (**C**$H_3$); $t_R$=13.5min; LRMS (*M/Z*): ($M^+$, 4%), 289 ($M^+$-$C_{10}H_{17}O$, 100%), 260 ($M^+$-$C_{11}H_{17}O_2$, 5%), 234 ($M^+$-$C_{13}H_{21}O_2$, 19%), 137 ($M^+$-$C_{18}H_{25}O_4$, 60%); HRMS (ES): Found 465.2976 $C_{28}H_{42}O_4Na$ required 465.2981.Section

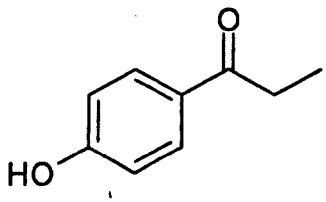
**The synthesis of 1-(4-Hydroxy-phenyl)-ethanone (130):**



Aluminium chloride ($AlCl_3$) (3.1g, 23.4mmol) was added to a solution of phenol (1.0g, 10.6mmol) in anhydrous dichloromethane (DCM) (10mL). The slurry was left to stir for 1h before adding acetyl chloride (0.8mL, 11.3mmol) in a dropwise manner. The solution was then left to stir for 14h. The reaction was quenched using ice-cold aqueous hydrochloric acid (HCl) (30mL, 1M) and extracted into diethyl ether (4x30mL). The combined organic layer was extracted with sodium hydroxide (NaOH) (4x30mL, 2M) and the aqueous layer combined and acidified to pH2 using concentrated HCl (20.6mL). The product was then extracted into diethyl ether (4x30mL) and the combined organic layer washed with water (2x50mL) and dried over anhydrous magnesium sulfate ($MgSO_4$), filtered and the solvent removed under vacuum to give a solid. The crude product was purified by flash chromatography to give (**130**) as a cream solid (1.0g, 69% yield); [m.p.= 108.9-109.3°C, lit. m.p.= 108.8-109.2°C (Ogata *et al*, 1972)]; $R_f$ = 0.30 diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3125.9 (OH), 1643.4 (C=O), 1603.4 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.63 (1H, s, O**H**), 7.9o (2H, d, J=8.8Hz, Ph-**H**), 6.96 (2H, d, J=8.8Hz, Ph-**H**), 2.58 (3H, s, $CH_3$); $\delta_C$ ($CDCl_3$): 199.50 (**C**=O), 162.04 (**C**-O), 131.54, 129.36, 115.83 (Ar, **C**), 26.46 (**C**$H_3$); GC: $t_R$=6.7min; LRMS (*M/Z*): 136 ($M^+$, 34%), 121 ($M^+$-100%), 93 ($M^+$-$C_2H_3O$, 28%); HRMS (ES): Found 136.0527 $C_8H_8O_2$ requires 136.0524.

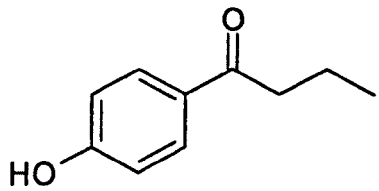
**The synthesis of 1-(4-Hydroxy-phenyl)-propan-1-one (131):**



Compound **(131)** was synthesised in a similar manner to **(130)** except that $AlCl_3$ (2.3g, 17.2mmol), phenol (0.7g, 7.4mmol) and propionyl chloride (0.7mL, 8.1mmol) were used. The crude product was purified via flash column chromatography to give **(131)** as a white solid (0.8g, 71% yield); [m.p.= 158.7-159.1°C, lit. m.p.= 152-153°C (Aulin-Erdtman *et al*, 1968)]. $R_f$ = 0.34, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3171.4 (OH), 1659.1 (C=O), 1603.1 (Ar, C=C); $\delta_H$ ($CDCl_3$): 10.23 (1H, s, O**H**), 7.85 (2H, d, J=8.4Hz, Ph-**H**), 6.84 (2H, d, J=8.4Hz, Ph-**H**), 2.92 (2H, q, J=7.1Hz, 7.3Hz, COC**H**$_2$), 1.06 (3H, t, J=7.1Hz, 7.3Hz, $CH_2$C**H**$_3$); $\delta_C$ ($CDCl_3$): 199.13 (**C**=O), 162.38 (**C**-O), 130.85, 128.86, 115.72 (Ar, **C**), 31.16 (CO**C**$H_2$), 8.97 ($CH_2$**C**$H_3$); GC: $t_R$=7.8min; LRMS (*M/Z*): 150 ($M^+$ ,16%), 121 ($M^+$-$C_2H_5$, 100%), 93 ($M^+$-$C_3H_5O$, 17%); HRMS (ES): Found 150.0684 $C_9H_{10}O_2$ requires 150.0681.

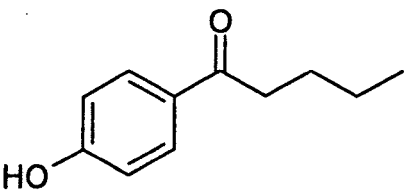
**The synthesis of 1-(4-Hydroxy-phenyl)-butan-1-one (132):**



Compound (**132**) was synthesised in a similar manner to (**130**) except that $AlCl_3$ (2.5g, 18.8mmol), phenol (0.8g, 8.5mmol) and butyryl chloride (0.9mL, 8.6mmol) were used. The crude product was purified via flash column chromatography to give (**132**) as a white solid (0.8g, 57% yield); [m.p.= 100.0-100.4°C, lit. m.p.= 93-94°C (Krausz *et al*, 1965)]; $R_f$ = 0.38, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3557.9 (OH), 1655.3 (C=O), 1600.7 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.64 (1H, s, **OH**), 7.91 (2H, d, J=8.9Hz, Ph-**H**), 6.97 (2H, d, J=8.9Hz, Ph-**H**), 2.92 (2H, t, J=7.3Hz, COC**H**$_2$), 1.75 (2H, sex, J=7.3Hz, 7.5Hz, C**H**$_2$CH$_3$), 0.97 (3H, t, J=7.3Hz, CH$_2$C**H**$_3$); $\delta_C$ ($CDCl_3$): 201.90 (**C**=O), 161.80 (**C**-O), 131.22, 129.24, 115.82 (Ar, **C**), 40.45 (CO**C**H$_2$), 18.55 (**C**H$_2$CH$_3$), 14.06 (CH$_2$**C**H$_3$); GC: $t_R$=8.8min; LRMS (*M/Z*): 164 ($M^+$, 15%), 136 ($M^+$-$C_2H_4$, 10%), 121 ($M^+$-$C_3H_7$, 100%), 93 ($M^+$-$C_3H_5O$, 14%); HRMS (ES): Found 164.0845 $C_{10}H_{12}O_2$ requires 164.0837.

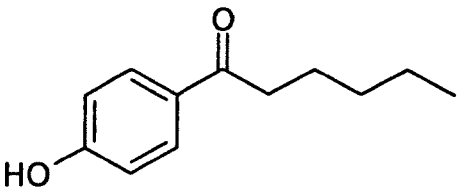
**The synthesis of 1-(4-Hydroxy-phenyl)-pentan-1-one (133):**



Compound **(133)** was synthesised in a similar manner to **(130)** except that $AlCl_3$ (2.7g, 20.2mmol), phenol (0.9g, 9.6mmol) and valeryl chloride (1.1mL, 9.3mmol) were used. The crude product was purified via flash column chromatography to give **(1353**as a white solid (1.0g, 59% yield); [m.p.= 61.8–62.3˚C, lit. m.p.= 62-63˚C (Coulthard *et al*, 1930)]; $R_f$ = 0.4, diethyl ether/petroleum ether 40-60˚C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3370.5 (OH), 1655.3 (C=O), 1598.9 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.52 (1H, s, **OH**), 7.90 (2H, d, J=8.8Hz, Ph-**H**), 6.96 (2H, d, J=8.8Hz, Ph-**H**), 2.93 (2H, t J=7.5Hz, $COCH_2$), 1.69 (2H, quin, J=7.5Hz, 7.7Hz, $COCH_2CH_2$), 1.38 (2H, sex, J=7.3Hz, 7.5Hz, $CH_2CH_3$), 0.91 (3H, t, J=7.3Hz, 7.5Hz, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 201.99 (**C**=O), 161.76 (**C**-O), 131.23, 129.26, 115.83 (Ar, **C**), 38.29 ($COCH_2$), 27.26 ($COCH_2CH_2$), 22.68 ($CH_2CH_3$), 14.05 ($CH_3$); GC: $t_R$=9.9min; LRMS (*M/Z*): 178 ($M^+$, 4%), 149 ($M^+$-$C_2H_5$, 4%), 136 ($M^+$-$C_3H_6$, 49%), 121 ($M^+$-$C_4H_9$, 100%), 93 ($M^+$-$C_5H_9O$, 23%); HRMS (ES): Found 178.0996 $C_{11}H_{14}O_2$ requires 178.0994.

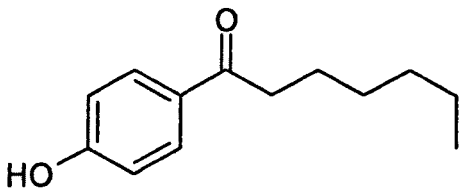
**The synthesis of 1-(4-Hydroxy-phenyl)-hexan-1-one (134):**



Compound (**134**) was synthesised in a similar manner to (**130**) except that $AlCl_3$ (2.7g, 20.2mmol), phenol (0.9g, 9.6mmol) and hexanoyl chloride (1.3mL, 9.3mmol) were used. The crude product was purified via flash column chromatography to give (**134**) as a white solid (1.0g, 54% yield); [m.p.= 63.9–64.3°C, lit. m.p.= 63-64°C (Coulthard *et al*, 1930)]; $R_f$ = 0.42, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3261.9 (OH), 1643.3 (C=O), 1596.5 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.50 (1H, s, **OH**), 7.90 (2H, d, J=8.9Hz, Ph-**H**), 6.96 (2H, d, J=8.9Hz, Ph-**H**), 2.92 (2H, t, J=7.5Hz, $COCH_2$), 1.71 (2H, quin, J=7.3Hz, 7.5Hz, $COCH_2CH_2$), 1.32 (4H, m, $CH_2$), 0.86 (3H, t, J=7.1Hz, $CH_3$); $\delta_C$ ($CDCl_3$): 202.00 (**C**=O), 161.76 (**C**-O), 131.21, 129.26, 115.82 (Ar, **C**), 38.54 ($COCH_2$), 31.72, 24.88, 22.63 ($CH_2$), 14.09 ($CH_3$); GC: $t_R$=11.1min; LRMS (*M/Z*): 192 ($M^+$, 2%), 149 ($M^+$-$C_3H_7$, 6%), 136 ($M^+$-$C_4H_8$, 58%), 121 ($M^+$-$C_5H_{11}$, 100%), 93 ($M^+$-$C_6H_{11}O$, 18%); HRMS (ES): Found 192.1163 $C_{12}H_{16}O_2$ requires 192.1150.

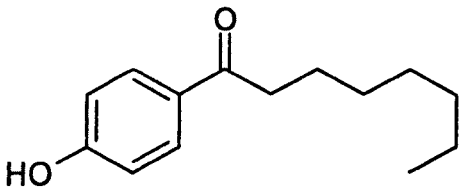
**The synthesis of 1-(4-Hydroxy-phenyl)-heptan-1-one (135):**



Compound **(135)** was synthesised in a similar manner to **(130)** except that $AlCl_3$ (2.5g, 18.7mmol), phenol (0.8g, 8.5mmol) and heptanoyl chloride (1.3mL, 8.4mmol) were used. The crude product was purified via flash column chromatography to give **(135)** as a white solid (0.9g, 51% yield); [m.p.= 94.7–95.2°C, lit. m.p.= 93-94°C (Coulthard *et al*, 1930)], $R_f$ = 0.45, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3308.8 (OH), 1660.2 (C=O), 1594.2 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.57 (1H, s, **OH**), 7.90 (2H, d, J=8.9Hz, Ph-**H**), 6.93 (2H, d, J=8.9Hz, Ph-**H**), 2.92 (2H, t, J=7.5Hz, $COCH_2$), 1.71 (2H, quin, J=7.3Hz, 7.7Hz, $COCH_2CH_2$), 1.31 (6H, m, $CH_2$), 0.84 (3H, t, J=7.1Hz, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 202.03 (**C**=O), 161.78 (**C**-O), 131.22, 129.25, 115.83 (Ar-**C**), 38.58 ($COCH_2$), 31.76, 29.25, 25.17, 22.67 ($CH_2$), 14.20 ($CH_3$); GC: $t_R$=12.2min; LMRS (*M/Z*): 206 ($M^+$, 3%), 149 ($M^+$-$C_4H_9$, 8%), 136 ($M^+$-$C_5H_{10}$, 38%), 121 ($M^+$-$C_6H_{13}$, 100%), 93 ($M^+$-$C_7H_{13}O$, 13%); HRMS (ES): Found 206.1315 $C_{13}H_{18}O_2$ requires 206.1307.

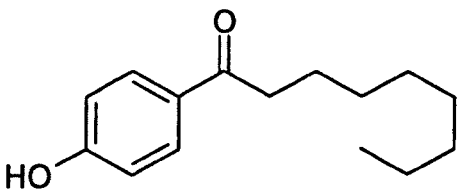
**The synthesis of 1-(4-Hydroxy-phenyl)-octan-1-one (136):**



Compound **(136)** was synthesised in a similar manner to **(130)** except that $AlCl_3$ (2.8g, 21.0mmol), phenol (0.9g, 9.6mmol) and octanoyl chloride (1.6mL, 9.4mmol) were used. The crude product was purified via flash column chromatography to give **(136)** as a white solid (0.9g, 43% yield); [m.p.= 72.3–72.8°C, lit. m.p.= 62.5-63.5°C (Ralston *et al*, 1940)], $R_f$ = 0.47, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3291.7 (OH), 1653.7 (C=O), 1601.1 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.03 (1H, s, **OH**), 7.90 (2H, d, J=8.8Hz, Ph-**H**), 6.94 (2H, d, J=8.8Hz, Ph-**H**), 2.91 (2H, t, J=7.5Hz, $COCH_2$), 1.70 (2H, quin, J=7.7Hz, 7.1Hz, $COCH_2CH_2$), 1.29 (8H, m, $CH_2$), 0.84 (3H, t, J=6.8Hz, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 201.64 (**C**=O), 161.49 (**C**-O), 131.16, 129.50, 115.78 (Ar, **C**), 38.60 ($COCH_2$), 31.88, 29.57, 29.28, 25.16, 22.80 ($CH_2$), 14.26 ($CH_3$). GC: $t_R$=13.1min; LMRS (*M/Z*): 220 ($M^+$, 3%), 149 ($M^+$-$C_5H_{11}$, 11%), 136 ($M^+$-$C_6H_{12}$, 83%), 121 ($M^+$-$C_7H_{15}$, 100%), 93 ($M^+$-$C_8H_{15}O$, 12%); HRMS (ES): Found 220.1477 $C_{14}H_{20}O_2$ requires 220.1463.

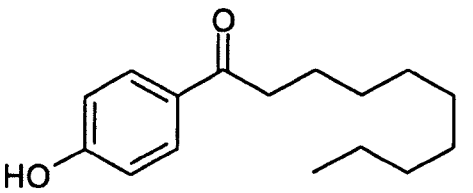
**The synthesis of 1-(4-Hydroxy-phenyl)-nonan-1-one (137)**




Compound (**137**) was synthesised in a similar manner to (**130**) except that $AlCl_3$ (2.8g, 21.0mmol), phenol (0.9g, 9.6mmol) and nonanoyl chloride (1.7mL, 9.4mmol) were used. The crude product was purified via flash column chromatography to give (**137**) as a white solid (0.9g, 40% yield); [m.p.= 57.1–57.6°C, lit. m.p.= 55.5-56.5°C (Kolobielski, 1968)], $R_f$ = 0.49, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3366.0 (OH), 1660.2 (C=O), 1602.3 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.14 (1H, s, **OH**), 7.89 (2H, d, J=8.8Hz, Ph-**H**), 6.94 (2H, d, J=8.8Hz, Ph-**H**), 2.92 (2H, t, J=7.5Hz, $COCH_2$), 1.70 (2H, quin, J=7.7Hz, 7.1Hz, $COCH_2CH_2$), 1.29 (10H, m, $CH_2$), 0.84 (3H, t, J=6.9Hz, $CH_3$); $\delta_C$ ($CDCl_3$): 201.71 (**C**=O), 161.57 (**C**-O), 131.19, 129.44, 115.79 (Ar, **C**), 38.60 ($COCH_2$), 32.01, 29.61, 29.58, 24.85, 29.33, 25.17, 22.83, ($CH_2$), 14.28 ($CH_3$); GC: $t_R$=14.1min; LMRS (*M/Z*): 234 ($M^+$, 3%), 149 ($M^+$-$C_6H_{13}$, 12%), 136 ($M^+$-$C_7H_{14}$, 93%), 121 ($M^+$-$C_8H_{17}$, 100%), 93 ($M^+$-$C_9H_{17}O$, 12%); HRMS (ES): Found 234.1607 $C_{15}H_{22}O_2$ requires 234.1620.

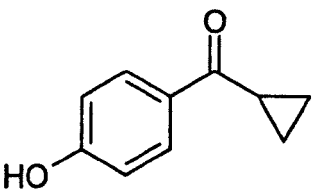
**The synthesis of 1-(4-Hydroxy-phenyl)-decan-1-one (138):**



Compound (**138**) was synthesised in a similar manner to (**130**) except that $AlCl_3$ (2.7g, 20.2mmol), phenol (0.9g, 9.6mmol) and decanoyl chloride (1.9mL, 9.2mmol) were used. The crude product was purified via flash column chromatography to give (**138**) as a white solid (0.8g, 34% yield); [m.p.= 66.7–67.2°C, lit. m.p.= 64°C (Woodcock, 1955)], $R_f$ = 0.51, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3296.1 (OH), 1655.1 (C=O), 1602.2 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.89 (2H, d, J=8.8Hz, Ph-**H**), 7.22 (1H, s, O**H**), 6.91 (2H, d, J=8.8Hz, Ph-**H**), 2.91 (2H, t, J=7.5Hz, $COCH_2$), 1.70 (2H, quin, J=7.3Hz, 7.5Hz, $COCH_2CH_2$), 1.28 (12H, m, $CH_2$), 0.85 (3H, t, J=6.8Hz, $CH_3$); $\delta_C$ ($CDCl_3$): 200.03 (**C**=O), 161.03 (**C**-O), 131.09, 129.85, 115.69 (Ar, **C**), 38.60 ($COCH_2$),, 32.08, 29.67, 29.64, 29.48, 25.07, 22.87 ($CH_2$), 14.32 ($CH_3$); GC: $t_R$=15.2min; LMRS (*M/Z*): 248 ($M^+$, 3%), 149 ($M^+$-$C_7H_{15}$, 13%, 136 ($M^+$-$C_8H_{16}$, 100%), 121 ($M^+$-$C_9H_{19}$, 96%), 93 ($M^+$-$C_{10}H_{19}O$, 11%); HRMS (ES): Found 248.1761 $C_{16}H_{24}O_2$ requires 248.1776.

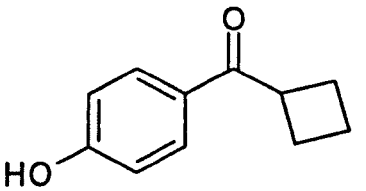
**The synthesis of Cyclopropyl-(4-hydroxy-phenyl)-methanone (139):**



Compound (**139**) was synthesised in a similar manner to (**130**) except that $AlCl_3$ (2.6g, 19.5mmol), phenol (0.8g, 8.5mmol) and cyclopropionyl chloride (0.8mL, 8.8mmol) were used. The crude product was purified via flash column chromatography to give (**139**) as a white solid (0.7g, 51% yield); [m.p.= 110.6-111.1°C, lit. M.p.= 95-99°C (Rastogi *et al*, 1972)]; $R_f$ = 0.35, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3133.7 (OH), 1641.3 (C=O), 1605.3 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.04 (1H, s, **OH**), 7.94 (2H, d, J=8.6Hz, Ph-**H**), 6.92 (2H, d, J= 8.6Hz, Ph-**H**), 2.66 (1H, m, COC**H**), 1.23 (2H, m, $CH_2$), 1.03 (2H, m, $CH_2$); $\delta_C$ ($CDCl_3$): 201.42 (**C**=O), 161.44 (**C**-O), 130.00, 130.38, 115.72 (Ar, **C**), 17.17 (CO**C**H), 12.13 ($CH_2$); GC: $t_R$=9.5min, LMRS (*M/Z*): 162 ($M^+$, 35%), 121 ($M^+$-$C_3H_5$, 100%), 93 ($M^+$-$C_4H_5O$, 15%); HRMS (ES): Found 162.0669 $C_{10}H_{10}O_2$ required 162.0681.

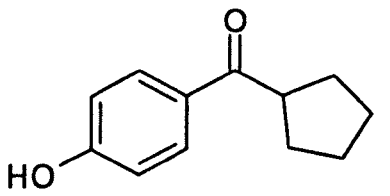
**The synthesis of Cyclobutyl-(4-hydroxy-phenyl)-methanone (140):**



Compound **(140)** was synthesised in a similar manner to **(130)** except that $AlCl_3$ (2.5g, 18.7mmol), phenol (0.8g, 8.5mmol) and cyclobutyl chloride (1.0mL, 10.9mmol) were used. The crude product was purified via flash column chromatography to give **(140)** as a white solid (0.6g, 38% yield); [m.p.= 113.0–113.5°C, lit. m.p.= 102.4-105.4°C (Patel, 2003); $R_f$ = 0.39, diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3167.9 (OH), 1648.0 (C=O), (1603.9 Ar, C=C); $\delta_H$ ($CDCl_3$): 10.30 (1H, s, **OH**), 7.56 (2H, d, J=8.6Hz, Ph-**H**), 6.83 (2H, d, J=8.6Hz, Ph-**H**), 4.01 (1H, quin, J=8.6Hz, 8.4Hz, COC**H**), 2.18 (4H, m, **$CH_2$**), 2.20 (1H, m, COCH**$CH_2$**), 1.76 (1H, m, COCH**$CH_2$**); $\delta_C$ ($CDCl_3$): 198.29 (**C**=O), 161.66(**C**-O), 130.69, 127.90, 115.24 (Ar, **C**), 41.60 (CO**C**H), 24.83 (CH**C**$H_2$), 17.82(CH$CH_2$**C**$H_2$); GC: $t_R$=10.6min, LMRS (*M/Z*): 176 ($M^+$, 8%), 121 ($M^+$-$C_4H_7$, 100%), 93 ($M^+$-$C_5H_7O$, 11%); HRMS (ES): Found 176.0834 $C_{11}H_{12}O_2$ requires 176.0837.

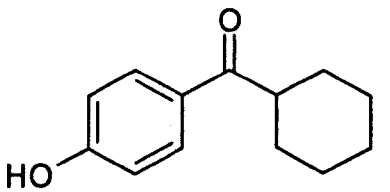
**The synthesis of Cyclopentyl-(4-hydroxy-phenyl)-methanone (141):**



Compound (**141**) was synthesised in a similar manner to (**130**) except that $AlCl_3$ (3.2g, 24.0mmol), phenol (1.0g, 10.6mmol) and cyclopentanoyl chloride (1.3mL, 10.7mmol) were used. The crude product was purified via flash column chromatography to give (**141**) as a white solid (0.8g, 39% yield); [m.p.= 123.1–123.6°C, lit. m.p.= 111.0-113.2°C (Patel, 2003); $R_f$ = 0.41, diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3158.6 (OH), 1637.9 (C=O), 1601.9 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.28 (1H, s, **OH**), 7.91 (2H, d, J=8.8Hz, Ph-**H**), 6.95 (2H, d, J=8.8Hz, Ph-**H**), 3.69 (1H, quin, J=8.1Hz, 7.9Hz, COC**H**), 1.89 (4H, m, **$CH_2$**), 1.65 (4H, m, **$CH_2$**); $\delta_C$ ($CDCl_3$): 204.05 (**C**=O), 161.46 (**C**-O), 131.46, 127.25, 115.76 (Ar, **C**), 46.34 (CO**C**H), 30.59 (CH**C**$H_2$), 26.50 ($CHCH_2$**C**$H_2$); GC: $t_R$=11.7min, LMRS (*M/Z*): 190 ($M^+$, 14%), 121 ($M^+$-$C_5H_9$, 100%), 93 ($M^+$-$C_6H_9O$, 10%); HRMS (ES): Found 190.0990 $C_{12}H_{14}O_2$ requires 190.0994.

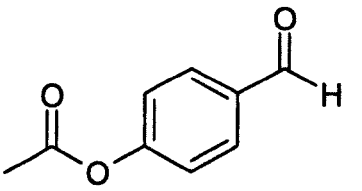
**The synthesis of Cyclohexyl-(4-hydroxy-phenyl)-methanone (142)):**



Compound (**142**) was synthesised in a similar manner to (**130**) except that $AlCl_3$ (2.4g, 18.0mmol), phenol (0.8g, 8.5mmol) and cyclohexanoyl chloride (1.2mL, 9.0mmol) were used. The crude product was purified via flash column chromatography to give (**142**) as a white solid (0.6g, 34% yield); [m.p.=103.8–104.3°C, lit. m.p.= 98°C (Sasse *et al*, 2001)]; $R_f$ = 0.46, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3370.1 (OH), 1656.2 (C=O), 1601.9 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.45 (1H, s, **OH**), 7.90 (2H, d, J=8.8Hz, Ph-**H**), 7.00 (2H, d, J=8.8Hz, Ph-**H**), 3.26 (1H, m , COC**H**), 1.84 (4H, m, $CH_2$), 1.41 (6H, m, $CH_2$); $\delta_C$ ($CDCl_3$): 205.05 (**C**=O), 161.64 (**C**-O), 131.34, 127.43, 115.88 (Ar, **C**), 45.63 (COCH), 29.87, 26.04 ($CH_2$); GC: $t_R$=10.7min, LMRS (*M/Z*): 204 ($M^+$, 16%), 121 ($M^+$-$C_6H_{11}$, 100%), 93 ($M^+$-$C_7H_{11}O$, 9%); HRMS (ES): Found 204.1137 $C_{13}H_{16}O_2$ required 204.1150.

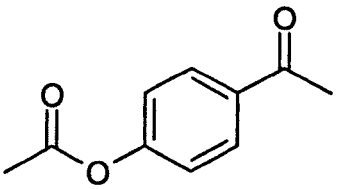
**The synthesis of acetic acid 4-formyl-phenyl ester (143):**



4-Hydroxybenzaldehyde (2.4g, 19.7mmol) was dissolved in anhydrous toluene (100mL) and acetyl chloride (2.8mL, 39.4mmol) added continually. The resulting solution was refluxed for 5h, cooled and washed with water (3x100mL). The organic phase was dried over $MgSO_4$, filtered and solvent was removed under vacuum. The crude product was purified via flash column chromatography to give **(143)** as a pale yellow oil (3.0g, 94% yield); $R_f$ = 0.24, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1763.2, 1702.2 (C=O), 1597.9 (Ar, C=C); $\delta_H$ ($CDCl_3$): 9.97 (1H, s, COH), 7.90 (2H, d, J=8.6$H_Z$, ph-**H**), 7.26 (2H, d, J=8.6$H_Z$, ph-**H**), 2.32 (3H, s, $CH_3$); $\delta_C$ ($CDCl_3$): 191.15 ($CH_3$**C**O), 168.91 (**C**OH), 155.50 (Ar-**C**O), 134.13 (Ar-**C**OH), 131.39, 122.55 (Ar-**C**), 21.32 (**C**$H_3$); GC: $t_R$=5.5min, LMRS (*M/Z*): 164 ($M^+$, 23%), 121 ($M^+$-$C_2H_3O$, 100%); 93 ($M^+$-$C_3H_3O_2$, 11%); HRMS (ES): Found 165.0559 $C_9H_9O_3$ required 165.0552.

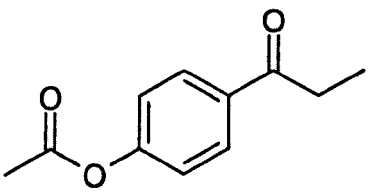
**The synthesis of acetic acid 4-acetyl-phenyl ester (144):**



Compound (**144**) was synthesised in similar manner to (**143**) except (**132**) (1.4g, 10.3mmol), acetyl chloride (1.5mL, 21.1mmol) were used. The crude product was purified via flash column chromatography to give (**144**) as an off-white solid (1.7g, 94% yield); m.p.= 58.7-59.4°C; $R_f$ = 0.20, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1760.5, 1683.7 (C=O), 1599.2 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.97 (2H, d, J=8.9$H_Z$, ph-**H**), 7.17 (2H, d, J=8.9$H_Z$, ph-**H**), 2.57 (3H, s, $CH_3CO_2$), 2.30 (3H, s, $CH_3CO$); $\delta_C$ ($CDCl_3$): 197.06 (**C**$OCH_3$), 169.08 ($CH_3$**C**OO), 154.53 (Ar-**C**O), 134.91 (Ar-**C**CO), 130.15, 121.97 (Ar-**C**), 26.81 (**C**$H_3$COO), 21.36 (**C**$H_3$CO); GC: $t_R$=6.4min, LMRS (*M/Z*): 178 ($M^+$, 16%), 136 ($M^+$-$C_2H_2O$, 28%), 121 ($M^+$-$C_3H_5O$, 100%), 93 ($M^+$-$C_4H_5O_2$, 7%); HRMS (ES): Found 179.0704 $C_{10}H_{11}O_3$ required 179.0708.

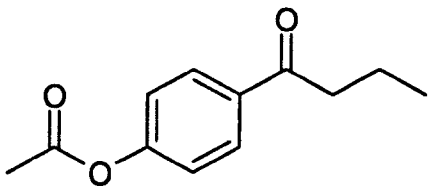
**The synthesis of acetic acid 4-propionyl-phenyl ester (145):**



Compound (**145**) was synthesised in similar manner to (**143**) except (**131**) (1.3g, 8.7mmol), acetyl chloride (1.2mL, 16.9mmol) were used. The crude product was purified via flash column chromatography to give (**145**) as an off-white solid (1.6g, 96% yield); m.p.= 57.6-58.4°C; $R_f$ = 0.29, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1757.0, 1684.2 (C=O), 1600.7 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.98 (2H, d, J=8.6$H_Z$, ph-**H**), 7.16 (2H, d, J=8.6$H_Z$, ph-**H**), 2.96 (2H, q, J=7.1$H_Z$, 7.3$H_Z$, $CH_2CH_3$), 2.30 (3H, s, $CH_3CO$), 1.20 (3H, t, J=7.3$H_Z$, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 199.73 (**C**$OCH_2$), 169.11 ($CH_3$**C**OO), 154.34 (Ar-**C**O), 134.70 (Ar-**C**CO), 129.79, 121.93 (Ar-**C**), 31.96 (CO**C**$H_2$), 21.35 (**C**$H_3$COO), 8.40 ($CH_2$**C**$H_3$); GC: $t_R$=7.0min, LMRS (*M/Z*): 192 ($M^+$, 8%), 163 ($M^+$-$C_2H_5$, 6%), 150 ($M^+$-$C_2H_3O$, 10%), 121 ($M^+$-$C_4H_7O$, 100%), 93 ($M^+$-$C_5H_7O_2$, 7%); HRMS (ES): Found 193.0861 $C_{11}H_{13}O_3$ required 193.0865.

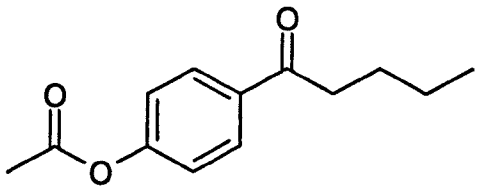
**The synthesis of acetic acid 4-butyl-phenyl ester (146):**



Compound (**146**) was synthesised in similar manner to (**143**) except (**132**) (1.2g, 7.3mmol), acetyl chloride (1.0mL, 14.1mmol) were used. The crude product was purified via flash column chromatography to give (**146**) as an off-white solid (1.4g, 93% yield); m.p.= 33.1-33.8°C; $R_f$ = 0.36, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1762.1, 1685.7 (C=O), 1599.8 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.97 (2H, d, J=8.6$H_Z$, ph-**H**), 7.16 (2H, d, J=8.6$H_Z$, ph-**H**), 2.90 (2H, t, J=7.3$H_Z$, $COCH_2$), 2.30 (3H, s, $CH_3CO$), 1.74 (2H, six, J=7.3$H_Z$, 7.5$H_Z$, $CH_2CH_3$), 0.97 (3H, t, J=7.3$H_Z$, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 199.30 (**C**$OCH_2$), 169.09 ($CH_3$**C**OO), 154.33 (Ar-**C**O), 134.85 (Ar-**C**CO), 40.65 (CO**C**$H_2$), 21.33 (**C**$H_3$COO), 17.90 ($CH_3$**C**$H_2$), 14.04 (**C**$H_3CH_2$); GC: $t_R$=7.6min, LMRS (*M/Z*): 206 ($M^+$, 0.4%), 163 ($M^+$-$C_3H_7$, 7%), 121 ($M^+$-$C_5H_9O$, 100%), 93 ($M^+$-$C_6H_9O_2$, 9%); HRMS (ES): Found 207.1011 $C_{12}H_{15}O_3$ required 207.1021.

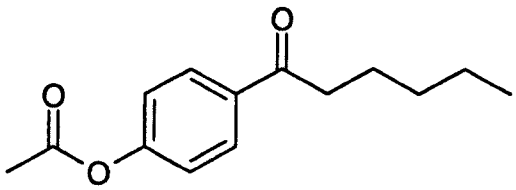
**The synthesis of acetic acid 4-pentanol-phenyl ester (147):**



Compound (**147**) was synthesised in similar manner to (**143**) except (**133**) (1.0g, 5.6mmol), acetyl chloride (0.8mL, 11.3mmol) were used. The crude product was purified via flash column chromatography to give (**147**) as a pale yellow oil (1.1g, 92% yield); $R_f$ = 0.40, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1760.1, 1684.0 (C=O), 1599.9 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.97 (2H, d, J=8.8$H_Z$, ph-**H**), 7.15 (2H, d, J=8.8$H_Z$, ph-**H**), 2.92 (2H, t, J=7.3$H_Z$, $COCH_2$), 2.29 (3H, s, $CH_3CO$), 1.69 (2H, m, $COCH_2CH_2$), 1.37 (2H, six, J=7.3$H_Z$, 7.5$H_Z$, $CH_2CH_3$), 0.92 (3H, t, J=7.3$H_Z$, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 199.45 (**C**$OCH_2$), 169.09 ($CH_3$**C**OO), 154.32 (Ar-**C**O), 134.84 9Ar-**C**CO), 129.85, 121.91 (Ar-**C**), 38.47 (CO**C**$H_2$), 26.60 ($COCH_2$**C**$H_2$), 22.63 (**C**$H_2CH_3$), 21.33 (**C**$H_3COO$), 14.12 ($CH_2$**C**$H_3$); GC: $t_R$=8.3min, LMRS (*M/Z*): 220 ($M^+$, 0.4%), 178 ($M^+$-$C_3H_6$, 11%), 163 ($M^+$-$C_4H_9$, 5%), 136 ($M^+$-$C_5H_8O$, 67%), 121 ($M^+$-$C_6H_{11}O$, 100%), 93 ($M^+$-$C_7H_{11}O_2$, 10%); HRMS (ES): Found 221.1176 $C_{13}H_{17}O_3$ required 221.1178.

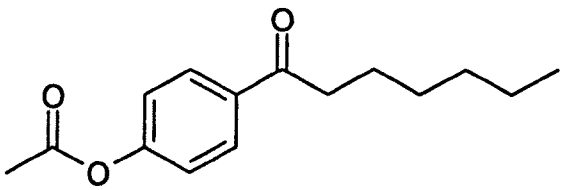
**The synthesis of acetic acid 4-hexanoyl-phenyl ester (148):**



Compound **(148)** was synthesised in similar manner to **(143)** except **(134)** (0.94g, 4.4mmol), acetyl chloride (0.6mL, 8.4mmol) were used. The crude product was purified via flash column chromatography to give **(148)** as an off-white solid (1.0g, 91% yield); m.p.= 44.1-44.8°C; $R_f$ = 0.42, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1761.5, 1684.6 (C=O), 1600.1 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.97 (2H, d, J=8.6Hz, ph-**H**), 7.16 (2H, d, J=8.6Hz, ph-**H**), 2.92 (2H, t, J=7.3Hz, $COCH_2$), 2.30 (3H, s, $CH_3CO$), 1.71 (2H, m, $CH_2$), 1.33 (4H, m, $CH_2$), 0.88 (3H, m, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 199.60 ($COCH_2$), 169.14 ($CH_3COO$), 154.35 (Ar-**C**O), 134.85 (Ar-**C**CO), 129.89, 121.94 (Ar-**C**), 38.76 ($COCH_2$), 31.71, 24.23, 22.72 ($CH_2$), 21.36 ($CH_3COO$), 14.15 ($CH_2CH_3$); GC: $t_R$=8.9min, LMRS (*M/Z*): 234 ($M^+$, 0.9%), 178 ($M^+$-$C_4H_8$, 16%), 163 ($M^+$-$C_5H_{11}$, 6%), 136 ($M^+$-$C_6H_{10}O$, 84%), 121 ($M^+$-$C_7H_{13}O$, 100%), 93 ($M^+$-$C_8H_{13}O_2$, 9%); HRMS (ES): Found 235.1325 $C_{14}H_{19}O_3$ required 235.1334.

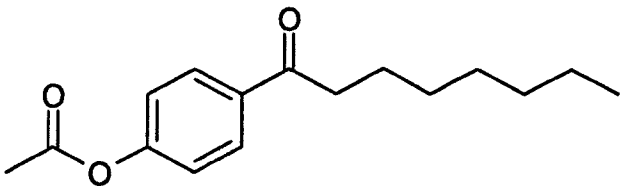
**The synthesis of acetic acid 4-heptanoyl-phenyl ester (149):**



Compound (**149**) was synthesised in similar manner to (**143**) except (**135**) (0.9g, 4.4mmol), acetyl chloride (0.6mL, 8.4mmol) were used. The crude product was purified via flash column chromatography to give (**149**) as an off-white solid (1.0g, 92% yield); m.p.= 48.9-49.7°C; $R_f$ = 0.44, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1759.3, 1681.2 (C=O), 1599.2 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.97 (2H, d, J=8.8$H_Z$, ph-**H**), 7.16 (2H, d, J=8.8$H_Z$, ph-**H**), 2.92 (2H, t, J=7.3$H_Z$, $COCH_2$), 2.30 (3H, s, $CH_3CO$), 1.70 (2H, quin, J=7.3$H_Z$, 7.5$H_Z$, $COCH_2CH_2$), 1.31 (6H, m, $CH_2$), 0.86 (3H, t, J=7.0$H_Z$, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 199.50 ($COCH_2$), 169.11 ($CH_3COO$), 154.33 (Ar-**C**O), 134.86 (Ar-**C**CO), 129.87, 121.92 (Ar-**C**), 38.79 ($COCH_2$), 31.85, 29.21, 24.49, 22.72 ($CH_2$), 21.35 ($CH_3COO$), 14.24 ($CH_2CH_3$); GC: $t_R$=9.5min, LMRS (*M/Z*): 248 ($M^+$, 0.8%), 178 ($M^+$-$C_5H_{10}$, 20%), 163 ($M^+$-$C_6H_{13}$, 6%), 136 ($M^+$-$C_7H_{12}O$, 99%), 121 ($M^+$-$C_8H_{15}O$, 100%), 93 ($M^+$-$C_9H_{15}O_2$, 10%); HRMS (ES): Found 249.1496 $C_{15}H_{21}O_3$ required 249.1491.

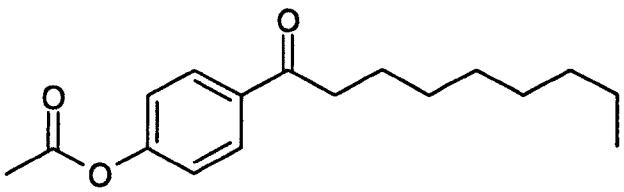
**The synthesis of acetic acid 4-octanoyl-phenyl ester (150):**



Compound (**150**) was synthesised in similar manner to (**143**) except (**136**) (1.1g, 5.0mmol), acetyl chloride (0.7mL, 8.9mmol) were used. The crude product was purified via flash column chromatography to give (**150**) as an off-white solid (1.2g, 92% yield); m.p.= 52.8-53.2°C; $R_f$ = 0.46, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1759.4, 1685.5 (C=O), 1600.0 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.97 (2H, d, J=8.8Hz, ph-**H**), 7.16 (2H, d, J=8.8Hz, ph-**H**), 2.91 (2H, t, J=7.3Hz, $COCH_2$), 2.30 (3H, s, $CH_3CO$), 1.70 (2H, quin, J=7.3Hz, $COCH_2CH_2$), 1.28 (8H, m, $CH_2$), 0.86 (3H, t, J=7.0Hz, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 199.50 ($CH_2$**C**=O), 169.10 ($CH_3$**C**OO), 154.33 (Ar-**C**O), 134.87 (Ar-**C**CO), 129.87, 121.92 (Ar-**C**), 38.79 (CO**C**$H_2$), 31.89, 29.50, 24.54, 22.82 (**C**$H_2$), 21.35 (**C**$H_3$COO), 14.27 ($CH_2$**C**$H_3$); GC: $t_R$=10.0min, LMRS (*M/Z*): 262 ($M^+$, 1%), 178 ($M^+$-$C_6H_{12}$, 24%), 163 ($M^+$-$C_7H_{15}$, 6%), 136 ($M^+$-$C_8H_{14}O$, 100%), 121 ($M^+$-$C_9H_{17}O$, 92%), 93 ($M^+$-$C_{10}H_{17}O_2$, 9%); HRMS (ES): Found 263.1641 $C_{16}H_{23}O_3$ required 263.1647.

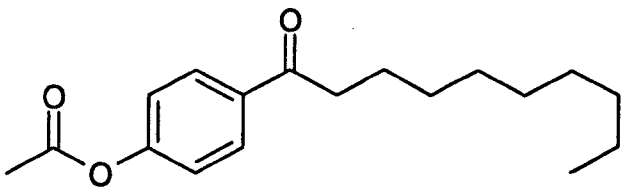
**The synthesis of acetic acid 4-nonanoyl-phenyl ester (151):**



Compound **(151)** was synthesised in similar manner to **(143)** except **(137)** (0.9g, 3.8mmol), acetyl chloride (0.6mL, 8.4mmol) were used. The crude product was purified via flash column chromatography to give **(151)** as an off-white solid (1.0g, 94% yield); m.p.= 46.1-46.8°C; $R_f$ = 0.47, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1759.7, 1684.3 (C=O), 1600.2 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.97 (2H, d, J=8.8$H_Z$, ph-**H**), 7.16 (2H, d, J=8.8$H_Z$, ph-**H**), 2.91 (2H, t, J=7.3$H_Z$, $COCH_2$), 2.30 (3H, s, $CH_3CO$), 1.70 (2H, quin, J-7.3$H_Z$, 7.1$H_Z$, $COCH_2CH_2$), 1.29 (12H, m, $CH_2$), 0.85 (3H, t, J=7.0$H_Z$, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 199.47 ($CH_2$**C**=O), 169.09 ($CH_3$**C**OO), 154.33 (Ar-**C**O), 135.85 (Ar-**C**CO), 129.86, 121.90 (Ar-**C**), 38.78 (CO**C**$H_2$), 32.01, 29.61, 29.54, 29.35, 2452, 22.83 (**C**$H_2$), 21.33 **C**$H_3$COO), 14.29 ($CH_2$**C**$H_3$); GC: $t_R$=10.6min, LMRS (*M/Z*): 276 ($M^+$, 0.9%), 178 ($M^+$-$C_7H_{14}$, 22%), 163 ($M^+$-$C_8H_{17}$, 6%), 136 ($M^+$-$C_9H_{16}O$, 100%), 121 ($M^+$-$C_{10}H_{19}O$, 85%), 93 ($M^+$-$C_{11}H_{19}O_2$, 8%); HRMS (ES): Found 277.1800 $C_{17}H_{25}O_3$ required 277.1804.

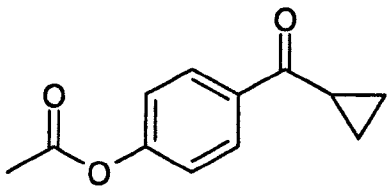
**The synthesis of acetic acid 4-decanoyl-phenyl ester (152):**



Compound **(152)** was synthesised in similar manner to **(143)** except **(138)** (0.8g, 3.2mmol), acetyl chloride (0.5mL, 7.0mmol) was used. The crude product was purified via flash column chromatography to give **(152)** as an off-white solid (0.9g, 96% yield); m.p.= 62.7-63.2°C; $R_f$ = 0.48, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1752.5, 1680.2 (C=O), 1599.5 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.97 (2H, t, J=8.8Hz, ph-**H**), 7.16 (2H, t, J=8.8Hz, ph-**H**), 2.91 (2H, t, J=7.5Hz, $CH_2CO$), 2.30 (3H, s, $CH_3CO$), 1.70 (2H, m, $COCH_2CH_2$), 1.29 (12H, m, $CH_2$), 0.85 (3H, t, J=7.0Hz, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 199.47 ($CH_2$**C**O), 169.07 ($CH_3$**C**OO), 154.33 (Ar-**C**O), 134.86 (Ar-**C**CO), 129.86, 121.90 (Ar-**C**), 38.79, 32.06, 29.65, 29.54, 29.47, 24.54, 22.85 (**C**$H_2$), 21.33 (**C**$H_3$CO), 14.30 ($CH_2$**C**$H_3$); GC: $t_R$=11.1min, LMRS (*M/Z*): 290 ($M^+$, 1%), 178 ($M^+$-$C_8H_{16}$, 29%), 163 ($M^+$-$C_9H_{19}$, 6%), 136 ($M^+$-$C_{10}H_{18}O$, 100%), 121 ($M^+$-$C_{11}H_{21}O$, 77%), 93 ($M^+$-$C_{12}H_{21}O_2$, 7%); HRMS (ES): Found 291.1967 $C_{18}H_{27}O_3$ required 291.1960.

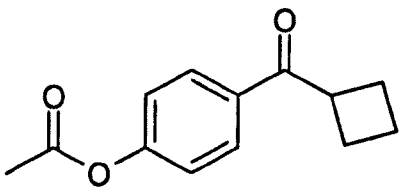
**The synthesis of acetic acid 4-cyclopropanecarbonyl-phenyl ester (153):**



Compound (**153**) was synthesised in similar manner to (**143**) except (**139**) (1.0g, 6.2mmol), acetyl chloride (0.9mL, 12.7mmol) were used. The crude product was purified via flash column chromatography to give (**153**) as a pale yellow oil (1.2g, 92% yield); $R_f$ = 0.27, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1759.1, 1667.5 (C=O), 1601.5 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.03 92H, d, J=8.8$H_Z$, ph-**H**), 7.18 (2H, d, J=8.8$H_Z$, ph-**H**), 2.62 (1H, m, COC**H**), 2.31 (3H, s, $CH_3$), 1.21 (2H, m, $CH_2$), 1.02 (2H, m, $CH_2$); $\delta_C$ ($CDCl_3$): 199.58 (**C**OCH), 169.13 ($CH_3$**C**OO), 154.28 (Ar-**C**O), 135.77 (Ar-**C**CO), 129.80, 121.19 (Ar-**C**), 21.37 (**C**$H_3$), 17.32 (CO**C**H), 11.88 (**C**$H_2$); GC: $t_R$=8.1min, LMRS (*M/Z*): 204 ($M^+$, 11%), 162 ($M^+$-$C_2H_2O$, 44%), 121 ($M^+$-$C_5H_7O$, 100%), 93 ($M^+$-$C_6H_7O_2$, 7%); HRMS (ES): Found 205.0857 $C_{12}H_{13}O_3$ required 205.0865.

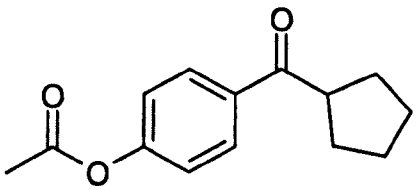
**The synthesis of acetic acid 4-cyclobutanecarbonyl-phenyl ester (154):**



Compound (**154**) was synthesised in similar manner to (**143**) except (**140**) (0.9g, 5.1mmol), acetyl chloride (0.7mL, 9.8mmol) were used. The crude product was purified via flash column chromatography to give (**154**) as a pale yellow oil (1.0g, 91% yield); $R_f$ = 0.33, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1759.7, 1678.0 (C=O), 1599.4 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.90 (2H, d, J=8.8$H_Z$, ph-**H**), 7.14 (2H, d, J=8.8$H_Z$, ph-**H**), 3.95 (1H, quin, J=8.8$H_Z$, 8.4$H_Z$, COC**H**), 2.39 (2H, m, $CH_2$), 2.64 (5H, m, $CH_2$, $CH_3$), 1.91 (2H, m, $CH_2$); $\delta_C$ ($CDCl_3$): 199.87 (**C**OCH), 169.12 ($CH_3$**C**OO), 154.28 (Ar-**C**O), 133.39 (Ar-**C**CO), 130.13, 121.94 (Ar-**C**), 42.35 (CO**C**H), 25.24 ($CH_2$), 21.35 ($CH_3$), 18.33 ($CH_2$); GC: $t_R$=8.7min, LMRS (*M/Z*): 218 ($M^+$, 6%), 163 ($M^+$-$C_4H_7$, 10%), 148 ($M^+$-$C_5H_9$, 5%), 121 ($M^+$-$C_6H_9O$, 100%), 93 ($M^+$-$C_7H_9O_2$, 5%); HRMS (ES): Found 219.1024 $C_{13}H_{15}O_3$ required 219.1021.

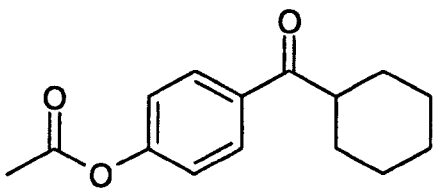
**The synthesis of acetic acid 4-cyclopentanecarbonyl-phenyl ester (155):**



Compound (**155**) was synthesised in similar manner to (**143**) except (**141**) (1.1g, 5.8mmol), acetyl chloride (0.8mL, 11.3mmol) were used. The crude product was purified via flash column chromatography to give (**155**) as a pale yellow oil (1.2g, 92% yield); $R_f$ = 0.38, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1761.1, 1681.0 (C=O), 1598.9 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.99 (2H, d, J=8.9$H_Z$, ph-**H**), 7.16 (2H, d, J=8.9$H_Z$, ph-**H**), 3.66 (1H, quin, J=8.06$H_Z$, 7.69$H_Z$, COC**H**), 2.30 (3H, s, $CH_3$), 1.89 (4H, m, $CH_2$), 1.65 (4H, m, $CH_2$); $\delta_C$ ($CDCl_3$): 201.67 (**C**OCH), 169.12 ($CH_3$**C**OO), 154.20 (Ar-**C**O), 134.64 (Ar-**C**CO), 130.25, 121.85 (Ar-**C**), 46.48 (**C**OCH), 30.13, 26.47 ($CH_2$), 21.34 ($CH_3$); GC: $t_R$=9.4min, LMRS (*M/Z*): 232 ($M^+$, 1%), 163 ($M^+$-$C_5H_9$, 20%), 148 ($M^+$-$C_6H_{11}$, 5%), 121 ($M^+$-$C_7H_{11}O$, 100%), 93 ($M^+$-$C_8H_{11}O_2$, 6%); HRMS (ES): Found 233.1173 $C_{14}H_{17}O_3$ required 233.1178.

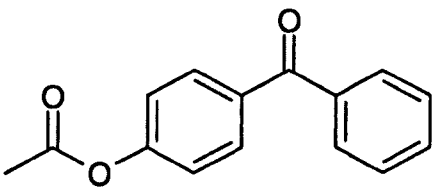
**The synthesis of acetic acid 4-cyclohexanecarbonyl-phenyl ester (156):**



Compound **(156)** was synthesised in similar manner to **(143)** except **(142)** (1.0g, 4.9mmol), acetyl chloride (0.7mL, 9.8mmol) were used. The crude product was purified via flash column chromatography to give **(156)** as a pale yellow oil (1.1g, 91% yield); $R_f$ = 0.40, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1961.1, 1680.8 (C=O), 1600.2 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.94 (2H, d, J=8.8$H_Z$, ph-**H**), 7.15 (2H, d, J=8.8$H_Z$, ph-**H**), 3.20 (1H, m, COC**H**), 2.28 (3H, s, $CH_3$), 1.50 (10H, m, $CH_2$); $\delta_C$ ($CDCl_3$): 202.69 (**C**OCH), 169.02 ($CH_3$**C**OO), 154.19 (Ar-**C**O), 133.99 (Ar-**C**CO), 130.00 (Ar-**C**), 121.89 (Ar-**C**), 45.72 (CO**C**H), 29.52, 26.06, 25.94 ($CH_2$), 21.28 ($CH_3$); GC: $t_R$=9.8min, LMRS (*M/Z*): 246 ($M^+$, 2%), 163 ($M^+$-$C_6H_{11}$, 22%), 149 ($M^+$-$C_7H_{13}$, 4%), 121 ($M^+$-$C_8H_{13}O$, 100%), 93 ($M^+$-$C_9H_{13}O_2$, 5%); HRMS (ES): Found 247.1329 $C_{15}H_{19}O_3$ required 247.1334.

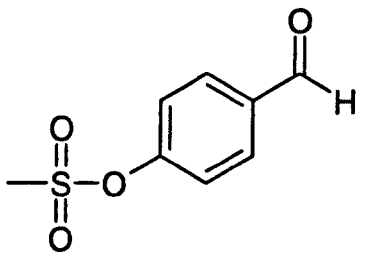
**The synthesis of acetic acid 4-benzoyl-phenyl ester (157):**



Compound **(157)** was synthesised in similar manner to **(143)** except 4-hydroxybenzophenone (3.0g, 15.2mmol), acetyl chloride (2.2mL, 30.9mmol) were used. The crude product was purified via flash column chromatography to give **(157)** as an off-white solid (3.6g, 97% yield); m.p.= 84.4-83.7°C; $R_f$= 0.29, ethyl acetate / petroleum ether 40-60°C (20:80).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1758.9, 1657.9 (C=O), 1598.2 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.82 (2H, d, J=808$H_Z$, ph-**H**), 7.77 (2H, d, J=7.9$H_Z$, ph-**H**), 7.56 (1H, t, J=7.5$H_Z$, ph-**H**), 7.45 (2H, t, J=7.7$H_Z$, ph-**H**), 7.19 (2H, d, J=8.8$H_Z$, ph-**H**), 2.30 (3H, s, $CH_3$); $\delta_C$ ($CDCl_3$): 195.63 ($CH_2$**C**O), 169.04 ($CH_3$**C**OO), 154.01 (Ar-**C**O), 137.58 (Ar-**C**CO), 135.15 (Ar-**C**CO), 132.61, 131.79, 130.06, 128.46, 121.67 (Ar-**C**), 21.29 (**C**$H_3$); GC: $t_R$=10.0min, LMRS (*M/Z*): 240 ($M^+$, 10%), 198 ($M^+$-$C_2H_2O$, 55%), 121 ($M^+$-$C_8H_7O$, 100%), 93 ($M^+$-$C_9H_7O_2$, 8%); HRMS (ES): Found 241.0869 $C_{15}H_{13}O_3$ required 241.0865.

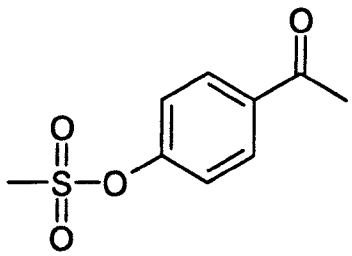
**The synthesis of methanesulfonic acid 4-formyl-phenyl ester (158):**



Methane sulfonyl chloride (MSC) (1.0mL, 12.9mmol) was added in a dropwise manner to a stirred solution of 4-hydroxy-benzaldehyde (1.3g, 10.7mmol) in triethylamine (TEA) (1.7mL, 12.2mmol) and anhydrous dichloromethane (DCM) (50mL) at room temperature. The resulting solution was then refluxed for 4h, After cool to room temperature, it was quenched in ice (50mL), washed with cold saturated sodium bicarbonate ($NaHCO_3$) (3x50mL), water (3x50mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum to give a solid. The crude product was purified via flash column chromatography to give **(158)** as a white solid (1.7g, 81% yield); [m.p.= 64.5-65.0°C, lit. m.p.= 64.1-64.6°C (Looker *et al*, 1957)]; $R_f$ = 0.33, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1693.8 (C=O), 1589.4 (Ar, C=C), 1361.6 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 9.96 (1H, s, OC**H**), 7.90 (2H, d, J=8.9Hz, Ph-**H**), 7.40 (2H, d, J=8.4Hz, Ph-**H**), 3.15 (3H, s, S-C**H**$_3$); $\delta_C$ ($CDCl_3$): 190.76 (**C**=O), 153.36 (**C**-O), 135.14, 131.71, 122.73 (Ar-**C**), 38.15 (S**C**$H_3$); GC: $t_R$=9.1min; LMRS (*M/Z*): 200 ($M^+$, 87%), 121 ($M^+$-$CH_3O_2S$, 100%), 93 ($M^+$-$C_2H_3O_3S$, 14%); HRMS (ES): Found 200.0143 $C_8H_8O_4S$ requires 200.0133.

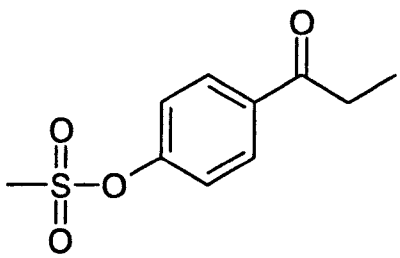
**The synthesis of methanesulfonic acid 4-acetyl-phenyl ester (159):**



Compound (**159**) was synthesised in a similar manner to (**158**) except that (**130**) (0.3g, 2.2mmol) was reacted with TEA (0.3mL, 2.2 mmol) and MSC (0.2mL, 2.6mmol). The crude product was purified via flash column chromatography to give (**159**) as a white solid (0.3g, 76% yield); [m.p.= 70.9-71.4°C, lit. m.p.= 70-71°C (Kametani *et al*, 1964)]; $R_f$ = 0.28, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1681.7 (C=O), 1593.8 (Ar, C=C), 1370.1 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.96 (2H, d, J=8.8Hz, Ph-**H**), 7.31 (2H, d, J=8.8Hz, Ph-**H**), 3.13 (3H, s, S-$CH_3$), 2.55 (3H, s $COCH_3$); $\delta_C$ ($CDCl_3$): 196.59 (**C**=O), 152.50 (**C**-O), 136.03, 130.50, 122.17 (Ar-**C**), 38.00 (S-$CH_3$), 26.74 ($COCH_3$); GC: $t_R$=10.3min, LMRS (*M/Z*): 214 ($M^+$, 26%), 199 ($M^+$-$CH_3$, 100%), 121 ($M^+$-$CH_3O_2S$, 83%), 92 ($M^+$-$C_2H_4O_3S$, 9%); HRMS (ES): Found 214.0308 $C_9H_{10}O_4S$ requires 214.0300.

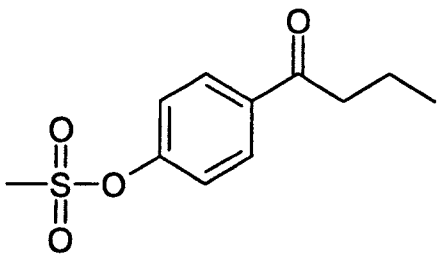
**The synthesis of methanesulfonic acid 4-propionyl-phenyl ester (160):**



Compound **(160)** was synthesised in a similar manner to **(158)** except that (131) (0.2g, 1.3mmol) was reacted with TEA (0.3mL, 2.2mmol) and MSC (0.2mL, 2.6mmol). The crude product was purified via flash column chromatography to give **(16-)** as a white solid (0.3g, 86% yield); [m.p.= 76.3-76.7°C, lit. m.p.= 78-79°C (Humora and Quick, 1979)]; $R_f$ =0.41, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1681.5 (C=O), 1597.5 (Ar, C=C), 1362.0 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 8.01(2H, d, J=8.7Hz, Ph-**H**), 7.35 (2H, d, J=8.6Hz, Ph-**H**), 3.17(3H, s, S-$CH_3$), 2.97 (2H, q, J=7.3Hz, 7.1Hz, COC**H**$_2$), 1.20 (3H, t, J=7.1Hz, 7.3Hz, $CH_2$C**H**$_3$); $\delta_C$ ($CDCl_3$): 199.31 (**C**=O), 152.34 (**C**-O), 136.86, 130.15, 122.15 (Ar-**C**), 37.96 (S-**C**$H_3$), 31.99 (CO**C**$H_2$), 8.20 ($CH_2$**C**$H_3$); GC: $t_R$=11.5min; LMRS (*M/Z*): 228 ($M^+$, 5%), 199 ($M^+$-$C_2H_5$, 100%), 121 ($M^+$-$C_3H_7O_2S$, 63%), 92 ($M^+$-$C_4H_8O_3S$, 9%); HRMS (ES): Found 228.0457 $C_{10}H_{12}O_4S$ requires 228.0456.

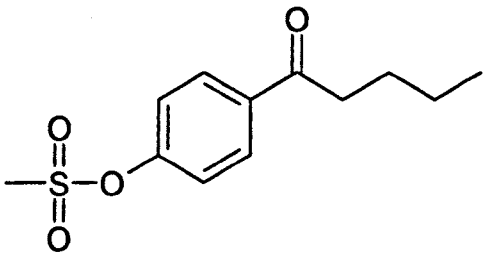
**The synthesis of methanesulfonic acid 4-butyryl-phenyl ester (161):**



Compound **(161)** was synthesised in a similar manner to **(158)** except that (132) (0.3g, 1.8mmol) was reacted with TEA (0.3mL, 2.2mmol) and MSC (0.2mL, 2.6mmol). The crude product was purified via flash column chromatography to give **(161)** as a white solid (0.4g, 88% yield); m.p.= 68.5-69.0°C; $R_f$ = 0.48, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1682.7 (C=O), 1595.8 (Ar, C=C), 1369.8 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.96 (2H, d, J=8.4Hz, Ph-**H**), 7.30 (2H, d, J=8.4Hz, Ph-**H**), 3.12 (3H, s, S-$CH_3$), 2.87 (2H, t, J=7.5Hz, 7.1Hz, $COCH_2$), 1.70 (2H, sex, J=7.3Hz, 7.5Hz, $CH_2CH_3$), 0.94 (3H, t, J=7.3Hz, 7.5Hz, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 198.90 (**C**=O), 165.14 (**C**-O), 136.04, 130.21, 122.13 (Ar-**C**), 40.65 ($COCH_2$), 37.96 (S-$CH_3$), 17.73 ($CH_3CH_2$), 13.90 ($CH_2CH_3$); GC: $t_R$=12.4min; LMRS (*M/Z*): 242 ($M^+$, 0.2%), 214 ($M^+$-$C_2H_4$, 10%), 199 ($M^+$-$C_3H_7$, 100%), 121 ($M^+$-$C_4H_9O_2S$, 62%), 92 ($M^+$-$C_5H_{10}O_3S$, 9%); HRMS (ES): Found 242.0598 $C_{11}H_{14}O_4S$ requires 242.0613.

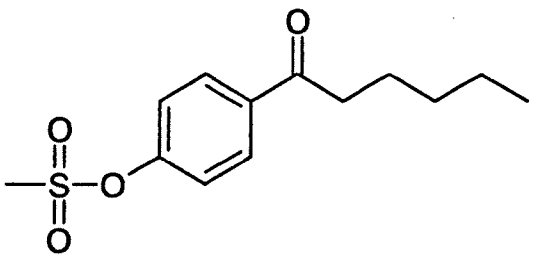
**The synthesis of methanesulfonic acid 4-pentanoyl-phenyl ester (162):**



Compound **(162)** was synthesised in a similar manner to **(158)** except that **(133)** (0.3g, 1.7mmol) was reacted with TEA (0.3mL, 2.2mmol) and MSC (0.2mL, 2.6mmol). The crude product was purified via flash column chromatography to give **(162)** as a white solid (0.3g, 81% yield); m.p.= 68.1-69.2°C; $R_f$ = 0.52, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1683.0 (C=O), 1595.6 (Ar, C=C), 1370.2 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.96 (2H, d, J=8.6Hz, Ph-**H**), 7.31 (2H, d, J=8.6Hz, Ph-**H**), 3.12 (3H, s, S-$CH_3$), 2.89 (2H, t, J=7.3Hz, 7.5Hz, $COCH_2$), 1.65 (2H, Quin, J=7.3Hz, 7.5Hz, 7.7Hz, $COCH_2CH_2$), 1.34 (2H, sex, J=7.3Hz, 7.7Hz, 7.5Hz, $CH_2CH_3$), 0.89 (3H, t, J=7.3Hz, $CH_3CH_2$); $\delta_C$ ($CDCl_3$): 199.05 (**C**=O), 152.33 (**C**-O), 136.03, 130.24, 12.13 (Ar-**C**), 38.48 ($COCH_2$), 37.97 (S-$CH_3$), 26.41, 22.51 ($CH_2$), 13.99 ($CH_2CH_3$); GC: $t_R$=13.3min; LMRS (*M/Z*): 256 ($M^+$, 0.2%), 227 ($M^+$-$C_2H_5$, 4%), 214 ($M^+$-$C_3H_6$, 91%), 199 ($M^+$-$C_4H_9$, 100%), 121 ($M^+$-$C_5H_{11}O_2S$, 87%); HRMS (ES): Found 256.0769 $C_{12}H_{16}O_4S$ requires 256.0769.

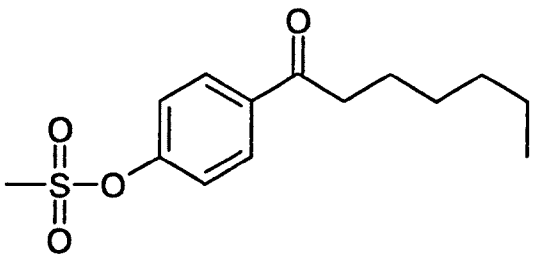
**The synthesis of methanesulfonic acid 4-hexanoyl-phenyl ester (163):**



Compound (**163**) was synthesised in a similar manner to (**158**) except that (**134**) (0.5g, 2.6mmol) was reacted with TEA (0.4mL, 2.9mmol) and MSC (0.2mL, 2.6mmol). The crude product was purified via flash column chromatography to give (**163**) as a white solid (0.6g, 84% yield); m.p.= 80.4-80.6°C; $R_f$ = 0.57, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1682.8 (C=O), 1596.4 (Ar, C=C), 1371.0 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 796 (2H, d, J=8.8Hz, Ph-**H**), 7.31 (2H, d, J=8.8Hz, Ph-**H**), 3.13 (3H, s, S-**CH**$_3$), 2.88 (2H, t, J=7.3Hz, COC**H**$_2$), 1.67 (2H, quin, J=7.3Hz, COCH$_2$C**H**$_2$), 1.29 (4H, m, C**H**$_2$), 0.85 (3H, t, J=6.9Hz, CH$_2$C**H**$_3$); $\delta_C$ ($CDCl_3$): 198.95 (**C**=O), 152.42 (**C**-O), 136.06, 130.24, 122.14 (Ar-**C**), 38.75 (CO**C**H$_2$), 37.97 (S-**C**H$_3$), 31.56, 24.01, 22.59 (**C**H$_2$), 14.03 (CH$_2$**C**H$_3$); GC: $t_R$=14.2min; LMRS (*M/Z*): 270 ($M^+$, 1%), 227 ($M^+$-$C_3H_7$, 6%), 214 ($M^+$-$C_4H_9$, 100%), 199 ($M^+$-$C_5H_{11}$, 91%), 121 ($M^+$-$C_6H_{13}O_2S$, 74%); HRMS (ES): Found 270.0934 $C_{13}H_{18}O_4S$ requires 270.0926.

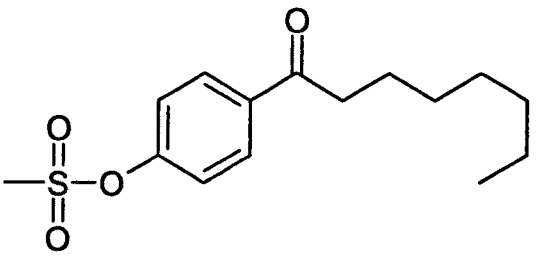
**The synthesis of methanesulfonic acid 4-heptanoyl-phenyl ester (164):**



Compound (**164**) was synthesised in a similar manner to (**158**) except that (**135**) (0.2g, 1.0mmol) was reacted with TEA (0.2mL, 1.4mmol) and MSC (0.1mL, 1.3mmol). The crude product was purified via flash column chromatography to give (**164**) as a white solid (0.3g, 79% yield); m.p.= 81.1-81.5°C; $R_f$ = 0.59, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1683.3 (C=O), 1596.0 (Ar, C=C), 1370.9 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 8.00 (2H, d, J=8.6Hz, Ph-**H**), 7.35 (2H, d, J=8.6Hz, Ph-**H**), 3.17 ( 3H, s, S-$CH_3$), 2.92 (2H, t, J=7.3Hz, $COCH_2$), 1.71 (2H, quin, J=7.5Hz, 7.3Hz, $COCH_2CH_2$), 1.32 (6H, m, $CH_2$), 0.87 (3H, t, J=6.9Hz, 6.9Hz, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 199.08 (**C**=O), 152.45 (**C**-O), 136.18, 130.35, 122.26 (Ar-**C**), 38.91 ($COCH_2$), 38.09 ($COCH_2CH_2$), 31.83 (S-$CH_3$), 29.17, 24.41, 22.72 ($CH_2$), 14.24 ($CH_2CH_3$); GC: $t_R$=15.3min; LMRS (*M/Z*): 284 ($M^+$, 1%), 227 ($M^+$-$C_4H_9$, 7%), 214 ($M^+$-$C_5H_{10}$, 100%), 199 ($M^+$-$C_6H_{13}$, 65%), 121 ($M^+$-$C_7H_{15}O_2S$, 49%); HRMS (ES): Found 284.1081 $C_{14}H_{20}O_4S$ requires 284.1082.

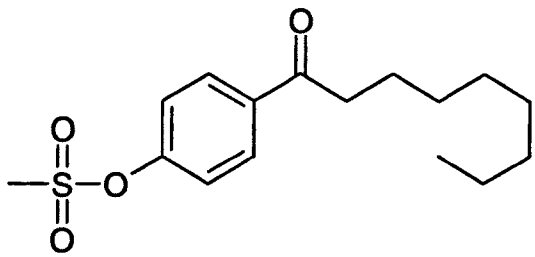
**The synthesis of methanesulfonic acid 4-octanoyl-phenyl ester (165):**



Compound (**165**) was synthesised in a similar manner to (**158**) except that (**136**) (0.6g, 2.7mmol) was reacted with TEA (0.4mL, 2.9mmol) and MSC (0.3mL, 3.9mmol). The crude product was purified via flash column chromatography to give (**165**) as a white solid (0.6g, 78% yield); m.p.= 89.8-90.3°C; $R_f$ = 0.61, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1682.9 (C=O), 1596.7 (Ar, C=C), 1371.9 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 8.01 (2H, d, J=8.8Hz, Ph-**H**), 7.35 (2H, d, J=8.8Hz, Ph-**H**), 3.17 (3H, s, S-$CH_3$), 2.93 (2H, t, J=7.3Hz, $COCH_2$), 1.71 (2H, quin, J=7.5Hz, 7.1Hz, $COCH_2CH_2$), 1.29 (8H, m, $CH_2$), 0.86 (3H, t, J=6.8Hz, $CH_2CH_3$); $\delta_C$ ($CDCl_3$): 199.21 (**C**=O), 152.45 (**C**-O), 136.16, 130.37, 122.26 (Ar-**C**), 38.92 ($COCH_2$), 38.09 (S-$CH_3$), 31.90, 29.48, 29.33, 24.46, 22.83 ($CH_2$), 14.29 ($CH_3$); GC: $t_R$=16.1min; LMRS (*M/Z*): 298 ($M^+$, 1%), 227 ($M^+$-$C_5H_{11}$, 9%), 214 ($M^+$-$C_6H_{12}$, 100%), 199 ($M^+$-$C_7H_{15}$, 58%), 121 ($M^+$-$C_8H_{17}O_2S$, 51%); HRMS (ES): Found 298.1238 $C_{15}H_{22}O_4S$ requires 298.1239.

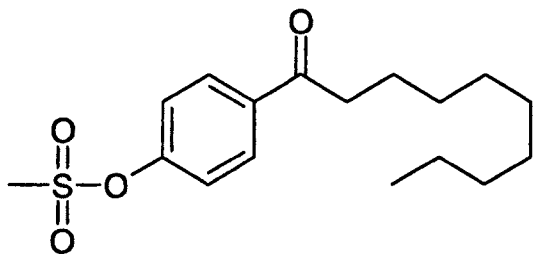
**The synthesis of methanesulfonic acid 4-nonanoyl-phenyl ester (166):**



Compound (**166**) was synthesised in a similar manner to (**158**) except that (**137**) (0.3g, 1.3mmol) was reacted with TEA (0.2mL, 1.4mmol) and MSC (0.2mL, 2.6mmol). The crude product was purified via flash column chromatography to give (**166**) as a white solid (0.3g, 79% yield); m.p.= 90.2-90.3°C; $R_f$ = 0.62, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1683.4 (C=O), 1596.9 (Ar, C=C), 1371.1 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.96 (2H, d, J=8.9Hz, Ph-**H**), 7.30 (2H, d, J=8.9Hz, Ph-**H**), 3.12 (3H, s, S-$\mathbf{CH_3}$), 2.88 (2H, t, J=7.1Hz, 7.5Hz, COC$\mathbf{H_2}$), 1.66 (2H, quin, J=7.5Hz, 7.1Hz, 7.3Hz, $COCH_2\mathbf{CH_2}$), 1.25 (10H, m, $\mathbf{CH_2}$), 0.81 (3H, t, J=6.9Hz, $CH_2\mathbf{CH_3}$); $\delta_C$ ($CDCl_3$): 199.07 (**C**=O), 152.33 (**C**-O), 136.04, 130.23, 122.12 (Ar-**C**), 38.79 (S-$\mathbf{C}H_3$), 37.97 (CO$\mathbf{C}H_2$), 31.90, 24.49, 29.40, 29.23, 24.33, 22.72 ($\mathbf{C}H_2$), 14.17 ($CH_2\mathbf{C}H_3$); GC: $t_R$=17.0min; LMRS (*M/Z*): 312 ($M^+$,1% ), 227 ($M^+$-$C_6H_{13}$, 8%), 214 ($M^+$-$C_7H_{14}$, 100%), 199 ($M^+$-$C_8H_{17}$, 50%), 121 ($M^+$-$C_9H_{19}O_2S$, 37%); HRMS (ES): Found 312.1404 $C_{16}H_{24}O_4S$ requires 312.1395.

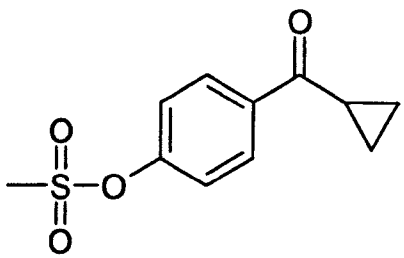
**The synthesis of methanesulfonic acid 4-decanoyl-phenyl ester (167):**



Compound (**167**) was synthesised in a similar manner to (**158**) except that (**138**) (0.3g, 1.2mmol) was reacted with TEA (0.2mL, 1.4mmol) and MSC (0.1mL, 1.3mmol). The crude product was purified via flash column chromatography to give (**167**) as a white solid (0.3g, 85% yield); m.p.= 94.3-94.8°C; $R_f$ = 0.63, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1683.0 (C=O), 1596.7 (Ar, C=C), 1370.7 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.96 (2H, d, J=8.8Hz, Ph-**H**), 7.31 (2H, d, J=8.8Hz, Ph-**H**), 3.12 (3H, s, S-$CH_3$), 2.88 (2H, t, J=7.3Hz, $COCH_2$), 1.66 (2H, quin, J=7.3Hz, 7.1Hz, 7.5Hz, $COCH_2CH_2$), 1.20 (12H, m, $CH_2$), 0.81 (3H, t, J=6.6Hz, 6.9Hz, $CH_2CH_3$). $\delta_C$ ($CDCl_3$): 199.09 (**C**=O), 152.33 (**C**-O), 136.02, 130.24, 122.14 (Ar-**C**), 38.79 ($COCH_2$), 37.96, 31.95 ($CH_2$), 29.53 (S-$CH_3$), 29.40, 29.36, 24.33, 22.75, 14.19 ($CH_2$)1.10 ($CH_3$); GC: $t_R$=17.8min; LMRS (*M/Z*): 326 ($M^+$, 1%), 227 ($M^+$-$C_7H_{15}$, 7%), 214 ($M^+$-$C_8H_{16}$, 100%), 199 ($M^+$-$C_9H_{19}$, 45%), 121 ($M^+$-$C10H_{21}O_2S$, 31%); HRMS (ES): Found 326.1541 $C_{17}H_{26}O_4S$ requires 326.1552.

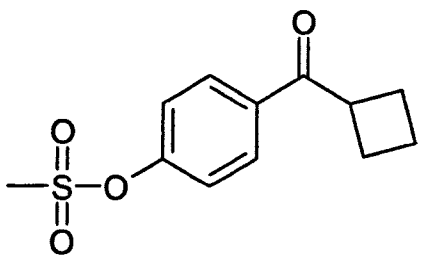
**The synthesis of methanesulfonic acid 4-cyclopropanecarbonyl-phenyl ester (168):**



Compound (**168**) was synthesised in a similar manner to (**158**) except that (**139**) (0.3g, 1.9mmol) was reacted with TEA (0.3mL, 2.2mmol) and MSC (0.2mL, 2.6mmol). The crude product was purified via flash column chromatography to give (**168**) as a white solid (0.3g, 71% yield); m.p.= 78.5-79.0°C; $R_f$ = 0.38, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1666.7 (C=O), 1596.1 (Ar, C=C), 1370.3 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 8.06 (2H, d, J=8.8Hz, Ph-**H**), 7.37 (2H, d , J=8.8Hz, Ph-**H**), 3.17 (3H, s, S-$CH_3$), 2.61 (1H, hept, J=4.6Hz, 3.3Hz, 3.1Hz, COC**H**), 1.23 (2H, quin, J=4.0Hz, 3.3Hz, 4.4Hz, COCHC**H**$_2$), 1.06 (2H, sex, J=3.3Hz, 3.8Hz, 3.5Hz, COCHC**H**$_2$); $\delta_C$ ($CDCl_3$): 199.26 (**C**=O), 152.27 (**C**-O), 136.96, 130.17, 122.09 (Ar-**C**), 37.92 (S-**C**$H_3$), 17.39 (CO**C**H), 12.05 (COCH**C**$H_2$); GC: $t_R$=12.9min; LMRS (*M/Z*): 240 ($M^+$, 47%), 199 ($M^+$-$C_3H_5$, 100%), 121 ($M^+$-$C_4H_7O_2S$, 75%), 92 ($M^+$-$C_5H_8O_3S$, 11%); HRMS (ES): Found 240.0452 $C_{11}H_{12}O_4S$ requires 240.0450.

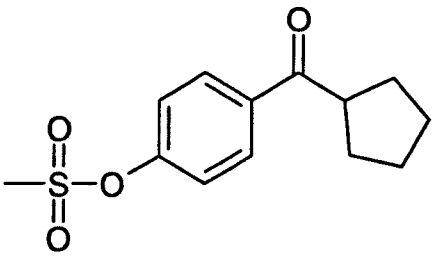
**The synthesis of methanesulfonic acid 4-cyclobutanecarbonyl-phenyl ester (169):**



Compound (**169**) was synthesised in a similar manner to (**158**) except that (**140**) (0.4g, 2.3mmol) was reacted with TEA (0.4mL, 2.9mmol) and MSC (0.2mL, 2.6mmol). The crude product was purified via flash column chromatography to give (**169**) as a white solid (0.5g, 83% yield); m.p.= 80.2-80.7°C; $R_f$ = 0.47, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1673.0 (C=O), 1594.1 (Ar, C=C), 1365.6 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.94 (2H, d, J=8.8Hz, Ph-**H**), 7.34 (2H, d, J=8.8Hz, Ph-**H**), 3.95 (1H, quin, J=8.1Hz, 8.6Hz, 8.4Hz, (COC**H**), 3.12 (3H, s, S-C**H**$_3$), 2.34 (4H, m, 2xC**H**$_2$), 1.95 (2H, m, COCHCH$_2$C**H**$_2$); $\delta_C$ ($CDCl_3$): 199.48 (**C**=O), 152.28 (**C**-O), 134.59, 130.50, 122.16 (Ar-**C**), 42.29 (S-**C**H$_3$), 37.96 (COCH), 25.10 (COCH**C**H$_2$), 18.22 (COCHCH$_2$**C**H$_2$); GC: $t_R$=14.0min; LMRS (*M/Z*): 254 ($M^+$, 3%), 199 ($M^+$-$C_4H_7$, 100%), 121 ($M^+$-$C_5H_9O_2S$, 45%), 92 ($M^+$-$C_5H_9O_3S$, 7%); HRMS (ES): Found 254.0613 $C_{12}H_{14}O_4S$ requires 254.0612.

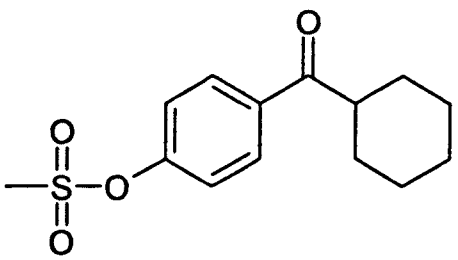
**The synthesis of methanesulfonic acid 4-cyclopentanecarbonyl-phenyl ester (170):**



Compound (**170**) was synthesised in a similar manner to (**158**) except that (**141**) (0.3g, 1.6mmol) was reacted with TEA (0.3mL, 2.2mmol) and MSC (0.2mL, 2.6mmol). The crude product was purified via flash column chromatography to give (**170**) as a white solid (0.3g, 74% yield); m.p.= 81.1-81.6°C; $R_f$ = 0.53, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1674.3 (C=O), 1594.2 (Ar, C=C), 1362.8 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.94 (2H, d, J=8.6Hz, Ph-**H**), 7.29 (2H, d, J=8.8Hz, Ph-**H**), 3.12 (3H, s, S-$CH_3$), 1.41 (11H, m, COC**H**, $CH_2$); $\delta_C$ ($CDCl_3$): 202.38 (**C**=O), 152.23 (**C**-O), 135.31, 130.43, 122.15 (Ar-**C**), 45.83 (S-$CH_3$), 37.95 (COCH), 29.40, 25.96, 25.85 ($CH_2$); GC: $t_R$=14.9min; LMRS (*M/Z*): 268 ($M^+$, 2%), 199 ($M^+$-$C_5H_9$, 100%), 121 ($M^+$-$C_6H_{11}O_2S$, 41%), 92 ($M^+$-$C_7H_{12}O_3S$, 6%); HRMS (ES): Found 268.0771 $C_{13}H_{16}O_4S$ requires 268.0769.

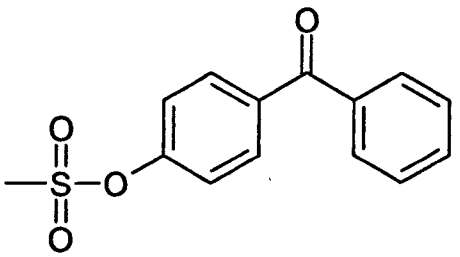
**The synthesis of methanesulfonic acid 4-cyclohexanecarbonyl-phenyl ester (171):**



Compound (**171**) was synthesised in a similar manner to (**158**) except that (**142**) (0.3g, 1.5mmol) was reacted with TEA (0.3mL, 2.2mmol) and MSC (0.2mL, 2.6mmol). The crude product was purified via flash column chromatography to give (**171**) as a white solid (0.3g, 85% yield); m.p.= 96.9-97.4°C; $R_f$ = 0.58, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1671.9 (C=O), 1594.9 (Ar, C=C), 1365.9 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.94 (2H, d, J=8.7Hz, Ph-**H**), 7.30 (2H, d, J=8.8Hz, Ph-**H**), 3.12 (3H, s, S-$CH_3$), 1.51 (11H, m, COC**H**, $CH_2$); $\delta_C$ ($CDCl_3$): 202.38 (**C**=O), 152.23 (**C**-O), 135.31, 130.43, 122.15 (Ar-**C**), 45.83 (COCH), 37.95 ($CH_2$), 29.40 (S-$CH_3$), 25.96, 25.85 ($CH_2$); GC: $t_R$=16.0min; LMRS (*M/Z*): 282 ($M^+$, 2%), 199 ($M^+$-$C_6H_{11}$, 100%), 121 ($M^+$-$C_7H_{13}O_2S$, 44%), 92 ($M^+$-$C_8H_{14}O_3S$, 6%); HRMS (ES): Found 282.0918 $C_{14}H_{18}O_4S$ requires 282.0919.

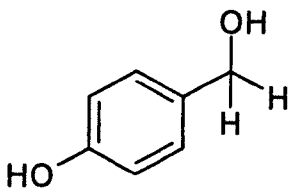
**The synthesis of methanesulfonic acid 4-benzoyl-phenyl ester (172):**



Compound (**172**) was synthesised in a similar manner to (**158**) except that 4-hydroxy benzophenone (1.3g, 6.6mmol) was reacted with TEA (1.0mL, 7.2 mmol) and MSC (0.6mL, 7.7mmol). The crude product was purified via flash column chromatography to give (**172**) as a white solid (1.5g, 85% yield); m.p.=105.9-106.3°C; $R_f$ = 0.49, diethyl ether/petroleum ether 40-60°C (70:30).

$\nu_{(max)}$ (Film) $cm^{-1}$: 1648.6 (C=O), 1593.2 (Ar, C=C), 1368.4 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.86 (2H, d, J=8.8Hz,Ph-**H**), 7.77 (2H, d, J= 7.0Hz, Ph-**H**), 7.59 (1H, t't, J=7.5Hz, Ph-**H**), 7.48 (2H, t, J=7.7Hz, 7.5Hz, Ph-**H**), 7.38 (2H, d, J=8.8Hz, Ph-**H**), 3.14 (3H, s, S-$CH_3$); $\delta_C$ ($CDCl_3$): 195.30 (**C**=O), 152.01 (Ar-**C**-O), 137.11, 136.61, 132.93, 132.14, 130.08, 128.56, 121.98 (Ar-**C**), 38.01 ($CH_3$); GC: $t_R$=16.2min; LMRS (*M/Z*): 276 ($M^+$, 100%), 199 ($M^+$-$C_6H_5$, 84%), 121 ($M^+$-$C_7H_7O_2S$, 50%), 92 ($M^+$-$C_8H_8O_3S$, 6%); HRMS (ES): Found 276.0446 $C_{14}H_{12}O_4S$ requires 276.0456.

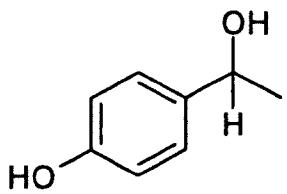
**The synthesis of 4-Hydroxymethyl-phenyl (173):**




Lithium borohydride ($LiBH_4$) (2M) in tetrahydrofuran (THF) (7.8mL, 15.6mmol) was added continually to a stirred solution of 4-hydroxy-benzaldehyde (1.9g, 15.6mmol) in anhydrous THF (100mL). The resulting mixture was then stirred for 24h. Then it was poured in ice (50mL) and acidified with HCl (0.1M) (10mL). The organic compound was mextracted into ethyl acetate (3x50mL) and washed with water (3x50mL) and then dried over anhydrous $MgSO_4$. The $MgSO_4$ was filtered and the solvent was removed under vacuum. The crude product was purified via flash column chromatography to give **(173)** as white solid (1.7g, 84% yield); m.p.= 25.3-26.2˚C; $R_f$ = 0.21, ethyl acetate/petroleum ether 40-60˚C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3384.7 (OH), 1598.0 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.30 (1H, s, COH), 7.22 (2H, d, J=8.6$H_Z$, Ph-**H**), 6.82 (2H, d, J=8.6$H_Z$, Ph-**H**), 4.55 (2H, s, $CH_2OH$), 4.14 (1H, s, $CH_2$**OH**); $\delta_C$ ($CDCl_3$): 156.54 (Ar, **C**OH), 133.34, 128.34, 114.98 (Ar, **C**), 63.79 (**C**$H_2OH$); GC: $t_R$=5.2min, LMRS (*M/Z*): 124 ($M^+$, 100%), 107 ($M^+$-OH, 38%), 95 ($M^+$-COH, 52%), 77 ($M^+$-$CH_3O_2$, 40%); HRMS (ES): Found 123.0439 $C_7H_7O_2$ requires 123.0446.

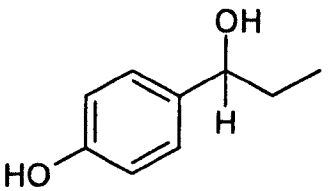
**The synthesis of 4-(1-Hydroxy-ethyl)-phenol (174):**




Compound **(174)** was synthesis in similar manner to **(173)** except that **(130)** (1.5g, 11.0mmol), $LiBH_4$ (5.5mL, 11.0mmol) were used. The crude product was purified via flash column chromatography to give **(174)** as a white solid (1.3g, 86% yield); m.p.= 30.8-31.8˚C; $R_f$ = 0.27, ethyl acetate/petroleum ether 40-60˚C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3383.9 (OH), 1599.6 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.21 (1H, s, COH), 7.24 (2H, d, J=8.2$H_Z$, Ph-**H**), 6.81 (2H, d, J=8.2$H_Z$, Ph-**H**), 4.79 (1H, m, CHOH), 4.04 (1H, s, CHO**H**), 1.40 (3H, d, J=6.4$H_Z$, **$CH_3$**); $\delta_C$ ($CDCl_3$): 157.14 (Ar, **C**OH), 139.15, 127.44, 115.68 (Ar, **C**), 69.70 (**C**HOH), 29.53 (**$CH_3$**); GC: $t_R$=7.1min, LMRS (*M/Z*): 138 ($M^+$, 47%), 123 ($M^+$-$CH_3$, 100%), 93 ($M^+$-$C_2H_5O$, 5%); HRMS (ES): Found 137.0595 $C_8H_9O_2$ requires 137.0603.

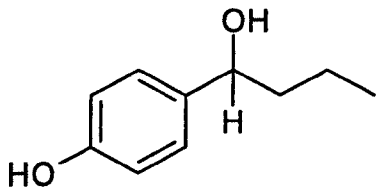
**The synthesis of 4-(1-Hydroxy-propyl)-phenol (175):**




Compound **(175)** was synthesis in similar manner to **(173)** except that **(131)** (2.3g, 15.3mmol), $LiBH_4$ (7.7mL, 15.4mmol) were used. The crude product was purified via flash column chromatography to give **(175)** as a white solid (2.0g, 84% yield); m.p.= 31.9-33.1°C; $R_f$= 0.36, ethyl acetate/petroleum ether 40-60°C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3333.4 (OH), 1599.5 (Ar, C=C); $\delta_H$ ($CDCl_3$): 7.18 (2H, d, J=8.6Hz, Ph-**H**), 6.77 (2H, d, J=8.6Hz, Ph-**H**), 5.44 (1H, s, O**H**), 4.52 (2H, t, J=6.8Hz, C**H**OH), 1.96 (1H, s, CHO**H**), 1.75 (2H, m, $CH_2$), 0.87 (3H, t, J=7.3Hz, $CH_3$); $\delta_C$ ($CDCl_3$): 155.34 (Ar, **C**OH), 136.68, 127.69, 115.47 (Ar, **C**), 76.09 (**C**HOH), 31.85 ($CH_2$), 10.43 ($CH_3$); GC: $t_R$=8.2min, LMRS (*M/Z*): 152 ($M^+$, 19%), 135 ($M^+$-OH, 3%), 123 ($M^+$-$CH_2CH_3$, 100%), 106 [$M^+$-$C_2H_6O$, 3%), 93 ($M^+$-$C_3H_7O$, 2%); HRMS (ES): Found 151.0762 $C_9H_{11}O_2$ requires 151.0759.

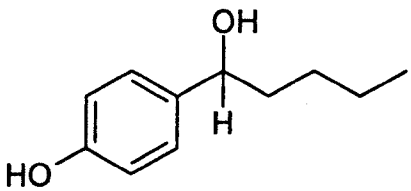
**The synthesis of 4-(1-Hydroxy-butyl)-phenol (176):**




Compound **(176)** was synthesis in similar manner to **(173)** except that **(132)** (1.9g, 11.6mmol), $LiBH_4$ (5.8mL, 11.6mmol) were used. The crude product was purified via flash column chromatography to give **(176)** as a white solid (1.8g, 95% yield); m.p.= 32.5-33.5°C; $R_f$ = 0.43, ethyl acetate/petroleum ether 40-60°C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3334.8 (OH), 1599.8 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.22 (1H, s, Ph-**OH**), 7.21 (2H,d, J=8.2$H_Z$, Ph-**H**), 6.81 (2H, d, J=8.2$H_Z$, Ph-**H**), 4.58 (1H, t, J=5.9$H_Z$, **CH**OH), 3.99 (1H, s, CHO**H**), 1.67 (2H, m, **$CH_2$**), 1.38 (2H, m, **$CH_2$**), 0.92 (3H, t, J=7.5$H_Z$, **$CH_3$**); $\delta_C$ ($CDCl_3$): 157.21 (Ar-**C**OH), 138.23, 127.97, 115.67 (Ar, **C**), 73.80 (**C**HOH), 42.82 (CHOH**C**$H_2$), 19.86 (**C**$H_2CH_3$), 14.44 (**C**$H_3$); GC: $t_R$=9.3min, LMRS (*M/Z*): 166 ($M^+$, 16%), 123 ($M^+$-$C_3H_7$, 100%), 106 ($M^+$-$C_3H_8O$, 3%), 94 ($M^+$-$C_4H_8$, 21%); HRMS (ES): Found 165.0906 $C_{10}H_{13}O_2$ requires 165.0916.

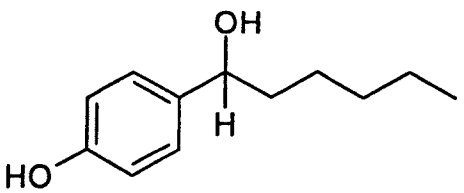
**The synthesis of 4-(1-Hydroxy-pentyl)-phenol (177):**




Compound **(177)** was synthesis in similar manner to **(173)** except that **(133)** (1.2g, 6.7mmol), $LiBH_4$ (3.4mL, 6.8mmol) were used. The crude product was purified via flash column chromatography to give **(177)** as a white solid (1.0g, 85% yield); m.p.= 33.1-34.2°C; $R_f$ = 0.46, ethyl acetate/petroleum ether 40-60°C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3340.0 (OH), 1600.0 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.23 (1H, s, Ph-**OH**), 7.21 (2H, d, J=8.4$H_Z$, Ph-**H**), 6.81 (2H, d, J=8.4$H_Z$, Ph-**H**), 4.57(1H, t, J=5.9$H_Z$, **CH**OH), 4.03 (1H, s, CHO**H**), 1.70 (2H, m, COH**CH$_2$**), 1.34 (4H, m, **CH$_2$**), 0.90 (3H, t, J=7.1$H_Z$, **CH$_3$**); $\delta_C$ ($CDCl_3$): 157.19 (Ar, **C**OH), 138.19, 127.97, 115.66 (Ar, **C**), 74.07 (**C**HOH), 40.29, 28.97, 23.41 (**CH$_2$**), 14.47 (**CH$_3$**); GC: $t_R$=10.5min, LMRS (*M/Z*): 180 ($M^+$, 10%), 123 ($M^+$-$C_4H_9$, 100%), 106 ($M^+$-$C_4H_{10}O$, 5%), 93 ($M^+$-$C_5H_{211}O$, 2%); HRMS (ES): Found 179.1073 $C_{11}H_{15}O_2$ requires 179.1072.

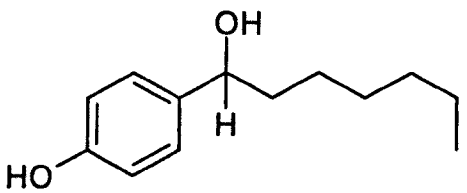
**The synthesis of 4-(1-Hydroxy-hexyl)-phenol (178):**




Compound **(178)** was synthesis in similar manner to **(173)** except that **(134)** (2.1g, 10.9mmol), $LiBH_4$ (5.5mL, 11.0mmol) were used. The crude product was purified via flash column chromatography to give **(178)** as a white solid (1.8g, 83% yield); m.p.= 33.9-35.0°C; $R_f$ = 0.48, ethyl acetate/petroleum ether 40-60°C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3334.3 (OH), 1599.8 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.21 (1H, s, Ph-COH), 7.21 (2H, d, J=8.2$H_Z$, Ph-**H**), 6.81 (2H, d, J=8.2$H_Z$, Ph-**H**), 4.57 (1H, t, J=7.9$H_Z$, CHOH), 4.00 (1H, s, CHO**H**), 1.69 (2H, m, CHOH$CH_2$), 1.36 (6H, m, $CH_2$), 0.90 (3H, t, J=6.7Hz, ,$CH_3$); $\delta_C$ ($CDCl_3$): 157.17 (Ar, **C**OH), 138.20, 127.95, 115.63 (Ar, **C**), 74.05 (**C**HOH), 40.55, 32.64, 26.41, 23.39 ($CH_2$), 14.39 ($CH_3$); GC: $t_R$=11.6min, LMRS (*M/Z*): 194 ($M^+$, 12%), 123 ($M^+$-$C_5H_{11}$, 100%), 94 ($M^+$-$C_6H_{12}O$, 13%); HRMS (ES): Found 193.1219 $C_{12}H_{17}O_2$ requires 193.1229.

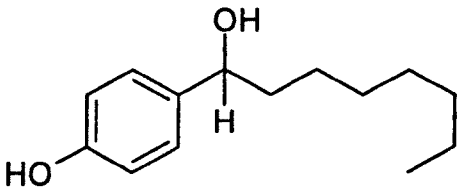
**The synthesis of 4-(1-Hydroxy-heptyl)-phenol (179):**




Compound **(179)** was synthesis in similar manner to **(173)** except that **(135)** (2.1g, 10.2mmol), $LiBH_4$ (5.1mL, 10.2mmol) were used. The crude product was purified via flash column chromatography to give **(179)** as a white solid (1.8g, 86% yield); m.p.= 36.6-37.1°C; $R_f$ = 0.50, ethyl acetate/petroleum ether 40-60°C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3395.8 (OH), 1599.8 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.22 (1H, s, Ph-**OH**), 7.21 (2H, d, J=8.2$H_Z$, Ph-**H**), 6.81 (2H, d, J=8.2$H_Z$, Ph-**H**), 4.57 (1H, t, J=6.0$H_Z$, **CH**OH) , 4.01 (1H, s, CHO**H**), 1.70 (2H, m, CHOH**CH$_2$**), 1.36 (8H, m, **CH$_2$**), 0.90 (3H, t, J=6.6$H_Z$, **CH$_3$**); $\delta_C$ ($CDCl_3$): 157.16 (Ar, **C**OH), 138.18, 127.94, 115.63 (Ar, **C**), 74.04 (**C**HOH), 40.58, 32.68, 30.08, 26.68, 23.33 (**CH$_2$**), 14.40 (**CH$_3$**); GC: $t_R$=12.6min, LMRS (*M/Z*): 208 ($M^+$, 11%), 123 ($M^+$-$C_6H_{13}$, 100%), 94 ($M^+$-$C_7H_{14}O$, 11%); HRMS (ES): Found 207.1389 $C_{13}H_{19}O_2$ requires 207.1385.

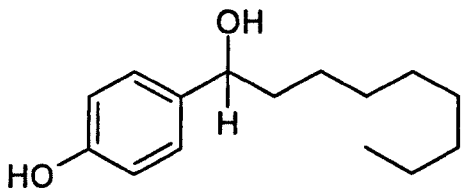
**The synthesis of 4-(1-Hydroxy-octyl)-phenol (180):**




Compound (**180**) was synthesis in similar manner to (**173**) except that (**136**) (1.9g, 8.6mmol), $LiBH_4$ (4.3mL, 8.6mmol) were used. The crude product was purified via flash column chromatography to give (**180**) as a white solid (1.5g, 80% yield); m.p.= 37.7-38.5˚C; $R_f$= 0.52, ethyl acetate/petroleum ether 40-60˚C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3342.2 (OH), 1599.6 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.26 (1H, s, Ph-**OH**), 7.21 (2H, d, J=8.2$H_Z$, Ph-**H**), 6.81 (2H, d, J=8.2$H_Z$, Ph-**H**), 4.57 (1H, t, J=6.6$H_Z$, **CH**OH), 4.01 (1H, s, CHO**H**), 1.70 (2H, m, **$CH_2$**), 1.39 (10H, m, **$CH_2$**), 0.90 (3H, t, J=6.8$H_Z$, $CH_3$); $\delta_C$ ($CDCl_3$): 157.20 (Ar, **C**OH), 138.1, 127.94, 115.64 (Ar, **C**), 74.05 (**C**HOH), 40.58, 32.64, 30.38, 30.13, 26.73, 23.36 (**$CH_2$**), 14.41 (**$CH_3$**); GC: $t_R$=13.6min, LMRS (*M/Z*): 222 ($M^+$, 9%), 123 ($M^+$-$C_7H_{15}$, 100%), 94 ($M^+$-$C_8H_{16}O$, 9%); HRMS (ES): Found 221.1532 $C_{14}H_{21}O_2$ requires 221.1542.

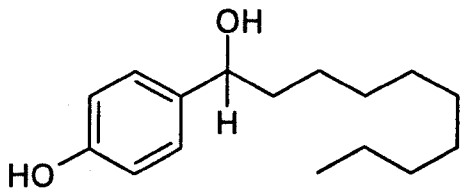
**The synthesis of 4-(1-Hydroxy-nonyl)-phenol (181):**




Compound (**181**) was synthesis in similar manner to (**173**) except that (**137**) (1.9g, 8.6mmol), LiBH4 (4.3mL, 8.6mmol) were used. The crude product was purified via flash column chromatography to give (**181**) as a white solid (1.5g, 80% yield); m.p.= 40.1-41.4°C; Rf = 0.54, ethyl acetate/petroleum ether 40-60°C (40:60).

ν(max) (Film) cm-1: 3334.0 (OH), 1599.7 (Ar, C=C); δH (CDCl3): 8.22 (1H, s, Ph-OH), 7.21 (2H, d, J=8.2HZ, Ph-H), 6.81 (2H, d, J=8.2HZ, Ph-H), 4.57 (1H, t, J=5.9HZ, CHOH), 4.01 (1H. s, CHOH), 1.70 (2H, m, CHOHCH2), 1.39 (12H, m, CH2), 0.91 (3H, t, J=6.9HZ, CH3); δC (CDCl3): 157.18 (Ar, COH), 138.18, 127.95, 115.64 (Ar, C), 74.05 (CHOH), 40.58, 32.66, 30.42, 30.08, 26.73, 23.37 (CH2), 14.42 (CH3); GC: tR=14.6min, LMRS (M/Z): 236 (M+, 9%), 123 (M+-C8H17, 100%), 94 (M+-C9H18O, 8%); HRMS (ES): Found 235.1704 $C_{15}H_{23}O_2$ requires 235.1698.

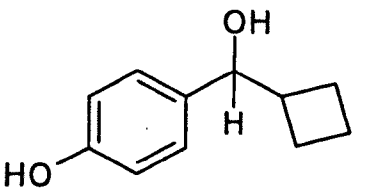
**The synthesis of 4-(1-Hydroxy-decyl)-phenol (182):**




Compound (**182**) was synthesis in similar manner to (**173**) except that (**138**) (1.1g, 4.7mmol), $LiBH_4$ (2.4mL, 4.8mmol) were used. The crude product was purified via flash column chromatography to give (**182**) as a white solid (0.9g, 80% yield); m.p.= 41.2-41.9°C; $R_f$ = 0.57, ethyl acetate/petroleum ether 40-60°C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3351.8 (OH), 1599.8 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.22 (1H, s, Ph-**OH**), 7.21 (2H, d, J=8.6Hz, Ph-**H**), 6.81 (2H, d, J=8.6Hz, Ph-**H**), 4.58 (1H, t, J=6.5Hz, **CH**OH), 3.96 (1H, s, CHO**H**), 1.64 (2H, m, CHOH**CH**$_2$), 1.29 (14H, m, **CH**$_2$), 0.86 (3H, t, J=6.7Hz, **CH**$_3$); $\delta_C$ ($CDCl_3$): 157.54 (Ar, **C**OH), 138.54, 128.31, 116.00 (Ar, **C**), 74.45 (**C**HOH), 40.95, 33.05, 30.84, 30.79, 30.74, 30.49, 27.10, 23.75 (**C**H$_2$), 14.81 (**C**H$_3$); GC: $t_R$=15.5min, LMRS (*M/Z*): 250 ($M^+$, 8.1%), 232 ($M^+$-$H_2O$, 4%), 133 ($M^+$-$C_7H_{15}$, 6%), 123 ($M^+$-$C_9H_{19}$, 100%), 43 ($M^+$-$C_{13}H_{19}O_2$, 3%); HRMS (ES): Found 249.1841 $C_{16}H_{25}O_2$ requires 249.1855.

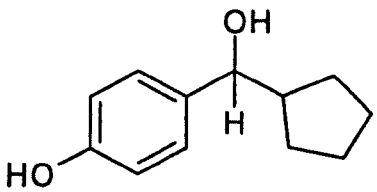
**The synthesis of 4-(Cyclobutyl-hydroxy-methyl)-phenol (183):**




Compound **(183)** was synthesis in similar manner to **(173)** except that **(140)** (1.0g, 5.7mmol), $LiBH_4$ (2.9mL, 5.8mmol) were used. The crude product was purified via flash column chromatography to give **(183)** as a white solid (0.9g, 90% yield); m.p.= 35.3-36.5˚C; $R_f$ = 0.40, ethyl acetate/petroleum ether 40-60˚C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3334.0 (OH), 1598.9 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.19 (1H, s, Ph-**OH**), 7.18 (2H, d, J=8.2$H_Z$, Ph-**H**), 6.79 (2H. d, J=8.2$H_Z$, Ph-**H**), 4.49 (1H, d, J=7.7$H_Z$, **CH**OH), 3.99 (1H, s, CHO**H**), 2.56 (1H, m, CHOH**CH**), 2.04 (2H, m, $CH_2$), 1.79 (4H, m, $CH_2$); $\delta_C$ ($CDCl_3$): 157.23 (Ar, **C**OH), 136.43, 128.25, 115.56 (Ar, **C**), 77.83 (**C**HOH), 4.96 (CHOH**C**H), 25.53, 25.03, 18.25 (**C**$H_2$); GC: $t_R$=11.1min, LMRS (*M/Z*): 178 ($M^+$,8%), 123 ($M^+$-$C_4H_7$, 100%), 93 ($M^+$-$C_5H_9O$, 4%); HRMS (ES): Found 177.0912 $C_{11}H_{13}O_2$ requires 177.0916.

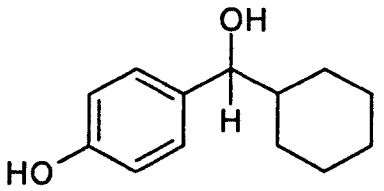
**The synthesis of 4-(Cyclopentyl-hydroxy-methyl)-phenol (184):**




Compound **(184)** was synthesis in similar manner to **(173)** except that **(141)** (1.2g, 6.3mmol), $LiBH_4$ (3.2mL, 6.4mmol) were used. The crude product was purified via flash column chromatography to give **(184)** as a white solid (1.0g, 84% yield); m.p.= 36.5-37.3°C; $R_f$ = 0.44, ethyl acetate/petroleum ether 40-60°C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3353.0 (OH), 1598.9 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.21 (1H, s, Ph-**OH**), 7.21 (2H, d, J=8.2$H_Z$, Ph-**H**), 6.80 (2H, d, J=8.2$H_Z$, Ph-**H**), 4.32 (1H, d, J=8.1$H_Z$, **C**HOH), 3.98 1H, s, CHO**H**), 2.18 (1H, six, J=7.9$H_Z$, 8.2$H_Z$, CHOH**CH**), 1.53 (8H, m, **CH2**); $\delta_C$ ($CDCl_3$): 157.20 (Ar, **C**OH), 137.80, 128.50, 115.54 (Ar, **C**), 78.36 (**C**HOH), 48.96 (CHOH**C**H), 30.15, 30.13, 26.19, 26.14 (**C**H2); GC: $t_R$=12.3min, LMRS (*M/Z*): 192 ($M^+$,3%), 123 ($M^+$-C5H9, 100%), 93 ($M^+$-$C_6H_{11}O$, 3%); HRMS (ES): Found 191.1079 $C_{12}H_{15}O_2$ requires 191.1072.

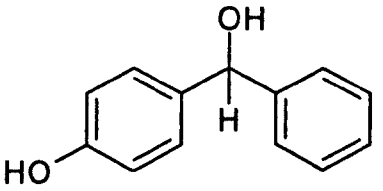
**The synthesis of 4-(Cyclohexyl-hydroxy-methyl)-phenol (185):**




Compound **(185)** was synthesis in similar manner to **(173)** except that **(142)** (1.6g, 7.8mmol), $LiBH_4$ (3.9mL, 7.8mmol) were used. The crude product was purified via flash column chromatography to give **(185)** as a white solid (1.4g, 86% yield); m.p.= 41.2-41.9°C; $R_f$ = 0.48, ethyl acetate/petroleum ether 40-60°C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3417.6 (OH), 1598.7 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.21 (1H, s, ph-COH), 7.16 (2H, d, J=8.2$H_Z$, ph-H), 6.81 (2H, d, J=8.2, ph-H), 4.27 (1H, d, J=6.8$H_Z$, CHOH), 3.96 (1H, s, CHOH), 2.03 (1H, m, CHOHCH), 1.64 (4H, m, CH2), 1.18 (6H, m, CH2); $\delta_C$ ($CDCl_3$): 157.15 (Ar-COH), 136.71 (Ar-CCHOH), 128.62, 115.43 (Ar-C), 78.80 (CHOH), 46.36 (CHOHCH), 30.22, 29.74, 27.37, 26.98, 26.93 (CH2); GC: $t_R$=13.5min, LMRS (*M/Z*): 206 ($M^+$, 5%), 123 ($M^+$,-$C_6H_{11}$, 100%), 93 ($M^+$,-$C_7H_{13}O$, 2%); HRMS (ES): Found 206.1307 $C_{13}H_{18}O_2$ requires 206.1307.

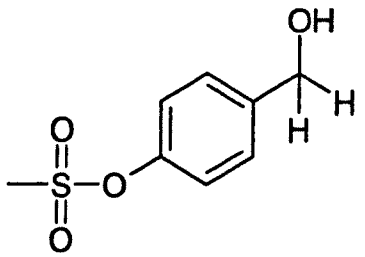
**The synthesis of 4-(Hydroxy-phenyl-methyl)-phenol (186):**




Compound **(186)** was synthesis in similar manner to **(173)** except that 4-hydroxybenzophenone (1.7g, 8.6mmol), $LiBH_4$ (4.3mL, 8.6mmol) were used. The crude product was purified via flash column chromatography to give **(186)** as a white solid (1.3g, 76% yield); m.p.= 42.3-44.1°C; $R_f$ = 0.42, ethyl acetate/petroleum ether 40-60°C (40:60).

$\nu_{(max)}$ (Film) $cm^{-1}$: 3334.0 (OH), 1599.6 (Ar, C=C); $\delta_H$ ($CDCl_3$): 8.27 (1H, s, ph-**OH**), 7.44 (2H, m, ph-**H**), 7.33 (2H, m, ph-**H**), 7.25 (3H, m, ph-**H**), 6.81 (2H, d, J=8.6$H_Z$, ph-**H**), 5.79 (1H, s, **CH**OH), 4.73 (1H, s, CHO**H**); $\delta_C$ ($CDCl_3$): 157.31 (Ar-**C**OH), 146.89 , 137.54 (Ar-**C**CHOH), 128.86, 128.73, 127.51, 127.24, 115.74 (Ar-**C**), 75.86 (**C**HOH); GC: $t_R$=13.9min, LMRS (*M/Z*): 200 ($M^+$, 54%), 121 ($M^+$,-$C_6H_7$, 100%), 94 ($M^+$,-$C_7H_6O$, 67%); HRMS (ES): Found 199.0769 $C_{13}H_{11}O_2$ requires 199.0758.

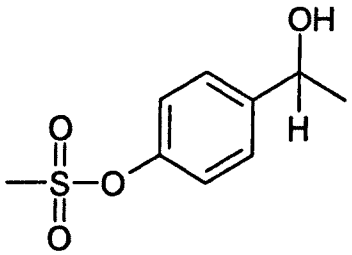
**The synthesis of methanesulfonic acid 4-hydroxymethyl-phenyl ester (187):**




Sodiumborohydride ($NaBH_4$) (0.2g, 5.3mmol) was added to stirred solution of **(158)** (0.8g, 4.3mmol) in ethanol (50mL) in small portions. The solution was stirred for 4h and then the organic solution was poured in ice (50mL) and acidified with HCl (0.1M) (10mL). The organic compound was extracted into diethyl ether (3x30mL). The combined ethereal solutions were washed with water (3x50mL) and died over anhydrous magnesium sulphate ($MgSO_4$). The solution was filtered and the solvent was removed in vacuum. The crude product was purified via flash column chromatography to give **(187)** as white solid (0.7g, 86% yield); [m.p. 50.1-50.9°C; $R_f$ = 0.13 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3373.7 (OH), 1603.8 (Ar, C=C), 1362.8 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.41 (2H, d, J=8.6Hz, Ph-**H**), 7.26 (2H, d, J=8.6Hz, Ph-**H**), 4.69 (2H, d, J=4.8Hz, $CH_2$), 3.13 (3H, s, $CH_3$), 2.01 (1H, t, J=5.4Hz, O**H**$CH_2$); $\delta_C$ ($CDCl_3$): 148.48 (Ar-**C**$CH_2OH$), 140.30 (Ar-**C**OS), 128.47 (Ar-**C**), 122.11 (Ar-**C**), 64.35 ($CH_2$), 37.38 ($CH_3$); GC: $t_R$=8.5min; LRMS (*M/Z*): 202 ($M^+$, 100%), 123 ($M^+$-$CH_3O_2S$, 83%), 106 ($M^+$-$CH_4O_3S$, 54%); HRMS (ES): Found 202.0313, $C_8H_{10}O_4S$ requires 202.0300.

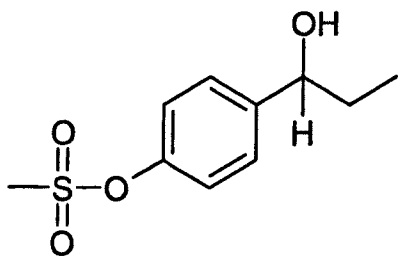
**The synthesis of methanesulfonic acid 4-(1-hydroxy-ethyl)-phenyl ester (188):**




Compound **(188)** was synthesised in similar manner to **(187)** except that **(159)** (0.9g, 4.5mmol) was reacted with $NaBH_4$ (0.2g, 5.3mmol). The crude product was purified via flash column chromatography to give **(188)** as a white solid (0.8g, 88% yield); [m.p. 64.5-66.1°C; $R_f$= 0.16 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3385.7 (OH), 1602.6 (Ar, C=C), 1364.0 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.36 (2H, d, J=8.8$H_Z$, Ph-**H**), 7.19 (2H, d, J=8.8$H_Z$, Ph-**H**), 4.84 (1H, d, J=5.1$H_Z$, **CH**OH), 3.07 (3H, s, **$CH_3$**CH), 2.49 (1H, s, **OH**), 1.41 (3H, q, J=2.4$H_Z$, 1.5, **$CH_3$**S); $\delta_C$ ($CDCl_3$): 148.29 (Ar-**C**OS), 145.39 (Ar-**C**COH), 127.17 (Ar-**C**), 122.09 (Ar-**C**), 69.59 (H**C**OH), 37.40 (COH**C**$H_3$), 25.44 (**C**$H_3$SO). GC: $t_R$=12.5min; LRMS (*M/Z*): 216 ($M^+$, 10%), 201 ($M^+$-$CH_3$, 100%), 173 ($M^+$-$C_2H_3O$, 15%), 137 ($M^+$-$CH_3O_2S$, 6%), 123 ($M^+$-$C_2H_6SO_2$, 67%); HRMS (ES): Found 216.0451, $C_9H_{12}O_4S$ requires 216.0456.

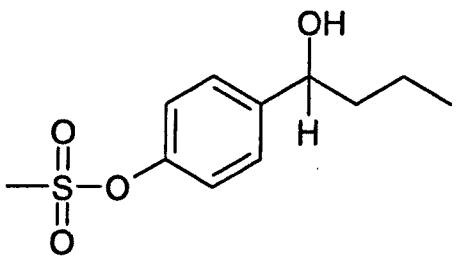
**The synthesis of methanesulfonic acid 4-(1-hydroxy-propyl)-phenyl ester (189):**




Compound **(189)** was synthesised in similar manner to **(187)** except that **(160)** (0.6g, 2.8mmol) was reacted with $NaBH_4$ (0.1g, 2.6mmol). The crude product was purified via flash column chromatography to give **(189)** as a white solid (0.5g, 84% yield); [m.p. 65.2-66.5°C; $R_f$ = 0.2 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3391.5 (OH), 1602.7 (Ar, C=C), 1364.8 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.37 (2H, d, J=8.6$H_Z$, Ph-**H**), 7.23 (2H, d, J=8.6$H_Z$, Ph-**H**), 4.59 (1H, t. J=5.3$H_Z$, **CH**OH), 3.11 (3H, s, $CH_3$S), 2.22 (1H, s, **OH**), 1.71 (2H, m. $CH_2CH_3$), 0.90 (3H, t, J=7.3$H_Z$, 7.5$H_Z$, $CH_2$**$CH_3$**). $\delta_C$ ($CDCl_3$): 148.41 (Ar-**C**OS), 144.19 (Ar-**C**COH), 127.73, 122.05 (Ar-**C**), 76.91 (H**C**OH), 37.45 ($CH_2$**$CH_3$**), 32.17 (**$CH_2$**$CH_3$), 10.18 (**$CH_3$**SO). GC: $t_R$=11.4min; LRMS (*M/Z*): 230 ($M^+$, 2%), 212 ($M^+$-$H_2O$, 4%), 201 ($M^+$-$C_2H_5$, 100%), 123 ($M^+$-$C_3H_7O_2S$, 55%); HRMS (ES): Found 230.0607 $C_{10}H_{14}O_4S$ requires 230.0613; Elemental analysis: Found C, 52.16%; H, 6.13%; $C_{10}H_{14}O_4S$ requires C, 52.18%; H, 6.10%.

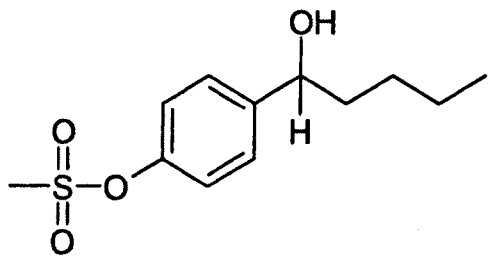
**The synthesis of methanesulfonic acid 4-(1-hydroxy-butyl)-phenyl ester (190):**




Compound (**190**) was synthesised in similar manner to (**187**) except that (**161**) (0.9g, 3.9mmol) was reacted with $NaBH_4$ (0.2g, 5.3mmol). The crude product was purified via flash column chromatography to give (**190**) as a white solid (0.8g, 88% yield); [m.p. 51.6-53.2°C; $R_f$ = 0.3 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3401.9 (OH), 1602.8 (Ar, C=C), 1365.4 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.38 (2H, d, J=8.4Hz, ph-**H**), 7.23 (2H, d, J=8.4Hz, ph-**H**), 4.68 (1H, m, **CH**OH), 3.11 (3H, s, **CH**$_3$S), 2.04 (1H, s, OH), 1.70 (2H, m, HCOHC**H**$_2$), 1.36 (2H, m, C**H**$_2$CH$_3$), 0.92 (3H, t, J=7.3Hz, CH$_2$C**H**$_3$); $\delta_C$ ($CDCl_3$): 148.45 (Ar-**C**OS), 144.54 (Ar-**C**COH), 127.68, 122.11 (Ar-**C**), 73.74 (**C**HOH), 41.54 (CHOH**C**H$_2$), 37.49 (**C**H$_3$S), 19.09 (**C**H$_2$CH$_3$), 14.08 (CH$_2$**C**H$_3$); $t_R$=13.4min; LRMS (*M/Z*): 244 ($M^+$, 2%), 226 ($M^+$-$H_2O$, 59%), 201 ($M^+$-$C_3H_7$, 100%), 147 ($M^+$-$CH_5O_3S$, 76%), 123 ($M^+$-$C_4H_9O_2S$, 44%); HRMS (ES): Found 244.0764, $C_{11}H_{16}O_4S$ requires 244.0769.

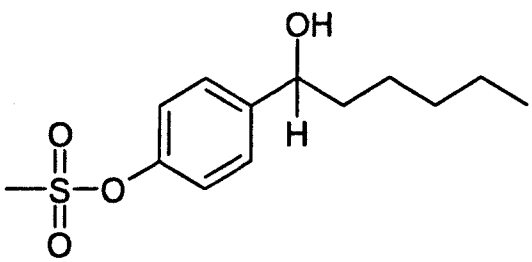
**The synthesis of methanesulfonic acid 4-(1-hydroxy-pentyl)-phenyl ester (191):**




Compound (**191**) was synthesised in similar manner to (**187**) except that (**162**) (0.9g, 3.7mmol) was reacted with $NaBH_4$ (0.2g, 5.3mmol). The crude product was purified via flash column chromatography to give (**191**) as a white solid (0.7g, 77% yield); [m.p. 48.5-49.9°C; $R_f$ = 0.3 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3387.2 (OH), 1602.4 (Ar, C=C), 1364.9 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.33 (2H, d, J=8.4Hz, Ph-**H**), 7.20 (2H, d, J=8.6Hz, Ph-**H**), 4.62 (1H, t, J=5.0Hz, **H**COH), 3.08 (3H, s, **CH**$_3$S), 2.31 (1H, s, **OH**), 1.66 (2H, m, HCOHC**H**$_2$), 1.28 (4H, m, **CH**$_2$), 0.85 (3H, t, J=6.9Hz, CH$_2$C**H**$_3$). $\delta_C$ ($CDCl_3$): 148.36 (Ar-**C**OS), 144.56 (Ar-**C**CHOH), 127.64 (Ar-**C**), 122.03 (Ar-**C**), 76.91 (H**C**OH), 39.06 (HCOH**C**H$_2$), 37.41 (**C**H$_3$CH$_2$), 27.95, 22.66 (**C**H$_2$), 14.12 (**C**H$_3$S). GC: $t_R$=14.8min. LRMS (*M/Z*): 258 ($M^+$, 1%), 240 ($M^+$-$H_2O$, 2%), 201 ($M^+$-$C_4H_9$, 100%), 131 ($M^+$-$C_3H_{11}O_3S$, 3%), 123 ($M^+$-$C_5H_{12}O_2S$, 43%); HRMS (ES): Found 258.0920, $C_{12}H_{18}O_4S$ requires 258.0926; Elemental analysis: Found C, 55.79%; H, 7.02%; $C_{12}H_{18}O_4S$ requires C, 56.01%; H, 7.10%.

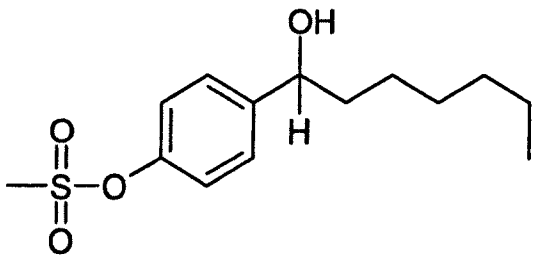
**The synthesis of methanesulfonic acid 4-(1-hydroxy-hexyl)-phenyl ester (192):**




Compound (**192**) was synthesised in similar manner to (**187**) except that (**163**) (1.0g, 3.9mmol) was reacted with $NaBH_4$ (0.2g, 5.3mmol). The crude product was purified via flash column chromatography to give (**192**) as a white solid (0.8g, 80% yield); [m.p. 44.5-44.9°C; $R_f$ = 0.4 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3393.3 (OH), 1602.4 (Ar, C=C), 1364.8 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.34 (2H, d, J=8.4$H_Z$, Ph-**H**), 7.21 (2H, d, J=8.4$H_Z$, Ph-**H**), 4.63 (1H, t, J=5.3$H_Z$, **H**COH), 3.08 (3H, s, $CH_3$S), 2.21 (1H, s, O**H**), 1.67 (2H, m, HCOHC**H**$_2$), 1.3 (6H, m, C**H**$_2$), 0.83 (3H, t, J=6.6$H_Z$, $CH_2$C**H**$_3$). $\delta_C$ ($CDCl_3$): 148.39 (Ar-**C**OS), 144.55 (Ar-**C**CHOH), 127.65 (Ar-**C**), 122.06 (Ar-**C**), 76.91(H**C**OH), 39.34 (HCOH**C**$H_2$), 37.43 ($CH_2$**C**$H_3$), 31.79, 25.52, 22.69 (**C**$H_2$), 14.18 (**C**$H_3$S). Gc: $t_R$=14.3min; LRMS (*M/Z*): 272 ($M^+$, 1%), 254 ($M^+$-$H_2O$, 3%), 201 ($M^+$-$C_5H_{11}$, 100%), 131 ($M^+$-$C_4H_{13}O_3S$, 5%), 123 ($M^+$-$C_6H_{13}O_2S$, 37%), HRMS (ES): Found 272.1077, $C_{13}H_{20}O_4S$ requires 272.1082; Elemental analysis: Found C, 57.33%; H, 7.40%; $C_{13}H_{20}O_4S$ requires C, 57.48%; H, 7.38%.

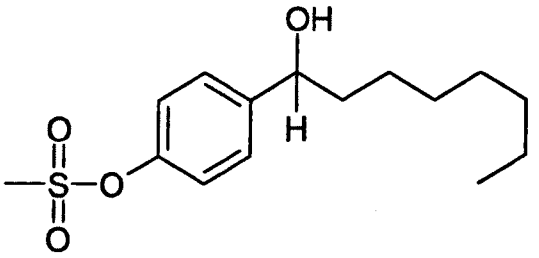
**The synthesis of methanesulfonic acid 4-(1-hydroxy-heptyl)-phenyl ester (193):**




Compound (**193**) was synthesised in similar manner to (**187**) except that (**164**) (1.1g, 4.1mmol) was reacted with $NaBH_4$ (0.2g, 5.3mmol). The crude product was purified via flash column chromatography to give (**193**) as a white solid (0.9g, 81% yield); [m.p. 34.1-35.7°C; $R_f$ = 0.4 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3395.8 (OH), 1602.5 (Ar, C=C), 1366.1 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.36 (2H, d, J=8.8$H_Z$, Ph-**H**), 7.22 (2H, d, J=8.8$H_Z$, Ph-**H**), 4.65 (1H, t, J=5.31$H_Z$, **H**COH), 3.10 (3H, s, **CH**$_3$SO), 2.03 (1H, s, **OH**), 1.68 (2H, m, HCOHC**H**$_2$), 1.29 (8H, m C**H**$_2$), 0.83 (3H, t, J=6.9$H_Z$, CH$_2$C**H**$_3$). $\delta_C$ ($CDCl_3$): 148.45 (Ar-**C**OS), 144.55 (Ar-**C**CHOH), 127.68 (Ar-**C**), 122.11 (Ar-**C**), 74.02 (H**C**OH), 39.44 (HOCH**C**H$_2$), 37.49 (**C**H$_2$CH$_3$), 31.90, 29.31, 25.85, 22.76 (**C**H$_2$), 14.24 (**C**H$_3$S); Gc: $t_R$=16.7min; LRMS (*M/Z*): 286 ($M^+$, 1%), 268 ($M^+$-$H_2O$, 2%), 201 ($M^+$-$C_6H_{13}$, 100%), 131 ($M^+$-$C_5H_{15}O_3S$, 4%), 123 ($M^+$-$C_7H_{15}O_2S$, 33%); HRMS (ES): Found 309.1131, $C_{14}H_{22}O_4NaS$ requires 309.1137.

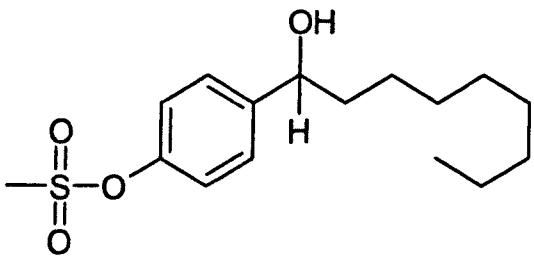
**The synthesis of methanesulfonic acid 4-(1-hydroxy-octyl)-phenyl ester (194):**




Compound **(194)** was synthesised in similar manner to **(187)** except that **(165)** (0.9g, 3.2mmol) was reacted with $NaBH_4$ (0.2g, 5.3mmol). The crude product was purified via flash column chromatography to give **(194)** as a white solid (0.7g, 77% yield); [m.p. 36.9-38.3°C; $R_f$ = 0.44 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3397.0 (OH), 1602.9 (Ar, C=C), 1366.7 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.39 (2H, d, J=8.42$H_Z$, Ph-**H**), 7.25 (2H, d, J=8.4$H_Z$, Ph-**H**), 4.68 (1H, t, J=5.5$H_Z$, **H**COH), 3.13 (3H, s, $CH_3$SO), 1.98 (1H, s, **OH**), 1.68 (2H, m, HCOHC**H**$_2$), 1.33 (10H, m, C**H**$_2$), 0.87 (3H, t, J=7.1$H_Z$, $CH_2$C**H**$_3$). $\delta_C$ ($CDCl_3$): 148.49 (Ar-**C**OS), 144.56 (Ar-**C**CHOH), 127.70 (Ar-**C**), 122.14 (Ar-**C**), 74.07 (H**C**OH), 39.47 (HCOH**C**H$_2$), 37.52 (**C**H$_3$CH$_2$), 31.98, 29.63, 29.38, 25.92, 22.82 (**C**H$_2$), 14.29 (**C**H$_3$S). GC: $t_R$=17.6min; LRMS (*M/Z*): 300 ($M^+$, .4%), 282 ($M^+$-$H_2O$, 2%), 201 ($M^+$-$C_7H_{15}$), 131 ($M^+$-$C_6H_{17}O_3S$, 4%), 123 ($M^+$-$C_8H_{17}O_2S$, 28%); HRMS (ES): Found 300.1395 $C_{15}H_{24}O_4S$ requires 300.1395.

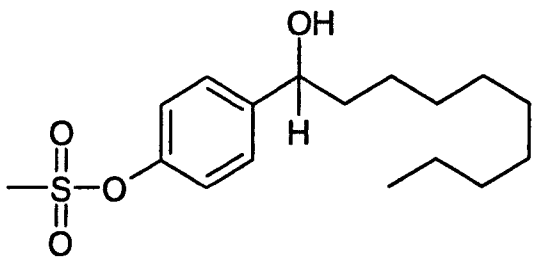
**The synthesis of methanesulfonic acid 4-(1-hydroxy-nonyl)-phenyl ester (195):**




Compound **(195)** was synthesised in similar manner to **(187)** except that **(166)** (1.0g, 3.4mmol) was reacted with $NaBH_4$ (0.2g, 5.3mmol). The crude product was purified via flash column chromatography to give **(195)** as a white solid (0.8g, 80% yield); [m.p. 45.6-47.1°C; $R_f$= 0.46 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3386.8 (OH), 1602.7 (Ar, C=C), 1366.5 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.39 (2H, d, J=8.6$H_Z$, Ph-**H**), 7.25 (2H, d, J=8.6$H_Z$, Ph-**H**), 4.68 (1H, t, J=5.3$H_Z$, 5.13, **H**COH), 3.13 (3H, s, $CH_3$S), 1.97 (1H, s, O**H**), 1.71 (2H, m, HCOH$CH_2$), 1.33 (12H, m, $CH_2$), 0.86 (3H, t, J=6.6$H_Z$, CH2$CH_3$). $\delta_C$ ($CDCl_3$): 148.49 (Ar-**C**OS), 144.55 (Ar-**C**CHOH), 127.70 (Ar-**C**), 122.14 (Ar-**C**), 74.07 (H**C**OH), 39.47 (HCOH$CH_2$), 37.52 ($CH_2CH_3$), 32.03, 29.67, 29.43, 25.92, 22.84 ($CH_2$), 14.30 ($CH_3$S). GC: $t_R$=18.5min. LRMS (*M/Z*): 314 ($M^+$, 1%), 296 ($M^+$-$H_2O$, 2%), 201 ($M^+$-$C_8H_{17}$, 100%), 131 ($M^+$-$C_7H_{19}O_3S$, 4%), 123 ($M^+$-$C_9H_{19}O_2S$, 24%); HRMS (ES): Found 314.1552 $C_{16}H_{26}O_4S$ requires 314.1552.

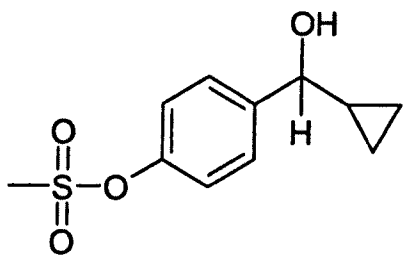
**The synthesis of methanesulfonic acid 4-(1-hydroxy-decyl)-phenyl ester (196):**




Compound **(196)** was synthesised in similar manner to **(187)** except that **(167)** (0.9g, 2.9mmol) was reacted with $NaBH_4$ (0.2g, 5.3mmol). The crude product was purified via flash column chromatography to give **(196)** as a white solid (0.7g, 77% yield); [m.p. 51.1-52.6°C; $R_f$ = 0.42 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3402.8 (OH), 1602.9 (Ar, C=C), 1367.0 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.35 (2H, d, J=8.6$H_Z$, Ph-**H**), 7.21 (2H, d, J=8.6$H_Z$, Ph-**H**), 4.64 (1H, t, J=6.4$H_Z$, 6.6$H_Z$, **H**COH), 3.09 (3H, s, $CH_3$S), 2.04 (1H, s, **OH**), 1.67 (2H, m, HCOHC$H_2$), 1.30 (14H, m, C$H_2$), 0.84 (3H, t, J=6.8$H_Z$, $CH_2CH_3$). $\delta_C$ ($CDCl_3$): 148.45 (Ar-**C**OS), 144.56 (Ar-**C**CHOH), 127.68 (Ar-**C**), 122.10 (Ar-**C**), 74.02 (H**C**OH), 39.44 (HCOH**C**$H_2$), 37.48 ($CH_2$**C**$H_3$), 32.05, 29.71, 29.65, 29.67, 25.90, 22.85 (**C**$H_2$), 14.30 (**C**$H_3$S). GC: 17.7min. LRMS (*M/Z*): 328 ($M^+$, 0.4%), 310 ($M^+$-$H_2O$, 1%), 201 ($M^+$-$C_9H_{19}$, 100%), 131 ($M^+$-$C_8H_{21}O_3S$, 4%), 123 ($M^+$-$C_{10}H_{21}O_2S$, 23%); HRMS (ES): Found 328.1703, $C_{17}H_{28}O_4S$ requires 328.1708.

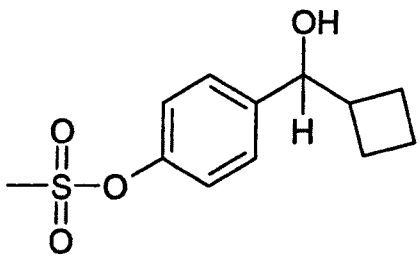
**The synthesis of methanesulfonic acid 4-(cyclopropyl-hydroxy-methyl)-phenyl ester (197):**




Compound **(197)** was synthesised in similar manner to **(187)** except that **(168)** (1.0g, 4.4mmol) was reacted with $NaBH_4$ (0.2g, 5.3mmol). The crude product was purified via flash column chromatography to give **(197)** as a white solid (0.8g, 80% yield); [m.p. 81.1-82.5°C; $R_f$= 0.21 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3384.1 (OH), 1602.5 (Ar, C=C), 1363.7 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.47 (2H, d, J=8.1$H_Z$, Ph-**H**), 7.25 (2H, d, J=8.1$H_Z$, Ph-**H**), 3.99 (1H, d, J=8.6$H_Z$, **CH**OH), 3.12 (3H, s, **CH**$_3$), 2.01 (1H, s, **OH**), 1.16 (1H, m, **CH**CHOH), 0.50 (4H, m, **CH**$_2$); $\delta_C$ ($CDCl_3$): 148.62 (Ar-**C**OS), 143.40 (Ar-**C**CHOH), 127.84, 122.09 (Ar-**C**), 78.03 (**C**HOH), 37.51 (**C**H$_3$), 19.64 (**C**HCHOH), 3.84, 3.30 (**C**H$_2$); LRMS (*M/Z*): 242 ($M^+$, 2%), 201 ($M^+$-$C_3H_5$ 100%), 123($M^+$-$C_4H_7O_2S$, 62%); HRMS (ES): Found 242.0607, $C_{11}H_{14}O_4S$ requires 242.0612.

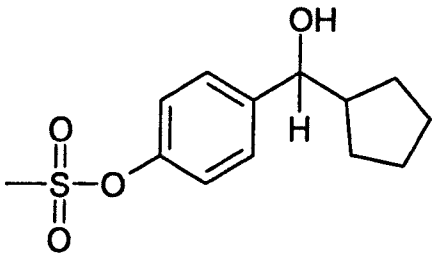
**The synthesis of methanesulfonic acid 4-(cyclobutyl-hydroxy-methyl)-phenyl ester (198):**




Compound **(198)** was synthesised in similar manner to **(187)** except that **(169)** (0.9g, 3.5mmol) was reacted with $NaBH_4$ (0.2g, 5.3mmol). The crude product was purified via flash column chromatography to give **(198)** as a white solid (0.7g, 77% yield); [m.p. 82.6-84.1°C; $R_f$ = 0.27 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3391.7 (OH), 1602.3 (Ar, C=C), 1364.7 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.34 (2H, d, J=8.6$H_Z$, ph-**H**), 7.21 (2H, d, J=8.6$H_Z$, ph-**H**), 4.57 (1H, d, J=8.2$H_Z$, **CH**OH), 3.10 (3H, s, **$CH_3$**), 2.56 (1H, six, J=8.1$H_Z$, 7.7$H_Z$, 7.9$H_Z$, **CH**CHOH), 1.90 (7H, m **$CH_2$**, **OH**); $\delta_C$ ($CDCl_3$): 148.55 (Ar-**C**OS), 142.70 (Ar-**C**CHOH), 127.94, 122.03 (Ar-**C**), 77.69 (**C**HOH), 42.62 (**C**HCHOH), 37.50 (**$CH_3$**), 24.77, 24.50, 17.86 (**$CH_2$**); GC: $t_R$=10.7min; LRMS (*M/Z*): 256 ($M^+$, 2%), 201($M^+$-$C_4H_7$, 100%), 123 ($M^+$-$C_5H_9O_2S$, 61%); HRMS (ES): Found 256.0764, $C_{12}H_{16}O_4S$ requires 256.0769.

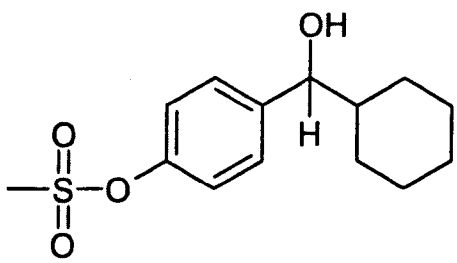
**The synthesis of methanesulfonic acid 4-(cyclopentyl-hydroxy-methyl)-phenyl ester (199):**




Compound **(199)** was synthesised in similar manner to **(187)** except that **(170)** (0.9g, 3.4mmol) was reacted with $NaBH_4$ (0.2g, 5.3mmol). The crude product was purified via flash column chromatography to give **(199)** as a white solid (0.7g, 77% yield); [m.p. 88.1-88.9°C; $R_f$ = 0.31 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3402.3 (OH), 1602.3 (Ar, C=C), 1365.7 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.37 (2H, d, J=8.6Hz, ph-**H**), 7.23 (2H, d, J=8.6Hz, ph-**H**), 4.42 (1H, d, J=8.0Hz, **C**HOH), 3.11 (3H, s, **C**$H_3$), 2.15 (1H, six, J=8.2Hz, 5.1Hz, 8.6Hz, **C**HCHOH), 1.94 (1H, s, **O**H), 1.54 (8H, m, **C**$H_2$); $\delta_C$ ($CDCl_3$): 148.55 (Ar-**C**OS), 144.02 (Ar-**C**CHOH), 128.26, 122.04 (Ar-**C**), 78.38 (**C**HOH), 47.97 (**C**HCHOH), 37.52 (**C**$H_3$), 29.61, 29.44, 25.65, 25.58 (**C**$H_2$); GC: $t_R$ =15.1min; LRMS (*M/Z*): 270 ($M^+$, 0.4%), 201 ($M^+$-$C_5H_9$, 100%), 123 ($M^+$-$C_6H_{11}O_2S$, 59%); HRMS (ES): Found 270.0920, $C_{13}H_{18}O_4S$ requires 270.0925.

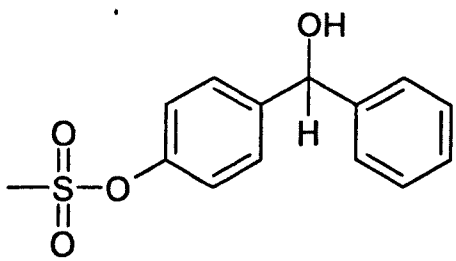
**The synthesis of methanesulfonic acid 4-(cyclohexyl-hydroxy-methyl)-phenyl ester (200):**




Compound (**200**) was synthesised in similar manner to (**187**) except that (**171**) (0.4g, 1.4mmol) was reacted with $NaBH_4$ (0.1g, 2.6mmol). The crude product was purified via flash column chromatography to give (**200**) as a white solid (0.3g, 75% yield); [m.p. 88.5-89.7°C; $R_f$ = 0.35 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3417.6 (OH), 1602.2 (Ar, C=C), 1366.0 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.33 (2H, d, J=8.6Hz, Ph-**H**), 7.23 (2H, d, J=8.6Hz, Ph-**H**), 4.39 (1H, d, J=6.9Hz, **CH**OH), 3.12 (3H, s, **CH**$_3$), 1.91 (2H, m, **CH**$_2$), 1.62 (4H, m, **CH**$_2$), 1.35 (1H, m, **OH**), 1.06 (5H, m, **CH**, **CH**$_2$); $\delta_C$ ($CDCl_3$): 148.49 (Ar-**C**OS), 143.18 (Ar-**C**CHOH), 128.40, 121.90 (Ar-**C**), 78.72 (**C**HOH), 45.22 (**C**HCHOH), 37.54 (**C**H$_3$), 29.45, 28.70, 26.53, 26.23, 26.13 (**C**H$_2$); GC: $t_R$ =16.6min; LRMS (*M/Z*): 284 ($M^+$, 0.5%), 201 ($M^+$-$C_6H_{11}$, 100%), 123 ($M^+$-$C_7H_{13}O_2S$, 54%); HRMS (ES): Found 284.1077, $C_{14}H_{20}O_4S$ requires 284.1082.

**The synthesis of methanesulfonic acid 4-(hydroxy-phenyl-methyl)-phenyl ester (201):**




Compound **(201)** was synthesised in similar manner to **(187)** except that **(172)** (1.0g, 3.6mmol) was reacted with $NaBH_4$ (0.2g, 5.3 mmol). The crude product was purified via flash column chromatography to give **(201)** as a white solid (0.8g, 80% yield); [m.p. 73.1-74.2°C; $R_f$ = 0.33 diethyl ether/petroleum ether 40-60°C (70:30)].

$\nu_{(max)}$ (Film) $cm^{-1}$: 3410.4 (OH), 1602.5 (Ar, C=C), 1364.5 ($O_3S$-$CH_3$); $\delta_H$ ($CDCl_3$): 7.40 (2H, d, J=8.6$H_Z$, ph-**H**), 7.33 (4H, d, J=4.4$H_Z$, ph-**H**), 7.28 (1H, t, J=4.4$H_Z$, ph-**H**), 7.21 (2H, d, J=8.6$H_Z$, ph-**H**), 5.80 (1H, s, **OH**), 3.07 (3H, s, $CH_3$), 2.50 (1H, s, **CH**OH); $\delta_C$ ($CDCl_3$): 148.51 (Ar-**C**O), 143.46, 143.30 (Ar-**C**CHOH), 128.86, 128.30, 128.11, 126.71, 122.16 (Ar-**C**), 75.66 (**C**HOH), 37.46 (**C**$H_3$); $t_R$=11.9min; LRMS (*M/Z*): 278 ($M^+$, 13%), 199 ($M^+$-$CH_3SO_2$, 26%), 182 ($M^+$-$CH_4SO_3$, 5%), 105 ($M^+$-$C_7H_9SO_3$, 100%); HRMS (ES): Found 278.0613, $C_{14}H_{14}O_4S$ requires 278.0612.

print

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