Synthesis and biochemical evaluation of novel non-steroidal inhibitors of the cytochrome P450 enzyme 17α-hydroxylase/17,20-lyase in the treatment of hormone-dependent prostate cancer

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BY

IMRAN SHAHID

DEPARTMENT OF PHARMACY
SCHOOL OF CHEMICAL AND PHARMACEUTICAL SCIENCES
PENRHYN ROAD
KINGSTON-UPON-THAMES
SURREY
KT1 2EE

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ABSTRACT

A high proportion of prostate cancers have been shown to be androgen-dependent. The biosynthesis of the androgens is catalysed by the cytochrome P450 enzyme 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$), which is responsible for the conversion of C_{21} steroids (for example pregnenolone and progesterone) to the androgens (for example dehydroepiandrosterone and androstenedione respectively). The inhibition of this enzyme would therefore lead to the overall reduction in the level of androgens and thus result in an overall decrease in the stimulation of androgen-dependent cancer cells.

The compounds synthesised within the current study were designed such that the compounds were able to donate a lone pair of electrons to the Fe atom within the haem group of the active site of $P450_{17\alpha}$. As such, compounds based on benzyl imidazole backbone were synthesised as the major range of compounds with a small number of phenyl alkyl imidazole based compounds synthesised in an effort to evaluate physicochemical factors such as hydrophobicity.

In general, the results of the study show that of the benzyl imidazole-based compounds were weak inhibitors of $P450_{17\alpha}$ in comparison to the standard compound, namely ketoconazole (**3**) (IC_{50}=1.66±0.15μM against 17,20-lyase and IC_{50}=3.76±0.01μM against 17α-hydroxylase). The most potent benzyl imidazole-based compounds synthesised were: 4-iodobenzyl imidazole (**224**) (IC_{50}=1.58±0.17μM against 17,20-lyase and IC_{50}=10.06±0.96μM against 17α-OHase); 1-(3,4-dichloro-benzyl)-1*H*-imidazole (**215**) (IC_{50}=2.07±0.07μM against 17,20-lyase and IC_{50}=12.22±0.88μM against 17α-hydroxylase); 1-(3,5-dichloro-benzyl)-1*H*-imidazole (**216**) (IC_{50}=3.34±0.11μM against 17,20-lyase and IC_{50}=22.56±0.34μM against 17α-hydroxylase); 1-(3,5-dibromo-benzyl)-1*H*-imidazole (**221**) (IC_{50}=3.16±0.11μM against 17,20-lyase and IC_{50}=25.95±0.91μM against 17α-hydroxylase). The phenyl alkyl imidazole based compounds were found to be more potent than the benzyl imidazole-based compounds and **3** and included: phenylheptyl imidazole (**318**) (IC_{50}=0.10±0.02μM against 17,20-lyase and IC_{50}=0.32±0.05μM against 17α-hydroxylase); phenyloctyl imidazole (**321**) (IC_{50}=0.21±0.02μM against 17,20-lyase and IC_{50}=0.25±0.01μM against 17α-hydroxylase); and phenylnonyl imidazole (**324**) (IC_{50}=0.35±0.01μM against 17,20-lyase and IC_{50}=1.06±0.03μM against 17α-hydroxylase).

Consideration of the structure-activity relationship determination and the molecular modeling of the synthesised compounds using the substrate-haem complex (SHC) approach shows that the disubstituted derivatives of benzyl imidazole were able to utilise both hydrogen bonding groups which are presumed to exist at the active site of $P450_{17\alpha}$. These compounds were found to be considerably more potent than the mono-substituted derivatives, as such, it suggests that the increase in the number of interactions between the inhibitor and the enzyme is the key feature which results in the increase in potency. The inhibitory data obtained for the phenyl alkyl imidazole-based compounds show that hydrophobicity (logP) of the inhibitor plays a major role in determining the overall inhibitory activity of these compounds. As such, the study suggests that in the design of further novel inhibitors of this enzyme, the interaction with the active site and logP are two factors which would allow for the synthesis of highly potent inhibitors of this enzyme.

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ABBREVIATIONS

PC	Prostate cancer
LHRH	Leuteinising hormone releasing hormone
LH	Leuteinising hormone
AR	Androgen receptor
DHEA	Dehydroepiandosterone
AD	Androstenedione
ATP	Adenosine triphosphate
DNA	Deoxyribose nucleic acid
P450	Family of enzymes absorbing UV light at 450nm [forms a pink (**P** in P450) coloured compound (also called cytochrome P450s)]
nm	Nano meters
UV	Ultra violet light
β	Beta
HSD	Hydroxysteroid dehydrogenase
α	Alpha
$P450_{17\alpha}$	17α-hydroxylase/17,20-lyase
17α-OHase	17α-hydroxylase component of enzyme $P450_{17\alpha}$
17,20-lyase	17,20-lyase component of enzyme $P450_{17\alpha}$
Δ	Delta
Å	Angstrom
KTZ	Ketoconazole
K_i	Constant of inhibition
nM	Nano molar
S	*Sinister* (anticlockwise configuration of groups in an enantiomer)
R	*Rectus* (clockwise configuration of groups in an enantiomer)
IC_{50}	Inhibitory concentration (causing 50% enzyme inhibition)
μM	Micro molars

TS*	Transition state
NI	No inihibition
ND	Not determined
[I]	Inhibitor concentration
mg	Milligram
Z	*Zusammen* [Refers to *cis*-isomer (i.e. *together*)]
E	*Entgegen* [Refers to *trans*-isomer (i.e. *opposite*)]
Arg	Arginine (amino acid residue)
His	Histidine (amino acid residue)
Lys	Lysine (amino acid residue)
Asn	Asparagine (amino acid residue)
Ala	Alanine (amino acid residue)
Met	Methionine (amino acid residue)
Ile	Isoleucine (amino acid residue)
Pro	Proline (amino acid residue)
Phe	Phenylalanine (amino acid residue)
SHC	Substrate-haem complex
pKa	Negative logarithm of acid dissociation constant
K_2CO_3	Potasium carbonate
THF	Tetrahydrofuran
$LiAlH_4$	Lithium aluminium hydride
PBr_3	Phosphorus tribromide
DCM	Dichlomethane
TEA	Triethylamine
LogP	logarithm of the partition coefficient
Bu^tOK	Potasium tertiarybutoxide
HCl	Hydrochloric acid
mmol	Millimole
g	Grams
mL	Millilitres
h	Hour

min	Minutes
M	Molar (moles/litre)
$NaHCO_3$	Sodium bicarbonate
Na_2CO_3	Sodium carbonate
$MgSO_4$	Magnesium sulfate
m.p.	Melting point (**lit. m.p.**: literature m.p.)
°C	Degree centigrade
$\nu_{(max)}$	Maximum frequency (infra-red radiation)
Cm^{-1}	Inverse if centimeter (infra-red radiation)
δ_H	^1H FT-NMR
δ_C	^{13}C FT-NMR
FT-NMR	Fourier Transform Nuclear Magnetic Resonance spectrometry
IR	Infra-red spectrometry
MHz	Mega hertz
$CDCl_3$	Deuturated chloroform
Ar	Aromatic
Ph	Phenyl
Im	Imidazole
J	Coupling constant
s	singlet
d	doublet
dd	doublet of doublets
t	triplet
q	quartet
m	multiplet
GC	Gas chromatography
t_R	Retention time
LRMS	Low resolution mass spectrum
EI	Electron ionisation (impact)
m/z	Mass to charge ratio

M^+	Molecular ion
HRMS	High resolution mass spectrum
CD_3OH	Deuturated Methanol
DMSO	Dimethylsulfoxide
NADPH	Nicotineadenine dinucleotide hydrogen phosphate
pH	Hydrogen ion concentration
µL	Micro litres
TLC	Thin layer chromatography
V	Velocity (rate of conversion)

CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

1.1 Cancer

Cancer is a disorder of cell growth that results in invasion and destruction of the healthy tissue by abnormal cells (Rang et al, 2003). A tumour or neoplasm (i.e. new growth) is considered as the initial stage of cancer, and involves the uncontrolled proliferation and multiplication of cells forming discrete lumps. Tumours can be divided into two types, with the first type being benign tumours. Here, the cell growth is localised within a specific tissue, and the rate of uncontrolled proliferation of abnormal cells can be faster or slower than normal cells (Rang et al, 2003). The second type of tumours are malignant tumours, which arise from cells which have lost the cell-specific functions and possess the ability to invade other tissues and travel to distant sites (i.e. metastasise).

Cancer appears to follow a specific route of development involving, in general, three main stages:

(1) *Loss of cell specific function*: one of the main characteristics of tumour cells is the loss of the capability to differentiate.

(2) *Invasiveness*: this is the stage when the tumour cells are able to invade their neighbouring tissues.

(3) *Metastasis*: this results in the formation of secondary tumours as primary tumour cells invade other organs and spread throughout the body via the blood and/or the lymphatic systems, or due to being shed into body cavities. As a result, tumour cells can spread throughout the body, thus reducing the survival rates of such patients.

1.2 Prostate Cancer

Prostate cancer is the second leading cause of cancer deaths in men in North America. It has been reported that about 220,000 new cases were diagnosed in 2007 in the USA, of whom, approximately 27,000 would die from the disease (Jemal et al, 2007). In Europe, however, prostate cancer is the third most common form of cancer deaths, after lung and colorectal cancer (Ferlay et al, 2007). The peripheral zone in the prostate gland has been reported to be mainly affected in prostate cancer (Figure 1) (De Marzo et al, 2007).

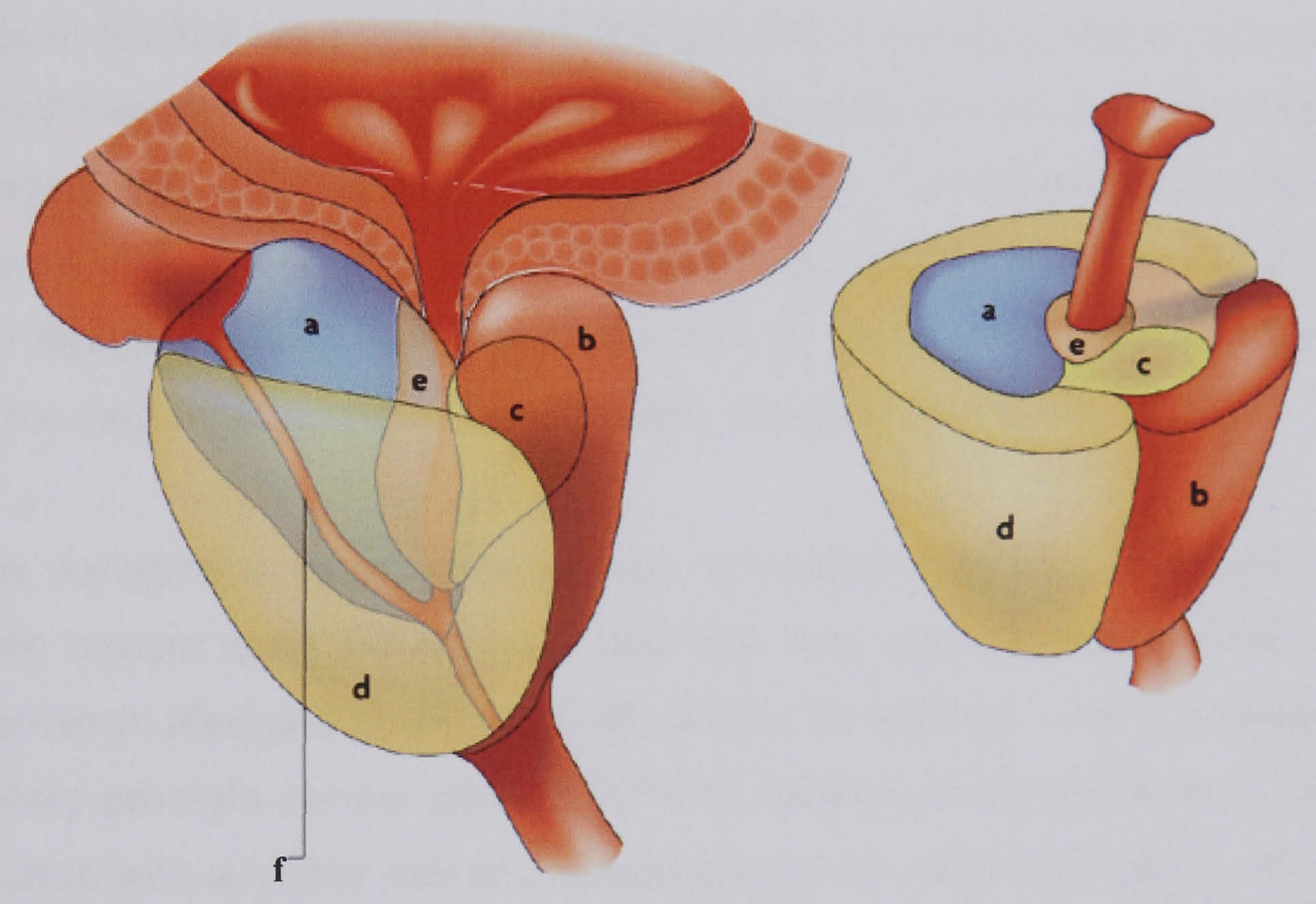


Figure 1. The different zones of the prostate gland (adapted from De Marzo et al, 2007) (a=central zone; b=fibromuscular zone; c=transitional zone; d=peripheral zone; e=periuretheral gland region; f=ejaculatory duct).

The aetiology of prostate cancer is as yet unclear, however, several factors have been postulated to increase the risk of developing the disease, for example

factors such as environment, age, diet, race and genetic factors have all been implicated (Colli and Amling, 2008).

For example, studies of migrant populations, and in particular of Asians in the USA, have shown that risk of prostate cancer is associated with prolonged exposure to the Western lifestyle. The incidence of prostate cancer in countries such as Japan and China is low, at 8·51 and 1·08 per 100,000 people, respectively, however, incidence rates for Asians who have migrated to the USA, in general, have shown to be as high as 95·1 per 100,000 people (Parkin et al, 1999).

Results of studies have also shown that the risk of prostate cancer increases with age (Franceschi and Vecchia, 2001). That is, prostate cancer is not common in men aged less than 50 years of age, however, the incidence of the disease increases with advancing age. It is proposed that the probability of prostate cancer developing in a man aged less than 40 years is 1 in 10,000 whereas it is 1 in 8 for men aged between 60–79 years (Swallow and Kirby, 2006).

African Americans have been shown to possess higher incidence rates of prostate cancer than Caucasians and this has been linked to both diet and differences in lifestyles (Colli and Colli, 2006). In addition, over-expression of the hereditary prostate cancer gene 1 (HPC1), located at chromosome 1, has been associated with a higher risk of prostate cancer in some families (Xu et al, 2001) (Table 1). Men with breast cancer gene (BRCA1) mutations have an estimated 3·33 higher risk of developing prostate cancer than those lacking mutations in this gene (Deutsch et al, 2004) (Table 1).

Several studies have shown that the risk of prostate cancer reduces significantly when men reduce their fat intake (Kolonel et al, 2000; Terry et al, 2001). In addition, diets low in selenium, vitamin E and lycopene have shown to increase

the risk of prostate cancer (Giovannucci et al, 2002; Wilkinson et al, 2003) (Table 1).

Risk Factor	Source/ origin	Role	Reference
Selenium (low levels in diet)	Fish, cereals	Apoptosis	Wilkinson & Chodak, 2003
Vitamin E (low levels in diet)	Lettuce	Antiproliferative	Wilkinson & Chodak, 2003
Zinc (low levels in diet)	Meat	Apoptosis	Ekane et al, 2001
Vitamin D (low levels in diet)	Vegetables UV	Antiproliferative	Chen and Holick, 2003
Lycopene (low levels in diet)	Tomatoes	Free radical scavenger	Giovannucci et al, 2002
Cruciferous (low levels in diet)	High Fat food	Cell cycle inhibition	Kolonel et al, 2000
HPC1 gene (activated in PC)	Chromosome 1	Anti-apoptotic	Deutsch et al, 2004
BRCA 1 gene (in-activated in PC)	Chromosome 17	Pro-apoptotic	Deutsch et al, 2004

Table 1. Dietary and genetic factors involved in prostate cancer (PC) aeitology.

1.3 Hormonal Control of Prostate Cancer

Androgens are vital for the growth and maintenance of cells of the prostate, however, they are also postulated to play a significant role in the initiation, development and progression of hormone-dependent prostate diseases. In particular, studies have shown that prolonged exposure to androgens is associated with a high risk of prostate cancer (Gann et al, 1996). Production of

androgens involves the release of leuteinising hormone releasing hormone (LHRH) from the hypothalamus which in turn stimulates the pituitary to release luteinising hormone (LH). LH travels via the vascular system and binds to specific receptors on the leydig cells in the testis, stimulating the production of testosterone (Konety et al, 2001). Testosterone binds to plasma albumin or sex hormone binding globulin (SHBG) and therefore can be transported throughout the body (Gann et al, 1996). It enters the prostate cells via passive diffusion, where it has been shown to initiate prostate cell/tumour proliferation, either directly or by being converted into its more active form, namely dihydrotestosterone (Klotz et al, 2007).

Cell proliferation and therefore tumour stimulation, is initiated through a series of complex interactions and involves interaction between the hormone responsive elements (i.e. specific binding sites on the DNA) and the complex which has resulted from the binding of the androgen (e.g. testosterone or dihydrotestosterone) with the androgen receptor (AR) (Haelens et al, 2001). The binding initiates transcription, leading to protein synthesis and subsequently to cell division, which can potentially result in an increase in the tumour mass. If untreated, this tumour can metastasise, resulting in the formation of secondary tumours in other parts of the body.

1.4 Treatments of Prostate Cancer

There are various methods employed for the treatment of prostate cancer which can be divided into surgical, radiation and hormonal based therapies; these will be discussed below.

1.4.1 Radical Prostatectomy

Radical prostatectomy is the surgical removal of the entire prostate gland and is one of the most common treatments for organ-confined prostate cancer.

Employing this procedure effectively removes the early stage tumour in most of the cases, however, urinary incontinence and impotence are the major drawbacks of this method (Remzi et al, 2005).

1.4.2 Radiation Therapy

Radiation therapy is also used in the treatment of early stage prostate cancer; it involves either targeting the cancerous area with external beams, or the implantation of a radioactive element (for example isotopic iodine, ^{127}I) in the prostate gland, either temporarily or permanently (known as brachytherapy). Although it is a better alternative for patients than radical prostatectomy, it can lead to non-targeted areas such as the bladder or rectum being affected by the radiation (Renaud et al, 2005).

1.4.3 Hormonal Therapy

Hormonal therapy is designed to reduce the production of the male hormones, or to prevent the androgenic hormones eliciting their effect. Commonly used methods employ LHRH analogues, which can either be agonists or antagonists (Isbarn et al, 2009). LHRH agonists, for example goserelin, bind to the same site on the LHRH receptor as the naturally occurring LHRH, initially enhancing the production of LH. This leads to eventual downregulation of LHRH receptors in the pituitary, leading to a reduction in LH release. LHRH antagonists, for example cetrorelix, act by turning off the LHRH receptor (in the pituitary) and by persistently blocking it, thus causing an immediate suppression of LH (Debruyne et al, 2008). LHRH agonists and antagonists, both lead eventually to the inhibition of testosterone/dihydrotestosterone production (Isbarn et al, 2009).

Another receptor which can be targeted by drugs is the AR. Anti-androgens, namely flutamide (**1**) and bicalutamide (**2**) (Figure 2) act by selectively binding to the AR, preventing the androgenic hormones (testosterone and

dihydrotestosterone) from binding, and thus preventing the androgen-AR complex being formed (Wakabayashi et al, 2005).

One major advantage of anti-androgen therapy is that side-effects observed with LHRH therapy, such as hot flushes and osteoporosis are reduced, however, high-doses of anti-androgens used alone, are not effective enough to cause the maximum (desired) reduction of the circulating androgens, therefore they are used in combination with LHRH based drugs (also known as combined androgen therapy). Common side-effects of anti-androgen therapy include breast enlargement and impotency (Tyrrell et al, 1998).

Flutamide (**1**) Bicalutamide (**2**)

Figure 2. Two anti-androgens available in the clinic for the treatment of prostate cancer.

1.4.4 Deprivation of Dihydrotestosterone and Testosterone in the Treatment of Prostate Cancer

Dihydrotestosterone is produced as a result of the reduction of testosterone in the cytoplasm of the prostate cell and is the principal stimulant of the tumour in hormone-dependent prostate cancer. It has been postulated that reducing the production of androgens by inhibiting enzymes in the steroidal cascade may lead to the treatment of prostate cancer (Festuccia et al, 2008). There are no such enzyme inhibitiors currently on the market for prostate cancer therapy, however, there is a lot of research being undertaken in this area and a number of

compounds have been produced which are in clinical trials, and these will be discussed in section 1.10.

The primary androgenic precursor of testosterone and dihydrotestosterone is androstenedione (AD), which is synthesised from cholesterol in a series of reactions known as the steroidal cascade. These steps of the cascade are governed by specific enzymes, most of which belong to the cytochrome P450 family of enzymes and these will be discussed in more detail below.

1.5 The Biosynthesis of Cholesterol and the Steroidal Cascade

All steroid hormones are derived from cholesterol and are produced primarily in tissues such as the adrenal cortex, the gonads and the placenta (in females) (Waterman and Keeney, 1996). The biosynthesis of cholesterol involves numerous steps (Figure 3), however, only a summary of these steps is mentioned here.

In summary, cholesterol synthesis is initiated when two molecules of acetyl-CoA react to form a dimer, which subsequently combines with another acetyl-CoA molecule forming a six carbon compound, 3-hydroxy-3-methyl glutaryl-CoA (HMG-CoA), which is reduced to give mevalonate (Figure 3). Three phosphate groups (via ATP) are added to mevalonate, which is subsequently converted to a 15 carbon intermediate, farnesyl pyrophosphate. Two molecules of farnesyl pyrophosphate join together with the elimination of the two pyrophosphate groups, resulting in the synthesis of squalene (a 30 carbon containing molecule). Squalene epoxidase catalyses the oxidation of squalene to form squalene-2,3-epoxide.

The double bonds of squalene-2,3-epoxide are positioned such that a concerted reaction leads to the cyclisation of squalene to give lanosterol, which is subsequently converted to cholesterol (Tacer et al, 2003).

Figure 3. A summary of the biosynthesis of cholesterol from acetyl co-A (not all steps shown; modified from Tacer et al, 2003).

Cholesterol is the primary precursor of three main families of steroid hormones, i.e. mineralocorticoids, glucocorticoids and sex steroids. These hormones are synthesised in a complex chain of reactions, called the steroidal cascade, whereby each step is catalysed by a specific enzyme (most of which belong to the cytochrome P450 family of enzymes).

All steroidogenic pathways begin in the mitochondrion with the conversion of cholesterol to pregnenolone by cholesterol side chain cleavage enzyme ($P450_{CSCC}$) (Hasler et al, 1999) (Figure 4). Pregnenolone enters the endoplasmic reticulum via passive diffusion, where it can act as the principal pregnane, leading to various metabolic pathways.

In the outer zone of the adrenal cortex, i.e. *zona glomerulosa* responsible for production of mineralocorticoids, pregnenolone is converted to progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD) which is then converted to 21-hydroxyprogesterone (deoxycorticosterone) by 21-hydroxylase ($P450_{21}$). Deoxycorticosterone leaves the endoplasmic reticulum and enters the mitochondria, where it is converted to corticosterone by 11β-hydroxylase ($P450_{11\beta}$). Another mitochondrial P450, 18-hydroxylase ($P450_{18}$) (also called aldosterone synthase) converts corticosterone to the major mineralocorticoid aldosterone, in a two step reaction involving C(18)-hydroxylation of corticosterone (Simpson and Waterman, 1995).

The inner zones of the adrenal cortex, i.e. *zona fasciculata* and *zona reticularis* produce glucocorticoids (Rang et al, 1999). Pregnenolone, after diffusing into the endoplasmic reticulum, can undergo two additional metabolic processes within the cell: either progesterone formation via 3β-HSD or, 17α-hydroxypregnenolone formation via the enzyme 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$). 17α-Hydroxyprogesterone is then converted to deoxycortisol by $P450_{21}$. Deoxycortisol leaves the endoplasmic reticulum, via passive diffusion, into the inner mitochondrial membrane where it is hydroxylated at the C(11) position (by

$P450_{11\beta}$) leading to the synthesis of the major glucocorticoid in humans cortisol (hydrocortisone), also known as the 'Stress Response' hormone, which controls the metabolism of fat and proteins, homeostasis and has anti-inflammatory and anti-allergy effects (Kagawa and Waterman, 1995) (Figure 4).

The enzyme $P450_{18}$ is not present in the *zona fasiculata* and the *zona reticularis* of the adrenals, ensuring that cortisol and not aldosterone is the major product. Since there is no glucocorticoid production in the testis, $P450_{17\alpha}$ serves solely to produce androgens in leydig cells (Fevold et al, 1989).

$P450_{17\alpha}$ has been proposed to be composed of two components, namely the 17α-hydroxylase (17α-OHase) component and the 17,20-lyase component (Zuber et al, 1986). The 17α-OHase component hydroxylates pregnenolone and progesterone to 17α-hydroxypregnenolone and 17α-hydroxyprogesterone respectively, as mentioned previously. The 17,20-lyase component cleaves the C(17)-C(20) bond of 17α-hydroxypregnenolone leading to the production of the androgen dehydroepiandrosterone (DHEA), whilst the same activity on 17α-hydroxyprogesterone leads to the formation of AD.

Alternatively, 3β-HSD can also convert DHEA to AD, which finally gets converted to the major male sex steroid testosterone by 17β-hydroxysteroid dehydrogenase (17β-HSD). As previously mentioned, testosterone is further reduced to its more active form, dihydrotestosterone, by the action of the cytoplasmic enzyme, 5α-steroid reductase (5α-SR) (Figure 4).

Testosterone, and more specifically dihydrotestosterone, have been reported to play a pivotal role in the stimulation of prostate cancer (Lynn et al, 2008).

Figure 4. The metabolism of cholesterol, leading to the synthesis of corticosteroids and androgens.

Hence the overall steroidal cascade is governed by two main families of enzymes, i.e. hydroxysteroid dehydrogenases and cytochrome P450 enzymes, however, only the latter will be discussed in greater detail in the sections below.

1.6 Catalytic Cycle of Cytochrome P450 Enzymes

The cytochrome P450 enzymes are a class of enzymes referred to as oxygenases, and more specifically, mono-oxygenases (Hayaishi, 1962) or mixed function oxidases (Mason, 1957). They possess unique spectrophotometric characteristics as they selectively absorb light at 450nm (Klingenberg, 1958). Microsomal P450 enzymes play a vital role in the metabolism of drugs, xenobiotics and natural substrates (e.g. cholesterol) (Omura and Sato, 1962). The cytochrome P450s are haem-containing metalloenzymes and catalyse a host of crucial biological oxidation reactions (Omura and Sato, 1964). The active sites of these enzymes have an iron protoporphyrin centre coordinated to a cysteine thiolate (Figure 5).

Figure 5. The Iron (III) protoporphryn with a cysteine residue as the axial ligand of the cytochrome P450 family of enzymes (Ortiz de Montellano, 2005).

Cytochrome P450 proteins are oxidoreductases that activate molecular oxygen at the iron centre and incorporate one of the oxygen atoms into the substrate (Sundaramoorty et al, 1995). The activation and transfer of molecular oxygen into

the substrate by an iron-containing enzyme was first demonstrated by Hayaishi et al (1955), however, it was later shown by Tchen and Block (1956) that only one atom of oxygen is derived from the molecular oxygen, whilst the other is transformed into water (Ortiz de Montellano, 2005).

The principal features of the mechanism of action of cytochrome P450 enzymes are summarised as follows (Figure 6):

(1) Binding of the substrate (R) to the enzyme, leading to the formation of the enzyme-substrate complex (complex II).

(2) Reduction of the ferric cytochrome P450 by an associated reductase to the ferrous cytochrome P450 (complex III).

(3) Binding of molecular oxygen to the ferrous moiety to give cytochrome P450-dioxygen complex (complex IV).

(4) A second one-electron reduction (complex V) and protonation occurs to arrive at the Fe(III) hydroperoxy complex (complexVI).

(5) Protonation and heterolytic cleavage of the O-O bond in complex VI with concurrent production of a molecule of water to form a reactive iron-oxo intermediate (complex VII).

(6) The oxygen-atom transfer from the iron-oxo complex VII to the bound substrate to form the oxygenated product (complex VIII).

(7) Product dissociation completes the cycle, replaced by a molecule of water, representing the resting state of haem (complex I) (Figure 6).

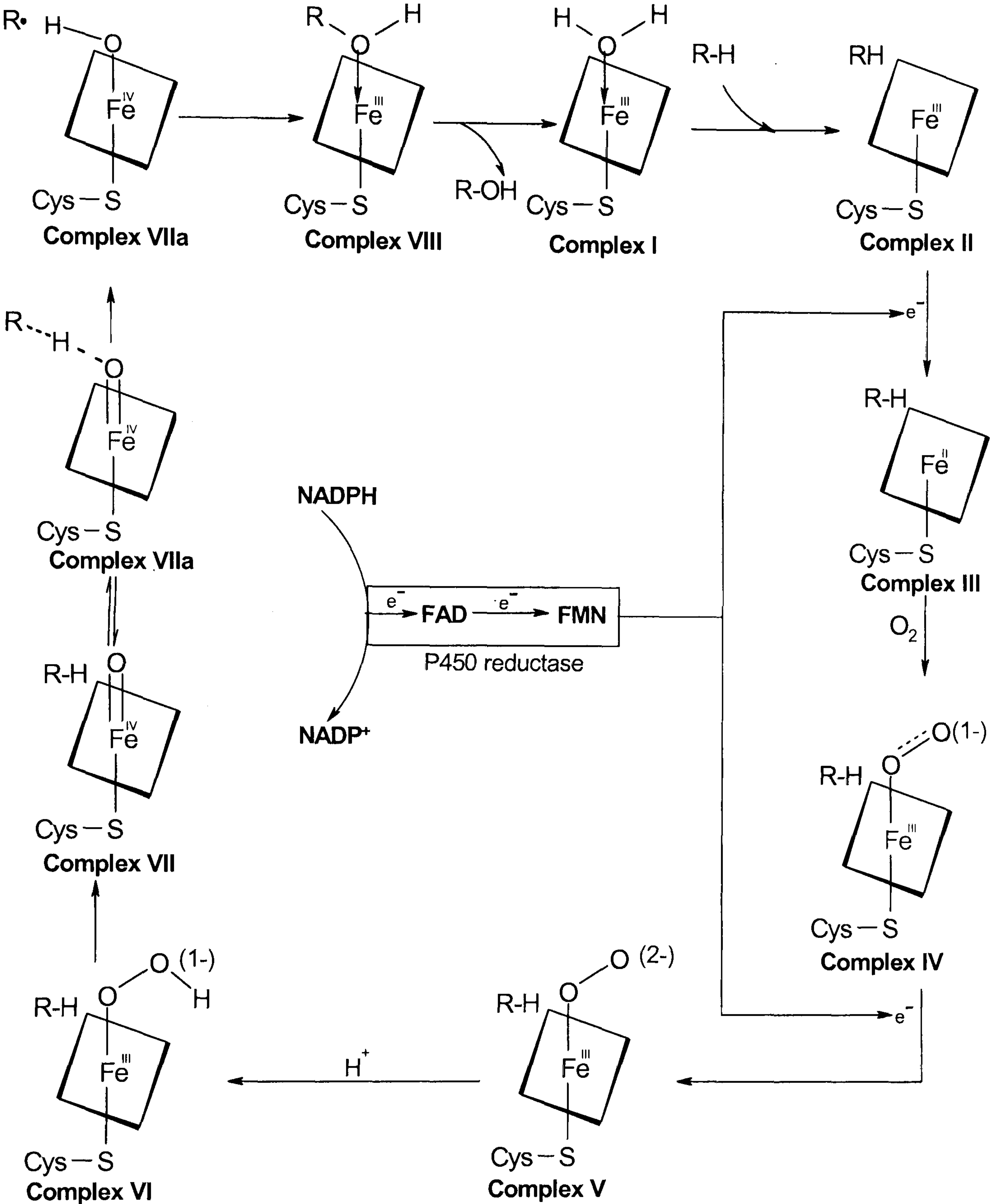


Figure 6. The catalytic cycle of cytochrome P450. NADPH=nicotinamide adenine dinucleotide hydrogen phosphate, FAD=flavin adenine dinucleotide, FMN=flavin mononucleotide (modified from Shaik and De Vesser, 2005).

1.7 $P450_{17\alpha}$

The cytochrome P450 enzyme $P450_{17\alpha}$ is a microsomal enzyme which has become the focus of research into the fight against hormone-dependent prostate cancer, due to the important role it plays in the metabolism of pregnanes and progestins leading to the synthesis of androgens, in particular, AD which is the primary precursor to the major male sex steroid, testosterone.

$P450_{17\alpha}$ has dual enzymatic functionalities (as mentioned previously in section 1.5); the first activity (17α-OHase) hydroxylates the C(17) position converting C_{21} steroids (e.g. pregnenolone and progesterone) into C_{21} hydroxy steroids (e.g. 17α-hydroxypregnenolone and 17α-hydroxyprogestrone respectively). The second activity (17,20-lyase) converts the C_{21} hydroxy steroids into C_{19} androgens (DHEA and AD respectively) by cleaving the C(17)-C(20) bond of the steroid, thus resulting in the loss of an acetate moiety (Imai et al, 1993; Zuber et al, 1986) (Figure 4).

1.8 Mechanisms for Mode of Action of $P450_{17\alpha}$

The specific mechanism of catalysis for $P450_{17\alpha}$ is still unclear, due to the enzyme being membrane bound, as such no crystal structure of $P450_{17\alpha}$ has yet been reported (Zurek et al, 2006).

For the hydroxylation step, the reported mechanism by Akhtar et al (1994) and Akhtar et al (1997) is generally well accepted. Complex I (Figure 7) (equivalent to the resting state of haem before substrate binding as in Figure 6) after various steps produces a ferric peroxy anion species (Fe^{III}-O-O^-) (equivalent to Complex IV in Figure 6) which gets protonated (by a nieghbouring amino acid residue) resulting in a ferro-hydro peroxy species (Fe^{III}-O-OH) (Figure 7).

An additional donation of a proton results in dehydration leading to the production of a feroxy radical species (Fe^{IV}-$O^{\cdot}$) (Lee-Robichaud et al, 1998) (Figure 7).

$$H_2O{-}Fe^{III} \xrightarrow{a} O^{-}{-}O{-}Fe^{III} \xrightarrow{H^+} HO{-}O{-}Fe^{III} \xrightarrow{H^+ \ OH_2} \cdot O{-}Fe^{IV}$$

Complex I   Complex IV

Figure 7. Various steps to show the production of the peroxy anion species and the ferroxy radical. Complex I and Complex IV shown here are equivalent to Complex I and Complex IV in Figure 6, a=(i) substrate binding, (ii) 1e$^-$ donation from NADPH, (iii) O_2 binding, (iv) 1e$^-$ donation from NADPH (modified from Lee-Robichaud et al, 1998).

The feroxy radical species (Fe^{IV}-O·) abstracts the hydrogen atom at the C(17) position of the substrate resulting in: (a) hydroxylation of the iron of the haem (producing Fe^{IV}-OH); and (b) radical formation at the C(17) position of the substrate (pregnenolone in this case) (Figure 8). The C(17) radical now attacks the hydroxyl group attached to the iron of the haem moiety thus completing the hydroxylation step (also called the oxygen rebound step) catalysed by $P450_{17\alpha}$ (Figure 8).

The hydroxylation step is followed by the lyase step resulting in the cleavage of the C(17)-C(20) bond (producing DHEA). This step, however, is not well understood but several mechanisms have been put forward. Lee-Robichaud et al (1998) have summarized the lyase step, as proposed by Akhtar et al (1994 and 1997), in which the step is initiated by an attack of another ferric peroxo anion species (Fe^{III}-O-O$^-$), which has not been protonated yet, on the C(20) carbonyl moiety of the C(17) hydroxylated substrate to an intermediate that subsequently fragments to: (a) produce an alkoxy radical at the oxygen attached to C(20) position of the substrate; and (b) a one-electron oxidised feroxy radical species (Fe^{III}-O·) (Figure 9).

Figure 8. 17α-hydroxylase step catalysed by $P450_{17\alpha}$. The ferroxy radical species abstracts the proton by attacking the C(17) moiety below the plane of the steroid (modified from Lee-Robichaud et al, 1998).

The alkoxy radical auto-disintegrates, leading to the synthesis of acetate (acetic acid) and produces a carbon radical, i.e. at C(17) position, which re-arranges itself to form the more stable carbonyl moiety at the C(17) position, leading to the production of DHEA, thus completing the 17,20 lyase step catalysed by $P450_{17\alpha}$ (Lee-Robichaud et al, 1998) (Figure 9).

$P450_{17\alpha}$ also catalyses the cleavage of the C(17)-C(20) bond to produce a number of minor products, through various mechanistic pathways (Shimizu, 1978) (Figure 10). These minor products, which arise due to the C(17)-C(20) bond cleavage, are proposed to originate as a result of an initial attack by the ferric peroxo anion species ($Fe\text{-}O\text{-}O^-$) directly on the carbonyl moiety [i.e. C(20)] before any kind of interaction with the C(17) carbon (Mak and Swinney, 1992).

Figure 9. The proposed mechanism for the 17,20 lyase step catalysed by $P450_{17\alpha}$ (modified from Lee-Robichaud et al, 1998).

Once the ferro peroxy-[C(20)-steroid backbone] complex is formed, it can undergo fragmentation to produce an alkoxy radical (pathway b, Figure 10), leading to a subsequent production of a C(17) radical, after losing acetic acid (as in the major pathway a) followed by either 17α-hydroxylation to give 17α-hydroxytetstosterone (in the case of progesterone) [pathway b (ii)] or an elimination to produce the $\Delta^{16,17}$-olefinic product [pathway b (ii)] (Figure 10).

In other studies, Ahmed and Owen (1998) and Ahmed et al (1999) have hypothesised the mode of action of $P450_{17\alpha}$, utilising novel molecular modeling techiques, namely the substrate haem complex (SHC) approach, to rationalise the role of the individual components of the active site (i.e. 17α-OHase and 17,20-lyase). They postulated that the initial ferroxy radical species catalyses both the steps undertaken by the enzyme, contrary to the mechanism proposed by Akhtar et al (1994) and Lee-Robichaud et al (1998) in which a feroxy radical species (Fe^{IV}-O•) takes part in the 17α-hydroxylation step whilst the 17,20-lyase step is predominantly undertaken by the ferric peroxo anion species (Fe^{III}-O-O$^-$).

Computer-based models were constructed by Ahmed and Owen (1998) representing the ferroxy- and the peroxy-based SHCs. It was hypothesised that the attacking oxygen species must be positioned within an approximate attacking distance (and angle) to the C(20) carbonyl of the substrate (17α-hydroxypregnenolone in this case) such that attack on the C(20) carbonyl group could take place. In the construction of the SHCs, the hydrogen abstraction from the hydroxyl group at the C(17) position, after the initial attack on the carbonyl at C(20) position, thus leading to the formation of the carbonyl at C(17) position, was considered to be an important step. Hence, their theory was based upon the feasibility of the abstraction of the C(17) hydrogen by the ferroxy- or peroxy-based SHC from a geometric point of view.

Progesterone

$Fe^{III}{=}O$

(Pathway a)

$-(CH_3COOH)$

AD

$Fe^{III}-O-O^-$ (Pathway b)

$Fe^{III}-O\bullet$

$Fe^{III}-O\bullet$

CH_3COOH

[Pathway b(i)]

17α-hydroxytestosterone

[Pathway b(ii)]

16,17-Olefin

Figure 10. Various types of reactions catalysed by $P450_{17\alpha}$ (Mak and Swinney, 1992).

(For convenience only the D ring of progesterone is drawn for pahways a and b).

The substrate (17α-hydroxypregnenolone) was initially fitted onto the peroxy SHC and it was observed that the hydrogen atom of the 17α-hydroxyl group was about 3.4Å away from the oxygen bound to the haem; the distance of 3.4Å was found to be too large for the pivotal H abstraction step to occur. The involvement of a peroxy radical in the carbon-carbon bond cleavage mechanism therefore was concluded to be unlikely, unless there is an extensive movement of the steroidal backbone in order for the 17α-hydroxyl group to approach the ferroxy radical.

Fitting of 17α-hydroxypregnenolone, on the other hand, onto the ferroxy SHC resulted in the ferroxy oxygen being positioned about 1.4Å away from the hydrogen atom of the 17α-hydroxyl group of 17α-hydroxypregnenolone, indicating a more favorable condition for the hydrogen abstraction step. In addition, the C(20) was also found to be in close proximity to the ferroxy species, i.e. about 1.7Å, in order to undergo side reactions resulting in other minor by-products produced by $P450_{17\alpha}$ (Figure 11).

This hypothesis was further supported by homology modeling based studies conducted by Auchus and Miller (1999) who argued that the involvement of the same iron oxene (Fe=O) species, involved in the hydroxylation step, could be theoretically involved in the lyase step as well. It was found that the hydrogen atom of the 17α-hydroxyl group, in the case of 17α-hydroxypregnenolone, remained much closer (1.4Å) to the oxene (Fe=O) than any other steroidal atoms. The study also gave a proposed mechanism of the 17,20-lyase step, which involved formation of a hydroxyl radical after the abstraction of the hydrogen atom from the 17α-hydroxyl group of the substrate (17α-hydroxypregnenolone) (Figure 12). It was further reported that such a process would result in an overwhelmingly favoured route of oxene catabolism, due both to proximity of the hydrogen atom with the iron oxene and to the greater stability of hydroxyl radicals over most carbon radicals (Auchus and Miller, 1999) (Figure 12).

Figure 11. Hypothetical mechanism for the formation of androst-5,16-diene-3β-ol using the ferroxy attacking radical (Ahmed and Owen, 1998).

Figure12. The proposed mechanism of the 17,20-lyase reaction. The ferryl oxene permits hydrogen abstraction at the C(17) hydroxyl of 17α-hydroxypregnenolone, to generate an alkoxy radical, that fragments to DHEA and an acetyl radical. The Fe-OH moiety autodisintegrates to produce resting state haem and a hydroxyl radical which combines with the acetyl radical to produce acetic acid (Auchus and Miller, 1999).

In order to further elucidate the mechanism of catalysis of $P450_{17\alpha}$, researchers have attempted to construct the hypothetical structure of the active site of $P450_{17\alpha}$. Various techniques, in particular homology-based molecular modeling techniques, have been employed by a number of study groups and has led to the design and synthesis of various inhibitors to determine and understand the active site of $P450_{17\alpha}$ (Lin et al, 1994; Ahmed and Owen, 1998; Ahmed, 1998, 1999 and 2004; Auchus and Miller,1999).

1.9 Computer Modeling of $P450_{17\alpha}$

To date crystal structures for very few P450s have been reported [for example cytochrome P450 camphor ($P450_{cam}$) (from *Pseudomonas putida*) (Poulos et al, 1987) and $P450_{BM3}$ (from *Bacillus megaterium*) (Ravichandran et al, 1993). There are, however, substantial differences between the bacterial $P450_{cam}$ and membrane-bound mammalian P450 enzymes of the endoplasmic reticulum, as $P450_{cam}$ generally has only about 14-15% amino acid sequence identity with mammalian P450s (Gonzalez, 1989). Nevertheless, $P450_{cam}$ and mammalian P450s still possess substantial three-dimensional similarities (Nelson and Strobe, 1989).

Lin and coworkers (1994) have attempted to model the active site of $P450_{17\alpha}$ on the established crystallographic structure of $P450_{cam}$ (Figure 13). The modeling predicts a bi-lobed hydrophobic pocket converging on the haem group. The 3-keto group in the A ring of pregnenolone is coordinated by Thr 227 (red), which allows the positioning of C(17) closest to the haem iron, facilitating 17α-hydroxylation. The study also indicates the presence of a large substrate-binding pocket within the active site of $P450_{17\alpha}$. This allows the steroid, pregnenolone in this case, to be positioned in other orientations, in such a way that only C(17) and C(20) come within 5Å of the haem iron, while C(16) comes within about 5.5 Å, explaining the low level of 16α-hydroxylase activity of $P450_{17\alpha}$ (Swart et al, 1993) (Figure 13).

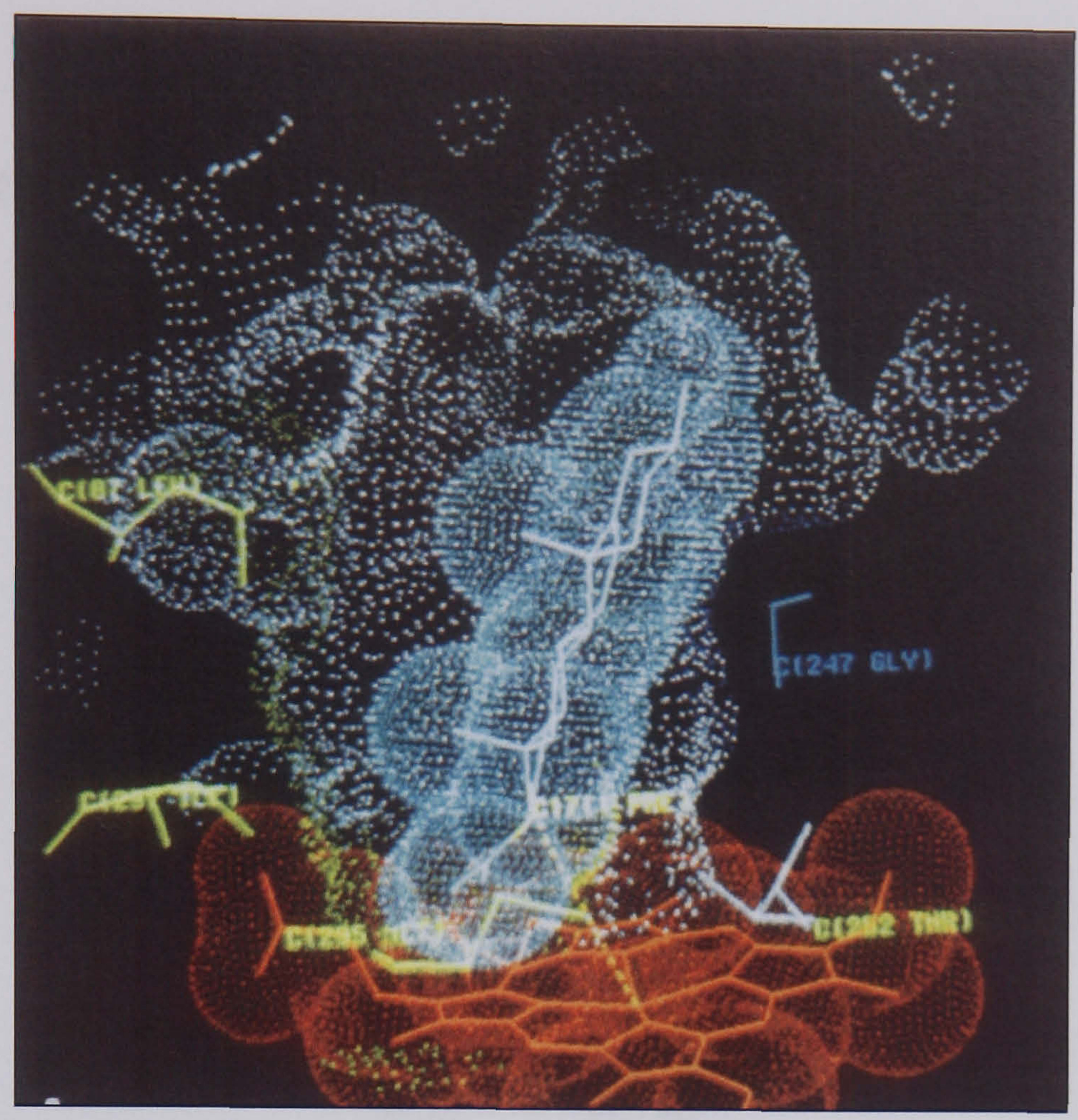
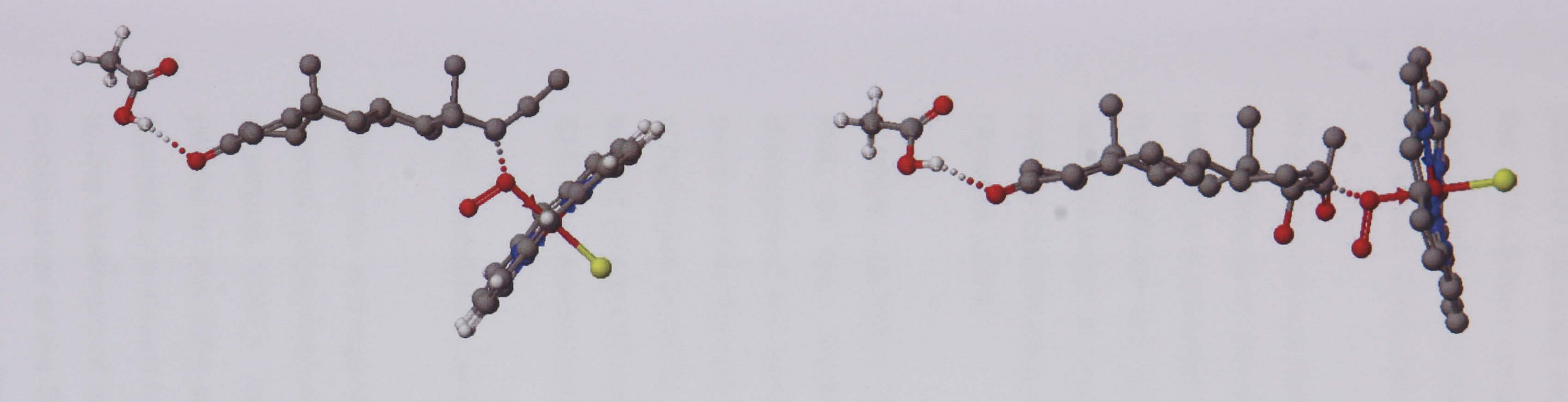


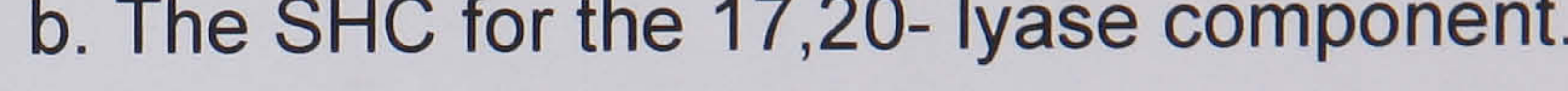
Figure 13. The homology modeling of the active site of human $P450_{17\alpha}$, showing pregnenolone situated in the smaller lobe of the substrate binding pocket of $P450_{17\alpha}$ viewed from the side. The substrate binding pocket is shown in white, space defined by the van der waals radii of the pregnenolene shown in blue and that of haem shown in red (Lin et al, 1994).

In the absence of the crystal structure of the overall enzyme complex, it is extremely difficult to predict the exact (or active) position of the haem and therefore the iron. Ahmed and Owen (1998) and Ahmed (1998, 1999 and 2004) have utilised an alternative approach to homology modeling, in order to elucidate the probable position of the haem of the individual components of $P450_{17\alpha}$ with respect to the substrate backbone in their SHC approach as the representation of the overall active site of 17α-OHase (using progesterone) and 17,20-lyase (using 17α-hydroxypregnenolone) (Figure 14).

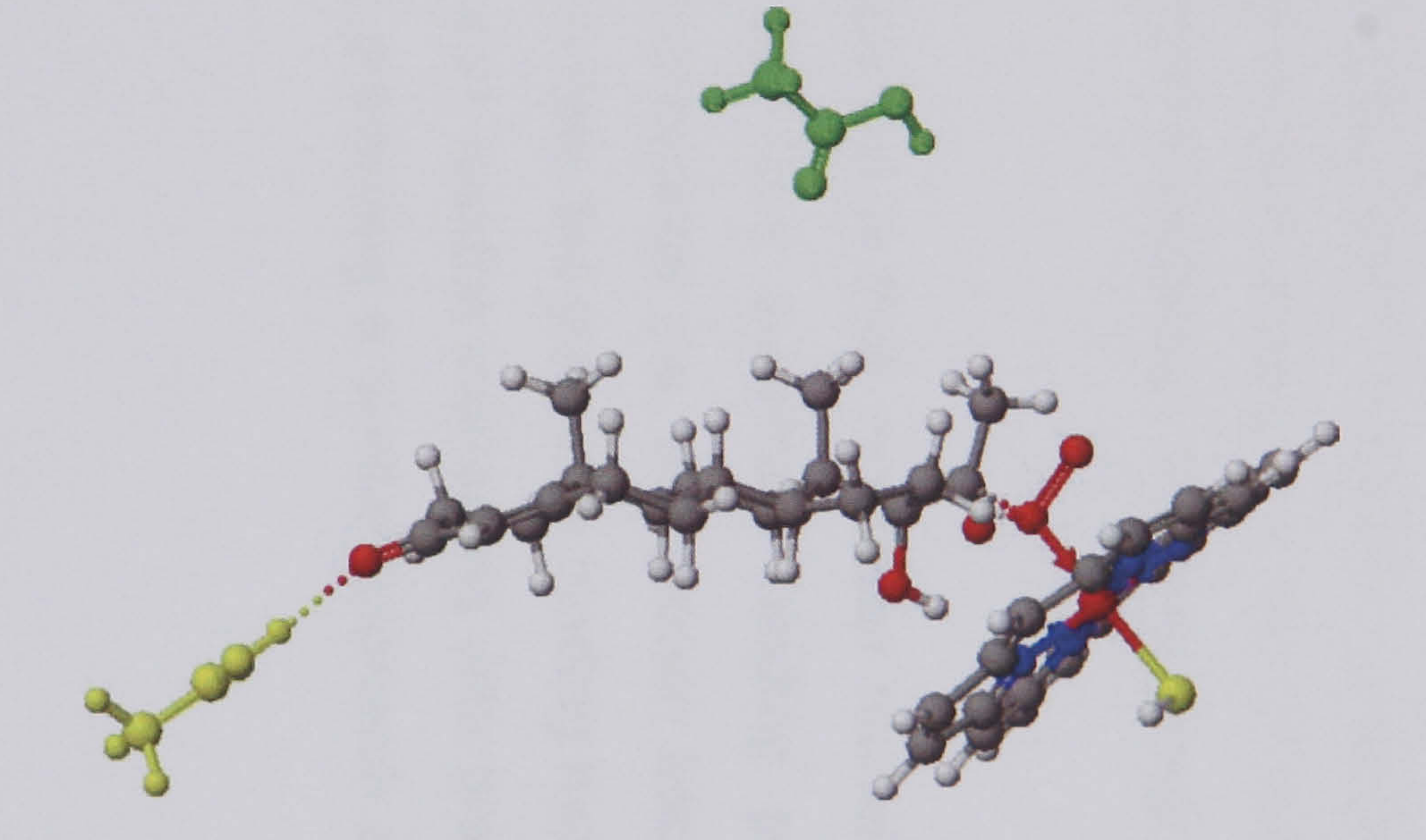
From the consideration of the SHC structures, it was found that there were two



a. The SHC for the 17α-OHase component



b. The SHC for the 17,20- lyase component.



c. The overall representation of the active site of $P450_{17\alpha}$.

Figure 14. Showing various structures utilised in the construction of the SHCs for the two components of $P450_{17\alpha}$ (Ahmed, 2004). (The green and yellow hydrogen bonding groups show the position of the hydrogen bonding group which is crucial to the binding of the non-hydroxylated and hydroxylated progestins, respectively).

potential substrate binding pockets within the active site, the smaller representing the 17α-OHase component, whilst the binding pocket for the lyase component was slightly larger, thus explaining the bi-lobed orientation of the enzyme (Ahmed and Owen, 1998; Ahmed, 1998 and 1999).

In addition, it was further observed that due to the specific orientations of the two components of the enzyme the substrate (progesterone in this case) would bind initially to a potential hydrogen bonding group to undergo the first activity, i.e. 17α-hydroxylation and then the hydroxylated progesterone would need to reorientate itself in order to undergo the second activity, i.e. the 17,20-lyase activity, again initially coordinating to a potential hydrogen bonding group via its C(3) position (Ahmed, 2004).

Auchus and Miller (1999) reported in their molecular modeling study of $P450_{17\alpha}$ that, in fact, there was only one substrate binding pocket. Their model disregarded the amino acid sequence (i.e. the protein backbone) and focused more on the (ferroxy) haem moiety, the substrate binding hydrophobic pocket and a hydrogen bonding amino acid residue interacting with the C(3) position of the steroid (pregnenolone), thus producing a similar approach as in the case of the SHC, mentioned previously.

1.10 Inhibitors of $P450_{17\alpha}$

The male androgens testosterone and dihydrotestosterone, in addition to their normal physiological function, also control prostatic disease in elderly men (Bertrand, 2007). Inhibitors of enzymes of androgen biosynthesis are therefore pivotal in the fight against prostatic carcinoma. The pathways leading to these steroids are shown in Figure 4. The enzyme $P450_{17\alpha}$ is of great interest in relation to the treatment of malignant prostatic disease, due the fact that it catalyses the crucial steps of the C(17) hydroxylation in pregnanes (for example pregnenolone) and then a subsequent cleavage of the C(17)-C(20) bond of 17α-

hydroxypregnenolone to form DHEA which after its conversion to AD, leads to the synthesis of androgens, namely tetstosterone and dihydrotestosterone (as mentioned in section 1.5). About 80% of patients with prostatic cancer have androgen-dependent disease and respond to hormonal ablation. Whereas dihydrotestosterone is the principal active androgen in the prostate, testosterone is also an active stimulant of the growth of prostatic cancer tissue. Hence inhibition of the synthesis or action of testosterone is necessary for the treatment of advanced hormone-dependent prostatic cancer (Bruno and Njar, 2007). Surgical castration or the medically equivalent use of LHRH agonists removes testicular androgens, however, approximately 5% of the tetstosterone is still being produced by the adrenals, which would continue to stimulate the growth of the prostatic tumour. This led to the concept of combined androgen blockade (as mentioned in section 1.4.3). The treatment includes combination of castration or a LHRH agonist with an antiandrogen to counteract the action of residual testosterone or dihydrotestosterone on the androgen receptor in the prostate cancer cell (Mikio et al, 2008). However, an inhibitor of cytochrome $P450_{17\alpha}$ could, by inhibiting the activity of the enzyme, inhibit the production of testosterone precursors (such as DHEA) in both the testes and the adrenals, and therefore achieve the same result as the combined therapy.

In order to stop the natural activity of $P450_{17\alpha}$, inhibitors need to be synthesised which mimic the characteristics of the natural substrate structurally and mechanistically, hence blocking the enzyme active site. This has led researchers to develop two main classes of inhibitors, namely the steroidal based and the non-steroidal based inhibitors (Figure 15).

The steroidal inhibitors (Figure 15-A) contain all the rings of the natural substrate and the A and D rings are generally modified. The non-steroidal inhibitors (Figure15-B) lack one or more rings of the natural substrate, however they may be designed as A-, AB-, AC-, BC-, ABD- and ACD-ring mimics (as shown by the dotted line).

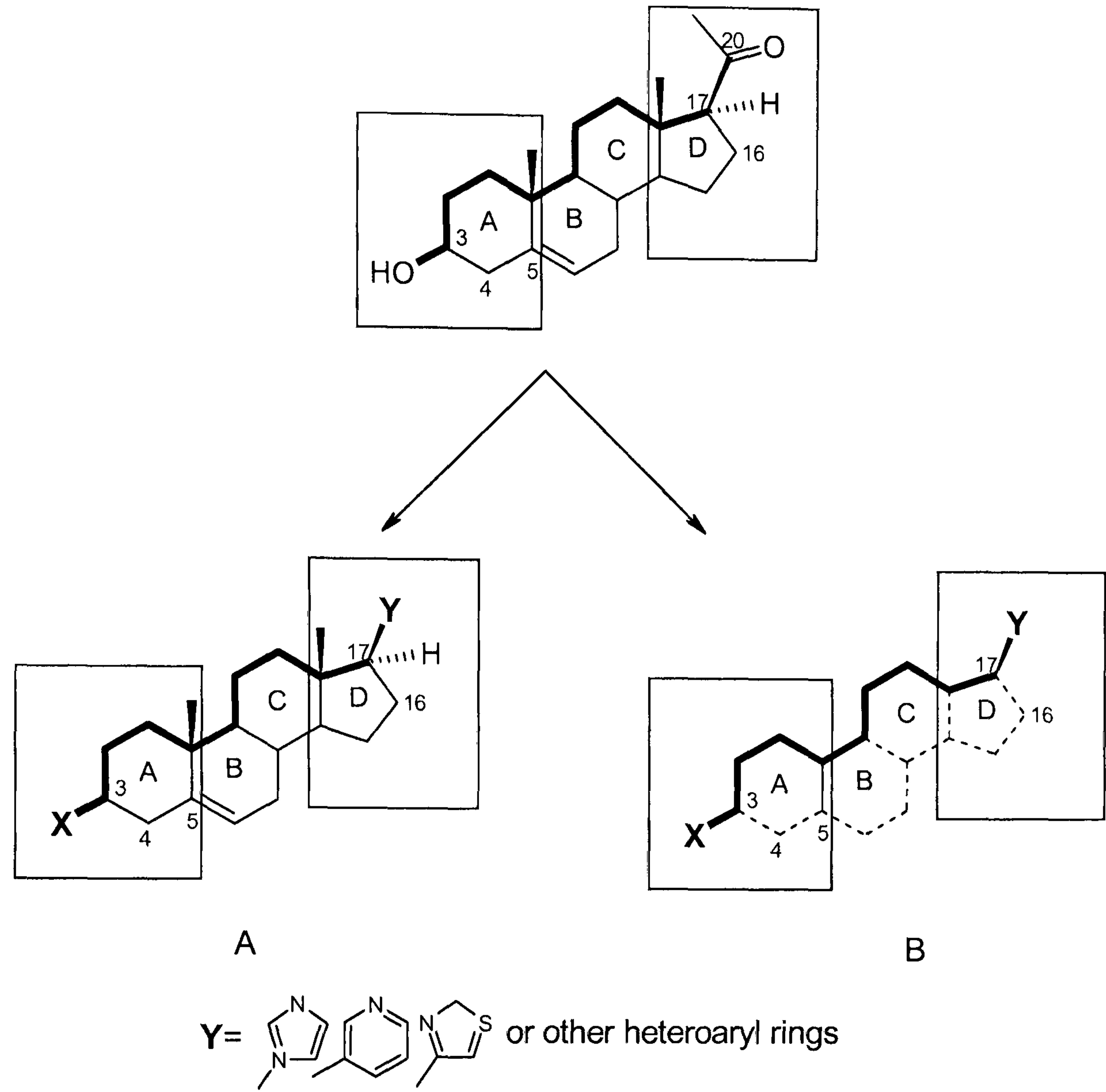


Figure 15. Structure based design of two main types of inhibitors of $P450_{17\alpha}$ mimicking the natural substrate (pregnenolone used here).

It is now well known that introducing a hetroaryl moiety (like imidazole, pyridine, thiazole etc.) at the C(17) or C(20) position of the D ring of the substrate and/or modifying the C(3) carbonyl or hydroxyl group in the A ring of the natural substrate (progesterone and pregnenolone respectively), leads to effective and selective inhibition of $P450_{17\alpha}$ and has resulted in the development of extremely potent steroidal inhibitors (Matsunaga et al, 2004). Non-steroidal inhibitors, on the other hand, lack one or more rings of the natural substrate, however may still mimic the potential hydrogen bonding groups and include a hetroaryl ring within

the structure. The rationale for introducing a heteroaryl ring lies in the fact that the hetero-atom (normally a sp^2 hybridised nitrogen) has been shown to interact with the haem Fe by forming a dative covalent bond, hence inhibiting the enzyme in a reversible fashion. The evidence of such an interaction was observed in the form of an absorption maximum of the UV soret band between 421nM to 430nM, and was called 'Type II' binding (Hall, 1986).

In rational drug design, selectivity of potential inhibitors for the target enzyme is difficult as many cytochrome P450 enzymes are involved in the steroidogenic pathway. In order to protect cortisol biosynthesis, it is aimed to design inhibitors showing selectivity for the lyase step over the hydroxylation step (so that the corticosteroid pathway remains unaffected, Figure 4).

Ketoconazole [KTZ (**3**)], an imidazole based antifungal agent, is a potent non-steroidal inhibitor of $P450_{17\alpha}$. Since its discovery as an anti-prostate cancer agent, KTZ has been extensively used as a standard by researchers in biochemical evaluation of novel inhibitors; in such cases, the biological activity of KTZ has been reported here along with the biological activity of the inhibitors under discussion. The various inhibitors of $P450_{17\alpha}$ will now be discussed.

1.10.1 Steroidal Inhibitors

Various research groups over the past decade have utilised structure based design using pregnenolone and progesterone as the template, and have produced extremely potent steroidal inhibitors.

1.10.1.1 Steroidal Inhibitors: Mechanism-based Compounds

Angelastro et al (1989) reported the mechanism-based inhibitor MDL 27302 (**4**), which was shown to be a competitive irreversible inhibitor of cynomolgus monkey testicular cytochrome $P450_{17\alpha}$, with a K_i value of 90nM, with good evidence of

selectivity as it did not inhibit steroid $P450_{21}$ or $P450_{CSCC}$. It was shown to be a mechanism-based inhibitor of $P450_{17\alpha}$ and was designed to be activated by enzymatic one-electron oxidation of the cyclopropylamino nitrogen followed by opening of the ring to form a β-iminium radical which could react covalently with the enzyme whilst the drug is bound to the active site (Angelastro et al, 1989) (Figure 16).

MDL 27302 (**4**)

Enzyme Inactivation

Figure 16. Proposed mechanism-based inactivation of $P450_{17\alpha}$ by MDL27302 (**4**), (Angelastro et al, 1989).

Other mechanism-based inhibitors include cyclopropyloxy derivatives (compounds **5** and **6**) (Table 2), which proved to be potent inhibitors of human testicular cytochrome $P450_{17\alpha}$. Inhibition was stronger after preincubation of enzyme with inhibitor, supporting the hypothesis of mechanism-based inactivation (Angelastro et al, 1996).

Njar et al (1996) has reported a range of aziridinyl- and amino-steroids (**7-10**, Table 3) designed as mechanism-based inhibitors. The aziridinyl derivatives **7** and **8**, and the amino derivative **9** and **10** were obtained as enantiomers. The inhibition proved markedly enantioselective, with the (*S*)-enantiomer (**7**) being much more potent (IC_{50}=0.21μM) than the (*R*)-enantiomer (**8**) (IC_{50}=34μM) (Table 3), however, **8** was twice as potent as KTZ (IC_{50}=67μM).

Compound No.	Structure	Biological Activity	Reference
4	NH HO	IC_{50}=0.09μM	Angelastro et al, 1989
5	O HO	%inhibition=64% (incubation for 30 min)	Angelastro et al, 1996
6	O O	%inhibition=55% (incubation for 40 min)	Angelastro et al, 1996

Table 2. Showing various mechanism-based inhibitors of $P450_{17\alpha}$.

Compound No.	Structure	IC_{50}
7	*S*-enantiomer	0.21μM
8	*R*-enantiomer	34μM
9	*S*-enantiomer	>125μM
10	*R*-enantiomer	>125μM
3	KTZ	67μM

Table 3. Showing various mechanism-based inhibitors of $P450_{17\alpha}$ (Njar et al, 1996).

In contrast, the amino steroids **9** and **10** (each with an IC_{50} value of >125μM) were very weak inhibitors, indicating the importance of the spacing of the amino group relative to C(17) for coordination with the haem iron. In addition, **7** (K_i=1.7nM) was shown to be tightly bound as the enzyme activity was restored very slowly after

preincubation with the inhibitor (Njar et al, 1996).

1.10.1.2 Steroidal Inhibitors: Pyridine- and Amide-based Compounds

Studies of the mechanism of the two enzymatic steps catalysed by $P450_{17\alpha}$, have led researchers to consider the role of the transition-state geometry for intermediates in the hydroxylase (Figure 17, TS*1) and lyase (Figure 17, TS*2) steps, which resulted in the prediction that attachment of a 2-, 3-, or 4-pyridyl substituent to the 17-position of a steroid carrying a double bond at the C(16)-C(17) position may lead to optimal binding to the haem iron in the respective transition states (Potter et al, 1995). This led to the synthesis of the 3-pyridyl based inhibitor abiraterone (**11**), which has been shown to be an irreversible inhibitor of $P450_{17\alpha}$ with an IC_{50} value of 2.9μM (17,20-lyase reaction), compared to KTZ (with an IC_{50} value of 26μM) (Table 4).

It was proposed that **11**, which possesses a 3-pyridyl ring at the C(17) postion of the steroid, interacts with the active site of $P450_{17\alpha}$ by potentially donating the lone pair of the sp^2 hybridised nitrogen atom within the pyridine ring, hence forming a dative covalent bond with the iron of the haem moiety (Barrie et al, 1994). **11** was substantially more potent than its 2-pyridyl counterpart **13**, whereas the 4-pyridyl derivative **12** was the least active (Table 4). Abiraterone acetate [esterification of the C(3) hydroxyl group of **11**] has successfully passed phase two clinical trials and is currently undergoing phase three clinical trials (Harris et al, 2004; Yap et al, 2008)

Haidar and Hartmann (2002) also reported various steroidal inhibitors bearing a pyridine ring or an amide group in the D ring of the steroid with a double bond at the C(16)-C(17) position (Tables 5 and 6). It was found that the position of the nitrogen in the pyridine ring and its position in the steroidal D ring, i.e. at C(16) or C(17), played a major role in the inhibitory activity of the compounds.

TS*1

TS*2

Figure 17. Proposed transition state geometeries for the the 17α-OHase (TS[*]1) and the C17,20-lyase (TS[*]2) steps utilised to design novel pyridyl based inhibitors such as abiraterone (**11**) (modified from Potter et al, 1995).

Compound No.	Structure	IC_{50}* 17α-OHase	IC_{50}* 17,20-lyase
11	X=Z=CH, Y=N	4.0μM	2.9μM
12	X=Y=CH, Z=N	270μM	76μM
13	Y=Z=CH, X=N	4000μM	1000μM

Table 4. Showing inhibitory activity of pyridyl based compound abiraterone (**11**) and its analogues (**12**) and (**13**) (Barrie et al, 1994). *(Human testicular $P450_{17\alpha}$).

Compound No.	Structure	Biological Activity (Human)	Biological Activity (Rat)
14	R=OCOCH$_3$,17-substituted	IC$_{50}$= 0.11μM	IC$_{50}$= 0.16μM
15	R=OCOCH$_3$,16-substituted	NI**	%inhibition= 19%*
16	R=OH, 17-substituted	IC$_{50}$= 0.074μM	IC$_{50}$= 0.20μM
17	R=OH,16-substituted	NI**	%inhibition= 18%*
18	17-substituted	IC$_{50}$= 0.003μM	ND
19	16-substituted	NI**	%inhibition= 24%
3	KTZ	IC$_{50}$= 0.74μM	IC$_{50}$= 0.67μM

Table 5. Showing pyridyl-based steroidal inhibitors. NI=no inhibition, ND=not determined, (Haidar and Hartmann, 2002).**([I]=2.5μM), *([I]=125μM).

For instance compounds **14**, **16** and **18**, where the pyridine ring is substituted at the C(17) position, showed good inhibitory activity, with IC$_{50}$ values of 0.11μM, 0.074μM and 0.003μM respectively in humans, as compared to their C(16) analogues (**15**, **17** and **19**), which exhibited almost no inhibition in humans and

poor inhibition in rats.

In addition, the attempt of introducing a 2-pyridylmethyl moiety at either position in the D ring, i.e. compound **20** and its C(16) analogue **21**, (Table 6) did not prove to be useful. Introduction of the 4-pyridyl-based moeity (compound **22**, IC_{50}=4.0μM for human), as well as an amide group (compound **24**, IC_{50}=0.29μM for human), at the C(17) position, produced better activity as compared to the 2-pyridyl-based inhibitors.

C(16) analogues of both **22** and **24** (compounds **23** and **25**), however produced extremely poor inhibition in human $P450_{17\alpha}$ (Table 6). The study concluded that the C(17) position within the steroid backbone plays a major role in the inhibitory process, and any substitution at this position is likely to result in a potent inhibitor (Haidar and Hartmann, 2002).

1.10.1.3 Steroidal Inhibitors: Furan-, Thiazolyl- and Oxazolyl-based Compounds

Burkhart et al (1996) have reported various steroidal inhibitors, with furan and aminothiazole rings attached at the C(17) position of the steroid, mimicking pregnenolone (Tables 7 and 8). The furan-based 16,17-dehydro compound **26** displayed extremely good activity against cynomolgus monkey testicular 17,20-lyase (%inhibition=91%) (Table 7). It was proposed that the double bond at the C(16)-C(17) position could have played a role in enhancing the inhibitory activity, as compared to compound **28** (%inhibtion=0%), which lacked the double bond at the C(16)-C(17) position (Table 8).

The thiazole based 16,17-dehydro compound **27** also showed good activity (%inhibition=40%), however its analogue **29** (%inhibition=0%) which did not have the double bond at C(16)-C(17) position, and where the thiazolyl ring was attached to the α-face of the steroid at the C(17) position, showed extremely poor

inhibiton. However when the thiazolyl ring was attached to the β-face of the steroid at the C(17) position as in the case of compound **30** (%inhibtion=39%), an increase in the potency was observed compared to **29** (Table 8).

Compound No.	Structure	Biological Activity (Human)	Biological Activity (Rat)
20	X=N, Y=CH, n=1, 17-substituted	IC_{50}= >10.0μM	ND
21	X=N, Y=CH, n=1, 16-substituted	%inhibition= 10%**	NI*
22	X=CH, Y=N, n=0, 17-substituted	IC_{50}= 4.0μM	ND
23	X=CH, Y=N, n=0, 16-substituted	NI**	%inhibition =22%*
24	17-substituted	IC_{50}= 0.29μM	%inhibition= 48%**
25	16-substituted	%inhibition= 21%**	%inhibition= 20%*
3	KTZ	IC_{50}= 0.74μM	IC_{50}= 0.67μM

Table 6. Showing pyridyl- and amide-based steroidal inhibitors. NI=no inhibition, ND=not determined (Haider and Hartmann, 2002). **([I]=2.5μM), *([I]=125μM).

Hence, inhibitors where the heterocycle was attached to the β-face of the steroid at the C(17) position (compound **30**) or through a trigonal C(17) position (compounds **26** and **27**) were proposed to be better positioned to interact with the haem, hence better inhibitors, in general, as compared to those where the heterocycle is attached to the α-face of the steroid through a C(17) tetrahedral centre (compound **28** and **29**) (Burkhart et al, 1996).

Compound No.	Structure	%inhibition*
26		91%
27		40%

Table 7. Showing furan- and aminothiazolyl-based steroidal inhibitors of $P450_{17\alpha}$. Values for cynomolgus monkey, testicular 17,20-lyase (Burkhart et al, 1996). *([I]=1μM).

Zhu and co-workers (2003) have also produced novel oxazolyl- and thiazolyl-based steroidal inhibitors showing moderate to good inhibition of $P450_{17\alpha}$ (Table 9). The results showed that the oxazolyl-based inhibitors, for example compound **31** (%inhibition=56%), and the thiazolyl-based inhibitors, for example **33**

(%inhibition=72%), demonstrated good inhibition against $P450_{17\alpha}$, however, they were less potent than the standard KTZ (%inhibition=100%) (Table 9).

Compound No.	Structure	%inhibition*
28	HO, OH (furan-substituted steroid)	0%
29	H_2N, S, N, OH, HO (aminothiazolyl steroid)	0%
30	H_2N, S, N, H, HO (aminothiazolyl steroid)	39%

Table 8. Showing furan- and aminothiazolyl-based steroidal inhibitors of $P450_{17\alpha}$. Values for cynomolgus monkey, testicular 17,20-lyase (Burkhart et al, 1996). *([I]=1μM).

The thiazolyl derivative **33** [where R represents a methyl group at the C(4) of the thiazolyl ring] showed very good inhibition (%inhibition=72%) and was the most potent inhibitor among this series (Table 9). Removal of substitiution at either the C(3) or C(4) position of the thiazole ring resulted in reduced potency (compound

34, %inhibition=42%), while introduction of a phenyl group as in **35** resulted in a significant decrease in activity (%inhibition=10%).

Compound No.	**Structure**	**%inhibition***
31	R=H, X=O	56%
32	R=4-CH_3, X=O	45%
33	R=4-CH_3, X=S	72%
34	R=H, X=S	42%
35	R=4-C_6H_5, X=S	10%
36	R=4-CH_3, X=S	67%
37	R=3-CH_3, X=S	28%
38	R=3-C_6H_5, X=O	29%
3	KTZ	100%

Table 9. Showing inhibitory activities of a range of oxazolyl- and thiazolyl-based steroidal inhibitors (Zhu et al, 2003). *(Human $P450_{17\alpha}$).

The oxazolyl-based compound **32** [where R represents a methyl group at the C(4) position of the oxazolyl ring], however, showed poorer inhibition (%inhibition=45%) compared to the parent compound **31** (where R represents a hydrogen atom) (Table 9).

Among the acetate-based steroidal inhibitors, compound **36** [where R represents a methyl group at the C(4) of the thiazolyl ring] showed good inhibition (%inhibition=67%), close to that of KTZ. However, its other analogue **37** [where R represents a methyl group at the C(3) position of the thiazolyl ring], as well as the oxazolyl-based inhibitor **38** [where R represents a phenyl group at C(3) position in the oxazolyl ring] showed poor inhibition (%inhibition=28% and 29% respectively) (Table 9).

1.10.1.4 Steroidal Inhibitors: 20-Hydroxy- and 20-Oxime-based Compounds

Ling et al (1998) have reported 20-hydroxy- (**39**-**46**) and 20-oxime-based steroidal inhibitors (**47**-**49**) (Tables 10 and 11). They found that compound **39** (IC_{50}=180nM) showed more potency against $P450_{17\alpha}$ than its 20α-counterpart (compound **40**, IC_{50}=720 nM). Compound **41** (IC_{50}=1547nM) showed a decrease in activity as compared to both **39** and **40** by the introduction of a carbonyl group at C(3) and double bond at the C(4)-C(5) position, however, its 20β-analogue **42** showed better inhibition (IC_{50}=204 nM) (Table 10).

The introduction of the double bond at C(16)-C(17), as in compounds **43** and **44** (IC_{50}=250nM and 255nM respectively) (Table 10), showed good inhibition and were equipotent to each other, while the 20α-hydroxy-4,16-pregnadien-3-one (**45**) and its 20β-hydroxy epimer (**46**) showed almost no inhibition (Table 11). The importance of the presence of an isomeric configuration at the C(20) position, together with the double bond at C(16)-C(17) position suggests that this area potentially interacts with the active site of the enzyme (Ling et al, 1998).

Compound No.	Structure	Human		Rat
		% inhibition*	IC_{50}	% inhibition**
39	20β-OH	13.4%	180nM	26.6%
40	20α-OH	16.4%	720nM	21.1%
41	20α-OH	8.4%	1547 nM	37.9%
42	20β-OH	23.3%	204nM	73.7%
43	R_1= CH_3, R_2= OH (*S*)	2.0%	250nM	4.8%
44	R_1=OH, R_2=CH_3 (*R*)	0.4%	255nM	8.7%
3	KTZ	69.5%	78nM	65.3%

Table 10. Showing 20α-hydroxy based steroidal inhibitors. NI=no inhibition, ND=not determined (Ling et al, 1998). *([I]=150μM), **([I]=500nM).

Compound No.	Structure	Human		Rat
		% inhibition*	IC_{50}	% inhibition**
45	20α-OH	15.5%	ND	58.0%
46	20β-OH	16.8%	ND	80.6%
47		80.3%	73nM	63.1%
48	$R=COCH_3$	83.8%	25nM	96.3%
49	R=OH	76.7%	43nM	91.7%
3	KTZ	69.5%	78nM	65.3%

Table 11. Showing 20α-hydroxy- and 20-oxime-based steroidal inhibitors, ND=not determined (Ling et al, 1998). *([I]=150μM), **([I]=500nM)

Pregnenolone-16-en-20-oxime (**47**) (Table 11) showed good activity (IC_{50}=73 nM) and its 4-en-3-one derivatives (**48** and **49**) showed much greater activity (IC_{50}=25nM and 43nM respectively) against human $P450_{17\alpha}$. Comparison of the

activities of the inhibitors indicates that introducing the 20-oxime group markedly increases the inhibitory activity (Ling et al, 1998).

1.10.1.5 Steroidal Inhibitors: Azole-based Compounds

Various researchers have reported numerous imidazole-based steroidal inhibitors, which proved to be potent inhibitors of $P450_{17\alpha}$, for example the 4-imidazolyl-based inhibitors **50** and **51** (IC_{50}=24nM and 21nM respectively) (Table 12). A key feature relating to the potency of these inhibitors was found to be the double bond present at the C(16)-C(17) position, which was proposed to play a significant role in the overall inhibitory process (Barrie at al, 1994).

It was also postulated that the sp^2 nitrogen in the imidazolyl ring was not sterically hindered, and that it was in an ideal orientation to interact with the iron of the haem moiety, as seen in the case of the 1-imidazolyl-based compound VN-87 (**53**) (IC_{50}=1.25nM) which showed much superior activity as compared to its 4-imidazolyl-based analogues **50** and **51**, as well the standard KTZ (IC_{50}=80.7nM) (Table 12). The progesterone-based compound VN-85 (**52**) also showed good activity (IC_{50}=2.96nM) similar to that of its pregnenolone-based analogue **53** leading to the conclusion that the prescence of the carbonyl or hydroxyl group in the A-ring of the steroid, as well as the double bond at either the C(4)-C(5) or C(5)-(6) position had little effect on the overall activity of the inhibitors (Nnane et al, 2001).

Introduction of a phenyl ring into the imidazolyl ring, however, resulted in a decrease in activity as in the case of VN-124 (**54**) (IC_{50}=300nM) (KTZ, IC_{50}=80nM) (Handratta et al, 2005). VN-124 proved to be a potent $P450_{17\alpha}$ inhibitor as well as an effective anti-androgen and showed superior activity *in vitro* as compared to the known anti-androgen bicalutamide (**2**) (Schayowitz et al, 2008). VN-124 has recently been licensed to Tokai Pharmaceuticals Inc (Boston, USA), with plans for clinical evaluation (Bruno and Njar, 2007).

Compound No.	Structure	IC_{50}	Reference
50	X=NH, Y=CH	24nM	Barrie et al, 1994
51	X=CH, Y=NH	21nM	
52		2.96nM	Nnane et al, 2001
53		1.25nM	
54		300nM	Handratta et al, 2005

Table 12. Showing various azole-based steroidal inhibitors.

1.10.2 Non-steroidal Inhibitors

Significant research has been undertaken to identify potent non-steroidal inhibitors of $P450_{17\alpha}$, since these would be expected to show reduced steriodogenic effects, an extremely beneficial factor in prostate cancer therapy, compared to steroidal $P450_{17\alpha}$ inhibitors. However, the only non-steroidal inhibitor used clinically to treat prostate cancer was KTZ, an azole antifungal agent. KTZ possesses two stereo centres and thus four possible diastereoisomers exist of which, the *cis*-2*S*,4*R* isomer (Figure 18) has been shown to be the most potent stereoisomer against porcine $P450_{17\alpha}$ (the 17,20-lyase component) (Rotstein et al, 1992).

2*S*,4*R* isomer of KTZ	IC_{50}=0.050μM, compared to 0.589μM, 2.04μM and 2.38μM for 2*S*,4*R*, 2*R*,4*R*, and 2*R*,4*S* isomers respectively for 17,20-lyase component of $P450_{17\alpha}$.

Figure 18. 2*S*,4*R* isomer of KTZ (**3**), its IC_{50} value compared with its other isomers (Rotstein et al, 1992).

Due to its poor selectivity (as it also effectively inhibits the enzyme $P450_{11\beta}$) leading to hepatotoxicity, KTZ was withdrawn from the market for the treatment of prostate cancer (Rotstein et al, 1992), however, it has since entered several clinical trials to study its effectiveness at lower doses [using one half the previously administered dose (i.e. 600 mg/day as 200mg three times a day, instead of 1200mg/day)] alone and in combination with other agents (Wilkinson and Chodak 2003; Nakabayashi et al, 2006).

The key structural feature with regards to the inhibitory activity of KTZ was found

to be the prescence of the azole (imidazole) based moiety which could undergo type II binding with the iron in the haem by forming a dative covalent bond (as mentioned previously). The lack of selectivity for $P450_{17\alpha}$, however, is still a major drawback in the use of KTZ to treat prostate cancer. Therefore in order for the future inhibitors to be more selective they need to mimic the natural substrates for $P450_{17\alpha}$, i.e. pregnenolone or progesterone. This has led various researchers to synthesise and develop a number of steroidal ring mimetics, bearing heteroaryl moieties (generally a pyridyl or azolyl group), which were named with respect to the number of rings of the steroid they mimic, for example A-, AB-, AC-, BC-, ABD- and ACD-ring mimics (Figure 19). These inhibitors will now be discussed.

1.10.2.1 Non-steroidal Inhibitors: Pyridyl-based Tetralones, Tetralines and Indanones

Sergejew and Hartmann (1994) published a series of pyridyl-based benzocycloalkanes as inhibitors of the enzyme $P450_{17\alpha}$ (Tables 13, 14 and 15). The inhibitors were designed mainly to mimic the AB and the BC rings of the steroid backbone.

The unsaturated tetralones, compounds **55-62**, caused inhibition ranging from 4-70%. The 3-pyridyl derivative **61** showed better activity (%inhibition=48%) than the 4-pyridyl analogue **55** (%inhibition=37%). The methoxy substituent at position 5 (compound **57**) increased the activity (%inhibition=53%), while introduction of hydroxyl substituents at 5-, 6- and 7-positions, i.e. compounds **58**, **59** and **60**, led to a decrease in activity (causing inhibition of 23%, 8% and 10% respectively). The *Z*-isomer (**56**) however showed nearly double the inhibition (%inhibition=70%) as compared to **55** and produced similar inhibition to KTZ (%inhibition=62%). In addition, the 5-methoxy compound **64** also showed extremely good inhibition (%inhibition=86%; IC_{50}=13.0μM) and showed more potency than KTZ (%inhibition=62%; IC_{50}=67.0μM) (Table 13).

Y= or other heteroaryl rings

X= hydrogen bonding groups, electron donating/withdrawing groups

n= 0-10

Figure 19. Design of inhibitors mimicking the steroid (pregnenolone) backbone leading to the development of A-, AB-, AC-, BC-, ABD- and ACD-ring mimics.

The 4-pyridyl-based hydrogenated tetralones, compounds **65-67** (Table 14) showed very good inhibition ranging from 67%-84%. Compound **65** (%inhibition=67%; IC_{50}=13μM) proved to be a stronger inhibitor than the corresponding unsaturated analogue **55** (%inhibition=37%). Introduction of a methoxy group at position 5 or 6 [compounds **66** and **67** respectively (where a 4-pyridyl moiety was utilised)] and a hydroxyl group at position 5 or 7 [compounds **68** and **70** respectively (where a 3-pyridyl moiety was utilised)] showed an increase in activity, whilst a hydroxyl group at position 6 for 3-pyridyl based inhibitors (compound **69**) showed a decrease in activity (Table 14).

Compound No.	Structure	%inhibition*
55	R=H (*E*)	37%
56	R=H (*Z*)	70%
57	R=5-OCH_3 (*E*)	53%
58	R=5-OH (*E*)	23%
59	R=6-OH (*E*)	8%
60	R=7-OH (*E*)	10%
61	X=CH, Y=N (*E*)	48%
62	X=N, Y=CH (*E*)	4%
63	R=H	71%
64	R=5-OCH_3	86%
3	KTZ	62%

Table 13. Showing various pyridyl-based non-steroidal inhibitors (Sergejew and Hartmann, 1994). *([I]=125μM, rat testicular $P450_{17\alpha}$).

The tetralines (compounds **71-74**, %inhibition=55%-85%, Table 14) showed better inhibition than the unsaturated tetralones **55** and **58-60** and similar activity as

compared to the saturated tetralones **65-67** (Table 14).

Compound No.	Structure	%inhibition*
65	R=H	67%
66	R=5-OCH_3	84%
67	R=6-OCH_3	83%
68	R=5-OH	83%
69	R=6-OH	34%
70	R=7-OH	79%
71	R=H	85%
72	R=5-OH	55%
73	R=6-OH	70%
74	R=7-OH	67%
3	KTZ	62%

Table 14. Showing various pyridyl-based non-steroidal inhibitors (Sergejew and Hartmann, 1994). *([I]=125μM, Rat testicular $P450_{17\alpha}$).

The unsubstituted analogue **71** was found to be a potent inhibitor of rat $P450_{17\alpha}$ (%inhibition=85%; IC_{50}=22μM) and showed better inhibition than the standard KTZ (%inhibition=62%; IC_{50}=67μM), however, addition of a hydroxyl group at position 5 or 7, in this case, decreased activity (Table 14). The most active compounds in this study proved to be the saturated tetralones **64** (Table 13) and **66** (Table 14) each with an IC_{50} value of 13μM (Sergejew and Hartmann, 1994).

Compound No.	Structure	%inhibition*
75	O, 6, R, 5, 4, N R=H	24%
76	R=4-OCH_3	27%
77	R=5-OCH_3	40%
78	R=4-OH	51%
79	R=5-OH	7%
80	O, 6, R, 5, 4, N R=H	66%
81	R=5-OCH_3	67%
82	R=5-OH	75%
3	KTZ	62%

Table 15. Various various pyridyl-based non-steroidal inhibitors (Sergejew and Hartmann, 1994). *([I]=125μM, Rat $P450_{17\alpha}$).

The unsaturated indanones, i.e. compounds **75-79**, proved, in general, to be

weaker than the standard, KTZ (Table 15). The unsubstituted compound (**75**) showed very weak activity (%inhibition=24%) for rat testicular $P450_{17\alpha}$. However the 5-methoxy and 4-hydroxy derivatives [compounds **77** and **78** (%inhibition of 40% and 51% respectively)] showed an increased activity compared to **75**.

The saturated indanones, i.e. compounds **80-82**, were more potent than the unsaturated indanones, for example, compound **80**, showed better activity (%inhibition=66%) than the corresponding unsaturated indanone **75** (%inhibition=24%) and was equipotent to the standard KTZ (%inhibition=62%) (Table 15). Introduction of a methoxy group at the 5-position, compound **81** (%inhibition=67%), did not alter the activity, whereas a hydroxy group at the 5-position did increase the activity, as in the case of compound **82** (%inhibition=75%) (Table 15).

1.10.2.2 Non-steroidal Inhibitors: Pyridyl-based Esters

Rowlands' group (1995) have reported the synthesis of various esters of 2-, 3- and 4-pyridyl acetic acid utilising various alcohols, namely isopinocampheol, (compounds **83-91**), 1-adamantanol, (compounds **92-94**), 2-methyl-2-adamantanol, (compounds **95** and **96**) and cedrol (compounds **97** and **98**) (Tables 16 and 17). The inhibitors were designed to mimic the steroidal backbone of pregnenolone (B ring) (Figure 20). The study showed that the 4-pyridyl based compounds were better inhibitors as compared to the 2- and 3-pyridyl-based compounds. In addition, the inhibitors where isopinocampheol was used as the alcohol (compounds **83-91**) showed, in general, good inhibition (with IC_{50} ranging from 14nM-1000nM and 5nM-1000nM for 17α-OHase and the 17,20-lyase components of the enzyme respectively).

In the case of the 4-pyridyl-based inhibitors, where isopinocampheol was used as the alcohol (compounds **83-87**), the unsubstituted analogue **83** showed extremely good inhibitory activity (IC_{50}=14nM and 5nM, for the 17α-OHase and the 17,20-

lyase components of the enzyme respectively), and was shown to be the most potent compound among this series of inhibitors (Table 16). However, the potency decreased with the introduction of methyl and ethyl groups at the R_1 and R_2 position, as in the case of compounds **84-87** (Table 16).

Figure 20. The structure based design of esters of pyridylacetic acid mimicking the B of pregnenolone, (Rowlands et al, 1995).

In the case of the 3-pyridyl-based inhibitors, where isopinocampheol was used as the alcohol (compounds **88-90**), introduction of a methyl group at R_1 increased the potency against both 17α-OHase and lyase [compound **89** (IC_{50}=82nM and 14nM respectively)]. This was further increased by the addition of another methyl group at R_2, although only for the 17α-OHase activity [**90** (IC_{50}=29nM and 15nM)] for the 17α-OHase and 17,20-lyase components of the enzyme respectively (Table 16).

Among the esters where 1-adamantanol (compounds **92-94**) and 2-methyl-2-adamantanol (compounds **95** and **96**) were used as the alcohols some activity was observed (Table 17).

An increase in potency was observed with the introduction of methyl groups at the R_1 and R_2 positions, as in the case of compounds **94** (IC_{50}=90nM and 13nM) and **96** (IC_{50}=75nM and 13nM) for the 17α-OHase and 17,20-lyase components

of the enzyme respectively, as compared to their non-substituted analogues, compounds **92** and **95** respectively (Table 17).

Compound No.	Structure	IC_{50}* 17α-OHase	IC_{50}* 17,20-lyase
83	R_1 R_2 O O N R_1=R_2=H	14nM	5nM
84	R_1=CH_3, R_2=H	19nM	6nM
85	R_1=R_2=CH_3	26nM	10nM
86	R_1=C_2H_5, R_2=H	34nM	9nM
87	R_1= R_2=C_2H_5,	140nM	35nM
88	R_1 R_2 O O N R_1=R_2=H	260nM	88nM
89	R_1=CH_3, R_2=H	82nM	14nM
90	R_1=R_2=CH_3	29nM	15nM
91	O O N	>1000nM	>1000nM
3	KTZ	65nM	26nM

Table 16. Showing pyridyl ester based non-steroidal inhibitors (Rowlands et al, 1995). *(Human testicular $P450_{17\alpha}$).

The 2-pyridyl based inhibitor (**91**) (Table 16), as well as inhibitors where cedrol was used as an alcohol (compounds **97** and **98**, Table 17), showed weaker inhibition of the enzyme compared to KTZ (Rowlands et al, 1995).

Compound No.	Structure	IC_{50}* 17α-OHase	IC_{50}* 17,20-lyase
92	R_1=R_2=H	930nM	130nM
93	R_1=CH_3, R_2=H	220nM	35nM
94	R_1=R_2=CH_3	90nM	13nM
95	R_1=R_2=H	1900nM	320nM
96	R_1=R_2=CH_3	75nM	13nM
97	X=N, Y=CH	230nM	38nM
98	X=CH, Y=N	270nM	52nM
3	KTZ	65nM	26nM

Table 17. Showing pyridyl ester based non-steroidal inhibitors (Rowlands et al, 1995). *(Human testicular $P450_{17\alpha}$).

1.10.2.3 Non-steroidal Inhibitors: Pyrrolidine-2,5-dione-based Compounds

Ahmed et al (1995) have reported the synthesis of a range of novel pyrrolidine-2,5-dione-based inhibitors of $P450_{17\alpha}$ (Table 18) designed to mimic the A ring of the steroid progesterone (Figure 21).

Figure 21. To show the structure-based design of pyrrolidine-2,5-dione-based inhibitors mimicking the A ring of progesterone (Ahmed et al, 1995).

The inhibitors (compounds **99-105**, Table 18), in general showed good inhibition, ranging from 58%-95%. The main feature in designing the inhibitors included introduction of an amino group at the *para*-position in the phenyl ring to potentially bind to the haem via a dative covalent bond (Figure 21). In addition the pyrrolidine-2,5-dione ring was utilised in order to mimic the A ring of progesterone, in particular, the carbonyl moeity at the C(3) position which is proposed to interact with a potential hydrogen bonding group within the active site of $P450_{17\alpha}$ (Figure 21). Other factors considered included increasing the alkyl chain length between the pyrrolidine-2,5-dione ring and the phenyl ring and *N*-alkylation of the pyrrolidinone nitrogen by an alkyl group. Addition of another amino group in the phenyl ring strongly increased the inhibitory activity (compounds **102-105**; %inhibition=75-95%). The inhibitors with the strongest activity were compounds **103** (%inhibition=95%) and **101** (%inhibition=88%; IC_{50}=26.4μM).

It can be concluded that among the mono-amino compounds studied, the compounds with a two carbon spacer group (**99-101**, Table 18) showed an increase in activity with an increase in the *N*-substituted alkyl chain [i.e. from butyl (compound **100**, IC_{50}=59.0μM) to octyl (compound **101**, IC_{50}=26.4μM)].

Compound No.	Structure	%inhibition*	IC_{50}
99	R=H	58%	112μM
100	R=*n*-butyl	61%	59.0μM
101	R=*n*-octyl	88%	26.4μM
102	R_1=R_2=H	62%	28.3μM
103	R_1=H, R_2=NH_2	95%	ND
104	R_1=methyl, R_2=NH_2	81%	ND
105	R_1=*n*-butyl, R_2=NH_2	75%	ND
3	KTZ	91.5%	12.1μM

Table 18. Showing pyrrolidine-2,5-dione based non-steroidal inhibitors, ND=not determined (Ahmed et al, 1995). *([I]=33μM, rat testicular $P450_{17\alpha}$).

Among the compounds with a three carbon spacer group (compounds **102-105**), the di-amino based compounds showed the best activity (compounds **103-105**), however, a slight decrease in activity was observed with an increase in the *N*-substituted alkyl chain, with **103** being the most potent inhibitor of the series (% inhibition= 95%) (Ahmed et al, 1995) (Table18).

1.10.2.4 Non-steroidal Inhibitors: Tetralone-based Di-heteroaryls

Wachter and co-workers (1996) explored the effect of different heteroaromatic ring systems (possessing two heteroatoms in the aromatic ring) attached to a tetralone (or dihydronaphthalene) moiety. The di-heteroaromatic ring systems studied included thiazolyl (compound **106**), pyrazinyl (compounds **107** and **109**), pyridazinyl (compounds **108** and **110**) and pyrimidinyl-based rings (compound **111**) (Table 19). The compounds in the study showed poor inhibition of $P450_{17\alpha}$ and the best result was found in the case of **106** (%inhibition=21%) where a thiazolyl ring was utilised, although the inhibition was poor as compared to KTZ (%inhibition=62%) (Wachter et al, 1996) (Table 19).

1.10.2.5 Non-steroidal Inhibitors: Pyridyl-based Phenylnaphthalenes

Mendieta and co-workers (2008) have reported the synthesis of various substituted phenylnaphthalenes mimicking the ACD- and ABD-rings of the steroid (pregnenolone) backbone, which have shown poor to average inhibitory activity of the enzyme $P450_{17\alpha}$ (Tables 20 and 21). The 3-pyridyl-substituted ACD-ring mimetics (compounds **112-114**) (Table 20), showed no or little inhibition (7-28%), in contrast to the reference compounds KTZ (IC_{50}=2780nM) and abiraterone (**11**) (IC_{50}=72nM) (Table 20). Among the ABD mimetics (Table 21), the 4-pyridyl-based compound **115** (%inhibition=66%) showed good activity, whilst the 3-pyridyl compound **117** was inactive (Table 21). A decrease in activity from compounds **115-117** (66%-8%) could be observed, with **115** being the most active compound of this study.

In order to better understand the potential interactions of the inhibitors with the enzyme active site, Mendieta and co-workers (2008) constructed a computer-based model for $P450_{17\alpha}$. Compound **115** and the steroidal inhibitor abiraterone (**11**) were docked into the computer-based simulation of the active site of $P450_{17\alpha}$ (Figure 22).

Compound No.	Structure	%inhibition*
106		21%
107	X=N, Y=CH	<10%
108	X=CH, Y=N	<10%
109	X=N, Y=Z=CH	<10%
110	X=Z=CH, Y=N	<10%
111	X=Y=CH, Z=N	<10%
3	KTZ	62%

Table 19. Showing di-heteroaryl-based non-steroidal inhibitors, (Wachter et al, 1996). *([I]=125μM; rat testicular $P450_{17\alpha}$).

The key interaction for compound **115** seems to be the H-bonding between the hydroxyl group on the naphthalene ring with the amino acids Arg109, His235, Lys231 and Asn202 within the the active site of $P450_{17\alpha}$. In addition, the 4-pyridyl moiety was also proposed to play an important role in the higher inhibitory activity of **115** as compared to **117** where a 2-pyridyl moiety was utilised (Mendieta et al, 2008).

Compound No.	Structure	Biological Activity
112		%inhibition=28%*
113		%inhibition=7%*
114		%inhibition=22%*
3	KTZ	IC_{50}=2780nM
11	abiraterone	IC_{50}=72nM

Table 20. Showing inhibitory activities of a range of phenylnaphthalene-based non-steroidal inhibitors (Mendieta et al, 2008).
*[([I]=2.0μM), %inhibition for **3** and **11** was not reported by the authors]

Compound No.	Structure	Biological Activity
115	X=CH, Y=N, R=OH	%inhibition=66%*
116	X=CH, Y=N, R=OCH_3	%inhibition=23%*
117	X=N, Y=CH, R=OH	%inhibition=8%*
3	KTZ	IC_{50}=2780nM
11	abiraterone	IC_{50}=72nM

Table 21. Showing inhibitory activities of a range of phenylnaphthalene-based non-steroidal inhibitors (Mendieta et al, 2008).
*[([I]=2.0μM), %inhibiton for **3** and **11** was not reported by the authors]

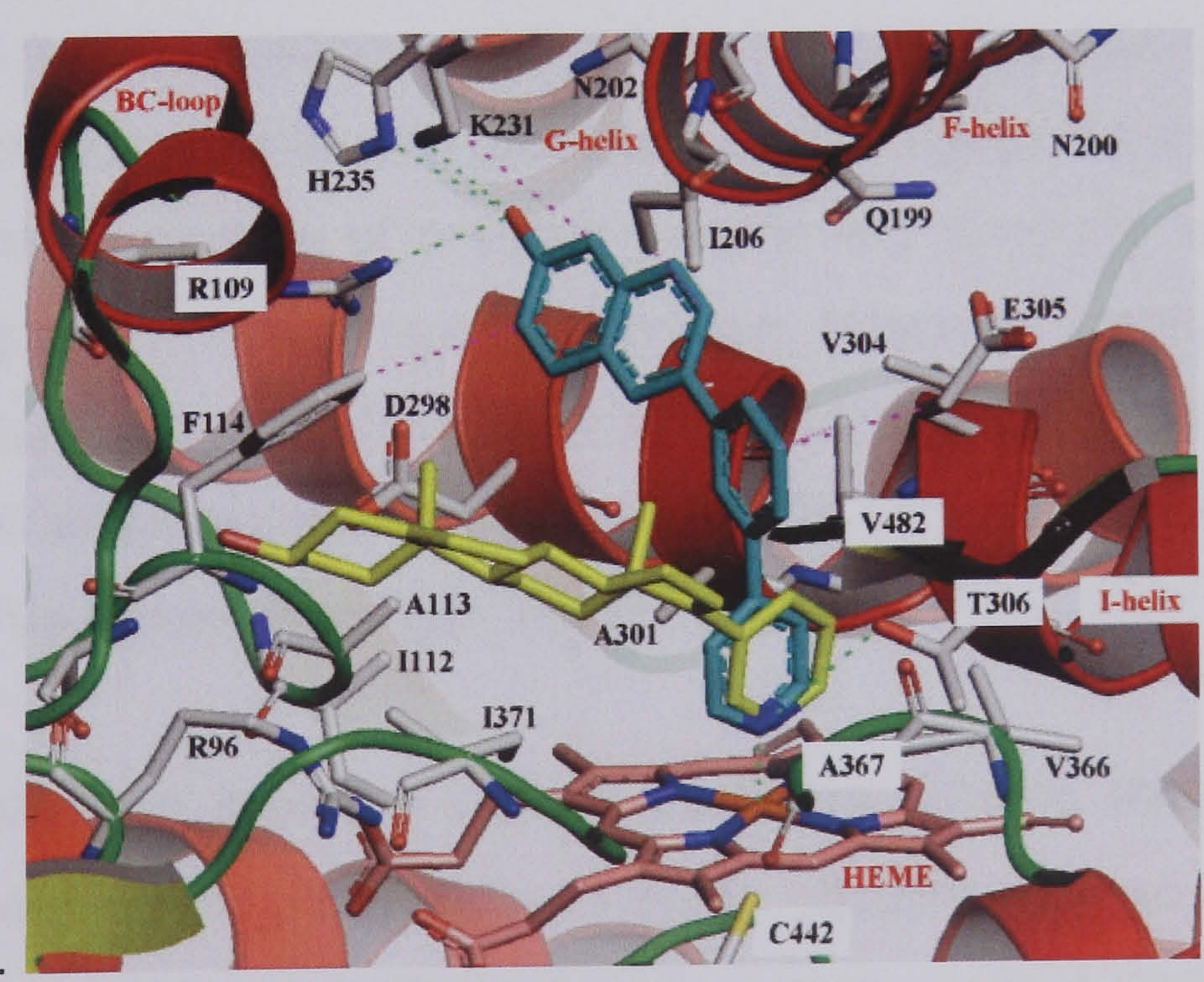


Figure 22. Docking of compound **115** (cyan) and abiraterone (**11**) (yellow) in the enzyme active site of $P450_{17\alpha}$ (Mendieta et al, 2008).

It was further proposed that introducing a halogen like chlorine or fluorine at the 1 position of the naphthalene ring, would increase the activity of **115** as it would facilitate the potentially hydrophobic interactions within the enzyme active site to interact with the inhibitor (Mendieta et al, 2008) (Figure 22).

1.10.2.6 Non-steroidal Inhibitors: Azole-based Compounds

Since the discovery of KTZ as an inhibitor of $P450_{17\alpha}$, other imidazole-based antifungals were tested against this enzyme, such as miconazole (**118**), econazole (**119**), bifonazole (**120**) and clotrimazole (**121**) (Ayub and Levell, 1987) (Table 22).

Bifonazole (**120**) was the most potent compound in the series with a K_i of 86nM and 56nM for the 17α-OHase and the 17,20-lyase components of $P450_{17\alpha}$, as compared to KTZ with a K_i of 160nM and 84nM respectively (Ayub and Levell, 1987) (Table 22).

In the study by Bruynseels and co-workers (1990), liarozole (**122**), an imidazole based non-steroidal inhibitor, showed an IC_{50} of 260nM for the rat $P450_{17\alpha}$ (Table 22). It is the first and only retinoic acid metabolism blocking agent (RAMBA) to be evaluated clinically in patients with prostate cancer. Liarozole showed promising results in pre-clinical models of prostate cancer and clinically as a second-line therapy following failure of androgen deprivation. However, liarazole's usefulness as a cancer therapy drug was limited by its lack of specificity (as it inhibits other cytochrome P450 enzymes as well), therefore it is no longer being developed as an anti-cancer drug (Bruno and Njar, 2007).

Researchers have also reported various inhibitors utilising the triazole moiety, which include compound **123** (IC_{50}=1.8μM) (Bruynseels et al, 1990) and compounds **124** and **125** which showed some activity against $P450_{17\alpha}$ (IC_{50}=11μM and 24μM respectively) (Zhuang et al, 2000) (Table 23), however they were poor

in their activity as compared to the standard KTZ (IC_{50}=0.74μM).

Compound No.	Structure	K_i	
		17α-OHase	17,20-lyase
118	R=H	668nM	325nM
119	R=Cl	599nM	243nM
120	R_1=R_2=H, R_3=phenyl	86nM	56.5nM
121	R_1=Cl, R_2=phenyl, R_3= H	170nM	81.5nM
122		260nM (Bruynseels et al, 1990)	ND
3	KTZ	160nM	84nM

Table 22. Showing various imidazole-based non-steroidal inhibitors of rat testicular microsomal $P450_{17\alpha}$ (Ayub and Levell, 1987).

In general, the imidazole-based compounds have been shown to be much superior in their activity as compared to triazole-based compounds, therefore

much of the research has been focused upon imidazole-based compounds and has resulted in the synthesis and development of extremely potent non-steroidal imidazole-based inhibitors of $P450_{17\alpha}$. One such inhibitor, for example, is YM116 (**126**) (Table 24) which proved to be a potent inhibitor of 17,20-lyase and is reported to be in clinical development, with an IC_{50} value of 4.2nM for the 17,20-lyase component of $P450_{17\alpha}$ compared to the standard KTZ with an IC_{50} value of 17nM for human $P450_{17\alpha}$ (Yoden et al, 1996).

Compound No.	Structure	IC_{50}	Reference
123		1.8μM	Bruynseels et al, 1990.
124		11μM	Zhuang et al, 2000
125		24μM	
3	KTZ	0.74μM	

Table 23. Various triazole-based non-steroidal inhibitors of $P450_{17\alpha}$.

It was also reported that **126** was a competitive inhibitor of rat 17,20-lyase with a K_i of 7nM and possesed an imidazole ring, like KTZ, which was proposed to form

a tight complex with the haem moiety (Ideyama et al, 1999). In addition to **126**, researchers, over the past ten years, have reported extremely potent and selective inhibitors of $P450_{17\alpha}$ which can be good candidates to enter clinical trials. These inhibitors (like the other non-steroidal inhibitors mentioned) are designed to mimic the A-, AB-, AC-, BC-, ABD- and ACD-ring of the steroidal backbone (Figure 19). These inhibitors will now be discussed.

Compound No.	**Structure**	**IC_{50}**	**K_i**
126		4.2 nM	7nM

Table 24. Showing inhibitory activity for YM116 (**126**), a potent inhibitor of the 17,20-lyase component of $P450_{17\alpha}$ (Yoden et al, 1996).

1.10.2.7 Non-steroidal inhibitors: Imidazole-based Biphenyls

Wachall et al (1999) reported various biphenyls where the 1-imidazolyl moiety was substituted at *para-* and *meta-*position (at one of the phenyl rings) while various other substituents were also utelised at the 3- and 4-position on the adjacent phenyl ring. The compounds showed moderate to excellent activity against rat and human $P450_{17\alpha}$ (Table 25). The inhibitors were designed to mimic the AC-rings of the steroidal backbone. The potency of the compounds where the 1-imidazolyl moiety was substituted at the *para-*position (compounds **127-129**) was shown to be superior to the corresponding *meta-*substituted biphenyls (compounds **130-132**) (Table 25). Introduction of lipophylic substituents (like fluorine or chlorine) at the 4-position in the phenyl ring, in the case of the *para-*substituted biphenyls, decreased the activity [compounds **128** (IC_{50}=0.96μM

for human $P450_{17\alpha}$) and **129** (IC_{50}=5.80μM for human $P450_{17\alpha}$)] as compared to the hydroxyl analogue **127** (IC_{50}=0.31μM for human $P450_{17\alpha}$). A similar pattern was observed for the *meta*-substituted biphenyls [compounds **130-133** (IC_{50}=13.0μM-0.087μM for human $P450_{17\alpha}$)] with the best activity observed for the di-hydroxyl analogue **133** (IC_{50}=0.087μM for human $P450_{17\alpha}$).

Compound No.	Structure	IC_{50} (Human)	IC_{50} (Rat)
127	R=OH	0.31μM	2.6μM
128	R=F	0.96μM	0.98μM
129	R=Cl	5.80μM	1.40μM
130	R_1=H, R_2=OH	0.86μM	2.9μM
131	R_1=H, R_2=F	2.90μM	6.10μM
132	R_1=H, R_2=Cl	13.0μM	5.00μM
133	R_1=OH, R_2=OH	0.087μM	3.10μM
3	KTZ	0.74μM	67μM

Table 25. Showing inhibitory activities of a range of imidazole-based non-steroidal inhibitors for human and rat $P450_{17\alpha}$ (Wachall et al, 1999).

It can be concluded that introduction of an additional hydroxyl group at the 3-position in the phenyl ring in the *meta*-substituted biphenyls (compound **133**),

increased the inhibitory activity substantially against the human $P450_{17\alpha}$ (IC_{50}=0.087μM) but not rat $P450_{17\alpha}$ (compound **133**, IC_{50}=3.10μM for rat $P450_{17\alpha}$) as compared to compound **130** where only one hydroxyl group is present in the phenyl ring (Wachall et al, 1999) (Table 25).

Zhuang and co-workers (2000) have reported the synthesis of various imidazolyl-based AC-ring mimics, showing strong inhibitory activity toward the human $P450_{17\alpha}$, for example, compounds **134** and **135** with IC_{50} values of 0.17μM and 0.24μM respectively for human $P450_{17\alpha}$. The compounds proved to be more potent than the standard KTZ (IC_{50}=67μM for human) against the human $P450_{17\alpha}$ (Table 26).

Compound No.	Structure	IC_{50} (Rat)	IC_{50} (Human)
134	R=H	1.2μM	0.17μM
135	R=F	0.54μM	0.24μM
3	KTZ	0.74μM	67 μM

Table 26. Showing inhibitory activities of imidazole-based non-steroidal inhibitors (Zhuang et al, 2000).

Against the rat enzyme, good inhibiton was also observed for both **134** and **135** showing IC_{50} values of 1.2μM and 0.54μM respectively (KTZ, IC_{50}=0.74 μM for rat).

Hutschenreuter et al (2004) have also published data from various biphenyl-based compounds mimicking the AC-rings of the steroidal backbone (compounds **136-141**) (Table 27) which showed poorer inhibitory activity as compared to the reference compound BW95 (**136**, %inhibition=67%), which had been shown in a pevious study to be the most potent compound of the series, except compound **138** (IC_{50}=0.12μM) (Hutschenreuter et al, 2004) (Table 27).

Compound No.	Structure	Biological Activity
136	R_2=R_3=R_5=H, R_4=OH (BW95)	%inhibition=67%*
137	R_2=R_5=OH, R_3=R_4=H	%inhibition=13%*
138	R_2=R_5=H, R_3=R_4=OH	IC_{50}=0.12μM
139	R_2=R_3=R_4=OH, R_5=H	%inhibition=30%*
140	R_2=R_5=H, R_3=R_4=OCH_3	%inhibition=37%*
141	R_2=R_3=R_4=OCH_3, R_5=H	%inhibition=5%*

Table 27. Showing inhibitory activities of a range of imidazole-based inhibitors (Hutschenreuter et al, 2004). *([I]=10μM).

The study was based upon the effect of substituted hydroxyl and methoxy groups at various positions in the phenyl ring. The best results were obtained in the case of hydroxyl-based compounds, in particular where the hydroxyl group was substituted at R_3 and R_4 positions (compound **138**, IC_{50}=0.12μM), however, the corresponding dimethoxy analogue **140** proved to be a weaker inhibitor of $P450_{17\alpha}$ (%inhibition=37%). The tri-hydroxy and methoxy based compounds (**139** and **141**

respectively) showed poor inhibition (Hutschenreuter et al, 2004) (Table 27).

1.10.2.8 Non-steroidal Inhibitors: Imidazole-based Phenylindanes

Zhuang et al (2000) have also reported novel imidazole-based phenylindanes, which were designed to mimic the ACD-ring of the steroidal backbone (compounds **142** and **143**, Table 28); they showed extremely potent inhibitory activity against human $P450_{17\alpha}$ compared to the standard KTZ. The unsubstituted compound **142** showed extremely good activity (IC_{50}=0.25μM) for human $P450_{17\alpha}$ as compared to the standard KTZ (IC_{50}=67μM), however, for rat $P450_{17\alpha}$ it showed poor inhibiton (IC_{50}=2.1μM) as compared to the standard KTZ (IC_{50}=0.74μM). Introduction of a fluoro group in the phenyl ring (compound **143**) decreased the activity against human $P450_{17\alpha}$ (IC_{50}=1.11μM), however, for rat $P450_{17\alpha}$ an increase in activity was seen (IC_{50}=1.7μM) as compared to **142** (Zhuang et al, 2000) (Table 23).

Compound No.	Structure	IC_{50} (Rat)	IC_{50} (Human)
142	R=H	2.1μM	0.25μM
143	R=F	1.7μM	1.11μM
3	KTZ	0.74μM	67 μM

Table 28. Showing inhibitory activities of a range of azole-based inhibitors (Zhuang et al, 2000).

1.10.2.9 Non-steroidal Inhibitors: Imidazole-based Naphthalenes

Hutschenreuter and co-workers (2004) have reported biological data from a range of azole-based dihydronaphthalenes (**144-147**), dihydronaphthalone (**149**) and naphthalene-based compound **148**, mimicking the BC-rings of the natural substrate (pregnenolone) (Table 29).

Compound No.	Structure	Biological Activity
144	R_1=H, R_2=OCH_3 (BW19)	IC_{50}= 0.11μM
145	R_1=OH, R_2=OCH_3	IC_{50}=1.10μM
146	R_1=R_2=OCH_3	IC_{50}=0.49μM
147	R_1=H, R_2=OH	%inhibition=65%*
148	HO, NH, N	%inhibition=57%*
149	O, HO, O, NH, N	%inhibition=33%*

Table 29. Showing inhibitory activities of a range of imidazole-based inhibitors (Hutschenreuter et al, 2004). *([I]=10μM).

The BC-ring mimics (compounds **144-149**) showed lower inhibitory activity against $P450_{17\alpha}$ as compared to the reference compound BW19 (**144**) (IC_{50}= 0.11μM), which had been shown in a pevious study to be the most potent compound of the

series (Hutschenreuter et al, 2004). The most potent compound among the novel BC-ring mimics was found to be **146**, with an IC_{50} value of 0.49μM, in which the two methoxy groups were thought to undrgo potential hydrophobic interactions within the enzyme active site (Hutschenreuter et al, 2004). This activity is reduced by the introduction of a hydroxyl group at the 5-position in the phenyl ring, as in compound **145**, which has an IC_{50} value of 1.10μM. Aromatisation of the B ring of the tetrahydronaphthalene, i.e. compound **148** (%inhibition=57%), resulted in decreased activity. In addition replacing the double bond and the methyl group in **145** by a carbonyl group (compound **149**, %inhibition=33%) also led to a decrease in inhibition (Table 29).

Matsunaga and co-wokers (2004) have also reported various naphthalene-based 1- and 4-imidazolyl derivatives mimicking the BC rings of the steroid substrate which have shown to be potent inhibitors of $P450_{17\alpha}$ (Table 30). Among the 1-imidazolyl derivatives, with small alkoxy substituents, compounds **151** and **152**, showed an increase in the inhibitory activity (IC_{50}=27nM and 23nM for human $P450_{17\alpha}$ respectively), as compared to the unsubstituted compound **150** (IC_{50}=43nM for human $P450_{17\alpha}$); this increase in activity was also observed in the rat $P450_{17\alpha}$. The isopropoxy compound (**153**) however showed an increase in activity only against the rat $P450_{17\alpha}$ (IC_{50}=24nM for rat) with a similar activity observed to **150** against human $P450_{17\alpha}$. The methyl sulfide-based inhibitor (**154**) also showed potent activity (IC_{50}=20nM for human and IC_{50}=18nM for rat $P450_{17\alpha}$) and was the most potent inhibitor of this series of compounds. Introduction of alkyl groups at the methylenic carbon, such as in **155** and **156**, resulted in the same level of activity as for **151** and had a negligible effect on the enzyme inhibition for both rat and human $P450_{17\alpha}$ (Table 30).

The 4-imidazolyl derivatives (**157-160**, Table 30) also showed good inhibition against human $P450_{17\alpha}$ with IC_{50} values ranging from 28nM-30nM for human $P450_{17\alpha}$. In the case of rat $P450_{17\alpha}$, good inhibiton of the enzyme was also observed, with IC_{50} values ranging from 9.5nM-26nM (Table 30).

Compound No.	Structure	IC_{50}* (Rat)	IC_{50}* (Human)
150	R_1=H, R_2=H	53nM	43nM
151	R_1=OCH_3, R_2=H	27nM	27nM
152	R_1=OC_2H_5, R_2=H	26nM	23nM
153	R_1=*iso*-OC_3H_7, R_2=H	24nM	46nM
154	R_1=SCH_3, R_2=H	18nM	20nM
155	R_1=OCH_3, R_2=CH_3	22nM	21nM
156	R_1=OCH_3, R_2=*iso*-CH_3	26nM	24nM
157		22nM	30nM
158	R=6-OCH_3	21nM	28nM
159	R=5,6-diOCH_3	26nM	29nM
160	R=6,7-diOCH_3	9.5nM	29nM

Table 30. Inhibitory activities of a range of imidazole-based non-steroidal inhibitors (Matsunaga et al, 2004). *(17,20-Lyase).

The 4-imidazolyl derivatives proved to be, in general, similar in their activity for human $P450_{17\alpha}$ as compared to the 1-imidazolyl derivatives. However, the 4-imidazolyl derivatives proved to be more potent in the case of rat $P450_{17\alpha}$, with compound **160** showing the best result (IC_{50}=9.5nM) (Table 30).

Computer modeling indicated a potential hydrogen bond formation between the methoxy oxygen of **158** (*S*-isomer) (Table 30) with the hydroxyl group of Thr10, which has been postulated to be present in the active site of $P450_{17\alpha}$ (Figure 23).

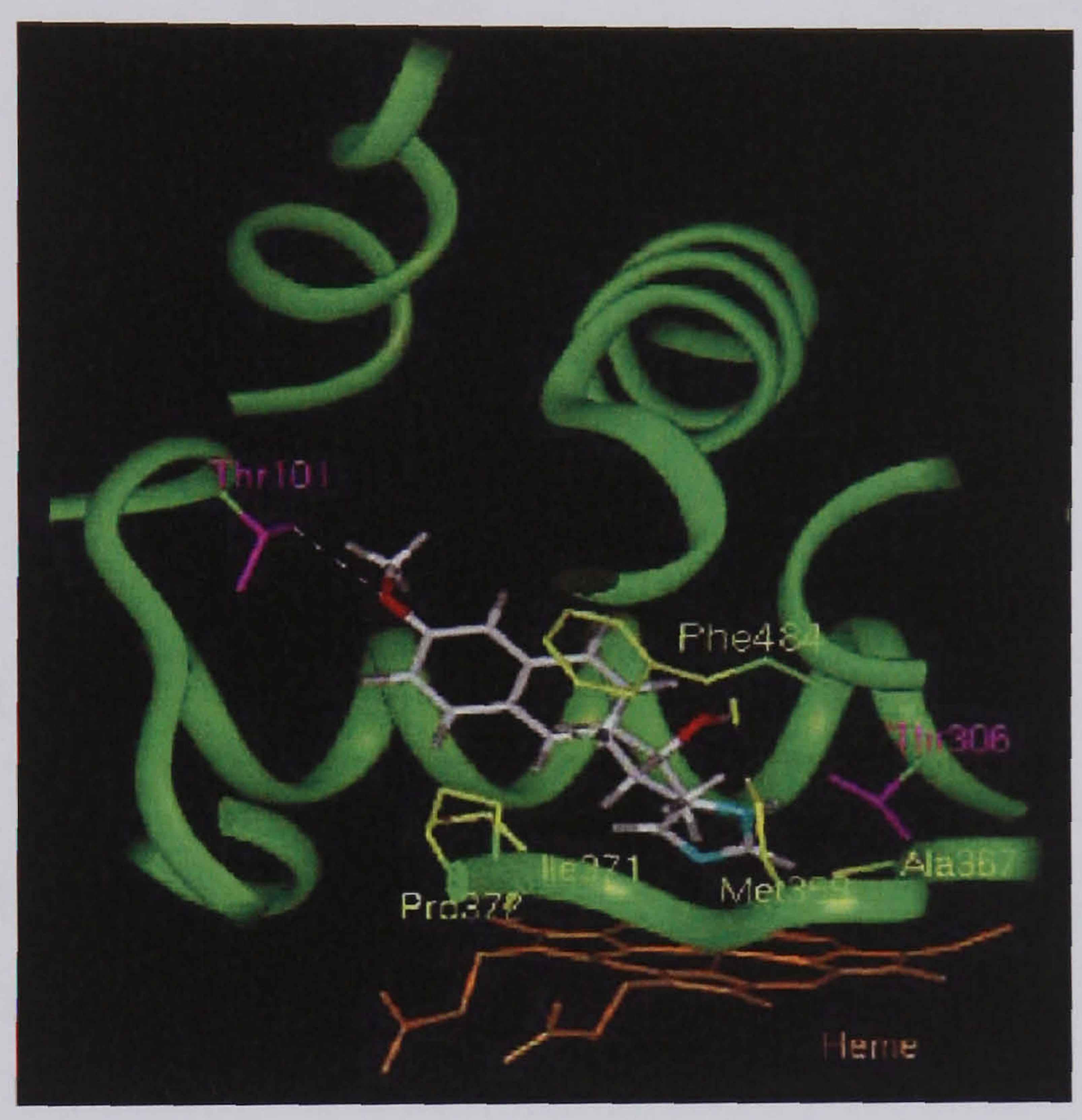


Figure 23. The proposed binding mode of **158** at the active site in a human 17,20-lyase model. Selected active site residues are labelled; orange, haem; magenta, polar residues; yellow, lipophilic residues (Matsunaga et al, 2004).

Furthermore, the docking mode suggests that the isopropyl group of **158** fits into the lipophilic pocket, which is constructed by Ala367, Met369, Ile371, Pro372 and Phe484 in the enzyme active site (Figure 23). Thus the introduction of a hydroxy group at the methylene carbon tends to increase the inhibitory activity, by interacting with a potential hydrogen bonding group. The best results were

achieved by the incorporation of a hydroxyl group and an isopropyl group, as described for **158** and **159** (Matsuanga et al, 2004) (Table 30).

Hartmann and coworkers (2004) have also reported various naphthalene-based BC-ring mimics and have shown the effect of fluorine substituted in the naphthalene ring (Table 31). The most potent inhibitor in their study was **162**, with an IC_{50} value of 0.27μM.

Compound No.	Structure	%inhibition*	IC_{50}
161	R1, R2, R3, R4 substituted naphthalene with CH2-imidazole (N) ; R_1=F, R_2=R_3=H, R_4=CH_3	74%	1.00μM
162	R_1=F, R_2=OCH_3, R_3=H, R_4=CH_3	88%	0.27μM
163	R_1=F, R_2=OCH_3, R_3=R_4=H	79%	0.41μM
164	R_1=F, R_2=OCH_3, R_3=F, R_4=CH_3	79%	0.50μM
165	R_1=F, R_2=OCH_3, R_3=F, R_4=H	70%	1.00μM

Table 31. Showing inhibitory activities of a range of imidazole-based non-steroidal inhibitors (Hartmann et al, 2004). *([I]=25μM), ND=not determined.

The study shows that there was an increase in potency with the introduction of fluorine and methoxy substituents (compounds **161-165**) with IC_{50} values ranging from 0.27μM-1.00μM. In addition, the mono-fluorinated inhibitors with a methoxy group (compounds **162** and **163**) proved to be more potent (with IC_{50} values of 0.27μM and 0.41μM respectively) than the di-fluorinated inhibitors with a methoxy group (compounds **164** and **165** with IC_{50} values of 0.50μM and 1.00μM respectively) (Hartmann et al, 2004) (Table 31).

Matsunaga and coworkers (2004) modified the naphthalene ring by adding a heteroatom, like nitrogen (quinoline, compound **167**) and oxygen (chromene, compound **168**) in the naphthalene ring, in an attempt to design inhibitors to mimic the AB-ring of the steroidal backbone (Table 32).

Compound No.	Structure	IC_{50}*
166	X=Y=CH	26nM
167	X=N, Y=CH	95nM
168	X=O, Y=CH_2 (ignore double bond between X and Y)	29 nM
3	KTZ	240nM

Table 32. Showing inhibitory activities of a range of imidazole-based non-steroidal inhibitors (Matsunaga et al, 2004). *(Rat 17,20-lyase).

The inhibitors showed good to excellent inhibiton of rat 17,20-lyase. Compound **166** (where the naphthalene ring was not modified using the heteroatom) proved to be the most potent inhibitor of 17,20-lyase among this series of compounds with an IC_{50} value of 26nM compared to the standard KTZ (IC_{50} value of 240nM).

The *2H*-chromene-based derivative, **168**, exhibited extremely good activity with an IC_{50} value of 29nM (similar to that of **166**), while the quinoline derivative **167** showed good inhibition (IC_{50}=95nM) as compared to the standard KTZ (IC_{50}= 240nM), though was much weaker as compared to **166** and **168** (Table 32).

1.10.2.10 Non-steroidal Inhibitors: Imidazole-based Benzothiophenes

Matsunaga and coworkers (2004) also produced a series of benzothiophene derivatives which showed excellent inhibition of $P450_{17\alpha}$ (Tables 33 and 34). Among the 1-imidazolyl-based inhibitors (Table 33), compound **169** proved to be a potent inhibitor (IC_{50}=16nM) as compared to the standard KTZ (IC_{50}= 240nM). In addition, the potency of **169** was increased when a fluoro group was introduced at the 7-position in the benzothiophene ring (compound **173**, IC_{50}=10nM). Introduction of a methyl group at the 2-position in the thiophene ring did not increase the activity (compounds **175-177**), with IC_{50} values ranging from 25-33nM (Table 33).

Among the 4-imidazolyl derivatives (Tables 34), compounds **178**, **179** and **181** showed strong inhibition of $P450_{17\alpha}$, with IC_{50} values of 4nM, 9nM and 6nM respectively. The key structural features required for the potency of these compounds were found to be the 5-fluoro group on the benzothiophene ring and the 4-imidazolyl moiety (compounds **178-181**). Compounds **182-185**, in particular, where a fluoro- group was attached at various positions in the phenyl ring of the benzothiophene moiety, were also found to be potent inhibitors of the human $P450_{17\alpha}$, with IC_{50} values ranging from 19-27nM (Matsuanga et al, 2004) (Table 34).

1.10.2.11 Non-steroidal Inhibitors: Imidazole-based Phenylthiophenes

Jagusch et al (2008) have reported a range of substituted phenylthiophenes [compounds **186-192**, (Table 35)] as inhibitors of $P450_{17\alpha}$. Introduction of an ethyl group at the methylene bridge (compound **189**, %inhibition=68%), led to an increase in the activity compared to compound **188** (%inhibition=25%). In addition compounds **191** and **192** proved to be highly active inhibitors of $P450_{17\alpha}$. Both the insertion of a fluorine atom into the phenyl ring (compound **191**, IC_{50}=236 nM) and a 5-chloro substituent in the thiophene ring (compound **192**, IC_{50}=263

nM) increased the potency and led to the most active inhibitors of this study (Table 35).

Compound No.	Structure	IC_{50}^{*}
169	R1, R2 substituted benzothiophene structure; $R_1=R_2=H$	16nM
170	R_1=4-F, R_2=H	20nM
171	R_1=5-F, R_2=H	25nM
172	R_1=6-F, R_2=H	26nM
173	R_1=7-F, R_2=H	10nM
174	R_1=5-Cl, R_2=H	29nM
175	R_1=5-F, $R_2=CH_3$	25nM
176	R_1=5-Cl, $R_2=CH_3$	26nM
177	$R_1=5\text{-}OCH_3$, $R_2=CH_3$	33nM
3	KTZ	240nM

Table 33. Showing inhibitory activities of a range of imidazole-based non-steroidal inhibitors (Matsunaga et al, 2004). *(Rat 17,20-lyase).

The structure-activity relationships obtained in this study demonstrate that an alkyl substituent at the methylene bridge potentially plays a role in fitting into the proposed hydrophobic pocket near the haem, and hence strongly contributes to the inhibitory activity of these compounds (Jagusch et al, 2008) (Table 35).

Compound No.	Structure	IC_{50}*
178	R_1=R_2=H, R_3=CH_3	4nM
179	R_1= CH_3, R_2=H, R_3=CH_3 (S)	9nM
180	R_1= CH_3, R_2=H, R_3=CH_3 (R)	8nM
181	R_1=R_2=R_3=CH_3	6nM
182	R=4-F	19nM
183	R=5-F	23nM
184	R=6-F	27nM
185	R=7-F	25nM
3	KTZ	240nM

Table 34. Showing inhibitory activities of a range of imidazole-based non-steroidal inhibitors (Matsunaga et al, 2004). *(Rat 17,20-lyase).

The compounds showed moderate to extremely good inhibitory activity with %inhibition ranging from 21-92% compared to the standard KTZ with a %inhibiton of 92% (Table 35).

Compound No.	Structure	Biological Activity
186	$R_1=R_2=R_3=H$	%inhibition=21%*
187	$R_1=OCH_3$, $R_2=R_3=H$	%inhibition=24%*
188	$R_1=R_2=F$, $R_3=H$	%inhibition=25%*
189	$R_1=R_2=F$, $R_3=C_2H_5$	%inhibition=68%*
190	$R_1=H$, $R_2=H$, $R_3=iso\text{-}C_3H_7$, $R_4=OH$	%inhibition=49%*
191	$R_1=2\text{-}CH_3$, $R_2=F$, $R_3=C_2H_5$, $R_4=H$	%inhibition=92%* IC_{50}=236nM
192	$R_1=5\text{-}Cl$, $R_2=F$, $R_3=C_2H_5$, $R_4=H$	%inhibition=85% * IC_{50}=263nM
3	KTZ	%inhibition=92%* IC_{50}=280nM

Table 35. Showing inhibitory activities of a range of imidazole-based non-steroidal inhibitors (Jagusch et al, 2008). *([I]=2.0μM).

1.10.2.12 Non-steroidal inhibitors: Imidazole-based Phenylnaphthalenes

Mendieta and co-workers (2008) have reported a range of substituted phenyl naphthalene-based compounds designed to mimic the ABD- and ACD-rings of the steroidal backbone (Tables 36 and 37).

The ABD mimetics (compounds **193** and **194**, Table 36) showed moderate activities (%inhibition=28% and 61% respectively) compared to KTZ (%inhibition=92%).

Compound No.	Structure	%inhibition*
193		28%
194		61%
3	KTZ	92%

Table 36. Showing inhibitory activities of imidazole-based non-steroidal inhibitors (Mendieta et al, 2008). *([I]=2.0μM).

Amongst the ACD mimetics, compounds **195-197** (Table 37), compound **197**, where the methylene-imidazolyl moiety was attached at position 8 in the naphthalene ring, showed the best inhibition (%inhibition=50%).

It can also be observed that the introduction of a 2-methoxy group, as in

compound **195**, leads to a moderate inhibition of the enzyme (%inhibition=45%) (Table37).

Compound No.	Structure	%inhibition*
195		45%
196		14%
197		50%
3	KTZ	92%

Table 37. Showing inhibitory activities of imidazole-based non-steroidal inhibitors (Mendieta et al, 2008). *([I]=2.0μM).

1.11 Basis of present investigation

The use of enzyme inhibitors in the treatment of hormone-dependent cancer has been shown to be of therapeutic use. As such, the synthesis of inhibitors of $P450_{17\alpha}$ may have potential benefits against hormone-dependent prostate cancer, where the long term exposure of the prostate tissue to androgens has been shown to increase the risk of developing prostate cancer. However, due to the location of the enzyme within the prostate cell (i.e. within the endoplasmic reticulum) it has not been possible to obtain a crystal structure for this enzyme, as such, workers have utilised molecular modeling techniques such as homology modeling (Laughton et al, 1990; Lewis and Lee-Robichaud, 1998) in an attempt to gain further information regarding the active site of this enzyme complex.

Ahmed and Davis (1995) have previously proposed an alternative technique which allows the determination of the approximate representation of the active site (for potentially all cytochrome P450 enzymes) through the consideration of the essential elements which are presumed to be at the active site, in particular, the haem, substrate and potential hydrogen bonding groups which would be expected to bind to specific areas of the substrate, as such, resulting in the 'substrate-haem complex' (SHC).

Using this novel approach, the SHC was produced for the two components of this overall enzyme complex, namely the 17,20-lyase component (Ahmed and Owen, 1998) and the 17α-OHase component (Ahmed, 1995). A number of compounds have previously been designed, synthesised and subsequently evaluated against the $P450_{17\alpha}$ enzyme complex, in particular, it was postulated that the initial (and more important) interaction was that between the heteroatom (e.g. N of groups such as imidazole and triazole) of the inhibitor and the Fe of the haem moiety within the enzyme. Once the initial Fe-N interaction had taken place, the remainder of the molecule then undergoes polar-polar/hydrogen bonding interaction with groups at the active site, as such, the combined interactions

between the active site and the inhibitor results in an overall inhibition of the enzyme. Furthermore, from the molecular modeling of poly-substituted compounds, it is postulated that the increase in the number of interactions between the inhibitor and the enzyme active site may lead to an increase in the level of inhibition observed.

From the molecular modeling study, benzyl imidazole-based compounds were proposed to possess a good inhibitory profile, as such, this study involves the synthesis (and subsequent biochemical evaluation) of a series of compounds based on those shown in Figure 24. That is, a range of substituted benzyl imidazole based compounds are the major targets in an effort to study the interaction(s) between the substituent R and the active site. A number of non-substituted phenyl alkyl imidazoles will also be studied in an effort to consider the role of physicochemical factors such as hydrophobicity in terms of the logarithm of the partition coefficient (logP).

Figure 24. Compounds under consideration within the present investigation (where R=various groups, including H, F, Cl, Br, I, NO_2, CH_3 etc; n=1 to 9).

CHAPTER 2

SYNTHESIS OF DERIVATIVES OF BENZYL IMIDAZOLES

2.0 SYNTHESIS OF DERIVATIVES OF BENZYL IMIDAZOLE

2.1 Discussion

Azole-based compounds have been investigated for some time as inhibitors of a number of cytochrome P450 enzymes such as 14α-demethylase (as anti-fungal agents), aromatase (as treatments against hormone-dependent breast cancer) and the enzyme complex $P450_{17\alpha}$ (as potential treatment for prostate cancer), resulting in the synthesis of a wide range of compounds, containing various structural features.

In an effort to investigate the SHC approach as a potential novel molecular modeling tool, a number of benzyl imidazole-based compounds were designed and their potential to inhibit the two components of the overall enzyme complex $P450_{17\alpha}$ was determined using the SHC technique. In the design process, the main interaction which was modeled was the initial Fe-N bond formation, however, potential interactions between the inhibitor and hydrogen bonding groups which are presumed to be at the active site [corresponding to the C(3) area of the steroid backbone] of the overall enzyme complex were also considered as the additional interaction with the active site is expected to increase the inhibition of the enzyme. As such, within the current study, the synthesis and biochemical evaluation of the designed compounds would therefore allow us to determine the usefulness of this theoretical model. The present investigation therefore involves the synthesis of a range of imidazole-based compounds as potential inhibitors of 17,20-lyase and 17α-OHase.

As discussed in the previous sections, a wide range of compounds have been investigated as inhibitors of the two components of the overall $P450_{17\alpha}$ enzyme complex, namely 17,20-lyase and 17α-OHase for some time. The compounds which have been shown to the more potent inhibitors of $P450_{17\alpha}$ are the imidazole-based, compounds where the azole functionality is postulated to

interact with the P450 haem moiety via a co-ordinate bond formation between the Fe of the haem and the sp^2 hybridised N(3) lone pair of electrons of the azole. In an effort to add further support to the SHC approach as a molecular modeling tool in the design of novel potential inhibitors of $P450_{17\alpha}$, a number of benzyl imidazole-based compounds were designed (Figure 25).

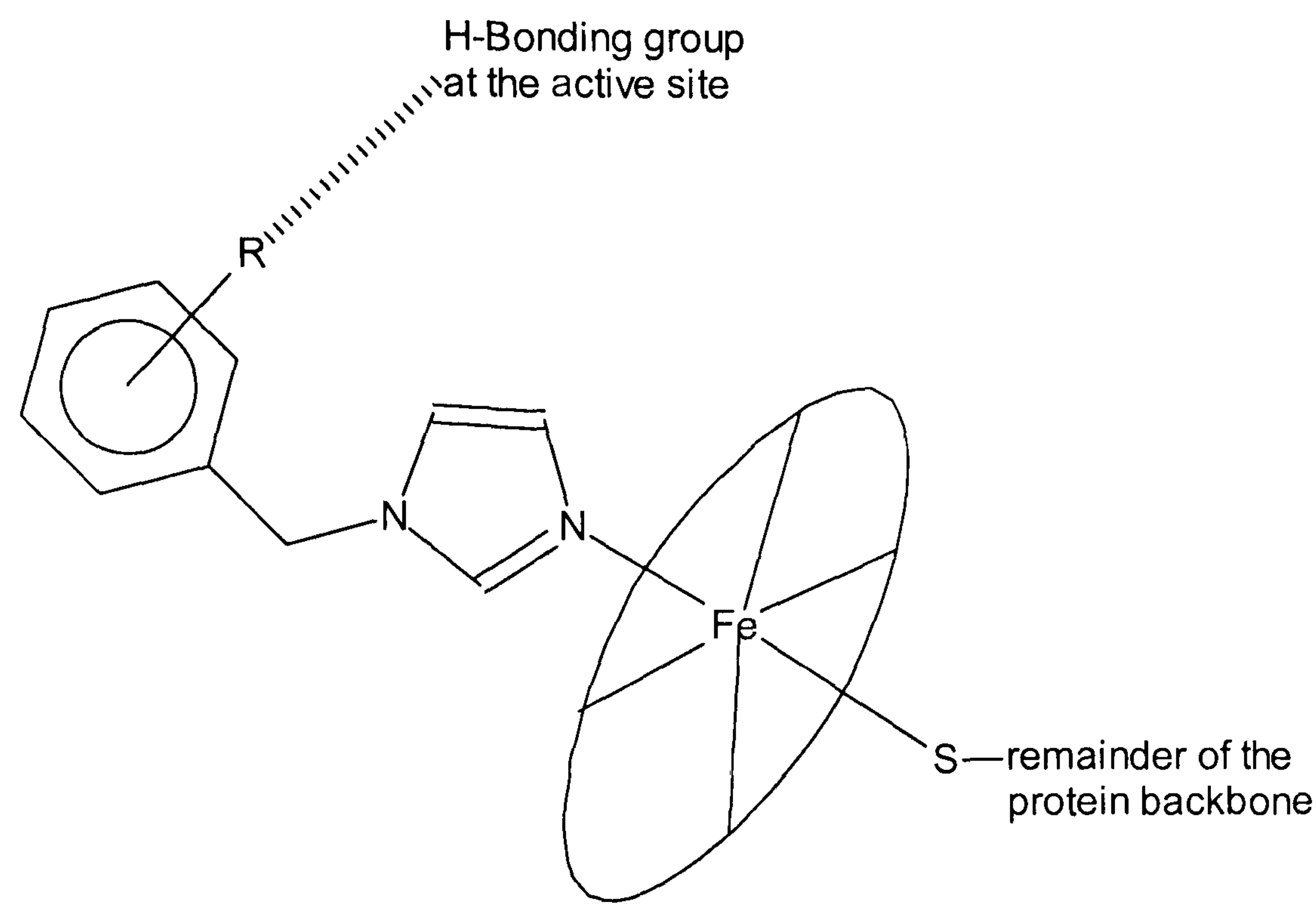


Figure 25. To show the potential interactions considered by the SHC in the design of potential inhibitors of $P450_{17\alpha}$.

In the design of novel benzyl imidazole-based compounds using the SHC, consideration of potential interactions between the inhibitor and hydrogen bonding group(s) were considered [the hydrogen bonding groups are presumed to be at the active site of the overall enzyme complex and are optimally positioned to be able to interact with the C(3) polar groups within pregnanes and progestins]. Within the current study, we report the synthesis of a series of derivatives of benzyl imidazole where the phenyl moiety was substituted so as to undergo potential interaction with the hydrogen bonding moieties which interact with the initial substrate (e.g. pregnenolone or progesterone) as well as the 17α-hydroxylated steroid (namely, 17α-hydroxypregnenolone and 17α-

hydroxyprogesterone respectively) – the subsequent biochemical evaluation of the synthesised compounds would therefore aid the determination of the structure-activity relationship of the compounds and therefore determine the usefulness of the SHC approach.

Two methods may be used in the synthesis of *N*-alkylated benzyl imidazole-based compounds. The first involves the synthesis of the imidazole moiety from the appropriate amine (Cannon et al, 1957; Zoorob et al, 1990) whilst the second method involves the *N*-alkylation of the azole ring (Baggaley et al, 1975; Ahmed, 1990; Ahmed et al, 1995a). The *N*-alkylation of the azole moiety using an alkyl halide in the presence of a base has been used extensively and has been shown to be the favoured method (Grimmett, 1970; Ahmed et al, 1995a). As such, in the synthesis of derivatives of benzyl imidazole, we considered the use of the reaction conditions outlined in Scheme 2.1.

Br a N N R R

Scheme 2.1 Synthesis of derivatives of benzyl imidazole-based compounds (a=imidazole/K_2CO_3/THF/Δ; R=various substituents including H, Ph, F, Cl, Br, I, NO_2, CN, OH, CF_3, OCH_3, SCH_3, alkyl moiety, and alternative substituents including various sulfonates).

In the synthesis of the non-sulfonated benzyl imidazole derivatives [e.g benzyl imidazole (**198**) to 1-(4-methyl-benzyl)-1*H*-imidazole (**241**) and 1-(3,5-dimethyl-benzyl)-1*H*-imidazole (**254**) to 1-(biphenyl-4-ylmethyl)-1*H*-imidazole (**258**)], the reactions where the appropriate benzyl bromide were commercially available, in general, proceeded in good yield (ranging from 25% to 70%) and without any major problems. It is proposed that the mechanism of the reaction in Scheme 2.1

involves an initial attack by the imidazole moiety on the benzyl bromide moiety as opposed to the deprotonation of the imidazole group by potassium carbonate (K_2CO_3). However in case of some of the fluorinated compounds, the NMR showed fluorine coupling, which led to a complicated spectra in particular the ^1H spectrum. The J values for the H-F splitting could not be determined as the signals overlapped with the H-H signals leading to the production of mulitplets (as reported in the experimental). In case of the C-F splitting, the signals were either too weak too be detected (forming very tiny shoulders with ^{13}C signals) or were overlapping with ^{13}C signals or they were too downfield to be detected (due to the electron withdrawing effect of the fluorine atom).

In the synthesis of 4-hydroxybenzyl imidazole (**263**), a different method was, however, required due to the potential presence of the labile proton present within 4-hydroxybenzyl bromide. That is, the introduction of even a mild base such as anhydrous K_2CO_3, would result in the deprotonation of the phenolic proton [pK_a of phenol has been shown to be 9.89 (Kato-Toma et al, 1998)] as opposed to the desired deprotonation of the imidazole moiety (pK_a of 6.95). Even if the imidazolyl ion had been formed prior to the addition of 4-hydroxybenzyl bromide, the labile proton would have been expected to neutralise the azolyl ion in preference to the alkylation reaction. Our search for a method for the synthesis of compound **263** led us to the work of Machin et al (1984). In their study, the authors showed that the heating of 4-hydroxybenzyl alcohol in the presence of excess imidazole, in the absence of any solvent, resulted in the production of the target compound in relatively high yield (Scheme 2.2). In our hands, the use of the reaction conditions resulted in compound **263** in excellent yield (80%).

HO–C6H4–CH2–OH —a→ HO–C6H4–CH2–N(imidazole)

Scheme 2.2 Synthesis of 4-hydroxybenzyl imidazole (a= imidazole/Δ)

As previously stated, in the synthesis of the benzyl imidazole-based compounds, the reactions outlined in Scheme 2.1 were attempted in the synthesis of the target compounds where the starting benzyl bromide was commercially available. For all other compounds where the appropriately substituted benzyl bromide was not commercially available, the synthesis of the initial bromide derivative was required. For example, in the synthesis of potential inhibitors where the phenyl moiety is substituted with an n-alkyl chain [e.g. 1-(4-propyl-benzyl)-1*H*-imidazole (**244**)], the appropriate benzyl bromide was not commercially available. However, we discovered that the 4-alkylated derivatives of benzoic acid were commercially available, for example, for compound **244**, 4-propyl-benzoic acid was available. As such, in the synthesis of compound **244**, we initially undertook a reaction to reduce the carboxylic acid moiety within 4-propyl-benzoic acid, using a reducing agent [e.g. lithium aluminium hydride ($LiAlH_4$)], to give the alcohol derivative [namely, (4-propyl)-phenyl methanol (**242**)] followed by the bromination of the hydroxy moiety to give the desired 4-n-alkylated-benzyl bromide [namely, 4-propylbenzyl bromide (**243**)] (Scheme 2.3).

R O OH a R OH b R Br

Scheme 2.3 Reactions undertaken in the synthesis of 4-n-alkyl derivatives of benzyl bromide (a=$LiAlH_4$/THF/Δ; b=PBr_3/diethyl ether/Δ).

The reactions, in general, proceeded in good yield with the 4-n-alkyl derivatives of benzyl bromide being obtained in yields ranging from 60% [for 4-butylbenzyl

bromide (**246**)] to 72% [for 4-heptylbenzyl bromide (**252**)] and without any major problems. In the synthesis of the target imidazole-based compounds [i.e. 1-(4-propyl-benzyl)-1*H*-imidazole (**244**), 1-(4-butyl-benzyl)-1*H*-imidazole (**247**), 1-(4-pentyl-benzyl)-1*H*-imidazole (**250**) and 1-(4-heptyl-benzyl)-1*H*-imidazole (**253**)], reaction conditions outlined in Scheme 2.1 were carried out between the appropriate benzyl bromide and imidazole and were found to proceed in good yield (ranging from 60% for compound **250** to 80% for compound **253**) and without any major problems.

In the synthesis of the 4-sulfonate derivatives of benzyl imidazole [compounds 4-(1*H*-imidazol-1-ylmethyl)phenyl methanesulfonate (**264**) to 4-(1*H*-imidazol-1-ylmethyl)phenyl 3,5-dimethylisoxazolesulfonate (**296**)], the reaction outlined in Scheme 2.4 was undertaken using anhydrous dichloromethane (DCM) as the reaction solvent and using triethylamine (TEA) as a weak base so as to enhance the production of the phenoxide ion, thereby aiding the nucleophilic substitution of the chloride moiety with the phenolic backbone.

The reaction was found to progress in moderate yield [44% for compound 4-(1*H*-imidazol-1-ylmethyl)phenyl 4-chlorobenzenesulfonate (**276**)] to excellent yield [76% for compound 4-(1*H*-imidazol-1-ylmethyl)phenyl-1-propanesulfonate (**267**)]. Whilst a number of target compounds were obtained in good yield, a number of compounds were obtained in poor yield due to problems in the purification of these compounds. That is, the purification of a number of 4-sulfonated derivatives of compound **263** required extensive column chromatography to be undertaken, thereby resulting in the loss of sample and therefore a lower yield.

HO–C6H4–CH2–(imidazol-1-yl) —a→ R–S(=O)2–O–C6H4–CH2–(imidazol-1-yl)

Scheme 2.4. Synthesis of sulfonate derivatives of 4-hydroxybenzyl imidazole (where a=DCM/sulfonyl chloride derivative/TEA; R=various substituents)

A series of phosphate derivatives were also attempted, however, in most cases the solubility of the target compounds in water was such that the compounds could not be obtained in sufficient yield, as a result only 4-(1*H*-imidazol-1-ylmethyl)phenyl diphenyl phosphate (**297**) was obtained. Due to a lack of time, the remaining range of compounds was not synthesised.

Synthesis of phenyl alkyl imidazole-based compounds

In an effort to take into consideration the effect of the logarithm of the partition coefficient (logP), we also undertook the synthesis of a range of phenyl alkyl imidazole-based compounds (Figure 26).

Figure 26. To show the phenyl alkyl imidazole-based compounds synthesised (n=1 to 8).

In the synthesis of the phenyl alkyl imidazole derivatives, where the starting phenyl alkyl bromide (in place of benzyl bromide) was commercially available, the reaction outlined in Scheme 2.1 was attempted, however, only phenyl ethyl bromide was found to be commercially available, as such, in the synthesis of phenyl ethyl imidazole (**298**) [where n=1 (Figure 26)], the reaction proceeded in good yield (84%) and without any major problems.

In the synthesis of phenyl propyl imidazole (**300**), the phenyl propyl bromide was not commercially available, however, phenyl alkyl alcohol was available. As such, in the synthesis of **300**, we undertook the last two steps outlined in Scheme 2.3 prior to following the reaction outlined in Scheme 2.1, that is, initial bromination of phenyl propyl alcohol gave compound **299**, which was then used in the alkylation of imidazole to give **300**.

For the remaining four non-substituted phenyl alkyl imidazole-based compounds, the synthesis of the appropriate phenyl alkyl bromide was required. In the case of phenyl butyl imidazole (compound **303**), this involved using the reactions previously outlined in Scheme 2.3. That is, in the synthesis of **303**, the initial step involved the reduction of 4-phenyl butanoic acid to give 4-phenyl butanol (**301**), followed by bromination to give 4-phenyl butyl bromide (**302**). The reactions, in general, proceeded in good yield (ranging from 69% to 76%) and without any major problems. In the synthesis of the target imidazole-based compound, the reaction outlined in Scheme 2.1 was attempted between the phenyl butyl bromide and imidazole and was found to proceed in good yield (69%).

In the synthesis of non-substituted phenyl pentyl bromide to phenyl heptyl bromide [i.e. where n=4 to n=6 (Figure 26)], we investigated reactions in an effort to extend the alkyl spacer group between the phenyl ring and the bromine atom. A general method used in alkyl chain extension involves the use of an appropriate alkyl bromide, where the bromo functionality undergoes nucleophilic substitution involving the use of CN^- followed by acid hydrolysis to give the corresponding carboxylic acid. The reactions outlined in Scheme 2.3 could then be utilised so as to derivatise the chain extended carboxylic acid derivative to the appropriate alcohol and subsequently to alkyl bromide.

Due to the carcinogenic nature of CN^- the KCN route was not preferred and we therefore considered the possibility of using a malonate functionality in extending the alkyl chains. The use of the malonate functionality is particularly useful since hydrolysis and decarboxylation are easily achieved during acid hydrolysis, resulting in the addition of the C_2 moiety. The synthesis of the phenyl pentyl bromide (**307**) to phenyl heptyl bromide (**317**) was therefore attempted using Scheme 2.5.

However, we discovered a number of problems involving the use of the reactions

outlined in Scheme 2.5. That is, the alkylation of the malonate moiety (step a, Scheme 2.5) resulted in di-substitution of the α-carbon, resulting in a mixture of compounds which could not be easily separated via column chromatography. The acid hydrolysis of the malonate derivatives to give the carboxylic acid derivative was also attempted, however, the acid hydrolysis did not yield sufficient amount of compound so as to attempt the separation of the target compound from the bis-phenyl derivative (Scheme 2.6).

Scheme 2.5 Reactions undertaken in the extension of the alkyl chain spacer group between the phenyl ring and the bromo functionality (a=diethyl malonate /Bu[t]OK/THF; b=HCl/H_2O/Δ; c=$LiAlH_4$/THF/Δ; d=PBr_3/diethyl ether/Δ; n= 3, 4 or 5].

Scheme 2.6. Synthesis of undesired bisphenyl derivative (a=diethyl malonate/Bu^{t}OK/THF).

We therefore considered the use of the triethylmethane tricarboxylate in place of diethyl malonate (Scheme 2.7). In general, the reactions leading to the bromo derivatives proceeded in moderate to good yield, ranging from 25% to 92%, and without major problems. The synthesis of the target imidazole containing compound from the appropriate phenyl alkyl bromide was therefore undertaken using the reaction outlined in Scheme 2.1; in general, the reactions were found proceed in good yield and without any major problems.

In the synthesis of the phenyl alkyl bromides containing longer alkyl chains, that is, for non-substituted phenyl octyl bromide to phenyl nonyl bromide [where n=7 to n=8 (Figure 26)], we discovered that the corresponding alcohols were commercially available. As such, we utilised the reaction conditions outlined in Scheme 2.5 (step d) in the conversion of the phenyl alkyl alcohol to the appropriate phenyl alkyl bromide. Again, the reactions proceeded in good yield, ranging from 40% to 90%, and without major problems.

Scheme 2.7. Reactions undertaken in the extension of the alkyl chain spacer group between the phenyl ring and the bromo functionality (a=triethylmethane tricarboxylate/Bu^{t}OK/THF; b=HCl/H_2O/Δ; c=$LiAlH_4$/THF/Δ; d=PBr_3/diethyl ether/Δ; n= 3, 4 or 5].

2.1.1 Compounds Synthesised

Various imidazole-based compounds were synthesised possessing the benzyl (**198-297**) and phenyl alkyl (**298-234**) backbones and are listed in Table 38a to 38t.

Compound NO	Compound Structure
198	
198	
200	
201	
202	

Table 38a. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
203	
204	
205	
206	
207	
208	

Table 38b. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
209	Cl, N, N
210	Cl, N, N
211	Cl, Cl, N, N
212	Cl, Cl, N, N
213	Cl, Cl, N, N
214	Cl, Cl, N, N
215	Cl, Cl, N, N

Table 38c. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
216	Cl, Cl, N, N
217	Br, N, N
218	Br, N, N
219	Br, N, N
220	Br, Br, N, N
221	Br, Br, N, N
222	I, N, N

Table 38d. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
223	I, N, N
224	N, N, I
225	NO_2, N, N
226	O_2N, N, N
227	N, N, O_2N
228	CN, N, N
229	NC, N, N

Table 38e. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
230	
231	
232	
233	
234	
235	

Table 38f. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
236	MeO, N, N
237	MeO, N, N
238	S, N, N
239	CH_3, N, N
240	H_3C, N, N
241	H_3C, N, N
242	OH

Table 38g. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
243	Br
244	N N
245	OH 3
246	Br 3
247	N N 3
248	OH 4
249	Br 4

Table 38h. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
250	N N 4
251	OH 6
252	Br 6
253	N N 6
254	H_3C N N CH_3
255	CH_3 H_3C H_3C N N CH_3

Table 38i. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
256	
257	Br
258	
259	Br Br
260	
262	

Table 38j. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
263	HO, N, N
264	$H_3C-S(=O)_2-O$, N, N
265	$H_5C_2-S(=O)_2-O$, N, N
266	$H_7C_3-S(=O)_2-O$, N, N
267	$H_9C_4-S(=O)_2-O$, N, N
268	$H_{17}C_8-S(=O)_2-O$, N, N

Table 38k. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
269	$H_{21}C_{10}$—SO_2—O— (4-(imidazol-1-ylmethyl)phenyl)
270	$H_{25}C_{12}$—SO_2—O— (4-(imidazol-1-ylmethyl)phenyl)
271	F_3C—SO_2—O— (4-(imidazol-1-ylmethyl)phenyl)
272	Ph—SO_2—O— (4-(imidazol-1-ylmethyl)phenyl)
273	F (3-fluorophenyl)—SO_2—O— (4-(imidazol-1-ylmethyl)phenyl)
274	F—(4-fluorophenyl)—SO_2—O— (4-(imidazol-1-ylmethyl)phenyl)

Table 38I. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
275	
276	
277	
278	
279	
280	

Table 38m. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
281	O_2N–C6H4–SO2–O–C6H4–CH2–(imidazol-1-yl)
282	3-F_3C–C6H4–SO2–O–C6H4–CH2–(imidazol-1-yl)
283	CF_3–C6H4–SO2–O–C6H4–CH2–(imidazol-1-yl)
284	H_3C–O–C6H4–SO2–O–C6H4–CH2–(imidazol-1-yl)
285	3-H_3C–C6H4–SO2–O–C6H4–CH2–(imidazol-1-yl)
286	H_3C–C6H4–SO2–O–C6H4–CH2–(imidazol-1-yl)

Table 38n. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
287	H_5C_2–C6H4–SO2–O–C6H4–CH2–imidazol-1-yl
288	H_7C_3–C6H4–SO2–O–C6H4–CH2–imidazol-1-yl
289	H_9C_4–C6H4–SO2–O–C6H4–CH2–imidazol-1-yl
290	tert-butyl–C6H4–SO2–O–C6H4–CH2–imidazol-1-yl
291	$H_{11}C_5$–C6H4–SO2–O–C6H4–CH2–imidazol-1-yl
292	C6H5–C6H4–SO2–O–C6H4–CH2–imidazol-1-yl

Table 38o. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
293	
294	
295	
296	
297	

Table 38p. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
298	
299	
300	
301	
302	
303	
304	

Table 38q. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
305	O OH 3
306	OH 3
307	Br 3
308	N N 3
309	COOEt COOEt COOEt 2
310	O OH 4
311	OH 4
312	Br 4

Table 38r. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
313	N, 4, N
314	3, COOEt, COOEt, COOEt
315	O, 5, OH
316	5, OH
317	5, Br
318	5, N, N

Table 38s. Table showing compounds synthesised in the research project.

Compound NO	Compound Structure
319	OH, 6
320	Br, 6
321	N, N, 6
322	OH, 7
323	Br, 7
324	N, N, 7

Table 38t. Table showing compounds synthesised in the research project.

2.2 Materials and Method

Chemicals were purchased from Sigma-Aldrich Company Ltd. (The Old Brickyard, Gillingham, UK), Lancaster Synthesis Ltd. (Newgate, Lancashire, UK) and Avocado Research Chemicals (Shore Road, Lancashire, UK). The purity of the starting materials was checked either by GC-MS or confirmed from the labels on containers. Structure elucidation was verified by ^{1}H and ^{13}CNMR [Jeol Eclipse+ 400 MHz and 100 MHz (FT-NMR) respectively]. Infrared spectrometry was carried out on a Perkin-Elmer Fourier Transform-Paragon 1000 infrared spectrometer and KBr disks were utelised for sample analysis. Gas chromatography-mass spectra were obtained using Hewlett 5890 Packard series II GCMS machines. Low resolution mass spectra obtained using Varian 1200L Quadrupole MS. TLC was undertaken using (normal phase) silica gel polyester plates and the mobile phase as listed in the experimental was used both for TLC and coloumn chromatography. Melting points are uncorrected and were carried out on a Stuart Scientific SMP3 melting point apparatus or Gallenkamp melting point instrument. High resolution mass spectroscopy was carried out at Chemistry Department Mass Spectrometry Sevice (King's College London, London, UK). Elemental analysis was carried out by the CHN microanalysis service (London School of Pharmacy, London, UK) using a Bruker Apex III System.

Benzyl imidazole (**198**)

Benzyl bromide (1.00g, 5.84mmol) was added to a mixture of anhydrous K_2CO_3 (0.96g, 7.02mmol) and imidazole (0.60g, 8.76mmol) in anhydrous tetrahydrofuran (THF) (50mL). The solution was then refluxed for 48h. After cooling, the THF was removed under vacuum to leave a creamy solid which was dissolved in dichloromethane (DCM) (40mL) and washed with water (3×100mL). The organic layer was then extracted using aqueous HCl (2M, 3×100mL) followed by water (2×50mL). The combined acid layer was neutralised with solid sodium bicarbonate ($NaHCO_3$) and extracted into DCM (2×50mL). The combined DCM layer was washed with water (3×50mL) and dried over anhydrous magnesium sulfate ($MgSO_4$). After filtration, DCM was removed under vacuum to give a solid which was purified using column chromatography to give **198** as a light yellow solid (0.61g, yield 67%); [m.p. 72.2-72.6°C (lit. m.p. 71.0-72.0°C; Begtrup et al, 1990)]; R_f=0.42 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3040 (Ar, C-H), 1962 (C=N), 1602 (Ar, C=C); δ_H (400MHz, $CDCl_3$): 7.70 (1H, s, NC<u>H</u>N, Im), 7.33 (3H, m, Ph-<u>H</u>), 7.16 (2H, m, Ph-<u>H</u>), 7.09 (1H, s, CH_2-NC<u>H</u>, Im), 6.90 (1H, s, NC<u>H</u>, Im), 5.13 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 137.36 (Im, NCN), 135.98, 129.12, 129.09, 127.47 (Ar, C), 128.46, 119.48 (Im, C), 51.08 (Ph-CH_2); GC: t_R 7.34min; LRMS (EI): m/z 158 (M^+, 40%), 91 (M^+-$C_3H_3N_2$, 100%), 77 (M^+-$C_4H_5N_2$, 7%); HRMS (EI): found m/z 159.09220, $C_{10}H_{11}N_2$, calculated m/z 159.09280.

1-(2-Fluoro-benzyl)-1*H*-imidazole (**199**)

Compound **199** was synthesised in a similar manner to **198**, except that

2-fluorobenzyl bromide (1.00g, 5.29mmol), anhydrous K_2CO_3 (0.87g, 6.35mmol) and imidazole (0.53g, 7.94mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **199** as a yellow oil (0.72g, yield 77%); R_f=0.42 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3113 (Ar, C-H), 2210 (Im, C=N), 1618 (Ar, C=C) ; δH (400MHz, $CDCl_3$): 7.57 (1H, s, NCHN, Im), 7.30 (1H, m, Ph-H), 7.10 (1H, s, CH_2-NCH, Im), 7.07 (3H, m, Ph-H), 6.93 (1H, s, NCH, Im), 5.15 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 161.83 (Ar, C), 137.47 (Im, NCN), 130.49, 129.57, 124.78, 123.67, 115.94 (Ar, C), 129.82, 119.32 (Im, C), 44.69 (Ph-CH_2); GC: t_R 7.15min; LRMS (EI): m/z 176 (M^+, 40%), 109 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 177.08260, $C_{10}H_{10}FN_2$, calculated m/z 177.08180.

1-(3-Fluoro-benzyl)-1*H*-imidazole (**200**)

Compound **200** was synthesised in a similar manner to **198**, except that 3-fluorobenzyl bromide (1.00g, 5.29mmol), anhydrous K_2CO_3 (0.87g, 6.35mmol) and imidazole (0.53g, 7.94mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **200** as a yellow oil (0.74g, yield 79%); R_f=0.42 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3113 (Ar, C-H), 2200 (C=N), 1617 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.70 (1H, s, NCHN, Im), 7.31 (1H, m, Ph-H), 7.10 (1H, s, CH_2-NCH, Im), 7.00 (1H, m, Ph-H), 6.92 (1H, m, Ph-H), 6.90 (1H, s, NCH, Im), 6.83 (1H, m, Ph-H), 5.13 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 164.39 (Ar, C), 138.58 (Ar, C), 137.43 (Im, NCN), 129.40, 122.91, 115.55 (Ar, C), 130.72, 119.44 (Im, C), 50.43 (Ph-CH_2); GC: t_R 15.72min; LRMS (EI): m/z 176 (M^+, 51%), 158 (M^+-F, 1%), 109 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 177.0820170, $C_{10}H_{10}FN_2$, calculated m/z 177.0822530.

1-(4-Fluoro-benzyl)-1*H*-imidazole (**201**)

Compound **201** was synthesised in a similar manner to **198**, except that 4-fluorobenzyl bromide (1.00g, 5.29mmol), anhydrous K_2CO_3 (0.87g, 6.35mmol) and imidazole (0.53g, 7.94mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **201** as a yellow oil (0.70g, yield 75%); R_f=0.45 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3113 (Ar, C-H), 2209 (Im, C=N), 1607 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.68 (1H, s, NC<u>H</u>N, Im), 7.14 (2H, m, Ph-<u>H</u>), 7.09 (1H, s, CH_2-NC<u>H</u>, Im), 7.03 (2H, m, Ph-<u>H</u>), 6.88 (1H, s, NC<u>H</u>, Im), 5.10 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 163.93 (Ar, C), 137.30 (Im, NCN), 131.84, 115.98 (Ar, C), 129.37, 119.29 (Im, C), 50.31 (Ph-CH_2); GC: t_R 7.29min; LRMS (EI): m/z 176 (M^+, 28%), 158 (M^+-F, 1%), 109 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 177.0819300, $C_{10}H_{10}FN_2$, calculated m/z 177.0822530.

1-(2,3-Difluoro-benzyl)-1*H*-imidazole (**202**)

Compound **202** was synthesised in a similar manner to **198**, except that 2,3-difluorobenzyl bromide (1.00g, 4.83mmol), anhydrous K_2CO_3 (0.80g, 5.80mmol) and imidazole (0.50g, 7.25mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **202** as a yellow oil (0.64g, yield 69%); R_f=0.50 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3113 (Ar, C-H), 2191 (C=N), 1629 (Ar, C=C); δH (400MHz,

$CDCl_3$): 7.73 (1H, s, NCHN, Im), 7.14 (1H, m, Ph-H), 7.07 (1H, s, CH_2-NCH, Im), 7.04 (1H, m, Ph-H), 6.93 (1H, s, NCH, Im), 6.84 (1H, m, Ph-H), 5.19 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 151.92 (Ar, C), 137.40 (Im, NCN), 125.92, 124.80, 124.22, 117.85, 117.68 (Ar, C), 129.40, 119.38 (Im, C), 44.37 (Ph-CH_2); GC: t_R 7.99min; LRMS (EI): m/z 194 (M^+, 63%), 175 (M^+-F, 1%), 127 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 195.0711660, $C_{10}H_9F_2N_2$, calculated m/z 195.0728311.

1-(2,4-Difluoro-benzyl)-1*H*-imidazole (**203**)

Compound **203** was synthesised in a similar manner to **198**, except that 2,4-difluorobenzyl bromide (1.00g, 4.83mmol), anhydrous K_2CO_3 (0.80g, 5.80mmol) and imidazole (0.50g, 7.25mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **203** as a yellow oil (0.66g, yield 71%); R_f=0.50 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3113 (Ar, C-H), 2238 (Im, C=N), 1618 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.70 (1H, s, NCHN, Im), 7.11 (1H, m, Ph-H), 7.07 (1H, s, CH_2-NCH, Im), 6.92 (1H, s, NCH, Im), 6.84 (2H, m, Ph-H), 5.13 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 162.01 (Ar, C), 137.17 (Im, NCN), 130.96, 112.25, 112.02, 104.50, 104.24 (Ar, C), 128.69, 119.35 (Im, C), 44.43 (Ph-CH_2); GC: t_R 7.59min; LRMS (EI): m/z 194 (M^+, 38%), 127 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 195.0712100, $C_{10}H_9F_2N_2$, calculated m/z 195.0728311.

1-(2,5-Difluoro-benzyl)-1*H*-imidazole (**204**)

Compound **204** was synthesised in a similar manner to **198**, except that 2,5-difluorobenzyl bromide (1.00g, 4.83mmol), anhydrous K_2CO_3 (0.80g, 5.80mmol) and imidazole (0.50g, 7.25mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **204** as a yellow oil (0.44g, yield 47%); R_f=0.50 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3112 (Ar, C-H), 1601 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.56 (1H, s, NC<u>H</u>N, Im), 7.08 (1H, s, CH_2-NC<u>H</u>, Im), 7.02 (2H, m, Ph-<u>H</u>), 6.91 (1H, s, NC<u>H</u>, Im), 6.71 (1H, m, Ph-<u>H</u>), 5.11 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 157.68 (Ar, C), 137.49 (Im, NCN), 117.15, 116.92, 116.67, 115.98, 115.73 (Ar, C), 130.16, 119.25 (Im, C), 44.39 (Ph-CH_2); GC: t_R 7.01min; LRMS (EI): m/z 194 (M^+, 44%), 127 (M^+-$C_3H_3N_2$, 100%), HRMS (EI): found m/z 195.0717140, $C_{10}H_9F_2N_2$, calculated m/z 195.0728311.

1-(2,6-Difluoro-benzyl)-1*H*-imidazole (**205**)

Compound **205** was synthesised in a similar manner to **198**, except that 2,6-difluorobenzyl bromide (1.00g, 4.83mmol), anhydrous K_2CO_3 (0.80g, 5.80mmol) and imidazole (0.50g, 7.25mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **205** as a yellow oil (0.58g, yield 62%); R_f=0.50 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3113 (Ar, C-H), 1627 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.67 (1H, s, NCHN, Im), 7.26 (1H, m, Ph-H), 6.99 (1H, s, CH_2-NCH, Im), 6.95 (1H, s, NCH, Im), 6.88 (2H, m, Ph-H), 5.14 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 162.44 (Ar, C), 137.09 (Im, NCN), 131.26, 111.80, 111.74 (Ar, C), 128.77, 119.33 (Im, C), 38.15 (Ph-CH_2); GC: t_R 13.15min; LRMS (EI): m/z 194 (M^+, 43%), 127 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 195.0719570, $C_{10}H_9F_2N_2$, calculated m/z 195.0728311.

1-(3,4-Difluoro-benzyl)-1*H*-imidazole (**206**)

Compound **206** was synthesised in a similar manner to **198**, except that 3,4-difluorobenzyl bromide (1.00g, 4.83mmol), anhydrous K_2CO_3 (0.80g, 5.80mmol) and imidazole (0.50g, 7.25mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **206** as a yellow oil (0.55g, yield 59%); R_f=0.50 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3118 (Ar, C-H), 2252 (Im, C=N), 1612 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.82 (1H, s, NCHN, Im), 7.11 (2H, m; 1H, Ph-H, 1H, CH_2-NCH, Im), 6.96 (1H, m, Ph-H), 6.89 (2H, m; 1H, Ph-H, 1H, NCH, Im), 5.11 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 137.30 (Im, NCN), 132.99, 123.57, 118.13, 117.97, 116.69, 116.50, (Ar, C), 129.12, 119.33 (Im, C), 50.04 (Ph-CH_2); GC: t_R 7.37min; LRMS (EI): m/z 194 (M^+, 36%), 127 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 195.0727690, $C_{10}H_9F_2N_2$, calculated m/z 195.0728311.

1-(3,5-Difluoro-benzyl)-1*H*-imidazole (**207**)

Compound **207** was synthesised in a similar manner to **198**, except that 3,5-difluorobenzyl bromide (1.00g, 4.83mmol), anhydrous K_2CO_3 (0.80g, 5.80mmol) and imidazole (0.50g, 7.25mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **207** as a yellow oil (0.64g, yield 69%); R_f=0.50 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3109 (Ar, C-H), 2200 (C=N), 1602 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.70 (1H, s, NC<u>H</u>N, Im), 7.11 (1H, s, CH_2-NC<u>H</u>, Im), 6.89 (1H, s, NC<u>H</u>, Im), 6.73 (1H, m, Ph-<u>H</u>), 6.63 (2H, m, Ph-<u>H</u>), 5.11 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 162.13 (Ar, C), 140.04 (Ar, C), 137.50 (Im, NCN), 110.27, 103.93 (Ar, C), 129.69, 119.43, (Im, C), 50.04 (Ph-CH_2); GC: t_R 13.36min; LRMS (EI): m/z 194 (M^+, 61%), 127 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 195.0711560, $C_{10}H_9F_2N_2$, calculated m/z 195.0728311.

1-(2-Chloro-benzyl)-1*H*-imidazole (**208**)

Compound **208** was synthesised in a similar manner to **198**, except that 2-chlorobenzyl bromide (1.00g, 4.88mmol), anhydrous K_2CO_3 (0.81g, 5.85mmol) and imidazole (0.50g, 7.32mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **208** as a yellow oil (0.74g, yield 78%); R_f=0.52 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3109 (Ar, C-H), 1594 (Ar, C=C); δH (400MHZ, $CDCl_3$): 7.50 (1H,

s, NC$\underline{H}$N, Im), 7.34 (1H, dd, J=1.46Hz, J=7.87Hz, Ph-$\underline{H}$), 7.18 (2H, m, Ph-$\underline{H}$), 7.03 (1H, s, CH_2-NC$\underline{H}$, Im), 6.87 (2H, m; 1H, Ph-$\underline{H}$, 1H, NC$\underline{H}$, Im), 5.15 (2H, s, Ph-C$\underline{H}_2$); δ_C (100MHz, $CDCl_3$): 137.74 (Im, NCN), 134.15, 133.14, 129.76, 129.01, 127.54 (Ar, C), 129.90, 119.46 (Im, C), 48.40 (Ph-CH_2); GC: t_R 8.41min; LRMS (EI): m/z 192 (M^+, 20%), 157 (M^+-Cl, 35%), 125 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 193.0533270, $C_{10}H_{10}ClN_2$, calculated m/z 193.0527025.

1-(3-Chloro-benzyl)-1*H*-imidazole (**209**)

Compound **209** was synthesised in a similar manner to **198**, except that 3-chlorobenzyl bromide (1.00g, 4.88mmol), anhydrous K_2CO_3 (0.81g, 5.85mmol) and imidazole (0.50g, 7.32mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **209** as a yellow oil (0.77g, yield 81%); R_f=0.52 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3109 (Ar, C-H), 2000 (C=N), 1599 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.53 (1H, s, NC$\underline{H}$N, Im), 7.27 (2H, m, Ph-$\underline{H}$), 7.11 (1H, m, Ph-$\underline{H}$), 7.09 (1H, s, CH_2-NC$\underline{H}$, Im), 7.01 (1H, m, (Ph-$\underline{H}$), 6.88 (1H, s, NC$\underline{H}$, Im), 5.08 (2H, s, Ph-C$\underline{H}_2$); δ_C (100MHz, $CDCl_3$): 138.29 (Ar, C), 137.52 (NCN, Im), 135.04, 130.18, 128.57, 127.39, 125.34 (Ar, C), 130.38, 119.33 (Im, C), 50.21 (Ph-CH_2); GC: t_R 8.66min; LRMS (EI): m/z 192 (M^+, 42%), 125 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 193.0525940, $C_{10}H_{10}ClN_2$, calculated m/z 193.0527025.

1-(4-Chloro-benzyl)-1*H*-imidazole (**210**)

Compound **210** was synthesised in a similar manner to **198**, except that 4-chlorobenzyl bromide (1.00g, 4.88mmol), anhydrous K_2CO_3 (0.81g, 5.85mmol)

and imidazole (0.50g, 7.32mmol) were used. Removal of the solvent under vacuum gave a brownish oil which was purified using column chromatography to give **210** as a yellow oil (0.71g, yield 75%); R_f=0.52 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3111 (Ar, C-H), 2250 (Im, C=N), 1640 (Ar, C=C); δH (400MHZ, $CDCl_3$): 7.67 (1H, s, NCHN, Im), 7.31 (2H, d, J=8.42Hz, Ph-H), 7.08 (2H, d, J=8.42Hz, Ph-H), 7.06 (1H, s, CH_2-NCH, Im), 6.87 (1H, s, NCH, Im), 5.09 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 137.37 (Im, NCN), 134.58, 134.38, 129.29, 128.76 (Ar, C), 129.48, 119.33 (Im, C), 50.30 (Ph-CH_2); GC: t_R 8.72min; LRMS (EI): m/z 192 (M^+, 30%), 125 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 193.0518120, $C_{10}H_{10}ClN_2$, calculated m/z 193.0527025.

1-(2,3-Dichloro-benzyl)-1*H*-imidazole (**211**)

Compound **211** was synthesised in a similar manner to **198**, except that 2,3-dichlorobenzyl bromide (1.00g, 4.17mmol), anhydrous K_2CO_3 (0.70g, 5.00mmol) and imidazole (0.42g, 6.26mmol) were used. Removal of the solvent under vacuum gave a brownish solid which was purified using column chromatography to give **211** as a yellow solid (0.66g, yield 70%); (m.p. 91.9-92.3°C); R_f=0.55 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3110 (Ar, C-H), 1640 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.57 (1H, s, NCHN, Im), 7.43 (1H, dd, J=1.46Hz, J=7.87Hz, Ph-H), 7.17 (1H, t, J=7.87Hz, Ph-H), 7.12 (1H, s, CH_2-NCH, Im), 6.93 (1H, s, NCH, Im), 6.76 (1H, dd, J=1.46, J=7.87, Ph-H), 5.25 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 137.79 (Im, NCN), 136.56, 133.83, 131.27, 130.41, 127.90, 126.71 (Ar, C), 130.12, 119.49 (Im, C), 48.92 (Ph-CH_2); GC: t_R 9.73min; LRMS (EI): m/z 226 (M^+, 19%), 191 (M^+-Cl, 50%), 159 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 227.0140070, $C_{10}H_9Cl_2N_2$, calculated m/z 227.0137301.

1-(2,4-Dichloro-benzyl)-1*H*-imidazole (**212**)

Compound **212** was synthesised in a similar manner to **198**, except that 2,4-dichlorobenzyl bromide (1.00g, 4.17mmol), anhydrous K_2CO_3 (0.70g, 5.00mmol) and imidazole (0.42g, 6.26mmol) were used. Removal of the solvent under vacuum gave a brownish solid which was purified using column chromatography to give **212** as a yellow solid (0.67g, yield 71%); [m.p. 56.3-56.7°C (lit. m.p. 49-50.0°C; Wellcome, 1979)]; R_f=0.55 [90/10 (diethyl ether/methanol)]. $\nu_{(max)}$(Film)cm^{-1}: 3113 (Ar, C-H), 1639 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.87 (1H, s, NC<u>H</u>N, Im), 7.40 (1H, d, J=8.24Hz, Ph-<u>H</u>), 7.24 (1H, d, J=2.56Hz, Ph-<u>H</u>), 7.12 (1H, s, CH_2-NC<u>H</u>, Im), 6.98 (1H, dd, J=2.56Hz, J=8.24Hz, Ph-<u>H</u>), 6.88 (1H, s, NC<u>H</u>, Im), 5.12 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 137.66 (Im, NCN), 135.10, 133.73, 132.75, 129.79, 127.89, 122.51 (Ar, C), 130.03, 119.34 (Im, C), 47.90 (Ph-CH_2); GC: t_R 9.52min; LRMS (EI): m/z 226 (M^+, 22%), 191 (M^+-Cl, 18%), 159 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 227.0137720, $C_{10}H_9Cl_2N_2$, calculated m/z 227.0137301.

1-(2,5-Dichloro-benzyl)-1*H*-imidazole (**213**)

Compound **213** was synthesised in a similar manner to **198**, except that 2,5-dichlorobenzyl bromide (1.00g, 4.17mmol), anhydrous K_2CO_3 (0.70g, 5.00mmol) and imidazole (0.42g, 6.26mmol) were used. Removal of the solvent under vacuum gave a light brown solid which was purified using column chromatography to give **213** as a yellow solid (0.69g, yield 73%); [m.p. 61.3-

61.8°C (lit. m.p. 59.0-60.0°C; Baggaley et al, 1975)]; R_f=0.55 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3082 (Ar, C-H), 1614 (Ar, C=C); δH (400MHZ, $CDCl_3$): 7.70 (1H, s, NCHN, Im), 7.31 (1H, d, J=8.42Hz, Ph-H), 7.24 (1H, dd, J=2.20, J=8.42, Ph-H), 7.12 (1H, s, CH_2-NCH, Im), 6.93 (1H, s, NCH, Im), 6.90 (1H, d, J=2.20, Ph-H), 5.20 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 137.61 (Im, NCN), 135.68, 133.61, 131.26, 131.03, 129.64, 128.94 (Ar, C), 129.89, 119.46 (Im, C), 48.17 (Ph-CH_2); GC: t_R 11.83min; LRMS (EI): m/z 226 (M^+, 25%), 191 (M^+-Cl, 63%), 159 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 227.0139910, $C_{10}H_9Cl_2N_2$, calculated m/z 227.0137301.

1-(2,6-Dichloro-benzyl)-1*H*-imidazole (**214**)

Compound **214** was synthesised in a similar manner to **198**, except that 2,6-dichlorobenzyl bromide (1.00g, 4.17mmol), anhydrous K_2CO_3 (0.70g, 5.00mmol) and imidazole (0.42g, 6.26mmol) were used. Removal of the solvent under vacuum gave a light brown oil which was purified using column chromatography to give **214** as a yellow oil (0.72g, yield 77%); R_f=0.55 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3120 (Ar, C-H), 2214 (Im, C=N), 1629 (Ar, C=C); δH (400MHZ, $CDCl_3$): 7.75 (1H, s, NCHN, Im), 7.36 (2H, d, J=7.32Hz, Ph-H), 7.24 (1H, t, J=7.32Hz, Ph-H), 7.04 (1H, s, CH_2-NCH, Im), 7.02 (1H, s, NCH, Im), 5.41 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 137.43 (Im, NCN), 136.39, 131.47, 130.87, 128.46 (Ar, C), 129.03, 119.36 (Im, C), 45.86 (Ph-CH_2); GC: t_R 9.73min; LRMS (EI): m/z 226 (M^+, 22%), 191 (M^+-Cl, 38%), 159 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 227.0136210, $C_{10}H_9Cl_2N_2$, calculated m/z 227.0137301.

1-(3,4-Dichloro-benzyl)-1*H*-imidazole (**215**)

Compound **215** was synthesised in a similar manner to **198**, except that 3,4-dichlorobenzyl chloride (1.00g, 5.12mmol), anhydrous K_2CO_3 (0.85g, 6.14mmol) and imidazole (0.51g, 7.67mmol) were used. Removal of the solvent under vacuum gave a light brown solid which was purified using column chromatography to give **215** as a yellow solid (0.59g, yield 63%); [m.p. 51.9.-52.8°C (lit. m.p. 50.0-51.0°C; Baggaley et al, 1975)]; R_f=0.55 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3109 (Ar, C-H), 2200 (Im, C=N); δH (400MHz, $CDCl_3$): 7.87 (1H, s, NCHN, Im), 7.39 (1H, d, J=8.42Hz, Ph-H), 7.24 (1H, d, J=2.56Hz, Ph-H), 7.12 (1H, s, CH_2-NCH, Im), 6.99 (1H, dd, J=2.56Hz, J=8.42Hz, Ph-H), 6.89 (1H, s, NCH, Im), 5.12 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 137.41 (Im, NCN), 136.52, 133.16, 132.46, 131.02, 130.00, 126.55 (Ar, C), 129.17, 119.30 (Im, C), 49.60 (Ph-CH_2); GC: t_R 17.25min; LRMS (EI): m/z 226 (M^+, 44%), 159 (M^+-$C_3H_3N_2$, 100%), 123 (M^+-$C_3H_3N_2Cl$, 13%); HRMS (EI): found m/z 227.0137180, $C_{10}H_9Cl_2N_2$, calculated m/z 227.0137301.

1-(3,5-Dichloro-benzyl)-1*H*-imidazole (**216**)

Compound **216** was synthesised in a similar manner to **198**, except that 3,5-dichlorobenzyl bromide (1.00g, 4.17mmol), anhydrous K_2CO_3 (0.70g, 5.00mmol) and imidazole (0.42g, 6.26mmol) were used. Removal of the solvent under vacuum gave a light brown solid which was purified using column chromatography to give **216** as a yellow solid (0.71g, yield 76%); (m.p. 55.9-

56.5°C); R_f=0.55 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3110 (Ar, C-H), 1630 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.55 (1H, s, NC<u>H</u>N, Im), 7.29 (1H, t, J=1.83, Ph-<u>H</u>), 7.11 (1H, s, CH_2-NC<u>H</u>, Im), 6.98 (2H, d, J=1.83, Ph-<u>H</u>), 6.88 (1H, s, NC<u>H</u>, Im), 5.06 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 139.63 (Ar, C), 137.52 (Im, NCN), 135.83, 128.64, 125.60 (Ar, C), 130.44, 119.29 (Im, C), 49.71 (Ph-CH_2); GC: t_R 9.56min; LRMS (EI): m/z 226 (M^+, 29%), 159 (M^+-$C_3H_3N_2$, 100%), 123 (M^+-$C_3H_3N_2Cl$, 11%); HRMS (EI): found m/z 227.0139310, $C_{10}H_9Cl_2N_2$, calculated m/z 227.0137301.

1-(2-Bromo-benzyl)-1*H*-imidazole (**217**)

Compound **217** was synthesised in a similar manner to **198**, except that 2-bromobenzyl bromide (1.00g, 4.00mmol), anhydrous K_2CO_3 (0.66g, 4.80mmol) and imidazole (0.40g, 6.00mmol) were used. Removal of the solvent under vacuum gave a light brown oil which was purified using column chromatography to give **217** as a yellow oil (0.56g, yield 59%); R_f=0.58 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3109 (Ar, C-H), 2361 (Im, C=N), 1590 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.51 (2H, m; 1H, NC<u>H</u>N Im, 1H, Ph-<u>H</u>), 7.20 (1H, m, Ph-<u>H</u>), 7.12 (1H, m, Ph-<u>H</u>), 7.04 (1H, s, CH_2-NC<u>H</u>, Im), 6.87 (1H, s, NC<u>H</u>, Im), 6.82 (1H, dd, J=1.46Hz, J=7.69Hz, Ph-<u>H</u>), 5.14 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 137.78 (Im, NCN), 135.76, 133.18, 129.90, 129.03, 128.17, 123.04 (Ar, C), 129.95, 119.47 (Im, C), 50.84 (Ph-CH_2); GC: t_R 9.09min; LRMS (EI): m/z 236 (M^+, 23%), 169 (M^+-$C_3H_3N_2$, 100%), 157 (M^+-Br, 72%); HRMS (EI): found m/z 237.0004060, $C_{10}H_{10}BrN_2$, calculated m/z 237.0021874.

1-(3-Bromo-benzyl)-1*H*-imidazole (**218**)

Compound **218** was synthesised in a similar manner to **198**, except that 3-bromobenzyl bromide (1.00g, 4.00mmol), anhydrous K_2CO_3 (0.66g, 4.80mmol) and imidazole (0.40g, 6.00mmol) were used. Removal of the solvent under vacuum gave a light brown oil which was purified using column chromatography to give **218** as a yellow oil (0.54g, yield 57%); R_f=0.58 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3111 (Ar, C-H), 2209 (Im, C=N), 1592 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.73 (1H, s, NCH̲N, Im), 7.39 (1H, d, J=7.87Hz, Ph-H̲), 7.25 (1H, br.s, Ph-H̲), 7.16 (1H, t, J=7.87Hz, Ph-H̲), 7.07 (1H, s, CH_2-NCH̲, Im), 7.03 (1H, d, J=7.87, Ph-H̲), 6.85 (1H, s, NCH̲, Im), 5.07 (2H, s, Ph-$C\underline{H_2}$); δ_C (100MHz, $CDCl_3$): 138.18 (Ar, C), 137.36 (Im, NCN), 131.67, 130.71, 130.44, 125.99, 123.19 (Ar, C), 129.14, 119.45 (Im, C), 50.40 (Ph-CH_2); GC: t_R 9.37min; LRMS (EI): m/z 236 (M^+, 39%), 169 (M^+-$C_3H_3N_2$, 100%), 90 (M^+-$C_3H_3N_2Br$, 36%); HRMS (EI): found m/z 237.0004030, $C_{10}H_{10}BrN_2$, calculated m/z 237.0021874.

1-(4-Bromo-benzyl)-1*H*-imidazole (**219**)

Compound **219** was synthesised in a similar manner to **198**, except that 4-bromobenzyl bromide (1.00g, 4.00mmol), anhydrous K_2CO_3 (0.66g, 4.80mmol) and imidazole (0.40g, 6.00mmol) were used. Removal of the solvent under vacuum gave a reddish brown oil which was purified using column chromatography to give **219** as a light brown solid (0.52g, yield 55%); [m.p. 82.3-84.2°C (lit. m.p. 81.0-82.0°C; Wellcome, 1979)]; R_f=0.58 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2252 (Im, C=N), 1600 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.75 (1H, s, NC<u>H</u>N, Im), 7.42 (2H, d, J=8.42 Hz, Ph-<u>H</u>), 7.05 (1H, s, CH_2-NC<u>H</u>, Im), 6.97 (2H, d, J=8.42Hz, Ph-<u>H</u>), 6.83 (1H, s, NC<u>H</u>, Im), 5.06 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 137.30 (Im, NCN), 134.90, 132.30 (Ar, C), 128.97, 122.58 (Ar, C), 129.14, 119.39 (Im, C), 50.50 (Ph-CH_2); GC: t_R 9.35min; LRMS (EI): m/z 236 (M^+, 38%), 169 (M^+-$C_3H_3N_2$, 100%), 90 (M^+-$C_3H_3N_2Br$, 35%); HRMS (EI): found m/z 237.0003720, $C_{10}H_{10}BrN_2$, calculated m/z 237.0021874.

1-(3,4-Dibromo-benzyl)-1*H*-imidazole (**220**)

Compound **220** was synthesised in a similar manner to **198**, except that 3,4-dibromobenzyl bromide (1.00g, 3.03mmol), anhydrous K_2CO_3 (0.50g, 3.64mmol) and imidazole (0.30g, 4.55mmol) were used. Removal of the solvent under vacuum gave a brown solid which was purified using column chromatography to give **220** as a yellow solid (0.52g, yield 53%); (m.p. 72.3-73.1°C); R_f=0.64 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3117 (Ar, C-H), 2223 (Im, C=N); δH (400MHZ, $CDCl_3$): 7.57 (1H, d, J=8.24Hz, Ph-<u>H</u>), 7.52 (1H, s, NC<u>H</u>N, Im), 7.37 (1H, d, J=2.20Hz, Ph-<u>H</u>), 7.09 (1H, s, CH_2-NC<u>H</u>, Im), 6.90 (1H, dd, J=2.20, J=8.24, Ph-H), 6.86 (1H, s, NC<u>H</u>, Im), 5.03 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 137.50 (Im, NCN), 137.24, 134.28, 132.34, 127.26, 125.61, 124.83 (Ar, C), 130.44, 119.22 (Im, C), 49.57 (Ph-CH_2); GC: t_R 20.36min; LRMS (EI): m/z 316 (M^+, 34%), 249 (M^+-$C_3H_3N_2$, 100%), 170 (M^+-Br, 12%); HRMS (EI): found m/z 316.91320, $C_{10}H_8Br_2N_2$, calculated m/z 316.90940.

1-(3,5-Dibromo-benzyl)-1*H*-imidazole (**221**)

Compound **221** was synthesised in a similar manner to **198**, except that 3,5-dibromobenzyl bromide (1.00g, 3.03mmol), anhydrous K_2CO_3 (0.50g, 3.64mmol) and imidazole (0.30g, 4.55mmol) were used. Removal of the solvent under vacuum gave a reddish brown solid which was purified using column chromatography to give **221** as a light brown solid (0.49g, yield 51%); (m.p. 70.2-71.1°C); R_f=0.62 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3086 (Ar, C-H), 2252 (Im, C=N); δH (400MHZ, $CDCl_3$): 7.90 (1H, s, NC<u>H</u>N, Im), 7.57 (1H, s, Ph-<u>H</u>), 7.19 (2H, m, Ph-<u>H</u>), 7.11 (1H, s, NC<u>H</u>, Im), 6.86 (1H, s, CH_2-NC<u>H</u>, Im), 5.09 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 139.60 (Im, NCN), 137.33, 134.34, 128.89, 123.76 (Ar, C), 129.16, 119.49 (Im, C), 49.89 (Ph-CH_2); GC: t_R 19.88min; LRMS (EI): m/z 316 (M^+, 37%), 249 (M^+-$C_3H_3N_2$, 100%), 168 (M^+-Br, 15%); HRMS (EI): found m/z 316.9109240, $C_{10}H_9Br_2N_2$, calculated m/z 316.9106533.

1-(2-Iodo-benzyl)-1*H*-imidazole (**222**)

Compound **222** was synthesised in a similar manner to **198**, except that 2-iodobenzyl bromide (1.00g, 3.37mmol), anhydrous K_2CO_3 (0.56g, 4.04mmol) and imidazole (0.34g, 5.06mmol) were used. Removal of the solvent under vacuum gave a light brown solid which was purified using column chromatography to give **222** as a yellow solid (0.68g, yield 71%); (m.p. 84.3-84.9°C) R_f=0.60 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3108 (Ar, C-H), 2229 (Im, C=N), 1583 (Ar, C-C); δH (400MHz,

$CDCl_3$): 7.80 (1H, dd, J=1.28Hz, J=7.51Hz, Ph-H), 7.50 (1H, s, NCHN, Im), 7.24 (1H, m, Ph-H), 7.04 (1H, s, CH_2-NCH, Im), 6.95 (1H, m, Ph-H), 6.86 (1H, s, NCH, Im), 6.77 (1H, dd, J=1.28Hz, J=7.51Hz, Ph-H), 5.08 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 139.87 (Im, NCN), 138.69, 137.82, 130.04, 129.01, 128.54, (Ar, C), 129.95, 119.46 (Im, C), 98.12 (Ar, C), 55.51 (Ph-CH_2); GC: t_R 9.83min; LRMS (EI): m/z 284 (M^+, 33%), 217 (M^+-$C_3H_3N_2$, 100%), 157 (M^+-I, 90%); HRMS (EI): found m/z 284.9865360, $C_{10}H_{10}IN_2$, calculated m/z 284.9883178.

1-(3-Iodo-benzyl)-1*H*-imidazole (**223**)

Compound **223** was synthesised in a similar manner to **198**, except that 3-iodobenzyl bromide (1.00g, 3.37mmol), anhydrous K_2CO_3 (0.56g, 4.04mmol) and imidazole (0.34g, 5.06mmol) were used. Removal of the solvent under vacuum gave a pale solid which was purified using column chromatography to give **223** as a off-white solid (0.67g, yield 70%); (m.p. 92.1-92.4°C); R_f=0.60 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3098 (Ar, C-H), 1625 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.92 (1H, s, NCHN, Im), 7.60 (1H, m, Ph-H), 7.47 (1H, s, CH_2-NCH, Im), 7.05 (3H, m, Ph-H), 6.86 (1H, s, NCH, Im), 5.08 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 137.89 (Im, NCN), 137.75, 137.20, 136.44, 130.87, 126.77 (Ar, C), 128.31, 119.53 (Im, C), 94.89 (Ar, C), 50.47 (Ph-CH_2); GC: t_R 18.45min; LRMS (EI): m/z 284 (M^+, 56%), 217 (M^+-$C_3H_3N_2$, 100%), 90 (M^+-$C_3H_3N_2I$, 25%); HRMS (EI): found m/z 284.9874810, $C_{10}H_{10}IN_2$, calculated m/z 284.9883178; Elemental analysis: found C 42.28%, H 3.19%, N 9.86%, $C_{10}H_9IN_2$, calculated C 42.21%, H 3.19%, N 9.85%.

1-(4-Iodo-benzyl)-1*H*-imidazole (**224**)

Compound **224** was synthesised in a similar manner to **198**, except that 4-iodobenzyl bromide (1.00g, 3.37mmol), anhydrous K_2CO_3 (0.56g, 4.04mmol) and imidazole (0.34g, 5.06mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **224** as a yellow solid (0.77g, yield 80%) (m.p. 69.3-71.2°C); R_f=0.60 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3084 (Ar, C-H), 1896 (Im, C=N), 1590 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.87 (1H, s, NCHN, Im), 7.68 (2H, d, J=8.42Hz, Ph-H), 7.11 (1H, s, CH_2-NCH, Im), 6.92 (2H, d, J=8.42Hz, Ph-H), 6.89 (1H, s, NCH, Im), 5.11 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 138.28 (Im, NCN), 137.27, 135.48, 128.72, (Ar, C), 129.34, 119.43 (Im, C), 94.18 (Ar, C), 50.65 (Ph-CH_2); GC: t_R 10.12min; LRMS (EI): m/z 284 (M^+, 44%), 217 (M^+-$C_3H_3N_2$, 100%), 90 (M^+-$C_3H_3N_2I$, 38%); HRMS (EI): found m/z 284.9864650, $C_{10}H_{10}IN_2$, calculated m/z 284.9883178.

1-(2-Nitro-benzyl)-1*H*-imidazole (**225**)

Compound **225** was synthesised in a similar manner to **198**, except that 2-nitrobenzyl bromide (1.00g, 4.62mmol), anhydrous K_2CO_3 (0.77g, 5.56mmol) and imidazole (0.47g, 6.93mmol) were used. Removal of the solvent under vacuum gave a light brown solid which was purified using column chromatography to give **225** as a light brown solid (0.66g, yield 70%); [m.p. 82.6-83.9°C (lit. m.p. 83.0-85.0°C; Stefancich et al, 1993)]; R_f=0.53 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3110 (Ar, C-H), 1610 (Ar, C=C); δH (400MHz, $CDCl_3$): 8.15 (1H,

dd, J=1.46Hz, J=8.06Hz, Ph-H), 7.60 (1H, s, NCHN, Im), 7.57 (1H, m, Ph-H), 7.49 (1H, m, Ph-H), 7.15 (1H, s, CH_2-NCH, Im), 6.94 (1H, s, NCH, Im), 6.81 (1H, dd, J=1.46Hz, J=8.06Hz, Ph-H), 5.57 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 147.16 (Ar, C), 138.11 (Im, NCN), 134.52, 132.89, 130.36, 128.73, 125.50, (Ar, C), 129.25, 119.84 (Im, C), 48.07 (Ph-CH_2); GC: t_R 18.96 min; LRMS (EI): m/z 203 (M^+, 88%), 136 (M^+-$C_3H_3N_2$, 100%), 90 (M^+-$C_3H_3N_3O_2$, 54%); HRMS (EI): found m/z 204.0765940, $C_{10}H_{10}N_3O_2$, calculated m/z 204.0767530.

1-(3-Nitro-benzyl)-1*H*-imidazole (**226**)

Compound **226** was synthesised in a similar manner to **198**, except that 3-nitrobenzyl bromide (1.00g, 4.62mmol), anhydrous K_2CO_3 (0.77g, 5.56mmol) and imidazole (0.47g, 6.93mmol) were used. Removal of the solvent under vacuum gave a light brown solid which was purified using column chromatography to give **226** as a yellow solid (0.64g, yield 68%); [m.p. 90.7-91.4°C (lit. m.p. 88.0-89.0°C; Baggaley et al, 1975)] R_f=0.54 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3097 (Ar, C-H), 1615 (Ar, C=C); δH (400MHz, $CDCl_3$): 8.15 (1H, m, Ph-H), 8.02 (1H, m, Ph-H), 7.74 (1H, s, NCHN, Im), 7.51 (1H, t, J=7.87, Ph-H), 7.43 (1H, m, Ph-H), 7.11 (1H, s, CH_2-NCH, Im), 6.91 (1H, s, NCH, Im), 5.25 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 148.64 (Ar, C), 138.32 (Im, NCN), 137.48, 133.21, 130.32, 123.49, 122.26 (Ar, C), 129.92, 119.33 (Im, C), 50.88 (Ph-CH_2); GC: t_R 18.66 min; LRMS (EI): m/z 203 (M^+, 78%), 136 (M^+-$C_3H_3N_2$, 100%), 90 (M^+ M^+-$C_3H_3N_3O_2$, 50%); HRMS (EI): found m/z 204.0768700, $C_{10}H_{10}N_3O_2$, calculated m/z 204.0767530.

1-(4-Nitro-benzyl)-1*H*-imidazole (**227**)

Compound **227** was synthesised in a similar manner to **198**, except that 4-nitrobenzyl bromide (1.00g, 4.62mmol), anhydrous K_2CO_3 (0.77g, 5.56mmol) and imidazole (0.47g, 6.93mmol) were used. Removal of the solvent under vacuum gave a light brown solid which was purified using column chromatography to give **227** as a yellow solid (0.72g, yield 76%); [m.p. 57.3-58.8°C (lit. m.p. 55.0-56.0°C; Street et al, 1995)]; R_f=0.55 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3116 (Ar, C-H), 2253 (Im, C=N), 1609 (Ar, C=C); δH (400MHz, $CDCl_3$): 8.20 (2H, d, J=8.97Hz, Ph-H), 7.91 (1H, s, NCHN, Im), 7.30 (2H, d, J=8.97Hz, Ph-H), 7.15 (1H, s, CH_2-NCH, Im), 6.92 (1H, s, NCH, Im), 5.30 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 147.96, 143.11 (Ar, C), 137.54 (Im, NCN), 128.04, 124.39 (Ar, C), 129.31, 119.49 (Im, C), 50.25 (Ph-CH_2); GC: t_R 19.26 min; LRMS (EI): m/z 203 (M^+-$C_{10}H_9N_3O_2$, 100%), 136 (M^+-$C_3H_3N_2$, 43%), 90 (M^+-$C_3H_3N_3O_2$, 24%); HRMS (EI): found m/z 204.0768120, $C_{10}H_{10}N_3O_2$, calculated m/z 204.0767530.

2-Imidazole-1-ylmethyl-benzonitrile (**228**)

Compound **228** was synthesised in a similar manner to **198**, except that 2-bromomethyl-benzonitrile (1.00g, 5.10mmol), anhydrous K_2CO_3 (0.85g, 6.12mmol) and imidazole (0.60g, 8.78mmol) were used. Removal of the solvent under vacuum gave a dark brown oil which was purified using column chromatography to give **228** as a brown oil (0.57g, yield 55%); R_f=0.43 [90/10

(diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3114 (Ar, C-H), 2226 (Im, C=N; Ph-CN), 1600 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.70 (1H, dd, J=1.46Hz, 7.69Hz, Ph-H), 7.62 (1H, s, NCHN, Im), 7.58 (1H, m, Ph-H), 7.44 (1H, m, Ph-H), 7.09 (2H, m; 1H, CH_2-NCH Im, 1H, Ph-H), 6.97 (1H, s, NCH, Im), 5.35 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 139.90 (CN), 137.63 (Im, NCN), 133.73, 133.27, 130.35, 128.28, 116.96, 111.51 (Ar, C), 129.01, 119.40 (Im, C), 48.78 (Ph-CH_2); GC: t_R 9.18 min; LRMS (EI): m/z 183 (M^+, 46%), 116 (M^+-$C_3H_3N_2$, 100%), 89 (M^+-$C_4H_4N_3$, 18%); HRMS (EI): found m/z 184.0872310, $C_{11}H_{10}N_3$, calculated m/z 184.0869238.

3-Imidazole-1-ylmethyl-benzonitrile (**229**)

Compound **229** was synthesised in a similar manner to **198**, except that 3-bromomethyl-benzonitrile (1.00g, 5.10mmol), anhydrous K_2CO_3 (0.85g, 6.12mmol) and imidazole (0.60g, 8.78mmol) were used. Removal of the solvent under vacuum gave a yellow solid which was purified using column chromatography to give **229** as a yellow solid (0.58g, yield 56%); [m.p. 66.6-67.2°C (lit. m.p. 64.0-66.0°C; Artico et al, 1992)]; R_f=0.41 [90/10 (diethyl ether/methanol)].

$\nu_{(Max)}$(Film)cm^{-1}: 3113 (Ar, C-H), 2231 (Im, C=N; Ph-CN), 1605 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.55 (1H, d, J=7.69Hz, Ph-H), 7.50 (1H, s, NCHN, Im) 7.41 (1H, t, J=7.69Hz, Ph-H), 7.35 (1H, br.s, Ph-H), 7.30 (1H, d, J=7.69Hz, Ph-H), 7.06 (1H, s, CH_2-NCH, Im), 6.83 (1H, s, NCH, Im), 5.11 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 138.04 (CN), 137.53 (Im, NCN), 132.04, 131.42, 130.56, 130.50, 118.25, 113.32 (Ar, C), 130.03, 119.27 (Im, C), 49.90 (Ph-CH_2); GC: t_R 9.58 min; LRMS (EI): m/z 183 (M^+, 31%), 116 (M^+-$C_3H_3N_2$, 100%), 89 (M^+-$C_4H_4N_3$, 29%); HRMS (EI): found m/z 184.0873110, $C_{11}H_{10}N_3$, calculated m/z 184.0869238.

4-Imidazole-1-ylmethyl-benzonitrile (**230**)

Compound **230** was synthesised in a similar manner to **198**, except that 4-bromomethyl-benzonitrile (1.00g, 5.10mmol), anhydrous K_2CO_3 (0.85g, 6.12mmol) and imidazole (0.60g, 8.78mmol) were used. Removal of the solvent under vacuum gave a brown solid which was purified using column chromatography to give **230** as a yellow soild (0.57g, yield 59%); [m.p. 108.9-109.5°C (lit. m.p. 106.0-108.0°C; Artico et al, 1992)]; R_f=0.44 [90/10 (diethyl ether/methanol)].

$\nu_{(Max)}$(Film)cm^{-1}: 3092 (Ar, C-H), 2229 (Im, C=N; Ph-CN), 1607 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.82 (1H, s, NC<u>H</u>N, Im), 7.59 (2H, d, J=8.42Hz, Ph-<u>H</u>), 7.19 (2H, d, J=8.42Hz, Ph-<u>H</u>), 7.09 (1H, s, CH_2-NC<u>H</u>, Im), 6.85 (1H, s, NC<u>H</u>, Im), 5.20 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 141.26 (<u>C</u>N), 137.53 (Im, NCN), 132.95, 127.85, 118.24, 112.57 (Ar, C), 129.33, 119.48 (Im, C), 50.48 (Ph-CH_2); GC: t_R 10.23 min; LRMS (EI): m/z 183 (M^+, 63%), 116 (M^+-$C_3H_3N_2$, 100%), 89 (M^+-$C_4H_4N_3$, 20%); HRMS (EI): found m/z 184.0867460, $C_{11}H_{10}N_3$, calculated m/z 184.0869238.

1-(2-Trifluoromethyl-benzyl)-1*H*-imidazole (**231**)

Compound **231** was synthesised in a similar manner to **198**, except that 2-trifluoromethyl-benzyl bromide (1.00g, 4.18mmol), anhydrous K_2CO_3 (1.04g, 7.55mmol) and imidazole (0.64g, 9.43mmol) were used. Removal of the solvent under vacuum gave yellow oil which was purified using column chromatography to give **231** as a clear oil (0.66g, yield 70%); R_f=0.48 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3115 (Ar, C-H), 2222 (Im, C=N), 1609 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.68 (1H, d, J=7.51Hz, Ph-H), 7.53 (1H, s, NCHN, Im), 7.47 (1H, t, J=7.51Hz, Ph-H), 7.41 (1H, t, J=7.51Hz, Ph-H), 7.10 (1H, s, CH_2-NCH, Im), 6.89 (2H, m; 1H, NCH Im, H, Ph-H), 5.31 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 137.90 (Im, NCN), 135.01, 132.72, 130.21, 128.56, 126.32 (Ar, C), 128.32, 119.65 (Im, C), 47.07 (Ph-CH_2); GC: t_R 12.45min; LRMS (EI): m/z 226 (M^+, 48%), 159 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 227.07704, $C_{11}H_{10}F_3N_2$, calculated m/z 227.07906.

1-(3-Trifluoromethyl-benzyl)-1*H*-imidazole (**232**)

Compound **232** was synthesised in a similar manner to **198**, except that 3-trifluoromethyl-benzyl bromide (1.00g, 6.29mmol), anhydrous K_2CO_3 (1.04g, 7.55mmol) and imidazole (0.64g, 9.43mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **232** as a clear oil (0.78g, yield 82%); R_f=0.50 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3112 (Ar, C-H), 2204 (Im, C=N), 1618 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.58 (1H, m, Ph-H), 7.56 (1H, s, Ph-H), 7.47 (1H, t, J=7.87Hz, Ph-H), 7.41(1H, s, NCHN, Im), 7.28 (1H, d, J=7.87Hz, Ph-H), 7.11 (1H, s, CH_2-NCH, Im), 6.90 (1H, s, NCH, Im), 5.14 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 137.54 (Im, NCN), 137.36, 131.66, 131.35, 130.50, 130.36, 125.29, 124.02 (Ar, C), 129.72, 119.27 (Im, C), 50.31 (Ph-CH_2); GC: t_R 12.99min; LRMS (EI): m/z 226 (M^+, 33%), 159 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 227.07960, $C_{11}H_{10}F_3N_2$, calculated m/z 227.07910.

1-(4-Trifluoromethyl-benzyl)-1*H*-imidazole (**233**)

Compound **233** was synthesised in a similar manner to **198**, except that 4-trifluoromethyl-benzyl bromide (1.00g, 6.29mmol), anhydrous K_2CO_3 (1.04g, 7.55mmol) and imidazole (0.64g, 9.43mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **233** as a clear oil (0.52g, yield 55%); R_f=0.50 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3053 (Ar, C-H), 2306 (Im, C=N), 1622 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.61 (1H, s, NC<u>H</u>N, Im), 7.58 (2H, d, J=8.06Hz, Ph-<u>H</u>), 7.22 (2H, d, J=8.06Hz, Ph-<u>H</u>), 7.11 (1H, s, CH_2-NC<u>H</u>, Im), 6.89 (1H, s, NC<u>H</u>, Im), 5.18 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 140.32 (Ar, C), 137.59 (Im, NCN), 130.87, 130.54, 127.45, 126.12 (Ar, C), 130.27, 119.40 (Im, C), 50.30 (Ph-CH_2); GC: t_R 13.35min; LRMS (EI): m/z 226 (M^+, 35%), 159 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 227.07980, $C_{11}H_{10}F_3N_2$, calculated m/z 227.07910.

1-[3,5-bis(trifluoromethyl)benzyl]-1*H*-imidazole (**234**)

Compound **234** was synthesised in a similar manner to **198**, except that 1-(bromomethyl)-3,5-bis(trifluoromethyl)benzene (1.00g, 3.26mmol), anhydrous K_2CO_3 (0.54g, 3.91mmol) and imidazole (0.33g, 4.90mmol) were used. Removal of the solvent under vacuum gave a light brown oil which was purified using column chromatography to give **234** as a yellow oil (0.61g, yield 64%); R_f=0.58 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2253 (Im, C=N), 1626 (Ar, C=C); δH (400MHZ, $CDCl_3$): 7.87 (1H,

s, NCHN, Im), 7.83 (1H, s, Ph-H), 7.58 (2H, s, Ph-H), 7.16 (1H, s, CH_2-NCH, Im), 6.92 (1H, s, NCH, Im), 5.31 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 138.72 (Ar, C), 137.50 (Im, NCN), 132.83, 132.52, 129.81 (Ar, C), 127.35, 119.27 (Im, C), 50.02 (Ph-CH_2); GC: t_R 5.23min; LRMS (EI): m/z 294 (M^+, 63%), 275 (M^+-F, 10%), 227 (M^+-$C_3H_3N_2$, 100%), 158 (M^+-$C_4H_3N_2F_3$, 5%); HRMS (EI): found m/z 295.0675570, $C_{12}H_9F_6N_2$, calculated m/z 295.0664439.

1-(2-Methoxy-benzyl)-1*H*-imidazole (**235**)

Compound **235** was synthesised in a similar manner to **198**, except that 2-methoxybenzyl bromide (1.00g, 4.97mmol), anhydrous K_2CO_3 (1.42g, 7.02mmol) and imidazole (0.50g, 7.50mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **235** as a yellow oil (0.69g, yield 69%); R_f=0.42 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3112 (Ar, C-H), 2941 (C-H), 2043 (Im, C=N), 1602 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.51 (1H, s, NCHN, Im), 7.25 (1H, m, Ph-H), 7.00 (1H, s, CH_2-NCH, Im), 6.95 (1H, d, J=7.32Hz, Ph-H), 6.89 (1H, s, NCH, Im), 6.87 (2H, m, Ph-H), 5.06 (2H, s, Ph-CH_2), 3.80 (3H, s, OCH_3); δ_C (100MHz, $CDCl_3$): 157.06 (Ar, C), 137.69 (Im, NCN), 129.83, 129.21, 124.81, 120.82, 110.62 (Ar, C), 129.09, 119.49 (Im, C), 55.44 (OCH_3), 46.09 (Ph-CH_2); GC: t_R 15.70min; LRMS (EI): m/z 188 (M^+, 48%), 121 (M^+-$C_3H_3N_2$, 100%), 91 (M^+-$C_4H_5N_2O$, 86%); HRMS (EI): found m/z 189.10223, $C_{11}H_{13}N_2O$, calculated m/z 189.10224.

1-(3-Methoxy-benzyl)-1*H*-imidazole (**236**)

Compound **236** was synthesised in a similar manner to **198**, except that 3-methoxybenzyl bromide (1.00g, 4.97mmol), anhydrous K_2CO_3 (1.42g, 7.02mmol) and imidazole (0.50g, 7.50mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **236** as a yellow oil (0.68g, yield 68%); R_f=0.40 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3111 (Ar, C-H), 2940 (C-H), 1943 (Im, C=N), 1601 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.51 (1H, s, NC<u>H</u>N, Im), 7.24 (1H, t, J=8.24, Ph-<u>H</u>), 7.05 (1H, s, Ph-<u>H</u>), 6.87 (1H, s, CH_2-NC<u>H</u>, Im), 6.81 (2H, dd, J=2.20, J=6.04, Ph-<u>H</u>), 6.71 (1H, d, J=8.24, Ph-<u>H</u>), 6.63 (1H, s, NC<u>H</u>, Im), 5.05 (2H, s, Ph-C<u>H</u>$_2$), 3.74 (3H, s, OC<u>H</u>$_3$); δ_C (100MHz, $CDCl_3$): 160.15, 137.80 (Ar, C), 137.54 (Im, NCN), 130.15, 119.55, 113.56, 113.09 (Ar, C), 129.86, 119.40 (Im, C), 55.34 (OCH_3) 50.78 (Ph-CH_2); GC: t_R 16.00min; LRMS (EI): m/z 188 (M^+, 33%), 121 (M^+-$C_3H_3N_2$, 100%), 91 (M^+-$C_4H_5N_2O$, 29%); HRMS (EI): found m/z 189.10260, $C_{11}H_{13}N_2O$, calculated m/z 189.10220.

1-(4-Methoxy-benzyl)-1*H*-imidazole (**237**)

Compound **237** was synthesised in a similar manner to **198**, except that 4-methoxybenzyl bromide (1.00g, 4.97mmol), anhydrous K_2CO_3 (1.42g, 7.02mmol) and imidazole (0.50g, 7.50mmol) were used. Removal of the solvent under vacuum gave a broen oil which was purified using column chromatography to give **237** as a yellow oil (0.72g, yield 72%); R_f=0.38 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3005 (Ar, C-H), 2939 (C-H), 1601 (Ar, C=C); δH (400MHz,

$CDCl_3$): 7.49 (1H, s, NC$\underline{H}$N, Im), 7.08 (2H, d, J=8.79Hz, Ph-$\underline{H}$), 7.04 (1H, s, CH_2-NC$\underline{H}$, Im), 6.85 (3H, m; 2H, Ph-$\underline{H}$, 1H, NC$\underline{H}$, Im), 5.01 (2H, s, Ph-C$\underline{H}_2$), 3.77 (3H, s, OC$\underline{H}_3$); δ_C (100MHz, $CDCl_3$): 159.60 (Ar, C), 137.33 (NCN), 129.79, 128.92, 114.41 (Ar, C), 128.18, 119.18 (Im, C), 55.41 (OCH_3) 50.42 (Ph-CH_2); GC: t_R 16.39min; LRMS (EI): m/z 188 (M^+, 15%), 121 (M^+-$C_3H_3N_2$, 100%), 91 (M^+-$C_4H_5N_2O$, 11%); HRMS (EI): found m/z 189.10280, $C_{11}H_{13}N_2O$, calculated m/z 189.10220.

1-(4-Methylthio-benzyl)-1*H*-imidazole (**238**)

Compound **238** was synthesised in a similar manner to **198**, except that 4-methylthio-benzyl bromide (1.00g, 4.60mmol), anhydrous K_2CO_3 (0.76g, 5.53mmol) and imidazole (0.46g, 6.90mmol) were used. Removal of the solvent under vacuum gave a light brown solid which was purified using column chromatography to give **238** as a yellow solid (0.65g, yield 69%); (m.p. 76.8-77.9); R_f=0.44 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3109 (Ar, C-H), 2920 (C-H), 2343 (Im, C=N), 1600 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.50 (1H, s, NC$\underline{H}$N, Im), 7.19 (2H, d, J=8.42Hz, Ph-$\underline{H}$), 7.06 (3H, m; 1H, CH_2-NC$\underline{H}$, Im), 2H, Ph-$\underline{H}$), 6.85 (1H, s, NC$\underline{H}$, Im), 5.04 (2H, s, Ph-C$\underline{H}_2$), 2.44 (3H, s, Ph-S-C$\underline{H}_3$); δ_C (100MHz, $CDCl_3$): 138.98 (Ar, C), 137.42 (NCN) 132.88, 127.94, 126.89 (Ar, C), 129.93, 119.24 (Im, C), 50.46 (Ph-CH_2), 15.73 (Ph-S-CH_3); GC: t_R 18.49min; LRMS (EI): m/z 204 (M^+, 20%), 137 (M^+-$C_3H_3N_2$, 100%), 122 (M^+-$C_4H_6N_2$, 17%); HRMS (EI): found m/z 204.07140, $C_{11}H_{12}N_2S_1$, calculated m/z 204.07160.

1-(2-Methyl-benzyl)-1*H*-imidazole (**239**)

Compound **239** was synthesised in a similar manner to **198**, except that 2-methylbenzyl bromide (1.00g, 5.85mmol), anhydrous K_2CO_3 (0.97g, 7.02mmol) and imidazole (0.60g, 8.78mmol) were used. Removal of the solvent under vacuum gave a light brown oil which was purified using column chromatography to give **239** as a yellow oil (0.69g, yield 69%); R_f=0.42 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3021 (Ar, C-H), 2208 (Im, C=N), 1606 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.46 (1H, s, NC<u>H</u>N, Im), 7.19 (3H, m, Ph-<u>H</u>), 7.06 (1H, s, CH_2-NC<u>H</u>, Im), 6.95 (1H, d, J=7.69Hz, Ph-<u>H</u>) 6.84 (1H, s, NC<u>H</u>, Im), 5.08 (2H, s, Ph-C<u>H</u>$_2$), 2.25 (3H, s, C<u>H</u>$_3$); δ_C (100MHz, $CDCl_3$): 137.51 (Im, NCN), 136.19, 134.05, 130.83, 128.61, 128.33, 126.65 (Ar, C), 129.62, 119.35 (Im, C), 49.05 (Ph-CH_2), 18.98 (CH_3); GC: t_R 8.05 min; LRMS (EI): m/z 172 (M^+, 21%), 105 (M^+-$C_3H_3N_2$, 100%), 77 (M^+-$C_5H_7N_2$, 14%); HRMS (EI): found m/z 173.1069380, $C_{11}H_{13}N_2$, calculated m/z 173.1073248.

1-(3-Methyl-benzyl)-1*H*-imidazole (**240**)

Compound **240** was synthesised in a similar manner to **198**, except that 3-methylbenzyl bromide (1.00g, 5.85mmol), anhydrous K_2CO_3 (0.97g, 7.02mmol) and imidazole (0.60g, 8.78mmol) were used. Removal of the solvent under vacuum gave a light brown oil which was purified using column chromatography to give **240** as a yellow oil (0.68g, yield 68%); R_f=0.40 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3026 (Ar, C-H), 2923 (C-H), 1950 (C=N), 1609 (Ar, C=C); δH

(400MHz, $CDCl_3$): 7.47 (1H, s, NCHN, Im), 7.17 (1H, m, Ph-H), 7.05 (1H, d, J=7.51, Ph-H), 7.01 (1H, s, CH_2-NCH, Im), 6.88 (2H, m, Ph-H), 6.82 (1H, s, NCH, Im), 5.00 (2H, s, Ph-CH_2), 2.25 (3H, s, CH_3); δ_C (100MHz, $CDCl_3$): 138.89 (Ar, C-CH_3), 137.52 (Im, NCN), 136.18, 129.08, 128.94, 128.06, 124.45 (Ar, C), 129.81, 119.40 (Im, C), 50.86 (Ph-CH_2), 21.44 (CH_3); GC: t_R 13.73min; LRMS (EI): m/z 172 (M^+, 50%), 105 (M^+-$C_3H_3N_2$, 100%), 77 (M^+-$C_5H_7N_2$, 15%); HRMS (EI): found m/z 173.1071860, $C_{11}H_{13}N_2$, calculated m/z 173.1073248.

1-(4-Methyl-benzyl)-1*H*-imidazole (**241**)

Compound **241** was synthesised in a similar manner to **198**, except that 4-methylbenzyl bromide (1.00g, 5.85mmol), anhydrous K_2CO_3 (0.97g, 7.02mmol) and imidazole (0.60g, 8.78mmol) were used. Removal of the solvent under vacuum gave a light brown solid which was purified using column chromatography to give **241** as a yellow solid (0.72g, yield 72%); [m.p. 52.7-53.6°C (lit. m.p. 51.0-52.0°C; Baggaley et al, 1975)]; R_f=0.38 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3024 (Ar, C-H), 2923 (C-H), 1900 (C=N), 1614 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.65 (1H, s, NCHN, Im), 7.13 (2H, d, J=7.87Hz, Ph-H), 7.06 (1H, s, CH_2-NCH, Im), 7.04 (2H, d, J=7.87Hz, Ph-H), 6.87 (1H, s, NCH, Im), 5.06 (2H, s, Ph-CH_2), 2.31 (3H, s, CH_3); δ_C (100MHz, $CDCl_3$): 138.31 (Ar, C), 137.30 (NCN) 133.04, 129.76, 127.53 (Ar, C), 129.05, 119.46 (Im, C), 50.88 (Ph-CH_2), 21.21 (CH_3); GC: t_R 14.70 min; LRMS (EI): m/z 172 (M^+, 37%), 105 (M^+-$C_3H_3N_2$, 100%), 77 (M^+-$C_5H_7N_2$, 10%); HRMS (EI): found m/z 173.1065700, $C_{11}H_{13}N_2$, calculated m/z 173.1073248.

4-(Propyl-phenyl)methanol (**242**)

To a stirred solution of 4-propylbenzoic acid (4.00g, 24.39mmol) in anhydrous THF (30 mL), lithium aluminium hydride ($LiAlH_4$) (6.10mL, 2M in THF) was added at room temperature. After 6h, conc. hydrochloric acid (HCl) was added drop wise until a pH of 2.0 was achieved. THF was removed under vacuum and water was added to the reaction mixture which was extracted with DCM (3×50mL). The DCM was further washed with water (3×50mL), dried over $MgSO_4$ and then filtered. Removal of the DCM under vacuum gave a yellow oil which was purified using column chromatography to give **242** as a clear oil (2.19g, yield 60%); R_f=0.36 [40/60 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1} : 3331 (O-H), 2929 (C-H), 1615 (Ar, C=C), 1015 (C-O); δH (400MHz, $CDCl_3$): 7.26 (2H, d, J=8.24Hz, Ph-H), 7.16 (2H, d, J=8.24Hz, Ph-H), 4.63 (2H, s, Ph-CH_2), 2.58 (2H, t, J=7.87Hz, Ph-CH_2-CH_2), 2.01 (1H, s, OH), 1.63 (2H, m, Ph-CH_2-CH_2), 0.93 (3H, t, J=7.51Hz, CH_3); δ_C (100MHz, $CDCl_3$): 142.34, 138.23, 128.77, 127.20, (Ar, C), 65.35 (Ph-CH_2), 37.83 (Ph-CH_2-CH_2), 24.70 (Ph-CH_2-CH_2), 13.92 (CH_3); GC: t_R 9.85min; LRMS (EI): m/z 150 (M^+, 67%), 121 (M^+-C_2H_4, 100%), 107 (M^+-C_3H_7, 90%), 91 (M^+-C_3H_7O, 81%).

4-Propylbenzyl bromide (**243**)

To a solution of **242** (2.00g, 13.33mmol) in anhydrous diethyl ether (50mL) PBr_3 (7.00g, 25.86mmol) was added in a dropwise manner. The reaction was refluxed for 12h. The diethyl ether was removed under vacuum to leave an orange oil, which was dissolved in DCM (50mL) and washed with saturated $NaHCO_3$ solution

(3×50mL), followed with water (3×50mL), dried over anhydrous $MgSO_4$ and filtered. Removal of the DCM under vacuum gave a brown oil which was purified using column chromatography to give **243** (1.92g, yield 68%) as a clear oil; R_f=0.76 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3024 (Ar, C-H), 2958 (C-H), 1613 (Ar, C=C), 673 (C-Br); δH (400MHz, $CDCl_3$): 7.29 (2H, d, J=8.24Hz, Ph-$\underline{H}$), 7.14 (2H, d, J=8.24Hz, Ph-$\underline{H}$), 4.48 (2H, s, Ph-C$\underline{H}_2$), 2.56 (2H, t, J=7.69Hz, Ph-C$\underline{H}_2$-CH_2), 1.61 (2H, m, Ph-CH_2-C$\underline{H}_2$), 0.92 (3H, t, J=7.32Hz, C$\underline{H}_3$); δ_C (100MHz, $CDCl_3$): 143.28, 135.11, 129.05, 129.01 (Ar, C), 37.86 (Ph-$\underline{C}H_2$-CH_2), 33.92 (Ph-CH_2), 24.53 (Ph-CH_2-$\underline{C}H_2$), 13.93 (CH_3); GC: t_R 11.00min; LRMS (EI): m/z 212 (M^+, 6%), 133 (M^+-Br, 100%), 104 (M^+-C_2H_5Br, 33%), 91 (M^+-C_3H_6Br, 30%).

1-(4-Propyl-benzyl)-1*H*-imidazole (**244**)

Compound **244** was synthesised in a similar manner to **198**, except that **243** (1.50g, 7.08mmol), anhydrous K_2CO_3 (1.25g, 9.06mmol) and imidazole (0.75g, 11.03mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **244** as a light yellow oil (0.89g, yield 64%); R_f=0.46 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3023 (Ar, C-H), 2958 (C-H), 1903 (Im, C=N), 1615 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.59 (1H, s, NC$\underline{H}$N, Im), 7.16 (2H, d, J=8.06Hz, Ph-$\underline{H}$), 7.08 (1H, s, CH_2-NC$\underline{H}$, Im), 7.06 (2H, d, J=8.06Hz, Ph-$\underline{H}$), 6.90 (1H, s, NC$\underline{H}$, Im), 5.08 (2H, s, Ph-C$\underline{H}_2$), 2.57 (2H, t, J=7.69Hz, Ph-C$\underline{H}_2$-CH_2), 1.63 (2H, m, Ph-CH_2-C$\underline{H}_2$-CH_3), 0.92 (3H, t, J=7.32Hz, C$\underline{H}_3$); δ_C (100MHz, $CDCl_3$): 143.08 (Ar, C) 137.34 (Im, NCN), 133.29, 129.39, 127.42 (Ar, C), 129.15, 119.40 (Im, C), 50.81 (Ph-CH_2), 37.72 (Ph-$\underline{C}H_2$-CH_2), 24.57 (Ph-CH_2-$\underline{C}H_2$), 13.87 (CH_3); GC: t_R 16.67min; LRMS (EI): m/z 200 (M^+, 29%), 133 (M^+-$C_3H_3N_2$, 100%), 91 (M^+-$C_6H_9N_2$, 35%), 77 (M^+-$C_7H_{11}N_2$, 7%); HRMS (EI): found m/z 200.13060, $C_{13}H_{16}N_2$, calculated m/z 200.13080; Elemental analysis: found C 77.96%, H 8.05%, N 13.99%, $C_{13}H_{15}N_2$,

calculated C 77.57%, H 8.07%, N 13.89% (0.04 mole of H_2O).

4-(Butyl-phenyl)methanol (**245**)

Compound **245** was synthesised in a similar manner to **242**, except that 4-butylbenzoic acid (3.00g, 16.85mmol) and $LiAlH_4$ (4.20mL, 2M in THF) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **245** as a clear oil (1.87g, yield 68%); R_f=0.39 [40/60 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1} : 3329 (O-H), 3013 (Ar, C-H), 2956 (C-H), 1615 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.25 (2H, d, J=8.06Hz, Ph-H), 7.16 (2H, d, J=8.06Hz, Ph-H), 4.62 (2H, d, J=5.86, Ph-CH_2), 2.60 (2H, t, J=7.87Hz, Ph-CH_2-CH_2), 1.72 (1H, t, J=5.86, OH), 1.57 (2H, m, Ph-CH_2-CH_2), 1.34 (2H, m, CH_2-CH_3), 0.91 (3H, t, J=7.32Hz, CH_3); δ_C (100MHz, $CDCl_3$): 142.59, 138.19, 128.72, 127.21, (Ar, C), 65.39 (Ph-CH_2), 35.44 (Ph-CH_2-CH_2), 33.78 (Ph-CH_2-CH_2), 22.43 (CH_2-CH_3), 14.04 (CH_3); GC: t_R 11.36min; LRMS (EI): m/z 164 (M^+, 35%), 121 (M^+-C_3H_7, 75%), 107 (M^+-C_4H_9, 100%), 91 (M^+-C_4H_9O, 68%).

4-Butylbenzyl bromide (**246**)

Compound **246** was synthesised in a similar manner to **243**, except that PBr_3 (5.00g, 18.47mmol) and **245** (1.50g, 9.15mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **246** (1.25g, yield 60%) as a clear oil; R_f=0.77 [10/90

diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3024 (Ar, C-H), 2956 (C-H), 1613 (Ar, C=C), 607 (C-Br); δH (400MHz, $CDCl_3$): 7.22 (2H, d, J=8.06Hz, Ph-H), 7.07 (2H, d, J=8.06Hz, Ph-H), 4.41 (2H, s, Ph-C$\underline{H}_2$), 2.52 (2H, t, J=7.69Hz, Ph-C$\underline{H}_2$-CH_2), 1.50 (2H, m, Ph-CH_2-C$\underline{H}_2$) 1.26 (2H, m, C$\underline{H}_2$-CH_3), 0.84 (3H, t, J=7.32Hz, C$\underline{H}_3$); δ_C (100MHz, $CDCl_3$): 143.51, 135.08, 129.07, 128.96, (Ar, C), 35.48 (Ph-$\underline{C}H_2$-CH_2), 33.93 (Ph-CH_2), 33.60 (Ph-CH_2-$\underline{C}H_2$), 22.45 ($\underline{C}H_2$-CH_3), 14.05 (CH_3); GC: t_R 5.19min; LRMS (EI): m/z 226 (M^+, 6%), 147 (M^+-Br, 100%), 104 (M^+-C_3H_7Br, 30%), 91(M^+-C_4H_8Br, 30%).

1-(4-Butyl-benzyl)-1*H*-imidazole (**247**)

Compound **247** was synthesised in a similar manner to **198**, except that **246** (1.00g, 4.72mmol), anhydrous K_2CO_3 (0.80g, 5.80mmol) and imidazole (0.50g, 7.35mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **247** as a light yellow oil (0.67g, yield 66%); R_f=0.56 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3023 (Ar, C-H), 2956 (C-H), 1904 (Im, C=N), 1614 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.53 (1H, s, NC$\underline{H}$N, Im), 7.09 (2H, d, J=8.24Hz, Ph-H), 7.02 (1H, s, CH_2-NC$\underline{H}$, Im), 7.00 (2H, d, J=8.24Hz, Ph-H), 6.83 (1H, s, NC$\underline{H}$, Im), 5.01 (2H, s, Ph-C$\underline{H}_2$), 2.51 (2H, t, J=7.69Hz, Ph-C$\underline{H}_2$-CH_2), 1.50 (2H, m, Ph-CH_2-C$\underline{H}_2$), 1.27 (2H, m, C$\underline{H}_2$-CH_3), 0.83 (3H, t, J=7.32Hz, C$\underline{H}_3$); δ_C (100MHz, $CDCl_3$): 143.32 (Ar, C), 137.33 (Im, NCN), 133.22, 129.34, 127.43 (Ar, C), 129.11, 119.40 (Im, C), 50.82 (Ph-CH_2), 35.35 (Ph-$\underline{C}H_2$-CH_2), 33.64 (Ph-CH_2-$\underline{C}H_2$), 22.39 ($\underline{C}H_2$-CH_3), 14.01 (CH_3); GC: t_R 17.68min; LRMS (EI): m/z 214 (M^+, 17%), 147 (M^+-$C_3H_3N_2$, 100%), 105 (M^+-$C_6H_9N_2$, 14%), 91 (M^+-$C_7H_{11}N_2$, 24%). HRMS (EI): found m/z 214.14610, $C_{14}H_{18}N_2$, calculated m/z 214.14650.

4-(Pentyl-phenyl) methanol (**248**)

Compound **248** was synthesised in a similar manner to **242**, except that 4-pentylbenzoicacid (3.00g, 15.63mmol) and $LiAlH_4$ (3.90mL, 2M in THF) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **248** as a clear oil (1.80g, yield 65%); R_f=0.39 [40/60 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3329 (O-H), 2956 (C-H), 1616 (Ar, C=C), 1015 (C-O); δH (400MHz, $CDCl_3$): 7.26 (2H, d, J=8.06Hz, Ph-H), 7.15 (2H, d, J=8.06Hz, Ph-H), 4.64 (2H, br.s, Ph-CH_2), 2.57 (2H, t, J=7.51Hz, Ph-CH_2-CH_2), 1.55 [5H, m; 4H, Ph-CH_2-(CH_2)$_2$, 1H, OH], 1.29 (2H, m, CH_2-CH_3), 0.87 (3H, t, J=6.77 Hz, CH_3); δ_C (100MHz, $CDCl_3$): 142.62, 138.19, 128.71, 127.21, (Ar, C), 65.39 (Ph-CH_2), 35.72 (Ph-CH_2-CH_2), 31.58 (Ph-CH_2-CH_2), 31.32 (CH_2-CH_2-CH_3), 22.64 (CH_2-CH_3), 14.14 (CH_3); GC: t_R 12.61min; LRMS (EI): m/z 178 (M^+, 58%), 121 (M^+-C_4H_9, 96%), 107 (M^+-C_5H_{11}, 100%), 91 (M^+-$C_5H_{11}O$, 52%).

4-Pentylbenzyl bromide (**249**)

Compound **249** was synthesised in a similar manner to **243**, except that PBr_3 (4.50g, 16.62mmol) and **248** (1.50g, 8.43mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **249** (1.36g, yield 67%) as a clear oil; R_f=0.83 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3024 (Ar, C-H), 2857 (C-H), 1613 (Ar, C=C), 607 (C-Br); δH (400MHz, $CDCl_3$): 7.31 (2H, d, J=8.06Hz, Ph-H), 7.16 (2H, d, J=8.06Hz, Ph-H),

4.50 (2H, s, Ph-CH_2), 2.60 (2H, t, J=7.87Hz, Ph-CH_2-CH_2), 1.61 (2H, m, Ph-CH_2-CH_2), 1.34 [4H, m, (CH_2)$_2$-CH_3], 0.90 (3H, t, J=7.14Hz, CH_3); δ_C (100MHz, CDCl$_3$): 143.54, 135.08, 129.07, 128.95, (Ar, C), 35.76 (Ph-CH_2-CH_2), 33.94 (Ph-CH_2), 31.59 (Ph-CH_2-CH_2), 31.15 [Ph-(CH_2)$_2$-CH_2], 22.64 (CH_2-CH_3), 14.14 (CH_3); GC: t_R 13.81min; LRMS (EI): m/z 242 (M^+, 2%), 161 (M^+-Br, 100%), 104 (M^+-C_4H_9Br, 34%), 91 (M^+-C_5H_{10}Br, 34%).

1-(4-Pentyl-benzyl)-1*H*-imidazole (**250**)

Compound **250** was synthesised in a similar manner to **198**, except that **249** (1.00g, 4.13mmol), anhydrous K_2CO_3 (0.70g, 5.07mmol) and imidazole (0.45g, 6.62mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **250** as a light yellow oil (0.56g, yield 60%); R_f=0.44 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3023 (Ar, C-H), 2955 (C-H), 1901 (Im, C=N), 1615 (Ar, C=C); δH (400MHz, CDCl$_3$): 7.56 (1H, s, NCHN, Im), 7.13 (2H, d, J=8.06Hz, Ph-H), 7.06 (1H, s, CH_2-NCH, Im), 7.04 (2H, d, J=8.06Hz, Ph-H), 6.88 (1H, s, NCH, Im), 5.06 (2H, s, Ph-CH_2), 2.56 (2H, t, J=7.69Hz, Ph-CH_2-CH_2), 1.57 (2H, m, Ph-CH_2-CH_2), 1.28 [4H, m, Ph-(CH_2)$_2$-(CH_2)$_2$], 0.86 (3H, t, J=7.14Hz, CH_3); δ_C (100MHz, CDCl$_3$): 143.34 (Ar, C), 137.37 (Im, NCN), 133.26 129.44, 127.42 (Ar, C), 129.09, 119.39 (Im, C), 50.80 (Ph-CH_2), 35.63 (Ph-CH_2-CH_2), 31.53 (Ph-CH_2-CH_2), 31.18 [Ph-(CH_2)$_2$-CH_2], 22.59 (CH_2-CH_3), 14.10 (CH_3); GC: t_R 18.97min; LRMS (EI): m/z 228 (M^+, 28%), 161 (M^+-$C_3H_3N_2$, 100%), 104 (M^+-$C_7H_{10}N_2$, 17%), 91 (M^+-$C_8H_{13}N_2$, 31%). HRMS (EI): found m/z 228.16210, $C_{15}H_{20}N_2$, calculated m/z 228.16210.

4-(Heptyl-phenyl)methanol (**251**)

Compound **251** was synthesised in a similar manner to **242**, except that 4-hepthylbenzoicacid (3.00g, 13.64mmol) $LiAlH_4$ and (3.40mL, 2M in THF) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **251** as a clear oil (1.88g, yield 67%); R_f=0.41 [40/60 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1} : 3327 (O-H), 3012 (Ar, C-H), 2955 (C-H), 1615 (Ar, C=C), 1015 (C-O); δH (400MHz, $CDCl_3$): 7.25 (2H, d, J=8.06Hz, Ph-H), 7.15 (2H, d, J=8.06Hz, Ph-H), 4.63 (2H, s, Ph-CH_2), 2.58 (2H, t, J=7.51Hz, Ph-CH_2-CH_2), 1.67 (1H, br.s, OH), 1.58 (2H, t, J=7.51Hz, Ph-CH_2-CH_2), 1.28 [8H, m, Ph-$(CH_2)_2$-$(CH_2)_4$], 0.86 (3H, t, J=6.96 Hz, CH_3); δ_C (100MHz, $CDCl_3$): 142.63, 138.19, 128.71, 127.20, (Ar, C), 65.41 (Ph-CH_2), 35.75 (Ph-CH_2-CH_2), 31.91 (Ph-CH_2-CH_2), 31.65 [Ph-$(CH_2)_2$-CH_2], 29.36 [Ph-$(CH_2)_3$-CH_2], 29.28 (CH_2-CH_2-CH_3), 22.76 (CH_2-CH_3), 14.20 (CH_3); GC: t_R 15.32min; LRMS (EI): m/z 206 (M^+, 23%), 121 (M^+-C_8H_9O, 83%), 107 (M^+-C_7H_{15}, 100%), 91 (M^+-$C_7H_{15}O$, 64%).

4-Heptylbenzyl bromide (**252**)

Compound **252** was synthesised in a similar manner to **243**, except that PBr_3 (4.00g, 14.76mmol) and **251** (1.50g, 7.28mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **252** (1.41g, yield 72%) as a clear oil; R_f=0.84 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3025 (Ar, C-H), 2928 (C-H), 1613 (Ar, C=C), 607 (C-Br); δH

(400MHz, $CDCl_3$): 7.28 (2H, d, J=8.06Hz, Ph-$\underline{H}$), 7.13 (2H, d, J=8.06Hz, Ph-$\underline{H}$), 4.48 (2H, s, Ph-$C\underline{H}_2$), 2.57 (2H, t, J=7.69Hz, Ph-$C\underline{H}_2$-CH_2), 1.60 (2H, m, Ph-CH_2-$C\underline{H}_2$), 1.28 [8H, m, Ph-$(CH_2)_2$-$(C\underline{H}_2)_4$], 0.86 (3H, t, J=6.96 Hz, $C\underline{H}_3$); δ_C (100MHz, $CDCl_3$): 143.55, 135.06, 129.06, 128.94, (Ar, C), 35.79 (Ph-$\underline{C}H_2$-CH_2), 33.92 (Ph-CH_2), 31.90 (Ph-CH_2-$\underline{C}H_2$), 31.46 [Ph-$(CH_2)_2$-$\underline{C}H_2$], 29.37 [Ph-$(CH_2)_3$-$\underline{C}H_2$], 29.26 ($\underline{C}H_2$-CH_2-CH_3), 22.76 ($\underline{C}H_2$-CH_3), 14.20 (CH_3); GC: t_R 16.32min; LRMS (EI): m/z 270 (M^+, 3%), 189 (M^+-Br, 100%), 105 (M^+-$C_6H_{12}Br$, 18%), 91 (M^+-$C_7H_{14}Br$, 14%).

1-(4-Heptyl-benzyl)-1*H*-imidazole (**253**)

Compound **253** was synthesised in a similar manner to **198**, except that **252** (0.70g, 3.51mmol), anhydrous K_2CO_3 (0.75g, 5.43mmol) and imidazole (0.50g, 7.35mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **253** as a light yellow oil (0.53g, yield 81%); R_f=0.46 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3024 (Ar, C-H), 2855 (C-H), 1901 (Im, C=N), 1614 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.50 (1H, s, $NC\underline{H}N$, Im), 7.13 (2H, d, J=8.24Hz, Ph-$\underline{H}$), 7.05 (1H, s, CH_2-$NC\underline{H}$, Im), 7.03 (2H, d, J=8.24Hz, Ph-$\underline{H}$), 6.87 (1H, s, $NC\underline{H}$, Im), 5.04 (2H, s, Ph-$C\underline{H}_2$), 2.56 (2H, t, J=7.87Hz, Ph-$C\underline{H}_2$-CH_2), 1.56 (2H, m, Ph-CH_2-$C\underline{H}_2$), 1.26 [8H, m, Ph-$(CH_2)_2$-$(C\underline{H}_2)_4$], 0.85 (3H, t, J=7.14Hz, $C\underline{H}_3$); δ_C (100MHz, $CDCl_3$): 143.27 (Ar, C), 137.47 (Im, NCN), 133.41, 129.79, 127.37, (Ar, C), 129.06, 119.34 (Im, C), 50.70 (Ph-CH_2), 35.67 (Ph-$\underline{C}H_2$-CH_2), 31.88 (Ph-CH_2-$\underline{C}H_2$), 31.52 [Ph-$(CH_2)_2$-$\underline{C}H_2$], 29.32 [Ph-$(CH_2)_3$-$\underline{C}H_2$], 29.23 ($\underline{C}H_2$-CH_2-CH_3), 22.74 ($\underline{C}H_2$-CH_3), 14.19 (CH_3); GC: t_R 20.97min; LRMS (EI): m/z 256 (M^+, 28%), 189 (M^+-$C_3H_3N_2$, 100%), 105 (M^+-$C_9H_{15}N_2$, 36%), 91 (M^+-$C_{10}H_{17}N_2$, 3%); HRMS (EI): found m/z 257.20170, $C_{17}H_{24}N_2$, calculated m/z 257.20030.

1-(3,5-Dimethyl-benzyl)-1*H*-imidazole (**254**)

Compound **254** was synthesised in a similar manner to **198**, except that 3,5-dimethylbenzyl bromide (1.00g, 5.02mmol), anhydrous K_2CO_3 (0.83g, 6.02mmol) and imidazole (0.50g, 7.53mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **254** as a light yellow oil (0.64g, yield 68%); R_f=0.55 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3010 (Ar, C-H), 2920 (C-H), 2209 (Im, C=N), 1606 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.54 (1H, s, NC<u>H</u>N, Im), 7.05 (1H, s, CH_2-NC<u>H</u>, Im), 6.92 (1H, s, Ph-<u>H</u>), 6.87 (1H, s, NC<u>H</u>, Im), 6.73 (2H, s, Ph-<u>H</u>), 5.00 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 138.76, (Ar, C), 137.44 (Im, NCN), 136.03, 129.96, 125.21 (Ar, C), 129.50, 119.45 (Im, C), 50.91 (Ph-CH_2), 21.30 [Ph-$(CH_3)_2$]; GC: t_R 15.39 min; LRMS (EI): m/z 186 (M^+, 26%), 119 (M^+-$C_3H_3N_2$, 100%), 91 (M^+-$C_5H_7N_2$, 18%); HRMS (EI): found m/z 186.11510, $C_{12}H_{14}N_2$, calculated m/z 186.11510.

1-(2,3,4,5-Tetramethyl-benzyl)-1*H*-imidazole (**255**)

Compound **255** was synthesised in a similar manner to **198**, except that 2,3,4,5-tetramethylbenzyl bromide (1.00g, 4.40mmol), anhydrous K_2CO_3 (0.73g, 5.28mmol) and imidazole (0.42g, 6.60mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **255** as a light yellow oil (0.37, yield 40%) (m.p. 74.1-75.2°C); R_f=0.55

[90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3051 (Ar, C-H), 2975 (C-H), 2307 (Im, C=N), 1621 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.33 (1H, s, NCHN, Im), 7.02 (1H, s, Ph-H), 7.00 (1H, s, CH_2-NCH, Im), 6.77 (1H, s, NCH, Im), 5.16 (2H, s, Ph-CH_2), 2.23 [6H, s, Ph-$(CH_3)_2$], 2.15 [6H, s, Ph-$(CH_3)_2$]; δ_C (100MHz, $CDCl_3$): 134.58 (Ar, C), 133.77, 132.52 (Im, NCN), 130.75, 128.93, (Ar, C), 118.81 (Im, C), 45.79 (Ph-CH_2), 20.55 (CH_3), 15.50 (CH_3); GC: t_R 18.25 min; LRMS (EI): m/z 214 (M^+, 17%), 91 (M^+-$C_7H_{11}N_2$, 6%), 147 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 214.14640, $C_{14}H_{18}N_2$, calculated m/z 214.14650.

1-(Biphenyl-3-ylmethyl)-1*H*-imidazole (**256**)

Compound **256** was synthesised in a similar manner to **198**, except that biphenyl-3-ylmethyl bromide (1.00g, 4.05mmol), anhydrous K_2CO_3 (0.67g, 4.86mmol) and imidazole (0.40g, 6.07mmol) were used. Removal of the solvent under vacuum gave a pale solid which was purified using column chromatography to give **256** as a white solid (0.58g, yield 61%), [m.p. 133.4-131.7°C (lit. m.p. 130.0-131.0°C; Baggaley et al, 1975)]; R_f=0.41 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3052 (Ar, C-H), 2930 (C-H), 2305 (Im, C=N), 1599 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.56 (1H, s, NCHN, Im) 7.50 (3H, m, Ph-H), 7.41 (3H, m, Ph-H), 7.33 (2H, m, Ph-H), 7.08 (2H, m; 1H, Ph-H, 1H, CH_2-NCH, Im), 6.91 (1H, s, NCH, Im), 5.15 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 142.18, 140.49, (Ar, C), 137.58 (Im, NCN), 136.85, 130.00, 129.54, 127.77, 127.23, 127.18, 126.17, 126.11 (Ar, C), 128.97, 119.42 (Im, C), 50.90 (Ph-CH_2); GC: t_R 21.34min; LRMS (EI): m/z 234 (M^+, 54%), 152 (M^+-CH_2, 24%), 167 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 235.12350, $C_{16}H_{14}N_2$, calculated m/z 235.12470.

4-(Bromomethyl)biphenyl (**257**)

Compound **257** was synthesised in a similar manner to **243**, except that PBr_3 (5.90g, 21.74mmol) and biphenyl-4-ylmethyl alcohol (2.00g, 10.87mmol) were used. Removal of the solvent under vacuum gave a brown solid which was purified using column chromatography to give **257** (1.42g, yield 53%) as a white solid [m.p. 83.3-83.7°C (lit. m.p. 82.0-83.0°C; Ghaddar et al, 2001)]; R_f=0.79 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3052 (Ar, C-H), 2971 (C-H), 1601 (Ar, C=C), 605 (C-Br); δH (400MHz, $CDCl_3$): 7.57 (4H, m, Ph-H), 7.43 (4H, m, Ph-H), 7.35 (1H, m, Ph-H), 4.54 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 141.46, 140.62, 140.54, 136.85, 129.60, 128.92, 127.65, 127.21 (Ar, C), 33.48 (Ph-CH_2); GC: t_R 16.93min; LRMS (EI): m/z 248 (M^+, 7%), 167 (M^+-$C_{13}H_{11}$, 100%), 152 (M^+-$C_{12}H_8$, 9%).

1-(Biphenyl-4-ylmethyl)-1*H*-imidazole (**258**)

Compound **258** was synthesised in a similar manner to **198**, except that **257** bromide (1.00g, 4.05mmol), anhydrous K_2CO_3 (0.67g, 4.86mmol) and imidazole (0.41g, 6.07mmol) were used. Removal of the solvent under vacuum gave a pale solid which was purified using column chromatography to give **258** as a white solid (0.73g, yield 77%), [m.p. 134.1-134.6°C (lit. m.p. 129.0-131.0°C; Baggey et al, 1975)]; R_f=0.44 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3051 (Ar, C-H), 2932 (C-H), 2305 (Im, C=N), 1601 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.50 (5H, m; 4H, Ph-H, 1H, NCH, Im), 7.36 (2H, m, Ph-H), 7.29 (1H, m, Ph-H), 7.15 (2H, d, J=8.60, Ph-H), 7.03 (1H, s, CH_2-NCH, Im), 6.86 (1H, s, NCH, Im), 5.08 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 141.37, 140.41, (Ar, C), 137.56 (Im, NCN), 135.22, 129.99, 127.81, 127.78, 127.68, 127.17 (Ar, C), 128.95, 119.39 (Im, C), 50.60 (Ph-CH_2); GC: t_R 21.78min; LRMS (EI): m/z 234 (M^+, 22%), 167 (M^+-$C_3H_3N_2$, 100%), 152 (M^+-CH_2, 14%), 77 (M^+-$C_{10}H_9N_2$, 4%); HRMS (EI): found m/z 235.12297, $C_{16}H_{14}N_2$, calculated m/z 235.12297.

1,4-bis(bromomethyl)benzene (**259**)

Compound **259** was synthesised in a similar manner to **243**, except that PBr_3 (7.85g, 28.99mmol) and 1,4-bis(bromomethanol) benzene (2.00g, 14.49mmol) were used. Removal of the solvent under vacuum gave a brown solid which was purified using column chromatography to give **259** (2.50g, yield 66%) as a white solid [144.6-145.7°C (lit. m.p. 146.0-147.0°C; Chen et al, 2003)]; R_f=0.82 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3053 (Ar, C-H), 2985 (C-H), 1603 (Ar, C=C), 612 (C-Br); δH (400MHz, $CDCl_3$): 7.35 (4H, s, Ph-H), 4.45 [4H, s, Ph-$(CH_2)_2$-$(Br)_2$], δ_C (100MHz, $CDCl_3$): 138.09, 129.58 (Ar, C), 32.90 [Ph-$(CH_2)_2$-$(Br)_2$]; GC: t_R 13.22min; LRMS (EI): m/z 264 (M^+, 5%), 185 (M^+-Br, 100%), 104 (M^+-Br_2, 91%), 77 (M^+-$C_2H_3Br_2$, 6%).

1,1'-[1,4-phenylenebis(methylene)]bis(1*H*-Imidazole) (**260**)

Compound **260** was synthesised in a similar manner to **260**, except that **259** (1.00g, 3.79mmol), anhydrous K_2CO_3 (0.78g, 5.68mmol) and imidazole (0.52g, 7.58mmol) were used. Removal of the solvent under vacuum gave a off-white solid which was purified using column chromatography to give **260** as a white solid (0.72g, yield 80%); [m.p. 132.8.-133.9°C (lit. m.p. 131.0-132.0°C; Baggaley et al, 1975)]; R_f=0.26 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3053 (Ar, C-H), 2985 (C-H), 2305 (Im,C=N), 1602 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.72 [2H, s, $(NC\underline{H}N)_2$, Im], 7.23 (4H, s, Ph-$\underline{H}$), 7.07 [2H, s, $(CH_2-NC\underline{H})_2$, Im], 6.96 [2H, s, $(NC\underline{H})_2$, Im], 5.20 [4H, s, Ph-$(C\underline{H}_2)_2$-$(Im)_2$]; δ_C (100MHz, $CDCl_3$): 137.30 (Im, NCN), 137.04, 128.04, (Ar, C), 127.84, 119.58 (Im, C), 49.80 [Ph-$(CH_2)_2$-$(Im)_2$]; GC: t_R 23.61min; LRMS (EI): m/z 238 (M^+, 91%), 171 (M^+-$C_3H_3N_2$, 100%), 104 (M^+-$C_6H_6N_4$, 76%); HRMS (EI): found m/z 239.12960, $C_{14}H_{14}N_4$, calculated m/z 239.12900.

1-(2-Naphthylmethyl)-1*H*-imidazole (**261**)

Compound **261** was synthesised in a similar manner to **198**, except that 2-naphthylmethyl bromide (1.00g, 4.52mmol), anhydrous K_2CO_3 (0.75g, 5.42mmol) and imidazole (0.46g, 6.78mmol) were used. Removal of the solvent under vacuum gave a yellow solid which was purified using column chromatography to give **261** as a light yellow solid (0.60g, yield 64%), [m.p. 85.7-86.2°C (lit. m.p. 82-85°C; Matsunaga et al, 2004)]; R_f=0.43 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3050 (Ar, C-H), 2976 (C-H), 2304 (Im, C=N), 1603 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.81 (3H, m; 1H, NCHN Im, 2H, NaPh-H), 7.58 (2H, d, J=7.87, NaPh-H), 7.50 (2H, m, NaPh-H), 7.25 (1H, dd, J=1.65, J=8.42, NaPh-H), 7.11 (1H, s, CH_2-NCH, Im), 6.93 (1H, s, NCH, Im), 5.25 (2H, s, Naph-CH_2); δ_C (100MHz, $CDCl_3$): 137.65 (NCN, Im), 133.66, 133.34, 133.07, 130.02, 127.94, 127.85, 126.75, 126.56, 126.36 124.94 (Naph, C), 129.05, 119.47 (Im, C), 51.04 (Naph-CH_2); GC: t_R 26.07min; LRMS (EI): m/z 208 (M^+, 29%), 141 (M^+-$C_3H_3N_2$, 100%); HRMS (EI): found m/z 208.10000, $C_{14}H_{12}N_2$, calculated m/z 208.09950.

1-(4-diphenylmethyl-benzyl)-1*H*-imidazole (**262**)

Compound **262** was synthesised in a similar manner to **198**, except that 4-diphenylmethylbenzyl bromide (1.00g, 4.05mmol), anhydrous K_2CO_3 (0.67g, 4.86mmol) and imidazole (0.41g, 6.07mmol) were used. Removal of the solvent under vacuum gave a light brown solid which was purified using column chromatography to give **262** as a yellow solid (0.62g, yield 65%), [m.p. 133.4-131.7°C (lit. m.p. 130.0-131.0°C; Njar et al, 2000)]; R_f=0.41 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3030 (Ar, C-H), 2343 (Im, C=N), 1601 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.39 (1H, s, NCHN, Im), 7.35 (1H, s, CH_2-NCH, Im), 7.33 (4H, m, Ph-H), 7.08 (6H, m, Ph-H), 6.83 (1H, s, NCH, Im), 6.95 (1H, s, $(Ph)_2$-CH), 6.50 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 139.19 (Ar, C), 137.49 (Im, NCN), 129.43, 128.96, 128.15 (Ar, C), 128.47, 119.47 (Im, C), 65.10 $(Ph)_2$-CH); GC: t_R 19.46min; LRMS (EI): m/z 234 (M^+, 9%), 167 (M^+-$C_3H_3N_2$, 100%), 77 (M^+-$C_{10}H_9N_2$, 5%); HRMS (EI): found m/z 234.11500, $C_{16}H_{14}N_2$, calculated m/z 234.11510.

1-(4-Hydroxy-benzyl)-1*H*-imidazole (**263**)

Imidazole (13.4g, 200mM) was thoroughly mixed with 4-hydroxybenzyl alcohol (5g, 40.00mM) in a conical flask (250 mL), and left at room temperature for 48h until a dark yellowish oil was produced. The reaction mixture was then heated at 160°C for 30 minutes and a dark brown oil was observed. The reaction mixture was then poured into 500mL of hot water resulting in the precipitation of a brown solid which was filtered and vacuum dried to give **263** as a light brown solid (6.08g, yield 87%); [m.p. 209.8-210.6°C (lit. m.p. 212.0-213.0°C; Kruse et al, 1990)]; R_f=0.35 [50/50 (diethyl ether/petroleum ether)].

$\nu_{(max)}$(Film)cm^{-1}: 3421 (Ph-OH), 1601 (Ar, C=C); δH (400MHz, DMSO): 9.45 (1H, s, Ph-O<u>H</u>), 7.66 (1H, s, NC<u>H</u>N, Im), 7.09 (1H, s, CH_2-NC<u>H</u>, Im), 7.06 (2H, d, J=8.79Hz, Ph-<u>H</u>), 6.83 (1H, s, NC<u>H</u>, Im), 6.68 (2H, d, J=8.79Hz, Ph-<u>H</u>); 4.99 (2H, s, Ph-C$\underline{H}_2$); δ_C (100MHz, DMSO): 157.55 (Ar, C-OH), 137.65 (Im, NCN), 129.65, 128.51, 115.88 (Ar, C), 129.09, 119.87 (Im, C), 49.67 (Ph-CH_2); GC: t_R 9.43 min; LRMS (EI): m/z 174 (M^+, 18%), 107 (M^+-$C_3H_3N_2$, 100%), 77 (M^+-$C_4H_5N_2O$, 14%); HRMS (EI): found m/z 175.0866300, $C_{10}H_{11}O_1$, calculated m/z 175.0866295.

4-(1*H*-imidazol-1-ylmethyl)phenyl methanesulfonate (**264**)

To a mixture of **263** (1.00g, 5.75 mmol) and triethylamine (0.70g, 6.89 mmol) in anhydrous DCM, methane sulfonyl chloride (0.72g, 6.32 mmol) was added and the reaction mixture refluxed for 12h. After cooling, the reaction mixture was poured on ice (100mL) and extracted with saturated Na_2CO_3 solution (2×50mL). The combined DCM layer was washed with water (3×50mL) and dried over

anhydrous $MgSO_4$. After filtration, DCM was removed under vacuum to give a dark brown oil which was purified using column chromatography to give **264** as a light yellowish oil (0.92g, yield 63%); R_f=0.22 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2515 (C-H), 2074 (Im, C=N), 1364 (S=O); δH (400MHz, $CDCl_3$): 7.76 (1H, s, NCHN, Im), 7.32 (4H, m, Ph-H), 7.12 (1H, s, CH_2-NCH, Im), 6.99 (1H, s, NCH, Im), 5.25 (2H, s, Ph-CH_2), 3.19 (3H, s, CH_3-S); δ_C (100MHz, $CDCl_3$): 149.33, (Ar, C), 137.36 (Im, NCN), 136.40, 128.95, 122.44 (Ar, C), 128.13, 119.60 (Im, C), 49.43 (Ph-CH_2), 36.24 (CH_3); GC: t_R 15.68 min; LRMS (EI): m/z 252 (M^+, 43%), 185 (M^+-$C_3H_3N_2$, 79%), 107 (M^+-$C_4H_7N_2O_2S$, 100%), 78 (M^+-C_{10} $H_{10}ON_2$, 16%); HRMS (EI): found m/z 253.0642330, $C_{11}H_{13}N_2O_3S$, calculated m/z 253.0641394.

4-(1*H*-imidazol-1-ylmethyl)phenyl ethanesulfonate (**265**)

Compound **265** was synthesised in a similar manner to **264**, except that ethane sulfonyl chloride (0.81g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **265** as a clear oil (1.04g, yield 68%); R_f=0.29 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2972 (C-H), 2359 (Im, C=N), 1603 (Ar, C=C), 1363 (S=O); δH (400MHz, $CDCl_3$): 7.66 (1H, s, NCHN, Im), 7.22 (2H, d, J=8.97Hz, Ph-H), 7.19 (2H, d, J=8.97Hz, Ph-H), 7.02 (1H, s, CH_2-NCH, Im), 6.89 (1H, s, NCH, Im), 5.15 (2H, s, Ph-CH_2), 3.26 (2H, q, J=7.32Hz, CH_2-CH_3), 1.35 (3H, t, J=7.32, CH_3); δ_C (100MHz, $CDCl_3$): 149.14, (Ar, C), 137.36 (Im, NCN), 136.26, 128.92, 122.36 (Ar, C), 128.17, 119.58 (Im, C), 49.41 (Ph-CH_2), 44.67 (CH_2-CH_3), 7.19 (CH_3); GC: t_R 18.74 min; LRMS (EI): m/z 266 (M^+, 31%), 199 (M^+-$C_3H_3N_2$, 61%), 107 (M^+-$C_5H_7N_2O_2S$, 100%), 78 (M^+-$C_{11}H_{12}ON_2$, 17%); HRMS (EI): found m/z 267.07980,

$C_{12}H_{15}N_2O_3S$, calculated m/z 267.07869; Elemental analysis: found C 54.12%, H 5.30%, N 10.52%, $C_{12}H_{14}N_2O_3S$, calculated C 54.13%, H 5.43%, N 10.60% (0.35 mole of H_2O).

4-(1*H*-imidazol-1-ylmethyl)phenyl-1-propanesulfonate (**266**)

Compound **266** was synthesised in a similar manner to **264**, except that 1-propane sulfonyl chloride (0.90g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **266** as a clear oil (1.13g, yield 70%); R_f=0.46 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2972 (C-H), 1647 (Ar, C=C), 1366 (S=O); δH (400MHz, $CDCl_3$): 7.68 (1H, s, NC<u>H</u>N, Im), 7.26 (2H, d, J=8.97Hz, Ph-<u>H</u>), 7.20 (2H, d, J=8.97Hz, Ph-<u>H</u>), 7.04 (1H, s, CH_2-NC<u>H</u>, Im), 6.92 (1H, s, NC<u>H</u>, Im), 5.17 (2H, s, Ph-C<u>H</u>$_2$), 3.26 (2H, m, C<u>H</u>$_2$-S), 1.89 (2H, m, C<u>H</u>$_2$-CH_2-S), 1.02 (3H, t, J=7.51, C<u>H</u>$_3$); δ_C (100MHz, $CDCl_3$): 149.11 (Ar, C), 137.36 (Im, NCN), 136.24, 128.92, 122.40 (Ar, C), 128.17, 119.59 (Im, C), 51.63 (Ph-CH_2), 49.42 (CH_2-S), 17.13 (<u>C</u>H_2-CH_2-S), 11.65 (CH_3); GC: t_R 22.92 min; LRMS (EI): m/z 280 (M^+, 28%), 213 (M^+-$C_3H_3N_2$, 60%), 107 (M^+-$C_6H_9N_2O_2S$, 100%), 78 (M^+-$C_{12}H_{14}ON_2$, 15%); HRMS (EI): found m/z 281.00552, $C_{13}H_{17}N_2O_3S$, calculated m/z 281.09540; Elemental analysis: found C 55.70%, H 5.75%, N 9.99%, $C_{13}H_{16}N_2O_3S$, calculated C 55.48%, H 5.77%, N 9.98%.

4-(1*H*-imidazol-1-ylmethyl)phenyl-1-propanesulfonate (**267**)

Compound **267** was synthesised in a similar manner to **264**, except that 1-butane sulfonyl chloride (1.00g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **267** as a light brownish oil (1.29g, yield 76%); R_f=0.49 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2968 (C-H), 2346 (Im, C=N), 1603 (Ar, C=C), 1364 (S=O); δH (400MHz, $CDCl_3$): 7.79 (1H, s, NC<u>H</u>N, Im), 7.37 (2H, d, J=8.79, Ph-<u>H</u>), 7.32 (2H, d, J=8.79, Ph-<u>H</u>), 7.15 (1H, s, CH_2-NC<u>H</u>, Im), 7.03 (1H, s, NC<u>H</u>, Im), 5.28 (2H, s, Ph-C<u>H</u>$_2$), 3.39 (2H, m, C<u>H</u>$_2$-S), 1.91 (2H, m, C<u>H</u>$_2$-CH_2-S), 1.53 [2H, m, C<u>H</u>$_2$-(CH_2)$_2$-S], 1.00 (3H, m, CH_3); δ_C (100MHz, $CDCl_3$): 149.12, (Ar, C), 137.36 (Im, NCN), 136.24, 128.92, 122.39 (Ar, C), 128.17, 119.59 (Im, C), 49.77 (Ph-CH_2), 49.42 (CH_2-S), 25.37 (<u>C</u>H_2-CH_2-S), 20.98 [<u>C</u>H_2-(CH_2)$_2$-S], 12.48 (CH_3); GC: t_R 23.91 min; LRMS (EI): m/z 294 (M^+, 7%), 227 (M^+-$C_{11}H_{15}O_3S$, 29%), 107 (M^+-C_7H_7O, 100%), 78 (M^+-CH_2O_2S, 12%); HRMS (EI): found m/z 295.11105, $C_{14}H_{19}N_2O_3S$, calculated m/z 295.11109; Elemental analysis: found C 57.12%, H 6.16%, N 9.52%, $C_{14}H_{18}N_2O_3S$, calculated C 57.38%, H 6.21%, N 9.54% (0.14 mole of H_2O).

4-(1*H*-imidazol-1-ylmethyl)phenyl-1-octanesulfonate (**268**)

Compound **268** was synthesised in a similar manner to **264**, except that

1-octane sulfonyl chloride (1.34g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **268** as a light yellow oil (1.35g, yield 67%); R_f=0.55 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2926 (C-H), 2345 (Im, C=N), 1605 (Ar, C=C), 1368 (S=O); δH (400MHz, $CDCl_3$): 7.67 (1H, s, NCHN, Im), 7.22 (2H, d, J=8.79Hz, Ph-H), 7.18 (2H, d, J=8.79Hz, Ph-H), 7.02 (1H, s, CH_2-NCH, Im), 6.90 (1H, s, NCH, Im), 5.14 (2H, s, Ph-CH_2), 3.24 (2H, t, J=7.69Hz, CH_2-S), 1.79 (2H, m, CH_2-CH_2-S), 1.37 [2H, m, CH_2-$(CH_2)_2$-S], 1.21 [8H, s, C_4H_8-$(CH_2)_2$-S], 0.79 (3H, t, J=7.14Hz, CH_3); δ_C (100MHz, $CDCl_3$): 149.14, (Ar, C), 137.33 (Im, NCN), 136.20, 128.95, 122.39 (Ar, C), 128.06, 119.62 (Im, C), 50.01 (Ph-CH_2), 49.45 (CH_2-S), 31.55 (CH_2-CH_2-S), 28.77 [CH_2-$(CH_2)_2$-S], 27.75 [CH_2-$(CH_2)_3$-S], 28.75 [CH_2-$(CH_2)_4$-S], 23.37 [CH_2-$(CH_2)_5$-S], 22.35 [CH_2-$(CH_2)_6$-S], 13.09 (CH_3); GC: t_R 31.13 min; LRMS (EI): m/z 350 (M^+, 2%), 283 (M^+-$C_3H_3N_2$, 14%), 174 (M^+-$C_8H_{16}O_2S$, 20%), 107 (M^+-$C_{11}H_{19}N_2O_2S$, 100%), 78 (M^+-$C_{17}H_{24}ON_2$, 16%); HRMS (EI): found m/z 351.17368, $C_{18}H_{27}N_2O_3S$, calculated m/z 351.17369; Elemental analysis: found C 61.69%, H 7.48%, N 7.99%, $C_{18}H_{26}N_2O_3S$, calculated C 61.90%, H 7.51%, N 8.29%.

4-(1*H*-imidazol-1-ylmethyl)phenyl-1-decanesulfonate (**269**)

Compound **269** was synthesised in a similar manner to **264**, except that 1-decane sulfonyl chloride (1.52g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **269** as a light yellow oil (1.60g, yield 68%); R_f=0.58 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2924 (C-H), 2348 (C=N), 1604 (Ar, C=C), 1369 (S=O); δH (400MHz, $CDCl_3$): 7.70 (1H, s, NCHN, Im), 7.27 (2H, d, J=8.79, Ph-H), 7.22 (2H,

d, J=8.79Hz, Ph-$\underline{H}$), 7.06 (1H, s, CH_2-NC$\underline{H}$, Im), 6.93 (1H, s, NC$\underline{H}$, Im), 5.18 (2H, s, Ph-C$\underline{H}_2$), 3.29 (2H, t, J=7.69Hz, C$\underline{H}_2$-S), 1.84 (2H, m, C$\underline{H}_2$-CH_2-S), 1.40 [2H, m, C$\underline{H}_2$-$(CH_2)_2$-S], 1.23 [12H, s, $C_6\underline{H}_{12}$-$(CH_2)_3$-S], 0.83 (3H, t, J=7.14Hz, C$\underline{H}_3$); δ_C (100MHz, $CDCl_3$): 149.13 (Ar, C), 137.33 (Im, NCN), 136.21, 128.93, 122.39 (Ar, C), 128.10, 119.59 (Im, C), 50.01 (Ph-CH_2), 49.44 (CH_2-S), 31.71 ($\underline{C}H_2$-CH_2-S), 29.25 [$\underline{C}H_2$-$(CH_2)_2$-S], 29.10 [$\underline{C}H_2$-$(CH_2)_3$-S], 29.08 [$\underline{C}H_2$-$(CH_2)_4$-S], 28.78 [$\underline{C}H_2$-$(CH_2)_5$-S], 27.75 [$\underline{C}H_2$-$(CH_2)_6$-S], 23.37 [$\underline{C}H_2$-$(CH_2)_7$-S], 22.39 [$\underline{C}H_2$-$(CH_2)_8$-S], 13.11 (CH_3); GC: t_R 35.38 min; LRMS (EI): m/z 378 (M^+, 2%), 349 (M^+-C_2H_5, 2%), 174 (M^+-$C_{10}H_{20}O_2S$, 33%), 107 (M^+-$C_{13}H_{23}N_2O_2S$, 100%), 78 (M^+-$C_{19}H_{28}ON_2$, 17%); HRMS (EI): found m/z 379.20457, $C_{20}H_{31}N_2O_3S$, calculated m/z 379.20499; Elemental analysis: found C 63.46%, H 7.99%, N 7.40%, $C_{20}H_{30}N_2O_3S$, calculated C 63.43%, H 7.79%, N 7.51%.

4-(1*H*-imidazol-1-ylmethyl)phenyl-1-dodecanesulfonate (**270**)

Compound **270** was synthesised in a similar manner to **264**, except that 1-dodecane sulfonyl chloride (1.60g, 5.61 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **270** as a light yellow oil (1.40g, yield 62%); R_f=0.63 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2926 (C-H), 2296 (C=N), 1605 (Ar, C=C), 1373 (S=O); δH (400MHz, $CDCl_3$): 7.76 (1H, s, NC$\underline{H}$N, Im), 7.31 (2H, d, J=8.79Hz, Ph-$\underline{H}$), 7.28 (2H, d, J=8.79Hz, Ph-$\underline{H}$), 7.11 (1H, s, CH_2-NC$\underline{H}$, Im), 6.99 (1H, s, NC$\underline{H}$, Im), 5.24 (2H, s, Ph-C$\underline{H}_2$), 3.33 (2H, t, J=7.69Hz, C$\underline{H}_2$-S), 1.88 (2H, m, C$\underline{H}_2$-CH_2-S), 1.46 [2H, m, C$\underline{H}_2$-$(CH_2)_2$-S], 1.28 [16H, s, $C_8\underline{H}_{16}$-$(CH_2)_3$-S], 0.88 (3H, t, J=7.14Hz, C$\underline{H}_3$); δ_C (100MHz, $CDCl_3$): 149.14, (Ar, C), 137.34 (Im, NCN), 136.21, 128.95, 122.40 (Ar, C), 128.10, 119.61 (Im, C), 50.02 (Ph-CH_2), 49.46 (CH_2-S), 31.75 ($\underline{C}H_2$-CH_2-S), 29.42, [sh.s, $\underline{C}_2H_4$-$(CH_2)_2$-S], 29.30 [$\underline{C}H_2$-$(CH_2)_4$-S], 29.15 [$\underline{C}H_2$-$(CH_2)_5$-S],

29.12 [CH_2-$(CH_2)_6$-S], 28.79 [CH_2-$(CH_2)_7$-S], 27.76 [CH_2-$(CH_2)_8$-S], 23.39 [CH_2-$(CH_2)_9$-S], 22.42 [CH_2-$(CH_2)_{10}$-S], 13.13 (CH_3); GC: t_R 51.26 min; LRMS (EI): m/z 406 (M^+, 1%), 349 (M^+-C_4H_9, 2%), 265 (M^+-$C_{10}H_{21}$, 2%), 174 (M^+-$C_{12}H_{24}O_2S$, 63%), 107 (M^+-$C_{15}H_{27}N_2O_2S$, 100%); HRMS (EI): found m/z 407.23717, $C_{22}H_{35}N_2O_3S$, calculated m/z 407.23629; Elemental analysis: found C 64.99%, H 8.43%, N 6.89%, $C_{22}H_{34}N_2O_3S$, calculated C 64.89%, H 8.36%, N 6.87%.

4-(1*H*-imidazol-1-ylmethyl)phenyl trifluoromethanesulfonate (**271**)

Compound **271** was synthesised in a similar manner to **264**, except that trifluoromethane sulfonyl chloride (1.06g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **271** as a light yellow oil (1.24g, yield 68%); R_f=0.29 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2072 (Im, C=N), 1616, (Ar, C=C), 1420 (S=O), 1121 (C-F); δH (400MHz, $CDCl_3$): 7.83 (1H, s, NCHN, Im), 7.42 (4H, m, Ph-H), 7.18 (1H, s, 1H, CH_2-NCH, Im), 7.05 (1H, s, NCH, Im), 5.34 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 198.87 (CF_3-S), 149.32, (Ar, C), 138.08 (Im, NCN), 137.43, 129.30, 121.66 (Ar, C), 128.23, 119.65 (Im, C), 49.20 (Ph-CH_2); GC: t_R 9.29 min; LRMS (EI): m/z 306 (M^+, 90%), 239 (M^+-$C_3H_3N_2$, 100%), 175 (M^+-$C_{10}H_{11}N_2O$, 76%), 69 (M^+-$C_{10}H_9N_2O_3S$, 38%); HRMS (EI): found m/z 307.0360410, $C_{11}H_{10}F_3N_2O_3S$, calculated m/z 307.358739.

4-(1*H*-imidazol-1-ylmethyl)phenyl benzenesulfonate (**272**)

Compound **272** was synthesised in a similar manner to **264**, except that benzene sulfonyl chloride (1.11g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **272** as a light yellow oil (1.29g, yield 71%); R_f=0.33 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3000 (Ar, C-H), 2241 (Im, C=N), 1635 (Ar, C=C), 1373 (S=O); δH (400MHz, $CDCl_3$): 7.81 (2H, d, J=8.60Hz, Ph-H), 7.72 (2H, m, Ph-H), 7.57 (2H, m, 1H, NCHN Im, 1H, Ph-H), 7.18 (2H, d, J=8.60Hz, Ph-H), 7.06 (1H, s, CH_2-NCH, Im), 6.97 (3H, m; 2H, Ph-H, 1H, NCH, Im), 5.18 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 149.36 (Ar, C), 137.32 (Im, NCN), 136.42, 135.21, 134.41, 129.21, 128.69, 128.26, 122.52 (Ar, C), 128.14, 119.56 (Im, C), 49.34 (Ph-CH_2); GC: t_R 17.24 min; LRMS (EI): m/z 314 (M^+, 33%), 247 (M^+-$C_3H_3N_2$, 100%), 141 (M^+-$C_{10}H_9N_2O$, 81%), 107 (M^+-$C_9H_9N_2O_2S$, 7%), 77 (M^+-$C_{10}H_9N_2O_3S$, 70%); HRMS (EI): found m/z 315.0799460, $C_{18}H_{27}N_2O_3S$, calculated m/z 315.0797895; Found C 61.13%, H 4.49%, N 8.91%, $C_{18}H_{26}N_2O_3S$, calculated C 61.28%, H 4.49%, N 8.91%.

4-(1*H*-imidazol-1-ylmethyl)phenyl 3-fluorobenzenesulfonate (**273**)

Compound **273** was synthesised in a similar manner to **264**, except that 3-fluorobenzene sulfonyl chloride (1.22g, 6.32 mmol) were used. Removal of the

solvent under vacuum gave a brown oil which was purified using column chromatography to give **273** as a light yellow oil (1.22g, yield 57%); R_f=0.47 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3000 (Ar, C-H), 2364 (Im, C=N), 1595 (Ar, C=C), 1320 (S=O), 1228 (F-Ph); δH (400MHz, $CDCl_3$): 8.00 (1H, s, NC<u>H</u>N, Im), 7.61 (1H, d, J=7.87Hz, Ph-<u>H</u>), 7.52 (2H, m, Ph-<u>H</u>), 7.38 (1H, m, Ph-<u>H</u>), 7.12 (3H, m; 2H, Ph-<u>H</u>, 1H, CH_2-NC<u>H</u> Im), 6.98 (2H, d, J=8.60Hz, Ph-<u>H</u>), 6.88 (1H, s, NC<u>H</u>, Im), 5.17 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 149.38 (Ar, C), 137.19 (Im, NCN), 135.10, 131.19, 128.95, 124.38, 123.00, 121.96, 121.75, 116.04, 115.79 (Ar, C), 128.16, 119.52 (Im, C), 50.46 (Ph-CH_2); GC: t_R 26.01 min; LRMS (EI): 332 (M^+, 20%); 265 (M^+-$C_3H_2N_2$, 100%), 159 (M^+-$C_{10}H_9N_2O$, 88%), 95 (M^+-$C_{10}H_9N_2O_3S$, 70%); HRMS (EI): found m/z 333.07090, $C_{16}H_{14}FN_2O_3S$, calculated m/z 333.07110

4-(1*H*-imidazol-1-ylmethyl)phenyl 4-fluorobenzenesulfonate (**274**)

Compound **274** was synthesised in a similar manner to **264**, except that 4-fluorobenzene sulfonyl chloride (1.22g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **274** as a light yellow oil (1.32g, yield 69%); R_f=0.45 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2241 (Im, C=N), 1592 (Ar, C=C), 1376 (S=O), 1200 (F-Ph); δH (400MHz, CD_3OD): 7.91 (2H, m, Ph-<u>H</u>), 7.75 (1H, s, NC<u>H</u>N, Im), 7.37 (2H, m, Ph-<u>H</u>), 7.25 (2H, m, Ph-<u>H</u>), 7.12 (1H, s, CH_2-NC<u>H</u>, Im), 7.03 (3H, m; 1H, NC<u>H</u> Im, 2H, Ph-<u>H</u>), 5.24 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, CD_3OD): 149.31 137.33 (Ar, C), 136.53 (Im, NCN), 131.42, 128.78, 122.52, 116.56, 116.33 (Ar, C), 128.16, 119.55 (Im, C), 49.33 (Ph-CH_2); GC: t_R 15.94 min; LRMS (EI): m/z 332 (M^+, 25%), 265 (M^+-$C_3H_2N_2$, 83%), 159 (M^+-$C_{10}H_9N_2O$, 100%), 95 (M^+-$C_{10}H_9N_2O_3S$, 67%); HRMS (EI): found m/z 333.07008, $C_{16}H_{14}FN_2O_3S$, calculated m/z 333.07008; Elemental

analysis: found C 57.82%, H 3.94%, N 8.43%, $C_{16}H_{13}FN_2O_3S$, calculated C 57.45%, H 4.01%, N 8.34% (0.05 mole of H_2O).

4-(1*H*-imidazol-1-ylmethyl)phenyl 3-chlorobenzenesulfonate (**275**)

Compound **275** was synthesised in a similar manner to **264**, except that 3-chlorobenzene sulfonyl chloride (1.32g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **275** as a light yellow oil (1.34g, yield 67%); R_f=0.43 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3090 (Ar, C-H), 2365 (Im, C=N), 1603 (Ar, C=C), 1376 (S=O), 1079 (Cl-Ph); δH (400MHz, $CDCl_3$): 7.79 (2H, m; 1H, NC<u>H</u>N Im, 1H, Ph-<u>H</u>), 7.69 (1H, d, J=7.87Hz, Ph-<u>H</u>), 7.61 (1H, d, J=7.87Hz, Ph-<u>H</u>), 7.45 (1H, t, J=7.87Hz, Ph-<u>H</u>), 7.09 (3H, br. d; 1H, CH_2-NC<u>H</u> Im, 2H, J=8.60Hz, Ph-<u>H</u>), 6.97 (2H, d, J=8.60Hz, Ph-<u>H</u>), 6.86 (1H, s, NC<u>H</u>, Im), 5.13 (2H, s, Ph-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 149.25 (Ar, C), 137.32 (Im, NCN), 136.94, 135.62, 135.41, 134.65, 130.62, 129.00, 128.84, 126.58, 122.97 (Ar, C), 128.47, 119.43 (Im, C), 50.26 (Ph-CH_2); GC: t_R 31.97 min; LRMS (EI): m/z 348 (M^+, 25%), 281 (M^+-$C_3H_2N_2$, 100%), 175 (M^+-$C_{10}H_9N_2O$, 57%), 111 (M^+-$C_{10}H_9N_2O_3S$, 48%); HRMS (EI): found m/z 349.04094, $C_{16}H_{14}ClN_2O_3S$, calculated m/z 349.04082; Elemental analysis: found C 55.10%, H 3.76%, N 8.03%, $C_{16}H_{13}ClN_2O_3S$, calculated C 55.28%, H 3.76%, N 7.98%.

4-(1*H*-imidazol-1-ylmethyl)phenyl 4-chlorobenzenesulfonate (**276**)

Compound **276** was synthesised in a similar manner to **264**, except that 4-chlorobenzene sulfonyl chloride (1.32g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **276** as a light yellow oil (0.89g, yield 44%); R_f=0.42 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3093 (Ar, C-H), 2290 (Im, C=N), 1640 (Ar, C=C), 1376 (S=O), 1088 (Cl-Ph); δH (400MHz, $CDCl_3$): 7.69 (2H, d, J=8.79Hz, Ph-H), 7.46 (1H, s, NCHN, Im), 7.44 (2H, d, J=8.79Hz, Ph-H), 7.01 (3H, m; 1H, CH_2-NCH Im, 2H, Ph-H), 6.91 (2H, d, J=8.79Hz, Ph-H), 6.80 (1H, s, NCH, Im), 5.03 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 149.26, 141.36 (Ar, C), 137.46 (Im, NCN), 135.68, 133.65, 130.14, 129.93, 129.73, 122.97 (Ar, C), 128.62, 119.05 (Im, C), 50.02 (Ph-CH_2); GC: t_R 19.87 min; LRMS (EI): m/z 348 (M^+, 20%), 281 (M^+-$C_3H_2N_2$, 70%), 175 (M^+-$C_{10}H_9N_2O$, 100%), 111 (M^+-$C_{10}H_9N_2O_3S$, 90%); HRMS (EI): found m/z 349.0400630, $C_{16}H_{14}ClN_2O_3S$, calculated m/z 349.0408171; Elemental analysis: found C 55.10%, H 3.76%, N 8.03%, $C_{16}H_{14}ClN_2O_3S$, calculated C 54.93%, H 3.93%, N 8.01% (0.57 mole of H_2O).

4-(1*H*-imidazol-1-ylmethyl)phenyl 4-bromobenzenesulfonate (**277**)

Compound **277** was synthesised in a similar manner to **264**, except that 3-bromobenzene sulfonyl chloride (1.61g, 6.32 mmol) were used. Removal of the

solvent under vacuum gave a brown oil which was purified using column chromatography to give **277** as a light brownish oil (1.15g, yield 51%); R_f=0.40 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2969 (Ar, C-H), 2345 (Im, C=N), 1596 (Ar, C=C), 1371 (S=O), 1093 (Ph-Br); δH (400MHz, $CDCl_3$): 8.21 (1H, s, Ph-H), 7.94 (1H, s, NCHN, Im), 7.77 (2H, m, Ph-H), 7.39 (1H, t, J=7.87Hz, Ph-H), 7.16 (3H, m; 1H, CH_2-NCH Im, 2H, Ph-H), 6.99 (2H, d, J=8.42Hz, Ph-H), 6.90 (1H, s, NCH, Im), 5.21 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 149.42 (Ar-C), 137.58 (Im, NCN), 137.05, 134.84, 131.29, 130.82, 129.14, 127.31, 123.10, (Ar, C), 127.01, 119.60 (Im, C), 50.67 (Ph-CH_2); GC: t_R 32.56 min; LRMS (EI): m/z 394 (M^+, 25%), 327 (M^+-$C_3H_2N_2$, 100%), 221 (M^+-$C_{10}H_9N_2O$, 48%), 157 (M^+-$C_{10}H_9N_2O_3S$, 44%); HRMS (EI): found m/z 392.97466, $C_{16}H_{14}BrN_2O_3S$, calculated m/z 392.97436.

4-(1*H*-imidazol-1-ylmethyl)phenyl 4-bromobenzenesulfonate (**278**)

Compound **278** was synthesised in a similar manner to **264**, except that 4-bromobenzene sulfonyl chloride (1.61g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a reddish brown solid which was purified using column chromatography to give **278** as a light brownish solid (1.05g, yield 46%); (m.p. 105.6-106.2°C); R_f=0.40 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3090 (Ar, C-H), 2290 (Im, C=N), 1603 (Ar, C=C), 1376 (S=O), 1090 (Ph-Br); δH (400MHz, $CDCl_3$): 7.60 (4H, s, Ph-H), 7.47 (1H, s, NCHN, Im), 7.02 (3H, m; 1H, CH_2-NCH Im, 2H, Ph-H), 6.91 (2H, d, J=8.79Hz, Ph-H), 6.80 (1H, s, NCH, Im), 5.03 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 149.21, (Ar, C), 137.46 (Im, NCN), 135.68, 134.32, 132.71, 130.09, 129.94, 129.90, 122.94 (Ar, C), 128.64, 119.32 (Im, C), 50.03 (Ph-CH_2) ; GC: t_R 23.74 min; LRMS (EI): m/z

392 (M^+, 19%), 327 (M^+-$C_3H_2N_2$, 100%), 219 (M^+-$C_{10}H_9N_2O$, 73%), 155 (M^+-$C_{10}H_9N_2O_3S$, 48%); HRMS (EI): found m/z 392.9896080, $C_{16}H_{14}BrN_2O_3S$, calculated m/z 392.9903020; Elemental analysis: found C 48.87%, H 3.33%, N 7.12%, $C_{16}H_{13}BrN_2O_3S$, calculated C 48.63%, H 3.34%, N 7.18%.

4-(1*H*-imidazol-1-ylmethyl)phenyl 4-iodobenzenesulfonate (**279**)

Compound **279** was synthesised in a similar manner to **264**, except that 4-iodobenzene sulfonyl chloride (1.91g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a pale solid which was purified using column chromatography to give **279** as a off-white solid (1.35g, yield 53%); (m.p. 134.6-135.1°C); R_f=0.36 60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3100 (Ar, C-H), 1610 (Ar, C=C), 1376 (S=O); δH (400MHz, $CDCl_3$): 7.87 (2H, d, J=8.60Hz, Ph-$\underline{H}$), 7.53 (1H, s, NC$\underline{H}$N, Im), 7.49 (2H, d, J=8.60Hz, Ph-$\underline{H}$), 7.07 (3H, m; 1H, CH_2-NC$\underline{H}$ Im, 2H, Ph-$\underline{H}$), 6.95 (2H, d, J=8.60Hz, Ph-$\underline{H}$), 6.85 (1H, s, NC$\underline{H}$, Im), 5.08 (2H, s, Ph-C$\underline{H_2}$); δ_C (100MHz, $CDCl_3$): 149.23, 138.67 (Ar, C), 137.47 (Im, NCN), 135.64, 135.01, 130.08, 129.70, 122.95 (Ar, C), 128.64, 119.32 (Im, C), 102.55 (Ar, C), 50.05 (Ph-CH_2) ; GC: t_R 26.92 min; LRMS (EI): m/z 440 (M^+, 18%), 373 (M^+-$C_3H_2N_2$, 100%), 267 (M^+-$C_{10}H_9N_2O$, 80%), 207 (M^+-$C_{10}H_6N_2O_3S$, 48%); HRMS (EI): found m/z 440.977360, $C_{16}H_{14}IN_2O_3S$, calculated m/z 440.9764324; Elemental analysis: found C 43.65%, H 2.98%, N 6.36%, $C_{16}H_{13}IN_2O_3S$, calculated C 43.62%, H 3.11%, N 6.47% (0.60 mole of H_2O).

4-(1*H*-imidazol-1-ylmethyl)phenyl 3-nitrobenzene sulfonate (**280**)

Compound **280** was synthesised in a similar manner to **264**, except that 3-nitrobenzene sulfonyl chloride (1.40g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **280** as a light yellow oil (1.41g, yield 68%); R_f=0.47 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2937 (Ar, C-H), 2305 (Im, C=N), 1606 (Ar, C=C), 1503 (Ar-NO_2), 1353 (S=O); δH (400MHz, CD_3OD): 8.59 (1H, m, Ph-H), 8.52 (1H, t, m, Ph-H), 8.20 (1H, m, Ph-H), 7.87 (1H, t, J=7.87Hz, Ph-H), 7.71 (1H, s, NCHN, Im), 7.22 (2H, d, J=8.60Hz, Ph-H), 7.06 (1H, s, CH_2-NCH, Im), 7.04 (2H, d, J=8.60Hz, Ph-H), 6.96 (1H, s, NCH, Im), 5.20 (2H, s, Ph-CH_2); δ_C (100MHz, CD_3OD): 149.08, 148.36 (Ar-C), 137.36 (Im, NCN), 136.96, 136.82, 133.79, 131.17, 128.95, 128.83, 123.12, 122.51, (Ar, C), 128.20, 119.53 (Im, C), 49.29 (Ph-CH_2); GC: t_R 33.96 min; LRMS (EI): m/z 359 (M^+, 30%), 292 (M^+-$C_3H_2N_2$, 100%), 186 (M^+-$C_{10}H_9N_2O$, 32%), 122 (M^+-$C_{10}H_9N_2O_3S$, 24%); HRMS (EI): found m/z 360.06487, $C_{16}H_{14}N_3O_5S$, calculated m/z 360.06487; Elemental analysis: found C 53.48%, H 3.65%, N 11.69%, $C_{16}H_{13}N_3O_5S$, calculated C 53.23%, H 3.68%, N 11.61% (0.11 mole of H_2O).

4-(1*H*-imidazol-1-ylmethyl)phenyl 4-Nitrobenzenesulfonate (**281**)

Compound **281** was synthesised in a similar manner to **264**, except that 4-nitrobenzene sulfonyl chloride (1.40g, 6.32 mmol) were used. Removal of the

solvent under vacuum gave a brown oil which was purified using column chromatography to give **281** as a yellow solid (0.98g, yield 48%); (m.p. 149.9-150.2°C); R_f=0.47 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3102 (Ar, C-H), 1605 (Ar, C=C), 1503 (Ar-NO_2), 1378 (S=O); δH (400MHz, $CDCl_3$): 8.35 (2H, d, J= 8.97Hz, Ph-H), 8.01 (2H, d, J=8.97Hz, Ph-H), 7.51 (1H, s, NCHN, Im), 7.06 (3H, m; 1H, CH_2-NCH Im, 2H, Ph-H), 6.97 (2H, d, J=8.60Hz, Ph-H), 6.85 (1H, s, NCH, Im), 5.08 (2H, s, Ph-CH_2); δ_C (100MHz, $CDCl_3$): 151.08, 148.93, 140.94 (Ar, C), 137.49 (Im, NCN), 136.16, 129.93, 128.79, 124.53, 122.76 (Ar, C), 130.17, 119.30 (Im, C), 49.95 (Ph-CH_2); GC: t_R 25.40 min; LRMS (EI): m/z 359 (M^+, 21%), 292 (M^+-$C_3H_2N_2$, 100%), 186 (M^+-$C_{10}H_9N_2O$, 40%), 122 (M^+-$C_{10}H_9N_2O_3S$, 34%); HRMS (EI): found m/z 360.0645080, $C_{16}H_{14}N_3O_5S$, calculated m/z 360.0648677; Elemental analysis: found C 53.48%, H 3.65%, N 11.69%, $C_{16}H_{13}N_3O_5S$, calculated C 53.21%, H 3.73%, N 11.51% (0.29 mole of H_2O).

4-(1*H*-imidazol-1-ylmethyl)phenyl 3-trifluoromethanebenzene sulfonate (**282**)

Compound **282** was synthesised in a similar manner to **264**, except that 3-trifluoromethanebenzene sulfonyl chloride (1.54g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **282** as a light yellow oil (1.19g, yield 54%); R_f=0.30 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3042 (Ar, C-H), 2302 (Im, C=N), 1595 (Ar, C=C), 1385 (S=O), 1231 (C-F); δH (400MHz, $CDCl_3$): 8.06 (1H, d, J=7.87Hz, Ph-H), 7.97 (1H, s, NCHN, Im) 7.95 (1H, s, Ph-H), 7.79 (1H, t, J=7.87Hz, Ph-H), 7.67 (1H, d, J=7.87Hz, Ph-H), 7.12 (3H, m; 2H, Ph-H , 1H, CH_2-NCH Im), 7.05 (2H, d, J=8.60Hz, Ph-H), 6.88 (1H, s, NCH, Im), 5.16 (2H, s, Ph-CH_2); δ_C (100MHz,

$CDCl_3$): 149.10 (Ar, C), 137.15 (Im, NCN), 135.04, 134.60, 134.44, 132.93, 132.45, 129.41, 129.01, 128.93, 122.94 (Ar, C), 128.10, 119.53 (Im, C), 50.47 (Ph-CH_2); GC: t_R 27.17 min; LRMS (EI): m/z 382 (M^+, 7%), 315 (M^+-$C_3H_2N_2$, 42%), 209 (M^+-$C_{10}H_9N_2O$, 61%), 145 (M^+-$C_{10}H_9N_2O_3S$, 100%); HRMS (EI): found m/z 383.06716, $C_{17}H_{14}F_3N_2O_3S$, calculated m/z 383.06717.

4-(1*H*-imidazol-1-ylmethyl)phenyl 4-trifluoromethanebenzenesulfonate (**283**)

Compound **283** was synthesised in a similar manner to **264**, except that 4-trifluoromethanebenzene sulfonyl chloride (1.54g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **283** as a light yellow oil (1.39g, yield 63%); R_f=0.27 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3000 (Ar, C-H), 2340 (Im, C=N), 1652 (Ar, C=C), 1320 (S=O), 1250 (C-F); δH (400MHz, CD_3OD): 8.02 (2H, d, J=8.24Hz, Ph-H), 7.92 (2H, d, J=8.24Hz, Ph-H), 7.72 (1H, s, NCHN, Im), 7.22 (2H, d, J=8.60Hz, Ph-H), 7.07 (1H, s, CH_2-NCH, Im), 7.02 (2H, d, J=8.60Hz, Ph-H), 6.97 (1H, s, NCH, Im), 5.19 (2H, s, Ph-CH_2); δ_C (100MHz, CD_3OD): 155.29, 149.17 (Ar, C), 138.99 (Im, NCN), 137.33, 136.78, 129.22, 128.88, 126.40, 122.43, 121.94 (Ar, C), 128.16, 119.55 (Im, C), 49.30 (Ph-CH_2); GC: t_R 14.78 min; LRMS (EI): m/z 382 (M^+, 30%), 315 (M^+-$C_3H_2N_2$, 100%), 209 (M^+-$C_{10}H_9N_2O$, 47%), 145 (M^+-$C_{10}H_9N_2O_3S$, 88%); HRMS (EI): found m/z 383.0689650, $C_{17}H_{14}F_3N_2O_3S$, calculated m/z 383.0671740.

4-(1*H*-imidazol-1-ylmethyl)phenyl 4-methoxybenzenesulfonate (**284**)

Compound **284** was synthesised in a similar manner to **264**, except that 4-methoxybenzene sulfonyl chloride (1.30g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **284** as a light yellow oil (1.09g, yield 56%); R_f=0.39 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3000 (Ar, C-H), 2200 (Im, C=N), 1610 (Ar, C=C), 1264 (Ph-OCH_3), 1373 (S=O); δH (400MHz, CD_3OD): 7.61 (2H, s, Ph-H), 7.59 (1H, s, NCHN, Im), 7.09 (2H, d, J=8.79Hz, Ph-H), 6.95 (3H, m; 2H, Ph-H, 1H, CH_2-NCH, Im), 6.87 (2H, m; Ph-H, 1H, NCH, Im), 5.08 (2H, s, Ph-CH_2), 3.76 (3H, s, Ph-OCH_3); δ_C (100MHz, CD_3OD): 164.65, 149.49 (Ar, C), 137.30 (Im, NCN), 136.22, 130.60, 128.66, 126.24, 122.62, 114.31 (Ar, C), 128.10, 119.57 (Im, C), 55.08 (OCH_3), 49.38 (Ph-CH_2); GC: t_R 24.05 min; LRMS (EI): m/z 344 (M^+, 20%), 277 (M^+-$C_3H_2N_2$, 14%), 171 (M^+-$C_{10}H_8N_2O$, 100%), 107 (M^+-$C_{10}H_8N_2O_3S$, 40%); HRMS (EI): found m/z 345.090430, $C_{17}H_{17}N_2O_4S$, calculated m/z 345.090350; Elemental analysis: found C 59.29%, H 4.68%, N 8.13%, $C_{17}H_{16}N_2O_3S$, calculated C 59.28%, H 4.78%, N 8.22% (0.35 mole of H_2O).

4-(1*H*-imidazol-1-ylmethyl)phenyl 3-toluenesulfonate (**285**)

Compound **285** was synthesised in a similar manner to **264**, except that 3-toluene sulfonyl chloride (1.20g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography

to give **285** as a light yellow oil (1.29g, yield 69%); R_f=0.39 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3066 (Ar, C-H), 2924 (CH_3), 1903 (Im, C=N), 1603 (Ar, C=C), 1371 (S=O); δH (400MHz, $CDCl_3$): 7.62 (1H, s, NCHN, Im), 7.56 (2H, m, Ph-H), 7.44 (1H, d, J=7.69Hz, Ph-H), 7.37 (1H, t, J=7.69Hz, Ph-H), 7.06 (1H, s, CH_2-NCH, Im), 7.04 (2H, d, J=8.79, Ph-H), 6.94 (2H, d, J=8.79, Ph-H), 6.84 (1H, s, NCH, Im), 5.07 (2H, s, Ph-CH_2), 2.38 (3H, s, CH_3); δ_C (100MHz, $CDCl_3$): 149.44, 139.72 (Ar, C), 137.40 (Im, NCN), 135.29, 135.15, 129.77, 129.13, 128.72, 125.67, 123.07 (Ar, C), 128.56, 119.35 (Im, C), 50.13 (Ph-CH_2), 21.37 (CH_3); GC: t_R 28.56 min; LRMS (EI): m/z 328 (M^+, 64%), 261 (M^+-$C_3H_3N_2$, 60%), 155 (M^+-$C_{10}H_9N_2O$, 64%), 91 (M^+-$C_{10}H_9N_2O_3S$, 100%); HRMS (EI): found m/z 328.08800, $C_{17}H_{16}N_2O_3S$, calculated m/z 328.08760.

4-(1*H*-imidazol-1-ylmethylphenyl 4-toluenesulfonate (**286**)

Compound **286** was synthesised in a similar manner to **264**, except that 4-toluene sulfonyl chloride (1.20g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **286** as a light yellow oil (1.07g, yield 57%); R_f=0.37 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3109 (Ar, C-H), 2303 (Im, C=N), 1596 (Ar, C=C), 1371 (S=O); δH (400MHz, $CDCl_3$): 7.61 (1H, s, NCHN, Im), 7.56 (2H, d, J=8.42, Ph-H), 7.28 (2H, d, J=8.42Hz, Ph-H), 7.07 (2H, d, J=8.79Hz, Ph-H), 6.97 (1H, s, CH_2-NCH, Im), 6.86 (3H; 2H, d, J=8.79Hz, Ph-H, 1H, NCH, Im), 5.08 (2H, s, Ph-CH_2), 2.32 (3H, s, CH_3); δ_C (100MHz, $CDCl_3$): 149.44, 145.97 (Ar, C), 137.32 (Im, NCN), 136.31, 132.20, 129.72, 128.66, 128.30, 122.55 (Ar, C), 128.14, 119.56 (Im, C), 49.35 (Ph-CH_2), 20.28 (CH_3); GC: t_R 28.65 min; LRMS (EI): m/z 328 (M^+, 52%), 261 (M^+-$C_3H_3N_2$, 52%), 155 (M^+-$C_{10}H_9N_2O$, 64%), 91 (M^+-$C_{10}H_9N_2O_3S$, 100%); HRMS (EI):

found m/z 329.0955060, $C_{17}H_{17}N_2O_3S$, calculated m/z 329.0954395.

4-(1*H*-imidazol-1-ylmethyl)phenyl 4-ethylbenzenesulfonate (**287**)

Compound **287** was synthesised in a similar manner to **264**, except that 4-ethylbenzene sulfonyl chloride (1.29g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **287** as a light yellow oil (1.5g, yield 76%); R_f=0.41 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3066 (Ar, C-H), 2934 (C-H), 2303 (Im, C=N), 1596 (Ar, C=C), 1368 (S=O); δH (400MHz, $CDCl_3$): 7.99 (1H, s, NCHN, Im), 7.70 (2H, d, J=8.42Hz, Ph-H), 7.31 (2H, d, J=8.42Hz, Ph-H), 7.11 (1H, s, CH_2-NCH, Im), 7.09 (2H, d, J=8.79Hz, Ph-H), 6.96 (2H, d, 8.79Hz, Ph-H), 6.88 (1H, s, NCH, Im), 5.14 (2H, s, Ph-CH_2), 2.72 (2H, q, J=7.51Hz, CH_2-CH_3), 1.22 (3H, t, J=7.51Hz, CH_3); δ_C (100MHz, $CDCl_3$): 151.76, 149.65 (Ar, C), 137.14 (Im, NCN), 134.63, 132.49, 128.83, 128.79, 128.63, 123.18 (Ar, C), 128.01, 119.55 (Im, C), 50.54 (Ph-CH_2), 29.00 (CH_2-CH_3), 15.02 (CH_3); GC: t_R 31.98 min; LRMS (EI): m/z 342 (M^+, 65%), 275 (M^+-$C_3H_3N_2$, 70%), 169 (M^+-$C_{10}H_9N_2O$, 100%), 105 (M^+-$C_{11}H_{13}N_2O_2S$, 80%); HRMS (EI): found m/z 343.11095, $C_{18}H_{19}N_2O_3S$, calculated m/z 343.11109; Elemental analysis: found C 63.14%, H 5.30%, N 8.18%, $C_{18}H_{18}N_2O_3S$, calculated C 62.98%, H 5.47%, N 8.48% (0.59 mole of H_2O).

4-(1*H*-imidazol-1-ylmethyl)phenyl-4-*n*-propylbenzenesulfonate (**288**)

Compound **288** was synthesised in a similar manner to **264**, except that 4-*n*-propylbenzene sulfonyl chloride (1.38g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **288** as a light yellow oil (1.32g, yield 65%); R_f=0.44 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3061 (Ar, C-H), 2960 (C-H), 1921 (Im, C=N), 1596 (Ar, C=C), 1374 (S=O); δH (400MHz, $CDCl_3$): 9.05 (1H, s, NCHN, Im), 7.71 (2H, d, J=8.24Hz, Ph-H), 7.60 (1H, s, CH_2-NCH, Im), 7.57 (1H, s, NCH, Im), 7.41 [4H, t (dd overlapped], J=8.60Hz, Ph-H), 7.05 (2H, d, J=8.24Hz, Ph-H), 5.43 (2H, s, Ph-CH_2), 1.65 (2H, m, CH_2-CH_2-Ph), 1.30 (2H, t, =7.14, CH_2-CH_2-Ph), 0.93 (3H, t, J=7.32Hz, CH_3); δ_C (100MHz, $CDCl_3$): 145.10, 140.82 (Ar, C), 135.35 (Im, NCN), 129.79, 128.33, 123.00, 122.91, 122.04, 120.26 (Ar, C), 129.28, 119.46 (Im, C), 51.64 (Ph-CH_2), 37.74 (CH_2-CH_2-Ph), 23.91 (CH_2-CH_2-Ph), 7.92 (CH_3); GC: t_R 33.09 min; LRMS (EI): m/z 356 (M^+, 68%), 289 (M^+-$C_3H_3N_2$, 78%), 183 (M^+-$C_{10}H_9N_2O$, 100%), 119 (M^+-$C_{11}H_{13}N_2O_2S$, 94%); HRMS (EI): found m/z 357.12660, $C_{19}H_{21}N_2O_3S$, calculated m/z 357.12730.

4-(1*H*-imidazol-1-ylmethyl)phenyl-4-*n*-butylbenzenesulfonate (**289**)

Compound **289** was synthesised in a similar manner to **264**, except that 4-*n*-butylbenzene sulfonyl chloride (1.47g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column

chromatography to give **289** as a light yellow oil (1.22g, yield 57%); R_f=0.47 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2957 (Ar, C-H), 2861 (C-H), 2302 (Im, C=N), 1596 (Ar, C=C), 1373 (S=O); δH (400MHz, $CDCl_3$): 7.70 (2H, d, J=8.42Hz, Ph-H), 7.53 (1H, s, NCHN, Im), 7.30 (2H, d, J=8.42Hz, Ph-H), 7.06 (1H, s, CH_2-NCH, Im), 7.04 (2H, d, J=8.79Hz, Ph-H), 6.95 (2H, d, J=8.79Hz, Ph-H), 6.84 (1H, s, NCH, Im), 5.07 (2H, s, Ph-CH_2), 2.66 (2H, t, J=7.87Hz, CH_2-CH_2-Ph), 1.59 (2H, m, CH_2-CH_2-Ph), 1.31 [2H, m, CH_2-$(CH_2)_2$-Ph], 0.91 (3H, t, J=7.32Hz, CH_3); δ_C (100MHz, $CDCl_3$): 150.53, 149.48 (Ar, C), 137.43 (Im, NCN), 135.20, 132.46, 129.86, 129.27, 128.56, 123.10 (Ar, C), 129.50, 119.34 (Im, C), 50.12 (Ph-CH_2), 35.72 (CH_2-CH_2-Ph), 33.09 (CH_2-CH_2-Ph), 22.33 [CH_2-$(CH_2)_2$-Ph], 13.93 (CH_3); GC: t_R 34.81 min; LRMS (EI): m/z 370 (M^+, 31%), 303 (M^+-$C_3H_3N_2$, 42%), 197 (M^+-$C_{10}H_9N_2O$, 100%), 133 (M^+-$C_{11}H_{13}N_2O_2S$, 72%); HRMS (EI): found m/z 371.14328, $C_{20}H_{23}N_2O_3S$, calculated m/z 371.14239; Elemental analysis: found C 64.84%, H 5.99%, N 7.56%, $C_{20}H_{22}N_2O_3S$, calculated C 64.91%, H 6.01%, N 7.58%.

4-(1*H*-imidazol-1-ylmethyl)phenyl-4-*iso*-butylbenzenesulfonate (**290**)

Compound **290** was synthesised in a similar manner to **264**, except that 4-*iso*-butylbenzene sulfonyl chloride (1.47g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **290** as a light yellow oil (1.12g, yield 53%); R_f=0.48 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3020 (Ar, C-H), 2966 (C-H), 2292 (Im, C=N), 1594 (Ar, C=C), 1373 (S=O); δH (400MHz, $CDCl_3$): 7.65 (2H, d, J=8.42Hz, Ph-H), 7.47 (1H, s,

NCHN, Im), 7.44 (2H, d, J=8.42Hz, Ph-H), 7.00 (1H, CH_2-NCH, Im), 6.98 (2H, d, J=8.79Hz, Ph-H), 6.89 (2H, d, J=8.79Hz, Ph-H), 6.78 (1H, s, NCH, Im), 5.01 (2H, s, Ph-CH_2), 1.25 [9H, $(CH_3)_3$-C]; δ_C (100MHz, $CDCl_3$): 158.61, 149.47 (Ar, C), 137.44 (Im, NCN), 135.20, 132.37, 128.53, 128.36, 126.32, 123.07 (Ar, C), 129.88, 119.34 (Im, C), 50.13 (Ph-CH_2), 35.46 (C-Ph), 31.09 [$(CH_3)_3$-C], GC: t_R 34.29 min; LRMS (EI): m/z 370 (M^+, 60%), 303 (M^+-$C_3H_3N_2$, 91%), 197 (M^+-$C_{10}H_9N_2O$, 88%), 133 (M^+-$C_{11}H_{13}N_2O_2S$, 100%); HRMS (EI): found m/z 371.14080, $C_{20}H_{23}N_2O_3S$, calculated m/z 371.14240; Elemental analysis: found C 64.84%, H 5.99%, N 7.56%, $C_{20}H_{22}N_2O_3S$, calculated C 64.68%, H 6.00%, N 7.53%.

4-(1*H*-imidazol-1-ylmethyl)phenyl-4-*n*-pentylbenzenesulfonate (**291**)

Compound **290** was synthesised in a similar manner to **264**, except that 4-*n*-pentylbenzene sulfonyl chloride (1.56g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **290** as a light yellow oil (1.02g, yield 63%); R_f=0.51 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3065 (Ar, C-H), 2928 (C-H), 2302 (Im, C=N), 1596 (Ar, C=C), 1371 (S=O); δH (400MHz, $CDCl_3$): 7.71 (2H, d, J=8.42Hz, Ph-H), 7.53 (1H, s, NCHN, Im), 7.31 (2H, d, J=8.42Hz, Ph-H), 7.08 (1H, s, CH_2-NCH, Im), 7.05 (2H, d, J=8.60Hz, Ph-H), 6.96 (2H, d, J=8.60Hz, Ph-H), 6.86 (1H, s, NCH, Im), 5.08 (2H, s, Ph-CH_2), 2.67 (2H, t, J=7.87Hz, CH_2-CH_2-Ph), 1.62 (2H, m, CH_2-CH_2-Ph), 1.30 [4H, m, C_2H_4-$(CH_2)_2$-Ph], 0.88 (3H, t, J=7.14Hz, CH_3); δ_C (100MHz, $CDCl_3$): 150.56, 149.45 (Ar, C), 137.44 (Im, NCN), 135.27, 132.43, 129.27, 128.55, 128.49, 123.08 (Ar, C), 129.94, 119.33 (Im, C), 50.08 (Ph-CH_2), 35.98 (CH_2-CH_2-Ph), 31.39 (CH_2-CH_2-Ph), 30.65 [CH_2-$(CH_2)_2$-Ph], 22.49 [CH_2-$(CH_2)_3$-Ph], 14.06 (CH_3); GC: t_R 37.03 min; LRMS (EI): m/z 384 (M^+, 40%), 317 (M^+-$C_3H_3N_2$, 46%),

211 (M^+-$C_{10}H_9N_2O$, 100%), 147 (M^+-$C_{11}H_{13}N_2O_2S$, 78%); HRMS (EI): found m/z 385.15840, $C_{21}H_{25}N_2O_3S$, calculated m/z 385.15800; Element analysis: found C 65.60%, H 6.29%, N 7.29%, $C_{21}H_{24}N_2O_3S$, calculated C 65.74%, H 6.35%, N 7.48% (0.23 mole of H_2O).

4-(1*H*-imidazol-1-ylmethyl)phenyl biphenylsulfonate (**292**)

Compound **292** was synthesised in a similar manner to **264**, except that biphenyl-4-sulfonyl chloride (1.59g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **292** as a light yellow oil (1.04g, yield 42%); R_f=0.36 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3000 (Ar, C-H), 2241 (Im, C=N), 1635 (Ar, C=C), 1373 (S=O); δH (400MHz, CD_3OD): 7.80 (2H, m, Ph-H), 7.70 (2H, m, Ph-H), 7.66 (1H,s, NCH, Im), 7.62 (2H, m, Ph-H), 7.41 (3H, m, Ph-H), 7.15 (2H, d, J=8.60Hz, Ph-H), 7.01 (1H, s, CH_2-NCH, Im), 6.97 (2H, d, J=8.60Hz, Ph-H), 6.91 (1H,s, NCH, Im), 5.13 (2H, s, Ph-CH_2); δ_C (100MHz, CD_3OD): 149.44, 147.31 (Ar, C), 138.70 (Im, NCN), 137.31, 136.39, 133.66, 128.92, 128.90, 128.66, 128.12, 127.52, 127.07, 122.57 (Ar, C), 128.76, 119.56 (Im, C), 49.37 (Ph-CH_2); GC: t_R 48.27 min; LRMS (EI): m/z 390 (M^+, 18%), 323 (M^+-$C_3H_2N_2$, 18%), 217 (M^+-$C_{10}H_9N_2O$, 80%), 153 (M^+-$C_{10}H_9N_2O_3S$, 100%); HRMS (EI): found m/z 391.1103200, $C_{22}H_{19}N_2O_3S$, calculated m/z 391.1110896.

4-(1*H*-imidazol-1-ylmethyl)phenyl naphthalene-1-sulfonate (**293**)

Compound **293** was synthesised in a similar manner to **264**, except that naphthalene-1-sulfonyl chloride (1.43g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **293** as a light yellow oil (1.42g, yield 62%); R_f=0.45 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3017 (Ar, C-H), 2295 (Im, C=N), 1623 (Ar, C=C), 1504 (substituted naphthalene), 1371 (S=O); δH (400MHz, CD_3OD): 8.73 (1H, d, J=8.60Hz, NaPh-H), 8.23 (1H, d, J=8.24Hz, NaPh-H), 8.04 (2H, m; 1H, NaPh-H, 1H, NCHN, Im), 7.78 (1H, m, NaPh-H), 7.70 (1H, m, NaPh-H), 7.50 (1H, t, J=7.87Hz, NaPh-H), 7.06 (2H, d, J=8.79Hz, Ph-H), 6.99 (1H, s, CH_2-NCH, Im), 6.93 (1H, s, NCH, Im), 6.81 (2H, d, J=8.79Hz, Ph-H), 5.10 (2H, s, Ph-CH_2); δ_C (100MHz, CD_3OD): 149.38, 136.35 (Ar, C), 135.89 (Im, NCN), 134.84, 134.34, 131.22, 130.45, 129.05, 128.72, 128.62, 127.27, 124.57, 123.90, 122.10, 117.33 (Ar, C), 128.42, 119.46 (Im, C), 49.25 (Ph-CH_2); GC: t_R 37.18min; LRMS (EI): m/z 364 (M^+, 4%), 297 (M^+-$C_3H_2N_2$, 10%), 191 (M^+-$C_{10}H_9N_2O$, 33%), 127 (M^+-$C_{10}H_9N_2O_3S$, 100%); Elemental analysis: found C 65.92%, H 4.43%, N 7.69%, $C_{20}H_{16}N_2O_3S$, calculated C 65.81%, H 4.43%, N 7.70%.

2.29 4-(1*H*-imidazol-1-ylmethyl)phenyl naphthalene-2-sulfonate (**294**)

Compound **294** was synthesised in a similar manner to **264**, except that

naphthalene-2-sulfonyl chloride (1.42g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **294** as a light yellow oil (1.37g, yield 60%); R_f=0.43 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3058 (Ar, C-H), 2295 (Im, C=N), 1625 (Ar, C=C), 1504 (substituted naphthalene), 1372 (S=O); δH (400MHz, CD_3OD): 8.29 (1H, wk. d, J=1.83Hz, NaPh-H), 8.02 (1H, d, J=8.79Hz, NaPh-H), 7.93 (2H, t, J=8.79Hz NaPh-H), 7.76 (1H, dd, J=1.83Hz, J=8.79Hz, NaPh-H), 7.62 (3H, m; 1H, NCHN Im, 2H, NaPh-H), 7.09 (2H, d, J=8.79Hz, Ph-H), 6.97 (1H, s, CH_2-NCH, Im), 6.92 (2H, d, J=8.79Hz, Ph-H), 6.89 (1H, s, NCH, Im), 5.09 (2H, s, Ph-CH_2); δ_C (100MHz, CD_3OD): 149.46 (Ar, C), 137.29 (Im, NCN), 136.39, 135.64, 131.98, 131.89, 130.37, 129.63, 129.57, 128.72, 128.14, 127.84, 122.55, 122.51 (Ar, C), 129.20, 119.51 (Im, C), 49.32 (Ph-CH_2); GC: t_R 37.39 min; LRMS (EI): m/z 364 (M^+, 3%), 297 (M^+-$C_3H_2N_2$, 11%), 191 (M^+-$C_{10}H_9N_2O$, 34%), 127 (M^+-$C_{10}H_9N_2O_3S$, 100%); Elemantal analysis: found C 65.92%, H 4.43%, N 7.69%, $C_{20}H_{16}N_2O_3S$, calculated C 64.73%, H 4.42%, N 7.68%.

2.29 4-(1*H*-imidazol-1-ylmethyl)phenyl thiophene-3-sulfonate (**295**)

Compound **295** was synthesised in a similar manner to **264**, except that thiophene-3-sulfonyl chloride (1.16g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **295** as a light yellow oil (1.46g, yield 72%); R_f=0.44 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3103 (Ar, C-H), 1900 (Im, C=N), 1632 (Ar, C=C), 1449 (Thiophene), 1377 (S=O); δH (400MHz, CD_3OD): 9.00 (1H, s, NCHN, Im), 7.63 (1H, m, Thioph-H), 7.59 (1H, s, CH_2-NCH, Im), 7.55 (1H, s, NCH, Im), 7.40 (3H,

m; 2H, Ph-H, 1H, Thioph-H)), 7.18 (1H, Thioph-H)), 7.10 (2H, d, J=8.60Hz, Ph-H), 5.44 (2H, s, Ph-CH_2); δ_C (100MHz, CD_3OD): 150.16 (Ar, C), 135.87 (Im, NCN), 135.68, 130.00, 127.73, 127.59, 122.85, 122.04, (Ar, C), 129.80, 120.53 (Im, C), 51.50 (Ph-CH_2); GC: t_R 17.17 min; LRMS (EI): m/z 320 (M^+, 21%), 253 (M^+-$C_3H_2N_2$, 46%), 147 (M^+-$C_{10}H_9N_2O$, 100%); HRMS (EI): found m/z 321.03624, $C_{14}H_{13}N_2O_3S_2$, calculated m/z 321.03621.

4-(1*H*-imidazol-1-ylmethyl)phenyl 3,5dimethylisoxazolesulfonate (**296**)

Compound **296** was synthesised in a similar manner to **264**, except that 3,5dimethylisoxazole sulfonyl chloride (1.24g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **296** as a light yellow oil (1.11g, yield 53%); R_f=0.34 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 1943 (Im, C=N), 1607 (Ar, C=C), 1367 (S=O); δH (400MHz, CD_3OD): 8.79 (1H, s, NCHN, Im), 7.51 (1H, s, CH_2-NCH, Im), 7.46 (1H, s, NCH, Im), 7.45 (2H, d, J=8.60Hz, Ph-H), 7.18 (2H, d, J=8.60Hz, Ph-H), 5.43 (2H, s, Ph-CH_2), 2.53 (3H, O-C-CH_3, isoxaz), 2.24 (3H, N-C-CH_3, isoxaz); δ_C (100MHz, CD_3OD): 176.11 (O-C, isoxaz), 168.41 (N-C, isoxaz), 149.31 (C-S, isoxaz), 135.77, 134.77 (Ar, C), 129.86, 121.44, (Ar, C), 123.06, 120.60 (Im, C), 51.07 (Ph-CH_2), 11.15 (O-C-CH_3, isoxaz), 10.81 (N-C-CH_3, isoxaz); GC: t_R 28.10 min; LRMS (EI): m/z 333 (M^+, 22%), 266 (M^+-$C_3H_2N_2$, 92%), 160 (M^+-$C_{10}H_9N_2O$, 100%); HRMS (EI): found m/z 334.08562, $C_{15}H_{16}N_3O_4S$, calculated m/z 334.08560.

4-(1*H*-imidazol-1-ylmethyl)phenyl diphenyl phosphate (**297**)

Compound **297** was synthesised in a similar manner to **264**, except that diphenyl chlorophosphate (1.69g, 6.32 mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **297** as a light yellow oil (1.09g, yield 47%); R_f=0.31 [60/30/10 (petroleum ether/diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 2228 (Im, C=N), 1654 (Ar, C=C), 1236 (P=O), 1027 (P-O); δH (400MHz, CD_3OD): 7.78 (1H, s, NCHN, Im), 7.31 (3H, m, Ph-H), 7.13 (11H, m, Ph-H), 7.05 (1H, s, CH_2-NCH, Im), 6.94 (1H, s, NCH, Im), 5.14 (2H, s, Ph-CH_2); δ_C (100MHz, CD_3OD): 137.07 (Im, NCN), 134.73, 129.89, 128.87, 127.23, 125.87, 120.37, 120.33, 119.81 (Ar, C), 129.31, 119.76 (Im, C), 49.66 (Ph-CH_2); GC: t_R 50.39 min; LRMS (EI): m/z 406 (M^+, 23%), 339 (M^+-$C_3H_2N_2$, 100%), 233 (M^+-$C_{10}H_9N_2O$, 5%); HRMS (EI): found m/z 407.1168150, $C_{22}H_{20}N_2O_4P$, calculated m/z 407.1155201.

Phenylethyl imidazole (**298**)

Compound **298** was synthesised in a similar manner to **198**, except that phenyethyl bromide (1.00g, 5.41mmol), anhydrous K_2CO_3 (0.90g, 6.50mmol) and imidazole (0.50g, 8.12mmol) were used. Removal of the solvent under vacuum

gave a yellow oil which was purified using column chromatography to give **298** as a clear oil (0.78g, yield 84%); R_f=0.45 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3027 (Ar, C-H), 2934 (C-H), 1955 (Im, C=N), 1603 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.29 (1H, s, NC<u>H</u>N, Im), 7.25 (3H, m, Ph-<u>H</u>), 7.04 (2H, m, Ph-<u>H</u>), 7.01 (1H, s, CH_2-NC<u>H</u>, Im), 6.81 (1H, s, NC<u>H</u>, Im), 4.15 (2H, t, J=7.14Hz, Ph-C<u>H</u>$_2$), 3.02 (2H, t, J=7.14Hz, Ph-CH_2C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 137.54 (Im, NCN), 137.17, 129.51, 128.84, 127.06 (Ar, C), 128.66, 118.83 (Im, C), 48.57 (Ph-CH_2), 37.93 (Ph-CH_2-<u>C</u>H_2); GC: t_R 14.48min; LRMS (EI): m/z 172 (M^+, 33%), 105 (M^+-$C_3H_3N_2$, 20%), 91 (M^+-$C_4H_5N_2$, 100%), 82 (M^+-$C_7H_7N_2$, 83%); HRMS (EI): found m/z 173.10732, $C_{11}H_{13}N_2$, calculated m/z 173.10732.

Phenylpropyl bromide (**299**)

Br

Compound **299** was synthesised in a similar manner to **243**, except that PBr_3 (123.40g, 441.35mmol) and phenyl propanol (31.00g, 227.94mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **299** (36.44g, yield 80%) as a clear oil; R_f=0.79 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3026 (Ar, C-H), 2938 (C-H), 1603 (Ar, C=C), 680 (C-Br); δH (400MHz, $CDCl_3$): 7.31 (2H, m, Ph-<u>H</u>), 7.21 (3H, m, Ph-<u>H</u>), 3.40 (2H, t, J=6.59Hz, Ph-C<u>H</u>$_2$), 2.79 [2H, t, J=7.32Hz, Ph-$(CH_2)_2$-C<u>H</u>$_2$], 2.18 (2H, m, Ph-CH_2-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 140.63, 128.64, 128.59, 126.25 (Ar, C), 34.25 (Ph-CH_2), 34.06 [Ph-$(CH_2)_2$-<u>C</u>H_2], 33.18 (Ph-CH_2-<u>C</u>H_2); GC: t_R 3.39min; LRMS (EI): m/z 198 (M^+, 21%), 105 (M^+-CH_2Br, 3%), 91 (M^+-C_2H_4Br, 100%), 77 (M^+-C_3H_6Br, 4%).

Phenylpropyl imidazole (**300**)

N
N

Compound **300** was synthesised in a similar manner to **198**, except that **299** (2.00g, 10.05mmol), anhydrous K_2CO_3 (1.65g, 11.96mmol) and imidazole (1.00g, 14.91mmol) were used. Removal of the solvent under vacuum gave a pale oil which was purified using column chromatography to give **300** as a light yellow oil (1.23g, yield 66%); R_f=0.54 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3026 (Ar, C-H), 2937 (C-H), 1952 (Im, C=N), 1602 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.44 (1H, s, NC<u>H</u>N, Im), 7.29 (2H, m, Ph-<u>H</u>), 7.21 (3H, m, Ph-<u>H</u>), 7.06 (1H, s, CH_2-NC<u>H</u>, Im), 6.90 (1H, s, NC<u>H</u>, Im), 3.91 (2H, t, J=6.96Hz, Ph-C<u>H</u>$_2$), 2.60 [2H, t, J=7.69Hz, Ph-$(CH_2)_2$-C<u>H</u>$_2$], 2.11 (2H, m, Ph-CH_2-C<u>H</u>$_2$); δ_C (100MHz, $CDCl_3$): 140.36 (Ar, C), 137.22 (Im, NCN), 129.65, 128.70, 126.42 (Ar, C), 128.44, 118.77 (Im, C), 46.20 (Ph-CH_2), 32.54 (Ph-CH_2-<u>C</u>H_2), 32.36(Ph-CH_2-CH_2-<u>C</u>H_2); GC: t_R 16.03min; LRMS (EI): m/z 186 (M^+, 29%), 117 (M^+-$C_3H_5N_2$, 17%), 91 (M^+-$C_5H_7N_2$, 31%), 82 (M^+-C_8H_8, 100%); HRMS (EI): found m/z 187.12212, $C_{12}H_{15}N_2$, calculated m/z 187.12297.

Phenylbutanol (**301**)

Compound **301** was synthesised in a similar manner to **242**, except that phenylbutanoic acid (20.00g, 121.95mmol) $LiAlH_4$ (30.50mL, 2M in THF) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **301** as a clear oil (13.89g, yield 76%); R_f=0.48 [40/60 diethyl ether/hexane].

$\nu_{(max)}$(ilm)cm^{-1}: 3334 (O-H), 2872 (C-H), 1615 (Ar, C=C); 1056 (C-O); δH (400MHz, $CDCl_3$): 7.26 (2H, m, Ph-<u>H</u>), 7.14 (3H, m, Ph-<u>H</u>), 3.60 (2H, t, J=6.59Hz, Ph-C<u>H</u>$_2$), 2.59 [2H, t, J=7.69Hz, Ph-$(CH_2)_3$-C<u>H</u>$_2$], 1.60 (2H, m, Ph-CH_2-C<u>H</u>$_2$), 1.38 [3H, m; 2H, Ph-$(CH_2)_2$-C<u>H</u>$_2$, 1H, -O<u>H</u>]; δ_C (100MHz, $CDCl_3$): 142.02, 127.86, 127.74, 125.13 (Ar, C), 62.38 (Ph-CH_2), 35.37 [Ph-$(CH_2)_3$-<u>C</u>H_2], 32.10 (Ph-CH_2-<u>C</u>H_2), 24.87 [Ph-$(CH_2)_2$-<u>C</u>H_2]; GC: t_R 4.10min; LRMS (EI): m/z 150 (M^+, 71%), 107

(M^+-C_2H_3O, 100%), 91 (M^+-C_3H_7O, 82%), 77 (M^+-C_4H_9O, 55%).

Phenylbutyl bromide (**302**)

Compound **302** was synthesised in a similar manner to **243**, except that PBr_3 (43.31g, 160.00mmol) and **301** (12.00g, 80mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **302** as a clear oil (11.88g, yield 70%); R_f=0.78 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3026 (Ar, C-H), 2936 (C-H), 1603 (Ar, C=C), 680 (C-Br); δH (400MHz, $CDCl_3$): 7.27 (2H, m, Ph-H), 7.17 (3H, m, Ph-H), 3.40 (2H, t, J=6.77Hz, Ph-CH_2), 2.63 [2H, t, J=7.51Hz, Ph-$(CH_2)_3$-CH_2], 1.88 (2H, m, Ph-CH_2-CH_2), 1.78 [2H, m, Ph-$(CH_2)_2$-CH_2]; δ_C (100MHz, $CDCl_3$): 141.93, 128.48, 126.00, (Ar, C), 35.07 (Ph-CH_2), 33.77 [Ph-$(CH_2)_3$-CH_2], 32.33 (Ph-CH_2-CH_2), 29.94 [Ph-$(CH_2)_2$-CH_2]; GC: t_R 11.28min; LRMS (EI): m/z 212 (M^+, 5%), 105 (M^+-C_2H_4Br, 4%), 91 (M^+-C_3H_6Br, 100%), 77 (M^+-C_4H_8Br, 4%).

Phenylbutyl imidazole (**303**)

Compound **303** was synthesised in a similar manner to **198**, except that **302** (1.50g, 7.08mmol), anhydrous K_2CO_3 (1.17g, 8.49mmol) and imidazole (0.72g, 10.60mmol) were used. Removal of the solvent under vacuum gave a pale oil which was purified using column chromatography to give **303** as a yellow oil (0.97g, yield 69%); R_f=0.66 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3025 (Ar, C-H), 2936 (C-H), 1951 (Im, C=N), 1602 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.41(1H, s, NCHN, Im), 7.26 (2H, m, Ph-H), 7.14 (3H, m, Ph-

H), 7.02 (1H, s, CH_2-NCH, Im), 6.85 (1H, s, NCH, Im), 3.90 (2H, t, J=7.14Hz, Ph-CH_2), 2.61 [2H, t, J=7.51Hz, Ph-CH_2-$(CH_2)_2$-CH_2], 1.78 (2H, m, Ph-CH_2-CH_2), 1.61 [2H, m, Ph-$(CH_2)_2$-CH_2]; δ_C (100MHz, $CDCl_3$): 141.56 (Ar, C), 137.12 (Im, NCN), 129.53, 128.52, 126.11 (Ar, C), 128.42, 118.81 (Im, C), 46.95 (Ph-CH_2), 35.35 [Ph-$(CH_2)_3$-CH_2], 30.65 (Ph-CH_2-CH_2), 28.31 [Ph-$(CH_2)_2$-CH_2]; GC: t_R 17.22min; LRMS (EI): m/z 200 (M^+, 3%), 91 (M^+-$C_6H_9N_2$, 95%), 82 (M^+-C_9H_{10}, 86%), 68 (M^+-$C_{10}H_{13}$, 100%); HRMS (EI): found m/z 201.13869, $C_{13}H_{17}N_2$, calculated m/z 201.13862.

Triethyl 4-(phenyl)butane-1,1,1-tricarboxylate (**304**)

COOEt
COOEt
COOEt

Triethylmethane tricarboxylate (56.00g, 241.21mmol) was added to abhydrous potassium tert-butoxide (Bu^tOK) (27.55g, 241.21mmol) and anhydrous THF. The mixture was stirred at room temperature for 15min and then **299** (32.00g, 160.80mmol) was added. The reaction was stirred under reflux for a further 12h. The THF was removed under vacuum to leave an oily solid, which was dissolved in DCM (500mL) and washed with saturated $NaHCO_3$ solution (3×500mL), followed with water (3×500mL), dried over anhydrous $MgSO_4$ and filtered. Removal of the DCM under vacuum gave **304** as a clear oil (50.30g, crude yield 89%), which was carried on to the next step without further purification.

$\nu_{(max)}$(Film)cm^{-1}: 2985 (C-H), 1738 (C=O), 1600 (Ar, C=C), 1447 (C-O, ester); GC: t_R 15.49min; LRMS (EI): m/z 350 (M^+, 10%), 304 (M^+-C_2H_6O, 12%), 230 (M^+-C_9H_{12}, 38%), 160 (M^+-$C_{12}H_{14}O_2$, 20%), 117 (M^+-$C_{10}H_{17}O_6$, 40%), 104 (M^+-$C_{11}H_{18}O_6$, 100%), 91 (M^+-$C_{12}H_{19}O_6$, 45%).

Phenylpentanoic acid (**305**)

Compound **304** (48.00g, 137.14mmol) was added to a stirred solution of concentrated HCl (300mL), water (300mL) and glacial acetic acid (150mL). The reaction was refluxed for 24h. After cooling to room temperature, the HCl, water and the glacial acetic acid was removed under vacuum to give a white solid. The crude product was washed with hot water (3×500mL), until no smell of the glacial acetic acid was noticed, dissolved in DCM and dried over $MgSO_4$. Removal of the DCM under vacuum gave a white solid which was purified using column chromatography to give **305** as a white solid (16.8g, yield 69%); [m.p. 57.5-58.8°C (lit. m.p. 58.0-59.0°C; Brady et al, 1979)]; R_f=0.70 [30/70 diethyl ether/petroleum ether (40-60^{0}C)].

$\nu_{(max)}$(Film)cm^{-1}: 3028 (Ar, C-H), 2938 (C-H), 2859 (COOH), 1700 (C=O), 1603 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.29 (2H, m, Ph-H), 7.19 (3H, m, Ph-H), 2.66 (2H, m, CH_2-COOH), 2.40 (2H, m, Ph-CH_2), 1.68 (4H, m, Ph-CH_2-$(CH_2)_2$); δ_C (100MHz, $CDCl_3$): 180.26 (C=O), 142.10, 128.48, 128.44, 125.92, (Ar, C), 35.63 (CH_2-COOH), 34.03 (Ph-CH_2), 30.87 (CH_2-CH_2-COOH), 24.37 (Ph-CH_2-CH_2); GC: t_R 12.81min; LRMS (EI): m/z 178 (M^+, 22%), 160 (M^+-OH, 16%), 104 (M^+-$C_3H_5O_2$, 27%), 91 (M^+-$C_4H_7O_2$, 100%).

Phenylpentanol (**306**)

Compound **306** was synthesised in a similar manner to **242**, except that **305** (15.00g, 84.26mmol) and $LiAlH_4$ (21mL, 2M in THF) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column

chromatography to give **306** as a clear oil (10.52g, yield 76%); R_f=0.57 [40/60 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3344 (O-H), 2857 (C-H), 1603 (Ar, C=C); 1054 (C-O); δH (400MHz, $CDCl_3$): 7.27 (2H, m, Ph-H), 7.17 (3H, m, Ph-H), 3.64 (2H, t, J=6.59Hz, Ph-CH$_2$), 2.62 (2H, t, J=7.69Hz, CH$_2$-OH), 1.65 [4H, m, Ph-CH$_2$-(CH$_2$)$_2$], 1.42 [3H, m; 2H, Ph-(CH$_2$)$_3$-CH$_2$, 1H, OH]; δC (100MHz, $CDCl_3$): 142.65, 128.49, 128.36, 125.76 (Ar, C), 63.01 (Ph-CH$_2$), 36.00 (CH$_2$-OH), 32.73 (Ph-CH$_2$-CH$_2$), 31.36 [Ph-(CH$_2$)$_2$-CH$_2$], 25.50 [Ph-(CH$_2$)$_3$-CH$_2$]; GC: t_R 11.72min; LRMS (EI): m/z 164 (M^+, 6%), 146 (M^+-OH, 32%), 117 (M^+-C_2H_7O, 56%), 105 (M^+-C_3H_7O, 80%), 91 (M^+-C_4H_9O, 100%), 77 (M^+-$C_5H_{11}O$, 9%).

Phenylpentyl bromide (**307**)

Compound **307** was synthesised in a similar manner to **243**, except that PBr_3 (33.00g, 121.96mmol) and **306** (10g, 60.98mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **307** (10.74g, yield 78%) as a clear oil; R_f=0.81 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3025 (Ar, C-H), 2856 (C-H), 1603 (Ar, C=C), 698 (C-Br); δH (400MHz, $CDCl_3$): 7.28 (2H, m, Ph-H), 7.18 (3H, m, Ph-H), 3.40 (2H, t, J=6.77Hz, Ph-CH$_2$), 2.62 (2H, t, J=7.69Hz, CH$_2$-Br), 1.90 (2H, m, Ph-CH$_2$-CH$_2$), 1.65 [2H, m, Ph-(CH$_2$)$_2$-CH$_2$], 1.48 [2H, m; Ph-(CH$_2$)$_3$-CH$_2$]; δC (100MHz, $CDCl_3$): 142.39, 128.47, 128.41, 125.85 (Ar, C), 35.82 (Ph-CH$_2$), 33.89 (CH$_2$-Br), 32.79 (Ph-CH$_2$-CH$_2$), 30.72 [Ph-(CH$_2$)$_2$-CH$_2$], 27.93 [Ph-(CH$_2$)$_3$-CH$_2$]; GC: t_R 12.61min; LRMS (EI): m/z 226 (M^+, 7%), 147 (M^+-Br, 9%), 91 (M^+-C_4H_8Br, 100%).

Phenylpentyl imidazole (**308**)

Compound **308** was synthesised in a similar manner to **198**, except that (1.00g, 4.41mmol), anhydrous K_2CO_3 (0.70g, 5.07mmol) and imidazole (0.45g, 6.60mmol) were used. Removal of the solvent under vacuum gave a pale oil which was purified using column chromatography to give **308** as a yellow oil (0.72g, yield 77%); R_f=0.62 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3025 (Ar, C-H), 2857 (C-H), 1950 (Im, C=N), 1602 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.41(1H, s, NCHN, Im), 7.26 (2H, m, Ph-H), 7.16 (3H, m, Ph-H), 7.03 (1H, s, CH_2-NCH, Im), 6.86 (1H, s, NCH, Im), 3.88 (2H, t, J=7.14Hz, Ph-CH_2), 2.58 (2H, t, J=7.69Hz, (CH_2-Im), 1.79 (2H, m, Ph-CH_2-CH_2), 1.63 [2H, m, Ph-$(CH_2)_2$-CH_2], 1.34 [2H, m, Ph-$(CH_2)_3$-CH_2]; δ_C (100MHz, $CDCl_3$): 142.17 (Ar, C), 137.12 (Im, NCN), 142.17 (Ar, C), 129.50, 128.63, 125.92 (Ar, C), 128.43, 118.81 (Im, C), 46.99 (Ph-CH_2), 35.76 (CH_2-Im), 31.07 (Ph-CH_2-CH_2), 30.94 [Ph-$(CH_2)_2$-CH_2], 26.22 [Ph-$(CH_2)_3$-CH_2]; GC: t_R 18.40min; LRMS (EI): m/z 214 (M^+, 2%), 91 (M^+-$C_7H_{11}N_2$, 67%), 82 (M^+-$C_{10}H_{12}$, 100%), 68 (M^+-$C_{11}H_{14}$, 35%); HRMS (EI): found m/z 215.15429, $C_{14}H_{19}N_2$, calculated m/z 215.15428.

Triethyl 5-(phenyl)pentane-1,1,1-tricarboxylate (**309**)

COOEt
COOEt
COOEt

Compound **309** was synthesised in a similar manner to **304**, except that **302** (9.00g, 42.25mmol), Bu^{t}OK (7.24g, 63.38mmol) and triethylmethane tricarboxylate (14.72g, 63.38mmol) were used. Removal of the solvent under vacuum gave **309** as a clear oil (13.28g, crude yield 87%) which was carried on to the next step

without further purification.

$\nu_{(max)}$(Film)cm^{-1}: 3062 (Ar, C-H), 2983 (C-H), 1740 (C=O), 1603 (Ar, C=C), 1447 (C-O, ester); GC: t_R 15.59min; LRMS (EI): m/z 364 (M^+, 10%), 232 (M^+-$C_{10}H_{12}$, 29%), 160 (M^+-$C_{13}H_{16}O_2$, 12%), 104 (M^+-$C_{12}H_{20}O_6$, 72%), 117 (M^+-$C_{11}H_{19}O_6$, 17%), 91 (M^+-$C_{13}H_{21}O_6$, 100%);

Phenylhexanoic acid (**310**)

Compound **310** was synthesised in a similar manner to **305**, except that **309** (12.50g, 34.34 mmol) was used. Removal of the solvent under vacuum gave an oil which was purified using column chromatography to give **310** as a clear oil (5.80g, yield 88%);R_f=0.72 [30/70 diethyl ether/petroleum ether (40-60^{0}C)].

$\nu_{(max)}$(Film)cm^{-1}: 3026 (Ar, C-H), 2934 (C-H), 2857 (COOH), 1700 (C=O), 1603 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.29 (2H, m, Ph-H), 7.19 (3H, m, Ph-H), 2.63 (2H, t, J=7.66Hz, CH_2-COOH), 2.37 (2H, t, J=7.52Hz, Ph-CH_2), 1.68 [4H, m; $(CH_2)_2$-(CH_2)-COOH], 1.41 (2H, m, Ph-CH_2-CH_2); δ_C (100MHz, $CDCl_3$): 180.42 (C=O), 142.54, 128.49, 128.39, 125.80, (Ar, C), 35.79 (CH_2-COOH), 34.10 (Ph-CH_2), 31.18 (CH_2-CH_2-COOH), 28.76 (Ph-CH_2-CH_2), 24.60 [CH_2-$(CH_2)_2$-COOH]; GC: t_R 14.21min; LRMS (EI): m/z 192 (M^+, 7%), 174 (M^+-OH, 4%), 105 (M^+-$C_4H_4O_2$, 11%), 91 (M^+-$C_5H_9O_2$, 100%).

Phenylhexanol (**311**)

Compound **311** was synthesised in a similar manner to **242**, except that **310** (4.00g, 20.83mmol) and $LiAlH_4$ (5.20mL, 2M in THF) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column

chromatography to give **311** as a clear oil (2.76g, yield 74%); R_f=0.59 [40/60 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3334 (O-H), 2856 (C-H), 1603 (Ar, C=C); 1054 (C-O); δH (400MHz, $CDCl_3$): 7.28 (2H, m, Ph-H), 7.18 (3H, m, Ph-H), 3.63 (2H, t, J=6.59Hz, Ph-CH_2), 2.62 (2H, t, J=7.51Hz, CH_2-OH), 1.65 [5H, m; 4H, Ph-CH_2-$(CH_2)_2$, 1H, OH], 1.39 [4H, m, Ph-$(CH_2)_3$-$(CH_2)_2$]; δ_C (100MHz, $CDCl_3$): 142.83, 128.48, 128.34, 125.70 (Ar, C), 63.08 (CH_2-OH), 35.98 (Ph-CH_2), 32.78 (Ph-CH_2-CH_2), 31.53 [Ph-$(CH_2)_2$-CH_2], 29.15 [Ph-$(CH_2)_3$-CH_2], 25.70 (CH_2-CH_2-OH); GC: t_R 12.85min; LRMS (EI): m/z 178 (M^+, 17%), 160 (M^+-OH, 15%), 117 (M^+-C_3H_9O, 34%), 105 (M^+-C_4H_9O, 99%), 91 (M^+-$C_5H_{11}O$, 100%).

Phenylhexyl bromide (**312**)

Compound **312** was synthesised in a similar manner to **243**, except that PBr_3 (6.08g, 22.47mmol) and **311** (2.00g, 11.23mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **312** (1.82g, yield 67%) as a clear oil; R_f=0.84 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3025 (Ar, C-H), 2855 (C-H), 1603 (Ar, C=C), 698 (C-Br); δH (400MHz, $CDCl_3$): 7.26 (2H, m, Ph-H), 7.16 (3H, m, Ph-H), 3.38 (2H, t, J=6.77Hz, Ph-CH_2), 2.59 (2H, t, J=7.69Hz, CH_2-Br), 1.84 (2H, m, Ph-CH_2-CH_2), 1.66 [2H, m, Ph-$(CH_2)_2$-CH_2], 1.41 [4H, m; Ph-$(CH_2)_3$-$(CH_2)_2$]; δ_C (100MHz, $CDCl_3$): 142.64, 128.46, 128.35, 125.75 (Ar, C), 35.88 (Ph-CH_2), 34.01 (CH_2-Br), 32.81 (Ph-CH_2-CH_2), 31.32 [Ph-$(CH_2)_2$-CH_2], 28.45 [Ph-$(CH_2)_3$-CH_2], 28.10 (CH_2-CH_2-Br); GC: t_R 14.11min; LRMS (EI): m/z 240 (M^+, 13%), 161 (M^+-Br, 2%), 151 (M^+-C_7H_7, 3%), 105 (M^+-C_4H_8Br, 10%), 91 (M^+-$C_5H_{10}Br$, 100%).

Phenylhexyl imidazole (**313**)

Compound **313** was synthesised in a similar manner to **198**, except that **312** (1.50g, 6.22mmol), anhydrous K_2CO_3 (1.30g, 9.42mmol) and imidazole (0.65g, 9.56mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **313** as a light yellow oil (1.12g, yield 79%); R_f=0.51 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3025 (Ar, C-H), 2856 (C-H), 1948 (Im, C=N), 1602 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.42 (1H, s, NC<u>H</u>N, Im), 7.26 (2H, m, Ph-<u>H</u>), 7.16 (3H, m, Ph-<u>H</u>), 7.03 (1H, s, CH_2-NC<u>H</u>, Im), 6.86 (1H, s, NC<u>H</u>, Im), 3.88 (2H, t, J=7.14Hz, Ph-C<u>H</u>$_2$), 2.58 (2H, t, J=7.51Hz, (C<u>H</u>$_2$-Im), 1.75 (2H, m, Ph-CH_2-C<u>H</u>$_2$), 1.60 [2H, m, Ph-$(CH_2)_2$-C<u>H</u>$_2$], 1.32 [4H, m; Ph-$(CH_2)_3$-(C<u>H</u>$_2$)$_2$]; δ_C (400MHz, $CDCl_3$): 142.44 (Ar, C), 137.12 (Im, NCN), 129.46, 128.43, 125.80 (Ar, C), 128.36, 118.81 (Im, C), 47.05 (Ph-CH_2), 35.82 (<u>C</u>H$_2$-Im), 31.22 (Ph-CH_2-<u>C</u>H$_2$), 31.05 [Ph-$(CH_2)_2$-<u>C</u>H$_2$], 28.67 [Ph-$(CH_2)_3$-<u>C</u>H$_2$], 26.48 (<u>C</u>H$_2$-CH_2-Im); GC: t_R 19.46min; LRMS (EI): m/z 228 (M^+, 33%), 137 (M^+-C_7H_7, 20%), 123 (M^+-C_8H_9, 100%), 91 (M^+-$C_8H_{13}N_2$, 98%), 82 (M^+-$C_{11}H_{14}$, 67%), 69 (M^+-$C_{12}H_{15}$, 30%); HRMS (EI): found m/z 229.16992, $C_{15}H_{21}N_2$, calculated m/z 229.16993; Elemental analysis: found C 78.91%, H 8.83%, N 12.27%, $C_{15}H_{20}N_2$, calculated C 78.70%, H 8.90%, N 12.01% (0.29 mole of H_2O).

Triethyl 6-(phenyl)hexane-1,1,1-tricarboxylate (**314**)

Compound **314** was synthesised in a similar manner to **304**, except that **307** (8.00g, 33.20mmol), Bu^{t}OK (0.20g, 1.78 mmol) and triethylmethane tricarboxylate (0.30g, 49.80mmol) were used. Removal of the solvent under vacuum gave **314**

as a clear oil (12.36g, crude yield 98%) which was carried on to the next step without further purification.

$\nu_{(max)}$(Film)cm^{-1}: 3062 (Ar, C-H), 2983 (C-H), 1739 (C=O), 1603 (Ar, C=C), 1453 (C-O, ester); GC: t_R 16.44min; LRMS (EI): m/z 378 (M^+, 48%), 333 (M^+-C_2H_5O, 20%), 305 (M^+-$C_3H_5O_2$, 30%), 287 (M^+-C_7H_7, 14%), 91 (M^+-$C_{14}H_{23}O_6$, 100%).

Phenylheptanoic acid (**315**)

Compound **315** was synthesised in a similar manner to **305**, except that **314** (11.00g, 29.10 mmol) was used. Removal of the solvent under vacuum gave a clear oil which was purified using column chromatography to give **315** as a clear oil (4.4g, yield 73%); R_f=0.76 [30/70 diethyl ether/petroleum ether (40-60^{0}C)].

$\nu_{(max)}$(Film)cm^{-1}: 3026 (Ar, C-H), 2931 (C-H), 2856 (COOH), 1700 (C=O), 1603 (Ar, C=C); δH (100MHz, $CDCl_3$): 7.27 (2H, m, Ph-$\underline{H}$), 7.16 (3H, m, Ph-$\underline{H}$), 2.59 (2H, t, J=7.66Hz, $C\underline{H}_2$-COOH), 2.33 (2H, t, J=7.52Hz, Ph-$C\underline{H}_2$), 1.60 [4H, m; $(C\underline{H}_2)_2$-(CH_2)-COOH], 1.35 [4H, m, Ph-CH_2-$(C\underline{H}_2)_2$]; δ_C (100MHz, $CDCl_3$): 180.38 ($\underline{C}$=O), 142.74, 128.49, 128.35, 125.73 (Ar, C), 35.95 ($\underline{C}H_2$-COOH), 34.13 (Ph-CH_2), 31.35 ($\underline{C}H_2$-CH_2-COOH), 28.99 (Ph-CH_2-$\underline{C}H_2$), 24.67 [$\underline{C}H_2$-$(CH_2)_2$-COOH], 28.96 [Ph-$(CH_2)_2$-$\underline{C}H_2$]; GC: t_R 5.19min; LRMS (EI): m/z 206 (M^+, 17%), 105 (M^+-$C_5H_6O_2$, 11%), 91 (M^+-$C_6H_{11}O_2$, 100%).

Phenylheptanol (**316**)

Compound **316** was synthesised in a similar manner to **242**, except that **315** (3.00g, 14.56mmol) and $LiAlH_4$ (3.65mL, 2M in THF) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column

chromatography to give **316** as a clear oil (2.2g, yield 79%); R_f=0.62 [40/60 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3343 (O-H), 2854 (C-H), 1603 (Ar, C=C); 1057 (C-O); δH (400MHz, $CDCl_3$): 7.27 (2H, m, Ph-$\underline{H}$), 7.15 (3H, m, Ph-$\underline{H}$), 3.61 (2H, t, J=6.59Hz, Ph-C$\underline{H}_2$), 2.58 (2H, t, J=7.51Hz, C$\underline{H}_2$-OH), 1.58 [5H, m; 4H, Ph-CH_2-(C$\underline{H}_2)_2$, 1H, O$\underline{H}$], 1.33 [6H, m, Ph-$(CH_2)_3$-(C$\underline{H}_2)_3$]; δ_C (100MHz, $CDCl_3$): 142.90, 128.47, 128.31, 125.67 (Ar, C), 63.11 ($\underline{C}H_2$-OH), 36.03 (Ph-CH_2), 32.83 (Ph-CH_2-$\underline{C}H_2$), 31.49 [Ph-$(CH_2)_2$-$\underline{C}H_2$], 29.36 [Ph-$(CH_2)_3$-$\underline{C}H_2$], 29.33 [$\underline{C}H_2$-$(CH_2)_2$-OH], 25.74 ($\underline{C}H_2$-CH_2-OH); GC: t_R 14.25min; LRMS (EI): m/z 192 (M^+, 7%), 117 (M^+-$C_4H_{11}O$, 27%), 104 (M^+-$C_5H_{12}O$, 80%), 91 (M^+-$C_6H_{13}O$, 100%).

Phenylheptyl bromide (**317**)

Compound **317** was synthesised in a similar manner to **243**, except that PBr_3 (6.00g, 22.14mmol) and **316** (2.00g, 10.41mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **317** (1.88g, yield 71%) as a clear oil; R_f=0.85 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3025 (Ar, C-H), 2854 (C-H), 1603 (Ar, C=C), 698 (C-Br); δH (400MHz, $CDCl_3$): 7.26 (2H, m, Ph-$\underline{H}$), 7.16 (3H, m, Ph-$\underline{H}$), 3.38 (2H, t, J=6.77Hz, Ph-C$\underline{H}_2$), 2.59 (2H, t, J=7.69Hz, C$\underline{H}_2$-Br), 1.83 (2H, m, Ph-CH_2-C$\underline{H}_2$), 1.59 [2H, m, Ph-$(CH_2)_2$-C$\underline{H}_2$], 1.35 [6H, m; Ph-$(CH_2)_3$-(C$\underline{H}_2)_3$]; δ_C (100MHz, $CDCl_3$): 142.82, 128.48, 128.34, 125.71 (Ar, C), 36.00 (Ph-CH_2), 34.10 ($\underline{C}H_2$-Br), 32.88 (Ph-CH_2-$\underline{C}H_2$), 31.46 [Ph-$(CH_2)_2$-$\underline{C}H_2$], 29.17 [Ph-$(CH_2)_3$-$\underline{C}H_2$], 28.72 [$\underline{C}H_2$-$(CH_2)_2$-Br], 28.18 ($\underline{C}H_2$-CH_2-Br); GC: t_R 15.70min; LRMS (EI): m/z 254 (M^+, 21%), 133 (M^+-C_3H_6Br, 6%), 105 (M^+-$C_5H_{10}Br$, 12%), 91 (M^+-$C_6H_{12}Br$, 100%).

Phenylheptyl imidazole (**318**)

Compound **318** was synthesised in a similar manner to **198**, except that **319** (1.00g, 3.92mmol), anhydrous K_2CO_3 (0.65g, 4.72mmol) and imidazole (0.40g, 5.90mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **318** as a light yellow oil (0.58g, yield 61%); R_f=0.57 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3024 (Ar, C-H), 2928 (C-H), 1944 (Im, C=N), 1602 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.43 (1H, s, NC<u>H</u>N, Im), 7.26 (3H, m, Ph-<u>H</u>), 7.16 (2H, m, Ph-<u>H</u>), 7.03 (1H, s, CH_2-NC<u>H</u>, Im), 6.87 (1H, s, NC<u>H</u>, Im), 3.89 (2H, t, J=7.14Hz, Ph-C<u>H</u>$_2$), 2.58 (2H, t, J=7.51Hz, C<u>H</u>$_2$-Im), 1.76 (2H, m, Ph-CH_2-C<u>H</u>$_2$), 1.59 [2H, m, Ph-$(CH_2)_2$-C<u>H</u>$_2$], 1.30 [6H, m; Ph-$(CH_2)_3$-(C<u>H</u>$_2$)$_3$]; δ_C (100MHz, $CDCl_3$): 142.69 (Ar, C), 137.16 (Im, NCN), 129.42, 128.44, 125.74 (Ar, C), 128.33, 120.69 (Im, C), 47.09 (Ph-CH_2), 35.94 (<u>C</u>H_2-Im), 31.30 (Ph-CH_2-<u>C</u>H_2), 31.07 [Ph-$(CH_2)_2$-<u>C</u>H_2], 29.09 [Ph-$(CH_2)_3$-<u>C</u>H_2], 28.96 [<u>C</u>H_2-$(CH_2)_2$-Im], 26.52 (<u>C</u>H_2-CH_2-Im); GC: t_R 20.53min; LRMS (EI): m/z 242 (M^+, 33%), 151 (M^+-C_7H_7, 41%), 137 (M^+-C_8H_9, 63%), 91 (M^+-$C_9H_{15}N_2$, 100%); HRMS (EI): found m/z 241.16980, $C_{16}H_{21}N_2$, calculated m/z 241.16990.

Phenyloctanol (**319**)

Compound **319** was synthesised in a similar manner to **242**, except that phenyloctanoic acid (3.50g, 15.91mmol) and $LiAlH_4$ (4mL, 2M in THF) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **319** as a clear oil (2.63g, yield 80%); R_f=0.54

[40/60 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3333 (O-H), 3025 (Ar, C-H), 2854 (C-H), 1603 (Ar, C=C); 1055 (C-O); δH (400MHz, $CDCl_3$): 7.28 (2H, m, Ph-H), 7.17 (3H, m, Ph-H), 3.63 (2H, t, J=6.59Hz, Ph-CH_2), 2.60 (2H, t, J=7.69Hz, CH_2-OH), 1.60 [4H, m, Ph-CH_2-$(CH_2)_2$], 1.33 [9H, m; 8H, Ph-$(CH_2)_3$-$(CH_2)_4$, 1H, OH]; δ_C (100MHz, $CDCl_3$): 142.97, 128.49, 128.32, 125.66 (Ar, C), 63.14 (CH_2-OH), 36.06 (Ph-CH_2), 32.86 (Ph-CH_2-CH_2), 31.59 [Ph-$(CH_2)_2$-CH_2], 29.55 [Ph-$(CH_2)_3$-CH_2], 29.44 [CH_2-$(CH_2)_3$-OH], 29.33 [CH_2-$(CH_2)_2$-OH] 25.81 [CH_2-CH_2-OH]; GC: t_R 5.87min; LRMS (EI): m/z 206 (M^+, 12%), 188 (M^+-OH, 5%), 117 (M^+-$C_5H_{13}O$, 28%), 104 (M^+-$C_6H_{14}O$, 92%), 91 (M^+-$C_7H_{15}O$, 100%).

Phenyloctyl bromide (**320**)

Compound **320** was synthesised in a similar manner to **243**, except that PBr_3 (5.26g, 19.42mmol) and **319** (2.00g, 9.71mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **320** (1.78g, yield 68%) as a clear oil; R_f=0.83 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3025 (Ar, C-H), 2854 (C-H), 1603 (Ar, C=C), 698 (C-Br); δH (400MHz, $CDCl_3$): 7.28 (2H, m, Ph-H), 7.17 (3H, m, Ph-H), 3.40 (2H, t, J=6.77Hz, Ph-CH_2), 2.60 (2H, t, J=7.69Hz, CH_2-Br), 1.85 (2H, m, Ph-CH_2-CH_2), 1.61 [2H, m, Ph-$(CH_2)_2$-CH_2], 1.42 [2H, m, Ph-$(CH_2)_3$-CH_2], 1.33 [6H, m; Ph-$(CH_2)_4$-$(CH_2)_3$]; δ_C (100MHz, $CDCl_3$): 142.94, 128.48, 128.32, 125.67 (Ar, C), 36.03 (Ph-CH_2), 34.14, CH_2-Br), 32.89 (Ph-CH_2-CH_2), 31.54 [Ph-$(CH_2)_2$-CH_2], 29.38 [Ph-$(CH_2)_3$-CH_2], 29.27 [CH_2-$(CH_2)_3$-Br], 28.77 [CH_2-$(CH_2)_2$-Br], 28.24 [CH_2-CH_2-Br]; GC: t_R 6.26min; LRMS (EI): m/z 268 (M^+, 20%), 133 (M^+-C_4H_7Br, 8%), 105 (M^+-$C_6H_{12}Br$, 11%), 91 (M^+-$C_7H_{14}Br$, 100%).

Phenyloctyl imidazole (**321**)

Compound **321** was synthesised in a similar manner to **198**, except that **320** (1.50g, 5.58mmol), anhydrous K_2CO_3 (0.90g, 6.52mmol) and imidazole (0.60g, 8.82mmol) were used. Removal of the solvent under vacuum gave a clear oil which was purified using column chromatography to give **321** as a clear oil (0.88g, yield 62%); R_f=0.57 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3025 (Ar, C-H), 2854 (C-H), 1946 (Im, C=N), 1602 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.43 (1H, s, NC<u>H</u>N, Im), 7.26 (2H, m, Ph-<u>H</u>), 7.16 (3H, m, Ph-<u>H</u>), 7.04 (1H, s, CH_2-NC<u>H</u>, Im), 6.88 (1H, s, NC<u>H</u>, Im), 3.89 (2H, t, J=7.14Hz, Ph-C$\underline{H}_2$), 2.58 (2H, t, J=7.69Hz, C$\underline{H}_2$-Im), 1.74 (2H, m, Ph-CH_2-C$\underline{H}_2$), 1.59 [2H, m, Ph-$(CH_2)_2$-C$\underline{H}_2$], 1.28 [8H, sh. s; Ph-$(CH_2)_3$-(C$\underline{H}_2)_4$]; δ_C (100MHz, $CDCl_3$): 142.83 (Ar, C), 137.14 (Im, NCN), 129.47, 128.46, 125.68 (Ar, C), 128.31, 118.82 (Im, C), 47.09 (Ph-CH_2), 35.98, ($\underline{C}H_2$-Im), 31.46 (Ph-CH_2-$\underline{C}H_2$), 31.13 [Ph-$(CH_2)_2$-$\underline{C}H_2$], 29.35 [Ph-$(CH_2)_3$-$\underline{C}H_2$], 29.20 [$\underline{C}H_2$-$(CH_2)_3$-Im], 29.05 [$\underline{C}H_2$-$(CH_2)_2$-Im], 29.60 [$\underline{C}H_2$-CH_2-Im]; GC: t_R 21.51min; LRMS (EI): m/z 256 (M^+, 38%), 123 (M^+-$C_{10}H_{13}$, 42%), 91 (M^+-$C_{10}H_{17}N_2$, 100%), 82 (M^+-$C_{13}H_{18}$, 72%), 69 (M^+-$C_{14}H_{19}$, 34%); HRMS (EI): found m/z 257.20109, $C_{17}H_{25}N_2$, calculated m/z 257.20123.

Phenylnonanol (**322**)

Compound **322** was synthesised in a similar manner to **242**, except that phenylnonanoic acid (4.00g, 17.09mmol) and $LiAlH_4$ (4.30mL, 2M in THF) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **322** as a clear oil (2.53g, yield 67%); R_f=0.48 [40/60 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3334 (O-H), 2854 (C-H), 1603 (Ar, C=C); 1056 (C-O); δH (400MHz, $CDCl_3$): 7.25 (2H, m, Ph-H), 7.15 (3H, m, Ph-H), 3.61 (2H, t, J=6.59Hz, Ph-CH_2), 2.58 (2H, t, J=7.69Hz, CH_2-OH), 1.57 [4H, m, Ph-CH_2-$(CH_2)_2$], 1.29 [11H, m; 10H, Ph-$(CH_2)_3$-$(CH_2)_5$, 1H, OH]; δ_C (100MHz, $CDCl_3$): 143.01, 128.49, 128.31, 125.64 (Ar, C), 63.15 (CH_2-OH), 36.07 (Ph-CH_2), 32.87 (Ph-CH_2-CH_2), 31.61 [Ph-$(CH_2)_2$-CH_2], 29.62 [Ph-$(CH_2)_3$-CH_2], 29.53 [Ph-$(CH_2)_4$-CH_2], 29.51 [CH_2-$(CH_2)_3$-OH], 29.40 [CH_2-$(CH_2)_2$-OH], 25.81 (CH_2-CH_2-OH); GC: t_R 5.67min; LRMS (EI): m/z 220 (M^+, 7%), 202 (M^+-OH, 6%), 117 (M^+-$C_6H_{15}O$, 26%), 104 (M^+-$C_7H_{15}O$, 98%), 91 (M^+-$C_6H_{13}O$, 100%).

Phenylnonyl bromide (**323**)

Compound **323** was synthesised in a similar manner to **243**, except that PBr_3 (4.92g, 18.18mmol) and **322** (2.00g, 9.09mmol) were used. Removal of the solvent under vacuum gave a brown oil which was purified using column chromatography to give **323** (1.94g, yield 75%) as a clear oil; R_f=0.85 [10/90 diethyl ether/hexane].

$\nu_{(max)}$(Film)cm^{-1}: 3025 (Ar, C-H), 2853 (C-H), 1603 (Ar, C=C), 698 (C-Br); δH (400MHz, $CDCl_3$): 7.27 (2H, m, Ph-H), 7.15 (3H, m, Ph-H), 3.39 (2H, t, J=6.77Hz, Ph-CH_2), 2.59 (2H, t, J=7.69Hz, CH_2-Br), 1.86 (2H, m, Ph-CH_2-CH_2), 1.57 [2H, m, Ph-$(CH_2)_2$-CH_2], 1.34 [10H, m, Ph-$(CH_2)_3$-$(CH_2)_5$]; δ_C (100MHz, $CDCl_3$): 142.64, 128.48, 128.31, 125.66 (Ar, C), 36.05 (Ph-CH_2), 34.16, (CH_2-Br), 32.90 (Ph-CH_2-CH_2), 31.57 [Ph-$(CH_2)_2$-CH_2], 29.47 [Ph-$(CH_2)_3$-CH_2], 29.44 [Ph-$(CH_2)_4$-CH_2], 29.35 [CH_2-$(CH_2)_3$-Br], 28.83 [CH_2-$(CH_2)_2$-Br], 28.24 [CH_2-CH_2-Br]; GC: t_R 6.02min; LRMS (EI): m/z 282 (M^+, 1%), 133 (M^+-C_5H_8Br, 6%), 105 (M^+-$C_7H_{14}Br$, 9%), 91 (M^+-$C_8H_{16}Br$, 100%).

Phenylnonyl imidazole (**324**)

Compound **324** was synthesised in a similar manner to **198**, except that **323** (1.50g, 5.32mmol), anhydrous K_2CO_3 (0.90g, 6.52mmol) and imidazole (0.60g, 8.82mmol) were used. Removal of the solvent under vacuum gave a yellow oil which was purified using column chromatography to give **324** as a clear oil (0.91g, yield 63%); R_f=0.58 [90/10 (diethyl ether/methanol)].

$\nu_{(max)}$(Film)cm^{-1}: 3025 (Ar, C-H), 2854 (C-H), 1945 (Im, C=N), 1603 (Ar, C=C); δH (400MHz, $CDCl_3$): 7.43 (1H, s, NC$\underline{H}$N, Im), 7.26 (2H, m, Ph-$\underline{H}$), 7.15 (3H, m, Ph-$\underline{H}$), 7.03 (1H, s, CH_2-NC$\underline{H}$, Im), 6.88 (1H, s, NC$\underline{H}$, Im), 3.89 (2H, t, J=7.14Hz, Ph-C$\underline{H}_2$), 2.58 (2H, t, J=7.69Hz, C$\underline{H}_2$-Im), 1.76 (2H, m, Ph-CH_2-C$\underline{H}_2$), 1.58 [2H, m, Ph-$(CH_2)_2$-C$\underline{H}_2$], 1.26 [10H, m, Ph-$(CH_2)_3$-(C$\underline{H}_2$)$_5$]; δ_C (100MHz, $CDCl_3$): 142.96 (Ar, C), 137.07 (Im, NCN), 129.47, 128.46, 125.65 (Ar, C), 128.30, 118.92 (Im, C), 47.10 (Ph-CH_2), 36.02 ($\underline{C}H_2$-Im), 31.50 (Ph-CH_2-$\underline{C}H_2$), 31.14 [Ph-$(CH_2)_2$-$\underline{C}H_2$], 29.41 [Ph-$(CH_2)_3$-$\underline{C}H_2$], 29.40 [Ph-$(CH_2)_4$-$\underline{C}H_2$], 29.28 [$\underline{C}H_2$-$(CH_2)_3$-Im], 29.11 [$\underline{C}H_2$-$(CH_2)_2$-Im], 26.61 [$\underline{C}H_2$-CH_2-Im]; GC: t_R 22.48min; LRMS (EI): m/z 270 (M^+, 35%), 137 (M^+-$C_{10}H_{13}$, 25%), 123 (M^+-$C_{11}H_{15}$, 52%), 91 (M^+-$C_{11}H_{19}N_2$, 81%), 82 (M^+-$C_{14}H_{20}$, 100%), 69 (M^+-$C_{15}H_{21}$, 30%); HRMS (EI): found m/z 271.21685, $C_{18}H_{27}N_2$, calculated m/z 271.21688.

3.0 BIOCHEMICAL EVALUATION AND STRUCTURE-ACTIVITY RELATIONSHIP DETERMINATION

A small range of the compounds previously synthesised were evaluated (by Dr. Sachin Dhanani at Kingston University) against rat testicular microsomal enzyme for $P450_{17\alpha}$ inhibitory activity, using the microsomal fraction obtained from rat testicular tissue (Sprague-Dawley). The assay was based on that of Owen (1995) and measures the effect of the novel compounds on the rate of conversion [also known as velocity (V)] of radiolabelled progesterone (for 17α-OHase activity) and 17α-hydroxyprogesterone (for lyase activity) to 17α-hydroxyprogesterone and androstenedione respectively.

In general, the assay procedure involved the incubation of the NADPH-generating system together with the enzyme, substrate and inhibitor in an aqueous medium at pH 7.4. Following the incubation, the reaction was quenched by the addition of diethyl ether to the incubation tube, the organic solvent therefore also acts as a solvent for the extraction of the radiolabelled substrate and products. The reaction products along with the starting material, were then separated involving the application of the spots to TLC plates, together with non-radiolabelled steroid carriers (17α-hydroxyprogesterone, androstenedione and testosterone) which were used for identification of the radiolabelled steroids from the assay mixture. The TLC plates were developed using a mixture of chloroform (80ml), cyclohexane (10mL), ethyl acetate (10mL) and methanol (4mL). After developing the plates, each steroid spot was identified under UV light and cut out, placed in individual scintillation tubes and counted for 3min in a cocktail of scintillation fluid and acetone. The percentage conversion was then determined.

In determining the IC_{50} values for the most potent compounds, the inhibitory activity was determined using the method outlined above, however, for each compound, five or more inhibitor concentrations were used and the inhibitory activity determined at each concentration. The IC_{50} values were then determined from a plot of percentage inhibition against log[I] using linear regression analysis.

In determining the K_i each assay tube contained substrate (of varying final concentration, 15μL), NADPH-generating system (25μL), phosphate buffer (445μL), enzyme (0.10mg/mL final concentration, 5μL) and inhibitor (of varying final concentration, 10μL). The K_i was then determined from a plot of I/V (inverse of the V) vs [I] using linear regression analysis.

At the time of submission of this report, only a small number of compounds have undergone biochemical evaluation, in particular, a range of 4-substituted and di-substituted benzyl imidazole-based compounds (Tables 39 and 40 respectively) and the range of phenyl alkyl imidazole-based compounds (Table 41).

Initial consideration of the inhibitory data for the mono- and di-substituted benzyl imidazole-based compounds shows that all of the benzyl compounds tested were weaker inhibitors against 17α-OHase than against lyase. This has been postulated to be a beneficial characteristic of these compounds since the 17α-OHase component is involved in the biosynthesis of glucocorticoids and mineralocorticoids – as such, it is presumed that weaker inhibitory activity (in comparison to the lyase component) against 17α-OHase would result in reduced side-effects. Furthermore, when compared to the standard compound, KTZ (**3**) (which was found to possess IC_{50} values of 3.76±0.01μM against 17α-OHase and 1.66±0.15μM against lyase), they possessed weaker inhibitory activity. The most potent compound was found to be 1-(4-iodo-benzyl)-1*H*-imidazole (**224**), which possessed IC_{50} values of 10.06±0.96μM against 17α-OHase and 1.58±0.17μM against lyase.

Detailed consideration of the mono- and di-substituted compounds does not show a clear structure-activity relationship. Indeed, consideration of the 4-substituted series of compounds show that there is no clear structure-activity relationship other than that all the substituted inhibitors {except 1-[3,5-bis(trifluoromethyl) benzyl]-1*H*-imidazole (**234**)} show greater potency against both 17α-OHase and lyase in comparison to benzyl imidazole (**198**) (which was found to possess IC_{50} values of 214.58±19.67μM against 17α-OHase and 39.06±1.22μM against lyase)

Compound number	IC_{50} 17α-OHase (μM)	IC_{50} 17,20-Lyase (μM)
3	3.76±0.01	1.66±0.15
198	214.58±19.67	39.06±1.22
201	86.58±5.21	18.44±0.17
210	31.63±3.86	2.81±0.27
219	53.40±4.40	6.67±0.36
224	10.06±0.96	1.58±0.17
227	25.38±1.65	7.17±0.13
230	40.26±3.49	7.67±0.05
241	50.56±8.01	8.81±0.43
263	72.05±3.95	8.22±0.97

Table 39. To show the IC_{50} values obtained for the 4-substituted benzyl imidazole-based compounds against both 17α-OHase and 17,20-lyase.

Compound number	IC_{50} 17α-OHase (μM)	IC_{50} 17,20-Lyase (μM)
3	3.76±0.01	1.66±0.15
206	70.66±6.72	9.60±0.14
207	83.10±7.05	11.80±0.41
215	12.22±0.88	2.07±0.07
216	22.56±0.34	3.34±0.11
221	25.95±0.91	3.16±0.11
234	244.85±24.45	19.46±0.75

Table 40. To show the IC_{50} values obtained for the di-substituted benzyl imidazole-based compounds against both 17α-OHase and 17,20-lyase.

and is therefore ~2.5 and ~2 times weaker respectively than 1-(4-fluoro-benzyl)-1*H*-imidazole (**201**), which was found to be one of the weakest mono-substituted derivatives, (IC_{50}=86.58±5.21μM against 17α-OHase and IC_{50}=18.44±0.17μM against lyase). As such, it would appear that the inclusion of a group into the phenyl ring system confers greater inhibitory activity than compounds devoid of substituents.

The modeling of the mono-substituted compounds within the SHC suggests that these compounds are able to undergo interaction with the hydrogen bonding groups which are presumed to exist at the active site. Figure 27 shows the compelxation of 1-(4-bromo-benzyl)-1*H*-imidazole (**219**) within the overall SHC for the overal enzyme complex and shows that this compound adopts a stable conformer and is able to undergo both the Fe-N ligation as well as undergoing polar-polar interaction with one of the hydrogen bonding groups at the active site. Figure 28 shows compound **219** bound to an additional potential hydrogen bonding group – both conformers were found to differ in energy by 3kcal.

Consideration of the di-substituted compounds shows that these inhibitors are, in general, more potent than the 4-substituted mono derivatives against both components of $P450_{17\alpha}$ (Table 39). For example, **201** (which is found to possess IC_{50} values of 86.58±5.21μM against 17α-OHase and 18.44±0.17μM against lyase) is weaker than both disubstituted derivatives evaluated, namely 1-(3,5-difluoro-benzyl)-1*H*-imidazole (**207**) (which has IC_{50} values of 83.10±7.05μM against 17α-OHase and 11.80±0.41μM against lyase) and 1-(3,4-difluoro-benzyl)-1*H*-imidazole (**206**) (which has IC_{50} values of 70.66±6.72μM against 17α-OHase and 9.60±0.14μM against lyase), with greater potency being observed where the substituent is positioned in the *meta* and *para* orientation compared to the mono-substituted derivatives, e.g. 1-(4-chloro-benzyl)-1*H*-imidazole (**210**) (which is found to possess IC_{50} values of 31.63±3.86μM against 17α-OHase and 2.81±0.27μM against lyase).

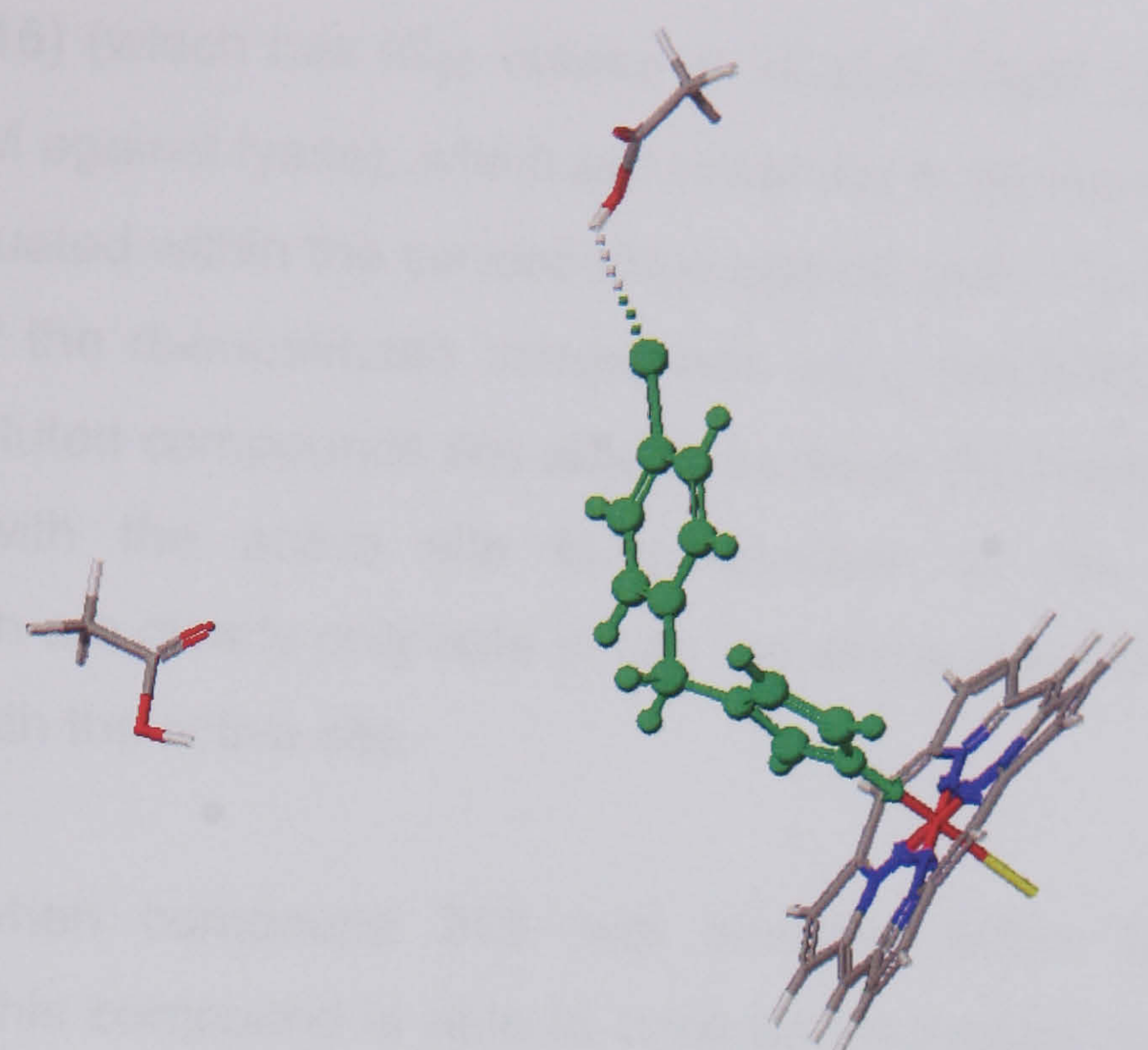


Figure 27. Proposed binding of **219** (in green) within the SHC for the overall enzyme complex

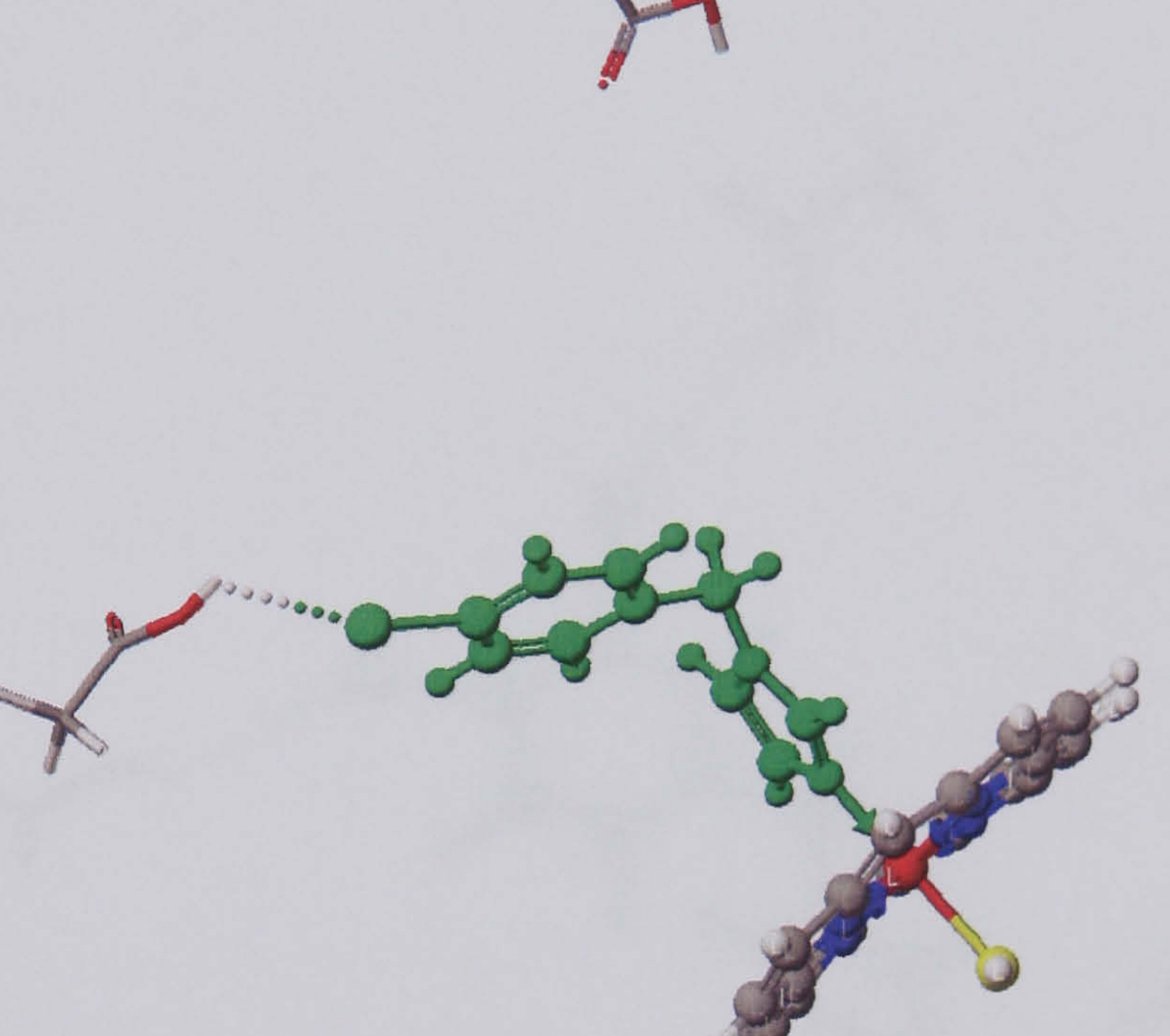


Figure 28. Proposed binding of **219** (in green) to the alternative hydrogen bonding group within the SHC for the overall enzyme complex

This trend is also observed within the chloro derivatives, namely compound 1-(3,4-dichloro-benzyl)-1*H*-imidazole (**215**) (which has IC_{50} values of 12.22±0.88μM against 17α-OHase and 2.07±0.07μM against lyase) and 1-(3,5-dichloro-benzyl)-

1*H*-imidazole (**216**) (which has IC_{50} values of 22.56±0.34μM against 17α-OHase and 3.34±0.11μM against lyase), which are observed to be two of the most potent compounds evaluated within the current study against both 17α-OHase and lyase. The modeling of the di-substituted compounds using the SHC approach shows that the di-substituted compounds are able to increase the number of interactions they undergo with the active site in comparison to the mono-substituted derivatives, which are clearly only able to use the single substituent on the phenyl ring to interact with the active site.

For example, when compound **215** was modeled within the SHC, it was discovered that this compound is able to undergo interaction with both hydrogen bonding groups which are presumed to exist within the active site of the enzyme complex [each group interacting with the C(3) hydrogen bonding group of the two substrates of each component (Figure 29)].

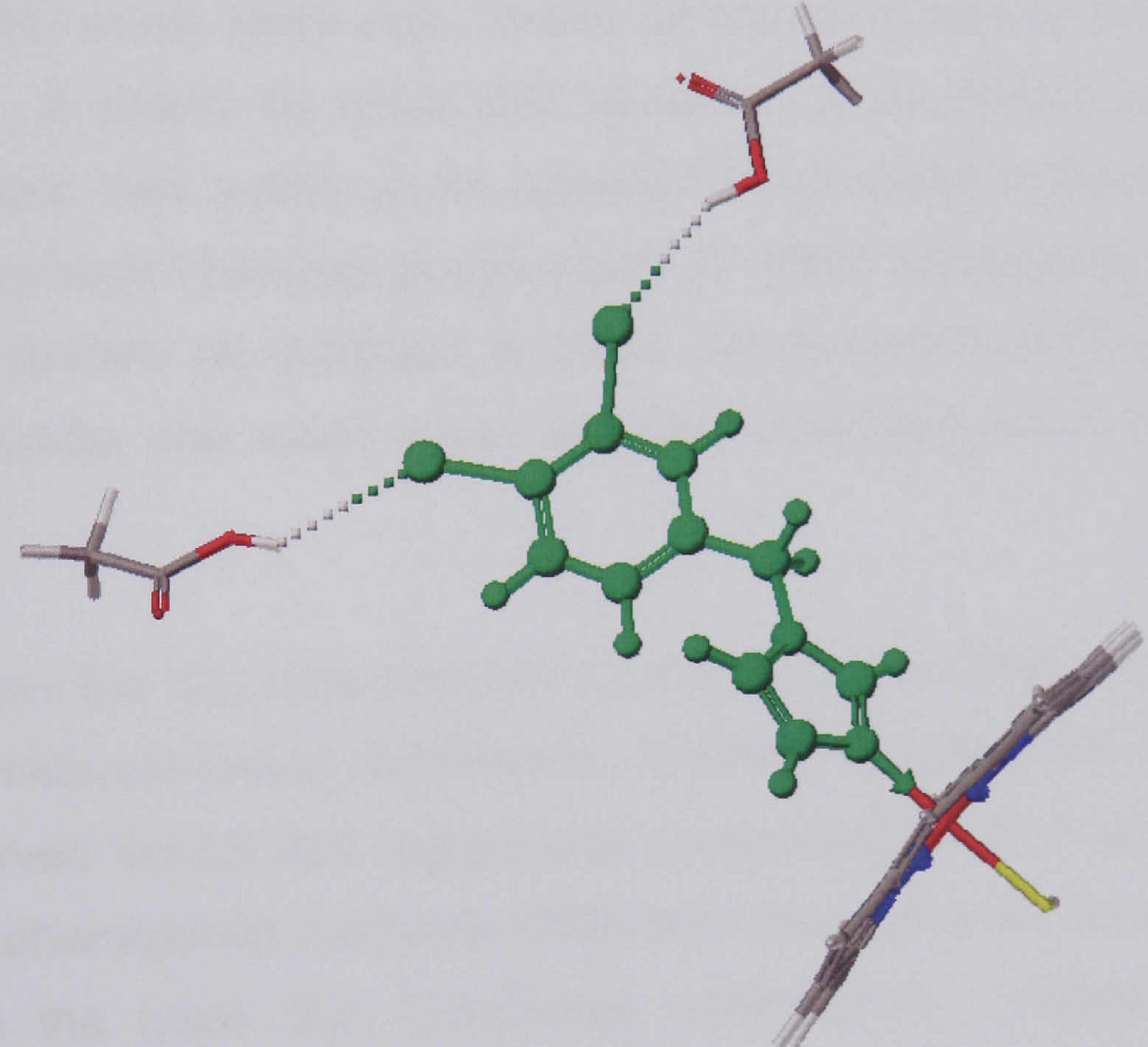


Figure 29. Proposed binding of **215** (in green) within the SHC showing interaction with both hydrogen bonding groups.

The mono-substituted compound, however, is only able to undergo interaction with one of the components at any given time (Figures 27 and 28). The results of the modeling study would therefore appear to suggest that the disubstituted (in particular, the 3,4-disubstituted) compounds are able to utilise both of the hydrogen bonding groups within the $P450_{17\alpha}$ active site resulting in increased stability of the inhibitor enzyme complex and therefore resulting in potent inhibitory activity.

In conclusion, the benzyl imidazole-based compounds are, in general, weaker inhibitors of the lyase component in comparison to the standard compound (**3**). Furthermore, the compounds are significantly weaker inhibitors of the 17α-OHase component and therefore would be expected to have less of an effect on corticosteroid biosynthesis. With regards to the selectivity of the above compounds against the lyase component, greater selectivity is observed for 1-(4-chloro-benzyl)-1*H*-imidazole (**210**) and 1-[3,5-bis(trifluoromethyl)benzyl]-1*H*-imidazole (**234**) which show over 10 fold difference in activity between the two components. It should be noted that whilst the di-substituted compounds are potent inhibitors, they would not be expected to be useful in the clinic since the use of both hydrogen bonding groups would, in effect, totally block the active site and would therefore be expected to affect the biosynthesis of glucocorticoids, mineralocorticoids, and which would therefore potentially result in severe side-effects.

Table 41 shows the IC_{50} data obtained against both 17α-OHase and lyase by the phenylalkyl imidazole-based compounds. Detailed consideration of the inhibitory activity observed shows that compounds possessing an alkyl spacer group ≥5 [compounds phenylpentyl imidazole (**308**) and above] are all more potent than **3** against both the lyase and 17α-OHase components. Indeed, phenylheptyl imidazole (**318**) is approximately 17 and 12 times more potent than **3** against the lyase and 17α-OHase components respectively whereas phenyloctyl imidazole (**321**) is approximately 8 and 15 times more potent than **3** against the two

components of P450$_{17\alpha}$.

In an attempt to determine the mode of action of the synthesised compounds, we undertook the determination of the K_i value of **318** and **3** against the lyase component. The K_i value for **318** was found to be 52nM (Figure 30) whilst **3** was found to possess a K_i of 680nM (Figure 31), showing that the phenylalkyl imidazole-based compounds are extremely potent inhibitors of the P450$_{17\alpha}$ enzyme complex. Furthermore, from the consideration of the Dixon plot, we observe that compound **318** inhibits P450$_{17\alpha}$ in a competitive and reversible manner.

Compound number	**IC_{50} 17,20-Lyase (μM)**	**IC_{50} 17α-OHase (μM)**
3	1.66±0.15	3.76±0.01
198	50.90±0.86	154.20±7.93
298	ND	ND
300	6.14±1.21	30.95±0.68
303	2.23±0.38	8.65±1.37
308	1.31±0.21	2.20±0.25
313	0.51±0.03	0.87±0.03
318	0.10±0.02	0.32±0.05
321	0.21±0.02	0.25±0.01
324	0.35±0.01	1.06±0.03

Table 41. To show the IC_{50} values obtained for the phenyl alkyl imidazole-based compounds against both 17α-OHase and 17,20-lyase.

Consideration of the biochemical data shows that the phenylalkyl imidazole-based compounds inhibit the lyase component to a greater extent than the 17α-OHase component.

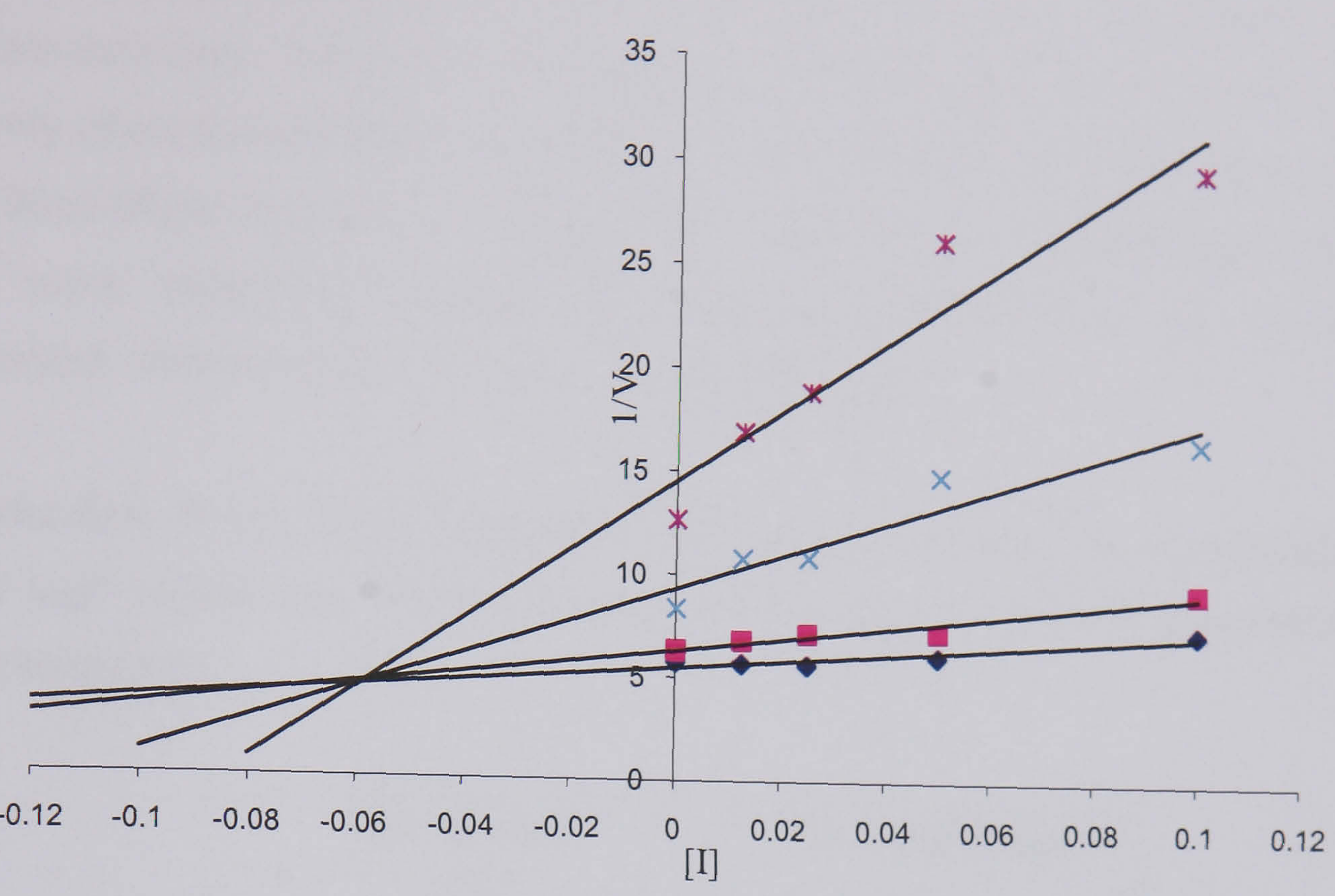


Figure 30. Dixon plot of 1/V versus [I] for **318** giving a K_i value of 52nM against lyase

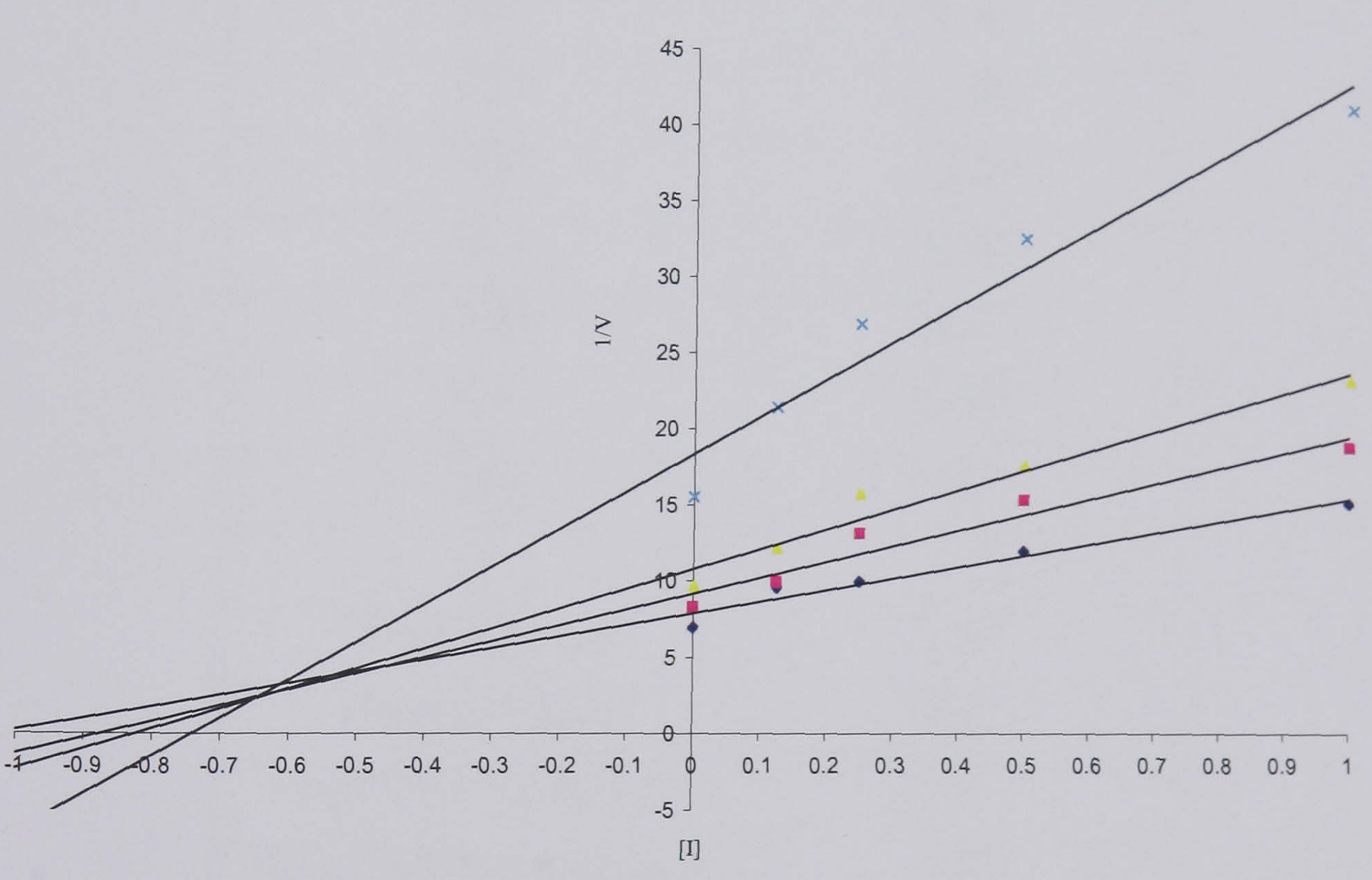


Figure 31. Dixon plot of 1/V versus [I] for **3** giving a K_i value of 680nM against lyase

As previously discussed, this is potentially an extremely useful and favourable feature since the data suggest that these compounds would possess weak

inhibitory activity with regards to the biosynthesis of the corticosteroids and the mineralocorticoids. However, the selectivity of these compounds is relatively poor with only phenylpropyl imidazole (**300**) (which was found to possess an IC_{50} value of 30.95±0.68μM against 17α-OHase and 6.14±1.21μM against lyase) showing 5 times more selectivity towards the lyase component than the 17α-OHase component - the compound is, however, weaker than **3**.

Consideration of the calculated logP (Table 42) shows that the compounds have similar logP values and which are similar to the logP of the natural substrates for each component.

Compound	Calculated logP
198	1.31
298	ND
300	1.96
303	2.36
308	2.75
313	3.15
318	3.54
321	3.94
324	4.34
3	3.18
Progesterone	3.69
Pregnenolone	4.08
17α-OHProgesterone	2.97
17α-OHPregnenolone	3.36

Table 42. Table showing the calculated logP values for a number of the synthesised compounds, **3** and for the four natural substrates of the overall enzyme complex, ND=not determined.

For example, the calculated logP values of pregnenolone and progesterone were found to be ~4.1 and ~3.7 respectively, whilst the most potent imidazole-based inhibitor against 17α-OHase, compound **321**, was found also to possess a logP value of ~3.9. When considering the natural substrates for the lyase component, i.e., 17α-hydroxypregnenolone and 17α-hydroxyprogesterone, the calculated logP values were found to be ~3.4 and ~3.0 respectively, whilst the most potent imidazole-based compound against lyase is found to possess a calculated logP value of ~3.5, compound **318**.

This would therefore appear to suggest that in the design of novel inhibitors of the two components of this enzyme complex, the logP factor should be considered as an important physicochemical factor and that inhibitors possessing logP values of approximately 3.9 may possess good inhibitory activity against the 17α-OHase component whereas compounds found to possess an approximate logP of 3.5 may show good inhibitory activity against the lyase component of the overall enzyme complex of $P450_{17\alpha}$-{The calculated logP was undertaken utelising the Quantum Cache Project leader [Quantum Cache Project Leader is a trademark of Oxford Molecular Ltd. (Fujitsu), Oxford Science Park, Oxford, UK]. The calculation includes an initial geometric minimization using PM5 forcefield (to get the lowest energy conformer) and the file is saved in the Quantum Cache Work Space. The file is then opened in Quantum Cache Project Leader where the logP calculation is undertaken (by slecting logP in the tools menu)}.

Conclusion

In conclusion, from the consideration of the biochemical evaluation of a range of compounds synthesised within the current study, the level of inhibition observed is disappointing as most of the compounds evaluated have shown weaker inhibitory activity than the standard compound, namely **3**. However, from the consideration of the structure-activity relationship determination of the ranges of compounds evaluated, a number of factors have been suggested which may be used in the

further design and subsequent synthesis of potential inhibitors of this important enzyme in the treatment of hormone-dependent prostate cancer.

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