SYNTHESIS OF SEROTONERGIC AGENTS

A Thesis Presented by

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To

those whom I love and so greatly miss.....

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ABSTRACT

Serotonin and its receptors have been the focus of intense research over the past decade. The identification of many serotonin receptor subtypes and the greater understanding of their physiological properties, has initiated many projects in search of potent and selective agents in the hope that novel therapeutic drugs may be identified. This is one such research project. This work has led to the discovery of several potent and selective 5-HT_{1A} agents. This was achieved by structural modification of the lead compounds, β-blockers, aryloxypropanolamines.

An historical review of the properties of serotonin, the classification and characterisation of serotonin receptors, and the structural requirements for activity at 5-HT_{1A} and 5-HT_{1B} receptors for many classes of compounds is presented. Structure-activity relationship for aryloxypropanolamines at β-adrenoceptors has also been discussed.

Structural features which are known to be essential for β-adrenergic activity of pindolol, were modified and compounds with moderate affinities and selectivities for 5-HT_{1A} receptors were obtained.

Introduction of electron withdrawing substituents at the 6-position of the naphthalene ring of propranolol significantly reduced affinity at 5-HT_{1A} and 5-HT_{1B} receptors. Other structural modifications in this series, also yielded compounds with less affinity than propranolol.

Novel analogues of 5-HT were also prepared and tested. These agents possessed reasonable affinities and selectivities for 5-HT_{1A} receptors and displayed weak mixed agonistic and antagonistic activities at 5-HT_{1B} receptors.

Recent publication on structure-activity relationships for a number of propranolol analogues enabled us to design a novel series of compounds, analogous to cyanopindolol. These agents are potent and highly specific partial agonists at 5-HT_{1A} receptors.

All of the target analogues were prepared via the condensation reaction of epichlorohydrin or an aminoalkyl halide with a hydroxyindole or a substituted naphthol, followed by amminolysis. An effective procedure for the synthesis of 5-hydroxy-2-naphthoic acid, the key intermediate for the preparation of propranolol analogues, is described. The procedure for the preparation of other key intermediates in the synthesis of the analogues is also presented.

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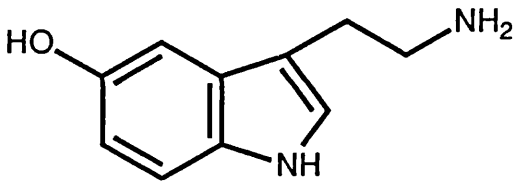
CHAPTER ONE

REVIEW: SEROTONIN AND SEROTONIN RECEPTORS

1.1. Introduction

The work described in this thesis is concerned with the synthesis of selective 5-HT_{1A} antagonists and selective 5-HT_{1B} agents. This introductory chapter contains a brief historical review of the discovery and the properties of serotonin and serotonin receptors. The object of the review is to highlight the background knowledge on which the work presented in this thesis is based. The review also contains published literature data which deals with the structure-activity correlation studies in the relevant parts of the serotonergic and adrenergic fields on which the present work is established .

1.2. Serotonin



(1)

Serotonin (5-hydroxytryptamine, 5-HT, **1**) is 3-(β-aminoethyl)-5-hydroxyindole. The discovery, isolation and subsequent characterisation of serotonin is associated with a number of names, famous in pharmacology. In 1940 Erspamer [1,2] isolated a pharmacologically-active substance from the salivary gland of octopus, which he named enteramine, believing it to be an indole alkylamine. In 1948 Rapport [3] isolated the vasoconstrictor substance serotonin, which was known to be present in blood serum and after isolation of the substance, he identified it as 5-HT. Erspamer realized that enteramine might be the same substance he had obtained and confirmed that it was. Serotonin was then prepared synthetically by Hamlin and Fischer (1951) and proved to have all the properties of natural serotonin [4]. It was later

found to be widely distributed in a variety of plant and animal species, for example in vertebrates, in tunicates, mollusks, arthropods and coelenterates, in fruits such as pineapples, bananas, plums and in various nuts. It is also present in stings and venoms of common nettle, cowhage, wasps and scorpions.

In the beginning of the fifties, Twarog and Page [5] and Gaddum and co-workers [6] found 5-HT in the mammalian brain and soon after Marrazzi and Hart [7] and Brodie et al [8] suggested that 5-HT could be a transmitter molecule within the central nervous system (CNS). This was not immediately accepted and as late as 1975 some concern was expressed regarding the concept of 5-HT as a neurotransmitter [9]. However, today, the neurotransmitter role of 5-HT is universally accepted [10].

Since its discovery, 5-HT has been implicated in the control of numerous central and peripheral pharmacological responses [11,12]. It stimulates or inhibits a variety of smooth muscles and nerves. It causes vasoconstriction or vasodilation by acting directly on vascular smooth muscle. Intravenous injections of 5-HT in the dog and man commonly causes a short-lived increase in the respiratory volume accompanied by variable changes in respiratory rate. Among its central effects, 5-HT has been linked with appetite, memory, sleep, sexual dysfunction, anxiety, depression, thermoregulation, hallucinogenic behaviour and many other processes. It is now believed that serotonin interacts with more than one 5-HT receptor. Over the last decade, intensive research in this field has yielded much information regarding 5-HT receptors. The interest in 5-HT and its receptors is now greater than ever since many believe that its interaction with different types of receptors might explain its various pharmacological actions.

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1.2.1. Biosynthesis and Catabolism of 5-HT

The biosynthesis and catabolism of 5-HT have been recently reviewed [13,14] and are out lined in figure 1.1.

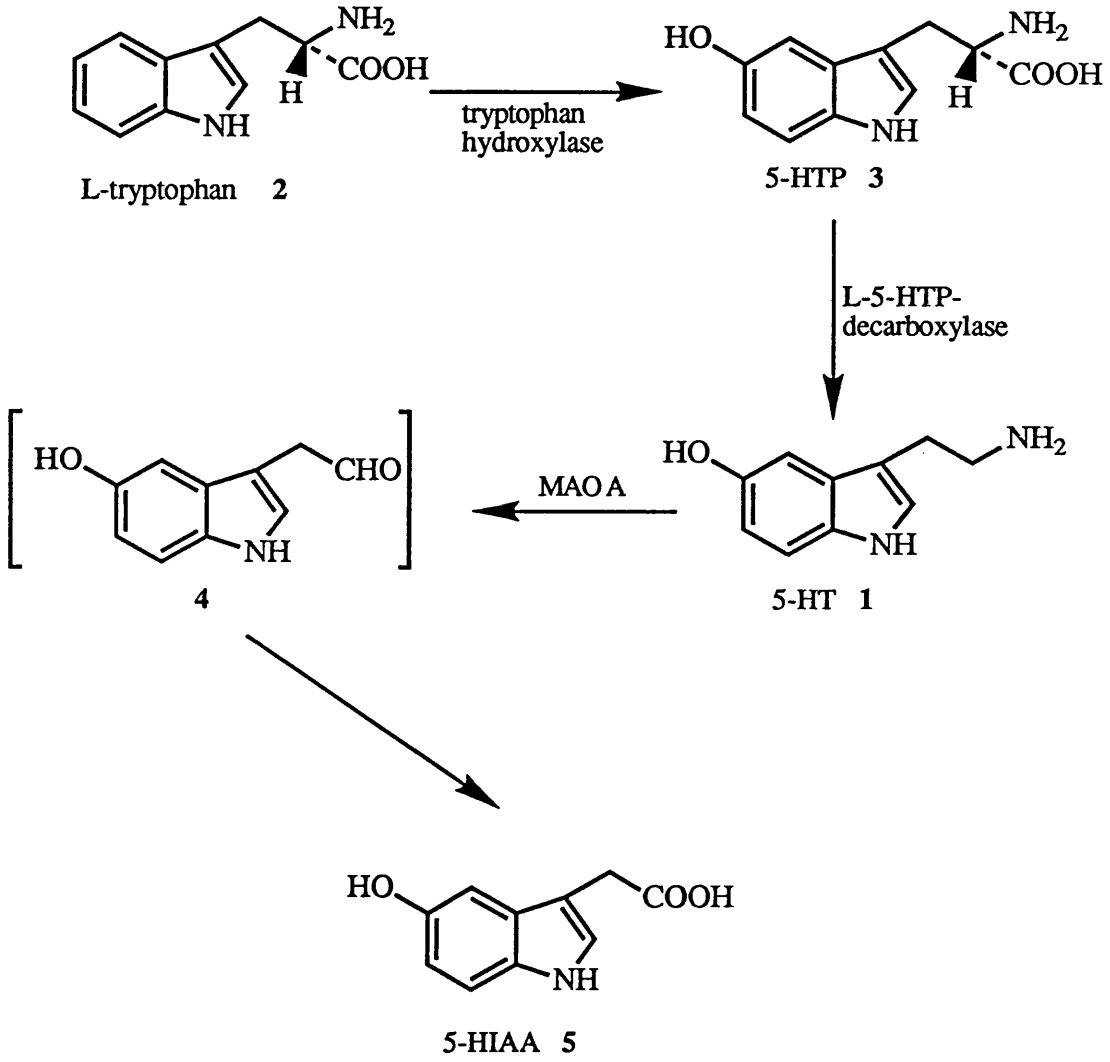

Figure 1.1. Biosynthesis and catabolism of 5-HT

Serotonin metabolism bears considerable similarities to that of the catacholamines. It is synthesised from the essential amino acid L-tryptophan 2 which is supplied by food. Tryptophan then enters the brain and is stored inside the neurons where it is converted to 5-HT via

the intermediate 5-hydroxytryptophan (5-HTP, **3**), by the enzymes tryptophan hydroxylase and L-5-HTP decarboxylase. The formation of 5-HTP is the rate determining step. The introduction of the 5-hydroxy group by tryptophan hydroxylase requires molecular oxygen and a protein cofactor as an electron donor. The synthesised 5-HT is stored in synaptic vesicles and upon nerve impulses, it is released by exocytosis and may interact with 5-HT receptors or it may be taken up by the 5-HT neurons. Inside the terminal, 5-HT can be degraded by monoamine oxidase type A (MAO A) to 5-hydroxyindole-3-acetic acid (5-HIAA, **5**) via the aldehyde intermediate **4**. This is the major catabolic pathways for 5-HT. Other minor metabolic pathways that have been reported are, for example, O-sulphate conjugation and formation of tetrahydro-β-carbolines [15].

1.3. Serotonin Receptors

The earliest pharmacological experiments devoted to the formulation of structure-activity relationship for serotonergic activity and to the development of novel 5-HT agonists and antagonists, using peripheral 5-HT receptor preparations soon indicated that multiple types of receptors might exist within the same tissue. Gaddum and co-workers were the first to provide evidence for this. They showed that drugs which acted as 5-HT antagonists gave different results when tested in different preparations. Thus, ergot derivatives such as LSD **6** and dihydroergotamine **6a** were potent antagonists of the action of 5-HT on smooth muscle of rat uterus and rabbit ear, but were not very potent as antagonists on the guinea-pig ileum preparations. In 1954, Gaddum and Hameed [16] postulated the existence of two types of 5-HT receptors to account for these effects of the antagonists and in 1957, Gaddum and Picarelli [17] published a more detailed account and gave the two

receptors the designation of D and M. The "D" receptors were located in the smooth muscle and the effects of 5-HT at this receptor were blocked with **D**ibenzyline **7**, while the "M" receptor was a neuronal receptor, located either in ganglia or nerve fibres of parasympathetic nerve, at which the effects of 5-HT were blocked by **M**orphine **8**. However, since its introduction, this classification has been largely criticized [18-22] because, on the one hand, dibenzyline is not a specific D receptor blocking drug, and also because morphine antagonizes the effect of 5-HT by blocking neuronal release of acetylcholine and not by blocking 5-HT receptors [23,24].

Some two decades later, reports describing high affinity [^{3}H] 5-HT binding to various brain preparations appeared in the literature and it was soon realized that certain radioligands [e.g. [^{3}H] 5-HT, [^{3}H] LSD, [^{3}H] spiperone **9**] did not label the same population of 5-HT recognition sites. Peroutka and Snyder [25], using radioligand binding studies, suggested the existence of 5-HT_1 and 5-HT_2 recognition sites. In 1986, Bradley et al [26] proposed a 5-HT_x system of nomenclature for the classification of functional 5-HT receptors which reconciled the Gaddum and Picarelli definition of peripheral 5-HT receptors with the several recognition sites identified in rat brain membranes by radioligand binding technology.

Three major categories of 5-HT receptors were proposed; designated as "5-$HT_{1\text{-like}}$", 5-HT_2 and 5-HT_3; the D and M receptors are incorporated in the 5-HT_2 and 5-HT_3 categories respectively. "5-$HT_{1\text{-like}}$" receptors were thought to include at least four subtypes [27], each with a distinct pharmacology, which were designated 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D}. The 5-HT_2 receptors were reported [28] to be a single site and had been well characterised using selective antagonists.

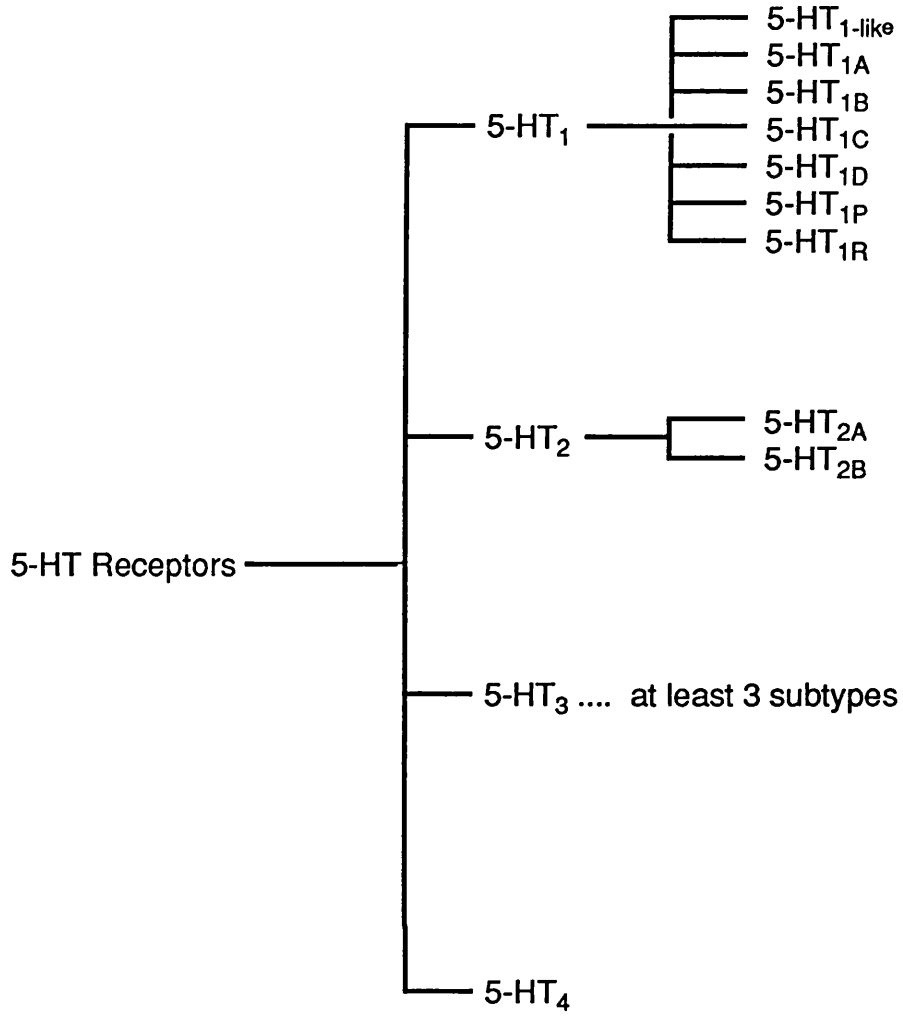

Figure 1.2. Classification of 5-HT Receptors

5-HT_3 receptors were reported [29] to represent a heterogeneous population of receptors with the possibility of at least three subtypes. Over the last three years, yet more 5-HT receptors have apparently been identified and reported. Subsequently, the proposed classification of Bradley et al [26] has been extended and / or modified to include the newly discovered receptors (see figure 1.2.). However, even this classification is not universally accepted since it is primarily based on the affinity of 5-HT or other agonists for these receptor sites, with subtypes defined by differences in antagonists profiles. Other 5-HT receptor classification based on second messenger systems or amino acid sequences have

also been proposed but they are only intended to offer an open forum for the debate until consensus is agreed.

1.3.1. "5-HT$_{1\text{-like}}$" Receptors: There is convincing evidence for the existence of 5-HT$_1$ receptor-mediated effect from biochemical, electrophysiological, behavioural and other functional studies. The receptors involved were termed "5-HT$_{1\text{-like}}$" by Bradley et al [26] and are those associated with for example, prejunctional inhibition of neuronal transmitter release, smooth muscle relaxation, and contraction of some cardiac and vascular smooth muscle (see Bradley et al [26]). There is some evidence that "5-HT$_{1\text{-like}}$" receptors are similar to 5-HT$_1$ sites and that both categories consist of the same 5-HT subtypes. However this remains to be determined and for the time being, "5-HT$_{1\text{-like}}$" receptors are included among the 5-HT receptor subtypes.

1.3.2. 5-HT$_{1A}$ and 5-HT$_{1B}$ Receptors: These receptors are the subject of the current study and will be discussed in full in sections 1.4. and 1.5.

1.3.3. 5-HT$_{1C}$ Receptors: 5-HT$_{1C}$ receptors were first discovered by Palacios and his colleagues [30,31] in the choroid plexus. Both receptor binding experiments using [^{3}H] mesulergine **10** and autoradiographic studies have since established that the choroid plexus of rat, pig and man are especially rich in 5-HT$_{1C}$ receptors [31,32]. Significant numbers also exist in other areas of brain, including frontal cortex and hippocampus. Functional rat 5-HT$_{1C}$ receptors have been cloned and expressed in Xenopus oocytes [34,35]. 5-HT$_{1C}$ receptors are a member of the G-protein linked super family [36,37], containing approximately four hundred and fifty amino acids arrayed as seven interconnected

transmembrane segments. 5-HT_{1C} receptors are positively coupled to phospholipase C and elevate inositol phosphates [33].

5-HT_2 receptors have also been cloned recently and a comparison between the protein structures of 5-HT_{1C} and 5-HT_2 receptors shows extensive amino acid homology (141 out of 180 amino acids are identical, 78% homology, see figure 1.3.) [37].

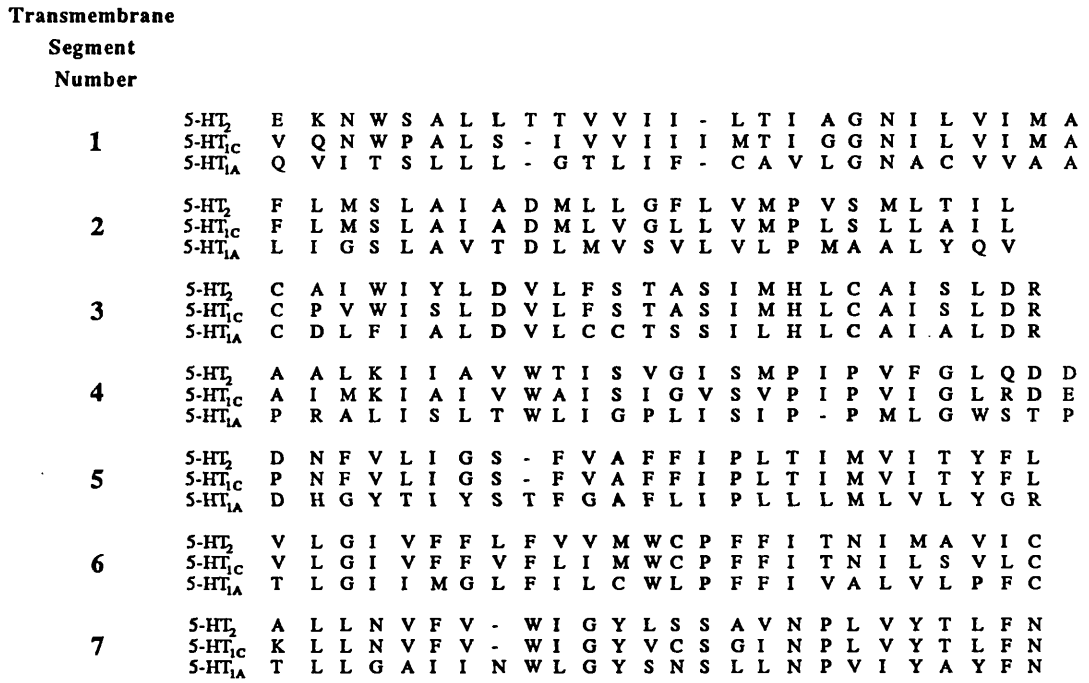

Figure 1.3. Amino acid sequences for the putative transmembrane segments of the 5-HT_{1C}, 5-HT_{1A} and 5-HT_2 receptor colnes.

Since the cloning of these two receptors, proposals have been made to rename 5-HT_{1C} receptors as a subtype of 5-HT_2 receptor. The argument is further fuelled since the 5-HT_{1C} receptor is unrelated in second messenger coupling and antagonists affinities with other 5-HT_1 subtypes [38].

1.3.4. 5-HT_{1D} Receptors: 5-HT_{1D} receptors were first labelled with [^{3}H] 5-HT in bovine brain [26] and later in pig and human brain preparations as well [39]. Recent autoradiographic studies of guinea-pig

brain indicate the predominant presence of 5-HT_{1D} receptors in most brain regions, the rank order of densities being, substantia nigra > globus pallidus > accumbens > caudate-putamen > internal neocortex. These sites are linked to adenylate cyclase [40]. Ligands with selectivity are not yet available at this site but yohimbine **11** and metergoline **12** are potent antagonists [41]. Recently, GR 43175 [100] **13**, a drug developed by Glaxo as an effective anti-migraine treatment has been reported to have preferential affinity for 5-HT_{1D} receptor [42], but no precise data regarding GR 43715 is yet available.

1.3.5. 5-HT_{1P} and 5-HT_{1R} Receptors: These are the most recently described 5-HT_1 receptors and data regarding these receptors is scarce. 5-HT_{1P} sites have been localised by autoradiography in enteric ganglia and mucosal nerve fibres. Among agonists at this site are the hydroxylated indalpines **14** while dipeptides of 5-hydroxy-tryptophan **15** and BRL 24924 **16** are antagonists of responses mediated by this receptor. The 5-HT_{1P} site has been linked to the mediation of slow (excitory post-synaptic potentials) EPSPS, prolonged membrane depolarisation accompanied by a decreased K^+ conductance, in response to the release of 5-HT from stimulated enteric neurons [43].
5-HT_{1R} sites are found in rabbit caudate nucleus (CN) [44]. The pharmacological profile of the 5-HT_{1R} sites is distinct from that of 5-HT_{1A}, 5-HT_{1B} , 5-HT_{1C} and 5-HT_2 receptors but similar to the 5-HT_{1D} site in brain caudate nucleus, however, compounds such as metergoline **12**, and methiothepin **17** show significantly different affinities for 5-HT_{1D} and 5-HT_{1R} sites . A more detailed study of both 5-HT_{1P} and 5-HT_{1R} sites awaits the identification of new and selective ligands.

1.3.6. 5-HT_2 Receptors: 5-HT_2 receptors were originally identified in rat frontal cortex homogenates with the use of

[^{3}H] spiperone [45] **9** and was later further characterised with the more selective ligand [^{3}H] ketanserine [46] **18**. 5-HT$_2$ receptors have been identified in brain tissue from a variety of mammalian species including human with the highest density being present in the frontal parts of the cortex. For a comprehensive discussion on the biochemical studies, ligands and binding affinities, cloning and characterisation, behavioural and other studies of the 5-HT$_2$ receptors, the reader is referred to several excellent reviews [11, 13, 26, 37, 47, 48].

Multiple investigators [49,50] have recently observed that 5-HT$_2$ receptors labelled by [^{3}H] antagonists display complex kinetic patterns in radioligand binding studies and conclude that radioligands such as ^{77}Br-R-(-)DOB **19** and [^{125}I](-)DOI **20** label a distinct subtype of 5-HT$_2$ receptors (i.e. 5-HT$_{2A}$) whereas [^{3}H] ketanserin **18** labels both 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors. It is therefore likely (with the renaming of 5-HT$_{1C}$ as a subtype of 5-HT$_2$ receptors) that three subtypes of 5-HT$_2$ receptors may exist. However, this remains to be determined.

1.3.7. 5-HT$_3$ Receptors: Identification of 5-HT$_3$ (M) receptor was for many years hampered due to the lack of selective antagonists [26] but continuous efforts by investigators [51, 52] led to the development of specific blocking drugs such as MDL 72222 **21** and ICS 205-930 **22**. With the advent of these and other compounds and their use in functional tests, three subtypes of 5-HT$_3$ receptor in the periphery were proposed [29,53]. Kilpatrick et al [54] (1987) then showed the existence of 5-HT$_3$ receptors in mammalian brain by using [^{3}H] GR 65630 **23**, a potent 5-HT$_3$ receptor antagonist. Because of the presence of 5-HT$_3$ receptors in the CNS and their localization in areas such as the entorinal cortex, amygdala and nucleus accumbens it has been suggested that they play a role in controlling behaviour. 5-HT$_3$ receptors are now the subject of

intense research and the reader is referred to several reviews for a more detailed discussion on 5-HT_3 receptors [26, 48, 55-57].

1.3.8. 5-HT_4 Receptors: The existence of 5-HT_4 receptors was recently proposed by Bockaert and colleagues [101]. 5-HT_4 receptors mediate the stimulation of adenylate cyclase activity in mouse embryo colliculi in primary culture. On this receptor, classical 5-HT_1, 5-HT_2 and 5-HT_3 receptor agonists and antagonists, except for some tryptamine derivatives, were inactive. Substituted benzamide derivatives are agonists of this receptor.

1.4. 5-HT_{1A} Receptors:

5-HT_{1A} receptors were first identified by Nelson et al [58] based on the observation of shallow competition curves with drugs such as spiperone (9) versus [^{3}H] 5-HT binding. Subsequently, the 5-HT_{1A} receptors were more fully characterised by Hamon et al [59] using [^{3}H] 8-OH-DPAT **24**. 5-HT_{1A} receptors have now been identified in the CNS of a variety of species, including; mouse, rat, guinea-pig, cow, pig, cat, and human. The pharmacological characteristics of 5-HT_{1A} receptors in the mammalian brain are similar from mice to man. This does not appear to be the case for other subtypes of 5-HT. In the human brain, the anatomical distribution of 5-HT_{1A} receptors is heterogenous. Like the rat, the hippocampal complex is the area most enriched in 5-HT_{1A} binding receptors throughout the human brain. Other areas of the human brain including the cortical areas and raphe nuclei are also rich in 5-HT_{1A} receptors, whereas the density of 5-HT_{1A} receptors range from intermediate to low in the thalamus, hypothalamus, amygdala and basal ganglia.

During the past five years, significant progress has been made in the molecular, pharmacological, biochemical and behavioural analysis of 5-HT_{1A} receptors [44]. The amino acid structure of the 5-HT_{1A} receptor was recently determined (figure 1.3.) [37] and found to closely resemble the β_2-adrenoceptor. The 5-HT_{1A} receptor is a member of G-protein linked family with a molecular weight of 63 KDa. Many potent and selective 5-HT_{1A} agonists such as 8-OH-DPAT **24**, 5-CT **25** and DP-5-CT **26** have now been described but the complex pharmacology mediated by this receptor has been largely hampered by the lack of selective 5-HT_{1A} antagonists. At the biochemical level, the 5-HT_{1A} receptor modulates adenylate cyclase activity. Although some report that 5-HT_{1A} receptors stimulate adenylate cyclase, others have observed an inhibition of forskolin-stimulated cyclase activity with 5-HT_{1A} agonists [44]. To date, the only agents consistently shown to behave as 5-HT_{1A} antagonists are certain β-adrenergic antagonists such as pindolol **27** and propranolol **28**. These agents can antagonise the hypothermic effects, discriminative stimulus effects, microiontophoretic effects and serotonin syndrome (e.g. forepaw treading and tremor in rats) produced by various 5-HT_{1A} agonists including 8-OH-DPAT **24**.

In summary, the 5-HT_{1A} receptors represent the most characterised of all known 5-HT receptor subtypes. It has been proposed that 5-HT_{1A} receptors may be involved in, for example, temperature regulation, sexual activity, appetite control, the mechanism of action of a new class of anxiolytic agents (i.e., second-generation arylpiperazine anxiolytics). However, a better definition of the pharmacology, biochemistry and potential clinical significance of 5-HT_{1A} receptors awaits the identification of new and selective antagonists.

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1.5. 5-HT_{1B} Receptors:

5-HT_1 receptors were subdivided into 5-HT_{1A} and 5-HT_{1B} subtypes in rat brain by means of radioligand binding studies performed with [^{3}H] 5-HT; the site with high affinity for spiperone **9** was termed 5-HT_{1A} whilst the site with low affinity for this compound was designated 5-HT_{1B} [97]. In 1985, Hoyer et al [66] then characterised the 5-HT_{1B} receptor in rat cortex membranes. The radioligand used for this purpose was the potent β-blocker R(-)[^{125}I] iodocyanopindolol {([^{125}I] CYP), **76**}. The experiment was performed in the presence of 30 uM isoprenaline **30** (a non-selective β–adrenoceptor agonist) to suppress binding to β–adrenoceptors. Hoyer and others then investigated the nature of the 5-HT terminal autoreceptor in rat cortex. They showed that the inhibition of electrically evoked [^{3}H] 5-HT release in rat frontal cortex by 5-HT agonists correlated very significantly with 5-HT_{1B} binding. Raiteri and colleagues [98, 99] reached the same conclusion when analysing high K^+ evoked release of [^{3}H] 5-HT from rat hippocampal and cerebellar synaptosomes. Further more, they showed that presynaptic heteroreceptors located on cholinergic terminals in rat hippocampus also belong to the 5-HT_{1B} receptor subtype. In 1986, Wyllie et al [93] showed the existence of 5-HT_{1B} subtypes in mouse urinary bladder, an invaluable assay system, enabling the determination of activity of chemical agents as agonists and antagonists (see section 1.5.1.). The distribution of 5-HT_{1B} receptors within the rat brain suggests that they represent important functional receptors. However, subsequent studies have clearly identified the 5-HT_{1B} site, only in rat, mouse, hamster and opposum brain or cell lines, and not in calf, pig, guinea-pig and human tissues. In vivo, 5-HT_{1B} agonists produce an anorectic effect. Ru 24969 **31**, an agonist with some selectivity for 5-HT_{1B} receptors induces certain

behavioural effects which are quite different from those induced by selective 5-HT_{1A} and other 5-HT agonists suggesting that its action may be mediated via the 5-HT_{1B} receptor.

However, as in the case of 5-HT_{1A} receptors, a better definition of the pharmacology and biochemistry of 5-HT_{1B} receptors awaits the identification of new and selective tools.

1.5.1. Characterisation of 5-HT_{1B} Receptors in Mouse Urinary Bladder

Over the past several years, Wyllie and others [91-93] have demonstrated that superfused mouse bladder responds to electrical stimulation (ES) by twitch contractions. Although 5-HT has no direct effect on this tissue, it potentiates the tissue response (twitch contractions) to ES, in concentrations sometimes as low as ten nanomolar. The effect is only seen with ES (see Figure 1.3.).

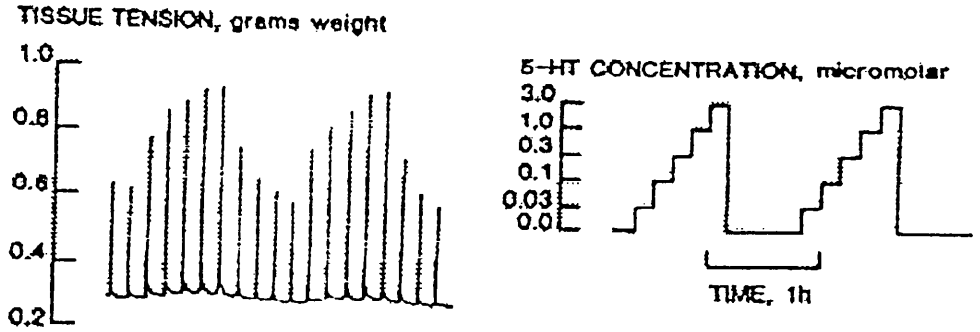

Figure 1.3. Data from Wyllie et al [91]. Contractions of mouse urinary bladder strip in response to trains of 162 ms pulses of 8 V at 10 Hz, delivered every 10 min. After the 2 control responses shown 5-HT was added to the Krebs solution in stepwise increments to concentrations of 0.03, 0.1, 0.3, 1.0 and 3.0 x 10^{-6} M. The concentration was changed immediately after each stimulus train; 5-HT produced a concentration-dependent increase in twitch tension which was reversible and reproducible on washing out and restoring the 5-HT. The EC_{50} for the effect was about 3 x 10^{-8} M. Vertical axis; tissue tension in g weight. Horizontal axis; time.

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Stimulation of tissue by acetylcholine or a stable analogue of ATP **74** stops the potentiating effects of 5-HT, suggesting that 5-HT has a presynaptic action mediated via a specific receptor. The receptor involved was characterised as the 5-HT_{1B} receptor subtype by using a variety of 5-HT agonists and 5HT, adrenergic and other antagonists.

Effects of 5-HT receptor agonists: Three analogues of 5-HT were examined as agonists for their ability to potentiate the response to ES and their relative values were compared. These included; 5-carboxamidotryptamine **25**, α-methyl 5-HT **35** and N-ω-methyl 5-HT **36**. 5-Carboxamidotryptamine a potent but nonselective 5-HT_{1B} agonist was about seven times more potent than 5-HT and evoked the same maximal effect as 5-HT. α-Methyl 5-HT was about five times less potent than 5-HT and N-ω-methyl 5-HT had approximately the same EC_{50} as 5-HT but it could not evoke the same maximal response as 8-OH-DPAT **24**, a selective 5-HT_{1A} agonist was inactive.

Effects of 5-HT receptor antagonists: Several antagonists including spiperone (**9**, 5-HT_1), ketanserine (**18**, 5-HT_2), methysergide (**75**, 5-HT_2), methiothepine (**76**, 5-HT_2) and MDL 72222 (**21**, 5-HT_3), ICS 205-930 (**22**, 5-HT_3), metoclopramide (**77**, 5-HT_3) and others were tested at concentrations as high as one micromolar but none exhibited any marked inhibitory effect except methiothepin **76** which blocked the effect of 5-HT when tested at ten micromolar concentration but its action was not surmountable.

The β–adrenoceptor antagonists, pindolol **27** and propranolol **28**, which are known to block 5-HT_{1A} and 5-HT_{1B} receptors were also tested. Pindolol was the most potent and displayed competitive antagonism

(Figure 1.4.), yielding a pA_2 value of 6.3 and a slope of Schild plot of 1.03.

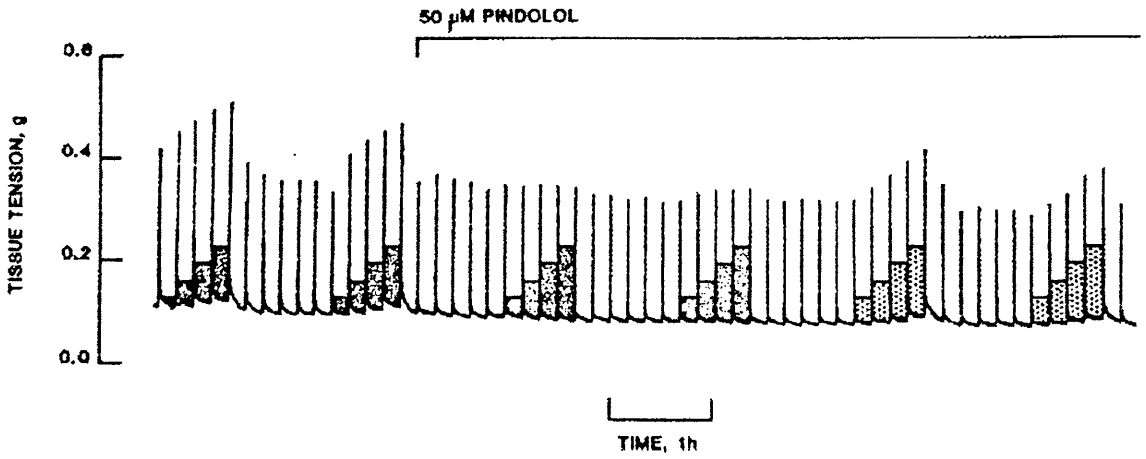

Figure 1.4. Data from Wyllie et al [91]. Effect of pindolol. Contractions of mouse bladder strip are shown as a continuous tracing. Each spike represents the tissue's response to ES (trains of 162 ms pulses of 8 V at 10 Hz) and the addition of 5-HT to the superfusate is indicated by hatched rectangles. The light dots in the first concentration-response curves represent 5-HT concentrations of 0.03, 0.1, 0.3 and 1μM. The heavy dots in the last two concentration-response curves represent concentrations of 3, 10, 30 and 100 μM. Pindolol was added as indicated. Pindolol abolished the response to the lower range of 5-HT concentrations, but augmenting the 5-HT concentration restored the responses. Vertical axis: tissue tension, g. Horizontal axis: time.

Propranolol also interfered with the response but its action was not surmountable by increasing the concentration of 5-HT. Other β–blockers atenolol **78**, metoprolol **79** and phentolamine **80** were essentially inactive.

The above results indicate that the receptor involved in the bladder is not the β, $5\text{-}HT_{1A}$, $5\text{-}HT_2$ or $5\text{-}HT_3$ receptors but strongly resembles the $5\text{-}HT_{1B}$ binding site.

This preparation was used by us to test the synthesised compounds in order to determine their activity as agonists and antagonists at $5\text{-}HT_{1B}$ receptors (see also chapter 6).

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1.6. 5-HT_{1A} and 5-HT_{1B} Receptor Ligands

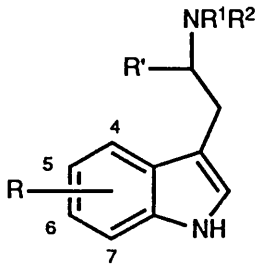
There are several classes of agents which are known to bind with high potencies at 5-HT_{1A} and 5-HT_{1B} receptor subtypes. These include; a) Indolylalkylamines, b) Aryloxypropanolamines, c) Aminotetralins, d) Arylpiperazines and other miscellaneous structures [12, 47, 48]. The work described in this thesis is mainly involved with compounds which are analogues of 5-HT i.e. those compounds in the indolylalkylamine and aryloxypropanolamine series. The following sections are intended to concentrate on the structure-activity relationships for these two series of compounds. Other classes of agents with less relevance to the work will also be briefly mentioned.

1.6.1. SAR for Indolylalkylamines at 5-HT_{1A} and 5-HT_{1B} Receptors

Since 5-HT itself is an indolylalkylamine and definitive for 5-HT_1 receptors, it might have been thought that this structural class would have been well-studied with respect to binding characteristics. Unfortunately, relatively little has been done and only a small number of compounds in this series has been reported, much of which became available relatively recently.

Some selected binding data are shown in table 1.1. The results of these and other recently published data [47, 48, 61], indicate the following trends in structure-activity relationship for indolylalkylamine series.

Table 1.1. Binding Properties of Some Indolylalkylamines

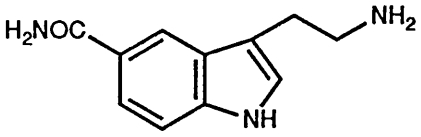


Agent	R^1	R^2	R^4	R^5	R^6	R'	Affinity*, nM 5-HT_{1A}	5-HT_{1B}
Tryptamine (32)	H	H	H	H	H	H	170	10200
4-Hydroxytryptamine (33)	H	H	OH	H	H	H	95	1050
5-Hydroxytryptamine (5-HT, 1)	H	H	H	OH	H	H	3	23
6-Hydroxytryptamine (34)	H	H	H	H	OH	H	1590	5890
α-Me-5-HT (35)	H	H	H	OH	H	Me	85	1000
N-Me-5-HT (36)	H	Me	H	OH	H	H	5	45
Bufotenine (37)	Me	Me	H	OH	H	H	25	910
5-OMe-tryptamine (38)	H	H	H	OMe	H	H	9	400
5-(Aminocarbonyl)tryptamine , 25)	H	H	H	$CONH_2$	H	H	0.2	5
RU 24969 (31)							5	4

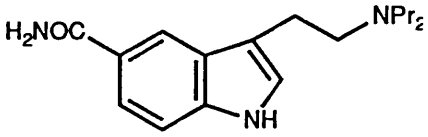
**Data are from Glennon [12], Engel et al [61] and Hoyer et al [27]. Values are approximate and are derived from pK_D values as reported by Engel et al .[61]*

1.6.1.1. Modification of the Terminal Amine: N-Mono-methylation of 5-HT has little effect at both 5-HT_{1A} and 5-HT_{1B} receptors, whereas N,N-dimethylation of 5-HT significantly reduces affinity at the 5-HT_{1B} receptor, indicating that while secondary amines are tolerated at 5-HT_{1A} and 5-HT_{1B} receptors, tertiary amines are only tolerated at the 5-HT_{1A} receptor. This observation is also supported by the fact that while 5-CT 25 binds with nanomolar affinity at both receptors, its congener

DP-5-CT **26** has no affinity for the 5-HT_{1B} receptor. It is also worth mentioning that 8-OH-DPAT **24**, a potent and selective 5-HT_{1A} agonist,



(5-CT, 25)



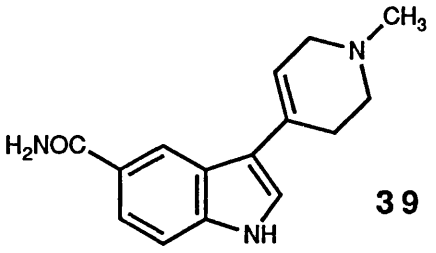
(DP-5CT, 26)

incorporates a tertiary dipropylamino group, a further evidence that tertiary amines are well tolerated at the 5-HT_{1A} receptors.

1.6.1.2. Modification of the Hydroxyl Group: Relocation of the OH group to the 4-position (i.e. 4-hydroxytryptamine) reduces affinity by 30 to 40-fold and even a greater fall in affinity is observed with the relocation of the hydroxyl group to the 6-position (i.e. 6-hydroxytryptamine). Removal of the hydroxyl group decreases 5-HT_{1A} affinity by about 50-fold and 5-HT_{1B} affinity by about 500-fold. Methylation of the hydroxyl group is tolerated at 5-HT_{1A} receptors but less so at 5-HT_{1B} receptors. Replacement of the hydroxyl group of 5-HT by a carboxamido group (i.e. 5-CT) increases affinity by about 10-fold at both receptors, whereas the replacement of the hydroxyl group with an amino group causes a significant loss of affinity. Incorporation of a second OH group into the 6 or 7-position of the indole ring is detrimental at both receptors.

1.6.1.3. Modification of the Side Chain: Introduction of a methyl group at either the α or the β–carbon of the side chain reduces 5-HT_{1A} and 5-HT_{1B} affinity considerably. Replacement of the aminoethyl chain of 5-HT with a cyclic ring such as piperidine (i.e. RU 24969, **31**) has no effect on affinity at either receptors. RU 24969 has been extensively used in the study and the classification of 5-HT receptors and

is the only potent agonist which displays some selectivity for 5-HT_{1B} receptors [62,63]. Various analogues of RU 24969 have now become available and their binding characteristics to 5-HT receptors determined [64,65]. The most potent compound in this series is the carboxamido analogue **39** of RU 24969.



39

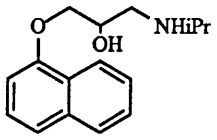
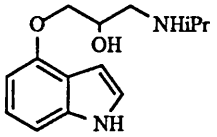
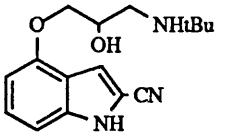
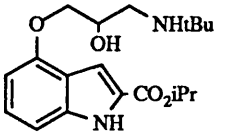
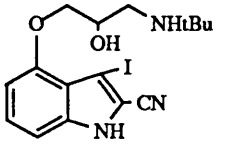
1.6.1.4. Other Structural Modifications: Methylation of 5-HT and RU 24969 at the 2-position of the indole ring markedly decreases potency at all 5-HT_1 receptors, probably by sterically induced deformation of the side chain.

It is also known that Indolylalkylamines display much lower affinities at 5-HT_2 and 5-HT_3 receptors.

1.6.2. Aryloxypropanolamines

As discussed in section 1.4. and 1.5., the only agents consistently shown to behave as 5-HT_{1A} and 5-HT_{1B} antagonists, other than a few nonselective 5-HT antagonists are certain β–adrenergic antagonists (see table 1.2.) commonly known as aryloxypropanolamines. These agents bind with high affinity at 5-HT_{1A} and 5-HT_{1B} receptors but are inactive at other 5-HT subtypes and can therefore serve as lead compounds for the development of new and selective 5-HT_{1A} and 5-HT_{1B} agents.

Table 1.2. Affinities of some β-adrenergic antagonists at 5-HT_{1A} and 5-HT_{1B} sites, (pK_D Values [a], -logmol/l).

Compound	Structure	pK_D values	
		5-HT_{1A}	5-HT_{1B}
(±)Propranolol (28)		6.48	7.07
(-)Pindolol (27)		7.66	7.19
(±)Cyanopindolol (40)		8.27	8.28
(±)SDZ 21009 (41)		7.76	8.53
(±)Iodocyanopindolol (76)		-	9.49

[a] Data are from Engel et al [61] except for iodocynaopindolol (42) binding results which is from Hoyer et al [66].

Propranolol **28** and pindolol **27** can antagonize for example, (i) the hypothermic effects, (ii) the discriminative stimulus effects, (iii) the microiontophoretic effects and (iv) the "serotonin syndrome" produced by various 5-HT_{1A} agonists including 8-OH-DPAT **24**. This antagonism is mediated via a serotonergic mechanism and there is no evidence of adrenergic involvement. Obviously these adrenergic agents are not selective for 5-HT receptors since they possess an even greater affinity for the β–adrenergic sites. Furthermore, these agents bind at 5-HT_{1A} and 5-HT_{1B} receptors with nearly comparable affinity. Nevertheless, they represent one of the few structural leads that may be exploited for the

development of 5-HT_{1A} selective antagonists and 5-HT_{1B} selective agents. In fact, very recently, Glennon et al [67] determined the binding affinities of a series of propranolol analogues in an attempt to develop such agents (discussed in section 1.6.2.2.).

1.6.2.1. SAR for Aryloxypropanolamines at 5-HT_{1A} and 5-HT_{1B} Receptors

To date, the only agents in the aryloxypropanolamine series which are known to possess potent serotonergic activity are listed in table 1.2. These agents are optically active and stereochemistry is important with regards to serotonergic affinity, S-(–) isomers being more potent than R-(+) enantiomers [68]. Pindolol **27** is also somewhat more potent than propranolol at the 5-HT_{1A} receptor, whereas they bind with equipotencies at the 5-HT_{1B} receptor [39]. These data suggest that indole is the preferred aromatic ring at 5-HT_{1A} site, while naphthalene as the aromatic ring is tolerated at both receptors. Cyanopindolol **40** and SDZ 21009 **41** are significantly more potent than pindolol at the 5-HT_{1B} receptor, indicating that the presence of electron-withdrawing groups such a cyano or an ester group in the 2-position of the indole ring of pindolol greatly increases 5-HT_{1B} affinity. However, these groups only slightly enhance 5-HT_{1A} affinity. Finally, the introduction of iodine into the 3-position of the indole ring as in Iodocyanopindolol **76** enhances 5-HT_{1B} affinity.

1.6.2.2. Analogues of Propranolol as 5-HT_{1A} and 5-HT_{1B} Agents

The β–adrenergic antagonist propranolol **28** binds stereoselectively at both 5-HT_{1A} and 5-HT_{1B} receptors (with several fold selectivity for the latter), and as such very recently, its structure was used by multiple investigators [67] for the development of new 5-HT_{1A} and 5-HT_{1B} agents. These analogues were tested at 5-HT_{1A} , 5-HT_{1B} and β–adrenergic receptors and the results are given in table 1.3.

Table 1.3. Affinities of propranolol analogues for 5-HT_{1A} , 5-HT_{1B} and β-adrenergic sites.

$ArX(CH_2)_nNRR'$

Compound No.	X	Ar[b]	n	R	R'	K_i Values[a], nM: 5-HT_{1A}	5-HT_{1B}	β-Adrenergic
42	CH_2	Ph	3	Me	Me	5000 (750)	>10000	-
43	NH	Ph	3	Me	Me	>25000	>10000	-
44	O	Ph	3	Me	Me	4850 (500)	>10000	-
44	O	Ph	3	Me	H	3300 (125)	3100 (250)	-
45	O	2-OMePh	3	Me	Me	3000 (350)	>10000	-
46	O	2-OHPh	3	Me	Me	4900 (400)	>10000	-
47	O	Ph	3	nPr	nPr	2750 (250)	>10000	-
48	O	NaPh	3	Me	Me	345 (20)	1000 (80)	2400 (235)
49	O	NaPh	3[c]	nPr	nPr	450 (20)	6100 (450)	-
50	O	NaPh	3	nPr	nPr	1325 (200)	8000 (700)	-
51	O	NaPh	4	Me	Me	400 (25)	1000 (125)	-
52	O	NaPh	2	Me	Me	80 (5)	1500 (200)	4500 (950)
53	O	NaPh	3	nBu	nBu	225 (25)	5650 (550)	8300 (850)
54	O	NaPh	2	nBu	nBu	150 (20)	9000 (900)	>10000
55	O	NaPh	2	Me	nPr	45 (10)	1800 (70)	5300 (980)
56	O	NaPh	2	Me	Bn	95 (5)	-	-
57	O	NaPh	2	Et	nBu	39 (2)	1100 (80)	5000 (300)
58	O	NaPh	2	H	H	60 (5)	-	-
59		NaPh	1	Me	Me	3420 (200)	-	-
60	C=O	NaPh	2	Et	nBu	3530 (390)	-	-
61	CH_2	NaPh	2	Et	nBu	300 (300)	-	-
62	CH_2	NaPh[d]	2	Et	nBu	540 (55)	-	-
(±) propranolol						90 (15)	50 (15)	2.4 (0.1)
(-) propranolol						55 (5)	17 (4)	-
(+) propranolol						1700 (120)	-	-

[a] Data are from Glennon et al [67b]. Ph = Phenyl, Naph = 1-naphthyl.[c] Hydroxylated chain, i.e. -$CH_2CH(OH)CH_2$-. [d] 1,2,3,4-Tetrahydronaphthyl.

The first series of compounds **42-44** demonstrate the importance of a phenolic oxygen atom for 5-HT_{1A} binding. It appears that methylene analogue **42** offers no advantage over the oxygen analogue **44** and that

the nitrogen analogue **43** is essentially inactive. However, unlike propranolol, these agents display some selectivity for 5-HT_{1A} receptors, probably due to being tertiary amines (which is known to be detrimental to 5-HT_{1B} activity [69]). The presence of a 2-methoxy or 2-hydroxy group has essentially no effect on affinity (i.e. **45** and **46**) whereas replacement of the dimethylamino groups of **44** with a di-n-propylamino group (i.e. **47**) appears to double affinity. The naphthyloxy derivatives **48**, **49** display a 10-fold greater affinity for 5-HT_{1A} receptors than their phenoxy counterparts **44**, **46**; however, there is no concomitant increase in affinity at 5-HT_{1B} receptors. Removal of the side chain hydroxyl group (i.e. compare **49** & **50**) increases 5-HT_{1A} potency by about 3-fold, and has no effect on 5-HT_{1B} affinity. The analogue **51** with a four-carbon chain separating the naphthyloxy group from the terminal amine is essentially equipotent with 48 at 5-HT_{1A} and 5-HT_{1B} receptors, whereas in comparison, the two carbon chain analogue 53, displays a 4-fold rise in affinity at 5-HT_{1A} receptors with no apparent increase at 5-HT_{1B} receptors.

Examination of **53** & **54** reveals that introduction of a di-n-butylamino group results in a decreased affinity for 5-HT_{1B} and β–adrenergic receptors but has little effect on affinity at 5-HT_{1A} receptors. Compound **54** also displays a 100-fold selectivity for 5-HT_{1A} receptors relative to 5-HT_{1B} and β–adrenergic sites. However, **55** and **57** seem to offer a compromise; whereas they possess a reasonable affinity for 5-HT_{1A} receptors (k_i = 45 and 39 nM, respectively) and a greater than a 100-fold selectivity for 5-HT_{1A} receptors relative to β–adrenergic sites, their selectivity for 5-HT_{1A} versus 5-HT_{1B} receptors is only 30 to 40-fold. Nevertheless, these agents possess a higher affinity / selectivity for 5-HT_{1A} receptors than does the lead compound propranolol. Other

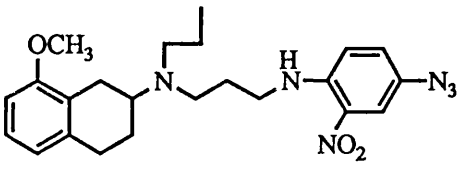
modifications of the terminal amine and the side chain (e.g. **56-58** and **59, 60** respectively) have no effect on affinity for 5-HT_{1A} receptors. Finally, the 1,2,3,4-tetrahydroderivative **62**, displays over one-tenth of the affinity of **57**.

These authors therefore have identified several key structural features which enhance affinity for 5-HT_{1A} receptors and selectivity for 5-HT_{1A} versus 5-HT_{1B} and β–adrenergic sites. However, because this was reported only recently, only some use of their findings was possible for the present study. This will be further discussed in chapter 5.

1.6.2.3. Miscellaneous Compounds with 5-HT_{1A} and 5-HT_{1B} Activity

There is a wide variety of ligand structures which bind with high affinity at 5-HT_{1A} and 5-HT_{1B} subtypes [12, 47, 48]. Most of these agents possess agonistic or as yet undetermined activity at the 5-HT_{1A} receptor. By far the most important compounds are in the aminotetraline and arylpiperazine series.

Aminotetralines: The most potent and selective member of this group is 8-OH-DPAT **24** which is a prototypic 5-HT_{1A} agonist. This series has been well studied and SAR knowledge has been summarized [47, 77, 83]. 8-OH-DPAT displays a 1000-fold selectivity for 5-HT_{1A} receptors relative to other 5-HT subtypes, and in tritiated form, is a useful ligand for binding studies. This agent has been used as a template to construct a photoaffinity label for 5-HT_{1A} receptors.



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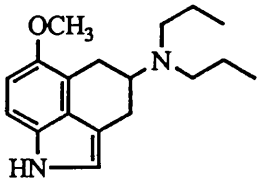

64

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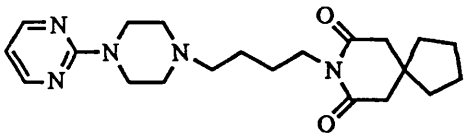
Another label **63** binds with high affinity (IC_{50} = 6.6 nM v's 8-OH-DPAT) to 5-HT_{1A} receptors in rat hippocampus, and has much lower affinity for 5-HT_{1B} receptors. After irradiation, the compound irreversibly blocks 55-60% of 5-HT_{1A} receptors, and the receptor appears to be a 63 KD protein with functionally important sulfhydryl groups [70-72]. Bay R 1531 **64**, an indole analogue of 8-OH-DPAT is an agonist with high affinity for 5-HT_{1A} receptors and displays antidepressant activity as well as stimulating sexual behaviour in castrated male rats [48].

Aryl and pyrimidinyl piperazines: This series of compounds has been investigated for a number of years. Buspirone **65**, a clinically effective anxiolytic drug, is an example of an aryl piperazine derivative with affinity and selectivity for 5-HT_{1A} receptors.



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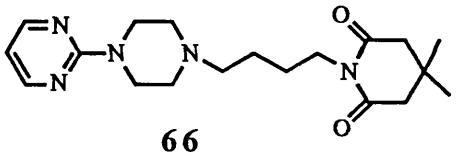

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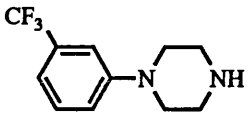
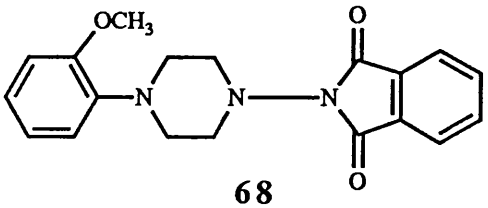


67

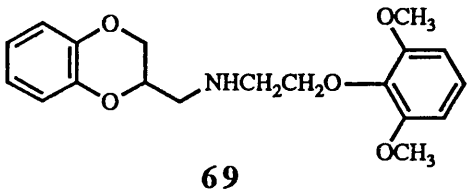
Buspirone has moderate effects on dopamine and α1-receptors, but negligible activity on α_2-, β–, muscarinic, 5-HT_2 receptors [73]. It has been suggested that the anxiolytic properties of buspirone arise through an interaction at 5-HT_{1A} receptors [74-76]. Many other analogues of buspirone have been prepared and structure activity studies have been reported [78-80, 82]. Gepirone **66**, an analogue of buspirone has recently been shown to have selective 5-HT_{1A} affinity, whereas, 1-[3-(trifluoromethyl) phenyl]-piperazine **67** has greater affinity for the 5-HT_{1B} receptors [47, 81].



68

Recently, another example of this series NAN-190 **68** was reported to bind with high potency (k_i = 0.6 nM) to 5-HT_{1A} receptors [82].

Benzodioxanes: ^{3}H-WB4101 **69**, a potent α_1 antagonist, selectively labels 5-HT_{1A} receptors when prazosin **70** is used to obliterate α_1 binding [85].



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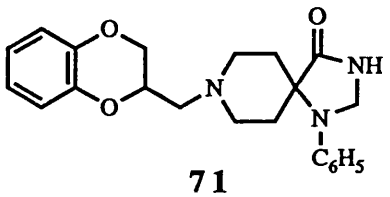
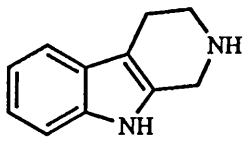


71

^{3}H-Spiroxatrine [86] **71** also labels the 5-HT_{1A} receptors, and in the canine basilar artery it is reported to be a 5-HT_{1A} antagonist [87, 88].

Miscellaneous structures: Tetrahydro-β–carboline **72** produces stimulus effects in rats by activating 5-HT_{1B} receptors [89].



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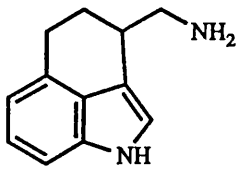

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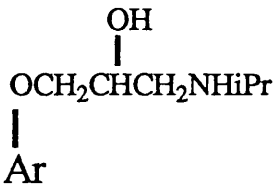
Compound **72** has a lower affinity for 5-HT_1 and 5-HT_2 binding sites than 5-HT itself. It may be that 5-HT binds to these receptors in a conformation that is different from that of tetrahydro-β–carboline. The conformationally restricted analogue of 5-HT **73**, has relatively low affinity for 5-HT receptors [90]. However, these agents do not incorporate

the hydroxyl group of 5-HT and only with such analogues is a better understanding of a favourable side chain conformation possible.

1.7. SAR for Aryloxypropanolamines at β-Adrenoceptors

There is a considerable body of classical structure-activity correlation studies for adrenergic agonists and antagonists in a variety of preparations [94, 95]. Of interest to this work are those structural features of aryloxypropanolamines which are fundamental for β–adrenergic activity, since manipulation of these structural features which are essential for β–adrenergic activity may not affect the serotonergic activity of aryloxypropanolamines.



Aryloxypropranolamine

Functional features which are very important to β–blockade have been well documented [82, 96] and are summarized as follows:

i) The catechol ring system can be replaced by a great variety of other ring systems, varying from phenylether (oxoprenolol, **81**) and sulphonamides (sotalol, **82**) to phenylamides (labetolol, **83**), indoles (pindolol, **27**) and naphthalene (propranolol, **28**).

ii) A terminal secondary amine. Primary and tertiary amines dramatically decrease affinity.

ii) N-substituents must be bulky to insure affinity to the β–receptors; isopropyl is the smallest effective substituent and smaller group such as methyl cause considerable loss of affinity.

iv) The side chain hydroxyl group is essential to the activity. Removal, alkylation or replacement (e.g. with halogens) will all lead to a substantial fall in affinity.

v) A four-atom chain separating the aromatic system from a terminal amine. Shortening or increasing the chain length leads to a fall in activity.

vi) Introduction of groups between the the amine and the carbinol group reduces potency.

vii) The phenolic oxygen also appears to be important for activity. Replacement with SCH_2, CH_2, C=O or NH diminishes β–affinity.

viii) Stereoconfiguration of the β–carbon hydroxyl is a major determinant of affinity hence, while the (S)(-) isomers are active, their respective enantiomers are devoid of any significant β–blocking activity.

1.8. Aims and Objectives

The neurotransmitter serotonin and its receptors have been intensively studied over the past several years. Nevertheless, there is still a paucity of selective agents particularly antagonists, at 5-HT_{1A} and 5-HT_{1B} receptors. The distribution of 5-HT_{1A} and 5-HT_{1B} receptors within the rat brain and the recent identification of 5-HT_{1A} receptors in human brain suggests that they represent important functional receptors (discussed in sections 1.4. and 1.5.) and therefore the synthesis of potent and selective ligands at these receptors may be of considerable significance. The present work has been concerned with the synthesis of such agents at 5-HT_{1A} and 5-HT_{1B} receptors.

Aryloxypropanolamines (discussed in sections 1.6.2. and 1.7.) are potent antagonists of 5-HT_{1A} and 5-HT_{1B} receptors. Structure-activity correlation studies for this series of agents in the relevant parts of the adrenergic and serotonergic fields were discussed in sections 1.6.2. and 1.7. The objective of the present work was to utilise these data in order to modify the structures of these agents in such a manner so as to reduce their β-adrenergic affinity while, at the same time retaining their affinity at 5-HT_{1A} or 5-HT_{1B} receptors. It was also our aim to introduce functional features which may provide selectivity between 5-HT_{1A} and 5-HT_{1B} receptors. The synthesised compounds were to be tested in both binding and functional assays by Professor J.H. Wyllie and Dr. M. Beer (see chapter 6 for details).

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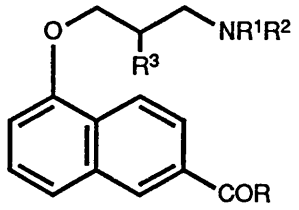
CHAPTER TWO

SYNTHESIS OF PROPRANOLOL ANALOGUES

2.1. Introduction



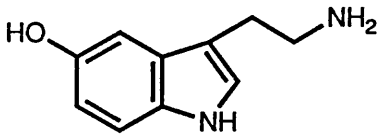
This chapter describes the strategy, synthesis and structure-activity correlation studies for some novel 1,6-disubstituted naphthalenes (shown above) analogous to propranolol. The strategy for designing this series of compounds is presented in section 2.2. The major part of this chapter describes the preparation of novel 1,6-disubstituted naphthalenes. The aim of this part of the work was to design selective 5-HT_{1B} antagonists. The analogues prepared were found to be weak antagonists at the 5-HT_{1B} receptor. These compounds were significantly less active than the lead compound propranolol, mainly due to the introduction of electron withdrawing substituents into the 6-position of the naphthalene ring. The final part of the chapter deals with the structure-activity relationship for these analogues.

2.2. Strategy

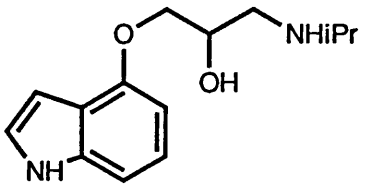
The β-adrenergic antagonist propranolol **28** binds stereoselectively (discussed in sections 1.6.2. & 1.6.3.) both at 5-HT_{1A} and 5-HT_{1B} receptors. However, propranolol is somewhat more selective for 5-HT_{1B} receptors and as such its structure was used as a template for the synthesis of novel potential 5-HT_{1B} agents. We synthesised a range of compounds of the general structure shown above, in which electron withdrawing groups such as esters or amides were incorporated into the

6-position of the naphthalene ring of propranolol. The design of this series of compounds was based on the following observations;

Choice of the Naphthalene Ring: A casual inspection of the structures of 5-HT and pindolol reveals that both compounds share an indole ring and an amino alkyl chain.

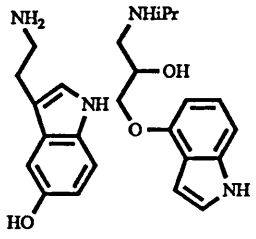


5-HT, 1

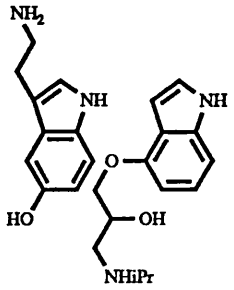


pindolol, 27

Since these agents have good affinities for 5-HT_1 sites, it is possible that they bind to a common site of the receptor. However, 5-HT and pindolol incorporate the amino alkyl chain in different positions of the indole ring, and therefore the question arises as to whether it is the amino alkyl chain or the indole ring of these agents that bind with the active site of the receptor.



type 1 binding



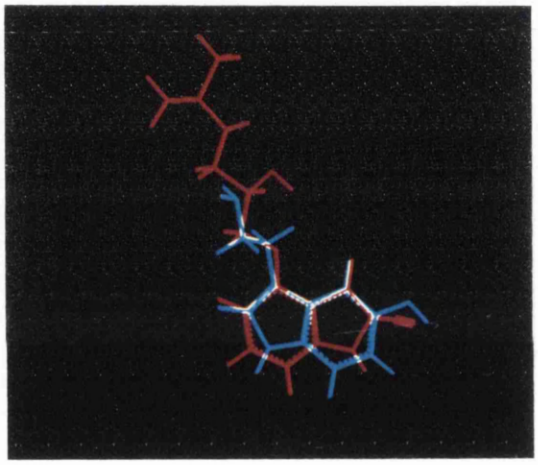
type 2 binding

Figure 2.1. Binding Modes of Interaction of 5-HT and Pindolol

If the aminopropoxy chain of pindolol and the aminoethyl chain of 5-HT bind with the same active site of the receptor (i.e. type 1 binding), it follows that the indole rings must bind differently and that the pyrrole

portion of the indole ring is not essential for activity and can be replaced by other rings such as benzene. However, if the indole rings of 5-HT and pindolol bind with the active site of the receptor (type 2), it follows that the amino alkyl chains of these agents can no longer interact with the same site of the receptor, since conformationally this would be impossible.

Of the two possible situations, the former represents a more likely mode of binding action for two reasons; firstly, cyanopindolol binds with similar affinity to that of 5-HT, so it is possible that the cyano group in the



Superimposition of 5-HT & Cyanopindolol

2-position of the indole ring (see above) mimics the action of the hydroxyl group of 5-HT and if so, the indole rings of these agents must bind differently to the receptor. Secondly, by analogy with the adrenergic system, it is well-known [1-3] that the amino alkoxy chain of β-adrenergic agents such as phenylethanolamines **84** and aryloxypropanolamines share a common active site of the receptor as the amino alkoxy chain portion of adrenaline **85**.

A further evidence for the likelihood of type 1 binding is that replacement of the indole ring of pindolol with a naphthalene ring as in propranolol is well tolerated at both 5-HT_{1A} and 5-HT_{1B} sites (see table 1.2.).

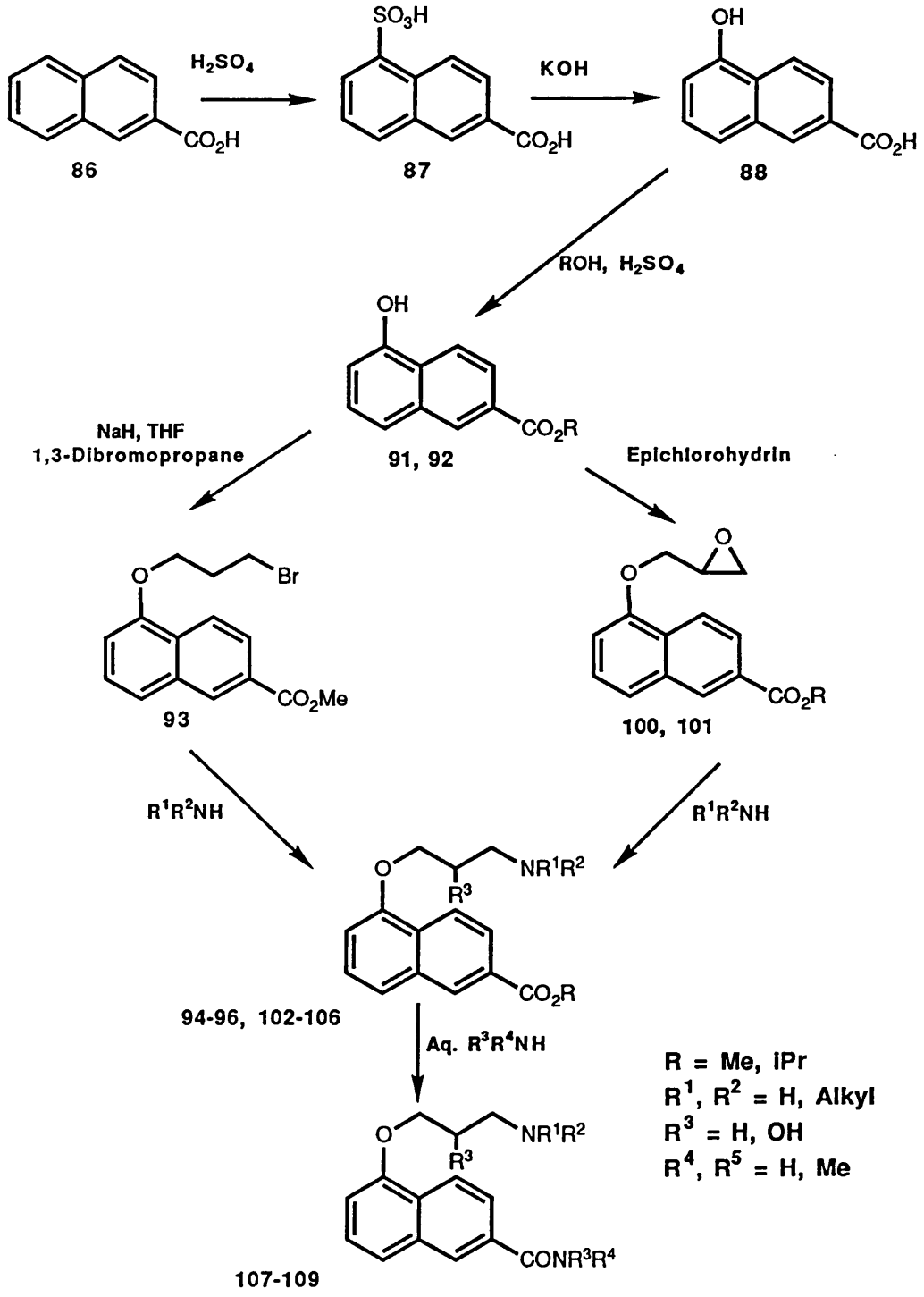
2-position of the indole ring of pindolol [see table 1.2., e.g. cyanopindolol **40** and SDZ 21009 **41**] significantly enhance affinity at the 5-HT_{1B} sites (see sections 1.6.3.). The indole-2-position of pindolol may be considered equivalent to either the 6 or 7-position of the naphthalene ring of propranolol. However, the synthesis of the 1,6-disubstituted naphthalene ring appeared to be easier, hence corresponding derivatives of propranolol with substituents in the 6-position of the naphthalene ring were synthesised to increase 5-HT_{1B} affinity.

Modification of the aminopropoxy chain: Structural features that are detrimental to β-adrenergic activity were discussed in section 1.8. Two features which are essential for β-blockade are a terminal secondary amine with a bulky substituent and a hydroxyl group on the side chain. It was therefore of interest to modify these functionalities in order to determine the importance of these groups with respect to 5-HT_{1A} and 5-HT_{1B} binding.

Following these observations, a series of 1,6-disubstituted naphthalenes was synthesised to provide structure-activity studies which would delineate the chemical requirements for specific interactions at 5-HT_{1B} receptors.

2.3. Synthesis of 1,6-Disubstituted Naphthalene

The overall synthetic routes to the 1,6-disubstituted naphthalenes is presented in scheme 2.1.



Scheme 2.1. Synthetic Pathway to 1,6-Disubstituted Naphthalenes

References, Page 102, Structures & Abbreviations, Page 205

The synthetic procedure for the preparation of propranolol and pindolol and some of their analogues has been extensively reported [4-11]. The starting material for the preparation of these analogues is 1-naphthol and 4-hydroxyindole respectively.

The key intermediate for the synthesis of 1,6-disubstituted naphthalenes is 5-hydroxy-2-naphthoic acid **88**. The procedure for the preparation of the acid was described by Butler and Royle [12, 13] in 1923. These authors, from the sulphonation product of 2-naphthoic acid **86** at ambient temperatures, were able to obtain pure 5-hydroxy-2-naphthoic acid **86**. Although this method has several drawbacks, (discussed in section 2.3.1.) one found that slight alteration of the reaction conditions gave an effective method for preparing the acid. Acid-catalysed esterification of 5-hydroxy-2-naphthoic acid in the appropriate alcohol gave the corresponding esters **91,92**.

Introduction of the 2-hydroxy-3-aminopropoxy chain was achieved by employing similar procedures to those described for pindolol and propranolol. The general method for the introduction of the chain is to generate the phenoxide ion with a metal base, followed by addition of epichlorohydrin. However, by employing a more convenient method, recently described [11], one was able to obtain the desired epoxides **100, 101** in good yields. The reaction of the epoxide intermediates with amines then gave the corresponding target analogues **102-106**.

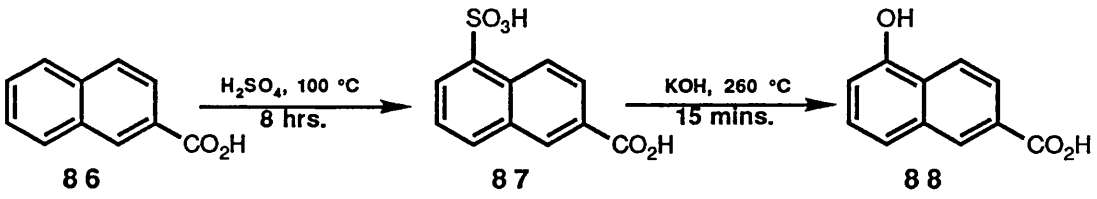
The analogues without the side chain hydroxyl group were prepared by reacting 5-hydroxy-2-naphthalene carboxylic esters **91, 92** in anhydrous conditions, with a large excess of 1,3-dibromo-propane in the presence of a metal base. Reaction of bromopropyl naphthalene intermediate **93**

with various amines in alcohol at refluxing temperatures yielded the desired analogues **94-96**.

The final step of the reaction was to synthesise a series of amides from the corresponding methyl ester **106**. The amides **107-109** were conveniently prepared in excellent yields by stirring the aqueous solution of the methyl ester and the corresponding amine in acidic conditions for several days at room temperature.

2.3.1. Preparation of 5-Hydroxy-2-naphthoic acid

The preparation of 5-hydroxy-2-naphthoic acid **88** was first described by Butler and Royle [12, 13] in 1923 as shown in scheme 2.2.



Scheme 2.2. Preparation of 5-Hydroxy-2-naphthoic Acid.

In their sulphonation of 2-naphthoic acid **86** with 98% sulphuric acid at 100 °C for 8 hours, they obtained 5-sulphonic-2-naphthoic acid **87** together with small amounts of 7- and 8-sulphonic-2-naphthoic acids. The isomers were separated by fractional recrystallisation of the acid barium salts. The fractions with similar crystalline appearances were combined and then converted to the corresponding hydroxy-2-naphthoic acid. Although, Butler and Royle successfully obtained pure hydroxynaphthoic acids, their method was extremely lengthy and laborious.

The method of Butler and Royle for the sulphonation step was repeated and successfully modified to yield pure 5-hydroxy-2-naphthoic acid. This was achieved by converting the isomeric mixture of sulphonic-2-naphthoic acids to their corresponding di-potassium salts (i.e. $SO_3^-K^+$-naphth-$CO_2^-K^+$). The mixture was dissolved in minimum quantities of hot anhydrous methanol and then allowed to stand at room temperature for 24 hours or until a white solid had deposited. After removal of the precipitate, the solution was concentrated and further fractions were obtained in this way. Each fraction obtained was

examined by ^1H N.M.R. in D_2O. Surprisingly, it was discovered that the majority of the fractions had similar ^1H N.M.R. spectra which were approximately first order. However, the ^1H N.M.R. spectra of various fractions also contained other signals arising from impurities or/and other minor isomers. The amount of impurities in various fractions ranged from 5-20%, as estimated from their ^1H N.M.R. spectra.

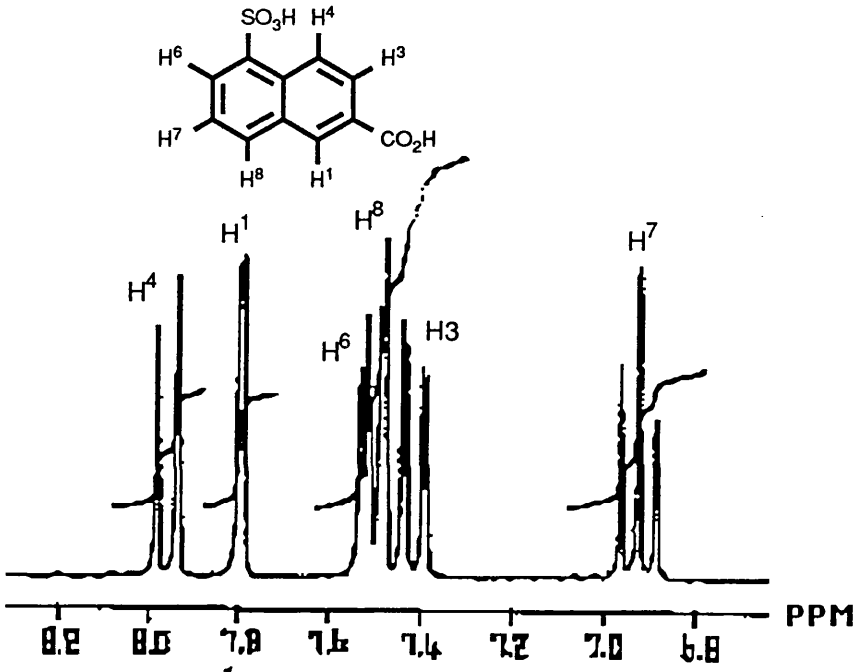


Figure 2.2 200 MHz ^1H N.M.R. of the di-potassium salt of 5-sulphonic-2-naphthoic acid (aromatic region).

The fractions with similar ^1H N.M.R. spectra were combined and further purified by recrystallisation from hot anhydrous methanol (may require more than one recrystallisation). The ^1H N.M.R. spectrum of the recrystallised product was recorded and found to be free of impurities (figure 2.2).

The potassium salt of 5-sulphonic-2-naphthoic acid **87a** was then converted to 5-hydroxy-2-naphthoic acid by an anion exchange reaction with molten potassium hydroxide. The reaction was carried out in a

stainless steel vessel for safety reasons. This procedure yielded pure 5-hydroxy-2-naphthoic acid in excellent yield.

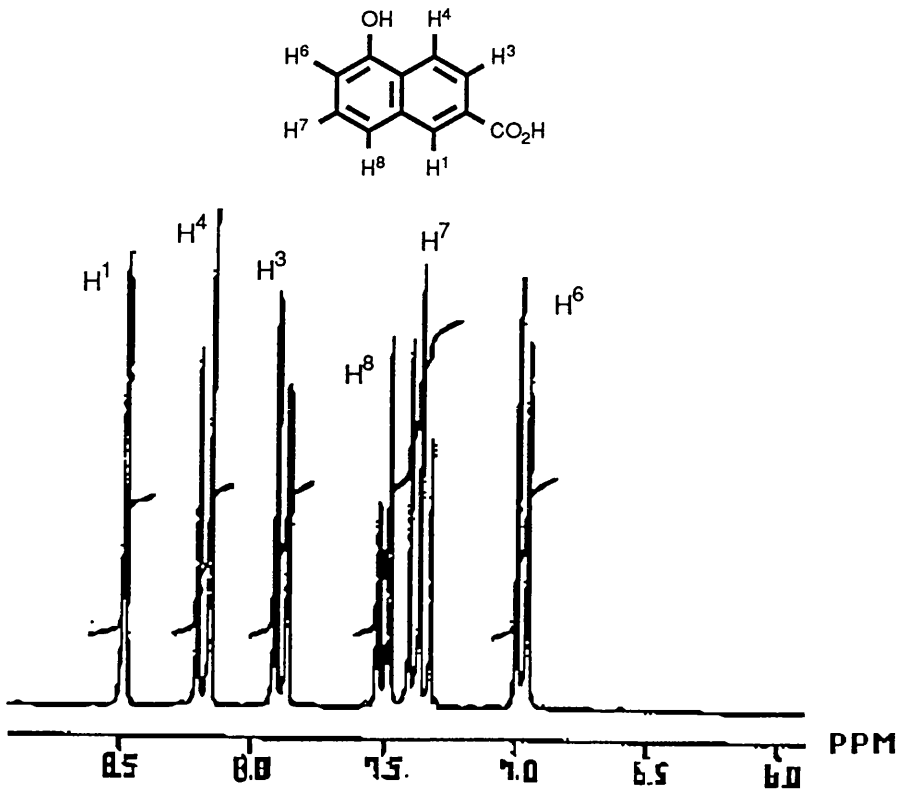


Figure 2.3. 200 MHz ^1H N.M.R. of 5-Hydroxy-2-naphthoic acid (Aromatic Region).

Examination of the product by ^1H N.M.R. revealed a first order spectrum. This is attributed to the opposite electronic nature of the two substituents in the rings. On the one hand, the electron donating hydroxyl group shielding the protons in one ring, while the protons in the adjacent ring are deshielded by the strong electron withdrawing carboxyl group. The ^1H N.M.R. spectrum exhibits one singlet, four doublets and one triplet which is consistent with the structure of the required product. The signals in the spectrum were easily assigned to the various naphthalene protons on the basis of their chemical shifts, multiplicity and coupling constants. The singlet at ca. δ 8.40-8.50 arises from proton H^1 which shows small long range coupling with H^8. H^1 is also involved in peri-interaction with

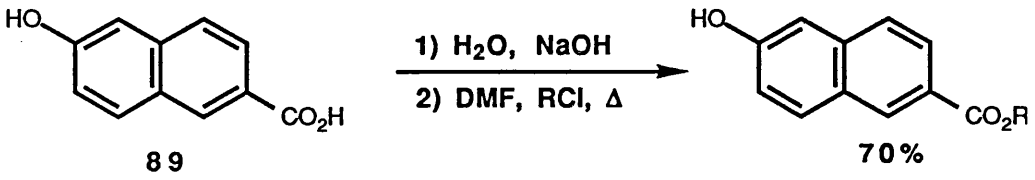
H^8, causing further deshielding of both protons. The AB quartet at ca. δ 7.80-8.25 is due to the protons H^3 and H^4, with H^3 showing further long range coupling with H^1. The pseudo-doublet at ca. δ 7.50 arises from H^8 coupling to H^7 at ca. d 7.35-7.45, itself giving rise to a triplet. Finally, the doublet at ca. δ 7.00 is assigned to H^6 showing long range coupling with H^8.

The preparation of 5-hydroxy-2-naphthoic acid as described by Butler and Royle involves a lengthy and laborious procedure. Due to the difficulties and poor yield, many have attempted to synthesize the acid using other procedures, but failed to obtain a more satisfactory one [14-16]. By slight modification of the reported reaction conditions and with the use of ^1H N.M.R. spectroscopy, it has been possible to obtain a convenient procedure for the preparation of 5-hydroxy-2-naphthoic acid.

References, Page 102, Structures & Abbreviations, Page 205

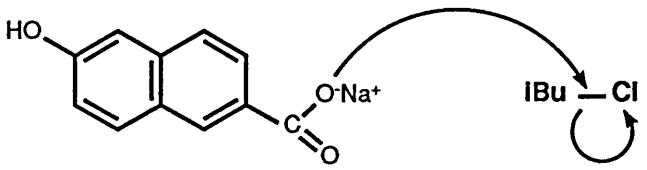
2.3.2. Esterification of 5-Hydroxy-2-naphthoic acid

5-Hydroxy-2-naphthoic acid was reacted with large excess amounts of alcohols in the presence of catalytic amounts of sulphuric acid to form the corresponding esters in good yields. However, at the start of this work, reports describing a high yield procedure for the esterification of 6-hydroxy-2-naphthoic acid **89** appeared in chemical abstracts [17] (scheme 2.3).



Scheme 2.3. Reported Esterification of 6-Hydroxy-2-naphthoic acid

Conversion of **89** to its sodium salt, followed by the addition of an alkyl chloride (e.g. isobutyl chloride) was claimed to give the corresponding ester in 70% yield. The ester is probably formed via a nucleophilic displacement reaction.

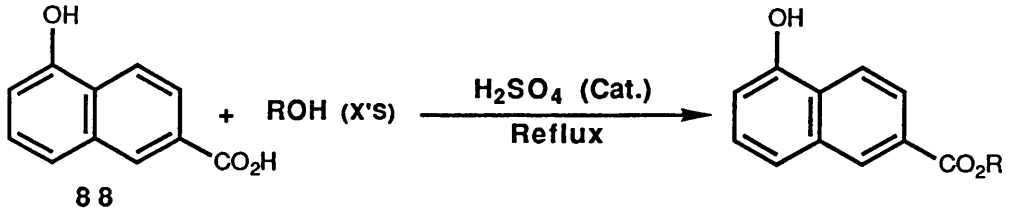


Following the above procedure, the esterification of 5-hydroxy-2-naphthoic acid **88** with isopropyl chloride was carried out but only a poor yield (25%) of the corresponding ispropyl ester **91** was obtained. The yield was somewhat improved by changing the partitioning solvent and replacing the isopropyl chloride with isopropyl bromide or isopropyl iodide (table 2.1). However, these methods gave unsatisfactory yields of the ester, probably due to a greater hindrance of the isopropyl group.

Reaction Condition	% yield of isopropyl ester
1) H_2O, NaOH 2) DMF, IPrCl, ∇	25
1) H_2O, KOH 2) Dioxane, IPrBr, ∇	50
1) H_2O, KOH 2) IPrOH, IPrI, ∇	55

Table 2.1. Esterification of 5-Hydroxy-2-naphthoic Acid

In view of these findings, it was decided to esterify **88** using acid-catalysed conditions [18].



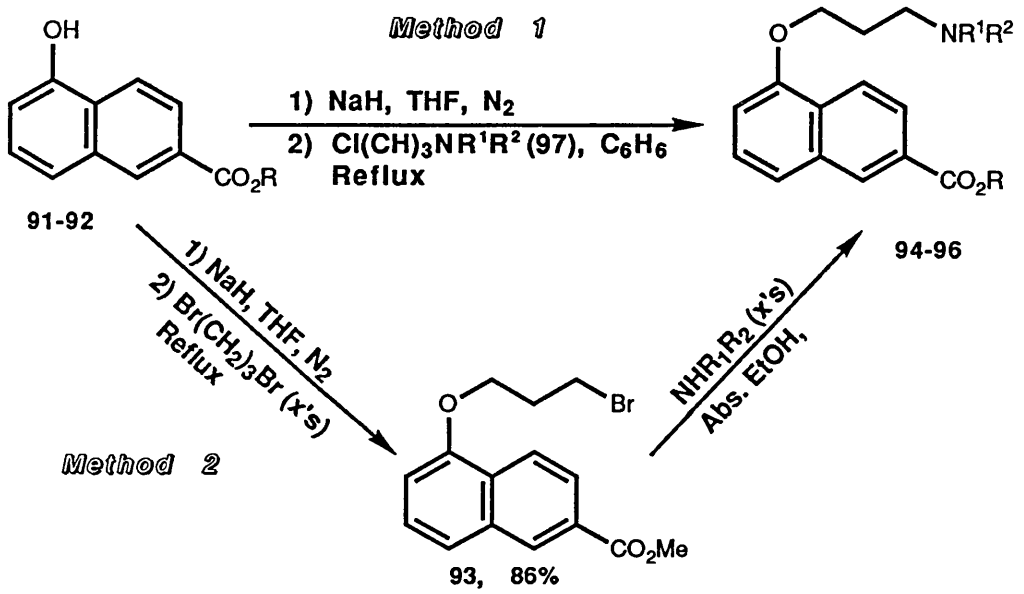
88

R = Me (90) 88%
R = IPr (91) 72%

The corresponding methyl and isopropyl esters **91, 92** were prepared in good yields by refluxing 88 in one hundred molar equivalents of the respective alcohol containing catalytic amounts of sulphuric acid. It was also possible to make full recovery of the unreacted acid by simple extraction procedures.

2.3.3. Preparation of 1-[3-(Alkylamino)propoxy]-6-(carboalkoxy)-naphthalenes

Analogues **94-96** were prepared from the condensation reactions of the corresponding esters **91-92** with halogenopropylamines **97**, via two different synthetic routes, depending on the availability of the halogenoalkylamines.



Scheme 2.4. Synthetic Route to Analogues 94-96.

The anions of **91, 92** was generated [19] with sodium hydride in anhydrous conditions, followed by gradual addition of the chloropropylamine (**97**, free base [20]) to afford **94** in 64% yield.

In the cases where the halogenopropylamines were not commercially available, it was envisaged that a nucleophilic attack by an amine on the bromopropyl intermediate **93** would yield the target analogues **95, 96**. The intermediate **93** was easily prepared in 86% yield by reacting the anion of **91** with twenty molar excess of 1,3-dibromopropane, in the presence of a base. The excess 1,3-dibromopropane was required to

overwhelm the relative concentration of **93**, hence minimising the potential formation of **93a**.

Scheme 2.5.

The nucleophilic displacement reaction of isopropylamine in absolute ethanol with **93** gave the hydrobromide salt of **95**. The product was purified by column chromatography, using 1% ammonia in a mixture of chloroform : methanol : pet. ether (1:4:5) as the solvent system. Addition of ammonia to the solvent system was necessary in order to reduce the tailing effect and achieve a better separation. In contrast, the reaction of dipropylamine with **93** under similar conditions afforded only the free base and not the hydrobromide salt of **96**.

Compound	Commercial Availability	Method	R	R^1	R^2	Overall % Yield
94	$Cl(CH_2)_3NMe_2$, (YES)	1	IPr	Me	Me	64
95	Hal.$(CH_2)_3NHiPr$, (NO)	2	Me	H	IPr	72
96	Hal.$(CH_2)_3NPr_2$, (NO)	2	Me	Pr	Pr	64

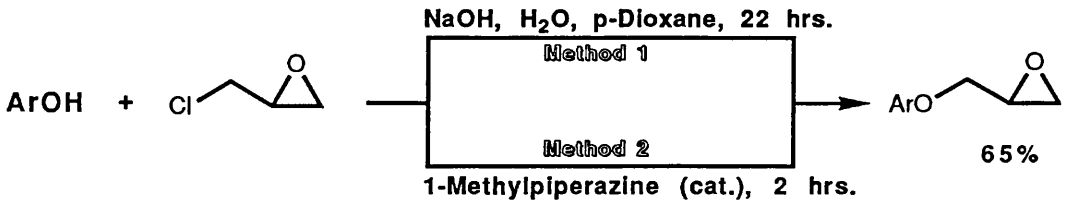
Table 2.2. Preparation of analogues 94-96

The findings are somewhat surprising, since these amines are of similar basicity and **96** should have also been obtained in its hydrobromide form. The free base of **96** (a gum) appeared to be hygroscopic and was converted to the oxalate salt on treatment with a saturated solution of oxalic acid in ethyl acetate.

References, Page 101, Structures & abbreviations, Page 205

2.3.4. Preparation of 1-(2,3-Epoxypropoxy)-6-(carboalkoxy)-naphthalenes

Scheme 2.6. illustrates the literature synthetic procedures for the epoxides reported.

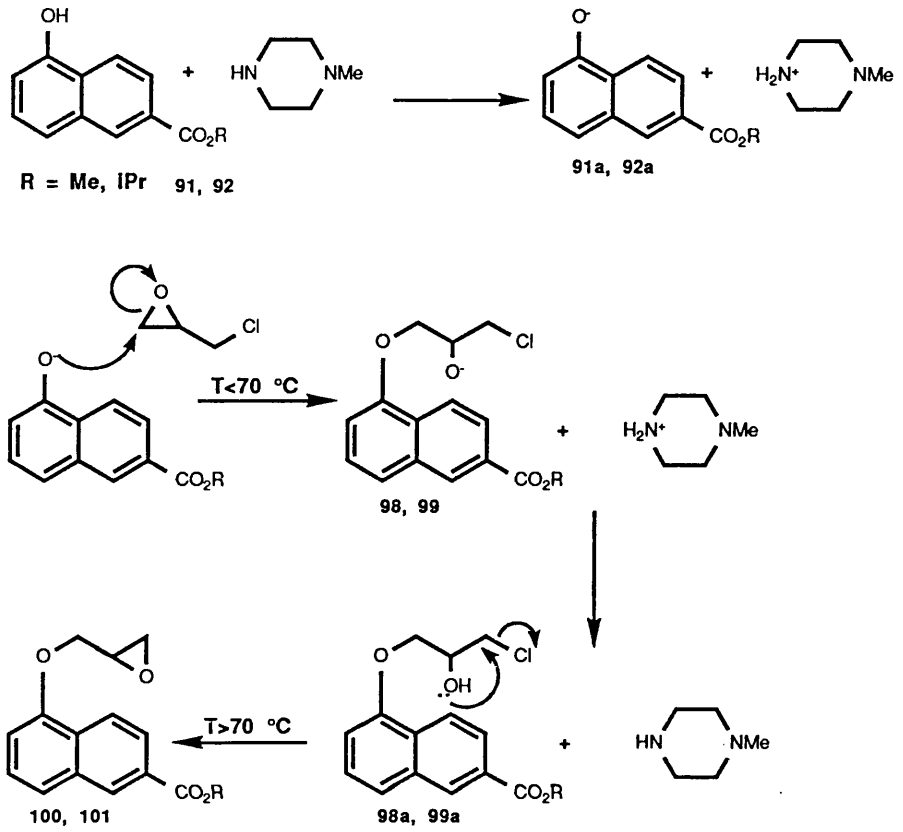


Ar = 1-Naphthol and 4-Hydroxyindole, Substituted Phenols.

Scheme 2.6.

The two methods [4, 11] illustrated above give the epoxides in similar yields. Of the two methods, the former appears to be more commonly used. However, in view of the longer reaction time and work up procedure of method 1, it was decided to prepare the epoxides **100, 101** via method 2 [4]. The hydroxy esters **91, 92** were dissolved in 17-25 molar equivalent of epichlorohydrin containing catalytic amounts of 1-methylpiperazine. The temperature was gradually increased to 100 °C and the reaction monitored by TLC. It was noticed that the formation of the epoxides **100, 101** does not proceed via a simple nucleophilic displacement reaction of the chloride ion as might have been expected but that the reaction proceeds via the intermediates **98a, 99a**. These intermediates are the sole product of the reaction at temperatures below 70 °C. On raising the temperature from 70 °C to 100 °C, the intermediates are rapidly converted to the desired epoxides. These were not isolated in this reaction, however, in a different set of reactions (see chapter 3), an analogous intermediate was obtained from the reaction of 4-hydroxyindole with epichlorohydrin. The intermediate was isolated and characterised by ^{1}H N.M.R. and M.S. It is believed that the

mechanism for the formation of the epoxides proceeds as illustrated in scheme 2.7.



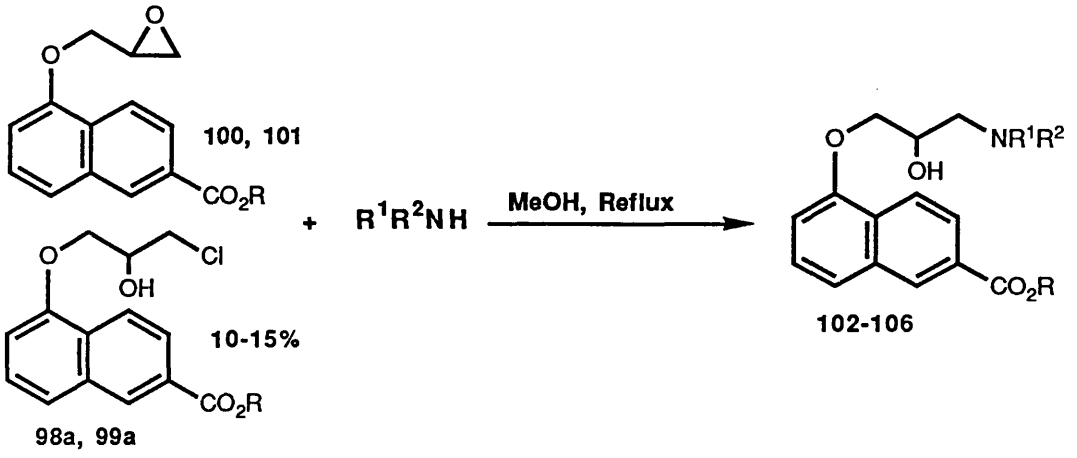
Scheme 2.7. Mechanism of the Reaction for the formation of epoxides 100, 101.

1-Methylpiperazine abstracts the acidic hydrogen, generating the anion of **91 or 92**, which in turn attacks the α-carbon of epichlorohydrin, generating the strong secondary alkoxide ion **98 or 99**. This in turn abstracts hydrogen from the protonated 1-methylpiperazine forming the secondary alcohol intermediates **98a or 99a** and hence regenerating the catalyst. Formation of the epoxides then proceeds via a simple internal SN_2 reaction.

The reaction of hydroxy esters with epichlorohydrin gave the corresponding epoxides in ca. 70% yield. The epoxides also contained 10-15% of **98a** or **99a** but no attempt was made to separate the mixture since both compounds react with amines to give the same product.

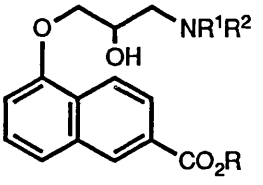
2.3.5. Preparation of 1-[3-(alkylamino)-2-hydroxypropoxy]-6-(carboalkoxy)naphthalenes

The desired amine analogues **102-106** were prepared [4] from the reaction of the epoxides **100, 101** with various primary and secondary amines (excess) in moderate to good yields as illustrated in scheme 2.8.



Scheme 2.8 Preparation of Analogues 102-106

The reaction of epoxides with the more volatile amines was safely carried out in a sealed pressure vessel.



Analogues	R	R^1	R^2	Base/Salt	% yield of the Free Base
102	IPr	H	Me	Oxalate	54
103	IPr	Me	Me	Oxalate	52
104	IPr	H	IPr	Base	61
105	IPr	Pr	Pr	Oxalate	81
106	Me	H	IPr	Base	74

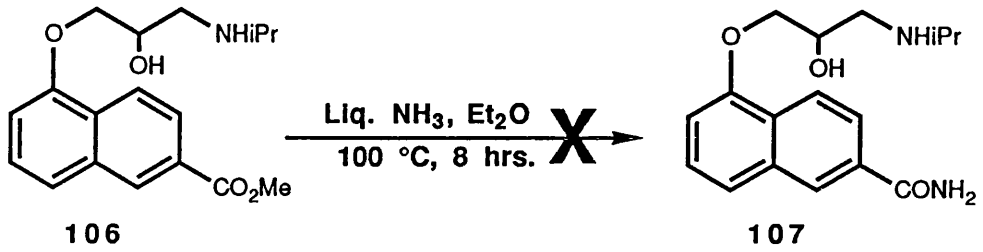
Table 2.3.

Although the amines were used in excess, no side-product arising from the transamination reactions of the amines with the ester portion of the epoxides (i.e. amide formation) was observed. The amines were obtained as racemates and no attempt was made to resolve the enantiomers.

Some of the analogues **102, 103, 105** were obtained as viscous oils and appeared to be extremely hygroscopic. These could not easily be solidified and were converted to their oxalate salts upon treatment with a saturated solution of oxalic acid (Table 2.3.). Compound **102** contained about 10% impurities, but no attempt was made to remove the impurity.

2.3.6. Amide Analogues of 102-106

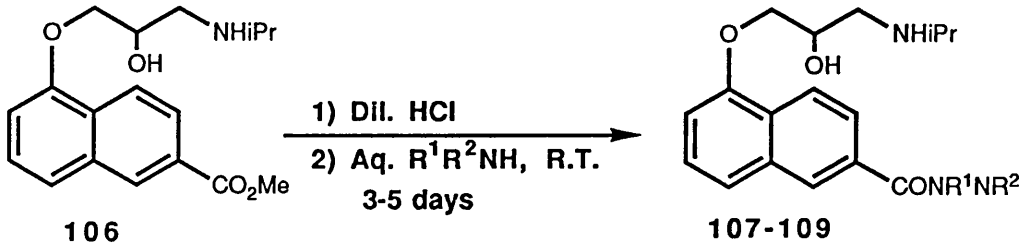
The synthesis of the primary amide analogue **107** was first attempted by heating the corresponding methyl ester **106** with a mixture of ether and liquid ammonia (equal volumes) in a sealed pressure vessel. However, **106** proved to be stable under these conditions and no reaction was observed.



106 **107**

Scheme 2.9.

In view of the above, an alternative method was investigated. It is known [21] that the aqueous solution of amines with less hindered alkyl esters at room temperature gives the corresponding amides in good yields. However, a practical difficulty was set upon us since the methyl ester **106** was insoluble in water. The problem was overcome by using a partitioning solvent such as methanol or dioxane. Although this procedure with conc. ammonia gave the amide **107** as the major product, other minor impurities were also observed.



106 **107-109**

Scheme 2.10.

A much better procedure was found when a solution of hydrochloride salt of the methyl ester was treated with large volumes of conc. amines at

room temperature for several days. This method gave the corresponding primary, secondary and tertiary amides (**107, 108** and **109** respectively) as a single product in high yields (scheme 2.10.).

Compound	R^1	R^2	Base/Salt	% Yield of Base
107	H	H	Base	88
108	H	Me	Base	82
109	Me	Me	Hydrochloride	79

Table 2.4.

The dimethyl amido analogue **109** was obtained as a gum and converted to its hydrochloride salt. The 200 MHz ^1H N.M.R. spectrum of the hydrochloride salt of **109** in $CDCl_3$ (figure 2.4.) is consistent with the structure of the compound. However, it has a few striking features, notably, the doublet due to the isopropyl group is further split into a doublet of doublets at ca. δ 1.5 and the two broad singlets at ca. δ 8.7 and 9.5. The former phenomenon has been observed before [8] with a similar compound (toliprolol, **110**) and is attributed to the fact that NH_2^+

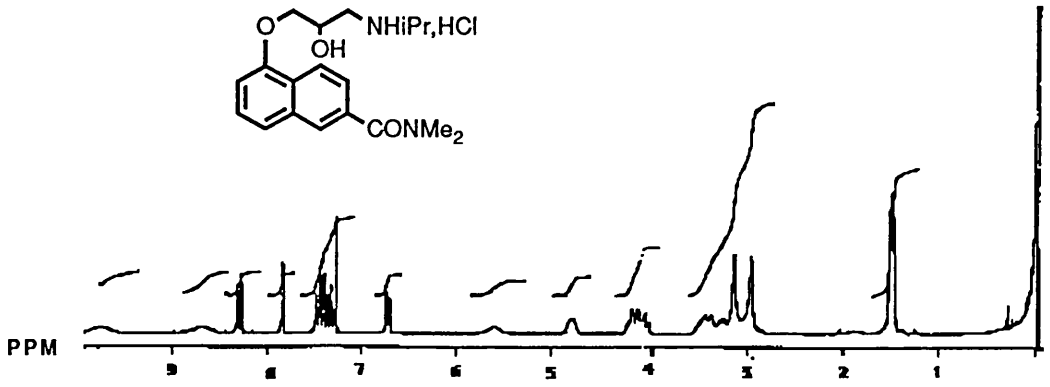
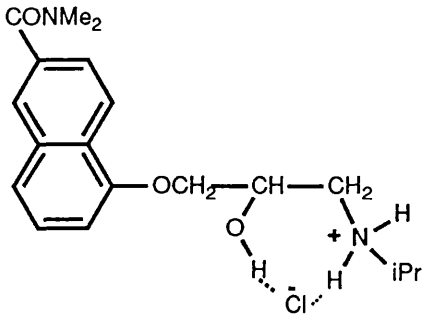


Figure 2.4. 200 MHz Spectrum of 109 in $CDCl_3$

protons are in direct interaction with the anion forming a seven membered ring (see below).



The two broad singlets at ca. δ 8.70 and 9.80 indicate a slow exchange of the two NH_2^+ protons which are anisocronous probably of the presence of the anion. The methyl protons of the isopropyl group are also non-equivalent, giving rise to two doublets. The non-equivalence of the methyl protons may arise from the possibility of slow inversion of the nitrogen atom. It is also possible that the above indicated interaction of the anion with both the OH and NH_2^+ protons could lead to restricted rotation about the C–N bond which might contribute to this non-equivalence.

The methyl protons of the amide group are also non-equivalent as expected and give rise to two singlets at ca. δ 2.98 and 3.14. Figure 2.5. shows the temperature dependence of the methyl protons of the amide group. In the spectra obtained, these two resonances have a coalescence temperature (T_c) of 40.1 °C and sharpen to a single resonance at 60 °C. Assuming the two environments are equally populated, i.e. a 1:1 singlet, it was possible to calculate the rate constant for the interconversion of the two conformations;

$$T_c = 313\ ^\circ K \qquad \delta\upsilon = 36\ Hz \qquad K_{(313\ ^\circ K)} = \frac{\Pi\delta\upsilon}{\sqrt{2}}\ s^{-1} \qquad K_{(313\ ^\circ K)} = \frac{\Pi}{\sqrt{2}}\ 36\ s^{-1} = 80\ s^{-1}$$

From the coalescence temperature, the free energy of activation (ΔG ‡) for the barrier to rotation about the C–N bond was evaluated according to the following equations;

$$DG^{\#}_{rotn.} = RT\,[22.96 + \ln (T/\delta\upsilon)]$$

$$DG^{\#}_{rotn.} = 8.314\ JK^{-1}Mol^{-1} \times 313K\,[22.96 + \ln (313K/36)]$$

$$DG^{\#}_{rotn.} = 65.38\ KJMol^{-1}$$

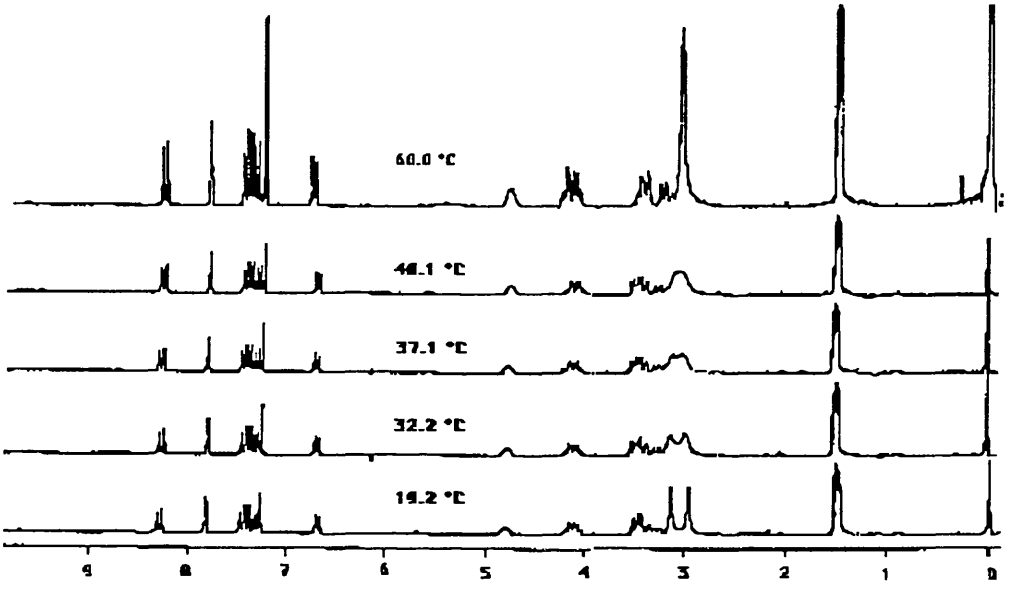
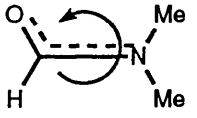


Figure 2.5. Temprature dependence of the resonance signal of the amido methyl protons.

The barrier to rotation about the C–N bond in DMF [23] is ca. 79 $KJMol^{-1}$, some 13 $KJMol^{-1}$ more than the calculated value for the rotational barrier in **109**.



The results reflect the fact that the partial double bond character in the dimethyl amide **109** is somewhat weaker than in DMF, probably due to electron donation from the naphthalene ring, stabilising the carbonyl group of the amide.

2.4. Structure-Activity Results and Discussion

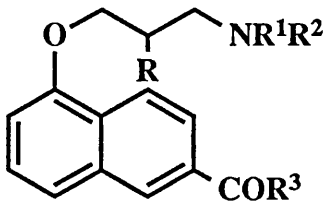
The object of the work presented in this chapter was to introduce electron withdrawing groups such as esters and amides into the 6-position of propranolol and simultaneously modify the side chain of propranolol in order to examine the effect on activity at 5-HT_{1A} and $5HT_{1B}$ receptors. It was hoped that this work would identify the key structural features involved in serotonergic binding interactions.

The activity of compounds as agonists and antagonists at 5-HT_{1B} receptors were determined using 5-HT potentiation of electrically stimulated twitch contractions of mouse urinary bladder strips in vitro. All compounds were tested at a concentration of 10^{-5} molar and in all cases a weak antagonistic activity was observed. The binding affinities of these analogues at 5-HT_{1A} and 5-HT_{1B} receptors were also determined (see also chapter 6).

The results (see table 2.5) clearly show that the effect of introducing esters or amides in the 6-position of propranolol is detrimental to the affinity at both receptors. These compounds are significantly less active than the lead compound propranolol. The strategy for the introduction of groups into the 6-position of propranolol was based on the proposition (discussed in section 2.2) that the indole 2-position might be considered to be equivalent to the naphthalene-6 or 7-position of either cyanopindolol or SDZ 21009. However the results of binding affinities strongly suggest that the indole-2-position is not equivalent to the 6-position of the naphthalene ring of propranolol.

References, Page 101, Structures & abbreviations, Page 205

Table 2.5. Affinities of propranolol analogues for 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, and effects at 5-HT$_{1B}$ receptors.



Compound	R	R^1	R^2	R^3	IC$_{50}$ values, nM 5-HT$_{1A}$ [8-OH-DPAT]	5-HT$_{1B}$ [^{125}I] CYP	Agonism at 5-HT$_{1B}$	Antagonism at 5-HT$_{1B}$
104	OH	H	iPr	OiPr	>10000	2138	NO	YES
102	OH	H	Me	OiPr	>10000	4464	NO	YES
103	OH	Me	Me	OiPr	>10000	>10000	NO	NO
105	OH	Pr	Pr	OiPr	>10000	>10000	NO	NO
106	OH	H	iPr	OMe	5623	870	-	-
107	OH	H	iPr	NH_2	4467	1230	NO	YES
108	OH	H	iPr	NHMe	>10000	4365	NO	YES
109	OH	H	iPr	NMe_2	>10000	2399	NO	YES
95	H	H	iPr	OMe	1230	>10000	YES	YES
94	H	Me	Me	OiPr	1862	18621	NO	YES
96	H	Pr	Pr	OMe	2138	>10000	NO	YES
±Propranolol*	OH	H	iPr	-	90	50	NO	YES

* Data from Glennon et al[22]

Superimposition and comparison of the structures of 5-HT, with 1,6 and 1,7-disubstituted naphthalene analogues of propranolol reveals that a much better molecular fit is obtained between 5-HT and 1,7-disubstituted

References, Page 102, Structures & Abbreviations, Page 205

naphthalenes (a) than with 5-HT and 1,6-disubstituted naphthalenes (b) (see figure 2.7.).

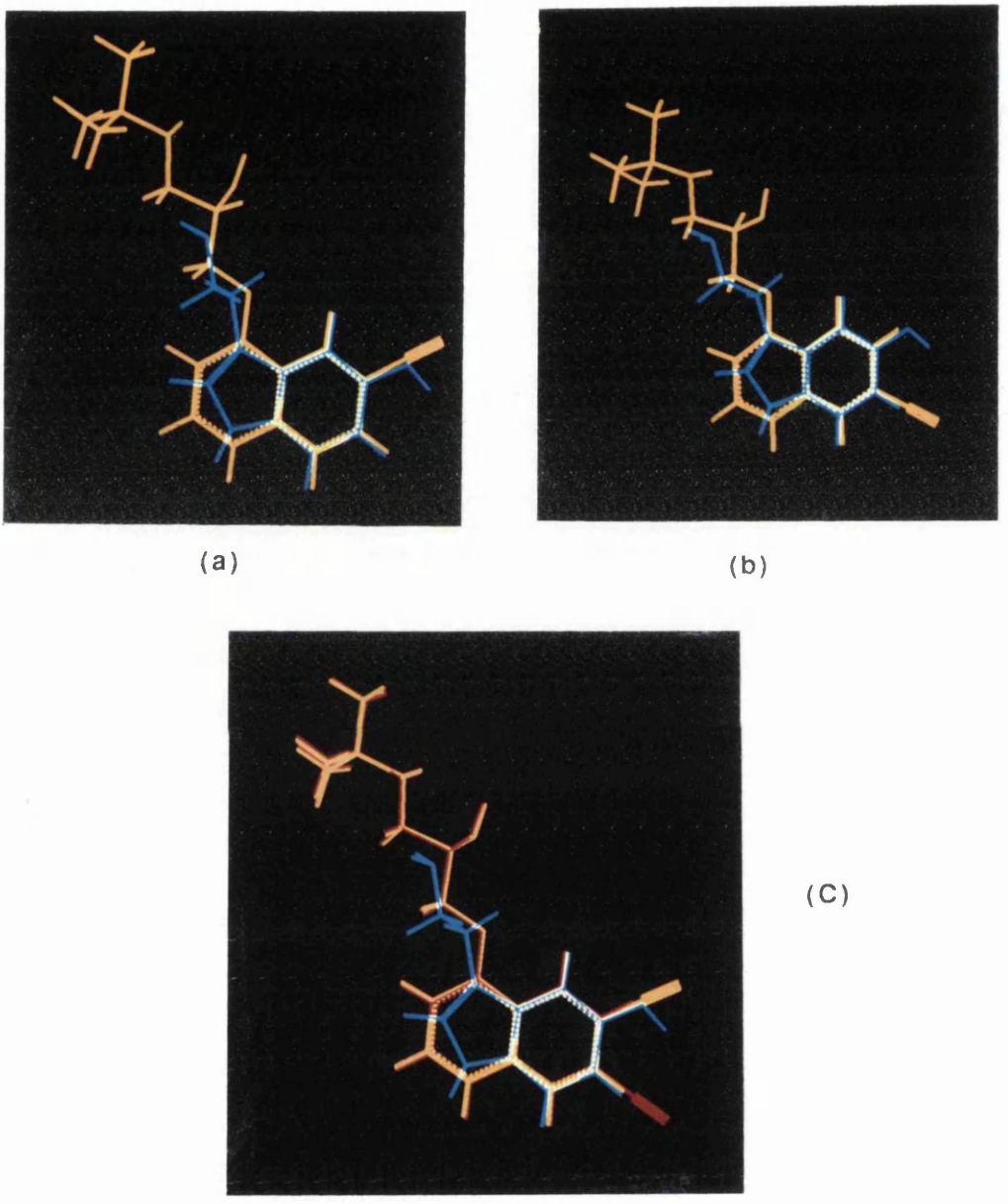


Figure 2.7. Superimposition of 5-HT with 1,6-disubstituted (b), 1,7-disubstituted (a),1,6-di-substituted & 1,7-disubstituted (c) naphthalene analogues of propranolol.

Indeed, if our initial observation that 5-HT, cyanopindolol and SDZ 21009 all interact with the receptor in the same manner is correct, it follows that the corresponding 1,7-disubstituted derivatives (a) of propranolol are more likely to be active.

Within the series, the following patterns are evident;

i) A comparison of **104** with **102, 103** and **105** reveals that while smaller secondary amines are tolerated at the 5-HT_{1B} receptor, tertiary amines reduce this affinity. In contrast, examination of **95** and **96** indicates that the introduction of a di-n-propylamino group has little effect on affinity at the 5-HT_{1A} receptor, a further conformation that tertiary amines are well tolerated at this receptor.

ii) The methyl ester **106** is more potent at both receptors than the corresponding isopropyl ester analogue **104**, indicating the preference of smaller alkyl groups. Replacement of the methyl ester of **106** by a primary amide (i.e. **107**) has virtually no effect on binding, however, secondary and tertiary amides (i.e. **108 and 109** respectively) bind with lower affinities, a further indication that the size of alkyl group may influence the binding.

iii) The chiral carbon atom and the hydroxyl group of propranolol are important for β-adrenergic activity. We find that the removal of the side chain hydroxyl group is not important for 5-HT_{1A} binding. In fact, the hydroxyl analogues possess only one-fifth the affinity of the nonhydroxylated compounds (i.e. **95** and **106, 94** and **103**). In contrast, the removal of the hydroxyl group reduces 5-HT_{1B} potency.

2.5. Experimental

General

Starting materials were prepared according to literature procedures as indicated and, if no reference is quoted, are available commercially.

solvents

Methanol, ethanol and isopropanol were dried with just a little more sodium than required to react with the water present and the alcohol was then distilled. Tetrahydrofuran (THF) was initially dried over sodium wire and later refluxed with Potassium metal in the presence of benzophenone (which forms a blue ketyl) and then distilled. Ether and toluene were dried over sodium wire. Ethyl acetate and di-isobutyl-ketone were dried over molecular sieves (5 °A) before distillation. In the text, petrol refers to petroleum ether (b.p. = 80-100 °C) and was distilled before use.

High Pressure Reactions

Reactions requiring the use of volatile amines or gases were carried out in a Berghof pressure vessel.

Melting Points

Melting points (M. P.) were carried out on an Electrothermal melting point apparatus and are uncorrected.

Chromatography

Thin layer chromatography (T.L.C.) was carried out on merck Kieselgel 60 F_{254} plates. The plates were visualised at 254 nm and 350 nm and

then with either gaseous iodine or potassium iodoplatinate spray reagent.

Column chromatography was carried out on Merck silica gel 60, particle size 0.063-0.200 mm (70-230 mesh ASTM) using gravity elution.

High performance liquid chromatography was carried out, using a Gilson Binary Gradient System with computerised control and data handling. Other details such as the type of column, solvent system, flow rate, detector wavelength, etc. are quoted in the experimental procedure for each target compound.

Elemental analysis were carried out by the microanalytical section of the Department of Chemistry at University College London.

Spectroscopy

The Infra-red (IR) spectra were recorded (1% w/w KBr discs) on a Perkin-Elmer 983 spectrophotometer.

The electronic spectra (UV) were determined on a Perkin-Elmer 554 recording spectrophotometer. Cells of 1 cm path length were used and the solvent was methanol.

Fourier transform proton magnetic resonance (^{1}H N.M.R.) were recorded on a Varian XL-200 MHz or a Varian XVR-400 spectrometer in deuterated chloroform ($CDCl_3$), deuterated methyl sulphoxide (DMSO-d_6) or in deuterated water (D_2O) and are reported in δ units relative to tetramethylsilane (TMS) or 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt (TSP).

Mass spectra (MS) were recorded on a VG 7070H mass spectrometer with Finnigan INCOS II data system.

The following abbreviations are used in the text:

TFA = trifluoroacetic acid, TEA = triethyl amine, M^+ = molecular ion, m/z = mass to charge ratio (for mass spectra), s = singlet, d = doublets, dd = doublet of doublets, t = triplets, q = quartet, m = multiplets, br. = broad, λ = wavelength and ε = extinction coefficient.

5-Sulphonic-2-naphthoic acid (87)

This procedure is a modification of the method by Butler and Royle [12,13]. 2-Naphthoic acid (33.0g, 0.192 mole, 86) was mixed with H_2SO_4 (98%, 96.0g, 0.96 mole). The semi-solid mixture was heated to 98-102 °C for 8 hours. The hot solution was poured into a conical flask and cooled to about 5 °C. Ice-cold water (200 ml) was carefully added with continuous stirring to dilute the reaction mixture. The solution was kept in the fridge overnight and the resulting precipitate filtered off, and washed several times with ice-cold water. The solid was redissolved in water (200 ml) and the pH of the solution adjusted to 11±1 with 40% KOH to convert the acid to its potassium salt form. The mixture was cooled and potassium sulphate filtered off. Methanol was added to the cold filtrate until the precipitation of potassium sulphate ceased. The mixture was cooled, filtered and the filtrate taken to dryness under reduced pressure. The resulting white solid was dried at 120 °C, 0.5 mm Hg for 4 hours and dissolved in minimum amounts of hot anhydrous methanol and left to crystallise. The first fraction was collected after 24 hours but found to be mainly potassium sulphate. Several more fractions were collected over a period of a week. All fractions were dried at 100 °C, 1 mmHg and then examined by ^{1}H N.M.R. Fractions that were identical by ^{1}H N.M.R. were combined and if necessary, recrystallised from hot anhydrous methanol.

This procedure yielded 15g of the pure di-potassium salt of the desired product.

^{1}H N.M.R. (D_2O-TSP, 200 MHz) : δ 6.93 (1H, H^7, t , J = 7.7 Hz), 7.42 (1H, H^3, dd, J = 8.5 Hz), 7.45 (1H, H^6, d, J = 7.7 Hz), 7.57 (2H, H^8, d, J = 7.7 Hz), 7.80 (1H, H^1, d, J = 1.7 Hz), 7.96 (1H, H^4, d, J = 8.5 Hz).

5-Hydroxy-2-naphthoic acid (88)

This procedure is a modification of the method by Butler and Royle [12,13]. Potassium hydroxide (127.5g, 3.19 mole) was placed in a stainless steel beaker fitted with a thermometer inside a stainless steel jacket, and a stainless steel stirrer. The metal beaker was carefully heated to 260 °C with vigorous stirring to melt the solid potassium hydroxide. Anhydrous powdered di-potassium salt of 5-sulphonic-2-naphthoic acid **87** (19.2g, 0.057 mole) was added to the molten KOH in small amounts with vigorous stirring. After each addition, the temperature was allowed to rise to 260 °C. When addition was complete, (~30 mins.) the yellow mixture was heated to 280 °C for a further 15 minutes. The semi-solid mixture was cooled to 0-5 °C and carefully diluted with ice-cold water. The solution was then neutralised to pH ~7 with dil. H_2SO_4 and the resulting mixture filtered. Conc. HCl was added to the filtrate and the product precipitated as a straw coloured solid. The crude acid was further purified by converting it to its sodium salt, the aqueous solution of which was boiled with charcoal. After filtration, the acid was reprecipitated with conc. HCl and recrystallised from boiling water as light tan needles. 5-Hydroxy-2-naphthoic acid was dried at 60 °C and 1 mmHg for 8 hours (9.5g, 89%).

M.P. 213-214 °C Lit. M.P. = 212-213 °C

M.S. m/z : 188 (M^+, 100%), 171 (M^+-17, 62%), 143 (M^+-45, 35%), 127 (M^+-61, 1%), 115 (M^+-73, 89%).

^{1}H N.M.R. (DMSO, 200 MHz) : δ 3.40 (1H, OH, br,), 6.98 (1H, H^6, dd, J = 6.1 Hz), 7.38 (1H, H^7, t, J = 6.1 Hz), 7.54 (1H, H^8, d, J = 6.1 Hz), 7.92 (1H, H^3, dd, J = 8.6 Hz), 8.21 (1H, H^4, d, J = 8.6 Hz), 8.44 (1H, H^1, d, J = 1.2 Hz).

I.R. (KBr) : 3359, 3252, 2551, 1688, 1628, 1518, 1462, 1267, 1192, 1035 and 773 cm^{-1}.

5-Hydroxy-2-naphthoic acid isopropyl ester (91)

Method 1 [17]

2-Chloropropane (0.22g, 2.7 x 10^{-3} mole) in DMF (5 ml) was added to a solution of 5-hydroxy-2-naphthoic acid **88** (0.5g, 2.7 x 10^{-3} mole) in water containing sodium hydroxide (0.11g, 2.7 x 10^{-3} mole). The solution was refluxed for 2 days, then cooled to R.T. and water (20 ml) was added. The aqueous solution was extracted with chloroform and the combined chloroform extracts were dried with $MgSO_4$. The resulting product, after removal of chloroform, was obtained as a straw coloured solid and after drying at 50 °C, 0.5 mmHg weighed 0.15g (25%). The unreacted hydroxynaphthoic acid was precipitated by addition of dilute HCl to the aqueous layer (0.2g recovery).

M.P. 118-119 °C

M.S. m/z : 230 ($M^{+\cdot}$ 76%), 188 (M^+-42, 100%), 171 (M^+-59, 59%), 149 (M^+-81, 24%), 143 (M^+-87, 13%), 115 (M^+-115, 42%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.26 (6H, d), 5.24 (1H, m), 6.87 (1H, d), 7.21 (1H, t), 7.37 (1H, d), 7.93 (1H, d), 8.17 (1H, d), 8.43 (1H, s).

I.R. (KBr) : 3312, 2928, 1681, 1578, 1371, 1277, 1197, 100 and 773 cm^{-1}.

Method 2

2-Bromopropane (1.0g, 8.1 x 10^{-3} mole) in dioxane (5 ml) was added to a solution of 5-hydroxy-2-naphthoic acid **88** (0.5g, 2.7 x 10^{-3} mole) in water (5 ml) containing potassium hydroxide (0.152g, 2.7 x 10^{-3} mole). The solution was refluxed for 2 days and the ester was worked up following the same procedure as in method 1, (0.3g, 50%). This product was identical by M.P., T.L.C. and ^{1}H N.M.R. to that obtained by method 1.

Method 3

2-Iodopropane (1.38g, 8.1 x 10^{-3} mole) in isopropanol (10 ml) was added to a solution of 5-hydroxy-2-naphthoic acid **88** (0.5g, 2.7 x 10^{-3} mole) containing potassium hydroxide (o.152g, 2.7 x 10^{-3} mole). The solution was refluxed for 2 days and then worked up as in method 1, (0.35g, 55%). This product was identical by M.P., T.L.C. and ^{1}H N. M.R. to that obtained by method 1.

Method 4

A solution of 5-hydroxy-2-naphthoic acid (0.5g, 2.7 x 10^{-3} mole, 88) in dry isopropanol (16.2g, 0.27 mole) containing H_2SO_4 (98%, 0.06g, catalytic amount) was refluxed for 18 hours. The solution was cooled to R.T. and its pH adjusted to ~5 with dilute KOH. The volume of the solution was halved in vacuo and water was then added. The aqueous solution was

extracted with chloroform and the combined extracts were washed several times with water to remove the remaining traces of the acid. The chloroform layer was dried with $MgSO_4$, filtered and the solvent removed to yield a straw coloured solid, (0.45g, 72%). The combined aqueous layers was acidified with conc. HCl to precipitate the unreacted acid, (0.1g). This product was identical by M.P., T.L.C. and ^1H N.M.R. to that obtained by method 1.

5-Hydroxy-2-naphthoic acid methylester (92)

A solution of 5-hydroxy-2-naphthoic acid **88** (1.0g, 5.53×10^{-3} mole) in dry methanol (25.0g, 0.78 mole) containing H_2SO_4 (98%, 0.1g, catalytic amounts) was refluxed for 10 hours. The solution was cooled to R.T. and its pH adjusted to ~5 with dilute KOH. The solution was concentrated to half its original volume and water (100 ml) was added. This was extracted with chloroform and the combined chloroform extracts were washed with water and then dried with $MgSO_4$. Chloroform was then removed under reduced pressure and the resulting product was obtained as a straw coloured solid, (0.95g, 88%).

M.P. 120-122 °C

M.S. m/z : 202 (M^+, 100%), 188 (M^+-14, 0.01%), 171 (M^+-31, 94%), 143 (M^+-59, 33%), 115 (M^+-87, 64%),

^1H N. M.R. ($CDCl_3$, 400 MHz) : δ 3.97 (3H, s), 6.16 (1H, s, br), 6.97 (1H, d), 7.36 (1H, t), 7.51 (1H, d), 8.05 (1H, d), 8.27 (1H, d), 8.58 (1H, s).

I.R. (KBr) 3340, 1721, 1691, 1580, 1290, 1276 and 772 cm^{-1}.

1-[3-(N,N-Dimethylamino)propoxy]-6-(carboisopropoxy)-naphthalene hydrogen oxalate (94)

A solution of 5-hydroxy-2-naphthoic acid isopropylester **91** (1.15g, 5 x 10^{-3} mole) in dry THF (15 ml) was prepared [19]. To this was added dropwise with stirring a suspension of NaH (60%, 0.3g, 7.5 x 10^{-3} mole) in THF (15 ml) under a slow stream of nitrogen. The mixture was warmed for a few minutes and then cooled to ~5 °C.

N,N-Dimethyl-3-chloropropylamine was liberated [20] from its hydrochloride salt (3.12g 0.02 mole) in benzene and added dropwise to the mixture. The reaction mixture was gradually warmed and then refluxed for 48 hours. After filtration, the solvent was removed and the residue chromatographed on silica, eluting with 40% $CHCl_3$ v/v in methanol to yield a straw coloured oil. The oil was dried at 50 °C and 0.1 mmHg for 6 hours, (1.0g, 64%). The oil (0.5g, 1.6 x 10^{-3} mole) was dissolved in absolute ethanol (5 ml). A saturated solution of oxalic acid in absolute ethanol was added dropwise with stirring until an excess of the acid was present. The white precipitate was collected by filtration and recrystallised from water to give colourless needles. The product was dried and weighed 0.5g (77%).

M.P. 186-188 °C

Analysis : Calculated for $C_{21}H_{27}NO_7$: C, 62.21, H, 6.71, N,3.45

Found: C, 62.04, H, 6.57, N, 3.20

M.S. m/z : 315 (M^+, 3%), 256 (M^+-59, 1%), 101 (M^+-214, 1%), 86 (M^+-229, 43%), 58 (M^+-257, 100%).

^{1}H N.M.R. (DMSO, 200 MHz) : δ 1.36 [6H,CH($C\underline{H}_3$)$_2$, d], 2.29 (2H, $CH_2C\underline{H}_2CH_2$, m), 2.81 [6H, N(CH_3)$_2$, s], 3.32 (2H, CH_2N, t),

4.25 (2H, OCH_2, t), 5.21 (1H, OCH, m), 7.12 (1H, naph-2-H, d), 7.51 (1H, naph-3-H, t), 7.69 (1H, naph-4-H, d), 7.97 (1H, naph-8-H, d), 8.32 (1H, naph-7-H, d), 8.55 (1H, naph-5-H, s).

I.R. (KBr) : 3426, 2965, 2638, 2464, 1701, 1594, 1277, 1180 and 1104 cm^{-1}.

U.V. (CH_3OH) λ, ε : (211 nm, 22979), (245 nm, 47440), (285 nm, 2965, Broad), (335 nm, 2594, Broad).

H.P.L.C. : Column: 250 mm x 4.6 mm spherisorb CN, 5 μM
Mobile Phase: Methanol/water 70:30 + 0.1% Et_3N.
Flow Rate: 1 ml/min.
Area of the main peak: 100% at 9.11 minutes.

1-(3-Bromopropoxy)-6-(carbomethoxy)naphthalene (93)

A solution of 5-hydroxy-2-naphthoic acid methylester 92 (0.80g, 3.96×10^{-3} mole) in dry THF (20 ml) was prepared [19]. To this was added dropwise with stirring a suspension of NaH (60%, 0.17g, 4.35×10^{-3} mole) in THF (15 ml) under a slow stream of nitrogen. The mixture was warmed for a few minutes and then cooled to room temperature.

1,3-Dibromopropane (12.00g, 5.94×10^{-2} mole) was dissolved in THF (20 ml) and added dropwise to the mixture. When the addition was complete, the mixture was refluxed for 12 hours. The mixture was then cooled to R. T. and NaBr filtered off. The solvent and the excess 1,3-dibromopropane was removed in vacuo and the residue was chromatogrphed on silica, eluting with 30% petrol in chloroform. After the removal of the solvent, 1.1g (86%) of a colourless oil was obtained which

solidified on standing at R.T. The product was dried at 50 °C and 0.1 mmHg for 3 hours.

M.P. 58-59 °C

M.S. m/z : 324 (M^+, 54%), 322 (M^+, 53%), 291 (M^+-31, 5%), 242 (M^+-80, 15%), 202 (M^+-120, 100%), 171 (M^+-151, 59%), 142 (M^+-180, 23%), 115 (M^+-207, 38%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 2.48 (2H, m), 3.70 (2H, t), 3.97 (3H, s), 4.30 (2H, t), 6.94 (1H, d), 7.43 (1H, t), 7.55 (1H, d), 8.04 (1H, dd), 8.28 (1H, d), 8.56 (1H, s).

I.R. (KBr) : 3426, 2965, 2638, 2464, 1701, 1594, 1277, 1180 and 1104 cm^{-1}.

1-[3-(N-Isopropylamino)propoxy]-6-(carbomethoxy)-naphthalene hydrogen bromide (95)

A solution of **92** (0.40g, 1.24 x 10^{-3} mole) and isopropylamine (14.6 ml) in absolute ethanol (75 ml) was refluxed for 10 hours. The solvent and the excess amine was then removed under reduced pressure and the resulting residue was chromatographed on silica, eluting with 1% ammonia (28%) in a mixture of chloroform:methanol:petrol (1:4:5). The solvent was removed and the resulting crude hydrobromide salt (0.40g, 84.5%) was recrystallised from a mixture of Absolute ethanol, ethyl acetate and petrol to yield the product as colourless needles. The salt was dried at 130 °C and 0.1 mmHg for 20 hours.

M.P. 175-177 °C

Analysis : Calculated for $C_{18}H_{24}BrNO_3$:C,56.55,H,6.33,Br,20.90,N,3.71

Found: C,56.64,H,6.38,Br,20.83 N,3.66

M.S. m/z : 301 (M^+, 9%), 286 (M^+-15, 26%), 270 (M^+-31, 3%), 186 (M^+-115, 7%), 143 (M^+-158, 7%), 127 (M^+-174, 14%), 100 (M^+-201, 73%), 72 (M^+-229, 100%), 58 (M^+-243, 53%).

^{1}H N.M.R. (DMSO, 200 MHz) : δ 1.57 [6H, $CH(C\underline{H}_3)_2$, d], 2.71 (2H, $OCH_2C\underline{H}_2$, m), 3.31 (2H, CH_2N, t), 3.50 (1H, NCH, m), 3.95 (3H, OCH_3, s), 4.22 (2H, OCH_2, t), 6.8 (1H, naph-2-H, d), 7.37 (1H, naph-3-H, t), 7.50 (1H, naph-4-H, d), 8.01 (1H, naph-8-H, d), 8.22 (1H, naph-7-H, d), 8.51 (1H, naph-5-H, s), 9.23 (1H, br, s).

I.R. (KBr) : 2965, 2791, 1714, 1598, 1580, 1274, 1227, 1114, 1090, and 770 cm^{-1}.

U.V. (CH_3OH) λ, ε : (215 nm, 22510, Sharp), (244 nm, 472722, Sharp), (284 nm, 3002, Broad), (330 nm, 1013, Broad).

H.P.L.C. : Column: 250 mm x 4 mm RP select B, 5μM
Mobile Phase: Methanol+0.1% Et_3N.
Flow Rate: 1ml/min.
Area of the main peak: 99.79% at 8.27 minutes.

1-[3-(N,N-Dipropylamino)propoxy]-6-(carbomethoxy)-naphthalene hydrogen oxalate (96)

A solution of **92** (0.50g, 1.55 x 10^{-3} mole), N,N-dipropylamine (15.66 ml) in absolute ethanol (75 ml) was refluxed for 10 hours. The solvent and the excess amine was then removed under reduced pressure and the resulting residue was chromatographed on silica, eluting with 15%

methanol v/v in a mixture of chloroform : petrol (4:6). Removal of solvent yielded the product as a colourless oil which was dried at R.T. and 0.1 mmHg for 5 hours.

To a solution of the product in ethyl acetate was added dropwise a saturated solution of oxalic acid in ether until an excess of the acid was present. The mixture was left overnight, filtered and washed twice with ether. The product was obtained pure and therefore not recrystallised but dried at 60 °C, 0.1 mmHg for 10 hours.

M.P. 174-176 °C

Analysis : Calculated for $C_{23}H_{31}NO_7$: C,63.73,H,7.21,,N,3.23

Found: C,63.91,H,7.23, N,3.23

M.S. m/z : 343 (M^+, 12%), 328 (M^+-15, 4%), 314 (M^+-29, 100%), 240 (M^+-103, 3%), 184 (M^+-159, 3%), 171 (M^+-172, 6%), 157 (M^+-186, 10%), 142 (M^+-201, 19%), 114 (M^+-229, 96%), 86 (M^+-257, 83%), 70 (M^+-273, 22%).

^{1}H N.M.R. (DMSO, 200 MHz) : δ 0.91 (6H, $CH_2C\underline{H}_3$, t), 1.67 (4H, $C\underline{H}_2CH_3$, m), 2.25 (2H, $OCH_2C\underline{H}_2$, m), 3.02 (4H, $C\underline{H}_2CH_2CH_3$, m), 3.28 (2H, $OCH_2CH_2C\underline{H}_2$, t), 3.91 (3H, OCH_3, s), 4.26 (2H, OCH_2, t), 7.14 (1H, naph-2-H, d), 7.54 (1H, naph-3-H, t), 7.71 (1H, naph-4-H, d), 7.99 (1H, naph-8-H, d), 8.29 (1H, naph-7-H, d), 8.58 (1H, naph-5-H, s), 7.40-8.80 (2H, br, s).

I.R. (KBr) : 2965, 2665, 1718, 1574, 1284, 1267, 1107 and 770 cm^{-1}.

U.V. (CH_3OH) λ, ε : (213, 25064, Sharp), (244, 48076, Sharp), (286, 4110, Broad), (338, 2876, Broad).

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H.P.L.C. : Column: 250 mm x 4 mm RP select B, 5 μM

Mobile Phase: Methanol+0.1% Et_3N.

Flow Rate: 1ml/min.

Area of the main peak: 98.96% and 0.5% at 3.09 and 2.65 minutes respectively.

1-(2,3-Epoxypropoxy)-6-(carboisopropoxy)naphthalene (100)

A solution of **91** (4.50g, 0.02 mole), epichlorohydrin (45.73g, 0.5 mole) and 1-methylpiperazine (0.10g, catalytic amount) was heated to 95-100 °C under nitrogen until no starting material was detected (~30 mins.) [4]. Epichlorohydrin was removed under reduced pressure to give a straw coloured viscous oil. The product was analysed by T.L.C. (2% CH_3OH in $CHCl_3$) and found to contain some of the by-product **98a** (10-15%). This was not further purified. The mixture was heated in isopropanol and then with ether under reduced pressure to azeotropically remove the remaining traces of epichlorohydrin. The resulting product, after drying at 50 °C and 0.5 mmHg for 4 hours, was obtained as a light brown solid, (0.34g).

M.S. m/z : 286 (M^+,100%) 244 (M^+-42, 6%), 227 (M^+-59, 32%), 201 (M^+-85, 7%), 188 (M^+-98, 71%), 171 (M^+-115, 18%), 159 (M^+-127, 16%), 142 (M^+-144, 15%), 115 (M^+-171, 35%).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.39 (6H, d), 2.75 (1H, m), 2.96 (1H, m), 3.48 (1H, m), 4.14 (1H, m), 4.42 (1H, dd), 5.31 (1H, m), 6.91 (1H, d), 7.42 (1H, t), 7.55 (1H, d), 8.05 (1H, d), 8.33 (1H, d), 8.54 (1H, s).

I.R. (KBr) : 2978, 2931, 1711, 1371, 1281, 1268, 1178, 1100 and 770 cm^{-1}.

1-(2,3-Epoxypropoxy)-6-(carbomethoxy)naphthalene (101)

A solution of **92** (0.90g, 4.46 x 10^{-3} mole) in epichlorohydrin (10.30g, 0.11 mole) containing 1-methylpiperazine (0.06g, catalytic amounts) was refluxed under nitrogen for 30 minutes [4]. Epichlorohydrin was removed in vacuo to give a straw coloured oil. This, after drying at high vacuum, solidified. The impure product was recrystallised from small amounts of hot methanol but still contained some side-product 99a, (10~15%). The epoxide was not further purified, but dried at R.T. and 0.1 mmHg for 4 hours (0.7g).

1-[3-(N-Isopropylamino)-2-hydroxypropoxy]-6-(carbo-isopropoxy)naphthalene (104)

A solution of **100** (0.60g, ~2.0 x 10^{-3} mole), isopropylamine (0.37g, 6.3 x 10^{-3} mole) and tri-butylamine (2 drops) in methanol (25 ml) was introduced into a Berghof pressure vessel. The vessel was closed and heated to 65 °C for 16 hours. The reaction was stopped and the pressure vessel allowed to cool to room temperature. The solvent and excess amine were removed under reduced pressure and the solid residue chromatographed on silica, eluting with 15% v/v CH_3OH in ethylacetate. The solvent was removed and the resulting residue recrystallised from petrol (20 ml/0.1g of compound) to give the amine as white needles. This was dried at 70 °C, 0.1 mmHg for 8 hours to weigh 0.44g (61%).

M.P. 113-115 °C

Analysis :	Calculated for $C_{20}H_{27}NO_4$:	C, 69.54, H, 7.88, N, 4.05
	Found:	C, 69.64, H, 7.80, N, 4.14

M.S. m/z : 345 (M^+, 1%), 330 (M^+- 15, 3%), 301 (M^+-44, 1%), 286 (M^+-59, 5%), 272 (M^+-73, 1%), 72 (M^+-173, 100%).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.11 [6H, $NCH(C\underline{H}_3)_2$, d], 1.42 [6H, $OCH(C\underline{H}_3)_2$, d], 2.82-2.91 (1H, NCH, m), 2.87-3.02 (2H, CH_2N, m), 4.13-4.24 (2H, OCH_2, m), 4.13-4.24 (1H, C<u>H</u>OH m), 5.32 (1H, OCH, m), 6,93 (1H, naph-2-H, d), 7.43 (1H, naph-3-H, t), 7.54 (1H, naph-4-H, d), 8.05 (1H, naph-8-H, d), 8.27 (1H, naph-7-H, d), 8.54 (1H, naph-5-H, s).

I. R. (KBr) : 2972, 1715, 1270, 1104 and 770 cm^{-1}.

U.V. (CH_3OH) λ, ε : (214 nm, 24066, Sharp), (244 nm, 49569, Sharp), (283 nm, 3951, Broad), (335 nm, 2694, Broad).

H.P.L.C. : Column: 100 mm x 4.9 mm, Spherisorb Ods2, 5 μM + 50 ml guard.
Mobile Phase: Pump B; CH_3OH + 4.9% HSA + 0.5g/100 ml citric acid + 0.1% Et_3N. Pump A; H_2O + 4.9% HSA + 0.5g/100 ml citric acid + 0.1 Et_3N. Pump B:Pump A, 30:70.
Flow Rate: 2 ml/min.
Area of the main peaks: 97.51% and 2.49% at 3.41 and 1.95 mins. respectively.

1-[3-(N,N-Dipropylamino)-2-hydroxypropoxy]-6-(carbo-isopropoxy)naphthalene hydrogen oxalate (105)

A solution of **100** (0.4g, 1.4 x 10^{-3} mole), di-n-propylamine (0.24g, 2.37 x 10^{-3} mole) and tributylamine (2 drops) in methanol (20 ml) was refluxed for 24 hours under nitrogen [4]. The solvent and the excess amine were removed in vacuo and the residue was chromatographed on

silica, eluting with 5% v/v CH_3OH in $CHCl_3$. The solvent was removed and a clear viscous oil was obtained, (0.40g, 74%). The oil was dissolved in anhydrous ether (30 ml). To this was added dropwise with stirring , a saturated solution of oxalic acid in ether until an excess of the acid was present. The mixture was cooled and the salt collected by filtration. Recrystallisation from ethyl acetate gave the compound as colourless needles, which was dried at 70 °C and 0.1 mmHg for 8 hours (0.40g, 81%).

M.P. 138-140 °C

Analysis : Calculated for $C_{25}H_{35}NO_8$: C, 62.88, H, 7.39, N, 2.98
Found: C, 62.66, H, 7.21, N, 2.78

M.S. m/z : 386 (M^+-1, 0.02%), 358 (M^+-29, 4%), 328 (M^+-49, 2%), 230 (M^+-157, 0.4%), 188 (M^+-199, 1.4%), 158 (M^+-229, 2%), 114 (M^+-273, 100%).

^{1}H N.M.R. (DMSO, 200 MHz) : δ 0.83 (6H, $CH_2CH_2C\underline{H}_3$, t), 1.38 [6H, $CH(C\underline{H}_3)_2$, d], 1.70 (4H, $CH_2C\underline{H}_2CH_3$, m), 3.06 (4H, $C\underline{H}_2CH_2CH_3$, m), 3.14-3.40 (2H, $CHC\underline{H}_2N$, m), 4.18 (2H, OCH_2, m), 4.40 (1H, $OCH_2C\underline{H}$, m), 5.20 [1H, $OC\underline{H}(CH_3)_2$, m], 7.12 (1H, naph-2-H, d), 7.52 (1H, naph-3-H, t), 7.72 (1H, naph-4-H, d), 7.96 (1H, naph-8-H, d), 8.34 (1H, naph-7-H, d), 8.54 (1H, naph-5-H, s).

I.R. (KBr) : 2971, 1715, 1608, 1268, 1198, 1110 and 772 cm^{-1}.

U.V. (CH_3OH) λ, ε : (212, 22450), (244, 49000, Sharp), 285, 2857, Broad), (388, 2296, Broad).

H.P.L.C. : Column: 250x4.9 mm Spherisorb CN + 5 μM

Mobile Phase: CH_3OH/H_2O, 80/20 + 0.1% Et_3N

Flow Rate: 2 ml/min.

Area of the main peak: 100% at 5.99 minutes.

1-[3-(N-Methylamino)-2-hydroxypropoxy]-6-(carbo-isopropoxy)naphthalene hydrogen oxalate (102)

A solution of **100** (0.30g, ~1.0 x 10^{-3} mole) in methanol (15 ml) was placed into the PTFE-liner of a Berghof pressure vessel and cooled to below -25 °C. Methylamine (10 ml, excess) was condensed in methanol (-30 °C) containing tri-butylamine (2 drops) and then transferred to the pressure vessel. The vessel was heated to 65 °C for 10 hours and then cooled to to R.T. The solvent was removed under reduced pressure and the resulting residue was chromatographed on silica, eluting with 20% v/v CH_3OH in ethyl acetate. The residue, after removal of solvent, appeared to be hygroscopic and was dried over P_2O_5 at R.T. and 0.1 mmHg for 4 hours. A solution of the solid (0.18g) in ethyl acetate (10 ml) was treated with a saturated solution of oxalic acid in ethyl acetate until an excess of the acid was present. The mixture was kept cold for several hours and the salt was collected by filtration. Recrystallisation from a mixture of ethyl acetate and absolute ethanol (and, if needed some ether) gave the product as colourless needles (0.06g, 40%).

M.P. 144-147 °C

Analysis : Calculated for $C_{20}H_{27}NO_8$: C, 59.45, H, 6.19, N, 3.44

Found: C, 60.46, H, 6.71, N, 3.92

M.S. m/z : 317 (M^+, 6%), 273 (M^+-44, 6%), 258 (M^+-59, 10%), 230 (M^+-87, 50%), 202 (M^+-115, 6%), 188 (M^+-129, 25%), 171 (M^+-148, 14%), 44 (M^+-273, 100%).

^{1}H N.M.R. (DMSO, 200 MHz) : δ 1.37 [6H, CH(C<u>H</u>$_3$)$_2$, d], 2.71 (3H, NCH$_3$, s), 3.09-3.30 (2H, CH$_2$N, m), 4.17 (2H, OCH$_2$, m), 4.36 (1H, C<u>H</u>CH$_2$, m), 5.21 (1H, C<u>H</u>(CH$_3$)$_2$, m), 7.13 (1H, naph-2-H, d), 7.53 (1H, naph-3-H, t), 7.62 (1H, naph-4-H, d), 7.97 (1H, naph-8-H, d), 8.38 (1H, naph-7-H, d), 8.57 (1H, naph-5-H, s).

I.R. (KBr) : 3362, 2981, 2757, 1710, 1433, 1386, 1370, 1273, 1194, 1178, 1105 and 774 cm^{-1}.

U.V. (CH$_3$OH) λ, ε : (214, 28460, Sharp), (244, 59478, Sharp), (284, 4880, Broad), (337, 3558, Broad).

H.P.L.C. : Column: 100x4.9 mm Spherisorb Ods2 5μM + 50 ml Guard column.
Mobile Phase: Pump B; CH$_3$OH + 4.9% HSA + 0.1% Et$_3$N + 0.5g/100 ml citric acid. Pump A; H$_2$O + 4.9% HSA + 0.1% Et$_3$N + 0.5g/100 ml citric acid, Pump B : Pump A, 65 : 35.
Flow Rate: 2 ml/min.
Area of the main peaks: 89.30% and 10.69% at 4.38 and 2.24 minutes respectively.

1-[3-(N,N-Dimethylamino)-2-hydroxypropoxy]-6-(carbo-isopropoxy)naphthalene hydrogen oxalate (103)

A solution of **100** (0.35g, ~1.2 x 10^{-3} mole), in methanol (15 ml) was chilled to 0 °C. To this was added, a cold solution of dimethylamine (10 ml, excess) in methanol (15 ml). The solution was introduced into a Berghof pressure vessel and heated to 65 °C. The solvent and the excess amine were removed under reduced pressure and the resulting

residue was chromatographed on silica, eluting with 40% CH_3OH in $CHCl_3$. The solvent was removed and a colourless oil obtained, (0.21g).

A solution of the oil (0.21g, 6.34 x 10^{-4} mole) in anhydrous ether (25 ml) was treated with a saturated solution of oxalic acid in isopropanol until an excess of the acid was present. The mixture was cooled and the salt collected by filtration. The product was recrystallised from isopropanol and dried at 80 °C and 0.1 mmHg for 8 hours, (0.18g, 67%).

M.P. 149-151 °C

Analysis : Calculated for $C_{21}H_{27}NO_4$: C, 59.85, H, 6.46, N, 3.32
Found: C, 59.79, H, 6.41, N, 3.25

M.S. m/z : 331 (M^+, 1%), 272 (M^+-59, 4%), 230 (M^+-101, 1%), 188 (M^+-143, 2%), 171 (M^+-160, 1%), 142 (M^+-185, 1%), 58 (M^+-273, 100%).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.38 [6H, $CH(C\underline{H}_3)_2$, d], 2.88 (6H, NCH_3, s), 3.22-3.48 (2H, CH_2N, m), 4.18 (2H, OCH_2, m), 4.46 (1H, C<u>H</u>OH, m), 5.22 (1H, OCH, m), 7.14 (1H, naph-2-H, d), 7.44 (1H, naph-3-H, t), 7.62 (1H, naph-4-H, d), 8.00 (1H, naph-8-H, d), 8.42 (1H, naph-7-H, d), 8.57 (1H, naph-5-H, s).

I.R. (KBr) : 3400, 2981, 1710, 1271, 1177, 1105 and 775 cm^{-1}.

U.V. (CH_3OH) λ, ε : (213, 24747, Sharp), (244, 49410, Sharp), (284, 4223, Broad), (337, 2787, Broad).

H.P.L.C. : Column: 100x4.9 mm Spherisorb Ods2 5μM + 50 ml Guard column.

Mobile Phase: Pump B; CH_3OH + 4.9% HSA + 0.1% Et_3N + 0.5g/100 ml citric acid. Pump A; H_2O + 4.9% HSA + 0.1% Et_3N + 0.5g/100 ml citric acid, Pump B : Pump A, 70 : 30.

Flow Rate: 1ml/min.

Area of the main peak: 98.42% at 7.41 min.

1-[3-(N-Isopropylamino)-2-hydroxypropoxy]-6-(carbomethoxy)naphthalene (106)

A solution of **101** (0.60g, ~2.3 x 10^{-3} mole), isopropylamine (0.41g, 7.0 x 10^{-3} mole, excess), tri-butylamine (2 drops) in methanol (15 ml) was introduced into a Berghof pressure vessel. The vessel was heated to 70 °C for 8 hours and then allowed to cool to R.T. The solvent and the excess amine were removed under reduced pressure and the resulting residue was chromatographed on silica, eluting with 30% v/v $CHCl_3$ in CH_3OH. After the removal of the solvent, the crude product was recrystallised from petrol to give colourless needles, (0.48g).

M.P. 104-105 °C

Analysis : Calculated for $C_{18}H_{23}NO_4$: C, 68.12, H, 7.30, N, 4.41

Found: C, 67.56, H, 7.02, N, 4.36

M.S. m/z : 317 (M^+, 2%), 302 (M^+-15, 3%), 286 (M^+-31, 2%), 273 (M^+-44, 2%), 202 (M^+-115, 5%), 171 (M^+-146, 6%), 142 (M^+-175, 3%), 114 (M^+-203, 7%), 72 (M^+-245, 100%).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.10 (6H, $CH(C\underline{H}_2)_2$, d), ? (2H, CH_2N, m), ? (1H, C<u>H</u>OH, m), ? (1H,NCH, m), 3.95 (3H, OCH_3, s), 4.16 (2H, $OC\underline{H}_2$, m), 6.92 (1H, naph-2-H, d), 7.42 (1H,

naph-3-H, t), 7.54 (1H, naph-4-H, d), 8.04 (1H, naph-8-H, d), 8.28 (1H, naph-7-H, d), 8.54 (1H, naph-5-H, s).

I.R. (KBr) : 3280, 2923, 1723, 1580, 1510, 1442, 1370, 1277, 1188, 1108, 1072 and 770 cm^{-1}.

U.V. (CH_3OH) λ, ε : (214 nm, 22464, Sharp), (244 nm, 47960, Sharp), (283 nm, 3209, Broad), (338 nm, 2246, Broad).

H.P.L.C. : Column: 100 x 4.9 mm Spherisrob Ods2, 5µM + 50 ml Guard column.
Mobile Phase: Pump B; CH_3OH + 4.9% HSA + 0.1% Et_3N + 0.5g/100 ml citric acid. Pump A; H_2O + 4.9% HSA + 0.1% Et_3N + 0.5g/100 ml citric acid.
Pump B : Pump A; 60 : 40
Flow rate: 1 ml/min.
Area of the main peak: 99.4% at 9.68 min.

1-[3-(N-Isopropylamino)-2-hydroxypropoxy]-6-(amino-carbonyl)naphthalene (107)

Method 1

1-[3-(N-Isopropylamino)-2-hydroxypropoxy]-6-(carbomethoxy)naphthalene **106** (0.050g, 1.60 x 10^{-4} mole), Liquid ammonia (25 ml) and ether (25 ml) were stirred at R.T. for 8 hours in a Berghof pressure vessel. However, no reaction was observed. The vessel was then heated to 50 °C, 75 °C and 100 °C for intervals of 8 hours, but the starting material remained unconsumed. This method was therefore abandoned.

Method 2

Conc. ammonia (sp. gr. 0.880, 100 ml) was added to a solution of **106** (0.30g, 9.4 x 10^{-4} mole) in dil. HCl (20 ml, 2 molar). The flask was stoppered and left to stand at R.T. for 5 days, with occasional shaking. The volume of the solution was halved under reduced pressure and its pH adjusted to 11±1 with dil. KOH. The solution was then extracted 4 times with ethyl acetate (150 ml portions) and the combined extracts dried with $MgSO_4$. Ethyl acetate was removed in vacuo and the resulting solid recrystallised from toluene to yield the product as a crystalline solid (0.25g, 88%).

M.P. 151-153 °C

Analysis : Calculated for $C_{17}H_{22}N_2O_3$: C, 67.53, H, 7.34, N, 9.26
Found: C, 67.54, H, 7.32, N, 9.01

M.S. m/z : 303 (MH^+, 4%), 302 (M^+, 4%), 287 (M^+-15, 7%), 270 (M^+-32, 1%), 258 (M^+-44, 9%), 187 (M^+-115, 12%), 142 (M^+-160, 6%), 72 (M^+-230, 100%).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.11 [6H, $CH(C\underline{H}_3)_2$, d], 2.78-2.94 (1H, NCH, m), 2.78-3.08 (2H, CH_2N, m), 4.11-4.33 (1H, C<u>H</u>OH, m), 4.11-4.23 (2H, OCH_2, m), 5.64-6.24 (2H, NH_2, br, m), 6.94 (1H, naph-2-H, d), 7.44 (1H, naph-3-H, t), 7.52 (1H, naph-4-H, d), 7.58 (1H, naph-8-H, d), 8.34 (1H, naph-7-H, d), 8.35 (1H, naph-5-H, s).

I.R. (KBr) : 3357, 3287, 3152, 2963, 1684, 1597, 1576, 1465, 1393 and 1271 cm^{-1}.

U.V. (CH3OH) λ, ε : (214, 27293, Sharp), (244, 43074, Sharp), (285, 3999, Broad), (356, 2324, Broad).

H.P.L.C. : Column: 100 x 4.9 mm Spherisorb Ods2 5 μM + 50 ml Guard column.
Mobile Phase: Pump B; CH_3OH + 4.9% HSA + 0.1% Et_3N + 0.5g/100 ml citric acid. Pump A; H_2O + 4.9% HSA + 0.1% Et_3N + 0.5g/100 ml citric acid, Pump B : Pump A, 30 : 70.
Flow Rate: 2 ml/min.
Area of the main peak: 99.24% at 9.28 minutes.

1-[3-(N-Isopropylamino)-2-hydroxypropoxy]-6-(methyl-aminocarbonyl)naphthalene (108)

25-30% Aqueous methylamine (150 ml) was added to a solution of **106** (0.50g, 1.58 x 10^{-3} mole) in dil. HCl (20 ml, 2 molar) in a R.B. flask. The flask was stoppered and left to stand at R.T. for 5 days, with occasional shaking. The volume of the solution was halved in vacuo and its pH was adjusted to 11±1 with dil. KOH. The solution was extracted 3 times with ethyl acetate (400 ml altogether) and the combined extracts dried with $MgSO_4$. The solvent was removed under reduced pressure and the resulting residue was recrystallised from a mixture of $CHCl_3$ and Et_2O to yield the product as a crystalline solid (0.4g, 82%).

M.P. 127-128 °C

Analysis : Calculated for $C_{18}H_{24}N_2O_3$: C, 68.65, H, 7.65, N, 8.85
Found: C, 68.06, H, 7.76, N, 8.72

M.S. m/z : 317 (M^+,7%), 316 (M^+-1, 4%), 302 (M^+-15, 6%), 201 (M^+-116, 18%), 171 (M^+-146, 15%), 142 (M^+-175, 11%), 114 (M^+-203, 21%), 72 (M^+-245, 100%).

^{1}H N.M.R. (DMSO, 400 MHz) : δ 1.10 [6H, $CH(C\underline{H}_3)_2$, d], 2.76-2.93 (1H, $C\underline{H}(CH_3)_2$, m), 2.76-3.06 (2H, CH_2N, m), 3.07 (3H, NCH_3, d), 4.04-4.26 (1H, C<u>H</u>OH, m), 4.04-4.26 (2H, OCH_2, m), 6.35 (1H, N<u>H</u>CH_3, br., s), 6.90 (1H, naph-2-H, d), 7.41 (1H, naph-3-H, t), 7.50 (1H, naph-4-H, d), 7.79 (1H, naph-8-H, d), 8.23 (1H, naph-5-H, s), 8.28 (1H, naph-7-H, d).

I.R. (KBr) : 3356, 3271, 2963, 1643, 1629, 1549, 1435, 1312, 1253, 1101 and 767 cm^{-1}.

U.V. (CH_3OH) λ, ε : (218 nm, 28838, Sharp), (240 nm, 42846, Sharp), (284 nm, 4394, Broad), (327 nm, 2307, Broad).

H.P.L.C. : Column: 100 x 4.9 mm Spherisorb Ods2, 5μM + 50 ml Guard column.
Mobile Phase: Pump B; CH_3OH + 4.9% HSA + 0.1% Et_3N + 0.5g/100 ml citric acid. Pump A; H_2O + 4.9% HSA + 0.1% Et_3N + 0.5g/100 ml citric acid, Pump B : Pump A, 30 : 70.
Flow Rate: 2 ml/min.
Area of the main peak: 99.02% at 9.00 minutes.

1-[3-(N-Isopropylamino)-2-hydroxypropoxy]-6-(N,N-dimethylamino carbonyl)naphthalene hydrochloride (109)

N,N-Dimethylamine (chilled to -5 °C,100 ml) was added to cold a solution of 106 (0.50g, 1.58 x 10^{-3} mole) in dil.HCl (20 ml, 2 mole) in a R.B. flask. The flask was stoppered and left to stand at R.T. for 5 days, with

occasional shaking. The volume of the solution was halved in vacuo and its pH was adjusted to 11±1 with dil. KOH. The solution was extracted twice with ethyl acetate (200 ml altogether) and the combined extracts dried with $MgSO_4$. Ethyl acetate was removed and the resulting residue was dried over P_2O_5 at R.T. and 0.1 mmHg for 24 hours (0.41g, 79%). A solution of the product (0.35g, 0.001 mole) in anhydrous ether was treated with a saturated solution of dry hydrogen chloride in ether until an excess of the acid was present. The mixture was kept cold for several hours and the hydrochloride salt was removed by filtration under a blanket of dry nitrogen since it appeared to be very hygroscopic. The hydrochloride salt was dried over P_2O_5 at 50 °C and 0.1 mmHg (0.28g, 76%).

M.P. Foamed at 75 °C onward

Analysis : Calculated for $C_{19}H_{27}ClN_2O_3$: C,61.00,H,7.49,Cl,9.48,N,7.49
Found: C,60.89,H,7.48,Cl,9.88,N,7.37

M.S. m/z : 331 (MH^+, 2%), 330 (M^+, 2%), 315 (M^+-15, 6%), 286 (M^+-44, 15%), 215 (M^+-115, 44%), 171 (M^+-159, 21%), 142 (M^+-188, 10%), 72 (M^+-258, 100%).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.52 [6H, $CH(C\underline{H}_3)_2$, m], 2.96 (3H, NCH_3, s), 3.14 (3H, NCH_3, s), 3.40 (2H, CH_2N, m), ? (1H, $CH(C\underline{H}_3)_2$, m), 4.13 (2H, OCH_2, m), 4.81 (1H, $C\underline{H}OH$, m), 5.68 (1H, OH, br., s) 6.70 (1H, naph-2-H, d), 7.26-7.50 (3H, naph-3, 4, 7-H, m), 7.83 (1H, naph-5-H, s), 8.30 (1H, naph-8-H, d), 8.68 (1H, br., s), 9.68 (1H, br., s).

I.R. (KBr) : 3370, 2973, 1612, 1573, 1431, 1394, 1271, 1175, 1109 and 768 cm^{-1}.

References, Page 102, Structures & Abbreviations, Page 205

U.V. (CH_3OH) λ, ε : (214, 32880, Sharp), (237, 35443, Sharp), (290, 4697, Broad), (324, 1708, Shoulder).

H.P.L.C. : Column: 100x4.9 mm Spherisorb Ods2 5μM + 50 ml Guard column.

Mobile Phase: Pump B; CH_3OH + 4.9% HSA + 0.1% Et_3N + 0.5g/100 ml citric acid. Pump A; H_2O + 4.9% HSA + 0.1% Et_3N + 0.5g/100 ml citric acid, Pump B : Pump A, 37.5 : 62.5.

Flow Rate: 2 ml/min.

Area of the main peaks: 96.31%,1.62%, 1.18%, and 0.88% at 9.80, 5.84, 3.81 and 12.29 mins. respectively.

2.5. References

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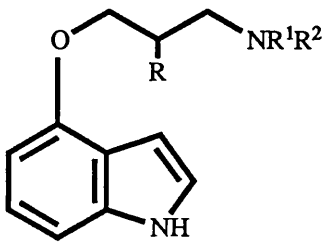
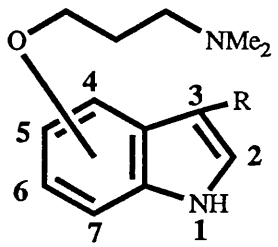
CHAPTER THREE

DESIGN, SYNTHESIS AND SAR OF PINDOLOL ANALOGUES

3.1. Introduction



TYPE 1 ANALOGUES



TYPE 2 ANALOGUES

This chapter is divided into parts A and B. The first part of the chapter is concerned with the design and synthesis of some novel analogues of pindolol (type 1), while the second part deals mainly with the synthesis of the 3-substituted pindolol derivatives (type 2). The purpose of the present investigation was to modify the structure of pindolol in such a manner as to abolish or decrease its affinity for β-adrenergic receptors, while at the same time, retaining its affinity for 5-HT_{1A} and 5-HT_{1B} receptors.

Initially, a series of pindolol analogues were prepared in a multi-step synthesis from 4-hydroxyindole **116**. This work led to the identification of our first selective 5-HT_{1A} agent **118**, possessing one tenth of affinity of pindolol.

Attempts were then made to increase 5-HT_{1A} affinity by preparing a series of type 2 analogues. This is discussed in part B together with structure-activity relationships found for all the analogues at 5-HT_{1A} and 5-HT_{1B} receptors.

References, Page 142, Structures & Abbreviations, Page 205

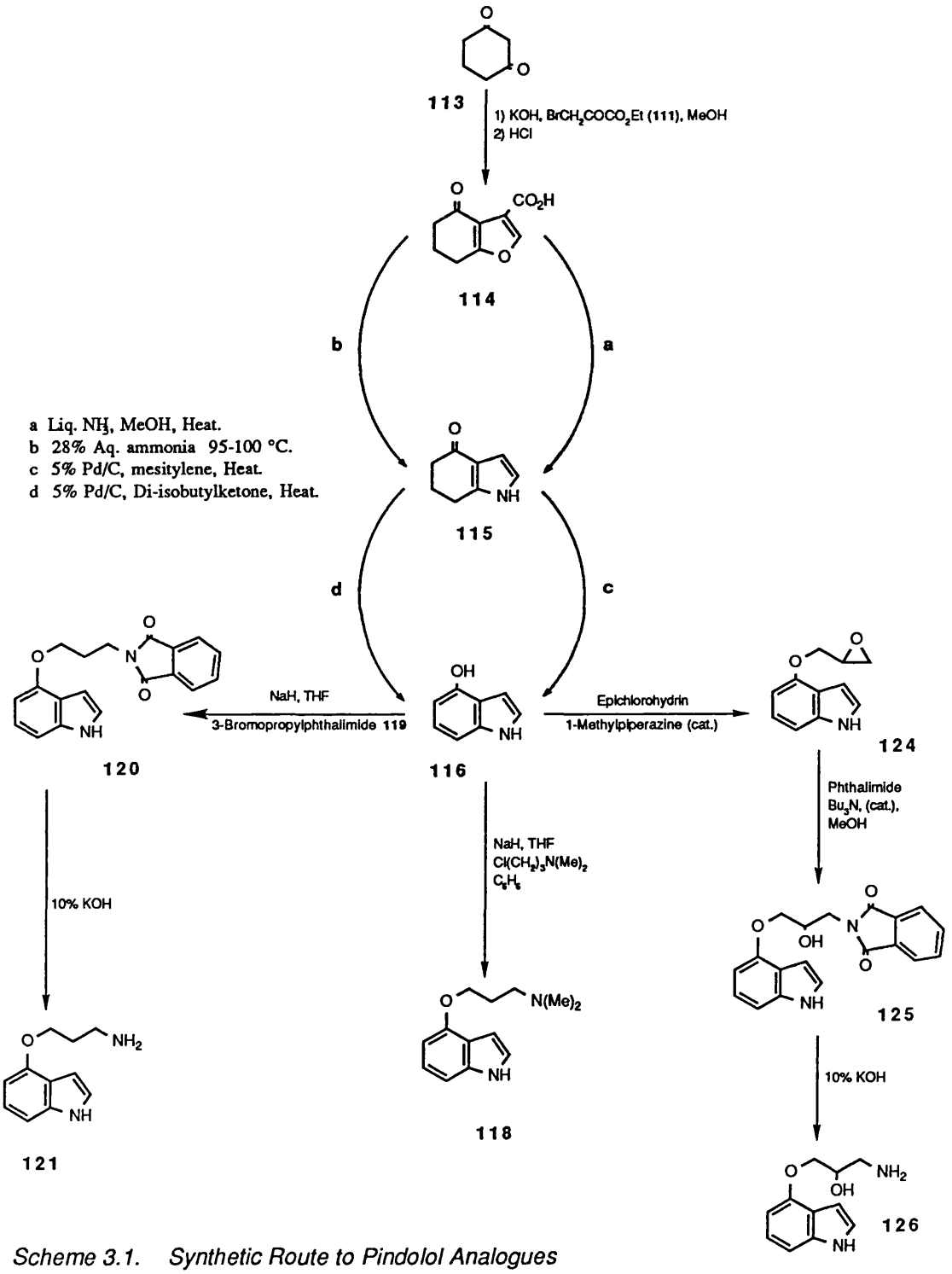
3.2. Part A : Strategy

The β-adrenergic antagonist pindolol **27** has been consistently shown to behave as a potent 5-HT_{1A} and 5-HT_{1B} antagonist (discussed in section 1.6.2.), although it binds with higher affinity (about 3 times) at β-adrenoceptors. Pindolol also binds with a higher affinity than propranolol at 5-HT_{1A} and 5-HT_{1B} sites. It is the only example of an antagonist which binds with some selectivity for 5-HT_{1A} v's 5-HT_{1B} receptors and as such represents one of the few structural leads that might be exploited for the development of novel selective 5-HT_{1A} antagonists.

Several analogues of pindolol (type 1) which incorporate terminal primary and tertiary amines, with and without the side chain hydroxyl group were synthesized and tested. The strategy for the modification of the aminopropoxy chain of pindolol was based on similar observations as those discussed for propranolol analogues (sections 1.6.2.1., 1.7. and 2.2.). For example, terminal tertiary amines have significantly reduced affinity at 5-HT_{1B} and β-adrenergic sites while they are well tolerated at 5-HT_{1A} sites. It was also of great interest to prepare analogues which incorporate a terminal primary amine, since this functionality is present in 5-HT.

3.3. Synthesis of Pindolol Analogues

Scheme 3.1. represents the overall synthetic pathway to the desired analogues of pindolol.



Scheme 3.1. Synthetic Route to Pindolol Analogues

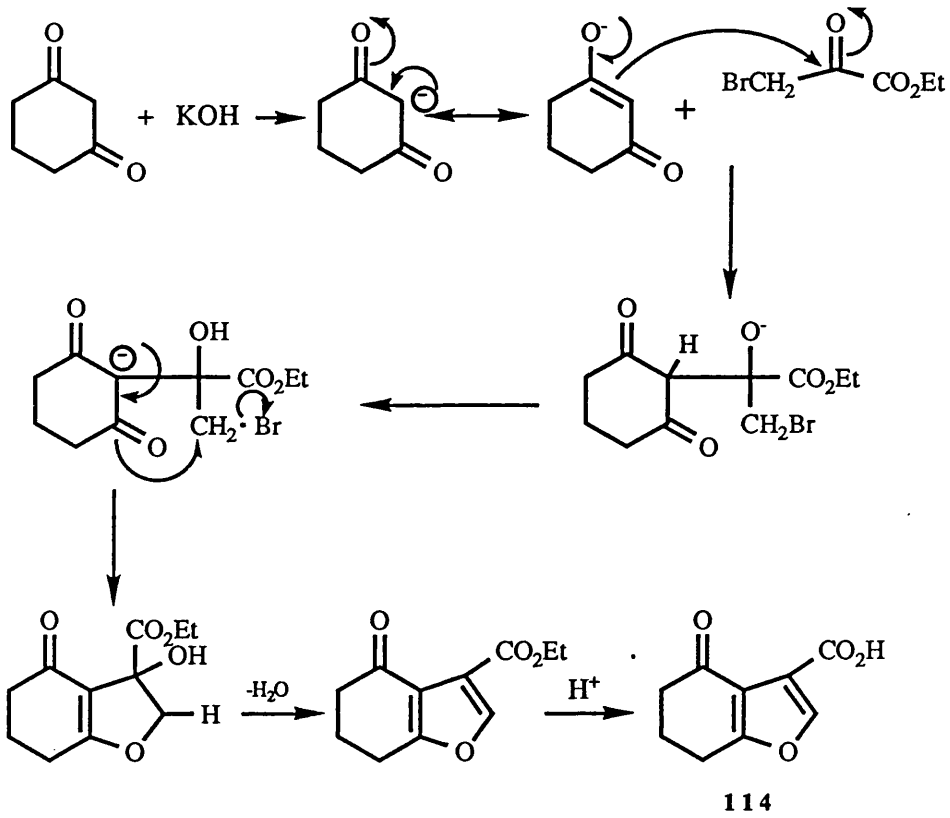
The key intermediate in the synthesis of these analogues is

4-hydroxyindole **116**. Although 4-hydroxyindole is commercially available, the high price for this reagent meant that its synthesis had to be investigated. The preparation of this reagent was successfully carried out in good yields, by employing recently described reaction conditions.

The analogues which incorporate terminal primary amine **121** and **126** were conveniently prepared via the Gabriel phthalimide synthesis. The analogues without the side chain hydroxyl group **118** and **120** were synthesized from the condensation reaction of 4-hydroxyindole and the respective haloalkylamine reagents.

3.3.1. Preparation of 4-Hydroxyindole

The synthesis of 4-hydroxyindole **116** has been extensively described [1,2,3], but the overall yields are generally reported to be poor. In 1948, Beer and Co-workers [1] achieved the synthesis of 4-hydroxyindole in 15% yield from β-dinitro-6-acetoxystyrene **112**. Later, Setter and Lauterbach [2], and Plienger and Kliga [3] reported an alternative synthetic route, starting from readily available cyclohexane-1,3-dione **113**. They obtained 4-hydroxyindole in 37% overall yield by employing the conditions described in methods a & b as shown in scheme 3.1. The mechanism for the formation of **114** is believed to proceed as shown in figure 3.1.



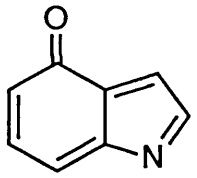
114

Figure 3.1. Reaction Mechanism for the Formation of 114.

We initially prepared **116** by repeating the procedure of Beer et al using the conditions a and c, but obtained lower yields than those reported for

all three compounds, with an overall yield of 25%. The preparation of 4-oxo-tetrahydroindole **115** was also difficult to achieve since this procedure requires the use of a suitable autoclave or a pressure vessel.

Soon after the preparation of 4-hydroxyindole by this method, reports by several Japanese patents, [4-7] describing modifications for the synthesis of the intermediates **114** and **115** appeared in Chemical Abstracts. These modifications (as described by methods b & d) were tried and found to greatly increase the overall percentage yield of 4-hydroxyindole. Thus addition of 28% aq. ammonia at 100 °C in place of liquid ammonia gave **115** in 89% yield. Replacing mesitylene with the higher boiling solvent diisobutyl ketone in the dehydrogenation step gave **116** in 83% yield. 4-Hydroxyindole was stable under these conditions and did not further dehydrogenate to the quinone form **117**.



117

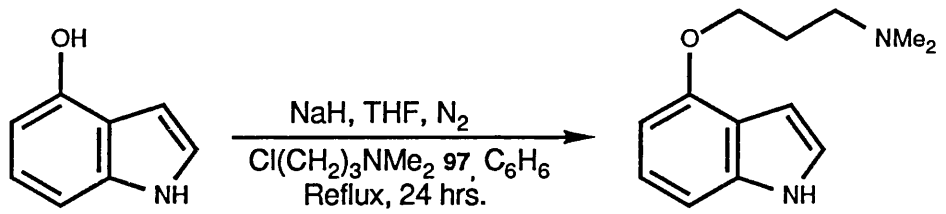
Purification of **116** was best achieved, either by recrystallisation from small quantities of chloroform or by column chromatography with chloroform : ether (3:2) as eluant. 4-hydroxyindole is light sensitive and therefore it was necessary to exclude light from all operations.

It is worth pointing out that the synthesis of 4-hydroxyindole was recently achieved [8] in one step from a radical hydroxylation of indole. Thus, treatment of indole with hydrogen peroxide in a phosphate buffer and EDTA di-sodium salt is claimed to give the compound in 50% yield. However, 5-, 6-, 7-hydroxyindole are also formed in this reaction and therefore purification may prove to be a problem.

References, Page 142, Structures & Abbreviations, Page 205

3.3.2. Preparation of 4-[3-(N,N-Dimethylamino)propoxy]-indole

The synthesis of the title compound **118** was achieved in 60% yield, from the condensation reaction of 4-hydroxyindole and N,N-dimethyl-3-chloropropylamine **97** in the presence of sodium hydride in anhydrous THF.



118

The amine hydrochloride **97** was neutralised with 40% sodium hydroxide and extracted with benzene prior to the addition. It was necessary to carry out the reaction with at least three molar equivalents of **97** in order to obtain reasonable yields of the product **118**. The use of excess amine could have given rise to the problem of N-alkylation, but this was avoided by using a 1:1 molar equivalence of NaH.

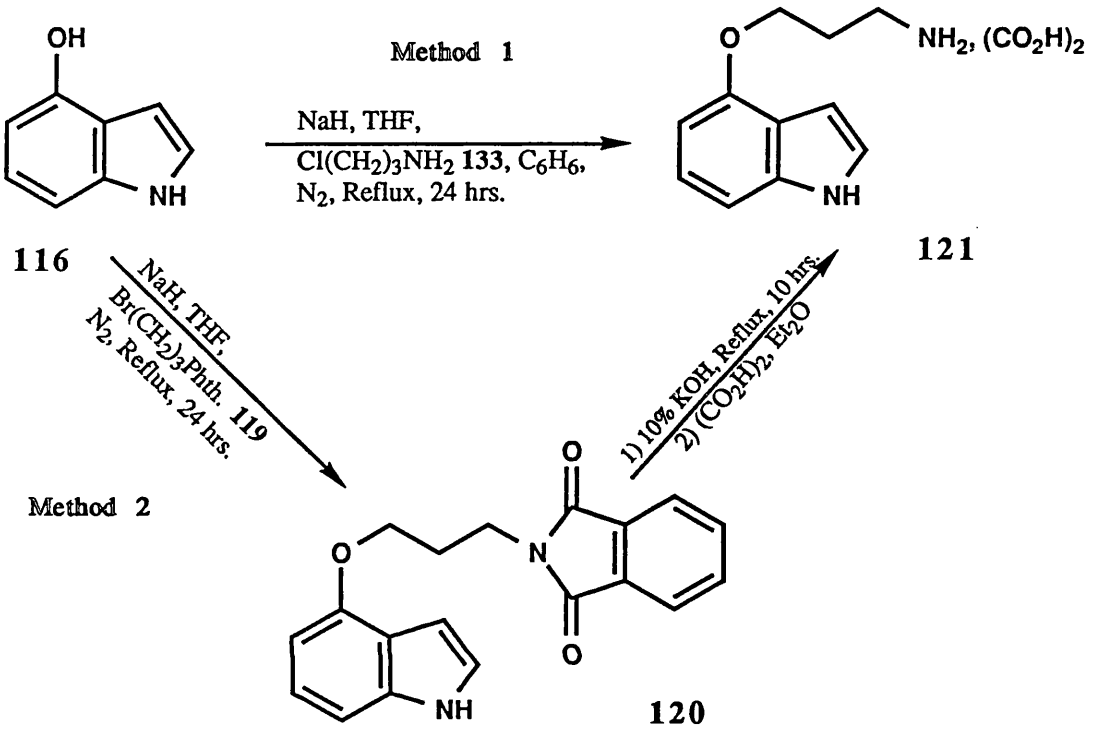
The alkylation of 4-hydroxyindole did not go to completion and about 30% remained unreacted. Attempts to increase the yield from 60% were unsuccessful since the reaction appeared to be independent of reaction time (> 24 hours), more than 3-fold excess amine, change of solvent and of the metal base. However, the unreacted 4-hydroxyindole was conveniently recovered either by extraction procedures or chromatography.

Attempts to increase the water solubility of the product by converting it into its hydrochloride salt also failed. The preparation of the

hydrochloride salt was attempted by treating an etheral solution of **118** with a saturated solution of hydrogen chloride in ether. On addition, a white solid precipitated out. The salt appeared to be extremely hygroscopic and on isolation quickly turned into a dark green paste, tlc analysis of which revealed a multi-component mixture.

3.3.3. Preparation of 4-[3-Aminopropoxy]indole

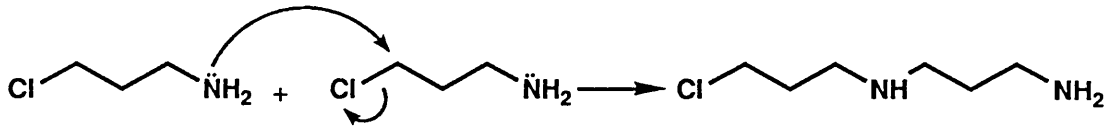
The synthesis of the title compound **121** was initially tried according to the procedure described for the preparation of **118**, i.e. method 1.



Scheme 3.2. Synthesis of 4-(3-Aminopropoxyindole)indole

However, under these conditions **116** failed to react with 3-chloro-propylamine **133** to generate **121**. A likely explanation for this is that 3-chloropropylamine is a better nucleophile than the corresponding tertiary amine **97** used in the previous reaction and consequently on liberation to the free base, it undergoes self condensation either by an intramolecular pathway;

or by an intermolecular pathway;

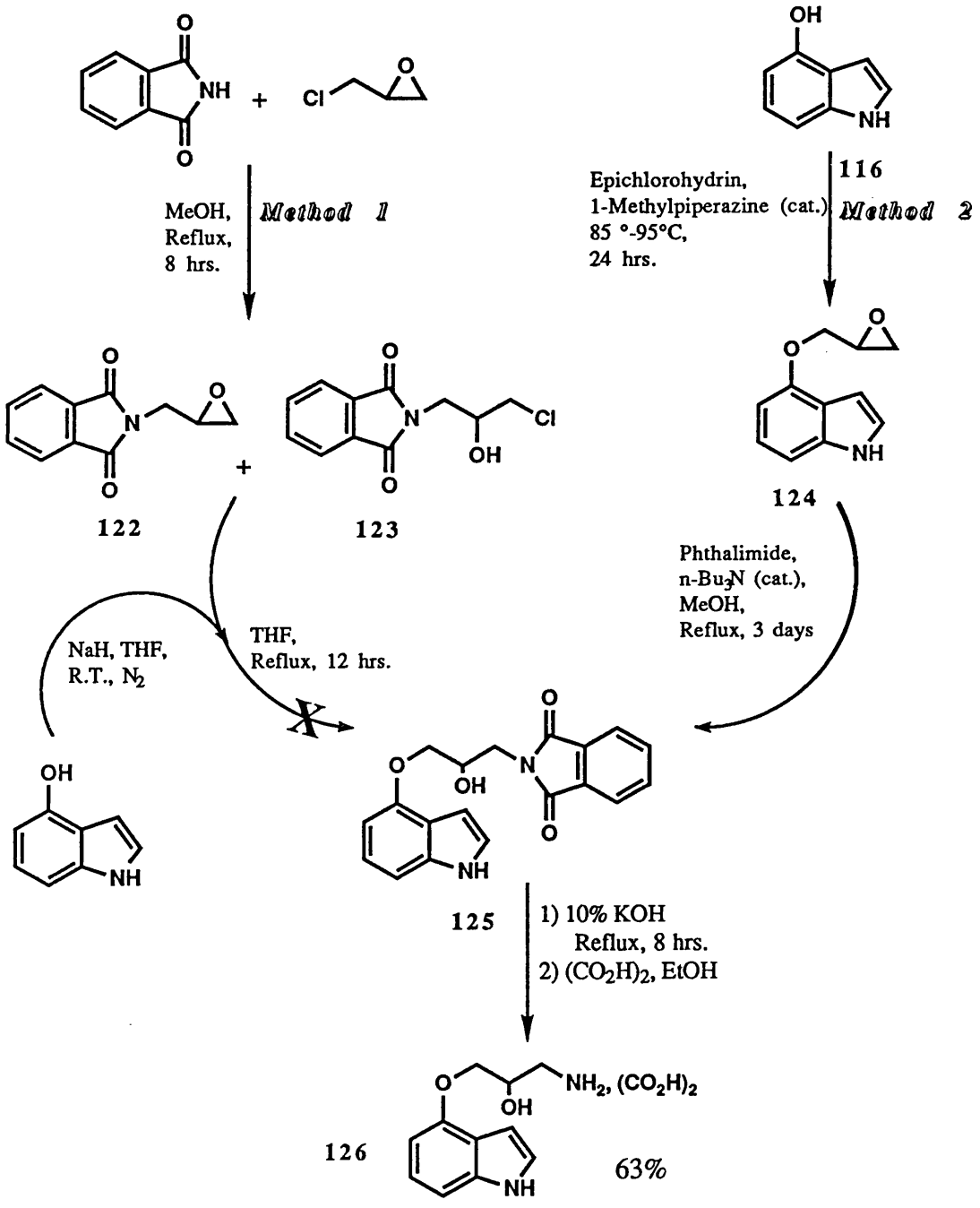


In view of the above, it was decided to employ the Gabriel phthalimide synthetic route for primary amines [9] in which phthalimide acts as a masking or protecting group. This is presented in scheme 3.2.

Treatment of 4-hydroxyindole with 3-bromopropylphthalimide **119** in anhydrous THF and in the presence of NaH afforded **120** in 60% yield. The product was obtained as yellow crystalline flakes. **120** is insoluble in water and alcohols but readily dissolves in DMSO. Hydrolysis of the phthalimide intermediate **120** was achieved in basic conditions following a Japanese procedure described in a Japanese patent [10]. Thus, refluxing **120** in 10% KOH for 12 hours afforded the amine **121** in 63% yield. The product appeared to be hygroscopic and was subsequently converted to its oxalate salt by treating a solution of it in absolute ethanol with a saturated solution of oxalic acid in ether.

3.3.4. Preparation of 4-(3-Amino-2-hydroxypropoxy)indole

The synthesis of 126 was initially attempted according to the procedure shown by method 1 in scheme 3.3. The rational behind this route was the fact that lesser amounts of 4-hydroxyindole are required to prepare the target compound in comparison with other synthetic routes.



Scheme 3.3. Synthesis of 4-[(3-Amino-2-hydroxy)propoxy]indole

Thus, reaction of phthalimide with one molar equivalence of epichlorohydrin in methanol gave the epoxide **122** together with **123** in the approximate ratio of 3 : 1 respectively in about 50% overall yield. The ^1H N.M.R spectrum also showed that the product was a mixture of two components, showing several overlapping signals in the aliphatic region. The E.I.M.S. provided further confirmation of the structure, showing the molecular ions of both components and their fragmentation patterns.

The mixture was not separated into its components, since both compounds were believed to react with 4-hydroxyindole in the next step of the reaction to give the same product (i.e. **125**). However, under similar alkylating conditions as those described for **120**, no reaction was observed. The reason for this is unclear.

In view of the above results, an alternative synthetic strategy was required. Our previous studies with the aminolysis of the epoxides in the naphthalene series indicated that the synthesis of **126** may be achieved by the reaction of phthalimide with **124** and this strategy was therefore adopted. **124** was prepared from the reaction of 4-hydroxyindole with epichlorohydrin as previously described in the naphthalene series. The epoxide **124** was then reacted successfully with excess phthalimide to give **125** in good yields. Recrystallisation of **125** from methanol gave an orange coloured crystalline solid, which contained small amounts of unreacted phthalimide. This was not further purified. The cleavage of the phthalimide was achieved by refluxing **125** in 10% KOH for ten hours. The crude product was purified by column chromatography, eluting with methanol to give a straw coloured gum. The amine was then treated with a saturated

solution of oxalic acid in absolute ethanol to give **126** as a white crystalline solid which appeared to be slightly hygroscopic.

3.4. Part B : Strategy

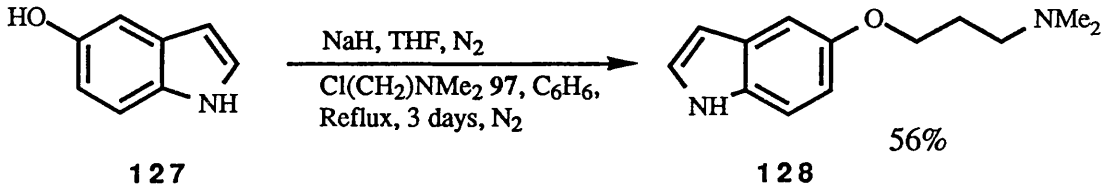
From the binding assays determined for the type 1 series of compounds prepared in the first part of this chapter, the dimethyl amino analogue **118** showed very encouraging results. This agent binds at 5-HT_{1A} receptors with an IC_{50} value of 100 nM, but is essentially inactive at 5-HT_{1B} and β-receptors. The high selectivity of **118** prompted us to use its structure as a template for the development of more potent 5-HT_{1A} agents (i.e. type 2 analogues).

In order to investigate the importance of the position of the dimethyl aminopropoxy chain at the indole ring with respect to 5-HT_{1A} affinity, we also prepared **128** where the side chain was relocated to the 5-position of the indole ring.

It was also decided to prepare analogues which incorporate electron withdrawing substituents such as esters at the 3-position of the indole ring. The latter strategy was based upon the observation that iodination of cyanopindolol at the 3-position increases 5-HT_{1B} binding by over 10-fold (see section 1.6.2.2.). In addition, this agent is a direct analogue of SDZ 21009, in which the ester group at the 2-position is relocated to the 3-position of the indole ring.

3.4.1. Preparation of 5-[3-(N,N-dimethylamino)propoxy]indole

The synthetic route and experimental procedure for the preparation of **128** was conducted in exactly the same manner as that previously described for **118** (see section 3.3.2.).



127 **128**

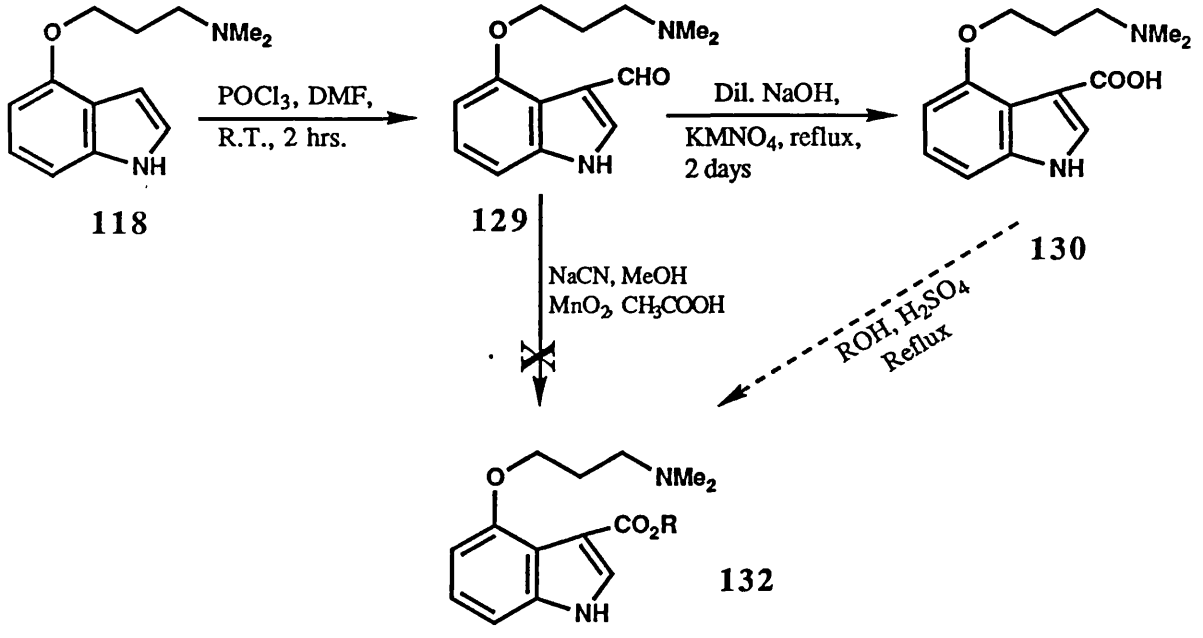
Thus, 5-hydroxyindole **127** was treated with N,N-dimethylchloro-propylamine **97** in the presence of sodium hydride in refluxing anhydrous THF in 56% yield. The yield was about 10% lower than that obtained for its corresponding isomer **118**. The difference in yields is real and not due to experimental error, since the yields quoted in both cases were maximised.

The product after recrystallisation from petroleum ether (80-100 °C) was obtained as colourless needles with an estimated purity of 98.3% as assessed by HPLC.

3.4.2. Preparation of 4-[3-(N,N-dimethylamino)propoxy]indole-3-carboxylic acid methyl ester.

The general procedure [11] for the introduction of a COOR group (R = H, alkyl) into the 3-position of the oxyindoles involves conversion into their Grignards reagents followed by addition of an alkylchloroformate at low temperatures. This procedure also gives the N-carboxylated isomer in equal yields and is therefore of limited use.

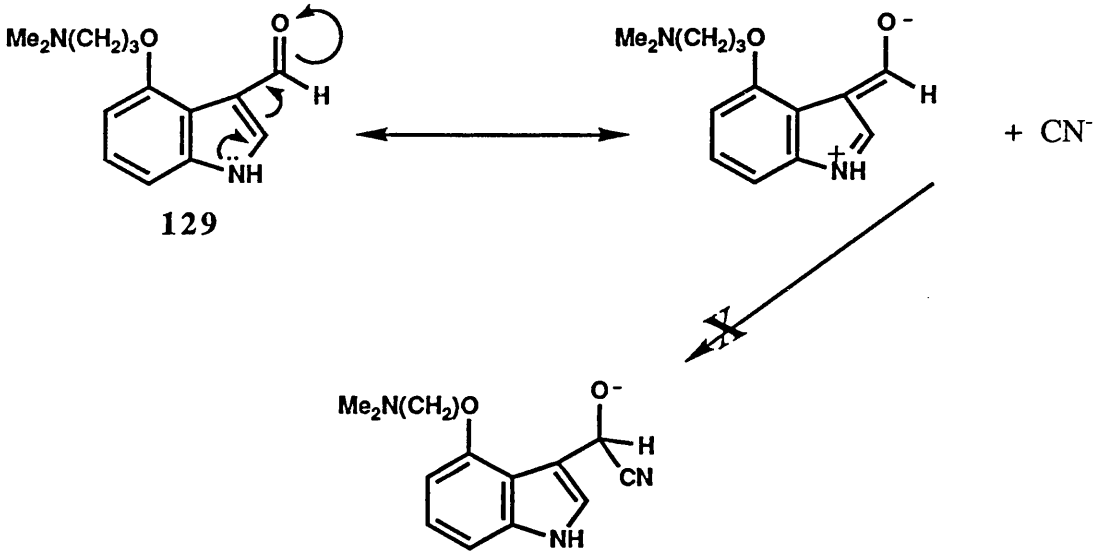
Somei et al [12] have reported the preparation of 3-formyl-4-benzyl-oxyindole from 4-benzyloxyindole via the Vilsmeier-Haack reaction. We applied the Vilsmeier-Haack reaction to the formylation of the aminopropoxyindole **118** and found it to be tolerant of the tertiary amine. This procedure gave the aldehyde **129** in pure form and in excellent yields. Thus, reaction of **118** with a solution of $POCl_3$ in DMF at room temperature gave the aldehyde **129** in 90% yield.



Scheme 3.4. Introduction of substituents into the indole 3-position

The Corey method [13] of esterification of aldehydes was initially employed to prepare the methyl ester **132** according to the reaction conditions

shown in scheme 3.4., but the aldehyde proved to be stable under these conditions and no reaction was observed. It is likely that the carbonyl group is stabilised towards nucleophiles such as CN^- via the nitrogen lone-pair conjugation (see figure 3.2.) and consequently the formation of cyanohydrin **131** from **129**, which is a necessary intermediate in this reaction, is unfavourable.



129 **131**

Figure 3.2.

The recently published modified Corey reaction [14] for aromatic ester synthesis, in which acetic acid is eliminated but instead the mixture is refluxed for several hours, was also tried without success.

In view of the above, it was decided to oxidise the aldehyde **129** to the corresponding carboxylic acid **131** and then esterify the acid. Oxidation of the aldehyde was achieved in about 60% with $KMNO_4$. The oxidation was only possible under basic conditions at refluxing temperatures. The use of other oxidizing agents resulted in, either a multi-component mixture (e.g. $K_2Cr_2O_7$) or no reaction at all (e.g. Ag_2O).

The oxidation reactions were carried out on a very small scale due to the unavailability of the aldehyde. The oxidation product was not therefore isolated or characterised. The evidence for the successful oxidation of the aldehyde is solely based on the tlc analysis of the reaction mixture, which showed the presence of a slower moving component with the Rf value of 0.45. Although, the correct route and reaction conditions for this oxidation was found, due to the unavailibility of time and the aldehyde precursor, this synthesis was not pursued.

3.5. Structure-Activity Results and Discussion

All compounds in this series were examined in binding studies for affinities to 5-HT_{1A}, 5-HT_{1B} and β-adrenergic receptors.

The results (table 3.1.) clearly demonstrate that these analogues with the exception of **126** are essentially inactive at 5-HT_{1B} and β-adrenergic receptors. In contrast, compound **118** possesses a good affinity for 5-HT_{1A} receptors (IC_{50} = 121 nM). This agent displays greater than 80-fold selectivity for 5-HT_{1A} receptors relative to 5-HT_{1B} and β-adrenergic receptors. The binding studies therefore indicate that replacement of the secondary isopropylamino group of pindolol with a tertiary amine together with simultaneous removal of the side chain hydroxyl group has marginal effect on 5-HT_{1A} affinity, while these modifications lead to about a 3000-fold drop in β-adrenergic antagonist activity.

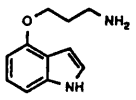
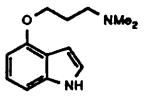
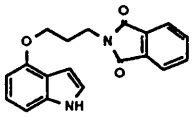
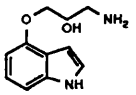
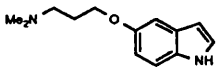
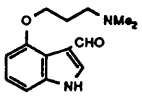
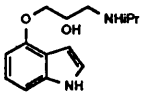
Attempts were then made to increase the 5-HT_{1A} binding of **118** by preparing a series of its analogues and derivatives. However, the following modifications lead to reduced 5-HT_{1A} affinities;

1) Replacement of the terminal tertiary amino group of **118** with either a primary amine (i.e. **121**) or a phthalimide group (i.e. **120** reduces affinity but the effect is more pronounced with the latter modification.

2) Relocation of the aminopropoxy chain from the 4-position to the 5-position of the indole ring reduces affinity by about 50-fold. The position of the chain therefore strongly influence the binding.

3) Introduction of an aldehyde into the 3-position of the indole ring reduces affinity by about 4-fold.

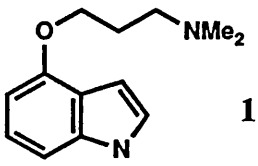
Table 3.1. Affinities of Pindolol Analogues for 5-HT_{1A}, 5-HT_{1B} and β-Adrenergic Receptors and Effects at 5-HT_{1B} Receptors.

Compound	Structure	IC_{50} values, nM 5-HT_{1A} [8-OH-DPAT]	5-HT_{1B} [^{125}I]CYP	β-Adrenoceptor [^{125}I]PIN.	Effects at 5-HT_{1B} Receptors
121		832	>10000	>10000	weak Antagonist
118		121	>10000	>10000	Weak Partial Agonist
120		>10000	>10000	>10000	weak Antagonist
126		1120	2870	110	Partial Agonist
128		10000	>10000	>10000	Weak Partial Agonist
129		500	>10000	>10000	Weak Partial Agonist
(±)pindolol		9.50	310	3.40	Antagonist

As in the case of propranolol series, the presence of the side chain hydroxyl group is only important with respect to 5-HT_{1B} binding and its presence has little or no effect on 5-HT_{1A} affinity.

Effects of compounds were also examined on the mouse urinary bladder strip preparation at concentrations of 10^{-5} molar for the test for antagonism and tested within a range of 1.0-100.0 μM (two experiments each) for agonism. The results indicate that unlike pindolol itself, which is a competitive antagonist at the 5-HT_{1B} receptor, compounds **118, 126, 128** and **129** are weak partial agonists. In contrast **120** and **121** are weak antagonists of the bladder tissue. However these agents bind with very low affinities toward 5-HT_{1B} receptors and any precise conclusion drawn from these results may be misleading.

The goal of this work was to modify the structure of pindolol so as to achieve 5-HT_{1A} selectivity. By removing the side-chain hydroxyl group and taking advantage of the fact that tertiary amines are not well tolerated by either 5-HT_{1B} or β-adrenergic receptors, we obtained an agent **118**, that to date, is the only known example of a modified β-blocker, which displays about 100-fold selectivity for 5-HT_{1A} relative



118

to 5-HT_{1B} and β-adrenergic receptors. Clearly, this compound provides a good lead for future work aimed at identifying analogues having increased affinity for 5-HT_{1A} receptors.

References, Page 142, Structures & Abbreviations, Page 205

3.6. Experimental

4-Oxo-4.5.6.7-tetrahydrocoumarone-3-carboxylic acid (114)

Ethyl bromopyruvate [1] (19.6g, 0.10 mole) was added to a solution of potassium hydroxide (5.6g, 0.10 mole) and 1,3-cyclohexanedione (11.2g, 0.10 mole) in methanol (15 ml). The reaction mixture was left to stir at room temperature for 12 hours. Water (100 ml) was then added and the solution acidified to pH 3 with dilute hydrochloric acid. The solution was concentrated in vacuo until the product began to crystallise out. The mixture was then cooled and the crude product removed by filtration. The residue was recrystallised from a mixture of water and methanol and dried at R.T. and 0.1 mmHg for 5 hours to give the product as a white crystalline solid, (11.2g , 62%).

M.P. 140-141 °C

M.S. m/z : 180 (M^+, 19%), 163 (M^+-17, 23%), 152 (M^+-28, 100%), 136 (M^+-44, 88%), 124 (M^+-54, 36%), 108 (M^+-72, 9%), 96 (M^+-84, 43%).

^{1}H N.M.R. ($CDCl_3$, 60 MHz) : δ 2.00-3.15 (6H, m), 8.00 (1H, s), 13.2 (1H, br., s).

I.R. (KBr) : 3147, 2957, 2633, 1720, 1622, 1549, 1414, 1270 and 1010 cm^{-1}.

4-Oxo-4.5.6.7-tetrahydroindole (115)

Method a

Liquid ammonia [1] (5.60g, 0.33 mole) was condensed in chilled methanol (40 ml). 4-Oxo-4.5.6.7-tetrahydrocoumarone-3-carboxylic

acid **114** (12.0g, 0.076 mole) was introduced into a Berghof pressure vessel fitted with a mechanical stirrer and a pressure gauge, and the methanolic solution of ammonia was then added. The pressure vessel was closed and heated to 100 °C for 5 hours. The pressure gauge registered 7 atmospheres at this temperature. The vessel was then heated to 150 °C for a further 40 hours with the pressure rising to 10 atmosphere. Heating was then stopped and the vessel allowed to cool to room temperature. The solvent was removed under reduced pressure and the resulting black residue was recrystallised from water containing some charcoal to yield the product as light tan crystals, (6.9g, 76%).

M.P. 187-188 °C

M.S. m/z : 135 (M^+, 100%), 107(M^+-28, 51%), 79 (M^+-56, 63%), 52 (M^+-85, 21%).

^{1}H N.M.R. ($CDCl_3$, 60 MHz) : δ 1.90-2.95 (6H, m), 6.32-6.71 (2H, m), 9.4 (1H, br., s).

I.R. (KBr) : 3171, 3092, 2940, 1493, 1496, 1306, 894 and 702 cm^{-1}.

Method b

4-Oxo-4.5.6.7-tetrahydrocoumarone-3-carboxylic acid [4] **114** (94.0g, 0.52 mole) was suspended in water (780g). The suspension was heated to 94-97 °C and aqueous ammonia (28%, 78.3g) was added dropwise over a period of one hour.

On completion of addition of ammonia, the mixture was heated for a further 4 hours at 95 °C. The solution was then allowed to cool and at

about 80 °C crystals began to form. The mixture was left overnight and cooled to 5 °C prior to the filtration. The product was washed several times with ice-water and dried at 50 °C and 0.1 mmHg for 8 hours, (62.5g, 89%). The product was identical by M.P., T.L.C., M.S. and ^{1}H N.M.R. to that obtained by method 1.

4-Hydroxyindole

Method c

A mixture [1] of 4-oxo-4,5,6,7-tetrahydroindole **115** (1.7g, 0.013 mole), 5% palladium on charcoal and mesitylene (75 ml) was refluxed for 3 hours under dry nitrogen. The reaction mixture was filtered hot and the residue washed 3 times with hot mesitylene. The solvent was removed in vacuo and the residue chromatographed on silica, eluting with 40% v/v ether in chloroform. The resulting product was recrystallised from water to give colourless needles, which were dried at R.T. and 0.1 mmHg for 5 hours (0.95g, 55%).

M.P. 96-98°C Lit M.P. 98-102°C

M.S. m/z : 133 (M^+, 100%), 104 ((M^+-29, 34%), 78 ((M^+-55, 5%), 51 ((M^+-82, 11%),

^{1}H N.M.R. (DMSO, 200 MHz) : δ 6.36 (1H, m), 6.49 (1H, m), 6.87 (2H, m), 7.15 (1H, m), 9.28 (1H, s), 10.90 (1H, s).

I.R. (KBr) : 3355, 3257, 1625, 1584, 1498, 1458, 1235, 1021 and 746 cm^{-1}.

Method d

A mixture [6] of 4-oxo-4.5.6.7-tetrahydroindole **115** (10.0g, 0.075 mole), 5% palladium on charcoal (1.25g) and re-distilled di-isobutylketone (200 ml) was refluxed for 15 hours under dry nitrogen in the dark. The hot solution was filtered and the palladium on charcoal was washed twice with di-isobutylketone (2 x 20 ml). The solvent was removed in vacuo and the dark green residue was recrystallised from a small quantity of chloroform (50 ml) to give 4-hydroxyindole as light green needles, (7.5g, 74%). The product was identical by M.P., T.L.C., and ^{1}H N.M.R. to that prepared by method 1.

Liberation of N,N-Dimethyl-3-chloropropylamine from its hydrochloride (97)

N,N-Dimethyl-3-chloropropylamine hydrochloride [15] (1.78g, 0.0113 mole) was finely ground and suspended in dry benzene (60 ml). Sufficient 40% sodium hydroxide was added until the aqueous layer of the mixture became semi-solid and benzene was removed by decantation. The residue was extracted 4 times with benzene and the benzene solutions were combined and dried over KOH. The total volume of benzene used was 12 ml and the drying time never exceeded 1 hour.

4-[3-(N,N-Dimethylamino)propoxy]indole (118)

4-Hydroxyindole **116** (0.50g, 0.004 mole) was dissolved in anhydrous THF (10 ml). The solution was cooled to 5 °C and 60% NaH in paraffin oil (0.15g, 0.004 mole) suspended in THF (10 ml) was added dropwise under dry nitrogen. The mixture was then warmed to 40°C until no more hydrogen evolved. The mixture was cooled to about 10°C and

the benzene solution containing the free N,N-dimethyl-3-chloropropyl-amine (1.37g, 0.0113 mole) was then added. The reaction mixture was refluxed for 18 hours . The solution was filtered, water (20 ml) was added to the filtrate and the mixture was extracted several times with ether. The etheral extracts were combined and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was chromatographed on silica, eluting with 40% v/v ether in methanol. After removal of the solvent, a white solid was obtained which was further purified by recrystallisation from petroleum ether to yield colourless needles. The product was dried at 60°C and 0.1 mmHg for 8 hours, (0.55g, 66%).

M.P. 79-81°C

Analysis Calculated for $C_{13}H_{18}N_2O$: C,71.52, H,8.32, N,12.83
Found : C,71.82, H,8.35, N,12.89

M.S. m/z : 218 (M^+, 3%), 132 (M^+-86, 2%), 116 (M^+-112, 3%), 104 (M^+-114, 6%), 86 (M^+-132, 54%), 58 (M^+-160, 100%).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.94-2.12 (2H, $CH_2C\underline{H}_2CH_2$, m), 2.25 (6H, NCH_3, s), 2.54 (2H, CH_2N, t), 4.18 (2H, OCH_2, t), 6.50-6.74 (2H, indole-2 and 3-H, m), 6.90-7.14 (3H, indole-5, 6 and 7-H, m), 8.68 (1H, NH, s).

I.R. (KBr) : 3117, 2940, 1591, 1519, 1451, 1252, 1098 and 729 cm^{-1}.

U.V. (CH_3OH), λ , ε : 217 nm (25951), 267 nm (6488), 282 nm, shoulder (4325), 293 nm, shoulder (2703).

H.P.L.C. : Column : 10 cm x 4.5 mm, spherisorb Ods 2, 5 μM.
Mobile phase : 40% CH_3OH in H_2O + 0.1% Et_3N +
4.9% HSA (0.5 mole) + 1g citric acid/100 ml
Area of the main peak : 98.75% at 3.49 mins.

4-[3-(N,N-Dimethylamino)propoxy]indole Hydrochloride

4-(3-N,N-Dimethylaminopropoxy)indole **118** (0.30g, 0.0013 mole) was dissolved in dry isopropanol (10 ml). Hydrogen chloride gas was bubbled through the solution until it became acidic. The solution was cooled and anhydrous ether was added dropwise until a white solid precipitated. The solid was extremely hygroscopic and on filtration turned into a dark green paste. This was examined by t.l.c. (ethyl acetate:ammonia:methanol; 25:1:1) revealing a multi-component mixture.

4-(3-Aminopropoxy)indole (121)

Method 1

4-Hydroxyindole **116** (0.50g, 3.76 x 10^{-3} mole) was dissolved in anhydrous THF (10 ml). The solution was cooled to 5 °C and to this was added dropwise a suspension of 60% NaH (0.173g, 4.23 x 10^{-3} mole) in THF (10 ml), under a slow stream of dry nitrogen. The mixture was warmed to about 40 °C until hydrogen evolution stopped and then cooled to 0-5 °C.

3-Chloropropylamine hydrochloride (1.47g, 1.13 x 10^{-2} mole) was liberated [15] in benzene (in exactly the same manner as described earlier for the N,N-dimethyl analogue) and added dropwise over a period of 20 minutes. The mixture was refluxed for 24 hours but the

starting material remained unconsumed. This procedure was therefore abandoned.

5-[3-(N,N-Dimethylamino)propoxy]indole (128)

A solution of 5-hydroxyindole **127** (0.50g, 3.76 x 10^{-3} mole) in dry THF (10 ml) was cooled to 0-5 °C. To this was added a suspension of 60% NaH (0.173g, 4.3 x 10^{-3} mole) in THF (10 ml) dropwise under a slow stream of nitrogen. This was warmed to 40 °C until hydrogen evolution stopped, and then cooled to 0-5 °C.

N,N-Dimethyl-3-chloropropylamine hydrochloride (1.78g, 1.13 x 10^{-3} mole) was liberated in benzene (in exactly the same manner as described earlier) and added to the mixture dropwise over a period of 20 minutes. The reaction mixture was gradually warmed and then refluxed for 24 hours. The hot mixture was filtered and the solvent removed under reduced pressure. The resulting brown oil was chromatographed on silica, eluting with 30% v/v methanol in ether. A clear oil was obtained after the removal of the solvent. The oil solidified on standing at room temperature and was further purified by recrystallisation from petroleum ether. The product was obtained as colourless needles, which were dried at 50 °C, 0.1 mmHg, 6 hours and weighed 0.46g, (56%).

M.P. 73-74 °C

Analysis : Calculated for $C_{13}H_{18}N_2O$: C,71.52, H,8.32, N,12.83
Found: C,71.39, H,8.48, N,12.60

M.S. m/z : 218 (M^+, 3%),173 (M^+-45, 1%), 132 (M^+-86, 2%), 116 (M^+-102, 3%), 86 (M^+-132, 46%), 58 (M^+-160, 100%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.88-2.05 (2H, $CH_2\underline{CH_2}CH_2$, m), 2.24 (6H, NCH_3, s), 2.50 (2H,CH_2N, t), 4.03 (2H, OCH_2, t), 6.42-6.90 (2H, indole-2 and 3-H, m), 7.08-7.18 (3H, indole-4, 6 and 7-H, m), 8.06 (1H, NH, br., s).

I.R. (KBr) : 3119, 2825, 2778, 1691, 1580, 1462, 1159 and 720 cm^{-1}.

U.V. (CH_3OH), λ, ε : 216 nm (23569), 268 nm (6896), 290 nm, shoulder, (4052), 303 nm, shoulder, (2755).

H.P.L.C. : Column: 10 cm x 4.5 mm spherisorb Ods 2, 5 μM.
Mobile Phase: 40% CH_3OH + 55% H_2O + 4.9% HSA (0.1 mole) + 0.1% Et_3N + 1g citric acid/100 ml.
Area of the main peak: 98.27% at 3.68 minutes.

4-[3-(N-phthalimido)propoxy]indole (120)

4-Hydroxyindole **116** (2.50g, 0.019 mole) was dissolved in dry THF (50 ml) and cooled to 0-5 °C. To this was added a suspension of 60% NaH (0.86g, 0.022 mole) in THF (30 ml) dropwise under a slow stream of nitrogen. The mixture was warmed to about 40 °C until the hydrogen evolution ceased and then cooled to 0-5 °C. A solution of N-(3-bromopropyl)phthalimide (15.04g, 0.047 mole) was added dropwise over a period of 20 minutes. The reaction mixture was refluxed for 5 hours, filtered hot and the filtrate taken to dryness under reduced pressure. The resulting crude residue was recrystallised twice from toluene to yield the product as yellow crystalline flakes, which were dried at 80°C, 0.1 mmHg for 10 hours and weighed 3.6g, (60%).

M.P. 179-182 °C

Analysis : Calculated for $C_{19}H_{16}N_2O_3$: C, 71.24, H, 5.03, N, 8.75

Found: C, 71.12, H, 4.99, N, 8.53

M.S. m/z : 320 (M^+, 35%), 188 (M^+-132, 100%), 160 (M^+-160, 85%), 148 (M^+-172, 4%), 104 (M^+-216, 36%), 133 (M^+-187, 40%).

^{1}H N.M.R. (DMSO, 200 MHz) : δ 2.15 (2H, $CH_2C\underline{H}_2CH_2$, m), 3.18 (2H, CH_2N, t), 4.13 (2H, OCH_2, t), 6.21 (1H, indole-3-H, m), 6.45 (1H, indole-2-H, t), 6.99 (3H, indole-5, 6 and 7-H, m), 7.84 (4H, phthalimide-H, m), 11.03 (1H, br, s).

I.R. (KBr) : 3305, 1762, 1702, 1508, 1401, 1090, 917, 750 and 716 cm^{-1}.

U.V. (CH_3OH), λ, ε : 219 nm (28827), 237 nm (5913), 261 nm, shoulder (4435), 286 nm, shoulder (2661).

H.P.L.C. : Column: 10 cm x 4.5 mm Spherisorb Ods 2, 5 μM.
Mobile Phase: 60% CH_3OH in H_2O.
Flow rate: 1 ml/min.
Area of the main peak: 98.8%.

4-(3-Aminopropoxy)indole (121)

Method 2

4-[3-(N-phthalimido)ropoxy]indole **120** (3.50g, 0.11 mole) was refluxed in 10% aqueous KOH (70 ml) for 10 hours. The solution was allowed to cool to room temperature and then extracted with ethyl acetate (100 ml). The solvent was removed in vacuo and the straw coloured

residue was recrystallised from toluene to yield the product as a white solid. This was dried at 50 °C and 0.2 mmHg for 8 hours, (1.1g, 53%). M.P. 124-126 °C

Analysis : Calculated for $C_{11}H_{14}N_2O$: C, 69.44, H, 7.42, N, 14.73
Found: C, 69.21, H, 7.45, N, 14.58

M.S. m/z : 190 (M^+, 46%), 133 (M^+-57, 100%)116 (M^+-74, 6%), 104 (M^+-86, 27%), 58 (M^+-132, 95%), .

^{1}H N.M.R. (DMSO, 200 MHz) : δ 1.85 (2H, NH_2, br, s), 1.85 (2H, $CH_2CH_2CH_2$, m), 2.75 (2H, CH_2N, t), 4.12 (2H, OCH_2, t), 6.50 (2H, indole-2 and 3-H, m), 6.99 (3H, indole-5, 6 and 7-H, m), 11.09 (1H, indole-NH, br, s).

I.R. (KBr) : 3352, 2912, 1584, 1448, 1364, 1251, 1091 and 743 cm^{-1}.

U.V. (CH_3OH), λ, ε : 217 nm (30934), 261 nm (8388), 274 nm, shoulder (5942), 283 nm (4544).

H.P.L.C. : Column: 10 cm x 4.5 mm spherisorb Ods 2, 5 μM.
Mobile Phase: 55% H_2O + 40% CH_3OH + 4.9% HSA (0.1 mole) + 0.1% Et_3N.
Area of the main peak:

N-(2-Hydroxy-3-chloropropoxy)phthalimide (123)

Epichlorohydrin (30.83g 0.33 mole), phthalimide (49.0g, 0.33 mole), tri-N-butylamine (0.50g, 0.003 mole) and methanol (250 ml) were refluxed for 8 hours. The solution was cooled to room temperature and the unreacted phthalimide removed by filtration. On concentrating the

filtrate more phthalimide crystallised out. This was removed by filtration and the filtrate taken to dryness under reduced pressure to yield a white gum. Crystallisation of the gum from toluene gave a white solid, (30g). Analysis of the product by t.l.c. (ethyl acetate:methanol:ammonia; 25:1:1) revealed the presence of two spots of about 3:1 intensity, the desired product being the major one and the other being N-(2,3-epoxypropyl) phthalimide **122**. The mixture was not separated.

M.P. 86-95 °C

M.S. m/z : 240 (M_A^+, 0.09%), 203 (M_B^+, 0.17%), 190 (7%), 60 (100%), 133 (15%), 104 (19%), 77 (23%).

^{1}H N.M.R. ($CDCl_3$, 60 MHz) : δ 3.10-4.4 (Complex Multiplet), 7.6 (Complex Multiplet)

4-[3-(N-phthalimido)-2-hydroxypropoxy]indole (125)

Method 1

A solution of 4-hydroxyindole **116** (3.5g, 0.0263 mole) in dry THF (60 ml) was cooled to about 5 °C. A suspension of 60% NaH (1.21g, 0.03 mole) in THF (30 ml) was added under a slow stream of nitrogen. The mixture was warmed until the hydrogen evolution ceased, and then cooled to 0-5 °C.

A solution of N-(2,3-epoxy)phthalimide mixture **122** (18.91g, excess) in THF (30 ml) was added dropwise and the mixture refluxed for 12 hours. No reaction was observed and the starting materials remained unconsumed. This procedure was abandoned.

4-(2,3-Epoxypropoxy)indole (124)

4-Hydroxyindole **116** (3.0g, 0.023 mole), epichlorohydrin (35.09g 0.383 mole) and 1-methylpiperazine were heated to 85-95 °C for 30 minutes. Excess epichlorohydrin was removed in vacuo and the resulting oily residue was chromatographed on silica, eluting with 2% v/v methanol in chloroform. Removal of the solvent gave a semi-solid paste. This was not purified but dried at R.T. and 0.1 mmHg for 4 hours (3.05g).

M.S. m/z : 189 (M^+, 100%), 146 (M^+-43, 17%), 133 (M^+-66, 75%), 116 (M^+-73, 18%), 104 (M^+-85,49%).

^1H N.M.R. ($CDCl_3$, 60 MHz) : δ 2.40-4.45 (6H, m), 6.24-7.40 (5H, m), 7.80-9.60 (1H, br, s).

Method 2

4-(2,3-Epoxypropoxy)indole **124** (2.95g), phthalimide (7.0g, 0.048 mole) and tri-N-butylamine (0.1g) were added to methanol (30 ml) and refluxed under a slow stream of nitrogen for 3 days. The mixture was filtered hot and on standing at R.T. for 12 hours an orange colour solid crystallised which was collected by filtration. This contained small amounts of the unreacted phthalimide and was not further purified. The product was dried at 40 °C and 0.1 mmHg for 8 hours (3.7g).

M.P. 98 °C went clear at 130 °C

M.S. m/z : 336 (M^+, 12%), 204 (M^+-132, 50%), 160 (M^+-176, 41%), 104 (M^+-232, 95%), 147 (M^+-189,100%), 76 (M^+-260, 93%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 3.04 (1H, d), 3.48 (1H, s), 4.04 (3H, m), 4.22 (3H, m), 4.40 (1H, m), 6.53 (1H, m), 6.66 (1H, m), 7.00-7.12 (3H, m), 7.66-7.92 (6H, m), 8.3 (1H, br, s).

4-(3-Amino-2-hydroxypropoxy)indole (126)

4-[3-{N-phthalimido(2-hydroxypropoxy}]indole **125** (3.7g, 0.011 mole) was added to 10% KOH (74g) and the mixture refluxed for 10 hours. The solution was cooled to R.T. and extracted with ethyl acetate (2 x 80 ml portions). The solvent was removed under reduced pressure and the residue was chromatographed on silica, eluting with methanol. The eluant was removed under reduced pressure to give a semi-solid paste which, after drying at 0.1 mmHg for several hours, solidified (0.8g, 44%).

4-(3-Amino-2-hydroxypropoxy)indole hydrochloride

4-(3-Amino-2-hydroxypropoxy)indole **126** (0.35g 0.0017 mole) was dissolved in absolute ethanol (10 ml). A saturated solution of hydrogen chloride gas in absolute ethanol was prepared and added dropwise until the solution became slightly acidic. On concentrating the resulting solution a white solid precipitated which immediately darkened on isolation. The preparation of the hydrochloride salt of this amine was therefore abandoned.

4-(3-Amino-2-hydroxypropoxy)indole hydrogen oxalate

4-(3-Amino-2-hydroxypropoxy) indole **126** (0.20g, 0.001 mole) was dissolved in absolute ethanol (10 ml). A saturated solution of oxalic acid in absolute ethanol was added dropwise until the solution was slightly acidic. The mixture was allowed to stand in the fridge for a few

hours before removing the solid by filtration. The resulting crystalline white product was dried at 80°C and 1 mmHg for 8 hours, (0.12g).

M.P. 192-193 °C

Analysis : Calculated for $C_{13}H_{16}N_2O_6$: C, 52.70, H, 5.44, N, 9.46
Found: C, 52.60, H, 5.70, N, 9.05

M.S. m/z : 206 (M^+, 70%), 147 (M^+-59%, 2%), 133 (M^+-73, 100%)
105 (M^+-101,13%), 74 (M^+-132, 65%).

^1H N.M.R. of the base ($CDCl_3$, 200 MHz) : δ 1.55-2,12 (2H, NH_2, br, s), 2.82-3.10 (2H, CH_2N, m), 4.00-4.20 (3H, OCH_2 and CHOH m), 6.53 (1H, indole-2-H, m), 6.62 (1H, indole-3-H, m), 7.00-7.20 (3H, indole 5, 6 and 7-H, m), 8.29 (1H, indole-NH, br., s).

I.R. (KBr) : 3389, 3208, 2927, 1719, 1608, 1501, 1363, 1250, 1130, 1092 and 742 cm^{-1}.

H.P.L.C. : column: 100 x 4.5 mm spherisorb Ods 2, 5 μM.
Mobile Phase: 65% H_2O + 30% CH_3OH + 4.9% HSA (0.5 mole) + 0.1% Et_3N + 0.2g/100 ml citric acid.
Flow Rate: 1 ml/min.
Area of the main peak: 97.1%

4-[3-(N,N-Dimethylamino)propoxy]indole-3-carboxaldehyde (129)

A solution of 4-[3-(N,N-dimethylamino)propoxy]indole **118** (2.50g, 1.20 x 10^{-2} mole) in DMF (19 ml) was made up. To this was added dropwise with stirring, a solution of $POCl_3$ (5 ml) in DMF (19 ml) while

keeping the temperature below 20 °C during the addition. Stirring was then continued for two hours at room temperature. The resulting dark brown solution was made alkaline (pH 12-13) with 40% NaOH. The mixture was filtered and extracted with three portions of chloroform. The combined extracts (120 ml in total) were dried with $MgSO_4$. The drying agent was filtered off and chloroform removed under reduced pressure to give a brown coloured residue. This was chromatographed on silica, eluting with methanol/chloroform; 1:1. The product was recrystallised from toluene as a pale crystalline powder which was dried at 50 °C and 0.2 mmHg for 4 hours, (2.40g, 90%).

M.P. 111-114 °C

Analysis : Calculated for $C_{14}H_{18}N_2O_6$: C, 68.27, H, 7.37, N, 11.37
Found: C, 68.07, H, 7.36, N, 11.32

M.S. m/z : 246 (M^+, 7%), 231 (M^+-15, 0.2%), 201 (M^+-45, 0.4%) 188 (M^+-58, 0.5%), 160 (M^+-86, 6%), 131 (M^+-115, 3%), 104 (M^+-142, 11%), 85 (M^+-161, 41%), 58 (M^+-188, 100%).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 2.06 (2H, $OCH_2C\underline{H}_2$, m), 2.25 (6H, NCH_3, s), 2.55 (2H, CH_2N, t), 4.19 (2H, OCH_2, t), ? (1H, d), ?(1H, d), 7.13 (1H, indole-6-H, t), 7.86 (1H, indole-2-H, s), 8.13 (1H, indole-NH, br. s), 8.49 (1H, CHO, s).

I.R. (KBr) : 3085, 2851, 1658, 1524, 1461, 1324, 1281, 1044 and 723 cm^{-1}.

U.V. (CH_3OH), λ, ε : 212 nm (22439), 241 nm (18438), 314 nm (7480).

H.P.L.C. : column: 250 x 4.5 mm spherisorb RP Select B, 5 μm + 40 x 4 mm guard column..

Mobile Phase: CH_3OH + 0.1% Et_3N

Flow Rate: 2 ml/min.

Area of the main peak: 99.73% at 5.63 minutes.

4-[3-(N,N-Dimethylamino)propoxy]indole-3-carboxylic acid methyl ester (132)

A mixture of 4-[3-(N,N-Dimethylamino)propoxy]indole-3-carboxaldehyde **129** (0.050g, 2.0 x 10^{-4} mole), sodium cyanide (0.082g, 1.7 x 10^{-3} mole), acetic acid (0.030g, 5.0 x 10^{-4} mole) and methanol (4 ml) was refluxed for 24 hours under an atmosphere of nitrogen. The mixture was cooled, filtered and taken to dryness under reduced pressure. However, the tlc analysis of the mixture only revealed the presence of the unreacted aldehyde. This was further confirmed by ^1H NMR spectrum of the residue which was identical to that of the starting material. This procedure was therefore abandoned.

4-[3-(N,N-Dimethylamino)propoxy]indole-3-carboxylic acid (130)

4-[3-(N,N-Dimethylamino)propoxy]indole-3-carboxaldehyde **129** (0.020g, 8.1 x 10^{-5} mole) was dissolved in water (1.10g) containing potassium hydroxide (0,0060g, 1.40 x 10^{-4} mole). To this was added in one portion a solution of potassium permanganate (9.1 ml, 0.05 molar). The solution was refluxed for 48 hours until no more starting material was consumed. TLC analysis (ethyl acetate:methanol:ammonia; 5:1:1) revealed the presence of two components in the approximate ratio of 3:1 with Rf values of 4.51 and 5.71 respectively. Due to lack of time, the components were not isolated or characterised.

3.7. References

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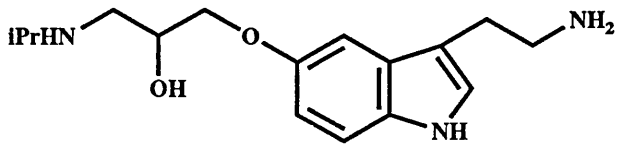
CHAPTER FOUR

A Novel Series of Serotonergic Agents

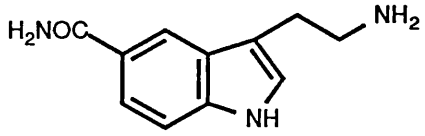
4.1. Design and Synthesis

This brief chapter describes the design, synthetic pathway and pharmacological activity of a novel derivative of serotonin, in which the aminopropoxy chain of the β-blockers, pindolol or propanolol is incorporated, i.e. **140.**

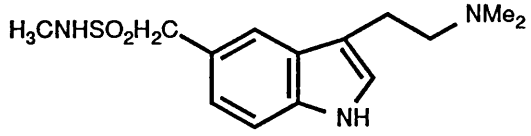


140

In binding assays [1], 5-HT has been shown to be a potent and definitive agent for 5-HT_1 receptors. In a limited series of its analogues, it has been demonstrated that affinity can be retained or increased by replacing its hydroxyl group on the aromatic ring with either an amide[1-5] or an alkylsulphonamide [6] **(25 & 13)**.



(5-CT, 25)



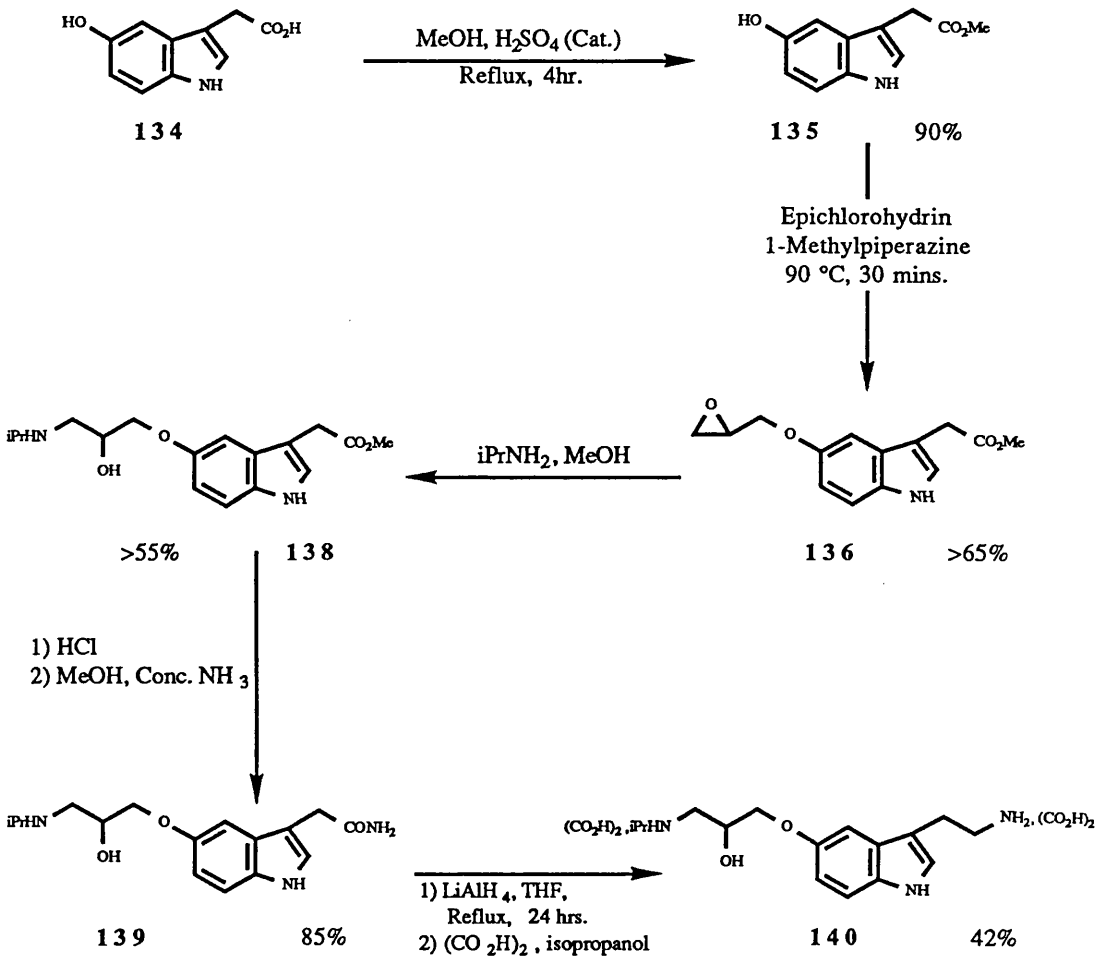
(GR 43175, 13)

The aminopropoxy chain is well-known to contribute to the antagonistic activity of the β–blockers under consideration. Based on these observations, we wished to couple the aminopropoxy functionality to 5-HT in the hope that this may render new 5-HT_{1A} and 5-HT_{1B} antagonists.

The synthesis of **140** was achieved via the synthetic pathway, shown in scheme 4.1. This may not be the shortest synthetic route to the target

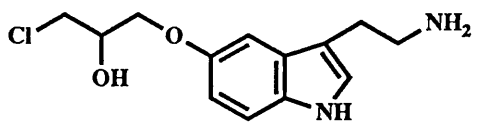
agent, but the starting material is cheap and readily available. Moreover, the scheme offers the opportunity of testing viable and potentially interesting intermediates.

Scheme 4.1. Derivatisation of 5-HT



Esterification of 5-hydroxyindole-3-acetic acid **134** with methanol, using acid catalysed conditions afforded the corresponding methyl ester **135** free of impurities and in almost quantitative yields. **135** was then reacted with excess epichlorohydrin [7] containing catalytic amounts of 1-methylpiperazine at elevated temperatures to give a mixture of the

required epoxide **136** and **137** in approximately 3:1 ratio (as analysed by tlc).



137

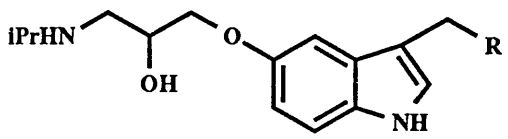
Without further purification or separation, the mixture was reacted with excess isopropylamine in methanol in a sealed Berghof pressure vessel at refluxing temperatures. After a simple work up procedure and purification by column chromatography, **138** was obtained pure as a white powder in better than a 65% yield. A solution of this in 3 molar hydrochloric acid was made up. This was treated with concentrated ammonia in a sealed flask at room temperature with occasional shaking for five days. This procedure afforded the amide **139** as its hydrochloride salt. Treatment of the salt with ion exchange resin gave the pure free base as a white solid.

Reduction [8,9] of the amide **139** to the primary amine **140** was only possible with five molar equivalence of $LiAlH_4$ and with the lower amounts of the reducing agent, only side-products were observed. The crude product was easily purified by column chromatography, eluting with 5% ammonia in methanol. The di-amine was obtained as a colourless oil. It was heated under reduced pressure several times with anhydrous isopropanol to azeotropically remove the remaining traces of water. The di-amine appeared to be extremely hygroscopic and was vigorously dried. This was dissolved in anhydrous isopropanol and then treated with a saturated solution of oxalic acid in ether to afford its di-oxalate salt as a white powder.

4.2. Results and Discussions

The binding affinities of the two intermediates **138** and **139**, and the target compound **140** at 5-HT_{1A}, 5-HT_{1B} and β–adrenoceptors were determined (see table 4.1.).

Table 4.1. Affinity of Some 5-HT Analogues at 5-HT_{1A}, 5-HT_{1B} & β-Receptors and Effects at 5-HT_{1B} Receptors.



Compound	R	IC_{50} values, nM 5-HT_{1A}	5-HT_{1B}	β	Agonism at 5-HT_{1B}	Antagonism at 5-HT_{1B}
138	COOMe	87	>10000	963	YES	NO
139	$CONH_2$	>10000	>10000	>10000	YES	YES
140	CH_2NH_2	102	610	>1000	YES	YES
5-HT*	-	3	25	-	YES	NO

* *Data from Engel et al [10].*

A comparison of **140** and 5-HT reveals that the introduction of the aminopropoxy chain into the 5-position of 5-HT decreases affinity by about 30-fold at both 5-HT_{1A} and 5-HT_{1B} receptors, but the compound displays a good selectivity between 5-HT_{1A} and β–adrenoceptors. Within the series, the following structure-binding relationships are evident ;

Replacement of the aminoethyl chain of **140** by methylacetate (i.e. **138**) has very little effect at 5-HT_{1A} receptor but reduces 5-HT_{1B} binding, whereas, its replacement by acetamide (i.e. **139**) essentially removes affinity at all receptors.

These findings are somewhat surprising and give no firm indication as to what type of receptor binding interactions might be involved with regards to the aminoethyl chain. However, the methylacetate **138** displays over a 100-fold selectivity between 5-HT_{1A} and 5-HT_{1B} receptors, and **140** shows about a 100-fold selectivity between 5-HT_{1A} and β–adrenoceptors.

These results are extremely encouraging and further synthetic work with these agents should lead to the identification of more potent and selective ligands.

The compounds were also assayed for their effect at 5-HT_{1B} receptors, using 5-HT potentiation of electrically stimulated twitch contractions of mouse urinary strips in vitro. These agents at concentrations of 10^{-5} molar, with the exception of **138** showed mixed agonistic and antagonistic activities.

References, Page 155, Structures & Abbreviations, Page 205

4.3. Experimental

Methyl 5-hydroxyindole-3-carboxylate (135)

A solution of 5-hydroxyindole-3-acetic acid **134** (3.0g, 1.50 x 10^{-2} mole) in anhydrous methanol (75 ml) containing 98% sulphuric acid (0.10g) was refluxed for four hours. The solution was cooled and its pH adjusted to 7 ± 1 with dilute potassium hydroxide solution. The mixture was concentrated under reduced pressure and then partitioned between water and chloroform. The chloroform layer was dried over magnesium sulphate, filtered and then removed under reduced pressure to give the pure product (2.73g, 90%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 3.75 (3H, s), 3.78 (2H, s), 4.95 (1H, s), 6.78 (1H, dd), 6.98 (1H, d), 7.16 (1H, t), 7.25 (1H, s), 8.04 (1H, br, s).

Methyl 5-(2,3-epoxypropoxy)indole-3-carboxylate (136)

A solution of methyl 5-hydroxyindole-3-carboxylate **135** (2.85g, 1.40 x 10^{-2} mole) in epichlorohydrin (33.0g, 0.36 mole) containing 1-methyl-piperazine (0.1g) was heated to 95 °C for 45 minutes under a slow stream of nitrogen. The solution was cooled and epichlorohydrin was removed in vacuo. The resulting residue was chromatographed on silica, eluting with 5% methanol in chloroform to give a straw coloured solid. The product contained some impurity but was not further purified (2.45g, 67.8%).

Methyl 5-(2-hydroxy-3-isopropylamino-propoxy)indole-3-carboxylate (138)

A solution of methyl 5-(2,3-epoxypropoxy)indole-3-carboxylate **136** (2.36g, 9.1 x 10^{-3} mole) and isopropylamine (5.3g, 0.09 mole) in

methanol (70 ml) was heated to 65 °C in a pressure vessel for 10 hours. The solvent and the excess amine were then removed under reduced pressure and the resulting oil was chromatographed on silica, eluting with ethyl acetate:methanol:ammonia (25:1:1). The solid obtained was dissolved in isopropranol (50 ml) and then filtered. Isopropranol was then removed in vacuo to give the product as a white solid (1.60g, 55.1%).

M.P. 99-101 °C

Analysis Calculated for $C_{17}H_{24}N_2O_4$: C,63.73, H,7.55, N,8.73
Found : C,64.02, H,7.75, N,8.57

M.S. m/z : 321 (MH^+,), 305 (M^+-15), 276 (M^+-44), 261 (M^+-59), 219 (M^+-101), 205 (M^+-115), 146 (M^+-174), 72 (M^+-248).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.12 [6H, $CH(C\underline{H}_3)_2$, d], 2.55-3.00 (1H, $C\underline{H}(CH_3)_2$, m), 2.55-3.00 (2H, CH_2N, m), 3.67 (3H, OCH_3, s), 3.74 (2H, CH_2CO, s), 3.98-4.15 (2H, OCH_2, m), 3.98-4.15 (1H, C<u>H</u>OH, m), 6.88 (1H, indole-6-H, dd), 7.06 (1H, indole-7-H, d), 7.14 (1H, indole-2-H, s), 7.26 (1H, indole-4-H, s), 8.08 (1H, indole-NH, br., s).

I.R. (KBr) : 3205, 2958, 1731, 1484, 1441, 1334, , 1024 and 937 cm^{-1}.

U.V. (CH_3OH), λ , ε : 218 nm (24645), 271 nm (6083), 290 nm, shoulder (4368), 303 nm, shoulder (2964).

H.P.L.C. : column: 250 x 4.5 mm Lichrosorb RP Select B, 5 µm + 40 x 4 mm guard column.

References, Page 155, Structures & Abbreviations, Page 205

Mobile Phase: Pump A; H_2O + 0.1% Et_3N, Pump B;
CH_3OH + 0.1% Et_3N, Ratio of Pump A : Pump B, 37 : 63.
Flow Rate: 1 ml/min.
Area of the main peaks: 98.78%, 0.72% and 0.50% at
9.53, 8.63 and 7.37 minutes respectively.

5-(2-Hydroxy-3-isopropylamino-propoxy)indole-3-acetamide (139)

Ammonia (0.88d, 150 ml) was added to a solution of methyl 5-(2-hydroxy-3-isopropylamino-propoxy)indole-3-carboxylate **138** (1.36g, 4.22 x 10^{-3} mole) in hydrochloric acid (50 ml, 3 molar) in a R. B. flask (250 ml). The flask was stoppered and allowed to stand at room temperature for 5 days. The solvent was removed using a freeze drier and the resulting residue dissolved in water (75 ml) containing ion-exchange resin (100g, 'amberlite' IRA-400). This was stirred for 1 hour, filtered, and the filtrate was run through an ion-exchange column, eluting with water. This procedure afforded the pure product as a white crystalline solid (1.1g, 85.5%).

M.P. 133-135 °C

Analysis Calculated for $C_{16}H_{23}N_3O_3$: C,62.93, H,7.59, N,13.76
Found : C,62.57, H,7.73, N,13.53

M.S. m/z : 306 (MH^+, 7%), 290 (M^+-15, 6%), 261 (M^+-44, 23%), 190 (M^+-115, 100%), 172 (M^+-133, 8%), 146 (M^+-159, 94%), 72 (M^+-233, 96%).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.12 (6H, d), 2.55-3.00 (1H, m), 2.55-3.00 (2H, m), 3.67 (3H, s), 3.74 (2H, s), 3.98-4.15

References, Page 155, Structures & Abbreviations, Page 205

(2H, m), 3.98-4.15 (1H, m), 6.88 (1H, dd), 7.06 (1H, d), 7.14 (1H, s), 7.26 (1H, s), 8.08 (1H, br., s).

I.R. (KBr) : 3285, 2911, 1731, 1654, 148, 1391, 1170, and 1023 cm^{-1}.

U.V. (CH_3OH), λ, ε : 221 nm (24856), 274 nm (5918), 293 nm, shoulder (4439), 304 nm, shoulder (2663).

H.P.L.C. : column: 250 x 4.5 mm Lichrosorb RP Select B, 5 µm + 40 x 4 mm guard column.
Mobile Phase: A gradient run from 10% CH_3CN in H_20 containing 0.1% Et_3N to 45% CH_3CN over 30 minutes.
Flow Rate: 1 ml/min.
Area of the main peaks: 99.06%, 0.34% and 0.27% at 15.99, 18.52 and 29.10 minutes respectively.

5-(2-Hydroxy-3-isopropylamino-propoxy)-3-(β–amino-ethyl)indole bis hydrogen oxalate (140)

A suspension of 60% lithium aluminium hydride [8, 9] (0.33g, 5.2 x 10^{-3} mole) in anhydrous THF (25 ml) was added dropwise with stirring, to a solution of 5-(2-hydroxy-3-isopropylamino-propoxy)indole-3-acetamide **139** (0.30g, 9.8 x10^{-4} mole) in THF (75 ml) under a slow stream of nitrogen. The mixture was refluxed for 24 hours, cooled, and crushed ice was then carefully added to quench the reaction mixture. The mixture was filtered and the solvent removed under reduced pressure. The resulting residue was chromatographed on silica, eluting with 5% ammonia (d = 0.88) in methanol to give a straw coloured paste. This was dissolved in anhydrous isopropranol (75 ml), and then filtered. Isopropranol was removed in vacuo, to give a colourless gum, which was further dried at 50 °C and 0.1 mmHg for 8 hours (0.12g, 42%).

References, Page 155, Structures & Abbreviations, Page 205

A solution of the base (0.12g) in anhydrous isopropanol (10 ml) was treated with a saturated solution of oxalic acid in anhydrous ether, until an excess of the acid was present. The white precipitate was collected by filtration and dried at 50 °C and 0.1 mmHg for four hours.

M.P. Foamed above 170 °C

Analysis Calculated for $C_{20}H_{29}N_3O_{10}$: C,50.95, H,6.20, N,8.91
Found : C,51.46, H,6.43, N,9.17

M.S. m/z : 291 (MH^+,3%), 276 (M^+-15, 0.1%), 262 (M^+-29, 33%), 230 (M^+-60, 5%), 176 (M^+-115, 32%), 147 (M^+-144, 95%), 137 (M^+-154, 24%), 130 (M^+-160, 6%),116 (M^+-175,15%), 72 (M^+-219, 81%).

^{1}H N.M.R. (DMSO, 200 MHz) : δ 0.95 [6H, $CH(C\underline{H}_3)_2$, d], 2.43-2.82 (7H, ?, m), 3.77-3.95 (3H, ?, m), 6.67 (1H, ?, dd), 6.97 (1H, ?, d), 7.05 (1H, ?, d), 7.18 (1H, ?, d).

I.R. (KBr) : 3425, 2975, 1640, 1400, 1220, 720 cm^{-1}.

U.V. (CH_3OH), λ , ε : 217 nm (26192), 272 nm (6366), 294 nm, shoulder (4730), 303 nm, shoulder (2910).

H.P.L.C. : column: 250 x 4.5 mm Lichrosorb RP Select B, 5 μm + 40 x 4 mm guard column.
Mobile Phase: A gradient run, starting from 5% CH_3OH in H_20 containing 0.5% ortho-phosphoric acid for 5 mins. then to 60% CH_3OH over 30 minutes.
Flow Rate: 1 ml/min.
Area of the main peaks: 97.32%, 1.09% at 8.51 and 19.02 minutes respectively.

References, Page 155, Structures & Abbreviations, Page 205

4.4. References

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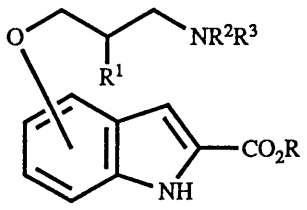
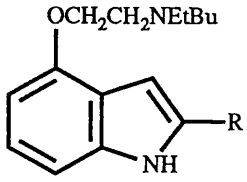
CHAPTER FIVE

Analogues of Cyanopindolol as Selective 5-HT_{1A} Agents.

5.1. Introduction

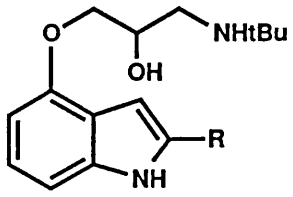


Type 1 Analogues



Type 2 Analogues

This chapter deals with the design, synthesis and structure-activity relationships for two series of novel compounds of the general structure shown above. The initial purpose of this investigation was to modify the structure of cyanopindolol **40** or SDZ 21009 **41** (i.e. type 1 analogues) in the anticipation that key structural features with regards to serotonergic binding may be identified. The second series of compounds were designed by utilising the knowledge gained from the recently published data on structure-binding correlations for a similar series of compounds.



R = CN, Cyanopindolol (40)

R = CO_2IPr, SDZ 21009 (41)

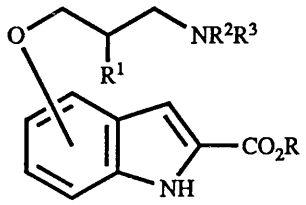
The bulk of the chapter is devoted to the synthesis of novel di-substituted indoles. These compounds were tested in various binding and functional assays. This work has led to the identification of some potent and selective 5-HT_{1A} agents. The synthesised compounds are the most potent and selective 5-HT_{1A} agents, ever to be derived from the β-blockers, aryloxypropanolamines.

References, Page 191, Structures & Abbreviations, Page 205

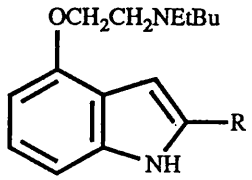
5.2. Strategy

Type 1 Analogues:

In 1986, Engel and co-workers [1] demonstrated that racemic cyanopindolol is more potent than either pindolol **27** or propranolol **28** and binds equally well to 5-HT_{1A} and 5-HT_{1B} receptors (see sections 1.6.2 and 1.6.2.1). The enhanced affinity of these agents is believed to be due to the presence of a strong electron withdrawing group in the indole 2-position (discussed in section 1.6.2.1 and 2.2).



Type 1 Analogues



Type 2 Analogues

From a pharmacological stand-point, it is unclear as to what type of activity these ligands possess. Evidence [2] suggests that they may act as 5-HT_{1A} and 5-HT_{1B} antagonists. Recent binding assays [3,4] in calf hippocampus and rat and calf substantia nigra suggest that cyanopindolol and SDZ 21009 also display partial agonism at 5-HT_{1D} receptors, although with over a hundred fold less potency than at 5-HT_{1A} and 5-HT_{1B} receptors. As discussed in section 1.6.2., these are non-selective agents for 5-HT receptors, and bind with an even greater affinity at the β-adrenoceptors.

As in our previous attempts with propranolol and pindolol, four analogues of SDZ 21009 of type 1 series were synthesised in order to verify the role of key functional features with regards to serotonergic binding. The following structural modifications to the lead compound were then carried out :

i) The side chain hydroxyl group was removed.

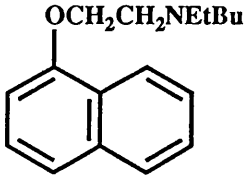
ii) The terminal t-butylamino was replaced with a smaller methyl amine.

iii) The isopropyl group of the ester was replaced with a methyl group.

iv) The aminopropoxy side chain was moved to the indole 5-position.

Type 2 Analogues:

The design of this series of compounds was largely based on the work carried out by Glennon and others. These authors, in a very recent publication [5] reported the binding affinities of a number of propranolol analogues at 5-HT_{1A}, 5-HT_{1B} and β-adrenoceptors (see section 1.6.2.2 for a full discussion on structure-activity studies). Using similar strategies as ours, they found that both the affinity and selectivity of propanolol for 5-HT_{1A} receptors is greatly improved with shortening the side chain from three carbon atoms to two carbon atoms, together with simultaneous replacement of the secondary isopropylamino group with a tertiary ethylbutylamine, i.e. **57**.



(57)

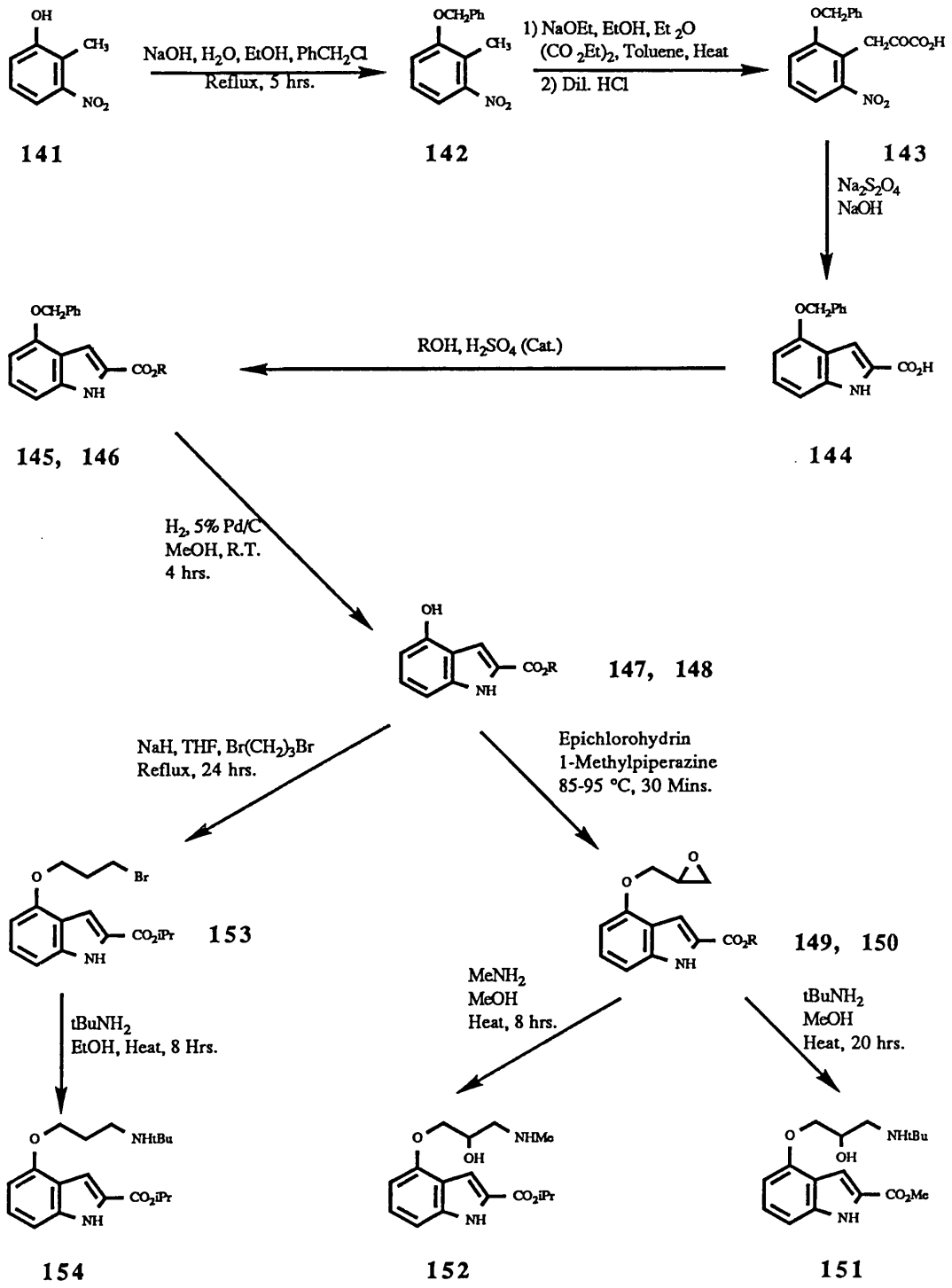
Thus, **57** was reported to be twice more potent than propranolol at 5-HT_{1A} receptors, with approximately 25-fold and 100-fold selectivity against 5-HT_{1B} and β-receptors respectively (see table 1.2). Their findings presented us with the opportunity of synthesising more potent and selective ligands at 5-HT_{1A} receptors. In section 1.6.2.1, the affinity and selectivity of pindolol and propanolol were discussed. Structural comparison of these agents indicate that indole is the preferred aromatic ring at the 5-HT_{1A} receptors.

Following these observations, we synthesised a number of compounds where the naphthalene ring of **57** was replaced with an indole ring containing various electron withdrawing substituents in the 2-position. The inclusion of the substituents was to further enhance 5-HT_{1A} affinity.

References, Page 191, Structures & Abbreviations, Page 205

5.3. Synthesis Of SDZ 21009 Analogues (Type 1)

Scheme 5.1. represents the overall synthetic pathway to the desired analogues of SDZ 21009.



Scheme 5.1. Synthetic Route to SDZ 21009 Analogues.

The synthesis of 4-(2-hydroxy-3-aminopropoxy)indoles with substituents in the 2-position has been well documented by several publications [6,7] and patents [8-11]. The key intermediate in the synthesis of these compounds is 4-benzyloxyindole-2-carboxylic acid **144** which was successfully prepared. The aminopropoxy side chain was then built up following the same synthetic pathway as discussed for pindolol and propanolol analogues and derivatives.

5.3.1. Preparation of 4-Benzyloxyindole-2-carboxylic acid

The synthesis of 4-benzyloxyindole-2-carboxylic acid was carried out using the method of Reissert [12] (see scheme 5.1.).

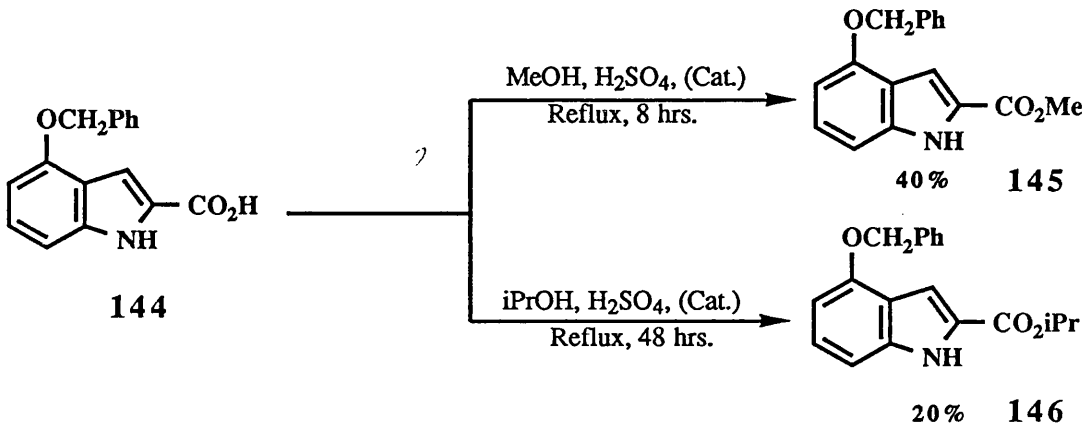
2-Methyl-3-nitrophenol **141** was benzylated, essentially following the literature procedure [13] described for the benzylation of 4-methyl-2-nitrophenol. The crude product obtained in this reaction contained many impurities but in small amounts. It was found that recrystallization of the crude product from petrol was extremely effective and gave the pure product as light yellow needles. We obtained 2-nitro-6-benzyloxytoluene **142** in 84% yield, 20% higher than that reported for the benzylation of 4-methyl-2-nitrophenol.

Following a procedure described by Stoll et al [12], **142** was reacted with diethyl oxalate in the presence of potassium ethoxide, which after hydrolysis with dilute acid gave **143** as a reddish brown solid (91%). The reaction requires two molar equivalent of potassium ethoxide and with one molar equivalent of the base, the yield was almost halved. The reason for the low yield is unclear. In the final step of the reaction, 4-benzyloxyindole-2-carboxylic acid **144** was prepared by reductive cyclisation of **143** with $Na_2S_2O_4$ as the reducing agent in about 60% yield. The final product contained some impurity but no purification attempt was carried out at this

stage. The acid was only slightly soluble in most solvents including alcohols.

5.3.2. Esterification of 4-Benzyloxyindole-2-carboxylic acid

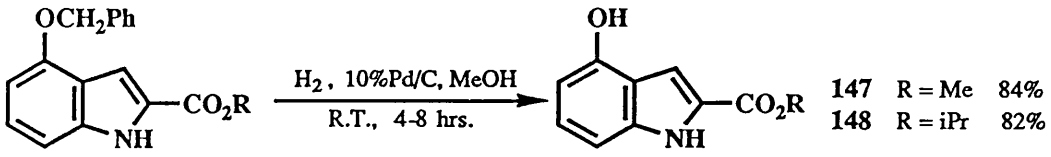
4-Benzyloxyindole-2-carboxylic acid was esterified using similar conditions as those described for the esterification of 5-hydroxy-2-naphthoic acid (see section 2.3.2.).



Thus, esterification of **144** was carried out in the respective alcohol, containing catalytic amounts of 98% H_2SO_4 at refluxing temperature. The methyl and isopropyl esters **145** and **146** were obtained in poor yields,40% and 20% respectively but a substantial recovery of the unreacted starting material was achieved by simple extraction procedures. In view of this, no attempts to investigate other esterification procedures were pursued. The esters were slightly more soluble than the corresponding acid in alcohols, nevertheless, large volumes of solvents were needed to fully dissolve them.

5.3.3. Debenzylation of 4-Benzyloxyindole-2-carboxylic acid esters

Debenzylation [12,14] of **145** and **146** were achieved via hydrogenolysis in methanol at room temperature using 10% Pd/C as catalyst.

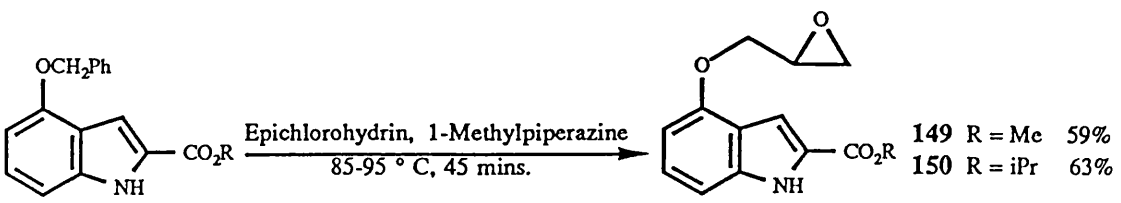


147 R = Me 84%
148 R = iPr 82%

Large volumes of methanol were required to dissolve the esters and therefore for safety reasons, the reaction was carried out in a Berghof pressure vessel under 5-10 atmospheres of hydrogen. Debenzylation can also readily occur by bubbling hydrogen through the solution at atmospheric pressure. This is probably a more convenient procedure for small scale synthesis. However, because of poor solubility of the esters in alcohols, problems may be encountered with the large scale debenzylation of **147** and **148**, using either of the above procedures.

5.3.4. Preparation of 4-(2,3-Epoxypropoxy)indole-2-carboxylic acid esters

The title compounds **149** and **150** were produced following similar reaction procedures as those described for the preparation of **100** and **136**.



149 R = Me 59%
150 R = iPr 63%

The hydroxyindole esters **147** and **148** were reacted with epichlorohydrin in the presence of catalytic amounts of 1-methylpiperazine at 90 °C to give **149** and **150** in 59% and 63% yield. The yields were not maximised. The

crude epoxides were obtained as a brown solid residue which contained small amounts of impurities. It was found that stirring the residues in small quantities of methanol dissolved all impurities and the product could be simply be collected by filtration. This is an effective procedure for the purification of the epoxides.

The products were obtained in good yields as light brown powders and appeared to be slightly air and light sensitive.

5.3.5. Preparation of 4-(Alkylamino-2-hydroxypropoxy)indole-2-carboxylic acid esters

The analogues of SDZ 21009, **151** and **152** were synthesised from the reaction of the epoxides **149** and **150** with the respective amines, (see scheme 5.1.) using similar procedures to those described in section 2.3.5.

Thus, heating the epoxides in methanol containing excess amounts of the respective amine gave the desired compounds in moderate yields. The yields were not maximised. Because of the low boiling point of methyl amine, amination of the epoxide **150** was carried out in a Berghof pressure vessel, but alternatively, an efficient cold condenser could have been used.

As with previous amination reactions, The reactions were clean and no transaminations products were formed. The compounds were obtained in their base form as white crystalline solids, stable under normal conditions.

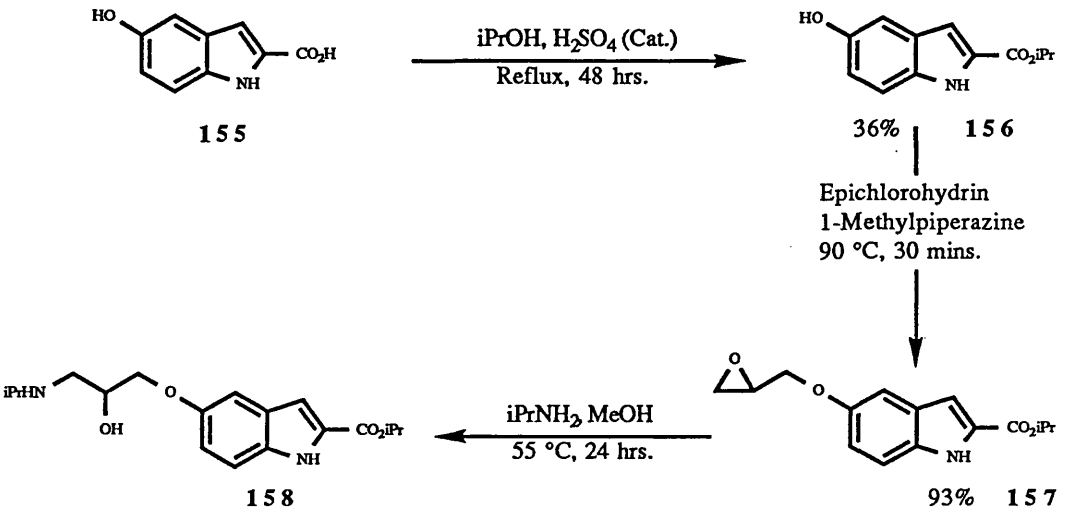
5.3.6. Preparation of 4-(3-t-Butylaminopropoxy)indole-2-carboxylic acid methyl ester

The title compound **154** was prepared from the condensation reaction of the ester **148** with 1,3-dibromopropane, followed by aminolysis with t-butylamine (see scheme 5.1.). The reaction conditions and procedures were similar to those described in section 2.3.3.

The bromo intermediate **153** was prepared by reacting **148** with 20 molar equivalence of 1,3-dibromopropane using 1 molar equivalence of NaH as base. After purification by column chromatography, **153** was obtain as a white crystalline solid in 67% yield. This was then reacted with excess t-butyl amine in absolute ethanol. The crude product was purified by column chromatography, eluting with EtOAc / MeOH / conc. ammonia (50:1:1). The above solvent system gave an excellent separation and the impurities were easily removed. However, on removing the solvent under reduced pressure, a white crystalline solid sublimed. The solid was suspected to be acetamide and this was later confirmed by ^{1}H N.M.R. and melting point. Acetamide is believed to have been formed during the purification procedure, from the reaction of ammonia with ethyl acetate. This was easily removed by sublimation under reduced pressure at 50 °C for a few hours. The product **154** was finally recrystallised from petrol as colourless needles in 79% yield.

5.3.7. Preparation of 5-(2-Hydroxy-3-isopropylaminopropoxy)indole-2-carboxylic acid isopropyl ester

The preparation of this agent **158** was achieved from the commercially available 5-hydroxyindole-2-carboxylic acid **155**, following a similar synthetic pathway as before, shown in scheme 5.2.

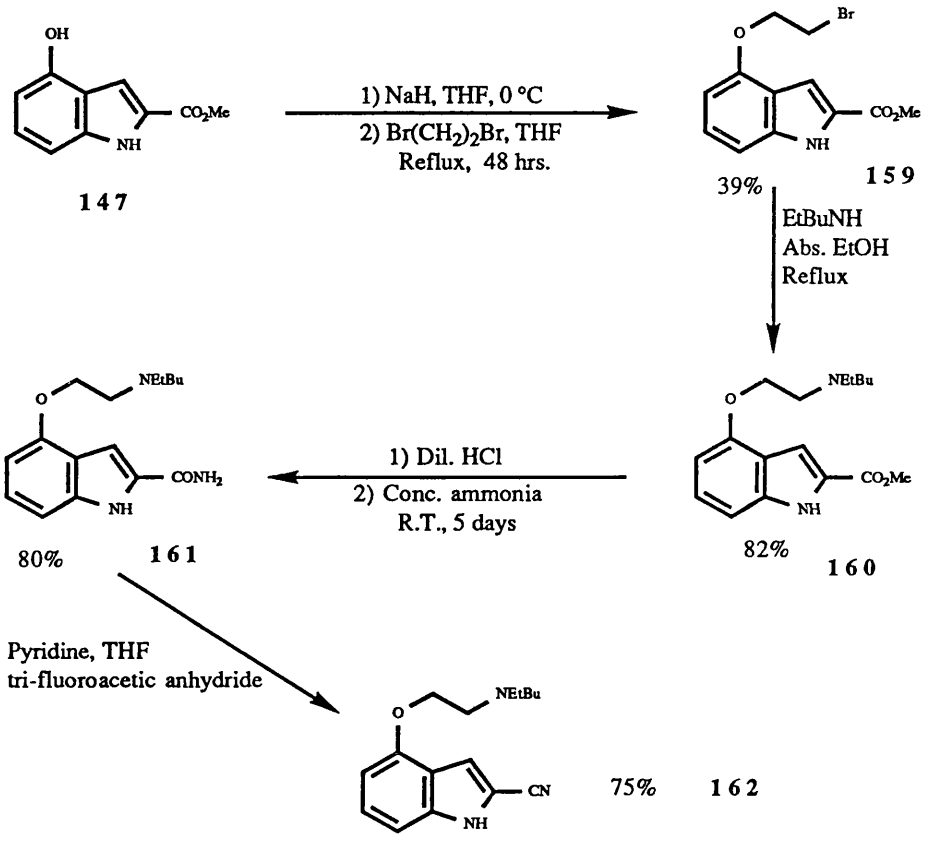


Scheme 5.2. Synthesis of 5-(2-Hydroxy-3-isopropylaminopropoxy)indole-2-carboxylic acid isopropyl ester

Thus, esterification of **155** with isopropanol containing catalytic amounts of 98% H_2SO_4, afforded **156** in 36%. This was reacted with excess epichlorohydrin to give the epoxide **157** in high yields. A solution of the epoxide in methanol was then refluxed with excess isopropyl amine in a Berghof pressure vessel. The crude product contained many small impurities and was subjected to purification by preparative HPLC. This was followed by recrystallisation from petrol to yield the amine **158** as colourless needles.

5.4. Preparation of 2-Substituted 4-[2-(N,N-Ethylbutyl)amino-ethoxy]indoles (Type 2 Analogues)

The synthetic strategy for the preparation of analogues **160-162** were similar as those described in previous chapters. The synthesis of the requisite starting material **147** was discussed in the early part of this chapter.



Scheme 5.3. Synthesis of 2-Substituted 4-[2-(N,N-Ethylbutylamino)ethoxy]indoles

The condensation reaction of **147** with 1,2-dibromoethane in THF in the presence of NaH afforded **159** in only 39% yield. The yield is about 30% lower than the corresponding reaction with 1,3-di-bromopropane. Attempts to increase the yield, including longer reaction time or use of excess amine were unfruitful. However, the reaction was clean and the starting material was easily recovered by column chromatography. The

bromo intermediate **159** was then reacted with ethylbutyl amine in ethanol and after purification by column chromatography and recrystallisation from petrol, **160** was obtained as colourless needles. The amide **161** was prepared by stirring a solution of **160** in methanol / conc. ammonia at room temperature for five days. The crude compound was recrystallised from petrol as colourless needles. Due to the poor solubility of the methyl ester, large volumes of the reaction solvent were necessary. This method may therefore limit the large scale preparation of the amide.

Dehydration [6] of the amide **161** to the corresponding nitrile **162** was achieved, following a procedure by Casini et al [15]. Thus, a solution of the amide in THF containing pyridine and tri-fluoroacetic anhydride was stirred at room temperature for three hours. The crude product was subjected to column chromatography and later recrystallised from petrol to give the nitrile as crystalline flakes.

5.5. Structure-Activity Results and Discussions

Type 1 series:

The compounds of this series were assayed at 5-HT_{1A}, 5-HT_{1B} and β-adrenergic receptors and their affinities were determined (see table 5.1). One of the objectives of this project was to determine the role of certain structural features of aryloxypropanolamines with regard to serotonergic binding. The following results from the binding experiment clearly fulfils this task;

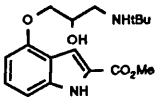
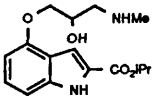
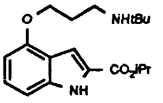
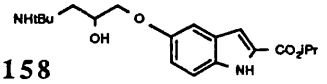
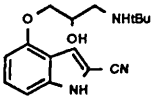
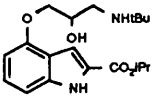
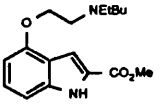
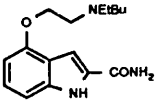
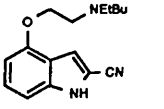
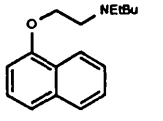
i) Replacement of the isopropyl of the ester with a methyl group enhances affinity at 5-HT_{1A} and β-adrenoceptors but slightly reduces it at 5-HT_{1B} receptors (i.e. **41 & 152**). The methyl ester is the best substituent for maximising affinity at 5-HT_{1A} receptors.

ii) In contrast to the above, replacement of the bulky t-butyl of the terminal amine with the smaller methyl group reduces binding at all receptors but more dramatically at β-adrenoceptors (i.e. **41 & 151**). This, further confirms other reports and our earlier observations in the naphthalene and pindolol series that a bulky alkyl group is crucial for β-adrenergic binding.

iii) Removal of the side chain hydroxyl group is detrimental to affinity at all receptors (i.e. **41 & 154**). This is in complete contrast to our earlier observation in the naphthalene series and to Glennon's findings [5] which suggested that removal of the hydroxyl group slightly favours 5-HT_{1A} and 5-HT_{1B} binding.

iv) Relocation of the aminopropoxy chain from the indole-4-position to the 5-position (i.e. **41 & 158**) completely abolishes affinity at serotonergic receptors but interestingly some affinity for the β-adrenoceptors remained.

Table 5.1. Affinities of SDZ 21009 Analogues for 5-HT_{1A}, 5-HT_{1B} and β-Adrenergic Receptors and Effects at 5-HT_{1A} and 5-HT_{1B} Receptors.

Compound	Structure	IC_{50} values, nM: 5-HT_{1A} [8-OH-DPAT]	5-HT_{1B} [^{125}I]CYP	β-Adrenoceptor [^{125}I]PIN.	Effects at 5-HT_{1A} Receptor	Effects at 5-HT_{1B} Receptor
Type 1						
152		2 (± 0.3)	5 (± 0.3)	0.7 (± 0.1)	N.D.	No Effect
151		226 (± 52)	34 (± 1)	91 (± 11)	N.D.	Antagonist
154		610 (± 110)	556 (±167)	295 (± 21)	N.D.	Antagonist
158		>10000	>10000	804 (± 28)	N.D.	N.D.
40 [a]		2.8 (± 0.9)	2.4 (± 0.7)	0.46 (± 0.05)	Antagonist	N.D.
41 [a]		22 (± 1)	2.3 (± 0.7)	2.6 (± 0.5)	Antagonist	N.D.
Type 2						
160		20 (± 6)	2754 (± 300)	3020 (± 680)	Partial Agonist	N.D.
161		34 (± 6)	>10000	>10000	Partial Agonist	N.D.
162		42 (± 1)	>10000	9225 (± 105)	Full Agonist	N.D.
57 [b]		39 (± 2)	1100 (± 80)	5000 (± 300)	N.D.	N.D.

[a] *Data from Engel et al* [1] *and* [b] *from Glennon et al* [5]

It can therefore be said, that all structural modifications, except in one case **152** yielded compounds with lower affinities than that of the lead agents at

all receptors. These modifications also failed to provide any selectivity for any of the agents.

These compounds were also tested for their effect at 5-HT_{1B} receptors. Compound **152** at concentrations of 10^{-5} molar, surprisingly showed no effect in two tests, but **151** and **154** displayed antagonism at concentrations as low as 10^{-7} moles.

Type 2 series:

Compounds **160-162** were also tested for the binding affinities at all receptors under consideration. The results are extremely encouraging. All three compounds show high affinity and specific binding for the 5-HT_{1A} receptors. The methyl ester **160** is the most potent one in the series with an affinity of 20 nM and a selectivity greater than 140-fold against 5-HT_{1B} and β-receptors. The amide **161** seem to offer a compromise; whereas it binds with slightly lower affinity (34 nM) than **160**, it has greater than 300-fold selectivity for 5-HT_{1A} receptors. Surprisingly, the cyano compound **162** is less potent (42 nM) than either of the two ligands in the series, but nevertheless it retains its high specificity for 5-HT_{1A} receptors.

These ligands were also tested in a 5-HT_{1A} functional assay for their ability to inhibit forskoline stimulated adenylate cyclase activity in the rat hippocampus. In this test, the methyl ester **160** showed an EC_{50} of only 16 nM and displayed partial agonistic activity at 5-HT_{1A} receptors. In this test, **161** and **162** appeared to be about 10-fold less active (200 nM and 130 nM respectively) than **160** and were classified as partial agonist and full agonist respectively.

5.6. Experimental

5-Hydroxyindole-2-carboxylic acid isopropyl ester (156)

A solution of 5-hydroxyindole-2-carboxylic acid **155** (2.0g, 1.13 x 10^{-2} mole) in anhydrous isopropranol (100g) containing 98% sulphuric acid (0.15g) was refluxed for four days. The volume of the solution was reduced in vacuo to a quarter and water (100 ml) was added. The pH of the solution was then adjusted to 10 ± 1 with dilute potassium hydroxide solution and the mixture was extracted with two portions of ether (200 ml in total). The combined extracts were dried over $MgSO_4$, filtered and then taken to dryness to give the product as a straw coloured solid (0.9g, 36.2%).

5-(2,3-Epoxypropoxy)indole-2-carboxylic acid isopropyl ester (157)

A solution of **156** (0.85g, 3.90 x 10^{-3} mole) in epichlorohydrin (9.5g, 0.10 mole) containing 1-methylpiperazine (0.1g) was heated to about 95 °C for 30 minutes. Epichlorohydrin was removed in vacuo, to give a straw coloured oil. The crude product was not further purified but heated in anhydrous isopropranol under reduced pressure to azeotropically remove the remaining traces of epichlorohydrin (1.05g, 93% crude).

M.S. m/z : 275 (M^+, 12%), 274 (M^+-1, 85%), 232 (M^+-42, 3%), 146 (M^+-129, 5%), 116 (M^+-159, 12%), 98 (M^+-177, 12%), 72 (M^+-203, 100%).

5-(2-Hydroxy-3-isopropylaminopropoxy)indole-2-carboxylic acid isopropyl ester (158)

A solution of **157** (0.50g, 1.82 x 10^{-3} mole) in methanol (40 ml) containing isopropylamine (1.10g 1.86 x 10^{-2} mole) was heated to 55 °C in a pressure vessel for 24 hours. The solvent and excess amine were removed under reduced pressure and the resulting solid was subjected to preparative HPLC, eluting with 30% water in methanol. The eluant (600 ml) was concentrated to a small volume and extracted 3 times with chloroform (150 ml in total). The combined extracts were dried over $MgSO_4$, filtered and then evaporated to dryness under reduced pressure. The product was recrystallised from petrol to give colourless needles (0.065g).

M.P. 122-125 °C

Analysis Calculated for $C_{18}H_{26}N_2O_4$: C,64.65, H,7.84, N,8.37
Found : C,64.93, H,7.99, N,8.24

M.S. m/z : 334 (M^+, 2%), 319 (M^+-15, 2%), 290 (M^+-44, 3%), 275 (M^+-59, 4%), 219 (M^+-115, 53%), 177 (M^+-157, 10%), 159 (M^+-175, 20%), 130 (M^+-204, 8%), 116 (M^+-218, 11%), 102 (M^+-232, 12%), 72 (M^+-262, 100%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.09 [6H, NCH($C\underline{H}_3$)$_2$, d], 1.39 (6H, OCH($C\underline{H}_3$)$_2$, d), 2.69-2.96 (1H, NCH, m), 2.69-2.96 (2H, CH_2N, m), 3.02 (2H, OCH_2, m), 3.02 (1H, C<u>H</u>OH, m), 5.25 [1H, OC<u>H</u>(CH_3)$_2$, m], 7.01 (1H, indole-6-H, dd), 7.09 (1H, indole-4-H, d), 7.25 (1H, indole-3-H, s), 7.30 (1H, indole-7-H, d), 8.78 (1H, br., s).

I.R. (KBr) : 3320, 2959, 1688, 1524, 1445, 1214, 1030, and 723 cm^{-1}.

U.V. (CH_3OH), λ, ε : 216 nm (26605), 291 nm (21890), 321 nm shoulder (5725).

H.P.L.C. : column: 250 x 4.5 mm Lichrosorb RP Select B, 5 µm + 40 x 4 mm guard column.
Mobile Phase: Pump A; H_2O + 0.1% Et_3N, Pump B; CH_3OH + 0.1% Et_3N, Pump A : Pump B; 30 : 70.
Flow Rate: 1 ml/min.
Area of the main peaks: 99.70%, 0.32% at 11.97, and 3.12 minutes respectively.

2-Nitro-5-benzyloxytoluene (142)

A solution of 2-methyl-3-nitrophenol [13] **141** (29.50g, 0.193 mole) in aqueous sodium hydroxide (8.50g, 70 ml) was made up. To this was added a solution of benzyl chloride (26.78g, 0.212 mole) in ethanol (200 ml) and the solution was refluxed for 8 hours. The solution was concentrated in vacuo to a small volume and water (200 ml) was then added. The mixture was extracted with ether and the combined extracts (400 ml) was dried over $MgSO_4$. Ether was removed and the residue was recrystallised from petrol to give the product as light yellow needles. (39.47g, 84%).

M.P. 60-61 °C

M.S. m/z : 243 (M^+, 19%), 152 (M^+-91, 3%), 105 (M^+-138, 7%), 91 (M^+-152, 100%), 77 (M^+-166, 22%), 65 (M^+-178, 57%).

I.R. (KBr) : 2871, 1601, 1517, 1454, 1361, 1264, 1210, 1054, 843, 764 and 703 cm^{-1}.

2-Nitro-6-benzyloxyphenylpyruvic acid (143)

Potassium metal [12] (12.65g, 0.324 mole) was dissolved in anhydrous methanol (70 ml). This was added dropwise with stirring under a slow stream of argon to a cold solution of 2-nitro-5-benzyloxytoluene **142** (37.46g, 0.154 mole) and diethyl oxalate (47.0g, 0.322 mole) in sodium dried toluene (80 ml) and anhydrous ether (250 ml). After the addition was complete, the reaction flask was stoppered and allowed to stand in the fridge at 10 °C for 12 hours and then at room temperature for a further 3 days. Ether (200 ml) was then added to the reaction mixture and the dark reddish residue filtered and washed with two portions of ether (2 x 100 ml). The residue was dissolved in water (540 ml) and aqueous sodium hydroxide (7.40g, 90 ml) was added.

The resulting reddish brown mixture was shaken with ether (310 ml) for at least one hour, where two layers were obtained. The layers were separated and the aqueous layer was extracted twice with ether (2 x 250 ml). The aqueous layer was acidified with conc. hydrochloric acid and a clear red oil precipitated out. water was decanted off and the oil was washed with ice-cold water. The product solidified, when dried at 50 °C and 0.1 mmHg for 40 hours (43.90g, 90%).

M.S. m/z : 315 (M^+, 1%), 298 (M^+-17, 0.1%), 290 (M^+-25, 0.1%), 281 (M^+-34, 0.1%), 270 (M^+-45, 2%), 255 (M^+-60, 3%), 239 (M^+-76, 3%), 225 (M^+-90, 3%), 209 (M^+-106, 2%), 165 (M^+-150, 3%), 152 (M^+-163, 15%), 105 (M^+-210, 10%), 91 (M^+-224, 100%), 77 (M^+-236, 35%), 65 (M^+-250, 57%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 4.61 (2H, s), 5.10 (2H, s), 6.43 (2H, br., s) 7.16-7.47 (7H, m), 7.66 (1H, dd).

References, Page 191, Structures & Abbreviations, Page 205

I.R. (KBr) : 3567, 2480, 1601, 1710, 1520, 1450, 1339, 1265, 1212, 1054, 843, 1050, 735 and 697 cm^{-1}.

4-Benzyloxyindole-2-carboxylic acid (144)

2-Nitro-6-benzyloxyphenylpyruvic acid [12] **143** (40.0g, 0.127 mole) was suspended in water (440 ml) and aqueous sodium hydroxide (2 M, ca. 60 ml) was added. The solution was stirred vigorously and sodium hydroxide (2 M, 60 ml) and $Na_2S_2O_4$ (51g) was added in small portions simultaneously. The addition approximately took one hour and the temperature never exceeded 40 °C. Conc. HCl (75 ml) was mixed with water (75 ml) and added dropwise to the solution. The mixture was cooled and the acid filtered, washed twice with cold water and dried (26.4g, 81%).

M.S. m/z : 267 (M^+, 53%), 249 (M^+-18, 2%), 223 (M^+-44, 1%), 176 (M^+-91, 7%), 158 (M^+-109, 17%), 142 (M^+-125, 1%), 130 (M^+-137, 13%), 114 (M^+-153, 2%), 102 (M^+-165, 14%), 91 (M^+-176, 100%), 69 (M^+-198, 9%), 65 (M^+-202, 24%).

^1H N.M.R. (DMSO, 200 MHz) : δ 5.21 (2H, s), 6.59 (1H, d), 7.00-7.44 (8H, m) 11.85 (1H, S).

I.R. (KBr) : 3325, 3038, 1619, 1614, 1581, 1511, 1424, 1257, 1197, 1060, 760 and 69 cm^{-1}.

4-Benzyloxyindole-2-carboxylic acid methyl ester (145)

A solution of 4-benzyloxyindole-2-carboxylic acid **144** (5.0g, 0.20 mole) in methanol (130g) containing 98% sulphuric acid (0.5g) was refluxed for ten hours. The solution was cooled and its pH was adjusted to 7 ± 1 with potassium hydroxide (0.01 molar). The solution was concentrated in vacuo and then partitioned between water (100 ml) and ether (100 ml).

The organic layer was separated and dried over $MgSO_4$. Ether was removed and the resulting residue was dried at 40 °C and 0.1 mmHg for 8 hours. (2.30g, 40%).

The aqueous layer was acidified with dilute hydrochloric acid and then extracted twice with ether. Ether was removed and 1.1g of pure acid was recovered.

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 4.91 (3H, s), 5.20 (2H, s), 6.56 (1H, d), 7.04 (1H, d), 7.17-7.54 (6H, m), 8.91 (1H, br., s).

I.R. (KBr) : 3332, 1691, 1578, 1514, 1437, 1354, 1240, 1200, 1080, 780 and 690 cm^{-1}.

4-Benzyloxyindole-2-carboxylic acid isopropyl ester (146)

A solution of **144** (10g, 0.037 mole) in anhydrous isopropanol (168g) containing 98% sulphuric acid (0.3g) was refluxed for 48 hours. The solution was cooled to below 5 °C and its pH was adjusted to 7 ± 1 with potassium hydroxide (0.01 molar). The mixture was concentrated in vacuo, and then partitioned between water (150 ml) and ether (250 ml). The organic layer was separated and dried over MgSO4. Ether was removed and the resulting residue was recrystallised from aqueous methanol and then from petrol to give colourless needles (2.30g, 19.90%).

The aqueous layer was acidified with dilute hydrochloric acid and the precipitate was collected by filtration (6.7g).

M.P. 162-165 °C

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.17 (6H, d), 5.20 (2H, s), 5.26 (1H, m), 6.57 (1H, d), 7.03 (1H, d), 7.18-7.53 (6H, m),8.90 (1H, br., s).

I.R. (KBr) : 3311, 1695, 1584, 1516, 1436, 1360, 1247, 1206, 1089, 758 and 690 cm^{-1}.

4-Hydroxyindole-2-carboxylic acid methyl ester (147)

4-Benzyloxyindole-2-carboxylic acid methyl ester [12-14] **145** (2.2g, 7.3 x 10^{-3} mole) was dissolved under reflux in methanol (500 ml). The solution was filtered and then transferred to a Berghof pressure vessel. Nitrogen was bubbled through the solution for 5 minutes and 10% Pd/C (1.0g) was carefully added. The vessel was sealed and hydrogen gas was then introduced. The pressure was maintained between 5-10 atmospheres for 8 hours with stirring. Nitrogen was then bubbled through the solution for 5 minutes and Pd/C was carefully filtered. Methanol was then removed in vacuo, and the product was obtained as light brown powder (1.25g, 83.6%).

M.P. 175-178 °C

M.S. m/z : 191 (M^+, 55%), 159 (M^+-31, 100%), 149 (M^+-42, 4%), 130 (M^+-61, 8%), 103 (M^+-88, 16%), 76 (M^+-115, 6%), 69 (M^+-122, 10%).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 3.93 (1H, s), 6.52 (1H, dd), 7.01 (1H, d) 7.17 (1H, t), 7.25 (1H, s), 7.31 (1H, s), 8.94 (1H, br., s).

I.R. (KBr) : 3325, 1695, 1628, 1584, 1528, 1438, 1351, 1240, 1207 and 756 cm^{-1}.

4-Hydroxyindole-2-carboxylic acid isopropyl ester (148)

4-Benzyloxyindole-2-carboxylic acid isopropyl ester [12-14] **146** (2.2g, 7.11 x 10^{-3} mole) was dissolved under reflux in methanol (400 ml). The solution was transferred to a Berghof pressure vessel and nitrogen was

bubbled through the solution for 5 minutes. 10% Pd/C (1.0g) was carefully added, maintaining the flow of nitrogen. The vessel was sealed and hydrogen gas was then introduced. The pressure was maintained between 5-10 atmospheres for 4 hours with stirring. Nitrogen was then bubbled through the solution for 5 minutes and Pd/C was carefully filtered. Methanol was then removed in vacuo, and the product was obtained as light brown powder (1.28g, 82%).

I.R. (KBr) : 3319, 1688, 1628, 1584, 1524, 1444, 1354, 1244, 1204 and 753 cm^{-1}.

4-(2,3-Epoxypropoxy)indole-2-carboxylic acid methyl ester (149)

A solution of 4-hydroxyindole-2-carboxylic acid methyl ester **147** (0.5g, 2.62 x 10^{-3} mole) in epichlorohydrin (6.0g, 0.65 mole) containing 1-methylpiperazine (0.04g) was heated to 90 °C for 45 minutes under a slow stream of nitrogen. Epichlorohydrin was removed in vacuo, and the resulting residue was heated in anhydrous isopropanol (50 ml) and then ether (50 ml) under reduced pressure to azeotropically remove the remaining traces of epiclorohydrin. The product was dissolved in small quantities of methanol and the insoluble materials filtered off. Methanol was removed and the product was obtained as a straw coloured solid (0.38g, 59%).

M.S. m/z : 247 (M^+, 100%), 233 (M^+-14, 0.1%), 215 (M^+-32, 20%), 204 (M^+-43, 7%), 190 (M^+-57, 18%), 174 (M^+-73, 6%), 159 (M^+-88, 67%), 142 (M^+-105, 6%), 130 (M^+-117, 24%), 114 (M^+-133, 5%), 102 (M^+-145, 18%), 76 (M^+-171, 15%), 62 (M^+-185, 5%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 2.85 (1H, dd), 2.94 (1H, t), 3.48 (1H, m)
3.90 (3H, s), 4.12 (1H, dd), 4.36 (1H, dd), 6.49 (1H, d),
7.05 (1H, d), 7.23 (1H, t), 7.37 (1H, s), 9.08 (1H, br., s).

4-(2,3-Epoxypropoxy)indole-2-carboxylic acid isopropyl ester (150)

A solution of 4-hydroxyindole-2-carboxylic acid isopropyl ester **148** (0.6g, 2.70 x 10^{-3} mole) in epichlorohydrin (6.33g, 0.68 mole) containing 1-methyl- piperazine (0.04g) was heated to 90 °C for 45 minutes under a slow stream of nitrogen. Epichlorohydrin was removed in vacuo, and the resulting residue was but heated in anhydrous isopropranol (50 ml) and anhydrous ether (50 ml) under reduced pressure to azeotropically remove the remaining traces of epichlorohydrin. The product was dissolved in small quantities of methanol and the insoluble materials filtered off. Methanol was removed and the product was obtained as a straw coloured solid (0.41g, 55%).

M.S. m/z : 275 (M^+, 96%), 260 (M^+-15, 0.1%), 233 (M^+-42, 44%),
215 (M^+-60, 44%), 198 (M^+-77, 2%), 185 (M^+-90, 8%),
177 (M^+-98, 41%), 159 (M^+-116, 100%), 142 (M^+-133,
7%), 130 (M^+-145, 30%), 114 (M^+-161, 7%), 102 (M^+- 173,
19%), 76 (M^+-199, 24%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.39 (6H, d), 2.80 (1H, dd), 2.94 (1H, t)
3.62 (1H, m), 4.12 (1H, dd), 4.35 (1H, dd), 5.25 (1H, m),
6.49 (1H, d), 7.04 (1H, d), 7.21 (1H, t), 7.36 (1H, s), 8.88
(1H, br., s).

4-(3-t-Butylamino-2-hydroxypropoxy)indole-2-carboxylic acid methyl ester (151)

A solution of 4-(2,3-epoxypropoxy)indole-2-carboxylic acid methyl ester **149** (0.18g, 7.3 x 10^{-3} mole) and t-butyl amine (0.32g, 4.40 x 10^{-3} mole) in methanol (30 ml) was heated to 55 °C for 16 hours. The solvent and excess amine were removed in vacuo, and the resulting residue was chromatographed on silica eluting with 1% ammonia (d = 0.88) in ethyl acetate/methanol (4:1). The crude product was recrystallised from from a mixture of petrol/toluene as a white crystalline solid. (0.14g, 60.0%).

M.P. 133-134 °C

Analysis Calculated for $C_{17}H_{24}N_2O_4$: C,63.73, H,7.55, N,8.74
Found : C,63.93, H,7.68, N,8.58

M.S. m/z : 320 (M^+, 6%), 305 (M^+-15, 42%), 289 (M^+-31, 2%), 276 (M^+-44, 18%), 198 (M^+-122, 4%), 191 (M^+-129, 34%), 174 (M^+-146, 3%), 159 (M^+-161, 25%), 130 (M^+-190, 25%), 114 (M^+-206, 30%), 102 (M^+-218, 13%), 91 (M^+-229, 14%), 86 (M^+-234, 99%), 71 (M^+-249, 34%), 57 (M^+-263, 65%) .

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.11 [9H, $C(CH_3)_3$, s], 2.71-2.99 (2H, CH_2N, m), 3.94 (3H, OCH_3, s), 3.98-4.24 (3H, OCH_2 and C<u>H</u>OH, m), 6.54 (1H, indole-5-H, d), 7.03 (1H, indole-7-H, m), 7.22 (1H, indole-6-H, m), 7.35 (1H, indole-3-H, s), 8.87 (1H, indole-NH, br., s).

I.R. (KBr) : 3506, 2965, 1688, 1528, 1431, 1352, 1210, 1120 and 723 cm^{-1}.

U.V. (CH_3OH), λ , ε : 207 nm (13350), 232 nm (26653), 288 nm shoulder (19280), 320 nm (5957).

H.P.L.C. : column: 250 x 4.5 mm Lichrosorb RP Select B, 5 μm + 40 x 4 mm guard column.
Mobile Phase: A gradient run starting from 40% CH_3OH + 0.1% Et_3N + 0.5% AcOH in H_2O to 80% over 20 minutes.
Flow Rate: 0.75 ml/min.
Area of the main peaks: 99.60%, 0.22% at 9.49 and 16.93 minutes respectively.

4-(3-Methylamino-2-hydroxypropoxy)indole-2-carboxylic acid isopropyl ester (152)

A solution of 4-(2,3-epoxypropoxy)indole-2-carboxylic acid isopropyl ester **149** (0.25g, 9.1 x 10^{-3} mole) in methanol (30 ml) was chilled and methyl amine (5.6g, 0.182 mole) was then added. The solution was transferred to a Berghof pressure vessel and heated to 55 °C for 24 hours. The solvent and excess amine were removed in vacuo, and the resulting residue was chromatographed on silica eluting with 2% ammonia (d = 0.88) in methanol. The crude product was recrystallised from from a mixture of petrol/toluene as white silky needles. (0.12g, 53.9%).

M.P. 179-180 °C

Analysis Calculated for $C_{16}H_{22}N_2O_4$: C,62.73, H,7.24, N,9.14
Found : C,62.65, H,7.28, N,8.99

M.S. m/z : 306 (M^+, 8%), 247 (M^+-59, 1%), 219 (M^+-87, 61%), 177 (M^+-129, 25%), 159 (M^+-147, 26%), 130 (M^+-176, 5%), 114 (M^+-192, 1%), 102 (M^+-204, 2%), 88 (M^+-218, 22%), 44 (M^+-262, 100%).

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.39 [6H, $CH(C\underline{H}_3]_2$, d), 2.50 (3H, NCH_3, s), 2.87 (2H, CH_2N, m), 4.15 (3H, OCH_2 and C<u>H</u>OH, m), 5.25

(1H, OC<u>H</u>$(CH_3)_2$, m), 6.52 (1H, indole-5-H, d), 7.02 (1H, indole-7-H, d), 7.20 (1H, indole-6-H, t), 7.34 (1H, indole-3-H, s), 8.92 (1H, indole-NH, br., s).

I.R. (KBr) : 3319, 2965, 1694, 1581, 1521, 1367, 1264, 1204, 1124 and 753 cm^{-1}.

U.V. (CH_3OH), λ, ε : 20 nm (12391), 232 nm (25865), 287 nm shoulder (19050), 315 nm (5576).

H.P.L.C. : column: 250 x 4.5 mm Lichrosorb RP Select B, 5 μm + 40 x 4 mm guard column.
Mobile Phase: A gradient run starting from 40% CH_3OH + 0.1% Et_3N + 0.5% AcOH in H_2O to 80% over 20 minutes.
Flow Rate: 0.75 ml/min.
Area of the main peaks: 98.37%, 1.63% at 11.01 and 6.68 minutes respectively.

4-(2-Bromoethoxy)indole-2-carboxylic acid methyl ester (159)

A suspension of 60% NaH (0.38g, 9.63 x 10^{-3} mole) in anhydrous THF (20 ml) was added dropwise to a chilled solution of 4-hydroxyindole-2-carboxylic acid methyl ester **147** (1.60g, 8.4 x 10^{-3} mole) in THF (20 ml) over a period of 20 minutes. A solution of 1,2-dibromoethane in THF (20 ml) was then added under a slow stream of nitrogen and the mixture was refluxed for 48 hours. The mixture was cooled, filtered and then taken to dryness. The resulting residue was chromatographed on silica, eluting with 30% petrol in chloroform and then with 5% methanol in chloroform. Removal of the solvent gave the pure product as white crystalline solid (0.98g, 39%).

M.P. 179-180 °C

M.S. m/z : 299 (M^+, 67%), 297 (M^+, 67%), 267 (M^+-32, 33%), 265 (M^+-34, 33%), 217 (M^+-82, 1%), 204 (M^+-95, 1%), 190 (M^+-109, 64%), 158 (M^+-141, 100%), 130 (M^+-169, 46%), 102 (M^+-197, 30%), 76 (M^+-223, 26%).

I.R. (KBr) : 3312, 1688, 1578, 1517, 1431, 1354, 1260, 1244, 1207 and 753 cm^{-1}.

4-(3-Bromopropoxy)indole-2-carboxylic acid isopropyl ester (153)

A suspension of 60% NaH (0.084g, 2.1 x 10^{-3} mole) in anhydrous THF (15 ml) was added dropwise to a chilled solution of 4-hydroxyindole-2-carboxylic acid isopropyl ester **148** (0.40g, 1.82 x 10^{-3} mole) in THF (15 ml) over a period of 20 minutes. A solution of 1,3-dibromopropane (7.37g, 0.036 mole) in THF (15 ml) was then added under a slow stream of nitrogen and the mixture was refluxed for 8 hours. The mixture was cooled, filtered and then taken to dryness. The resulting semi-solid residue was chromatographed on silica, eluting with a mixture of petrol / chloroform / ether (5:4:1). Removal of the solvent gave the product as a white solid (0.42g, 67.4%).

M.P. 179-180 °C

^1H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.41 (6H, d), 2.60 (2H, m), 3.31 (2H, t), 4.25 (2H, t), 5.27 (1H, m), 6.58 (1H, d), 7.04 (1H, d), 7.22 (1H, t), 7.31 (1H, s), 9.02 (1H, br., s).

I.R. (KBr) : 3319, 2971, 1688, 1581, 1521, 1381, 1247, 1214, 1104, 977 and 753 cm^{-1}.

4-[2-(N,N-Ethylbutyl)aminoethoxy]indole-2-carboxylic acid methyl ester (160)

A solution of 4-(2-bromoethoxy)indole-2-carboxylic acid methyl ester 159 (1.20g, 4.03 x 10^{-3} mole) and ethylbutyl amine (20.33g, 0.20 mole) in absolute ethanol (150 ml) were refluxed for 8 hours. The solvent and the excess amine were then removed in vacuo, and the resulting residue was chromatographed on silica, eluting with 10% methanol in chloroform. The product was obtained as colourless needles after recrystallisation from small amounts of petrol, cooling the solution to -25 °C for 24 hours before isolation (1.05g, 81.9 %).

M.P. 54-56 °C

Analysis Calculated for $C_{18}H_{26}N_2O_3$: C,68.23, H,8.45, N,8.64

Found : C,67.90, H,8.23, N,8.30

M.S. m/z : 318 (M^+, 6%), 303 (M^+-15, 0.3%), 287 (M^+-31, 0.3%), 275 (M^+-43, 10%), 243 (M^+-75, 0.2%), 200 (M^+-118, 0.1%), 186 (M^+-132, 3%), 158 (M^+-160, 6%), 138 (M^+-180, 5%), 128 (M^+-190, 28%), 114 (M^+-204, 100%), 102 (M^+-216, 4%), 83 (M^+-235, 6%), 70 (M^+-248, 48%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 0.93 (3H, $CH_2CH_2C\underline{H}_3$, t), 1.10 (3H, $NCH_2C\underline{H}_3$, t), 1.39 (2H, $CH_2C\underline{H}_2CH_3$, m), 1.49 (2H, $NCH_2C\underline{H}_2$, m), 2.57 (2H, $NC\underline{H}_2CH_2$, m), 2.67 (2H, $NC\underline{H}_2CH_3$, q), 2.98 (2H, $OCH_2C\underline{H}_2N$, t), 3.92 (3H, OCH_3, s), 4.19 (2H, OCH_2, t), 6.50 (1H, indole-5-H, d), 7.00 (1H, indole-7-H d), 7.22 (1H, indole-6-H, t), 7.32 (1H, indole-3-H, s), 8.82 (1H, indole-NH, br., s).

H.P.L.C. : column: 250 x 4.5 mm Lichrosorb RP Select B, 5 μm + 40 x 4 mm guard column.

Mobile Phase: CH_3CN : H_2O (30 : 70), both solvents contain 0.1% TFA.

Flow Rate: 1 ml/min.

Area of the main peaks: 100%, at 14.84 minutes.

4-(3-t-Butylaminopropoxy)indole-2-carboxylic acid isopropyl ester (154)

A solution of 4-(3-bromopropoxy)indole-2-carboxylic acid isopropyl ester **153** (0.30g, 8.82 x 10^{-4} mole) and t-butyl amine (12.90g, 0.176 M in absolute ethanol (50 ml) was heated to 55 °C for 24 hours. The solvent and the excess amine were removed in vacuo, and the resulting residue was chromatographed on silica, eluting with a mixture of ethyl acetate / ammonia (d = 0.88) / methanol (50:1:1). The crude product was recrystallised from petrol as colourless needles (0.23, 79%).

M.P. 106-107 °C

Analysis Calculated for $C_{19}H_{28}N_2O_3$: C,68.65, H,8.49, N,8.43

Found : C,68.80, H,8.55, N,8.32

M.S. m/z : 332 (M^+, 17%), 317 (M^+-15, 100%), 299 (M^+-33, 0.4%), 275 (M^+-57, 14%), 257 (M^+-75, 3%), 219 (M^+-113, 3%), 203 (M^+-129, 4%), 159 (M^+-173, 15%), 129 (M^+-203, 24%), 114 (M^+-218, 72%), 98 (M^+-234, 70%), 86 (M^+-246, 43%), 71 (M^+-261, 47%), 58 (M^+-274, 60%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 1.10 [9H, $C(CH_3)_3$, s], 1.38 [6H, $CH(C\underline{H}_3)_2$, d], 2.03 (2H, $OCH_2C\underline{H}_2$, m), 2.83 (2H, CH_2N, t), 4.19 (2H, OCH_2, t), 5.26 (1H, OCH, m), 6.50 (1H, indole-5-H, d), 7.00 (1H, indole-7-H, d), 7.20 (1H, indole-6-H, t), 7.34 (1H, indole-3-H, s), 8.86 (1H, indole-NH, br., s).

I.R. (KBr) : 3305, 2965, 1688, 1578, 1514, 1361, 1267, 1244, 1204, 1117, 980 and 723 cm^{-1}.

U.V. (CH_3OH), λ, ε : 206 nm (13203), 233 nm (28155), 289 nm shoulder (20837), 315 nm (6203).

H.P.L.C. : column: 250 x 4.5 mm Lichrosorb RP Select B, 5 µm + 40 x 4 mm guard column.
Mobile Phase: A gradient run starting from 40% CH_3OH + 0.1% Et_3N + 0.5% AcOH in H_2O to 80%, over 20 minutes.
Flow Rate: 0.75 ml/min.
Area of the main peaks: 100%, at 17.39 minutes.

4-[2-(N,N-Ethylbutyl)aminoethoxy]indole-2-acetamide (161)

A solution of 4-[2-(N,N-ethylbutyl)aminoethoxy]indole-2-carboxylic acid methyl ester **160** (0.98g, 3.08 x 10^{-3} mole) in conc. hydrochloric acid (100 ml), Methanol (500 ml) and ammonia (d = 0.88, 1L) was stoppered and allowed to stand at room temperature for 5 days. The volume of the solution was halved in vacuo, and the solution extracted with two portions of chloroform (2 x 150 ml). The combined extracts were dried over $MgSO_4$ and chloroform was then removed. The crude product was recrystallised from petrol as colourless needles (0.75g, 80.1%).

M.P. 110-112 °C

Analysis Calculated for $C_{17}H_{25}N_3O_2$: C,67.30, H,8.31, N,13.85
Found : C,67.09, H,8.50, N,13.81

M.S. m/z : 303 (M^+, 6%), 288 (M^+-15, 0.4%), 274 (M^+-29, 0.2%), 260 (M^+-43, 10%), 243 (M^+-60, 1%), 202 (M^+-101, 0.3%), 158 (M^+-145, 7%), 142 (M^+-161, 3%), 128 (M^+-175, 31%),

122 (M^+-181, 15%), 114 (M^+-189, 100%), 102 (M^+-201, 6%), 83 (M^+-217, 8%), 70 (M^+-233, 63%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 0.92 (3H, $CH_2CH_2C\underline{H}_3$, t), 1.08 (3H, $NCH_2C\underline{H}_3$, t), 1.34 (2H, $CH_2C\underline{H}_2CH_3$, m), 1.49 (2H, $C\underline{H}_2CH_2CH_3$, m), 2.57 (2H, $NC\underline{H}_2CH_2$, m), 2.67 (2H, $NC\underline{H}_2CH_3$, q), 2.97 (2H, CH_2N, t), 4.19 (2H, OCH_2, t), 5.85 (2H, NH_2, br., s), 6.51 (1H, indole-5-H, d), 7.01 (1H, indole-3-H, s), 7.20 (1H, indole-7-H, d), 7.25 (1H, indole-6-H, t), 9.25 (1H, indole-NH, br., s).

I.R. (KBr) : 3359, 3132, 2952, 2911, 2858, 1658, 1608, 1581, 1524, 1504, 1417, 1401, 1357, 1317, 1251, 1130, 1087 and 723 cm^{-1}.

H.P.L.C. : column: 250 x 4.5 mm Lichrosorb RP Select B, 5 μm + 40 x 4 mm guard column.
Mobile Phase: CH_3OH : H_2O (65 :35), both solvents contain 0.1% Et_3N.
Flow Rate: 1 ml/min.
Area of the main peaks: 100%, at 14.84 minutes.

4-[2-(N,N-Ethylbutyl)aminoethoxy]indole-2-nitrile (162)

A solution of 4-[2-(N,N-ethylbutyl)aminoethoxy]indole-2-acetamide [15] **161** (0.48g, 1.58 x 10^{-3} mole), pyridine (0.156g, 1.95 x 10^{-3} mole) in THF (50 ml) was cooled to 0 °C. To this was added dropwise with stirring, a chilled solution of tri-fluoroacetic anhydride (0.44g, 1.58 x 10^{-3} mole) in THF (20 ml) under a slow stream of nitrogen. The reaction mixture was stirred at room temperature for 3 hours. The solvent was removed in vacuo, and the residue chromatographed on silica, eluting with 7% methanol in

chloroform. The resulting colourless oil was dried at 0.1 mmHg for 8 hours before it solidified. The product was recrystallised from petrol as colourless flakes (0.38g, 84%).

M.P. 82-83 °C

Analysis Calculated for $C_{17}H_{23}N_3O$: C,71.55, H,8.12, N,14.72

Found : C,71.33, H,8.21, N,14.74

M.S. m/z : 285 (M^+, 3%), 270 (M^+-15, 1%), 256 (M^+-29, 0.1%), 242 (M^+-43, 16%), 185 (M^+-60, 2%), 157 (M^+-101, 3%), 141 (M^+-145, 2%), 121 (M^+-161, 5%), 114 (M^+-175, 100%), 102 (M^+-181, 2%), 83 (M^+-189, 5%), 70 (M^+-201, 43%).

^{1}H N.M.R. ($CDCl_3$, 200 MHz) : δ 0.93 (3H, $CH_2CH_2C\underline{H}_3$, t), 1.10 (3H, $NCH_2C\underline{H}_3$, t), 1.35 (2H, $CH_2C\underline{H}_2CH_3$, m), 1.52 (2H, $C\underline{H}_2CH_2CH_3$, m), 2.58 (2H, $NC\underline{H}_2CH_2$, m), 2.69 (2H, $NC\underline{H}_2CH_3$, q), 2.99 (2H, $OCH_2C\underline{H}_2$, t), 4.19 (2H, OCH_2, t), 6.53 (1H, indole-5-H, d), 6.98 (1H, indole-7-H, d), 7.18-7.34 (2H, indole3 and 6-H, m), 8.86 (1H, indole-NH, br., s).

I.R. (KBr) : 3052, 2951, 2852, 2671, 2210, 1614, 1584, 1529, 1507, 1377, 1254, 1160, 1140, 960, 767 and 723 cm^{-1}.

H.P.L.C. : column: 250 x 4.5 mm Lichrosorb RP Select B, 5 µm + 40 x 4 mm guard column.
Mobile Phase: A gradient run starting from 80% CH_3OH in H_2O containing 0.1% Et_3N to 100% MeOH + 0.1% Et_3N, over 15 minutes.
Flow Rate: 0.75 ml/min.
Area of the main peaks: 99.50% and 0.50% at 10.13 and 7.37 minutes respectively.

5.7. References

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CHAPTER 6

Biological Methods

6.1. Binding Assays

The affinity of all the synthesised compounds for 5-HT_{1A}, 5-HT_{1B} and β-adrenergic receptors (except for those in the naphthalene series, chapter 2) in vitro was assessed by their abilities to displace [^{3}H]8-OH-DPAT, [^{125}I]ICYP and [^{125}I]PIN respectively. 5-HT_{1A} and 5-HT_{1B} binding was assessed in rat hippocampal tissue, whereas β-adrenergic binding was measured in cerebral cortex membranes. The binding and 5-HT_{1A} functional assays were carried out by Dr. M. Beer at the laboratories of Merck Sharp & Dohme in Harlow.

6.1.1. 5-HT_{1A} Binding Assay

Membrane Preparation: On the day of the assay male Sprague Dawley rats (200g-300g) were killed, their brains removed and frontal cortices placed on ice. These were then transferred to 10-15 volumes of ice-cold sucrose (0.32 M) and homogenised with 20 strokes of a motor driven teflon/glass homogeniser (Janke and Kunkel) at 500 r.p.m. The homogenate was centrifuged (10 min., 900g, 4 °C). The supernatant was then removed and respun (21 min., 48000g, 4 °C). The supernatant was discarded and the pellet resuspended in 10-15 volumes of Tris HCl (50 mM) buffer (pH 7.5, room temperature) and rehomogenised with 10 strokes of the homogeniser. The homogenate was then incubated at 37 °C in a shaking water bath for 15 minutes to remove endogenous 5-HT before being centrifuged again (21 min., 48000g, 4 °C). Finally the supernatant was discarded and the pellet resuspended in assay buffer [TrisHCl (50 mM), $CaCl_2$ (5.7 mM), pargyline (10 μM), 0.1% ascorbic acid, pH 7.7 at room temperature], one gram wet weight per 120 ml of buffer. [^{3}H]8-OH-DPAT binding were carried out using methods based on those described by Gozlan et al [1] (1983) and by Pazos et al [2] (1984). All

assays were performed in duplicate. Membranes, [^{3}H]8-OH-DPAT (Amersham int.) and 5-HT (30 μM) which was used to define specific binding, and displacing drugs, were prepared in assay buffer. A 300 μl aliquot of the membrane suspension (approximately 50-150 μg protein) was incubated at 37 °C in a shaking water bath with [^{3}H]8-OH-DPAT (1-2 nM) and displacing drugs to give a final volume of 500 μl. The reaction was started by adding the membrane suspension and was terminated after 15 minutes by rapid filtration through Whatman GF/B glass fibre filters using a Brandel M-24R cell harvester. Each tube was then washed three times with Tris (4 ml, 50 mM), and the washings passed through the filters. The filters had previously been soaked in 0.3% Polyethylenimine (PEI)/0.5% Triton X 100 to reduce non-specific binding. The filters were then transferred to scintillation vials containing Beckman Ready Gel (10 ml), and the radioactivity determined by liquid scintillation spectrometry on a model 1219 Rack Beta, LKB scintillation counter.

6.1.2. 5-HT$_{1B}$ Binding Assay

Membrane preparation was performed as that described for 5HT$_{1A}$ binding assay. Final resuspension 1g wet weight of starting material to 50 ml of assay buffer.

Assay Buffer: Tris (10 mM), NaCl (154 mM), Pargyline 10 μM, pH 7.7 at room temperature.

All assays were performed in duplicate. [^{125}I]CYP (100-150 pM, specific activity, 2200 Ci\mM from NEN research products, NEX-189) was used to label 5HT$_{1B}$ recognition sites, 5-HT (30 μM) was used to define specific binding and isoprenaline (30 μM) to block out β-adrenoreceptors.

Membranes, [^{125}I]CYP, 5-HT, isoprenaline and displacing drugs were made up in assay buffer. An aliquot (150 µl) of the membrane suspension was incubated at 37 °C in a shaking water bath with the remaining drugs to give a final assay volume of 300 µl. The reaction was initiated by the addition of the membrane suspension and was terminated after 20 minutes by rapid filtration through Whatman GF\B glass fibre filters using a Brandel M-24R cell harvester. Each tube was then washed 3 times with 4 ml ice cold 50 mM Tris HCl (pH 7.5 at room temperature) and the washings pressed through the filters. The filters had previously been soaked in 0.3% PEI/0.5% Triton X 100 to reduce non-specific binding. The filters were then transferred to scintillation vials containing Beckman Ready Gel (10 ml), and the radioactivity determined by liquid scintillation spectrometry on a model 1219 Rack Beta, LKB scintillation counter.

6.1.3. β-Adrenoceptor Binding Assay

For a full description of material and methods and membrane preparation, see M. Beer et al [3] (1988).

6.2. Functional Assays

In addition to the binding experiments, many of the compounds were also tested for their activity as agonist and antagonist at 5-HT_{1A} and 5-HT_{1B} receptors.

6.2.1. 5-HT_{1A} Activity

EC_{50} values were determined from inhibition of forskolin stimulated adenylate cyclase activity in the rat hippocampus for four of the synthesised compounds (section 5.5.)

Membrane Preparation: Male, Sprague-Dawley rats weighing between 200g and 250g were decapitated and the brains quickly removed on ice. The hippocampus was quickly dissected out,weighed and transferred to 1:10 w/v ice-cold Tris (20 mM), sucrose (300 mM), DTT (5 mM), EGTA (5 mM), pH 7.4 at room temperature and homogenised using a Teflon/glass motor driven homogeniser at 500 r.p.m. for 20 strokes. The homogenate was centrifuged at 900g (2750 r.p.m.) for 10 minutes at 4 °C after which the pellet was discarded and the pellet recentrifuged at 20000g (13000 r.p.m.) for 20 minutes at 4 °C to give a P2 pellet. This was resuspended 1:10 w/v in ice-cold Tris (10 mM), EGTA (1 mM), pH 7.6 at room temperature as before and the homogenate incubated on ice for between 15 and 60 minutes. Following this it was centrifuged at 20000g (13000 r.p.m.) at 4 °C for 20 minutes and the pellet finally suspended in 1:20 w/v ice cold Tris (50 mM), EDTA (5 mM) at pH 7.6 at room temperature.

Assay Procedure: The reaction cocktail was made up as outlined below;

Reagent	Reagent Conc. mM	Reagent Vol. µl	Final Assay Conc. mM
TRIS-HCl pH 7.6	1000	250	50.00
NaCl	2000	250	100.00
GTP	3	50	0.03
cAMP	10	25	0.05
DTT	100	50	1.00
ATP	100	25	0.50
$MgCl_2$	1000	25	5.00
Pargyline	2000	25	10.00
[^{32}P]ATP		100	1-2 uCi
[^{3}H]cAMP		40	20000 DPM
H_2O		260	

Creatine phosphate sodium salt (6.4 mg), creatine phosphokinase (pinch).

The assay was set up in glass tubes on ice as follows;
EGTA (10 µl, 5 mM), IBMX solution (5 µl, pH 7.6), reaction cocktail (10 µl), 5-HT or test compounds (10 µl, 50 µM). All drugs were diluted in 0.1% ascorbate.

The reaction was initiated by the addition of membrane preparation (10 µl) to the tubes in quadruplicate, previously incubated at 30 °C for five minutes in a shaking water bath. The tubes were incubated with the membranes for a further 10 minutes at the same temperature. The reaction was terminated by the addition of ATP (100 µl, 100 mM), cAMP (2.5 µl) and sodium lauryl sulphate (150 µl) pH 7.5 at room temperature,

and the tubes returned to ice. Water (1 ml) was added to each tube before the ion exchange chromatography took place.

Controls without forskolin and controls in the absence of any drug were also incubated to determine maximum stimulation with no inhibitory drugs present.

Ion Exchange Chromatography: The ion exchange procedure using Dowex and Alumina columns were calibrated to show the separation of cAMP from ATP using radiolabels, [^{3}H]cAMP and [^{32}P]ATP. This enabled the optimum separation conditions for the two compounds to be determined.

A mixture of [^{3}H]cAMP and [^{32}P]ATP was prepared by adding [^{3}H]cAMP (40 µl, 20000 DPM), [^{32}P]ATP (100 µl, 1-2 µCi) and water (860 µl) to a glass vial. A sample (10 µl) was placed into a glass tube and water (1 ml) was added. This was decanted onto a Dowex column and allowed to drain completely. A second sample (10 µl) prepared in a similar way was decanted onto an Alumina column. A third sample (10 µl) with water (1000 µl) was placed in a scintillation vial and Hydrofluor (10 ml) was added to the vial counted over a two minute period on a ^{32}P/^{3}H dual label counting program to determine the total concentrations of radiolabels and therefore the total concentrations of cAMP and ATP used. Each procedure was repeated in triplicate.

Samples of water (0.5 ml) were added to the Dowex columns and collected in plastic scintillation vials, to which Hydrofluor (10 ml) was added. Values of ^{3}H and ^{32}P from the counting program gave a pattern for the elution of the two radiolabels and hence the two compounds from the Dowex columns.

Samples of imidazole (0.5 ml, 0.1 M, pH 7.3 at room temperature) were added to the Alumina columns and collected in plastic scintillation vials below and counted with Hydrofluor (10 ml) as above. This gave a pattern for the elution of the two radiolabels and therefore of the two compounds from the Alumina columns. Using these two procedures the volumes of water and imidazole necessary to achieve maximum elution of cAMP could be determined.

On the day of use, Dowex columns were regenerated with HCl (10 ml, 1 M) followed by distilled water (10 ml) and Alumina columns were regenerated with imidazole (10 ml, 1 M) pH 7.3 at room temperature. Tubes were decanted onto the Dowex columns, allowed to drain completely and a further 1 ml of distilled water added and drained. The Dowex columns were placed over the Alumina columns and distilled water (5 ml) added to the Dowex columns and allowed to drain completely through both sets of columns. The Alumina columns were washed with imidazole (1 ml, 0.1 M, pH 7.3 at room temperature) and allowed to drain completely. The Alumina columns were then placed over plastic scintillation vials and imidazole (4 ml, 0.1 M) added to the columns and collected in the vials. Hydrofluor (10 ml) was added to each scintillation vial and each was counted over a 2 minute period on a $^{32}P/^{3}H$ dual counting program.

Inhibition by each of the compounds was calculated by measuring the decrease in cAMP produced in the presence of the compound under test. This is shown by a decrease in [^{32}P]cAMP produced. The values of [^{3}H]cAMP gave a measure of recovery allowing for the correction of the [^{32}P]cAMP DPM obtained.

To test the ability of compounds to act as antagonists in the assay, their ability to reduce the effect of a standard agonist, 5-HT, was measured. The antagonist was included in the incubation together with the agonist and again percentage inhibition measured.

6.2.2. 5-HT_{1B} Activity

5-HT_{1B} functional assays were carried out by Prof. J. H. Wyllie at Whittington Hospital. Superfused mouse bladder strip responds to electrical stimulation (ES) by twitch contractions. These contractions are potentiated by 5-HT in the range 0.03-3.0 μM. The synthesised compounds (usually at concentration of 10^{-5} molar) were tested for their ability to potentiate the responses to ES or to block the 5-HT potentiation.

For a full description of methods, material and membrane preparation, see J. H. Wyllie et al [4-6] and section 1.5.1.

6.3. References

1) Gozlan H., El Mestikaway S., Pichat L., Glowinski J. and Hamon M., Nature, 305, 140, 1983.

2) Pazos A., Hoyer D. and Palacios J. M., Eur. J. Pharm., 106, 539, 1984.

3) Beer M., Richardson A., Poat J., Iverson L. and Stahl S. M., Biochemical Pharmacology, 37, No. 6, 1145, 1988.

4) Cooper M., Please N. M. and Wyllie J. H., Br. J. Pharmacol., 78, 85P, 1983.

5) Holt S. E., Cooper M. and Wyllie J. H., Eur. J. Pharmacol., 116, 105, 1985.

6) Holt S. E., Cooper M. and Wyllie J. H., Naunyn-Schmeideberg's Arch. Pharmacol., 334, 1986.

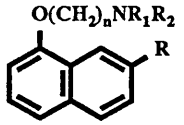
7.0 Summary and Conclusion

The purpose of the present study was to synthesise selective 5-HT_{1A} and 5-HT_{1B} agents. The β-adrenergic antagonists propranolol, pindolol and cyanopindolol are amongst the most potent 5-HT_{1A} and 5-HT_{1B} antagonists known. Our strategy was to remove or modify functional features which were essential for β-blocking activity and identify structural groups which provide selectivity between 5-HT_{1A} and 5-HT_{1B} receptors. For this purpose, analogues of each of the lead compounds and other novel serotonegic agents were prepared and their affinities evaluated from binding experiments.

Structure-binding relationships, within each series of analogues, together with their syntheses were fully discussed. The following is a summary of the findings and conclusions of this project;

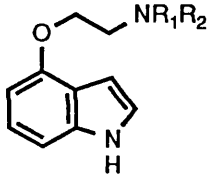
i) All attempts to identify structural groups which either enhance affinity or selectivity at 5-HT_{1B} v's β-receptors were unfruitful. 5-HT_{1B} receptors now appear to be less important than originally thought, mainly because they are specific to only a few species such as mice and rats and not humans. However, a potent and selective 5-HT_{1B} agent would be an invaluable pharmacological tool.

ii) In the propranolol series, we synthesised a range of novel compounds and learned that affinity is abolished with electron withdrawing substituents in the naphthalene 6-position. It was then evident that 5-HT_{1A} and 5-HT_{1B} receptors are extremely sensitive to the position of the substituents on the aromatic ring. A comparison of the structures of the neurotransmitter 5-HT, with other derivatives of propranolol suggested that derivatives with substituents in the 7-position are more likely to be active (see next page).



n = 2,3,4
R = CN, COOR', CONR'$_2$, SONR'$_2$
R' = H, Alkyl
R_1, R_2 = Me, Et, nPr, nBu,...

ii) A number of pindolol analogues were then synthesised in order to delineate the receptor requirements with respect to the functional features present in the lead molecule. The results indicated that the side chain hydroxyl and large bulky amino groups are not essential for 5-HT_{1A} affinity and that they can be removed or modified. These were major findings since these functional groups are essential to β-receptor and 5-HT_{1B} binding. 4-[3-(N,N-Dimethylamino)propoxy]indole **113** displayed the greatest 5-HT_{1A} affinity and selectivity in this series. It is strongly believed that further chemical modifications of **113**, (see below) would yield compounds with even greater 5-HT_{1A} affinity and selectivity.

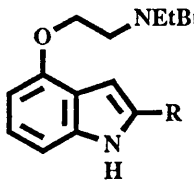


R_1, R_2 = H, Me, Et,....

iv) In chapter 4, a novel derivative of serotonin was prepared. it was demonstrated that derivatisation of 5-HT at the hydroxyl group with large bulky functional groups is indeed possible without serious loss of 5-HT_{1A} affinity. This agent therefore offers a great deal of scope for further structural modifications to achieve higher affinity and selectivity.

v) Finally two series of compounds analogous to the structures of SDZ 21009 or cyanopindolol were synthesised. In the first series, simple modifications to the structure of SDZ 21009 yielded compounds with much lower affinities. Removal of the side chain hydroxyl group abolished affinity at all receptors. This was surprising since in the pindolol series the hydroxyl group appeared to be unimportant for 5-HT_{1A} affinity.

Based on the findings of Glennon and co-workers, a second series of compounds, of the general structure shown below, were synthesised.



R = CN, $CONH_2$, CO_2Me

160-162

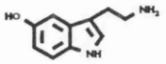
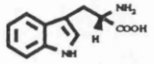
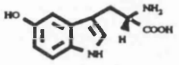
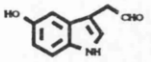
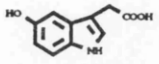
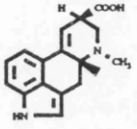
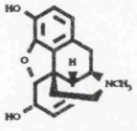
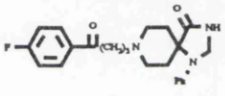
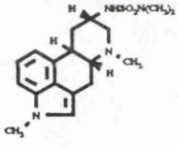
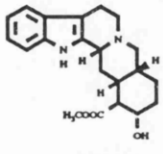
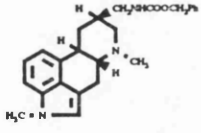
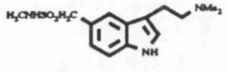
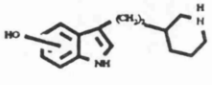
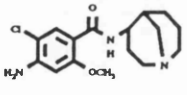
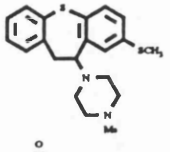
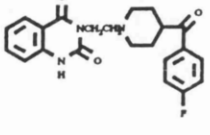
These agents were tested in binding and functional assays and found to be potent and selective 5-HT_{1A} partial agonists. This, by far, is the major finding of the project.

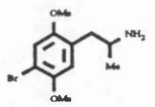
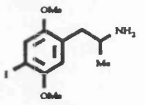
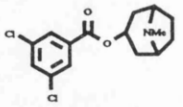
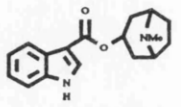
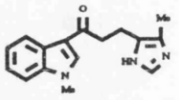
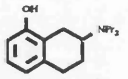
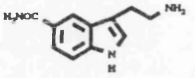
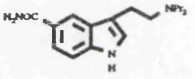
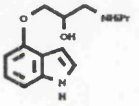
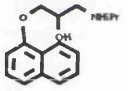
Since the late 1970's, there have been numerous attempts to design drugs which interact at 5-HT receptors. Two such compounds, namely ketanserine (anti-hypertensive, 5-HT_2) and GR 43715 (known as sumatriptan, anti-migraine, 5-$HT_{1\text{-like}}$) are now being successfully marketed. To date, the synthesised compounds **160-162** are the most potent 5-HT_{1A} agents to be derived by structural manipulation of the β-blockers. It has been reported (M. Hibert and P. Moser, Drugs of the Future, Vol. 15, No. 2, 159-170, 1990) that partial agonism may be a necessary criteria for compounds to act as anxiolytics, a criteria met by compounds **160-162**. Further more, these compounds are highly likely to cross the blood-brain barrier, because of the presence of lipophilic groups and potentially a few hydrogen bonding sites in two of the molecules.

Compounds 160-162 are therefore excellent candidates for further pharmacological and physiological evaluations.

The present results validate the utility of our approach, and continued studies with the above series should result in newer agonists and antagonists with even greater affinity and selectivity for 5-HT_{1A} and 5-HT_{1B} receptors.

Structures & Abbreviations

Compound No.	Name	Abbreviations	Structure
1	5-Hydroxytryptamine	5-HT	
2	L-Tryptophan	-	
3	5-Hydroxytryptophan	5-HTP	
4	5-Hydroxyindole-3-acetaldehyde	-	
5	5-Hydroxyindole-3-acetic acid	5-HIAA	
6	Lysergic acid	LSD	
6a	Dihydroergotamine	-	-
7	Dibenzyline	-	-
8	Morphine	-	
9	Spiperone	-	
10	Mesulergine	-	
11	Yohimbine	-	
12	Metergoline	-	
13	Sumatriptan	GR 43 175	
14	Hydroxylate indalpines	-	
15	Dipeptides of 5-Hydroxytryptophan	-	-
16	-	BRL 24 924	
17	Methiothepin	-	
18	Ketanserine	-	

19	-	Br-R-(-)DOB	
20	-	I-R-(-)DOI	
21	-	MDL 7222	
22	-	ICS 205-930	
23	-	GR 65 630	
24	8-Hydroxy-di-n-propylaminotetraline	8-OH-DPAT	
25	5-Carboxymidotryptamine	5-CT	
26	Di-n-propyl-5-carboximidotryptamine	DP-5-CT	
27	Pindolol	Pin.	
28	Propranolol	-	
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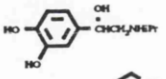
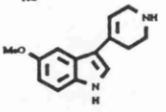
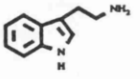
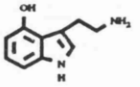
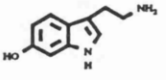
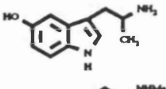
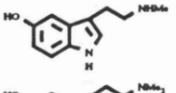
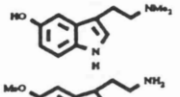
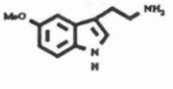
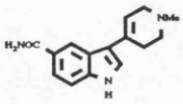
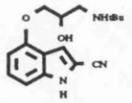
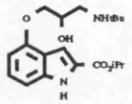
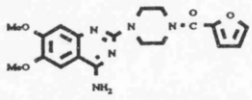
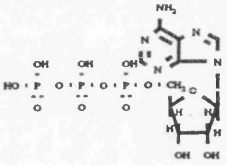
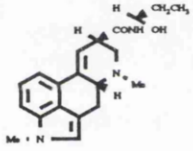
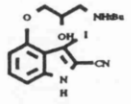
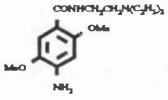
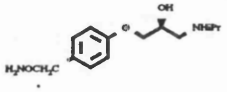
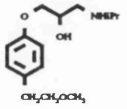
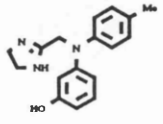
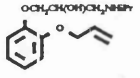
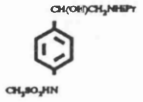
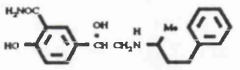
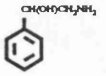
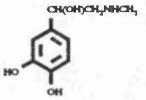
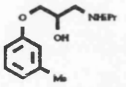
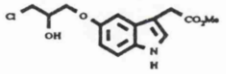
30	Isoprenaline	-	
31	-	RU 24 969	
32	Tryptamine	-	
33	4-Hydroxytryptamine	4-HT	
34	6-Hydroxytryptamine	6-HT	
35	α-Methyl-5-hydroxytryptamine	α -Me-5-HT	
36	N-Methyl-5-hydroxytryptamine	N-Me-5-HT	
37	Bufotenine	-	
38	5-Methoxytryptamine	-	

Compound No.	Name	Abbreviations	Structure
39	-	RU 24969	
40	Cyanopindolol	CYP	
41	-	SDZ 21 009	
42-62	See Chapter 1, Table 1.3., Page 33	-	-
63-69	See Chapter 1, Pages 27-28	-	-
70	Prazosin	-	
71-73	See Chapter 1, Page 28	-	-
74	Adenosine triphosphate	ATP	
75	Methysergide	-	
76	Iodocyanopindolol	ICYP	
77	Metoclopramide	-	
78	Atenolol	-	
79	Metoprolol	-	
80	Phentolamine	-	
81	Oxoprenolol	-	
82	Sotalol	-	

Compound No.	Name	Abbreviations	Structure
83	Labetolol	-	
84	Phenylethanolamine	-	
85	Adrenaline	-	
86-109	See Chapter 2, Pages 53, 62, 65	-	-
110	Tolprolol	-	
111-116	See Chapter 2, Pages 107	-	-
117	See Chapter 2, Page 110	-	-
118-121	See Chapter 2, Page 107	-	-
122-126	See Chapter 2, Page 115	-	-
127-128	See Chapter 2, Page 119	-	-
129-132	See Chapter 2, Pages 120, 121	-	-
133	3-Chloropropylamine	-	$ClCH_2CH_2CH_2NH_2$
134-136	See Chapter 4, Page 146	-	-
137	-	-	
138-140	See Chapter 4, Page 146	-	-
141-154	See Chapter 5, Page 161	-	-
155-158	See Chapter 5, Page 167	-	-
159-162	See Chapter 5, Page 168	-	-