The medicinal chemistry of aryl triflates
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Chapter 1

Introduction

1.1 History

Serotonin (5-hydroxytryptamine, 5-HT, 1), a neurotransmitter present in most species including man, plays a role in a variety of physiological functions such as pain, appetite, sex, emotion, sleep, memory and their associated pathological states.\(^1\) 5-HT was isolated for the first time from the blood in 1948 and characterized shortly thereafter by Rapport et al.,\(^2\) who named the endogenous compound after its source (serum) and action (tonus). This ‘peripheral hormone’ was also found in the intestinals but had yet another accommodation, the brain. This finding, and the synthesis of 5-HT in 1951, initiated an extensive research on the functioning and dysfunctions of (central) serotonergic systems. Although less than 5% of the total amount of 5-HT in the body resides in the central nervous system (CNS), 5-HT is an important factor in normal brain function. Serotonin was recognized to be a neurotransmitter substance.\(^3\) In 1957, Gaddum and Picarelli\(^4\) found indications of more than one 5-HT receptor site. According to different antagonizing effects of dibenzyline and morphine on the action of 5-HT in smooth muscle cells in guinea-pig ileum on the one hand, and acetylcholine release on the other, the receptors were initially designated as D- and M-receptors, respectively. The introduction of in vitro radioligand binding techniques in 1979 by Peroutka and Snyder allowed accurate discrimination between various 5-HT receptor subtypes on the basis of different ligand binding characteristics.\(^5\)

1.2 Serotonin Receptors

Classification. Molecular biology techniques accounted for the cloning of 5-HT receptor genes, and it became evident that multiple subtypes of this receptor protein exist. Recently, Hoyer et al.\(^6\) proposed operational (selectivity and affinity for agonists and antagonists), structural (protein homology) and transductional (intracellular mechanisms) criteria which a receptor should meet in order to become part of the 5-HT
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receptor superfamily. To date, 5-HT receptors can be classified to at least three, possibly seven, groups of receptors. They comprise the 5-HT\textsubscript{1}, 5-HT\textsubscript{2} and 5-HT\textsubscript{3} classes, as well as the uncloned 5-HT\textsubscript{4} receptor. The 5-ht\textsubscript{5}, 5-ht\textsubscript{6} and 5-ht\textsubscript{7} receptor genes have been cloned but these so-called ‘orphan’ receptors have yet to be fully characterized with respect to their pharmacological function and selectivity for certain drugs. All 5-HT receptor (sub)types belong to the G-protein coupled receptor superfamily, except the 5-HT\textsubscript{3} receptor, which is a ligand gated ion channel. In Table 1.1, the nomenclature and characteristics of the 5-HT receptor subtypes are summarized.
### Table 1.1. Current Status of 5-HT Receptor Characteristics

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Location</th>
<th>Response</th>
<th>Agonists</th>
<th>Antagonists</th>
<th>Clinical Implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>Mainly CNS</td>
<td>Neuronal hyperpolarization, hypotension</td>
<td>8-OH-DPAT, buspirone, 5-CT</td>
<td>WAY100635</td>
<td>Anxiety, depression</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1B&lt;/sub&gt;</td>
<td>CNS and some peripheral nerves</td>
<td>Neurotransmitter release ↓</td>
<td>CP93129, 5-CT</td>
<td>SDZ21009</td>
<td>Appetite disorders</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1D α&lt;/sub&gt;</td>
<td>Mainly CNS</td>
<td>Neurotransmitter release ↓</td>
<td>Sumatriptan, L694247, 5-CT</td>
<td>GR127935, GR55562</td>
<td>Migraine, depression</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1D β&lt;/sub&gt;</td>
<td>Mainly CNS</td>
<td>Neurotransmitter release ↓</td>
<td>Sumatriptan, L694247, 5-CT</td>
<td>GR127935</td>
<td>Migraine, depression</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1E&lt;/sub&gt;</td>
<td>Only CNS</td>
<td>cAMP ↓</td>
<td>5-HT</td>
<td>None</td>
<td>??</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1F&lt;/sub&gt;</td>
<td>Mainly CNS</td>
<td>cAMP ↓</td>
<td>5-HT</td>
<td>None</td>
<td>??</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>Vascular smooth muscle, platelets, lung, CNS, gastrointestinal tract</td>
<td>Vasoconstriction, platelet aggregation, bronchoconstriction</td>
<td>α-Methyl-5-HT, DOI</td>
<td>Ketanserine, cinanserine, pirenperone</td>
<td>Sexual and sleep disorders</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;2B&lt;/sub&gt;</td>
<td>Mainly peripheral?</td>
<td>Rat stomach fundic muscle contraction</td>
<td>α-Methyl-5-HT, DOI</td>
<td>SB200646</td>
<td>??</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;2C&lt;/sub&gt;</td>
<td>CNS</td>
<td>Phosphoinositide turnover ↑</td>
<td>α-Methyl-5-HT, DOI</td>
<td>Mesulergine</td>
<td>Anxiety, migraine</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Peripheral and central neurones</td>
<td>Depolarization</td>
<td>2-Methyl-5-HT, m-chlorophenylbiguanide</td>
<td>Ondansertron, tropisetron</td>
<td>Emesis, anxiety, depression, memory disorders</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Gastrointestinal tract, CNS, heart, urinary bladder</td>
<td>acetylcholine release in gut ↑, tachycardia, cAMP ↑ in CNS neurones</td>
<td>Metoclopramide, enzapride</td>
<td>GR113808, SB204070 Cisapride</td>
<td>??</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;5A&lt;/sub&gt; and 5-HT&lt;sub&gt;5B&lt;/sub&gt;</td>
<td>CNS</td>
<td>??</td>
<td>5-HT</td>
<td>Methiothepin</td>
<td>??</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;6&lt;/sub&gt;</td>
<td>CNS</td>
<td>cAMP ↑</td>
<td>5-HT</td>
<td>Methiothepin</td>
<td>Anxiety?</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;7&lt;/sub&gt;</td>
<td>CNS</td>
<td>cAMP ↑</td>
<td>5-HT</td>
<td>Methiothepin</td>
<td>??</td>
</tr>
</tbody>
</table>

Adapted with minor modifications from refs 1, 5 and 6. Orphan receptors are denoted in small characters (see text).

**Evolutionary Perspectives.** Protein receptors that mediate the actions of 5-HT have existed in the membranes of a variety of animal cell types for millions of years, which likely explains the multiplicity of these receptors. Peroutka and Howell⁷
performed a molecular evolution analysis which was based on the amino acid sequence homology of 5-HT and other G-protein coupled receptors. By correlating the percent amino acid homology between various species and the dates of evolutionary divergence of the species, the rate of molecular evolution was estimated to be approximately 1% every 10 million years.

![Phylogenetic tree of 5-HT receptors](image)

Figure 1.1. A phylogenetic tree of 5-HT receptors, adapted from Peroutka and Howell. The length of each ‘branch’ of the tree correlates with the evolutionary distance between receptor subpopulations. 5-HT subpopulations of other species have been excluded for the sake of clarity.

The sequences, which were aligned according to the method of Feng and Doolittle, were used to construct a phylogenetic tree (Figure 1.1). The length of each
‘branch’ correlates with the evolutionary distance between receptor subpopulations. Data indicate that the ‘primordial’ 5-HT receptor evolved over 750 million years ago. During such a long period of time, there has been ample opportunity for mutation and consequent evolutionary acceptance of multiple variants of receptors for the neurotransmitter 5-HT. The number of 5-HT receptor subtypes partly explains the amount of pathological pathways 5-HT is involved in. Although each receptor can be potently activated by 5-HT itself, the differences in protein structure offer medicinal chemists opportunities to design selective ligands for each receptor subtype. The challenge for (molecular) pharmacologists is to define the role of each receptor and to elucidate their function and distribution. These efforts should provide (selective) 5-HT receptor ligands with therapeutic utility and a better understanding of the clinical relevance of each receptor variant and vice versa.

1.3 5-HT1A Receptors

Distribution and Function. The distribution and function of central 5-HT1A receptors has been extensively studied in a number of vertebrates the past two decades. Among 5-HT receptors, the 5-HT1A receptor became the first and the best characterized receptor due to the development of high affinity 5-HT1A receptor ligands. Autoradiography studies revealed common distribution patterns in the brain of rats, guinea-pigs, cats, primates and humans. Recently, these patterns were confirmed with PET-studies utilizing radiolabelled WAY100635, which is the first silent and selective 5-HT1A receptor antagonist (for chemical structure see section 1.4). High densities of 5-HT1A receptors were shown to be located in the raphe nuclei and in limbic structures such as hippocampus, lateral septum and amygdala. The cerebellum was reported to be essentially devoid of 5-HT1A receptors.

The presence of 5-HT1A receptor populations in the dorsal and median raphe nuclei indicates that 5-HT can modulate the activity of serotonergic neurones. Activation of these somatodendritic 5-HT1A receptors causes inhibition of the cell firing activity and consequently reduction of 5-HT synthesis and neurotransmission in terminal brain areas. On the other hand, activation of the postsynaptic 5-HT1A receptors results in neuronal inhibition of particular parts of the limbic system, an area which has been implicated in the modulation of emotion. This ‘double-face’ character of the 5-HT1A receptor makes this receptor an interesting therapeutic target in the treatment of mood-disorders such as anxiety and depression (Figure 1.2). It has been suggested that the presence of a large receptor reserve at somatodendritic receptors and the lack of a receptor reserve at postsynaptic receptors, combined with the intrinsic
activity of 5-HT₁A receptor ligands at these receptors, may determine the anxiolytic/antidepressant profile.¹⁷

Typically, activation of central 5-HT₁A receptors causes a lower lip retraction (LLR)¹⁸ and the so-called ‘5-HT syndrome’ in rats, which is characterized by flat body posture, abducted hind-limbs, fore-paw treading (piano playing) and head weaving.¹⁹ Several pharmacological studies performed with 5-HT₁A receptor agonists showed effects on body temperature, feeding behaviour and sexual activity, which all could be effectively blocked by antagonists. A variety of 5-HT₁A receptor agonists produce anxiolytic effects in animal models of anxiety, although the clinically effective compounds (such as buspirone) show additional anti-depressant activity.¹ᵇ

Structural Aspects. The gene encoding the human 5-HT₁A receptor was successfully cloned in 1987 by Kobilka et al.,²⁰ although its identity remained unelucidated until 1988.²¹ The corresponding protein consists of 421 amino acid residues, which were shown to contain seven hydrophobic regions, reflecting seven transmembrane spanning regions (TMRs), in analogy with the structure of the G-protein coupled receptor bacteriorhodopsin.²² The rat 5-HT₁A receptor gene has also been cloned and
expressed, having 99% sequence homology with the human equivalent in the putative TMRs. Presumably, the TMRs are arranged in α-helices, which are connected with intra- and extracellular loops. According to mutagenicity experiments, certain conserved carboxylate and hydroxy bearing residues are important for the binding of a 5-HT₁₅ receptor agonist. Amino acid substitution in the TMRs, Asp⁸²→Asn⁸² (TM2), Asp¹¹⁶→Asn¹¹⁶ (TM3) and Ser¹₉₈→Ala¹₉₈ (TM5) all resulted in a decrease in affinity for 5-HT by 60-100 fold, whereas mutant Thr¹⁹⁹→Ala¹⁹⁹ (TM5) showed virtually no binding (Figure 1.3). Another mutation study revealed that Ser³⁹² and Asn³⁹⁵ on TM7 may also be crucial for ligand binding. In summary, the agonist binding is proposed to be facilitated through ion-pair formation between the protonated amino group of the ligand and the carboxylate groups of the aspartate residues in the 2nd and 3rd helix, and an interaction between the hydrogen-bonding group (hydroxy) and the hydroxy group of a serine or threonine residue (Figure 1.4). In addition, the interaction is probably stabilized by (π-π interactions with) surrounding aromatic groups.

Figure 1.4. Representation of the human 5-HT₁₅ receptor showing 7 putative trans-membrane regions, embedded in the lipid bilayer (gray). The TM-regions are connected by intra- and extracellular loops. The dark-grey amino acid residues have been implicated in receptor-ligand interactions.

**Pharmacophore of the 5-HT₁₅ Receptor.** Taking these considerations into account, two pharmacophoric models for 5-HT₁₅ receptor agonists proposed by Hibert et al. (left) and Mellin et al. (right) are shown (Figure 1.5). Adapted from refs 28 and 29, respectively.
account, several groups have attempted to define the pharmacophore of a 5-HT_{1A} receptor agonist or antagonist.\textsuperscript{27,28,29,30} Mostly, common structural elements of minimized (or crystal) conformations of known semi-rigid 5-HT_{1A} receptor ligands, such as 2-aminotetralins (see Section 1.4), were fitted in order to find this pharmacophore. According to the resulting models, pharmacophoric elements are an aromatic plane and (a specific direction of) the lone pair of a nitrogen atom at a fixed distance of approximately 5-6 Å from the midpoint of the aromatic plane (Figure 1.5).

Nilsson et al. stressed the importance of the hydrogen bond accepting oxygen atoms by fitting several hydroxy and methoxy analogues of 8-OH-DPAT,\textsuperscript{31} and developed a model with two different nitrogen lone-pairs or nitrogen dummy sites at a distance of 2.6 Å (Figure 1.6). This model resembles the Mellin model but additionally explains the affinity of some partial 5-HT_{1A} receptor agonists. However, the structure-activity relationship (SAR) is complicated and no model has been able to accommodate 5-HT_{1A} receptor ligands from different structural classes.

1.4 5-HT_{1A} Receptor Agonists and Antagonists (SAR)

A variety of compounds, representing different structural classes, have been presented in the literature\textsuperscript{32} to have considerable affinity for 5-HT_{1A} sites with varying degrees of intrinsic efficacy. The following section deals with the most important structures, including some structure-affinity-relationships (SAR).

Indolealkylamines. Modification of serotonin (1), the endogenous 5-HT_{1A} receptor ligand, was the first approach for a number of groups. Removal of the hydroxy moiety resulted in a 50 fold decrease in affinity and moving the hydroxy from the 5- to the 4- or 6-position had a similar effect.\textsuperscript{33} Dimethyl- and di-n-propylation of the primary amine group resulted in slightly lower affinities but neither compound displayed selectivity for 5-HT_{1A} vs 5-HT_{2} sites.\textsuperscript{34} Several tryptamine derivatives were shown to level the affinity of 5-HT as exemplified by 5-carboxamidotryptamine (5-CT, 4) and its N,N-di-n-propyl analogue DP-5-CT (5). Conformationally restricted tryptamine analogues such as RU28253 (6) and RU24969 (7) were shown to have good affinity for
the 5-HT\textsubscript{1A} receptor, but also were reported to show considerable affinity for the 5-HT\textsubscript{1B} sites.\textsuperscript{35} All tryptamine derivatives reported to date are agonists.

The tetracyclic ergolines constituted a special class of serotonergic agents, which possess high affinity but low selectivity for 5-HT receptor subtypes.\textsuperscript{36} (\textpm)-LSD (8) shows a Ki of 2.6 nM for 5-HT\textsubscript{1A} sites and, when tritiated, has proven its use as a radioligand for 5-HT\textsubscript{1} and 5-HT\textsubscript{2} receptor types.\textsuperscript{3} Molecular modification of the ergoline framework has led to the development more selective compounds such as 10, showing an IC\textsubscript{50} value of 5 nM for 5-HT\textsubscript{1} sites.\textsuperscript{37} Interestingly, trans-(\textpm)-2,3-dihydrofestuclavines, such as compound 11, are essentially inactive stressing the importance of the double bond in these type of structures. Omission of the D-ring of the ergoline skeleton led to the development of the (S)-enantiomer of compound 12 (LY228729),\textsuperscript{38} which is a fused structure of an ergoline and DP-5-CT and displays high affinity (Ki = 0.13 nM) and selectivity for the 5-HT\textsubscript{1A} receptor but was recently withdrawn from the clinic due to adverse effects.\textsuperscript{39}

Aminotetralins and Analogues. Beside an indolealkylamine structure, the above compounds also possess a 2-aminotetralin moiety within their multicyclic framework. In 1981, Arvidsson et al. provided a breakthrough in the search for selective 5-HT receptor ligands with 8-hydroxy-N,N-di-n-propyl-2-aminotetralin (8-
OH-DPAT, 13), which was the first nonindole-containing agent with full agonist properties.\textsuperscript{40} It was reported to induce the 5-HT behavioural syndrome and to decrease the cerebral 5-HT turnover very potently. But it was not until 1984, after extensive pharmacological evaluations, that the 5-HT\textsubscript{1A} receptor was found to be the mediator of these effects.\textsuperscript{41} Ever since this finding, 8-OH-DPAT (Ki = 1.2 nM) has been serving as an important pharmacological and structural tool in the development of novel 5-HT\textsubscript{1A} receptor agonists. Due to poor pharmacokinetic properties, 8-OH-DPAT itself failed to be of any clinical interest.

Thorberg et al. prepared racemic 3-aminochromanes, which were predicted to have better brain penetration than 8-OH-DPAT. 5-OH-DPAC (14) levelled the affinity of 8-OH-DPAT for 5-HT\textsubscript{1} sites but was less potent in the inhibition of 5-HTP accumulation.\textsuperscript{42} The (R)-enantiomer of its orally available (21\% in the cat) carboxamido congener (Ebaltozan\textsuperscript{®}, 15) displays a Ki value of 8.5 nM for the 5-HT\textsubscript{1A} site and is subjected to clinical trials.\textsuperscript{43}

\[
\begin{align*}
13 & \quad \text{OH} \\
14 & \quad R_1 = \text{OH}, R_2 = \text{n-Pr} \\
15 & \quad R_1 = \text{CONH(i-Pr)}, R_2 = \text{i-Pr}
\end{align*}
\]

Although aporphines are typically associated with dopaminergic activity, (R)-(\textendash\textendash)-10-methyl-11-hydroxyaporphine (16) unexpectedly was shown to be a high affinity 5-HT\textsubscript{1A} receptor agonist.\textsuperscript{44} It is difficult to reconcile the affinity of 16, since it bears the hydroxy group in the ‘wrong’ position, compared to 13.

(1S,2R)-Cis-1-methylated 2-aminotetralin derivative (17) was shown to be equipotent to 13. The (R)-enantiomer of 13 is only twice as potent as the (S)-enantiomer, whereas the (1R,2S)-antipode of 17 and the respective trans-isomers are inactive.\textsuperscript{45,46} This improvement in stereoselectivity has prompted considerable structure activity work. Comparison of semi-rigid cis- and trans-octahydrobenzoquinoline (OHBQ) derivatives in binding, biochemical assays and conformational calculations (MM2) led to the observation that trans-(4aS,10bS)-isomer 18 was the most active one (Ki = 3.87 nM).\textsuperscript{47} The nitrogen lone-pair is in the opposite direction, as compared to compound 17, but this fits in the pharmacophore model of Nilsson et al. (see Section 1.3). In 5-membered fused ring-systems, the cis-isomers were the most active, as exemplified by benz[e]indole derivative 19 and the orally active analogue 20 (Ki values 0.1 and 1.9 nM, respectively). The activities of these compounds reside in the
enantiomers which have the same configuration at the carbon resembling the 2-position of compound 17.\textsuperscript{48} It should be noted that the C-1 methyl group of compound 17 and the C-1 methylene groups of compounds 18 and 19, respectively, coincide and occupy the same space in the receptor.\textsuperscript{49} In the above mentioned studies, methylation or ring fusion at the 4-position provided inactive 8-OH-DPAT analogues.\textsuperscript{29}

Interestingly, substitution of the aromatic ring had quite dramatic consequences for the potency and/or intrinsic activity of the resulting compounds. Introduction of a C-5-fluoro substituent into the S-enantiomer of 8-OH-DPAT ((S)-UH301, 21) was reported to abolish the intrinsic efficacy and to some extent the affinity (Ki = 52 nM), presumably due to electronic effects.\textsuperscript{50} Liu et al. described a method to prepare a variety of 8-substituted 2-aminotetralins of 8-OH-DPAT via palladium-catalyzed reactions, utilizing the triflate analogue (22) as the key-intermediate.\textsuperscript{51} The highest affinities for the 5-HT\textsubscript{1A} receptor were observed for the R-enantiomers, except for the derivatives containing an acetyl or methylester moiety at the 8-position. The stereoselectivity of the latter compounds was reversed when cis-1-methyl analogues were tested.\textsuperscript{52}

Benz[e]indole derivative 25 was reported to exhibit mixed 5-HT\textsubscript{1A} and D\textsubscript{2} receptor stimulating properties.\textsuperscript{53} Extremely potent 5-HT\textsubscript{1A} receptor agonists with high oral bioavailabilities were obtained by introducing a formyl (OSU191, 26)\textsuperscript{54} on the C-1 position, or a nitrile group on the C-1 (27) or C-2 (28) position of the N,N-di-n-propyl derivative, respectively.\textsuperscript{55} Stjernlöf et al. postulated that the electronic density of the
nitrile groups and the lone-pairs of the formyl moiety interact with the hydroxy groups of a serine or threonine residue on TM5 of the 5-HT$_{1A}$ receptor protein (Figure 1.6).\textsuperscript{56}

![Figure 1.6. Possible hydrogen-bonding interactions between compound 26, 27 or 28 and the 5-HT$_{1A}$ receptor. Adapted from ref. 56.](image)

The examples given so far, among other things, suggest an important positive contribution of linear hydrophobic N-substituents to binding, giving optimal affinity for the 5-HT$_{1A}$ receptor in case of two n-propyl groups. Lengthening of unfunctionalized alkyl chains results in loss of affinity and, in case of the N,N-dibutyl analogue of 8-OH-DPAT, inversion of stereoselectivity, as was reported by Björk et al.\textsuperscript{57} However, a number of functionalized alkyl chains (such as ethylene, propylene and butylene chains) were shown to retain high affinity for this receptor subtype. Naiman et al.\textsuperscript{58} were the first to show that a phenyl substituent at one of the n-propyl terminals did not attenuate the affinity. Recently, Podona et al.\textsuperscript{59} prepared a number of aminochromane derivatives and explored the length of alkyl spacers and their substituents. Their best compounds, exemplified by compounds 30 and 31, possess imido or sulfonamido functional groups with a preferential length of four methylene for the side chain and were proven to be full agonists. In line with this observation Ennis et al.\textsuperscript{60} presented a number of viable (heteroaromatic) ring substituents, such as 2-thiophene and 2-methoxy- or 3-chloro-benzene, on the alkyl chain terminal employing the basic structure of compound 26, resulting in subnanomolar Ki values ranging from 0.02-2.8 nM. Taken together, these observations suggest the presence of at least two lipophilic pockets in the receptor, which can accommodate an n-propyl group and a reasonable flat moiety, respectively. The latter may contribute in stabilizing the ligand-receptor complex by means of hydrogen-bonding and/or π-π-interactions.\textsuperscript{26}
Arylpiperazines and Analogues. The prototypical arylpiperazine buspirone (32, $K_i = 30$ nM) and the more selective ipsapirone (33, $K_i = 7$ nM) and gepirone (34, $K_i = 181$ nM), were the first anxiolytic agents that directly stimulated the serotonergic system. Of these partial 5-HT$_{1A}$ receptor agonists buspirone, recently classified as anti-depressant, is widely used as an anti-anxiety agent.

The spacer length and the nature of the aryl substituent and the terminal moiety play important roles in determining the affinity, the selectivity and the degree of intrinsic efficacy for 5-HT$_{1A}$ receptors. NAN190 (35), a ligand which was initially reported to be an antagonist ($K_i = 0.6$ nM), was later shown to have agonistic effects in some assays. All above-mentioned compounds behave as partial agonists or antagonists at receptors localized postsynaptically, but stimulate the somatodendritic receptors. Evaluation of compounds belonging to the class of benzodioxyn-5-ylpiperazines led to the discovery of 5-HT$_{1A/1B}$ receptor agonist eltoprazine (36) and the selective 5-HT$_{1A}$ receptor agonist flesinoxan (37), which is investigated in clinical trials in depression. Another potent, selective and full agonist at 5-HT$_{1A}$ receptors in vitro and in vivo is tetrahydropyridine SR57747A (38), having a $K_i$ of 2.0 nM. Until recently, no full antagonists were known at both somatodendritic and postsynaptic 5-HT$_{1A}$ receptors. In 1994, Fletcher et al., reported WAY100635 (39) to be a silent and selective antagonist, which is now being frequently used as a pharmacological tool and as PET-ligand in clinical studies (see Section 1.3 and Chapter 7).
Aryloxyalkylamines. Pindolol (40, Ki = 35 nM) and propanolol (41, Ki = 90 nM) belong to a whole different, but important structural class of compounds. In addition to being β₁-adrenergic antagonists, these compounds are known to bind at 5-HT₁₄ sites, having low intrinsic efficacies. The β-hydroxy groups of 40 and 41 do not contribute to 5-HT₁₄ receptor binding and their removal actually enhances 5-HT₁₄ affinity, whereas it reduces β-adrenergic affinity. Guan et al. reported that amino acid residue Asn³⁸⁶ is responsible for the binding of these aryloxyalkylamines, while mutation of this residue produced only minor changes in the binding of other 5-HT receptor agonists. Conformationally constrained 1,4-benzodioxanes, such as the antagonist spiroxatrine (42), possess good affinity (Ki = 1.9 nM) and reasonable selectivity for 5-HT₁₄ receptors. Indolodioxane derivative U86192A (43) is another representative of this type of compounds and was shown to have antihypertensive effects in the cat. The latter examples, including S14063 (44), have a shorter alkyl spacer in between the oxygen and nitrogen atom, compared to 40 and 41, which enhances the 5-HT₁₄ receptor affinity.
1.5 5-HT\textsubscript{1D} Receptors

Distribution and Function. 5-HT\textsubscript{1D} receptors were first defined in bovine caudate and subsequently in the brain of a variety of other species, including man.\textsuperscript{73,74} Initially, the anatomical distribution of 5-HT\textsubscript{1D} receptors has been studied using quantitative autoradiography using nonselective 5-HT\textsubscript{1} receptor ligands, such as [\textsuperscript{3}H]5-CT (4) or [\textsuperscript{3}H]5-HT, which required saturation of the non-5-HT\textsubscript{1D} sites. The highest densities have been found in the substantia nigra, basal ganglia and nigrostriatal pathway, whereas lower densities were reported in the hippocampus, raphé nuclei and cortex.\textsuperscript{75} The introduction of serotonin-5-O-carboxymethyl-glycyl[\textsuperscript{125}I]tyrosinamide ([\textsuperscript{125}I]GTI, 50) allowed for the direct visualization of 5-HT\textsubscript{1D} sites, confirming the distribution patterns previously reported.\textsuperscript{76} The 5-HT\textsubscript{1D} receptor was shown to exist as a presynaptic heteroreceptor or a terminal autoreceptor, activation of which inhibits neurotransmitter release.\textsuperscript{77,78} Starkey and Skingle\textsuperscript{79} were the first to demonstrate the presence of functional 5-HT\textsubscript{1D} autoreceptors in the guinea-pig dorsal raphé nucleus, using the technique of fast-cyclic voltametry. The cloning of two distinct human genes encoding for two highly homologous proteins, designated 5-HT\textsubscript{1D\textsubscript{a}}\textsuperscript{80} and 5-HT\textsubscript{1D\textsubscript{b}} (alias 5-HT\textsubscript{1B}),\textsuperscript{81} accounted for a temporary confusion. Which of the two receptors was responsible for the pharmacological effects reported? In 1992, Adham et al.,\textsuperscript{82} shed light on this problem by showing that the human 5-HT\textsubscript{1D\textsubscript{b}} receptor, although operationally distinct, constitutes the counterpart of the rodent 5-HT\textsubscript{1B} receptor. The CNS distribution of the latter receptor, notably absent in mammals and birds, indeed parallels the regional distribution of 5-HT\textsubscript{1D} receptors in non-rodent species.\textsuperscript{83} Growing evidence suggests that the 5-HT\textsubscript{1D\textsubscript{a}} receptors are predominant, for instance, the distribution of 5-HT\textsubscript{1D\textsubscript{b}} receptor mRNA is consistently more widespread than that of the co-distributing 5-HT\textsubscript{1D\textsubscript{a}} receptor mRNA.\textsuperscript{84} Activation of 5-HT\textsubscript{1D} receptors induces inhibition of forskolin-stimulated adenylyl cyclase in the substantia nigra of calf and guinea pig. This observation is substantiated with studies performed in cells transfected with either 5-HT\textsubscript{1D\textsubscript{a}} or 5-HT\textsubscript{1D\textsubscript{b}} receptors.\textsuperscript{85} Since 5-HT\textsubscript{1B} receptors are implicated in the regulation of 5-HT release,\textsuperscript{86} and on the basis of the topographical similarities, centrally acting 5-HT\textsubscript{1D} receptor antagonists may well produce both antidepressant and anxiolytic effects, alone or in combination with SSRIs, and thus may constitute an attractive new drug research target.

5-HT\textsubscript{1D} receptors seem to have a prominent position within the final common pathway of the mechanisms involved in migraine, which is presumably manifested through dilation of cerebral arteries.\textsuperscript{87} Stimulation of these receptors by 5-HT\textsubscript{1D} receptor
agonists, such as sumatriptan (GR43175, 45), rapidly relieve the symptoms of the headache phase. Four mechanism have been suggested for the anti-migraine action of 5-HT₁D receptor agonists (Figure 1.8): (1) Vasoconstriction of cranial blood vessels,⁸⁸ (2) inhibition of release of vasoactive neuropeptides,⁸⁹ (3) blockade of trigeminal nerve terminal depolarization,⁹⁰ and (4) central inhibition with the trigeminal nucleus caudatus in the brainstem.⁹¹

![Diagram of proposed intervention pathways of sumatriptan](image)

Figure 1.8. Proposed intervention pathways of sumatriptan. Adapted from refs. 87 and 89.

Hamel et al.⁹² reported the presence mRNA for the 5-HT₁Dβ receptor in cerebral arteries of humans, suggesting that constriction of these vessels results from 5-HT₁Dβ receptor activation. However, selective gene-expression for the 5-HT₁Dα receptor was found in the human trigeminal ganglia,⁹³ implying that subtype-selective agonists are still needed to determine the contribution of each receptor subtype in the abortion of migraine-attacks.

Structural Aspects. The 5-HT₁Dα and the 5-HT₁Dβ receptor subtypes were shown to contain 377 and 390 amino acids, respectively. Both receptors are G-protein-coupled and consist of seven transmembrane spanning segments connected by extra- and intracellular loops (Figure 1.9).⁹⁴ The amino acid sequence identity in the membrane
spanning domain of both receptors is approximately 77%, whereas the human 5-HT\textsubscript{1D\beta} receptor differs only 4% from its rodent homologue, represented by eight amino acid residues (Table 1.2).

By replacing Thr\textsuperscript{355} of the human 5-HT\textsubscript{1D\beta} receptor with a corresponding Asn\textsuperscript{355} found in rodent 5-HT\textsubscript{1B} receptors, Oksenberg et al.\textsuperscript{67} showed that the major pharmacological difference between these species homologues confers a one single amino acid residue. This implies that the 5-HT\textsubscript{1D\beta} and 5-HT\textsubscript{1B} receptors are likely to have the same biological functions, while exhibiting distinct binding profiles for various compounds. Other important residues for binding to 5-HT\textsubscript{1D\alpha} and 5-HT\textsubscript{1D\beta} receptors are
likely the ones which are conserved in most 5-HT receptor proteins, such as an Asp (TM3) and Ser/Thr on TM5. Additionally, the serine residue on helix 4, which is suggested to be important for the binding of 5-HT to the 5-HT$_2$ receptor, is also present in the 5-HT$_{1D}$ receptor subtypes.\textsuperscript{95}

<table>
<thead>
<tr>
<th>Table 1.2. Amino acid sequence identities (%) in the TM domain of cloned 5-HT receptors.</th>
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<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>Human</td>
</tr>
<tr>
<td>Rat</td>
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<tr>
<td>Human</td>
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<td>Human</td>
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The 5-HT$_{1D}$ Receptor Pharmacophore. To date, only one pharmacophore model has been proposed. Glen et al.\textsuperscript{96} superimposed computed conformations of active molecules using known ligands, such as methysergide (9), as template molecules. The resulting pharmacophore hypothesis is composed of a protonated amine site, an aromatic region, a hydrophobic pocket and two hydrogen-bonding sites (Figure 1.9). However, this pharmacophore has yet to be challenged by (future) conformationally restricted analogues which bind to the 5-HT$_{1D}$ receptor.

1.6 5-HT$_{1D}$ Receptor Agonists and Antagonists (SAR)

Due to the recent discovery of the 5-HT$_{1D}$ receptor subtypes, the collection of known ligands that bind to these receptors is much smaller, for instance, compared to 5-HT$_{1A}$ receptor ligands. Many of the tryptamine derivatives mentioned in section 1.4, such as 5-CT (4; $K_i = 1.1$ nM), show considerable affinity for the 5-HT$_{1D}$ receptor subtypes. Simple modifications of 5-HT ($K_i = 2.2$ nM), such as removal or methylation of the hydroxy group and dimethylation of the primary amine function resulted in an approximately ten, two and four fold attenuation of the affinity, respectively.\textsuperscript{97} Larger
lipophilic moieties in the 5-position of the indole nucleus, such as a p-chlorobenzyl oxy group, are well-tolerated. The non-selective compound (+)-LSD (8) also binds to the 5-HT$_{1D}$ receptor with a Ki of 11 nM. However, none of the above-cited compounds can discriminate between the 5-HT$_{1A}$ and 5-HT$_{1D}$ receptor subtypes and were superceded by sumatriptan (GR43175, 45), which was identified as the first 5-HT$_{1D}$ receptor agonist with reasonable selectivity. This compound was recently successfully introduced for the treatment of migraine. From then on, novel compounds marched swiftly after each other. Mostly hydrogen-bond accepting, (aromatic) heterocycles in the 5-position of tryptamine have proven to be viable moieties, exemplified by compounds 46 (MK462) and 47 (311C90). Both compounds are clinically effective in the treatment of migraine, thus confirming the therapeutic utility of this class of compounds.

Soon, it became evident that a 5-substituent with considerable length can easily be accommodated by the 5-HT$_{1D}$ receptor. This was demonstrated by Glennon et al., who introduced hydrophobic tails with various lengths on this position resulting in 5-nonyloxy-tryptamine (48), a compound which binds with higher affinity to 5-HT$_{1D\beta}$ receptors than 5-HT$_{1D\alpha}$ receptors (Ki 1.2 vs 16 nM, respectively). Other representatives bearing a large group in this direction are L694,247 (49), GTI (50) and the arylpiperazide derivative 51.
Others embarked on the synthesis of conformationally restricted tryptamine analogues. Both, the sumatriptan analogue 52 (CP122,288)\textsuperscript{103} and the (3-nitro-pyridin-2-yl)amino derivative 53 (CP124,439)\textsuperscript{104} are constrained in the ethylamino side chain by the introduction of a pyrrolidine ring. King et al.\textsuperscript{105} developed the 3-aminotetrahydrocarbazole derivative BRL56905 (54), which is a conformationally restricted analogue of 5-CT, exhibiting a Ki of 10 nM for the 5-HT\textsubscript{1D\beta} receptor. However, this carbon skeleton seems to be valid only with the carboxamido substituent but not for alkyloxy substituents.\textsuperscript{106}

Although most 5-HT\textsubscript{1D} receptor agonists are of the tryptamine type, few compounds were reported having a structurally different composition. LeBoulluec et al. demonstrated that bivalent indoles, represented by compound 55, bind in the low-nanomolar range to the 5-HT\textsubscript{1D} receptor subtype (IC\textsubscript{50} = 0.05 nM).\textsuperscript{107} The optimal chain linkage was found to be seven methylene units but was also found to have high affinity for the 5-HT\textsubscript{1A} site.
Arylpiperazine 56 was shown to have a high affinity for 5-HT\textsubscript{1D} receptors (Ki = 2 nM), but is a high affinity ligand for 5-HT\textsubscript{1A} receptors as well (Ki = 3.3 nM).\textsuperscript{108} Alniditan (57) is a structurally different antimigraine agent which binds in de nanomolar range to 5-HT\textsubscript{1D} and 5-HT\textsubscript{1A} receptors, possessing full agonist properties for both 5-HT\textsubscript{1Da} and 5-HT\textsubscript{1Db} receptors in vitro.\textsuperscript{109}

Several of the benz[e]indoles 26, 27 and 28 and their derivatives (see Section 1.4), in addition to being 5-HT\textsubscript{1A} receptor ligands, were reported to have considerable affinity for both 5-HT\textsubscript{1D} receptor subtypes, showing a preference of 20-37 fold for the 5-HT\textsubscript{1Da} over the 5-HT\textsubscript{1Db} site.\textsuperscript{56,60} Few compounds, such as compound 58 (Ki 13 nM 5-HT\textsubscript{1A}; 18 nM 5-HT\textsubscript{1Da}) and 58 (Ki 0.6 nM 5-HT\textsubscript{1A}; 13 nM 5-HT\textsubscript{1Da}) were shown to be inactive for the 5-HT\textsubscript{1Db} receptor subtype.

The first disclosures of selective 5-HT\textsubscript{1D} antagonists were made by researchers at Glaxo, who based their structures upon the phenylpiperazinylbenzanilide moiety. The antagonism was determined by the inhibitory response of the 5-HT-induced contraction
of the dog saphenous vein and hypothermia in guinea-pigs. This strategy provided the
oxadiazole derivative 60 (GR127935), which now serves as a widely used
pharmacological tool (Ki 5-HT_{1A} = 1.3 nM, Ki 5-HT_{1Dβ} = 0.13 nM).\textsuperscript{110} Despite its
recognized antagonist profile in animal isolated tissues and behavioural models,
GR127935 was shortly thereafter shown to be a partial agonist at cloned human 5-HT_{1A} receptors.\textsuperscript{111} Unlike GR127935, the (dimethylamino)propyl benzanilide 61 (GR55562) behaved as a silent antagonist at the 5-HT_{1A} and 5-HT_{1Dβ} receptors in a similar study.\textsuperscript{112}

1.7 Objective and Outline

The trifluoromethanesulfonate (triflate) group is known for its electron-
withdrawing properties indicated by the positive Hammett \( \sigma_p \) (+0.37) and Taft \( \sigma_I \) (+0.84) constants.\textsuperscript{113} Consequently, the aliphatic triflate functionality is an excellent
leaving group, and therefore widely used in Organic Chemistry. However, the
properties of aryl triflates are considerably different as indicated by its chemical
stability with respect to solvolysis.\textsuperscript{114} Chemical stablity is an advantageous property in
drug-development and therefore, the aryl triflate group may be employed as a
bioisostere of some of the aryl substituents discussed in this chapter. In addition, the
electron-withdrawing effect of aryl triflates may prevent aromatic in vivo
hydroxylation, resulting in particularly metabolic stable compounds. The research
presented in this thesis describes a survey of the aryl triflate concept, as applied to 5-
HT_{1A}, 5-HT_{1Dα} and 5-HT_{1Dβ} receptor ligands. Consequently, this class of compounds may
have potential therapeutic applications in treatment of depression, anxiety disorders or
migraine. The emphasis is put on the structure-affinity relationships (SAFIR) and the
structure-activity relationships (SAR). In addition, the bioavailability of some of the
newly synthesized compounds is considered.

As indicated in this chapter, much is known about the brain function and
localization of 5-HT_{1A} receptors and moreover, selective and potent 5-HT_{1A} receptor
agonists such as 8-hydroxy-2-(di-n-propylamino)tetratin (8-OH-DPAT) are available.
However, due to poor pharmacokinetic properties the clinical potential of this compound is low. Chapter 2 deals with the efforts to develop structural analogues of 8-OH-DPAT which retain selectivity and potency for the 5-HT$_{1A}$ receptor but display an improved (oral) bioavailability. The hydroxy group is sensitive to O-glucuronidation and additionally, the low oral bioavailability of 8-OH-DPAT was found to be caused by N-depropylation. For this reason, the phenol portion was masked as an aryl triflate and the N-monopropyl substituted 2-aminotetralins were selected as a starting-point. The newly synthesized compounds were screened for their affinity to 5-HT$_{1A}$ receptors and the most promising compounds were evaluated by means of 5-hydroxytryptophan (5-HTP) accumulation in the rat brain, behavioural experiments and hypothermia. Especially, the (R)-enantiomer of 8-OSO$_2$CF$_3$-PAT was found to be very potent, however, the oral availability was comparatively low (7.6%). In addition, a drastic increase in affinity for 5-HT$_{1D}$ receptors was observed, as compared to 8-OH-DPAT. Methylation of the C1-position of the tetralin system (cis-8-OSO$_2$CF$_3$-MPAT) resulted in a slight decrease in affinity for 5-HT$_{1A}$ and 5-HT$_{1D\alpha}$ receptors. Cis-(1S,2R)-8-OSO$_2$CF$_3$-MPAT was shown to be the most potent enantiomer for 5-HT$_{1A}$ sites in the rat after subcutaneous or oral administration. The (1R,2S)-enantiomer exhibited a low intrinsic efficacy but an increased selectivity towards 5-HT$_{1A}$ receptors, as compared to its optical antipode. The cis-N-methylamino derivative (cis-8-OSO$_2$CF$_3$-MMAT) was found to be a nonselective ligand, whereas the trans-analogues were shown to be inactive.

The pharmacological profile of the 5-HT$_{1A}$ receptor agonist (R)-8-OSO$_2$CF$_3$-PAT gave rise to the investigation of its potential anxiolytic properties by means of animal models. Chapter 3 describes the effects of acute administration of (R)-8-OSO$_2$CF$_3$-PAT on rats in the conditioned defensive burying, the elevated plus-maze and the inescapable footshock model. In addition, the 5-HT turnover was determined in homogenates of various brain areas after administration of (R)-8-OSO$_2$CF$_3$-PAT at the doses that were used in the behavioural models. (R)-8-OSO$_2$CF$_3$-PAT was found to be active in the burying model and the plus-maze but not in the footshock paradigm. The 5-HT turnover significantly decreased in parts of the limbic system of the rat brain.

Inspired by the positive influence of the triflate group on the 5-HT$_{1D}$ receptor affinity of 2-aminotetralins the SAFIR and SAR of tryptamines was investigated. In Chapter 4 , the N-methylaminosulfonylmethylene group of the effective anti-migraine agent sumatriptan, was replaced by a triflate group. A series of N,N-dialkyl substituted 5-triflated tryptamines was prepared and screened for affinity and activity at 5-HT$_{1D\alpha}$ en 5-HT$_{1D\beta}$ sites. Forskolin-stimulated cAMP inhibition was employed as a measure for the 5-HT$_{1D}$ receptor-agonist properties of the compounds. The primary amines and small
N,N-dialkyl substituents were well-tolerated by the 5-HT1Dα and 5-HT1Dβ receptor subtype, resulting in fairly potent compounds. All derivatized tryptamines displayed moderate affinity for the 5-HT1A receptor. The most promising compound, the N,N-dimethyl-5-triflate-substituted tryptamine, induced hypothermia and a decreased 5-HT turnover in the brain of the guinea pig. The inactivity of this compound for 5-HT1A sites in the rat was confirmed by means of 5-HTP accumulation and intracerebral microdialysis.

The receptograms of the 2-aminotetralins described in Chapter 2 indicate that selectivity may be induced by ethylamino side chain restriction of serotonin analogues. Chapter 5 deals with other rigidification possibilities, exemplified by the synthesis of a triflate-substituted 3-aminocarbazole and 4-indol-3-ylpiperidines. Both classes of compounds displayed a strong preference for 5-HT1D receptors. This chapter also describes the preparation and testing of other sulfonic acid ester derivatized tryptamines, however, all compounds were found to have a lower affinity relative to the triflate analogue.

The 5-HT1A receptor antagonists ORG13502 and WAY100635 both possess an ortho-methoxyphenylpiperazine structure. In Chapter 6, the affinity and intrinsic activity for 5-HT1A receptors of ortho-methoxy, hydroxy and triflate substituted phenylpiperazines are compared. The triflate analogues were found to have a comparatively lower affinity than ORG13502 and WAY100635, along with an strongly enhanced intrinsic activity. With the aid of molecular modelling and a crystal database search we tried to find an explanation for this phenomenon.

Finally, Chapter 7 describes the radiochemical synthesis and biodistribution studies of [11C]ORG13502 and the previously reported [11C]WAY100635 by means of positron emission tomography (PET) in the rat brain. The regional uptake of [11C]WAY100635, but not of [11C]ORG13502, reflected the known 5-HT1A receptor density. In addition, the experiments were repeated with adrenalectomized (ADX) animals which are known to have an increased 5-HT1A receptor density, as compared to normal animals. Small differences were observed in the uptake of [11C]WAY100635 between normal and ADX rats, however, these differences were not significant.

Taken together, this thesis presents some interesting 5-HT1A and 5-HT1D receptor agonists. Follow-up studies will have to determine whether these compounds have any therapeutic potential. In the Concluding remarks the author states that the electron-withdrawing aryl triflate group is an interesting bioisostere for a number of aryl substituents. Depending on the nature of the ligand and the receptor, the aryl triflate group may successfully be applied in future drugs.
1.8 References


