1 Introduction

1.1 Scope of thesis

The interaction of an effector - ranging from small ions to large polypeptides - with a transmembrane protein forms the essence of the general mechanism to transmit signals from the exterior of a cell to the interior. This mechanism is generally characterized by specificity and reversibility, and involves a conformational change of the membrane bound protein as a result of molecular interactions with an effector molecule. Consequently, this conformational change can induce a cascade of intracellular modifications via second messenger mechanisms that might result in a number of additional effects, such as the production of peptides, cell reproduction, in- or efflux of ions, or release of active substances from the cell.

In nerve cells, membrane bound proteins called receptors, regulate the signaling pathways that enable cells to communicate and to transport signals over a long distance. Among these transmembrane proteins, the majority belongs to a super family of receptors that are coupled to heterotrimeric guanine nucleotide binding proteins, and which consists of a peptide chain with 7 transmembrane domains (seven-transmembrane G-protein Coupled Receptors, 7-TM GPCRs). The receptors are activated upon binding an endogenous neurotransmitter molecule (agonist) within the region of the transmembrane domains. The conformational change that results from this receptor-ligand interaction leads to the association of an intracellular G-protein, which in turn is linked to a second messenger pathway. Changes in concentration of second messenger may generate an action potential, if it leads to the opening of ion channels, depending on the nature of the distinct member of the GPCR family recognizing its ligand in a highly specific manner. This is the classical and most widely accepted model used to describe the activation of GPCRs by means of a ternary complex formed by a receptor, an agonist, and a G-protein[35]. Recently, this view has been extended in order to explain the observation that many receptors are able to activate G-proteins in the absence of an extracellular ligand as well[28,102,103,121,194].

To understand the principles of these signal transduction systems in living organisms at a molecular level is the key to developping novel pharmacologically active compounds. An estimated 1-3% of the human genome encodes for G-protein coupled receptors. As a result of their great abundance, GPCRs are major targets of current research on novel therapeutic agents for a large number of pathologies. Approximately 50-60% of the drugs that are on the market elicit their therapeutic effects through GPCRs[153,247]. Ranging from therapeutics for cardiopulmonary diseases interacting with members of the adrenoreceptor, to compounds that are related to disorders of the central nervous system, these drugs accounted for a worldwide market potential of approximately € 200 billion by the end of the 20th century.

Most of the endogenous ligands for GPCRs are small molecules and their binding can be mimicked or blocked by analogues that are either rationally designed or discovered through screening of extensive databases consisting of natural and synthetic compounds. These analogues are very often structurally related to the original signaling molecules, but (minor) alterations of the chemical structure can exert influence on affinity, efficacy, and metabolic stability. With the purpose to visualize the structures of both proteins and drugs, and to interpret the effects of structural modifications on protein-drug interactions and pharmacological activity, scientists can take advantage of modern computational methods. Based on quantitative structure-activity relationships of comprehensive and structurally diverse sets of ligands, important information can be deduced which subsequently can assist the development of novel drug candidates: the ligand-based approach. Alternatively, in the process of designing new or improved pharmacologically active substances, construction of three-dimensional models of the biological target can help to understand the effects of mutations causing malfunction of the protein, and achieve perception of the molecular architecture of the peptide being a host for a chemical messenger: the structure based approach. Models build to serve this latter method rely on the presumed sequential and structural similarities within the super family of G-protein coupled receptors. Rhodopsin, the only member of this super family of GPCRs which structure has been elucidated by X-ray crystallography, often serves this purpose.

Both techniques have been implemented in this thesis to investigate structure as well as function of a recently discovered receptor that is activated by the neurotransmitter serotonin. The design, synthesis, and pharmacological evaluation of supposed ligands that interact with this receptor subtype will be evaluated, as well as a number of computational strategies to explore structural features of these ligands and their biological target. This resulted in two valuable models that can attribute to the development of novel compounds to medicate disorders related to the (mal-) function of this receptor.

1.2 Serotonin

1.2.1 Early receptor subtype classification

The neurotransmitter serotonin, 5-hydroxytryptamine (5-HT, ***1c***, Figure 1.1), is involved in numerous functions of vertebrates, insects, and plants. In mammals, it is abundantly present in both the central nervous system (CNS) and in the periphery. Its name originates from the early time of discovery as a chemical component in the blood (serum) which was known to cause contraction (tonus) of blood vessels[182,183].

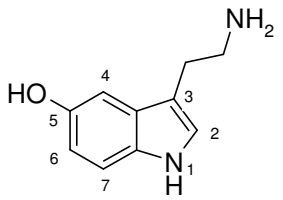

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Figure 1.1: ***Chemical structure of serotonin.***

As a result of extensive studies on its function in the periphery and the central nervous system[49,60,238,254], the compound was acknowledged in 1964 to be a neurotransmitter[34]. This discovery ushered a new era in which the development of therapeutic agents which modified 5-HT functions evolved tremendously. During the 1950s, efforts were devoted to the determination of structure-activity relationships for serotonergic activity with the use of peripheral tissue preparations. Unsurprisingly, the interpretation of these inquiries led to the assumption that multiple types of serotonergic receptors might exist. Morphine and atropine appeared to be able to block functional responses of the guinea pig ileum only partially, whilst dibenzylene, lysergide, 2-bromolysergide, and dihydro-ergotamine inhibited the remainder of the response on the tissue. This resulted in the first classification of M and D receptors, which were present on enteric cholinergic neurons and on smooth muscle cells, respectively[61]. Re-examination of this classification turned out to be necessary when it became obvious that the mode of action of the ligands used to discriminate between receptor subtypes was far from selective[126]. With he use of radioligands ([^{3}H]-5-HT, [^{3}H]-spiperone, and [^{3}H]-LSD) that could be displaced by 5-HT only, a more explicit receptor classification system of 5-HT_1 and 5-HT_2 (formerly D receptor) subtypes was founded[12,171]. Conversely, the subtype that was previously known as the M receptor did not correspond pharmacologically to either of these 2 subtypes. As a consequence, the distinct characteristics were acknowledged in 1986 and resulted in a revised classification of 5-HT_1-like, 5-HT_2 and 5-HT_3 receptors[19,66]. 5-HT_1-like receptors were primarily associated with prejunctional inhibition of neuronal transmitter release, smooth muscle relaxation, contraction of cardiac and vascular smooth muscles and tachycardia in cats, and characteristically exhibited low affinity for spiperone. In contrast, spiperone sensitive stimulation of 5-HT_2 receptors was related to gastrointestinal and vascular smooth muscle contraction and platelet aggregation, and could be inhibited by dibenzylene, lysergide, 2-bromolysergide, and dihydro-ergotamine (the former D receptors). Finally, the class of 5-HT_3 receptors was constituted of those previously known as M receptors. Further characterization in the following years revealed that this was in fact the only member of the family of serotonin receptors that is not a GPCR, but a ligand-gated ion channel[56].

1.2.2 Further characterization of 5-HT$_1$-like serotonin receptors

The class of 5-HT$_1$-like receptors was provisionally compiled on the basis of certain common pharmacological features of the receptors that were found to bind the radioligand [^{3}H]-5-HT with high affinity[171]. Their response to 5-carboxamidotryptamine (5-CT) was more intense than that to the endogenous ligand 5-HT and could be blocked by methiothepin and methysergide; however, the receptors were not susceptible to blockade by selective 5-HT$_2$ and 5-HT$_3$ antagonists[19]. With the development of many novel and selective compounds in the subsequent years of this early classification, further heterogeneity within the [^{3}H]-5-HT binding site could be identified. This initially accounted for the discovery of the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors[143,170] and subsequently the 5-HT$_{1C}$[169], 5-HT$_{1D}$[84,88,89], 5-ht$_{1E}$[123], and 5-ht$_{1F}$[1,2,4] subtypes (lower case notification for orphan receptors: receptor has been cloned, but no functional correlation described). Later, once the receptor was cloned and more information about its characteristics became available (i.e. activation of protein kinase C via increased phosphoinositide metabolism), the 5-HT$_{1C}$ receptor was reclassified as 5-HT$_{2C}$[86,95], while the 5-HT$_{1D}$ receptor was recognized as a combination of the species variant of the 5-HT$_{1B}$ (formerly known as 5-HT$_{1D\beta}$) receptor and the closely related 5-HT$_{1D}$ (formerly known as 5-HT$_{1D\alpha}$) receptor. It was now clear that the 5-HT$_{1A}$ mediated behavioral changes and centrally evoked hypotension and inhibition of acetylcholine release from enteric neurons, while the 5-HT$_{1B}$ receptor mediated inhibition of serotonin release from 5-HT containing neurons in the rat cortex[51,198,199]. Thus, from that moment the term 5-HT$_1$-like was used for the remaining functional receptors that met the operational criteria of this class (i.e. susceptibility to antagonism by methiothepin and/or methysergide, resistance to 5-HT$_2$ and 5-HT$_3$ antagonists and potent agonism by 5-CT), mediating contraction or relaxation of vascular and non-vascular smooth muscle, sympathic neuroinhibition and tachycardia in the cat, but could not be treated as equals of the 5-HT$_1$ recognition sites known at that time[197]. Once more, further exploration led to the observation that the class of 5-HT$_1$-like receptors consisted of distinct members as well. It turned out that some of the 5-HT$_1$-like receptors, mediating vascular contraction and sympathic neuroinhibition, were 10-100 times less susceptible to activation by 5-CT than receptors mediating vascular relaxation and tachycardia in cats[51]. This discrepancy was confirmed by the use of sumatriptan, at that time known as a selective 5-HT$_1$-like receptor agonist[91-94]: it stimulated only those receptors mediating vascular constriction and sympathic neuroinhibition, but not those mediating vasodilatation. The distinct members were arbitrarily denoted 5-HT$_{1X}$ (sumatriptan sensitive) and 5-HT$_{1Y}$ (sumatriptan insensitive)[198,199]. Very soon it was recognized as well, that the 5-HT$_{1X}$ receptor was negatively coupled to adenylyl cyclase, while activation of the 5-HT$_{1Y}$ receptor stimulated the formation of cyclic adenosine monophosphate (cAmp)[216,217].

The Serotonin Club Receptor Nomenclature Committee of the IUPHAR (International Union of Pharmacology) originally founded in 1984 as a working party and adopted by the Serotonin Club formed in 1986, became part of the IUPHAR in 1987. It has been assigned the task of providing a

classification system based on operational (drug related), structural (primary amino acid sequence) and transductional (receptor coupling) information[95]. The need for unambiguous guidelines for classification was greatly brought about by the rapid and extensive advances in the development and application of cloning techniques and profited from the expanding number of compounds that showed selectivity for one or more receptor subtypes. Consequently, the classification of 5-HT_{1X} and 5-HT_{1Y} was regarded as no longer appropriate: the 5-HT_{1X} receptor had been reclassified according to the new guidelines as 5-HT_1-like as a result of its negative coupling to adenylyl cyclase, and was further characterized by the use of GR-127935, a novel piperazinyl-benzanilide compound that was able to block the effects of sumatriptan with high potency[30,39,166-168,206,245]. Indeed, these characteristics very soon resulted in acknowledgement of the hypothesis that this 5-HT_1-like receptor was identical with the 5-HT_{1D} receptor subtype. However, by means of cloning techniques and minor differences in pharmacological profiles (i.e. response to ketanserine[87]), this receptor appeared to be encoded by two structurally different genes in rat and human tissue[249], and was therefore divided into 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ subtypes. Subsequently, reconsideration of the pharmacological profiles of these two distinct subtypes resulted finally in reclassification of these receptors: the human homologue of the 5-HT_{1B} receptor (5-$HT_{1D\beta}$) was logically renamed as 5-HT_{1B}, while the 5-$HT_{1D\alpha}$ receptor is now recognized as 5-HT_{1D}[77].

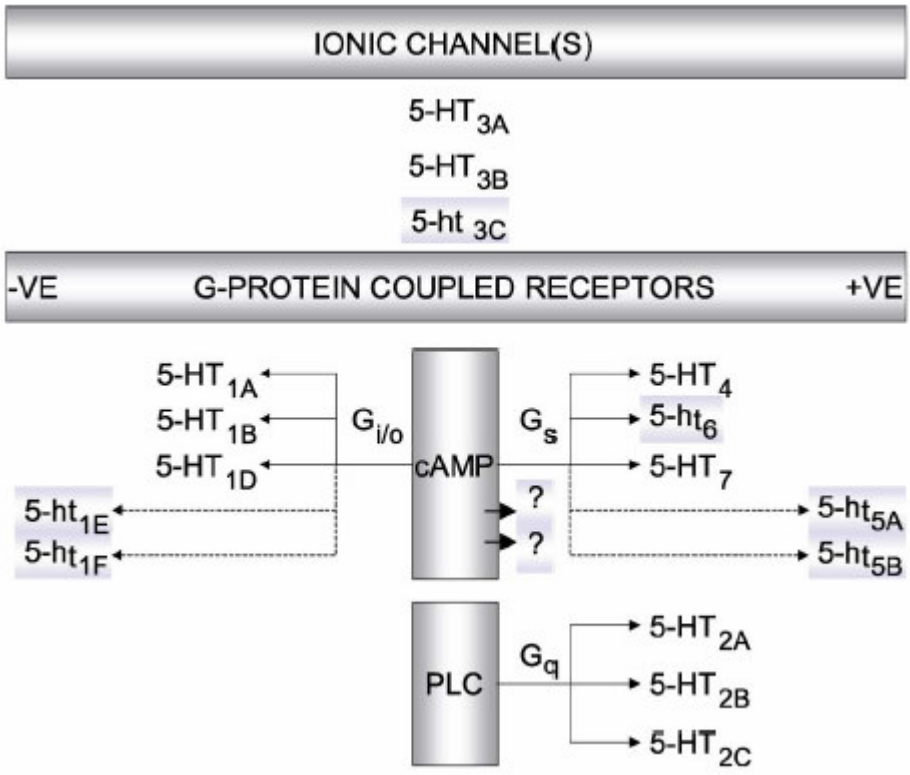


Figure 1.2: ***Graphical representation of the current classification of 5-HT receptors.*** *Receptor subtypes represented by shaded boxes and lower case designate receptors that have not been demonstrated to definitively function in native systems. Abbreviations: 3'-5' cyclic adenosine monophosphate (cAMP); phospholipase C (PLC); negative (-ve); positive (+ve).*

The 5-HT_{1Y} receptor that mediated smooth muscle relaxation and tachycardia in cats was transferred to the category of orphan receptors to await further characterization[87]. Initially it showed to be hardly distinguishable from the 5-HT_{1B} and 5-HT_{1D} receptor subtypes with respect to some operational characteristics: the potency rank order of agonists was identical (5-CT > 5-HT ≥ 5-methoxytryptamine (5-MeOT)).

Nomenclature	Previous name	Selective agonists	Selective antagonists (pK_B)	Radioligands	G-protein effector
5-HT_{1A}	–	8-OH-DPAT	WAY 100635 (8.7)	[^{3}H]WAY100635 [^{3}H]8-OH-DPAT	$G_{i/o}$
5-HT_{1B}	5-$HT_{1Dβ}$	Sumatriptan L 694247	GR 55562 (7.4) SB-224289 (8.5) SB-236057 (8.9)	[^{125}I]GTI [^{125}I]CYP (rodent) [^{3}H]Sumatriptan [^{3}H]GR 125743	$G_{i/o}$
5-HT_{1D}	5-$HT_{1Dα}$	Sumatriptan PNU 109291	BRL 15572 (7.9)	[^{125}I]GTI [^{3}H]Sumatriptan [^{3}H]GR 125743	$G_{i/o}$
5-ht_{1E}	–	–	–	[^{3}H]5-HT	$G_{i/o}$
5-ht_{1F}	5-$ht_{1Eβ}$, 5-HT_6	LY 334370	–	[^{125}I]LSD [^{3}H]LY 334370	$G_{i/o}$
5-HT_{2A}	D, 5-HT_2	DOI	Ketanserin (8.5-9.5) MDL-100907 (9.4)	[^{125}I]DOI [^{3}H]Ketanserin [^{3}H]MDL 100907	G_q
5-HT_{2B}	5-HT_{2F}	BW 723C86	SB-200646 (7.5) SB-204741 (7.8)	[^{3}H]5-HT	G_q
5-HT_{2C}	5-HT_{1C}	Ro 600175	Mesulergine (9.1) SB-242084 (9.0) RS-102221 (8.4)	[^{125}I]LSD [^{3}H]Mesulergine	G_q
5-HT_3	M	SR 57227 m-chlorophenyl-biguanide	granisetron (10) ondansetron (8-10) tropisetron (10-11)	[^{3}H](S)-zacopride [^{3}H]tropisetron [^{3}H]granisetron [^{3}H]GR 65630 [^{3}H]LY 278584	Na^+/K^+-channel
5-HT_4	–	BIMU 8 RS 67506 ML 10302	GR-113808 (9-9.5) SB-204070 (10.8) RS-100235 (11.2)	[^{125}I]SB 207710 [^{3}H]GR 113808 [^{3}H]RS 57639	G_s
5-ht_{5A}	5-$HT_{5α}$	–	–	[^{125}I]LSD [^{3}H]5-CT	$G_{i/o}$
5-ht_{5B}	–	–	–	[^{125}I]LSD [^{3}H]5-CT	None identified
5-ht_6	–	2-Ethyl-5-MeO-*N,N*-DMT	Ro-630563 (7.9) SB-271046 (7.8) SB-357134 (8.5)	[^{125}I]SB 258585 [^{125}I]LSD [^{3}H]5-HT	G_s
5-HT_7	5-HT_X, 5-HT_1-like	–	SB-258719 (7.9) SB-269970 (9.0)	[^{125}I]LSD [^{3}H]SB 269970 [^{3}H]5-CT [^{3}H]5-HT	G_s

Table 1.1: ***Currently identified 5-HT receptors and their summarized characteristics*[90]*.***

On the other hand, it was already known that this class of receptors was insensitive to sumatriptan and it appeared not to be subject to blockade of the agonists by GR-127935 as well[37,40,223,242,244]. With the use of cloning techniques, two novel recombinant receptors positively coupled to adenylyl cyclase were identified which could account for this profile: 5-ht$_6$[146,190] and 5-ht$_7$[9,134,176,191,203]. However, a striking discrepancy of the cloned 5-ht$_6$ receptor subtype compared to the cloned 5-ht$_7$ receptor was its submicromolar affinity for 5-CT[146]. Additionally, the relative selectivity of mesulergine[87] (245-fold selectivity for cloned 5-ht$_7$ over cloned 5-ht$_6$), and the distribution of mRNA of the cloned receptors[20,63,240] eventually indicated that the 5-HT$_{1Y}$ receptor profile resembled the profile of the cloned 5-ht$_7$ receptor both structurally, transductionally, and operationally (IUPHAR criteria). Based on these findings, this cloned receptor was identified from that moment as the 5-HT$_7$ receptor. A summarizing graphical representation of the current classification of 5-HT receptors, according to the criteria of the IUPHAR is depicted in Figure 1.2, and a summary of the serotonin receptors known to date with an overview of their selective ligands, the radioligands used for characterization of the receptor subtype, and their effectors is listed in Table 1.1.

1.3 Common knowledge on 5-HT$_7$ receptors

1.3.1 Introduction

The 5-HT$_7$ receptor is the most recent addition to the large family of G-protein coupled serotonin receptors. Since its identification in the early 1990's, much effort has been made to investigate its nature and biological function.

receptor subtype	overall percentage similarity	overall percentage homology
5-HT$_{1A}$	49	38
5-HT$_{1B}$	46	37
5-HT$_{1D}$	46	38
5-ht$_{1E}$	48	39
5-ht$_{1F}$	48	38
5-HT$_{2A}$	38	28
5-HT$_{2B}$	37	28
5-HT$_{2C}$	37	28
5-HT$_3$	<<10	<<10
5-HT$_4$	42	32
5-ht$_{5A}$	42	33
r5-ht$_{5B}$	44	34
5-ht$_6$	40	33
5-HT$_{7A}$	100	100

Table 1.2: ***Homology of the 5-HT$_{7(a)}$ receptor with other serotonin receptors****. Amino acid similarity: identical or similar amino acids. Amino acid homology: identical amino acids only. Data derived from literature refer to human receptors, except 5-ht$_{5B}$ (rat)*[11,90].

The receptor has been cloned from the genomes of human[9,80,100,116,211], rat[32,80,81,134,142,191,203], mouse[176], pig[13], guinea pig[46,237], rabbit[177], and frog[155]. Additionally, several groups reported the identification of a transcribed human 5-HT_7 receptor pseudogene[144] that possesses over 90% homology with the other known 5-HT_7 receptor sequences[119,161,162,181]. Although the primary structure (amino acid sequence) of the receptor exhibits a high degree of interspecies homology (ca. 95%), the resemblance with other members of the serotonin receptor family appears to be considerably lower (Table 1.2).

1.3.2 Distribution of 5-HT_7 receptors

Initial publications describing the cloning of the 5-HT_7 receptor (5-$HT_{7(a)}$ isoform) gave somewhat conflicting descriptions of mRNA distribution, perhaps due to differences in methodologies used to measure mRNA. Nevertheless, using a number of different techniques (reverse transcriptase polymerase chain reaction (RT-PCR), *in situ* hybridization, northern-blot analysis, immunocytochemistry, specific radiolabeling), the expression of mRNA encoding the 5-HT_7 receptor is detected at high levels in the brain and generally lower levels in a variety of peripheral tissues: lung[116], kidney[116], liver[116], pancreas[116], placenta[116], spleen[116], testis[116], ovary[116], retina[177], heart[116], coronary, pulmonary and uterine arte-ries[9,13,158,201,240], superior vena cava[13], saphenous vein[13], and various regions of the gastro-intestinal tract[116], including the stomach[9], colon[9,116], and ileum[9,82,83]. Within the CNS, the expression of the 5-HT_7 receptor is relatively high within regions of the cortex[191,237], sep-tum[16,71], cerebellum[13,237], striatum[191], thalamus[71,109,156,234,237], hypothalamus[16,71,109,156,191,203], the olfactory complex[156,203], trigeminal ganglia[13,226], mesencephalon[203], and the hippocampus[71,109,156,191,203,234,237], while generally lower levels of expression are detected in areas such as the cerebral cortex[72,109,203,234], basal ganglia[234], midbrain[234], hindbrain[234], and amygdala[71,234]. Notably, a number of research groups[11,16,43,81,156] do find expression of 5-HT_7 receptors in the suprachiasmatic nucleus (SCN), while others[71,109,134,152,191], using different techniques for detection of serotonin receptors in the brain, do not (specifically) report detectable levels of 5-HT_7 receptors in the same area. The impact of these findings will be discussed later in this chapter, since it was hypothesized that the 5-HT_7 receptor might play an important role in the regulation of circadian rhythm and sleep disorders via the SCN located in the hypothalamus[15,134,260].

Recent studies on the characterization of 5-HT autoreceptors in the CNS, using 5-HT_7 receptor selective antagonist, revealed that this receptor subtype is not likely to act as an autoreceptor[188]. Despite their distribution throughout the raphe, SB 269970, a potent and selective 5-HT_7 receptor antagonist was unable to attenuate serotonin agonist responses in either the cortex or the dorsal raphe nuclei. On the contrary, evidence has been obtained that shows 5-HT_7 receptors being responsible for a positive feedback regulation of [^{3}H]-5-HT release from neuronal stores of 5-HT in bovine and human iris ciliary bodies[76]. This study also made use of selective

5-HT_7 receptor antagonists to support this hypothesis. However, it should be noted that the use of ligands with relatively high affinity for the 5-HT_3 receptor warrants further research to identify the role of this receptor subtype in regulating 5-HT release from anterior uveal tissue.

1.3.3 5-HT_7 receptor isoforms

The 5-HT_7 receptor gene is located on human chromosome 10 (10q21–q24)[62] and contains two introns[48,80,191]. The presence of one of these introns corresponds to the predicted second intracellular loop and, therefore, any variants arising at this site as a result of alternative splicing of receptor pre-mRNA –a mechanism observed in many receptor subtypes to further increase the number of receptor isoforms from a single receptor gene– are probably ineffectual[48,80,203]. However, alternative splicing of receptor pre-mRNA can produce at least 4 isoforms of the 5-HT_7 receptor that differ primarily in their intracellular carboxyl terminals due to a the presence of a second intron (Figure 1.3)[80,81,100,134,203,211]. The 5-$HT_{7(a)}$ receptor, the first splice variant cloned from human, has a predicted length of 445 amino acids. A truncated splice variant resulting from alternative splicing of the receptor pre-mRNA counts 432 amino acids (5-$HT_{7(b)}$). Retention of additional exons (C and D) in the receptor gene result in two additional isoform (5-$HT_{7(c)}$ counting 467 amino acids, and 5-$HT_{7(d)}$ counting 477 amino acids respectively)[80]. Additionally, all three rat isoforms and the human 5-$HT_{7(a)}$ and 5-$HT_{7(b)}$ splice variants do not show known consensus potential phosphorylation sites in the predicted intracellular C-terminal protein sequence. On the other hand, the 5-$HT_{7(d)}$ splice variant has two additional phosphorylation consensus sites, one for protein kinase C and one for casein kinase II[80]. Since desensitization and/or internalization of some GPCRs is shown to be dependent on receptor phosphorylation[59,196,250], this could also be the case for 5-HT_7 receptor isoforms. However, this has not been demonstrated to date. Two of the isoforms, the 5-$HT_{7(a)}$ and 5-$HT_{7(b)}$ were observed in both human and rat, while the expression of the other isoforms appears to be specific for rat (5-$HT_{7(c)}$) and human (5-$HT_{7(d)}$), due to the species related exons in the DNA sequences coding for the 5-HT_7 receptor gene[80,81]. Recently, a 5th isoform has been identified in rat, provisionally named 5-$HT_{7(e)}$, that might be the product of expression of another exon[129]. The relative abundance of all 5-HT_7 receptor isoforms is different among humans and rats, possibly indicating different functional properties and physiological roles for each of these isoforms. RT-PCR studies have identified the 5-$HT_{7(a)}$ receptor as the most widely distributed variant in both human and rat (49-58% and 76-89% respectively), while the 5-$HT_{7(b)}$ receptor is generally expressed at lower levels (human: 32-46%, rat: 9-21%), although this isoform appears to be the primary transcript in human smooth muscle tissue[73]. The species dependent 5-$HT_{7(c)}$ and 5-$HT_{7(d)}$ isoforms both show only very low levels of expression (1-5% and 4-10%, respectively), leading to the suggestion that these isoforms may result from inaccurate transcription and that they have no physiological relevance[80,81,116,129]. In all species studied, the 5-HT_7 receptor isoforms are positively coupled to adenylyl cyclase through $G\alpha_s$[9,81,116,134,176,229,237].

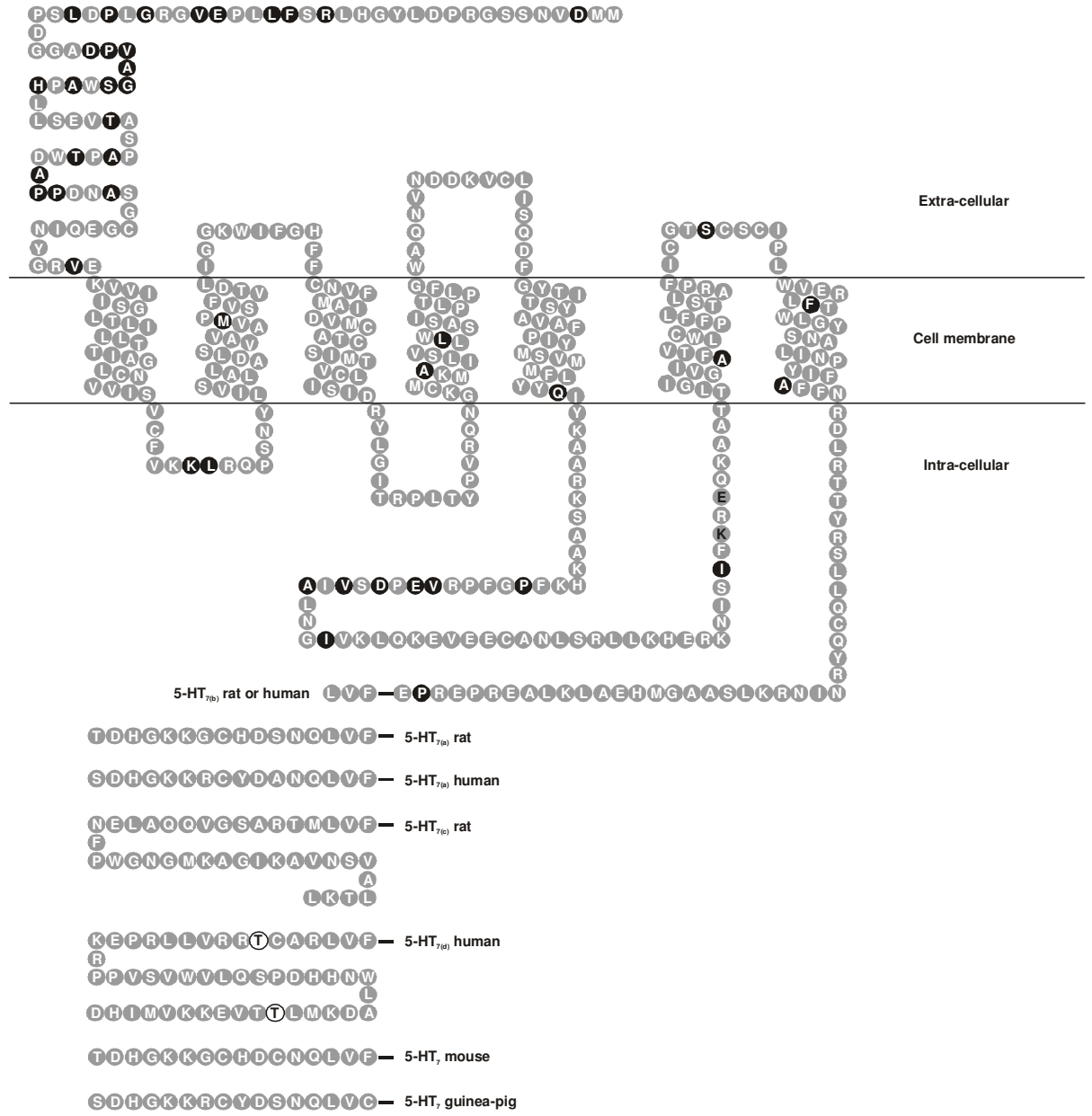


Figure 1.3: ***Snake-plot of 5-HT$_7$ receptor isoforms.*** *Full sequence with the 7 trans-membrane domains and C-termini of either rat or human 5-HT$_{7(b)}$ in grey dots/white fonts. Other C-termini are depicted starting from corresponding C-terminus of 5-HT$_{7(b)}$. Phosphorylation sites in C-terminus of human 5-HT$_{7(d)}$ are depicted in white dots/black fonts. Grey dots/black fonts represent Glu325 and Lys327 that have been identified as important residues for Gα_s-coupling by mutational studies. Black dots/white fonts represent amino acids that exhibit differences among mammals. Adapted from literature*[80].

1.3.4 Pharmacological properties of 5-HT$_7$ receptors

Despite the fact that alternative splicing of the 5-HT$_7$ gene predicts at least four isoforms that differ primarily in their C-terminal tail, suggesting different G-protein coupling efficiency and downstream signaling, in general, the pharmacological and functional properties of the 4 isoforms are very indistinguishable. Studies analyzing possible different pharmacological profiles of the

isoforms demonstrate that binding affinities of small series of 5-HT agonists and antagonists do not differ significantly and that 5-HT stimulation of cAMP production by the three isoforms of both human and rat expressed in HEK293 and JEG-3 cells is indistinguishable, although some differences in relative efficacies of a number of the antagonists tested on the human isoforms could be observed (Table 1.3)[81,116].

	5-HT$_{7(a)}$		5-HT$_{7(b)}$		5-HT$_{7(d)}$	
	pK_i	Relative efficacy	pK_i	Relative efficacy	pK_i	Relative efficacy
Agonists						
5-CT	9.91 ± 0.21	1.02 ± 0.01	9.94 ± 0.13	1.01 ± 0.01	9.93 ± 0.07	1.05 ± 0.01
5-MeOT	9.15 ± 0.13	1.07 ± 0.01	8.96 ± 0.06	1.03 ± 0.02	9.09 ± 0.12	1.04 ± 0.03
5-HT	8.93 ± 0.36	1.00	8.80 ± 0.30	1.00	8.78 ± 0.33	1.00
(*R*)-8-OH-DPAT	7.37 ± 0.26	0.78 ± 0.05	7.39 ± 0.08	0.80 ± 0.04	7.58 ± 0.15	0.76 ± 0.01
Antagonists						
Methiothepin	9.36 ± 0.15	1.00	9.39 ± 0.19	1.00	9.29 ± 0.29	1.00
Metergoline	8.57 ± 0.19	0.26 ± 0.07	8.53 ± 0.07	0.36 ± 0.04	8.45 ± 0.20	0.20 ± 0.03
Mesulergine	8.11 ± 0.07	0.00 ± 0.05	8.16 ± 0.07	0.47 ± 0.18	8.11 ± 0.14	-0.10 ± 0.09
Clozapine	7.66 ± 0.09	0.96 ± 0.08	7.80 ± 0.12	0.91 ± 0.06	7.62 ± 0.14	0.96 ± 0.03
Methysergide	7.58 ± 0.16	0.18 ± 0.09	7.75 ± 0.19	0.19 ± 0.07	7.74 ± 0.14	-0.02 ± 0.06
Spiperone	7.57 ± 0.04	1.03 ± 0.21	7.80 ± 0.04	1.01 ± 0.08	7.57 ± 0.09	0.97 ± 0.05
Ritanserin	6.82 ± 0.01	1.10 ± 0.19	6.89 ± 0.03	1.00 ± 0.08	6.83 ± 0.03	0.93 ± 0.05
Ketanserin	6.33 ± 0.02	1.06 ± 0.17	6.45 ± 0.12	0.95 ± 0.07	6.31 ± 0.11	0.93 ± 0.05

Table 1.3: ***Binding affinities and efficacies in adenylyl cyclase activation of recombinant human 5-HT$_7$ receptors expressed in HEK293 cells***[116,117]***.*** *Efficacies of agonist increase of basal AC activity relative to 5-HT. Efficacies of antagonist (inverse agonist) inhibition of basal AC activity relative to methiothepin.*

Furthermore, it is suggested that an inherent property of 5-HT$_7$ receptors is poor receptor-effector coupling to adenylyl cyclase, since a low potency of agonists to elicit functional responses has been demonstrated for all isoforms, but in particular the human 5-HT$_{7(d)}$ isoform[116]. Mutational studies (mouse 5-HT$_7$) suggest the importance of the C-terminal region of the third intracellular loop in receptor-Gα_s-protein interaction and that specific charged residues, Glu325 and Lys327, may play a critical role in this interaction[159]. However, these amino acid residues are highly conserved among all isoforms of the 5-HT$_7$ receptor and therefore cannot contribute to the relatively poorer coupling of the 5-HT$_{7(d)}$ isoform to adenylyl cyclase.

Many GPCRs have multifunctional signaling potential when expressed in a given cell type, either through activation of more than one type of G-protein or through pleiotropic effects of a single G-protein (e.g. coupling to different isoforms of adenylyl cyclase). Additional studies revealed that stimulation of the 5-HT$_{7(a)}$ receptor not only caused activation of adenylyl cyclase 5 (AC5) through coupling with Gα_s, but also AC1 and AC8[8]. Since these Ca^{2+}/calmodulin-stimulated isoforms of adenylyl cyclase are insensitive to Gα_s *in vivo* –this in contrast with the 7 other isoforms which are sensitive to Gα_s *in vitro*–, are neural specific and expressed in areas of the brain were 5-HT$_7$ receptors are localized (hypothalamus and hippocampus)[256], it is hypothesized that 5-HT

might regulate intracellular cAMP in certain areas of the brain by mobilizing intracellular Ca^{2+} after activation of 5-$HT_{7(a)}$ receptors.

From the initial categorization of 5-HT receptors, some operational characteristics for the 5-HT_7 receptor were known: the potency rank order of agonists was identical with that for the 5-HT_{1B} and 5-HT_{1D} receptor subtypes (5-CT > 5-HT ≥ 5-methoxytryptamine (5-MeOT). On the other hand, it was already recognized that this class of receptors was insensitive to sumatriptan and it appeared not to be subject to blockade of agonists by GR-127935 as well[37,40,223,242,244]. Strikingly, the high affinity of the 5-HT_7 receptor for 5-HT and 5-CT, and the moderate affinity for 8-OH-DPAT – a compound that was previously considered to be a selective 5-HT_{1A} receptor agonist – implies that responses previously attributed to other receptor subtypes, might also have been, at least partly, 5-HT_7 mediated responses. Furthermore, the pharmacological profile of this serotonin receptor is marked by high affinities for a number of drugs with different chemical structures that are known for their treatment of psychosis and schizophrenia[9,189,191,234], depression[33,176,234], hypertension[203,234], Parkinsons disease[191],[203,234] and migraine[9,191,203,234]. This suggests once more that the 5-HT_7 receptor might be involved, at least to some extent, in the pathofysiology of these disorders.

In the process of exploring the most recent addition to the family of serotonin receptors, several radioligands, such as [^{3}H]-5-HT[3,9,29,67,71,81,114,116,117,159,176,191,203,232,237], [^{3}H]-5-CT[16,72,105-108,116,117,130,133,164,213,229,234,251], [^{3}H]-mesulergine[82,83], [^{3}H]-D-lysergic acid diethylamide (LSD)[3,98,186,189,203], [^{125}I]-LSD[159,203], and [^{3}H]-8-OH-DPAT[16,232] have been used, but none of these ligands is selective. For this reason, application of these radioligands to examine 5-HT_7 receptor pharmacology had to be combined always with masking ligands to rule out other receptor subtypes that might be subjective to binding of the radioligand as well. More selective (radio-) ligands that have been developed in recent years can contribute to further elucidate the pharmacological profile of this receptor and its isoforms. A first step in this direction was made by the development of SB 258719, the first reported 'cold' ligand that binds the 5-HT_7 receptor with an affinity (pK_i = 7.5) at least 100 times higher than a broad range of serotonergic, adrenergic, and dopaminergic receptors[54]. A chemical structure analogue to that of SB 258719 was found by high-throughput screening of compound databases, and appeared to potently inhibit the effect of 5-CT-stimulated adenylyl cyclase activity in HEK293 cells expressing the human 5-HT_7 receptor. Optimization by conformational restraint of the side chain of SB 258719 led to the more potent (pK_i = 8.9) and even more selective structure SB 269970 (>250 compared to broad range, except 5-ht_{5A} (50 fold)) that was also developed as tritiated radioligand[133]. [^{3}H]-SB 269970 has been evaluated in binding to the human 5-$HT_{7(a)}$ receptor expressed in HEK293 cells and homogenized guinea pig brain cerebral cortex membranes in comparison with the profile for [^{3}H]-5-CT[231]. In a parallel study, [^{3}H]-SB 269970 was used to radiolabel 5-HT_7 receptors in mouse, rat, pig, marmoset, and human brain tissues and represented a valuable tool with which the distribution and function of 5-HT_7 receptors

in native tissues can be further characterized and their potential role in disease states can be elucidated[230]. Another compound that also attracted attention during screening of compound libraries because of its high affinity for the 5-HT_7 receptor led to the development of DR4446; likewise a potent antagonist (p*K*i = 8.0), but with little lower selectivity with respect to other serotonin receptors (>100 fold selectivity, except 5-HT_{1A} (80 fold))[106,107] and possibly α-adrenoceptors, dopamine, and histamine receptors (since a close analogue of DR4446, namely DR4004, also showed considerable affinity for these receptor subtypes) [114]. Nevertheless, the presence of a chemically readily accessible methyl group in this compound made it a good candidate for radioligand binding as well as positron emitting tomography (PET) studies[262] that contribute to the examination of the pharmacological profile and the biological role of 5-HT_7 receptors in the CNS and the periphery.

1.3.5 Constitutive activity

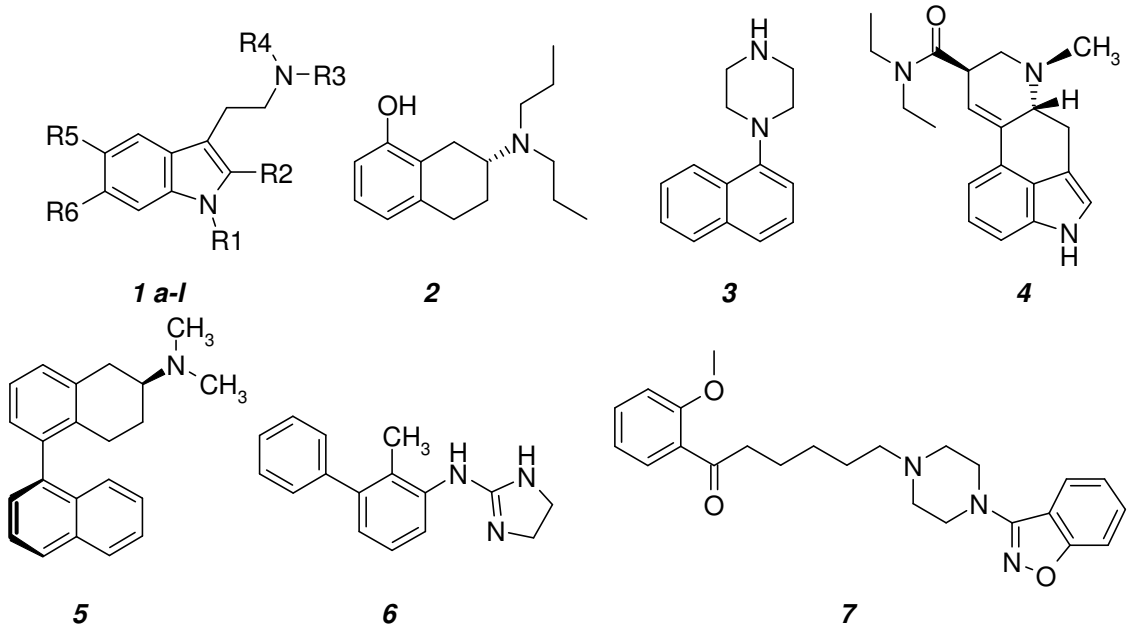
As described in the scope of this thesis, the classical view of signal transduction by means of receptor activation by an agonist or blockade by an antagonist, has been extended recently as a result of the observation that many receptors exhibit constitutive activity[28,102-104,121,194]. Even in the absence of an agonist, these receptors are able to activate G proteins and mutations in different structural domains of the receptor can enhance this agonist independent activity[117,187,194,210]. The extended model of receptor activation, or the allosteric ternary model, describes (at least) two states of the receptor that are in equilibrium: an active and an inactive state. In the absence of agonists, the level of basal receptor activity is determined by the equilibrium of the two functionally distinct states. It is hypothesized that the efficacy of ligands is a reflection of their ability to alter the equilibrium between these two states. Agonists would favor to bind the receptor in the active state. This results in enhancement of the basal activity. On the contrary, inverse agonists would favor to bind and lock the receptor in the inactive state, thereby changing the level of receptor activity to sub-basal and causing decoupling of the initial complex of receptor and G-protein. Partial agonists would bind a conformation of the receptor that also leads to G protein activation, but with less effectiveness than full agonists. The classical, neutral antagonists would bind both states with similar affinity, thereby leaving the equilibrium of basal activity unchanged.

Strikingly, basal adenylyl cyclase activity was reduced by increased concentrations of 5-HT antagonists (inverse agonists) in membranes of stable cell lines expressing the human 5-HT_7 receptors, indicating that these splice variants are constitutively active, a phenomenon that has been determined by several groups[117,133,229], but not all[3]. The latter may be inherent to the different types of cell lines used to express the 5-HT_7 receptor, although this is not established. In general, both the basal constitutive activity and agonist efficacy can be influenced by the ratio of expressed receptors and the concentration of G-protein on one side, and by over-expression of AC on the other[117]. Constitutive activity of 5-HT_7 receptors has not been reported to date *in vivo*.

1.4 Current knowledge of chemical structures that interact with the 5-HT$_7$ receptor

1.4.1 Early 5-HT$_7$ receptor agonists

As discussed earlier, the 5-HT$_7$ receptor pharmacology was characterized by its high affinity for a number of ligands (Figure 1.4, Table 1.4). The potency rank order of agonists was identical with that for the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes (5-CT (***1j***) > 5-HT (***1c***) ≥ 5-methoxytryptamine (5-MeOT, ***1g***). Additionally, the classical and until then known as selective 5-HT$_{1A}$ receptor agonist (*R*)-8-OH-DPAT (***2***), also displayed above moderate affinity, albeit in some cases with only moderate efficacy (about 80-90%) compared to the endogenous ligand serotonin (***1c***)[116,136,232,253]. The low response to sumatriptan (***1l***) by the 5-HT$_7$ receptor was one of the major criteria to draw a distinction between this and other functional 5-HT$_1$-like receptor subtypes[87]. Furthermore, a limited number of other, primarily tryptamine-based ligands were tested which appeared to behave as agonists. The simplified structure activity relationship (SAR) of this set of tryptamines (***1a-l***) indicates that a hydrogen bond accepting group at the 5-position, and to a lesser extent at the 6-position, is preferred (compare ***1a***, ***1b***, ***1c*** and ***1g***), but larger substituents at the oxygen reduce affinity (***1i***). Methylation of the indole nucleus is well tolerated at the nitrogen atom (***1f***), but not at the 2-position (***1e***). If the indole nucleus is equipped with a carboxamide group at the 5-position, a 5-HT$_7$ receptor agonist is obtained with the highest affinity among this series of tryptamines. This could be explained in terms of double hydrogen bond formation (with the carboxamide oxygen atom acting as acceptor, and the amine acting as donor). Another possible reason for increased affinity is the longer distance of the hydrogen bond accepting oxygen relative to the centroid of the six-membered aromatic ring, which facilitates interaction with a more distant amino acid residue at the binding site of the receptor. Finally, substitution of the nitrogen atom of the ethylamine side chain with 2 methyl or propyl groups is tolerated; nevertheless, this reduces the affinity approximately 5-10 fold (compare ***1c*** with ***1d*** and ***1g*** with ***1h***). The conformationally restricted ergoline (+)-LSD (***4***) also shows high affinity for the 5-HT$_7$ receptor and stimulates the formation of cAMP[191]. This is remarkable, because many other ergoline-based ligands exhibit antagonistic behavior. The absence of a hydrogen bond accepting group at the six-membered aromatic ring of the ergoline skeleton appears to be surmountable. Possibly, this deficiency is compensated by the presence of the ethylamide moiety at the heterocyclic six-membered ring. As a result of its rigid structure, (***4***) appears to be a valuable tool for molecular modeling studies in determining the active conformation of more flexible agonists that bind to the receptor as will be discussed later on. During the period of the research project described in this thesis, a number of novel compounds has been developed, which show intrinsic agonist activity in functional essays (***5-7***). These compounds will be discussed in the next section.



1 a-l ***2*** ***3*** ***4***

5 ***6*** ***7***

Figure 1.4: ***Chemical structures of early and novel 5-HT$_7$ receptor agonists.***

		Substituents					
Agonist	**Trivial name**	**R1**	**R2**	**R3,R4**	**R5**	**R6**	**pK_i**
1a	Tryptamine	H	H	H	H	H	6.8-7.8
1b	6-MeOT	H	H	H	H	CH_3O	7.4
1c	Serotonin	H	H	H	HO	H	8.1-9.6
1d	Bufotenine	H	H	CH_3	HO	H	7.7
1e	2-Methylserotonin	H	CH_3	H	HO	H	5.9
1f	5-HO-*N*(ω)-MeT	CH_3	H	H	HO	H	9.0
1g	5-MeOT	H	H	H	CH_3O	H	8.3-9.3
1h	5-MeO-DMT	H	H	CH_3	CH_3O	H	7.9-8.1
1i	5-BeOT	H	H	H	BnzO	H	6.6
1j	5-CT	H	H	H	H_2NCO	H	9.0-9.9
1k	*N,N*-dipropyl-5-CT	H	H	*n*-Pr	H_2NCO	H	8.3
1l	Sumatriptan	H	H	CH_3	$CH_3NHSO_2CH_2$	H	6.0-6.6
2	(*R*)-8-OH-DPAT						6.3-7.5
3	1-Naphthylpiperazine						7.1-7.7
4	(+)-LSD						7.8-8.0
5	AS4						>9.5
6							7.8
7							9.0

Table 1.4: ***Binding affinities of early and novel 5-HT$_7$ receptor agonists.*** *Data derived from literature*[9,164,172,176,191,195,203,234].

1.4.2 Novel 5-HT$_7$ receptor agonists

A series of (*S*)-dimethyl-aminotetralins with various aryl substituents at the 5-position was developed as structural analogues of 11-substituted (*R*)-phenylaporphines[127]. Several members of this series, like compound ***5*** (Figure 1.4, Table 1.4), exhibit very high affinity for the 5-HT$_7$ receptor, some of them with moderate selectivity over the 5-HT$_{1A}$ receptor. Strikingly, instead of possessing a hydrogen bond accepting moiety like most members of the series of tryptamines, compounds of this kind are characterized by the presence of a flat aromatic ring system that is incapable of

forming hydrogen bonds. Nevertheless, these ligands show affinities for the 5-HT_7 receptor equal to, or even higher than 5-CT (***1j***). Apparently, the aryl-moieties can compensate for the absence of a hydrogen bond accepting functional group by occupying a hypothesized lipophilic pocket at the binding site of the receptor. It remains unclear, however, what determines the intrinsic activity of these compounds, since minor differences in the aryl substituents can shift the activity from full agonist to antagonist (and vice versa), and the compounds should be tested for a full range of receptor subtypes to determine their overall selectivity[195].

The development of a series of (4,5-dihydroimidazol-2-yl)-biphenylamines (analogues of ***6***) as 5-HT_7 receptor agonists was initiated by high-throughput screening of compound libraries. Inherent in the 2-aminoimidazoline moiety, these compounds also show α_1 and α_2 receptor activity and cause pronounced hypertension after *intravenous* administration in rats[164]. Substituents at the *meta* position of the free phenyl ring tend to lower the affinity for the 5-HT_7 receptor, while replacement of this ring by a thiophene moiety does not alter its affinity for the serotonin receptor significantly, but increases the affinity for the α_2-adrenergic receptor. For this compound, the considerable affinity can be argued for by the hypothesis that the free phenyl ring occupies a lipophilic pocket at the binding site of the receptor to compensate the absence of a hydrogen bond accepting moiety.

Within a series of 1-[ω-(4-aryl-1-piperazinyl)alkyl]-1-aryl ketones, compound ***7*** surprisingly acted as an agonist at the 5-HT_7 receptor as well[172]. As an analogue of a series of compounds developed as antagonists at this receptor[105-108], sharing structural features such as “long-chain” arylpiperazines with other 5-HT_{1A} or D_4 receptor ligands, it is remarkable to observe that compound ***7*** produced dose-dependent guinea-pig ileum relaxation of substance-P induced contraction. The effect of ***7*** could be reverted by the selective 5-HT_7 antagonist SB-269970. However, the presence of the risperidone-like 3-(1-piperazinyl)-1,2-benzioxazole moiety also results in good affinity for the 5-HT_{2A} receptor, and additionally, the compound exhibits moderate to high affinity for dopamine and α_1 receptors.

1.4.3 Early 5-HT_7 receptor antagonists

Since its discovery, moderate to high affinity for a number of miscellaneous compounds marks the pharmacological profile of the 5-HT_7 receptor. Some of these compounds are known for longer times as therapeutics for the treatment of numerous disorders of the human body, and are supposed to interact with receptor subtypes other than the 5-HT_7 receptor. Among these compounds, an extensive number of ergoline derivatives is present (Figure 1.5, Table 1.5) with affinities up to sub-nanomolar in case of lisuride (***11***), which has been launched as a drug in the early 1980’s as a D_2 receptor agonist for the treatment of Parkinson’s disease. Furthermore, dihydro-ergotamine (**9**), showing nanomolar affinity for the 5-HT_7 receptor, has been on the market for a very long time for the prophylactic treatment of migraine. In general it can be stated among

the series of ergoline derivatives that neither the stereochemistry of the ergoline skeleton, nor the presence of a double bond in the six-membered heterocyclic ring (*e.g.* ***8***, ***11***, ***13***) seem to affect the binding affinity dramatically. Most ergolines possess an (sulfon-) amide moiety at the six-membered heterocyclic ring, but this feature seems not to be essential for binding to the receptor (*e.g.* ***16***).

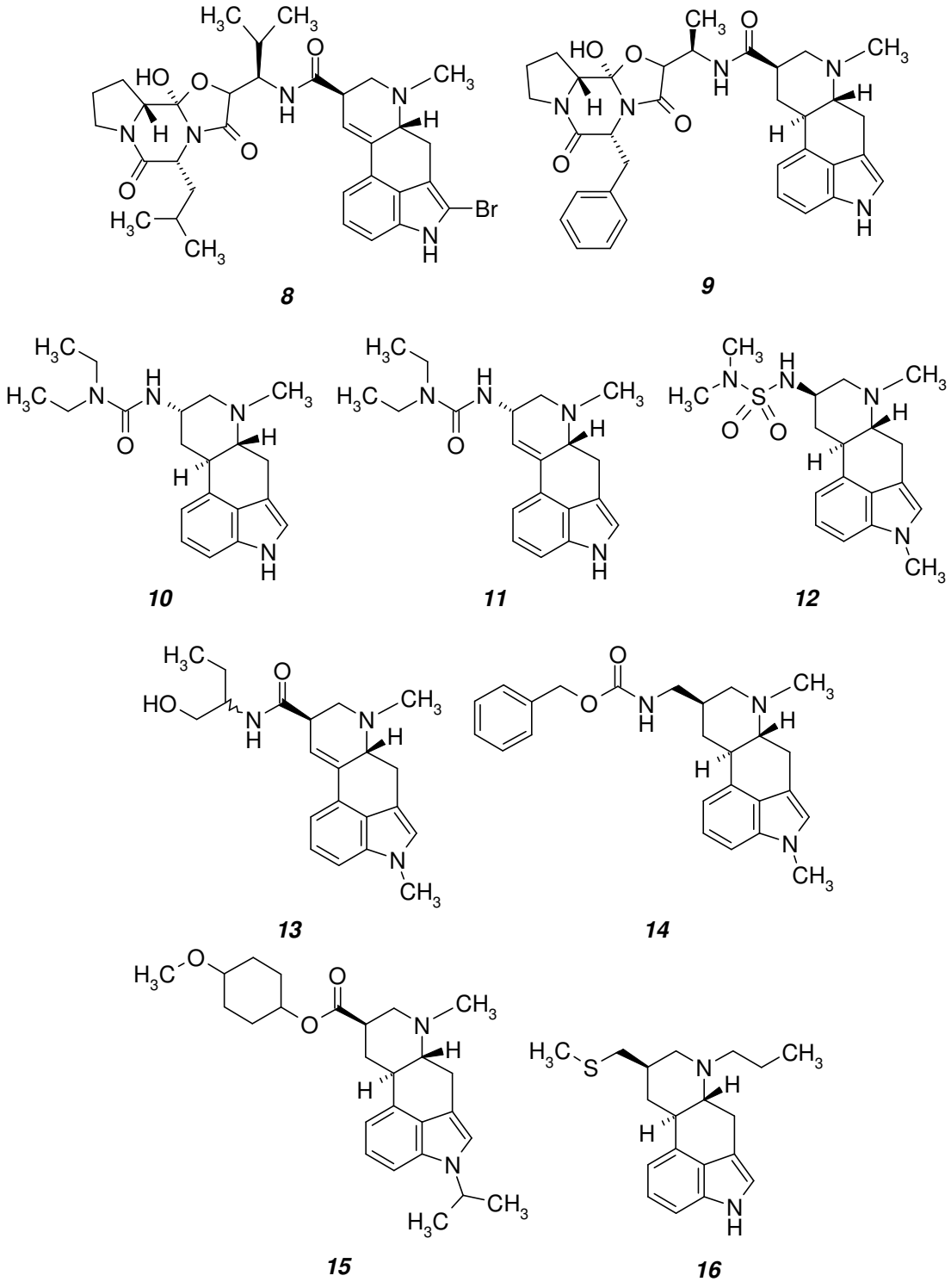


Figure 1.5: ***Chemical structures of ergoline based 5-HT$_7$ receptor antagonists.***

Larger substituents at the six-membered heterocyclic ring are well tolerated (*e.g.* ***8***, ***9***, ***14***, ***15***), as well as longer alkyl chains attached to the nitrogen atom of this ring (*e.g.* ***16***), and substitution of the 5-membered heterocyclic ring (*e.g.* ***8, 12, 13, 14, 15***). Strikingly these ergolines act as antagonists at the 5-HT_7 receptor, while the structurally closely related ***4*** acts as an agonist. Apparently, the substitution of the regular amid moiety by a urea (***4*** compared with ***11***), or replacement of one ethyl group by a hydroxy-methylene group combined with methylation of the nitrogen of the 5-membered heterocyclic ring (***4*** compared with ***13***) can cause this dramatic difference in efficacy. The effect of these subtle structural differences with respect to conformational changes at the receptor binding site, altering the efficacy from agonistic to antagonistic, remains unclear at present.

Another comprehensive series of compounds that interact with the 5-HT_7 receptor include a number of typical and atypical antipsychotic agents such as phenothiazines (***26, 27***), thioxanthenes (***25***) and other heterocyclic compounds (***17, 18, 19, 20, 21, 24***), among which the very potent antagonist methiothepin (***20***).

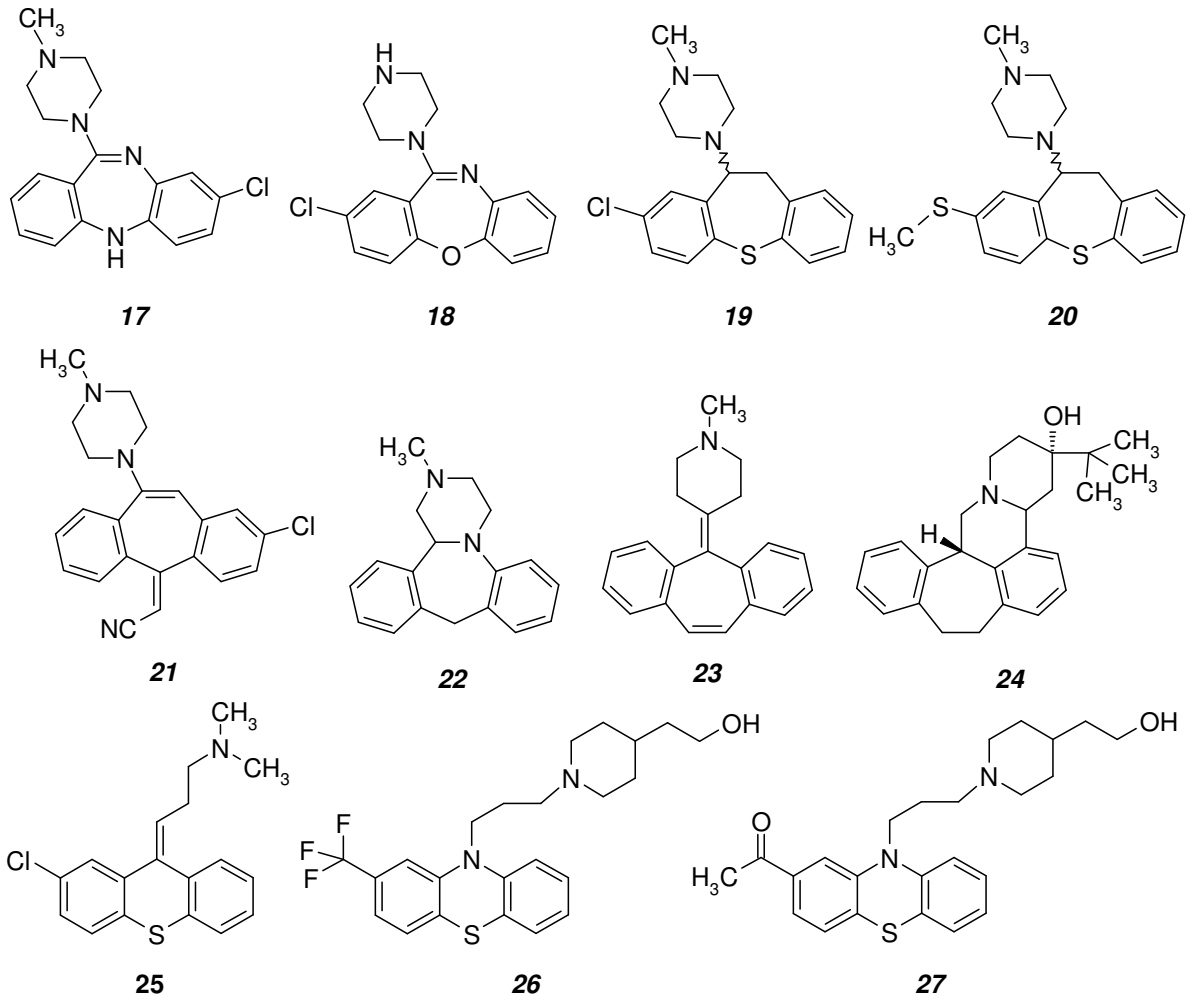


Figure 1.6: ***Chemical structures of hetero- and polycyclic 5-HT_7 receptor antagonists.***

In addition, many other substances with related chemical structures, that have proven their therapeutic use for many disorders are depicted in Figure 1.6 and listed in Table 1.5. This series, like the series of ergolines, shows once more a broad scope of tolerable chemical variation. All compounds share a general structural similarity with two aromatic rings bridged by a 6- or 7-membered (hetero-) cycle, which forces the molecule into a non-planar V-shape. A protonatable nitrogen atom, in most cases part of a piperidine or piperazine ring system, is also present at a distance comparable with a C_3 alkyl chain. In addition, the presence of a polarizing substituent at either one of the aromatic rings seems to enhance receptor affinity (compare ***22***-***24*** with rest of series). Derivatives of ***17*** (Fluperlapine: F instead of Cl, and Clothiapine: NH instead of S; structures not shown) demonstrate a 10-fold increase of affinity (pK_i = 8.3 and 8.4, respectively) compared to ***17***. Furthermore, in some cases, stereochemistry seems to influence binding affinity marginally (*e.g.* (+)-***19*** pK_i = 9.4, (-)-***19*** pK_i = 8.9).

Antagonist	Trivial name	Therapeutic indications	pK_i
8	Bromocryptine	a, b,c, d	6.9-7.3
9	Dihydroergotamine	e, f	6.8-8.1
10	Terguride	b, c, d	8.1
11	Lisuride	a, b, g, d	8.2-9.2
12	Mesulergine	a, b	7.1-7.8
13	Methysergide	e	7.1-7.9
14	Metergoline	f, d, h	7.2-8.2
15	Sergolexole	e, i, j	7.0
16	Pergolide	a, b	8.5-9.0
17	Clozapine	k, l	7.2-7.4
18	Amoxapine	k, m	6.7-7.4
19	Octoclothepin	k, n	8.6
20	Methiothepin	k	8.1-9.4
21	Rilapine	k, o	8.6
22	Mianserine	m	7.0-7.4
23	Cyproheptadine	e	6.9-7.5
24	(+)-Butaclamol	k	6.7-7.5
25	Chlorprothixene	k	8.3
26	Fluphenazine	m, k, o	8.1
27	Acetophenazine	k	8.6
28	Tiosperone	e, k	9.2
29	GR-127935	p, m	6.2
30	Pimozide	k	9.3
31	Ritanserin	j, m, k, o, q	7.3-7.8
32	Pirenperone	j, o	7.7
33	Ketanserin	i, j	5.9-6.6
34	Spiperone	k	7.0-7.3
35	Risperidone	i, k, r, n	8.1-9.0
36	WAY-100635	o, s	6.8

Table 1.5: ***Binding affinities of early 5-HT$_7$ receptor antagonists.*** *Data derived from literature*[9,18,33,157,176,189,191,203,219,234]*. Therapeutic indications (a) Parkinson's disease, (b) D_2 Agonist, (c) Prolactin Secretion Inhibitors, (d) Hyperprolactinemia, (e) Migraine, (f) Migraine prophylaxis, (g) Treatment of Gynecological Disorders, (h) Vasodilatation, (i) 5-HT$_{2A}$ Antagonists, (j) Hypertension, (k) Psychosis, (l) D_4 Antagonists, (m) Depression, (n) D_2 Antagonists, (o) Anxiety, (p) 5-HT$_{1B/1D}$ Antagonists, (q) Treatment of Alcohol Dependency, (r) Treatment of Bipolar Disorder, (s) 5-HT$_{1A}$ antagonist.*

Finally, a number of miscellaneous compounds (***28***-***36***, Figure 1.7, Table 1.5) was analyzed for their binding affinity in the early years after the discovery of the 5-HT_7 receptor. The series comprises relatively large and flexible molecules showing even more structural freedom. Many compounds are mono- (***32***-***34***) or di-fluoronated (***30***-***31***), which would make them valuable tools for PET studies, if only they would have been more selective.

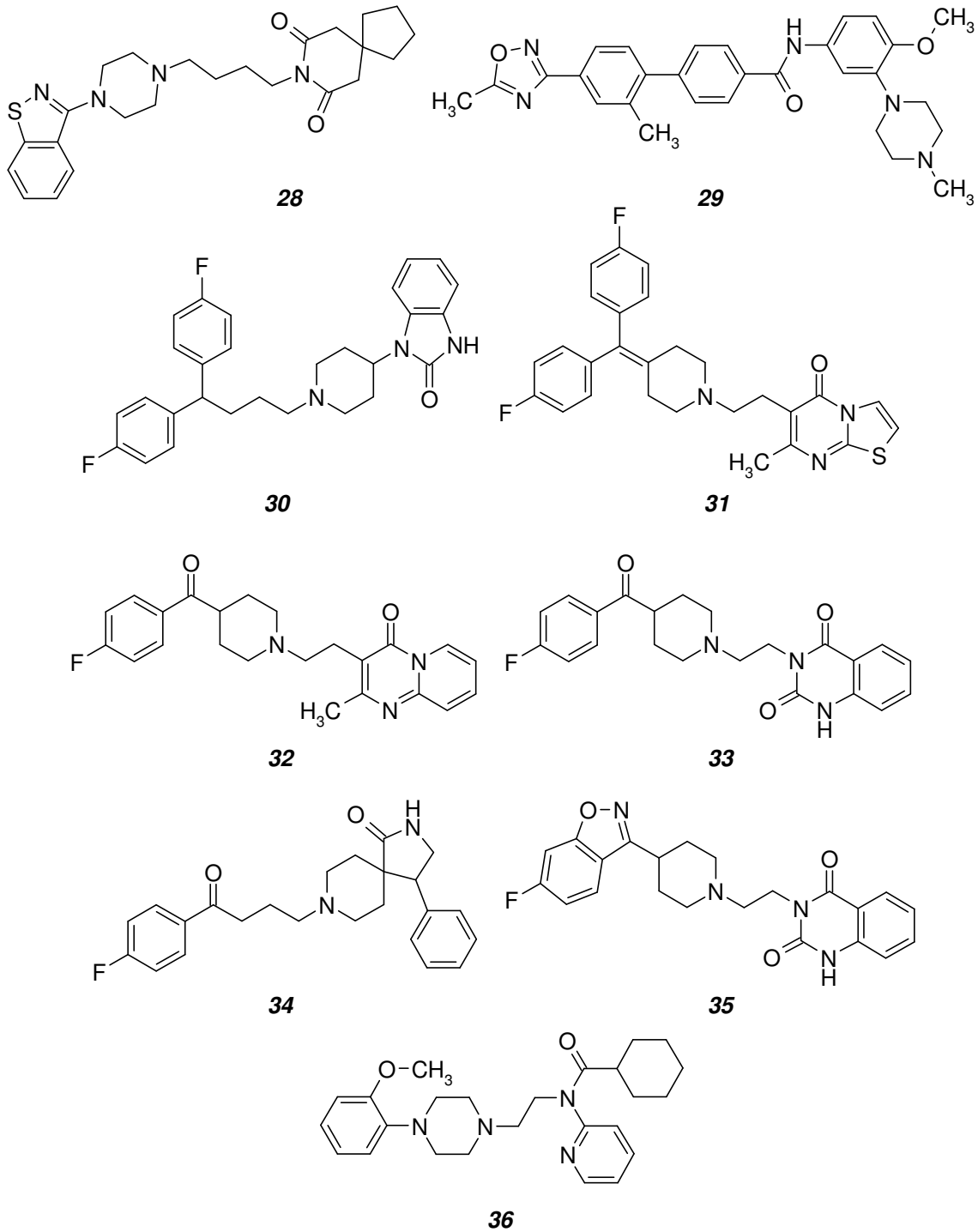


Figure 1.7: ***Chemical structures of miscellaneous 5-HT_7 receptor antagonists.***

From the initial categorization of serotonin receptors, it was already recognized that the 5-HT_7 receptor was not subject to blockade of agonists by GR-127935 (***29***). Furthermore, ketanserin (***33***) and WAY-100635 (***36***) – the latter is known as selective 5-HT_{1A} receptor antagonist – are also incapable of blocking the receptor with high affinity. Of the remaining ligands of Figure 1.7, some differences in binding affinity are eye-catching, like the closely related ***33*** and ***35***: the benzoxazole group, a ring-closed isostere of the aromatic ketone, causes an almost 100-fold increase of 5-HT_7 receptor affinity. Likewise, a comparable difference in affinity can be observed when the two bis-(4-fluoro-phenyl) containing ligands (***30*** and ***31***) are compared that are known for their therapeutic effect as anitpsychotics. Another example of the effect of different isosteres is given by ***32*** with the 2-methyl-pyrido[1,2-a]pyrimidin-4-one moiety on one hand, and ***33*** with the 1H-quinazoline-2,4-dione moiety on the other, which results in an at least 10 fold increase of affinity in favor of the former.

Clearly, the diversity of compounds capable of blocking the action of agonists at the 5-HT_7 receptor is enormous. Roughly, they are divided in three main groups: ergolines, hetero- and polycyclic 5-HT_7 receptor antagonists, and a series of flexible miscellaneous antagonists. However, all of these compounds lack selectivity for the 5-HT_7 receptor, since they were initially designed to interact with other subtypes of the serotonin receptor family, or even other kinds of GPCRs like dopaminergic or α-adrenergic receptors. During the late 1990's, several groups started to report the development of more selective 5-HT_7 receptor antagonists. These are discussed in the next section.

1.4.4 Novel 5-HT_7 receptor antagonists

In 1997, the first selective 5-HT_7 receptor antagonist, SB-258719 (***44a,*** Figure 1.8**,** Table 1.6) was published as a derivative of a positive hit resulting from high-throughput screening of the SmithKline Beecham Compound database against the cloned human 5-HT_7 receptor[54]. SAR studies with numerous analogous chemical structures indicated the importance of the (*R*)-configuration of the chiral center in the sulfonamide tail with respect to receptor affinity. Additionally, it was found that a wide range of aromatic nuclei was tolerated by the receptor binding site. Binding profiles of the best compounds within this series indicated impressive selectivity (100 fold) over a wide range of other receptors. Subsequent publications[133] reported improved affinity combined with higher selectivity in case the sulfonamide tail was conformationally restricted by means of a heterocyclic 5- or 6-membered ring (***44b***). Such modifications, and replacement of the 3-methylphenyl by a 3-hydroxyphenyl moiety resulted in SB-269970 (***44c***). The latter appeared to be highly selective as well (>250 fold), if tested against the same range of receptors used for ***44a***. However, ***44c*** also showed moderate binding affinity for the 5-ht_{5A} receptor (pK_i = 7.2), and as a consequence the overall selectivity was repressed to a less impressive 50 fold. Nevertheless, this compound was developed as a radioligand as well[72,74,231], and proved to be a valuable tool with

which the distribution and function of the 5-HT_7 receptor in recombinant and native tissues can be further characterized, and the potential role in disease states can be elucidated. Attempts to reduce the extremely high *in vivo* blood clearance of the sulfonamides in rats, resulting from the presence of the phenolic hydroxyl group and Phase II metabolism, succeeded by replacement of the 3-hydroxy-phenyl moiety by a metabolically more stable bioisostere[55]. SB-656104 (***44d***), a conformationally restricted analogue of ***44a*** with an indol-6-yl moiety instead of a phenol, and a 4-chlorophenoxy substituent at the 4-position of the piperidine ring does not show the highest affinity for the 5-HT_7 receptor within this series, nor the highest selectivity over a wide range of receptors. Nonetheless the compound has acceptable low affinity for adrenergic receptors combined with increased oral bioavailability (16%) compared to the earlier lead ***44c***, which makes it an interesting candidate for future research.

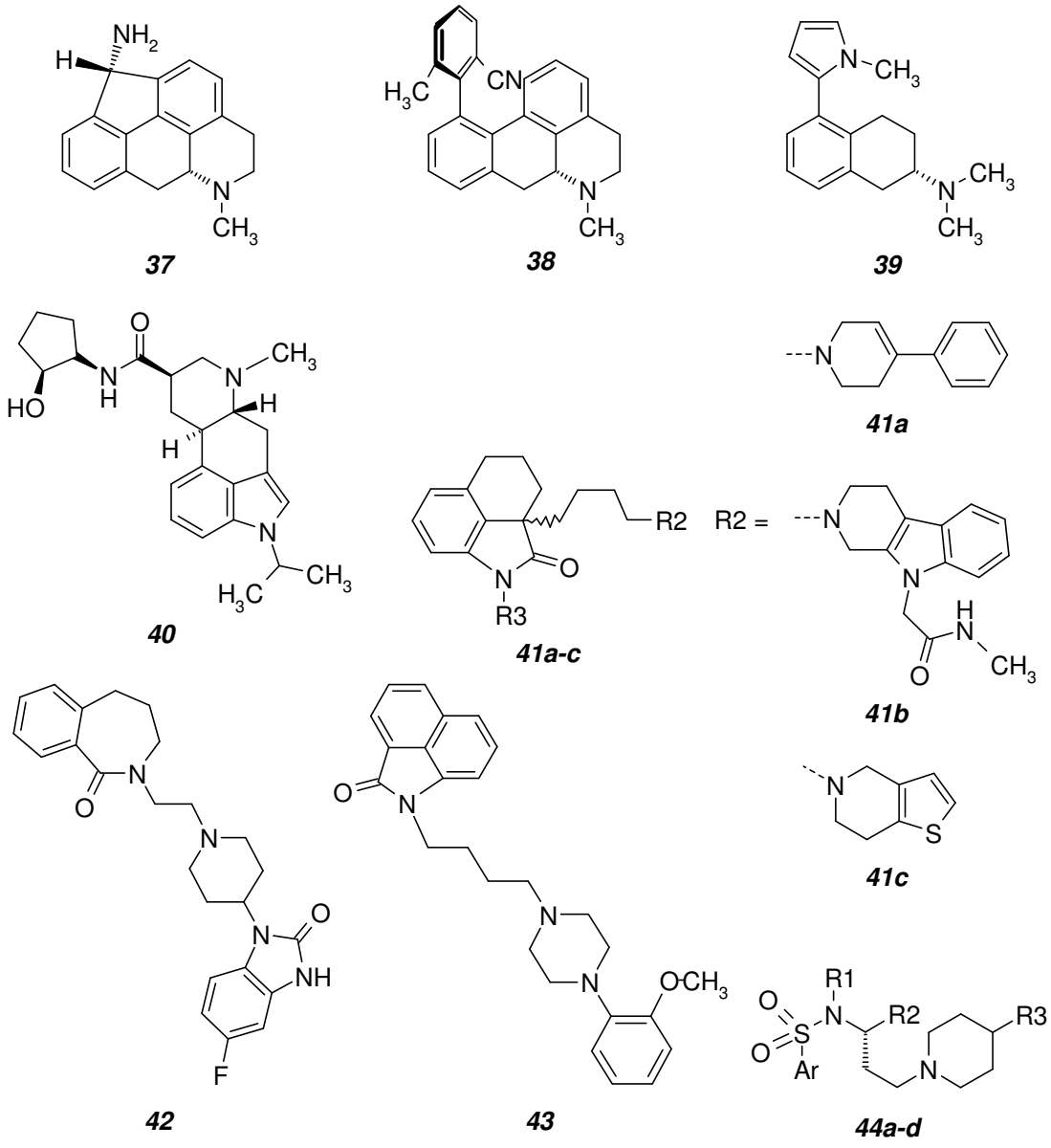


Figure 1.8: ***Chemical structures of novel 5-HT_7 receptor antagonists.***

Antagonist	Trivial name	Ar	R_1, R_2	R_3	pK_i	Selectivity
37					9.0	20 fold[§]
38					8.4	37 fold[§]
39	AS-23				9.2	0.3 fold[§]
40	LY-215840				7.8	n.d.
41a	DR-4004			H	8.5	46 fold[§]
41b	DR-4365			H	8.5	36 fold[§]
41c	DR-4446			CH_3	8.0	80 fold
42	SB-691673				8.7	105 fold
43					7.2	n.d.
44a	SB-258719	3-CH_3-phenyl	CH_3, CH_3	CH_3	7.5	100 fold
44b	SB-258714	3-CH_3-phenyl	$(-CH_2-)_3$	CH_3	8.5	316 fold[§]
44c	SB-269970	3-HO-phenyl	$(-CH_2-)_3$	CH_3	8.9	50 fold
44d	SB-656104	Indol-6-yl	$(-CH_2-)_3$	4-chlorophenoxy	8.7	13 fold

Table 1.6: ***Binding affinities of novel 5-HT$_7$ receptor antagonists.*** *Data derived from literature*[53-55,105-108,114,127,128,130,133,262]. *(§)Not tested against full range of receptor subtypes.*

Compounds ***41a-c*** also originate from screening of a compound library against the human 5-HT_7 receptor[105-107]. The series comprises several racemic tetrahydrobenzindoles with tetrahydropyridyl analogues connected by C_4-alkyl spacers. The therefore highly flexible ligands generally show good affinity and selectivity for the 5-HT_7 receptor, although the range of receptors that was used to screen the pharmacological profile of these compounds is less comprehensive compared to the studies performed with the previously discussed sulfonamides. Besides, ***41a*** also shows functional activity at the dopamine D_2 receptor and the α_1-adrenoceptor[114] making this a less interesting tool for selective blockade of the 5-HT_7 receptor *in vivo*. Attempts to reduce the flexibility of the tetrahydropyridyl by replacement with a substituted tetrahydropyridindole resulted in a compound with comparable affinity, but slightly reduced selectivity over a limited range of receptor subtypes (***41b***). To prevent rapid metabolization (aromatic hydroxylation) of these compounds due to the presence of a unsubstituted phenyl ring, several halogenated derivatives were synthesized and successfully tested for their resistance to biodegradation in the presence of human and rat liver microsomes[108]. Moreover, the increased metabolic stability did not significantly alter receptor affinity. The improved selectivity of ***41c*** in combination with the presence of a chemically readily accessible methyl group made this compound a good candidate for radioligand binding studies as well as positron emitting tomography (PET) studies[262]. Thereby it contributes to the examination of the pharmacological profile and the biological role of 5-HT_7 receptors in the CNS and the periphery. The non-chiral analogue ***43*** was part of a series compounds synthesized for the development of a pharmacophoric hypothesis for 5-HT_7 antagonism[130]. Compared to ***41a-c***, compounds in this series showed lower affinity for the 5-HT_7 receptor – ***43*** being the most potent antagonist among them –, while no details are known as far as selectivity is concerned.

Yet another example of cross-screening of compound data bases is given by ***42***[53]. It was identified as the most promising analogue within a series, blocking 5-CT stimulated adenylyl cyclase activity, confirming its antagonist profile. However, the profile of ***42*** is consistent with an inverse agonist as the basal response of adenylyl cyclase was reduced in the absence of 5-CT.

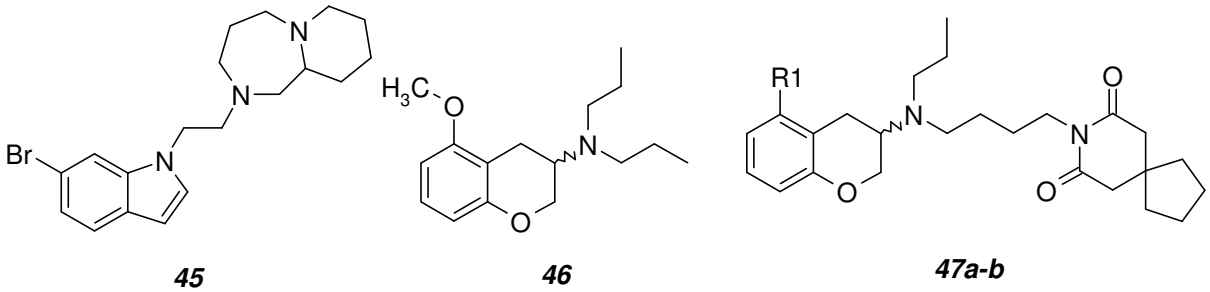
This latter quality is also true for compounds ***44a-d***[133]. The selectivity over a range of closely related receptors is high. Moreover, the compound is devoid of cytochrome P450 metabolism issues, making it a promising new tool for characterization of the functional role of the 5-HT_7 receptor.

Many of the physiological effects of aporphines are related to dopaminergic receptors, since it embraces the rigidified conformation of dopamine. Nevertheless, several apomorphine derivatives showing selectivity for 5-HT receptors are known for several years[21,22]. In many cases this radical change in selectivity from dopaminergic to serotonergic is due to introduction of a steric group at the 10-position of the apomorphine nucleus. The efficacy of these derivatives ranges from full agonists to full antagonists. With the aim to study the pharmacological profiles of pentacyclic ring systems based on aporphine, and investigate SARs of 11-substituted (*R*)-aporphines, compounds ***37***, ***38*** and the direct spin-off ***39*** were developed among many other analogues[127,128,195]. These compounds revealed interesting and diverse pharmacological profiles through binding studies at 5-HT_7 and 5-HT_{1A} receptors as well as at dopamine D_{2A} receptors. Compound ***37*** is not the most potent structure within the series of pentacyclic ring systems interacting with the 5-HT_7 receptor. On the other hand, it shows moderate preference for this receptor subtype over the 5-HT_{1A} receptor and even higher selectivity with respect to the dopamine D_{2A} receptors (125 fold). Introduction of a C11-phenyl substituent at the aporphine nucleus had proven to increase the affinity for serotonin 5-HT_{1A} receptors considerably[78]. On expanded profiling of such analogues it was observed that they also exhibit interesting affinity for the 5-HT_7 receptor. The 2'-CN,6'-Me-substituted analogue ***38*** is the most interesting of this series as it is a potent 5-HT_7 receptor antagonist which exhibits selectivity versus 5-HT_{1A} and D_{2A} receptors[127]. Noteworthy is the fact that the compound is a stable atropisomer and displays pharmacological stereoselectivity. An aminotetralin analogue of this series (***39***) has also proven to be a very potent 5-HT_7 receptor antagonist[195]. However, the compound lacks selectivity over the 5-HT_{1A} receptor. Some of its analogues do show increased selectivity, but this gain is compensated with reduced affinity. Moreover, as discussed earlier in this chapter, it is unclear within this series what determines the compound's efficacy, since closely related structures (e.g. ***5***) appear to be full agonists instead of antagonists.

The ergoline ***40***, structurally a close relative to ***13***, exhibits, like many earlier developed ergolines, also good affinity for the 5-HT_7 receptor[33]. Nevertheless, the compound lacks selectivity for this receptor subtype. As a matter of fact, the compound shows more affinity for several 5-HT_2 receptors and comparable affinity for the 5-HT_{1A} receptor. Therefore, this ergoline is not a suitable ligand for the investigation of the functional profile of the 5-HT_7 receptor *in vitro* let alone *in vivo*, without co-administration of selective blockers of the competing receptor subtypes.

1.4.5 Other compounds with high affinity for the 5-HT$_7$ receptor

Several ligands with affinity for the 5-HT$_7$ receptor worth to be mentioned are depicted in Figure 1.9 and listed in Table 1.7. Despite their interesting potencies, no accurate data are reported on their efficacy. Being part of a larger series of iso-indoles, ***45*** proved to be the most promising ligand with high selectivity over other serotonin receptor subtypes, but with considerable potency at muscarinic and dopaminergic receptors as well[98].



45 ***46*** ***47a-b***

Figure 1.9: ***Chemical structures of other 5-HT$_7$ receptor ligands.***

Ligand	Trivial name	R1	pK_i	Selectivity
45			8.5	3 fold
46	5-MeO-DPAC		7.3	0.1 fold[§]
47a	S20244	CH_3O	8.3	4 fold[§]
47b		CH_3CO	8.5	0.1 fold[§]

Table 1.7: ***Binding affinities of other 5-HT$_7$ receptor ligands.*** *Data derived from literature*[98,186].

With the aim to design potent and selective 5-HT$_7$ receptor ligands as derivatives of compounds that had already proven to be potent ligands at the 5-HT$_{1A}$ receptor, ***46*** and ***47a-b*** were reported to be the most interesting outcome of this research[186]. Some of the racemic compounds exhibit nanomolar affinity for the 5-HT$_7$ receptor, but also sub-nanomolar affinity for the 5-HT$_{1A}$ receptor. In a light-dark test, the anxiolytic-like activity of ***47b*** was observed, while it was devoid of sedative or excitatory effects. However, due to the lack of selectivity of this compound, no conclusions can be drawn from these results with respect to 5-HT$_7$ receptor interactions.

1.4.6 Pharmacophore modeling of 5-HT$_7$ receptor ligands

Based on a set of 30 structurally diverse 5-HT$_7$ receptor ligands originating from all the classes of compounds discussed in the previous sections, the first pharmacophoric hypothesis for 5-HT$_7$ antagonism was presented that offers structural insight to aid the development of novel 5-HT$_7$ antagonists (Figure 1.10)[130]. According to this hypothesis, the minimal structural requirements for 5-HT$_7$ antagonism consist of an aromatic ring, a basic nitrogen (positive ionizable centre), a H-bonding acceptor group and a hydrophobic region at 4.9-5.9 Å apart from the basic centre. For all the molecules in the training set, reasonable low-energy conformations that align on

the model were found. Furthermore, the model is characterized by a good r^2 value (r^2 = 0.921) and validated using analogues of ***41a-c***, ***43***, ***44a-d***. At the end of 2003, an optimized version of this model, incorporating more recently reported ligands, was published[131]. This model will be discussed in more detail in Chapter 7 (Section 7.2).

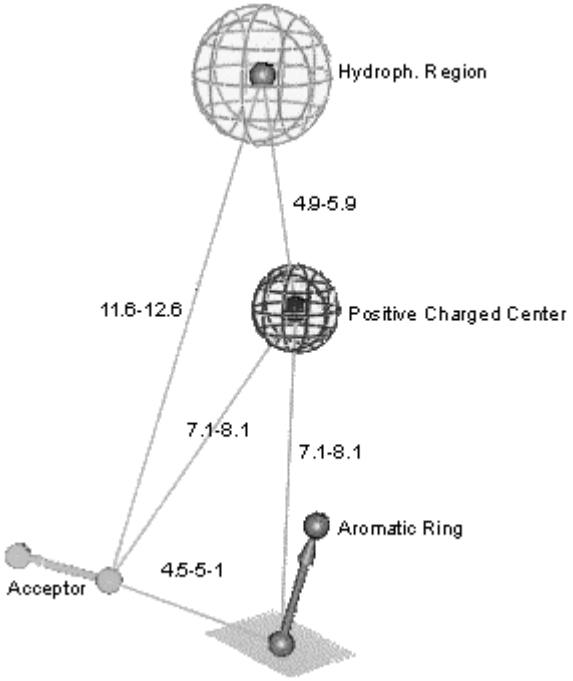


Figure 1.10: ***Proposed pharmacophore for 5-HT$_7$ antagonism.***

A second model has been presented, that is based on comparative molecular field analysis (CoMFA) of a mixed set of both agonists and antagonists of predominantly ergoline based ligands[251]. Though a valuable tool for the development of novel compounds with high affinity for the 5-HT$_7$ receptor, this model does not discriminate between agonistic and antagonistic properties. Furthermore, neither model offers a revelation in receptor-ligand interactions, nor do they indicate which amino acids may be involved in antagonist binding. In general, this additional information forms an important tool in understanding the mechanism of action at the binding site of the receptor, and the design of new compounds.

1.4.7 Summary of current knowledge of 5-HT$_7$ receptor ligands

Summarizing the previous sections on ligands with affinity for the 5-HT$_7$ receptor, it can be stated that the number of ligands that is tested for their 5-HT$_7$ receptor affinity is already impressive, and the chemical structures are highly diverse. Some compounds are especially interesting due to their proven selectivity, which makes them valuable tools for further characterization of the newest addition of the family of serotonin receptors *in vitro* and so much more *in vivo*. Furthermore, in general the importance of data base screening in order to find new

leads for drug discovery becomes increasingly more important[135]. Many of the compounds discussed in the previous sections originate from this strategy that is especially useful in cases of drug design independent of the structural features of the endogenous ligand of the receptor concerned. A major additional advantage is the reduced problem of ligand specificity: starting from the structure of the endogenous ligand, new drugs often suffer from being non-selective which is true to a much lesser extent for ligands found by screening of compound libraries. On the other hand, techniques that facilitate structure-based design of new ligands are also improving and become indispensable[14]. Because these techniques are highly dependent on the quality of the models of the functional target bio-molecule, validation of the models is essential. The quest for structural characterization of the target by means of spectroscopic methods (e.g. X-ray and nuclear magnetic resonance (NMR)) is growing bigger with the increasing number of possible new targets. For the characterization of GPCRs, the elucidation of the structure of bovine rhodopsin was an important progression[163]. As a result, the view emerged that it would now be easy to automatically obtain realistic models for any GPCR by homology modeling. The structure is widely used as a template for computational models of GPCRs, being the first elucidated high-resolution crystal structure of a mammalian homologue. Nevertheless, signals are spread that the crystal structure is not the Holy Grail in receptor modeling[5,132], and that construction of realistic models of certain GPCRs remains time-consuming and requires many refinements of the models in close association with experiments.

1.5 Function of the 5-HT$_7$ receptor

1.5.1 Introduction

As a result of its widespread distribution throughout the brain and peripheral tissues, and its affinity for numerous drugs that have been discussed previously in this chapter, it has been suggested that the 5-HT$_7$ receptor is implicated in multiple disorders of the brain and other regulatory processes. These suggested involvements will be discussed according to the numerous papers that have been published over the last decade. However, conclusive experimental evidence for many of these implications is yet to be acquired. Novel pharmacologically interesting ligands with improved selectivity for this receptor subtype that have been developed over the recent years form a powerful tool for further explanation of its functional and pathophysiological relevance.

1.5.2 Regulation of circadian rhythm

The suprachiasmatic nucleus (SCN) of the hypothalamus is an integral component of the mammalian circadian system responsible for generating behavioral and physiological circadian rhythms[113,138,147,193]. The timing of the SCN is synchronized to the day-night cycle by photic input supplied directly from the retina to the SCN via the retinohypothalamic tract (RHT) and indirectly

via the projection from the intergeniculate leaflet (IGL), the geniculohypothalamic tract[23,75,150,173,192]. The SCN also receives serotonergic input from the raphe nuclei[6,17,65,70,141,148,212]. Although efforts to demonstrate the presence of 5-HT_7 receptor in the SCN using several different techniques are ambiguous, various groups reported moderate levels of 5-HT_7 receptor mRNA in the SCN[11,16,43,81,156].

The hypothesis that 5-HT_7 receptors are involved in the regulation of circadian rhythms is based upon the observation that the non-selective 5-HT_7 receptor agonists 5-HT (***1c***), 5-CT (***1j***) and 8-OH-DPAT (***2***) are able to inhibit light induced phase shifting of circadian rhythms when injected directly into the SCN of hamsters[134,248,260]. This effect could be blocked by metergoline (***14***) and ritanserin (***31***), but not by pindolol, cyanopindolol, or WAY-100635 (***36***), thus ruling out interference of the 5-HT_{1A} receptor[42,185]. Comparable conclusions were drawn from a study on the arousal induced phase shifts of the circadian rhythm of wheel-running hamsters[218], and in vitro studies on the spontaneous discharge of SCN neurons[261], since ritanserin was capable of inhibiting these phase shifts and the inhibitory effect of 5-HT and 8-OH-DPAT on the spontaneous discharge, respectively. Again, the 5-HT_2 receptor ketanserin and the 5-HT_1 receptor antagonist pindolol were ineffective, suggesting that the 5-HT_7 receptor is the mediating subtype. Using mice to explore the retinal input to the suprachiasmatic nucleus, it was established that activation by 5-HT or 8-OH-DPAT of 5-HT_7 receptors in the SCN, and not 5-HT_{1A} receptors, result in a reduction of the amplitude of the optic nerve-evoked excitatory postsynaptic currents (EPSCs), while presynaptic 5-HT_{1B} receptors mediate reduction of RHT input to the SCN upon 5-HT stimulation[209]. Although ritanserin is a mixed 5-$HT_{2/7}$ receptor antagonist with little affinity for the 5-HT_{1A} receptor[134], a 5-HT_2 effect was ruled out by the fact that 8-OH-DPAT virtually has no affinity for the receptor subtype[87]. Similar to the preceding studies, WAY-100635 was not able to block the effect evoked by the 5-$HT_{1A/7}$ agonist. It has been established that electrical stimulation of the dorsal or median raphe nuclei (DRN and MRN, respectively) induced 5-HT release in the SCN[65]. Notably, DRN- but not MRN-stimulated 5-HT release was blocked by the 5-$HT_{1/2/7}$ antagonist, metergoline, suggesting that the DRN signals to the SCN indirectly via the activation of a 5-HT-responsive multisynaptic pathway. Pretreatment with the 5-$HT_{2/7}$ antagonist ritanserin also significantly inhibited DRN-electrically stimulated SCN 5-HT release. However, pretreatment with the 5-HT_{1A} antagonist, NAN-190, or the 5-HT_2 antagonists ketanserin and cinanserin had little suppressive effect on this DRN-stimulated 5-HT release. In complementary behavioral trials, electrical stimulation of the DRN during subjective midday caused an advance in the free-running circadian activity rhythm under constant darkness, which was inhibited by metergoline. Collectively, these results are evidence that DRN-stimulated 5-HT release in the SCN requires the activation of an intermediate target with receptors having 5-HT_7 pharmacological characteristics. The finding that aging induces a marked decrease in specific binding of [^{3}H]-8-OH-DPAT to the 5-HT_7 receptors in the DRN during the mid-subjective day suggests that this region and this receptor subtype play

important roles in 8-OH-DPAT induction of circadian phase shifts in vivo and that they constitute an important locus of aging in the circadian timing system[43]. Besides, age related decrease of 5-HT_7 mRNA expression was also found in specific areas of the hippocampus[115].

While all the reports discussed in this section apply indirect pharmacology by means of non-selective ligands to investigate a possible role for 5-HT_7 receptors in the regulation of circadian rhythms, the use of a more selective 5-HT_7 receptor antagonist, DR-4004 (***41a***), also indicated that phase advances induced by 8-OH-DPAT perfusion into the SCN were inhibited, implicating the 5-HT_7 receptor in mediating this phase resetting[47]. Concurrent exposure to light during the perfusion of 8-OH-DPAT abolished the phase advances, while co-perfusion of the SCN with TTX, which blocks in vivo serotonin release, does not suppress these 8-OH-DPAT-induced phase advances. This latter report is the first to directly assign a possible role for 5-HT_7 receptors in the regulation of circadian rhythms using a selective 5-HT_7 receptor antagonist. Future experiments with other recently developed selective antagonists should reveal whether 5-HT_7 receptors play a role in circadian rhythm regulation and constitute a possible target for treatment of jet lag or sleep disorders.

1.5.3 Depression

Preliminary evidence has implicated a possible role for the 5-HT_7 receptor in depression[207,208]. Impairment of the efficiency of rhythm maintenance or rhythm desynchronization has been suggested by many to lead to mental fatigue and depression[68,165,202]. The timing and the structure of circadian rhythms in physiological, behavioral, and endocrinological functions seem to be abnormal in depression, [31,44]. Chronic treatment of rats with antidepressants can produce functional effects in the SCN in a pharmacological manner consistent with activation of the 5-HT_7 receptor: it induces significant functional *Fos* immunoreactivity in the SCN, which indicates neuronal activation. Chronic administration of *Fos* inducing agents and the selective serotonin reuptake inhibitor fluoxetine produces a neuroadaptive down-regulation of the 5-HT_7 receptor in the hypothalamus[154,208]. Thus, these studies provide further evidence to support the role of the 5-HT_7 receptor in the mechanism of antidepressant action and in the regulation of circadian rhythms controlled by the SCN.

Both the brain serotonin system and the hypothalamic-pituary-adrenocortical axis (HPA) are implicated in depressive illness[25,124]. Under normal conditions, circulating glucocorticoids exert a negative feedback on the HPA system through specific glucocorticoid receptors. In patients suffering from severe depression, this feedback is defective resulting in an increased activity of the HPA axis and a premature escape from the cortisol-suppressing action of dexamethasone[10,145]. Since adrenocortical synthesis blockers have been reported successfully to reverse resistant depression[71,139], up-regulation of 5-HT_7 mRNA in certain areas of the hippocampus by means of pharmacological adrenalectomy also suggests an implication of 5-HT_7 receptors in depression and

affective disorders[120,259]. Consistent with the previous observations, it was shown that dexamethasone decreases expression of the 5-HT_7 receptor gene in rat astrocytes and receptor-mediated adenylyl cyclase activity in the frontal cortex.[204]. Additionally, it was demonstrated that acute restraint stress results in an increase of 5-HT_7 receptor mRNA expression in rat hippocampus (CA2 and CA3 subregions), which suggests that acute, but not chronic stress regulates 5-HT_7 receptor mRNA expression in a manner that is likely to be independent of its glucocorticoid actions[258]. Within this context, it is interesting that results indicate that expression of the glucocorticoid receptor in the hippocampus is mediated through 5-HT_7 receptor activation[118].

Encapsulating, this implies that a failure in the glucocorticoid-induced down-regulation of 5-HT_7 receptors is involved in depression. Previously mentioned decreases in hypothalamic 5-HT_7 binding sites as a result of chronic antidepressant treatment are also in line with this hypothesis[208].

1.5.4 Affective behavior

The results of the early characterization of the pharmacological profile of the 5-HT_7 receptor with use of numerous typical and atypical antipsychotic drugs imply a possible role for the receptor subtype in the processes that give rise to disrupted affective behavior[189]. The high affinities of clozapine and related second generation antipsychotics in combination with the in vitro up-regulation of the expression of the 5-HT_7 receptor seem to confirm this hypothesis[263]. The localization of the 5-HT_7 receptor at the level of limbic structures also supports this theory[20,72,120]. Furthermore, levels of mRNA encoding for this receptor subtype in the dorsolateral prefrontal cortex appear to be reduced in schizophrenics[45]. These regional decreases may contribute to the overall serotonergic alterations that occur in the disorder, in part through their interactions with other neurotransmitter systems including glutamate and acetylcholine.

On the contrary, experiments with the selective 5-HT_7 receptor antagonist ***44b*** demonstrate that no antipshychotic action is to be expected after treatment of selective 5-HT_7 receptor antagonists[178]. Compared to ***35*** (risperidone), it showed no beneficial effect in a model for the negative symptoms, and brought a clear outcome in only one of the three models used for testing the effects on the positive symptoms of schizophrenia.

Summarizing, it can be stated that a functional role for the 5-HT_7 receptor in the pathophysiology of schizophrenia is doubtful. The effect of drugs that lack selectivity for this receptor subtype, which has led to this hypothesis, is now refuted by the use of selective compounds. The fact that many of the non-selective compounds also exhibit high affinity for the 5-HT_{2A} and the dopamine D_2 receptors that are thought to play a role in the treatment of the negative and positive symptoms respectively is very important to remember.

1.5.5 Cardiovascular and peripheral smooth muscle relaxation

Since its isolation form blood serum, serotonin was known to cause contraction of blood vessels[182,183,240]. However, the effects of ***1c*** in the cardiovascular system are complex because they consist of bradycardia or tachycardia, hypotension or hypertension, and vasodilatation or vasoconstriction[198]. As a result, its physiological and pathophysiological importance remains unclear.

The resemblance of the pharmacological profile of the 5-HT receptor mediating smooth muscle relaxation in canine coronary arteries with that of the new member of the 5-HT receptor family was an important finding described in one of the early reports on the involvement of the 5-HT_7 receptor in the regulation of blood pressure and blood flow[223]. The experiments were conducted in the presence of GR-127935 (***29***), the potent 5-HT_{1D} receptor antagonist that completely abolished the initial agonists induced vasoconstrictor phase of the responses[222,243]. Several drugs with high affinity for the relaxant 5-HT receptor subtype competitively antagonized the relaxant affects of ***1c***, ***1g*** and ***1j***. A series of ergolines with high affinity for the 5-HT_2 and the 5-HT_7 receptor, among which ***40***, also proved to block the 5-HT induced relaxation in the same tissues[33]. Based on the fact that the 5-HT_2 receptor was ruled out by the ineffectiveness of ***31*** and ***33*** to antagonize the relaxant effect in combination with the 100 fold higher potency of ***40*** despite its equal affinity for the 5-HT_2 receptor – thereby more resembling 5-HT_7 pharmacology – implies a relationship between the 5-HT_7 receptor and the relaxation of canine coronary arteries. On the other hand, in human coronary arteries, only low levels of mRNA encoding for the 5-HT_7 receptor were expressed, casting doubt on a role for this receptor subtype in coronary vasodilatation[158]. However, it must be stated that these results were obtained using human coronary arteries with intact endothelium in contrast with the previously reported studies on canine coronary arteries, and others have reported high levels of mRNA encoding for the 5-HT_7 receptor in the same human areas[9]. More evidence of the involvement of the 5-HT_7 receptor in smooth muscle relaxation within the cardiovascular system comes from experiments on cerebral arteries[227] and the external carotid bed of dogs[242,246], jugular veins of monkeys[125], pial veins and carotid arteriovenous anastomoses of pigs[41,99], and pulmonary arteries of rabbits[149]. Further pharmacological analysis revealed as well the association of the 5-HT_7 receptor with tachycardia[244] and the 5-HT induced late hypotensive response in vagosympathectomized cats via vascular smooth muscle relaxation[241]. Intravenous (i.v.) administration of serotonin produces a triphasic blood pressure response in rats[101]. This response consists of a short lasting depressor phase via the von Bezold-Jarisch reflex that is mediated by 5-HT_3 receptors, a pressor phase mediated by vascular 5-HT_{2A} receptors and a long lasting hypotensive phase[198]. After bilateral vagosympathectomy and 5-HT_2 receptor blockade using ritanserin, serotonin exclusively produces the late vasodepressor response, which has previously been ascribed to an action at vascular 5-HT_1-like receptors[198]. Evidence has now been accumulated that this effect is mediated by the 5-HT_7 receptor, also because activation of this

receptor causes an increase in cAMP, which is associated with vasodilatation and concomitant hypotensive responses[38,40,87,224].

Additionally, numerous reports have been published collecting evidence for 5-HT_7 receptor mediated responses in several peripheral tissues like the porcine myometrium[110-112] and oviduct[96], canine antral longitudinal muscle of the stomach[179], guinea pig ileum[24,82] and human colonic circular smooth muscle[180]. As a result of this latter observation, it has been suggested that the receptor could play a role in the pathophysiology of irritable bowel syndrome[36].

1.5.6 Migraine

Serotonin has been long implicated in the pathophysiology of migraine, although the precise mechanisms involved are still a matter of debate[137,205]. Traditionally, migraine is widely described as a vascular headache, which is based on the idea that diameter changes in extracranial, and most likely intracranial arteries are the cause of headache[92,93,199]. More recently, given the evidence that nitric oxide (NO) may be involved in migraine headaches, it was hypothesized that release of endogenous serotonin stimulates endothelial 5-HT_{2B} receptors in cerebral blood vessels to release NO[57,58,160,200]. If cerebrovascular vasodilatation does underlie the development of headache, as suggested by the vascular hypothesis of migraine, and 5-HT is involved in this process, it seems plausible that the 5-HT_7 receptor may be involved in the putative vasodilator effect induced by a massive discharge of 5-HT in the cerebral vasculature[199]. This discharge would arise from an increased activation of the dorsal raphe nucleus and locus coerulus resulting as a consequence of a hypothalamic dysfunction probably situated in the SCN. The increased amounts of serotonin would target specific 5-HT receptors similar to the 5-HT_7 type located in the smooth muscle cells of large conduit vessels to cause vasodilatation. Large wall distension would then lead to activation of trigeminal sensory nerves by antidromic stimulation causing the release of pro-inflammatory peptides, i.e. substance P and calcitonin gene related peptide (CGRP). The 5-HT_7 receptors located in the trigeminal fibers that neighbor cerebrovascular serotonergic terminals in meningeal vessels may in turn produce hyperalgesia and trigger/facilitate the release of neuropeptide at this level[228]. The observation that most of the migraine prophylactic drugs exhibit moderate to high affinity for the 5-HT_7 receptor (Table 1.5) and have been shown to antagonize functional 5-HT_7 receptors mediating vasodilatation in several vascular smooth muscle preparations also supports this theory[33,125,223-225,239,242]. Their therapeutic efficacy could be explained by blockade of 5-HT_7 receptors mediating craniovascular vasodilatation and activation of perivascular trigeminal nerve endings. Additionally, high expression levels of 5-HT_7 receptor transcripts in porcine cerebral blood vessels and human meningeal tissues have been demonstrated[200,240]. Studies also demonstrate that the endothelium independent relaxant responses in major conduit cerebral arteries are produced by 5-HT, and that on basis of operational criteria the 5-HT_7 receptor is the receptor most likely to be involved[227].

1.5.7 Pain perception

Many studies have indicated that serotonin is involved in the modulation of the transmission of nociceptive messages in the CNS, and that 5-HT released from platelets or mast cells in peripheral tissues is a potent pro-inflammatory and noxious agent to cause hyperalgesia in both human and rodent[50,52,214,221]. It has been suggested as well that stimulation of 5-HT_{1A}-like receptors with "5-HT_{1A} selective ligands", specifically those on C-fiber neurons, produced this hyperalgesia through activation of the adenylyl cyclase system[220,221,235]. Since the 5-HT_7 receptor is positively coupled to adenylyl cyclase and this subtype also shows high affinity for the so-called "5-HT_{1A} selective ligand" 8-OH-DPAT, it is to be expected that this receptor subtype mediates 5-HT induced hyperalgesia. Furthermore, PCR studies demonstrated the presence of 5-HT_7 receptor mRNA in both rat and human dorsal root ganglia (DRG), and that unilateral injection of complete Frend's adjuvant (CFA) into the rat hind paw resulted in a significant increase in mRNA levels of this receptor subtype among others[174,175,255]. This raises the possibility that peripheral inflammation increases the biosynthesis of some 5-HT receptor subtypes in primary sensory neurons.

1.5.8 Hypothermia

It has been recognized that systemic administration of serotonin causes a hypothermic response in rodents[215,252,257]. Nonetheless, the serotonergic receptor subtype(s) causing this effect remains ambiguous. Early evidence suggested the involvement of the 5-HT_1 receptor subtype[257], while further investigations also indicated a possible role for 5-HT_2 receptors[215]. However, this latter hypothesis was rebutted by a study showing that the 5-HT_{2A} receptor most likely was not involved in the regulation of body temperature[151]. Additionally, the capacity of 8-OH-DPAT to induce hypothermia supported the early theory that this was mediated through activation of the 5-HT_{1A} receptor[85]. When it became clear that 8-OH-DPAT was not interacting selectively with this receptor subtype, but also showed considerable affinity for the 5-HT_7 receptor, it was hypothesized that 5-HT mediated thermoregulation could be the result of interactions with this novel receptor subtype. In fact, this idea was supported by the results obtained with the selective 5-HT_7 receptor antagonist ***44c***, which was capable of blocking the hypothermic response in guinea pigs caused by 5-CT, while pindolol and GR-125743 –antagonists with selectivity for 5-$HT_{1A/1B}$ and 5-$HT_{1B/1D}$ receptors respectively– appeared to be ineffective[72]. Using mice lacking 5-HT_7 receptors, further evidence was acquired that hypothermia is mediated by this subtype, while 5-HT and 5-CT failed to induce hypothermia in these 5-HT_7 receptor knock-out animals[79]. However, the fact that oleamide was equally effective in inducing hypothermia in 5-HT_7 receptor knock-out mice as in wild-type mice suggests involvement of another independent mechanism. Interference of cannabinoid receptors cannot be excluded[27,140,184].

1.5.9 Depolarization and ion-channels

Several publications report a linkage between the 5-HT_7 receptor and membrane depolarization. By comparison to other GPCRs, 5-HT_7 receptors may modulate the activity of numerous ion channels, as well as greatly influence intracellular biochemistry, which could have major consequences for synaptic function and the output of neuronal circuits. In this respect, it has been shown that this receptor subtype can inhibit the pharmacologically isolated slow calcium activated potassium channel in hippocampal CA3 pyramidal neurons[7]. Since disinhibition of CA3 neuronal networks occurs during epileptic states, or during repetitive activity in septal or hippocampal GABAergic inputs during learning behavior, it is relevant to study the exact mechanism and physiological role of the 5-HT_7 receptor in this perspective[233,236]. It turned out that GABA receptor antagonists induce low frequency bursting activity in CA3 hippocampal slices that was influenced by 5-CT and 8-OH-DPAT[64]. SB-269970 (***44c***) was able to reverse the 5-HT agonist induced increase in bursting activity. The accompanied shortening of the burst event wave form and a reduction in the after-hyperpolarization (AHP) following each bursting event were both inhibited by ***44c*** as well. Mechanistically, the 5-HT_7 receptor mediated inhibition of the slow AHP appeared to occur via direct inhibition of a Ca^{2+}-activated K^+ channel. Others suggest that this receptor attenuates the AHP and enhances the after-depolarization (ADP) through activation of protein kinase A (PKA), and the attenuation of AHP through the 5-HT_7 receptors in enhanced under raised Ca^{2+} levels by activation of protein kinase C (PKC)[97]. In the anterodorsal nucleus of the thalamus, the 5-HT_7 receptor is identified to mediate depolarization of cell membranes by increasing the nonselective hyperpolarization-activated cation current I_h through a cAMP-dependent, PKA-independent mechanism[26].

Ca^{2+} influx through T-type Ca^{2+} channels and increase in adenylyl cyclase activity in rat glomerulosa cells is associated with activation of the 5-HT_7 receptor as well[122]. These results were obtained as a result of the observation that the secretion of aldosterone from the rat adrenal gland by stimulation of serotonin on glomerulosa cells was also mediated through this receptor subtype[32]. Noteworthy, this was the first report describing the occurrence of 5-HT_7 receptors in endocrine glands and demonstrating their involvement in the control of hormonal secretions, a characteristic that has been demonstrated by others as well with regard to steroidogenesis via adenylyl cyclase activation by 5-HT_7 receptors in human granulosa cells[69].

1.5.10 Summary

From the previous sections it becomes evident that the 5-HT_7 receptor could play a role in a variety of pharmacological processes. However, the ground for these implications is based in more than one case on investigations performed without selective ligands. Despite this deficiency, using indirect pharmacology and modern molecular biology techniques, substantial evidence has accumulated that this novel receptor subtype plays a significant role in many of the suggested

processes and disorders. With the more selective 5-HT_7 receptor antagonist now available, future experiments will further demystify the involvement of this receptor subtype. Additionally, this will contribute to the characterization of the isoforms and their unexplored pharmacological differences.

1.6 Aim of this thesis

The objective of the present study is to contribute to the further characterization of the 5-HT_7 receptor by means of medicinal chemistry techniques. The design and synthesis of novel ligands that show affinity for this serotonin receptor subtype will gain further insight in the molecular interactions between ligand and receptor, based on the structure-activity relationships. Using a combination of modern molecular modeling techniques –pharmacophore determination by ligand alignment, 3-dimensional quantitative structure activity relationship determination (3D-QSAR), comparative molecular field analysis (CoMFA), and homology modeling of the transmembrane domains of the receptor – we aim to develop pharmacophore models for 5-HT_7 receptor agonism and inverse agonism, which may be helpful to future design of novel ligands. The design of a model of the 7 transmembrane domains of the 5-HT_7 receptor should provide us with relevant information revealing the boundaries of the binding site of the receptor. By means of sophisticated docking procedures of both agonists and inverse agonists into this receptor model, we hope to identify the relevant molecular interactions between ligands and the amino acid residues at the binding site. These molecular interactions might lead to a better understanding of the mechanism of activation of the 5-HT_7 receptor and provide useful information for the development of target specific ligands.

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