Journal of Medicinal Chemistry

Volume 46, Number 14 July 3, 2003

Perspective

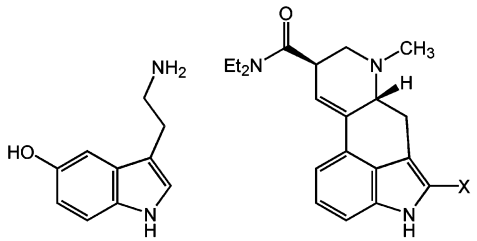
Higher-End Serotonin Receptors: 5-HT$_5$, 5-HT$_6$, and 5-HT$_7$

Richard A. Glennon[†]

Department of Medicinal Chemistry, School of Pharmacy, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, Virginia 23298-0540

Received January 17, 2003

Serotonin (5-hydroxytryptamine, 5-HT; **1**) is a major neurotransmitter that is believed to produce many of its effects via interaction at seven populations of 5-HT receptors, 5-HT$_1$ to 5-HT$_7$, and the serotonin transporter (SERT). Serotonin was discovered in the late 1940s, and the first 5-HT receptors were identified in the periphery shortly thereafter. It was in the 1970s that 5-HT binding sites were definitively identified in the brain, and in 1979 two populations of receptors, 5-HT$_1$ and 5-HT$_2$ receptors, were proposed (reviewed in ref 1). Over the ensuing decade or so, by use of techniques such as radioligand binding to brain homogenates, autoradiography, and molecular biology, seven families of 5-HT receptors were described.[2] Some families consist of multiple subpopulations, excluding splice variants and species homologues, and at least 14 different types of 5-HT receptors have been reported. Nearly all these 5-HT receptors now have been cloned and expressed (see Kroeze et al.[3] for a recent review of those 5-HT receptors cloned to date). Work on 5-HT receptors has led to the introduction of various agents currently in clinical use, or now in clinical trials, here and abroad. Among some of the first were the anxiolytic agent buspirone (5-HT$_{1A}$ partial agonist), the antimigraine agent sumatriptan (5-HT$_{1D/1F}$ agonist), the antipsychotic agent risperidone (5-HT$_2$/D$_2$ antagonist), and the antiemetic agent ondansetron (5-HT$_3$ antagonist). Three of the newest populations of 5-HT receptors are the 5-HT$_5$, 5-HT$_6$, and 5-HT$_7$ receptors. Given the enormous effort devoted to 5-HT$_1$−5-HT$_4$ receptors, various selective agonists and/or antagonists have been developed, and significant progress has been made toward elucidating their functional properties. In contrast, 5-HT$_5$−5-HT$_7$ receptors have been less extensively investigated and much less is known about them. Because these receptors could be involved in, for example, schizophrenia, convulsive disorders, memory, cognition, sensory processing, circadian rhythm, brain development, and cardiovascular function, they would seem to represent potential, yet hitherto unexploited, targets for drug development. Owing to the paucity of information on these latter receptor populations, it was not so long ago that they were collectively referred to as "orphan serotonin receptors". But with efforts made in the past few years, this situation is rapidly changing.



1

2 X = -H
3 X = -Br
4 X = -I

5-HT$_5$ Receptors

5-HT$_5$ receptors, although now more than a decade old, have been referred to as one of the "orphan" serotonin receptors because so little is known about them.[2] Serotonin receptors are classified on the basis of operational, structural, and transductional criteria.[4] The transductional mechanisms associated with 5-HT$_5$

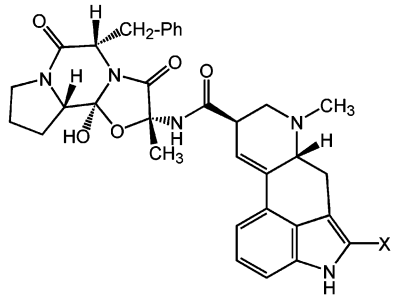
[†] Phone: 804-828-8487. Fax: 804-828-7404. E-mail: glennon@hsc.vcu.edu.

10.1021/jm030030n CCC: $25.00
Published on Web 06/11/2003

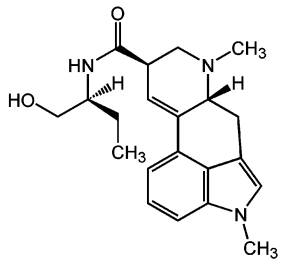
receptors are still in question.[2,5] Furthermore, because 5-HT_5 receptors have not yet been demonstrated to "function" in native systems, they are most correctly referred to by the use of a lower-case designator (i.e., 5-ht_5 receptors).[2,5] Mouse 5-HT_5 receptors were first identified in 1992;[6] a related receptor protein was identified shortly thereafter.[7] These were termed 5-HT_{5A} and 5-HT_{5B}, respectively.[7] Since then, rat 5-HT_{5A} and 5-HT_{5B} receptors have been identified,[8,9] as have human 5-HT_{5A} receptors.[10] The mouse, rat, and human 5-HT_{5A} receptors comprise 357 amino acid residues, and the human receptor gene is located on chromosome 7.[5] A human 5-HT_{5B} gene, isolated from a human genomic library, does not encode a functional protein because its coding sequence is interrupted by stop codons.[11] Hydropathy analysis of the predicted amino acid sequence of 5-HT_{5A} receptors suggests that they belong to the 7-transmembrane G-protein-coupled receptor superfamily; however, as mentioned above, a second messenger system has yet to be unequivocally defined.[5] 5-HT_{5A} receptors have been detected in human forebrain, cerebellum, and spinal cord. The structure and distribution of 5-HT_{5A} and 5-HT_{5B} receptors, and investigations of possible second messenger systems, have been recently reviewed.[2,5]

5-HT_5 Receptor Function. In part because of a lack of selective agonists or antagonists, the function of 5-HT_5 receptors is unknown. Early on, it was shown that there exists significant similarity between 5-HT_{5A} receptors and 5-HT_{1D} receptors,[6] leading to speculation that some actions ascribed to the latter population (i.e., feeding behavior, anxiety, motor control) might actually involve the newer 5-HT_{5A} receptors.[6,10] Indeed, 5-HT_{5A} receptor knock-out mice displayed increased locomotor activity and exploratory behavior.[11] Because 5-HT_{1D} receptors are also believed to be involved in migraine, the role of 5-HT_5 receptors in dural neurogenic inflammation has been studied.[12] It has been speculated that mutations in the gene encoding 5-HT_{5A} receptors might be detrimental to brain development.[7] On the basis of their localization, for example, it has been speculated that 5-HT_{5A} receptors might be involved in certain central nervous system (CNS) disorders, including Alzheimer's disease,[13] and that carotid 5-HT_{5A} receptors might play a fundamental role in arterial chemoreception.[14] Some of the psychotropic effects of LSD could be modulated by 5-HT_{5A} receptors.[15] In fact, it has been suggested that genetic variation in human 5-HT_{5A} receptors might be involved in susceptibility to psychosis or depression,[16] but a subsequent study failed to identify any relationship between 5-HT_{5A} receptor gene polymorphism and the pathogenesis of effective disorders in humans.[17] Clearly, 5-HT_5 pharmacology could benefit from the availability of selective ligands.

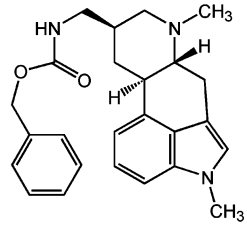
5-HT_{5A} Receptor: Ligand Binding Profile. To date, there are no agents, agonists, or antagonists that show selectivity for 5-HT_5 receptors. Nevertheless, given the appropriate assay system, nonselective agents can still provide a wealth of information. A number of "standard" nonselective serotonergic agents bind at 5-HT_5 receptors, but most studies have been conducted using rodent receptors, and there is actually surprisingly little binding data available for the human form of the receptor. The NIMH Psychoactive Drug Screening Program database (PDSP database)[18] provides binding data on a variety of agents at rodent 5-HT_{5A} and 5-HT_{5B} receptors. Unless a specific reference is provided, binding data discussed herein are from the PDSP database. Also, because human 5-HT_{5B} receptors have not been identified, the data discussed will all be 5-HT_{5A} data. Serotonin itself binds at 5-HT_{5A} receptors with modest affinity ($K_i \approx 125-250$ nM); Rees et al.[10] reported a K_i value of 126 nM for human 5-HT_{5A} receptors. The ergoline lysergic acid diethylamide (LSD; **2**) and 2-halogenated derivatives of LSD such as 2-bromo LSD (BOL; **3**) and 2-iodo LSD (**4**) bind with high affinity ($K_i < 10$ nM), and [^{125}I]-2-iodo LSD is commonly employed to radiolabel 5-HT_{5A} receptors. Other ergolines also bind at 5-HT_{5A} receptors including ergotamine (**5**) ($K_i \approx 4-40$ nM), methysergide (**6**) ($K_i \approx 60-200$ nM), and metergoline (**7**) ($K_i \approx 600-1000$ nM).



5



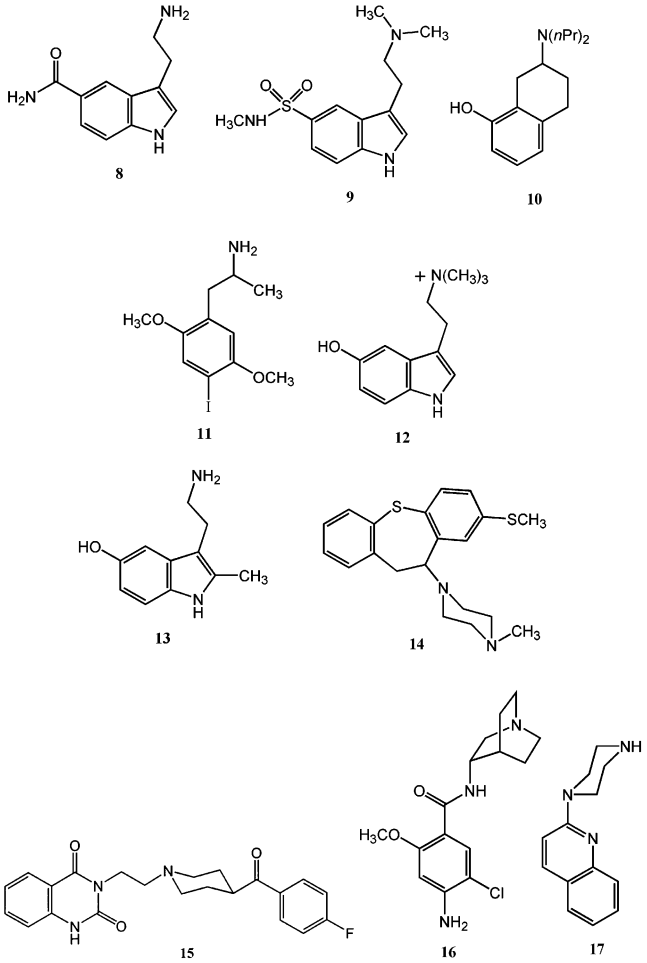
6



7

Typical 5-HT "agonists" examined at human 5-HT_{5A} receptors include 5-carboxamidotryptamine (5-CT; **8**) ($K_i = 20$ nM) and the 5-HT_{1D} agonist sumatriptan (**9**) ($K_i \approx 5000$ nM).[10] The low affinity of the latter agent clearly distinguishes 5-HT_{5A} from 5-HT_{1D} receptors. Although not very common, tritiated 5-carboxamidotryptamine ([^{3}H]-5-CT) has been used on occasion to label 5-HT_{5A} receptors. The 5-HT_{1A} agonist 2-(*N*,*N*-di-*n*-propylamino)-8-hydroxytetralin (8-OH DPAT; **10**) binds at rodent 5-HT_{5A} receptors ($K_i \approx 400-1000$ nM) and at human 5-HT_{5A} receptors ($K_i = 2500$ nM).[10] However, higher affinities were reported for 8-OH DPAT (and also for 5-HT) for rodent 5-HT_{5A} receptors when [^{3}H]-5-CT was used as radioligand. The 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; **11**) binds with low affinity at 5-HT_{5A} receptors ($K_i > 1000$ nM), as does the 5-HT_3 agonist 5-HTQ (**12**) and 2-methyl-5-HT (**13**) ($K_i > 10\,000$ nM).[19] The serotonin "antagonist" methiothepin (**14**) ($K_i \approx 1$ nM) binds at 5-HT_{5A} receptors.[10] Interestingly, methiothepin displays lower affinity for rodent receptors ($K_i \approx 100$ nM) than for human 5-HT_{5A} receptors and is one of the only agents to show such selectivity. The 5-HT_2 antagonist ketanserin (**15**) ($K_i \approx 20\,000$ nM) and the 5-HT_3 antagonist zacopride (**16**) (K_i

≈ 16 000 nM) bind at human 5-HT$_{5A}$ receptors with very low affinity.[10]



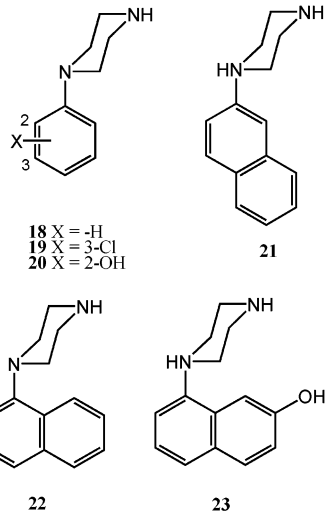
Agents such as quipazine (**17**) and *m*-chlorophenylpiperazine (*m*CPP; **19**) are relatively nonselective but widely used serotonergic agents. They behave as agonists at certain 5-HT receptor populations and as antagonists at others. Quipazine (K_i > 10 000 nM) and *m*CPP (K_i = 8700 nM) bind with low affinity at murine 5-HT$_{5A}$ receptors.[19] Interestingly, the nonheterocyclic counterpart of quipazine (i.e., 2-naphthylpiperazine, 2-NP; **21**) (K_i = 2100 nM) binds with higher affinity, and a positional isomer of 2-NP (i.e., 1-naphthylpiperazine, 1-NP; **22**) (K_i = 40 nM) binds with yet higher affinity.[19] One of the highest affinity 5-HT$_{5A}$ agents identified thus far is the 7-hydroxy analogue of 1-NP (i.e., 7-hydroxy-1-NP; **23**) (K_i = 3 nM).[19] Although a selectivity profile was not reported for **23**, this agent is very likely to be nonselective for 5-HT$_{5A}$ receptors based on the high affinity of its *O*-methyl ether for several other populations of 5-HT receptors. 1-Phenylpiperazine (**18**) lacks affinity for murine 5-HT$_{5A}$ receptors; nevertheless, certain other piperazine derivatives including 1-(2-hydroxyphenyl)piperazine (**20**; K_i = 215 nM) bind with enhanced affinity,[19] suggesting that piperazines might be worthwhile templates for further development of 5-HT$_5$ ligands. Some binding data comparisons are shown in Table 1.

Almost no efforts to develop a 5-HT$_{5A}$-selective agent have been reported. Screening of a large number of compounds at murine 5-HT$_{5A}$ receptors identified γ-carboline **24** (K_i = 5300 nM) as a modest-affinity ligand.[20] Although the affinity of **24** was not particularly promising, it was of interest because, with the exception of

Table 1. Comparison of the Receptor Affinities of Some Common Serotonergic Ligands at 5-HT$_{5A}$, 5-HT$_6$, and 5-HT$_7$ Receptors

	receptor affinity (approximate K_i, nM)		
	5-HT$_{5A}$[a]	5-HT$_6$[b]	5-HT$_7$[c]
5-HT (**1**)	126	65	8.1
2-Br LSD (**3**)	2	17	30
methysergide (**6**)	63	180	83
metergoline (**7**)	630	400	6.4
5-CT (**8**)	20	720	0.9
sumatriptan (**9**)	5000	2600	950
8-OH DPAT (**10**)	2500	>10000	466
2-methyl 5-HT (**13**)	>10000	400	1258
methiothepin (**14**)	1	0.4	3.7
ketanserin (**15**)	20000	2800	1334
quipazine (**17**)	>10000	3600	3033
*m*CPP (**19**)	8700	2300	304
1-NP (**22**)	40	104	83
spiperone (**27**)	63000	1595	110[d]
clozapine (**28**)	1000	9.5	40
mianserin (**29**)		55	8010

[a] Data from Rees et al.,[10] using human 5-HT$_{5A}$ receptors labeled with [^{3}H]-5-CT, except for **3**,[18] **6** (mouse, [^{125}I]-2-iodo LSD),[7] and **13**, **17**, **19**, **22** (mouse, [^{3}H]LSD).[19] [b] Data from Kohen et al.,[24] using human 5-HT$_6$ receptors with [^{3}H]LSD as radioligand except for **13**,[23] **3**, **19**, and **22**[22] where [^{125}I]-2-iodo LSD was used as radioligand. [c] Data from Bard et al.,[60] using human 5-HT$_7$ receptors labeled with [^{3}H]-5-HT, except for compounds **13**, **17**, **19**, **28**, and **29**, which represent average K_i values (where possible) from the PDSP database.[18] [d] The 5-HT$_7$ K_i value for spiperone represents human data. Spiperone (**27**) binds with somewhat higher affinity at rat (K_i = 10−20 nM) and mouse (K_i = 63 nM) 5-HT$_7$ receptors.[18]



5-HT$_2$ receptors, γ-carbolines are not known to bind at most populations of 5-HT receptors. Subsequent studies resulted in compound **25** (K_i = 13 nM), which binds only with about a 3-fold selectivity for 5-HT$_{5A}$ versus 5-HT$_{2A}$ receptors.[20] Later studies examined the affinity of various γ-carbolines at human 5-HT$_{5A}$ receptors, and for a series of 15 carboline derivatives, there was a significant correlation (r = 0.962) between mouse and human 5-HT$_{5A}$ K_i values.[21] For a limited series of standard agents, the pharmacology of murine and human 5-HT$_{5A}$ receptors has been suggested to be rather similar.[10] The above-mentioned correlation supports this contention.

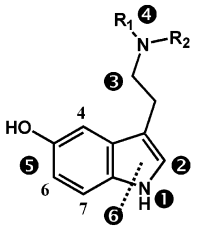
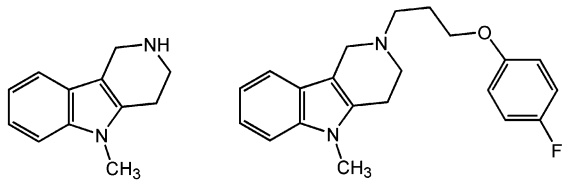


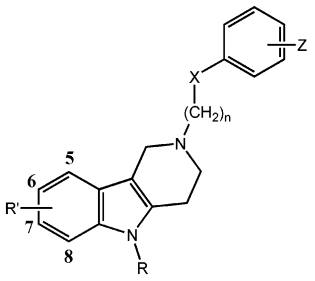
Figure 1. Influence of structural modification on the binding of 5-HT and simple tryptamines at 5-HT_{5A} receptors. (1) Small alkyl substituents at the N_1-position dramatically decrease affinity. (2) A methyl group is not tolerated at the 2-position. (3) An ergoline-like extended conformation is preferred for binding, introduction of an α-methyl group decreases affinity, and shortening or lengthening of the side chain by a methylene group decreases affinity. (4) *N*-Monomethyl substitution is tolerated whereas *N*,*N*-dimethyl substitution decreases affinity somewhat. Larger alkyl substituents further decrease affinity, and quaternization abolishes affinity. (5) Replacement of the 5-hydroxy group of 5-HT with −H decreases affinity by 7-fold, whereas O-methylation doubles affinity. With respect to methoxy substitution, both the 4-OCH_3 and 6-OCH_3 derivatives lack affinity, and a 5-OCH_3 group can be replaced with 5-SCH_3 with little effect on affinity. (6) Reduction to an indoline and conversion to an indane abolish affinity.[19]

5-HT_{5A}: Structure−Affinity Relationships (SAFIR). Very little has been published on the structure−affinity relationships of 5-HT_{5A} ligands. Although a variety of agents has been examined at rodent 5-HT_{5A} receptors,[16] the structural diversity of these agents is such that it is nearly impossible to relate them to one another, making formulation of SAFIR virtually impossible. One of the very few SAFIR studies reported to date is that by Teitler et al.,[19] which investigated the binding of various tryptamine derivatives. Although there is no reason to believe that tryptamine derivatives all bind at 5-HT_{5A} receptors in a common manner, there is greater likelihood of such than there is among a group of structurally diverse and seemingly unrelated agents. The general conclusions of the study (summarized in Figure 1) were that (a) tryptamines bind with an ergoline-like conformation, (b) the 5-hydroxy group of 5-HT contributes to binding (its replacement by a hydrogen atom reduced affinity by approximately 10-fold), (c) quaternization of the terminal amine of 5-HT abolishes affinity, (d) lengthening or shortening of the tryptamine side chain reduces affinity, (e) 5-methoxy substitution is optimal compared to other aryl methoxy derivatives, (f) 2-methylation is not tolerated, and (g) alkyl substitution on the terminal amine results in progressively reduced affinity, with primary amines being optimal for high affinity.[19] A QSAR (i.e., CoMFA) investigation of these tryptamines was also reported.[19]

The structure−affinity relationships of various carbolines have been examined. Random screening identified γ-carboline **24** (K_i = 5300 nM) as binding at mouse 5-HT_{5A} receptors,[20] and tetrahydro-β-carboline (K_i = 2470) binds with similar affinity.[19] A systematic SAFIR investigation of **24** led to structures such as **26**.[20] Variation of substituents in the benzenoid ring (R′), and on the indolic nitrogen atom (i.e., R), revealed that affinity was optimal when R′ = H and R = $-CH_3$. Enhanced affinity was associated with analogues that contained at N_3 an ω-(phenyl)alkyl substituent. Although substituent X could be a methylene group or a carbonyl group, highest affinity was associated with X = O. Substituents on the terminal phenyl ring had little influence on 5-HT_{5A} receptor affinity, and ring opening of the piperidine ring and deletion of the indole moiety resulted in loss of affinity. The highest affinity compound emerging from this series was **25** (K_i = 13 nM).[20] Subsequent studies have shown that **25** (K_i = 55 nM) binds with severalfold lower affinity at human 5-HT_{5A} receptors than at mouse 5-HT_{5A} receptors.[21] Related β-carboline derivatives were also examined but displayed lower affinities than their corresponding γ-carboline counterparts.



24 **25**



26

Because γ-carbolines are known to bind at 5-HT_2 receptors, derivatives of **26** were examined at 5-HT_{2A} and 5-HT_{2C} receptors. All analogues displayed appreciable affinity, with some binding with >50-fold selectivity for 5-HT_{2A} versus 5-HT_{5A} receptors. However, structural modification has resulted in compounds with severalfold selectivity for 5-HT_{5A} receptors. Compound **25** was reported to bind only with about 3-fold and 5-fold selectivity versus 5-HT_{2A} and 5-HT_{2C} receptors, respectively.[20] Even though these compounds cannot be termed selective for 5-HT_{5A} receptors, they represent the first lead structures for the development of such agents.

5-HT_{5A} Receptors: Summary. Human 5-HT_{5A} receptors represent somewhat of an enigma in that neither a function nor a second messenger system has yet been identified. Whereas other populations of 5-HT receptors have generated widespread interest, 5-HT_5 receptors remain a curiosity and have attracted little attention. No selective agents exist for 5-HT_5 receptors, and very little has been published on SAFIR, particularly human SAFIR. As has been the case with many other populations of 5-HT receptors, interest in 5-HT_5 receptors will only follow the discovery of a therapeutically useful function for the receptors or the development of selective ligands.

5-HT_6 Receptors

Less than 5 years ago, 5-HT_6 receptors were as obscure as 5-HT_5 receptors are today. The advent of selective agents has greatly benefited 5-HT_6 studies, and this field of research has recently exploded. Rat[22] and mouse[23] 5-HT_6 receptors were described in 1993 and 1994, respectively, and human 5-HT_6 receptors were

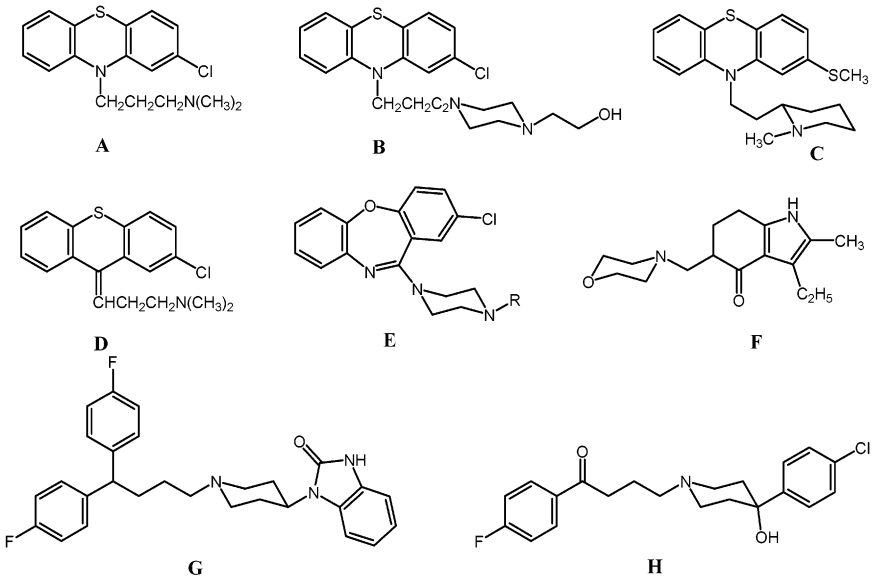
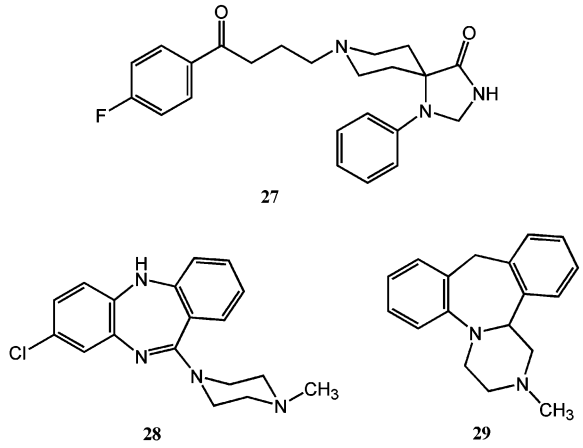


Figure 2. Structures of representative classical antipsychotics found to bind at 5-HT_6 and/or 5-HT_7 serotonin receptors: the phenothiazines chlorpromazine (A), perphenazine (B), and thioridazine (C), the thioxanthene chlorprothixene (D), the tricyclic antipsychotics amoxapine (E, R = −H) and loxapine (E, R = $-CH_3$), the tetrahydroindolylmethylmorpholine molindone (F), the diphenylbutylpiperidine pimozide (G), and the butyrophenone haloperidol (H). See text for discussion and K_i values.

first reported in 1996.[24] The deduced structure of 5-HT_6 receptors corresponds to a seven-transmembrane-spanning G-protein-coupled arrangement that is positively coupled to an adenylate cyclase second messenger system. Human 5-HT_6 receptors, a 440 amino acid polypeptide, display 89% overall sequence homology with the rat receptors and are nearly exclusively localized in the central nervous system.[24] Tritiated 5-HT (**1**), tritiated LSD (**2**), and [^{125}I]-2-iodo LSD have been used to radiolabel 5-HT_6 receptors. 5-HT binds with moderately high affinity (K_i = 50−150 nM).

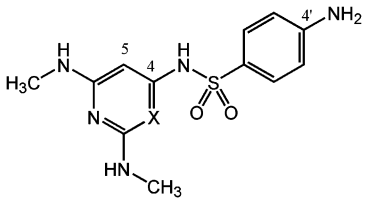
5-HT_6 Receptors: Ligand Binding Profile. The first paper describing 5-HT_6 receptors demonstrated that tricyclic antipsychotic agents and some antidepressants bind with significant affinity.[22] A related investigation examined antipsychotics in greater detail and found that representative members of several classes of antipsychotics bind with high affinity. Examples include (see Figure 2 for structures) the phenothiazine chlorpromazine (K_i = 4 nM), the thioxanthene chlorprothixene (K_i = 3 nM), the diphenylbutylpiperidine pimozide (K_i = 71 nM), and heterocyclic antipsychotic agent loxapine (K_i = 15 nM).[25] Not all antipsychotics displayed high affinity. Butyrophenones such as spiperone (**27**) (K_i = 1595) and haloperidol (K_i > 5000 nM) (Figure 2) showed much lower affinity. In contrast, the atypical antipsychotic agent clozapine (**28**) (K_i = 4 nM) displayed high affinity.[25] These results led to suggestions that 5-HT_6 receptors might play a role in certain types of psychoses and that they might represent significant targets for the atypical antipsychotics in particular. Similar results were obtained with human 5-HT_6 receptors.[24] It was such findings that spurred initial interest in this receptor population. A comparison of the binding properties of some standard serotonergic agents across the three different receptor populations discussed in this review is shown in Table 1.

Until selective ligands were developed, exploration of 5-HT_6 pharmacology was largely dependent on the use of nonselective agents. Because 5-HT_6 receptors are positively coupled to adenylate cyclase, stimulation of adenylate cyclase was employed to identify potential agonists, and antagonists could be identified by their ability to attenuate 5-HT-induced cAMP formation. In the first such investigation of this kind, certain nonselective agents were characterized as 5-HT_6 agonists with the following rank order of potency: 5-methoxytryptamine > 5-HT (**1**) > 2-methyl 5-HT (**13**) > 5-CT (**8**) ≫ α-methyltryptamine.[23] (+)-LSD (**2**) and 2-bromo LSD (**3**) were characterized as partial agonists, and methiothepin (**14**), clozapine (**28**), and mianserin (**29**) behaved as antagonists.[23] Agents that lacked significant agonist or antagonist action included 8-OH DPAT (**10**), DOI (**11**), ketanserin (**15**), *m*CPP (**19**), and spiperone (**27**).[23]

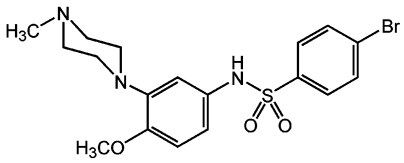


5-HT_6 Selective Agents. The above-mentioned agents were useful for investigating the pharmacology of 5-HT_6 systems in preparations where other 5-HT receptors were absent (e.g., cAMP assays); however, owing to their lack of selectivity, they were of limited value for most other pharmacological studies. It was not until 1998 that the first 5-HT_6-selective antagonist was described, and this prompted others to quickly report their efforts

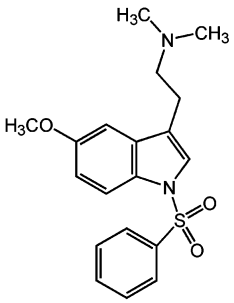
in this area. Sleight et al.[26] identified the bisaryl sulfonamides **30** (**a**, X = N, Ro 04-6790; **b**, X = CH, Ro 63-0563) as very selective 5-HT_6 antagonists. Shortly thereafter, compounds **31**[27] and MS-245 (**32**)[28,29] were reported. Interestingly, although they represented independent discoveries, all three were identified by random screening methods and all three possess a sulfonamide moiety. The first nonsulfonamide showing 5-HT_6 antagonist character was 5-methoxy-2-phenyl-*N*,*N*-dimethyltryptamine (PMDT; **33**).[29] These will be discussed below in further detail.



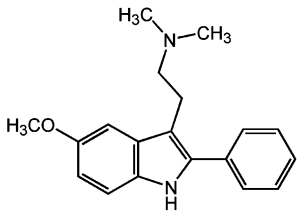
30a X = N
30b X = CH



31



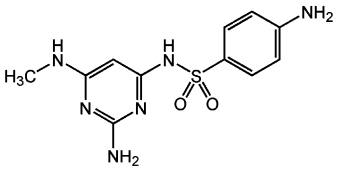
32



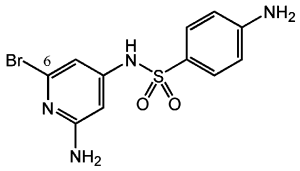
33

Ro 04-6790 (**30a**) and Ro 63-0563 (**30b**) (h5-HT_6 K_i = 55 and 12 nM, respectively) were described by Hoffman-La Roche as being very selective 5-HT_6 antagonists.[26] Compound **30a** displayed >100-fold selectivity for [^{3}H]-LSD-labeled 5-HT_6 receptors over other 5-HT receptors and lacked measurable affinity for 23 other receptor populations, whereas **30b** showed >100-fold selectivity over a total of 69 other receptor/binding sites. Both behaved as competitive antagonists of 5-HT-induced cAMP accumulation (pA_2 = 6.75 and 7.10, respectively) and lacked agonist or inverse agonist actions.

One problem associated with these antagonists was their low penetration of the CNS. A structure−activity study was undertaken to identify related analogues that would be more brain-penetrant.[30] The 4′-amino group of **30a** seems critical for binding and its omission or replacement by halogen or an alkyl group caused a loss in potency. Methyl or ethyl substitution (mono- or disubstitution) of the 2-amino substituent afforded compounds that retained (within 10-fold) the affinity of **30a** for 5-HT_6 receptors; alkyl substituents possessing more than three carbon atoms (cyclic or acyclic) resulted in reduced affinities.[30] Interestingly, demethylation of **30a** to the primary amine **34** ($K_i \approx$ 1000 nM) resulted in a dramatic decrease in affinity. In contrast, the 6-amino group did not seem to be quite so important. For example, replacement of the amine substituent of **30b** with a bromo group (**35**; K_i = 55 nM) had little effect on affinity.[30]

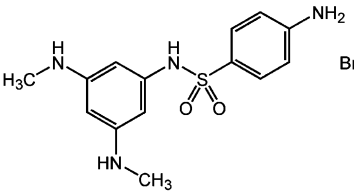


34

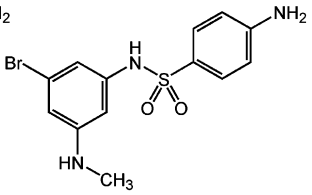


35

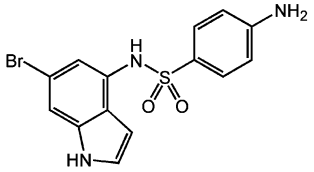
The necessity of the aryl nitrogen atoms was also examined. From a comparison of **30b** with **30a**, it is apparent that both ring nitrogen atoms are not required for binding. Removal of both nitrogen atoms reduced affinity considerably (**36**; $K_i \approx$ 200 nM); however, compound **37** (K_i = 20 nM) essentially retained the affinity of **35**.[30] Cyclic versions of **37** were explored, and compounds such as **38** (K_i = 50 nM) were shown to retain affinity. These studies provided a number of compounds with varying lipophilicities, and it was concluded that compounds with log *P* values between 2 and 3.5 were most favorably suited for brain penetration.[30] Nevertheless, none of the analogues examined possessed significantly higher affinity than **30b**.



36



37

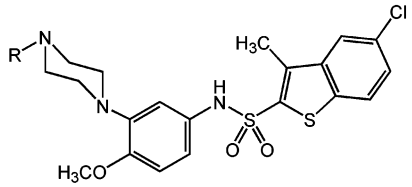


38

Tritiated Ro 63-0563 (**30b**) was introduced in 1998 as the first radioligand selective for 5-HT_6 receptors.[31] In rat brain, [^{3}H]Ro 63-0563 was displaced by a variety of ligands with the following rank order of affinities: methiothepin (**14**) > LSD (**2**) > clozapine (**28**) ≈ Ro 63-0563 (**30b**) > ergotamine (**5**) ~ Ro 04-6790 (**30a**) > 5-HT (**1**) > metergoline (**7**) ~ mianserin (**29**) > methysergide (**6**).[31]

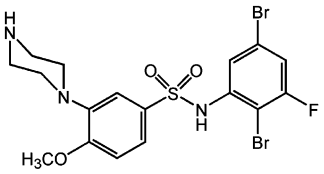
Compound **31** was identified by (at the time) Smith-Kline Beecham via high-throughput screening. It displayed high affinity (K_i = 5 nM) for 5-HT_6 receptors and >50-fold selectivity over 10 other 5-HT receptors and no measurable affinity for 50 other receptor/binding sites.[27] Compound **31** was a pure antagonist of cAMP accumulation (pK_b = 7.8). It was moderately brain-penetrant (25%) but subject to rapid blood clearance resulting in low bioavailability. An ensuing structure−activity study identified **39** (K_i = 0.6 nM; pK_b = 8.5) as

an antagonist that was 18% brain-penetrant and that displayed low blood clearance but was rapidly metabolically demethylated. The demethylated metabolite (**40**; $K_i = 1$ nM) retained antagonist activity. Compound **40** (SB-271046) displayed >200-fold selectivity over 50 other receptors, and although less brain-penetrant (10%), it showed excellent (>80%) oral bioavailability.[27]

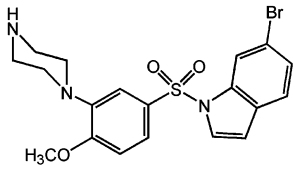


39 R = CH_3
40 R = H

Subsequent studies by this group showed that "reverse sulfonamides" retain 5-HT_6 affinity.[32] Structure−affinity investigations ultimately led to SB-357134 (**41**; $K_i = 3$ nM). Compound **41** was an antagonist with a low clearance rate and excellent oral bioavailability. One of the highest affinity compounds identified during this investigation was **42** ($K_i = 0.3$ nM).[32]

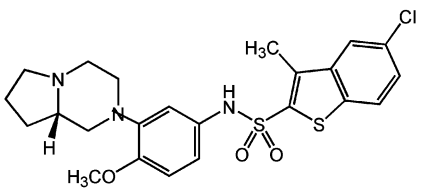


41

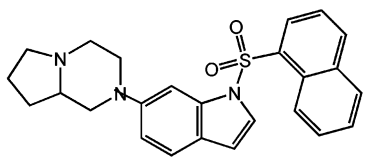


42

In attempts to develop even more brain-penetrant analogues of SB-271046 (**40**), Bromidge et al.,[33] explored a series of constrained bicyclic systems. The *S*-isomer of hexahydropyrrolo[1,2-*a*]pyrazine derivative **43** displayed an affinity ($K_i = 0.8$ nM) identical to its *R*-enantiomer. **43** was a 5-HT_6 antagonist ($pK_b = 7.7$; cAMP assay) and was slightly more brain-penetrant (24%) than **40** but was subject to increased blood clearance. The corresponding racemic octahydropyrido-[1,2-*a*]pyrazine ($K_i = 5$ nM) was shown to bind with somewhat reduced affinity. Slassi et al.[34] at Allelix Biopharmaceuticals independently reported a series of structurally related compounds. Both isomers of **44** were



43



44

shown to bind with comparably high affinity ($K_i \approx 0.7$ nM).[34]

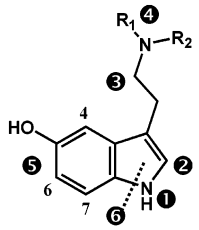
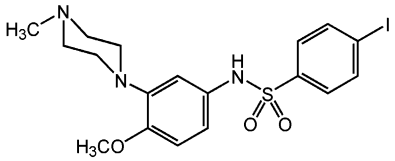


Figure 3. Influence of structural modification on the binding of 5-HT and simple tryptamines at 5-HT_6 receptors. (1) Small alkyl substituents (e.g., methyl, ethyl, *n*-propyl) at the N_1-position dramatically decrease affinity but N_1-benzyl and N_1-SO_2Ph enhance affinity. (2) A methyl group is tolerated at the 2-position, as are an ethyl group and a phenyl group. (3) An ergoline-like extended conformation seems preferred for the binding of N_1-unsubstituted tryptamines, and introduction of an α-methyl group decreases affinity. (4) *N*-Monomethyl and *N*,*N*-dimethyl substitution slightly increase affinity, *N*,*N*-diethyl substitution is tolerated, *N*,*N*-di-*n*-propyl substitution decreases affinity, and quaternization dramatically decreases affinity. (5) Replacement of the 5-hydroxy group of 5-HT with −H halves affinity, whereas the *O*-methyl and *O*-benzyl ethers retain affinity. Of the four possible methoxy derivatives, affinity decreases as 5 > 4 > 6 > 7, and replacement of 5-OCH_3 by 5-SCH_3 enhances affinity. (6) Conversion to an indane abolishes affinity.[29,36] N_1-Benzenesulfonyltryptamine derivatives do not necessarily follow this same SAFIR.

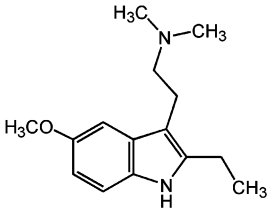
SB-258585 (**45**) was identified as a high-affinity 5-HT_6 ligand, and this led to the development of [^{125}I]SB-258585.[35] [^{125}I]SB-258585 labeled 5-HT_6 sites in human, rat, and porcine brain, and the rank order of affinities for [^{125}I]SB-258585-labeled sites in human caudate putamen membranes was the following: SB-271046 (**40**) > SB-258585 (**45**) > methiothepin (**14**) > clozapine (**28**) > 5-methoxytryptamine > 5-HT (**1**) > Ro 04-6790 (**30a**) > mianserin (**29**) > 5-CT (**8**).[35]



45

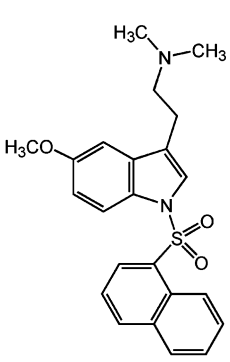
In 1999, Glennon et al.[36] undertook a structure−affinity investigation of the binding of tryptamine derivatives at human 5-HT_6 receptors. A summary of the results is shown in Figure 3. One of the more interesting findings to emerge from this study was that 5-HT_6 receptors tolerated an indolic 2-methyl substituent. Until that time, 2-methyl 5-HT (**13**) had been considered a 5-HT_3-selective agonist. Compound **13** was shown to bind at 5-HT_6 receptors ($K_i = 46$ nM) with 20 times the affinity it displayed for 5-HT_3 receptors. Furthermore, the affinity of **13** at 5-HT_6 receptors was nearly twice that of 5-HT itself.[36] Taking advantage of the finding that 5-HT_3 receptors do not tolerate a 5-methoxy group on the tryptamine ring, **13** was O-methylated to afford a more selective agent (i.e., 5-methoxy-2-methyltryptamine; 5-HT_6 $K_i = 80$ nM and 5-HT_3 K_i > 10 000 nM). To further increase lipophilicity and metabolic stability, two *N*-methyl groups were incorporated to provide the first compound specifically designed to be a 5-HT_6-selective agent (i.e., 5-methoxy-2-methyl-*N*,*N*-dimethyltryptamine or MMDT; 5-HT_6 $K_i = 60$

nM).[29] Subsequent studies showed that other substituents were tolerated at the 2-position, including an ethyl group (EMDT, **46**; K_i = 16 nM) and a phenyl group (PMDT, **33**; K_i = 20 nM). EMDT behaved as a 5-HT$_6$ agonist (cAMP assay) with potency comparable to that of 5-HT. In contrast, PMDT was without agonist action but displayed antagonist character.[29]

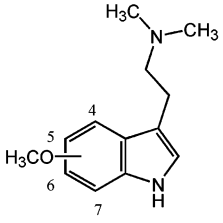


46

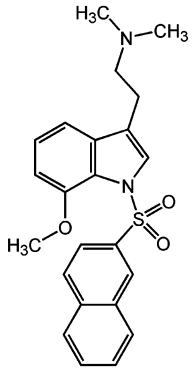
During the synthesis of some of these analogues, N_1-benzenesulfonyl derivatives were prepared to protect the indolic nitrogen atom as the 2-position substituents were being elaborated. Random screening of one of these intermediates, BS/5-OMe DMT (MS-245, **32**; K_i = 2.3 nM), revealed that it displayed high affinity for 5-HT$_6$ receptors.[29] Subsequently, **32** was shown to act as a 5-HT$_6$ antagonist (pA_2 = 8.88).[28] Substituents on the benzenesulfonyl aromatic ring had relatively little impact on affinity, with the highest affinity analogue being **47** (K_i = 0.9 nM).[28] An earlier structure−affinity investigation had shown that a 5-methoxy substituent was optimal for the binding of *N*,*N*-dimethyltryptamines; that is, the K_i values for the variously substituted methoxy derivatives **48** are the following: 5-OMe (16 nM) > 4-OMe (154 nM) > 6-OMe (8000 nM) > 7-OMe (19 600 nM).[36] However, incorporation of an N_1-benzenesulfonyl substituent influenced affinity in a different manner. For example, with an N_1-(2,5-dimethoxybenzene)sulfonyl group present, the affinity of the 5-methoxy analogue was enhanced by 12-fold, that of the 4-methoxy analogue by 20-fold, and that of the 6- and 7-methoxy analogues by 840- and 107-fold, respectively.[28] Indeed, although 7-methoxy-*N*,*N*-dimethyltryptamine (K_i = 19 600 nM) binds with very low affinity, its N_1-(2-naphthalenesulfonyl) analogue **49** (with a K_i of 5 nM) binds with nearly a 4000-fold enhanced affinity. It was results such as these that prompted the suggestion that simple tryptamines and their corresponding N_1-sulfonyl analogues do not bind at 5-HT$_6$ receptors in a comparable fashion.



47

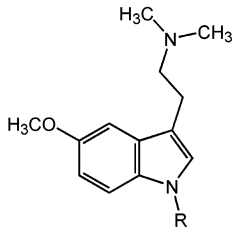


48

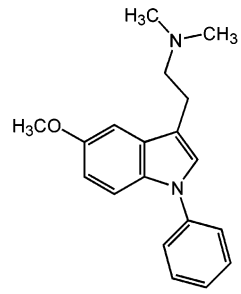


49

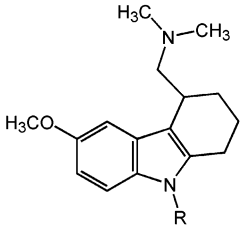
The benzenesulfonyl group is not a requirement for the binding of these compounds at 5-HT$_6$ receptors.[37] Replacement of the sulfonyl group of **32** (K_i = 2.1 nM) with a carbonyl group (**50a**, K_i = 18 nM) or a methylene group (**50b**, K_i = 6.5 nM) resulted in retention of affinity. Indeed, elimination of the sulfonyl group, providing the corresponding N_1-phenyl analogue **51** (K_i = 33 nM), was also tolerated. Nevertheless, the presence of the sulfonyl group seemed optimal.



50a R = -C(O)-Ph
50b R = -CH_2-Ph



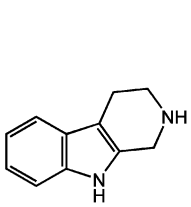
51



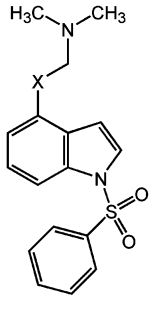
52 R = -H
53 R = -$S(O)_2$Ph
54 R = -CH_2Ph

The conformationally constrained tryptamine **52** (K_i = 168 nM) binds with modest affinity at 5-HT$_6$ receptors.[36] Here too, incorporation of an N_1-benzenesulfonyl substituent resulted in a dramatic increase in affinity (**53**; K_i = 1.5 nM).[37] Interestingly, unlike what was seen in the tryptamine series, the N_1-benzyl analogue **54** (K_i = 136 nM) did not display enhanced affinity.[37]

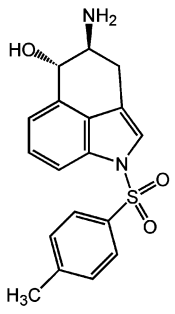
It was argued, on the basis of the high affinity of ergolines at 5-HT$_6$ receptors and the low affinity of 1,2,3,4-tetrahydro-β-carboline (**55**, K_i > 5000 nM), that tryptamines likely bind in an ergoline-like extended conformation.[36] Russell et al.[38] at Merck have demonstrated that compounds such as **56a** (K_i = 7.9 nM), **56b** (K_i = 1.5 nM), and **57** (K_i = 7.2 nM) bind at 5-HT$_6$



55



56a X = -CH_2CH_2-
56b X = -CH_2-

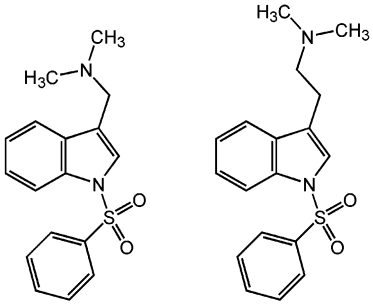


57

receptors and seemingly support this concept. It might be noted that the *O*-methyl ether of **57** displayed substantially reduced affinity (K_i = 130 nM) and the affinity of **57** was, in part, ascribed to a possible

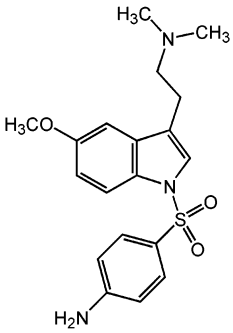
hydrogen-bonding interaction between a receptor feature and the benz[*c,d*]indole hydroxyl group.

If the benzenesulfonyltryptamines bind via an ergoline-like conformation, shortening of the tryptamine aminoethyl chain might be reasonably expected to result in reduced affinity. This was not found to be the case. Gramine analogue **58** (1-benzenesulfonylgramine, BSG; K_i = 3.1 nM) displayed an affinity comparable to tryptamine counterpart **59** (K_i = 4.1 nM).[39] Furthermore, **58** was found to be a 5-HT$_6$ antagonist (IC_{50} = 0.8 μM; cAMP assay).[39] This led to speculation that an amine at this position might not even be required for binding. Removal of the dimethylamino portion of **58** would most likely result in a compound with little aqueous solubility. A study was conducted to determine where a solubilizing group might be appended to the molecule, and **60** (K_i = 2 nM) and **61** (K_i = 0.8 nM) provided reasonable leads.[39] The high affinities of both compounds indicated that a 4′-amino group was tolerated by the receptors. The amino function was also incorporated into **58** (i.e., **62**; K_i = 6.9 nM) with little effect on affinity, and then the dimethylamino portion of **62** was eliminated to provide **63**. Compound **63**

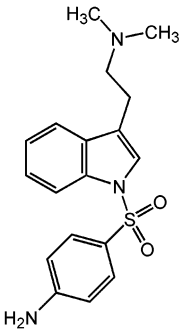


58

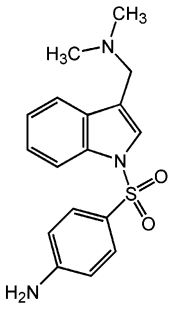
59



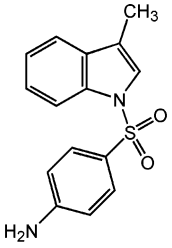
60



61



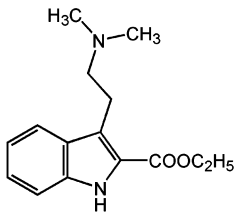
62



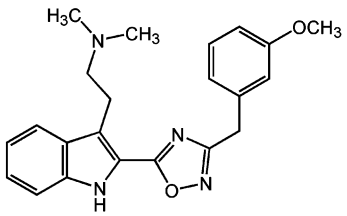
63

(aminoBSS; K_i = 12 nM) displayed slightly reduced affinity but retained antagonist activity (IC_{50} = 0.3 μM, pA_2 = 7.0; cAMP assay).[39] Although there is no assurance that each of the compounds is binding in a comparable fashion, it would appear that the "tryptamine side chain" is not required for binding at 5-HT$_6$ receptors when an indolic N_1-benzenesulfonyl group is present. Furthermore, these results support the idea that not all tryptamines bind in an ergoline-like conformation. It may be that simple tryptamines (perhaps those with agonist action) bind in an extended ergoline-like conformation, but when a benzenesulfonyl substituent is present at the indole N_1-position, the tryptamines might bind in an altogether different manner.

PMDT (**33**) binds at 5-HT$_6$ receptors with high affinity (K_i = 20 nM) and possesses a reasonably selective binding profile but binds only with about 25-fold selectivity for 5-HT$_6$ versus 5-HT$_{2A}$ receptors.[29] Russell et al.[38] demonstrated that a carboethoxy group (i.e., **64**; K_i = 20 nM) is also tolerated at the indole 2-position but that the resulting compound now displayed only about 6-fold selectivity over 5-HT$_2$ receptors.[38] Nevertheless, like **33**, **64** behaved as a 5-HT$_6$ antagonist (cAMP assay). Further investigation led to the oxadiazolyl derivative **65** (5-HT$_6$ K_i = 1.3 nM).[38]

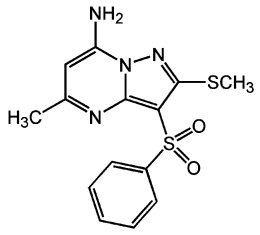


64

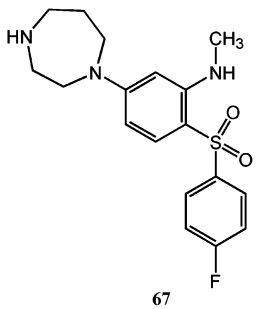


65

Slassi et al.[34] recently reviewed the patent literature and have described a number of novel 5-HT$_6$ agents. Nearly all of the agents presented are sulfonamides or derivatives of tryptamines with the exception of several sulfones recently revealed by Hoffmann-LaRoche (i.e., **66**) and Pharmacia-Upjohn (i.e., **67**; K_i = 1.4 nM).[34]



66



67

5-HT$_6$ Receptor Function. The high affinity of certain antipsychotic agents initially suggested that 5-HT$_6$ receptors might be involved in schizophrenia. Atypical antipsychotics, in particular, display high affinity at these receptors (vide supra). In addition, the tritiated atypical antipsychotic agent [^{3}H]clozapine was shown to label two populations of receptors in rat brain and one population was thought to represent 5-HT$_6$ receptors.[40] Vogt et al.[41] performed a systematic mutation scan of the coding region of the 5-HT$_6$ receptor gene of 137 individuals (including schizophrenic and depressed patients) and concluded that the gene might be

involved in bipolar affective disorder. In contrast, SB-271046 (**40**) was examined in several animal models of schizophrenia, and with a clear positive outcome in only one of the models and no beneficial effect in a model for negative symptoms of schizophrenia, the conclusion reached was that SB-271046 (**40**) was not expected to possess antipsychotic efficacy.[42] By use of [^{125}I]SB-258585 ([^{125}I]**45**), 5-HT$_6$ receptor binding site densities were measured in dorsolateral prefrontal cortex of 20 patients with chronic schizophrenia and compared with 17 control subjects.[43] No differences were seen between the two groups. Also, [^{125}I]SB-258585 binding site densities were unaffected in the frontal cortex and in the striatum of rats following a 2-week administration of the typical antipsychotic drugs haloperidol, chlorpromazine, or the atypical antipsychotics clozapine (**28**), olanzapine (**68**), and risperidone (**69**). Although [^{125}I]-SB-258585 might be a useful radioligand for studying human brain 5-HT$_6$ receptors and although it was shown that their distribution is broadly similar to that of the rodent, the lack of effect of schizophrenia or antipsychotic drug administration on [^{125}I]SB-258585 binding indicated that altered receptor density did not contribute to involvement that the 5-HT$_6$ receptors might have in the disease or its treatment.[43] A role for 5-HT$_6$ receptors in mental disorders has yet to be firmly established but is nevertheless still being pursued. For example, a recently reported novel approach is to investigate the actions of antipsychotic agents on constitutively active mutant 5-HT$_6$ receptors.[44]

Prior to the identification of 5-HT$_6$-selective agents, Bourson et al.[45] demonstrated that intracerebroventricular administration of antisense oligonuceotides to reduce expression of functional 5-HT$_6$ receptors produced in rats a syndrome of yawning, stretching, and chewing. Because the effect could be antagonized by atropine, involvement of a cholinergic mechanism was implicated. With the advent of the first 5-HT$_6$ antagonist, Sleight et al.[26] demonstrated that Ro 04-6790 (**30a**) was capable of inducing this same effect. Owing to a relationship between cholinergic function and cognition, this led to speculation that 5-HT$_6$ receptors might be involved in memory and cognitive dysfunction.[46,47] In addition, because antisense oligonucleotide pretreatment and Ro 04-6790 administration both led to decreased food intake by rats, it was suggested that 5-HT$_6$ receptors might be involved in the regulation of feeding.[46] However, it has been argued that the yawning, stretching, and chewing syndrome following Ro 04-6790 administration might be merely a response to an intraperitoneal-induced irritant because SB-271046 (**40**) and MS-245 (**32**) failed to produce this effect.[48] Furthermore, Russell and Dias[48] have questioned the postulate that 5-HT$_6$ antagonists increase cholinergic transmission. Despite the mechanistic disagreement, there is evidence for the involvement of 5-HT$_6$ receptors in learning and memory. When a water maze was used with rats as subjects, SB-271046 (**40**) and SB-357134 (**41**) had no effect on learning per se but produced significant improvement in retention of a previously learned task.[47] Furthermore, SB-271046 (**40**) produced no changes in basal levels of dopamine, norepinephrine, or serotonin in rat striatum, frontal cortex, hippocampus, or nucleus accumbens and no changes in excitatory amino acid levels in striatum or nucleus accumbens but increased by severalfold extracellular glutamate levels in frontal cortex and dorsal hippocampus, leading to the conclusion that selective enhancement of excitatory neurotransmission by SB-271046 supports a role for 5-HT$_6$ receptor antagonists in the treatment of cognitive disorders and memory dysfunction.[49]

SB-357134 (**41**), a 5-HT$_6$ antagonist shown to bind at [^{125}I]SB-258585- and [^{3}H]LSD-labeled human 5-HT$_6$ receptors with high affinity ($K_i \approx 3$ nM), was not only active in enhancing memory and learning in a rat water maze but produced a potent and dose-dependent increase in seizure threshold (rat maximal electroseizure threshold) following oral administration, suggesting possible therapeutic utility in convulsive disorders.[50] These findings are consistent with an earlier finding that SB-271046 (**40**) and Ro 04-6790 (**30a**) possess anticonvulsant activity.[51]

EMDT (**46**) was reported to be the first 5-HT$_6$ agonist; however, follow-up studies with this agent have not yet appeared in the literature. There also exist 5-HT$_6$ partial agonists. **56**, for example, produced 49% of the effect of 5-HT (**1**) in a cAMP assay, whereas the *des*-methoxy analogue of MS-245 (**32**) (i.e., **59**) produced 59% of the effect of 5-HT.[48] Much remains to be done with these agents.

5-HT$_6$ Receptors: Summary. Human and other mammalian 5-HT$_6$ receptors have been cloned. Various useful 5-HT$_6$-selective antagonists have been identified, and a fairly selective 5-HT$_6$ agonist has been reported. Newer agents continue to be developed in attempts to improve pharmacokinetic and pharmacodynamic properties. Now that some tools are available, attention is focusing more and more on the function of 5-HT$_6$ receptors. Overall, there is some evidence to suggest that 5-HT$_6$ receptors could be involved in psychosis. There is still more evidence that these receptors are involved in cognition and learning and additional evidence that they might have a role in convulsive disorders and appetite control. Although additional studies are certainly warranted, particularly with some of the newer 5-HT$_6$ antagonists that are more brain-penetrant than the earlier agents, the future of 5-HT$_6$ receptor ligands as potential therapeutic agents is quite exciting.

5-HT$_7$ Receptors

Like 5-HT$_5$ receptors, 5-HT$_7$ receptors were once considered "orphan" 5-HT receptors. Then in 1997 Eglen et al.[52] published a paper aptly entitled "The 5-HT$_7$ Receptor: Orphan Found" that reviewed the significant advances made up to that time and helped this population shed its ignominy. Vanhoenacker et al.[53] published a more extensive review shortly thereafter. However, it has only been in the past several years that 5-HT$_7$ selective antagonists have been identified and the first lead structures for 5-HT$_7$ agonists have been described.

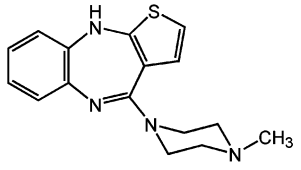
Rat[54−56] and mouse[57] 5-HT$_7$ receptors were first cloned in 1993 by several groups of investigators. Guinea pig,[58] porcine,[59] and human[60] 5-HT$_7$ receptors also have been cloned. Hydropathy analysis was indicative of seven hydrophobic domains suggestive of a transmembrane-spanning helical structure consistent with that of other G-protein-coupled receptors. 5-HT$_7$ receptors, like 5-HT$_6$ receptors, were found to be posi-

tively coupled to an adenylate cyclase second messenger system. The greatest abundance of 5-HT$_7$ receptors is found in the brain (thalamus, hypothalamus, limbic, and cortical regions), but 5-HT$_7$ receptors are also found in the periphery (e.g., spleen, kidney, heart, coronary artery, gastrointestinal tract).

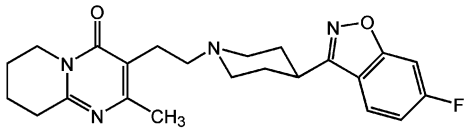
Almost immediately after the discovery of h5-HT$_7$ receptors, splice variants of 5-HT$_7$ receptors were identified. The human 5-HT$_7$ receptor gene contains at least two introns and encodes a 445 amino acid 5-HT receptor. A truncated splice variation of the human 5-HT$_7$ receptor was isolated from a human placental cDNA library, and the long and short forms were termed h5-HT$_{(7a)}$ and h5-HT$_{(7b)}$, respectively.[61] Others independently obtained similar results.[62] Further investigation revealed that alternative splicing in rat and human tissue results in four 5-HT$_7$ receptor isoforms that vary in structure with respect to the length of their C-terminal chains. In rat, the isoforms are termed 5-HT$_{(7a)}$, 5-HT$_{(7b)}$, and 5-HT$_{(7c)}$. Two of the isoforms are homologous in rat and human (5-HT$_{(7a)}$ and 5-HT$_{(7b)}$); the third human isoform is termed 5-HT$_{(7d)}$. Of obvious importance are questions regarding the location, density, coupling mechanisms, and ligand selectivity of the different isoforms. Such factors could have a major impact on function, therapeutic possibilities, and drug design. In a series of investigations from several laboratories,[63−65] it was determined that alternative splicing of 5-HT$_7$ receptor mRNAs is quite different in rat and human, that the expression and relative distribution of the different isoforms can vary from tissue to tissue, but (although coupling efficiency might vary) that all are positively coupled to adenylate cyclase in a similar manner. Most importantly, it has been concluded that the three human splice variants are pharmacologically indistinguishable.[46] That is, length differences in the C-termini of 5-HT$_7$ receptors did not appear to influence agonist binding affinity, and the isoforms showed almost identical affinity for various other ligands.[53]

Another feature that complicates 5-HT$_7$ receptors is evidence for constitutive activity. Thomas et al.[66] examined the functional profile (cAMP assay) of cloned h5-HT$_{(7a)}$ receptors using a variety of agonist and antagonist ligands, and certain of these displayed partial inverse agonist effects. Krobert and Levy[67] later examined all three h5-HT$_7$ isoforms and compared their abilities to constitutively activate adenylate cyclase. Constitutive activity was significantly higher for the 5-HT$_{(7b)}$ splice variant than the h5-HT$_{(7a)}$ or h5-HT$_{(7d)}$ isoforms in stable cell lines. The serotonin antagonist methiothepin (**14**), for example, displayed inverse agonist effects, whereas other antagonists did not; nevertheless, the different isoforms displayed similar constitutive activity and inverse agonist properties.[67] This will be further discussed below.

5-HT$_7$ Receptor: Ligand Binding Profile. As with the 5-HT$_6$ receptors, certain antipsychotic agents and antidepressants bind with high affinity at rat 5-HT$_7$ receptors. Representative examples include (see Figure 2 for structures) the phenothiazine chlorpromazine (K_i = 21 nM), the thioxanthene chlorprothixene (K_i = 5.6 nM), the diphenylbutylpiperidine pimozide (K_i = 0.5 nM), and heterocyclic antipsychotic agent loxapine (K_i = 43 nM).[25] Unlike what was seen at 5-HT$_6$ receptors, some butyrophenones bind at 5-HT$_7$ receptors including haloperidol (K_i = 263 nM) (Figure 2) and spiperone (**27**; K_i = 9.9 nM).[25] Atypical antipsychotic agents such as olanzepine (**68**) (K_i = 104 nM), clozapine (**28**) (K_i = 6.3 nM), and risperidone (**69**) (K_i = 1.4 nM) also bind.[25] Others obtained similar results and further found that certain tricyclic antidepressants display affinity for 5-HT$_7$ receptors.[54,56,60] The high affinities of some of these agents reveal the structural diversity that can be accommodated by 5-HT$_7$ receptors.

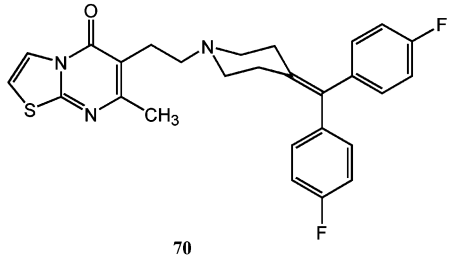


68

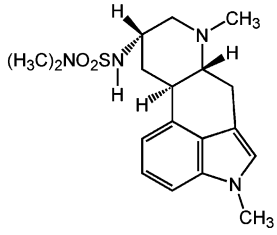


69

Standard serotonin "agonists" initially examined at 5-HT$_7$ receptors included 5-HT (**1**; $K_i \approx 1-10$ nM), 5-CT (**8**; $K_i \approx 0.1-0.9$ nM), and 8-OH DPAT (**10**; $K_i \approx 35-150$ nM).[54,56,60] Up until that time, 5-CT (**8**) had been used to identify "5-HT$_1$-like" receptors, and 8-OH DPAT (**10**) was considered a prototypical 5-HT$_{1A}$ agonist. Not unexpectedly, several ergolines displayed high affinity, LSD (**2**; K_i = 2 nM), methysergide (**6**; K_i = 12−80 nM), metergoline (**7**; K_i = 2−60 nM), as did several standard nonselective serotonin "antagonists" including methiothepin (**14**; K_i = 1−4 nM) and mianserin (**29**; $K_i \approx 50-100$ nM).[54,56,60] Interestingly, two antagonists thought to be reasonably selective (within the 5-HT family) for 5-HT$_2$ receptors were demonstrated to bind at h5-HT$_7$ receptors, ritanserin (**70**; K_i = 45 nM) and mesulergine (**71**; K_i = 18 nM),[60] and at rat 5-HT$_7$ receptors with



70



71

similarly high affinity (K_i = 15 and 21 nM, respectively),[54] and the 5-HT$_{1A}$/5-HT$_{2A}$ antagonist spiperone

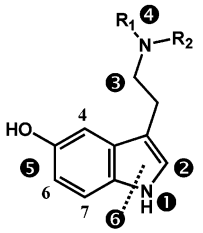
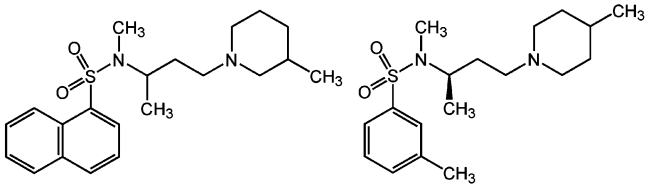


Figure 4. The influence of structural modification on the binding of 5-HT and simple tryptamines at 5-HT_7 receptors has not been systematically examined. By use of composite data from the PDSP database,[18] the following SAFIR are evident: 5-HT binds at 5-HT_7 receptors with high affinity ($K_i \approx 4$ nM). (1) N-methylation has no effect on affinity. (2) A methyl group is not tolerated at the 2-position (i.e., 2-methyl-5-methoxy-*N*,*N*-dimethyltryptamine; $K_i = 1260$ nM). (3) An ergoline-like extended conformation is probably preferred because ergolines bind with high affinity. (4) Amine substitution has not been investigated; however, N,N-dimethylation reduces the affinity of 5-HT by about 10-fold and 5-methoxy-*N*,*N*-dimethyltryptamine ($K_i \approx 30$ nM) binds with about 15-fold lower affinity than its corresponding primary amine, 5-methoxytryptamine ($K_i = 2$ nM). (5) Replacement of the 5-hydroxy group of 5-HT with −H reduces affinity by approximately 15-fold, O-methylation of 5-HT doubles affinity, O-benzylation of 5-HT decreases affinity by about 170-fold, and moving the methoxy group of 5-methoxytryptamine from the 5- to the 6-position reduces affinity by approximately 100-fold. Replacement of the 5-OH group of 5-HT with a carboxamido group has been reported to result in (i.e., 5-CT; **8**) an agent with reported K_i values ranging from <0.1 to about 1 nM. (6) Modifications of the pyrrole portion of the indole nucleus have not been investigated.

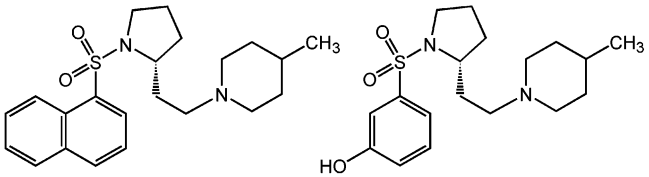
(**27**) (which also binds with even higher affinity at dopamine D_2 receptors) also displayed high affinity for rat[56] and human[60] 5-HT_7 receptors ($K_i = 20$ and 110 nM, respectively). KML-010, an analogue of spiperone where the triazaspirodecanone phenyl moiety of spiperone (**27**) is replaced by a methyl group (leading to an agent with greater selectivity for 5-HT_{2A} versus 5-HT_{2C}, 5-HT_{1A}, and dopamine D_2 receptors than spiperone), binds ($K_i = 140$ nM) with an affinity comparable to that of spiperone.[68] So, another of the interesting results to emerge from these early studies is that the selectivity of certain relatively "selective" agents (e.g., 8-OH DPAT, ritanserin, mesulergine, spiperone)—agents widely used in various pharmacological studies—was called into question. Table 1 provides additional information on the binding of various ligands at 5-HT_7 receptors, and Figure 4 summarizes known tryptamine SAFIR.

Progress toward the Development of 5-HT_7-Selective Agents. The first 5-HT_7-selective compound, **72**, was identified by high-throughput screening.[69] The compound possesses two chiral centers, and all four isomers were prepared and evaluated. The isomeric mixture binds with high affinity ($K_i \approx 65$ nM), but interestingly, none of the individual isomers displayed higher affinity than the mixture. Nevertheless, the *R*,*R*-isomer possessed the highest affinity ($K_i = 125$ nM). To reduce stereochemical considerations, the ring methyl group was moved to the piperidine 4-position. The resulting *R*-isomer ($K_i = 32$ nM) retained affinity. Structure−activity relationships for the aryl moiety were explored, and **73** (SB-258719; $K_i = 32$ nM) was identified for subsequent evaluation. SB-258719 (**73**), with >100-fold selectivity over other 5-HT receptors), antagonized 5-CT-stimulated adenylate cyclase accumulation ($pK_B = 7.0$) and acted as a competitive antagonist.[69]



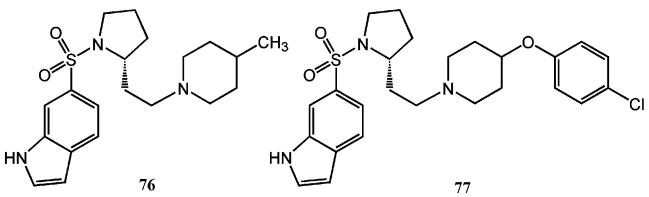
72 **73**

A subsequent investigation by the same group of investigators attempted to optimize the activity of **73**. Molecular modeling studies identified what was a low-energy conformer, and conformationally restricted analogues were prepared where the freely rotating side chain was incorporated into a piperidine or pyrrolidine ring system.[70] The *R*-isomer **74** ($K_i = 10$ nM) was found to bind with higher affinity than its *S*-enantiomer ($K_i \approx 400$ nM). Further exploration of SAR resulted in SB-269970 (**75**; $K_i = 1.3$ nM). SB-269970 (**75**) displayed an excellent selectivity profile with >250-fold selectivity over other 5-HT receptors except that it had some affinity ($K_i \approx 65$ nM) for 5-HT_{5A} receptors and behaved as a 5-HT_7 antagonist ($pK_B = 8.3$ cAMP assay).[70] Actually, both SB-258719 (**73**) and SB-269970 (**75**) produced small reductions in basal levels of adenylate cyclase activity and appeared to be inverse agonists.[66,70]



74 **75**

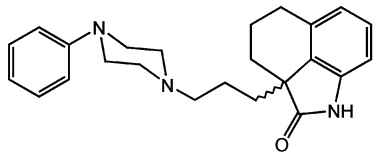
Studies with SB-269970 (**75**) were hampered by its extremely high in vivo blood clearance that was primarily attributed to the presence of the phenolic hydroxyl group, and a program was embarked to develop metabolically more stable bioisosteres. In essence, the hydroxyl group was replaced with a nitrogen atom that was tethered to the benzene ring in the form of various heterocyclic moieties to afford a series of indoles, indazoles, benzotriazoles, benzimidazoles, and benzoxazoles. The analogues displayed affinities ranging from $K_i \approx 2$ to $K_i \approx 165$ nM.[71] One of the higher affinity analogues was **76** ($K_i = 2.4$ nM), but **76** lacked bioavailability in the rat. Metabolism studies suggested that the piperidinylmethyl group likely undergoes hydroxylation in vivo. Further exploration of piperidine 4-position substituents identified **77** (SB-656104; $K_i = 2$ nM).[71] SB-656104 (or its hydrochloride salt SB-656104-A) antagonized 5-CT-mediated adenylate cyclase activity ($pA_2 = 8.1$), showed >100-fold selectivity over most other 5-HT



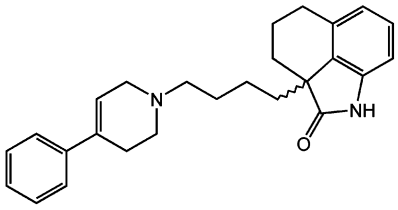
76 **77**

receptors (except 5-HT_{2A}, 30-fold; 5-HT_{2B}, 50-fold; 5-HT_{1D}, 12-fold), showed reduced affinity for 5-HT_{5A} receptors (K_i = 168 nM), and showed a greatly improved pharmacokinetic profile. The presence of the chlorophenoxy substituent also conferred oral bioavailability.[71]

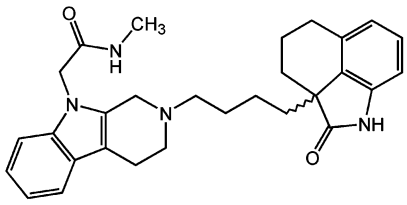
Shortly after the introduction of **72**, Kikuchi et al.[72] described a series of tetrahydrobenzindoles identified by high-throughput screening of a compound library. Compound **78**, for example, displayed high affinity for 5-HT_7 receptors (K_i = 5 nM) but also showed good affinity for 5-HT_2 receptors (K_i = 16 nM). To enhance selectivity, a range of modifications was explored, leading to DR4004 (**79**; 5-HT_7 K_i = 2 nM; 5-HT_2 K_i = 98 nM), a compromise between high affinity and improved selectivity.[72] DR4004 (**79**) did not stimulate basal adenylate cyclase activity on its own and acted as an antagonist. Because **79** displayed only about 50-fold selectivity for 5-HT_7 versus 5-HT_2 receptors, a structure−affinity investigation was undertaken to improve selectivity. Structural modification resulted in about 40 analogues with varying affinity and selectivity, of which **80** (DR4365) was found to be optimal (5-HT_7 K_i = 4 nM; 5-HT_2 K_i > 1000 nM).[73] DR4365 (**80**) acted as a 5-HT_7 antagonist (cAMP assay) with no evidence of inverse agonism.[73]



78

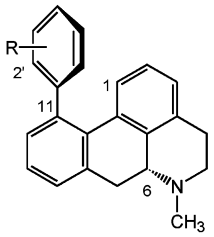


79

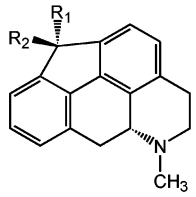


80

Various aporphine analogues are known to bind at dopamine and at 5-HT_{1A} receptors. Linnamen et al.[74] found that introduction of a C_{11}-phenyl substituent not only enhances the 5-HT_{1A} affinity of certain aporphines but also imparts affinity for 5-HT_7 receptors. Compound **81** (R = H), for example, binds both at 5-HT_{1A} receptors (K_i = 1.8 nM) and 5-HT_7 receptors (K_i = 9.78 nM). Various substituted-phenyl analogues were examined, and that bearing a 2′-cyano-6′-methyl group (i.e., **81** where R = 2-cyano-6-methyl) was shown to display some selectivity for 5-HT_7 receptors. The nonsymmetrically substituted phenyl derivatives display atropisomerism resulting from restricted rotation about the aporphine−phenyl bond. Hence, the 2′-cyano-6′-methyl derivative exists as 6a*R*,a*S* and 6a*R*,a*R* isomers, and the two isomers bind with different affinities (5-HT_7 K_i = 3.79 and 20.8 nM). The higher affinity isomer binds with 37-fold and 130-fold selectivity over 5-HT_{1A} and D_2 receptors, respectively, and behaved as 5-HT_7 antagonists (cAMP assay) without evidence of inverse agonist action.[74]



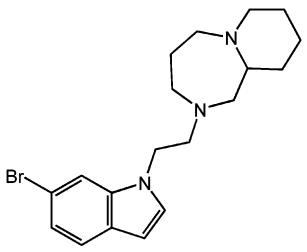
81



82

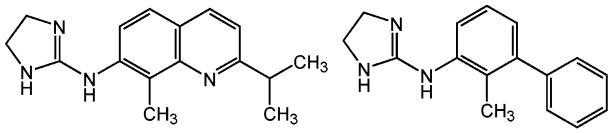
Certain aporphine analogues, 1,11-methyleneaporphines **82**, bind with high affinity at 5-HT_7 receptors. Compounds **82a** (R_1 = H, R_2 = NH_2) and **82b** (R_1 = NH_2, R_2 = H), K_i = 13.4 and 18.0 nM, respectively, bind with about 10- to 20-fold selectivity over 5-HT_{1A} receptors, whereas **82c** (R_1 = CH_3, R_2 = OCH_3) and **82d** (R_1 = OCH_3, R_2 = CH_3), K_i = 1.1 nM in both cases, bind with higher affinity at 5-HT_7 receptors but with about similar (i.e., ca. 17-fold) selectivity.[75] The four derivatives of **82** display varying affinities for dopamine D_2 receptors (K_i = 261, 2250, 7.1, and 71 nM, respectively). Preliminary studies indicated that **82b** and **82d** are 5-HT_7 antagonists and that **82b** also binds at 5-HT_{2A} (K_i = 469 nM), α_1-adrenergic (K_i = 373 nM), and α_2-adrenergic (K_i = 92.8 nM) receptors.[75] Although the aporphine analogues represent interesting lead structures and might also find utility in pharmacophoric investigations because of their stereochemically defined and conformationally constrained nature, the current compounds probably lack sufficient selectivity to be useful as pharmacological tools.

NPS Pharmaceuticals made the serendipitous discovery that certain isotryptamines (i.e., indoles bearing an 2-aminoethyl moiety at the indolic N_1-position) bind at 5-HT_7 receptors. About 60 isotryptamines were synthesized where substituents on the indole ring were explored and the terminal amine was varied. Emerging from this investigation was bicyclohomopiperazine **83** (K_i = 3 nM).[76] The bromo substituent of **83** was replaced by chloro (K_i = 10 nM) and trifluoromethyl (K_i = 7 nM) with little effect on affinity.[76] Although **83** displayed reasonable selectivity for 5-HT_7 receptors, stereochemistry was not defined and functional data were not reported.



83

Pfizer has very recently reported the first 5-HT_7 agonist (i.e., **84**), another ligand identified by high-throughput screening.[77] Manipulation of the structure resulted in **85** (K_i = 16 nM). **85** also displayed affinity for α_1- and α_2-adrenergic receptors (K_i = 200 and 19 nM, respectively).[77] Electron-donating and electron-withdrawing substituents were introduced to the appended phenyl ring of **85**, but none appreciably improved affinity or selectivity. Replacement of the phenyl group with a thiophene or pyridine ring had relatively little effect on 5-HT_7 affinity, but both enhanced adrenergic affinity. The project apparently has been terminated because of the profound blood pressure and heart rate changes (probably consequences of adrenergic actions) seen after administration of **85** to animals. Although these imidazolines represent novel 5-HT_7 agonists (cAMP assay), binding profiles were not obtained, likely because of the observed adrenergic character of the compounds evaluated. Although **85** can hardly be called a 5-HT_7-selective agonist, it currently represents one of the only lead structures for the development of such agents.



84 **85**

The first QSAR (i.e., CoMFA) study on 5-HT_7 ligands recently has been reported and might aid in the future design of novel 5-HT_7 agents.[78]

5-HT_7 Receptor Function. Atypical antipsychotic agents, including clozapine (**28**), olanzepine (**68**), and risperidone (**69**) (vide supra), putative atypical antipsychotics (e.g., aripiprazole; K_i = 14 nM),[18,79] and antipsychotic agents such as perphenazine (K_i = 23 nM), thioridazine (K_i = 70 nM), molindone (K_i = 265 nM), amoxepine ($K_i \approx$ 500 nM) (see Figure 2 for structures), and others[18,25] bind at 5-HT_7 receptors. Upon examining the effect of clozapine on receptor regulation in rats, Zhukovskaya and Neumaier[80] found that clozapine (**28**) down-regulates 5-HT_6 receptors but up-regulates 5-HT_7 receptors. Yet a role for these receptors in schizophrenia is far from certain. For example, SB-258741 (i.e., **75** where the 3-OH group is replaced by a methyl group; K_i = 3 nM), a selective 5-HT_7 antagonist, was examined and compared to risperidone (**69**) in three animal models for the positive symptoms of schizophrenia and in a model for negative symptoms, and it was found to be active in only one of the positive models.[81] SB-258741 was not expected to have an antipsychotic effect on its own in the clinic.[81]

Serotonin can reset or phase-shift circadian rhythms in the suprachiasmatic nuclei (SCN) of the hypothalamus, an effect thought to involve, at least in part, 5-HT_{1A} receptors because of the actions of 8-OH DPAT (reviewed in ref 53). Intriguing is that 8-OH DPAT (**10**), in addition to being a 5-HT_{1A} agonist, is also a 5-HT_7 agonist. Recently, 5-HT_7 receptors have been identified in the SCN,[82] leading to speculation that these receptors might be involved in the regulation of circadian rhythm and that 5-HT_7 agents might be useful for the treatment of jet lag and certain sleep disorders.[53] Repeated dosing of the designer drug MDMA (Ecstasy) can influence circadian rhythm in rats and might be responsible for some of the changes in mood and sleep patterns following MDMA use in humans.[83] Phase advances to 8-OH DPAT were attenuated by pretreatment with MDMA leading to speculation that 5-HT_{1A} or 5-HT_7 receptors might be involved.[83] Oleamide is an endogenous lipid that accumulates during sleep deprivation in rodents. Evidence suggests that oleamide is an allosteric modulator of 5-HT_7 receptors.[84,85] Vanhoenacker et al.[53] have also discussed possible connections among 5-HT_7 receptors, circadian rhythm, adrenal steroids, and depression. Recently, 5-HT_7 receptors have been implicated in the regulation of hippocampal glucocorticoid receptor expression.[86]

5-HT_7 receptors can mediate smooth muscle relaxation.[53] It has been speculated that 5-HT_7 receptors might be involved in irritable bowel syndrome,[87] and a smooth muscle inhibitory 5-HT receptor that mediates relaxation of human colon circular muscle resembles the 5-HT_7 receptor.[88] 5-HT_7-like receptors have also been implicated in long-lasting vasodepressor effects of 5-HT in rat[89] and have been shown to mediate smooth muscle relaxation in canine cerebral arteries.[90] The latter finding has implications regarding a possible role for 5-HT_7 receptor ligands in the treatment of migraine[91] and could be involved in hyperalgesic pain and neurogenic inflammation.[92] Because these receptors are expressed in rat primary afferent nociceptors that terminate in the spinal cord, they could also play a role in the actions of noxious stimuli.[93] However, caution has been urged when developing 5-HT_7 agents with agonist activity because of possible untoward cardiovascular effects.[53] For example, activation of what might be 5-HT_7 receptors has been demonstrated to produce tachycardia in the cat.[94]

Audiogenic seizures can be induced in a certain strain of mice following intense auditory stimulation; several neurotransmitters, including 5-HT, are believed to be involved. Examination of several nonselective agents led to the conclusion that 5-HT_7 receptor antagonism best correlated with protection against the sound-induced seizures.[95]

As mentioned above, the 5-HT_{1A} agonist 8-OH DPAT (**10**) binds at 5-HT_7 receptors. Various actions once attributed to 5-HT_{1A} receptors might actually involve 5-HT_7 receptors. Very few studies have gone back to reassess this possibility. For example, 8-OH DPAT (**10**) enhances learning consolidation and evidence suggests that both 5-HT_{1A} and 5-HT_7 receptors might be involved in this action.[96]

Thus far, a number of interesting therapeutic applications have been proposed, but additional work is required. Pouzet[97] has critically reviewed the role of 5-HT_7 receptors in schizophrenia, depression, migraine, epilepsy, pain, and memory impairment.

One final issue that should be mentioned is the concept of inverse agonism. Upon examination of a series of antagonist ligands on h5-$HT_{(7a)}$ adenylate cyclase activity, it was shown that methiothepin (**14**), ketanserin (**15**), clozapine (**28**), olanzepine (**68**), and risperidone (**69**) behaved as antagonists whereas mesulergine (**71**) and SB-258719 (**73**) displayed partial inverse agonist action.[66] In another study using human 5-$HT_{(7a)}$, 5-$HT_{(7b)}$, and 5-$HT_{(7d)}$ receptors, although

inverse agonism was observed and efficacy was not receptor level dependent and varied for several antagonists between membrane preparations of transiently and stably transfected cells, the splice variants displayed similar constitutive activity and inverse agonist character. In this investigation, basal adenylate cyclase activity was reduced by methiothepin (**14**) and this effect was blocked by mesulergine (**71**).[67] All of the eight 5-HT antagonists tested inhibited constitutive adenylate cyclase activity in all splice variants in a concentration-dependent manner, and the rank order of inverse agonist potencies was highly correlated with antagonist potencies: methiothepin (**14**) > metergoline (**7**) > mesulergine (**71**) ≥ clozapine ≥ spiperone (**27**) ≥ ritanserin (**70**) > methysergide (**6**) > ketanserin (**15**).[67]

5-HT$_7$ Receptor Summary. To date, three isoforms of human 5-HT$_7$ receptors have been identified: 5-HT$_{(7a)}$, 5-HT$_{(7b)}$, and 5-HT$_{(7d)}$. These receptors are positively coupled to an adenylate cyclase second messenger system. Although the coupling mechanisms may not be exactly identical, they appear quite similar. Binding profiles of various agents also suggest that there are no significant differences in affinity among the isoforms. Several reasonably selective antagonists now have been identified, and a number of leads for other structure types have been discovered. No selective agonist is yet available.

5-HT$_7$ receptors have been implicated in a wide variety of pharmacological functions. However, much of the speculation, implications, and supporting evidence have appeared in the literature only within the past several years, and in some instances, the results remain to be fully substantiated. In any event, even if only some of the claims can be supported, 5-HT$_7$ receptors should be an attractive target for future drug development efforts.

Summary

5-HT$_5$, 5-HT$_6$, and 5-HT$_7$ receptors were first discovered (i.e., cloned and expressed) within the past 10 years. Most of the 1990s was devoted to characterizing these receptors (or subtypes or splice variants). It was only in the very late 1990s that clues began to emerge regarding the development of selective agents, and this work for the most part continues today. It is perhaps appropriate that a Perspective article on these receptor populations follows a most recent Perspective article on 5-HT$_4$ receptors and their ligands.[98] As 2003 begins, no 5-HT$_5$-selective agonists or antagonists have been identified. Progress has been slow on 5-HT$_{5A}$ receptors, and that on 5-HT$_{5B}$ receptors is virtually nonexistent. In fact, there is still some controversy regarding the 5-HT$_5$ second messenger,[99] and a function for 5-HT$_5$ receptors has yet to be clearly demonstrated. 5-HT$_6$ receptors, of the three receptor types discussed in this article, have seen the most progress. A sufficient number of useful and selective antagonists (and radioligands) have been identified, and the function of these receptors is now a matter of serious investigation. The one agonist showing some selectivity for 5-HT$_6$ receptors has seen little investigation. A novel 5-HT$_6$ agonist with a conformationally constrained side chain, 5-chloro-2-methyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (IC_{50} = 6 nM) was recently reported in an abstract by Merck.[100] 5-HT$_7$ receptors are beginning to emerge from the shadows, and identification of a putative 5-HT$_7$ pharmacophore model[101] might aid further progress in this area. 5-HT$_7$ receptor splice variants and their constitutive activity require further investigation. Nevertheless, some interesting antagonists have been reported and time has arrived for greater application of these agents in functional studies. To date, no 5-HT$_7$-selective agonist has been reported. In general, as described above, some progress has been made toward the development of selective agents for these three receptor populations, and where such agents are unavailable, certain lead structures have been identified. Site-directed mutagenesis and construction and evaluation of chimeric 5-HT receptors[3] could provide additional leads for synthesis of selective agents.

The three receptor types discussed herein have been implicated in a broad variety of physiological processes and possible disease states, both central and peripheral. Because most of these have been implications based on receptor localization studies or perhaps the results of individual empirical studies or studies using nonselective agents with different binding profiles and pharmacologies, greater effort is required to substantiate the claims and proposed therapeutic applications. With the recent emergence of novel chemical tools and with continued development of required selective agonist and/or antagonist ligands, answers should be forthcoming in the relatively near future.

Acknowledgment. Work from the authors' laboratories was supported in part by U.S. Public Health Service, Grant MH 60599.

Biography

Richard A. Glennon obtained his Bachelors degree in Pharmacy and his Masters degree in Medicinal Chemistry from Northeastern University, Boston, MA, and his Doctoral degree in Medicinal Chemistry from the State University of New York at Buffalo. He was an NIH (ADAMHA) postdoctoral fellow, and after a postdoctoral position in psychopharmacology at the Department of Pharmacology, School of Medicine, SUNY at Buffalo, he joined the faculty at the School of Pharmacy at Virginia Commonwealth University in 1975. He is currently Professor and Vice Chairman of Medicinal Chemistry at this institution. His research interests are primarily in the CNS area with particular emphasis on the medicinal chemistry of serotonin receptors, nicotinic acetylcholinergic receptors, and drugs of abuse.

References

(1) Glennon, R. A.; Dukat, M. Serotonin receptors and drugs affecting serotonergic neurotransmission. In *Foye's Principles of Medicinal Chemistry*; Williams, D. A., Lemke, T. M., Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, 2002; pp 315−337.
(2) Hoyer, D.; Hannon, J. P.; Martin, G. R. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.* **2002**, *71*, 533−554.
(3) Kroeze, W. K.; Kristiansen, K.; Roth, B. L. Molecular biology of serotonin receptors−Structure and function at the molecular level. *Curr. Top. Med. Chem.* **2002**, *2*, 507−528.
(4) Humphrey, P. P. A.; Hartig, P. R.; Hoyer, D. A new nomenclature for 5-HT receptors. *Trends Pharmacol. Sci.* **1993**, *14*, 233−236.
(5) Barnes, N. M.; Sharp, T. A review of central 5-HT receptors and their function. *Neuropharmacology* **1999**, *38*, 1083−1152.
(6) Plassat, J.-L.; Boschert, U.; Amlaiky, N.; Hen, R. The house 5-HT$_5$ receptor reveals a remarkable heterogeneity within the 5-HT$_{1D}$ family. *EMBO J.* **1992**, *11*, 4779−4786.

(7) Matthes, H.; Boschert, U.; Amlaiky, N.; Grailhe, R.; Plassat, J. L.; Muscatelli, F.; Mattei, M. G.; Hen, R. Mouse 5-hydroxytryptamine$_{5A}$ and 5-hydroxytryptamine$_{5B}$ receptors define a new family of serotonin receptors: cloning, functional expression, and chromosomal localization. *Mol. Pharmacol.* **1993**, *43*, 313−319.

(8) Erlander, M. G.; Lovenberg, T. W.; Baron, B. M.; deLeccea, L.; Danielson, P. E.; Racke, M.; Slone, A. L.; Siegel, B. W.; Foye, P. E.; Cannon, K.; Burns, J. E.; Sutcliffe, J. G. Two members of a distinct subfamily of 5-hydroxytryptamine receptors differentially expressed in rat brain. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3452−3456.

(9) Wisden, W.; Parker, E. M.; Mahle, C. D.; Grisel, D. A.; Nowak, H. P.; Yocca, F. D.; Felder, C. C.; Seeburg, P. H.; Voight, M. M. Cloning and characterization of the rat 5-HT$_{5B}$ receptor. *FEBS Lett.* **1993**, *333*, 25−31.

(10) Rees, S.; den Daas, I.; Foord, S.; Goodson, S.; Bull, D.; Kilpatrick, G.; Lee, M. Cloning and characterization of the human 5-HT$_{5A}$ serotonin receptor. *FEBS Lett.* **1994**, *355*, 242−246.

(11) Grailhe, R.; Grabtree, G. W.; Hen, R. Human 5-HT$_5$ receptors: the 5-HT$_{5A}$ receptor is functional but the 5-HT$_{5B}$ receptor was lost during mammalian evolution. *Eur. J. Pharmacol.* **2001**, *418*, 157−167.

(12) Waeber, C.; Grailhe, R.; Yu, X. J.; Hen, R.; Moskowitz, M. A. Putative 5-ht5 receptors: localization in the mouse CNS and lack of effect in the inhibition of dural protein extravasation. *Ann. N. Y. Acad. Sci.* **1998**, *861*, 85−90.

(13) Carson, M. J.; Thomas, E. J.; Danielson, P. A.; Sutcliffe, J. G. *Glia* **1996**, *17*, 317−326.

(14) Wang, Z. Y.; Keith, I. M.; Beckman, M. J.; Brownfield, M. S.; Vidruk, E. H.; Bisgard, G. E. 5-HT$_{5a}$ receptors in the carotid body chemoreception pathway of rat. *Neurosci. Lett.* **2000** *278*, 9−12.

(15) Grailhe, R.; Waeber, C.; Dulawa, S. C.; Hornung, J. P.; Zhuang, X.; Brunner, D.; Geyer, M. A.; Hen, R. Increased exploratory activity and altered response to LSD in mice lacking the 5-HT$_{5A}$ receptor. *Neuron* **1999**, *22*, 581−591.

(16) Birkett, J. T.; Arranz, M. J.; Munro, J.; Osbourn, S.; Kerwin, R. W.; Collier, D. A. Association analysis of the 5-HT$_{5A}$ gene in depression, psychosis and antipsychotic response. *NeuroReport* **2000**, *11*, 2017−2020.

(17) Arias, B.; Collier, D. A.; Gasto, C.; Pintor, L.; Gutierrez, B.; Valles, V.; Fananas, L. Genetic variation in the 5-HT$_{5A}$ receptor gene in patients with bipolar disorder and major depression. *Neurosci. Lett.* **2001**, *303*, 111−114.

(18) The Psychoactive Drug Screening Program Database can be found at http://pdsp.cwru. edu/pdsp.asp.

(19) Teitler, M.; Scheick, C.; Howard, P.; Sullivan, J. E.; Iwamura, T.; Glennon, R. A. 5-HT$_{5A}$ serotonin receptor binding: A preliminary structure−affinity investigation. *Med. Chem. Res.* **1997**, *7*, 207−218.

(20) Khorana, N.; Purohit, A.; Herrick-Davis, K.; Teitler, M.; Glennon, R. A. γ-Carbolines: Binding at 5-HT$_{5A}$ serotonin receptors. *Bioorg. Med. Chem. Lett.*, in press.

(21) Khorana, N.; Dukat, M.; Teitler, M.; Glennon, R. A. γ-Carboline binding at human 5-HT$_{5A}$ receptors. Presented at the 80th Annual Virginia Academy of Sciences Meeting, Hampton, VA, May 22−24, 2002 (abstract in the following: *Va. J. Sci.* **2002**, *53*, 98.).

(22) Monsma, F. J.; Shen, Y.; Ward, R. P.; Hamblin, M. W.; Sibley, D. R. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.* **1993**, *43*, 320−327.

(23) Unsworth, C. D.; Molinoff, P. B. Characterization of a 5-hydroxytryptamine receptor in mouse neuroblastoma N18TG2 cells. *J. Pharmacol. Exp. Ther.* **1994**, *269*, 246−255.

(24) Kohen, R.; Metcalf, M. A.; Khan, N.; Druck, T.; Huebner, K.; Lachowicz, J. E.; Meltzer, H. Y.; Sibley, D. R.; Roth, B. L.; Hamblin, M. W. Cloning, characterization, and chromosomal localization of a human 5-HT$_6$ serotonin receptor. *J. Neurochem.* **1996**, *66*, 47−56.

(25) Roth, B. L.; Craigo, S. C.; Choudhary, M. S.; Uluer, A.; Monsma, F. J.; Shen, Y.; Meltzer, H. Y.; Sibley, D. R. Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 1403−1410.

(26) Sleight, A. J.; Boess, F. G.; Bös, M.; Levet-Trafit, B.; Riemer, C.; Bourson, A. Characterization of Ro 04-6790 and Ro 63-0563: Potent and selective antagonists at human and rat 5-HT$_6$ receptors. *Br. J. Pharmacol.* **1998**, *124*, 556−562.

(27) Bromidge, S. M.; Brown, A. M.; Clarke, S. E.; Dodgson, K.; Gager, T.; Grassam, H. L.; Jeffrey, P. M.; Joiner, G. F.; King, F. D.; Middlemiss, D. N.; Moss, S. F.; Newman, H.; Riley, G.; Routledge, C.; Wyman, P. 5-Chloro-*N*-(4-methoxy-3-piperazin-1-ylphenyl)-3-methyl-2-benzothiophenesulfonamide (SB-271046): A potent, selective, and orally bioavailable 5-HT$_6$ receptor antagonist. *J. Med. Chem.* **1999**, *42*, 202−205.

(28) Tsai, Y.; Dukat, M.; Slassi, A.; MacLean, N.; Demchyshyn, L.; Savage, J. E.; Roth, B. L.; Hufesein, S.; Lee, M.; Glennon, R. A. N_1-(Benzenesulfonyl)tryptamines as novel 5-HT$_6$ antagonists. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2295−2299.

(29) Glennon, R. A.; Lee, M.; Rangisetty, J. B.; Dukat, M.; Roth, B. L.; Savage, J. E.; McBride, A.; Rauser, L.; Hufesien, L.; Lee, D. K. H. 2-Substituted tryptamines: Agents with selectivity for 5-HT$_6$ serotonin receptors. *J. Med. Chem.* **2000**, *43*, 1011−1018.

(30) Bös, M.; Sleight, A. J.; Godel, T.; Martin, J. R.; Riemer, C.; Stadler, H. 5-HT6 receptor antagonists: Lead optimization and biological evaluation of *N*-aryl and *N*-heteroaryl 4-aminobenzene sulfonamides. *Eur. J. Med. Chem.* **2001**, *36*, 165−178.

(31) Boess, F. G.; Riemer, C.; Bös, M.; Bentley, J.; Bourson, A.; Sleight, A. J. The 5-hydroxytryptamine$_6$ receptor-selective radioligand [^{3}H]Ro 63-0563 labels 5-hydroxytryptamine receptor binding sites in rat and porcine striatum. *Mol. Pharmacol.* **1998**, *54*, 577−583.

(32) Bromidge, S. M.; Clarke, S. E.; Gager, T.; Griffith, K.; Jeffrey, P.; Jennings, A. J.; Joiner, G. F.; King, F. D.; Lovell, P. J.; Moss, S. F.; Newman, H.; Riley, G.; Rogers, D.; Routledge, C.; Serafinowska, H.; Smith, D. R. Phenyl benzenesulfonamides are novel and selective 5-HT6 antagonists: Identification of *N*-(2,5-dibromo-3-fluorophenyl)-4-methoxy-3-piperazin-1-ylbenzenesulfonamide (SB-357134). *Bioorg. Med. Chem. Lett.* **2001**, *11*, 55−58.

(33) Bromidge, S. M.; Clarke, S. E.; King, F. D.; Lovell, P. J.; Newman, H.; Riley, G.; Routledge, C.; Serafinowska, H.; Smith, D. R.; Thomas, D. R. Bicyclic piperazinylbenzenesulfonamides are potent and selective 5-HT$_6$ receptor antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1357−1360.

(34) Slassi, A.; Isaac, M.; O'Brien, A. Recent progress in 5-HT6 receptor antagonists for the treatment of CNS diseases. *Expert Opin. Ther. Pat.* **2002**, *12*, 513−527.

(35) Hirst, W. D.; Minton, J. A.; Bromidge, S. M.; Moss, S. F.; Latter, A. J.; Riley, G.; Routledge, C.; Middlemiss, D. N.; Price, G. W. Characterization of [^{125}I]-SB-258585 binding to human recombinant and native 5-HT$_6$ receptors in rat, pig and human brain tissue. *Br. J. Pharmacol.* **2000**, *130*, 1597−1605.

(36) Glennon, R. A.; Bondarev, M.; Roth, B. L. 5-HT$_6$ serotonin receptor binding of indolealkylamines: A preliminary structure−affinity investigation. *Med. Chem. Res.* **1999**, *9*, 108−117.

(37) Lee, M.; Rangisetty, J. B.; Dukat, M.; Slassi, A.; MacLean, N.; Lee, D. K. H.; Glennon, R. A. 5-HT$_6$ serotonin receptor binding affinities of N1-benzenesulfonyl and related tryptamines. *Med. Chem. Res.* **2000**, *10*, 230−242.

(38) Russell, M. G. N.; Baker, R. J.; Barden, L.; Beer, M. S.; Bristow, L.; Broughton, H. B.; Knowles, M.; McAllister, G.; Patel, S.; Castro, J. L. *N*-Arylsulfonylindole derivatives as serotonin 5-HT$_6$ receptor ligands. *J. Med. Chem.* **2001**, *44*, 3881−3895.

(39) Pullagurla, M.; Setola, V.; Roth, B. L.; Glennon, R. A. N_1-Benzenesulfonylgramine and N_1-benzenesulfonylskatole: Novel 5-HT$_6$ Receptor Ligand Templates. *Bioorg. Med. Chem. Lett.*, in press.

(40) Glatt, C. E.; Snowman, A. M.; Sibley, D. R.; Snyder, S. H. Clozapine: Selective labeling of sites resembling 5-HT$_6$ serotonin receptors may reflect psychoactive profile. *Mol. Med.* **1995**, *1*, 398−406.

(41) Vogt, I. R.; Shimron-Abarbanell, D.; Neidt, H.; Erdmann, J.; Cichon, S.; Schulze, T. G.; Muller, D. J.; Maier, W.; Albus, M.; Borrmann-Hassenbach, M.; Knapp, M.; Rietschel, M.; Propping, P.; Nothen, M. M. Investigation of the human serotonin-6 (5-HT$_6$) receptor gene in bipolar affective disorder and schizophrenia. *Am. J. Med. Genet.* **2000**, *96*, 217−221.

(42) Pouzet, B.; Didriksen, M.; Arnt, J. Effects of the 5-HT$_6$ receptor antagonist, SB-271046, in animal models for schizophrenia. *Pharmacol. Biochem. Behav.* **2002**, *71*, 635−643.

(43) East, S. Z.; Burnet, P. W.; Leslie, R. A.; Roberts, J. C.; Harrison, P. J. 5-HT$_6$ receptor binding sites in schizophrenia and following antipsychotic drug administration: autoradiographic studies with [^{125}I]SB-258585. *Synapse* **2002**, *45*, 191−199.

(44) Purohit, A.; Herrick-Davis, K.; Teitler, M. Creation, expression, and characterization of a constitutively active mutant of the human serotonin 5-HT$_6$ receptor. *Synapse* **2003**, *47*, 218−224.

(45) Bourson, A.; Borroni, E.; Austin, R. H.; Monsma, F. J.; Sleight, A. J. Determination of a role of the 5-HT$_6$ receptor in the rat brain: A study using antisense oligonucleotides. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 173−180.

(46) Woolley, M. L.; Bentley, J. C.; Sleight, A. J.; Marsden, C. A.; Fone, K. C. A role for 5-ht$_6$ receptors in retention of spatial learning in the Morris water maze. *Neuropharmacology* **2001**, *41*, 210−219.

(47) Rogers, D. C.; Hagan, J. J. 5-HT$_6$ receptor antagonists enhance retention of a water maze task in the rat. *Psychopharmacology (Berlin)* **2001**, *158*, 114−119.

(48) Russell, M. G. N.; Dias, R. Memories are made of this (perhaps): A review of serotonin 5-HT$_6$ receptor ligands and their biological functions. *Curr. Top. Med. Chem.* **2002**, *2*, 643−654.

(49) Dawson, L. A.; Nguyen, H. Q.; Li, P. The 5-HT_6 receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. *Neuropsychopharmacology* **2001**, *25*, 662−668.

(50) Stean, T. O.; Hirst, W. D.; Thomas, D. R.; Price, G. W.; Rogers, D.; Riley, G.; Bromidge, S. M.; Serafinowska, H. T.; Smith, D. R.; Bartlett, S.; Deeks, N.; Duxon, M.; Upton, N. Pharmacological profile of SB-357134: a potent, selective, brain penetrant, and orally active 5-HT_6 receptor antagonist. *Pharmacol. Biochem. Behav*. **2002**, *71*, 645−654.

(51) Routledge, C.; Bromidge, S. M.; Moss, S. F.; Price, G. W.; Hirst, W.; Newman, H.; Riley, G.; Gager, T.; Stean, T.; Upton, N.; Clarke, S. E.; Brown, A. M.; Middlemiss, D. N. Characterization of SB-271046: a potent, selective and orally active 5-HT_6 receptor antagonist. *Br. J. Pharmacol*. **2000**, *130*, 1606−1612.

(52) Eglin, R. M.; Jasper, J. R.; Chang, D. J.; Martin, G. R. The 5-HT_7 receptor: Orphan found. *Trends Pharmacol. Sci*. **1997**, *18*, 104−107.

(53) Vanhoenacker, P.; Haegeman, G.; Leysen, J. E. 5-HT_7 receptors: Current knowledge and future prospects. *Trends Pharmacol. Sci*. **2000**, *21*, 70−77.

(54) Shen, Y.; Monsma, F. J., Jr.; Metcalf, M. A.; Jose, P. A.; Hamblin, M. W.; Sibley, D. R. Molecular cloning and expression of a 5-hydroxytryptamine$_7$ serotonin receptor subtype. *J. Biol. Chem*. **1993**, *268*, 18200−18204.

(55) Lovenberg, T. W.; Baron, B. M.; de Lecea, L.; Miller, J. D.; Prosser, R. A.; Rea, M. A.; Foye, P. E.; Racke, M.; Slone, A. L.; Siegel, B. W.; Danielson, P. E.; Sutcliffe, J. G.; Erlander, M. G. A novel adenylyl cyclase-activating serotonin receptor (5-HT_7) implicated in the regulation of mammalian circadian rhythms. *Neuron* **1993**, *11*, 449−458.

(56) Ruat, M.; Traiffort, E.; Leurs, R.; Tardivel-Lacombe, J.; Diaz, J.; Arrang, J. M.; Schwartz, J. C. Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT_7) activating cAMP formation. *Proc. Natl. Acad. Sci. U.S.A*. **1993**, *90*, 8547−8551.

(57) Plassat, J.-L.; Amlaiky, N.; Hen, R. Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. *Mol. Pharmacol*. **1993**, *44*, 229−236.

(58) Tsou, A. P.; Kosaka, A.; Bach, C.; Zuppan, P.; Yee, C.; Tom, L.; Alvarez, R.; Ramsey, S.; Bonhaus, D. W.; Stefanich, E.; Jakeman, E.; Eglen, R. M.; Chan, H. W. Cloning and expression of a 5-hydroxytryptamine$_7$ receptor positively coupled to adenylyl cyclase. *J. Neurochem*. **1994**, *63*, 456−464.

(59) Bhalla, P.; Saxena, P. R.; Sharma, H. S. Molecular cloning and tissue distribution of mRNA encoding porcine 5-HT_7 receptor and its comparison with the structure of other species. *Mol. Cell. Biochem*. **2002**, *238*, 81−88.

(60) Bard, J. A.; Zgombick, J.; Adham, N.; Vaysse, P.; Brancheck, T. A.; Weinshank, R. L. Cloning of a novel human serotonin receptor (5-HT_7) positively linked to adenylate cyclase. *J. Biol. Chem*. **1993**, *268*, 23422−23426.

(61) Jasper, J. R.; Kosaka, A.; To, Z. P.; Chang, D. J.; Eglen, R. M. Cloning, expression and pharmacology of a truncated splice variant of the human 5-HT_7 receptor (h5-HT_{7b}). *Br. J. Pharmacol*. **1997**, *122*, 126−132.

(62) Stam, N. J.; Roesink, C.; Dijcks, F.; Garritsen, A.; van Herpen, A.; Olijve, W. Human serotonin 5-HT_7 receptor: Cloning and pharmacological characterization of two receptor variants. *FEBS Lett*. **1997**, *413*, 489−494.

(63) Heidmann, D. E.; Metcalf, M. A.; Kohen, R. Hamblin, M. W. Four 5-hydroxytryptamine$_7$ (5-HT_7) receptor isoforms in human and rat produced by alternative splicing: species differences due to altered intron−exon organization. *J. Neurochem*. **1997**, *68*, 1372−1381.

(64) Heidmann, D. E.; Szot, P.; Kohen, R.; Hamblin, M. W. Function and distribution of three rat 5-hydroxytryptamine$_7$ (5-HT_7) receptor isoforms produced by alternative splicing. *Neuropharmacology* **1998**, *37*, 1621−1632.

(65) Krobert, K. A.; Bach, T.; Syversveen, T.; Kvingedal, A. M.; Levy, F. O. The cloned human 5-HT_7 receptor splice variants: a comparative characterization of their pharmacology, function and distribution. *Naunyn-Schmiedeberg's Arch. Pharmacol*. **2001**, *363*, 620−632.

(66) Thomas, D. R.; Gittins, S. A.; Collin, L. L.; Middlemiss, D. N.; Riley, G.; Hagan, J.; Gloger, I.; Ellis, C. E.; Forbes, I. T.; Brown, A. M. Functional characterisation of the human cloned 5-HT_7 receptor (long form); antagonist profile of SB-258719. *Br. J. Pharmacol*. **1998**, *124*, 1300−1306.

(67) Krobert, K. A.; Levy, F. O. The human 5-HT_7 serotonin receptor splice variants: constitutive activity and inverse agonist effects. *Br. J. Pharmacol*. **2002**, *135*, 1563−1571.

(68) Glennon, R. A.; Metwally, K.; Dukat, M.; Ismaiel, A. M.; De los Angeles, J.; Herndon, J.; Teitler, M.; Khorana, N. Ketanserin and spiperone as templates for novel serotonin 5-HT_{2A} antagonists. *Curr. Top. Med. Chem*. **2002**, *2*, 539−558.

(69) Forbes, I. T.; Dabbs, S.; Duckworth, D. M.; Jennings, A. J.; King, F. D.; Lovell, P. J.; Brown, A. M.; Collin, L.; Hagan, J. J.; Middlemiss, D. N.; Riley, G. J.; Thomas, D. R.; Upton, N. (*R*)-3,*N*-dimethyl-*N*-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzenesulfonamide: the first selective 5-HT_7 receptor antagonist. *J. Med. Chem*. **1998**, *41*, 655−657.

(70) Lovell, P. J.; Bromidge, S. M.; Dabbs, S.; Duckworth, D. M.; Forbes, I. T.; Jennings, A. J.; King, F. D.; Middlemiss, D. N.; Rahman, S. K.; Saunders, D. V.; Collin, L. L.; Hagan, J. J.; Riley, G. J.; Thomas, D. R. A novel, potent, and selective 5-HT_7 antagonist: (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl)phenol (SB-269970). *J. Med. Chem*. **2000**, *43*, 342−345.

(71) Forbes, I. T.; Douglas, S.; Gribble, A. D.; Ife, R. J.; Lightfoot, A. P.; Garner, A. E.; Riley, G. J.; Jeffrey, P.; Stevens, A. J.; Stean, T. O.; Thomas, D. R. SB-656104-A: A novel 5-HT_7 receptor antagonist with improved in vivo properties. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 3341−3344.

(72) Kikuchi, C.; Nagaso, H.; Hiranuma, T.; Koyama, M. Tetrahydrobenzindoles: Selective antagonists of the 5-HT_7 receptor. *J. Med. Chem*. **1999**, *42*, 533−535.

(73) Kikuchi, C.; Ando, T.; Watanabe, T.; Nagaso, H.; Okuno, M.; Hiranuma, T.; Koyama, M. 2a-[4-(Tetrahydropyridoindol-2-yl)-butyl]tetrahydrobenzindole derivatives: New selective antagonists of the 5-hydroxytryptamine$_7$ receptor. *J. Med. Chem*. **2002**, *45*, 2197−2206.

(74) Linnanen, T.; Brisander, M.; Unelius, L.; Rosqvist, S.; Nordvall, G.; Hacksell, U.; Johansson, A. M. Atropisomeric derivatives of 2′,6′-disubstituted (*R*)-11-phenylaporphine: selective serotonin 5-HT_7 receptor antagonists. *J. Med. Chem*. **2001**, *44*, 1337−1340.

(75) Linnanen, T.; Brisander, M.; Unelius, L.; Sundholm, G.; Hacksell, U.; Johansson, A. M. Derivatives of (*R*)-1,11-methyleneaporphine: synthesis, structure, and interactions with G-protein coupled receptors. *J. Med. Chem*. **2000**, *43*, 1339−1349.

(76) Isaac, M. B.; Xin, T.; O'Brien, A.; St-Martin, D.; Naismith, A.; MacLean, N.; Wilson, J.; Demchyshyn, L.; Tehim, A.; Slassi, A. 1-(Bicyclopiperazinyl)ethylindoles and 1-(homopiperazinyl)ethylindoles as highly selective and potent 5-HT_7 receptor ligands. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 2451−2454.

(77) Parikh, V.; Welch, W. M.; Schmidt, A. W. Discovery of a series of (4,5-dihydroimidazol-2-yl)biphenylamine 5-HT_7 agonists. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 269−271.

(78) Wilcox, R. E.; Ragan, J. E.; Pearlman, R. S.; Brusniak, M. Y.; Eglen, R. M.; Bonhaus, D. W.; Tenner, T. E.; Miller, J. D. High-affinity interactions of ligands at recombinant guinea pig 5-HT7 receptors. *J. Comput.-Aided Mol. Des*. **2001**, *15*, 883−909.

(79) Lawler, C. P.; Prioleau, C.; Lewis, M. M.; Mak, C.; Jiang, D.; Schertz, J. A.; Gonzalez, A. M.; Sibley, D. R.; Mailman, R. B. Interactions of the novel antipsychotic aripiprazole (OPC-14597) with dopamine and serotonin receptor subtypes. *Neuropsychopharmacology* **1999**, *20*, 612−627.

(80) Zhukovskaya, N. L.; Neumaier, J. F. Clozapine downregulates 5-hydroxytryptamine$_6$ (5-HT_6) and upregulates 5-HT_7 receptors in HeLa cells. *Neurosci. Lett*. **2000**, *288*, 236−240.

(81) Pouzet, B.; Didriksen, M.; Arnt, J. Effects of the 5-HT_7 receptor antagonist SB-258741 in animal models for schizophrenia. *Pharmacol. Biochem. Behav*. **2002**, *71*, 655−665.

(82) Jovanovska, A.; Prosser, R. A. Translational and transcriptional inhibitors block serotonergic phase advances of the suprachiasmatic nucleus circadian pacemaker in vitro. *J. Biol. Rhythms* **2002**, *17*, 137−146.

(83) Biello, S. M.; Dafters, R. I. MDMA and fenfluramine alter the response of the circadian clock to a serotonin agonist in vitro. *Brain Res*. **2001**, *920*, 202−209.

(84) Thomas, E. A.; Cravatt, B. F.; Sutcliffe, J. G. The endogenous lipid oleamide activates serotonin 5-HT_7 neurons in mouse thalamus and hypothalamus. *J. Neurochem*. **1999**, *72*, 2370−2378.

(85) Hedlund, P. B.; Carson, M. J.; Sutcliffe, J. G.; Thomas, E. A. Allosteric regulation by oleamide of the binding properties of 5-hydroxytryptamine$_7$ receptors. *Biochem. Pharmacol*. **1999**, *58*, 1807−1813.

(86) Laplante, P.; Diorio, J.; Meaney, M. J. Serotonin regulates hippocampal glucocorticoid receptor expression via a 5-HT_7 receptor. *Dev. Brain Res*. **2002**, *139*, 199−203.

(87) De Ponti, F. Tonini, M. Irritable bowel syndrome: new agents targeting serotonin receptor subtypes. *Drugs* **2001**, *61*, 317−332.

(88) Prins, N. H.; Briejer, M. R.; Van Bergen, P. J.; Akkermans, L. M.; Schuurkes, J. A. Evidence for 5-HT_7 receptors mediating relaxation of human colonic circular smooth muscle. *Br. J. Pharmacol*. **1999**, *128*, 849−852.

(89) Terron, J. A. Role of 5-ht_7 receptors in the long-lasting hypotensive response induced by 5-hydroxytryptamine in the rat. *Br. J. Pharmacol*. **1997**, *121*, 563−571.

(90) Terron, J. A.; Falcon-Neri, A. Pharmacological evidence for the 5-HT_7 receptor mediating smooth muscle relaxation in canine cerebral arteries. *Br. J. Pharmacol.* **1999**, *127*, 609−616.
(91) Villalon, C. M.; Centurion, D.; Valdivia, L. F.; De Vries, P.; Saxena, P. R. An introduction to migraine: From ancient treatment to functional pharmacology and antimigraine therapy. *Proc. West. Pharmacol. Soc.* **2002**, *45*, 199−210.
(92) Terron, J. A. Is the 5-HT_7 receptor involved in the pathogenesis and prophylactic treatment of migraine? *Eur. J. Pharmacol.* **2002**, *439*, 1−11.
(93) Meuser, T.; Pietruck, C.; Gabriel, A.; Xie, G. X.; Lim, K. J.; Palmer, P. 5-HT7 receptors are involved in mediating 5-HT-induced activation of rat primary afferent neurons. *Life Sci.* **2002**, *71*, 2279−2289.
(94) Villalon, C. M.; Centurion, D.; Bravo, G.; De Vries, P.; Saxena, P. R.; Ortiz, M. I. Further pharmacological analysis of the orphan 5-HT receptors mediating feline vasodepressor responses: close resemblance to the 5-HT_7 receptor. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *361*, 665−671.
(95) Bourson, A.; Kapps, V.; Zwingelstein, C.; Rudler, A.; Boess, F. G.; Sleight, A. J. Correlation between 5-HT_7 receptor affinity and protection against sound-induced seizures in DBA/2J mice. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1997**, *356*, 820−826.
(96) Menses, A.; Terron, J. A. Role of 5-HT_{1A} and 5-HT_7 receptors in the facilatory response induced by 8-OH-DPAT on learning consolidation. *Behav. Brain Res.* **2001**, *121*, 21−28.
(97) Pouzet, B. SB-258741: A 5-HT_7 receptor antagonist of potential clinical interest. *CNS Drug Rev.* **2002**, *8*, 90−100.
(98) Langlois, M.; Fischmeister, R. 5-HT_4 receptor ligands: Applications and new prospects. *J. Med. Chem.* **2003**, *46*, 321−344.
(99) Noda, M.; Yasuda, S.; Okada, M.; Higashida, H.; Shimada, A.; Iwata, N.; Ozaki, N.; Nishikawa, K.; Shirasawa, S.; Uchida, M.; Aoki, S.; Wada, K. Recombinant human serotonin$_{5A}$ receptors stably expressed in C6 glioma cells couple to multiple signal transduction pathways. *J. Neurochem.* **2003**, *84*, 222−232.
(100) Mattsson, C.; Sandal, A.; Ludvigsson, E.; Sonesson, C.; Bottcher, H. Novel, potent and selective 2-alkyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole at 5-HT_6 receptor agonists. *Drugs Fut.* **2002**, *27 (Suppl. A)*, 369.
(101) Lopez-Rodriguez, M. L.; Porras, E.; Benhamu, B.; Ramos, J. A.; Morcillo, M. J.; Lavandera, J. L. First pharmacophoric hypothesis for 5-HT_7 antagonism. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1097−1100.

JM030030N