5-HT$_7$ Receptor Ligands: Recent Developments and Potential Therapeutic Applications

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Abstract: The 5-HT$_7$ receptors (5-HT$_7$Rs) are the most recent classified members of the serotonin family. Characterized in 1993, they belong to the G protein-coupled receptor family. Since their discovery, they have been the subject of intense research due to their widespread distribution in the brain, suggestive of multiple central roles. The focus of this review is to discuss the literature concerning recent advances on 5-HT$_7$Rs and their ligands.

Key Words: Serotonin, serotonin receptor subtypes, 5-HT$_7$Rs, 5-HT$_7$ ligands, G protein-coupled receptors, agonists, antagonists.

1. INTRODUCTION

The biogenic amine serotonin (5-hydroxytryptamine, 5-HT) is one of the most studied and abundant neurotransmitters in the central nervous system (CNS). It acts through the activation of at least fourteen 5-HT receptor subtypes. According to International Union of Pharmacology these receptors are classified, basing on structural, operational, and transductional information, in seven subfamilies: 5-HT$_1$ (including 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_{1F}$ subtypes), 5-HT$_2$ (5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$), 5-HT$_3$, 5-HT$_4$, 5-HT$_{5A}$, 5-HT$_6$, 5-HT$_7$ [1]. All these receptors possess seven transmembrane domains (TMs) and belong to the G protein-coupled receptor family with the exception of 5-HT$_3$ receptor subtype (further divided into 5-HT$_{3A}$, and 5-HT$_{3B}$ subtypes) that is a ligand-gated ion channel and it appears to be located mainly in neuronal tissue where it mediates fast depolarization [2]. Serotonin receptors are located both centrally and peripherally, are involved in a wide variety of physiological functions, and in a number of pathological states.

The 5-HT$_7$Rs are the most recent classified members of the serotonin family. In 1993 at least three different laboratories reported the discovery of this new serotonergic receptor [3-5]. Subsequently, the protein has been cloned from a number of species including guinea pig, rat, mouse, pig, human and recent additions to this list include *Caenorhabditis elegans*, *Aedes aegypti*, *Apis mellifera,* and *Aphis gossypi* [3-14]. Despite showing high interspecies homology, 5-HT$_7$Rs possess a low overall amino acid homology (<50%) with other members of the 5-HT receptor family [15]. The gene encoding for human 5-HT$_7$R is located on chromosome 10q23.3-q24.4, the full length expressed protein being positively coupled with adenylyl cyclase activity through the activation of G_s proteins [4,5,16]. 5-HT$_7$Rs are defined pharmacologically by their high affinity for 5-HT, 5-carboxytryptamine (5-CT), 5-methoxytryptamine, and methiothepin, moderate affinity for 8-OH-DPAT and ritanserin and low affinity for pindolol, sumatriptan, and buspirone [3-5,17]. Moreover, 5-HT$_7$Rs as well as a number of other G protein-coupled receptors are functionally regulated by membranous cholesterol. Indeed, depletion of this membrane lipid decreases binding of both agonist and antagonist radioligands to 5-HT$_7$Rs and counteracts 5-HT$_7$Rs-mediated intracellular signalling [18].

Alternative splicing of the 5-HT$_7$R gene has been reported to generate different isoforms, namely: 5-HT$_{7(a),\,(b),\,(c)}$ in rat and 5-HT$_{7(a),\,(b),\,(d)}$ in human which differ only in the length and amino acid composition of their carboxyl-terminal tail [19-22]. In human, the most abundant isoform, the 5-HT$_{7(a)}$ receptor, consists of 445 amino acids with a long carboxy terminus. In previous studies these isoforms did not show significant differences in their pharmacological profile, signal transduction or tissue distribution [22-24]. All the three human splice variants constitutively activate adenylyl cyclase and numerous 5-HT$_7$R antagonists displayed either full or partial inverse agonist activity [24]. However, it was recently demonstrated that the 5-HT$_{7(d)}$ isoform exhibits receptor trafficking distinct from that of 5-HT$_{7(a)}$ or 5-HT$_{7(b)}$ [25]. In this study, human 5-HT$_{7(d)}$ receptors displayed agonist-independent internalization and indeed internalization even in the presence of antagonist. The consequence of this removal of 5-HT$_{7(d)}$ receptors from the surface is a reduced efficacy of cAMP-responsive reporter gene activity. Agonist-independent internalization provides a possible mechanism for the previous observation that the human 5-HT$_{7(d)}$ isoform has a reduced ability to stimulate the second messenger pathway [23]. These data further show that the 5-HT$_{7(d)}$ carboxy tail may mediate an interaction with a trafficking pathway for internalization other than that used by 5-HT$_{7(a)}$ and 5-HT$_{7(b)}$ receptors. However, the functional significance of the reduced signalling efficacy of the 5-HT$_{7(d)}$ receptor on the regulation of serotonin signalling through the 5-HT$_7$Rs is unclear. Moreover, in a recent study using the three recombinant human 5-HT$_7$R splice variants some peculiar properties for the 5-HT$_7$Rs were reported: antagonist-mediated homologous desensitization, antagonist-mediated heterologous desensitization and the absence of receptor down-regulation in the presence of homologous or heterolo-

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gous desensitization. These properties distinguish 5-HT$_7$Rs from other receptors [26].

During a systematic mutation screening on human 5-HT$_{7(a)}$ receptors, two naturally occurring variants emerged: Pro^{279}Leu (located in the third intracellular loop) and Thr^{92}Lys (located in the first TM) [27]. The first variant showed a strong impairment of signal transduction, leaving receptor affinity of agonists and antagonists unaffected [28]. This finding is compatible with the location of the mutation in the third intracellular loop which plays an important role in G protein coupling. On the other hand, no change in signal transduction was expected studying the Thr^{92}Lys variant of the 5-HT$_{7(a)}$ receptor but, due to the importance of TMs for ligand binding, a modification of agonist or antagonist binding was expected when compared to the wild-type receptor [29,30]. It was indeed observed that the affinity of the 5-HT$_7$R agonists 5-HT, 5-CT, RU-24969, and 8-OH-DPAT but not of the 5-HT$_7$R antagonists SB-269970, risperidone, mesulergine, and clozapine is considerably decreased compared to the wild-type receptor [31].

The distribution of the 5-HT$_7$ binding sites was studied, in the presence of suitable pharmacological agents for non-5-HT$_7$ receptor binding sites blockade, by autoradiography using [^{3}H]5-HT, [^{3}H]5-CT, [^{3}H]mesulergine, [^{3}H]8-OH-DPAT, and [^{3}H]SB-269970, and by immunohistochemical or immunocytochemical techniques [15,17,32-52]. At the mRNA level, *in situ* hybridization, Northern-blot analysis, and reverse-transcriptase polymerase chain reaction were used to study these sites [3-6,9,14,15,33,42,47,53-56]. Those reports indicate that the distribution of 5-HT$_7$ sites along with their splice variants is largely consistent with that reported for 5-HT$_7$R mRNA and consistent across species. The highest density has been observed in the brain, mainly in the hypothalamus (including suprachiasmatic nucleus), thalamus, hippocampus, brainstem, and cortex. In peripheral tissues 5-HT$_7$Rs are present on smooth muscle cells in blood vessels, heart, coronary artery, gastrointestinal tract, spleen, and kidney. Recently, 5-HT$_7$Rs mRNA was identified in the ciliary body, choroid, conjunctiva, and iris, with much lower levels in the retina and ciliary epithelium and optic nerve tissue [56].

The characterization and distribution of 5-HT$_7$Rs along with their ligands have been reviewed up to 2004 [57-66]. The aim of this review is to discuss the literature concerning recent advances on 5-HT$_7$Rs, from a medicinal chemistry perspective. Indeed, during the last three years new and significant progresses have been made in the field of novel selective ligands, a number of patents have been filed, and new pharmacological studies, made with more selective ligands, helped to clarify their functional roles.

2. 5-HT$_7$ RECEPTOR LIGANDS

During the first decade after the cloning and characterization of 5-HT$_7$Rs, a number of non-selective ligands showing high affinity toward these receptors were identified. Moreover, the high affinity for the 5-HT$_7$Rs of a wide range of psychoactive drugs, such as typical and atypical antipsychotic, some antidepressant, together with almost exclusive expression of these receptors in the CNS has stimulated significant research interest. Different classes of non selective 5-HT$_7$R ligands were soon identified, featuring: ergolines (2-Br-LDS, methergoline, mesulergine, methysergide, amesergide, sergolexole, ergotamine, bromocriptine, LY-215480, LY-53857), aporphine derivatives, antipsychotic tricyclic analogues (clozapine, (*S*)-methiothepin, mianserin, amitryptiline, maprotiline, (+)-butaclamol, cyproheptadine, phenotiazines, thioxanthenes), piperidine derivatives (spiperone, ritanserin, risperidone, 9-OH-risperidone) [3,5,6,8,15,67-70]. Although the action of these substances has been known for a long-time, it was only recently demonstrated a particular mechanism of action for risperidone, 9-OH-risperidone, and methiothepin [71]. It seems that these ligands interact in an irreversible or pseudo-irreversible manner with the 5-HT$_7$Rs when expressed in intact cells but not in membrane preparations, producing an inhibition of the receptor activity that seems to be rapid, potent, and essentially complete. The implications for these findings, considering that risperidone is a highly prescribed drug, are noteworthy.

Over the last ten years considerable efforts have been focused on the development of novel and selective 5-HT$_7$R agonists and antagonists to better study the physiological and functional role of these receptors. The first selective 5-HT$_7$R antagonist (**1**) was identified, in 1997, as a mixture of four diastereoisomers in a high-throughput screening (HTS) at GlaxoSmithKline and belongs to the sulfonamide class [72, 73]. SAR studies around this compound led to the identification of SB-258719 (**2**, 5-HT$_7$ K_i = 32 nM) selected for further evaluation [72,73] and, subsequently, to SB-269970 (**3**, 5-HT$_7$ K_i = 1 nM); both compounds behave as antagonists at the 5-HT$_7$Rs [74-77]. However, it was recently demonstrated that SB-258719 and SB-269970 possess inverse agonist activities at human recombinant 5-HT$_7$Rs being SB-258719 closest to a neutral antagonist, while SB-269970 is the most robust inverse agonist [78]. At the time of its discovery, SB-269970 showed an excellent selectivity profile but its high *in vivo* blood clearance led to further studies aimed at ameliorating its pharmacokinetic profile [79]. Compound **4** (SB-656104) was selected as the most promising analogue for further studies. SB-691673 (**5**) came from the optimization of a back-up series and features a diverse chemical structure from the above-mentioned sulfonamides [80]. Another important class of 5-HT$_7$R ligands, the tetrahydrobenzindoles, emerged from HTS of the Meiji Seika Kaisha Ltd. library [81,82]. The parent compound is the well known DR-4004 (**6**) behaving as 5-HT$_7$R antagonist. Further SAR studies and attempts to optimize the pharmacokinetic profile led to the identification of DR-4446 (**7**), DR-4485 (**8**), and DR-4365 (**9**) [83-88]. Similar compounds were patented also by GlaxoSmithKline [89].

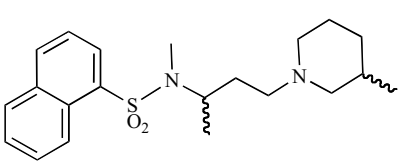
Soon after the discovery of the above-mentioned classes of antagonists, Shionogi & Co. reported the first patent claiming the identification of 5-HT$_7$R agonists possessing a pyridine structure [90]. Among them, the most potent compound is **10** with a K_i value of 9.2 nM. Later on, researchers from Pfizer filed a patent application claiming novel 4-(pyridin-2-yl)piperazine derivatives acting as agonists at 5-HT$_7$Rs [91]. Soon after, in 2003, HTS of Pfizer library and SAR studies on a novel class of (4,5-dihydroimidazol-2-yl) biphenylamines led to the identification of compound **11**,

being the most potent of the series, with a pK_i values of 7.79 for 5-HT$_7$Rs and endowed with affinity for α_1 and α_2 adrenoceptors too (pK_i = 6.68 and 7.71, respectively) [92].

Subsequently a number of pharmaceutical companies and academic research groups reported the identification of selective 5-HT$_7$R ligands and excellent reviews in the field were provided during 2004 [62,64]. Briefly, some of the most interesting ligands worth of mention are: tetrahydroquinolines from Roche, aporphine derivatives from Uppsala University, aminoalkylpirrolidines and amidinoureas from Pfizer, 6-bromo-1-substituted-ethylindoles from NPS Pharmaceuticals, carbocyclic sulfonamide derivatives and dimethyl-(2-arylsulfanylethyl)amines from Merck & Co. Inc. [93-101].

Researchers at Bristol-Myers Squibb, in 2001, disclosed a patent application covering the discovery of about a hundred aminopyrimidine and aminotriazine derivatives with IC_{50} values < 50 nM, useful as 5-HT$_7$R antagonists and their use for treating CNS and ocular disorders [102]. More recently, they reported in two papers binding data and SAR for these classes of 5-HT$_7$R ligands [103,104]. Aminotriazine derivatives are represented by the general chemical structure **12** [103]. The starting *hit compound* (**13**) has a K_i value of 60 nM. SAR studies around compound **13** led the phenoxyethyl amine analogue **14** (* = ±, K_i = 8 nM) with improved affinity and determined that all the 5-HT$_7$R affinity resided in the *S* isomer (K_i = 3 nM). The role of the R substituent was studied demonstrating that the unsubstituted triazine (R = H) is as potent as the primary amine (**14**), while the presence of fluoro, methyl or dimethylamino substituent seems to be detrimental for affinity. High affinity for 5-HT$_7$Rs is maintained when X = F instead of H (as an example see compound **15** possessing K_i value of 2 nM). Further SAR studies on the side chain (Y) showed high binding affinity with 4-fluorophenethyl residue (**16**, K_i = 2 nM), 2-pyridylethyl moiety (**17**, K_i = 9 nM) and 2-thienylethyl portion (**18**, K_i = 2 nM). The presence of a fluorine residue at the 4-position of the phenoxy moiety (**15**) or the phenylethyl residue (**16**) protects compounds from metabolic hydroxylation, and consequently improves oral bioavailability in rat. Regarding the selectivity, compounds **14** and **15** possess moderate affinity for the α_1 adrenoceptors, but no affinity for 5-HT$_6$ or 5-HT$_{2C}$ receptors; compound **16** is endowed with moderate affinity for 5-HT$_6$ and α_1 receptors, and high affinity for the 5-HT$_{2C}$ receptor (K_i = 20 nM); compound **17** shows moderate affinity for the 5-HT$_{2C}$ receptor. Finally, compound **18** possesses moderate affinity for the 5-HT$_6$ receptor and high affinity for the 5-HT$_{2C}$ receptor (K_i = 10 nM). None of these compounds (**14**-**18**) exhibit affinity for the 5-HT$_{1A}$ and the D$_{2L}$ receptors or the 5-HT transporter. These derivatives, when tested in the 5-HT$_7$R adenylyl cyclase assay, behave as antagonists. In the second paper SAR studies are reported on diaminopyrimidine and diaminopyridine ligands (general formula **19**) for 5-HT$_7$Rs in order to explore the importance of the number and the placement of ring nitrogens [104]. Almost all the disubstituted pyrimidines (nitrogen in positions W and Y, or in position W and V, or Y and V) showed moderate to good affinity. Compound **20** possesses the highest affinity in these series with a K_i value of 0.3 nM. Replacement of a second ring nitrogen by a CH group led to the diaminopyridines. This time, changes in location of ring nitrogen caused significant effects on 5-HT$_7$R binding affinity. A loss of affinity was observed when V = N or when the pyridine ring was substituted with a phenyl ring, and moderate affinity when X = N, whereas excellent binding affinities were obtained with the 2,4-disubstituted pyridines (W = N or Y = N). Compound **21** possesses the highest affinity in these series with a K_i value of 0.2 nM; unfortunately, its selectivity was unsatisfactory possessing good affinity towards α_1 adrenoceptors (K_i = 8 nM) and 5-HT$_{2C}$ receptors (K_i = 15 nM). Better results in term of selectivity were obtained with compound **22** possessing K_i value for the 5-HT$_7$Rs of 4 nM, no affinity for 5-HT$_{1A}$, D$_{2L}$ receptors, and 5-HT transporter, low affinity for α_1 adrenoceptors (K_i = 379 nM), and 5-HT$_{2C}$ receptors (K_i = 476 nM). However no functional data on these series of compounds were reported.

Perrone and coworkers during 2003 identified several 1-[ω-(4-aryl-1-piperazinyl)alkyl]-1-aryl ketones possessing good 5-HT$_7$R affinity and represented by the general formula **23** [105]. Compound **24** and its methoxy analogue **25** exhibited high 5-HT$_7$R affinities (K_i = 2.93 and 0.90 nM, respectively) and agonist properties when tested for 5-HT$_7$ receptor-mediated relaxation of substance P-induced guinea pig ileum contraction. Compounds **24** and **25** when tested against a panel of receptors showed moderate to high affinity for 5-HT$_{1A}$, 5-HT$_{2A}$, D$_{2L}$, D$_3$, D$_4$, α_1, σ_1, and σ_2 receptors. In a subsequent paper the same research group identified a series of 4-(2-phenylsubstituted-ethyl)-1-substituted-piperazines endowed with moderate affinity for the 5-HT$_7$Rs and somewhat higher affinity for 5-HT$_{1A}$ receptors [106]. Among them, compound **26** (5-HT$_7$ K_i = 8.2 nM, 5-HT$_{1A}$ K_i = 3.63 nM) behaved as agonist when tested for 5-HT$_7$ receptor-mediated relaxation of substance P-induced guinea pig ileum contraction. Screening of a series of 1-(2-methoxyphenyl) piperazine derivatives endowed with 5-HT$_{1A}$ affinity led to the identification of a new class of 5-HT$_7$R ligands with general chemical structure **27** [107]. SAR of this series suggest that the optimal alkyl chain length is five methylenes and an unsubstituted 1,2,3,4-tetrahydronaphthalenyl nucleus is preferred. Compounds **28** and **29** possess high affinity for the 5-HT$_7$Rs (K_i = 6.05 and 6.64 nM, respectively) as well as for the 5-HT$_{1A}$ receptors (K_i = 9 and 8.6 nM, respectively). Replacement of the aromatic ring attached to the piperazine residue with a bicyclic aromatic system was detrimental for 5-HT$_7$R affinity. Further optimization of the series is represented by compounds of general formula **30** [107]. Substitution pattern of the aryl ring linked to the piperazine plays a crucial role in term of affinity and selectivity. Compound **31** shows high and comparable affinity at both 5-HT$_7$ and 5-HT$_{1A}$ receptors (K_i = 4.14 and 3.8 nM, respectively), a similar profile is showed by compound **32** (5-HT$_7$ K_i = 11.4, 5-HT$_{1A}$ K_i = 24 nM). Its 2-methylthio analogue (**33**) possesses the best profile in terms of affinity towards 5-HT$_7$Rs (K_i = 0.22 nM) and selectivity (5-HT$_{1A}$ K_i = 52.7 nM, 5-HT$_{2A}$ K_i = 326 nM). In functional assay compounds **29** and **33** behaved as full agonists, while **31** as partial agonist and **32** as antagonist. However this class of compounds showed high lipophilicity and to overcome this limitation, the parent structure **27** was simplified and compounds of general formula **34** were synthesized [108].



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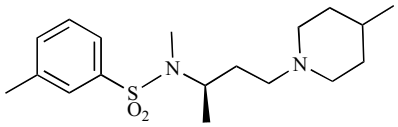

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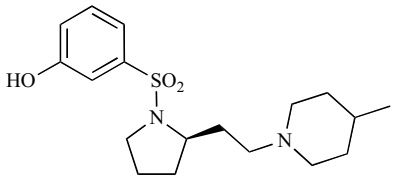

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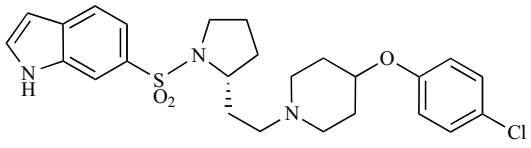

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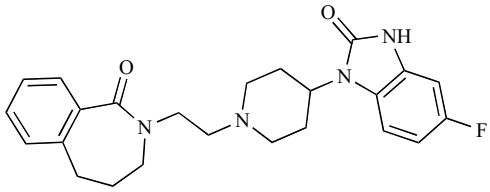

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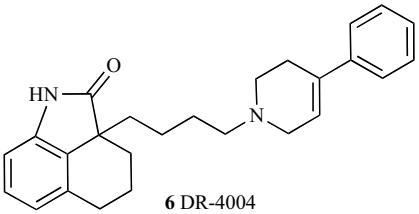

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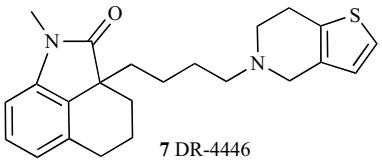

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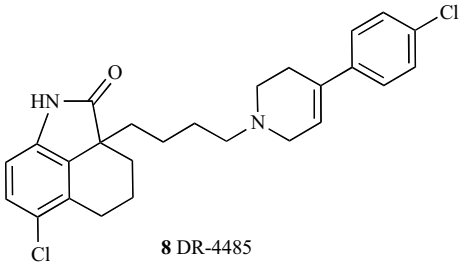

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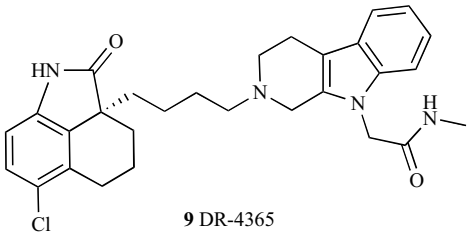

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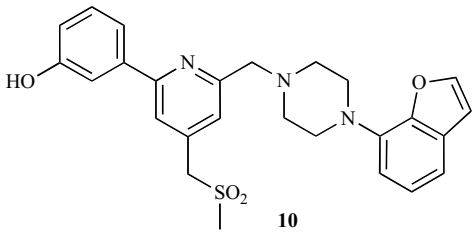

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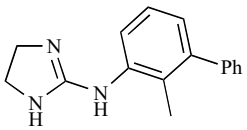

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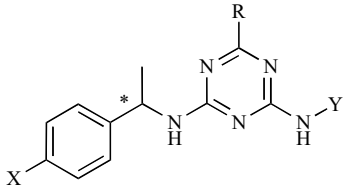
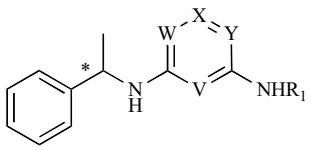


12 general structure
13 * = ±, R = NH_2, X = H, Y = $(CH_2)_3$Ph
14 * = *S*, R = NH_2, X = H, Y = $(CH_2)_2$OPh
15 * = *S*, R = NH_2, X = F, Y = $(CH_2)_2$O(4-FPh)
16 * = *S*, R = NH_2, X = F, Y = $(CH_2)_2$(4-FPh)
17 * = *S*, R = NH_2, X = F, Y = $(CH_2)_2$(2-pyridyl)
18 * = ±, R = NH_2, X = F, Y = $(CH_2)_2$(2-thienyl)



19 general structure
20 * = *S*, V = N, W = CH, X = CH, Y = N, R_1 = $(CH_2)_2$O(4-FPh)
21 * = *S*, V = CH, W = CH, X = CH, Y = N, R_1 = $(CH_2)_2$O(4-FPh)
22 * = *S*, V = CH, W = CH, X = N, Y = CH, R_1 = $(CH_2)_2$O(4-FPh)

Chart (1).

Holmberg and coworkers reported the synthesis of novel 2-aminotetralin and 3-aminochroman derivatives as ligands for the 5-HT$_7$Rs exemplified by the general formula **35** [109]. The interaction of the novel ligands with 5-HT$_7$Rs is stereospecific preferring the (*R*)-chromans (X = O), corresponding to the (*S*)-tetralins (X = CH_2). Introduction of 2,6-dimethoxy substituents on the aryl ring, substantially reduces the affinity for the 5-HT$_{1A}$ receptors while affecting only marginally the binding at 5-HT$_7$Rs. Interestingly, shortening the length of the *N*-alkyl-substituents from propyl to methyl (for example: **36** *vs* **37**, and **38** *vs* **39**) increases the selectivity for the 5-HT$_7$Rs 6- or 7-fold (**36**, 5-HT$_7$ K_i = 7.9 nM, 5-

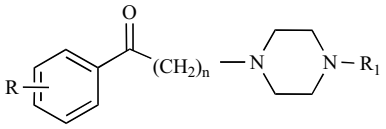
HT_{1A} K_i = 347 nM; **37**, 5-HT_7 K_i = 2.55 nM, 5-HT_{1A} K_i = 1420 nM; **38**, 5-HT_7 K_i = 6.44 nM, 5-HT_{1A} K_i = 174 nM; **39**, 5-HT_7 K_i = 5.29 nM, 5-HT_{1A} K_i = >1000 nM). Again interestingly, the N-alkyl-substituents influence intrinsic activity of these compounds; indeed **36** and **38** behave as full agonists and the corresponding dimethylamino derivatives **37** and **39** behave as antagonists or weak partial agonists. These results indicate that the occupation of a propyl pocket is needed, at least within these classes of compounds, for activation of the 5-HT_7Rs. Compounds **36** and **37** were tested against a panel of about forty G protein-coupled receptors and ion channels. The results show high selectivity over other receptors and ion channels except for a few targets such as 5-HT_{1A} (**36**, K_i = 347 nM), 5-HT_5 (**37**, K_i = 65 nM), D_{2A} (**37**, K_i = 241 nM), α_2 (**37**, K_i = 188 nM). In a subsequent paper, the same research group presented a new series of 3-aminochromans regioisomers of **35** exemplified by the general formula **40** [110]. Whereas the phenyl derivative (Ar = Ph) does not bind the 5-HT_7Rs, the corresponding 2,6-dimethoxyderivative (Ar = 2,6-$(CH_3O)_2$Ph) shows only moderate affinity (K_i = 75.9 nM). The best compound of this series is the dimethyl analogue (Ar = 2,6-$(CH_3)_2$Ph) which possesses K_i affinity value for the 5-HT_7Rs of 13.4 nM and behaves as partial agonist. However, selectivity towards 5-HT_{1A} receptors is not achieved (K_i = 75.1 nM). Some conformational studies were performed to rationalize the difference in stereoselectivity and activity between compound **39** (the (*R*)-isomer) and compound **40** (Ar = 2,6-$(CH_3)_2$Ph, (*S*)-isomer). In the proposed fit with the receptor binding site the 8-aryl and 6-aryl substituents overlap.

Researchers from the Polish Academy of Sciences in 1994 reported the synthesis of MM-77, a potent and, by that time, selective ligand for the 5-HT_{1A} receptors (K_i = 6.4 nM) [111]. More recently they demonstrated that this derivative and its analogue NAN-190 possess moderate affinity for the 5-HT_7Rs (K_i = 90 and 87 nM, respectively) [112]. Moreover, to further investigate the influence of the spacer structure on 5-HT_7 and 5-HT_{1A} receptors, novel MM-77 and NAN-190 analogues were synthesized. An increase of 5-HT_7R affinity, but again a preference for the 5-HT_{1A} receptors, was observed for derivatives with a (*E*)-2-butenyl spacer chain **41** (5-HT_7 K_i = 63 nM, 5-HT_{1A} K_i = 29 nM) and **42** (5-HT_7 K_i = 36 nM, 5-HT_{1A} K_i = 5 nM) [112]. Moreover, in an attempt to optimize MM-77, a novel series of 1-aryl-4-(4-succinimidobutyl)piperazines was synthesized [113]. This class shows again preferential binding affinity in the low nanomolar range for the 5-HT_{1A}, but the compound 1-[4-(4-phenyl-1-piperazinyl)butyl]-2,5-pyrrolidinedione shows also moderate affinity for the 5-HT_7Rs (5-HT_7 K_i = 82 nM *versus* 5-HT_{1A} K_i = 7.4 nM).

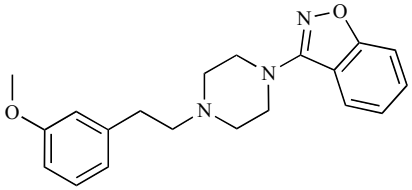
The first pharmacophoric hypothesis for 5-HT_7R antagonism was proposed from Lopez-Rodriguez and coworkers and was subsequently confirmed by the synthesis of novel naphtolactam and naphtosultam derivatives [29,114]. Moreover, Wilcox and coworkers reported a CoMFA study on a set of seventeen 5-HT_7R ligands using *R*-lisuride as template [115]. These models were reviewed in 2004 [61]. A pharmacophore model for 5-HT_7R agonism based on twenty agents belonging to different classes of 5-HT_7R agonists was proposed by Vermeulen and coworkers [116]. Moreover two CoMFA-based modeling of the agonist binding site were developed basing on different alignment of the agonists. Both models show good correlation between the experimental and predictive pK_i values and possess a high degree of similarity [116]. Subsequently, the same research group reported the synthesis and evaluation of novel 2-methoxyphenylpiperazine (2-MPP) and 1,2,3,4-tetrahydroisoquinoline (THIQ)-based arylsulfonamides (**43**) behaving as inverse agonists [117]. *N*-Ethyl-substituted sulfonamides with a connecting alkyl chain of three carbon atom and a properly substituted THIQ residue **45**-**47** showed the best affinities (K_i values comprised between 11 and 29 nM), with the exception of compound **44** possessing a 2-MPP residue (K_i = 19 nM). Moreover, computational studies on the basis of these novel inverse agonists and a selection of previously published ligands resulted in a pharmacophore model that shows similarities with the previously published model for 5-HT_7R agonism and antagonism and a CoMFA model with good correlation between experimental and predicted pK_i values [29,114,116].

Based on the above-mentioned models, pharmacophores were created by using genetic algorithm similarity program, which were used as queries to validate targeted libraries focused on 5-HT_7R ligands [118].

Lepailleur and coworkers defined a pharmacophore model for the 5-HT_7R antagonism taking into account the structure of selective 5-HT_7R ligands and comparing the obtained results with pharmacophores created for other serotonin receptors (5-HT_3, 5-HT_4, and 5-HT transporter) [119]. The proposed 3D pharmacophore consists of one basic centre, three hydrophobic groups, and one hydrogen-bond acceptor (HBA). This model led to the synthesis of compounds with general formula **49** based on the structure of compound **48**, a mixed 5-HT_7/5-HT_3 receptor ligand. The presence of a flexible chain linked to the tricyclic ring should allow a correct fit. A sulphur residue (X = S) in place of NH, CH_2 or O, seems to be optimal for binding with pK_i values at 5-HT_7Rs of 9.05 for compounds **50** and **51**, and 8.25 for compound **52**. Selectivity against 5-HT_3 and 5-HT_4 receptors was satisfactory; however, compounds **50** and **52** showed good affinity for the 5-HT transporter. The same research group reported the identification of new phenylpyrrole derivatives as 5-HT_7R ligands emerged from chemolibrary virtual screening [120, 121]. Some phenylpyrroles were obtained with a similarity value greater than 0.90, using the Tanimoto similarity coefficient performing 2D similarity search using 1-(1-naphthalenyl)piperazine as the query molecule (5-HT_7 K_i = 83 nM). Binding assays for this class of compounds were performed on rat 5-HT_7Rs and compound **53** possesses a percentage of inhibition of 91% at 10^{-6} M. Structural modifications were performed to improve the 5-HT_7R affinity. Best results were obtained with 3-substituted arylpyrroles (**54**-**56**) and the presence of a 2-methoxyphenyl residue instead of 1-naphthalenyl moiety led to further increase of affinity. These compounds were evaluated for their affinity on human 5-HT_7Rs (**54**, K_i = 4.7 nM; **55**, K_i = 5.4 nM; **56**, K_i = 18 nM). In functional assays compounds **55** and **56** behaved as antagonists, in contrast compound **54** was a partial agonist. With regard to selectivity, compounds **54**-**56** were found to be selective towards 5-HT_7 *versus* 5-HT_6 receptors; however they exhib-



23 general structure
24 R = 2-OH, n = 5, R_1 = 1,2-benzisoxazol-3-yl
25 R = 2-OCH_3, n = 5, R_1 = 1,2-benzisoxazol-3-yl



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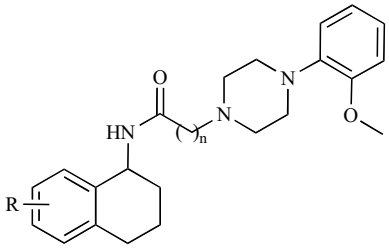
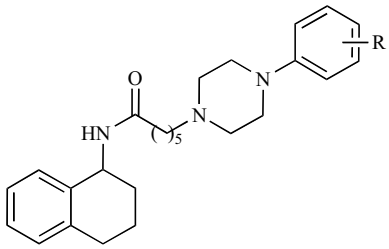
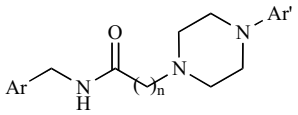


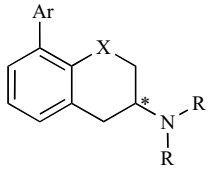
27 general structure
28 R = H, n = 4
29 R = H, n = 5



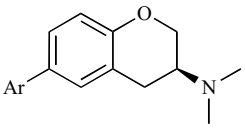
30 general structure
31 R = 2-$COCH_3$
32 R = 2-OH
33 R = 2-SCH_3



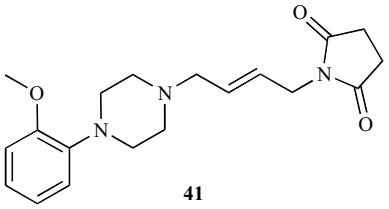
34 general structure



35 general structure
36 * = S, Ar = 2,6-$(CH_3O)_2$Ph, X = CH_2, R = $(CH_2)_2CH_3$
37 * = *S*, Ar = 2,6-$(CH_3O)_2$Ph, X = CH_2, R = CH_3
38 * = *R*, Ar = 2,6-$(CH_3O)_2$Ph, X = O, R = $(CH_2)_2CH_3$
39 * = *R*, Ar = 2,6-$(CH_3O)_2$Ph, X = O, R = CH_3



40 general structure



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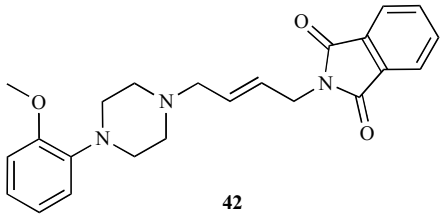

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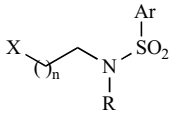
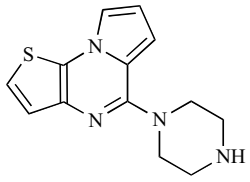


43 general structure
44 X = 2-MPP, R = CH_3CH_2, Ar = 4-CH_3Ph, n = 2
45 X = 6-CH_3O-THIQ, R = CH_3CH_2, Ar = 4-CH_3Ph, n = 2
46 X = 6-CH_3O-THIQ, R = CH_3CH_2, Ar = 4-CH_3OPh, n = 2
47 X = 6-CH_3O-THIQ, R = CH_3CH_2, Ar = 1-naphthyl, n = 2



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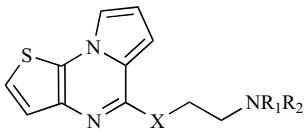


49 general structure
50 X = S, R_1 = R_2 = H
51 X = S, R_1 = CH_3, R_2 = H
52 X = S, R_1 = R_2 = CH_3

Chart (2).

ited high affinity for 5-HT_{1A} receptors. Moreover, optimization of 5-HT_7R pharmacophore model and molecular modeling studies focused on 5-HT_7 *versus* 5-HT_{1A} receptor selectivity were reported [121].

Very recently, Bojarski reviewed all the pharmacophore models for metabotropic 5-HT receptor ligands, including 5-HT_7Rs and proposed two receptor-based pharmacophores for serotonin 5-HT_7R antagonism [122]. For the generation of these pharmacophores, ligand-receptor interactions of representatives of different classes of 5-HT_7R antagonists with a repertoire of 5-HT_7R models were investigated using a fully flexible docking approach [122a]. This approach allows the ligand to adopt its best conformation fitting into a receptor binding site with the most suitable conformations of amino-acid side chains. The thirty-one 5-HT_7R antagonists used in this study were divided into six different chemical classes: (1) ergolines; (2) aporphines; (3) tricyclic psycotropic agents

such as clozapine; (4) arylpiperidines, arylpiperazines, and β-carbolines; (5) arylsulfonamidoalkylamines; (6) diaminopyridines, diaminopyrimidines, diaminotriazines, 2-aminotetralines. The 5-HT$_7$R homology model was generated using bovine rhodopsin (in its inactive state) as a template for the transmembrane heptahelical bundle of the receptor protein. During docking, for all the ligands, the essential ionic interaction with Asp3.32 (according to Ballesteros-Weinstein's nomenclature) was constrained [123]. From the analysis of top-scored ligand-receptor complexes, two different pharmacophore models were generated: the first one describes the features necessary for affinity and the second defines those critical for selectivity of 5-HT$_7$R antagonists. The binding site, in which Asp3.32 provides the main anchoring interaction with the ligand, can be subdivided into two fairly symmetrical pockets: one is positioned between TMs 4-6 and the other is encompassed by TMs 7-3. "Affinity" pharmacophore is constituted by six features: a protonated nitrogen (positive ion, PI, forming a salt bridge with Asp3.32), three hydrophobic aromatic regions (AR1, located in the pocket between TMs 7-3; AR2 and AR3 placed in the pocket between TMs 4-6), and two HBA (HBA1 and HBA2, one for each pocket). At least three of these features are needed for an appreciable 5-HT$_7$R affinity. PI and one of the ARs are essential whereas one HBA or another AR may be the third. However, this "affinity" model was generated using non-selective 5-HT$_7$R antagonists and, consequently, it shares much with other pharmacophore models for monoamine receptors. On the other hand the selectivity pharmacophore was based on selective antagonists only and defines three essential features providing exclusive binding at 5-HT$_7$Rs. Two of them are PI and AR1 (each other arranged 6.9-7.7 Å apart) whereas the third can be either HBA1 (H-bond acceptor close to Tyr7.43) set at 3.7-5.1 Å from PI or AR2 placed in the pocket between TMs 4-6 and 4.3-7.0 Å apart from PI. According to this "selectivity" hypothesis, the spatial arrangement of the ligand moiety containing AR1 is critical for an optimal fit with the binding site. The aromatic plane of the ligand should be suitably placed to allow an aromatic π-π stacking interaction with Phe3.21 and, optimally, an ion-π interaction with Arg7.36. This is the case, for example, of some selective 5-HT$_7$R antagonists belonging to the arylsulfonamidoalkylamine class. In fact, in SB-269970 (**3**) the protonated piperidine nitrogen forms a salt bridge with Asp3.32 whereas the arylsulfonamido moiety is thought to enter the pocket between TMs 7-3; thus, the aryl ring is able to form π-π and ion-π interactions with Phe3.21 and Arg7.36, respectively, and sulfone oxygen is H-bonded to Tyr7.43. The "selectivity" model presented in Bojarski's study suggests that only three pharmacophoric features (PI, AR1, and HBA1 or AR2) in the structure of the ligand are enough for a selective binding to the 5-HT$_7$R; moreover, this model highlights the importance, in the ligand-receptor complex, of some specific interactions with the side chain of residues, such as Arg7.36, located into the pocket between TMs 7-3, giving clues for the design of novel selective 5-HT$_7$R antagonists.

Besides to the publication of papers regarding the identification of novel ligands for the 5-HT$_7$Rs and the development of pharmacophore models, a number of patents have been filed during the last three years. This continued interest from pharmaceutical companies underscores the significance of 5-HT$_7$Rs as potential therapeutic target above all in the treatment of CNS diseases.

Novel quinoline derivatives represented by the general formula **57** were claimed by Pfizer as 5-HT$_7$R agonist for the treatment of CNS disorders, including depression and disorders that can be treated by modulating circadian rhythms [124]. About 40 compounds are stated to behave as full or partial agonists and bind with moderate to high affinity (K_i values ranging from approximately 3.5 nM to 5 μM) to rat 5-HT$_7$Rs expressed into HEK-293 cells. Among them, 3-methyl-8-piperidin-3-yl-quinoline (**58**) showed a K_i value of about 7.6 nM. Specific pharmacological tests to evaluate antidepressant properties of the claimed compounds are reported.

An application from Merck&Co. reported the discovery of novel heterocycloalkyl sulfones having 5-HT$_7$R affinity for the treatment and/or prevention of adverse conditions of the central nervous system, such as depression, anxiety, sleep disorders, and psychotic disorders such as schizophrenia [125]. These compounds possess typically a K_i of 100 nM or less and are endowed with at least 10-fold selective affinity and preferably 50-fold affinity for the human 5-HT$_7$Rs over other human 5-HT receptors (in particular 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{2A}$) and to the human dopamine D$_2$ receptors. However, no specific affinity or selectivity data were disclosed. In a subsequent paper, the same research group reported binding data for a novel series of aminoalkylphenyl sulfones [126]. Compounds of general formula **59** were synthesized basing on modelling studies on compound **60** suggesting an overlap with one of the low energy conformation of SB-269970 (**3**). SAR studies on this cyclobutyl series (**59**) showed remarkable improvement in binding affinity in the presence of a phenyl residue linked to a piperazine or pyrrolidine ring (**62** and **63**, K_i = 22 and 9 nM, respectively) as well as with THIQ residue (**61**, K_i = 8 nM) with respect to aliphatic amines such as piperidine, morpholine, dimethylamine, etc. (K_i > 500 nM). The three carbon linker afforded best compounds and was chosen for further SAR evaluation. In functional assay compound **61** behaves as antagonist, but its selectivity against related human serotonin receptors was unsatisfactory (5-HT$_{1A}$ K_i = 35 nM, 5-HT$_{2A}$ K_i = 65 nM). An interesting improvement in selectivity was obtained by substituting THIQ residue with (4a*S*,8a*S*)decahydroisoquinoline (**64**, 5-HT$_7$ K_i = 6 nM, 5-HT$_{1A}$ K_i = 210 nM, 5-HT$_{2A}$ K_i = 570 nM). Substitution of the cyclobutyl ring with hydrogens was detrimental for affinity, while *gem*-dimethyl substitution resulted in compounds with excellent affinity and selectivity profile. As such an example, compound **65** compared with **64** while maintaining 5-HT$_7$Rs affinity (K_i = 8 nM) is endowed with remarkably higher selectivity (5-HT$_{1A}$ K_i = 1500 nM, 5-HT$_{2A}$ K_i = 2000 nM, D$_2$ K_i = 900 nM, 5-HT$_{1B}$ K_i > 7000, α_1 K_i > 3500, hERG K_i > 4000). However compound **65** behaves as partial agonist. High binding affinity towards 5-HT$_7$Rs was maintained by introducing different Y substituents affording compounds like **66** (K_i = 15 nM) or **67** (K_i = 7 nM) both possessing interesting selectivity toward 5-HT$_7$Rs among tested receptors (5-HT$_{1A}$, 5-HT$_{2A}$, D$_2$, 5-HT$_{1B}$, α_1, hERG) and endowed with antagonistic properties.

Researchers from Ajinomoto Co. claimed the discovery of novel 4-benzylidenopiperidine derivatives **68**-**73** endowed

with high affinity for the 5-HT$_7$Rs [127]. Best results were obtained with compounds in which X = OCH_3 and Y = 3-chlorophenyl residue (**68**, pIC_{50} = 8.0; **69**, pIC_{50} = 7.7; **70**, pIC_{50} = 8.3). Nevertheless, a 2-thienyl residue (**71**) gave interesting results (pIC_{50} = 8.2). When tested in functional assay, compounds **68**-**70** showed respectively EC_{50} values of 99, 87, and 23 nM. Other interesting compounds are **72** and **73** which possess pIC_{50} values of 8.2 and 8.0, respectively.

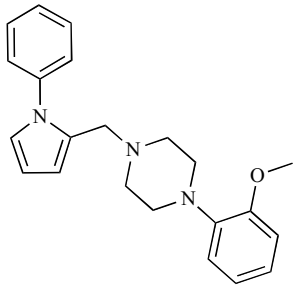
At Mitsubishi Pharma Corp. new derivatives with mixed 5-HT$_7$R antagonist and muscarinic M4 receptor agonist activity were developed [128]. These compounds were claimed as useful agents to a novel approach for the treatment of schizophrenia and/or bipolar disorder. Indeed there is some evidence that M4 agonists may show antipsychotic-like activity in some tests. In addition, some of the more effective atypical antipsychotic drugs have significant 5-HT$_7$R affinity as part of their complex pharmacological profile, such as clozapine. In view of this assumption, compounds were synthesized with M4/5-HT$_7$ receptor mixed affinity, such as **74** and **75** which show 5-HT$_7$Rs K_i values of 0.4 and 2.7 μM, respectively, and M4 K_i values of 0.32 and 2.8 μM, respectively, with no interference with dopaminergic D_2 receptors (K_i > 300 μM). Moreover compound **74** was able to reduce cAMP levels, and the effect was blocked by the muscarinic antagonist atropine.

In a recent paper, subsequent to a patent application from the same group, Lattmann and coworkers claimed the synthesis of a novel series of *N*-substituted-*N*-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]arylsulfonamides and their use as antidepressants [129,130]. SB-258719 (**2**) was used as starting template for variation, and the sulfonamide moiety was modified in a combinatorial fashion (**76**). A convergent synthesis of the sulfonamide derivatives was set up. About twentyfour amines were combined with fifteen sulfonyl chlorides to afford intermediates that were alkylated by the means of the chemically reactive 1-(3-iodobutyl)-4-methylpiperidine. Initial screening showed some interesting compounds that were purified by chromatography and their IC_{50} were determined. Best results were obtained when the methyl group of SB-258719 was substituted with an aromatic moiety. Compounds **77** and **78** (IC_{50} values of 61 and 83 nM, respectively) were selected for further evaluation in mice. Both sulfonamides, **77** and **78**, were found efficacious in antidepressant activity animal models.

Researchers at Solvay Pharmaceuticals filed a patent application claiming the use of 1-[2*H*]-1-benzopyran-2-one-8-yl]piperazine derivatives for the treatment of movement disorders [131]. Surprisingly, the non-selective 5-HT$_{1A}$ agonist **79** was found, in a test for antiepileptic activity, more active than selective derivatives. Based on results from other *in vivo* tests for specific 5-HT$_{1A}$ agonism, the authors suggest a non 5-HT$_{1A}$ mechanism of action in anti-seizure activity of **79**. Screening against a panel of approximately a hundred receptors and channels for compound **79** revealed high affinity for the serotonin 5-HT$_{1B}$ (K_i = 2.0 nM), 5-HT$_{1D}$ (K_i = 13 nM), and 5-HT$_7$ (K_i = 3.2 nM) receptors. In functional assays compound **79** behaves as 5-HT$_{1A}$ receptor agonist, 5-HT$_{1D}$ receptor antagonist and, as 5-HT$_7$R agonist [131,132]. It was demonstrated that antiepileptic activity of compound **79** was partially antagonized by the use of a selective 5-HT$_{1D}$ receptor agonist (sumatriptan) as well as the selective 5-HT$_7$R antagonist SB-258741. The authors suggest that this unique pharmacological profile for **79** makes this compound a good drug candidate for the treatment of epilepsy with reduced side effects and able to be used in patients resistant to antiepileptic drug therapy.

Researchers from Egis Gyogyszergyar Pharmaceuticals filed three patent applications claiming the discovery of novel piperazine or pyridine derivatives of alkyl oxindoles as 5-HT$_7$R active agents useful in the treatment of CNS or cardiovascular disorders [133-135]. These series of compounds (**80**, **84**, and **85**) can be regarded as analogues of the abovementioned Meiji Seika Kaisha Ltd compounds (**6**-**9**), with somewhat easier chemical accessibility, but with lower selectivity. However, only limited biological data were provided in the three patents and no functional data. Regarding the general structures (**80**, **84**, and **85**), although variation in the spacer length is claimed, only *n*-butyl derivatives were reported as example and the nitrogen of the oxindole ring is always unsubstituted. Thirteen compounds of general formula **80**, claimed in the first application, are stated to be ligands for the 5-HT$_7$Rs with K_i values < 100 nM [133]. Some of them are also ligands for the α_1 adrenoceptors with K_i values < 50 nM (**81** and **82**) and inhibitor of the 5-HT re-uptake with K_i < 100 nM (**81**-**83**) [133]. Analogously, seven compounds of general formula **84**, claimed in the second application patent, behaved as mixed 5-HT$_7$/α_1 receptor ligands with IC_{50} values < 100 nM at both receptors [134]. R is typically hydrogen or a fluorine residue placed at the 5- or 6-position, Q is a nitrogen; when Q is a CH residue the obtained phenyl ring is variously substituted, preferably R_1 and R_2 taken together form ethylenedioxy chain and R_3 is a chlorine at the 7-position. Finally, ten compounds represented by the general formula **85**, and object of the third patent application, behaved as 5-HT$_7$R ligands and, two of them, showed inhibition of 5-HT re-uptake (**86** and **87**) with IC_{50} values < 100 nM [135,136].

A patent application from Janssen Pharmaceutica claims the discovery of fused heterocyclic compounds as 5-HT$_7$R modulators, represented by the general formulae **88**-**90** [137]. The authors report more than three hundreds of examples, and affinity data toward 5-HT$_7$Rs for almost a hundred compounds with noteworthy K_i values (< 10 nM). Only a few examples of derivatives possessing a 4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine or 4,5,7,8-tetrahydro-1*H*-pyrrolo [2,3-*d*]azepine scaffolds are described. The most interesting compound is **91** with a K_i value of 18 nM, but no selectivity data are reported (Table **1**). In general, tested compounds are represented mainly by 1,4,5,6,7,8- or 2,4,5,6, 7,8-hexahydro-1,2,6-triaza-azulene scaffolds (**89** and **90**, n = 2) with unsubstituted nitrogen at the 6-position (R_1 = H). Substitution of this nitrogen with an arylmethyl residue is, generally, detrimental for affinity; smaller alkyl substituents (such as methyl, ethyl, or propyl chain) are well tolerated. Among compounds with general formula **89**, the most interesting derivatives mainly present a 4-chlorophenyl residue at the 3-position of the pyrazole ring and a substituted phenyl ring (CYC) with $(ALK)_q$ = CH_2 (Table **1**). As such an example, compound **92** possesses satisfactory profile in term of affinity towards 5-HT$_7$Rs and selectivity over other tested sero-



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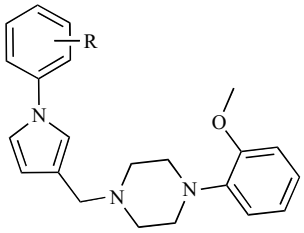
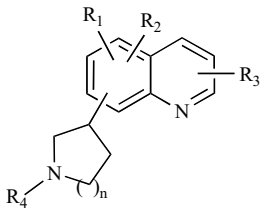


54 R = 2-CH_3
55 R = 2-CN
56 R = 2-$COOCH_3$, 5-CH_3



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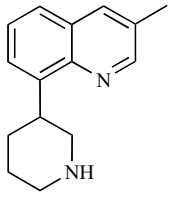

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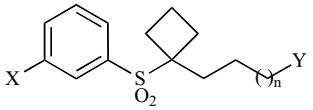
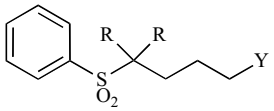
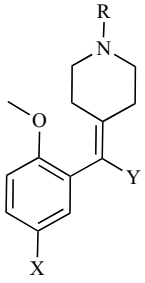


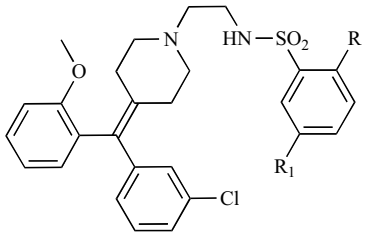
59 general structure
60 X = OH, Y = 4-methylpiperidin-1-yl, n =1
61 X = H, Y = THIQ, n = 1
62 X = H, Y = 4-phenylpiperazin-1-yl, n = 1
63 X = H, Y = 3-phenylpyrrolidin-1-yl, n = 1
64 X = H, Y = (4a*S*,8a*S*)-decahydroisoquinolin-1-yl, n = 1



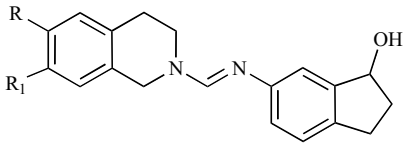
65 R = CH_3, Y = (4a*S*,8a*S*)-decahydroisoquinolin-1-yl
66 R = CH_3, Y = (3-oxazol-5-yl)piperidin-1-yl
67 R = CH_3, Y = 3-phenylpiperidin-1-yl



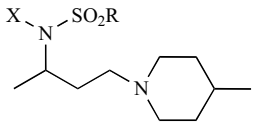
68 R = CH_3, Y = 3-ClPh, X = OCH_3
69 R = H, Y = 3-ClPh, X = OCH_3
70 R = *n*Pr, Y = 3-ClPh, X = OCH_3
71 R = CH_3, Y = 2-thienyl, X = OCH_3



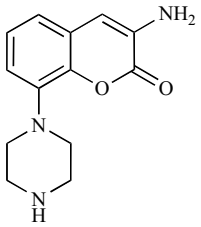
72 R = R_1 = OCH_3
73 R = R_1 = Cl



74 R = R_1 = OCH_3
75 R = R_1 = H



76 general structure
77 X = 4-CH_3Ph, R = biphenyl
78 X = 1-naphtyl, R = 3,4-$(OCH_3)_2$Ph



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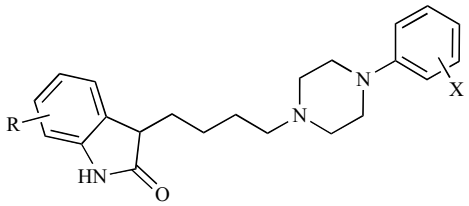


80 general structure
81 R = H, X = 3-Cl
82 R = H, X = 4-Cl
83 R = 6-F, X = 4-Cl

Chart (3).

tonergic receptors. The introduction of substituents on the phenylmethyl residue (compounds **93**-**96**) while maintaining the affinity for 5-HT_7Rs is favourable for selectivity. Analogously, the methylation of the azepine nitrogen is favourable for selectivity (**97**); ethylation is a bit detrimental for selectivity towards 5-HT_{2A} receptors (**98**). Compounds represented by the general formula **90**, while affording derivatives with interesting K_i values for the 5-HT_7Rs, in general, possess even higher affinity for the other tested 5-HT_2 receptor subtypes (as such an example derivative **99**). The compounds in the above-mentioned invention are stated to behave as 5-HT_7R modulators and many are claimed to behave as

Table 1. Binding Affinities for Compounds 91-99[a]

Compound	K_i (nM) 5-HT$_7$	K_i (nM) 5-HT$_{2A}$	K_i (nM) 5-HT$_{2B}$	K_i (nM) 5-HT$_{2C}$
91	18	NT	NT	NT
92	6	280	160	74
93	5	100	94	180
94	7	200	100	320
95	8	210	350	690
96	7	300	350	3500
97	4	100	310	180
98	8	80	560	590
99	6	1.1	1.4	12

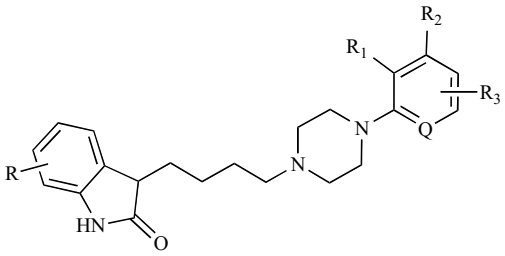
[a]Receptors and radioligand used in binding assays: rat 5-HT$_7$ ([^{3}H]-5-CT); human recombinant 5-HT$_{2A}$ ([^{3}H]ketanserin); human recombinant 5-HT$_{2B}$ ([^{3}H]mesulergine); human recombinant 5-HT$_{2A}$ ([^{3}H]mesulergine).

antagonists; however only for compound **92** functional data are reported. Where the compounds possess substantial 5-HT$_7$R antagonist activity, the authors affirm that they might be useful in the treatment or prevention of depression/anxiety, sleep/wake disturbances, jetlag, migraine, urinary incontinence, gastric motility, and irritable bowel disorders.

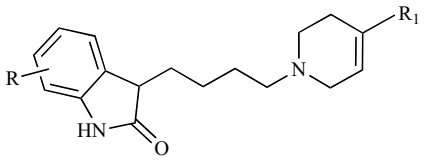
More recently, Torrens and coworkers disclosed novel 5-HT$_7$R antagonists characterized by a THIQ scaffold linked to a sulfonyl residue through an alkylamino linker or a methylene to the 3- or 4-position of a piperidine ring (general formulae **100** and **103**) [138-140]. Concerning compounds with general structure **100**, claimed in the first application, the alkyl spacer (n ≥ 2) is preferably a *n*-butyl chain, although some examples with n = 3 or 4 are reported; good affinities were obtained when R_1 and R_2 are hydrogens or methoxy residues and R_3 is an aryl or heteroaryl ring. More than three hundreds compounds are described, but IC_{50} values for 5-HT$_7$Rs are reported for a limited number of compounds and are comprised between 31 and 80 nM. In these compounds n is always 2, and R_3 is a 2-naphtyl or a substituted phenyl ring. The best compound is **101** and possesses an IC_{50} value of 31 nM. Tested compounds are claimed to be selective over a number of receptors such as serotonin 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_4$, and 5-HT$_{5A}$, dopamine D_l, D_2, D_3, and D_4, adrenergic α_{lA}, α_{lB}, β_l, and β_2 receptors, tachykinin NK-1, opiate GABA, estrogen, glutamate, adenosine, nicotinic, muscarinic receptors, and calcium, potassium, and sodium channels and neurotransmitter transporters (serotonin, dopamine, norepinephrine, GABA). Object of the second patent are compounds with general formula **100** differing from the previous application only for the alkyl chain spacer between the THIQ and the sulfonamide residue. This spacer is always a propyl residue (n = 1) [139]. More than a hundreds of compounds are described, but IC_{50} values for 5-HT$_7$Rs are reported for five compounds only and are comprised between 65 and 380 nM. The best compound is **102** possessing IC_{50} value of 65 nM. The shortening of the alkyl chain, when compared with the previous series of compounds, is detrimental for affinity (compare **102** *vs* **101**). Tested compounds are claimed to be selective over a number of other receptors. The third application patent is represented by compounds of general formula **103** [140]. IC_{50} values for the 5-HT$_7$Rs are reported only for eight derivatives and are comprised between 17 and 84 nM. In this series afforded compound **104** with a 4-methylpiperidine linker possesses the highest affinity for 5-HT$_7$Rs among the three application patent (IC_{50} = 16.8 nM). Tested compounds are claimed to be selective towards a number of receptors such as serotonin 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_4$, 5-HT$_{5A}$ receptors, dopaminergic D_1, D_2, D_3, D_4 receptors and α_{1A}, α_{1B}, α_{1D}, β_1, and β_2 adrenoceptors. No affinity data are reported for 3-methylpiperidine spacer. Derivatives with general structures **100** and **103** are stated to be antagonists for the 5-HT$_7$Rs, although no functional data are reported.

3. POTENTIAL THERAPEUTIC IMPLICATIONS FOR 5-HT$_7$ RECEPTOR LIGANDS

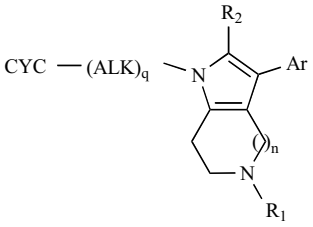
The widespread distribution of 5-HT$_7$Rs in the brain is suggestive of multiple central roles, though investigation of functional significance of 5-HT$_7$Rs has been hampered for many years by the relative lack of specific tool drugs. Initial progresses in the elucidation of potential therapeutic function of 5-HT$_7$Rs have been made using unselective ligands in the presence of masking agents. The pharmacological aspects of 5-HT$_7$Rs have been reviewed in 2004 [62,63,65,66]. Subsequently, the use of selective 5-HT$_7$Rs ligands, such as SB-269970 (**3**), helped to clarify the pharmacological profile and the functional role of these receptors. During the last three years, 5-HT$_7$Rs have been proposed to be involved in learning and memory [141-143], sleep circadian rhythms [144-147], depression, anxiety, and mood [147-150], psychiatric disorders [151,152], hippocampal activity [153,154], thermoregulation [155-159], nociception [160-162], inflammatory processes [163-167], epilepsy [131,168], locomotor



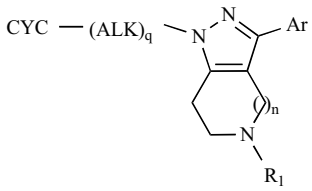
84 general structure



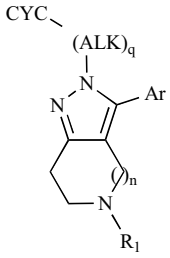
85 general structure
86 R = H, R_1 = 4-ClPh
87 R = 5-F, R_1 = 3-CF_3Ph



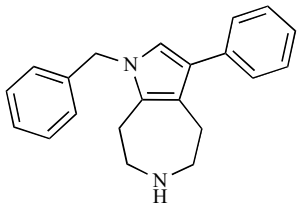
88 general structure



89 general structure



90 general structure



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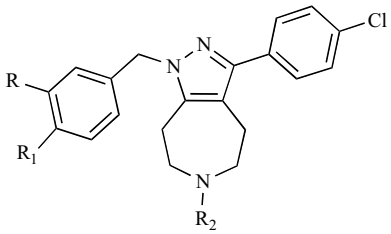
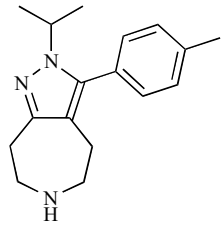


92 R = R_1 = H, R_2 = H
93 R = CH_3, R_1 = H, R_2 = H
94 R = H, R_1 = F, R_2 = H
95 R = F, R_1 = H, R_2 = H
96 R = F, R_1 = OH, R_2 = H
97 R = R_1 = H, R_2 = CH_3
98 R = R_1 = H, R_2 = CH_2CH_3



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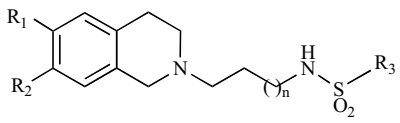
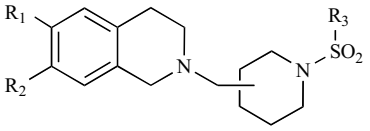
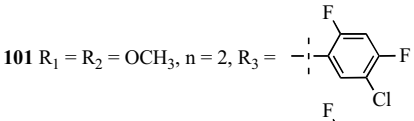


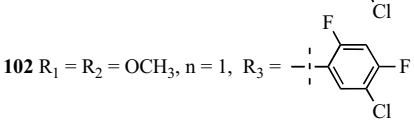
100 general structure



103 general structure
104 R_1 = R_2 = H, R_3 = 2-BrPh



101 R_1 = R_2 = OCH_3, n = 2, R_3 =



102 R_1 = R_2 = OCH_3, n = 1, R_3 =

Chart (4).

activity [169], endocrine and cardiovascular function [170-173], ileum peristalsis [174,175], and micturition reflex [176-178]. However, for sake of shortness, only some pharmacological aspect of 5-HT_7Rs will be reviewed.

3.1. Depression, Anxiety, Mood Diseases, and 5-HT_7R Antagonists

The affinity of several antidepressant and antipsychotic drugs for the 5-HT_7Rs, along with their peculiar distribution

in the CNS, suggested the involvement of 5-HT$_7$Rs in the physiopathology of depression. However, it was only recently demonstrated the direct involvement of these receptors in depression, anxiety and mood diseases [124,129,130, 147-150]. In two different behavioural models of depression, the Porsolt forced swim test and the tail suspension test, 5-HT$_7$R knockout mice showed in both tests a significant decrease in immobility [148]. Interestingly, wild types treated with the selective 5-HT$_7$R antagonist SB-258719 (**2**), did not exhibited a significant change in immobility, unless animals were tested in the dark. This peculiar behaviour further confirm an involvement of 5-HT$_7$Rs in circadian rhythm. Indeed, administration of the antagonist SB-269970 (**3**) showed antidepressant-like activity in both tests [147]. This indicates that the observed effects are truly dependent on the 5-HT$_7$Rs and not the result of compensatory mechanisms during development in the 5-HT$_7$R knockout mice. Moreover, 5-HT$_7$R knockout mice showed an altered sleep pattern, spending less time in REM, and this altered sleep pattern is also predictive of an antidepressant-like activity of 5-HT$_7$R antagonists [147]. Finally, Wesełowska and coworkers, besides reconfirming the involvement of 5-HT$_7$Rs in depression, suggested that 5-HT$_7$R antagonists may play an important role in the treatment of anxiety [149]. Indeed, SB-269970 produced an anxiolytic-like effect in different behavioural tests such as rat conflict drinking tests, rat elevated plus-maze test, and mouse four-plate test [149]. This latter effect was observed at lower doses (0.5 or 1 mg/kg) of SB-269970 whereas antidepressant-like activity appeared at higher doses (5 or 10 mg/kg). The authors suggest that this difference may result from interaction with diversely localized 5-HT$_7$Rs and/or by different mechanism of action. More recently they demonstrated that the hippocampus is one of the neuroanatomical structures involved in the potential anxiolytic and, in particular, antidepressant activity of SB 269970 [150]. Moreover, SB-269970 seems to be devoid of any visibly unfavourable motor effects [149]. Thus the use of 5-HT$_7$R antagonists may be useful in the treatment of depression, anxiety, and mood diseases.

3.2. Thermoregulation and 5-HT$_7$R Agonists

The hypothermic response to 5-HT is mediated, among others serotonergic receptors, by the action at 5-HT$_7$Rs [155,156]. In support, there are high levels of 5-HT$_7$Rs in the anteroventral preoptic region. More recently, Gargaglioni and coworkers, by the use of SB-269970, demonstrated the involvement of 5-HT$_7$Rs in models of hypoxia-induced hypothermia and demonstrated that 5-HT$_7$Rs in the anteroventral preoptic region exert an inhibitory modulation on the hypoxic ventilatory response [157,158]. This inhibitory action together with decreased body temperature is a very effective way of reducing hypoxia-induced cell damage and the understanding of the mechanisms underlying this phenomenon may give rise to novel therapeutic approaches to diseases such as stroke or cerebral ischemia. Moreover, it was demonstrated the ability of 8-OH-DPAT, a mixed 5-HT$_{1A}$/5-HT$_7$ receptor agonist, to reverse opioid-induced respiratory depression, by improving pulmonary circulation [159]. Thus the use of 5-HT$_7$R agonists may be useful in the treatment of stroke, cerebral ischemia and opioid induced respiratory depression.

3.3. Motor Activity of the Gastrointestinal Tract and 5-HT$_7$R Antagonists

The involvement of 5-HT in motor activity of the gastrointestinal tract is well documented and enterochromoaffin cells of the enteric mucosa are the main source in the body of this neurotransmitter. In guinea pig ileum, 5-HT produces muscle relaxation through the direct activation of 5-HT$_7$Rs present in the smooth muscle cells of gut and indirectly through the stimulation of neuronal 5-HT$_7$Rs [174]. Overstimulation of these receptors might contribute to abdominal symptoms in inflammatory bowel diseases and targeting 5-HT$_7$Rs with antagonists could offer new therapeutic options. The role of serotonergic receptors in gastrointestinal disorders was recently reviewed by Neal and Bornstein [175].

3.4. Micturition and 5-HT$_7$R Antagonists

Recent studies reported that 5-HT$_7$Rs are involved in the micturition reflex, having an excitatory physiological role in the control of bladder function [176,177]. Administration in anaesthetized rats of SB-269970, a 5-HT$_7$R antagonist, caused an increase in bladder pressure and volume threshold and at higher doses abolished the micturition reflex. SB-656104 had a similar effect. Taken together these results suggest that 5-HT$_7$Rs play an essential role in micturition along with other serotonergic receptor subtypes. Moreover, D'Agostino and coworkers demonstrated the presence of peripheral excitatory 5-HT$_7$Rs that control parasympathetic drive in the human detrusor and suggested the possible use of 5-HT$_7$R ligands in the control of bladder overactivity dysfunctions [178].

3.5. Epilepsy and 5-HT$_7$R Antagonists

An involvement of 5-HT$_7$Rs in epilepsy has been proposed in 1997 based on the capability of a number of non-selective 5-HT$_7$R antagonists, including mianserin, mesulergine, and ritanserine, to protect DBA/J2 mice from audiogenic seizures [67]. Later on, Graf and coworkers studied the behaviour of selective 5-HT$_{1A}$ and 5-HT$_7$R antagonists (WAY-100635 and SB-258719, respectively) in WAG/Rij rat model of absence epilepsy [168]. In general, both compounds reduced epileptic activity when compared to vehicle. Particularly, SB-258719 significantly reduced the cumulative duration of spike-wave discharges as well as the number and the average of paroxysms. Thus the use of 5-HT$_7$R antagonists may be useful in the treatment of absence epilepsy. However, the role of 5-HT$_7$Rs in epilepsy seems to be complex and not yet clear. Indeed, researchers at Solvay Pharmaceuticals suggested a possible beneficial role of compound **79**, a mixed 5-HT$_{1A}$ receptor agonist, 5-HT$_{1D}$ receptor antagonist and, 5-HT$_7$R agonist, in DBA/2 mice. They reported the ability of this compound to antagonize the tonic and the clonic phase of audiogenic seizures. In the same experiment, flesinoxan (a 5-HT$_{1A}$ receptor agonist) did not significantly antagonized audiogenic seizures; 8-OH-DPAT and SB-258741 antagonized only the tonic phase at a dose of 15 mg/kg. Interaction studies in DBA/2 mice with compound **79** and sumatripam (a selective 5-HT$_{1D}$ receptor agonist) or SB-258741 (a selective 5-HT$_7$ antagonist) showed that the pretreatment with either SB-258741 or sumatripam was able to shift the dose-response curve of **79** to the right, being suma-

tripam more effective than SB-258741. This experiment suggests a possible beneficial role for 5-HT$_7$R agonists in the treatment of epilepsy.

CONCLUDING REMARKS

Over the last three years 5-HT$_7$Rs have seen many progresses. These progresses, made in the discovery of selective ligands and in the development of novel and more specific pharmacophore models for agonism and antagonism along with a number of studies dedicated to clarify their functional role, reconfirm the high interest in this field.

Nowadays a sufficient number of useful and selective antagonists have been identified and the wide variety of functions in which these receptors are implicated make the 5-HT$_7$Rs a fascinating target for future therapeutic agents.

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ABBREVIATIONS

AR = Aromatic region

CNS = Central nervous system

5-CT = 5-Carboxyamidotryptamine

DR-4004 = 2*a*-[4-(3,6-Dihydro-4-phenyl-1(2*H*)-pyridinyl)butyl]-2*a*,3,4,5-tetrahydrobenz[*cd*]indol-2(1*H*)-one

DR-4446 = (1-Methyl-2*a*-[4-(4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-5-yl)butyl]-2*a*,3,4,5-tetrahydro-1*H*-benz[*cd*]indole-2-one)

DR-4485 = 6-Chloro-2*a*-[4-[4-(4-chlorophenyl)-3,6-dihydro-1(2*H*)-pyridinyl]butyl]-2*a*,3,4,5-tetrahydrobenz[*cd*]indol-2(1*H*)-one

DR-4365 = 2*a*-[4-(6,7-Dihydro-3-methylthieno[3,2-*c*]pyridin-5(4*H*)-yl)butyl]-2*a*,3,4,5-tetrahydrobenz[*cd*]indol-2(1*H*)-one

HBA = Hydrogen-bond acceptor

5-HT = Serotonin

5-HT$_7$R = 5-HT type-7 receptor

HTS = High-throughput screening

LY-215480 = [8β(1*S*,2*R*)]-*N*-(2-Hydroxycyclopentyl)-6-methyl-1-(1-methylethyl)-ergoline-8-carboxamide

LY-53857 = (8β)-6-Methyl-1-(1-methylethyl)-ergoline-8-carboxylic acid 2-hydroxy-1-methylpropyl ester, (2Z)-butanedioic acid salt

MM-77 = 1-[4-[4-(2-Methoxyphenyl)-1-piperazinyl]butyl]-2,5-pyrrolidinedione

NAN-190 = 2-[4-[4-(2-Methoxyphenyl)-1-piperazinyl]butyl]-1*H*-isoindole-1,3(2*H*)-dione monohydrobromide

8-OH-DPAT = 7-(Dipropylamino)-5,6,7,8-tetrahydro-1-naphthalenol

PI = Positive ion

RU-24969 = 5-Methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-indole butanedioic acid salt

SAR = Structure activity relationships

SB-258719 = (*R*)-*N*,3-Dimethyl-*N*-[1-methyl-3-(4-methyl-1-piperidinyl)propyl]benzenesulfonamide

SB-258741 = (2*R*)-1-[(3-Methylphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine

SB-269970 = (2*R*)-1-[(3-Hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine

SB-656104 = (2*R*)-2-[2-[4-(4-Chlorophenoxy)-1-piperidinyl]ethyl]-1-(1*H*-indol-6-ylsulfonyl)pyrrolidine

SB-691673 = 2-[2-[4-(5-Fluoro-2,3-dihydro-2-oxo-1*H*-benzimidazol-1-yl)-1-piperidinyl]ethyl]-2,3,4,5-tetrahydro-1*H*-2-benzazepin-1-one

THIQ = 1,2,3,4-Tetrahydroisoquinoline

TMs = Transmembrane domains

WAY-100635 = N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexanecarboxamide, hydrochloride

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