Polish Journal of Pharmacology
Pol. J. Pharmacol., 2002, 54, 327–341
ISSN 1230-6002

REVIEW

IN THE SEARCH FOR SELECTIVE LIGANDS OF 5-HT_5, 5-HT_6 AND 5-HT_7 SEROTONIN RECEPTORS

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In the search for selective ligands of 5-HT_5, 5-HT_6 and 5-HT_7 serotonin receptors. A. WESOŁOWSKA. Pol. J. Pharmacol., 2002, 54, 327–341.

In recent years much attention has been focused on the functional importance of 5-HT_5, 5-HT_6 and 5-HT_7 receptors in the pathogenesis of neuropsychiatric and other diseases. In this connection, intensive studies with ligands of these receptors are currently in progress. Recognition of the structural characteristics responsible for the binding of a ligand molecule to an appropriate receptor, and development of an active complex have reached an advanced stage in the search for selective compounds. This review was undertaken to summarize the results of structure-activity relationship studies with ligands of 5-HT_5, 5-HT_6 and 5-HT_7 receptors. Additionally, some data on localization, pharmacological properties and the functional role of those receptors were reported.

Key words: *structure-activity relationship, 5-HT_5 ligands, 5-HT_6 ligands, 5-HT_7 ligands*

Receptors through which serotonin (5-HT) produces its physiological and pathological effects have been the subject of thorough investigation, initially using both *in vitro* and *in vivo* pharmacological methods, and later on by means of radioligand binding. However, a large body of new data were accumulated in the past years, also thanks to molecular techniques including *in situ* hybridization, which makes modification of the above classification justifiable. The Serotonin Club Receptor Nomenclature Committee has recently proposed new classification of 5-HT receptors, which requires three fundamental properties of a receptor to be described to ensure a comprehensive classification: its *operational* (drug-related), *transductional* (receptor-coupling) and *structural* (primary amino acid sequence) characteristics. When applied to the currently recognized 5-HT receptors, the above criteria indicate the existence of up to seven receptor classes in the central nervous system (CNS) [4, 12]. To date, the least time has been spent on 5-HT_5, 5-HT_6 and 5-HT_7 receptor studies, mostly due to the lack of selective ligands of these receptors. This review is focused on structure-activity relationship investigations with 5-HT_5, 5-HT_6 and 5-HT_7 receptor ligands. Additionally, some data on localization, pharmacological properties and functional role of 5-HT_5, 5-HT_6 and 5-HT_7 receptors as well as their potential contribution to neuropsychiatric and other diseases have been presented.

5-HT_5 receptors and their ligands

Two 5-HT_5 receptor subtypes, i.e. 5-HT_{5A} and 5-HT_{5B}, have been recognized. Human and rodent 5-HT_{5A} complementary deoxyribonucleic acid (cDNA) encodes a protein of 357 amino acid residues with seven hydrophobic domains, two putative N-linked glycosylation sites and several potential phosphorylation sites for protein kinase C and 3',5'-cyclic adenosine monophosphate (cAMP)-dependent protein kinase. Murine 5-HT_{5B} receptor cDNA codes for a protein of 370 amino acids with one putative site for N-linked glycosylation and consensus sites for phosphorylation by protein kinase C (PKC) and cAMP-dependent protein kinase. 5-HT_{5A} and 5-HT_{5B} receptors display a 68% amino acid identity [4, 38, 45].

The expression of murine and human 5-HT_5 receptors has already been shown in various cell systems, however, most of the described studies failed to demonstrate effects on signal transduction systems such as adenylate cyclase (AC) or phospholipase C [38, 44, 63]. Although no second messenger coupling could be detected for mouse 5-HT_{5A} receptor, Francken et al. [18] and Hurley et al. [29] reported functional coupling of the human 5-HT_{5A} receptor to G proteins and receptor-mediated inhibition of AC activity in HEK293 cells expressing human 5-HT_{5A} receptors.

The nothern blot analysis, quantitative polymerase chain reaction (PCR) and *in situ* hybridization experiments showed the presence of 5-HT_{5A} messenger ribonucleic acid (mRNA) in human brain cortex, hippocampus (dentate gyrus, CA_1, CA_2 and CA_3), hypothalamic area, amygdala and cerebellum. The 5-HT_{5A} receptor mRNA is expressed in many regions of the rat brain, the highest levels being found in the hippocampus and hypothalamus, with lower concentrations in the cortex, thalamus and striatum. The presence of 5-HT_{5A} mRNA in the mouse brain was shown in the cerebral cortex, hippocampus, cerebellum and olfactory bulb, but not in the kidney, liver, spleen, lung or heart [4, 41, 42, 44, 45].

The physiological function of 5-HT_{5A} receptors is still unclear. On the basis of their localization it is proposed that 5-HT_{5A} receptors may be involved in multiple functions of forebrain 5-HT, such as regulation of affective states, cognition, anxiety (and related behaviors), sensory perception and neuroendocrine functions [41]. Limbic distribution of 5-HT_{5A} mRNA seems to suggest its role in learning, memory and emotional behavior [42, 44]. Expression of 5-HT_{5A} immunoreactive cells in the substantia nigra points to involvement of this receptor in the regulation of nigrostriatal dopaminergic transmission and sensorimotor integration. The above assumption is evidenced by an increased locomotor and exploratory behavior in 5-HT_{5A} receptor knockout mice [23]. Interestingly, 5-HT_{5A} immunoreactive cells are in abundance in the suprachiasmatic nucleus, which suggests its potential involvement in circadian rhythms [41]. The results of genetic studies seem to testify to a dysfunction of the 5-HT system in complex psychiatric disorders, since Birkett et al. [3] found that an allelic variation in human 5-HT_{5A} receptor gene may play a role in the development of schizophrenia and affective disorder.

5-HT_{5B} receptor mRNA was present exclusively in the CA_1 field of the hippocampus, the habe-

nula and dorsal raphe in murine brain, but not in peripheral organs such as, e.g. the kidney, heart, lung or liver [4, 38]. The recent findings of Grailhe et al. [22] showed that, in contrast to mouse 5-HT$_{5B}$ gene, human 5-HT$_{5B}$ gene does not encode a functional protein because its coding sequence is interrupted by stop codons. Hence, the latter authors suggested that the 5-HT$_{5B}$ receptor was lost during evolution, after rodents and primates have diverged. The 5-HT$_{5B}$ receptor is the first example of a brain-specific protein not found in humans.

COS-7 and NIH-3T3 cells expressing murine 5-HT$_{5A}$ or 5-HT$_{5B}$ receptors displayed high affinity binding of [^{125}J]-2-iodo-lysergic acid diethylamide (LSD), that could be displaced by various non-selective serotonergic substances including 2-bromo--LSD, ergotamine, methysergide and methiothepin (Tab. 1) [4, 22, 44].

Table 1. The affinity of some compounds for 5-HT$_{5A}$ and 5-HT$_{5B}$ receptors [4]

Compound	pK$_i$	
	5-HT$_{5A}$ (mouse COS-7)	5-HT$_{5B}$ (mouse COS-7)
2-Bromo-LSD	8.7	8.5
Ergotamine	8.4	7.4
5-Carboxyamidotryptamine	7.8	6.9
Methysergide	7.2	7.8
Methiothepin	7.0	6.6
5-HT	6.6	6.4
RU24969	6.5	6.4

Up to the present, only Teitler et al. [54] have conducted preliminary structure-5-HT$_{5A}$ receptor affinity relationship studies. Beginning with the structure of 5-HT, which binds only with modest affinity to 5-HT$_{5A}$ receptors (K$_i$ = 170 nM), minor structural modifications were examined in a stepwise fashion. Methylation of 5-HT in position 2, homologation of its side chain, dimethylation of its amine group or removal of the pyrrole moiety yielded substances with a reduced affinity for 5-HT$_{5A}$ receptors (K$_i$ = 1290–10000 nM). Only methylation of 5-HT in position 5 improved almost twofold the affinity of 5-methoxy-5-HT (K$_i$ = 98 nM) in relation to 5-HT itself [54]. 5-HT likely binds at 5-HT$_{5A}$ receptors with its side chain being in a fully extended conformation. The above assumption is supported by the high affinity of ergolines, such as LSD (K$_i$ = 0.9 nM), which contain an embedded conformationally-constrained tryptamine moiety. Ibogaine, which represents a different conformation of the tryptamine moiety, does not bind at 5-HT$_{5A}$ receptors (K$_i$ > 10000 nM); however, its lack of affinity may be associated with a bulk of the bridged ring (Fig. 1).

Successive structure-5-HT$_{5A}$ receptor affinity investigations consisted in modifications of a molecule of dimethyltryptamine (DMT) which showed no significant affinity for 5-HT$_{5A}$ receptors (K$_i$ = 2815 nM) [54]. N$_1$-methylation, transposition of the nitrogen atom within the pyrrole moiety or its removal, reduction of the pyrrole moiety as well as homologation of a side chain of DMT did not yield substances showing affinity for 5-HT$_{5A}$ receptors (K$_i$ = 3000–25000 nM). Only introduction of a 5-methoxy group, but not a 4- or 6-methoxy one, into DMT enhanced about threefold the affinity (K$_i$ = 850 nM for 5-methoxyDMT). Introduction of more bulky substituents to position 5 of DMT (e.g. for 5-benzyloxy-DMT K$_i$ = 2660 nM), as well as to the amine group (K$_i$ = 5075–10330 nM) yielded substances with a reduced affinity for 5-HT$_{5A}$ receptors. In contrast, arylpiperazines showed a diverse affinity for 5-HT$_{5A}$ receptors (Fig. 2). In particular, 1-(1-naphthyl)piperazines represent high-affinity 5-HT$_{5A}$ ligands (K$_i$ = 40 and 3 nM for 1-(1-naphthyl)piperazine (1-NP) and 7-hydroxy-1-NP, respectively). Although insufficient information is available to permit a conclusion that the naphthyl ring of 1-NP binds to 5-HT$_{5A}$ receptors in the same

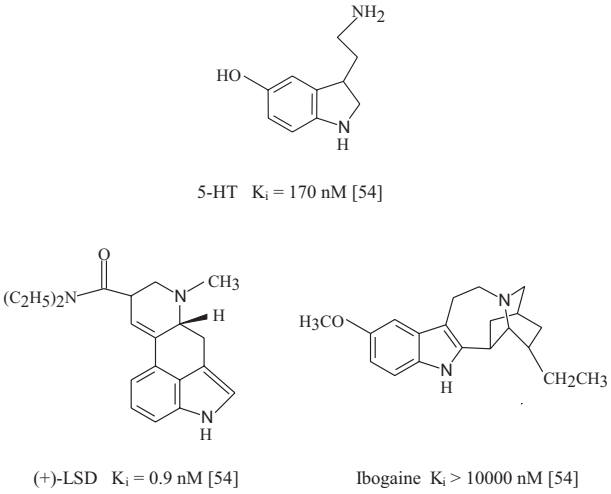


Fig. 1. Chemical structures of 5-HT, (+)-LSD and ibogaine and their affinity for 5-HT$_{5A}$ receptors

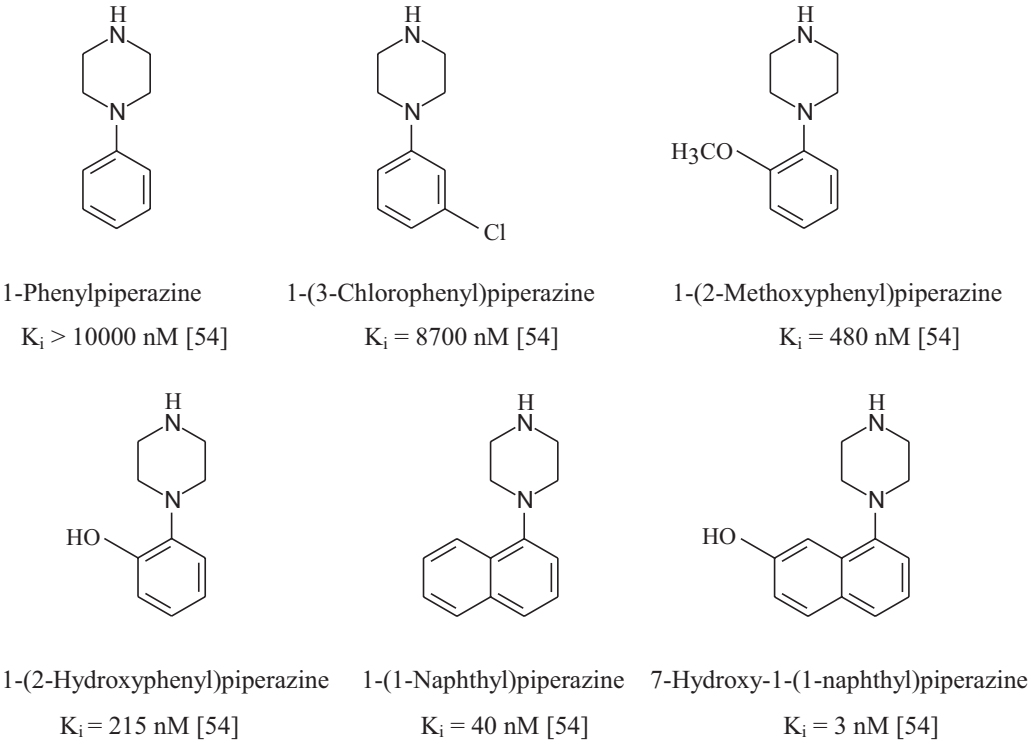


Fig. 2. Chemical structures of arylpiperazine derivatives and their affinity for 5-HT_{5A} receptors

manner as does the indole nucleus of 5-HT, the high affinity of these agents supports the notion that an indole ring is not essential for 5-HT_{5A} binding. Naphthylpiperazines, such as 7-methoxy-1-NP, were previously reported to bind to multiple populations of 5-HT receptors; for example, 7-methoxy-1-NP binds to 5-HT_{1A} receptors with K_i = 3.2 nM [21] and thus cannot be considered selective for 5-HT_{5A} receptors. However, due to their high affinity, 1-NPs may serve as a suitable non-indolic template for the prospective development of agents with a greater 5-HT_{5A} selectivity.

5-HT_6 receptors and their ligands

Monsma et al. [39] isolated the first cDNA coding for a 5-HT_6 receptor from rat striatum. The cDNA obtained, by this approach encodes a protein of 437 amino acid residues with seven hydrophobic regions, one potential N-linked glycosylation site and several potential sites for phosphorylation by cAMP-dependent PKC in the predicted third cytoplasmatic loop and the carboxyl-terminal tail. Within the transmembrane regions, this receptor showed homologies of 36–41% to other 5-HT receptors. Human 5-HT_6 cDNA encodes a 440-amino-acid polypeptide which sequence significantly diverges from that described for rat 5-HT_6 receptor [32]. 5-HT_6 receptors are positively linked to AC *via* $G_{S\alpha}$ protein, since 5-HT and 5-HT_6 agonists increase cAMP levels in cells which stably expressed 5-HT_6 receptors [32, 39, 49, 50].

The presence of 5-HT_6 mRNA was showed in several human and rat brain regions, most distinctly in the striatum, low levels were found in the cortex, nucleus accumbens, olfactory tubercle, hippocampus, hypothalamus, amygdala and cerebellum, while no signal was detected in peripheral organs [32, 49, 62].

Bourson et al. [7] attempted to determine the physiological role of 5-HT_6 receptors using oligonucleotide antisenses (AOs) to selectively prevent translation of the 5-HT_6 receptor *in vivo* and to measure various behavioral parameters such as food intake, body weight, body temperature, locomotor activity, nociception and free behavior. Repeated intracerebroventricular treatment with AOs, but not with a scrambled form of the antisense sequence, gave rise to a specific behavioral syndrome of yawning, stretching and chewing. Some well-known

stereotypic effects are induced, first of all, by dopamine D_2 receptor agonists, yet in this case the specific behavioral syndrome did not seem to be caused by modulation of dopaminergic transmission, since no changes in tissue levels of either dopamine or its metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid were observed nor did haloperidol (a D_2 receptor antagonist) reduce the number of yawns or stretches. On the other hand, atropine (a muscarine receptor antagonist) dose-dependently antagonized the behavioral syndrome induced by AOs [7] or Ro 04-6790 (a selective 5-HT$_6$ receptor antagonist) [53]. Ro 04-6790 also inhibited the rotational behavior of unilaterally 6-hydroxydopamine lesioned rats, induced by the muscarinic antagonists, scopolamine and atropine [6]. Hence, one of the functions of 5-HT$_6$ receptors appears to be the control of cholinergic neurotransmission which is increased by 5-HT$_6$ antagonists. Therefore, it is interesting to speculate about a possible clinical role of selective 5-HT$_6$ antagonists as potential drugs to be used in pathological states when a change of cholinergic neurotransmission is indicated. This would include depression, maybe memory deficits, and anxiety. The results obtained by Yoshioka et al. [65] indicated that 5-HT$_6$ receptors may be involved in certain states of anxiety. The latter authors used conditioned fear stress (CFS) to induce both an increase in 5-HT release from the prefrontal cortex and freezing behavior in rats. Treatment with AOs for 5-HT$_6$ receptor mRNA suppressed the CFS-induced 5-HT release, but not the freezing behavior. After infusion with AOs, Hamon et al. [25] observed a decrease in 5-HT$_6$ receptor-like immunostaining of the nucleus accumbens and an anxiogenic behavior of rats in social interaction and elevated plus maze tests.

The evidence obtained from positron emission tomography indicates that the limbic prefrontal cortex containing cells which make connections to the nucleus accumbens may be involved in the etiology of schizophrenia itself [13]. Demonstration of the presence of 5-HT$_6$ receptors in the nucleus accumbens, prefrontal cortex and other limbic areas [62] and high affinity of some neuroleptics for them [47] give support to the concept that 5-HT$_6$ receptors may play an important role in mediating the effects of some antipsychotic agents.

COS-7 cells, transiently expressing rat 5-HT$_6$ receptors, displayed high affinity binding sites for [^{125}J]LSD and [^{3}H]5-HT. Various non-selective serotonergic substances, including 2-bromo-LSD, dihydroergotamine, methysergide and methiothepin, typical and atypical antipsychotic drugs (e.g. chlorprothixene, clozapine), as well as some antidepressants (e.g. mianserin, clomipramine) showed relatively high affinities for those receptors (Tab. 2). Tryptamine and ergoline derivatives are functional agonists, while antipsychotic and antidepressant drugs are regarded as antagonists of 5-HT$_6$ receptors [32, 47, 50]. It should be stressed here that the abovementioned drugs and substances have a diversified chemical structure.

5-HT$_6$ receptors have a characteristic pharmacological profile that distinguishes them from other 5-HT receptors. Boess et al. [5] attempted to identify specific interactions between 5-HT$_6$ receptors and nonselective ligands. They showed that 5-HT and N_1-unsubstituted ergolines (e.g. LSD, ergotamine) bind to threonine 196 (Thr196) in the transmembrane region V, having created a hydrogen bond between the hydroxyl group of threonine and the indole nitrogen of such ligands. In contrast, removal of a potential hydrogen bond-forming site in transmembrane helix of five of the 5-HT$_6$ receptors by changing Thr196 to alanine, selectively reduced the affinity of the natural N_1-unsubstituted indoleamine and ergolines without affecting or increasing

Table 2. The affinity of some compounds for 5-HT$_6$ receptors [4, 47]

Compound	pK_i 5-HT$_6$ (rat COS-7)
Agonists:	
Dihydroergotamine	7.9
2-Bromo-LSD	7.8
5-Methoxytryptamine	7.4–7.7
5-HT	6.8–7.3
5-Carboxyamidotryptamine	6.7
Antagonists:	
Methiothepin	8.7–9.4
Chlorprothixene	8.5
Chlorpromazine	8.4
Clozapine	7.9–8.4
Risperidone	6.4
Amoxypine	7.5
Mianserin	7.4
Amitriptyline	7.2
Clomipramine	7.3
Imipramine	6.7

the affinity of N_1-methylated compounds (e.g. metergoline, methysergide and mesulergine). The mentioned increased affinity of N_1-methylated ligands may be the result of elimination of an unfavorable steric interaction between the methyl group in the N_1 position of these ligands and Thr196 of 5-HT_6 receptors.

The abovementioned 5-HT_6 receptor antagonist Ro 04-6790 and its analog Ro 63-0563 are benzenesulfonamide derivatives with high affinities for 5-HT_6 receptors (pK_i = 7.3 and 7.9, respectively, [53]) (Fig. 3). Both these compounds have an over hundredfold higher selectivity for 5-HT_6 receptors with respect to the other 23 binding sites studied, however, Ro 63-0563 is characterized by some affinity for 5-HT_{2A} and 5-HT_{2C} receptors (pK_i = 5.3 and 5.7, respectively) [23]. When they were tested by functional assays, neither compound had any significant effect on basal cAMP accumulation, which suggests that they are neither agonists nor inverse agonists. At the same time, both Ro 04-6790 and Ro 63-0563, behaved like competitive antagonists in the HeLa cells expressing human 5-HT_6 receptors, since they inhibited the 5-HT-stimulated AC activity. Ro 04-6790 given intraperitoneally (3–30 mg/kg) produced a dose-dependent behavioral syndrome similar to that produced by 5-HT_6 AOs, which consisted in yawning, stretching and chewing [53].

The next described 5-HT_6 antagonist was compound **1**, a benzenesulphonamide derivative (Fig. 3). It showed an excellent affinity for 5-HT_6 receptors (pK_i = 8.3) and more than fiftyfold higher selectivity for more than 50 receptors, enzymes or ion channels tested so far [8]. After examination of the whole series of compound **1** analogs (whose benzene moiety was enlarged), it turned out that compound **2** with a benzothiophene ring had the highest affinity for 5-HT_6 receptors (pK_i = 9.2) (Fig. 3). However, a pharmacokinetic study indicated that compound **2** was rapidly metabolically N-dealkylated in rats and its CNS penetration was poor [8]. Consequently, the N-demethylated derivative was synthesized (compound SB-271046) (Fig. 3) and was found to be a high-affinity (pK_i = 8.9), selective (over 200 times more selective for the 5-HT_6 receptor versus 55 other receptors, binding sites or ion channels) and orally active 5-HT_6 receptor antagonist [48]. SB-271046 produced a potent and long-lasting anticonvulsant activity in a rat maximal electroshock seizure threshold test. However, the magnitude of those antiseizure effects was modest in comparison with that of some well-known antiepileptic drugs, e.g. carbamazepine. Such a low

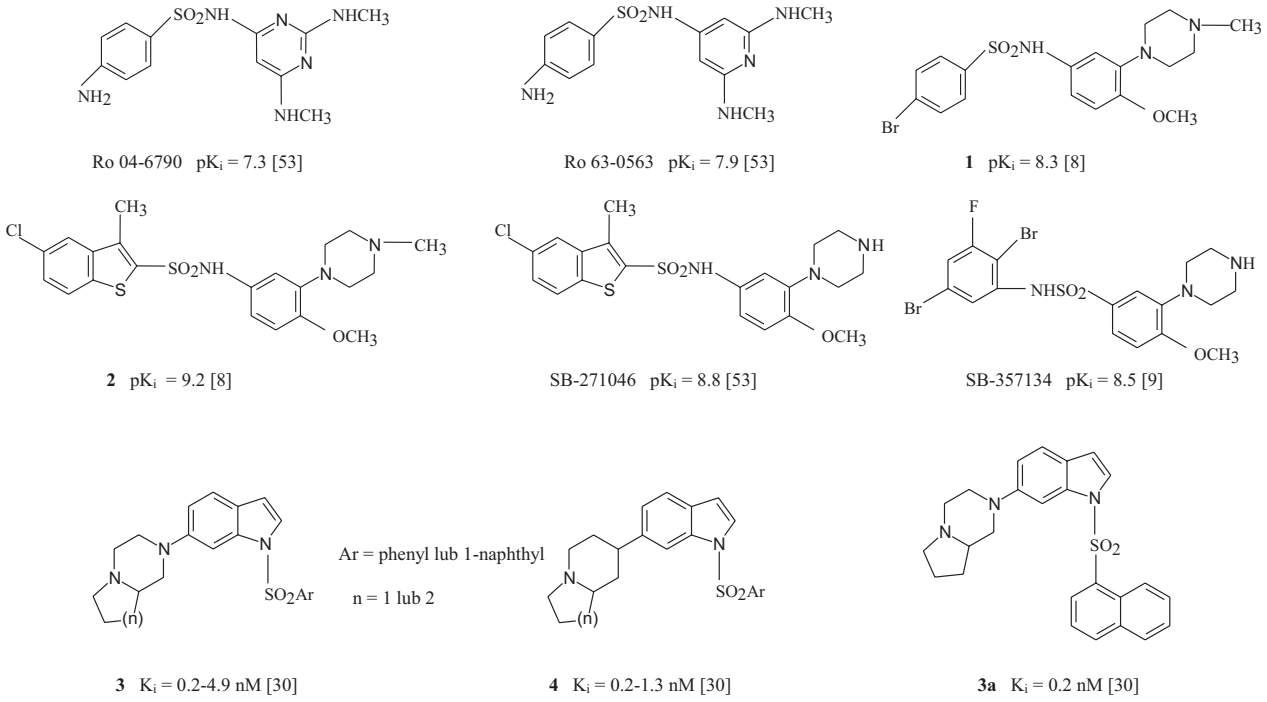


Fig. 3. Chemical structures of sulfonamide derivatives and their affinity for 5-HT_6 receptors

level of the anticonvulsant efficacy, associated with 5-HT$_6$ receptor blockade, probably contributes to the apparent absence of dose-dependence of SB-271046 in the latter test. Therefore, a possible clinical use of SB-271046 as a drug in the treatment of epilepsy is still controversial at this stage of experimentation. In separate behavioral [46] and neurochemical [15] studies, SB-271046 produced indications of improvement in cognitive function. At present, it is in the first phase of clinical trials for the treatment of cognitive disorders [9].

The aim of successive studies was to further explore the scope of activity of SB-271046-related structures. Therefore, a number of 4-piperazinyl quinoline derivatives connected with the benzothiophene moiety, showing excellent 5-HT$_6$ binding affinities (pK_i = 7.6-9.2) and > 100 higher selectivity in comparison with a range of other receptors, were identified. It was shown that the selectivity of 2-substituted benzothiophene derivatives depended on the size of 3-substituent, and the preferred point of attachment of the piperazine ring to quinoline was at position 4. In a functional model, the tested compounds were found to reverse the 5-HT-stimulated AC activity and were classified as 5-HT$_6$ receptor antagonists. These compounds are under further investigation for their potential utility in the treatment of CNS disorders [10].

In the course of consecutive studies with sulfonamide derivatives into the structure-activity relationship, compound SB-357134 (Fig. 3) was synthesized and examined. It was found to be a high affinity (pK_i = 8.5 [9]) and selective 5-HT$_6$ receptor antagonist with good oral bioavailability in rats. All its analogs (with different halogen substituens within the benzene moiety) also demonstrated an excellent 5-HT$_6$ affinity (pK_i = 7.5–9.3 [9]), but diverse selectivities. In contrast to 2,4-, 2,5- or 3,5-disubstituted derivatives of SB-357134, monosubstituted compounds were very rapidly cleared *in vivo* and their brain to blood ratios were very low. In an effort to combine the metabolic stability with enhanced CNS penetration, a series of 2,3,5-trisubstituted analogs of SB-357134 was prepared. Most of those compounds had good 5-HT$_6$ affinity (pK_i = 6.8–8.7 [9]) and an exceptional selectivity, but their pharmacokinetic profile was similar to that of 3,5-disubstituted derivatives. In an attempt to further increase brain penetration of these compounds, conformationally restricted indoline, tetrahydroquinoline and tetrahydroisoquinoline analogs were synthesized in the hope that replacement of polar sulfonamide NH would increase their CNS penetration. A number of these compounds maintained an excellent 5-HT$_6$ receptor affinity (pK_i = 8.4–9.5) and diversified selectivity. Generally in that series, attempts to increase the brain penetration by augmenting lipophilicity also led to an enhanced *in vivo* clearance in the rat [9].

Isaac et al. [30] described a new series of 6-bicyclopiperazinyl-1-arylsulfonylindole (**3**) and 6-bicyclopiperidinyl-1-arylsulfonylindole (**4**) derivatives (Fig. 3) as new 5-HT$_6$ antagonists. In general, all the derivatives were found to be very potent ligands of 5-HT$_6$ receptors, whose K_is were lower than 10 nM. Compound **3a** with the 1-naphthyl group and n = 1 had the highest 5-HT$_6$ receptor affinity (K_i = 0.2 nM) and a good binding selectivity compared to other key receptors. In a functional AC assay, it was found to be an antagonist, since it decreased AC activity measured in HEK293 cells expressing human 5-HT$_6$ receptors. Those findings make it a promising candidate for the possible treatment of schizophrenia, depression and memory dysfunctions. Compound **3a** is currently being further evaluated for its therapeutic potential [30].

The possibility that the 5-HT$_6$ receptor population may play a role in neuropsychiatric disorders has attracted considerable attention, but to date relatively little is known about structural requirements for the binding at 5-HT$_6$ receptors and ligands which may possibly be selective for this population of receptors. Glennon et al. [19] attempted to determine structural characteristics of tryptamine derivatives, which would decide the affinity of these compounds for 5-HT$_6$ receptors. In the first phase of their studies with tryptamine (K_i = 180 nM for 5-HT$_6$ receptors), as a starting point they investigated the role of aminoethyl side chain and substitution mode of the indole fragment. However, methylation in position 1 or α, methoxylation in position 4, 6 or 7, transposition of the nitrogen atom or its removal resulted in a dramatic reduction of the affinity for 5-HT$_6$ receptors (K_i = 350–20000 nM), whereas introduction of the methyl group to position 2 (K_i = 46 nM for 2-methyl-5-HT) or of the hydroxyl group to position 5 of tryptamine molecule, as well as dimethylation of the amine group (K_i = 30 nM for N,N-dimethyltryptamine) yielded derivatives with significantly higher 5-HT$_6$ affinities. Those studies indicated that the presence of a lateral substituent in position

5 of dimethyltryptamine was an optimum arrangement (K_i = 16 nM for 5-methoxy-N,N-dimethyltryptamine, and K_i = 11 nM for 5-methylthio-N,N-dimethyltryptamine). Those findings also gave support to an earlier, advanced hypothesis [28] that hydrogen bond formation by the 5-OH group is not important for the 5-HT_6 binding. Methylthio derivatives tend to form weaker hydrogen bonds with 5-HT_6 receptors, and in *in vitro* studies 5-methylthio-N,N,-dimethyltryptamine (K_i = 11 nM) showed a higher affinity for 5-HT_6 receptors than its 5-hydroxy derivative (K_i = 95 nM) [19].

On the grounds of the abovementioned results, Glennon et al. [20] synthesized several 2-alkyl-5-methoxytryptamines with affinities at least comparable to that of 5-HT itself (K_i = 75 nM). In particular, 2-ethyl-5-methoxy-N,N-dimethyltryptamine (EMDT) (Fig. 4) showed a high 5-HT_6 receptor affinity (K_i = 16 nM) and a reasonable selectivity for 5-HT_6 versus other 5-HT subtype receptors. In functional studies, EMDT was demonstrated to behave like a 5-HT_6 agonist (it stimulated AC activity) with a potency at least equal to that of 5-HT. A 2-phenyl derivative (PMDT) (K_i = 20 nM) (Fig. 4), which has a binding profile slightly different from that of EMDT, also seems of interest. Compound PMDT lacks an agonist activity and may thus be regarded as a novel 5-HT_6 antagonist, since it inhibited the 5-HT-stimulated AC measured in HEK293 cells expressing human 5-HT_6 receptors [20]. While discussing these analogs, it is also noteworthy that even small changes in the molecule structure may completely alter the intrinsic activity.

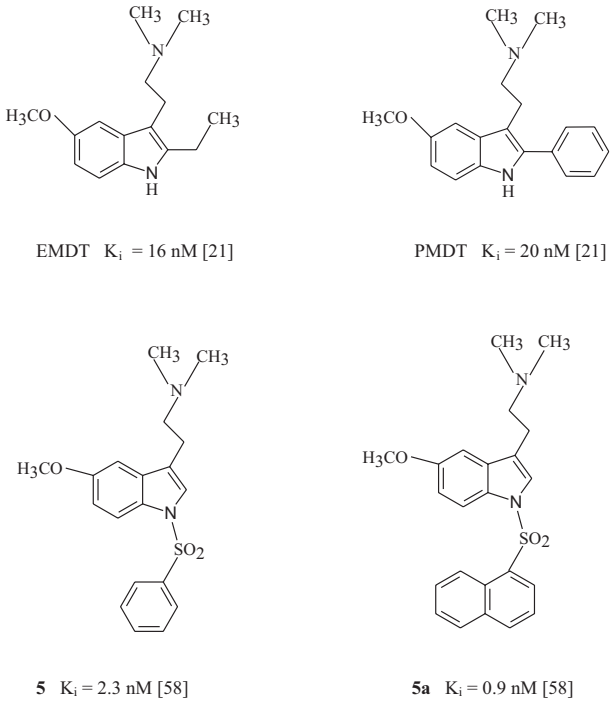


Fig. 4. Chemical structures of tryptamine derivatives and their affinity for 5-HT_6 receptors

To continue the structure-activity relationship studies of this class of ligands, a series of N_1-(arylsulfonyl)-N,N-dimethyltryptamine derivatives was synthesized and investigated [58]. While changing aromatic substituents at the suflonyl group and the position of the methoxy group within the indole moiety, it was found that compound **5** (Fig. 4) with an unsubstituent phenyl ring bound with a high affinity (K_i = 2.3 nM) to 5-HT_6 receptors, but also showed a significant affinity for 5-HT_{2A} (K_i = 130 nM) and 5-HT_{2C} (K_i = 23 nM) receptors. The structure of compound **5** was modelled using SYBYL, and three families of low-energy conformations were identified. The results of those molecular modeling investigations indicated that members of the three conformational families of **5** were superimposed with the corresponding members of families of the selective 5-HT_6 receptor antagonist Ro 63-0563. Functional studies brought support to that observation. Compound **5** produced inhibition of the 5-HT-stimulated AC activity in a dose-dependent manner and was lacking an agonist character [58]. Other modifications of the phenyl ring or its replacement with 1- or 2-naphthalene, as well as a simultaneous change of the methoxy group position in the indole moiety yielded novel 5-HT_6 receptor ligands with a high, but diversified, affinity (K_i = 0.9–93 nM) [58]. Although the obtained compounds (e.g. **5a** (Fig. 4)) showed a higher affinity for 5-HT_6 receptors than did compound **5**, they were not subjected to further functional *in vitro* or *in vivo* studies; therefore, it is not possible to conclude what was the impact of the introduced structural modifications on the functional activity of these ligands.

The role of the sulfonyl group in N_1-benzenesulfonyl tryptamines has also been investigated. The obtained results indicate that, as a rule, the sulfonyl group enhances the 5-HT_6 receptor affinity of tryptamines and although the presence of the sulfoxide moiety is optimal, it is unnecessary for binding [34]. The lack of functional and pharmacokinetic studies with these ligands limits evaluation of the effect of the sulfonyl group on the intrinsic activity and metabolism of compounds of this group.

5-HT$_7$ receptors and their ligands

Heidmann et al. [26] reported that alternative splicing in human and rat tissues produced several 5-HT$_7$ receptor isoforms which differed in amino acid sequences of their carboxyl terminal tails. In rat tissues, three 5-HT$_7$ isoforms, called 5-HT$_{7(a)}$, 5-HT$_{7(b)}$, and 5-HT$_{7(c)}$ in line with the NC-IUPHAR nomenclature [60], were found. Rat 5-HT$_{7(a)}$ (448--amino acid) and 5-HT$_{7(b)}$ (435-amino acid) forms arise from alternative splice donor sites. A third new isoform found in the rat, 5-HT$_{7(c)}$ (470-amino acid), results from a retained exon cassette. Three 5-HT$_7$ mRNA isoforms were also identified in human tissues. Two human isoforms represent 5-HT$_{7(a)}$ (a long form) and 5-HT$_{(7b)}$ (a short form) forms (445- and 432-amino acid, respectively), but the third one does not correspond to rat 5-HT$_{7(c)}$. Instead, it constitutes a distinct isoform, 5-HT$_{7(d)}$ (479-amino acid), resulting from retention of a separate exon cassette. 5-HT$_{7(d)}$ transcripts are not present in the rat, because a 5-HT$_{7(d)}$-specifying exon is absent from rat 5-HT$_7$ gene [26, 27]. 5-HT$_7$ receptor protein is unique, with a low (< 40%) overall homology with other 5-HT receptors [16]. It has seven hydrophobic domains, two putative sites for N-linked glycosylation in the amino-terminal region, and two cysteine residues which may form a structurally important disulfide bond. One putative recognition site for PKC, and another one for both cAMP-dependent protein kinase and multifunctional calmodulin-dependent protein kinase II are conserved in the carboxyl-terminal domain of murine 5-HT$_7$ receptors [4]. 5-HT$_7$ receptors preferentially activate AC, supposedly *via* coupling to $G_S\alpha$ [1, 2, 43]. 5-HT and nonselective agonists of these receptors increase the cAMP level measured in different cells expressing human, rat and mouse 5-HT$_7$ receptors [27, 33, 59].

A northen blot analysis of various mammalian tissues has shown the highest levels of 5-HT$_{7(a)}$ mRNA in the hypothalamus and thalamus, its high amount in the brainstem and hippocampus, and lower levels in the cerebral cortex, striatum, olfactory bulb and olfactory tubercle. The 5-HT$_{7(a)}$ isoform also predominates in the spleen, kidney, heart and coronary artery [4, 16, 27, 59]. The isoform of the 5-HT$_{7(b)}$ receptor comprises 31–45% of 5-HT$_7$ receptor mRNA in the caudate, hippocampus and spleen [59]. *In situ* hybridization studies indicate generalized low levels of the expression of 5-HT$_{7(c)}$ (hindbrain, cerebellum, spleen) and 5-HT$_{7(d)}$ (spleen, caudate nucleus) isoforms (Tab. 3) [27, 59]. The small amount of 5-HT$_{7(c)}$ and 5-HT$_{7(d)}$ suggests that these isoforms result from a "leaky" transcription, and that they have no physiological relevance [27].

Table 3. The number of amino acids and regional distribution of 5-HT$_7$ receptor isoforms [4, 33, 59]

Receptor	Number of amino acids	Regional distribution
5-HT$_{7a}$	445 (human) 448 (rat) 448 (mouse) 446 (gwinea pig)	thalamus, hypothalamus, hippocampus, brain stem, cortex, striatum, olfactory bulb, olfactory tubercle, spleen, kidney, heart, coronary artery
5-HT$_{7b}$	432 (human) 435 (rat)	caudate nucleus, hippocampus, spleen
5-HT$_{7c}$ (rat)	470 (rat)	cerebellum, hindbrain, spleen
5-HT$_{7d}$ (human)	479 (human)	caudate nucleus, spleen

On the grounds of 5-HT$_7$ receptor localization in the suprachiasmatic nuclei (SCN) of the hypothalamus [27, 33], it has been postulated that 5-HT$_7$ receptors may play an important role in circadian rhythms. Hence, selective 5-HT$_7$ receptor compounds may be useful in the treatment of jet lag and sleep disorders of a circadian nature. It is also believed that a disregulated circadian rhythm may lead to mental fatigue and depression. Recent results show that administration of antidepressant drugs according to a profile consistent with the activity of the 5-HT$_7$ receptor (fluoxetine pK_i = 6.0, amitriptyline pK_i = 6.2) induces the immediate early gene Fos in the SCN, which is indicative of neuronal activation. That effect was diminished upon chronic exposure which, in turn, was correlated with down-regulation of 5-HT$_7$ receptors [40]. Thus, one of the consequences of antidepressant treatment may be modulation of a possible dysrhythmic circadian function in depression, in which 5-HT$_7$ receptors might be one of the key factors. Further evidence for the role of 5-HT$_7$ receptors in depression comes from the fact that they are under inhibitory regulation exerted by adrenal steroids [14, 52, 64] whose concentration also undergoes day-night variations. Both the hypothalamic-

pituitary-adrenocortical axis and 5-HT are implicated in depressive illnesses [11], therefore, further studies with selective 5-HT_7 ligands are necessary to elucidate possible involvement of the receptor in depression and antidepressant therapeutic response. The expression of mRNA for 5-HT_7 receptors in thalamic and limbic structures points to their role in affective behavior. The fact that such antipsychotics as risperidone (pK_i = 8.9) and clozapine (pK_i = 8.2) show a high affinity for 5-HT_7 receptors and have features of antagonists [47] has led to an assumption that these receptors may be important for mediating the unique actions of certain antipsychotic drugs. The role of 5-HT_7 receptors in schizophrenia is corroborated by the observation that a decrease in 5-HT_7 mRNA is observed in the prefrontal cortex of schizophrenic patients [59]. Pharmacological data show that 5-HT_7 receptors mediate the spasmolytic action of 5-HT on the smooth muscle of cerebral and peripheral vessels [55, 61]. Thus, ligands of these receptors could be used in migraine prophylaxis. The presence of these receptors in the periphery may suggest their involvement in immunological reactions, inflamatory processes, hypertension and peripheral vessels diseases [59].

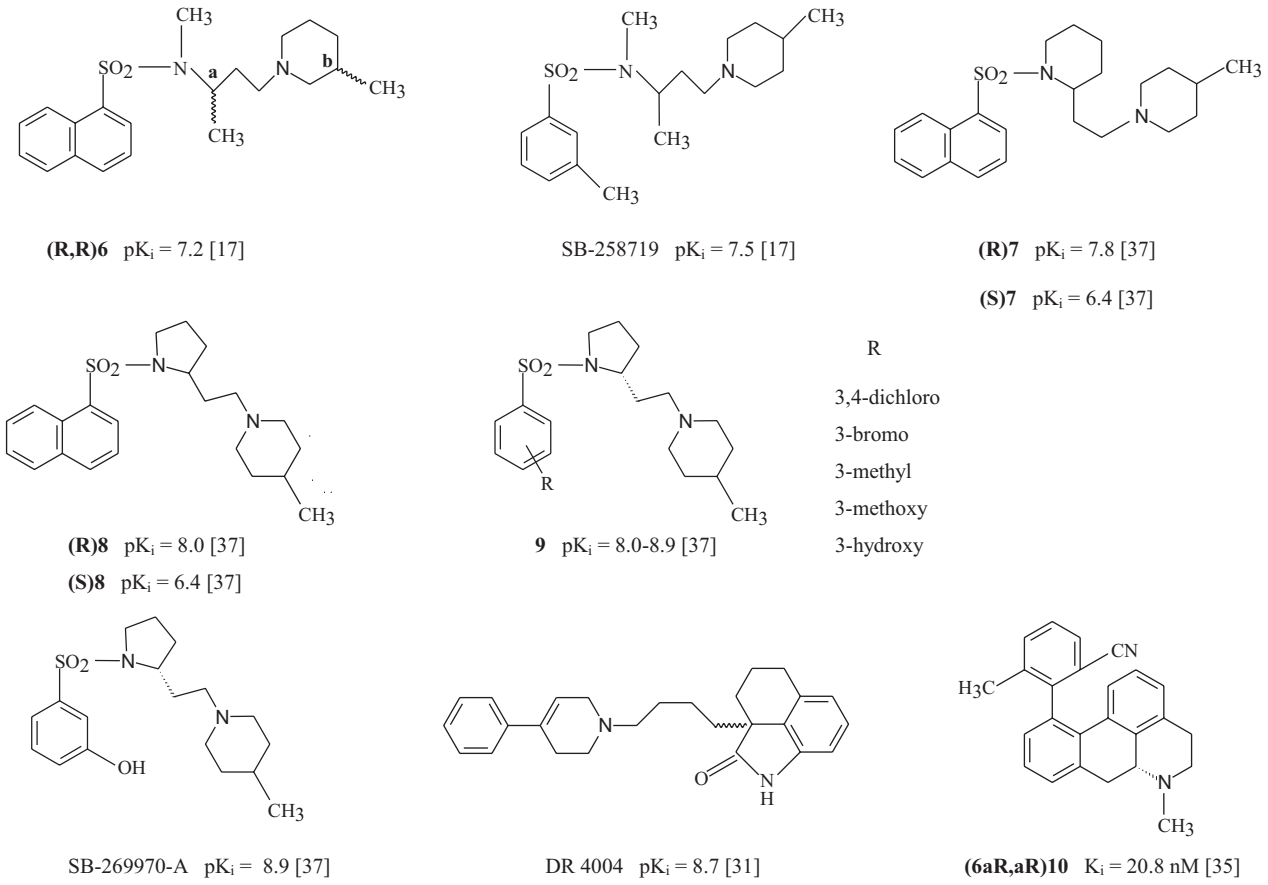
At this stage of knowledge, it may only be stated that further studies with selective ligands of 5-HT_7 receptors are necessary to elucidate involvement of these receptors in the pathogenesis and treatment of schizophrenia and other emotional and psychic disturbances, as well as vascular disorders.

High affinity binding sites for [^{3}H]5-HT were found in COS-7 cells expressing mouse 5-HT_7 receptors or rat and human homologs [4, 16, 47]. Recently, a number of substances with a differentiated chemical structure have been reported to show high affinity for 5-HT_7 receptors. Among them there are 5-carboxyamidotryptamine, 5-methoxytryptamine and 8-hydroxy-2-(di-n-propylamino)tetraline (8-OH-DPAT, a 5-HT_{1A} ligand), all of them being agonists of 5-HT_7 receptors. Methiothepin, mesulergine, a few typical (e.g. chlorprothixene) and atypical (e.g. clozapine) antipsychotic drugs and some antidepressants (e.g. mianserin, amitriptyline) are antagonists of these receptors (Tab. 4). All the abovementioned substances are nonselective, since they also have an excellent affinity for other 5-HT and non-5-HT receptors [4, 16, 47, 51], therefore, studies are in progress at present to develop new, selective ligands of these receptors.

Table 4. The affinity of some compounds for 5-HT_7 receptors [4, 16, 47]

Compound	pK_i 5-HT_7 (rat HEK293)
Agonists:	
5-Methoxytryptamine	8.3–8.8
5-HT	8.1–8.7
5-Carboxyamidotryptamine	9.0–9.5
8-OH-DPAT	6.3–7.4
Antagonists:	
Methiothepin	8.4–9.0
Mesulergine	7.7–8.2
Pimozide	9.3
Chlorprothixene	8.3
Chlorpromazine	7.6
Risperidone	8.9
Clozapine	7.9–8.2
Mianserin	6.9–7.3
Amitriptyline	6.2
Fluoxetine	6.0

The results of structure-activity relationship studies with benzenesulfonamide derivatives, 5-HT_7 receptor ligands [17], showed that the chirality center **a** (e.g. in the isomer **(R,R)6**, pK_i = 7.2 [17]) (Fig. 5) was essential for the binding to 5-HT_7 receptors, whereas the chirality center **b** was apparently less important. Additionally, it was found that moving the methyl group to position 4 of piperidine, as well as replacement of the bulk aromatic moiety in the sulfonamide group (compound **6**) with a mono- or disubstituted phenyl or thienyl moiety resulted in improvement of the affinity for 5-HT_7 receptors. Following the abovementioned suggestions, compound SB-258719 (pK_i = 7.5 for 5-HT_7 receptors) (Fig. 5) was designed and synthesized. SB-258719 inhibited the 5-carboxyamidotryptamine-stimulated activity of AC in HEK293 cells expressing human 5-HT_7 receptors, and was classified as a 5-HT_7 antagonist [17, 57]. A conformational analysis of compound SB-258719 using MACROMODEL revealed that all bonds were relatively free to rotate, except for the S-N and N(Me)-C(Me) bonds. The Ramachandran plot showing rotation around these two bonds in SB-258719 molecule reveals an energy minimum when the two methyl groups (one in the chirality center **a**, and the another in nitrogen atom of the sulfona-



(R,R)6 pK_i = 7.2 [17]

SB-258719 pK_i = 7.5 [17]

(R)7 pK_i = 7.8 [37]

(S)7 pK_i = 6.4 [37]

(R)8 pK_i = 8.0 [37]

(S)8 pK_i = 6.4 [37]

9 pK_i = 8.0-8.9 [37]

SB-269970-A pK_i = 8.9 [37]

DR 4004 pK_i = 8.7 [31]

(6aR,aR)10 K_i = 20.8 nM [35]

Fig. 5. Chemical structures of benzenesulfonamide, tetrahydrobenzindole and apomorphine derivatives and their affinity for 5-HT$_7$ receptors

mide group) adopt a gauche orientation (60°). This suggested the synthesis of analogs in which both methyl groups have been tied together into a piperidine (**7**) or pyrrolidine (**8**) ring (Fig. 5). The R enantiomers of both these derivatives are potent 5-HT$_7$ ligands [37]. Using an optimized R-pyrrolidinylethyl side chain, further investigations were carried out into the effect of the 3-substituted phenyl ring (i.e. **9** derivative) (Fig. 5) on 5-HT$_7$ receptor affinity and selectivity. Suprisingly, introduction of a polar 3-hydroxy group afforded SB-269970-A (Fig. 5) with the highest 5-HT$_7$ receptor affinity (pK_i = 8.9) and an excellent selectivity profile (> 100 times) compared with a total of 50 other receptors, enzymes, or ion channels, except for 5-HT$_{5A}$ receptors (50 times). In a functional model, that compound showed features of an antagonist, since it inhibited 5-HT- or 5-carboxyamidotryptamine-stimulated AC activity in HEK293 cells stably expressing human 5-HT$_7$ receptors [17, 24, 37]. Pharmacokinetic studies demonstrated that SB-269970-A showed good CNS penetration, however, was rapidly cleared from the blood in the rat. In *in vivo* experiments, SB-269970-A dose-dependently inhibited the 5-carboxyamidotryptamine-induced hypothermia in guinea pigs and paradoxical sleep in rats without effects on other sleep stages. These data suggest that 5-HT$_7$ receptors play a role in sleep control. These observations support the hypothesis that 5-HT$_7$ receptor antagonists have potential utility for the treatment of depression (in which disturbances of sleep are present) and/or circadian rhythm disturbances [24, 56].

Of the tetrahydrobenzindole derivatives, compound DR 4004 is a highly potent ligand of 5-HT$_7$ receptors (pK_i = 8.7 [31]) (Fig. 5), with at least 47-fold higher selectivity over the 5-HT$_2$ (pK_i = 7.0 [31]) and other receptors. DR 4004 inhibits the 5-HT-induced stimulation of cAMP accumulation in COS-7 cells transfected with human 5-HT$_7$ receptors, hence, it is regarded as a 5-HT$_7$ receptor antagonist [31].

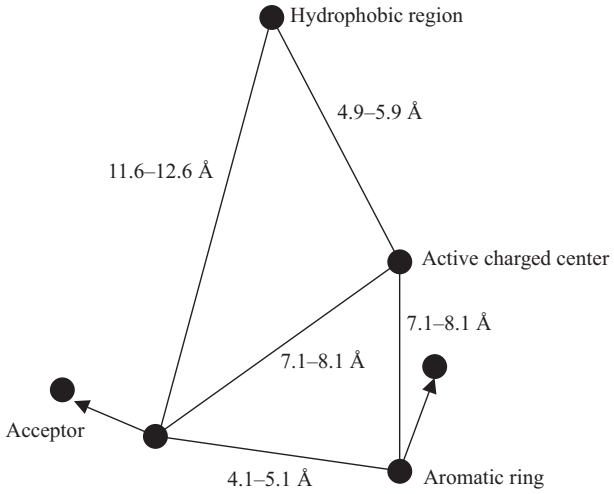


Fig. 6. Proposed pharmacophore for 5-HT_7 antagonists according to López-Rodríguez et al. [36]

Of the series of atropisomeric biaryl derivatives of (R)-aporphine, Linnanen et al. [35] synthesized compounds which interacted in a stereoselective manner with 5-HT_7, 5-HT_{1A}, and D_2 receptors. The novel derivative, compound **(6aR,aR)10** (Fig. 5), showed preference for the 5-HT_7 receptor subtype (K_i = 20.8 nM) and – in contrast to its analogs – a significantly lower affinity for 5-HT_{1A} (K_i = 778 nM) and D_2 (K_i = 2470 nM) receptors. **(6aR,aR)10** turned out to be 5-HT_7 receptor antagonist, since it inhibited the 5-HT-stimulated cAMP production in CHO cells transfected with rat 5-HT_7 receptors [35].

Using the software package Catalyst, López-Rodríguez et al. [36], performed a study with a set of thirty 5-HT_7 antagonists, structurally different from a chemical feature standpoint, which was selected from the reported data as the target training set for Catalyst analysis. The results of their study indicated that the minimal structural requirements for 5-HT_7 antagonism consist of an aromatic ring, a basic nitrogen atom (a positive ionizable center), a H-bonding acceptor group and a hydrophobic region at 4.9-5.9 Å away from the basic center (Fig. 6). It is very likely that compounds synthesized according to these principles will show an antagonistic action towards 5-HT_7 receptors. This pharmacophore model for 5-HT_7 antagonists represents the first contribution to the rational design of agents acting on this receptor type. It offers a structural insight to aid the development of novel 5-HT_7 ligands which are esesential for the knowledge of the (patho)physiological role of 5-HT_7 receptors.

However, it is should be stressed here that the above-described studies bore fruit, having developed 5-HT_7 receptor ligands which are characterized by a significant affinity, distinct selectivity and activity typical of antagonists. On the other hand, no selective agonist of these receptors has been available as yet.

As it has been mentioned elsewhere in this paper, intensive studies with ligands of serotonin receptors of the 5-HT_5, 5-HT_6 and 5-HT_7 type are currently in progress, and they are concerned with the structure-affinity and intrinsic activity relationships. Recognition of the structural features responsible for the binding of a ligand molecule to an appropriate receptor and the creation of an active complex, in which information is encoded that is later passed into higher organizational levels of an organism, is an important stage in the search for selective compounds. Such selective ligands, especially agonists of 5-HT_5, 5-HT_6 and 5-HT_7 receptors, would certainly be helpful in determining their functional importance and involvement in the pathogenesis of diseases, not exclusively of the CNS. However, it should be kept in mind that altough selective receptor ligands are an important and indispensable research tool, they rarely happen in practice to be drugs.

REFERENCES

1. Adham N., Zgombick J.M., Bard J.A., Branchek T.A.: Functional characterization of the recombinant human 5-hydroxytryptamine7(a) receptor isoform coupled to adenylate cyclase stimulation. J. Pharmacol. Exp. Ther., 1998, 287, 508–514.
2. Bard J.A., Zgombick J.M., Adham N., Vaysse P., Branchek 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.
3. 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.
4. Boess F.G., Martin I.L.: Molecular biology of 5-HT receptors. Neuropharmacology, 1994, 33, 275–317.
5. Boess F.G., Monsma F.J., Mayer V., Zwingelstin C., Sleight A.J.: Interaction of tryptamine and ergoline compounds with threonine 196 in the ligand binding site of the 5-hydroxytryptamine$_6$ receptor. Mol. Pharmacol., 1997, 52, 515–523.
6. Bourson A., Boess F.G., Bös M., Sleight A.J.: Involvement of 5-HT_6 receptors in nigro-striatal function in rodents. Brit. J. Pharmacol., 1998, 125, 1562–1566.

7. Bourson A., Borroni E., Austin R.H., Monsma F.J., Sleight A.J.: Determination of the role of the 5-HT_6 receptor in the rat brain: a study using antisense oligonucleotides. J. Pharmacol. Exp. Ther., 1995, 274, 173–180.
8. 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-yl-phenyl)-3-methyl-2-benzothiophenesulfonamide (SB-271046): a potent selective, and orally bioavailable 5-HT_6 receptor antagonist. J. Med. Chem., 1999, 42, 202–205.
9. 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-HT_6 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.
10. Bromidge S.M., Griffith K., Heightman T.D., Jennings A., King F.D., Moss S.F., Newman H., Riley G., Routledge C., Serafinowska H.T., Thomas D.R.: Novel (4-piperazin-1-ylquinolin-6-yl)arylsulfonamides with high affinity and selectivity for the 5-HT_6 receptor. Bioorg. Med. Chem. Lett., 2001, 11, 2843–2846.
11. Chaouloff F.: Regulation of 5-HT receptors by corticosteroids: where do we stand? Fundam. Clin. Pharmacol., 1995, 9, 219–233.
12. Chojnacka-Wójcik E.: Serotonin receptors. In: Receptors, Structure, Characteristics, Function (Polish). Eds. Nowak J.Z., Zawilska J.B., PWN, Warszawa, 1997, 83–104.
13. Cohen R.M., Semple W.E., Gross M., Nordahl T.E.: From syndrome to illness: delineating the pathophysiology of schizophrenia with PET. Schizophr. Bull., 1988, 14, 169–179.
14. Le Corre S., Sharp T., Young A.H., Harrison P.J.: Increase of 5-HT7 (serotonin-7) and 5-HT1A (serotonin-1A) receptor mRNA expression in rat hippocampus after adrenalectomy. Psychopharmacology, 1997, 130, 368–374.
15. Dawson L.A., Nguyen H.Q., Li P.: In vivo effects of the 5-HT(6) antagonist SB-271046 on striatal and frontal cortex extracellular concentrations of noradrenaline, dopamine, 5-HT, glutamate and aspartate. Brit. J. Pharmacol., 2000, 130, 23–26.
16. Eglen 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.
17. 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-methyl-piperidin-1-yl)propyl]benzenesulfonamide: the first selective 5-HT_7 receptor antagonist. J. Med. Chem., 1998, 41, 655–657.
18. Francken B.J.B., Jurzak M., Vanhauwe J.F.M., Luyten W.H.M.L., Leysen J.E.: The human 5-ht_{5A} receptor couples to G_i/G_o proteins and inhibits adenylate cyclase in HEK 293 cells. Eur. J. Pharmacol., 1998, 361, 299–309.
19. Glennon R.A., Bondarev M., Roth B.: 5-HT_6 serotonin receptors binding of indolealkylamines: a preliminary structure-affinity investigation. Med. Chem. Res., 1999, 9, 108–117.
20. Glennon R.A., Lee M., Rangisetty J.B., Dukat M., Roth B.L., Savage J.E., McBride A., Rauser L., Hufeisen S., Lee D.K.H.: 2-Substituted tryptamines: agents with selectivity for 5-HT_6 serotonin receptors. J. Med. Chem., 2000, 43, 1011–1018.
21. Glennon R.A., Naiman N.A., Pierson. M.E., Smith J.D., Ismaiel A.M., Titeler M., Lyon R.A.: *N*-(Phthalimidoalkyl) derivatives of serotonergic agents: a common interaction at 5-HT_{1A} serotonin binding sites? J. Med. Chem., 1989, 32, 1921–1926.
22. Grailhe R., Grabtree G.W., Hen R.: Human 5-HT_5 receptors: the 5-HT_{5A} receptor is functional but the 5HT_{5B} receptor was lost during mammalian evolution. Eur. J. Pharmcol., 2001, 418, 157–167.
23. 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.
24. Hagan J.J., Price G.W., Jeffrey P., Deeks N.J., Stean T., Piper D., Smith M.I., Upton N., Medhurst A.D., Middlemiss D.N., Riley G.J., Lovell P.J., Bromidge S.M., Thomas D.R.: Characterization of SB-269970-A, a selective 5-HT_7 receptor antagonist. Brit. J. Pharmacol., 2000, 130, 539–548.
25. Hamon M., Doucet E., Lefevre K., Miquel M.C., Lanfumey L., Insausti R., Frechilla D., Del Rio J., Vergé D.: Antibodies and antisense oligonucleotide for probing the distribution and putative functions of central 5-HT_6 receptors. Neuropsychopharmacology, 1999, 21, 68–76.
26. Heidmann D.E.A., Metcalf M.A., Kohen R., Hamblin M.W.: Four 5-hydroxytryptamine7 (5-HT7) receptor isoforms in human and rat produced by alternative splicing: species differences due to altered intron-exon organization. J. Neurochem., 1997, 68, 1372–1381.
27. Heidmann D.E.A., 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.
28. Herndon, J.L., Glennon R.A.: Serotonin receptors, agents, and actions. In: Drug Design for Neuroscience. Ed. Kozikowski A.P., Raven Press, NY, 1993, 167–212.
29. Hurley P.T., McMahon R.A., Fanning P., O'Boyle K.M., Rogers M., Martin F.: Functional coupling of a recombinant human 5-HT_{5A} receptor to G-proteins in HEK 293 cells. Brit. J. Pharmacol., 1998, 124, 1238–1244.

30. Isaac M., Slassi A., Xin T., MacLean N., Wilson J., McCallum K., Wang H., Demchyshyn L.: 6-Bicyclopiperazinyl-1-arylsulfonylindoles and 6-bicyclopiperidinyl-1-arylsulfonyl-indoles derivatives as novel, potent, and selective 5-HT_6 receptor antagonists. Bioorg. Med. Chem. Lett., 2000, 10, 1719–1721.
31. Kikuchi C., Nagaso H., Hiranuma T., Koyama M.: Tetrahydrobenzindoles: selective antagonists of the 5-HT_7 receptor. J. Med. Chem., 1999, 42, 533–535.
32. 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.
33. 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-Schmied. Arch. Pharmacol., 2001, 363, 620–632.
34. 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 N_1-benzenesulfonyl and related tryptamines. Med. Chem. Res., 2000, 10, 230–242.
35. 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.
36. López-Rodríguez M.L., Porras E., Benhamú 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.
37. 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.
38. 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.
39. Monsma F.J. Jr., 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.
40. Mullins U.L., Gianutsos G., Eison A.S.: Effects of antidepressants on 5-HT_7 receptor regulation in the rat hypothalamus. Neuropsychopharmacology, 1999, 21, 352–367.
41. Oliver K.R., Kinsey A.M., Wainwright A., Sirinathsinghji D.J.S.: Localization of 5-HT_{5A} receptor-like immunoreactivity in the rat brain. Brain Res., 2000, 867, 131–142.
42. Pasqualetti M., Ori M., Nardi I., Castagne M., Cassano G.B., Marazziti D.: Distribution of the 5-HT_{5A} serotonin receptor mRNA in the human brain. Mol. Brain Res., 1998, 56, 1–8.
43. Plassat J.-L., Amlaiky N., Hen R.: Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. Mol. Pharmacol., 1993, 44, 229–236.
44. Plassat J.-L., Boschert U., Amlaiky N., Hen R.: The mouse 5-HT_5 receptor reveals a remarkable heterogeneity within the 5-HT_{1D} receptor family. EMBO J., 1992, 11, 4779–4786.
45. Rees S., den Daas I., Foord S., Goodson S., Bull D., Kilpatrick G., Lee M.: Cloning and characterization of the human 5-HT_{5A} receptor. FEBS Lett., 1994, 355, 242–246.
46. Rogers D.C., Robinson T.L., Quilter C.A., Hunter A.J., Routledge C., Hagan J.J.: Cognitive enhancement effects of the selective 5-HT_6 antagonist SB-271046. Brit. J. Pharmacol., 1999, 127, 22P.
47. Roth B.L., Craigo S.C., Choudhary M.S., Uluer A., Monsma F.J.Jr., 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.
48. 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. Brit. J. Pharmacol., 2000, 130, 1606–1612.
49. Ruat M., Traiffort E., Arrang J.-M., Tardivel-Lacombe J., Diaz J., Leurs R., Schwartz J.-C.: A novel rat serotonin (5-HT_6) receptor: molecular cloning, localization, and stimulation of cAMP accumulation. Biochem. Biophys. Acta, 1993, 193, 268–276.
50. Sebben M., Ansanay H., Bockaert J., Dumuis A.: 5-HT_6 receptor positively coupled to adenylyl cyclase in striatal neurones in culture. NeuroReport, 1994, 5, 2553–2557.
51. 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.
52. Shimizu M., Nishida A., Zensho H., Miyata M., Yamawaki S.: Down-regulation of 5-hydroxytryptamine7 receptors by dexamethasone in rat frontocortical astrocytes. J. Neurochem., 1997, 68, 2604–2609.
53. 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. Brit. J. Pharmacol., 1998, 124, 556–562.
54. Teitler M., Scheick C., Howard P., Sullivan J.E. III, Iwamura T., Glennon R.A.: 5-HT_{5A} serotonin receptor

binding: a preliminary structure-affinity investigation. Med. Chem. Res., 1997, 7, 207–218.
55. Terrón J.A., Falcon-Neri A.: Pharmacological evidence for the 5-HT_7 receptor mediating smooth muscle relaxation in canine cerebral arteries. Brit. J. Pharmacol., 1999, 127, 609–616.
56. Thomas D.R., Atkinson P.J., Ho M., Bromidge S.M., Lovell P.J., Villani A.J., Hagan J.J., Middlemiss D.N., Price G.W.: [^{3}H]-SB-269970 – a selective antagonist radioligand for 5-HT_7 receptors. Brit. J. Pharmacol., 2000, 130, 409–417.
57. Thomas D.R., Gittins S.A., Collin L.L., Middlemiss D.N., Riley G., Hagan J.J., Gloger I., Ellis C.E., Forbes I.T., Brown A.M.: Functional characterization of the human cloned 5-HT_7 receptor (long form); antagonist profile of SB-258719. Brit. J. Pharmacol., 1998, 124, 1300–1306.
58. 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.
59. Vanhoenacker P., Haegeman G., Leysen J.E.: 5-HT_7 receptors: current knowledge and future prospects. Trends Pharmacol. Sci., 2000, 21, 70–77.
60. Vanhoutte P.M., Humphrey P.P.A., Spedding M.: X International Union of Pharmacology recommendations for nomenclature of new receptor subtypes. Pharmacol. Rev., 1996, 48, 1–2.
61. De Vries P., Visser P.A., Heiligers J.P., Villalon C.M., Saxena P.R.: Changes in systemic and regional haemodynamics during 5-HT_7 receptor-mediated depressor responses in rats. Naunyn-Schmied. Arch. Pharmacol., 1999, 359, 331–338.
62. Ward R.P., Hamblin M.W., Lachowicz J.E., Hoffman B.J., Sibley D.R., Dorsa D.M.: Localization of serotonin subtype 6 receptor messenger RNA in the rat brain by *in situ* hybridization histochemistry. Neuroscience, 1995, 64, 1105–1111.
63. Wisden W., Parker E.M., Mahle C.D., Grisel D.A., Nowak H.P., Yocca F.D., Felder C.C., Seeburg P.H., Voigt M.M.: Cloning and characterization of the rat 5-HT_{5B} receptor. FEBS Lett., 1993, 333, 25–31.
64. Yau J.L., Noble J., Widdawson J., Seckl J.R.: Impact of adrenalectomy on 5-HT_6 and 5-HT_7 receptor gene expression in the rat hippocampus. Mol. Brain Res., 1997, 45, 182–186.
65. Yoshioka M., Matsumoto M., Togashi H., Mori K., Saito H.: Central distribution and function of 5-HT_6 receptor subtype in the rat brain. Life Sci., 1998, 62, 1473–1477.

Received: January 25, 2002; in revised form: March 18, 2002.