Synthesis of New Arylpiperazinylalkylthiobenzimidazole, Benzothiazole, or Benzoxazole Derivatives as Potent and Selective 5-HT_{1A} Serotonin Receptor Ligands[†]

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A series of new compounds containing a benzimidazole, benzothiazole, or benzoxazole nucleus linked to an arylpiperazine by different thioalkyl chains was prepared. They were tested in radioligand binding experiments to evaluate their affinity for 5-HT_{1A} and 5-HT_{2A} serotonergic, α_1 adrenergic, D_1, and D_2 dopaminergic receptors. Many of tested compounds showed an interesting binding profile; in particular, **36** displayed very high 5-HT_{1A} receptor affinity and selectivity over all the other investigated receptors. Selected compounds, evaluated in functional assays, showed antagonistic or partial agonistic activity at 5-HT_{1A} receptor. An extensive conformational research using both NMR and modeling techniques indicated that extended conformations predominated in vacuum, in solution and during interactions with 5-HT_{1A} receptor. Finally, the elaborated binding mode of selected compounds at 5-HT_{1A} receptor was used to explain the influence of spacer length on ligands affinity.

Introduction

Serotonin (5-HT)[a] is an important neurotransmitter that mediates various physiological and pathological processes in the peripheral and central nervous system by interaction with several different receptors. To date 14 serotonin receptor (5-HTR) subtypes have been identified and grouped into seven subfamilies (5-HT_{1-7}) on the basis of molecular cloning, amino acid sequence, pharmacology, and signal transduction.[1,2] The 5-HTRs are members of the superfamily of G protein-coupled receptors (GPCRs), with the exception of the 5-HT_3, which is a ligand-gated cation channel receptor. Among the 5-HTRs, the 5-HT_{1A} subtype is one of the most studied and it is generally accepted that it is involved in anxiety and depression;[3,4] recently it has been suggested that 5-HT_{1A}R agonists have neuroprotective properties,[5,6] whereas 5-HT_{1A}R antagonists could be useful in the treatment of Alzheimer disease.[7] Several potent 5-HT_{1A}R ligands belong to different chemical classes[8] such as aminotetralines,[9] indolylalkylamines, ergolines, arylpiperazines,[10,11] aporphines, and aryloxyalkylamines. Among aminotetralines, 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT, **1**) (Chart 1) is the most prominent member, being a potent and selective 5-HT_{1A}R ligand, and its tritiated form is used to label the 5-HT_{1A}Rs.

Long-chain arylpiperazine (LCAP) derivatives represent one of the most important classes of 5-HT_{1A}R ligands. In general, arylpiperazine moiety is a good template for many different biological targets, especially central nervous system receptors. As a consequence, several compounds containing arylpiperazine portion bind with high affinity at 5-HT_{1A}R, but few of them show also high selectivity for 5-HT_{1A}R over other receptors. Buspirone (**2a**) (Chart 1), one of the most known member of this class, shows high affinity for 5-HT_{1A}R but poor selectivity over α_1-adrenergic receptors (α_1-ARs). In general, LCAPs contain an alkyl chain, constituted by two to four carbon atoms, linked to the *N*-1 of piperazine moiety and to a terminal fragment usually containing an amide or imide function. A large number of studies have been devoted to explore the role of the terminal part in ligand−receptor interaction, therefore several structural modifications have been carried out in the terminal fragment.[12]

For many years, our research group has been interested in the synthesis of arylpiperazine derivatives as 5-HT_{1A}R and α_1-AR ligands. Among them, thieno[2,3-*d*]pyrimidine derivatives **3** and **4**[13] (Chart 1) showed high affinity for the 5-HT_{1A}R coupled to a reduced affinity for α_1-AR. More recently, we reported structure−affinity relationship (SAR) studies on a class of 5-phenyl[1,2,4]triazoles structurally related to **3** and **4**, in which the aryl[1,2,4]triazole replaced the 3-amino-6-ethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one moiety.[14,15] Some of these compounds showed K_i values in the nanomolar range, in particular, compound **5** exhibited good selectivity for 5-HT_{1A}R over both the α_1 and D_2 receptors. These results confirmed the hypothesis suggested in the literature that the terminal amide function of LCAPs is not critical for binding.[16,17] On the basis of the above results and with the aim to improve affinity and selectivity for 5-HT_{1A}R, in this work we describe the synthesis and the binding data for 5-HT_{1A}Rs, α_1-ARs, 5-HT_{2A} serotonergic, D_1, and D_2 dopaminergic receptors of a new class of arylpiperazinylthioalkyl derivatives (**16**−**38**) (Chart 1). Derivatives **16**−**38**, as the most of LCPAs, can be divided, from a structural point of view in three principal parts: (i) a pharmacophoric portion constituted by a substituted arylpiperazine, (ii)

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[a] Abbreviations: 5-HT, serotonin; 5-HTR, serotonin receptor; GPCRs, G protein-coupled receptors; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetraline; 5-HT_{1A}R, 5-HT_{1A} serotonin receptor; LCAP, long-chain arylpiperazine; α_1-ARs, α_1-adrenergic receptors; [^{35}S]GTPγS, guanosine-5′-*O*-(γ-thio)triphosphate; DMSO, dimethylsulfoxide; EC_{50}, half-maximum effective inhibitory concentration; PMF, potential mean force; IC_{50}, median inhibitory concentration; K_i, inhibitor constant; TMS, tetramethylsilane; TFA, trifluoroacetic acid.

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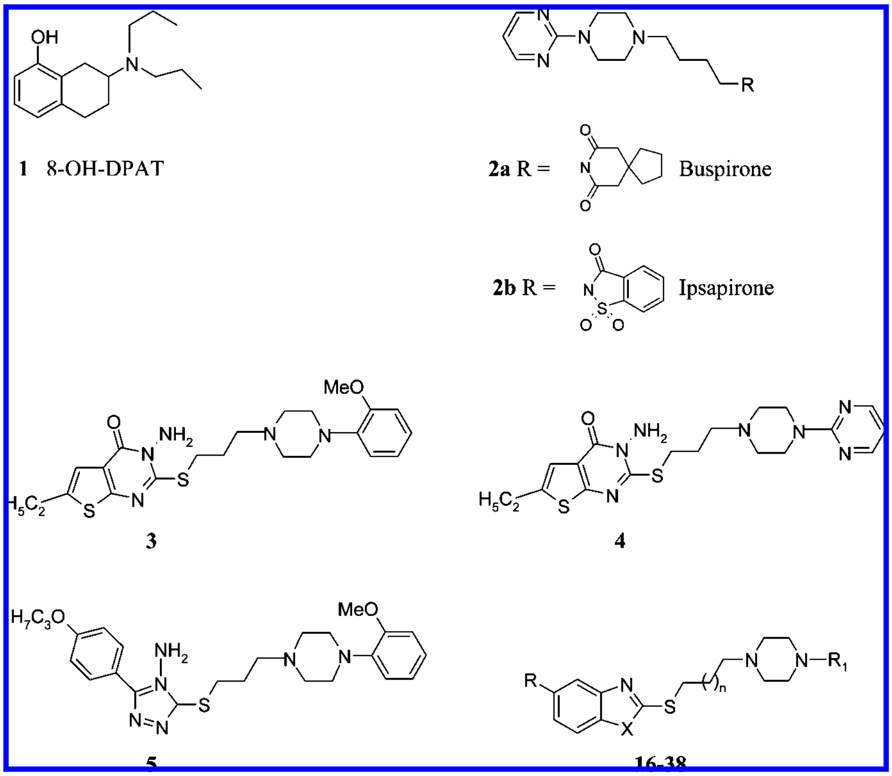
Chart 1. 5-HT_{1A} Receptor Ligands



a terminal fragment constituted by a heterobicyclic system, (iii) a linker between these two substructures. In these new molecules, the pharmacophoric portion was represented by a 2-nitrophenyl-, 2-methoxyphenyl-, pyridin-2-yl-, or pyrimidin-2-yl-1-piperazine; the terminal fragment was represented by a benzimidazole, benzothiazole, or benzoxazole ring systems (often present in the structure of ligands for receptors of biogen amines),[18] the linker connecting arylpiperazines and bicyclic nucleus was a flexible alkylthio chain of different length (n = 0, 1, 2, 4) (Chart 1). To get information on functional activity at 5-HT_{1A}R, selected compounds were also tested in [^{35}S]GTPγS binding assays. Furthermore, NMR experiments, conformational analysis, and molecular dockings were conducted to understand conformational preferences of studied compounds in different environments. Finally, the previously elaborated full flexible docking methodology[19] was used for explanation of ligand–receptor interactions important for affinity.

Chemistry

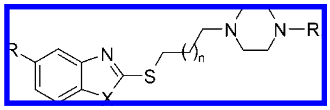
Compounds **16–38** (Table 1) were obtained by a simple one- or two-steps condensation as outlined in Scheme 1. The commercially available compounds **6–10** were reacted with appropriate 1-(ω-chloroalkyl)-4-arylpiperazine in acetone at reflux for 24 h, in the presence of potassium carbonate and potassium iodide, to give desired products **16–34** in good yields. Since efforts devoted to prepare compound **32** by direct methylation of **22** with CH_3I were unsuccessful, it was synthesized using as starting material *N*-methylbenzimidazoline-2-thione (**11**), prepared according to literature.[20] Reaction of 1-(2-methoxyphenyl)piperazine with 1-bromo-4-chlorobutane did not give the desired alkylating agent requested for the preparation of compounds **35** and **36**, but a quaternary spiro structure[21] that did not react in the standard conditions described for the preparation of compounds **16–34**. Therefore, to synthesize compounds **35–38**, we used the reaction condition described by Mokrosz et al. for *N*-alkylation of benzotriazole.[22] In brief, 2-mercaptobenzoxazole (**7**) or 2-mercaptobenzothiazole (**8**) were alkylated with 1-bromo-4-chlorobutane or 1-bromo-6-chlorohexane in acetonitrile at reflux for 1 h in the presence of potassium fluoride/aluminum oxide. The intermediates 2-[(4-chlorobutyl)thio]benzothiazole or benzoxazole and 2-[(6-chlorohexyl)thio]benzothiazole or benzoxazole **12–15** were then reacted with 1-(2-methoxyphenyl)piperazine in acetonitrile at reflux for 1 h in the presence of potassium carbonate to give desired final products **35–38**.

Results and Discussion

Compounds **16–38** were tested in binding assays to evaluate their affinity and selectivity for 5-HT_{1A}Rs over α_1-ARs. For the derivatives that showed the highest affinity and selectivity toward the 5-HT_{1A}R over α_1-ARs, affinities for 5-HT_{2A} serotonergic, D_1, and D_2 dopaminergic receptors were also evaluated. Binding data, expressed as K_i (nM), are summarized in Tables 1 and 2 along with selectivities reported as ratios of K_i values.

The first step of our investigation was the synthesis of compounds **16–27** in which a 2-methoxy- or a 2-nitrophenylpiperazine was linked to a benzoxazole, benzothiazole, or benzimidazole system by an ethylthio or propylthio unit. In general, derivatives **19–21** and **25–27**, which possess the 2-nitrophenylpiperazine, showed lower affinity for 5-HT_{1A}R with respect to the 2-methoxyphenylpiperazine analogues **16–18** and **22–24**, as previously described for analogues containing 5-phenyl[1,2,4]triazole nucleus.[14,15] In addition, compounds characterized by a thiopropyl chain between the terminal fragment and the arylpiperazine portion (**22–27**) exhibited higher affinity toward 5-HT_{1A}R with respect to the analogues **16–21** containing a thioethyl chain as linker. In particular, among thiopropyl derivatives, **23** was the most interesting because it showed an affinity for 5-HT_{1A}R in the subnanomolar range (K_i = 0.29 nM) coupled to a high selectivity over α_1-AR

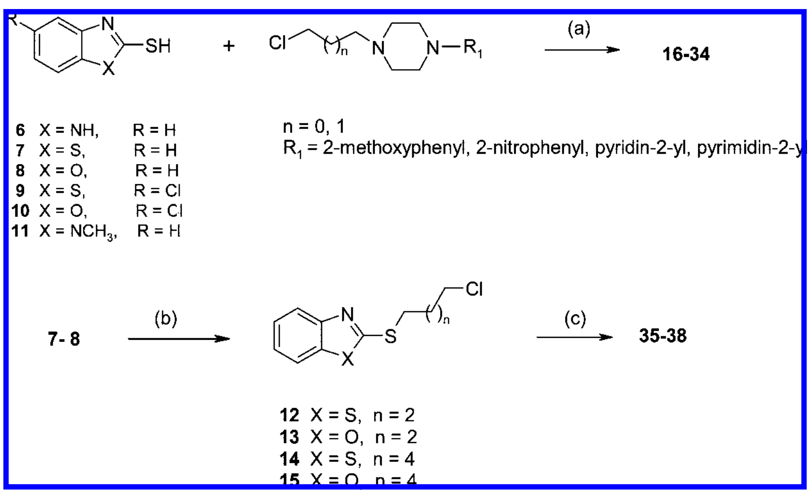
Table 1. 5-HT$_{1A}$ and α_1-Adrenergic Receptors Binding Data for Compounds **16**−**38**



compd	X	*n*	R	R$_1$	K_i (nM)[a] 5-HT$_{1A}$	α_1	α_1/5-HT$_{1A}$[b]
16	NH	0	H	2-$CH_3OC_6H_4$	33.1 ± 5.8	240 ± 31	7.25
17	S	0	H	2-$CH_3OC_6H_4$	31.2 ± 3.4	207 ± 51	6.63
18	O	0	H	2-$CH_3OC_6H_4$	67.2 ± 17.1	276 ± 34	4.11
19	NH	0	H	2-$NO_2C_6H_4$	95.2 ± 13.0	457 ± 33	4.8
20	S	0	H	2-$NO_2C_6H_4$	1702 ± 398	>10000	>5.87
21	O	0	H	2-$NO_2C_6H_4$	326 ± 91	1915 ± 200	5.87
22	NH	1	H	2-$CH_3OC_6H_4$	1.0 ± 0.1	25.9 ± 5	26
23	S	1	H	2-$CH_3OC_6H_4$	0.29 ± 0.06	33.2 ± 5.4	114
24	O	1	H	2-$CH_3OC_6H_4$	0.55 ± 0.05	21.5 ± 4.5	39
25	NH	1	H	2-$NO_2C_6H_4$	16.6 ± 2.22	77.4 ± 5.9	4.66
26	S	1	H	2-$NO_2C_6H_4$	9.6 ± 0.6	249 ± 30	26
27	O	1	H	2-$NO_2C_6H_4$	14.1 ± 2.0	127 ± 15	9
28	S	1	H	pyridin-2-yl	0.78 ± 0.02	77.7 ± 7.6	100
29	O	1	H	pyridin-2-yl	1.60 ± 0.12	98.0 ± 5.6	61
30	S	1	H	pyrimidin-2-yl	2.5 ± 0.1	328 ± 44	131
31	O	1	H	pyrimidin-2-yl	14.9 ± 1.7	1651 ± 122	111
32	NCH_3	1	H	2-$CH_3OC_6H_4$	0.270 ± 0.002	20.0 ± 2.9	74
33	S	1	Cl	2-$CH_3OC_6H_4$	1.0 ± 0.2	21.9 ± 0.7	22
34	O	1	Cl	2-$CH_3OC_6H_4$	0.88 ± 0.01	46.7 ± 4.6	53
35	S	2	H	2-$CH_3OC_6H_4$	0.27 ± 0.01	16.6 ± 1.4	61
36	O	2	H	2-$CH_3OC_6H_4$	0.094 ± 0.008	78.0 ± 23.2	830
37	S	4	H	2-$CH_3OC_6H_4$	1.30 ± 0.004	46.0 ± 7.3	53
38	O	4	H	2-$CH_3OC_6H_4$	0.52 ± 0.01	52.5 ± 7.4	101
buspirone[c]					15	600	40
ipsapirone[c]					5.5	200	36

[a] K_i values were calculated as described in the Experimental Section and are the mean ± SD of three separate experiments. [b] Ratio K_i α_1/K_i 5-HT$_{1A}$. [c] Data taken from literature.[31]

Scheme 1[a]



[a] Reagents and conditions: (a) K_2CO_3, KI, CH_3COCH_3, reflux, 24 h; (b) 1-bromo-4-chlorobutane or 1-bromo-6-chlorohexane, CH_3CN, KF/Al_2O_3, KI, reflux, 1 h; (c) 1-(2-methoxyphenyl)piperazine, CH_3CN, K_2CO_3, reflux, 1 h.

(K_i α_1-AR/K_i 5-HT$_{1A}$R = 114). With regard to the terminal fragment, compounds bearing a benzothiazole or benzoxazole (**17**, **18**, **20**, **21**, **23**, **24**, **26**, and **27**) showed higher affinity than those bearing their isoster benzimidazole (**16**, **19**, **22**, and **25**). On the basis of these results and in order to further study SARs within this series, we decided to carry out additional work. In particular, considering **23** as "lead compound", the following structural modifications were realized: (i) replacement of the 2-methoxyphenyl with other aromatic rings, such as pyridin-2-yl or pyrimidin-2-yl nucleus present in the buspirone and ipsapirone, to obtain compounds **28**−**31**; (ii) introduction of a chlorine atom at the 5-position of the benzothiazole or benzoxazole nucleus to obtain compounds **33** and **34**; (iii) methylation on *N*-1 of the benzimidazole nucleus of **22** to obtain compound **32**. Compounds **28**−**31** showed a slight decrease in affinity at 5-HT$_{1A}$R, if compared to related compounds **22**−**24**. In particular, compounds bearing a pyridin-2-yl residue (**28** and **29**) still remained potent ligands at the 5-HT$_{1A}$R, whereas pyrimidin-2-yl derivatives **30** and **31**, despite their lower affinity at 5-HT$_{1A}$R, maintained good selectivity over α_1-AR (K_i α_1-AR/K_i 5-HT$_{1A}$R = 132 and 111, respectively); therefore, the presence of the pyrimidin-2-yl ring in the pharmacophoric portion seems to play an unfavorable role in the interaction of these compounds with α_1-AR binding site. The 5-HT$_{1A}$R affinity

Table 2. D_1, D_2, and 5-HT_{2A} Receptors Binding Data for Selected Compounds

compd	K_i (nM)[a] D_1	D_2	5-HT_{2A}	D_1/5-HT_{1A}[b]	D_2/5-HT_{1A}[b]	5-HT_{2A}/5-HT_{1A}[b]
23	1280 ± 161	25.8 ± 3.1	96.1 ± 7.9	4415	89	331
28	5630 ± 925	462 ± 51	78.8 ± 8.8	7218	592	101
29	>10.000	429 ± 86	174 ± 35	6250	268	109
30	>10.000	532 ± 36	183 ± 18	4000	213	73
35	2005 ± 388	58.3 ± 12.2	106 ± 23	7426	216	393
36	1600 ± 82	37.3 ± 8.3	298 ± 44	17021	397	3170
38	922 ± 85	46.6 ± 7.0	207 ± 19	1773	90	398

[a] K_i values were calculated as described in the Experimental Section and are the mean ± SD of three separate experiments. [b] Ratio K_i D_1/K_i 5-HT_{1A}, K_i D_2/K_i 5-HT_{1A}, and K_i 5-HT_{2A}/K_i 5-HT_{1A}.

of *N*-methyl derivative **32** was greater than that of the parent compound **22**, suggesting that the presence of an unsubstituted nitrogen at the imidazole nucleus is detrimental for affinity toward 5-HT_{1A}R. The introduction of a chlorine atom in the benzoxazole or benzothiazole at the 5-position caused only a slight decrease in the 5-HT_{1A}R affinity. As reported in the literature, the length of the alkyl chain greatly influences affinity and selectivity for 5-HT_{1A}R.[11,13] Therefore, with the aim to investigate the effect of the length of the connecting chain on binding properties, we synthesized compounds **35**−**38**, characterized by a butylthio or a hexylthio linker, having a connecting chain between the arylpiperazine moiety and the bicyclic heteroaromatic system equivalent to 5 or 7 methylene units, respectively. A comparison of the binding data of compounds **35**−**38** with those of related **23** indicated that affinity for 5-HT_{1A}R was very similar for **35**, **37**, and **38**, whereas selectivity over α_1-AR was reduced for **35** and **37**. On the contrary, 2-[[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]ylthio]benzoxazole (**36**) results were more potent and selective (K_i 5-HT_{1A}R = 0.094 nM, K_i α_1-AR/K_i 5-HT_{1A}R = 830) than **23**, thus being the most interesting compound within this series.

Compounds **23**, **28**−**30**, **35**, **36**, and **38** that showed high affinity and selectivity were tested for their affinities at D_1, D_2 dopaminergic, and 5-HT_{2A} serotonergic receptors. Results clearly demonstrated that these compounds possess a very good binding profile, preferring 5-HT_{1A}Rs over all other evaluated receptors. In particular, with regard to dopaminergic receptors, all the tested compounds exhibited no affinity for D_1 receptor, with K_i values in the range 922−10000 nM; affinity for D_2 receptor was in some cases moderate (**23**, **35**, **36**, and **38**), with K_i values in the range 25−58 nM, or low (**28**, **29**, and **30**), with K_i values in the range 429−532 nM. The 5-HT_{2A} receptor affinity of the tested compounds was generally lower than those observed for 5-HT_{1A}R, and so they exhibited a favorable 5-HT_{2A}/5-HT_{1A} selectivity ratio.

The functional activity of selected compounds **23**, **28**, **36**, and **38** along with compounds **3** and **4** was determined in functional [^{35}S]GTPγS binding assays in rat hippocampal membranes, and results are reported in Table 3. Compounds **3**, **23**, **36**, and **38** behave as 5-HT_{1A}R antagonists being able to block the increase of [^{35}S]GTPγS binding induced by 10 μM 5-HT in a dose-dependent manner, with a K_i value in the nanomolar range. Compounds **4** and **28** behave as partial agonists because also at the high concentration tested (10 μM), they did not reach the maximum effect elicited by 5-HT but only a 30−40% enhancement of [^{35}S]GTPγS binding, with EC_{50} values of 1210 and 204 nM, respectively.

To gain insight into structural properties of compounds **16**−**38**, we selected benzoxazole derivatives **18**, **24**, **36**, and **38**, where the sole structural difference lies in the length of the connecting chain. At this purpose, ^{1}H NMR experiments of the

Table 3. Effect of 5-HT and Compounds **4**, **28**, **3**, **23**, **36**, and **38** on [^{35}S]GTPγS Binding to 5-HT_{1A}R[a]

	E_{max} (%)	EC_{50} ± SD, nM	K_i ± SD, nM
5-HT (*agonist*)	100 ± 5.6	323 ± 47	
4 (*partial agonist*)	38 ± 1.5	1210 ± 26	
28 (*partial agonist*)	33 ± 2.3	204 ± 45	
3 (*antagonist*)			7.4 ± 2.4
23 (*antagonist*)			1.7 ± 0.4
36 (*antagonist*)			6.6 ± 1.9
38 (*antagonist*)			1.3 ± 0.4

[a] [^{35}S]GTPγS binding in rat hippocampus. Hippocampal homogenates were incubated in the presence of graded concentrations of 5-HT or compounds **4** and **28**. The antagonists were tested at different concentrations in presence of 10 μM 5-HT. IC_{50}, EC_{50}, and E_{max} were obtained using the "Allfit" program and K_i were derived from the IC_{50} values using the Cheng and Prusoff equation.[29] Data are the mean ± SD of three separate experiments.

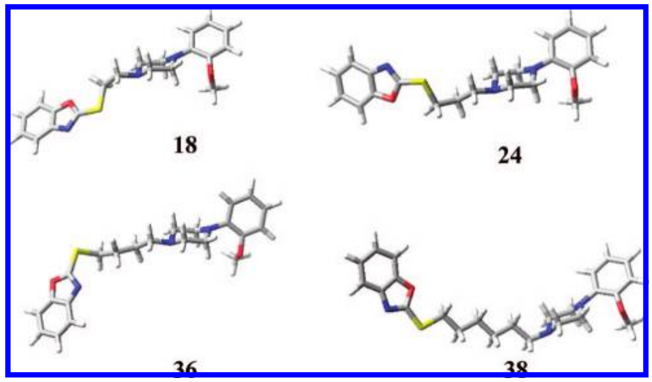


Figure 1. Computer calculated structures of compounds **18**, **24**, **36**, and **38** in their fully extended conformation.

free bases in DMSO solution and molecular modeling studies were performed. The 500 MHz ^{1}H NMR spectra consist of relatively simple and well resolved patterns of resonances straightforwardly assigned by inspection of COSY maps. Moreover, at 300 K, the independence of chemical shifts from concentration was verified by excluding possible aggregation effects. 2D-NOE experiments showed the cross peaks expected for intramolecular interactions in agreement with extended conformations in solution. Molecular modeling studies of compounds **18**, **24**, **36**, and **38** were undertaken using MacroModel (version 8.6) with the MM2* force field.[23] Conformational preferences were explored using the parameters for either isolated "gas-phase" or water continuum. Monte Carlo searches were conducted by allowing all flexible bonds to rotate, the simulations collected 800 iterations each, and conformations that were within 4 kJ of the lowest energy conformation were examined. Among the variety of structures produced, the free base energetically favored conformations (in the range 178.81−187.02 kJ mol^{-1}) showed the methylene bridging groups in an antiperiplanar arrangement with aromatic portions enough far each other, consistent with NMR experimental data (Figure 1). Higher energy folded structures were also generated, which were about 30 kJ above the extended ones, on the maximum. This feature occurs, in particular, for compound **38**, where the longest spacer separates the pharmacophoric portion and the aromatic terminal group.

As folded conformations could not be precluded, exploration of the conformational properties of **38** was extended to its salt in solution. In fact, optimized folded structures showed the space proximity of the benzoxazole ring and piperazine, wherein the protonation of the nitrogen might engender an intramolecular hydrogen bond interaction with the heteroatoms of benzoxazole

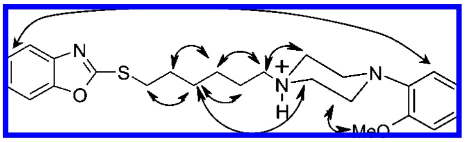


Figure 2. Significant NOE signals of protonated **38** in DMSO/TFA solution.

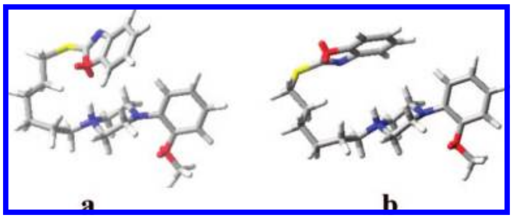


Figure 3. Folded low-energy conformation of the protonated form of **38** in vacuum (a) and in aqueous medium (b).

moiety, useful to stabilize folded conformations. Addition of TFA in DMSO solution of **38** caused a sensible variation of the ^{1}H NMR spectrum easily interpreted with the protonation of the piperazine nitrogen N-1. Inspection of the NOESY map evidenced significant NOE cross peaks represented in Figure 2.

Besides the expected strong intramolecular NOE signals, weak dipolar contacts were observed between methylenes of the alkyl chain and piperazine protons and between the benzoxazole protons and the aromatic proton located on the 2-methoxyphenyl ring, suggesting that folded conformational structures were also present in solution in equilibrium with extended ones.

The results of conformational analysis of protonated form of the compound **38** gave, in vacuum, the folded lowest-energy conformation (219.7 kJ mol^{-1}), shown in Figure 3a, where an intramolecular hydrogen bond (2.006 Å) between the protonated piperazine nitrogen and the properly oriented lone pair on the nuclear oxygen of the benzoxazole ring may contribute to stabilize the structure, whereas the two aromatic moieties were found in an edge-to-face disposition.

The continuous aqueous medium generated lowest energy conformation (Figure 3b) showed a larger distance (4.04 Å) between protonated piperazine and the nuclear oxygen lone pair of the benzoxazole ring, indicating that hydrogen bonding interaction found in "gas phase" could be overestimated with respect to a more realistic model in a polar solvent. Despite this finding, the folded structure (in equilibrium with extended ones) is retained with the aromatic moieties in close contact (ca. 3 Å), in agreement with the existence of restricted conformations evidenced by NMR NOE data.

To complete an examination of conformational preferences of studied compounds (**18**, **24**, **36**, and **38**), a fully flexible docking approach with the use of 100 5-HT$_{1A}$R models,

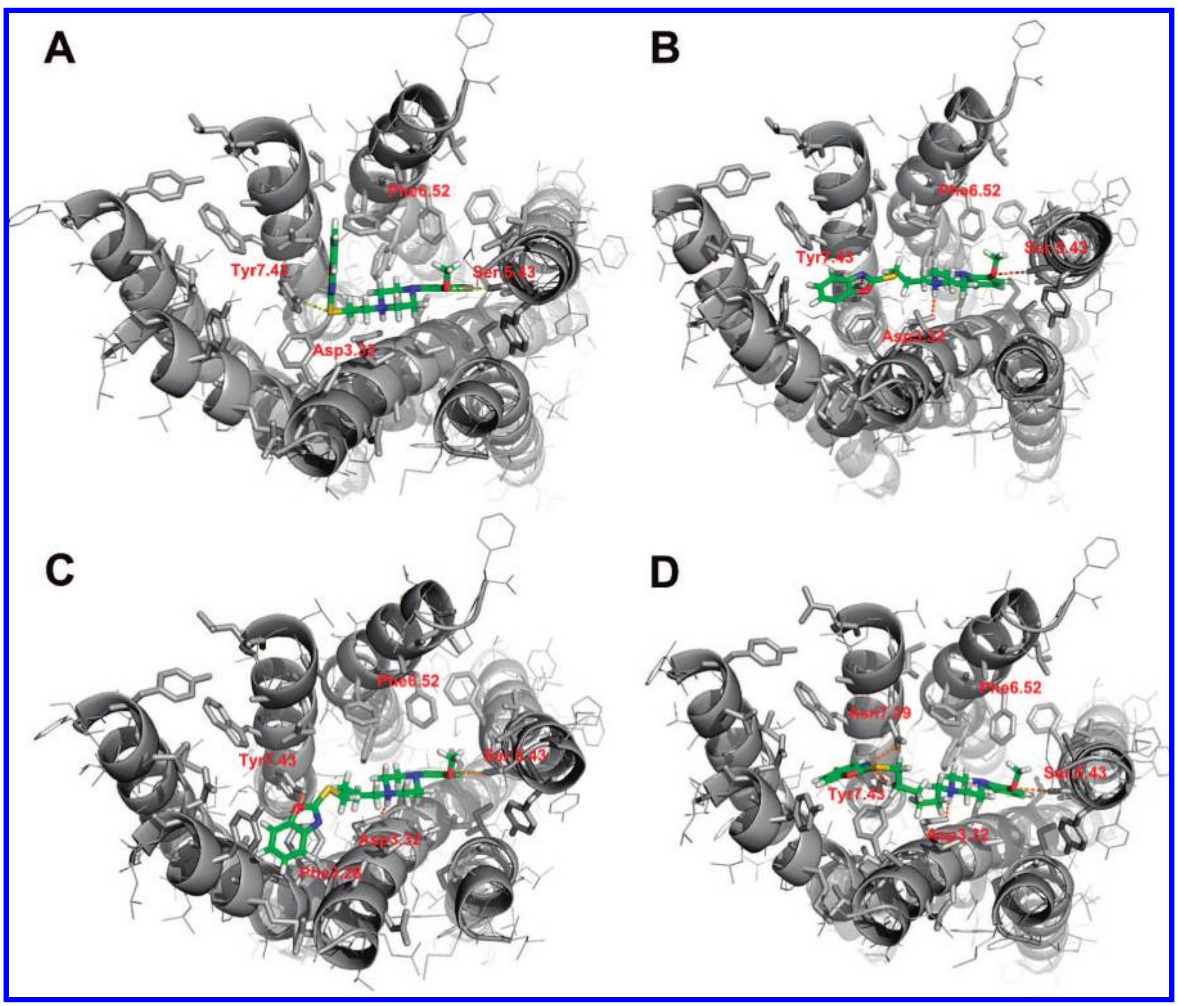


Figure 4. Top scored ligand–receptor complexes for compounds studied by means of ligand–receptor docking: (A) **18**, (B) **24**, (C) **36**, (D) **38**. Helical bundle is presented from the extracellular side. Sticks representation depicts residues used as "active site" in FlexX docking. Amino acids forming specific interactions with ligands were labeled. Dashed yellow line shows H-bonding.

Table 4. 5-HT_{1A}R Experimental Binding Affinity: PMF Score for (a) the Top Solutions of the Entire Set of Receptor Models and for (b) the Receptor Model 82[a]

		top solution		receptor model no. 82[a]	
compd	K_i 5-HT_{1A} (nM)	PMF score	receptor model no.	PMF score	rank[b]
18	67.22	−85.8	82	−85.8	1
24	0.55	−106.2	67	−106.0	3
36	0.094	−110.9	82	−110.9	1
38	0.52	−111.0	23	−102.5	28

[a] The best receptor model identified according to the lowest value of summed PMF scores of complexes with analyzed compounds. [b] Position in the ranking of the PMF scores for the complex of ligand−receptor model 82.

previously "tuned" for arylpiperazine ligands,[19] was applied. Pharmacophoric constraints imposed on 2-methoxyphenylpiperazine fragment facilitated reproduction of the proposed LCAPs binding mode. The resulting 4 × 100 L−R complexes were subjected to a consensus scoring procedure, and the PMF (potential mean force) score was used for further analysis. Among all the docked poses, the extended conformations predominated and no folded arrangements were found. For the three-unit spacer compound, however, partly bent (hockey stick-like) conformations were also observed because even one torsion angle in synclinal position caused such a shape of a molecule. It has to be stressed that in all the best-scored complexes, the analyzed molecules existed in extended conformations (Figure 4, Table 4), which is in general agreement with NMR experimental data as well as with results of conformational analysis for free bases (Figure 1).

A careful inspection of preferable ligand binding poses generated in our models gives additional information that can be discussed in regard to the observed differences in affinity. As depicted in Figure 4, apart from principal interactions coming from pharmacophoric arylpiperazine fragment, benzoxazole moiety of **24**, **36**, and **38** formed H-bonds with Tyr7.43 and/or Asn7.39. Additionally, for the best binder in the group (**36**), a remarkable $\pi-\pi$ stacking with Phe3.28 was observed (Figure 4C). This last finding could be considered as a possible explanation of outstanding high affinity of this compound, especially when compared to binding results obtained for derivatives **24** and **38**. The most visible difference in K_i values characterized compound **18** that was at least 100-fold less active than the others. In the scored solution for this compound, only weak H-bond interaction was present between Tyr7.43 and thioether fragment of the spacer (Figure 4C). In a certain number of solutions, H-bonds between benzoxazole moiety of **18** and residues on helix 7 were formed (results not shown) but at the cost of weakening interactions from arylpiperazine part. On the other hand, when this pharmacophoric portion of **18** occupied more optimal position (common for the remaining compounds) as was found, e.g., for the best receptor model 82, the benzoxazole fragment has lost its specific interactions with helix 7 and is pointed toward the exterior of the binding site (Figures 4A and 5).

Because of significant simplification of scoring functions, quantitative correlations of PMF scores with binding data are not justified (Table 4); nevertheless, our molecular docking experiments brought qualitative rationalization of ligand−receptor interactions important for affinity.

Conclusions

In this study, a new series of arylpiperazine derivatives **16**−**38** were synthesized as 5-HT_{1A}R ligands. Generally, most of compounds showed very high affinity for the 5-HT_{1A}R (with a K_i values in the nanomolar range) and good selectivity over 5-HT_{2A}, α_1, D_1, and D_2 receptors.

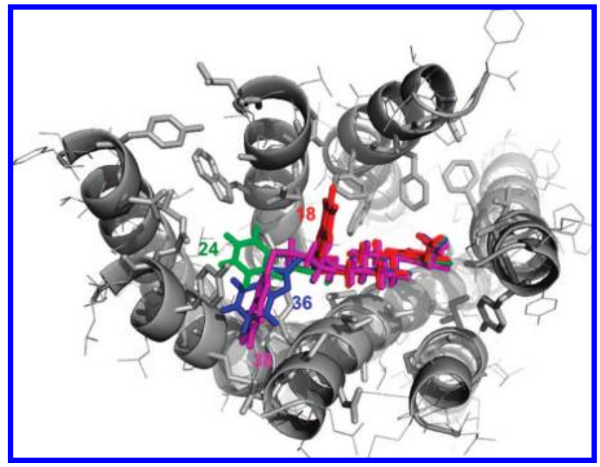


Figure 5. The best receptor model (no. 82) with top scored poses of all four ligands. Arylpiperazine part was constrained to interact with Phe6.62, Ser5.43, and Asp3.32.

SAR analyses pointed out the importance of some structural features for an optimal interaction of these molecules with the 5-HT_{1A}R binding site. With regard to the arylpiperazine pharmacophoric substructure, 2-methoxyphenyl and pyridin-2-yl moieties gave compounds endowed with the best affinities; 2-nitrophenyl and pyrimidin-2-yl derivatives were poorer ligands, although the latter still showed remarkable selectivity over the α_1-AR. Considering the terminal fragment, benzoxazole and benzothiazole derivatives invariably were more potent ligands than corresponding benzimidazole analogues. As far as the length of the spacer between pharmacophoric and terminal moieties is concerned, thiobutyl and thiopropyl chains gave the best contribution to affinity at 5-HT_{1A}R and selectivity over the other receptors. Among tested compounds, 2-[[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]lthio]benzoxazole (**36**) displayed very high affinity at 5-HT_{1A}R (K_i in the subnanomolar range) and the best selectivities over 5-HT_{2A}, α_1, D_1, and D_2 receptors. This binding profile makes **36** the most striking compound within this series and one of the most interesting 5-HT_{1A}R ligand so far reported. The nature of the pharmacophoric portion greatly influences the functional activity at 5-HT_{1A}R of these compounds. In fact, in [^{35}S]GTPγS binding assay, 2-methoxyphenyl derivatives behaved as antagonists, whereas pyridin-2-yl and pyrimidin-2-yl derivatives showed partial agonistic activity.

An extensive conformational research carried out for a representative set of derivatives **18**, **24**, **36**, and **38** showed a strong preference of extended molecular arrangements. Although folded conformations were detected in NMR experiments and classic conformational analysis, they were not found during thorough flexible docking to a set of 100 models of 5-HT_{1A}R. The applied approach allowed the identification of important interactions within receptor binding site, which seems to determine ligands affinity. Longer spacer (4−7 units) of compounds with nanomolar activity enabled very strong interactions of the terminal benzoxazole moiety with respective residues on helix 7 or 3 and arylpiperazine pharmacophoric part with Asp3.32, Phe6.62, and Ser5.43. On the other hand, a three-unit spacer seems too short to form both contacts in optimal mode, explaining significantly lower affinity of compound **18**.

In the end, obtained results appear to be coherent with observed differences in experimental binding affinities and the usefulness of previously elaborated full flexible docking methodology[19] was additionally attested.

Experimental Section

Chemistry. Melting points were determined in a Gallemkamp apparatus with an MFB-59 digital thermometer in glass capillary tubes and are uncorrected. Elemental analyses for C, H, N, and S were within ± 0.4% of theoretical values and were performed on a Carlo Erba elemental analyzer model 1108 apparatus. ^{1}H NMR spectra were determined with a Varian Inova Unity 200 (200 MHz), and 2D-NOE and COSY spectra were determined with a Varian Inova Unity 500 (500 MHz) instrument in DMSO-d_6. Chemical shifts are in δ values (ppm) using tetramethylsilane as the internal standard; coupling constants (J) are given in Hz. Signal multiplicities are characterized as s (singlet), d (doublet), t (triplet), q (quartet), qu (quintet), m (multiplet), and br (broad signal). All the synthesized compounds were tested for purity on TLC on Merck plates (Kieselgel 60 F_{254}), and spots were visualized under the UV light (λ = 254 and 366 nm). Preparative column chromatography was performed using Merck silica gel (0.040−0.063 mm). All chemicals and solvents were reagent grade and were purchased from commercial sources. 1-(ω-Chloroalkyl)-4-arylpiperazines were synthesized according to literature.[24]

General Procedure for the Preparation of Compounds 16−34. A solution of 3 mmol of suitable 1-(ω-chloroalkyl)-4-arylpiperazine in 20 mL of acetone was slowly added dropwise to a suspension in acetone (20 mL) of compounds **6−11** (3 mmol), potassium carbonate (3 mmol), and a catalytic amount of potassium iodide. The mixture was refluxed for 24 h. After this period, the mixture was concentrated, and the residue was diluted with brine (30 mL) and extracted with ether (4 × 20 mL). The combined ethereal phases were washed with brine, dried, and the solvent removed in vacuo. The obtained residue was purified by flash column chromatography on silica gel using ethyl acetate/cyclohexane (1:1) mixtures as eluent. Using this procedure, the following products were synthesized.

2-[[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]thio]1H-benzimidazole (16). The title compound was isolated as white powder (65%); mp 136−138 °C. ^{1}H NMR (DMSO-d_6): δ 2.55- 2.67 (m, 4H, piperazine), 2.74 (t, J = 6.6 Hz, 2H, CH_2N), 2.91−2.99 (m, 4H, piperazine), 3.46 (t, J = 6.6 Hz, 2H, SCH_2), 3.76 (s, 3H, CH_3), 6.86−6.93 (m, 4H, aromatic), 7.08−7.12 (m, 2H, aromatic), 7.40−7.45 (m, 2H, aromatic), 12.53 (br s, 1H, NH, which exchanges with D_2O). Anal. ($C_{20}H_{24}N_4OS$) C, H, N, S.

2-[[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]thio]benzothiazole (17). The title compound was isolated as white powder (70%); mp 77−78 °C. ^{1}H NMR (DMSO-d_6): δ 2.58- 2.65 (m, 4H, piperazine), 2.78 (t, J = 6.8 Hz, 2H, CH_2N), 2.93−2.99 (m, 4H, piperazine), 3.56 (t, J = 6.8 Hz, 2H, SCH_2), 3.77 (s, 3H, CH_3), 6.85−6.93 (m, 4H, aromatic), 7.31−7.50 (m, 2H, aromatic), 7.83−7.88 (m, 1H, aromatic), 7.99−8.03 (m, 1H, aromatic). Anal. ($C_{20}H_{23}N_3OS_2$) C, H, N, S.

2-[[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]thio]benzoxazole (18). The title compound was isolated as light-yellow powder (77%); mp 75−76 °C. ^{1}H NMR 500 MHz (DMSO-d_6): δ 2.60 (br m, 4H, piperazine), 2.79 (t, J = 6.0 Hz, 2H, CH_2N), 2.93 (br m, 4H, piperazine), 3.54 (t, J = 6.0 Hz, 2H, SCH_2), 3.76 (s, 3H, CH_3), 6.85 (m, 2H, aromatic), 6.92 (m, 2H, aromatic), 7.31 (m, 2H, aromatic), 7.63 (m, 2H, aromatic). Anal. ($C_{20}H_{23}N_3O_2S$) C, H, N, S.

2-[[2-[4-(2-Nitrophenyl)-1-piperazinyl]ethyl]thio]1*H*-benzimidazole (19). The title compound was isolated as bright-orange powder (68%); mp 174−175 °C. ^{1}H NMR (DMSO-d_6): δ 2.50- 2.67 (m, 4H, piperazine), 2.74 (t, J = 7.0 Hz, 2H, CH_2N), 2.92−3.05 (m, 4H, piperazine), 3.46 (t, J = 7.0 Hz, 2H, SCH_2), 7.02−7.23 (m, 3H, aromatic), 7.28−7.62 (m, 4H, aromatic), 7.71−7.82 (m, 1H, aromatic), 12.55 (br s, 1H, NH, which exchanges with D_2O). Anal. ($C_{19}H_{21}N_5O_2S$) C, H, N, S.

2-[[2-[4-(2-Nitrophenyl)-1-piperazinyl]ethyl]thio]benzothiazole (20). The title compound was isolated as bright-orange powder (68%); mp 79−81 °C. ^{1}H NMR (DMSO-d_6): δ 2.54−2.65 (m, 4H, piperazine), 2.79 (t, J = 7 Hz, 2H, CH_2N), 2.93−3.04 (m, 4H, piperazine), 3.55 (t, J = 7.0 Hz, 2H, SCH_2), 7.06−7.15 (m, 1H, aromatic), 7.27−7.39 (m, 2H, aromatic), 7.42−7.49 (m, 1H, aromatic), 7.53−7.61 (m,1H, aromatic), 7.74−7.88 (m, 2H, aromatic), 7.97−8.03 (m, 1H, aromatic). Anal. ($C_{19}H_{20}N_4O_2S_2$) C, H, N, S.

2-[[2-[4-(2-Nitrophenyl)-1-piperazinyl]ethyl]thio]benzoxazole (21). The title compound was isolated as bright-orange oil (68%). ^{1}H NMR (DMSO-d_6): δ 2.51−2.55 (m, 4H, piperazine), 2.80 (t, J = 6.6 Hz, 2H, CH_2N), 2.90−3.05 (m, 4H, piperazine), 3.53 (t, J = 6.6 Hz, 2H, SCH_2), 7.06−7.17 (m, 1H, aromatic), 7.26−7.39 (m, 3H, aromatic), 7.53−7.69 (m, 3H, aromatic), 7.75−7.82 (m, 1H, aromatic). Anal. ($C_{19}H_{20}N_4O_3S$) C, H, N, S.

2-[[3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl]thio]1*H*-benzimidazole (22). The title compound was isolated as cream powder (59%); mp 153 °C. ^{1}H NMR (DMSO-d_6): δ 1.86−1.95 (m, 2H, $CH_2CH_2CH_2$), 2.41- 2.54 (m, 2H + 4H, CH_2N + piperazine), 2.89−3.03 (m, 4H, piperazine), 3.31 (t, J = 7.0 Hz, 2H, SCH_2), 3.76 (s, 3H, CH_3), 6.80−6.96 (m, 4H, aromatic), 7.05−7.18 (m, 2H, aromatic), 7.33−7.55 (m, 2H, aromatic), 12.52 (s, 1H, NH, which exchanges with D_2O). Anal. ($C_{21}H_{26}N_4OS$) C, H, N, S.

2-[[3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl]thio]benzothiazole (23). The title compound was isolated as brown semisolid (59%). ^{1}H NMR (DMSO-d_6): δ 1.91−2.05 (m, 2H, $CH_2CH_2CH_2$), 2.42−2.55 (m, 2H + 4H, CH_2N + piperazine), 2.85−3.02 (m, 4H, piperazine), 3.41 (t, J = 7.2 Hz, 2H, SCH_2), 3.76 (s, 3H, CH_3), 6.82−6.95 (m, 4H, aromatic), 7.28−7.56 (m, 2H, aromatic), 7.80−7.87 (m, 1H, aromatic) 7.90−8.05 (m, 1H, aromatic). Anal. ($C_{21}H_{25}N_3OS_2$) C, H, N, S.

2-[[3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl]thio]benzoxazole (24). The title compound was isolated as light-yellow powder (73%); mp 58−59 °C. ^{1}H NMR 500 MHz (DMSO-d_6): δ 1.99 (br m, 2H, $CH_2CH_2CH_2$), 2.45−2.59 (m, 2H + 4H, CH_2N + piperazine), 2.95 (m, 4H, piperazine), 3.37 (t, J = 7.2 Hz, 2H, SCH_2), 3.76 (s, 3H, CH_3), 6.80−6.95 (m, 4H, aromatic), 7.26−7.38 (m, 2H, aromatic), 7.57−7.68 (m, 2H, aromatic). Anal. ($C_{21}H_{25}N_3O_2S$) C, H, N, S.

2-[[3-[4-(2-Nitrophenyl)-1-piperazinyl]propyl]thio]1*H*-benzimidazole (25). The title compound was isolated as yellow powder (84%); mp 119−121 °C. ^{1}H NMR (DMSO-d_6): δ 1.85−1.99 (m, 2H, $CH_2CH_2CH_2$), 2.41- 2.52 (m, 2H + 4H, CH_2N + piperazine), 2.95−3.05 (m, 4H, piperazine), 3.31 (t, J = 7.2 Hz, 2H, SCH_2), 7.05−7.18 (m, 3H, aromatic), 7.24−7.62 (m, 4H, aromatic), 7.75−7.82 (m, 1H, aromatic), 12.51 (br s, 1H, NH, which exchanges with D_2O). Anal. ($C_{20}H_{23}N_5O_2S$) C, H, N, S.

2-[[3-[4-(2-Nitrophenyl)-1-piperazinyl]propyl]thio]benzothiazole (26). The title compound was isolated as bright-orange powder (76%); mp 73−76 °C. ^{1}H NMR (DMSO-d_6): δ 1.90−2.03 (m, 2H, $CH_2CH_2CH_2$), 2.49−2.59 (m, 2H + 4H, CH_2N + piperazine), 2.85−3.10 (m, 4H, piperazine), 3.36 (t, J = 7.2 Hz, 2H, SCH_2), 7.06−7.17 (m, 1H, aromatic), 7.26−7.62 (m, 4H, aromatic), 7.51−7.84 (m, 2H, aromatic), 7.98−8.05 (m, 1H, aromatic). Anal. ($C_{20}H_{22}N_4O_2S_2$) C, H, N, S.

2-[[3-[4-(2-Nitrophenyl)-1-piperazinyl]propyl]thio]benzoxazole (27). The title compound was isolated as bright-orange semisolid (81%). ^{1}H NMR (DMSO-d_6): δ 1.90−2.05 (m, 2H, $CH_2CH_2CH_2$), 2.45−2.58 (m, 2H + 4H, CH_2N + piperazine), 2.86−3.10 (m, 4H, piperazine), 3.37 (t, J = 7.2 Hz, 2H, SCH_2), 7.09−7.19 (m, 1H, aromatic), 7.26−7.38 (m, 3H, aromatic), 7.49−7.68 (m, 3H, aromatic), 7.78−7.82 (m, 1H, aromatic). Anal. ($C_{20}H_{22}N_4O_3S$) C, H, N, S.

2-[[3-[4-(Pyridin-2-yl)-1-piperazinyl]propyl]thio]benzothiazole (28). The title compound was isolated as white powder (65%); mp 85−86 °C. ^{1}H NMR (DMSO-d_6): δ 1.92−2.03 (m, 2H, $CH_2CH_2CH_2$), 2.42−2.50 (m, 2H + 4H, CH_2N + piperazine), 3.41−3.50 (m, 2H + 4H, SCH_2 + piperazine), 6.58−6.66 (m, 1H, aromatic), 6.78−6.84 (m, 1H, aromatic), 7.31−7.57 (m, 3H, aromatic), 7.82−7.88 (m, 1H, aromatic), 7.98−8.12 (m, 2H, aromatic). Anal. ($C_{19}H_{22}N_4S_2$) C, H, N, S.

2-[[3-[4-(Pyridin-2-yl)-1-piperazinyl]propyl]thio]benzoxazole (29). The title compound was isolated as cream powder (70%); mp 84−85 °C. ^{1}H NMR (DMSO-d_6): δ 1.94−2.09 (m, 2H, $CH_2CH_2CH_2$), 2.43−2.59 (m, 2H + 4H, CH_2N + piperazine), 3.39−3.52 (m, 2H + 4H, SCH_2 + piperazine), 6.58−6.66 (m, 1H, aromatic), 6.78−6.84 (m, 1H, aromatic), 7.29−7.68 (m, 5H, aromatic), 8.08−8.11 (m, 1H, aromatic). Anal. ($C_{19}H_{22}N_4OS$) C, H, N, S.

2-[[3-[4-(Pyrimidin-2-yl)-1-piperazinyl]propyl]thio]benzothiazole (30). The title compound was isolated as light-yellow powder (76%); mp 70−72 °C. ^{1}H NMR (DMSO-d_6): δ 1.88−2.04 (m, 2H, $CH_2CH_2CH_2$), 2.37−2.50 (m, 2H + 4H, CH_2N + piperazine), 3.41 (m, 2H, SCH_2), 3.67−3.75 (m, 4H, piperazine), 6.57−6.63 (m, 1H, aromatic), 7.30−7.50 (m, 2H, aromatic), 7.82−8.02 (m, 2H, aromatic), 8.32−8.36 (m, 2H, aromatic). Anal. ($C_{18}H_{21}N_5S_2$) C, H, N, S.

2-[[3-[4-(Pyrimidin-2-yl)-1-piperazinyl]propyl]thio]benzoxazole (31). The title compound was isolated as white powder (74%); mp 75−77 °C. ^{1}H NMR (DMSO-d_6): δ 1.95−2.03 (m, 2H, $CH_2CH_2CH_2$), 2.30−2.51 (m, 2H + 4H, CH_2N + piperazine), 3.39 (t, J = 7.2 Hz, 2H, SCH_2), 3.69−3.75 (m, 4H, piperazine), 6.58−6.66 (m, 1H, aromatic), 7.30−7.36 (m, 2H, aromatic), 7.60−7.68 (m, 2H, aromatic), 8.32−8.37 (m, 2H, aromatic). Anal.($C_{18}H_{21}N_5OS$) C, H, N, S.

2-[[3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl]thio]-1-methyl-1*H*-benzimidazole (32). The title compound was isolated as light-yellow powder (52%); mp 83−84 °C. ^{1}H NMR (DMSO-d_6): δ 1.87−1.98 (m, 2H, $CH_2CH_2CH_2$), 2.41−2.52 (m, 2H + 4H, CH_2N + piperazine), 2.89−3.02 (m, 4H, piperazine), 3.36 (t, J = 7.2 Hz, 2H, SCH_2), 3.67 (s, 3H, NCH_3), 3.75 (s, 3H, OCH_3), 6.86−6.91 (m, 4H, aromatic), 7.13−7.19 (m, 2H, aromatic), 7.43−7.56 (m, 2H, aromatic). Anal. ($C_{22}H_{28}N_4OS$) C, H, N, S.

5-Chloro-2-[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio]benzothiazole (33). The title compound was isolated as white powder (65%); mp 77−78 °C. ^{1}H NMR (DMSO-d_6): δ 1.90−1.99 (m, 2H, $CH_2CH_2CH_2$), 2.43- 2.52 (m, 2H + 4H, CH_2N + piperazine), 2.88−2.91 (m, 4H, piperazine), 3.38 (t, J = 7.0 Hz, 2H, SCH_2), 3.76 (s, 3H, CH_3), 6.85−6.93 (m, 4H, aromatic), 7.39−7.45 (m, 1H, aromatic), 7.90−8.09 (m, 2H, aromatic). Anal. ($C_{21}H_{24}ClN_3OS_2$) C, H, N, S.

5-Chloro-2-[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio]benzoxazole (34). The title compound was isolated as light-yellow powder (72%); mp 78−80 °C. ^{1}H NMR (DMSO-d_6): δ 1.93−2.02 (m, 2H, $CH_2CH_2CH_2$), 2.43−2.52 (m, 2H + 4H, CH_2N + piperazine), 2.88−3.02 (m, 4H, piperazine), 3.39 (t, J = 7.0 Hz, 2H, SCH_2), 3.76 (s, 3H, CH_3), 6.85−6.93 (m, 4H, aromatic), 7.32−7.39 (m, 1H, aromatic), 7.65−7.78 (m, 2H, aromatic). Anal. ($C_{21}H_{24}ClN_3O_2S$) C, H, N, S.

General Procedure for the Preparation of Compounds 35−38. Compounds **7** or **8** (10 mmol), 1-bromo-4-chlorobutane or 1-bromo-6-chlorohexane (30 mmol), potassium fluoride/aluminum oxide (10 g), potassium iodide (0.1 g), and acetonitrile (100 mL) were refluxed for 1 h and then allowed to stand overnight at room temperature. The mixture was filtered to eliminate inorganic material and then evaporated under reduced pressure to give a residue which was taken up in water.

The solution was extracted with dichloromethane (3 × 50 mL) and the combinated extracts were washed with water, dried, and evaporated to give intermediates 2-(4-chlorobutyl)thiobenzothiazole or benzoxazole and 2-(6-chlorohexyl)thiobenzothiazole or benzoxazole that were used without further purification for the final step.

A suspension of 2-(ω-chloroalkylthio)benzothiazole or 2-(ω-chloroalkyl)thiobenzoxazole **12−15** (10 mmol), 1-(2-methoxyphenyl)piperazine (12 mmol)), and potassium carbonate (12 mmol) in acetonitrile (30 mL) was refluxed for 24 h. After this period, the mixture was concentrated and the residue was diluted with water and extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and removed in vacuo. The obtained residue was purified by flash column cromatography on silica gel using ethyl acetate/cyclohexane (1:1) mixture as eluent.

2-[[4-[4-(2-Methoxyphenyl)-1-piperazinyl]butyl]thio]benzothiazole (35). The title compound was isolated as light-yellow semisolid (61%). ^{1}H NMR (DMSO-d_6): δ 1.61−1.86 (m, 4H, $CH_2CH_2CH_2$ CH_2), 2.33−2.50 (m, 2H + 4H, CH_2N + piperazine), 2.85−2.99 (m, 4H, piperazine), 3.39 (t, J = 7.2 Hz, 2H, SCH_2), 3.76 (s, 3H, CH_3), 6.82−6.94 (m, 4H, aromatic), 7.32−7.50 (m, 2H, aromatic), 7.83−8.02 (m, 2H, aromatic). Anal. ($C_{22}H_{27}N_3OS_2$) C, H, N, S.

2-[[4-[4-(2-Methoxyphenyl)-1-piperazinyl]butyl]thio]benzoxazole (36). The title compound was isolated as yellow semisolid (69%). ^{1}H NMR 500 MHz (DMSO-d_6): δ 1.60 (qu, 2H, $CH_2CH_2CH_2$ CH_2), 1.82 (qu, 2H, $CH_2CH_2CH_2CH_2$), 2.36 (t, J = 6.6 Hz, 2H, CH_2N), 2.48 (br m, 4H, piperazine), 2.92 (br m, 4H, piperazine), 3.37 (t, J = 6.8 Hz, 2H, SCH_2), 3.75 (s, 3H, CH_3), 6.82−6.92 (m, 4H, aromatic), 7.31−7.35 (m, 2H, aromatic), 7.62−7.67 (m, 2H, aromatic). Anal. ($C_{22}H_{27}N_3O_2S$) C, H, N, S.

2-[[6-[4-(2-Methoxyphenyl)-1-piperazinyl]hexyl]thio]benzothiazole (37). The title compound was isolated as light-yellow oil (58%). ^{1}H NMR (DMSO-d_6): δ 1.37−1.81 (m, 8H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 2.32 (t, J = 6.2 Hz, 2H, CH_2N), 2.48−2.52 (m, 4H, piperazine), 2.89−2.91 (m, 4H, piperazine), 3.36 (t, J = 6.2 Hz, 2H, SCH_2), 3.76 (s, 3H, CH_3), 6.83−6.91 (m, 4H, aromatic), 7.32−7.52 (m, 2H, aromatic), 7.82−8.03 (m, 2H, aromatic). Anal. ($C_{24}H_{31}N_3OS_2$) C, H, N, S.

2-[[6-[4-(2-Methoxyphenyl)-1-piperazinyl]hexyl]thio]benzoxazole (38). The title compound was isolated as light-yellow semisolid (60%). ^{1}H NMR 500 MHz (DMSO-d_6): δ 1.34 (qu, 2H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.44 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.79 (qu, 2H, $CH_2CH_2CH_2CH_2CH_2CH_2$), 2.29 (t, 2H, CH_2N), 2.46 (br m, 4H, piperazine), 2.92 (br m, 4H, piperazine), 3.33 (t, J = 7.0 Hz, 2H, SCH_2), 3.76 (s, 3H, CH_3), 6.85−6.93 (m, 4H, aromatic) 7.28−7.32 (m, 2H, aromatic), 7.32−7.67 (m, 2H, aromatic). Anal. ($C_{24}H_{31}N_3O_2S$) C, H, N, S.

Pharmacology: Binding Assays. Male CRL:CD(SD)BR-COBS rats weighing about 150 g were killed by decapitation,[25] and their brains were rapidly dissected (hippocampus for 5-HT_{1A}R; striatum for D_1 and D_2 receptors; cortex for α_1-AR and 5-HT_{2A} receptor), frozen, and stored at −80 °C until the day of assay.

Tissue was homogenized in about 50 volumes of ice-cold 50 mM Tris·HCl buffer (pH 7.4) using an Ultra Turrax TP-1810 (2 × 20 s) and centrifuged at 50000*g* for 10 min (Beckman model J-21B refrigerated centrifuge). The pellet was resuspended in the same volume of fresh buffer, incubated at 37 °C for 10 min, and centrifuged again at 50000*g* for 10 min. The pellet was then washed once by resuspension in fresh buffer and centrifuged as before. The pellet was then resuspended in the appropriate incubation buffer (50 mM Tris·HCl, pH 7.7 containing 10 μM pargyline and 4 mM $CaCl_2$ for 5-HT_{1A}Rs; 50 mM Tris·HCl, pH 7.7 containing 10 μM pargyline and 0.1% ascorbic acid for α_1-ARs; 50 mM Tris·HCl, pH 7.4 containing 10 μM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% ascorbic acid for D_1 and D_2 receptors; 50 mM Tris·HCl, pH 7.7 for 5-HT_{2A} receptors) just before the binding assay.

Binding assays were done as previously described.[26] Briefly, the following incubation conditions were used. 5-HT_{1A}: [^{3}H]-8-OH-DPAT (specific activity 157 Ci/mmol, NEN) final concentration 1 nM, 30 min, 25 °C (nonspecific binding: 5-HT 10 μM). D_1: [^{3}H]SCH23390 (specific activity 71.1 Ci/mmol, NEN) final concentration 0.4 nM, 15 min, 25 °C (nonspecific binding: (−)-*cis*-flupentixol 10 μM). D_2: [^{3}H]spiperone (specific activity 19.0 Ci/mmol, NEN) final concentration 0.2 nM, 15 min, 37 °C (nonspecific binding: (−)-sulpiride 100 μM). α_1-ARs: [^{3}H]prazosin (specific activity 71.8 Ci/mmol, NEN) final concentration 0.2 nM, 30 min, 25 °C (nonspecific binding: phentolamine 3 μM). 5-HT_{2A}: [^{3}H]ketanserin (specific activity 63.3 Ci/mmol, Amersham) final concentration 0.35 nM, 15 min, 37 °C (nonspecific binding: methisergide 1 μM).[27]

For [^{35}S]GTPγS binding assay to 5-HT_{1A}Rs, tissue was prepared as described above for 5-HT_{1A}R binding. Binding was performed as follows: [^{35}S]GTPγS (specific activity 1064 Ci/mmol, Amersham) final incubation volume of 1.04 mL, consisting of 1 mL of membrane suspension from rat hippocampus (200 μg of protein/

sample), 20 μL of [^{35}S]GTPγS (final concentration 0.1 nM), and 20 μL of drugs or solvent. Nonspecific binding was obtained in presence of 10 μM GTPγS. Samples were preincubated for 20 min at 37 °C without [^{35}S]GTPγS and then for 45 min at 37 °C with [^{35}S]GTPγS.

Incubations were stopped by rapid filtration under vacuum through GF/B filters, which were then washed with 12 mL of ice-cold 50 mM Tris HCl, pH 7.4 using a Brandel M 48-R cell harvester.

The radioactivity trapped on the filters was counted in 4 mL of Ultima Gold MV (Packard) in a Wallac 1409 DSA liquid scintillation counter with a counting efficiency of 50% for [^{3}H] or 90% for [^{35}S].[26] Dose−response curves were analyzed by the "Allfit" program.[28] The K_i values were derived from the IC$_{50}$ values.[29]

Molecular Docking. The population of 100 5-HT$_{1A}$R models described previously[19] was reused in the present work. Models were constructed on the basis of slightly modified bovine rhodopsin template. All the docking experiments were conducted using the FlexX software (www.biosolveit.de) with FlexX-Pharm extension. Pharmacophore constraints were applied: H-bond acceptor at the carboxylic oxygen of Asp3.32, CH−π edge-to-face interaction with Phe6.62 and H-bond donor at hydroxylic group of Ser5.43. The results were rescored with 4 additional scoring functions and subjected to consensus scoring procedure, as implemented in SYBYL (www.tripos.com). Highest PMF scored solutions out of those having consensus score 5 were considered representative. PMF score was used for final scoring of docking solutions, as it was proved to give the best enrichment factors in virtual screening experiments.[30]

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Supporting Information Available: Elemental analysis data of compounds **16**−**36**, NOESY (DMSO/TFA) of compound **38**, and ^{1}H NMR spectra at 500 MHz of compounds **18**, **24**, **36**, and **38**. This material is available free of charge via the Internet at http://pubs.acs.org..

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