5186 J. Med. Chem. 2010, 53, 5186–5196
DOI: 10.1021/jm100350r

Journal of Medicinal Chemistry Article

(3-Phenylsulfonylcycloalkano[*e* and *d*]pyrazolo[1,5-*a*]pyrimidin-2-yl)amines: Potent and Selective Antagonists of the Serotonin 5-HT$_6$ Receptor

Alexandre V. Ivachtchenko,[†,§] Dmitri E. Dmitriev,[†] Elena S. Golovina,[‡] Madina G. Kadieva,[†] Angela G. Koryakova,[‡] Volodymyr M. Kysil,[§] Oleg D. Mitkin,[†] Ilya M. Okun,*[,§] Sergey E. Tkachenko,[§] and Anton A. Vorobiev[†]

[†]*Department of Organic Chemistry, and* [‡]*Department of Molecular Pharmacology, Chemical Diversity Research Institute, 114401 Khimki, Moscow Reg, Russia, and* [§]*ChemDiv, Inc., 6605 Nancy Ridge Drive, San Diego, California 92121*

Received April 1, 2010

5-HT$_6$ receptors are exclusively localized in the CNS and have high affinity with many psychotropic agents. Though the role of this receptor in many CNS diseases is widely anticipated, lack of definite progress in the development of 5-HT$_6$ receptor-oriented drugs indicates a need for further discoveries of novel chemotypes with high potency and high selectivity to the receptor. Here we present preparations and biological evaluation of a series of (3-phenylsulfonylcycloalkano[*e* and *d*]pyrazolo[1,5-*a*]pyrimidin-2-yl)amines. Phenylsulfonylcyclopentapyrazolopyrimidine **7** was found to be a highly selective 5-HT$_6$ receptor antagonist with high affinity (low picomolar range) and potency. **7** and a few of its analogues were further tested for biological effect on 5-HT$_{2B}$ receptors and hERG potassium channels, potential liability targets. Such liability appears to be minimal, based on the *in vitro* data.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT[a]) receptor (5-HT$_6$R) belongs to a group of G$_s$-protein-coupled receptors controlling cellular levels of cAMP. Rat and then human receptor were reported to be cloned by Monsma et al.[1] and Kochen et al.[2] in 1993 and 1996, respectively. The receptor is almost exclusively localized in the central neural system (CNS). The receptor attracted a good deal of attention as a potential target for medicinal chemistry for such indications as anxiety,[3,4] cognition,[5] learning and memory,[6] and mood.[3,7] It was shown[8] that 5-HT$_6$R can modulate cholinergic, noradrenergic, glutamatergic, and dopaminergic neurotransmitter systems. Given the fundamental role of these systems in normal cognitive processes and in neurodegeneration, it became an apparent necessity to elucidate the role of 5-HT$_6$R in the processes controlling normal or "pathologic" memory. Development of 5-HT$_6$R antagonists facilitated discovery that blockage of these receptors led to a significant enhancement of memory consolidation in various animal models of learning and memorization, reproduction,[9−11] and improvement of cognitive function in old rats.[9]

In recent years, an understanding of the role of 5-HT$_6$R in cognitive processes has improved. Possible pharmacophore properties of their ligands were formulated,[12] which helped in developing quite potent and selective antagonists. It was also shown that many typical and atypical antipsychotic agents bind to 5-HT$_6$R with high affinity.[13] Use of the 5-HT$_6$R antagonists as therapeutic tools revealed that these receptors are promising targets for the development of new drug candidates for treatment of various CNS diseases such as schizophrenia, Alzheimer's disease, and other neurodegenerative diseases and cognitive disorders.[14−17] In particular, the successful ongoing clinical trials of new highly selective and potent 5-HT$_6$R antagonists, AVN-211 and AVN-322, have recently been announced for treatment of some CNS diseases.[18,19]

Interest in the development of 5-HT$_6$R antagonists is growing (Figure 1), and the role of the receptors in metabolic diseases, such as obesity, is suggested.[14] However, in spite of the ever growing number of articles, proof that the antagonists actually work through the 5-HT$_6$R antagonism is still elusive and novel, more selective and potent molecules are needed.

Numerous selective antagonists of 5-HT$_6$R have been disclosed, most of them representing the heterocyclic compounds bearing a sulfonyl or sulfamide group.[14−16,20−24] On the basis of available structurally diverse 5-HT$_6$R antagonists, pharmacophore models for this type of receptor antagonist have been suggested.[25−29] In general, the models entail positive ionizable atom (PI, usually secondary or tertiary amino group), strong multiple hydrogen bond acceptor group (mHBA, usually sulfonyl or sulfamide group), a hydrophobic site (HYD, for example, phenyl), and π-electron donor aromatic or heterocyclic ring (AR). These important recognition elements are represented in a simplified form of a model **A** in Figure 2.[22]

The most typical representatives of the 5-HT$_6$R antagonists conforming to the pharmacophore **A** model are 3-benzenesulfonyl-8-piperazin-1-ylquinoline (SB-742457) with K_i = 0.234 nM[10,30] and 3-benzenesulfonyl-2-methylsulfanyl-8-piperazin-1-yl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrazolo[1,5-*a*]pyrimidine (Ro-65-7674) with K_i = 0.85 nM (Figure 3).[11,31] The SB-742457 is currently in phase II clinical trials for the treatment of Alzheimer's disease.[12,32] The Ro-65-7674, though a highly

*To whom correspondence should be addressed. Phone: 1-858-794-4860. Fax: 858-794-4931. E-mail: iokun@chemdiv.com.

[a] Abbreviations: CNS, central nervous system; GSPCR, G$_s$-protein-coupled receptor; 5HT, 5-hydroxytryptamine; 5-HT$_6$R, serotonin type 6 receptor; PI, positive ionizable atom; mHBA, multiple hydrogen bond acceptor group; HYD, a hydrophobic site; AR, π-electron donor aromatic or heterocyclic ring; K_i, equilibrium antagonist binding constant; IC$_{50}$, half maximal inhibition concentration.

 Published on Web 06/18/2010

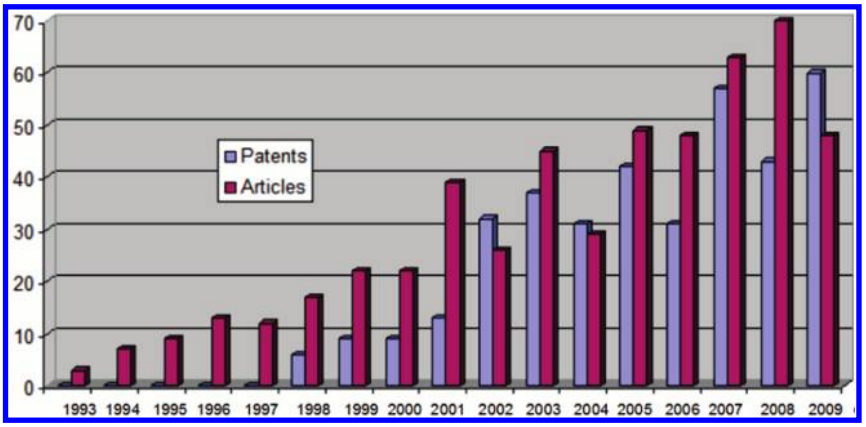


Figure 1. Number of publications and patents in the 5-HT6R field between the years 1993 and 2009. Data taken from the SciFinder database searching the key word 5-HT6R, December 01, 2009.

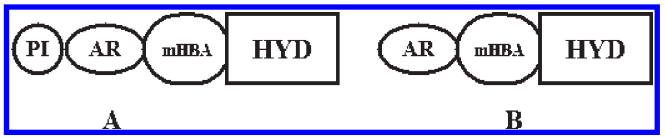


Figure 2. Simplified recognition elements representation of pharmacophores **A** and **B** characteristic for 5-HT_6 receptor antagonists.[22,28]

potent and selective 5-HT_6R antagonist with good DMPK properties, did not reach the clinical trials stage because of its high affinity to the hERG channel.[31]

Recently, we have described the synthesis of cycloalkane-annelated 3-phenylsulfonylpyrazoles (**C** and **D** in Figure 3), which are highly potent and highly specific antagonists of the 5-HT_6R.[33] In spite of their high affinity (IC_{50} in a single-digit nanomolar range), the **C** and **D** models do not include the positive ionizable group, PI, and could be described by the model **B** of the recognition elements pharmacophore (Figure 2). The most active 5-HT_6R antagonist in this series of cycloalkane-annelated 3-phenylsulfonylpyrazolo[1,5-*a*]pyrimidines (**C** and **D**, Figure 3) was 5-methyl-2-methylsulfanyl-3-phenylsulfonyl-6,7,8,9-tetrahydropyrazolo[1,5-*a*]quinazoline **C** (R^1 = SMe, R^2 = Me, n = 2) with 5-HT_6R antagonistic IC_{50} = 6 nM.

Results

In this paper, we describe further development of the 5-HT_6R antagonists based on pharmacophore **D** containing a pyrazolo[1,5-*a*]pyrimidine template. The new synthesized 5-HT_6R antagonists, (3-phenylsulfonyl-cycloalkano[*d*]pyrazolo[1,5-*a*]pyrimidin-2-yl)amines **1**−**6** and (3-phenylsulfonylcycloalkano[*e*]pyrazolo[1,5-*a*]pyrimidin-2-yl)amines **7**−**16**, are shown in Figure 4.

The synthesis was carried out as shown in Scheme 1 with benzenesulfonylacetonitrile **17** as a starting material. Reaction of **17** with CH_3NCS in KOH/dioxane for 2 h at room temperature, step a, and with MeI for 1 h at room temperature, step b, resulted in formation of 2-phenylsulfonyl-3-methylamino-3-methylsulfanylacrylonitrile **18**, which then led, in step c, to formation of the N^3-methyl-4-phenylsulfonyl-1*H*-pyrazole-3,5-diamine **19**. N^3,N^3-Dimethyl-4-(phenylsulfonyl)-1*H*-pyrazole-3,5-diamine **20** was obtained by reacting **17** with CS_2 in a mixture of KOH/dioxane at room temperature for 2 h in step g. The reaction product, 3,3-bis-(methylthio)-2-(phenylsulfonyl)acrylonitrile, was then reacted with dimethylamine in ethanol for an additional 12 h in step h to produce 3-(dimethylamino)-3-(methylthio)-2-(phenylsulfonyl)acrylonitrile **25**. The **25** was then reacted with $N_2H_4 \cdot H_2O$ in an *i*-PrOH/water mixture, step c, which yielded 1*H*-pyrazole-3,5-diamine **20**.

The reaction of **19** and **20** with 2-formylcyclopentanone **21** in acetic acid at room temperature, condition d, or at 100 °C, condition e, or in acetic acid in the presence of HCl at room temperature, condition f, exclusively yielded (3-phenylsulfonyl-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidin-2-yl)methylamine**7** and N^2,N^2-dimethyl-3-phenylsulfonyl-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidin-2-amine **11**.

When sodium 2-formylcyclohexanone **22** was used in condition d, the reaction produced (3-phenylsulfonyl-5,6,7,8-tetrahydropyrazolo[5,1-*b*]quinazolin-2-yl)methylamine **1**. The same reaction performed in condition f produced (3-phenylsulfonyl-6,7,8,9-tetrahydropyrazolo[1,5-*a*]quinazolin-2-yl)methylamine **8**. Our data agree well with those of the synthesis of 3-cyanocycloalkano[*e* and *d*]pyrazolo[1,5-*a*]pyrimidines.[34]

Reaction of the 3,5-diamino-1*H*-pyrazole **19** with 2-acetylcycloalkanones **23** and **24** (Scheme 1) was less selective than that with 2-formylcycloalkanones **21** and **22**. The reaction of **19** with 2-acetylcyclopentanone **23** in condition a, b, or c yielded a 1:9 mixture of linear **3** and angular **9** products (Figure 5). Reaction of **19** with 2-acetylcyclohexanone **24** yielded a mixture of products **4** and **10**. When being performed in condition a + c, this reaction produced the mixture of **4** and **10** with a ratio of ~7:3. In the condition b + c, the **4**/**10** ratio was 6:5.

5-Methyl-3-phenylsulfonyl-2-piperazin-1-yl derivatives **15** and **16** were obtained from 2-phenylsulfonyl-3,3-bis-methylsulfanylacrylonitrile **26**.[35] The latter was transformed into 2-phenylsulfonyl-3-mercapto-3-piperazin-1-ylacrylonitrile **27** by reaction with 1-Boc-piperazine. Subsequent reaction of **27** with $N_2H_4 \cdot H_2O$ in H_2O yielded 4-(5-amino-4-phenylsulfonyl-1*H*-pyrazol-3-yl)piperazine-1-carboxylic acid *tert*-butyl ester **28**. The reaction of the 5-amino-1*H*-pyrazol **28** with diketones **23** proceeded to yield a mixture of both the linear **5** and angular **13** products in a mass ratio of 15:85. In the reaction with 2-acetylcyclohexanone **24**, angular product **14** was obtained without impurity of the linear product **6**. As a result of removing the Boc protection from **13** and **14**, we obtained the individual 3-piperazin-1-yl derivatives **15** and **16** (Scheme 2).

The structures of the synthesized compounds **9** and **12** were confirmed with X-ray crystallography and with LC/MS and NMR analyses.

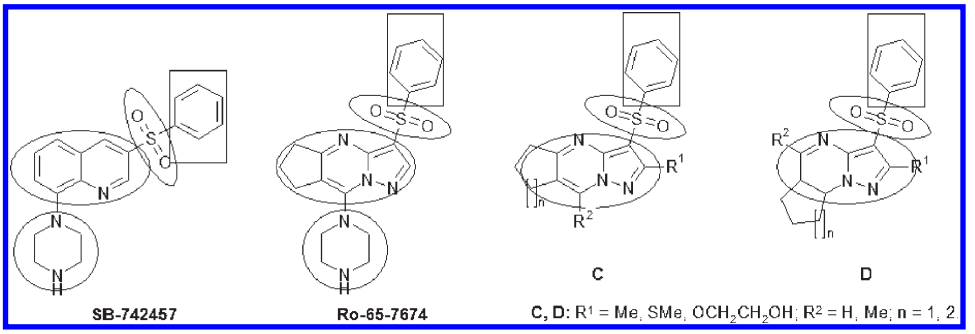


Figure 3. Antagonists of 5-HT$_6$R with structures conforming either to model **A** (SB-742457 and Ro-65-7674) or to model **B** (**C** and **D**) in Figure 2.

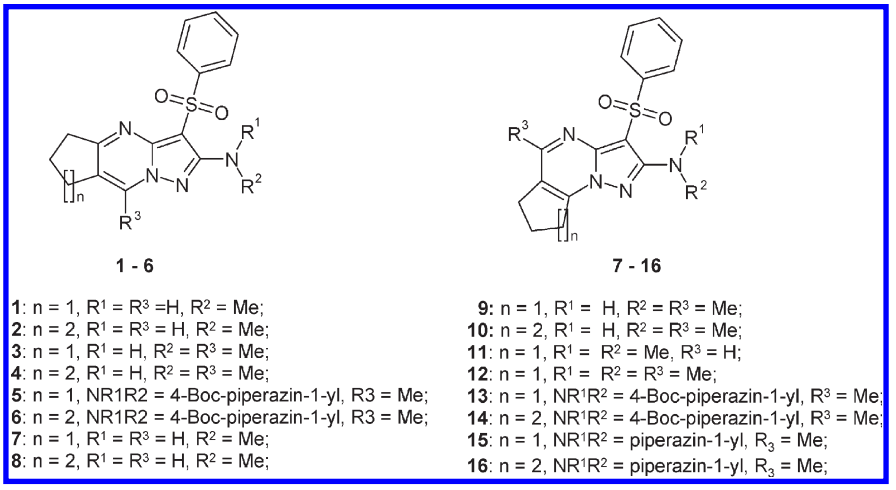


Figure 4. Novel synthesized 5-HT$_6$R antagonists **1**−**16**.

Discussion

In Figure 6, we show the 3D structures of cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidine derivatives **9** and **12**, which were minimized (convergence criterion of 0.000 01) using Accelrys DS ViewerPro 6.0 software. The molecules have three rotatable bonds, and the energy minimization showed existence of at least two local energy minima defined by position of the oxygen atoms O(1) and O(2) relative to the nitrogen atom N(4), to which either a methyl (in **9**) or dimethyl (in **12**) moiety is attached. The distances between the oxygen and nitrogen atoms, torsion angles on the rotatable bond, S−C(10) for **9** or S−C(8) for **12**, and minimized energies of the molecule conformation are shown in Table 1.

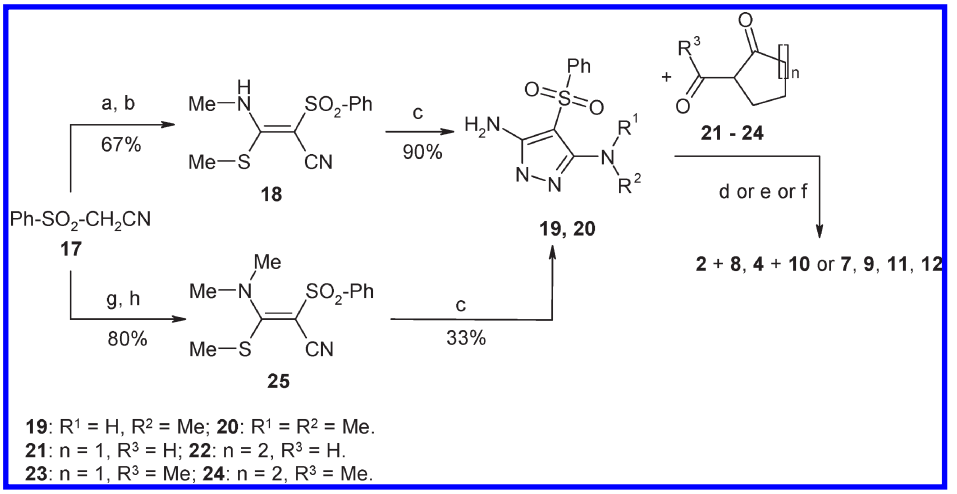
As one can see, the minimal free energies of the two **9** conformations, O(2)N(4) and O(1)N(4), are practically the same. The major difference between the two conformers is the torsion angle around C(10)−S bond, which defines the benzene ring position relative to the plane of the cyclopenta[*e*]pyrazolo-[1,5-*a*]pyrimidine core. In the O(1)N(4) conformation, the ring is directed toward the viewer, while in the O(2)N(4) conformation, the ring is directed away from the viewer (Figure 6). It is important to note that in both conformations, the distance between one of the two oxygen atoms and a proton of N(4) comprises only 2.1 Å, which indicates formation of the hydrogen bond and, hence, stabilization of the molecule in either conformation. The X-ray data indicate that in the crystal state, **9** exists in a conformation closer to the O(2)N(4) conformation (Figure 7A), with the benzene ring directed away from the viewer. These data also support formation of the hydrogen bond between the oxygen atom O(2) and the amine proton HN(4).

N,*N*,5-Trimethyl-3-(phenylsulfonyl)-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidin-2-amine **12** could also have at least two conformations, O(2)N(4) and O(1)N(4) (Figure 6 and Table 1), though with the local free energy minima much higher than those of **9** conformations. This can be explained by the absence of the stabilizing hydrogen bond in **12**. The two **12** conformations differ from each other at a substantially higher degree than those of the **9**. As shown in Figure 6, the benzene ring occupies a drastically different position relative to the cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidine core in the two conformations and the energy of the O(2)N(4) is 1.2 kcal/mol as low as that of O(1)N(4). X-ray data confirm that in the crystal form, **12** exists in a conformation closer to the O(2)N(4) conformation (Figure 7B) with the benzene ring facing up and away from the cyclopenta-[*e*]pyrazolo[1,5-*a*]pyrimidine core.

The ratio of the corresponding compound pairs **1**−**6** and **7**−**16** in the reaction mixtures was determined by the ratio of integral signal intensities in pairs of protons of methylene groups, 2.64−2.79 (m) and 2.58−2.63 (m), and methyl groups, 2.57 (s) and 2.50 (s).

The structural assignments of **1**−**6** and **7**−**16** were made on the basis of 2D NMR experiment analyses (NOESY and HMBC) for all synthesized compounds **1**−**16** and confirmed for compounds **9** and **12** based on the X-ray data (Figures S1 and S2 of Supporting Information). The data obtained are in a good agreement with the published NMR spectra and quantum chemical calculations for known analogues.[34,36,37]

Scheme 1. Preparation of Compounds **2**, **4**, **7**−**12**[a]



[a] Reagents and conditions: (a) CH_3NCS, KOH, dioxane, room temp 2 h; (b) MeI, room temp 12 h; (c) $N_2H_4 \cdot H_2O$, *i*-PrOH, room temp 1 h, then reflux 2 h; (d) AcOH, room temp 12 h; (e) AcOH, 100 °C, 3 h; (f) AcOH, HCl, room temp 12 h; (g) KOH, dioxane, CS_2, room temp 2 h; (h) Me_2NH, EtOH, room temp 12 h.

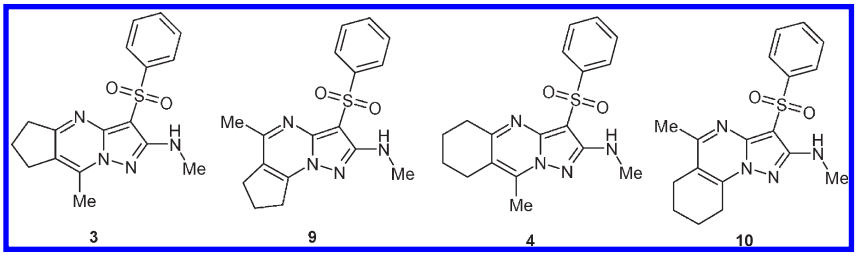
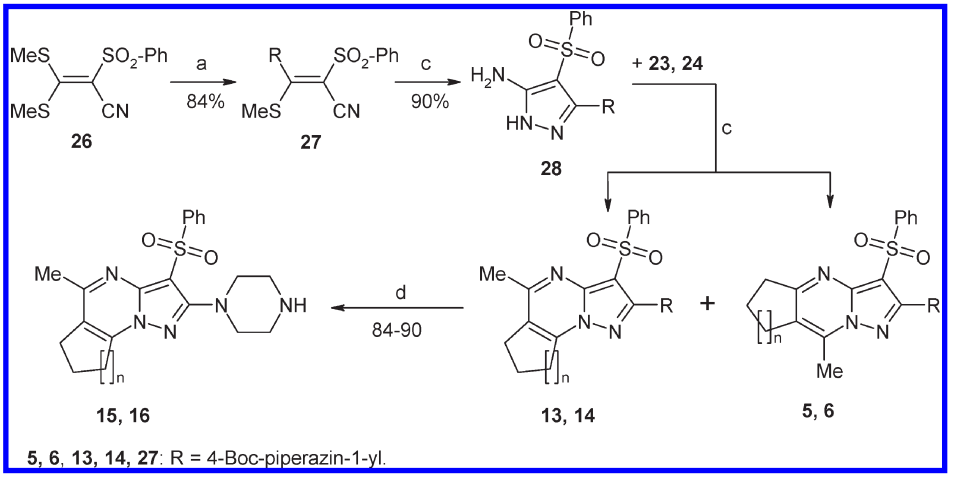


Figure 5. Products **3**, **9** and **4**, **10** of the reaction of 3,5-diamino-1*H*-pyrazole **19** with 2-acetylcycloalkanones **23** and **24**.

Scheme 2. Preparation of Compounds **15** and **16**[a]



[a] Reagents and conditions: (a) 1-Boc-piperazine, *i*-PrOH, reflux 1 h, then room temp for 12 h; (b) $N_2H_4 \cdot H_2O$, *i*-PrOH, room temp for 0.5 h; (c) AcOH, 100°C, 3 h; (d) 6 N AcCl in EtOH, room temp 1 h.

The synthesized compounds **1**−**16** were tested in a 5-HT_6R radioligand binding assay for their ability to compete with [^{3}H]lysergic acid diethylamide and in functional cell-based assays for their ability to block serotonin-induced cAMP production in HEK293 cells expressing human 5-HT_6R (Table 2). For comparison, some of the compounds were also assessed for their ability to interact with targets with potential liability: 5-HT_{2B} receptor (blockage of αMe-5-HT-induced $[Ca^{2+}]_i$ mobilization in HEK293 cells expressing 5-HT_{2B} receptor) and hERG potassium channels (whole cell patch clamp).

The synthesized compounds **1**−**16** showed very high affinity to the 5-HT_6R, with K_i values in the picomolar range and functional IC_{50} values in a single-digit nanomolar range. In the pair of linear molecules **2** and **4**, substitution of a proton with Me in R^3 position led to a 2-fold increase in the affinity. Similar substitution in the angular molecules, **8** and **10**, exhibited even more profound effect, 4-fold increase in the

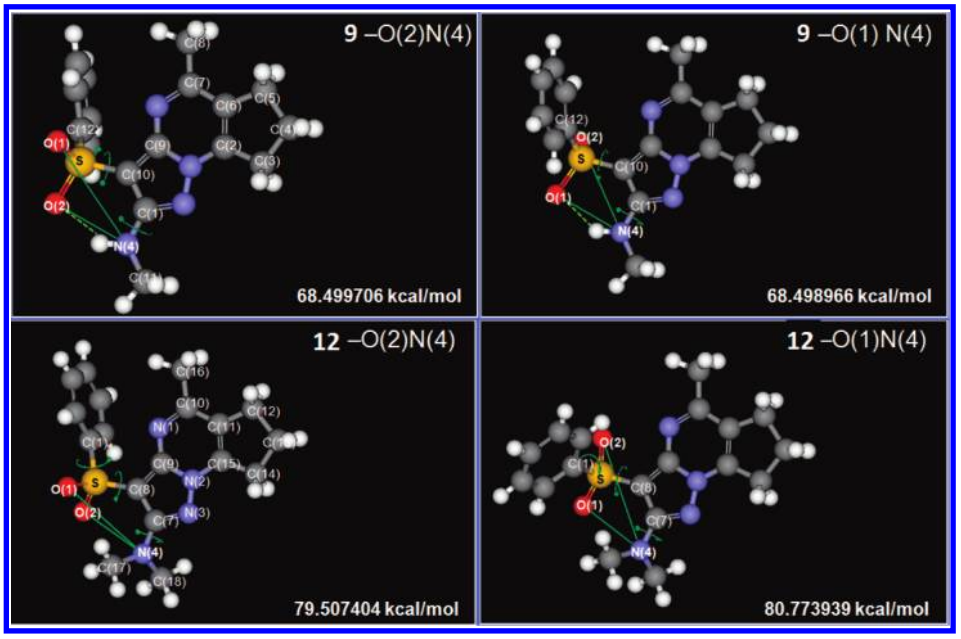


Figure 6. Free energy minimized 3D structures for the compounds **9** and **12**. For each compound, the energy minimization was performed for each of the two conformations with shortest distances between N(4) and either O(1), O(1)N(4), or O(2), O(2)N(4), atoms shown with solid green lines. The green dotted line shows the hydrogen bond (distance less than 2.5 Å) between either O(2) or O(1) and a proton of N(4).

Table 1. Parameters of the Energy Minimized 3D Structures of (5-Methyl-3-phenylsulfonyl-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidin-2-yl)methylamine, **9**, and (5-Dimethyl-3-phenylsulfonyl-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidin-2-yl)methylamine, **12**

	9 conformation						**12** conformation					
	O(2)N(4)		O(1)N(4)		crystal[a]		O(2)N(4)		O(1)N(4)		crystal[a]	
parameter	distance, Å	angle, deg	distance, Å	angle, deg	distance, Å	angle, deg	distance, Å	angle, deg	distance, Å	angle, deg	distance, Å	angle, deg
O(2)···HN(4)	2.104		3.923		2.218							
O(1)···HN(4)	3.924		2.104		4.23							
O(2)···HN(4)	2.926		4.312		2.882		3.244		4.808		4.383	
O(1)···N(4)	4.313		2.926		4.561		3.937		3.211		3.129	
S−O···HN(4)		96.6		96.6		103.3						
torsion S−C		52.25		−52.22		86.05		−154.03		95.09		−125.39
energy	68.499 706		68.498 966		209.097 534		79.507 404		80.773 939		271.264 518	

[a] Parameters were calculated on the basis of X-ray-determined positions of the atoms.

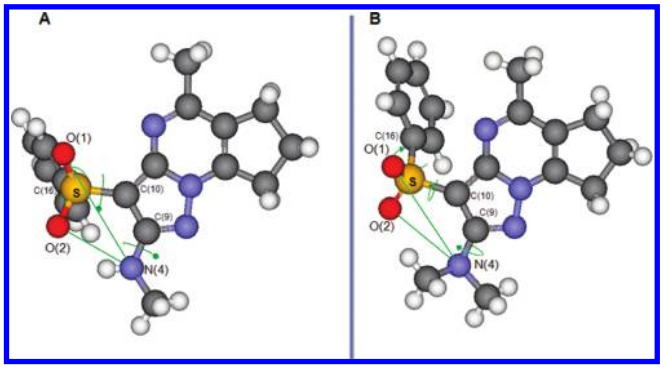


Figure 7. 3D structures deduced from the X-ray data, of (A) (5-methyl-3-phenylsulfonyl-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo-[1,5-*a*]pyrimidin-2-yl)methylamine, **9**, and (B) (5-dimethyl-3-phenylsulfonyl-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidin-2-yl)-methylamine, **12**.

affinity. When comparing affinities of linear analogues with their angular counterparts, **2** vs **8** and **4** vs **10**, one can see that in the pair of R^3 proton-substituted compounds, the angular molecule has more than 4-fold higher affinity whereas in the pair of R^3 methyl-substituted, the linear molecule has slightly higher affinity. In a cell-based functional assay, the angular **8** was also more potent than its linear **2** counterpart. In the R^3 proton-substituted compounds, the cycloalkane ring size does not seem to play a substantial role in either the affinity or potency of the compounds to bind to and block 5-HT_6R response (compare **7** with **8**). In R^3 Me-substituted compounds, **9** and **10**, cyclopentane derivative shows slightly (2.5-fold) higher affinity than the cyclohexane derivative.

To the best of our knowledge, the 3-phenylsulfonyl-2-methylamino-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]-pyrimidine, **7**, and 3-phenylsulfonyl-2-methylamino-7,8-dihydro-6*H*-cyclohexa[*e*]pyrazolo[1,5-*a*]pyrimidine, **8**, have the highest affinity to the 5-HT_6R known among all 5-HT_6R antagonists measured.

Noteworthy, dimethyl-substituted cyclopenta[*e*]pyrazolo-[1,5-*a*]pyrimidines **11** and **12** exhibit 40- to 70-fold lesser potency to antagonize the 5-HT_6R (Table 2) than their respective 2-methylamino-substituted counterparts **7** and **9**. This agrees well with the above-discussed ability of the 2-methylamino-substituted molecules to form an intramolecular hydrogen bond that can limit the molecular rotational freedom in an advantageous for the receptor binding conformation. The 2-(piperazin-1-yl)-substituted derivatives **15** and **16** were even less potent (IC_{50} in the micromolar range) relative to

Table 2. Biological Activity of (3-Phenylsulfonylcycloalkano[*e* and *d*]pyrazolo[1,5-*a*]pyrimidin-2-yl)amines **2**, **4**, **7**−**12**, **15**, **16**

compd	*n*	NR^1R^2	R^3	K_i, nM (binding)[a]	K_i, nM (functional)[b]		% inhibition ± SEM (conc)[c]
				5-HT_6	5-HT_6	5-HT_{2B}	HERG
2	2	NHMe	H	0.499	0.542	169	
4	2	NHMe	Me	0.256			12.5 ± 10.1 (3 μM)
7	1	NHMe	H	0.088	0.375	464	29.5 ± 3.9 (90 μM)
8	2	NHMe	H	0.112	0.259	153	
9	1	NHMe	Me	0.202	0.413	188	4.3 ± 2.4 (3 μM)
10	2	NHMe	Me	0.490			52.4 ± 2.8 (10 μM)
11	1	NMe_2	H		28.8		
12	1	NMe_2	Me		25.5		
15	1	piperazin-1-yl	Me		869		
16	2	piperazin-1-yl	Me		1764		

[a] K_i was determined in radioligand receptor binding assay (see Supporting Information) by concentration-dependent displacement of [^{3}H]lysergic acid diethylamide. Each curve was measured in duplicate. [b] K_i was determined in cell-based functional assays (see Experimental Section) by concentration-dependent inhibition of serotonin-induced (5-HT_6R) or αMe-serotonin-induced (5-HT_{2B}R) responses. The values are the geometric mean of three to four independent experiments. Each concentration curve was measured in duplicate. [c] Inhibition of hERG channel was determined in triplicate at several concentrations. The mean values ± SEM for the highest concentration tested for each compound (shown in parentheses) are presented.

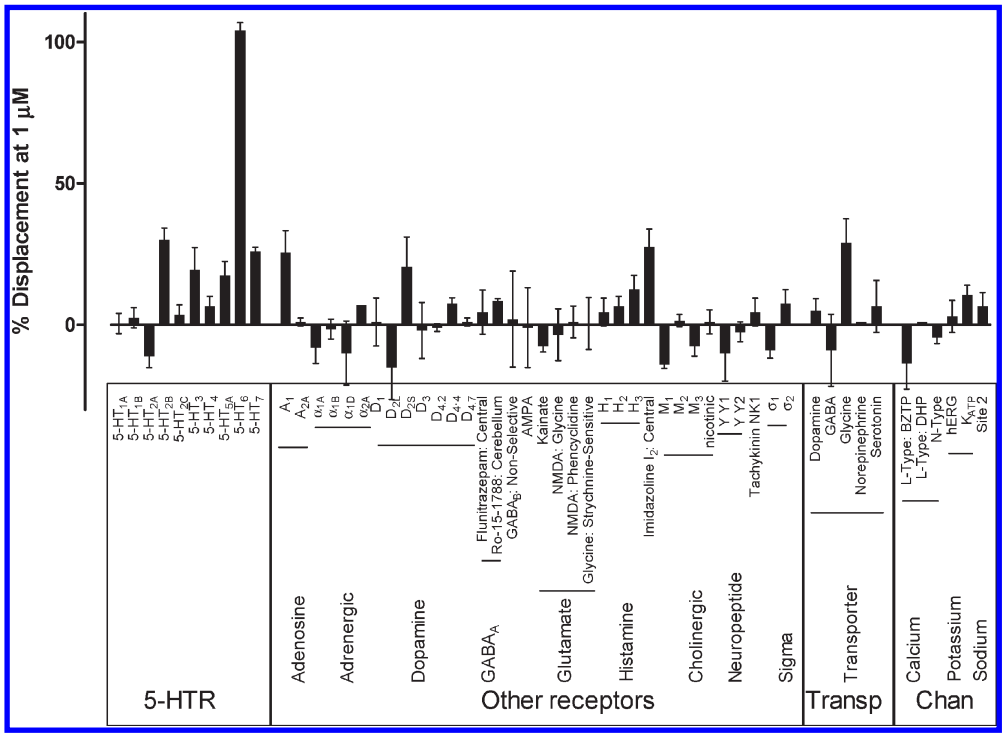


Figure 8. Specificity profile of (3-phenylsulfonyl-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidin-2-yl)methylamine **7**. Displacement of target-specific radiolabeled ligands was measured at a compound concentration of 1 μM.[38] Shown is a typical experiment performed in duplicate; mean values ± SD are presented.

their analogues **9** and **10**. The most plausible explanation of much stronger binding of the 2-methylamino-substituted cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidines with the 5-HT_6R compared to their 2-dimethylamino or 2-(piperazin-1-yl) analogues **11**, **12**, **15**, **16** can be attributed to the stabilization of the **7**, **9**, **10** molecules in an advantageous binding conformation assisted by the intramolecular hydrogen bond. The bulky 2-(piperazin-1-yl) group may additionally impose a steric hindrance for binding within the 5-HT_6R site.

The antagonist **7**, one of the compounds with the highest affinity (Table 1), was then tested on a panel of 55 therapeutic targets consisting of GPCRs, ion channels, and neurotransmitter transporters. The specificity profile of **7** was determined by its ability to compete (at 1 μM) with radiolabeled ligands for the targets studied (Figure 8).

The data of Figure 8 show that the (3-phenylsulfonyl-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidin-2-yl)methylamine, **7**, has a very good profile with high specificity and selectivity toward 5-HT_6R. Some interaction of **7** with the 5-HT_{2B} receptor known to be responsible for drug-induced valvular heart disease (VHD)[39] prompted us to test **7** for its potential 5-HT_{2B} receptor agonistic and antagonistic activity (for details, see Experimental Section). At concentrations of up to 10 μM tested, **7** had no agonistic activity (data not shown) and exhibited rather weak antagonistic activity (IC_{50} = 3.5 μM) (Figure 9). These data suggest the safety of **1** in relation to potentially causing VHD. Besides, **7** has 3 orders of magnitude selectivity index between the 5-HT_6R and 5-HT_{2B}R (Figure 9; also see Table 1).

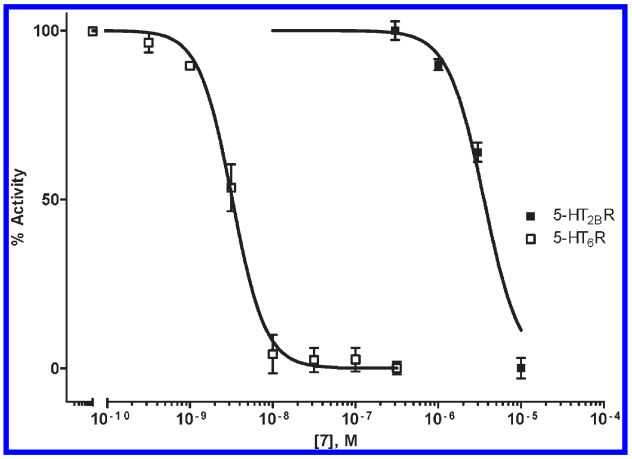


Figure 9. Compound **7** concentration-dependently inhibits both the 5-HT_{2B}R and 5-HT_6R-induced functional responses in HEK293 cells exogenously expressing corresponding receptors. Each point is an average of two replicates ± SD.

7 interacted with hERG (human ether-a-go-go-related gene) potassium channel neither in binding[38] (Figure 8) nor in functional (Table 1) assays. The hERG is another target with potential liability associated with QT prolongation and torsade de pointes,[40] which was implicated in withdrawal of previously approved drugs and in preventing others from gaining FDA approval. Our data clearly indicate the safety of **7** and make it an ideal candidate as a therapeutic tool in probing the role of the 5-HT_6R in different diseases.

Conclusion

(3-Phenylsulfonylcycloalkano[*e* and *d*]pyrazolo[1,5-*a*]pyrimidin-2-yl)amines **1**−**16** represent a new type of highly potent (K_i < 1 nM) 5-HT_6R antagonists with the basic secondary amino group located next to (rather than apart from) the sulfonyl group. This closeness of the basic amine and sulfonyl groups makes it possible for formation of an intramolecular hydrogen bond, which stabilizes the molecule in an appropriate conformation and thus provides gain in the receptor binding energy. The 2-methylamino-3-phenylsulfonyl-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidine, **7**, to the best of our knowledge possesses the highest affinity (K_i = 88 pM) to the 5-HT_6R and is the most selective, based on a panel of 55 therapeutic targets, among known antagonists of the receptor. The data also indicate the absence of potential liability coupled with either 5-HT_{2B} receptor or hERG channel. This makes it an excellent candidate for development of a therapeutic tool to probe the role of 5-HT_6R in different diseases of the CNS.

Experimental Section

Chemistry. General Methods. ^{1}H NMR spectra of solutions of the investigated compounds in DMSO-d_6 or $CDCl_3$ were recorded on a Bruker DPX-400 spectrometer (400 MHz, 27 °C), and ^{13}C NMR and two-dimensional spectra were obtained on a Bruker DPX-300 (75 MHz, 27 °C).

LC−MS data were obtained using a Shimadzu HPLC equipped with a Waters XBridge C_{18} 3.5 mm column (4.6 mm × 150 mm), PE SCIEX API 150 EX mass detector, and Shimadzu spectrophotometric detector (λ, 220 and 254 nm).

HR mass spectra (ESI-TOF, positive) were obtained on a Waters Qtof API US instrument in the positive mode.

In all cases, the end of the reaction was determined by conversion of the substrate (LC−MS control). Evaporation of solvents and drying of products were carried out only at reduced pressure. Separation of reaction products was performed using a HPLC system using Shimadzu LC-8A on the chromatographic column Reprosil-Pur C-18-AQ 10 mm, 250 mm × 20 mm (column Reprosil-Pur C-18-AQ 10 mm, 50 mm × 20 mm) at a flow rate of 25 mL/min in gradient mode with mobile phase MeCN/water + 0.05% CF_3COOH.

According to LC−MS, the purity of the obtained compounds **1** and **2** exceeded 98.0%.

Preparation of (3-Phenylsulfonylcycloalkano[*e* and *d*]pyrazolo[1,5-*a*]pyrimidin-2-yl)amines (1−16 and Their Mixtures). Procedure A. Stirred for 1 h at 0 °C were *N*(3)-methyl-4-(phenylsulfonyl)-1*H*-pyrazole-3,5-diamine **19**, **20** (1 mmol) and sodium 2-oxocycloalkylidenmethylate **21**, **22** (2 mmol) or 2-acetylcycloalkanone **23**, **24** (2 mmol) and 3.5 mL of glacial AcOH. The mixture was allowed to stir overnight at room temperature. The resulting precipitate was filtered, washed with AcOH, hexane, and dried in a vacuum. Product **2** was obtained in 63% yield, or a mixture of products **3** and **9** (ratio 1:9) or products **4** and **10** (ratio 1:3) was obtained.

Procedure B. Stirred for 3 h at 100 °C were aminopirazole **19**, **20** (0.5 mmol) and sodium 2-oxocycloalkylidenmethylate **21**, **22** (2 mmol) or 2-acetylcycloalkanone **23**, **24** (2 mmol) in 1 mL of AcOH. Evaporation of solvent was done in a vacuum. The residue was mixed with 20 mL of *i*-PrOH at 0 °C, and the resulting precipitate was separated, washed twice with *i*-PrOH, hexane, and dried in a vacuum. Products **9** and **13** were obtained in a yield of 80−90%, or a mixture of products was obtained: **4** and **10** (ratio ∼6: 5), which were separated by using HPLC; a mixture of products **3** and **9** (ratio ∼1:10)

Procedure C. To a solution of 0.3 g (2 mmol) of sodium 2-oxocyclohexylidenmethylate in 3.5 mL of AcOH was added 1 mL of 6 M HCl solution in EtOAc−EtOH (prepared by dissolving AcCl in EtOH). The reaction mixture was cooled in a water−ice bath to ∼0 °C, and 0.252 g (1 mmol) of *N*(3)-methyl-4-(phenylsulfonyl)-1*H*-pyrazole-3,5-diamine **19** was added. Stirring continued for 1 h at this temperature and then 12 h at room temperature. The precipitate was filtered, washed on the filter with AcOH, hexane, and dried in a vacuum. Target products were purified by column chromatography (eluent, $CHCl_3$). Product **8** was obtained in 67% yield, or product mixtures of **3** and **9** (ratio 1: 4) or of **4** and **10** (ratio ∼2:1; but when the reaction was carried out in 2 weeks at room temperature, the ratio was ∼4:1) were obtained.

***N*-Methyl-3-(phenylsulfonyl)-5,6,7,8-tetrahydropyrazolo[5,1-*b*]quinazolin-2-amine (2).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (s, 1H, 9-CH), 8.00 (m, 2H, 2-CH-Ph), 7.59 (m, 1H, 4-CH-Ph), 7.55 (m, 2H, 3-CH-Ph), 6.32 (q, J = 4.8 Hz, 1H, NH), 2.88 (d, J = 4.8 Hz, 3H, NCH_3), 2.86 (t, J = 6.4 Hz, 2H, 5-CH_2), 2.69 (t, J = 6.2 Hz, 2H, 8-CH_2), 1.82 (m, 2H, 6-CH_2), 1.72 (m, 2H, 7-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.82 (4a-C), 158.06 (2-C), 145.67 (3a-C), 144.14 (1-Ph-C), 133.80 (9-C), 132.69 (4-Ph-C), 129.13 (2-Ph-C), 125.62 (3-Ph-C), 118.98 (8a-C), 88.75 (3-C), 32.43 (5-C), 29.05 (NCH_3), 25.09 (8-C), 21.93 (6-C), 21.56 (7-C). HRMS calculated for $C_{17}H_{17}N_3O_2S_2$ (M + H) 343.1229, found 343.1230. MS-ESI calculated for $C_{17}H_{17}N_3O_2S_2$ (M + H) 343, found m/z 343. LC−MS (UV-254) purity: 98%.

***N*,9-Dimethyl-3-(phenylsulfonyl)-5,6,7,8-tetrahydropyrazolo-[5,1-*b*]quinazolin-2-amine (4).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (m, 2H, 2-CH-Ph), 7.55 (m, 3H, 3,4-CH-Ph), 6.31 (br m, 1H, NH), 2.91 (s, 3H, NCH_3), 2.84 (t, J = 5.2 Hz, 2H, 5 or 8-CH_2), 2.64 (t, J = 5.2 Hz, 2H, 5 or 8-CH_2), 2.53 (s, 3H, 9-CH_3), 1.77 (m, 4H, 6,8-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.32 (4a-C), 157.63 (2-C), 145.47 (3a-C), 144.43 (9-C), 144.05 (1-Ph-C), 132.46 (4-Ph-C), 128.92 (2-Ph-C), 125.73 (3-Ph-C), 116.76 (8a-C), 89.12 (3-C), 33.07 (5-C), 28.96 (NCH_3), 24.02 (8-C), 21.88, 21.70 (6-C, 7-C), 12.81 (9-CH_3). HRMS calculated for $C_{18}H_{20}N_4O_2S$ (M + H) 357.1380, found 357.1377. MS-ESI calculated for $C_{18}H_{20}N_4O_2S$ (M + H) 357, found m/z 357. LC−MS (UV-254) purity: 98%.

Mixture (~6:5) of *N*,9-Dimethyl-3-(phenylsulfonyl)-5,6,7,8-tetrahydropyrazolo[5,1-*b*]quinazolin-2-amine (4) and *N*,5-Dimethyl-3-(phenylsulfonyl)-6,7,8,9-tetrahydropyrazolo[5,1-*a*]quinazolin-2-amine (10). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11−8.17 (m, 2H), 7.39−7.49 (m, 3H), 5.91−6.00 (br m, 1H), 2.94−3.05 (m, 5H), 2.64−2.79 (m, 0.8H), 2.58−2.63 (m, 1.2H), 2.57 (s, 0.96H), 2.50 (s, 1.60H), 1.79−1.90 (m, 4H). MS-ESI calculated for $C_{18}H_{20}N_4O_2S$ (M + H) 357, found *m*/*z* 357.

Mixture (15:85) of *tert*-Butyl 4-[8-Methyl-3-(phenylsulfonyl)-6,7-dihydro-5*H*-cyclopenta[*d*]pyrazolo[1,5-*a*]pyrimidin-2-yl]piperazine-1-carboxylate (5) and *tert*-Butyl 4-[5-Methyl-3-(phenylsulfonyl)-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidin-2-yl]piperazine-1-carboxylate (13). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07−8.18 (m, 2H), 7.39−7.53 (m, 3H), 3.57−3.64 (m, 4H), 3.39−3.47 (m, 4H), 3.24 (t, J = 7.6 Hz, 1.7H), 3.10 (t, J = 7.8 Hz, 0.3H), 2.07 (t, J = 7.6 Hz, 1.7H), 2.93 (t, J = 7.8 Hz, 0.3H), 2.58 (s, 0.45H), 2.54 (s, 2.55H), 2.18−2.32 (m, 2H), 1.48 (s, 9H). MS-ESI calculated for $C_{25}H_{31}N_5O_4S$ (M + H) 498, found *m*/*z* 498.

***N*-Methyl-(3-phenylsulfonyl)-7,8-dihydro-6*H*-cyclopenta[*e*]-pyrazolo[1,5-*a*]pyrimidin-2-amine (7).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 1H, 5-CH), 8.00 (m, 2H, 2-CH-Ph), 7.59 (m, 1H, 4-CH-Ph), 7.54 (m, 2H, 3-CH-Ph), 6.44 (q, J = 4.8 Hz, 1H, NH), 3.17 (t, J = 7.6 Hz, 2H, 8-CH_2), 2.96 (t, J = 7.6 Hz, 2H, 6-CH_2), 2.92 (d, J = 4.8 Hz, 3H, NCH_3), 2.15 (p, J = 7.6 Hz, 2H, 7-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.67 (2-C), 150.77 (8a-C), 147.58 (5-C), 146.92 (3a-C), 144.25 (1-Ph-C), 132.61 (4-Ph-C), 129.03 (2-Ph-C), 125.67 (3-Ph-C), 124.53 (5a-C), 90.23 (3-C), 29.49, 29.11, 28.32 (NCH_3, 6-C, 8-C), 22.19 (7-C). HRMS calculated for $C_{16}H_{16}N_4O_2S$ (M + H) 329.1072, found 329.1077. MS-ESI calculated for $C_{16}H_{16}N_4O_2S$ (M + H) 329, found *m*/*z* 329. LC−MS (UV-254) purity: 99%.

***N*-Methyl-(3-phenylsulfonyl)-6,7,8,9-tetrahydropyrazolo[1,5-*a*]quinazolin-2-amine (8).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 1H, 5-CH), 7.99 (m, 2H, 2-CH-Ph), 7.59 (m, 1H, 4-CH-Ph), 7.54 (m, 2H, 3-CH-Ph), 6.36 (q, J = 4.8 Hz, 1H, NH), 2.92 (m, 5H, NCH_3, 9-CH_2), 2.67 (t, J = 5.8 Hz, 2H, 6-CH_2), 1.83 (m, 2H, 8-CH_2), 1.72 (m, 2H, 7-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.51 (2-C), 151.71 (5-C), 146.04 (3a-C), 145.01 (9-C), 144.09 (1-Ph-C), 132.73 (4-Ph-C), 129.16 (2-Ph-C), 125.60 (3-Ph-C), 118.52 (5a-C), 90.14 (3-C), 29.07 (NCH_3), 23.89, 23.65 (6-C, 9-C), 21.20 (7-C), 20.51 (8-C). HRMS calculated for $C_{17}H_{18}N_4O_2S$ (M + H) 343.1229, found 343.1227. MS-ESI calculated for $C_{17}H_{18}N_4O_2S$ (M + H) 343, found *m*/*z* 343. LC−MS (UV-254) purity: 98%.

***N*,5-Dimethyl-3-(phenylsulfonyl)-7,8-dihydro-6*H*-cyclopenta-[*e*]pyrazolo[1,5-*a*]pyrimidin-2-amine (9).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (m, 2H, 2-CH-Ph), 7.54 (m, 3H, 3,4-CH-Ph), 6.19 (q, J = 6.4 Hz, 1H, NH), 3.18 (t, J = 6.0 Hz, 2H, 6 or 8-CH_2), 2.95 (d, J = 6.4 Hz, 3H, NCH_3), 2.92 (t, J = 6.0 Hz, 2H, 6 or 8-CH_2), 2.45 (s, 3H, 5-CH_3), 2.18 (p, J = 6.0 Hz, 2H, 7-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.92 (2-C), 157.11 (5-C), 149.01, 146.43 (3a-C, 8a-C), 143.90 (1-Ph-C), 132.04 (4-Ph-C), 128.47 (2-Ph-C), 125.27 (3-Ph-C), 122.91 (5a-C), 89.68 (3-C), 29.16 (NCH_3), 28.62, 28.33 (6-C, 8-C), 21.93, 21.19 (7-C, 5-CH_3). HRMS calculated for $C_{17}H_{18}N_4O_2S$ (M + H) 343.1229, found 343.1234. MS-ESI calculated for $C_{17}H_{18}N_4O_2S$ (M + H) 343, found *m*/*z* 343. LC−MS (UV-254) purity: 99%.

***N*,5-Dimethyl-3-(phenylsulfonyl)-6,7,8,9-tetrahydropyrazolo-[1,5-*a*]quinazolin-2-amine (10).** ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (m, 2H, 2-CH-Ph), 7.54 (m, 3H, 3,4-CH-Ph), 6.20 (q, J = 5.6 Hz, 1H, NH), 2.91 (d, J = 5.6 Hz, 3H, NCH_3), 2.90 (br m, 2H, 9-CH_2), 2.59 (br m, 2H, 6-CH_2), 2.44 (s, 3H, 5-CH_3), 1.78 (br m, 4H, 7-CH_2, 8-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.29 (5-C), 157.28 (2-C), 145.07, 144.26 (3a-C, 9a-C), 143.98 (1-Ph-C), 132.58 (4-Ph-C), 129.03 (2-Ph-C), 125.67 (3-Ph-C), 117.24 (5a-C), 89.57 (3-C), 29.05 (NCH_3), 24.16, 23.73 (6-C, 9-C), 22.56 (5-CH_3), 21.31, 20.23 (7-C, 8-C). HRMS calculated for $C_{18}H_{20}N_4O_2S$ (M + H) 357.1380, found 357.1382. MS-ESI calculated for $C_{18}H_{20}N_4O_2S$ (M + H) 357, found *m*/*z* 357. LC−MS (UV-254) purity: 98%.

***N*,*N*-Dimethyl-3-(phenylsulfonyl)-7,8-dihydro-6*H*-cyclopenta-[*e*]pyrazolo[1,5-*a*]pyrimidin-2-amine (11).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H, 5-H), 8.14 (m, 2H, 2-CH-Ph), 7.50 (m, 1H, 4-CH-Ph), 7.45 (m, 2H, 3-CH-Ph), 3.29 (t, J = 7.6 Hz, 2H, 8-CH_2), 3.10 (s, 6H, $N(CH_3)_2$), 3.09 (t, J = 8.0 Hz, 2H, 6-CH_2), 2.31 (p, J = 7.6 Hz, 2H, 7-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.45 (2-C), 149.55 (8a-C), 148.48 (3a-C), 147.32 (5-C), 144.29 (1-Ph-C), 131.63 (4-Ph-C), 128.06 (2-Ph-C), 125.92 (3-Ph-C), 124.03 (5a-C), 95.25 (3-C), 42.03 ($N(CH_3)_2$), 29.19, 28.27 (6-C, 8-C), 22.03 (7-C). HRMS calculated for $C_{17}H_{18}N_4O_2S$ (M + H) 343.1380, found 357.1223. MS-ESI calculated for $C_{17}H_{18}N_4O_2S$ (M + H) 343, found 343. LC−MS (UV-254) purity: 98.8%.

***N*,*N*,5-Trimethyl-3-(phenylsulfonyl)-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidin-2-amine (12).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (m, 2H, 2-CH-Ph), 7.50 (m, 1H, 4-CH-Ph), 7.45 (m, 2H, 3-CH-Ph), 3.26 (t, J = 7.6 Hz, 2H, 8-CH_2), 3.10 (s, 6H, $N(CH_3)_2$), 2.98 (t, J = 7.6 Hz, 2H, 6-CH_2), 2.53 (s, 3H, 5-CH_3), 2.28 (p, J = 7.6 Hz, 2H, 7-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.39 (2-C), 157.77 (5-C), 148.29, 148.27 (3a-C, 8a-C), 144.18 (1-Ph-C), 131.66 (4-Ph-C), 127.96 (2-Ph-C), 126.52 (3-Ph-C), 123.14 (5a-C), 94.86 (3-C), 42.35 ($N(CH_3)_2$), 29.37, 28.92 (6-C, 8-C), 22.61, 21.59 (7-C, 5-CH_3). HRMS calculated for $C_{18}H_{20}N_4O_2S$ (M + H) 357.1380, found 357.1379. MS-ESI calculated for $C_{18}H_{20}N_4O_2S$ (M + H) 357, found 357. LC−MS (UV-254) purity: 99%.

***tert*-Butyl 4-[5-Methyl-3-(phenylsulfonyl)-6,7,8,9-tetrahydropyrazolo[1,5-*a*]quinazolin-2-yl]piperazine-1-carboxylate (14).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, J = 7.7 Hz, 2H), 7.40−7.52 (m, 3H), 3.58−3.63 (m, 4H), 3.41−3.46 (m, 4H), 2.99 (t, J = 5.7 Hz, 2H), 2.64 (t, J = 5.9 Hz, 2H), 2.53 (s, 3H), 1.82−1.95 (m, 4H), 1.49 (s, 9H). MS-ESI calculated for $C_{26}H_{33}N_5O_4S$ (M + H) 512, found 512. LC−MS (UV-254) purity: 99%.

5-Methyl-3-phenylsulfonyl-2-piperazin-1-yl-7,8-dihydro-6*H*-cycloalkane[*e*]pyrazolo[1,5-*a*]pyrimidines, Hydrochlorides (15, 16). An amount of 0.3 mmol of Boc derivative mixture **5** and **13** or Boc derivative **15** was added to 1.5 mL of 6 N HCl solution of AcOH in EtOH. The reaction mixture was stirred for 1 h at room temperature before an excess of ether was added. Then hydrochloride precipitates of **15** and **16** were separated, successively washed with AcOEt (twice), acetone (twice), and hexane (once), and dried in a vacuum to provide colorless crystalline products **15** and **16**.

5-Methyl-3-(phenylsulfonyl)-2-piperazin-1-yl-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo[1,5-*a*]pyrimidine, Hydrochloride (15). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (br m, 2H, NH_2^+), 8.03 (m, 2H, 2-CH-Ph), 7.55 (m, 3H, 3,4-CH-Ph), 3.64 (br m, 4H, 2-piperazin-CH_2), 3.24 (br m, 4H, 3-piperazin-CH_2), 3.20 (t, J = 10.4 Hz, 2H, 8-CH_2), 2.92 (t, J = 9.6 Hz, 2H, 6-CH_2), 2.47 (s, 3H, 5-CH_3), 2.16 (m, 2H, 7-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.57, 159.20 (2-C, 5-C), 149.77 (8a-C), 147.22 (3a-C), 143.65 (1-Ph-C), 132.85 (4-Ph-C), 128.96 (4-Ph-C), 126.43 (3-Ph-C), 124.92 (5a-C), 95.85 (3-C), 47.48 (2-piperazin-CH_2), 42.52 (3-piperazin-CH_2), 29.56, 28.82 (6-C, 8-C), 22.75 (5-CH_3), 21,56 (7-C). HRMS calculated for $C_{20}H_{23}N_5O_2S$ (M + H) 398.1651, found 398.1646. MS-ESI calculated for $C_{20}H_{23}N_5O_2S$ (M + H) 398, found 398. LC−MS (UV-254) purity: 99%.

5-Methyl-3-(phenylsulfonyl)-2-piperazin-1-yl-6,7,8,9-tetrahydropyrazolo[1,5-*a*]quinazoline, Hydrochloride (16). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (br m, 2H, NH_2^+), 8.03 (m, 2H, 2-CH-Ph), 7.57 (m, 3H, 3,4-CH-Ph), 3.63 (br m, 4H, 2-piperazin-CH_2), 3.26 (br m, 4H, 3-piperazin-CH_2), 2.95 (br m, 2H, 9-CH_2), 2.63 (br m, 2H, 6-CH_2), 2.47 (s, 3H, 5-CH_3), 1.79 (m, 4H, 7,8-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.81 (5-C), 158.47 (2-C), 145.47, 144.22, 143.71 (3a-C, 9a-C, 1-Ph-C), 132.86 (4-Ph-C), 128.98 (2-Ph-C), 126.44 (3-Ph-C), 118.76 (5a-C), 95.88 (3-C), 47.48 (2-piperazin-CH_2), 42.57 (3-piperazin-CH_2), 23.94, 23.68 (6-C, 9-C), 22.84 (5-CH_3), 21.18 (8-C), 20.12 (7-C). HRMS calculated for $C_{21}H_{25}N_5O_2S$ (M + H) 412.1807, found 412.1811. MS-ESI calculated for $C_{21}H_{25}N_5O_2S$ (M + H) 412, found 412. LC−MS (UV-254) purity: 99%.

N^3-Methyl-4-(phenylsulfonyl)-1*H*-pyrazole-3,5-diamine (19). With vigorous stirring at room temperature, 6.16 g (110 mmol) of finely triturated KOH was added to a solution of 9 g (50 mmol) of sulfonylacetonitrile **17** and 4.0 g (55 mmol) of MeNCS in 80 mL of dioxane. The reaction mixture was stirred for 2 h before adding 7.1 g (50 mmol) of MeI, and stirring continued at room temperature for 12 h. The resultant mixture was poured onto 400 mL of a mixture of ice and H_2O and stirred for 1 h. The precipitate was filtered, washed with H_2O, and freeze-dried. Obtained was 4.1 g of 2-phenylsulfonyl-3-methylamino-3-methylsulfanylacrylonitrile **18**. In addition, 4.74 g of the acrylonitrile **18**, identical according to LC−MS and NMR with the first portion, was obtained after neutralization of the filtrate with AcOH, followed by filtering of the precipitate, washing with H_2O, and drying. The overall yield of **18** was 67%, considering the source acetonitrile **17**. When using an excess of MeI (> 50 mmol), one gets the product **18** containing, according to LC−MS, 2-phenylsulfonyl-3-dimethylamino-3-methylsulfanylacrylonitrile **26** in the amount of the corresponding excess of MeI. To 8.84 g (0.033 mol) of acrylonitrile **18** in 70 mL of *i*-PrOH was added 2.5 g (0.05 mol) of $N_2H_2 \cdot H_2O$, and the mixture was stirred for 1 h at room temperature, then boiled for 2 h, and diluted with 70 mL of H_2O. The precipitate was filtered and dried in a vacuum. Obtained was 7.5 g (90%) of pyrazole **19**. LC−MS purity was 97%+. MS-ESI calculated for $C_{10}H_{12}N_4O_2S$ (M +1) 253, found 253. When using 10−15% excess MeI in the synthesis of acrylonitrile **18**, pyrazole **19** was obtained containing, according to LC−MS, a corresponding excess of 4-benzenesulfonyl-*N*(3), *N*(3)-dimethyl-1*H*-pyrazole-3,5-diamine **20**. MS-ESI calculated for $C_{11}H_{14}N4O_2S$ (M +1) 267, found 267.

N^3,N^3-Dimethyl-4-(phenylsulfonyl)-1*H*-pyrazole-3,5-diamine (20). To a solution of 5.00 g (27 mmol) of phenylsulfonylacetonitrile **17** and 2.31 g (30 mmol) of CS_2 in 50 mL of dioxane, 3.99 g (55 mmol) of finely grounded KOH was added with vigorous stirring. The mixture was stirred under Ar for 2 h. Then 8.61 g (55 mmol) of MeI was added and the stirring was continued for 3 h. The resultant mixture was poured into 100 mL of ice−water and stirred for 1 h. The precipitate was filtered, washed with H_2O, hexane, and freeze-dried to give 6.24 g (80%) of 3,3-bis(methylthio)-2-(phenylsulfonyl)acrylonitrile. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (m, 2H), 7.70 (m, 1H), 7.59 (m, 2H), 2.72 (s, 3H), 2.57 (s, 3H). To a solution of 571 mg (2.0 mmol) of the obtained 3,3-bis(methylthio)-2-(phenylsulfonyl)acrylonitrile in 10 mL of MeOH, 0.32 mL (2.5 mmol) of 40% aqueous Me_2NH was added. The mixture was stirred at ambient temperature for 12 h and then concentrated and dried under vacuum to afford 565 mg (100%) of 3-(dimethylamino)-3-(methylthio)-2-(phenylsulfonyl)acrylonitrile **25**, configuration undetermined. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (m, 2H), 7.60 (m, 1H), 7.54 (m, 2H), 3.37 (s, 6H), 2.49 (s, 3H). To 565 mg (2.0 mmol) of acrylonitrile **25** in 10 mL of *i*-PrOH was added 200 mg (4.0 mmol) of $N_2H_2 \cdot H_2O$. The mixture was stirred for 1 h at room temperature, then refluxed for 2 h, concentrated under vacuum, treated with water, and extracted with CH_2Cl_2. The organic phase was washed with 10% K_2CO_3 solution, dried over Na_2SO_4, and concentrated under vacuum. The resulting mixture (450 mg) consisted of **20** and 3-(methylthio)-4-(phenylsulfonyl)-1*H*-pyrazol-5-amine, 2:1 ratio according to LC−MS. The pyrazole **20** was isolated by HPLC. Isolated yield was 175 mg (33%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.30 (br s, 0.6H), 7.81 (m, 2H), 7.60 (m, 1H), 7.55 (m, 2H), 5.93 (br s, 2H), 2.65 (s, 6H). MS-ESI calculated for $C_{11}H_{14}N_4O_2S$ (M +1) 267, found 267.

4-(5-Amino-4-phenylsulfonyl-1*H*-pyrazol-3-yl)piperazine-1-carboxylic Acid *tert*-Butyl Ester (28). An amount of 1.74 g (9.35 mmol) of 1-Boc-piperazine was added to a mixture of 2.67 g (9.35 mmol) of 2-phenylsulfonyl-3,3-bis-methylsulfanylacrylonitrile **27** in 15 mL of *i*-PrOH. The resulting mixture was heated with stirring for 1 h and sat for 12 h at room temperature. Then the precipitate was filtered, washed with *i*-PrOH, hexane, and dried. Product obtained was 3.32 g (84%) of 4-(2-phenylsulfonyl-2-cyano-1-methylsulfanylvinyl)piperazine-1-carboxylic acid *tert*-butyl ester **10**. An amount of 3.32 g (7.85 mmol) of this product in 15 mL of *i*-PrOH was stirred with 0.48 g (9.5 mmol) of $N_2H_2 \cdot H_2O$ with heating. The reaction mixture was poured into a mixture of H_2O with ice, and the precipitated oil quickly solidified. The solid was filtered and washed with H_2O, cold *i*-PrOH, and hexane to provide 2.65 g (83%) of the target product **28**. MS-ESI calculated for $C_{18}H_{25}N_5O_4S$ (M +1) 408, found 408.

Biological Assays. Cell-Based Functional Assays. 5-HT_{2B} Receptor Functional Assay. The 5-HT_{2B}-HEK cells were grown in T-175 flasks at 37 °C in an atmosphere of air/CO_2 (95%:5%) in DMEM (Sigma, MO) supplemented with 10% FBS, 1% AAS, blasticidine S, and phleomycin (Invitrogen, Carlsbad, CA). The T-Rex/5-HT_{2B} receptor expression was activated by addition of tetracycline, as recommended by the manufacturer, a day before the experiments. The cells were dissociated with TrypLE Express (Invitrogen, Carlsbad, CA), washed twice with PBS, and loaded at room temperature with 4 μM calcium-sensitive dye, Fura-2AM (Invitrogen, Carlsbad, CA), for 30 min. After the loading, the cells were washed once with PBS, resuspended into protein free hybridoma media without phenol red (Sigma, St. Louis, MO), and allowed to incubate for an additional 30 min with gentle shaking at room temperature. All loading procedures were performed in dark conditions. The loaded cells were washed twice with PBS and resuspended into the hybridoma media at a cell density of (3−4) × 10^6 cells/mL for subsequent experiments. Fura-2 ratiometric fluorescence signal was registered at 510 nm upon alternate excitation at 340 and 380 nm using spectrofluorometer RF5301PC (Shimadzu, Columbia, MD). In a square (1 cm) optical cuvette with a magnetic stirring bar, 100 μL aliquots of the loaded cells were diluted into 2.4 mL of buffer containing (mM) NaCl (145), KCl (5.4), $MgSO_4$ (0.8), $CaCl_2$ (1.8), HEPES (30), and D-glucose (11.2). The fluorescence signal was allowed to stabilize for 20−30 s before addition of a test compound or vehicle to assess potential agonistic activity of the compounds, with subsequent addition of serotonin (2.5 μL, 10 mM) to assess the compounds' blocking activity.

5-HT_6 Receptor Functional Assay. The 5-HT_6-HEK cells were grown in Corning 384-well plates (Lowell, MA) at 37 °C in an atmosphere of air/CO_2 (95%:5%) in DMEM supplemented with 10% FBS, 1% AAS, blasticidine S, and phleomycin (Invitrogen, Carlsbad, CA). T-Rex/5-HT_6 receptor expression was activated by addition of tetracycline, as recommended by the manufacturer, a day before the experiments. On the day of the experiment, the medium in the wells was substituted with phenol red-, calcium-, and magnesium-free HBSS (Invitrogen, Carlsbad, CA), supplemented with 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM HEPES, pH 7.4, and 100 μM IBMX. The test compounds were added at different concentrations while maintaining constant final DMSO concentration of 0.1%. After 15 min of incubation, serotonin hydrochloride (Sigma, MO) was added to a final concentration of 10 nM and incubation continued for additional 30 min at room temperature. The cells were treated as described in the cAMP LANCE assay kit protocol (PerkinElmer, Waltham, MA) as recommended by the manufacturer. The LANCE signal was measured in white 384-well plates (Corning, MA) using multimode plate reader VICTOR^{2}V (PerkinElmer, Waltham, MA) with built-in settings for the LANCE detection.

hERG Channel Functional Assay. Human recombinant hERG channel was stably expressed in HEK-297 cells. Intracellular solution consisted of 130 mM KCl, 5 mM EGTA, 5 mM Mg Cl_2, 10 mM HEPES, 5 mM Na-ATP, pH 7.2. Extracellular solution consisted of 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 11 mM dextrose, 10 mM HEPES, pH 7.4. Voltage clamp measurements were performed at 25 °C using PatchXpress 7000A (Molecular Devices, Sunnyvale, CA). hERG channels were activated by 2 s pulses to +20 mV from

a holding potential of −80 mV, and peak tail currents were recorded upon repolarization to −50 mV. This voltage-clamp pulse protocol was performed continuously during the experiment (vehicle control, test compound, washout, and positive control additions). An interpulse interval of 15 s allowed recovery from any residual inactivation. Test compounds were incubated with cells until the current reached a steady state level (3−8 min). After the final test compound concentration was tested, test compound was washed out with continuous perfusion of extracellular solution for 3 min, followed by application of positive control (10 μM cisapride). Data were analyzed using DataXpress software. Percent of control values were calculated on the basis of current peak at each compound concentration relative to maximal tail current in the presence of vehicle control.

Competitive Radioligand Displacement Assays. The assays were performed by MDS Pharma Services (currently Ricerca) in accordance with their internal protocols briefly described in Supporting Information.

Acknowledgment. The authors thank Dr. Norman Lee from Chemical Instrumentation Center, Boston University, for HR-MS measurements.

Supporting Information Available: X-ray crystallographic data for compounds **9** and **12** (including two files in cif format) and a description of the competitive radioligand displacement assays. This material is available free of charge via the Internet at http://pubs.acs.org. CCDC-769754 and CCDC-769755 contain supplementary crystallographic data of **9** and **12** for this paper, and these data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif by emailing data_request@ccdc.cam.ac.uk or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, U.K. (fax, +44 1223 336033).

References

(1) 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.
(2) 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-HT6 serotonin receptor. *J. Neurochem.* **1996**, *66*, 47–56.
(3) Wesolowska, A. The anxiolytic-like effect of the selective 5-HT6 receptor antagonist SB-399885: the impact of benzodiazepine receptors. *Eur. J. Pharmacol.* **2008**, *580*, 355–360.
(4) Wesolowska, A.; Nikiforuk, A. Effects of the brain-penetrant and selective 5-HT6 receptor antagonist SB-399885 in animal models of anxiety and depression. *Neuropharmacology* **2007**, *52*, 1274–1283.
(5) Hirst, W. D.; Stean, T. O.; Rogers, D. C.; Sunter, D.; Pugh, P.; Moss, S. F.; Bromidge, S. M.; Riley, G.; Smith, D. R.; Bartlett, S.; Heidbreder, C. A.; Atkins, A. R.; Lacroix, L. P.; Dawson, L. A.; Foley, A. G.; Regan, C. M.; Upton, N. SB-399885 is a potent, selective 5-HT6 receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. *Eur. J. Pharmacol.* **2006**, *553*, 109–19.
(6) Perez-García, G.; Meneses, A. Oral administration of the 5-HT6 receptor antagonists SB-357134 and SB-399885 improves memory formation in an autoshaping learning task. *Pharmacol., Biochem. Behav.* **2005**, *81*, 673–682.
(7) Wesolowska, A.; Nikiforuk, A. The selective 5-HT(6) receptor antagonist SB-399885 enhances anti-immobility action of antidepressants in rats. *Eur. J. Pharmacol.* **2008**, *582*, 88–93.
(8) Dawson, L. A.; Nguyen, H. Q.; Li, P. The 5-HT(6) receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. *Neuropsychopharmacology* **2001**, *25*, 662–668.
(9) Foley, A. G.; Murphy, K. J.; Hirst, W. D.; Gallagher, H. C.; Hagan, J. J.; Upton, N.; Walsh, S.; Regan, C.,M. The 5-HT(6) receptor antagonist SB-271046 reverses scopolamine-disrupted consolidation of a passive avoidance task and ameliorates spatial task deficits in aged rats. *Neuropsychopharmacology* **2004**, *29*, 93–100.
(10) Riemer, C.; Borroni, E.; Levet-Trafit, B.; Martin, J. R.; Poli, S.; Porter, R. H.; Bos, M. Influence of the 5-HT6 receptor on acetylcholine release in the cortex: pharmacological characterization of 4-(2-bromo-6-pyrrolidin-1-ylpyridine-4-sulfonyl)phenylamine, a potent and selective 5-HT_6 receptor antagonist. *J. Med. Chem.* **2003**, *46*, 1273–1276.
(11) King, M. V.; Woolley, M. L.; Topham, I. A.; Sleight, A. J.; Marsden, C. A.; Fone, K. C. 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation—an effect sensitive to NMDA receptor antagonism. *Neuropharmacology* **2004**, *47*, 195–204.
(12) Holenz, J.; Pauwels, P. J.; Diaz, J. L.; Merce, R.; Codony, X.; Buschmann, H. Medicinal chemistry strategies to 5-HT_6 receptor ligands as potential cognitive enhancers and antiobesity agents. *Drug Discovery Today* **2006**, *11*, 283–299.
(13) 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.
(14) Heal, D. J.; Smith, S. L.; Fisas, A.; Codony, X.; Buschmann, H. Selective 5-HT6 receptor ligands: progress in the development of a novel pharmacological approach to the treatment of obesity and related metabolic disorders. *Pharmacol. Ther.* **2008**, *117*, 207–231.
(15) Davies, S. L. Drug discovery targets: 5-HT_6 receptor. *Drugs Future* **2005**, *30*, 479–495.
(16) Woolley, M. L. 5-HT6 receptors. *Curr. Drug Targets: CNS Neurol. Disord.* **2004**, *3*, 59–79.
(17) Vicker, S. P.; Dourish, C. T. Serotonin receptor ligands and the treatment of obesity. *Curr. Opin. Invest. Drugs.* **2004**, *5*, 377–388.
(18) http://www.biospace.com/news_story.aspx?NewsEntityId=141148.
(19) Tkachenko, S.; Ivachtchenko, A.; Khvat, A.; Okun, I.; Lavrovsky, Y.; Salimov, R. Discovery and Preclinical Studies of AVN-322 Highly Selective and Potent 5-HT6 Antagonist for Cognition Enhancement in Treating Neurodegenerative Diseases. *Abstracts of Papers*, 9th International Conference AD/PD 2009, Prague, Czech Republic, 2009; Karger: Basel, Switzerland, 2009; p 945.
(20) 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-HT6 antagonists. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2295–2299.
(21) Kim, H.-J.; Doddareddy, M. R.; Choo, H.; Cho, Y. S.; No, K. T.; Park, W.-K.; Pae, A. N. *J. Chem. Inf. Model.* **2008**, *48*, 197–206.
(22) López-Rodríguez, M. L.; Benhamu, B.; de la Fuente, T.; Sanz, A.; Pardo, L.; Campillo, M. A three-dimensional pharmacophore model for 5-hydroxytryptamine6 (5-HT6) receptor antagonists. *J. Med. Chem.* **2005**, *48*, 4216–4219.
(23) Bromidge, S. M. Potent and selective 5-HT6 receptor antagonists. *Spec. Publ. R. Soc. Chem.* **2001**, *264*, 101–119.
(24) Hirst, W. D.; Minton, J. A.; Bromidge, S. M.; Moss, S. F.; Latter, A. J.; Riley, G.; Routledge, C.; Middlemiss, D. N.; Price, G. W. Characterization of [^{125}I]-SB-258585 binding to human recombinant and native 5-HT_6 receptors in rat, pig and human brain tissue. *Br. J. Pharmacol.* **2000**, *130*, 1597–1605.
(25) Pullagurla, M. R.; Westkaemper, R. B.; Glennon, R. A. Possible differences in modes of agonist and antagonist binding at human 5-HT_6 receptors. *Bioorg. Med. Chem. Lett.* . **2004**, *14*, 4569–4573.
(26) Dukat, M.; Mosier, P. D.; Kolanos, R.; Roth, B. L.; Glennon, R. A. Binding of serotonin and *N*1-benzenesulfonyltryptamine-related analogs at human 5-HT6 serotonin receptors: receptor modeling studies. *J. Med. Chem.* **2008**, *51*, 603–611.
(27) Singer, J. M.; Wilson, M. W.; Johnson, P. D.; Graham, S. R.; Cooke, L. W.; Roof, R. L.; Boxer, P. A.; Gold, L. H.; Meltzer, L. T.; Janssen, A.; Roush, N.; Campbell, J. E.; Su, T. Z.; Hurst, S. I.; Stoner, C. L.; Schwarz, J. B. Synthesis and SAR of tolylamine 5-HT6 antagonists. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2409–2412.
(28) Ivachtchenko, A. V.; Mitkin, O. D.; Tkachenko, S. E.; Okun, Kysil, V. M. 8-Sulfonyl-substitutedtetrahydro-1*H*-pyrido[4,3-*b*]-indolesas 5-HT receptor antagonists. *Eur. J. Med. Chem.* **2010**, *45*, 782–789.
(29) Seong, C.,M.; Park, W. K.; Park, C. M.; Kong, J. Y.; Park, N. S. Discovery of 3-aryl-3-methyl-1*H*-quinoline-2,4-diones as a new class of selective 5-HT6 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 738–743.
(30) Upton, N; Chuang, T. T.; Hunter, A. J.; Virley, D. J. 5-HT6 receptor antagonists as novel cognitive enhancing agents for Alzheimer's disease. *Neurotherapeutics* **2008**, *5*, 458–469.
(31) Zhao, S.-H.; Berger, J.; Clark, R. D.; Sethofer, S. G.; Krauss, N. E.; Brothers, J. M.; Martin, R. S.; Misner, D. L.; Schwabd, D.;

Alexandrova, L. 3,4-Dihydro-2*H*-benzo[1,4]oxazine derivatives as 5-HT6 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3504–3507.
(32) http://clinicaltrials.gov/ct2/show/NCT00348192.
(33) Ivachtchenko, A. V.; Dmitriev, D. E.; Golovina, E. S.; Dubrovskaya, E. S.; Kadieva, M. G.; Koryakova, A. G.; Kysil, V. M.; Mitkin, O. D.; Tkachenko, S. E.; Okun, I. M.; Vorobiov, A. A. Synthesis of cycloalkane-annelated 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines and their evaluation as 5-HT_6 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2133–2136.
(34) Bajwa, J. S.; Sykes, P. J. Synthesis and structure of same azolo-[*a*]pyrimidines, 2,6,7,8-tetrahydro-1*H*-cyclopenta[*e*]azolo[*a*]pyrimidines, 6,7-dihydro-5*H*-ceclopenta[*f*]azolo[*a*]pyrimidines, 7,8-dihydro-6*H*-cyclopenta[*f*]-*s*-triazolo[4,3-*b*]pyrimidine, 5,6,7,8-tetrahydroazolo[*b*]quinozalines, 6,7,8,9-tetrahydroazolo[*a*]quinozalines, and 7,8,9,10-tetrahydro-*s*-triazolo[3,4-*a*]phthalazine. *J. Chem. Soc., Perkin Trans. 1* **1979**, *1*, 3085–3094.
(35) Augustin, M.; Schmidt, R.; Rudors, W.-D. Synthese und reaktivitat von phenylsulfonyl-cyan-keten-*S*,*S*-acetalen. *Z. Chem.* **1977**, *17*, 289–290.
(36) Pugmire, R. J.; Robins, M. J.; Grant, D. M.; Robins, R. K. Carbon-13 magnetic resonance. XX. 4-Azaindene (pyrrocoline) and related bridgehead nitrogen heterocycles. *J. Am. Chem. Soc.* **1971**, *93*, 1887–1893.
(37) Vartanyan, M. A.; Eliseev, O. L.; Skov, Kh. R.; Karakhanov, R. A. Synthesis of acetonylmalonaldehyde and its interaction with aminoazoles. *Russ. Chem. Bull.* **1997**, *46*, 1178–1179.
(38) Radioligand binding assays were performed by MDS Pharma Services (Taipei, Taiwan) in accordance with their internally developed assays, briefly described in the Supporting Information.
(39) Rothman, R. B.; Baumann, M. H.; Savage, J. E.; Rauser, L.; McBride, A.; Hufeisen, S. J.; Roth, B. L. Evidence for possible involvement of 5-HT2B receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation* **2000**, *102*, 2836–2841.
(40) Yao, X.; Anderson, D. L.; Ross, S. A.; Lang, D. G.; Desai, B. Z.; Cooper, D. C.; Wheelan, P.; McIntyre, M. S.; Bergquist, M. L.; MacKenzie, K. I.; Becherer, J. D.; Hashim, M. A. Predicting QT prolongation in humans during early drug development using hERG inhibition and an anaesthetized guinea-pig model. *Br. J. Pharmacol.* **2008**, *154*, 1446–1456.