Synthesis and Structure-Activity Relationship (SAR) of (5,7-Disubstituted 3-phenylsulfonyl-pyrazolo[1,5-a]pyrimidin-2-yl)-methylamines as Potent Serotonin 5-HT6 Receptor (5-HT6R) A...

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Synthesis and Structure–Activity Relationship (SAR) of (5,7-Disubstituted 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methylamines as Potent Serotonin 5-HT$_6$ Receptor (5-HT$_6$R) Antagonists

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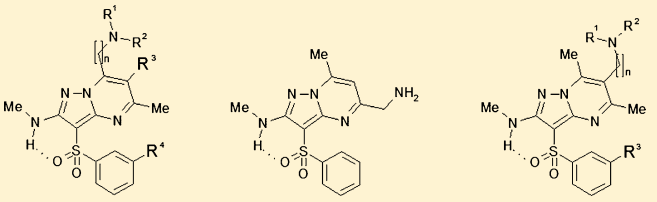
Supporting Information

ABSTRACT: Syntheses, biological evaluation as 5-HT$_6$ receptor (5-HT$_6$R) antagonists, and structure–activity relationships for a series of novel 5,7-disubstituted (3-arylsulfonyl-pyrazolo[1,5-*a*]pyrimidins are disclosed. The molecule conformational flexibility in the series is restricted by formation of the intramolecular hydrogen bond between 3-sulfo and 2-methylamino groups, which renders high potency and high selectivity to block serotonin-induced responses in HEK-293 cells stably expressing human 5-HT$_6$R. In this work, we tested the hypothesis if addition of a positively ionizable group (PI) to the pyrimidine ring of the scaffold members in positions 5, 6, or 7 could further increase their 5HT$_6$R blocking potency. We show that the presence of the PI group with small substituents does not substantially affect either potency or selectivity of the ligands while causing substantial changes in their cLogP values. This provides a possibility for designing of the 5HT$_6$R ligands with modified ADME characteristics without grossly affecting efficiency of their interaction with the receptor. In respect to the structure–activity relationship (SAR), among other physiochemical parameters, only the molecule size and shape (described by gyration radii) showed a clear tendency for more compact molecules to be more potent antagonists of this receptor.



INTRODUCTION

The 5-HT$_6$ receptors (5-HT$_6$R) were first described in 1993 (rat),[1] 1994 (mouse),[2] and 1996 (human).[3] Almost exclusive localization of these receptors in the central nervous system (CNS),[3] as well as their relatively high affinity (though not selectivity) toward some tricyclic antipsychotics,[1,3] made the 5-HT$_6$R a particularly intriguing potential therapeutic target for treatment of many CNS disorders.[4] Currently, several 5-HT$_6$R antagonists, for example, *N*,*N*-dimethyl-3-[naphthalen-1-ylsulfonyl)-1*H*-indazol-5-yloxy]propan-1-amine (SAM-531, PF-5212365, WAY-262531),[5−7] [2-(6-fluoro-1*H*-indol-3-yl)-ethyl]-[3-(2,2,3,3-tetrafluoro-propoxy)-benzyl]-amine (Lu-AE-58054),[6−8] 3-benzenesulfonyl-8-piperazin-1-yl-quinoline (SB-742457, GSK-742457) (Figure 1), as well as AVN-211[6,7,9] and AVN-322 (Avineuro Pharmaceuticals),[6,7,10] for which structures are not disclosed, are being tested in phase II clinical trials for treatment of different cognitive and mental disorders including Alzheimer's disease and schizophrenia. Phase IIa double blind clinical trials performed with AVN-211 (Avineuro Pharmaceuticals) on 50 patients stabilized on an atypical antipsychotic therapy showed positive results as an augmentation therapy to improve cognition in schizophrenia patients.[9] It should also be noted, however, that two other drug candidates, SB-742457 (for the treatment of Alzheimer's disease[11]) and Lu-AE-58054 (for the treatment of schizophrenia[12]), have failed to produce significant improvements over the placebo.

The first potent 5-HT$_6$R-selective antagonists were discovered through both synthetic design and random screening.[16−18] Since then, a wealth of potent and selective 5-HT$_6$R antagonists have been discovered that possess a common feature, the sulfonamide or sulfonyl moiety. A broad range of sulfonamide/sulfonyl derivatives containing aromatic and heterocyclic systems with high affinity to the 5-HT$_6$R have been described as potential drug candidates.[4,19−23] On the basis of analysis of a few selective 5-HT$_6$R ligands known at that time, Holenz et al.[24] suggested a conceptual framework model (Figure.2), which they used for the design and synthesis of four classes of novel series of sulfonamides of indoles with different substitution patterns. This hypothetical model was confirmed and further developed into a three-dimensional pharmacophore model based on the structural analysis of 5-HT$_6$R antagonists from a diverse group of 45 sulfonamide/sulfonyl containing compounds (Figure 2).[25,26]

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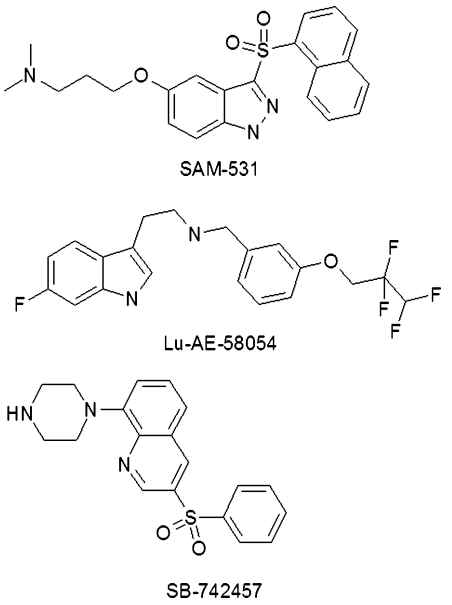

Figure 1. Structures of 5-HT$_6$ receptor antagonists, SAM-531,[13] Lu-AE-58054,[14] and SB-742457,[15] currently in phase II clinical trials for multiple mental disorders.

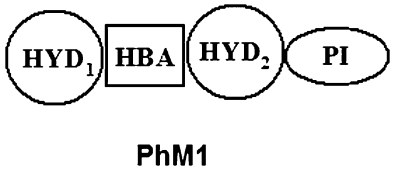

Figure 2. Conceptual pharmacophore model, PhM1, adapted from Holenz et al.[24] and López-Rodrigues et al.[25] The HYD$_1$ and HYD$_2$ groups (circles) represent aromatic or the heterocyclic hydrophobic moieties. HBA (in square) is a hydrogen bond acceptor group[25] or double electron acceptor,[24] represented by either a sulfonamide or sulfonyl group. PI (oval) is a positive ionizable atom[25] or proton donor,[21] mainly represented by an amine group.

Many 5-HT$_6$R antagonists with either sulfonamide[15,26,27] or sulfonyl[25−30] group as a hydrogen bond acceptor (HBA), which conform well to this model, were tested as potential cognitive enhancers[16] and as drugs for treating obesity[16] and Alzheimer's disease.[31]

Recently, we have described several series of highly potent 5-HT$_6$R antagonists represented by substituted 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines.[32−35] Unlike previously disclosed analogues, for example, 3-benzenesulfonyl-2-methylsulfanyl-8-piperazin-1-yl-1,5,6,7-tetrahydro-cyclopenta[*d*]pyrazolo[1,5-*a*]-pyrimidine (Ro-65−7674, Figure 3A),[26,27] which fit into the conceptual model PhM1, the disclosed series lacked a PI group. The most potent antagonists from the 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines series[30−35] were those with 2-methylamino substitution (Figure 3B), which could contribute in stabilization of the molecule structure by forming intramolecular hydrogen bond (IHB) between the methyl-amine hydrogen bond donor (HBD) group and oxygen of the sulfo group, HBA. The likelihood of the hydrogen bond formation is reasonable to suggest as the calculated with DS ViewerPro 6.0 (Accelrys, San Diego, CA) distance between the methyl-amine hydrogen and one of the sulfo group oxygen in energy minimized molecules **1** and **2** is 2.10 and 2.11 Å, respectively. Examples of such constricted 5-HT$_6$R antagonists, represented by (3-benzenesulfonyl-7,8-dihydro-6*H*-cyclopenta[*e*]pyrazolo-[1,5-*a*]pyrimidin-2-yl)-methyl-amine (**1**) and (3-benzenesulfonyl-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine (**2**) are shown in Figure 3B.

In spite of the absence of PI group, **1** and **2** are more potent 5-HT$_6$R antagonists than Ro-65−7674. Taking this into consideration, a new conceptual pharmacophore model, PhM2, can be hypothesized (Figure 4) which reflects the

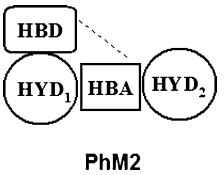

Figure 4. Conceptual pharmacophore model, PhM2, which illustrates known potent 5-HT$_6$R antagonists lacking a positively ionizable group but carrying a hydrogen bond donor group, HBD (round cornered square), capable of forming intramolecular hydrogen bond (dotted line) with a hydrogen bond acceptor group, HBA (square). The HYD$_1$ and HYD$_2$ (circles) are hydrophobic moieties separated by the HBA.

essential physiochemically distinct moieties in the active 5-HT$_6$R ligands. This model does not require the PI group as a

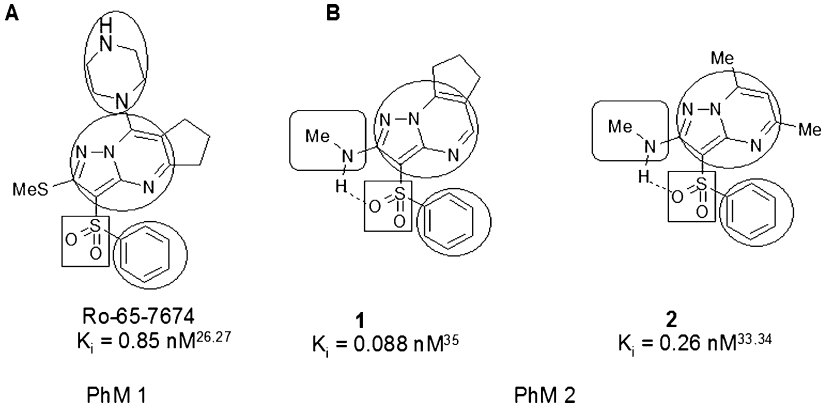

Figure 3. Structures of potent 5-HT$_6$ receptor antagonists with outline shapes representing corresponding functional groups (see description in caption to Figure 2). (A) Ro-65−7674 structurally conforms to the PhM1 model. (B) **1** and **2** (Avineuro Pharmaceticals) without positively ionizable (PI) group of PhM 1 but with hydrogen bond donor group (HBD, square with rounded corners) capable of forming intramolecular hydrogen bond with HBA (square) group in accordance with PhM 2.

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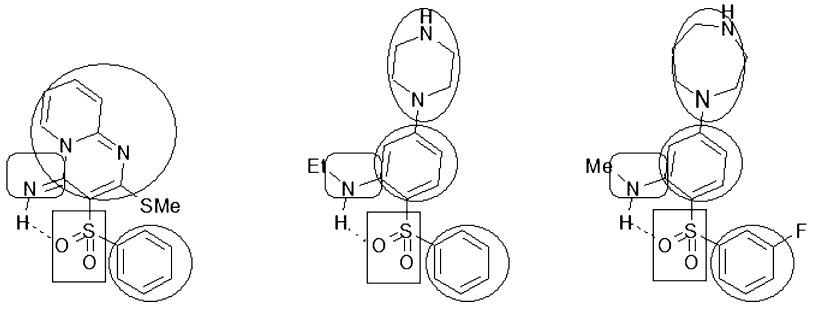


3 IC_{50} = 4 nM[33] **4** K_i = 2.7 nM[37] **5** K_i = 1.4 nM[37]

Figure 5. Potent and selective 5-HT_6 receptor antagonists with restricted conformational flexibility due to intramolecular hydrogen bond formation which conform to PhM 2. The hydrogen bond formation was confirmed upon energy minimization of the molecules using DS Viewer Pro 6.0 (Accelrys, San Diego).

necessary feature for the high potency. Instead, the model includes the hydrogen bond donor group (HBD, a round-cornered square in Figures 3 and 4) which can form intramolecular hydrogen bond with the hydrogen bond acceptor group (HBA, square) and thus serve as a "conformational restrictor". One can also argue that HBD as well as HBA groups of the discussed molecules could alternately participate in formation of an intermolecular, ligand–receptor interaction and, hence, increase the affinity of the ligands to the receptor.

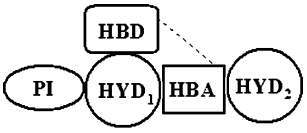
Another potent 5-HT_6R ligand, (*E*)-3 benzenesulfonyl-2-methylsulfanyl-pyrido[1,2-*a*]pyrimidin-4-ylideneamine (**3**), with the structure conforming to the model PhM 2, absence of PI group, and presence of the intramolecular hydrogen bond, was described in the literature[33] (Figure 5). However, the authors did not specifically investigate the role the intramolecular hydrogen bond could play in the ligand receptor potency.

Previously described[37] ligands, (2-benzenesulfonyl-5-piperazin-1-yl-phenyl)-ethyl-amine (**4**) and [5-[1,4]diazepan-1-yl-2-(3-fluoro-benzenesulfonyl)-phenyl]-methyl-amine (**5**), with high affinity toward 5-HT_6R (Figure 5) similar to **3**, also contain characteristic to the PhM 2 hydrogen bond donor–acceptor pair, HBD (round-cornered square) and HBA (square), as well as a positively ionizable group (oval) adjacent to the HYD_1 group (circle).

Taken together, this data indicates that the presence of the PI group does not seem to significantly improve the potency and its essentiality for the receptor–ligand interaction seems questionable.

In this work, we set out to investigate in more detail the question if addition of the positively ionizable group to the conformationally restricted ligands could further improve their affinity to the receptor. We have synthesized and evaluated a new series of the 5-HT_6R ligands, substituted 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines. This set of molecules structurally could be illustrated by a pharmacophore model PhM 3, which combines features characteristic of both the PhM 1 and PhM 2 models (Figure 6).

Using this conceptual framework model, we have synthesized a series of novel compounds **6**–**32** (Figure 7). The potencies of the compounds were assessed in a cell-based assay by their ability to block serotonin-induced stimulation of adenylate cyclase in HEK-293 cells stably expressing human recombinant 5-HT_6 receptor.



PhM 3

Figure 6. Conceptual pharmacophore model, PhM 3, which illustrates known and new potent 5-HT_6R antagonists. The HYD_1, HYD2, HBA, PI, and HBD are as defined in the captions to Figures 2 and 4.

CHEMISTRY

3-(Arylsulfonyl)-N^2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-2,7-diamines **6** and **7** were obtained with a yield of 22–35% by interaction of 4-(arylsulfonyl)-N^3-methyl-1*H*-pyrazole-3,5-diamines **33** and **34** with 3-aminocrotononitrile **35** in acetic acid at elevated temperature (Scheme 1).

Similar conditions were used for the synthesis of 3-(arylsulfonyl)-N^2-methylpyrazolo[1,5-*a*]pyrimidin-2-amines **23** and **36**–**45**. The compounds were synthesized using, as starting reagents, both the 1*H*-pyrazole-3,5-diamines **33**, **34**, and **46** and *β*-dicarbonyl compounds **47**–**53** (Scheme 2).

With asymmetric *β*-diketone **50**, we have predominantly obtained N^2,5-dimethyl-3-(arylsulfonyl)pyrazolo[1,5-*a*]-pyrimidin-2-amine **41**. However, with 2-(2,4-dioxopentyl)-isoindole-1,3-dione **49**, we observed formation of a mixture of the isomeric products **39** and **40** with the ratio of 87:13. The mixture was separated by fractional crystallization and then transformed by a hydrazinolysis reaction into 7-(aminomethyl)-*N*,5-dimethyl- (**17**) and 5-(aminomethyl)-*N*,7-dimethyl-3-(phenylsulfonyl)pyrazolo[1,5-*a*]pyrimidin-2-amine (**21**).

3-(Arylsulfonyl)-5-methyl-2-(methylamino)pyrazolo[1,5-*a*]-pyrimidin-7-ols **36**–**38** were converted to corresponding 7-chloro derivatives **54**–**56** by treatment with $POCl_3$. Subsequent reaction in dioxane or in dioxane–methanol with either ammonia or primary or secondary amines led to desired compounds **8**–**16** with 58–86% yield (Scheme 3).

N-(5,7-Dimethyl-2-methylamino-3-(phenylsulfonyl)-pyrazolo[1,5-*a*]pyrimidin-6-yl)-acetamide **23** was converted into the compound **22** by alkaline hydrolysis, which then was selectively methylated by a reductive amination to yield compound **24** (Scheme 4).

7-(2-Aminoethyl)-*N*,5-dimethyl-3-(phenylsulfonyl)- (**19**), 6-(aminomethyl)-3-(arylsulfonyl)-*N*,5,7-trimethyl- (**25**–**27**), and

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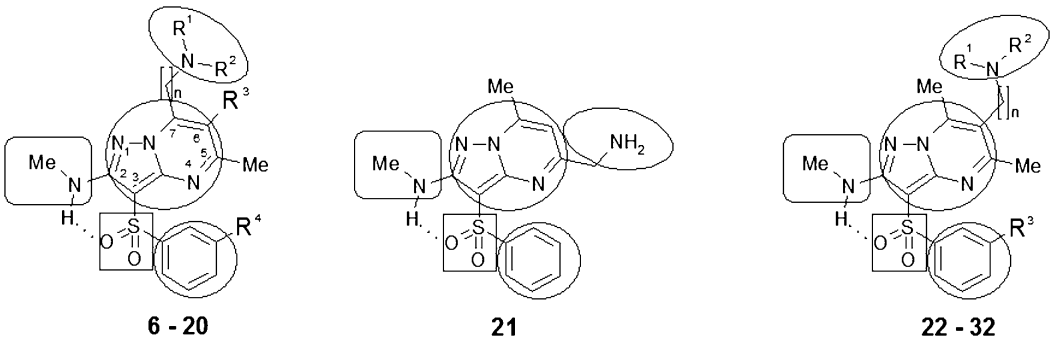
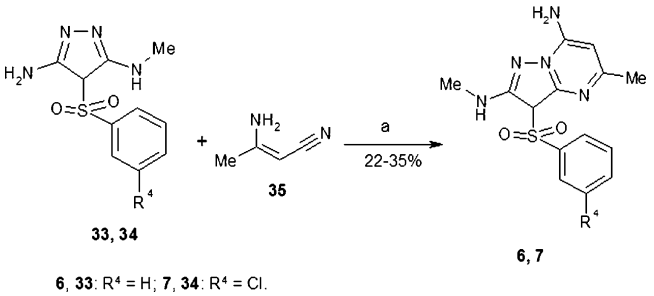


Figure 7. A series of highly potent 5-HT$_6$R antagonists synthesized and tested in this work. Outline shapes around the characteristic groups are as defined in captions to Figures 2 and 4.

Scheme 1. Synthesis of 3-(Arylsulfonyl)-*N*2,5-dimethylpyrazolo-[1,5-*a*]pyrimidin-2,7-diamines 6 and 7 [a]



6, **33**: R^4 = H; **7**, **34**: R^4 = Cl.

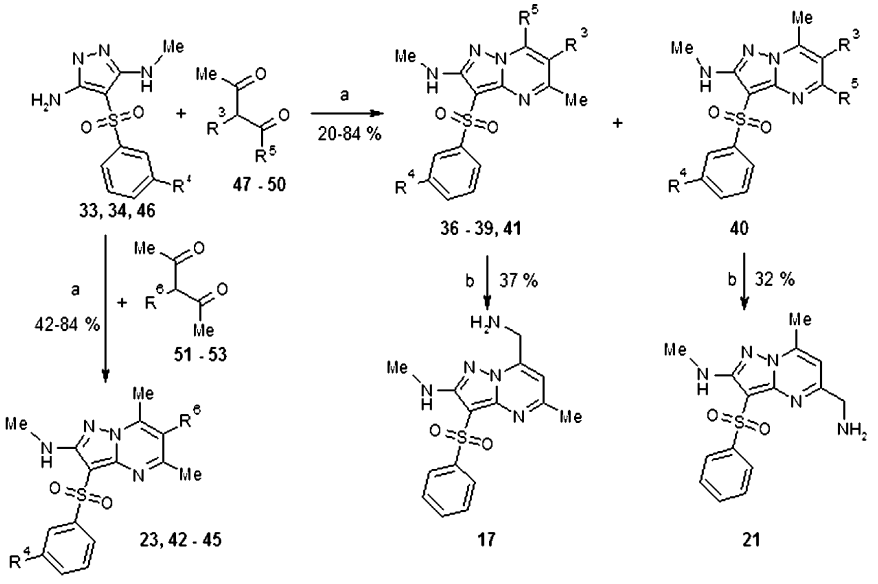
[a]Reagents and conditions: (a) AcOH, 100 °C, 15 h.

6-(2-aminoethyl)-3-(phenylsulfonyl)-*N*,5,7-trimethylpyrazolo-[1,5-*a*]pyrimidin-2-amine (**31**) were synthesized in five steps (Scheme 5) from the corresponding esters **41**−**45**. The compounds **41**−**45** were converted into acids **57**−**61** by alkaline hydrolysis, which were then transformed by successive treatment with ethyl chloroformate and sodium azide into acylazides **62**−**66** and further, by heating, to isocyanates **67**−**71**. The latter were hydrolyzed to the desired ligands **19**, **25**−**27**, and **31**. The ligands **20**, **28**−**30**, and **32** were synthesized by reductive amination of **19**, **25**−**27**, and **31** in the presence of formalin and sodium triacetoxyborohydride.

Similarly, ligand **18** was prepared by reductive amination of amine **17** in the presence of formalin with NaBH(OAc)$_3$ in DCM (Scheme 6).

The structures of synthesized compounds were confirmed with LC-MS and NMR spectra. MS and UV detectors indicated a purity of 98% and higher. The NMR spectra of the compounds were in a good agreement with their expected structures and purity.

Scheme 2. Synthesis of Substituted 3-Arylsulfonyl-pyrazolo[1,5-*a*]pyrimidine-2-amines 17, 21, 23, 36−45 [a]



23: R^4 = H, R^6 = NHCOMe.
36: R^3 = R^4 = H, R^5 = OH.
37: R^3 = Cl, R^4 = H, R^5 = OH.
38: R^3 = R^4 = Cl, R^5 = OH.
39, **40**: R^3 = R^4 = H, R^5 = (1,3-dioxo-isoindol-2-yl)methyl
41: R^3 = R^4 = H, R^5 = $CH_2CH_2CO_2Et$.
42: R^4 = H, R^6 = CH_2CO_2Et.
43: R^4 = F, R^6 = CH_2CO_2Et.
44: R^4 = Cl, R^6 = CH_2CO_2Et.
45: R^4 = H, R^6 = $CH_2CH_2CO_2Et$.
46: R^4 = F.
47: R^3 = H, R^5 = OEt.
48: R^3 = Cl, R^5 = OEt.
49: R^3 = H, R^5 = (1,3-dioxo-isoindol-2-yl)methyl
50: R^3 = H, R^5 = $CH_2CH_2CO_2Et$.
51: R^6 = NHCOMe
52: R^6 = CH_2CO_2Et.
53: R^6 = $CH_2CH_2CO_2Et$.

[a]Reagents and conditions: (a) AcOH, 80−100 °C, 12 h; (b) $N_2H_4 \cdot H_2O$, EtOH, reflux, 4 h.

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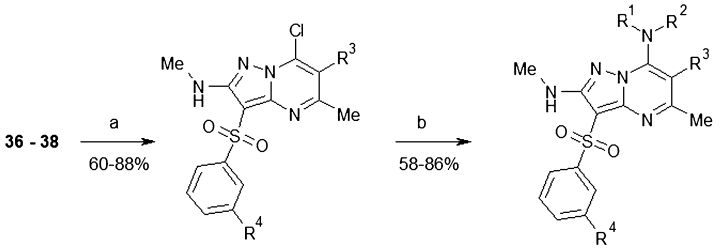
Scheme 3. Synthesis of *N*7-Substituted 3-(Arylsulfonyl)-*N*2,5-dimethylpyrazolo[1,5-*a*]pyrimidine-2,7-diamines 8−16[a]

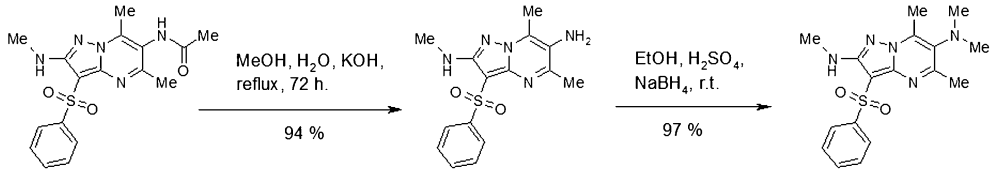


54: $R^3 = R^4 = H$
55: $R^3 = Cl$, $R^4 = H$
56: $R^3 = R^4 = Cl$.

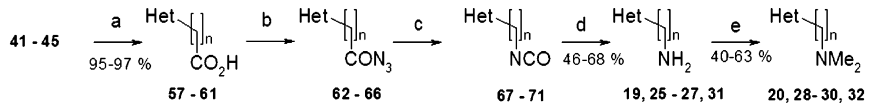
8: $R^1 = R^2 = H$, $R^3 = Cl$, $R^4 = H$
9: $R^1 = R^2 = H$, $R^3 = R^4 = Cl$
10: $R^1 = H$, $R^2 = Me$, $R^3 = R^4 = H$
11: $R^1 = R^2 = Me$, $R^3 = R^4 = H$
12: $R^1 = H$, $R^2 = CH_2CH_2NMe_2$, $R^3 = R^4 = H$
13: $R^1 = Me$, $R^2 = CH_2CH_2NMe^2$, $R^3 = R^4 = H$
14: $R^1 = H$, $R^2 = CH_2CH_2CH_2NMe_2$, $R^3 = R^4 = H$
15: R^1 - $R^2 = CH_2CH_2OCH_2CH_2$, $R^3 = R^4 = H$
16: R^1 - $R^2 = CH_2CH_2N(Me)CH_2CH_2$, $R^3 = R^4 = H$

[a]Reagents and conditions: (a) $POCl_3$, sulfolane, 70 °C, 3 h; (b) one of the following: ammonia, primary amine, secondary amine, dioxane or dioxane−MeOH, 70 °C, 3 h.

Scheme 4. Synthesis of 3-Benzenesulfonyl-5,7,N^2-trimethyl-pyrazolo[1,5-*a*]pyrimidine-2,6-diamine 22 and 3-Benzenesulfonyl-5,7,N^2,N^6,N^6-pentamethyl-pyrazolo[1,5-*a*]pyrimidine-2,6-diamine 24



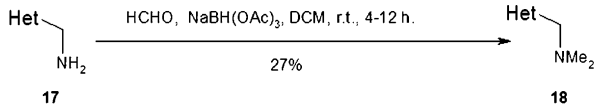
Scheme 5. Synthesis of target compounds 19, 20, 25−32 [a]



71, 76, 81: n = 2, Het = 5-methyl-2-(methylamino)-3-(phenylsulfonyl)pyrazolo[1,5-*a*]pyrimidin-7-yl.
72, 77, 82: n = 1, Het = 5,7-dimethyl-2-(methylamino)-3-(phenylsulfonyl)pyrazolo[1,5-*a*]pyrimidin-6-yl.
73, 78, 83: n = 1, Het = 5,7-dimethyl-2-(methylamino)-3-(3-fluorophenylsulfonyl)pyrazolo[1,5-*a*]pyrimidin-6-yl.
74, 79, 84: n = 1, Het = 5,7-dimethyl-2-(methylamino)-3-(3-chlorophenylsulfonyl)pyrazolo[1,5-*a*]pyrimidin-6-yl.
75, 80, 85: n = 2, Het = 5,7-dimethyl-2-(methylamino)-3-(phenylsulfonyl)pyrazolo[1,5-*a*]pyrimidin-6-yl.

[a]Reagents and conditions: (a) KOH, H_2O, EtOH, 80 °C, 3 h; (b) Et_3N, acetone, $ClCO_2Et$, 0 °C, 0.5 h, then NaN_3, H_2O, 0 °C, 1 h; (c) DCM, dioxane reflux, 1 h, then (d) 20% HCl, 70−80 °C, 0.5 h; (e) DCM or DCE, HCHO, $NaBH(OAc)_3$, rt, 15 h.

Scheme 6. Synthesis of Target Compound 18



17, 18: Het = 5-methyl-2-methylamino-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-7-yl.

RESULTS AND DISCUSSION

Potencies of the compounds **6**−**32** to block serotonin-induced responses were investigated in a cell-based assay using HEK 293 cells stably expressing recombinant human 5-HT_6 receptor (5-HT_6R-HEK) and are shown in Table 1. The K_i were calculated from IC_{50} values using the Cheng−Prusoff[38] equation, and pA_2 values were calculated from Schild transformation.[39] The K_i values were transformed into pK_i (negative logarithm of K_i) to directly reflect the compound antagonist potencies.

Using Schild experimental set up (Figure 8), we show that the compound-mediated inhibition of the receptor activity is competitive (a parallel rightword shift of the 5-HT-induced activation curves in the presence of increasing antagonist concentrations, with Schild coefficient close to unity). Figure 8A illustrates such an experiment for compound **6**. pA_2 values were determined for compounds **6**, **19**, and **22** as an intersection of the *X*-axis at a $\mathrm{Log}(A'/A - 1)$ value equal to zero (Figure 8B).[39] As one can see (Table 1), the pA_2 from Schild analysis and pK_i values from Cheng−Prusoff analysis were close to each other, which confirms usability of a simpler Cheng−Prussoff approach for assessment of the compound potencies.

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Table 1. SAR Analysis of the 5-HT$_6$R Antagonist Potencies (pK_i and pA_2) of Substituted 5,N^2-Dimethyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine-2-amines 6−32

ligand	R^1	R^2	R^3	R^4	*n*	pK_i[a]	pA_2[b]	ALogP98[c]
2[d]			H	H		9.58		2.38
6	H	H	H	H	0	9.52	9.3	1.89
7	H	H	H	Cl	0	9.66		2.55
8	H	H	Cl	H	0	9.85		2.55
9	H	H	Cl	Cl	0	7.73		3.22
10	H	Me	H	H	0	9.24		2.44
11	Me	Me	H	H	0	9.46		2.80
12	H	$CH_2CH_2NMe_2$	H	H	0	7.16		2.58
13	Me	$CH_2CH_2NMe_2$	H	H	0	7.65		2.94
14	H	$CH_2CH_2CH_2NMe_2$	H	H	0	6.95		2.65
15	$CH_2CH_2OCH_2CH_2$		H	H	0	7.78		2.49
16	$CH_2CH_2N(Me)CH_2CH_2$		H	H	0	8.36		2.76
17	H	H	H	H	1	8.73		1.42
18	Me	Me	H	H	1	8.74		2.39
19	H	H	H	H	2	8.51	8.4	1.67
20	Me	Me	H	H	2	7.47		2.63
21						9.01		1.42
22	H	H	H		0	9.28	9.4	1.63
23	H	MeCO	H		0	8.33		1.50
24	Me	Me	H		0	9.68		2.54
25	H	H	H		1	9.19		1.49
26	H	H	F		1	8.85		1.69
27	H	H	Cl		1	9.26		2.15
28	Me	Me	H		1	9.15		2.45
29	Me	Me	F		1	9.05		2.66
30	Me	Me	Cl		1	9.38		3.12
31	H	H	H		2	8.86		1.81
32	Me	Me	H		2	8.68		2.77

[a] pK_i values to block serotonin-induced cyclic adenosine monophosphate (cAMP) production in 5-HT$_6$R-HEK were calculated in accordance with the Cheng−Prusoff equation.[38] IC_{50} values (and, hence pK_i) are averages of at least two independent experiments performed in duplicates with less than 2-fold difference in IC_{50} between the two experiments. [b] pA_2 values were calculated from Schild type experiments[39] as shown in Figure 8. [c] ALogP98 was calculated using DS Viewer Pro 6 (Accelrys Software Inc., CA). [d] The data is from ref 34.

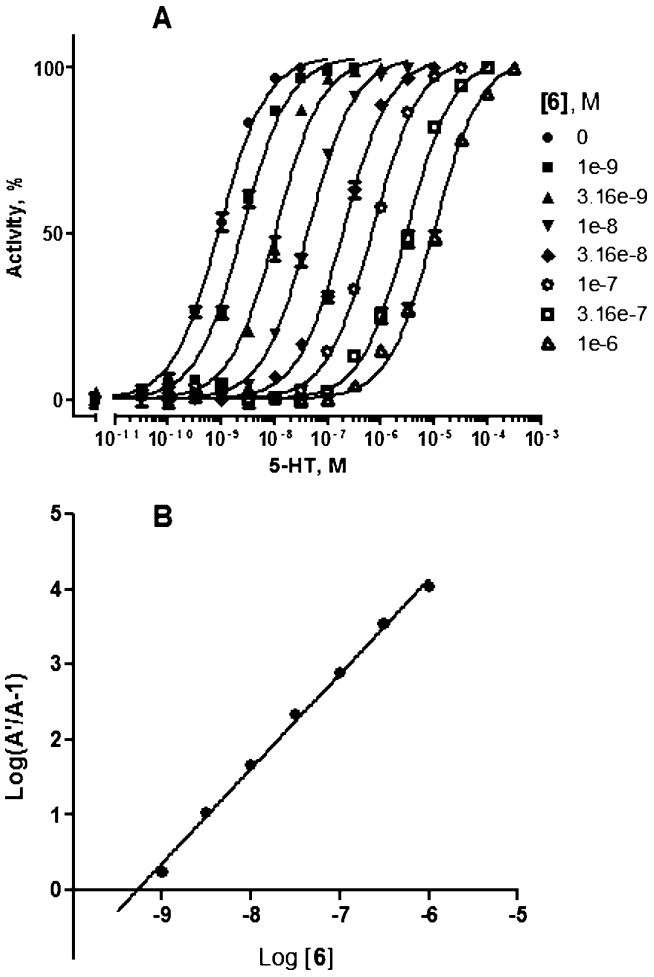

Figure 8. (A) Concentration-dependent serotonin-induced activation of cAMP production in HEK-5-HT$_6$R cells in the presence of different concentrations of **6**. (B) Schild plot of the panel (A) data expressed in units of Log(A'/A − 1) as a function of the Log concentration of the blocker,[39] where A and A' are, respectively, serotonin-induced EC_{50} values measured without and in the presence of specified antagonist concentration. Each concentration point represents a mean of triplicates ± SE.

In the series of 5,N^2-dimethyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]-pyrimidine-2,7-diamines (**6**−**16**), substituent groups greatly affect the ability of the compounds to antagonize 5-HT$_6$R. Thus, depending on the substitute, the K_i values vary 3 orders of magnitude from K_i = 0.14 nM (pK_i = 9.85) (**8**) to K_i = 112 nM (pK_i = 6.95) (**14**) (Table 1).

Addition of a positively ionizable group in position 7, 7-amino (**6**), 7-methylamino (**10**), and 7-dimethylamino (**11**) produced a negligible, if any, effect on the antagonistic potency (pK_i, correspondingly, are 9.52, 9.24, 9.46) as compared to the prototype compound **2** (pK_i = 9.58). The secondary amine in the position 7 renders slightly lower potency than corresponding tertiary amine (compare pK_i values of **10** vs **11** and **12** vs **13** in Table 1).

Bulkier substituents of the 7-amino group, on the other hand, cause substantial reduction in the 5-HT$_6$R antagonistic potencies. Thus, 7-(2-dimethylaminoethyl)-amino derivative **12** and 7-(3-dimethylaminopropyl)-amino derivative **14** are, respectively, 120-fold and 190-fold less potent antagonists than 7-methylamino derivative **10**.

Substitution of the 7-amino group in **6** with heterocyclic derivatives 7-morpholyn-4-yl (**15**) or 7-(4-methylpiperidine-1-yl) (**16**) leads to a substantially reduced antagonistic potency (Table 1).

Introduction of a chlorine atom either in position 6 of the pyrazolopyrimidine or in position 3 of the phenyl ring leads to a small increase in the potency (pK_i = 9.66 (**7**) and pK_i = 9.85 (**8**)) relative to its unsubstituted analogue **6**, 5,N^2-dimethyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine-2,7-diamine (pK_i = 9.52). Unexpectedly, introduction of the chlorine atoms into both positions (**9**) led to 60-fold reduction of the antagonistic potency relative to its unsubstituted analogue **6**.

Moving the 7-amino group away from the pyrazolopyrimidine core produced compounds with substantially diminished 5-HT$_6$R antagonistic potencies. Thus, transition from 3-benzenesulfonyl-5,N^2-dimethyl-pyrazolo[1,5-*a*]pyrimidine-2,7-diamine (**6**) to (7-aminomethyl-3-benzenesulfonyl-5-methyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine (**17**) or to [7-(2-amino-ethyl)-3-benzenesulfonyl-5-methyl-pyrazolo-[1,5-*a*]pyrimidin-2-yl]-methyl-amine (**19**) led, respectively, to a 6.2-fold and 10.3-fold increase in K_i values (decrease in pK_i,

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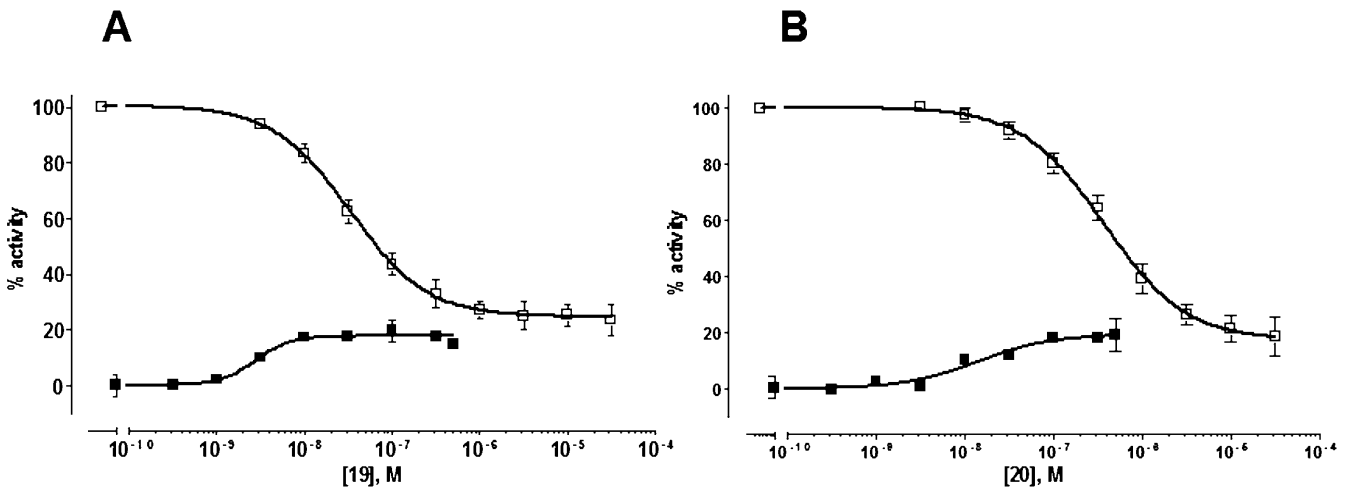

Figure 9. Concentration-dependent effect of **19** (A) and **20** (B) on serotonin-induced (open squares) and basal (black squares) levels of cAMP in the 5-HT_6R-HEK cells. Mean values (± SD, triplicates) of a typical experiment are shown.

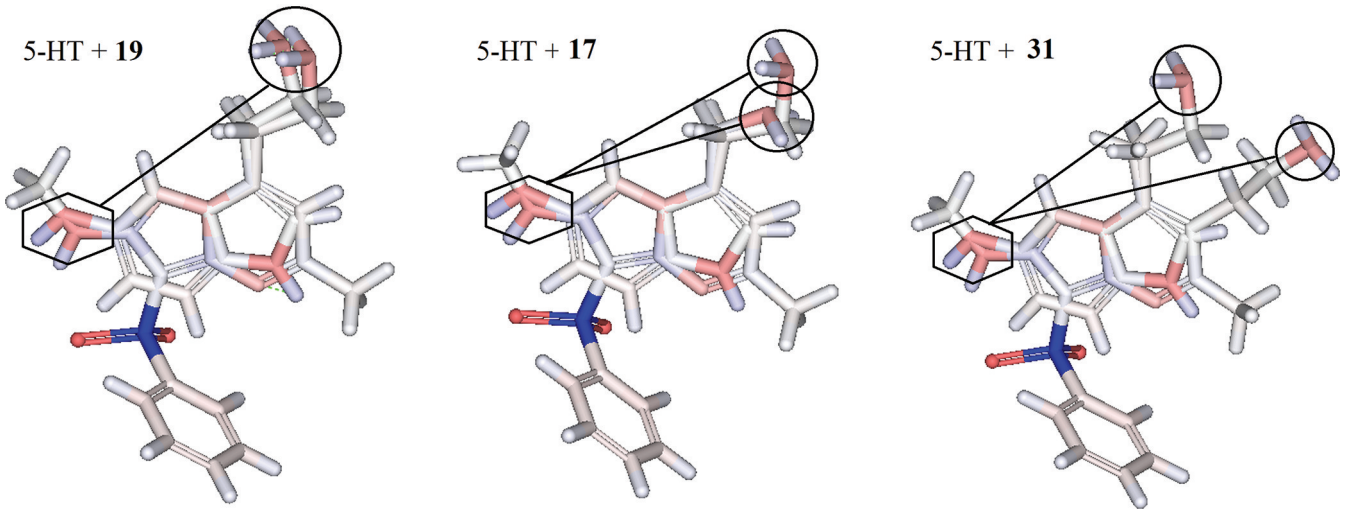

Figure 10. Topological overlay of 5-HT with **19** (A), **17** (B), and **31** (C). The molecules were aligned by tethering their cyclic systems together after energy minimization performed with DS ViewerPro 6.0. The atoms with partial negative and positive charges are shown, respectively, in red and blue.

Table 1). A similar trend was also observed for the 7-dimethylaminomethyl group in **11** being moved away from the pyrazolopyrimidin core (compare **11** with **18** and **20**).

Interestingly, compounds **19** and **20** (panels A and B of Figure 9, respectively), which have the amino or dimethyl amino group in the 7 position spaced by two carbon atoms from the core, exhibited a partial blockage of the serotonin-induced cyclic adenosine monophosphate (cAMP) production in the cells. The partial inhibitory activity of **19** and **20** indicated a possibility that those compounds could be partial agonists. Indeed, when the cells were treated with **19** (Figure 9A) or **20** (Figure 9B) without serotonin present, we observed partial agonism. The maximal compound-stimulated cAMP level was fairly close to that of the maximal compound-inhibited serotonin-stimulated level, which is in line with the mechanism characteristic for partial agonists.

Contrary to **19** and **20**, the compounds **17** and **18** with the amino- or dimethyl amino- group set apart one atom from the core, and compounds **6** and **11** with the amino- or dimethyl amino- group directly attached to the core, showed full antagonistic activity. Compounds **31** and **32** with the amine- or dimethyl amine- group also set apart two atoms from the core but located in 6- position, showed full antagonistic activity (data not shown). One could rationalize the appearance of the partial agonism in **19** and **20** based on a similarity of their molecular topology with that of 5-HT. In Figure 10, the overlay of 5-HT with **19**, **17**, or **31** is shown. Only **19** has a good topological correspondence of its 7-(2-dimethylamino-ethyl) group with that of 5-HT.

Relative positioning of the amine group on the pyrimidine ring did not substantially affect the antagonistic potency. Thus (5-aminomethyl-3-benzenesulfonyl-7-methyl-pyrazolo[1,5-*a*]-pyrimidin-2-yl)-methyl-amine (**21**) and (6-aminomethyl-3-benzenesulfonyl-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine (**25**) were, respectively, only 1.9-fold and 2.9-fold more potent than their analogue 7-aminomethyl-3-benzenesulfonyl-5- methyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine (**17**) (Table 1). In the group of 6-substituted derivatives, like with the 7-substituted ones, lengthening of the amino group distance from the pyrazolo[1,5-*a*]pyrimidine core led to

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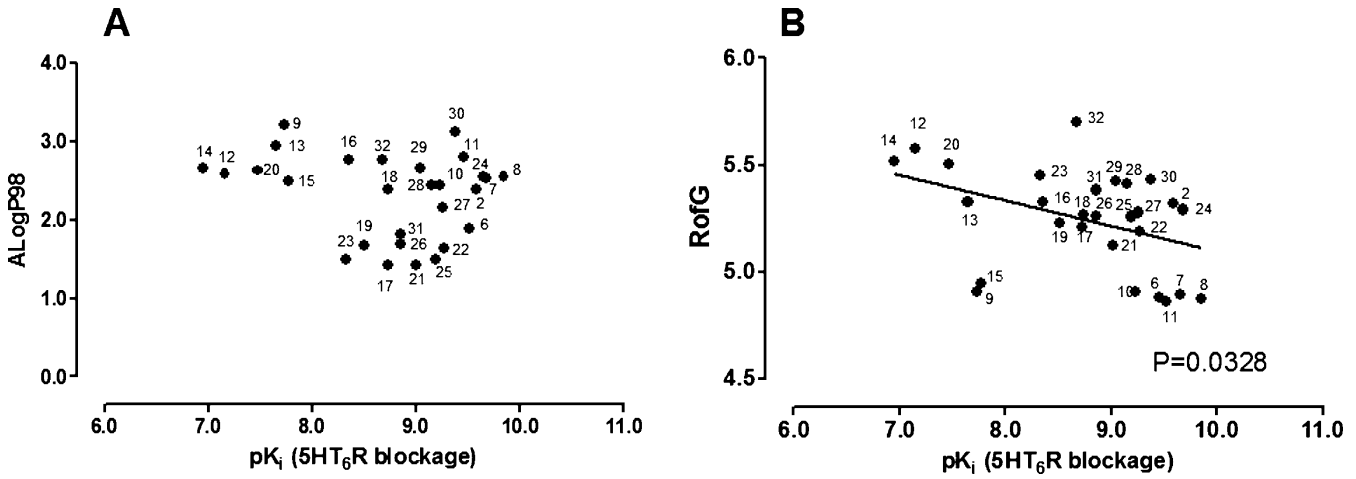

Figure 11. Correlation between compound potencies (pK_i) to block 5-HT$_6$R and their calculated octanol/water partition coefficients, ALogP98 (A) and radii of gyration, RofG (B).

a decrease in the compound potencies (**22** = **25** > **31** for 6-aminomethyl derivatives and **24** ≥ **28** > **32** for 6-dimethylaminomethyl derivatives). Acetylation of the 6-amino group in **23** substantially reduced its potency relative to **22** (Table 1).

In the series of 6-aminomethyl and 6-dimethylaminomethyl derivatives **25** and **28**, substitution with 3-(3-chlorophenylsulfonylchlorphenylsulfonyl) in **27** and **30**, respectively, led to a small increase in pK_i values. When a more negatively charged fluorine atom was introduced, compounds **26** and **29**, a small decrease in the potency was observed.

As we reported earlier for the pyrazolopyrimidines,[33] the newly synthesized compounds in this series showed no correlation between the potencies and ALogP98 values (Table 1) (Figure 11A). At the same time, similar to 3-arylsulfonylpyrazolo[1,5-*a*]pyrimidines,[34] a statistically significant trend (P = 0.033) of increase in potency with decrease in the radius of gyration was observed (Figure 11B). At this moment, it is still to be defined why smaller (more compact) molecules exhibit higher potency to block the receptor. One of the possibilities is the assumption of a rather narrow pocket in the receptor that accommodates better fit, and hence tighter binding, of the smaller ligands.

Independence of the compound potencies on the ALogP98 opens a possibility to design highly potent 5-HT$_6$R antagonists with different levels of lipophilicity. For example compounds **6**, **7**, **8**, **11**, **22**, **24**, **25**, and **27**−**30** all have potencies in a submicromolar range, while their lipophilicities, ALogP98, varied from low 1.89 to high 3.12, which could substantially affect pharmacokinetic behavior.

We have also tested selectivity/specificity of a few highly potent 5-HT$_6$R ligands with the PI moiety located in either fifth (**21**), sixth, (**22** and **24**), or seventh (**6**) position. A panel of 32 GPCR targets belonging to six classes (adenosinergic, adrenergic, dopaminergic, histaminergic, muscarinic, and serotoninergic) was used for the assessment. Also, a potassium hERG channel has been included in the panel for the assessment of potential liability of the compounds. The data are presented in Figure 12, where values of the equilibrium displacement of corresponding radiolabeled ligands from related receptors with the compounds **6**, **21**, **22**, and **24** are shown.

As one can see, at a concentration of 1 μM, **6** (PI in the position 7) exhibits quite a clean profile with 100% inhibition of the 1.5 nM [^{3}H]lysergic acid diethylamide binding to the 5-HT$_6$R. Besides the 5-HT$_6$R, out of 32 receptors tested, only one other receptor, 5-HT$_{2B}$R, weakly interacts with **6** (approximately 50% inhibition of the 1.2 nM [^{3}H]lysergic acid diethylamide binding at this concentration (IC_{50} ∼ 1 μM). Shift of the amino group into position 5 (**21**) or 6 (**22**) seems to even improve the selectivity by reducing the ability of these compounds to interact with the 5-HT$_{2B}$R. Interestingly, substitution of the amino group in position 6 with the dimethyl amino group (**24**) leads to a restoration of the weak interaction with the 5-HT$_{2B}$R.

Neither compound of the series showed potential liability associated with inhibition of hERG channel (Figure 12).

CONCLUSION

SAR studies of new 5-HT$_6$R ligands **6**−**32** described by the pharmacophore model **PhM3** (Figure 6) showed that all of the tested compounds have high antagonistic potencies ranging from 141 pM to 112 nM. The compounds from this conformationally restricted series seem to have high selectivity toward 5-HT$_6$ receptors. The potency of 7-aminosubstituted compounds did not significantly depend on either basicity or the hydrogen bond donor capability of the nitrogen atom (similar pK_i for primary, secondary, and tertiary amines in, correspondingly, **6**, **10**, and **11**) nor did it depend on the presence of the basic nitrogen atom at all (compare pK_i of **6**, **10**, and **11** with that of **2** (Table 1), which does not possess the corresponding amino group and can be described with the PhM2 model, Figure 4). The major negative effect on the potency is caused by introduction of a bulky substituent into the 7-amine group. In agreement with our earlier published work,[32,33,35] this data indicates that the positively ionizable group in positions 5-, 6-, or 7- does not further improve the 5-HT$_6$R potency relative to the parent compounds lacking such a group as long as the core contains 2-amino substitution capable of forming intra- and/or intermolecular hydrogen bonding. SAR analysis of the tryptamine derivatives as 5HT$_6$R ligands led Pullagurla et al. to the same conclusion of a nonessential contribution of the positively ionizable amino group to the binding energy.[40] While the ligands of this series have a very clean selectivity/specificity profile, presence of the well tolerated

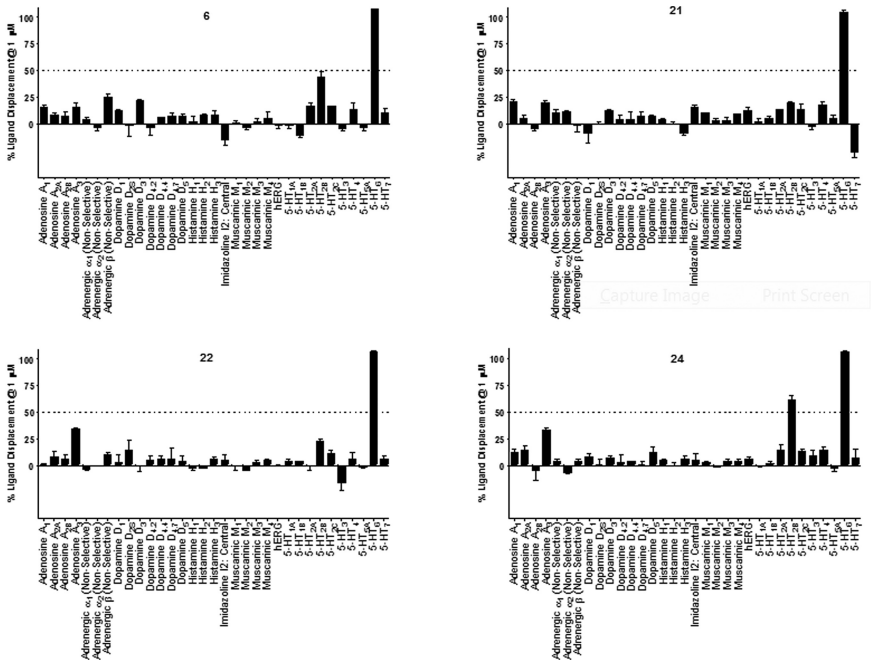

Figure 12. Specificity profiles of 5-HT$_6$R antagonists **6**, **21**, **22**, and **24** were measured on a panel of 33 receptors by competitive displacement of corresponding radiolabeled probes specific to each of the receptors. The compounds were tested at a concentration of 1 μM in duplicates and average values of radioligand displacement ± SD are plotted.

relatively small PI group at the 5-, 6-, or 7-postion of the pyrazolopyrimidine system does not seem to substantially affect the specificity and could be used to modulate other physiochemical parameters, which play an important role in defining the ligand ADME characteristics. Thus, the PhM3 model can be useful for the designing of 5-HT$_6$R antagonists with improved pharmacokinetic parameters.

EXPERIMENTAL SECTION

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

^{1}H NMR and ^{13}C NMR spectra of the investigated compounds were recorded in solutions of DMSO-d_6 or $CDCl_3$, respectively, using a Bruker DPX-400 spectrometer (400 MHz, 27 °C) for collecting ^{1}H NMR spectra and Bruker DPX-300 spectrometer (300 75 MHz, 27 °C) to collect ^{13}C NMR and two-dimensional spectra.

LC-MS spectra were obtained using Shimadzu HPLC liquid chromatograph, equipped with 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_{max} 220 and 254 nm).

Purity of the synthesized compounds was determined using the LC-MS method with UV detector at the absorption wavelength of 254 nm. Purity of the compounds was ≥95%.

Synthesis of Tested Compounds. N^2,5-Dimethyl-3-(phenylsulfonyl)pyrazolo[1,5-*a*]pyrimidine-2,7-diamines **6** and **7** (Scheme 1).

To a suspension of N^5-methyl-4-(phenylsulfonyl)-1*H*-pyrazole-3,5-diamines **33** and **34** (3.17 mmol each) in acetic acid (3 mL) and 3-aminocrotononitrile, **35** (520 mg, 6.34 mmol) was added. The reaction mixtures were heated at 100 °C for 15 h. The precipitates of **6** and **7** formed after cooling, were filtered, washed with a minimum amount of a mixture of acetic acid and ether (1:1), and dried.

6: N^2,5-Dimethyl-3-(phenylsulfonyl)pyrazolo[1,5-*a*]pyrimidine-2,7-diamine (yield: 22%). ^{1}H NMR (DMSO-d_6) δ 8.00 (m, 2H), 7.55 (m, 5H), 6.16 (q, J = 4.8 Hz, 1H), 6.02 (s, 1H), 2.91 (d, J = 4.8 Hz, 3H), 2.30 (s, 3H). HRMS calculated for $C_{14}H_{15}N_5O_2S$ (M + H) 318.102470, found 318.1021. ESI-MS calculated for $C_{14}H_{15}N_5O_2S$ (M + H) 318, found m/z 318. LC-MS (UV-254), purity: 98%.

7: N^2,5-Dimethyl-3-(3-chlorophenylsulfonyl)pyrazolo[1,5-*a*]-pyrimidine-2,7-diamine (yield: 35%). ^{1}H NMR (DMSO-d_6) δ 8.14 (m, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.96 (brs, 2H), 7.68 (d, J = 7.2 Hz, 1H), 7.60 (t, J = 8.0 Hz, 1H), 6,27 (brm, 1H), 6.10 (s, 1H), 2.91 (s, 3H), 2.37 (s, 3H). MS-ESI calculated for $C_{14}H_{14}ClN_5O_2S$ (M + H) 352, found m/z 352. LC-MS (UV-254), purity: 98%.

***N*^{2},5-Dimethyl-3-(phenylsulfonyl)-pyrazolo[1,5-*a*]-pyrimidine-2,7-diamines 8 and 9 (Scheme 3).** Suspensions of 7-chloro-*N*,5-dimethyl-3-(phenylsulfonyl)pyrazolo[1,5-*a*]pyrimidin-2-amines **55** and **56** (0.54 mmol each) in 2 mL of THF were burbled with a flow of ammonia. The corresponding mixtures were stirred for 12 h at room temperature. The formed precipitates of **8** and **9** were filtered, washed with THF, hot acetone, and dried.

8: 6-Chloro-N^2,5-dimethyl-3-(phenylsulfonyl)pyrazolo[1,5-*a*]-pyrimidine-2,7-diamine (yield: 66%). ^{1}H NMR (DMSO-d_6) δ 7.99 (d, J = 6.8 Hz, 2H), 7.91 (s, 2H), 7.56 (m, 3H), 6.22 (q, J = 4.8 Hz, 1H), 2.93 (d, J = 4.8 Hz, 3H), 2.43 (s, 3H). MS-ESI calculated for $C_{14}H_{14}ClN_5O_2S$ (M + H) 352, found m/z 352. LC-MS (UV-254), purity: 99%.

9: 6-Chloro-3-(3-chlorophenylsulfonyl)-N^2,5-dimethylpyrazolo-[1,5-*a*]pyrimidine-2,7-diamine (yield: 64%). ^{1}H NMR (DMSO-d_6) δ 8.10 (s, 1H), 7.90 (d, J = 7.6 Hz, 1H), 7.60 (d, J = 7.2 Hz, 1H), 7.53 (t, J = 7.6 Hz, 1H), 7.28 (s, 2H), 5.68 (m, 1H), 2.81 (d, J = 4.8 Hz, 3H), 2.29 (s, 3H). MS-ESI calculated for $C_{14}H_{13}Cl_2N_5O_2S$ (M + H) 487, found m/z 487. LC-MS (UV-254), purity: 98%.

***N*^{2},5-Dimethyl-3-(phenylsulfonyl)-pyrazolo[1,5-*a*]-pyrimidine-2,7-diamines 10–16 (Scheme 3).** Compounds **54**–**56** (1 mmol each) were stirred separately in a mixture with dioxane (1 mL) and were treated with primary or secondary amines (5 mmol) (or solutions of methylamine or dimethylamine in MeOH). The mixtures were stirred at 70 °C for 1 h (reaction monitored by LC-MS) and

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dispersed into ice/water (6 mL). Resulting mixtures were treated with 0.5 mL of 5N aq NaOH and stirred for 0.5 h. Formed precipitates were separated by centrifugation, twice washed with water, *i*-PrOH (twice), and dried. To obtain HCl salts, the products **10**−**16** were dissolved in $CHCl_3$ and treated with an excess of 6N solution of HCl (as AcCl in EtOH) and then diluted with EtOAc. The formed precipitates were separated by centrifugation, washed with EtOAc (twice), acetone (twice), and dried. Compounds **10**−**16** were thus obtained as hydrochlorides.

10·HCl: 3-Phenylsulfonyl-5,N^2,N^7-trimethyl-pyrazolo[1,5-*a*]-pyrimidine-2,7-diamine hydrochloride (yield: 76%). ^{1}H NMR (DMSO-d_6) δ 8.00 (d, J = 6.4 Hz, 2H), 7.55 (m, 4H), 6.15 (m, 1H), 6.13 (s, 1H), 2.90 (m, 6H), 2.36 (s, 3H). HRMS calculated for $C_{15}H_{17}N_5O_2S$ (M + H) 332.118120, found 332.1186. MS-ESI calculated for $C_{15}H_{17}N5O_2S$ (M + H) 332, found m/z 332. LC-MS (UV-254), purity: 98%.

11·HCl: 3-Phenylsulfonyl-5,N^2,N^7,N^7-tetramethyl-pyrazolo[1,5-*a*]-pyrimidine-2,7-diamine hydrochloride (yield: 82%). ^{1}H NMR (DMSO-d_6) δ 8.02 (d, J = 68 Hz, 2H), 7.55 (m, 3H), 6.22 (s, 1H), 6.16 (m, 1H), 3.24 (s, 6H), 2.88 (d, J = 5.2 Hz, 3H), 2.37 (s, 3H). HRMS calculated for $C_{16}H_{19}N_5O_2S$ (M + H) 346.133770, found 346.1326. MS-ESI calculated for $C_{16}H_{19}N_5O_2S$ (M + H) 346, found m/z 346. LC-MS (UV-254), purity: 98%.

12·HCl: N^7-(2-Dimethylamino-ethyl)-5,N^2-dimethyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine-2,7-diamine hydrochloride (yield: 65%). ^{1}H NMR (DMSO-d_6), δ: 10.07 (brs, 1H), 8.02 (d, J = 8.0 Hz, 2H), 7.52−7.62 (m, 3H), 6.95 (s, 1H), 6.72 (brt, J = 5.7 Hz, 1H), 3.69−3.76 (m, 2H), 3.29−3.35 (m, 2H), 2.80 (s, 6H), 2.54 (s, 3H), 2.47 (s, 3H). HRMS calculated for $C_{18}H_{24}N_6O_2S$ (M + H) 389.175969, found 389.1765. MS-ESI calculated for $C_{18}H_{24}N_6O_2S$ (M + H) 389, found m/z 389. LC-MS (UV-254), purity: 98%.

13·HCl: N^7-(2-Dimethylamino-ethyl)-5,N^2,N^7-trimethyl 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine-2,7-diamine hydrochloride (yield: 69%). ^{1}H NMR (DMSO-d_6), δ: 10.46 (brs, 1H), 8.01 (d, J = 8.0 Hz, 2H), 7.52−7.62 (m, 3H), 7.04 (s, 1H), 3.73 (t, J = 6.6 Hz, 2H), 3.36 (t, J = 6.6 Hz, 2H), 3.03 (s, 3H), 2.78 (s, 6H), 2.58 (s, 3H), 2. 48 (s, 3H). HRMS calculated for $C_{19}H_{26}N_6O_2S$ (M + H) 403.191619, found 403.1906. MS-ESI calculated for $C_{19}H_{26}N_6O_2S$ (M + H) 403, found m/z 403. LC-MS (UV-254), purity: 99%.

14·HCl: N^7-(3-Dimethylamino-propyl)-5,N^2-dimethyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine-2,7-diamine hydrochloride (yield: 58%). ^{1}H NMR (DMSO-d_6), δ: 10.52 (s, 1H), 8.29 (s, 1H), 8.06 (d, J = 6.8 Hz, 2H), 7.60 (m, 3H), 6.53 (m, 1H), 6.44−6.17 (brm, 1H), 3.51 (m, 2H), 3.06 (m, 2H), 2.93 (s, 3H), 2.69 (d, J = 4.8 Hz, 5H), 2.45 (s, 3H), 2.01 (m, 2H). HRMS calculated for $C_{19}H_{26}N_6O_2S$ (M + H) 403.191619, found 403.1920. MS-ESI calculated for $C_{19}H_{26}N_6O_2S$ (M + H) 403, found m/z 403. LC-MS (UV-254), purity: 98%.

15·HCl: (5-Methyl-7-morpholin-4-yl-3-phenylsulfonyl-pyrazolo-[1,5-*a*]pyrimidin-2-yl)-methyl-amine hydrochloride (yield: 84%). ^{1}H NMR (DMSO-d_6), δ: 8.00 (d, J = 6.4 Hz, 2H), 7.56 (m, 3H), 6.40 (s, 1H), 6.24 (m, 1H), 3.73 (s, 4H), 3.71 (s, 4H), 2.87 (d, J = 5.2 Hz, 3H), 2.41 (s, 3H). MS-ESI calculated for $C_{18}H_{21}N_5O_3S$ (M + H) 388, found m/z 388. LC-MS (UV-254), purity: 98%.

16·HCl: *N*,5-Dimethyl-7-(4-methylpiperazin-1-yl)-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-amine hydrochloride (yield: 86%). ^{1}H NMR (DMSO-d_6), δ: 11.24 (brs, 1H), 7.99 (d, J = 8.0 Hz, 2H), 7.50−7.60 (m, 3H), 6.54 (s, 1H), 6.24 (brs, 1H), 4.50−4.60 (m, 4H), 3.40−3.50 (m, 4H), 2.87 (s, 3H), 2.75 (d, J = 4.3 Hz, 3H), 2.41 (s, 3H). HRMS calculated for $C_{19}H_{17}N_5O_2S$ (M + H) 380.118120, found 380.1175. MS-ESI calculated for $C_{19}H_{17}N_5O_2S$ (M + H) 380, found m/z 380. LC-MS (UV-254), purity: 98%.

7-Aminomethyl-5-methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]-pyrimidin-2-yl)-methyl-amine (17) and 5-Aminomethyl-7-methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine (21) (Scheme 2). A solution of N^5-methyl-4-phenylsulfonyl-1*H*-pyrazole-3,5-diamine (**33**) (0.60 g, 2.38 mM) and 2-(2,4-dioxopentyl)-isoindol-1,3-dione (**49**) (1.17 g, 4.76 mM) in acetic acid (20 mL) was heated at 80 °C for 3 h. A mixture of isomers 2-(3-phenylsulfonyl-5-methyl-2-methylamino-pyrazolo[1,5-*a*]pyrimidin-7-ylmethyl)-isoindole-1,3-dione (**39**) and 2-(3-phenylsulfonyl-7-methyl-2-methylamino-pyrazolo[1,5-*a*]pyrimidin-5-ylmethyl)-isoindole-1,3-dione (**40**) was obtained with a corresponding ratio of 87:13 (ratio was determined by ^{1}H NMR analysis). After cooling, the formed precipitate was filtered, washed with ethanol and ether, and dried. The yield of obtained isomer **40** was 65%. The mother liquor was evaporated in vacuo, 2-propanol was added and the formed precipitate was filtered, washed with hexane, and dried. The other isomer was isolated by recrystallization from MeCN. The yield of obtained isomer **39** was 9%. The phthalimido-derivatives **39** and **40** (1 mmol each) were refluxed separately with 4 mmol of hydrazine hydrate in 5 mL of EtOH for 4 h. After cooling, each mixture was filtered and evaporated in vacuo. The residues were treated with DCM, filtered, and evaporated in vacuo. The 7-aminomethyl-5-methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine (**17**) and 5-aminomethyl-7-methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine (**21**) were converted to hydrochlorides **17·HCl** and **21·HCl** by an equivalent amount of 6 M ethanolic HCl.

17·HCl: (7-Aminomethyl-5-methyl-3-phenylsulfonylpyrazolo-[1,5-*a*]pyrimidin-2-yl)-methyl-amine hydrochloride (yield: 37%). ^{1}H NMR (DMSO-d_6), δ 8.76 (brs, 3H), 8.03 (m, 2H), 7.59 (m, 3H), 7.15 (s, 1H), 6.51 (brm, 1H), 4.39 (s, 2H), 2.95 (d, J = 2.4 Hz, 3H), 2.56 (s, 3H). HRMS calculated for $C_{15}H_{17}N_5O_2S$ (M + H) 332.118120, found 332.1176. MS-ESI calculated for $C_{15}H_{17}N_5O_2S$ (M + H) 332, found m/z 332. LC-MS (UV-254), purity: 98%.

21·HCl: (5-Aminomethyl-7-methyl-3-phenylsulfonylpyrazolo-[1,5-*a*]pyrimidin-2-yl)-methyl-amine hydrochloride (yield: 32%). ^{1}H NMR (DMSO-d_6), δ 8.50 (s, 3H), 8.14 (d, J = 7.6 Hz, 2H), 7.62 (t, J = 7.2 Hz, 1H), 7.56 (t, J = 7.6 Hz, 2H), 7.12 (s, 1H), 6.50 (d, J = 4.0 Hz, 1H), 4.24 (s, 2H), 2.93 (d, J = 4.4 Hz, 3H), 2.62 (s, 3H). HRMS calculated for $C_{15}H_{17}N_5O_2S$ (M + H) 332.118120, found 332.1191. MS-ESI calculated for $C_{15}H_{17}N_5O_2S$ (M + H) 332, found m/z 332. LC-MS (UV-254), purity: 98%.

[7-Dimethylaminomethyl-5-methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methyl-amine Hydrochloride (18) (Scheme 6) and [7-(2-Dimethylamino-ethyl)-5-methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methyl-amine Hydrochloride (20) (Scheme 5). Formalin (83 mL of 37% , 91 mg, 1.12 mmol) was added to solutions of amines **17** and **19** (the synthesis procedure of **19** is described below) (0.28 mmol each) in 3 mL of DCM, followed by the addition of $NaBH(OAc)_3$ (159 mg, (0.84 mmol). The mixture was stirred for 4 h at ambient temperature, treated with 10% K_2CO_3 solution, and extracted with DCM. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was dissolved in acetone and treated with an excess of 6 M ethanolic HCl. Subsequent evaporation and recrystallization from EtOH yielded compounds **18·HCl** and **20·HCl**, correspondingly.

18·HCl: (7-Dimethylaminomethyl-5-methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine hydrochloride (yield: 27%). ESI-MS 360. ^{1}H NMR (DMSO-d_6), δ: 10.94 (brs, 1H), 8.04 (m, 2H), 7.55−7.64 (m, 3H), 7.37 (s, 1H), 6.51 (brm, 1H), 4.69 (s, 2H), 2.95 (brm, 3H), 2.86 (s, 6H), 2.56 (s, 3H). HRMS calculated for $C_{17}H_{21}N_5O_2S$ (M + H) 360.14942, found 360.1489. MS-ESI calculated for $C_{17}H_{21}N_5O_2S$ (M + H) 360, found m/z 360. LC-MS (UV-254), purity: 99%.

20·HCl: [7-(2-Dimethylamino-ethyl)-5-methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methyl-amine hydrochloride (yield: 18%). ^{1}H NMR (DMSO-d_6), δ: 10.40 (brs, 1H), 8.03 (m, 2H), 7.54−7.63 (m, 3H), 7.01 (s, 1H), 6.44 (q, J = 4.8 Hz, 1H), 3.49 (t, J = 7.0 Hz, 2H), 3.41 (t, J = 7.0 Hz, 2H), 2.93 (d, J = 4.8 Hz, 3H), 2.81 (s, 6H), 2.51 (s, 3H). HRMS calculated for $C_{18}H_{23}N_5O_2S$ (M + H) 374.16507, found 374.165. MS-ESI calculated for $C_{18}H_{23}N_5O_2S$ (M + H) 374, found m/z 374. LC-MS (UV-254), purity: 98%.

***N*^{2},5,7-Trimethyl-3-(phenylsulfonyl)pyrazolo[1,5-*a*]-pyrimidine-2,6-diamine (22) (Scheme 4).** To a suspension of *N*-(5,7-dimethyl-2-methylamino)-3-phenylsulphonyl)-pyrazolo[1,5-*a*]-pyrimidine-6-yl)acetamide (**23**) (100 g, 0.37 M) in 1350 mL of 3:1 methanol−water mixture, KOH (135 g, 2.4 M) was added. The reaction mixture was refluxed for 72 h. After completion of the reaction (LC-MS control), the formed precipitate was thoroughly dispersed with rotary dispergator or ultrasound, filtered, and first washed with an alkaline solution and then with water.

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22: N^2,5,7-Trimethyl-3-(phenylsulfonyl)pyrazolo[1,5-*a*]-pyrimidine-2,6-diamine (yield 94%). ^{1}H NMR (DMSO-d_6), δ: 7.98 (d, J = 8.4 Hz, 2H), 7.51−7.59 (m, 3H), 4.59 (brm, 3H), 2.89 (s, 3H), 2.55 (s, 3H), 2.47 (s, 3H). HRMS calculated for $C_{15}H_{17}N_5O_2S$ (M + H) 332.11812, found 332.1175. MS-ESI calculated for $C_{15}H_{17}N_5O_2S$ (M + H) 332, found m/z 332. LC-MS (UV-254), purity: 99%.

5-Methyl-2-methylamino-3-phenylsulfonyl-pyrazolo[1,5-*a*]-pyrimidine (23) (Scheme 2). N^3-Methyl-4-(phenylsulfonyl)-1*H*-pyrazole-3,5-diamine **33** (27.8 mmol) was mixed with a 1,3-dicarbonyl compound, **51** (83.4 mmol), and AcOH (20 mL). The mixture was stirred overnight at ambient temperature with subsequent heating for 1−2 h at 80−100 °C and cooling down. Precipitate was filtered, washed with AcOH and *i*-PrOH, and dried to produce 5-methyl-2-methylamino-3-phenylsulfonyl-pyrazolo[1,5-a]pyrimidine (**23**) as colorless solid compound (yields: 63−75%).

23: *N*-(5,7-Dimethyl-2-methylamino-3-phenylsulfonyl-pyrazolo-[1,5-*a*]pyrimidin-6-yl)-acetamide (yield 83%). ^{1}H NMR (DMSO-d_6), δ: 9.66 (s, 1H), 8.02 (d, J = 6.8 Hz, 2H), 7.56 (m, 3H), 6.39 (q, J = 4.8 Hz, 1H), 2.92 (d, J = 4.8 Hz, 3H), 2.45 (s, 3H), 2.39 (s, 3H), 2.08 (s, 3H). HRMS calculated for $C_{17}H_{19}N_5O_3S$ (M + H) 374.128685, found 374.1291. MS-ESI calculated for $C_{17}H_{19}N_5O_3S$ (M + H) 374, found m/z 374. LC-MS (UV-254), purity: 98%.

***N*^{2},*N*^{6},*N*^{6},5,7-Pentamethyl-3-(phenylsulfonyl)pyrazolo[1,5-*a*]pyrimidine-2,6-diamine (24) (Scheme 4).** To a suspension of N^2,5,7-trimethyl-3-(phenylsulphonyl)pyrazolo[1,5-*a*]pyrimidine-2,6-diamine (**22**) (75 g, 0.23 mol;) in 3750 mL of ethanol, a 3 M solution of 370 mL of H_2SO_4 was added. The mixture was heated to 90 °C and then cooled to a room temperature. Formalin solution (35%, 116.5 g, 1.4 mol) was added, the mixture was stirred for 30 min, and $NaBH_4$ (56 g, 1.5 mol) was added portionwise to maintain the temperature below 25 °C. After the reaction was complete (LC-MS control), the precipitate was filtered, thoroughly washed with water, and dried. Ethanol from the mother liquor was evaporated in vacuo, and the additional precipitate of **24** was filtered, washed with water, and dried (yield: 97%).

24: ^{1}H NMR (DMSO-d_6), δ: 8.02 (m, 2H), 7.53−7.61 (m, 3H), 6.29 (br, 1H), 2.91 (s, 3H), 2.74 (s, 6H), 2.57 (s, 3H), 2.49 (s, 3H). HRMS calculated for $C_{17}H_{21}N_5O_2S$ (M + H) 360.14942, found 360.1493. MS-ESI calculated for $C_{17}H_{21}N_5O_2S$ (M + H) 360, found m/z 360. LC-MS (UV-254), purity: 98%.

6-Substituted ω-[5,7-Dimethyl-2-methylamino-3-arylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2 or 6-yl]alkylamines 19, 25−27, and 31 (Scheme 5). To a suspension of a corresponding ester **41**−**45** (0.73 mmol each) in ethanol (20 mL), a solution of potassium hydroxide (96 mg, 1.5 mmol) in water (2 mL) was added. The mixtures were stirred for 3 h at 80 °C. Then ethanol was evaporated in vacuo, and water (10 mL) was added. The obtained solutions were acidified with 20% HCl (270 mL). The residues were filtered, washed with water, and dried in air to obtain acids **57**−**61** (yield: 95−97%). To a suspension of each starting acid **57**−**61** (1.6 mmol each) in acetone (12 mL), a solution of Et_3N (263 mL, 1.9 mmol) in acetone (1 mL) was added at 0 °C. After dissolution of the acid, a solution of ethyl chloroformate (197 mL, 2.1 mmol) in acetone (1 mL) was added. The mixture was stirred for 30 min at 0 °C, and then a solution of sodium azide (0.16 g, 2.45 mmol) in water (450 mL) was added, maintaining the same temperature. The mixture was stirred for 1 h at 0 °C, and then water (20 mL) was added. The obtained acylazides **62**−**66** were extracted with DCM, the organic layer was dried with sodium sulfate, and the solvent was evaporated in vacuo at 30 °C to obtain acylazides **62**−**66**, which were used in the next step without further purification. Solutions of each acylazide **62**−**66** in a mixture of DCM and dioxane (5 mL:5 mL) were added dropwise to a boiled dioxane (30 mL). The reaction mixture was refluxed for 1 h, cooled to 70−80 °C, and 20% solution of hydrochloric acid (450 mL) was added. In 20−30 min, hydrochlorides of the desired compounds, **19·HCl**, **25·HCl**−**27·HCl**, **31·HCl**, began to precipitate. The reaction mixture was stirred for 2 h at 70−80 °C, cooled to room temperature, the residue was filtered and washed with dioxane and dried in vacuo to obtain compounds **19·HCl**, **25·HCl**−**27·HCl**, **31·HCl** (yield: 46−68%).

19 HCl: 7-(2-Aminoethyl)-5-methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methylamine hydrochloride (yield: 46%). ^{1}H NMR (DMSO-d_6), δ: 8.03 (brs, 3H), 8.03 (m, 2H), 7.59 (m, 3H), 6.96 (s, 1H), 6.41 (m, 1H), 3.29 (brm, 4H), 2.92 (d, J = 3.6 Hz, 3H), 2.51 (s, 3H). HRMS calculated for $C_{16}H_{19}N_5O_2S$ (M + H) 346.13377, found 346.1340. MS-ESI calculated for $C_{16}H_{19}N_5O_2S$ (M + H) 346, found m/z 346. LC-MS (UV-254), purity: 98%.

25 HCl: 6-Aminomethyl-5,7-dimethyl-3-phenylsulfonyl-pyrazolo-[1,5-*a*]pyrimidin-2-yl]-methylamine hydrochloride (yield: 52%). ^{1}H NMR (400 MHz, DMSO-d_6), δ: 8.36 (brs, 3H), 8.03 (m, 2H), 7.54−7.63 (m, 3H), 6.43 (q, J = 4.8 Hz, 1H), 2.93 (d, J = 4.8 Hz, 3H), 2.74 (s, 3H), 2.66 (s, 3HH). HRMS calculated for $C_{16}H_{19}N_5O_2S$ (M + H) 346.13377, found 346.1339. MS-ESI calculated for $C_{16}H_{19}N_5O_2S$ (M + H) 346, found m/z 346. LC-MS (UV-254), purity: 98%.

26 HCl: 6-Aminomethyl-5,7-dimethyl-3-(3-fluorophenylsulfonyl)-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methylamine hydrochloride (yield: 54%). ^{1}H NMR (400 MHz, DMSO-d_6), δ: 8.06 (brs, 3H), 7.85 (m, 2H), 7.63 (dt, J_1 = 8 Hz, J_2 = 5.6 Hz, 1H), 7.48 (dt, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 6.47 (q, J = 4.8 Hz, 1H), 4.17 (s, 2H), 2.92 (d, J = 5.2 Hz, 3H), 2.72 (s, 3H), 2.65 (s, 3H). HRMS calculated for $C_{16}H_{18}FN_5O_2S$ (M + H) 364.124348, found 364.1244. MS-ESI calculated for $C_{16}H_{18}FN_5O_2S$ (M + H) 364, found m/z 364. LC-MS (UV-254), purity: 98%.

27 HCl: 6-Aminomethyl-5,7-dimethyl-3-(3-chlorophenylsulfonyl)-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methylamine hydrochloride (yield: 55%). ^{1}H NMR (400 MHz, DMSO-d_6), δ: 8.34 (brs, 3H), 8.10 (t, J = 1.6 Hz, 1H), 7.97 (ddd, J_1 = 7.6 Hz, J_2 = 1.6 Hz, J_3 = 1.2 Hz, 1H), 7.69 (ddd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, J_3 = 1.2 Hz, 1H), 7.61(t, J = 8.0 Hz, 1H), 6.48 (q, J = 4.8 Hz, 1H), 4.14 (s, 2H), 2.92 (d, J = 4.8 Hz, 3H), 2.75 (s, 3H), 2.67 (s, 3H). HRMS calculated for $C_{16}H_{18}ClN_5O_2S$ (M + H) 380.094797, found 380.0948. MS-ESI calculated for $C_{16}H_{18}ClN_5O_2S$ (M + H) 380, found m/z 380. LC-MS (UV-254), purity: 99%.

31 HCl: 6-(2-Aminoethyl)-5,7-dimethyl-3-phenylsulfonyl-pyrazolo-[1,5-*a*]pyrimidin-2-yl]-methylamine hydrochloride (yield: 68%). ^{1}H NMR (400 MHz, DMSO-d_6), δ: 8.17 (brs, 3H), 8.01 (d, J = 7.6 Hz, 2H), 7.53−7.61 (m, 3H), 2.98 (m, 2H), 2.92 (s, 3H), 2.89 (brs, 2H), 2.64 (s, 3H), 2.56 (s, 3H). HRMS calculated for $C_{17}H_{21}N_5O_3S$ (M + H) 360.14942, found 360.1501. MS-ESI calculated for $C_{17}H_{21}N_5O_3S$ (M + H) 360, found m/z 360. LC-MS (UV-254), purity: 98%.

[6-(2-Dimethylaminomethyl)-5,7-dimethyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methylamines 28−30 and [6-Dimethylaminoethyl)-5,7-dimethyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methylamine (32) (Scheme 5). To a solution of amine **25**−**27** and **31** (1 mmol each) in 10 mL of DCM, formalin (3 mmol) and sodium triacetoxyborohydride (0.53 g, 2.5 mmol) were added. The mixture was stirred at room temperature for 3 h, and then more formalin (3 mmol) and sodium triacetoxyborhydride (0.53 g, 2.5 mmol) were added and the stirring continued for 12 h. Mixtures were diluted with water, extracted with DCM, washed with 10% potassium carbonate solution, dried over Na_2SO_4, and evaporated in vacuo. The product was isolated by column chromatography (eluent: hexane/ethylacetate/triethylamine in ratio of 30:10:1). Hydrochlorides were obtained by addition of 3 M HCl solution in dioxane to a solution of amine in acetone.

28 HCl: [6-Dimethylaminomethyl)-5,7-dimethyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methylamine hydrochloride (yield: 57%). ^{1}H NMR (DMSO-d_6), δ: 10.10 (brs, 1H), 8.04 (m, 2H), 7.54−7.63 (m, 3H), 6.47 (q, J = 4.4 Hz, 1H), 2.93 (d, J = 4.4 Hz, 3H), 2.80 (s, 6H), 2.76 (s, 3H), 2.72 (s, 3H). MS-ESI calculated for $C_{18}H_{23}N_5O_2S$ (M + H) 374, found m/z 374. LC-MS (UV-254), purity: 98%.

29 HCl: [6-Dimethylaminomethyl)-5,7-dimethyl-3(3-fluorophenyl-sulfonyl)-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methylamine hydrochloride (yield: 37%). ^{1}H NMR (DMSO-d_6), δ: 10.3 (brs, 1H), 7.87 (t, J = 7.6 Hz, 2H), 7.64 (m, 1H), 7.49 (m, 1H), 6.48 (m, 1H), 4.46 (d, J = 5.6 Hz, 2H), 2.93 (d, J = 4.4 Hz, 3H), 2.80 (d, J = 2.8 Hz, 6H), 2.77 (s, 3H), 2.73 (s, 3H). HRMS calculated for $C_{18}H_{22}FN_5O_2S$ (M + H) 392.155648, found 392.1555. MS-ESI calculated for $C_{18}H_{22}FN_5O_2S$ (M + H) 392, found m/z 392. LC-MS (UV-254), purity: 98%.

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30 HCl: [6-Dimethylaminomethyl)-5,7-dimethyl-3-(3-chlorophenylsulfonyl)-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methylamine hydrochloride (yield: 56%). ^{1}H NMR (DMSO-d_6), δ: 10.1 (brs, 1H), 8.10 (t, J = 2.0 Hz, 1H), 7.99 (dt, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 7.70 (ddd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, J_3 = 0.8 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 6.50 (q, J = 4,8 Hz, 1H), 4.47 (d, J = 5.6 Hz, 2H), 2.93 (d, J = 4.8 Hz, 3H), 2.82 (s, 3H), 2.81 (s, 3H), 2.77 (s, 3H), 2.73 (s, 3H). MS-ESI calculated for $C_{18}H_{22}ClN_5O_2S$ (M + H) 409, found m/z 409. LC-MS (UV-254), purity: 98%.

32 HCl: [6-(2-Dimethylaminoethyl)-5,7-dimethyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methylamine hydrochloride (yield: 61%). ^{1}H NMR (DMSO-d_6), δ: 10.78 (brs, 1H), 8.02 (d, J = 13.2 Hz, 2H), 7.53−7.62 (m, 3H), 6.34 (q, J = 4.8 Hz, 1H), 3.09 (brm, 4H), 2.92 (d, J = 4.8 Hz, 3H), 2.82 (s, 6H), 2.67 (s, 3H), 2.60 (s, 3H). MS-ESI calculated for $C_{19}H_{25}N_5O_2S$ (M + H) 388, found m/z 388. LC-MS (UV-254), purity: 99%.

Cell-Based 5-HT$_6$ Receptor Functional Assay. The 5-HT$_6$R was subcloned into T-Rex system (Invitrogen, Carlsbad, CA) and expressed into HEK (5-HT$_6$R-HEK) cells. The cells were grown in DMEM supplemented with 10% FBS, 1%AAS, blasticidine S, and zeocin (all from Invitrogen, Carlsbad, CA) in a T-175 cell culture flask. T-Rex/5-HT$_6$ receptor expression was activated by addition of tetracycline (1 μg/mL), as recommended by the T-Rex system manufacturer (Invitrogen, Carlsbad, CA), a day before the experiments. On the day of the experiment, the cells were harvested from the flask using 6 mM EDTA/HBSS solution, gently triturated by passing through a pipet tip several times to break down cell aggregates, washed with Serum Free Medium, and counted. The cells were resuspended to 0.67×10^6 cells/mL in SB2 buffer, HBSS, supplemented with 5 mM HEPES, pH 7.4, 0.05% BSA, and 1 mM IBMX (SigmaAldrich, St. Louis, MO) containing Alexa Fluor 647-anti cAMP antibody (from LANCE cAMP 384 kit, PerkinElmer, Waltham, MA). Then 6 μL of the cell suspension (~4000 cells) were aliquoted into the wells of 384-well assay plates (PerkinElmer White OptiPlates). The test compounds were premixed at different concentrations with serotonin hydrochloride (Sigma, MO), and the mixtures were added to the assay plates with the cells (final concentrations: serotonin, 10 nM; DMSO, 0.32%; IBMX, 500 mM). Each assay plate contained serotonin and cAMP standard concentration curves for quality assurance. Cells, after 2 h of incubation with the compound/serotonin mixtures, were treated as described in the cAMP LANCE assay kit protocol (PerkinElmer, Waltham, MA). The LANCE signal was measured using the multimode plate reader, VICTOR 3 (PerkinElmer, Waltham, MA), with built-in settings for the LANCE detection.

Curve Fitting. The concentration-dependent cell response data were fitted with Prism 5 (Graph-Pad, CA) using built-in 4-parametric equation to calculate IC$_{50}$ values. All experiments were performed in duplicates or triplicates. Standard errors (SE) were calculated with Prism built-in statistical package.

Determination of K_i Values. K_i values from the cell-based functional 5-HT$_6$ receptor inhibition assays were calculated using modified Cheng−Prusoff's equation,[38] $K_i = IC_{50}/(1 + [Ag]/EC_{50})$, where IC$_{50}$ is the concentration of antagonist causing 50% inhibition of the serotonin-induced cell response, and [Ag] is a concentration of serotonin (10 nM), at which inhibition was measured and EC$_{50}$ is serotonin concentration causing 50% stimulation of the cell response, measured simultaneously with the test compounds on the same plate. The mean EC$_{50}$ value for serotonin-induced cAMP production in 5-HT$_6$R-HEK cells was 1.91 ± 0.13 nM as determined from four independent experiments (different days) with three to five repeats (separate plates during the day run), each in quadruplicates (on each plate).

Determination of pA_2 Values. The pA_2 values were calculated from a Schild-type experiments[39] in the cell-based functional 5-HT$_6$ receptor inhibition assay using Prism 5 as described by H. Motulsky.[41] The cell responses to incremental concentrations of serotonin were measured in the presence of different concentrations of an antagonist. At zero and at each antagonist concentration, the EC$_{50}$ values (A and A'_i, respectively) of the serotonin-induced responses were calculated and the Log($A'_i/A - 1$) as a function of the Log blocker concentration have been plotted. This yielded the Schild plots for the tested compounds, from which pA_2 values were determined as an intersection of the straight line with the X-axis.

Receptor Selectivity/Specificity Profiling. The panel of 33 different receptors expressed in cell membranes was used for the profiling. The compounds were tested at a concentration of 1 μM in duplicates at equilibrium binding conditions with radiolabeled ligands specific to each of the receptor. The testing was performed by Ricerka (Taiwan) in accordance with their internally optimized protocols.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis procedures, analytical and spectral data of the synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

5-HT$_6$R, serotonin 5-HT$_6$ receptor; cAMP, cyclic adenosine monophpsphate; CNS, central nervous system; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; HEK 293, human embryonic kidney cell line; HYD, hydrophobic region; IHB, intramolecular hydrogen bond; IC$_{50}$, concentration of half-maximal inhibition; IHB, intramolecular hydrogen bond; K_i, activity constant; PD, proton donor; PhM, pharmacophore model; PI, positively ionizable group; SAR, structure−activity relationship

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