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Synthesis and SAR of 3-arylsulfonyl-pyrazolo[1,5-*a*]pyrimidines as potent serotonin 5-HT$_6$ receptor antagonists

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ABSTRACT

Syntheses of a series of novel 3-sulfonyl-pyrazolo[1,5-*a*]pyrimidines and their 5-HT$_6$ receptor antagonistic structure–activity relationship are disclosed. The nature and position of substituents, which affect their receptor antagonistic activity, are analyzed. Among all synthesized derivatives, {3-(3-chlorophenylsulfonyl)-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidin-2-yl}-methyl-amine **33** (K_i = 190 pM), (3-phenylsulfonyl-7-methyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine **44** (K_i = 240 pM), (3-phenylsulfonyl-5-metoxymethyl-7-methyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine **50** (K_i = 270 pM), and (3-phenylsulfonyl-5-methyl-7-metoxymethyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine **52** (K_i = 280 pM) are the most potent antagonists of the 5-HT$_6$ receptors.

1. Introduction

Exclusive localization of 5-HT$_6$ receptors (5-HT$_6$R) in the CNS, and high affinities of many psychiatric drugs to this receptor,[1–3] have attracted considerable attention of researchers in a quest for the discovery of new drug candidates for treating diseases of the central nervous system (CNS) including cognitive desorders.[4–8] There are only a few selective 5-HT$_6$R antagonists that are being tested in Phase I and Phase II clinical trials as drug candidates for treating various CNS diseases.[9–11] Recently, we reported on the synthesis and structure–activity relationship (SAR) of 2-substituted 5,7-dimethyl-[12] and cycloalkane-annelated[13,14] 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines, arylsulfonylpyrazolopyrimidines (ARSPP), as exceptionally potent and selective 5-HT$_6$R antagonists (Fig. 1). We have concluded that at least three factors could be responsible for the high potency of the compounds: (i) torsion angles between the phenylsulfonyl moiety relative to the heterocycle core plane, which define the conformation of the molecule, (ii) the nature and size of a substituent in the 2-position,[12] and (iii) the formation of an intramolecular hydrogen bond between the basic amino group located next to the sulfonyl group, which may stabilize an advantageous binding conformation.[14]

In particular, we have shown[12–14] that in the corresponding pairs of potent and selective 2-methylamino- and methylthio-substituted ARSPPs **A**, **B** and **C**, **D** (Fig. 1), the methylamino-substituted **B** and **D**, which can form intramolecular hydrogen bonds, exhibit substantially higher potencies as 5-HT$_6$R antagonists.

In this paper, we further explore the SAR of 5,7-substituted 2-methylthio- and 2-methylamino-3-arylsulfonylpyrazolopyrimidines for their ability to antagonize 5-HT$_6$R (Table 1).

2. Results and discussion

2.1. Chemistry

2-Methylthio (**9**–**12**) and 2-methylamino (**13**–**15**) ARSPPs were obtained in the reaction of 3-amino-4-arylsulfonylpyrazoles **1**–**7** with 1,1,3,3-tetramethoxypropane **8** in acetic acid at 100 °C (Scheme 1).

5,7-Dimethyl-2-methylthio ARSPPs **23**–**28** and (5,7-dimethyl-2-methylamino ARSPPs **29**–**33** were obtained in the reaction of corresponding 3-amino-4-arylsulfonylpirazoles **2**–**4**, **6**–**7**, and **16**–**21** with acetylacetone **22** in acetic acid (Scheme 2) in conditions similar to ones described earlier.[14]

Abbreviations: 5-HT$_6$R, serotonin 5-HT$_6$ receptor; CNS, central nervous system; SAR, structure–activity relationship; ARSPP, arylsulfonylpyrazolopyrimidines.

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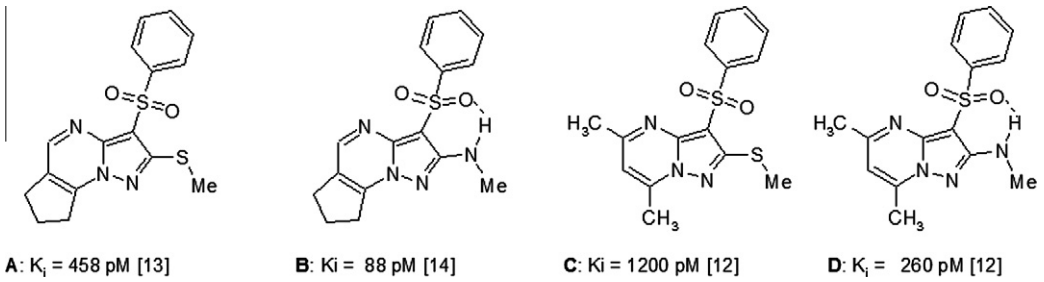
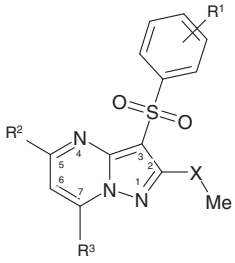


Figure 1. Substituted 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines **A**–**D** as potent 5-HT_6R antagonists.

Table 1
Synthesized 5,7-substituted 2-methylthio- and 2-methylamino-ARSPPs **9**–**15**, **23**–**36**, **38**–**43**, **45**–**55**, and their 5-HT_6R antagonistic activity



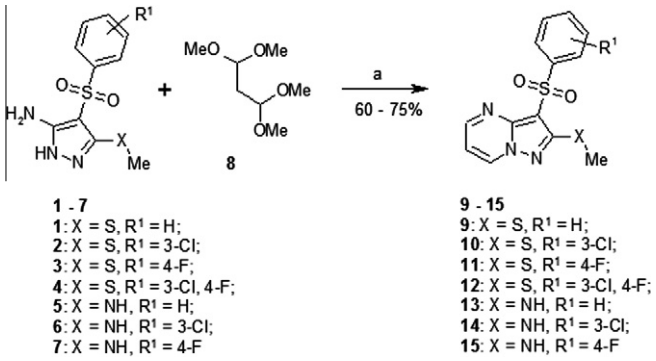
Compd[a]	Substituent			pK_i	
	R^1	R^2	R^3	X = S	X = NH
9, **13**	H	H	H	6.94	8.41
54, **55**[b]	H	Me	Me	8.92[b]	9.59[b]
38, **40**	H	Me	H	7.78	8.84
41, **43**	H	H	Me	7.64	9.62
46, **47**	H	Me	CH_2OMe	6.81	8.12
48, **49**	H	CH_2OMe	Me	8.49	9.57
50, **51**	H	Me	CH_2OH	9.25	9.55
52, **53**	H	CH_2OH	Me	8.67	9.32
10, **14**	3-Cl	H	H	7.82	8.93
26, **31**	3-Cl	Me	Me	9.12	9.73
25, **30**	3-F	Me	Me	8.97	9.30
24	4-Cl	Me	Me	8.50	NT[c]
23, **29**	4-F	Me	Me	8.17	9.40
11, **15**	4-F	H	H	6.88	7.93
35, **36**	4-HO	Me	Me	8.4	8.7
28, **33**	4-MeO	Me	Me	7.43	8.02
34	4-Me_2N	Me	Me	7.43	NT[c]
12	3-Cl, 4-F	H	H	7.35	NT[c]
27, **32**	3-Cl, 4-F	Me	Me	8.42	9.22
39	3-Cl, 4-F	Me	H	8.1	NT[c]
42	4-F, 3-Cl	H	Me	NT	NT

[a] First number in each pair is for the compounds with X = S and the second one is for compounds with X = NH.
[b] Compounds **54** and **55** correspond to compounds **3** and **4**, respectively, in 12. The data is taken from Ref. 12.
[c] NT—Not tested.

5,7-Dimetyl-3-(4-dimethylaminophenylsulfonyl)-2-methylthiopyrazolo[1,5-*a*]pyrimidine **34** was synthesized from the corresponding 4-chlorophenylsulfonyl derivative **24** under reaction with dimethylamine (Scheme 3).

3-(4-Methoxyphenyl) ARSPPs **28** and **33** were further transformed into 4-(5,7-dimethyl-2-methylthio- (**35**) and 4-(5,7-dimethyl-2-methylamino- (**36**) pyrazolo[1,5-*a*]pyrimidine-3-sulfonyl)-phenols under treatment with HBr (Scheme 4).

5-Methyl- (**38**–**40**) and 7-methyl- (**41**–**43**) substituted ARSPPs were obtained from reaction of corresponding 3-amino-4-arylsulfonylpyrazoles **1**, **4**, and **5** with 4,4-dimethoxy-butan-2-one **37** (Scheme 5), similar to the synthesis of ARSPPs **9**–**15**. In these reactions, mixtures of 5-methyl- and 7-methyl-substituted ARSPPs, **38** and **41**, **39** and **42**, and **40** and **43**, were formed.



Scheme 1. Synthesis of 2-methylthio ARSPPs **9**–**12** and 2-methylamino ARSPPs **13**–**15**. Reagents and conditions: (a) AcOH, 100 °C, 6 h.

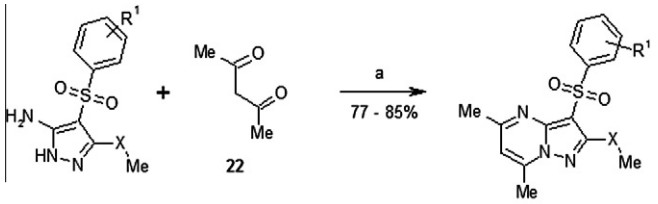
^{1}H NMR data showed that in the corresponding mixtures, products **38** and **39** are formed in a ratio of ~1.4:1 relative to their corresponding counterparts **41** and **42**. ARSPPs **40** and **43** were formed in the ratio of ~1:2.5. ARSPPs **40** and **43** were separated chromatographically on a silica gel column with DCM as eluent. ARSPPs **39** and **42** were separated using HPLC. Using HPLC, we were able to separate **41** with a purity of >95% from its mixture with **38**, though we failed to separate **38** with adequate purity. Instead, we obtained ARSPP **38** by hydrogenation of 7-chloro-5-methyl-2-methylthio-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine **44**[15] (Scheme 6).

3-Amino-4-arylsulfonylpyrazoles **1** and **5** reacted with the sodium salt of 1-methoxy-2,4-pentanedione[16] **45** in AcOH at 100 °C (Scheme 7) similar to the reaction described above in Scheme 4. These reactions have also yielded approximately equimolar mixtures of, respectively, **46** and **48** or **47** and **49**. Using silica gel chromatography, we separated the mixtures and transformed the individual compounds **46**–**49** into corresponding 3-phenylsulfonyl-heterocyclyl-methanols **50**–**53** using BBr_3 in DCM.

Structures of synthesized ARSPPs were confirmed by LC–MS and NMR spectra, including 2D NMR NOESY experiments. According to the LC–MS spectra, all of the synthesized materials had purity above 98%. The NMR spectra are in good agreement with the structures illustrated (see Section 4).

2.2. Inhibition of 5-HT_6 receptors

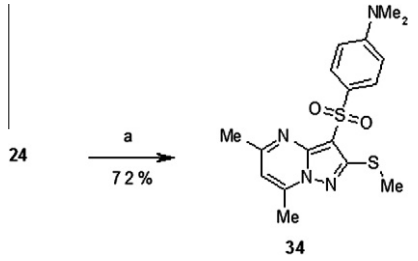
Experiments were performed as was described earlier[14] in a functional assay using recombinant human 5-HT_6 receptor exogenously expressed into HEK 293 cells, which responded to serotonin with increase in cAMP synthesis. Interaction of the ARSPPs with the receptor was competitive with serotonin (data not shown) and the potencies (pK_i) of the compounds to block serotonin-induced cAMP accumulation 5-HT_6R are summarized in Table 1. It can be seen that the novel ARSPPs synthesized are very potent



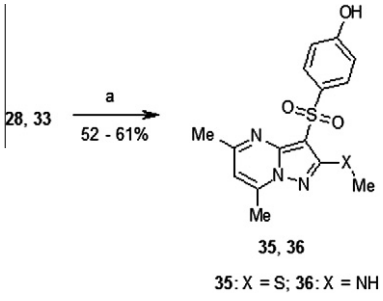
16: X = S, R^1 = 4-Cl;
17: X = S, R^1 = 3-F;
18: X = S, R^1 = 4-OMe;
19: X = NH, R^1 = 3-F;
20: X = NH, R^1 = 3-Cl, 4-F;
21: X = NH, R^1 = 4-OMe

23: X = S, R^1 = 4-F;
24: X = S, R^1 = 4-Cl;
25: X = S, R^1 = 3-F;
26: X = S, R^1 = 3-Cl;
27: X = S, R^1 = 3-Cl, 4-F;
28: X = S, R^1 = 4-OMe
29: X = NH, R^1 = 4-F;
30: X = NH, R^1 = 3-F;
31: X = NH, R^1 = 3-Cl;
32: X = NH, R^1 = 3-Cl, 4-F;
33: X = NH, R^1 = 4-OMe

Scheme 2. Synthesis of 5,7-dimethyl-2-methylthio ARSPPs **23**–**28** and 5,7-dimethyl-2-methylamino ARSPPs **29**–**33**. Reagents and conditions: (a) AcOH, 100 °C, 6 h.



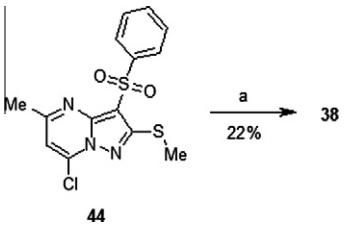
Scheme 3. Synthesis of 5,7-dimetyl-3-(4-dimethylaminophenylsulfonyl)-2-methylthiopyrazolo[1,5-*a*]pyrimidine (**34**). Reagents and conditions: (a) 40% Me_2NH, DMF, 140 °C, 48 h.



35: X = S; **36**: X = NH

Scheme 4. Synthesis of 4-(5,7-dimethyl-2-methylthio- (**35**) and 4-(5,7-dimethyl-2-methylamino-(**36**) pyrazolo[1,5-*a*]pyrimidine-3-sulfonyl)-phenols. Reagents and conditions: (a) 40% HBr, reflux, 24 h.

5-HT_6R antagonists with K_i values ranging from 0.19 nM (compound **31**) to 155.7 nM (compound **46**). The nature of the substituent groups in positions 2, 5, and 7 determines a SAR range of more than two orders of magnitude. It is noticeable that 2-thiomethyl

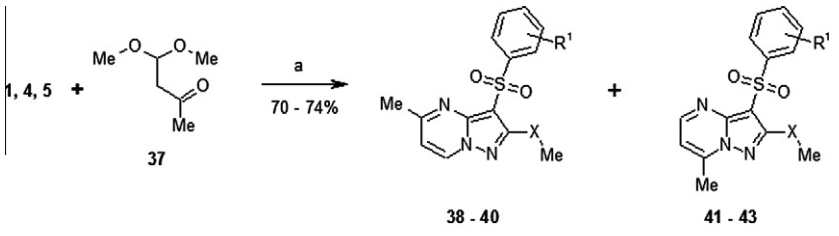


Scheme 6. Synthesis of 5-methyl-2-methylthio-ARSPP **38**. Reagents and conditions: (a) H_2, 10% Pd/C, benzene, MeOH, rt, 2 h.

compounds (X = S) have higher K_i values (lower potency) than their corresponding analogs in the 2-aminomethyl (X = NH) series. When all the compounds were analyzed as a whole set, no significant correlation was observed between their potencies to block 5-HT_6R and the most common physicochemical molecular descriptors, such as MW, molecular volume, polar surface area, lipophilicity (*A* Log *P*98), polarizability, dipole moment, and radius of gyration (data not shown) calculated using DS ViewerPro 6.0 (Accelrys Software Inc., San Diego).

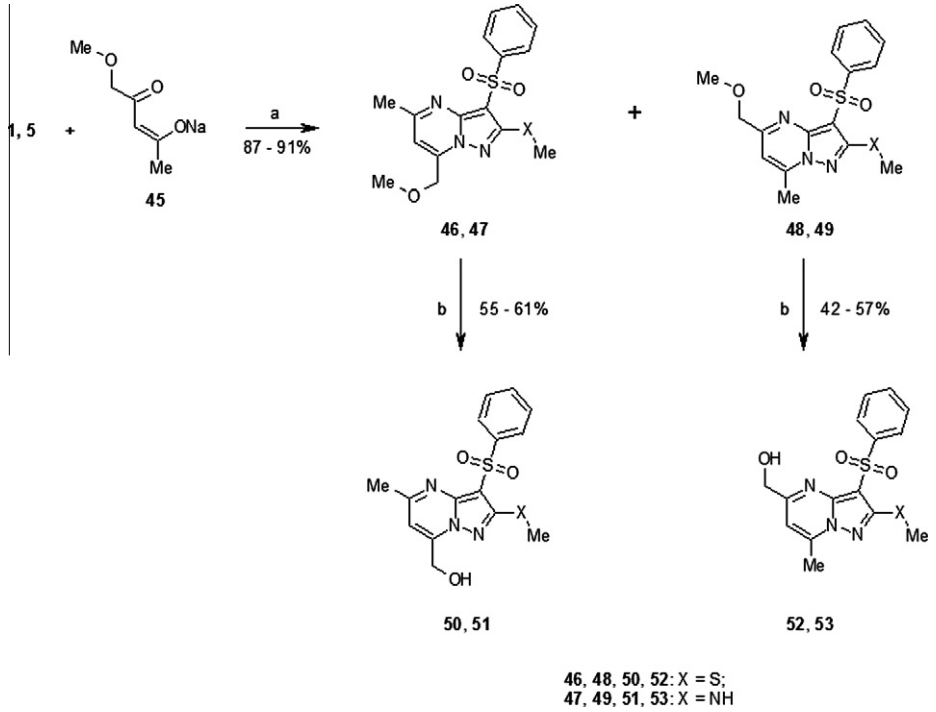
This is in agreement with our earlier data obtained with 2-substituted 5,7-dimethyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine analogs.[12] However, when the compounds were separated into two groups, namely, the 2-thiomethyl (2-SMe) and the 2-aminomethyl (2-NHMe)-substituted ARSPPs, there was a significant correlation between pK_i and the radius of gyration within each group (Fig. 2A and B, respectively).

In both series of compounds, the larger gyration radii of the molecules are associated with lower compound potencies, pK_i. As the radius of gyration reflects hydrodynamic size and shape of the molecule,[17] it seems reasonable to assume from the reciprocal correlation that the binding pocket of the 5-HT_6 receptor is more suitable for accommodation of smaller molecules.



38, **41**: X = S, R^1 = H;
39, **42**: X = S, R^1 = 3-Cl-4-F;
40, **43**: X = NH, R^1 = H

Scheme 5. Synthesis of 5-methyl- (**38**, **39**), 7-methyl- (**41**, **42**) 2-methylthio-ARSPPs and 5-methyl- (**40**), and 7-methyl- (**43**) 2-methyl-amine ARSPPs. Reagents and conditions: (a) AcOH, 100 °C, 6 h.



Scheme 7. Synthesis of methoxymethyl-3-phenylsulfonyl-pyrazolopyrimidines **46–49** and methylmethanol-3-phenylsulfonyl-pyrazolopyrimidines **50–53**. Reagents and conditions: (a) AcOH, 100 °C, 12 h. (b) DCM, BBr_3, rt, 12 h.

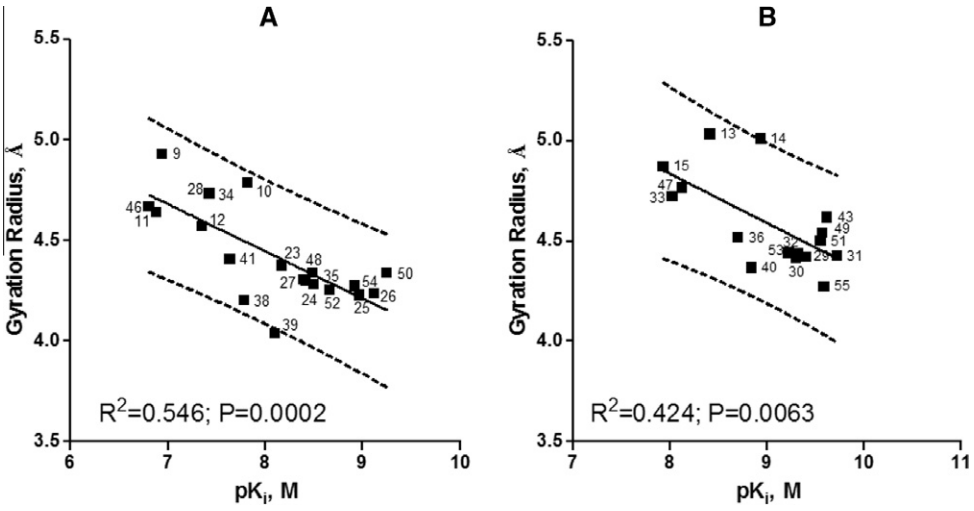


Figure 2. Correlation between radius of gyration (calculated using DS ViewerPro 6.0) and pK_i values of the compounds in 2-SMe (**A**) or 2-NHMe (**B**) subgroups. Dotted lines represent confidence interval of 95%.

The 5,7-substituents as well as those on the phenyl moiety (R^1) affect compound potencies in a similar rank-order independently of the nature of the 2-substituent group, 2-SMe or 2-NHMe. However, the latter substituents significantly affect overall potency of the compounds in each of the two series, compounds in the 2-NHMe series being approximately an order of magnitude more potent than their 2-SMe counterparts (Fig. 3).

A plausible explanation of this increase in 5-HT_6R blocking potency attributable to the 2-NHMe group could be that it forms an intramolecular hydrogen bond between the proton of the 2-methylamine group and one of the two oxygens of the 3-arylsulfonyl group. This restricts the molecule mobility in a conformation advantageous for the binding. The energy minimization (Accelrys DS ViewerPro 6.0 software; minimization cycles were being repeated until the convergence occured at a convergence criterion of 0.00001) showed that all of the 2-NHMe-substituted molecules have two stable conformations, as exemplified in Figure 4 by compound **55**. Depending on the oxygen atom participating in the hydrogen bond formation, O(1) or O(2), the two conformations differ in their torsion angles between the planes formed by the *ab* and *bc* bonds.

The 2-SMe-substituted compounds, exemplified in Figure 5 by compound **54**, unlike 2-NHMe-substituted ones, accept at least three major conformations defined by the torsion angle between the planes formed by the same *ab* and *bc* bonds (see Fig. 4). With symmetrical derivatization of the phenyl ring, there are three additional conformations with 2-SMe facing in opposite to shown direction (total of six conformations). With asymmetric derivatiza-

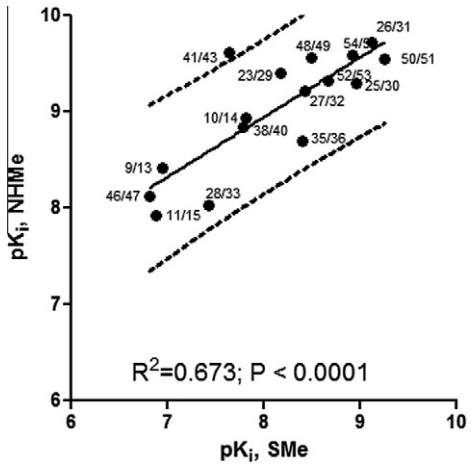


Figure 3. Corellation between pK_i values for blocking serotonin-induced cAMP production in HEK cells expressing 5-HT$_6$ receptor by 2-aminomethyl- and 2-thiomethyl-substituted ARSPPs. Dotted lines represent confidence interval of 95%.

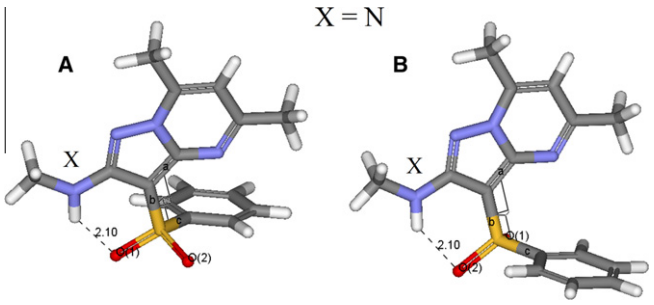


Figure 4. Calculated stable conformations (DS ViewerPro 6.0) of the compound **55**. The torsion angle between planes formed by bonds ab and bc are: (**A**) −52.65° (E = 58.876635 kcal/mol) and (**B**) +52.47° (E = 58.875965 kcal/mol).

tion of the phenyl ring, there are up to six additional conformations with local minima. However, the global free energy minimum calculated with the DS ViewerPro 6.0, corresponds to the conformation with the *ab*/*bc* torsion angle close to zero. This conformation is characteristic for all of the 2-SMe-substituted compounds (Fig. 5C). Thus, the generally lower receptor affinity of the compounds within the 2-SMe-substituted series compared with 2-NHMe-substituted one could result from the entropy factor, higher conformational freedom, which effectively reduces the concentration of the compounds in an appropriate 'active' binding conformation.

Alternative explanation of the higher potencies of compounds with the 2-NHMe substitution could be that the 2-NHMe group provides an additional binding energy by forming intermolecular hydrogen bonding with Ser193 and/or Thr196, which are located in the transmembrane region V of the 5-HT6R receptor.[18]

The compound potencies are substantially affected by the ature and position of the substituents in the 5- and 7-positions. As compared with previously described[12] 5,7-dimethyl substituted compounds **54** and **55**, removal of both methyl moieties in compounds **9** and **13** leads to a substantial (one to two orders of magnitude) increase in K_i (see Table 1). 5-Me (**38**) or 7-Me (**41**) substitution in the 2-SMe series leads to, respectively, 4.9-fold and 6.9-fold decrease in K_i (compared with **9**). Similar substitutions in 2-NHMe series lead to 2.7-fold (**40**) and 16.2-fold (**43**) decrease in K_i (compared with **13**). In both series, methyl substitution of the 7-position is more effective than that of 5-position in increasing the compound potencies.

Substitution of the 5-Me group in **54** and **55** with methoxymethyl (correspondingly, **48** and **49**), engendered a minimal, if any, effect on the compound potencies. On the other hand, similar substitution in position 7 (**46** and **47**) led to a substantial, 130-fold and 32-fold, decrease in the compound potencies. Interestingly, the methyl substitution in either position with a more hydrophilic moiety, hydroxymethyl, does not cause substantial changes in the compound K_i values (compare **50** and **52** with **54** (2-SMe series) and **51** and **53** with **55** (2-NHMe series)).

3-Cl-phenyl derivatized compounds (R^1 in Table 1) show the same K_i trend as with the compounds having unsubstituted phenyl. 5,7-dimethyl substituted compounds **26** (2-SMe series) and **31** (2-NHMe series) are 20-fold and 6-fold more potent than their 5,7-unsubstituted analogs, **10** and **14**, respectively. 3-F-phenyl substitution for 3-Cl-phenyl, **25** and **30**, leads to a moderate, 1.4-fold to 2.6-fold, reduction in the compound potencies (compare, correspondingly, with **26** and **31**).

Derivatizing the *para*-position in the phenyl ring with a chlorine atom (in **24)** instead of the *meta*-position (in **26)** leads to 4.2-fold increase in K_i value. Substitution of the 4-Cl with 4-F leads to further reduction in the potency (compare **23** and **24**). 4-F-phenyl-substituted 5,7 dimethyl ARSPPs **23** and **29** were 5.7-fold and 1.5-fold less potent than their corresponding unsubstituted analogs **54** and **55**. 4-Fluoro substitution of 5,7-unsubstituted 2-SMe-ARSPP **11** does not affect its potency (compare with **9**), whereas the same substitution of 2-NHMe-ARSPP (**13**) with 4-F (**15**) leads to a threefold decrease in the potency (increase in K_i).

In a series of 5,7-dimethyl-substituted compounds, there is a clear trend in decreasing potency (pK_i values) with increase in a partial negative charge, δ, on the 4-phenyl substituent (Fig. 6). The δ values were calculated with the DS ViewerPro 6.0 using method of Gasteiger. The potency rank order of compounds in the 5,7-dimethyl-2-SMe series is **54** (δ = +0.062) > **24** (δ = −0.084) > **23** (δ = −0.207) > **28** (δ = −0.349) = **34** (δ = −0.378). The trend indicates that the binding site of the receptor could have a preference to a hydrophobic moiety in this position. However, almost one order of

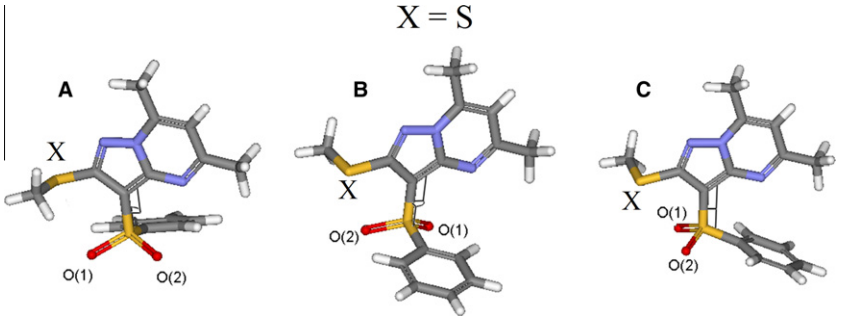


Figure 5. Calculated stable conformations (DS ViewerPro 6.0) of the compound **54**. The characteristic torsion angles are: (**A**) −85.21° (E = 60.91859 kcal/mol), (**B**) +85.23° (E = 60.91838 kcal/mol), and (**C**) −0.35° (E = 60.132609 kcal/mol).

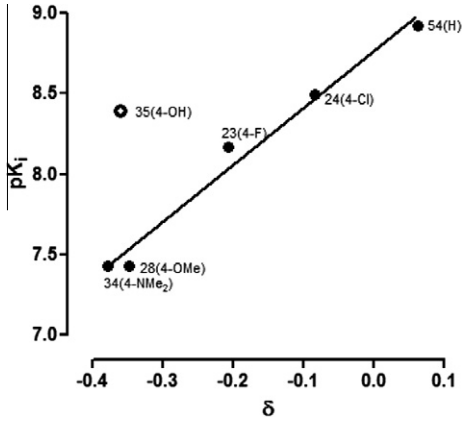


Figure 6. Dependence of ARSPPs potencies (pK_i) on a partial charge of the 4-phenyl-substitute atom (H, Cl, F, O, or N). Compound **6** with R^1 = 4-OH is shown as open symbol. Numbers denote compound IDs.

magnitude higher potency than the one expected from the partial atom charge trend (Fig. 6) was observed for compound **35** having 4-OH substituent. This substantial deviation could indicate a possibility of the hydroxyl group forming a hydrogen bond in the receptor binding pocket and thus increasing binding energy. Though a fewer number of 4-substituted 5,7-dimethyl-2-NHMe ARSPPs was tested (Table 1), the same rank-order trend in pK_i values (see Table 1) was observed: **55** (4-H, δ = +0.062) > **29** (4-F, δ = −0.207) > **33** (4-MeO, δ = −0.349).

3-Cl-phenyl substitution leads to increased compound potency (pK_i): **10** > **9** and **26** > **54** in 2-SMe series; and **14** > **13** and **31** > **55** in 2-NHMe series. However, additional 4-phenyl substitution with partially negative fluorine atom leads to a decrease in pK_i values for both the 2-SMe- and 2-NHMe-substituted series: **12** < **10**, **27** < **26**, and **32** < **31** (see Table 1).

3. Conclusions

Here, we have described syntheses of novel 3-arylsulfonyl-pyrazolo[1,5-*a*]pyrimidines, which are competitive 5-HT_6R antagonists with a range of potency (pK_i) between 9.72 (compound **31**) and 6.81 (compound **46**). SAR of the synthesized compounds established several structural trends affecting the compounds' potencies towards blocking 5-HT_6 receptors. First, structurally restricted, due to intramolecular hydrogen bonding, 2-aminomethyl substituted compounds show substantially increased 5-HT_6R antagonistic potency compared with less restricted 2-thiomethyl substituted ones. Secondly, derivatization of both the 5- and 7-positions in the pyrimidine cycle as well as that of 3- and 4-positions in the phenyl ring leads to similar potency trends for both the 2-aminomethyl and 2-thiomethyl substituted series of compounds (Fig. 3). Thirdly, the compound potencies (in both the 2-aminomethyl and 2-thiomethyl substituted series) reversely depend on a radius of molecule gyration, which reflects better compatibility of the receptor binding site with more compact molecules (Fig. 2). Finally, in both the 2-aminomethyl-5,7-dimethyl and 2-thiomethyl-5,7-dimethyl substituted series of compounds, a clear reciprocal correlation between pK_i and partial negative charge of the 4-phenyl substituent was observed (Fig. 6).

4. Experimental section

4.1. Chemistry

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

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

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 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 micron, 20 × 250 mm (precolumn Reprosil-Pur C-18-AQ 10 micron, 20 × 50 mm) at a flow rate of 25 mL/min in gradient mode with mobile phase MeCN/water + 0.05% CF_3COOH.

According to the LC–MS, the purity of the obtained compounds exceeded 98.0%.

4.1.1. Aminopyrazoles 1–7, 16–21

Aminopyrazoles **1**–**7**, **16**–**21** were synthesized from corresponding arylsulfonylacetonitriles according to the procedures described previously.[14]

4.1.1.1. 3-(Methylthio)-4-(phenylsulfonyl)-1*H*-pyrazol-5-amine 1. ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.00 (br s, 1H), 7.91 (m, 2H), 7.63 (m, 1H), 7.57 (m, 2H), 6.10 (s, 2H), 2.32 (s, 3H). MS-ESI *m*/*z* 270 (M+H).

4.1.1.2. 4-(3-Chlorophenylsulfonyl)-3-(methylthio)-1*H*-pyrazol-5-amine 2. ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.04 (s, 1H), 7.94 (s, 1H), 7.88 (d, *J* = 7.6 Hz, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 6.24 (s, 2H), 2.34 (s, 3H). MS-ESI *m*/*z* 304, 306 (M+H).

4.1.1.3. 4-(4-Fluorophenylsulfonyl)-3-(methylthio)-1*H*-pyrazol-5-amine 3. MS-ESI *m*/*z* 288 (M+H).

4.1.1.4. 4-(3-Chloro-4-fluorophenylsulfonyl)-3-(methylthio)-1*H*-pyrazol-5-amine 4. MS-ESI *m*/*z* 322, 324 (M+H).

4.1.1.5. N^3-Methyl-4-(phenylsulfonyl)-1*H*-pyrazole-3,5-diamine 5. ^{1}H NMR (DMSO-d_6, 400 MHz), δ 10.94 (br m, 1H), 7.90 (m, 2H), 7.60 (m, 1H), 7.54 (m, 2H), 5.83 (br m, 1.5H), 5.00 (br s, 1.3H), 2.68 (d, *J* = 2.8 Hz, 3H). MS-ESI *m*/*z* 253 (M+H).

4.1.1.6. 4-(3-Chlorophenylsulfonyl)-N^3-methyl-1*H*-pyrazole-3,5-diamine 6. ^{1}H NMR (DMSO-d_6, 400 MHz), δ 10.97 (br s, 1H), 7.96 (s, 1H), 7.87 (d, *J* = 7.6 Hz, 1H), 7.67 (d, *J* = 7.6 Hz, 1H), 7.58 (t, *J* = 8.0 Hz, 1H), 5.82 (br s, 2H), 5.25 (br m, 1H), 2.68 (d, *J* = 4.4 Hz, 3H). MS-ESI *m*/*z* 287, 289 (M+H).

4.1.1.7. 4-(4-Fluorophenylsulfonyl)-N^3-methyl-1*H*-pyrazole-3,5-diamine 7. MS-ESI *m*/*z* 271 (M+H).

4.1.1.8. 4-(4-Chlorophenylsulfonyl)-3-(methylthio)-1*H*-pyrazol-5-amine 16. MS-ESI *m*/*z* 304, 306 (M+H).

4.1.1.9. 4-(3-Fluorophenylsulfonyl)-3-(methylthio)-1*H*-pyrazol-5-amine 17. ^{1}H NMR (DMSO-d_6, 400 MHz), δ 12.06 (br s, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 7. 72 (m, 1H), 7. 65 (td, J_1 = 8.0 Hz, J_2 = 5.6 Hz, 1H), 7.51 (td, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 6.19 (s, 2H), 2.34 (s, 3H). MS-ESI *m*/*z* 288 (M+H).

4.1.1.10. 4-(4-Methoxyphenylsulfonyl)-3-(methylthio)-1*H*-pyrazol-5-amine 18. MS-ESI *m*/*z* 300 (M+H).

4.1.1.11. 4-(3-Fluorophenylsulfonyl)-N^3 -methyl-1*H*-pyrazole-3,5-diamine 19. ^{1}H NMR (DMSO-d_6, 400 MHz), δ 10.99 (br s, 1H), 7.75 (m, 2H), 7.62 (td, J_1 = 8.8 Hz, J_2 = 6.4 Hz, 1H), 7.47 (td, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H), 5.89 (s, 2H), 5.06 (m, 1H), 2.49 (d, J = 3.6 Hz, 3H). MS-ESI m/z 271 (M+H).

4.1.1.12. 4-(3-Chloro-4-fluorophenylsulfonyl)-N^3 -methyl-1*H*-pyrazole-3,5-diamine 20. MS-ESI m/z 305, 307 (M+H).

4.1.1.13. 4-(4-Methoxyphenylsulfonyl)-N^3-methyl-1*H*-pyrazole-3,5-diamine 21. MS-ESI m/z 283 (M+H).

4.1.2. General procedure for synthesis of pyrazolo[1,5-*a*]pyrimidines 9–15, 23–33, 38–43, 46–49

A solution of 10 mmol of an aminopyrazole **1**–**7**, **16**–**21** and 11 mmol of either 1,1,3,3-tetramethoxypropane **8**, acetylacetone **22**, 4,4-dimethoxybutan-2-one **38** or sodium 1-methoxy-2,4-pentanedionate[16] **45** in 10–15 mL of AcOH was heated for 0.5–6 h at 100 °C. After reaction completion (LC–MS control), 5 mL of water was added and the heating continued for 1 h. The reaction mixture was cooled, poured into 150 mL of ice water and stirred for 1 h. The formed precipitate was filtered, washed with water, *i*-PrOH, hexane, and dried to obtain ARSPPs **9**–**15**, **23**–**33**, **38**–**43**, **46**–**49** with 60–91% yield. The formed mixtures of ARSPPs **39** and **42**, **40** and **43**, **46** and **48**, **47** and **49** were separated by column chromatography on silica gel (eluent—hexane/AcOEt = 2:1–0:1) or by HPLC.

4.1.2.1. 2-Methylthio-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine 9. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 8.70 (dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H), 8.59 (dd, J_1 = 1.6 Hz, J_2 = 6.8 Hz, 1H), 8.20 (m, 2H), 7.49–7.55 (m, 3H), 6.97 (dd, J_1 = 4.4 Hz, J_2 = 6.8 Hz, 1H), 2.62 (s, 3H). MS-ESI m/z 306 (M+H). LC–MS (UV-254) purity: 98%. Anal. Calcd for $C_{13}H_{11}N_3O_2S_2$: C, 51.13; H, 3.53; N, 13.76. Found: C, 50.91; H, 3.62; N, 13.70.

4.1.2.2. 3-(3-Chloro-phenylsulfonyl)-2-methylthio-pyrazolo[1,5-*a*]pyrimidine 10. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.73 (dd, J_1 = 1.2 Hz, J_2 = 4.4 Hz, 1H), 8.62 (dd, J_1 = 1.2 Hz, J_2 = 6.8 Hz, 1H), 8.19 (s, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.43 (t, J = 8.0 Hz, 1H), 7.01 (dd, J_1 = 4.4 Hz, J_2 = 6.8 Hz, 1H), 2.63 (s, 3H). MS-ESI m/z 340 (M+H). LC–MS (UV-254) purity: 98%. Anal. Calcd for $C_{13}H_{10}ClN_3O_2S_2$: C, 45.95; H, 2.97; N, 12.37. Found: C, 45.88; H, 2.99; N, 12.31.

4.1.2.3. 3-(4-Fluoro-phenylsulfonyl)-2-methylthio-pyrazolo[1,5-*a*]pyrimidine 11. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.71 (dd, J_1 = 4.4 Hz, J_2 = 2.0 Hz, 1H), 8.61 (dd, J_1 = 6.8 Hz, J_2 = 2.0 Hz, 1H), 8.22 (m, 2H), 7.16 (m, 2H), 7.00 (dd, J_1 = 6.8 Hz, J_2 = 4.4 Hz, 1H), 2.63 (s, 3H). MS-ESI m/z 324 (M+H). LC–MS (UV-254) purity: 98%. Anal. Calcd for $C_{13}H_{10}FN_3O_2S_2$: C, 48.29; H, 3.12; N, 12.99. Found: C, 48.36; H, 3.08; N, 13.04.

4.1.2.4. 3-(3-Chloro-4-fluoro-phenylsulfonyl)-2-methylthio-pyrazolo[1,5-*a*]pyrimidine 12. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.73 (m, 1H), 8.63 (d, J = 7.2 Hz, 1H), 8.29 (dd, J_1 = 6.8 Hz, J_2 = 2.0 Hz, 1H), 8.11 (ddd, J_1 = 6.8 Hz, J_2 = 4.4 Hz, J_3 = 2.0 Hz, 1H), 7.25 (t, J = 8.8 Hz, 1H), 7.02 (dd, J_1 = 6.0 Hz, J_2 = 4.4 Hz, 1H), 2.63 (s, 3H). MS-ESI m/z 358 (M+H). LC–MS (UV-254) purity: 98%. Anal. Calcd for $C_{13}H_{10}ClFN_3O_2S_2$: C, 43.64; H, 2.54; N, 11.74. Found: C, 43.47; H, 2.61; N, 11.68.

4.1.2.5. (3-Phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine 13. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 8.96 (dd, J_1 = 0.8 Hz, J_2 = 6.8 Hz, 1H), 8.55 (dd, J_1 = 1.2 Hz, J_2 = 4.4 Hz, 1H), 8.02 (m, 2H), 7.54–7.64 (m, 3H), 7.07 (dd, J_1 = 4.4 Hz, J_2 = 6.8 Hz, 1H), 6.47 (q, J = 4.8 Hz, 1H), 2.91 (d, J = 4.8 Hz, 3H). MS-ESI m/z 289 (M+H). LC–MS (UV-254) purity: 98%. Anal. Calcd for $C_{13}H_{12}N_4O_2S$: C, 54.15; H, 4.20; N, 19.43. Found: C, 54.19; H, 4.11; N, 19.44.

4.1.2.6. [3-(3-Chloro-phenylsulfonyl)-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methyl-amine 14. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.53 (dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H), 8.46 (dd, J_1 = 1.6 Hz, J_2 = 6.4 Hz, 1H), 8.13 (s, 1H), 8.04 (d, J = 7.6 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.43 (t, J = 8.0 Hz, 1H), 6.87 (dd, J_1 = 4.4 Hz, J_2 = 6.4 Hz, 1H), 6.07 (q, J = 5.2 Hz, 1H), 3.06 (d, J = 5.2 Hz, 3H). MS-ESI m/z 323 (M+H). LC–MS (UV-254) purity: 98%. Anal. Calcd for $C_{13}H_{11}ClN_4O_2S$: C, 48.37; H, 3.44; N, 17.36. Found: C, 48.44; H, 3.46; N, 17.41.

4.1.2.7. [3-(4-Fluoro-phenylsulfonyl)-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methyl-amine 15. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.50 (dd, J_1 = 4.4 Hz, J_2 = 1.6 Hz, 1H), 8.45 (dd, J_1 = 6.8 Hz, J_2 = 1.6 Hz, 1H), 8.14–8.18 (m, 2H), 7.13–7.18 (m, 2H), 6.85 (dd, J_1 = 6.8 Hz, J_2 = 4.4 Hz, 1H), 6.08 (q, J = 4.8 Hz, 1H), 3.06 (d, J = 4.8 Hz, 3H). MS-ESI m/z 307 (M+H). LC–MS (UV-254) purity: 98%. Anal. Calcd for $C_{13}H_{11}FN_4O_2S$: C, 50.97; H, 3.62; N, 18.29. Found: C, 50.81; H, 3.67; N, 18.22.

4.1.2.8. 3-(4-Fluoro-phenylsulfonyl)-5,7-dimethyl-2-methylthio-pyrazolo[1,5-*a*]pyrimidine 23. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.23 (m, 2H), 7.13 (t, J = 4.6 Hz, 3H), 6.69 (s, 1H), 2.69 (s, 3H), 2.64 (s, 3H), 2.62 (s, 3H) MS-ESI m/z 352 (M+H). LC–MS (UV-254) purity: 98%. Anal. Calcd for $C_{15}H_{14}FN_3O_2S_2$: C, 51.27; H, 4.02; N, 11.96. Found: C, 51.10; H, 4.15; N, 11.93.

4.1.2.9. 3-(4-Chloro-phenylsulfonyl)-5,7-dimethyl-2-methylthio-pyrazolo[1,5-*a*]pyrimidine 24. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 8.01 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 8.8 Hz, 2H), 7.13 (s, 1H), 2.66 (s, 3H), 2.58 (m, 6H). MS-ESI m/z 368 (M+H). LC–MS (UV-254) purity: 98%. Anal. Calcd for $C_{15}H_{14}ClN_3O_2S_2$: C, 48.97; H, 3.84; N, 11.42. Found: C, 49.02; H, 3.80; N, 11.43.

4.1.2.10. 3-(3-Fluoro-phenylsulfonyl)-5,7-dimethyl-2-methylthio-pyrazolo[1,5-*a*]pyrimidine 25. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.00 (d, J = 8.0 Hz, 1H), 7.96 (dt, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H), 7.45 (td, J_1 = 8.0 Hz, J_2 = 5.2 Hz, 1H), 7.21 (tdd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, J_3 = 0.4 Hz, 1H), 6.70 (s, 1H), 2.70 (s, 3H), 2.66 (s, 3H), 2.63 (s, 3H). MS-ESI m/z 352 (M+H). LC–MS (UV-254) purity: 98%. Anal. Calcd for $C_{15}H_{14}FN_3O_2S_2$: C, 51.27; H, 4.02; N, 11.96. Found: C, 51.13; H, 4.09; N, 11.89.

4.1.2.11. 3-(3-Chloro-phenylsulfonyl)-5,7-dimethyl-2-methylthio-pyrazolo[1,5-*a*]pyrimidine 26. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 8.26 (m, 1H), 8.09 (d, J = 7.6 Hz, 1H), 7.48 (m, 1H), 7.41 (t, J = 7.6 Hz, 1H), 6.70 (s, 1H), 2.70 (s, 3H), 2.66 (s, 3H), 2.63 (s, 3H). MS-ESI m/z 368 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.12. 3-(3-Chloro-4-fluoro–phenylsulfonyl)-5,7-dimethyl-2-methylthio-pyrazolo[1,5-*a*]pyrimidine 27. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.35 (dd, J_1 = 6.8 Hz, J_2 = 2.0 Hz, 1H), 8.11 (ddd, J_1 = 6.8 Hz, J_2 = 4.4 Hz, J_3 = 2.0 Hz, 1H), 7.22 (t, J = 8.8 Hz, 1H), 6.71 (d, J = 0.8 Hz, 1H), 2.71 (d, J = 0.8 Hz, 3H), 2.66 (s, 3H), 2.63 (s, 3H). MS-ESI m/z 386 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.13. [4-(5,7-Dimethyl-2-methylthio-pyrazolo[1,5-*a*]pyrimidine-3-sulfonyl)-phenyl]-dimethyl-amine 28. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 7.77 (d, J = 9.2 Hz, 2H), 7.06 (s, 1H), 6.73 (d, J = 8.8 Hz, 2H), 2.95 (s, 6H), 2.64 (s, 3H), 2.56 (m, 6H). MS-ESI m/z 377 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.14. 5,7-Dimethyl-2-methylthio-3-(4-methoxy-phenylsulfonyl)-pyrazolo[1,5-*a*]pyrimidine 30. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 7.94 (d, J = 8.8 Hz, 2H), 7.09 (m, 3H), 3.79 (s, 3H),

2.65 (m, 3H), 2.57 (s, 6H). MS-ESI *m*/*z* 364 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.15. 5,7-Dimethyl-[3-(4-fluoro-phenylsulfonyl)-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methyl-amine 29. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.18 (m, 2H), 7.13 (m, 2H), 6.56 (s, 1H), 5.99 (q, J = 4.8 Hz, 1H), 3.05 (d, J = 4.8 Hz, 3H), 2.61 (s, 1H), 2.56 (s, 1H). MS-ESI *m*/*z* 335 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.16. 5,7-Dimethyl-[3-(3-fluoro-phenylsulfonyl)-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methyl-amine 30. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 7.85 (m, 2H), 7.63 (dt, J_1 = 7.6 Hz, J_2 = 6.0 Hz, 1H), 7.47 (dt, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H), 6.94 (s, 1H), 6.37 (q, J = 4.4 Hz, 1H), 2.91 (d, J = 4.4 Hz, 3H), 2.55 (s, 3H), 2.49 (s, 3H). MS-ESI *m*/*z* 335 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.17. [3-(3-Chloro-phenylsulfonyl)-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methyl-amine 31. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 8.22 (s, 1H), 8.04 (d, J = 7.6 Hz, 1H), 7.44 (m, 1H), 7.40 (t, J = 7.6 Hz, 1H), 6.57 (s, 1H), 5.97 (q, J = 5.2 Hz, 1H), 3.05 (d, J = 5.2 Hz, 3H), 2.61 (s, 3H), 2.58 (s, 3H). MS-ESI *m*/*z* 351 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.18. [3-(3-Chloro-4-fluoro-phenylsulfonyl)-5,7-dimethyl-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methyl-amine 32. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.32 (dd, J_1 = 6.8 Hz, J_2 = 2.4 Hz, 1H), 8.11 (ddd, J_1 = 6.8 Hz, J_2 = 4.4 Hz, J_3 = 2.4 Hz, 1H), 7.21 (t, J = 8.8 Hz, 1H), 6.58 (s, 1H), 5.95 (q, J = 5.2 Hz, 1H), 3.05 (d, J = 5.2 Hz, 3H), 2.62 (s, 3H), 2.57 (s, 3H). MS-ESI *m*/*z* 369 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.19. [5,7-Dimethyl-3-(4-methoxy-phenylsulfonyl)-pyrazolo[1,5-*a*]pyrimidin-2-yl]-methyl-amine 33. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 7.94 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 8.8 Hz, 2H), 6.89 (s, 1H), 6.31 (q, J = 4.8 Hz, 1H), 3.79 (s, 3H), 2.91 (d, J = 4.8 Hz, 3H), 2.54 (s, 3H), 2.47 (s, 3H). MS-ESI *m*/*z* 347 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.20. Mixture of 5-methyl-2-methylthio-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine 38 and of 7-methyl-2-methylthio-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine 41 ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.58 (d, J = 4.8 Hz, 1H), 8.41 (d, J = 7.2 Hz, 1.4H), 8.21 (m, 4.8H), 7.50 (m, 7.2H), 6.82 (d, J = 4.8 Hz, 1H), 6.80 (d, J = 7.2 Hz, 1.4H), 2.76 (s, 3H), 2.69 (s, 4.2H), 2.64 (s, 3H), 2.59 (s, 4.2H). The most distinguished are the doublet signals at 8.58 and 8.41 ppm corresponding to the protons in position 5 and 7 of ARSPPs **38** and **41**. Their integrals ratio is 1:1.4. At the same time, the chemical shift of the doublet at 8.41 ppm is identical to those in the spectrum of independently synthesized ARSPP **38**.

4.1.2.21. 3-(3-Chloro-4-fluoro-phenylsulfonyl)-5-methyl-2-methylthio-pyrazolo[1,5-*a*]pyrimidine 39. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.60 (d, J = 4.4 Hz, 1H), 8.28 (dd, J_1 = 6.4 Hz, J_2 = 2.0 Hz, 1H), 8.11 (ddd, J_1 = 6.4 Hz, J_2 = 4.4 Hz, J_3 = 2.0 Hz, 1H), 7.23 (t, J = 8.8 Hz, 1H), 6.85 (d, J = 4.4 Hz, 1H), 2.79 (s, 3H), 2.66 (s, 3H). MS-ESI *m*/*z* 372 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.22. (5-Methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine 40. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.38 (d, J = 4.4 Hz, 1H), 8.13–8.15 (m, 2H), 7.45–7.54 (m, 3H), 6.69 (d, J = 4.4 Hz, 1H), 6.08 (q, J = 3.6 Hz, 1H), 3.08 (d, J = 5.2 Hz, 3H), 2.67 (s, 3H). MS-ESI *m*/*z* 303 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.23. 3-(3-Chloro-4-fluoro-phenylsulfonyl)-7-methyl-2-methylthio-pyrazolo[1,5-*a*]pyrimidine 42. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.44 (d, J = 7.2 Hz, 1H), 8.35 (dd, J_1 = 6.8 Hz, J_2 = 2.0 Hz, 1H), 8.11 (ddd, J_1 = 6.4 Hz, J_2 = 4.0 Hz, J_3 = 2.0 Hz, 1H), 7.24 (t, J = 8.8 Hz, 1H), 6.85 (d, J = 7.2 Hz, 1H), 2.71 (s, 3H), 2.61 (s, 3H). MS-ESI *m*/*z* 372 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.24. (7-Methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine 43. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.26 (d, J = 7.2 Hz, 1H), 8.16–8.18 (m, 2H), 7.46–7.56 (m, 3H), 6.67 (d, J = 6.8 Hz, 1H), 6.06 (q, J = 4.4 Hz, 1H), 3.03 (d, J = 5.2 Hz, 3H), 2.61 (s, 3H). MS-ESI *m*/*z* 303 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.25. 7-Methoxymethyl-5-methyl-2-methylthio-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine 46. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.22 (m, 2H), 7.45–7.55 (m, 3H), 6.96 (s, 1H), 4.85 (s, 2H), 3.59 (s, 3H), 2.70 (s, 3H), 2.59 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ: 162.89, 156.15, 147.27, 145.14, 142.98, 132.30, 128.30, 126.45, 106.39, 105, 81, 66.88, 59.35, 25.08, 12.88. MS-ESI *m*/*z* 364 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.26. (7-Methoxymethyl-5-methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine 47. ^{1}H NMR ($CDCl_3$, 400 *M*Hz), δ: 8.17 (m, 2H), 7.45–7.55 (m, 3H), 6.84 (s, 1H), 6.05 (d, J = 4.8 Hz, 1H), 4.78 (s, 2H), 3.57 (s, 3H), 3.03 (d, J = 4.8 Hz, 3H), 2.62 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ: 161.41, 158.09, 147.29, 144.78, 143.55, 132.01, 128.26, 126.04, 105.17, 90.58, 67.16, 59.25, 28.68, 24.78. MS-ESI *m*/*z* 347 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.27. 5-Methoxymethyl-7-methyl-2-methylthio-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine 48. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.20 (d, J = 7.2 Hz, 2H), 7.45–7.55 (m, 3H), 7.05 (s, 1H), 4.66 (s, 2H), 3.52 (s, 3H), 2.75 (s, 3H), 2.63 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ: 162.37, 156.32, 147.11, 146.25, 142.99, 132.27, 128.29, 126.51, 106.43, 106.15, 74.42, 58.70, 16.74, 12.87. MS-ESI *m*/*z* 364 (M+H). LC–MS (UV-254) purity: 98%.

4.1.2.28. (5-Methoxymethyl-7-methyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-2-yl)-methyl-amine 49. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.14 (m, 2H), 7.44–7.54 (m, 3H), 6.90 (s, 1H), 6.06 (q, J = 5.2 Hz, 1H), 4.59 (s, 2H), 3.49 (s, 3H), 3.07 (d, J = 5.2 Hz, 3H), 2.66 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ: 160.74, 158.05, 147.13, 145.86, 143.49, 132.03, 128.25, 126.01, 105.66, 90.74, 74.40, 58.61, 28.66, 16.94. MS-ESI *m*/*z* 347 (M+H). LC–MS (UV-254) purity: 98%.

4.1.3. Synthesis of [4-(5,7-dimethyl-2-methylthio-pyrazolo[1,5-*a*]pyrimidine-3-sulfonyl)-phenyl]-dimethyl-amine 34

To a solution of 87 mg (0.24 mmol) of 3-[(4-chlorophenyl)sulfonyl]-5,7-dimethyl-2-(methylthio)pyrazolo[1,5-*a*]pyrimidine (**24**) in 0.5 mL of DMF was added 1 mL 40% aqueous dimethylamine, and the mixture was stirred in a closed tube at 140 °C for 48 h. After the cooling the formed precipitate was separated by centrifugation, suspended in hot MeOH, cooled and centrifuged again, washed with *i*-PrOH, hexane and dried in vacuum. Yield 72%. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 7.77 (d, J = 9.2 Hz, 2H), 7.06 (s, 1H), 6.73 (d, J = 8.8 Hz, 2H), 2.95 (s, 6H), 2.64 (s, 3H), 2.56 (m, 6H). MS-ESI *m*/*z* 377 (M+H). LC–MS (UV-254) purity: 98%.

4.1.4. General procedure for synthesis of 5,7-dimethyl-3-(4-hydroxyphenyl)-ARSPP 35, 36

A solution of 0.5 mmol of methoxyderivative **28** or **33** in 10 mL of 40% HBr was refluxed for 24 h. After reaction completion (LC–MS control), the solvent was stripped off in vacuo and the residue was separated by column chromatography on silica gel (eluent—hexane/AcOEt = 1:1 for ARSPP **35**, 1:2 for ARSPP **36**). Yield 52–61%.

4.1.4.1. 4-(5,7-Dimethyl-2-methylthio-pyrazolo[1,5-*a*]pyrimidine-3-sulfonyl)-phenol 35. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 10.46 (br, 1H), 7.84 (d, J = 8.0 Hz, 2H), 7.36 (s, 1H), 7.07 (s, 1H), 6.87 (d, J = 8.0 Hz, 2H), 2.64 (s, 3H), 2.56 (s, 6H). MS-ESI m/z 350 (M+H). LC–MS (UV-254) purity: 98%.

4.1.4.2. 4-(5,7-Dimethyl-2-methylamino-pyrazolo[1,5-*a*]pyrimidine-3-sulfonyl)-phenol 36. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 10.37 (s, 1H), 7.83 (d, J = 8.8 Hz, 2H), 6.87 (s, 1H), 6.85 (d, J = 8.8 Hz, 2H), 6.28 (q, J = 4.8 Hz, 1H), 2.91 (d, J = 4.8 Hz, 3H), 2.54 (s, 3H), 2.47 (s, 3H). MS-ESI m/z 333 (M+H). LC–MS (UV-254) purity: 98%.

4.1.5. Synthesis of 5-methyl-2-methylthio-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidine 38

A solution of 354 mg (1 mmol) of 7-chloro-5-methyl-2-methylthio-3-phenylsulfonylpyrazolo[1,5-*a*]pyrimidine **44** in 15 mL of methanol and 5 mL of benzene was stirred under hydrogen with 100 mg of 10% Pd/C. The mixture was filtered through Celite and solvent was removed by roto-evaporation. ARSPP **38** was isolated by column chromatography on silica gel (eluent—DCM) with a yield of 22%. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.41 (d, J = 7.2 Hz, 1H), 8.20–8.23 (m, 2H), 7.54 (m, 1H), 7.49 (m, 2H), 6.80 (d, J = 7.2 Hz, 1H), 2.69 (s, 3H), 2.59 (s, 3H). MS-ESI m/z 320 (M+H). LC–MS (UV-254) purity: 98%.

4.1.6. General procedure for synthesis of (3-phenyl-ARSPP-yl)-methanols 50–53

A solution of 1.2 mmol of BBr_3 in 10 mL of DCM was added to a solution of 0.4 mmol of ARSPP **46**–**49** in 10 mL of DCM and the mixture was stirred for 12 h at ambient temperature. The mixture was quenched by the addition of 20 mL of water and stirred vigorously for 30 min. The mixture was extracted three times with DCM, the combined extracts were washed with water, dried over Na_2SO_4 and evaporated in vacuo. The residue was separated by column chromatography on silica gel (eluent—$CHCl_3$/AcOEt = 10:1) to obtain ARSPPs **50**–**53** with a yield of 42–61%.

4.1.6.1. (5-Methyl-2-methylthio-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-7-yl)-methanol 50. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 8.02 (m, 2H), 7.56–7.65 (m, 3H), 7.18 (s, 1H), 5.98 (t, J = 5.6 Hz, 1H), 4.90 (d, J = 5.6 Hz, 2H), 2.64 (s, 3H), 2.55 (s, 3H). ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.16 (m, 2H), 7.53 (m, 1H), 7.47 (m, 23H), 6.88 (s, 1H), 5.00 (d, J = 6.8 Hz, 2H), 3.70 (t, J = 6.8 Hz, 1H), 2.63 (s, 3H), 2.58 (s, 3H). MS-ESI m/z 350 (M+H). LC–MS (UV-254) purity: 98%.

4.1.6.2. (5-Methyl-2-methylamino-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-7-yl)-methanol 51. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.15 (d, J = 7.2 Hz, 2H), 7.54 (m, 1H), 7.48 (m, 2H), 6.68 (s, 1H), 6.11 (q, J = 4.8 Hz, 1H), 4.89 (d, J = 6.8 Hz, 2H), 3.92 (t, J = 6.8 Hz, 1H), 3.03 (d, J = 4.8 Hz, 3H), 2.59 (s, 3H). MS-ESI m/z 333 (M+H). LC–MS (UV-254) purity: 98%.

4.1.6.3. (7-Methyl-2-methylthio-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-5-yl)-methanol 52. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.16 (m, 2H), 7.46–7.56 (m, 3H), 6.81 (s, 1H), 4.83 (d, J = 4.8 Hz, 2H), 3.73 (t, J = 4.8 Hz, 1H), 2.73 (s, 3H), 2.64 (s, 3H). MS-ESI m/z 350 (M+H). LC–MS (UV-254) purity: 98%.

4.1.6.4. (7-Methyl-2-methylamino-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidin-5-yl)-methanol 53. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.11 (m, 2H), 7.53 (m, 1H), 7.48 (m, 2H), 6.64 (s, 1H), 6.05 (q, J = 4.8 Hz, 1H), 4.74 (d, J = 5.2 Hz, 2H), 3.49 (t, J = 5.2 Hz, 1H), 3.07 (d, J = 4.8 Hz, 3H), 2.65 (s, 3H). MS-ESI m/z 333 (M+H). LC–MS (UV-254) purity: 98%.

4.2. Biology

4.2.1. Cell handling

The 5-HT_6R was sub-cloned into T-Rex system (Invitrogen, Carlsbad, CA) and expressed into HEK (5-HT6R-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-HT6 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.

4.2.2. Measurements of the 5-HT_6R-HEK cell responses

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 pipette tip several times to break down cell aggregates, washed with Serum Free Medium, and counted. The cells were re-suspended to 0.67×10^6 cells/mL in an HBSS buffer supplemented with 5 mM HEPES, pH 7.4, 0.05% BSA, and 1 mM IBMX (Sigma–Aldrich, St. Louis, MO) containing Alexa Fluor 647-anti cAMP antibody (from LANCE cAMP 384 kit, PerkinElmer, Waltham, MA). 6-μL (~4000 cells/well) aliquots were then transferred into 384-well assay plates (PerkinElmer White OptiPlates). The test compounds, at different concentrations, were premixed with serotonin hydrochloride (Sigma, MO) and added to the cells (final serotonin concentration 10 nM, final DMSO concentration 0.32%, final IBMX concentration 500 μM). Each assay plate contained serotonin and cAMP standard concentration curves for the assay quality control. After 2 h of incubation with the mixture of compound/serotonin, cells were treated as described in the cAMP LANCE assay kit protocol (PerkinElmer, Waltham, MA). The LANCE signal was measured using multimode plate reader VICTOR 3 (PerkinElmer, Waltham, MA) with built-in settings for the LANCE detection.

4.3. Curve fitting and statistical analysis

The concentration curve data were fitted with Prism 5 (GraphPad, CA) using built-in 4-parametric equation to calculate IC_{50} values. All experiments were performed in duplicate. Standard deviations (SD) were calculated with Prism built-in statistical package. K_i values for functional 5-HT_6 receptor inhibition assays were calculated using Cheng–Prusoff's[19] modified equation, $K_i = IC_{50}/(1 + [Ag]/EC_{50})$. Where IC_{50} is the concentration of antagonist causing 50% inhibition of serotonin-induced cell response; [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 plates. The mean EC_{50} value for serotonin-induced cAMP production in 5-HT_6R-HEK cells was 1.91 ± 0.13 nM as determined from 4 independent experiments with three to five repeats (separate plates), each in quadruplicates.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.12.055. These data include MOL files and InChiKeys of the most important compounds described in this article.

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