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Synthesis of cycloalkane-annelated 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines and their evaluation as 5-HT$_6$ receptor antagonists

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ABSTRACT

Synthesis and biological evaluation of 1 ('angular') and 2 ('linear') cycloalkane-annelated 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines as novel ligands of the 5-HT$_6$ receptors are disclosed. The new compounds 1 and 2 are highly selective antagonists of the receptor with sub-nanomolar affinities (K_i <1 nM). In its structure, this new chemotype lacks a basic ionizable side chain, which is considered as the characteristic feature of the 5-HT$_6$ receptor antagonists pharmacophore model.

Since the discovery of serotonin 5-HT$_6$ receptors (5-HT$_6$R), multiple selective antagonists were synthesized. Majority of these are based on heterocyclic structures containing a sulfonyl substituent.[1–3] 3-Phenylsulfonyl-8-piperazin-1-yl-quinoline (SB-742457, GlaxoSmithKline, Fig. 1)[4] is in an advanced stage of clinical development—currently in phase II of clinical trials for the treatment of Alzheimer's disease.[5]

Another series of 5-HT$_6$R antagonists,[6] substituted 8-amino-2-methylsulfanyl-3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines, has been developed by Roche. The leader in this series of compounds is 2-methylsulfanyl-3-phenylsulfonyl-8-piperazin-1-yl-6,7-dihydro-5*H*-cyclopenta[d]pyrazolo[1,5-*a*]pyrimidine (Ro-65-7674, Roche, Fig. 1) with good DMPK properties in preclinical studies. However, this molecule was shown to exhibit quite a high potency hERG channel inhibition activity with IC$_{20}$ of 0.2 μM.[7]

It should be noted that the role of 5-HT$_6$R in the etiology of different diseases is still elusive, mainly due to a lack of safe, highly selective and potent ligands with a good blood–brain barrier permeability. Therefore, the quest for discovery of new potent and selective antagonists of 5-HT$_6$R as potential drugs for the treatment of various diseases of the central nervous system, continues unabated.[1,8,9]

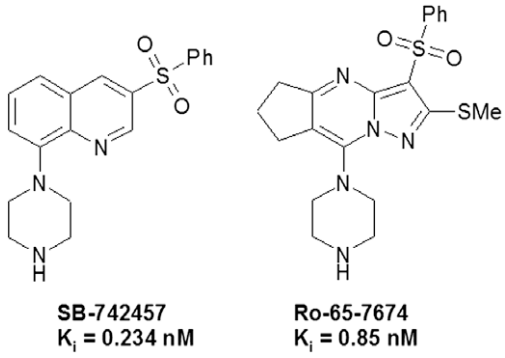

Figure 1. Structure of sulfonyl-substituted drug candidates currently in clinical trials.

The pharmacophore model (Fig. 2A) of 5-HT$_6$R ligands has been developed based on 3D analysis of 45 structurally diverse antagonists.[10] The model includes a central aromatic or heterocyclic scaffold, AR, separating a positive ionizable group, PI (usually secondary or tertiary amino-group), from a strong multiple hydrogen

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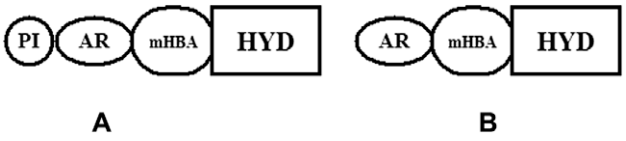
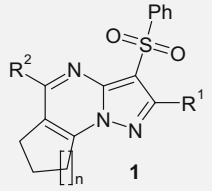
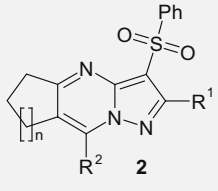


Figure 2. (A) Simplified schematic representation of a 5-HT$_6$R pharmacophore model,[10] which does not reflect the interatomic distances, vectors, and 3D atom locations. (B) Representation of a new pharmacophore model. AR—aromatic or heterocyclic scaffold; PI—positive ionizable group; mHBA—multiple hydrogen bond acceptor group; HYD—hydrophobic site.

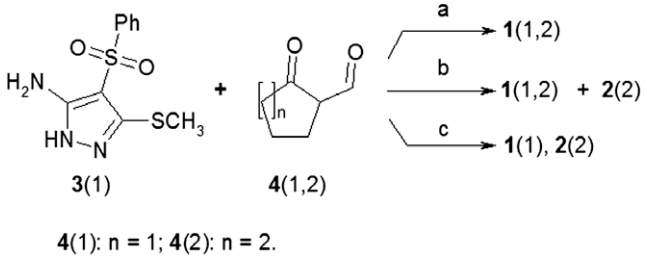
Table 1
Structure of 'angular', **1**, and 'linear', **2**, sulfonyl-substituted cycloalakane-annelated 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines





Compd	*n*	R^1	R^2
1(1)	1	SMe	H
1(2)	2	SMe	H
1(3)	1	Me	Me
1(4)	2	Me	Me
1(5)	1	SMe	Me
1(6)	2	SMe	Me
1(7)	2	OCH_2CH_2OH	Me
2(2)	2	SMe	H
1(3)*	1	Me	Me
2(4)	2	Me	Me
1(5)*	1	SMe	Me
2(6)	2	SMe	Me
2(7)	2	OCH_2CH_2OH	Me

* The compound was not separated from the mixture.



Scheme 1. Synthesis of cycloalkane-annelated 2-methylsulfanyl-PSPP **1**(1,2) and **2**(2).

bond acceptor group, mHBA, (usually sulfonyl or sulfamide group), and a hydrophobic site, HYD (for example, phenyl). The basic ionizable group, PI, is considered a common pharmacophoric element of most of the ligands that interact with biogenic amine GPCRs.

However, we have found no publications, which would convincingly indicate the necessity of the basic side chain for effective interaction with the receptor. Therefore, the purpose of this communication is to establish if the basic side chain is essential for the high affinity binding of the ligands to the 5-HT$_6$R. For this, we have synthesized several analogs of the Ro-65-7674. The synthesized compounds represent a family of previously unknown cycloalkane-annelated 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines (PSPP), **1** ('angular') and **2** ('linear') analogs (Table 1) and examined their affinity to the 5-HT$_6$R. The characteristic feature of these molecules is the absence of the basic side chain, PI, in their structures.

The PSPPs **1**, **2** were synthesized by the interaction of the 3-aminopyrazoles **3** with the corresponding β-dicarbonyl compounds **4** (Schemes 1 and 2).

We found that the interaction of 3-amino-5-methylsulfanyl-4-phenylsulfonyl-2*H*-pyrazole **3**(1) with 2-formylcyclopentanone **4**(1) in acetic acid with or without HCl at either room temperature or 100 °C led to synthesis of the 2-methylsulfanyl-3-phenylsulfonyl-7,8-dihydro-6*H*-cyclopenta[e]pyrazolo[1,5-*a*]pyrimidine **1**(1) only (yield 42%). The reaction of **3**(1) with 2-formylcyclohexanone **4**(2) in acetic acid, at 100 °C, led to synthesis of both the 2-methylsulfanyl-3-phenylsulfonyl-6,7,8,9-tetrahydro-pyrazolo[1,5-*a*]quinazoline, **1**(2), and 2-methylsulfanyl-3-phenylsulfonyl-5,6,7,8-tetrahydro-pyrazolo[5,1-*b*]quinazoline, **2**(2), in approximately equal proportion. At the same time, this reaction performed in a mixture of acetic acid and HCl at room temperature, produced PSPP **1**(2) with a yield of 59%. When performed at room temperature in acetic acid only, the reaction produced PSPP **2**(2) with a yield of 53%.

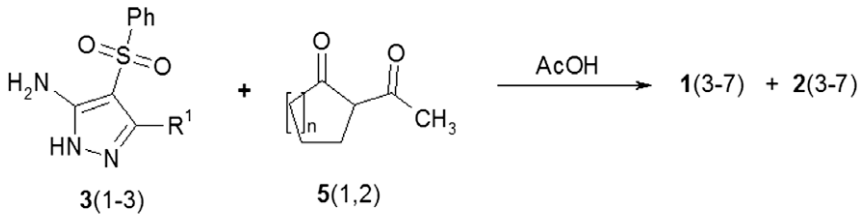
The reaction of 3-amino-2*H*-pyrazoles **3**(1–3) with β-diketones 5(1,2), regardless of the conditions presented in Scheme 1 as well as in alcohol in presence of triethylamine, led to synthesis of a mixture of angular **1**(3–7) and linear **2**(3–7) products (Scheme 2).

In reactions with 2-acetylcyclopentanone **5**(1), the main products were represented by angular compounds **1**(3,5) (>90%). Pure 5-methyl-3-phenylsulfonyl-7,8-dihydro-6*H*-cyclopenta[e]pyrazolo[1,5-*a*]pyrimidines **1**(3,5) were isolated from the mixtures by repeated re-crystallization.

In reactions with 2-acetylcyclohexanone **5**(2), a barely separable mixture comprising 50–70% of the linear products was formed. Using HPLC, we were able to separate individual angular **1**(6) and linear **2**(6) compounds.

The structures of the synthesized compounds **1**, **2** were confirmed by LC–MS and NMR data. Structural assignments, angular versus linear, of the synthesized compounds **1**, **2** were made based on 2D NMR experiments (NOESY and HMBC) using published data[11–13] for the NMR spectra and quantum-mechanical calculations of such heterocyclic compounds.

We investigated the effects of the substitutions in the R^1 and R^2 positions as well as those of the cycloalkane size on the affinity of the compounds **1** and **2** to the 5-HT$_6$R. The compound affinities



Scheme 2. Synthesis of cycloalkane-annelated 5-methyl-PSPP **1**(3–7) and 7-methyl-PSPP **2**(3–7).

Table 2
Comparison of binding affinities (K_i) and antagonistic potencies (IC_{50}) of angular, **1**, and linear, **2**, compounds

Compd #	K_i,[a] nM (binding)	IC_{50},[b] nM (functional)	
		5-HT_6	5-HT_{2B}
1(1)	0.458	6.0	118.0
1(2)	0.549	7.5	411.0
2(2)	7.97	98.5	
1(4) + **2**(4)	4.42	26.8	
1(6)	0.440	16.8	161.0
2(6)	0.680	7.8	127.0
1(6) + **2**(6)	0.479	16.9	

[a] Values are averages from single experiment performed in duplicates.
[b] Values are geometric means of three independent experiments. There was less than twofold deviation of IC_{50} values from the geometric means of the experiments.

(Table 2) were measured in a competitive radioligand binding assay as described in the Supplementary data. Affinities of the angular molecules were not affected by either ring expansion from cyclopentyl (**1**(1)) to cyclohexyl (**1**(2)) or by substitution of proton (**1**(2)) for CH_3 (**1**(6)) in R^2 position. Contrary, linear molecules seemed to be quite sensitive to the substitution in R^2 position. Inclusion of a methyl group in **2**(6) led to 10-fold increase in the affinity compared to **2**(2) (Table 2). The affinity of a product mixture **1**(6) + **2**(6), obtained from the reaction with 2-acetylcyclohexanone **5**(2), was similar (K_i = 0.48 nM) to those of the individual components. Substitution of SCH_3 group in R^1 position with CH_3 group in compounds of a mixture **1**(4) + **2**(4) also led to almost 10-fold reduction in the affinity as compared with the respective **1**(6) + **2**(6) product.

Similar SAR trends were observed in a cell-based functional assay[14] where the potency, IC_{50}, of the compounds of series **1** and **2** to block serotonin-induced increase of cAMP level in HEK293 cells expressing 5-HT_6 receptor was measured. For example, the linear ligand **2**(2) was order of magnitude less potent than its angular analog **1**(2) (IC_{50} = 98.5 nM and 7.5 nM, respectively, Table 2). The ring expansion from cyclopentyl to cyclohexyl in the series of angular compounds (**1**(1) versus **1**(2)) affected neither binding affinity nor functional potency of the compounds.

The product **1**(6) + **2**(6) was tested in radioligand competition binding assays (as described in the Supplementary data) on a panel of therapeutic targets comprising enzyme, GPCR, ion channel, and transporter families (Fig. 3). This allowed us to simultaneously assess specificity of both the linear and angular forms. The **1**(6) + **2**(6) combination was highly specific and selective towards 5-HT_6R. At 1 μM (approx. 0.5 μM each), the product **1**(6) + **2**(6) besides 5-HT_6R (100 ± 7%) strongly interacted, with only two other targets, 5-HT_{2B}R (85 ± 3%) and peripheral benzodiazepine receptor (PBR) (66 ± 7%).

Ligands, **1**(1,2,6), and **2**(6), were also examined for their 5-HT_{2B}R functional activity[15] (Table 2). Stimulation of this receptor is thought to be potentially involved in cardiac valvulopathy[16] and represents potential liability. Effect of the compounds was assessed in a cell-based functional assay in both the agonistic and

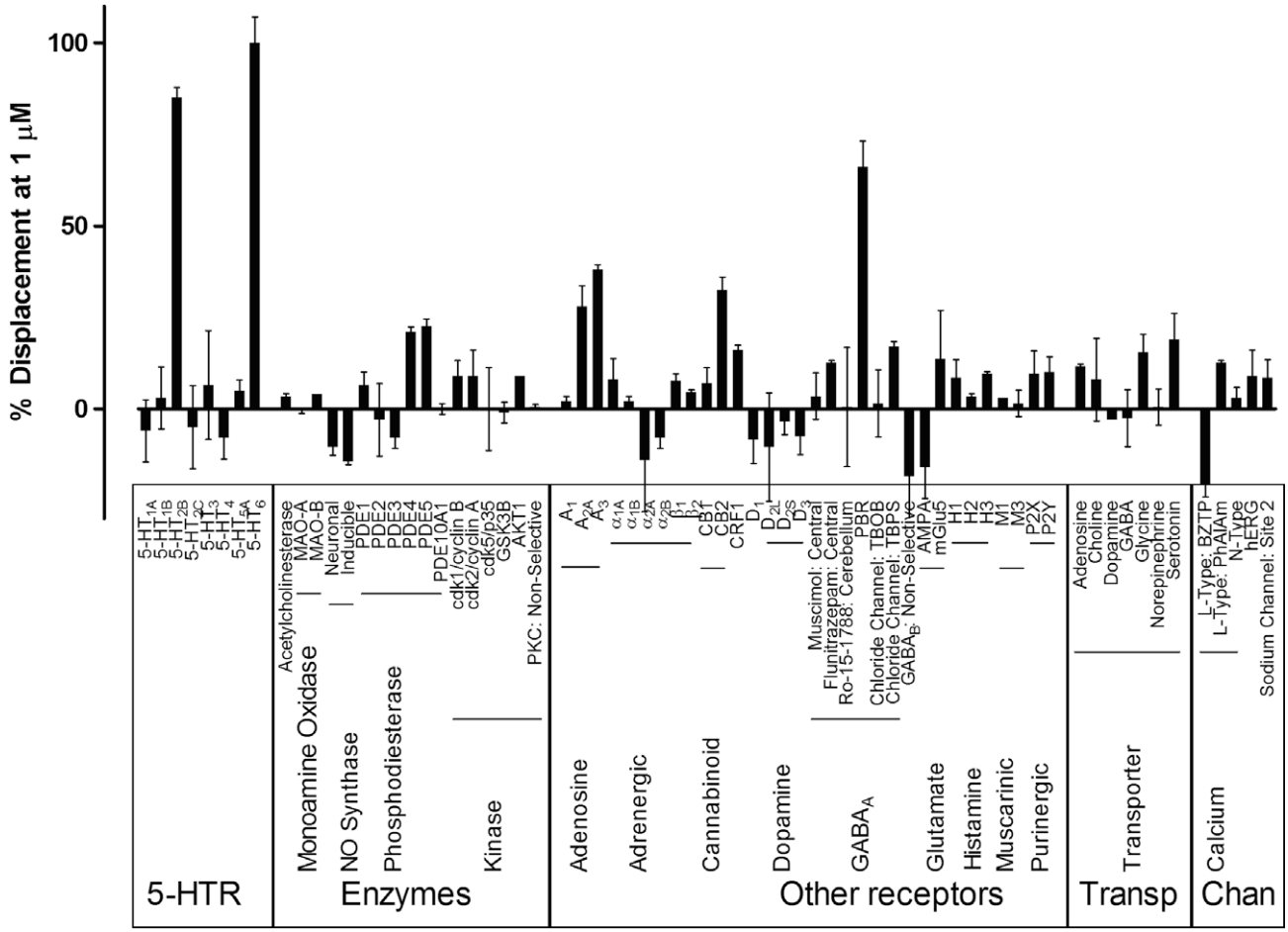


Figure 3. Target-specific profile determined for a **1**(6) + **2**(6) product consisting of 1:1 mixture of 5-methyl-5-methylsulfanyl-3-phenylsulfonyl-6,7,8,9-tetrahydro-pyrazolo[1.5-*a*]quinazoline , **1**(6), and 5-methyl-5-methylsulfanyl-3-phenylsulfonyl-9-methyl-5,6,7,8-tetrahydro-pyrazolo[5.1-*b*]quinazoline, **2**(6). Displacement of corresponding radio-labeled ligands was measured at the compound concentration of 1 μM. Shown is a typical experiment performed in duplicates, where the radio-labeled ligand displacement values ± SD are presented.

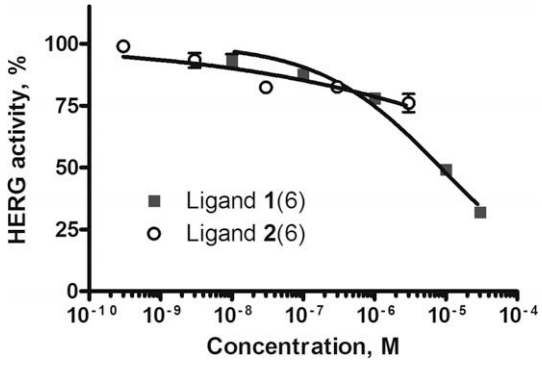


Figure 4. HERG potassium channel blocking activity of ligands **1**(6) and **2**(6) as measured in Patch clamp assay. Shown are averages of three measurements ± SD.

antagonistic mode.[17] In short, the compounds were added into a 1 cm cuvette containing HEK293 cells exogenously expressing h5-HT_{2B} receptor, 5-HT_{2B}R-HEK293 cells. The cells were loaded with a ratiometric fluorescent dye, Fura-2 AM, and the fluorescent signals were registered with the spectrofluorometer RF-5301PC (Shimadzu, Japan) at 510 nm upon alternative excitation at 340 nm and 380 nm. The ratios were expressed as free intracellular calcium ion concentrations,[18] $[Ca^{2+}]_i$, with a built in Super Ion Probe Software. For antagonistic activity, αMe-5-HT (5 nM final) was added to the cuvette 15 sec after the compound addition and the compound effect is assessed as the inhibition of the αMe-5-HT-induced $[Ca^{2+}]_i$ mobilization. The compounds tested, **1**(1,2,6) and **2**(6), did not cause any noticeable activation of the 5-HT_{2B} receptor. As the 5-HT_{2B} receptor blockers, these compounds were 10–55-fold less potent compared to the 5-HT_6 receptor (Table 2).

The ligands **1**(6) and **2**(6) at 1 μM did not noticeably interact with another target of potential liability, hERG channel (Fig. 3). In a functional patch clamp assay (CHO cells with exogenously expressed hERG channel)[16], the compounds showed very low channel blocking activity (Fig. 4). The angular ligand **1**(6) blocked hERG channel with IC_{50} = 8.4 μM. Its linear analog, **2**(6), reduced the channel permeability only by approx. 25% at 10.0 μM.

In conclusion, to the best of our knowledge, the cycloalkane-annelated 3-phenylsulfonyl-pyrazolo[1,5-*a*]pyrimidines **1** and **2** are the first in class 5-HT_6R antagonists lacking the basic ionizable side chain. This new chemotype of potent (binding K_i in a pM range and antagonistic IC_{50} in low nM range) and highly selective 5-HT_6R antagonists represent a simpler pharmacophore model than the one suggested earlier.[10] The lack of the basic ionizable amino group shows a promise for a development of 5-HT_6R ligands with reduced bias towards other biogenic amine receptors.

Supplementary data

Supplementary data (description of compound syntheses and biological assay methods) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.046.

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14. *5-HT_6 receptor functional assay*. The 5-HT_6-HEK cells were grown in Corning 384-well plates (Lowell, MA) at 37 °C in atmosphere of air:CO_2 (95%:5%) in DMEM supplemented with 10% FBS, 1% AAS, blasticidine S, and phleomycin (Invitrogen, Carlsbad, CA). T-Rex/5-HT_6 receptor expression was activated by addition of tetracycline, as recommended by the manufacturer, a day before the experiments. On the day of the experiment, the medium in the wells was substituted with phenol red-, calcium- and magnesium-free HBSS, (Invitrogen, Carlsbad, CA), supplemented with 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM HEPES, pH 7.4, and 100 μM IBMX. The test compounds were added at different concentrations while maintaining constant final DMSO concentration of 0.1%. After 15 min incubation, serotonin hydrochloride (Sigma, MO) was added to a final concentration of 10 nM and incubation continued for additional 30 min at rt. The cells were treated as described in cAMP LANCE assay kit protocol (Perkin–Elmer, Waltham, MA) as recommended by the manufacturer. The LANCE signal was measured in white 384-well plates (Corning, MA) using multimode plate reader VICTOR^{2}V (Perkin–Elmer, Waltham, MA) with built-in settings for the LANCE detection.
15. *5-HT_{2B} receptor functional assay*. The 5-HT_{2B}-HEK cells were grown in T-175 flasks at 37 °C in atmosphere of air:CO_2 (95%:5%) in DMEM (Sigma, MO) supplemented with 10% FBS, 1% AAS, blasticidine S, and phleomycin (Invitrogen, Carlsbad, CA). The T-Rex/5-HT_{2B} receptor expression was activated by addition of tetracycline, as recommended by the manufacturer, a day before the experiments. The cells were dissociated with TrypLE™ Express (Invitrogen, Carlsbad, CA), washed twice with PBS and loaded at room temperature with 4 μM calcium-sensitive dye, Fura-2AM (Invitrogen, Carlsbad, CA) for 30 min. After the loading, the cells were washed once with PBS, re-suspended into protein free Hybridoma media without phenol red (Sigma, St. Louis, MO) and allowed to incubate for additional 30 min with gentle shaking at room temperature. All loading procedures were performed in dark conditions. The loaded cells were washed twice with PBS and re-suspended into the Hybridoma media at a cell density of 3–4 × 10^6 cells/mL for subsequent experiments. Fura-2 ratiometric fluorescence signal was registered at 510 nm upon alternate excitation at 340 nm and 380 nm using spectrofluorometer RF-5301PC (Shimadzu, Columbia, MD). In a square (1 cm) optical cuvette with a magnetic stirring bar, 100 μL aliquots of the loaded cells were diluted into 2.4 mL buffer containing (mM): NaCl (145), KCl (5.4), $MgSO_4$ (0.8), $CaCl_2$ (1.8), HEPES (30), D-glucose (11.2). The fluorescence signal was allowed to stabilize for 20–30 s before addition of a test compound or vehicle to assess potential agonistic activity of the compounds, with subsequent addition of serotonin (2.5 μL, 10 mM) to assess the compounds' blocking activity.
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