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Indole-3-piperazinyl derivatives: Novel chemical class of 5-HT$_6$ receptor antagonists

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ABSTRACT

N_1-Arylsulfonyl-3-piperazinyl indole derivatives were designed and identified as a novel class of 5-HT$_6$ receptors ligands. All the compounds have high affinity and antagonist activity towards 5-HT$_6$ receptor. The compound 7a (K_i = 3.4 nM, functional assay IC_{50} = 310 nM) shows enhanced cognitive effect when tested in NORT and Morris water maze models. Synthesis, SAR and PK profile of these novel compounds constitute the subject matter of this Letter.

5-Hydroxytryptamine$_6$ receptor (5-HT$_6$R), the family member of G-protein coupled receptors (GPCR)[1,2] is present in various regions of the brain and is associated with learning and memory. Blockade of their function increases acetylcholine and glutamate-mediated neurotransmission and enhances the cognitive processes, which amply demonstrate the therapeutic usefulness of this receptor for CNS mediated disorders such as schizophrenia, AD and also in obesity and eating disorders.[3–12]

As it was shown by various in vivo studies that 5-HT$_6$R active ligands play important role in cognition and memory formation,[13–20] since then the tremendous progress has been made in identifying novel 5-HT$_6$ receptor ligands. A number of 5-HT$_6$ receptor antagonists are in advanced stage of clinical development (Fig. 1). SAM-531[21], GSK-742457, LY-483518 and SB-271046[8,22–24] are the phase II clinical candidates, where as Lu AE58054[25], PRX-07034, SYN-114[8,22–24] are in phase I study. SUVN-502, our in house discovered clinical candidate, has recently completed its phase I study for cognitive impairment in Schizophrenia and AD.[26]

All the reported ligands have some common pharmacophoric features like basic ionisable amine functionality and two hydrophobic aromatic sites, which are the basic requirements needed for binding at 5-HT$_6$ receptor.[4] In our recent publications, we have disclosed a series of 2-arylsulfonylmethyl-3-piperazinylmethylindole derivatives[27] and aryl amino sulfonamide derivatives[28] as the 5-HT$_6$ receptor ligands. The prior series belongs to the non-sulfonamide class of compounds, which have mild to moderate affinity towards the receptor while the latter sulfonamide class of compounds are potent and selective 5-HT$_6$ receptor antagonists

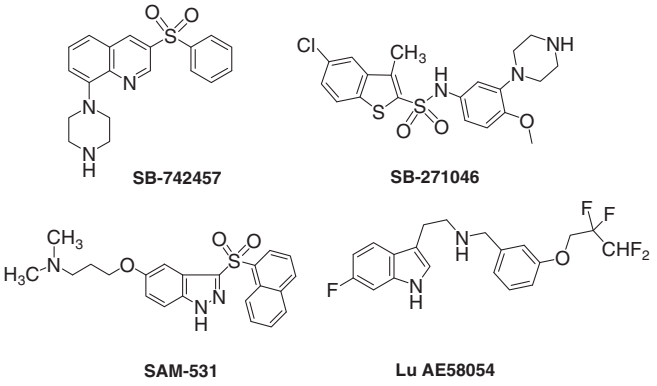

Figure 1. 5-HT$_6$ receptor antagonists.

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with the good ADME profile. Another recent publication from the Korea research institute of chemical technology[29] has highlighted the sulfonamide compounds with 5-HT_{2C}/5-HT_6 dual antagonist activity.

Also in our earlier effort, we disclosed a series of N_1-arylsulfonyl-3-piperazinylmethyl indole derivatives[30] as potent 5-HT_6 receptor antagonists (Fig. 2). In this particular series both the nitrogen are highly basic in nature. In continuation to explore the SAR study around these derivatives and to establish the impact of removal of 'methylene' spacers in the side-chain with respect to 5-HT_6 receptor binding affinity, the new series of N_1-arylsulfonyl-3-piperazinyl indole derivatives were conceived. Thus the present series was designed to understand the impact of reduced basicity of one of the nitrogens of piperazine attached to the third position of the indole nucleus on the binding affinities as well as on the ADME profile of these molecules.

The general synthetic strategy used for the title Compounds **7a–7t** was summarized in Scheme 1. Various substituted N_1-acetyl indoxyl derivatives **4** were synthesized according to the literature methods.[31,32] More often, the indoxyls were synthesized starting from variously substituted anthranilic acids and chloro acetic acid or from 2-chloro benzoic acid and glycine to obtain 2-carboxyphenylglycine derivatives **2**, which were further cyclized in acetic anhydride to 1-acetyl-3-acetyloxy indole derivatives **3**. Selective hydrolysis of these derivatives resulted in indoxyl derivatives **4**.

Substituted N_1-acetyl indoxyl derivatives **4** were treated with titanium isopropoxide, *N*-alkyl piperazines and sodium triacetoxyborohydride to obtain the corresponding substituted N_1-acetyl-3-piperazinyl indole derivatives **5**. These indole derivatives were then deacetylated using inorganic bases like KOH or NaOH in polar protic solvents under reflux conditions to obtain the deacetylated product **6**. Various alternative methods were used for the N_1 sulfonylation of indole. The best system was found to be the sodium hydride and DMF. Alternatively, potassium hydroxide and THF was also found to be appropriate for obtaining target compounds **7a–7t** in good yields (see Supplementary data).

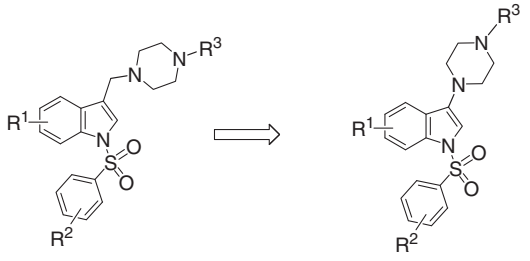
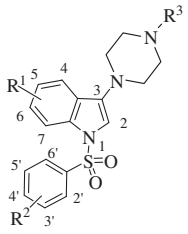


Figure 2. Genesis of ligands.

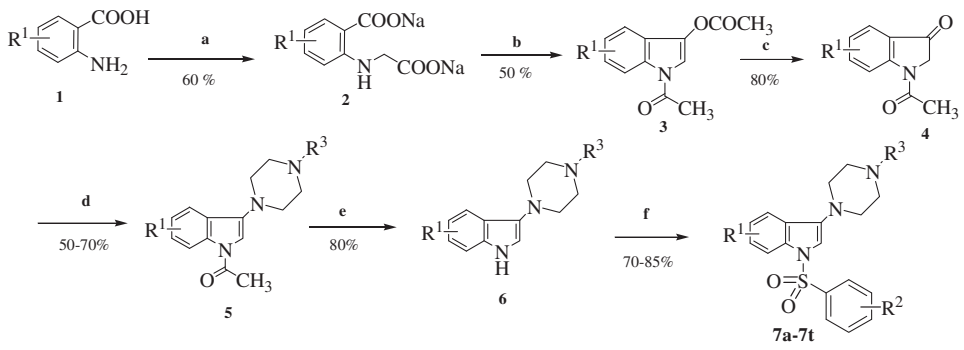
Table 1
5-HT_6 receptor binding data[a]



Compound	R^1	R^2	R^3	5-HT_6R K_i (nM)
7a	H	4′-*i*Pr	CH_3	3.4
7b	H	4′-F	CH_3	11.8
7c	H	3′-Cl	CH_3	10.8
7d	H	2′-Br	CH_3	15.2
7e	H	H	CH_3	15.9
7f	H	4′-*i*Pr	C_2H_5	8.6
7g	5-Br	3′-Cl	CH_3	7.8
7h	5-Br	4′-F	CH_3	9.2
7i	5-Br	4′-F	C_2H_5	10.6
7j	5-Br	4′-*i*Pr	CH_3	23.2
7k	5-Br	4′-*i*Pr	C_2H_5	30.1
7l	5-Br	2′-Br	CH_3	10.2
7m	5-Cl	4′-F	C_2H_5	10.7
7n	5-Cl	2′-Br	C_2H_5	20.3
7o	5-Cl	4′-*i*Pr	C_2H_5	30.5
7p	6-Cl	4′-OMe	C_2H_5	82.3
7q	6-Cl	4′-*i*Pr	CH_3	55.3
7r	6-Cl	2,5-di-OMe	C_2H_5	63.5
7s	6-Cl	2′-Naphthyl	C_2H_5	25.5
7t	6-Cl	2′-Br	CH_3	30.2

[a] Displacement of [^{3}H]-LSD binding to cloned human 5-HT_6 receptors stably expressed in HeLa cells, K_i values were determined in duplicates and mean values are reported.

All the synthesized compounds were tested in a standard radioligand-binding assay using human-cloned 5-HT_6 receptors stably transferred in HeLa cells to determine affinity for 5-HT_6 receptor. K_i values are summarized in Table 1. For the initial SAR study, the unsubstituted indole moiety was undertaken first. Different substituted sulfonyl chlorides were used for sulfonylation at N_1 of indole nucleus. Among the derivatives synthesized, compound **7a**, which is 4′-isopropyl benzene sulfonyl derivative, was found to have the highest affinity (K_i = 3.4 nM), while its unsubstituted sulfonyl analog (**7e**, K_i = 15.9 nM) has shown fivefold decrease in binding affinity at 5-HT_6 receptor. Replacement of isopropyl group in compound **7a** with fluoro (**7b**, K_i = 11.8 nM) reduced the binding affinity by three fold. The analogs **7c** (K_i = 10.8 nM) and **7d** (K_i = 15.2 nM), with halo substitution in the benzene sulfonamide ring, show reduction in the binding affinities by 4–5-fold as compared to **7a**. Replacement of methyl group at piperazinyl nucleus



Scheme 1. Synthesis of compounds **7a–7t**. Reagents and conditions: (a) $ClCH_2COOH$, water, NaOH, heat; (b) acetic anhydride, sodium acetate, H_2O, DMF, heat; (c) Na_2SO_3, abs ethanol, heat; (d) titanium isopropoxide, *N*-alkyl piperazine, $NaBH(OAc)_3$, ethanol, rt; (e) KOH, ethanol, reflux; (f) NaH, DMF, $ArSO_2Cl$, rt.

Table 2
Functional activity data[a]

Compound	K_b (nM)	IC_{50} (nM)
7a	0.53	310
7b	0.5	292
7h	0.6	322
7g	3.0	1528
7m	3.5	1783

[a] K_b values were determined using non-radioactive cell-based assay. IC_{50} and K_b values are the mean of two experiments.

Table 3
Human cytochrome P450 inhibitory data and microsomal metabolic stability

Compound	IC_{50} (μM)		% Metabolism in liver microsomes	
	CYP2D6	CYP3A4	Human	Rat
7a	39.2	5.54	80	95
7b	26.4	4.77	85	99
7h	28.8	4.61	96	99

The cytochrome P450 inhibitory potential was determined using isoform-selective assays and heterologously expressed human CYP2D6 and CYP3A4. These values are the mean of duplicate determinations. Microsomal metabolic stability in Wistar rat and human at 0.5 h, Concn 2.5 μM.

with ethyl (**7f**, K_i = 8.6 nM) resulted in twofold decrease in binding affinity.

Substitutions at 5 and 6-position of indole nucleus with different halo groups like bromo and chloro were well tolerated. In the case of 5-bromo substituted derivatives, the compounds with halo substitution at ortho, *meta*-and para-position in benzene sulfonamide ring have shown better affinity compared to the compounds with alkyl groups. Compounds **7g** (R^2 = 3′-Cl), **7h** (R^2 = 4′-F) and **7l** (R^2 = 2′-Br) had K_i values 7.8, 9.2 and 10.2 nM, respectively, while compound **7j** (R^2 = 4′-*i*Pr) has a K_i value of 23.2 nM. Almost similar pattern was observed for the 5-chloro and 6-chloro substituted indole derivatives **7m**, **7n**, **7o** and **7t**.

Replacement of R^3 methyl group with ethyl, in 5-halo and 6-halo substituted indole sulfonamide derivatives, shows marginal reduction or no effect on binding affinity as can be seen by comparing the K_i values of compounds **7h** (K_i = 9.2 nM) and **7j** (K_i = 23.2 nM) with **7i** (K_i = 10.6 nM) and **7k** (K_i = 30.1 nM), respectively.

By comparing the SAR data of these derivatives, we came to the conclusion that in unsubstituted indole derivatives, electron donating groups in benzene sulfonamide ring (R^2) were more preferred for 5-HT_6R binding over the halogens, while the reverse was the case for the 5-halo and 6-halo substituted indole derivatives.

The most potent compounds**7a, 7b, 7h, 7g** and **7m** from the series, were further evaluated for their functional activity at 5-HT_6 receptor using non-radioactive CHO cell based assay. All the tested compounds have shown antagonist activity by inhibiting the 5-HT stimulated cAMP accumulation as can be seen from the IC_{50} and K_b values (Table 2).

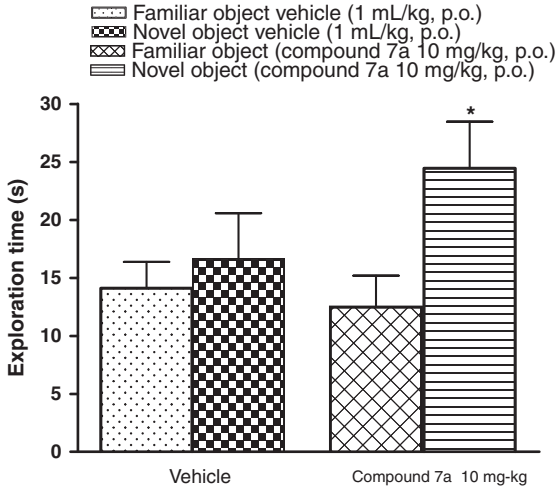
These compounds were further profiled for their selectivity against a panel of receptors, inclusive of other 5-HT receptor subtypes, dopamine D_2, histamine H_1 and the transporters like SERT and NET. All the compounds are 50–100-fold selective over all the tested receptors (data not shown).

Compounds **7a**, **7b** and **7h** were further evaluated for their metabolic stability and CYP liabilities. All the three compounds have extensively metabolized in rat and human liver microsomes. The values were summarized in Table 3. The effect of compounds **7a**, **7b** and **7h** on cytocrome 450 enzymes were determined in liver

Table 4
PK profile in male Wistar rats[a]

			Compound **7a**					
Route	*n*	Dose (mg/kg)	C_{max} (ng/mL)	AUC (ng h/mL)	$t_{1/2}$ (h)	V_z (L/kg)	Cl (mL/min/kg)	*F* (%)
Oral	3	5	40 ± 30	59 ± 25	2.35 ± 0.21	436 ± 203	2200 ± 1100	8 ± 0.7
iv	3	5	365 ± 90	674 ± 257	4.47 ± 0.3	51.8 ± 19.7	132.8 ± 44.2	

[a] Fasted male wistar rats, vehicle used: water for injection for both oral and iv routes. Dosing volumes: 10 mL/kg for oral and 2 mL/kg for iv.



Data represents mean ± SEM of exploration time (Paired 't' test), *P<0.05). Vehicle-PEG 400 50% v/v; 1 mL/kg, p.o. n=6-9 /group, *p.o.*, dosing vehicle/drug: 60 min prior to test (*p.o.*).

Figure 3. Novel object recognition test for compound **7a** in male Wistar rats.

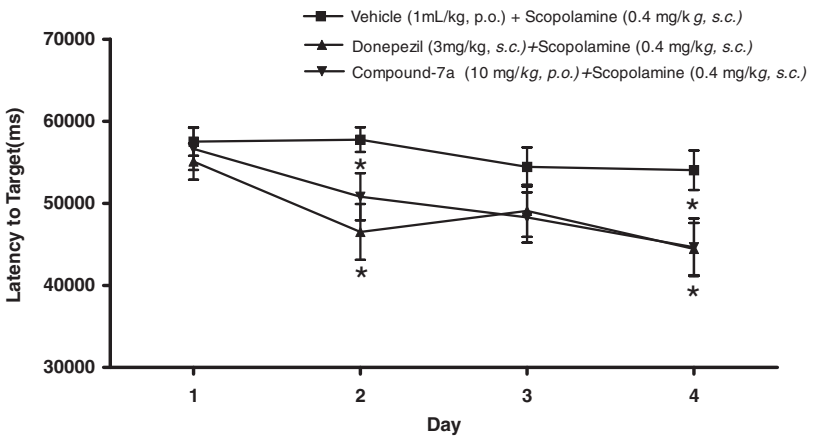


Figure 4. Effect of acute treatment of compound **7a** in Morris water maze for Latency to target in rats. Data represents Mean ± SEM of latency to target, $^{*}p$ <0.05, (One Way ANOVA, Dunnett's post hoc analysis).

microsomes, the IC_{50} values for all three compounds were found to be >25 μM for 2D6, while for 3A4 it was nearly 5 μM (Table 3).

Compound **7a** was further selected for determining its pharmacokinetics profiling in male Wistar rats. Compound **7a** displayed lower oral exposure with AUC 59 ng h/mL, moderate half-life ($t_{1/2}$ = 2.35 h), high clearance (2200 mL/min/kg) with large volume of distribution (436 L/kg), which resulted in moderate bioavailability (%*F*) of 8% following the oral administration of 5 mg/kg dose (Table 4). The low oral bioavailability of these compounds may be because of their poor metabolic stability in rat liver microsomes.

Compound **7a** was further evaluated for its cognitive potential in novel object recognition (NORT) paradigm and Morris water maze. Compound **7a** has shown improvement in cognitive performance at 10 mg/kg oral dose in NORT model (Fig. 3) while in Morris water maze model, it has significantly reversed the scopolamine induced memory deficit at 10 mg/kg ip dose, which was apparent from lesser target latency (Fig. 4).

In summary, a novel series of 3-piperazinyl-N_1-indolesulfonamide derivatives were designed and identified as 5-HT_6 receptor antagonist ligands. Compound **7a** has shown pro-cognitive potential when tested in NORT and Morris water maze models. Most of these derivatives are 50–100-fold selective over the other tested receptors. High metabolism of these compounds could be one of the reasons for low bioavailability. So the further efforts are in progress towards the prediction of metabolism sites and blocking them with appropriate substitutions so as to improve upon the metabolism and thereby make these molecules more bioavailable while maintaining the affinity towards 5-HT_6 receptor.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.001.

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