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N_1-arylsulfonyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole derivatives are potent and selective 5-HT$_6$ receptor antagonists

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Abstract—A series of N_1-arylsulfonyl-3-(1,2,3,6-tetrahydropyridin-4-yl)indole derivatives was designed and synthesized. These compounds were shown to have high affinity for the 5-HT$_6$ receptor. Two analogs, 4-[3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole-1-sulfonyl]-phenylamine **15g** and 4-[3-(1,2,3,6-tetrahydropyridin-4-yl)-5-methoxy-1*H*-indole-1-sulfonyl]-phenylamine **15y**, had 0.4 and 3.0 nM affinity, respectively, and antagonized the production of adenylate cyclase at sub-nanomolar concentrations.

The serotonin (5-HT) receptors have been divided into seven subclasses (5-HT$_{1-7}$).[1] The rat 5-HT$_6$ receptor was cloned in 1993 and found to consist of a 438 residue peptide chain with <40% protein sequence homology with the other 5-HT receptors.[2] The human receptor was subsequently cloned in 1996 and consists of 440 amino acid residues and shares 89% homology with the rat.[3] The 5-HT$_6$ receptor belongs to a group, including 5-HT$_4$ and 5-HT$_7$, which is positively coupled to adenylyl cyclase.[4]

Expression of the 5-HT$_6$ receptor mRNA is exclusive to the central nervous system, with highest density in the cerebral cortex, nucleus accumbens, caudate-putamen and hippocampus, with moderate densities in the thalamus and substantia nigra.[5] Several antipsychotic agents and antidepressants have high affinity for the 5-HT$_6$ receptor.[6] These two findings have sparked considerable effort to understand the role of this receptor in treatment of CNS disorders, including schizophrenia, depression, and impaired learning and memory.

In 1998, the first 5-HT$_6$ antagonists, a series of bismethylaminopyrimidinyl- and bismethylaminopyridinyl-sulfonamides, Ro-04-6790 (**1**) and Ro-63-0563 (**2**) were described (Fig. 1).[7] Subsequently, a series of piperazinyl-benzenesulfonamide antagonists SB-271046 (**3**),[8] SB- 258585 (**4**),[9] and SB-357134 (**5**) were disclosed.[10] The agonist 2-ethyl-5-methoxy-*N*,*N*-dimethyltryptamine (**6**) was reported by Glennon et al.[11] Tsai and co-workers first described N_1-benzenesulfonyltryptamines as 5-HT$_6$ antagonists[12] and *N*,*N*-dimethyl-N_1-benzenesulfonyl-5-methoxytryptamine (**7**) was reported by Russell et al.[13] The first non-sulfonamide antagonist, 4-(2-bromo-6-pyrrolidine-1-ylpyridine-4-sulfone)phenylamine (**8**) was reported by Riemer et al.[14] In the course of our work we also discovered that N_1-arylsulfonyltryptamine derivatives had high affinity for the 5-HT$_6$ receptor.[12,13] Exploration of the SAR led to the replacement of the tryptamine aminoethyl group with the more rigid tetrahydropyridinyl group, as had previously been reported for 5-HT$_{1A}$ and 5-HT$_2$ receptors (Fig. 2).[15] Herein, we describe the synthesis and biological activities of this class of 5-HT$_6$ receptor ligands[16,17].

1. Chemistry

The synthesis of N_1-arylsulfonyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole compounds **12**, **15**, and **18** was carried out as shown in Scheme 1. Condensation of an indole with a 4-methyl- or 4-benzyl-piperidone in the presence of potassium hydroxide in refluxing methanol

Keywords: 5-HT$_6$ antagonists; 5-Hydroxytryptamine$_6$ antagonists.
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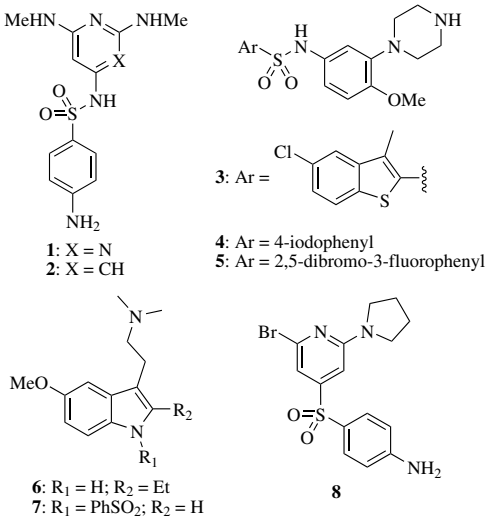


Figure 1. Structures of 5-HT_6 antagonists (**1**–**5**, **7**–**8**) and agonist (**6**).

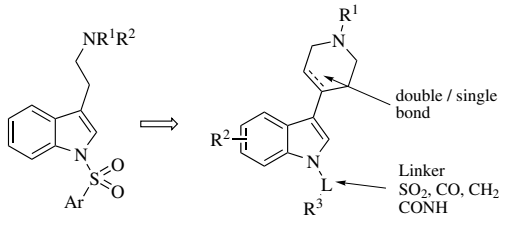
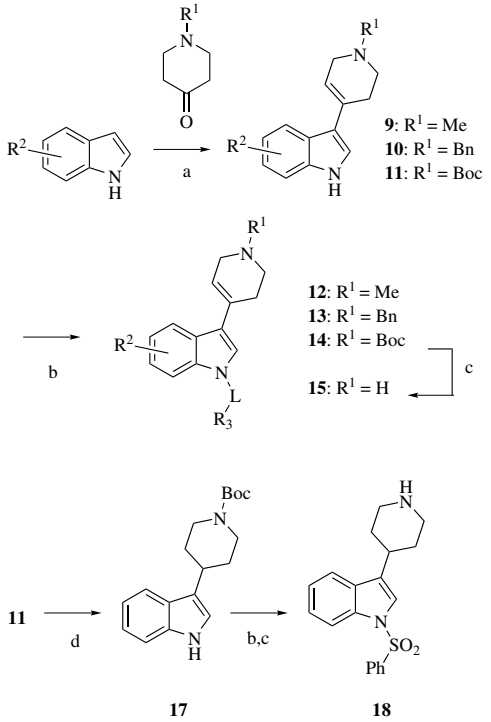


Figure 2. Design of N_1-arylsulfonyl-(1,2,3,6-tetra-hydropyridin-4-yl)-1*H*-indoles.



Scheme 1. Reagents and conditions: (a) KOH, MeOH, 60 °C; (b) R_3SO_2Cl, BnBr, PhCOCl, or PhNCO, *t*-BuOK, THF, rt; (c) 2 N HCl, dioxane, rt; (d) H_2, EtOH, palladium on carbon.

afforded 3-(1-methyl- and 3-(1-benzyl-1,2,3,6-tetrahydropyridinyl)-indole, **9** and **10**, respectively.[15] Sulfonylation by reaction with the appropriate sulfonyl chloride in THF in the presence of potassium *tert*-butoxide gave the desired N_1-sulfonylindoles **12** and **13**. The N_1-benzylindole, N_1-benzoylindole, and N_1-phenylindolecarboxamide compounds (**12i**–**k**; L = CH_2, CO, CONH) were prepared by coupling the indole **9** with benzyl bromide, benzoyl chloride, and phenylisocyanate in the presence of potassium *tert*-butoxide.

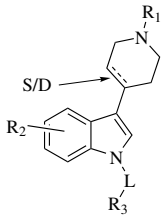
The *N–H* tetrahydropyridinyl compounds (**15**) were prepared by coupling the substituted indoles with *N*-Boc-piperidinone, to give the 3-(*N*-Boc-1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles **11**, followed by sulfonylation and removal of the Boc group by stirring with HCl in dioxane. When 4-acetylaminophenylsulfonyl chloride was used, treatment with HCl to remove the Boc group led to simultaneous removal of the acetyl group affording the corresponding 4-aminophenylsulfonyl derivatives. Hydrogenation of 3-(*N*-Boc-1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole **11** over palladium on carbon in ethanol gave the reduced 3-(*N*-Boc-piperidin-4-yl)-1*H*-indole **17**, which was sulfonylated and deprotected under the conditions described above. The *N*-acyl piperidine compound **16** was prepared by acylation of **15a** with acetyl chloride.

2. Biology

All of the 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles were evaluated for their ability to displace [^{3}H]-LSD from cloned human 5-HT_6 receptors stably expressed in HeLa cells.[18,19] In general, the N_1-arylsulfonylindoletetrahydropyridinyl *N–Me* and *N–H* derivatives (**12**, **15**) had excellent affinity for the 5-HT_6 receptor and higher affinity than the N_1-unsubstituted indole derivative (**9**; K_i = 98 nM). The larger *N*-benzyl derivative (**13a**; K_i = 246 nM) had lower affinity, also the requirement for a basic amine was confirmed by the low affinity of the *N*-Ac (**14a**; K_i = 54 nM) and *N*-Boc (**16**; 64% inhibition at 1 μM) derivatives. The N_1-phenylsulfonyl-3-piperidin-4-yl-1*H*-indole derivatives (**18a** and **18f**; K_i = 12 and 20 nM) had about 6-fold lower affinity than the corresponding unsaturated 3-(tetrahydropyridin-4-yl) derivatives (**15a** and **15f**; K_i = 2 and 2.4 nM) while in the 4-aminophenylsulfonyl series the saturated analogs (**18h** and **18w**; K_i = 3 and 2 nM) had similar affinity to the unsaturated analogs (**15h** and **15w**; K_i = 3 and 1 nM). Substitution of the sulfonyl linker with a methylene (**12i**; K_i = 10 nM), acyl (**12j**; K_i = 99 nM) or amido (**12k**; K_i = 118 nM) group led to 5-, 50-, and 60-fold reductions in affinity. Similar outcomes were observed in the N_1-phenylsulfonyltryptamine series where replacement of the phenylsulfonyl moiety with benzyl and benzoyl groups resulted in 3- and 11-fold loss in affinity, respectively[13,20] (Table 1).

In general, unsubstituted phenyl, and 2-, 3- and 4-fluoro- and chlorophenylsulfonyl analogs had high affinity, as did the 5-chlorothienyl derivative (**15l**;

Table 1. 5-HT_6 binding affinity of 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles



Compd	R_1	S/D[b]	L	R_2	R_3	5-HT_6[a] K_i (nM)
9	Me	D	Bond	H	H	98
12a	Me	D	SO_2	H	Ph	2 ± 0.1
12b	Me	D	SO_2	H	2-Cl–Ph	10 ± 1
12c	Me	D	SO_2	H	2-F–Ph	10 ± 1
12d	Me	D	SO_2	H	3-Cl–Ph	1 ± 1
12e	Me	D	SO_2	H	4-F–Ph	5 ± 1
12i	Me	D	CH_2	H	Ph	10 ± 1
12j	Me	D	CO	H	Ph	99 ± 2
12k	Me	D	CONH	H	Ph	118 ± 7.7
13a	Bn	D	SO_2	H	Ph	246 ± 26
14a	Boc	D	SO_2	H	Ph	54 ± 1.4
15a	H	D	SO_2	H	Ph	2 ± 1
15b	H	D	SO_2	H	2-Cl–Ph	0.4 ± 0.1
15c	H	D	SO_2	H	2-F–Ph	1 ± 0.2
15f	H	D	SO_2	5-F	Ph	2.4 ± 0
15g	H	D	SO_2	H	4-NH_2–Ph	2 ± 0.4
15h	H	D	SO_2	6-Cl	4-NH_2–Ph	3 ± 1
15l	H	D	SO_2	H	5-Cl-thiophen-2-yl	3 ± 0.3
15m	H	D	SO_2	5-F	4-F–Ph	3 ± 0.1
15n	H	D	SO_2	5-F	4-Cl–Ph	14 ± 1
15o	H	D	SO_2	H	4-CF_3-Ph	20 ± 2
15p	H	D	SO_2	H	4-MeO-Ph	18 ± 3
15q	H	D	SO_2	5-F	1-Naphthyl	4 ± 0.3
15r	H	D	SO_2	6-NO_2	4-NH_2–Ph	31 ± 2
15s	H	D	SO_2	H	4-Benzo[1,2,5]thiadiazolyl	5 ± 1
15t	H	D	SO_2	H	2-Naphthyl	27 ± 6
15u	H	D	SO_2	5-MeO	2-Naphthyl	19 ± 5
15v	H	D	SO_2	H	*n*-Bu	77 ± 2.4
15w	H	D	SO_2	5-F	4-NH_2–Ph	1 ± 0.1
15x	H	D	SO_2	5-Br	4-NH_2–Ph	7 ± 1
15y	H	D	SO_2	5-MeO	4-NH_2–Ph	3 ± 1
15z	H	D	SO_2	5-BnO	4-NH_2–Ph	50 ± 2.4
15aa	H	D	SO_2	5-NO_2	4-NH_2–Ph	29 ± 2
15ab	H	D	SO_2	5,6-Methylenedioxy	4-NH_2–Ph	1 ± 0.4
16	Ac	D	SO_2	H	Ph	64%[c]
18a	H	S	SO_2	H	Ph	12 ± 1
18f	H	S	SO_2	5-F	Ph	20 ± 1
18h	H	S	SO_2	6-Cl	4-NH2–Ph	3 ± 0.2
18w	H	S	SO_2	5-F	4-NH2–Ph	2 ± 0.2

[a] Displacement of [^{3}H]-LSD binding to cloned h5-HT_6 receptors stably expressed in HeLa cells.[18,19] Mean of three determinations.
[b] S/D = single/double bond.
[c] % inhibition @ 1 μM.

K_i = 3 nM), while larger groups, for example, 4-trifluoromethyl, and 4-methoxy (**15o**–**p**; K_i = 20 and 18 nM) had weaker affinity. Replacement of the N_1-phenylsulfonyl group with 1-naphthylenesulfonyl (**15q**; K_i = 4 nM) and 4-benzo[1,2,5]thiadiazolylsulfonyl (**15s**; K_i = 5 nM) led to compounds with similar affinity, while the 2-naphthylenesulfonyl (**15t**, **15u**; K_i = 27, 19 nM) and *n*-butylsulfonyl (**15v**; K_i = 77 nM) derivatives had significantly lower affinity. This is in contrast to the N_1-sulfonyltryptamine series where the 1- and 2-naphthylenesulfonyl derivatives had similar affinity to the N_1-phenylsulfonyl analogs.[12,21]

Published data for the N_1-arylsulfonyltrpytamine series show the 4-amino analogs bind with between 1- and 15-fold enhanced affinity, relative to the phenylsulfonyl parent, and a proposed binding mode places the amino group proximal to Asp^{303} on TM7.[21] In this N_1-arylsulfonyl-3-(tetrahydropyridin-4-yl)-1*H*-indole series the 4-aminophenylsulfonyl derivatives bind with similar affinity as the phenylsulfonyl parents (**15g** and **12a**; K_i = 2 nM).

The effect of indole substitution can be observed in the 4-aminophenylsulfonyl series (**15g**; K_i = 2 nM). Halo

Table 2. Antagonism of cAMP production

Compd	cAMP assay	
	IC_{50} (nM)	I_{max} (%)
12a	13.8 ± 2.6	100 ± 0
12b	0.7 ± 0.1	83 ± 2
12c	3.9 ± 1.6	87 ± 1
12d	9.4 ± 0.4	95 ± 2
12e	42.7 ± 8.9	83 ± 1
12i	14.8 ± 0.1	76 ± 0
15a	43 ± 2	96 ± 1
15b	8.8 ± 0.4	97 ± 1
15c	2.7 ± 0.7	100 ± 0
15g	0.8 ± 0.1	97 ± 0
15h	11.2 ± 3.7	105 ± 0
15l	14.1 ± 1.8	83 ± 1
15m	31 ± 4	87 ± 1
15q	9.0 ± 1.4	85 ± 2
15s	169 ± 6	65 ± 2
15u	4.1 ± 0.6	97 ± 1
15w	9.7 ± 0.4	100 ± 0
15x	33.7 ± 1.2	102 ± 1
15y	0.4 ± 0.1	100 ± 0
15ab	1.7 ± 0.1	99 ± 1
18a	19.5 ± 2.5	54 ± 1
18h	8.5 ± 1.1	98 ± 1
18w	1.3 ± 0.2	100 ± 0

Inhibition of 5-HTstimulated cAMP production in HeLa cells stably transfected with human 5-HT_6 receptors. Mean of three determinations.

(**15h**, **15w** and **15x**; K_i = 3, 1, and 7 nM) or methoxy (**15y**; K_i = 3 nM) groups are tolerated in the 5- and 6-positions, as is the 5,6-methylenedioxy group (**15ab**; K_i = 1 nM). Larger groups such as 5-benzyloxy (**15z**; K_i = 50 nM) and 5- and 6-nitro (**15aa** and **15r**; K_i = 29 and 31 nM) led to lower affinity. Similar results were reported for the N_1-benzenesulfonyltryptamine series.[12,13]

Compounds which had high affinity for the 5-HT_6 receptor were tested in the cyclase assay to determine if they could modulate 5-HT_6 function. None of the compounds tested showed any agonist activity.[22]

Many N_1-arylsulfonyl-3-(tetrahydropyridin-4-yl)-1*H*-indole compounds inhibited 5-HT stimulated cAMP production at low nanomolar concentrations, suggesting that they are antagonists (Table 2).[22] Interestingly, although 4-aminophenylsulfonyl derivatives had similar affinity to their phenylsulfonyl parents, they are significantly more potent antagonist. In particular, **15g** and **15y** showed complete inhibition of cAMP production at sub-nanomolar concentrations.

The published literature does not contain enough data on the functional activity of 4-aminophenylsulfonyl derivatives relative to their phenylsulfonyl parents to indicate that the 4-amino moiety generally enhances antagonist activity. However, it is interesting to note the 4-aminophenylsulfonyl moiety is present in the lead compounds (**1**, **2**, and **8**) in several different series.

The binding affinity of selected compounds for a panel of receptors is shown in Table 3. In general, this class of 5-HT_6 antagonists displayed low affinity for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2c}, 5-HT_7, dopamine D_2, D_3, and D_4 receptors.

In conclusion, an interesting class of N_1-arylsulfonyltryptamine analogs in which the aminoethyl chain is rigidified into a tetrahydropyridinyl ring were designed. Synthesis and biological evaluation indicate they are selective 5-HT_6 receptor antagonists. The N_1-4′-aminophenylsulfonyl moiety appears to be important for enhanced functional activity, with 4-[3-(1,2,3,6-tetrahydro-pyridin-4-yl)-1*H*-indole-1-sulfonyl]-phenylamine **15g** and 4-[3-(1,2,3,6-tetrahydro-pyridin-4-yl)-5-methoxy-1*H*-indole-1-sulfonyl]-phenylamine **15y** having sub-nanomolar inhibition of adenylate cyclase production.

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Table 3. Binding affinity for other serotonin and dopamine receptors

Compd	K_i (nM)									
	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{1F}	5-HT_{2A}	5-HT_{2C}	5-HT_7	D_2	D_3	D_4
15b	>2000	12%[a]	6%[a]	2%[a]	599 ± 59	383 ± 18	NT	>2000	627 ± 39	>5000
15c	875 ± 16	11%[a]	9%[a]	53%[a]	50%[a]	57%[a]	NT	>2000	741 ± 22	>5000
15g	1670 ± 191	33%[a]	NT	NT	NT	NT	1555 ± 9	NT	NT	NT
15y	97 ± 6	27%[a]	32%[a]	NT	NT	NT	>5000	NT	NT	NT
18h	115 ± 15	30%[a]	10%[a]	16%[a]	65%[a]	15%[a]	>2000	10%[a]	7%[a]	7%[a]

Receptors were all human clones stably expressed in CHO cells (5-HT receptors) or CHO-K1 Cells (D receptors). Radioligands were as follows: 5-HT_{1A}: DPAT; 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2C}: [^{3}H]-5-HT; 5-HT_{2A}: [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ([^{125}I]DOI); 5-HT_6, 5-HT_7: [^{3}H]LSD; dopamine D_2, D_3, and D_4: [^{3}H]spiperone].

NT = not tested.

[a] % inhibition @ 1 μM.

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18. 5-HT_6 membrane preparation: cultured HeLa cells expressing h5-HT6 receptors are harvested and centrifuged at low speed ($1000 \times g$) for 5 min to remove the culture media. The harvested cells are suspended in 1 volume of fresh physiological phosphate buffered saline (PBS) solution and recentrifuged at the same speed. This operation is repeated once more. The collected cells are then homogenized in 10 volumes of 50 mM Tris–HCl, pH 7.4 and 0.5 mM EDTA. The homogenate is centrifuged at $900 \times g$ for 10 min and the supernatant collected. The supernatant is then centrifuged at $40{,}000 \times g$ for 30 min. The pellet is resuspended in 10 volumes of Tris–HCl buffer and recentrifuged at the same speed. The final pellet is suspended in a small volume of Tris–HCl buffer and the tissue protein content is determined in aliquots of 10–25 μL volumes. The volume of the suspended cell membranes is adjusted to give a tissue protein concentration of 1.0 mg/mL of suspension. The prepared membrane suspension (10 times concentrated) is aliquoted in 1.0 mL volumes and stored at −70 °C until used in subsequent binding experiments.
19. 5-HT_6 binding assay: total bound well: 80 μL binding buffer, 20 μL of 3 μM [^{3}H]-LSD, 100 μL membrane protein (20–50 μg protein). Non-specific well: 60 μL buffer, 20 μL of 1 μM cold LSD, 20 μL of 3 μM [^{3}H]-LSD, 100 μL of membrane protein (20–50 μg protein). Compound well: containing 60 μL buffer, 20 μL test compounds at different concentrations, 20 μL of 3 μM [^{3}H]-LSD, 100 μL membrane protein (20–50 μg protein). Incubate at room temperature for 2 h and harvest reaction mixture by using Packard 96 well harvest system followed by counting the plate in Top Count machine (Packard).
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22. Determination of 5-HT_6 compound intrinsic activity using cAMP accumulation: intracellular cAMP levels are measured using 24-well plates containing the human 5-HT_6 receptor stably transfected into HeLa cells. Upon initiation of the assay, the media from the cell maintenance is aspirated and the cells are preincubated at 37 °C for 15 min in KREBS buffer. Following this primary incubation, the buffer is aspirated and an additional incubation is performed at 37 °C for 5 min in KREBS buffer containing 500 μM IBMS (3-isobutyl-1-methylxanthine). Subsequently, the cells are incubated with the test compound at concentrations ranging from 10^{-5} to 10^{-10} M for 10 min at 37 °C (antagonist assay require a second incubation with the addition of 100 nM 5HT). The assay is terminated by the addition of 0.5 M percloric acid. Intracellular cAMP levels were determined by radioimmunoassay through the cAMP SPA screening kit. Data were analyzed graphically with GraphPad Prism (GraphPad Software, San Diego, CA).