

***N*₁-arylsulfonyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole derivatives are potent and selective 5-HT₆ 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-HT₆ 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.

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The serotonin (5-HT) receptors have been divided into seven subclasses (5-HT_{1–7}).¹ The rat 5-HT₆ 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-HT₆ receptor belongs to a group, including 5-HT₄ and 5-HT₇, which is positively coupled to adenylyl cyclase.⁴

Expression of the 5-HT₆ 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-HT₆ 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-HT₆ antagonists, a series of bismethylaminopyrimidinyl- and bismethylaminopyridinyl-sul-

fonamides, 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-HT₆ antagonists¹² 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-sulfonyl)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-HT₆ 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-HT₂ receptors (Fig. 2).¹⁵ Herein, we describe the synthesis and biological activities of this class of 5-HT₆ 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-HT₆ antagonists; 5-Hydroxytryptamine₆ antagonists.

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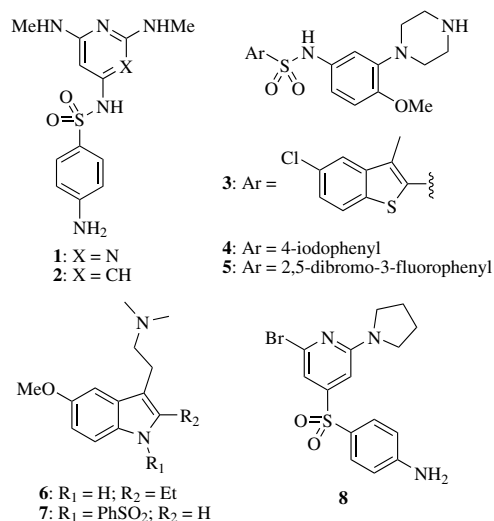


Figure 1. Structures of 5-HT₆ antagonists (1–5, 7–8) and agonist (6).

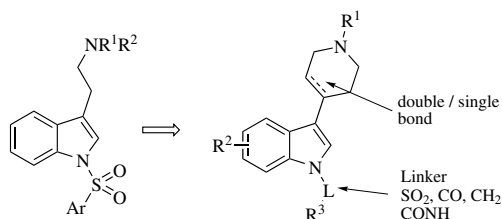
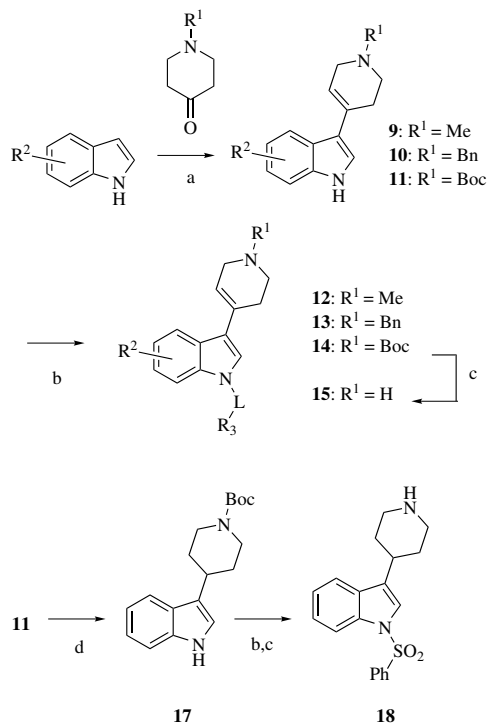


Figure 2. Design of *N*₁-arylsulfonyl-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles.



Scheme 1. Reagents and conditions: (a) KOH, MeOH, 60°C; (b) R₃SO₂Cl, BnBr, PhCOCl, or PhNCO, *t*-BuOK, THF, rt; (c) 2*N* HCl, dioxane, rt; (d) H₂, EtOH, palladium on carbon.

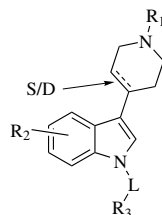
afforded 3-(1-methyl- and 3-(1-benzyl-1,2,3,6-tetrahydropyridinyl)-indole, **9** and **10**, respectively.¹⁵ Sulfonylation by reaction with the appropriate sulfonyl chloride in THF in the presence of potassium *tert*-butoxide gave the desired *N*₁-sulfonylindoles **12** and **13**. The *N*₁-benzylindole, *N*₁-benzoylindole, and *N*₁-phenylindolecarboxamide compounds (**12i–k**; L = CH₂, 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-acetylamino phenylsulfonyl 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 [³H]-LSD from cloned human 5-HT₆ receptors stably expressed in HeLa cells.^{18,19} In general, the *N*₁-arylsulfonylindole tetrahydropyridinyl *N*-Me and *N*-*H* derivatives (**12**, **15**) had excellent affinity for the 5-HT₆ 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-chlorothiophenyl derivative (**15i**);

Table 1. 5-HT₆ 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	<i>n</i> -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-NH ₂ -Ph	3 ± 0.2
18w	H	S	SO ₂	5-F	4-NH ₂ -Ph	2 ± 0.2

^a Displacement of [³H]-LSD binding to cloned h5-HT₆ 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 N₁-phenylsulfonyl group with 1-naphthylsulfonyl (**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-naphthylsulfonyl (**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 N₁-sulfonyltryptamine series where the 1- and 2-naphthylsulfonyl derivatives had similar affinity to the N₁-phenylsulfonyl analogs.^{12,21}

Published data for the N₁-arylsulfonyltryptamine 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 Asp³⁰³ on TM7.²¹ In this N₁-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-HT-stimulated cAMP production in HeLa cells stably transfected with human 5-HT₆ 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-HT₆ receptor were tested in the cyclase assay to determine if they could modulate 5-HT₆ 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-HT₆ antagonists displayed low affinity for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₇, dopamine D₂, D₃, and D₄ receptors.

In conclusion, an interesting class of *N*₁-arylsulfonyl-tryptamine analogs in which the aminoethyl chain is rigidified into a tetrahydropyridinyl ring were designed. Synthesis and biological evaluation indicate they are selective 5-HT₆ 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}: [³H]-5-HT; 5-HT_{2A}: [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ([¹²⁵I]DOI); 5-HT₆, 5-HT₇: [³H]LSD; dopamine D₂, D₃, and D₄: [³H]piperone].

NT = not tested.

^a % inhibition @ 1 μM.

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18. 5-HT₆ membrane preparation: cultured HeLa cells expressing h5-HT₆ receptors are harvested and centrifuged at low speed (1000×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 50mM Tris–HCl, pH 7.4 and 0.5mM EDTA. The homogenate is centrifuged at 900×g for 10 min and the supernatant collected. The supernatant is then centrifuged at 40,000×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 1.0mg/mL of suspension. The prepared membrane suspension (10 times concentrated) is aliquoted in 1.0mL volumes and stored at –70°C until used in subsequent binding experiments.
19. 5-HT₆ binding assay: total bound well: 80 µL binding buffer, 20 µL of 3 µM [³H]-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 [³H]-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 [³H]-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-HT₆ compound intrinsic activity using cAMP accumulation: intracellular cAMP levels are measured using 24-well plates containing the human 5-HT₆ 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 0.5M perchloric 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).