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Novel brain penetrant benzofuropiperidine 5-HT$_6$ receptor antagonists

Babu G. Sundar *, Thomas R. Bailey, Derek D. Dunn, Edward R. Bacon, Joseph M. Salvino, George C. Morton, Lisa D. Aimone, Huang Zeqi, Joanne R. Mathiasen, Amy Dicamillo, Mark J. Huffman, Beth A. McKenna, Karla Kopec, Lily D. Lu, Rebecca Brown, Jie Qian, Thelma Angeles, Thomas Connors, Chrysanthe Spais, Beverly Holskin, Deborah Galinis, Emir Duzic, Herve Schaffhauser, Gerard C. Rosse

Worldwide Discovery Research, Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380, USA

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ABSTRACT

7-Arylsulfonyl substituted benzofuropiperidine was discovered as a novel scaffold for 5HT$_6$ receptor antagonists. Optimization by substitution at C-1 position led to identification of selective, orally bioavailable, brain penetrant antagonists with reduced hERG liability. An advanced analog tested in rat social recognition model showed significant activity suggesting potential utility in the enhancement of short-term memory.

The 5-hydroxytryptamine-6 receptor (5-HT$_6$R) is one of 14 known sub-types from the receptors of neurotransmitter, serotonin. With the exception of 5-HT$_3$R, which is a ligand-gated ion channel, all of them are G-protein coupled receptors (GPCRs).[1] The rat 5-HT$_6$R was first identified in 1993 followed by the identification of human 5-HT$_6$R in 1996.[2,3] 5-HT$_6$R is positively coupled to adenylyl cyclase, and the receptor activation leads to increased production of cyclic adenosine monophosphate (cAMP).[4] The 5-HT$_6$R is found to be almost exclusively expressed in the central nervous system (CNS) of mammals and is suggested to play a post synaptic role.[5,6] Because of their distribution in cerebral cortex and limbic areas, these receptors were proposed to be involved in cognitive processes. The 5-HT$_6$R antisense oligonucleotide studies suggested potential application of 5-HT$_6$R antagonists in cognition.[7] This hypothesis was further supported by the enhanced cholinergic[8] and glutamatergic neurotransmission[9] by selective small molecule 5-HT$_6$R antagonists in preclinical animal studies. In addition to their application in treating cognitive impairment in Alzheimer's disease (AD), Schizophrenia and Depression, 5-HT$_6$R antagonists have demonstrated preclinical utility in treating obesity.[10,11] With the development of ligand pharmacophore and 5-HT$_6$R homology models[12,13] over the last few years, several selective 5-HT$_6$ receptor antagonists have been disclosed (Fig. 1).[10,11,14] A majority of these compounds possess either indole or arylpiperazine as a core structural feature. Some of the earlier clinically investigated compounds such as SB-271046 suffered from poor blood–brain barrier (BBB) penetration which may be the reason for its discontinuation from further development. Eli Lilly's LY-483518 was licensed in by Saegis (SGS-518) and its clinical development was discontinued after Phase I trials for cognition in Schizophrenia.

Currently GSK's piperazinyl quinoline, SB-742457 is in Phase II development for AD. Also under active clinical development are the former Wyeth compounds SAM-531 (PF-05212365) in Phase II, and SAM-760 (PF-05212377) in Phase I for AD. Cephalon's early objective in this project was to discover novel, potent 5-HT$_6$R antagonists with acceptable selectivity, oral bioavailability, brain penetration and establish preclinical proof of concept. By a de novo design, we identified arylsulfonyl substituted benzofuropiperidine

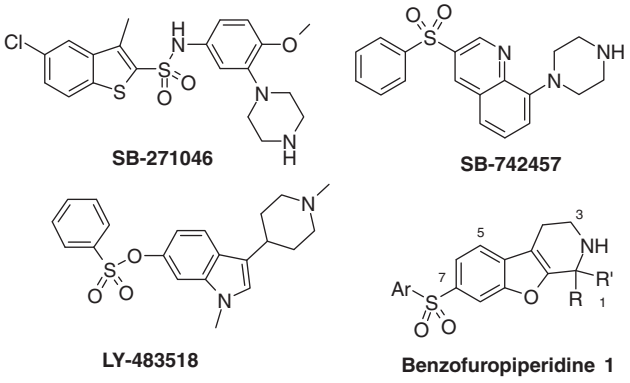

Figure 1. Clinically investigated 5-HT$_6$R antagonists and benzofuropiperidine.

* Corresponding author. Tel.: +1 610 738 6348; fax: +1 610 738 6558.
E-mail address: bsundar@cephalon.com (B.G. Sundar).

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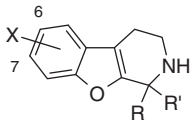
1 as a novel patentable scaffold. Our early structure activity (SAR) studies had demonstrated weaker binding affinity for compounds with substitution of arylsulfone at C-6 position of the benzofuropiperidine core. For example, compound **1a** was >15-fold weaker than **1b** in binding affinity toward human and rat receptors (Table 1). With arylsulfonyl at C-7, aromatic substitution was briefly examined. *Meta*-fluoro substitution afforded more potent compound compared to *para*-fluoro substitution (**1c** vs **1d**). Substitution of electron releasing group such as OMe led to drop in potency (compound **1e**). Compound **1c**, despite showing high affinity (h5-HT_6 K_i = 1.6 nM, r5-HT_6 K_i = 6.9 nM) with potent functional activity (IC_{50} = 3.6 nM),[15] displayed poor metabolic stability in the rat and dog liver microsomes ($t_{1/2}$ = <5 min and 10.3 min, respectively). Guided by the in vitro metabolic identification study in liver microsomes, blocking the C1 position from metabolism was expected to improve the stability. To test this, 1,1-*gem* diMe compounds **1f–1h** were prepared. As previously observed, *meta*-fluoro substitution gave better affinity (compound **1g**) than *meta*-OMe (**1h**) or unsubstituted phenyl compound (**1f**). Compound **1g** retained affinity (h5-HT_6 K_i = 2.9 nM, r5-HT_6 K_i = 19 nM) and functional activity (cAMP IC_{50} = 11.7 nM). Also compared to **1c**, the liver microsome stability showed overall improvement with $t_{1/2}$ (min) = 16, 32, >40 rat, dog, and human, respectively. This was reflected in a measurable oral exposure in the rat PK (Table 3).

But both the compounds **1c** and **1g** inhibited the hERG channel (IC_{50} of 1–3 μM). To mitigate the hERG inhibition risk, a strategy of reducing the secondary amine basicity was adopted. Compounds with electron withdrawing groups (OMe, CF_3 and CF_2H) at the β-position to the amine were synthesized (Table 2). The fluoroalkyl substitutions led to significant reduction in the amine pK_a and greater separation from hERG inhibition. But unfortunately, this also led to reduced human receptor binding affinity with the expected increase in log*D*. For example, the CF_3 compound (**1i**) with *c*Log*D* of 3.6, had h5-HT_6 K_i of 139 nM, and hERG IC_{50} of >30 μM. The less lipophilic CF_2H compound **1j** (*c*Log*D* of 3.0) had better binding affinity compared to compound **1k** (h5-HT_6 K_i = 16 nM) and good hERG selectivity (IC_{50} = >30 μM). But still, the rat 5-HT_6 affinity (K_i = 183 nM) and functional potency (cAMP IC_{50} = 54 nM) of **1j** were weaker than those of compound **1g**. As a next step, we focused on decreasing the amine basicity and maintain the potency.

The initial profile of OMe compound (**1k**) (*c*Log*D* = 2.3, pK_a = 7.5, h5-HT_6 K_i = 18 nM, r5-HT_6 K_i = 113 nM), provided a balance between potency and calculated properties. Based on this result, a small set of cyclic ether targets was prepared. The 4-spiropyran analog **1l** (4-spiro THP) displayed improved binding affinity towards human and rat receptors with a good hERG separation (*c*Log*D* = 2.4, h5-HT_6 K_i = 2.1 nM, r5-HT_6 K_i = 13.9 nM, hERG IC_{50}

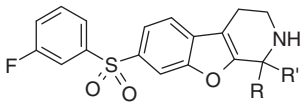
Table 1
5-HT_6R binding and functional activity for benzofuropiperidines



Compound	R/R′	X	h5-HT_6R[a] (K_i, nM)	r5-HT_6R [a] (K_i, nM)	h5-HT_6R[b] cAMP-(IC_{50}, nM)
1a	H/H	6-($PhSO_2$)–	46	156	–
1b	H/H	7-($PhSO_2$)–	2.7	9.8	8.5
1c	H/H	7-(*m*-F–$PhSO_2$)–	1.6	6.9	3.6
1d	H/H	7-(*p*-F–$PhSO_2$)–	22	–	–
1e	H/H	7-(*m*-OMe–$PhSO_2$)–	5.6	34	21
1f	Me/Me	7-($PhSO_2$)–	7.7	41	34
1g	Me/Me	7-(*m*-F–$PhSO_2$)–	2.9	19	11.7
1h	Me/Me	7-(*m*-OMe–$PhSO_2$)–	17.5	–	68.6

[a] [^{3}H]LSD binding in membranes prepared from cells expressing full length human or rat 5-HT_6R. The K_i values are the mean of duplicate values from three experiments with the exception of **1f** (*n* = 1).
[b] Mean of 3 functional activity experiments from human astrocytoma cell line stably expressing the human 5-HT_6R.

Table 2
5-HT_6R and hERG activity of 1,1-disubstituted benzofuropiperidines



Compound	R/R′	h5-HT_6R[a] (K_i, nM)	r5-HT_6R[a] (K_i, nM)	h5-HT_6R[b] cAMP-(IC_{50}, nM)	hERG[c] (IC_{50}, μM)	pK_a/*c*Log*D*[d]
1i	Me/CF_3	139	–	–	>30	5.7/3.6
1j	Me/CF_2H	16	183	54	>30	6.2/3.0
1k	Me/CH_2OMe	18	113	–	–	7.5/2.3
1l	4-THP	2.1	14	6.8	>30	7.4/2.4
1m	(±)3-THF	22.3	131.5	32	–	7.5/1.8
1n	(±)3-THP	6.5	38.3	–	13.6	7.5/2.1
2	(*ent-1*)-3-THP	4.4	16.9	9.8	12	7.5/2.1
3	(*ent-2*)-3-THP	130	–	–	9.6	7.5/2.1

[a] [^{3}H]LSD binding in membranes prepared from cells expressing full length human or rat 5-HT_6R. The K_i values are the mean of duplicate values from three experiments with the exception of **1i** (*n* = 1), **2** and **3** (*n* = 2).
[b] Mean of 3 functional activity experiments on human astrocytoma cell lines stably expressing the human 5-HT_6R.
[c] Determined by the patchXpress assay at MDS Pharma Services.
[d] Calculated values from ACD software.

Table 3
Rat pharmacokinetic data for select benzofuropiperidines

Rat pharmacokinetic data	**1g**	**1l**	**2**
i.v. (1 mg/kg)			
$t_{½}$ (h)	0.9 ± 0.2	0.9 ± 0.2	1.4 ± 0.4
Vd (L/kg)	5.5 ± 0.2	1.4 ± 0.2	4 ± 1.3
CL (mL/min/kg)	82 ± 16	17 ± 3	32 ± 4
p.o. (5 mg/kg)			
C_{max} (ng/mL)	35 ± 3	84 ± 7	210 ± 25
6 h-AUC (ng/h/mL)	149 ± 9	368 ± 31	974 ± 119
% *F*	15 ± 1	6	36 ± 4
i.p. (5 mg/kg)			
1 h-Brain conc. (nM/g)	1625 ± 604	979 ± 79	1659 ± 350
Brain/plasma	7.7 ± 0.4	3.3 ± 0.1	2 ± 0.5

i.v. Vehicle: 3% DMSO, 30% Solutol, 67% PBS.
p.o. Vehicle: Tween 80:propylene carbonate:propylene glycol (5:4:1).

>30 μM). The 3-spirotetrahydrofuran **1m** (3-spiroTHF) had binding affinities (h5-HT_6 K_i = 22.3 nM, r5-HT_6 K_i = 131.5 nM), comparable to those of the acyclic ether (**1k**). The 3-spirotetrahydropyran **1n** (3-spiroTHP) analog exhibited superior binding affinity (h5-HT_6 K_i = 6.5 nM, r5-HT_6 K_i = 38.3 nM). Anticipating potential activity differences between the enantiomers, the racemate spiroether **1n** was resolved by chiral supercritical fluid chromatography.[16] As illustrated in Table 2, the two 3-spiroTHP enantiomers displayed a large difference in binding affinity (**2**, h5-HT_6 K_i = 4.4 nM and **3**, h5-HT_6 K_i = 130 nM) with an eudismic ratio of 29.5. Based on the balanced potency, hERG separation and physical properties, the compounds **1l** and **2** were selected for further profiling. The liver microsome stability for **1l** ($t_{1/2}$ (min) = 25, >40, 19 rat, dog, and human) was comparable with that of **2** ($t_{1/2}$ (min) = 25, >40, 30 rat, dog, and human) except that **1l** had relatively inferior human metabolic stability.

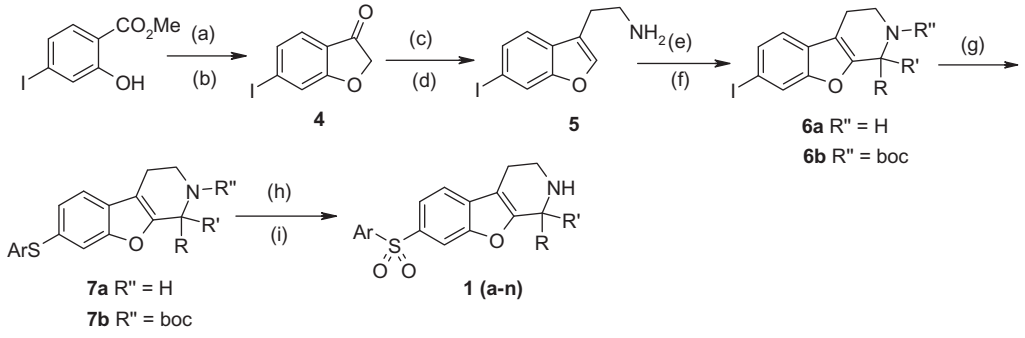
Due to their overall favorable attributes, compound **1l** and **2** advanced to rat pharmacokinetic experiments (Table 3) After a single i.v. bolus dose of 1 mg/kg, compound **1l** had a $t_{½}$ of 0.9 h with moderate clearance (17 mL/min/kg) and volume of distribution (1.4 L/kg). Oral administration of a single 5 mg/kg dose showed low oral bioavailability (F = 6%) based on 6hAUC (386 ng/h/mL) and C_{max} (84 ng/mL). On the other hand, compound **2** displayed an i.v. half life of 1.4 h, with acceptable plasma clearance (32 mL/min/kg) and volume of distribution of 4.0 L/kg. In the p.o. arm of the rat pharmacokinetic study, after an oral dose of 5 mg/kg, compound **2** had superior plasma exposure with an estimated oral bioavailability of 36% based on 6hAUC (974 ng/h/mL) and C_{max} (210 ng/mL). Compound **2** also had BBB penetration with a brain to plasma ratio (b/p) of 2.0. Based on its overall superior rat PK profile, compound **2** progressed to further characterization.

Compound **2** was further profiled for in house serotonin subtype selectivity, hERG and receptor selectivity and cytochrome P450 (CYP) inhibition. Compound **2** with MDSP patchXpress hERG IC_{50} of 12 μM showed >2700-fold separation from its h5-HT_6 affinity (K_i = 4.4 nM). In the CEREP lead profile testing, compound was highly selective, showing >50% inhibition at 10 μM for only 8 out of 95 tested targets (5-HT_{2A}, 5-HT_{2B}, 5-HT_6, α_{2A}, α_{2B}, D_2S, D_3 and rat L-type Ca-channel). The CYP IC_{50} was >30 μM for all isoforms (1A2, 2C19, 2D6, 3A4) except for CYP2C9 (IC_{50} = 1.9 μM). Compound **2**, tested for serotonin subtype selectivity in the counter screen binding assays was found to be very selective. Considering the h5-HT_6 K_i of 4.4 nM for compound, the binding selectivity against the closely related serotonin sub-types were determined to be 258-fold versus 5-HT_{2A}, 100-fold versus 5-HT_{2B} and >10,000-fold versus 5-HT_{2C}. Follow up testing for 5-HT_{2B} functional activity at CEREP showed that compound **2** was an antagonist with an IC_{50} of 1.5 μM.

Following the pharmacokinetic and the selectivity profiling, compound **2** advanced to establishing preclinical in vivo proof of concept. Based on previous published work, rat social recognition model[17] was chosen for this project for evaluation of short-term memory. In this model, compound **2** produced a significant activity at doses between 0.1 and 3.0 mg/kg i.p. compared to the vehicle. Detailed biological characterization and in vivo testing of this novel 5-HT_6 antagonist **2** will be described in due course.

The benzofuropiperidines were prepared as described in Scheme 1.[18] The commercially available iodosalicylate was O-alkylated with ethylbromo acetate and the resultant ether product was subjected to Claisen condensation to provide the benzofuranone **4**. Horner–Emmons olefination followed by borane reduction of the vinylnitrile intermediate afforded the key furan-3-ethylamine intermediate **5** in 50% overall yield. Pictet–Spengler cyclization with **5** and paraformaldehyde was readily performed in aq. HCl. Cyclization with ketones required stronger conditions such as warm trifluroacetic acid.[18] Boc-protection of tricyclic amines followed by copper catalyzed thiolation reaction resulted in the thioether derivatives **7**. Oxidation of the sulfide group was accomplished with *m*-CPBA in methylene chloride and the boc-group was removed using HCl in dioxane. Sterically hindered Pictet–Spengler products with weakly basic amines were subjected to thiolation, oxidation sequence without the N-boc-protective group.

In summary, we have disclosed here a novel fused piperidine scaffold for 5-HT_6 receptor antagonists. Scaffold optimization with 1,1-*gem* disubstitution resulted in compounds with improved pharmacokinetic profile. Further elaboration of 1,1-disubstitution into spirocyclic ethers provided an overall balance in physical properties, potency, ADME and hERG selectivity profile. A potent, selective functional antagonist from this study, compound **2**



Scheme 1. Preparation of benzofuropiperidines. Reagents and conditions: (a) $BrCH_2CO_2Et$, K_2CO_3, KI, acetone, 50 °C, 5 h, 90–95%; (b) (i) LiHMDS, toluene; 0–25 °C, 2 h; (ii) aq. NaOH, EtOH, 0–80 °C, 1.5 h; (iii) aq. HCl, 25–60 °C, 2.5 h (85%, 3 steps); (c) NaH, $CNCH_2PO(OEt)_2$, THF, 15–25 °C, 1 h; (d) BH_3-THF, 0–5 °C, 20 h (70–80% 2 steps); (e) carbonyl compound, 1 N aq. HCl, 70 °C, 2 h or DCE, TFA, 80 °C, 15 h, 50–85%; (f) $(Boc)_2O$, Et_3N, CH_2Cl_2 or THF; (g) ArSH, NaO^{t}Bu, CuI, Neocuproine, DMF, 100 °C, 15 h (60–90% 2 steps); (h) *m*-CPBA, CH_2Cl_2, 0–25 °C, 15 h or oxone, H_2O, MeOH, rt, 3 h; (i) 4 M HCl-dioxane (50–95%).

showed acceptable oral bioavailability (estimated $F = 36\%$) with brain penetration ($b/p = 2.0$) and established preclinical proof of concept in rat short-term memory model.

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15. (a) For binding assay details, see Ref. 18; (b) Antagonist assay: cAMP level in the cells were determined as previously described (Gabriel et al., *Assay Drug Dev. Technol.* **2003**, *1*, 291–303) using a homogenous time-resolved fluorescent (HTRF®) assay, (Cat # 62AM4PEC, Cisbio, Bedford, MA) in cell line stably expressing h5-HT_6, receptor. Briefly, 18 μL of cells in the cell density of 5000 cells per well were plated to white 384-well OptiPlates (Cat # 600727, PerkinElmer Life Sciences, Boston, MA). 100 nL of test compounds were added to cells and the plate was pre-incubated at room temperature for 10 min (final DMSO concentration was 0.5%). The cells were then stimulated with 6 μL of 5-HT at its EC_{80} concentration of 10 nM for 30 min at room temperature. The reaction was stopped by the addition of the 12 μL of cAMP-d2 in lysis buffer, followed by addition of 12 μL of anti cAMP-cryptate in lysis buffer. The plate was incubated at room temperature for 1 h and read on multi-labeled plate reader EnVision 2102 or 2104 (Perkin Elmer Life Sciences, Boston, MA). The fluorescence ratio (665 nm/590 nm $\times 10^4$) was calculated, which was inversely proportional to the level of cAMP in the sample.
16. Supercritical fluid chromatography chiral separation conditions: Chiral Pak AS-H column (10 × 250 mm), 6.0 ml/min 80% CO_2/20% *i*PrOH/0.1% Et_2NH, λ = 220 nm, compound **2**(*ent-1*): 20 min, compound **3**(*ent-2*): 26 min. Compound **2**: 7-[(3-fluorophenyl)sulfonyl]-3,4,5′,6′-tetrahydro-2*H*,4′*H*-spiro[1-benzofuro[2,3-c]pyridine-1,3′-pyran].
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.7 (d, *J* = 12.6 Hz, 1H), 1.83 (d, *J* = 14.2 Hz, 1H), 2.0 (m, 2H), 2.3 (m, 1H), 2.7 (m, 2H), 3.2 (m, 2H), 3.6 (m, 1H), 3.7 (d, *J* = 11.5 Hz, 1H), 3.85 (d, *J* = 12 Hz, 1H), 4.0 (d, *J* = 10.6 Hz, 1H), 7.2 (m, 2H), 7.4 (m, 1H), 7.57 (d, *J* = 8.1 Hz, 1H), 7.66 (d, *J* = 8.1 Hz, 1H), 7.76 (d, *J* = 8.1 Hz, 1H), 7.82 (d, *J* = 8.2 Hz, 1H), 8.08 (s, 1H), MS (*m*/*e*): 403 (M+H); m.p. (HCl salt) = 300–320 °C dec.
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