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New spiro-piperidines as 5-HT$_{2B}$ receptor antagonists

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Abstract—A functional screening highlighted a series of spiro-piperidines as 5-HT$_{2B}$ receptor antagonists. Preliminary structure–activity relationship has been explored driving to potent antagonists (IC_{50} = 1 nM) and indicating directions for further explorations.

Serotonin (5-hydroxytryptamine, 5-HT) is a well-known monoamine neurotransmitter, hormone, and mitogenic factor which mediates a wide range of physiological activities in different cells by binding to multiple receptor subtypes. With the exception of the 5-HT$_3$ which is a ligand-gated ion channel, all of these receptors are known to be G-protein coupled receptors (GPCRs).

The 5-HT$_{2B}$ receptor was first identified in rat stomach fundus.[1,2] Although originally termed 5-HT$_{2F}$ or 5-HT$_1$-like receptor,[3] it was reclassified as 5-HT$_{2B}$ to become the third member of the 5-HT$_2$ receptor family.[4–7] 5-HT$_{2B}$ receptors are widely expressed in peripheral tissues of various species. 5-HT$_{2B}$ receptor mRNA or protein immunoreactivity has been found throughout the gastrointestinal tract including smooth muscle of the stomach fundus, esophagus, small intestine, and colon. The receptor has also been found in the placenta, uterus, lung, and prostate, and 5-HT$_{2B}$ receptor antagonist activities were reported in several diseases such as prostatic cancer,[8] gastrointestinal diseases, migraine,[9] CNS disorders, urinary incontinence and bladder dysfunction,[10] and pulmonary hypertension.[11] Regulating 5-HT$_{2B}$/5-HT interaction appears to be a promising approach in the development of small-molecule drugs.

Among many 5-HT$_{2B}$ antagonists, **SB204741**,[12,13] **LY266097**,[14] and **RS127445**[15] (Fig. 1) are described as selective antagonists at the human receptor. We present here the identification of a new series of 5-HT$_{2B}$ antagonists.

Hit identification was realized by screening a set of 1250 small molecules selected for their structural relevance.[16] The functional test performed corresponds to the inhibition of calcium release after activation of 5-HT$_{2B}$ receptor by an agonist (α-methyl-5-hydroxytryptamine) in CHO cells.[17] A series of spiro-piperidine compounds exhibited potent antagonistic effects, **1** and **2** (Fig. 2) showing IC_{50} of 95 and 30 nM, respectively.

In this study, all the spiro compounds were either purchased commercially or prepared using a 3-step synthesis (Scheme 1). According to the literature, the formation of 2′-hydroxychalcone involved the Claisen–Schmidt condensation of a 2-hydroxybenzaldehyde **I** with an acetophenone **II** in the presence of base as catalyst.[18,19] Subsequently, hydrazine hydrate was

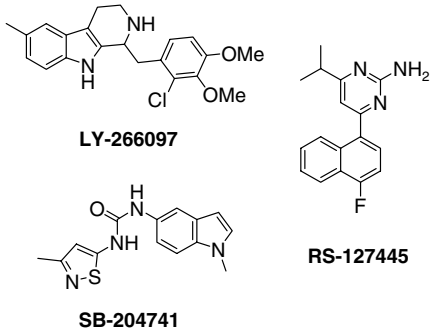

Figure 1. 5-HT$_{2B}$ receptor ligands.

Keywords: HTR2B antagonists; G-protein coupled receptors; Spiro-piperidines; Pyrazoline.
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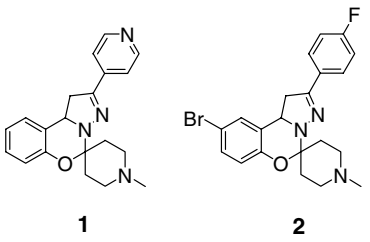
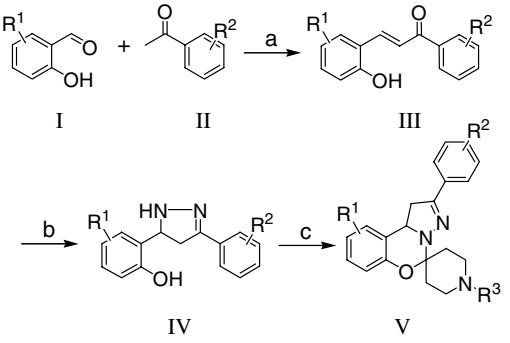


Figure 2. Lead compounds.



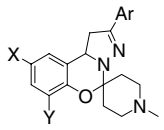
Scheme 1. Reagents and conditions: (a) KOH, aqueous ethanol, 16 h, rt; (b) NH_2NH_2, MeOH, rt; (c) N-substituted piperidin-4-one, neat, 50 °C.

added to the chalcone **III** in methanol and furnished the corresponding pyrazoline **IV** in good yield.[19] Finally, the spiro compound **V** was obtained by heating the pyrazoline with a N-substituted piperdin-4-one at 50 °C.

The 5-HT_{2B} antagonist activity for compounds **1**–**24** is shown in Table 1. The most potent compound is the 4-fluorophenyl **3** with an IC_{50} below 10 nM. Generally, both the hydrogen (**3**, **4**, **7**, and **9**) and bromine (**2**, **5**, **8**, and **10**) substitutions at the position *X* had similar potency on the target. On the contrary, when the hydrogen in position *Y* (**3**, **6**, and **7**) was replaced with electron-rich methoxy group (**22**, **23**, and **24**), a markedly reduced activity was observed.

For the aromatic group (Ar), the inhibition was decreased by using bulkier groups such as naphthyls (**20** and **21**) or with heteroaryl groups such as 2-furyl (**18**) and 2-thienyl (**19**). Interestingly, compared to the lead compound **1**, which has an $IC_{50} = 95$ nM, the 3-pyridyl analog (**17**) completely lost its activity. The effect of varying the pattern on the phenyl group was explored. Among the 4-halophenyl compounds, 4-fluoro substitution was the preferred one (comparing **3**, **6**, and **7**, also **22**, **23**, and **24** activities). In addition, shifting the halogen from the 4-position (**6** and **8**) to the 2-position (**9** and **10**) resulted in approximately 2-fold loss in activity. Introduction of an electron-rich methoxy group to the 4-position of the phenyl ring led to a decrease in potency. Compounds without any substitution (**4** and **5**) did not show any improvement compared to the hit compounds. The 3,4-dichlorophenyl (**15**) and 3,4-dimethoxyphenyl (**16**) compounds retained similar activity when compared to their mono-4-substituted analogs (**8** and **12**). These results disclosed that the

Table 1. Biological assay results for compounds **1**–**24**



Compound[a]	Ar	X	Y	Activity[b] IC_{50}[c] (nM)
1	4-Pyridyl	H	H	95
2	4-Fluorophenyl	Br	H	30
3	4-Fluorophenyl	H	H	<10[d]
4	Phenyl	H	H	50–100
5	Phenyl	Br	H	92
6	4-Chlorophenyl	H	H	280
7	4-Bromophenyl	H	H	426
8	4-Chlorophenyl	Br	H	100–500
9	2-Chlorophenyl	H	H	753
10	2-Chlorophenyl	Br	H	856
11	2-Methoxyphenyl	Br	H	5000
12	4-Methoxyphenyl	Br	H	5000
13	2-Difluoromethoxyphenyl	Br	H	427
14	4-Difluoromethoxyphenyl	Br	H	175
15	3,4-Dichlorophenyl	Br	H	542
16	3,4-Dimethoxyphenyl	Br	H	5000
17	3-Pyridyl	H	H	na
18	2-Furyl	Br	H	5000
19	2-Thienyl	Br	H	5000
20	1-Naphthyl	Br	H	>10000
21	2-Naphthyl	Br	H	5000
22	4-Fluorophenyl	H	MeO	46
23	4-Chlorophenyl	H	MeO	1470
24	4-Bromophenyl	H	MeO	1281

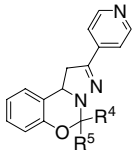
[a] Racemates were used.
[b] Inhibition of calcium release generated by α-methyl-5-HT (Ref. 17).
[c] na, not active. Average value from one experiment in duplicate.
[d] 90% inhibition at 10 nM.

3-phenyl substitution was acceptable but does not seem to be essential. Replacing the methoxy group of **11** or **12** with a difluoromethoxy group, **13** and **14** showed a 10- to 20-fold increase in biological activity. These results further showed the importance of the 4-fluoro substitution pattern for 5-HT_{2B} receptor affinity. From all these results, both 4-fluorophenyl and 4-pyridyl groups exhibited a high affinity with 5-HT_{2B} receptor.

The modification of the spiro-piperidine moiety (R^4 and R^5) was investigated. Replacement by groups such as dimethyl (**25**), cyclohexyl (**26**), 4-methylcyclohexyl (**27**), and tetrahydropyranyl (**28**) resulted in the complete loss of activity (Table 2).

Consequently, our efforts were addressed to the optimization of the N-substitution of the spiro-piperidine scaffold (Table 3). Attachment of an electron-withdrawing acetyl group to the nitrogen atom led to the loss of the biological activity (**29**, **30**, and **31**). The bulky benzyl group (comparison between **34** and **42**) was also not suitable for the nitrogen substitution, but the 2-phenylethyl substitution was tolerated (comparison between **43** and **51**). When the *N*-alkyl chain evolved from methyl to ethyl and to *n*-propyl, the activity was increased by 2-fold for each

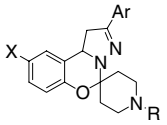
Table 2. Variations at the R^4 and R^5 positions



Compound[a]		Activity[b] IC_{50}[c] (nM)
25		5000
26		na
27		na
28		na

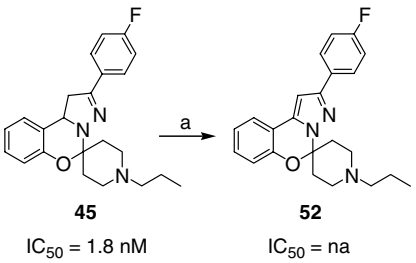
[a] Racemates were prepared by heating pyrazoline **IV** with corresponding ketone at 50 °C.
[b] Inhibition of calcium release generated by α-methyl-5-HT (Ref. 17).
[c] na, not active. Average value from one experiment in duplicate.

Table 3. Variations at the R position



Compound[a]	Ar	X	R	Activity[b] IC_{50}[c] (nM)
29	Phenyl	Br	Acetyl	na
30	4-Fluorophenyl	Br	Acetyl	na
31	4-Chlorophenyl	Br	Acetyl	na
32	2-Chlorophenyl	Br	Benzyl	na
33	4-Chlorophenyl	H	Benzyl	350
34	4-Methoxyphenyl	Br	Benzyl	na
35	4-Pyridyl	H	Ethyl	42
36	4-Pyridyl	H	*i*-Butyl	21
37	Phenyl	Br	*i*-Propyl	188
38	4-Chlorophenyl	H	*i*-Propyl	133
39	4-Bromophenyl	H	*i*-Propyl	>10,000
40	4-Tolyl	Br	*n*-Propyl	5000
41	4-Bromophenyl	Br	*n*-Propyl	791
42	4-Methoxyphenyl	Br	*n*-Propyl	179
43	4-Pyridyl	H	*n*-Propyl	15
44	Phenyl	H	*n*-Propyl	<10[d]
45	4-Fluorophenyl	H	*n*-Propyl	1.8
46	4-Fluorophenyl	Me	*n*-Propyl	115
47	4-Fluorophenyl	F	*n*-Propyl	26
48	4-Fluorophenyl	Cl	*n*-Propyl	32
49	4-Fluorophenyl	Br	*n*-Propyl	29
50	2-Chlorophenyl	H	*n*-Propyl	500
51	4-Pyridyl	H	2-Phenylethyl	16

[a] Racemates were used.
[b] Inhibition of calcium release generated by α-methyl-5-HT (Ref. 17).
[c] na, not active. Average value from one experiment in duplicate.
[d] 90% inhibition at 10 nM.



Scheme 2. Reagents and conditions: (a) CAN, CH_3CN-water (9:1), 16 h.

homologation (**1**, **35**, and **43**). All the *n*-propyl series generally provided the best activity (comparison between **12**–**42** and **4**–**44**). Concerning the substitution at the *X*-position, the halo-substitution gave similar activities between **47** and **49** (IC_{50} = 26 to 32 nM) but the most active one was the compound without any substitution (X = H). Therefore, 2-(4-fluorophenyl)-1,10b-dihydro-benzo[*e*]pyrazolo[1,5-*c*][1,3]oxazine-5-spiro-4′-(1′-propylpiperidine) (**45**)[20] was selected for chiral resolution (one chiral center is present in the pyrazoline ring). From the racemate mixture, both enantiomers were separated by chromatography using a chiral column.[21] Each enantiomer exhibited very different activity. The enantiomer with the shorter retention time, $\mathbf{45}t_{\mathbf{R1}}$, was the most active with an IC_{50} = 1.0 nM, while the second one (longer retention time, $\mathbf{45}t_{\mathbf{R2}}$ was inactive up to 500 nM. Therefore, the stereochemistry of the compound was important for the target affinity.

In order to avoid the chirality issue, pyrazole **52** was synthesized from the pyrazoline **45**. Several oxidizing agents like MnO_2, H_2O_2, and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) were attempted but failed. Finally, the reaction was carried out successfully using ammonium cerium (IV) nitrate (CAN) and gave 50% yield of **52** which was found to be inactive (Scheme 2).

In summary, a novel series of 5-HT_{2B} receptor ligand was identified, 2-aryl-1,10b-dihydro-benzo[*e*]pyrazolo [1,5-*c*][1,3]oxazine-5-spiro-4′-(1′-alkylpiperidine). We found that variations of aromatic substitution (Ar) and N-substitution of the piperidyl can influence the activity and allowed to highlight potent antagonists (IC_{50} < 10 nM in a functional assay). The most dramatic structural modification observed was the required basic nitrogen of the piperidine ring, indicating a strong proton acceptor interaction, and the stereospecificity on the pyrazoline ring which seems related to a specific protein pocket for the aromatic group. The established S.A.R. pattern gave the opportunity for further optimization. Further studies are needed to find out the selectivity of 5HT receptor sub-types for these spiro-piperidines.

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16. The selection has been made creating a chemofilter of autocorrelative vectors (Oto program) by discriminant analysis from a set of 167 published selective and not selective 5-HT_{2B} ligands extracted from Aureus Pharma database. The confidence of the assay was 92% well identified compared to 63% in the random case. A virtual screening was conducted with this filter on an internal collection of 30,000 compounds driving to the set of 1250 small molecules.
17. Screening protocol (Euroscreen): CHO cells expressing 5-HT_{2B} receptors, aequorin, and Gα16 were seeded in poly-D-lysine coated plates with coelentterazine and fetal serum albumin. After 15-h incubation at rt, the cell suspension was incubated for 30 min with the tested compound followed by the addition of the agonist (α-methyl-5-HT) at a concentration corresponding to its EC_{80} of the day. The luminescence, related to calcium release, was measured for 30 s following the agonist addition. Inhibition percentage of the luminescence was measured and IC_{50} evaluated for active compounds.
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20. Characterization data for compound **45**: (mp 130.1–131.3 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (*ppm*), 7.62–7.58 (m, 2H, CH_{arom}), 7.12–7.08 (m, 1H, CH_{arom}), 7.04–6.97 (m, 3H, CH_{arom}), 6.91 (dt, 1H, CH_{arom}, J_1 = 1.01 Hz, J_2 = 7.58 Hz), 6.77 (dd, 1H, CH_{arom}, J_1 = 1.01 Hz, J_2 = 8.08 Hz), 5.08 (d, 1H, $CH_{pyrazoline}$, J = 9.35 Hz), 3.51–3.45 (m, 1H, $CH_{pyrazoline}$), 3.23 (dd, 1H, $CH_{pyrazoline}$, J_1 = 1.26 Hz, J_2 = 15.92 Hz), 2.84–2.79 (m, 1H, CH), 2.68–2.59 (m, 3H, CH), 2.50 (m, 1H, CH), 2.40–2.36 (m, 3H, CH), 2.11–2.01 (m, 2H, CH_2), 1.60–1.51 (m, 2H, CH_2), 0.92 (t, 3H, CH_3, J = 7.33 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ (*ppm*), 163.1 (d, J = 248.82 Hz), 152.2, 150.7, 129.1, 129.1, 128.2, 128.1, 127.0, 126.4, 123.3, 121.3, 117.5, 115.4, 115.2, 88.1, 60.5, 56.5, 49.6, 49.4, 41.0, 34.8, 32.4, 20.4, 12.1; LC/MS (EI, *m*/*z*): (M+l) 380.28; R_f (10% MeOH in DCM) = 0.52.
21. HPLC experiments were carried out using a Waters HPLC system equipped with a *Quaternary Pump 600 E Multisolvent Delivery System*, a *Waters 996 Photodiode Array Detector*, a*Waters 2767 Sample Manager*, a make-up pump *Waters Reagent Manager* (100% MeOH, flow rate 1 ml/min), and a preparative/analytical splitter from *LCPackings* with a 1/1000 split. The system was run with MassLynx 3.5 configured with OpenLynx 3.5 and FractionLynx 3.5. The chiral stationary phases (CSP) were Chiralpak AD-H, 5 μm. Analytical and preparative assays were carried out on 250 × 4.6 mm ID and 250 × 20 mm ID columns, respectively. Flow rates were 1 ml/min and 19 ml/min, respectively. Samples were dissolved in MeOH (36 mg/ml). Enantiomers were resolved on a semi-preparative scale. Fraction collections were triggered by the DAD TIC signal (210–500 nm). The HPLC analytical assay was performed using mixtures of acetonitrile/2-propanol/diethylamine (85:15:0.1) as eluent. The retention time of the 2 enantiomers was t_{R1} = 4.2 min and t_{R2} = 5.2 min.

t_{R1}	t_{R2}	k'_1	k'_2	α	w_1	w_2	Rs
4.2	5.2	0.24	0.53	2.2	0.30	0.47	1.53