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Identification of *tris*-(phenylalkyl)amines as new selective h5-HT$_{2B}$ receptor antagonists

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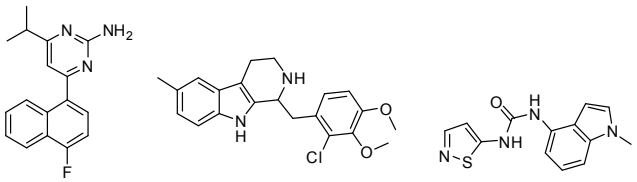
A series of *tris*-(phenylalkyl)amines was synthesized and evaluated for affinity to human 5-HT$_2$ receptors. In general, the compounds displayed high affinity (4 of 11 analogs had K$_i$ values < 10 nM) and good selectivity for the 5-HT$_{2B}$ receptor vs other 5-HT$_2$ receptors. Functional assays revealed that the compounds are 5-HT$_{2B}$ antagonists.

The 5-HT$_{2B}$ receptor is involved in regulation of the CNS, gastric and intestinal motility and cardiovascular function. 5-HT$_{2B}$ antagonists have been explored as potential pharmacotherapies for migraine,[1] irritable bowel syndrome,[2-4] pulmonary hypertension[5] and heart failure.[6] 5-HT$_{2B}$ receptor agonists display antidepressant activity and 5-HT$_{2B}$ receptor activation is required for antidepressant actions of selective serotonin reuptake inhibitors (SSRI's).[7] However, 5-HT$_{2B}$ agonism is known to be associated with the development of valvular heart disease (VHD) and as such is regarded as an anti-target in most drug discovery programs.[8-10]

Figure 1. Selective 5-HT$_{2B}$ antagonists



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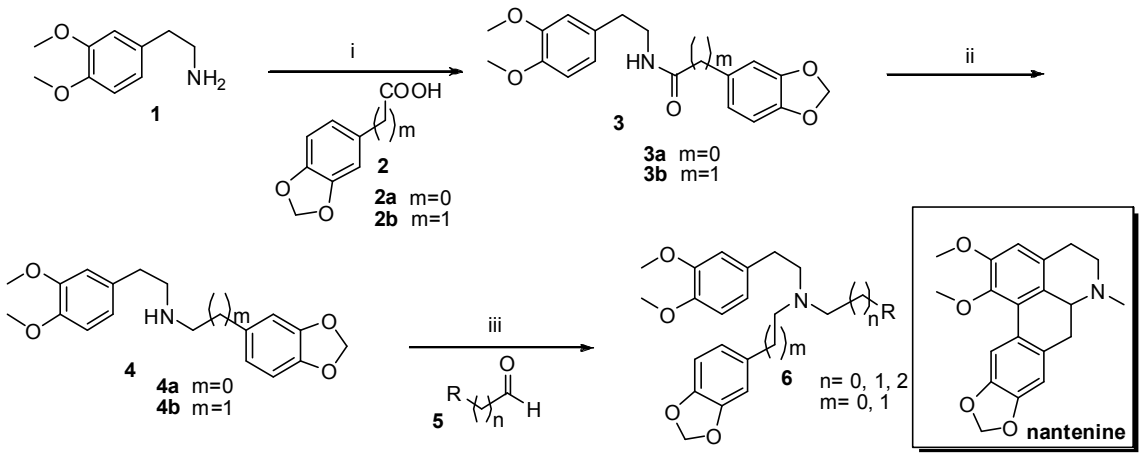
Despite the promise of 5-HT$_{2B}$ antagonists as useful therapeutics, there are no 5-HT$_{2B}$ antagonists that are clinically approved for the clinical indications mentioned previously. This is partly because many known ligands are not truly 5-HT$_{2B}$ selective (5-HT$_{2B}$ ligands often also have affinity for the related 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors) and even when selective there are issues related to ADME properties of the compounds that prohibit clinical translational studies. Figure 1 shows some selective 5-HT$_{2B}$ antagonists that are commercially available; these compounds are predominantly used as biological tools.[11-14] The identification of new 5-HT$_{2B}$ preferring scaffolds is critical in the pursuit of novel chemical entities that may be developed as useful 5-HT$_{2B}$ antagonist therapeutics. We describe herein the serendipitous discovery of a new series of ligands bearing a *tris*-(phenylalkyl)amine scaffold with high affinity and selectivity for the 5-HT$_{2B}$ receptor. The ease of synthesis of this scaffold makes it particularly attractive for further structure-activity work to optimize 5-HT$_{2B}$ affinity, selectivity and antagonist activity in the quest for 5-HT$_{2B}$ antagonist drugs.

Our research team has been investigating aporphines based on the natural product nantenine (see inset, Scheme 1) as ligands for the 5-HT$_{2A}$ receptor and this program has resulted in the identification of a number of new aporphine-based 5-HT$_{2A}$ antagonists.[15-17] As part of those efforts, we decided to investigate the importance of molecular rigidity of the aporphine template on 5-HT$_{2A}$ antagonism. In that regard, we decided to explore whether the replacement of the *N*-methyl group of nantenine with an *N*-phenylalkyl moiety and concomitant increase in flexibility would affect 5-HT$_{2A}$ antagonist activity. We considered that this approach might allow the ligands multiple possibilities for interaction of the receptor with *N*-phenylalkyl groups which seem to be important pharmacophoric recognition elements in 5-HT$_{2A}$ ligands, thus leading to increase in 5-HT$_{2A}$ receptor affinity. Additionally, we reasoned that this approach could lead to more diverse series of analogs and a much shorter synthetic route to the compounds, precluding laborious synthesis of the aporphine template. Thus we engaged the synthesis of compounds **6a - 6k** as shown in Scheme 1.

The preparation of analogs **6a – 6k** was readily accomplished in 3 steps. In the first step, the commercially available amine **1** was coupled to acids **2a** and **2b** to furnish compounds **3a** and **3b**. Reduction of amides **3a** and **3b** with $LiAlH_4$ gave the secondary amines **4a** and **4b**. Reductive amination of secondary amines **4a** and **4b** with various aldehydes (**5**) provided the target molecules **6a – 6k** (see Supplementary Information for experimental procedures).

Analogs **6a - 6k** were submitted to the Psychoactive Drug Screening Program (PDSP)[18] for evaluation of their affinity to 5-HT$_2$ receptors. Here, the submitted compounds were first screened in a primary radioligand binding assay (in quadruplicate) at a concentration of 10 μM at the three human 5-HT$_2$ receptor sites. Compounds which displayed a minimum of 50% inhibition for a particular receptor in this preliminary assay were then evaluated in secondary radioligand binding assays (11 concentrations; each in triplicate) to determine K$_i$ values. These K$_i$ values are compiled in Table 1. Complete details of the assays performed may be found in the PDSP assay protocol book (http://pdsp.med.unc.edu/PDSP%20Protocols%20II%202013-03-28.pdf).

As mentioned before, the motivation behind the design and synthesis of this set of compounds was due to our interest in identification of 5-HT$_{2A}$ receptor ligands and so we were a bit surprised at the outcome of the assays. In general, this series of compounds displays high affinity for the 5-HT$_{2B}$ receptor and a range of selectivity (from 2 to almost 90-fold) vs the 5-HT$_{2A}$ and 5-HT$_{2C}$ subtypes. Most of the analogs had 5-HT$_{2B}$ affinities that were similar or superior to the standard ligand used – SB206553, which had 5-HT$_{2B}$ affinity of 21 nM (see Supplementary Information for typical binding curve).



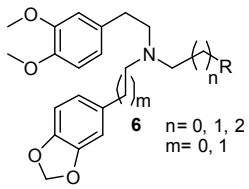
Scheme 1. *Reagents and conditions*: (i) **2**, CDI,THF, 0° C - rt, 12h; (ii) $LiAlH_4$, THF, 0° C - rt, 12h; (iii) **5**, $NaBH(OAc)_3$, DCM, rt, 12h.

Compound **6a** showed good affinity (59 nM, see Table 1) for the 5-HT_{2B} receptor. This affinity improved upon addition of one or two methylene groups between the nitrogen atom and the benzene ring (ie compounds **6b** and **6c**; 17 and 26 nM respectively). In the case of compound **6b**, as compared to compound **6a**, the increase in 5-HT_{2B} affinity was accompanied by increases in 5-HT_{2A} and 5-HT_{2C} affinities as well. However, the selectivity for 5-HT_{2B} vs 5-HT_{2A} and 5-HT_{2C} receptors improved (from 60 and 19-fold respectively for **6a** to 87 and 41-fold for **6b**). For compound **6c**, there was also an increase in 5-HT_{2A} and 5-HT_{2C} affinities as compared to **6a**. However, the selectivity for 5-HT_{2B} was lower than both **6a** and **6b** (6 and 15-fold respectively for 5-HT_{2A} and 5-HT_{2C} selectivities). Thus it appears that a 2 carbon chain between the nitrogen atom and the unsubstituted aryl ring is well tolerated for 5-HT_{2B} selectivity. As compared to compound **6c**, the 2-, 3- and 4-methoxy derivatives **6d** – **6f** showed higher affinity for the 5-HT_{2B} receptor (5.8, 4.6 and 6.8 nM respectively), indicating excellent tolerance for these substituents on the scaffold. In general it appears that the position of the methoxy group on the aromatic ring does not impact 5-HT_{2B} affinity among this subset of compounds given the similar affinities observed. Among **6d - 6f**, the highest 5-HT_{2B} selectivity vs 5-HT_{2A} was seen for the 3-methoxy derivative, **6e** (43-fold). The 2-methoxy derivative **6d** had the lowest 5-HT_{2B} selectivities (24 and 21-fold for 5-HT_{2A} and 5-HT_{2C} respectively) in the **6d - 6f** mono-methoxy series. A 2,5-dimethoxy substitution pattern did not improve affinity as is evident from the comparison of **6c** (26 nM) and **6g** (36 nM). Furthermore, **6g** had reduced 5-HT_{2B} affinity when compared to the 2-methoxy derivative **6d** (36 vs 5.8 nM) indicating that a 2-methoxy substitution is preferred to 2,5-dimethoxy substitution for affinity. Low 5-HT_{2B} selectivities were also seen for compound **6g** (26 and 8-fold for 5-HT_{2A} and 5-HT_{2C}). When compared to the unsubstituted benzene derivative **6c**, a 3,4,5-trimethoxy substitution pattern (ie **6h**) gave higher 5-HT_{2B} affinity (4.1 nM) - comparable to that seen in the mono-methoxy derivatives **6d** – **6f**. 5-HT_{2B} selectivity for **6h** vs the 5-HT_{2A} receptor was comparable to that seen for **6e** and **6f** and selectivity vs 5-HT_{2C} was improved. In fact, **6h** had the highest 5-HT_{2B} vs 5-HT_{2C} selectivity (47-fold) of all the compounds tested.

For compounds **6i - 6j** in which the nitrogen atom is separated from the methylendioxyphenyl moiety by only one methylene group, the highest 5-HT_{2B} affinity was seen for compound **6i**. Unlike the case where the 3,4,5-trimethoxyphenyl analog **6h** and 3-methoxyphenyl derivative **6e** diplayed similar 5-HT_{2B} affinities, significantly lower 5-HT_{2B} affinity was seen for the 3,4,5-trimethoxyphenyl derivative **6j** when compared to 3-methoxyphenyl derivative **6i**. A comparison of **6i** with its methylene homologue **6e**, shows a reduction in 5-HT_{2B} affinity for **6i** (59 vs 4.6 nM). Comparison of 5-HT_{2B} affinities for **6j** and its homologue **6h** also shows a similar trend (231 vs 4.1 nM). These pieces of data taken together indicate that the presence of an ethyl linker between the nitrogen atom and the methylenedioxyphenyl unit is more desirable for 5-HT_{2B} affinity. Interestingly, the styryl derivative **6k** maintained very good 5-HT_{2B} affinity despite the absence of an ethyl linker unit as seen in **6c** – **6h**. Indeed, the 5-HT_{2B} affinity for **6k** was similar to **6c** which is tending to suggest that the presence of a *cis* double bond locks the phenylpropyl unit into a favorable conformation for binding to the 5-HT_{2B} receptor. However, even though good 5-HT_{2B} affinity was retained in **6k**, this was not accompanied by any improvement in selectivity vs the other 5-HT_2 receptors. Thus the styryl moiety is not preferred for 5-HT_{2B} selectivity.

To further characterize the pharmacological properties of the analogs, selected compounds were evaluated for functional activity at the 5-HT_{2B} receptor in calcium mobilization assays. Here, the analogs were first tested in a primary assay for agonist and antagonist activity at a single concentration (10 μM). For each compound, a secondary assay was performed if the compound was active in the primary assay. For agonists identified in the primary assay, concentration-response curves were run to determine EC_{50} values in a secondary assay. In the case of antagonists, concentration-response curves were performed in the presence of the agonist 5-HT at a concentration of 3 nM to determine IC_{50} values.

Table1. Binding affinities and 5-HT_{2B} selectivities of compounds **6a-6k** at h5-HT_2 receptors



Cmpd.	R	n	m	K_i (nM)[a]			Selectivity	
				5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_{2A}/5-HT_{2B}	5-HT_{2C}/5-HT_{2B}
6a	Phenyl	0	1	3531 ± 460	59 ± 8.8	1091 ± 140	60	19
6b	Phenyl	1	1	1472 ± 190	17 ± 2.5	690 ± 100	87	41
6c	Phenyl	2	1	165 ± 25	26 ± 2.3	399 ± 75	6	15
6d	2-Methoxyphenyl	2	1	140 ± 15	5.8 ± 0.6	123 ± 16	24	21
6e	3-Methoxyphenyl	2	1	200 ± 22	4.6 ± 0.5	108 ± 14	43	24
6f	4-Methoxyphenyl	2	1	267 ± 34	6.8 ± 0.7	206 ± 27	39	30
6g	2,5-Dimethoxyphenyl	2	1	919 ± 120	36 ± 4.6	273 ± 35	26	8
6h	3,4,5-Trimethoxyphenyl	2	1	146 ± 19	4.1 ± 0.5	194 ± 25	36	47
6i	3-Methoxyphenyl	2	0	1507 ± 190	59 ± 7.6	103 ± 19	26	2
6j	3,4,5-Trimethoxyphenyl	2	0	2234 ± 290	231 ± 25	na[b]	10	-
6k	(*Z*)-Styryl	0	0	226 ± 29	21 ± 2.3	241 ± 45	11	12
Clozapine				15				
SB206553					21			
Ritanserin						1.8		

[a] Radioligands are [^{3}H]ketanserin, [^{3}H]LSD and [^{3}H]mesulergine for 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} respectively

[b] na - not active defined as: % inhibition at 10 μM < 50% in primary assay

Table 2. pIC_{50} Data for 5-HT_{2B} antagonist assays

Compound	5-HT_{2B}
6b	5.0
6c	6.1
6d	5.0
6e	5.9
6f	5.4
6g	5.1
6h	5.9
6i	nd[a]
6j	4.9
6k	5.2

[a] not determined - inactive in primary assay

No significant agonist activity was detected for the compounds in the primary assay. Compound **6i** did not display antagonist activity in the primary assay and so was not tested in the secondary functional assay. The other compounds examined were all found to be 5-HT_{2B} receptor antagonists in the primary assay with pIC_{50} values ranging from 4.9 to 6.1 in the subsequent secondary assays (Table 2).

In order to gauge the selectivity of the scaffold against other CNS targets and to determine the mode of antagonist action, compound **6c** (as the compound with the highest 5-HT_{2B} antagonist activity and as a representative of the set of analogues), was submitted for further pharmacological characterization.

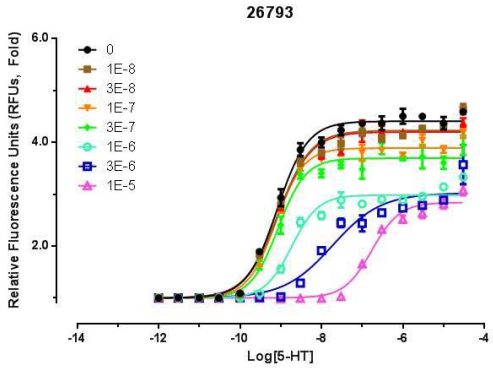
The following nanomolar affinities for **6c** were returned from the PDSP broad panel screening: [5-HT_{1A} (821); 5-HT_{1D} (451); 5-HT_7 (700); α_{1A} (333); α_{1D} (467); α_{2A} (102); α_{2B} (29); α_{2C} (429); β_1 (1885); D_1 (1682); D_2 (1729); D_3 (498); D_4 (853); DAT (498); H_1 (1297); kappa opioid receptor (363); mu opioid receptor (341); NET (11); SERT (1001); $sigma_1$ (176); $sigma_2$ (242)]. No appreciable affinity was seen for the following sites: 5-HT_{1B}, 5-

HT_{1e}, 5-HT_3, 5-HT_{5A}, 5-HT_6, α_{1B}, β_2, β_3, BZP; D_5, delta opioid receptor, $GABA_A$, H_3, M_1-M_5 and PBR. Further functional assays on **6c** revealed that it is also an antagonist at the other 5-HT_2 receptor subtypes with pIC_{50} values of 5.3 and 4.9 nM for 5-HT_{2A} and 5-HT_{2C} receptors respectively. No appreciable agonist activity was observed at these receptors.

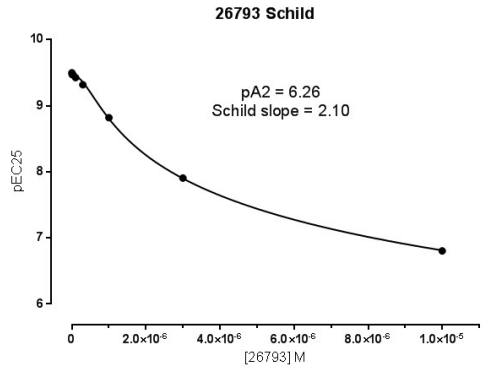
The pIC_{50} values obtained for the compounds did not seem to be in line with the affinities (assuming that the compounds are competitive antagonists). We considered that one possibility for this apparent discrepancy was that the compounds are non-competitive antagonists. To shed some light on this issue, compound **6c** was submitted for a Schild analysis to clarify the mode of antagonism. The result of this analysis is presented in Figure 2. As shown in figure 2a, increasing concentrations of compound **6c** (PDSP code 26793), caused a dextral shift in the dose-response curve with a depression in the maximum response observed in the absence of antagonist. The slope of the Schild plot was significantly different from unity. This indicates that compound **6c** is a non-competitive 5-HT_{2B} antagonist. The pA2 value as determined by modified Schild analysis[19] was 6.26.

Figure 2. a) Schild analysis on compound **6c** (PDSP compound code 26793) b) Schild slope regression

a)



b)



Conclusions

In summary, we have identified a new series of *tris*-(phenylalkyl)amine ligands with high affinity and good selectivity for the h5-HT_{2B} receptor. Of the analogs tested, compound **6b** displayed the highest selectivity vs the 5-HT_{2A} receptor, while compound **6h** shows the highest selectivity vs 5-HT_{2C}. Compound **6c** showed moderate (>100 nM) or no appreciable affinity for a number of other receptor sites in a broad panel screening (excepting for α_{2B} and NET where affinities of <30 nM were obtained). We anticipate that the other analogs will display a similar profile but this needs to be confirmed in future. The affinity data reveals that various alkyl chain lengths (between N and the aromatic rings), as well as a variety of methoxylated aromatic ring substitution patterns can be tolerated for good 5-HT_{2B} affinity. However, the best 5-HT_{2B} affinities are seen for compounds that feature a propyl linker between the nitrogen atom and one aromatic moiety and an ethyl unit between the nitrogen atom and a methylenedioxyphenyl moiety. Functional activity testing revealed that most of these compounds are h5-HT_{2B} receptor antagonists. Schild analysis revealed that compound **6c** is a non-competitive 5-HT_{2B} antagonist; it is possible that the other analogues also display a similar mode of antagonism given the data obtained and the structural similarities among the series.

The synthetic tractability of this newly identified *tris*-(phenylalkyl)amine template (only 3, high-yielding synthetic steps from commercially available materials) provides this scaffold with a significant advantage for the synthesis of larger libraries of analogs and promise for optimization of 5-HT_{2B} affinity and selectivity. Additional exploration of the scaffold should provide new tool compounds that will be useful for mapping the binding surfaces of the 5-HT_{2B} receptor. Further *in vitro* as well as *in vivo* pharmacological characterization of these compounds is an exciting dimension for future work. We are continuing with these synthetic and biological investigations and will furnish our findings in this regard in due course.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Typical binding curve for 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} binding experiments (shown for compound **6h** only) and 5-HT_{2B} antagonist functional data. Typical synthetic procedures and NMR spectra for analogs **6a-6k**. See DOI: 10.1039/b000000x/

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