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New halogenated tris-(phenylalkyl)amines as *h*5-HT$_{2B}$ receptor ligands

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ABSTRACT

A series of compounds in which various halogen substituents were incorporated into a phenyl ring of a tris-(phenylalkyl)amine scaffold, was synthesized and evaluated for affinity to *h*5-HT$_2$ receptors. In general, all compounds were found to have good affinity for the 5-HT$_{2B}$ receptor and were selective over 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. Compound **9i** was the most selective compound in this study and is the highest affinity 5-HT$_{2B}$ receptor ligand bearing a tris-(phenylalkyl)amine scaffold to date.

5-HT$_{2B}$ receptors are known to play an important role in cardiac function,[1–4] regulation of gastrointestinal motility,[5,6] growth and differentiation[7,8] and in regulation of the CNS.[9–11] Selective 5-HT$_{2B}$ receptor antagonists are pursued as therapeutics for the treatment of migraine,[12] irritable bowel syndrome,[6,13] pulmonary hypertension[14] and cardiac failure.[2,15–17] Antagonism at the 5-HT$_{2B}$ receptor is also suggested as a therapeutic approach for the treatment of MDMA abuse.[10] Although a few selective 5-HT$_{2B}$ antagonists have been advanced to clinical trials, there are no such compounds clinically approved up to now. SB-200646 and RS-127445 (Fig. 1) are perhaps the most well-known selective 5-HT$_{2B}$ receptor antagonists; these compounds have found utility as biological tools rather than as drugs. Identification of new selective 5-HT$_{2B}$ antagonists is of current importance because of their potential clinical applications. However, identifying selective 5-HT$_{2B}$ antagonists is challenging because most 5-HT$_{2B}$ receptor ligands also have affinity for the closely related 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.

We recently reported the discovery of tris-(phenylalkyl)amines as a novel 5-HT$_{2B}$ receptor-preferring antagonist scaffold.[18] This fortuitous finding, revealed a number of compounds with high 5-HT$_{2B}$ receptor affinity and good selectivity versus 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. The present report describes follow-up structure-affinity relationship (SAR) studies in order to glean further insights into the structural features of the tris-(phenylalkyl)amine template that are of importance to 5-HT$_{2B}$ receptor affinity and selectivity.

From the previous study, we found that the introduction of methoxy substituents at the *ortho*, *meta* or *para* positions in ring C of compound **1** (Fig. 2), resulted in compounds with higher affinity for the 5-HT$_{2B}$ receptor as compared to **1**. Indeed, these compounds had very similar 5-HT$_{2B}$ affinities (4.6–6.8 nM). Since only methoxy substituents were investigated, it remains unclear how other functionalities with varied electronic and steric properties in ring C may influence 5-HT$_{2B}$ affinity. Therefore, we decided to synthesize and evaluate a series of ring C halogenated compounds in order to decipher the tolerance of ring C for such substituents.

Scheme 1 outlines our synthetic approach to the target compounds. We followed a similar route as described in our previous report. Here, commercially available amine **3** was coupled with acid **4** to afford amide **5**; compound **5** was subsequently reduced to amine **6**. EDCI coupling of **6** with various halo-acids (**7a–7l**) furnished amides **8a–8l**. Reduction of **8a–8l** afforded the target compounds **9a–9l**.

Compounds **9a–9l** were submitted to the Psychoactive Drug Screening Program (PDSP), where they were evaluated for affinity at *h*5-HT$_2$ receptor sites.[19] Details on the experimental procedures employed by the PDSP may be found in the assay protocolbook: https://pdspdb.unc.edu/pdspWeb/content/PDSP%20Protocols%20II%202013-03-28.pdf. The data for affinity (K_i) measurements are compiled in Table 1. A discussion of the structure-affinity results follows, with a focus on comparisons to compound **1**.

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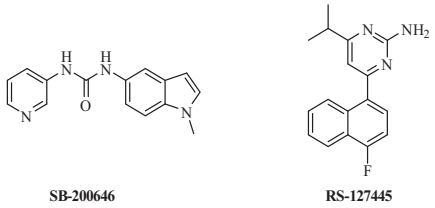


Figure 1. Structures of the selective 5-HT_{2B} antagonists SB-200646 and RS-127445.

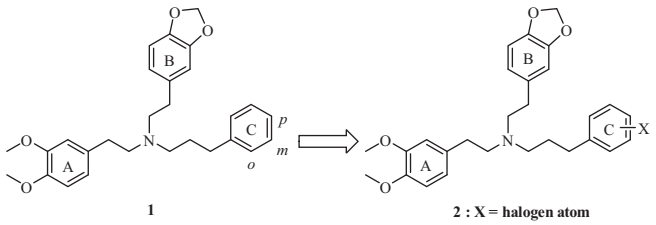


Figure 2. SAR strategy.

Compound **9a** which has a fluorine atom in the *para*-position of ring C, displayed slightly improved affinity (K_i = 14 nM) at the 5-HT_{2B} receptor as compared to compound **1** (K_i = 26 nM). This change also led to an increase in selectivity over the 5-HT_{2A} receptor (13-fold vs 7-fold), but a decrease in selectivity over the 5-HT_{2C} receptor (9-fold vs 15-fold). Interestingly, the *para*-chloro (**9b**, K_i = 54 nM), *para*-bromo (**9c**, K_i = 45 nM) and *para*-iodo (**9d**, K_i = 51 nM) compounds, had a slight reduction in 5-HT_{2B} receptor affinity. Compounds **9b**–**9d** also showed lower selectivity over 5-HT_{2A} and 5-HT_{2C} receptors as compared to **1**.

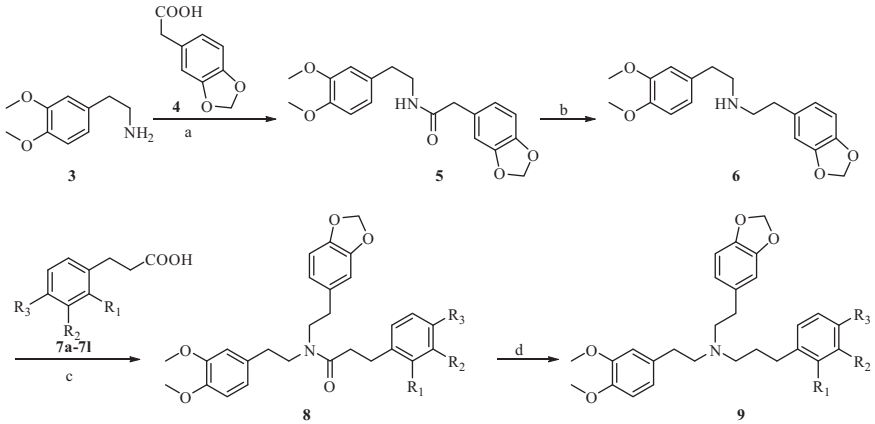
The reason for this trend in affinity for the *para*-substituted halo derivatives is not clear at the moment (assuming that the compounds all have similar binding orientations in the receptor). On steric grounds, it is tempting to postulate that the *para*-fluoro group is smaller than the other *para*-halo groups and thus may be accommodated more readily in the binding pocket into which the halo group protrudes. However, the corresponding *para*-methoxy compound (identified previously) has a higher 5-HT_{2B} affinity than **9a** (K_i = 6.8 nM). Thus a steric reasoning of the trend is elusive. The trend also does not fit any obvious rationale based on the electronic properties of the halogen groups.

Introduction of a halogen atom at the *meta* position in ring C (**9e**–**9h**) resulted in a moderate increase in affinity at the 5-HT_{2B} receptor relative to compound **1**. The *meta*-fluoro (**9e**, K_i = 13 nM) and *meta*-iodo (**9h**, K_i = 14 nM) derivatives exhibited a 2-fold increase in affinity at the 5-HT_{2B} receptor. However, in most cases, an increased affinity was also observed for 5-HT_{2A} and 5-HT_{2C} receptors in this series. Thus, **9e**–**9h** are generally less selective for the 5-HT_{2B} receptor than **1**. All the analogues in the **9e**–**9h** series had similar affinities, indicative of a good tolerance for halogen atoms at this position.

In the *ortho*-substituted series (**9i**–**9l**), introduction of a fluorine atom resulted in a 15-fold increase (**9i**, K_i = 1.7 nM) in affinity at the 5-HT_{2B} receptor, when compared with compound **1**. In a similar comparison at 5-HT_{2A} and 5-HT_{2C} receptors, the affinity of **9i** was found to increase by 2.5 and 5-fold respectively. Moreover, the selectivity of **9i** at both these receptors was better as compared to **1** (38- vs 6-fold over 5-HT_{2A} and 46- vs 15-fold over 5-HT_{2C}). Analogously, **9j** (5-HT_{2B} K_i = 3.1 nM) and **9k** (5-HT_{2B} K_i = 3.4 nM) were found to be 8 times more potent than the parent compound **1,** whereas **9l** (5-HT_{2B} K_i = 13 nM) was found to be 2 times potent than **1** at the 5-HT_{2B} receptor. Notably, **9i** is 12 times more potent than the standard 5-HT_{2B} ligand SB-206553 and was the strongest binder identified in this series.

The affinity of compound **9i** at the 5-HT_{2B} receptor was found to be comparable to methoxyphenyl-containing analogues from our previous study (e.g. the 3,4,5-trimethoxyphenyl analogue, K_i = 4.1 nM and the 2-methoxyphenyl analogue, K_i = 5.8 nM). Similar bioisosteric effects between C–F and C–OMe groups have been reported before; this biological resemblance may have its origins in the similarity in polarity between fluorine and oxygen.[20]

Although no clear trend was seen in the *para* and *meta* series of compounds, a trend was observed in the *ortho* series **(9i–9l)**. Here,

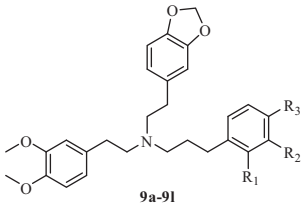


8a: R_1 = F, R_2 = R_3 =H
8b: R_1 = Cl, R_2 = R_3 =H
8c: R_1 = Br, R_2 = R_3 =H
8d: R_1 = I, R_2 = R_3 =H
8e: R_2 = F, R_1 = R_3 =H
8f: R_2 = Cl, R_1 = R_3 =H
8g: R_2 = Br, R_1 = R_3 =H
8h: R_2 = I, R_1 = R_3 =H
8i: R_3 = F, R_2 = R_3 =H
8j: R_3 = Cl, R_2 = R_3 =H
8k: R_3 = Br, R_2 = R_3 =H
8l: R_3 = I, R_2 = R_3 =H

9a: R_1 = F, R_2 = R_3 =H
9b: R_1 = Cl, R_2 = R_3 =H
9c: R_1 = Br, R_2 = R_3 =H
9d: R_1 = I, R_2 = R_3 =H
9e: R_2 = F, R_1 = R_3 =H
9f: R_2 = Cl, R_1 = R_3 =H
9g: R_2 = Br, R_1 = R_3 =H
9h: R_2 = I, R_1 = R_3 =H
9i: R_3 = F, R_2 = R_3 =H
9j: R_3 = Cl, R_2 = R_3 =H
9k: R_3 = Br, R_2 = R_3 =H
9l: R_3 = I, R_2 = R_3 =H

Scheme 1. Reagents & conditions: (a) 3,4-methylenedioxyphenylacetic acid, CDI, THF, 0 °C-rt, 16 h; (b) BH_3–THF, $BF_3{\cdot}Et_2O$, THF, rt-reflux, 4 h; (c) appropriate acid, EDCI, HOBt, DMF, 0 °C-rt, 6 h; (d) BH_3-THF, $BH_3{\cdot}Et_2O$, THF, rt-reflux, 4 h.

Table 1
Binding affinities of **9a–9l** at the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors



9a-9l

Compounds				K_i[a,b] (nM)			Selectivity	
	R_1	R_2	R_3	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_{2A}/5-HT_{2B}	5-HT_{2C}/5-HT_{2B}
1	H	H	H	165 ± 25	26 ± 2.3	399 ± 75	6	15
9a	H	H	F	185 ± 24	14 ± 1.8	126 ± 1.6	13	9
9b	H	H	Cl	365 ± 47	54 ± 7.0	235 ± 3.0	7	4
9c	H	H	Br	221 ± 29	45 ± 5.8	219 ± 2.8	5	5
9d	H	H	I	288 ± 37	51 ± 6.6	224 ± 2.9	6	4
9e	H	F	H	185 ± 24	18 ± 2.3	167 ± 2.2	10	9
9f	H	Cl	H	125 ± 16	17 ± 2.2	206 ± 27	7	12
9g	H	Br	H	103 ± 13	14 ± 1.8	143 ± 18	7	10
9h	H	I	H	169 ± 22	18 ± 2.3	154 ± 20	9	9
9i	F	H	H	65 ± 8.4	1.7 ± 0.2	79 ± 10	38	46
9j	Cl	H	H	43 ± 5.5	3.1 ± 0.4	92 ± 12	14	30
9k	Br	H	H	54 ± 7.0	3.4 ± 0.4	124 ± 16	16	36
9l	I	H	H	75 ± 9.7	13 ± 1.7	68 ± 8.8	6	5
Clozapine				15 ± 1.9	nd[c]	nd[c]		
SB-206553				nd[c]	21 ± 2.7	nd[c]		
Ritanserin				nd[c]	nd[c]	1.8 ± 0.2		

[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] Values represent mean of three experiments.
[c] nd = not determined.

5-HT_{2B} receptor affinity was found to increase with decreasing size/increasing electronegativity of the halogen atom. With regards to the position of substitution in ring C, with the exception of the fluoro substituted compounds (**9a**, **9e** and **9i**), affinity at the 5-HT_{2B} receptor was in the following order: *ortho*-substituted compounds > *meta*-substituted compounds > *para*-substituted compounds. For example, **9j** (K_i = 3.1 nM) was 5 times potent than **9f** (K_i = 17 nM), which in turn was 3 times more potent than **9b** (K_i = 54 nM). This trend was also observed for the affinities of the same compounds at the 5-HT_{2A} and 5-HT_{2C} receptors.

To characterize the 5-HT_{2B} activity of the analogues, the most potent compounds, **9i–9k** were tested in 5-HT_{2B} functional assays by the PDSP. These assays revealed that all three compounds were 5-HT_{2B} antagonists with the following IC_{50} values: **9i** (135 nM); **9j** (99 nM); **9k** (326 nM).

Introduction of a halogen atom can have beneficial effects on the affinities of a ligand. There are several plausible ways in which halogen atoms can interact with a protein or other relevant target. Classically halogens are considered to be hydrophobic moieties and Lewis bases in accordance with their electronegativities.[21] However, the halogen atom can also act as a Lewis acid (via σ-hole) and interact with an electron donating residue of a protein. Bromine and chlorine atoms may be involved in bonding interactions with backbone amino acids that contain carbonyl oxygen atoms.[4] Additionally, halogen atoms are also involved in bond formation with side chain groups such as hydroxyls in serine, threonine and tyrosine, carboxylate groups in aspartate and glutamate, sulfur atoms in cysteine and methionine, nitrogen atoms in histidine and the π surfaces of phenylalanine, tyrosine, histidine and tryptophan.[4] In the case of tris-(phenylalkyl)amines in this study, it is not clear what receptor ligand interactions are responsible for the enhanced affinity of the *ortho* halo-substituted ring C derivatives. Molecular docking studies may shed light on their possible modes of interaction and aid in ligand design efforts in future. In that regard, deciphering the bioactive conformation of these highly flexible ligands is a goal.

In conclusion, we report the synthesis and evaluation of a series of halo-substituted analogues of the tris-(phenylalkyl)amine **1** at human 5-HT_2 receptors. All the analogues displayed high affinities for the 5-HT_{2B} receptor and had good selectivity over 5-HT_{2A} and 5-HT_{2C} receptors. In general, compounds with *ortho*–halo substitutions in ring C displayed higher 5-HT_{2B} affinities than the corresponding *meta* or *para* analogues. Compounds **9i–9k** were found to be 5-HT_{2B} receptor antagonists. Compound **9i** is the most selective 5-HT_{2B} ligand identified in this study and has the highest 5-HT_{2B} receptor affinity of the tris-(phenylalkyl)amines evaluated up to now.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.05.079.

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