Synthesis and pharmacological evaluation of \( N \)-benzyl substituted 4-bromo-2,5-dimethoxyphenethylamines as 5-HT\(_{2A/2C}\) partial agonists

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**ABSTRACT**

\( N \)-benzyl substitution of phenethylamine 5-HT\(_{3A}\) receptor agonists has dramatic effects on binding affinity, receptor selectivity and agonist activity. In this paper we examine how affinity for the 5-HT\(_{2A/2C}\) receptors are influenced by \( N \)-benzyl substitution of 4-bromo-2,5-dimethoxyphenethylamine derivatives. Special attention is given to the 2' and 3'-position of the \( N \)-benzyl as such compounds are known to be very potent. We found that substitutions in these positions are generally well tolerated. The 2'-position was further examined using a range of substituents to probe the hydrogen bonding requirements for optimal affinity and selectivity, and it was found that small changes in the ligands in this area had a profound effect on their affinities. Furthermore, two ligands that lack a 2'-benzylsubstituent was also found to have high affinity contradicting previous held notions. Several high-affinity ligands were identified and assayed for functional activity at the 5-HT\(_{2A}\) and 5-HT\(_{3C}\) receptor, and they were generally found to be less efficacious agonists than previously reported \( N \)-benzyl phenethylamines.

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1. Introduction

The 5-HT\(_{3A}\) receptor plays a significant role in the regulation and control of various physiological mechanisms in both the central and peripheral nervous system. In the CNS, 5-HT\(_{3A}\) receptors are implicated in the regulation of cognitive states, associative learning, mood and circadian rhythm.\(^2\) Peripheral 5-HT\(_{3A}\) receptors mediate processes such as vasoconstriction, platelet aggregation and intra-ocular pressure.\(^2\) Drugs targeting the 5-HT\(_{3A}\) receptor are used in the treatment of various neurological disorders. Atypical antipsychotics such as risperidone and clozapine are inverse agonists at the 5-HT\(_{3A}\) receptor.\(^3\) Neutral antagonists such as 2-bromolysergic acid diethylamide (BOL-148) have been investigated for the treatment of cluster headaches.\(^4\) Peripherally selective agonists are being investigated for the treatment of glaucoma and other affictions related to intra-ocular pressure.\(^5\) Agonism of central 5-HT\(_{3A}\) receptors is generally accepted to be responsible for the psychopharmacological effects of the hallucinogens lysergic acid diethylamide (LSD) and psilocybin.\(^5\) Some of these compounds have in the past and the present been explored in the psychotherapy of people with fear and anxiety related to terminal illness or post-traumatic stress disorder (PTSD).\(^7\) Recently, 5-HT\(_{2A}\) agonists have also been investigated as potent inhibitors of tumor necrosis factor alpha (TNF-\(\alpha\)) mediated inflammation and may one day lead to novel treatments of inflammatory diseases and conditions.\(^8\) Despite this fact, legislative impediments still hinder the exploration of the clinical potential of 5-HT\(_{2A}\) agonists.\(^9\)

The 5-HT\(_{2}\) receptor subtypes 2A, 2B and 2C share a high degree of sequence homology which makes the development of selective ligands difficult. Selective antagonists exist for all three 5-HT\(_{2}\) receptor subtypes, while selective agonists have been discovered only recently for the 5-HT\(_{2A}\) and 5-HT\(_{2C}\) receptor.\(^10,11\) and 5-HT\(_{2C}\) receptor.\(^12\)

Of the 5-HT\(_{2A}\) agonists developed so far, only a few are selective for the 5-HT\(_{2A}\) receptor. 5-HT\(_{2A}\) agonists from the ergoline family (e.g. LSD) generally have a broad pharmacological profile that encompasses most of the 5-HT receptor subtypes as well as dopaminergic and adrenergic receptors. Tryptamines (e.g. psilocybin) are slightly more selective, but are known to also activate 5-HT\(_{2A}\) receptors. The most selective group of compounds is the phenethylamines, which bind preferentially to 5-HT\(_{2}\) receptor subtypes A, B, and C.\(^15\)
$N$-substitution of the phenethylamine series of 5-HT$_{2A}$ agonists has a dramatic effect on the affinity for the 5-HT$_{2A}$ receptor. Simple alkylation generally results in a decrease of affinity, whereas benzylamine restores affinity to the level of the unsubstituted derivative. Introduction of an ortho methoxy (or hydroxy) group on the $N$-benzyl substituent had a dramatic effect on the affinity giving access to the most potent family of 5-HT$_{2A}$ agonists reported to date — often referred to as NBOMe’s. This discovery paved the way for the development of selective agonists: Juncosa et al. reported that conformational restriction of the known agonist 25B-NBOMe lead to a 100 fold selective 5-HT$_{2A}$ agonists: Juncosa et al. reported that conformational restriction of the known agonist 25B-NBOMe lead to a 100 fold selective 5-HT$_{2A}$ receptor.

As part of a research program directed towards the development of selective agonist PET-ligands for the 5-HT$_{2A}$ receptor, we recently reported a systematic survey of different combinations of 4- and 2'-substituents of the $N$-benzyl phenethylamine chemotype wherein 12 different phenethylamines (with different 4-substituents) were joined with four different benzyl groups. As previous studies indicated that the 2'-position of the $N$-benzyl substituent is an important determinant for 5-HT$_{2A-C}$ affinity and functional potency, we limited our investigation to include substituents of that nature. Although we discovered a new potent and selective 5-HT$_{2A}$ ligand we also saw an erratic picture with respect to the structure activity relationships for the entire ligand set as no correlation between the substitution pattern and affinity/selectivity could be identified. To shed further light on this issue we wish to report a more in-depth investigation into the effect of changing the substituent on the $N$-benzyl group while keeping the phenethylamine core static.

In our previous study we noticed a general trend that 2',3'-methyleneoxy substitution gave rise to a moderate selectivity for the 5-HT$_{2A}$ receptor, see figure 1 for a selected example. Compound 1a is a very potent agonist both in binding and functional assays. To explore this in more detail we targeted ligands with various heteratoms occupying the 2' and/or 3' position, see Figure 1.

![Figure 1. Structure of previously reported 5-HT$_{2A}$ agonist](image)

<table>
<thead>
<tr>
<th>pK$_A$</th>
<th>pK$_{EC50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.22</td>
<td>7.95</td>
</tr>
<tr>
<td>7.98</td>
<td>6.66</td>
</tr>
<tr>
<td>19</td>
<td>18</td>
</tr>
</tbody>
</table>

**Ligands targeted in this study**

The final compounds were all synthesized by indirect reductive amination of 4-bromo-2,5-dimethoxyphenethylamine with the required aldehydes, as detailed in the supporting information. The aldehydes were either commercially available or synthesized as detailed below.

**Chemistry**

![Scheme 1. a) NaH, BrCH$_2$CH(OEt)$_2$, DMF, 100 °C b) PPA, PhCl, 130 °C. c) Mg at 80 °C then DMF at 0 °C, THF. d) K$_2$CO$_3$, BrCH$_2$CH(OEt)$_2$, DMF. rt.](image)

**Scheme 1.**

- a) NaH, BrCH$_2$CH(OEt)$_2$, DMF, 100 °C
- b) PPA, PhCl, 130 °C
- c) Mg at 80 °C then DMF at 0 °C, THF
- d) K$_2$CO$_3$, BrCH$_2$CH(OEt)$_2$, DMF, rt.

Benzofurane 2 and benzothiophene 3 were synthesized using a similar sequence; alkylation with 2-bromoacetaldehyde diethyl acetal followed by Friedel-Craft style ring closure to give the benzo furan and benzothiophene. Halogen-metal exchange with $n$-BuLi followed by a DMF quench led to products formylated in the 2-position as the organolithium intermediate rapidly rearranges. However, reductive metatllation with Mg followed by DMF resulted in the desired 7-formylated products.

![Scheme 2. a) (1) NH$_2$, H$_2$O (2) Br$_2$, KOH, H$_2$O (3) H$_2$, Pd(C), MeOH. b) (4) HCOOH, HCl, H$_2$SO$_4$, MeOH (6) LiAlH$_4$, THF/1,4-dioxane (7) MnO$_2$, CH$_2$Cl$_2$/DMF.](image)

**Scheme 2.**

- a) (1) NH$_2$, H$_2$O (2) Br$_2$, KOH, H$_2$O (3) H$_2$, Pd(C), MeOH
- b) (4) HCOOH, HCl, H$_2$SO$_4$, MeOH (6) LiAlH$_4$, THF/1,4-dioxane (7) MnO$_2$, CH$_2$Cl$_2$/DMF

Aldehyde 4 was synthesized from 3-nitrophthalic anhydride in seven steps in 8 % overall yield. Regioselective ammoniolyis afforded the phthalamic acid which was converted to anthranilic acid by Hofmann rearrangement. The nitro group was reduced by catalytic hydrogenation to give the diaminobenzoic acid. Treatment with formic acid in aqueous HCl assembled the phthalamic acid by Hofmann rearrangement. The nitro group was reduced by catalytic hydrogenation to give the diaminobenzoic acid. Treatment with formic acid in aqueous HCl assembled the benzimidazole. The carboxylic acid was converted to the methyl ester to improve solubility and then reduced with LiAlH$_4$ to give the benzyl alcohol. Subsequent oxidation to the required aldehyde 4 was accomplished with MnO$_2$.

![Scheme 3. a) (1) NaNO$_2$, aq. HBF$_4$, (2) KOAc, 18-crown-6, CHCl$_3$. b) (3) LiAlH$_4$, THF/4-MeOCH$_2$Cl/DMF.](image)

**Scheme 3.**

- a) (1) NaNO$_2$, aq. HBF$_4$, (2) KOAc, 18-crown-6, CHCl$_3$
- b) (3) LiAlH$_4$, THF/4-MeOCH$_2$Cl/DMF

The indazole aldehyde was accessed by diazotation followed by ring closure of methyl 2-amino-3-methylbenzoate. A two-step reduction-oxidation sequence furnished aldehyde 5.

![Scheme 4. a) (1) H$_2$SO$_4$, MeOH (2) H$_2$, Pd(C), THF/EtOAc (3) Yb(OTf)$_3$, HC(OEt)$_2$, Toluene. b) (3) LiAlH$_4$, THF (4) MnO$_2$, CH$_2$Cl$_2$/DMF.](image)

**Scheme 4.**

- a) (1) H$_2$SO$_4$, MeOH (2) H$_2$, Pd(C), THF/EtOAc (3) Yb(OTf)$_3$, HC(OEt)$_2$, Toluene
- b) (3) LiAlH$_4$, THF (4) MnO$_2$, CH$_2$Cl$_2$/DMF

2. Results and discussion

All compounds were prepared as detailed below and screened for binding affinity at 5-HT$_{3A}$ and 5-HT$_{3C}$ receptors at the NIMH Psychoactive Drug Screening Program (PDS). Selected compounds were subsequently evaluated in functional assays measuring Gq-mediated inositol monophosphate production.
Benzoxazole 6 was synthesized in five steps from 3-nitrosalicylic acid. Fischer esterification gave the methyl ester and then the nitro group was reduced by catalytic hydrogenation to give the corresponding aminophenol. Cyclization was accomplished using triethyl orthoformate in the presence of a catalytic amount of Lewis acid to yield the benzoxazole. LiAlH$_4$ reduction of the methyl ester followed by final oxidation with TPAP/NMO gave the desired aldehyde 6.

Scheme 5. a) (1) LiTMP, HCOOEt, THF, -78 °C. b) cat. H$_2$O$_2$, aq. HCl c) MeONa, MeOH.

Aldehydes 7 and 8 were both made from 2-chloropyridine. Lithiation with LiTMP followed by a quench with ethyl formate yielded the 2-chloronicotinaldehyde. Treatment with either HCl and a catalytic amount of H$_2$O$_2$ or MeONa gave pyridone 7 and 2-methoxypyridine 8 respectively.

Pharmacology - binding studies

Initially we focused on heterocyclic derivatives where a heteroatom was preserved in the 2'-position on the N-benzyl core.

![Figure 2. Structure of ligands 1b-h containing heteroatoms fused to the 2'-3'-benzylposition and their affinities for 5-HT$_{2A}$/2C.](image)

The benzofuran- and benzothiophene derivatives 1b and 1c maintain high affinity at both 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor subtypes. Adding a second heteroatom in the 3'-position was known to confer a modest selectivity in the case of the 2',3'-methylenedioxybenzyl derivative 1a, but this effect was not seen with the benzoxazole derivative 1d. Saturation of the benzofuran moiety to give coumaran analogue 1e gave a N-benzyl phenethylamine with one of the highest affinities we have assayed to date with similar selectivity towards 5-HT$_{2C}$ as for 1a.

We then analyzed derivatives that in principle could act as combined hydrogen bond donors and acceptors comparable to the 2'-hydroxybenzyl substituted compound which in previous studies also resulted in potent agonists.

The benzimidazole 1f, indazole 1g and indole 1h all retain affinity for the 5-HT$_{2A}$ but they compare unfavorably with 1a with a ten-fold loss of affinity and negligible selectivity for either receptor subtype.

The pyridine derivatives were included to determine the effect of a ring nitrogen in the benzylic substituent. First, we examined derivatives of 1i and 1j with nitrogen in the 3'-position.

![Figure 3. Structure of pyridine substituted ligands 1i-m and their affinities for 5-HT$_{2A}$/2C (pK$_i$).](image)

Both derivatives exhibited a significant loss of affinity (>100 fold) at both 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor subtypes. The reason for this is unclear; one explanation could be a diminished capability of forming hydrogen bonds due to the electron withdrawing effect of the nitrogen-containing ring.

The unsubstituted regioisomeric pyridines 1k-m were tested for comparison and they performed in a similar fashion - despite lacking the hydrogen bond acceptor in the 2' position. Taken together, the presence of a nitrogen ring likely dampens the electronic-rich ring interaction with the receptor resulting in lower affinity.

Finally, we decided to perform a methoxy-scan on the benzyl group while keeping the 2'-methoxy substituent resulting in the four derivatives shown in scheme 4.

![Figure 4. Structure of dimethoxy substituted ligands 1n-q and their affinities for 5-HT$_{2A}$/2C (pK$_i$).](image)

Only the 2'-3'-dimethoxy substituted derivative 1n showed high affinity at the 5-HT$_{2A}$ receptor while the 2',4' and 2',5'-disubstituted derivatives 1o and 1p both displayed lower affinity.
The 2',6'-dimethoxy compound 1q was even less potent and exhibited a 17 fold selectivity for the 5-HT$_{2C}$ receptor.

As both 1e, 1n and 1a are high-affinity compounds (confirming the spatial availability of these positions), we decided to add two additional compounds acting as hybrids between these compounds.

![Structure of ligands 1r-u and their affinities for 5-HT$_{2A/C}$ (pK$_i$). 1n included for comparison.](Figure 5)

Compound 1r, where the 2'-methoxy substituent is replaced with a fluorine atom, had an affinity comparable to 1n but with increased selectivity. Compound 1s with a hydroxy group in the ortho-position gives a compound which has identical affinity for 5-HT$_{2A}$ as 1e whilst being almost twice as selective.

Thus, it is clearly evident that subtle modifications in this part of the ligands have a large impact on the affinity and selectivity. To further probe this region of the molecules, the 3'-methoxyderivate 1t without a hydrogen bond acceptor in the 2'-position was evaluated and it retains a surprising level of affinity as is the case for the 3'-hydroxyderivate 1u – both being more potent and selective than the 2',3'-dimethoxyderivate 1n. These results question the previous accepted notion that a hydrogen bond acceptor in the 2'-position is required if the ligands are to have higher affinity for the 5-HT$_{2A}$ receptor.

**Pharmacology - functional studies**

Four of the compounds, 1e, 1q, 1r and 1s, were examined for their ability to activate human 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors stably expressed in HEK-293 cells in vitro using an inositol monophosphate assay (IP-One, Cisbio) as a measure of G$_Q$ function (Table 1) as previously described. Although 1q was moderately selective for 5-HT$_{2C}$ in the binding assay, this compound was considerably more potent at 5-HT$_{2A}$ in the functional assay being 16 fold selective for 5-HT$_{2A}$.

The 2'-F,3'-OMe derivative 1r was slightly less potent than the structurally very similar 2'-OH,3'-OMe derivative 1s - with negligible selectivity for 5-HT$_{2A}$. 1s on the other hand proved very selective for the 5-HT$_{2A}$ receptor with more than 500-fold higher potency than at 5-HT$_{2C}$. Interestingly, 1s showed a modest 32 fold selectivity for 5-HT$_{2A}$ in the binding assay, and this lack of correlation between the binding and functional data mirrors our previous study.

**Table 1. Functional characterization of selected compounds at 5-HT$_{2A}$ and 5-HT$_{2C}$ (inositol monophosphate accumulation).**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>pEC$_{50}$ ± S.E.M.</th>
<th>Selectivity</th>
<th>Maximal response ± S.E.M. (% of 5-HT$_{2A}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>6.52 ± 0.06</td>
<td>6.72 ± 0.07</td>
<td>0.63</td>
</tr>
<tr>
<td>DOI</td>
<td>8.20 ± 0.03</td>
<td>7.34 ± 0.08</td>
<td>7.2</td>
</tr>
<tr>
<td>1a'</td>
<td>7.98 ± 0.17</td>
<td>6.66 ± 0.06</td>
<td>18</td>
</tr>
<tr>
<td>1e</td>
<td>8.34 ± 0.06</td>
<td>7.49 ± 0.15</td>
<td>7.1</td>
</tr>
<tr>
<td>1q</td>
<td>5.27 ± 0.06</td>
<td>4.06 ± 0.21</td>
<td>16</td>
</tr>
<tr>
<td>1r</td>
<td>6.98 ± 0.10</td>
<td>6.55 ± 0.06</td>
<td>2.7</td>
</tr>
<tr>
<td>1s</td>
<td>7.47 ± 0.04</td>
<td>4.81 ± 0.11</td>
<td>457</td>
</tr>
</tbody>
</table>

*Data from ref. 11.

The compounds were tested in at least three independent experiments in triplicate at each cell line.

3. Conclusion

A total of 21 N-benzylated derivatives of 4-bromo-2,5-dimethoxyphenethylamine where synthesized and affinities were characterized using binding assays at 5-HT$_{2A}$ and 5-HT$_{2C}$ in an attempt to uncover the structural features that govern affinity and selectivity at these receptors. Based on the previous finding that a methylenedioxy substitution in the 2',3'-position of N-benzyl phenethylamine agonists provided ligands with slight selectivity for 5-HT$_{2A}$, these ligands were selected in order to explore this region of the receptor-ligands interaction in further detail.

Affinity results from the binding assays show that the ligands generally can be manipulated in this area while maintaining affinity. Replacement of the N-benzyl moiety with a pyridine-derivative gave compounds with lower affinity, indicating that an electron rich aromatic system is beneficial for binding. Indeed, ligands 1b, 1e, 1s and 1t all have pK$_i > 9$, thus being ligands with some of the highest affinities reported for the 5-HT$_{2A}$ receptor to date, but no clear picture has emerged yet with respect to their structure activity relationship.

Functional characterization of selected ligands complicates the analysis further as no clear picture yet emerges with respect to the binding studies. Very minor manipulations lead to dramatic effects both with respect to selectivity and intrinsic activity. Thus, further studies are needed to understand the behavior of this class of ligands at the 5-HT$_{2A}$ receptors, and the compounds presented herein could prove to be important tools for mapping the N-benzyl binding pocket in the 5-HT$_{2A}$ receptor.
4. Experimental

General procedure for the synthesis of N-benzylation phenethylamines 1b-u.

Et₃N (1.0 equiv.) was added to a suspension of 2-(4-bromo-2,5-dimethoxyphenyl)ethanamine hydrochloride (2C-B HCl, 1.0 mmol) and the required aldehyde (1.1 equiv.) in EtOH (10 mL) and the reaction was stirred until formation of the imine was complete according to TLC or GC (between 30 minutes and 3 hrs depending on the aldehyde). NaBH₄ (2.0 mmol) was then added and the reaction was stirred for another 30 minutes. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between CH₂Cl₂ and water (30 mL, 1:1). The organic layer was isolated and the aqueous layer was extracted with CH₂Cl₂ (2 × 15 mL). The combined organic extracts were dried over Na₂SO₄, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (CH₂Cl₂/MeOH/NH₄OH 98:2:0.04). The purified free base was dissolved in EtOH (2 mL) and HCl (1M in EtOH, 2 mL) was added and the resulting solution was diluted with Et₂O until a precipitate was formed. The crystals were collected by filtration and dried under reduced pressure to provide the desired products.

Functional characterization at 5-HT₂A and 5-HT₂C Receptors

Functional characterization was performed on HEK-293 cell lines stably expressing human 5-HT₂A and human 5-HT₂C respectively by using the IP-One assay (Cisbio, Codolet, France). Subconfluent cells were detached from the cell culture dish by using cell dissociation solution (Sigma-Aldrich, St. Louise, MO), and cell suspensions of 1 x 10⁷ cells/mL were prepared in 37°C using cell dissociation solution (Sigma-Aldrich, St. Louise, MO), and the plate was sealed and incubated at 37°C for 1 hour, followed by 15 minutes incubation at room temperature. Detection solution was prepared (IP-One Conjugate & Lysis buffer with 2.5% anti-IP1 cryptate Tb conjugate and 2.5% IP1-d2 conjugate), and 10 µl/well was added to the OptiPlate, which was then incubated for 1 hour at room temperature away from light. The plate was read on an Envision multilabel reader (PerkinElmer, Waltham, MA, USA), in which the plate was excited at 340 nm and emission was measured at 615 nm and 665 nm. The fluorescence resonance energy transfer (FRET) 665 nm/615 nm ratio is inversely proportional to the isositol monophosphate (IP1) accumulation produced upon activation of 5-HT₂A, and 5-HT₂C. FRET ratios were converted to IP1 concentrations by interpolating values from an IP1 standard curve generated from a calibrator provided by the manufacturer (Cisbio, Codolet, France).

Acknowledgments

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References and notes


Supplementary Material

Supplementary data associated with this article (full experimental detail and description of the assays) can be found, in the online version, at: DOI: XXX