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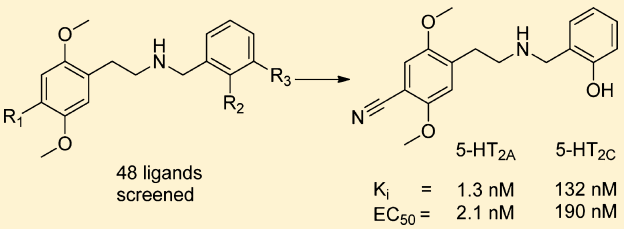
Synthesis and Structure–Activity Relationships of *N*-Benzyl Phenethylamines as 5-HT$_{2A/2C}$ Agonists

Martin Hansen, Karina Phonekeo, James S. Paine, Sebastian Leth-Petersen, Mikael Begtrup, Hans Bräuner-Osborne, and Jesper L. Kristensen*

Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 København Ø, Denmark

Supporting Information

ABSTRACT: *N*-Benzyl substitution of 5-HT$_{2A}$ receptor agonists of the phenethylamine structural class of psychedelics (such as 4-bromo-2,5-dimethoxyphenethylamine, often referred to as 2C-B) confer a significant increase in binding affinity as well as functional activity of the receptor. We have prepared a series of 48 compounds with structural variations in both the phenethylamine and *N*-benzyl part of the molecule to determine the effects on receptor binding affinity and functional activity at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. The compounds generally had high affinity for the 5-HT$_{2A}$ receptor with **8b** having the highest affinity at 0.29 nM but with several other compounds also exhibiting subnanomolar binding affinities. The functional activity of the compounds was distributed over a wider range with **1b** being the most potent at 0.074 nM. Most of the compounds exhibited low to moderate selectivity (1- to 40-fold) for the 5-HT$_{2A}$ receptor in the binding assays, although one compound **6b** showed an impressive 100-fold selectivity for the 5-HT$_{2A}$ receptor. In the functional assay, selectivity was generally higher with **1b** being more than 400-fold selective for the 5-HT$_{2A}$ receptor.



KEYWORDS: *Serotonin, 5-HT$_{2A}$ receptor agonist, N-benzyl phenethylamines, selectivity*

Serotonin (5-HT) receptors are widely distributed in both the CNS and the peripheral nervous system and are involved in the regulation of a plethora of physiological responses such as cognition, memory processing, mood, circadian behavior, and appetite.[1] The 5-HT$_{2A}$ receptor appears to play a key role in a number of disease states such as addiction,[2] schizophrenia,[3] obsessive compulsive disorder,[4,5] depression,[6,7] pain,[8] inflammation,[9] migraine,[10] and cluster headaches,[11] and in the manifestation of mystical/religious-type experiences as well as in altered states of consciousness.[3,12−15] Agonist activation of 5-HT$_{2A}$ receptors in the cortex is believed to be responsible for the remarkable psychopharmacological effects exerted by hallucinogens such as lysergic acid diethylamide (LSD) and psilocybin; see Figure 1.[16] Neutral antagonists such as 2-bromolysergic acid diethylamide (BOL-148) have been investigated as a prophylactic treatment of cluster headaches, and inverse agonists such as risperidone and clozapine are well-known atypical antipsychotics.[17,18]

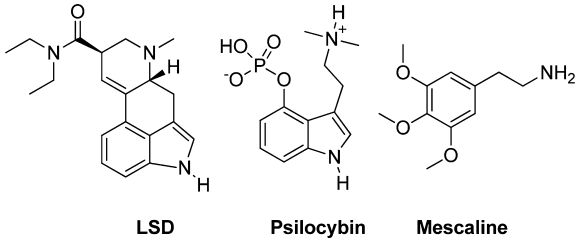


Figure 1. Representative members of the three structural groups of 5-HT$_{2A}$ agonists. LSD (an ergoline), psilocybin (a tryptamine), and mescaline (a phenethylamine).

5-HT$_{2A}$ agonists have traditionally been divided into three structural groups: Ergolines, tryptamines, and phenethylamines.[19] The ergoline group is named after the ergot fungus which was the initial source of ergot alkaloids, but ergolines are also known to occur naturally in several other species of plant and fungi.[20] The ergolines comprise a varied group of compounds with a rich pharmacology and bind to many types of monoamine receptors both serotonergic and non-serotonergic. Not all ergolines are 5-HT$_{2A}$ agonists and several are used clinically in the treatment of migraine,[21] Parkinson's disease and in obstetrics.[22,23]

Tryptamines are slightly more selective compounds but usually have strong affinity for several 5-HT receptors. Serotonin itself is an agonist at all 5-HT receptors, whereas the classical hallucinogens psilocybin and *N*,*N*-dimethyltryptamine (DMT) activate 5-HT$_{1A}$ receptors as well as the 5-HT$_2$ subtypes.

Phenethylamines are generally selective for the 5-HT$_2$ receptor subtypes but lack selectivity between the individual subtypes.[24] The optimal substitution pattern and molecular configuration for simple phenethylamines have been refined

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and improved during the past 40 years and have resulted in some very potent agonists.[25,26]

Initially, *N*-substituted phenethylamines were thought to be inferior compared to the parent phenethylamines because early studies involving *N*-alkylation with simple substituents (e.g., methyl, ethyl, propyl) produced compounds with significantly diminished activity.[27,28] Therefore, it was surprising when it was discovered that *N*-benzyl and specifically *N*-(2-methoxy)benzyl substitution dramatically improved both binding affinity and functional activity and in vivo 5-HT_{2A} activation of simple phenethylamines such as 2C-I (4-iodo-2,5-dimethoxyphenethylamine).[29,30] This discovery was later expanded upon to include conformationally restricted *N*-benzylphenethylamines which resulted in the first truly selective 5-HT_{2A} agonist.[31]

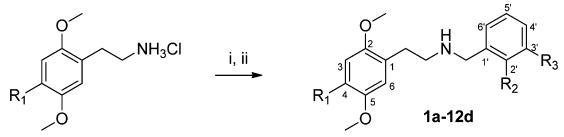
As part of our efforts to develop an agonist PET-tracer for the 5-HT_{2A} receptor, we have examined a number of different *N*-benzylphenethylamines for their ability to bind to and activate 5-HT_{2A} receptors in the search for a 5-HT_{2A} selective agonist.[32] Agonists are of special interest in PET studies because agonist PET-ligands only label receptors in the active, high-affinity state which provides more functionally relevant imaging. Furthermore, agonist PET-ligands are potentially more sensitive to changes in synaptic serotonin levels due to the higher affinity of 5-HT for agonist versus antagonist labeled receptors. Several ligands from the study have been investigated as PET-ligands, and **5a** (under the alias Cimbi-36) is currently being evaluated in humans.[33] Herein we wish to report the full details on the medicinal chemistry development of these ligands. We wanted to study the impact of the 4-substituent on *N*-benzylphenethylamines with a small set of *N*-benzyl substituents and determine the effects on both binding affinity and functional activity at the 5-HT_{2A} receptor as well as the 5-HT_{2C} receptor. Extensive structure−activity relationship (SAR) studies have previously been performed on the 4-position of psychedelic phenethylamines and amphetamines.[27,34] These studies revealed that 4-substituents containing hydrogen bond donors such as −COOH, −OH, and $-NH_2$ decrease affinity by several orders of magnitude while nonpolar substituents such as halogens and alkyl groups increased affinity. Thus, there seems to be a clear correlation between the lipophilic nature of the substituent in the 4-position and binding affinity; however, only halogens and short alkyl chains (1−4 carbons) are agonists while longer alkyl chains, aryl and benzyl groups are antagonists at the 5-HT_{2A} receptor.

Herein we report our efforts to investigate the SAR of this compound family via a systematic variation of substituents on the phenethylamine and benzyl rings. Twelve phenethylamines were paired with 4 benzaldehydes to give a set of 48 *N*-benzyl phenethylamines that subsequently were evaluated on the 5-HT_{2A} and 5-HT_{2C} receptors in binding and functional assays.

CHEMISTRY

The targeted 48 compounds were all synthesized by indirect reductive amination of the respective phenethylamines and benzaldehydes as shown in Scheme 1. The *N*-benzylphenethylamines were precipitated as their hydrochloride salts in 46−94% isolated yields. The syntheses of the parent 12 phenethylamines have been described previously; see the Supporting Information for details.

Scheme 1. Synthesis of *N*-Benzyl Phenethylamines[a]



R_2/R_3 \ R_1	I	Br	Cl	F	CF_3	CN	Me	Et	Pr	SMe	SEt	SPr
2'-MeO	**1a**	**2a**	**3a**	**4d**	**5a**	**6a**	**7a**	**8a**	**9a**	**10a**	**11a**	**12a**
2'-OH	**1b**	**2b**	**3b**	**4b**	**5b**	**6b**	**7b**	**8b**	**9b**	**10b**	**11b**	**12b**
2'-F	**1c**	**2c**	**3c**	**4c**	**5c**	**6c**	**7c**	**8c**	**9c**	**10c**	**11c**	**12c**
2',3'-MD	**1d**	**2d**	**3d**	**4d**	**5d**	**6d**	**7d**	**8d**	**9d**	**10d**	**11d**	**12d**

[a]Reaction conditions: (i) aldehyde, EtOH, rt; (ii) $NaBH_4$, EtOH, rt.

IN VITRO PHARMACOLOGY

All compounds were assessed in a radioligand competition binding assay for affinity at human 5-HT_{2A} receptors and rat 5-HT_{2C} receptors using displacement of antagonist radioligands [^{3}H]Ketanserin and [^{3}H]Mesulergine via the NIMH Psychoactive Drug Screening Program (PDSP). The results are summarized in Figure 2. In Figure 3, the 5-$HT_{2A/2C}$ selectivities based on the data in Figure 2 are presented.

Functional assays were performed on all compounds to determine the ability of the ligands to activate downstream cellular signaling pathways. The compounds were examined for their efficacy at stimulating phospholipase C mediated production of inositol phosphates (IP_{1-3}) at both human 5-HT_{2A} and 5-HT_{2C} receptors. The results are summarized in Figure 4; see the Supporting Information for full details.

RESULTS AND DISCUSSION

The results from the binding affinity measurements showed that the majority of the compounds bind to both 5-HT_{2A} and 5-HT_{2C} receptors in the low nanomolar range with several compounds having subnanomolar affinities (pK_i above 9) at the 5-HT_{2A} receptor. Previously, a smaller subset of compounds were subjected to a broader screen which showed the *N*-benzyl phenethylamines are highly selective for the 5-HT_2 receptor subtypes over a wide selection of other neuroreceptors.[32] Ligands substituted with F, CN, or Me at the 4-position of the phenethylamine core (**4a−d**, **6a−d**, and **7a−d**) have slightly lower affinities which is in accordance with affinity data published previously on simple/primary phenethylamines and amphetamines.[35] In general, the ligands with a *N*-(2-fluorobenzyl) substituent (**1c−12c**) have lower affinities than the other *N*-benzyl substituents which could be due to a diminished hydrogen bond acceptor capability.

While all compounds were generally 5-HT_{2A}-selective in the binding assays, the selectivity varied with the nature of both the 4-substituents and the *N*-benzyl substituents. 2,3-Methylenedioxy substitution on the *N*-benzyl part results in a general increase in the 5-HT_{2A}-selective binding with most 4-substituents; see Figure 3. The influence of the other *N*-benzyl substituents on 5-$HT_{2A/2C}$-selectivity was too erratic to show any general trends. The same applies to the 4-substituents which do not display any general trends. In Figure 5, **5b**, **5d**, **6b**, and **6d** are shown for comparison. Compound **5b** is a very potent agonist with 85-fold selectivity in the functional assays, but low selectivity (3-fold) in the binding assay. In **5d**, the methylenedioxy moiety in the *N*-benzyl group increases the selectivity to a factor of 226 and 18, respectively, while maintaining potency. Exchanging the CF_3-group for a CN-

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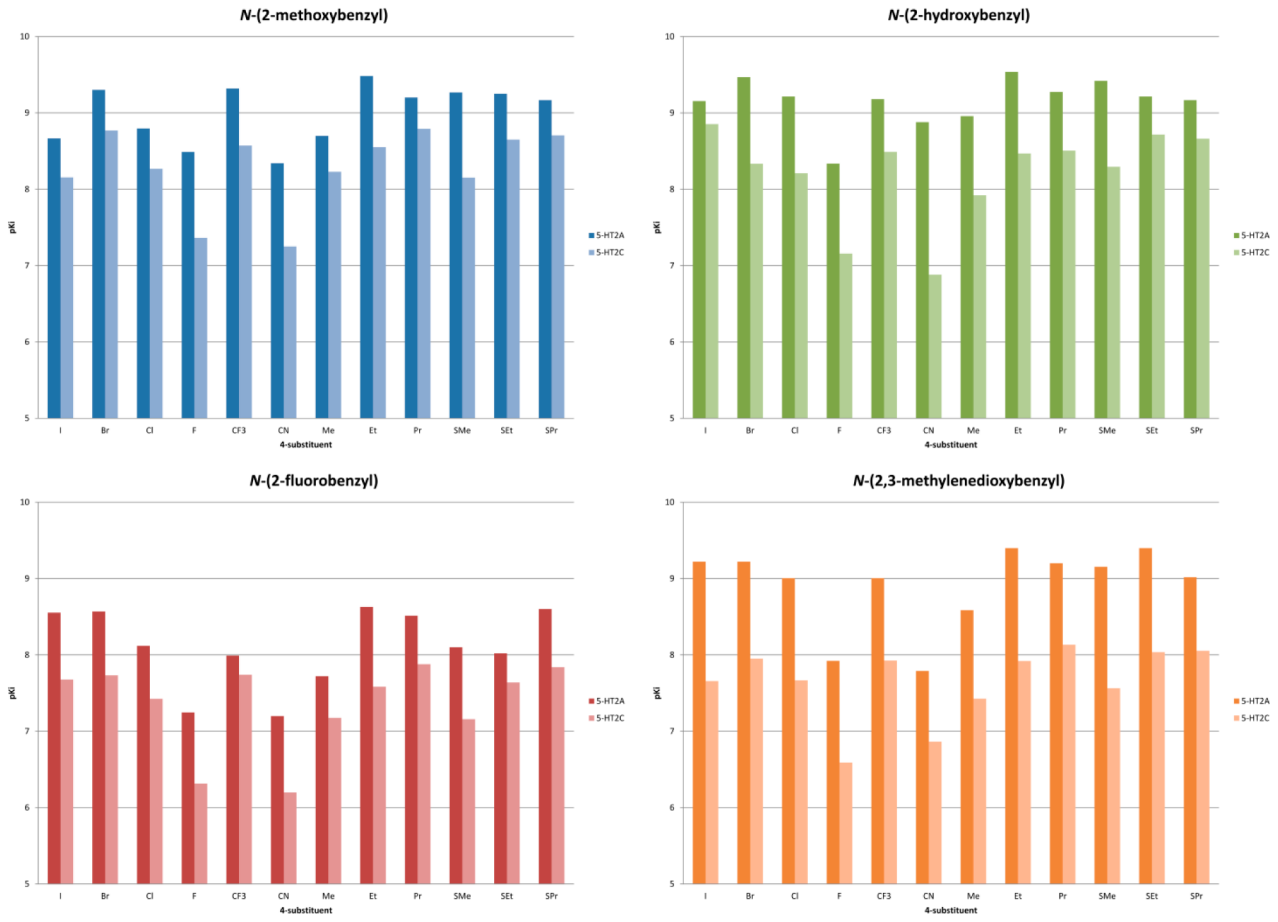

Figure 2. Binding affinities (pK_i) of *N*-benzylphenethylamines at the 5-HT_{2A} and 5-HT_{2C} receptor. See the Supporting Information for tables with all data.

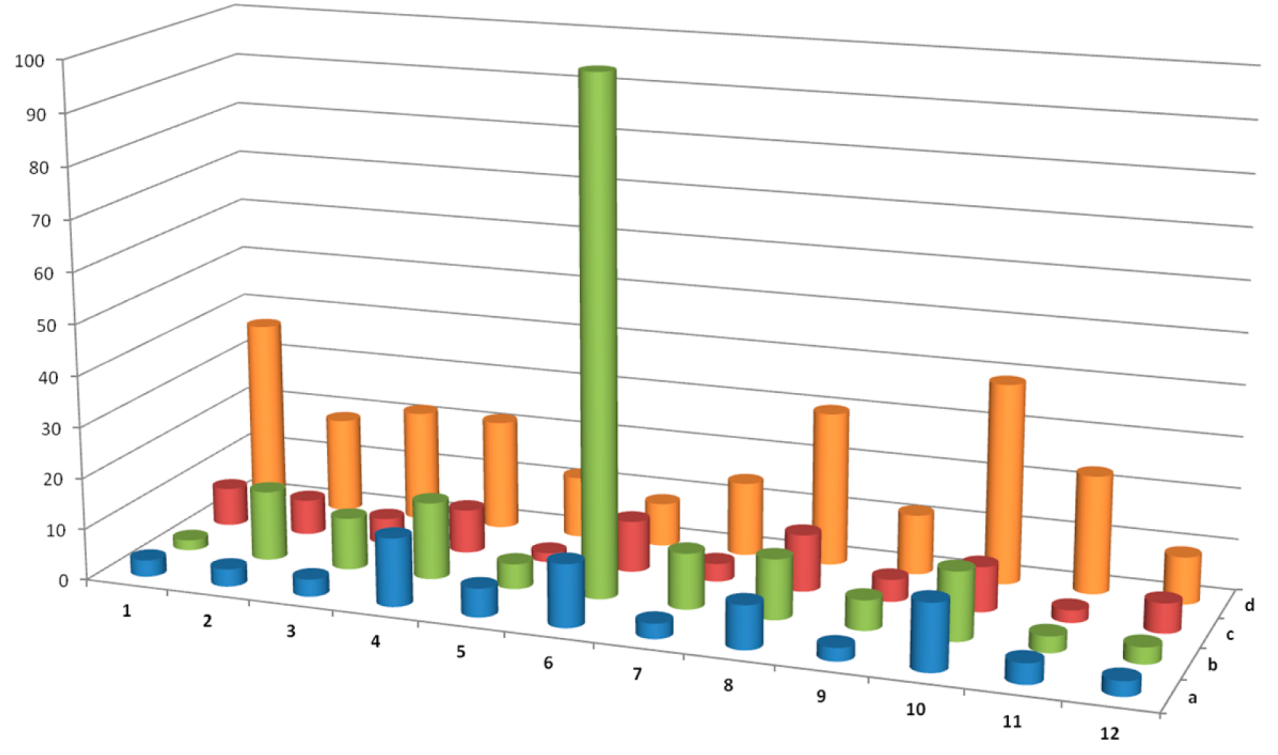

Figure 3. Graph showing the 5-HT_{2A}/5-HT_{2C} selectivities based on binding affinities.

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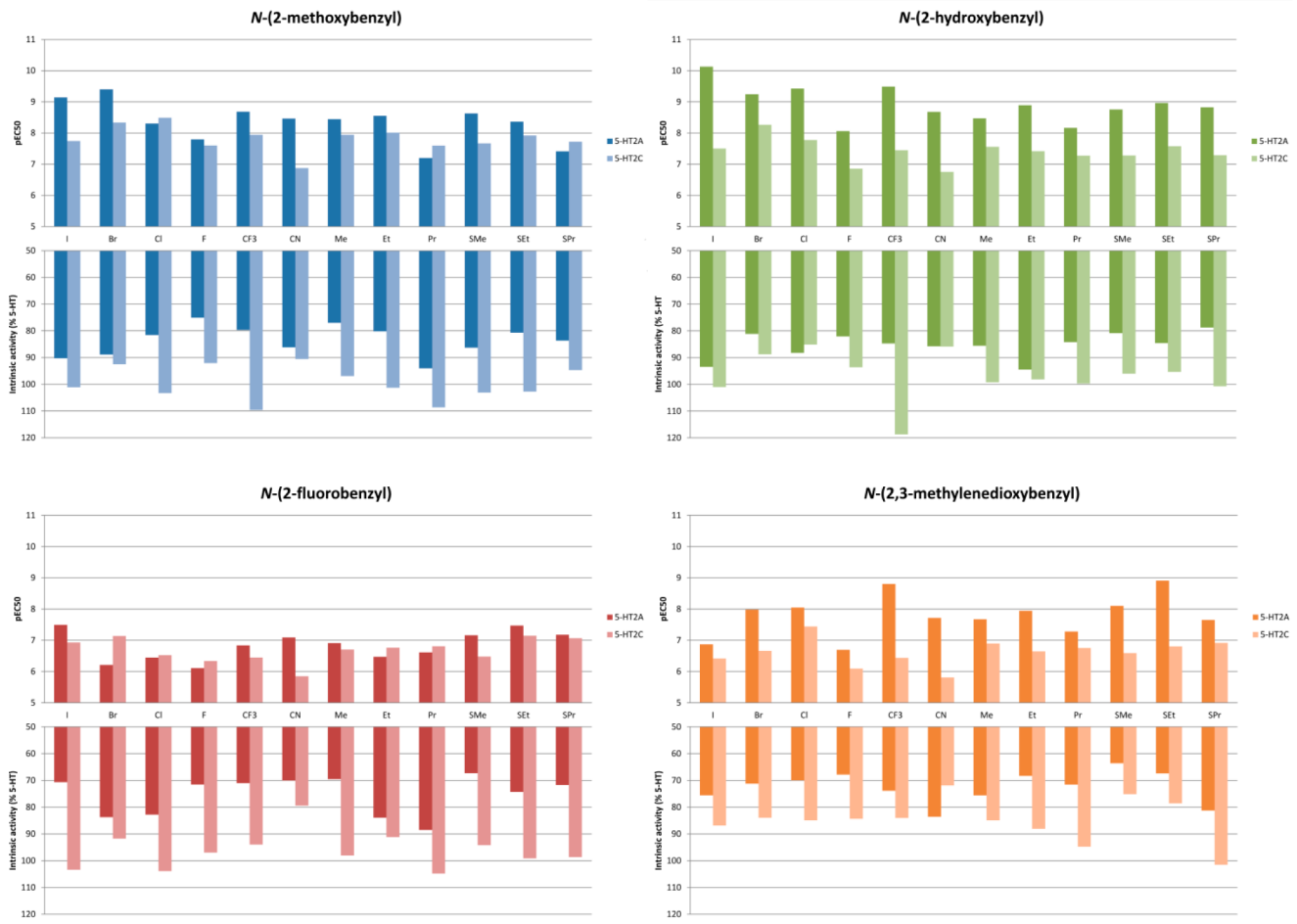
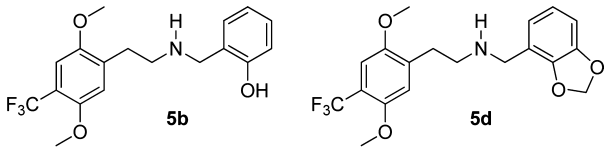
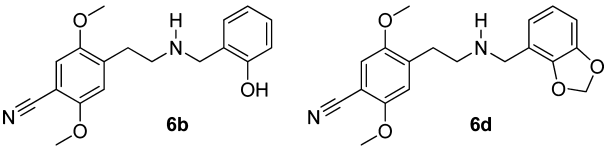


Figure 4. Functional characterization of *N*-benzylphenethylamines at human 5-HT_{2A} and 5-HT_{2C} receptors. Top half shows pEC_{50}, whereas bottom half represents intrinsic activity.



	2A	2C	2A/2C
pK_i	9.18	8.49	3
pEC_{50}	9.49	7.45	85

	2A	2C	2A/2C
pK_i	9.00	7.93	18
pEC_{50}	8.80	6.43	226



	2A	2C	2A/2C
pK_i	8.88	6.88	100
pEC_{50}	8.68	6.76	90

	2A	2C	2A/2C
pK_i	7.79	6.86	9
pEC_{50}	7.72	5.81	78

Figure 5. Structure and in vitro pharmacological profile of **5b**, **5d**, **6b**, and **6d**.

group in these two compounds gives two very different results: **6b** retains the high affinity for 5-HT_{2A}, while affinity for 5-HT_{2C} diminishes, giving a 100-fold selective compound, whereas in **6d** the same CF_3 to CN substitution leads to an erosion of both affinity and selectivity for 5-HT_{2A}. In Figure 6, the concentration−response curves for **6b** and **6d** on 5-HT_{2A} and 5-HT_{2C} are compared with DOI. As can be seen in Figure 3, the combination of the 4-CN and *N*-(2-hydroxybenzyl) substituents in **6b** gives the most selective compound in the series with respect to binding affinities. In simple amphetamines with the same substitution pattern, the 4-CN substitution results in a moderate (20-fold) selectivity toward 5-HT_{2A}, and it appears that this property is augmented by the 2′-hydroxybenzyl substituent but not by the other *N*-benzyl groups examined in this study. Attempts to rationalize the structure−activity relationships via docking of the ligand set in previously reported 5-$HT_{2A/2C}$ homology-models were unsuccessful.[35]

In the functional assay, the *N*-(2-hydroxybenzyl) substituted compounds generally showed the highest activity at the 5-HT_{2A} receptor with moderate to good selectivity. With very few exceptions, the intrinsic activity was above 70% for all compounds on both 5-HT_{2A} and 5-HT_{2C}. *N*-(2-Methoxybenzyl) compounds (**1**−**12a**) were less active and also less selective 5-HT_{2A} agonists. From the *N*-(2-hydroxybenzyl) compounds, **1b** emerged as the most functionally potent of all ligands tested with an EC_{50} of 0.074 nM with more than 400-fold selectivity for the 5-HT_{2A} receptor. The *N*-(2,3-methylenedioxybenzyl) substituted compounds (**1d**−**12d**) were generally less potent. The *N*-(2-fluorobenzyl) compounds (**1c**−**12c**) were inferior in terms of affinity, efficacy, and selectivity compared to the other

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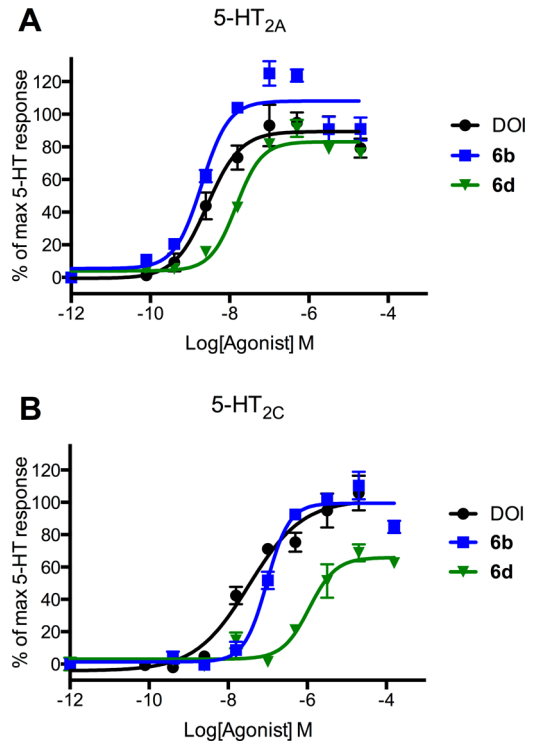
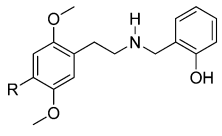


Figure 6. Concentration–response curves of the agonists DOI, **6b** and **6d** at 5-HT_{2A} (A) and 5-HT_{2C} (B) receptors, respectively. Concentration–response curves were generated from stimulation of inositol phosphate formation in tsA cells transiently expressing either 5-HT_{2A} (A) or 5-HT_{2C} (B) receptors. The formation of inositol phosphate was determined as described in Methods and calculated as percent response compared to a full 5-HT response. Data shown are mean ± SD of a single representative experiment performed in triplicate. Two additional experiments gave similar results.

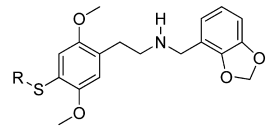
three series, mirroring the results from the binding assays, but comparable to the parent phenethylamines.

In simple phenethylamines, the functional activity drops as the size of the 4-substituent is increased. This is mirrored in our result: Starting from 4-methyl (**7b**), there is a drop in activity going from ethyl (**8b**) to propyl (**9b**) with the *N*-(2-hydroxybenzyl) substituent. In the *N*-(2,3-methylendioxybenzyl) series, the trend is similar; in the 4-thioalkyl series, **10d**–**11d**–**12**, where **11d** is the most potent of the three; see Figure 7. Thus, it appears that the substituent on the *N*-benzyl somehow influences the interaction of the 4-substituent as well.

In conclusion, we have investigated the structure activity relationship of 48 closely related *N*-benzyl phenethylamines as 5-$HT_{2A/2C}$ agonists. From that study, several interesting



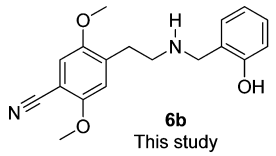
		pK_i			pEC_{50}		
R		2A	2C	2A/2C	2A	2C	2A/2C
7b	Me	8.96	7.92	11	8.47	7.56	7
8b	Et	9.54	8.47	12	8.89	7.42	31
9b	Pr	9.28	8.51	6	8.16	7.28	9



		pK_i			pEC_{50}		
R		2A	2C	2A/2C	2A	2C	2A/2C
10d	Me	9.15	7.56	39	8.10	6.59	29
11d	Et	9.40	8.04	23	8.91	6.81	97
12d	Pr	9.02	8.06	9	7.65	6.92	6

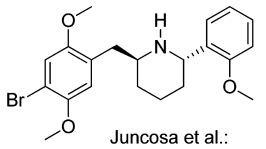
Figure 7. Structure and in vitro pharmacological profile of **7b**–**9b** and **10d**–**12d**.

compounds emerged. Several compounds displayed affinities and potencies in the picomolar range with varying levels of selectivity. Although the structure activity relations of the 48 ligands were erratic, the effect of the cyano substituent in the 4-position and the general trend of the *N*-(2,3-methylenedioxybenzyl) substituted phenethylamines on the selectivity is an interesting new observation. The most selective compound (when taking both binding and functional data into account) was **6b** being 100- and 90-fold selective; see Figure 8. In 2013,



6b
This study

	2A	2C	2A/2C
pK_i	8.88	6.88	100
pEC_{50}	8.68	6.76	90



Juncosa et al.:

	2A	2C	2A/2C
pK_i	8.60	6.51	124
pEC_{50}	7.13	-	-

Figure 8. Comparison between **5d** and 5-HT_{2A} selective agonist published by Juncosa et al. in 2013.

Juncosa et al.[31] published a conformationally restrained *N*-benzyl phenethylamine with more than 100-fold selectivity for the 5-HT_{2A} receptor; see Figure 8. We are currently pursuing ligands where these structural motifs are merged in the search for even more selective 5-HT_{2A} agonists.

METHODS

Synthesis of Secondary Amines. Et_3N (1.0 equiv) was added to a suspension of the phenethylamine hydrochloride (1.0 mmol) and 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 (30 min to 3 h). $NaBH_4$ (2.0 mmol) was added, and the reaction was stirred for another 30 min. The reaction mixture was concentrated under reduced pressure and partitioned between CH_2Cl_2/H_2O (30 mL, 1:1). The organic layer was isolated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH/NH_3 98:2:0.04). The purified free base was dissolved in EtOH (2 mL), there was added ethanolic HCl (1M, 2 mL), and the solution was diluted with Et_2O until crystals formed. The crystals were collected by filtration and dried under reduced pressure.

Functional Pharmacology. Cell culturing, transfection, and inositol phosphate turnover assay were adopted from a previously published procedure.[36]

Cell Culture and Transfections. tsA201 cells (a transformed HEK293 cell line) were cultured in GlutaMAX-I Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% dialyzed fetal bovine serum, penicillin (100 U mL^{-1}), and streptomycin (100 mg mL^{-1}) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Constructs encoding human 5-HT_{2A} and 5-HT_{2C} in pcDNA3.1 were obtained from the Missouri S&T cDNA Resource Center (www.cdna.org) and transiently transfected into cells using PolyFect according to the manufacturer's protocol (Qiagen, West Sussex, U.K.).

Inositol Phosphate (IP) Turnover Assay. The day after transfection, tsA201 cells were split into poly-D-lysine-coated 96-well tissue culture plates in inositol-free DMEM supplemented with 10% dialyzed fetal bovine serum, penicillin (100 U mL^{-1}), streptomycin (100 mg mL^{-1}), and 4 μCi mL^{-1} *myo*-[2-^{3}H]inositol (GE Healthcare, Buckinghamshire, U.K.). Two days after transfection, cells were washed with assay buffer 1 (Hanks' balanced saline solution (HBSS) containing 20 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mg mL^{-1} BSA, pH 7.4) and preincubated in 100 μL assay buffer 1 for 4 h at 37 °C, where the buffer was replaced after 2 h. The cells were then

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washed and subsequently incubated in 50 μL of assay buffer 2 (HBSS containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 20 mM LiCl) for 30 min at 37 °C. Following this incubation, the cells were stimulated with 50 μL of the indicated agonists in assay buffer 2 for 30 min at 37 °C.

The reactions were stopped by exchanging the buffer with 50 μL ice-cold 10 mM formic acid and incubating the cells at 4 °C for at least 30 min. Yttrium silicate scintillation proximity assay beads (GE Healthcare, Buckinghamshire, U.K.) were used for measuring radioactivity from generated [^{3}H]-IP essentially as previously described.[37] In brief, 20 μL of the formic acid cell extracts were transferred to white 96-well plates, and 1 mg of yttrium silicate scintillation proximity assay beads suspended in 80 μL water added to each well. The plates were sealed, shaken vigorously for 1 h, and centrifuged at 1500 rpm for 5 min. The radioactivity was quantified in a Wallac Microbeta scintillation counter, and responses read as counts per minute (CPM). All experiments were performed in triplicate and repeated in at least three independent experiments.

■ ASSOCIATED CONTENT

Supporting Information

NMR-data, including copies of ^{1}H and ^{13}C NMR spectra and full details on the pharmacological characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +45 3533 6487. E-mail: jesper.kristensen@sund.ku.dk.

Author Contributions

J.K., M.H., M.B., and H.B.O. conceived the experiments. M.H. and J.S.P. synthesized the compounds. M.H. and S.L.P. characterized the compounds. K.P. and H.B.O. performed the functional assays. M.H., S.L.P., and J.L.K. prepared the manuscript.

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Notes

The authors declare no competing financial interest.

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Radioligand competition binding assay for affinity at human 5-HT_{2A} receptors and rat 5-HT_{2C} receptors using displacement of antagonist radioligands [^{3}H]Ketanserin and [^{3}H]Mesulergine was generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda MD.

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