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Investigation of the 2,5-dimethoxy motif in phenethylamine Serotonin 2A receptor agonists

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KEYWORDS: Phenethylamine, Serotonin 2A receptor, agonists, Structure activity relationship.

ABSTRACT: The 2,5-dimethoxyphenethylamine (2, 5-PEA) scaffold is recognized as a motif conferring potent agonist activity at the serotonin 2A receptor (5-HT_{2A}R). The 2,5-dimethoxy motif is present in several classical phenethylamine psychedelics such as mescaline, TMA-2, DOM, DOI, DOB, 2C-B and 2C-I, and it has previously been suggested that this structural motif is essential for 5-HT_{2A}R activation. In the present study we present data that challenges this assumption. The 2- and 5-desmethoxy derivatives of 2C-B and DOB were synthesized and their pharmacological profiles evaluated *in vitro* at 5-HT_{2A}R and 5-HT_{2C}R in binding and functional assays and *in vivo* by assessing their induction of Head Twitch Response in mice. Elimination of either the 2- or 5-methoxy leads to a modest drop in binding affinity and functional potency at 5-HT_{2A}R and 5-HT_{2C}R, which was more pronounced upon removal of the 5-methoxy. However, this trend was not mirrored *in vivo*, as removal of either methoxy group resulted in significant reduction in the compounds ability to induce the Head Twitch Response in mice. Thus, the 2,5-dimethoxyphenethylamine motif appears to be important for *in vivo* potency of phenethylamine 5-HT_{2A}R agonists, but this does not correlate to the relative affinity and potency of the ligands at the recombinant 5-HT_{2A}R.

For the past five decades, agonistic activity at the serotonin 2A receptor (5-HT_{2A}R) has been viewed primarily as an undesirable off-target effect within the field of drug-development. This subclass of serotonin receptors has been linked to (and is thought to be primarily responsible for inducing) - the psychedelic experience induced by hallucinogenic drugs in man[1,2]. Compounds known to work as agonists at 5-HT_{2A}R include the classic psychedelics lysergic acid diethylamide ((+)-LSD), psilocybin and 2C-B, each representing one of the three primary structural classes interacting with this target receptor, ergolines, the tryptamines and phenethylamines respectively[3].

Recently several classic psychedelic drugs have been investigated as potential therapeutic agents for treating multiple psychiatric disorders[4–6]. In light of this recent development the effects and mechanisms of action of 5-HT_{2A}R agonists are now receiving considerable attention. As this new avenue of treatment is explored to address several psychiatric illnesses, including treatment-resistant depression, interest in the development of new 5HT_{2A}R ligands is gaining momentum. Therefore elucidating the key structural elements required for agonist activity at this receptor target is of high importance. Among 5HT_{2A}R ligands one of the most prevalent chemical motifs is the 2, 5-dimethoxyphenthylamine scaffold, which is present in many well characterized high potency compounds[7].

The current study analyzes the role that the 2,5-dimethoxy motif plays in the activity of the classic 5-HT_{2A}R agonists 2,5-dimethoxy-4-bromophenethylamine (2C-B, **1**, Fig 1) and 4-bromo-2,5-dimethoxyamphetamine (DOB, **2**, Fig 1). Specifically, compounds **3-6** (Fig 1), the desmethoxy derivatives of DOB and 2C-B, were synthesized, analyzed, and characterized.

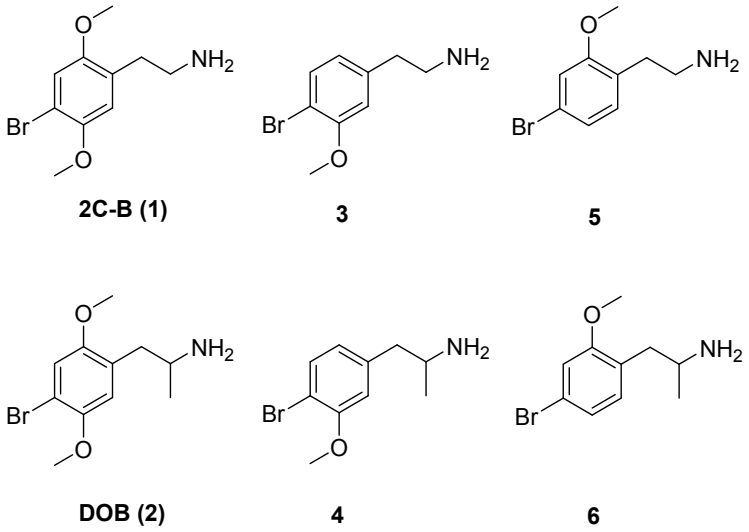

Figure 1. Structures of the phenethylamines **1-6** investigated in the present study.

Inspired by the prototypical phenethylamine psychedelic mescaline (**7**, Fig 2), many studies have examined the structure-activity relationships (SAR) of phenethylamine hallucinogens at 5-HT2 receptors[8]. Phenethylamines containing a 2,5-dimethoxy-substitution pattern and a lipophilic 4-substituent (such as halogen, alkyl, alkoxy or alkylthio) have relatively high potency at those receptor subtypes. Methylation of the α-position further increases *in vivo* potency, with amphetamines such as, **2**, 2,4,5-trimethoxyamphetamine (TMA-2, **8**, Fig 2), and 2,5-dimethoxy-4-methylamphetamine (DOM, **9**, Fig 2) being prominent examples[9,10].

Numerus potent analogs of **2** have been synthesized by varying the 4-position substituent[11,12]. The presence of methoxy groups in the 2- and 5-positions has been reported to be important for 5-HT_{2A}R activation and thus has been preserved in most investigations. Shulgin observed that replacement of either the 2- (**10**, Fig 2) or the 5-position (**11**, Fig 2) oxygen in **9** with a sulfur atom reduces its hallucinogenic potency by approximately 15- or 10-fold, respectively[13]. Replacing both oxygen atoms with sulfur (**12**, Fig 2) completely abolished activity. Removal of the 2- or 5-position methoxy-group in 2,4,5-substituted compounds is also detrimental for their *in vivo* activity. For example, 2,4-dimethoxyamphetamine(**13**, Fig 2) and 3,4-dimethoxyamphetamine(**14**, Fig 2) are less potent than **8** in humans and in rats trained to discriminate **9**.[14,15] Likewise, **6** is significantly less potent than **2** and produces only partial generalization in DOM-trained rats.[16] Other studies examining the stimulus effects of **9** and 2-desmethoxy-DOM(**15**, Fig 2) in rats trained to discriminate LSD has found the former to produce full substitution with an ED_{50} of 0.148 mg/kg, whereas the latter is almost an order of magnitude less potent and does not fully substitute for LSD.[15,17]

Evidence strongly indicates that the psychedelic effects produced by serotonergic hallucinogens are mediated by activation of 5-HT_{2A}R[18,19]. Due to a high degree of homology between 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, compounds active at 5-HT_{2A}R often show little to no selectivity at the two other subtypes. **2** displays high affinity for 5-HT_{2A}Rs in rat cortical homogenates in a [^{3}H]ketanserin binding assay (Ki = 63 nM), whereas **4** (Ki > 25,000 nM) and **6** (Ki = 4,870 nM) displayed markedly lower 5-HT_{2A}R affinity [16,20] Nichols and collaborators found that the affinity and potency of **9** at 5-HT_{2A}R is reduced if one of the methoxy groups is replaced with an ethyl group, giving 2-Et-DOM (**16**, Fig 2) and 5-Et-DOM (**17**, Fig 2), which is further corroborated by the very low 5-HT_{2A}R affinity exhibited by the DOB analog 4-bromo-2,5-diflouroamphetamine (**18**, Fig 2) [21,22].

Westkaemper and Glennon have proposed that the 2,5-dimethoxy substitution pattern may enhance 5-HT_{2A}R affinity because of hydrogen bond interactions between the methoxy groups and serine residues in the binding pocket, and subsequent mutagenesis and homology modelling studies identified Ser-159 and Ser-239 as likely candidates to interact with the 2- and 5-methoxy groups, respectively[21,23,24].

Molecular modeling studies published in 2017 by Roth *et al.* based on the 5-HT_{2B}R receptor crystal structure (-which shares significant homology to the 2A subtype), have led to significant insight into the binding mode of ligands in the orthosteric pocket of 5-HT_{2A}R[25]. However, a crystal structure of the 5-HT_{2A}R bound to a phenethylamine-based agonist ligand remains elusive; -therefore relevant interactions within the orthosteric binding pocket have yet to be fully elucidated.

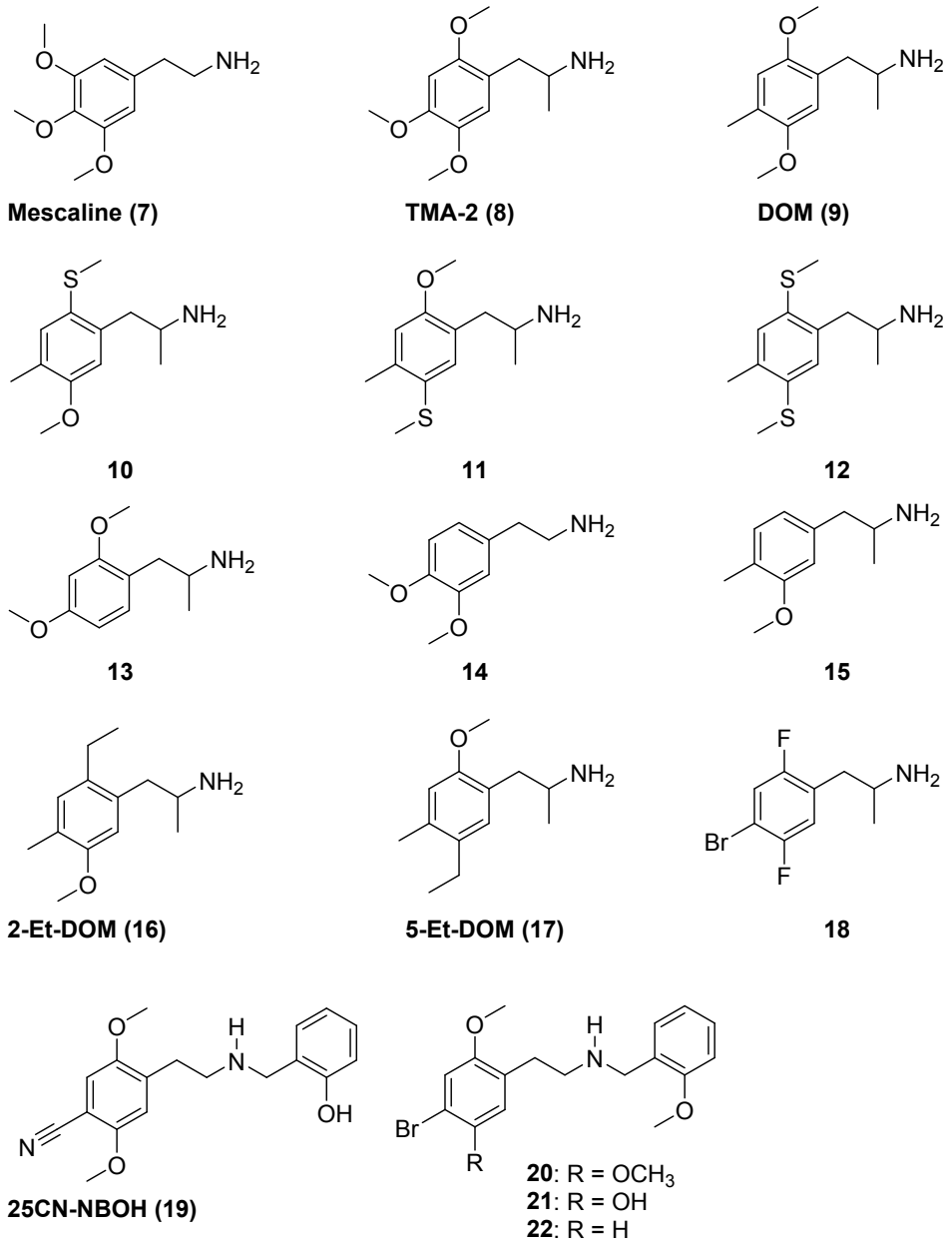

Figure 2. Structure and analogs of prototypical phenethylamine derived 5-HT_{2A}R agonists.

The search for 5-HT_{2A}R selective compounds, using the 2,5 dimethoxy phenethylamine scaffold as a starting point, led to the discovery of the NBOMe class of benzylated phenethylamines[26–28]. While N-methylation of phenethylamines causes a significant reduction of 5-HT_{2A}R binding affinity, N-benzylation yields highly potent 5-HT_{2A}R agonists[29–32]. 25CN-NBOH(**19**, Fig 2) emerged from this compound class as a selective 5-HT_{2A}R agonist amenable for *in vivo* investigations[33–37].

Investigations into the metabolism of the NBOMe class of ligands using 25B-NBOMe (**20**, Fig 1) lead us to conclude that the 5-position methoxy-group is a metabolic soft spot with 2-bromo-4-methoxy-5-(2-((2-methoxybenzyl)amino)ethyl)phenol (**21**, Fig 2) being the primary phase 1 metabolite[38]. This prompted us to synthesize and evaluate the 5-desmethoxy analog 2-(4-bromo-2-methoxyphenyl)-N-(2-methoxybenzyl)ethan-1-amine (**22**, Fig 2)[39]. To our surprise, removal of the 5-methoxy-group from the NBOMe-scaffold resulted in only a 10-fold decrease in 5-HT_{2A}R binding affinity compared to the parent compound **20** (pK_i 9.68 and 8.68, respectively), whereas the effect on efficacy was even smaller (**20**: pEC_{50} = 9.04, R_{max} = 83%; **22**: pEC_{50} = 8.87, R_{max} = 70%). This observation motivated us to re-investigate the importance of the 2,5-dimethoxy motif on the activity of the parent phenethylamine and amphetamine scaffold.

Due to the large degree of homology between 5HT2 subtypes numerous scientific studies have investigated 5HT_{2A}R over 5HT_{2C}R selectivity in this drug class[33,40,41]. Historically, the functional properties of 5HT_{2A}R agonists have been characterized by their selectivity for the 2A over the 2C subtype. For this reason we were prompted to assess the implications of modifying

this structural motif, with regards to the selectivity profile of these compounds. Thus, in the present study, the des-methoxy analogs of **1** and **2** were synthesized and evaluated pharmacologically *in vitro* and *in vivo*.

Synthesis of **1** and **2** was carried out using the procedures previously reported by Heim and Aldous, respectively[26,42]. Synthesis of the des-methoxy analogs **3-6** was achieved in two steps by condensing the appropriate brominated anisaldehyde with either nitromethane or nitroethane. Subsequent reduction of the formed nitroalkenes (**23-26**, Fig 3) using diisopropylaluminium hydride yielded the desired phenethylamine and amphetamines (**3-6**, Fig **3**)(see supporting information for full experimental details). The chiral amphetamine compounds were used as racemic mixture and not separated further as it has been established that the activity of **2** and several other chiral amphetamines resides primarily at in the R-enantiomer, which boasts 6-fold higher potency at the $5HT_{2A}R$ as compared to its corresponding S-enantiomere.[43,44]

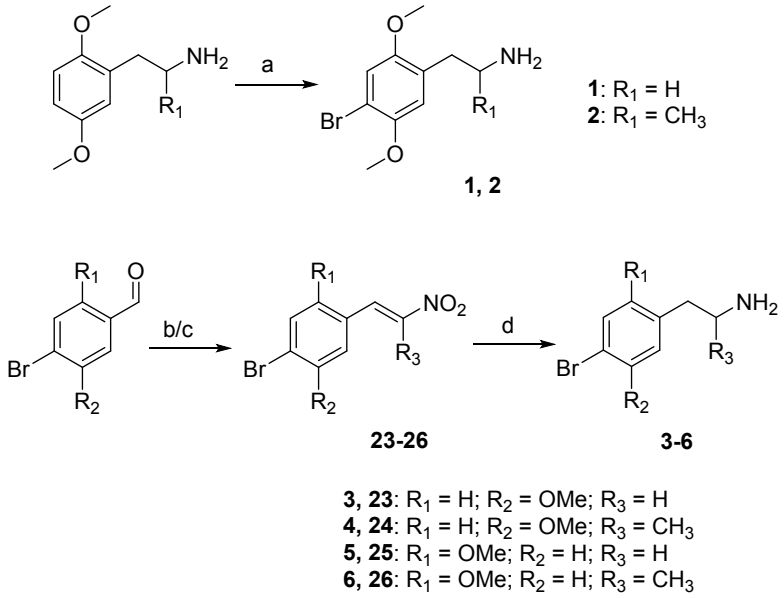

Figure 3. (a)Br_2, AcOH, 3 h, rt; (b) $MeNO_2$, AcOH, $Et(NH_2)_2$, i-PrOH, 75 ^{0}C, 1 h; (c) $EtNO_2$, AcOH, $Et(NH_2)_2$, i-PrOH, 75^{0}C, 1 h; (d) DIBAL-H, THF, 60 ^{0}C, 2 h.

The binding properties of compounds **1-6** at human 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R were characterized using membranes from tsA201 cells transiently expressing the three receptors in a [^{3}H]LSD competition binding assay (Table 1.)**.** **1** and **2** were found to display comparable K_i values at the three receptors in the assay. For 5-HT_{2A}R, removal of the 2-methoxy(**4**) and the 5-methoxy group(**6**) in **2** resulted in 9- and 26-fold lower binding affinity respectively, whereas removal of the 2-methoxy(**3**) and the 5-methoxy(**5**) group in **1** resulted in 17- and 31-fold lower binding affinities, respectively. Similar reductions in binding affinity were observed for the des-methoxy analogs compared to their parent compounds at 5-HT_{2B}R and 5-HT_{2C}R (Table 1.).

Compound	5-HT_{2A}R K_i (nM) [pK_i ± SEM]	5-HT_{2B}R K_i (nM) [pK_i ± SEM]	5-HT_{2C}R K_i (nM) [pK_i ± SEM]	Binding selectivity (2A/2B)	Binding selectivity (2A/2C)
1 (2C-B)	32 [7.50 ± 0.04]	97 [7.01 ± 0.08]	32 [7.49 ± 0.12]	3	1
2 (DOB)	25 [7.61 ± 0.07]	48 [7.32 ± 0.04]	34 [7.47 ± 0.07]	1.9	1.4
3	1,000 [5.98 ± 0.04]	590 [6.23 ± 0.11]	380 [6.42 ± 0.09]	0.6	0.4
4	650 [6.19 ± 0.09]	300 [6.52 ± 0.07]	330 [6.48 ± 0.06]	0.5	0.5
5	540 [6.27 ± 0.12]	710 [6.15 ± 0.08]	760 [6.12 ± 0.05]	1.3	1.4
6	230 [6.63 ± 0.10]	260 [6.58 ± 0.13]	1,100 [5.97 ± 0.11]	1.1	4.7

Table 1. Binding affinities of compounds **1-6** at 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R, based on[^{3}H]LSD competition binding assays (affinities are provided as Ki values in nM, with pKi ± SEM in brackets). The receptors were expressed in tsA201 cells. The data are based on 3 independent experiments.

The functional agonist activities of compounds **1-6** were next characterized at human 5-HT_{2A}R and 5-HT_{2C}R stably expressed in HEK293 cells in a fluorescence-based calcium flux assay (Table 2).[45] Overall, the four des-methoxy analogs exhibited significantly lower agonist potencies compared to their respective parent compounds at both 5-HT_2R subtypes, whereas their agonist efficacy was largely unaffected, with all analogs acting as high-efficacy partial agonists, bordering to full agonism, at both receptors (Table 2.). For example, analogs **3** and **5** activated 5HT_{2A}R with 27- and 6-fold lower potency than **1**, and analogs **4** and **6** activated 5HT_{2A}R with 70- and 17-fold lower potency than **2**. Thus, in both series of compounds removal of the 2-methoxy group had a notably greater effect on 5-HT_{2A}R potency compared to removal of the 5-methoxy group, which aligns well with the binding affinity data. Neither the modest 2A/2C selectivity exhibited by **2** nor the equipotent activity of **1** at the two receptors were influenced substantially by the removal of the methoxy groups (Table 2.).

	5-HT_{2A}R			5-HT_{2C}R			
Compound	**EC50 (nM) [pEC_{50}]**	**R_{max}**	**n**	**EC50 (nM) [pEC_{50}]**	**R_{max}**	**n**	**Functional selectivity (2A/2C)**
1 (2C-B)	1.6 [8.79 ± 0.08]	68 (± 6)	4	4.1 [8.36 ± 0.02]	74 (± 3)	4	2.8
2 (DOB)	0.2 [9.71 ± 0.16]	94 (± 6)	3	4.1 [8.38 ± 0.18]	92 (± 7)	3	20.7
3	42 [7.38 ± 0.08]	80 (± 3)	4	64 [7.19 ± 0.07]	85 (± 3)	4	1.5
4	14 [7.86 ± 0.05]	93 (± 7)	3	91 [7.04 ± 0.04]	81 (± 3)	4	6.5
5	11 [7.97 ± 0.04]	72 (± 4)	4	21 [7.68 ± 0.11]	79 (± 2)	4	1.9
6	3.4 [8.47± 0.09]	94 (± 5)	4	38 [7.42± 0.06]	97 (± 4)	4	11.2

Table 2. Functional properties of compounds 1-6 at stable 5-HT_{2A}R- and 5-HT_{2C}R-HEK293 cell lines in the Ca2+/Fluo-4 assay given as EC50 values in nM (with pEC50 ± SEM in brackets) and Rmax ± SEM values (in % of Rmax for 5-HT tested at the same plate).

Next, the functional properties of the compounds were assessed by investigation of their ability to induce the head twitch response (HTR) in mice, a well-established measure of 5-HT_{2A}R activation *in vivo* (**Figure 4** and Table S1).[46,47] When tested previously under equivalent conditions, **1** and **2** induced the HTR with ED50 values of 0.75 and 2.43 μmol/kg, respectively[48]. In concordance with the reduced 5-HT_{2A}R agonist potency displayed by analogs **3-6** in the Ca^{2+} flux assay, removal of the 2- or the 5-position methoxy groups reduced the potency of **1** and **2** in mice. Both **4** (ED50 = 2.26 μmol/kg) and **5** (ED50 = 2.55 μmol/kg) were threefold less potent than the parent compound, meaning they induce the HTR with about the same potency as **1**.

In addition to reducing the potency of **2**, loss of the 2- or 5-methoxy also produced a threefold reduction in the magnitude of the HTR (Figure 2). In comparison to **4** and **6**, the reduction of potency was more pronounced in the 2C-B derivatives **3** and **5**. Removal of the 2-methoxy-group in **1** resulted in a 25-fold reduction in potency (**3**: ED50 = 61.1 μmol/kg). In contrast, removal of the 5-methoxy-group was not tolerated; **5** did not induce the HTR at doses up to 100 mg/kg. In fact, **5** actually reduced the HTR below baseline levels, displaying an ID50 = 36.0 mg/kg (95% CI 17.0–76.3 mg/kg), which is equivalent to 135 μmol/kg.

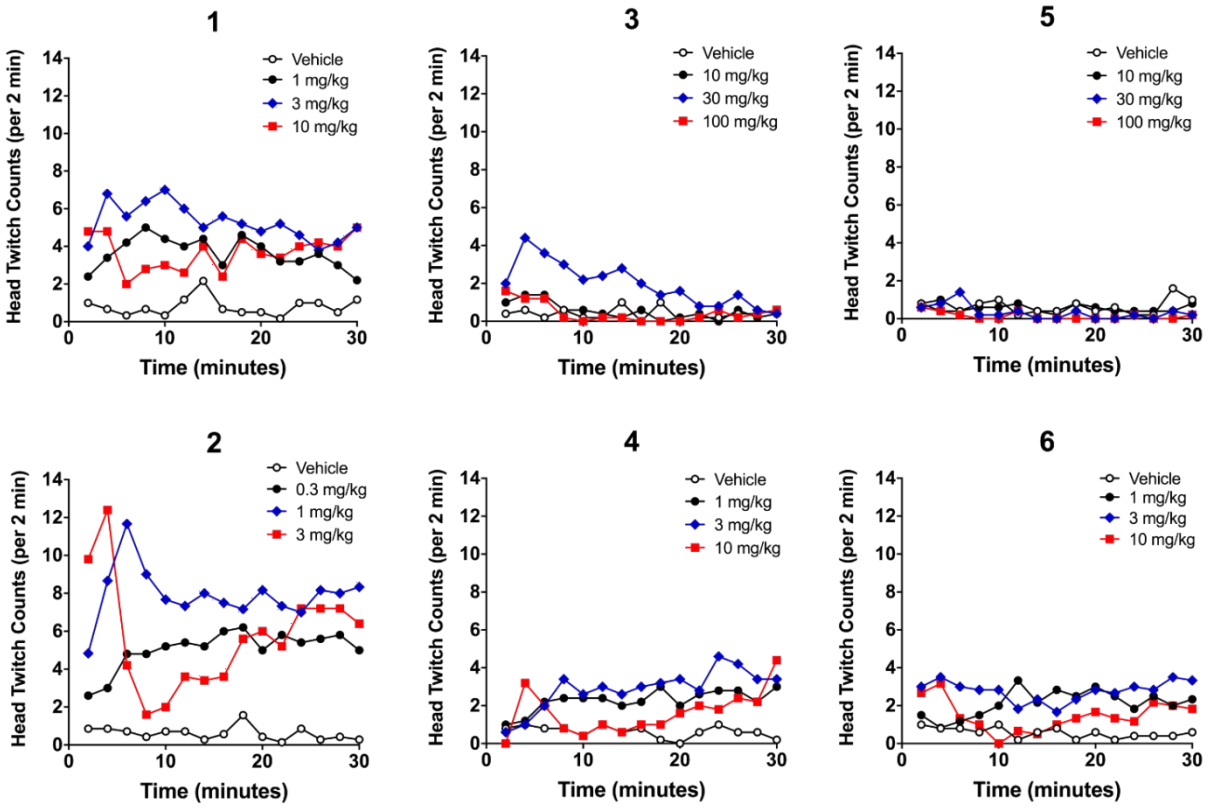

Figure 4. Time-course of the head-twitch response (HTR) induced by compounds 1-6 in C57BL/6J mice. The mean response during successive 2-minute time blocks is shown.

In the present study, we have shown that removing either the 2- or 5-methoxy group in the 2,5-dimethoxyphenethylamine scaffold produces a substantial loss of potency at 5-HT_{2A}R *in vivo*, in contrast to the more modest effect on 5-HT_{2A}R affinity and agonist potency. The results of the study show that removing the 5-methoxy substituent is more detrimental to 5-HT_{2A}R activity compared to removing the 2-methoxy group. This supports anecdotal accounts regarding the influence of the 5-position O-alkyl group on potency and duration of action.[49] These results, however, are not consistent with evidence that the potency of 5-HT_{2A}R agonists *in vivo* is correlated with their *in vitro* activity at the receptor level[50–52]. Therefore, caution is warranted when evaluating the importance of this structural motif for potent *in vivo* agonist activity at 5-HT_{2A}R.

Although the phenethylamine scaffold still merits further investigation as a starting point for developing potent 5-HT_{2A}R compounds, this study shows that our understanding of the SAR for 5-HT_{2A}R is not complete. There is clearly considerable room for variation within the classic 5-HT_{2A}R agonist structural motifs.

Methods.

[^{3}H]LSD binding assay. Binding affinity was measured in competition binding assays using [^{3}H]LSD (PerkinElmer, Waltham, MA) and membranes from tsA201 cells transiently transfected with cDNA for human 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R. 40-48 h after transfection, the transfected tsA201 cells were scraped into ice-cold assay buffer (50 mM Tris–HCl, 10 mM $MgCl_2$, 0.1 mM EDTA, pH 7.4), homogenized on ice using an IKA T18 Basic Ultra-Turrax for 10 s, and centrifuged for 20 min at 50.000 g at 4 °C. Cell pellets were re-suspended in fresh assay buffer, homogenized on ice, and centrifuged at 50.000 *g* at 4 °C for another 20 min, after which the membranes were stored at –80 °C. On the day of the assay, the cell membranes were re-suspended in assay buffer and incubated with a fixed concentration of [^{3}H]LSD (0.4-0.6 nM for 5-HT_{2A}R and 1.0-1.5 nM for 5-HT_{2B}R and 5-HT_{2C}R) and various concentrations of the test compounds in a 96-well plate at 37 °C for 2 h. The filters in UniFilter 96-well GF/C plates (PerkinElmer, Waltham, MA) were presoaked for 1h in assay buffer supplemented with 0.5% BSA, and the binding reactions were terminated by rapid filtration through the plates using a FilterMate Harvester (PerkinElmer), followed by four washes with ~300 µL of ice-cold wash buffer (0.9% w/v NaCl, 50 mM Tris-HCl, pH 7.4). Next, the filters were dried for at least 1 h at 50°C, 25 µL Microscint-0 (PerkinElmer) was added to each well in the filter, which were then shaken gently over-night. The following day, the amount of bound radioactivity on the filters was determined in a TopCount NXT scintillation counter (PerkinElmer). Bound radioligand was plotted as a function of log[ligand], and the data were analyzed using a one-site Ki model built into Graphpad Prism 7.0 (Graphpad, La Jolla, CA). The determined IC_{50}

values were used to calculate the K_i values for the compounds using the Chen-Prusoff equation: $K_i = IC_{50}/(1+([RL]/K_D))$, where [RL] and K_D are the [^{3}H]LSD concentration used in that experiment and the dissociation constant for [^{3}H]LSD determined in a saturation binding experiment performed with the respective receptor.

Ca^{2+}/Fluo-4 assay. The agonist activity of the compounds was assessed using stable 5-HT_{2A}R- and 5-HT_{2C}R-HEK293 cell lines in a fluorescence-based Ca^{2+}/Fluo-4 assay, essentially as previously described[45]. Briefly, the cells were split into poly-D-lysine-coated black 96-well plates with clear bottoms (6 x 10^4 cells/well). The following day, the culture medium was aspirated and the cells were incubated in 50 µL assay buffer [Hanks Buffered Saline Solution containing 20 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 2.5 mM probenecid, pH 7.4] supplemented with 6 mM Fluo–4/AM at 37 °C for 1 h. Next, the buffer was aspirated, the cells were washed once with 100 µL assay buffer, and then 100 µL assay buffer was added to the cells. The 96-well plates was assayed in a FLEXStation^3 (Molecular Devices, Crawley, UK) measuring emission [in fluorescence units (FU)] at 525 nm in response to excitation at 485 nm before and up to 90 s after addition of 33.3 µL of assay buffer containing the test compound. Each compound was tested at each cell line in three independent experiments performed in duplicate. Data were normalized to the Rmax value for 5-HT from the same plate and analyzed using log [agonist] versus response in Graphpad Prism 7.0, and the EC_{50} and R_{max} values for test compounds were estimated based on the concentration-response curves.

Animals. Male C57BL/6J mice (6-8 weeks old) obtained from Jackson Laboratories (Bar Harbor, ME, USA) were housed in a vivarium at the University of California San Diego, an AAALAC-approved animal facility that meets all Federal and State requirements for care and treatment of laboratory animals. Mice were housed up to four per cage in a climate-controlled room on a reverse-light cycle (lights on at 1900 h, off at 0700 h) and were provided with ad libitum access to food and water, except during behavioral testing. Testing was conducted between 1000 and 1800 h. All animal experiments were carried out in accordance with NIH guidelines and were approved by the UCSD animal care committee.

Head-Twitch Response Studies. The head-twitch response (HTR) was assessed using a head-mounted magnet and a magnetometer detection coil[53,54]. Briefly, mice were anesthetized, a small incision was made in the scalp, and a small neodymium magnet was attached to the dorsal surface of the cranium using dental cement. Following a two-week recovery period, HTR experiments were carried out in a well-lit room with at least 7 days between sessions to avoid carryover effects. Test substances were dissolved in saline and injected intraperiotoneally (IP) at a volume of 5 mL/kg. Mice (n = 5-7/group) were injected with drug or vehicle and then HTR activity was recorded in a glass cylinder surrounded by a magnetometer coil for 30 min. Coil voltage was low-pass filtered (2–10 kHz cutoff frequency), amplified, and digitized (20 kHz sampling rate) using a Powerlab/8SP with LabChart v 7.3.2 (ADInstruments, Colorado Springs, CO, USA), then filtered off-line (40–200 Hz band-pass). Head twitches were identified manually based on the following criteria: 1) sinusoidal wavelets; 2) evidence of at least three sequential head movements (usually exhibited as bipolar peaks) with frequency ≥ 40 Hz; 3) amplitude exceeding the level of background noise; 4) duration < 0.15 s; and 5) stable coil voltage immediately preceding and following each response.

Data Analysis. Head-twitch counts were analyzed using one-way analyses of variance (ANOVA). Post hoc pairwise comparisons between selected groups were performed using Tukey's studentized range method. Median effective doses (ED50 values) and 95% confidence intervals (95% CI) for HTR dose-response experiments were calculated by nonlinear regression (Prism 7.00, GraphPad Software, San Diego, CA, USA). A gaussian distribution was used to fit biphasic HTR dose-response data[48,55].

ASSOCIATED CONTENT

Supporting Information. Experimental procedures for the synthesis of compounds **1-6** and extended pharmacological data are available free of charge at http://pubs.acs.org

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Author Contributions

All authors have contributed to the writing of and given approval to the final version of the manuscript.

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ABBREVIATIONS

2,5-PEA 2,5-dimethoxyphenethylamine, 5-HT_{2A}R serotonin 2A receptor, , LSD (+)-Lysergic Acid diethylamide, TMA-2 2,4,5-trimethoxyamphetamine, DOM 2,5-dimethoxy-4-methylamphetamine, DOI 2,5.dimethoxy-4-iodoamphetamine, DOB 2,5-dimethoxy-4-bromoamphetamine, 2C-B 2,5-dimethoxy-4-bromophenethylamine, 2C-I 2,5-dimethoxy-4-iodophenethylamine SAR Structure activity relationship2-Et-DOM 2-ethyl,5-methoxy-4-methylamphetamine, , 5-Et-DOM 2-methoxy,5-ethyl-4-methylamphetamine, Ser-159 Serine 159, Ser-239 Serine 239, NBOMe N-benzylphenethylamine, 25CN-NBOH 4-[2-[[(2-Hydroxyphenyl)methyl]amino]ethyl]-2,5-dimethoxybenzonitril, 25B-NBOMe 2-(4-bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethan-1-amine, HTR Head-twitch Response

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SYNOPSIS TOC. The 2,5-dimethoxy phenethylamine scaffold is widely associated with agonistic activity at 5-HT_{2A}R and other 5-HT2Rs. Herein we challenge this dogma via the synthesis and *in vitro* and *in vivo* evaluation of ligands where the methoxy groups are systematically eliminated and the effects of this on their pharmacological profiles at 5-HT_{2A}R and 5-HT_{2C}R are evaluated along with their binding affinity for the 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R.

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