

## Receptor interaction profiles of novel *N*-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs)



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### ABSTRACT

**Background:** *N*-2-methoxybenzyl-phenethylamines (NBOMe drugs) are newly used psychoactive substances with poorly defined pharmacological properties. The aim of the present study was to characterize the receptor binding profiles of a series of NBOMe drugs compared with their 2,5-dimethoxy-phenethylamine analogs (2C drugs) and lysergic acid diethylamide (LSD) *in vitro*.

**Methods:** We investigated the binding affinities of 2C drugs (2C-B, 2C-C, 2C-D, 2C-E, 2C-H, 2C-I, 2C-N, 2C-P, 2C-T-2, 2C-T-4, 2C-T-7, and mescaline), their NBOMe analogs, and LSD at monoamine receptors and determined functional 5-hydroxytryptamine-2A (5-HT<sub>2A</sub>) and 5-HT<sub>2B</sub> receptor activation. Binding at and the inhibition of monoamine uptake transporters were also determined. Human cells that were transfected with the respective human receptors or transporters were used (with the exception of trace amine-associated receptor-1 [TAAR<sub>1</sub>], in which rat/mouse receptors were used).

**Results:** All of the compounds potently interacted with serotonergic 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub> receptors and rat TAAR<sub>1</sub> (most K<sub>i</sub> and EC<sub>50</sub>: <1 μM). The *N*-2-methoxybenzyl substitution of 2C drugs increased the binding affinity at serotonergic 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, adrenergic α<sub>1</sub>, dopaminergic D<sub>1-3</sub>, and histaminergic H<sub>1</sub> receptors and monoamine transporters but reduced binding to 5-HT<sub>1A</sub> receptors and TAAR<sub>1</sub>. As a result, NBOMe drugs were very potent 5-HT<sub>2A</sub> receptor agonists (EC<sub>50</sub>: 0.04–0.5 μM) with high 5-HT<sub>2A</sub>/5-HT<sub>1A</sub> selectivity and affinity for adrenergic α<sub>1</sub> receptors (K<sub>i</sub>: 0.3–0.9 μM) and TAAR<sub>1</sub> (K<sub>i</sub>: 0.06–2.2 μM), similar to LSD, but not dopaminergic D<sub>1-3</sub> receptors (most K<sub>i</sub>: >1 μM), unlike LSD.

**Conclusion:** The binding profile of NBOMe drugs predicts strong hallucinogenic effects, similar to LSD, but possibly more stimulant properties because of α<sub>1</sub> receptor interactions.

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**Abbreviations:** 25B-NBOMe, 2-(4-bromo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25C-NBOMe, 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25D-NBOMe, 2-(4-methyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25E-NBOMe, 2-(4-ethyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25H-NBOMe, 2-(2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25I-NBOMe, 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25N-NBOMe, 2-(4-nitro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25P-NBOMe, 2-(4-propyl-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25T2-NBOMe, 2-(2,5-dimethoxy-4-ethylthiophenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25T4-NBOMe, 2-(2,5-dimethoxy-4-isopropylthiophenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 25T7-NBOMe, 2-(2,5-dimethoxy-4-*n*-propylthiophenyl)-*N*-[(2-methoxyphenyl)methyl] ethanamine; 2C-B, 4-bromo-2,5-dimethoxyphenethylamine; 2C-C, 2-(4-chloro-2,5-dimethoxy)ethanamine; 2C-D, 2-(2,5-dimethoxy-4-methyl)ethanamine; 2C-E, 1-(2,5-dimethoxy-4-ethylphenyl)-2-aminoethane; 2C-H, 2,5-dimethoxyphenethylamine; 2C-I, 4-iodo-2,5-dimethoxyphenethylamine; 2C-N, 2-(2,5-dimethoxy-4-nitro)ethanamine; 2C-P, 2-(2,5-dimethoxy-4-propylphenyl)ethanamine; 25CN-NBOH, 2-[(2-(4-cyano-2,5-dimethoxyphenyl)ethylamino)-methyl]phenol; 2C-T-2, 2-[2,5-dimethoxy-4-(ethylthio)phenyl]ethanamine; 2C-T-4, 2,5-dimethoxy-4-isopropylthiophenethylamine; 2C-T-7, 2-[2,5-dimethoxy-4-(propylthio)phenyl]ethanamine; 5-HT, 5-hydroxytryptamine (serotonin); DA, dopamine; DAT, dopamine transporter; mescaline, 2-(3,4,5-trimethoxyphenyl)ethanamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; NBOMe, *N*-(2-methoxy)benzyl; NE, norepinephrine; NET, norepinephrine transporter; SERT, serotonin transporter; TAAR, trace amine-associated receptor; LSD, lysergic acid diethylamide.

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## 1. Introduction

New psychoactive substances are constantly emerging on the illicit drug market and typically sold via the Internet. Of particular interest are *N*-2-methoxybenzyl-phenethylamines (NBOMe drugs), which are novel and reportedly very potent hallucinogens that have been increasingly used recreationally (Forrester, 2014; Hill et al., 2013; Ninnemann and Stuart, 2013; Rose et al., 2013; Walterscheid et al., 2014; Wood et al., 2015; Zuba, 2012), with additional potential use as radiotracers (Ettrup et al., 2011, 2010). Recreationally used NBOMe drugs include 25I-NBOMe, 25C-NBOMe, 25B-NBOMe, and 25D-NBOMe (Armenian and Gerona, 2014; Poklis et al., 2014; Rose et al., 2013), which are derivatives of 2,5-dimethoxy-4-substituted phenethylamines (2C drugs; Dean et al., 2013; Hill and Thomas, 2011; Shulgin and Shulgin, 1991) (see Fig. 1). *N*-2-methoxybenzyl substitution enhances the potency of 2C drugs at serotonergic 5-hydroxytryptamine-2A (5-HT<sub>2A</sub>) receptors, resulting in exceptionally potent 5-HT<sub>2A</sub> receptor agonists (Braden et al., 2006; Heim, 2004; Nichols et al., 2015) with strong hallucinogenic properties in animals and humans (Halberstadt and Geyer, 2014; Srisuma et al., 2015). Pharmacological interactions between NBOMe drugs and 5-HT<sub>2</sub> receptors have been well characterized for some compounds of this novel drug family (Blaazer et al., 2008; Braden et al., 2006; Ettrup et al., 2011, 2010; Hansen et al., 2014; Nichols et al., 2008). However, systematic characterizations of the effects of a larger series of NBOMe drugs at a wider range of relevant human receptors and comparisons with their 2C parent drugs are lacking. Importantly, NBOMe drugs have been reported to produce psycho- and cardiovascular stimulant effects, in addition to hallucinations. Specifically, sympathomimetic toxicity, including tachycardia, hypertension, mydriasis, agitation, and hyperthermia, is commonly reported in cases of acute NBOMe drug intoxication (Hill et al., 2013; Rose et al., 2013; Srisuma et al., 2015; Stellpflug et al., 2014; Wood et al., 2015). Pharmacologically, compounds of the 2C series, including 2C-C, 2C-E, and 2C-I, inhibit the norepinephrine (NE) and serotonin transporters (NET and SERT, respectively), similar to amphetamines, although with only very low potency (Eshleman et al., 2014; Nagai et al., 2007). These findings raise the question of whether NBOMe drugs may have similar but more potent stimulant-type pharmacological properties, including inhibition of the NET, dopamine (DA) transporter (DAT), and SERT, or interactions with adrenergic  $\alpha_1$  receptors that lead to vasoconstriction.

We assessed the *in vitro* pharmacology of a series of NBOMe drugs compared with their 2C parent drugs. We characterized the binding affinity profiles at monoamine receptors and DAT, NET, and SERT inhibition potencies. We also determined the functional 5-HT<sub>2A</sub> receptor activation potencies because 5-HT<sub>2A</sub> receptors mediate hallucinogenic effects (Nichols, 2004). The prototypical serotonergic hallucinogen lysergic acid diethylamide (LSD) was included as a comparator drug (Nichols, 2004; Passie et al., 2008).

## 2. Methods

### 2.1. Drugs

2C-B, 2C-C, 2C-D, 2C-E, 2C-H, 2C-I, 2C-N, 2C-P, 2C-T-2, 2C-T-4, 2C-T-7, mescaline, 25B-NBOMe, 25C-NBOMe, 25D-NBOMe, 25E-NBOMe, 25H-NBOMe, 25I-NBOMe, 25N-NBOMe, 25P-NBOMe, 25T2-NBOMe, 25T4-NBOMe, 25T7-NBOMe, and mescaline-NBOMe were synthesized by Lipomed (Arlesheim, Switzerland) for this study at no cost. All of the compounds were used as hydrochloride salts. Purity was >98% for all of the substances. [<sup>3</sup>H]NE and [<sup>3</sup>H]DA were obtained from Perkin–Elmer (Schwerzenbach, Switzerland), and [<sup>3</sup>H]5-HT was obtained from Anawa (Zürich, Switzerland).

### 2.2. Radioligand receptor and transporter binding assays

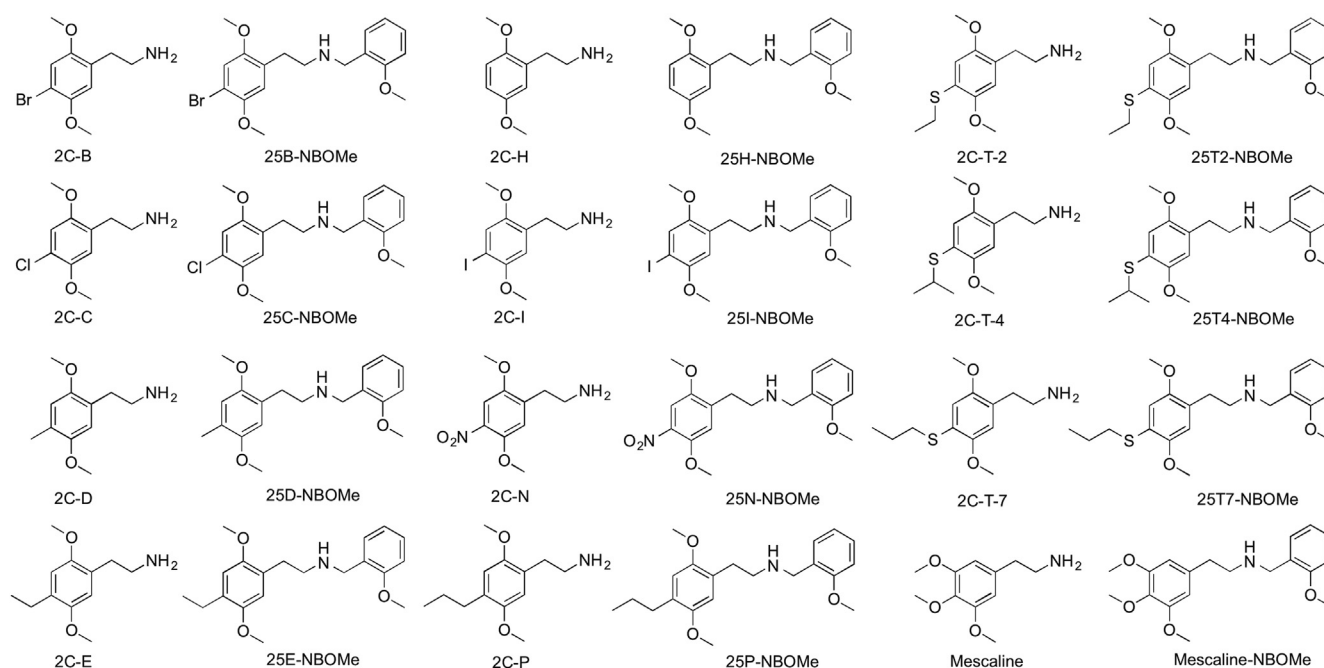
The radioligand binding assays were performed as described previously (Hysek et al., 2012; Simmler et al., 2013). Briefly, membrane preparations of human embryonic kidney (HEK) 293 cells (Invitrogen, Zug, Switzerland) that overexpress the respective transporters (Tatsumi et al., 1997) or receptors (human genes, with the exception of rat and mouse genes for trace amine-association receptor 1 [TAAR<sub>1</sub>]; (Revel et al., 2011)) were incubated with the radiolabeled selective ligands at concentrations equal to K<sub>d</sub>, and ligand displacement by the compounds was measured. Specific binding of the radioligand to the target receptor was defined as the difference between the total binding and nonspecific binding that was determined in the presence of selected competitors in excess. The following radioligands and competitors, respectively, were used: *N*-methyl-[<sup>3</sup>H]-nisoxetine and indatraline (NET), [<sup>3</sup>H]citalopram and indatraline (SERT), [<sup>3</sup>H]WIN35,428 and indatraline (DAT), [<sup>3</sup>H]8-hydroxy-2-(di-*n*-propylamine)tetralin and indatraline (5-HT<sub>1A</sub> receptor), [<sup>3</sup>H]ketanserin and spiperone (5-HT<sub>2A</sub> receptor), [<sup>3</sup>H]mesulgerine and mianserin (5-HT<sub>2C</sub> receptor), [<sup>3</sup>H]prazosin and risperidone (adrenergic  $\alpha_1$  receptor), [<sup>3</sup>H]rauwolscine and phentolamine (adrenergic  $\alpha_2$  receptor), [<sup>3</sup>H]SCH 23390 and butaclamol (D<sub>1</sub> receptor), [<sup>3</sup>H]spiperone and spiperone (D<sub>2</sub> and D<sub>3</sub> receptors), [<sup>3</sup>H]pyrilamine and clozapine, (histaminergic H<sub>1</sub> receptor), and [<sup>3</sup>H]RO5166017 and RO5166017 (TAAR<sub>1</sub>). IC<sub>50</sub> values were determined by calculating non-linear regression curves for a one-site model using three to five independent 10-point concentration–response curves for each compound. K<sub>i</sub> (affinity) values, which correspond to the dissociation constants, were determined using the Cheng–Prusoff equation.

### 2.3. Activity at serotonin 5-HT<sub>2A</sub> receptor

Human 5-HT<sub>2A</sub> receptor-expressing NIH-3T3 cells were incubated in HEPES–Hank's Balanced Salt Solution (HBSS) buffer (70'000 cells/100  $\mu$ l) for 1 h at 37 °C in 96-well poly-D-lysine-coated plates. To each well 100  $\mu$ l of Dye solution (FLIPR calcium 5 assay kit; Molecular Devices, Sunnyvale, CA, USA) was added and plates were incubated for 1 h at 37 °C. The plates were then placed in a fluorescence imaging plate reader (FLIPR), and 25  $\mu$ l of the test substances diluted in HEPES–HBSS buffer containing 250 mM probenidol were added online. The increase in fluorescence was then measured. EC<sub>50</sub> values were derived from the concentration–response curves using nonlinear regression. Efficacy (maximal activity) is expressed relative to the activity of 5-HT, which was used as a control set to 100%.

### 2.4. Activity at serotonin 5-HT<sub>2B</sub> receptor

Human 5-HT<sub>2B</sub> receptor-expressing HEK293 cells were incubated in growth medium (DMEM high glucose [Invitrogen, Zug, Switzerland], 10 ml/l PenStrep [Gibco, Life Technologies, Zug, Switzerland]), 10% FCS non dialyzed heat inactivated and 250 mg/l geneticin) at a density of 50'000 cells/well at 37 °C in 96-well poly-D-lysine-coated plates over-night. On the next day the growth medium was removed by snap inversion, and 100  $\mu$ l of Fluo-4 solution (calcium indicator; Molecular Probes, Eugene, OR, USA) was added to each well. The plates were incubated for 45 min at 31 °C. The Fluo-4 solution was removed by snap inversion, and 100  $\mu$ l of Fluo-4 solution was added a second time. The cells were then incubated for another 45 min at 31 °C. Immediately before testing, the cells were washed with HBSS (Gibco) and 20 mM HEPES (assay buffer; Gibco) using an EMBLA cell washer, and 100  $\mu$ l assay buffer was added. The plate was placed in a fluorescence imaging plate reader (FLIPR), and 25  $\mu$ l of the test substances diluted in assay



**Fig. 1.** Chemical structures of 2,5-dimethoxyphenethylamines (2C drugs) and their *N*-2-methoxybenzyl-substituted analogs (NBOME drugs).

buffer was added online. The increase in fluorescence was then measured.  $EC_{50}$  values were derived from the concentration–response curves using nonlinear regression. Efficacy (maximal activity) is expressed relative to the activity of 5-HT, which was used as a control set to 100%.

### 2.5. Monoamine uptake transporter inhibition

Inhibition of the human NET, DAT, and SERT was assessed in HEK 293 cells that were stably transfected with transporters as specified previously (Hysek et al., 2012). Briefly, the cells were suspended in uptake buffer and incubated for 10 min with different concentrations of the test substances. The corresponding radiolabeled [ $^3$ H] monoamine (5 nM final concentration) was then added at room temperature. After 10 min, uptake was stopped by separating the cells from the buffer using centrifugation through silicone oil (Hysek et al., 2012). The centrifugation tubes were frozen in liquid nitrogen and cut to separate the cell pellet from the silicone oil and assay buffer layers. The cell pellet was then lysed. Scintillation fluid was added, and radioactivity was counted on a  $\beta$ -counter. Nonspecific uptake was determined for each experiment in the presence of 10  $\mu$ M fluoxetine for SERT cells, 10  $\mu$ M nisoxetine for NET cells, and 10  $\mu$ M mazindol for DAT cells and subtracted from the total counts to yield specific uptake (100%). The data were fitted by non-linear regression to variable slope sigmoidal dose–response curves (bottom = 0%), and  $IC_{50}$  values were calculated using Prism software (GraphPad, San Diego, CA, USA).

### 2.6. Cytotoxicity

To confirm cell integrity during the pharmacological assays, cytotoxicity was assessed using the ToxiLight bioassay (Lonza, Basel, Switzerland) according to the manufacturer's instructions. The assay quantitatively measures the release of adenylate kinase from damaged cells, providing a highly sensitive method of measuring cytolysis (Crouch et al., 1993). Cells that were grown in 96-well plates were exposed to the compounds at a high

concentration of 100  $\mu$ M. All of the test conditions contained 0.1% (v:v) dimethylsulfoxide, which is non-toxic at this concentration and was also used as a negative control. Triton X-100 (0.1%, Sigma–Aldrich, Buchs, Switzerland) lyses cells and was used as a positive control. After 4 h incubation at 37 °C, 10  $\mu$ l of the supernatant per well was removed and combined with 50  $\mu$ l of ToxiLight reagent, and luminescence was recorded using a Tecan Infinite 200 Pro plate reader (Tecan, Männedorf, Switzerland).

## 3. Results

### 3.1. Interactions with serotonin receptors

Table 1 shows binding to serotonin 5-HT $_1A$ , 5-HT $_2A$ , and 5-HT $_2C$  receptors, activation potency and efficacy at 5-HT $_2A$  and 5-HT $_2B$  receptors, and 5-HT receptor binding ratios. All of the compounds exhibited high binding affinity for 5-HT $_2A$  and 5-HT $_2C$  receptors ( $K_i < 1 \mu$ M, with the exception of 2C-H and mescaline). *N*-2-methoxybenzyl substitution further increased the average binding affinity for both 5-HT $_2A$  and 5-HT $_2C$  receptors 26- and 14-fold (range: 6–100 and 8–32, respectively), leading to compounds with up to 8.4-fold higher affinity for these receptors compared with LSD. Moderate 5-HT $_2A$  over 5-HT $_2C$  receptor binding preference was observed, with 5-HT $_2A$ /5-HT $_2C$  receptor binding ratios of 3–16 for the 2C drugs and slightly more selective ratios of 5–26 for the NBOME drugs. All of the compounds also potently activated 5-HT $_2A$  receptors and typically more potently than LSD ( $EC_{50} < 1 \mu$ M, with the exception of 2C-H, mescaline, and mescaline-NBOMe). However, in contrast to the robust effect on binding to 5-HT $_2A$  receptors, *N*-2-methoxybenzyl substitution did not consistently change the activation potency at 5-HT $_2A$  receptors and even reduced the activation efficacy, with the exception of 2C-H. All of the compounds potently activated the 5-HT $_2B$  receptor ( $EC_{50} < 1 \mu$ M, with the exception of 2C-H, mescaline, mescaline-NBOMe, and LSD). *N*-2-methoxybenzyl substitution increased 5-HT $_2B$  receptor activation 5-fold (range: 0.8–18) but reduced activation efficacy. All of the 2C drugs potently bound to 5-HT $_1A$

**Table 1**  
Serotonin receptor interactions.

	5-HT <sub>1A</sub>		5-HT <sub>2A</sub>		5-HT <sub>2B</sub>		5-HT <sub>2C</sub>	Selectivity (binding ratios)	
	Receptor binding K <sub>i</sub> ± SD [μM]	Receptor binding K <sub>i</sub> ± SD [μM]	Activation potency EC <sub>50</sub> ± SD [μM]	Activation efficacy % maximum ± SD	Activation potency EC <sub>50</sub> ± SD [μM]	Activation efficacy % maximum ± SD	Receptor binding K <sub>i</sub> ± SD [μM]	5-HT <sub>2A</sub> / 5-HT <sub>1A</sub>	5-HT <sub>2A</sub> / 5-HT <sub>2C</sub>
<b>2Cs</b>									
2C-B	0.24 ± 0.04	0.0086 ± 0.003	0.08 ± 0.02	45 ± 7	0.13 ± 0.06	89 ± 13	0.047 ± 0.009	28	4.7
2C-C	0.19 ± 0.01	0.0130 ± 0.005	0.20 ± 0.06	49 ± 10	0.28 ± 0.11	81 ± 14	0.090 ± 0.026	15	6.9
2C-D	0.44 ± 0.01	0.0324 ± 0.005	0.35 ± 0.18	41 ± 3	0.23 ± 0.07	77 ± 17	0.15 ± 0.03	14	4.6
2C-E	0.36 ± 0.04	0.0105 ± 0.001	0.11 ± 0.03	40 ± 2	0.19 ± 0.04	66 ± 7	0.10 ± 0.02	34	10
2C-H	0.07 ± 0.02	1.6 ± 0.3	9.4 ± 0.5	28 ± 5	6.2 ± 2.8	46 ± 18	4.1 ± 0.9	0.04	2.6
2C-I	0.18 ± 0.01	0.0035 ± 0.001	0.06 ± 0.03	45 ± 8	0.15 ± 0.10	70 ± 18	0.040 ± 0.009	51	11
2C-N	2.2 ± 0.1	0.0235 ± 0.011	0.17 ± 0.04	48 ± 10	0.73 ± 0.09	74 ± 20	0.37 ± 0.02	94	16
2C-P	0.11 ± 0.04	0.0081 ± 0.001	0.09 ± 0.06	63 ± 5	0.13 ± 0.01	72 ± 18	0.040 ± 0.005	14	4.9
2C-T-2	0.37 ± 0.04	0.0090 ± 0.002	0.08 ± 0.03	67 ± 16	0.13 ± 0.09	75 ± 14	0.069 ± 0.018	41	7.7
2C-T-4	0.47 ± 0.13	0.0279 ± 0.012	0.22 ± 0.13	87 ± 7	0.16 ± 0.06	68 ± 10	0.18 ± 0.07	17	6.5
2C-T-7	0.52 ± 0.05	0.0065 ± 0.002	0.13 ± 0.05	76 ± 10	0.35 ± 0.25	45 ± 10	0.039 ± 0.013	80	6.0
Mescaline	4.6 ± 0.4	6.3 ± 1.8	10 ± 1.8	56 ± 15	>20	NA	17 ± 2.0	0.73	2.7
<b>N-benzylphenylethylamines (NBOMes)</b>									
25B-NBOMe	3.6 ± 0.3	0.0005 ± 0.0000	0.04 ± 0.01	28 ± 7	0.01 ± 0.01	19 ± 5	0.0062 ± 0.0022	7200	12
25C-NBOMe	5.0 ± 0.1	0.0007 ± 0.0002	0.15 ± 0.06	32 ± 2	0.10 ± 0.13	16 ± 5	0.0052 ± 0.0026	7143	7.4
25D-NBOMe	7.1 ± 0.5	0.0010 ± 0.0004	0.09 ± 0.03	27 ± 7	0.10 ± 0.07	22 ± 6	0.013 ± 0.004	7100	13
25E-NBOMe	3.5 ± 0.2	0.0006 ± 0.0001	0.16 ± 0.11	28 ± 15	0.06 ± 0.03	26 ± 10	0.0072 ± 0.0029	5833	12
25H-NBOMe	6.0 ± 0.7	0.0164 ± 0.0014	0.49 ± 0.07	38 ± 10	0.34 ± 0.14	11 ± 5	0.13 ± 0.02	366	7.9
25I-NBOMe	1.8 ± 0.3	0.0006 ± 0.0002	0.24 ± 0.12	27 ± 7	0.13 ± 0.08	32 ± 12	0.0046 ± 0.0020	3000	7.7
25N-NBOMe	4.2 ± 0.6	0.0008 ± 0.0002	0.07 ± 0.03	34 ± 3	0.07 ± 0.03	26 ± 14	0.021 ± 0.003	5250	26
25P-NBOMe	1.8 ± 0.1	0.0011 ± 0.0002	0.22 ± 0.11	42 ± 7	0.17 ± 0.13	23 ± 8	0.0060 ± 0.0015	1636	5.5
25T2-NBOMe	2.2 ± 0.2	0.0006 ± 0.0002	0.10 ± 0.03	38 ± 6	0.04 ± 0.04	31 ± 12	0.0065 ± 0.0006	3667	11
25T4-NBOMe	2.5 ± 0.3	0.0016 ± 0.0004	0.13 ± 0.05	46 ± 8	0.20 ± 0.10	27 ± 11	0.016 ± 0.005	1563	10
25T7-NBOMe	1.8 ± 0.2	0.0011 ± 0.0002	0.26 ± 0.16	41 ± 6	0.31 ± 0.23	14 ± 5	0.0064 ± 0.0013	1636	5.8
Mescaline-NBOMe	21 ± 5.7	0.14 ± 0.03	3.0 ± 0.6	33 ± 11	>20	NA	0.64 ± 0.04	147	4.5
<b>LSD</b>	0.0030 ± 0.0005	0.0042 ± 0.0013	0.26 ± 0.15	28 ± 10	12 ± 0.35	71 ± 31	0.015 ± 0.003	0.71	3.6

Values are K<sub>i</sub> given as μM (mean ± SD); NA, not assessed.

receptors (K<sub>i</sub> < 0.52 μM, with the exception of 2C-N and mescaline), although none exhibited the very high affinity of LSD. *N*-2-methoxybenzyl substitution decreased binding to 5-HT<sub>1A</sub> on average 17-fold (range: 2–86). The 2C drugs preferentially bound to 5-HT<sub>2A</sub> over 5-HT<sub>1A</sub> receptors with binding ratios of 14–94, with the exception of 2C-H and mescaline (Table 1). Receptor selectivity was markedly increased for 5-HT<sub>2A</sub> over 5-HT<sub>1A</sub> receptors for all of the compounds with *N*-2-methoxybenzyl substitution, with 5-HT<sub>2A</sub>/5-HT<sub>1A</sub> ratios >100 for 25H-NBOMe and mescaline-NBOMe and >1000 for all of the other NBOMe drugs.

### 3.2. Binding to monoamine receptors and transporters

Table 2 shows the binding affinities for monoamine receptors and transporters. Compared with the 2C drugs, the NBOMe analogs exhibited higher binding affinities for all receptors and transporters, with the exception of TAAR<sub>1</sub>. Specifically, all of the NBOMe drugs and LSD showed high-affinity binding to adrenergic α<sub>1A</sub> receptors (K<sub>i</sub> < 1 μM, with the exception of mescaline-NBOMe) and 19-fold (range: 11–38) higher binding affinity compared with the 2C drugs (not including mescaline). Most of the compounds also potentially bound to α<sub>2A</sub> receptors (K<sub>i</sub> < 1 μM, with the exception of 2C-H, 2C-N, and mescaline). *N*-2-methoxybenzyl substitution did not appreciably alter α<sub>2A</sub> receptor binding. LSD was the only substance that exhibited high-affinity binding to dopamine D<sub>1</sub>–D<sub>3</sub> receptors. Most of the 2C and NBOMe drugs showed low-affinity binding to D<sub>2</sub> receptors, and NBOMe drugs also showed low-affinity binding to D<sub>2</sub> and D<sub>3</sub> receptors. *N*-2-methoxybenzyl substitution also increased histamine H<sub>1</sub> receptor binding 65-fold (range: 2–267) compared with the 2C analogs, resulting in high-affinity binding for several NBOMe drugs (Table 2). All of the 2C and NBOMe drugs showed high-affinity binding to TAAR<sub>1rat</sub> (K<sub>i</sub> < 1 μM,

with the exception of mescaline, 25-H-NBOMe, 25-N-NBOMe, and mescaline-NBOMe). *N*-2-methoxybenzyl substitution decreased binding to TAAR<sub>1rat</sub> 4-fold (range: 2–9). Binding affinity to monoamine transporters was low for 2C drugs (K<sub>i</sub> > 10 μM). *N*-2-methoxybenzyl substitution increased binding to all monoamine transporters, resulting in low-affinity interactions for most of the NBOMe drugs (K<sub>i</sub> < 1–10 μM, with the exception of mescaline-NBOMe). LSD did not interact with any of the monoamine transporters.

### 3.3. Monoamine uptake transporter inhibition

IC<sub>50</sub> values for monoamine uptake inhibition are listed in Table 3. The 2C drugs did not inhibit or only very weakly inhibited (IC<sub>50</sub> > 10 μM) monoamine uptake. *N*-2-methoxybenzyl substitution consistently enhanced monoamine uptake inhibition potency approximately two- to 15-fold for the NET, two- to five-fold for the DAT, and two- to 26-fold for the SERT. As a result, 25B-NBOMe, 25C-NBOMe, 25D-NBOMe, 25E-NBOMe, 25H-NBOMe, and 25I-NBOMe blocked the NET and/or SERT at 5–10 μM concentrations. LSD did not inhibit any of the monoamine transporters.

### 3.4. Cytotoxicity

None of the compounds produced cytotoxicity after 4 h incubation at 37 °C, with the exception of 25T7-NBOMe. 25T7-NBOMe became toxic after 4 h incubation at 100 μM (but not 10 μM). Because the assays lasted less than 4 h, this toxicity did not affect the data.



**Table 2**  
Monoamine transporter and receptor-binding affinities.

	$\alpha_{1A}$	$\alpha_{2A}$	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	H <sub>1</sub>	TAAR <sub>1rat</sub>	TAAR <sub>1mouse</sub>	NET <sup>a</sup>	DAT <sup>b</sup>	SERT <sup>c</sup>
<b>2C-series</b>											
2C-B	8.2 ± 2.2	0.32 ± 0.01	12 ± 1.2	2.2 ± 0.3	10 ± 2.0	14 ± 0.5	0.09 ± 0.01	3.0 ± 0.3	31 ± 6.6	>30	9.7 ± 0.3
2C-C	13 ± 1.9	0.53 ± 0.06	13 ± 1.0	2.1 ± 0.4	17 ± 0.3	24 ± 0.9	0.11 ± 0.02	4.1 ± 0.3	>30	>30	24 ± 4.1
2C-D	12 ± 3.2	0.29 ± 0.03	24 ± 5.2	7.1 ± 1.7	>17	>25	0.15 ± 0.03	3.5 ± 0.1	>30	>30	31 ± 2.2
2C-E	7.4 ± 2.8	0.10 ± 0.02	15 ± 0.6	3.2 ± 1.0	19 ± 4.4	>25	0.07 ± 0.01	1.2 ± 0.1	33 ± 2.7	>30	29 ± 4.4
2C-H	7.9 ± 1.8	1.0 ± 0.05	>14	9.0 ± 1.5	>17	>25	0.90 ± 0.16	11 ± 2.2	>30	>30	>30
2C-I	5.1 ± 1.1	0.07 ± 0.01	13 ± 4.1	2.7 ± 0.58	5.0 ± 0.1	6.1 ± 0.5	0.12 ± 0.02	3.3 ± 0.1	15 ± 3.5	>30	4.9 ± 0.3
2C-N	>15	1.3 ± 0.2	19 ± 5.2	6.1 ± 2.7	20 ± 3.1	>25	0.34 ± 0.02	>20	>30	>30	32 ± 3.1
2C-P	3.5 ± 0.5	0.09 ± 0.01	8.4 ± 0.9	2.3 ± 0.7	5.2 ± 0.5	21 ± 3.2	0.02 ± 0.01	0.28 ± 0.03	18 ± 2.4	40 ± 4.0	19 ± 0.2
2C-T-2	17 ± 6.4	0.23 ± 0.01	15 ± 1.7	5.1 ± 1.0	11 ± 0.6	>25	0.04 ± 0.01	2.2 ± 0.6	>30	>30	13 ± 0.6
2C-T-4	11 ± 4.4	0.13 ± 0.04	20 ± 6.3	16 ± 2.1	19 ± 1.4	>25	0.05 ± 0.01	4.5 ± 0.9	17 ± 1.1	>30	>30
2C-T-7	13 ± 5.0	0.18 ± 0.001	15 ± 3.1	5.0 ± 0.8	7.5 ± 0.3	>25	0.03 ± 0.01	0.56 ± 0.1 2	27 ± 9.8	34 ± 6.2	12 ± 0.7
Mescaline	>15	1.4 ± 0.2	>14	>10	>17	>25	3.3 ± 0.5	11 ± 3.6	>30	>30	>30
<b>N-benzylphenylethylamines (NBOMes)</b>											
25B-NBOMe	0.43 ± 0.10	0.43 ± 0.03	9.3 ± 2.0	0.84 ± 0.27	2.7 ± 0.3	0.08 ± 0.02	0.28 ± 0.002	4.5 ± 1.7	1.1 ± 0.3	7.2 ± 0.5	0.84 ± 0.06
25C-NBOMe	0.81 ± 0.26	0.56 ± 0.08	12 ± 1.6	1.6 ± 0.4	3.5 ± 0.3	0.09 ± 0.01	0.52 ± 0.10	15 ± 1.9	1.6 ± 0.6	14 ± 3	1.5 ± 0.1
25D-NBOMe	0.70 ± 0.26	0.37 ± 0.05	8.7 ± 1.4	2.6 ± 0.4	6.4 ± 0.9	0.63 ± 0.06	0.81 ± 0.10	13 ± 4.4	2.2 ± 0.3	14 ± 2.4	1.4 ± 0.2
25E-NBOMe	0.53 ± 0.20	0.26 ± 0.07	4.9 ± 0.9	1.5 ± 0.2	3.2 ± 0.2	1.4 ± 0.2	0.26 ± 0.03	1.1 ± 0.3	3.0 ± 0.2	8.1 ± 0.6	1.7 ± 0.1
25H-NBOMe	0.55 ± 0.05	0.53 ± 0.04	14 ± 2.4	7.7 ± 1.7	20 ± 4.5	4.1 ± 0.4	1.4 ± 0.2	>20	5.5 ± 0.9	35 ± 1.7	2.3 ± 0.1
25I-NBOMe	0.37 ± 0.02	0.32 ± 0.01	6.7 ± 1.1	0.90 ± 0.13	2.1 ± 0.2	0.09 ± 0.01	0.44 ± 0.07	4.0 ± 0.8	1.3 ± 0.5	5.4 ± 0.5	1.0 ± 0.2
25N-NBOMe	0.85 ± 0.11	0.59 ± 0.07	18 ± 6.7	2.4 ± 0.1	4.5 ± 0.8	0.21 ± 0.04	2.2 ± 0.1	>20	7.2 ± 0.5	13 ± 1.2	5.1 ± 0.3
25P-NBOMe	0.31 ± 0.08	0.41 ± 0.07	3.1 ± 0.1	0.87 ± 0.08	2.3 ± 0.3	1.7 ± 0.2	0.06 ± 0.01	0.24 ± 0.03	2.8 ± 0.3	4.7 ± 0.4	5.2 ± 0.4
25T2-NBOMe	0.55 ± 0.17	0.45 ± 0.04	7.7 ± 0.4	1.6 ± 0.3	3.0 ± 0.4	0.49 ± 0.04	0.35 ± 0.02	4.2 ± 0.6	5.9 ± 0.4	8.6 ± 1.8	5.0 ± 0.2
25T4-NBOMe	0.58 ± 0.25	0.26 ± 0.03	4.9 ± 0.5	1.7 ± 0.5	1.9 ± 0.3	5.4 ± 0.3	0.12 ± 0.02	1.6 ± 0.4	4.3 ± 0.8	6.2 ± 1.5	8.1 ± 0.3
25T7-NBOMe	0.34 ± 0.06	0.36 ± 0.02	4.1 ± 0.2	1.0 ± 0.2	1.4 ± 0.2	1.2 ± 0.1	0.09 ± 0.03	1.0 ± 0.2	3.7 ± 1.1	4.8 ± 1.4	3.2 ± 0.2
Mescaline-NBOMe	3.0 ± 1.2	0.81 ± 0.05	>14	9.6 ± 2.6	>17	14 ± 1.2	13 ± 5.6	>20	46 ± 7.5	>30	24 ± 1.3
<b>LSD</b>	0.67 ± 0.18	0.012 ± 0.002	0.31 ± 0.1	0.025 ± 0.0004	0.096 ± 0.005	1.1 ± 0.2	0.45 ± 0.05	10 ± 2.9	>30	>30	>30

<sup>a</sup> Values are K<sub>i</sub> given as μM (mean ± SD). Comparative K<sub>i</sub> values for known monoamine transporter inhibitors were: 0.015 ± 0.01 μM for reboxetine at the NET.

<sup>b</sup> 0.06 ± 0.01 μM for methylphenidate at the DAT.

<sup>c</sup> 0.005 ± 0.001 μM for citalopram at the SERT.

**Table 3**  
Monoamine transporter inhibition.

	NET	DAT	SERT
	IC <sub>50</sub> [μM] (95% CI)	IC <sub>50</sub> [μM] (95% CI)	IC <sub>50</sub> [μM] (95% CI)
<b>2C-series</b>			
2C-B	44 (33–58)	231 (196–271)	18 (12–27)
2C-C	93 (64–137)	305 (243–383)	74 (58–95)
2C-D	45 (28–72)	626 (536–730)	77 (60–98)
2C-E	26 (18–37)	275 (221–343)	62 (52–74)
2C-H	125 (97–161)	857 (752–976)	311 (238–408)
2C-I	22 (16–31)	126 (103–155)	13 (10–16)
2C-N	287 (223–369)	>900	154 (112–213)
2C-P	94 (73–120)	198 (136–287)	30 (22–41)
2C-T-2	153 (152–154)	332 (332–332)	62 (62–62)
2C-T-4	134 (92–195)	294 (242–357)	113 (92–138)
2C-T-7	135 (115–163)	261 (210–324)	44 (36–52)
Mescaline	>900	841 (590–1200)	367 (291–462)
<b>N-benzylphenylethylamines (NBOMes)</b>			
25B-NBOMe	6.7 (5.6–8.1)	117 (89–154)	7.1 (5.7–8.8)
25C-NBOMe	5.9 (4.4–7.8)	70 (56–87)	7.3 (5.6–9.6)
25D-NBOMe	4.0 (3.0–5.3)	106 (81–140)	3.9 (2.6–5.7)
25E-NBOMe	11 (8.3–14)	100 (88–112)	8.3 (6.2–11)
25H-NBOMe	10 (7.8–13)	120 (101–144)	12 (9.7–14)
25I-NBOMe	10 (7.4–14)	65 (46–89)	6.8 (4.8–9.5)
25N-NBOMe	33 (25–44)	245 (194–310)	20 (15–26)
25P-NBOMe	14 (11–16)	82 (61–110)	12 (9.3–16)
25T2-NBOMe	25 (15–42)	67 (54–84)	20 (14–29)
25T4-NBOMe	28 (22–35)	58 (43–80)	14 (11–18)
25T7-NBOMe	34 (29–40)	55 (45–68)	17 (13–23)
Mescaline-NBOMe	89 (61–130)	449 (303–665)	85 (63–116)
<b>LSD</b>	>900	>900	>900
<b>Monoamine transporter inhibitors</b>			
Reboxetine	0.036 (0.030–0.044)	ns	ns
Methylphenidate	ns	0.12 (0.09–0.16)	ns
Citalopram	ns	ns	0.045 (0.037–0.057)

Values are means of three to four independent experiments and 95% confidence intervals (CI). ns, not shown.

#### 4. Discussion

We pharmacologically characterized the *in vitro* receptor interaction profiles of novel recreationally abused hallucinogenic *N*-2-methoxybenzyl-substituted phenethylamines compared with their 2C phenethylamine analogs. Both the NBOMe and 2C drugs potently interacted with serotonin 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub> receptors and TAAR<sub>1rat</sub>. We also found several consistent and potentially important structure-affinity relationships for the NBOMe drugs, their 2C analogs, and several targets. Specifically, *N*-2-methoxybenzyl substitution increased the binding affinity for and/or activation potency at serotonergic 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub> receptors, adrenergic  $\alpha_1$  receptors, dopaminergic D<sub>1-3</sub> receptors, histaminergic H<sub>1</sub> receptors, and monoamine transporters but reduced binding to 5-HT<sub>1A</sub> receptors and TAAR<sub>1</sub>.

The 5-HT<sub>2A</sub> receptor mediates hallucinogenic drug properties (Halberstadt and Geyer, 2011; Nelson et al., 1999; Nichols, 2004; Vollenweider et al., 1998) and is therefore considered the key target of hallucinogenic phenethylamines, including 2C and NBOMe drugs (Braden et al., 2006; Halberstadt, 2015; Halberstadt and Geyer, 2014). *N*-2-methoxybenzyl substitution consistently increased the already high *in vitro* affinity of 2C drugs for 5-HT<sub>2A</sub> receptors, in agreement with data on 25H-NBOMe and 25I-NBOMe vs. 2C-H and 2C-I, respectively (Braden et al., 2006; Heim, 2004). All of the NBOMe drugs exhibited low nanomolar or even subnanomolar affinity for 5-HT<sub>2A</sub> receptors, confirming studies on 25B-NBOMe, 25C-NBOMe, 25H-NBOMe, 25I-NBOMe, and 25B-NBOMe that used rat receptors (Braden et al., 2006; Ettrup et al., 2011, 2010; Nichols et al., 2015) or human receptors (Braden et al., 2006; Hansen et al., 2014; Nichols et al., 2015). Generally, 5-HT<sub>2A</sub> receptor affinity correlates with hallucinogenic drug potency in humans (Halberstadt, 2015; Titeler et al., 1988), and NBOMe drugs can be expected to be extremely potent hallucinogens *in vivo*. Indeed, higher incidences of hallucinations and delusions have been reported in patients with NBOMe compared with 2C drug intoxication (Forrester, 2013, 2014; Srisuma et al., 2015).

Surprisingly, the consistent six- to 100-fold increase in 5-HT<sub>2A</sub> receptor affinity that was produced by *N*-2-methoxybenzyl substitution did not translate into a similar increase in 5-HT<sub>2A</sub> receptor activation potency, and the activation efficacy was even reduced compared with the 2C drugs in our functional assay. In contrast, others found that *N*-2-methoxybenzyl substitution in 2C-H or 2C-I increased the potency for rat or human 5-HT<sub>2A</sub> receptor activation in the inositol phosphate hydrolysis assay *in vitro* (Braden et al., 2006). However, high-affinity agonist binding does not correlate well with inositol phosphate turnover (Acuna-Castillo et al., 2002; Roth et al., 1997), suggesting that additional ligand–receptor interactions contribute to receptor activation (Halberstadt, 2015; Nichols, 2004). Additionally, marked discrepancies between inositol phosphate hydrolysis activation and other *in vitro* assays and the *in vivo* effects of hallucinogens in laboratory animals or humans are well recognized (Nichols, 2004; Saez et al., 1994; Villalobos et al., 2004). Thus, although most of the effects of hallucinogens are clearly mediated by 5-HT<sub>2A</sub> receptor activation (Halberstadt, 2015; Nichols, 2004), the signaling pathways that mediate these effects have not yet been conclusively identified (Halberstadt, 2015).

Currently unknown pharmacokinetic characteristics of NBOMe drugs may also influence drug potency *in vivo*. For example, differences in the *in vivo* brain binding properties of *N*-2-methoxybenzyl-substituted positron emission tomography tracers were reported for substances with similar *in vitro* 5-HT<sub>2A</sub> receptor binding properties (Ettrup et al., 2011). Most importantly, NBOMe drugs are used recreationally at higher doses than LSD (Bersani et al., 2014; Halberstadt and Geyer, 2014), despite their higher 5-HT<sub>2A</sub> receptor binding affinities. The lower *in vivo* potency of

orally administered NBOMe drugs could be explained by their lower hepatic stability that reduced oral bioavailability compared with 2C drugs (Leth-Petersen et al., 2014). Thus, high 5-HT<sub>2A</sub> receptor binding or activation *in vitro* is only one factor that potentially predicts hallucinogen potency *in vivo*. In the first *in vivo* studies that evaluated NBOMe drugs in mice, 25I-NBOMe was 14-times more potent than its analog 2C-I in inducing 5-HT<sub>2A</sub> receptor-mediated head-twitch responses (Halberstadt and Geyer, 2014), consistent with the higher 5-HT<sub>2A</sub> receptor binding in the present study. In contrast, 25I-NBOMe was slightly less potent in inducing head twitches than expected, based on its high 5-HT<sub>2</sub> binding potency (Nichols et al., 2015) and compared with LSD (Halberstadt and Geyer, 2013, 2014), consistent with the similar 5-HT<sub>2A</sub> receptor activation potency of the two compounds in the present study but not reflecting the higher receptor binding potency of 25I-NBOMe compared with LSD. Additionally, 2-([2-(4-cyano-2,5-dimethoxyphenyl)ethylamino]-methyl)phenol (25CN-NBOH), which is structurally similar to the NBOMe drugs that were tested in the present study, was a more potent 5-HT<sub>2A</sub> receptor agonist than 2,5-dimethoxy-4-iodoamphetamine (DOI) *in vitro* (Hansen et al., 2014) but less effective in inducing head-twitch responses in mice (Fantegrossi et al., 2015). Thus, more *in vivo* studies are needed to determine the *in vivo* potency of novel NBOMe drugs.

Within the 2C or NBOMe drug series, para-phenyl substitutions compared with 2C-H or 25H-NBOMe, respectively, enhanced 5-HT<sub>2</sub> receptor binding and activation potency, which was expected based on previous studies (Blaazer et al., 2008; Eshleman et al., 2014; Hansen et al., 2014; Shulgin and Shulgin, 1991). Interestingly, 5-HT<sub>2A</sub> receptor activation potency increased with the size of the 4-substituent (2C-D < 2C-E < 2C-P) within the 2C series (Blaazer et al., 2008; Eshleman et al., 2014), whereas it decreased within the NBOMe series (25D-NBOMe > 25E-NBOMe > 25P-NBOMe). Similarly, activation potency increased with halogen size for the 4-halogen-substituted 2C drugs (2C-C < 2C-B < 2C-I) but not consistently for the NBOMe analogs. Thus, *N*-2-methoxybenzyl substitution interacted with 4-phenyl substitution to affect 5-HT<sub>2A</sub> receptor activation potency.

In the present study, all of the compounds were partial agonists at 5-HT<sub>2A</sub> receptors, but receptor activation efficacy was consistently decreased for the *N*-2-methoxybenzyl-substituted compounds in the assay used in the present study. The high 5-HT<sub>2A</sub> receptor affinity and reduction of partial activation efficacy of the NBOMe drugs suggest 5-HT<sub>2A</sub> antagonistic properties of these compounds, as similarly described for LSD (Nichols, 2004). In fact, 2C drugs have been shown to act as 5-HT<sub>2A</sub> receptor antagonists that inhibit 5-HT-induced currents in *Xenopus laevis* oocytes (Villalobos et al., 2004). Therefore, 5-HT<sub>2A</sub> receptor antagonism has been suggested to also play a role in the mechanism of action of hallucinogens (Villalobos et al., 2004). Alternatively, other receptors, such as 5-HT<sub>2C</sub> and 5-HT<sub>1</sub> receptors, may contribute to the mechanism of action of hallucinogens, or signaling pathways other than inositol phosphate hydrolysis may be involved (Nichols, 2004). Consistently, *N*-2-methoxybenzyl substitution increased binding affinity for 5-HT<sub>2C</sub> receptors. All of the NBOMe drugs very potently bound to 5-HT<sub>2C</sub> receptors, with only low (five- to 26-fold) selectivity for 5-HT<sub>2A</sub> receptors over 5-HT<sub>2C</sub> receptors in the binding assay, as previously shown for some NBOMe drugs (Ettrup et al., 2010; Hansen et al., 2014) and generally observed with hallucinogenic phenethylamines (Eshleman et al., 2014; Glennon et al., 1992). *N*-2-methoxybenzyl substitution only slightly increased 5-HT<sub>2A</sub> over 5-HT<sub>2C</sub> receptor binding selectivity. In contrast, *N*-2-methoxybenzyl substitution consistently decreased 5-HT<sub>1A</sub> receptor binding, thus markedly altering 5-HT<sub>1A</sub> over 5-HT<sub>2A</sub> receptor binding ratios for the NBOMe drugs compared with the 2C drugs.

Thus, NBOMe drugs are unlike LSD, which is a potent 5-HT<sub>1A</sub> receptor ligand and full agonist at 5-HT<sub>1A</sub> receptors (Nichols, 2004). Importantly, 5-HT<sub>1A</sub> receptors have been shown to contribute to the discriminative stimulus effects of some hallucinogens (Halberstadt, 2015; Nichols, 2004). Additionally, 5-HT<sub>1A</sub> antagonism markedly enhanced the hallucinogenic effects of DMT in humans (Strassman, 1996). Accordingly, 5-HT<sub>1A</sub> receptor stimulation has been hypothesized to counteract hallucinogenic activity (Halberstadt and Geyer, 2011; Nichols, 2004), and lower 5-HT<sub>1A</sub> receptor stimulation for the NBOMe drugs may further enhance their hallucinogenic drug properties. *N*-2-methoxybenzyl substitution increased 5-HT<sub>2B</sub> activation, but this is likely not relevant for the psychotropic properties of the NBOMe drugs (Blaazer et al., 2008). However, 5-HT<sub>2B</sub> receptors have been implicated in substance-induced heart valve fibrosis (Bhattacharyya et al., 2009; Setola et al., 2003), and the 2C and NBOMe drugs may therefore have cardiac toxicity if used chronically.

Because NBOMe drugs produce marked sympathomimetic cardiovascular effects in humans (Wood et al., 2015), we tested whether these drugs interact with monoamine transporters similarly to cocaine or amphetamines (Simmler et al., 2013, 2014a) and other novel psychoactive substances (Rickli et al., 2015a, 2015b; Simmler et al., 2014a; Simmler et al., 2014b). *N*-2-methoxybenzyl substitution enhanced monoamine transporter inhibition compared with the 2C drugs. However, the potency of even the most potent NBOMe drugs at the NET and SERT was low and only in the 5–10 μM range, indicating that amphetamine-type monoamine transporter interactions contribute only little to the cardio-stimulant effects of NBOMe drugs.

In addition to their very high 5-HT<sub>2A</sub> binding affinity, we found that the NBOMe drugs and LSD had high binding affinity for adrenergic α<sub>1A</sub> receptors. 2C drugs have been shown to contract blood vessels (Saez et al., 1994) through direct interactions with serotonergic 5-HT<sub>2</sub> and adrenergic α<sub>1</sub> receptors (Lobos et al., 1992). The vasoconstrictive potency of 2C drugs does not appear to correlate well with hallucinogenic potency in humans (Saez et al., 1994) or 5-HT<sub>2A</sub> receptor activation. For example, 2C-D had higher affinity for 5-HT<sub>2A</sub> receptors compared with 2C-H in the present study but lower potency in contracting the rat aorta (Saez et al., 1994). Additionally, 2C-N, which exhibited high affinity for 5-HT<sub>2A</sub> receptors but not α<sub>1</sub> receptors in the present study, did not present vasoconstrictive activity (Saez et al., 1994). These findings and the relatively high affinity of the NBOMe drugs for adrenergic α<sub>1</sub> receptors indicate that these receptors might contribute to the stimulant-type cardiovascular effects that are typically seen in cases of NBOMe drug intoxication (Srisuma et al., 2015; Wood et al., 2015). Additionally, the behavioral effects of 25I-NBOMe in mice showed a rapid peak (within minutes), whereas the response to 2C-I was relatively flat (Halberstadt and Geyer, 2014). Thus, such substance characteristics as the higher lipophilicity of NBOMe drugs may further accentuate the clinical drug response. As a result, there is likely a high risk of overdose with NBOMe drugs, and several fatalities have been reported (Hill et al., 2013; Srisuma et al., 2015; Walterscheid et al., 2014; Wood et al., 2015).

Both the 2C and NBOMe drugs bound to TAAR<sub>1</sub>, with few exceptions. *N*-2-methoxybenzyl substitution slightly decreased TAAR<sub>1</sub> binding affinity as previously shown for other *N*-substitutions in phenethylamines (Lewin et al., 2008). TAAR<sub>1</sub> modulates psychotropic drug actions. Importantly, methylenedioxymethamphetamine inhibits its own stimulant effects via TAAR<sub>1</sub> activation (Di Cara et al., 2011). Whether similar TAAR<sub>1</sub>-mediated “auto-inhibition” exists for hallucinogens remains to be determined. One hypothesis is that the lower TAAR<sub>1</sub> activity that is associated with *N*-2-methoxybenzyl substitution may also enhance psychostimulant drug properties *in vivo*.

LSD exhibited high affinity for D<sub>1</sub>, D<sub>2</sub> and D<sub>3</sub> receptors, as previously shown (Watts et al., 1995) and in contrast to phenethylamines. D<sub>2</sub> receptors have been shown to contribute to the interoceptive effects of LSD in rats (Halberstadt and Geyer, 2013, 2014). Although *N*-2-methoxybenzyl substitution increased D<sub>1-3</sub> receptor binding affinity compared with 2C drugs, NBOMe drugs were less potent at D<sub>1-3</sub> receptors compared with LSD, indicating that LSD has a unique mixed dopaminergic-serotonergic binding profile.

In summary, NBOMe drugs are highly potent 5-HT<sub>2A</sub> receptor ligands and partial 5-HT<sub>2A</sub> receptor agonists, similar to the classic hallucinogen LSD, but with 5-HT<sub>2</sub> over 5-HT<sub>1</sub> receptor selectivity, unlike LSD. NBOMe drugs bind to adrenergic α<sub>1</sub> receptors and TAAR<sub>1</sub>, similar to LSD, but do not bind to dopaminergic D<sub>1-3</sub> receptors, unlike LSD. The *in vitro* binding profiles of NBOMe drugs suggest that they have higher hallucinogenic effects and potency compared with their parent 2C drugs and are similar to the very potent hallucinogen LSD because of their similar or even higher potency at 5-HT<sub>2A</sub> receptors. At higher doses, NBOMe drugs may also exhibit additional stimulant properties through α<sub>1</sub> receptor interactions.

### Conflicts of interest

M.C.H. is an employee of F. Hoffmann-La Roche.

### Authorship contributions

*Participated in research design:* Rickli, Liechti.

*Conducted experiments:* Rickli, Luethi, Reinisch, Buchy.

*Performed data analysis:* Rickli, Hoener, Liechti.

*Wrote or contributed to the writing of the manuscript:* Rickli, Liechti.

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