

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

Anna Rickli,¹ Dino Luethi,¹ Julian Reinisch,¹ Danièle Buchy,² Marius C. Hoener,²
and Matthias E. Liechti^{1,*}

¹*Division of Clinical Pharmacology and Toxicology, Department of Biomedicine, University Hospital Basel and University of Basel, Basel, Switzerland;* ²*Neuroscience Research, pRED, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland*

*Corresponding author: Prof. Dr. med. Matthias E. Liechti, Division of Clinical Pharmacology and Toxicology, University Hospital Basel, Hebelstrasse 2, Basel, CH-4031, Switzerland. Tel: +41 61 328 68 68; Fax: +41 61 265 45 60; E-mail: matthias.liechti@usb.ch (M.E. Liechti)

Word counts:

Abstract: 246

References: 59

Tables: 3

Figures: 1

Running title: Pharmacology of NBOMes

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_{2A}) and 5-HT_{2B} 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 [TAAR₁], in which rat/mouse receptors were used).

Results: All of the compounds potently interacted with serotonergic 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} receptors and rat TAAR₁ (most K_i and EC₅₀: <1μM). The *N*-2-methoxybenzyl substitution of 2C drugs increased the binding affinity at serotonergic 5-HT_{2A}, 5-HT_{2C}, adrenergic α₁, dopaminergic D₁₋₃, and histaminergic H₁ receptors and monoamine transporters but reduced binding to 5-HT_{1A} receptors and TAAR₁. As a result, NBOMe drugs were very potent 5-HT_{2A} receptor agonists (EC₅₀: 0.04-0.5μM) with high 5-HT_{2A}/5-HT_{1A} selectivity and affinity for adrenergic α₁ receptors (K_i: 0.3-0.9μM) and TAAR₁ (K_i: 0.06-2.2μM), similar to LSD, but not dopaminergic D₁₋₃ receptors (most K_i: >1μM), unlike LSD.

Conclusion: The binding profile of NBOMe drugs predicts strong hallucinogenic effects, similar to LSD, but possibly more stimulant properties because of α₁ receptor interactions.

Keywords: phenethylamines, hallucinogens, novel psychoactive substances, receptor, affinity

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.

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; Ettrup et al., 2010). Recreationally used NBOMe drugs include 25I-NBOMe, 25C-NBOMe, 25B-NBOMe, and 25D-MBOMe (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). *N*-2-methoxybenzyl substitution enhances the potency of 2C drugs at serotonergic 5-hydroxytryptamine-2A (5-HT_{2A}) receptors, resulting in exceptionally potent 5-HT_{2A} 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-HT₂ 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; Ettrup et al., 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 α_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_{2A} receptor activation potencies because 5-HT_{2A} 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).

Methods

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. [³H]NE and [³H]DA were obtained from Perkin-Elmer (Schwerzenbach, Switzerland), and [³H]5-HT was obtained from Anawa (Zürich, Switzerland).

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 [TAAR₁]; (Revel et al., 2011)) were incubated with the radiolabeled selective ligands at concentrations equal to K_d , 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-[³H]-nisoxetine and indatraline (NET), [³H]citalopram and indatraline (SERT), [³H]WIN35,428 and indatraline (DAT), [³H]8-hydroxy-2-(di-*n*-propylamine)tetralin and indatraline (5-HT_{1A} receptor), [³H]ketanserin and spiperone (5-HT_{2A} receptor), [³H]mesulgerine and mianserin (5-HT_{2C} receptor), [³H]prazosin and risperidone (adrenergic α_1 receptor), [³H]rauwolscine and phentolamine (adrenergic α_2 receptor), [³H]SCH 23390 and butaclamol (D₁ receptor), [³H]spiperone and spiperone (D₂ and D₃ receptors), [³H]pyrilamine and clozapine, (histaminergic H₁ receptor), and [³H]RO5166017 and RO5166017 (TAAR₁). IC₅₀ 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_i (affinity) values, which correspond to the dissociation constants, were determined using the Cheng-Prusoff equation.

Activity at serotonin 5-HT_{2A} receptor

Human 5-HT_{2A} receptor-expressing NIH-3T3 cells were incubated in HEPES-Hank's Balanced Salt Solution (HBSS) buffer (70'000 cells/ 100 μ l) for 1 h at 37°C in 96-well poly-D-lysine-coated plates. To each well 100 μ 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 µl of the test substances diluted in HEPES-HBSS buffer containing 250 mM probenidol were added online. The increase in fluorescence was then measured. EC₅₀ 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%.

Activity at serotonin 5-HT_{2B} receptor

Human 5-HT_{2B} 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 dialysed 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 µl 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 µl 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 µl assay buffer was added. The plate was placed in a fluorescence imaging plate reader (FLIPR), and 25 µl of the test substances diluted in assay buffer was added online. The increase in fluorescence was then measured. EC₅₀ 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%.

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 [³H] 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 β -counter. Nonspecific uptake was determined for each experiment in the presence of 10 μ M fluoxetine for SERT cells, 10 μ M nisoxetine for NET cells, and 10 μ 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 IC₅₀ values were calculated using Prism software (GraphPad, San Diego, CA, USA).

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 μ 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 μ l of the supernatant per well was removed and combined

with 50 μ l of ToxiLight reagent, and luminescence was recorded using a Tecan Infinite 200 Pro plate reader (Tecan, Männedorf, Switzerland).

Results

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 ($K_i < 1 \mu\text{M}$, 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 ($EC_{50} < 1 \mu\text{M}$, 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 ($EC_{50} < 1 \mu\text{M}$, 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} receptors ($K_i < 0.52 \mu\text{M}$, 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_{1A} on average 17-fold (range: 2-86). The 2C drugs preferentially bound to 5-HT_{2A} over 5-HT_{1A} 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_{2A} over 5-HT_{1A} receptors for all of the compounds with *N*-2-methoxybenzyl substitution, with 5-HT_{2A}/5-HT_{1A} ratios > 100 for 25H-NBOMe and mescaline-NBOMe and > 1000 for all of the other NBOMe drugs.

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 TAAR₁. Specifically, all of the NBOMe drugs and LSD showed high-affinity binding to adrenergic α_{1A} receptors ($K_i < 1 \mu\text{M}$, 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 potently bound to α_{2A} receptors ($K_i < 1 \mu\text{M}$, with the exception of 2C-H, 2C-N, and mescaline). *N*-2-methoxybenzyl substitution did not appreciably alter α_{2A} receptor binding. LSD was the only substance that exhibited high-affinity binding to dopamine D₁-D₃ receptors. Most of the 2C and NBOMe drugs showed low-affinity binding to D₂ receptors, and NBOMe drugs also showed low-affinity binding to D₂ and D₃ receptors. *N*-2-methoxybenzyl substitution also increased histamine H₁ 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_{1rat} ($K_i < 1 \mu\text{M}$, with the exception of mescaline, 25-H-NBOMe, 25-N-NBOMe, and mescaline-NBOMe). *N*-2-methoxybenzyl substitution decreased binding to TAAR_{1rat} 4-fold (range: 2-9). Binding affinity to monoamine transporters was low for 2C drugs ($K_i > 10 \mu\text{M}$). *N*-2-methoxybenzyl substitution increased binding to all monoamine transporters, resulting in low-affinity interactions for most of the NBOMe drugs ($K_i < 1\text{-}10 \mu\text{M}$, with the exception of mescaline-NBOMe). LSD did not interact with any of the monoamine transporters.

Monoamine uptake transporter inhibition

IC₅₀ values for monoamine uptake inhibition are listed in Table 3. The 2C drugs did not inhibit or only very weakly inhibited (IC₅₀ > 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.

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.

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_{2A}, 5-HT_{2B}, 5-HT_{2C} receptors and TAAR_{1rat}. 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_{2A}, 5-HT_{2B}, 5-HT_{2C} receptors, adrenergic α₁ receptors, dopaminergic D₁₋₃ receptors, histaminergic H₁ receptors, and monoamine transporters but reduced binding to 5-HT_{1A} receptors and TAAR₁.

The 5-HT_{2A} 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_{2A} 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_{2A} 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; Ettrup et al., 2010; Nichols et al., 2015) or human receptors (Braden et al., 2006; Hansen et al., 2014; Nichols et al., 2015). Generally, 5-HT_{2A} 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_{2A} receptor affinity that was produced by *N*-2-methoxybenzyl substitution did not translate into a similar increase in 5-HT_{2A} 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_{2A} 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*

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_{2A} 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_{2A} 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_{2A} 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_{2A} 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_{2A} receptor-mediated head-twitch responses (Halberstadt and Geyer, 2014), consistent with the higher 5-HT_{2A} 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-HT₂ binding potency (Nichols et al., 2015) and compared with LSD (Halberstadt and Geyer, 2013, 2014), consistent with the similar 5-HT_{2A} 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_{2A} 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-HT₂ 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_{2A} 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 > 25-E-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_{2A} receptor activation potency.

In the present study, all of the compounds were partial agonists at 5-HT_{2A} 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_{2A} receptor affinity and reduction of partial activation efficacy of the NBOMe drugs suggest 5-HT_{2A} antagonistic properties of these compounds, as similarly described for LSD (Nichols, 2004). In fact, 2C drugs have been shown to act as 5-HT_{2A} receptor antagonists that inhibit 5-HT-induced currents in *Xenopus laevis* oocytes (Villalobos et al., 2004). Therefore, 5-HT_{2A} 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_{2C} and 5-HT₁ 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_{2C} receptors. All of the NBOMe drugs very potently bound to 5-HT_{2C} receptors, with only low (five- to 26-

fold) selectivity for 5-HT_{2A} receptors over 5-HT_{2C} 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_{2A} over 5-HT_{2C} receptor binding selectivity. In contrast, *N*-2-methoxybenzyl substitution consistently decreased 5-HT_{1A} receptor binding, thus markedly altering 5-HT_{1A} over 5-HT_{2A} receptor binding ratios for the NBOMe drugs compared with the 2C drugs. Thus, NBOMe drugs are unlike LSD, which is a potent 5-HT_{1A} receptor ligand and full agonist at 5-HT_{1A} receptors (Nichols, 2004). Importantly, 5-HT_{1A} receptors have been shown to contribute to the discriminative stimulus effects of some hallucinogens (Halberstadt, 2015; Nichols, 2004). Additionally, 5-HT_{1A} antagonism markedly enhanced the hallucinogenic effects of DMT in humans (Strassman, 1996). Accordingly, 5-HT_{1A} receptor stimulation has been hypothesized to counteract hallucinogenic activity (Halberstadt and Geyer, 2011; Nichols, 2004), and lower 5-HT_{1A} receptor stimulation for the NBOMe drugs may further enhance their hallucinogenic drug properties. *N*-2-methoxybenzyl substitution increased 5-HT_{2B} activation, but this is likely not relevant for the psychotropic properties of the NBOMe drugs (Blaazer et al., 2008). However, 5-HT_{2B} 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; Simmler et al., 2014a) and other novel psychoactive substances (Rickli et al., 2015a; Rickli et al., 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 cardiostimulant effects of NBOMe drugs.

In addition to their very high 5-HT_{2A} binding affinity, we found that the NBOMe drugs and LSD had high binding affinity for adrenergic α_{1A} receptors. 2C drugs have been shown to contract blood vessels (Saez et al., 1994) through direct interactions with serotonergic 5-HT₂ and adrenergic α_1 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_{2A} receptor activation. For example, 2C-D had higher affinity for 5-HT_{2A} 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_{2A} receptors but not α_1 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_1 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 TAAR₁, with few exceptions. *N*-2-methoxybenzyl substitution slightly decreased TAAR₁ binding affinity as previously shown for other *N*-substitutions in phenethylamines (Lewin et al., 2008). TAAR₁ modulates psychotropic drug actions. Importantly, methylenedioxymethamphetamine inhibits its own stimulant effects via TAAR₁ activation (Di Cara et al., 2011). Whether

similar TAAR₁-mediated “auto-inhibition” exists for hallucinogens remains to be determined. One hypothesis is that the lower TAAR₁ activity that is associated with *N*-2-methoxybenzyl substitution may also enhance psychostimulant drug properties *in vivo*.

LSD exhibited high affinity for D₁, D₂ and D₃ receptors, as previously shown (Watts et al., 1995) and in contrast to phenethylamines. D₂ 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 D₁₋₃ receptor binding affinity compared with 2C drugs, NBOMe drugs were less potent at D₁₋₃ receptors compared with LSD, indicating that LSD has a unique mixed dopaminergic-serotonergic binding profile.

In summary, NBOMe drugs are highly potent 5-HT_{2A} receptor ligands and partial 5-HT_{2A} receptor agonists, similar to the classic hallucinogen LSD, but with 5-HT₂ over 5-HT₁ receptor selectivity, unlike LSD. NBOMe drugs bind to adrenergic α₁ receptors and TAAR₁, similar to LSD, but do not bind to dopaminergic D₁₋₃ 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_{2A} receptors. At higher doses, NBOMe drugs may also exhibit additional stimulant properties through α₁ receptor interactions.

Conflict of interest

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

Acknowledgements

This work was supported by the Federal Office of Public Health (no. 13.006497) and Translational Medicine Hub Innovation Fund of F. Hoffmann-La Roche and the University of Basel. The authors thank Sylvie Chaboz for technical

assistance, Lipomed (Arlesheim, Switzerland) for providing the 2C and NBOMe drugs at no cost, and Michael Arends for text editing.

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

References

- Acuna-Castillo, C., Villalobos, C., Moya, P.R., Saez, P., Cassels, B.K., Huidobro-Toro, J.P., 2002. Differences in potency and efficacy of a series of phenylisopropylamine/phenylethylamine pairs at 5-HT_{2A} and 5-HT_{2C} receptors. *Br J Pharmacol* 136, 510-519.
- Armenian, P., Gerona, R.R., 2014. The electric Kool-Aid NBOMe test: LC-TOF/MS confirmed 2C-C-NBOMe (25C) intoxication at Burning Man. *Am J Emerg Med* 32, 1444.e3-1444.e5.
- Bersani, F.S., Corazza, O., Albano, G., Valeriani, G., Santacroce, R., Bolzan Mariotti Posocco, F., Cinosi, E., Simonato, P., Martinotti, G., Bersani, G., Schifano, F., 2014. 25C-NBOMe: preliminary data on pharmacology, psychoactive effects, and toxicity of a new potent and dangerous hallucinogenic drug. *Biomed Res Int* 2014, 734749.
- Bhattacharyya, S., Schapira, A.H., Mikhailidis, D.P., Davar, J., 2009. Drug-induced fibrotic valvular heart disease. *Lancet* 374, 577-585.
- Blaazer, A.R., Smid, P., Kruse, C.G., 2008. Structure-activity relationships of phenylalkylamines as agonist ligands for 5-HT_{2A} receptors. *ChemMedChem* 3, 1299-1309.

- Braden, M.R., Parrish, J.C., Naylor, J.C., Nichols, D.E., 2006. Molecular interaction of serotonin 5-HT_{2A} receptor residues Phe339^(6.51) and Phe340^(6.52) with superpotent *N*-benzyl phenethylamine agonists. *Mol Pharmacol* 70, 1956-1964.
- Crouch, S.P., Kozlowski, R., Slater, K.J., Fletcher, J., 1993. The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. *J Immunol Methods* 160, 81-88.
- Dean, B.V., Stellpflug, S.J., Burnett, A.M., Engebretsen, K.M., 2013. 2C or not 2C: phenethylamine designer drug review. *J Med Toxicol* 9, 172-178.
- Di Cara, B., Maggio, R., Aloisi, G., Rivet, J.M., Lundius, E.G., Yoshitake, T., Svenningsson, P., Brocco, M., Gobert, A., De Groote, L., Cistarelli, L., Veiga, S., De Montrion, C., Rodriguez, M., Galizzi, J.P., Lockhart, B.P., Coge, F., Boutin, J.A., Vayer, P., Verdouw, P.M., Groenink, L., Millan, M.J., 2011. Genetic deletion of trace amine 1 receptors reveals their role in auto-inhibiting the actions of ecstasy (MDMA). *J Neurosci* 31, 16928-16940.
- Eshleman, A.J., Forster, M.J., Wolfrum, K.M., Johnson, R.A., Janowsky, A., Gatch, M.B., 2014. Behavioral and neurochemical pharmacology of six psychoactive substituted phenethylamines: mouse locomotion, rat drug discrimination and in vitro receptor and transporter binding and function. *Psychopharmacology (Berl)* 231, 875-888.
- Ettrup, A., Hansen, M., Santini, M.A., Paine, J., Gillings, N., Palner, M., Lehel, S., Herth, M.M., Madsen, J., Kristensen, J., Begtrup, M., Knudsen, G.M., 2011. Radiosynthesis and in vivo evaluation of a series of substituted ¹¹C-phenethylamines as 5-HT_{2A} agonist PET tracers. *Eur J Nucl Med Mol Imaging* 38, 681-693.
- Ettrup, A., Palner, M., Gillings, N., Santini, M.A., Hansen, M., Kornum, B.R., Rasmussen, L.K., Nagren, K., Madsen, J., Begtrup, M., Knudsen, G.M., 2010. Radiosynthesis and evaluation of ¹¹C-CIMBI-5 as a 5-HT_{2A} receptor agonist radioligand for PET. *J Nucl Med* 51, 1763-1770.

- Fantegrossi, W.E., Gray, B.W., Bailey, J.M., Smith, D.A., Hansen, M., Kristensen, J.L., 2015. Hallucinogen-like effects of 2-([2-(4-cyano-2,5-dimethoxyphenyl)ethylamino]methyl)phenol (25CN-NBOH), a novel *N*-benzylphenethylamine with 100-fold selectivity for 5-HT_{2A} receptors, in mice. *Psychopharmacology (Berl)* 232, 1039-1047.
- Forrester, M.B., 2013. 2C series phenethylamine derivative exposures in Texas. *Subst Abus* 34, 81-82.
- Forrester, M.B., 2014. NBOMe designer drug exposures reported to Texas poison centers. *J Addict Dis* 33, 196-201.
- Glennon, R.A., Raghupathi, R., Bartyzel, P., Teitler, M., Leonhardt, S., 1992. Binding of phenylalkylamine derivatives at 5-HT_{1C} and 5-HT₂ serotonin receptors: evidence for a lack of selectivity. *J Med Chem* 35, 734-740.
- Halberstadt, A.L., 2015. Recent advances in the neuropsychopharmacology of serotonergic hallucinogens. *Behav Brain Res* 277, 99-120.
- Halberstadt, A.L., Geyer, M.A., 2011. Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. *Neuropharmacology* 61, 364-381.
- Halberstadt, A.L., Geyer, M.A., 2013. Characterization of the head-twitch response induced by hallucinogens in mice: detection of the behavior based on the dynamics of head movement. *Psychopharmacology (Berl)* 227, 727-739.
- Halberstadt, A.L., Geyer, M.A., 2014. Effects of the hallucinogen 2,5-dimethoxy-4-iodophenethylamine (2C-I) and superpotent *N*-benzyl derivatives on the head twitch response. *Neuropharmacology* 77, 200-207.
- Hansen, M., Phonekeo, K., Paine, J.S., Leth-Petersen, S., Begtrup, M., Brauner-Osborne, H., Kristensen, J.L., 2014. Synthesis and structure-activity relationships of *N*-benzyl phenethylamines as 5-HT_{2A/2C} agonists. *ACS Chem Neurosci* 5, 243-249.

- Heim, R., 2004. Synthesis and pharmacology of potent 5-HT_{2A} receptor agonists which have a partial N-2-2-methoxybenzyl structure. These, Department of Chemistry. Freie Universität Berlin, Germany, Berlin.
- Hill, S.L., Doris, T., Gurung, S., Katebe, S., Lomas, A., Dunn, M., Blain, P., Thomas, S.H., 2013. Severe clinical toxicity associated with analytically confirmed recreational use of 25I-NBOMe: case series. *Clin Toxicol* 51, 487-492.
- Hill, S.L., Thomas, S.H., 2011. Clinical toxicology of newer recreational drugs. *Clin Toxicol* 49, 705-719.
- Hysek, C.M., Simmler, L.D., Nicola, V., Vischer, N., Donzelli, M., Krähenbühl, S., Grouzmann, E., Hoener, M.C., Liechti, M.E., 2012. Duloxetine inhibits effects of MDMA ("ecstasy") in vitro and in humans in a randomized placebo-controlled laboratory study. *PLoS One* 7, e36476.
- Jensen, N.H., Rodriguiz, R.M., Caron, M.G., Wetsel, W.C., Rothman, R.B., Roth, B.L., 2008. *N*-desalkylquetiapine, a potent norepinephrine reuptake inhibitor and partial 5-HT_{1A} agonist, as a putative mediator of quetiapine's antidepressant activity. *Neuropsychopharmacology* 33, 2303-2312.
- Leth-Petersen, S., Bundgaard, C., Hansen, M., Carnerup, M.A., Kehler, J., Kristensen, J.L., 2014. Correlating the metabolic stability of psychedelic 5-HT_{2A} agonists with anecdotal reports of human oral bioavailability. *Neurochem Res* 39, 2018-2023.
- Lewin, A.H., Navarro, H.A., Mascarella, S.W., 2008. Structure-activity correlations for β -phenethylamines at human trace amine receptor 1. *Bioorg Med Chem* 16, 7415-7423.
- Lobos, M., Borges, Y., Gonzalez, E., Cassels, B.K., 1992. The action of the psychoactive drug 2C-B on isolated rat thoracic aorta. *Gen Pharmacol* 23, 1139-1142.

- Nagai, F., Nonaka, R., Satoh Hisashi Kamimura, K., 2007. The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. *Eur J Pharmacol* 559, 132-137.
- Nelson, D.L., Lucaites, V.L., Wainscott, D.B., Glennon, R.A., 1999. Comparisons of hallucinogenic phenylisopropylamine binding affinities at cloned human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors. *Naunyn Schmiedebergs Arch Pharmacol* 359, 1-6.
- Nichols, D.E., 2004. Hallucinogens. *Pharmacol Ther* 101, 131-181.
- Nichols, D.E., Frescas, S.P., Chemel, B.R., Rehder, K.S., Zhong, D., Lewin, A.H., 2008. High specific activity tritium-labeled *N*-(2-2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine (INBMeO): a high-affinity 5-HT_{2A} receptor-selective agonist radioligand. *Bioorg Med Chem* 16, 6116-6123.
- Nichols, D.E., Sassano, M.F., Halberstadt, A.L., Klein, L.M., Brandt, S.D., Elliott, S.P., Fiedler, W.J., 2015. *N*-Benzyl-5-methoxytryptamines as potent serotonin 5-HT₂ receptor family agonists and comparison with a series of phenethylamine analogues. *ACS Chem Neurosci* in press.
- Ninnemann, A., Stuart, G.L., 2013. The NBOMe series: a novel, dangerous group of hallucinogenic drugs. *J Stud Alcohol Drugs* 74, 977-978.
- Passie, T., Halpern, J.H., Stichtenoth, D.O., Emrich, H.M., Hintzen, A., 2008. The pharmacology of lysergic acid diethylamide: a review. *CNS Neurosci Ther* 14, 295-314.
- Poklis, J.L., Nanco, C.R., Troendle, M.M., Wolf, C.E., Poklis, A., 2014. Determination of 4-bromo-2,5-dimethoxy-N-[(2-methoxyphenyl)methyl]-benzeneethanamine (25B-NBOMe) in serum and urine by high performance liquid chromatography with tandem mass spectrometry in a case of severe intoxication. *Drug Test Anal* 6, 764-769.
- Revel, F.G., Moreau, J.L., Gainetdinov, R.R., Bradaia, A., Sotnikova, T.D., Mory, R., Durkin, S., Zbinden, K.G., Norcross, R., Meyer, C.A., Metzler, V., Chaboz, S.,

- Ozmen, L., Trube, G., Pouzet, B., Bettler, B., Caron, M.G., Wettstein, J.G., Hoener, M.C., 2011. TAAR1 activation modulates monoaminergic neurotransmission, preventing hyperdopaminergic and hypoglutamatergic activity. *Proc Natl Acad Sci U S A* 108, 8485-8490.
- Rickli, A., Hoener, M.C., Liechti, M.E., 2015a. Monoamine transporter and receptor interaction profiles of novel psychoactive substances: para-halogenated amphetamines and pyrovalerone cathinones. *Eur Neuropsychopharmacol* 25, 365-376.
- Rickli, A., Kopf, S., Hoener, M.C., Liechti, M.E., 2015b. Pharmacological profile of novel psychoactive benzofurans. *Br J Pharmacol* 172, 3412-3425.
- Rose, S.R., Poklis, J.L., Poklis, A., 2013. A case of 25I-NBOMe (25-I) intoxication: a new potent 5-HT_{2A} agonist designer drug. *Clin Toxicol* 51, 174-177.
- Roth, B.L., Choudhary, M.S., Khan, N., Uler, A.Z., 1997. High-affinity agonist binding is not sufficient for agonist efficacy at 5-hydroxytryptamine_{2A} receptors: evidence in favor of a modified ternary complex model. *J Pharmacol Exp Ther* 280, 576-583.
- Saez, P., Borges, Y., Gonzalez, E., Cassels, B.K., 1994. α -Adrenergic and 5-HT₂-serotonergic effects of some β -phenylethylamines on isolated rat thoracic aorta. *Gen Pharmacol* 25, 211-216.
- Setola, V., Hufeisen, S.J., Grande-Allen, K.J., Vesely, I., Glennon, R.A., Blough, B., Rothman, R.B., Roth, B.L., 2003. 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. *Mol Pharmacol* 63, 1223-1229.
- Shulgin, A.T., Shulgin, A., 1991. PiHKAL - A chemical love story. Berkeley: Transform Press.

- Simmler, L.D., Buser, T.A., Donzelli, M., Schramm, Y., Dieu, L.H., Huwyler, J., Chaboz, S., Hoener, M.C., Liechti, M.E., 2013. Pharmacological characterization of designer cathinones in vitro. *Br J Pharmacol* 168, 458-470.
- Simmler, L.D., Rickli, A., Hoener, M.C., Liechti, M.E., 2014a. Monoamine transporter and receptor interaction profiles of a new series of designer cathinones. *Neuropharmacology* 79, 152-160.
- Simmler, L.D., Rickli, A., Schramm, Y., Hoener, M.C., Liechti, M.E., 2014b. Pharmacological profiles of aminoindanes, piperazines, and pipradrol derivatives. *Biochem Pharmacol* 88, 237-244.
- Srisuma, S., Bronstein, A.C., Hoyte, C.O., 2015. NBOMe and 2C substitute phenylethylamine exposures reported to the National Poison Data System. *Clin Toxicol* in press.
- Stellpflug, S.J., Kealey, S.E., Hegarty, C.B., Janis, G.C., 2014. 2-(4-Iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25I-NBOMe): clinical case with unique confirmatory testing. *J Med Toxicol* 10, 45-50.
- Strassman, R.J., 1996. Human psychopharmacology of *N,N*-dimethyltryptamine. *Behav Brain Res* 73, 121-124.
- Tatsumi, M., Groshan, K., Blakely, R.D., Richelson, E., 1997. Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur J Pharmacol* 340, 249-258.
- Titeler, M., Lyon, R.A., Glennon, R.A., 1988. Radioligand binding evidence implicates the brain 5-HT₂ receptor as a site of action for LSD and phenylisopropylamine hallucinogens. *Psychopharmacology (Berl)* 94, 213-216.
- Villalobos, C.A., Bull, P., Saez, P., Cassels, B.K., Huidobro-Toro, J.P., 2004. 4-Bromo-2,5-dimethoxyphenethylamine (2C-B) and structurally related phenylethylamines are potent 5-HT_{2A} receptor antagonists in *Xenopus laevis* oocytes. *Br J Pharmacol* 141, 1167-1174.

- Vollenweider, F.X., Vollenweider-Scherpenhuyzen, M.F., Babler, A., Vogel, H., Hell, D., 1998. Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *Neuroreport* 9, 3897-3902.
- Walterscheid, J.P., Phillips, G.T., Lopez, A.E., Gonsoulin, M.L., Chen, H.H., Sanchez, L.A., 2014. Pathological findings in 2 cases of fatal 25I-NBOMe toxicity. *Am J Forensic Med Pathol* 35, 20-25.
- Watts, V.J., Lawler, C.P., Fox, D.R., Neve, K.A., Nichols, D.E., Mailman, R.B., 1995. LSD and structural analogs: pharmacological evaluation at D1 dopamine receptors. *Psychopharmacology (Berl)* 118, 401-409.
- Wood, D.M., Sedefov, R., Cunningham, A., Dargan, P.I., 2015. Prevalence of use and acute toxicity associated with the use of NBOMe drugs. *Clin Toxicol* 53, 85-92.
- Zuba D, S.K., 2012. Analytical characterization of three hallucinogenic *N*-(2-methoxy)benzyl derivatives of the 2C-series of phenethylamine drugs. *Drug Test Anal* 5, 634-645.

Figure Legend

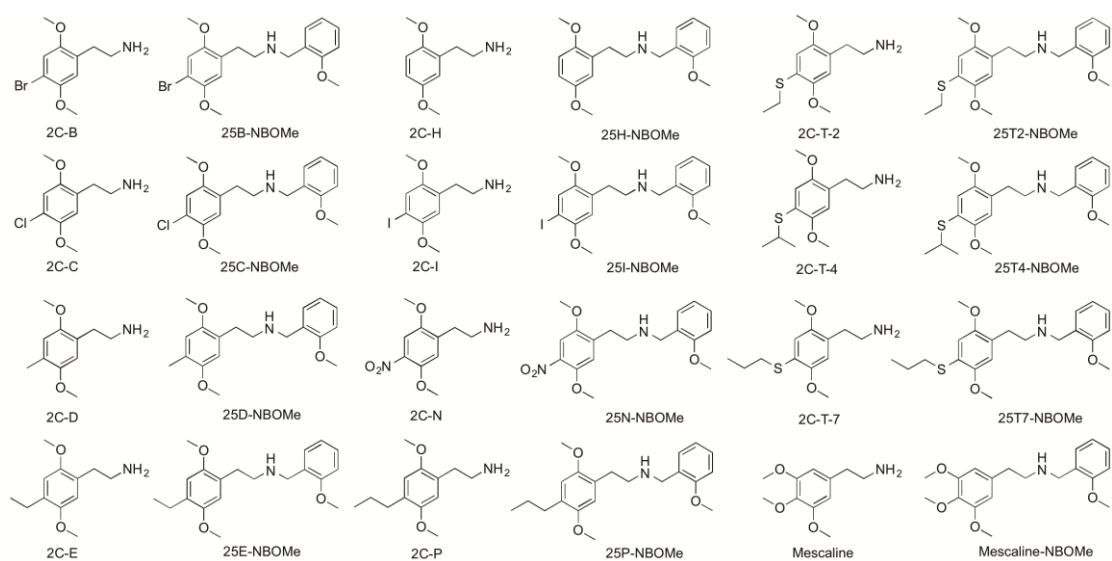


Figure 1. Chemical structures of 2,5-dimethoxyphenethylamines (2C drugs) and their *N*-2-methoxybenzyl-substituted analogs (NBOME drugs).

Table 1. Serotonin receptor interactions

	5-HT _{1A}	5-HT _{2A}		5-HT _{2B}		5-HT _{2C}	Selectivity (binding ratios)		
	receptor binding K _i ± SD [μM]	receptor binding K _i ± SD [μM]	activation potency EC ₅₀ ± SD [μM]	activation efficacy % maximum ± SD	activation potency EC ₅₀ ± SD [μM]	activation efficacy % maximum ± SD	receptor binding K _i ± SD [μM]	5-HT _{2A} /5-HT _{1A}	5-HT _{2A} /5-HT _{2C}
2Cs									
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
N-benzylphenylethylamines (NBOMes)									
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
LSD	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_i given as μM (mean ± SD); NA, not assessed

Table 2. Monoamine transporter and receptor-binding affinities

	a_{1A}	a_{2A}	D ₁	D ₂	D ₃	H ₁	TAAR _{1rat}	TAAR _{1mouse}	NET ^a	DAT ^b	SERT ^c
2C-series											
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 ± 0.12	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
N-benzylphenylethylamines (NBOMes)											
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
LSD	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

Values are K_i given as μM (mean ± SD). Comparative K_i values for known monoamine transporter inhibitors were: 0.015 ± 0.01 μM for reboxetine at the NET^a, 0.06 ± 0.01 μM for methylphenidate at the DAT^b, and 0.005 ± 0.001 μM for citalopram at the SERT^c.

Table 3. Monoamine transporter inhibition

	NET	DAT	SERT
	IC ₅₀ [μM] (95% CI)	IC ₅₀ [μM] (95% CI)	IC ₅₀ [μM] (95% CI)
2C-series			
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)
N-benzylphenylethylamines (NBOMes)			
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)
LSD	>900	>900	>900
Monoamine transporter inhibitors			
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.