Pharmacological profile of novel psychoactive benzofurans

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Word counts: abstract: 243; main text: 4417

References: 64

Tables: 4

Figures: 3

Author contribution
A Rickli and ME Liechti designed the research study
A Rickli, S Kopf, and MC Hoener performed the research
A Rickli, MC Hoener, and ME Liechti analysed the data
A Rickli and ME Liechti wrote the paper
Abstract

Background and purpose: Benzofurans are newly used psychoactive substances, but their pharmacology is unknown. The aim of the present study was to pharmacologically characterize benzofurans in vitro.

Experimental approach: We assessed the effects of the benzofurans 5-APB, 5-APDB, 6-APB, 6-APDB, 4-APB, 7-APB, 5-EAPB, and 5-MAPDB and benzodifuran 2C-B-FLY on the human noradrenaline (NA), dopamine (DA), and serotonin (5-HT) uptake transporters using HEK 293 cells that express the respective transporters. We also investigated the release of NA, DA, and 5-HT from monoamine-preloaded cells, monoamine receptor binding affinity, and 5-HT\textsubscript{2A} and 5-HT\textsubscript{2B} receptor activation.

Key results: All of the benzofurans inhibited NA and 5-HT uptake more than DA uptake, similar to methylenedioxyethylamphetamine (MDMA) and unlike methamphetamine. All of the benzofurans also released monoamines and interacted with trace amine-associated receptor 1 (TAAR\textsubscript{1}), similar to classic amphetamines. Most benzofurans were partial 5-HT\textsubscript{2A} agonists similar to MDMA, but also 5-HT\textsubscript{2B} receptor agonists, unlike MDMA and methamphetamine. The benzodifuran 2C-B-FLY very potently interacted with 5-HT\textsubscript{2} receptors and also bound to TAAR\textsubscript{1}.

Conclusions and implications: Despite very similar structures, differences were found in the pharmacological profiles of the different benzofurans and compared with their amphetamine analogues. Benzofurans acted as indirect monoamine agonists that interact with transporters similarly to MDMA. The benzofurans also interacted with serotonergic receptors. This pharmacological profile likely results in MDMA-like entactogenic psychoactive properties. However, benzofurans produce 5-HT\textsubscript{2B} receptor activation associated with heart valve fibrosis. The pharmacology of 2C-B-FLY indicates predominant hallucinogenic properties and a risk for vasoconstriction.

Keywords: Novel psychoactive substances, benzofuran, pharmacology, monoamine transporter, receptor
**Abbreviations:** 5-APB, 5-(2-aminopropyl)benzofuran; 6-APB, 6-(2-aminopropyl)benzofuran; 5-APDB, 5-(2-aminopropyl)-2,3-dihydrobenzofuran; 6-APDB, 6-(2-aminopropyl)-2,3-dihydrobenzofuran; 4-APB, 4-(2-aminopropyl)benzofuran; 7-APB, 7-(2-aminopropyl)benzofuran; bromo-dragonFLY, 1-(8-bromobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminoprop-ane; β-keto-MDA, β-keto-3,4-methylenedioxyamphetamine; 2C-B-fly, 8-bromo-2,3,6,7-benzo-dihydro-difuran-ethylamine; DA, dopamine; DAT, DA transporter; 5-EAPB, 5-(2-ethylaminopropyl)benzofuran; 5-HT, 5-hydroxytryptamine or serotonin; 5-MAPDB, 1-(2,3-dihydrobenzofuran-5-yl)-N-methylpropan-2-amine; MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxymethamphetamine; NA, noradrenaline; NET, noradrenaline transporter; SERT, serotonin transporter; TAAR, trace amine-associated receptor.
Introduction

Novel psychoactive substances are newly used designer drugs ("internet drugs," “research chemicals,” “legal highs”) that potentially pose similar health risks to classic illicit substances. In recent years, the number of newly detected psychoactive substances on the illicit drug market has dramatically increased. In the European Union, 41 novel psychoactive substances were identified for the first time in 2010, 49 were identified in 2011, 73 were identified in 2012, and 81 were identified in 2013 within the European Early Warning System (EMCDDA, 2014).

Benzofurans are a group of novel psychoactive substances (King, 2014) of particular interest because they are structurally very similar to the popular recreational drug 3,4-methylenedioxymethamphetamine (MDMA) and its active metabolite 3,4-methylenedioxymethamphetamine (MDA; (Greene, 2013). 5-(2-Aminopropyl)benzofuran (5-APB) and 6-(2-aminopropyl)benzofuran (-APB) are benzofuran analogues of MDA (Fig. 1). 5-(2-Aminopropyl)-2,3-dihydrobenzofuran (5-APDB) and 6-(2-aminopropyl)-2,3-dihydrobenzofuran (6-APDB) are dihydrobenzofuran analogues (Fig. 1) that were originally synthesized for research purposes (Monte et al., 1993). 4-(2-Aminopropyl)benzofuran (4-APB) and 7-(2-aminopropyl)benzofuran (7-APB) are positional isomers of 5-APB and 6-APB. 1-(2,3-Dihydrobenzofuran-5-yl)-N-methylpropan-2-amine (5-MAPDB) is a dihydrobenzofuran analogue of MDMA, and 5-(2-ethylaminopropyl)benzofuran (5-EAPB) is a benzofuran analogue of MDMA but with an N-ethyl group (Fig. 1).

5-APB and 6-APB appeared on the drug market in 2010-2011 (Chan et al., 2013; Jebadurai et al., 2013; Stanczuk et al., 2013; Archer et al., 2014; Elliott et al., 2014; King, 2014), with reports of intoxication (Chan et al., 2013; Greene, 2013; Jebadurai et al., 2013; Seetohul et al., 2013). 4-APB was first reported to the EMCDDA in 2010 (King, 2014) and is typically detected in products that are sold as 6-APB as byproduct (Stanczuk et al., 2013; Strano Rossi et al., 2014). Users report that the effects of 5-APB and 6-APB are comparable to MDMA but more intense (Greene, 2013; Jebadurai et al., 2013). Adverse effects include nausea, sympathomimetic stimulation, and agitation (Chan et al., 2013; Greene, 2013). 5-
APDB and 6-APDB were first reported to the EMCDDA in 2012, and another three benzofurans, including 5-EAPB, were first reported in 2013 (King, 2014). Presently, no published studies have reported the psychotropic and toxic effects of these benzofurans, but 5-APDB, 6-APDB, and 5-EAPB are being discussed in drug user forums (Bluelight, 2013a; Bluelight, 2013b; Drugs-Forum, 2013). Little is known about the pharmacology of benzofurans. 5-APB and 6-APB have been shown to inhibit the human dopamine (DA), noradrenaline (NA), and serotonin (5-hydroxytryptamine [5-HT]) transporters (DAT, NET, and SERT, respectively; (Iversen et al., 2013) and are agonists at the rat 5-HT2A receptor (Dawson et al., 2014) and human and rat 5-HT2B receptor (Iversen et al., 2013; Dawson et al., 2014). Additionally, fast cyclic voltammetry experiments in rat brain slices indicated that 5-APB releases DA at high concentrations (Dawson et al., 2014). 5-APDB and 6-APDB also inhibited the monoamine transporters with greater affinity for the SERT over the DAT compared with MDA in crude rat synaptosome preparations (Monte et al., 1993).

The benzodifurans 8-bromo-2,3,6,7-benzo-dihydro-difuran-ethylamine (2C-B-FLY) and 1-(8-bromobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminoprop-ane (bromo-dragonFLY) are known as “fly” drugs because of their chemical structures (Fig. 1). A series of benzodifurans were originally synthesized to study 5-HT2A receptor function (Monte et al., 1997; Parker et al., 1998; Chambers et al., 2001). The recreational use of 2C-B-FLY and bromo-dragonFLY began to be reported in 2007 (Andreasen et al., 2009; Greene, 2013; King, 2014), and there are case reports of severe agitation, hallucinations, seizures, and fatalities associated with bromo-dragonFLY (Andreasen et al., 2009; Wood et al., 2009; Nielsen et al., 2010). 2C-B-FLY and bromo-dragonFLY are potent 5-HT2A agonists (Monte et al., 1996; Chambers et al., 2001), but interactions with other monoamine receptors and their transporters have not been tested.

Systematic evaluations of the pharmacological profiles of benzofurans are lacking. We determined the potencies of a series of benzofurans and the benzodifuran 2C-B-FLY to inhibit the DAT, NET, and SERT and tested transporter-mediated monoamine release in vitro. We also characterized the binding profiles at monoamine receptors and assessed 5-
HT$_{2A}$ and 5-HT$_{2B}$ receptor activation. The 5-HT$_{2A}$ receptor mediates hallucinogenic effects (Nichols, 2004), and the 5-HT$_{2B}$ receptor has been implicated in drug-associated endocardial fibrosis (Roth, 2007). MDMA, MDA, β-keto-MDA, and methamphetamine were included as comparator substances.

**Methods**

**Drugs**

MDMA, MDA, β-keto-MDA, methamphetamine, and 2C-B-Fly were obtained from Lipomed (Arlesheim, Switzerland). 6-APB, 6-APDB, 5-APB, 5-APDB, 4-APB, 7-APB, and 5-MAPDB were obtained from Cayman Chemicals (Ann Arbor, MI, USA). 5-EAPB was obtained from the Forensic Institute (Zurich, Switzerland). All of the drugs were used as racemic hydrochloride salts. Purity was at least 98% for all of the substances, with the exception of 2C-B-Fly, whose purity was approximately 95% as determined by HPLC.

**Monoamine uptake transport inhibition**

Inhibition of the human NET, DAT, and SERT was assessed in human embryonic kidney (HEK) 293 cells that were stably transfected with the transporters as specified previously (Hysek et al., 2012c). Briefly, the cells were suspended in uptake buffer. We incubated the cells for 10 min with different concentrations of the test compounds and then added the corresponding [$^3$H]monoamine (5 nM final concentration) at room temperature. After 10 min, we stopped uptake by separating the cells from the buffer using centrifugation through silicone oil (Hysek et al., 2012c). 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 μM fluoxetine for SERT cells, 10 μM nisoxetine for NET cells, and 10 μM mazindol for DAT cells and subtracted from the total counts to yield specific uptake (100%). DAT:SERT inhibition ratios were calculated as 1/DAT IC$_{50}$:1/SERT IC$_{50}$. Higher relative potency at the
DAT indicates a higher abuse potential, whereas relatively increased activity of the 5-HT system is linked to a reduction of abuse potential and more MDMA-like psychotropic effects (Wee et al., 2005). Stimulant amphetamines, such as methamphetamine, have a DAT:SERT inhibition ratio > 10, whereas MDMA and other substances with MDMA-like psychotropic effects have a DAT:SERT inhibition ratio close to 0.1 (Baumann et al., 2012; Simmler et al., 2013; Simmler et al., 2014a; Simmler et al., 2014b).

Transporter-mediated monoamine release

We studied the effects of a single high dose (100 µM) of the test compounds on transporter-mediated NE, 5-HT, and DA efflux in HEK 293 cells that overexpressed the respective human monoamine transporter as previously reported in detail (Simmler et al., 2013). Briefly, adherent cells were incubated with the respective radiolabeled monoamine (10 nM [3H]NA and 10 µM unlabelled NA, 10 nM [3H]DA and 1 µM unlabelled DA, and 10 nM [3H]5-HT) for 20 min at 37°C. We then washed the cells twice with buffer and added 1 ml of buffer that contained the test compound (100 µM final concentration). We stopped [3H]5-HT and [3H]DA release after 15 min and [3H]NA release after 45 min by washing twice with ice-cold buffer. We quantified the radioactivity that remained in the cells. Nonspecific "pseudo-efflux," which arises from nonspecific substrate release and subsequent reuptake inhibition (Scholze et al., 2000), was assessed for each experiment using the transporter inhibitors nisoxetine (NET cells), citalopram (SERT cells), and mazindol (DAT cells) at 10 µM as negative control conditions.

Radioligand binding assays

The radioligand binding assays were performed as described previously (Hysek et al., 2012c; Simmler et al., 2013). Briefly, membrane preparations of HEK 293 cells (Invitrogen, Zug, Switzerland) that overexpress the respective transporters (Tatsumi et al., 1997) or receptors (human genes, except rat and mouse genes for TAAR1; Revel et al., 2011) were incubated with the radiolabeled selective ligands at concentrations equal to KD.
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 determined in the presence of selected competitors in excess. The following radioligands and competitors, respectively, were used: \(N\)-methyl-[\(^3\)H]-nisoxetine and indatraline (NET), [\(^3\)H]citalopram and indatraline (SERT), [\(^3\)H]WIN35,428 and indatraline (DAT), [\(^3\)H]8-hydroxy-2-(di-n-propylamino)tetralin and indatraline (5-HT\(_{1A}\) receptor), [\(^3\)H]ketanserin and spiperone (5-HT\(_{2A}\) receptor), [\(^3\)H]mesulergine and mianserin (5-HT\(_{2C}\) receptor), [\(^3\)H]prazosin and risperidone (\(\alpha_1\) adrenergic receptor), [\(^3\)H]rauwolscine and phentolamine (\(\alpha_2\) adrenergic receptor), [\(^3\)H]SCH 23390 and butaclamol (D\(_1\) receptor), [\(^3\)H]spiperone and spiperone (D\(_2\) and D\(_3\) receptors), [\(^3\)H]pyrilamine and clozapine (histaminergic H\(_1\) receptor), and [\(^3\)H]RO5166017 and RO5166017 (TAAR\(_1\)).

**Functional serotonin 5-HT\(_{2A}\) and 5-HT\(_{2B}\) activity**

The 5-HT\(_{2B}\) receptor functional assay was performed as described previously (Jensen et al., 2008). Briefly, human 5-HT\(_{2B}\) receptor-expressing HEK 293 cells were incubated at 37°C in 96-well plates coated with poly-D-lysine. 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 Hank’s Buffered Saline Solution (Gibco) and 20 mM HEPES (assay buffer; Gibco) using an EMLA 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. Efficacy (maximal activity) is expressed relative to the activity of 5-HT, which was used as a control set to 100%.
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 for measuring cytolysis (Crouch et al., 1993; Hysek et al., 2012c; Felser et al., 2014). Cells grown in 96-well plates were exposed to the compounds at a high concentration of 100 μM. All test conditions contained dimethyl sulfoxide (DMSO) 0.1% (v:v) which is non-toxic and was also used as negative control. Triton™ X-100 (0.1%) lyses cells and was used as positive control. After 4 h of incubation at 37°C 10 μl of supernatant per well was removed and combined with 50 μl of ToxiLight™ reagent and luminescence recorded using a Tecan Infinite™ 200 Pro (Tecan, Männedorf, Switzerland) plate reader.

Statistical analyses

The uptake transporter inhibition data were fit by non-linear regression to variable-slope sigmoidal dose-response curves, and IC$_{50}$ values were calculated using Prism software (GraphPad, San Diego, CA, USA). Analysis of variance followed by the Holm-Sidak test was used to compare compound-induced release with the negative controls. Substances that induced significantly higher monoamine efflux compared with the negative control were considered monoamine releasers. IC$_{50}$ values for radioligand binding were determined by calculating nonlinear 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. EC$_{50}$ values for the 5-HT$_2$ receptor activation were derived from the concentration-response curves using nonlinear regression.

Results

Monoamine uptake transporter inhibition
Uptake inhibition curves are depicted in Fig. 2, and the corresponding IC<sub>50</sub> values and DAT:SERT inhibition ratios are listed in Table 1. All of the benzofurans inhibited the NET at submicromolar concentrations, similar to MDMA, MDA, and methamphetamine. All of the benzofurans were weak DAT inhibitors compared with methamphetamine and more similar to MDMA, which was also a weak DAT inhibitor. Only 5-APB, 6-APB, and 5-EAPB were more potent at the DAT compared with MDA and MDMA. In contrast, the dihydrobenzofurans 5-APDB, 6-APDB, and 5-MAPDB were inactive at the DAT (IC<sub>50</sub> > 30 μM). 5-APB, 5-APDB, 6-APB, and 5-EAPB inhibited the SERT at submicromolar concentrations and more potently than MDMA. 6-APDB and 5-MAPDB inhibited the SERT in the 1-3 micromolar concentration range, similar to MDMA. 4-APB and 7-APB exhibited low potency at the SERT, more similar to methamphetamine. The DAT:SERT inhibition ratio for all of the benzofurans was low, consistent with greater serotonergic vs. dopaminergic activity that is overall similar to MDMA. The dihydrobenzofurans (5-APDB, 6-APDB, and 5-MAPDB) and 5-APB exhibited the lowest DAT:SERT inhibition ratios (lower than MDMA). In contrast, 4-APB and 7-APB exhibited the highest DAT:SERT inhibition ratios, consistent with their low potency at the SERT and showing a profile that is between MDMA and methamphetamine with regard to serotonergic vs. dopaminergic activity. In terms of structure-activity relationships, the dihydro-compounds 5-APDB and 6-APDB had similar noradrenergic and serotonergic activities compared with their analogues 5-APB and 6-APB but were markedly less potent at the DAT. The monoamine transporter inhibition potencies of the positional isomers 4-APB and 7-APB were reduced, particularly for the SERT, compared with their analogues 5-APB and 6-APB. Additionally, the oxygen in the para-position for 5-APB and 5-APDB resulted in higher absolute and relative potency at the SERT compared with 6-APB and 6-APDB, respectively. Beta-keto-substitution in the β-keto-MDA vs. MDA structures increased dopaminergic vs. serotonergic activity. The benzodifuran 2C-B-FLY was inactive at all of the monoamine transporters (IC<sub>50</sub> > 50 μM).

*Monoamine release*
At high concentrations, all of the benzofurans released at least one of the monoamines through the respective monoamine transporter, similar to the amphetamines (Fig. 3). In contrast, 2C-B-FLY was not a monoamine releaser.

**Binding affinities**

The benzofurans interacted with the monoamine transporters but also with several monoamine receptors (Table 2 and 3). All of the benzofurans exhibited submicromolar affinity for TAAR₁, except for 5-EAPB, which was inactive at mouse TAAR₁. Benzofurans showed mostly higher potency at TAAR₁ than the classic amphetamines. All of the benzomonofurans exhibited binding affinities for the 5-HT₂ₐ receptor in the micromolar range (0.8-3.4 μM). Functionally, most of them acted as low-potency partial agonists similar to MDMA and MDA but unlike methamphetamine. Most of the benzofurans were also partial agonists at the 5-HT₂₉ receptor. In contrast MDMA and methamphetamine did not stimulate 5-HT₂₉ receptors. With the exception of 7-APB and 5-EAPB, the benzofurans exhibited submicromolar binding affinities at the 5-HT₂₄ receptor. Binding potencies at the 5-HT₁ receptor varied among the different benzofurans. Only 7-APB showed submicromolar binding affinity. Potent binding to most of the assessed 5-HT receptor subtypes distinctly discriminated the benzofurans from the pharmacological profiles of their related amphetamines, which exhibited no or low 5-HT₁ₐ affinity and did not bind to 5-HT₂₉ or 5-HT₂₄ receptors except for MDA with a Kᵢ value of 3 μM at 5-HT₂₄. Most of the benzofurans bound to α₁- and α₂-adrenergic receptors in the 3-12 μM and 0.1-6 μM ranges, respectively. There was no binding to DA receptors and only low-affinity binding to histamine H₁ receptors (> 10 μM for most of the drugs). The benzodifuran 2C-B-FLY did not bind to the monoamine transporters but interacted with all of the receptors tested in the present study and particularly exhibited high affinity for TAAR₁ and all of the 5-HT₂ receptors. Importantly, 2C-B-FLY was a very potent agonist at the 5-HT₂ₐ receptor. 2C-B-FLY thus exhibited a pharmacological profile that was distinct from the mono-benzofurans and related amphetamines.
Cytotoxicity

None of the investigated compounds produced cytotoxicity, thus confirming cell integrity during the functional assays in this study.

Discussion

We determined the in vitro pharmacological profiles of new benzofurans that are recreationally abused compared with their well-known amphetamine analogues. The benzofurans blocked monoamine transporters and induced transporter-mediated monoamine release similarly to MDMA. More than MDMA and methamphetamine, the benzofurans also directly stimulated adrenergic and 5-HT receptors. The benzodifuran 2C-B-FLY was a potent agonist at 5-HT\textsubscript{1A}, 5-HT\textsubscript{2A}, 5-HT\textsubscript{2B}, and 5-HT\textsubscript{2C} receptors, consistent with the reported hallucinogenic properties of 2C-B-FLY.

Monoamine uptake transporter inhibition and monoamine release

All of the benzofurans inhibited the NET at submicromolar concentrations, similar to MDMA, MDA, and methamphetamine. Noradrenaline mediates sympathomimetic stimulation (Hysek et al., 2011), and this finding predicts the cardiostimulant and psychostimulant properties of these benzofurans, similar to MDMA and methamphetamine. Unlike the relatively constant NET inhibition, the potencies of the benzofurans to inhibit the DAT and SERT notably varied, resulting in DAT:SERT inhibition ratios that ranged from 0.01 to 0.65. Specifically, the dihydrobenzofurans 5-APDB and 5-MAPDB exhibited the highest preference for the SERT vs. DAT (more selective than MDMA), followed by 5-APB, 6-APDB, and 5-EAPB, which exhibited a DAT:SERT inhibition ratio similar to MDMA. With DAT:SERT ratios of 0.46 and 0.65, 4-APB and 7-APB were the benzofurans with the most dopaminergic profiles and were relatively more dopaminergic than MDMA. Stimulants like methamphetamine exhibit a DAT:SERT ratio > 10, whereas MDMA and other entactogens exhibit a DAT:SERT ratio of 0.01-1 (Simmler et al., 2013; Liechti, 2014b; Simmler et al., 2014a; Simmler et al., 2014b).
Accordingly, based on their DAT:SERT inhibition ratios, all of the benzofurans can be expected to produce MDMA-like entactogenic subjective effects in humans. In contrast to the benzofurans, the benzodifuran 2C-B-FLY blocked monoamine transporters only at very high concentrations but had high affinity for 5-HT receptors. Thus, monoamine transporter inhibition is unlikely to contribute to the mechanism of action of 2C-B-FLY as is also the case for the structurally similar substance 2C-B and related compounds of the 2C phenethylamine series containing methoxy-groups at positions 2 and 5 of the benzene ring (Acuna-Castillo et al., 2002; Hill et al., 2011; Eshleman et al., 2014).

Only a few other studies determined the monoamine transporter inhibition profiles of some of the benzofurans. Consistent with our findings, 5-APDB and 6-APDB inhibited the SERT more potently than the DAT in rat synaptosomes (Monte et al., 1993). The oxygen in the para position in the 5-APDB and 5-APB structures enhanced the serotonergic vs. dopaminergic properties compared with 6-APDB and 6-APB, respectively, as shown in the present study and previously for 5-APDB vs. 6-APDB in rat synaptosomes (Monte et al., 1993). Drug discrimination studies in rats showed that 5-APDB and 6-APDB substituted for MDMA-like serotonergic drugs but not the more dopaminergic stimulant amphetamine (Monte et al., 1993). These behavioural findings support our hypothesis that 5-APDB and 6-APDB produce subjective effects that are similar to MDMA, and entactogenic effects have been reported by users (Bluelight, 2013a; Bluelight, 2013b; Drugs-Forum, 2013). The monoamine transporter inhibition profiles for 5-APB and 6-APB were determined in one previous study (Iversen et al., 2013). In contrast to our results, this study showed that 5-APB and 6-APB inhibited the DAT more potently than the SERT (Iversen et al., 2013). However, MDMA did not show the serotonergic preference that is typically reported by others (Rothman et al., 2001; Han et al., 2006; Hysek et al., 2012c; Simmler et al., 2013). Consistent with the present results, the inhibition profiles for 5-APB and 6-APB were similar to MDMA and unlike methamphetamine (Iversen et al., 2013). The reinforcing effects of benzofurans have not yet been studied in drug self-administration studies. There is a decrease in reinforcing potency and efficacy among monoamine releasing agents when 5-
HT releasing potency is increased relative to DA (Wee et al., 2005). The relatively serotonergic in vitro properties of the benzofurans would indicate lower addictive properties (Wee et al., 2005; Liechti, 2014b), more similar to MDMA, which is not a strong reinforcer in self-administration studies (Lamb et al., 1987; Cole et al., 2003), than to methamphetamine.

All of the benzofurans also released 5-HT, NA, and DA through their respective transporters, similar to their amphetamine analogues and other amphetamine derivatives (Simmler et al., 2013; Simmler et al., 2014a; Simmler et al., 2014b). Dopamine release has also been previously documented for 5-APB in voltammetric studies of rat brain slices (Dawson et al., 2014). In contrast to the benzofurans, 2C-B-FLY did not release monoamines. Our release assay was designed to qualitatively assess monoamine release because we used only one high concentration of the substances to induce transporter-mediated monoamine efflux. Additional studies that include the assessment of transporter-mediated ionic currents and in vivo microdialysis could be useful to further characterize and quantify monoamine release and its contribution to the mechanism of action of the benzofurans.

Receptor binding profiles

The present study found several relevant high-potency interactions between the benzofurans and various monoamine receptors. 6-APB, 6-APDB, 4-APB, 7-APB, and 2C-B-FLY all bound to α2A-adrenergic receptors, which are known to modulate NA release and sympathomimetic activity (Hysek et al., 2012a). As expected (Monte et al., 1996), 2C-B-FLY potently interacted with 5-HT2 receptors. Specifically, 2C-B-FLY potently bound to the human 5-HT2A receptor (Kᵢ = 0.01 μM), consistent with the previously documented nanomolar affinity for rat cortical 5-HT2A receptors (Monte et al., 1996). Even higher-potency binding to 5-HT2A receptors has been shown for the benzodifuran bromo-dragonFLY in rat (Monte et al., 1996; Chambers et al., 2001) and human (Monte et al., 1996) 5-HT2A receptors. In the present study, 2C-B-FLY was also a very potent functional 5-HT2A receptor agonist. 2C-B-FLY resembles the structures of the 2C series phenethylamines, which are
also potent 5-HT$_{2A}$ receptor agonist (Nelson et al., 1999; Acuna-Castillo et al., 2002; Hansen et al., 2014).

Consistent with the predicted lysergic acid diethylamide (LSD)-like properties of substances with high 5-HT$_{2A}$ receptor affinity, both 2C-B-FLY and bromo-dragonFLY completely substituted for LSD in drug discrimination studies (Monte et al., 1996). The affinity of 2C-B-FLY for the 5-HT$_{1A}$ receptor was relatively low, which has also been shown for rat 5-HT$_{1A}$ receptors (Monte et al., 1996). The 5-HT$_{2A}$ receptor is thought to mediate the alterations in perception induced by hallucinogens (Vollenweider et al., 1998; Nelson et al., 1999; Nichols, 2004) and therefore is likely the key target in the mechanism of action of benzodifuran hallucinogens. Interestingly, some of the benzofurans also exhibited micromolar affinity for the 5-HT$_{2A}$ receptor and were low-potency 5-HT$_{2A}$ receptor partial agonists similar to MDMA and MDA, but in contrast to methamphetamine. Binding to 5-HT$_{2A}$ receptors at micromolar concentrations has also been previously shown for 5-APB and 6-APB (Iversen et al., 2013). 5-APB also constricted rat aorta via 5-HT$_{2A}$ agonist action (Dawson et al., 2014). Thus, some benzofurans could have hallucinogenic properties because of 5-HT$_{2A}$ receptor stimulation, in addition to their MDMA-like entactogenic subjective effects. Psychosis and hallucinations have been reported after the use of 6-APB (Chan et al., 2013; Greene, 2013). However, in drug discrimination studies, 5-APDB and 6-APDB did not substitute for LSD in rats (Monte et al., 1993), consistent with their lower binding affinity compared with 5-APB and 6-APB. In terms of clinical toxicity, the 5-HT$_{2A}$ agonist and possible $\alpha_1$-adrenergic agonist action could enhance the risk for vasoconstriction, hyperthermia, and hypertension. Both $\alpha_1$ and 5-HT$_{2A}$ receptors are implicated in substance-induced vasoconstriction (Blessing et al., 2003; Docherty et al., 2010; Dawson et al., 2014) and associated hypertension (Hysek et al., 2013) and hyperthermia (Liechti et al., 2000; Hysek et al., 2012b; Liechti, 2014a) in humans. In fact, hypertension, hyperpyrexia, and cases with severe limb ischemia have been reported after use of bromo-dragonFLY (Thorlaciuss et al., 2008; Wood et al., 2009; Nielsen et al., 2010), a benzodifuran structurally similar to 2C-B-FLY. Direct agonist actions at the 5-HT$_{2A}$ receptor compared with an indirect action via 5-HT release can also be expected to result in
longer-lasting effects as described for serotonergic hallucinogens (Schmid et al., 2014) and compared with MDMA (Hysek et al., 2012c).

In humans, MDMA is mainly inactivated by O-demethylation but also N-demethylated to the minor but active metabolite MDA (Hysek et al., 2013). Similarly, 5-MAPDB and 5-EAPB are N-dealkylated (Welter et al., 2014) to 5-APDB and 5-APB, respectively. As shown in the present study, the N-dealkylated substances MDA, 5-APDB, and 5-APB activate 5-HT\textsubscript{2A} and 5-HT\textsubscript{2B} receptors more potently and also more potently bind to 5-HT\textsubscript{2C} receptors than their parent compounds MDMA, 5-MAPDB, and 5-EAPB, respectively. Thus, the formation of active metabolites likely adds enhanced 5-HT\textsubscript{2A} and 5-HT\textsubscript{2B}-receptor-associated toxicity in these cases.

2C-B-FLY and several of the benzofurans acted as partial agonists at the 5-HT\textsubscript{2B} receptor as previously shown for 5-APB (Iversen et al., 2013; Dawson et al., 2014) and 6 APB (Iversen et al., 2013). In contrast, no such 5-HT\textsubscript{2B} agonist properties were observed for the classic amphetamines MDMA and methamphetamine in the present study. 5-HT\textsubscript{2B} receptors have been implicated in substance-induced heart valve fibrosis (Setola et al., 2003; Bhattacharyya et al., 2009). 5-HT\textsubscript{2B} receptor activation by 2C-B-FLY, 5-APB, 6-APB, 6-APDB, and 7-APB occurred at submicromolar concentrations that are likely present when these drugs are used by drug users to induce subjective effects.

All of the benzofurans bound to TAAR\textsubscript{1}, many at even higher potency than MDMA or methamphetamine. MDMA and methamphetamine inhibit their own neurochemical and locomotor stimulant effects via TAAR\textsubscript{1} activation (Di Cara et al., 2011). Similar TAAR\textsubscript{1}-mediated “auto-inhibition” may therefore modulate the effects of benzofurans. In contrast, for cathinones (e.g., β-keto-amphetamines), more stimulant-like and addictive properties would be expected based on their lower affinity for TAAR\textsubscript{1} compared with their amphetamine analogues (Simmler et al., 2013; Simmler et al., 2014a).

In terms of structure-activity relationships, 3,4-substitution on the benzene ring (methylenedioxy-group in MDMA and MDA, furans, or dihydrofurans) strongly reduced the DAT/SERT inhibition ratio confirming previous studies (Nichols, 1994; Han et al., 2006;
Iversen et al., 2013; Simmler et al., 2013). Additionally, the DAT/SERT inhibition ratio depended on the position of the oxygen on the benzene ring and was lowest for compounds with the oxygen in the para-(4)-position and highest for those with the oxygen in the ortho-(2)-position. This finding was consistent with the high serotonergic activity of other para-substituted amphetamines (Nichols, 1994; Rickli et al., 2015). The dihydrobenzofurans (5-APDB, 6-APDB, and 5-MAPDB) exhibited reduced monoamine transporter inhibition potency in particular at the DAT resulting in relatively more serotonergic properties compared to their furan analogues. N-alkylation (MDMA, 5-MAPDB, 5-EAPB, methamphetamine) moderately reduced activity at 5-HT$_{2A/B}$ receptors and binding at 5-HT$_{2C}$ receptors. This has previously been shown for other phenethylamines for simple N-alkylation (e.g., methyl, ethyl) (Nelson et al., 1999). N-alkylation had no relevant effect on the interactions with the monoamine transporter as previously noted for related amphetamines (Nichols, 1994). 2,5-substitution on the benzene ring strongly increased activity at the 5-HT$_2$ receptors and reduced interactions with the monoamine transporters as seen in 2C-B-FLY in the present study and many other 2,5-substituted phenethylamines (2C series) (Hill et al., 2011; Eshleman et al., 2014). Transporter inhibition potency was also moderately reduced when the oxygen was in the ortho-(2)-position (similar to the 2 series) at the benzene ring as in 7-APB compared with 5-APB, 6-APB, or 4-APB. Beta-keto-substitution increased dopaminergic vs. serotonergic activity extending previous similar findings (Simmler et al., 2013; Simmler et al., 2014a).

**Conclusions**

Benzofurans are monoamine transporter blockers and monoamine releasers, similar to MDMA, but they also interact with serotonergic receptors. This mechanism of action predicts psychotropic and clinical toxicological effects that are similar to the entactogen MDMA but with additional hallucinogenic properties. The benzodifuran 2C-B-FLY is a potent hallucinogen, likely also associated with a risk for clinical complications related to vasoconstriction (e.g., ischemia and hypertension). Although structure-activity relationships
exist, the present study showed that structurally very similar compounds may exhibit distinct pharmacological profiles, illustrating the need for pharmacotoxicological profiling of each novel psychoactive substance.

**Conflict of interest**

None.

**Acknowledgements**

This work was supported by the Federal Office of Public Health (no. 13.006497) and the Translational Medicine Hub Innovation Fund of F. Hoffmann-La Roche and the University of Basel. The authors thank Daniele Buchy and Sylvie Chaboz for technical assistance, Linda Simmler for critical comments on the manuscript, Lipomed (Arlesheim, Switzerland) for providing 2C-B-Fly at no cost, and Michael Arends for text editing.
References


Jensen NH, Rodriguiz RM, Caron MG, Wetsel WC, Rothman RB, Roth BL (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(10): 2303-2312.


Figure 1. Chemical structures of benzofurans and related amphetamines.
Figure 2. Monoamine uptake transporter inhibition. Concentration-response curves show the uptake inhibition of $[^3]$H]NA, $[^3]$H]DA, and $[^3]$H]5-HT in HEK 293 cells transfected with the respective monoamine transporter. The data are expressed as the mean ± SEM of 3-4 independents experiments. Curves were fitted to the data with non-linear regression. The corresponding IC$_{50}$ values are shown in Table 1.
Figure 3. Monoamine release. Monoamine release was induced by a high concentration of the compound (100 µM) after preloading the transporter-transfected cells with the respective radiolabeled monoamine. All of the benzofurans released NA, DA, and 5-HT similarly to methamphetamine and MDMA. In contrast, the benzodifuran 2CB-FLY was not a monoamine releaser. Transporter blockers induced nonspecific “pseudo-efflux” (horizontal dashed line, open bars), which arises from substrate that diffuses out of the cells and from subsequent reuptake inhibition. Compounds that produced significantly more monoamine efflux (*P < 0.05, **P < 0.01, ***P < 0.001) compared with the respective non-releasing uptake inhibitors (negative controls, open bars) were considered monoamine releasers. The data are expressed as the mean ± SEM of 3-4 independent experiments.
Table 1. Monoamine transporter inhibition.

<table>
<thead>
<tr>
<th>NET</th>
<th>DAT</th>
<th>SERT</th>
<th>DAT/SERT inhibition ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ [µM] (95% CI)</td>
<td>IC₅₀ [µM] (95% CI)</td>
<td>IC₅₀ [µM] (95% CI)</td>
<td>Ratio (95% CI)</td>
</tr>
</tbody>
</table>

**Benzofurans**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>5-APB</td>
<td>0.16 (0.08-0.3)</td>
<td>6.1 (4-9)</td>
<td>0.29 (0.17-0.5)</td>
<td>0.05 (0.02-1.2)</td>
</tr>
<tr>
<td>5-APDB</td>
<td>0.29 (0.2-0.5)</td>
<td>49 (33-73)</td>
<td>0.58 (0.4-0.9)</td>
<td>0.01 (0.005-0.03)</td>
</tr>
<tr>
<td>6-APB</td>
<td>0.19 (0.1-0.3)</td>
<td>3.3 (2.4-4.5)</td>
<td>0.93 (0.7-1.3)</td>
<td>0.29 (0.16-0.54)</td>
</tr>
<tr>
<td>6-APDB</td>
<td>0.56 (0.4-0.8)</td>
<td>33 (25-43)</td>
<td>2.3 (1.4-3.9)</td>
<td>0.07 (0.03-0.16)</td>
</tr>
<tr>
<td>5-MAPDB</td>
<td>0.96 (0.5-1.7)</td>
<td>77 (62-96)</td>
<td>1.2 (0.7-2)</td>
<td>0.02 (0.01-0.03)</td>
</tr>
<tr>
<td>4-APB</td>
<td>0.24 (0.2-0.3)</td>
<td>12 (9-16)</td>
<td>5.5 (3.4-8.7)</td>
<td>0.46 (0.21-1.0)</td>
</tr>
<tr>
<td>7-APB</td>
<td>0.27 (0.2-0.3)</td>
<td>20 (16-26)</td>
<td>13 (9-18)</td>
<td>0.65 (0.35-1.1)</td>
</tr>
<tr>
<td>5-EAPB</td>
<td>0.56 (0.4-0.7)</td>
<td>4.9 (3-8)</td>
<td>0.72 (0.5-1.1)</td>
<td>0.15 (0.07-0.35)</td>
</tr>
</tbody>
</table>

**Benzodifuran**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>2C-B-FLY</td>
<td>94 (72-124)</td>
<td>187 (161-217)</td>
<td>73 (58-92)</td>
<td>0.39 (0.27-0.57)</td>
</tr>
</tbody>
</table>

**Related amphetamines**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>MDMA</td>
<td>0.36 (0.2-0.6)</td>
<td>16.7 (16.3-17)</td>
<td>2.4 (1.4-3.0)</td>
<td>0.14 (0.08-0.18)</td>
</tr>
<tr>
<td>MDA</td>
<td>0.42 (0.3-0.6)</td>
<td>20.5 (20.3-20.6)</td>
<td>4.9 (3.5-6.8)</td>
<td>0.24 (0.17-0.33)</td>
</tr>
<tr>
<td>beta-keto-MDA</td>
<td>1.6 (1.1-2.3)</td>
<td>14 (10-18)</td>
<td>21 (15-28)</td>
<td>1.5 (0.8-2.8)</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>0.14 (0.09-0.2)</td>
<td>0.87 (0.84-0.91)</td>
<td>13.6 (13.5-13.8)</td>
<td>15.6 (14.8-16.4)</td>
</tr>
</tbody>
</table>

Values are means of three to four independent experiments and 95% confidence intervals (CI).

DAT/SERT inhibition ratio = 1/DAT IC₅₀ : 1/SERT IC₅₀.
Table 2. Monoamine transporter and receptor binding affinities.

<table>
<thead>
<tr>
<th></th>
<th>NET</th>
<th>DAT</th>
<th>SERT</th>
<th>1A</th>
<th>1A</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>H1</th>
<th>TAAR1rat</th>
<th>TAAR1mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benzofurans</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5-APB</td>
<td>3.1 ± 0.2</td>
<td>2.6 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>3.5 ± 0.5</td>
<td>2.9 ± 0.1</td>
<td>&gt; 14</td>
<td>&gt; 10</td>
<td>&gt; 17</td>
<td>8.4 ± 0.8</td>
<td>0.04 ± 0.01</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>5-APDB</td>
<td>28 ± 5</td>
<td>&gt; 30</td>
<td>4.0 ± 0.3</td>
<td>11 ± 3</td>
<td>4.2 ± 0.5</td>
<td>&gt; 14</td>
<td>&gt; 10</td>
<td>&gt; 17</td>
<td>21 ± 2</td>
<td>0.49 ± 0.05</td>
<td>0.77 ± 0.06</td>
</tr>
<tr>
<td>6-APB</td>
<td>1.8 ± 0.4</td>
<td>0.60 ± 0.05</td>
<td>12 ± 1</td>
<td>7.3 ± 3.4</td>
<td>0.38 ± 0.02</td>
<td>&gt; 14</td>
<td>&gt; 10</td>
<td>&gt; 17</td>
<td>15 ± 2</td>
<td>0.05 ± 0.02</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>6-APDB</td>
<td>18 ± 1</td>
<td>&gt; 30</td>
<td>23 ± 1</td>
<td>&gt; 15</td>
<td>0.65 ± 0.07</td>
<td>&gt; 14</td>
<td>&gt; 10</td>
<td>&gt; 17</td>
<td>&gt; 25</td>
<td>1.0 ± 0.04</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>5-MAPDB</td>
<td>26 ± 5</td>
<td>&gt; 30</td>
<td>6.3 ± 0.6</td>
<td>4.9 ± 1.5</td>
<td>6.4 ± 1.8</td>
<td>23 ± 3</td>
<td>&gt; 10</td>
<td>&gt; 17</td>
<td>4.9 ± 0.1</td>
<td>0.67 ± 0.09</td>
<td>3.5 ± 0.1</td>
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<tr>
<td>4-APB</td>
<td>3.9 ± 0.5</td>
<td>7.4 ± 0.6</td>
<td>7.7 ± 0.5</td>
<td>12 ± 3</td>
<td>0.87 ± 0.22</td>
<td>&gt; 14</td>
<td>&gt; 10</td>
<td>&gt; 17</td>
<td>16 ± 1</td>
<td>0.11 ± 0.02</td>
<td>2.08 ± 0.14</td>
</tr>
<tr>
<td>7-APB</td>
<td>5.3 ± 0.1</td>
<td>14 ± 2</td>
<td>14 ± 1</td>
<td>9.6 ± 2.4</td>
<td>0.14 ± 0.02</td>
<td>&gt; 14</td>
<td>8.2 ± 3.2</td>
<td>&gt; 17</td>
<td>25 ± 5</td>
<td>0.07 ± 0.01</td>
<td>0.13 ± 0.02</td>
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<tr>
<td>5-EPAPB</td>
<td>1.0 ± 0.3</td>
<td>0.34 ± 0.02</td>
<td>0.52 ± 0.03</td>
<td>3.3 ± 0.5</td>
<td>2.7 ± 0.7</td>
<td>16 ± 3</td>
<td>&gt; 10</td>
<td>&gt; 17</td>
<td>2.4 ± 0.4</td>
<td>0.81 ± 0.08</td>
<td>&gt; 15</td>
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<tr>
<td><strong>Benzodifuran</strong></td>
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</tr>
<tr>
<td>2C-B-FLY</td>
<td>17 ± 4</td>
<td>&gt; 26</td>
<td>10 ± 3</td>
<td>11 ± 1</td>
<td>0.78 ± 0.3</td>
<td>1.4 ± 0.2</td>
<td>1.9 ± 0.3</td>
<td>6.8 ± 1.2</td>
<td>3.4 ± 0.5</td>
<td>0.03 ± 0.01</td>
<td>0.71 ± 0.23</td>
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<td><strong>Related amphetamines</strong></td>
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</tr>
<tr>
<td>MDMA</td>
<td>27 ± 9</td>
<td>8.4 ± 3.3</td>
<td>13 ± 2</td>
<td>&gt; 5</td>
<td>15 ± 10</td>
<td>&gt; 12</td>
<td>&gt; 20</td>
<td>&gt; 17</td>
<td>&gt; 13</td>
<td>0.37 ± 0.12</td>
<td>2.4 ± 1.1</td>
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<tr>
<td>MDA</td>
<td>13 ± 3.7</td>
<td>&gt; 26</td>
<td>5.6 ± 1.5</td>
<td>&gt; 5</td>
<td>1.1 ± 0.1</td>
<td>&gt; 12</td>
<td>&gt; 20</td>
<td>&gt; 17</td>
<td>&gt; 13</td>
<td>0.25 ± 0.04</td>
<td>0.16 ± 0.01</td>
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<tr>
<td>beta-keto-MDA</td>
<td>&gt; 30</td>
<td>11 ± 2</td>
<td>&gt; 30</td>
<td>&gt; 5</td>
<td>15 ± 2</td>
<td>&gt; 12</td>
<td>&gt; 20</td>
<td>&gt; 17</td>
<td>&gt; 13</td>
<td>4.8 ± 0.9</td>
<td>6.5 ± 2.8</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>3.0 ± 2.2</td>
<td>1.8 ± 0.7</td>
<td>25 ± 10</td>
<td>&gt; 5</td>
<td>6.1 ± 1.6</td>
<td>&gt; 12</td>
<td>&gt; 20</td>
<td>&gt; 17</td>
<td>&gt; 13</td>
<td>0.35 ± 0.12</td>
<td>0.55 ± 0.24</td>
</tr>
</tbody>
</table>

Values are $K_i$ given as microM (mean ±SD); a values are from Simmler et al. 2014a.
### Table 3. Serotonin receptor interactions.

<table>
<thead>
<tr>
<th>Benzofurans</th>
<th>5-HT&lt;sub&gt;1A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2A&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2B&lt;/sub&gt;</th>
<th>5-HT&lt;sub&gt;2C&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>receptor binding</td>
<td>receptor binding</td>
<td>activation potency</td>
<td>activation efficacy</td>
<td>receptor binding</td>
</tr>
<tr>
<td>Ki (µM)</td>
<td>Ki (µM)</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>% maximum</td>
<td>Ki (µM)</td>
</tr>
<tr>
<td>5-APB</td>
<td>3.3 ± 0.2</td>
<td>0.84 ± 0.27</td>
<td>6.3 ± 2.1</td>
<td>54 ± 35</td>
</tr>
<tr>
<td>5-APDB</td>
<td>20 ± 4</td>
<td>3.4 ± 1.0</td>
<td>11 ± 2</td>
<td>24 ± 17</td>
</tr>
<tr>
<td>6-APB</td>
<td>1.5 ± 0.2</td>
<td>0.97 ± 0.23</td>
<td>5.9 ± 1.8</td>
<td>43 ± 23</td>
</tr>
<tr>
<td>6-APDB</td>
<td>9.2 ± 1.5</td>
<td>2.0 ± 1.0</td>
<td>5.9 ± 1.1</td>
<td>62 ± 36</td>
</tr>
<tr>
<td>5-MAPDB</td>
<td>26 ± 6</td>
<td>4.8 ± 2.1</td>
<td>&gt; 20</td>
<td>0</td>
</tr>
<tr>
<td>4-APB</td>
<td>1.2 ± 0.1</td>
<td>0.96 ± 0.17</td>
<td>13 ± 2</td>
<td>30 ± 9</td>
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<tr>
<td>7-APB</td>
<td>0.28 ± 0.05</td>
<td>0.91 ± 0.20</td>
<td>5.7 ± 2.0</td>
<td>43 ± 21</td>
</tr>
<tr>
<td>5-EAPB</td>
<td>3.1 ± 0.6</td>
<td>2.7 ± 1.5</td>
<td>7.6 ± 3.2</td>
<td>29 ± 7</td>
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<tr>
<td>Benzodifuran</td>
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<tr>
<td>2C-B-FLY</td>
<td>0.35 ± 0.04</td>
<td>0.011 ± 0.002</td>
<td>0.0015 ± 0.0002</td>
<td>82 ± 12</td>
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<tr>
<td>MDMA</td>
<td>12 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.3 ± 2.4</td>
<td>6.1 ± 0.3</td>
<td>55 ± 9</td>
</tr>
<tr>
<td>MDA</td>
<td>4.9 ± 0.9</td>
<td>3.3 ± 0.8</td>
<td>0.63 ± 0.24</td>
<td>77 ± 16</td>
</tr>
<tr>
<td>beta-keto-MDA</td>
<td>&gt; 17</td>
<td>&gt; 13</td>
<td>&gt; 20</td>
<td>0</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>8.1 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt; 13</td>
<td>&gt; 20</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are Ki given as microM (mean±SD). <sup>a</sup>Values are from Simmler et al. 2014a and were included for comparison.
<table>
<thead>
<tr>
<th>Structure-activity relationships</th>
<th>Present in</th>
<th>Not present in</th>
<th>Pharmacological (clinical) activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-substitution on benzene ring (methylene dioxide or furan)</td>
<td>MDMA, 5-MAPDB, 5-EAPB, methamphetamine</td>
<td>7-APB, 4-APB, 6-APB, 6-APDB</td>
<td>reduced DAT/SERT inhibition ratio, (^a)increased potency to release 5-HT, (^a)reduced potency to release DA (more entactogenic, less stimulant)</td>
</tr>
<tr>
<td>Oxygen in para-(4)-position</td>
<td>MDA, 5-APB, 6-APB, 5-APDB, 6-APDB</td>
<td>5-APB, 6-APB, 4-APB, 7-APB</td>
<td>reduced DAT/SERT inhibition ratio (more serotonergic) (^b)</td>
</tr>
<tr>
<td>Dihydrobenzofuran</td>
<td>5-APDB, 6-APDB, 5-MAPDB</td>
<td>5-APB, 6-APB, 4-APB, 7-APB</td>
<td>reduced DAT/SERT inhibition ratio (more serotonergic) (^b)</td>
</tr>
<tr>
<td>N-alkyl group</td>
<td>MDMA, 5-EAPB, 5-MAPDB</td>
<td>MDA, 5-APB, 5-APDB</td>
<td>reduced 5-HT(<em>{2A/B}) receptor activation and 5-HT(</em>{2C}) receptor binding potency (less hallucinogenic) (^b)</td>
</tr>
<tr>
<td>2,5-oxy-substitution on benzene ring</td>
<td>2C-B-FLY</td>
<td>all other compounds</td>
<td>strongly increased 5-HT(<em>{2A/B}) receptor activation, strongly increased 5-HT(</em>{2C}) receptor binding potency (more hallucinogenic) (^b)</td>
</tr>
<tr>
<td>(\beta)-keto group</td>
<td>(\beta)-keto MDA</td>
<td>MDA</td>
<td>(^a,b)increased DAT/SERT ratio (more dopaminergic) (^b)</td>
</tr>
</tbody>
</table>

\(^{a}\)Simmler et al 2013, \(^{b}\)Simmler et al. 2014a