# Pharmacological profile of mephedrone analogs and related new psychoactive substances

Dino Luethi[a], Karolina E. Kolaczynska[a], Luca Docci[a], Stephan Krähenbühl[a], Marius C. Hoener[b], Matthias E. Liechti[a]

[a]Division of Clinical Pharmacology and Toxicology, Department of Biomedicine, University Hospital Basel and University of Basel, Basel, Switzerland

[b]Neuroscience Research, pRED, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland

**Running title:** Mephedrone-like designer drugs

*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

Word count: Manuscript: 3842, Abstract: 222, References: 59, Tables: 2, Figures: 3

**Abstract**

*Background:* Mephedrone is a synthetic cathinone and one of the most popular recreationally used new psychoactive substances. The aim of the present study was to characterize the *in vitro* pharmacology of novel analogs of mephedrone and related newly emerged designer stimulants.

*Methods:* We determined norepinephrine, dopamine, and serotonin transporter inhibition potencies and monoamine release in transporter-transfected human embryonic kidney 293 cells. We also assessed monoamine receptor and transporter binding affinities.

*Results:* Mephedrone analogs potently inhibited the norepinephrine transporter and, with the exception of 3-methylmethcathinone (3-MMC), inhibited the serotonin transporter more potently than the dopamine transporter. Similar to classic amphetamines, mephedrone analogs were substrate-type monoamine releasers. 5-(2-Aminopropyl)indole (5-IT) was a highly potent monoamine transporter inhibitor and a releaser of dopamine and serotonin. 4-Methylamphetamine (4-MA) mediated efflux of all three monoamines and inhibited the serotonin transporter more potently than the dopamine transporter, unlike amphetamine. *N*-methyl-2-aminoindane (*N*-methyl-2-AI) was a selective norepinephrine transporter inhibitor and norepinephrine releaser, whereas 5-methoxy-6-methyl-2-aminoindane (MMAI) was a selective serotonin transporter inhibitor and serotonin releaser. All of the drugs interacted with monoamine receptors.

*Conclusion:* The predominant actions on serotonin *vs.* dopamine transporters suggest that dimethylmethcathinones, 4-MA, and MMAI cause entactogenic effects similar to 3,4-methylenedioxymethamphetamine, whereas 3-MMC, 5-IT, and *N*-methyl-2-AI have more stimulant-type properties like amphetamine. Because of pharmacological and structural similarity to mephedrone, similar health risks can be expected for these analogs.

**Keywords:** mephedrone, new psychoactive substances, monoamine, receptors, transporters.

**Abbreviations**

2,3-DMMC, 2,3-dimethylmethcathinone; 2,4-DMMC, 2,4-dimethylmethcathinone; 3,4-DMMC, 3,4-dimethylmethcathinone, 3-MMC, 3-methylmethcathinone; 4-MA, 4-methylamphetamine; 4-MMC, 4-methylmethcathinone (mephedrone); 5-IT, 5-(2-aminopropyl)indole; 5-HT, 5-hydroxytryptamine (serotonin); DA, dopamine; DAT, dopamine transporter; FLIPR, fluorescence imaging plate reader; HPLC, high-performance liquid chromatography; MDMA, 3,4-methylenedioxymethamphetamine; MMAI, 5-methoxy-6-methyl-2-aminoindane; NE, norepinephrine; NET norepinephrine transporter; *N*-methyl-2-AI, *N*-methyl-2-aminoindane; NPS, new psychoactive substances; SERT, serotonin transporter; TAAR, trace amine-associated receptor.

## 1. Introduction

4-Methylmethcathinone (4-MMC, mephedrone) is a substituted synthetic cathinone (β-keto amphetamine) that has recently become popular as a party drug (Dargan et al., 2010; Green et al., 2014). Mephedrone was widely sold as a "legal high" and continued to be available on the illicit drug market after being classified as illegal (Green et al., 2014; Wood et al., 2012). Structurally and pharmacologically similar new psychoactive substances (NPS) have emerged on the drug market as legal alternatives to the newly banned mephedrone (Brandt et al., 2010). Knowledge of the effects and toxicity of NPS is often solely based on user reports and clinical intoxication cases, and pharmacological and toxicological data are mostly lacking. Therefore, the assessment of *in vitro* pharmacological profiles of NPS is a first approach to better understand their clinical effects and toxicology. In the present study, we assessed monoamine transporter and receptor interaction profiles of a new series of mephedrone analogs and related designer drugs (Fig. 1) and compared them to mephedrone. Several of the tested substances were first described in the 20th century, but the widespread availability and recreational use of these substances is a rather recent phenomenon (Baumeister et al., 2015; Brandt et al., 2014; King, 2014; Liechti, 2015). The substituted cathinones 2,3-dimethylmethcathinone (2,3-DMMC), 2,4-dimethylmethcathinone (2,4-DMMC), and 3,4-dimethylmethcathinone (3,4-DMMC) have received relatively little attention to date. 3,4-DMMC has recently been sold and confiscated in various countries (Locos and Reynolds, 2012; Odoardi et al., 2016; Zancajo et al., 2014). 3-Methylmethcathinone (3-MMC) has become one of the most popular NPS in various European countries after the ban of mephedrone, and it has been associated with clinical toxicity and several fatal cases (Adamowicz et al., 2016; Adamowicz et al., 2014; Backberg et al., 2015; European Monitoring Centre for Drugs and Drug Addiction, 2015). 5-(2-Aminopropyl)indole (5-IT) is an indole derivative and stimulant NPS that has been

associated with numerous fatal and non-fatal intoxications in recent years (Backberg et al., 2014; Katselou et al., 2015; Kronstrand et al., 2013; Seetohul and Pounder, 2013). 5-IT has been shown to be a substrate at the transporter for norepinephrine (NET), dopamine (DAT), and serotonin (SERT) in rat brain synaptosomes with greater potency for release at NET and DAT over SERT (Marusich et al., 2016). Moreover, 5-IT produced locomotor stimulation and stimulant effects similar to 3,4-methylenedioxymethamphetamine (MDMA) in mice (Marusich et al., 2016). 4-Methylamphetamine (4-MA) is an NPS that has been detected in street amphetamine ("speed") samples across Europe and was linked to several fatalities in combination with amphetamine (Blanckaert et al., 2013). In a study comparing the monoamine releasing potencies of a series of amphetamines analogs *in vitro*, 4-MA and *d*-amphetamine had similar potencies as releasers of norepinephrine (NE) and dopamine (DA), but 4-MA was a more potent releaser of serotonin (5-HT) (Wee et al., 2005). 4-MA was self-administered at a lower rate by rhesus monkeys compared to *d*-amphetamine (Wee et al., 2005). *N*-methyl-2-aminoindane (*N*-methyl-2-AI) and 5-methoxy-6-methyl-2-aminoindane (MMAI) are two psychoactive aminoindanes that have been sold as designer drugs online. MMAI has previously been shown to have effects on the SERT similar to MDMA (Rudnick and Wall, 1993) and a high selectivity for 5-HT *vs.* NE and DA uptake inhibition (Johnson et al., 1991).

## 2. Material and methods

### *2.1. Drugs*

MDMA, mephedrone, and 4-MA were purchased from Lipomed (Arlesheim, Switzerland) with high-performance liquid chromatography (HPLC) purity > 98.5%. 2,3-DMMC, 2,4-DMMC, 3,4-DMMC, 3-MMC, 5-IT, and MMAI were purchased from Cayman Chemicals (Ann Arbor, MI, USA) with purity > 98%. *N*-methyl-2-AI was provided by Dr.

Christian Bissig (Forensic Institute, Zürich, Switzerland) with purity > 98%. 5-IT was obtained as racemic base; the remaining compounds were obtained as racemic hydrochlorides. Radiolabelled norepinephrine and dopamine ([$^3$H]-NE and [$^3$H]-DA, respectively) were obtained from Perkin-Elmer (Schwerzenbach, Switzerland). Radiolabeled serotonin ([$^3$H]-5-HT) was purchased from Anawa (Zürich, Switzerland).

*2.2. Monoamine uptake transport inhibition*

Inhibition of the human NE, DA, and 5-HT transporter (hNET, hDAT, and hSERT, respectively) was assessed in human embryonic kidney (HEK) 293 cells (Invitrogen, Zug, Switzerland) stably transfected with the respective human transporter as previously described (Hysek et al., 2012; Tatsumi et al., 1997). Briefly, cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Life Technologies, Zug, Switzerland) with 10% fetal bovine serum (Gibco) and 250 μg/ml Geneticin (Gibco) to 70-90% confluence, detached, and then resuspended ($3 \times 10^6$ cells/ml) in Krebs-Ringer Bicarbonate Buffer (Sigma-Aldrich, Buchs, Switzerland). For [$^3$H]-DA uptake experiments, the uptake buffer was supplemented with 0.2 mg/ml ascorbic acid. The cell suspension (100 μl) was incubated with 25 μl buffer containing the test drugs, vehicle control, or monoamine-specific inhibitors (10 μM nisoxetine for NET, 10 μM mazindol for DAT, and 10 μM fluoxetine for SERT) for 10 min in a round bottom 96-well plate at room temperature by shaking at 450 rotations per minute on a rotary shaker. To initiate uptake transport, 50 μl of [$^3$H]-NE, [$^3$H]-DA, or [$^3$H]-5-HT dissolved in uptake buffer were added at a final concentration of 5 nM for additional 10 min. Thereafter, 100 μl of the cell suspension was transferred to 500 μl microcentrifuge tubes that contained 50 μl of 3 M KOH and 200 μl silicon oil (1:1 mixture of silicon oil types AR 20 and AR 200; Sigma-Aldrich). The tubes were centrifuged for 3 min at 16,550g to transport the cells through the silicone oil into the KOH. The tubes were frozen in liquid nitrogen and

the cell pellet was then cut into 6 ml scintillation vials (Perkin-Elmer) that contained 0.5 ml lysis buffer (0.05 M TRIS-HCl, 50 mM NaCl, 5 mM EDTA, and 1% NP-40 in water). The samples were shaken for 1 h before 5 ml scintillation fluid (Ultimagold, Perkin Elmer, Schwerzenbach, Switzerland) was added. Monoamine uptake was then quantified by liquid scintillation counting on a Packard Tri-Carb Liquid Scintillation Counter 1900 TR. Nonspecific uptake in the presence of selective inhibitors was subtracted from the total counts.

*2.3. Transporter-mediated monoamine release*

Transporter-mediated monoamine efflux was assessed in HEK 293 cells stably expressing the respective transporter as previously described (Simmler et al., 2013; Simmler et al., 2014a). Briefly, 100,000 cells per well were cultured overnight in a poly-D-lysine coated XF24 cell culture microplate (Seahorse Biosciences, North Billerica, MA, USA). Thereafter, the cells were preloaded with 10 nM [$^3$H]-NE, [$^3$H]-DA, or [$^3$H]-5-HT diluted in 85 µl Krebs-HEPES buffer (130 mM NaCl, 1.3 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM $KH_2PO_4$, 10 mM HEPES, 10 mM D-glucose, pH 7.5) containing 10 µM pargyline and 0.2 mg/mL ascorbic acid for 20 min at 37 °C, washed twice, and treated with 1000 µl Krebs-HEPES buffer containing 100 µM of the test drugs for 15 min (DAT and SERT) or 45 min (NET) at 37 ºC by shaking at 300 rotations per minute on a rotary shaker. The cells were then washed again with cold buffer and lysed in 50 µl lysis buffer during 1 h. Thereafter, 40 µl of the cell lysate was transferred into 4 ml scintillation vials with 3.5 ml scintillation fluid and the radioactivity inside the cells was quantified by liquid scintillation counting as described for the monoamine uptake inhibition assay. Monoamine transporter blockers (10 µM nisoxetine for NET, 10 µM mazindol for DAT, and 10 µM citalopram for SERT) were included in the experiment to determine "pseudo-efflux" caused by nonspecific monoamine

release and subsequent reuptake inhibition (Scholze et al., 2000). The use of a single high concentration and the release durations were based on kinetic evaluation of the release-over-time curves for substrate-releasers in previous studies (Hysek et al., 2012; Simmler et al., 2014a).

*2.4. Radioligand receptor and transporter binding assays*

The radioligand binding assays were performed as previously described in detail for transporters (Hysek et al., 2012) and receptors (Revel et al., 2011). Briefly, HEK 293 cell membrane preparations (Invitrogen, Zug, Switzerland) overexpressing the respective transporters (Tatsumi et al., 1997) or receptors (human genes except rat and mouse genes for trace amine-associated receptors [TAARs]) (Revel et al., 2011) were incubated with radiolabeled selective ligands at concentrations equal to $K_d$ and ligand displacement by the compounds was measured. The difference between the total binding and nonspecific binding that was determined in the presence of the selected competitors in excess, was defined as specific binding of the radioligand to the target. 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-propylamine)tetralin and indatraline (5-$HT_{1A}$ receptor), [$^3$H]ketanserin and spiperone (5-$HT_{2A}$ receptor), [$^3$H]mesulgerine 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]spiperone and spiperone ($D_2$ receptor), and [$^3$H]RO5166017 and RO5166017 (TAAR1).

*2.5. Activity at the serotonin 5-$HT_{2A}$ receptor*

Mouse embryonic fibroblasts (NIH-3T3 cells) expressing the human 5-$HT_{2A}$ receptor were incubated in HEPES-Hank's Balanced Salt Solution (HBSS) buffer (Gibco; 70,000 cells/100 µl) for 1 h at 37 °C in 96-well poly-D-lysine-coated plates. To each well, 100 µl dye solution (fluorescence imaging plate reader [FLIPR] calcium 5 assay kit; Molecular Devices, Sunnyvale, CA, USA) was added and the plates were incubated for 1 h at 37 °C. The plates were placed in a FLIPR and 25 µl of the test drugs diluted in HEPES-HBSS buffer containing 250 mM probenicid were added online. The increase in fluorescence was then measured and $EC_{50}$ values were derived from the concentration-response curves using nonlinear regression. The maximal receptor activity (efficacy) is expressed relative to 5-HT activity, which was set to 100%.

*2.6. Activity at the serotonin 5-$HT_{2B}$ receptor*

HEK 293 cells expressing the human 5-$HT_{2B}$ receptor were incubated in growth medium (DMEM high glucose [Invitrogen, Zug, Switzerland], 10 ml/l PenStrep [Gibco], 10% fetal calf serum [non-dialysed, heat-inactivated], and 250 mg/l Geneticin) at a density of 50,000 cells/well at 37 °C in poly-D-lysine-coated 96-well plates overnight. The growth medium was then removed by snap inversion, and 100 µl of the calcium indicator Fluo-4 solution (Molecular Probes, Eugene, OR, USA) was added to each well. The plates were incubated for 45 min at 31 °C before the Fluo-4 solution was removed by snap inversion, and 100 µl of Fluo-4 solution was added a second time for 45 min at 31 °C. The cells were washed with HBSS and 20 mM HEPES (assay buffer) immediately before testing using an EMBLA cell washer, and 100 µl assay buffer was added. The plates were placed in a FLIPR, and 25 µl of the test substances diluted in assay buffer was added online. The increase in fluorescence was then measured and $EC_{50}$ values were derived from the concentration-

response curves using nonlinear regression. The maximal receptor activity (efficacy) is expressed relative to 5-HT activity, which was set to 100%.

*2.7. Cytotoxicity*

Cytotoxicity in hSERT-, hDAT-, and hNET-transfected HEK 293 cells was assessed with the ToxiLight bioassay kit (Lonza, Basel, Switzerland) according to the manufacturer's protocol. The cells were treated for 1 h at room temperature with the drugs at the highest assay concentrations. Adenylate kinase release as a result of cell membrane integrity loss was then quantified and compared to control.

*2.8. Statistical analysis*

Monoamine uptake data were fit by nonlinear regression to variable-slope sigmoidal dose-response curves and $IC_{50}$ values were assessed with Prism software (version 7.0a, GraphPad, San Diego, CA, USA). The DAT/SERT ratio is expressed as 1/DAT $IC_{50}$ : 1/SERT $IC_{50}$. Analysis of variance followed by the Holm-Sidak test was used to analyze drug-induced release of five independent experiments. The drugs were considered monoamine releasers if they caused significantly higher ($*p < 0.05$) efflux than the selective inhibitors. $IC_{50}$ values of radioligand binding were determined by calculating nonlinear regression curves for a one-site model using three independent 10-point concentration-response curves for each substance. $K_i$ (affinity) values, which correspond to the dissociation constants, were calculated using the Cheng-Prusoff equation. Nonlinear regression concentration-response curves were used to determine $EC_{50}$ values for 5-$HT_{2A}$ and 5-$HT_{2B}$ receptors activation. Efficacy (maximal activity) is expressed relative to the activity of 5-HT, which was used as a control set to 100%.

## 3. Results

### *3.1. Monoamine uptake transporter inhibition*

$IC_{50}$ values for NET, DAT, and SERT inhibition are listed in Table 1, and the corresponding uptake inhibition curves are presented in Fig. 2. Mephedrone analogs potently inhibited the NET and, with the exception of 3-MMC, were more potent SERT *vs.* DAT inhibitors. 5-IT was a highly potent inhibitor of the NET and a potent inhibitor of the DAT and SERT. 4-MA and MMAI inhibited the SERT at submicromolar concentrations but were only weak inhibitors of the DAT. *N*-methyl-2-AI was a selective NET inhibitor with only very weak inhibition of the SERT and DAT.

### *3.2. Monoamine release*

Monoamine efflux at a 100 μM concentration of the test drugs is shown in Fig. 3. All of the cathinones were releasers of all three monoamines, with the exception of 3,4-DMMC, for which 5-HT release was not significantly higher than the inhibitor control. 5-IT caused DA and 5-HT efflux. 4-MA caused NE, DA, and 5-HT efflux. *N*-methyl-2-AI was a selective NE releaser. MMAI was a selective 5-HT releaser.

### *3.3. Monoamine receptor and transporter binding affinities*

The monoamine receptor and transporter binding affinities and receptor activation potentials of the mephedrone analogs and related designer drugs are shown in Table 2. None of the drugs interacted with the dopamine $D_2$ receptor, but all of the drugs had low micromolar or submicromolar affinity for $\alpha_{1A}$ or $\alpha_{2A}$ adrenergic receptors. 4-MA and *N*-methyl-2-AI interacted with the $\alpha_{2A}$ receptor but not the $\alpha_{1A}$ receptor. All other compounds interacted with the $\alpha_{1A}$ and the $\alpha_{2A}$ receptor. 3-MMC, *N*-methyl-2-AI, and MMAI had low micromolar affinities for the serotonin $5\text{-}HT_{1A}$ receptor, and the other drugs had only low or

no affinity for this receptor. All of the drugs bound to the 5-$HT_{2A}$ receptor, but only 2,3-DMMC, 5-IT, 4-MA, and mephedrone activated the receptor. Only 5-IT and 4-MA activated the 5-$HT_{2B}$ receptor. *N*-methyl-2-AI did not bind to the 5-$HT_{2C}$ receptor, whereas the other drugs bound with affinities of 1.3–8.1 μM. All of the drugs interacted with rat and mouse TAARs.

### *3.4. Cytotoxicity*

None of the drugs were cytotoxic up to 1 h at the investigated concentrations, thus confirming cell integrity during the functional assays.

## 4. Discussion

### *4.1. Monoamine uptake transporter inhibition*

Similar to mephedrone, the novel mephedrone analogs potently inhibited the NET, which likely results in similar sympathomimetic stimulation (Hysek et al., 2011). The crucial role of NE in the acute effects of psychostimulants is supported by the finding that the release of NE but not DA correlates with human doses of amphetamine-type stimulants (Rothman et al., 2001). Additionally, NET inhibition potency values strongly correlated with the psychotropic effective doses of psychostimulants including cathinones in humans (Simmler et al., 2013). Furthermore, NE has been shown to contribute to the acute subjective stimulation and cardiovascular effects of MDMA in humans (Hysek et al., 2011). (Hysek et al., 2011)

3-MMC more potently inhibited the DAT than the SERT. Mephedrone (4-MMC) had similar potency at the DAT and SERT as previously shown in some other studies (Baumann et al., 2012; Hadlock et al., 2011; Simmler et al., 2013), while others found 5–10-fold higher potency at the DAT *vs.* SERT (Eshleman et al., 2013; Mayer et al., 2016; Pifl et al., 2015).

Moreover, the present high NET *vs.* DAT selectivity of mephedrone was not or less observed in some other studies (Eshleman et al., 2013; Mayer et al., 2016; Pifl et al., 2015). While the selectivity of mephedrone for the NET over the SERT in our study is similar to other *in vitro* studies (Eshleman et al., 2013; Mayer et al., 2016; Pifl et al., 2015), the NET over DAT selectivity appears to be higher compared with other labs. This has been observed for mephedrone in previous studies of our lab (Rickli et al., 2015a; Simmler et al., 2013), suggesting that those differences may be explained by differences in the experimental design or the transfected cell line.

The dimethylmethcathinones inhibited the SERT more potently than the DAT. These results suggest that 3-MMC has stronger amphetamine-like stimulant properties compared with mephedrone and especially the other more serotonergic dimethylmethcathinones. Stimulant toxicity was reported to be the main clinical feature in patients with recreational 3-MMC intoxication, although often combined with other drugs (Backberg et al., 2015). Dimethylmethcathinones presumably have entactogenic properties that are similar to MDMA because of greater activation of the 5-HT system (Hysek et al., 2012; Simmler et al., 2013). High selectivity for the SERT *vs*. DAT was also observed for the para-substituted 4-MA, whereas previous studies found high inhibition selectivity for the DAT *vs.* SERT for amphetamine (Rickli et al., 2015a; Simmler et al., 2013). The strong serotonergic activity of 4-MA has been hypothesized to decrease its reinforcing potency compared with other amphetamine analogs (Baumann et al., 2011; Wee et al., 2005). However, the strong serotonergic activity of 4-MA may have led to several fatal cases when combined with the strong dopaminergic activity of amphetamine in users of 4-MA contaminated “speed” (Blanckaert et al., 2013). Moreover, the extreme hyperthermia that is observed in such patients may be explained by the strong serotonergic potency of 4-MA, which is not shared by amphetamine (Blanckaert et al., 2013). 5-IT was a very potent inhibitor of the NET, with

potent inhibition also of the DAT and SERT. 5-IT has been associated with sympathomimetic and serotonergic toxicity and was involved in numerous deaths across Europe (Backberg et al., 2014; Katselou et al., 2015; Kronstrand et al., 2013; Seetohul and Pounder, 2013). *N*-methyl-2-AI selectively inhibited the NET, with very weak inhibition potency for the DAT and SERT, suggesting mild psychoactive effects that are similar to 2-aminoindane (2-AI) (Simmler et al., 2014b). MMAI had NET inhibition potencies that were similar to *N*-methyl-2-AI. Unlike *N*-methyl-2-AI, however, MMAI potently inhibited the SERT at submicromolar concentrations.

*4.2. Monoamine release*

Consistent with previous studies, mephedrone caused efflux of all three monoamines (Baumann et al., 2012; Eshleman et al., 2013; Mayer et al., 2016). The cathinone analogs of mephedrone were also monoamine releasers, indicating that they are monoamine transporter substrates like most amphetamines (Sitte and Freissmuth, 2015). One exception was 3,4-DMMC, which was a potent inhibitor of the SERT but did not cause significant 5-HT efflux. The monoamine transporter inhibition profile of 3,4-DMMC is similar to MDMA (Simmler et al., 2013), but their differences in 5-HT release may partially explain their different subjective effects and potency. 4-MA released all three monoamines as described for amphetamine (Rickli et al., 2015a). 5-IT was a very potent inhibitor of the NET, but NE release was not observed. *N*-methyl-2-AI selectively inhibited the NET and was also a selective NE releaser. MMAI was a highly selective 5-HT releaser, consistent with previous reports (Marona-Lewicka and Nichols, 1994, 1998). The high serotonergic activation by MMAI suggests entactogenic effects. However, the lack of any effect on the DA or NE system indicates that the psychopharmacology of MMAI differs from typical entactogens like MDMA (Marona-Lewicka and Nichols, 1994).

*4.3. Receptor-binding profiles*

All of the drugs potently bound to adrenergic receptors, which are known to modulate stimulant-induced behavior (Schmidt and Weinshenker, 2014). Furthermore, the drugs interacted with several serotonin receptors. All of the compounds bound to the 5-$HT_{2A}$ receptor as previously shown for mephedrone and MDMA (Eshleman et al., 2013; Simmler et al., 2013) and typically for serotonergic hallucinogens (Eshleman et al., 2014; Nichols, 2016; Rickli et al., 2015c; Rickli et al., 2016). Additionally, 2,3-DMMC, mephedrone, and 5-IT were potent functional 5-$HT_{2A}$ agonists in our calcium mobilization assay like MDMA (Rickli et al., 2015b) and classic serotonergic hallucinogens (Rickli et al., 2016) known to produce their psychotropic effects at least in part via 5-$HT_{2A}$ receptor activation (Liechti et al., 2000; Preller et al., 2017; Vollenweider et al., 1998). Another study documented 5-$HT_{2A}$ receptor antagonistic properties for mephedrone in another 5-HT-induced inositol monophosphate formation assay (Eshleman et al., 2013). However, MDMA had both agonist (Eshleman et al., 2014) and antagonist effects (Eshleman et al., 2013) in this assay indicating that the 5-$HT_{2A}$ ligands may act as agonist and antagonists depending on assay set-up. Certain hallucinogenic properties have been described for mephedrone (Kasick et al., 2012; Schifano et al., 2011) and our results suggest that 2,3-DMMC could have hallucinogen-like properties as well. 5-IT is a positional isomer of the psychedelic tryptamine α-methyltryptamine (αMT). 5-IT has been previously suggested to also have hallucinogenic properties (Marusich et al., 2016), and its potent 5-$HT_{2A}$ receptor activation supports this possibility. All of the substances interacted with rat and mouse TAARs. Many stimulant NPS interact with TAARs (Simmler et al., 2016), which have a modulatory role on monoaminergic activity (Revel et al., 2012; Revel et al., 2011). In a recent screening of a large set of NPS, cathinones were described as poor TAAR1 ligands (Simmler et al., 2016).

Our results suggest that this does not apply to all cathinones as submicromolar affinity for rat and mouse TAARs was observed for 2,4-DMMC and 2,3-DMMC, respectively.

The present study has limitations. First, we did not investigate the effects of the drugs on intracellular targets such as the vesicular monoamine transporter 2 (VMAT2). Lower potency VMAT2 interactions have been reported for methcathinones compared to MDMA and methamphetamine (Eshleman et al., 2013; Fleckenstein et al., 2009; Pifl et al., 2015). It was therefore concluded that mephedrone is unlikely to cause neurotransmitter release form synaptic vesicles (Eshleman et al., 2013). Second, the static monoamine release assay used in the present study was only useful to qualitatively determine whether a drug is a substrate releaser or not, but the assay was not suitable to assess the potency of the releasers. Superfusion assays would be more suitable to also determine the potency of the substances to release monoamines (Eshleman et al., 2013). However, the potency of the substances to release monoamine is reflected by their potency to inhibit monoamine uptake in the uptake assay used in the present study (Simmler et al., 2013). Finally, we included no *in vivo* data. However, *in vivo* microdialysis studies showed that the cathinones mephedrone and methylone markedly released both 5-HT and DA at similar potencies reflecting their *in vitro* pharmacological profiles (Baumann et al., 2012; Kehr et al., 2011). Additionally, methcathinone was a more potent inhibitor of the DAT than SERT *in vitro*, more potently released monoamines via the DAT than SERT (Cozzi et al., 2013; Simmler et al., 2013), and consistently also more potently increased extracellular DA than 5-HT in rat brain nucleus accumbens dialysate (Cozzi et al., 2013). Vice versa, the more potent *in vitro* SERT than DAT inhibitor and predominant 5-HT releaser 4-trifluoromethylmethcathinone (4-TFMAP) increased 5-HT but not DA *in vivo* (Cozzi et al., 2013). Thus, for several cathinones the *in vitro* profiles accurately predicted the *in vivo* neurochemical effects.

## 5. Conclusion

The present study characterized a series of novel mephedrone analogs that potently interacted with monoamine transporters and receptors, suggesting their potential abuse liability, which has been previously observed for synthetic cathinones. 4-MA is a potent inhibitor of the SERT, which may explain its higher toxicity when combined with the potent DAT inhibitor amphetamine. 5-IT is a highly potent monoamine transporter inhibitor that has been associated with sympathomimetic toxicity and numerous fatalities across Europe. *N*-methyl-2-AI is a selective NET inhibitor and NE releaser, and MMAI is a selective SERT inhibitor and 5-HT releaser.

## Author contributions

D.L., S.K., and M.E.L. designed the research. D.L., K.E.K., L.D., and M.C.H. performed the research. D.L., M.C.H., and M.E.L. analyzed the data. D.L. and M.E.L. wrote the manuscript with input from all of the other authors.

## Acknowledgements

This work was supported by the Federal Office of Public Health (no. 16.921318). The authors thank Christian Bissig for providing several test substances, Sylvie Chaboz and Danièle Buchy for technical assistance, and Michael Arends for text editing.

## Conflict of Interest

M.C.H. is an employee of F. Hoffmann-La Roche. The other authors do not have any conflicts of interest to declare for this work.

## References

Adamowicz, P., Gieron, J., Gil, D., Lechowicz, W., Skulska, A., Tokarczyk, B., 2016. 3-Methylmethcathinone: interpretation of blood concentrations based on analysis of 95 cases. J Anal Toxicol 40, 272-276.

Adamowicz, P., Zuba, D., Byrska, B., 2014. Fatal intoxication with 3-methyl-N-methylcathinone (3-MMC) and 5-(2-aminopropyl)benzofuran (5-APB). Forensic Sci Int 245, 126-132.

Backberg, M., Beck, O., Hulten, P., Rosengren-Holmberg, J., Helander, A., 2014. Intoxications of the new psychoactive substance 5-(2-aminopropyl)indole (5-IT): a case series from the Swedish STRIDA project. Clin Toxicol (Phila) 52, 618-624.

Backberg, M., Lindeman, E., Beck, O., Helander, A., 2015. Characteristics of analytically confirmed 3-MMC-related intoxications from the Swedish STRIDA project. Clin Toxicol (Phila) 53, 46-53.

Baumann, M.H., Ayestas, M.A., Jr., Partilla, J.S., Sink, J.R., Shulgin, A.T., Daley, P.F., Brandt, S.D., Rothman, R.B., Ruoho, A.E., Cozzi, N.V., 2012. The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. Neuropsychopharmacology 37, 1192-1203.

Baumann, M.H., Clark, R.D., Woolverton, W.L., Wee, S., Blough, B.E., Rothman, R.B., 2011. In vivo effects of amphetamine analogs reveal evidence for serotonergic inhibition of mesolimbic dopamine transmission in the rat. J Pharmacol Exp Ther 337, 218-225.

Baumeister, D., Tojo, L.M., Tracy, D.K., 2015. Legal highs: staying on top of the flood of novel psychoactive substances. Ther Adv Psychopharmacol 5, 97-132.

Blanckaert, P., van Amsterdam, J., Brunt, T., van den Berg, J., Van Durme, F., Maudens, K., van Bussel, J., 2013. 4-Methyl-amphetamine: a health threat for recreational amphetamine users. J Psychopharmacol 27, 817-822.

Brandt, S.D., King, L.A., Evans-Brown, M., 2014. The new drug phenomenon. Drug Test Anal 6, 587-597.

Brandt, S.D., Sumnall, H.R., Measham, F., Cole, J., 2010. Analyses of second-generation 'legal highs' in the UK: initial findings. Drug Test Anal 2, 377-382.

Cozzi, N.V., Brandt, S.D., Daley, P.F., Partilla, J.S., Rothman, R.B., Tulzer, A., Sitte, H.H., Baumann, M.H., 2013. Pharmacological examination of trifluoromethyl ring-substituted methcathinone analogs. Eur J Pharmacol 699, 180-187.

Dargan, P.I., Albert, S., Wood, D.M., 2010. Mephedrone use and associated adverse effects in school and college/university students before the UK legislation change. QJM 103, 875-879.

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.

Eshleman, A.J., Wolfrum, K.M., Hatfield, M.G., Johnson, R.A., Murphy, K.V., Janowsky, A., 2013. Substituted methcathinones differ in transporter and receptor interactions. Biochem Pharmacol 85, 1803-1815.

European Monitoring Centre for Drugs and Drug Addiction, 2015. New psychoactive substances in Europe: an update from the EU Early Warning System. March 2015.

Luxembourg: European Monitoring Center for Drugs and Drug Addiction. (http://www.emcdda.europa.eu/attachements.cfm/att_235958_EN_TD0415135ENN.pdf; accessed March 19, 2017).

Fleckenstein, A.E., Volz, T.J., Hanson, G.R., 2009. Psychostimulant-induced alterations in vesicular monoamine transporter-2 function: neurotoxic and therapeutic implications. Neuropharmacology 56 Suppl 1, 133-138.

Green, A.R., King, M.V., Shortall, S.E., Fone, K.C., 2014. The preclinical pharmacology of mephedrone; not just MDMA by another name. Br J Pharmacol 171, 2251-2268.

Hadlock, G.C., Webb, K.M., McFadden, L.M., Chu, P.W., Ellis, J.D., Allen, S.C., Andrenyak, D.M., Vieira-Brock, P.L., German, C.L., Conrad, K.M., Hoonakker, A.J., Gibb, J.W., Wilkins, D.G., Hanson, G.R., Fleckenstein, A.E., 2011. 4-Methylmethcathinone (mephedrone): neuropharmacological effects of a designer stimulant of abuse. J Pharmacol Exp Ther 339, 530-536.

Hysek, C.M., Simmler, L.D., Ineichen, M., Grouzmann, E., Hoener, M.C., Brenneisen, R., Huwyler, J., Liechti, M.E., 2011. The norepinephrine transporter inhibitor reboxetine reduces stimulant effects of MDMA ("ecstasy") in humans. Clin Pharmacol Ther 90, 246-255.

Hysek, C.M., Simmler, L.D., Nicola, V.G., Vischer, N., Donzelli, M., Krähenbühl, S., Grouzmann, E., Huwyler, J., 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.

Johnson, M.P., Conarty, P.F., Nichols, D.E., 1991. [3H]monoamine releasing and uptake inhibition properties of 3,4-methylenedioxymethamphetamine and p-chloroamphetamine analogues. Eur J Pharmacol 200, 9-16.

Kasick, D.P., McKnight, C.A., Klisovic, E., 2012. "Bath salt" ingestion leading to severe intoxication delirium: two cases and a brief review of the emergence of mephedrone use. Am J Drug Alcohol Abuse 38, 176-180.

Katselou, M., Papoutsis, I., Nikolaou, P., Spiliopoulou, C., Athanaselis, S., 2015. 5-(2-Aminopropyl)indole: a new player in the drama of 'legal highs' alerts the community. Drug Alcohol Rev 34, 51-57.

Kehr, J., Ichinose, F., Yoshitake, S., Goiny, M., Sievertsson, T., Nyberg, F., Yoshitake, T., 2011. Mephedrone, compared to MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and serotonin levels in nucleus accumbens of awake rats. Br J Pharmacol 164, 1949-1958.

King, L.A., 2014. New phenethylamines in Europe. Drug Test Anal 6, 808-818.

Kronstrand, R., Roman, M., Dahlgren, M., Thelander, G., Wikstrom, M., Druid, H., 2013. A cluster of deaths involving 5-(2-aminopropyl)indole (5-IT). J Anal Toxicol 37, 542-546.

Liechti, M.E., 2015. Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signaling. Swiss Med Wkly 145, w14043.

Liechti, M.E., Saur, M.R., Gamma, A., Hell, D., Vollenweider, F.X., 2000. Psychological and physiological effects of MDMA ("Ecstasy") after pretreatment with the 5-HT(2) antagonist ketanserin in healthy humans. Neuropsychopharmacology 23, 396-404.

Locos, O., Reynolds, D., 2012. The characterization of 3,4-dimethylmethcathinone (3,4-DMMC). J Forensic Sci 57, 1303-1306.

Marona-Lewicka, D., Nichols, D.E., 1994. Behavioral effects of the highly selective serotonin releasing agent 5-methoxy-6-methyl-2-aminoindan. Eur J Pharmacol 258, 1-13.

Marona-Lewicka, D., Nichols, D.E., 1998. Drug discrimination studies of the interoceptive cues produced by selective serotonin uptake inhibitors and selective serotonin releasing agents. Psychopharmacology (Berl) 138, 67-75.

Marusich, J.A., Antonazzo, K.R., Blough, B.E., Brandt, S.D., Kavanagh, P.V., Partilla, J.S., Baumann, M.H., 2016. The new psychoactive substances 5-(2-aminopropyl)indole (5-IT) and 6-(2-aminopropyl)indole (6-IT) interact with monoamine transporters in brain tissue. Neuropharmacology 101, 68-75.

Mayer, F.P., Wimmer, L., Dillon-Carter, O., Partilla, J.S., Burchardt, N.V., Mihovilovic, M.D., Baumann, M.H., Sitte, H.H., 2016. Phase I metabolites of mephedrone display biological activity as substrates at monoamine transporters. Br J Pharmacol 173, 2657-2668.

Nichols, D.E., 2016. Psychedelics. Pharmacol Rev 68, 264-355.

Odoardi, S., Romolo, F.S., Strano-Rossi, S., 2016. A snapshot on NPS in Italy: distribution of drugs in seized materials analysed in an Italian forensic laboratory in the period 2013-2015. Forensic Sci Int 265, 116-120.

Pifl, C., Reither, H., Hornykiewicz, O., 2015. The profile of mephedrone on human monoamine transporters differs from 3,4-methylenedioxymethamphetamine primarily

by lower potency at the vesicular monoamine transporter. Eur J Pharmacol 755, 119-126.

Preller, K.H., Herdener, M., Pokorny, T., Planzer, A., Kraehenmann, R., Stampfli, P., Liechti, M.E., Seifritz, E., Vollenweider, F.X., 2017. The fabric of meaning and subjective effects in LSD-induced states depend on serotonin 2A receptor activation. Curr Biol 27, 451-457.

Revel, F.G., Meyer, C.A., Bradaia, A., Jeanneau, K., Calcagno, E., Andre, C.B., Haenggi, M., Miss, M.T., Galley, G., Norcross, R.D., Invernizzi, R.W., Wettstein, J.G., Moreau, J.L., Hoener, M.C., 2012. Brain-specific overexpression of trace amine-associated receptor 1 alters monoaminergic neurotransmission and decreases sensitivity to amphetamine. Neuropsychopharmacology 37, 2580-2592.

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.

Rickli, A., Luethi, D., Reinisch, J., Buchy, D., Hoener, M.C., Liechti, M.E., 2015c. Receptor interaction profiles of novel N-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs). Neuropharmacology 99, 546-553.

Rickli, A., Moning, O.D., Hoener, M.C., Liechti, M.E., 2016. Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. Eur Neuropsychopharmacol 26, 1327-1337.

Rothman, R.B., Baumann, M.H., Dersch, C.M., Romero, D.V., Rice, K.C., Carroll, F.I., Partilla, J.S., 2001. Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. Synapse 39, 32-41.

Rudnick, G., Wall, S.C., 1993. Non-neurotoxic amphetamine derivatives release serotonin through serotonin transporters. Mol Pharmacol 43, 271-276.

Schifano, F., Albanese, A., Fergus, S., Stair, J.L., Deluca, P., Corazza, O., Davey, Z., Corkery, J., Siemann, H., Scherbaum, N., Farre, M., Torrens, M., Demetrovics, Z., Ghodse, A.H., 2011. Mephedrone (4-methylmethcathinone; 'meow meow'): chemical, pharmacological and clinical issues. Psychopharmacology (Berl) 214, 593-602.

Schmidt, K.T., Weinshenker, D., 2014. Adrenaline rush: the role of adrenergic receptors in stimulant-induced behaviors. Mol Pharmacol 85, 640-650.

Scholze, P., Zwach, J., Kattinger, A., Pifl, C., Singer, E.A., Sitte, H.H., 2000. Transporter-mediated release: a superfusion study on human embryonic kidney cells stably expressing the human serotonin transporter. J Pharmacol Exp Ther 293, 870-878.

Seetohul, L.N., Pounder, D.J., 2013. Four fatalities involving 5-IT. J Anal Toxicol 37, 447-451.

Simmler, L.D., Buchy, D., Chaboz, S., Hoener, M.C., Liechti, M.E., 2016. In vitro characterization of psychoactive substances at rat, mouse, and human trace amine-associated receptor 1. J Pharmacol Exp Ther 357, 134-144.

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.

Sitte, H.H., Freissmuth, M., 2015. Amphetamines, new psychoactive drugs and the monoamine transporter cycle. Trends Pharmacol Sci 36, 41-50.

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.

Vollenweider, F.X., Vollenweider-Scherpenhuyzen, M.F., Bäbler, A., Vogel, H., Hell, D., 1998. Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. Neuroreport 9, 3897-3902.

Wee, S., Anderson, K.G., Baumann, M.H., Rothman, R.B., Blough, B.E., Woolverton, W.L., 2005. Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. J Pharmacol Exp Ther 313, 848-854.

Wood, D.M., Measham, F., Dargan, P.I., 2012. 'Our favourite drug': prevalence of use and preference for mephedrone in the London night-time economy 1 year after control. J Subst Use 17, 91-97.

Zancajo, V.M., Brito, J., Carrasco, M.P., Bronze, M.R., Moreira, R., Lopes, A., 2014. Analytical profiles of "legal highs" containing cathinones available in the area of Lisbon, Portugal. Forensic Sci Int 244, 102-110.

**Figures**

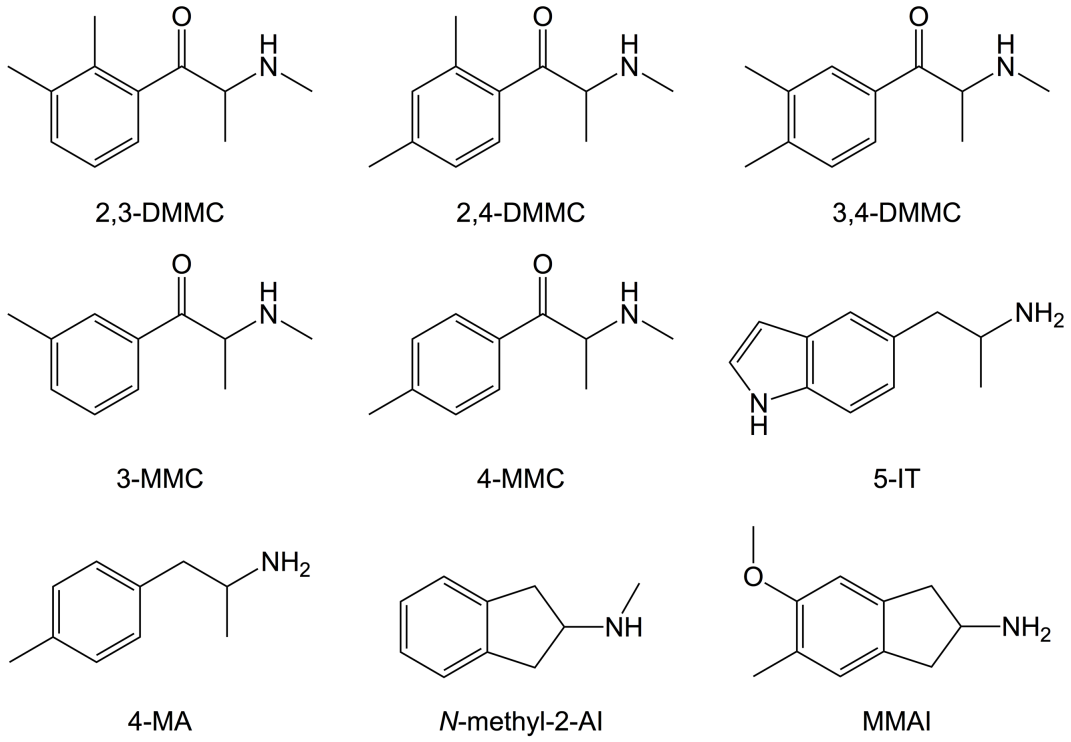


**Fig. 1.** Chemical structures of mephedrone analogs and related designer drugs.

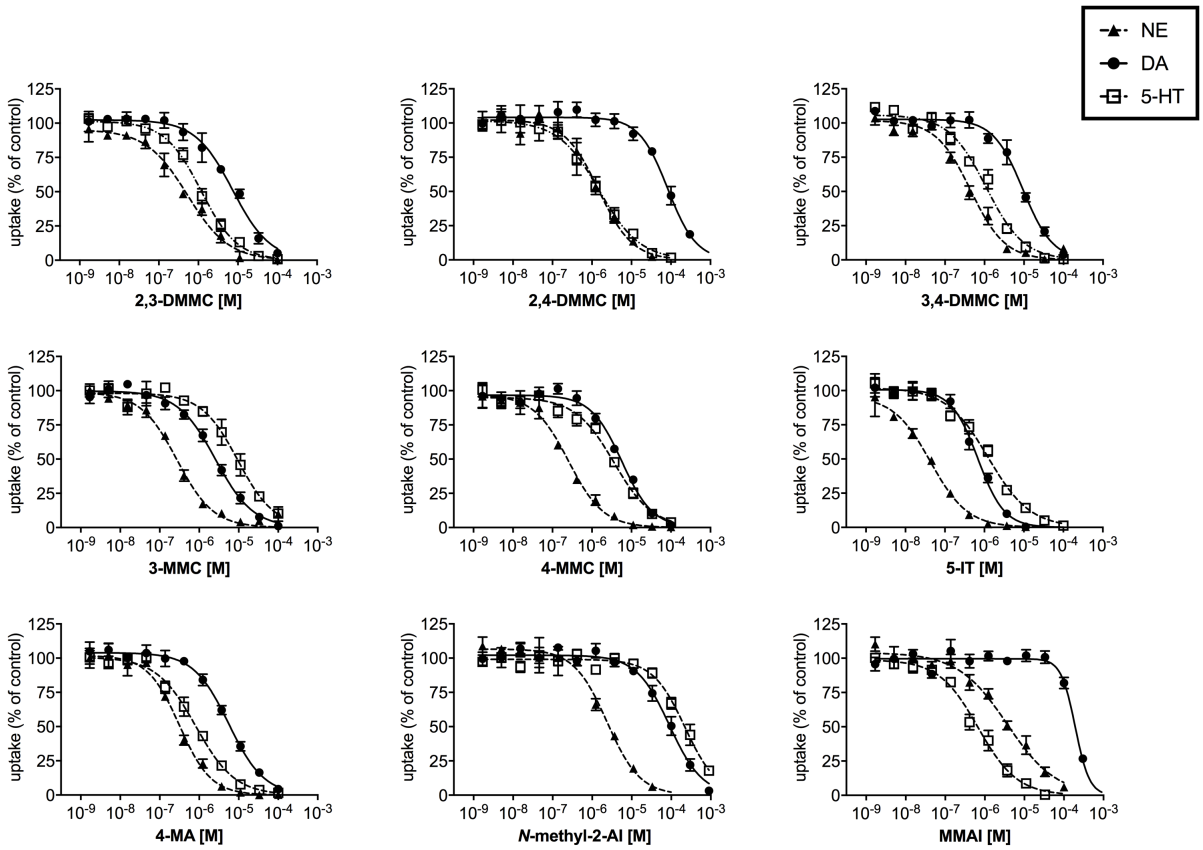


**Fig. 2.** Monoamine uptake inhibition in stably transfected HEK 293 cells that expressed the hNET, hDAT, or hSERT. Curves were fitted by non-linear regression, and corresponding $IC_{50}$ values are shown in Table 1. The data are presented as the mean ± SEM. Numbers in parentheses indicate the number of individual experiments performed in triplicate (hNET/hDAT/hSERT): 2,3-DMMC (3/3/7), 2,4-DMMC (4/6/3), 3,4-DMMC (4/3/3), 3-MMC (3/3/3), 4-MMC (3/3/3), 5-IT (3/4/3), 4-MA (4/3/4), *N*-methyl-2-AI (3/6/5), MMAI (4/6/5).

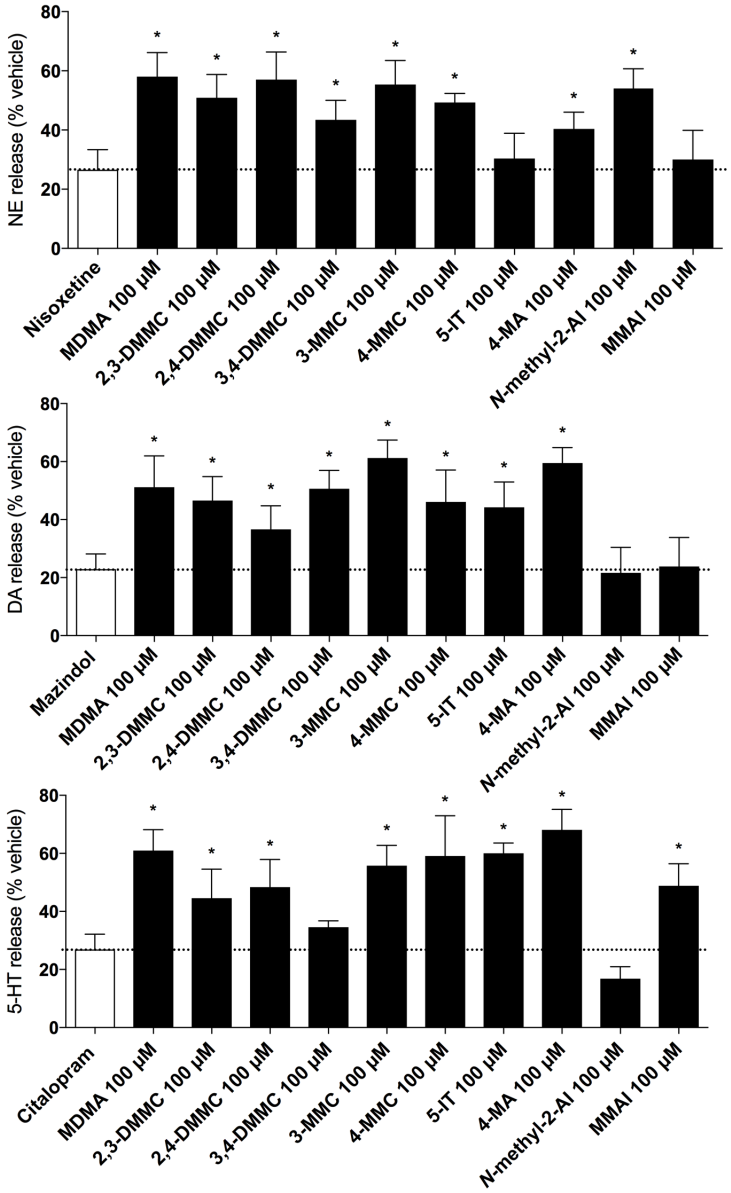


**Fig. 3.** Monoamine release induced by 100 µM of the drugs after preloading hNET-, hDAT-, or hSERT-expressing HEK 293 cells with radiolabeled monoamines. "Pseudo-efflux" that arose from monoamine diffusion and subsequent reuptake inhibition is marked with a dashed line. Substances that caused significantly higher monoamine efflux (*$p < 0.05$) than pure uptake inhibitors (open bars) were determined to be monoamine releasers. The data are presented as the mean ± SEM of five independent experiments.

# Tables

**Table 1.** Monoamine transport inhibition.

| | NET | DAT | SERT | DAT/SERT |
|---|---|---|---|---|
| | $IC_{50}$ [μM] (95% CI) | $IC_{50}$ [μM] (95% CI) | $IC_{50}$ [μM] (95% CI) | ratio (95% CI) |
| Cathinones | | | | |
| 3-MMC | 0.27 (0.21-0.36) | 2.6 (2.0-3.3) | 9.5 (6.9-13.2) | 3.7 (2.1-6.6) |
| 4-MMC | 0.26 (0.19-0.35) | 5.7 (4.5-7.2) | 3.6 (2.8-4.6) | 0.63 (0.39-1.02) |
| 2,3-DMMC | 0.53 (0.36-0.78) | 7.4 (5.4-10.1) | 1.2 (1.0-1.4) | 0.16 (0.10-0.26) |
| 3,4-DMMC | 0.45 (0.33-0.60) | 9.4 (7.6-11.7) | 1.1 (0.9-1.4) | 0.12 (0.08-0.18) |
| 2,4-DMMC | 1.5 (1.1-2.0) | 83 (65-105) | 1.5 (1.0-2.2) | 0.02 (0.01-0.03) |
| Phenethylamines | | | | |
| 5-IT | 0.04 (0.03-0.06) | 0.68 (0.55-0.85) | 1.3 (0.9-1.7) | 1.9 (1.1-3.1) |
| 4-MA | 0.31 (0.24-0.42) | 5.6 (4.5-6.9) | 0.82 (0.64-1.05) | 0.15 (0.09-0.23) |
| Aminoindanes | | | | |
| *N*-methyl-2-AI | 2.4 (1.9-3.1) | 90 (71-113) | 223 (175-284) | 2.5 (1.5-4.0) |
| MMAI | 3.6 (2.5-5.3) | 193 (167-225) | 0.68 (0.50-0.92) | 0.004 (0.002-0.006) |

Values are means and 95% confidence intervals (CI). DAT/SERT ratio = 1/DAT $IC_{50}$ : 1/SERT $IC_{50}$.

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

| | NET | DAT | SERT | $D_2$ | $\alpha_{1A}$ | $\alpha_{2A}$ | 5-$HT_{1A}$ | 5-$HT_{2A}$ | 5-$HT_{2A}$ | | 5-$HT_{2B}$ | | 5-$HT_{2C}$ | $TA1_{rat}$ | $TA1_{mouse}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Ki* | *Ki* | *Ki* | *Ki* | *Ki* | *Ki* | *Ki* | *Ki* | $EC_{50}$ | $E_{max}$ | $EC_{50}$ | $E_{max}$ | *Ki* | *Ki* | *Ki* |
| Cathinones | | | | | | | | | | | | | | | |
| 2,3-DMMC | 8.4±0.3 | 4.2±0.6 | 6.1±0.5 | >11 | 0.78±0.10 | 3.0±0.1 | >17 | 0.64±0.19 | 0.13±0.02 | 84±12 | >10 | | 2.4±0.9 | 1.2±0.1 | 0.88±0.06 |
| 2,4-DMMC | >26 | >26 | 17±1 | >11 | 0.16±0.02 | 3.0±0.3 | 15±3 | 1.3±0.1 | >10 | | >10 | | 1.3±0.3 | 0.59±0.08 | 3.1±0.2 |
| 3,4-DMMC | 12±2 | 7.6±0.6 | 5.7±0.3 | >11 | 1.9±0.3 | 3.5±0.2 | >17 | 1.9±0.3 | >10 | | >10 | | 1.5±0.2 | 2.6±0.2 | 4.5±0.4 |
| 3-MMC | 5.6±1.5 | 3.2±0.6 | >22 | >12 | 7.9±0.2 | 1.1±0.1 | 4.8±0.5 | 3.4±0.8 | >20 | | >20 | | 3.6±1.0 | 5.7±1.4[a] | 10±1[a] |
| 4-MMC | >26 | 2.9±0.2 | >22 | >11 | 1.1±0.1 | 11±1 | >17 | 1.6±0.2 | 0.36±0.19 | 79±20 | >10 | | 8.1±5.4 | 5.0±0.1 | 12±1 |
| Phenethylamines | | | | | | | | | | | | | | | |
| 5-IT | 1.3±0.3 | 0.92±0.13 | 10±2 | >25 | 5.4±0.5 | 1.7±0.1 | 11±2 | 0.38±0.11 | 0.49±0.17 | 42±9 | 1.5±0.6 | 36±5 | 3.0±0.8 | 0.15±0.02[a] | 0.36±0.15[a] |
| 4-MA | 9.4±1.2 | 9.4±0.9 | 13±3 | >25 | >12 | 2.1±0.4 | 18±6 | 3.3±0.5 | 3.3±1.0 | 71±4 | 0.86±0.38 | 54±8 | 6.3±1.1 | 0.10±0.01[a] | 0.15±0.07[a] |
| Aminoindanes | | | | | | | | | | | | | | | |
| *N*-methyl-2-AI | >30 | >30 | >30 | >25 | >12 | 0.49±0.07 | 3.6±0.1 | 5.4±0.9 | >20 | | >20 | | >15 | 0.53±0.04[a] | 2.6±0.1[a] |
| MMAI | >26 | >26 | 11±1 | >11 | 4.0±0.2 | 1.0±0.1 | 1.6±0.2 | 8.3±1.3 | | | >10 | | 5.4±1.4 | 0.14±0.02 | 4.9±1.1 |

*Ki* and $EC_{50}$ values are given as μM (mean±SD); activation efficacy ($E_{max}$) is given as percentage of maximum±SD.

[a]Values are from Simmler et al., 2016.