

## **Pharmacological profile of methylphenidate-based designer drugs**

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

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## **Abstract**

*Background:* Methylphenidate-based designer drugs are new psychoactive substances (NPS) that are used outside medical settings and their pharmacology is largely unexplored. The aim of the present study was to characterize the pharmacology of methylphenidate-based substances *in vitro*.

*Methods:* We determined the potencies of the methylphenidate-based NPS *N*-benzylethylphenidate, 3,4-dichloroethylphenidate, 3,4-dichloromethylphenidate, ethylnaphthidate, ethylphenidate, 4-fluoromethylphenidate, isopropylphenidate, 4-methylmethylphenidate, methylmorphenate, and propylphenidate and the potencies of the related compounds cocaine and modafinil with respect to norepinephrine, dopamine, and serotonin transporter inhibition in transporter-transfected human embryonic kidney 293 cells. We also investigated monoamine efflux and monoamine receptor and transporter binding affinities. Furthermore, we assessed the cell integrity under assay conditions.

*Results:* All methylphenidate-based substances inhibited the norepinephrine and dopamine transporters 4 to >1,000-fold more potently than the serotonin transporter. Similar to methylphenidate and cocaine, methylphenidate-based NPS did not elicit transporter-mediated efflux of monoamines. Besides binding to monoamine transporters, several test drugs had affinity for adrenergic, serotonergic, and rat trace amine-associated receptors but not for dopaminergic or mouse trace amine-associated receptors. No cytotoxicity was observed after drug treatment at assay concentrations.

*Conclusion:* Methylphenidate-based substances had pharmacological profiles similar to methylphenidate and cocaine. The predominant actions on dopamine transporters vs. serotonin transporters may be relevant when considering abuse liability.

**Keywords:** methylphenidate, cocaine, new psychoactive substances, monoamine, receptor, transporter.

**Abbreviations:** 5-HT, 5-hydroxytryptamine (serotonin); DA, dopamine; DAT, dopamine transporter; FLIPR, fluorescence imaging plate reader; HPLC, high-performance liquid chromatography; MDMA, 3,4-methylenedioxymethamphetamine; MPH, methylphenidate; NE, norepinephrine; NET norepinephrine transporter; NPS, new psychoactive substances; SERT, serotonin transporter; TAAR, trace amine-associated receptor.

## **1. Introduction**

The psychostimulant methylphenidate (MPH; Ritalin<sup>®</sup>) is used for the treatment of attention-deficit/hyperactivity disorder and narcolepsy but it also has a history of being misused as a ‘smart drug’ and ‘cognitive enhancer’ (Arria et al., 2008; Liakoni et al., 2015; Maier et al., 2013). In recent years, an increasing number of MPH-based new psychoactive substances (NPS; Fig. 1) (Brandt et al., 2014) have become available as alternatives to MPH (Bailey et al., 2015; European Monitoring Centre for Drugs and Drug Addiction, 2015) and have been associated with several fatalities (Krueger et al., 2014; Maskell et al., 2016; Parks et al., 2015). Characteristic for the NPS phenomenon, many of the currently circulating MPH analogs originated from drug development efforts (Deutsch et al., 1996; Markowitz et al., 2013; Misra et al., 2010), which subsequently appeared on the streets. The pharmacological and subjective-effect profiles of MPH are very similar to cocaine (Simmler et al., 2014; Vogel et al., 2016; Volkow et al., 1999). Furthermore, some of these substances are either sold in their own right or offered in the form of branded products (Bailey et al., 2015; Parks et al., 2015). Methylphenidate predominantly inhibits the norepinephrine (NE) and dopamine (DA) transporters (NET and DAT, respectively), thus, possibly contributing to its abuse

potential (Simmler et al., 2014; Vogel et al., 2016). Correspondingly, questions arise about the extent to which MPH analogs might share MPH-like characteristics. Assessing the pharmacological profile of NPS *in vitro* is an initial step to gain a better understanding of the potential clinical effects and toxicology of these substances. For this reason, the present study reports on the transporter interaction profiles of the MPH-related NPS *N*-benzylethylphenidate, 3,4-dichloroethylphenidate, 3,4-dichloromethylphenidate, and isopropylphenidate and the transporter and receptor interaction profiles of ethylnaphthidate, ethylphenidate, 4-fluoromethylphenidate, 4-methylmethylphenidate, methylmorphenate, and propylphenidate. Modafinil, a stimulant prescribed for the treatment of narcolepsy, which is frequently offered for sale as a ‘neuroenhancer’ (Ghahremani et al., 2011; Maier et al., 2013; Mereu et al., 2013; Müller et al., 2013), has also been included in this investigation. Stimulants may act as transporter inhibitors or as transporter substrates that cause monoamine efflux into the synaptic cleft (Rothman and Baumann, 2003; Sitte and Freissmuth, 2015). Therefore, additionally to the transporter inhibition potencies of the substances, their mechanism of action (reuptake inhibitor or transporter substrate) was determined.

## **2. Material and methods**

### *2.1. Drugs*

Cocaine, 3,4-methylenedioxymethamphetamine (MDMA) and MPH were purchased from Lipomed (Arlesheim, Switzerland), with high-performance liquid chromatography (HPLC) purity > 98.5%. Modafinil was purchased from Cayman Chemicals (Ann Arbor, MI, USA), with purity > 98%. Methylmorphenate and propylphenidate were obtained from reChem Labs (Ontario, Canada) and afterwards identified and tested for purity using nuclear magnetic resonance (NMR) and HPLC, which revealed purity > 95%. *N*-Benzylethylphenidate, 3,4-dichloroethylphenidate, 3,4-dichloromethylphenidate,

ethylnaphthidate, 4-fluoromethylphenidate, isopropylphenidate, and 4-methylmethylphenidate were part of confiscations by German authorities and test purchases (Klare et al., 2017). The substances were fully characterized in a previous study (Klare et al., 2017) and purity values were estimated at > 95% based on spectroscopic and chromatographic methods of analysis. Ethylphenidate was provided by Dr. Christian Bissig (Forensic Institute, Zurich, Switzerland) after being confiscated by Swiss authorities and being tested for purity of > 98%. Modafinil was obtained as racemic base. The other drugs were obtained as racemic hydrochloride salts. Radiolabeled [<sup>3</sup>H]-NE (13.1 Ci/mmol) and [<sup>3</sup>H]-DA (30.0 Ci/mmol) were obtained from Perkin-Elmer (Schwerzenbach, Switzerland). Radiolabeled [<sup>3</sup>H]-5-HT (80 Ci/mmol) was purchased from Anawa (Zürich, Switzerland).

## 2.2. Monoamine uptake transport inhibition

Monoamine uptake inhibition was assessed using human embryonic kidney (HEK) 293 cells that stably expressed the human SERT, DAT, or NET (Tatsumi et al., 1997) as previously described (Hysek et al., 2012). Briefly, the cells were cultured to 70-90% confluence, detached, and resuspended in Krebs-Ringer Bicarbonate Buffer (Sigma-Aldrich, Buchs, Switzerland). For [<sup>3</sup>H]-DA uptake experiments, the uptake buffer was supplemented with 1.14 mM ascorbic acid. The cells were then treated with vehicle control and drug in the range of 1 nM to 900 μM for 10 min at room temperature. Additionally, monoamine-specific inhibitors were added (10 μM fluoxetine for SERT, 10 μM mazindol for DAT, and 10 μM nisoxetine for NET). To initiate uptake transport, [<sup>3</sup>H]-5-HT, [<sup>3</sup>H]-DA, or [<sup>3</sup>H]-NE were added at a final concentration of 5 nM for an additional 10 min. The cells were then separated from the uptake buffer by centrifugation through silicone oil, and the tubes were frozen in liquid nitrogen. The cell pellet was cut into scintillation vials and lysed. The samples were shaken for 1 h before 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. Uptake in the presence of the selective inhibitors was determined to be nonspecific and subtracted from the total counts.

### *2.3. Transporter-mediated monoamine efflux*

The potential of the drugs to initiate transporter-mediated NE, DA, or 5-HT efflux was assessed in HEK 293 cells that overexpressed the respective transporter as previously described (Simmler et al., 2013). Briefly, the cells were first preloaded with [<sup>3</sup>H]-NE, [<sup>3</sup>H]-DA, or [<sup>3</sup>H]-5-HT dissolved in Krebs-HEPES buffer for 20 min at 37°C. The cells were then washed and treated with 100 μM of the drugs for 15 min (DAT and SERT) or 45 min (NET). The treatment durations for [<sup>3</sup>H]-NE, [<sup>3</sup>H]-DA, and [<sup>3</sup>H]-5-HT efflux experiments were based on kinetic evaluation of the efflux-over-time curves of MDMA (Simmler et al., 2014). The cells were washed again, and the remaining radioactivity inside the cells was quantified. The monoamine transporter blockers citalopram (SERT), mazindol (DAT), and nisoxetine (NET) were added as a negative control at a concentration of 10 μM to determine “pseudo-efflux” that was caused by nonspecific monoamine efflux and subsequent reuptake inhibition (Scholze et al., 2000).

### *2.4. Radioligand receptor and transporter binding assays*

The radioligand binding assays were performed as previously described for transporters (Hysek et al., 2012) and receptors (Revel et al., 2011). Briefly, membrane preparations of HEK 293 cells (Invitrogen, Zug, Switzerland) that overexpressed the respective transporters (Tatsumi et al., 1997) or receptors (human genes, with the exception of rat and mouse genes for trace amine-associated receptors [TAARs]) (Revel et al., 2011)

were incubated with the radiolabeled selective ligands at concentrations equal to  $K_d$ , and ligand displacement by the compounds was measured. Specific binding of the radioligand to the target receptor was defined as the difference between the total binding and nonspecific binding that was determined in the presence of the selected competitors. The following radioligands and competitors, respectively, were used: *N*-methyl-[<sup>3</sup>H]-nisoxetine and 10  $\mu$ M indatraline (NET), [<sup>3</sup>H]citalopram and 10  $\mu$ M indatraline (SERT), [<sup>3</sup>H]WIN35,428 and 10  $\mu$ M indatraline (DAT), [<sup>3</sup>H]8-hydroxy-2-(di-*n*-propylamine)tetralin and 10  $\mu$ M pindolol (5-HT<sub>1A</sub> receptor), [<sup>3</sup>H]ketanserin and 10  $\mu$ M spiperone (5-HT<sub>2A</sub> receptor), [<sup>3</sup>H]mesulgerine and 10  $\mu$ M mianserin (5-HT<sub>2C</sub> receptor), [<sup>3</sup>H]prazosin and 10  $\mu$ M chlorpromazine ( $\alpha_{1A}$  adrenergic receptor), [<sup>3</sup>H]rauwolscine and 10  $\mu$ M phentolamine ( $\alpha_{2A}$  adrenergic receptor), [<sup>3</sup>H]spiperone and 10  $\mu$ M spiperone (D<sub>2</sub> receptor), and [<sup>3</sup>H]RO5166017 and 10  $\mu$ M RO5166017 (TAAR<sub>1</sub>).

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

Activity at the 5-HT<sub>2B</sub> receptor was assessed as previously described (Rickli et al., 2016). Briefly, human 5-HT<sub>2B</sub> receptor-expressing HEK 293 cells were incubated in a cell culture plate overnight. The next day, the growth medium was removed by snap inversion, and calcium indicator Fluo-4 solution (Molecular Probes, Eugene, OR, USA) was added to each well. The plates were then incubated for 45 min at 31°C. The Fluo-4 solution was removed by snap inversion and then added a second time. The cells were then incubated for another 45 min at 31°C. Immediately before testing, the cells were washed with HBSS and 20 mM HEPES (assay buffer; Gibco) using an EMBLA cell washer, and assay buffer was added. The plates were placed in a FLIPR. Test substances that were diluted in assay buffer were added online, and the increase in fluorescence was measured.

### 2.6. Cytotoxicity

Cytotoxicity was assessed with the ToxiLight bioassay kit (Lonza, Basel, Switzerland) according to the manufacturer's protocol. The kit measures adenylate kinase release as a result of cell membrane integrity loss. Human SERT-, DAT-, and NET-transfected HEK 293 cells were treated for 1 h at room temperature with the drugs at the highest assay concentrations.

### 2.7. Statistical analysis

Calculations were performed using Prism 7.0a software (GraphPad, San Diego, CA, USA). Monoamine transporter inhibition data were fit by nonlinear regression to variable-slope sigmoidal dose-response curves and IC<sub>50</sub> values were assessed. The DAT/SERT ratio is expressed as 1/DAT IC<sub>50</sub>:1/SERT IC<sub>50</sub>. Compound-induced monoamine efflux of five independent experiments was compared with negative controls using analysis of variance followed by the Holm-Sidak test. *P* values lower than 0.05 were considered significant and substances were considered transporter substrates if they caused significantly higher efflux than the negative controls. IC<sub>50</sub> 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 compound. *K<sub>i</sub>* (affinity) values, which correspond to the dissociation constants, were determined using the Cheng-Prusoff equation. Nonlinear regression concentration-response curves were used to calculate the EC<sub>50</sub> values for the 5-HT<sub>2B</sub> receptor activation.

## 3. Results

### 3.1. Monoamine uptake transporter inhibition

Monoamine uptake inhibition curves are shown in Fig. 2, and the corresponding IC<sub>50</sub> values and DAT/SERT inhibition ratios are listed in Table 1. Methylphenidate was a potent



inhibitor of the NET and DAT at submicromolar concentrations and a weak inhibitor of the SERT. 3,4-dichloromethylphenidate inhibited the NET more than 10-fold more potently than MPH, whereas the inhibition potency for the DAT was more than 2-fold increased. The NET and DAT inhibition potencies of 3,4-dichloroethylphenidate, ethylnaphthidate, 4-fluoromethylphenidate, and 4-methylmethylphenidate were similar to MPH in the range of 0.04–0.42  $\mu\text{M}$  for the NET and 0.08–0.34  $\mu\text{M}$  for the DAT. *N*-Benzylethylphenidate, ethylphenidate, isopropylphenidate, methylmorphenate, and propylphenidate inhibited the NET with 6 to 800-fold lower potency compared to MPH and the DAT with 4 to 500-fold lower potency. The SERT inhibition potency for all MPH-based NPS was lower than the NET and DAT inhibition potencies. Ethylnaphthidate inhibited the SERT at 1.7  $\mu\text{M}$  with a DAT/SERT ratio of 5. The remaining compounds inhibited the SERT 40 to >1000-fold weaker than the DAT and 26 to >1000-fold weaker than the NET. Modafinil was a weak inhibitor of monoamine transporters with an  $\text{IC}_{50}$  value >10  $\mu\text{M}$  for the DAT and no relevant NET or SERT inhibition ( $\text{IC}_{50}$  values >100  $\mu\text{M}$ ). Unlike the MPH-based substances and modafinil, cocaine inhibited all three transporters with similar potency in the range of 0.5–1.5  $\mu\text{M}$ .

### 3.2. Monoamine efflux

Similar to cocaine, MPH and the MPH-based NPS and related compounds did not cause monoamine efflux (Fig. 3) and are therefore not transporter substrates.

### 3.3. Monoamine receptor and transporter binding affinities

The interactions between MPH-based NPS and related compounds with monoamine receptors and transporters are shown in Table 2. All MPH-based NPS bound to the NET and DAT but only *N*-benzylethylphenidate, 3,4-dichloroethylphenidate, 3,4-

dichloromethylphenidate, and ethylnaphthidate bound to the SERT in the concentration range tested. 3,4-Dichloroethylphenidate, 3,4-dichloromethylphenidate, 4-methylmethylphenidate, and ethylnaphthidate bound to the  $\alpha_{1A}$  receptor in the range of 1.7–6.5  $\mu\text{M}$  and additionally to the  $\alpha_{2A}$  receptor in the range of 7–10  $\mu\text{M}$ . Ethylphenidate and propylphenidate bound to the  $\alpha_{2A}$  receptor with 14  $\mu\text{M}$  and 8.7  $\mu\text{M}$ , respectively, but did not bind to the  $\alpha_{1A}$  receptor in the investigated concentration range. 3,4-Dichloroethylphenidate, 3,4-dichloromethylphenidate, ethylnaphthidate, 4-methylmethylphenidate, and propylphenidate, had affinities of 1–17  $\mu\text{M}$  for the 5-HT<sub>1A</sub> receptor. Ethylnaphthidate was the only drug to bind to the 5-HT<sub>2A</sub> receptor with an IC<sub>50</sub> value of 4.9  $\mu\text{M}$  and only 3,4-dichloromethylphenidate and ethylnaphthidate bound to the 5-HT<sub>2C</sub> receptor, both with an IC<sub>50</sub> of 12  $\mu\text{M}$ . None of the compounds activated the 5-HT<sub>2B</sub> receptor or bound to the mouse TAAR<sub>1</sub>, and only 3,4-dichloroethylphenidate, 3,4-dichloromethylphenidate, and isopropylphenidate bound to the rat TAAR<sub>1</sub> with affinities in the range of 6–13  $\mu\text{M}$ . None of the MPH-based NPS had relevant affinity for D<sub>2</sub> receptors. Modafinil and cocaine bound to the monoamine transporters but did not interact with monoamine or trace amine receptors.

### 3.4. Cytotoxicity

Cytotoxicity was not observed for any of the drugs in the functional assays at the concentrations tested, thus confirming cell integrity during the assays.

## 4. Discussion

We characterized the *in vitro* pharmacological profiles of MPH-based NPS and compared them with MPH and cocaine. All compounds inhibited the DAT substantially more potently than the SERT, suggesting predominantly stimulant-type effects similar to amphetamine and a high abuse liability (Liechti, 2015; Simmler et al., 2013).

#### 4.1. Monoamine uptake transporter inhibition and monoamine efflux

Methylphenidate and MPH-based NPS, with the exception of *N*-benzylethylphenidate, isopropylphenidate, and methylmorphenate, inhibited the NET at submicromolar concentrations, suggesting cardiostimulant and psychostimulant properties, similar to amphetamines (Hysek et al., 2011; Simmler et al., 2013). Moreover, the NET and DAT inhibition potencies but not the SERT inhibition potency correlate with the psychotropic effective doses of psychostimulants in human (Simmler et al., 2013).

The MPH-based NPS were only monoamine transporter inhibitors and not monoamine transporter substrates, indicating a mechanism of action similar to cocaine but not amphetamines (Fleckenstein et al., 2007; Torres et al., 2003). Ethylnaphthidate inhibited the SERT at low micromolar concentrations, but the remaining MPH-based NPS displayed a clear preference for DAT over SERT, resulting in high DAT/SERT ratios frequently reported for locomotor stimulants (Simmler et al., 2013). Our results are consistent with other studies that reported potent NET and DAT inhibition for MPH (DAT/SERT ratio = 2207) and triple uptake inhibition for cocaine (DAT/SERT ratio = 3.2) (Han and Gu, 2006). Modafinil was a moderate and relatively selective DAT inhibitor, with an  $IC_{50}$  value of 11  $\mu$ M. This finding is consistent with previous *in vitro* studies that reported  $IC_{50}$  values of 4–13  $\mu$ M (Karabacak et al., 2015; Loland et al., 2012; Madras et al., 2006; Zolkowska et al., 2009). The interaction between modafinil and DAT is also thought to modulate the pharmacological effects of the drug (Wisor, 2013). The psychopharmacological profiles and cognitive-enhancing properties of MPH and modafinil may be different. Modafinil has been shown to improve attention and wakefulness, whereas MPH has been shown to improve memory (Repantis et al., 2010).

#### 4.2. Transporter and receptor binding profiles

Compared with ethylphenidate, replacement of the benzene ring with naphthalene (ethylnaphthidate) increased the potency in inhibiting the SERT and increased the affinity for 5-HT receptors. Many stimulant NPS interact with TAARs (Simmler et al., 2016); however, no potent TAAR interactions were found for MPH-based NPS. 3,4-Dichloromethylphenidate and ethylnaphthidate interacted with the  $\alpha_{1A}$  and 5-HT<sub>1A</sub> receptor in the low micromolar range. The remaining MPH-based NPS did not potently interact with monoamine receptors, indicating that they exert their primary effects by inhibiting uptake transporters, similar to MPH and cocaine (Ritz et al., 1987, 1988; Volkow et al., 2002). Consistent with the monoamine uptake data, 3,4-dichloroethylphenidate, 3,4-dichloromethylphenidate, and ethylnaphthidate potently bound to the NET and DAT and had affinity for the SERT as well. 4-Fluoromethylphenidate, 4-methylmethylphenidate, and methylphenidate bound potently to the NET and DAT but had no affinity to the SERT in the tested concentration range. *N*-Benzylethylphenidate, ethylphenidate, isopropylphenidate, and propylphenidate showed high affinity for the DAT but not for the NET or SERT. Methylnormphenate did not potently bind to any transporter. Cocaine potently bound to all transporters but not to receptors. No interaction between modafinil and monoamine receptors was observed. To date, no single site of action for modafinil has been identified (Gerrard and Malcolm, 2007).

#### *4.3. Comparison of transporter binding and transporter inhibition*

No drug-mediated monoamine efflux was observed for any of the MPH-based compounds, strengthening the argument that they are pure uptake blockers. For uptake blockers, a correlation between the monoamine uptake and radioligand binding affinities has been previously described for the NET (Cheetham et al., 1996; Lee et al., 1982), the DAT (Javitch et al., 1984; Schoemaker et al., 1985), and the SERT (D'Amato et al., 1987; Langer et al., 1980). However, discrepancies between monoamine uptake inhibition and

radioligand binding have been observed for cocaine-like drugs and proposed for MPH-like drugs, when the conditions for the binding and uptake inhibition assays varied (Reith et al., 2005; Rothman et al., 1993).

Highest NET and DAT binding affinities were observed for the most potent NET and DAT inhibitor 3,4-dichloromethylphenidate. However, the increase in potency compared to MPH was much more pronounced with a 76-fold and 12-fold increase for NET and DAT binding, respectively. 3,4-Dichloroethylphenidate bound more than 10-fold more potently to the NET and DAT whereas the NE and DA uptake inhibition was similar to MPH. Ethylnaphthidate, 4-fluoromethylphenidate, and 4-methylmethylphenidate inhibited the NET and DAT with similar potency as MPH. These substances bound to the NET with affinity in the range of 0.22–0.31  $\mu\text{M}$  and to the DAT with affinity in the range of 0.026–0.040  $\mu\text{M}$ . MPH bound to the NET and DAT with 0.50  $\mu\text{M}$  and 0.070  $\mu\text{M}$ , respectively. Thus, unlike for the dichloro substituted compounds, the  $\text{IC}_{50}$  values and the  $K_i$  values for ethylnaphthidate, 4-fluoromethylphenidate, and 4-methylmethylphenidate correlate well. *N*-Benzylethylphenidate, ethylphenidate, isopropylphenidate, methylmorphenate, and propylphenidate inhibited the NET with 6 to 800-fold lower potency compared to MPH and the DAT with 4 to 500-fold lower potency. While the binding affinities for the NET were 8 to 48-fold decreased, the DAT binding affinities were decreased only for *N*-benzylethylphenidate, methylmorphenate, and propylphenidate (5 to 46-fold) whereas the DAT binding affinities of ethylphenidate and isopropylphenidate were close to MPH. Remarkably, *N*-benzylethylphenidate was by far the weakest transporter inhibitor, it did however not have the lowest NET and DAT binding affinities. In the investigated concentration range, only *N*-benzylethylphenidate, 3,4-dichloroethylphenidate, 3,4-dichloromethylphenidate, and ethylnaphthidate bound to the SERT. 3,4-dichloroethylphenidate, 3,4-dichloromethylphenidate, and ethylnaphthidate, were the most

potent SERT inhibitors, *N*-benzylethylphenidate was however the weakest SERT inhibitor. Thus, as observed for the NET and DAT, the SERT binding affinity of *N*-benzylethylphenidate was much higher than might be expected from the uptake inhibition data.

To conclude, the rank order of potency of the radioligand binding and uptake inhibition was similar with the 3,4-substituted and 4-substituted compounds being among the most potent MPH-based NPS; the relative potencies of the uptake inhibition and transporter binding varied however to a certain extent.

Besides cocaine, ethylnaphthidate was the only compound to have considerable inhibition potencies and affinities for all transporters. The inhibition and binding potencies generally decreased with increasing size of the carbon side chain. Compared to MPH, the steric ring-substitution of *N*-benzylethylphenidate substantially decreased the inhibition potency for all transporters and the binding to the NET and DAT. However, higher binding affinity for the SERT was observed. Modafinil selectively inhibited and bound to the SERT.

The present study has limitations. Possible potent contaminants could theoretically have influenced the results for some drugs with lower purity. Substance-induced efflux was only tested at a high substance concentration. The absence of monoamine efflux could be the result of bell-shaped concentration-efflux curves as it has been demonstrated for amphetamine analogs with known monoamine releasing properties, including MDMA, in different *in vitro* assays (Seidel et al., 2005). However, such bell-shaped efflux curves were not observed in the assay used in the present study as previously documented (Hysek et al., 2012), strengthening the argument that the MPH-based NPS are in fact pure uptake inhibitors. Moreover, in this study the focus was laid on the NET, DAT, and SERT, as they are main targets of amphetamines and presumably many stimulant NPS (Sitte and Freissmuth, 2015). Other possible mechanisms that may contribute to the effects of NPS,

such as VMAT2 inhibition (Sulzer et al., 2005), calcium-triggered exocytosis of monoamines (Mundorf et al., 1999; Sulzer et al., 2005), mRNA regulation (Douglass et al., 1995), or ion channel blockage (Bauman and DiDomenico, 2002; O'Leary and Hancox, 2010), were not investigated in this study.

## **5. Conclusion**

Similar to MPH and cocaine, MPH-based NPS are potent inhibitors of the NET and DAT. Furthermore, they are not monoamine transporter substrates and have only minor interactions with monoamine receptors. The high selectivity for the DAT vs. SERT suggests that these emerging drugs may have abuse potential. Modafinil is a weak but selective inhibitor at DAT but does not present monoamine receptor interactions.

## **Author contributions**

D.L., S.D.B., S.K., and M.E.L. designed research. D.L., P.J.K., and M.C.H. performed research. D.L., M.C.H., and M.E.L. analysed data. D.L. and M.E.L. wrote the paper with input from all other authors.

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## **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

- Arria, A.M., Caldeira, K.M., O'Grady, K.E., Vincent, K.B., Johnson, E.P., Wish, E.D., 2008. Nonmedical use of prescription stimulants among college students: associations with attention-deficit-hyperactivity disorder and polydrug use. *Pharmacotherapy* 28, 156-169.
- Bailey, G.P., Ho, J.H., Hudson, S., Dines, A., Archer, J.R., Dargan, P.I., Wood, D.M., 2015. Nopaine no gain: recreational ethylphenidate toxicity. *Clin Toxicol (Phila)* 53, 498-499.
- Bauman, J.L., DiDomenico, R.J., 2002. Cocaine-induced channelopathies: emerging evidence on the multiple mechanisms of sudden death. *J Cardiovasc Pharmacol Ther* 7, 195-202.
- Brandt, S.D., King, L.A., Evans-Brown, M., 2014. The new drug phenomenon. *Drug Test Anal* 6, 587-597.
- Cheetham, S.C., Viggers, J.A., Butler, S.A., Prow, M.R., Heal, D.J., 1996. [3H]nisoxetine--a radioligand for noradrenaline reuptake sites: correlation with inhibition of [3H]noradrenaline uptake and effect of DSP-4 lesioning and antidepressant treatments. *Neuropharmacology* 35, 63-70.
- D'Amato, R.J., Largent, B.L., Snowman, A.M., Snyder, S.H., 1987. Selective labeling of serotonin uptake sites in rat brain by [3H]citalopram contrasted to labeling of multiple sites by [3H]imipramine. *J Pharmacol Exp Ther* 242, 364-371.
- Deutsch, H.M., Shi, Q., Gruszecka-Kowalik, E., Schweri, M.M., 1996. Synthesis and pharmacology of potential cocaine antagonists. 2. Structure-activity relationship studies of aromatic ring-substituted methylphenidate analogs. *J Med Chem* 39, 1201-1209.



Douglass, J., McKinzie, A.A., Couceyro, P., 1995. PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. *J Neurosci* 15, 2471-2481.

European Monitoring Centre for Drugs and Drug Addiction, 2015. Europol 2015 Annual Report on the implementation of Council Decision 2005/387/JHA, <http://www.emcdda.europa.eu/system/files/publications/2880/TDAS16001ENN.pdf>. Accessed 09 October 2016.

Fleckenstein, A.E., Volz, T.J., Riddle, E.L., Gibb, J.W., Hanson, G.R., 2007. New insights into the mechanism of action of amphetamines. *Annu Rev Pharmacol Toxicol* 47, 681-698.

Gerrard, P., Malcolm, R., 2007. Mechanisms of modafinil: A review of current research. *Neuropsychiatr Dis Treat* 3, 349-364.

Ghahremani, D.G., Tabibnia, G., Monterosso, J., Helleman, G., Poldrack, R.A., London, E.D., 2011. Effect of modafinil on learning and task-related brain activity in methamphetamine-dependent and healthy individuals. *Neuropsychopharmacology* 36, 950-959.

Han, D.D., Gu, H.H., 2006. Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC Pharmacol* 6, 6.

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.

Javitch, J.A., Blaustein, R.O., Snyder, S.H., 1984. [3H]mazindol binding associated with neuronal dopamine and norepinephrine uptake sites. Mol Pharmacol 26, 35-44.

Karabacak, Y., Sase, S., Aher, Y.D., Sase, A., Saroja, S.R., Cicvaric, A., Höger, H., Berger, M., Bakulev, V., Sitte, H.H., Leban, J., Monje, F.J., Lubec, G., 2015. The effect of modafinil on the rat dopamine transporter and dopamine receptors D1-D3 paralleling cognitive enhancement in the radial arm maze. Front Behav Neurosci 9, 215.

Klare, H., Neudorfl, J.M., Brandt, S.D., Mischler, E., Meier-Giebing, S., Deluweit, K., Westphal, F., Laussmann, T., 2017. Analysis of six "neuro-enhancing" phenidate analogs. Drug Test Anal.

Krueger, J., Sachs, H., Musshoff, F., Dame, T., Schaeper, J., Schwerer, M., Graw, M., Roider, G., 2014. First detection of ethylphenidate in human fatalities after ethylphenidate intake. Forensic Sci Int 243, 126-129.

Langer, S.Z., Moret, C., Raisman, R., Dubocovich, M.L., Briley, M., 1980. High-affinity [3H]imipramine binding in rat hypothalamus: association with uptake of serotonin but not of norepinephrine. Science 210, 1133-1135.

Lee, C.M., Javitch, J.A., Snyder, S.H., 1982. Characterization of [3H]desipramine binding associated with neuronal norepinephrine uptake sites in rat brain membranes. J Neurosci 2, 1515-1525.

Liakoni, E., Schaub, M.P., Maier, L.J., Glauser, G.V., Liechti, M.E., 2015. The use of prescription drugs, recreational drugs, and "soft enhancers" for cognitive enhancement among Swiss secondary school students. *PLoS One* 10, e0141289.

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

Loland, C.J., Mereu, M., Okunola, O.M., Cao, J., Prisinzano, T.E., Mazier, S., Kopajtic, T., Shi, L., Katz, J.L., Tanda, G., Newman, A.H., 2012. R-modafinil (armodafinil): a unique dopamine uptake inhibitor and potential medication for psychostimulant abuse. *Biol Psychiatry* 72, 405-413.

Madras, B.K., Xie, Z., Lin, Z., Jassen, A., Panas, H., Lynch, L., Johnson, R., Livni, E., Spencer, T.J., Bonab, A.A., Miller, G.M., Fischman, A.J., 2006. Modafinil occupies dopamine and norepinephrine transporters in vivo and modulates the transporters and trace amine activity in vitro. *J Pharmacol Exp Ther* 319, 561-569.

Maier, L.J., Liechti, M.E., Herzig, F., Schaub, M.P., 2013. To dope or not to dope: neuroenhancement with prescription drugs and drugs of abuse among Swiss university students. *PLoS One* 8, e77967.

Markowitz, J.S., Zhu, H.J., Patrick, K.S., 2013. Isopropylphenidate: an ester homolog of methylphenidate with sustained and selective dopaminergic activity and reduced drug interaction liability. *J Child Adolesc Psychopharmacol* 23, 648-654.

Maskell, P.D., Smith, P.R., Cole, R., Hikin, L., Morley, S.R., 2016. Seven fatalities associated with ethylphenidate. *Forensic Sci Int* 265, 70-74.

Mereu, M., Bonci, A., Newman, A.H., Tanda, G., 2013. The neurobiology of modafinil as an enhancer of cognitive performance and a potential treatment for substance use disorders. *Psychopharmacology (Berl)* 229, 415-434.

Misra, M., Shi, Q., Ye, X., Gruszecka-Kowalik, E., Bu, W., Liu, Z., Schweri, M.M., Deutsch, H.M., Venanzi, C.A., 2010. Quantitative structure-activity relationship studies of threo-methylphenidate analogs. *Bioorg Med Chem* 18, 7221-7238.

Müller, U., Rowe, J.B., Rittman, T., Lewis, C., Robbins, T.W., Sahakian, B.J., 2013. Effects of modafinil on non-verbal cognition, task enjoyment and creative thinking in healthy volunteers. *Neuropharmacology* 64, 490-495.

Mundorf, M.L., Hochstetler, S.E., Wightman, R.M., 1999. Amine weak bases disrupt vesicular storage and promote exocytosis in chromaffin cells. *J Neurochem* 73, 2397-2405.

O'Leary, M.E., Hancox, J.C., 2010. Role of voltage-gated sodium, potassium and calcium channels in the development of cocaine-associated cardiac arrhythmias. *Br J Clin Pharmacol* 69, 427-442.

Parks, C., McKeown, D., Torrance, H.J., 2015. A review of ethylphenidate in deaths in east and west Scotland. *Forensic Sci Int* 257, 203-208.

Reith, M.E., Wang, L.C., Dutta, A.K., 2005. Pharmacological profile of radioligand binding to the norepinephrine transporter: instances of poor indication of functional activity. *J Neurosci Methods* 143, 87-94.

Repantis, D., Schlattmann, P., Laisney, O., Heuser, I., 2010. Modafinil and methylphenidate for neuroenhancement in healthy individuals: A systematic review. *Pharmacol Res* 62, 187-206.

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., 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.

Ritz, M.C., Lamb, R.J., Goldberg, S.R., Kuhar, M.J., 1987. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 237, 1219-1223.

Ritz, M.C., Lamb, R.J., Goldberg, S.R., Kuhar, M.J., 1988. Cocaine self-administration appears to be mediated by dopamine uptake inhibition. *Prog Neuropsychopharmacol Biol Psychiatry* 12, 233-239.

Rothman, R.B., Baumann, M.H., 2003. Monoamine transporters and psychostimulant drugs. *Eur J Pharmacol* 479, 23-40.

Rothman, R.B., Becketts, K.M., Radesca, L.R., de Costa, B.R., Rice, K.C., Carroll, F.I., Dersch, C.M., 1993. Studies of the biogenic amine transporters. II. A brief study on the use of [3H]DA-uptake-inhibition to transporter-binding-inhibition ratios for the in vitro evaluation of putative cocaine antagonists. *Life Sci* 53, P1267-272.

Schoemaker, H., Pimoule, C., Arbilla, S., Scatton, B., Javoy-Agid, F., Langer, S.Z., 1985. Sodium dependent [3H]cocaine binding associated with dopamine uptake sites in the rat striatum and human putamen decrease after dopaminergic denervation and in Parkinsons disease. *Naunyn Schmiedebergs Arch Pharmacol* 329, 227-235.

Scholze, P., Zwach, J., Kattinger, A., Piffl, 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.

Seidel, S., Singer, E.A., Just, H., Farhan, H., Scholze, P., Kudlacek, O., Holy, M., Koppatz, K., Krivanek, P., Freissmuth, M., Sitte, H.H., 2005. Amphetamines take two to tango: an oligomer-based counter-transport model of neurotransmitter transport explores the amphetamine action. *Mol Pharmacol* 67, 140-151.

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., Schramm, Y., Hoener, M.C., Liechti, M.E., 2014. 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.

Sulzer, D., Sonders, M.S., Poulsen, N.W., Galli, A., 2005. Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol* 75, 406-433.

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.

Torres, G.E., Gainetdinov, R.R., Caron, M.G., 2003. Plasma membrane monoamine transporters: structure, regulation and function. *Nat Rev Neurosci* 4, 13-25.

Vogel, M., Bucher, P., Strasser, J., Liechti, M.E., Krähenbühl, S., Dürsteler, K.M., 2016. Similar and different? Subjective effects of methylphenidate and cocaine in opioid-maintained patients. *J Psychoactive Drugs* 48, 93-100.

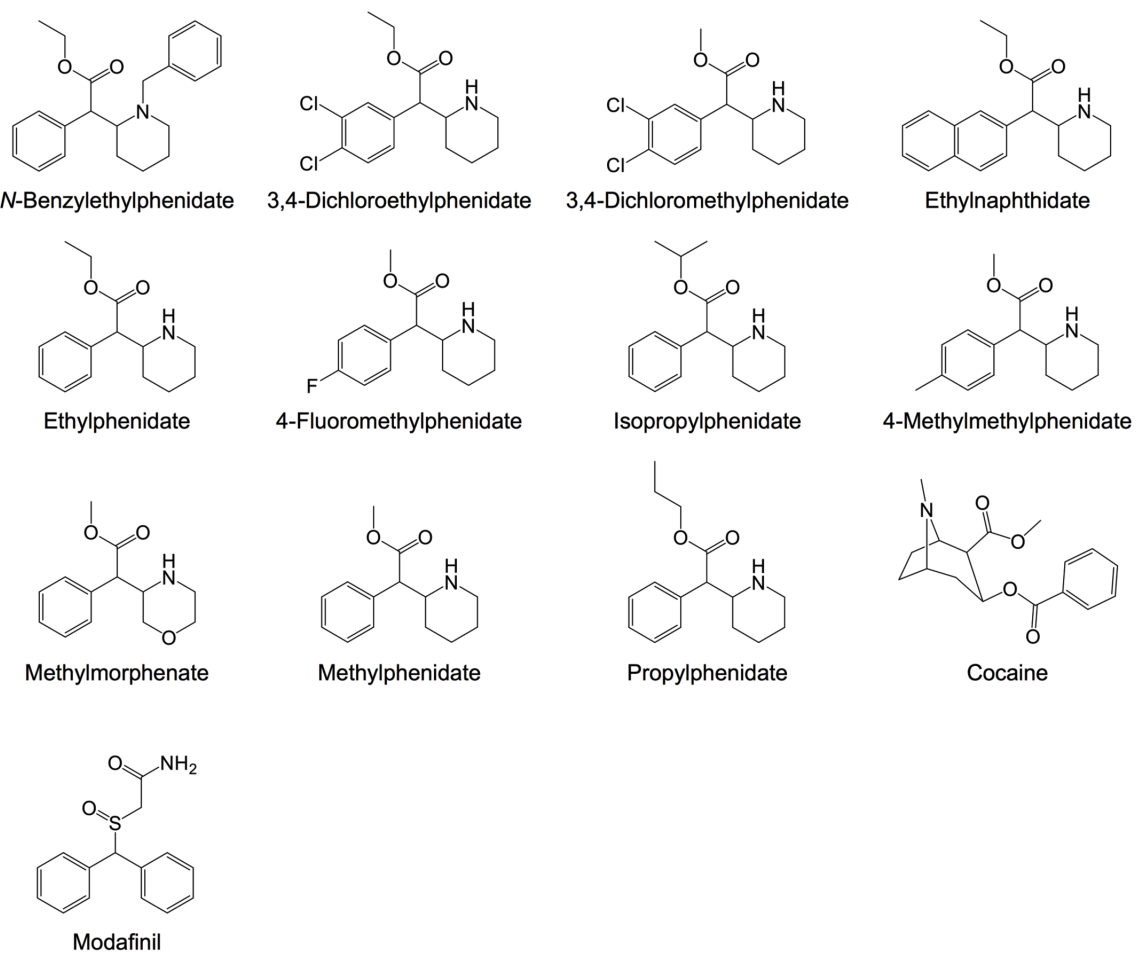
Volkow, N.D., Fowler, J.S., Wang, G., Ding, Y., Gatley, S.J., 2002. Mechanism of action of methylphenidate: insights from PET imaging studies. *J Atten Disord* 6 Suppl 1, S31-43.

Volkow, N.D., Wang, G.J., Fowler, J.S., Fischman, M., Foltin, R., Abumrad, N.N., Gatley, S.J., Logan, J., Wong, C., Gifford, A., Ding, Y.S., Hitzemann, R., Pappas, N., 1999. Methylphenidate and cocaine have a similar in vivo potency to block dopamine transporters in the human brain. *Life Sci* 65, PL7-12.

Wisor, J., 2013. Modafinil as a catecholaminergic agent: empirical evidence and unanswered questions. *Front Neurol* 4, 139.

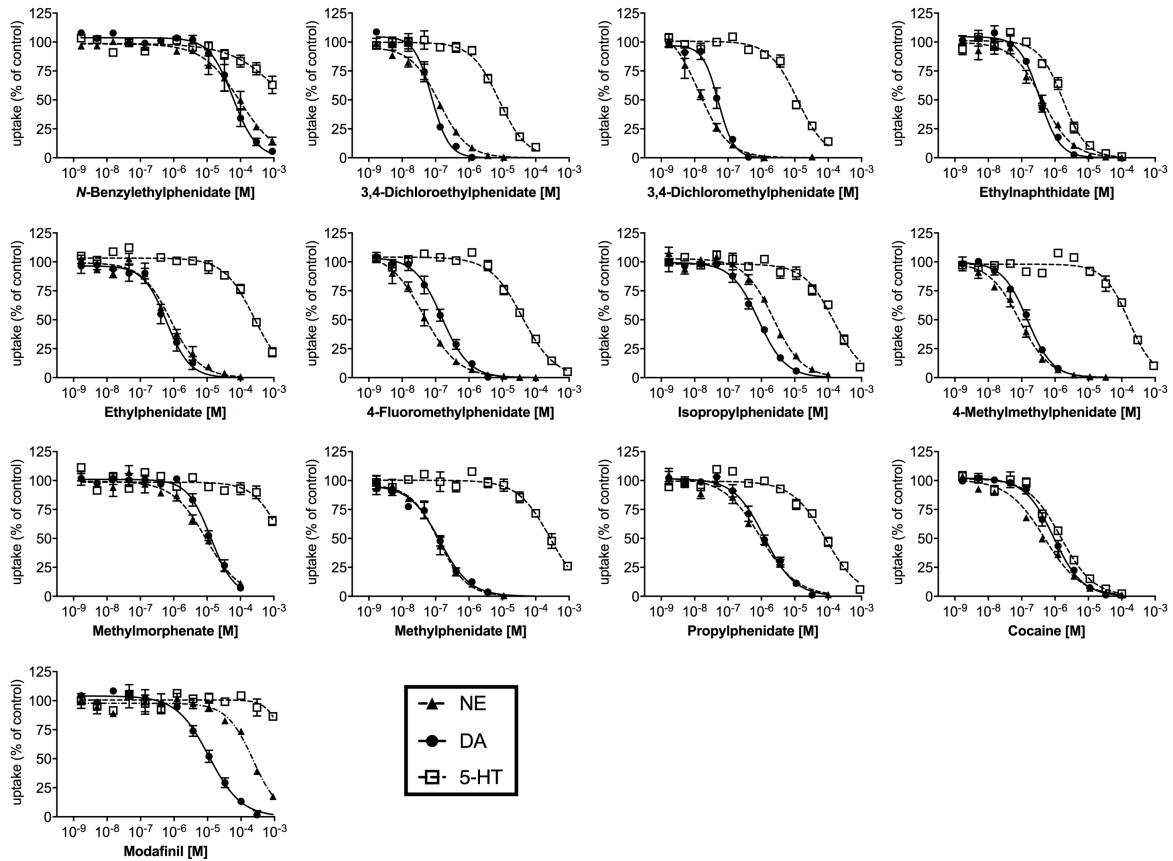
Zolkowska, D., Jain, R., Rothman, R.B., Partilla, J.S., Roth, B.L., Setola, V., Prisinzano, T.E., Baumann, M.H., 2009. Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. *J Pharmacol Exp Ther* 329, 738-746.

## Figures

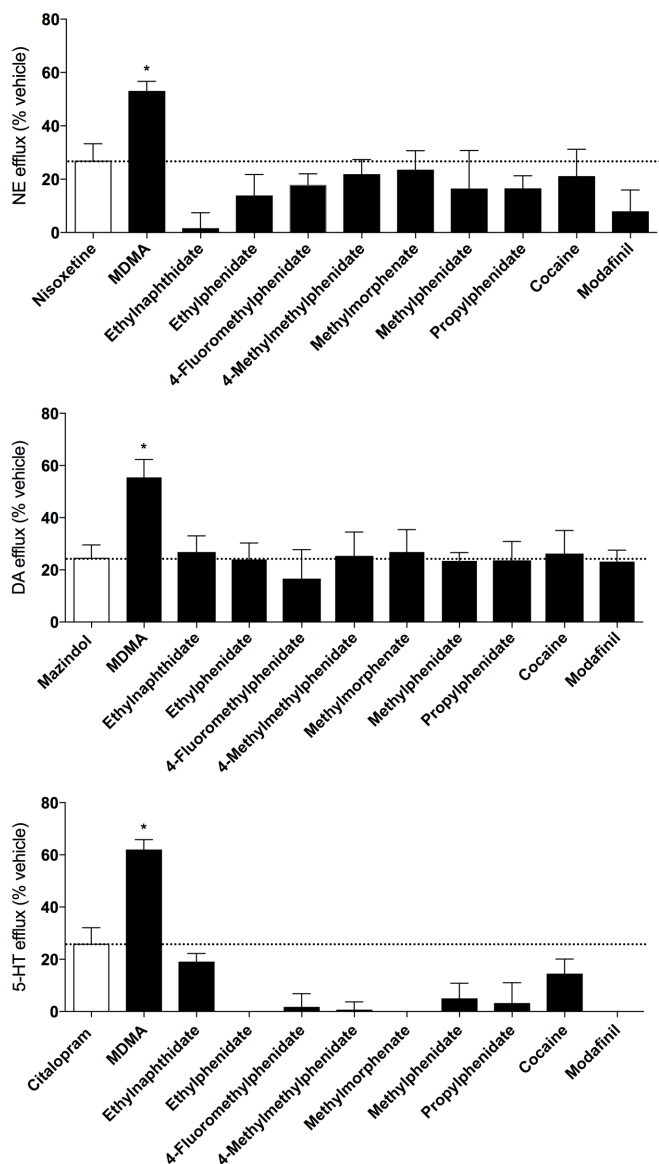


**Fig. 1.** Chemical structures of MPH-based NPS and related compounds.





**Fig. 2.** Monoamine uptake inhibition in stably transfected HEK 293 cells that expressed the human NET, DAT, or SERT. Curves were fitted by non-linear regression, and corresponding  $IC_{50}$  values are shown in Table 1. The data are presented as the mean  $\pm$  SEM and numbers in parentheses indicate the number of individual experiments performed in triplicate (NET/DAT/SERT): *N*-benzylethylphenidate (4/4/4), 3,4-dichloroethylphenidate (3/3/5), 3,4-dichloromethylphenidate (3/3/4), ethylnaphthidate (3/3/3), ethylphenidate (3/3/5), 4-fluoromethylphenidate (4/4/4), isopropylphenidate (3/3/4), 4-methylmethylphenidate (4/3/4), methylnorphenate (3/3/4), methylphenidate (3/3/6), propylphenidate (3/3/4), cocaine (3/4/5), modafinil (6/7/6).



**Fig. 3.** Monoamine efflux induced by 100  $\mu$ M of the compounds after preloading HEK 293 cells that expressed the human NET, DAT, or SERT with radiolabeled monoamine. The efflux is expressed as percentage of [ $^3$ H]-NE, [ $^3$ H]-DA, or [ $^3$ H]-5-HT decrease in monoamine preloaded cells compared to vehicle control. The dashed line marks nonspecific “pseudo-efflux” that arises from monoamine diffusion and subsequent reuptake inhibition. Substances that caused significantly more monoamine efflux ( $*p < 0.05$ ) than pure uptake inhibitors (open bars) were determined to be monoamine transporter substrates. The data are presented as the mean  $\pm$  SEM of five independent experiments.

## Tables

**Table 1.** Monoamine transport inhibition.

	NET	DAT	SERT	DAT/SERT
	IC <sub>50</sub> [μM] (95% CI)	IC <sub>50</sub> [μM] (95% CI)	IC <sub>50</sub> [μM] (95% CI)	ratio (95% CI)
<i>Methylphenidate-based</i>				
Methylphenidate	0.12 (0.09–0.16)	0.13 (0.10–0.18)	274 (204–366)	2108 (1133–3660)
4-Methylmethylphenidate	0.09 (0.07–0.11)	0.15 (0.12–0.18)	164 (132–204)	1093 (733–1700)
Ethylphenidate	0.81 (0.62–1.06)	0.61 (0.45–0.84)	257 (205–322)	421 (244–716)
4-Fluoromethylphenidate	0.04 (0.03–0.06)	0.15 (0.12–0.20)	40 (33–48)	267 (165–400)
3,4-Dichloromethylphenidate	0.01 (0.01–0.02)	0.05 (0.04–0.06)	12 (9–15)	240 (150–375)
Isopropylphenidate	2.3 (1.8–2.9)	0.82 (0.68–1.00)	147 (112–193)	179 (112–284)
Methylmorphenate	9.3 (7.0–12.3)	13 (11–16)	1831 (932–3600)	141 (58–327)
3,4-Dichloroethylphenidate	0.13 (0.10–0.16)	0.08 (0.06–0.09)	8.0 (6.9–9.3)	100 (77–155)
Propylphenidate	0.94 (0.71–1.25)	1.2 (1.0–1.6)	84 (67–106)	70 (42–106)
<i>N</i> -Benzylethylphenidate	95 (59–154)	60 (41–86)	2515 (958–6605)	42 (11–161)
Ethylinaphthidate	0.42 (0.32–0.54)	0.34 (0.28–0.42)	1.7 (1.3–2.1)	5.0 (3.1–7.5)
<i>Other</i>				
Modafinil	231 (177–300)	11 (9–14)	2616 (250–27300)	238 (28–1950)
Cocaine	0.48 (0.36–0.64)	0.90 (0.75–1.08)	1.5 (1.2–1.9)	1.7 (1.1–2.5)

Values are means and 95% confidence intervals (CI). DAT/SERT ratio = 1/DAT IC<sub>50</sub> : 1/SERT IC<sub>50</sub>.

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

	NET	DAT	SERT	D <sub>2</sub>	α <sub>1A</sub>	α <sub>2A</sub>	5-HT <sub>1A</sub>	5-HT <sub>2A</sub>	5-HT <sub>2B</sub>	5-HT <sub>2C</sub>	TA <sub>1rat</sub>	TA <sub>1mouse</sub>
	K <sub>i</sub>	K <sub>i</sub>	K <sub>i</sub>	K <sub>i</sub>	K <sub>i</sub>	K <sub>i</sub>	K <sub>i</sub>	K <sub>i</sub>	EC <sub>50</sub>	K <sub>i</sub>	K <sub>i</sub>	K <sub>i</sub>
<i>Methylphenidate-based</i>												
Methylphenidate	0.50±0.17	0.070±0.020	>22	>4.4	>8.9	>15	>17	>13	>10	>15	>5.0	>4.7
4-Methylmethylphenidate	0.31±0.10	0.033±0.007	>22	>4.4	6.5±0.3	10±1	9.9±0.7	>13	>10	>15	>5.0	>4.7
Ethylphenidate	4.9±0.7	0.081±0.007	>30	>25	>12	14±1	>25	>12	>20	>15	>15 <sup>a</sup>	>15 <sup>a</sup>
4-Fluoromethylphenidate	0.22±0.08	0.040±0.007	>22	>4.4	>8.9	>15	>17	>13	>10	>15	>5.0	>4.7
3,4-Dichloromethylphenidate	0.0066±0.0006	0.0060±0.0005	3.0±0.1	>4.4	1.7±0.1	7.0±0.6	1.8±0.1	>13		>15	6.2±0.6	>14
Isopropylphenidate	4.2±0.4	0.097±0.014	>23	>4.4	>11	>15	>17	>13		>15	13±2	>14
Methylmorphenate	24±1	3.2±0.3	>22	>4.4	>8.9	>15	>17	>13	>10	>15	>5.0	>4.7
3,4-Dichloroethylphenidate	0.028±0.003	0.0065±0.0002	1.5±0.2	>4.4	4.3±0.1	7.5±0.3	4.5±0.4	>13		12±0.3	6.6±1.3	>14
Propylphenidate	3.8±1.3	0.33±0.07	>22	>4.4	>8.9	8.7±0.5	17±1	>13	>10	>15	>5.0	>4.7
<i>N</i> -Benzylethylphenidate	5.5±0.5	0.33±0.01	8.4±1.0	>4.4	>11	>15	>17	>13		>15	>15	>14
Ethyl-naphthidate	0.27±0.06	0.026±0.003	0.58±0.05	>4.4	1.8±0.2	8.6±0.5	1.3±0.2	4.9±0.5	>10	12±3	>5.0	>4.7
<i>Other</i>												
Modafinil	>26	4.0±0.7	>22	>4.4	>8.9	>15	>17	>13	>10	>15	>5.0	>4.7
Cocaine	1.6±0.3	0.20±0.02	0.87±0.04	>4.4	>8.9	>15	>17	>13	>10	>15	>5.0	>4.7

Values are given as μM (mean±SD).

<sup>a</sup>From Simmler et al., 2016