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Full Length Article

Pharmacological characterization of the aminorex analogs 4-MAR, 4,4′-DMAR, and 3,4-DMAR

Anna Rickli[a], Karolina Kolaczynska[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

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ABSTRACT

4,4′-Dimethylaminorex (4,4′-DMAR) is a novel psychoactive substance (NPS) that appeared on the illicit drug market in addition to the psychostimulant 4-methylaminorex (4-MAR). Both substances are methylated derivatives of aminorex, an amphetamine-like anorectic used in the 1960ies and withdrawn from the marked due to severe cardiovascular toxicity. The aim of the present study was to characterize the *in vitro* pharmacological profiles of 4-MAR, 4,4′-DMAR, and 3,4-dimethylaminorex (3,4-DMAR, direx). We assessed norepinephrine (NE), dopamine (DA), and serotonin (5-HT) transporter inhibition potencies and monoamine release in transporter-transfected human embryonic kidney (HEK) 293 cells. We also assessed monoamine receptor and transporter binding affinities. 4,4′-DMAR potently inhibited all monoamine transporters (IC_{50} < 1 μM) with greater potency than 3,4-methlyenedioxymethamphetaime (MDMA) and displayed a higher serotonergic over dopaminergic preference, relatively similar to MDMA (DA transporter / 5-HT transporter inhibition ratio of 0.4 and 0.08 for 4,4′-DMAR and MDMA, respectively). In contrast, 4-MAR preferentially inhibited the NE and DA transporter, exhibiting a pharmacological profile more similar to amphetamine. Both 4-MAR and 4,4′-DMAR were also substrate releasers at the DAT. 3,4-DMAR only weakly inhibited the NE transporter and showed no relevant activity at the DA and 5-HT transporter. Binding affinities of all three aminorex derivatives at various monoamine receptors were negligible (K_i values > 2 μM). The *in vitro* pharmacological profiles indicate that 4,4′-DMAR has comparable psychoactive properties and serotonergic toxicity to MDMA and may be more potent. 4-MAR is a psychostimulant similar to amphetamine or methamphetamine. 3,4-DMAR likely has only weak psychostimulant properties.

1. Introduction

Aminorex (2-amino-5-phenyl-2-oxazoline) is an amphetamine-type psychostimulant (Hofmaier et al., 2014; Rothman et al., 2001). It was marketed in the mid 1960ies (Kay et al., 1971) as an anorectic drug in Switzerland, Germany, and Austria. In the late 1960ies, it was withdrawn from the market (Kay et al., 1971; Langleben, 1998) because it was related to cases of pulmonary hypertension (Gaine et al., 2000). Aminorex was consecutively listed as a controlled substance. Due to its known toxicity, aminorex does not seem to be widely used as a recreational substance. However, it may play a role as psychoactive and potentially toxic metabolite of the frequent cocaine-adulterant levamisole (Bertol et al., 2011; Hess et al., 2013; Hofmaier et al., 2014; Karch et al., 2012). Additionally, derivatives of aminorex such as 4-methylaminorex (4-MAR) and 4,4′-dimethylaminorex (4,4′-DMAR) are misused as psychoactive substances. Awareness of 4-MAR (street name "U4Euh" or "ice") increased after a fatality in the late 1980ies (Davis and Brewster, 1988). Pulmonary hypertension caused by 4-MAR use was also reported in some cases (Gaine et al., 2000). 4,4′-DMAR was first detected in Europe in 2012, and 31 deaths were associated with 4,4′-DMAR consumption between 2013 and 2014 (EMCDDA, 2015). 4,4′-DMAR is a novel psychoactive substance (NPS), not listed under the convention on psychotropic substances 1971, in comparison to 4-MAR and aminorex which have previously been scheduled (UN, 1971) but also 4,4′-DMAR it is now scheduled as controlled substance in many countries. Other derivatives of aminorex, such as the 3,4-dimethylaminorex (3,4-DMAR) which is the methamphetamine-type isomer of 4,4′-DMAR, are so far not subject of control despite a potential for abuse. Drug users have discussed that 3,4-DMAR has only weak psychoactive effects (Sciencemadness, 2007). Based on chemical structure (Fig. 1), aminorex derivatives are amphetamine-type substances considered to interact mainly with monamine transporters (Maier et al.,

* Corresponding author at: Division of Clinical Pharmacology and Toxicology, University Hospital Basel, Schanzenstrasse 55, Basel, CH-4031, Switzerland.
E-mail address: matthias.liechti@usb.ch (M.E. Liechti).

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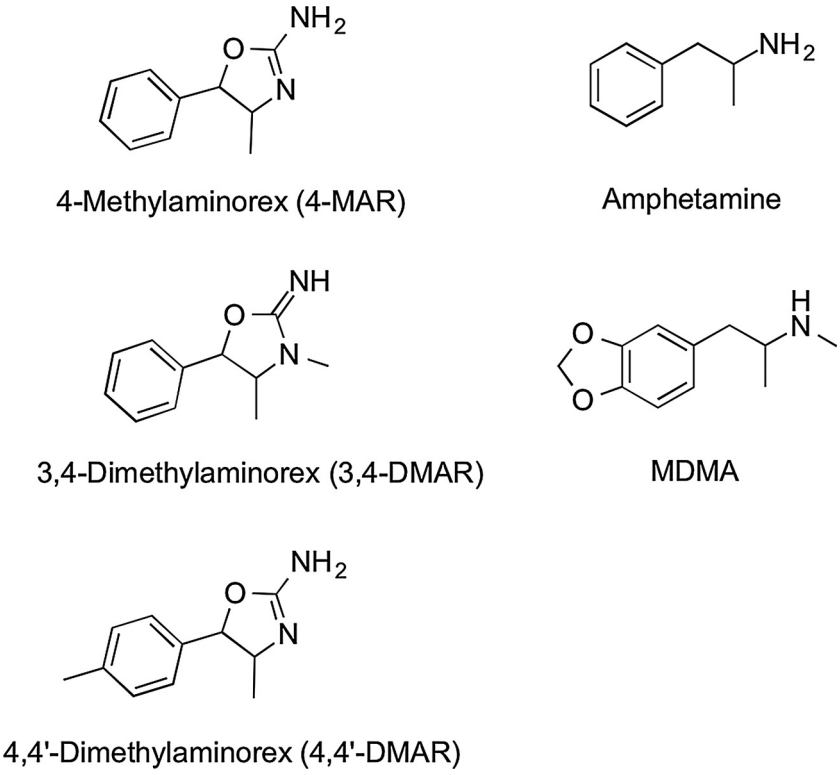

Fig. 1. Chemical structures of aminorex derivatives compared with amphetamine and MDMA.

2018a). Studies using rat brain synaptosomes *in vitro,* confirmed potent dopamine (DA), norepinephrine (NE), and serotonin (5-HT) release for aminorex, 4-MAR, and 4,4′-DMAR (Brandt et al., 2014). The interaction profile of 4,4′-DMAR with human monoamine transporters and receptors has also recently been published (Maier et al., 2018b). However, there is no data on 3,4-DMAR. Comparisons of the pharmacological profiles of different aminorex derivatives at human targets are also lacking. Therefore, the aim of the present study was to evaluate the three methylated aminorex derivatives 4-MAR, 4,4′-DMAR, and 3,4-DMAR regarding their potency to interact with human monoamine reuptake transporters and with monoamine receptors. D-Amphetamine and MDMA were included as comparator substances. The goal was to use identical *in vitro* assays as previously used to characterize classic and novel amphetamine-type substances and related NPS (Luethi et al., 2018a, b; Luethi et al., 2018c; Luethi and Liechti, 2018; Rickli et al., 2015a, b; Simmler et al., 2013, 2014). Thus, the present data can be directly compared with the pharmacological profiles of many other NPS and may be useful to predict the psychoactivity of these compounds in humans (Luethi and Liechti, 2018). The receptor binding data of 4,4′-DMAR, but not the transporter inhibition profile determined in the present study, has previously been published (Maier et al., 2018b).

2. Material and methods

2.1. Drugs

4-MAR, cis-(±)-4,4′-DMAR and 3,4-DMAR were obtained from Lipomed (Arlesheim, Switzerland) with high-performance liquid chromatography purities of > 98.5%. Radiolabelled [^{3}H]NE and [^{3}H]DA were purchased from Perkin-Elmer (Schwerzenbach, Switzerland). Radiolabeled [^{3}H]5-HT was obtained from Anawa (Zürich, Switzerland).

2.2. Monoamine uptake transporter inhibition

Inhibition of the human NE, DA, and 5-HT transporters (NET, DAT, and SERT, respectively) was assessed in transfected human embryonic kidney (HEK) 293 cells, stably expressing the monoamine transporters as previously described in (Hysek et al., 2012; Luethi et al., 2018c). In brief, cells were suspended in uptake buffer and incubated with the test drug for 10 min before [^{3}H]NE, [^{3}H]DA, or [^{3}H]5-HT was added at a final concentration of 5 nM for an additional 10 min to initiate uptake transport. Thereafter, the cells were separated from the uptake buffer by centrifugation through silicone oil. The centrifugation tubes were then frozen in liquid nitrogen and the cell pellet was cut into scintillation vials containing lysis buffer. Scintillation fluid was added to the vials and uptake was quantified by liquid scintillation counting. Transporter inhibitors (10 µM nisoxetine for NET, 10 µM mazindol for DAT, and 10 µM fluoxetine for SERT) were added to assess for non-specific monoamine uptake which was subtracted from the total counts to yield specific uptake (Hysek et al., 2012; Mayer et al., 2016; Rothman et al., 2000; Verrico et al., 2007). Nonspecific uptake was < 10% of total uptake (Hysek et al., 2012).

2.3. Drug-induced monoamine efflux

To assess whether the test drugs act as pure transporter inhibitors or as transporter substrates, drug-induced monoamine efflux was assessed at a drug concentration of 100 µM in NET-, DAT-, or SERT-transfected HEK 293 cells as previously described (Luethi et al., 2018c; Simmler et al., 2013). Briefly, cells were cultured in a poly-D-lysine coated microplate. Thereafter, the cells were preloaded with 10 nM [^{3}H]NE, [^{3}H]DA, or [^{3}H]5-HT for 20 min at 37 °C, washed twice, and treated with the test drugs for 15 min (DAT and SERT) or 45 min (NET) at 37 °C on a rotary shaker. Thereafter, 300 µl of the assay buffer was transferred into scintillation vials, scintillation fluid was added, and the amount of monoamine efflux was then quantified by liquid scintillation counting. Transporter inhibitors (10 µM nisoxetine for NET, 10 µM mazindol for

DAT, and 10 µM citalopram for SERT) were included to determine "pseudo-efflux" caused by nonspecific monoamine efflux and subsequent reuptake inhibition (Scholze et al., 2000). The assay set-up was based on previous kinetic evaluation of the efflux-over-time curves for monoamine transporter substrates (Hysek et al., 2012).

2.4. Binding at monoamine transporters and receptors

Radioligand binding affinities to transporters and receptors were assessed as previously described in detail (Luethi et al., 2018c, d; Maier et al., 2018b). In brief, membrane preparation that overexpressed the respective transporter or receptor (human genes with the exception of rat and mouse genes for trace amine-associated receptors 1, $TAAR_1$, (Revel et al., 2012)) were incubated with the radiolabeled selective ligand at concentrations equal to K_d and ligand displacement by the test 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 at a concentration of 10 µM. The following radioligands and competitors, respectively, were used: *N*-methyl-[^{3}H]nisoxetine and indatraline (NET), [^{3}H]WIN35,428 and indatraline (DAT), [^{3}H]citalopram and indatraline (SERT), [^{3}H]8-hydroxy-2-(di-*n*-propylamine)tetralin and pindolol (serotonin 5-HT_{1A} receptor), [^{3}H]ketanserin and spiperone (serotonin 5-HT_{2A} receptor), [^{3}H]mesulergine and mianserin (serotonin 5-HT_{2C} receptor), [^{3}H]prazosin and chlorpromazine (α_1 adrenergic receptor), [^{3}H]rauwolscine and phentolamine (α_2 adrenergic receptor), [^{3}H]-spiperone and spiperone (dopamine D_2 receptor), and [^{3}H]RO5166017 and RO5166017 ($TAAR_1$ receptor).

2.5. Activity at the serotonin 5-HT_{2B} receptor

Activation of 5-HT_{2B} receptors was assessed by measuring calcium flux in HEK 293 cells expressing the human 5-HT_{2B} receptor as previously described in detail (Luethi et al., 2018c). Briefly, cells were incubated over night in growth medium at 37 °C in poly-D-lysine-coated 96-well plates. 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, the Fluo-4 solution was removed by snap inversion, and 100 µl of Fluo-4 solution was added a second time for 45 min 31 °C. The cells were washed with HBSS and 20 mM HEPES (assay buffer) using an EMBLA cell washer, and 100 µl assay buffer was added. The plates were then placed in a FLIPR, and 25 µl of the test substances that were diluted in assay buffer was added online. The increase in fluorescence was measured, and EC_{50} values were derived from the concentration-response curves using nonlinear regression. The maximal receptor activity (efficacy) is expressed relative to the activity of 5-HT, which was set to 100%.

2.6. Cytotoxicity

To confirm cell membrane integrity during the pharmacological assays, cytotoxicity was assessed using the ToxiLight™ bioassay kit (Lonza, Basel, Switzerland) according to the manufacturer's instructions. The assay quantitatively measures the increase of adenylate kinase release from damaged cells, providing a highly sensitive method for measuring cytolysis (Crouch et al., 1993). Cells that were grown in 96-well plates were exposed to the compounds at the highest assay concentration of 100 µM. All of the test conditions contained 0.1% (v:v) dimethylsulfoxide, which is non-toxic at this concentration and was also used as a negative control. Triton X-100 (0.1%, Sigma-Aldrich, Buchs, Switzerland) lyses cells and was used as a positive control. After 1 h incubation at 37 °C, 10 µl of the supernatant per well was removed and combined with 50 µl of ToxiLight™ reagent. Luminescence was recorded using a Tecan Infinite 200 Pro plate reader (Tecan, Männedorf, Switzerland), quantified and compared to control.

2.7. Statistical analyses

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}. Drug-induced monoamine efflux of three independent experiments was analyzed using analysis of variance followed by the Holm-Sidak test. The drugs were considered monoamine transporter substrates, if they caused significantly higher ($^* P < 0.05$) efflux than the selective inhibitors. IC_{50} values of the radioligand binding assays were assessed by calculating nonlinear regression curves for a one-site model using three independent 10-point concentration-response curves for each substance. Affinity (K_i) values, which correspond to the dissociation constants, were calculated using the Cheng-Prusoff equation. EC_{50} values for serotonin 5-HT_{2B} receptor activation were determined using nonlinear regression concentration-response curves.

3. Results

3.1. Monoamine uptake transporter inhibition

IC_{50} values for the inhibition of the NET, DAT, and SERT are shown in Table 1. 4-MAR and 4,4′-DMAR potently inhibited the NET and DAT similar to amphetamine. 4,4′-DMAR also potently blocked the SERT, similar to MDMA but at 5-fold lower concentrations, whereas 4-MAR was a weaker inhibitor of the SERT. 3,4-DMAR was only a low potency NET inhibitor with no relevant activity at the DAT and SERT ($IC_{50} > 50$ µM). The DAT/SERT inhibition ratio was 7.2 for 4-MAR indicating greater dopaminergic *vs.* serotonergic activity similar to amphetamine (ratio > 10). The DAT/SERT inhibition ratio for 4,4′-DMAR was 0.40, indicating greater serotonergic *vs.* dopaminergic activity similar to MDMA, although the latter has an even greater serotonergic *vs.* dopaminergic potency (ratio = 0.08).

3.2. Drug-induced monoamine efflux

4-MAR and 4,4′-DMAR were substrate-type releasers similar to

Table 1
Monoamine transporter inhibition.

	NET IC_{50} [µM] (95% CI)	DAT IC_{50} [µM] (95% CI)	SERT IC_{50} [µM] (95% CI)	DAT/SERT ratio (95% CI)
4-MAR	0.11 (0.08-0.17)	0.96 (0.73-1.3)	6.9 (5.0 - 9.6)	7.2 (3.9-13)
4,4'-DMAR	0.08 (0.06-0.12)	0.70 (0.55-0.89)	0.28 (0.20-0.40)	0.40 (0.22-0.73)
3,4-DMAR	7.0 (5.2-9.5)	54 (37-79)	> 100	> 1.9 (1.3-2.7)
D-Amphetamine	0.09 (0.06-0.14)[a]	1.3 (0.83-2.0)[a]	> 10[a]	> 10[a]
MDMA	0.45 (0.33-0.60)[a]	17 (12-24)[a]	1.4 (1.0-2.0)[a]	0.08 (0.04-0.16)[a]

Values are means of three to four independent experiments and 95% confidence intervals (CI). DAT/SERT ratio = 1/DAT IC50 : 1/SERT IC50.
[a] Values were previously published in Simmler et al. (2013).

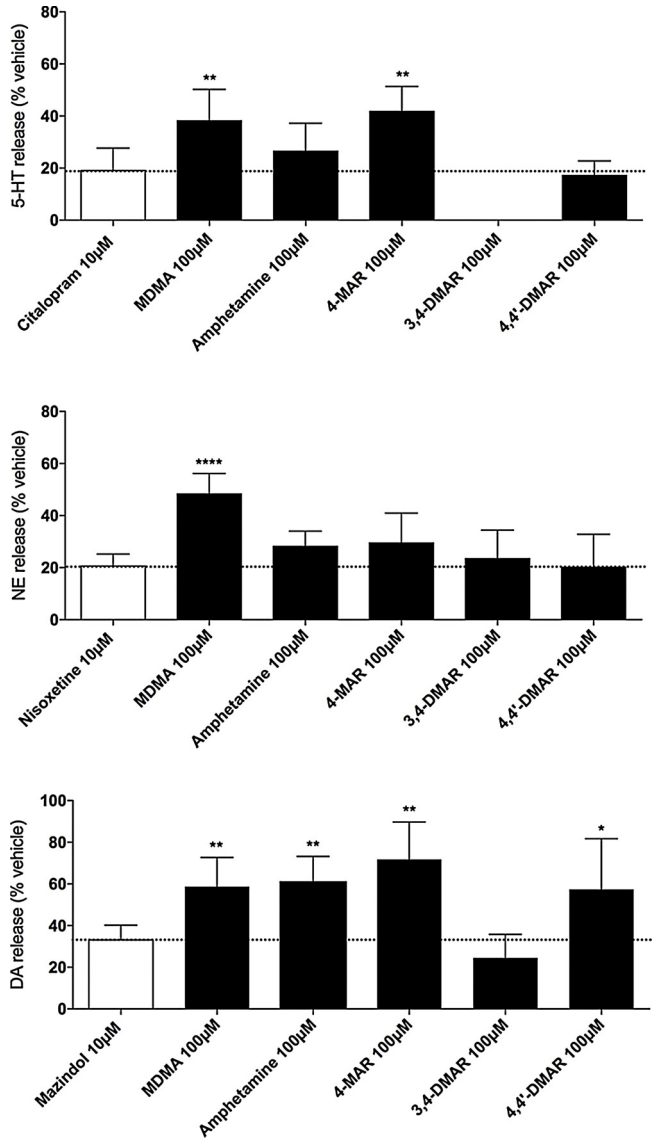

Fig. 2. Monoamine release induced by 100 µM of the compounds after pre-loading hNET-, hDAT-, or hSERT-expressing HEK 293 cells with radiolabeled monoamines. "Pseudo-efflux" that arose from monoamine diffusion and subsequent reuptake inhibition is marked with a dashed line. Substances that caused significantly 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 ± SD of five independent experiments.

MDMA and amphetamine induced significant monoamine efflux through at least one of the transporters compared with pure transporter inhibitors (Fig. 2). 4-MAR induced significant monoamine release at the SERT and DAT. 4,4′-DMAR only induced significant monoamine release at the DAT. In contrast, 3,4-DMAR was found not to be a releaser at all three of the monoamine transporters.

3.3. Binding at monoamine receptors and transporters

4-MAR and 4,4′-DMAR potently bound to the DAT and NET, and 4,4′-DMAR also to the SERT, consistent with the uptake inhibition of these transporters. 3,4-DMAR interacted weakly with the DAT (K_i = 5.63 µM; Table 2). The aminorex derivatives showed no relevant binding (K_i < 2 µM) to any of the monoamine receptors tested. There were only low-potency interactions of 4-MAR with the α_{2A} (K_i = 2.1 µM) and 5-HT_{2C} (K_i = 9.2 µM) receptors, and of 4,4′-DMAR with the 5-HT_{2A} receptor (K_i = 8.9 µM). In contrast to the aminorex derivatives,

Table 2
Monoamine transporter and receptor binding affinities and 5-HT2B receptor activation potency.

	NET K_i	DAT K_i	SERT K_i	5-HT_{1A} K_i	5-HT_{2A} K_i	5-HT_{2B} EC_{50}	5-HT_{2C} K_i	D_2 K_i	α_{2A} K_i	α_{1A} K_i	$TAAR_{1rat}$ K_i	$TAAR_{1mouse}$ K_i
Aminorex derivatives												
4-MAR	0.10 ± 0.01	0.09 ± 0.01	> 7	> 10	> 10	> 10	9.2 ± 5.3	> 10	2.1 ± 0.2	> 2	> 5	> 5
4,4′-DMAR	0.27 ± 0.06[b]	0.53 ± 0.04[b]	1.9 ± 0.2[b]	> 10[b]	8.9 ± 0.9[b]	> 10[b]	> 10	> 10	> 5.0[b]	> 2[b]	> 5[b]	> 5[b]
3,4-DMAR	> 7	5.6 ± 0.6	> 7	> 10	> 10	> 10	> 10	> 10	> 5.0	> 2	> 5	> 5
Phenethylamines												
D-Amphetamine	1.0 ± 0.6[a]	5.7 ± 3.8[a]	> 25[a]	6.7 ± 1.4[a]	> 10[a]	9.5 ± 1.6	> 10[a]	> 10[a]	2.8 ± 0.8[a]	> 2[a]	0.23 ± 0.2[a]	0.09 ± 0.06[a]
MDMA	> 7	> 7	> 7	> 10	4.6 ± 1.1	> 10	4.4 ± 0.8	> 10	4.6 ± 0.1	> 2	0.37 ± 0.1[a]	2.4 ± 1.1[a]

Receptor binding (Ki) and activation potency (EC50) values are given as µM (mean ± SD).
[a] Values were previously published in Simmler et al., 2013.
[b] values were previously published in Maier et al., 2018a,b.

both d-amphetamine and MDMA exhibited binding to rat and mouse $TAAR_1$ (Table 2).

3.4. Activity at the serotonin 5-HT_{2B} receptor

None of the compounds tested activated the 5-HT_{2B} receptor in the investigated concentration range besides d-amphetamine, which showed weak activity (EC_{50} = 9.5).

3.5. Cytotoxicity

None of the compounds showed release of adenylate kinase at a concentration of 100 μM and after an incubation time of 1 h.

4. Discussion

The main finding of this study was that 4-MAR and 4,4′-DMAR potently inhibited monoamine uptake by the human NET and DAT, indicating that these substances are active as psychostimulants at low doses in humans (Luethi and Liechti, 2018; Rothman et al., 2001). The DAT/SERT inhibition potency ratio of 7.2 for 4-MAR suggests that it has mainly amphetamine-type stimulant properties, whereas the ratio of 0.4 for 4,4′-DMAR indicates more empathogenic effect properties similar to MDMA, due to the more serotonergic pharmacology (Liechti, 2015). Additionally, the lower DAT/SERT ratio of 4,4′-DMAR may be associated with a lower abuse liability compared with more dopaminergic amphetamines (Liechti, 2015; Negus and Banks, 2017; Suyama et al., 2016; Wee et al., 2005), while serotonergic toxicity could be enhanced (Liechti, 2015; Maier et al., 2018b). In line with possible serotonergic toxicity, cardiac arrest, brain oedema, elevated body temperature, bleeding, and seizures were the most common adverse events/autopsy findings in fatalities associated with 4,4′-DMAR (EMCDDA, 2015). 3,4-DMAR was only weakly active at the NET and even less active at the DAT, consistent with weak stimulant effects reported by recreational users (Drugs-Forum, 2009; Sciencemadness, 2007).

4,4′-DMAR inhibited the NET, DAT, and SERT with similar potency, largely consistent with a study using rat brain synaptosomes (Brandt et al., 2014) and a previous study from another laboratory also using HEK293 cells and human transporters (Maier et al., 2018b). In the present study, 4,4′-DMAR showed a DAT/SERT ratio of 0.4, indicating higher serotonergic preference compared with the previously published DAT/SERT ratio of 1.7 in another study (Maier et al., 2018b). Similarly, the present data show that MDMA was more serotonergic (DAT/SERT ratio of 0.1) compared to the study by Maier et al. (ratio of 1), indicating that the assays used in our laboratory may yield generally higher potency values at the SERT compared to some other studies on human transporters and likely due to methodological differences (Maier et al., 2018b; Verrico et al., 2007). 4,4′-DMAR was consistently more potent than MDMA to inhibit all monoamine transporters in the present study and in the study of Maier et al. (Maier et al., 2018b). Consistently, 4,4′-DMAR was also the most potent releaser of 5-HT from rat brain synaptosomes compared with d-amphetamine, aminorex, and 4-MAR (Brandt et al., 2014), supporting the view that 4,4′-DMAR is relatively more serotonergic than amphetamine and other aminorex derivatives and pharmacologically more similar to MDMA. Recreational user reports indicate long-lasting and potent psychoactive effects for 4-MAR and 4,4′-DMAR at oral doses of 10 mg and higher (Coppola and Mondola, 2015; Drugs-Forum, 2017; EMCDDA, 2014; Erowid, 2003; Glanville et al., 2015; Loi et al., 2017; TripSit, 2018). Consistent with the present pharmacological data, substance users reported 4,4′-DMAR (street name “Serotoni”) to be more potent than MDMA or 6-APB and having longer-lasting empathogenic effects (EMCDDA, 2014; Loi et al., 2017; reddit, 2014). The high relative serotonergic potency of 4,4′-DMAR is likely attributed to its *para*-methylation at the phenyl ring, similar to the increased serotonergic activity in other *para*-halogenated amphetamines (Glennon and Dukat, 2017; Negus and Banks, 2017; Rickli et al., 2015a). In contrast, substance users report effects of 4-MAR to be more like methamphetamine than MDMA (Erowid, 2002), consistent with the present *in vitro* pharmacological data. *In vivo* studies with 4-MAR in rats and primates also revealed amphetamine-like psychostimulant properties (Batsche et al., 1994; Glennon and Misenheimer, 1990; Mansbach et al., 1990). Furthermore, 4-MAR and its isomers produced conditioned place preference, which was mediated via dopamine D_1 and D_2 receptors (Meririnne et al., 2005) in line with its dopaminergic action.

The present study did not include the parent substance aminorex. However, it has previously been shown in monoamine-preloaded rat brain synaptosomes that aminorex is a potent releaser of NE and DA, and a less potent releaser of 5-HT. The pharmacological profile of aminorex is therefore similar to d-amphetamine, but with a moderately higher additional serotonergic activity (Brandt et al., 2014; Rothman et al., 2001). In the present study we showed that 4-MAR and 4,4′-DMAR are also substrate-type monoamine releasers similar to aminorex.

Both 4,4′-DMAR and MDMA interacted with the serotonin 5-HT_{2A} receptor known to mediate the mind-altering effects of hallucinogen (Preller et al., 2017; Vollenweider et al., 1998). Serotonin 5-HT_{2A} receptor activation is also thought to underlie the mild hallucinogenic properties of MDMA (Liechti et al., 2000). The 4,4′-DMAR binding at the 5-HT_{2A} receptor suggests similar mild hallucinogenic effects. The methylated aminorex derivatives investigated in the present study did not interacted with $TAAR_1$ receptors in contrast to amphetamine, MDMA, and several other phenethylamine derivatives (Revel et al., 2012; Simmler et al., 2016). Other aminorex-like ring-substituted 2-aminooxazolines have been shown to interact with $TAAR_1$ receptors (Galley et al., 2016). However, they did not contain a 4-methyl group in contrast to the currently investigated compounds. Activity at $TAAR_1$ may have auto-inhibitory effects on the monoaminergic action of amphetamine-type substances (Di Cara et al., 2011; Simmler et al., 2016). Therefore, the presently investigated compounds that did not bind to $TAAR_1$ may exhibit greater stimulant properties compared to other amphetamines that also bind to $TAAR_1$. The compounds investigated in this study did not relevantly interact with monoaminergic receptors indicating that their primary sites of action are the monoamine transporters.

In conclusion, 4-MAR and 4,4′-DMAR potently interacted with monoamine transporters. 4-MAR mainly acted on the NET and DAT similar to d-amphetamine, indicating stimulant-like properties. 4,4′-DMAR potently interacted with the SERT similar to MDMA, suggesting more MDMA-like empathogenic clinical effects. 3,4-DMAR only weakly interacted with the NET, predicting weak stimulant-type effects.

Author contributions

A.R. and M.E.L. designed the research. A.R., K.K. and M.C.H. performed the research. A.R., K.K., M.C.H., and M.E.L. analyzed data. A.R. and M.E.L. wrote the manuscript.

Conflict of interest

None. M.C.H. is an employee of F. Hoffmann-La Roche.

Transparency document

The Transparency document associated with this article can be found in the online version.

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