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Pharmacological profiles of aminoindanes, piperazines, and pipradrol derivatives

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ABSTRACT

Aminoindanes, piperazines, and pipradrol derivatives are novel psychoactive substances found in "Ecstasy" tablets as replacements for 3,4-methylenedioxymethamphetamine (MDMA) or substances sold as "ivory wave." The pharmacology of these MDMA- and methylphenidate-like substances is poorly known. We characterized the pharmacology of the aminoindanes 5,6-methylenedioxy-2-aminoindane (MDAI), 5-iodoaminoindane (5-IAI), and 2-aminoindane (2-AI), the piperazines meta-chlorophenylpiperazine (m-CPP), trifluoromethylphenylpiperazine (TFMPP), and 1-benzylpiperazine (BZP), and the pipradrol derivatives desoxypipradrol (2-diphenylmethylpiperidine [2-DPMP]), diphenylprolinol (diphenyl-2-pyrrolidinemethanol [D2PM]), and methylphenidate. We investigated norepinephrine (NE), dopamine (DA), and serotonin (5-hydroxytryptamine [5-HT]) uptake inhibition using human embryonic kidney 293 (HEK 293) cells that express the respective human monoamine transporters (NET, DAT, and SERT). We also evaluated the drug-induced efflux of NE, DA, and 5-HT from monoamine-preloaded cells and the binding affinity to monoamine transporters and receptors, including trace amine-associated receptor 1 ($TAAR_1$). 5-IAI and MDAI preferentially inhibited the SERT and NET and released 5-HT. 2-AI interacted with the NET. BZP blocked the NET and released DA. m-CPP and TFMPP interacted with the SERT and serotonergic receptors. The pipradrol derivatives were potent and selective catecholamine transporter blockers without substrate releasing properties. BZP, D2PM, and 2-DPMP lacked serotonergic activity and $TAAR_1$ binding, in contrast to the aminoindanes and phenylpiperazines. In summary, all of the substances were monoamine transporter inhibitors, but marked differences were found in their DAT *vs.* SERT inhibition profiles, release properties, and receptor interactions. The pharmacological profiles of D2PM and 2-DPMP likely predict a high abuse liability.

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1. Introduction

New psychoactive substances [1] are constantly emerging on the illicit drug market. Many of these novel designer substances are amphetamine derivatives and typically marketed as "bath salts", "research chemicals" or "legal highs" via the Internet [2]. Pharmacological information is typically not available for these newly emerging designer substances. Interactions with the norepinephrine (NE), dopamine (DA), and serotonin (5-hydroxytryptamine [5-HT]) transporters (NET, DAT, and SERT, respectively) to block or release monoamines can be expected based on the amphetamine-like core structure of many of these substances. In addition, chemical modifications typically alter absolute or relative potencies at the NET and DAT relative to the SERT or substrate release properties, thereby affecting stimulant-like and reinforcing properties [3,4]. Additional interactions with the $5\text{-}HT_{2A}$ receptor may result in hallucinogenic-like actions. Substances that predominantly act on the NET and DAT have stimulant-like properties similar to amphetamine, whereas substances that mostly act on

Abbreviations: 2-AI, 2-aminoindane; BZP, 1-benzylpiperazine; DA, dopamine; DAT, dopamine transporter; D2PM, diphenyl-2-pyrrolidinemethanol; 2-DPMP, desoxypipradrol or 2-diphenylmethylpiperidine; HEK, human embryonic kidney; 5-IAI, 5-iodoaminoindane; m-CPP, meta-chlorophenylpiperazine; MDAI, 5,6-methylenedioxy-2-aminoindane; MDMA, 3,4-methylenedioxymethamphetamine; NE, norepinephrine; NET, norepinephrine transporter; 5-HT, 5-hydroxytryptamine (serotonin); SERT, serotonin transporter; TAAR, trace amine-associated receptor; TFMPP, trifluoromethylphenylpiperazine..

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the SERT may have more "empathogenic" properties similar to 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) [4,5]. Assessing the *in vitro* pharmacological profiles of novel substances is a relatively rapid approach for gaining a first impression of their potential clinical effects and toxicology, in addition to user reports. Accordingly, the pharmacology of many novel designer cathinones ("bath salts" and "research chemicals") has recently been characterized *in vitro* [4,6–10]. The aim of the present study was to describe the effects on monoamine uptake and release of novel psychoactive substances that are not cathinones, but have been introduced into the illicit drug market as "legal highs" to typically mimic the subjective effects of MDMA or amphetamine-type stimulants. Aminoindanes, such as 5,6-methylenedioxy-2-aminoindane (MDAI) and 5-iodoaminoindane (5-IAI), became increasingly available over the Internet starting in 2010 as legal and, in the case of MDAI, allegedly less-neurotoxic alternatives to MDMA [11–13]. Piperazines have been used for more than a decade [14] and are commonly found in Ecstasy pills as substitutes for MDMA [15,16]. Toxicity associated with the use of "ivory wave," which contains the pipradrol derivatives desoxypipradrol (2-diphenylmethylpiperidine [2-DPMP]) or diphenylprolinol (diphenyl-2-pyrrolidinemethanol [D2PM]) was increasingly reported starting in 2010 [17–19]. The present study investigated the aminoindanes 2-aminoindane (2-AI), 5-IAI, and MDAI, the piperazines meta-chlorophenylpiperazine (m-CPP), trifluoromethylphenylpiperazine (TFMPP), and 1-benzylpiperazine (BZP), and the pipradrol derivatives D2PM and 2-DPMP (Fig. 1). Similar data on MDMA and other novel psychoactive substances have previously been published [4,6]. We determined the potencies of the compounds to inhibit the human NET, DAT, and SERT. We tested whether the compounds induce the transporter-mediated release of NE, DA, and 5-HT and characterized the binding affinities of the compounds for monoamine transporters, α_1 and α_2 adrenergic receptors, dopamine D_1-D_3 receptors, 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors, the histamine H_1 receptor, and trace amine-associated receptor 1 ($TAAR_1$). Most of the substances examined herein were previously studied using rodent transporters, but only a few were also studied using human transporters and receptors [7]. However, more comprehensive analyses are needed at both human transporters and receptors. Similar data on novel designer cathinones and classic stimulants, including amphetamine, methamphetamine, MDMA, and cocaine have previously been obtained using identical methods [4,6].

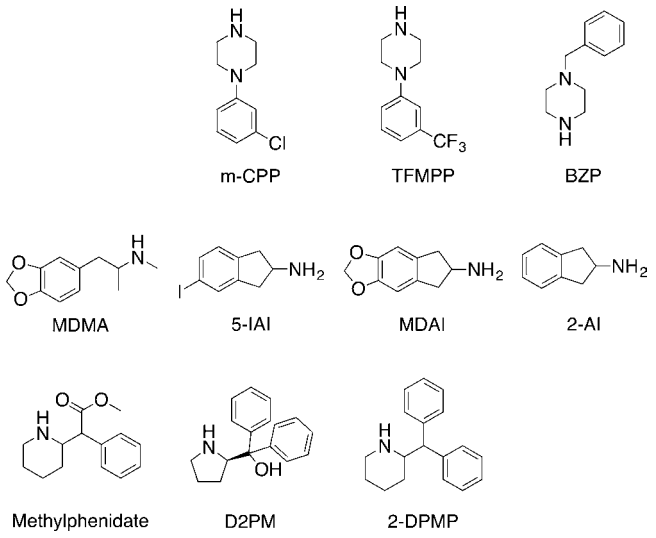


Fig. 1. Structures of novel psychoactive substances that mimic the effects of 3,4-methylenedioxymethamphetamine (MDMA) or methylphenidate. 2-Aminoindane (2-AI), 5-iodo-2-aminoindane (5-IAI), and 5,6-methylenedioxy-2-aminoindane (MDAI) are recreationally used aminoindanes. Meta-chlorophenylpiparazine (m-CPP), trifluoromethylphenylpiperazine (TFMPP), and 1-benzylpiperazine (BZP) are piperazines commonly found in pills sold as Ecstasy. Diphenylprolinol (diphenyl-2-pyrrolidinemethanol [D2PM]) and desoxypipradrol (2-diphenylmethylpiperidine [2-DPMP]) are pipradrol derivatives sold as "legal highs" ("ivory wave") and structurally similar to methylphenidate.

2. Methods

2.1. Chemicals

MDMA, methylphenidate, m-CPP, TFMPP, and BZP were supplied by Lipomed (Arlesheim, Switzerland), and 5-IAI, 2-AI, 2-DPMP, and D2PM were supplied by Cayman Chemicals (Ann Arbor, MI, USA) as racemic hydrochloride salts (purity > 98.5%). MDAI was synthesized as a racemic hydrochloride salt in our laboratory according to Nichols et al. [20]. Radiochemicals (^{3}H-isotopes) were obtained from Anawa (Wangen, Switzerland) or Perkin Elmer (Schwerzenbach, Switzerland), with the exception of [^{3}H]RO5166017, which was synthesized at Roche (Basel, Switzerland).

2.2. Monoamine uptake transport inhibition

The inhibition of the NET, SERT, and DAT was assessed in human embryonic kidney 293 (HEK 293) cells that stably expressed the human NET, SERT, and DAT [21] as previously described in detail [22]. Cultured cells were detached and resuspended in uptake buffer. We incubated the cells with various concentrations of the test compounds and the vehicle control for 10 min and then added [^{3}H]DA, [^{3}H]NE, or [^{3}H]5-HT (5 nM final concentrations) to initiate the uptake transport of the labeled monoamines at room temperature. Uptake was stopped after 10 min by separation of the cells from the buffer by rapid centrifugation at high speed through silicone oil [22]. The uptake times were based on kinetic evaluations showing that uptake is complete after 5 min [22]. The centrifugation tubes were frozen in liquid nitrogen and cut to separate the cell pellet from the silicone oil and assay buffer layers. The cell pellet was lysed. Scintillation fluid was added, and radioactivity was counted on a beta-counter. Nonspecific uptake was determined for each experiment in the presence of 10 μM fluoxetine for SERT cells, 10 μM nisoxetine for NET cells, and 10 μM mazindol for DAT cells and subtracted from the total counts to yield specific uptake (100%). Nonspecific uptake was <15% of total uptake. The data were fit by non-linear regression to variable-slope sigmoidal dose-response curves, and IC_{50} values were calculated using Prism (GraphPad, San Diego, CA, USA). DAT/SERT ratios were calculated as 1/DAT IC_{50}:1/SERT IC_{50}. The DAT/SERT ratio is considered useful to predict the characteristics of the psychoactive effects of novel psychoactive substances [4,23–25]. Higher relative potency at the DAT may indicate a higher abuse potential while relatively increased activity on the 5-HT system is linked to reduced abuse potential and more MDMA-like psychotropic effects [25]. Stimulant amphetamines such as methamphetamine exhibit a DAT/SERT ratio >10, while MDMA and other substances with MDMA-like psychotropic effects exhibit a DAT/SERT ratio close to 0.1 [4,26].

2.3. Transporter-mediated monoamine release

We studied the effects of 100 μM of the test compounds on transporter-mediated NE, 5-HT, and DA efflux in HEK 293 cells that overexpressed the respective human monoamine transporter as previously reported in detail [4]. Briefly, we preloaded the cells by incubating SERT cells with 10 nM [^{3}H]5-HT, DAT cells with 10 nM [^{3}H]DA and 1 μM unlabeled DA, and NET cells with 10 nM [^{3}H]NE and 10 μM unlabeled NE for 20 min. The cells were then washed twice, and release was induced by adding 1000 μl of release buffer

that contained the test compounds at concentrations of 100 μM. We incubated the SERT and DAT cells for 15 min and NET cells for 45 min at 37 °C by shaking at 300 rotations per minute on a rotary shaker. The release times were based on kinetic evaluation of the release-over-time curves for MDMA. After 15 min for [^{3}H]5-HT and [^{3}H]DA and 45 min for [^{3}H]NE, a sufficient amount of radioactivity was released to allow for comparisons with the control conditions. We then stopped release by removing the buffer and gently washing the cells twice with cold buffer. We quantified the radioactivity that remained in the cells. Nonspecific "pseudo-efflux," which arises from substrate that diffuses out of the cells and reuptake inhibition [27,28], was assessed for each experiment using the transporter inhibitors nisoxetine (NET cells), citalopram (SERT cells), and mazindol (DAT cells) at 10 μM as negative control conditions. We then used analysis of variance followed by Dunnett's test to compare test drug-induced monoamine release with nisoxetine, citalopram, and mazindol (negative controls). Compounds that induced significantly higher maximal monoamine efflux compared with the respective transporter inhibitors, which induced slight nonspecific release, were considered monoamine releasers. MDMA was used as a positive control condition in each experiment. Previously published data on cathinones [6] were obtained from the same experiments and tested along-side with the drugs described here. Therefore the data on MDMA are the same as previously published [6] and data on cathinones [6] can be compared with those obtained with the data shown here. All of the conditions were normalized to radioactive counts of the assay buffer control condition. The assays allowed qualitative classification of a drug as a releaser or non-releaser at 100 μM, but not quantitative comparisons between transporters.

2.4. Radioligand binding assays

The radioligand binding assays were performed as described previously [4,22,29]. Briefly, membrane preparations of HEK 293 cells (Invitrogen, Zug, Switzerland) that overexpress the respective transporters [21] or receptors (human genes, with the exception of $TAAR_1$ receptors that were rat/mouse; [29]) 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 determined in the presence of selected competitors in excess. The following radioligands and competitors, respectively, were used: *N*-methyl-[^{3}H]-nisoxetine and indatraline (NET), [^{3}H]citalopram and indatraline (SERT), [^{3}H]WIN35,428 and indatraline (DAT), [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin and indatraline (5-HT_{1A} receptor), [^{3}H]ketanserin and spiperone (5-HT_{2A} receptor), [^{3}H]mesulergine and mianserin (5-HT_{2C} receptor), [^{3}H]prazosin and risperidone (α_1 adrenergic receptor), [^{3}H]rauwolscine and phentolamine (α_2 adrenergic receptor), [^{3}H]SCH 23390 and butaclamol (DA D_1 receptor), [^{3}H]spiperone and spiperone (DA D_2 and D_3 receptors), [^{3}H]pyrilamine and clozapine (histaminergic H_1 receptor), and [^{3}H]RO5166017 and RO5166017 ($TAAR_1$). IC_{50} values were determined by calculating nonlinear regression curves for a one-site model using three to five independent 10-point concentration-response curves for each compound. K_i (affinity) values, which correspond to the dissociation constants, were determined using the Cheng-Prusoff equation. Similarly obtained data on MDMA has previously been published [4,6].

3. Results

3.1. Monoamine uptake transporter inhibition

The effects of the test compounds on monoamine transporter function are presented in Fig. 2. The corresponding IC_{50} values for monoamine transport inhibition and DAT/SERT inhibition ratios are shown in Table 1. With the exception of m-CPP and TFMPP, all of the tested compounds inhibited NET with IC_{50} values of 0.1–1 μM. For comparison, clinically used NET inhibitors such as reboxetine, indatraline, or duloxetine are slightly more potent and inhibited NET with IC_{50} values of 0.036, 0.43 and 0.126 μM in the same or similar assays [22].

DAT and SERT inhibition potencies varied considerably, resulting in a wide range of DAT/SERT inhibition ratios. Both ring-substituted aminoindanes, 5-IAI and MDAI, and both phenylpiperazines, m-CPP and TFMPP, preferentially inhibited the SERT over the DAT, similar to MDMA [4,6]. The pipradrol derivatives D2PM, 2-DPMP, and methylphenidate were all considerably more potent DAT *vs.* SERT inhibitors. 2-AI and BZP showed only low potency as DAT or SERT inhibitors (IC_{50} values > 10 μM).

3.2. Transporter-mediated monoamine release

The effects of the test compounds on the transporter-mediated release of NE, DA, and 5-HT from transmitter-preloaded cells are depicted in Fig. 3. As expected, MDMA induced significant efflux of NE, DA, and 5-HT compared with the nonspecific "release" observed with the pure uptake inhibitors nisoxetine, mazindol, and citalopram, respectively. The aminoindanes were releasers of at least one monoamine. 5-IAI released 5-HT and DA. MDAI

Table 1
Monoamine uptake transport inhibition.

	NET	DAT	SERT	DAT/SERT ratio
	IC50 [μM] (95% CI)	IC50 [μM] (95% CI)	IC50 [μM] (95% CI)	Ratio (95% CI)
Aminoindans				
5-IAI	0.76 (0.60–0.98)	23 (15–35)	2.5 (1.9–3.4)	0.11
MDAI	0.65 (0.50–0.84)	31 (23–41)	8.3 (3.2–22)	0.2
2-AI	0.54 (0.42–0.69)	58 (4–905)	>100	>1
Piparazines				
m-CPP	1.67 (1.2–2.4)	31 (25–38)	1.2 (0.9–1.6)	0.04
TFMPP	17.5 (8–39)	>100	5.2 (3.8–7.0)	< 0.05
BZP	0.41 (0.33–0.53)	17 (15–19)	57 (40–81)	3.39
Pipradrol derivatives				
D2PM	0.41 (0.34–0.50)	0.86 (0.74–1.0)	38 (4.7–307)	44.36
2-DPMP	0.14 (0.11–0.18)	0.07 (0.06–0.08)	>10	>100
Methylphenidate	0.13 (0.10–0.16)	0.12 (0.09–0.16)	>100	>100

Values are means of three to four independent experiments and 95% confidence intervals (CI). DAT/SERT ratio = 1/DAT IC_{50}: 1/SERT IC_{50}.

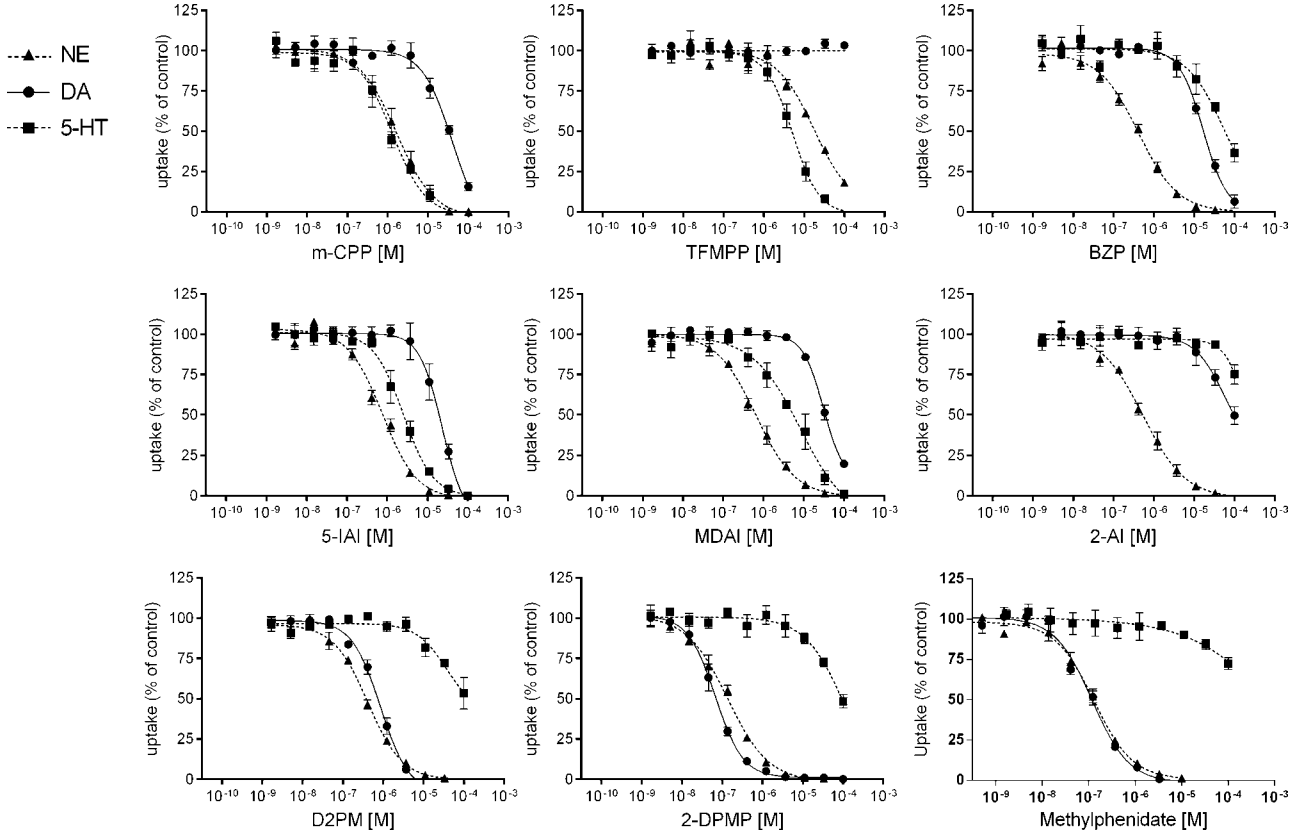


Fig. 2. Monoamine uptake inhibition presented as dose-response curves for the inhibition of [^{3}H]NE, [^{3}H]DA, and [^{3}H]5-HT into NET-, DAT-, and SERT-transfected HEK 293 cells, respectively. The data are expressed as the mean ± SEM of 3-4 independent experiments. The data were fit by nonlinear regression. The corresponding IC_{50} values are shown in Table 2.

released 5-HT and NE. 2-AI released NE and DA. Among the piperazines, BZP released DA, m-CPP released 5-HT, and TFMPP did not induce the efflux of any monoamine. None of the pipradrol derivatives or methylphenidate was a substrate releaser.

3.3. Binding affinities

Table 2 shows the binding profiles of the test compounds expressed as the potencies of the compounds (K_i) to inhibit radioligand binding to the NET, DAT, and SERT and different monoamine receptors. Among the aminoindanes, the binding profile of MDAI was similar to MDMA [4,6], whereas 5-IAI exhibited submicromolar affinities (<1 μM) for the 5-HT_{1A}, 5-HT_{2A}, α_{2A}, and D_3 receptors. In contrast to MDMA [4,6], the phenylpiperazines m-CPP and TFMPP showed submicromolar (<1 μM) binding to many monoamine receptors, including the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, α_{2A}, and D_{1-3} receptors. The pipradrol derivatives and methylphenidate potently bound to the DAT, but not to any other sites. The aminoindanes, and the phenylpiperazines showed affinity for the rat and mouse $TAAR_1$, similar to MDMA [4,6]. Binding potencies at the monoamine transporters were typically weak, except for the high-affinity (<100 nM) binding of the pipradrol derivatives at the DAT.

4. Discussion

All of the novel substances characterized in the present study interacted with the monoamine transporters. High potency of a compound to inhibit the catecholamine transporter NET and DAT *in vitro* is associated with greater psychostimulant potency in humans [4]. These compounds typically exhibit a DAT/SERT ratio >1 and a high abuse potential [4]. Predominant drug activity at the SERT [22] and a DAT/SERT inhibition ratio of typically 0.01–0.1 are expected to result in subjective drug effects similar to those of MDMA or other empathogens [4,6]. These serotonergic compounds produce subjective well-being and enhanced empathy and sociability in humans without marked psychostimulation [5,30]. Additionally, compounds which predominantly act on SERT and NET [6] have been associated with 5-HT syndrome, hyperthermia and resulting organ failure. Furthermore, compounds which act as monoamine releasers (i.e., MDMA or methamphetamine [4,6]) enter the intracellular space via the transporter. In contrast to pure transporter blockers (i.e., cocaine), monoamine releasers are expected to have more subsequent intracellular pharmacological and neurotoxic consequences [31,32].

The *in vitro* pharmacological profiles of the compounds studied herein may be useful to predict the clinical effects according to the associations noted above. The profiles can also be compared with those of cocaine and a series of recreationally used amphetamine and cathinone derivatives previously characterized using the same *in vitro* assays [4,6].

4.1. Aminoindanes

The aminoindanes 5-IAI and MDAI preferentially inhibited the NET and SERT and less potently inhibited the DAT, similar to MDMA [4,6], but with approximately two-fold lower potency. 5-IAI and MDAI released 5-HT through the SERT, similar to MDMA. MDAI also shared the NE-releasing property and receptor binding profile of MDMA [4,6]. Similar inhibitory effects of 5-IAI and MDAI on human monoamine transporters have recently been shown [7],

Table 2
Monoamine transporter and receptor binding affinities.

	NET	DAT	SERT	5-HT$_{1A}$	5-HT$_{2A}$	5-HT$_{2C}$	α_{1A}	α_{2A}	D_1	D_2	D_3	H_1	TAAR$_{1rat}$	TAAR$_{1mouse}$
Aminoindanes														
5-IAI	6.3 ± 1.4	5.6 ± 1.5	34 ± 16	0.28 ± 0.08	0.73 ± 0.14	1.2 ± 0.6	>6	0.87 ± 0.33	>12	1.2 ± 0.6	0.68 ± 0.09	7.4 ± 1.3	0.03 ± 0.01	1.1 ± 0.3
MDAI	18 ± 2	12 ± 4	22 ± 12	>17	>12	>12	>6	1.36 ± 0.51	>12	>10	14 ± 2	>13	0.57 ± 0.19	1.8 ± 0.1
2-AI	20 ± 7	21 ± 5	>30	4.0 ± 0.8	>12	>12	>6	0.45 ± 0.10	>12	>10	7.6 ± 2.9	>13	0.31 ± 0.09	2.1 ± 0.4
Piperazines														
m-CPP	3.0 ± 0.4	5.8 ± 1.4	0.63 ± 0.1	0.14 ± 0.01	0.06 ± 0.02	0.13 ± 0.02	0.52 ± 0.01	0.26 ± 0.02	4.0 ± 0.1	2.2 ± 0.8	2.4 ± 0.6	1.5 ± 0.2	0.05 ± 0.01	6.6 ± 1.1
TFMPP	13 ± 2	>25	1.7 ± 0.04	0.17 ± 0.02	0.06 ± 0.01	0.13 ± 0.01	>6	0.73 ± 0.2	>12	1.4 ± 1.0	0.54 ± 0.05	3.3 ± 0.7	0.38 ± 0.06	2.3 ± 0.6
BZP	8.1 ± 0.7	11 ± 4	24 ± 8	> 17	>12	>12	>6	16 ± 5	>12	>10	>16	>13	>10	>10
Pipradrol derivatives														
D2PM	8.2 ± 2.8	0.07 ± 0.03	8.4 ± 1.3	>17	>12	>12	>6	>30	>12	>10	>16	>13	>10	>10
2-DPMP	38 ± 11	0.007 ± 0.001	>30	>17	>12	5.5 ± 0.1	>6	27 ± 9	>12	>10	>16	>13	>10	>10
Methylphenidate	3.3 ± 3.6	0.06 ± 0.01	21 ± 9	NA	>12	NA	>6	20 ± 9	NA	>10	NA	NA	>10	>10

NA, not assessed. Values are K_i given as μM (mean ± SD).

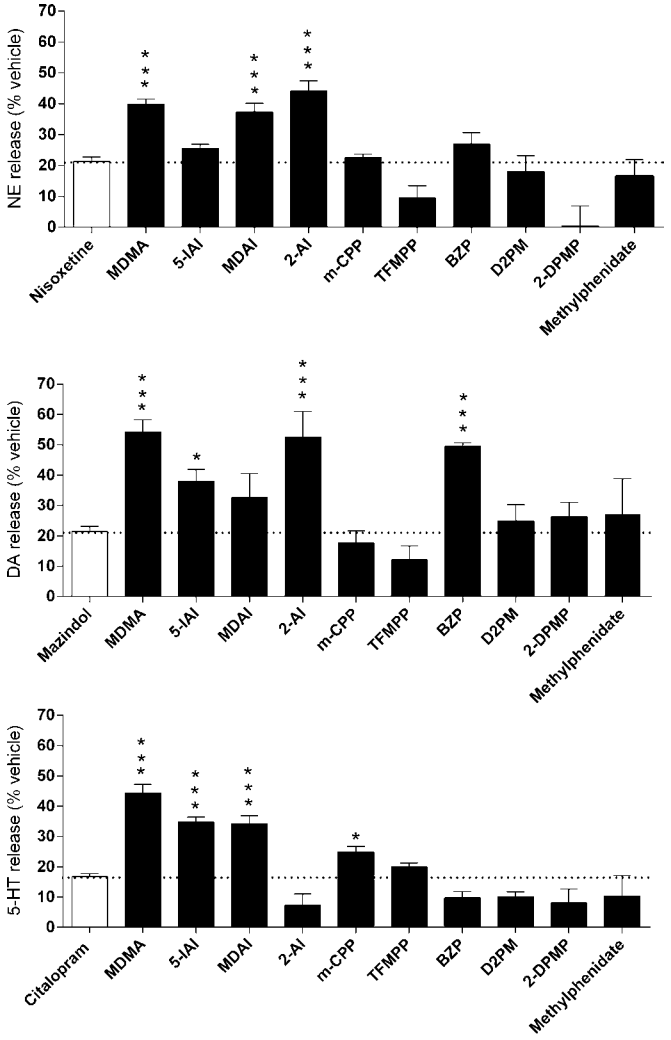


Fig. 3. Monoamine release induced by 100 μM of test compound. HEK 293 cells that expressed NET, DAT, and SERT were loaded with [^{3}H]NE, [^{3}H]DA, and [^{3}H]5-HT, respectively, washed, and incubated with a high concentration of the compounds (100 μM). Monoamine release is expressed as the percent reduction of monoamine cell content compared with vehicle (0% = no release). 100% release would indicate that all of the monoamine was released from the cells. In such a batch assay, non-releasing monoamine transporter blockers induce nonspecific "pseudo-efflux" (dashed line, open bars), which arises from substrate that diffuses out of the cells and reuptake inhibition. Only compounds that produced significantly more monoamine efflux ($^*p < 0.05$, $^{***}p < 0.001$) compared with the non-releasing uptake inhibitors (negative controls, open bars) nisoxetine (HEK-NET cells), mazindol (HEK-DAT cells), and citalopram (HEK-SERT cells) were considered monoamine releasers. The known monoamine releaser MDMA served as a positive control condition for each experiment. The data are expressed as the mean ± SEM of 3–4 independent experiments (with negative and positive controls added in each experiment).

but no comparable data on monoamine release are available. In contrast to the human transporter studies, both MDAI and 5-IAI were relatively more potent SERT and DAT *vs.* NET inhibitors in rat brain synaptosomes [33]. Similar to our data, MDAI released 5-HT, but not DA, and 5-IAI released both 5-HT and DA from rat brain synaptosomes [33]. 5-IAI and MDAI substituted for MDMA in drug discrimination studies [20,34], but were considered less neurotoxic than MDMA [20,34,35]. This profile may increase the popularity of these aminoindanes [13]. The comparable monoamine transporter inhibition and release profile to MDMA [4,6] would predict that MDAI has very similar subjective effects to MDMA, and this is supported by user reports [12,36]. Rare severe complications include serotonin syndrome and hyperthermia [36],

also similar to MDMA. In contrast to MDAI and MDMA [4,6], 5-IAI exhibited relevant binding to 5-HT receptors, including the 5-HT_{2A} receptor that is implicated in the action of hallucinogens [37]. 5-IAI is also considered a less potent MDMA substitute, but dysphoria, anxiety, and hallucinations have also been reported [13]. In contrast to the substituted aminoindanes, 2-AI selectively inhibited the NET, but not the DAT or SERT. This profile is relatively similar to BZP in the present study, but most other amphetamines also typically more potently inhibit the DAT [4,6]. 2-AI also released NE and DA. No comparable data on the pharmacology of 2-AI have been reported. Based on the profile in the present study, 2-AI likely has only mild psychostimulant effects in humans.

4.2. Piperazines

Although piperazines have been widely used since the 1990s, and their pharmacology and toxicology have been reviewed [14,38–41], only few and conflicting original data are available on their pharmacological mechanism. In the present study, BZP inhibited the NET and released DA. Early studies in rats found that BZP inhibits the uptake of not only NE and DA, but also 5-HT [42], which is very inconsistent with our data obtained with human transporters and recent rat studies [43]. Similar to the present study, BZP produced the transporter-mediated release of DA, but not 5-HT from rat synaptosomes *in vitro* [43]. BZP enhanced electrically induced NE release from rabbit arteries [44], likely reflecting its NET-inhibiting properties. BZP also induced a robust increase in extracellular DA *in vivo*, but only weakly increased 5-HT dialysate levels at higher doses [43]. Speculations that BZP may act as an α_2-adrenergic antagonist [44] in humans seem unlikely, given the lack of binding to this and other monoamine receptors in the present study. We also did not confirm the results of an early rat study that reported the 5-HT antagonistic properties of BZP [45]. Thus, our data indicate that BZP is an indirect DA and NE agonist without serotonergic properties. In animals, BZP induced place preference in rats [46] and was self-administered in monkeys, and it substituted for amphetamine in discrimination studies [47]. In humans, 100 mg BZP produced subjective and cardiostimulant effects similar to 7.5–10 mg amphetamine [48,49], consistent with the five- to 10-fold lower potency of BZP at the NET and DAT compared with amphetamine [4]. In healthy women, a dose of 200 mg BZP produced cardiostimulant and subjective effects that were considered similar to those generally seen with stimulants [50], but a direct comparison with other compounds is lacking. The clinical toxicity of BZP mainly includes hallucinations, agitation, seizures, and hyperthermia [40]. Drug users associated more unpleasant effects and hallucinations with BZP than with MDMA [51]. The phenylpiperazines TFMPP and m-CPP preferentially inhibited the SERT as previously reported [52,53]. TFMPP did not act as a 5-HT releaser, and m-CPP only weakly released 5-HT in the present study. SERT-mediated 5-HT release from rat brain synaptosomes or slices has previously been documented for both TFMPP [43,54] and m-CPP [54–56]. Further studies are needed to determine whether the phenylpiperazines differentially interact with the human and rat SERT and whether additional proteins present in the synaptosomal preparations, but not in transfected HEK-293 cells may explain this discrepancy. Also needing clarification is the extent to which the *in vivo* serotonergic action of m-CPP is linked to 5-HT release *vs.* uptake inhibition. In fact, m-CPP has been shown to bind more potently to the SERT than the 5-HT releaser fenfluramine and not to induce long-term 5-HT depletion [53], which are both characteristics of SERT inhibitors rather than 5-HT releasers. m-CPP did not release DA or NE from synaptosomes [56], consistent with our data. Furthermore, we confirmed the previously documented binding of TFMPP and m-CPP to rat 5-HT receptors [52] for the human 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors. In rhesus monkeys, TFMPP has no reinforcing properties and does not maintain responding for amphetamine [47]. Additionally, TFMPP reduced the self-administration of BZP and responding for cocaine [47]. Altogether, the preclinical data indicate that both m-CPP and TFMPP are both indirect and direct serotonergic agonists without relevant dopaminergic activity. However, their precise interaction with the human SERT and the nature of their serotonergic action *in vivo* require further investigations. m-CPP is frequently found in Ecstasy pills as a replacement for MDMA [57,58]. Recreational users consider m-CPP to have less desirable psychotropic effects and more adverse effects, including nausea, compared with MDMA [51,58]. In experimental studies in humans, m-CPP produced mostly dysphoria, weakness, dizziness, anxiety, and nausea [59–61] and less, if any, positive subjective effects, drug liking, and cardiovascular stimulation in direct comparisons with MDMA [62]. The lower clinical potency and efficacy of m-CPP compared with MDMA may be explained by its lower potency as a DAT and NET inhibitor compared with MDMA [4,6] or by its lower efficacy to induce the release of 5-HT. The effects of TFMPP have not been directly compared with other psychoactive substances in humans. TFMPP alone produced moderate dysphoria and amphetamine-type stimulation [63], but not the usual increases in euphoria seen after MDMA administration [64] using the same psychometric scale. Unsurprisingly, therefore, the use of TFMPP alone does not appear to be common [51]. In contrast, BZP in combination with either m-CPP or TFMPP is sometimes sold as Ecstasy [16,41]. Because BZP releases DA, and m-CPP and TFMPP are direct and indirect serotonergic agonists, their combination would be expected to mimic the psychoactive profile of MDMA. In rats, the combination of BZP and TFMPP elevated brain DA and 5-HT levels similarly to MDMA [43]. In humans, the combination of BZP and TFMPP produced stimulation and "good" drug effects, but no euphoria [65]. The BZP-TFMPP combination was not well tolerated at higher doses and frequently produced agitation, anxiety, hallucinations, and vomiting [66], whereas these adverse effects were infrequently observed after MDMA administration in a similar laboratory study [67]. As noted above, the BZP-TFMPP combination has reduced reinforcing properties compared with BZP alone [47], consistent with the abuse-lowering effects of 5-HT.

4.3. Pipradrol derivatives

D2PM and 2-DPMP were selective catecholamine transporter inhibitors without transporter-mediated substrate-releasing properties, similar to methylphenidate. 2-DPMP was a DAT/NET inhibitor that was equally potent to methylphenidate, whereas D2PM was less potent. Consistent with our findings, 2-DPMP has been previously shown to inhibit the human NET and DAT, but not SERT [7], and block the uptake of DA and NE into synaptic rat brain vesicles [68,69]. 2-DPMP also blocked NE uptake into rabbit aortic strips, but did not induce NE release [70], also consistent with our results. Compared with classic stimulants, 2-DPMP was a 10-fold more potent DAT blocker than cocaine [4]. Consistent with the greater DAT-inhibiting potency, 2-DPMP also more potently increased electrically evoked DA release in rat brain slices compared with cocaine [71]. We found no other data on the monoamine uptake and releasing properties of D2PM. The pharmacological profile of the pipradrol derivatives was very similar to the pyrovalerone cathinones MDPV and naphyrone that were characterized in the same assays [4], although naphyrone also inhibits the SERT. MDPV and naphyrone rather than 2-DPMP have been found in some samples of "ivory wave" [72]. Similar to MDPV [4] and naphyrone [73], 2-DPMP and D2PM are highly

lipophilic. Compared with methylphenidate, 2-DPMP lacks polar groups that are typically targeted by metabolic enzymes, resulting in a longer half-life [74,75]. The clinical toxicity of 2-DPMP and D2PM is long-lasting (24–72 h) and involves sympathomimetic stimulation and predominantly psychiatric symptoms, including agitation, hallucinations, and insomnia [17,18]. Altogether, the pipradrol derivatives are potent and selective catecholamine uptake inhibitors, consistent with their potent and prolonged psychostimulant actions. The pharmacological profile is also likely associated with high abuse liability and an increased risk of psychiatric complications.

4.4. $TAAR_1$ binding

The aminoindanes and phenylpiperazines, but not BZP or pipradrol derivatives, exhibited potent $TAAR_1$ binding affinity comparable to MDMA [4,6]. In the present series, all of the serotonergic compounds also bound $TAAR_1$, whereas the affinity for $TAAR_1$ has previously been documented for amphetamine and methamphetamine [4], which only weakly interact with the SERT. Drug activity at the SERT and $TAAR_1$ are both considered to counteract the abuse liability associated with dopaminergic drug properties. Higher serotonergic *vs*. dopaminergic activity has been associated with a lower abuse potential of a drug [4,23–25]. Amphetamines such as MDMA and methamphetamine have been shown to inhibit their own neurochemical and locomotor stimulant effects via $TAAR_1$ activation [76]. The lack of serotonergic activity and lack of $TAAR_1$-mediated "auto-inhibition" in particular with the pipradrol derivatives may contribute to the more stimulant-like and addictive properties of this class of designer compounds compared with classic amphetamines, including MDMA [4].

4.5. Limitations

Knowing the mechanism of action of novel compounds *in vitro* helps to predict potential clinical effects and abuse potential. However, many additional factors also play a role such as brain tissue penetration and pharmacokinetics which need to be further assessed *in vivo*.

5. Conclusion

In summary, the aminoindanes, 5-IAI and MDAI inhibited the SERT and released 5-HT, similar to MDMA [4]. Among the piperazines, BZP interacted with the DAT and NET, and m-CPP and TFMPP interacted with the SERT and serotonergic receptors. The pipradrol derivatives were all potent and selective catecholamine transporter blockers without substrate-releasing properties. The predominant actions of D2PM and 2-DPMP on DAT likely predict a high abuse liability. Further studies are needed to determine potential differences between data obtained with human or rodent transporter studies and to further validate predictions of clinical effects based on such data.

Conflict of interest

The authors do not have any conflicts of interest to declare for this work.

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References

[1] Dargan PI, Wood DM. Novel Psychoactive Substances: Classification, Pharmacology and Toxicology. Amsterdam: Elsevier Academic Press; 2013.
[2] Hill SL, Thomas SH. Clinical toxicology of newer recreational drugs. Clin Toxicol (Phila) 2011;49:705–19.
[3] Dal Cason TA, Young R, Glennon RA. Cathinone: an investigation of several *N*-alkyl and methylenedioxy-substituted analogs. Pharmacol Biochem Behav 1997;58:1109–16.
[4] Simmler L, Buser T, Donzelli M, Schramm Y, Dieu LH, Huwyler J, et al. Pharmacological characterization of designer cathinones *in vitro*. Br J Pharmacol 2013;168:458–70.
[5] Hysek CM, Schmid Y, Simmler LD, Domes G, Heinrichs M, Eisenegger C, et al. MDMA enhances emotional empathy and prosocial behavior. Soc Cogn Affect Neurosci 2013 (in press).
[6] Simmler LD, Rickli A, Hoener MC, Liechti ME. Monoamine transporter and receptor interaction profiles of a new series of designer cathinones. Neuropharmacology 2014;79:152–60.
[7] Iversen L, Gibbons S, Treble R, Setola V, Huang XP, Roth BL. Neurochemical profiles of some novel psychoactive substances. Eur J Pharmacol 2013;700:147–51.
[8] Baumann MH, Partilla JS, Lehner KR, Thorndike EB, Hoffman AF, Holy M, et al. Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive bath salts products. Neuropsychopharmacology 2013;38:552–62.
[9] Lopez-Arnau R, Martinez-Clemente J, Pubill D, Escubedo E, Camarasa J. Comparative neuropharmacology of three psychostimulant cathinone derivatives: butylone, mephedrone and methylone. Br J Pharmacol 2012;167:407–20.
[10] Eshleman AJ, Wolfrum KM, Hatfield MG, Johnson RA, Murphy KV, Janowsky A. Substituted methcathinones differ in transporter and receptor interactions. Biochem Pharmacol 2013;85:1803–15.
[11] Sainsbury PD, Kicman AT, Archer RP, King LA, Braithwaite RA. Aminoindanes: the next wave of legal highs. Drug Test Anal 2011;3:479–82.
[12] Gallagher CT, Assi S, Stair JL, Fergus S, Corazza O, Corkery JM, et al. 5,6-Methylenedioxy-2-aminoindane: from laboratory curiosity to legal high. Hum Psychopharmacol 2012;27:106–12.
[13] Coppola M, Mondola R. 5-Iodo-2-aminoindan (5-IAI): Chemistry, pharmacology, and toxicology of a research chemical producing MDMA-like effects. Toxicol Lett 2013;218:24–9.
[14] Monteiro MS. Bastos Mde L, Guedes de Pinho P, Carvalho M. Update on 1-benzylpiperazine (BZP) party pills. Arch Toxicol 2013;87:929–47.
[15] Wilkins C, Sweetsur P, Girling M. Patterns of benzylpiperazine/trifluoromethylphenylpiperazine party pill use and adverse effects in a population sample in New Zealand. Drug Alcohol Rev 2008;27:633–9.
[16] Wood DM, Button J, Lidder S, Ramsey J, Holt DW, Dargan PI. Dissociative and sympathomimetic toxicity associated with recreational use of 1-(3-trifluoromethylphenyl) piperazine (TFMPP) and 1-benzylpiperzine (BZP). J Med Toxicol 2008;4:254–7.
[17] Murray DB, Potts S, Haxton C, Jackson G, Sandilands EA, Ramsey J, et al. Ivory wave toxicity in recreational drug users; integration of clinical and poisons information services to manage legal high poisoning. Clin Toxicol 2012;50:108–13.
[18] Wood DM, Puchnarewicz M, Johnston A, Dargan PI. A case series of individuals with analytically confirmed acute diphenyl-2-pyrrolidinemethanol (D2PM) toxicity. Eur J Clin Pharmacol 2012;68:349–53.
[19] Corkery JM, Elliott S, Schifano F, Corazza O, Ghodse AH. 2-DPMP (desoxypipradrol, 2-benzhydrylpiperidine, 2-phenylmethylpiperidine) and D2PM (diphenyl-2-pyrrolidin-2-yl-methanol, diphenylprolinol): A preliminary review. Prog Neuropsychopharmacol Biol Psychiatry 2012;39:253–8.
[20] Nichols DE, Brewster WK, Johnson MP, Oberlender R, Riggs RM. Nonneurotoxic tetralin and indan analogues of 3,4-(methylenedioxy)amphetamine (MDA). J Med Chem 1990;33:703–10.
[21] Tatsumi M, Groshan K, Blakely RD, Richelson E. Pharmacological profile of antidepressants and related compounds at human monoamine transporters. Eur J Pharmacol 1997;340:249–58.
[22] Hysek CM, Simmler LD, Nicola V, Vischer N, Donzelli M, Krähenbühl S, et al. Duloxetine inhibits effects of MDMA (ecstasy) in vitro and in humans in a randomized placebo-controlled laboratory study. PLoS One 2012;7:e36476.
[23] Baumann MH, Clark RD, Woolverton WL, Wee S, Blough BE, Rothman RB. In vivo effects of amphetamine analogs reveal evidence for serotonergic inhibition of mesolimbic dopamine transmission in the rat. J Pharmacol Exp Ther 2011;337:218–25.
[24] Rothman RB, Baumann MH. Balance between dopamine and serotonin release modulates behavioral effects of amphetamine-type drugs. Ann N Y Acad Sci 2006;1074:245–60.
[25] Wee S, Anderson KG, Baumann MH, Rothman RB, Blough BE, Woolverton WL. Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. J Pharmacol Exp Ther 2005;313:848–54.
[26] Baumann MH, Ayestas Jr MA, Partilla JS, Sink JR, Shulgin AT, Daley PF, et al. The designer methcathinone analogs, mephedrone and methylone, are substrates

for monoamine transporters in brain tissue. Neuropsychopharmacology 2012;37:1192–203.
[27] Scholze P, Zwach J, Kattinger A, Pifl C, Singer EA, Sitte HH. Transporter-mediated release: a superfusion study on human embryonic kidney cells stably expressing the human serotonin transporter. J Pharmacol Exp Ther 2000;293:870–8.
[28] Rosenauer R, Luf A, Holy M, Freissmuth M, Schmid R, Sitte HH. A combined approach using transporter-flux assays and mass spectrometry to examine psychostimulant street drugs of unknown content. ACS Chem Neurosci 2013;4:182–90.
[29] Revel FG, Moreau JL, Gainetdinov RR, Bradaia A, Sotnikova TD, Mory R, et al. TAAR1 activation modulates monoaminergic neurotransmission, preventing hyperdopaminergic and hypoglutamatergic activity. Proc Natl Acad Sci U S A 2011;108:8485–90.
[30] Hysek CM, Simmler LD, Schillinger N, Meyer N, Schmid Y, Donzelli M, et al. Pharmacokinetic and pharmacodynamic effects of methylphenidate and MDMA administered alone and in combination. Int J Neuropsychopharmacol 2013 (in press).
[31] Fleckenstein AE, Gibb JW, Hanson GR. Differential effects of stimulants on monoaminergic transporters: pharmacological consequences and implications for neurotoxicity. Eur J Pharmacol 2000;406:1–13.
[32] Baumann MH, Wang X, Rothman RB. 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. Psychopharmacology 2007;189:407–24.
[33] Johnson MP, Conarty PF, Nichols DE. [3H]monoamine releasing and uptake inhibition properties of 3,4-methylenedioxymethamphetamine and p-chloroamphetamine analogues. Eur J Pharmacol 1991;200:9–16.
[34] Nichols DE, Johnson MP, Oberlender R. 5-Iodo-2-aminoindan, a nonneurotoxic analogue of p-iodoamphetamine. Pharmacol Biochem Behav 1991;38:135–9.
[35] Johnson MP, Huang XM, Nichols DE. Serotonin neurotoxicity in rats after combined treatment with a dopaminergic agent followed by a nonneurotoxic 3,4-methylenedioxymethamphetamine (MDMA) analogue. Pharmacol Biochem Behav 1991;40:915–22.
[36] Corkery JM, Elliott S, Schifano F, Corazza O, Ghodse AHMDAI. (5,6-methylenedioxy-2-aminoindane; 6,7-dihydro-5H-cyclopenta[f][1,3]benzodioxol-6-amine; 'sparkle'; 'mindy') toxicity: a brief overview and update. Hum Psychopharmacol 2013;28:345–55.
[37] Nichols DE. Hallucinogens. Pharmacol Ther 2004;101:131–81.
[38] Schep LJ, Slaughter RJ, Vale JA, Beasley DM, Gee P. The clinical toxicology of the designer party pills benzylpiperazine and trifluoromethylphenylpiperazine. Clin Toxicol 2011;49:131–41.
[39] European Monitoring Centre for Drugs and Drug Addiction. Report on the risk assessment of BZP in the framework of the Council decision on new psychoactive substances. Issue 8. Lisbon: European Monitoring Centre for Drugs and Drug Addiction; 2009.
[40] Gee P, Gilbert M, Richardson S, Moore G, Paterson S, Graham P. Toxicity from the recreational use of 1-benzylpiperazine. Clin Toxicol 2008;46:802–7.
[41] Sheridan J, Butler R, Wilkins C, Russell B. Legal piperazine-containing party pills: a new trend in substance misuse. Drug Alcohol Rev 2007;26:335–43.
[42] Tekes K, Tothfalusi L, Malomvolgyi B, Herman F, Magyar K. Studies on the biochemical mode of action of EGYT-475, a new antidepressant. Pol J Pharmacol Pharm 1987;39:203–11.
[43] Baumann MH, Clark RD, Budzynski AG, Partilla JS, Blough BE, Rothman RB. N-substituted piperazines abused by humans mimic the molecular mechanism of 3,4-methylenedioxymethamphetamine (MDMA, or 'Ecstasy'). Neuropsychopharmacology 2005;30:550–60.
[44] Magyar K, Fekete MI, Tekes K, Torok TL. The action of trelibet, a new antidepressive agent on [3H]noradrenaline release from rabbit pulmonary artery. Eur J Pharmacol 1986;130:219–27.
[45] Malomvolgyi B, Tothfalusi L, Tekes K, Magyar K. Comparison of serotonin agonistic and antagonistic activities of a new antidepressant agent Trelibet (EGYT-475) and its metabolite EGYT-2760 on isolated rat fundus. Acta Physiol Hung 1991;78:201–9.
[46] Meririnne E, Kajos M, Kankaanpaa A, Seppala T. Rewarding properties of 1-benzylpiperazine, a new drug of abuse, in rats. Basic Clin Pharmacol Toxicol 2006;98:346–50.
[47] Fantegrossi WE, Winger G, Woods JH, Woolverton WL, Coop A. Reinforcing and discriminative stimulus effects of 1-benzylpiperazine and trifluoromethylphenylpiperazine in rhesus monkeys. Drug Alcohol Depend 2005;77:161–8.
[48] Bye C, Munro-Faure AD, Peck AW, Young PA. A comparison of the effects of 1-benzylpiperazine and dexamphetamine on human performance tests. Eur J Clin Pharmacol 1973;6:163–9.
[49] Campbell H, Cline W, Evans M, Lloyd J, Peck AW. Proceedings Comparison of the effects of dexamphetamine and 1-benzylpiperazine in former addicts. Br J Pharmacol 1972;44:70P–369P.
[50] Lin JC, Bangs N, Lee H, Kydd RR, Russell BR. Determining the subjective and physiological effects of BZP on human females. Psychopharmacology 2009;207:439–46.
[51] Brunt TM, Koeter MW, Niesink RJ, van den Brink W. Linking the pharmacological content of ecstasy tablets to the subjective experiences of drug users. Psychopharmacology 2012;220:751–62.
[52] Owens MJ, Morgan WN, Plott SJ, Nemeroff CB. Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. J Pharmacol Exp Ther 1997;283:1305–22.
[53] Baumann MH, Ayestas MA, Dersch CM, Rothman RB. 1-(m-chlorophenyl)-piperazine (mCPP) dissociates in vivo serotonin release from long-term serotonin depletion in rat brain. Neuropsychopharmacology 2001;24:492–501.
[54] Auerbach SB, Kamalakannan N, Rutter JJ. TFMPP and RU24969 enhance serotonin release from rat hippocampus. Eur J Pharmacol 1990;190:51–7.
[55] Pettibone DJ, Williams M. Serotonin-releasing effects of substituted piperazines *in vitro*. Biochem Pharmacol 1984;33:1531–5.
[56] Rothman RB, Baumann MH. Therapeutic and adverse actions of serotonin transporter substrates. Pharmacol Ther 2002;95:73–88.
[57] Brunt TM, Poortman A, Niesink RJ, van den Brink W. Instability of the ecstasy market and a new kid on the block: mephedrone. J Psychopharmacol 2011;25:1543–7.
[58] Bossong MG, Brunt TM, Van Dijk JP, Rigter SM, Hoek J, Goldschmidt HM, et al. mCPP: an undesired addition to the ecstasy market. J Psychopharmacol 2010;24:1395–401.
[59] Broocks A, Briggs NC, Pigott TA, Hill JL, Canter SK, Tolliver TJ, et al. Behavioral, physiological and neuroendocrine responses in healthy volunteers to m-chlorophenylpiperazine (m-CPP) with and without ondansetron pretreatment. Psychopharmacology 1997;130:91–103.
[60] Feuchtl A, Bagli M, Stephan R, Frahnert C, Kolsch H, Kuhn KU, et al. Pharmacokinetics of m-chlorophenylpiperazine after intravenous and oral administration in healthy male volunteers: implication for the pharmacodynamic profile. Pharmacopsychiatry 2004;37:180–8.
[61] Gijsman HJ, Van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari MD, et al. Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. J Clin Psychopharmacol 1998;18:289–95.
[62] Tancer M, Johanson CE. Reinforcing, subjective, and physiological effects of MDMA in humans: a comparison with d-amphetamine and mCPP. Drug Alcohol Depend 2003;72:33–44.
[63] Jan RK, Lin JC, Lee H, Sheridan JL, Kydd RR, Kirk IJ, et al. Determining the subjective effects of TFMPP in human males. Psychopharmacology 2010;211:347–53.
[64] Hysek CM, Domes G, Liechti MEMDMA. enhances mind reading of positive emotions and impairs mind reading of negative emotions. Psychopharmacology 2012;222:293–302.
[65] Lin JC, Jan RK, Lee H, Jensen MA, Kydd RR, Russell BR. Determining the subjective and physiological effects of BZP combined with TFMPP in human males. Psychopharmacology 2011;214:761–8.
[66] Thompson I, Williams G, Caldwell B, Aldington S, Dickson S, Lucas N, et al. Randomised double-blind, placebo-controlled trial of the effects of the party pills BZP/TFMPP alone and in combination with alcohol. J Psychopharmacol 2010;24:1299–308.
[67] Liechti ME, Gamma A, Vollenweider FX. Gender differences in the subjective effects of MDMA. Psychopharmacology 2001;154:161–8.
[68] Ferris RM, Tang FL. Comparison of the effects of the isomers of amphetamine, methylphenidate and deoxypipradrol on the uptake of l-[3H]norepinephrine and [3H]dopamine by synaptic vesicles from rat whole brain, striatum and hypothalamus. J Pharmacol Exp Ther 1979;210:422–8.
[69] Ferris RM, Tang FL, Maxwell RA. A comparison of the capacities of isomers of amphetamine, deoxypipradrol and methylphenidate to inhibit the uptake of tritiated catecholamines into rat cerebral cortex slices, synaptosomal preparations of rat cerebral cortex, hypothalamus and striatum and into adrenergic nerves of rabbit aorta. J Pharmacol Exp Ther 1972;181:407–16.
[70] Maxwell RE, Chaplin E, Eckhardt SB, Soares JR, Hite G. Conformational similarities between molecular models of phenethylamine and of potent inhibitors of the uptake of tritiated norepinephrine by adrenergic nerves in rabbit aorta. J Pharmacol Exp Ther 1970;173:158–65.
[71] Davidson C, Ramsey J. Desoxypipradrol is more potent than cocaine on evoked dopamine efflux in the nucleus accumbens. J Psychopharmacol 2012;26:1036–41.
[72] Durham M. Ivory wave: the next mephedrone. Emerg Med J 2011;28:1059–60.
[73] Derungs A, Schietzel S, Meyer MR, Maurer HH, Krahenbuhl S, Liechti ME. Sympathomimetic toxicity in a case of analytically confirmed recreational use of naphyrone (naphthylpyrovalerone). Clin Toxicol 2011;49:691–3.
[74] Coppola M, Mondola R. Research chemicals marketed as legal highs: the case of pipradrol derivatives. Toxicol Lett 2012;212:57–60.
[75] Tripod J, Sury E, Hoffmann K. Zentralerregende Wirkung eines neuen Piperidinderivates [Analeptic effect of a new piperidine derivative]. Experientia 1954;10:261–2.
[76] Di Cara B, Maggio R, Aloisi G, Rivet JM, Lundius EG, Yoshitake T, et al. Genetic deletion of trace amine 1 receptors reveals their role in auto-inhibiting the actions of ecstasy (MDMA). J Neurosci 2011;31:16928–40.