ABSTRACT
Ecstasy samples often contain byproducts of the illegal, uncontrolled synthesis of N-methyl-3,4-methylenedioxy-amphetamine or 3,4-methylenedioxy-methamphetamine (MDMA). MDMA and eight chemically defined byproducts of MDMA synthesis were investigated for their interaction with the primary sites of action of MDMA, namely the human plasmalemmal monamine transporters for norepinephrine, serotonin, and dopamine [(norepinephrine transporter (NET), serotonin transporter (SERT), and dopamine transporter (DAT)]. SK-N-MC neuroblastoma and human embryonic kidney cells stably transfected with the transporter cDNA were used for uptake and release experiments. Two of the eight compounds, 1,3-bis(3,4-methylenedioxyphenyl)-2-propanamine (12) and N-formyl-1,3-bis(3,4-methylenedioxyphenyl)-prop-2-yl-amine (13) had uptake inhibitory potencies with IC \(_{50}\) values in the low micromolar range similar to MDMA. Compounds with nitro instead of amino groups and a phenylethenyl instead of a phenylethyl structure or a formamide or acetamide modification had IC \(_{50}\) values beyond 100 \(\mu\)M. MDMA, 12, and 13 were examined for induction of carrier-mediated release by superfusion of transporter expressing cells preloaded with the metabolically inert transporter substrate \([3H]1\)-methyl-4-phenylpyridinium. MDMA induced release mediated by NET, SERT, or DAT with EC \(_{50}\) values of 0.64, 1.12, and 3.24 \(\mu\)M, respectively. 12 weakly released from NET- and SERT-expressing cells with maximum effects less than one-tenth of that of MDMA and did not release from DAT cells. 13 had no releasing activity. 12 and 13 inhibited release induced by MDMA, and the concentration dependence of this effect correlated with their uptake inhibitory potency at the various transporters. These results do not support a neurotoxic potential of the examined ecstasy synthesis byproducts and provide interesting structure-activity relationships on the transporters.

ECSTASY IS THE POPULAR OR STREET NAME FOR A SUBSTANCE IDENTIFIED CHEMICALLY AS N-METHYL-3,4-METHYLENEDIOXY-AMPHETAMINE OR 3,4-METHYLENEDIOXY-METHAMPHETAMINE (MDMA; FIG. 1), WHICH ARE THE NAMES COMMONLY USED IN THE CLINICAL AND RESEARCH LITERATURE. LIKE AMPHETAMINE, MDMA IS A COMPLETELY SYNTHETIC SUBSTANCE THAT DOES NOT EXIST IN NATURE. IT WAS FIRST SYNTHESIZED MANY DECADES AGO IN 1912 AND WAS PATENTED IN 1914 AS AN APPETITE SUPPRESSANT, BUT IT WAS NEVER PRODUCED COMMERCIAL, NOR DID IT.

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ABREVIATIONS: MDMA, N-methyl-3,4-methylenedioxy-amphetamine or 3,4-methylenedioxy-methamphetamine; SERT, serotonin transporter; 3, benzo[1,3]dioxol-5-yl-propane-2-one; 12, 1,3-bis(3,4-methylenedioxyphenyl)-2-propanamine; 13, N-formyl-1,3-bis(3,4-methylenedioxyphenyl)-prop-2-yl-amine; 9, 5-(2-nitro-vinyl)-benzo[1,3]dioxole; 4, MDMA; 5, N-(2-benzo[1,3]dioxol-5-yl-1-methyl-ethyl)-N-methyl-formamide; 8, N-(2-benzo[1,3]dioxol-5-yl-1-methyl-ethyl)-formamide; 10, 5-(2-nitro-ethyl)-benzo[1,3]dioxole; 11, 2-nitro[1,3-bis-benzo[1,3]dioxol-5-yl-propene; 6, N-(2-benzo[1,3]dioxol-5-yl-1-methyl-ethyl)-N-methyl-acetamide; HEK, human embryonic kidney.
studies have shown serotonin content of the brain and serotonin transporter (SERT) uptake or binding still reduced weeks after acute administration of MDMA (Battaglia et al., 1987; Schmidt, 1987; Ricaurte et al., 1988, 1992). In addition, in studies on subhuman primates, highly abnormal reinnervation patterns of serotoninergic axonal sprouting were observed 18 months after MDMA (Fischer et al., 1995).

One of the uncertainties in assessing the cause of toxicity in human subjects who have taken MDMA is the purity of the ingested substance. Synthesis of MDMA is relatively simple, and the illegal drug is made in illicit laboratories, with little regard for quality and purity of the product. The impurities can be composed of precursors, intermediates, and byproducts. The amount of these constituents particularly depends on the temperature and time of reaction, the purity of starting materials, and the purification process used for the final product. Since blockade of the SERT by the selective serotonin uptake inhibitors citalopram and fluoxetine prevents not only the acute but also the long-term effects induced by MDMA (Schmidt et al., 1987; Malberg et al., 1996), the excessive neurotransmitter release in serotoninergic and, possibly, dopaminergic neurons seems to be decisive for neurotoxicity. Therefore, we thought that it might be interesting to investigate MDMA synthesis byproducts for potential releasing effects via the human monoamine transporters.

After reviewing the extensive literature related to the clandestine synthesis of amphetamine, methamphetamine, and ecstasy-type compounds, three commonly used synthesis routes, the so called Leuckart-Wallach, reductive amination, and bromopropane methods emerged. A number of synthesis impurities specific to these routes were characterized and described by previous authors (Lukaszewski, 1982; Noggle et al., 1991; Bohn et al., 1993; Renton et al., 1993; Palhol et al., 2002). The main precursor or intermediate for the most popular Leucart-Wallach and reductive amination pathways is the benzo[1,3]dioxol-5-yl-propane-2-one (3), which can be prepared from piperonal by the Knoevenagel/nitropropane route (Fig. 1), or from benzo[1,3]dioxol-5-yl-acetic acid (Fig. 2). In the last case, a side reaction produces a diarylacetone (Bohn et al., 1993), which can be transformed to compounds 1,3-bis(3,4-methylenedioxyphenyl)-2-propanamine and N-formyl-1,3-bis(3,4-methylenedioxyphenyl)-prop-2-yl-amine (13) by the Leucart-Wallach pathway (Fig. 2). Because the synthesis of 12 and 13 was not described in the literature, we elaborated a convenient procedure starting from piperonal and using the Knoevenagel method (Fig. 1). Until the late 1990s, the results of the analysis of illicit ecstasy samples seized worldwide expressed the prominence of the Leuckart-Wallach and reductive amination routes.

On the basis of the above, we felt that it was reasonable to deal with the impurities produced by the two most popular methods. For reasons of structure-activity investigations, we also included two compounds [5-(2-nitro-vinyl)-benzo[1,3]dioxole (9) and 5-(2-nitro-ethyl)-benzo[1,3]dioxole (10)], which are intermediates of the synthesis we used to produce 12 and 13. For characterization of
the pharmacological effects of MDMA and these defined impurities, we used cell lines heterologously expressing the cloned human dopamine transporter (DAT), norepinephrine transporter (NET), or SERT in uptake and superfusion experiments. These experiments allow a clear distinction between a transport-inhibiting and a carrier-mediated outward transport activity of drugs (Pifl et al., 1995; Scholze et al., 2000).

Materials and Methods

Materials. Media, sera, and other tissue culture reagents were obtained from Invitrogen (Carlsbad, CA). [7-3H]Dopamine (22 Ci/mmol), levo-[7-3H]norepinephrine (15 Ci/mmol), 5-[1,2-3H][N]) hydroxytryptamine (21 Ci/mmol) and [3H]-methyl-4-phenylpyridinium (MPP+); 79.9 Ci/mmol) were obtained from PerkinElmer Life and Analytical Sciences (Boston, MA), and mazindol was obtained from Novartis (Basel, Switzerland). The other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Merck (Darmstadt, Germany). 1H NMR spectra were recorded on a Bruker WP 200 SY spectrometer (Bruker, Newark, DE). Chemical shifts are reported in parts per million (δ).

Synthesis of Substances. The following compounds were prepared according to the described methods, such as 5-(2-nitropropionyl)-benzo[1,3]dioxole (2) and 2-benzo[1,3]dioxol-5-yl-1-methy-ethyl-N-methyl-formamide (5) and N-(2-benzo[1,3]dioxol-5-yl-1-methy-ethyl)formamide (8) (Nichols et al., 1986) and 12-(2-benzo[1,3]dioxol-5-yl)-1-methy-ethyl (Kodukulla et al., 1994) (Fig. 1).

Preparation of 5-(2-Benzyl[1,3]dioxol-5-yl-1-methy-ethyl-N-methyl-acetamide (6). A mixture of 1.3 g (6.79 mmol) of 4 acetic anhydride (6 ml) and pyridine (3 ml) was heated at 120°C for 2 h. The mixture was diluted with cold water (100 ml) and extracted with ethyl acetate (3 × 15 ml). The organic solution was concentrated by rotary evaporation and purified by column chromatography (Kieselgel 60, toluene/methanol, 4:1) to yield 1.4 g (88.6%) as an oil.

1H NMR (CDCl3) δ 6.05–6.28 (m, 3, ArH), 2.75 (m, 3, ArH), 2.55 (d, 2, CH2), 2.35 (d, 2, CH2).

Preparation of 12. A solution of 0.98 g (3.38 mmol) of nitroolefin was extracted with 2 N HCl (3 × 10 ml), and the combined aqueous extracts were basified with NaOH. The free base was extracted with ethyl acetate (3 × 10 ml), dried (MgSO4), filtered, and concentrated by rotary evaporation. The residual oil was dissolved in absolute ethanol, acidified with concentrated HCl, diluted with Et3O, and cooled to yield 0.45 g (41.3%) of white crystalline 12-HCl (melting point, 230–233°C).

1H NMR (CDCl3) δ 6.61–6.85 (m, 6, ArH), 5.95 (s, 4, OCH3), 2.75 (dd, 2, CH2), 2.55 (dd, 2, CH2).

Preparation of 13. A solution of 1.09 g (4.0 mmol) of 12-HCl salt dissolved in H2O was neutralized to the free base with excess NaOH. The free base was extracted into ether. The ether solution was dried (MgSO4) and filtered, and the ether was removed by rotary evaporation to give the free base as an oil. The oil was dissolved in 30 ml of methyl formate, placed into a 100-ml high-pressure Parr bomb, and heated on steam bath overnight. The bomb was cooled, and the reaction mixture was concentrated by rotary evaporation. The residue was crystallized from ethanol-hexane mixture to give 0.52 g (53%) (melting point, 155–156°C).

1H NMR (CDCl3) δ 6.55–6.72 (m, 3, ArH), 6.15–6.35 (m, 3, ArH), 6.05 (s, 2, OCH2O), 5.55 (s, 2, OCH2O), 2.75 (dd, 2, CH2), 2.35 (dd, 2, CH2).

The structure of prepared compounds was determined by comparison of their 1H NMR and electron ionization-mass spectroscopy data with those reported in the literature. The synthetic preparation of 12 and 13 has not been reported earlier. These were previously identified as impurities of illegal MDA synthesis in seized ecstasy tablets (Bohn et al., 1993).

Cell Culture. SK-N-MC (human neuroblastoma) and human embryonic kidney (HEK) 293 cells were grown in minimum essential medium with Earle’s salts and 1-glutamine, 10% heat-inactivated fetal bovine serum, and 50 mg/liter gentamicin. Cells were grown in 100-mm-diameter tissue culture dishes (polystyrene; Falcon; BD Biosciences Discovery Labware, Bedford, MA) at 37°C under an atmosphere of 5% CO2/95% air. The human DAT or NET cDNA was stably expressed in SK-N-MC cells using methods as described recently (Pifl et al., 1996). The human SERT was similarly expressed in HEK 293 cells using the vector pRc/CMV and selection by 1 g/liter G418 in the medium.

Uptake Experiments. The cells were seeded in poly-d-lysine-coated 24-well plates (2 × 105 SK-N-MC or 1 × 106 HEK cells/well; 1 day later, each well was washed with 0.5 ml of uptake buffer and incubated with 0.5 ml of buffer containing various concentrations of the drugs. Uptake was started by addition of [3H]dopamine, [3H]norepinephrine, or [3H]serotonin at a final concentration of 1 μM (specific activity 0.14 Ci/mmol) after 2 min of preincubation. After
incubation for 2.5 min at 25°C, it was stopped by aspirating the uptake buffer and washing each well twice with 1 ml of ice-cold buffer. Nonspecific uptake was determined in the presence of 10 μM mazindol (DAT and NET cells) or 3 μM clomipramine (SERT cells). The radioactivity remaining in each well was determined by incubating with 0.4 ml of 1% sodium dodecyl sulfate and transferring this solution into scintillation vials containing 3 ml of scintillation cocktail (Ultima Gold MV, PerkinElmer Life and Analytical Sciences, Boston, MA). The uptake buffer consisted of 4 mM Tris-HCl, 6.25 mM HEPES, 120 mM NaCl, 5 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgSO₄, 5.6 mM d-glucose, and 0.5 mM ascorbic acid, pH 7.1.

Superfusion Experiments. Cells were seeded onto poly-D-lysine-coated 5-mm-diameter glass cover slips in 96-well tissue culture plates (7 × 10⁴ SK-N-MC cells/well and 3 × 10⁴ HEK cells/well). On the following morning, cells were loaded with [³H]MPP⁺ in uptake buffer at 37°C: DAT cells, 6 μM with 0.2 Ci/mm; 20 min; NET cells, 0.1 μM with 29 Ci/mm; 20 min; and SERT cells, 10 μM with 0.4 Ci/mm; 30 min. Coverslips were then transferred to small chambers and superfused (25°C, 1.0 ml/min) with the uptake buffer mentioned above in a setup as described recently (Pifl et al., 1995; Scholze et al., 2000). After a washout period of 45 min to establish a stable efflux of radioactivity, the experiment was started with the collection of 4-min fractions. At the end of the experiment, cells were lysed by superfusion with 4 ml of 1% SDS. The radioactivity in the superfusate fractions and the SDS-lysates was determined by liquid scintillation counting. Release of tritium was expressed as fractional release rate; i.e., the radioactivity released during a fraction was expressed as percentage of the total radioactivity present in the cells at the beginning of that fraction.

Data Analysis. Uptake data of each separate experiment were fitted to the equation $f = \left(1 - \frac{\mathrm{IC}_{50}}{\mathrm{EC}_{50}}\right) + \frac{\max \times f}{(1 + \frac{\mathrm{IC}_{50}}{\mathrm{EC}_{50}})}$, min being nonspecific uptake, max the uptake in the absence of inhibiting drug, x the molar concentration of the inhibiting drug, and IC₅₀, the drug concentration that inhibits 50% of specific uptake. Since max and min did not differ significantly from 100% and nonspecific uptake, respectively, they were constrained to these values to accurately estimate IC₅₀ and Hill slope. From the fitted IC₅₀ and Hill slopes of each experiment mean values ± S.D. were calculated. Dose-response dependence of the release stimulation was generated from mean values by fitting the increment over baseline (baseline = mean of the first three fractions) of the 20-min fraction to the equation $f = \frac{E_{\text{max}} \times (E_{\text{IC}_{50}} + x)}{E_{\text{max}}}$, where E_max being maximal efflux over baseline, x the molar concentration of MDMA, and EC₅₀ the MDMA concentration that stimulates 50% of maximal efflux. Fitting was performed by the nonlinear curve-fitting computer program SigmaPlot (Systat Software, Inc., Point Richmond, CA). All results were expressed as means ± S.D.

Results

Inhibition of NET-, SERT-, and DAT-Mediated Uptake by MDMA and Ecstasy Synthesis Impurities. MDMA concentration-dependently inhibited norepinephrine, serotonin, and dopamine uptake in cells expressing the human NET, SERT, or DAT, respectively (Fig. 3). MDMA was nearly four times more potent on the NET (Fig. 3A) than on the SERT (Fig. 3B), which again was blocked by MDMA approximately two times more potently than the DAT (Fig. 3C). Of the ecstasy synthesis impurities, only compounds 12 and 13 blocked monoamine uptake by more than 50% in concentrations up to 100 μM. For these two compounds, concentration-dependent inhibition of uptake by the NET, SERT, or DAT is shown in Fig. 3, A through C, respectively. The Hill slopes of MDMA, 12, and 13 were around 1 with mean values between 0.93 and 1.24, except the slopes by 13 on the NET and DAT. The high values of 1.43 ± 0.03 and

![Fig. 3. Effects of MDMA and two defined byproducts of uncontrolled MDMA synthesis, 12 and 13, on uptake by the NET, SERT, and DAT. Concentration-inhibition curves for their effects on [³H]norepinephrine, [³H]serotonin, and [³H]dopamine uptake in SK-N-MC or HEK293 cells stably expressing NET (A), SERT (B), or DAT (C). The cells were incubated in 24-well plates for 2.5 min at 25°C with 1 μM of the tritiated monoamines in the absence (control) or presence of MDMA (circle), 12 (square), or 13 (triangle) at the concentrations indicated, and uptake was determined as described under Methods. Symbols represent means ± S.D. of three to five independent experiments, each in duplicates. Control uptake was 15 ± 2, 49 ± 17, and 31 ± 12 pmol/min/10⁴ cells for NET, SERT, and DAT cells, respectively. Dashed line, nonspecific uptake. The data of each experiment were fitted by nonlinear regression, and the means of the IC₅₀ values ± S.D. are inserted into the panels.](https://jpet.aspetjournals.org/article.aspx?doi=10.1124/jpet.117.242262)
weak effect of the compounds 2, 5, 6, 8, 9, and 10 on the uptake by NET, SERT, and DAT is shown in Table 1 (in percentage of vehicle treated cells).

Stimulation of NET-, SERT-, and DAT-Mediated Release by MDMA. To measure carrier-mediated release, we used a technique in which transporter-expressing cells grown on coverslips were prelabeled with the metabolically inert transporter substrate [3H]MPP+ and superfused in microchambers. The releasing activity of a drug added to the superfusion buffer was discerned by an increase of radioactivity in the fractionated perfusates. As shown in Fig. 4, MDMA, tested at a concentration of 3 μM, increased [3H]MPP+-efflux from NET-, SERT-, and DAT-expressing cells. This releasing action was transporter-mediated since it was blocked by 10 μM of the NET- and DAT-blocking drug mazindol in NET- and DAT-cells (Figs. 4, A and C, respectively) and by 3 μM of the SERT uptake inhibitor clomipramine in SERT cells (Fig. 4B). MDMA concentration-dependently stimulated release from NET, SERT, and DAT cells (Fig. 5, A–C, respectively), with the highest potency on the NET (EC50, 0.64 ± 0.05 μM; Emax, 9.0 ± 0.26%) and the lowest on the DAT (EC50, 3.2 ± 0.6 μM; Emax, 3.6 ± 0.16%), and a potency on the SERT in between (EC50, 1.1 ± 0.9 μM; Emax, 8.8 ± 0.76%).

Releasing Activity of Compounds 12 and 13. 12 and 13 did not affect efflux from cells expressing the NET or SERT in concentrations up to 100 μM and weakly suppressed efflux from DAT-cells at a concentration of 30 and 100 μM (data not shown).

Blocking Action of Compounds 12 and 13 on MDMA-Induced Release. If added 4 min before MDMA to the superfusion buffer, 12 and 13 concentration-dependently suppressed the release induced by 3 μM MDMA in NET-, SERT-, and DAT-expressing cells (Figs. 7 and 8). Compound 12 (100 μM) suppressed MDMA-induced release mediated by the DAT to levels slightly beneath basal efflux (Figs. 7C and 8C). The potency of 13 in its inhibitory action on MDMA-induced release was in agreement with its potency in uptake inhibition experiments: 13 suppressed MDMA-induced release more potently than NET-mediated release, whereas MDMA-induced release mediated by the DAT was only weakly inhibited in concentrations up to 100 μM (Fig. 8C). The compounds 2, 5, 6, and 8 to 10 were not active in concentrations of 100 μM in superfusion experiments, neither in induction nor in suppression of release (data not shown).

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<td>10</td>
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* P < 0.05 vs. control by paired Student’s t test.
The main finding of this study is that two defined byproducts of illegal MDMA synthesis interact in low micromolar concentrations with the human plasmalemmal monoamine transporters. These membrane proteins, NETs, SERTs, and DATs, are the main molecular sites of action of MDMA. To the best of our knowledge, this is also the first study comparing the activity of MDMA on the three human transporters by a technique that allows a clear distinction between an inhibitory action on uptake and an induction of reverse transport. In our experimental set up, only releasers, but not pure uptake inhibitors, stimulate efflux from transporter-expressing cells, and transporter-mediated release induced by a releasing drug can be blocked by a drug with pure uptake inhibitory activity.

**Discussion**

The main finding of this study is that two defined byproducts of illegal MDMA synthesis interact in low micromolar concentrations with the human plasmalemmal monoamine transporters. These membrane proteins, NETs, SERTs, and DATs, are the main molecular sites of action of MDMA. To the best of our knowledge, this is also the first study comparing the activity of MDMA on the three human transporters by a technique that allows a clear distinction between an inhibitory action on uptake and an induction of reverse transport. In our experimental set up, only releasers, but not pure uptake inhibitors, stimulate efflux from transporter-expressing cells, and transporter-mediated release induced by a releasing drug can be blocked by a drug with pure uptake inhibitory activity.
MDMA had a higher affinity to the NET than to the SERT and DAT in our uptake blocking and release experiments, respectively. These findings, obtained on the human transporter proteins, confirm and extend the study by Rothman et al. (2001) in which the most potent effect of amphetamine-type stimulants was to release norepinephrine from rat synaptosomal preparations. It further supports the hypothesis that the action of MDMA on the noradrenergic system might contribute to the subjective effects of MDMA in humans.

Long-term toxicity of ecstasy preparations in humans is a big concern considering the widespread abuse of this drug. Since the discovery of the dramatic effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a byproduct of illegal synthesis of a meperidine analog (Langston, 1985), such potential hazard of the impurities in illegally synthesized ecstasy preparations cannot be ruled out. The clandestine synthesis
of MDMA is based foremost on two methods, the Leukart-Wallach (3 → 5 → MDMA and 3 → 8 → MDMA in Fig. 2) and the reductive amination (3 → MDMA in Fig. 1) routes. Of the ecstasy impurities investigated in this study, 5, 8, 12, and 13 relate to the Leukart-Wallach route (Fig. 2), 5 and 8 being intermediates of MDMA synthesis and 12 and 13 being side products of a reaction intended to produce a precursor of this synthetic pathway. Compounds 2 and 6 relate to both routes: 2 is produced as an intermediate of a synthesis (the Knoevenagel/nitropropane route) intended to produce the precursor 3 of both routes, and 6 may be produced as a side product of the final synthetic step to MDMA.

Compounds 2, 5, 6, 8, 12, and 13 have been found in seized samples of illegally synthesized ecstasy preparations. However, there are no quantitative data allowing a reliable estimation of the amounts to be expected in humans taking a street preparation of ecstasy. On the other hand, it is noteworthy that in a reaction analogous to that in Fig. 2, the amount of diarylacetonone byproduct in the reaction of phenylacetic acid and acetic acid was reported 20% compared with 70% arylmethyl keton (Herbst and Manske, 1947). Although a separation by fractionated distillation is possible, this is a not too effective method. The ratio of product and byproduct and the separation difficulties may be similar in the synthesis of compound 3, the precursor of the pharmacologically active compounds 12 and 13 that may be consequently in more than just trace amounts in preparations following the Leukart-Wallach route.

There are two reasons why our findings on cultured cells are relevant for the abuse of illegally produced ecstasy by humans. First, even if the ultimate mechanism is still not clarified, a releasing activity is necessary if not sufficient for amphetamine-type neurotoxicity (Baumann et al., 2001), whereas blocking without releasing activity is potentially inhibitory on in vivo effects of amphetamine whether they are acute behavioral or chronic neurotoxic ones (Schmidt et al., 1995; Rothman et al., 2001). This can explain our finding that 13, containing two 3,4-methylenedioxyphenyl groups compared with that of 3,4-methylenedioxyamphetamine (not functionally different from MDMA; see Wichems et al., 1995), and 8 containing only one of them: the 3,4-methylenedioxy substitution increased the affinity of phenylethylamines to the SERT and decreased it to the NET and DAT (Wall et al., 1995; Rothman et al., 2001). This can explain our finding that 13, containing two 3,4-methylenedioxyphenyl groups, was equipotent with MDMA at the SERT, whereas it displayed less affinity to NET and DAT than MDMA. Nevertheless, it is surprising how the second 3,4-methylenedioxyphenyl groups in 13 restored the transporter affinity by masking the effect of the formamide residue in the simple 3,4-methylenedioxyphenyl derivative 8, which had no transporter affinity. That the formamide structure is unstable and 13 was hydrolyzed on the NET and DAT and by the persistently low potency of 8, which by the same token should have been converted to the potent 3,4-methylenedioxyamphetamine.

Finally, although MDMA is a potent releasing drug on NET, SERT and DAT, the compounds 12 and 13 blocked MDMA-induced release. In a way, the second 3,4-methylenedioxyphenyl group in 12 and 13 seems to switch the mode of interaction with the transporters from induction of transporter-mediated release to transporter inhibition. Based on the concept that a drug acts as a releasing agent by being a transporter substrate that is actively moved from the outside to the inside of the cell, where it exchanges with a different
of release, argues against a neurotoxic potential of the substances investigated.

References