1521-0103/391/1/22–29$35.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

dx.doi.org/10.1124/jpet.123.001837
J Pharmacol Exp Ther 391:22–29, October 2024

Novel Benzofuran Derivatives Induce Monoamine Release and Substitute for the Discriminative Stimulus Effects of 3,4-Methylenedioxymethamphetamine

Candace B. Johnson, Donna Walther, Matthew J. Baggott, Lisa E. Baker, and Michael H. Baumann

Department of Psychology, Western Michigan University, Kalamazoo, Michigan (C.B.J., L.E.B.); Designer Drug Research Unit, National Institute on Drug Abuse Intramural Research Program, Baltimore, Maryland (D.W., M.H.B.); and Tactogen Inc., Palo Alto, California (M.J.B.)

Received July 13, 2023; accepted January 18, 2024

ABSTRACT

3,4-Methylenedioxymethamphetamine (MDMA) has shown efficacy as a medication adjunct for treating post-traumatic stress disorder (PTSD). However, MDMA is also used in nonmedical contexts that pose risk for cardiovascular and neurologic complications. It is well established that MDMA exerts its effects by stimulating transporter-mediated release of the monoamines 5-hydroxytryptamine (5-HT), norepinephrine, and dopamine. Current research efforts are aimed at developing MDMA-like monoamine releasers with better efficacy and safety profiles. To this end, we investigated neurochemical and behavioral effects of novel analogs of the designer drug 5-(2-methylaminopropyl)benzofuran (5-MAPB). We used in vitro transporter assays in rat brain synaptosomes to examine transmitter uptake inhibition and releasing properties for enantiomers of 5-(2-methylaminobutyl)benzofuran (5-MABB) and 6-(2-methylaminobutyl)benzofuran (6-MABB) compared with MDMA. We then tested these same compounds in male Sprague-Dawley rats trained to discriminate MDMA (1.5 mg/kg) from saline. In vitro results revealed that *S* isomers of 5- and 6-MABB are efficacious releasing agents at transporters for 5-HT (SERT), norepinephrine (NET), and dopamine (DAT). By contrast, *R* isomers are efficacious releasers at SERT and partial releasers at NET but lack releasing activity at DAT. In vivo results showed that all compounds produce dose-dependent increases in MDMA-lever responding and full substitution at the highest dose tested. The diminished NET and DAT releasing activities for *R* isomers of 5- and 6-MABB are associated with reduced potency for inducing behavioral effects. Collectively, these findings indicate that the aminoalkyl benzofuran scaffold may be a viable template for developing compounds with MDMA-like properties.

SIGNIFICANCE STATEMENT

Despite the clinical utility of 3,4-methylenedioxymethamphetamine (MDMA), the drug is associated with certain cardiovascular risks and metabolic side effects. Developing a therapeutic alternative with MDMA-like monoamine releasing activity is of interest. Our in vitro and in vivo findings indicate that the aminoalkyl benzofuran scaffold may be useful for developing compounds with MDMA-like properties.

Introduction

3,4-Methylenedioxymethamphetamine (MDMA), also known as "Ecstasy," is a phenethylamine derivative with structural similarity to the psychostimulant methamphetamine and the psychedelic mescaline (Green et al., 2003; Luethi and Liechti, 2018). Recently, MDMA-assisted psychotherapy has received considerable attention, and phase III clinical trials with MDMA are ongoing for the treatment of post-traumatic stress disorder (PTSD) (https://clinicaltrials.gov/ct2/show/NCT03537014). Individuals with PTSD experience intrusive memories of traumatic events that evoke emotional reactions, flashbacks, hypervigilance, and negative thinking (Boeckel et al., 2017; Ford 2018). The increased empathy and prosocial effects of MDMA serve to reduce anxiety and facilitate conversation, which help patients to confront traumatic experiences (Carhart-Harris et al., 2014; Boeckel et al., 2017). In phase II clinical trials, where PTSD symptoms were assessed using the Clinician Administered PTSD Scale-IV, results demonstrated that symptom severity was significantly decreased for up to

This work was supported by Tactogen Inc. and National Institutes of Health National Institute on Drug Abuse [Grant DA000522-16] (to M.H.B.). This research was supported in part by the Intramural Research Program of National Institutes of Health National Institute on Drug Abuse.

M.J.B. is employed by and holds equity in Tactogen Inc., which is developing benzofuran derivatives as medicines. None of the other authors has any actual or perceived conflicts of interest with the contents of this article.

Part of this work was previously presented as follows: Johnson C (2022) Benzofuran derivatives substitute for the discriminative stimulus effects of MDMA in rats (poster presentation). *14th Annual Behavior, Biology, and Chemistry Conference*; 2022 Feb 25–27; San Antonio, TX.

dx.doi.org/10.1124/jpet.123.001837.

ABBREVIATIONS: 5-APB, 5-(2-aminopropyl)benzofuran; CI, confidence interval; DAT, dopamine transporter; DOM, 2, 5-dimethoxy-4-methylphenyl isopropylamine; Emax, maximal release; FR, fixed ratio; 5-MABB, 5-(2-methylaminobutyl)benzofuran; 6-MABB, 6-(2-methylaminobutyl)benzofuran; 5-MAPB, 5-(2-methylaminopropyl)benzofuran; MDMA, 3, 4-methylenedioxymethamphetamine; MPP+, 1-methyl-4-phenylpyridinium; NET, norepinephrine transporter; NIDA, National Institute on Drug Abuse; PTSD, post-traumatic stress disorder; SERT, 5-HT transporter.

12 months after MDMA assisted-psychotherapy (Mithoefer et al., 2018; Jerome et al., 2020).

Though clinical outcomes with MDMA are promising, there remains cause for concern. The nonmedical (i.e., recreational) use of MDMA has a well documented history of cardiovascular and neurologic complications (Simmler and Liechti, 2018; Mitchell et al., 2021). Prolonged nonmedical use of MDMA can induce altered 5-hydroxytryptamine (5-HT) receptor densities and 5-HT depletions in the brain [see Parrott (2013)]. Neuroimaging studies reveal decreases in 5-HT–related proteins in human MDMA users that may reflect loss of nerve terminals (Reneman et al., 2006; Roberts et al., 2016). Although the risk for 5-HT deficits is greatest after high-dose MDMA exposure, even low-dose administration in controlled settings can produce elevations in blood pressure, heart rate, and stress hormones in humans (Mas et al., 1999; Kolbrich et al., 2008). Additionally, the metabolism of MDMA causes irreversible inhibition of cytochrome 2D6, which leads to nonlinear accumulation of the drug (de la Torre et al., 2000; O'Mathúna et al., 2008). Thus, there is room for improvement in developing new MDMA-like medications (Oeri, 2021; Heal et al., 2023).

MDMA exerts its pharmacological effects by interacting with monoamine transporter proteins expressed on nerve cells (Sandtner et al., 2016; Simmler and Liechti, 2018). In particular, MDMA acts as a transportable substrate at transporters for 5-HT (SERT), norepinephrine (NET), and dopamine (DAT), thereby stimulating transporter-mediated release of 5-HT, norepinephrine, and dopamine (Rothman et al., 2001; Verrico et al., 2007). Studies in rodents reveal that prosocial effects of MDMA are related to SERT-mediated release of 5-HT (Heifets et al., 2019), whereas cardiovascular and reinforcing effects likely involve NET and DAT (Schindler et al., 2014; Schenk and Highgate, 2021). Clinical investigations show that blockade of SERT with 5-HT selective reuptake inhibitors can significantly blunt subjective effects of MDMA in humans, supporting involvement of SERT-mediated 5-HT release in the therapeutic attributes of the drug (Liechti et al., 2000; Farré et al., 2007; Tancer and Johanson, 2007).

Aminoalkyl benzofuran derivatives such as 5-(2-aminopropyl)benzofuran (5-APB) and its *N*-methylated counterpart 5-(2-methylaminopropyl)benzofuran (5-MAPB) (see Fig. 1) are new psychoactive substances (NPS) that act as transporter substrates analogous to MDMA (Rickli et al., 2015; Eshleman et al., 2019). Members of our team previously showed that 5-APB and 5-MAPB are releasers at SERT, NET, and DAT, with 3-fold greater potency than MDMA (Brandt et al., 2020). Consistent with their transporter releasing properties, 5-APB and 5-MAPB elevate extracellular 5-HT and dopamine in rodent brain (Cha et al., 2016; Fuwa et al., 2016; Brandt et al., 2020) and engender MDMA-like discriminative stimulus effects in rats (Dolan et al., 2017). 5-APB and 5-MAPB also exhibit agonist activity at 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors, which may contribute to their pharmacology (Shimshoni et al., 2017).

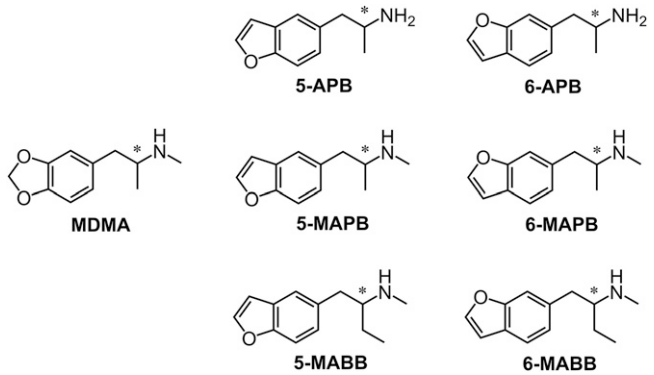

Fig. 1. Chemical structures of 5-(2-aminopropyl)benzofuran (5-APB), 6-(2-aminopropyl)benzofuran (6-APB), 5-(2-methylaminopropyl)benzofuran (5-MAPB), 6-(2-methylaminopropyl)benzofuran (6-MAPB), 5-(2-methylaminobutyl)benzofuran (5-MABB), and 6-(2-methylaminobutyl)benzofuran (6-MABB) compared with MDMA. Asterisks indicate the location of the asymmetric chiral alpha-carbon for each molecule.

With regard to medication development, one potential attribute of benzofurans is that they are metabolized differently than MDMA and may not induce inhibition of cytochrome 2D6 (Welter et al., 2015a,b). Moreover, benzofuran compounds have more sustained acute effects when compared with MDMA [see Brandt et al. (2020)], which might be advantageous in some clinical circumstances. In the present study, we examined the pharmacology of novel derivatives of 5-MAPB and its positional isomer 6-(2-methylaminopropyl)benzofuran (6-MAPB). We used in vitro transporter assays in rat brain synaptosomes to evaluate uptake inhibition and releasing activity for enantiomers of 5-(2-methylaminobutyl)benzofuran (5-MABB) and 6-(2-methylaminobutyl)benzofuran (6-MABB) (Fig. 1). In separate experiments, in vivo drug discrimination methods were used to characterize interoceptive stimulus effects of the drugs in rats trained to discriminate MDMA from saline. Based on previous findings with cathinone-based compounds (Saha et al., 2019), we hypothesized that the increased alpha-carbon chain lengths for 5- and 6-MABB would produce 5-HT releasers with reduced activity at NET and DAT.

Materials and Methods

Drugs and Reagents

R,S-3,4-methylenedioxymethamphetamine HCl (MDMA) was generously provided by the National Institute on Drug Abuse (NIDA) Drug Supply Program (Rockville, MD). *R* and *S* enantiomers of 5-(2-methylaminobutyl)benzofuran HCl (5-MABB) and 6-(2-methylaminobutyl)-benzofuran HCl (6-MABB) were supplied by Tactogen (Palo Alto, CA). For in vitro assays, compounds were diluted in DMSO at a concentration of 10 mM and stored at −80°C. On the day of an experiment, aliquots of 10 mM stock solution were diluted in Krebs-phosphate buffer to construct concentration-response curves. The radioligands [^{3}H]5-HT, [^{3}H]norepinephrine, [^{3}H]dopamine, and [^{3}H]1-methyl-4-phenylpyridinium ([^{3}H]MPP+) were purchased from PerkinElmer (Waltham, MA). For in vivo drug discrimination experiments, MDMA and benzofuran compounds were dissolved in 0.9% saline and injected intraperitoneally (i.p.) at a volume of 1 ml/kg. Doses are expressed as the salt. All other chemicals and reagents were purchased from MilliporeSigma (St. Louis, MO) unless noted otherwise.

Animals

For the in vitro transporter assays, 24 adult male Sprague-Dawley rats (Envigo, Frederick, MD) were used. Rats weighing 250–300 g were group housed at the NIDA Intramural Research Program (IRP) animal facility in a temperature (22.2 ± 1.1°C) and humidity (45% ± 10%) controlled room on a 12-hour light/dark cycle (lights on at 0700) with free access to food and water. For the in vivo drug discrimination experiments, eight adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used. Rats were individually housed at the Western Michigan University animal facility in a temperature

(20 ± 2°C) and humidity (50% ± 5%) controlled room on a 12-hour light/dark cycle (lights on at 0700). For the drug discrimination studies, animals were provided free access to water in their home cages and fed restricted diets of commercial rodent chow (Purina, Richmond, IN) to maintain body weights at approximately 90% of free-feeding weights (380–460 g). All procedures were reviewed and approved by the Institutional Animal Care and Use Committees of the NIDA IRP (Baltimore, MD) and Western Michigan University (Kalamazoo, MI) and were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011). It is noteworthy that we used only male rats in this study to allow for comparison with our previous work with benzofuran compounds. Future studies will examine the effects of these compounds in female rats.

In Vitro Transporter Assays

Synaptosome Preparation. Rats were killed by CO_2 narcosis, and synaptosomes were prepared from brains using standard procedures. In brief, synaptosomes were prepared from caudate tissue for DAT assays or from whole brain minus caudate and cerebellum for NET and SERT assays. Brain tissue was homogenized in ice-cold 10% sucrose followed by centrifugation at 1000 *g* for 10 minutes. The resulting supernatant containing crude synaptosomes was kept on ice until use in uptake or release procedures. Uptake inhibition and release assays were carried out as previously described (Saha et al., 2019; Brandt et al., 2020).

Uptake Inhibition Assays. For uptake inhibition assays, 5 nM [^{3} 10 nM [^{3}H]norepinephrine, or 5 nM [^{3}H]5-HT was used as the radiolabeled transmitter for DAT, NET, or SERT, respectively (Rothman et al., 2001). To optimize uptake for a single transporter, unlabeled blockers were included that prevented uptake of [^{3}H]transmitter by competing transporters. Specifically, 50 nM 1-(2-(diphenylmethoxy)ethyl)-4-(3-phenylpropyl)piperazine (GBR12935) was added to the NET and SERT assays to block DAT, whereas 100 nM nomifensine was added to the SERT assays to block NET. Uptake assays were initiated by adding 100 μl of tissue to 900 μl Krebs-phosphate buffer (126 mM NaCl, 2.4 mM KCl, 0.83 mM $CaCl_2$, 0.8 mM $MgCl_2$, 0.5 mM KH_2PO_4, 0.5 mM Na_2SO_4, 11.1 mM glucose, 0.05 mM pargyline, 1 mg/ml bovine serum albumin, and 1 mg/ml ascorbic acid, pH 7.4) containing test drug and [^{3}H]transmitter. Uptake assays were terminated by rapid vacuum filtration, and retained radioactivity was quantified with liquid scintillation counting.

Release Assays. For release assays, 9 nM [^{3}H]MPP+ was used as the radiolabeled substrate for DAT and NET, whereas 5 nM [^{3}H]5-HT was used for SERT. All buffers used in the release assay contained 1 μM reserpine to block vesicular uptake of substrates. The selectivity of release assays was optimized for a single transporter by including unlabeled blockers to prevent the uptake of [^{3}H]MPP+ or [^{3}H]5-HT by competing transporters. Specifically, 100 nM desipramine was added to DAT assays to block NET, whereas 100 nM citalopram was added to DAT and NET assays to block SERT. Synaptosomes were preloaded with radiolabeled substrate in Krebs-phosphate buffer for 1 hour. Release assays were initiated by adding 850 μl of preloaded synaptosomes to 150 μl of test drug. Release was terminated by vacuum filtration, and retained radioactivity was quantified by liquid scintillation counting. Effects of test drugs on release are expressed as percent maximum release, with maximal release (i.e., 100% Emax) defined as the release produced by tyramine at doses that evoke the efflux of all 'releasable' tritium by synaptosomes (10 μM tyramine for DAT and NET assay conditions and 100 μM tyramine for SERT assay conditions).

Data Analysis. Effects of test drugs on release and uptake inhibition were analyzed by nonlinear regression using GraphPad Prism 7 (GraphPad Scientific, San Diego, CA). Dose-response values for the uptake inhibition and release were fit to the equation $Y(x) = Ymin+(Ymax - Ymin)/(1 + 10exp[(logP_{50} - logx)] \times n)$, where x is the concentration of the compound tested, Y(x) is the response measured, Ymax is the maximal response, P_{50} is either IC_{50} (the concentration that yields half-maximal uptake inhibition response) or EC_{50} (the concentration that yields half-maximal release response), and n is the Hill slope parameter. Compounds displaying <30% of Emax releasing efficacy were considered inactive in the release assay since uptake inhibitors can evoke this degree of partial release.

Drug Discrimination Experiments

Apparatus. Training and testing were conducted in eight sound-attenuated operant conditioning chambers (ENV-001; Med Associates Inc., St. Albans, VT) equipped with three retractable levers, a food pellet delivery mechanism, a house light, and a fan. All experimental events and data collection were programmed with Med-PC software (versions IV and V; Med Associates Inc.). Food reinforcers were 45-mg Dustless Precision Pellets (product F0021; Bio-Serv Inc., Flemington, NJ).

Preliminary Training. Rats were initially acclimated to operant conditioning chambers for a single 60-minute session in which no levers were present, and food pellets were dispensed on a 60-second fixed time interval. Subsequently, rats were trained to lever press the center lever on a fixed ratio (FR) 1 schedule that was gradually incremented to FR 20. Once animals consistently responded on an FR 20 schedule, errorless training commenced, in which either the left or right lever was extended. Subjects were injected with MDMA (1.5 mg/kg, i.p.) or saline 15 minutes before each training session, and training sessions lasted 20 minutes. The assignment of left or right lever to drug or vehicle conditions was counterbalanced among subjects.

Discrimination Training. Drug (D) and vehicle (V) training sessions followed a repeating alternating schedule of V, V, D, D, V, D. Both left and right levers were extended for these and all remaining sessions. The criteria for stimulus control were a minimum of eight out of 10 consecutive training sessions with 80% or greater correct lever responses on the first FR and for the remainder of each training session.

Stimulus Substitution Tests. Test sessions were conducted similar to training sessions, with the exception that no reinforcers were delivered, and tests ended upon completion of the first FR 20 or after 20 minutes elapsed, whichever occurred first. Substitution tests were conducted once or twice per week provided that animals maintained adequate stimulus control during intervening training sessions. A minimum of one saline and one MDMA training session was conducted between tests. Prior to the assessment of novel compounds, substitution tests were conducted with a range of MDMA doses (0.19, 0.38, 0.75. 1.5 mg/kg, i.p.) or saline administered 15 minutes prior to the session to determine a dose effect curve with the training drug. Substitution tests were then conducted with *R* and *S* isomers of 5-MABB and 6-MABB, administered at 0.32, 0.64, 1.28, and 2.56 mg/kg, i.p. 30 minutes prior to the session. The order of doses for each compound was counterbalanced among rats.

Data Analysis. The number of sessions to criteria for each subject was calculated as the number of training sessions completed to meet the aforementioned criteria for stimulus control. Percent drug lever was calculated by dividing the number of responses emitted on the MDMA-associated lever by the total number of responses on both levers and multiplying by 100. Response rates were expressed as the number of responses emitted per second throughout a test session. Full substitution was defined as 80% or greater responses on the MDMA-associated lever; partial substitution was defined as 20%–79% responses on the MDMA lever; no substitution was defined as less than 20% responses on the MDMA lever. Dose-response curves were plotted for each compound and nonlinear regressions were performed to determine ED_{50}s. For each test compound assessed, response rate was analyzed with a repeated measures analysis of variance. Statistical significance was determined at alpha of $P < 0.05$. All statistical and graphical analyses were performed using the GraphPad Prism version 7 software (GraphPad Software, Inc., La Jolla, CA).

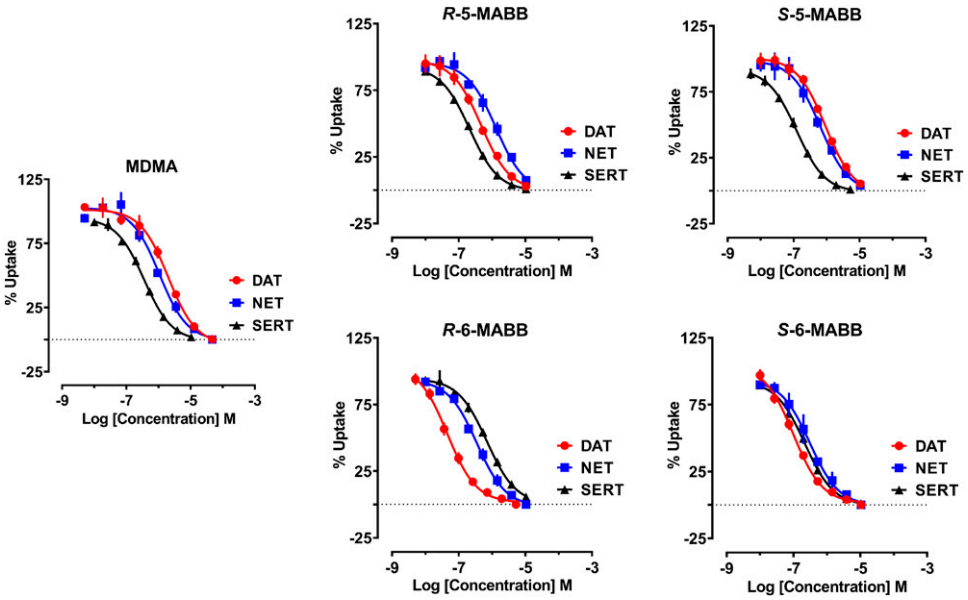

Fig. 2. Concentration-response curves for MDMA, *R*-5-MABB, *S*-5-MABB, *R*-6-MABB and *S*-6-MABB to inhibit [^{3}H] neurotransmitter uptake into rat brain synaptosomes. [^{3}H]dopamine, [^{3}H]norepinephrine, or [^{3}H]5-HT were used to evaluate transmitter uptake via DAT, NET, or SERT, respectively. Data are percentage of control uptake, expressed as mean ± S.E.M. for $N = 3$ experiments performed in triplicate.

Results

In Vitro Transporter Assays

We first characterized the effects of MDMA and the enantiomers of 5- and 6-MABB in uptake inhibition assays carried out in rat brain synaptosomes. Figure 2 depicts the concentration-response curves for the test compounds to inhibit uptake of [^{3}H]neurotransmitters at DAT, NET and SERT, whereas Table 1 summarizes potency estimates for each compound at each transporter (i.e., IC_{50} values). In previous publications (e.g., Brandt et al., 2020), we have highlighted the relationship between potency at DAT versus SERT using the DAT/SERT ratio. For uptake inhibition assays, this metric is defined as $(\text{DAT } IC_{50})^{-1}/(\text{SERT } IC_{50})^{-1}$. As shown previously, MDMA acted as a fully efficacious uptake inhibitor at DAT, NET, and SERT, with somewhat greater potency (i.e., left-shifted concentration-response curve) for SERT (Rothman et al., 2001; Sandtner et al., 2016). *R*- and *S*-5-MABB had uptake inhibition profiles analogous to MDMA, with more potent effects at SERT relative to DAT and NET. The 5-MABB enantiomers displayed potencies that were generally similar to MDMA at all transporters. The enantiomers of 6-MABB also acted as fully efficacious uptake inhibitors at DAT, NET, and SERT, but these compounds were more potent at inhibiting uptake at DAT. In particular, *R*-6-MABB had a DAT/SERT ratio of 15.4, indicative of substantial selectivity for DAT over SERT.

Although uptake inhibition assays are useful for identifying compounds that interact with monoamine transporters, such assays cannot differentiate between nontransportable uptake inhibitors versus transportable substrates that evoke neurotransmitter release (Sitte and Freissmuth 2015; Baumann et al., 2018). Thus, we next examined the effects of MDMA and benzofuran compounds in release assays designed to detect drug-induced efflux of [^{3}H]substrates from preloaded synaptosomes. Figure 3 depicts the effects of MDMA and the enantiomers of 5- and 6-MABB in release assays optimized for DAT, NET, and SERT, whereas Table 2 summarizes potency estimates for each compound at each transporter (i.e., EC_{50} values). The efficacy of the compounds is also noted in Table 2, expressed as a percentage of maximal release, Emax. For the release assays, the DAT/SERT ratio is defined as $(\text{DAT } EC_{50})^{-1}/(\text{SERT } EC_{50})^{-1}$. The *S* enantiomers of 5- and 6-MABB acted as efficacious releasing agents at DAT, NET, and SERT. *S*-5-MABB displayed selectivity toward SERT, whereas *S*-6-MABB was nonselective across the three transporters. It is noteworthy that the release efficacies for *S*-6-MABB at DAT and NET ranged from 72%–81% of maximal, which is somewhat less than the full efficacy effects of *S*-5-MABB and MDMA at these transporters. Importantly, the *R* enantiomers of 5- and 6-MABB displayed hybrid transporter activity, characterized by full efficacy releasing effects at SERT (102%–104% Emax), partial releasing activities at NET (58%–66% Emax), and minimal release at DAT (<30% Emax). We previously showed that increasing the alpha-carbon chain length of cathinone compounds, from methyl to ethyl, produces hybrid transporter compounds that exhibit fully

TABLE 1

Potency of MDMA and benzofuran analogs to inhibit of [^{3}H]neurotransmitter uptake in rat brain synaptosomes
Data are mean ± S.E.M. for $N = 3$ experiments performed in triplicate, as determined from nonlinear regression of the concentration-response curves shown in Fig. 2.

Compound	Inhibition of DAT-Mediated [^{3}H]DA Uptake IC_{50} (nM)	Inhibition of NET-Mediated [^{3}H]NE Uptake IC_{50} (nM)	Inhibition of SERT-Mediated [^{3}H]5-HT Uptake IC_{50} (nM)	DAT/SERT Uptake Inhibition Ratio[a]
MDMA	2008 ± 147	1067 ± 98	356 ± 16	0.2
R-5-MABB	472 ± 35	1305 ± 129	212 ± 6	0.4
S-5-MABB	921 ± 55	641 ± 59	124 ± 4	0.2
R-6-MABB	46 ± 4	352 ± 18	703 ± 44	15.4
S-6-MABB	99 ± 5	306 ± 29	217 ± 8	2.2

[a]DAT/SERT ratio is $(\text{DAT } IC_{50})^{-1}/(\text{SERT } IC_{50})^{-1}$.

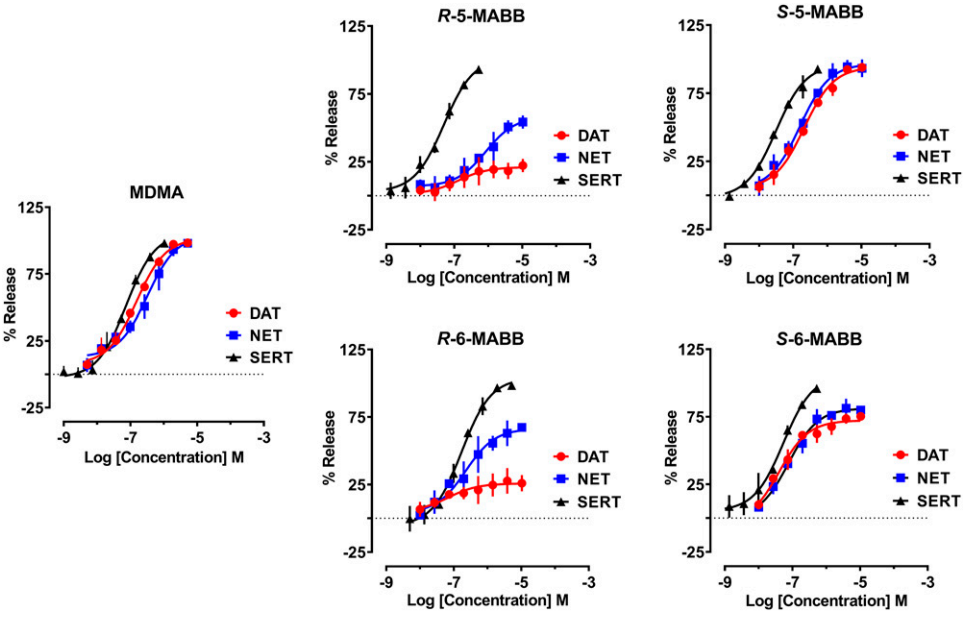

Fig. 3. Concentration-response curves for MDMA, *R*-5-MABB, *S*-5-MABB, *R*-6-MABB, and *S*-6-MABB to evoke transporter-mediated release of [^{3}H]substrates from preloaded rat brain synaptosomes. [^{3}H]MPP+ was used as the radiolabeled substrate for DAT and NET, whereas [^{3}H]5-HT was used as the radiolabeled substrate for SERT. Data are percentage of maximal release, expressed as mean ± S.E.M. for N = 3 to 4 experiments performed in triplicate.

efficacious releasing effects at SERT coupled with lack of releasing activity at DAT (Saha et al., 2019).

Drug Discrimination

In drug discrimination experiments using MDMA as the training drug, all eight rats met the initial criteria for stimulus control with an average of 34 (±15) sessions. Figure 4 displays the dose-response curves generated from stimulus substitution tests with MDMA and the enantiomers of 5- and 6-MABB. MDMA produced a dose-dependent increase in MDMA-lever responses, with full substitution after 0.75 mg/kg and 1.5 mg/kg. Response rates were not significantly different among MDMA doses or in comparison with saline control levels [F (4, 28) = 1.03, P = 0.41]. The MDMA ED_{50} was 0.37 [confidence intervals (CI): 0.27 to 0.49].

R-5-MABB produced a dose-dependent increase in MDMA-lever responses, with partial substitution at 1.28 mg/kg and full substitution after 2.56 mg/kg. *S*-5-MABB produced a dose-dependent increase in MDMA-lever responses, with full substitution at the 1.28 mg/kg and 2.56 mg/kg doses. The ED_{50}s for *R*- and *S*-5-MABB were 0.74 (CI: 0.44 to 1.14) and 0.35 (CI: 0.02 to 0.62), respectively. Neither enantiomer of 5-MABB affected response rates compared with vehicle control. A repeated measures ANOVA across response rates indicated no statistically significant effect of either *R*-5-MABB [F (4, 24) = 0.26, P = 0.90] or *S*-5-MABB [F (4, 28) = 0.43, P = 0.79].

The 6-MABB enantiomers also produced dose-dependent increases in MDMA-lever responses, with full substitution after 1.28 mg/kg. The ED_{50} values for *R*- and *S*-6-MABB were 0.45 (CI: 0.31 to 0.61) and 0.21, respectively. Due to the irregular shape of the *S*-6-MABB dose-response function, the lower confidence interval could not be estimated by the nonlinear regression model used. Response rates displayed a possible dose-dependent decrease with both enantiomers, but these effects were not statistically significant for either *R*-6-MABB [F (4, 24) = 1.48, P = 0.24] or *S*-6-MABB [F (4, 28) = 0.09, P = 0.38].

Discussion

In recent years, MDMA has gained attention as an effective adjunct for treating PTSD (Mithoefer et al., 2018; Mitchell et al., 2021), and therapeutic effects of the drug are thought to involve transporter-mediated release of monoamine neurotransmitters, especially 5-HT (Simmler and Liechti, 2018; Heifets et al., 2019; Oeri, 2021). Despite the promising clinical outcomes with MDMA, there are legitimate health concerns with the medical use of the drug, including cardiovascular stimulation and metabolic inhibition of cytochrome P450

TABLE 2

Potency of MDMA and benzofuran analogs to evoke the release of [^{3}H]MPP+ via DAT and NET, or [^{3}H]5-HT via SERT, in rat brain synaptosomes Data are mean ± S.E.M. for N = 3 to 4 experiments performed in triplicate, as determined from nonlinear regression of the concentration-response curves shown in Fig. 3.

Compound	DAT-Mediated [^{3}H]MPP+ Release EC_{50} (nM) [%Emax][a]	NET-Mediated [^{3}H]MPP+ Release EC_{50} (nM) [%Emax][a]	SERT-Mediated [^{3}H]5-HT Release EC_{50} (nM) [%Emax][a]	DAT/SERT Release Ratio[b]
MDMA	154 ± 17 [102%]	323 ± 53 [104%]	75 ± 9 [105%]	0.5
R-5-MABB	N.A.	850 ± 204 [58%]	49 ± 6 [102%]	—
S-5-MABB	210 ± 25 [94%]	158 ± 22 [97%]	31 ± 3 [96%]	0.1
R-6-MABB	N.A.	227 ± 55 [66%]	172 ± 19 [104%]	—
S-6-MABB	41 ± 9 [72%]	77 ± 12 [81%]	54 ± 8 [106%]	1.3

Dash indicates ratio that can not be calculated.
N.A., not active, which indicates release efficacy below 30% of Emax.
[a]Percentage of maximal release, %Emax, is denoted in brackets.
[b]DAT/SERT ratio is defined as $(DAT\ EC_{50})^{-1}/(SERT\ EC_{50})^{-1}$.

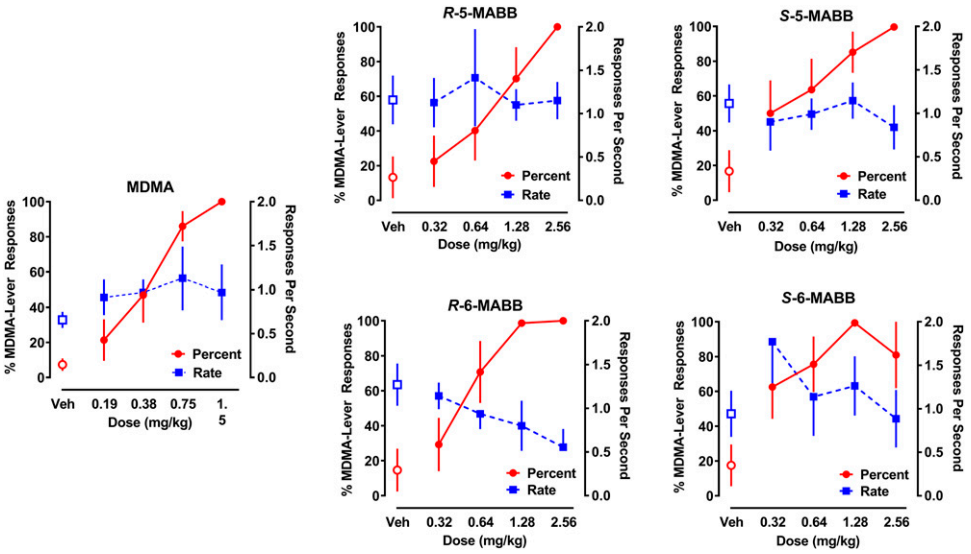

Fig. 4. Dose-response curves for MDMA, *R*-5-MABB, *S*-5-MABB, *R*-6-MABB, and *S*-6-MABB to engender MDMA-lever responses in male rats. Percent MDMA-lever responses are shown on the left *y*-axis, whereas response rates are shown on the right *y*-axis. Data are mean ± S.E.M. for $N = 6$ rats per group.

enzymes. Accordingly, current medication development efforts are aimed at finding new chemical scaffolds with MDMA-like monoamine releasing activity [see Heal et al. (2023)]. As an example, a number of research groups have suggested that ring-substituted cathinone derivatives might be effective MDMA-like treatments for PTSD and other indications such as depression and substance use disorders (López-Arnau et al., 2022; Mayer et al., 2023; Unterwald and Rawls, 2023). Here, we examined the neurochemical and behavioral effects of benzofuran derivatives structurally related to the monoamine releasers 5- and 6-MAPB (Eshleman et al., 2019; Brandt et al., 2020). We found that *S* isomers of 5- and 6-MABB are efficacious releasing agents at SERT, NET, and DAT, similar to the mechanism of MDMA. By contrast, the *R* isomers of 5- and 6-MABB are efficacious releasers only at SERT, with reduced substrate activity at NET and DAT. Results from drug discrimination experiments in rats demonstrated that enantiomers of 5- and 6-MABB fully substitute for the MDMA stimulus cue, and the diminished NET and DAT releasing activities for *R* isomers coincide with reduced potency in vivo. Taken together, the findings point to the further exploration of aminoalkyl benzofuran analogs as MDMA-like monoamine releasers with therapeutic potential.

Our uptake inhibition assays in rat brain synaptosomes reveal for the first time that both enantiomers of 5- and 6-MABB are fully efficacious inhibitors at DAT, NET, and SERT. The *R* and *S* enantiomers of 5-MABB are more potent at inhibiting SERT-mediated uptake relative to DAT-mediated uptake, with DAT/SERT ratios less than 1. By contrast, the *R* and *S* isomers of 6-MABB are more potent as uptake inhibitors at DAT relative to SERT, and this selectivity profile is especially true for *S*-6-MABB, which exhibits a DAT/SERT ratio of 15.4. Our uptake inhibition results with MABB compounds are consistent with prior findings examining the effects of various benzofuran new psychoactive substances (NPS) in cells transfected with human DAT, NET, or SERT (Rickli et al. 2015; Shimshoni et al., 2017; Eshleman et al., 2019). In particular, Rickli et al. (2015) demonstrated that racemic 6-APB has higher DAT/SERT selectivity when compared with racemic 5-APB, and Eshleman et al. (2019) showed similar results for 6-MAPB versus 5-MAPB. Thus, the more potent effects on DAT-mediated uptake inhibition seem to be conserved for 6-APB, 6-MAPB, and 6-MABB.

Although uptake inhibition assays are useful for identifying transporter ligands, such assays are unable to discriminate nontransportable uptake inhibitors from transportable substrate-type releasers (Sitte and Freissmuth, 2015; Baumann et al., 2018). Here, we used release assays in rat brain synaptosomes to characterize transporter-mediated releasing activities for the enantiomers of 5- and 6-MABB. We found that the *S* isomers of 5- and 6-MABB are fully efficacious releasers at DAT, NET, and SERT, whereas the *R* isomers are fully efficacious releasers only at SERT. The *R* isomers of 5- and 6-MABB act as partial releasers at NET and are devoid of releasing activity at DAT (i.e., <30% efficacy). The release findings with *R* isomers of MABB are consistent with data showing that increased alpha carbon chain length of ring-substituted cathinones (e.g., butylone) can decrease releasing efficacy at NET and eliminate release activity at DAT [see Saha et al. (2019)]. Moreover, the findings suggest that decreased releasing activity of certain racemic compounds (i.e., partial releasers) may be driven chiefly by their *R* isomers, as suggested by the findings of others (Mayer et al., 2023). For instance, Mayer et al. (2023) recently demonstrated that *S* enantiomers of ring-substituted cathinones are fully efficacious releasers at human monoamine transporters, whereas their *R* counterparts are not. With regard to the development of novel MDMA-like medications, more studies are needed to examine the enantiomer-specific releasing effects of phenalkylamines, cathinones, and benzofuran ligands.

Our study is the first to examine the discriminative stimulus effects of 5- and 6-MABB. We found that both enantiomers of 5- and 6-MABB engender MDMA-like discriminative stimulus effects, and *S* enantiomers are slightly more potent than *R* enantiomers in this regard. Although no published drug discrimination studies have examined effects of racemic 5- or 6-MABB, Dolan et al. (2017) investigated the effects of racemic 5-APB in groups of rats trained to discriminate MDMA, methamphetamine, cocaine, or the psychedelic compound 2,5-dimethoxy-4-methylphenyl isopropylamine (DOM) from saline. They found that 5-APB fully substituted for MDMA and partially substituted for methamphetamine, cocaine, and DOM. Based on their

results, Dolan et al. (2017) concluded that 5-APB discrimination is mediated by both dopamine and serotonin neuronal systems (Dolan et al., 2017). To further assess the duration of action and active dose range of benzofuran compounds, Dolan et al., (2017) also measured the effects of these substances on locomotor activity in mice. Their findings indicated that 5-APB has a slightly different locomotor activity profile from MDMA, with 5-APB inducing more rapid and sustained increases in motor activity when compared with MDMA.

In the present study, we observed greater behavioral potency for *S* enantiomers of 5- and 6-MABB when compared with their *R* enantiomers. It is tempting to speculate that reduced NET and DAT releasing activities for *R* enantiomers might contribute to their reduced potency in drug discrimination experiments. However, the *R* enantiomers of both 5- and 6-MABB are also somewhat less potent at inducing SERT-mediated 5-HT release when compared with the *S* enantiomers. Our drug discrimination findings showing more potent effects of *S* enantiomers of MABB compounds align with the known behavioral profile of MDMA enantiomers, where *S*-MDMA is more potent than *R*-MDMA (Schechter, 1987; Baker and Taylor, 1997). In a study where rats were trained to discriminate MDMA enantiomers from saline, the serotonergic psychedelics DOM, lysergic acid diethylamide (LSD), and mescaline partially substituted for *S*-MDMA, whereas the psychostimulants amphetamine and cocaine failed to substitute for either enantiomer (Baker et al., 1995). Given the pharmacological specificity of drug discrimination, the prior findings with MDMA indicate that serotonergic neural systems are more salient to the discriminative stimulus effects of *S*-MDMA than to those of *R*-MDMA, whereas dopaminergic actions seem less relevant to the discrimination of either isomer. More studies are warranted to determine the precise neurobiological underpinning of the discriminative stimulus effects of MABB enantiomers and other MDMA-like entactogen compounds.

In summary, the results obtained from the present study affirm that enantiomers of 5- and 6-MABB are monoamine releasing agents that produce MDMA-appropriate lever responding and fully substitute for MDMA at sufficient doses. Further research is needed to discern which transporters and receptors are most important for the discriminative stimulus effects of these benzofuran derivatives and their enantiomers, but our current findings reveal that SERT-mediated release of 5-HT is a prominent feature shared by all compounds tested. The assessment of selective 5-HT or dopamine receptor antagonists would help differentiate the relative importance of 5-HT versus dopamine receptors in modulating the MDMA-like stimulus effects of benzofuran derivatives. Based on the structural and pharmacological similarities between MDMA and various benzofurans, it would also be of interest to assess the stimulant effects of the compounds. Objective assessments of locomotor activity and drug self-administration would be valuable to determine if the *R* and *S* enantiomers of 5- and 6-MABB differ with respect to central nervous system stimulant effects. Finally, it must be mentioned that we only studied the effects of drugs in male rats, and future investigations are warranted to investigate the pharmacology of these compounds on female rats. The present study contributes to the growing literature on the in vivo pharmacological actions of benzofuran molecules. Further evaluation of these substances is warranted and may aid in development of medications that retain MDMA-like properties with fewer adverse effects.

Acknowledgments

Compounds were provided by Tactogen and the NIDA Drug Supply Program.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Authorship Contributions

Participated in research design: Johnson, Baggott, Baker, Baumann.

Conducted experiments: Johnson, Walther.

Contributed new reagents or analytic tools: Johnson, Baggott, Baker, Baumann.

Performed data analysis: Johnson, Baker, Baumann.

Wrote or contributed to the writing of the manuscript: Johnson, Baggott, Baker, Baumann.

References

Baker LE, Broadbent J, Michael EK, Matthews PK, Metosh CA, Saunders RB, West WB, and Appel JB (1995) Assessment of the discriminative stimulus effects of the optical isomers of Ecstasy (3,4-methylenedioxymethamphetamine; MDMA). *Behav Pharmacol* **6**:263–275.

Baker LE and Taylor MM (1997) Assessment of the MDA and MDMA optical isomers in a stimulant-hallucinogen discrimination. *Pharmacol Biochem Behav* **57**:737–748.

Baumann MH, Walters HM, Niello M, and Sitte HH (2018) Neuropharmacology of synthetic cathinones, in *New Psychoactive Substances: Pharmacology, Clinical, Forensic and Analytical Toxicology* (Maurer HH and Brandt SD, eds) pp 113–142, Springer, Cham, Switzerland.

Boeckel MG, Wagner A, and Grassi-Oliveira R (2017) The effects of intimate partner violence exposure on the maternal bond and PTSD symptoms of children. *J Interpers Violence* **32**:1127–1142.

Brandt SD, Walters HM, Partilla JS, Blough BE, Kavanagh PV, and Baumann MH (2020) The psychoactive aminoalkylbenzofuran derivatives, 5-APB and 6-APB, mimic the effects of 3,4-methylenedioxyamphetamine (MDA) on monoamine transmission in male rats. *Psychopharmacology (Berl)* **237**:3703–3714.

Carhart-Harris RL, Wall MB, Erritzoe D, Kaelen M, Ferguson B, De Meer I, Tanner M, Bloomfield M, Williams TM, Bolstridge M, et al. (2014) The effect of acutely administered MDMA on subjective and BOLD-fMRI responses to favourite and worst autobiographical memories. *Int J Neuropsychopharmacol* **17**:527–540.

Cha HJ, Lee KW, Eom JH, Kim YH, Shin J, Yun J, Han K, and Kim HS (2016) 5-(2-Aminopropyl)benzofuran and phenazepam demonstrate the possibility of dependence by increasing dopamine levels in the brain. *Pharmacol Biochem Behav* **149**:17–22.

de la Torre R, Farré M, Ortuño J, Mas M, Brenneisen R, Roset PN, Segura J, and Camí J (2000) Non-linear pharmacokinetics of MDMA ('Ecstasy') in humans. *Br J Clin Pharmacol* **49**:104–109.

Dolan SB, Forster MJ, and Gatch MB (2017) Discriminative stimulus and locomotor effects of para-substituted and benzofuran analogs of amphetamine. *Drug Alcohol Depend* **180**:39–45.

Eshleman AJ, Nagarajan S, Wolfrum KM, Reed JF, Swanson TL, Nilsen A, and Janowsky A (2019) Structure-activity relationships of bath salt components: substituted cathinones and benzofurans at biogenic amine transporters. *Psychopharmacology (Berl)* **236**:939–952.

Farré M, Abanades S, Roset PN, Peiró AM, Torrens M, O'Mathúna B, Segura M, and de la Torre R (2007) Pharmacological interaction between 3,4-methylenedioxymethamphetamine (Ecstasy) and paroxetine: pharmacological effects and pharmacokinetics. *J Pharmacol Exp Ther* **323**:954–962.

Ford JD (2018) Trauma memory processing in posttraumatic stress disorder psychotherapy: a unifying framework. *J Trauma Stress* **31**:933–942.

Fuwa T, Suzuki J, Tanaka T, Inomata A, Honda Y, and Kodama T (2016) Novel psychoactive benzofurans strongly increase extracellular serotonin level in mouse corpus striatum. *J Toxicol Sci* **41**:329–337.

Green AR, Mechan AO, Elliott JM, O'Shea E, and Colado MI (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"). *Pharmacol Rev* **55**:463–508.

Heal DJ, Gosden J, Smith SL, and Atterwill CK (2023) Experimental strategies to discover and develop the next generation of psychedelics and entactogens as medicines. *Neuropharmacology* **225**:109375.

Heifets BD, Salgado JS, Taylor MD, Hoerbelt P, Cardozo Pinto DF, Steinberg EE, Walsh JJ, Sze JY, and Malenka RC (2019) Distinct neural mechanisms for the prosocial and rewarding properties of MDMA. *Sci Transl Med* **11**:6435.

Jerome L, Feduccia AA, Wang JB, Hamilton S, Yazar-Klosinski B, Emerson A, Mithoefer MC, and Doblin R (2020) Long-term follow-up outcomes of MDMA-assisted psychotherapy for treatment of PTSD: a longitudinal pooled analysis of six phase 2 trials. *Psychopharmacology (Berl)* **237**:2485–2497.

Kolbrich EA, Goodwin RS, Gorelick DA, Hayes RJ, Stein EA, and Huestis MA (2008) Physiological and subjective responses to controlled oral 3,4-methylenedioxymethamphetamine administration. *J Clin Psychopharmacol* **28**:432–440.

Liechti ME, Baumann C, Gamma A, and Vollenweider FX (2000) Acute psychological effects of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") are attenuated by the serotonin uptake inhibitor citalopram. *Neuropsychopharmacology* **22**:513–521.

López-Arnau R, Camarasa J, Carbó ML, Nadal-Gratacós N, Puigseslloses P, Espinosa-Velasco M, Urquizu E, Escubedo E, and Pubill D (2022) 3,4-Methylenedioxy methamphetamine, synthetic cathinones and psychedelics: From recreational to novel psychotherapeutic drugs. *Front Psychiatry* **13**:990405.

Luethi D and Liechti ME (2018) Monoamine transporter and receptor interaction profiles in vitro predict reported human doses of novel psychoactive stimulants and psychedelics. *Int J Neuropsychopharmacol* **21**:926–931.

Mas M, Farré M, de la Torre R, Roset PN, Ortuño J, Segura J, and Camí J (1999) Cardiovascular and neuroendocrine effects and pharmacokinetics of 3, 4-methylenedioxymethamphetamine in humans. *J Pharmacol Exp Ther* **290**:136–145.

Mayer FP, Niello M, Cintulova D, Sideromenos S, Maier J, Li Y, Bulling S, Kudlacek O, Schicker K, Iwamoto H, et al. (2023) Serotonin-releasing agents with reduced off-target effects. *Mol Psychiatry* **28**:722–732.

Mitchell JM, Bogenschutz M, Lilienstein A, Harrison C, Kleiman S, Parker-Guilbert K, Ot'alora G M, Garas W, Paleos C, Gorman I, et al. (2021) MDMA-assisted therapy for severe PTSD: a randomized, double-blind, placebo-controlled phase 3 study. *Nat Med* **27**:1025–1033.

Mithoefer MC, Mithoefer AT, Feduccia AA, Jerome L, Wagner M, Wymer J, Holland J, Hamilton S, Yazar-Klosinski B, Emerson A, et al. (2018) 3,4-methylenedioxymethamphetamine (MDMA)-assisted psychotherapy for post-traumatic stress disorder in military veterans, firefighters, and police officers: a randomised, double-blind, dose-response, phase 2 clinical trial. *Lancet Psychiatry* **5**:486–497.

National Research Council (2011) *Guide for the Care and Use of Laboratory Animals*, 8th ed, National Academies Press, Washington, DC.

Oeri HE (2021) Beyond Ecstasy: alternative entactogens to 3,4-methylenedioxymethamphetamine with potential applications in psychotherapy. *J Psychopharmacol* **35**:512–536.

O'Mathúna B, Farré M, Rostami-Hodjegan A, Yang J, Cuyàs E, Torrens M, Pardo R, Abanades S, Maluf S, Tucker GT, et al. (2008) The consequences of 3,4-methylenedioxymethamphetamine induced CYP2D6 inhibition in humans. *J Clin Psychopharmacol* **28**:523–529.

Parrott AC (2013) MDMA, serotonergic neurotoxicity, and the diverse functional deficits of recreational 'Ecstasy' users. *Neurosci Biobehav Rev* **37**:1466–1484.

Reneman L, de Win MM, van den Brink W, Booij J, and den Heeten GJ (2006) Neuroimaging findings with MDMA/Ecstasy: technical aspects, conceptual issues and future prospects. *J Psychopharmacol* **20**:164–175.

Rickli A, Kopf S, Hoener MC, and Liechti ME (2015) Pharmacological profile of novel psychoactive benzofurans. *Br J Pharmacol* **172**:3412–3425.

Roberts CA, Jones A, and Montgomery C (2016) Meta-analysis of molecular imaging of serotonin transporters in Ecstasy/polydrug users. *Neurosci Biobehav Rev* **63**:158–167.

Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI, and Partilla JS (2001) Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse* **39**:32–41.

Saha K, Li Y, Holy M, Lehner KR, Bukhari MO, Partilla JS, Sandtner W, Sitte HH, and Baumann MH (2019) The synthetic cathinones, butylone and pentylone, are stimulants that act as dopamine transporter blockers but 5-HT transporter substrates. *Psychopharmacology (Berl)* **236**:953–962.

Sandtner W, Stockner T, Hasenhuetl PS, Partilla JS, Seddik A, Zhang YW, Cao J, Holy M, Steinkellner T, Rudnick G, et al. (2016) Binding mode selection determines the action of Ecstasy homologs at monoamine transporters. *Mol Pharmacol* **89**:165–175.

Schechter MD (1987) MDMA as a discriminative stimulus: isomeric comparisons. *Pharmacol Biochem Behav* **27**:41–44.

Schenk S and Highgate Q (2021) Methylenedioxymethamphetamine (MDMA): Serotonergic and dopaminergic mechanisms related to its use and misuse. *J Neurochem* **157**:1714–1724.

Schindler CW, Thorndike EB, Blough BE, Tella SR, Goldberg SR, and Baumann MH (2014) Effects of 3,4-methylenedioxymethamphetamine (MDMA) and its main metabolites on cardiovascular function in conscious rats. *Br J Pharmacol* **171**:83–91.

Shimshoni JA, Winkler I, Golan E, and Nutt D (2017) Neurochemical binding profiles of novel indole and benzofuran MDMA analogues. *Naunyn Schmiedebergs Arch Pharmacol* **390**:15–24.

Simmler LD and Liechti ME (2018) Pharmacology of MDMA- and amphetamine-like new psychoactive substances. *Handb Exp Pharmacol* **252**:143–164.

Sitte HH and Freissmuth M (2015) Amphetamines, new psychoactive drugs and the monoamine transporter cycle. *Trends Pharmacol Sci* **36**:41–50.

Tancer M and Johanson CE (2007) The effects of fluoxetine on the subjective and physiological effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. *Psychopharmacology (Berl)* **189**:565–573.

Unterwald EM and Rawls SM (2023) Bath salts to therapies: can separation of adverse and therapeutic effects of substituted cathinones lead to a medication for psychostimulant use disorder? *J Pharmacol Exp Ther* **385**:159–161.

Verrico CD, Miller GM, and Madras BK (2007) MDMA (Ecstasy) and human dopamine, norepinephrine, and serotonin transporters: implications for MDMA-induced neurotoxicity and treatment. *Psychopharmacology (Berl)* **189**:489–503.

Welter J, Brandt SD, Kavanagh P, Meyer MR, and Maurer HH (2015a) Metabolic fate, mass spectral fragmentation, detectability, and differentiation in urine of the benzofuran designer drugs 6-APB and 6-MAPB in comparison to their 5-isomers using GC-MS and LC-(HR)-MS(n) techniques. *Anal Bioanal Chem* **407**:3457–3470.

Welter J, Kavanagh P, Meyer MR, and Maurer HH (2015b) Benzofuran analogues of amphetamine and methamphetamine: studies on the metabolism and toxicological analysis of 5-APB and 5-MAPB in urine and plasma using GC-MS and LC-(HR)-MS(n) techniques. *Anal Bioanal Chem* **407**:1371–1388.

Address correspondence to: Dr. Michael H. Baumann, Designer Drug Research Unit, National Institute on Drug Abuse, 333 Cassell Drive Suite 4400, Baltimore, MD 21224. E-mail: mbaumann@mail.nih.gov; or Dr. Matthew J. Baggott, Chief Executive Officer, Tactogen, Inc., 3790 El Camino Real Unit 510, Palo Alto, CA 94306. E-mail: matt@tactogen.com