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Deconstructed Analogues of Bupropion Reveal Structural Requirements for Transporter Inhibition versus Substrate-Induced Neurotransmitter Release

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Abstract

Bupropion (**1**), an α -aminophenone uptake inhibitor at plasma membrane transporters for dopamine (DAT) and norepinephrine (NET), is a widely prescribed antidepressant and smoking cessation aid. Cathinone (**2**), a structurally simpler α -aminophenone, is a substrate-type releasing agent at the same transporters and a recognized drug of abuse. Our goal was to identify the structural features of α -aminophenones that govern the mechanistic transition from uptake inhibition to substrate-induced release. Deconstructed analogues of **1** were synthesized and compared for their ability to interact with DAT, NET, and the serotonin transporter (SERT) using in vitro assay methods. Bulky amine substituents resulted in compounds that function as DAT uptake inhibitors but not release agents, whereas smaller amine substituents result in relatively nonselective releasing agents at DAT and NET. Our findings add to empirical evidence supporting distinct molecular determinants for α -aminophenone- (i.e., cathinone-) related agents acting as transporter inhibitors versus those acting as releasers.

Graphical Abstract

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Author Contributions

The project was proposed by R.A.G. A.R.S. synthesized and characterized all target compounds under the supervision of R.A.G., and D.W. conducted all transporter assays under the supervision of M.H.B. M.H.B. and R.A.G. analyzed the data. All authors had an opportunity to contribute to the preparation of, and to review, the submitted manuscript.

The authors declare no competing financial interest.

Bupropion

Keywords

Dopamine transporter; norepinephrine transporter; serotonin transporter; cathinone analogues; synthetic cathinones; drug abuse

INTRODUCTION

Bupropion (**1**), a widely prescribed antidepressant and smoking cessation aid,¹ is an α -aminophenone very closely related in chemical structure to the illicit and controlled (U.S. Schedule I) substance cathinone (**2**), a central nervous system stimulant. The structural difference between the two α -aminophenones is (i) the presence of a *tert*-butyl amine group and (ii) an aromatic *meta*-chloro substituent, present in the former but not the latter. Bupropion (**1**) is a nontransported inhibitor of uptake at dopamine (DAT) and norepinephrine (NET) transporters, whereas cathinone (**2**) acts as a substrate at DAT and NET thereby inducing transporter-mediated neurotransmitter release.^{1,2}

Based on its relatively selective actions at DAT and NET, bupropion (**1**) might be expected to possess at least some abuse liability. Indeed, although its abuse does not constitute a major problem, bupropion is the most commonly misused of all available antidepressants.³ The topic of bupropion abuse has been reviewed.^{3,4} Human subjects who abuse bupropion describe subjective effects as being much like those produced by cocaine.^{5,6} The euphoria associated with bupropion (**1**) is observed upon insufflation or intravenous injection of crushed material (i.e., crushed bupropion tablets), but not upon oral administration, suggesting slower kinetics or substantial metabolism following oral administration.^{4,7} Consequently, the pharmacokinetic character of oral bupropion might limit its abuse.^{7,8} On the other hand, the unavailability of more traditionally abused stimulants to certain populations (e.g., those in correctional facilities) seems to make bupropion abuse attractive and more prevalent in these situations.⁴

Because bupropion (**1**) is a documented DAT/NET uptake inhibitor whereas cathinone (**2**) is a DAT/NET releasing agent, we “deconstructed” bupropion, piece-by-piece, to determine at what point, structurally, a nontransported uptake inhibitor (i.e., bupropion; **1**) is converted to a transportable substrate (i.e., cathinone; **2**) that induces transporter-mediated neurotransmitter release. That is, we addressed what structural features of bupropion (**1**) make it an uptake inhibitor at DAT/NET as opposed to the simpler α -aminophenone cathinone (**2**), which is a releasing agent. To this end, we prepared and examined specific analogues, each of which bears a single structural modification relative to the parent agent bupropion (**1**): *des*-ketobupropion (**3**), *des*-chlorobupropion (**4**), and the *N*-isopropyl, *N*-methyl, and primary amine counterparts of bupropion (i.e., **5–7**, respectively), that might answer this question.

This study examined the ability of bupropion (**1**) and its deconstructed analogues **3–7** to inhibit neurotransmitter uptake and stimulate release at DAT and NET. The action of the compounds at the serotonin transporter (SERT) was also examined for comparison. The more behaviorally potent isomer of cathinone (**2**), *S*(-)-cathinone, was included as a comparator of known pharmacology.

SYNTHESIS

Compound **3** was prepared in a one-pot reaction from 1-(3-chlorophenyl)-2-propanone and *tert*-butylamine using a reductive amination procedure ($\text{TiCl}_4/\text{NaCNBH}_3$) developed by Barney et al.⁹ for the synthesis of hindered amines. The *des*-chloro analogue **4** was obtained by the simple reaction of α -chloropropiophenone with *tert*-butylamine using conditions comparable to what we have previously reported for the synthesis of related compounds, and **5** was prepared by a literature method,¹⁰ but isolated as its (previously unknown, and more water-soluble) HCl salt. The α -methylaminopropiophenone **6** was not known at the time we prepared it (Scheme 1), but was subsequently reported by Blough et al.¹¹ using a somewhat different method of preparation involving an N-protected amine. Primary amine **7** was obtained by reduction of azide **9** (Scheme 1) which was prepared following the general method of Pulici et al.¹² for a related compound. Although **7** has been identified as a possible bupropion metabolite in rabbit liver homogenates by spectroscopic methods,¹³ this finding has not been confirmed in other animal species,⁸ and **7** has not been hitherto synthesized or characterized.

RESULTS

Uptake Inhibition Findings.

Figure 1 depicts the effects of test compounds on inhibition of uptake at DAT, NET and SERT in rat brain synaptosomes. Table 1 shows potency estimates expressed as IC_{50} values; IC_{50} represents the drug concentration required to inhibit uptake by 50%. Consistent with previous reports, bupropion (**1**) was found to act as a fully efficacious uptake inhibitor at DAT ($\text{IC}_{50} = 305 \text{ nM}$). Given differences in species and techniques across previous studies, the DAT potency for bupropion (**1**) obtained here is comparable to what others have reported: $\text{IC}_{50} = 550$,¹⁴ 630,¹⁵ and 945 nM.¹⁰

It would appear that the various bupropion substituents make only a minor contribution to DAT uptake potency, with compounds **5–7** being equipotent with bupropion (**1**), and compounds **3** and **4** being about three to four times less potent. Carroll et al.¹⁰ have previously found that **4** and **5** are half as potent as **1** at inhibiting DAT uptake.

At NET, bupropion (**1**) was about 10-fold less potent as an uptake inhibitor ($\text{IC}_{50} = 3,715 \text{ nM}$) compared to its action at DAT ($\text{IC}_{50} = 305 \text{ nM}$; Table 1). Here too, given differences in species and techniques, the NET potency of bupropion we found is reasonably analogous to what has been previously reported: $\text{IC} = 443$,¹⁰ 1900,¹⁴ 50 2300 nM.¹⁵ In general, compounds with bulky terminal amine substituents were more potent as uptake inhibitors at DAT than at NET (i.e., **1**, **3–5**), whereas compounds with reduced bulk at this position displayed nearly equal potency at DAT and NET (e.g., **6** and **7**).

Consistent with prior literature,^{15,16} bupropion (**1**) was essentially inactive as a 5-HT uptake inhibitor at SERT ($\text{IC}_{50} > 10\,000 \text{ nM}$; Table 1). Compound **3** was weakly active at SERT while **4** was inactive. Compounds **5–7** displayed efficacious inhibition at SERT but at lower potency when compared to effects at DAT. Finally, the behaviorally more potent isomer of

cathinone, *S*(-)**2**, was weak at SERT producing only partial inhibition of uptake at 10 μ M (Table 1).

Release Findings.

Figure 2 illustrates the effects of test compounds on transporter-mediated release of tritiated substrates at DAT, NET and SERT. Table 1 shows potency estimates as EC₅₀ values; EC₅₀ is the drug concentration required to evoke a release response that is 50% of maximum. Interestingly, bupropion (**1**) and compounds **3–5** lacked activity as DAT releasing agents as demonstrated by the flattened appearance of their release curves. For these experiments, release efficacy <30% of maximum was considered inactive since this type of low-level partial release is often evoked by uptake inhibitors.¹⁷ In contrast, the *N*-methyl counterpart of **1**, compound **6** (EC₅₀ = 29 nM), and its corresponding primary amine **7** (EC₅₀ = 64 nM), were fully efficacious releasers at DAT, with greater potency for DAT-mediated release than uptake inhibition (IC₅₀ = 342 and 399 nM, respectively). The same was true of *S*(-)**2** (IC₅₀ = 357 nM; EC₅₀ = 18 nM) (Table 1). In agreement with the present data, Blough et al.¹¹ reported that **6** is a DAT releaser (EC₅₀ = 47 nM).

Similar to the findings with DAT release, bupropion (**1**) and compounds **3–5** failed to act as releasing agents at NET (Table 1). In contrast, compounds **6** and **7** (EC₅₀ = 40 and 105 nM, respectively) were more potent at NET-mediated release than uptake inhibition. *S*(-)-Cathinone, *S*(-)**2**, was a potent and efficacious releaser at NET (EC₅₀ = 26 nM).

With regard to activity at SERT, compounds **1**, **3**, and **4** were inactive as SERT releasing agents, **5** was weakly active (EC₅₀ = 1,228 nM), whereas **6** and **7** were moderately potent at SERT (EC₅₀ = 212 and 567 nM, respectively). It is noteworthy that *N*-isopropyl compound **5** displayed efficacious releasing action at SERT, but not at DAT and NET. Compounds **6** and **7** were approximately five times more potent as SERT releasing agents than as uptake inhibitors. Blough et al.¹¹ demonstrated that **6** acts as a releasing agent at SERT (EC₅₀ = 410 nM).

DISCUSSION

In the present investigation of bupropion (**1**) and its deconstructed analogs, we were particularly interested in the molecular mechanism(s) of action at major monoamine transporters (i.e., uptake inhibition versus substrate-induced release), potency, and transporter selectivity. In agreement with previous literature we found that bupropion (**1**) is an efficacious uptake inhibitor at DAT and NET, but very weak at SERT. Although bupropion (**1**) is not especially potent as an inhibitor of DAT and NET, its potency is in the same range or within 1 order of magnitude as cocaine (IC₅₀ = 198.8 and 395.9 nM at DAT and NET, respectively).¹⁸ This might explain the sporadic abuse of bupropion. On the other hand, to put this in perspective, the potency of bupropion (**1**) at DAT and NET is much less than that of the structurally related α -aminophenone central stimulant α -pyrrolidinovalerophenone (α -PVP; **10**; Figure 3) (IC₅₀ ca. 15 nM both at DAT and NET^{19,20}), a potent and controlled (U.S. Schedule I) drug of abuse. Importantly, bupropion (**1**) is devoid of releasing action at all three transporters indicating that its bulky amine substituent precludes the drug from serving as a transporter substrate.

Deconstruction of bupropion (**1**) to analogues **3-7** had little influence on potency as DAT uptake inhibitors, and systematic elimination of various structural features decreased potency by no more than 4-fold. These data indicate that none of the substituents of bupropion examined in this study plays a dominant role in its potency as a DAT uptake inhibitor. In contrast, bupropion analogues bearing an *N*-methyl group or a primary amine (i.e., **6** and **7**) acted as substrate-type releasing agents at DAT and were more potent as releasers than as uptake inhibitors. Thus, reducing the size of the amine substituent in this series of compounds converted DAT uptake inhibitors to substrate-type releasers.

It is noteworthy that **6** and **7**, compounds with the smallest (i.e., least sterically bulky) amine substituents, were also the least selective across DAT, NET, and SERT and behaved both as uptake inhibitors and releasing agents at all three neurotransmitter transporters, though there was a tendency for them to be 5- to 10-fold more potent as releasing agents at DAT/NET over SERT. Previous results have shown that abused α -aminophenones with *N*-methyl substituents, such as 4-methyl-*N*-methylecathinone (mephedrone; **11**) and 3,4-methylenedioxy-*N*-methylecathinone (methyline, MDMC; **12**) (Figure 3), are transporter releasing agents with similar potency at DAT, NET and SERT.²¹ In contrast, compound **5** with an intermediate-sized *N*-isopropyl substituent was a weak release agent at SERT but inactive at DAT and NET.

Consistent with prior observations regarding the pharmacology of other α -aminophenones,^{20,22,23} bulky amine substituents enhance DAT versus SERT selectivity, and favor DAT uptake inhibition over DAT release. It would appear, from the results shown in Table 1, that the amine bulk of **1** renders it more potent at DAT versus SERT (i.e., greater DAT/SERT selectivity) and limits its actions to uptake inhibition (i.e., precludes releasing activity). Similar but less dramatic results were obtained when investigating effects at NET. Bulkier amine analogues resulted in compounds that acted as NET uptake inhibitors rather than releasing agents (i.e., **1**, **3**, **5**). Again, analogues bearing an *N*-methyl group or a primary amine (i.e., **6** and **7**) also behaved as releasing agents and were more potent at release than uptake inhibition. Analogues with bulky amine substituents displayed little to no action at SERT either as uptake inhibitors or releasing agents (i.e., **1-3**), whereas those with smaller amine substituents were only modestly active as SERT uptake inhibitors. Nevertheless, analogues bearing an *N*-methyl group or primary amine (i.e., **6** and **7**) yet again behaved as releasing agents and were several times more potent as such than as reuptake inhibitors.

These findings provide useful information on the structural requirements of α -aminopropiophenones (i.e., synthetic cathinones) regarding neurotransmitter release versus uptake inhibition and DAT/NET/SERT transporter selectivity. But, there does not appear to be a simple and orderly structural progression across this series of compounds in terms of molecular mechanism or selectivity; nevertheless, compounds with bulky (i.e., relatively large) amine substituents favor inhibition of DAT/NET uptake over SERT uptake, and are inactive at DAT and NET release when the remainder of the molecule remains unchanged. These findings suggest that the steric volume of the amine substituent limits the ability of certain compounds to serve as transportable substrates for DAT and NET. Indeed, compounds with small amine substituents are (weak) inhibitors of DAT uptake but are more potent as releasing agents; the same can be said for NET. The bulkier amine-substituted

compounds were not particularly potent as inhibitors of SERT uptake and lacked action as releasing agents. Interestingly, the *N*-isopropyl analogue **5** was a weak releaser at SERT but failed to induce release at DAT and NET. Thus, this compound displays a different molecular mechanism of action at SERT (i.e., release) versus DAT and NET (i.e., uptake inhibition). This so-called “hybrid” transporter activity has been described previously for *N*-cyclopropyl¹¹ and *N*-ethyl cathinone analogues.²³ The findings with compound **5** suggest that SERT is more tolerant of amine steric bulk in its substrates when compared to DAT and NET.

The present data add to the growing body of evidence demonstrating distinct and strict structural requirements for compounds acting as transporter inhibitors versus those acting as releasers. For the α -aminophenones studied here, *N*-alkyl substituents larger (i.e., bulkier) than a methyl group tend to favor transporter inhibition over transporter-mediated release. The current results also provide hints that there are unique structural features for drugs favored by each of the three monoamine transporters, but additional study is required to test this hypothesis. Future studies should investigate the pharmacology of other structural templates known to interact with monoamine transporter proteins. Such studies could aid in the design and development of novel pharmacotherapies for psychiatric disorders and drug addiction. For example, Blough et al.¹¹ have suggested that hybrid transporter compounds might represent novel lead drug molecules with therapeutic potential for the treatment of drug abuse.

METHODS

Synthesis.

Melting points were taken on a Thomas-Hoover melting point apparatus in glass capillary tubes and are uncorrected. ¹H NMR spectra were recorded with a Bruker ARX 400 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Peak positions are given in parts per million (δ). Elemental analyses were performed by Atlantic Microlab Inc. (Norcross, GA) for the indicated elements and results are within 0.4% of calculated values. Reactions were monitored by thin-layer chromatography on silica gel GHLF plates (250 μ , 2.5 $\times$ 10 cm; Analtech Inc., Newark, DE).

N-tert-Butyl-1-(3-chlorophenyl)propan-2-amine (or des-Ketobupropion) Hydrogen Oxalate (3).—Triethylamine (5.3 mL, 38 mmol) and *tert*-butylamine (1.5 mL, 12 mmol) were added at room temperature to a stirred solution of 1-(3-chlorophenyl)-2-propanone²⁴ (1.5 g, 9 mmol) in CH₂Cl₂ (75 mL) under an N₂ atmosphere. A solution of TiCl₄ in CH₂Cl₂ (1M, 6.2 mL) was added, and the reaction mixture was allowed to stir at room temperature for 20 h, slowly quenched with a solution of NaCNBH₃ (2.3 g, 38 mmol) in MeOH (30 mL), and allowed to stir for 15 min. The mixture was basified with NaOH (5 N, to pH 13) and extracted with EtOAc (2 $\times$ 150 mL). The combined organic portion was dried (Na₂SO₄) and evaporated under reduced pressure. A saturated solution of oxalic acid in Et₂O was added in a dropwise manner to a solution of the free base in Et₂O (30 mL). The precipitate was collected by filtration to yield a yellow-white solid that was recrystallized from MeOH/Et₂O to yield 0.9 g (31%) of **3** as a white solid: mp 195 °C; ¹H NMR (DMSO-

d_6) δ 1.13 (d, 3H, CH₃), 1.37 (s, 9H, CH₃), 2.69–2.75 (m, 1H, CH₂), 3.18–3.22 (m, 1H, CH₂), 3.59–3.64 (m, 1H, CH), 7.28 (d, 1H, ArH), 7.33–7.40 (m, 2H, ArH), 7.44 (s, 1H, ArH); Anal. Calcd for (C₁₃H₂₀ClN·C₂H₂O₄) C, 57.05; H, 7.02; N, 4.44. Found: C, 56.92; H, 6.90; N, 4.35.

2-(N-tert-Butylamino)propiofenone (or des-Chlorobupropion) Hydrochloride (4).—

tert-Butylamine (9 mL, 86 mmol) was added to α -chloropropiofenone (i.e., 2-chloro-1-phenylpropan-1-one) (1.0 g, 4 mmol) and the reaction mixture was heated in a sealed tube at 100 °C for 3 days. The reaction mixture was allowed to cool to room temperature, filtered, and the collected solid was washed with EtOAc. The filtrate was washed with H₂O (2 × 10 mL) and extracted with HCl (6N, 3 × 10 mL). The aqueous portion was washed with EtOAc (10 mL), basified with NaOH (20%) to pH 12, and extracted with EtOAc (3 × 20 mL). The combined organic portion was washed with H₂O (10 mL) and brine (10 mL), dried (Na₂SO₄), and solvent was removed under reduced pressure. The hydrochloride salt was prepared by adding a saturated solution of gaseous HCl in Et₂O to the solution of the free base in Et₂O. Recrystallization of the salt from MeOH/Et₂O afforded 0.4 g (25%) of **4** as a white solid: mp 231–235 °C (lit.²⁵ mp 231–235 °C).

1-(3-Chlorophenyl)-2-(isopropylamino)propan-1-one Hydrochloride (5).—

The free base of **5** was prepared by the method of Carroll et al.¹⁰ but then converted to a water-soluble salt. A saturated solution of gaseous HCl in Et₂O (10 mL) was diluted with Et₂O (40 mL) and added in a dropwise manner at 0 °C (ice-bath) to the solution of the free base of **5** in Et₂O (40 mL). The precipitate was collected by filtration and recrystallized from EtOH/Et₂O to afford 0.3 g (32%) of **5** as a white solid: mp 215–220 °C; ¹H NMR (DMSO-*d*₆) δ 1.27 (d, 6H, CH₃), 1.44 (d, 3H, CH₃), 3.29–3.36 (m, 1H, CH), 5.19 (q, 1H, CH), 7.65 (t, 1H, ArH), 7.82 (d, 1H, ArH), 8.07 (d, 1H, ArH), 8.16 (s, 1H, ArH); Anal. Calcd for (C₁₂H₁₆ClNO·HCl) C, 54.97; H, 6.54; N, 5.34. Found: C, 54.77; H, 6.48; N, 5.36.

1-(3-Chlorophenyl)-2-(methylamino)propan-1-one Hydrochloride (6).—

Bromo-1-(3-chlorophenyl)propan-1-one (**8**) (0.8 g, 3 mmol) in 95% EtOH (5 mL) was added in a dropwise manner at 0 °C (ice-bath) to a stirred solution of methylamine (0.2 g, 6 mmol) in 1:1 EtOH/H₂O (2 mL) over 20 min, and the reaction mixture was allowed to stir for 2 h. The solvent was evaporated under reduced pressure. Water (25 mL) was added and the mixture was acidified with HCl (1N, to pH 1). The aqueous portion was washed with EtOAc (5 × 30 mL), basified with NaOH (5N, to pH 12) and extracted with EtOAc (3 × 10 mL). The combined organic portion was washed with H₂O (10 mL) and brine (20 mL), dried (Na₂SO₄), and solvent was evaporated under reduced pressure. A saturated solution of gaseous HCl in Et₂O (5 mL) was diluted with Et₂O (495 mL), and added in a dropwise manner at 0 °C (ice-bath) to a solution of the free base in Et₂O (30 mL). The precipitate was collected by filtration to yield 0.08 g (11%) of a crude yellow-white solid. Recrystallization of the salt from *i*-PrOH/Et₂O afforded **6** as a white solid: mp 193 °C (lit.¹¹ 181–183 °C); ¹H NMR (DMSO-*d*₆) δ 1.45 (d, 3H, CH₃), 2.61 (s, 3H, CH₃), 5.20 (q, 1H, CH), 7.66 (t, 1H, ArH), 7.85 (d, 1H, ArH), 8.00 (d, 1H, ArH), 8.09 (s, 1H, ArH), 9.34 (s, 1H, NH₂⁺). Due to the 10 °C discrepancy in melting point for **6** relative to the literature, it was submitted for

microanalysis. Anal. Calcd for (C₁₀H₁₂ClNO·HCl) C, 51.30; H, 5.60; N, 5.98. Found: C, 51.32; H, 5.54; N, 5.91.

2-Amino-1-(3-chlorophenyl)propan-1-one Hydrogen Oxalate (7).—Stannous chloride dihydrate (1.7 g, 8 mmol) was added in a single portion at 0 °C (ice-bath) to a stirred solution of **9** (0.8 g, 4 mmol) in EtOH (5 mL). The ice bath was removed and the mixture was allowed to stir at room temperature overnight. The solvent was evaporated under reduced pressure. The reaction mixture was diluted with H₂O (20 mL), filtered, and the collected solid was washed with H₂O. The filtrate was basified with NaOH (5N, to pH 12) and extracted with EtOAc (3 × 30 mL). The combined organic portion was washed with brine (2 × 5 mL), dried (Na₂SO₄), and solvent was evaporated under reduced pressure. A saturated oxalic acid solution in Et₂O was added in a dropwise manner to the solution of the free base in Et₂O (30 mL). The precipitate was collected by filtration to yield 0.35 g (34%) of **7** as a white solid: mp 175–180 °C; ¹H NMR (DMSO-*d*₆) δ 1.41 (d, 3H, CH₃), 5.16 (q, 1H, CH), 7.65 (t, 1H, ArH), 7.83 (d, 1H, ArH), 8.02 (d, 1H, ArH), 8.09 (s, 1H, ArH); Anal. Calcd for (C₉H₁₀ClNO·C₂H₂O₄) C, 48.28; H, 4.42; N, 5.12. Found: C, 47.98; H, 4.57; N, 5.24.

2-Azido-1-(3-chlorophenyl)propan-1-one (9).—Sodium azide (0.4 g, 6 mmol) (*Caution! Explosive hazard.*) was added to a stirred solution of 2-bromo-1-(3-chlorophenyl)propan-1-one (**8**) (1 g, 4 mmol) in acetone (8 mL), and the reaction mixture was allowed to stir at room temperature for 20 h. The solvent was evaporated under reduced pressure. Diethyl ether (20 mL) was added and the mixture was washed with H₂O (5 mL), brine (5 mL), dried (Na₂SO₄), and evaporated to dryness under reduced pressure to yield 0.81 g (95%) of **9** as a yellow oil which was used in the preparation of **7** without further characterization.

In Vitro Reuptake and Release Assays.

Subjects.—Male Sprague–Dawley rats (Harlan Laboratories, Frederick, MD) weighing 250–350 g were housed three per cage with free access to food and water and maintained on a 12 h light/dark cycle with lights on from 7:00 a.m. to 7:00 p.m. Animal facilities were accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, and procedures were carried out in accordance with the Institutional Animal Care and Use Committee and the National Institutes of Health guidelines on care and use of animal subjects in research (National Research Council, 2011).

Uptake Assays.—[³H]Dopamine, [³H]norepinephrine, and [³H]5-HT (specific activity ranging from 30 to 50 Ci/mmol) were purchased from PerkinElmer (Shelton, CT). All other chemicals and reagents were acquired from Sigma-Aldrich (St. Louis, MO). Rats were euthanized by CO₂ narcosis, and brains were processed to yield synaptosomes as previously described.¹⁷ Rat caudate tissue was used for DAT assays, whereas rat whole brain minus caudate and cerebellum was used for NET and SERT assays. Transport activity at DAT, NET, and SERT was assessed using 5 nM [³H]dopamine, 10 nM [³H]norepinephrine, and 5 nM [³H]5-HT, respectively. The selectivity of uptake assays was optimized for a single transporter by including unlabeled blockers to prevent uptake of [³H]transmitter by

competing transporters. Uptake inhibition assays were initiated by adding 100 μL of tissue suspension 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 [^3H]transmitter. Assays were terminated by rapid vacuum filtration through Whatman GF/B filters, and retained radioactivity was quantified by liquid scintillation counting. Statistical analyses were carried out using GraphPad Prism (v. 6.0; GraphPad Scientific, San Diego, CA). IC_{50} values for uptake inhibition were calculated based on nonlinear regression analysis.

Release Assays.—Rats were euthanized by CO_2 narcosis, and brains were processed to yield synaptosomes. Synaptosomes were prepared from striatum for DAT assays, whereas synaptosomes were prepared from whole brain minus striatum and cerebellum for the NET and SERT assays. [^3H]1-Methyl-4-phenylpyridinium ([^3H]MPP+) (9 nM) was used as the radiolabeled substrate for DAT and NET, whereas [^3H]5-HT (5 nM) was used as a substrate for SERT. All buffers used in the release assays contained 1 μM reserpine to block vesicular uptake of substrates. The selectivity of assays was optimized for a single transporter by including unlabeled compounds to prevent the uptake of [^3H]MPP+ or [^3H]5-HT by competing transporters. Synaptosomes were preloaded with radiolabeled substrate in Krebs-phosphate buffer for 1 h (steady state). Assays were initiated by adding 850 μL of preloaded synaptosomes to 150 μL of test drug. Assays were terminated by vacuum filtration, and retained radioactivity was quantified by liquid scintillation counting. Effects of test drug concentrations were expressed as percent of maximum release, with maximum release (i.e., 100% E_{max}) defined as the release produced by 10 nM tyramine for DAT and NET assay conditions, and 100 nM tyramine for SERT assay conditions. These doses of tyramine evoke the efflux of all “releasable” tritium from synaptosomes as determined previously.¹⁷ EC_{50} values were determined using nonlinear least-squares curve fitting (GraphPad Prism, San Diego, CA).

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REFERENCES

- (1). Stahl SM, Pradko JF, Haight BR, Modell JG, Rockett CB, and Learned-Coughlin S (2004) A review of the neuropharmacology of bupropion, a dual norepinephrine and dopamine reuptake inhibitor. *Prim. Care Companion J. Clin. Psychiat* 6, 159–166.
- (2). Fava M, Rush AJ, Thase ME, Clayton A, Stahl SM, Pradko JF, and Johnston JA (2005) 15 years of clinical experience with bupropion HCl: from bupropion to bupropion SR to bupropion XL. *Prim. Care Companion J. Clin. Psychiat* 7, 106–113.
- (3). Evans EA, and Sullivan MA (2014) Abuse and misuse of antidepressants. *Subst. Abuse Rehabil* 5, 107–120. [PubMed: 25187753]
- (4). Hilliard WT, Barloon L, Farley P, Penn JV, and Koranek A (2013) Bupropion diversion and misuse in the correctional facility. *J. Correct. Health Care* 19, 211–217. [PubMed: 23788587]
- (5). Yoon G, and Westermeyer J (2013) Intranasal bupropion abuse case report. *Am. J. Addict. Psychiat* 22, 180.

- (6). Steele LS, Macdonald EM, Gomes T, Hollands S, Paterson JM, Mamdani MM, and Juurlink DN (2015) Rates of anomalous bupropion prescriptions in Ontario, Canada. *Ann. Fam. Med* 13, 343–346. [PubMed: 26195679]
- (7). Baribeau D, and Araki KF (2013) Intravenous bupropion: a previously undocumented method of abuse of a commonly prescribed antidepressant agent. *J. Addict. Med* 7, 216–217. [PubMed: 23519045]
- (8). Butz RF, Schroeder DH, Welch RM, Mehta NB, Phillips AP, and Findlay JW (1981) Radioimmunoassay and pharmacokinetic profile of bupropion in the dog. *J. Pharmacol. Exp. Ther* 217, 602–610. [PubMed: 6785419]
- (9). Barney CL, Huber EW, and McCarthy JR (1990) A convenient synthesis of hindered amines and α -trifluoromethylamines from ketones. *Tetrahedron Lett.* 31, 5547–5550.
- (10). Carroll FI, Blough BE, Abraham P, Mills AC, Holleman JA, Wolckenhauer SA, Decker AM, Landavazo A, McElroy KT, Navarro HA, Gatch MB, and Forster MJ (2009) Synthesis and biological evaluation of bupropion analogues as potential pharmacotherapies for cocaine addiction. *J. Med. Chem* 52, 6768–6781. [PubMed: 19821577]
- (11). Blough BE, Landavazo A, Partilla JS, Baumann MH, Decker AM, Page KM, and Rothman RB (2014) Hybrid dopamine uptake blocker-serotonin releaser ligands: a new twist on transporter-focused therapeutics. *ACS Med. Chem. Lett* 5, 623–627. [PubMed: 24944732]
- (12). Pulici M, Quartieri F, and Felder ER (2005) Trifluoroacetic anhydride-mediated solid-phase version of the Robinson-Gabriel synthesis of oxazoles. *J. Comb. Chem* 7, 463–473. [PubMed: 15877475]
- (13). Milena P (1987) The in vitro metabolism of bupropion. *The Third European. Congr. Biopharm. Pharmacokinet. Proc* 2, 313–317.
- (14). Damaj MI, Carroll FI, Eaton JB, Navarro HA, Blough BE, Mirza S, Lukas RJ, and Martin BR (2004) Enantioselective effects of hydroxy metabolites of bupropion on behavior and on function of monoamine transporters and nicotinic receptors. *Mol. Pharmacol* 66, 675–682. [PubMed: 15322260]
- (15). Richelson E, and Pfenning M (1984) Blockade by antidepressants and related compounds of biogenic amine uptake into rat brain synaptosomes: Most antidepressants selectively block norepinephrine uptake. *Eur. J. Pharmacol* 104, 277–286. [PubMed: 6499924]
- (16). Jørgensen S, Nielsen EØ, Peters D, and Dyhring T (2008) Validation of a fluorescence-based high-throughput assay for the measurement of neurotransmitter transporter uptake activity. *J. Neurosci. Methods* 169, 168–176. [PubMed: 18222006]
- (17). Baumann MH, Partilla JS, Lehner KR, Thorndike EB, Hoffman AF, Holy M, Rothman RB, Goldberg SR, Lupica CR, Sitte HH, Brandt SD, Tella SR, Cozzi NV, and Schindler CW (2013) Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive ‘bath salts’ products. *Neuropsychopharmacology* 38, 552–562. [PubMed: 23072836]
- (18). Kolanos R, Partilla JS, Baumann MH, Hutsell BA, Banks ML, Negus SS, and Glennon RA (2015) Stereoselective actions of methylenedioxypyrovalerone (MDPV) to inhibit dopamine and norepinephrine transporters and facilitate intracranial self-stimulation in rats. *ACS Chem. Neurosci* 6, 771–777. [PubMed: 25688761]
- (19). Marusich JA, Antonazzo KR, Wiley JL, Blough BE, Partilla JS, and Baumann MH (2014) Pharmacology of novel synthetic stimulants structurally related to the “bath salts” constituent 3,4-methylenedioxypyrovalerone (MDPV). *Neuropharmacology* 87, 206–213. [PubMed: 24594476]
- (20). Kolanos R, Sakloth F, Jain AD, Partilla JS, Baumann MH, and Glennon RA (2015) Structural modification of the designer stimulant α -pyrrolidinovalerophenone (α -PVP) influences potency at dopamine transporters. *ACS Chem. Neurosci* 6, 1726–1731. [PubMed: 26217965]
- (21). (a) Baumann MH, Ayestas MA Jr., Partilla JS, Sink JR, Shulgin AT, Daley PF, Brandt SD, Rothman RB, Ruoho AE, and Cozzi NV (2012) The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. *Neuropsychopharmacology* 37, 1192–1203. [PubMed: 22169943] (b) Bonano JS, Banks ML, Kolanos R, Sakloth F, Barnier ML, Glennon RA, Cozzi NV, Partilla JS, Baumann MH, and Negus SS (2015) Quantitative structure-activity relationship analysis of the pharmacology of

para-substituted methcathinone analogues. Br. J. Pharmacol 172, 2433–2444. [PubMed: 25438806]

- (22). Kolanos R, Solis E Jr., Sakloth F, De Felice LJ, and Glennon RA (2013) ‘Deconstruction’ of the abused synthetic cathinone methylenedioxypyrovalerone (MDPV) and an examination of effects at the human dopamine transporter. ACS Chem. Neurosci 4, 1524–1529. [PubMed: 24116392]
- (23). Saha K, Partilla JS, Lehner KR, Seddik A, Stockner T, Holy M, Sandtner W, Ecker GF, Sitte HH, and Baumann MH (2015) ‘Second-generation’ mephedrone analogs, 4-MEC and 4-MePPP, differentially affect monoamine transporter function. Neuropsychopharmacology 40, 1321–1331. [PubMed: 25502630]
- (24). Counsell RE, Desai P, Smith TD, Chan PS, Weinhold PA, Rethy VB, and Burke D (1970) Aromatic amino acid hydroxylase inhibitors. 1. Halogenated phenylalanines. J. Med. Chem 13, 1040–1042. [PubMed: 5479837]
- (25). Foley KF, and Cozzi NV (2003) Novel amino-propiofenones as potential antidepressants. Drug Dev. Res 60, 252–260.

Figure 1.

Effects of bupropion analogues on inhibition of [^3H]transmitter uptake via DAT, NET, and SERT in rat brain synaptosomes. Uptake of [^3H]dopamine (DAT assays), [^3H]norepinephrine (NET assays), or [^3H]5-HT (SERT assays) was examined in the absence or presence of test drugs across eight different drug concentrations to construct dose–response curves. Data are mean $\pm$ SD for $N=3$ experiments each performed in triplicate, expressed as percent of maximal uptake. All compounds were examined as their salts; see Methods for specific salts used.

Figure 2.

Effects of bupropion analogues on transporter-mediated release via DAT, NET and SERT in rat brain synaptosomes. Synaptosomes were preloaded with [^3H]MPP $^+$ (DAT and NET assays) or [^3H]5-HT (SERT assays) to achieve steady state. Preloaded synaptosomes were incubated in the absence or presence of test drugs across 8 different concentrations to construct dose–response curves. Data are mean $\pm$ SD for $N=3$ experiments performed in triplicate, expressed as a % of maximal release. Analogues producing release responses $<30\%$ of maximum are considered inactive. All compounds were examined as their salts; see Methods for specific salts used.

Figure 3.
Structures of α -PVP (**10**), mephedrone (**11**), and methylene (**12**).

Scheme 1. Synthesis of Compounds 6 and 7^a^aReagents and conditions: (i) CH₃NH₂, room temperature, 2 h; (ii) NaN₃, room temperature, 20 h; (iii) SnCl₂, room temperature, 2 h.

Table 1.

IC₅₀ Values for the Test Agents to Block Uptake and EC₅₀ Values for Test Agents to Act as Releasing Agents, at DAT, NET, and SERT^a

	uptake inhibition IC ₅₀ , nM (mean ± SD)			release EC ₅₀ , nM (mean ± SD)		
	DAT	NET	SERT	DAT	NET	SERT
1	305 (55)	3715 (545)	>10 000	IA ^b	IA	IA
3	938 (130)	2513 (420)	5113 (467)	IA	IA	IA
4	1308 (317)	>10 000	>10 000	IA	IA	IA
5	537 (65)	3288 (434)	3414 (462)	IA	IA	1228 (189)
6	342 (46)	290 (36)	1104 (79)	29 (3)	40 (9)	212 (32)
7	399 (87)	551 (50)	2779 (454)	64 (10)	105 (20)	567 (75)
8(-) ²	357 (46)	224 (37)	>10 000	18 (4)	28 (5)	4741 (1035)

^a All compounds were examined as their salts; see experimental section for specific salts used.

^b IA = Inactive as defined by <30% efficacy in release response.