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Synthetic Cathinone Analogs Structurally Related to the Central Stimulant Methylphenidate as Dopamine Reuptake Inhibitors

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ABSTRACT

Synthetic cathinones are, primarily, stimulant drugs of abuse that act at monoamine transporters (e.g. the dopamine transporter or DAT) as releasing agents or as reuptake inhibitors. In the past few years the emergence of >150 new synthetic cathinones has attracted considerable attention from medical and law enforcement communities. *threo*-Methylphenidate (*t*MP), used clinically for the treatment of ADHD and narcolepsy, is also a DAT reuptake inhibitor. *t*MP is somewhat structurally similar to abused cathinone stimulants and the structure-activity relationships (SAR) of *t*MP have been well defined. Hence, available *t*MP literature might assist in understanding the SAR of synthetic cathinones, about which less is known. In the present study we synthesized and examined eight 2-benzoylpiperidine analogs (**4**, **6**-**12**) to determine if *t*MP SAR might be applicable to cathinone SAR. The benzoylpiperidine analogs were evaluated in a competition assay using live-cell imaging against APP^+ in HEK293 cells stably expressing hDAT and in cells co-expressing DAT and voltage-gated Ca^{2+} channels. All compounds were found to be DAT reuptake inhibitors and a significant correlation was obtained between the potency of the benzoylpiperidines and *t*MP binding data (r = 0.91) suggesting that the SAR of *t*MP analogs might be directly applicable to certain synthetic cathinones as DAT reuptake inhibitors.

KEYWORDS: Structure-activity relationships, DAT reuptake inhibitors, α-PVP, drugs of abuse, synthetics cathinones, NPS

INTRODUCTION

Methylphenidate, specifically *threo*-methylphenidate (*t*MP; **1**), is an FDA-approved U.S.

Schedule II drug commonly prescribed to treat attention-deficit hyperactivity disorder

(ADHD) in children and narcolepsy in adults.[1,2] Low methylphenidate doses appear to be

responsible for its clinical effect in the treatment of ADHD, which primarily involves

reuptake inhibition at the norepinephrine transporter.[3-5] Methylphenidate is a recognized

central stimulant with a mechanism of action somewhat resembling that of cocaine.[6,7]

Although cocaine is a reuptake inhibitor at all three monoamine transporters (i.e., the

dopamine, norepinephrine, and serotonin transporters DAT, NET, and SERT,

respectively), *t*MP (**1**) has little to no effect at SERT.[8,9] Reuptake inhibition by *t*MP (**1**)

and cocaine at DAT is believed to be associated with their central stimulant properties

and abuse liability.[3,10] The structure-activity (SAR) and structure-affinity (SAFIR)

relationships of methylphenidate analogs for their ability to block DAT reuptake and bind

at DAT, respectively, have been extensively investigated (for example, see references 7

and 11). Found, with respect to the β-substituent, is that the methyl ester of *t*MP (**1**) is

not essential for its actions and can be replaced with other β-substituents (e.g. $-CH_2-OH$

and $-CH_2-OCH_3$).[11] However, the β-methyl ester of **1** was found optimal for DAT

transporter affinity and DAT reuptake action amongst a series of β-substituted *t*MP (**1**)-

related analogs.[7,11,12]

1   **2**   **3**   **4**   **5**
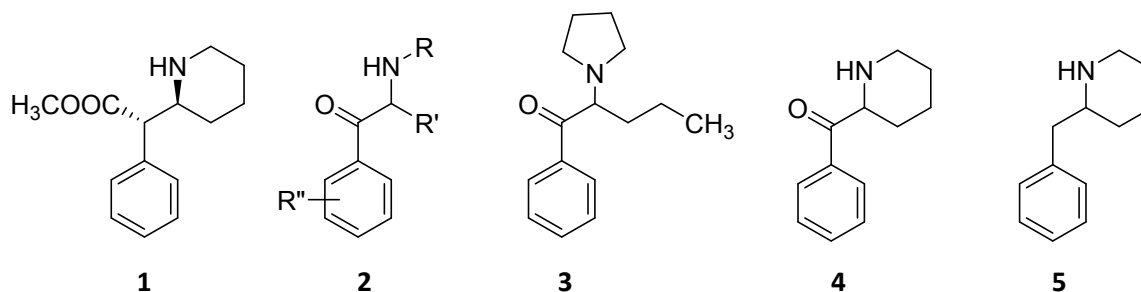
The phenylalkylamine methylphenidate has been well documented as an abused central stimulant. Interestingly, *t*MP (**1**) is remotely structurally related to another novel series of abused phenylalkylamine stimulants derived from cathinone (**2**, R=R"=H, R'=CH$_3$). Cathinone is a naturally occurring constituent of the shrub *Catha edulis*, and >150 synthetic cathinone analogs have been identified on the clandestine market as abused substances.[13] *Simple synthetic cathinones*, that is, cathinone analogs where R and R' = –H or –CH$_3$, typically act as releasing agents at DAT, NET and/or SERT (potency and selectivity dictated to some extent by the nature of the aryl (or R") substituent.[14] In contrast, when cathinone analogs bear a tertiary amine or a bulky secondary amine, and/or an extended α-(i.e., R')-substituent, these more *complex synthetic cathinones* generally act as selective DAT/NET reuptake inhibitors.[13] An example of the latter is α-pyrolidinovalerophenone (α-PVP, *flakka*; **3** – currently, a U.S. Schedule I controlled substance).[15,16]

Benzoylpiperidine **4** is a novel cathinone-related analog (i.e., a methylphenidate/cathinone hybrid, or a methylphenidate analog bearing a β-keto rather than a β-methyl ester function) that has the extended side chain of α-PVP (**3**) ligated to the terminal amine [i.e., **4** is a secondary amine with a bulky (i.e., R > –CH$_3$) terminal

amine substituent and an extended α- or R'-side chain]. As such, if **4** was to be active at DAT, it would be expected to behave as a reuptake inhibitor. Subsequently, we prepared **4** and found, in a preliminary electrophysiological study at a single concentration of 10 μM, that **4** behaved as a DAT reuptake inhibitor (i.e., it produced hyperpolarization; unpublished data). This prompted the current study.

Much is known about the SAR and SAFIR of methylphenidate (i.e., **1**) analogs.[7,11] Less is known about the SAR of synthetic cathinone (i.e., **2**) analogs.[13] Yet, *t*MP (**1**) and **4** bear some structural similarity, both behave as DAT reuptake inhibitors, and it is known that the methyl ester of **1**, although optimal, is not essential for its DAT reuptake action (*vide supra*). Can methylphenidate SAR/SAFIR be utilized to inform/forecast the SAR of synthetic cathinones? If so, this would assist the identification and potential Scheduling of novel synthetic cathinones that have yet to be prepared or that might eventually be found on the "street". Thus, we prepared a small series of analogs **4** (i.e., **6**-**12**) with various aryl substituents common to known *t*MP (**1**) analogs already reported in the literature. In addition, we prepared and examined the *des*-keto analog of **4** (i.e., **5**) to determine if the keto group of **4** (or if the ester function of **1**) is important for DAT transporter activity. The compounds were examined as DAT reuptake inhibitors competing the uptake of the fluorescent non-selective DAT substrate APP[+] as previously reported.[17] Additional testing confirmed that none behaved as releasing agents; all behaved as DAT reuptake inhibitors.

RESULTS AND DISCUSSION

Synthesis

The parent compound **4** was prepared by reducing 2-benzoylpyridine (**13**) to the alcohol

14 as previously reported.[7] Intermediate **14** was then oxidized using Jones reagent as

outlined in Scheme 1. Further reduction of intermediate **14** in the presence of perchloric

acid gave compound **5** in quantitative yields.

Scheme 1. Synthesis of compounds **4** and **5**.[a]

13   **14**   **4**

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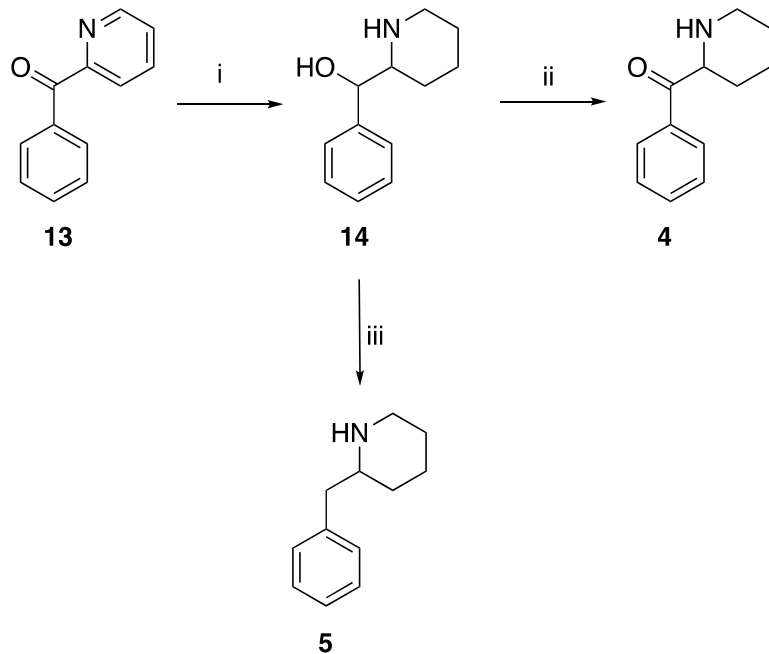
[a]Reagents and conditions: (i) AcOH, 5% Pt/C, 30-40 psi, rt, 6h; (ii) Jones reagent, 0 ^{0}C, 1h, rt, 18h (iii) AcOH, HClO$_4$, 5% Pt/C, 50-55 psi, rt, 72h.

Compounds **6**-**9** were prepared using a one-pot Friedel-Crafts acylation reaction

(Scheme 2). Compounds **6** and **7** were prepared in one step with *N*-Boc-pipecolic acid

(**15**) by reaction with toluene and ethylbenzene, respectively.

For compounds **8** and **9**, pipecolic acid (**16**) was protected with a formyl group using acetic formic anhydride. Reaction of **17** for the *in situ* formation of the acid chloride was accomplished using thionyl chloride, but the reaction mixture quickly turned to a black solid. IR spectra were obtained on the solid and did not show the presence of the acid chloride peak suggesting that the reaction had failed. Phosphorus trichloride was then used as the chlorinating reagent instead of thionyl chloride which resulted in the successful generation of the acid chlorides *in situ*, which were subsequently converted to **8** and **9** via Friedel-Crafts acylation with chlorobenzene and bromobenzene respectively.

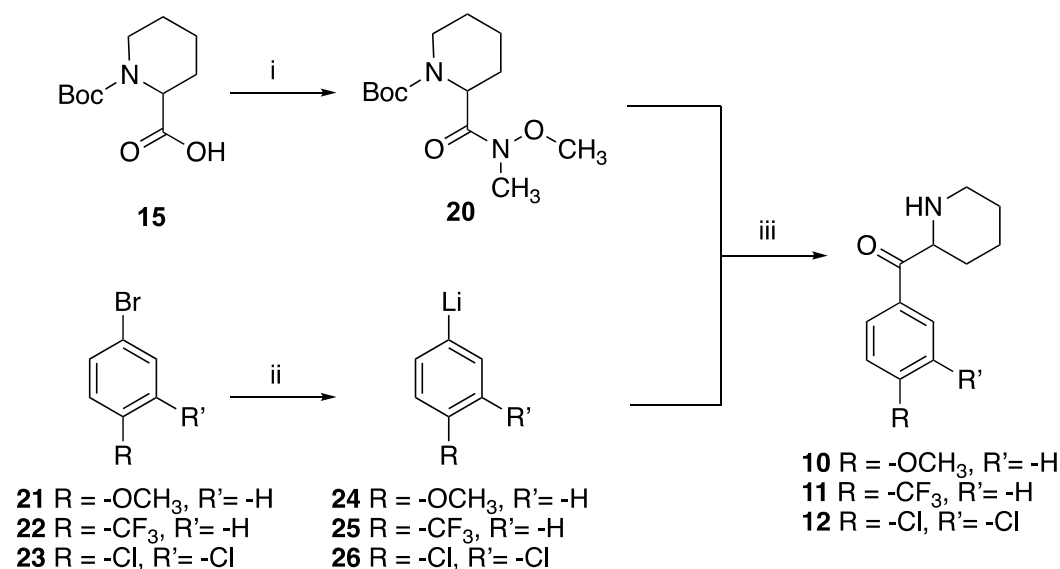**Scheme 2.** Friedel-Crafts acylation for the synthesis of compounds **6-9**.[a]
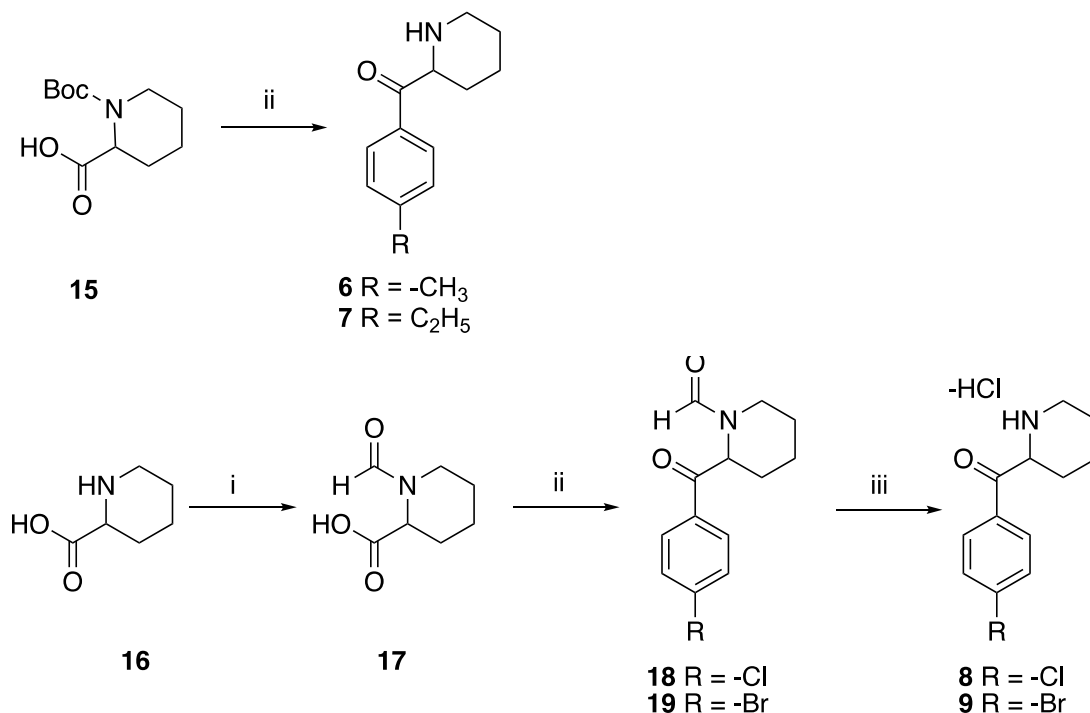
^{a}Reagents and conditions: (i) $(CH_3CO_2)_2O$, HCOOH, rt, 1h; (ii) (a) *p*-substituted benzene, PCl_3, 60 ^{0}C, 2h, (b) $AlCl_3$, rt, 16-18h; (iii) HCl/EtOH, reflux, 3h.

The reaction of *N*-Boc-pipecolic acid (**15**) with *N,O*-dimethylhydroxylamine

hydrochloride, TEA, and a coupling agent (benzotriazol-1-yloxy)tris(dimethylamino)-

phosphonium hexafluorophosphate (BOP) gave intermediate **20** as described in the

literature.[2] The organolithium reagents (**24**, **25**) were prepared using the appropriately

p-substituted bromobenzene and *n*-BuLi. The obtained organolithium reagent was

added to intermediate **20** at –23 °C resulting is compounds **10** and **11** (Scheme 3).

Compound **12** was synthesized in a manner similar to that shown in Scheme 3 using 1-

bromo-3,4-dichlorobenzene and *n*-BuLi to obtain the organolithium intermediate **26**

which was then reacted with **20**.

Scheme 3. Synthesis of compounds **10**-**12**.[a]

21 R = -OCH$_3$, R'= -H
22 R = -CF$_3$, R'= -H
23 R = -Cl, R'= -Cl

24 R = -OCH$_3$, R'= -H
25 R = -CF$_3$, R'= -H
26 R = -Cl, R'= -Cl

10 R = -OCH$_3$, R'= -H
11 R = -CF$_3$, R'= -H
12 R = -Cl, R'= -Cl

[a]Reagents and conditions: (i) *N,O*-dimethylhydroxylamine hydrochloride, TEA, BOP, rt, 3h; (ii) 2.5 M *n*-BuLi in hexane, -40 °C, 3h; (iii) Et$_2$O, -23 °C.

Activity at the dopamine transporter

APP$^+$ uptake assays were performed in FlpIn TRex HEK293 cells stably expressing the human dopamine transporter (hDAT).[18] Compounds **4**-**12**, *t*MP (**1**), and cocaine (as comparator) were tested in the assay. All compounds inhibited APP$^+$ uptake in a dose-dependent manner (Figure 1) and their potencies are listed in Table 1. In these experiments, inhibition of APP$^+$ uptake indicates activity at DAT, but it cannot discriminate whether a test compound works as substrate or as an uptake inhibitor. Given the structural similarity of these compounds with *t*MP, they are, presumably, uptake inhibitors. To assess this presumption, experiments were performed to test the electrical effect of these compounds in cells co-expressing DAT and voltage-gated Ca^{2+} channels. It is well accepted that substrate transport through DAT is associated with inward electrical currents that depolarize the plasma membrane.[19] Uptake inhibitors, on the other hand, although they interact with the transporter, cannot induce the structural transitions that activate this depolarizing conductance.[19,20] As shown previously, voltage-gated Ca^{2+} channels are excellent sensors of membrane depolarization that can be used as sensitive biosensors to detect substrate activity at DAT.[18,21] In this experimental setting, substrates of DAT (e.g. DA) produce fast and reversible Ca^{2+} signals that can be measured using fluorescence microscopy, and inhibitors do not produce such responses, but can interfere with the substrate-induced signal if applied together (Figure 2). None of the compounds (i.e., **4**-**11**) produced Ca^{2+} signals when perfused alone, strongly suggesting that all are uptake inhibitors (data not shown).

Table 1. Dopamine transporter reuptake inhibition by compounds **4-12,** *t*MP (**1**), with cocaine as reference.

4, 6-12   5
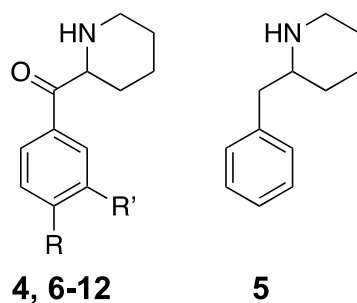
Ligand	-R	-R'	IC$_{50}$ (nM) ± S.E.M.	pIC$_{50}$
4	-H	-H	1080 ± 190	5.96
5	-	-	3780 ± 1200	5.42
6	-CH$_3$	-H	380 ± 80	6.42
7	-C$_2$H$_5$	-H	6900 ± 1900	5.16
8	-Cl	-H	520 ± 60	6.28
9	-Br	-H	590 ± 100	6.22
10	-OCH$_3$	-H	2340 ± 400	5.63
11	-CF$_3$	-H	14300 ± 4000	4.84
12	-Cl	-Cl	47 ± 6	7.32
*t*MP (**1**)			72 ± 10	7.14
Cocaine			170 ± 20	6.76

Figure 1 shows the dose response curves of the compounds for their ability to inhibit

DAT-mediated uptake in cells expressing hDAT. All the compounds were found to be

DAT reuptake inhibitors with the dichloro compound **12** being most potent (IC$_{50}$ = 47

nM; Table 1) and the trifluoromethyl compound **11** being least potent (IC_{50} = 14,300 nM).
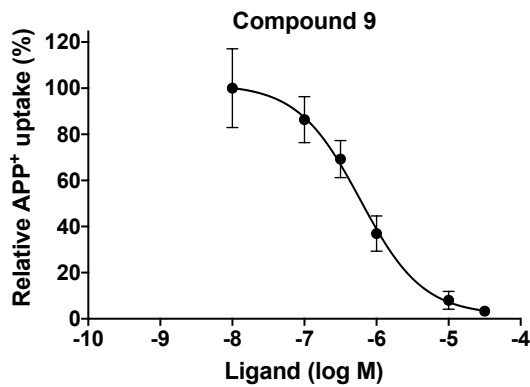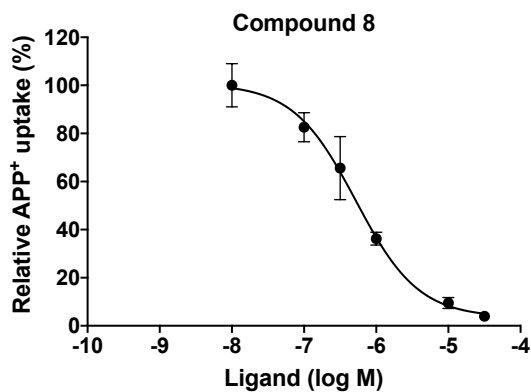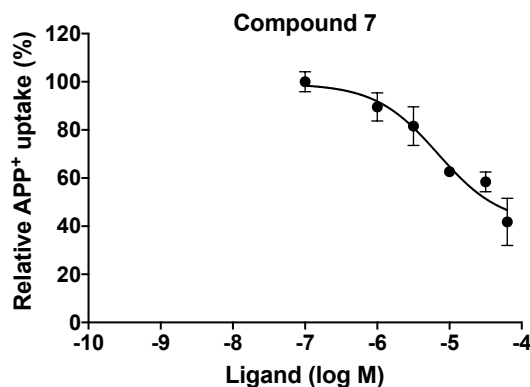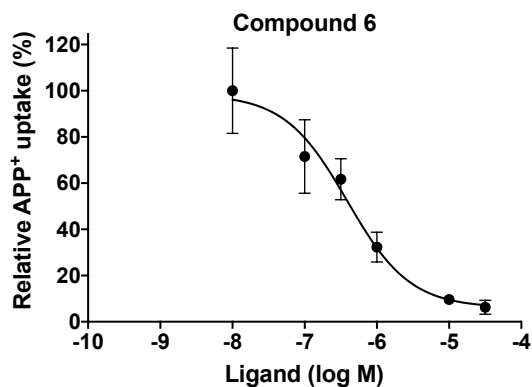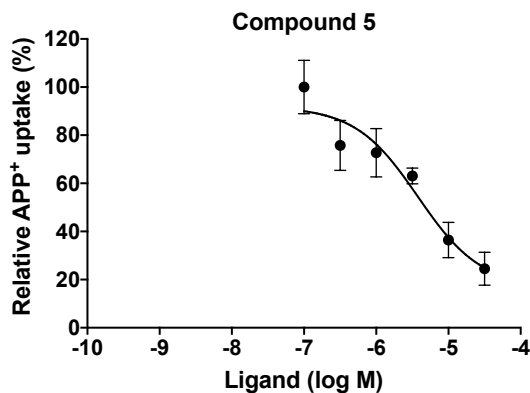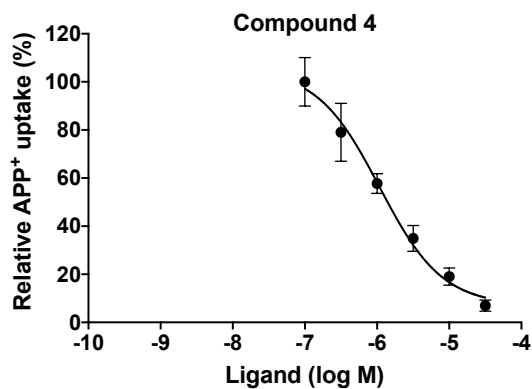
Figure 1. APP+ uptake assay curves of the hybrid compounds, tMP (**1**), and cocaine for comparison in HEK293 cells stably transfected with hDAT.

Figure 2. The depolarization induced by hDAT activation, electrically coupled to Ca$_v$1.2. Hybrid compounds did not produce a depolarization current when perfused alone.
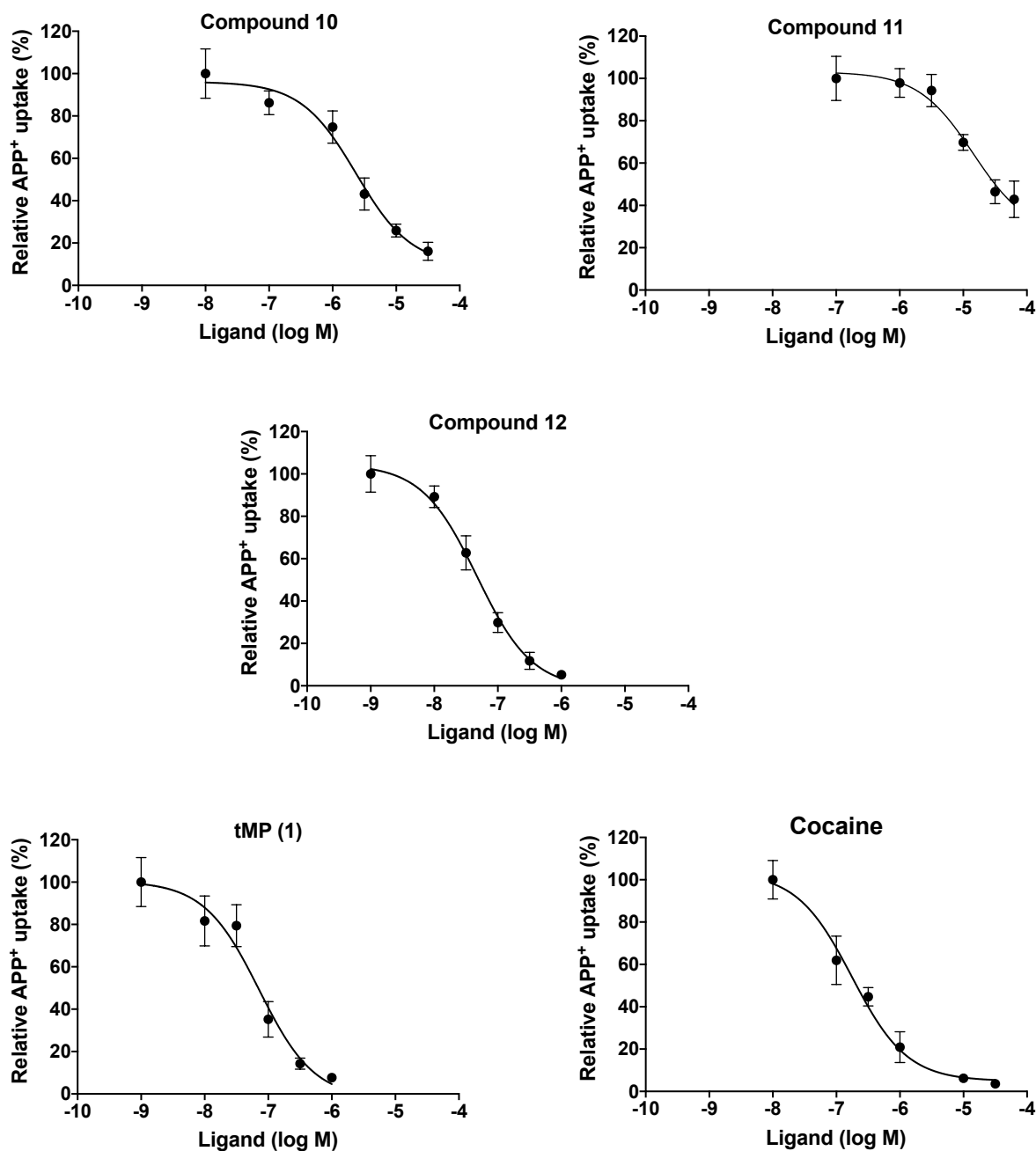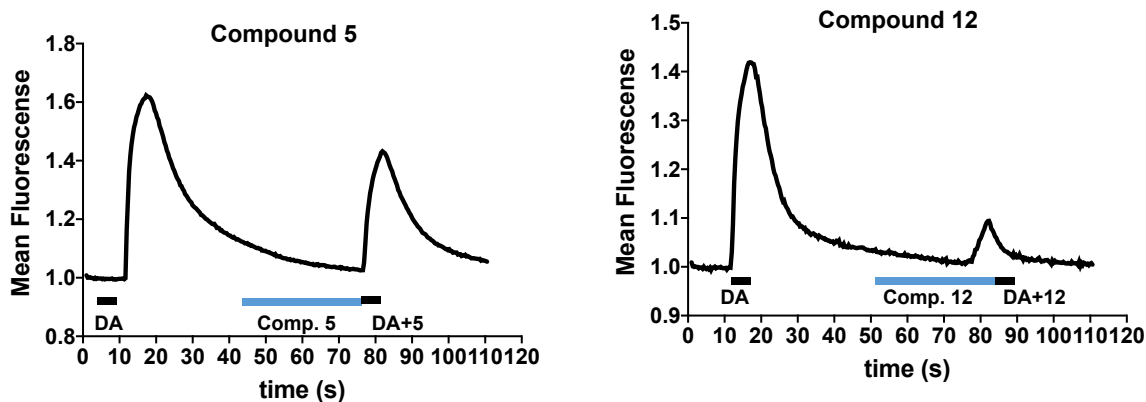
Cathinone analogs bearing a bulky terminal amine and/or an extended α-side chain have been demonstrated to act as reuptake inhibitors at the dopamine transporter.[13,22] Due to their structural similarity to *t*MP (**1**), a series of cathinone-related benzoylpiperidines (i.e., **4**, **6**-**12**) was prepared and examined. The specific aryl substituents were selected because their corresponding *t*MP analogs already had been studied[7,11] and displayed a range in potencies from the potent 3,4-dichloro analog of *t*MP to the less potent 4-trifluoromethyl analog. All the present compounds behaved as DAT reuptake inhibitors, although none, with the exception of the dichloro analog **12,** was more potent than *t*MP (**1**).

It has been reported that the methyl ester of the *t*MP (**1**) can be replaced with an amide, hydroxymethyl, or methoxymethyl group.[11] Compound **5** has also, apparently, been previously prepared and examined as a DAT reuptake inhibitor by Kim et al.[6] However, there is a potential problem. Although Kim et al.[6] showed the correct chemical structure for 2-benzylpiperidine, their experimental write-up suggests they might have

inadvertently prepared 2-phenylpiperidine. Furthermore, their melting point for the target is different from that previously reported by others for the target compound.[23,24] Obviously, apart from the different melting point, this might have been a typographical error. Nevertheless, given this anomaly, we prepared and examined 2-benzylpiperidine (**5**). Here, we showed that the carbonyl oxygen atom of **4** can be removed altogether (i.e., the carbonyl group can be replaced with a methylene function; **5**) with <4-fold decreased potency. As such, neither the carbonyl oxygen atom of benzoylpiperidine **4** (i.e., **5**), nor the ester function of methylphenidate, is requisite for these compounds to behave as dopamine reuptake inhibitors. Nevertheless, for these analogs, the methyl ester function of *t*MP (**1**) would seem optimal for DAT reuptake inhibition as previously concluded by Deutsch and co-workers.[11]

A comparison was made between DAT reuptake functional potency[7] and radioligand binding data[11] for 25 *t*MP (2-, 3-, and 4-substituted) analogs (see Figure S1 in Supporting Information), and a significant correlation was obtained (r = 0.98) between the two parameters indicating that the potency of these analogs as DAT reuptake inhibitors parallels their affinity for the transporter. This allowed us to compare the literature binding data for *t*MP analogs with the functional data we obtained in the APP[+] uptake assay for the corresponding benzoylpiperidines. For the eight benzoylpiperidines examined, there was a significant relationship between their potencies and the DAT affinity of their corresponding *t*MP analogs (r = 0.91) (Figure 3).
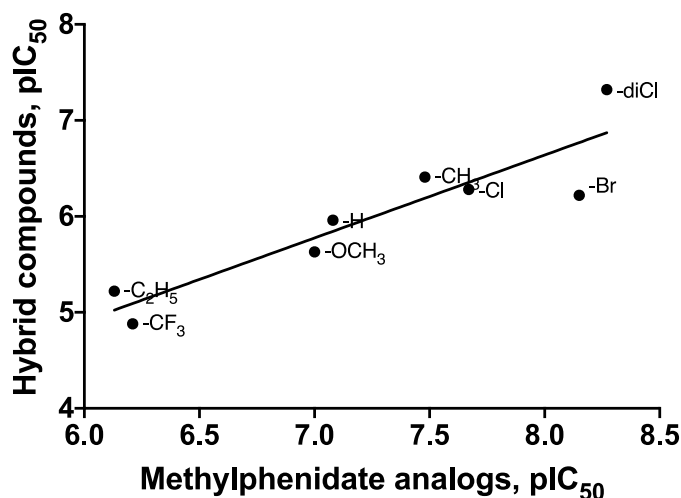
Figure 3. Correlation between the binding data of *t*MP analogs[11] (X-axis) and APP$^+$ uptake assay data (Y-axis) for the corresponding benzoylpiperidines (r = 0.91, n = 8).

The present findings show that the 2-benzoylpiperidines (i.e., MP/cathinone hybrids) investigated here act as DAT reuptake inhibitors, that the carbonyl oxygen atom is not required for this action (although the carbonyl group or its corresponding methyl ester contribute to activity), and suggest that the SAR of *t*MP analogs might be applied to the present synthetic cathinone-related compounds as DAT reuptake inhibitors.

Methods

Synthesis:

Melting points were measured in Thomas-Hoover or MEL TEMP apparatus using glass capillaries. The compounds were characterized by ^{1}H NMR, mass spectrometry (MS), and IR spectroscopy. ^{1}H NMR spectra were recorded using a Bruker AXR 400 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. IR spectra were recorded using a Thermo Nicolet iS10 FT-IR and MS was recorded using a Waters

Acquity tandem quadrupole (TQD) instrument with electrospray ionization. Reactions

were monitored by thin-layer chromatography (TLC) using silica gel GHLF plates (250

mm, 2.5 x 10 cm; Analtech Inc. Newark, DE) and flash chromatography was performed

on a CombiFlash Companion/TS (Teledyne Isco Inc. Lincoln, NE). All final compounds

were prepared as their water soluble hydrochloride salts. The purity of the novel

compounds was determined by elemental analysis for C, H and N (Atlantic Micolab Inc.;

Norcross, GA) and the results were within 0.4% of the calculated values. *threo*-

Methylphenidate and cocaine as their HCl salts were purchased from Sigma-Aldrich and

used as supplied. Compounds **17**[25] and **20**[2] as their HCl salts were prepared according

to literature procedures.

2-Benzoylpiperidine hydrochloride (4). Jones reagent (1.78 mL, 4.47 mmol) prepared

from chromium(VI) oxide (2.50 g), concentrated H_2SO_4 (2.50 mL), and H_2O (7.50 mL)

was added in a dropwise manner to a solution of **14** in a mixture of acetone (10 mL) and

H_2O (2 mL) at 0 °C. The reaction mixture was allowed to stir at 0 °C for 1 h and then at

room temperature for 18 h. The reaction mixture was basified by addition of saturated

solution of $NaHCO_3$. The aqueous portion was extracted with EtOAc (3 x 30 mL) and the

combined organic portion was dried (Na_2SO_4), and then evaporated to dryness under

reduced pressure to yield a yellow solid. The solid was dissolved in Et_2O and HCl gas

was allowed to slowly bubble through the solution yielding a white solid. The solid was

collected by filtration and recrystallized from *i*-PrOH to yield 0.11 g (16%) of **4** as a white

solid: mp 220-221 °C (lit.[26] mp 225-227 °C). IR (diamond, cm^{-1}): 1685 (C=O). ^{1}H NMR

(DMSO-d_6) δ: 1.44-1.47 (m, 1H, CH), 1.50-1.77 (m, 4H, 2 X CH_2), 1.99-2.18 (m, 1H,

CH), 2.34-2.40 (m, 1H, CH), 3.18-3.24 (m, 1H, CH), 4.92-5.12 (m, 1H, CH), 7.59 (t, 2H,

J = 7.9 Hz, Ar-H), 7.72 (m, 1H, J = 8.5 Hz, Ar-H), 8.05 (dd, J = 7.0 Hz, 2H, ArH), 9.98

(br s, 2H, NH^+).

2-Benzylpiperidine hydrochloride (5). To a solution of **14** (0.88 g, 4.68 mmol) in

AcOH (40 mL) and perchloric acid (2 mL), 5% Pt/C (0.35 g) was added. The mixture

was treated with H_2 gas on a Parr hydrogenator at 50-55 psi for 72 h. The reaction

mixture was filtered through celite and evaporated to dryness to yield a yellow oil. The

oily residue was basified with NaOH (3M, to pH ~ 12) and extracted with methylene

chloride (3 x 50 mL). The combined organic portion was dried (Na_2SO_4) and evaporated

to dryness under reduced pressure to yield a white solid. The solid was dissolved in

Et_2O and HCl gas was allowed to slowly bubble through the solution yielding a white

solid. The solid obtained was filtered and dried to give a solid which upon

recrystallization from i-PrOH yielded 0.45 g (45%) of compound **5** as a white solid: mp

135-136 °C (lit.[23] mp 130-135 °C). ^{1}H NMR (DMSO-d_6) δ: 1.08-1.85 (m, 6H, 3 x CH_2),

2.36-2.83 (m, 4H, 2 X CH_2), 3.27-3.34 (m, 1H, CH), 7.14 (t, 2H, J = 6.0 Hz, Ar-H), 7.20

(m, 1H, J = 7.2 Hz, Ar-H), 7.37 (dd, J = 8.5 Hz, 2H, ArH).

2-(4-Methylbenzoyl)piperidine hydrochloride (6). In a 2-neck flask, PCl_3 (1.31 g, 9.59

mmol) was added to a solution of N-Boc-dl-pipecolic acid (2 g, 8.72 mmol) in anhydrous

toluene (50 mL) under an N_2 atmosphere and the reaction mixture was stirred for 2 h at

60 °C. Aluminum trichloride (3.48 g, 26.16 mmol) was added portionwise at 0 °C and

the mixture was allowed to stir at room temperature overnight. The reaction mixture was

quenched by careful pouring into ice-cold H_2O (50 mL) and extracted with EtOAc (50

mL). The aqueous portion was basified with NaOH (3 M, to pH 12), and extracted with

EtOAc (3 x 50 mL). The combined organic portion was dried (Na_2SO_4), filtered, and the

filtrate evaporated to dryness under reduced pressure to yield a yellow oil. The oily

residue was dissolved in Et_2O and converted to the HCl salt by the addition of a

saturated solution of HCl(g) in Et_2O. The solid material was collected by filtration and

recrystallized from EtOH/Et_2O to give 0.49 g (23%) of compound **6** as a white solid: mp

258-261 °C. ^1H NMR (DMSO-d_6) δ: 1.29-1.55 (m, 1H, CH), 1.56-1.90 (m, 4H, 2 X CH_2),

1.95-2.20 (m, 1H, CH), 2.42 (s, 3H, CH_3), 2.79-3.10 (m, 1H, CH), 3.27-3.51 (m, 1H,

CH), 4.79-5.27 (m, 1H, CH), 7.43 (d, 2H, J = 8.0 Hz, Ar-H), 7.96 (d, 2H, J = 8.2 Hz, Ar-

H), 8.87 (br s, 1H, NH), 9.36 (br s, 1H, NH^+); IR (diamond, cm^{-1}): 1677 (C=O), 3453

(NH). Anal. Calcd ($C_{13}H_{17}NO \cdot HCl \cdot 0.1\ H_2O$) C, 64.64; H, 7.59; N, 5.79. Found C,

64.61; H, 7.60; N, 5.84.

2-(4-Ethylbenzoyl)piperidine hydrochloride (7). Compound **7** was synthesized

following the procedure for compound **6** and utilized ethylbenzene instead of toluene as

the starting material. Compound **7** (14%) was obtained as a white solid. mp 238-240 °C;

^1H NMR (DMSO-d_6) δ: 1.07-1.12 (m, 3H, CH_3), 1.20-1.23 (m, 2H, CH_2), 1.45-1.47 (m,

1H, CH), 1.49-1.77 (m, 4H, 2 X CH_2), 2.08-2.12 (m, 1H, CH), 2.96-2.99 (m, 2H, CH_2),

5.05-5.11 (m, 1H, CH), 7.46 (d, 2H, J = 8.6 Hz, Ar-H), 7.98 (d, 2H, J = 8.2 Hz, Ar-H),

8.87 (br s, 1H, NH), 9.37 (br s, 1H, NH^+); IR (diamond, cm^{-1}): 1668 (C=O), 3026 (NH).

Anal. Calcd for ($C_{14}H_{19}NO \cdot HCl \cdot 0.1\ H_2O$) C, 66.26; H, 7.94; N, 5.52. Found: C, 65.07;

H, 7.87; N, 5.63.

2-(4-Chlorobenzoyl)piperidine hydrochloride (8). In a 3-neck flask, PCl_3 (0.69 g, 5.03 mmol) was added to a solution of **17**[25] (0.71 g, 4.57 mmol) in chlorobenzene (50 mL) under an N_2 atmosphere and the reaction mixture was stirred for 2 h at 60 °C. Aluminum trichloride (1.82 g, 13.72 mmol) was added portionwise at 0 °C and the reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was quenched by carefully pouring into ice-cold H_2O (50 mL) and washed with EtOAc. The aqueous portion was basified with NaOH (3 M, to pH 12) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic portion was washed with brine (30 mL), dried (Na_2SO_4), filtered, and then evaporated to dryness under reduced pressure to yield a crude residue which was purified by column chromatography (silica gel; hexane/EtOAc; 10:0 to 5:5) to afford 0.25 g (21%) of compound **18** as a yellow oil; ^1H NMR ($CDCl_3$) δ: 1.20-1.90 (m, 4H, 2 X CH_2), 1.94-2.07 (m, 2H, CH_2), 3.15-3.25 (m, 1H, CH), 3.59-3.70 (m, 1H, CH), 5.70-5.80 (m, 1H, CH), 7.61 (d, 2H, J = 2.0 Hz, Ar-H), 7.94 (d, 2H, J = 4.8 Hz, Ar-H), 8.1 (s, 1H, H); IR (diamond, cm^{-1}): 1660 (C=O).

Compound **18** in EtOH (2 mL) and HCl (3 N, 2 mL) was heated at reflux for 3 h, cooled to room temperature and evaporated to dryness under reduced pressure to give 0.26 g (21%) of a crude white solid which was recrystallized from *i*-PrOH to give 0.03 g (11%) **of** compound **8** as a white solid: mp 282-284 °C. ^1H NMR ($CDCl_3$) δ: 1.33-1.38 (m, 1H, CH), 1.89-2.07 (m, 4H, 2 X CH_2), 2.48-2.54 (m, 1H, CH), 3.06-3.17 (m, 1H, CH), 3.60-3.51 (m, 1H, CH), 4.88-4.78 (m, 1H, CH), 7.38 (d, 2H, J = 8.1 Hz, Ar-H), 7.79 (d, 2H, J = 8.1 Hz, Ar-H), 9.20 (br s, 1H, NH), 10.54 (br s, 1H, NH^+); IR (diamond, cm^{-1}): 1681

(C=O). Anal. Calcd for (C$_{12}$H$_{14}$ClNO · HCl) C, 55.40; H, 5.81; N, 5.38. Found: C, 55.10;

H, 5.91; N, 5.42.

2-(4-Bromobenzoyl)piperidine hydrochloride (9). Compound **9** was synthesized

following the procedure for compound **8**, using bromobenzene as a starting material, to

give compound **19** as a yellow oil. The oily residue was heated at reflux in EtOH (2 mL)

and HCl (3 N, 2 mL) for 3 h, cooled to room temperature and the solution evaporated to

dryness under reduced pressure to give a crude white solid which was later

recrystallized from *i*-PrOH to give 0.12 g (13%) of compound **9** as a white solid: mp 250-

252 °C. ^{1}H NMR (DMSO-d_6) δ: 1.06-2.03 (m, 4H, 2 X CH$_2$), 2.18-2.39 (m, 2H, CH),

3.01-3.21 (m, 1H, CH), 3.43-3.60 (m, 1H, CH), 4.85-5.95 (m, 1H, CH), 7.74 (d, 2H, *J* =

7.0 Hz, Ar-H), 7.88 (d, 2H, *J* = 7.8 Hz, Ar-H), 8.76 (br s, 1H, NH), 9.45 (br s, 1H, NH$^+$);

IR (diamond, cm^{-1}): 1678 (C=O), 3456 (NH). Anal. Calcd for (C$_{12}$H$_{14}$BrNO · HCl) C,

47.31; H, 4.96; N, 4.59. Found: C, 47.52; H, 5.08; N, 4.60.

2-(4-Methoxybenzoyl)piperidine hydrochloride (10). Using a 3-neck flask, 4-

bromoanisole (**21**) (0.54 g, 2.93 mmol) was stirred in anhydrous Et$_2$O (5 mL) and cooled

to -78 °C. To the reaction mixture, 2.5 M solution of *n*-BuLi in hexane (2.33 mL, 5.84

mmol) was carefully added in a dropwise manner and the reaction mixture was warmed

to -40 °C and stirred for 3 h to give intermediate **24**. In another flask, a solution of **20**[2]

(0.80 g, 2.93 mmol) in anhydrous ether (10 mL) was brought to -23 °C under an N$_2$

atmosphere, and compound **24** was added dropwise via syringe over 15 min. The

reaction mixture was allowed to stir at -23 °C for 3 h, warmed to room temperature, and

quenched by carefully pouring into an ice-cold solution of 1M KH_2PO_4 (30 mL). The

aqueous layer was extracted with EtOAc (3 x 30 mL) and the combined organic portion

was dried (Na_2SO_4), filtered, and then evaporated to dryness under reduced pressure to

yield a crude residue which was purified by column chromatography (silica gel;

hexane/EtOAc; 10:0 to 2:8) to afford a yellow oil. The oily residue was stirred in

methanolic HCl overnight and evaporated to dryness to yield a yellow solid which upon

which upon recrystallization from *i*-PrOH yielded 0.03 g (3%) of compound **10** as a white

solid: mp 218-220 °C. ^{1}H NMR (DMSO-d_6) δ: 1.03-1.05 (m, 1H, CH), 1.41-1.49 (m, 4H,

2 X CH_2), 1.78-1.76 (m, 1H, CH), 2.95-2.97 (m, 1H, CH), 3.81-3.89 (m, 1H, CH), 5.03-

5.07 (m, 1H, CH), 7.12-7.14 (d, 2H, *J* = 6.7 Hz, Ar-H), 8.03-8.05 (d, 2H, *J* = 8.8 Hz, Ar-

H), 9.01 (br s, 1H, NH), 9.37 (br s, 1H, NH[+]); IR (diamond, cm[-1]): 1677 (C=O). Anal.

Calcd ($C_{13}H_{17}NO_2 \cdot$ HCl $\cdot$ 0.2 H_2O) C, 61.05; H, 7.09; N, 5.48. Found: C, 59.81; H, 6.76;

N, 5.34.

2-(4-Trifluoromethylbenzoyl)piperidine hydrochloride (11). Using a 3-neck flask, 4-

bromo trifluoromethylbenzene (**21**) (0.29 g, 1.32 mmol) was stirred in anhydrous Et_2O (5

mL) and cooled to -78 °C. To the reaction mixture, a 2.5 M solution of *n*-BuLi in hexane

(1.05 mL, 2.64 mmol) was carefully added in a dropwise manner and the reaction

mixture was warmed to -40 °C and stirred for 3 h to give intermediate **25**. In another

flask a solution of **20**[2] (0.39 g, 1.32 mmol) in anhydrous ether (5 mL) was brought to -23

°C under an N_2 atmosphere, and intermediate **25** was added dropwise via syringe over

15 min. The reaction mixture was allowed to stir at -23 °C for 3 h, warmed to room

temperature, and quenched by carefully pouring into an ice-cold solution of 1M KH_2PO_4

(20 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL) and the combined

organic portion was dried (Na$_2$SO$_4$), filtered, and then evaporated to dryness under

reduced pressure to yield a crude residue which was purified by column

chromatography (silica gel; hexane/EtOAc; 10:0 to 2:8) to afford a yellow oil. The oily

residue was stirred in methanolic HCl overnight and evaporated to dryness to yield a

yellow solid which upon which upon recrystallization from *i*-PrOH yielded 0.12 g (26%)

of compound **11** as a white solid: mp 273-275 °C. ^{1}H NMR (DMSO-d_6) δ: 1.03-1.05 (m,

1H, CH), 1.48-1.51 (m, 4H, 2 X CH$_2$), 1.71-1.73 (m, 1H, CH), 1.81-2.12 (m, 1H, CH),

3.76-3.79 (m, 1H, CH), 5.19-5.22 (m, 1H, CH), 7.99-8.01 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.25-

8.27 (d, 2H, *J* = 8.2 Hz, Ar-H), 9.02 (br s, 1H, NH), 9.54 (br s, 1H, NH$^+$); IR (diamond,

cm^{-1}): 1689 (C=O). Anal. Calcd (C$_{13}$H$_{14}$F$_3$NO · HCl) C, 53.16; H, 5.15; N, 4.77. Found: C,

53.25; H, 5.22; N, 4.78.

2-(3,4-Dichlorobenzoyl)piperidine hydrochloride (12). Using a 3-neck flask, 1-

bromo-3,4-dichlorobenzene (**23**) (0.34 g, 1.52 mmol) was stirred in anhydrous Et$_2$O (5

mL) and cooled to -78 °C. To the reaction mixture, a 2.5 M solution of *n*-BuLi in hexane

(1.21 mL, 3.04 mmol) was carefully added in a dropwise manner and the reaction

mixture was warmed to -40 °C and stirred for 3 h to give intermediate **26**. In another

flask a solution of **20**[2] (0.34 g, 1.26 mmol) in anhydrous ether (5 mL) was brought to -23

°C under an N$_2$ atmosphere, and compound **26** was added dropwise via syringe over 15

min. The reaction mixture was allowed to stir at -23 °C for 3 h, warmed to room

temperature, and quenched by carefully pouring into an ice-cold solution of 1M KH$_2$PO$_4$

(20 mL). The aqueous layer was extracted with EtOAc (3 x 30 mL) and the combined

organic portion was dried (Na_2SO_4), filtered, and then evaporated to dryness under

reduced pressure to yield a crude residue which was purified by column

chromatography (silica gel; hexane/EtOAc; 10:0 to 2:8) to afford a yellow oil. The oily

residue was stirred in methanolic HCl overnight and evaporated to dryness to yield a

yellow solid which upon recrystallization from *i*-PrOH yielded 0.02 g (13%) of compound

12 as a beige solid: mp 273-275 °C. ^{1}H NMR (DMSO-d_6) δ: 1.05-1.09 (m, 1H, CH),

1.50-1.55 (m, 4H, 2 X CH_2), 1.71-1.75 (m, 1H, CH), 1.89-2.10 (m, 1H, CH), 3.71-3.78

(m, 1H, CH), 5.25-5.28 (m, 1H, CH), 7.85-7.88 (d, 1H, *J* = 7.7 Hz, Ar-H), 7.91-7.95 (d,

1H, *J* = 7.9 Hz, Ar-H), 8.30 (s, 1H, Ar-H), 9.02 (br s, 1H, NH), 9.54 (br s, 1H, NH^+); IR

(diamond, cm^{-1}): 1683 (C=O). HRMS (ESI-TOF) *m/z*: $[M + H]^+$ calcd for $C_{12}H_{14}Cl_2NO$,

258.0448; found, 258.0452.

Phenyl (2-piperidinyl)methanol (14). Pt/C (5%, 0.32 g) was added to a solution of 2-

benzoylpyridine (1 g, 5.45 mmol) in AcOH (50 mL). The mixture was treated with H_2 gas

on a Parr hydrogenator at 30-40 psi for 6 h. The reaction mixture was filtered through

celite and the filtrate was evaporated to dryness to yield a yellow oil. The oily residue

was basified with NaOH (3M, to pH ~ 12) and extracted with methylene chloride (3 x 50

mL). The combined organic portion was dried (Na_2SO_4), filtered, and the filtrate

evaporated to dryness under reduced pressure to yield a white solid which upon

recrystallization with *i*-PrOH yielded 0.83 g (80%) of compound **14** as a white solid: mp

135-136 °C (lit.[27] mp 137 °C); ^{1}H NMR (DMSO-d_6) δ: 1.40-1.48 (m, 1H, CH), 1.56-1.81

(m, 4H, 2 X CH_2), 1.91-2.23 (m, 2H, CH_2), 2.39-2.81 (m, 1H, CH), 3.27-3.34 (m, 1H,

CH), 4.71-4.92 (m, 2H, CH$_2$), 7.49 (t, 2H, J = 8.1 Hz, Ar-H), 7.54 (m, 1H, J = 7.5 Hz, Ar-H), 8.1 (dd, J = 7.0 Hz, 2H, ArH); IR (diamond, cm^{-1}) 3267 (OH).

APP$^+$ Uptake Studies:

4-(4-(Dimethylamino)phenyl)-1-methylpyridinium (APP$^+$) acts as a substrate at the monoamine transporters[17] and therefore was used to examine the effects of the compounds on APP$^+$-induced signals (3 μM) at hDAT as previously described.[17] APP$^+$ does not fluoresce on its own but only when it is taken up by cells and undergoes interactions with intracellular components.[17] The fluorescence intensity was measured in FlpIn-TRex HEK293 cells stably expressing hDAT and plated in 96-well imaging plates. Cells were transiently transfected with a DsRED expression plasmid. The expression of DAT was induced using 1 µg/mL doxycycline.[18] An epifluorescence microscope (Olympus IX70) equipped with a monochromator (Polychrome V), digital EMCCD camera (Andor) and a pressurized perfusion system (Automate Scientific) was used to monitor the activity of DAT in live cells which were exposed to APP$^+$ as described previously.[28] The DsRed fluorescent signal was used to find the focal plane of the cell monolayer, and then the APP$^+$ signal was measured using wavelengths of 460 nm for excitation and 540 nm for emission at a 10Hz acquisition rate.[17,28] The imaging solution (IS) used for the experiment consisted of: 130 NaCl, 4 KCl, 1 MgCl$_2$, 2 CaCl$_2$, 5 glucose, 10 Hepes, in mM, and the pH was adjusted to 7.3 using NaOH. Cells were exposed to IS for 10s, then exposed to the test compound for 30s, and then exposed to the test compound plus APP$^+$ for 30s. Each well was exposed to a single concertation of the test compound and control wells without test compound were run every experimental day to

define the 100% APP$^+$ uptake. Cell fluorescence maximal intensities were measured

using Fiji (Image J) 2.0 at the end of the experiment (30 sec of APP$^+$ exposure) for each

well. The results are expressed as % of the APP+ uptake measured in the control

wells, the dose-response curves were plotted using GraphPad Prism 8.0. Each plotted

point corresponds to mean ± SEM of n > 6 wells per concentration.

Intracellular Ca^{2+} determinations:

FlpIn TRex permanently expressing hDAT cells were plated in 96-well imaging plates

and cotransfected with voltage-gated Ca^{2+} channels (Ca$_V$1.2) and EGFP expression

plasmids as described previously.[18,29] Intracellular Ca^{2+} was measured using Fura2-AM

Ca^{2+} indicator, and ratiometric Ca^{2+} determinations were performed using fluorescence

microscopy identical to method described before.[18,29] Experiments were performed

under constant perfusion at ~35 ^{o}C; cells were exposed for 5s to 10 µM DA to have a

positive control signal, then were washed for 30 s and exposed to the test compound at

ten times the IC$_{50}$ concentration measured for APP$^+$ uptake inhibition (Table 1), followed

by exposing the cells for 5s to DA and the test compound combined. Each well was

exposed to a single concentration of test compound, n > 18 cells analyzed per

compound from at least 5 different wells were averaged and plotted. None of the tested

compounds showed Ca^{2+} signals, indicating that all are reuptake inhibitors.

Supporting Information: Supplementary information includes a plot between the

binding data and *t*MP [³H]DA reuptake data of tMP analogs.

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Author Contributions:

BJY conducted the synthesis under the supervision of RAG, and the biological assays

under the supervision of JME. RAG conceived of the project and all coauthors had an

opportunity to contribute to the final manuscript.

ABBREVIATIONS USED

tMP, *threo*-methylphenidate; ADHD, attention deficit hyperactivity disorder; BOP, (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; DA, dopamine; DAT, dopamine transporter; NET, norepinephrine transporter; SERT, serotonin transporter; α-PVP, α-pyrolidinovalerophenone; HEK, human embryonic kidney; TLC, thin layer chromatography; APP$^+$, 4-(4-(dimethylamino)phenyl)-1-methylpyridinium.

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TOC Graphic

Methylphenidate
Analogs

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Synthetic
Cathinones