



Published in final edited form as:

*ACS Chem Neurosci.* 2019 January 16; 10(1): 740–745. doi:10.1021/acschemneuro.8b00524.

## Systematic structure-activity studies on selected 2-, 3-, and 4-monosubstituted synthetic methcathinone analogs as monoamine transporter releasing agents

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### Abstract

Methcathinone analogs are appearing on the clandestine market at a rate nearly out-pacing the ability of investigators to examine them on an individual basis. In order to formulate structure-activity relationship (SAR) generalities, we examined releasing ability of several simple methcathinone analogs at the three monoamine transporters (i.e., the dopamine, norepinephrine, and serotonin transporters, DAT, NET, and SERT, respectively) using in vitro assay methods. The analogs included methcathinone, and 14 other compounds mono-substituted at either the 2-, 3-, or 4-position. In general, a) the 2-substituted analogs were less potent than either the 3- or 4-substituted analogs, b) the 3- and 4-substituted analogs were relatively similar in potency, c) methcathinone was the most selective as a DAT releasing agent, and d) the 3- and 4-CF<sub>3</sub> analogs were the least DAT-selective. For the 15 compounds, there was a significant correlation ( $r > 0.9$ ) between DAT and NET potency suggesting relatively similar structure-activity relationships (at least for the compounds examined here). Several of the compounds have appeared on the clandestine market since our studies were initiated and the present results provide new information on how they might act.

### INTRODUCTION

Over the last several years, >150 methcathinone (**1**) analogs, or “synthetic cathinones” have appeared on the clandestine market as abused substances.<sup>1,2</sup> They vary with respect to their terminal amine substituents, length and nature of their  $\alpha$ -alkyl group, and aryl substitution pattern. In theory, many more synthetic cathinone analogs are possible. Some synthetic cathinones behave, primarily, as central stimulants, and a few have been controlled as U.S. Schedule I substances.<sup>2</sup> Yet, many more remain to be pharmacologically and mechanistically characterized. Their pharmacological actions can vary from agent to agent, and synthetic

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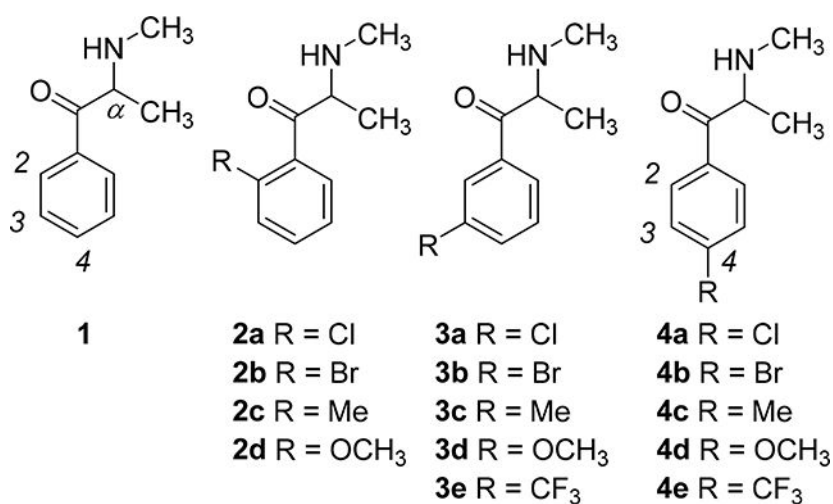
Author contributions

ARS performed the synthesis under the direction of RAG, and DW measured synaptosomal release under the supervision of MHB. RAG prepared the first draft of the manuscript.

The authors declare no competing financial interest.

cathinones cannot be considered as being an homogeneous group of similar-acting agents.<sup>1</sup> Those synthetic cathinones bearing a bulky terminal amine substituent and/or an extended  $\alpha$ -alkyl side chain are fairly selective reuptake inhibitors (i.e., blockers) at membrane dopamine and norepinephrine transporters (DAT and NET, respectively).<sup>Reviewed: 3</sup> Agents with an N-methyl terminal amine and an  $\alpha$ -methyl substituent, termed here as “*simple methcathinone analogs*”, typically behave as DAT and NET releasing agents (i.e., substrates), but some also act as releasing agents at the serotonin transporter (SERT) depending upon the nature of their 4-position aryl substituent.<sup>1,3,4</sup>

The appearance on the illicit market of so many synthetic cathinones is out-pacing the ability of investigators (and law enforcement officials) to examine them on an individual basis. An approach we have previously used to circumvent this problem is to systematically examine small sets of methcathinone analogs that vary with regard to specific substituents at a specific location to formulate structure-activity relationship (SAR) generalities that might be useful to better understand larger groups of agents – including agents that have yet to appear on the “street”. In the present investigation, we further define the SAR of simple N-methyl cathinone or methcathinone analogs (i.e., cathinone analogs that possess an N-methyl and  $\alpha$ -methyl group).



Methcathinone (**1**) itself, as well as a number of other simple methcathinone analogs, act as releasing agents (i.e., as substrates) at DAT and NET.<sup>1-3</sup> However, variation of the aryl 4-position substituent not only influences methcathinone potency, but can shift action from a relatively selective DAT vs SERT releaser, to a much less DAT versus SERT-selective agent, and even to agents with some selectivity for SERT.<sup>4</sup> For example, methcathinone (**1**) displays greater >300-fold selectivity for DAT vs SERT as a releasing agent (see Table 1 for reference), whereas the 4-trifluoromethyl analog of methcathinone (i.e., **4e**) displays 14-fold SERT selectivity.<sup>4</sup> In an attempt to better understand the role of 4-position substituents on potency and selectivity, we conducted a quantitative structure-activity relationship (QSAR) study and found that SERT better accommodates bulky 4-position substituents than does DAT.<sup>5</sup> That is, methcathinone analogs with small 4-position substituents are selective

releasing agents at DAT, but as the size of the 4-position substituent is increased, selectivity shifts in favor of SERT.<sup>5</sup>

Here, we investigate a series of 2- and 3-substituted methcathinone analogs (i.e., **2a-d** and **3a-e**, respectively) to determine their potency and selectivity as releasing agents at DAT and SERT with the intent of conducting a QSAR study much in the same way we examined the 4-substituted analogs.<sup>5</sup> Furthermore, we examined each of the compounds for their actions at NET; we have not previously reported NET release data for **4a-4e**.

## SYNTHESIS

Compounds **1**, **3a**, and **4a-4e** as their water-soluble HCl salts were on-hand from earlier studies<sup>4,6</sup> Some of the products and/or intermediates also have been reported by others, but none of the synthetic targets was prepared as shown in Scheme 1.

Reaction of bromopropiophenones **5a-d** and **6b-d** with *N*-benzyl-*N*-methylamine provided *N*-benzyl analogs **7a-d** and **8b-d**, respectively. Deprotection by treatment with 1-chloroethyl chloroformate afforded targets **2a-d** and **3b-d**. Compound **3e** was prepared in the same manner from the known **8e**. The synthesis of **3b** is described in the Methods section; all other targets were obtained using comparable reaction conditions.

## RESULTS

The potency of methcathinone (**1**) as a releasing agent at DAT and NET was found to be similar ( $EC_{50} = 21\text{--}25$  nM; Table 1), and comparable, to its previously reported potency as a DAT releasing agent.<sup>4</sup> Its potency as a releasing agent at SERT was substantially lower (i.e., by 100- to >300-fold; see Table 1). Representative plots for methcathinone (**1**) and several 3-substituted methcathinone analogs are shown in Supporting Information (Figure S1).

### 4-Substituted Analogs.

DAT and SERT release potencies have been previously reported by us for **1** and **4a-4e**<sup>4</sup> and are reproduced in Table 1 for comparison with the new NET release data. NET potency for these compounds appeared to co-vary with their DAT potency (correlation coefficient  $r > 0.9$ ;  $n = 6$ ).

### 2-Substituted Analogs.

In general, where comparisons could be made, the 2-substituted analogs displayed approximately 2- to 10-fold reduced potency at DAT than their corresponding 4-substituted counterparts (Table 1). Potencies at NET closely mirrored potencies at DAT ( $r > 0.9$ ;  $n = 5$ ). 2-Substituted methcathinone analogs showed reduced potencies at SERT as compared to DAT release, and were from 4- to 47-fold lower than their corresponding 4-substituted analogs.

### 3-Substituted Analogs.

We previously reported the potency of **3a** as a DAT, NET, and SERT releasing agent ( $EC_{50} = 29, 40, \text{ and } 212$  nM, respectively).<sup>6</sup> The current study provided comparable values ( $EC_{50} =$

26, 19, and 211 nM) (Table 1). As with data for the 4-substituted and 2-substituted series, potencies of the 3-substituted analogs at DAT mirrored their potency at NET ( $r > 0.9$ ). In general, the potencies of the 3-substituted compounds as DAT releasing agents were 2- to 5-fold higher than those of their corresponding 4-substituted counterparts whereas, in contrast, potencies at SERT were consistency lower by about half, and only spanned a 5-fold range from the most potent (i.e., **3b**) to the least potent (**3d**).

As with the 4-substituted methcathinone derivatives (i.e., analogs **4**), the high DAT potency of the 3-substituted analogs was associated with an unsubstituted ring (i.e., **1**). However, whereas the 4-Cl, 4-Br, and 4-Me analogs **4a-4c**, respectively, showed several-fold reduced potency relative to methcathinone (**1**), their 3-substituted counterparts were very similar in potency to the parent. Nevertheless, as seen in the 4-substituted series, the 3-OMe and 3-CF<sub>3</sub> analogs **3d** and **3e**, respectively, were the least potent.

## DISCUSSION

We have now examined a number of 2-, 3-, and 4-substituted simple methcathinone analogs in a fairly systematic manner at the three monoamine transporters (present investigation and reference 4). Each compound examined was a releasing agent at DAT, NET, and SERT. In general, the agents ( $n = 15$ ) displayed similar potencies at DAT and NET (Figure 1). However, such a relationship was not apparent when DAT pEC<sub>50</sub> values were plotted against SERT EC<sub>50</sub> values ( $r = 0.209$ ,  $n = 15$ ; data not shown). Hence, aryl substitution had a decided influence on DAT/NET versus SERT potency and selectivity.

As releasing agents, methcathinone analogs with substituents at the 2-position (i.e. **2** analogs) were not as potent as their 3- or 4-positional isomers (i.e., **3** and **4** analogs, respectively). This is particularly true at SERT where, with one exception, **2c**, EC<sub>50</sub> values are in the  $\mu\text{M}$  range. Given this trend, we did not examine the 2-CF<sub>3</sub> analog. In fact, a prior investigation had already demonstrated that the 2-CF<sub>3</sub> analog of methcathinone to be >10-fold less potent than **3e** as a releasing agent at DAT and SERT.<sup>7</sup> In contrast, with the exception of the OMe and CF<sub>3</sub> analogs **3d** and **3e**, substituents at the 3-position had little effect on the potency of substituted methcathinone analogs at DAT and NET, but decreased potency at SERT, relative to their 4-substituted counterparts.

On the basis of functional QSAR studies, we found that there are limitations to the amount of steric bulk associated with the 4-position of simple methcathinone analogs tolerated by DAT as releasing agents.<sup>5</sup> In the present investigation, this same trend was evident with the 3-substituted analogs; however, due the narrow potency range, a QSAR study was not feasible here for these compounds. That is, four of the analogs (i.e., **3a-3d**) showed only a 4-fold range in potency, and the entire range in potency was only 34-fold (when **3e** is considered). Nevertheless, the two lowest potency compounds as DAT releasing agents are the 3-OMe and 3-CF<sub>3</sub> analogs **3d** and **3e**, which are the two largest analogs (or analogs with the largest 3-position substituents) of those examined. Regardless of the steric metric employed (see Supporting Information for a comparison of modified steric  $E'_s$  values, Charton's steric parameter  $\nu$ , and the Kier third-order steric shape index  $^3\kappa_a$  for the

described substituents, and the calculated volume of the molecules as a whole), the 3-OMe and 3-CF<sub>3</sub> groups appear to be the most bulky.

DAT does not readily accommodate large substituents at the aryl 4-position of the simple methcathinones as releasing agents;<sup>5</sup> this seems to be echoed here by the findings with 3-position substituents (although no QSAR studies were conducted due to the constricted findings). Nevertheless, it would seem that DAT does not appreciate steric bulk on or around the 3- or 4-position of the simple methcathinone analogs as DAT releasing agents; that is, as bulk around the 3- or 4-position increases, DAT release potency decreases. This is not unreasonable considering that these are adjacent positions.

The abuse character of 4-substituted methcathinone analogs has been described;<sup>1,4</sup> but, much less is known about the 2- and 3-substituted compounds shown in Table 1. 3-Chloromethcathinone (**3a**) has been found on the clandestine market,<sup>8</sup> but its mechanism of action has not been investigated. Likewise, there have been seizures of 3-bromomethcathinone (**3b**).<sup>9</sup> 3-Bromomethcathinone (**3b**) acts as a reuptake inhibitor at NET and SERT (IC<sub>50</sub> = 158 and 2,000 nM, respectively)<sup>10</sup> at concentrations higher than those reported here for neurotransmitter release. Because releasing agents can behave as reuptake inhibitors at high concentrations,<sup>6</sup> the present results suggest that **3b** might produce its effects primarily as a releasing agent. 2-Methylmethcathinone (**2c**) and, especially, its positional isomer, 3-methylmethcathinone (**3c**), are fast becoming two of the most popular synthetic cathinones in Europe.<sup>11-15</sup> Compound **3c** has been reported to be a reuptake inhibitor at DAT, NET, and SERT (IC<sub>50</sub> = 2,600, 270, and 9,500 nM, respectively)<sup>14</sup> at concentrations well above those shown here to serve as a releasing agent at all three transporters.

In conclusion, 2-substituted methcathinone analogs are not well tolerated by DAT, NET, and SERT as determined by their reduced potency as releasing agents; however, the 3-substituted analogs are nearly equivalent in potency to their 4-substituted counterparts as DAT and NET releasing agents, but somewhat less potent at SERT. As a consequence, the 3-substituted analogs are slightly more DAT selective than the corresponding 4-substituted compounds. Nevertheless, none is as selective for DAT/NET versus SERT as is methcathinone (**1**) itself. Also, there is no discernable difference in DAT and NET release potencies suggesting a similar SAR for the simple methcathinone analogs at these two transporters. A final, yet unintended, consequence of the present investigation was to shed light on the transporter actions of some newer agents currently found on the clandestine market.

## METHODS

### Synthesis.

A Thomas-Hoover melting point apparatus was used for the determination of melting points and the values are uncorrected. A Bruker ARX 400 MHz spectrometer was used for recording <sup>1</sup>H NMR spectra with tetramethylsilane (TMS) as an internal standard and IR spectra were obtained using a Thermo Nicolet iS10 FT-IR. Elemental analysis was performed by Atlantic Microlab Inc. (Norcross, GA) and observed values were within 0.4 % of theory. A CombiFlash Companion/TS (Telodyne Isco Inc., Lincoln, NE) was used for

automated flash chromatography. Silica Gel (230–400 mesh) was used as adsorbent, and RediSep Rf Normal-phase Silica Flash Columns (Teledyne Isco Inc., Lincoln, NE) were used as the stationary phase. Thin-layer chromatography on silica gel GHLF plates (250  $\mu$ , 2.5  $\times$  10 cm; Analtech Inc., Newark, DE) was used for monitoring reactions. Note: Although all compounds described here are methcathinone analogs, this section identifies them by their correct chemical names.

**1-(3-Bromophenyl)-2-(methylamino)propan-1-one Hydrochloride (3b).**—

*N*-Benzyl-*N*-methylamine (0.6 mL, 5 mmol) was added at room temperature to a stirred solution of **6b**<sup>16</sup> (0.67 g, 2 mmol) in anhydrous THF (10 mL). The stirred reaction mixture was heated at reflux for 18 h, allowed to cool to room temperature, and solvent was removed under reduced pressure. The residue was dissolved in EtOAc (20 mL), washed with saturated NaHCO<sub>3</sub> solution (5 mL), H<sub>2</sub>O (5 mL), brine (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to dryness under reduced pressure to yield a crude product that was purified by column chromatography (silica gel; hexane/EtOAc; 95:5) to afford 0.28 g (36%) of **8b** as a yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.22 (d, 3H, CH<sub>3</sub>), 2.11 (s, 3H, CH<sub>3</sub>), 3.56 (s, 2H, CH<sub>2</sub>), 4.13 (q, 1H, CH), 7.16–7.26 (m, 6H, ArH), 7.59 (d, 1H, ArH), 7.82 (d, 1H, ArH), 8.09 (t, 1H, ArH).

1-Chloroethyl chloroformate (0.2 mL, 2.0 mmol) was added at room temperature to a stirred solution of **8b** (0.28 g, 0.8 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL). The stirred reaction mixture was heated at reflux for 2 h, cooled to room temperature, and concentrated under reduced pressure. The residue was dissolved in CH<sub>3</sub>OH (5 mL), heated at reflux for 1 h, cooled to room temperature, and evaporated to dryness under reduced pressure. The residue was stirred in Et<sub>2</sub>O for 18 h. The solid material was collected by filtration to yield a brown solid that was recrystallized from *i*-PrOH/Et<sub>2</sub>O to yield 0.09 g (39%) of **3b** as a pale brown solid: mp 185 °C (lit.<sup>17</sup> mp 178–181 °C, *i*-PrOH/Et<sub>2</sub>O). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.42 (d, 3H, CH<sub>3</sub>), 2.57 (s, 3H, CH<sub>3</sub>), 5.18 (d, 1H, CH), 7.57 (t, 1H, ArH), 7.95 (d, 1H, ArH), 8.02 (d, 1H, ArH), 8.19 (t, 1H, ArH), 9.16 (br s, 1H, NH), 9.62 (br s, 1H, NH<sup>+</sup>).

The following targets were prepared in the same manner as **3b**.

**1-(2-Chlorophenyl)-2-(methylamino)propan-1-one Hydrochloride (2a).**—

The product was obtained from bromopropiophenone **5a**<sup>18</sup> as a white solid in 35% yield after recrystallization from MeOH/EtOAc: mp 172–174 °C (lit.<sup>19</sup> mp 159–161 °C). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.37 (d, 3H, CH<sub>3</sub>), 2.66 (s, 3H, CH<sub>3</sub>), 4.99 (q, 1H, CH), 7.55–7.59 (m, 1H, ArH), 7.66 (d, 2H, ArH), 7.90 (d, 1H, ArH), 9.36 (br s, 2H, NH<sub>2</sub><sup>+</sup>); Due to the discrepancy with the literature melting point, **2a** was submitted for elemental analysis. Anal. Calcd for (C<sub>10</sub>H<sub>12</sub>ClNO·HCl) C, 51.30; H, 5.60; N, 5.98. Found: C, 51.08; H, 5.51; N, 5.94.

**1-(2-Bromophenyl)-2-(methylamino)propan-1-one Hydrochloride (2b).**—

The product was obtained from **5b**<sup>20</sup> as a white solid in 29% yield after recrystallization from MeOH/EtOAc: mp 172–174 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  1.36 (d, 3H, CH<sub>3</sub>), 2.66 (s, 3H, CH<sub>3</sub>), 4.98 (q, 1H, CH), 7.54–7.62 (m, 2H, ArH), 7.82–7.84 (m, 1H, ArH), 7.88 (d, 1H, ArH), 9.44 (br s, 2H, NH<sub>2</sub><sup>+</sup>); Anal. Calcd for (C<sub>10</sub>H<sub>12</sub>BrNO·HCl) C, 43.12; H, 4.70; N, 5.03. Found: C, 43.21; H, 4.84; N, 4.91.

**2-(Methylamino)-1-(2-methylphenyl)propan-1-one Hydrochloride (2c).**—The product was obtained from **5c**<sup>18</sup> as a white solid in 53% yield after recrystallization from MeOH/EtOAc: mp 160 °C (lit.<sup>21</sup> mp 163–165 °C). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.36 (d, 3H, CH<sub>3</sub>), 2.44 (s, 3H, CH<sub>3</sub>), 2.62 (s, 3H, CH<sub>3</sub>), 5.06 (q, 1H, CH), 7.39–7.42 (m, 2H, ArH), 7.53–7.57 (m, 1H, ArH), 7.89–7.92 (m, 1H, ArH), 9.24 (br s, 1H, NH), 9.61 (br s, 1H, NH<sup>+</sup>).

**1-(2-Methoxyphenyl)-2-(methylamino)propan-1-one Hydrochloride (2d).**—The product was obtained from **5d**<sup>22</sup> as a white solid in 70% yield after recrystallization from MeOH/EtOAc: mp 184 °C (lit.<sup>22</sup> mp 175–177 °C). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.40 (d, 3H, CH<sub>3</sub>), 2.60 (s, 3H, CH<sub>3</sub>), 3.96 (s, 3H, CH<sub>3</sub>), 4.83 (q, 1H, CH), 7.13 (t, 1H, ArH), 7.28 (d, 1H, ArH), 7.70 (t, 1H, ArH), 7.80 (d, 1H, ArH), 9.22 (br s, 2H, NH<sub>2</sub><sup>+</sup>).

**2-(Methylamino)-1-(3-methylphenyl)propan-1-one Hydrochloride (3c).**—The product was obtained from **6c**<sup>23</sup> as a grey solid in 70% yield after recrystallization from MeOH/EtOAc: mp 193–195 °C (lit.<sup>21</sup> mp 188–190 °C). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.42 (d, 3H, CH<sub>3</sub>), 2.39 (s, 3H, CH<sub>3</sub>), 2.57 (s, 3H, CH<sub>3</sub>), 5.13 (q, 1H, CH), 7.45–7.57 (m, 2H, ArH), 7.81–7.85 (m, 2H, ArH).

**2-(Methylamino)-1-(3-methoxyphenyl)propan-1-one Hydrochloride (3d).**—The product was obtained from **6d**<sup>24</sup> as a white solid in 70% yield after recrystallization from EtOH: mp 174–175 °C. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.42 (d, 3H, CH<sub>3</sub>), 2.57 (s, 3H, CH<sub>3</sub>), 3.83 (s, 3H, CH<sub>3</sub>), 5.17 (q, 1H, CH), 7.29–7.33 (m, 1H, ArH), 7.49–7.54 (m, 2H, ArH), 7.60–7.63 (m, 1H, ArH), 9.34 (br s, 2H, NH<sub>2</sub><sup>+</sup>); Although the compound was previously reported,<sup>25</sup> it was not characterized. Anal. Calcd for (C<sub>11</sub>H<sub>15</sub>NO<sub>2</sub>·HCl·0.7 H<sub>2</sub>O) C, 54.52; H, 7.24; N, 5.78. Found: C, 54.63; H, 7.20; N, 5.85.

**2-(Methylamino)-1-(3-(trifluoromethyl)phenyl)propan-1-one Hydrochloride (3e).**—The product was prepared from amine **8e**<sup>21</sup> and obtained as a yellow-white solid in 71% yield after recrystallization from MeOH/EtOAc: mp 171 °C (lit.<sup>7</sup> mp 163.5–165 °C). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.54 (d, 3H, CH<sub>3</sub>), 2.70 (s, 3H, CH<sub>3</sub>), 5.38 (d, 1H, CH), 7.96 (t, 1H, ArH), 8.22 (d, 1H, ArH), 8.42–8.43 (m, 2H, ArH), 9.28 (br s, 1H, NH), 9.76 (br s, 1H, NH<sup>+</sup>).

### In vitro release assay.

Assays were performed as previously described.<sup>6</sup> Male Sprague-Dawley rats (Harlan Laboratories, Frederick, MD, USA) 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).

Rats were euthanized by CO<sub>2</sub> 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. [<sup>3</sup>H]1-Methyl-4-phenylpyridinium ([<sup>3</sup>H]MPP+) (9 nM) was used as the radiolabeled substrate for DAT and NET, whereas [<sup>3</sup>H]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 [<sup>3</sup>H]MPP+ or [<sup>3</sup>H]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 % Maximum Release, with maximum release (i.e., 100% E<sub>max</sub>) defined as the release produced by 10 nM tyramine for DAT and NET assay conditions, and 100 nM tyramine for SERT assay conditions. These concentrations of tyramine evoke the efflux of all “releasable” tritium from synaptosomes as determined previously.<sup>26</sup> EC<sub>50</sub> values were determined using nonlinear least-squares curve fitting (GraphPad Prism, v. 6.0; GraphPad Scientific, San Diego, CA, USA).

### Correlation analysis.

The correlation shown as Figure 1 was obtained using the linear regression analysis feature of GraphPad Prism, v. 7.01 (GraphPad Scientific, San Diego, CA, USA). EC<sub>50</sub> values in Table 1 were converted to their pEC<sub>50</sub> values prior to analysis.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

### Funding

This work was supported in part by DA033930 and by the National Institute on Drug Abuse Intramural Research Program Grant DA000523.

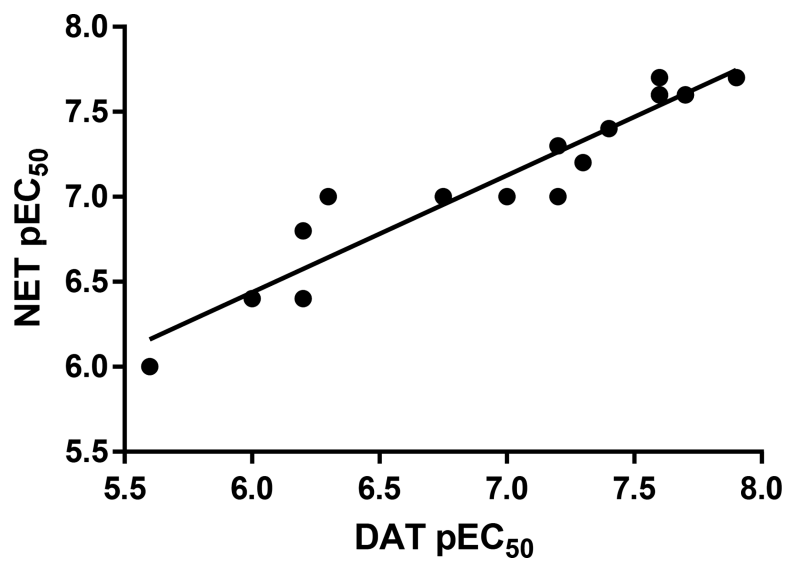
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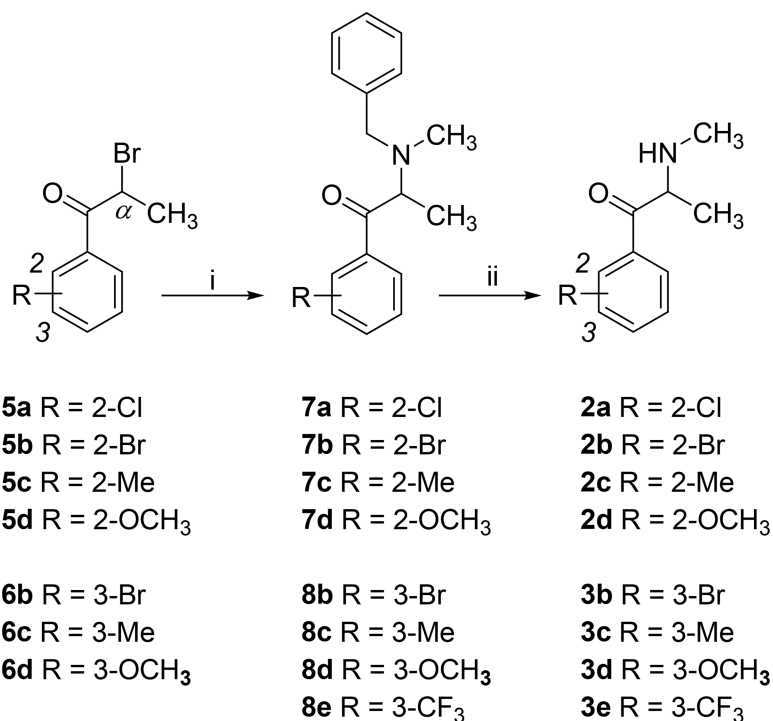


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**Figure 1.** A comparison of DAT and NET release potencies for compounds shown in Table 1 ( $n = 15$ ,  $r = 0.950$ ); DAT and NET data for methcathinone were only considered once,  $IC_{50} = 21$  and  $22$  nM, respectively).

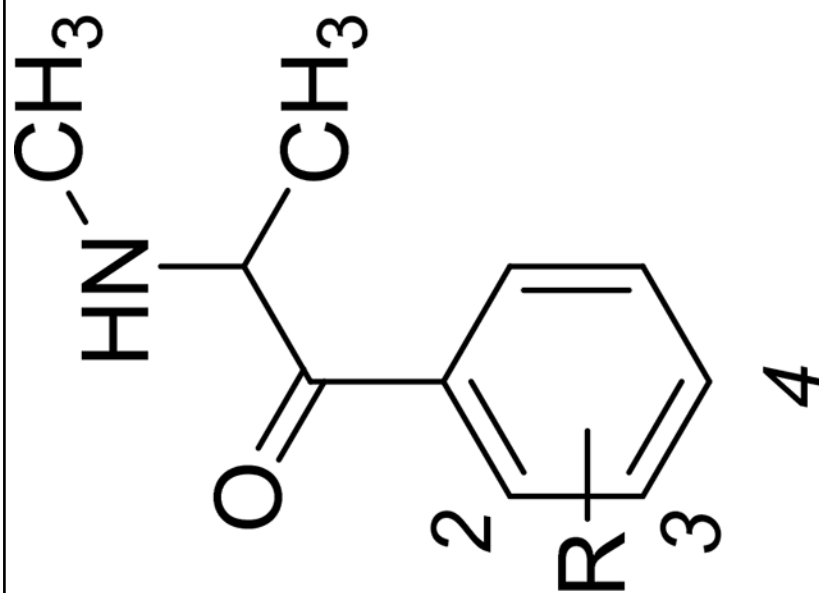
**Scheme 1.**

Synthesis of 2- and 3-substituted methcathinone analogs.<sup>a</sup>

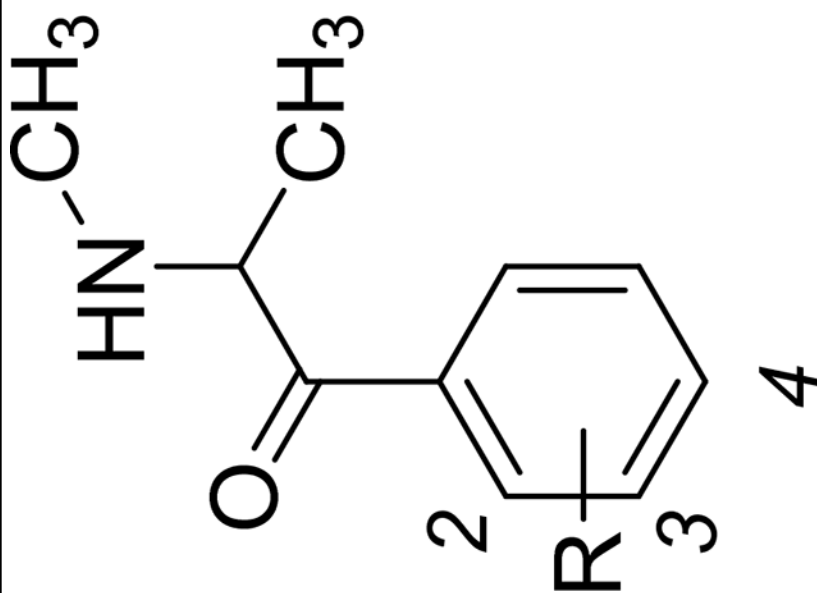
<sup>a</sup>Reagents and conditions: (i) *N*-benzyl-*N*-methylamine, THF, reflux, 18–23 h; (ii) (a) 1-chloroethyl chloroformate, CH<sub>2</sub>Cl<sub>2</sub>, reflux, 2 h; (b) CH<sub>3</sub>OH, reflux, 1 h; (c) Et<sub>2</sub>O, room temperature, 18 h.

Table 1.

Potencies of aryl-substituted methcathinone analogs on transporter-mediated release in rat brain synaptosomes.<sup>a</sup>

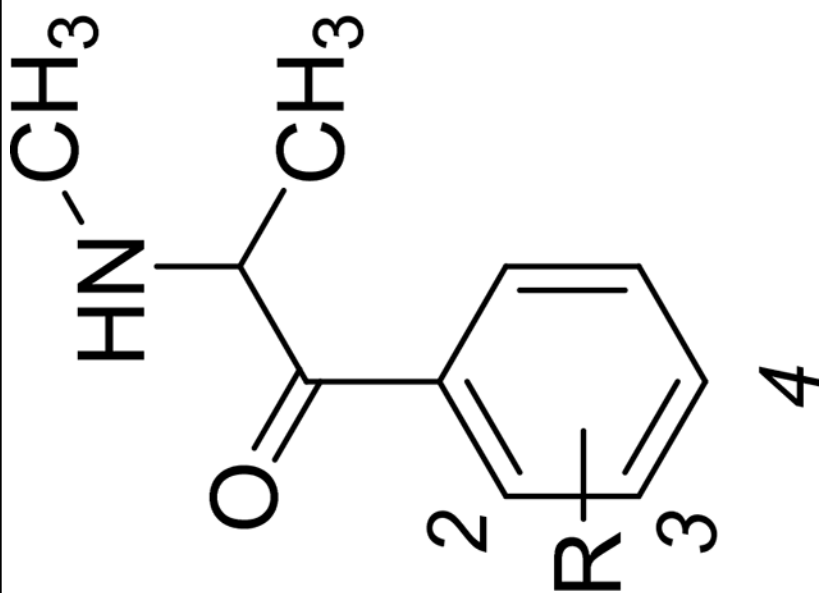


		EC <sub>50</sub> nM (SD)									
		2-Substituted:			3-Substituted:			4-Substituted: <sup>b</sup>			
		NET	SERT	DAT vs SERT Selectivity <sup>c</sup>	NET	SERT	DAT vs SERT Selectivity <sup>c</sup>	NET	SERT	DAT vs SERT Selectivity <sup>c</sup>	
<b>H</b>	<b>(1)</b>	25 (3)	2592 (1000)	103	21 (3)	5853 (1250)	279	12.5	22 (4)	3860	309



EC<sub>50</sub> nM (SD)

	2-Substituted:			3-Substituted:			4-Substituted: <sup>b</sup>			DAT vs SERT Selectivity <sup>c</sup>
	DAT	NET	SERT	DAT	NET	SERT	DAT	NET	SERT	
<b>Cl</b>	179 (22)	93 (6)	2815 (620)	26 (4)	19 (3)	211 (33)	42.2	44 (9)	144	3.4
<b>Br</b>	650 (80)	156 (12)	2837 (260)	21 (3)	25 (4)	136 (24)	59.4	100 (16)	60	1.0
<b>Me</b>	81 (8)	53 (4)	490 (15)	28 (6)	27 (4)	268 (25)	49.1	63 (16)	118	2.4
<b>OMe</b>	920 (30)	339 (12)	7220 (900)	109 (18)	111 (12)	683 (92)	506	111 (24)	120	0.24



**EC<sub>50</sub> nM (SD)**

2-Substituted:				3-Substituted:				4-Substituted: <sup>b</sup>				
CF <sub>3</sub>	NET	SERT	DAT vs SERT Selectivity <sup>c</sup>	NET	SERT	DAT vs SERT Selectivity <sup>c</sup>	NET	SERT	DAT vs SERT Selectivity <sup>c</sup>	NET	SERT	DAT vs SERT Selectivity <sup>c</sup>
-	-	-	-	714 (73)	370 (49)	281 (57)	0.4	2700	900 (300)	190	0.07	

<sup>a</sup>All data are expressed as mean ± SD for n = 3 experiments performed in triplicate.

<sup>b</sup>DAT and SERT data previously published.<sup>4</sup>

SERT EC50 ÷ DAT EC50

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