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The dopamine, serotonin and norepinephrine releasing activities of a series of methcathinone analogs in male rat brain synaptosomes

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Abstract

Rationale.—Novel synthetic ‘bath salt’ cathinones continue to appear on the street as abused and addictive drugs. The range of subjective experiences produced by different cathinones suggests that some compounds have primarily dopaminergic activity (possible stimulants) while others have primarily serotonergic activity (possible empathogenics). An understanding of the structure activity relationships (SARs) of these compounds will help in assessing the likely behavioral effects of future novel structures, and to define potential therapeutic strategies to reverse any reinforcing effects.

Objectives.—A series of methcathinone analogues was systematically studied for their activity at the dopamine and serotonin transporters. Compound structures varied at the aromatic group, either by substituent or by replacement of the phenyl ring with a naphthalene or indole ring.

Methods.—A novel, high yielding synthesis of methcathinone hydrochlorides was developed which avoids isolation of the unstable free bases. Neurotransmitter transporter release activity was determined in rat brain synaptosomes as previously reported. Compounds were also screened for activity at the norepinephrine transporter.

Results.—Twenty-eight methcathinone analogs were analyzed and fully characterized in dopamine and serotonin transporter release assays. Compounds substituted at the 2-position (ortho) were primarily dopaminergic. Compounds substituted at the 3-position (meta) were found to be much less dopaminergic, with some substituents favoring serotonergic activity. Compounds substituted at the 4-position (para) were found to be far more serotonergic, as were disubstituted compounds and other large aromatic groups. One exception was the fluoro substituted analogs which seem to favor the dopamine transporter.

Conclusions.—The dopaminergic to serotonergic ratio can be manipulated by choice of substituent and location on the aromatic ring. It is therefore likely possible to tweak the subjective

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and reinforcing effects of these compounds by adjusting their structure. Certain substituents like a fluoro group tend to favor the dopamine transporter, while others like a trifluoromethyl group favor the serotonin transporter.

Keywords

biogenic amine transporters; rat brain synaptosomes; “bath salts”; methcathinones

Introduction

Synthetic ‘bath salt’ cathinones continue to be a serious abuse problem due to the powerful stimulant, hallucinogenic, and addictive effects they produce in humans. The CY 2017 DEA “Emerging Threats” report indicates that there were 369 confirmed reports of ‘bath salts’, the most prevalent being N-ethylpentylone. Structurally smaller ‘bath salts’ were also observed including N-ethyl-4-chloromethcathinone, ethylone, and dibutylone. Methcathinone analogs, including the original ‘bath salt’ methylone, along with newer methcathinones such as methylmethcathinone, and chloromethcathinone were also found. The evidence shows that clandestine laboratories continue to make and distribute novel cathinone analogs making the study of their properties imperative.

As a class of abused drugs, cathinones are a recent phenomenon exploding on the street in the early 2000s, but bioactive compounds have actually been studied for many years. Methcathinone (**1**) was first synthesized in 1928 as an ephedrine homolog (Hyde et al. 1928). It was later patented by Parke Davis in 1957 for its analeptic or “restorative” properties (L’Italien et al. 1957). In 1975, the UN found that the N-demethylated analog cathinone (**2**) was the active ingredient in *Catha edulis*, a plant used widely in Africa and the Middle East as a stimulant (Patel 2000). In the early 1980s, methcathinone was examined as a stimulant and was found to substitute for amphetamine in drugdiscrimination assays in rats (Glennon et al. 1987). Methcathinone was also found to dose-dependently maintain self-administration in baboons (Kaminski and Griffiths 1994). Several methcathinone analogs were reported in the literature prior to 2004, including bromomethcathinones (Foley and Cozzi 2003) and methylone (Peyton and Shulgin 1996), all of which were primarily studied as potential antidepressants. These efforts may have been a follow-up on the success of the cathinone antidepressant and smoking cessation drug bupropion (Wellbutrin®, Zyban®) (Carroll et al. 2014). Interestingly, as an extension of the bupropion success, cathinone analogs of bupropion have been studied as potential therapeutics for cocaine and nicotine abuse (Carroll et al. 2009; Carroll et al. 2010).

Since the explosion of the cathinones as a popular class of recreational drug around 2010, laboratories around the world began to characterize these compounds pharmacologically, resulting in numerous reports identifying mechanism of action and SAR (Baumann et al. 2013; Bonano et al. 2015; Del Bello et al. 2015; Eshleman et al. 2017; Glennon and Dukat 2017; Gregg et al. 2015; Hutsell et al. 2016; Luethi et al. 2018; Rickli et al. 2015; Saha et al. 2015; Schindler et al. 2016; Shalabi et al. 2017; Shekar et al. 2017; Simmler et al. 2013; Simmler et al. 2014). The primary mechanism of action of the cathinones as a class appears to be their interactions at the biogenic amine transporters (BATs), which help regulate

monoaminergic signaling in the central nervous system (CNS). The primary function of BATs is to transport previously released monoamine neurotransmitters – dopamine, norepinephrine, and serotonin (DA, NE, and 5-HT transported via DAT, NET, and SERT, respectively) – from the synapse back to the neuronal cytoplasm. (Masson et al. 1999) BATs can also participate in release of the same neurotransmitters in a non-calcium dependent manner in the opposite direction (Sulzer et al. 2005). Ligands that interact with BATs are classified as either reuptake inhibitors or substrate-type releasers; both types of ligands act to elevate extracellular neurotransmitter concentrations but they act via different mechanisms (Rothman et al. 2008). Reuptake inhibitors bind to transporters and block transporter-mediated reuptake of neurotransmitters without being transported, (Rudnick and Clark 1993) while substrate-type releasers bind to the substrate site on the transporters, are transported inside the neuron, and promote neurotransmitter efflux by transporter-mediated exchange. (Fleckenstein et al. 2007) While both types of compounds elevate synaptic neurotransmitters, a releaser also depolarizes the membrane as ions move back through the transporter, while reuptake inhibitors do not (Sulzer et al. 2005). The SERT and NET also have voltage-dependent transient currents with ion channel-like properties that may be relevant to their function, and these currents can be blocked by reuptake inhibitors such as fluoxetine and cocaine (Li et al. 2002). These differences in mechanism are substantial, likely induce divergent effects between mechanistic classes, and probably underlie the differences in therapeutic potential of releasers and reuptake inhibitors.

Given the potential abuse liability of “bath salts” along with the ever-changing illicit drug market, it is becoming increasingly important to understand the pharmacology of these drugs at BATs. An understanding of the mechanism of action of these emerging bath salts, and their specific BAT profiles, might also aid in the discovery of treatment medications. Our research laboratories have studied neurotransmitter releasers as a potential therapeutic for cocaine addiction and abuse since 1999 (Rothman et al. 2008). These efforts included the discovery of novel N-cyclopropyl and N-cyclobutylcathinones as hybrid dopamine reuptake inhibitor/serotonin releasers (Blough et al. 2014a), and the pursuit of the DA releasing stimulant phenmetrazine as a potential pharmacotherapy (Banks et al. 2013; Rothman et al. 2002). Many compound classes, including methcathinones, have been considered as potential therapeutics and were studied as part of our research (Reith et al. 2015). Herein we report the systematic study of a series of methcathinones, and their interactions at the DAT, SERT, and NET.

Methods

Compounds

Compounds **1** and **2** were acquired from the NIDA Drug Supply. Phenacylamine was purchased from Sigma-Aldrich and converted to its hydrochloride salt. All other compounds were synthesized following the route described in Scheme 1. The synthesis and analytical information of all novel compounds is provided in the electronic supplementary information (ESI). All compounds were >95% pure when tested.

DAT, SERT, and NET Rat Brain Synaptosome Assays

The DAT, SERT, and NET reuptake inhibition and release assays were conducted according to the methods of Rothman and Baumann (Partilla et al. 2016). Male CD Sprague-Dawley rats (300–400g) were maintained in facilities fully accredited by the AAALAC and experiments were performed in accordance with the Institutional Animal Care and Use Committee at Mispro Biotech. A total of 25 rats were used for the experiments. Rats were euthanized by CO₂ narcosis and brains were processed to yield synaptosomes as previously described. Synaptosomes were prepared from rat striatum for the DAT assays, whereas synaptosomes were prepared from whole brain minus striatum and cerebellum for the SERT and NET assays. Initial screening assays were conducted at a final test compound concentration of 10 µM.

For neurotransmitter reuptake inhibition assays, 5 nM [³H]DA, 5 nM [³H]5-HT, and 10 nM [³H]NE were used to assess activity at the DAT, SERT, and NET, respectively. The selectivity of the SERT assay was optimized for SERT by including unlabeled blockers to prevent uptake of [³H]5-HT by competing transporters (100 nM nomifensine to block NET, 50 nM GBR12935 to block DAT). The selectivity of the NET assay was optimized for NET by including unlabeled blockers to prevent uptake of [³H]NE by competing transporters (50 nM GBR12935 to block DAT). No blockers are needed for the DAT assay. The assay was initiated by adding 100 µL of the synaptosomal prep to 900 µL of Krebs-phosphate buffer (12.6 mM NaCl, 0.24 mM KCl, 0.05 mM KH₂PO₄, 0.11 mM CaCl₂, 0.083 mM MgCl₂, 0.05 mM Na₂SO₄, 11.1 mM glucose, 0.05 mM pargyline, 13.8 mM Na₂HPO₄, 1 mg/mL ascorbic acid, and 1 mg/mL BSA, pH 7.4) containing test compound and [³H]neurotransmitter. Non-specific binding (NSB) was determined in the presence of 1 µM indatraline and total binding (TB) was determined in the presence of vehicle. After 15 min at RT (DAT), 30 min at RT (SERT), or 10 min at 37°C (NET), the assay was terminated by rapid vacuum filtration/washing through GF/B filters (Brandel harvester). The retained radioactivity was quantified by a PerkinElmer TopCount.

For neurotransmitter release assays, 5 nM [³H]DA, 2 nM [³H]5-HT, and 5 nM [³H]MPP⁺ were used to assess activity at the DAT, SERT, and NET, respectively. All buffers used in the release assays contained 1 µM reserpine to block vesicular uptake of substrates. The same blockers are used for the SERT assay to optimize SERT selectivity as listed in the reuptake inhibition methods. The selectivity of the NET assay was optimized for NET by including unlabeled blockers to prevent uptake of [³H]MPP⁺ by competing transporters (100 nM GBR12935 to block DAT, 100 nM citalopram to block SERT). No blockers are needed for the DAT release assay. Synaptosomes were preloaded (steady state) with radiolabeled substrate in Krebs-phosphate buffer (12.6 mM NaCl, 0.24 mM KCl, 0.05 mM KH₂PO₄, 0.11 mM CaCl₂, 0.083 mM MgCl₂, 0.05 mM Na₂SO₄, 11.1 mM glucose, 0.05 mM pargyline, 13.8 mM Na₂HPO₄, 1 mg/mL ascorbic acid, pH 7.4) for 30 min (DAT, NET) or 1 h (SERT). Release assays were initiated by adding 850 µL of preloaded synaptosomes to 150 µL of test compound prepared in Krebsphosphate buffer containing 1 mg/mL BSA. NSB was determined in the presence of tyramine (10 µM for DAT/NET and 100 µM for SERT) and TB was determined in the presence of vehicle. Assays were terminated by rapid vacuum

filtration/washing through GF/B filters on a Brandel (Gaithersburg, MD, USA) harvester and retained radioactivity was quantified by a PerkinElmer TopCount.

Substrate activity for releasers was confirmed by detecting a significant reversal of the releasing effect of the test compound in the presence of reuptake inhibitors (at least N=2). The release assays were run in the same manner as described except that the EC₈₀ concentration of each test compound was tested in the presence and absence of a known reuptake inhibitor (100x IC₅₀). The reuptake inhibitor will bind to the transporter and block the releaser from being transported, thus the releaser's ability to release neurotransmitter will be diminished, as shown by a reduction in % release in the assay. GBR12935 (82 nM) is the known blocker for the DAT substrate reversal assay, while citalopram (450 nM) is the known blocker for the SERT substrate reversal assay.

Data Analysis

Maximal binding (MB) was calculated with the equation $MB = TB - NSB$. Specific bound (SB) was calculated with the equation $SB = \text{test compound signal} - NSB$. Percent of MB was calculated with the equation $\% MB = (1 - SR/MR) \times 100$. To determine compound potencies in the release assays, % MR was plotted against the log of compound concentration. Data were fit to a three-parameter logistic curve to generate EC₅₀ values (GraphPad Prism, GraphPad Software, Inc., San Diego, CA). For the substrate reversal assays, % MB was calculated for the test compound with and without the known reuptake inhibitor. Student's t-test was performed to determine if the release values were significant.

Results

Methcathinone Synthesis

The methcathinone analogs were synthesized as shown in Scheme 1. The synthesis is described for the parent scaffold methcathinone (**1**) but was the same for all of the methcathinones (see Supplemental Data). Acetophenone was quantitatively brominated alpha to the ketone to form bromoketone (**4**), followed by amination with methylbenzylamine to form ketoamine (**5**). The benzyl group was then removed using 1-chloroethyl chloroformate (ACE-Cl) to directly form methcathinone hydrochloride analogs in high yields and purity. Overall yields of the methcathinone hydrochloride salts from their respective substituted phenylpropiophenones ranged between 50–97%, depending on the phenyl substitution.

While amination of bromoketones like **4** with large primary alkylamines, such as t-butylamine, is high yielding and scalable, in our experience, amination with smaller primary alkylamines like methylamine results in highly colored solutions and much lower yields. These observations suggest that free base dimerization occurs followed by various oxidative processes. Large α -alkyl groups prevent dimerization and free base decomposition. Synthesis of the benzyl protected methcathinones by amination with the secondary amine N-benzylmethylamine, followed by debenzylation with ACE-Cl, allowed us to form the hydrochloride salts directly and completely avoid isolation of the unstable free base.

Dopamine, Serotonin, and Norepinephrine Transporter Activity.

The synthesized methcathinones were evaluated for DAT, SERT, and NET activity according to the protocol developed by Rothman and co-workers (Partilla et al. 2016) using rat brain synaptosomes prepared from rat brain homogenates. Compounds were initially screened in DA, 5-HT, NE reuptake inhibition and release assays to assess mechanism of action.

According to the protocol, compounds active in both the reuptake inhibition and release assays are binned mechanistically as releasers while compounds only active in the reuptake inhibition assay are binned as reuptake inhibitors. We have never observed compounds only active in the release assay. All of the compounds in this study were found to be releasers or were inactive. Active releasers at DA and 5-HT were then fully characterized for release potency at each transporter by running 8-point concentration response curves; substrate reversal experiments were conducted to validate substrate activity. NE release was not fully characterized; therefore, the screening data is presented as % release at 10 μ M. Since the ratio of DAT and SERT release has been shown throughout the literature to be important for abuse liability and psychoactive behavioral effects, we calculated this ratio with the equation $\text{DAT selectivity} = \text{SERT EC}_{50} / \text{DAT EC}_{50}$.

Table 1 shows DAT, SERT and NET release activity for phenacylamine (**6**), cathinone (**2**), methcathinone (**1**), and several 2-substituted methcathinones. Methcathinone **1** was 86-fold more selective for releasing DA relative to 5-HT with EC_{50} values of 49.9 nM and 4270 nM, respectively. The unsubstituted cathinones phenacylamine (**6**) and cathinone (**2**) were both potent DA releasers with EC_{50} values of 208 nM and 83.1 nM, respectively, which are 4.2-fold and 1.7-fold less potent compared to **1**. Adding substituents at the 2-position led to a variety of results. Compared to methcathinone **1**, DAT activity remained unchanged for the 2-F analog (**7**, EC_{50} = 48.7 nM) while SERT activity was completely lost. The 2-CH₃ analog **10** was 2-fold less potent as a DA releaser with an EC_{50} value of 97.9 nM, but was 12-fold more potent as a 5-HT releaser (EC_{50} = 347 nM). Interestingly, addition of a CF₃ group (**8**) or a OCF₃ group (**9**) to the 2-position resulted in a complete loss of transporter substrate activity at both DAT and SERT. All of the compounds were active as potent NET releasers, except for the 2-OCF₃ analog (**9**) which was weak. Surprisingly, the 2-CF₃ analog (**8**) appears active as a NE releaser without having DAT or SERT activity suggesting it may be a selective NET releaser.

Table 2 shows DAT, SERT and NET release activity for 3-substituted methcathinones. Compared to the parent methcathinone **1**, adding a bromide to the 3-position (**11**) led to an almost 2fold more potent compound at DAT (EC_{50} = 28.0 nM) and a 31-fold more potent compound at SERT (EC_{50} = 137 nM). The 3-Cl analog **12** was approximately the same potency as **1** at DAT with an EC_{50} = 46.8 nM, but was about an order of magnitude more potent as a 5-HT releaser with an EC_{50} = 410 nM. The 3-F analog **13** was equipotent at releasing DA compared to **1** but was 2.9-fold more potent at releasing 5-HT (EC_{50} = 1,460 nM). The trifluoromethyl analog **14** was 26-fold less potent at releasing DA but was 14-fold more potent at releasing 5-HT (EC_{50} = 297 nM) compared to **1**. The 3-OCF₃ analog **15** was also less potent at DAT but only by 15-fold, and kept the increase in SERT potency with an EC_{50} value of 188 nM. Compared to **1**, the 3-CH₃ analog **16** was equipotent at the DAT with an EC_{50} value of 70.6 nM but was 14.6-fold more potent at releasing 5-HT with an EC_{50}

value of 292 nM. Having a methoxy group at the 3-position (**17**) resulted in a 2.6-fold increase in potency ($EC_{50} = 129$ nM) at DAT and a 14-fold decrease in potency ($EC_{50} = 306$ nM) at SERT. The 3-nitro analog **18** was 25-fold less potent and 3-fold more potent at the DAT and the SERT, respectively, compared to **1**. All of the compounds were active as NET releasers. While difficult to accurately predict based on the screening data, the 3- CF_3 analog **14** and possibly the 3- OCF_3 analog **15** appear to be SERT/NET selective releasers. The 3-nitro analog **18** appears to be a selective NE releaser.

Table 3 shows DAT, SERT and NET release activity data for a series of 4-substituted methcathinones. Compared to the parent methcathinone **1**, the 4-Br analog **19** was equipotent at DAT and 100-fold more potent at SERT with an EC_{50} value of 42.5 nM. The 4-Cl analog **20** had similar release potencies at DAT and SERT compared to the 4-Br analog **19**. Relative to **1**, the 4-F analog **21** was 2.4-fold less potent at DAT and 2.9-fold more potent at SERT with EC_{50} values of 119 nM and 1,450 nM, respectively. The trifluoromethyl analog **22** and trifluoromethoxy analog **23** resulted in weak DA releasers ($EC_{50} > 4$ μ M) but 15-fold and 36-fold more potent 5-HT releasers, respectively, indicating that large groups are not well tolerated at the 4-position for DAT translocation. Placing a methoxy group at the 4-position forming **25** resulted in an 18-fold less potent DA releaser ($EC_{50} = 881$ nM) and a 22-fold more potent 5-HT releaser ($EC_{50} = 195$ nM). The 4-methyl substituted compound known as mephedrone (**24**) was not examined as part of this study but was included in the Bonano et al. study (Bonano et al. 2015). Clearly, the methyl substituent results in equipotent DA release and 36-fold more potent 5-HT release compared to **1**; however because compound **24** was not tested as part of the current study, making such a comparison has limitations. All of the compounds were potent NE releasers.

Table 4 shows the DAT, SERT and NET release activity data for 1-naphthylmethcathinone, 2-naphthylmethcathinone, 3-indoylmethcathinone, and several disubstituted methcathinone analogs. The analogs with other aromatic groups were evaluated for transporter activity and all were found to be almost equipotent at the DAT and >100-fold more potent at the SERT compared to **1**. The 1-naphthyl analog **26** was equipotent at the DAT compared to **1** with an EC_{50} of 55.2 nM, but was 198-fold more potent at the SERT with an EC_{50} of 21.6 nM. Similarly, the 2-naphthyl analog **27** was equipotent at the DAT with an EC_{50} of 33.8 but was 158-fold more potent at the SERT with an EC_{50} of 27.1 nM. The 3-indole analog **28** was almost 2-fold less potent at the DAT with an EC_{50} of 92.8 nM but was 103-fold more potent at the SERT with an EC_{50} of 41.3 nM. Several di-substituted analogs were synthesized and tested in the rat brain synaptosome assays. Compared to methcathinone **1**, the 3,4-dichloro analog **29** was 3.6-fold less potent at releasing DA and was 57-fold more potent at releasing 5-HT. The 3,4-difluoro analog **30** was 4.5-fold less potent at DAT and was 4-fold more potent at releasing 5-HT. The 3-chloro, 4-methyl analog **31** was 2.5-fold less potent at DAT and was 102-fold more potent at SERT. All of the compounds except for the 3-indoyl analog **28** appear to be potent NE releasers.

Discussion

All of the BAT active methcathinones were neurotransmitter releasers. These observations fit with the mechanistic theory that neurotransmitter releasers are transporter substrates, since

all of these compounds are small molecular weight, sterically small phenyl amines (Reith et al. 2015; Sulzer et al. 2005). The location and type of phenyl substituent had a large effect on the potency of a compound as a DAT or SERT releaser. For the 2-substituted compounds (Table 1), the DAT rank order was $H = F > CH_3 \gg CF_3 \sim OCF_3$ indicating that sterically smaller groups are preferred by the DAT compared to the bulkier groups. The SERT rank order was $CH_3 \gg H, F, CF_3, OCF_3$, indicating that SERT translocation requires small electron donating groups at the 2-position. In the case of 3-substituted analogs (Table 2), the DAT rank order was $Br > Cl = H > F \sim CH_3 > OCH_3 > OCF_3 > NO_2 \sim CF_3$, indicating that small electron withdrawing groups are preferred most by the DAT. The SERT rank order was $Br \sim OCF_3 > Cl \sim CH_3 \sim OCH_3 \sim CF_3 > NO_2 \sim F \gg H$. This rank order provides no clear preference for steric or ionic character in a substituent at the 3-position at the SERT, other than the finding that all of the substitutions were more potent as 5-HT releasers than **1**. The DAT activity rank order for the 4-substituted analogs (Table 3) was $H = CH_3 > Br \sim Cl > F > OCH_3 \gg CF_3 > OCF_3$, indicating that at the 4-position, small electron withdrawing groups are preferred for DAT translocation. The SERT rank order was $Br > Cl > OCH_3 > CH_3 = OCF_3 \sim CF_3 > F \gg H$, indicating that SERT translocation also prefers small electron withdrawing groups but can tolerate larger electron donating groups as well. As with the 3-substituted analogs, all were much more potent as 5-HT releasers than methcathinone.

As observed with other releaser scaffolds (Blough 2008; Blough et al. 2014a; Blough et al. 2014b) the DAT/SERT ratio was greatly influenced by the choice of phenyl substituent and location of substitution. Methcathinone, cathinone, and phenylacetylamine were all DAT selective, as were both 2-substituted compounds with transporter activity, the 2-fluoro compound (**7**) and the 2-methyl compound (**10**). The 3-substituted compounds were much less DAT selective with all but the 3-F analog (**13**) being less than 10-fold DAT selective. Two of the compounds were selective 5-HT releasers, the 3- CF_3 analog (**14**) and the 3- OCF_3 compound (**15**). Interestingly these two substituents rendered the scaffold inactive at both transporters when substituted at the 2-position. All but two of the 4-substituted compounds (**21** and **24**) were selective 5-HT releasers. The same two substituents that were inactive when at the 2-position were the most serotonergic, the 4- CF_3 analog (**22**) and the 4- OCF_3 analog. These trends suggest that the serotonin transporter can accommodate and translocate 3,4-substituted phenyl analogs better than the dopamine transporter but that the serotonin transporter cannot accommodate a 2-substituent very well due to steric and/or electronic effects.

Within a specific substituent, the trend shows that there is relatively more activity as a 5-HT releaser going from 2- to 3- to 4-substitution. For example, the DAT selectivity decreased for the fluoro compounds from 205 to 22 to 12. The 3-bromo and 4-bromo compounds have a DAT selectivity of 4.9 and 0.6 respectively, as do the same chloro analogs, which have ratios of 8.8 and 0.9. The CF_3 and OCF_3 analogs were inactive as 2-substituted compounds, but had DAT selectivities of ~ 0.2 at the 3-position and ~ 0.04 at the 4-position. These changes in DAT selectivity generally reflect weaker release activity at the DAT. The CF_3 analog data were found to be roughly the same as that reported by Cozzi et al. and show the same substitution pattern trend (Cozzi et al. 2013). The 2-substituted compounds were not very active at any of the transporters, with EC_{50} values of 8 μM . The relative selectivity for the 3-

CF₃ and 4-CF₃ analogs was 0.6 and <<0.04. One substituent that did not appear to vary much in selectivity was the CH₃ group, which had a DAT selectivity of 3.5, 4.1, and 2.4 at the 2-, 3-, and 4-positions respectively. The EC₅₀ data did not differ substantially between the 3 regioisomers and the compounds were all slightly dopaminergic.

Table 4 lists several di-substituted compounds as well as compounds in which the phenyl ring is replaced by an indole or naphthalene ring. Interestingly, all of the compounds with the exception of the 3,4-difluoro analog, had roughly the same DAT selectivity, ranging from 0.3 to 0.8. The 3,4-difluoro compound (**29**) was the only DAT selective compound, which correlates with observations that fluoro compounds are generally selective DA releasers. These results indicate that di-substitution at the 3- and 4-positions results in a small loss of DA release potency but a large increase in 5-HT release potency. This observation substantiates the notion that the SERT has additional room at the site of translocation than the DAT.

DAT selectivity of neurotransmitter releasers is important because it appears to affect their abuse potential. Wee et al showed that the reinforcing properties of releasers in rhesus monkeys varied with DAT selectivity, the most selective DAT compounds being more reinforcing compared to analogs that were non-selective (Wee et al. 2005). A similar trend was observed when studying the same set of releasers in locomotor stimulation in rats, with the more DAT selective compounds demonstrating more forward locomotor activity than less selective compounds (Baumann et al. 2011). These observed trends continue to be validated as novel releaser bath salts have emerged (Glennon and Dukat 2017; Simmler et al. 2013; Simmler et al. 2014). DAT selectivity also appears to affect the psychoactive effects of a releaser. Highly DAT selective releasers such as methamphetamine are potent locomotor stimulants. Releasers more selective for SERT can have empathogenic or entactogenic properties, such as 3,4-methylenedioxymethamphetamine (MDMA). DAT selectivity can therefore be used as a guide for both abuse liability as well as potential psychoactive effects.

Based on the in vitro data, one might expect that 3-fluoromethcathinone (**13**) would be a locomotor stimulant similar to methamphetamine because it is a highly potent, DAT selective compound (Simmler et al. 2013). Indeed, 3-fluoromethcathinone was found to induce locomotor stimulation and produced similar discriminative effects as methamphetamine in rats (Gatch et al. 2015).

Methcathinones such as the 4-CF₃ analog (**22**) and 4-OCF₃ analog (**23**) with low DAT selectivity might be less abused empathogens similar to MDMA. Such compounds have yet to be explored to our knowledge, but may generalize behaviorally to MDMA. The behavioral outcomes of analogs with similar DAT and SERT activity are difficult to predict. The 2-naphthyl analog of methcathinone (**27**) is roughly equipotent at DAT and SERT, and one might predict weak reinforcing and rewarding properties. Compound **27** was modestly self-administered in rats but induced conditioned place preference in mice similar to methamphetamine which suggests more complicated behavioral effects (Botanas et al. 2017).

While most of the focus of these compounds has been on their DAT and SERT activity, the noradrenergic properties of methcathinones might also play a significant role in their pharmacology (Hysek et al. 2011; Rothman et al. 2001). NE releasers have been shown to affect cocaine selfadministration (Banks et al. 2014; Kohut et al. 2017) and drugs elevating NE do not appear to be reinforcing (Wee and Woolverton 2004). In our experience with rat brain synaptosomes, NET release potencies generally vary in a similar fashion as DAT release potencies and compounds tend to be slightly more potent as NE releasers (unpublished). We generally observed the same trends with the methcathinones based on percent release, but with several notable exceptions. The 2-CF₃ analog **8** and 3-nitro analog **18** both appear to be selective NE releasers. This was unexpected but they may represent probe compounds for the study of the effects of NE release in various aspects of drug seeking behavior such as reinforcement and reward. Finally, the NE release potency of the 3-indoyl analog **28** appears to be much weaker than its DAT release potency compared to other bioisosteric analogs such as the 2-naphthyl analog **27**. This trend was also observed in a study of tryptamines (Blough et al. 2014b) and remains the only releaser scaffold which consistently releases DA more potently than NE.

Conclusion

The work presented herein is an SAR study of the DAT, SERT and NET properties of a set of twentyeight methcathinone analogs. All of the active compounds were found to be neurotransmitter releasers, as opposed to reuptake inhibitors. The choice of phenyl substituent had a dramatic effect on the DAT, SERT, and NET releasing activity of the analogs, as did the site of phenyl substitution. These differences demonstrate an ability to manipulate the DAT, SERT, and NET releasing potencies as well as the DAT selectivity of each analog, which plays a critical role in abuse liability and potential psychoactive effects. A rapid, high-yielding, and scalable synthesis of methcathinone hydrochloride analogs was also presented.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.
General Synthesis of Methcathionones

Table 1.

DAT-, SERT- and NET-mediated release activity of 2-phenyl substituted methcathinones.

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>phenacylamine (6)</p> </div> <div style="text-align: center;"> <p>cathinone (2)</p> </div> <div style="text-align: center;"> </div> </div>					
Compound	R	DA Release EC ₅₀ , nM ^a	5-HT Release EC ₅₀ , nM ^a	NE Release % @ 10 μM ^b	DAT Selectivity ^c
1	methcathinone (R = H)	49.9 ± 3.1	4,270 ± 270	100%	86
2	cathinone	83.1 ± 6.8	6,100 ± 750	95%	73
6	phenylacetylamine	208 ± 13	>10,000	96%	>48
7	F	48.7 ± 8.8	>10,000	85%	>205
8	CF ₃	>10,000	>10,000	69%	n/a
9	OCF ₃	>10,000	>10,000	33%	n/a
10	CH ₃	97.9 ± 11	347 ± 130	ND ^d	3.5

^aValues are mean ± S.D. of at least N=3 performed in duplicate as described in the methods.

^bValues are the mean % release of N=2 10 μM screens performed in duplicate.

^cDAT selectivity is calculated as SERT EC₅₀ / DAT EC₅₀.

^dND = not determined

Table 2.

DAT-, SERT- and NET-mediated release activity of 3-phenyl substituted methcathinones.

Compound	R	DA Release EC50, nM ^a	5-HT Release EC50, nM ^a	NE Release % @ 10 μM ^b	DAT Selectivity ^c
11	Br	28.0 ± 4.1	137 ± 75	112%	4.9
12	Cl	46.8 ± 4.0	410 ± 38	94%	8.8
13	F	64.8 ± 8.6	1,460 ± 640	100%	22
14	CF ₃	1,290 ± 370	297 ± 81	78%	0.2
15	OCF ₃	729 ± 150	188 ± 35	79%	0.3
16	CH ₃	70.6 ± 22	292 ± 39	94%	4.1
17	OCH ₃	129 ± 8	306 ± 42	68%	2.4
18	NO ₂	1,230 ± 290	1,400 ± 590	86%	1.1

^aValues are mean ± S.D. of at least N=3 performed in duplicate as described in the methods.

^bValues are the mean % release of N=2 10 μM screens performed in duplicate.

^cDAT selectivity is calculated as SERT EC50 / DAT EC50.

Table 3.

DAT-, SERT- and NET-mediated release activity of 4-phenyl substituted methcathinones.

Compound	R	DA Release EC50, nM ^a	5-HT Release EC50, nM ^a	NE Release % @ 10 μM ^b	DAT Selectivity ^c
19	Br	67.8 ± 12	42.5 ± 26	78%	0.6
20	Cl	74.7 ± 15	71.1 ± 12	102%	0.9
21	F ^d	119 ± 18	1,450 ± 360	107%	12
22	CF ₃ ^d	4,230 ± 1,500	270 ± 97	ND ^f	0.06
23	OCF ₃	7,510 ± 2,100	118 ± 71	ND ^f	0.02
24	CH ₃	49.1 ± 8.3 ^e	118 ± 26 ^e	94%	2.4
25	OCH ₃ ^d	881 ± 220	195 ± 86	101%	0.2

^aValues are mean ± S.D. of at least N=3 performed in duplicate as described in the methods.

^bValues are the mean % release of N=2 10 μM screens performed in duplicate.

^cDAT selectivity is calculated as SERT EC50 / DAT EC50.

^dIn the course of our studies, these compounds were included as part of a separate methcathinone study (Bonano et al. 2015). Values reported in this Table were generated by our laboratory.

^eValues are from (Bonano et al. 2015).

^fND = not determined

Table 4.

DAT-, SERT- and NET-mediated release activity of other methcathinones.

Compound	X	Y	DA Release EC ₅₀ , nM ^a	5-HT Release EC ₅₀ , nM ^a	NE Release % @ 10 μM ^b	DAT Selectivity ^c
26	-		55.2 ± 12	21.6 ± 4.4	92%	0.4
27	-		33.8 ± 3.6	27.1 ± 14	94%	0.8
28	-		92.8 ± 21	41.3 ± 10	55%	0.4
29	Cl	Cl	178 ± 33	74.4 ± 31	103%	0.4
30	F	F	227 ± 44	960 ± 450	85%	4.2
31	Cl	CH ₃	124 ± 7.4	41.7 ± 14	94%	0.3

^aValues are mean ± S.D. of at least N=3 performed in duplicate as described in the methods.^bValues are the mean % release of N=2 10 μM screens performed in duplicate.^cDAT selectivity is calculated as SERT EC₅₀ / DAT EC₅₀.