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Pharmacological examination of trifluoromethyl ring-substituted methcathinone analogs

Nicholas V. Cozzi [a,c,*], Simon D. Brandt [b], Paul F. Daley [c], John S. Partilla [d], Richard B. Rothman [d], Andreas Tulzer [e], Harald H. Sitte [e], Michael H. Baumann [d]

[a] Neuropharmacology Laboratory, 2695 Medical Sciences Center, Department of Cell and Regenerative Biology, University of Wisconsin School of Medicine and Public Health, 1300 University Avenue, Madison, WI 53706, USA
[b] School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK
[c] Alexander Shulgin Research Institute, 1483 Shulgin Road, Lafayette, CA 94549, USA
[d] Translational Pharmacology Section, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, 333 Cassell Drive, Suite 4500, Baltimore, MD 21224, USA
[e] Center for Physiology and Pharmacology, Institute of Pharmacology, Waehringerstrasse 13a, Vienna A-1090, Austria

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ABSTRACT

Cathinones are a class of drugs used to treat various medical conditions including depression, obesity, substance abuse, and muscle spasms. Some "designer" cathinones, such as methcathinone, mephedrone, and methylone, are used nonclinically for their stimulant or entactogenic properties. Given the recent rise in nonmedical use of designer cathinones, we aimed to improve understanding of cathinone pharmacology by investigating analogs of methcathinone with a CF_3 substituent at the 2-, 3-, or 4-position of the phenyl ring (TFMAPs). We compared the TFMAPs with methcathinone for effects on monoamine uptake transporter function *in vitro* and *in vivo*, and for effects on locomotor activity in rats. At the serotonin transporter (SERT), 3-TFMAP and 4–TFMAP were 10-fold more potent than methcathinone as uptake inhibitors and as releasing agents, but 2-TFMAP was both a weak uptake inhibitor and releaser. At the norepinephrine and dopamine transporters (NET and DAT), all TFMAP isomers were less potent than methcathinone as uptake inhibitors and releasers. *In vivo*, 4-TFMAP released 5-HT, but not dopamine, in rat nucleus accumbens and did not affect locomotor activity, whereas methcathinone increased both 5-HT and dopamine and produced locomotor stimulation. These experiments reveal that TFMAPs are substrates for the monoamine transporters and that phenyl ring substitution at the 3- or 4-position increases potency at SERT but decreases potency at NET and DAT, resulting in selectivity for SERT. The TFMAPs might have a therapeutic value for a variety of medical and psychiatric conditions and may have lower abuse liability compared to methcathinone due to their decreased DAT activity.

1. Introduction

Cathinones are phenylisopropylamine compounds with a carbonyl oxygen at the benzylic position of the molecule. The prototype drug in this class is cathinone itself, a naturally-occurring substance that has been used for centuries in plant form for its stimulant effects (Alles et al., 1961). Other drugs in this class include the psychostimulant methcathinone (Fig. 1), the antidepressant and stop-smoking aid bupropion, antispasmodic agents eperisone and tolperisone, and the anorectic agent diethylpropion. Cathinones with a wide range of activities have been synthesized and some may be useful as new therapeutic agents with improved adverse effect profiles for treating depression, obesity, cocaine and nicotine addiction, and as centrally acting muscle relaxants (Carroll et al., 2009, 2010; Cozzi et al., 2005; Foley and Cozzi, 2003; Lukas et al., 2010; Nisijima et al., 1998; Shiozawa et al., 1995). Identified biological targets for cathinones include acetylcholine, serotonin, dopamine, norepinephrine, histamine, and sigma-1 receptors, voltage-gated sodium and calcium ion channels, plasma membrane transporters for serotonin, norepinephrine, and dopamine (SERT, NET, and DAT, respectively) and the vesicle monoamine transporter 2 (VMAT2) (Baumann et al., 2012; Carroll et al., 2009, 2010; Cozzi and Foley, 2003; Cozzi et al., 1999, 2005, 2007; Foley and Cozzi, 2003; Fujioka and Kuriyama, 1985; Hofer et al., 2006; Kehr et al., 2011; Kocsis et al., 2005; Slemmer et al., 2000).

Lately, psychoactive "designer" cathinones, marketed as "bath salts", have become commercially available for purchase over the Internet and in retail shops. These compounds are used outside of

* Corresponding author at: University of Wisconsin School of Medicine and Public Health, Department of Cell and Regenerative Biology, Neuropharmacology Laboratory, 2695 Medical Sciences Center, 1300 University Avenue, Madison, WI 53706, USA. Tel.: +1 608 262 7596.
E-mail address: cozzi@wisc.edu (N.V. Cozzi).

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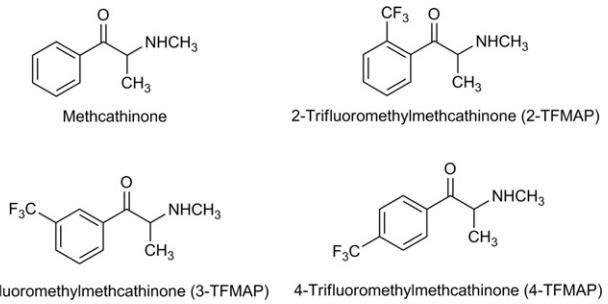

Fig. 1. Chemical structures of methcathinone and trifluoromethylmethcathinone (TFMAP) positional isomers.

medical settings for personal experimentation, mood elevation, and for entertainment purposes as "legal highs". Some of the most popular designer cathinones include methylone (3,4-methylenedioxymethcathinone), mephedrone (4-methylmethcathinone), and 3,4-methylenedioxypyrovalerone (MDPV) (Brandt et al., 2010, 2011; EMCDDA, 2011; Kelly, 2011; Vardakou et al., 2011). The effects of these agents range from amphetamine-like stimulation (alertness, loss of fatigue, increased locomotor activity) to entactogenic effects (increased empathy, reduced fear) resembling those produced by 3,4-methylenedioxymethamphetamine (MDMA) (Mithoefer et al., 2011; Nichols et al., 1986). The emergence of designer cathinones on the recreational drug market represents an approach to scientific data mining of the existing patent literature and contemporary medicinal chemistry literature. This approach is certainly not new, but it is worth noting that a large number of cathinone derivatives have appeared very recently, especially in the European Union. An annual monitoring exercise, carried out by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), revealed that synthetic cathinones represent a key substance class, with 34 new designer cathinones reported between 2005 and 2011 (EMCDDA, 2012). In the United States, mephedrone, methylone, and MDPV were classified as Schedule I controlled substances in October 2011(Anonymous, 2011).

We recently prepared three trifluoromethyl ring-substituted positional isomers of methcathinone (trifluoromethyl-methylaminopropiophenones; TFMAPs) as reference standards for the analytical characterization of new cathinones for forensic and clinical purposes (Brandt et al., 2012). These new compounds incorporate a CF_3 substituent at the 2-, 3-, or 4-position of the phenyl ring (2-TFMAP, 3-TFMAP, and 4-TFMAP, respectively; Fig. 1). Here, we examine the TFMAPs *in vitro* and *in vivo* for their neurochemical and behavioral effects. We tested the TFMAPs for their abilities to inhibit neurotransmitter uptake *via* the human transporters hSERT, hNET, and hDAT. We then tested the TFMAPs and methcathinone as releasing agents at the rat SERT (rSERT) using rat brain synaptosomes preloaded with [^{3}H]5-HT, and at the rNET and rDAT using synaptosomes preloaded with [^{3}H]1-methyl-4-phenylpyridin-1-ium ([^{3}H]MPP$^+$), a model catecholamine transporter substrate (Wall et al., 1995). Lastly, we used *in vivo* microdialysis to compare the effects of intravenously-administered 4-TFMAP and methcathinone on extracellular dopamine and 5-HT levels in nucleus accumbens, while simultaneously monitoring spontaneous locomotor activity in awake, freely-moving rats.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 300–350 g were housed under conditions of controlled temperature (22 ± 2 °C) and humidity (45 ± 5%) with food and water available *ad libitum* and under a 12 h light–dark cycle. Rats were maintained in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, and procedures were carried out in accordance with the Animal Care and Use Committee of the NIDA IRP. Rats were housed in pairs upon receipt and were allowed at least two weeks for acclimatization to the vivarium conditions before being used in experiments.

2.2. Drugs and reagents

Methcathinone was prepared *via* the permanganate oxidation of racemic ephedrine as described (Zhingel et al., 1991). To synthesize the new TFMAP compounds (Fig. 1), commercially available 2-, 3-, or 4-positional isomers of trifluoromethylpropiophenones (Sigma-Aldrich, St. Louis, MO, USA) were used as precursors to prepare 2-trifluoromethylmethcathinone (2-TFMAP; 2-(methylamino)-1-(2-trifluoromethylphenyl)propan-1-one), 3-trifluoromethylmethcathinone (3-TFMAP; 2-(methylamino)-1-(3-trifluoromethylphenyl)propan-1-one), and 4-trifluoromethylmethcathinone (4-TFMAP; 2-(methylamino)-1-(4-trifluoromethylphenyl)propan-1-one), respectively. The TFMAPs were synthesized using a modification of the method of Schmidt and Eberhard (1915). This consists of side-chain α-bromination of the appropriate trifluoromethylpropiophenone with elemental bromine in dichloromethane, followed by displacement of the bromine with a 20% solution of methylamine in 1:1 water:ethanol to generate the respective trifluoromethyl-substituted methylaminopropiophenone. After work-up, all target compounds were isolated as the hydrochloride salts. Chemical purity and identity of these compounds were verified by melting-point determination, thin-layer chromatography, proton nuclear magnetic resonance, and mass spectrometry. All analytical data were consistent with the assigned structures and analytical details have been published elsewhere (Brandt et al., 2012). [^{3}H]Serotonin ([^{3}H]5-HT, specific activity = 20–30 Ci/mmol), [^{3}H]dopamine ([^{3}H]DA, specific activity = 27.5 Ci/mmol), and [^{3}H]norepinephrine ([^{3}H]NE, specific activity = 71.7 Ci/mmol) were purchased from Perkin-Elmer (Shelton, CT, USA). [^{3}H]1-methyl-4-phenylpyridin-1-ium acetate ([^{3}H]MPP$^+$, specific activity = 85 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). Cell culture medium and antibiotics were obtained from Life Technologies (Gaithersburg, MD, USA). Fetal bovine serum was purchased from Hyclone (Logan, UT, USA). Citalopram, desipramine, GBR12935, nomifensine, pargyline, buffer components, and other miscellaneous chemicals were acquired from multiple commercial sources.

2.3. Inhibition of hSERT-mediated [^{3}H]5-HT uptake into human platelets

Human platelets, which naturally express the human SERT (hSERT), were used to assess drug activity on [^{3}H]5-HT uptake. Outdated human platelets were obtained from the blood bank at the University of Wisconsin Clinical Sciences Center (Madison, WI, USA). Platelets from 5 to 10 donors were pooled, 10% dimethylsulfoxide was added, and aliquots were stored frozen at −80 °C until use. For assays, 5 mL of platelets were thawed and added to 20 mL ice-cold Krebs-Ringer-HEPES (KRH) buffer containing (mM): NaCl (124.0), KCl (2.9), $MgSO_4$ (1.3), KH_2PO_4 (1.2), $CaCl_2$ (2.4), D-glucose (5.2), HEPES (25.0), sodium ascorbate (0.1), pargyline (0.1), pH = 7.4. The platelet suspension was subjected to centrifugation (4340 × *g*, 4 °C, 10 min) and the supernatant was discarded. The pellet was washed twice by resuspension in KRH followed by centrifugation. The final pellet was suspended in 20 mL ice-cold KRH and stored on ice until use. The ability of platelets to accumulate [^{3}H]5-HT was measured in the absence and presence of various concentrations of TFMAP isomers and methcathinone as follows: a 400 μL aliquot of