Short communication

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Analytical characterization of three trifluoromethyl-substituted methcathinone isomers

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Cathinone derivatives display a wide range of pharmacological activities and uses; some of them are used as prescription medicines, while others are encountered within a recreational context and are available without a prescription over the Internet and in retail shops around the world. One of the difficulties involved in the unambiguous identification of these new psychoactive substances is the lack of suitable reference standards, particularly when dealing with unreported derivatives and positional isomers. In order to address this need, three trifluoromethyl analogues of the psychostimulant methcathinone, with a CF_3 substituent at the 2-, 3- and 4-position of the phenyl ring (2-TFMAP 1, 3-TFMAP 2 and 4-TFMAP 3), have been prepared for analytical characterization using ATR-FTIR, ^1H and ^{13}C NMR, and GC-(EI/CI)-ion trap-MS. Differentiation among isomers was feasible by IR, for example when assessing the carbonyl stretch at 1711 (1), 1693 (2) and 1688 (3) cm^{-1}, respectively. In addition to the expected iminium base peak at *m/z* 58, EI-MS displayed key ions at *m/z* 173, 145, 125, 95, and 75. Separation of isomers was possible under GC conditions. A characteristic feature under CI conditions was the loss of water from the $[M+H]^+$ yielding *m/z* 214 in addition to *m/z* 58. Studies currently underway show that the three CF_3-methcathinone analogues have central nervous system effects and that the 4-CF_3 isomer 3 is more potent as a serotonin uptake inhibitor and releasing agent than the 3-CF_3 and 2-CF_3 counterparts. Copyright © 2012 John Wiley & Sons, Ltd.

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Introduction

The cathinone nucleus provides access to a diverse array of bioactive compounds that have been explored for a range of clinical applications including as adjuncts in smoking-cessation programmes and as antidepressants.[1–4] On the other hand, the emergence of substituted cathinones and other new psychoactive substances on the recreational drug market[5,6] is a challenging phenomenon for scientific communities, legislators, and policy makers.[6] The term 'legal highs', although a misnomer in cases where these derivatives fall under legislative control, has caught the attention of the media and scientific circles. The identification and full characterization of substituted cathinones continues to play a key role in assessing their prevalence of use as recreational drugs available from Internet sources and retail shops.[7–9]

One of the difficulties involved in the unambiguous identification of these new psychoactive substances is the lack of suitable reference standards, particularly when dealing with unknown derivatives or positional isomers.[10] In order to address this need, three trifluoromethyl analogues of methcathinone have been prepared with a CF_3 substituent at the 4-, 3- and 2-position of the phenyl ring (2-TFMAP **1**, 3-TFMAP **2** and 4-TFMAP **3**), respectively. The rationale behind this approach was based on the requirement for new derivatives used for pharmacological studies and to extend existing knowledge regarding the analytical characterization of new cathinones used for forensic and clinical purposes.

Experimental

Three trifluoromethyl ring-substituted methcathinone analogues **1**–**3** were prepared using an adapted standard procedure.[11] Bromination of 2-, 3-, and 4-trifluoromethylpropiophenone was carried out in dichloromethane by dropwise addition of a 20% (*v/v*) solution of bromine in dichloromethane. A few drops of acetic acid were used to initiate the reaction. The resulting α-bromoketones were dissolved in ethanol, then condensed at 0 °C with 20% aqueous *N*-methylamine to generate the respective trifluoromethyl methcathinone positional isomers. Isolation of products followed precipitation as hydrochloride salts.

Instrumentation

Melting points were determined with a Mel-Temp® capillary melting point apparatus (Barnstead/Thermolyne, Dubuque, IA, USA) and are uncorrected.

Samples dissolved in methanol (1 µl at 0.5 mg/ml) were subjected to both electron ionization (EI) and chemical ionization (CI) modes. Both EI and CI mass spectra (scan range *m/z* 40–m/z 500) were obtained on a Varian 220-MS ion trap MS equipped

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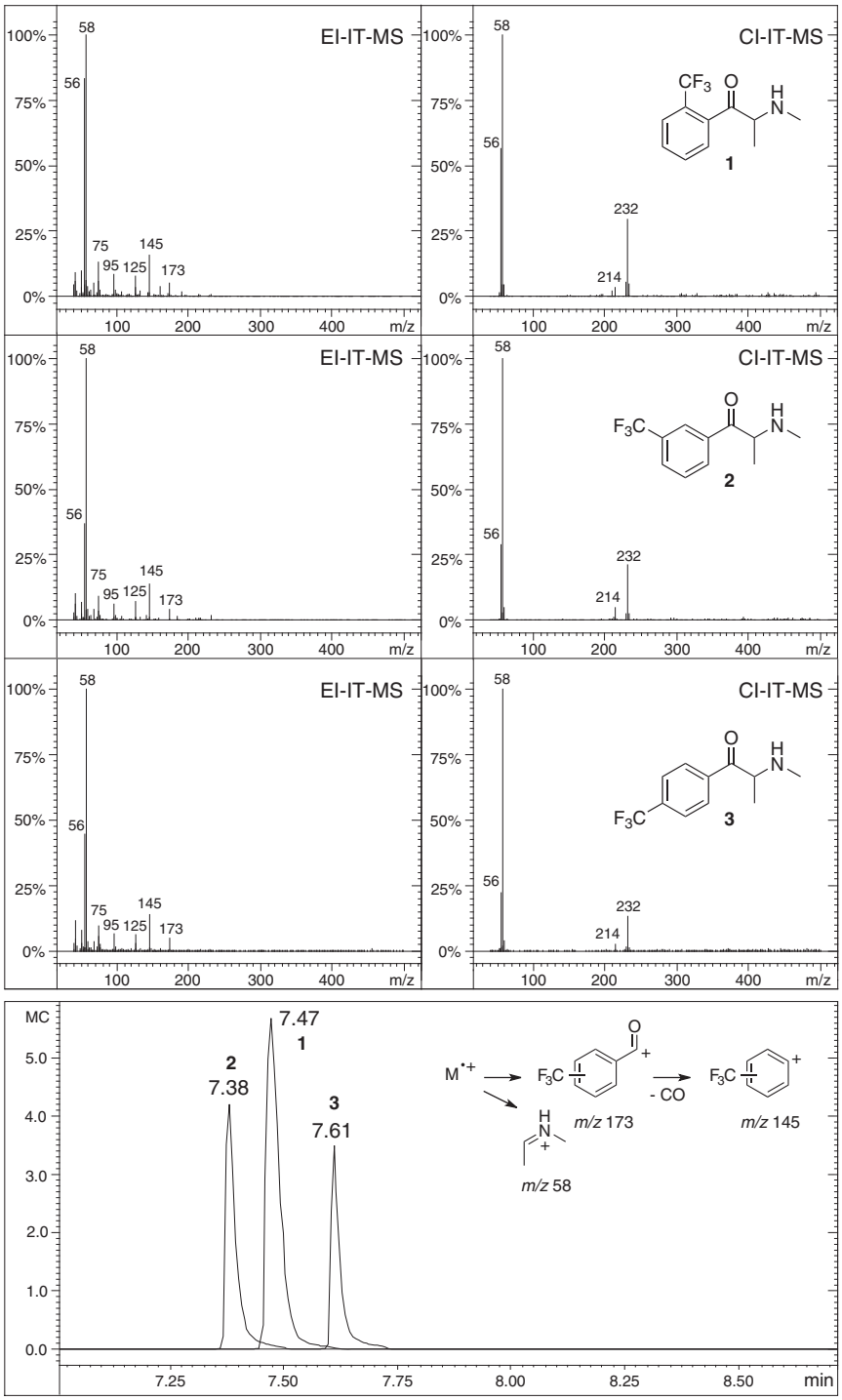

Figure 1. Electron ionization and chemical ionization ion trap mass spectra of TFMAP isomers **1**–**3**. Separation was observed under GC conditions.

with a Varian 450-GC gas chromatograph and a Varian 8400 autosampler. Data handling was carried out with the workstation, Version 6.91 software. The carrier gas was helium at a flow rate of 1 ml/min using the EFC constant flow mode. A CP-1177 injector (275 °C) was used in split mode (1:50). Transfer line, manifold and ion trap temperatures were set at 280, 80, and 220 °C, respectively. High performance liquid chromatography (HPLC) grade methanol was used as the liquid CI reagent. CI ionization parameters (0.5 s/scan): CI storage level 19.0 *m/z*; ejection amplitude 15.0 *m/z*; background mass 55 *m/z*; maximum ionization time 2000 μs; maximum reaction time 40 ms; target TIC 5000 counts. The number of ions in the trap was controlled by an automatic gain control function. Separations were carried out using 30 m × 0.25 mm (0.25 μm film thickness) Factor Four capillary column (VF-5 ms, Varian). The column temperature was programmed as follows: 100 °C held for 1 min, then heated at 20 °C/min to 280 °C and held constant for 10 min; total run time was 20 min.

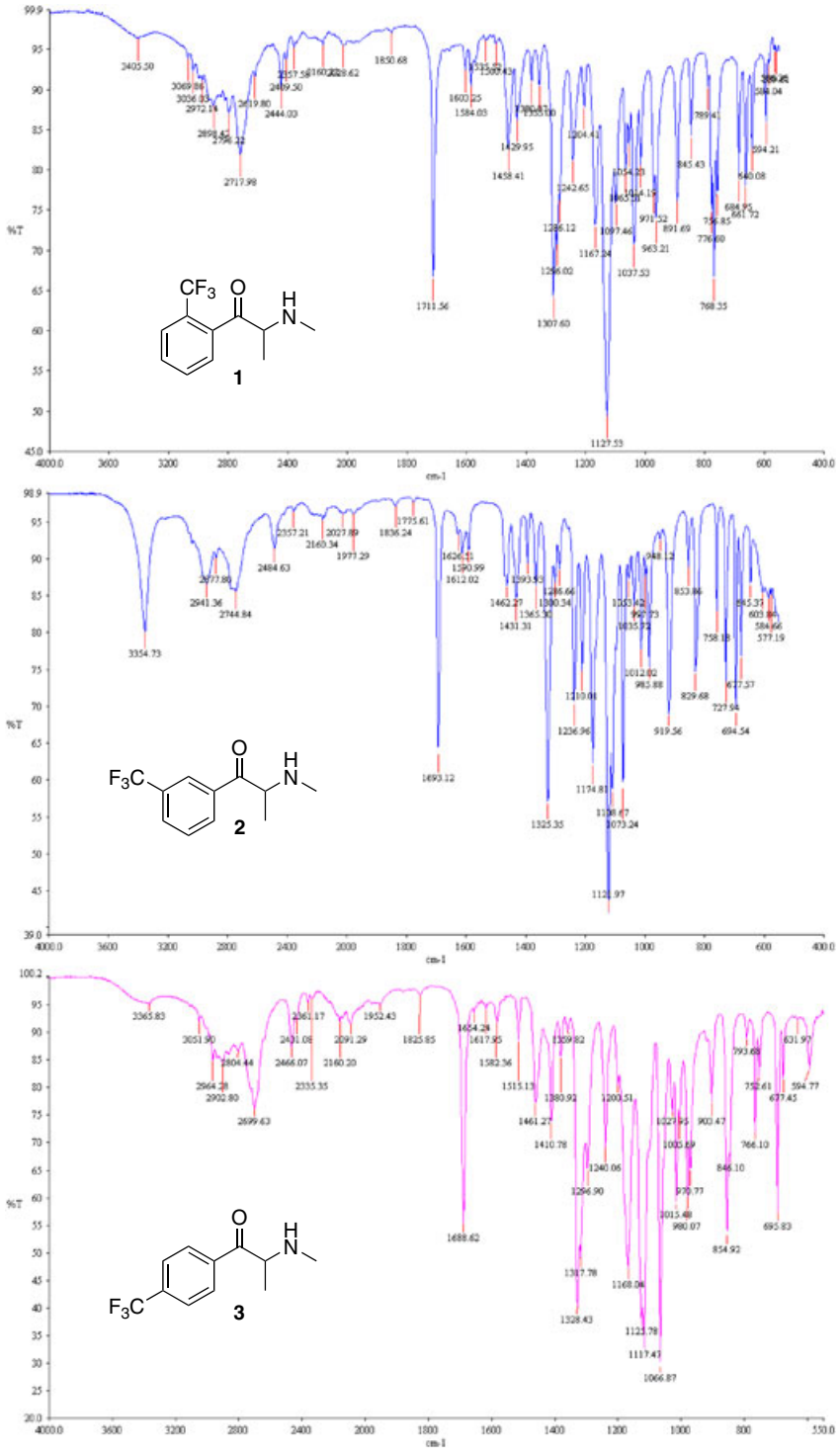

Figure 2. ATR-FTIR spectra of TFMAP isomers **1**–**3**.

Nuclear magnetic resonance (NMR) spectra were recorded using a 300 MHz Bruker Avance 300 spectrometer. ^{1}H and ^{13}C and DEPT-135 (and ^{13}C APT) NMR spectra were recorded in CD_3OD and chemical shifts are reported relative to TMS at $\delta = 0$ ppm.

Infrared (IR) spectra were obtained on a Perkin Elmer Spectrum BX FTIR model using a Pike MIRacle ATR system. Data were acquired with the Spectrum v5.01 software (scan range 4000−400 cm^{-1}, resolution 4 cm^{-1}, 16 scans).

Results and discussion

NMR data for 1−3

*2-Trifluoromethylmethcathinone **1** hydrochloride (2-TFMAP):*

M.p. 173–175 °C. ^{1}H NMR (CD_3OD): 7.95-7.91 (2H, m, Ar-H), 7.85-7.82 (2H, m, Ar-H), 5.01 (1H, q, α-CH, J 7.4 Hz), 2.83 (3H, s, *N*-CH_3), 1.46 (3H, d, α-CH_3, J 7.3 Hz). ^{13}C NMR: 199.2, 135.7, 133.9, 133.8, 129.8, 128.9 (q, J 5.3 Hz Ar-CH), 126.6, 123.0, 62.9, 31.6, 14.1.

*3-Trifluoromethylmethcathinone **2** hydrochloride (3-TFMAP):*

M.p. 163.5-165 °C. ^{1}H NMR (CD_3OD): 8.33-8.31 (2H, m, Ar-H), 8.05 (1H, d, Ar-H, J 7.9 Hz), 7.84 (1H, t, Ar-H, J 8.1 Hz), 5.20 (1H, q, α-CH, J 7.3 Hz), 2.80 (3H, s, *N*-CH_3), 1.59 (3H, d, α-CH_3, J 7.3 Hz). ^{13}C NMR: 196.3, 135.1, 133.7, 133.1, 132.3 (q, J 3.3 Hz), 131.6, 126.5 (q, J 3.8 Hz), 123.3, 60.8, 31.7, 15.9.

*4-Trifluoromethylmethcathinone **3** hydrochloride (4-TFMAP):*

M.p. 189–192 °C. ^{1}H NMR (CD_3OD): 8.24 (2H, d, H-2/6, J 8.7 Hz), 7.92 (2H, d, H-3/5, J 8.7 Hz), 5.17 (1H, q, α-CH, J 7.2 Hz), 2.80 (3H, s, *N*-CH_3), 1.59 (3H, d, α-CH_3, J 7.3 Hz). ^{13}C NMR: 196.6, 137.3, 136.5, 130.8, 127.3 (q, J 3.8 Hz), 123.2, 61.0, 31.8, 15.8.

Mass spectrometry

For those who operate within a forensic or clinical environment, the ability to differentiate among positional isomers of bioactive compounds can be hampered by the lack of reference materials. Recent reports, involving the preparation and characterization of 2-, 3- and 4-substituted isomers of methedrone (methoxymethcathinones),[12] flephedrone (fluoromethcathinones)[13] and mephedrone (methylmethcathinones),[14] have provided important data to tackle this need. In order to address the requirement for an extended range of new cathinone derivatives, three trifluoromethyl analogues of methcathinone have been prepared with a CF_3 substituent at the 2-, 3- and 4-position of the phenyl ring (2-TFMAP **1**, 3-TFMAP **2** and 4-TFMAP **3**).

Inspection of electron ionization ion trap mass spectra (EI-IT-MS) revealed the presence of the typical iminium base peak at *m/z* 58 found with ethylamine-type side chains. The even-electron counterpart following α-cleavage of $M^{\bullet+}$ was the trifluoromethyl benzoyl species at *m/z* 173 followed by the classical neutral loss of CO which gave rise to *m/z* 145. Extensive formation of an *m/z* 56 ion was also observed which pointed towards a potential degradation of the parent molecule following exposure to gas chromatography-mass spectrometry (GC-MS) conditions. Similar artifact formation has been observed by Jacob and Shulgin in 1996 when discussing the analytical profile of methylone (3,4-methylenedioxymethcathinone).[15] A similar phenomenon was then reported during the characterization of fluorinated methcathinones[13] and naphthylpyrovalerone isomers.[10] Under CI-IT-MS conditions, the protonated molecule $[M+H]^+$ was detected at *m/z* 232, in addition to the *m/z* 58 base peak. The presence of a fragment at *m/z* 214 was also observed which indicated a potential loss of water from the $[M+H]^+$. This has been observed previously during analysis of other *N*-monoalkylated cathinones but not with derivatives carrying a pyrrolidine ring instead.[8,9] The inability to differentiate the three trifluoromethyl isomers by mass spectrometry was expected but adequate separation was, apart from nuclear magnetic resonance spectroscopy, obtained under GC conditions (Figure 1). Although separation was deemed acceptable for the purpose of successful differentiation future studies might reveal further improvement of peak shapes by implementation of appropriate derivatization procedures. One recent example was reported for the detection of 4-methylmethcathinone (mephedrone) when using 2,2,2-trichloroethyl chloroformate which resulted in formation of the corresponding carbamate derivative.[16]

Infrared spectroscopy

The corresponding attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectra are summarized in Figure 2. Differentiation among isomers appeared to be feasible under the conditions used, for example, when comparing the carbonyl absorption bands alone at 1711 (2-TFMAP **1**), 1693 (3-TFMAP **2**) and 1688 (4-TFMAP **3**) cm^{-1}, respectively. An additional example included inspection of the region between 1000 and1200 cm^{-1}. For example, a comparison between the most intense bands offered distinguishable signals at 1127 (**1**), 1121 (**2**) and 1066 (**3**) cm^{-1}.

The ability to inhibit uptake and to affect release of biogenic amines via uptake transporters for dopamine (DAT), noradrenaline (NET), and serotonin (SERT) influences the psychoactive properties of methcathinone and related cathinone derivatives. Detailed investigations into the pharmacological properties of the three synthesized TFMAP derivatives **1**−**3** at the uptake transporters are currently underway. Initial data indicate that the 4-CF_3 isomer **3** is more potent as a serotonin uptake inhibitor and releasing agent than the 3-CF_3 and 2-CF_3 counterparts or methcathinone.[17] However, at the NET and DAT, all trifluoromethyl ring-substituted isomers have decreased potency compared to methcathinone, both as uptake inhibitors and releasing agents. Interestingly, substitution at the 2-position shifted selectivity towards catecholamine transporters vs. serotonin transporters.[17]

Conclusion

The analytical characterization of three trifluoromethyl ring-substituted methcathinones in this communication adds to a growing body of information that is hoped to be of use to both the clinical and forensic communities. The availability of these new isomers will enable further studies to be carried out to improve understanding about the pharmacological properties of cathinones and to elucidate structure-activity relationships when developing medicinal products. The implementation of ATR-FTIR and NMR spectroscopy and GC-(EI/CI)-MS provided sufficient data to distinguish between these three positional isomers.

References

[1] K. F. Foley, N. V. Cozzi. Novel aminopropiophenones as potential antidepressants. *Drug Dev. Res.* **2003**, *60*, 252.
[2] P. C. Meltzer, D. Butler, J. R. Deschamps, B. K. Madras. 1-(4-Methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (pyrovalerone) analogues: a promising class of monoamine uptake inhibitors. *J. Med. Chem.* **2006**, *49*, 1420.
[3] F. I. Carroll, B. E. Blough, P. Abraham, A. C. Mills, J. A. Holleman, S. A. Wolckenhauer, *et al*. Synthesis and biological evaluation of bupropion analogues as potential pharmacotherapies for cocaine addiction. *J. Med. Chem.* **2009**, *52*, 6768.
[4] F. I. Carroll, B. E. Blough, S. W. Mascarella, H. A. Navarro, J. B. Eaton, R. J. Lukas, *et al*. Synthesis and biological evaluation of bupropion analogues as potential pharmacotherapies for smoking cessation. *J. Med. Chem.* **2010**, *53*, 2204.
[5] EMCDDA-Europol 2010 Annual Report on the Implementation of Council Decision 2005/387/JHA, European Monitoring Centre for Drugs and Drug Addiction, Lisbon, **2011**.
[6] H. R. Sumnall, M. Evans-Brown, J. McVeigh. Social, policy, and public health perspectives on new psychoactive substances. *Drug Test. Analysis* **2011**, *3*, 515.
[7] J. Ramsey, P. I. Dargan, M. Smyllie, S. Davies, J. Button, D. W. Holt, *et al*. Buying 'legal' recreational drugs does not mean that you are not breaking the law. *QJM* **2010**, *103*, 777.
[8] S. D. Brandt, H. R. Sumnall, F. Measham, J. Cole. Analyses of second-generation 'legal highs' in the UK: initial findings. *Drug Test. Analysis* **2010**, *2*, 377.
[9] S. D. Brandt, S. Freeman, H. R. Sumnall, F. Measham, J. Cole. Analysis of NRG 'legal highs' in the UK: identification and formation of novel cathinones. *Drug Test. Analysis* **2011**, *3*, 569.

[10] S. D. Brandt, R. C. R. Wootton, G. De Paoli, S. Freeman. The naphyrone story: The alpha or beta-naphthyl isomer? *Drug Test. Analysis* **2010**, *2*, 496.
[11] E. Schmidt, A. Eberhard. Ueber das Ephedrin und verwandte Verbindungen. *Arch. Pharm.* **1915**, *253*, 62.
[12] T. Belal, T. Awad, J. DeRuiter, C. R. Clark. GC-IRD methods for the identification of isomeric ethoxyphenethylamines and methoxymethcathinones. *Forensic Sci. Int.* **2009**, *184*, 54.
[13] R. P. Archer. Fluoromethcathinone, a new substance of abuse. *Forensic Sci. Int.* **2009**, *185*, 10.
[14] J. D. Power, P. McGlynn, K. Clarke, S. D. McDermott, P. Kavanagh, J. O'Brien. The analysis of substituted cathinones. Part 1: Chemical analysis of 2-, 3- and 4-methylmethcathinone. *Forensic Sci. Int.* **2011**, *212*, 6.
[15] P. Jacob III, A. T. Shulgin. Preparation of novel N-substituted-2-amino-3',4'-methylenedioxypropiophenones as anti-depressant and anti-parkinsonism agents. US Patent WO9639133(A1), **1996**.
[16] G. Frison, M. Gregio, L. Zamengo, F. Zancanaro, S. Frasson, R. Sciarrone. Gas chromatography/mass spectrometry determination of mephedrone in drug seizures after derivatization with 2,2,2-trichloroethyl chloroformate. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 387.
[17] N. V. Cozzi, P. F. Daley, D. L. Evans, J. S. Partilla, R. B. Rothman, A. E. Ruoho, *et al*. Trifluoromethyl ring-substituted methcathinone analogs: Activity at monoamine uptake transporters. *FASEB J.* **2011**, *1083*, 1.