Synthesis, analytical characterization, and monoamine transporter activity of the new psychoactive substance 4-methylphenmetrazine (4-MPM), with differentiation from its *ortho*- and *meta*- positional isomers

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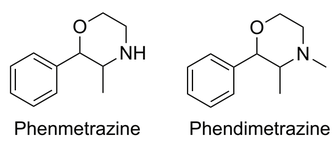
Introduction

The European drug market continues to evolve, with a wide range of new psychoactive substances (NPS) emerging over the last decade. By the end of 2017, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) was monitoring more than 670 NPS that have been identifed in Europe, 51 new substances were first identifed in 2017.[1] Phenmetrazine (3-methyl-2-phenylmorpholine) and an array of its analogs form a class of psychostimulants that are well documented in the patent and scientific literature. Phenmetrazine (Figure 1) is a synthetic amphetamine derivative that consists of a phenylisopropylamine skeleton with the terminal amine incorporated into a morpholine ring.[2] Phenmetrazine and its *N*-methylated analog phendimetrazine (Figure 1) were introduced as an anoretic medications in the 1950s.



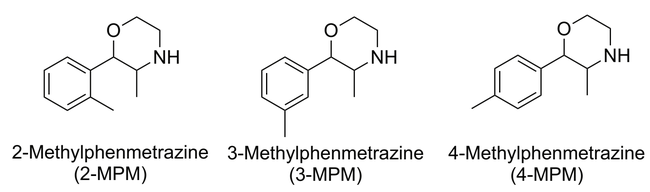


Figure 1. Chemical structures of phemetrazine, its *N*-methyl derivative phendimetrazine and the methylphenmetrazine (MPM) positional isomers.

Phenmetrazine is a potent substrate-type releaser at dopamine transporters (DAT) and norepinephrine transporters (NET), with less potent effects at serotonin transporters (SERT).[3] Phendimetrazine exerts its pharmacological effects via *N*-demethylation to phenmetrazine. Both substances are listed in the United Nations Convention on Psychotropic Substances 1971.[4] The manipulation of the phenmetrazine structure by substitution on the phenyl or morpholine ring creates a variety of suitable candidates for the NPS market. In 2014, 3-FPM was first notified by the EMCDDA .[5] A recent study on FPM provided the analytical profile of all three positional isomers (2-, 3- and 4-FPM) and identified 3-FPM in a number of samples obtained from Internet Vendors.[6] Pharmacological studies on the three FPM isomers revealed that 3-FPM and its positional isomers are substrate type releasing agents at monoamine transporters with marked potency at DAT and NET.[7] The present study reports on two phenmetrazine analogs that have been encountered on the NPS drug market following the introduction of 3-FPM, namely 4-MPM and 3-MPM. This study describes the syntheses, analytical characterization and pharmacological evaluation of the positional isomers of MPM.

Synthesis

The synthesis scheme employed for the preparation of the MPM positional isomers was adapted from McLaughlin et al (Figure 2).[6] Three samples, two advertised as 4-MPM and one as 3-MPM, were obtained from two different Internet retailers and subjected to extensive characterization using a variety of analytical platforms. One of the vendor samples of 4-MPM consisted of crystals whereas the other was a powder. The 3-MPM vendor sample was in powder form.

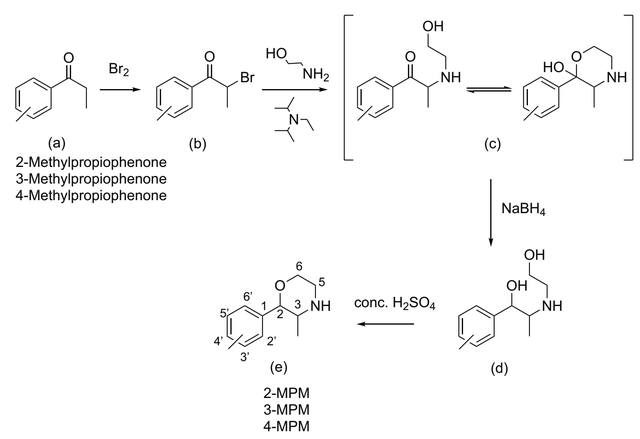


Figure 2. The synthesis pathway employed for the preparation of 2-, 3- and 4-MPM isomers.

Characterization (GC-MS)

Analytical characterizations employed various chromatographic, spectroscopic, and mass spectrometric platforms. For gas chromatography mass spectrometry (GC-MS) analysis, derivatization of the samples with trifluoroacetic anhydride (TFAA) was employed and retention times of 15.85, 16.05, and 16.34 minutes were recorded for 3-, 2-, and 4-MPM isomers, respectively (Figure 3A). The electron ionization (EI) mass spectral data for the MPM-TFAA isomers were identical (Figure 3B). A proposed fragmentation pattern for the MPM-TFAA isomers is outlined in Figure 3C. In the EI mass spectra of each MPM-TFAA isomer, the molecular ion was detected at *m/z* 287. The fragment observed at *m/z* 218 might have been the result of radical loss of CF_3 via cleavage of the nitrogen in the morpholine ring. A further loss of carbon monoxide would be consistent with *m/z* 190. Two dominant fragments were observed at *m/z* 167 and *m/z* 70. The *m/z* 167 indicated a potential loss of the oxonium species, which is suggested to give rise to a TFAA-azetidine species ($C_6H_8F_3NO^{\bullet+}$). The base peak at *m/z* 70 may be accounted for by the loss of the ring substituted methylbenzyl alcohol, which is proposed to form an azete species (2-methyl-2,3-dihydroazete, $C_4H_8N^+$). In addition, the loss of a TFAA-diethylamine entity from the molecular ion leads to the formation of an oxonium ion at *m/z* 119 ($C_8H_7O^+$). The fragment at *m/z* 54 may reflect the formation of an aziridine species ($C_3H_6N^+$). The GC-MS data recorded for the vendor samples of 3- and 4-MPM were consistent with the data of the respective synthesized reference standard.

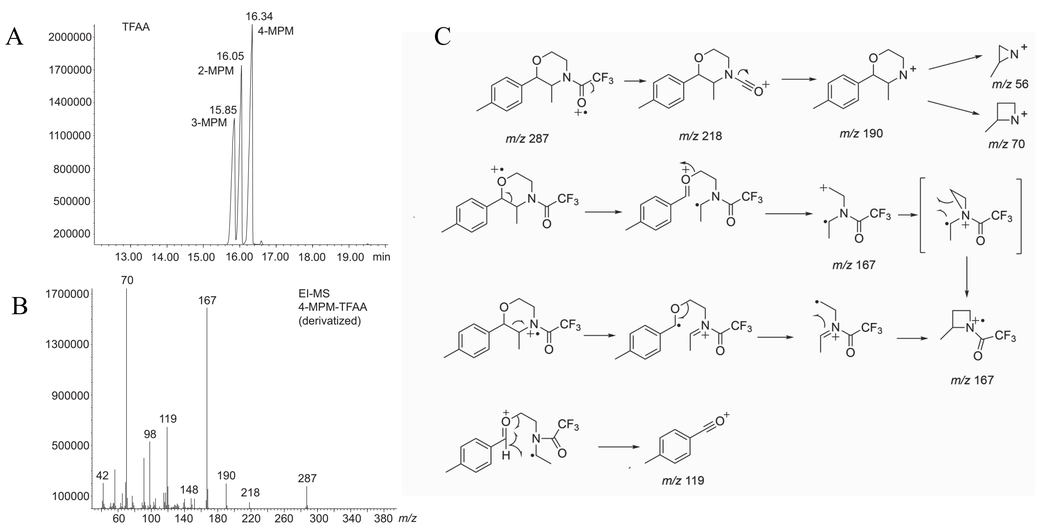


Figure 3. A, Gas chromatographic (GC) separation achieved for the MPM-TFAA derivatives. B, The electron ionization (EI) mass spectra recorded for the MPM-TFAA derivatives. C, A proposed fragmentation pattern for the MPM-TFAA isomers under EI-MS conditions.

Characterization (LC-MS)

Satisfactory separation of all three MPM isomers was achieved using liquid chromatography mass spectrometry (LC-MS) with retention times of 13.06 mins, 16.70 mins, and 17.33 mins recorded for 2-MPM, 3-MPM, and 4-MPM, respectively. The 2-MPM isomer was completely separated from the other two positional isomers; however, only partial separation was achieved for the 3- and 4-MPM isomers. Electrospray ionization (ESI) single quadrupole mass spectra were obtained from in-source collision induced dissociation (CID) at increased fragmentor voltage (150 V) (Figure 4A) and the suggested dissociation pathways are shown in Figure 4B. The fragmentation pattern of the MPM isomers were consistent with the fragmentation pattern also recorded for the FPM isomers.[6] The protonated molecule $[M+H]^+$ was present in all spectra at *m/z* 192. The formation of *m/z* 174 might have represented a loss of methanol from the protonated molecule, presumably consistent with $C_{12}H_{16}N^+$. The *m/z* 148 ion might have reflected a loss of ethylene oxide from the protonated molecule to form an aziridine species. The product ion at *m/z* 131 may be represented by the formation of a stabilized allylic cation and might have formed following the loss of ethenamine (C_2H_5N) from *m/z* 174 and/or the loss of NH_3 from the aziridine species at *m/z* 148. The fragments at *m/z* 105 and 91 may be represented by the formation of a methylium and tropylium species, respectively. The LC–ESI–MS data recorded for the vendor samples of 3- and 4-MPM were consistent with the data recorded for the respective synthesized reference standard.

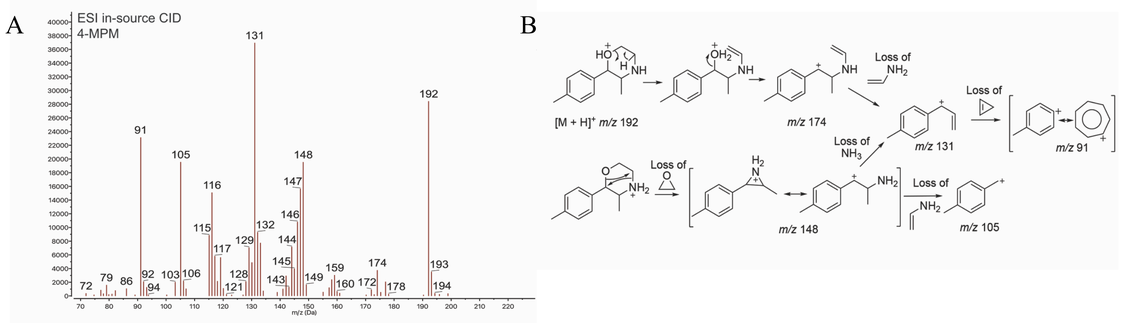


Figure 4 (A) The product ion spectra obtained for 4-MPM from in-source CID at 150 V. (B) A suggested dissociation pathway for the 4-MPM isomer.

Monoamine Transporter Assays

Male Sprague-Dawley rats were euthanised by CO_2 narcosis and brains were processed to yield synaptosomes.[8-10] For release assays, 9 nM [^{3}H]-1-methyl-4-phenylpyridinium ([^{3}H]MPP^+) was used as the radiolabeled substrate for DAT and NET, whereas 5 nM [^{3}H]5-HT was used as the radiolabeled substrate for SERT. Synaptosomes were preloaded with radiolabeled substrates in Krebs-phosphate buffer for 1 hour (steady state). Release assays were initiated by adding 850 µL of preloaded synaptosomes to 150 µL of test drug. The release assays were terminated by vacuum filtration and retained radioactivity was quantified by scintillation counting.

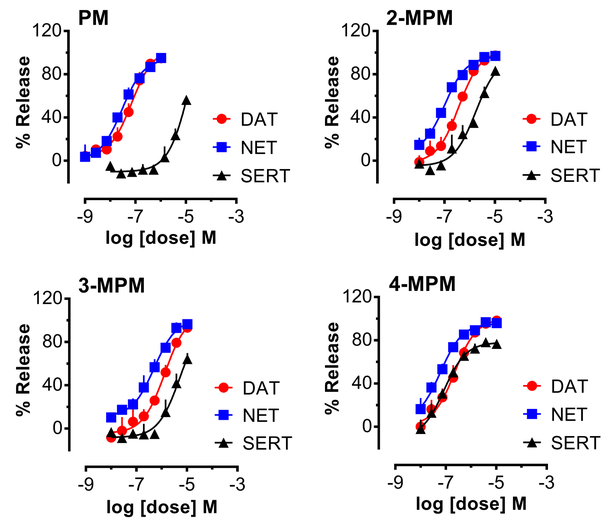


Figure 5. Dose–response effects of phenmetrazine (PM), 2-MPM, 3-MPM, and 4-MPM on release of [^{3}H]substrates by DAT, NET, and SERT in rat brain synaptosomes. Data are expressed as % of maximal release (mean ± SD) for n = 3 experiments performed in triplicate.

Figure 5 shows the effects of 2-MPM, 3-MPM, and 4-MPM on release of [^{3}H]MPP^+ via DAT and NET, and [^{3}H]serotonin via SERT. Consistent with prior findings, we found that phenmetrazine is a potent releaser at DAT and NET, with negligible activity at SERT. The data obtained for the 2-, 3-, and 4-MPM isomers exhibited sigmoidal dose–response curves indicative of fully efficacious release activity across all three transporters. Importantly, the addition of a methyl moiety to the phenmetrazine molecular structure increased the relative potency at SERT. Moving the methyl group from the *ortho*- or *meta*- position to the *para*- position leads to a substantial increase in the selectively for SERT, so much so that 4-MPM is essentially a nonselective monoamine releaser, with similar potency at DAT (227 nM), NET (62 nM), and SERT (86 nM). In this regard, the release data are generally consistent with the uptake inhibition data, which show greater relative potency toward SERT for 4-MPM when compared to 2- and 3-MPM. The non-selective profile of monoamine release produced by 4-MPM is similar to the club drug 3,4-methylenedioxymethamphetamine (MDMA) and the synthetic cathinone methylone.[11] Previous studies on the pharmacological activity of 2-, 3- and 4-FPM found that addition of the fluorine atom to the phenyl ring increased activity at SERT, but none of the FPM isomers were able to affect SERT releasing activity to the same extent as 4-MPM.[7]

Conclusion

The availability of NPS on the recreational drug market continues to create challenges for scientists in the forensic, clinical and toxicology fields. This study provides comprehensive analytical and pharmacological data on *ortho*-, *meta*-, and *para*-substituted methylphenmetrazine isomers. The combination of test purchases, analytical characterization, targeted organic synthesis, and pharmacological evaluation of NPS and their isomers is an effective approach for the provision of data on these substances as they emerge in the marketplace. The analytical characterization of three vendor samples revealed the presence of 4-MPM in two of the samples and 3-MPM in the third sample, which agreed with the product labels. The pharmacological findings suggest that 2-MPM and 3-MPM will exhibit stimulant properties similar to the parent compound phenmetrazine, whereas 4-MPM may display entactogen properties more similar to MDMA.

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