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. In 2017, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) was monitoring more than 670 NPS that have not been identified in Europe. 51 new substances were first identified in 2017.1,2 Phenmetrazine (3-methyl-2-phenylmorpholine) and an array of its analogs constitute a family 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.1,2 Phenmetrazine and its N-methylated analog phenmetrazine (Figure 1) were introduced as an anorectic medications in the 1950s.

Phenmetrazine is a potent substrate-type releaser at dopamine transporters (DAT) and noradrenergic transporters (NET), with less potent effects at serotonin transporters (SERT).2 Phendimetrazine exerts its pharmacological effects via β3-adrenergic receptor. Both substances are listed in the United Nations Convention on Psychotropic Substances 1971.3,4 The manipulation of the phenmetrazine skeleton by substitution of a chloro or fluorine or phenoxy group 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-FPM and 3-MPM. This study describes the synthesis, 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).3,6 There 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.

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 trimethylsilyl (TMS) was employed and retention times of 15.83, 16.05, and 16.84 minutes were recorded for 2, 3-, and 4-MPM isomers, respectively (Figure 3A). The electron ionization (EI) mass spectral data for the MPM-TFAA isomers are 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 CF3 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-oxonium species (C20H20FNO+). 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 azetidene species (2-methyl-2,3-dihydroazepine, C19H15NO). In addition, the loss of a TFAA-dimethylamine entity from the molecular ion leads to the formation of an oxonium ion at m/z 119 (C10H15NO+). The fragment at m/z 354 may reflect the formation of an azidine species (C19H17N2O+). 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.

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 min, 17.33 min, and 19.70 min, respectively for 3-MPM, 4-MPM, and 3-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 recorded for the 4-MPM isomers.9 The protonated molecule [M+H]+ was present in all spectra at m/z 174. The formation of m/z 174 might have represented a loss of methyl from the protonated molecule, presumably consistent with C5H5N+. The m/z 148 ion might have reflected a loss of ethylene oxide from the protonated molecule to form an azidine species. The product ion at m/z 131 may be explained by the formation of a stabilized allylic cation and might have formed following the loss of ethaneoxime C3H4N from m/z 174 and the loss of N2H from the azidine species at m/z 148. The fragments at m/z 105 and 91 may be represented by the formation of a methylaziridine and tropolone 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.

Monoamine Transporter Assays
Male Sprague-Dawley rats were euthanized by CO2, narcosis and brains were processed to yield synaptosomes.8,9 For release assays, 9 µM [1]-methyl-4-phenylpyridinium ([1]MPP+) was used as the radioisolate substrate for DAT and NET, whereas 5 µM [3H]-HT was used as the radioisolated substrate for SERT. Synaptosomes were incubated with radiolabeled substrates in Krebs-phosphate buffer at 37°C 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.

In a recent study, McLaughlin et al.9 2-Methylphenmetrazine (2-MPM), 3-Methylphenmetrazine (3-MPM) and 4-Methylphenmetrazine (4-MPM) on release of [3H]meproperidine ([3H]MPD) was used as the radioisolated substrate for DAT and NET, whereas 5 µM [3H]-HT was used as the radioisolated substrate for SERT. Synaptosomes were incubated with radiolabeled substrates in Krebs-phosphate buffer at 37°C 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.

In the current study, 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 methylphenmetrazines. The combination of test purchases, analytical characterization, targeted organic synthesis, and pharmacological evaluation of NPS and their isomers is an effective approach to the provision of data on these substances as they emerge onto 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. 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.

Conclusion

References

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

Figure 4. A. The product ion spectra obtained for MPM-TFAA n=4 at m/z 174 at CID 150 V. B. A suggested dissociation pathway for the 4-MPM isomer.

Figure 5. Dose-response effects of phenmetrazine (Pm), 2-MPM, 3-MPM, and 4-MPM on release of [3H]Harotonin by DAT, NET, and SERT in rat brain synaptosomes. Data are expressed as % of maximal release (max) ± SEM for 1 µM iproniazid pretreatment. Inset: Figure 5 shows the effects of 2-25, 3-MPM, 3-MPM, and 4-MPM on release of [3H]meproperidine ([3H]MPD) for DAT and NET, [3H]Harotonin 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 are displayed as dose-response curves for a fixed concentration of 25 nM [3H]-MPD. The dose-response curves for the 2-, 3-, and 4-MPM isomers also show evidence of full dose-response curves with their respective IC50 (50% inhibitory effect) values being calculated.

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