Analytical characterisation and pharmacological evaluation of the new psychoactive substance 4-fluoromethylphenidate (4F-MPH) and differentiation between the (±)-*threo* and (±)-*erythro* diastereomers

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Introduction

Over the past number of years in Europe, there has been an unprecedented increase in the number, types and seizures of chemicals frequently referred to as new psychoactive substances (NPS). The nature of substances available for purchase is not limited to compounds derived from illicit drugs as increasing ranges of compounds derived from medicinal products have also joined the catalogues of NPS suppliers.[1] In 2016, 66 NPS were detected for the first time via the European Union Early Warning System (EWS), controlled by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).[2] Although this figure indicates a decrease in the number of new substances being introduced onto the market – 98 substances were detected in 2015 – the overall number of substances now available remains high and continues to be a considerable health challenge in Europe. By the end of 2016, the EMCDDA were monitoring more than 620 NPS.

Methylphenidate & 4-Fluoromethylphenidate

Methylphenidate (MPH; Ritalin®) is a substituted phenethylamine that was first synthesised in 1944 and subsequently recognised as a psychostimulant (Figure 1).[3,4] MPH acts primarily by inhibiting the reuptake of dopamine and norepinephrine resulting in elevated concentrations of these monoamine neurotransmitters within the synaptic cleft.[5,6] The pharmacological activity of MPH is attributed to the (±)-*threo* diastereomers. MPH is widely used in the treatment of attention deficit hyperactivity disorder (ADHD), as the symptoms of this condition are believed to be associated with suppressed levels of these neurotransmitters.[7,8] Misuse of MPH has been observed among recreational drug users, presumably due to its psychostimulant properties. MPH is listed as a Schedule II substance in the 1971 United Nations Convention on Psychotropic Substances.[9]

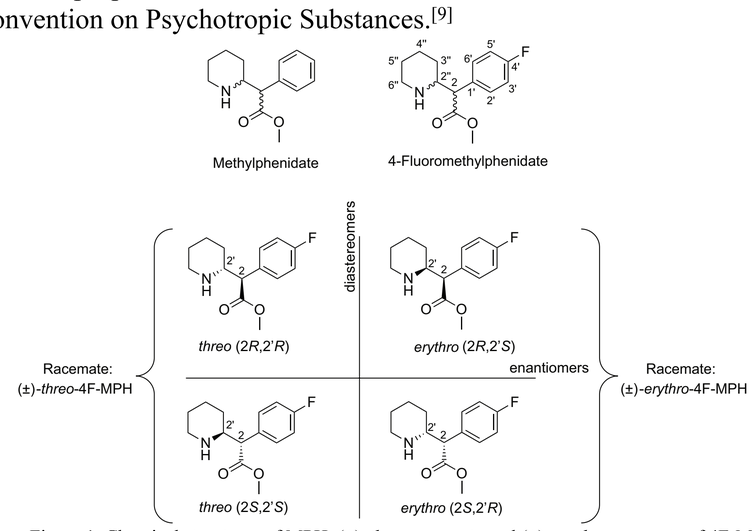

Figure 1. Chemical structures of MPH, (±)-*threo* racemate and (±)-*erythro* racemate of 4F-MPH

In the last decade, several unregulated substances that are closely related to MPH have been launched on the NPS market. As with the majority of NPS launched by vendors, many of these MPH analogues have already been described in the pharmaceutical research literature. 4-Fluoromethylphenidate (4F-MPH; Figure 1) is a MPH analogue that was developed within the pharmaceutical setting. In November 2015, 4F-MPH was first notified by the EMCDDA EWS following its detection on the recreational drug market.[10]

Methods

The present study describes the analytical characterisation of two powdered samples and a set of tablets of 4F-MPH that were obtained from the same vendor based in the United Kingdom in 2015. Various chromatographic, spectroscopic and mass spectrometric analysis methods were employed, followed by x-ray crystal structure analysis. In addition, the ability of all test drugs to inhibit uptake of [^{3}H]dopamine, [^{3}H]norepinephrine and [^{3}H]serotonin was investigated using synaptosomal preparations from rat brain.[11,12]

Characterisation

Preliminary chemical analysis found that one of the vendor samples contained (±)-*threo*-4F-MPH, which was consistent with the expected racemate based on the current knowledge of the biological activity of (±)-*threo*-MPH. Unexpectedly, the analysis of the second sample revealed that it consisted of a mixture of (±)-*threo* and (±)-*erythro*-4F-MPH. This suggested that the two powdered vendor products might have originated from different batches. From examination of the MPH synthesis literature, it seemed plausible that the presence of the (±)-*erythro* racemate might have reflected a lack of purification at the end of the synthesis procedure. The two racemates were isolated from the mixture, purified, and converted to HCl salts prior to full extensive analytical characterisation. In addition, the set of 4F-MPH tablets were found to be consistent with the (±)-*threo* racemate.

Gas Chromatography Mass Spectrometry (GC-MS)

The GC method used was able to achieve satisfactory separation between the (±)-*threo* (18.13 min) and (±)-*erythro* racemates (18.04 min) (Figure 2A). The electron ionisation (EI) mass spectra obtained for both racemates were identical (Figure 2B) and the suggested fragments are shown in Figure 2C.

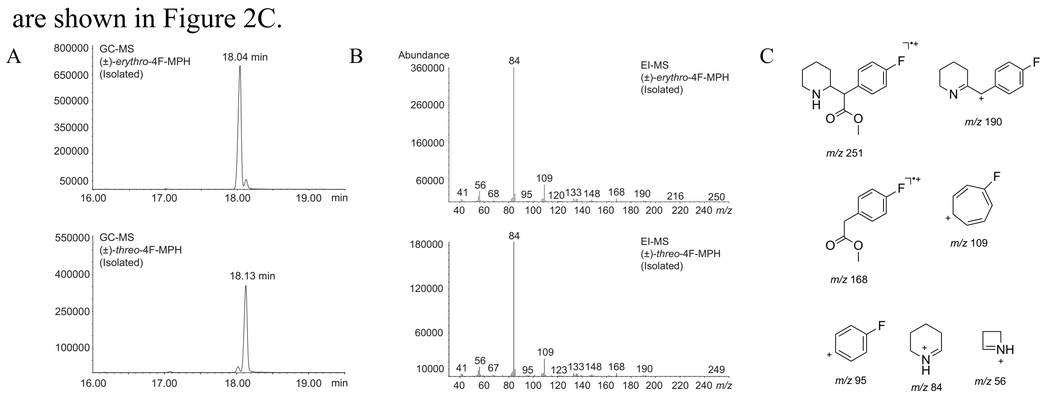

Figure 2 (A) GC separation obtained for the isolated (±)-*erythro* and (±)-*threo* racemates of 4F-MPH. (B) EI mass spectra obtained for the isolated (±)-*erythro* and (±)-*threo* racemates of 4F-MPH. (C) Proposed fragmentation pattern for both racemates.

The base peak was observed at *m/z* 84 and it is suggested that this was due to the formation of a tetrahydropyridinium species ($C_5H_{10}N^+$), following the loss of a methyl 4-fluorophenylacetate moiety from the molecular ion. This fragment was consistent with the EI mass spectral data for MPH, ethylphenidate (EPH) and 4F-MPH available in the literature. The fragment observed at *m/z* 190 was consistent with the formation of a 4-fluorophenyl(piperidin-2-ylidene)methylium species ($C_{12}H_{13}FN^+$). The detection of *m/z* 168 may be rationalised by the loss of a tetrahydropyridine moiety from the parent structure, which gave rise to a radical cation and formation of a methyl fluorophenylacetate species ($C_9H_8FO_2^{\bullet+}$). The fragment at *m/z* 109 can be described by the formation of a tropylium ion. The fragment at *m/z* 56 could represent the formation of a dihydroazetium ion.

Liquid Chromatography Mass Spectrometry (LC-MS)

Analysis of both (±)-*threo* and (±)-*erythro* racemates of 4F-MPH using LC-MS confirmed satisfactory separation for identification purposes. A retention time of 8.90 min was obtained for the (±)-*erythro* form, whereas a retention time of 9.81 min was obtained for the (±)-*threo* racemate (Figure 3A). The electrospray ionisation (ESI) single quadrupole mass spectra obtained from in-source collision induced dissociation (CID) (fragmentor 110 V) of the 4F-MPH racemates shared two key ions (Figure 3B). The suggested pathways are shown in Figure 3C. In the mass spectra of both racemates, the fragment observed at *m/z* 252 represented the protonated molecule (100% abundance). The product ion observed at *m/z* 84 might have represented a loss of the methyl 4-fluorophenylacetate moiety from the protonated molecule resulting in the formation of a tetrahydropyridinium species ($C_5H_{10}N^+$) (95% abundance).

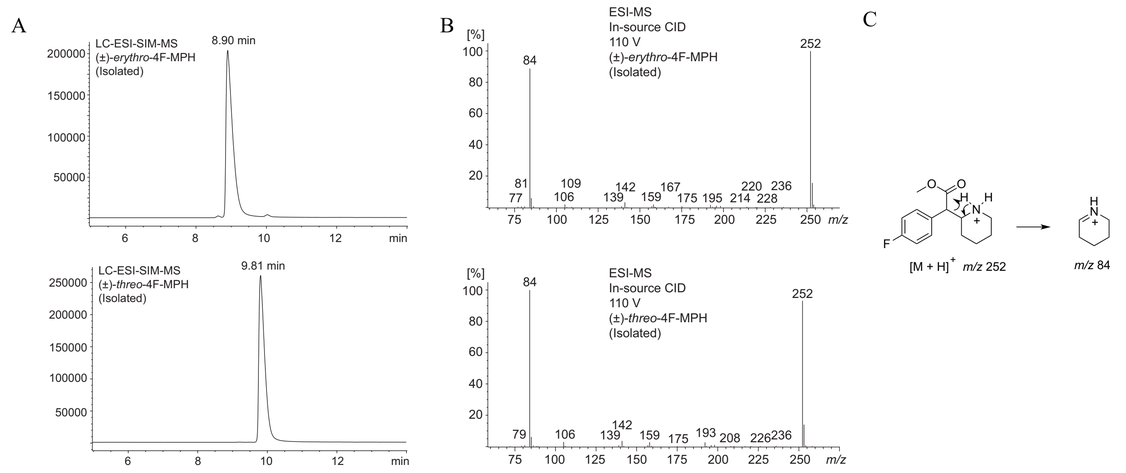

Figure 3 (A) LC separation obtained for the isolated (±)-*erythro* and (±)-*threo* racemates of 4F-MPH. (B) Product ion spectra obtained for the isolated (±)-*erythro* and (±)-*threo* racemates of 4F-MPH obtained from in-source CID at 110 V. (C) Proposed fragments for both racemates.

Monoamine Transporter Assays

Male Sprague-Dawley rats were euthanised by CO_2 narcosis and brains were processed to yield synaptosomes.[11,12] For uptake assays, synaptosomes were incubated with different concentrations of test drugs in the presence of 5 nM [^{3}H]dopamine, 10 nM [^{3}H]norepinephrine, or 5 nM [^{3}H]serotonin. The uptake assays were terminated by vacuum filtration and retained radioactivity was quantified by scintillation counting.

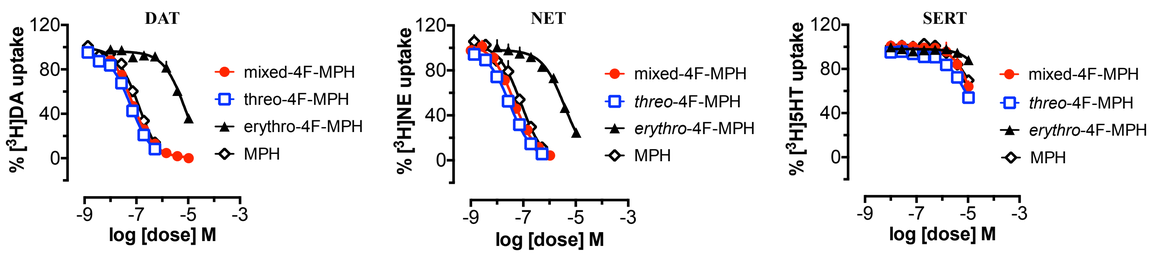

Figure 4. Dose response curves depicting the effects of 4F-MPH isomers and comparison with methylphenidate (MPH) on inhibition of uptake at DAT, NET, and SERT in rat brain synaptosomes. Data are percentage of [^{3}H] transmitter uptake expressed as mean ± s.e.m. for n = 3 experiments.

Figure 4 shows the effects of the (±)-*threo*/*erythro* mixture, (±)-*threo*-4F-MPH, (±)-*erythro*-4F-MPH and MPH (reference standard) on the uptake of [^{3}H]dopamine (DA), [^{3}H]norepinephrine (NE) and [^{3}H]serotonin (5-HT) by their respective transporters DAT, NET and SERT. As revealed from the dose response curves, all test drugs were fully effective uptake inhibitors at DAT and NET, with little activity at SERT, thus displaying catecholamine selectivity. The 4F-MPH mixture was about twice as potent as MPH at DAT and NET. The data (Table 1) suggests that the biological activity of the 4F-MPH mixture predominately resided with the (±)-*threo* and not the (±)-*erythro* isomers given that higher potencies were determined for DA uptake (IC_{50} (±)-*threo* = 61 nM vs. IC_{50} (±)-*erythro* = 8,528 nM) and NE uptake (IC_{50} (±)-*threo* = 31 nM vs. IC_{50} (±)-*erythro* = 3,779 nM) at DAT and NET, respectively. MPH was three times less potent than (±)-*threo*-4F-MPH at DAT and approx. 2.5 times less potent at NET. These findings suggest that the psychostimulant properties of (±)-*threo*-4F-MPH might be more potent in humans than MPH. Overall, it appears that the acetate group and the piperidine ring must be oriented in the opposite direction for the drugs to interact optimally with the transporter proteins.

Table 1. Effects of 4F-MPH and MPH on transporter-mediated uptake in rat brain synaptosomes[a]

Test Drug	[^{3}H]DA uptake via DAT IC_{50} (nM)	[^{3}H]NE uptake via NET IC_{50} (nM)	[^{3}H]5-HT uptake via SERT IC_{50} (nM)
Diastereomeric mixture of 4F-MPH	66.35 ± 3.27	44.6 ± 4.17	>10,000
(±)-*threo*-4F-MPH	60.96 ± 4.6	30.68 ± 2.64	8,805 ± 2475
(±)-*erythro*-4F-MPH	8,528 ± 1753	3,779 ± 570.5	>10,000
MPH	131.0 ± 14.2	82.85 ± 11.15	>10,000

[a] Data are expressed as nM concentrations (mean ±SD) for n = 3 separate experiments performed in triplicate.

Conclusion

The presence of isomers and the absence of reference materials can cause difficulties in the day-to-day operation of forensic work. This study provided essential analytical data for 4F-MPH and its diastereomeric racemates, which could be utilised in a forensic science laboratory. The pharmacological findings suggest that the psychostimulant properties of (±)-*threo*-4F-MPH might be more potent in humans than MPH. Since the biological activity resides in the (±)-*threo* form, it is anticipated that other MPH-derived NPS on the market might also display this configuration.

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