The synthesis and characterisation of the 'research chemical' *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1*H*-pyrazole-5-carboxamide (3,5-AB-CHMFUPPYCA) and differentiation from its 5,3-regioisomer

G. McLaughlin[1,2], N. Morris[1], P.V. Kavanagh[2], J.D. Power[2,3], G. Dowling[2], B. Twamley[4], J. O'Brien[4], B. Talbot[5] and S.D. Brandt[6]

Introduction

Over the past decade, the phenomenon linked to new psychoactive substances (NPS) has attracted great interest from various communities and stakeholders that are concerned with public health, law enforcement, and a range of fundamental sciences. The European Monitoring Centre for Drug and Drug Addiction (EMCDDA) is currently monitoring over 450 NPS via the European Union (EU) early warning system (EWS) on NPS and reports have indiciated that the number of synthetic cannabinoids has risen to over 130.[1,2]

Synthetic Cannabinoids

Synthetic cannabinoids (SCs), or synthetic cannabinoid receptor agonists (SCRAs), are synthetic chemicals that mimic the effects of the natural psychoactive substances found in cannabis (e.g. tetrahydrocannabinol) (Figure1). 'Legal high' products containing SCs have been sold as herbal smoking mixtures since at least 2006.[3] In recent times, novel SC products have emerged in 'resin' format resembling cannabis resin and liquids suitable for use in electronic cigarettes.[3]

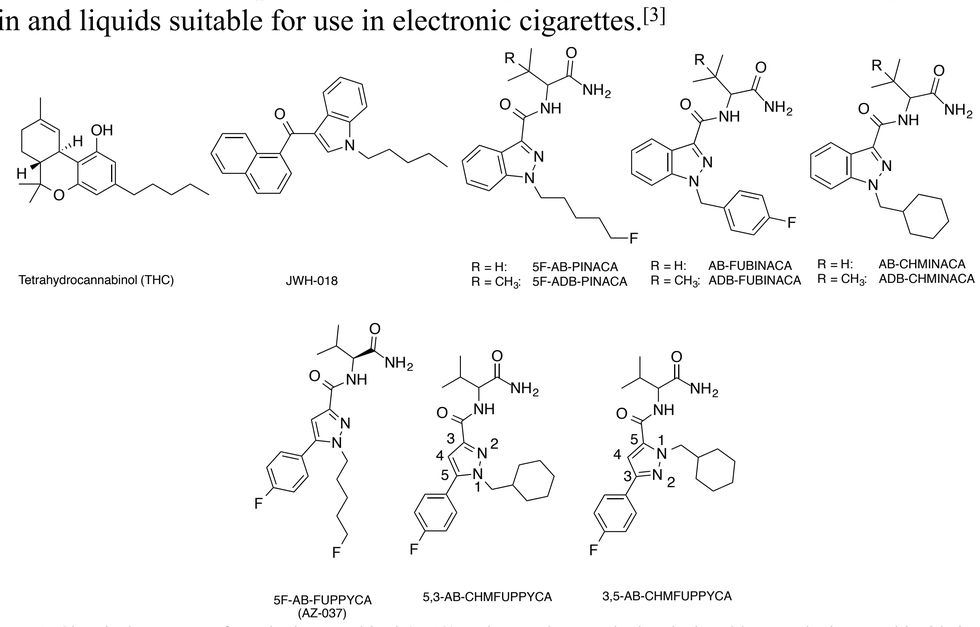

Figure 1. Chemical structure of tetrahydrocannabinol (THC) and several research chemicals sold as synthetic cannabinoids including JWH-018, the PINACA, FUBINACA and CHMINACA series, and the more recent AZ-037 and AB-CHMFUPPUCA.

The number of substances, the diversity in their chemical structure, and the rate of their emergence make SC one of the largest family of NPS monitored at European level.[2] Recent SCs carrying a pyrazole moiety instead of the more established indole, benzimidazole, pyrrole or indazole core, have been advertised on a number of 'research chemicals' (RC) websites (Figure 1).

Experimental

This study presents the identification of *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1*H*-pyrazole-5-carboxamide (3,5-AB-CHMFUPPYCA). This compound was obtained from a UK-based internet vendor, who erroneously advertised this 'RC' as ((*S*)-*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1*H*-pyrazole-3-carboxamide (AZ-037). The vendor sample was subjected to analytical characterisation by gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled to various forms of mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR). These investigations revealed that the material was inconsisent with the structural features associated with AZ-037. The substance was characterised as 3,5-AB-CHMFUPPYCA.

Synthesis and Characterisation

Further confirmation was obtained from organic synthesis of this substance along with its alternative regioisomer, 5,3-AB-CHMFUPPYCA. Both isomers were synthesised using two specific routes (Figure 2) and subjected to extensive analytical characterisation using chromatographic, spectroscopic, mass spectrometric platforms as well as crystal structure analysis.

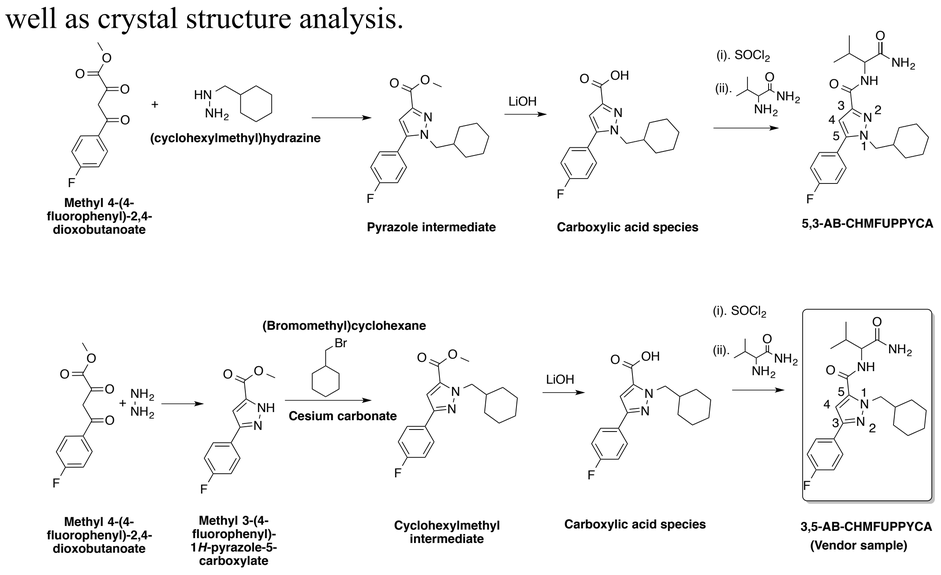

Figure 2. The synthesis pathways employed for the preparation of the AB-CHMFUPPYCA isomers.

Separation of both isomers was successfully achieved using GC, achieving retention times of 14.74 and 15.20 min for 5,3-AB-CHMFUPPYCA and 3,5-AB-CHMFUPPYCA, respectively, and a comparison with the vendor sample was in agreement with the identity of the latter (Figure 3A-C). A comparison of both electron ionisation (EI) mass spectra also provided sufficient evidence that both isomers could be differentiated using the fragmentation patterns (Figure 3D-G). For example, in the EI mass spectrum (MS) for 5,3-AB-CHMFUPPYCA, the base peak was observed at *m/z* 285, which represents the formation of an oxonium species. In the EI-MS of 3,5-AB-CHMFUPPYCA, the base peak was observed at *m/z* 257, which was thought to represent a pyrazole species and its formation might have been facilitated by the position of the double bonds within the pyrazole ring of the 3,5-AB-CHMFUPPYCA isomer (Figure 3H).

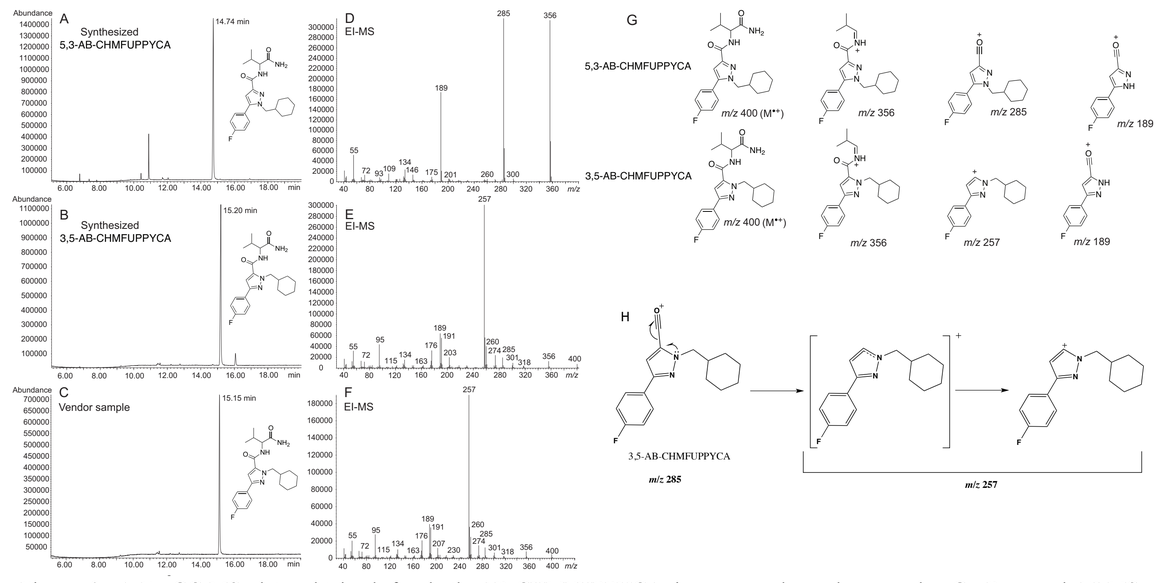

Figure 3. A-F: GC-MS data obtained for both AB-CHMFUPPYCA isomers and vendor sample. G: Proposed EI-MS fragmentation patterns for AB-CHMFUPPYCA isomers. H: Proposed mechanism for the formation of pyrazole species at m/z 257 (base peak) in EI-MS of 3,5-AB-CHMFUPPYCA.

Analysis of both synthesised isomers and the vendor sample by HPLC achieved satisfactory separation and provided further evidence that the vendor samples true identity was 3,5-AB-CHMFUPPYCA. The electrospray ionisation (ESI) single quadrupole mass spectra obtained from in-source collision-induced dissociation (CID) of the synthesised regioisomers (150 V fragmentor voltage) shared similar product ions but key features that allowed for differentiation between the two substances were also apparent (Figure 4A-B). For example, the in-source CID spectrum of 5,3-AB-CHMFUPPYCA displayed the sodiated adduct $[M + Na]^+$ at *m/z* 423 as the base peak, which was not the case with 3,5-AB-CHMFUPPYCA where the relative abundance was around 40%. A major difference, however, was observed in the mass spectrum of 3,5-AB-CHMFUPPYCA that formed a base peak at *m/z* 260 and which was absent in the 5,3-AB-CHMFUPPYCA. A proposed mechanism of its formation that may be rationalized by the loss of 2-cyclohexylacetamide from the protonated molecule.

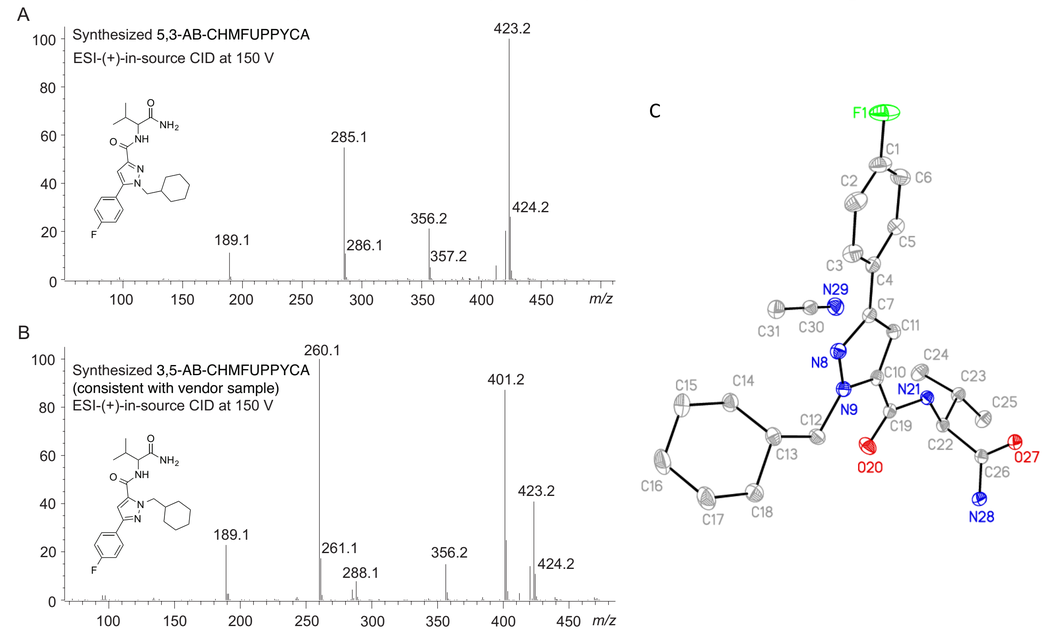

Figure 4. A-B: ESI-mass spectra obtained for each isomer following in-source CID at 150 V. C: Molecular structure of 3,5-AB-CHMFUPPYCA ascertained using x-ray crystallography.

The solid-state structure of the 3,5-AB-CHMFUPPYCA isomer was also elucidated by single crystal X-ray diffraction and is shown in Figure 4C. The structure is a solvate with a co-crystallized molecule of acetonitrile present in the asymmetric unit.

Conclusion

The 'RC' obtained from the Internet vendor was consistent with that of the 3,5-AB-CHMFUPPYCA synthesised standard. Incidentally, members of the EU EWS on NPS have just reported the detection of 3,5-AB-CHMFUPPYCA to the EMCDDA.[4] The structural diversity associated with substances labelled as synthetic cannabinoids can create challenges for forensic, clinical, law enforcement and regulatory communities. The recently occurring indazole derivatives are based on patent literature which point towards appreciable CB_1 receptor affinity and [^{35}S]GTPγS activity[5,6] but others remain to be fully explored to assess the extent to which these substances show psychopharmacological similarities to compounds present in cannabis. The syntheses and analytical characterisation of both AB-CHMFUPPYCA isomers are reported for the first time and serve as a reminder that the possibility of mislabelling of RCs cannot be excluded. The pharmacological activities of both AB-CHMFUPPYCA isomers remain to be explored, however, it may be surmised that the pyrazole derivatives share similar psychopharmacological effects as their indazole counterparts. Further in vitro metabolism and thermolytic stability studies on the AB-CHMFUPPYCA isomers are currently on-going.

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Affililations : **1.** Department of Life and Physical Sciences, School of Science, Athlone Institute of Technology, Co. Westmeath, Ireland. **2.** Department of Pharmacology & Therapeutics, School of Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8, Ireland. **3.** Forensic Science Laboratory, Garda Headquarters, Dublin 8, Ireland. **4.** School of Chemistry, Trinity College Dublin, Dublin 2, Ireland. **5.** School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland. **6.** School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L33, UK.