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

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

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Keywords:	New Psychoactive Substances, Synthetic Cannabinoids, AB-CHMFUPPYCA isomers, Pyrazole-carboxamide derivatives
Abstract:	This study presents the identification of N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1H-pyrazole-5-carboxamide that was termed 3,5-AB-CHMFUPPYCA. This compound was obtained from a UK-based Internet vendor, who erroneously advertised this 'research chemical' as AZ-037 and which would have been associated with (S)-N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-5-(4-fluorophenyl)-1H-pyrazole-3-carboxamide. The presence of the pyrazole core indicates a bioisosteric replacement of an indazole ring that is frequently associated with synthetic cannabinoids of the "PINACA", "FUBINACA" and "CHMINACA" series. The pyrazole ring system present in 3,5-AB-CHMFUPPYCA gives rise to the regioisomer N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-5-(4-fluorophenyl)-1H-pyrazole-3-carboxamide (named 5,3-AB-CHMFUPPYCA) and both isomers were synthesized using two specific routes which supported the correct identification of the 'research chemical' as 3,5-AB-CHMFUPPYCA. Both isomers could be conveniently differentiated. Interestingly, a route specific chlorine-containing by-product also was observed during the synthesis of 3,5-AB-CHMFUPPYCA and identified as N-(1-amino-3-methyl-1-oxobutan-2-yl)-4-chloro-1-

(cyclohexylmethyl)-3-(4-fluorophenyl)-1H-pyrazole-5-carboxamide. An extensive analytical characterization included chromatographic, spectroscopic, mass spectrometric platforms as well as crystal structure analysis. The syntheses and analytical characterizations of both AB-CHMFUPPYCA isomers are reported for the first time and it serves as a reminder that the possibility of mislabeling of 'research chemicals' cannot be excluded. The pharmacological activities of both AB-CHMFUPPYCA isomers remain to be explored.

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3 **The synthesis and characterization of the 'research chemical' *N*-(1-**
4 **amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-3-(4-fluorophenyl)**
5 **-1*H*-pyrazole-5-carboxamide (3,5-AB-CHMFUPPYCA) and differentiation**
6 **from its 5,3-regioisomer.**
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53 **Running title:** Syntheses and characterization of AB-CHMFUPPYCA isomers
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56 CHMFUPPYCA isomers. Pyrazole-carboxamide derivatives.
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Abstract

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 that was termed 3,5-AB-CHMFUPPYCA. This compound was obtained from a UK-based Internet vendor, who erroneously advertised this 'research chemical' as AZ-037 and which would have been associated with (*S*)-*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-5-(4-fluorophenyl)-1*H*-pyrazole-3-carboxamide. The presence of the pyrazole core indicates a bioisosteric replacement of an indazole ring that is frequently associated with synthetic cannabinoids of the "PINACA", "FUBINACA" and "CHMINACA" series. The pyrazole ring system present in 3,5-AB-CHMFUPPYCA gives rise to the regioisomer *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-5-(4-fluorophenyl)-1*H*-pyrazole-3-carboxamide (named 5,3-AB-CHMFUPPYCA) and both isomers were synthesized using two specific routes which supported the correct identification of the 'research chemical' as 3,5-AB-CHMFUPPYCA. Both isomers could be conveniently differentiated. Interestingly, a route specific chlorine-containing by-product also was observed during the synthesis of 3,5-AB-CHMFUPPYCA and identified as *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-4-chloro-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1*H*-pyrazole-5-carboxamide. An extensive analytical characterization included chromatographic, spectroscopic, mass spectrometric platforms as well as crystal structure analysis. The syntheses and analytical characterizations of both AB-CHMFUPPYCA isomers are reported for the first time and it serves as a reminder that the possibility of mislabeling of 'research chemicals' cannot be excluded. The pharmacological activities of both AB-CHMFUPPYCA isomers remain to be explored.

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 diversity of compounds that show, or are suspected to show, psychoactive properties in humans and that are made available to the general public by various routes include synthetic replacements for controlled substances, designer drugs in the traditional sense, regulated and unregulated medicinal products and derivatives of various biological activities developed by the pharmaceutical industry.^[1] The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is currently monitoring over 450 NPS via the European Union (EU) Early warning system (EWS) on NPS and it has become clear that the number of notified synthetic cannabinoids has risen to over 130.^[2,3] The number of substances, the diversity in their chemical structure and the rate of their emergence make synthetic cannabinoids one of the largest family of NPS monitored at European level and it is equally obvious that this is a more widespread phenomenon (e.g.^[4,5]). A number of recently occurring synthetic cannabinoids included the emergence of substituted indazole core structures that carry a valinamide component. Examples may be found in the so-called "PINACA", "FUBINACA" and "CHMINACA" series (Figure 1A) and a range of studies have been published^[6-20] in addition to recent developments in the United States of America where some of these newly emerging substances were subjected to temporary placement into Schedule 1.^[21,22]

A more recent example in which the indazole core is replaced by a pyrazole ring, thus creating a bioisosteric system that mimics the indazole ring system, can be found in (S)-N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-5-(4-fluorophenyl)-1H-pyrazole-3-carboxamide that is currently advertised by a number of Internet retailers as the synthetic cannabinoid AZ-037. Following previously suggested conventions in naming structurally related substances, the name 5,3-5F-AB-FUPPYCA is suggested (Figure 1B). Although the identification of the structurally related 3,5-5F-ADB-FUPPYCA has been recently reported^[23] (Figure 1B), detailed data on AZ-037 have not yet been described. The present study describes the characterization of a sample advertised as AZ-037 that was donated in January 2015 by an online retailer based in the United Kingdom. This sample was subjected to analytical characterization by gas chromatography (GC) and high performance liquid

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3 chromatography (HPLC) coupled to various forms of mass spectrometry (MS) and
4 nuclear magnetic resonance spectroscopy (NMR). These investigations revealed that
5 the material was inconsistent with the structural features associated with AZ-037.
6 Instead, the substance was characterized as *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-
7 1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1*H*-pyrazole-5-carboxamide, which was
8 termed 3,5-AB-CHMFUPPYCA. Incidentally, the detection of this compound has just
9 been reported to the EMCDDA by members of the EU EWS on NPS, and was
10 termed AB-CHMFUPPYCA.^[24] Further confirmation was obtained from organic
11 synthesis of this substance along with its alternative regioisomer *N*-(1-amino-3-
12 methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-5-(4-fluorophenyl)-1*H*-pyrazole-3-
13 carboxamide (5,3-AB-CHMFUPPYCA) that arises from presence of the pyrazole core
14 structure. Both isomers were synthesized using two specific routes and subjected to
15 extensive analytical characterization using chromatographic, spectroscopic, mass
16 spectrometric platforms as well as crystal structure analysis. The 'research chemical'
17 obtained from the Internet vendor was consistent with a compound isolated from
18 plant material in Japan where it was termed AB-CHFUPPYCA although further
19 confirmation by synthesis of the two possible isomers was not obtained.^[25]
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33 **Experimental**

34 ***Reagents and standards***

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37 All reagents and dry solvents used in the syntheses were obtained from Sigma
38 Aldrich Ltd. (Arklow, Ireland). LC-MS grade solvents were obtained from Fisher
39 Scientific (Dublin, Ireland). A sample, advertised as AZ-037, was donated by a UK
40 based 'research chemical' vendor in January 2015.
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47 ***Syntheses***

48 ***Methyl 4-(4-fluorophenyl)-2,4-dioxobutanoate***

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51 Potassium *tert*-butoxide (11.20 g, 100 mmol) was added to a solution of dimethyl
52 oxalate (11.80 g, 100 mmol) in tetrahydrofuran (75 mL). The mixture was stirred at
53 room temperature for 30 min. A solution of 4-fluoroacetophenone (6.4 g, 50 mmol) in
54 tetrahydrofuran (25 mL) was added and stirring was continued for 1 h. The reaction
55 mixture was then added to 0.5 M aqueous hydrochloric acid (600 mL) and the
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precipitated solid was collected by filtration to afford a light yellow solid (9.54 g, 43 mmol, 86 %): ESI-HRMS m/z found 225.0568 (m/z theor. for M+H, C₁₁H₁₀O₄F, 225.0558). ¹H NMR (d₆-CDCl₃) δ 7.77-7.69 (m, 2H, 2 x Ar-CH), 7.23-7.14 (m, 2H, 2 x Ar-CH), 7.05 (s, 1H, OH), 3.96 (d, J = 1.2 Hz, 3H), ¹³C NMR (d₆-CDCl₃) δ 189.71 (C=O), 168.67-167.05 (Ar-CF), 165.35 (C(OH)), 162.53 (C=O), 131.27 (Ar-C), 130.50 (Ar-CH), 116.34 (Ar-CH), 53.20 (CH₃), ¹⁹F (d₆-CDCl₃) δ -103.91 ppm.

Methyl 5-(4-fluorophenyl)-1H-pyrazole-3-carboxylate

A mixture of methyl 4-(4-fluorophenyl)-2,4-dioxobutanoate (4.48 g, 20 mmol) and hydrazine hydrate (1.10 g, 22 mmol) in acetic acid (20 mL) was heated (oil bath at 100 °C) for 2 h. The mixture was allowed to cool to room temperature and the product was collected by filtration to afford colorless crystals that were used without further purification (3.08 g, 14.0 mmol, 70 %): ESI-HRMS m/z found 221.0725 (m/z theor. for M+H, C₁₁H₁₀O₂N₂F, 221.0721).

Cyclohexylmethyl)hydrazine trifluoroacetate salt

A mixture of *tert*-butyl carbazate (5.89 g, 44.6 mmol) and cyclohexanecarboxaldehyde (5.00 g, 44.6 mmol) in methanol (140 mL) was stirred at room temperature for 1 h. The solution was then evaporated to dryness. Aqueous 50 % v/v aqueous acetic acid (125 mL) was added and, with constant mixing, sodium cyanoborohydride (2.80 g, 44.6 mmol) was added. The mixture was stirred for 2 h at room temperature, diluted with water, neutralized with sodium hydroxide and extracted with dichloromethane. Drying (anhydrous magnesium sulfate) and removal of the solvent afforded a colorless oil, which was dissolved in a mixture of dichloromethane (30 mL) and trifluoroacetic acid (30 mL). The mixture was stirred for 1 h at room temperature and the volatiles were removed under vacuum to afford a light brown, viscous oil (15.27 g which was used without further purification).

N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-5-(4-fluorophenyl)-1H-pyrazole-3-carboxamide and *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1H-pyrazole-5-carboxamide (5,3-AB-CHMFUPPYCA) (Figure 2A)

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3 A mixture of crude cyclohexylmethyl)hydrazine trifluoroacetate (2.00 g) and methyl 4-
4 (4-fluorophenyl)-2,4-dioxobutanoate (1.12 g, 10 mmol) in methanol (20 mL) was
5 refluxed for 3 h. The mixture was allowed to cool to room temperature, the volatiles
6 removed under vacuum and the residue partitioned between aqueous sodium
7 carbonate and dichloromethane. Drying (anhydrous magnesium sulfate) followed by
8 solvent removal gave a yellow oil. Tetrahydrofuran (20 mL), water (10 mL) and
9 lithium hydroxide (2.00 g) were added. The mixture was heated (oil bath at 80 °C) for
10 2 h and then allowed to stir overnight at room temperature. The mixture was diluted
11 with water, washed with diethyl ether, made acidic (concentrated aqueous
12 hydrochloric acid) and extracted with dichloromethane. Drying (anhydrous
13 magnesium sulfate) and removal of the solvent afforded light a light yellow solid (936
14 mg). Thionyl chloride (5 mL) was added to the solid (836 mg) and the mixture was
15 refluxed for 1 hr. The volatiles were then removed under vacuum. *N,N*-
16 Dimethylformamide (5 mL), *L*-valinamide hydrochloride (0.50 g, 3.3 mmol) and *N,N*-
17 diisopropylethylamine (3 mL) were added, and the mixture was stirred at room
18 temperature for 4 h. The mixture was diluted with water and extracted with
19 dichloromethane. Drying (anhydrous magnesium sulfate) and removal of the solvent
20 afforded a light a brown oil (689 mg). This was purified by preparative thin layer
21 chromatography (silica gel; ethyl acetate/hexane, 9/1; 2 runs) to afford a beige solid
22 (74 mg, 0.19 mmol, 2.1 % from methyl 4-(4-fluorophenyl)-2,4-dioxobutanoate): ESI-
23 HRMS *m/z* found 401.2346 (*m/z* theor. for M+H, C₂₂H₃₀O₂N₄F, 401.2347); Melting
24 point: 58-62 °C. ¹H NMR (d₆-DMSO) δ 7.63 (s; 1 H; one H from NH₂), 7.82-7.85 (m; 3
25 H; Ar H and one H from NH₂), 7.36 (t; *J* = 8.8 Hz; 2 H; Ar H), 6.73 (s; 1 H; pyrazole H),
26 4.36 (dd; *J* = 9.0, 6.3 Hz; NH-CH-CO), 4.02 (d; *J* = 7.2 Hz; 2 H; CyCH₂N), 2.03-2.10
27 (m; 1 H; CH(CH₃)₂), 1.71-1.79 (m; 1 H; cyclohexyl H), 1.49-1.59 (m; 3 H; cyclohexyl
28 H), 1.34-1.42 (m; 2 H; cyclohexyl H), 1.02-1.14 (m; 3 H; cyclohexyl H), 0.92 (d; *J* =
29 6.8 Hz; 3 H; CH₃), 0.88 (d; *J* = 6.8 Hz; 3 H; CH₃) and 0.70-0.8 (m; 2 H; cyclohexyl H)
30 ppm; ¹³C NMR (d₆-DMSO) δ 172.54 (C=O), 162.26 (d; ¹*J*_{CF} = 245 Hz; Ar C), 160.63
31 (C=O), 144.95 (pyrazole C), 144.21 (pyrazole C), 131.23 (d; ³*J*_{CF} = 8 Hz; Ar CH),
32 126.19 (Ar C), 115.86 (d; ²*J*_{CF} = 22 Hz; Ar CH), 106.31 (pyrazole CH), 56.78 (CO-CH-
33 NH), 55.34 (CyCH₂N), 38.09 (cyclohexyl CH), 30.53 (CH(CH₃)₂), 29.72/29.67 (2 x
34 cyclohexyl CH₂), 25.67 (cyclohexyl CH₂), 24.98/24.95 (2 x cyclohexyl CH₂), 19.29
35 (CH₃) and 17.92 (CH₃) ppm; ¹⁹F NMR (d₆-DMSO) δ -112.98 ppm.

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55 *N*-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-5-(4-fluorophenyl)-1H-
56 pyrazole-3-carboxamide and *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-
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(cyclohexylmethyl)-3-(4-fluorophenyl)-1H-pyrazole-5-carboxamide (3,5-AB-CHMFUPPYCA) (Figure 2B)

A mixture of methyl 5-(4-fluorophenyl)-1H-pyrazole-3-carboxylate (250 mg, 1.14 mmol), (bromomethyl)cyclohexane (404 mg, 2.28 mmol), cesium carbonate (371 mg, 2.28 mmol) in dimethylformamide (3 mL) was stirred overnight at room temperature. The mixture was then diluted with saturated aqueous ammonium chloride solution and extracted with dichloromethane. Drying (anhydrous magnesium sulfate) and removal of the solvent afforded a colorless oil (428 mg). Lithium hydroxide (480 mg) and tetrahydrofuran (5 mL) were added, the mixture refluxed for 6 h and then allowed to stir overnight at room temperature. The mixture was diluted with water, washed with diethyl ether, made acidic (conc. aqueous hydrochloric acid) and extracted with dichloromethane. Drying (anhydrous magnesium sulfate) and removal of the solvent afforded light a colorless solid (230 mg, 0.76 mmol). Thionyl chloride (3 mL) was added to the solid and the mixture was refluxed for 1 h. The volatiles were then removed under vacuum. *N,N*-Dimethylformamide (2.5 mL), *L*-valinamide hydrochloride (304 mg, 2 mmol) and *N,N*-diisopropylethylamine (1 mL) were added, and the mixture was stirred at room temperature for 4 h. This was then diluted with water and extracted with dichloromethane. Drying (anhydrous magnesium sulfate) and removal of the solvent afforded a beige solid (257 mg). This was purified by preparative thin layer chromatography (silica gel; ethyl acetate/hexane, 9/1) to afford an almost colorless solid (115 mg, 0.29 mmol, 25 % from methyl 5-(4-fluorophenyl)-1H-pyrazole-3-carboxylate). This was recrystallized (ethanol) to give a colorless solid (49 mg): ESI-HRMS *m/z* found 401.2344 (*m/z* theor. for M+H, C₂₂H₃₀O₂N₄F, 401.2347); Melting point: 172-178 °C. ¹H NMR (d₆-DMSO) δ 8.20 (d; *J* = 8.8 Hz; 1 H; NH), 7.82-7.85 (m; 2 H; Ar H), 7.48 (s; 1 H; one H from NH₂), 7.40 (s; 1 H; pyrazole H), 7.27 (tr; *J* = 8.9 Hz; 2 H; Ar H), 7.13 (s; 1 H; one H from NH₂), 4.37 (dd; *J* = 7.2, 2.4 Hz; 2 H; CyCH₂N), 4.26 (dd; *J* = 8.4, 8.1 Hz; NH-CH₂-CO), 2.07-2.14 (m; 1 H; CH(CH₃)₂), 1.77-1.85 (m; 1 H; cyclohexyl H), 1.47-1.67 (m; 5 H; cyclohexyl H), 1.10-1.17 (m; 3 H; cyclohexyl H) and 0.91-0.99 (m; 8 H; 2 cyclohexyl H and 2 CH₃) ppm; ¹³C NMR (d₆-DMSO) δ 172.87 (C=O), 161.10 (d; ¹*J*_{CF} = 243 Hz; Ar C), 159.71 (C=O), 147.62 (pyrazole C), 137.40 (pyrazole C), 129.64 (Ar C), 127.27 (d; ³*J*_{CF} = 8 Hz; Ar CH), 115.96 (d; ²*J*_{CF} = 21 Hz; Ar CH), 104.84 (pyrazole CH), 58.49 (CO-CH-NH), 56.47 (CyCH₂N), 39.94 (cyclohexyl CH), 30.35 (CH(CH₃)₂), 30.23 (cyclohexyl CH₂), 26.19 (cyclohexyl CH₂), 25.50 (cyclohexyl CH₂), 19.68 (CH₃) and 18.73 (CH₃) ppm; ¹⁹F NMR (d₆-DMSO) δ -115.00 ppm. A sample of the product was recrystallized from ethyl acetate/cyclohexane for x-ray crystallography.

Instrumentation

Liquid chromatography - mass spectrometry

LC-MS analyses, equipped with an electrospray ionization source, were performed on an Agilent 1100 LC system. The column (Allure PFP Propyl, 5 μm , 50 \times 2.1 mm) was from Restek (Bellefonte, PA, USA) and the aqueous mobile phase A consisted of 0.1% formic acid in water whereas mobile phase B was prepared from 0.1% formic acid in acetonitrile, respectively. The Agilent LC-MSD settings were as follows: positive electrospray mode, capillary voltage 3000 V, drying gas (N_2) 12 L/min at 350 $^\circ\text{C}$, nebulizer gas (N_2) pressure 60 psi, m/z 50-500, fragmentor voltage either 50 V, 130 V or 150 V. Samples for LC-MS analysis (2 μL injection volume) were dissolved in acetonitrile/water (1:1, containing 0.1% formic acid) at a concentration of 5 $\mu\text{g}/\text{mL}$. The following gradient elution program was used: 0-5 min 12% A and then increased to 35% over 30 min using a linear gradient. The flow rate was 1 mL/min and the column temperature was 30 $^\circ\text{C}$.

Gas chromatography – mass spectrometry

An Agilent 6980 GC coupled to an Agilent 5973 MSD (HP-5ms column, 30 m \times 0.25 mm \times 0.25 μm) using helium as the carrier gas at a constant flow of 1 mL/min was employed in splitless mode. Injection port and transfer line temperatures were set at 250 $^\circ\text{C}$ and 280 $^\circ\text{C}$, respectively. Oven temperature: 150 $^\circ\text{C}$ held for 2 min, ramped at 20 $^\circ\text{C}/\text{min}$ to 300 $^\circ\text{C}$ and held for 10.5 min. The total run time was 20 min. The samples for analysis were dissolved in acetonitrile and the injection volume was 1 μL .

X-Ray Crystallography

Intensity data were collected at 100(2) K using a MiTeGen micromount on a Bruker APEX Duo CCD diffractometer equipped with an Oxford Cobra cryosystem. Data were collected using ω and ϕ scans, corrected for Lorentz and polarization effects, and integrated using the Bruker APEX program suite. Structures were solved by direct methods and refined with least squares procedures. All non-hydrogen atoms were refined anisotropically and hydrogen atoms were placed geometrically in the calculated positions using a riding model except for H21, H28a, and H28b which were located and refined.

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4 Data collected using Cu K α radiation (1.54178 Å) for a colorless plate crystal 0.26 ×
5 0.11 × 0.08mm³, C₂₄H₃₂FN₅O₂, M = 441.54, Monoclinic, P2₁, a = 12.1979(5),
6 b=7.0566(3), c = 14.6230(6)Å, β = 111.6180(10)°, V = 1170.15(8)Å³, Z=2, ρ =
7 1.253mg/m³, μ =0.709mm⁻¹, Reflections collected 18953 (θ_{\max} = 68.49°), independent
8 reflections 4247, R_(int) = 0.0321, S = 1.025, R1 = 0.0290, wR2 = 0.0753.* CCDC
9 deposition number 1405051. (* R1= $\Sigma||F_o|-|F_c|| / \Sigma |F_o|$ and wR2 = $\Sigma w(|F_o|^2-|F_c|^2)^2 /$
10 $\Sigma w|F_o|^2$)^{1/2})
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18 *Nuclear magnetic resonance spectroscopy*

19 Samples were prepared in d₆-DMSO and ¹H (600 MHz) and ¹³C (150 MHz) NMR
20 spectra were recorded on a Bruker AV600 NMR spectrometer using a 5 mm TCI
21 cryoprobe. ¹H NMR spectra were referenced to an external TMS reference at δ = 0
22 ppm.
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27 *High-resolution electrospray mass spectrometry*

28 HR-ESI mass spectra were recorded by direct injection into a LTQ Orbitrap
29 Discovery (Thermo Fisher, UK). Samples were dissolved in acetonitrile/water
30 (1:1, containing 0.1% formic acid) and infused at a rate of 5 μ L/min. Full
31 accurate high-resolution (30000) mass scans were performed in positive
32 electrospray mode. Measured accurate masses were within \pm 5ppm of the
33 theoretical masses. The following conditions were used: drying gas (N₂) 10000
34 mL/min, capillary temperature 310 °C, spray voltage 4 V, capillary voltage 22 V and
35 tube lens 77 V.
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43 **Results and discussion**

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46 The structural diversity associated with substances considered as so-called
47 synthetic cannabinoids can create challenges to forensic, clinical, law enforcement
48 and regulatory communities.^[2-5,26-28] Some of the recently occurring indazole
49 derivatives (Figure 1A) are based on the patent literature which point towards
50 appreciable CB₁ receptor affinity and [³⁵S]GTP γ S activity^[29,30] but others remain to
51 be fully explored to assess the extent to which these substances show
52 psychopharmacological similarities to compounds present in cannabis. A compound
53 that appeared to deviate from currently reported core structures, i.e. carrying a
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3 pyrazole moiety instead of a more established indole, benzimidazole, pyrrole or
4 indazole core, has been advertised on a number of 'research chemicals' websites
5 as AZ-037, which represents (S)-N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-
6 fluoropentyl)-5-(4-fluorophenyl)-1*H*-pyrazole-3-carboxamide (Figure 1B). Any
7 information about its psychoactive or pharmacological properties seems to be
8 currently unavailable although it appears to be advertised as a synthetic
9 cannabinoid on various websites. The EMCDDA has recently received a notification
10 on its detection in an EU Member State via the EU EWS on NPS.^[31]
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17 The present investigation was initiated following the donation of a vendor sample to
18 the authors' laboratories labeled as AZ-037. During initial characterization it was
19 revealed that the analytical data were inconsistent with the expected structure.
20 Inspection of the UK-based website also indicated that the suggested IUPAC name
21 for AZ-037 was also not in agreement with the shown structure. The shown
22 structure associated with AZ-037 was 1-(5-fluoropentyl)-1*H*-indole-3-carboxylic acid
23 8-quinolinyl ester, i.e. commonly referred to as the synthetic cannabinoid 5F-PB-22.
24 Interestingly, the analytical characterization of a substance very closely related to
25 AZ-037 has been published recently. In this case, the reported compound was *N*-
26 (1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-3-(4-fluorophenyl)-
27 pyrazole-5-carboxamide and the suggested name for this substance is 3,5-5F-ADB-
28 FUPPYCA (Figure 1B). The presence of the pyrazole ring can give rise to a number
29 of isomers, which complicates matters significantly and this meant that the
30 chromatographic separation and characterization of isomers was deemed
31 necessary. The preparation of both isomers associated with positions 5 and 3
32 confirmed unambiguously that the sample received from the Internet supplier was
33 consistent with 3,5-AB-CHMFUPPYCA (Figure 2).
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44 Synthesis

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47 Both regioisomers 5,3-AB-CHMFUPPYCA and 3,5-AB-CHMFUPPYCA were
48 synthesized using two specific routes that are outlined in Figure 2. The synthesis of
49 5,3-AB-CHMFUPPYCA involved the reaction of methyl 4-(4-fluorophenyl)-2,4-
50 dioxobutanoate (a) with (cyclohexylmethyl)-hydrazine (b), which gave rise to the
51 pyrazole intermediate (c). This intermediate was then reacted with lithium
52 hydroxide, which provided the carboxylic acid species (d). This entity was reacted
53 with thionyl chloride and subsequently *N,N*-dimethylformamide, *L*-valinamide
54 hydrochloride and *N,N*-diisopropylethylamine to yield the 5,3-AB-CHMFUPPYCA
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3 product (Figure 2A). The preparation of 3,5-AB-CHMFUPPYCA involved reacting 5-
4 (4-fluorophenyl)-1*H*-pyrazole-3-carboxylate (e) with (bromomethyl)-cyclohexane (f)
5 and cesium carbonate yielding the cyclohexylmethyl intermediate (g). This
6 intermediate was reacted with lithium hydroxide, which induced the formation of the
7 carboxylic acid species (h). This entity was reacted with thionyl chloride and
8 subsequently *N,N*-dimethylformamide, *L*-valinamide hydrochloride and *N,N*-
9 diisopropylethylamine, which yielded the 3,5-AB-CHMFUPPYCA product (Figure
10 2B).
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17 The structural differences in the regioisomers included the positioning of the
18 substituents around the pyrazole core, which were dictated by the positioning of the
19 double bonds within the pyrazole ring structure. In the 5,3-AB-CHMFUPPYCA isomer
20 (Figure 2A) the 4-fluorophenyl component was attached to position 5 whereas the
21 secondary carboxamide structure was attached to position 3 of the pyrazole ring. In
22 case of the 3,5-AB-CHMFUPPYCA isomer, the opposite arrangement was present. A
23 route specific chlorine containing by-product, *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-
24 4-chloro-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1*H*-pyrazole-5-carboxamide, was
25 encountered during the synthesis of the 3,5-AB-CHMFUPPYCA isomer
26 (supplemental information) and this was not observed during the preparation of the
27 regioisomeric form.
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38 *Gas chromatography mass spectrometry*

39 Separation of both isomers was successfully achieved using gas chromatography
40 (GC). The retention times for 5,3-AB-CHMFUPPYCA and 3,5-AB-CHMFUPPYCA
41 were 14.74 and 15.20 min, respectively, and a comparison with the vendor sample
42 was in agreement with the identity of the latter (Figure 3A-C). A comparison of both
43 EI mass spectra also provided sufficient evidence that both isomers could be
44 differentiated based on fragmentation patterns (Figure 3E-F). In the EI mass spectrum
45 for 5,3-AB-CHMFUPPYCA, the base peak was observed at *m/z* 285 and indicated
46 the loss of 115 Da from the parent molecule, which represented the formation of an
47 oxonium species (Figure 3G). Another dominant peak was observed at *m/z* 356 that
48 may be described by the loss of formamide from the molecular ion. Another minor
49 fragment encountered in the EI mass spectrum was *m/z* 189, which might have been
50 consistent with the loss of cyclohexylmethyl species from the oxonium ion at *m/z* 285.
51 In the EI mass spectrum of 3,5-AB-CHMFUPPYCA, the base peak was observed at
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3 m/z 257, which was thought to represent the 1-(cyclohexylmethyl)-3-(4-fluorophenyl)-
4 1*H*-pyrazole fragment and its formation might have been facilitated by the position of
5 the double bonds within the pyrazole ring of the 3,5-AB-CHMFUPPYCA isomer
6 (Figure 3G). The positioning of the double bonds in the pyrazole ring might have
7 facilitated the formation of a temporary double bond between the nitrogen at position
8 1 of the pyrazole ring and the carbon at position 5, which might have resulted in the
9 formation of a thermodynamically stable species (Figure 4). The formation of this
10 species would not have been possible with the 5,3-AB-CHMFUPPYCA isomer due to
11 the positioning of the double bonds within the pyrazole ring. Also present in the EI
12 mass spectrum of the 3,5-AB-CHMFUPPYCA isomer was the detection of the
13 molecular ion at m/z 400. The fragment at m/z 356 was presumably formed by the
14 loss of formamide (Figure 3G). The chlorinated by-product formed during the
15 synthesis of 3,5-AB-CHMFUPPYCA was detected under GC-MS analysis conditions
16 and a retention time of 16.07 min was obtained for this compound (supplemental
17 data).
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28 *Liquid chromatography mass spectrometry*

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30 Analysis of both synthesized isomers and the vendor sample by high performance
31 liquid chromatography (HPLC) confirmed satisfactory separation. A retention time of
32 13.41 min was obtained for the 5,3-AB-CHMFUPPYCA isomer, whereas a retention
33 time of 13.85 min was obtained for the 3,5-AB-CHMFUPPYCA isomer (Figure 5).
34 The electrospray ionization (ESI) single quadrupole mass spectra obtained from in-
35 source collision-induced dissociation (CID) of the synthesized regioisomers (150 V
36 fragmentor voltage) shared similar product ions but key features that allowed for
37 differentiation between the two substances were also apparent (Figure 6). For
38 example, the in-source CID spectrum of 5,3-AB-CHMFUPPYCA displayed the
39 sodiated adduct $[M + Na]^+$ at m/z 423 as the base peak (Figure 6A), which was not
40 the case with 3,5-AB-CHMFUPPYCA where the relative abundance was around 40%
41 (Figure 6B). A major difference, however, was observed in the mass spectrum of 3,5-
42 AB-CHMFUPPYCA that formed a base peak at m/z 260 and which was absent in the
43 5,3-AB-CHMFUPPYCA (Figure 6A). Figure 6C shows a proposed mechanism of its
44 formation that may be rationalized by the loss of 2-cyclohexylacetamide from the
45 protonated molecule. Product ions common in both spectra included m/z 356 by way
46 of cleaving formamide from the protonated molecule and the subsequent formation of
47 the m/z 285 oxonium ion. The m/z 189 ion may have then been formed by the loss of
48 the cyclohexylmethyl species from the oxonium fragment, resulting in a species with
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3 chemical formula $C_{10}H_6FN_2O^+$. The chlorinated by-product was observed during the
4 HPLC-ESI-MS analysis of 3,5-AB-CHMFUPPYCA. A retention time of 14.49 min was
5 obtained for this compound (supplemental data). Analysis by high-resolution mass
6 spectrometry was employed, which yielded formation of identical product ions for
7 each isomer (supplemental data).
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10 11 12 13 14 *Nuclear magnetic resonance spectrometry*

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16 In addition to one-dimensional proton (1H), carbon (^{13}C) and fluorine (^{19}F) NMR
17 analyses, the implementation of two-dimensional experiments proved helpful for the
18 characterization of both isomers and the vendor sample. For example, in the case of
19 5,3-AB-CHMFUPPYCA, heteronuclear multiple-bond correlation (HMBC)
20 experiments revealed a 3-bond $^1H/^{13}C$ correlation between the respective carbon
21 atom at position 5 of the pyrazole ring and the protons at the *ortho* position of the
22 fluorinated phenyl ring. A correlation was also observed between the aforementioned
23 carbon and the protons of the methylene group attached to the cyclohexyl ring
24 (Figure 7A). As far as 3,5-AB-CHMFUPPYCA was concerned, the 3-bond correlation
25 between the carbon atom at position 5 of the pyrazole ring and the protons on the
26 methylene group attached to the cyclohexyl ring (Figure 7B) was also present. As
27 expected, the 3-bond correlation with the protons on the fluorinated phenyl ring was
28 not detected. The vendor sample was also subjected to HMBC analysis and
29 confirmed the 3,5-AB-CHMFUPPYCA assignment (supplemental data). Further
30 information was obtained from nuclear Overhauser effect spectroscopy (NOESY),
31 which provided additional insights into distinguishing features between the two
32 isomers. A qualitative nuclear Overhauser experiment (NOE) showed connectivity
33 between the methylene group attached to the cyclohexyl ring and the protons
34 attached to the *ortho* position of the fluorinated phenyl ring present in the 5,3-AB-
35 CHMFUPPYCA isomer. This nOe was not observed with 3,5-AB-CHMFUPPYCA
36 (supplemental data).
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48 49 50 51 *X-Ray crystallography*

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53 The solid-state structure of the 3,5-AB-CHMFUPPYCA isomer was elucidated by
54 single crystal X-ray diffraction and is shown below in Figure 8. The structure is a
55 solvate with a co-crystallized molecule of acetonitrile present in the asymmetric unit.
56 The molecule crystallizes in the chiral monoclinic space group $P2_1$ and the chiral
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3 center C22 has been determined to be the S-enantiomer. The packing is dominated
4 by the amide donor groups (N21, N28), which form hydrogen bonds with the terminal
5 amido oxygen O27 (N28...O27 range 2.856(2) - 2.963(2) Å). These link the
6 molecules into a strongly associated chain. A channel exists between the chains and
7 the acetonitrile solvent molecules lies within it. A weak NH...N interaction (N28...N29,
8 3.039 Å) exists between 3,5-AB-CHMFUPPYCA and the solvent (supplemental
9 information).

17 Conclusion

19 An in-depth analytical characterization of a 'research chemical' advertised as AZ-037
20 revealed mislabeling when it transpired that it was consistent with 3,5-AB-
21 CHMFUPPYCA instead. The combination of analytical techniques and confirmation
22 of compound identification by organic synthesis provided an effective approach to
23 tackling an increasingly complex area of investigation where increasing demands are
24 placed on investigators in the field of new psychoactive substances.
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Figures

Figure 1. Chemical structures of synthetic cannabinoids from (A) the PINACA, FUBINACA, CHMINACA series and (B) the FUPPYCA series.

Figure 2. Synthesis protocol for the AB-CHMFUPPYCA isomers. (A) Specific synthetic route for 5,3-AB-CHMFUPPYCA. (B) Specific synthetic route for 3,5-AB-CHMFUPPYCA.

Figure 3. (A-F) GC-MS data obtained for both AB-CHMFUPPYCA isomers and vendor sample. (G) Proposed EI-MS fragmentation patterns for the AB-CHMFUPPYCA isomers.

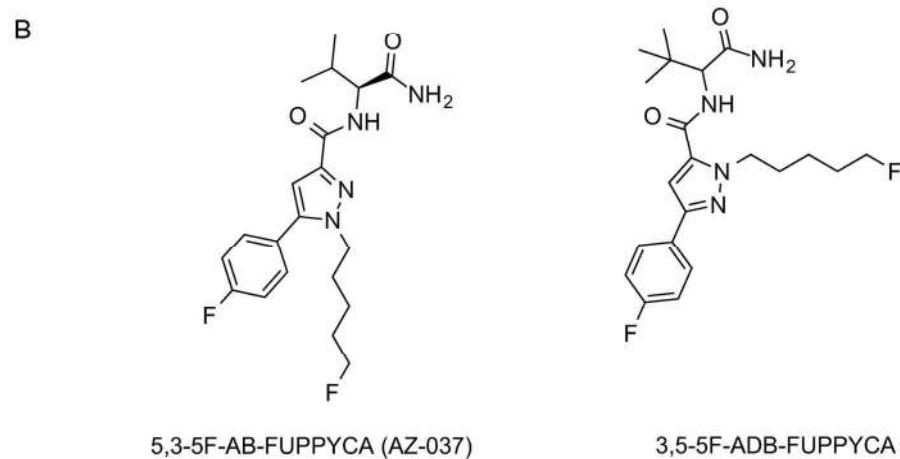
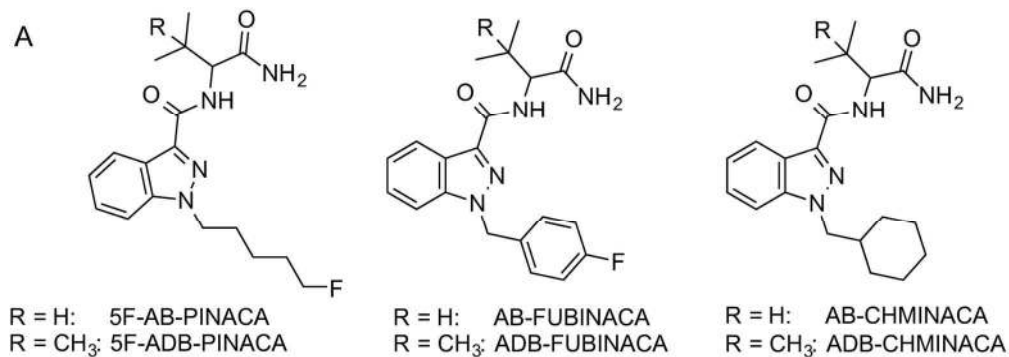
Figure 4. Proposed mechanism for the formation of 1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1*H*-pyrazole species observed at m/z 257 in the EI-mass spectrum of 3,5-AB-CHMFUPPYCA.

Figure 5. (A-F) Analysis of synthesized AB-CHMFUPPYCA isomers and vendor sample using high performance liquid chromatography selected ion monitoring (SIM) mass spectrometry at 50V.

Figure 6. (A-B) Electrospray ionization single quadrupole mass spectra following in-source collision-induced dissociation at 150 V. (C) Proposed mechanism for the formation of the species with m/z 260 that may be rationalized by the loss of 2-cyclohexylacetamide from the protonated molecule.

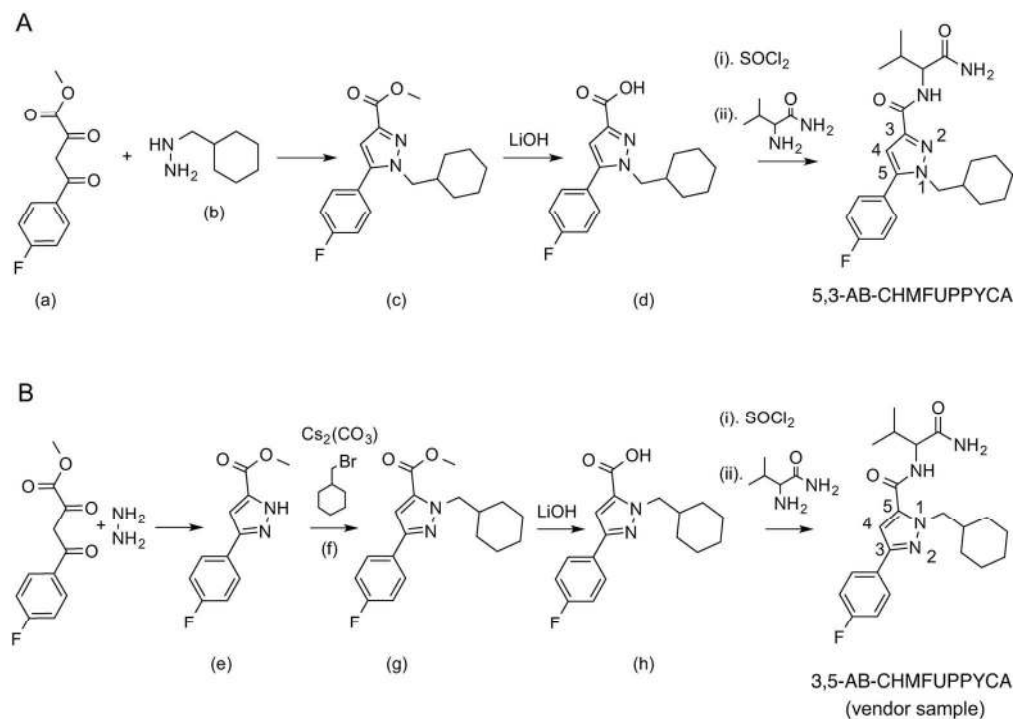
Figure 7. Heteronuclear multiple-bond correlation NMR spectra for (A) the 5,3-AB-CHMFUPPYCA isomer and (B) the 3,5-AB-CHMFUPPYCA isomer.

Figure 8. Molecular structure of 3,5-AB-CHMFUPPYCA (thermal displacement at 50% probability) with hydrogen atoms omitted for clarity.



Chemical structures of synthetic cannabinoids from (A) the PINACA, FUBINACA, CHMINACA series and (B) the FUPPYCA series.

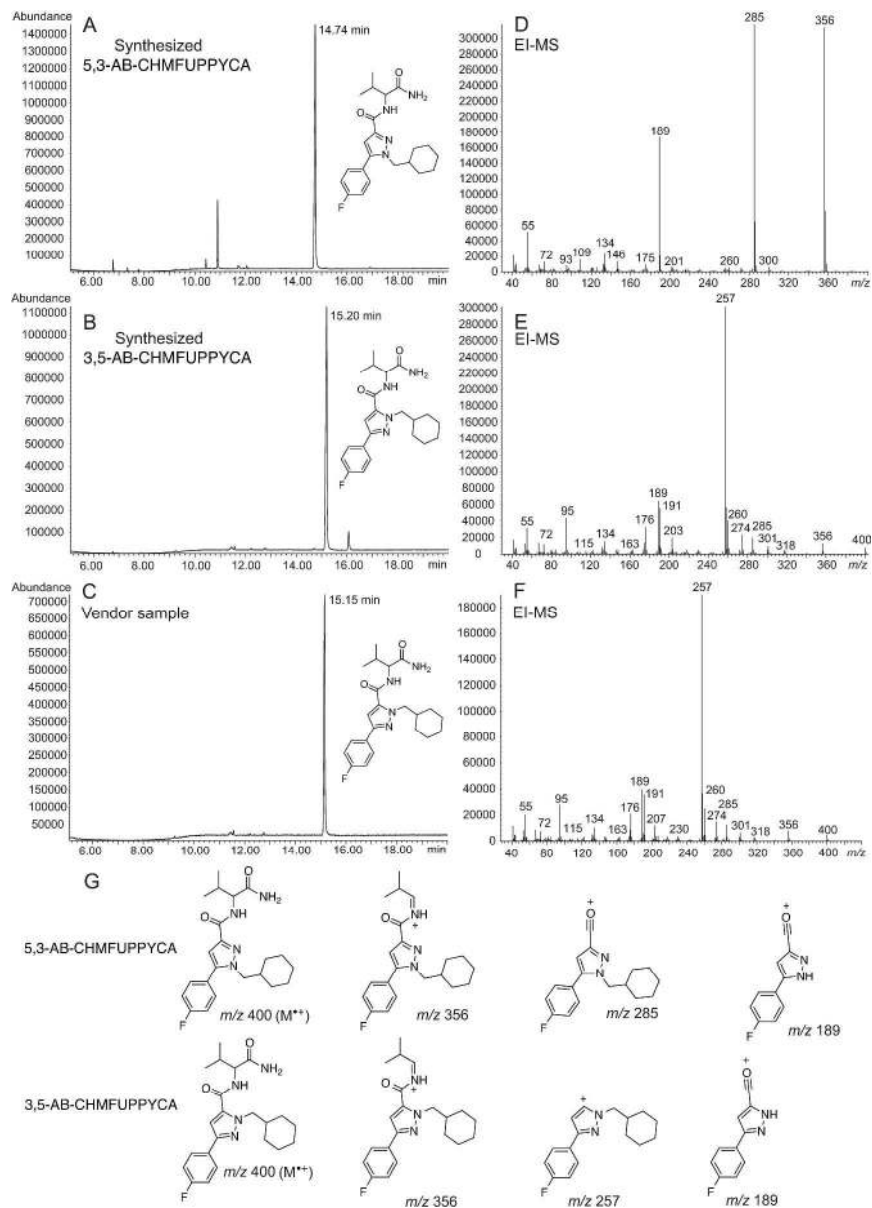
153x130mm (300 x 300 DPI)



Synthesis protocol for the AB-CHMFUPPYCA isomers. (A) Specific synthetic route for 5,3-AB-CHMFUPPYCA.

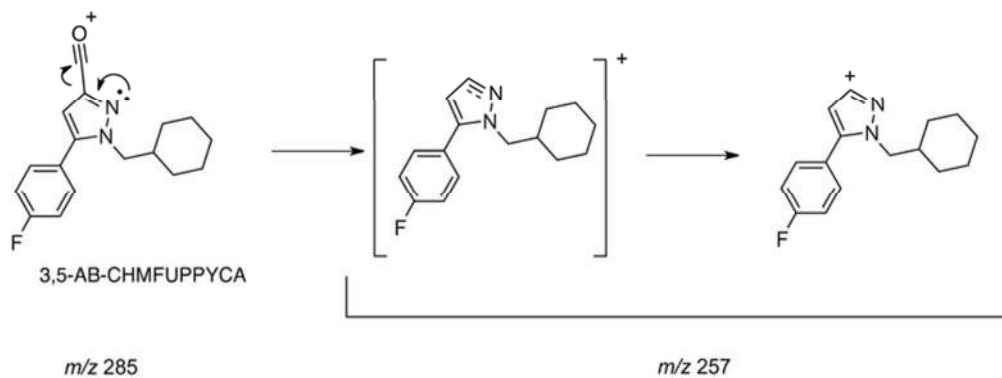
(B) Specific synthetic route for 3,5-AB-CHMFUPPYCA.

142x101mm (300 x 300 DPI)



(A-F) GC-MS data obtained for both AB-CHMFUPPYCA isomers and vendor sample. (G) Proposed EI-MS fragmentation patterns for the AB-CHMFUPPYCA isomers.

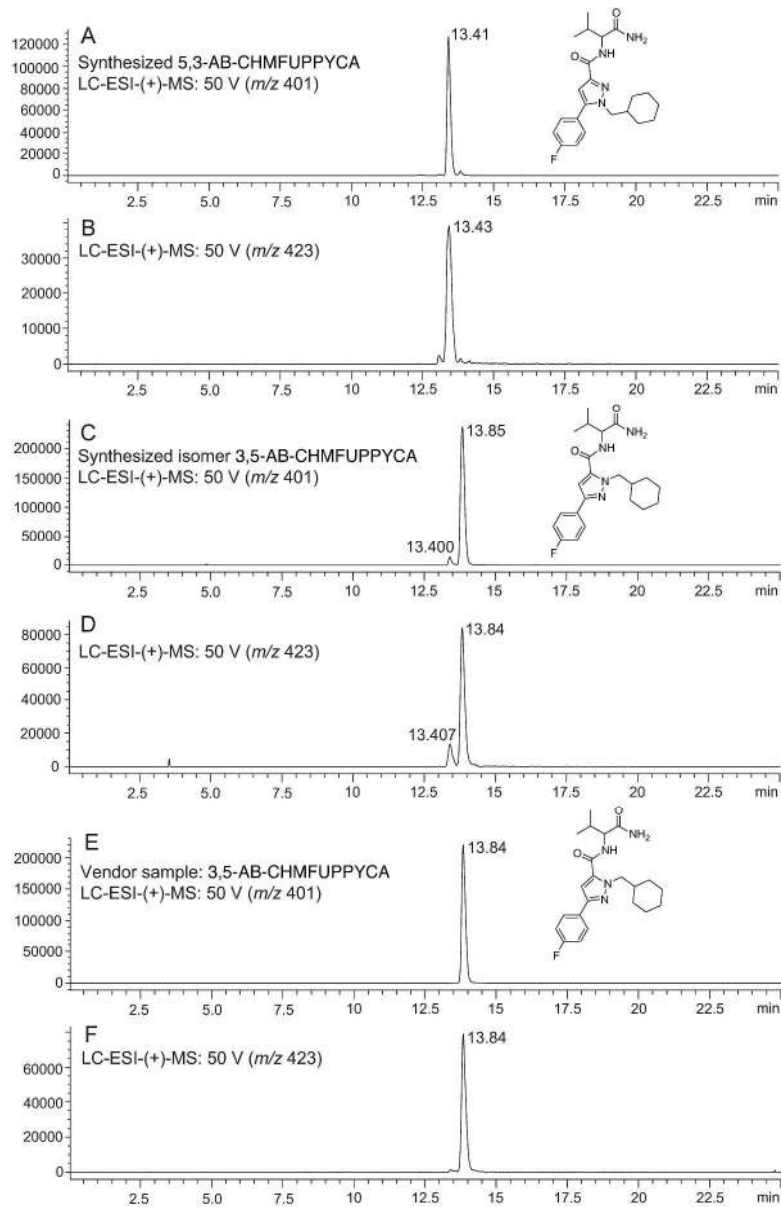
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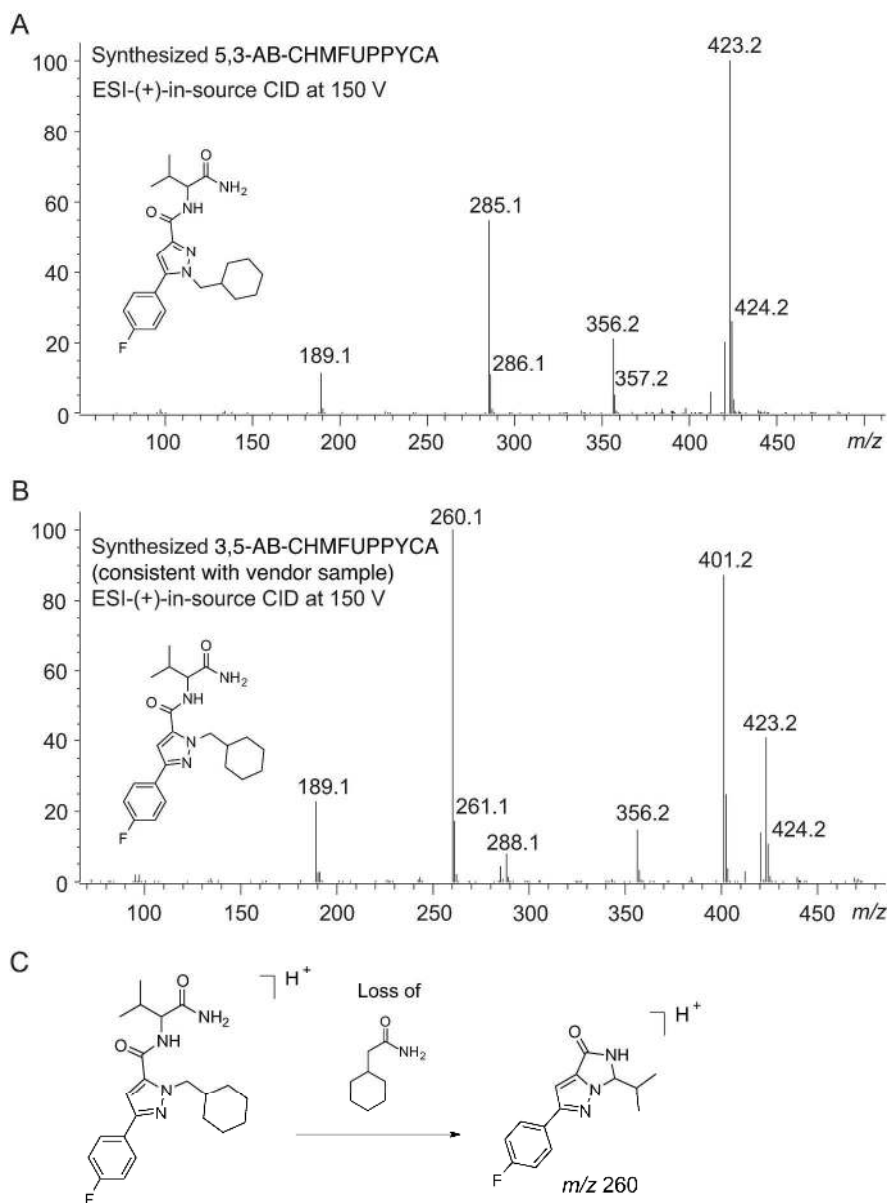
20 Proposed mechanism for the formation of 1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1H-pyrazole species
21 observed at m/z 257 in the EI-mass spectrum of 3,5-AB-CHMFUPPYCA.
22 66x24mm (300 x 300 DPI)

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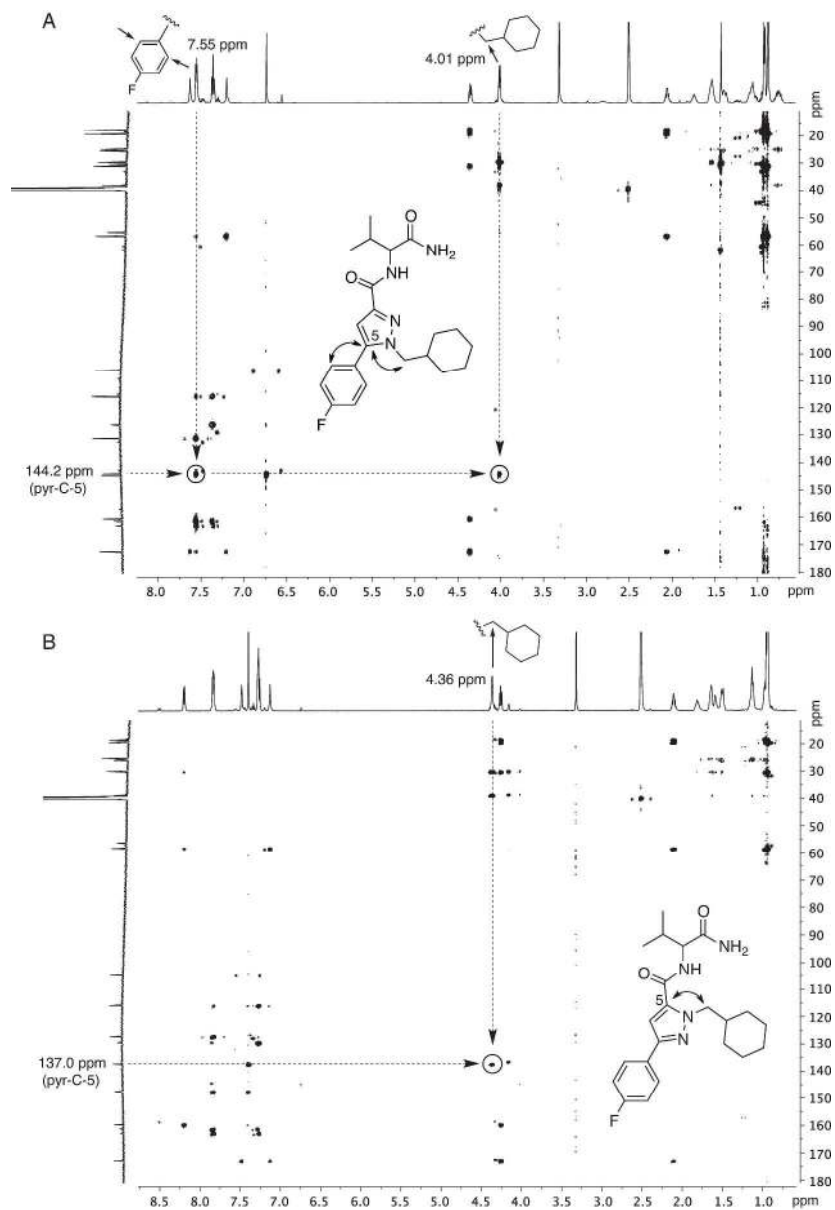
Peer Review



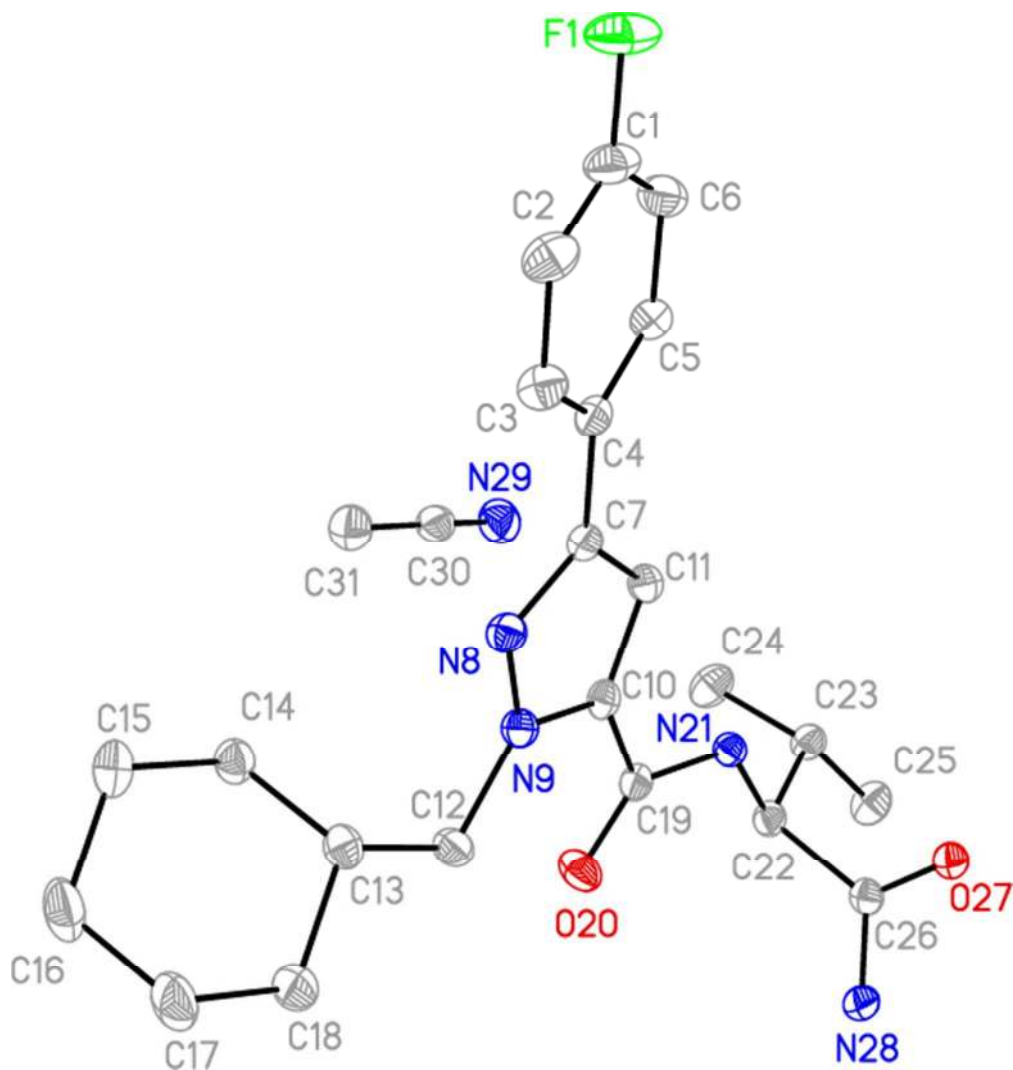
(A-F) Analysis of synthesized AB-CHMFUPPYCA isomers and vendor sample using high performance liquid chromatography selected ion monitoring (SIM) mass spectrometry at 50V.
266x415mm (300 x 300 DPI)



(A-B) Electrospray ionization single quadrupole mass spectra following in-source collision-induced dissociation at 150 V. (C) Proposed mechanism for the formation of the species with m/z 260 that may be rationalized by the loss of 2-cyclohexylacetamide from the protonated molecule.
215x294mm (300 x 300 DPI)



Heteronuclear multiple-bond correlation NMR spectra for (A) the 5,3-AB-CHMFUPPYCA isomer and (B) the 3,5-AB-CHMFUPPYCA isomer.
288x422mm (300 x 300 DPI)



Molecular structure of 3,5-AB-CHMFUPPYCA (thermal displacement at 50% probability) with hydrogen atoms omitted for clarity.

159x168mm (96 x 96 DPI)