Correspondence case report

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Identification and characterization of a putative new psychoactive substance, 2-(2-(4-chlorophenyl)acetamido)-3-methylbutanamide, in Spain

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Introduction

The term 'new psychoactive substances' (NPS) refers to synthetically changed natural compounds or newly designed compounds intended to elicit a psychotropic response such as stimulation, hallucination or sedation.[1] According to the European Monitoring Centre for Drugs and Drug Addiction of the European Union, more than 560 NPS are currently being monitored, and 98 of these substance were reported for the first time in 2015.[2,3] Despite that, there are still many NPS that are not being monitored, and therefore their health effects are not yet studied. Hence, many cases of intoxication and death related to NPS have been reported in the past few years, highlighting the public health risks of these substances.[4] One of the challenges of monitoring NPS is the continuous structural evolution to evade regulation, so when one substance is banned or controlled, several new compounds replace it in the market.

NPS are usually found with various appearances, like incenses, bath salts, herbal blends or party pills, sold through an unregulated market, different websites or smart-shops.[4,5] These products are typically known as 'legal highs'. Synthetic cannabinoids, synthetic cathinones and amphetamines constitute the largest groups of NPS, although opioids, tryptamines, benzodiazepines, piperazines and phenethylamines are also common.[2,3] Synthetic cathinones and amphetamines are sold as replacements for stimulants, and have been reported in pills, crystal and sniff powder.[6] Synthetic cannabinoids are commonly sold as herbal blends or spices, replacing cannabis.[7]

Forensic laboratories, universities, research institutes, public health centres and law enforcement agencies play an important role in monitoring these types of substances, which are also encountered in customs seizures and medical emergencies.[8] Various analytical approaches have been reported for the analysis of NPS in legal highs samples. Gas chromatography coupled to mass spectrometry (GC–MS) using electron ionization (EI) is a fast and reliable technique for the identification of these compounds based on the use of spectral libraries.[9] However, fragmentation spectra of novel NPS may not be available. Moreover, some of these compounds are non-volatile and thermolabile, requiring additional derivatization steps in GC–MS analysis. Liquid chromatography (LC) coupled to high-resolution MS (HRMS) has proven to be a powerful technique for the screening of NPS in legal highs samples.[4,10–12] Moreover, HRMS allows screening analyses without reference standards being available (tentative identification).[11,13,14] HRMS/MS also has potential application in the compound structural elucidation of unknown substances by using accurate-mass fragmentation data.[15,16]

As complementary tools to HRMS instruments, additional spectroscopic techniques can be used for the unequivocal characterization of NPS. Fourier transform infrared (FTIR) spectroscopy and Raman spectroscopy are useful for a fast evaluation of molecules, identifying functional groups without destroying the sample and with reasonable running costs.[10] Nuclear magnetic resonance (NMR) spectroscopy has proved to be one of the most powerful techniques for the structural elucidation and characterization of organic molecules, and has been applied to the identification and characterization of synthetic cannabinoids[17] and synthetic cathinones.[18] X-ray crystallography, especially single-crystal X-ray diffraction, allows an unequivocal characterization of the structure of a molecule, but only if the compound can be formed as X-ray-suitable crystals. Thus, the combination of spectroscopic and mass spectrometric techniques provides a versatile workflow for structural elucidation, characterization and identification of unknown substances.[19]

The aim of the work reported here was the determination of the main compound present in a crystal sample from an anonymous Spanish consumer. The structure of a new designer drug derivate was elucidated after an exhaustive analysis using various analytical techniques. Characterization of the molecule was performed by GC–MS, LC-HRMS using hybrid quadrupole time-of-flight (QTOF) mass analyser, NMR and single-crystal X-ray diffraction. Additionally, melting point determination, FTIR and ultraviolet

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(UV) analyses were performed. Current information reported in this article will be useful for other laboratories in order to monitor the presence of this novel NPS or other related compounds in seizures and biological samples.

Experimental

Legal high sample

The sample, consisting of small crystals, was submitted by an anonymous consumer to Energy Control for analysis. According to the information provided by the consumer, it was purchased from the Internet, but no information was received about the consumption or psychoactive effects of this product. Energy Control is a project based on risk reduction, belonging to the Spanish non-governmental organization Asociación Bienestar y Desarrollo. Energy Control offers an anonymous drug testing service. Consumers can bring their samples to one of the four Energy Control headquarters (located in Madrid, Catalonia, Balearic Islands and Andalucía), send them by mail or submit during outreach work in nightlife settings, such as music festivals, clubs or underground raves. This service allows the monitoring of illegal drug markets, and the detection of trends in substances and their use, making this information available to all stakeholders. Additionally, analyses of both national and international samples received contribute to understanding of what is happening at street level.

Reagents and chemicals

For GC–MS analysis, HPLC-grade methanol (MeOH) was purchased from Panreac (Barcelona, Spain). For LC-HRMS analysis, HPLC-grade MeOH, HPLC-grade acetonitrile (ACN), acetone, formic acid (HCOOH) and sodium hydroxide (NaOH) were purchased from Scharlau (Scharlab, Barcelona, Spain). HPLC-grade water was obtained by purifying demineralized water using a Milli-Q system from Millipore (Bedford, MA, USA). Leucine encephalin was acquired from Sigma-Aldrich (St Louis, MO, USA). For NMR analysis, deuterated dimethylsulfoxide (DMSO-d_6), deuterated chloroform ($CDCl_3$) and deuterated water (D_2O) were purchased from Sigma-Aldrich.

Sample treatment

For GC–MS analysis, 10 mg of sample was extracted with 10 mL of MeOH assisted with sonication during 15 min, due to the compound being soluble in MeOH. Finally, the extract was centrifuged to remove insoluble material and afterwards directly injected into the GC–MS system.

For LC-HRMS analysis, 25 mg of sample was weighed in 2 mL propylene tubes and extracted with 1 mL of acetone in an ultrasonic bath for 15 min. Acetone has been successfully used for extracting NPS from legal highs samples.[11,14] After centrifugation at 12 000 rpm during 12 min, the supernatant was diluted 100-fold with HPLC-grade water, and 10 μL of the extract was injected in the LC-HRMS system, using MS^E acquisition mode (see Instrumentation section for details of this acquisition mode).

For NMR analysis, 10 mg of sample was extracted in 0.6 mL of DMSO-d_6. An additional sample treatment was performed by addition of D_2O to the DMSO-d_6 extract, in order to avoid amide signals in the ^1H NMR experiment. Additionally, 10 mg of sample was treated with 0.6 mL of $CDCl_3$ in order to study diastereotopic hydrogens.

For single-crystal X-ray diffraction, an adequate needle-shaped crystal was selected from the sample.

For FTIR analysis, a sample was prepared with 5% of the unknown compound and 95% of potassium bromide, homogenized in an agate mortar and compressed under a pressure of 5000 kg/cm^2.

UV analysis was performed using the extract prepared for LC-HRMS analysis.

Instrumentation

For GC–MS analysis, an Agilent 7890B gas chromatograph was coupled to a 5977A quadrupole mass spectrometer detector (Agilent, Santa Clara, CA, USA). The gas chromatograph was fitted with a G4513A auto-sampler injector. Insert liners packed with silanized glasswool were used, and the injector and the interface were operated at 280°C. An amount of 1 μL of sample was injected in split mode, with a split ratio of 1:10, into a 30 m, 0.25 mm i.d., 0.25 μm film thickness 5% phenylmethylsilicone column (HP-5MS, Agilent Technologies). Helium was used as carrier gas at a flow rate of 1 mL/min. The oven temperature was initially maintained at 90°C for 2 min and programmed to reach 320°C at 20°C/min. It was finally maintained at 320°C for 9.5 min (total run time was 21.5 min). The mass spectrometer was operated in EI mode at 70 eV. MS system worked in SCAN acquisition mode, acquiring from *m/z* 40 to 400 Da. In order to identify the unknown compound, the fragmentation spectrum was compared with four spectral libraries: the searchable mass spectral library NIST/EPA/NIH Mass Spectral Library, Data Version NIST 14; Searchable Mass Spectral Library Version 2.3 (http://www.swgdrug.org/ms.htm); the searchable mass spectral library Cayman Spectral Library (CSL) (https://www.caymanchem.com/app/template/SpectralLibrary.vm); and Energy Control's internal mass spectral library. Analytical data were acquired and processed using MassHunter B.06.00 (Agilent) operation software.

LC-HRMS analysis was performed using an ACQUITY UPLC ultrahigh-performance liquid chromatography (UHPLC) system (Waters, Milford, MA, USA) coupled to a XEVO G2 hybrid QTOF mass spectrometer (Waters Micromass, Manchester, UK) with an orthogonal Z-spray electrospray ionization interface operating in positive ionization mode. The chromatographic separation was performed using a Cortecs C18 (Waters) 2.7 μm particle size analytical column (100 × 2.1 mm) at a flow rate of 0.3 mL/min. The column temperature was set to 40°C. The mobile phases used were H_2O with 0.01% HCOOH (A) and MeOH with 0.01% HCOOH (B). The mobile phase gradient was performed as follows: 10% of B at 0 min, 90% of B at 14 min linearly increased, 90% of B at 16 min, and finally 10% B at 18 min in order to return to initial conditions. The injection volume was 10 μL. Nitrogen (Praxair, Valencia, Spain) was used as desolvation and nebulizing gas. The desolvation gas flow was set at 1000 L/h. The TOF resolution was *ca* 20 000 at FWHM at *m/z* 556. The range acquired by the MS system was from *m/z* 50 to 1000. A capillary voltage of 0.7 kV and a cone voltage of 10 V were used during all the chromatographic runs. Argon (99.995%; Praxair, Valencia, Spain) was used as a collision gas. The interface temperature was set to 650°C and the source temperature to 120°C. For MS^E experiments, two acquisition functions with different collision energy were created. The low-energy function (LE) used a collision energy of 4 eV in order to obtain information about the protonated molecule and adducts (if present), while the high-energy function (HE) applied a collision energy ramp from 15 to 40 eV, in order to promote fragmentation

of the compounds. For further details, see Ibañez *et al.*[14] Calibration of the mass axis was performed daily from *m/z* 50 to 1000 Da using a 1:1 mixture of 0.05 M NaOH:5% HCOOH, diluted 1:25 with ACN:H_2O 80:20 mixture. For accurate mass measurement, a 2 μg/mL leucine encephalin solution in ACN:H_2O 50:50 with 0.1% HCOOH was used as lock-mass, pumped at a flow rate of 20 μL/min. The leucine encephalin protonated molecule (*m/z* 556.2771) was used for recalibrating the mass axis and to ensure an accurate mass during all the chromatographic runs. MS data were acquired in centroid mode using MassLynx data station operation software, version 4.1 (Waters).

NMR analyses were performed using a Varian VNMRS 500 MHz spectrometer at 303 K using DMSO-d_6 (Varian Medical Systems, Palo Alto, CA, USA). The residual solvent signals at δ = 2.50 ppm for ^{1}H (DMSO) and at δ = 39.51 ppm for ^{13}C (DMSO-d_6) were used as internal references. Characterization of the compound was performed using five experiments: ^{1}H NMR, ^{13}C NMR, correlated spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC). NMR experiment data were collected using Varian Vnmr 2.2c software (Varian Medical Systems, Palo Alto, CA, USA).

For single-crystal X-ray diffraction, an Agilent SuperNova diffractometer (Agilent Technologies) was used. The diffractometer was equipped with an Atlas CCD detector (Agilent Technologies), and Cu Kα radiation (λ = 1.54184 Å) was used. Sample was kept at 199.95 K during data collection. Experimental data were acquired using SHELXS-2013 software (Yale University, New Haven, CT, USA), using the OLEX software package (Olex AS, Trondheim, Norway).

For FTIR analysis, a Jasco FT/IR-6200 FTIR spectrometer (Jasco Inc., Easton, MD, USA) was used. Data acquisition was performed at 23°C between 4000 and 400 cm^{-1}, with a resolution of 4 cm^{-1} and performing 32 acquisitions.

UV analysis was performed using a Waters Alliance 2795 LC system (Waters, Milford, MA, USA) equipped with a Waters 2998 photodiode array detector. The LC separation was performed using an Atlantis C18 column (5 μm, 2.1 × 50 mm; Waters) at a flow rate of 0.2 mL/min. The isocratic mobile phase used was a mixture of H_2O: ACN 70:30. The injection volume was 20 μL. UV acquisition was performed between 200 and 500 nm.

Melting point range was determined using a Techne Stuart digital melting point apparatus (Stuart, Stone, UK) using open capillary tubes.

Results and discussion

The complete analysis of the experimental data obtained by LC-HRMS and NMR can be found in the Supporting Information (Experimental data analysis).

Gas chromatography–mass spectrometry

Initially, the sample was analysed by GC–MS. The total ion chromatogram showed an intense and single peak at 7.20 min, showing that the sample contained a highly pure compound. The application of different spectral libraries did not retrieve results. At this point, the interpretation of fragmentation in EI spectra was performed. The EI spectrum of the chromatographic peak at 7.20 min showed four intense *m/z* ions (Figure 1). Ions at *m/z* 224 and 125 presented the characteristic ^{35}Cl/^{37}Cl isotopic pattern, which indicates the presence of a chlorine atom in the structure.

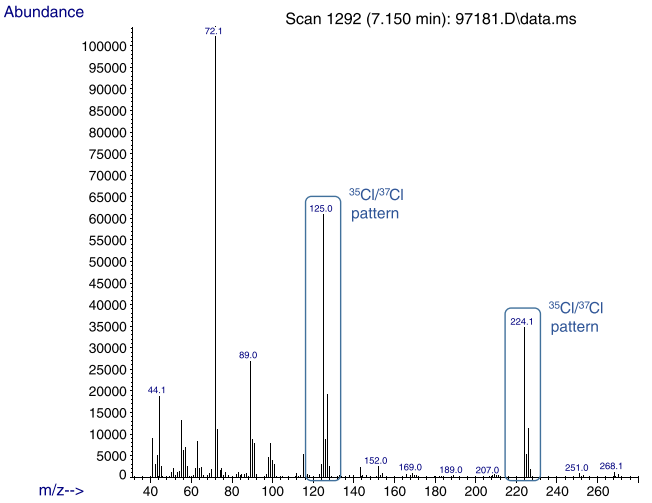

Figure 1. EI mass spectrum of the unknown compound. [Colour figure can be viewed at wileyonlinelibrary.com]

Additional fragment ions were observed at *m/z* 44, 72 and 89, the fragment ion at *m/z* 72 being the base peak. Ions above *m/z* 224 were also observed. However, it was impossible to determine if these ions came from the compound or not due to their low intensity. Ion at *m/z* 224 could correspond to the molecular ion of the compound; however, due to the high fragmentation obtained under EI, this could not be confirmed. Thus, additional analytical techniques were necessary for the elucidation of the structure.

Liquid chromatography–high-resolution mass spectrometry

Figure 2 (left) shows the LE and HE spectra of the chromatographic peak at 8.17 min, corresponding to the unknown compound. Three ions were observed in the LE function at *m/z* 224.0835, 252.0786 and 269.1053. The use of a soft ionization technique showed that ions at *m/z* 224 and 252 were in-source fragments, demonstrating that the protonated molecule was the ion at *m/z* 269. The use of UHPLC provides narrow chromatographic peaks, which are very useful for determining if a fragment ion comes from a selected protonated molecule (Figure 2, right). The elemental composition of the $[M + H]^+$ ion was calculated to be $C_{13}H_{18}ClN_2O_2^+$, with a mass error of −1.5 ppm (*m/z* 269.1053). Other feasible molecular formulas were rejected due to the associated high mass error. The elemental compositions of the two in-source fragments were also obtained ($C_{13}H_{15}ClNO_2^+$ for the fragment at *m/z* 252.0786, and $C_{12}H_{15}ClNO^+$ for *m/z* 224.0835), corresponding to the loss of an ammonia molecule, followed by carbon monoxide loss. These fragments might indicate the presence of a terminal amide group loss. The characteristic ^{35}Cl/^{37}Cl isotopic pattern was also observed for the three ions.

Regarding HE spectra, three collision-induced fragments were observed at *m/z* 125.0151, 89.0386 and 72.0803. After calculating their elemental composition, the presence of a chlorophenyl group bonded to a methylene group, known as chlorotropylium (fragment at *m/z* 125.0151, $C_7H_6Cl^+$), was supposed. The fragment at *m/z* 89.0386 ($C_7H_5^+$) would therefore correspond to the loss of HCl molecule from the chlorotropylium ion (*m/z* 125). Finally, the fragment at *m/z* 72.0803 corresponded to an amine functionalized with four carbon atoms ($C_4H_{10}N^+$).

In order to enhance the confidence in the results obtained, MS/MS experiments were additionally performed, showing the

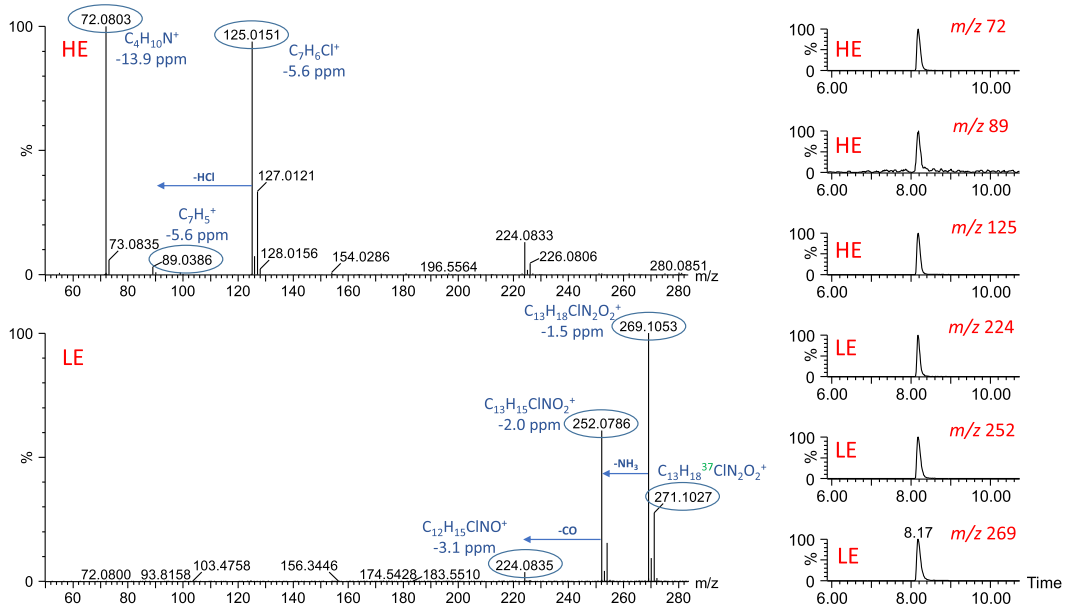

Figure 2. MS^E collision spectra of the unknown compound. Right: Extracted ion chromatograms (0.02 Da mass window) for protonated molecule and fragment ions in LE and HE functions. Left: LE (bottom) and HE (top) spectra of the unknown compound. [Colour figure can be viewed at wileyonlinelibrary.com]

same fragmentation as that observed after performing MS^E acquisition (Supporting Information, SI.1).

LC-HRMS data analysis determined the presence of a terminal amide and a chlorotropylium group. Nevertheless, additional spectroscopic techniques were required for the complete identification and characterization of the molecule.

Nuclear magnetic resonance

Various NMR experiments were carried out. Firstly, ^{1}H NMR spectrym (500 MHz, DMSO-d_6) was obtained. Three broad signals corresponding to amine protons (related to terminal amide group predicted during LC-HRMS analysis) were observed. In order to remove these signals and obtain a cleaner spectrum, deuterated water was added to the extract and ^{1}H NMR spectrum (500 MHz, DMSO-d_6 and D_2O) was acquired again (SI.2 top). The addition of D_2O removed amide and amine ^{1}H NMR signals produced by proton transfer between amine/amide hydrogens and solvent deuterium. The ^{1}H NMR signal analysis determined the presence of a methyl group bonded to a CH group, and the presence of aromatic hydrogens. Considering the fragment at *m/z* 125, corresponding to a chlorotropylium fragment, substituents of the phenyl ring could be a chlorine atom and a methylene group. The other ^{1}H NMR signals could not be assigned until the complete elucidation of the molecule. For more details about ^{1}H NMR signals, see Supporting Information.

Secondly, ^{13}C NMR spectra (500 MHz, DMSO-d_6 and D_2O) were acquired to estimate the functional groups of the molecule based on the type of carbon atom (SI.2 bottom). Two methyl groups were identified, related to the methyl ^{1}H NMR signals. Three CH_2 or CH groups were also identified, and based on the chemical shift, one of them could be bonded to an oxygen or nitrogen atom. These three CH_2/CH groups corresponded to the three unassigned signals in ^{1}H NMR spectra (SI.3). Aromatic carbon signals were also identified according to the four aromatic signals obtained. Two of these carbons would be functionalized according to the chemical shift observed in ^{13}C NMR, indicating the presence of a quaternary assignment. These aromatic signals were in accordance with the aromatic hydrogens detected in ^{1}H NMR spectra and the chlorotropylium fragment (*m/z* 125) observed during LC-HRMS analysis. The last two additional signals corresponded to amide or ester carbons. As previously commented, LC-HRMS analysis determined the presence of a terminal amide group. Considering that the molecular formula indicated the presence of two oxygen atoms and two nitrogen atoms, and based on the ^{13}C NMR spectra, the presence of a second amide group in the molecule was undeniable. For more details about ^{13}C NMR signals, see Supporting Information.

^{1}H NMR and ^{13}C NMR experiments did not allow the complete characterization of the molecule, so additional NMR experiments were performed. COSY is a two-dimensional NMR (2D NMR) technique that allows the determination of ^{1}H correlations present in the compound (which is a homonuclear through-bond correlation method). COSY can be complemented with HSQC experiments (heteronuclear through-bond correlation method), which match ^{1}H NMR and ^{13}C NMR signals, differentiating CH_3/CH groups (in red and yellow spots) from CH_2 groups (blue spots). The combination of COSY and HSQC (both spectra were acquired at 500 MHz, DMSO-d_6 and D_2O) provides enough information for the tentative identification of this compound.[19] Figure 3 shows COSY and HSQC for the sample, identifying NMR signals based on the structure of the compound. COSY (Figure 3, top) shows the correlation between the hydrogens of the molecule, while HSQC (Figure 3, bottom) indicates which proton corresponds to each carbon signal. The correlation between both methyl groups (C1 and C2, doublet at δ = 0.77 ppm in ^{1}H NMR) and two CH groups (C3 and C4, multiplet at δ = 1.92 ppm and doublet at δ = 3.97 ppm in ^{1}H NMR, respectively) suggested the presence of an isopropyl group bonded to another CH, according to C4 ^{1}H NMR signal multiplicity (Figure 3, top). The chemical shift of C4 (δ = 63.09 ppm in ^{13}C NMR; Figure 3, bottom) indicated that this carbon was bonded to a nitrogen or oxygen atom. Regarding LC-HRMS analysis, the fragment at *m/z* 72 corresponded to an amine bonded to four carbon atoms. This would be in concordance

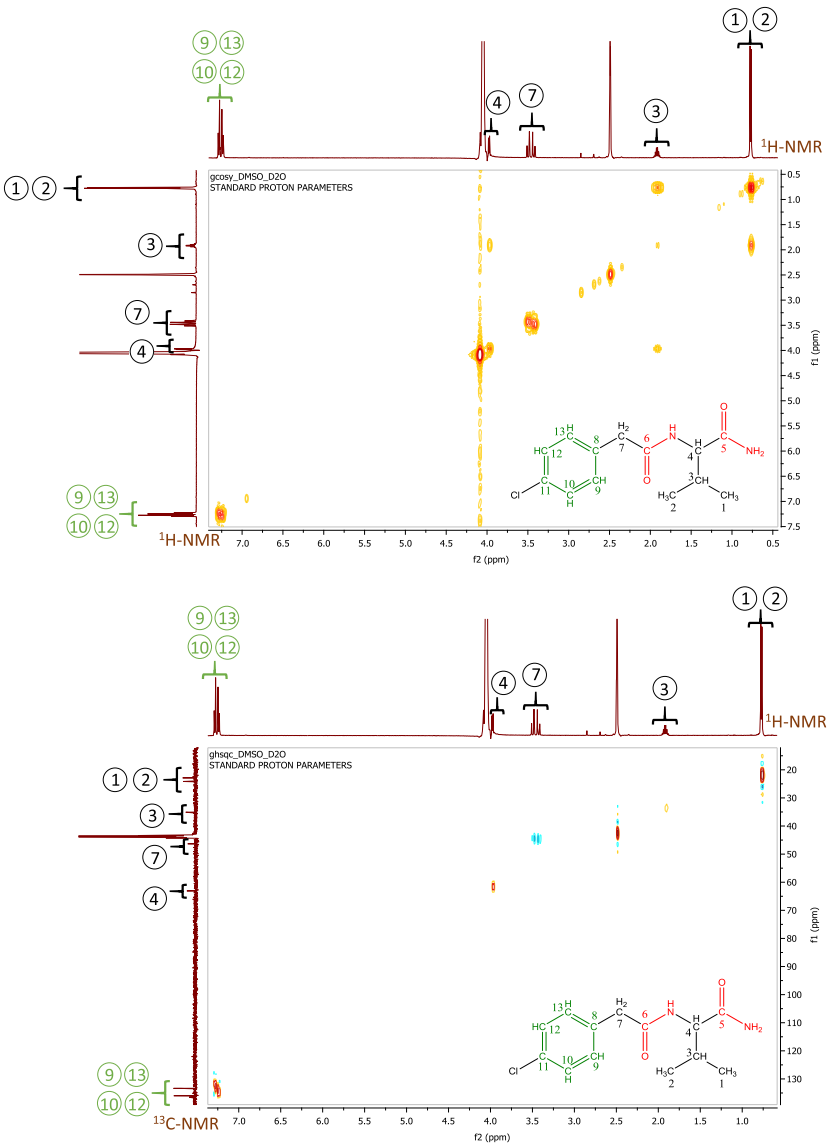

Figure 3. 2D NMR spectra of the unknown substance. Top: COSY spectra, showing the correlation between hydrogens of the molecule. Bottom: HSQC spectra, linking ^{1}H NMR and ^{13}C NMR signals. CH_3 and CH groups appear as red and yellow spots, and CH_2 groups as blue spots. [Colour figure can be viewed at wileyonlinelibrary.com]

with the structure composed of a CH group (C4) bonded to a nitrogen atom and an isopropyl group (C1, C2 and C3), predicted from NMR experiments. The combination of the information obtained using NMR and LC-HRMS could establish that the fragment at *m/z* 72 corresponded to a 2-methylpropanamine group, which in combination with fragments at *m/z* 72, 224, 252 and 269 (see Figure 2) and the presence of two amide groups determined by NMR (see Figure 3) suggested that the molecule presented an *N*-isobutylacetamide group.

The double of doublets at $\delta = 3.45$ ppm in ^{1}H NMR corresponded to a methylene bond between the amide and the chlorophenyl group, because no proton correlations were observed in COSY spectra. The multiplicity of this signal indicates that the two hydrogens in the CH_2 group were diastereotopic; therefore these hydrogens had different surrounding electron density (for more information, see Experimental data analysis and SI.3 in Supporting Information). Finally, the other substituent of the CH group linked to the amide was bonded to the carbonyl of the terminal amide group present in the molecule (considering that the presence of this group was predicted during LC-HRMS analysis).

After this thorough interpretation of both 2D NMR spectra, the compound could be identified as 2-(2-(4-chlorophenyl) acetamido)-3-methylbutanamide, a putative new psychoactive substance.

After compound identification, NMR signals were assigned. ^{1}H NMR and ^{13}C NMR signal assignment can be found in Supporting Information (SI.4). In order to enhance the confidence in the structure, HMBC (a heteronuclear through-bond correlation method) 2D NMR experiment was performed. In this experiment, correlations over 2–4 bonds were observed, confirming the identity of the compound. HMBC spectrum can be found in Supporting Information (SI.5).

The characterized compound has a valinamide group (*N*-(1-carbamoyl-2-methylprop-1-yl) group), present in some third-generation synthetic cannabinoids such as 5F–AB-PINACA, AB-CHMFUPPYCA, AB-CHMINACA, AB-FUBINACA or 5F–AB-

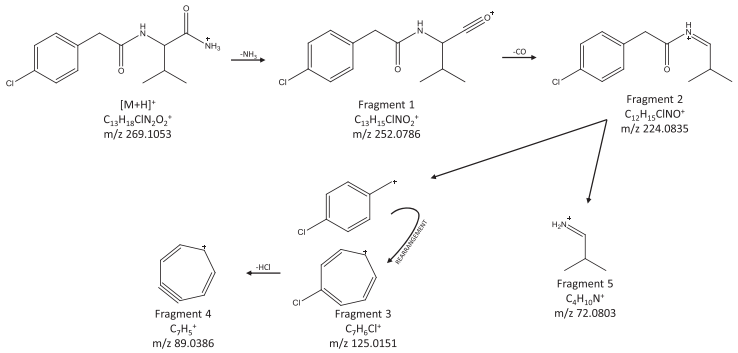

Figure 4. Proposed collision-induced dissociation fragmentation pathway for 2-(2-(4-chlorophenyl)acetamido)-3-methylbutanamide.

FUPPYCA.[20–22] However, the valinamide group can be found in vast diversity of compounds. Therefore, the presence of this moiety in the compound under discussion is not enough to assume similarity with synthetic cannabinoids as no information about its psychoactive effects is available. Once identified, the fragmentation pathway of the new compound obtained by LC-HRMS was proposed (Figure 4).

Table 1. Crystallographic data for the characterized compound

Parameter	Data
Empirical formula	$C_{13}H_{17}ClN_2O_2$
Formula weight	268.74
Temperature (K)	199.95(10)
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	
a, Å	4.81884(8)
b, Å	22.1502(2)
c, Å	6.83798(9)
α, °	90.00
β, °	108.6266(15)
γ, °	90.00
Volume (Å^3)	691.643(16)
Z	2
ρ_{calc} (mg/mm^3)	1.290
Absorption coefficient, μ (mm^{-1})	2.421
F(000)	284.0
Crystal size (mm^3)	0.368 × 0.146 × 0.083
2θ range for data collection (°)	7.98 to 133.18
Index ranges	$-5 \le h \le 5$
	$-26 \le k \le 26$
	$-8 \le l \le 8$
Reflections collected	12 265
Independent reflections	2448 [*R*(int) = 0.0199]
Absorption correction	Multi-scan
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2448/1/170
Goodness of fit on F^2	1.070
Final *R* indices [$I > 2\sigma(I)$]	R_1 = 0.0318
	wR_2 = 0.0804
R indices (all data)	R_1 = 0.0320
	wR_2 = 0.0807
Largest difference in peak/hole (e·Å^{-3})	0.35/−0.44
Flack parameter	0.003(14)

Single-crystal X-ray diffraction

In order to unequivocally confirm the structure of the compound, single-crystal X-ray diffraction analysis was also performed. Using the OLEX2 software package,[23] the structure was solved by using the Superflip structure solution program[24] for charge-flipping methods and refined by the full-matrix least-squares method using the ShelXL refinement package,[25] applying multi-scan method to perform absorption corrections. Table 1 lists collected data and refinement parameters.

Crystals suitable for single-crystal X-ray diffraction experiments were selected directly from the sample (Figure 5). The compound structure was refined in the monoclinic space group $P2_1$, with the following unit cell dimensions: a = 4.81884(8) Å, b = 22.1502(2) Å, c = 6.83798(9) Å, $\alpha = \gamma = 90.00°$ and β = 108.6266(15)°. Figure 6 shows the structure predicted after performing the refinement. The Flack parameter was 0.003, which indicates that this compound was crystallized with a high enantiomeric purity of one of the plausible isomers. Thus, the unknown compound was

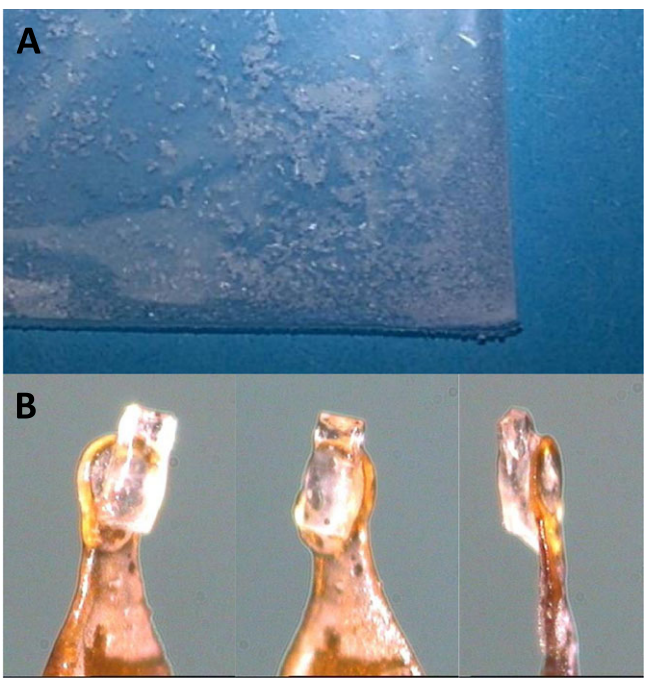

Figure 5. (A) Bulk sample containing the newly characterized compound. Small needle-shaped crystals can be observed. (B) Single crystal selected from sample observed through X-ray diffractometer. [Colour figure can be viewed at wileyonlinelibrary.com]

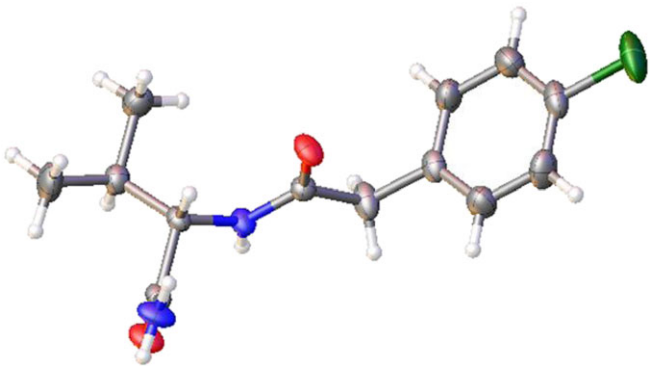

Figure 6. Predicted structure after processing single-crystal X-ray diffraction data. Grey spheres, carbon atoms; white spheres, hydrogen atoms; red spheres, oxygen atoms; blue spheres, nitrogen atoms; green sphere, chlorine atom. [Colour figure can be viewed at wileyonlinelibrary.com]

unequivocally confirmed to be 2-(2-(4-chlorophenyl)acetamido)-3-methylbutanamide that, to our knowledge, has not been registered in the IUPAC and CAS database.

In order to facilitate data sharing, X-ray diffraction data and structure refinement were checked and included in the Cambridge Crystallographic Data Centre (CCDC). Supplementary crystallographic data for this compound can be found in file CCDC 1522776, available free of charge at the CCDC webpage: www.ccdc.cam.ac.uk/data_request/cif.

Additional characterization

In order to complete the characterization of the compound, the melting point range was determined and established between 223 and 225°C. FTIR and UV absorption spectra and instrumental conditions can also be found in Supporting Information (SI.6 and SI.7, respectively).

Conclusions

In this work, a putative new psychoactive substance identified as 2-(2-(4-chlorophenyl)acetamido)-3-methylbutanamide ($C_{13}H_{17}ClN_2O_2$) was characterized. The complete identification of the compound required the combination of different analytical techniques, such as GC–MS, LC-HRMS, NMR and X-ray crystallography. The fragmentation pathway of this compound in LC-HRMS has also been proposed, in order to make easier the future identification of related compounds by common fragmentation analysis. Unfortunately, the psychoactive effects and toxicity have not been evaluated yet. The strategy applied in this work has proven to be a powerful workflow for the identification and characterization of novel NPS. The information obtained about this new compound will be useful for forensic laboratories, toxicological studies or to enhance early warning systems.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.