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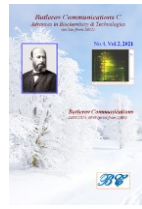

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Identification and analytical profiles of synthetic cannabinoid 3,3-dimethyl-2-(2-(1-(4-fluoro-benzyl)-1H-indol-3-yl)aceta-mido)butanamide (ADB-FUBIATA, FUB-ACADB)

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Abstract

The research work presents the results of identification of 3,3-dimethyl-2-(2-(1-(4-fluorobenzyl)-1*H*-indol-3-yl)acetamido)butanamide (ADB-FUBIATA, FUB-ACADB) using modern highly reliable physicochemical methods for the structural identification of organic compounds, namely gas chromatography with mass spectrometric detection (GC–MS), nuclear magnetic resonance spectroscopy (^{1}H and ^{13}C NMR) and infrared (IR) spectroscopy.

The practical experience of competent specialists and forensic scientific institutions indicate that the appearance among traditional objects of forensic chemical, criminalistics and toxicological examinations of substances with new chemical structures and similar in their psychoactive effect to previously identified and controlled substances and drugs based on them, have become commonplace. Modern recreational substances are very diverse, and

the introduction of structural changes in them allows some time to exclude their circulation from special legislative control. However, structural modification of a substance is often accompanied by an increase in the psychoactive effect.

Paramount importance is therefore given to the correct identification of the chemical structure of new psychoactive substances (NPS) detected in the objects of forensic chemical and criminalistics examinations. The data on structural identification obtained contribute to the establishment of analytical NPS profiles and provide a timely and adequate response of the competent authorities to the emergence of new potential threats to life safety.

Over the past few years, the research groups periodically reported on the detection and identification of cannabinoid CB_1 and CB_2-receptor agonists in plant matrices and individual substrates, as well as in biofluids –synthetic cannabimimetics of the cyclohexylphenol group, naphthoylindoles, phenylacetylindoles, benzoylindoles, indole- and indazole-3-carboxamides, indole-3-carboxylates and a number of other synthetic cannabinoids and their derivatives.

The data presented in the research work on the establishment of the chemical structure of 3,3-dimethyl-2-(2-(1-(4-fluorobenzyl)-1H-indol-3-yl)acetamido)butanamide suggest that its structure has a certain similarity with chemical structure of indolecarboxamide derivatives.

Introduction

The term new psychoactive substances (NPS) is now widely used in place of the term "designer" drugs. It means a range of recreationally used substances that have been designed to mimic established illicit drugs. A significant part of NPS are synthetic cannabinoids – cannabimimetics [1-6], which reproduce the activity of Δ^9-tetrahydrocannabinol contained in the *Cannabis* L. plant. Synthetic cannabinoids are substances with a high affinity for cannabinoid receptors. To date, two types of cannabinoid receptors are well known and studied: CB_1- and CB_2-receptors. Cannabinoid CB_1 receptors are involved in the formation of various kinds of ecstatic and emotional reactions, such as fear and anxiety, persistent and characterological actions (expansiveness, stereotype), the formation of cognitive and educational processes, the mechanism of pain, and efferent skills. Receptors of the second type (CB_2) take part in the normalization of various immune responses [7].

Studies on laboratory animals using some synthetic cannabinoids have shown the activation of presynaptic CB_1 receptors in the mesolimbic reward system, which promotes the stimulation of dopaminergic neurons in the medial forebrain bundles [8]. At the same time, there was a "triggering" of opioid receptors responsible for euphoria, as in the case of human consumption of natural cannabis products [9]. Studies have demonstrated that the orexin-hypocretin system, which is the most important regulatory mechanism in the brain, plays one of the leading roles in the formation of the addiction effect when using synthetic cannabinoids [10]. The interaction of synthetic cannabinoids with other receptor systems, as well as their direct influence on complex behavioral acts and the consequences of chronic use, is currently poorly understood. The experiments on laboratory rodents showed that the mechanism of upregulation of 5-HT2A receptors in the hypothalamus is directly related to the activation of CB_1 receptors after administration of a synthetic cannabinoid, which causes increased anxiety-like behaviours [11].

In Russia, as in most countries of the European Union, NPS from the group of synthetic cannabinoids take a leading position among recreational substances abuse and are often the reason for urgent medical hospitalization in specialized institutions [12-16].

Therefore, the implementation of strict control in the area of NPS trafficking is a necessary government measure to suppress new potential threats to life safety.

The purpose of this work was to establish the analytical profiles of a new synthetic cannabinoid 2-(2-(1-(4-fluorobenzyl)-1*H*-indol-3-yl)acetamido)-3,3-dimethylbutanamide by the methods of structural identification of organic compounds (GC-MS , ^{1}H, ^{13}C NMR and IR spectroscopy).

Experimental part

Equipment. GC-MS studies were performed on a gas chromatograph with a mass selective detector *Agilent* 6890N/5975 Inert MSD (*Agilent Technologies*, USA). Electron ionization mass spectra (EI, 70 eV) were recorded in the range m/z = 30-550. *HP-5 MS* quartz capillary column (30m × 0.25mm × 0.25μm) was used. GC separation conditions: injector temperature – 280 °C, interface temperature – 280 °C, ion source temperature – 250 °C, electron multiplier voltage 1717 V, initial thermostat temperature – 100 °C (2 min.), rise rate column temperature – 20 °C/min., final temperature of the column thermostat – 290 °C (25 min.). Sample injection with flow division 1:40, sample volume – 1 μl, volumetric carrier gas flow rate (He, 99.999%) – 1.0 ml/min – constant flow.

For GC-MS studies solutions containing 10-15 mg of the test substance in 1 ml of methanol were used.

The GC-MS system was adjusted and the chromatomass-spectral data were processed using the software *"MassHunter"* (*Agilent Technologies*, USA), the electronic library of mass spectra EI *"NIST'17", "NIST MS Interpreter"* (NIST, USA) and *AIPSIN ™ AntiNarcotics* (*BelHardGroup*, Belarus).

^{1}H and ^{13}C NMR spectra were recorded on a *Bruker Avance III 600* spectrometer (*Bruker*, Germany) with an operating frequency of 600 MHz ($_{1}$H) and 151 MHz (^{13}C) in the Collective use center of the Federal Research Center «Krasnoyarsk Scientific Center of the Siberian Branch of the Russian Academy of Sciences». For this, 15 mg of the test substance was placed in an ampoule 5 mm in diameter and dissolved in C_3D_6O. Chemical shifts were measured on a δ (ppm) scale at 23 °C using deuterium field stabilization.

^{1}H NMR spectra were obtained with acquisition of 16 scans separated by a relaxation interval of 8 s, ^{13}C NMR spectra were obtained using the method of pulse decoupling of spin-spin interactions with protons, with 4096 scans separated by a delay of 3.5 s.

The spectrum of homonuclear ^{1}H-^{1}H-correlations (COSY) was recorded using a variant with a two-quantum filter (cosygpmfqf from the *Bruker* library). To obtain a spectrum, 128 sections of 8 scans with a delay between scans of 5 s were sequentially recorded with a resolution of 1024 points. The spectrum of heteronuclear single quantum coherence (HSQC) was obtained using a sequence with editing by the number of addition of protons (hsqcedetgpsisp 2.3 from the *Bruker* library). For this, 128 sections of 8 scans with a resolution of 2048 points were registered. The spectrum of heteronuclear multiple bond correlations (HMBC) (hmbcgplpndqf from the Bruker library) was obtained by acquisition of 128 sections of 16 scans with a resolution of 2048 points. The experiment was optimized for 5 Hz spin-spin interactions.

IR spectra were recorded using an Inspect IR-microscope (*SpectraTech*, USA) combined with a *Nicolet 380* IR Fourier spectrometer with the *Omnic 8.1.11* software (*Thermo Scientific*, USA) under the following conditions: resolution – 4, gain – 1, number of scans – 32 (reflection mode), wave number range 4000-650 cm^{-1}.

Reagents. Organic solvents with gradation “for chromatography” were used.

Results and discussion

During GC-MS screening of an extract of a substance received in July 2021 for expert examination at the Forensic Center of the Main Directorate of the Ministry of

Internal Affairs of Russia for the Krasnoyarsk region, a chromatogram of total ion current (TIC) was recorded, and a dominant peak with retention time of 16.40 min was detected. (fig. 1a).

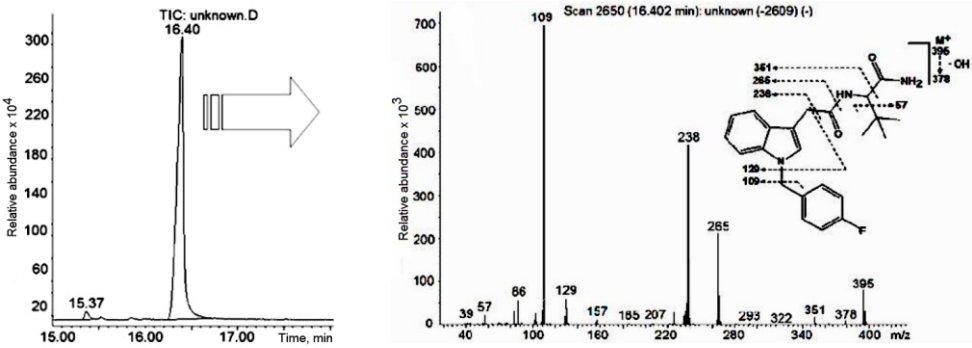

Fig. 1. The total ion current (TIC) chromatogram (a) and mass-spectrum(EI) (b) ofthe test substance

The mass-spectrum of the peak with a retention time 16.40 min has the following main ions with relative intensities (m/z(%)): 57(5), 86(8), 109(100), 110(6), 129(8), 238(57), 239(12), 265(26), 266(5), 351(4), 378(2), 395(M+, 7). Analysis of the mass-spectrum of the test substance suggested a characteristic fragmentation pattern (fig. 1b). In particular the dominant fragment with m/z = 109 ($C7H6F^+$) indicates the presence of fluorobenzyl group as it was already reported for different synthetic cannabinoids [17, 18], and the ion with m/z = 395 apparently represents the molecular ion of the substance. It should be noted that the EI mass-spectrum of this substance was not provided at the time of the study in commercially available electronic mass-spectral libraries.

In order to establish the chemical structure of the substance under investigation and correlate it with the results obtained by GC-MS (EI), ^1H and ^{13}C NMR studies were carried out. The analysis of NMR-spectra makes it possible to ascribe to the test substance the structure of 2-(2-(1-(4-fluorobenzyl)-1*H*-indol-3-yl)acetamido)-3,3-dimethylbutanamide, which matches well with the proposed dissociative ionization pattern under EI conditions (70 eV), shown in Fig. 1b.

The proposed structure of 3,3-dimethyl-2-(2-(1-(4-fluorobenzyl)-1H-indol-3-yl)acetamido)butanamide is evidenced by the presence of an intense singlet signal in the ^1H NMR spectrum at 0.85 ppm, that belongs to nine equivalent protons of the tert-butyl group (see table). The signal of the methine proton of the side chain is observed as two doublets of equal intensity at 4.3 and 4.31 ppm. This type of signal indicates that the test substance exists in solution as a mixture of two rotamers in a 1:1 ratio. The small value of the vicinal spin-spin coupling constants (SSC) with the proton of the neighboring amide group (~3.5 Hz) also indicates the hindered rotation around the C-N bond, which is typical for substituted carboxylic acid amides.

Signals of diastereotopic protons of the methylene group of the side chain are observed as two doublets at 3.7 and 3.74 ppm, demonstrating a characteristic geminal coupling with 15.9 Hz. The proton signal of the internal amide group is observed in the region of 6.78-6.87 ppm. The assignment of side chain proton

signals is confirmed by the presence of the corresponding correlation signals in the HH COSY, HSQC, and HMBC spectra (fig. 2).

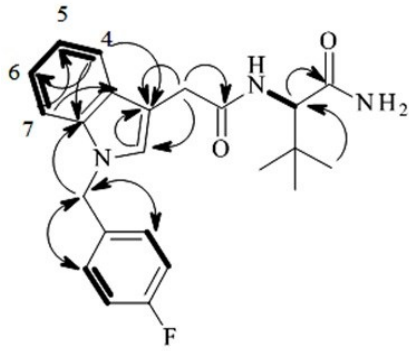

Fig. 2. Selected correlations: HH COSY ( ) и HMBC ( )
of 3,3-dimethyl-2-(2-(1-(4-fluorobenzyl)-1H-indol-3-yl)acetamido)butanamide

The protons of the benzylic methylene group of the *N*-(4-fluorophenyl)methyl fragment of the structure are observed as a broadened singlet at 5.42 ppm.

The structure of the test substance contains two aromatic units – indole and benzyl ones. The proton signal at the 4-position of the indole fragment is observed as a broadened doublet at 7.64 ppm. The proton signal in the seventh position of the indole system is observed in the range 7.34-7.39 ppm. The signals of the *ortho*-protons of the *N*-fluorobenzyl group appear as a doublet of doublets at 7.22 ppm, and the signal of the proton in the sixth position of the indole system is observed as a triplet-like multiplet in the region of 7.09-7.15 ppm. The proton signal in the 2nd position of the indole system is observed at 7.36 ppm. The signals of two meta-protons of the *N*-fluorobenzyl group, as well as the proton in the 5th position of the indole system, are observed in the region of 6.99-7.08 ppm. The assignment of the signals of the protons of the aromatic fragments of the structure is also confirmed by the presence of correlation signals in the spectra of HH COSY, HSQC, and HMBC.

The ^{13}C NMR spectrum of the test substance contains 22 resolved signals (see table). In the high-field region, there is a degenerate signal of three methyl groups of the *tert*-butyl fragment at 26.96 ppm. The signal of the quaternary carbon atom is observed at 35.07 ppm Signals of carbon atoms of the methine and methylene groups of the side chain are observed at 60.36 and 33.66 ppm respectively. The signal of the carbon atom in the benzyl position of the *N*-fluorobenzyl fragment is observed at 49.50 ppm, which is confirmed by the presence of corresponding correlations in the HSQC spectrum. The signal of the carbon atom bonded to the fluorine atom appears as a doublet with a characteristic SSC constant of 243.5 Hz in a low field at 162.93 ppm. Signals of a pair of *m*-atoms and *o*-carbon atoms of the 1,4-disubstituted phenyl group are observed at 129.69 and 116.12 ppm also in the form of the corresponding doublets. The study of the HSQC and HMBC spectra makes it possible to assign the signals of the remaining carbon atoms in the structure proposed above. Thus, signals of carbon atoms in the 4th, 5th, 6th and 7th positions of the indole system are observed at 119.99 120.04, 122.62, and 110.78 ppm respectively. The carbon signal in the 2nd position of the indole system is at 128.62

ppm. Signals of other carbon atoms not bonded to hydrogen atoms appear at 135.45 (C-1-benzyl), 137.46, 129.19 and 110.17 ppm (C-3-indole).

Clear correlations in the HMBC spectrum make it possible to reliably assign the signals of carbon atoms to the inner and outer carboxamide groups at 170.89 and 173.02 ppm respectively.

Table. ^{1}H and ^{13}C spectra of the compound under investigation

^{1}H NMR spectrum (600 MHz, C_3D_6O)	δ 0.85 (c, 9H), 3.70 (d, J = 15.9 Hz, 1H), 3.74 (d, J = 15.9 Hz, 1H), 4.30 (d, J = 3.6 Hz, 0.5H), 4.31 (d, J = 3.4 Hz, 0.5H), 5.42 (s, 2H), 6.78-6.87 (m, 1H), 6.99-7.08 (m, 4H), 7.09-7.15 (m, 1H), 7.22 (dd, J = 8.5, 5.5 Hz, 2H), 7.34-7.39 (m, 1H), 7.64 (d, J = 7.9 Hz, 1H),
^{13}C NMR spectrum (151 MHz, C_3D_6O)	δ 26.96, 30.22, 33.66, 35.07, 49.50, 60.36, 110.17, 110.78, 116.05, 116.20, 119.99, 120.04. 122.62, 128,62, 129.19, 129.67, 129.72, 135.45, 137.48, 162.12, 163.73, 173.02

In the IR spectrum of the test substance, there are characteristic for carboxylic acid amides bands of stretching vibrations of the non-conjugated carbonyl group and the N-H group in the range of 1660-1600 cm^{-1}, which is quite consistent with the proposed structure. There are also bands of bending vibrations of C-H-carbo- and heterocyclic aromatic fragments in the region of 1224 and 1513 cm^{-1} (fig. 3).

Thus, the study by IR spectroscopy in combination with the results of studies by GC-MS (EI) and NMR spectroscopy allows us to assign the structure of the compound under investigation to be 3,3-dimethyl-2-(2-(1-(4-fluorobenzyl)-1H-indol-3-yl)acetamido)butanamide.

The substance with the structure of 3,3-dimethyl-2-(2-(1-(4-fluorobenzyl)-1H-indol-3-yl)acetamido)butanamide established in the current work, has not previously been encountered as an object of forensic examinations of NPS. It should be noted that at the time of the study, mass spectral and other analytical characteristics of this substance were not available in commercial expert reference databases. At this stage of research, it was decided to assign the name ADB-FUBIATA (FUB-ACADB) to this substance in accordance with one of the international nomenclatures.

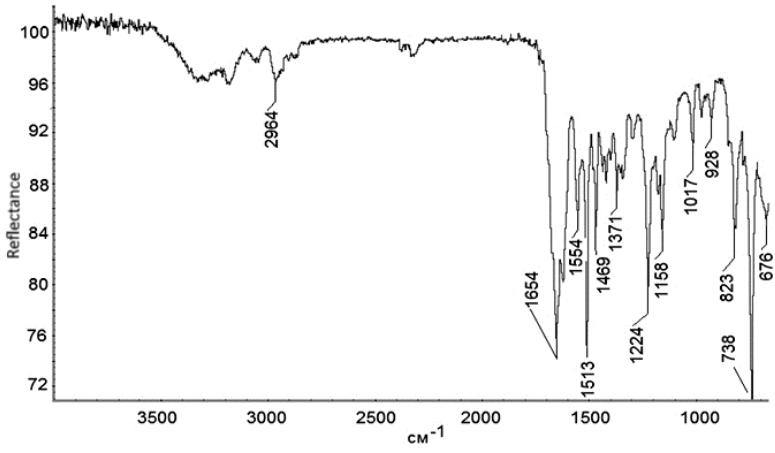

Fig. 3. IR-spectrum of 3,3-dimethyl-2-(2-(1-(4-fluorobenzyl)-1H-indol-3-yl)acetamido)butanamide

The structure of 3,3-dimethyl-2-(2-(1-(4-fluorobenzyl)-1H-indol-3-yl)acetamido)butanamide – ADB-FUBIATA (FUB-ACADB) has a certain similarity to the structures of known and widespread synthetic cannabimimetics. Thus, the presence of a fluorobenzyl substituent in the first position of the indole system is found in such synthetic cannabinoids as MBA-BZ-F, MMBA-BZ-F, CUMYL-FUBICA, MDMB-BZ-F, which are the objects of forensic examinations. The *tert*-leucine residue is present in the structure of the synthetic cannabinoid MMBA-BZ-F (synonym: *N*-(1-carbamoyl-2,2-dimethylprop-1-yl)-1-(4-fluorobenzyl)-1H-indole-3-carboxamide) – a derivative of the psychoactive substance *N*-(1-carbamoyl-2-methylpropyl)-1-(phenylmethyl)-1H-indole-3-carboxamide [19].

Noting the above, the authors of this work believe that the compound ADB-FUBIATA (FUB-ACADB) on its chemical structure, is a homologue of the synthetic cannabinoid MMBA-BZ-F with one additional methylene group. The above structural difference between ADB-FUBIATA (FUB-ACADB) does not allow us to extend to it the concept of a derivative of the "basic structure" – N-(1-carbamoyl-2-methylpropyl)-1-(phenylmethyl)-1H-indole-3-carboxamide [20]. Thereby the elucidation of psychoactive properties of ADB-FUBIATA (FUB-ACADB) during its effect on cannabinoid receptors of the central nervous system requires further investigations in order to determine and prevent its social danger and to include tis compound to the lists of controlled substances.

The Russian version of this article was published in the journal *Butlerov Communications* [21].

Conclusions

The chemical structure of 3,3-dimethyl-2-(2-(1-(4-fluorobenzyl)-1H-indol-3-yl)acetamido)butanamide (ADB-FUBIATA, FUB-ACADB), first identified in the objects of forensic examinations on the territory of Russia, was established by methods of structural identification of organic compounds (GC-MS(EI), NMR-spectroscopy and IR-spectrometry).

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