ORIGINAL ARTICLE

Identification and structure characterization of five synthetic opioids: 3,4-methylenedioxy-U-47700, *o*-methyl-acetylfentanyl, 2-thiophenefentanyl, benzoylfentanyl and benzoylbenzylfentanyl

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Abstract

Purpose The number of new synthetic opioids, especially fentanyl analogs, on the illicit market is increasing each year and becoming a disturbing phenomenon. Due to the very high potency, ease of addiction and high risk of overdose with the fatal effect, they pose a serious threat to public health. The ever-changing drug market, lack of reference standards and low doses make this class of new psychoactive substances (NPS) a continual analytical challenge in terms of detection and identification. Structure elucidations of five synthetic opioids are described in this paper.

Methods Unequivocal identifications were performed using liquid chromatography–high resolution tandem mass spectrometry with quadrupole time-of-flight analyzer (LC–QTOF-MS/MS) and nuclear magnetic resonance (NMR) spectroscopy with prior chromatographic purification of the sample in each case.

Results Identified NPS included one non-fentanyl opioid belonging to “U-compounds group”—3,4-methylenedioxy-U-47700 and four fentanyl analogs: *o*-methyl-acetylfentanyl, benzoylfentanyl, 2-thiophenefentanyl and benzoylbenzylfentanyl.

Conclusions Complementary LC–QTOF-MS/MS and NMR techniques enabled the unambiguous identification of new synthetic opioids. To the best of our knowledge, this is the first comprehensive characterization of these five NPS with complete data which are published to facilitate their detection and identification by forensic and clinical laboratories.

Keywords Synthetic opioids · Non-fentanyl and fentanyl analogs · Structure identification · New psychoactive substance (NPS) · Liquid chromatography–high resolution tandem mass spectrometry with quadrupole time-of-flight analyzer (LC–QTOF-MS/MS) · Nuclear magnetic resonance (NMR) spectroscopy

Introduction

New psychoactive substances (NPS) available on European drug market pose a public health problem and challenge not only for policymakers but also analysts. By the end of 2018, over 730 NPS have been reported to the European Monitoring Center for Drugs and Drug Addiction (EMCDDA) through the Early Warning System (EWS). As many as 55 NPS were detected for the first time in Europe in 2018 [1]. Among all the groups of substances monitored, the most

numerous are still synthetic cannabinoids, followed by derivatives of cathinone and phenylethylamine. A disturbing phenomenon is the appearance of substances from the group of opioids, especially fentanyl derivatives, and the fact that the number of new analogs identified is increasing each year. By the end of 2018, 49 new opioids were detected on the European drug market, including 34 fentanyl derivatives, as many as 10 fentanyl derivatives of which were first reported in 2018. They are unregistered derivatives of active pharmaceutical substance—fentanyl, used in authorized medicinal products. Considering their very high potency, ease of addiction and high risk of overdose with the fatal effect (due to respiratory depression), they pose a serious threat to public health [2, 3]. Other synthetic opioids, which are non-fentanyl derivatives, belong mainly to the group of the *N*-substituted benzamides and acetamides colloquially called “U-compounds” or the group of *N*-substituted cyclohexylmethylbenzamides known as the “AH-compounds” [4]. Majority of

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these fentanyl and non-fentanyl analogs were synthesized and patented as potential non-addictive analgesics. Although they have been discarded on the next stage of drug development (mainly due to unsatisfying results of toxicity tests, side effects or potency), they are easily available on the illicit drug market. Novel synthetic opioids are distributed in various forms: powders, tablets, herbal mixtures and liquids.

Over the past 10 years, in National Medicines Institute (NMI), Poland, we have examined seven thousand samples of designer drugs in which about two hundred NPS have been detected by liquid chromatography–electrospray ionization–quadrupole time-of flight mass spectrometry (LC–ESI–QTOF–MS) and nuclear magnetic resonance (NMR) spectroscopy. Thirty-two of them were new on the EU market and were reported to the EWS-EMCDDA. Among 13 synthetic opioids which were identified in our laboratory, as many as 8 have been reported to the EWS-EMCDDA for the first time (Table 1).

In Polish legislation, the problem of psychoactive substances is regulated by the Act of July 29, 2005 on counteracting drug addiction, which has been amended several times in recent years, first of all to control more NPS. In 2018, the amendment of the Act introduced a generic character for

four NPS groups containing the basic core of the molecule and defined possible substituents, covering most substances from derivatives of 2-phenylethylamine, cathinone, synthetic cannabinoids (cannabinomimetics), and fentanyl derivatives. According to the last amendment of 2019, the fifth open group of benzodiazepines has been added.

A lot of new synthetic opioids have been identified and characterized recently [5–7], including a fentanyl derivative: furanylfentanyl [8] and a non-fentanyl: U-47700 [9, 10]. The identification of novel opioids is a continual analytical challenge due to their variety, low dose needed to cause desired effects, the lack of analytical data available for these substances and the lack of reference standards.

Here we describe the identification of five synthetic opioids detected in four illegal products (sample A, B, C and D) investigated in NMI laboratory. Analyses based on complementary methods LC–QTOF–MS/MS and NMR spectroscopy allowed elucidating their structures. Sample A contained a single compound belonging to “U-compounds group” identified as 3,4-methylenedioxy-U-47700 (compound 1), in sample B two fentanyl analogs were detected: *o*-methyl-acetylfentanyl (compound 2) and benzoylfentanyl (compound 3). Herbal sample C was enhanced with

Table 1 Years of the first identification of the synthetic opioid in samples by National Medicines Institute (NMI) in Poland, the years of the first reports to the Early Warning System—the European Moni-

toring Centre for Drugs and Drug Addiction (EWS-EMCDDA) and the years since they are controlled in Poland

Year	Substance	Chemical name	EMCDDA	Controlled in Poland since
2014	Butyrfentanyl (BF)	<i>N</i> -Phenyl- <i>N</i> -[1-(2-phenylethyl)piperidin-4-yl]butanamide	2014 ^a	2015
	4-Fluorobutyrfentanyl (4F-BF)	<i>N</i> -(4-Fluorophenyl)- <i>N</i> -[1-(2-phenylethyl)piperidin-4-yl]butanamide	2014 ^a	2015
	Furanylfentanyl (FU-F)	<i>N</i> -Phenyl- <i>N</i> -[1-(2-phenylethyl)piperidin-4-yl]furan-2-carboxamide	2015	2018
2017	U-47700	3,4-Dichloro- <i>N</i> -[2-(dimethylamino)cyclohexyl]- <i>N</i> -methylbenzamide	2015	2018
	Cyclopropylfentanyl	<i>N</i> -Phenyl- <i>N</i> -[1-(2-phenylethyl)piperidin-4-yl]cyclopropanecarboxamide	2017	2018
	2-Thiophenefentanyl	<i>N</i> -Phenyl- <i>N</i> -[1-(2-phenylethyl)piperidin-4-yl]thiophene-2-carboxamide	2017 ^a	2018 (generic law)
	Benzylfentanyl	<i>N</i> -(1-Benzylpiperidin-4-yl)- <i>N</i> -phenylpropanamide	2017 ^a	2018
	Acetylbenzylfentanyl (<i>N</i> -benzyl-acetylfentanyl)	<i>N</i> -(1-Benzylpiperidin-4-yl)- <i>N</i> -phenylacetamide	2017 ^a	2018 (generic law)
	Benzoylbenzylfentanyl (<i>N</i> -benzyl-benzoylfentanyl)	<i>N</i> -(1-Benzylpiperidin-4-yl)- <i>N</i> -phenylbenzamide	2017 ^a	2018 (generic law)
2018	4-Fluoro-isobutyrfentanyl (4F-iBF)	<i>N</i> -(4-Fluorophenyl)-2-methyl- <i>N</i> -[1-(2-phenylethyl)piperidin-4-yl]propanamide	2016	2018
	Benzoylfentanyl	<i>N</i> -Phenyl- <i>N</i> -[1-(2-phenylethyl)piperidin-4-yl]benzamide	2017	2018 (generic law)
	<i>o</i> -Methyl-acetylfentanyl (2-methyl-acetylfentanyl)	<i>N</i> -(2-Methylphenyl)- <i>N</i> -[1-(2-phenylethyl)piperidin-4-yl]acetamide	2018 ^a	2018 (generic law)
	3,4-Methylenedioxy-U-47700 (3,4-MDO-U-47700)	<i>N</i> -[2-(Dimethylamino)cyclohexyl]- <i>N</i> -methyl-1,3-benzodioxole-5-carboxamide	2018 ^a	Not controlled

^aReported for the first time to EWS-EMCDDA by NMI, Poland

2-thiophenefentanyl (compound **4**) and sample D contained, among several fentanyl analogs, one unknown compound identified as benzoylbenzylfentanyl (compound **5**).

Materials and methods

Materials and reagents

Samples were collected directly from the users by NMI in Warsaw in 2017 and 2018 as a part of the research project. The pilot testing of psychoactive substances obtained directly from users of "designer drugs" in Poland was implemented within the framework of tasks defined in the National Health Program. Three samples consisted of white powders (samples A, B, D) and one sample was in a form of herbal material (sample C). A reference spectrum of fentanyl was extracted from the data obtained for the original medicinal product Fentanyl WZF from Polfa Warszawa S.A. (Warsaw, Poland).

Methanol and acetonitrile, both of purity suitable for LC–MS, were purchased from Merck Millipore (LiChrosolv; Darmstadt, Germany); formic acid from Honeywell (Metropolis, IL, USA); acetonitrile Chromasolv™ from Honeywell [high-performance liquid chromatography (HPLC) grade]; dimethyl sulfoxide (DMSO) pure p.a. from POCH SA (Gliwice, Poland); 85% formic acid p.a. from Chempur (Piekary Śląskie, Poland); methanol-*d*₄ [methyl-*d*₃ alcohol-*d*, 99.8 atom %D, containing 0.03%(v/v) tetramethylsilane (TMS)] (CD₃OD) from Armar Isotopes (Döttingen, Switzerland). Doubly distilled water additionally purified in the Nanopure Diamond UV Deionization System from Barnstead (Dubuque, IA, USA) was used throughout.

Sample preparation

For LC–QTOF-MS/MS analysis, ca. 0.5 mg of powder or ca. 5 mg of herbal mixture was dissolved or extracted with a 1:1:1 (v/v/v) mixture of water/methanol/acetonitrile, with the assistance of ultrasonication for 10 min, and afterwards filtered by Whatman 0.2 µm pore size polytetrafluoroethylene filter media (GE Healthcare, Chicago, IL, USA). If necessary, the filtrate was further diluted to a suitable concentration.

For NMR analysis, several milligrams of powder (samples A, D) were dissolved in 0.7 mL of CD₃OD and ca. 30 mg of herbal mixture (sample C) was extracted in 1.5 mL of CD₃OD. Several milligrams of powder (sample B) were dissolved in 1.5 mL of DMSO and purified using HPLC method. Fraction at retention time 13.4 min (compound **2**) and 16.3 min (compound **3**) was collected, lyophilized and next dissolved in 0.7 mL of CD₃OD. The solutions were transferred to 5 mm NMR tube.

Instruments and methods

High-performance liquid chromatography–quadrupole time-of-flight tandem mass spectrometry

Identifications of new compounds were performed by means of a high-resolution tandem mass spectrometer with time-of-flight analyzer micrOTOF-QII from Bruker Daltonik (Bremen, Germany) coupled to high-performance liquid chromatography Ultimate 3000 system from Dionex (a part of Thermo Fisher Scientific, Waltham, MA, USA) applying a standard screening method which is used in the laboratory. Chromatographic separations were carried out on Hypersil GOLD C18 analytical column (100×2.1 mm i.d., 3 µm particle size; Thermo Fisher Scientific) with a guard column Hypersil GOLD (10×2.1 mm i.d., 3 µm particle size; Thermo Fisher Scientific) at 25 °C. Linear gradient elution using solvent A consisting of water/acetonitrile/formic acid (90/10/0.1, v/v/v) and solvent B consisting of methanol/acetonitrile/formic acid (90/10/0.1, v/v/v) was applied with the flow rate set at 0.15 mL min^{−1} and the following program was used: 0–2 min, 10%B; 2–7 min, 10–90%B; 7–9 min, 90%B; 9–12 min, 90–10%B; 12–14 min, 10%B. MS settings were as follows: ESI, positive ion mode; capillary voltage, 4500 V; end plate offset, −500 V; dry gas flow rate, 8.0 L min^{−1}; dry heater, 180 °C; MS data, full scan mode (from *m/z* 50 to 1500). High mass accuracies were ensured by a calibration of TOF analyzer using a solution of sodium formate prior to each sample. Collision energy was linearly ramped as a function of *m/z* ratio in the fragmentation experiments. For values from *m/z* 200 to 400, the voltage gradient linearly increased from 20 to 25 eV, for values from *m/z* 400 to 800, it increased from 25 to 35 eV and for values from *m/z* 800 to 1500, it held at 35 eV. Product ions were obtained by the collision induced dissociation.

HPLC purification for NMR experiments

Purification of sample B was performed on HPLC system from Shimadzu USA Manufacturing Inc. (Canby, OR, USA) consisting of a low pressure gradient flow LC-20AT pump, a DGU-20A on line solvent degasser, a photodiode array detector SPD-M20A, an SIL-10AF sample injector and an FRC-10A fraction collector. The data were monitored using Shimadzu LabSolution system. Separations were carried out on Bionacom Velocity C18 LPH HPLC column (150×10 mm i.d., 3 µm particle size; Genore Chromatography, Nagłowice, Poland) using 0.1% formic acid as an aqueous solvent (A) and acetonitrile as an organic solvent (B), in the following gradient: 0–10 min, 20–50%B; 10–15 min, 50–80%B. Flow rate of the mobile phase was set at 3 mL min^{−1}. The course of the chromatography was

monitored using ultraviolet detection at a wavelength of 254 nm and peaks of interest were collected by the fraction collector.

NMR spectroscopy

The NMR spectra were recorded at 298 K on Varian VNMRS-500 spectrometer (Varian, Inc., Palo Alto, CA, USA) operated at 499.8 and 125.7 MHz for ^1H and ^{13}C , respectively. The spectrometer was equipped with an inverse $^1\text{H}\{^{31}\text{P}-^{15}\text{N}\}$ the 5 mm Z-SPEC Nalorac IDG 500-5HT probe with an actively shielded z-gradient coil. The high power ^1H and ^{13}C $\pi/2$ pulses were 7.6 and 11.6 μs , respectively. The NMR experiments were run using the standard Varian software.

The ^1H NMR spectra and the ^1H dimension in 2D heteronuclear spectra were referenced to the internal reference of TMS. The 1D $^{13}\text{C}\{^1\text{H}\}$ spectra and ^{13}C dimension in 2D heteronuclear spectra were referenced to the solvent (CD_3OD , $\delta_{\text{c}} = 49.0$ ppm), which was treated as a secondary standard.

A standard single-pulse experiment was used to acquire the ^1H spectrum using an 8000 Hz spectral window, 30° pulse width, an acquisition time of 4 s, relaxation delay of 2 s, and 64,000 complex data points.

Gradient-selected correlation spectroscopy (COSY): spectral widths, 6000 Hz in both dimensions; 1024 complex points in t_2 , 512 increments in t_1 ; 1–4 scans per increment; relaxation delay, 1 s; sine-bell squared apodization functions used in processing.

The 1D ^{13}C NMR spectra were run using a spectral range of 32,000 Hz, 30° pulse width, an acquisition time of 1 s, a relaxation delay of 1 s, and by collecting 32,000 complex data points.

The phase sensitive adiabatic heteronuclear single quantum coherence ($^1\text{H}\{^{13}\text{C}\}$ HSQC): spectral widths, 6000 Hz in F2 and 21,300 Hz in F1; 1024 complex points in t_2 , 512 complex points in t_1 ; 4 scans per increment; relaxation delay of 1 s; $^1J_{(\text{C,H})} = 146$ Hz. The data were linearly predicted to 1 K and zero filled to 4 K complex data points in F1 and processed using cosine window function in both dimensions prior to Fourier transformation.

The phase sensitive gradient-selected adiabatic heteronuclear multiple bond correlation ($^1\text{H}\{^{13}\text{C}\}$ HMBC): spectral widths, 6000 Hz in F2 and 25,100 Hz in F1; 1024 complex points in t_2 , 512 complex points in t_1 ; 4–16 scans per increment; relaxation delay of 1 s; $^nJ_{(\text{C,H})} = 8$ Hz. The data were processed using sine-bell square multiplication in F2 and Gaussian window function in F1 dimensions prior to Fourier transformation.

The signals in the ^1H and ^{13}C NMR spectra of studied compounds were assigned to the proton and carbon atoms in the appropriate structural fragments with the aid of the proton-proton coupling pattern and the proton-proton and

proton-carbon correlations obtained from $^1\text{H}\{^1\text{H}\}$ COSY, $^1\text{H}\{^{13}\text{C}\}$ HSQC and $^1\text{H}\{^{13}\text{C}\}$ HMBC experiments. The presence of oxygen, nitrogen and sulfur ions in structures of studied compounds and the molecular formulae were determined on the basis of QTOF-MS results.

Results

LC–MS/MS chromatogram obtained for sample A displayed a single peak at 6.9 min corresponding to compound **1**, the structure of which was further investigated on the basis of mass spectrometric data. Two main peaks at 8.4 min for compound **2** and at 8.9 min for compound **3** were detected in sample B. A peak at the retention time of 8.7 min was recorded for the extract of sample C. It corresponded to compound **4** and its structure elucidation is described below. Sample D was a mixture of several compounds. Two main compounds were identified as *N*-benzylfentanyl at 7.8 min and 1-benzyl-4-propionyloxypiperidine at 6.7 min. There were two other less intense peaks. The first one at 7.4 min was derived from acetylbenzylfentanyl, and the last one at 8.2 min was unknown compound **5**, the structure of which was characterized using QTOF-MS/MS data.

Except for the LC–MS/MS spectrum of compound **3** and some ^1H NMR spectra without assignments, all presented analytical data had not been previously described in any scientific articles, reports, EMCDDA notifications or databases at the time points of our identification (Table 1).

Accurately measured masses, exact (theoretical) masses, the error, relative intensities and elemental compositions for precursor ions and product ions are displayed in Table 2. Proposed fragmentations of identified compounds are presented in Figs. 1, 2, 3, 4, 5, 6. The mass accuracies for precursor ions in MS spectra were largely below 5 ppm, although for some product ions of very low intensity the mass error was higher.

Identification of compound 1: 3,4-methylenedioxy-U-47700

LC–QTOF-MS/MS results

The protonated molecular ion $[\text{M} + \text{H}]^+$ at m/z 305.1848 with predicted chemical formula $\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_3^+$ (error 3.7 ppm) was recorded. The most intense product ion at m/z 260.1286 ($\text{C}_{15}\text{H}_{18}\text{NO}_3^+$) was formed by the cleavage of dimethylamine. Subsequent product ions in the MS/MS spectrum (Fig. 1) derived from the cleavage of C–N bonds on the nitrogen from the amide bond. The amide bond breakage formed the product ion at m/z 149.0234, whereas the cleavage of dimethylaminocyclohexane moiety resulted in two product ions corresponding to dimethylaminocyclohexane

Table 2 Cumulative data of electrospray ionization-quadrupole time-of-flight-tandem mass spectra obtained for investigated new synthetic opioids

Compound name	Precursor ion ^a [M+H] ⁺ Product ions (<i>m/z</i>)	Chemical formula	Theoretical value (<i>m/z</i>)	Error (mDa)	Error (ppm)	Relative intensity (%)
3,4-Methylenedioxy-U -47700 (compound 1)	305.1848 ^a	C ₁₇ H ₂₅ N ₂ O ₃	305.1860	1.1	3.7	2.2
	260.1286	C ₁₅ H ₁₈ NO ₃	260.1281	−0.5	−1.9	100
	180.0656	C ₉ H ₁₀ NO ₃	180.0655	−0.1	−0.3	6.4
	162.0546	C ₉ H ₈ NO ₂	162.0550	0.3	2.1	0.1
	149.0234	C ₈ H ₅ O ₃	149.0233	−0.1	−0.6	8.5
	126.1274	C ₈ H ₁₆ N	126.1277	0.3	2.3	0.4
	123.0448	C ₇ H ₇ O ₂	123.0441	−0.7	−5.7	0.5
	81.0699	C ₆ H ₉	81.0699	−0.1	−0.9	0.1
<i>o</i> -Methyl-acetylfentanyl (compound 2)	337.2265 ^a	C ₂₂ H ₂₉ N ₂ O	337.2274	0.9	2.8	72.2
	295.2139	C ₂₀ H ₂₇ N ₂	295.2169	3	10	0.2
	245.1652	C ₁₅ H ₂₁ N ₂ O	245.1648	−0.3	−1.4	0.1
	216.1380	C ₁₄ H ₁₈ NO	216.1383	0.3	1.2	5.2
	188.1434	C ₁₃ H ₁₈ N	188.1434	−0.1	−0.3	100
	186.1268	C ₁₃ H ₁₆ N	186.1277	1	5.2	0.6
	176.1057	C ₁₁ H ₁₄ NO	176.1070	1.3	7.2	0.3
	160.1112	C ₁₁ H ₁₄ N	160.1121	0.9	5.4	0.3
	150.0897	C ₉ H ₁₂ NO	150.0913	1.6	10.6	0.4
	146.0960	C ₁₀ H ₁₂ N	146.0964	0.4	2.8	1.5
	134.0963	C ₉ H ₁₂ N	134.0964	0.2	1.1	1.5
	105.0702	C ₈ H ₉	105.0699	−0.4	−3.4	5.4
	84.0811	C ₅ H ₁₀ N	84.0808	−0.3	−3.8	0.1
Benzoylfentanyl (compound 3)	385.2253 ^a	C ₂₆ H ₂₉ N ₂ O	385.2274	2.1	5.5	28.1
	279.1814	C ₁₉ H ₂₃ N ₂	279.1856	4.2	15.1	0.1
	264.1385	C ₁₈ H ₁₈ NO	264.1383	−0.3	−1	0.8
	238.1197	C ₁₆ H ₁₆ NO	238.1226	2.9	12.3	0.3
	198.0900	C ₁₃ H ₁₂ NO	198.0913	1.3	6.8	0.6
	188.1436	C ₁₃ H ₁₈ N	188.1434	−0.3	−1.4	100
	160.1127	C ₁₁ H ₁₄ N	160.1121	−0.6	−4	0.5
	146.0962	C ₁₀ H ₁₂ N	146.0964	0.3	1.8	0.9
	134.0971	C ₉ H ₁₂ N	134.0964	−0.7	−4.9	0.8
	105.0340	C ₇ H ₅ O	105.0335	−0.5	−4.6	11.1
	84.0817	C ₅ H ₁₀ N	84.0808	−0.9	−11	0.07
2-Thiophenefentanyl (compound 4)	391.1831 ^a	C ₂₄ H ₂₇ N ₂ OS	391.1839	0.8	2	30.7
	270.0944	C ₁₆ H ₁₆ NOS	270.0947	0.3	1.2	0.9
	244.0783	C ₁₄ H ₁₄ NOS	244.0791	0.8	3.2	0.2
	204.0468	C ₁₁ H ₁₀ NOS	204.0478	0.9	4.7	0.8
	188.1439	C ₁₃ H ₁₈ N	188.1434	−0.5	−2.8	100
	160.1106	C ₁₁ H ₁₄ N	160.1121	1.5	9.2	0.3
	146.0963	C ₁₀ H ₁₂ N	146.0964	0.1	0.5	0.7
	134.0966	C ₉ H ₁₂ N	134.0964	−0.2	−1.4	0.9
	110.9903	C ₅ H ₃ OS	110.9899	−0.4	−3.7	4.8
	105.0702	C ₈ H ₉	105.0699	−0.3	−2.7	5.1
	84.0796	C ₅ H ₁₀ N	84.0808	1.2	14	0.1
Benzoylbenzylfentanyl (compound 5)	371.2105 ^a	C ₂₅ H ₂₇ N ₂ O	371.2118	1.3	3.4	29.6
	264.1388	C ₁₈ H ₁₈ NO	264.1383	−0.5	−2	0.5
	198.0903	C ₁₃ H ₁₂ NO	198.0913	1	5.1	0.8
	188.1066	C ₁₂ H ₁₄ NO	188.1070	0.4	2	1.1

Table 2 (continued)

Compound name	Precursor ion ^a [M+H] ⁺ Product ions (<i>m/z</i>)	Chemical formula	Theoretical value (<i>m/z</i>)	Error (mDa)	Error (ppm)	Relative intensity (%)
	174.1281	C ₁₂ H ₁₆ N	174.1277	−0.4	−2.4	100
	146.0959	C ₁₀ H ₁₂ N	146.0964	0.5	3.8	0.6
	120.0806	C ₈ H ₁₀ N	120.0808	0.2	1.6	0.4
	105.0337	C ₇ H ₅ O	105.0335	−0.2	−2.2	14.3
	91.0538	C ₇ H ₇	91.0542	0.4	4.4	10.3
	82.0652	C ₅ H ₈ N	82.0651	−0.1	−1.3	0.2

Fig. 1 Product ion spectra by liquid chromatography–electrospray ionization–quadrupole time-of-flight tandem mass spectrometry (LC–ESI–QTOF–MS/MS) for 3,4-methylenedioxy-U-47700 (compound **1**) with assigned fragmentation pattern; auto-scaled view (upper) and zoomed view (lower)

at *m/z* 126.1274 and *N*-methyl-1,3-benzodioxole-5-carboxamide fragment at *m/z* 180.0656. Two minor signals at *m/z* 81.0699 and 123.0448 are characteristic of rings which are present in the molecule: cyclohexyl ring and methylenedioxyphenyl ring, respectively. The structure of compound **1** was further elucidated by NMR spectra.

NMR spectroscopy

The NMR data for compound **1** are collected in Table 3. In the ¹H NMR spectrum of compound **1**, there were signals belonging to 24 protons (three singlets of CH₃ groups, seven multiplets in the range of 1.4–4.7 ppm, one signal at 6.02 ppm of CH₂ group and three signals of aromatic protons). The ¹³C NMR spectrum consisted of seventeen signals, thirteen of which on the basis of the ¹H{¹³C}HSQC spectrum were assigned to CH₃ (three signals), CH₂ (five signals) and CH (two signals in aliphatic and three in aromatic part of spectrum) groups. The other four signals belong to quaternary carbon atoms.

The occurrence in ¹H NMR spectrum of the aliphatic spin-spin coupling pattern in the range of 1.4–4.7 ppm belonging to ten protons and simultaneously the presence in ¹³C NMR spectrum of carbon atom signals belonging to four CH₂ groups and two CH groups confirmed the presence of the double-substituted cyclohexyl ring.

The ¹H and ¹³C chemical shifts of signals of three CH₃ groups as well as of two CH groups of the double-substituted cyclohexyl ring may suggest that these groups are connected to the heteroatom, e.g., nitrogen atom. The connection of CH₃ groups to the nitrogen atoms which are simultaneously connected to a cyclohexyl ring was confirmed by the occurrence of strong cross-peaks through three bonds in ¹H{¹³C}HMBC spectrum: H10–C11, H17–C12, H12–C17 and H12–C18.

The occurrence of the signals of three aromatic protons in the ¹H NMR spectrum as well as the presence of the three signals of CH group and three signals of quaternary carbon atom in the aromatic part of the ¹³C NMR spectrum indicated the presence of double substituted phenyl ring.

Additionally, the ^{13}C chemical shifts (about 150 ppm) of quaternary carbon atoms C1a and C3a of this ring suggested that these carbon atoms were connected to the oxygen atom. The presence of strong cross-peaks in the $^1\text{H}\{^{13}\text{C}\}$ HMBC spectrum: H2-C3a and H2-C1a confirmed that CH_2 group is connected to the double substituted phenyl group. Moreover, the ^1H and ^{13}C chemical shifts of the CH_2 group (6.02/103.1 ppm) indicated that this group is connected to phenyl ring via oxygen atom. Based on above information, the presence of the 1,3-benzodioxole moiety could be confirmed in the structure of the tested compound.

Moreover, in the ^{13}C NMR spectrum, there was a signal of quaternary carbon atom located at 175.1 ppm (C8) which could be attributed to the amide group. The complex proton spectral pattern around C11-C12 bond did not allow the assessment of *cis/trans* geometry along this bond, although, considering the bulky substituents on both carbon atoms, *trans* geometry should be favored.

Finally, the structure of compound **1** was determined as *N*-[2-(dimethylamino)cyclohexyl]-*N*-methyl-1,3-benzodioxole-5-carboxamide, so-called 3,4-methylenedioxy-U-47700 or 3,4-MDO-U-47700.

Identification of compound 2: *o*-methyl-acetylfentanyl

LC-QTOF-MS/MS results

The mass spectrum of the analyte with the precursor ion $[\text{M} + \text{H}]^+$ at m/z 337.2265, corresponding to the protonated molecule $\text{C}_{22}\text{H}_{29}\text{N}_2\text{O}^+$, was similar to MS data obtained for fentanyl. Furthermore, almost all product ions, both main fragments (at m/z 188.1434 originated from

N-phenylethylpiperidine, at m/z 105.0702 corresponding to phenylethyl moiety and at m/z 216.1380 formed by cleavage of C–N bonds in piperidine ring) and those of relative intensity below 2%, could be found in the MS/MS spectrum of fentanyl. However, the cleavage of an amide bond formed distinctive fragments for fentanyl precursor ion at m/z 281.2018 corresponding to $\text{C}_{19}\text{H}_{25}\text{N}_2^+$ and for *o*-methyl-acetylfentanyl at m/z 295.2139 which matches with elemental composition $\text{C}_{20}\text{H}_{27}\text{N}_2^+$. Characteristic difference of 14 Da and confirmed presence of *N*-phenylethylpiperidine indicated that the methyl group is attached to *N*-phenyl ring. The product ion spectrum of compound **2** is depicted in Fig. 2. Comparison of fentanyl and compound **2** fragmentation spectra is shown in Fig. 3. However, to determine the position of the methyl group at the phenyl ring, the NMR experiment was necessary to be conducted.

NMR spectroscopy

The NMR data for compound **2** are collected in Table 4. In the ^1H NMR spectrum, there were: two singlets of CH_3 groups, an AA'BB' aliphatic spin system ($-\text{CH}_2-\text{CH}_2-$ moiety), nine multiplets in the range of 1.4–4.5 ppm and signals of nine aromatic protons. The ^{13}C NMR spectrum consisted of twenty signals belonging to carbon atoms of CH_3 (two signals), CH_2 (six signals) and CH groups (one signal in aliphatic and seven in aromatic part of spectrum) as well as to the four of quaternary carbon atom.

The spin-spin coupling pattern in the range of 1.4–4.5 ppm belonging to nine protons was assigned to *para* substituted piperidine ring on the basis of the $^1\text{H}\{^1\text{H}\}$ COSY and $^1\text{H}\{^{13}\text{C}\}$ HSQC spectra (the protons are connected with five carbon atoms of four CH_2 groups and one CH group).

Table 3 Experimental 1 dimensional (1D) and 2D nuclear magnetic resonance (NMR) spectroscopic data of compound **1** in CD_3OD

	Numbering of atom position (see figure)	^1H NMR δ [ppm] (multiplicity, number of protons J_{HH} [Hz])	^{13}C NMR δ [ppm]	$^1\text{H}/^1\text{H}$ COSY	$^1\text{H}\{^{13}\text{C}\}$ HMBC
	1a (C)	-	150.8	-	-
	2 (CH₂)	6.02 (m, 2H)	103.1	-	C1a, C3a
	3a (C)	-	149.1	-	-
	4 (CH)	7.09 (brs, 1H)	109.3	-	-
	5 (C)	-	130.5	-	-
	6 (CH)	7.09 (d, 1H, J_{HH} =8.5 Hz)	123.3	H7	-
	7 (CH)	6.90 (d, 1H, J_{HH} =8.5 Hz)	109.0	H6	C1a, C3a, C5
	8 (C=O)	-	175.1	-	-
	10 (CH₃)	2.97 (s, 3H)	33.5	-	C8, C11
	11 (CH)	4.70 (brs, 1H)	55.3	H12, H16a, H16b	-
	12 (CH)	3.71 (td, 1H, J_{HH} =11.6, 3.7 Hz)	65.4	H11, H13a, H13b	C11, C17, C18, C13
	13a (CH₂)	1.65 (m, 1H)	24.2	H12, H13a/13b, H14a, H14b	-
	13b	2.23 (m, 1H)	-	-	-
	14a (½ CH₂)	1.44 (m, 1H)	25.0	Strongly coupled system	-
	14b (½ CH₂)	1.96 (m, 1H)	-	-	-
	15a (½ CH₂)^a	1.45 (m, 1H)	25.6	Strongly coupled system	-
	15b (½ CH₂)	1.88 (m, 1H)	-	-	-
	16a (½ CH₂)^a	1.85 (m, 1H)	30.3	Strongly coupled system	-
	16b (½ CH₂)	1.96 (m, 1H)	-	-	-
	17 (CH₃)	2.95 (s, 3H)	43.5	-	C12, C18
	18 (CH₃)	2.90 (brs, 3H)	38.1	-	-

brs broad singlet, COSY correlation spectroscopy, δ chemical shift [ppm], d doublet, HMBC heteronuclear multiple bond correlation, J_{HH} proton-proton coupling constant [Hz], m multiplet, s singlet, td triplet of doublets

^aprotons H15 and H16 can be interchange

Fig. 2 Product ion spectra by LC–ESI–QTOF–MS/MS for *o*-methyl-acetylfentanyl (compound **2**) with assigned fragmentation pattern; auto-scaled view (upper) and zoomed view (lower)

The symmetrical *para* substitution of piperidine ring was confirmed by the identical chemical shifts of carbon atoms C2 and C6. However, the unsymmetrical remote environment, flanking the 3-CH₂ and 5-CH₂ groups, adjacent to a substitution center, may induce observed geminal nonequivalence in proton and carbon spectra (see Table 4) of these groups. The ¹H and ¹³C chemical shifts of the CH group of piperidine ring (4.51/54.8 ppm) indicated that this group is connected with heteroatom e.g., nitrogen atom.

The occurrence of the coupling system of nine aromatic protons in the ¹H NMR spectrum as well as the presence of the seven signals of CH group and three signals of quaternary carbon atom in the aromatic part of the ¹³C NMR spectrum indicated the presence of one phenyl group and one substituted phenyl group. The substituent was a CH₃ group, attached in *ortho*-position, confirmed by the long-range proton-carbon correlations obtained from ¹H{¹³C} HMBC spectrum; the CH₃ group located at 2.27 ppm

Fig. 3 Product ion spectra by LC–ESI–QTOF–MS/MS for fentanyl and *o*-methyl-acetylfentanyl (compound **2**) with assigned distinctive fragments; auto-scaled views of: fentanyl (**a**), *o*-methyl-acetylfentanyl (**b**); zoomed views of: fentanyl (**c**), *o*-methyl-acetylfentanyl (**d**)

Table 4 Experimental 1D and 2D NMR spectroscopic data of compound **2** in CD₃OD

Numbering of atom position (see figure)	¹ H NMR δ [ppm] (multiplicity, number of protons J_{HH} [Hz])	¹³ C NMR δ [ppm]	¹ H/ ¹ H COSY	¹ H/ ¹³ C HMBC
2a (½ CH ₂)	2.41 (1H, <i>m</i>)		H3a, H3b, H2b	C7, C4, C6, C3
2b (½ CH ₂)	3.15 (1H, <i>dm</i> , J_{HH} =12.2 Hz)	53.8	H3a, H3b, H2a	-
3a (½ CH ₂)	1.39 (1H, <i>qd</i> , J_{HH} =12.6, 4.0 Hz)		H3b, H2a, H2b, H4	C4, C2, C5
3b (½ CH ₂)	1.82 (1H, <i>m</i>)	29.4	H3a, H2a, H2b, H4	-
4 (CH)	4.51 (1H, <i>tt</i> , J_{HH} =12.2, 4.2 Hz)	54.8	H5b, H3b, H5a, H3a	C22, C16, C2, C6, C3, C5
5a (½ CH ₂)	1.76 (1H, <i>qd</i> , J_{HH} =12.4, 4.0 Hz)		H5b, H6a, H6b, H4	C4, C6, C3
5b (½ CH ₂)	2.13 (1H, <i>m</i>)	30.9	H5a, H6a, H6b, H4	-
6a (½ CH ₂)	2.44 (1H, <i>m</i>)		H5a, H5b, H6b	C7, C4, C2, C5
6b (½ CH ₂)	3.23 (1H, <i>dm</i> , J_{HH} =12.2 Hz)	53.8	H6a, H5b, H5a	-
7 (CH ₂)	2.73 (2H, <i>m</i>)	60.7	H8	C2, C6, C8, C9
8 (CH ₂)	2.80 (2H, <i>m</i>)	33.4	H7	C9, C10/C14, C7
9 (C)	-	140.2	-	-
10/14 (CH)	7.18 (2H, <i>m</i> , overlapped)	129.7	-	C12, C10/14, C8
11/13 (CH)	7.25 (2H, <i>m</i>)	129.6	-	C9, C11/13
12 (CH)	7.17 (1H, <i>m</i> , overlapped)	127.5	-	C10/14
16 (C)	-	139.6	-	-
17 (CH)	7.17 (1H, <i>dd</i> , J_{HH} =7.5, 1.3 Hz)	131.1	-	C21, C19, C16
18 (CH)	7.31 (1H, <i>td</i> , J_{HH} =7.5, 1.9 Hz)	128.3	H19, H17	C16, C20
19 (CH)	7.35 (1H, <i>td</i> , J_{HH} =7.5, 1.3 Hz)	130.2	H18, H20	C21, C17
20 (CH)	7.40 (1H, <i>dd</i> , J_{HH} =7.5, 1.9 Hz)	132.8	H19	C24, C16, C18
21 (C)	-	138.2	-	-
22 (C=O)	-	173.1	-	-
23 (CH ₃)	1.71 (3H, <i>s</i>)	23.0	-	C22
24 (CH ₃)	2.27 (3H, <i>s</i>)	18.3	-	C16, C20, C21

dd doublet of doublets, *dm* doublet of multiplets, *qd* quartet of doublets, *tt* triplet of triplets (for other abbreviations, see Table 3)

(H24) gave the strong cross-peaks to two signals of quaternary carbon atoms at 138.2 ppm (C21) and 139.6 ppm (C16) and to one carbon atom of CH group at 132.8 ppm (C20).

The NMR data also confirmed the presence of an ethylene group –CH₂–CH₂–. That group is connected to the phenyl ring as well as to piperidine ring via nitrogen atom as evidenced by occurring strong cross-peaks in the ¹H{¹³C}HMBC spectrum: H10/14–C8, H8–C10/14, H8–C9, H2a–C7, H6a–C7, H7–C2, and H7–C6. The ¹H and ¹³C chemical shifts of the ethylene group (2.80/33.4 ppm and 2.73/60.7 ppm) also confirmed that this group is connected to piperidine ring via nitrogen atom.

Moreover, in ¹³C NMR spectrum, there was a signal of quaternary carbon atom located at 173.1 ppm (C22) and signal of CH₃ group at 23.0 ppm (C23) which could be attributed to the acetamide group. The piperidine ring as well as *ortho*-substituted phenyl group were connected to the nitrogen atom of acetamide group as evidenced by occurring strong cross-peaks in the ¹H{¹³C}HMBC spectrum: H4–C22 and H4–C16.

Finally, compound **2** was determined as *N*-(2-methylphenyl)-*N*-[1-(2-phenylethyl)piperidin-4-yl]acetamide (*ortho*-methyl-acetylfentanyl).

Identification of compound **3**: benzoylfentanyl

LC–QTOF–MS/MS results

The LC–QTOF–MS/MS data of compound **3** displayed the protonated monoisotopic ion [M + H]⁺ at *m/z* 385.2253

(C₂₆H₂₉N₂O⁺, error 5.5 ppm). MS/MS experiment formed two main product ions (Fig. 4). The most intense ion at *m/z* 188.1436 corresponded to *N*-phenylethylpiperidine moiety. The exact mass of the second product ion at *m/z* 105.0340 determined the most possible formula C₇H₅O⁺ and indicated that this product ion derived rather from the benzoyl group than from phenylethyl moiety that is also included in fentanyl or *o*-methyl-acetylfentanyl (Figs. 2, 3). Phenylethyl product ion of the latter two drugs gave a theoretical value at *m/z* 105.0699, but its low intensity with the limited resolution of mass spectrometer did not allow observing it as a completely separated ion. Apart from the benzoyl product ion, the cleavage of amide bond resulted in the fragment at *m/z* 279.1814 corresponding to *N*-phenyl-1-(2-phenylethyl)piperidin-4-amine. An ion at *m/z* 198.0900 corresponded to *N*-phenylbenzamide substructure. Formation of several minor fragments at *m/z* 134.0971, 146.0962, 160.1127, 264.1385 and 238.1197 was a consequence of cleavages of piperidine ring bonds. The structure of compound **3** was further revealed by NMR spectra.

NMR spectroscopy

The NMR data for compound **3** are collected in Table 5. In the ¹H NMR spectrum there were signals belonging to thirteen aliphatic and fifteen aromatic protons. In the ¹³C NMR spectrum there were eighteen signals, from which four can be assigned to the carbon atoms of the CH₂ groups and ten to the CH groups (one signal in aliphatic and nine in

Fig. 4 Product ion spectra by LC-ESI-QTOF-MS/MS for benzoylfentanyl (compound **3**) with assigned fragmentation pattern; auto-scaled view (upper) and zoomed view (lower)

aromatic part of spectrum). The other four signals belonged to quaternary carbon atoms.

The five multiples of nine protons in the range of 1.7–4.7 ppm in the ^1H NMR spectrum were assigned to *para* substituted piperidine ring. In this compound in contrast to compound **2**, equivalent protons at positions 2a and 6a, 2b and 6b, 3a and 5a, and 3b and 5b were observed. Also in the ^{13}C NMR spectrum only two carbon atom signals connected with those protons were observed pointing to equivalent

carbon atoms at positions 2 and 6, and 3 and 5. Additionally, the ^1H and ^{13}C chemical shifts of the CH group of piperidine (4.72/55.0 ppm) indicated that this group is connected with nitrogen atom like for compounds **2**.

The occurrence of the coupling system of fifteen aromatic protons in the ^1H NMR spectrum as well as the presence of the nine signals of CH group and three signals of quaternary carbon atom in the aromatic part of the ^{13}C NMR spectrum indicated the presence of three phenyl groups.

Table 5 Experimental 1D and 2D NMR spectroscopic data of compound **3** in CD_3OD

	Numbering of atom position (see figure)	^1H NMR δ [ppm] (multiplicity, number of protons J_{HH} [Hz])	^{13}C NMR δ [ppm]	$^1\text{H}/^1\text{H}$ COSY	$^1\text{H}/^{13}\text{C}$ HMBC
	2a ($\frac{1}{2}$ CH_2)	2.42 (2H, brs)		H3a/5a, H3b/5b, H2b/6b	-
	6a ($\frac{1}{2}$ CH_2)		53.9		
	2b ($\frac{1}{2}$ CH_2)	3.20 (2H, d, J_{HH} =11.7 Hz)		H3a/5a, H3b/5b, H2a/6a	-
	6b ($\frac{1}{2}$ CH_2)				
	3a ($\frac{1}{2}$ CH_2)	1.69 (2H, qd, J_{HH} =12.5, 3.7 Hz)		H3b/5b, H2a/6a, H2b/6b, H4	C2/C6, C3/C5, C4
	5a ($\frac{1}{2}$ CH_2)		30.7		
	3b ($\frac{1}{2}$ CH_2)	2.05 (2H, d, J_{HH} =12.5 Hz)		H3a/5a, H2a/6a, H2b/6b, H4	-
	5b ($\frac{1}{2}$ CH_2)				
	4 (CH)	4.72 (1H, brs)	55.0	H3a/5a, H3b/5b	-
	7 (CH_2)	2.69 (2H, m)	60.9	H8	C2/C6, C8
	8 (CH_2)	2.79 (2H, m)	33.7	H7	C9, C10/C14, C7
	9 (C)	-	140.6	-	-
	10/14 (CH)	7.19 (2H, m, overlapped)	129.7	-	C12, C10/14, C8
	11/13 (CH)	7.25 (2H, m, overlapped)	129.6	-	C9, C11/13
	12 (CH)	7.17 (1H, m, overlapped)	127.4	-	C10/14
	16 (C)	-	140.5	-	-
	17/21 (CH)	7.14 (2H, m, overlapped)	132.1	-	C17/21, C19, C16
	18/20 (CH)	7.26 (2H, m, overlapped)	130.0	-	C18/20, C16
	19 (CH)	7.22 (1H, m, overlapped)	129.2	-	C17/C21
	22 (C=O)	-	173.4	-	-
	23 (C)	-	138.1	-	-
	24/28 (CH)	7.24 (2H, m, overlapped)	129.0	-	C24/28, C26, C22
	25/27 (CH)	7.16 (2H, m, overlapped)	128.8	-	C25/27, C23
	26 (CH)	7.19 (1H, m, overlapped)	130.4	-	C24/28

(for abbreviations, see Tables 3 and 4)

Fig. 5 Product ion spectra by LC–ESI–QTOF–MS/MS for 2-thiophenefentanyl (compound **4**) with assigned fragmentation pattern; auto-scaled view (upper) and zoomed view (lower)

Like in compound **2**, an ethylene group $-\text{CH}_2-\text{CH}_2-$ is connected to the one of phenyl group as well as to piperidine ring via nitrogen atom.

Moreover, in ^{13}C NMR spectrum there was signal of quaternary carbon atom located at 173.4 ppm (C22) which could be attributed to the amide group. The second phenyl group was attached directly to carbonyl atom as evidenced by occurring a strong cross-peak in the $^1\text{H}\{^{13}\text{C}\}$ HMBC spectrum: H24/28–C22. The piperidine ring as well as the next phenyl group was connected to the nitrogen atom of amide group.

Finally, the structure of compound **3** was determined as *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]benzamide, so-called benzoylfentanyl or phenylfentanyl.

Identification of compound **4**: 2-thiophenefentanyl

LC–QTOF–MS/MS results

The LC–QTOF–MS/MS data of compound **4** displayed the protonated molecular ion $[\text{M} + \text{H}]^+$ at m/z 391.1831 with the isotope pattern characteristic to a compound containing one sulphur atom in the molecule and suggested formula $\text{C}_{24}\text{H}_{27}\text{N}_2\text{OS}^+$ (calculated m/z 391.1839, error 2.0 ppm). Similarly to fragmentation spectra of fentanyl and fentanyl analogs with the unsubstituted phenylethylpiperidine group, two main characteristic fragments at m/z 188.1439 and at m/z 105.0702 were observed in MS/MS spectrum of compound **4** (Fig. 5). The third relatively intense ion at m/z 110.9903 ($\text{C}_5\text{H}_3\text{OS}^+$ thienoyl moiety) arose from the amide bond cleavage. A less intense ion at m/z 204.0468 was associated with the cleavage between the piperidine ring

and *N*-phenyl-amide moiety. Several minor fragments were formed due subsequent bond-breaking in the piperidine ring. To verify position in which the thiophene ring is linked to carboxamide group, measurements using NMR had to be performed.

NMR spectroscopy

The NMR data for compound **4** were collected in Table 6. In the ^1H NMR spectrum, there were signals from thirteen aliphatic and thirteen aromatic protons. In the ^{13}C NMR spectrum, there were eighteen signals, fourteen of which were assigned to proton-bearing carbon atoms of CH_2 (four signals) and CH groups (one signal in aliphatic and nine in aromatic part of spectrum). The other four signals belonged to quaternary carbon atoms.

The aliphatic parts of ^1H as well as ^{13}C NMR spectra of compound **4** were very similar to analogous fragments of spectra of compounds **3** although slight differences in chemical shifts were observed. Based on the NMR data the presence of the *para* substituted piperidine ring and ethylene group were confirmed.

The analysis of the coupling pattern of signals of thirteen aromatic protons in the ^1H NMR and $^1\text{H}\{^1\text{H}\}$ COSY spectra as well as the aromatic part of the ^{13}C NMR spectrum (the nine signals of CH group and three signals of quaternary carbon atom) confirmed the presence of two phenyl and 2-thiophene groups.

The signal of quaternary carbon atom located in ^{13}C NMR spectrum at 164.4 ppm (C22) could be attributed to the amide group. The 2-thiophene group was attached directly to carbonyl atom as evidenced by occurring a

Table 6 Experimental 1D and 2D NMR spectroscopic data of compound **4** in CD₃OD

	Numbering of atom position (see figure)	¹ H NMR δ [ppm] (multiplicity, number of protons <i>J</i> _{HH} [Hz])	¹³ C NMR δ [ppm]	¹ H/ ¹ H COSY	¹ H/ ¹³ C HMBC
2a (½ CH ₂) 6a (½ CH ₂) 2b (½ CH ₂) 6b (½ CH ₂)	3.08 (2H, <i>td</i> , <i>J</i> _{HH} =12.6, 2.1 Hz)	53.4	H3a/5a, H3b/5b, H2b/6b	C7, C2/6
	3a (½ CH ₂) 5a (½ CH ₂) 3b (½ CH ₂) 5b (½ CH ₂)	1.82 (2H, <i>qd</i> , <i>J</i> _{HH} =12.9, 3.9 Hz)	29.0	H3b/5b, H2a/6a, H2b/6b, H4	C2/6, C3/5, C4
	4 (CH) 7 (CH ₂) 8 (CH ₂) 9 (C)	4.89 (1H, <i>m</i>) 3.20 (2H, <i>m</i>) 2.96 (2H, <i>m</i>) -	53.5 59.2 31.9 138.0	H3a/5a, H3b/5b H8 H7 -	C16, C2/6, C3/5, C22 C9, C2/6, C8 C9, C10/14, C7 -
	10/14 (CH) 11/13 (CH) 12 (CH)	7.25 (2H, <i>m</i>) 7.31 (2H, <i>m</i>) 7.24 (1H, <i>m</i>)	129.7 129.9 128.2	H11/13 H10/14, H12 H11/13	C12, C10/14, C8 C9, C11/13 C10/14
	16 (C) 17/21 (CH) 18/20 (CH) 19 (CH)	- 7.33 (2H, <i>m</i>) 7.53 (2H, <i>m</i>) 7.53 (1H, <i>m</i>)	139.6 132.4 131.0 130.8	- H18/20 H17/21 -	- C17/21, C18/20, C19 C18/20, C16 C17/21
	22 (C=O) 23 (C) 24 (CH) 25 (CH) 26 (CH)	- - 6.66 (1H, <i>dd</i> , <i>J</i> _{HH} =3.9, 1.2 Hz) 6.80 (1H, <i>dd</i> , <i>J</i> _{HH} =5.1, 3.9 Hz) 7.47 (1H, <i>dd</i> , <i>J</i> _{HH} =5.1, 1.2 Hz)	164.4 139.0 133.7 127.8 132.7	- - H25 H24, H26 H25	- - C22, C26, C25, C23 C23, C24, C26 C24, C23, C25, C22

(for abbreviations, see Tables 3 and 4)

strong cross-peak in the ¹H{¹³C}HMBC spectrum: H24–C22. The piperidine ring as well as phenyl group was connected to the nitrogen atom of amide group as evidenced by occurring a strong cross-peaks in the ¹H{¹³C}HMBC spectrum: H4–C22 and H4–C16.

Like in compounds **2** and **3**, the ethylene group was connected to the other phenyl group as well as to piperidine ring via nitrogen atom.

Finally, based on mass spectra and NMR data, compound **4** was determined as *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]thiophene-2-carboxamide, and named as 2-thiophenefentanyl. It is structurally related to furanylfentanyl. 2-Thiophenefentanyl differs from furanylfentanyl by replacement of the oxygen atom in the furan ring with a sulphur atom.

Identification of compound **5**: benzoylbenzylfentanyl

LC–QTOF-MS/MS results

The protonated molecular ion at *m/z* 371.2105 with the predicted chemical formula C₂₅H₂₇N₂O⁺ (error 3.4 ppm) was detected in LC–QTOF-MS/MS spectrum. Elimination of the benzyl moiety and the cleavage between the piperidine ring and *N*-phenyl-amide moiety resulted in the formation of two most intense product ions in MS/MS spectrum at *m/z* 91.0538 and 174.1281, respectively (Fig. 6). Similarly to compound **3** a product ion at *m/z* 105.0337 which derived from benzoyl moiety elimination was present. Less intense product ions were formed by the piperidine ring degradation. An ion at *m/z* 198.0903 was associated with the cleavage

between the piperidine ring and *N*-phenyl-amide moiety. The breaking of C–N bonds in piperidine ring and further loss of the phenyl group generated a product ion at *m/z* 188.1066. Due to limited amount of sample D, which was a mixture of several benzylfentanyl analogs and low content of investigated compound **5** in this mixture, structural confirmation by means of NMR spectroscopy was not possible.

On the basis of mass spectra, a structure of compound **5** was proposed as *N*-(1-benzylpiperidin-4-yl)-*N*-phenylbenzamide (benzoylbenzylfentanyl).

Discussion

Except for compound **3**, which has been seized and detected for the first time in Sweden in March 2017 [11], all of them were identified for the first time on the illicit EU market and reported to the EWS-EMCDDA by NMI [12–15]. Such notification includes names, identifiers of the substance, sample origin (seizure by law enforcement agencies, poisonings, samples collected from NPS users or test purchases), chemical and physical properties, analytical methodologies applied for structure elucidation or harm caused by the NPS. Analytical data are also provided, because they can substitute the reference data for other laboratories (in the absence of reference standards at the time of the first detection). All these information are collected in The European Database on New Drugs (EDND). However, access to the EDND is restricted only to the members of the Network and selected members of the national early warning systems, which implies that, except this limited group of users, other European scientists as well as control laboratories or scientific communities

Fig. 6 Product ion spectra by LC–ESI–QTOF–MS/MS for benzoylbenzylfentanyl (compound **5**) with assigned fragmentation pattern; auto-scaled view (upper) and zoomed view (lower)

from other regions of the world cannot reach the contents of EDND system. Therefore, the publication of this manuscript in an international journal is essential to make the analytical information for these five NPS widespread in the world.

Compounds **1** and **2** have never been detected before but the structure of compound **2** was taken into account as a potential component of illicit preparation even in the 1980s [16]. After our notification, the detection of compounds **3** and **5** on the US market using gas chromatography–mass spectrometry (GC–MS) was published [17]. To the best of our knowledge, our paper is the first comprehensive report that characterized these newly identified NPSs by means of LC–QTOF–MS/MS and 1D and 2D NMR spectroscopy. Some GC–MS and ^1H NMR spectra without any assignments were attached to the SWGDRUG monographs [18, 19] or commercial websites providing product information of analytical reference standards [20–23]. In the case of compound **3**, available literature or reports [24] contained only selected analytical information, therefore, we present complete LC–QTOF–MS/MS and NMR data for easier identification and characterization of benzoylfentanyl.

The molecule of compound **1** is structurally related to internationally controlled U-47700. Two chlorine groups at the 3- and 4-positions on the phenyl ring in U-47700 structure are replaced with a methylenedioxy group in 3,4-methylenedioxy-U-47700 molecule. The modification based on methylenedioxy group introduction to positions 3 and 4 of the aromatic ring was observed also in other NPS classes like phenylethylamines, cathinones, aminoindanes, piperazines and even synthetic opioids which belong to fentanyl analogs (benzodioxole fentanyl).

In case of compound **4**, if one based the identification on LC–MS/MS results only, it should be performed with

caution, because its isomer may have similar MS/MS spectrum. In this study, 2-position of thiophene moiety was verified by the NMR, however, the reference standards of 3-thiophene isomer is currently also available and potentially may appear on the illicit market.

Compound **5** differs from compound **3** by the removal of single carbon in a chain of *N*-substituent of piperidine ring. According to the literature [25], such a structure modification dramatically reduces the potency of fentanyl *N*-benzyl analogs, leaving them inactive. However, Polish authorities responsible for NPS supervision, reported fatal intoxications associated with the products of the same name as sample D, in which fentanyl with benzylfentanyl were found. In sample D fentanyl was not detected. Results of this sample analysis, in which four compounds including two *N*-benzylfentanyl, two *N*-benzylfentanyl derivatives and 1-benzyl-4-propionyloxypiperidine were identified, indicated that it was most probably the effect of the failed synthesis. One of the major compound in sample D 1-benzyl-4-propionyloxypiperidine was reported as a manufacturing impurity and a marker of Jenssen synthesis route of illicit fentanyl [26].

Conclusions

In this study, identification and analytical characterization of five synthetic opioids, including one non-fentanyl opioid belonging to “U-compounds group” and four fentanyl analogs were presented. Structure elucidation was based on LC–QTOF–MS/MS and NMR data. These synthetic opioids were detected in samples collected in Warsaw by NMI during last two years that confirmed the trend of increasing popularity of this group of NPS in Europe.

The data presented in this article will be useful for identifying the 5 synthetic opioids included in illicit products or human specimens.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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