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Detection and identification of the new potential synthetic cannabinoids 1-pentyl-3-(2-iodobenzoyl)indole and 1-pentyl-3-(1-adamantoyl)indole in seized bulk powders in Hungary

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ABSTRACT

3-Naphthoyl- and 3-phenylacetylindoles represent a group of substances of cannabimimetic activity with affinities – strongly influenced by their functional groups – to cannabinoid receptors CB1 and CB2. Some of them have been described as ingredients of herbal blends also known as "smart products" by several research groups. Recently further cannabimimetic substances possessing new chemical structures like benzoylindoles and adamantoylindoles have emerged. In Hungary, two powder samples were seized by the authorities and identified as 1-pentyl-3-(2-iodobenzoyl)indole (AM-679) and 1-pentyl-3-(1-adamantoyl)indole. Structure elucidation was carried out by LC–UV–MS/MS, LC–TOF–MS, GC–MS and NMR. The benzoylindole AM-679 is a known agonist of cannabinoid receptors while the adamantoylindole derivative also carries chemical features typical for cannabimimetics. It is thus assumed that both substances might be detected in "smart products" in the future.

1. Introduction

3-Naphthoyl- and 3-phenylacetylindoles have been reported to be used for their cannabimimetic effects by several research groups recently [1–7]. A high number of these substances were originally synthesized by J.W. Huffman et al. who carried out a series of pharmacodynamic studies to investigate their affinity to cannabinoid (CB1 and CB2) receptors [8–15]. Thus, the molecules are usually referred to as JWH-substances identified with a 3-digit number linked to the abbreviation.

As a result of the pharmacodynamic studies the affinity of the indoles to cannabinoid receptors was explained by a three-point attachment for each compound with regions of the natural ligand Δ9-tetrahydrocannabinol (Δ9-THC) the three key regions being the naphthalene ring, the carbonyl group and the *N*-alkyl substituent of the indole moiety [13]. A more recent publication also claimed that replacement of the naphthalene by a methyl-, methoxy-, fluoro-, chloro- or bromo-substituted phenylacetyl group resulted in an increased selectivity for the CB1 receptor depending on the nature and location of substituent at the aromatic ring [12]. Reports on benzoylindoles, pyrroles and indenes with potential cannabimimetic activity are also available [10,16,17].

According to scientific literature, JWH-018 and JWH-073 were frequently detected in herbal drug products [1–3,6,7]. Furthermore, JWH-015, -081, -200, -250 and -251 were also identified as adulterants [4,5,17].

In 2010 four stocks of bulk powders were seized by the authorities at a Hungarian international airport. Three of them were labeled as calcium stearate and the fourth as malic acid. The results of a chemical analysis demonstrated that all the powders were of high purity. Powders **1** and **2** were identified as JWH-018 and JWH-122, respectively (Fig. 1a and b), while **3** and **4** were deemed to be closely related to them. After structure elucidation by LC–MS/MS, LC–TOF–MS, GC–MS and NMR compound **3** was identified as 1-pentyl-3-(2-iodobenzoyl)indole (Fig. 1c) and compound **4** as 1-pentyl-3-(1-adamantoyl)indole (Fig. 1d). It was found that **3** was identical with AM-679, a benzoylindole with known cannabimimetic activity [18]. In case of compound **4**, close structural relationship to the cannabinoid

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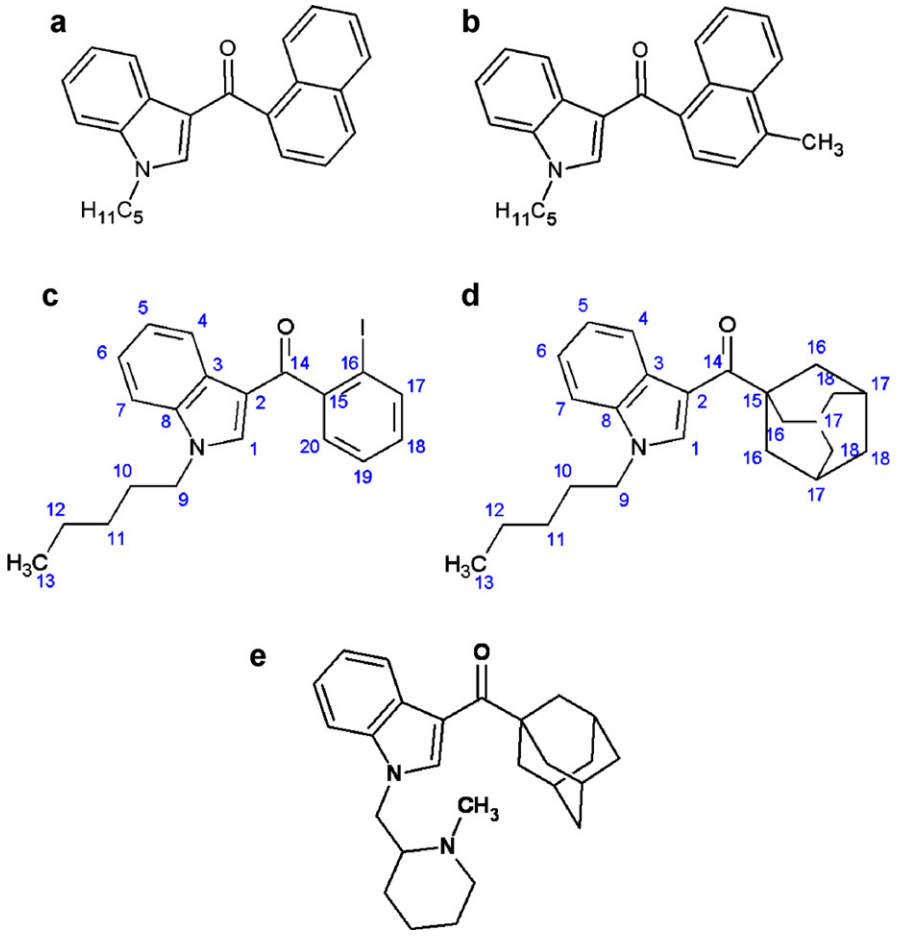

Fig. 1. Chemical structures of (a) JWH-018 (**1**); (b) JWH-122 (**2**); (c) 1-pentyl-3-(2-iodobenzoyl)indole (**3**); (d) 1-pentyl-3-(1-adamantoyl)indole (**4**) and (e) AM-1248. The numbering indicates the corresponding carbon atoms in Table 1.

receptor agonist AM-1248 [19] (Fig. 1e) was established. AM-679 is a benzoylindole with moderate affinity to both CB receptors [18]. On the other hand, no data about the cannabimimetic activity of 1-pentyl-3-(1-adamantoyl)indole were found in public databases. The findings were reported to the Hungarian Customs and Finance Guard. The results of detailed characterization of both compounds are presented in this paper.

Table 1
NMR chemical shifts for structures **3** and **4** (see Fig. 1 for numbering of the carbon atoms).

Atom number	Compound **3**		Compound **4**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	7.30 s 1H	138.19	7.92 s 1H	133.16
2		126.88		128.66
3		115.49		112.95
4	8.33 d 1H	122.93	8.51 m 1H	123.64
5	7.33 m 1H	123.06*	7.26 m 1H	122.39*
6	7.33 m 1H	123.79*	7.26 m 1H	123.11*
7	7.38 d 1H	110.16	7.32 m 1H	109.50
8		137.26		135.69
9	4.10 t 2H	47.40	4.15 t 2H	47.14
10	1.84 m 2H	29.61	1.88 m 2H	29.79
11	1.30 m 2H	29.05	1.33 m 2H	29.11
12	1.32 m 2H	22.32	1.35 m 2H	22.34
13	0.87 t 3H	14.03	0.90 t 3H	14.04
14		191.42		202.04
15		146.56		47.01
16		92.77	2.13 brs 6H	40.70
17	7.92 s 1H	139.77	2.12 brs 3H	28.71
18	7.15 ddd 1H	130.59	1.81 brs 6H	37.08
19	7.43 ddd 1H	127.85		
20	7.39 m 1H	128.19		

* Signals in any column may be reversed.

2. Experimental

2.1. Materials

Powder samples **1**, **2**, **3** and **4** were submitted to the National Institute of Pharmacy by the Hungarian Customs and Finance Guard following a seizure. Identity and purity of powders **1** and **2** were confirmed by LC–UV–MS/MS and NMR. The two substances were used as standards to support structure elucidation of the unknown compounds **3** and **4** in the LC–MS/MS studies.

2.2. Solvents and reagents

Water was produced by a Millipore Elix3 (Billerica, MA, USA) water purifying system. Acetonitrile (ACN), methanol (MeOH) (both from Merck KGaA, Darmstadt, Germany) and formic acid (Sigma–Aldrich GmbH, Seelze, Germany) were of LC–MS grade. For NMR studies samples were dissolved in $CDCl_3$ of 99.8% isotopic purity containing 0.03% TMS (Sigma–Aldrich GmbH, Seelze, Germany).

2.3. Apparatus

LC–UV–MS/MS: Agilent Technologies 1200 Series HPLC equipped with an UV-VIS diode array detector (DAD) and coupled with an Agilent Technologies 6410A Triple Quad mass spectrometer equipped with a multimode ion source (MMI).

LC–TOF–MS: Agilent Technologies 1260 Infinity HPLC coupled with Agilent Technologies 6230 Time of Flight MS equipped with a Jet Stream ion source operated in positive ion mode.

GC–MS: Shimadzu GCMS-QP2010 system.

NMR: ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectra: 600 MHz Varian VNMRS spectrometer; δ in ppm rel. to TMS as internal standard.

2.4. Methods

All MS studies were carried out using dilute methanolic solutions of each powder.

For LC–MS/MS a mixture of water, ACN and formic acid (50:50:0.1 v/v) was used as mobile phase at a flow rate of 0.4 mL min^{-1}. The following MS parameters were employed: fragmentor voltage: 135 V; charging voltage: 2000 V; capillary voltage: 2500 V; nebulizer pressure: 60 psi; gas temperature: 325 °C; gas flow rate: 5 L min^{-1}; collision energy (for product ion spectra): 30 V. Product ion spectra were obtained after direct injection.

For acquiring full scan MS and UV-DAD spectra as well as for purity assessment samples were analyzed on an Agilent Zorbax SB C-18 30 mm × 2.1 mm column (particle size: 3.5 μm). A gradient elution program was applied at a flow rate of 0.4 mL min^{-1} with mobile phase A consisting of 95% water, 5% ACN and 0.1% formic acid and mobile phase B consisting of 95% ACN, 5% water and 0.1% formic acid. In the gradient elution program the ratio of mobile phase B was held at 0% from 0 to 1 min, then linearly increased to 100% from 1 to 11 min and held at 100% from 11 to 15 min. UV chromatograms at 210 nm and UV spectra in the range of 200–400 nm were recorded by the diode array detector (DAD).

In case of LC–TOF–MS two reference masses (*m/z* 121.050873 and 922.009798) were used. Mass spectra were acquired by direct injection using a mixture of water, ACN and formic acid (50:50:0.1) as mobile phase at a flow rate of 0.5 mL min^{-1}. The most relevant MS parameters were set as follows: fragmentor voltage: 175 V; capillary voltage: 3500 V; nebulizer pressure: 30 psi; gas temperature: 325 °C; gas flow rate: 10 L min^{-1}. Data analysis was carried out using Agilent MassHunter Workstation Software B.01.03.

GC–MS: samples were analyzed on a Zebron ZB-5 30 m × 0.25 mm capillary column with a film thickness of 0.25 μm. Helium was applied as carrier gas with a linear velocity of 55 cm s^{-1} and a split ratio of 1:10. Injection temperature was 310 °C. Oven temperature was linearly increased from 120 °C to 250 °C at a rate of 10 °C min^{-1} and was held at 250 °C for 27 min. The following ion source parameters were applied: accelerating voltage: 4 kV; ion current: 400 mV; ionization voltage: 70 eV; ion source temperature: 250 °C.

Log *P* (octanol/water) values of iodobenzene, naphthalene, 1-methylnaphthalene and adamantane were estimated using EpiWeb 4.1. free on-line software (copyright: U.S. Environmental Protection Agency). Experimental log *P* values were taken from the database of the same software.

2.5. Confirmation of identity and purity of powder samples 1 and 2

ESI positive full scan spectra of both powders showed a single mass peak each at *m/z* 342 for **1** and *m/z* 356 for **2**, both corresponding to the respective $[M+H]^+$. Product ion spectrum of **1** was similar to that presented in a previous paper on the analysis of JWH-018 with LC–MS/MS [20] while in case of **2** the product ion spectrum obtained using identical parameters could be easily explained based on the fragmentation of **1**.

The powders were subjected to ^1H NMR and ^{13}C NMR spectroscopy which confirmed the chemical structures for both samples. No signs of any contaminating compound or impurity were detected. All peaks except for those of the solvent and water were related to the substances.

Further purity assessment of the powders was carried out by LC–UV–MS/MS. Neither the UV chromatograms at 210 nm nor the total ion chromatograms obtained with a mass range of *m/z* 110–1000 showed any significant peaks apart from the main peaks. The recorded UV spectrum of **1** was in agreement with the spectrum presented for JWH-018 in a previous publication [3]. The UV spectrum of **2** was similar indicating a chemical structure closely related to **1**.

3. Results and discussion

3.1. LC–UV–MS/MS

In the ESI positive full scan mass spectra a single peak at *m/z* 418 for **3** and at *m/z* 350 for **4** appeared corresponding to the $[M+H]^+$ ions, respectively. Similar spectra were obtained in APCI positive mode even though peak intensities were lower. By the use of negative polarity no ionization of the compounds was observed.

The four compounds appeared at different retention times on the chromatograms in the following elution order: **3**, **1**, **2** and **4**

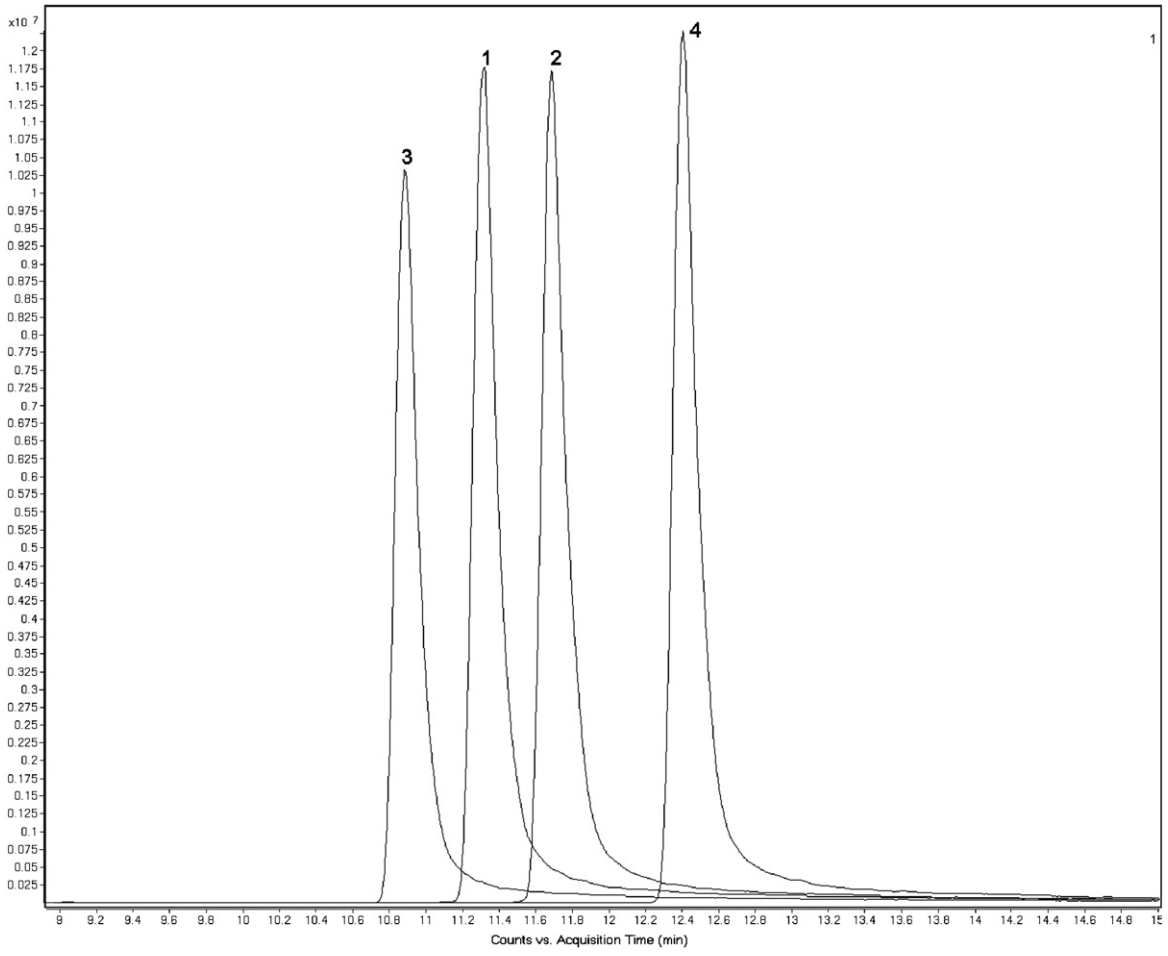

Fig. 2. Overlaid extracted ion chromatograms (EIC) of the four compounds. Retention times in the order of elution: 10.9 min; 11.3 min; 11.7 min and 12.7 min.

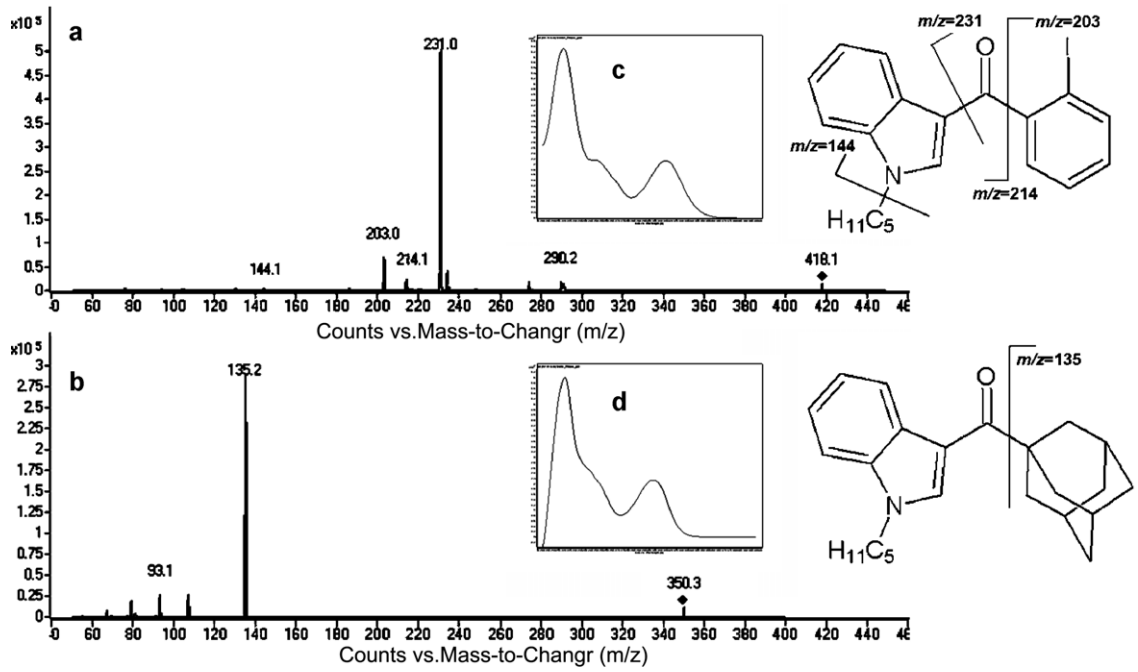

Fig. 3. Product ion spectra of (a) compound **3** and (b) compound **4**. UV spectra extracted from the maxima of the peaks in the UV chromatograms: (c) compound **3** and (d) compound **4**.

(Fig. 2). Hence, it was assumed that **3** was a more polar and **4** was a less polar molecule compared to JWH-018 and JWH-122. The elution order was verified by the calculated and experimental log *P* values of iodobenzene (calculated: 3.16; experimental: 3.25), naphthalene (cal: 3.17; exp: 3.30), 1-methylnaphthalene (cal: 3.72; exp: 3.87) and adamantane (cal: 3.94; exp: 4.24).

In the product ion spectrum of **3** (Fig. 3a) the most obvious common feature with those of **1** and **2** was the presence of the fragment at *m/z* 214 indicating that the molecule may contain a 1-pentylindole-3-carbonyl moiety. The fragment at *m/z* 203 – representing the remaining part of the molecule – equaled the mass of an iodophenyl ion while the most intensive one at *m/z* 231 was probably due to the iodobenzoyl ion. The fragment at *m/z* 290 was explained by the loss of the neutral particle HI.

In case of **4** the *m/z* 214 fragment was completely missing (Fig. 3b). The difference of 215 mass units between the precursor ion and the most abundant fragment, however, suggested the presence of a 1-pentylindole-3-carbonyl group. The fragment at *m/z* 135 was attributed to the adamantane ion. The series of less intensive fragments in the lower mass region with successive differences of 14 mass units also confirmed the presence of an aliphatic structure.

It was found that UV spectra of both compounds **3** and **4** (Fig. 3c and 3d) were similar to those of **1** and **2** with respect to the wavelength and ratio of UV absorption maxima and minima. The hypsochromic shift of the UV maximum of **4** from about 315 nm to about 305 nm may be explained by the lack of the aromatic chromophore at the carbonyl group.

3.2. GC–MS

On the GC–MS chromatograms compound **3** appeared with a retention time of about 26 min while the peak of **4** was observed at about 31 min. EI mass spectra and proposed fragmentation pathways of the molecules are presented in Figs. 4, 5 and 6. Fragmentation patterns of both molecules were in close agreement with their presumed chemical structures. For compound **4** the 1-pentylindole-3-carbonyl fragment at *m/z* 214 which was missing in the LC–MS product ion spectrum appeared as the base peak in the EI mass spectrum.

Furthermore, EI mass spectra of 3-indole aldehyde, adamantane (NIST/EPA/NIH Mass Spectral Library) and 3-iodobenzene (Wiley Registry™ of Mass Spectral Data) were sought out from spectral databases and compared with mass spectra of **3** and **4**. All the

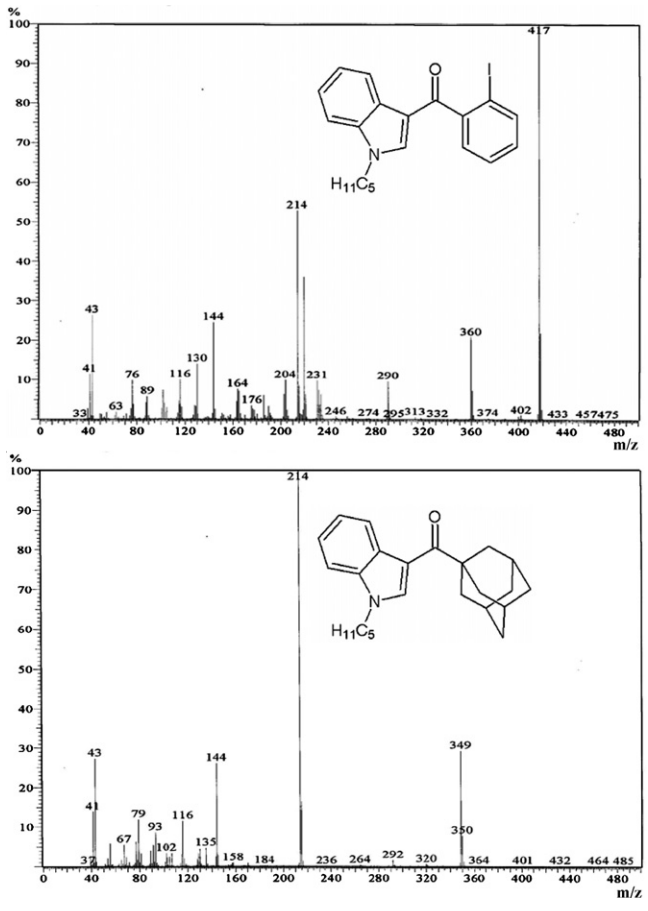

Fig. 4. GC–MS electron impact mass spectra of **3** and **4**.

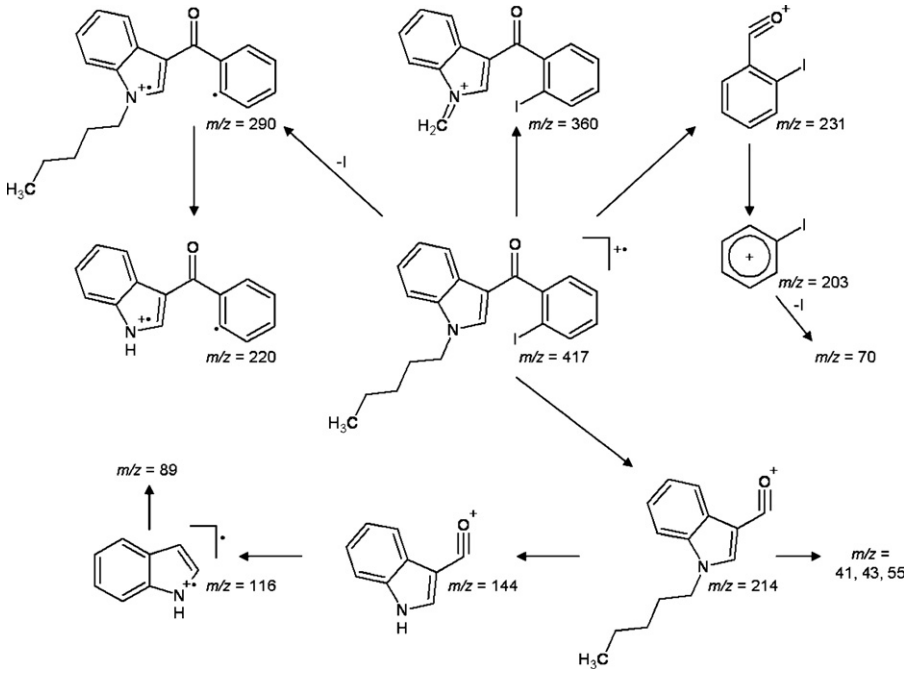

Fig. 5. Proposed GC–MS fragmentation of compound **3.**

relevant fragments of the corresponding molecules were detected in the mass spectra of the samples.

3.3. NMR

Assignment of NMR spectral data was completed on the basis of one- and two-dimensional homo- and heteronuclear measurements (HSQC, HMBC, COSY and NOESY). ^{1}H and ^{13}C NMR chemical shifts for the atoms of **3** and **4** are listed in Table 1. The presence of four distinct non-singlet peaks attributed to the iodophenyl group in the proton spectrum of **3** along with their respective NOESY and COSY cross-peaks unambiguously prove the 2-iodo substitution. The 1-adamantyl moiety in **4** was identified by its three characteristic broad singlets at 2.13 ppm (6 H, $3 \times CH_2$), 2.12 ppm (3H, $3 \times CH$) and 1.81 ppm (6H, $3 \times CH_2$).

3.4. LC–TOF–MS

In LC–MS–TOF analysis besides the protonated molecule ions sodium and potassium adducts appeared for both compounds and were thus involved in the accurate mass calculations. Results presented in Table 2 demonstrate that the generated

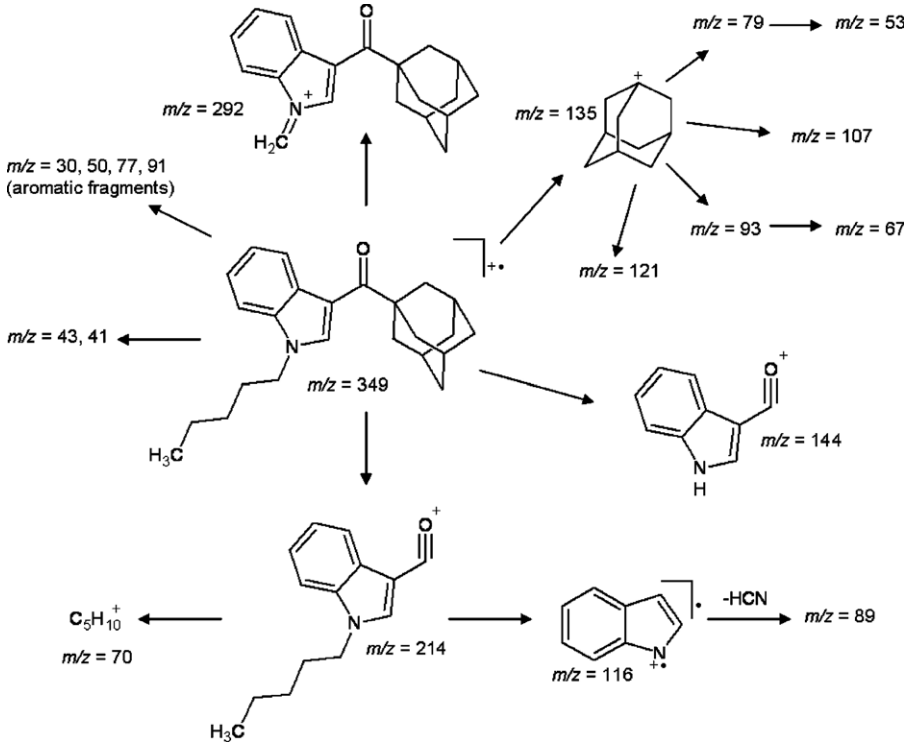

Fig. 6. Proposed GC–MS fragmentation of compound **4**.

Table 2
Results of the accurate mass measurement by LC–TOF–MS. Mass spectra were extracted from the peaks obtained after direct injection of methanolic solutions of the powders.

	$[M+H]^+$	$[M+Na]^+$	$[M+K]^+$
Compound **3**			
Measured mass	417.05953	417.05890	417.05901
Calculated mass	417.05896	417.05896	417.05896
Absolute difference	1.4 ppm	0.1 ppm	0.1 ppm
Isotope abundance score	98.8%	86.4%	89.8%
Generated formula	$C_{20}H_{20}INO$	$C_{20}H_{20}INO$	$C_{20}H_{20}INO$
Compound **4**			
Measured mass	349.24047	349.24033	349.24005
Calculated mass	349.24056	349.24056	349.24056
Absolute difference	0.3 ppm	0.7 ppm	1.5 ppm
Isotope abundance score	92.4%	98.1%	91.1%
Generated formula	$C_{24}H_{31}NO$	$C_{24}H_{31}NO$	$C_{24}H_{31}NO$

formulae supported the results of LC–MS/MS, GC–MS and NMR analyses. Differences between calculated and measured mass values were never greater than 1.5 ppm. Isotope abundance scores were above 85% for all ion species. The presented formulae were the only results generated within ±5 ppm accuracy, when restricting possible elements to C, H, N, O and I.

4. Conclusions

Two new potential cannabimimetic molecules were identified and characterized by several analytical techniques. The substances were seized in the form of pure powders by the customs office in Hungary. LC–MS/MS and UV spectra of JWH-018 and JWH-122 were used as a basis of structure elucidation.

Taking into account the circumstances of their seizure, it was suggested that both powder samples might have been intended to be used as designer drugs in their pure form or as ingredients of "smart products". Even though the cannabimimetic activity of 1-pentyl-3-(1-adamantoyl)indole was not reported adamantoylindoles are not unknown as a class within synthetic cannabinoids. It is therefore assumed that 1-pentyl-3-(1-adamantoyl)indole may represent a new potent member of this family.

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