Forensic Toxicol
DOI 10.1007/s11419-011-0108-3

ORIGINAL ARTICLE

Identification and quantitation of two benzoylindoles AM-694 and (4-methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone, and three cannabimimetic naphthoylindoles JWH-210, JWH-122, and JWH-019 as adulterants in illegal products obtained via the Internet

Jun'ichi Nakajima · Misako Takahashi · Takako Seto · Chieko Kanai · Jin Suzuki · Masao Yoshida · Tomoko Hamano

Received: 19 January 2011 / Accepted: 16 February 2011

Abstract During our careful surveillance of unregulated drugs, we found five new compounds used as adulterants in herbal and drug-like products obtained via the Internet. These compounds were identified by liquid chromatography–mass spectrometry, gas chromatography–mass spectrometry, accurate mass spectrometry, and nuclear magnetic resonance spectroscopy. The first compound identified was a benzoylindole AM-694, which is 1-[(5-fluoropentyl)-1*H*-indol-3-yl]-(2-iodophenyl)methanone (**1**). The second compound was (4-methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone (**2**), which was also classified as a benzoylindole. The three other compounds were identified as naphthoylindoles JWH-210 (4-ethylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone; **3**), JWH-122 (4-methylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone; **4**), and JWH-019 (1-hexyl-3-(naphthalen-1-oyl)-indole; **5**). All compounds except compound **2** had been reported to be cannabinoid receptor agonists. For quantitation of the five compounds and previously reported compounds, each product was extracted with methanol under ultrasonication to prepare a test solution for analysis by liquid chromatography with ultraviolet detection. Each compound detected in 43 commercial products showed large variation in content ranging from 4.0 to 359 mg per pack.

Keywords AM-694 · 4-(Methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone · JWH-210 · JWH-122 · JWH-019 · Cannabimimetic indole

J. Nakajima (✉) · M. Takahashi · T. Seto · C. Kanai · J. Suzuki · M. Yoshida · T. Hamano
Division of Drugs, Tokyo Metropolitan Institute of Public Health, 3-24-1 Hyakunin-cho, Shinjuku-ku, Tokyo 169-0073, Japan
e-mail: Junichi_Nakajima@member.metro.tokyo.jp

Introduction

Various types of herbal or drug-like products with psychotropic actions are commercially available via the Internet [1–3]. In 2009, Uchiyama et al. [1, 2] identified (1*RS*,3*SR*)-3-[4-(1,1-dimethyloctyl)-2-hydroxyphenyl]cyclohexan-1-ol and an aminoalkylindole JWH-018 in dubious commercial products. In Japan, these compounds became regulated substances (Shitei-Yakubutsu) under the Pharmaceutical Affairs Law in 2009. In addition, JWH-073 [4] and JWH-250 [5] became regulated by the same law in 2010. Although such measures have been taken, continuous surveillance and rapid development of new methods for analysis of new drugs are necessary. In our surveillance in September to November 2010, we found five new adulterants in herbal and drug-like products. Although these compounds had been synthesized for research purposes, no scientific reports for their identification, isolation, or quantitation in dubious products have appeared to our knowledge. This study deals with identification of these five compounds (Fig. 1) and their quantitation together with the compounds previously reported [4, 5] in 43 commercial products to observe the trend of the illegal drug market in Tokyo.

Materials and methods

Chemicals and reagents

Pravadoline was purchased from BIOMOL International (Polymouth Meeting, PA, USA); JWH-200, WIN-55212-2, JWH-073, and JWH-019 from Cayman Chemical (Ann Arbor, MI, USA); JWH-015, formic acid in acetonitrile

Published online: 29 March 2011

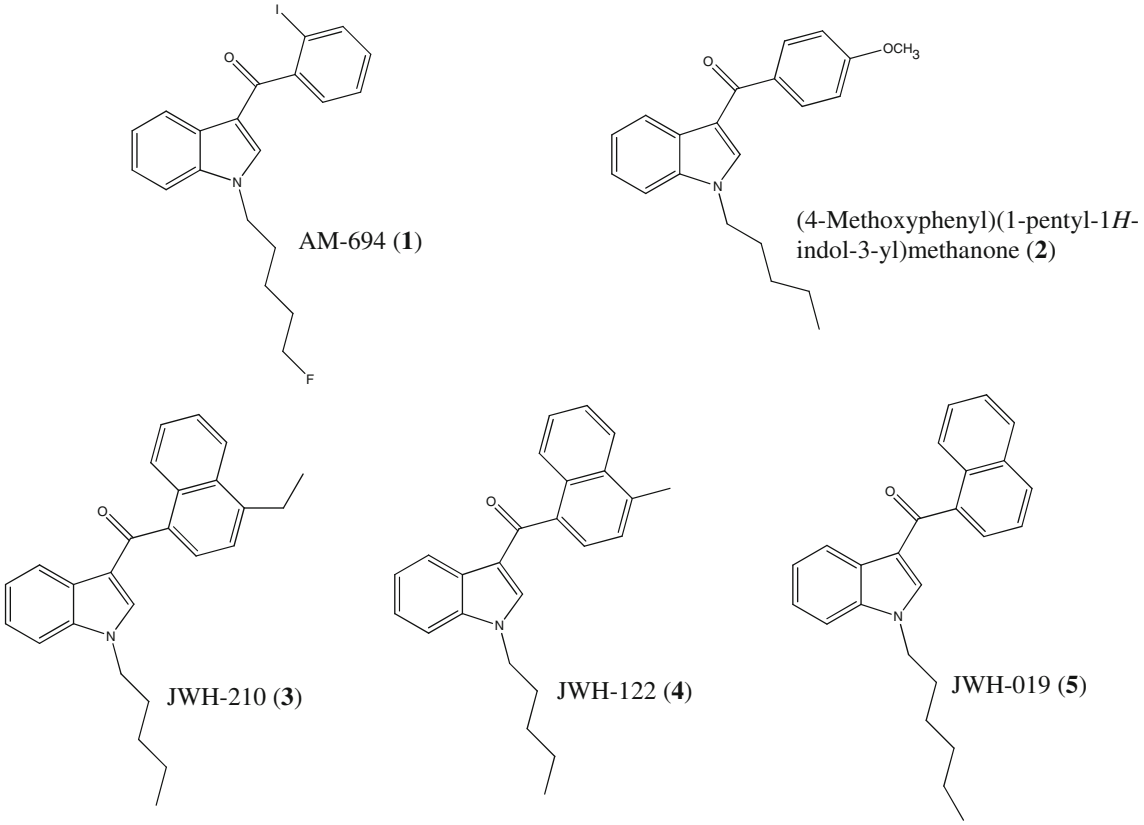

Fig. 1 Chemical structures of compounds **1–5**

[0.1%, v/v, liquid chromatography–mass spectrometry (LC–MS) grade], and $CDCl_3$ (99.8%) for nuclear magnetic resonance (NMR) analysis from Wako (Osaka, Japan); silica gel for chromatography (40–50 μm, spherical, neutral:X and 60 μm, spherical:Y) from Kanto Chemical (Tokyo, Japan) and Mitsubishi Chemical Medience (Tokyo, Japan), respectively. JWH-018, JWH-250, JWH-251, and JWH-081 were isolated from commercial products and identified by comparison with published data [2, 4, 5]. All other common chemicals and solvents used were of analytical grade or high-performance liquid chromatography (HPLC) grade.

Product samples and preparation of test solutions

Commercial product samples analyzed in this report were purchased via the Internet from September 2010 to December 2010.

To prepare a test solution from each herbal product, the content of each package was powdered and about a quarter of it was accurately weighed and extracted with 20 ml of methanol under sonication for 10 min. After centrifugation at 3,000 rpm for 10 min, the supernatant solution was transferred to a 50-ml volumetric flask. The precipitate was re-extracted using the same procedure as described above, and the supernatant fractions were combined and diluted with methanol to 50 ml. After gently shaking the volumetric flask, the solution was passed through a 0.20-μm filter (Millex LG; Millipore, Bedford, MA, USA) to obtain the test solution, which was mainly subjected to quantitation by liquid chromatography (LC)-ultraviolet (UV) detection. If necessary, the test solution was diluted with methanol to an adequate concentration before analysis.

Instrumental analyses

LC–MS in electrospray ionization (ESI) mode was performed on an ACQUITY LC instrument connected to a photodiode array (PDA) detector and to a quadrupole mass detector (Waters, Milford, MA, USA). LC conditions were: separation column, ACQUITY UPLC HSS T3 column (50 mm × 2.1 mm i.d., particle size 1.8 μm; Waters) at 40°C; LC gradient solutions, mobile phase A [5 mM ammonium formate buffer (pH 3.5) in water/acetonitrile (95:5, v/v)] and mobile phase B (0.1% formic acid in acetonitrile); flow rate, 0.6 ml/min; gradient program, 70% A/30% B held for 2 min, changed to 50% A/50% B over 2–6 min with a linear gradient, 6-min hold, changed to 20% A/80% B over 12–20 min with a linear gradient, and final change to 100% B over 20–26 min with a linear

gradient; injection volume, 1 µl. The scan range of the PDA detector was 190–400 nm. MS conditions were: ESI, positive mode; desolvation gas, nitrogen at 400°C; ion source temperature, 150°C; cone voltage, 40 V; mass spectral range, *m*/*z* 40–600.

The accurate mass spectrum of each target compound was measured in the positive ion mode by direct flow injection of the isolated extract on a time-of-flight (TOF) mass spectrometer (micro TOF LC, Bruker Daltonics, Bremen, Germany). The TOF-MS conditions were: nitrogen gas flow, 8.0 l/min; capillary voltage, 4,500 V; drying temperature, 200°C; internal standard, sodium formate.

Gas chromatography (GC)–MS was performed on an Agilent (Palo Alto, CA, USA) 6890 Network GC system with a 5973 mass-selective detector. The GC–MS conditions were: ionization, electron impact (EI) mode; electron energy, 70 eV; GC column, HP-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent); carrier gas, helium; column temperature program, 50°C followed by an increase at a rate of 10°C/min to 315°C (5-min hold); MS scan range, *m*/*z* 20–600.

The NMR spectra were recorded on an ECA-500 spectrometer (JEOL, Tokyo, Japan) for the isolated target compounds. Assignments were made using ^{1}H and ^{13}C NMR, heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), and total correlation spectroscopy (TOCSY) spectra.

Isolation of compounds **1** and **2**

About 3 g of a herbal product (No. 22) was extracted with 100 ml of methanol under ultrasonication for 30 min. The methanol solution was evaporated to dryness. The residue was dissolved in 1 ml of chloroform and loaded onto a silica gel (X) column (27 cm × 25 mm i.d.). Chromatographic separation was performed by gradient elution using hexane (A) and ethyl acetate (B): 100A:0B (v/v), 50 ml each for fractions 1–7; 100A:10B, 30 ml each for fractions 8–25; 100A:15B, 30 ml each for fractions 26–43; 100A:20B, 30 ml each for fractions 44–61; 100A:30B, 30 ml each for fractions 62–79. Compound **1** was obtained as a pale brown oil (15 mg) from fractions 70–72; compound **2** was obtained as a pale brown oil (18 mg) from fractions 29–31.

Isolation of compounds **3** and **4**

About 1.5 g of herbal product (No. 17 or 34) was extracted with 50 ml of methanol under ultrasonication for 30 min. The methanol solution was evaporated to dryness.

The residue of No. 17 was dissolved in 1 ml of chloroform and loaded onto a silica gel column (26 cm × 25 mm i.d.) packed with silica gel (X). Chromatographic separation was performed by gradient elution using the same solvents (A:hexane; B:ethyl acetate): 100A:0B (v/v), 100 ml each for

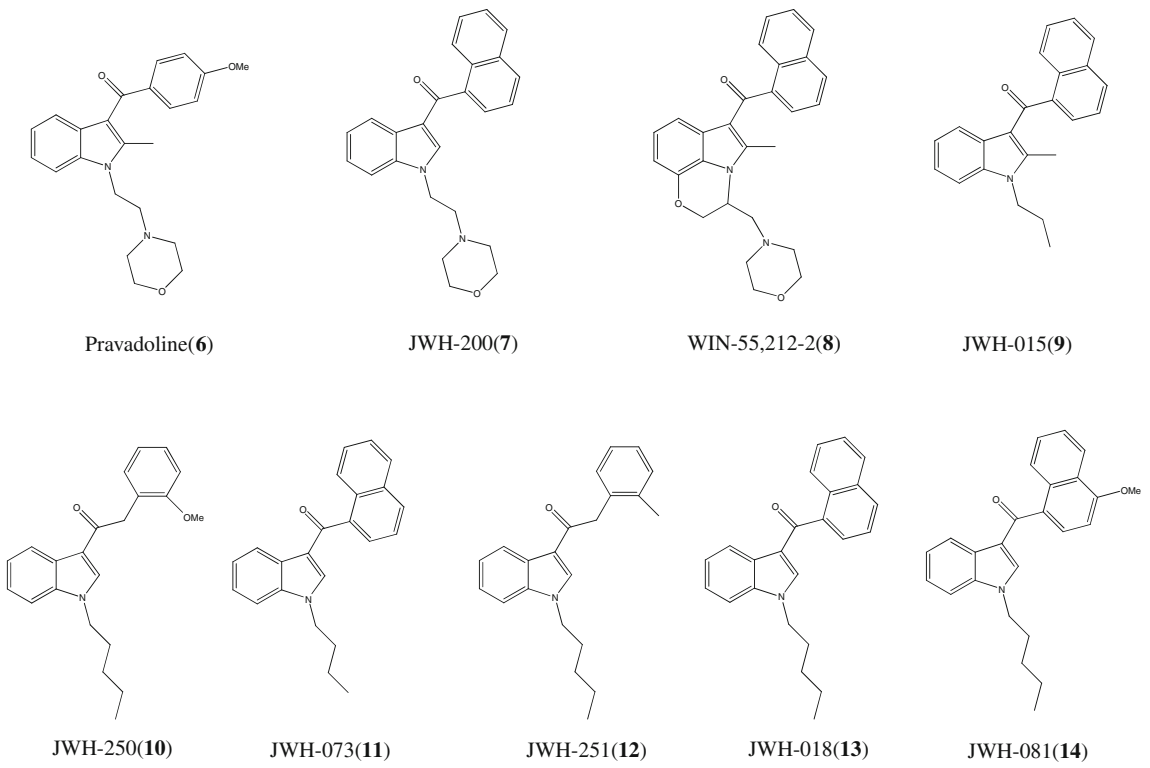

Fig. 2 Chemical structures of known cannabimimetic indoles (**6–14**)

fractions 1 and 2; 100A:25B, 100 ml each for fractions 3–17. Compound **3** was obtained as a colorless oil (42 mg) from fractions 5 and 6.

The residue of No. 34 was dissolved in 1 ml of 0.1 M aqueous ammonium solution and dehydrated with sodium sulfate. A 100-ml volume of chloroform was added to the extract, mixed and evaporated to dryness. The resulting residue was reconstructed in 1 ml of chloroform and loaded onto a silica gel (Y) column (30 cm × 20 mm i.d.). Chromatographic separation was performed by gradient elution using the same solvents as above: 100A:0B (v/v), 100 ml for fraction 1; 100A:5B, 100 ml for fraction 2; 100A:10B, 30 ml each for fractions 3 and 4; 100A:20B, 50 ml each for fractions 5–8. Compound **4** was obtained as a colorless oil (60 mg) from fraction 6.

Standard solutions

For qualitative analysis, standard solutions were prepared in methanol at 50 μg/ml for compounds **1**–**4** isolated as above, and JWH-019 (**5**), pravadoline (**6**), JWH-200 (**7**), WIN-55212-2 (**8**), JWH-015 (**9**), JWH-250 (**10**), JWH-073 (**11**), JWH-251 (**12**), JWH-018 (**13**), and JWH-081 (**14**). The structures of compounds **6**–**14** are shown in Fig. 2.

Calibration curves

Calibration curves using an external calibration method were constructed by LC-PDA detection with peak heights at 315 nm for compounds **1**, **3**, and **4**, 320 nm for compounds **2**, **5**, **7**, **9**, **11**, and **14**, and at 305 nm for compounds **10** and **12**. All compounds were diluted with methanol to prepare calibration solutions at concentrations of 10, 50, 100, 250, and 500 μg/ml.

Selection of extraction solvent

Fine powders were prepared from each commercial product. About 100 mg of powder was accurately weighed and extracted using ten different solvents (20 ml) with sonication for 10 min. After centrifugation at 3,000 rpm for 10 min, each supernatant fraction was transferred to a 50-ml volumetric flask. The precipitate was re-extracted

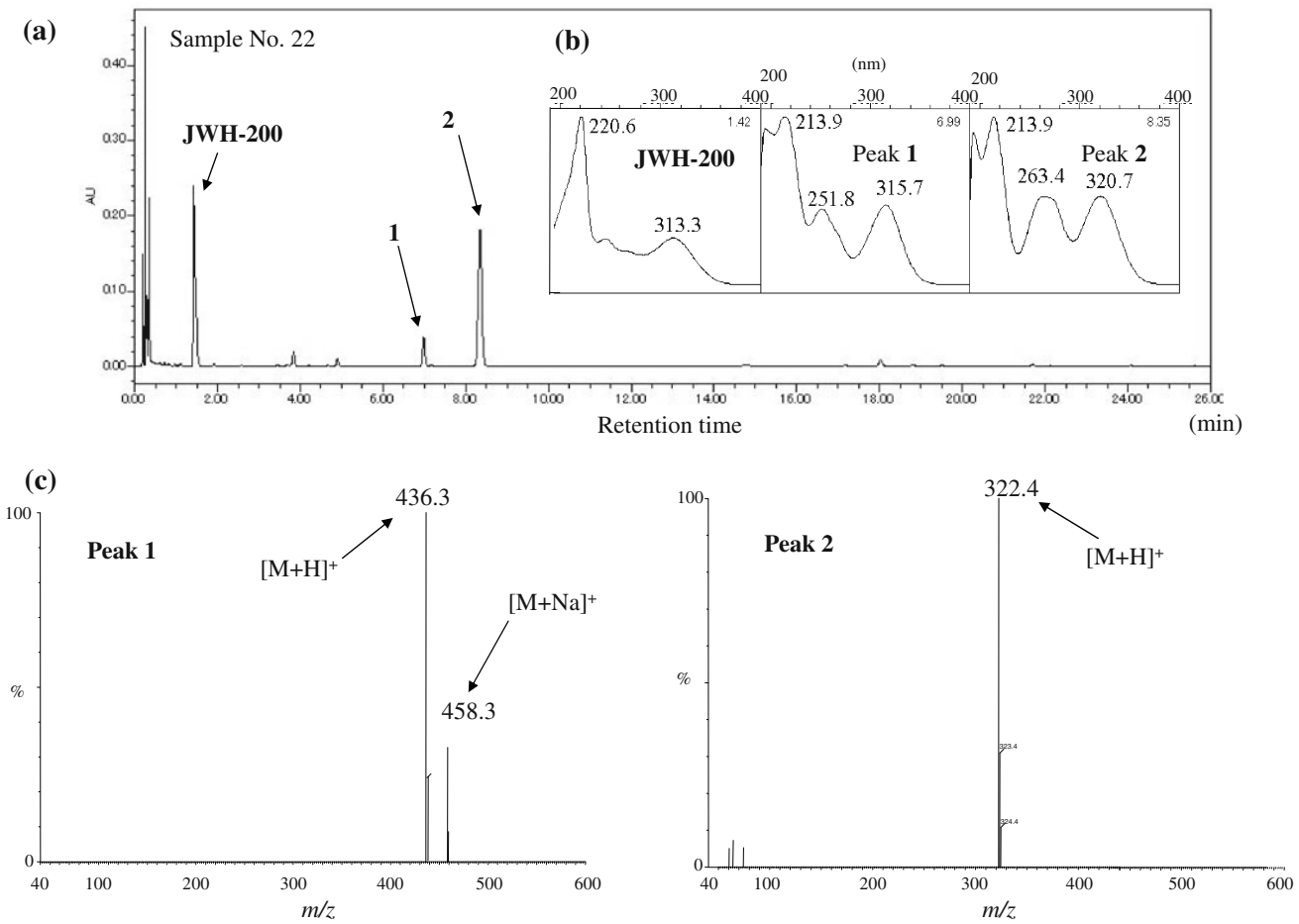

Fig. 3 Liquid chromatography (LC)-ultraviolet (UV) detection chromatogram at 275 nm (**a**), photodiode array (PDA) UV spectra for the three peaks (**b**), and electrospray ionization (ESI) mass spectra for the two peaks appearing at 6.99 min (**1**) and 8.35 min (**2**) (**c**) for sample No. 22

using the same procedure as described above, and the supernatant fractions were combined and diluted with methanol to a final volume of 50 ml. After shaking the volumetric flask gently, the solution was passed through a 0.20-μm filter (Millex LG; Millipore) to obtain test solution, which was analyzed by LC-PDA detection.

Results and discussion

Identification of unknown peaks **1** and **2**

For the test solution of herbal product No. 22, three major peaks appeared at 1.42, 6.99, and 8.35 min by LC-UV detection (Fig. 3a). The first peak was easily identified as JWH-200 by LC–MS and GC–MS data according to Uchiyama et al. [4]. The second (**1**) and third (**2**) peaks seemed to be new compounds. The PDA-sliced UV spectrum of unknown peak **1** showed maxima at 213.9, 251.8, and 315.7 nm (Fig. 3b); the LC–MS spectrum showed the base peak at *m/z* 436 $[M+H]^+$ in the positive scan mode (Fig. 3c). The PDA-sliced UV spectrum of peak **2** showed maxima at 213.9, 263.4, and 320.7 nm (Fig. 3b); the mass spectrum showed the base peak at *m/z* 322 $[M+H]^+$ in the positive scan mode (Fig. 3c).

The total ion chromatogram (TIC) by GC–MS for the test solution of product No. 22 showed peaks **2** and **1** at 26.75 and 27.49 min, respectively (Fig. 4a). Peak **1** gave a mass spectrum with ion peaks at *m/z* (relative intensity) 435(65), 232(100), and 220(50) (Fig. 4b). Peak **2** gave a mass spectrum with ion peaks at *m/z* 321(100), 264(70), and 135(60) (Fig. 4b).

The accurate MS of isolated compound **1** revealed $[M+H]^+$ at *m/z* 436.0575 in the positive scan mode, suggesting the molecular formula of $C_{20}H_{19}FINO$. The error between the observed and theoretical mass number of $[M+H]^+$ was 0.7 mDa. The accurate MS of isolated compound **2** revealed $[M+H]^+$ at *m/z* 322.1797, suggesting the molecular formula of $C_{21}H_{23}NO_2$. The error between the observed and theoretical mass number of $[M+H]^+$ was 0.5 mDa.

The ^1H NMR spectrum of isolated compound **1** showed 19 nonexchangeable protons, including signals for 9 aromatic protons at 7.29 (1H, s), 7.34 (2H, overlapped), 7.35, 7.39 7.45 (each 1H, m), 7.14 (1H, td, $J = 7.4$, 2.3 Hz), 7.91 (1H, brd, $J = 8.0$ Hz), and 8.34 (1H, m) as shown in Table 1. Furthermore, there were 3 methylene proton

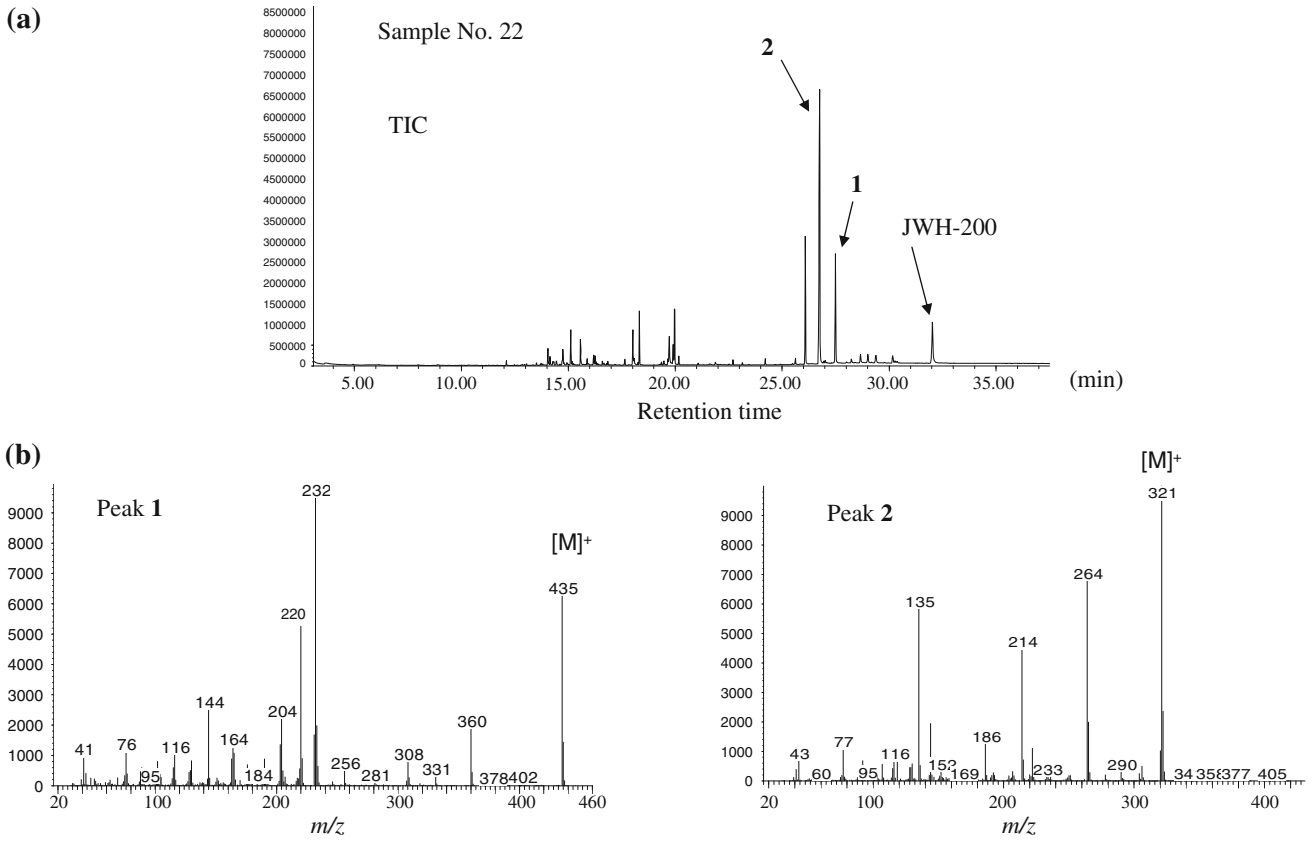

Fig. 4 Total ion chromatogram (TIC) by gas chromatography–mass spectrometry (GC–MS) (**a**), and EI mass spectra of the peaks **2** and **1** detected at 26.75 and 27.49 min (**b**) for sample No. 22

Table 1 Nuclear magnetic resonance (NMR) data for compounds **1** and **2** in $CDCl_3$ (ppm)

No.	Compound **1**			Compound **2**		
	^{13}C	^{1}H	HMBC	^{13}C	^{1}H	HMBC
1	191.2	–	–	189.8	–	–
2′	137.9	7.29, 1H, s	1, 3′, 3′a, 7′a, 1‴	136.1	7.58, 1H, s	1, 3′, 3′a, 7′a, 1‴
3′	115.4	–	–	115.6	–	–
3′a	126.7	–	–	127.5	–	–
4′	122.9	8.34, 1H, m	6′, 7′a	122.7	8.37, 1H, m	6′, 7′a
5′	122.8	7.34, 1H, m, overlapped	7′	122.3	7.32, 1H, m, overlapped	7′
6′	123.7	7.34, 1H, m, overlapped	7′a	123.3	7.32, 1H, m, overlapped	7′a
7′	109.9	7.39, 1H, m, overlapped	3′a, 5′, 7′a	109.8	7.39, 1H, m, overlapped	3′a, 5′, 7′a
7′a	137.0	–	–	136.8	–	–
1″	146.3	–	–	133.5	–	–
2″	92.5	–	–	131.0[b]	7.84, 1H, brd, $J = 8.6$ Hz, overlapped	1, 4″, 6″
3″	139.6	7.91, 1H, brd, $J = 8.0$ Hz	1″, 5″	113.5[c]	6.99, 1H, brd, $J = 8.6$ Hz, overlapped	1″, 5″
4″	130.5	7.14, 1H, td, $J = 7.6$, 2.3 Hz	2′, 6″	162.2	–	–
5″	127.7	7.45, 1H, m, overlapped	1″, 3″	113.5[c]	6.99, 1H, brd, $J = 8.6$ Hz, overlapped	1″, 3″
6″	128.0	7.35, 1H, m, overlapped	1, 4″	131.0[b]	7.84, 1H, brd, $J = 8.6$ Hz, overlapped	1, 2″, 4″
1‴	47.0	4.12, 2H, t, $J = 7.5$ Hz	2′, 7′a, 2‴, 3‴	47.1	4.15, 2H, t, $J = 7.5$ Hz	2′, 7′a, 2‴, 3‴
2‴	29.4	1.89, 2H, quint, $J = 7.4$ Hz	1‴, 3‴, 4‴	29.6	1.88, 2H, quint, $J = 7.5$ Hz	1‴, 3‴, 4‴
3‴	22.7 (d, $J = 4$ Hz[a])	1.44, 2H, m, overlapped	1‴, 2‴, 4‴, 5‴	29.0	1.34, 2H, m, overlapped	1‴, 2‴, 4‴, 5‴
4‴	29.8 (d, $J = 20$ Hz[a])	1.68, 2H, m, overlapped	2‴, 3‴, 5‴	22.2	1.26, 2H, m, overlapped	2‴, 3‴, 5‴
5‴	83.5 (d, $J = 165$ Hz[a])	4.45, 1H, t, $J = 5.7$ Hz 4.35, 1H, t, $J = 5.7$ Hz	3‴, 4‴	13.9	0.87, 3H, t, $J = 6.9$ Hz	3‴, 4‴
1⁗	–	–	–	55.4	3.89, 3H, s	4″

[a] C–F coupling

[b, c] Overlapped

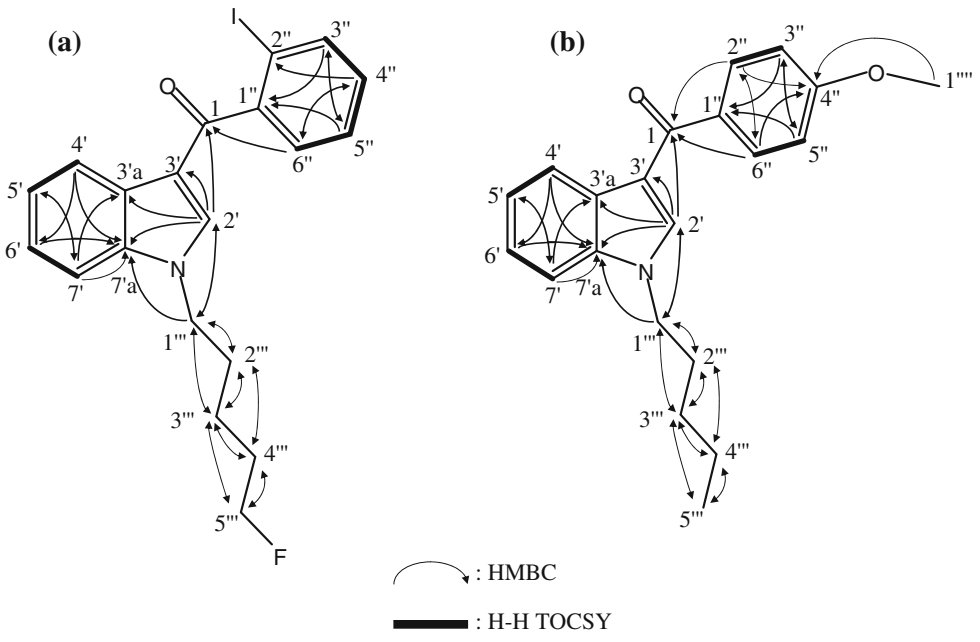

Fig. 5 Heteronuclear multiple-bond correlation (HMBC) and total correlation spectroscopy (TOCSY) for isolated compound **1** to be identified as AM-694 (**a**) and for isolated compound **2** to be identified as (4-methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone (**b**)

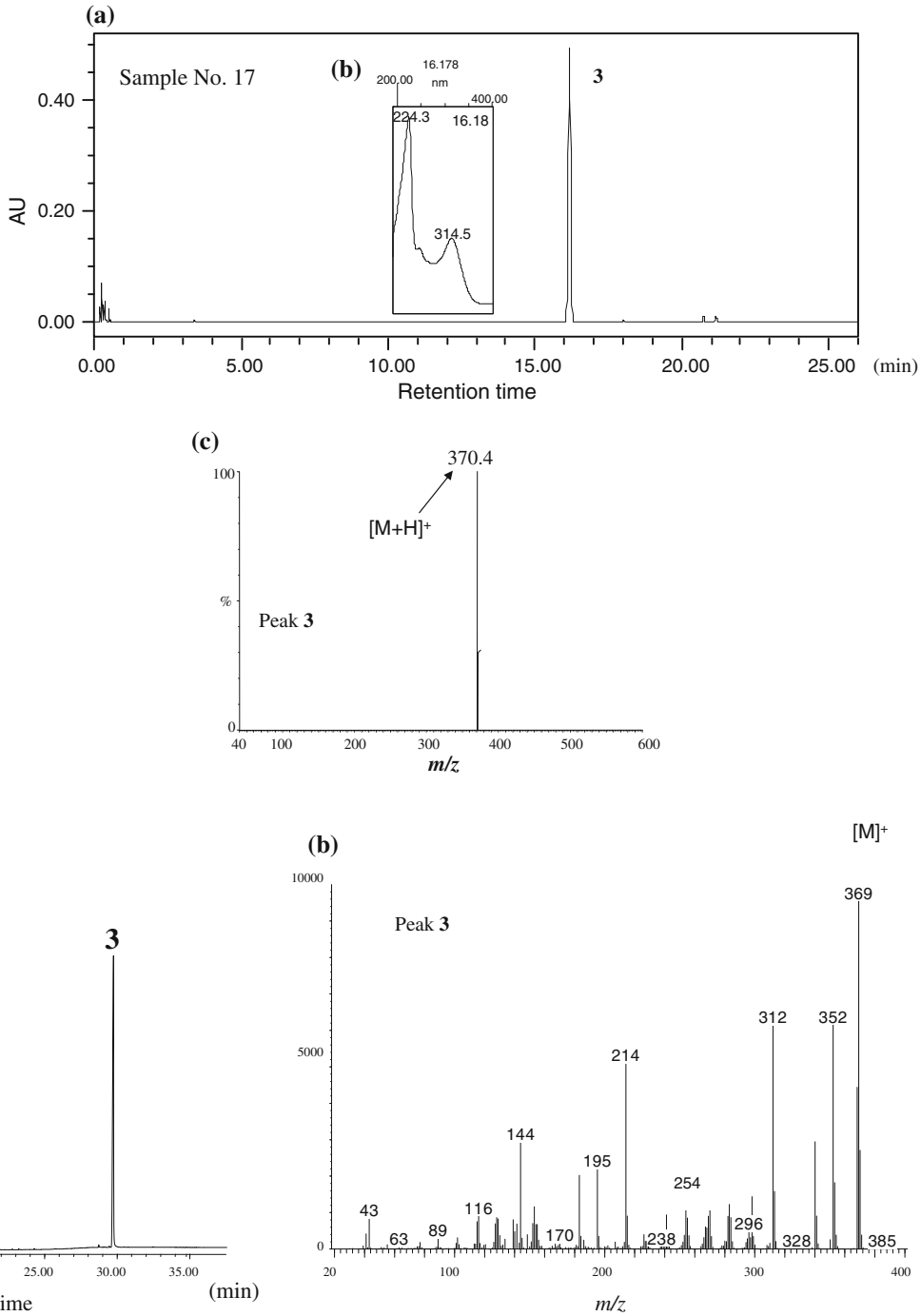

Fig. 6 LC-UV detection (275 nm) chromatogram (**a**), UV spectrum (**b**), and ESI–MS spectrum (**c**) for peak **3** appearing at 16.18 min for sample No. 17

Fig. 7 TIC by GC–MS analysis (**a**) and the EI mass spectrum of peak **3** detected at 29.7 min (**b**) for sample No. 17

signals at δ 1.44, 1.68 (each 2H, m), and 1.89 (2H, quint, $J = 7.4$ Hz), a methylene signal connected to a nitrogen atom at δ 4.12 (2H, t, $J = 7.4$ Hz), and methylene signals coupled with a fluorine atom at 4.35 and 4.45 (2H, t, $J = 5.7$ Hz), but no methyl signals were observed. According to the ^{13}C NMR spectrum, 20 carbon signals were detected, suggesting the existence of 1 methylene with its carbon (δ 83.5, d, $J = 165$ Hz) connected to a fluorine atom, 2 methylene carbons (δ 29.8, d, $J = 20$ Hz and δ 22.7, d, $J = 4$ Hz) coupled with the same fluorine, 1 methylene carbon connected to a nitrogen (δ 47.0), 5 aromatic quaternary carbons (δ 92.5, 115.4, 126.7, 137.0, and 146.3), 9 aromatic carbons (δ 109.9, 122.8, 122.9, 123.7, 127.7, 128.0, 130.5, 137.9, and 139.6), and a carbonyl carbon (δ 191.2). The existence of an indole group, iodo-benzyl group, and fluoro-*n*-pentyl group was

Table 2 NMR data for compounds **3** and **4** in $CDCl_3$ (ppm)

No.	Compound **3**			Compound **4**		
	^{13}C	^{1}H	HMBC	^{13}C	^{1}H	HMBC
1	192.3	–	–	192.3	–	–
2′	137.5	7.37, 1H, s, overlapped	1, 3′, 3′a, 7′a, 1‴	137.9	7.37, 1H, brs, overlapped	1, 3′, 3′a, 7′a, 1‴
3′	117.7	–	–	117.8	–	–
3′a	127.1	–	–	127.2	–	–
4′	123.0	8.49, 1H, m	3′, 3′a, 5′, 6′, 7′a	122.9	8.49, 1H, m	3′, 3′a, 5′, 6′, 7′a
5′	122.7	7.35, 1H, m, overlapped	7′	123.1	7.36, 1H, m, overlapped	7′
6′	123.8	7.35, 1H, m, overlapped	7′a	123.6	7.36, 1H, m, overlapped	7′a
7′	109.9	7.39, 1H, m, overlapped	5′, 7′a	110.0	7.39, 1H, m, overlapped	5′, 7′a
7′a	137.0	–	–	137.1	–	–
1″	137.8	–	–	137.7	–	–
2″	125.9	7.59, 1H, brd, $J = 6.9$ Hz	1, 4″, 8″a	125.9	7.57, 1H, brd, $J = 6.9$ Hz	1, 4″, 8″a
3″	123.5	7.38, 1H, m, overlapped	1″, 4″a, 1⁗	125.4	7.38, 1H, m, overlapped	1″, 4″a, 1⁗
4″	142.5	–	–	136.7	–	–
4″a	132.0	–	–	132.9	–	–
5″	125.8	8.13, 1H, d, $J = 8.0$ Hz	4″, 7″, 8″a	124.3	8.08, 1H, brd, $J = 8.6$ Hz	4″, 7″, 8″a
6″	126.2	7.54, 1H, td, $J = 7.8$, 1.7 Hz	4″a, 8″	126.2	7.56, 1H, td, $J = 6.9$, 1.1 Hz	4″a, 8″
7″	126.8	7.46, 1H, td, $J = 7.7$, 1.8 Hz	5″, 8″a	126.5	7.48, 1H, ddd, $J = 7.7$, 6.9, 1.1 Hz	5″, 8″a
8″	126.0	8.25, 1H, d, $J = 8.0$ Hz	1″, 4″a, 6″	126.7	8.25, 1H, brd, $J = 8.0$ Hz	1″, 4″a, 6″
8″a	131.2	–	–	131.0	–	–
1‴	47.1	4.07, 2H, t, $J = 7.5$ Hz	2′, 7′a, 2‴, 3‴	47.2	4.07, 2H, t, $J = 7.4$ Hz	2′, 7′a, 2‴, 3‴
2‴	29.5	1.80, 2H, quint, $J = 7.5$ Hz	1‴, 3‴, 4‴	29.6	1.82, 2H, quint, $J = 7.4$ Hz	1‴, 3‴, 4‴
3‴	28.9	1.26, 2H, m, overlapped	1‴, 2‴, 4‴, 5‴	29.1	1.32, 2H, m, overlapped	1‴, 2‴, 4‴, 5‴
4‴	22.2	1.28, 2H, m, overlapped	2‴, 3‴, 5‴	22.3	1.27, 2H, m, overlapped	2‴, 3‴, 5‴
5‴	13.8	0.85, 3H, t, $J = 7.5$ Hz	3‴, 4‴	14.0	0.86, 3H, t, $J = 7.4$ Hz	3‴, 4‴
1⁗	26.2	3.18, 2H, quartet, $J = 7.5$ Hz	2⁗, 3″, 4″	19.9	2.79, 3H, s	3″, 4″
2⁗	14.9	1.44, 3H, t, $J = 7.4$ Hz	1⁗, 4″	–	–	–

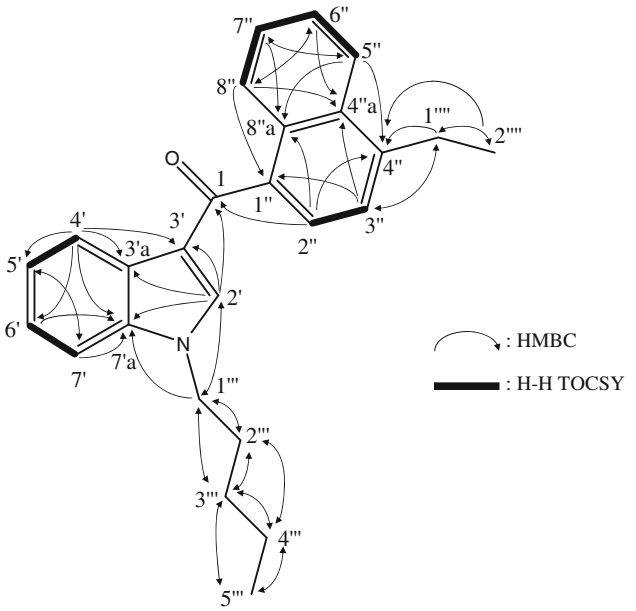

Fig. 8 HMBC and TOCSY for isolated compound **3** to be identified as JWH-210

suggested from the TOCSY, HMQC, and HMBC spectra (Table 1). The relationships of these groups were assigned from the HMBC spectra: the aromatic proton, which is a characteristic signal of the indole group at δ 7.29 (H-2′), correlated to the carbonyl carbon at δ 191.2 (C-1); the methine protons at δ 7.35 (C-6″) also correlated to the carbonyl carbon as shown in Fig. 5a. On the basis of these instrumental data, the chemical structure of compound **1** was elucidated as 1-[(5-fluoropentyl)-1*H*-indol-3-yl]-(2-iodophenyl)methanone. This compound had been reported as a cannabimimetic indole derivative acting as an agonist for the cannabinoid receptor CB_1 with a Ki value of 0.08 nM, and named AM-694 [6, 7].

The ^{1}H NMR spectrum of isolated compound **2** showed 23 nonexchangeable protons, including 2 methyl signals at δ 0.87 (3H, t, $J = 6.9$ Hz) and 3.89 (3H, s), signals for 9 aromatic protons at δ 6.99 (2H, brd, $J = 8.6$ Hz), 7.32 (2H, m, overlapped), 7.39 (1H, m, overlapped), 7.58 (1H, s), 7.84 (2H, brd, $J = 6.9$ Hz), and 8.37 (1H, m), 3 methylene proton signals at δ 1.26, 1.34 (each 2H, m), and 1.88 (2H,

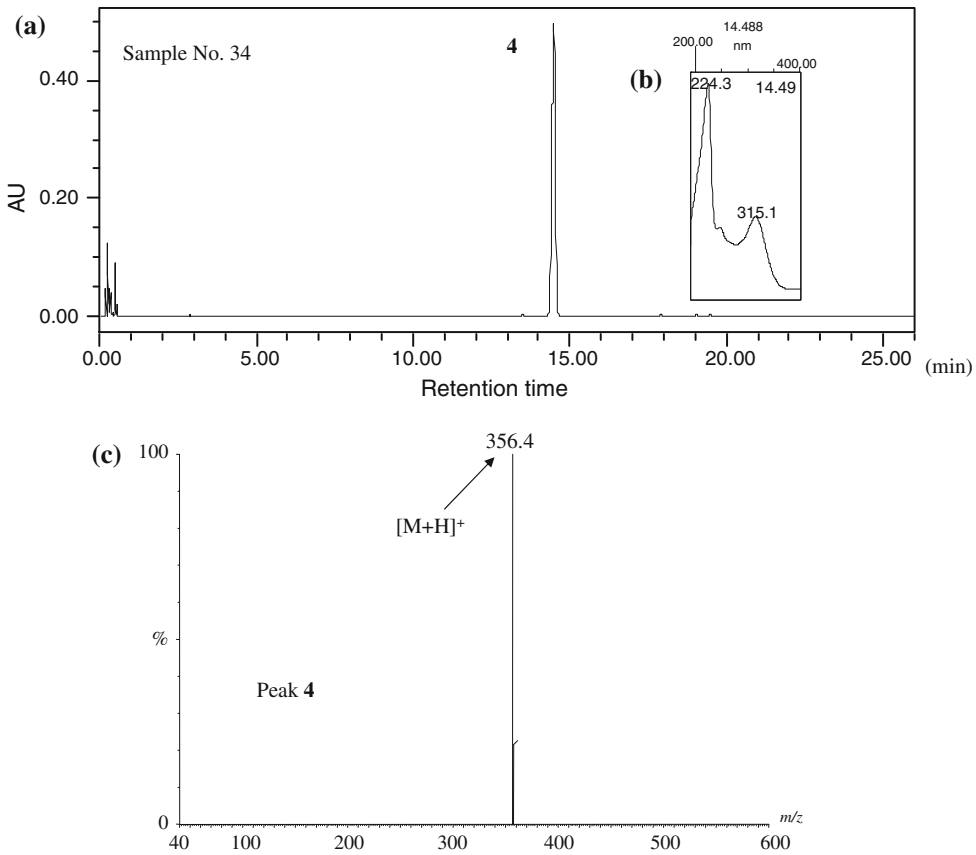

Fig. 9 LC-UV detection (275 nm) chromatogram (**a**), UV spectrum (**b**), and ESI–MS spectrum for peak **4** appearing at 14.49 min (**c**) for sample No. 34

quint, $J = 7.5$ Hz), and a signal for a methylene group connected to a nitrogen atom at δ 4.15 (2H, t, $J = 7.5$ Hz), as shown in Table 1. According to the ^{13}C NMR spectrum, 21 carbon signals were detected, suggesting the existence of 2 methyls (δ 13.9 and 55.4), 3 methylene carbons (δ 22.2, 29.0, and 29.6), 1 methylene carbon connected to a nitrogen (δ 47.1), 5 aromatic quaternary carbons (δ 115.6, 127.5, 133.5, 136.8, and 162.2), 9 aromatic carbons (δ 109.8, 113.5 overlapped, 122.3, 122.7, 123.3, 131.0 overlapped, and 136.1), and a carbonyl carbon (δ 189.8). The existence of an indole group, benzyl group, and *n*-pentyl group was also suggested from the TOCSY, HMQC, and HMBC spectra like compound **1**. The relationships of these groups were assigned from the HMBC spectra: the

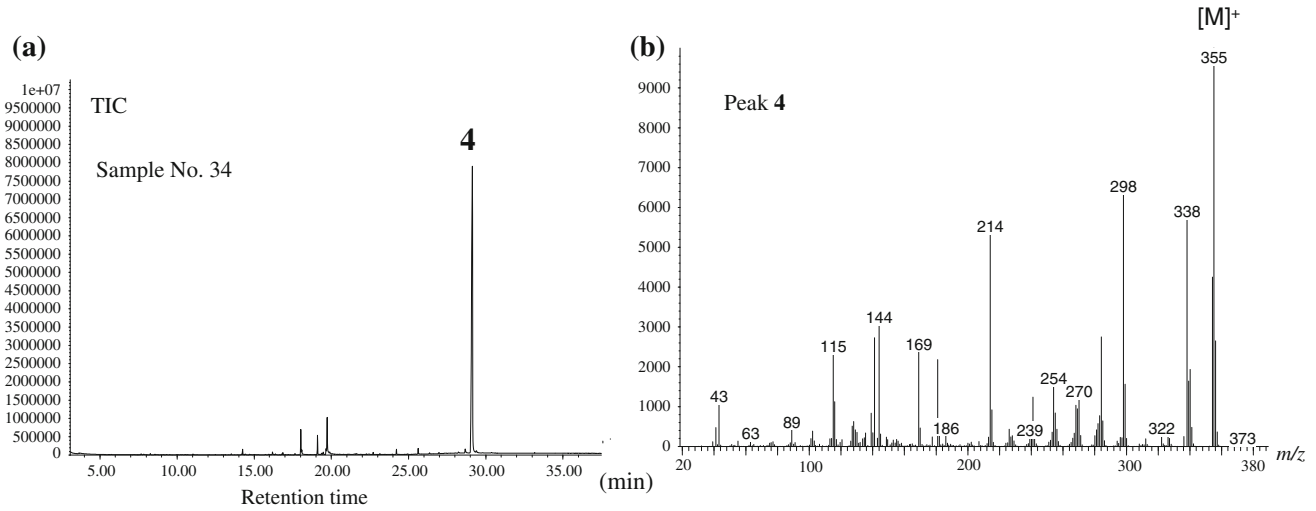

Fig. 10 TIC by GC–MS analysis (**a**) and the EI mass spectrum of peak **4** detected at 29.1 min (**b**) for sample No. 34

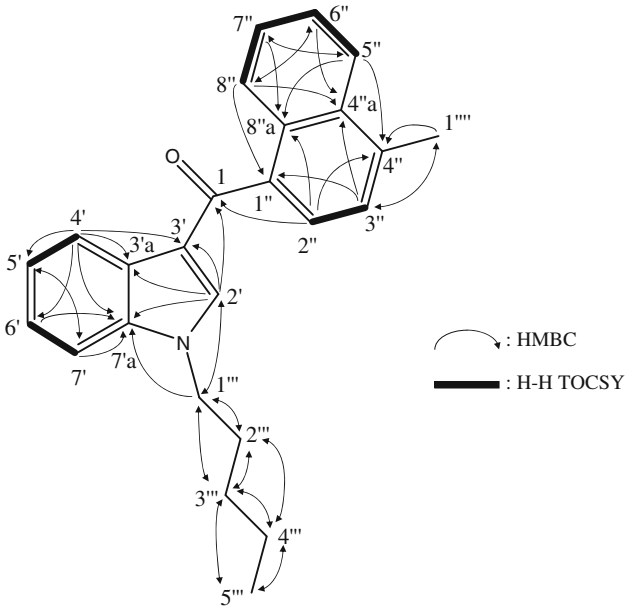

Fig. 11 HMBC and TOCSY for isolated compound **4** to be identified as JWH-122

aromatic proton, which is a characteristic signal of the indole group at δ 7.58 (H-2′), correlated to the carbonyl carbon at δ 189.8 (C-1); the methine protons at δ 7.84 (C-2″, 6″) also correlated to the carbonyl carbon as shown in Fig. 5b. On the basis of these instrumental data, the chemical structure of compound **2** was elucidated as (4-methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone. At present, the biological activity of compound **2** has not been reported to our knowledge, but we estimate that this compound may have a cannabimimetic activity judging from its chemical structure.

Identification of unknown peaks **3** and **4**

For the test solution of herbal product No. 17, an intense peak of an unknown compound was detected at 16.18 (**3**) min by LC-UV detection (Fig. 6a). The PDA-sliced UV spectrum of unknown peak **3** showed maxima at 224.3 and 314.5 nm (Fig. 6b); the mass spectrum showed the base peak at *m*/*z* 370 $[M+H]^+$ in the positive scan mode (Fig. 6c).

The TIC by GC–MS also showed an unknown intense peak of compound **3** appearing at 29.67 min (Fig. 7a), and the EI mass spectrum of compound **3** showed peaks at *m*/*z* (relative intensity) 369(100), 214(50), and 144(30) (Fig. 7b).

The accurate MS of isolated compound **3** revealed $[M+H]^+$ at *m*/*z* 370.2163 in the positive scan mode, suggesting the molecular formula of $C_{26}H_{27}NO$. The error

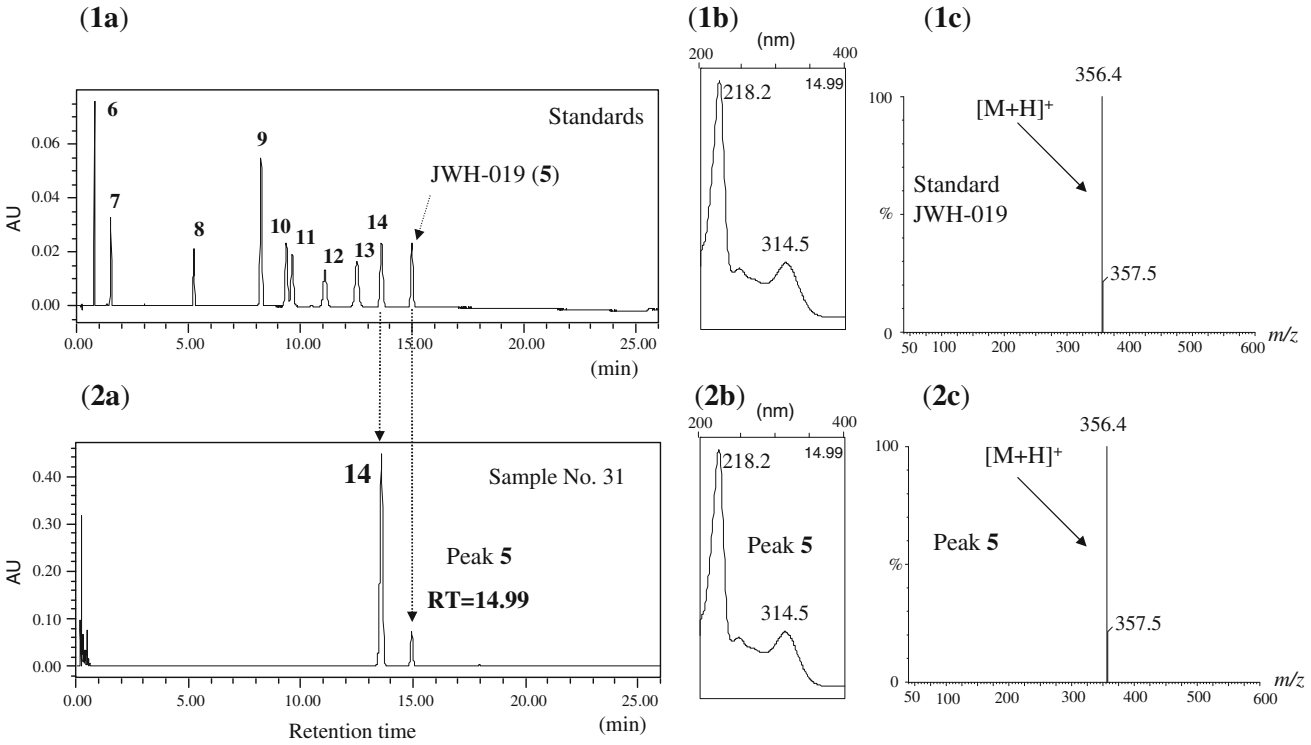

Fig. 12 LC-UV detection (275 nm) chromatograms of the standard cannabimimetic indoles **5**–**14** (**1a**) and of sample No. 31 (**2a**), UV spectra (**1b**, **2b**) and ESI–MS spectra (**1c**, **2c**) of standard JWH-019 and peak **5** obtained from sample No. 31. **6**, Pravadoline; **7**, JWH-200; **8**, WIN-55212-2; **9**, JWH-015; **10**, JWH-250; **11**, JWH-073; **12**, JWH-251; **13**, JWH-018; **14**, JWH-081

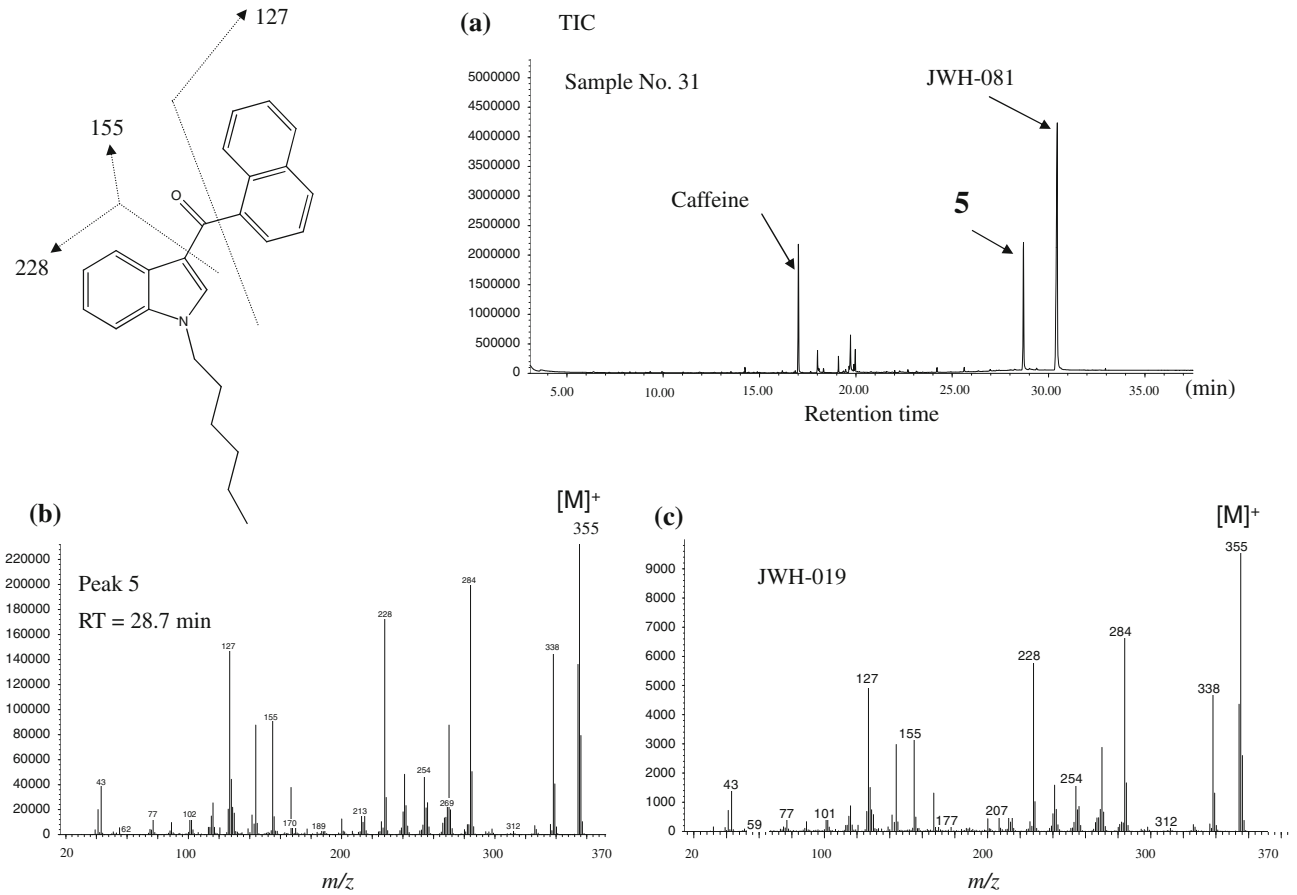

Fig. 13 GC–MS analysis for sample No. 31. TIC (**a**) and EI mass spectra of peak **5** detected at 28.7 min (**b**) and of standard JWH-019 (**c**)

Table 3 Relative efficiencies of extraction of detected compounds (**1–5**, **7**, **9–12**, **14**) as a function solvent type

Solvent	AM-694 (**1**)	(4-Methoxyphenyl) (1-pentyl-1*H*-indol-3-yl) methanone (**2**)	JWH-210 (**3**)	JWH-122 (**4**)	JWH-019 (**5**)	JWH-200 (**7**)	JWH-015 (**9**)	JWH-250 (**10**)	JWH-073 (**11**)	JWH-251 (**12**)	JWH-081 (**14**)
MeOH	100	100	100	100	100	100	100	100	100	100	100
90% MeCN	89	91	92	88	89	100	100	99	97	99	98
MeCN	87	93	93	93	92	90	100	100	98	100	100
EtOH	84	89	91	91	93	96	99	98	100	98	99
50% MeCN	85	94	88	89	90	93	91	90	88	89	87
50% EtOH	83	86	64	68	76	91	90	57	80	80	34
50% MeOH	72	67	7	13	29	72	60	9	34	33	3
0.1 M HCl	0	0	0	0	0	29	0	0	0	0	0
H_2O	0	0	0	0	0	0	0	0	0	0	0
0.1 M NH_3	0	0	0	0	0	0	0	0	0	0	0

The maximum value was indicated as 100%

MeOH methanol, *MeCN* acetonitrile, *EtOH* ethanol

between the observed and theoretical mass number of $[M+H]^+$ was 0.2 mDa.

The ^1H NMR spectrum of isolated compound **3** showed 27 nonexchangeable protons, including signals for 11 aromatic protons at δ 7.35 (2H, overlapped), 7.37 (1H, s), 7.38, 7.39 (each 1H, m), 7.46 (1H, td, $J = 7.4$, 1.8 Hz), 7.54 (1H, td, $J = 7.8$, 1.7 Hz), 7.59 (1H, brd, $J = 6.9$ Hz), 8.13 (1H, d, $J = 8.0$ Hz), 8.25 (1H, d, $J = 8.0$ Hz), and

Table 4 Calibration linearities for quantitation of compounds (**1–5**, **7**, **9–12**, **14**) detected in commercial products by LC-UV detection

Peak no.	Compound	Wavelength for quantification	Linear range (μg/ml)	Regression equation	Correlation coefficient (r^2)
1	AM-694	315	10–500	$y = 856.86x - 623.11$	1.0000
2	(4-Methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone	320	10–500	$y = 646.79x - 1281.7$	0.9999
3	JWH-210	315	10–500	$y = 665.88x - 837.14$	1.0000
4	JWH-122	315	10–500	$y = 665.88x - 837.14$	1.0000
5	JWH-019	320	10–500	$y = 446.6x + 1163$	0.9999
7	JWH-200	320	10–500	$y = 771.0x + 30491$	0.9935
9	JWH-015	320	10–500	$y = 547.7x + 13348$	0.9937
10	JWH-250	305	10–500	$y = 622.0x + 939.28$	0.9998
11	JWH-073	320	10–500	$y = 844.8x + 7969$	0.9995
12	JWH-251	305	10–500	$y = 441.2x + 865.6$	0.9999
14	JWH-081	320	10–500	$y = 446.6x + 1163$	0.9999

8.49 (1H, m), 4 methylene proton signals at δ 1.26, 1.28 (each 2H, m, overlapped), 1.80 (2H, quint, $J = 7.5$ Hz), and 3.18 (2H, quartet, $J = 7.5$ Hz), a signal for a methylene connected to a nitrogen atom at δ 4.07 (2H, t, $J = 7.5$ Hz), and a methyl signal at δ 1.44 (3H, t, $J = 7.4$ Hz) as shown in Table 2. According to the ^{13}C NMR spectrum, 26 carbon signals were detected, suggesting the existence of 2 methyls (δ 13.8 and 14.9), 4 methylene carbons (δ 22.2, 26.2, 28.9, and 29.5), 1 methylene carbon connected to a nitrogen (δ 47.1), 7 aromatic quaternary carbons (δ 117.7, 127.1, 131.2, 132.0, 137.0, 137.8, and 142.5), 11 aromatic carbons (δ 109.9, 122.7, 123.0, 123.5, 123.8, 125.8, 126.0, 126.2, 126.8, and 137.5), and a carbonyl carbon (δ 192.3). The existence of an indole group, ethylnaphthalene group, and *n*-pentyl group was also suggested from the NMR spectra (Table 2; Fig. 8). The relationships of these groups were assigned from the HMBC spectra. The aromatic proton, which is a characteristic signal of the indole group at δ 7.37 (H-2′), correlated to the carbonyl carbon at δ 192.3 (C-1); the methine protons at δ 7.59 (C-2″) also correlated to the carbonyl carbon as shown in Fig. 8. On the basis of these instrumental data, the chemical structure of compound **3** was elucidated as 4-ethylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone. This compound had been reported as a cannabimimetic indole derivative acting as an agonist for the cannabinoid receptor CB_1 with a Ki value of 0.46 nM and for CB_2 with a Ki value of 0.69 nM, and named JWH-210 [8].

For the test solution of herbal product No. 34, an intense peak of unknown compound was detected at 14.49 min (**4**) by LC-UV detection (Fig. 9a). The PDA-sliced UV spectrum of unknown peak **4** showed maxima at 224.3 and 315.1 nm; the spectrum was quite similar to that of compound **3** (Fig. 9b), and the mass spectrum showed the base peak at *m/z* 356 $[M+H]^+$ in the positive scan mode (Fig. 9c).

The TIC by GC–MS also showed an unknown intense peak **4** appearing at 29.13 min (Fig. 10a), which showed a mass spectrum with ion peaks at *m/z* (relative intensity) 355(100), 214(64), and 144(40) (Fig. 10b). The accurate MS of isolated compound **4** revealed $[M+H]^+$ at *m/z* 356.2017 in the positive scan mode, suggesting the molecular formula of $C_{25}H_{25}NO$. The error between the observed and theoretical mass number of $[M+H]^+$ was 0.8 mDa. Judging from these data, it was suggested that compound **4** was smaller than compound **3** by one methylene unit. The above estimation was confirmed by NMR experiments, and their data and assignments are shown in Table 2 and Fig. 11. On the basis of these instrumental data, the chemical structure of compound **4** was elucidated as 4-methylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone. This compound had also been reported as a cannabimimetic indole derivative acting as an agonist for the cannabinoid receptor CB_1 with a Ki value of 0.69 nM and for CB_2 with a Ki value of 1.2 nM, and named JWH-122 [8].

Identification of unknown peak **5**

Nowadays, various types of synthetic cannabinoids can be purchased as reagents. We are in the process of collecting them from commercial sources together with the previously isolated compounds to construct a database using LC-PDA/MS and GC–MS for rapid identification. Our laboratory now has the capability for rapid detection of the chemical structures shown in Figs. 1 and 2, and the LC chromatogram of these compounds except compounds **1–4** is shown in Fig. 12(1a). An unknown peak **5** was detected at 14.99 min in sample No. 31 (Fig. 12(2a)). Compound **5** showed the same retention time, the same PDA spectrum, and the same LC–ESI–MS spectrum as those of JWH-019, which had been purchased from a manufacturer (Fig. 12).

Table 5 Content of cannabimimetic indoles in products obtained via the Internet

Sample no.	Sample form	Acquisition date	Net weight (g/pack)	Content (mg/pack)										
				AM-694 (**1**)	(4-Methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone (**2**)	JWH-210 (**3**)	JWH-122 (**4**)	JWH-019 (**5**)	JWH-200 (**7**)	JWH-015 (**9**)	JWH-250 (**10**)	JWH-073 (**11**)	JWH-251 (**12**)	JWH-081 (**14**)
1	Herbal product (cutting)	September 2010	3.05	–	–	–	54.8	–	11.5	–	–	–	–	–
2	Herbal product (cutting)	September 2010	2.15	–	–	–	–	–	–	–	83.8	–	–	127
3	Herbal product (cutting)	September 2010	3.04	–	–	–	–	–	–	–	359	29.8	–	90.1
4	Herbal product (cutting)	September 2010	2.08	–	–	–	–	–	–	–	75.1	–	–	104
5	Herbal product (cutting)	September 2010	3.06	–	–	–	–	–	–	–	108	–	–	154
6	Herbal product (cutting)	September 2010	3.07	–	–	–	–	–	–	–	77.8	53.5	–	154
7	Powder	September 2010	0.04	–	–	–	–	–	–	–	12.2	11.0	–	–
8	Powder	September 2010	0.09	–	–	–	–	–	–	–	30.9	–	–	44.7
9	Herbal product (cutting)	September 2010	3.02	–	–	–	–	–	–	–	73.5	55.0	–	152
10	Herbal product (cutting)	September 2010	2.99	–	–	–	–	–	45.9	–	8.9	13.0	–	23.9
11	Herbal product (cutting)	September 2010	2.95	–	–	–	–	–	17.9	–	7.8	10.2	–	41.0
12	Herbal product (cutting)	September 2010	3.04	–	–	–	–	–	67.8	15.5	232	43.5	–	–
13	Herbal product (cutting)	September 2010	3.03	–	–	–	–	–	91.1	24.7	246	–	–	–
14	Herbal product (cutting)	September 2010	3.08	–	–	–	–	–	61.9	19.9	247	4.0	–	–
15	Herbal product (cutting)	September 2010	3.00	–	–	–	–	–			79.7	55.5	–	61.9
16	Herbal product (cutting)	September 2010	3.05	–	–	–	–	–	98.0		185	–	–	–
17	Herbal product (cutting)	October 2010	3.08	–	–	166	–	–	–	–	–	–	–	–
18	Herbal product (cutting)	October 2010	2.95	–	–	178	–	–	–	–	–	–	–	–
19	Powder	October 2010		–	–	–	56.5	–	–	–	–	–	–	68.6
20	Powder	October 2010		–	–	–	58.8	–	–	–	–	–	–	7.7

Table 5 continued

Sample no.	Sample form	Acquisition date	Net weight (g/pack)	Content (mg/pack)										
				AM-694 (**1**)	(4-Methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone (**2**)	JWH-210 (**3**)	JWH-122 (**4**)	JWH-019 (**5**)	JWH-200 (**7**)	JWH-015 (**9**)	JWH-250 (**10**)	JWH-073 (**11**)	JWH-251 (**12**)	JWH-081 (**14**)
21	Herbal product (cutting)	October 2010	1.95	–	–	59.5	58.2	–	–	–	–	–	–	25.2
22	Herbal product (cutting)	October 2010	3.02	18.3	52.7	–	–	–	70.2	–	–	–	–	–
23	Herbal product (cutting)	October 2010	3.04	–	–	–	–	18.3	–	–	–	–	–	164
24	Herbal product (cutting)	October 2010	3.03	–	–	–	125	–	–	–	–	–	–	–
25	Powder	October 2010	0.10	–	–	–	16.1	–	–	–	–	–	–	24.8
26	Powder	October 2010	0.10	–	–	–	23.2	–	–	–	–	–	–	36.1
27	Powder	October 2010	0.14	–	–	–	33.9	–	–	–	–	–	–	53.2
28	Herbal product (cutting)	October 2010	2.19	–	–	–	110	–	–	–	–	–	–	15.9
29	Herbal product (cutting)	October 2010	3.04	21.1	5.1	–	–	–	65.8	–	–	–	–	60.6
30	Herbal product (cutting)	October 2010	3.03	–	–	–	119	–	–	–	–	–	–	–
31	Herbal product (cutting)	October 2010	3.04	–	–	–	–	22.4	–	–	–	–	–	216
32	Herbal product (cutting)	October 2010	3.01	–	–	–	–	116	–	–	–	–	–	150
33	Herbal product (cutting)	October 2010	3.03	–	–	–	16.0	28.1	–	–	–	–	–	43.1
34	Herbal product (cutting)	October 2010	3.01	–	–	–	143	–	–	–	–	–	–	–
35	Herbal product (cutting)	October 2010	3.07	–	–	–	–	30.6	–	–	–	–	–	252
36	Herbal product (cutting)	October 2010	2.99	–	–	–	73.0	–	–	–	–	–	–	100
37	Herbal product (cutting)	October 2010	3.11	–	–	–	148	–	–	–	–	–	–	–
38	Herbal product (cutting)	October 2010	3.11	–	–	–	–	–	–	–	–	–	–	282
39	Herbal product (cutting)	October 2010	3.14	–	–	–	–	130	–	–	–	–	–	168
40	Herbal product (cutting)	November 2010	2.99	–	–	76.4	76.3	–	–	–	–	–	–	–

Table 5 continued

Sample no.	Sample form	Acquisition date	Net weight (g/pack)	Content (mg/pack)										
				AM-694 (**1**)	(4-Methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone (**2**)	JWH-210 (**3**)	JWH-122 (**4**)	JWH-019 (**5**)	JWH-200 (**7**)	JWH-015 (**9**)	JWH-250 (**10**)	JWH-073 (**11**)	JWH-251 (**12**)	JWH-081 (**14**)
41	Herbal product (cutting)	November 2010	2.98	–	–	192	–	–	–	–	–	–	–	–
42	Herbal product (cutting)	November 2010	3.04	–	–	–	–		–	–	–	–	172	104
43	Herbal product (cutting)	November 2010	3.03	–	–	126	10.7	–	–	–	–	–	–	–

–, Not detected

We also confirmed its presence by GC–MS. According to the GC–MS analysis of sample No. 31, peak **5** was identified as JWH-019 in the presence of caffeine and JWH-081 (Fig. 13). JWH-019 had been reported as a cannabimimetic indole having high affinity for both CB_1 and CB_2 receptors [9].

Selection of extraction solvent, calibration curves

For quantitation of the compounds in products, the most effective solvent for extraction was found to be methanol among the ten solvents tested (Table 3). Therefore, the test solutions were prepared in methanol, and two methanol extractions were confirmed to be sufficient (data not shown). The linearities of the calibration curves were satisfactory for their quantitation (Table 4).

Trends in contents of cannabimimetic indoles in commercial products

The levels of cannabimimetic indoles in 43 commercial products are summarized in Table 5. Around September 2010, compounds **7**–**11** and **14** were detected. Compounds **10** and **11** have been controlled as designated substances (Shitei-Yakubutsu) under the Pharmaceutical Affairs Law in Japan since September 2010. Therefore, compounds **10** and **11** were not detected after the regulation was enacted. On the other hand, compounds **1**–**5** began to be detected from October 2010. The contents and combinations of compounds greatly varied from product to product. However, the powder-type products (Nos. 19–20, 25–27) only contained JWH-122 and JWH-081 from October 2010.

Conclusions

Many cannabimimetic compounds have been synthesized for research purposes, but recently these agents have begun to be used as adulterants in herbal products that are sold on the Internet. There is an urgent need to develop analytical methods and grasp the trends of their abuse as a first step of the administrative response. In this study, we isolated and identified five new compounds (**1**–**5**) in commercial drug products obtained from September 2010 to November 2010, and quantitated them together with previously reported compounds. In early 2009, cannabicyclohexanol, CP47497, and JWH-018 were detected [1, 2, 10], and other cannabimimetic compounds (**7**, **9**–**14**) were reported online in late 2010 [4, 5]. Most of the detected compounds including compounds **3**–**5** have higher affinities for CB_1 receptors than does Δ^9-THC (Ki = 41 nM) [8, 9, 11–15]; it should also be noted that compound **1** (Ki = 0.08 nM) [6, 7] shows an affinity for the CB_1 receptor that is about 500-fold that shown by Δ^9-THC. The cannabimimetic

effects of compound **2** have not been reported at the present time; we are planning to evaluate them by biological methods. All compounds detected in the products were found to be at relatively high concentrations, suggesting the risk of serious health damage upon their abuse.

References

1. Uchiyama N, Kikura-Hanajiri R, Kawahara N, Haishima Y, Goda Y (2009) Identification of a cannabinoid analog as a new type of designer drug in a herbal product. Chem Pharm Bull 57:439–441
2. Uchiyama N, Kikura-Hanajiri R, Kawahara N, Goda Y (2009) Identification of a cannabimimetic indole as a designer drug in a herbal product. Forensic Toxicol 27:61–66
3. Kikura-Hanajiri R, Kawamura M, Maruyama T, Kitajima M, Takayama M, Goda Y (2009) Simultaneous analysis of mitragynine, 7-hydroxymitragynine, and other alkaloids in the psychotropic plant "kratom" (*Mitragyna speciosa*) by LC–ESI–MS. Forensic Toxicol 27:67–74
4. Uchiyama N, Kawamura M, Kikura-Hanajiri R, Goda Y (2011) Identification and quantitation of two cannabimimetic phenylacetylindoles JWH-251 and JWH-250, and four cannabimimetic naphthoylindoles JWH-081, JWH-015, JWH-200, and JWH-073 as designer drugs in illegal products. Forensic Toxicol 29:25–37
5. Nakajima J, Takahashi M, Seto T, Suzuki J (2011) Identification and quantitation of cannabimimetic compound JWH-250 as an adulterant in products obtained via the Internet. Forensic Toxicol 29:51–55
6. Makriyannis A, Deng H (2007) Cannabimimetic indole derivatives. United States Patent, US 7,241,799 B2
7. Willis PG, Katoch-Rouse R, Horti AG (2003) Regioselective F-18 radiolabeling of AM694, a CB1 cannabinoid receptor ligand. J Label Compd Radiopharm 46:799–804
8. Huffman JW, Zengin G, Wu MJ, Lu J, Hynd G, Bushell K, Thompson ALS, Bushell S, Tartal C, Hurst DP, Reggio PH, Selley DE, Cassidy MP, Wiley JL, Martin BR (2005) Structure-activity relationships for 1-alkyl-3-(1-naphthoyl)indoles at the cannabinoid CB_1 and CB_2 receptors: steric and electronic effects of naphthoyl substituents. New highly selective CB_2 receptor agonists. Bioorg Med Chem 13:89–112
9. Aung MM, Griffin G, Huffman JW, Wu MJ, Keel C, Yang B, Showalter VM, Abood ME, Martin BR (2000) Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB_1 and CB_2 receptor binding. Drug Alcohol Depend 60:133–140
10. Auwärter V, Dresen S, Weinmann W, Müller M, Pütz M, Ferreirós N (2009) 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? J Mass Spectrom 44:832–837
11. Huffman JW, Mabon R, Wu MJ, Lu J, Hart R, Hurst DP, Reggio PH, Wiley JL, Martin BR (2003) 3-Indoyl-1-naphthylmethanes: new cannabimimetic indoles provide evidence for aromatic stacking interactions with the CB1 cannabinoid receptor. Bioorg Med Chem 11:539–549
12. Wiley JL, Compton DR, Dai D, Lainton JA, Phillips M, Huffman JW, Martin BR (1998) Structure–activity relationships of indole and pyrrole-derived cannabinoids. J Pharmacol Exp Ther 285:995–1004
13. Huffman JW (1999) Cannabimimetic indoles, pyrroles and indenes. Curr Med Chem 6:705–720
14. EMCDDA (2009) Thematic papers: understanding the "Spice" phenomenon. http://www.emcdda.europa.eu/attachements.cfm/att_80086_EN_Spice%20Thematic%20paper%20%E2%80%94%20final%20version.pdf. Accessed January 2011
15. ACMD (2009) Consideration of the major cannabinoid agonists. http://www.homeoffice.gov.uk/publications/drugs/acmd1/acmd-report-agonists?view=Binary. Accessed January 2011