Identification of two new-type synthetic cannabinoids, N-(1-adamantyl)-1-pentyl-1H-indole-3-carboxamide (APICA) and N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (APINACA), an...

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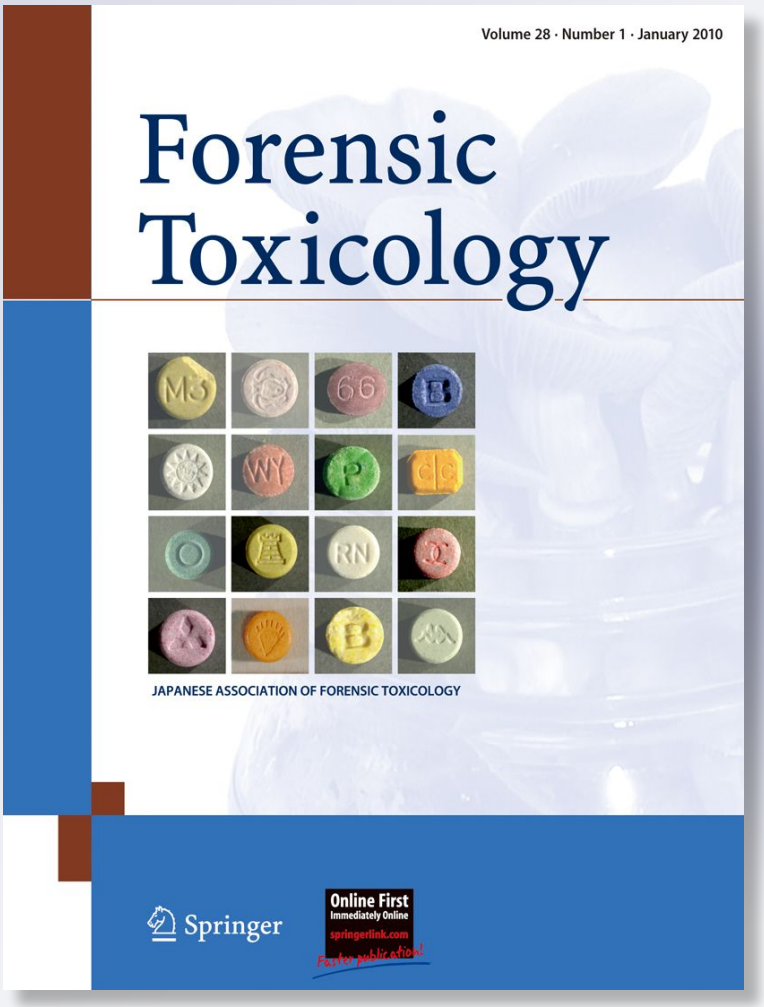

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ORIGINAL ARTICLE

Identification of two new-type synthetic cannabinoids, *N*-(1-adamantyl)-1-pentyl-1*H*-indole-3-carboxamide (APICA) and *N*-(1-adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide (APINACA), and detection of five synthetic cannabinoids, AM-1220, AM-2233, AM-1241, CB-13 (CRA-13), and AM-1248, as designer drugs in illegal products

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Abstract Two new-type synthetic cannabinoids, *N*-(1-adamantyl)-1-pentyl-1*H*-indole-3-carboxamide (APICA, **1**) and *N*-(1-adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide (APINACA, **2**), have been identified as designer drugs in illegal products being sold in Japan. The identification was based on liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), high-resolution MS and nuclear magnetic resonance (NMR) analyses. Both mass and NMR spectrometric data revealed that **1** was 1-pentyl-*N*-tricyclo[3.3.1.1^{3,7}]dec-1-yl-1*H*-indole-3-carboxamide, and **2** was 1-pentyl-*N*-tricyclo[3.3.3.1.^{3,7}]dec-1-yl)-1*H*-indazole-3-carboxamide. Although many of the synthetic cannabinoids detected in illegal products, such as JWH-018, have a 3-carbonyl indole moiety, compounds **1** and **2** are a new type of synthetic cannabinoid having an amide and an adamantyl group, and **2** also has an indazole group in place of an indole group. There has been no synthetic, chemical, or biological information about **1** or **2** until now, making this the first report of these cannabimimetic compounds (**1** and **2**) as designer drugs. In addition, five synthetic cannabinoids, AM-1220, AM-2233, AM-1241, CB-13 (CRA-13), and AM-1248, are also described herein as newly distributed designer drugs in Japan.

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Keywords *N*-(1-adamantyl)-1-pentyl-1*H*-indole-3-carboxamide · *N*-(1-adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide · AM-1220 · AM-2233 · AM-1241 · AM-1248

Introduction

Many herbal products with brand names such as "Spice" and "Herbal Incense" began to appear in the illegal drug market in 2006 [1]. Following the identification of synthetic cannabinoids as the psychoactive ingredients of these products by our group and a group in Germany in 2009 [2–4], these illegal herbal products, adulterated with synthetic cannabinoids, have received broad global attention due to their cannabimimetic effects. Since then, more than 20 synthetic cannabinoids have been detected as psychoactive ingredients in herbal products or chemical powders around the world [1–21].

The cannabinoids detected so far belong to six different groups: cyclohexylphenol [such as cannabicyclohexanol (CCH) and CP-47497], classical cannabinoids (such as HU-210), naphthoylindoles (such as JWH-018 and JWH-073), phenylacetylindoles (such as JWH-250 and JWH-203), benzoylindoles (such as AM-694 and RCS-4), and naphthoylnaphthalenes (such as CB-13) [9, 15]. In addition, most of these agents have been shown to have binding affinity for cannabinoid CB_1 (central type) and/or CB_2 (peripheral type) receptors [22].

A number of countries began to control these compounds in 2009, in consideration of their potential health problems [1, 15, 16]. In Japan, 16 synthetic cannabinoids (CCH, CP-47,497, JWH-018, JWH-073, JWH-250, JWH-

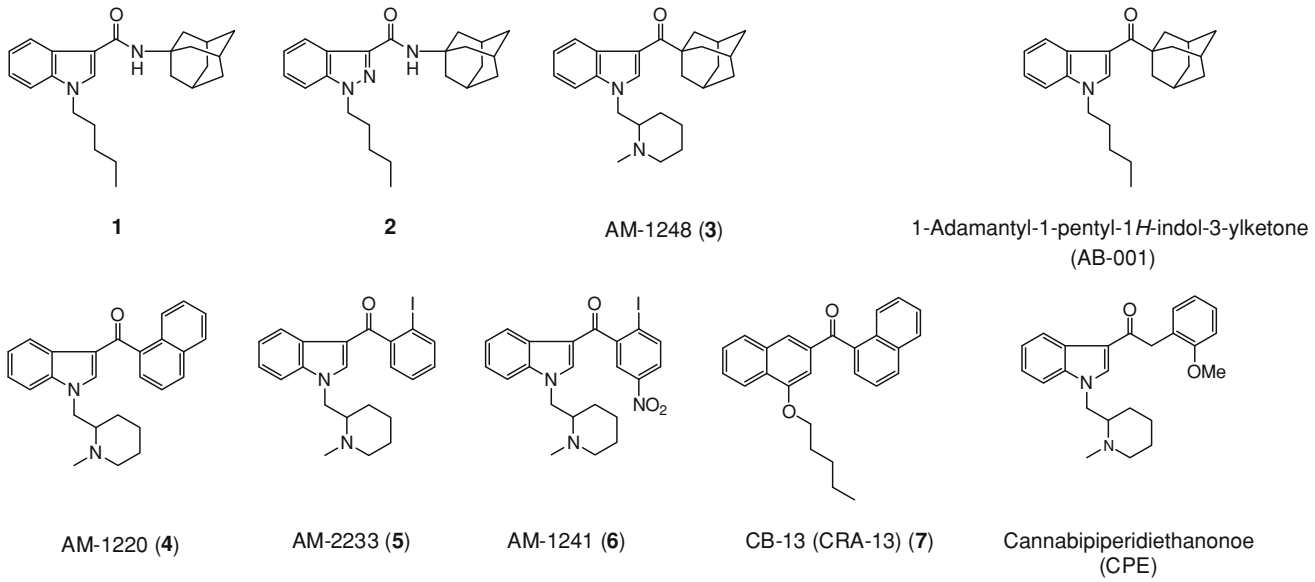

Fig. 1 Structures of the detected (**1–7**) and related synthetic cannabinoids

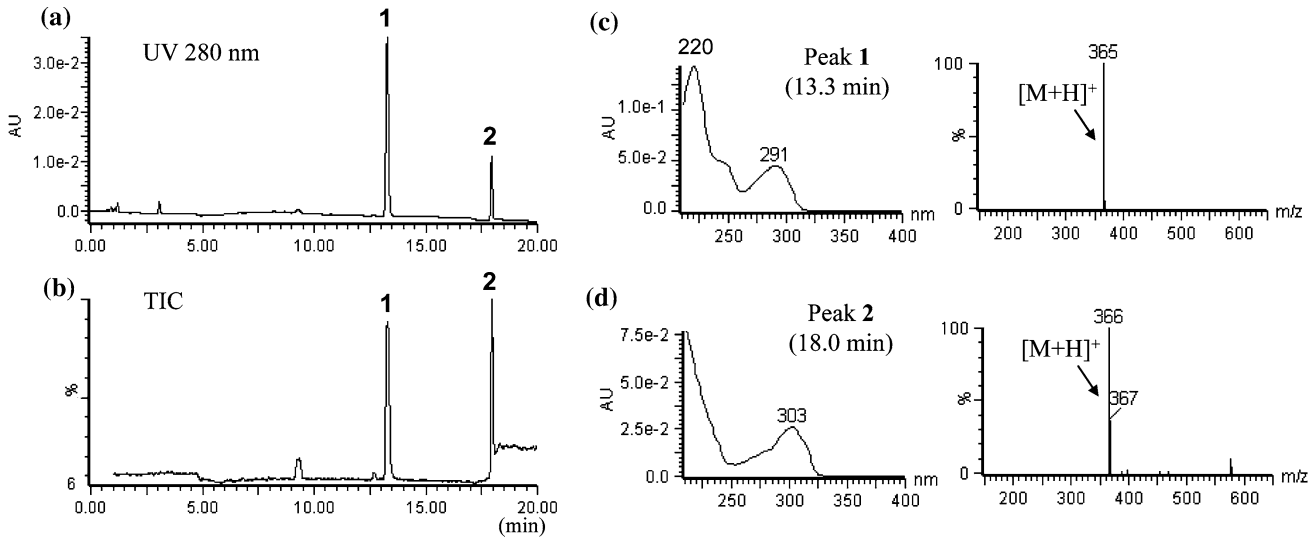

Fig. 2 HPLC UV and total ion chromatograms (**a**, **b**, respectively) of the extract of product A and UV and ESI mass spectra of peaks **1** and **2** (**c**, **d**, respectively)

015, JWH-122, JWH-081, JWH-200, JWH-251, JWH-019, JWH-203, JWH-210, AM-2201, AM-694, and RCS-4) were controlled as designated substances (Shitei-Yakubutsu) under the Pharmaceutical Affairs Law as of October 2011. However, in response to such control measures, new analogs of the controlled substances subsequently appeared as other ingredients in these herbal and chemical products.

As described in our earlier reports [2, 3, 6, 8–10], we have been conducting an ongoing survey of designer drugs in illegal markets in Japan, and we have found two synthetic cannabinoids of a new type, *N*-(1-adamantyl)-1-pentyl-1*H*-indole-3-carboxamide (APICA, **1**) and *N*-(1-adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide (APINACA, **2**), which cannot be classified into the previous six cannabinoid groups (Fig. 1). In this study, we describe our identification of these two new compounds (**1** and **2**) in a chemical product along with the detection of five newly distributed synthetic cannabinoids, AM-1248 (**3**), AM-1220 (**4**), AM-2233 (**5**), AM-1241 (**6**), and CB-13 (CRA-13, **7**) in herbal products (Fig. 1).

Materials and methods

Samples for analysis

The analyzed samples were purchased via the Internet from August to December 2011 as chemical or herbal products

being sold in Japan. The chemical product (A), which was called "Fragrance Powder," consisted of about 400 mg of a pale brown powder. Each of the herbal products (B–D) contained about 3 g of mixed dried plants.

Chemicals and reagents

Authentic AM-1248 (**3**), AM-1220 (**4**), AM-2233 (**5**), and CB-13 (**7**) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). AM-1241 (**6**) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other common chemicals and solvents were of analytical reagent grade or HPLC grade. As solvents for nuclear magnetic resonance (NMR) analysis, pyridine-d_5 (99.96 %), benzene-d_6 (99.96 %), CD_3OD (99.96 %), and CD_3OH (99.8 %) were purchased from the ISOTEC division of Sigma-Aldrich.

Preparation of sample solution

For qualitative analyses, 2 mg of the chemical product was used for extraction with 1 ml of methanol under ultrasonication for 10 min. For the herbal products, 10 mg of each product was crushed into powder and extracted with 1 ml of methanol under ultrasonication for 10 min. After centrifugation (5 min, 3000 rpm) of the extract, the supernatant solution was passed through a centrifugal filter (Ultrafree-MC, 0.45 μm filter unit; Millipore, Bedford, MA, USA) to afford sample solution. If necessary, the solution was diluted with methanol to a suitable concentration before instrumental analyses.

Analytical conditions

Ultra-performance liquid chromatography-electrospray ionization-mass spectrometry (UPLC-ESI-MS) was

Table 1 NMR data of compounds **1** and **2**

Position	APICA (**1**) in pyridine-d_5[a]			APINACA (**2**) in benzene-d_6[a,b]		
	^{13}C	^{1}H	HMBC[c]	^{13}C	^{1}H	HMBC[c]
1	165.2	–	–	161.8	–	–
2′	130.7	8.14, 1H, s	1, 3′, 3′a, 7′a, 1″,		–	–
3′	112.6	–	–	139.3	–	–
3′a	127.8	–	–	123.8	–	–
4′	122.4	8.86, 1H, m	3′, 3′a, 5′, 6′, 7′a	124.2	8.97, 1H, dd-like, J = 7.9, 1.0 Hz	3′, 3′a, 5′, 6′, 7′a
5′	121.3	7.36, 1H, td, J = 6.9, 1.4 Hz, overlapped	3′a, 7′	122.5	7.07, 1H, td, J = 7.9, 1.0 Hz, overlapped	3′a, 6′, 7′
6′	122.6	7.34, 1H, td, J = 6.9, 1.4 Hz, overlapped	4′, 7′, 7′a	126.6	7.10, 1H, td, J = 7.9, 1.0 Hz, overlapped	4′, 5′, 7′a
7′	110.5	7.48, 1H, dd-like, J = 6.9, 1.4 Hz	3′a, 5′, 6′	109.1	6.95, 1H, dd, J = 7.9, 1.0 Hz	3′a, 5′
7′a	137.1	–	–	141.3	–	–
1″	46.5	3.92, 2H, t, J = 7.2 Hz	2′, 7′a, 2″, 3″	49.1	3.87, 2H, t, J = 7.2 Hz	7′a, 2″, 3″
2″	29.9	1.53, 2H, quintet, J = 7.6 Hz	1″, 3″, 4″	29.6	1.60, 2H, m, overlapped	1″, 3″, 4″
3″	29.0	0.99, 2H, m	2″, 4″, 5″	29.0	0.98, 2H, m	1″, 2″, 4″, 5″
4″	22.4	1.05, 2H, m	2″, 3″, 5″	22.4	1.06, 2H, m	2″, 3″, 5″
5″	13.9	0.66, 3H, t, J = 7.2 Hz	3″, 4″	13.9	0.71, 3H, t, J = 7.2 Hz	3″, 4″
1‴	52.0	–	–	51.7	–	–
2‴/8‴/9‴	42.3	2.36, 6H, brs	1‴, 3‴/5‴/7‴, 4‴/6‴/10‴	42.1	2.21, 6H, brs	1‴, 3‴/5‴/7‴, 4‴/6‴/10‴
3‴/5‴/7‴	30.0	1.99, 3H, brs	1‴	29.9	1.92, 3H, brs	1‴, 4‴/6‴/10‴
4‴/6‴/10‴	36.8	1.65, 3H, brd, J = 11.3 Hz	3‴/5‴/7‴	36.7	1.59, 3H, brd, J = 12.4 Hz, overlapped	3‴/5‴/7‴, 2‴/8‴/9‴
		1.57, 3H, brd, J = 11.3 Hz	3‴/5‴/7‴, 2‴/8‴/9‴		1.51, 3H, brd, J = 12.4 Hz	3‴/5‴/7‴, 2‴/8‴/9‴
NH	–	7.20, 1H, brs	1, 1‴, 2‴/8‴/9‴	–	6.92, 1H, brs	1, 1‴, 2‴/8‴/9‴

[a] Recorded at 600 MHz (^{1}H) and 150 MHz (^{13}C), respectively; data in δ ppm (J in Hz)

[b] Recorded at 30 °C

[c] J = 8 or 4 Hz; the proton signal correlated with the indicated carbons

performed on an ACQUITY UPLC system with a mass detector and a photodiode array (PDA) detector (Waters, Milford, MA, USA) [6, 8]. The sample solutions were separated with an ACQUITY UPLC HSS T3 column (100 mm × 2.1 mm i.d., particle size 1.8 μm; Waters) protected by a Van Guard column (5 mm × 2.1 mm i.d., 1.8 μm; Waters) at 40 °C. Each analysis was carried out with a binary mobile phase consisting of solvent A (0.1 % formic acid in

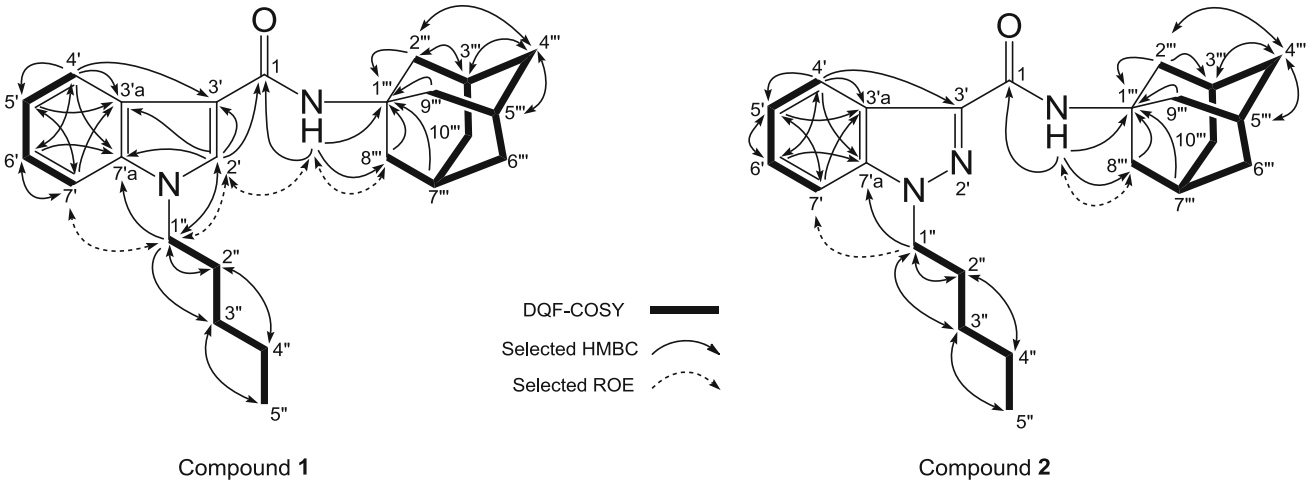

Fig. 3 DQF-COSY, selected HMBC, and selected ROE correlations of **1** and **2**

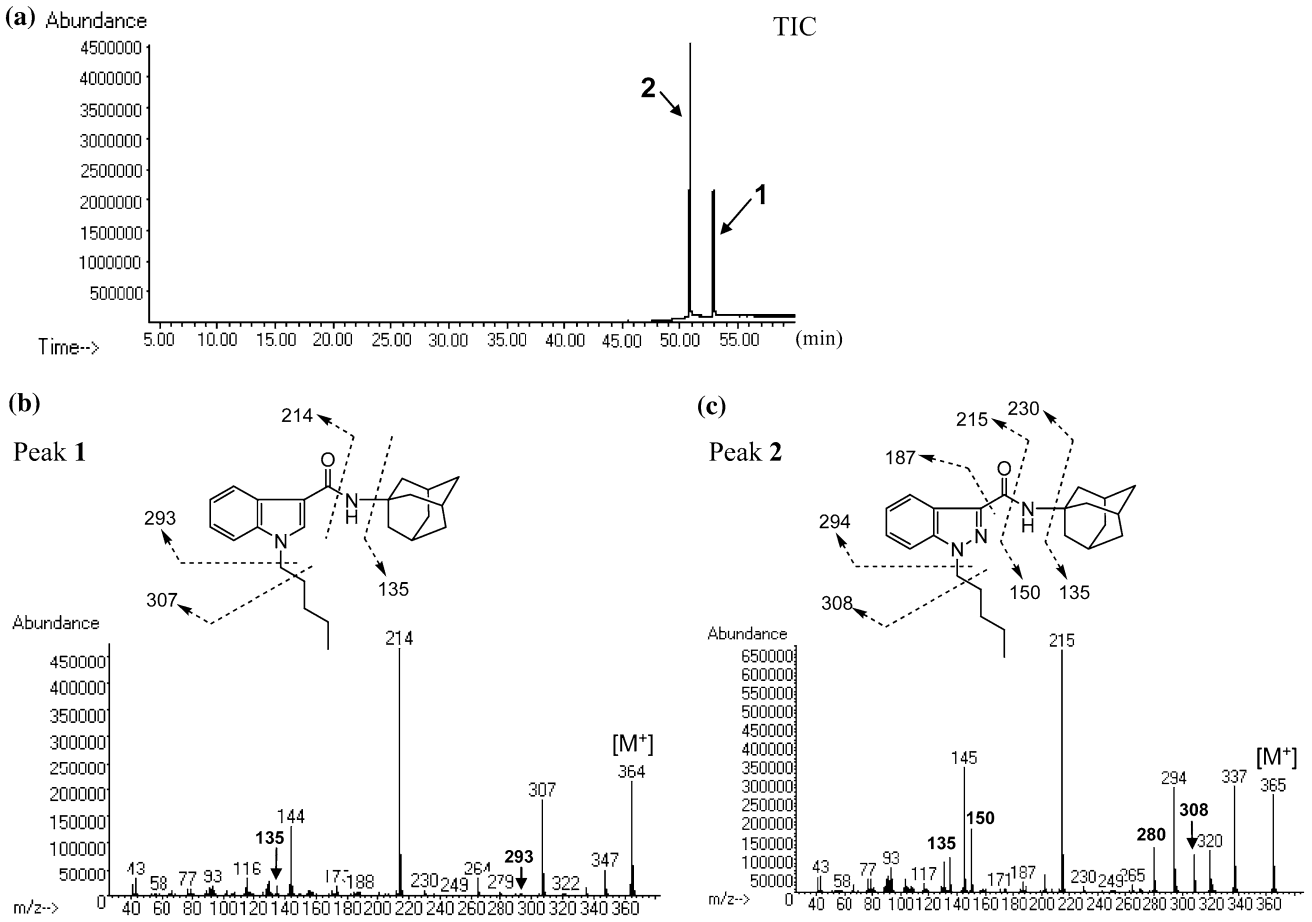

Fig. 4 GC-MS analysis of product A. Total ion chromatogram (**a**); EI mass spectra of the detected peaks at 52.85 min (**b**, **1**) and 50.82 min (**c**, **2**)

water) and solvent B (0.1 % formic acid in acetonitrile). The linear gradient elution program was as follows: 35 % B (4-min hold) and 65 % B to 75 % B (4–16 min), and up to 90 % B (16–17 min, 5 min hold) at a flow rate of 0.3 ml/min. The injection volume was 1 μl and the wavelength of the PDA detector for screening was set from 210 to 450 nm. The MS conditions for the LC-ESI-MS were: ionization, positive; desolvation gas, nitrogen at a flow rate of 650 l/h at 350 °C; capillary and cone voltages, 3000 V and 30 V, respectively; mass spectral range, *m/z* 150–650.

The sample solutions were also analyzed by gas chromatography-mass spectrometry (GC-MS) in electron ionization (EI) mode according to our previous report [6, 8]. GC-EI-MS was performed on an Agilent 6890N GC with a 5975 mass-selective detector (Agilent, Santa Clara, CA, USA) using a capillary column (HP-1MS capillary, 30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent) with helium gas as a carrier at 0.7 ml/min. The conditions were: electron energy, 70 eV; injector temperature, 200 °C; injection, splitless mode for 1.0 min; oven temperature program, 80 °C (1.2-min hold) increased at 5 °C/min to 190 °C (15-min hold), followed by an increase at 10 °C/min to 310 °C (10-min hold); mass selective detector temperature, 280 °C; scan range, *m/z* 40–650.

The accurate mass spectrum of the target compound was measured using a direct analysis in real time (DART) ion source coupled to a time-of-flight (TOF) mass spectrometer (AccuTOF JMS-100LC; JEOL, Tokyo, Japan) operated in positive ion mode. The measurement conditions were: ion guide peak voltage, 500 V; reflectron voltage, 950 V; orifice 1 voltage, 15 V; orifice 2 voltage, 5 V; ring lens voltage, 5 V; orifice 1 temperature, 80 °C; mass range, *m/z* 100–1000. The conditions of the DART ion source were:

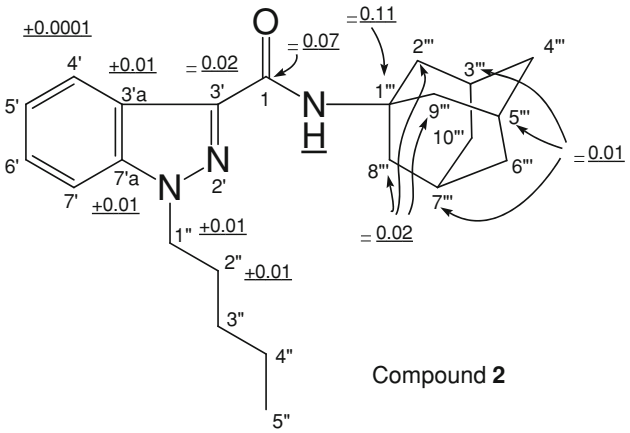

Fig. 5 Deuterium-induced isotope shifts (ppm) of NH proton for the ^{13}C-NMR signals of **2** in CD_3OD

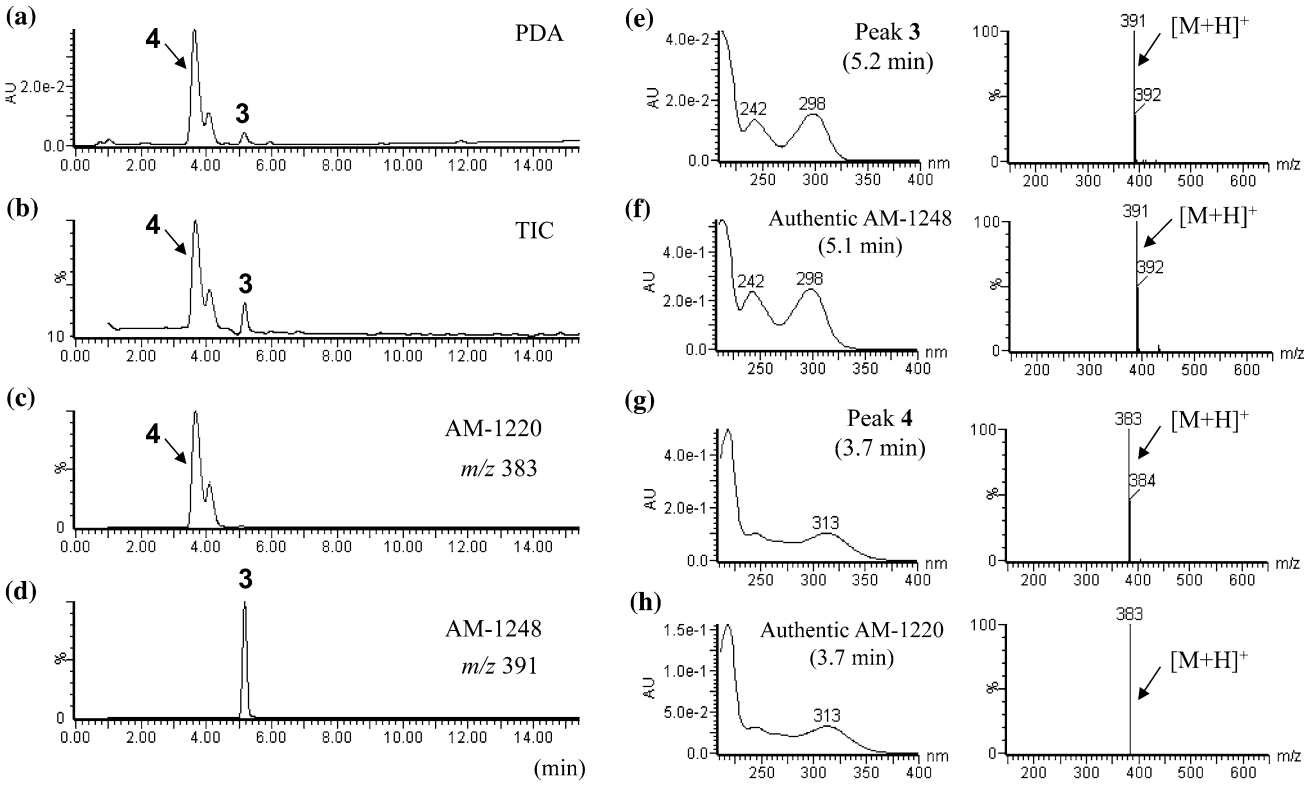

Fig. 6 HPLC UV and total ion chromatograms (**a**, **b**, respectively) and mass chromatograms of *m/z* 383 and *m/z* 391 (**c**, **d**, respectively) of product B and UV and ESI mass spectra of peaks **3** and **4** and authentic AM-1248 and AM-1220 (**e**, **g** and **f**, **h**, respectively)

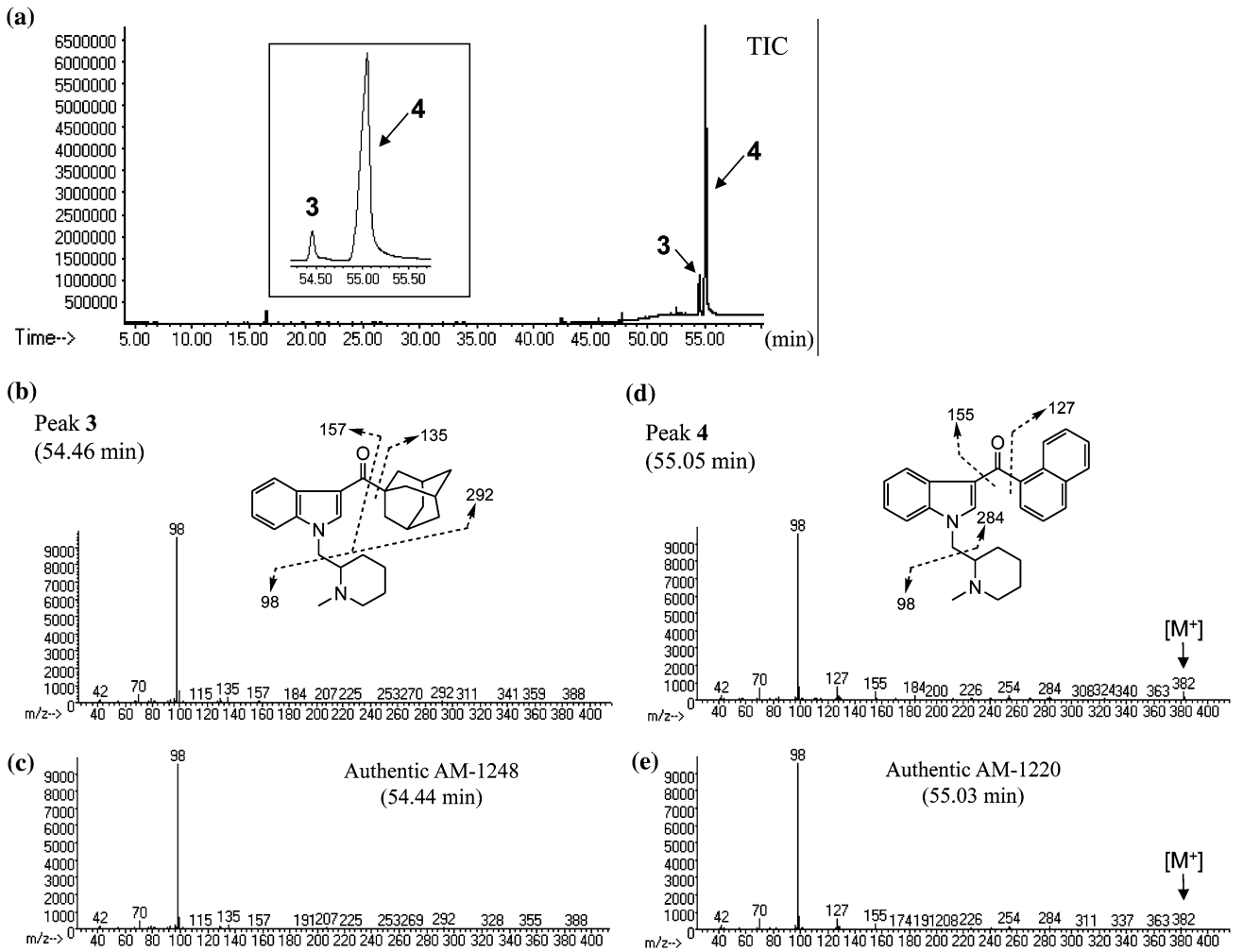

Fig. 7 GC-MS analysis of product B. Total ion chromatogram (**a**); EI mass spectra of the detected peaks **3** (54.46 min) and **4** (55.05 min) and authentic AM-1248 and AM-1220 (**b**, **d** and **c**, **e**, respectively)

helium gas flow rate, 2.0 l/min; gas heater temperature, 250 °C; discharge electrode needle voltage, 3200 V; and voltages of electrodes 1 and 2, 100 and 250 V, respectively. Internal mass number calibration was achieved using PEG600, and diphenhydramine ($C_{17}H_{21}NO$) and verapamil ($C_{27}H_{38}N_2O_4$) were used as internal standards for each accurate mass analysis. The product itself or an extract was directly exposed to the vicinity of the DART ion source.

The NMR spectra were obtained on ECA-600 spectrometers (JEOL). Assignments were made via ^{1}H-NMR, ^{13}C-NMR, heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), double quantum filtered correlation spectroscopy (DQF-COSY), and rotating frame nuclear Overhauser effect (ROE) spectra.

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

A 400-mg sample of a pale brown powder was extracted with 20 ml of methanol by ultrasonication for 10 min. The extractions were repeated three times, and the supernatant fractions were combined and evaporated to dryness. The extract was placed on a preparative silica-gel thin-layer chromatography (TLC) plate (Silica Gel 60, 20 × 20 cm, 2 mm; Merck, Darmdstadt, Germany), which was then developed using hexane/ethyl acetate (3:1). A portion of the silica gel in the TLC plate that contained the target compound, as detected by UV 254 nm and DART-TOF-MS, was scraped from the plate to get fractions 1 and 2. Each fraction was eluted with chloroform to obtain compound **1** (55 mg) as a white solid, and compound **2** (26 mg) as a yellow oil, respectively.

Results and discussion

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

By UPLC-MS analysis of the chemical product A, two peaks **1** and **2**, both having UV280 absorption, were mainly

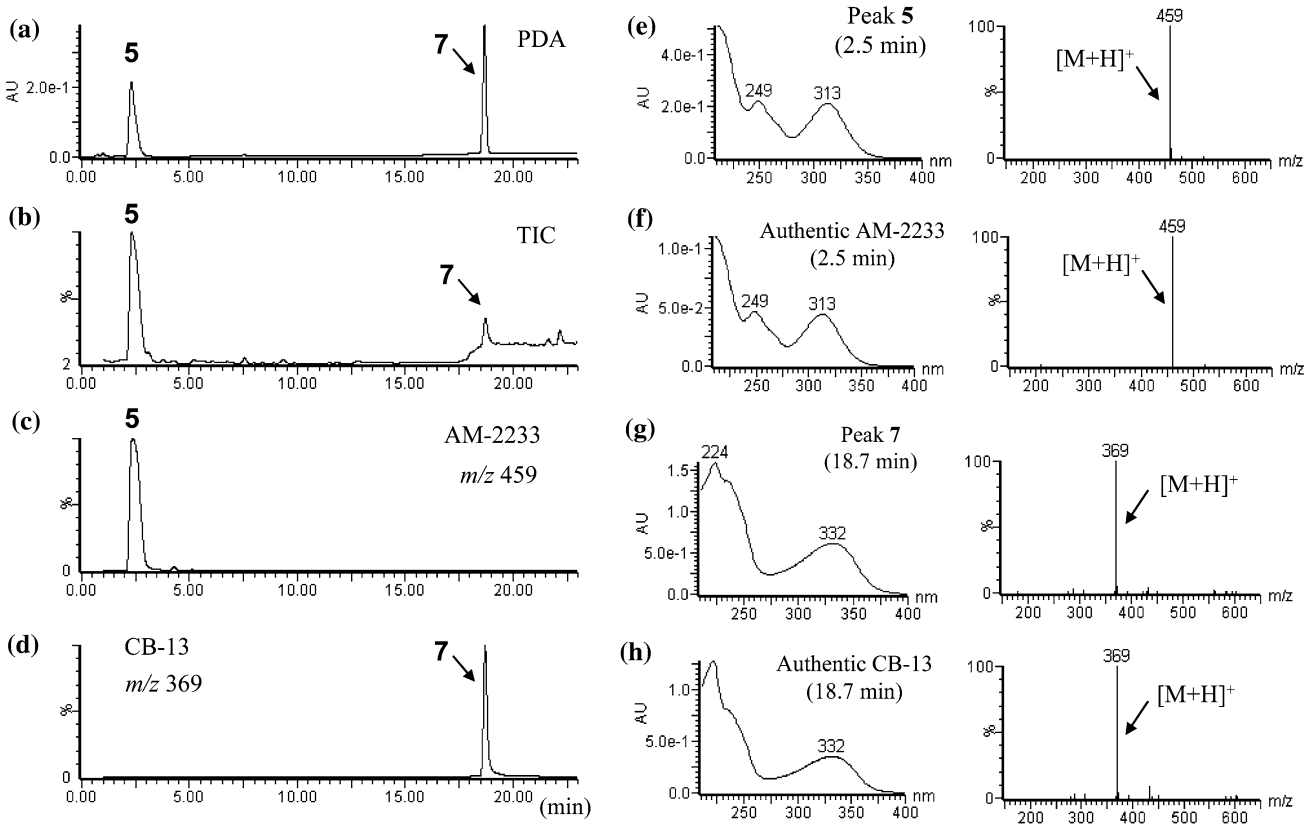

Fig. 8 HPLC UV and total ion chromatograms (**a**, **b**, respectively) and mass chromatograms of *m/z* 459 and *m/z* 369 (**c**, **d**, respectively) of product C and UV and ESI mass spectra of peaks **5** and **7** and authentic AM-2233 and CB-13 (**e**, **g** and **f**, **h**, respectively)

observed in the total ion chromatogram (TIC) (Fig. 2a, b). Their quasi-molecular ion $[M+H]^+$ signals were *m/z* 365 and 366, respectively (Fig. 2c, d).

After isolation of compounds **1** and **2**, their accurate mass spectra were measured by DART-TOF-MS (positive mode). The observed ion peaks at 365.2590 and 366.2553 suggested that the quasi-molecular formulae of **1** and **2** were $C_{24}H_{33}N_2O$ (calcd. 365.2593) and $C_{23}H_{32}N_3O$ (calcd. 366.2545), respectively.

Next, we elucidated the chemical structure of compound **1** by NMR analyses (Table 1; Fig. 3). The ^{1}H- and ^{13}C-NMR spectra of **1** suggested 32 protons and 24 carbons as shown in Table 1. These NMR data were very similar to those of 1-adamantyl-1-pentyl-1*H*-indol-3-yl ketone (AB-001; molecular formula: $C_{24}H_{31}NO$) (Fig. 1) [19] except for the quarternary carbon signal at δ_c 165.2 (C-1), which was high-field shifted from δ_c 202.0 and for the additional broad proton signal at δ_H 7.20 (1H, brs). The difference in molecular formula between AB-011 and compound **1** was NH, and HMQC analysis of the broad proton signal suggested that the hydrogen atom connected to a heteroatom. These data strongly suggested that compound **1** possessed an amide group instead of the keto group of AB-011. Therefore, the structure was deduced to be *N*-(1-adamantyl)-1-pentyl-1*H*-indole-3-carboxamide (IUPAC name: 1-pentyl-*N*-tricyclo[3.3.3.1.^{3,7}]dec-1-yl)-1*H*-indole-3-carboxamide) and named APICA. The fragment ions by GC-MS analysis of peak **1**, as shown in Fig. 4b, and the observed DQF-COSY, HMBC, and ROE correlations in Fig. 3 further confirmed the structure.

The difference between the molecular formula of compound **2** and that of compound **1** is the additional nitrogen atom in the place of the absent CH. The comparison of ^{1}H-NMR and ^{13}C-NMR data between compounds **2** and **1** revealed the loss of the methine unit at the 2′-position of the indole group of compound **1** in the structure of compound **2**. Furthermore, the major fragment ions at *m/z* 215, 294, and 308 of peak **2** instead of those at *m/z* 214, 293, and 307 of peak **1** (Fig. 4b, c) by GC-MS analyses strongly suggested the replacement of the methine unit with a nitrogen atom. Therefore, the structure of compound **2** was elucidated as *N*-(1-adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide.

The clear difference of the UV spectrum of peak **2** compared with that of peak **1** (Fig. 2c, d), also supports the structural change from an indole to an indazole moiety. To confirm the structure, DQF-COSY, HMBC, and ROE NMR analyses were carried out and the observed

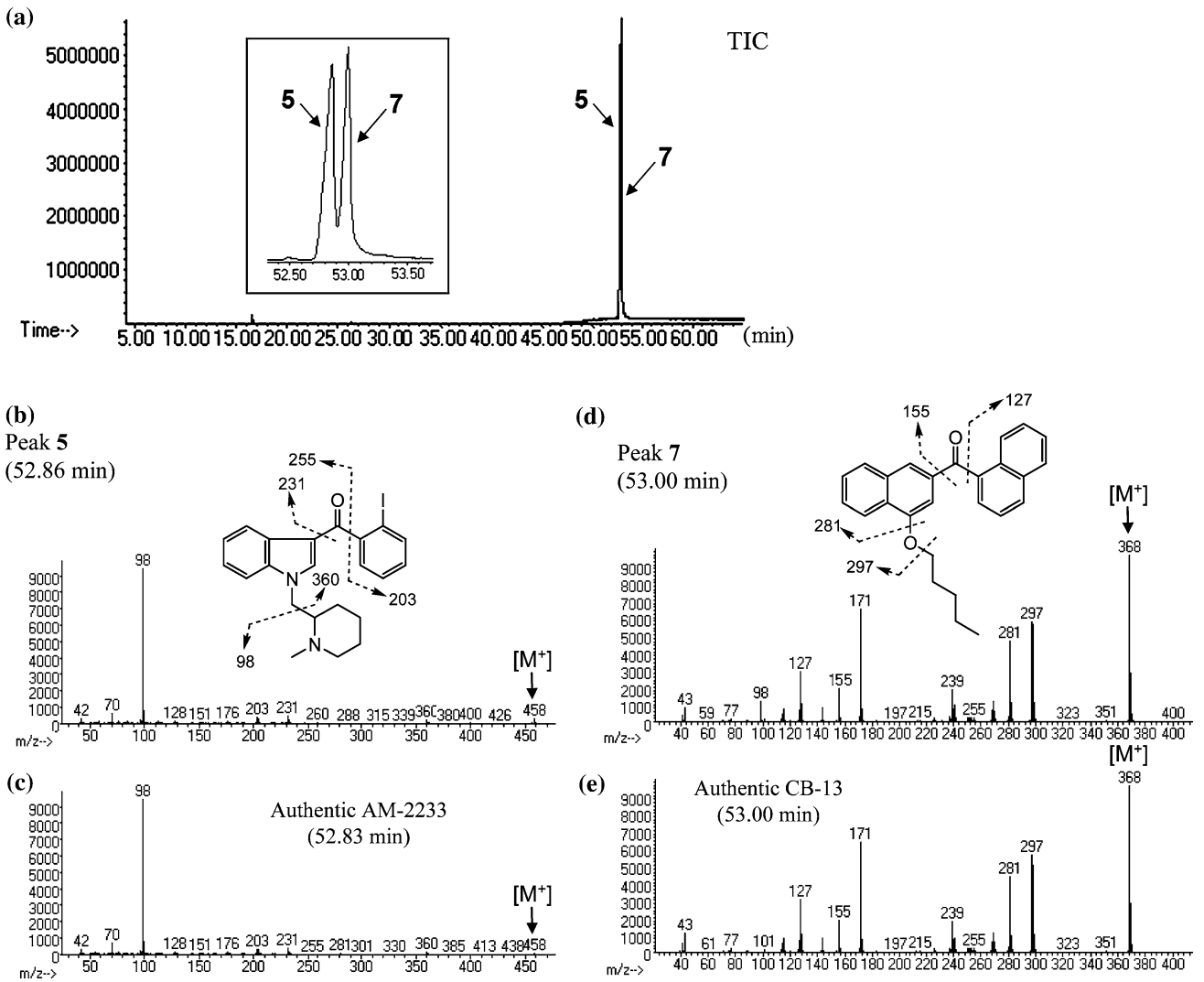

Fig. 9 GC-MS analysis of product C. Total ion chromatogram (**a**); EI mass spectra of the detected peaks **5** (52.86 min) and **7** (53.00 min) and authentic AM-2233 and CB-13 (**b**, **d** and **c**, **e**, respectively)

correlations shown in Table 1 and Fig. 3 are well consistent with it.

Finally, the deuterium isotope effect of the NH amide proton on the ^{13}C chemical shift was measured to definitively determine the connection between the 1-pentyl-1*H*-indazole moiety and the 1-adamantyl-carboxyamide moiety. The ^{13}C-NMR spectrum of **2**, recorded in CD_3OH, was compared with that recorded in CD_3OD. The isotope shift values for the ^{13}C-NMR signals of **2** are shown in Fig. 5. The largest and second largest deuterium shifts (0.11 and 0.07 ppm) were observed at the C-1‴ position of the adamantyl moiety and the C-1 position of the carbonyl moiety, respectively. The third largest shift of 0.02 ppm, which was attributed to the three-bond deuterium isotope effects of the NH proton on each carbon, was observed in the ^{13}C-NMR signal at C-3′ of the indazole group or C-2‴/8‴/9‴ of the adamantyl group. These results strongly suggested that the 1-pentyl-1*H*-indazole moiety was connected at the 3′-position of the indazole to the carboxyamide. Therefore, compound **2** was definitively identified as *N*-(1-adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide (IUPAC name: 1-pentyl-*N*-tricyclo[3.3.3.1.^{3,7}]dec-1-yl)-1*H*-indazole-3-carboxamide) and named APINACA.

Compounds **1** and **2** are novel cannabimimetic substances and their chemical or pharmacological information has not been reported, although the analogs of compound **2** in which the Cl-, I-, or CN-group was substituted for the terminal methyl of the pentyl moiety (position 5″ in Fig. 3) were synthesized as cannabimimetic ligands [23].

Identification of unknown peaks **3**–**7**

Unknown peaks **3** and **4** were detected in the LC-MS and GC-MS chromatograms of product B, as shown in Figs. 6

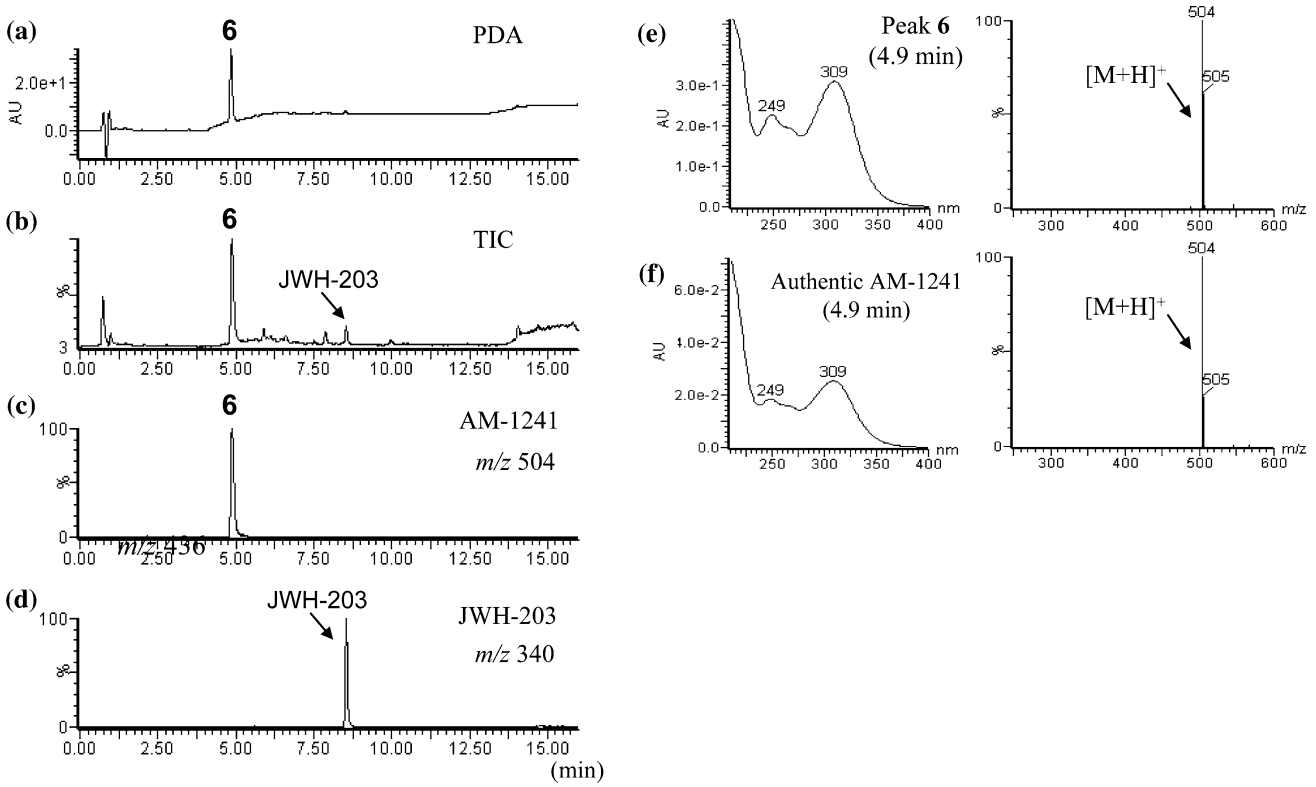

Fig. 10 HPLC UV and total ion chromatograms (**a**, **b**, respectively) and mass chromatograms of *m/z* 504 and *m/z* 340 (**c**, **d**, respectively) of product D and UV and ESI mass spectra of peak **6** and authentic AM-1241 (**e**, **f**, respectively)

and 7. The LC-MS chromatograms demonstrated that peak **3** at 5.2 min (Fig. 6d, e) and peak **4** at 3.7 min (Fig. 6c, g) showed protonated ion signals ($[M+H]^+$) at *m/z* 391 and 383, respectively. In the GC-MS chromatogram of peaks **3** and **4** (Fig. 7), the most obvious common feature was the presence of the fragment at *m/z* 98, which indicated that these compounds (**3** and **4**) may contain an *N*-methylpiperidine-2-yl-methyl group as seen in cannabipiperidiethanone (CPE), as shown in Fig. 1. CPE was previously reported by us as a cannabimimetic compound used in designer drugs and shown to have binding affinity to cannabinoid CB_1/CB_2 receptors [10]. The proposed fragment patterns and the presumed structures of **3** (AM-1248) and **4** (AM-1220) are also shown in Fig. 7b, d. The LC-MS and GC-MS spectra of the purchased authentic AM-1248 and AM-1220, the molecular weights of which were 390 and 382, are shown (Figs. 6f, h, 7c, e); compounds **3** and **4** were found to be identical to AM-1248 and AM-1220, respectively. It is worth noting in this context that AM-1220 (**4**) has been detected in herbal products present on the German market [20].

As shown in Figs. 8 and 9, unknown peaks **5** and **7** were detected in LC-MS and GC-MS chromatograms of product C. The LC-MS chromatograms showed that peaks **5** and **7** exhibited protonated ion signals ($[M+H]^+$) at *m/z* 459 and 369, respectively (Fig. 8e, g). The proposed fragment patterns and the presumed structures of **5** (AM-2233) and **7** (CB-13) from the GC-MS analysis are also shown in Fig. 9b, d. LC-MS and GC-MS analyses of the purchased authentic AM-2233 and CB-13, the molecular weights of which were 458 and 368, were performed (Figs. 8f, h, 9c, e), and compounds **5** and **7** were identified as AM-2233 and CB-13, respectively. CB-13 (**7**) has also been detected in herbal products present in European countries [20].

Similar analyses were used to determine the unknown peak **6** with the known peak of JWH-203, which is controlled as a designated substance (Shitei-Yakubutsu), in the chromatograms of product D (Figs. 10, 11). First, it was deduced that peak **6** was nitro-AM2233 (**5**) based on the LC-MS and GC-MS data (Figs. 10e, 11b), and this peak was finally attributed to AM-1241 by direct comparison of the data to those of the purchased authentic AM-1241 (Figs. 10f, 11c). Although AM-1241 is known as a selective CB_2 receptor agonist [24], this is the first report to detect AM-1241 as an ingredient in illegal products.

Features of synthetic cannabinoids detected in illegal products

The pharmacological activities of the two novel compounds **1** and **2** have not been reported. However, analogs of **2** in which a Cl-, I-, or CN-group is substituted for the

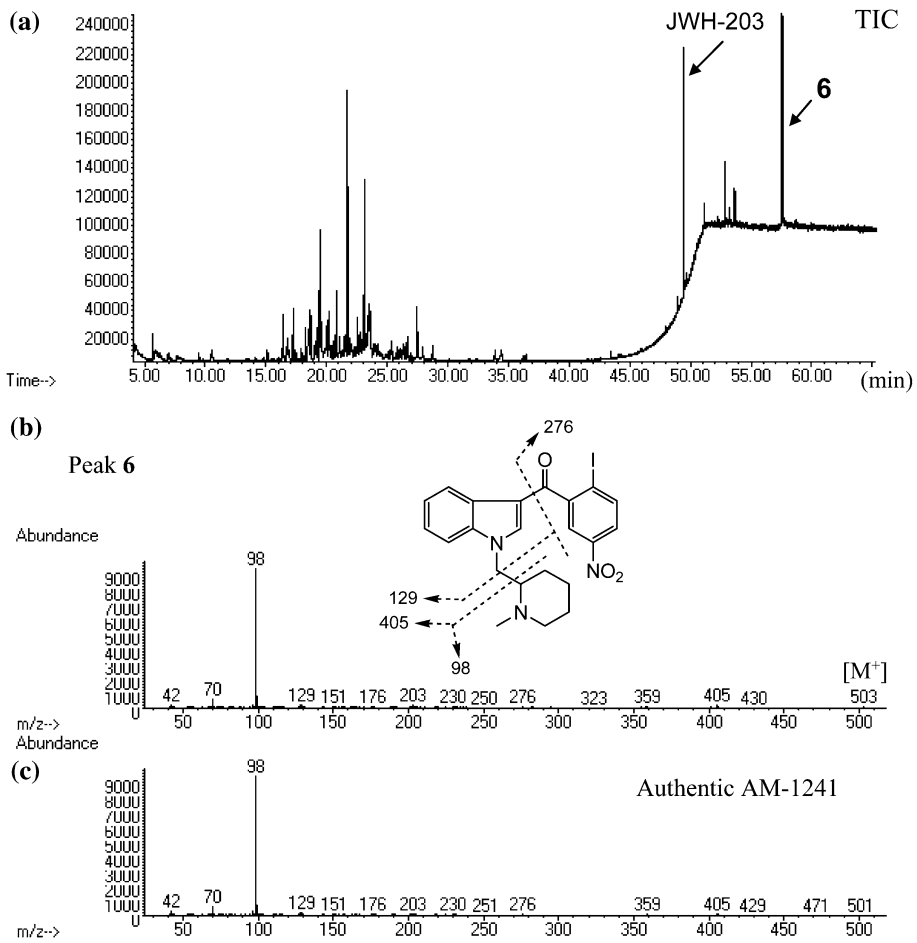

Fig. 11 GC-MS analysis of product D. Total ion chromatogram (**a**); EI mass spectra of the detected peak **6** (57.57 min) and authentic AM-1241 (**b**, **c**, respectively)

terminal methyl of the pentyl moiety (position 5″ in Fig. 3) have been reported to exhibit potent affinity for cannabinoid CB_1/CB_2 receptors; their Ki (nM) values for CB_1/CB_2 are 1.7/1.3, 0.6/0.2, and 2.3/0.3, respectively [23]. Therefore, it is assumed that compounds **1** and **2** may have similar cannabimimetic activities. On the other hand, compounds **3**–**7** have been reported as synthetic cannabinoids possessing affinity for cannabinoid CB_1 and/or CB_2 receptors. Makriyannis and Deng [25] described the high affinity of AM-1248 (**3**) for CB_1/CB_2 receptors [Ki (nM) = 11.9/4.8]. AM-1220 (**4**) and AM-2233 (**5**) were first synthesized as candidates for radioactive ligands for imaging the neuronal cannabinoid receptor, and their binding activities to the CB_1 receptor were reported [Ki (nM) values = 0.8 and 2.8 nM, respectively] [26, 27]. AM-2233 (**5**) was also reported to have the effect of drug discrimination for rats together with JWH-018 [28]. CB-13 (CRA-13, **7**) was reported as a dual agonist for CB_1/CB_2 receptors with cannabimimetic pharmaceutical effects [29]. On the other hand, AM-1241 (**6**) acts as a selective agonist for CB_2 receptor [24].

The detected compounds (**1**–**6**) have bulky structures such as an adamantyl group and *N*-methyl-piperidine-2-methyl group, although CB-13 (**7**) does not (Fig. 1). Adamantyl-based compounds are used clinically for the treatment of neurological conditions. It has been reported that the steric bulk of the adamantyl group can: (1) restrict or modulate intramolecular reactivity; and (2) impede the access of hydrolytic enzymes, thereby increasing drug stability and plasma half-life [30]. AM-411, in which an adamantyl group is appended to the C-3 position of Δ^8-tetrahydrocannabinol (Δ^8-THC), was reported to have sevenfold higher selectivity for CB_1 receptor than its des-adamantyl analog, Δ^8-THC, and its in vivo cannabimimetic activity was shown to have greater potency than that of Δ^9-tetrahydrocannabinol [30]. The *N*-methyl-piperidine-2-methyl group also may be an important moiety for controlling the lipophilicity of the aminoalkylindole ligands such as AM-1220 and AM-2233 in binding to CB_1/CB_2 receptors [26, 27]. Therefore, there is a worrisome possibility that the related analogs of compounds **1**–**6** will appear as new designer drugs in illegal products.

Conclusions

In the present study, we have identified two novel cannabimimetic compounds (**1** and **2**) having an amide and an

adamantyl group in illegal products being sold in Japan. Compound **2** also has an indazole group in place of an indole group. In addition, five newly distributed cannabimimetic cannabinoids, AM-1248 (**3**), AM-1220 (**4**), AM-2233 (**5**), AM-1241 (**6**), and CB-13 (CRA-13, **7**) were found in illegal products. The five compounds (**3**–**7**) have previously been shown to have binding affinity for cannabinoid CB_1 and/or CB_2 receptors and limited cannabimimetic effects, albeit only in animal models [24–29]. However, there are no pharmacological data on compounds **1** and **2**. Because of the limited pharmacological and toxicological information for most of these cannabimimetic compounds, it is difficult to predict the health risks associated with their use. Therefore, continuous monitoring of illegal products, rapid identification of unknown ingredients, and sharing of the information globally will be needed to prevent their abuse worldwide.

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References

1. EMCDDA (2009) EMCDDA action on new drugs briefing paper: understanding the 'Spice' phenomenon (a report from an EMCDDA expert meeting, 6 March 2009, Lisbon). http://www.emcdda.europa.eu/attachements.cfm/att_80086_EN_Spice%20Thematic%20paper%20—%20final%20version.pdf. Accessed April 2010
2. 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
3. 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
4. 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
5. Lindigkeit R, Boehme A, Eiserloh I, Luebbecke M, Wiggermann M, Ernst L, Beuerle T (2009) Spice: a never ending story? Forensic Sci Int 191:58–63
6. Uchiyama N, Kikura-Hanajiri R, Ogata J, Goda Y (2010) Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. Forensic Sci Int 198:31–38
7. Dresen S, Ferreirós N, Pütz M, Westphal F, Zimmermann R, Auwärter V (2010) Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. J Mass Spectrom 45:1186–1194
8. 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
9. Kikura-Hanajiri R, Uchiyama N, Goda Y (2011) Survey of current trends in the abuse of psychotropic substances and plants in Japan. Legal Med 13:109–115
10. Uchiyama N, Kikura-Hanajiri R, Goda Y (2011) Identification of a novel cannabimimetic phenylacetylindole, cannabipiperidiethanone, as a designer drug in a herbal product and its affinity for cannabinoid CB_1 and CB_2 receptors. Chem Pharm Bull 59:1203–1205
11. Nakajima J, Takahashi M, Seto T, Kanai C, Suzuki J, Yoshida M, Hamano T (2011) Identification and quantitation of two benzoylindoles AM-694 and (4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone, and three cannabimimetic naphthoylindoles JWH-210, JWH-122, and JWH-019 as adulterants in illegal products obtained via the Internet. Forensic Toxicol 29:95–110
12. Nakajima J, Takahashi M, Seto T, Kanai C, Suzuki J, Yoshida M, Hamano T (2011) Identification and quantitation of a benzoylindole(2-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone and a naphthoylindole 1-(5-fluoropentyl-1H-indol-3-yl)-(naphthalene-1-yl)methanone (AM-2201) found in illegal products obtained via the Internet and their cannabimimetic effects evaluated by in vitro [35S]GTPγS binding assays. Forensic Toxicol 29:132–141
13. Ernst L, Schiebel HM, Theuring C, Lindigkeit R, Beuerle T (2011) Identification and characterization of JWH-122 used as new ingredient in "Spice-like" herbal incenses. Forensic Sci Int 208:e31–e35
14. Bononi M, Belgi P, Tateo F (2011) Analytical data for identification of the cannabimimetic phenylacetylindole JWH-203. J Anal Toxicol 35:360–363
15. EMCDDA (2011) EMCDDA–Europol 2010 Annual Report on the implementation of Council Decision 2005/387/JHA EMCDDA-Europol, Lisbon, May 2011. http://www.emcdda.europa.eu/attachements.cfm/att_132857_EN_EMCDDA-Europol%20Annual%20Report%202010A.pdf. Accessed Dec 2011
16. United Nations Office on Drugs and Crime (UNODC) (2011) Synthetic Cannabinoids in Herbal Products. April 2011. http://www.unodc.org/documents/scientific/Synthetic_Cannabinoids.pdf. Accessed May 2011
17. EMCDDA (2011) Online sales of new psychoactive substances/'legal highs': summary of results from the 2011 multilingual snapshots., Lisbon, November 2011. http://www.emcdda.europa.eu/attachements.cfm/att_143801_EN_SnapshotSummary.pdf. Accessed Nov 2011
18. Nakajima J, Takahashi M, Seto T, Yoshida M, Kanai C, Suzuki J, Hamano T (2012) Identification and quantitation of two new naphthoylindole drugs-of-abuse, (1-(5-hydroxypentyl)-1*H*-indol-3-yl)(naphthalen-1-yl)methanone (AM-2202) and (1-(4-pentenyl)-1*H*-indol-3-yl)(naphthalen-1-yl)methanone, with other synthetic cannabinoids in unregulated "herbal" products circulated in the Tokyo area. Forensic Toxicol 30:33–44
19. Jankovics P, Váradi A, Tölgyesi L, Lohner S, Németh-Palotás J, Balla J (2012) 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. Forensic Sci Int 214:27–32
20. Kneisel S, Westphal F, Moosmann B, Brecht V, Bisel P, Vidal C, Jacobsen-Bauer A, Bork WR, Auwärter V (2011) Cannabimimetics II: mass spectra and ATR-IR spectra of new compounds between the end of 2010 and late 2011. TIAFT Bull 41:29–38
21. Grigoryev A, Kavanagh P, Melnik A (2011) The detection of the urinary metabolites of 3-[(adamantan-1-yl)carbonyl]-1-pentylindole (AB-001), a novel cannabimimetic, by gas chromatography-mass spectrometry. Drug Test Anal. doi:10.1002/dta.350
22. Huffman JW (2009) Cannabimimetic indoles, pyrroles, and indenes: structure-activity relationships and receptor interactions. In: Reggio PH (ed) The cannabinoid receptors. Humana Press, New York, pp 49–94
23. Makriyannis A, Lui Q (2003) Patent: WO/2003/035005 May, 2003. Heteroindanes: a new class of potent cannabimimetic ligands
24. Bingham B, Jones PG, Uveges AJ, Kotnis S, Lu P, Smith VA, Sun SC, Resnick L, Chlenov M, He Y, Strassle BW, Cummons

TA, Piesla MJ, Harrison JE, Whiteside GT, Kennedy JD (2007) Species-specific in vitro pharmacological effects of the cannabinoid receptor 2 (CB2) selective ligand AM1241 and its resolved enantiomers. Br J Pharmacol 151:1061–1070

25. Makriyannis A, Deng H (2002) Patent: WO/2002/060447, August 2002. Receptor selective cannabimimetic aminoalkylindoles
26. Willis PG, Pavlova OA, Chefer SI, Vaupel DB, Mukhin AG, Horti AG (2005) Synthesis and structure–activity relationship of a novel series of aminoalkylindoles with potential for imaging the neuronal cannabinoid receptor by positron emission tomography. J Med Chem 48:5813–5822
27. Deng H, Gifford AN, Zvonok AM, Cui G, Li X, Fan P, Deschamps JR, Flippen-Anderson JL, Gatley SJ, Makriyannis A (2005) Potent cannabinergic indole analogues as radioiodinatable brain imaging agents for the CB1 cannabinoid receptor. J Med Chem 48:6386–6392
28. Järbe TU, Deng H, Vadivel SK, Makriyannis A (2011) Cannabinergic aminoalkylindoles, including AM678 = JWH018 found in 'Spice', examined using drug (Δ^9-tetrahydrocannabinol) discrimination for rats. Behav Pharmacol 22:498–507
29. Dziadulewicz EK, Bevan SJ, Brain CT, Coote PR, Culshaw AJ, Davis AJ, Edwards LJ, Fisher AJ, Fox AJ, Gentry C, Groarke A, Hart TW, Huber W, James IF, Kesingland A, La Vecchia L, Loong Y, Lyothier I, McNair K, O'Farrell C, Peacock M, Portmann R, Schopfer U, Yaqoob M, Zadrobilek J (2007) Naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone: a potent, orally bioavailable human CB1/CB2 dual agonist with antihyperalgesic properties and restricted central nervous system penetration. J Med Chem 50:3851–3856
30. Liu J, Obando D, Liao V, Lifa T, Codd R (2011) The many faces of the adamantyl group in drug design. Eur J Med Chem 46:1949–1963