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ORIGINAL ARTICLE

Two new-type cannabimimetic quinolinyl carboxylates, QUPIC and QUCHIC, two new cannabimimetic carboxamide derivatives, ADB-FUBINACA and ADBICA, and five synthetic cannabinoids detected with a thiophene derivative α-PVT and an opioid receptor agonist AH-7921 identified in illegal products

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Abstract We identified two new-type cannabimimetic quinolinyl carboxylates, quinolin-8-yl 1-pentyl-(1*H*-indole)-3-carboxylate (QUPIC, **1**) and quinolin-8-yl 1-(cyclohexylmethyl)-1*H*-indole-3-carboxylate (QUCHIC, **2**); and two new cannabimimetic carboxamide derivatives, *N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (ADB-FUBINACA, **3**) and *N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1*H*-indole-3-carboxamide (ADBICA, **4**), as designer drugs in illegal products. Compound **3** was reported to have a potent affinity for cannabinoid CB_1 receptor by Pfizer in 2009, but this is the first report of its detection in illegal products. No chemical or pharmacological data for compounds **1**, **2**, and **4** have appeared until now, making this the first report on these compounds. We also detected synthetic cannabinoids, APICA *N*-(5-fluoropentyl) analog (**5**), APINACA *N*-(5-fluoropentyl) analog (**6**), UR-144 *N*-(5-chloropentyl) analog (**7**), JWH-122 *N*-(5-chloropentyl) analog (**8**), and AM-2201 4-methoxynaphthyl analog (4-MeO-AM-2201, **9**) herein as newly distributed designer drugs in Japan. It is of interest that compounds **1** and **2** were detected with their synthetic component, 8-quinolinol (**10**). A stimulant thiophene analog, α-pyrrolidinovalerothiophenone (α-PVT, **11**), and an opioid receptor agonist, 3,4-dichloro-*N*-([1-(dimethylamino)cyclohexyl]methyl)benzamide (AH-7921, **12**), were also detected as new types of designer drugs coexisting with several synthetic cannabinoids and cathinone derivatives in illegal products.

Keywords Quinolin-8-yl 1-pentyl-(1*H*-indole)-3-carboxylate (QUPIC) · Quinolin-8-yl 1-(cyclohexylmethyl)-1*H*-indole-3-carboxylate (QUCHIC) · *N*-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (ADB-FUBINACA) · *N*-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1*H*-indole-3-carboxamide (ADBICA) · Synthetic cannabinoids · α-Pyrrolidinovalerothiophenone (α-PVT)

Introduction

The number of new psychotropic substances—not only synthetic cannabinoids but also other types of substances such as cathinone derivatives—has been increasing in Japan and in European countries year by year [1–10]. To prevent the abuse of these drugs, a total of 106 substances, including 35 synthetic cannabinoids, 17 cathinone derivatives, 26 phenethylamines, 13 tryptamines, 4 piperazines, 10 others, and 1 plant extract, were controlled as designated substances (Shitei-Yakubutsu) under the Pharmaceutical Affairs Law in Japan as of January 2013. Moreover, among them, 3 of the phenethylamines (2C-I, 2C-T-2, and 2C-T-4) have been strictly regulated as narcotic substances in Japan since January 2008. In August 2012, 2 synthetic cannabinoids (cannabicyclohexanol and JWH-018) and 2 cathinone derivatives (MDPV and mephedrone) were also classified as new narcotic substances.

We have been conducting an ongoing survey of designer drugs in the Japanese illegal market, and our

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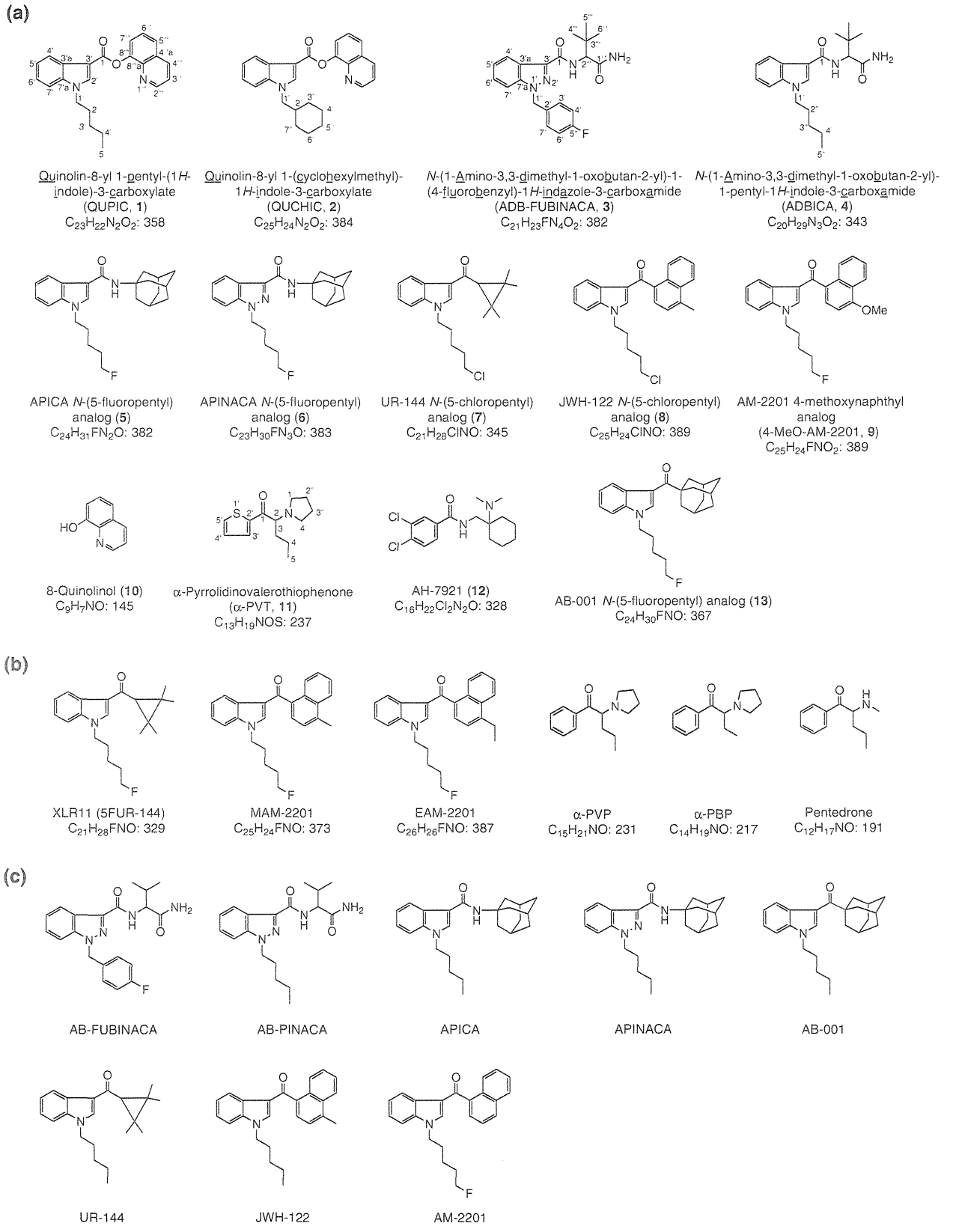

Fig. 1 Structures of newly detected (**1**–**12**, **a**), detected but known (**b**), and related compounds (**c**)

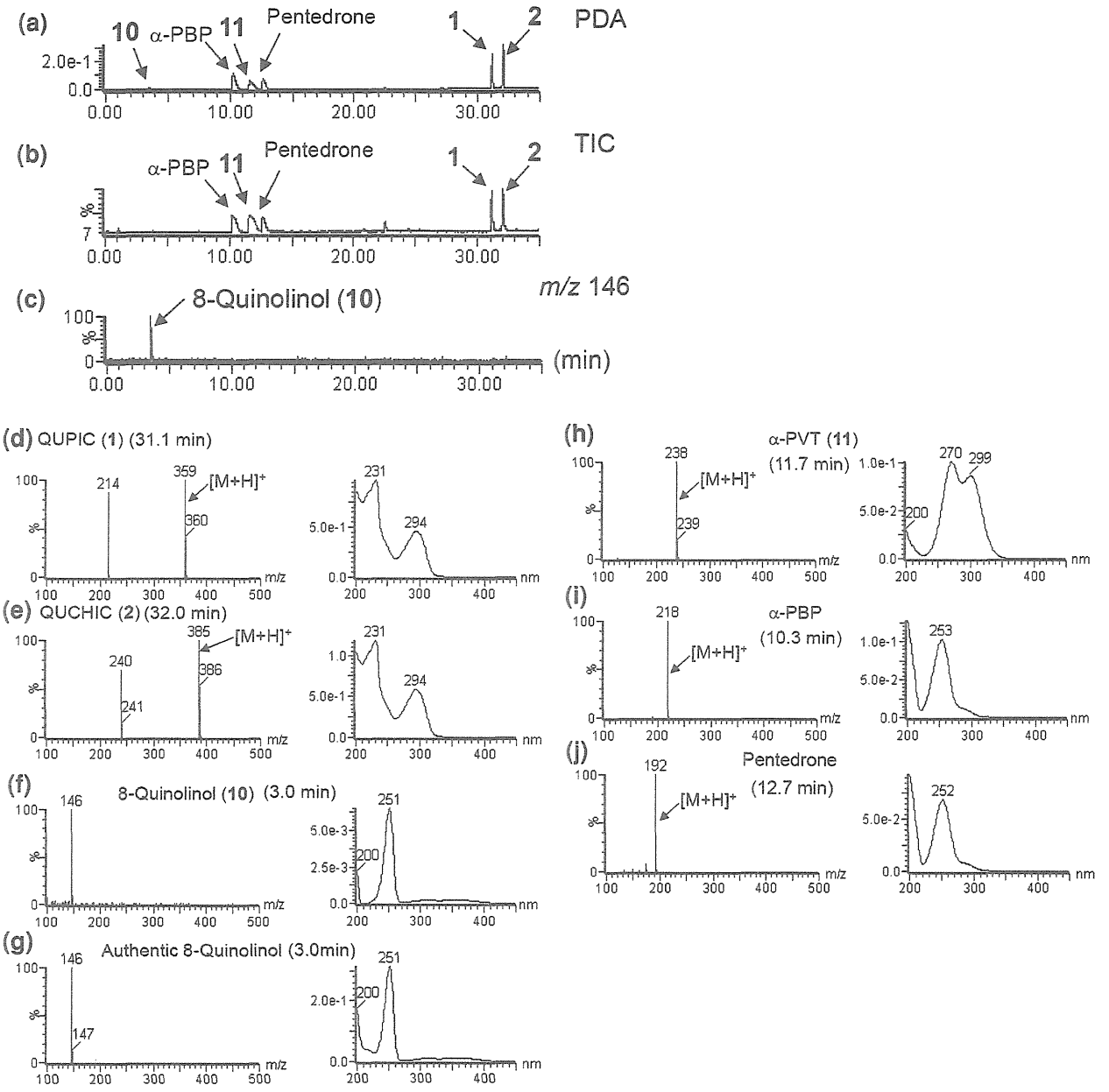

Fig. 2 Liquid chromatography–mass spectrometry (LC–MS) analysis of product A. Liquid chromatography–ultraviolet-photodiode array (LC–UV-PDA) chromatogram (**a**), total ion chromatogram (TIC) (**b**), and mass chromatogram at *m/z* 146 (**c**) using elution program (2). Ultraviolet (UV) and electrospray ionization (ESI) mass spectra of peaks **1** (**d**), **2** (**e**), **10** (**f**), **11** (**h**), α-PBP (**i**), pentedrone (**j**), and authentic 8-quinolinol (**g**) obtained by LC–MS

survey has revealed nine synthetic cannabinoids (**1**–**9**), a substance (**10**) that is a synthetic component of **1** or **2**, and two other psychotropic substances (**11** and **12**) newly detected in the present study (Fig. 1a). In this article, we describe our identification of these newly detected compounds in detail.

Materials and methods

Samples for analysis

The analyzed samples were purchased on the Internet between July 2012 and January 2013 as chemical-type or

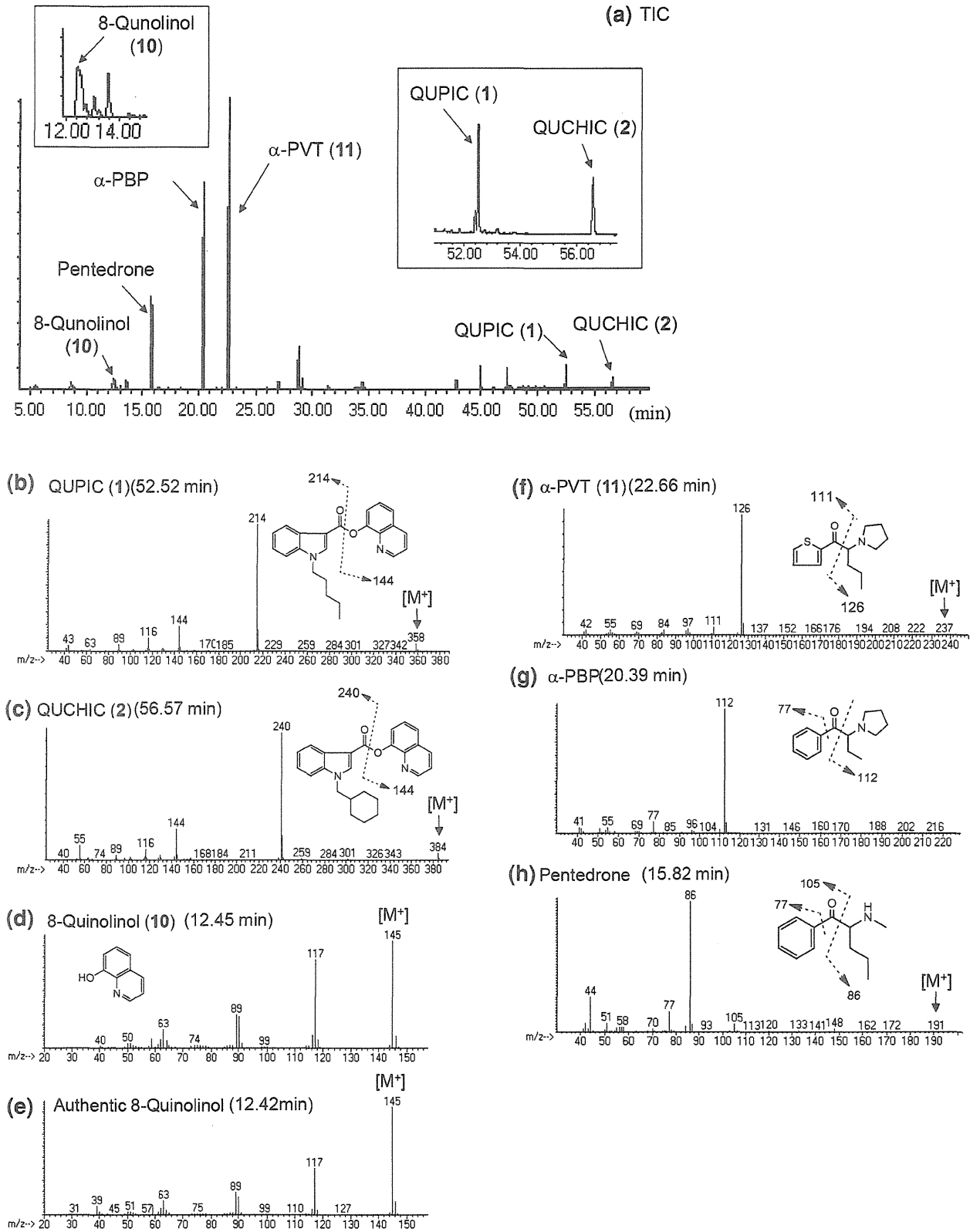

Fig. 3 Gas chromatography–mass spectrometry (GC–MS) analysis of product A. TIC (**a**) and electron ionization (EI) mass spectra of peaks **1** (**b**), **2** (**c**), **10** (**d**), **11** (**f**), α-PBP (**g**), pentedrone (**h**), and authentic 8-quinolinol (**e**)

herbal-type products being sold in Japan. Each of the herbal-type products (A–J) contained about 3 g of mixed dried plants. The chemical product K, which was called "Fragrance Powder," consisted of about 400 mg of white powder.

Chemicals and reagents

Authentic APICA *N*-(5-fluoropentyl) analog (**5**), APINACA *N*-(5-fluoropentyl) analog (**6**), UR-144 *N*-(5-chloropentyl) analog (**7**), α-PBP, pentedrone, α-PVP, XLR11 (5FUR-144), MAM-2201, AH-7921 (**12**), and EAM-2201 were purchased from Cayman Chemical (Ann Arbor, MI, USA). 8-Quinolinol (**10**) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Other compounds (**1**–**4**, **8**, and **9**) were isolated from herbal or chemical products. All other common chemicals and solvents were of analytical reagent grade or HPLC grade. As solvents for nuclear magnetic resonance (NMR) spectroscopy, CD_3OD (99.96 %), CD_3OH (99.8 %), $CDCl_3$ (99.96 %), benzene-d_6 (99.96 %), and dimethyl sulfoxide (DMSO)-d_6 (99.96 %) were purchased from the ISOTEC division of Sigma-Aldrich (St. Louis, MO, USA).

Preparation of sample solution

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

Table 1 Nuclear magnetic resonance (NMR) data for QUPIC (**1**) and QUCHIC (**2**)

No.	QUPIC (**1**)		QUCHIC (**2**)	
	^{13}C	^{1}H	^{13}C	^{1}H
1	165.2	–	165.2	–
2′	137.7	8.33, 1H, s	138.2	8.28, 1H, s
3′	106.3	–	106.2	–
3′a	128.6	–	128.5	–
4′	122.4	8.13, 1H, brd, J = 7.9 Hz	122.3	8.13, 1H, d, J = 7.9 Hz
5′	123.1	7.24, 1H, ddd, J = 7.9, 6.9, 1.0 Hz	123.0	7.23, 1H, td, J = 7.9, 1.0 Hz
6′	124.0	7.31, 1H, ddd, J = 7.9, 6.9, 1.0 Hz	124.0	7.30, 1H, td, J = 7.9, 1.0 Hz
7′	111.7	7.57, 1H, brd, J = 7.9 Hz, overlapped	111.9	7.55, 1H, d, J = 7.9 Hz, overlapped
7′a	138.3	–	138.6	–
1″	48.0	4.33, 2H, t, J = 7.2 Hz	54.2	4.15, 2H, d, J = 7.2 Hz
2″	30.9	1.95, 2H, q, J = 7.2 Hz	39.9	1.97, 1H, m
3″	30.1	1.37, 2H, m, overlapped	–	–
4″	23.4	1.40, 2H, m, overlapped	–	–
5″	14.3	0.92, 3H, t, J = 7.2 Hz	27.4	1.69, 2H, m, overlapped
3″/7″	–	–	31.9	1.66, 2H, m, overlapped
				1.09, 2H, m
4″/6″	–	–	26.8	1.28, 2H, m, overlapped
				1.75, 2H, m
1‴	–	–	–	–
2‴	151.4	8.83, 1H, dd, J = 4.1, 1.7 Hz	151.4	8.83, 1H, dd, J = 4.2, 1.7 Hz
3‴	123.1	7.56, 1H, m, overlapped	123.1	7.52, 1H, dd, J = 8.3, 4.2 Hz
4‴	138.1	8.41, 1H, dd, J = 8.3, 1.4 Hz	138.1	8.41, 1H, dd, J = 8.3, 1.7 Hz
4‴a	131.2	–	131.2	–
5‴	127.0	7.89, 1H, dd, J = 7.6, 1.4 Hz	127.0	7.89, 1H, brdd, J = 7.6, 1.4 Hz
6‴	127.8	7.67, 1H, t, J = 7.6 Hz	127.8	7.66, 1H, t, J = 7.6 Hz
7‴	123.7	7.63, 1H, dd, J = 7.6, 1.4 Hz	123.7	7.63, 1H, dd, J = 7.6, 1.4 Hz
8‴	148.5	–	148.5	–
8‴a	142.8	–	142.8	–

Recorded in CD_3OD at 600 MHz (^{1}H) or 150 MHz (^{13}C); data in δ ppm

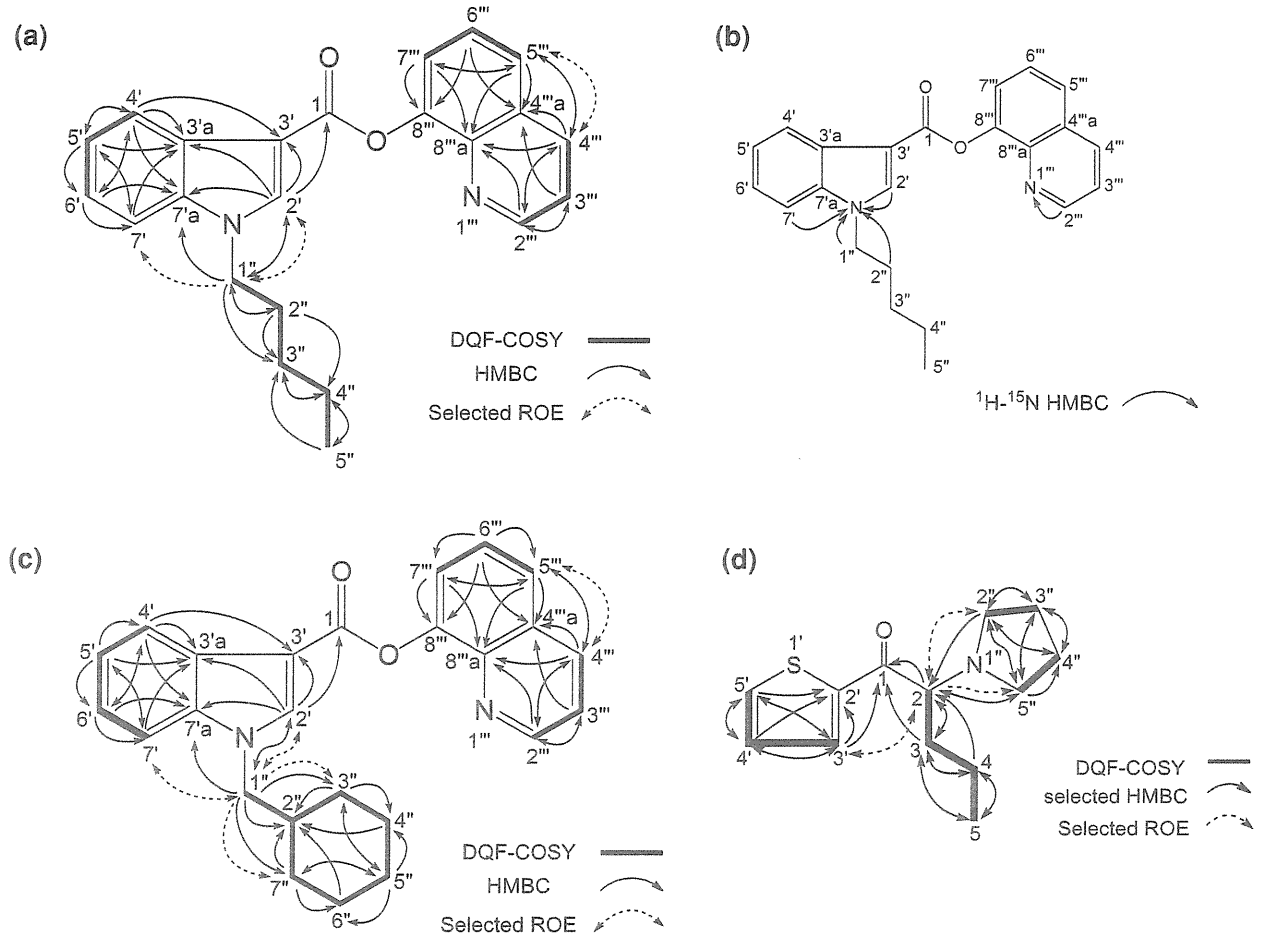

Fig. 4 Double quantum filtered correlation spectroscopy (DQF-COSY), selected heteronuclear multiple-bond correlation (HMBC), and selected rotating frame nuclear Overhauser effect (ROE) correlations (**a**) and ^{1}H–^{15}N HMBC (**b**) for compound **1** (QUPIC), and DQF-COSY, selected HMBC, and selected ROE correlations for compound **2** (QUCHIC, **c**) and compound **11** (α-PVT, **d**)

Analytical conditions

Each sample solution was analyzed by ultra-performance liquid chromatography–electrospray ionization–mass spectrometry (UPLC–ESI–MS) and gas chromatography–mass spectrometry (GC–MS) in the electron ionization (EI) mode according to our previous report [11]. Two elution programs were used in the LC–MS analysis. Programs (1) and (2) were used for synthetic cannabinoids and for the other compounds including cathinone derivatives, respectively [11]. The obtained GC mass spectra were compared to those of an EI-MS library [Mass Spectra of Designer Drugs 2012 (Wiley-VCH, Weinheim, Germany)]. In addition, our in-house EI-MS library of designer drugs obtained by our continuous survey of illegal products and commercially available reagents was also used for structural elucidation.

The accurate mass numbers of the target compounds were measured by liquid chromatography–quadrupole time-of-flight–mass spectrometry (LC–QTOF–MS) system consisting of an Acquity UPLC and Xevo QTOFMS (Waters, Milford, MA, USA) with a photodiode array (PDA) detector (Waters). The sample solutions were separated with an ACQUITY UPLC HSS C18 column (150 mm × 2.1 mm i.d., particle size 1.8 μm; Waters) at 50 °C. Each analysis was carried out with a binary mobile phase consisting of solvent A (10 mM ammonium formate in water, pH 3.0) and solvent B (0.1 % formic acid in acetonitrile). The elution program was: 87 % A/13 % B (0.5-min hold) to 50 % A/50 % B (0.5–10 min), and up to 10 % A/90 % B (10–15 min, 5-min hold) at a flow rate of 0.4 ml/min. The injection volume was 1 μl, and the wavelength of the PDA detector for screening was set from 210 to 400 nm. The MS conditions were: ion source, ESI in the positive mode; ion source temperature, 120 °C; desolvation gas, nitrogen at a flow rate of 800 l/h at 400 °C; capillary and cone voltages, 3000 and 30 V, respectively; collision energy, 27 V; mass spectral range,

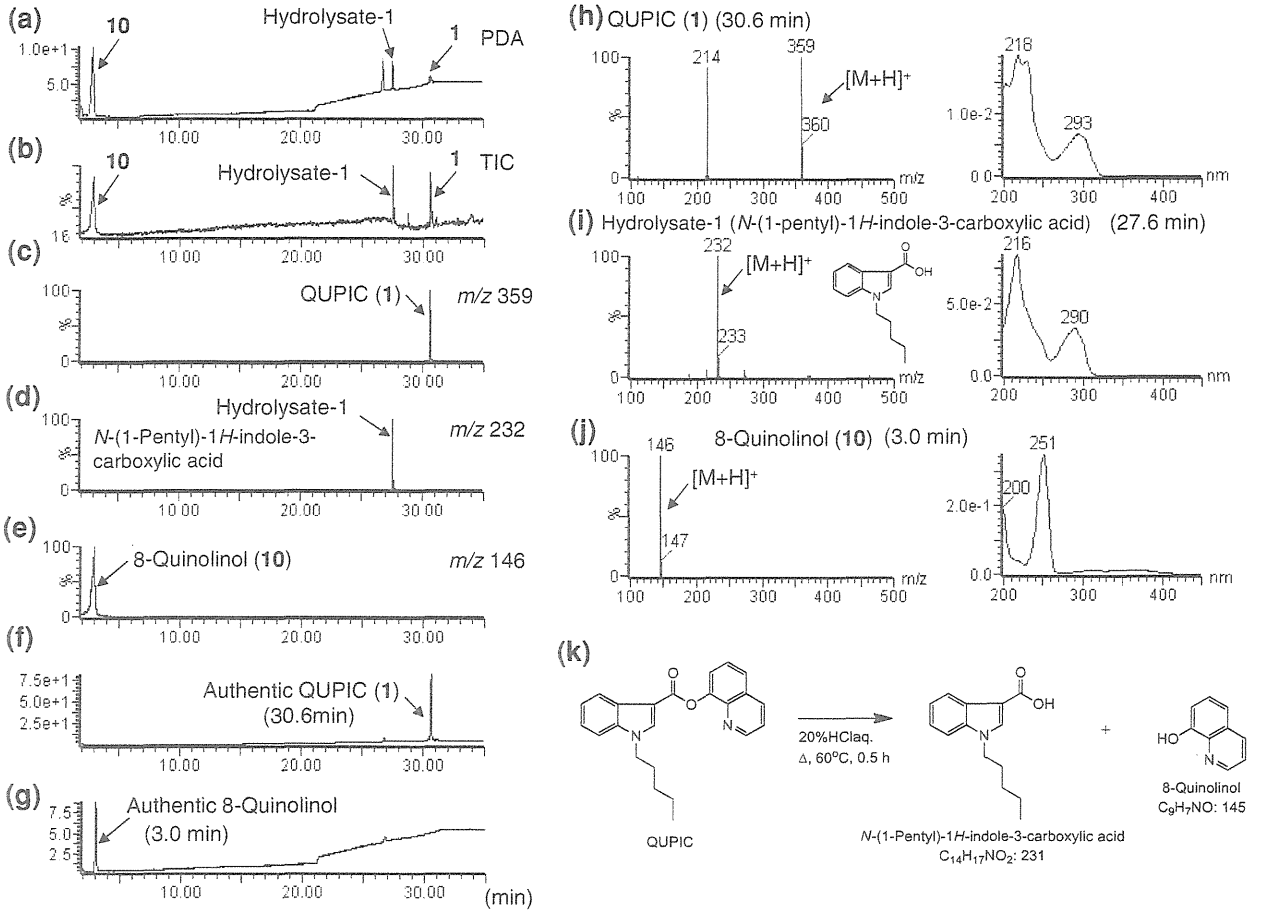

Fig. 5 LC–MS analysis of hydrolysates of QUPIC (**1**). LC–UV-PDA chromatogram (**a**), TIC (**b**), and mass chromatograms at *m/z* 359 (**c**), 232 (**d**), and 146 (**e**) of the reaction mixture of QUPIC (**1**) after acid hydrolysis using elution program (2). LC–UV-PDA chromatograms of authentic QUPIC (**1**, **f**) and authentic 8-quinolinol (**g**). UV and ESI mass spectra of peaks **1** (**h**), hydrolysate-1 (**i**), and **10** (**j**). The putative hydrolysis mechanism of QUPIC (**1**) in acidic conditions (**k**)

m/z 50–1000. Leucine enkephalin [*m/z* 278.1141 and 508.20783 ($[M+H]^+$)] was used as a substance for lock mass ions during the measurements.

The NMR spectra were obtained on ECA-800 and 600 spectrometers (JEOL, Tokyo, Japan). Assignments were made via ^{1}H NMR, ^{13}C NMR, heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), ^{15}N HMBC, double quantum filtered correlation spectroscopy (DQF-COSY), and rotating frame nuclear Overhauser effect (ROE) spectra.

Isolation of compound 1

A 3-g sample of mixed dried plants (product I) was extracted with 250 ml of chloroform by ultrasonication for 30 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, Darmstadt, Germany), which was then developed using hexane/ethyl acetate (3:1). A portion of the silica gel containing a target compound in the TLC plate was detected under ultraviolet (UV) light (254 nm). It was then scraped from the plate and eluted with chloroform to give fraction 1, which was further purified by repeated preparative TLC with toluene/chloroform (1:1) and then recrystallized by methanol. Finally, compound **1** (130 mg) was obtained as a brown oil.

Isolation of compound 2

A 3-g sample of mixed dried plants (product A) was extracted with 250 ml of chloroform by ultrasonication for 30 min. The extractions were repeated three times, and the supernatant fractions were combined and evaporated to dryness. Separation of the extract by repeated preparative TLC [hexane/acetone (2:1) and toluene/ethyl acetate (10:1)] and silica gel column chromatography [toluene/

ethyl acetate (10:1, 9:1, 8:2, 7:3, 6:4)] gave compound **2** (13 mg) as a yellow oil.

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

Each 3-g sample of mixed dried plants (products J and B) was extracted with 250 ml of chloroform by ultrasonication for 30 min. The extractions were repeated three times, and the supernatant fractions were combined and evaporated to dryness. Each extract was purified by preparative TLC [hexane/ethyl acetate (1:2)] to obtain compound **3** (65 mg) as a yellow solid and compound **4** (81 mg) as a pale yellow solid.

Isolation of compound **8**

A 3-g sample of mixed dried plants (product F) was extracted by the same method as described above. The final separation of the extract by silica gel column chromatography [toluene/chloroform (5:5, 6:4)] resulted in the isolation of compound **8** (10 mg) as a yellow solid.

Isolation of compound **9**

A 3-g sample of mixed dried plants (product G) was extracted by the same method described above. Separation of the extract by preparative TLC [hexane/ethyl acetate (3:1)] and recrystallization in methanol gave compound **9** (145 mg) as a pale yellow solid.

Hydrolysis of compounds **1** and **2**

A 2-mg sample of each compound was dissolved in 20 % HCl aqueous solution and heated at 60 °C for 30 min. The reaction mixture was evaporated under a nitrogen stream, and the residue was redissolved in methanol. The solution was then analyzed by LC–MS and LC–QTOF–MS.

Results and discussion

Identification of unknown peaks **1**, **2**, **10**, and **11**

Four unknown peaks, **1**, **2**, **10**, and **11**, were detected along with known cathinone derivatives, α-PBP, and pentedrone in the LC–MS and GC–MS chromatograms for product A, as shown in Figs. 2a, b, 3a. In the LC–MS chromatograms using elution program (2) for the analysis of cathinones and others [11], two unknown peaks (**1** and **2**) at 31.1 and 32.0 min showed protonated molecular ion $[M+H]^+$ signals at *m/z* 359 and 385, respectively (Fig. 2d, e). The UV spectra of both compounds showed the same absorbance maxima at 231 and 294 nm (Fig. 2d, e).

In addition, peaks **1** and **2** were detected at 10.2 and 12.2 min under elution program (1) for the analysis of cannabinoids (data not shown) [11]. The total ion chromatogram (TIC) by GC–MS showed peak **1** at 52.52 min and peak **2** at 56.57 min (Fig. 3a), which indicated putative molecular ion signals at *m/z* 358 and 384, respectively (Fig. 3b, c). Unknown peak **10** was presumed to be 8-quinolinol, based on the fragment patterns of the GC–MS analysis (Fig. 3d) and LC–MS analysis (Fig. 2c, f). Peak **10** was confirmed to be identical to 8-quinolinol by direct comparison of the data to those of the authentic compound (Figs. 2g, 3e). After isolation of compounds **1** and **2**, their accurate mass spectra were measured by LC–QTOF–MS in the positive mode. The ion peaks observed at *m/z* 359.1764 and 385.1908 suggested that the protonated molecular formulae of compounds **1** and **2** were $C_{23}H_{23}N_2O_2$ (calcd. 359.1760) and $C_{25}H_{25}N_2O_2$ (calcd. 385.1916), respectively.

The structure of compound **1** was elucidated by NMR analysis (Table 1; Fig. 4a, b). The ^1H and ^{13}C NMR spectra of compound **1** suggested the existence of 22 protons and 23 carbons as shown in Table 1. The analyses by DQF-COSY, HMQC, HMBC, and one-dimensional (1D) ROE spectra for compound **1** revealed the presence of an *N*-(1-pentyl)-1*H*-indole-3-carbonyl moiety (Fig. 4a). In addition, the NMR spectra of the remaining C_9H_6NO unit

Table 2 NMR data for α-PVT (**11**)

No.	^{13}C	^1H
1	188.7	–
2	63.1	4.89, 1H, t, J = 4.8 Hz
3	32.9	2.31, 1H, m
		2.03, 1H, m, overlapped
4	19.8	1.47, 1H, m
		1.30, 1H, m
5	13.9	0.93, 3H, t, J = 7.2 Hz
1′	–	–
2′	143.4	–
3′	134.5	7.88, 1H, d, J = 4.8 Hz
4′	129.4	7.23, 1H, t, J = 4.8 Hz
5′	137.7	7.85, 1H, d, J = 4.8 Hz
2″	52.9	3.77, 1H, m
		2.82, 1H, m
3″	23.9	2.15, 1H, m, overlapped
		1.98, 1H, m, overlapped
4″	23.6	2.18, 1H, m, overlapped
		2.08, 1H, m, overlapped
5″	48.6	3.89, 1H, m
		3.63, 1H, m

Recorded in $CDCl_3$ at 600 MHz (^1H) or 150 MHz (^{13}C); data in δ ppm

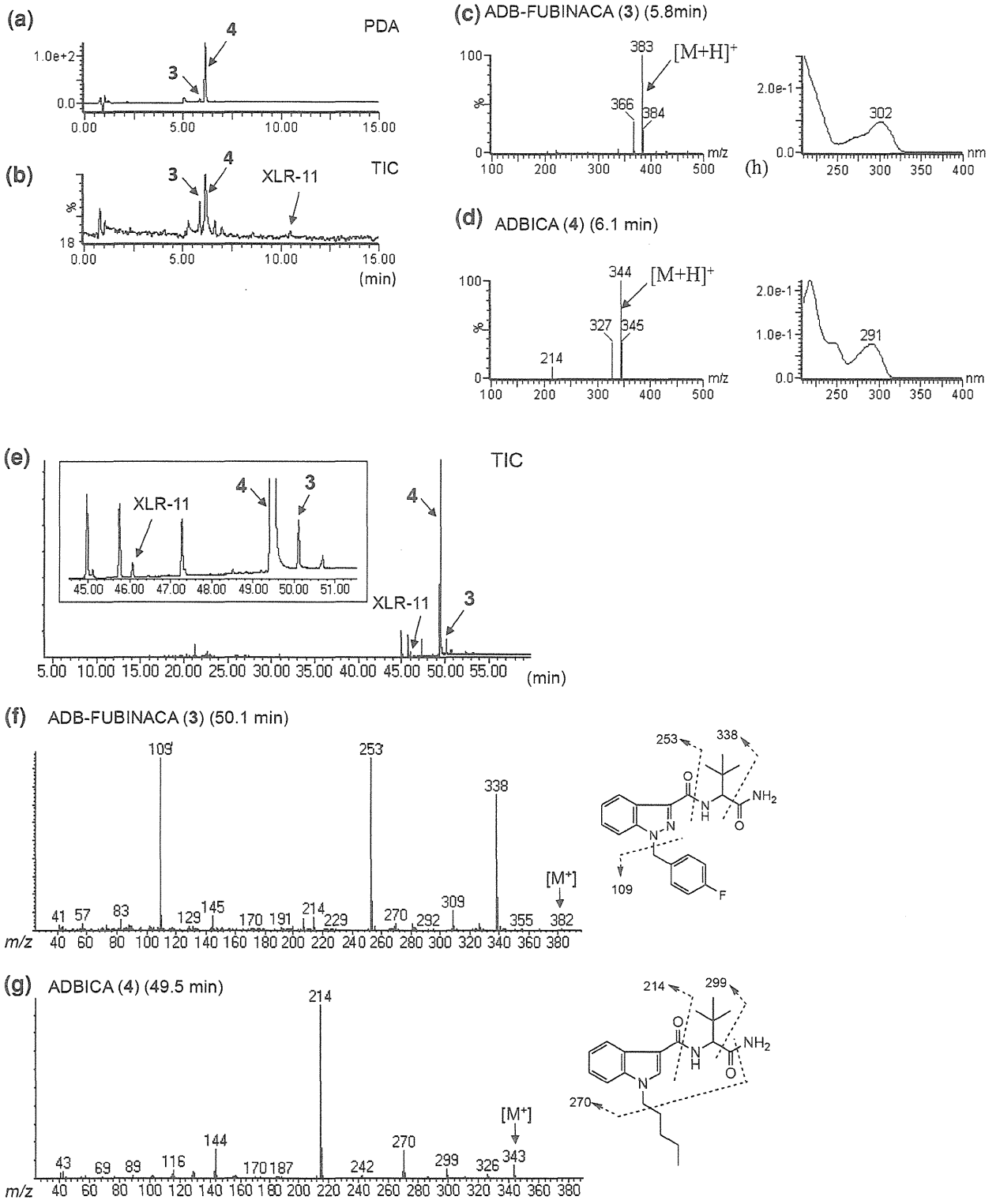

Fig. 6 LC–MS and GC–MS analyses of product B. LC–UV-PDA chromatogram (**a**) and TIC (**b**) using elution program (1) obtained by LC–MS. UV and ESI mass spectra of peaks **3** (**c**) and **4** (**d**). TIC (**e**) and EI mass spectra of peaks **3** (**f**) and **4** (**g**) obtained by GC–MS

suggested the presence of a quinolinol group, and the observed ^{15}N HMBC correlations (Fig. 4b) and the fragment ions at *m/z* 214 and 144 of peak **1** revealed by the GC–MS analysis (Fig. 3b) supported the existence of *N*-(1-pentyl)-1*H*-indole-3-carbonyl and quinolinol moieties.

On the basis of the two-dimensional (2D) NMR correlations and the quaternary carbon signals at δ_C 165.2 (C-1) and δ_C 148.5 (C-8‴), we concluded that the *N*-(1-pentyl)-1*H*-indole moiety was attached to the 8-quinolinol moiety by an ester linkage at position-1. However, the ester bond between the *N*-(1-pentyl)-1*H*-indole-3-carbonyl and quinolinol moieties was not clear. To determine the structure of compound **1**, we hydrolyzed it under acidic conditions, and then analyzed the reaction mixture by LC–MS (Fig. 5). The peak at 3.0 min was identified as 8-quinolinol (**10**) by direct comparison of its spectral data with those of the purchased compound (Fig. 5e, g, j). The other peak at 27.6 min showed the ion signal at *m/z* 232 (Fig. 5d, i). Its LC–QTOF–MS analysis showing the ion signal at 232.1353 suggested the protonated molecular formula of $C_{14}H_{18}NO_2$ (calcd. 232.1338) for the expected hydrolysate, *N*-(1-pentyl)-1*H*-indole-3-carboxylic acid (Fig. 5k). Thus, the structure of compound **1** was determined as quinolin-8-yl 1-pentyl-(1*H*-indole)-3-carboxylate and was named QUPIC (**1**).

Given the estimated protonated molecular formulae of compounds **2** and **1** of $C_{25}H_{25}N_2O_2$ and $C_{23}H_{23}N_2O_2$, respectively, the elemental difference between them was C_2H_2. The ^1H and ^{13}C NMR spectra of compound **2** were very similar to those of compound **1** except for the

Table 3 NMR data for ADB-PINACA (**3**) and ADBICA (**4**)

No.	AB-FUBINACA[a,c]	ADB-PINACA (**3**)[a]		ADBICA (**4**)[b]	
	^{13}C	^{13}C	^1H	^{13}C	^1H
1	161.2	160.6	–	165.0	–
2′	–	–	–	131.5	7.70, 1H, s
3′	137.1	136.9	–	110.3	–
3′a	122.3	122.2	–	125.4	–
4′	121.8	121.8	8.17, 1H, d, J = 7.9 Hz	120.2	7.99, 1H, m
5′	122.8	122.8	7.29, 1H, t, J = 7.9 Hz	121.6	7.24, 1H, m, overlapped
6′	127.0	127.1	7.46, 1H, brt, J = 7.9 Hz	122.5	7.26, 1H, m, overlapped
7′	110.6	110.7	7.79, 1H, d, J = 7.9 Hz	110.3	7.36, 1H, m
7′a	140.6	140.6	–	136.6	–
1″	51.6	51.6	5.78, 2H, s	46.9	4.10, 2H, t, J = 7.2 Hz
2″	133.0, J = 2.9 Hz	133.0, J = 2.9 Hz	–	29.7	1.84, 2H, q, J = 7.2 Hz
3″/7″	129.5, J = 8.7 Hz	129.5, J = 8.7 Hz	7.31, 2H, dd, J = 7.2, 1.3 Hz, overlapped	–	–
4″/6″	115.5, J = 21.7 Hz	115.6, J = 21.7 Hz	7.16, 1H, brd, J = 8.6 Hz, 7.15, 1H, brd, J = 8.6 Hz	–	–
3″	–	–	–	29.0	1.29, 2H, m, overlapped
4″	–	–	–	22.3	1.32, 2H, m, overlapped
5″	161.6, d, J = 242.8 Hz	160.9, d, J = 244.2 Hz	–	13.9	0.87, 3H, t, J = 6.9 Hz
1‴	172.6	171.7	–	173.2	–
2‴	56.9	58.7	4.45, 1H, d, J = 9.6 Hz	59.7	4.68, 1H, d, J = 9.3 Hz
3‴	31.2	34.6	–	34.7	–
4‴	19.4	–	–	–	–
5‴	18.1	–	–	–	–
4‴/5‴/6‴	–	26.6	0.98, 9H, s	26.8	1.13, 9H, s
1-CONH	–	–	7.60, 1H, d, J = 9.6 Hz	–	6.7, 1H, d, J = 8.9 Hz
1‴-CONH$_{2a}$	–	–	7.27, 1H, brs, overlapped	–	6.41, 1H, brs
1‴-CONH$_{2b}$	–	–	7.72, 1H, brs	–	5.63, 1H, brs

Recorded at 600 MHz (^1H) or 150 MHz (^{13}C); data in δ ppm

[a] Recorded in dimethyl sulfoxide (DMSO)-d_6

[b] Recorded in $CDCl_3$

[c] From Uchiyama et al. [15]

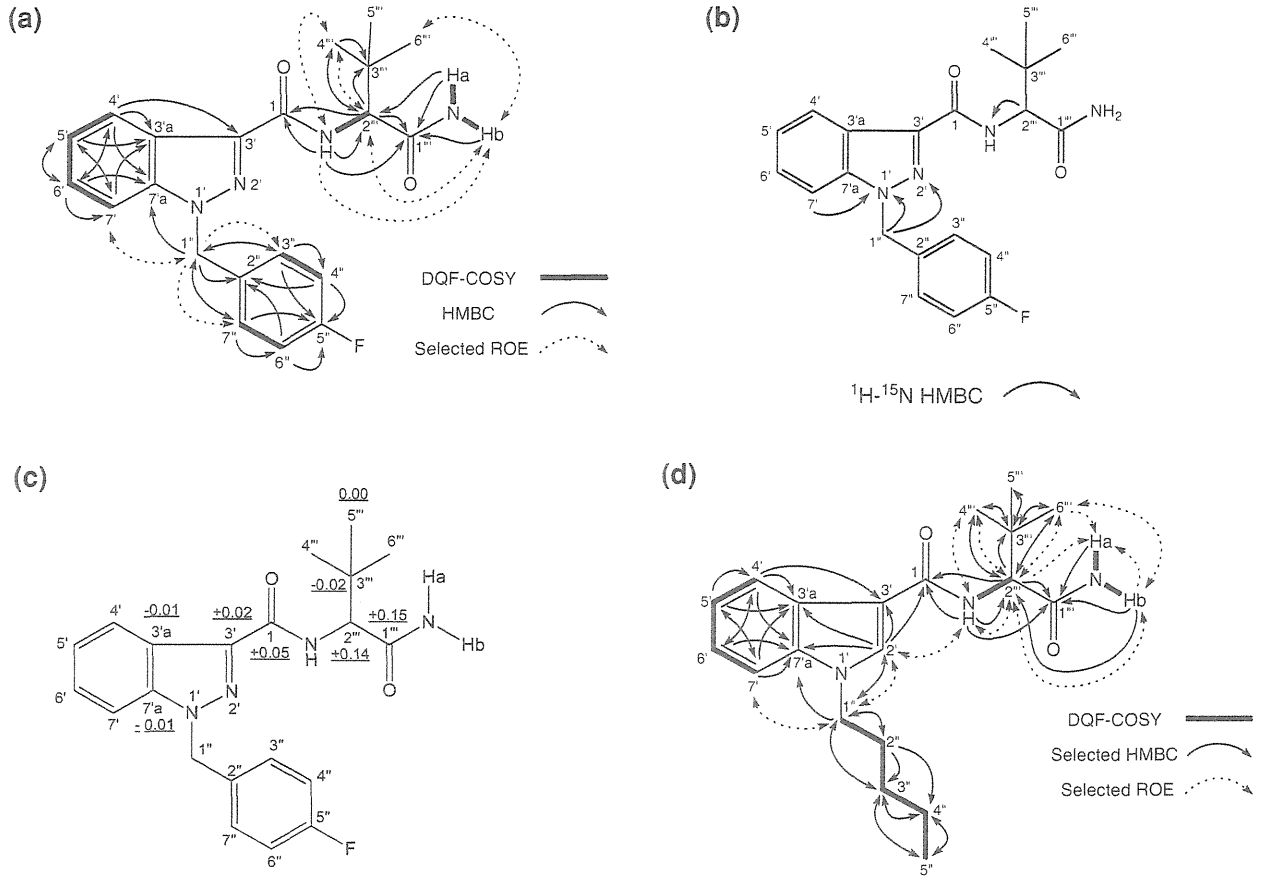

Fig. 7 DQF-COSY, selected HMBC, and selected ROE correlations (**a**) and ^{1}H–^{15}N HMBC correlations (**b**) for compound **3** (ADB-FUBINACA); DQF-COSY, selected HMBC, and selected ROE correlations for compound **4** (ADBICA, **d**), and deuterium-induced isotope shifts of NH protons for the ^{13}C NMR signals of compound **3** in CD_3OD (**c**)

N-cycloalkyl moiety (position-1″ to 7″) as shown in Table 1. The observed DQF-COSY, HMQC, HMBC, 1D ROE correlations and the quarternary carbon signals at δ_C 165.2 (C-1) and δ_C 148.5 (C-8‴) for compound **2** suggested the presence of the *N*-(1-cyclohexylmethyl)-1*H*-indole-3-carbonyl and quinolinol moieties, and an ester linkage between these two moieties at position-1, similar to compound **1** (Fig. 4c; Table 1). Compound **2** was similarly hydrolyzed under acidic conditions to determine the structure (Supplementary material, Fig. S1). The structure of compound **2** was deduced to be quinolin-8-yl 1-(cyclohexylmethyl)-1*H*-indole-3-carboxylate and was named QUCHIC (**2**).

The chemical and pharmacological data on compounds **1** and **2** have not been reported, although the quinoline derivatives have been synthesized as cannabinoid receptor ligands [12]. Even though compounds **1** and **2** are being sold under the names "PB-22" and "BB-22," respectively, on illegal drug markets on the Internet, we named these compounds QUPIC and QUCHIC, respectively, taking into account the IUPAC (International Union of Pure and Applied Chemistry) naming system and regulatory action.

Unknown peak **11** was detected together with two other peaks by LC–MS and GC–MS analyses of the same product A (Figs. 2h–j, 3f–h). The latter two peaks were readily found to be identical to α-PBP and pentedrone by direct comparison of the data with those of the purchased authentic compounds (data not shown). Both compounds were detected in European countries in 2011 [9, 13]. The LC–MS chromatogram demonstrated that unknown peak **11** at 11.7 min showed a protonated ion signal ($[M+H]^+$) at *m/z* 238 and absorbance maxima at 270 and 299 nm in the UV spectrum (Fig. 2h). The accurate mass spectrum was measured by LC–QTOF–MS in the positive mode. The ion peak observed at *m/z* 238.1252 suggested the protonated molecular formula of compound **11** to be $C_{13}H_{20}NOS$ (calcd. 238.1266). The LC–MS and GC–MS analyses revealed that product K mainly contained compound **11**. Therefore, product K was directly dissolved in $CDCl_3$ and analyzed by NMR spectroscopy. The ^{1}H and ^{13}C NMR

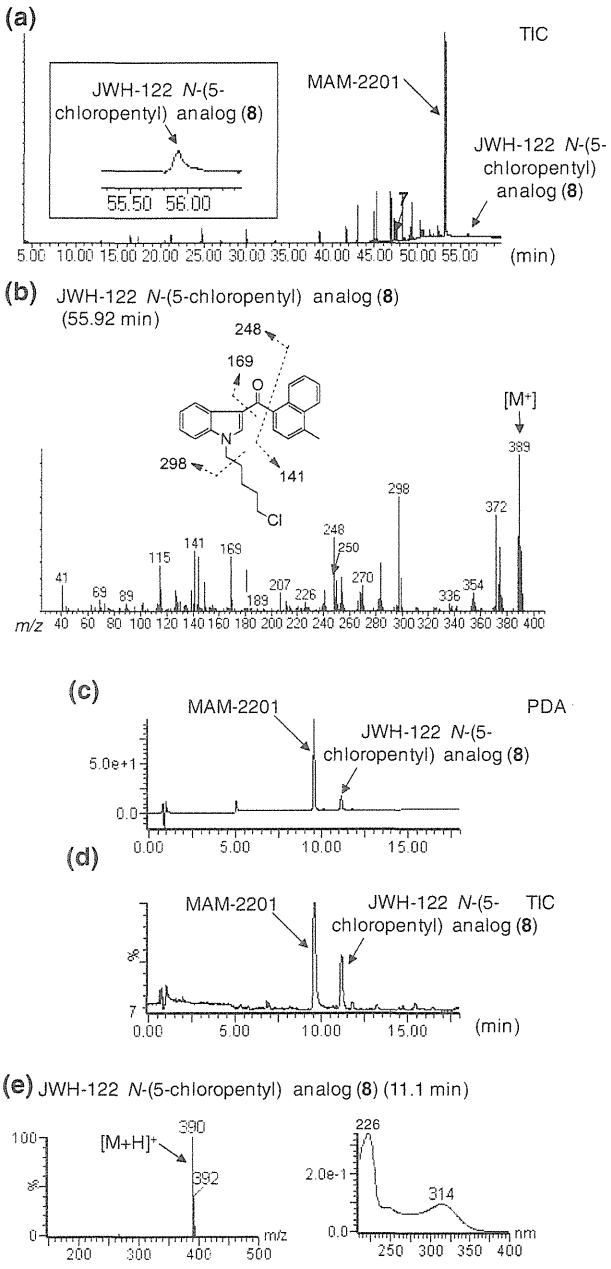

Fig. 8 GC–MS and LC–MS analyses of product F. TIC (**a**) and EI mass spectra of peak **8** (**b**) obtained by GC–MS analysis. LC–UV-PDA chromatogram (**c**) and TIC (**d**) using elution program (1) obtained by LC–MS. UV and ESI mass spectra of peak **8** (**e**)

spectra of compound **11** suggested the existence of 19 protons and 13 carbons (Table 2). The analyses by DQF-COSY, HMQC, HMBC, and 1D-ROE spectra for compound **11** suggested the presence of a 2-(pyrrolidin-1-yl)pentanoyl moiety (Fig. 4d). The ^{1}H, ^{13}C NMR, and 2D NMR spectra of the remaining C_4H_3S unit suggested the existence of a 2-substituted thiophene moiety (position-1′ to 5′) as shown in Fig. 4d. The connection of the thiophene moiety to the carbonyl group was shown by HMBC correlations to be from the aromatic proton (H-3′) to the carbonyl carbon (C-1) (Fig. 4d). Additionally, the major fragment ions at *m/z* 126 and 111 of peak **11** in GC–MS spectra suggested the presence of 1-butylpyrrolidine and thiophene-2-carbonyl moieties, respectively (Fig. 3f). Therefore, the structure of **11** was determined as α-pyrrolidinovalerothiophenone [α-PVT, IUPAC: 2-(pyrrolidin-1-yl)-1-(thiophen-2-yl)pentan-1-one]. Compound **11** (α-PVT) is a novel designer drug, and its chemical and pharmacological data have not been reported. However, chloro- or methyl-substituted (position-5′ in Fig. 4d) thiophene analogs of α-PVT have been reported as monoamine uptake inhibitors [14]. It is thus possible that compound **11** has similar inhibitory activity.

Identification of unknown peaks 3 and 4

Two unknown peaks **3** and **4** were detected together with the known synthetic cannabinoid XLR-11 (Fig. 1b, [11]) in the GC–MS and LC–MS chromatograms of product B (Fig. 6a, b, e). In the LC–MS chromatogram, two unknown peaks **3** and **4** at 5.8 and 6.1 min showed protonated ion signals at *m/z* 383 and 344 and absorbance maxima at 302 and 291 nm in UV spectra, respectively (Fig. 6c, d). In the GC–MS chromatogram, peaks **3** and **4** at 50.1 and 49.5 min showed putative molecular ion signals at *m/z* 382 and 343, respectively (Fig. 6f, g). After the isolation of compounds **3** and **4**, the accurate mass spectra were measured by LC–QTOF–MS in the positive mode. The observed ion peaks at *m/z* 383.1891 and 344.2347 suggested the protonated molecular formulae of compounds **3** and **4** to be $C_{21}H_{24}FN_4O_2$ (calcd. 383.1883) and $C_{20}H_{30}N_3O_2$ (calcd. 344.2338), respectively.

The ^{13}C NMR spectra of compound **3** was very similar to that of a known synthetic cannabinoid, AB-FUBINACA, except for a dimethylpropyl moiety (position-2‴ to 6‴) as shown in Table 3 and Fig. 1a, c [15]. The difference between the molecular formula of compound **3** and that of AB-FUBINACA ($C_{20}H_{21}FN_4O_2$) is the additional CH_2. The observed DQF-COSY, HMQC, HMBC, ^{15}N HMBC, and 1D ROE correlations of compound **3** suggested the presence of 1-(4-fluorobenzyl)-1*H*-indazole and (1-amino-3,3-dimethyl-1-oxobutan-2-yl)-carboxamide moieties (Fig. 7a, b). That is, compound **3** may have an additional methyl group at the 3‴-position in the structure of AB-FUBINACA. However, no HMBC correlation between the two moieties was observed. We therefore measured the deuterium isotope effect of the NH amide proton on the ^{13}C chemical shift to determine the connection between the two moieties. The ^{13}C NMR spectrum of compound **3**, measured in CD_3OH, was compared with that in CD_3OD. The isotope shift values for the ^{13}C NMR signals of this compound are

shown in Fig. 7c. The first, second, and third largest deuterium shifts (0.15, 0.14, and 0.05 ppm) were observed at the C-1‴ and C-2‴ positions of the 1-amino-3,3-dimethyl-1-oxobutane moiety and the C-1 position of the carboxamide moiety, respectively. The fourth largest shift of 0.02 ppm was attributed to the three-bond deuterium isotope effects of the NH amide proton on the indazole carbon (C-3′) and the 1-amino-3,3-dimethyl-1-oxobutane carbon (C-3‴). These results strongly indicate that the 1-(4-fluorobenzyl)-1*H*-indazole moiety is connected at the 3′-position of the indazole to the carboxyamide (1-CONH). In addition, the major fragment ion signals at *m/z* 109, 253, and 338 revealed by the GC–MS analyses (Fig. 6f) supported the presumed structure of compound **3**. Therefore, compound **3** was identified as *N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (Fig. 1a).

The *S*-form of compound **3** has been reported to have an affinity for the cannabinoid CB_1 receptor (K_i = 0.36 nM) that is 25- and 2.5-fold more potent than those of JWH-018 (K_i = 9.0 nM) and AB-FUBINACA (K_i = 0.9 nM), respectively [16, 17]. Considering its general properties, we propose a new name for this compound, ADB-FUBINACA (**3**), with agreement from Pfizer. This is the first case in which compound **3** has been detected in an illegal product.

The major fragment ion signals at *m/z* 214 and 144 of peak **4** (Fig. 6g) shown by the GC–MS analysis suggested the presence of a 1-pentyl-1*H*-indole-3-carbonyl moiety from comparison of mass fragment patterns of known

Table 4 NMR data for JWH-122 *N*-(5-chloropentyl) analog (**8**) and AM-2201 4-methoxynaphthyl analog (4-MeO-AM-2201) (**9**)

No.	JWH-122[a]	JWH-122 *N*-(5-Chloropentyl) analog (**8**)[b]		4-MeO-AM-2201 (**9**)[c]	
	^{13}C	^{13}C	^{1}H	^{13}C	^{1}H
1	192.2	191.5	–	190.5	–
2′	137.8	137.1	6.96, 1H, s, overlapped	138.9	7.83, 1H, s
3′	117.7	118.6	–	116.2	–
3′a	127.0	127.0	–	126.6	–
4′	122.9	123.9	9.13, 1H, d, J = 7.9 Hz	121.7	8.30, 1H, d, J = 7.6 Hz
5′	122.7	123.2	7.34, 1H, t, J = 7.9 Hz	122.3	7.29, 1H, t, J = 7.6 Hz, overlapped
6′	123.5	123.7	7.22, 1H, brt, J = 7.9 Hz	123.2	7.32, 1H, t, J = 7.6 Hz, overlapped
7′	109.9	109.9	6.95, 1H, d, J = 7.9 Hz	110.9	7.64, 1H, d, J = 7.6 Hz
7′a	137.0	137.2	–	136.7	–
1″	47.1	46.2	3.05, 2H, t, J = 7.2 Hz	46.0	4.23, 2H, t, J = 6.9 Hz
2″	29.5	28.8	0.93, 2H, m	29.0	1.76, 2H, quintet, J = 6.2 Hz
3″	28.9	23.9	0.71, 2H, m	21.9, d, J = 5.8 Hz	1.28, 2H, quintet, J = 6.2 Hz
4″	22.2	31.9	1.04, 2H, m	29.3, d, J = 18.8 Hz	1.63, 1H, quintet, J = 6.2 Hz, 1.58, 1H, quintet, J = 6.2 Hz
5″	13.9	44.3	2.85, 2H, t, J = 6.5 Hz	83.6, d, J = 161.8 Hz	4.40, 1H, t, J = 6.2 Hz, 4.32, 1H, t, J = 6.2 Hz
1‴	137.5	138.9	–	125.0	–
2‴	125.8	125.8	7.54, 1H, d, J = 6.9 Hz	127.9	7.70, 1H, d, J = 7.9 Hz
3‴	125.2	125.6	7.09, 1H, d, J = 6.9 Hz	103.1	7.06, 1H, d, J = 7.9 Hz
4‴	136.6	136.3	–	156.1	–
4‴a	132.8	133.3	–	130.8	–
5‴	124.2	124.5	7.83, 1H, brd, J = 8.4 Hz	121.7	8.25, 1H, m
6‴	126.1	126.4	7.29, 1H, m	125.7	7.55, 1H, m, overlapped
7‴	126.4	126.7	7.26, 1H, m	127.2	7.54, 1H, m, overlapped
8‴	126.6	127.4	8.61, 1H, brd, J = 7.6 Hz	125.4	8.12, 1H, m
8‴a	130.9	131.7	–	131.5	–
4‴-Me	19.8	19.6	2.42, 3H, s	–	–
4‴-OMe	–	–	–	55.9	4.06, 3H, s

Recorded at 600 MHz (^{1}H) or 150 MHz (^{13}C); data in δ ppm

[a] Recorded in $CDCl_3$ at 150 MHz (^{13}C)

[b] Recorded in benzene-d_6

[c] Recorded in DMSO-d_6

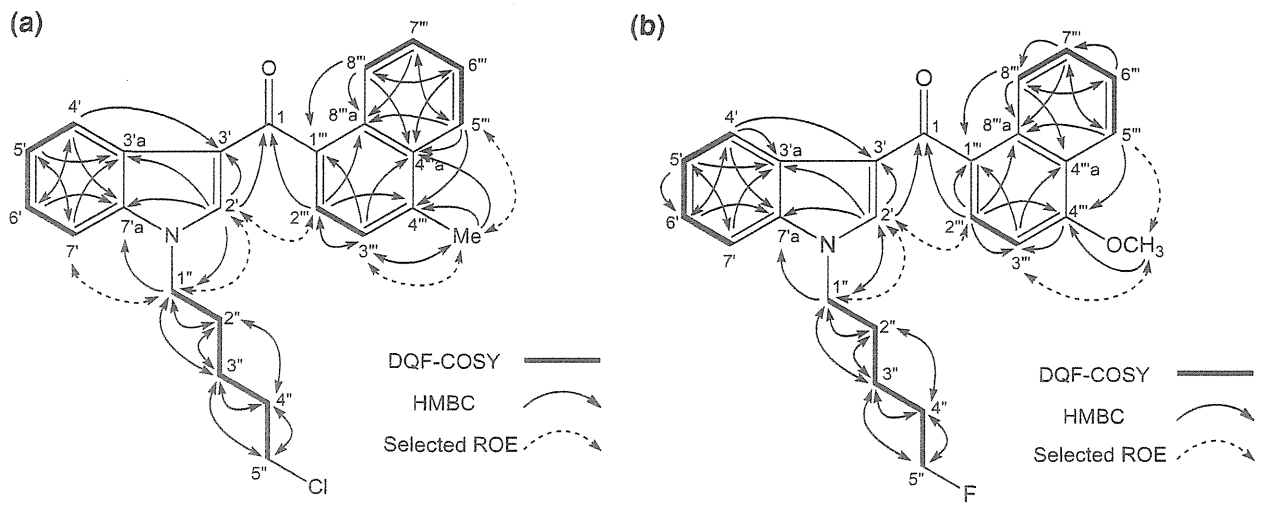

Fig. 9 DQF-COSY, selected HMBC, and selected ROE correlations for compound **8** [JWH-122 *N*-(5-chloropentyl) analog, **a**] and compound **9** [AM-2201 4-methoxynaphthyl analog (4-MeO-AM-2201), **b**]

synthetic cannabinoids that have the same moiety, such as JWH-018 and JWH-122 [18, 19].

The ^{1}H and ^{13}C NMR spectra of compound **4** suggested the existence of 29 protons and 20 carbons as shown in Table 3. The fragment ions at *m/z* 214 and 299 of peak **4** by GC–MS analysis (Fig. 6g) and the observed DQF-COSY, HMQC, HMBC, and 1D ROE spectra of compound **4** suggested the presence of 1-pentyl-1*H*-indole and (1-amino-3,3-dimethyl-1-oxobutan-2-yl)-carboxamide moieties (Fig. 7d; Table 3). In addition, the HMBC correlations from the amide proton (1-CONH) and the indole proton (H-2′) to the carboxyamide carbon (C-1) suggested that the carboxyamide carbon (C-1) in the (1-amino-3,3-dimethyl-1-oxobutan-2-yl)carboxamide moiety was attached to the carbon at the 3′-position of the 1-pentyl-1*H*-indole moiety (Fig. 7d). On the basis of the mass spectral and NMR data, the structure of compound **4** was determined to be *N*-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1*H*-indole-3-carboxamide. We named the compound ADBICA (Fig. 1a). This is the first study in which compound **4** has been detected in an illegal product. Compound **4** is a novel cannabimimetic substance and its chemical and pharmacological data have not been reported, although its structure is similar to that of a known indazole derivative, ABPINACA (Fig. 1c, [15]).

Identification of the unknown peaks 5–7

GC–MS and LC–MS analyses were performed to identify the unknown peaks **5**, **6**, and **7** in products C (Suppl., Fig. S2), D (Fig. S3), and E (Fig. S4), respectively. Based on the GC–MS and LC–MS data, the three peaks were identified as APICA *N*-(5-fluoropentyl) analog (Fig. S2a, b, d–f), APINACA *N*-(5-fluoropentyl) analog (Fig. S3a, b, d–f), and UR-144 *N*-(5-chloropentyl) analog (Fig. S4a, b, d–f) by direct comparison of the data with those of the purchased authentic compounds, respectively (Fig. S2c, g; Fig. S3c, g; Fig. S4c, g). In addition, compound **7** [UR-144 *N*-(5-chloropentyl) analog] was detected along with XLR-11 and a cathinone derivative, α-PVP, which are controlled as designated substances (Shitei-Yakubutsu) in Japan, in product E (Fig. 1b; Fig. S4a, d, e).

Compounds **5**, **6**, and **7** were detected as newly distributed designer drugs in Japan. These compounds are analogs of known cannabimimetic substances, APICA, APINACA, and UR-144, respectively, which have been controlled as designated substances (Shitei-Yakubutsu) in Japan since 2012 [5, 11, 20].

Identification of unknown peaks 8 and 9

Unknown peak **8** was detected together with known synthetic cannabinoid MAM-2201 (Fig. 1b) in the GC–MS and LC–MS chromatograms of product F (Fig. 8a, c, d). The proposed fragment patterns and presumed structure of peak **8** obtained by GC–MS analysis are shown in Fig. 8b. The LC–MS data revealed that peak **8** showed absorbance maxima at 226 and 314 nm in the UV spectrum, a protonated ion signal at *m/z* 390 ($[M+H]^+$), and an isotopic ion signal at *m/z* 392 ($[M+2+H]^+$) due to the presence of chlorine atom (Fig. 8e). After the isolation of compound **8**, the accurate mass spectrum obtained by LC–QTOF–MS gave an ion peak at *m/z* 390.1632, suggesting the protonated molecular formula of $C_{25}H_{25}ClNO$ (calcd. 390.1625).

The ^{13}C NMR spectrum of compound **8** was very similar to that of JWH-122 (Fig. 1c) except for the chlorine-

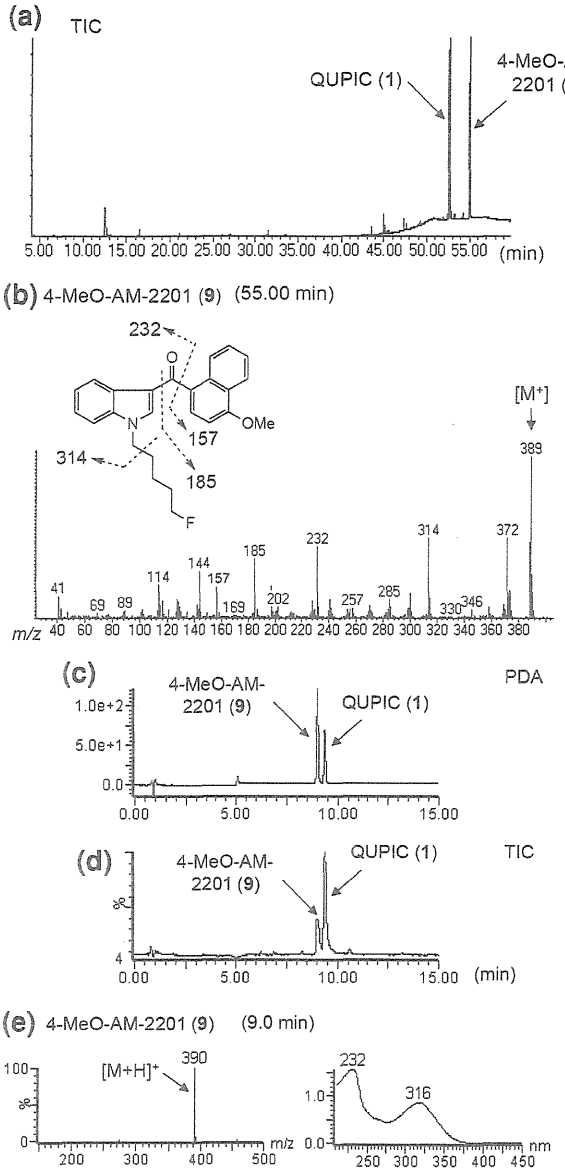

Fig. 10 GC–MS and LC–MS analyses of product G. TIC (**a**) and EI mass spectra of peak **9** (**b**) obtained by GC–MS analysis. LC–UV-PDA chromatogram (**c**) and TIC (**d**) using elution program (1) obtained by LC–MS. UV and ESI mass spectra of peak **9** (**e**)

substituted moiety (C-5″) of compound **8**, as shown in Table 4. On the basis of the mass spectra and the observed DQF-COSY, HMBC, and 1D ROE correlations shown in Fig. 9a, the structure of compound **8** was identified as JWH-122 *N*-(5-chloropentyl) analog (IUPAC: [1-(5-chloropentyl)-1*H*-indol-3-yl](4-methylnaphthalen-1-yl)methanone) (Fig. 1a).

In the GC–MS and LC–MS chromatograms of product G, unknown peak **9** was detected along with the peak of QUPIC (**1**) (Fig. 10a, c, d). Peak **9** at 55.00 min showed a putative molecular ion signal at *m/z* 389 by GC–MS analysis (Fig. 10b). The proposed fragment patterns and presumed structure of compound **9** by GC–MS analysis are also shown in Fig. 10b. The LC–MS data revealed that peak **9** showed absorbance maxima at 232 and 316 nm in the UV spectrum and a protonated ion signal at *m/z* 390 ($[M+H]^+$) (Fig. 10e). After isolation of compound **9**, the accurate mass spectrum obtained by LC–QTOF–MS showed an ion peak at *m/z* 390.1859, suggesting the protonated molecular formula of $C_{25}H_{25}FNO_2$ (calcd. 390.1869).

The ^1H and ^{13}C NMR spectra of compound **9** suggested the presence of *N*-(5-fluoropentyl)-3-carbonyl indole and 4-methoxynaphthyl moieties as shown in Fig. 9b. The fragment ions at *m/z* 232 and 157 of compound **9** in the GC–MS spectrum also supported the presence of these moieties (Fig. 10b). The connection of the two moieties was revealed by HMBC correlations from the indole proton (H-2′) and the naphthyl proton (H-2‴) to the carbonyl carbon (C-1) as shown in Fig. 9b. Therefore, the structure of compound **9** was identified as AM-2201 4-methoxynaphthyl analog (4-MeO-AM-2201, IUPAC: [1-(5-fluoropentyl)-1*H*-indol-3-yl](4-methoxynaphthalen-1-yl)methanone) (Fig. 1a). Compounds **8** and **9**, which have no reported pharmacological data, are analogs of the known synthetic cannabinoids JWH-122 and AM-2201, respectively.

Identification of unknown peaks 12 and 13

In the GC–MS and LC–MS analyses using elution program (2) [11], unknown peaks **12** and **13** were detected together with α-PBP and known synthetic cannabinoid EAM-2201 (Fig. 1b, [11]) in product H (Fig. 11a, e, f). Based on the GC–MS and LC–MS data, peak **12** was identified as opioid receptor agonist AH-7921 [3,4-dichloro-*N*-([1-(dimethylamino)cyclohexyl]methyl)benzamide] (Figs. 1a, 11b, g) by direct comparison of the data with those of the purchased authentic compound (Fig. 11c, h). AH-7921 (**12**) has been classified as an opioid analgesic with high addictive liability and has been reported to act as a selective μ-opioid receptor agonist [21, 22]. The present study is the first reported case in which AH-7921 has been detected in an illegal product.

Unknown peak **13** was presumed to be AB-001 *N*-(5-fluoropentyl) analog, from the proposed fragment patterns obtained by GC–MS analysis (Fig. 11d). The LC–MS chromatograms showed that peak **13** exhibited protonated ion signals ($[M+H]^+$) at *m/z* 368 and showed absorbance maxima at 220, 246, and 303 nm (Fig. 11i). Peak **13** was detected at 12.8 min under elution program (1) (data not shown [11]). The accurate mass spectrum was measured by LC–QTOF–MS. The observed ion peak at *m/z* 368.2389 suggested that the protonated molecular formula of

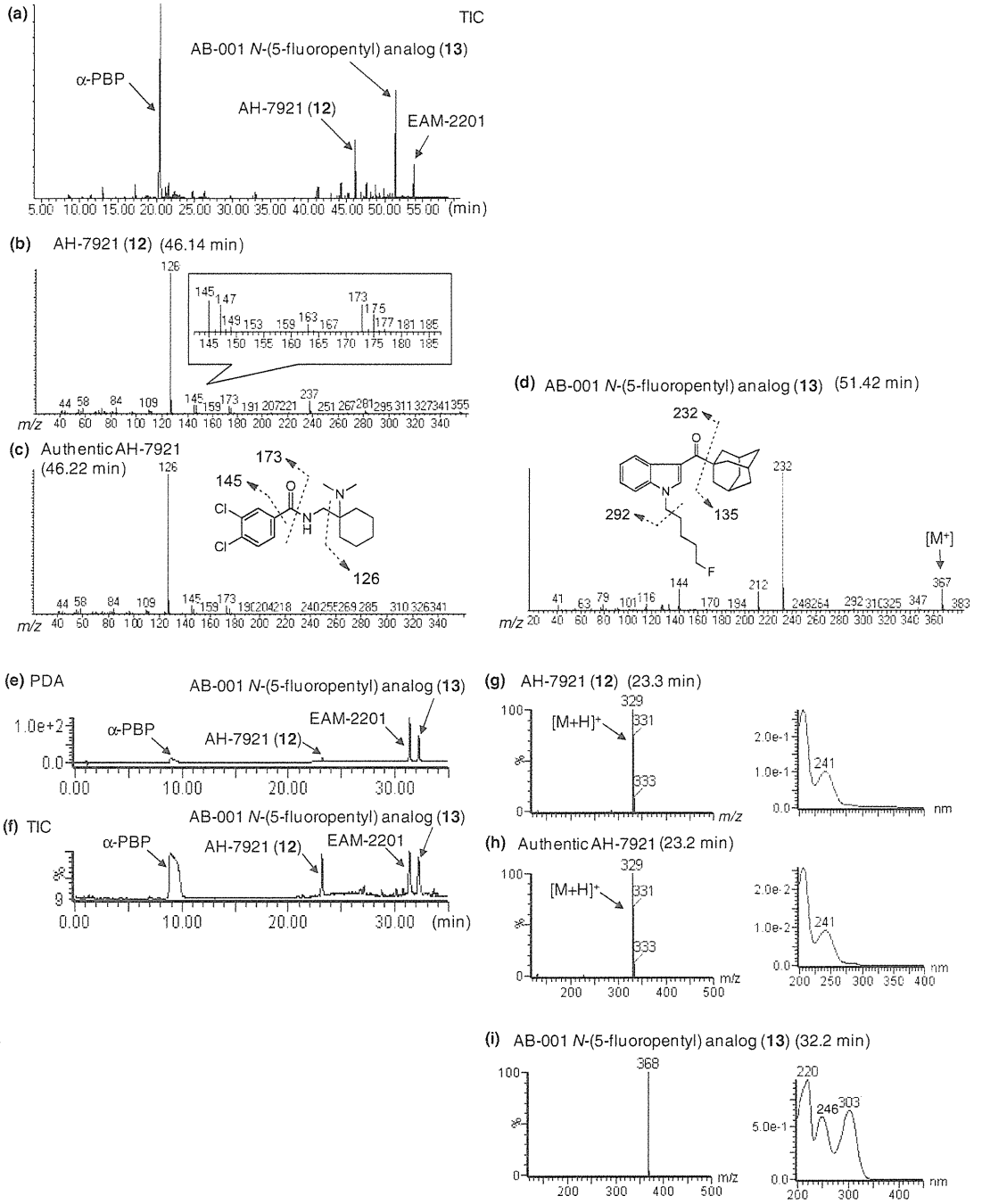

Fig. 11 GC–MS and LC–MS analyses of product H. TIC (**a**) and EI mass spectra of peaks **12** (**b**) and **13** (**d**) and authentic AH-7921 (**c**) obtained by GC–MS. LC–UV-PDA chromatogram (**e**) and TIC (**f**) using elution program (2) obtained by LC–MS. UV and ESI mass spectra of peaks **12** (**g**) and **13** (**i**) and authentic AH-7921 (**h**)

compound **13** was $C_{24}H_{31}FNO$ (calcd. 368.2389). A product ion peak at *m/z* 135.1169 indicating the presence of an adamantyl group [$C_{10}H_{15}$ (calcd. 135.1174)] in the structure of compound **13** was also detected by LC–QTOF–MS-MS analysis (data not shown). The above results supported the putative structure of compound **13** as AB-001 *N*-(5-fluoropentyl) analog. A strict confirmation analysis of the structure using NMR spectroscopy is now in progress.

To the best of our knowledge, the chemical and/or pharmacological data of most of the new detected compounds (except compounds **3** and **12**) have not been

reported. We are, therefore, conducting the following two examinations: (1) the affinities of the abused synthetic cannabinoids for cannabinoid CB_1/CB_2 receptors as described in our previous reports [11, 23], and (2) the inhibitory activities of the cathinones and their related derivatives on the neuronal uptake of the monoamines. The results will be reported in the near future (Kikura-Hanajiri et al., in preparation).

Of the new designer drugs distributed since late 2011 in Japan, the new synthetic cannabinoids belong to chemically diverse families, such as the naphthoylpyrroles (to which JWH-307 and JWH-030 belong [11]), the adamantyl-indoles/indazoles (to which APICA and APINACA belong [5]), and the dicarboxamide-indazoles (to which AB-PINACA and AB-FUBINACA belong [15]). With the marked increase in the detection of new cathinone derivatives in illegal products, other substances belonging to an expanding range of chemical families that are derivatives of controlled drugs such as aminoindanes (5-IAI), tryptamines (4-OH-DET), and arylcyclohexylamines (methoxetamine) have begun to appear in illegal drug markets [7–11, 24].

Our ongoing survey of designer drugs in the illegal market in Japan has revealed that a recent trend is the supply of a mixture of different designer drugs, such as cathinones (stimulants) and tryptamines (hallucinogens) with synthetic cannabinoids in one illegal product [11]. More recently, several potent hallucinogenic *N*-(2-methoxy)benzyl phenethylamine derivatives, 25I-NBOMe, 2C-C-NBOMe, and 25H-NBOMe [25, 26], were detected together with synthetic cannabinoids, AM-2201 and MAM-2201, in an illegal herbal product distributed in Japan (unpublished observation).

Conclusions

In this study, we detected two new-type cannabimimetic quinolinyl carboxylates, QUPIC (**1**) and QUCHIC (**2**); two new cannabimimetic carboxamide derivatives, ADB-FUBINACA (**3**) and ADBICA (**4**); and five new distributed synthetic cannabinoids, APICA *N*-(5-fluoropentyl) analog (**5**), APINACA *N*-(5-fluoropentyl) analog (**6**), UR-144 *N*-(5-chloropentyl) analog (**7**), JWH-122 *N*-(5-chloropentyl) analog (**8**), and AM-2201 4-methoxynaphthyl analog (4-MeO-AM-2201, **9**) in illegal products in Japan. In addition, 8-quinolinol (**10**), a synthetic component of compound **1** or **2** was detected. Moreover, a stimulant thiophene analog, α-PVT (**11**), and an opioid receptor agonist, AH-7921 (**12**), were detected as new types of designer drugs together with several synthetic cannabinoids and cathinone derivatives in illegal products. The types of designer drugs and their combinations in illegal products seem to be diversifying, and more serious health risks will be associated with their use than ever before. Therefore, continuous monitoring and rapid identification of newly distributed designer drugs are essential to prevent their abuse.

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Conflict of interest There are no financial or other relations that could lead to a conflict of interest.

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Warning against co-administration of 3,4-methylenedioxymethamphetamine (MDMA) with methamphetamine from the perspective of pharmacokinetic and pharmacodynamic evaluations in rat brain

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ABSTRACT

3,4-Methylenedioxymethamphetamine (MDMA) and methamphetamine often cause serious adverse effects (e.g., rhabdomyolysis, and cardiac disease) following hyperthermia triggered by release of brain monoamines such as dopamine and serotonin. Therefore, evaluation of brain monoamine concentrations is useful to predict these drugs' risks in human. This study aimed to evaluate risks of co-administration of MDMA and methamphetamine, both of which are abused frequently in Japan, based on drug distribution and monoamine level in the rat brain. Rats were allocated to three groups: (1) sole MDMA administration (12 or 25 mg/kg, intraperitoneally), (2) sole methamphetamine administration (10 mg/kg, intraperitoneally) and (3) co-administration of MDMA (12 mg/kg, intraperitoneally) and methamphetamine (10 mg/kg, intraperitoneally). We monitored pharmacokinetic and pharmacodynamic variables for drugs and monoamines in the rat brain. Area under the curve for concentration vs. time until 600 min from drug administration (AUC_{0-600}) increased from 348.0 to 689.8 μg min/L for MDMA and from 29.9 to 243.4 μM min for dopamine in response to co-administration of methamphetamine and MDMA compared to sole MDMA (12 mg/kg) administration. After sole methamphetamine or that with MDMA administration, AUC_{0-600} of methamphetamine were 401.8 and 671.1 μg min/L, and AUC_{0-600} of dopamine were 159.9 and 243.4 μM min. In conclusion, the brain had greater exposure to MDMA, methamphetamine and dopamine after co-administration of MDMA and methamphetamine than when these two drugs were given alone. This suggests co-administration of MDMA with methamphetamine confers greater risk than sole administration, and that adverse events of MDMA ingestion may increase when methamphetamine is co-administered.

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1. Introduction

Recreational use of illegal drugs, particularly stimulants such as amphetamine derivatives, is a serious global problem. Among them methamphetamine called as "speed", "meth", and "ice" is one of amphetamine derivatives and most frequently abused in Japan (National Police Agency of Japan, 2011). Methamphetamine are reported to cause many kinds of effects such as euphoria, rush, increased wakefulness physical activity, decreased fatigue, cardiovascular problem, hyperthermia, and convulsion (NIDA, 2006a; Quinton and Yamamoto, 2006). This drug remains as an unchanged form in the body for long time, which makes stimulant effects prolonged. The stimulant effects of methamphetamine are mainly appeared by increase of dopamine levels in brain. The possible mechanism is believed that methamphetamine inhibits dopamine re-uptake and increase in release of dopamine (Han and Gu, 2006).

3,4-Methylenedioxymethamphetamine (MDMA) is a synthesized stimulant and hallucinogen drug having a similar structure to methamphetamine and mescaline. MDMA has been widely spread in tablet form, and commonly known as "Ecstasy" and "Adam". Because the colorful MDMA tablets have a familiar logo (Teng et al., 2006), abusers of this drug, many of whom are young people, take few precautions when taking it. Hence, the issue of

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; CL, clearance; DIB-Cl, 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride; ECD, electrochemical detection; HPLC, high performance liquid chromatography; MRT, mean residence time; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; ODS, octadecylsilica; C_{max}, peak concentration; T_{max}, time to peak concentration.

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drug abuse is a grave concern for society. After taking of MDMA, abusers experience euphoria, feelings of mental stimulation, empathy toward others, and decreased anxiety (Verheyden et al., 2003). Also, MDMA causes many physical symptoms such as circulatory problems, heart disease, muscle tension, nausea, hyperthermia, and multi-organ failure (NIDA, 2006b; Quinton and Yamamoto, 2006). The mechanism of these effects is thought that MDMA increases serotonin levels in the brain via inhibition of serotonin re-uptake. And, MDMA enters the serotonin neurons by the transporter and cause increased releases of serotonin by exchange diffusion (Hall and Henry, 2006).

As described previously, methamphetamine and MDMA have a central action by influencing neurotransmitter regulation in the brain, and in particular, methamphetamine triggers dopaminergic neurons to release dopamine and MDMA causes serotonergic neurons to release serotonin (Quinton and Yamamoto, 2006). Therefore, changes in dopamine and serotonin levels should be useful as an indicator to evaluate the influence of these drugs on the central nervous system.

Hyperthermia is one of the main adverse effects of methamphetamine and MDMA. Several studies have reported complications such as rhabdomyolysis and cardiac disease following hyperthermia in abusers of these drugs (Numachi et al., 2007; Sano et al., 2009), and at worst this disorder can be fatal. The mechanism of hyperthermia remains unclear, but some researchers consider monoamines such as dopamine and serotonin to be involved (Mechan et al., 2002; Cole and Sumnall, 2003; Lyles and Cadet, 2003; Docherty and Green, 2010; Tohmas et al., 2010). Hall and Henry (2006) also suggest that MDMA activate both conservation and generation of heat, and both serotonin and dopamine are involved in central control of thermoregulation. The serotonin syndrome is the most extreme of these effects. In this syndrome, confusion, diaphoresis, and muscle tone and rigidity are appeared. The severe muscle contraction is estimated to lead to hyperthermia and death. As seen above, serotonin and dopamine contribute the acute hyperthermia and following adverse reactions caused by MDMA intake. Therefore, evaluation of monoamine levels such as dopamine and serotonin is useful to predict the risks of these drugs to humans.

Users of MDMA and methamphetamine vary their drug intake from time to time. In particular, MDMA abusers tend to have widely differing habits in terms of number of tablets ingested at parties (Greene et al., 2003; Irvine et al., 2006; Silins et al., 2009), and the MDMA content in tablets varies widely (Makino et al., 2003; Teng et al., 2006; Morefield et al., 2011). Estimation of the influences and risks of MDMA intake is therefore complicated. Moreover, in many cases, more than two illicit drugs are knowingly taken simultaneously (Fox et al., 2002; Kaye et al., 2009; Silins et al., 2009), and some MDMA tablets contain other drugs, for example, methamphetamine, amphetamine, 3,4-methylenedioxyamphetamine (MDA), and caffeine (Teng et al., 2006).

In order to predict risks caused by drugs of abuse, it is accordingly important to evaluate the influence of different dosages of these drugs and clarify drug-drug interactions.

This study aimed to evaluate the risks of sole administration of methamphetamine or MDMA versus a combination of these drugs. To achieve this goal, we monitored drug distribution and monoamine release over time using microdialysis sampling from the brains of anesthetized rats. Pharmacodynamic parameters indicate a drug's effect on the central nervous system and pharmacokinetic parameters indicates its distribution in the central nervous system. As a pharmacokinetic parameter, we determined drug concentration by high performance liquid chromatography (HPLC)-fluorescence detection using the methods reported by Tomita et al. (2007) with some modification. As a pharmacodynamic parameter we determined concentrations of the monoamines, dopamine and serotonin, are involved in effects and adverse reactions of drugs, by HPLC–electrochemical detection (ECD) (Ikeda et al., 2011).

2. Materials and methods

2.1. Animals

Male Wistar rats (7 weeks old, 250–260 g) were purchased from Kyudo Co. Ltd., Saga, Japan and housed in plastic cages containing wood-chip bedding material under standard environmental conditions (ambient temperature 22 ± 1 °C, humidity 55 ± 5% and 12 h light-dark cycle). They had free access to food and water. Animals were used for experiments at 8 weeks of age and weighing 270–320 g. Each group consisted of four rats. All animal studies were approved by the Nagasaki University Animal Care and Use Committee.

2.2. Drugs and reagents

MDMA tablets were kindly donated by the National Institute of Health Sciences (Tokyo, Japan). MDMA hydrochloride, MDA hydrochloride and amphetamine sulfate were obtained as narcotics for research from the Ministry of Health, Labour and Welfare (Tokyo). Methamphetamine hydrochloride (Philopon®) was purchased from Dainippon Pharma (Osaka, Japan). 4-(4,5-Diphenyl-1*H*-imidazol-2-yl)benzoyl chloride (DIB-Cl) was synthesized in our laboratory (Nakashima et al., 1995). Dopamine, HPLC-grade acetonitrile and 2-propanol were obtained from Wako Pure Chemical Industries (Osaka). Water was deionized and passed through a water purification system (Pure Line WL21P, Yamato Kagaku, Tokyo). Serotonin and HPLC-grade methanol were purchased from Kanto Chemical (Tokyo). 1-Methyl-3-phenylpropylamine (MPPA, used for an internal standard to determine the drug concentrations and sodium 1-decanesulfonate were purchased from Sigma Aldrich Japan (Tokyo), and ethylenediamine-*N,N,N′,N′*-tetraacetic acid disodium salt dehydrate (EDTA·2Na) was purchased from Dojindo Laboratories (Kumamoto, Japan).

2.3. Standard solutions

Stock solutions of MDMA (molecular weight, 193.24; Log P, 2.2 (from the PubChem Project)), MDA (molecular weight, 179.22; Log P, 1.6 (from the PubChem Project)), methamphetamine (molecular weight, 149.23; Log P, 2.1 (from the PubChem Project)), and amphetamine (molecular weight, 135.21; Log P, 1.8 (from the PubChem Project)) were prepared at 1 mg/mL with methanol, and stored at −4 °C. Dopamine (molecular weight, 153.18; Log P, -1 (from the PubChem Project)) was dissolved in 0.1 M hydrochloride containing 100 mg/L EDTA·2Na, and serotonin (molecular weight, 176.22; Log P, 0.2 (from the PubChem Project)) was dissolved in 0.1 M acetic acid containing 100 mg/L EDTA·2Na to prepare 10 mM stock solutions, which were stored at −4 °C. When using these chemicals in experiments, we created serial dilutions of these solutions.

2.4. Microdialysis

Rats were anesthetized with ethylcarbamate (1.5 g/kg, intraperitoneally (i.p.) and fixed on a stereotaxic system (SR-5R, Narishige Scientific Instrument, Tokyo). A CMA microdialysis system (Carnegie Medicine, Stockholm, Sweden) was used. An MAB6 microdialysis probe with a 4-mm, 15 kDa cutoff polyethersulfone membrane (ALS/Microbiotech, Stockholm) was implanted in the left striatum (coordinates: *A*, +0.6 mm; *L*, +3.0 mm from bregma; *H*, −7.0 mm from the skull surface; Paxinos and Watson, 2007) and was per-