

A synthetic cannabinoid FDU-NNEI, two 2*H*-indazole isomers of synthetic cannabinoids AB-CHMINACA and NNEI indazole analog (MN-18), a phenethylamine derivative *N*-OH-EDMA, and a cathinone derivative dimethoxy- α -PHP, newly identified in illegal products

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Abstract Six new psychoactive substances were identified together with two other substances (compounds **1–8**) in illegal products by our ongoing survey in Japan between January and July 2014. A new synthetic cannabinoid, FDU-NNEI [1-(4-fluorobenzyl)-*N*-(naphthalen-1-yl)-1*H*-indole-3-carboxamide, **2**], was detected with the newly distributed synthetic cannabinoid FDU-PB-22 (**1**). Two 2*H*-indazole isomers of synthetic cannabinoids, AB-CHMINACA 2*H*-indazole analog (**3**) and NNEI 2*H*-indazole analog (**4**), were newly identified with 1*H*-indazoles [AB-CHMINACA and NNEI indazole analog (MN-18)]. In addition, 2-methylpropyl *N*-(naphthalen-1-yl) carbamate (**5**) and isobutyl 1-pentyl-1*H*-indazole-3-carboxylate (**6**) were detected in illegal products. Compound **6** is considered to be a by-product of the preparation of NNEI indazole analog from compound **5** and 1-pentyl-1*H*-indazole. A phenethylamine derivative, *N*-OH-EDMA [*N*-hydroxy-3,4-ethylenedioxy-*N*-methylamphetamine, **7**], and a cathinone derivative, dimethoxy- α -PHP (dimethoxy- α -pyrrolidinohexanophenone, **8**), were newly identified in illegal products. Among them, compounds **1** and **8** have been controlled as designated substances (Shitei-Yakubutsu) under the Pharmaceutical Affairs Law in Japan since August and November 2014, respectively.

Keywords 2*H*-indazole isomer · Synthetic cannabinoid · FDU-NNEI · *N*-OH-EDMA · Dimethoxy- α -PHP · Cathinone derivative

Introduction

The consumption of new psychoactive substances (NPSs) including synthetic cannabinoids and cathinone derivatives has become widespread despite regulatory control measures [1–7]. The EMCDDA (European Monitoring Centre for Drugs and Drug Addiction) reported that 81 NPSs were identified by the EU early warning system in 2013, with 37 NPSs reported from January to May 2014 [2]. Ninety-seven NPSs were reported to the UNODC (United Nations Office on Drugs and Crime) in 2013 alone [3].

We previously reported the appearance of 45 newly distributed substances in Japan between January 2013 and May 2014, by our ongoing survey of NPSs in the illegal drug market in Japan [8–10]. The detected compounds were 18 synthetic cannabinoids, 13 cathinone derivatives, five phenethylamines, and nine other substances, including the *N*-methyl-D-aspartate (NMDA) channel blocker diphenidine [8–10]. In the present study, we describe the identification of eight newly distributed compounds: four synthetic cannabinoids (**1–4**), two other substances (**5** and **6**), a phenethylamine derivative (**7**), and a cathinone derivative (**8**) in illegal products purchased from January to July 2014 (Fig. 1a).

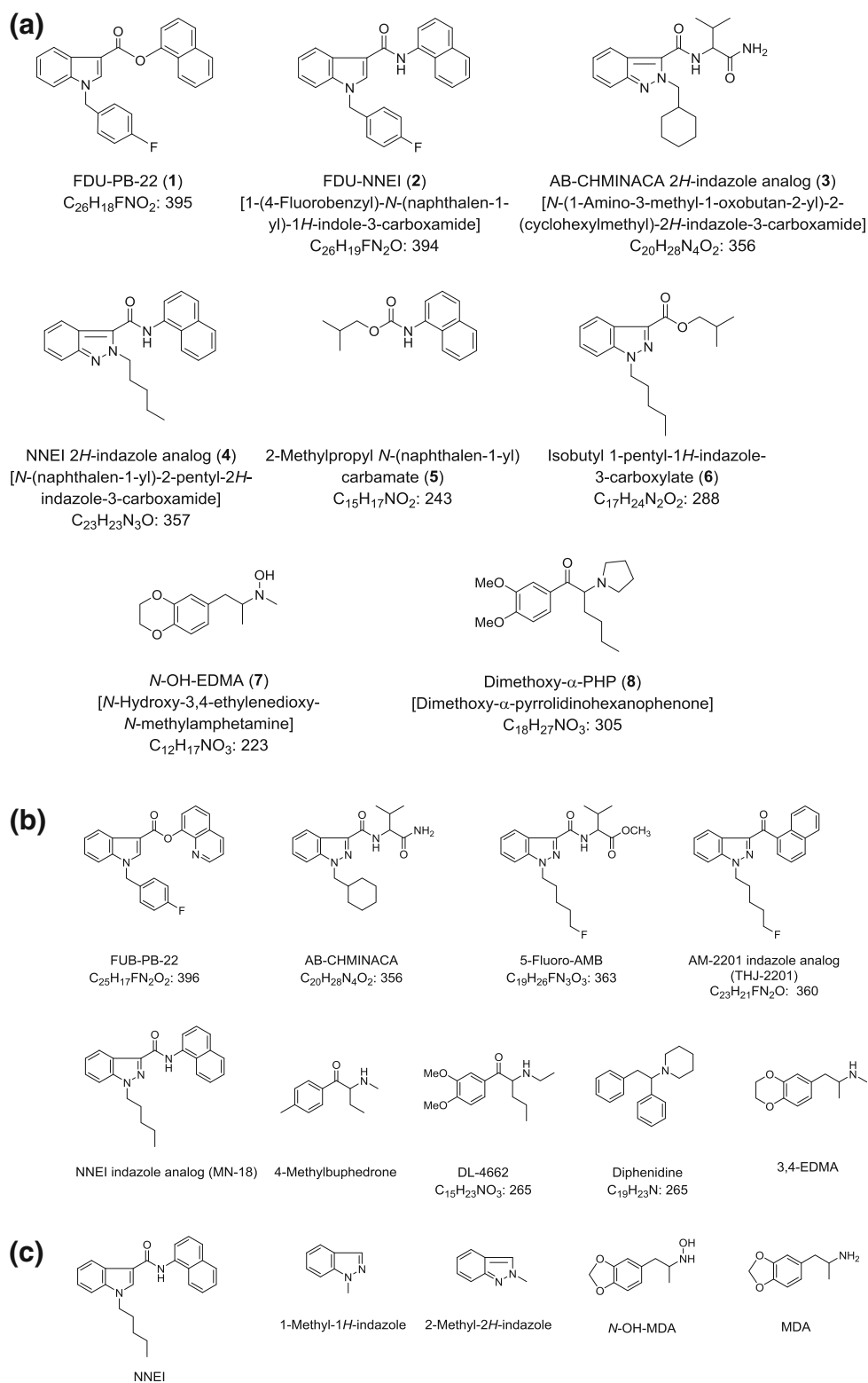
Materials and methods

Samples for analyses

The analyzed samples were purchased via the Internet between January and July 2014 as 241 chemical-type or herbal-type products being sold in Japan. Among them, we

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Fig. 1 Structures of the newly detected compounds (**1–8**, **a**), detected, but known compounds (**b**), and related compounds (**c**)



show the analysis data of five products (A–E) for describing the identification of compounds **1–8** in this paper. Each of the herbal-type products (A–D) contained

approximately 3 g of mixed dried plants. The single powder-type product called “fragrance powder” consisted of 400 mg of a brown powder (E).

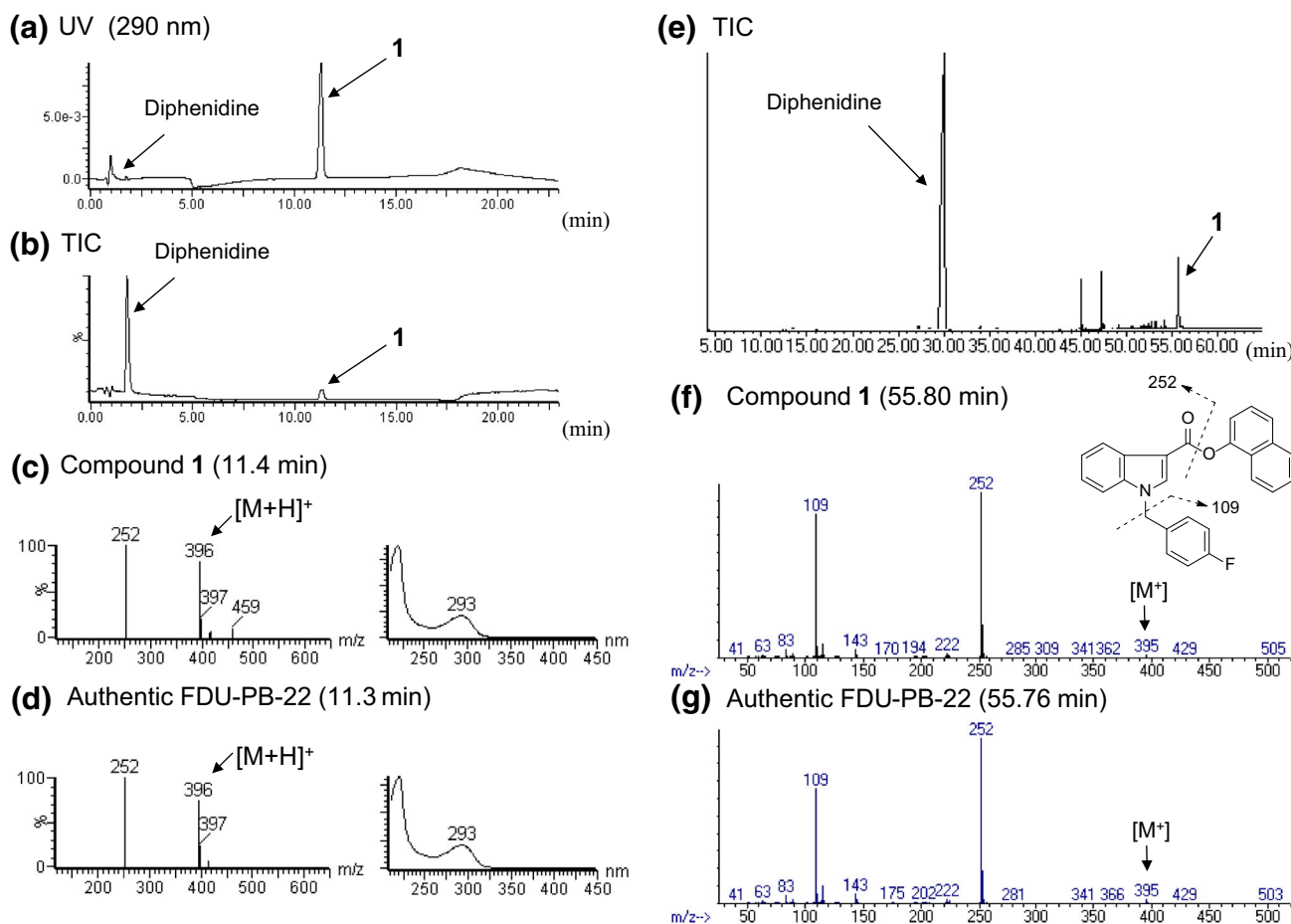


Fig. 2 Liquid chromatography–mass spectrometry (LC–MS) and gas chromatography–mass spectrometry (GC–MS) analyses of product A. The liquid chromatography–ultraviolet–photodiode array (LC–UV–PDA) chromatogram (a), total ion chromatogram (TIC) (b), and

electrospray ionization (ESI) mass and ultraviolet (UV) spectra of peaks **1** (c) and the authentic FDU-PB-22 (d) are shown. TIC (e), electron ionization (EI) mass spectra of peaks **1** (f) and the authentic FDU-PB-22 (g) obtained by GC–MS are also shown

Chemicals and reagents

FDU-PB-22 (**1**), FUB-PB-22, AB-CHMINACA, 5-fluoro-AMB, AM-2201 indazole analog (THJ-2201), NNEI indazole analog (MN-18), 4-methylbuphedrone, DL-4662, and 3,4-EDMA were purchased from Cayman Chemical (Ann Arbor, MI, USA); 2-methylpropyl *N*-(naphthalen-1-yl) carbamate (**5**) and diphenidine from Otava Ltd. (Toronto, Canada) and Tocris Bioscience (Bristol, UK), respectively. All other common chemicals and solvents were of analytical reagent grade or high-performance liquid chromatography (HPLC) grade. As solvents for nuclear magnetic resonance (NMR) analysis, chloroform- d_3 (99.96 %), methanol- d_4 (99.96 %), methanol- d_3 (99 %), pyridine- d_5 (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 solutions

For the qualitative analyses, 10 mg of each herbal-type product was crushed into 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 (3,000 rpm, 5 min) of each extract, the supernatant solution was passed through a centrifugal filter (Ultrafree-MC, 0.45- μ m filter unit; Millipore, Bedford, MA, USA) to serve as the sample solution for the analyses. If necessary, the solution was diluted with methanol to a suitable concentration before instrumental analyses.

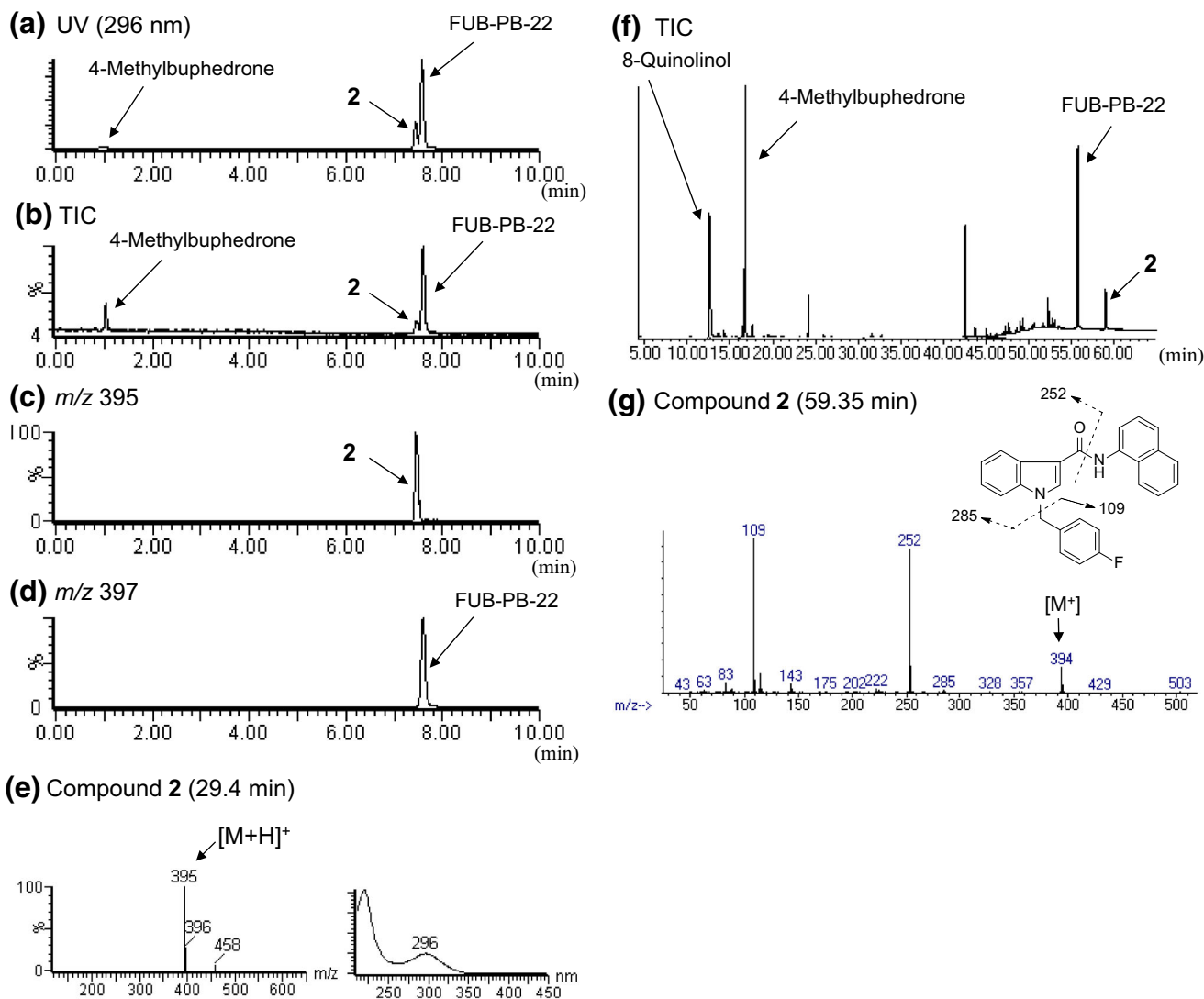


Fig. 3 LC-MS and GC-MS analyses of product B. LC-UV-PDA chromatogram (a), TIC (b), extracted-ion chromatograms at m/z 395 (c) and 397 (d), and ESI mass and UV spectra of peak 2 (e) are

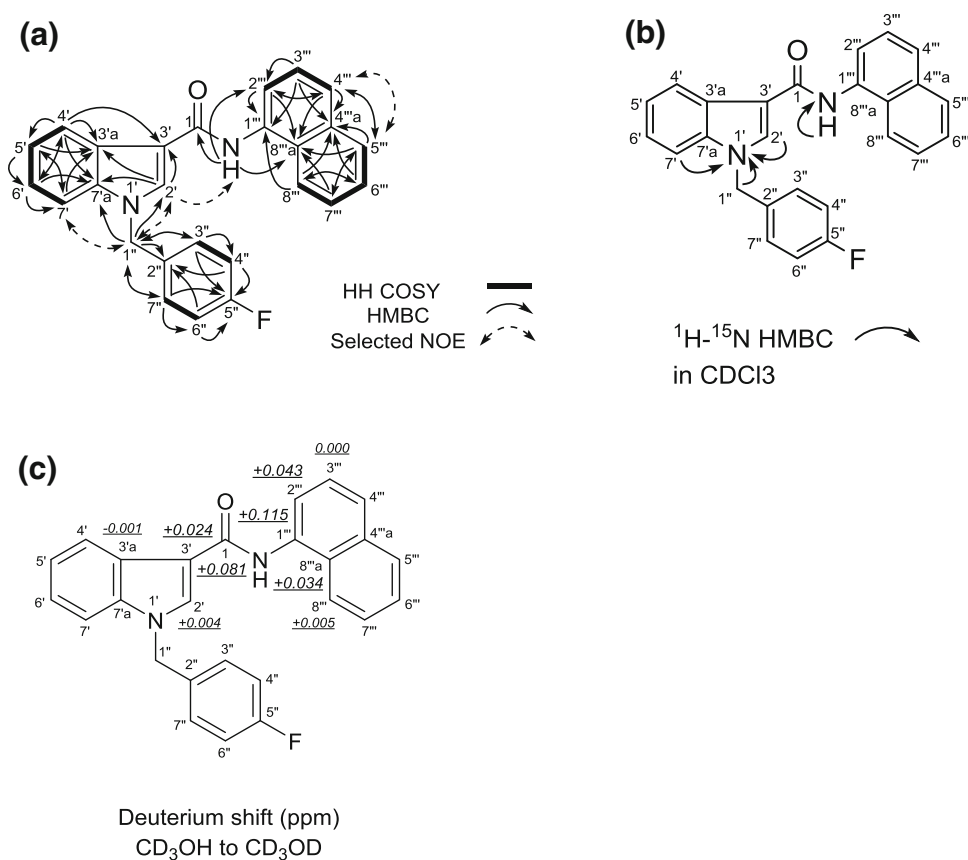
shown. TIC (f) and EI mass spectrum of peak 2 (g) obtained by GC-MS are also presented

Analytical conditions

Each sample solution was analyzed by ultra-performance liquid chromatography-electrospray ionization-mass spectrometry (UPLC-ESI-MS) and by 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. Each analysis was carried out with a binary mobile phase consisting of solvent A (0.1 % formic acid in water) and solvent B (0.1 % formic acid in acetonitrile). The elution program (1) used for analysis of cannabinoids was as follows: 35 % B (4-min hold), 65 % B to 75 % B (4–16 min), and up to 90 % B (16–17 min, 6-min hold) at a flow rate of 0.3 ml/

min. The elution program (2) used for the analysis of cathinone derivatives and other compounds was as follows: 5 % B to 20 % B (0–20 min), and up to 80 % B (20–30 min, 10-min hold). In this study, products A, B, and D were analyzed using program (1), and products C and E analyzed using program (2). GC-EI-MS was performed on an Agilent 6890N GC with a 5975 mass selective detector (Agilent Technologies, Santa Clara, CA, USA) using a capillary column (HP-1MS capillary, 30 m \times 0.25 mm i.d., 0.25- μ m film thickness; Agilent Technologies) with helium gas as a carrier at 0.7 ml/min. The conditions were: electron energy, 70 eV; injector temperature, 220 $^{\circ}$ C; injection, splitless mode for 1.0 min; oven temperature program, 80 $^{\circ}$ C (1-min hold) and increase at a rate of

Fig. 4 Results of HH correlation spectroscopy (HH COSY), heteronuclear multiple-bond correlation (HMBC), selected nuclear Overhauser effect (NOE) correlations (a), ^1H - ^{15}N HMBC correlations (b) and deuterium-induced isotope shift of NH protons for the ^{13}C nuclear magnetic resonance (NMR) signals of compound **2** (FDU-NNEI) in CD_3OD (c)



5 °C/min to 190 °C (15-min hold) followed by increase at 10 °C/min up to 310 °C (15-min hold); transfer line temperature, 280 °C; scan range, m/z 40–550.

The obtained GC mass spectra were compared to those of an EI-MS library (Mass Spectra of Designer Drugs 2013; WILEY-VCH, Weinheim, Germany). We also used our in-house EI-MS library of designer drugs obtained by our ongoing survey of illegal products and commercially available reagents for the structural elucidation.

We measured the accurate mass numbers of the target compounds by liquid chromatography–quadrupole-time-of-flight–mass spectrometry (LC–QTOF–MS) in the ESI mode according to our previous report [12].

For the isolation of each compound, we performed two preparative methods. One was recycling preparative HPLC on a JAI (Japan Analytical Industry, Tokyo, Japan) LC-9201 instrument with gel permeation JAIGEL-1H columns (JAI) and chloroform as an eluent. The other was silica gel or ODS column chromatography (CC) on a Biotage (Stockholm, Sweden) Isorela One instrument with a SNAP KP-sil column (particle size: 50 μm), SNAP Ultra column (particle size: 25 μm), or SNAP KP-C18-HS column (particle size: 50 μm) (Biotage).

The nuclear magnetic resonance (NMR) spectra were obtained on ECA-800 and 600 spectrometers (JEOL, Tokyo, Japan). Assignments were made via ^1H NMR, ^{13}C NMR, heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), ^{15}N HMBC, HH correlation spectroscopy (HH COSY), nuclear Overhauser effect (NOE), and incredible natural abundance double-quantum transfer experiment (INADEQUATE) spectra.

Isolation of compound **2**

A 3.0-g sample of mixed dried plants (product B) was extracted with 250 ml of chloroform by ultrasonication for 30 min. The extraction was repeated three times, and the supernatant fractions were combined and evaporated to dryness. The extract was separated by silica gel CC (SNAP KP-sil, 25 g; Biotage) [hexane/ethyl acetate (100:0–75:25)] and ODS CC (SNAP KP-C18-HS, 12 g; Biotage) [water/methanol (30:70–0:100)]. The extract was then recrystallized in ethyl acetate/hexane, which gave compound **2** (28 mg) as a pale yellow solid.

Isolation of compound 3

A 2.8-g sample of mixed dried plants (product C) was extracted by the same method as that used for compound 2. The separation of extract by silica gel CC (SNAP KP-sil, 50 g; Biotage) [hexane/ethyl acetate (50:50)] and ODS CC (SNAP KP-C18-HS, 12 g; Biotage) [water/methanol (35:65)] resulted in the isolation of compound 3 (8 mg) as a white solid.

Isolation of compounds 4 and 6

A 5.6-g sample of mixed dried plants (product D) was extracted by the same method described above. Separation of extract by silica gel CC (SNAP Ultra, 25 g; Biotage) [hexane/ethyl acetate (87:13–0:100)] gave fractions 1 and 2. Fraction 1 was further separated by silica gel CC (SNAP KP-sil, 50 g; Biotage) [hexane/ethyl acetate (90:10)] to obtain compound 4 (5 mg) as a white solid. Fraction 2 was dissolved in chloroform and purified by recycling preparative HPLC (eluent: chloroform) to give compound 6 (1 mg) as a white solid.

Isolation of compounds 7 and 8

A 600-mg sample of powder (product E) was dissolved in 2.5 % triethylamine (TEA) in hexane/ethyl acetate (50:50) and then loaded onto a silica gel CC (SNAP KP-sil, 50 g; Biotage), which was then eluted with a stepwise gradient of 2.5 % TEA in hexane/2.5 % TEA in ethyl acetate (90:10–0:100) to give compound 7 (73 mg) and compound 8 (302 mg) as a colorless oil, respectively.

Results and discussion

Identification of an unknown peak 1

An unknown peak 1 was detected with an NMDA receptor channel blocker, diphenidine (Fig. 1b) [13] in the LC–MS and GC–MS for product A (Fig. 2a, b, e). Based on the LC–MS and GC–MS data, peak 1 was finally identified as a synthetic cannabinoid FDU-PB-22 (Figs. 1a, 2c, f) by direct comparison of the data to those of the purchased authentic compound (Fig. 2d, g). Compound 1 was thus detected as a newly distributed NPS in Japan. In addition, FDU-PB-22 (1) has been controlled as a designated substance (Shitei-Yakubutsu) in Japan since August 2014.

Identification of an unknown peak 2

In the LC–MS and GC–MS analyses, an unknown peak 2 was detected with a synthetic cannabinoid (FUB-PB-22),

Table 1 NMR data of compound 2

No.	¹³ C	¹ H
1	167.5	–
2'	133.0	8.25, 1H, brs
3'	111.5	–
3'a	128.6	–
4'	122.7	8.22, 1H, d, <i>J</i> = 7.8 Hz
5'	122.7	7.20, 1H, t, <i>J</i> = 7.8 Hz
6'	124.0	7.24, 1H, t, <i>J</i> = 7.8 Hz
7'	111.6	7.45, 1H, d, <i>J</i> = 7.8 Hz
7'a	138.2	–
1''	50.7	5.48, 2H, s
2''	134.5, d, <i>J</i> = 2.8 Hz	–
3''/7''	130.3, d, <i>J</i> = 7.7 Hz	7.30 and 7.29, each 1H, d, <i>J</i> = 8.7 Hz, overlapped
4''/6''	116.6, d, <i>J</i> = 22.2 Hz	7.08 and 7.07, each 1H, d, <i>J</i> = 8.7 Hz, overlapped
5''	163.8, d, <i>J</i> = 244.7 Hz	–
1'''	134.9	–
2'''	125.2	7.61, 1H, d, <i>J</i> = 6.9 Hz
3'''	126.6	7.53, 1H, m, overlapped
4'''	127.8	7.82, 1H, d, <i>J</i> = 8.3 Hz
4'''a	135.9	–
5'''	129.3	7.91, 1H, m
6'''	127.1	7.51, 1H, m, overlapped
7'''	127.2	7.51, 1H, m, overlapped
8'''	124.1	8.06, 1H, m
8'''a	131.3	–
NH	–	9.96, 1H, s

Recorded in CD₃OH at 800 MHz (¹H) and 200 MHz (¹³C), respectively; data in δ ppm (*J* in Hz)

its synthetic component 8-quinolinol, and a cathinone derivative (4-methylbuphedrone) in product B (Figs. 1b, 3a, b, d, f). By GC–MS analysis, peak 2 showed a molecular ion at *m/z* 394 (Fig. 3g). The major fragment ions of peak 2 (*m/z* 109 and 252) are the same as those of FDU-PB-22 (1) except for the molecular ion of compound 1 at *m/z* 395 (Fig. 2g). In the LC–MS analysis, peak 2 showed the protonated molecular ion at *m/z* 395 ([M + H]⁺) (Fig. 3c, e). However, the major fragment ion at *m/z* 252 of FDU-PB-22 (1) given by cleavage of an ester group (Fig. 2d) was not observed in the mass spectrum of peak 2 (Fig. 3e). The accurate mass spectrum obtained by LC–QTOF–MS gave an ion peak at *m/z* 395.1565, suggesting that the protonated molecular formula of compound 2 was C₂₆H₂₀N₂O_F (calcd. 395.1560).

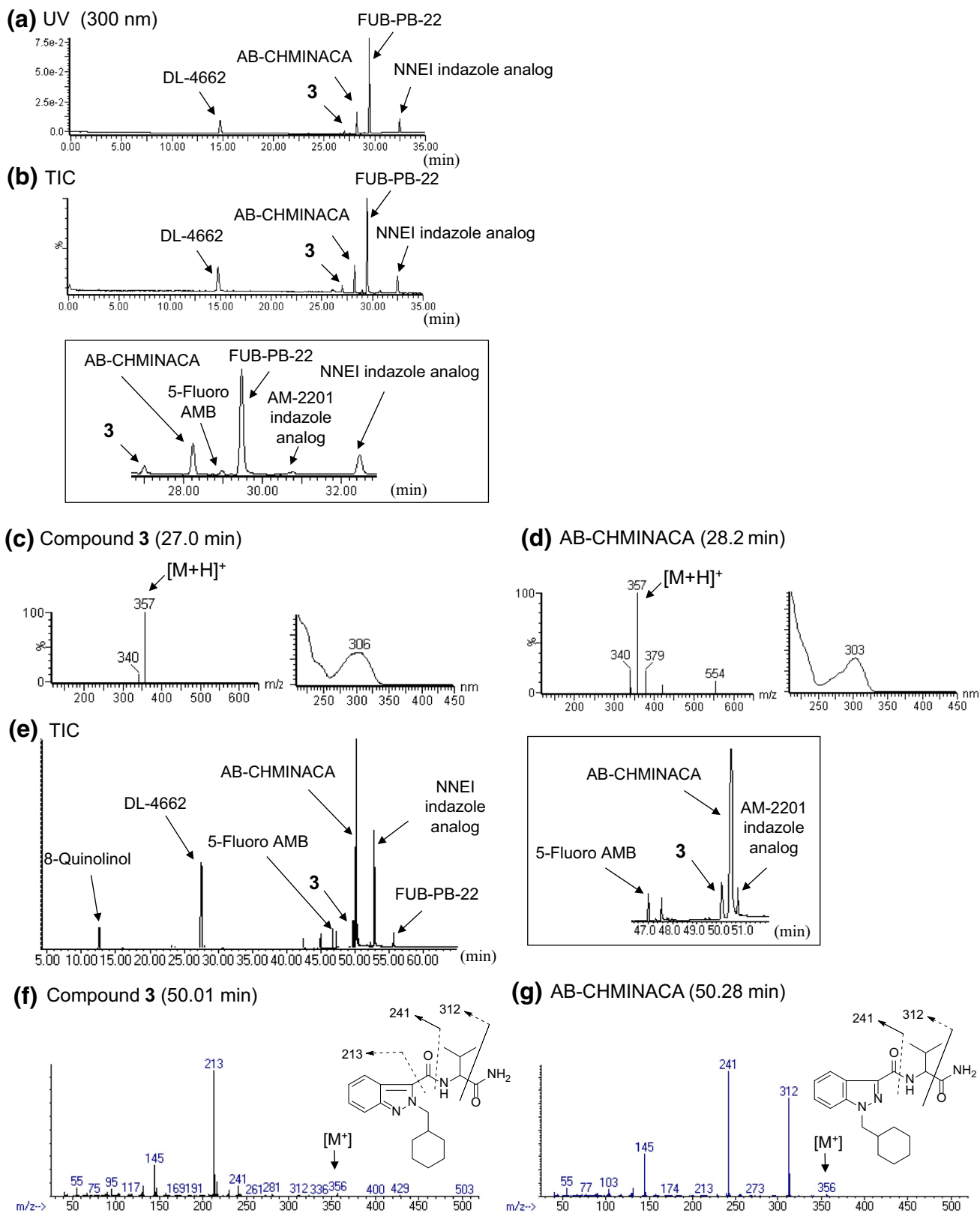


Fig. 5 LC-MS and GC-MS analyses of product C. The LC-UV-PDA chromatogram (a), TIC (b), and ESI mass and UV spectra of peak 3 (c) and the authentic AB-CHMINACA (d) are shown. TIC (e),

and EI mass spectra of peaks 3 (f) and the authentic AB-CHMINACA (g) obtained by the GC-MS analysis are also shown

The structure of compound **2** was elucidated by NMR analysis (Fig. 4; Table 1). The analyses by ^1H and ^{13}C NMR, HH COSY, HMQC, HMBC, ^{15}N HMBC and 1D-NOE spectra of compound **2** revealed the presence of a 1-(4-fluorobenzyl)-1*H*-indole (positions 1' to 7'a and positions 1'' to 7'') and a *N*-(naphthalen-1-yl)-carboxamide moieties (position 1 and positions 1''' to 8'''a) as shown in Fig. 4a, b. However, no HBMC correlation between the two moieties was observed. We, therefore, measured the deuterium isotope effect on the NH amide proton on the ^{13}C chemical shift to determine the connection between the two moieties.

We compared the ^{13}C NMR spectrum of compound **2**, measured in CD_3OH , with that in CD_3OD . The isotope shift values for the ^{13}C NMR signals of this compound are shown in Fig. 4c. The first- to fourth-largest deuterium shifts (0.115, 0.081, 0.043, 0.034 ppm) were observed at the positions of C-1''', C-1, C-2''', and C-8'''a of the *N*-(naphthalen-1-yl)carboxamide moiety. The fifth-largest deuterium shift of 0.024 ppm was attributed to the three-bond deuterium isotope effect of the NH amide proton on the indole carbon (C-3'). These results strongly suggested that the 1-(4-fluorobenzyl)-1*H*-indole moiety is connected at the 3'-position of the indole to the carboxamide (1-CONH).

On the basis of the above data, compound **2** was finally identified as 1-(4-fluorobenzyl)-*N*-(naphthalen-1-yl)-1*H*-indole-3-carboxamide (Fig. 1a). Compound **2** is a novel compound, and therefore, we named it FDU-NNEI because the structure of compound **2** consists of a [1-(4-fluorobenzyl)-1*H*-indolyl]carbonyl moiety and a (naphthalen-1-yl)amino moiety, which are characteristic parts of two synthetic cannabinoids, FDU-PB-22 (**1**) and NNEI, respectively (Fig. 1a–c) [10].

Identification of an unknown peak **3**

We detected an unknown peak **3** together with seven peaks of five known synthetic cannabinoids (AB-CHMINACA, 5-fluoro-AMB, FUB-PB-22, AM-2201 indazole analog and NNEI indazole analog), a known cathinone derivative DL-4662, and 8-quinolinol in the LC–MS and GC–MS chromatograms for product C (Fig. 5a, b, e). In the LC–MS and GC–MS analysis, the unknown peak **3** showed a protonated molecular ion signal at m/z 357 [$\text{M} + \text{H}^+$] (Fig. 5c) and a molecular ion signal at m/z 356 [M^+] (Fig. 5f). The accurate mass spectrum obtained by LC–QTOF–MS gave an ion signal at m/z 357.2282, suggesting that the protonated molecular formula of compound **3** was $\text{C}_{20}\text{H}_{29}\text{N}_4\text{O}_2$ (calcd. 357.2291). The presumed molecular formula of compound **3** ($\text{C}_{20}\text{H}_{28}\text{N}_4\text{O}_2$: 356) was thus the same as that of AB-CHMINACA (Fig. 1b). However, the LC–MS and GC–MS spectra patterns, in addition to each retention time, were different (Fig. 5c, d, f, g).

Table 2 NMR data of compound **3** and AB-CHMINACA

No.	AB-CHMINACA		Compound 3	
	^{13}C		^{13}C	^1H
1	172.6		172.6	–
2	56.7		58.4	4.38, 1H, dd, $J = 8.7, 6.9$ Hz
3	31.2		30.1	2.15, 1H, m
4	19.3		19.3	0.99, 3H, d, $J = 6.4$ Hz, overlapped
5	17.9		18.2	0.96, 3H, d, $J = 6.9$ Hz, overlapped
1'	–		–	–
2'	–		–	–
3'	136.4		129.1	–
3'a	121.8		120.3	–
4'	121.6		120.2	7.81, 1H, d, $J = 8.3$ Hz
5'	122.4		122.7	7.20, 1H, t, $J = 7.3$ Hz
6'	126.6		125.7	7.31, 1H, t, $J = 7.3$ Hz
7'	110.6		117.4	7.68, 1H, d, $J = 8.7$ Hz
7'a	141.2		146.4	–
1''	54.5		57.3	4.58 and 4.49, each 1H, dd, $J = 12.8, 7.3$ Hz
2''	38.3		38.9	1.90, 1H, m
3''	30.0		30.0	0.97, 2H, m, overlapped
4''	25.0		25.1	1.11, 2H, m, overlapped
5''	25.7		25.7	1.56, 1H, m
				1.09, 1H, m, overlapped
6''	25.1		25.1	1.61, 2H, m
7''	29.9		29.9	1.47, 2H, d, $J = 12.4$ Hz
1'''-CONH	161.3		159.8	8.38, 1H, d, $J = 8.7$ Hz
1-NH ₂	–		–	7.54 and 7.16, each 1H, brs

Recorded in $\text{DMSO}-d_6$ at 800 MHz (^1H) and 200 MHz (^{13}C), respectively; data in δ ppm (J in Hz)

The observed ^1H and ^{13}C NMR spectra (Table 2), HH COSY, HMQC, HMBC and ^{15}N HMBC correlations for compound **3** indicated the presence of *N*-cyclohexylmethyl-indazole and *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-carboxamide moieties (Fig. 6a, b). Additionally, 2D-INADEQUATE correlation revealed that the cyclohexylmethyl-indazole moiety is connected at the 3'-position of the indazole to the carboxamide (C-1'''). Nevertheless, the ^{13}C NMR chemical shifts of compound **3** were significantly different at the C-3' (δ_{C} 129.1), C-7' (δ_{C} 117.4) and C-7'a (δ_{C} 146.4) positions from those of AB-CHMINACA [C-3' (δ_{C} 136.4), C-7' (δ_{C} 110.6) and C-7'a (δ_{C} 141.2) as shown in Table 2.

We next compared the ^{15}N NMR chemical shifts of compound **3** with those of 1*H*-indazole derivatives (Table 3). The ^{15}N chemical shifts at the *N*-1' (δ_{N} –85.5) and *N*-2' (δ_{N} –153.9) of the indazole moiety in compound

Fig. 6 Results of incredible natural abundance double-quantum transfer experiment (INADEQUATE), HMBC, selected NOE correlations (a) and ^1H - ^{15}N HMBC correlations (b) for compound **3** (AB-CHMINACA 2*H*-indazole analog)

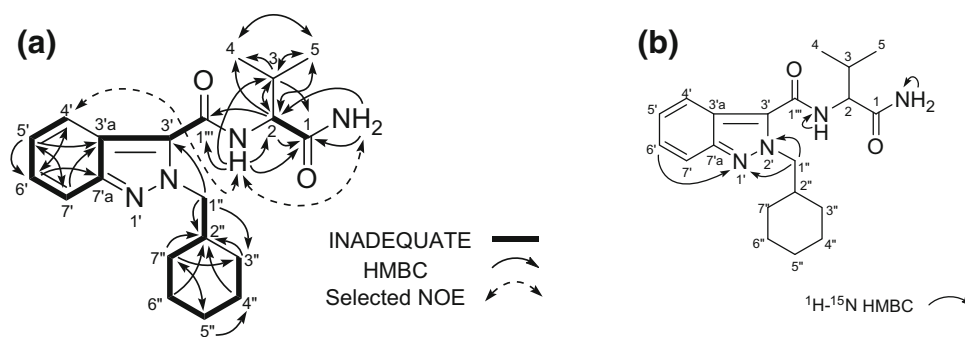


Table 3 ^{15}N NMR data of detected and related compounds

Type	1 <i>H</i> -Indazole				2 <i>H</i> -Indazole		
	1-Methyl-1 <i>H</i> -indazole ^a	AB-CHMINACA ^b	NNEI indazole analog ^c	Compd. 6 ^c	2-Methyl-2 <i>H</i> -indazole ^a	Compd. 3 ^b	Compd. 4 ^c
<i>N</i> -1'	-202.8	-191.4	-187.8	-185.5	-91.2	-85.5	-82.7
<i>N</i> -2'	-56.6	-62.8	-62.0	-53.0	-161.0	-153.9	-147.4
CONH	-	-273.3	-262.0	-	-	-257.2	-256.5
CONH ₂	-	-273.3	-	-	-	-274.0	-

^{15}N NMR chemical shifts, which observed in ^1H - ^{15}N HMBC spectrum, are referenced to the signal of CH_3NO_2 (δ_{N} 0.0 ppm)

^a Ref. [11], recorded in $\text{DMSO}-d_6$

^b Recorded in $\text{DMSO}-d_6$

^c Recorded in CDCl_3

3 were different from those of the 1*H*-indazole moiety in 1-methyl-1*H*-indazole [*N*-1' (δ_{N} -202.8) and *N*-2' (δ_{N} -56.6)] and AB-CHMINACA [*N*-1' (δ_{N} -191.4) and *N*-2' (δ_{N} -62.8)], respectively (Table 3; Fig. 1c). However, their shifts of compound **3** were similar to those of 2-methyl-2*H*-indazole [*N*-1' (δ_{N} -91.2) and *N*-2' (δ_{N} -161.0)] (Table 3; Fig. 1c) [14]. Therefore, the structure of compound **3** was determined as AB-CHMINACA 2*H*-indazole analog [*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-2-(cyclohexylmethyl)-2*H*-indazole-3-carboxamide] (Fig. 1a).

It was reported that different forms of tautomerism are very common in nitrogen compounds. For indazoles, in most cases the 1*H*-tautomer is the most stable; however, sometimes several indazoles of the 2*H*-tautomer can be more stable than 1*H*-tautomer [14]. The differences in energy between the 1*H*- and 2*H*-tautomers were interpreted in terms of substituent effects [14], and we have assumed that the minor component AB-CHMINACA 2*H*-indazole analog (**3**) is generated via tautomerization from AB-CHMINACA (1*H*-indazole). This is the first report of the identification of 2*H*-indazole isomers of synthetic cannabinoids in illegal products to our knowledge. The chemical characterization, pharmacological activity and toxicological activity of 2*H*-indazole isomers of synthetic cannabinoids as NPSs have never been reported before.

Identification of unknown peaks 4–6

Three unknown peaks **4**, **5**, and **6** were detected along with a synthetic cannabinoid NNEI indazole analog in the LC-MS and GC-MS chromatograms for product D (Figs. 7a, b, i, 1b). In the LC-MS and GC-MS analysis, the unknown peak **4** showed a protonated molecular ion signal at m/z 358 [$\text{M} + \text{H}^+$] (Fig. 7c, d) and a molecular ion signal at m/z 357 [M^+] (Fig. 7j). The accurate mass spectrum obtained by LC-QTOF-MS gave an ion signal at m/z 358.1916, suggesting that the protonated molecular formula of compound **4** was $\text{C}_{23}\text{H}_{24}\text{N}_3\text{O}$ (calcd. 358.1919). Hence, the presumed molecular formula of compound **4** ($\text{C}_{23}\text{H}_{23}\text{N}_3\text{O}$: 357) was the same as that of NNEI indazole analog. However, the GC-MS and LC-MS spectra patterns and each retention time of both compounds were different (Fig. 7d, e, j, k).

The ^1H and ^{13}C NMR spectra (Table 4), HH COSY, HMQC, HMBC and ^{15}N HMBC correlations of compound **4** suggested the presence of *N*-pentyl-indazole and *N*-(naphthalen-1-yl)carboxamide moieties (Fig. 8a, b). Therefore, the connectivity of the two moieties (positions 1 and 3') was not observed. On the other hand, the ^{13}C NMR chemical shifts of compound **4** at the C-3' (δ_{C} 127.1), C-7' (δ_{C} 119.0) and C-7'a (δ_{C} 147.3) positions were different from those of

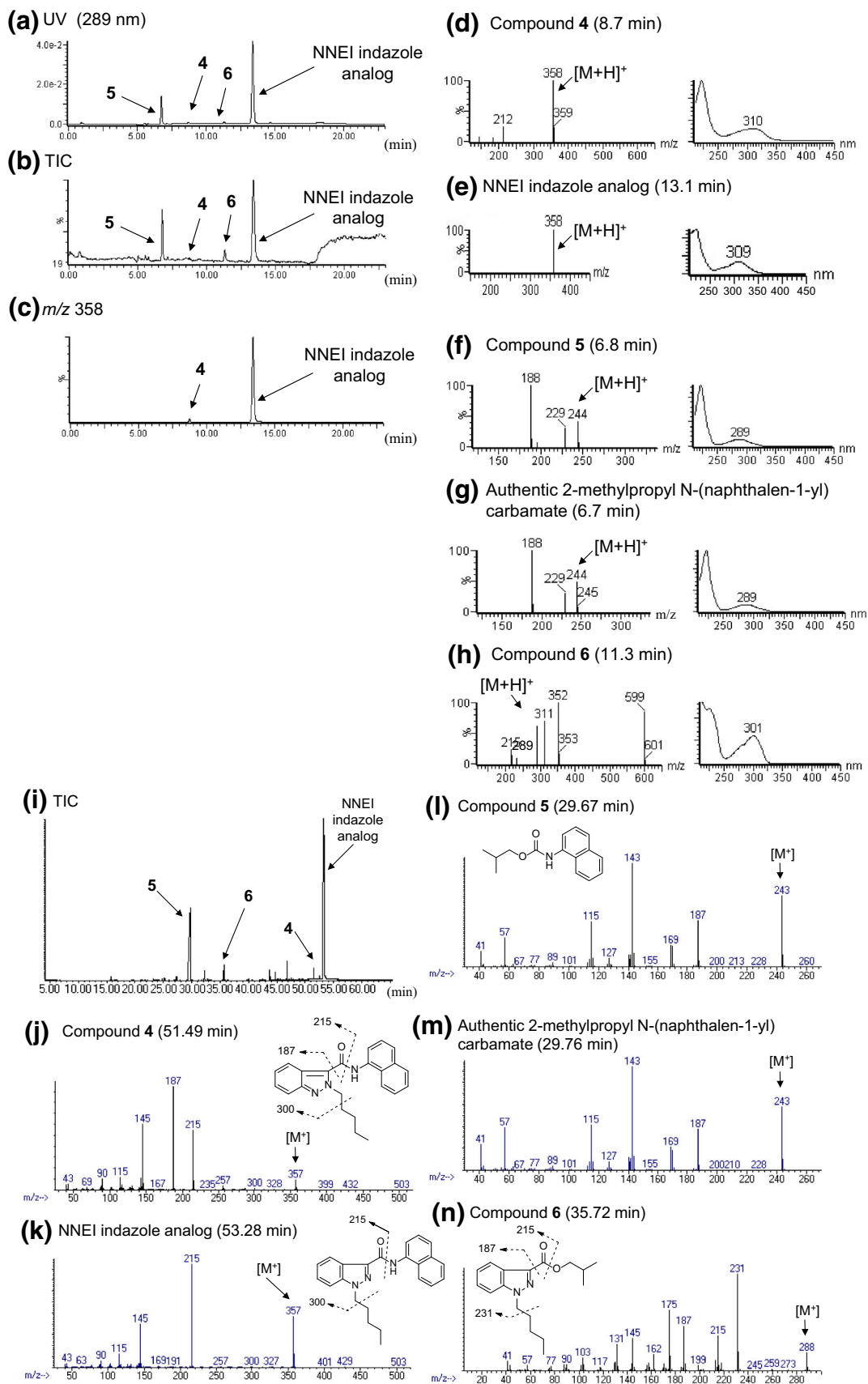


Fig. 7 LC–MS and GC–MS analyses of product D. The LC–UV–PDA chromatogram (a), TIC (b) and an extracted-ion chromatogram at m/z 358 (c) are shown, along with the ESI mass and UV spectra of peaks **4** (d), **5** (f), **6** (h), the authentic NNEI indazole analog (e) and the authentic 2-methylpropyl *N*-(naphthalen-1-yl) carbamate (g). TIC (i) and EI mass spectra of peaks **4** (j), **5** (l), **6** (n), the authentic NNEI indazole analog (k) and the authentic 2-methylpropyl *N*-(naphthalen-1-yl) carbamate (m) obtained by the GC–MS analysis are also indicated

NNEI indazole analog [C-3' (δ_c 137.3), C-7' (δ_c 109.4), and C-7'a (δ_c 141.1)] (Table 4). Comparing the ^{15}N NMR chemical shifts between the two compounds showed that the ^{15}N chemical shifts at the *N*-1' (δ_N -82.7) and *N*-2' (δ_N -147.4) of the indazole moiety in compound **4** were different from those of the 1*H*-indazole moiety in NNEI indazole analog [*N*-1' (δ_N -187.8) and *N*-2' (δ_N -62.0)] (Table 3). However, their shifts of compound **4** were similar to those of 2-methyl-2*H*-indazole [*N*-1' (δ_N -91.2) and *N*-2' (δ_N -161.0)] (Table 3) [14]. Therefore, it was revealed that compound **4** has a 2-pentyl-2*H*-indazole moiety.

We measured the deuterium isotope effect in compound **4** in the same way as that used for compound **2** (Fig. 4c) to determine the connection between the two moieties. The isotope shift values for the ^{13}C NMR signals of this compound are shown in Fig. 8c. The first- to fourth-largest deuterium shifts (0.115, 0.087, 0.048, 0.038 ppm) were observed at the C-1''', C-1, C-2''', and C-8'''a positions of the *N*-(naphthalen-1-yl)-carboxamide moiety. The fifth-largest deuterium shift of 0.029 ppm was attributed to the three-bond deuterium isotope effect of the NH amide proton on the indazole carbon (C-3'). These results suggested that the 2-pentyl-2*H*-indazole moiety is connected at the 3'-position of the 2*H*-indazole to the carboxamide (1-CONH). Finally, the structure of compound **4** was determined as NNEI 2*H*-indazole analog [*N*-(naphthalen-1-yl)-2-pentyl-2*H*-indazole-3-carboxamide] (Fig. 1a).

The unknown peak **5** was identified as a 2-methylpropyl *N*-(naphthalen-1-yl) carbamate (Fig. 7f, l) by direct comparison of the GC–MS and LC–MS data to those of the purchased authentic compound (Fig. 7g, m). This compound has not been reported as any cannabimimetic- or cannabinoid-related substance.

The GC–MS and LC–MS spectra of the unknown peak **6** are shown in Fig. 7h, n. A molecular ion signal of compound **6** was observed at m/z 288 in the GC–MS analysis (Fig. 7n). The accurate mass spectrum obtained by LC–QTOF–MS gave an ion peak at m/z 289.1906, suggesting that the protonated molecular formula of compound **6** was $\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_2$ (calcd. 289.1916). One-dimensional (1D)- and 2D-NMR analyses revealed that compound **6** has isobutyl-carboxylate and *N*-pentyl-indazole moieties, as shown in Fig. 8d and Table 5. The ^{15}N HMBC correlations of *N*-1' with H-6' and H-2'' (Fig. 8e) and the ^{15}N chemical shifts of

Table 4 NMR data of compound **4** and NNEI indazole analog

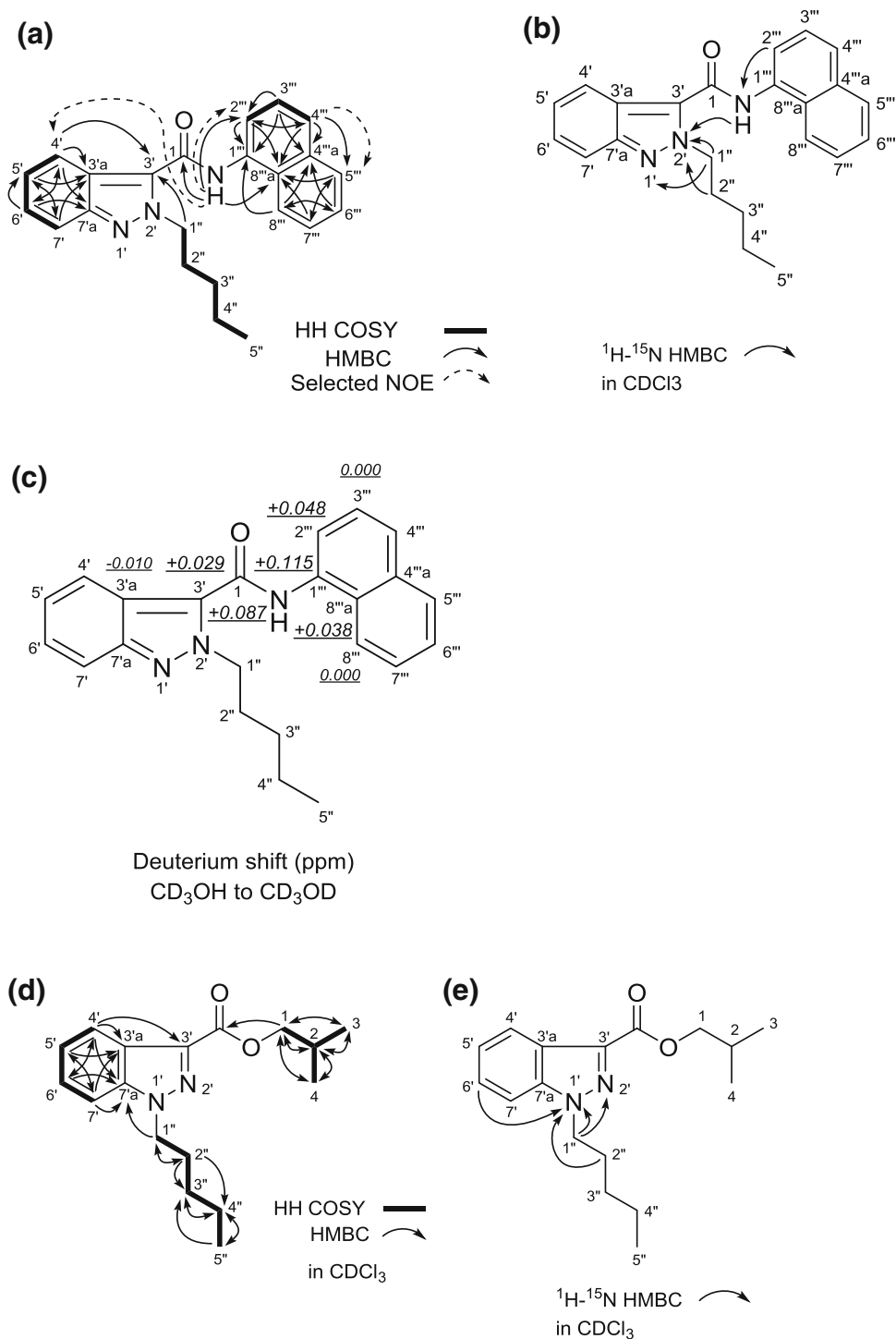
No.	NNEI 1 <i>H</i> -indazole analog ^a ^{13}C	Compound 4 ^b	
		^{13}C	^1H
1	160.9	159.0	–
2'	–	–	–
3'	137.3	127.1	–
3'a	123.0	120.6	–
4'	122.9	118.2	7.94, 1H, d, $J = 9.6$ Hz, overlapped
5'	122.9	124.9	7.34, 1H, td, $J = 7.6, 0.7$ Hz
6'	126.9	126.3	7.40, 1H, ddd, $J = 7.6, 6.5, 1.0$ Hz
7'	109.4	119.0	7.88, 1H, d, $J = 8.9$ Hz
7'a	141.1	147.3	–
1''	49.6	53.4	4.89, 2H, t, $J = 7.2$ Hz
2''	29.5	31.0	2.04, 2H, m
3''	29.0	28.9	1.35, 2H, m, overlapped
4''	22.3	22.3	1.35, 2H, m, overlapped
5''	14.0	14.0	0.87, 3H, t, $J = 7.2$ Hz
1'''	132.4	131.8	–
2'''	119.4	121.1	8.08, 1H, d, $J = 7.6$ Hz
3'''	126.0	126.2	7.55, 1H, m, overlapped
4'''	125.0	126.6	7.79, 1H, d, $J = 8.3$ Hz
4'''a	134.2	134.3	–
5'''	128.8	129.0	7.93, 1H, m, overlapped
6'''	125.9	126.8	7.54, 1H, m, overlapped
7'''	126.2	125.8	7.57, 1H, m, overlapped
8'''	120.5	120.4	7.92, 1H, m, overlapped
8'''a	126.7	127.2	–
NH	–	–	8.25, 1H, brs

^a Recorded in CDCl_3 at 800 MHz (^1H) and 200 MHz (^{13}C), respectively; data in δ ppm (J in Hz)

^b Recorded in CDCl_3 at 600 MHz (^1H) and 150 MHz (^{13}C), respectively; data in δ ppm (J in Hz)

compound **6** at the *N*-1' (δ_N -185.5) and *N*-2' (δ_N -53.0) of the indazole moiety, which were similar to those of the 1*H*-indazole moiety in NNEI indazole analog [*N*-1' (δ_N -187.8) and *N*-2' (δ_N -62.0)], suggested the presence of 1*H*-indazole (Table 3). No HMBC correlation was observed between the two moieties at position-3' and ester group of compound **6**. However, the major GC–MS fragment ion signal at m/z 187 was probably caused by the cleavage of a bond between the 1*H*-indazole and the ester group (Fig. 7n). In addition, another fragment ion signal at m/z 215 was probably caused by the cleavage of a bond of the ester group (Fig. 7n). Therefore, the structure of compound **6** was elucidated as isobutyl 1-pentyl-1*H*-indazole-3-carboxylate (Fig. 1a). Compound **6**, which is a novel substance, was not reported to have any pharmacological and toxicological activity.

Fig. 8 Results of HH COSY, HMBC, selected NOE correlations (a), ^1H - ^{15}N HMBC correlations (b) and deuterium-induced isotope shift of NH protons for the ^{13}C NMR signals of compound **4** (NNEI 2*H*-indazole analog) in CD_3OD (c). HH COSY and HMBC correlations (d) and ^1H - ^{15}N HMBC correlations (e) for compound **6** (isobutyl 1-pentyl-1*H*-indazole-3-carboxylate), are also shown



As a result, two major components, i.e., NNEI indazole analog and 2-methylpropyl *N*-(naphthalene-1-yl) carbamate (**5**), and three minor components, i.e., isobutyl 1-pentyl-1*H*-indazole-3-carboxylate (**6**), NNEI 2*H*-

indazole analog (**4**) and a presumed 1-pentyl-1*H*-indazole (elucidated by GC-MS, data not shown) were detected in product D. On the basis of these minor components, we expected the following reaction mechanism for the

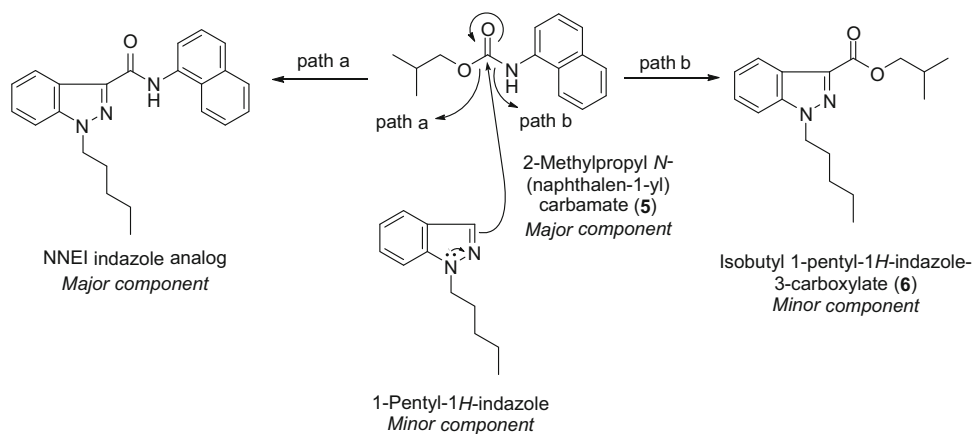
Table 5 NMR data of compound **6**

No.	¹³ C	¹ H
COO	162.9	–
2'	–	–
3'	134.8	–
3'a	123.5	–
4'	122.2	8.17, 1H, d, <i>J</i> = 8.3 Hz
5'	122.9	7.29, 1H, t, <i>J</i> = 8.3 Hz
6'	126.6	7.41, 1H, t, <i>J</i> = 8.3 Hz
7'	109.7	7.46, 1H, d, <i>J</i> = 8.3 Hz
7'a	140.5	–
1''	49.9	4.45, 2H, t, <i>J</i> = 7.3 Hz
2''	29.6	1.94, 2H, m
3''	28.9	1.29, 2H, m, overlapped
4''	22.3	1.33, 2H, m, overlapped
5''	13.9	0.86, 3H, t, <i>J</i> = 7.3 Hz
1	71.0	4.24, 2H, t, <i>J</i> = 6.4 Hz
2	27.9	2.19, 1H, m
3/4	19.3	1.05, 6H, d, <i>J</i> = 6.9 Hz

Recorded in CDCl₃ at 800 MHz (¹H) and 200 MHz (¹³C), respectively; data in δ ppm (*J* in Hz)

preparation of the major NNEI indazole analog; compound **5** is likely to react with 1-pentyl-1*H*-indazole to yield the major component NNEI indazole analog (path a) and the minor component **6** (path b), as shown in Fig. 9. In addition, the 1*H*- and 2*H*-indazole tautomerism of the starting material or the reaction product accounted for the existence of the minor 2*H*-indazole product (**4**).

Fig. 9 Possible reaction mechanisms for the preparation of NNEI indazole analog and compound **6**



Identification of unknown peaks **7** and **8**

Unknown peaks **7** and **8** were detected in the LC–MS chromatogram for product E (Fig. 10a, b, e). In the LC–MS chromatogram, the unknown peak **7** at 12.2 min showed a protonated molecular ion signal at *m/z* 224 ([*M* + *H*]⁺) (Fig. 10c). The accurate mass spectrum obtained by LC–QTOF–MS gave an ion peak at *m/z* 224.1280, suggesting that the protonated molecular formula of compound **7** was C₁₂H₁₈NO₃ (calcd. 224.1287).

The observed ¹H and ¹³C NMR (Table 6), HH COSY, HMQC, HMBC and 1D-NOE revealed that the structure of compound **7** was *N*-hydroxy-3,4-ethylenedioxy-*N*-methylamphetamine (*N*-OH-EDMA), as shown in Fig. 11a. In addition, Noggle et al. [15] reported that *N*-hydroxy-3,4-methylenedioxyamphetamine (*N*-OH-MDA), an analog of *N*-OH-EDMA (**7**), was unstable at high temperatures in a GC–MS analysis, and that *N*-OH-MDA (Fig. 1c) decomposed to MDA (Fig. 1c) [15]. In the present GC–MS TIC chromatogram, peak **7** (*N*-OH-EDMA) was not observed (Fig. 10e). However, the peak of its decomposed compound, which was finally identified as 3,4-ethylenedioxy-*N*-methylamphetamine (3,4-EDMA, Fig. 1b) by direct comparison of the GC–MS data to those of the purchased authentic compound (Fig. 10g), was detected (Fig. 10e, f). No pharmacological information is available for *N*-OH-EDMA (**7**); however, 3,4-EDMA has been reported to stimulate the release of serotonin and dopamine from rat brain synaptosomes at 1 μM [16].

The proposed fragment pattern and the presumed structure of peak **8** obtained by the GC–MS analysis

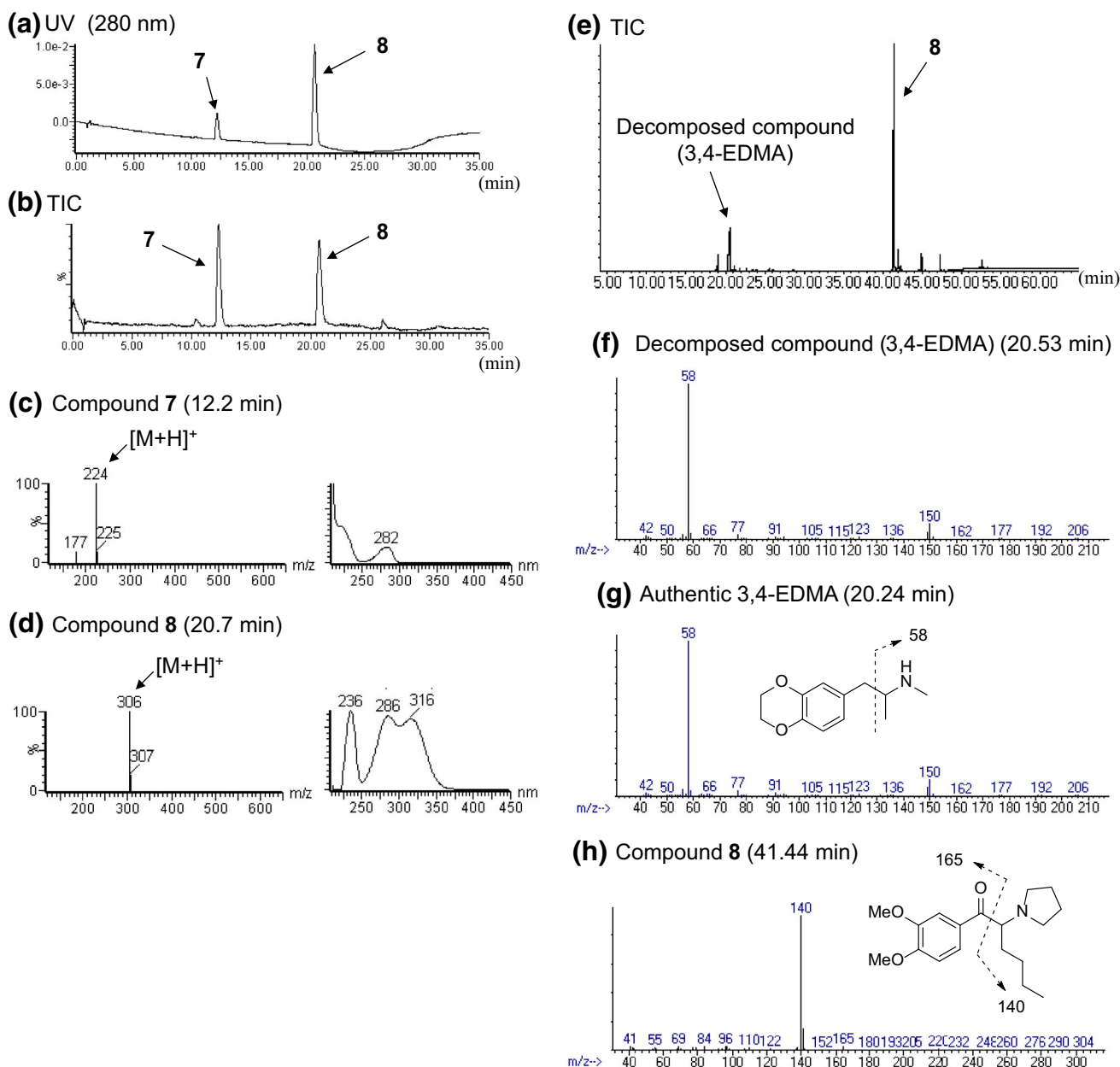


Fig. 10 LC–MS and GC–MS analyses of product E. The LC–UV–PDA chromatogram (a), TIC (b), ESI mass and UV spectra of peaks 7 (c) and 8 (d) are shown. TIC (e) and EI mass spectra of peaks of

decomposed compound [3,4-ethylenedioxy-*N*-methylamphetamine (3,4-EDMA)] (f), compound 8 (h) and the authentic 3,4-EDMA (g) obtained by GC–MS analysis are also presented

are shown in Fig. 10h. The LC–MS data revealed that peak 8 gave a protonated ion signal at m/z 306 ($[M + H]^+$) (Fig. 10d). The accurate mass spectrum obtained by LC–QTOF–MS gave an ion peak at m/z 306.2072, suggesting that the protonated molecular

formula of compound 8 was $C_{18}H_{28}NO_3$ (calcd. 306.2069).

The ^{13}C NMR spectrum of compound 8 was similar to a combination of two known cathinone derivatives: an α -pyrrolidinohexanone moiety of α -PHP and a 3,4-

Table 6 NMR data of compound **7**

No.	¹³ C	¹ H
1	38.3	2.84, 1H, dd, <i>J</i> = 13.1, 4.5 Hz 2.26, 1H, dd, <i>J</i> = 13.4, 8.6 Hz
2	64.2	2.67, 1H, m
3	14.1	0.83, 3H, d, <i>J</i> = 6.5 Hz
1'	133.2	–
2'	117.6	6.66, 1H, d, <i>J</i> = 1.7 Hz
3'	142.9	–
4'	141.4	–
5'	116.5	6.71, 1H, d, <i>J</i> = 7.9 Hz
6'	121.9	6.61, 1H, dd, <i>J</i> = 8.3, 1.7 Hz
–O–(CH ₂) ₂ –O–	64.0	4.18, 4H, m
	63.9	
N–CH ₃	44.2	2.46, 3H, s
N–OH	–	7.64, 1H, brs

Recorded at 600 MHz (¹H) and 150 MHz (¹³C) in DMSO-*d*₆, respectively; data in δ ppm (*J* in Hz)

dimethoxyphenyl moiety of DL-4662 (Table 7) [13]. The observed ¹H and ¹³C NMR (Table 7), HH COSY, HMQC, HMBC, and 1D-NOE correlations (Fig. 11b) suggested that the structure of compound **8** is dimethoxy- α -pyrrolidinohexanophenone (dimethoxy- α -PHP), as shown in Fig. 1a. The fragment ions at *m/z* 140 and 165 of compound **8** in the GC–MS spectrum corroborated the structure (Fig. 10h). Compound **8** was detected as a newly distributed designer drug, and its chemical and pharmaceutical data have not been reported. Dimethoxy- α -PHP (**8**) has been controlled as a designated substance (Shitei-Yakubutsu) in Japan since November 2014.

Conclusions

Four synthetic cannabinoids: FDU-PB-22 (**1**), FDU-NNEI (**2**), two 2*H*-indazole isomers of synthetic cannabinoids, i.e., AB-CHMINACA 2*H*-indazole analog (**3**) and NNEI

Table 7 NMR data of compound **8**

No.	¹³ C	¹ H
1	199.1	–
2	68.7	4.08, 1H, dd, <i>J</i> = 9.3, 4.8 Hz
3	30.0	2.08, 1H, m 1.83, 1H, m
4	28.9	1.32, 2H, m
5	23.1	1.26, 2H, m
6	14.1	0.78, 3H, t, <i>J</i> = 7.2 Hz
1'	130.6	–
2'	111.9	8.03, 1H, d, <i>J</i> = 1.7 Hz
3'	149.5	–
4'	154.1	–
5'	111.1	7.00, 1H, d, <i>J</i> = 8.6 Hz
6'	123.7	8.15, 1H, dd, <i>J</i> = 8.6, 1.7 Hz
2''/5''	50.7	2.75, 2H, m 2.64, 2H, m
3''/4''	23.9	1.63, 4H, m
3'-MeO	55.9/55.7	3.78, 3H, s
4'-MeO	55.7/55.9	3.78, 3H, s

Recorded at 600 MHz (¹H) and 150 MHz (¹³C) in pyridine-*d*₅, respectively; data in δ ppm (*J* in Hz)

2*H*-indazole analog (**4**), a phenethylamine derivative *N*-OH-EDMA (**7**), and a cathinone derivative dimethoxy- α -PHP (**8**) were newly identified in illegal products obtained in Japan. In addition, 2-methylpropyl *N*-(naphthalen-1-yl) carbamate (**5**) and isobutyl 1-pentyl-1*H*-indazole-3-carboxylate (**6**), which is probably a by-product in the synthesis of NNEI indazole analog from 1-pentyl-1*H*-indazole and compound **5** (Fig. 9), were detected. Among them, compounds **3**, **4**, and **6** were detected as minor components; their pharmacological and toxicological properties have never been reported. This appears to be the first report to estimate the synthetic pathway of illegal synthetic cannabinoids on the basis of detailed analytical data of illegal products.

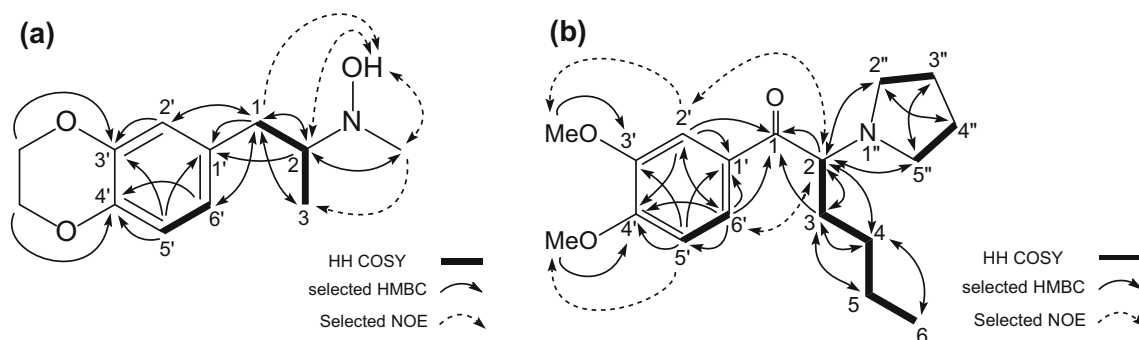


Fig. 11 HH COSY, HMBC, and selected NOE correlations for compound **7** (*N*-OH-EDMA, **a**) and for compound **8** (dimethoxy- α -PHP, **b**)

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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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