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ORIGINAL ARTICLE

Identification of a cannabimimetic indole as a designer drug in a herbal product

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Abstract A cannabimimetic indole has been identified as a new adulterant in a herbal product being sold illegally in Japan for its expected narcotic effect. Liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry analyses indicated that the product contained two major compounds. One was identified as a cannabinoid analog (1*RS,*3*SR*)-3-[4-(1,1-dimethyloctyl)-2-hydroxyphenyl]cyclohexan-1-ol (**1**) by direct comparison with the authentic compound, which we reported previously. The other compound (**2**) showed a molecular weight of 341 daltons, and accurate mass spectral measurements showed its elemental composition to be $C_{24}H_{23}NO$. Both mass and nuclear magnetic resonance spectrometric data revealed that **2** was 1-pentyl-3-(1-naphthoyl)indole [or naphthalen-1-yl-(1-pentylindol-3-yl)methanone] being identical to JWH-018, which was synthesized by Wiley and coworkers in 1998. This compound was reported as a potent cannabinoid receptor agonist possessing a pharmacological cannabimimetic activity.

Keywords 1-Pentyl-3-(1-naphthoyl)indole · Naphthalen-1-yl-(1-pentylindol-3-yl)methanone · JWH-018 · Cannabimimetic indole · Designer drug · Herbal product

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Introduction

Various psychotropic substances are being sold and distributed around the world via the Internet. Most recently, we found a synthetic cannabinoid analog (1*RS,*3*SR*)-3-[4-(1,1-dimethyloctyl)-2-hydroxyphenyl]cyclohexan-1-ol (**1**) [1], which contains no amino groups (Fig. 1), as an adulterant in a herbal product being commercially sold as an incense. This was the first report to identify a synthetic cannabinoid analog in a herbal product distributed on the illegal drug market for its expected narcotic effect. At almost the same time, we found another compound (**2**) that was also thought to be an adulterant in the same type of herbal products. This compound was finally found to be identical to JWH-018, a cannabimimetic aminoalkyl naphthoyl indole derivative; it had been first synthesized by Huffman and coworkers in 1998, and was reported as a potent cannabinoid receptor agonist possessing a cannabimimetic pharmacological activity in vivo [2–5]. Also, in January 2009, the Health Minister of Germany announced that **2** is an active component in a mislabeled mixture of herbs; **2** has been a controlled substance in Germany since 22 January 2009 [6]. However, no scientific report describing the isolation and identification of this compound from herbal products has been published. The present report deals with the details of its identification in a herbal product by various instrumental analyses.

Materials and methods

Materials and preparation

Acetonitrile (high-performance liquid chromatography grade) and all other chemicals (analytical grade) were

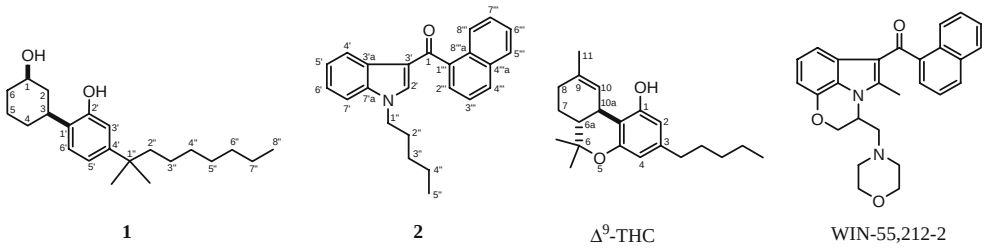

Fig. 1 Structures of detected compounds **1**, **2** and related compounds [Δ^9-tetrahydrocannabinol (Δ^9-THC) and WIN-55,212-2]

obtained from Wako (Osaka, Japan). A product, described as a herbal mixture and having the appearance of dried plants, was purchased via the Internet (December 2008). A 10-mg portion of the product was crushed into powder and extracted with 1 ml of methanol under ultrasonication for 10 min. After centrifugation for 5 min at 3000 rpm, the supernatant solution was passed through a centrifugal filter (Ultrafree-MC, 0.45 μm filter unit, Millipore, Bedford, MA, USA).

Instrumental analyses

Gas chromatography-mass spectrometry (GC-MS) was used in the electron impact (EI) mode at 70 eV of electron energy. The analysis was performed on a Hewlett-Packard 6890N GC with a 5975 mass-selective detector (Agilent, Palo Alto, CA, USA) using a capillary column (HP1-MS capillary, 30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent) and helium as carrier gas. An initial column temperature of 80°C was employed, and it was increased at a rate of 5°C/min to 190°C and then at a second rate of 10°C/min up to 310°C. The data were obtained in the full scan mode with a scan range of *m*/*z* 40–550. The analysis was performed under the same conditions as used in the analysis of designated drugs (Shitei-Yakubutsu) controlled by the Pharmaceutical Affairs Law of Japan [7].

The MS analysis was also made by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). The instrument consisted of an ACQUITY ultra-performance LC system connected with a single quadrupole mass detector and a photodiode array (PDA) detector (Waters, Milford, MA, USA). The sample solutions were separated using an ACQUITY UPLC HSS T3 column (2.1 mm i.d. × 100 mm, 1.8 μm; Waters) protected by a Van Guard column (2.1 mm i.d. × 5 mm, 1.8 μm; Waters) at 40°C. The following gradient system was used with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile) delivered at 0.3 ml/min; 50% A/50% B for 3 min, changing to 20% A/80% B over 2 min and held with the final composition over 5 min. The injection volume was 1 μl. The wavelength of the PDA detector for screening was set from 190 to 500 nm, and chromatographic peaks were monitored at 275 nm. Mass analysis by ESI was used in both positive and negative modes. Nitrogen gas was used for desolvation at a flow rate of 650 l/h at 350°C. The capillary and cone voltages were 3000 V and 30 V, respectively. MS data were recorded in the full scan mode (*m*/*z* 150–700).

The accurate mass spectrum of the target compound was measured using a direct analysis in real time (DART) ion source coupled to a time-of-flight (TOF) mass spectrometer (AccuTOF JMS-100LC, JEOL, Tokyo, Japan) operated in the positive ion mode. The measurements were made with the ion guide peak voltage set at 500 V, the reflectron voltage at 950 V, orifice 1 voltage at 15 V, orifice 2 voltage at 5 V, ring lens voltage at 5 V, and the orifice 1 temperature at 80°C. The mass range was 100–500 daltons. The DART ion source was used at a helium gas flow rate of 2.0 l/min, the gas heater temperature at 250°C, the discharge electrode needle setting at 3200 V, electrode 1 at 100 V, and electrode 2 at 250 V. Internal mass number calibration was achieved using PEG600, and diphenhydramine was used as an internal standard for each analysis.

For nuclear magnetic resonance (NMR) analysis, $CDCl_3$ (99.96%) was purchased from ISOTEC, a part of Sigma-Aldrich (St. Louis, MO, USA). The NMR spectra were obtained on ECA-600 and ECA-800 spectrometers (JEOL). Assignments were made via ^{1}H NMR, ^{13}C NMR, heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), double quantum filtered correlation spectroscopy (DQF-COSY), and rotating frame nuclear overhauser effect (ROE) spectra.

Isolation of compound **2**

A 3-g portion of the herbal product was extracted with 100 ml of methanol by ultrasonication for 1 h. After the extraction was repeated three times, the combined supernatant was evaporated to dryness. The extract was loaded on a preparative silica gel thin layer chromatography (TLC) plate (Silica Gel 60, 20 × 20 cm, 2 mm, Merck, Darmdstadt, Germany) using hexane/acetone

(4 : 1) as developing solvent. A portion of the silica gel in the TLC plate was taken and eluted with CH_2Cl_2/methanol (2 : 1) to give fraction 1. Repeated fractionation of fraction 1 by preparative silica gel TLC with hexane/CH_2Cl_2 (1 : 20) gave compound **2** (15 mg) as an off-white solid.

Results and discussion

In the sample solution of the product, two major peaks were detected by LC-ESI-MS analysis (Fig. 2a–d). One peak, detected at 7.8 min, exhibited two ion peaks at *m/z* 333 $[M+H]^+$ and at 315 $[M+H-18]^+$ in the positive scan mode (Fig. 2e). A comparison with the mass spectrum of the authentic compound revealed that this peak was (1*RS,*3*SR*)-3-[4-(1,1-dimethyloctyl)-2-hydroxyphenyl]cyclohexan-1-ol (**1**) (Fig. 1), which was reported as an adulterant in a herbal product in our previous study [1]. Another unknown peak (**2**) detected at 7.5 min showed a major peak at *m/z* 342 $[M+H]^+$ (Fig. 2f). The PDA-sliced ultraviolet (UV) spectrum of the peak (**2**) exhibited maxima at 218, 247, and 314 nm and minima at 239 and 285 nm (Fig. 2h). These characteristics were completely different from those of **1** (UV λ_{max} 220, 275 nm; λ_{min} 212, 249 nm, Fig. 2g).

GC-EI-MS analysis showed two major peaks with a peak of α-tocopherol, which had been added as an antioxidant (Fig. 3a). One peak, detected at 47.9 min, showed a mass spectrum with four ion peaks at *m/z* (relative intensity) 332 (16), 314 (14), 233 (80), and 215 (100) as shown in Fig. 3b, which was identical to the mass spectrum of the authentic compound (**1**). An unknown peak (**2**), detected at 51.7 min, showed a mass spectrum with five ion peaks at *m/z* 341 (100), 324 (43), 284 (58), 214 (52), and 127 (32), as shown in Fig. 3c.

The accurate mass spectrum measured by TOF-MS showed a protonated molecular ion peak ($[M+H]^+$) at *m/z* 342.18579 in the positive mode, suggesting that the molecular formula of **2** was $C_{24}H_{24}NO$. The error between

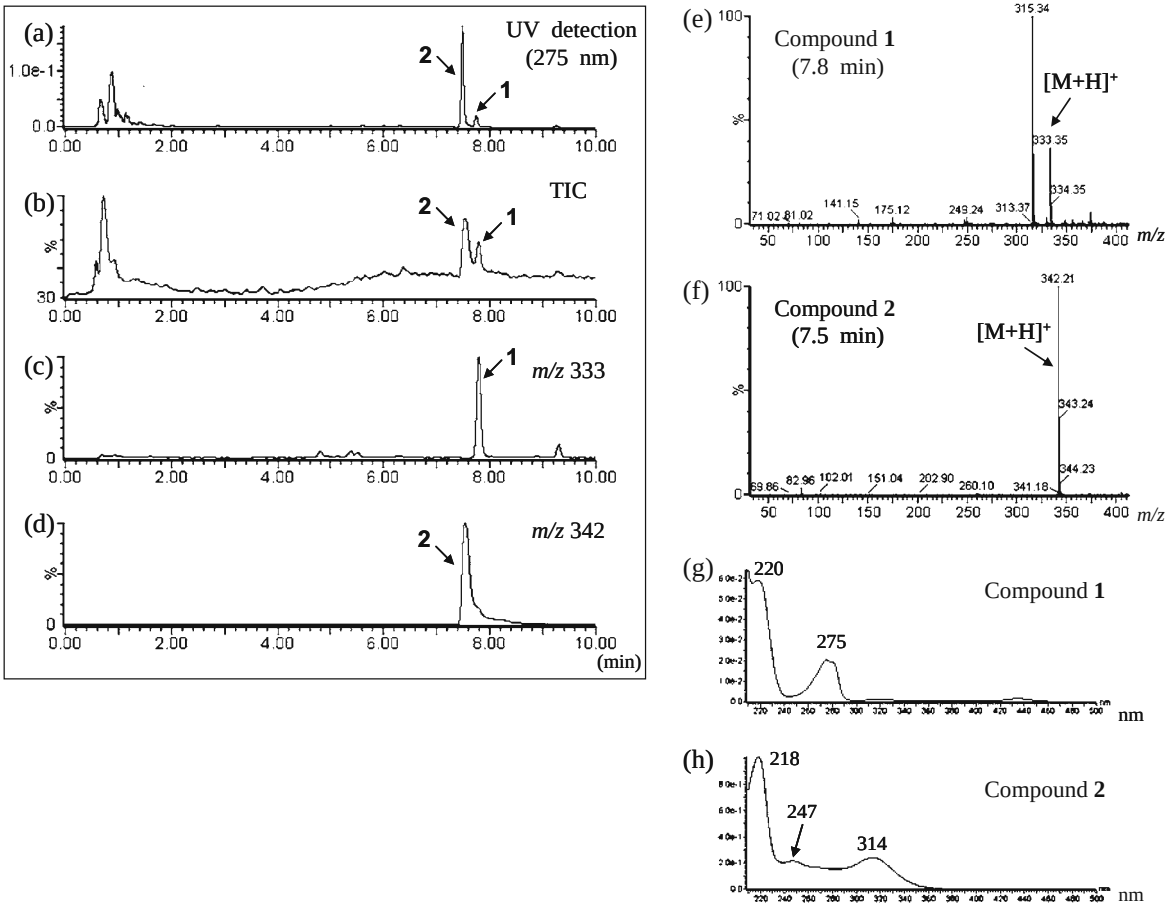

Fig. 2a–h Data from high-performance liquid chromatography with ultraviolet detection (**a**, **g**, **h**) and liquid chromatography-electrospray ionization-mass spectrometry (**b–f**) for the extract of the sample. Total ion chromatogram (**b**), mass chromatograms at *m/z* 333 (**1**) (**c**) and *m/z* 342 (**2**) (**d**), electrospray ionization mass spectra (**e**, **f**) and ultraviolet spectra (**g**, **h**) of each peak are shown

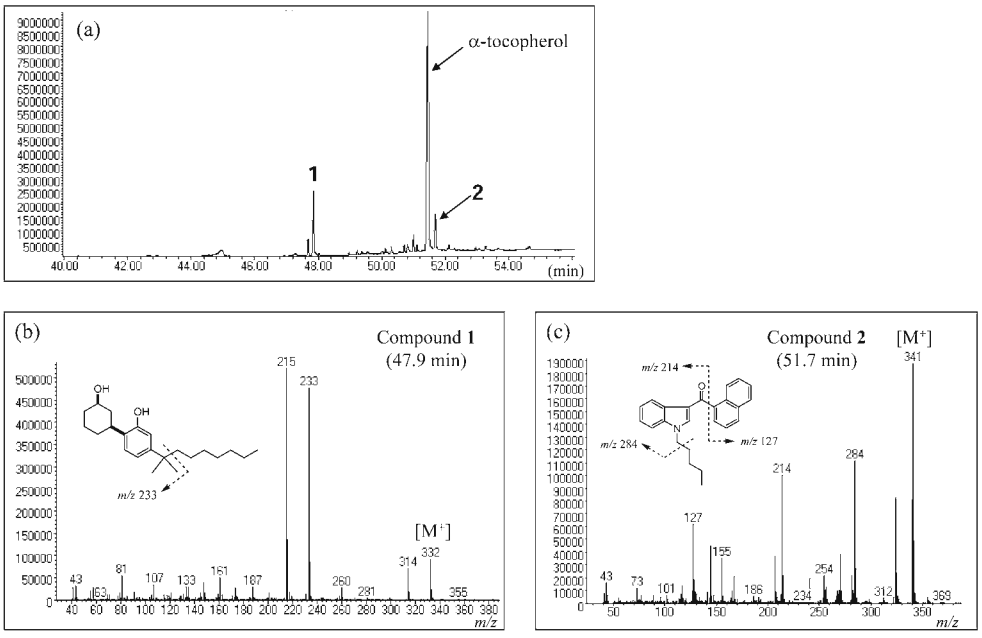

Fig. 3 Total ion chromatogram (**a**) and electron impact mass spectra of the peaks detected at 47.9 min (**1**) (**b**) and 51.7 min (**2**) (**c**) measured by gas chromatography-mass spectrometry

Table 1 Nuclear magnetic resonance data of compound **2**

No.	^{13}C	^{1}H	HMBC[a]
1	192.0	–	–
2’	137.9	7.33, 1H, s, overlapped	1, 3’, 3’a, 7’a, 1”
3’	117.5	–	–
3’a	127.0	–	–
4’	122.9	8.47, 1H, m	3’, 3’a, 6’, 7’a
5’	122.8	7.35, 1H, m, overlapped	7’
6’	123.6	7.35, 1H, m, overlapped	7’a
7’	110.0	7.38, 1H, m, overlapped	3’a, 5’, 7’a
7’a	137.0	–	–
1”	47.2	4.05, 2H, t, J = 7.4 Hz	2’, 7’a, 2”, 3”
2”	29.5	1.79, 2H, quint, J = 7.4 Hz	1”, 3”, 4”
3”	28.9	1.24, 2H, m, overlapped	1”, 4”, 5”
4”	22.2	1.28, 2H, m, overlapped	2”, 3”, 5”
5”	13.8	0.83, 3H, t, J = 7.0 Hz	3”, 4”
1”’	139.1	–	–
2”’	125.8	7.64, 1H, dd, J = 7.1, 1.3 Hz	1, 3”’, 4”’, 8”’a
3”’	124.5	7.51, 1H, dd, J = 8.3, 7.1 Hz, overlapped	1”’, 2”’, 4”’a
4”’	129.9	7.95, 1H, brd, J = 8.3 Hz	2”’, 4”’a, 5”’, 8”’a
4”’a	133.7	–	–
5”’	128.1	7.90, 1H, brd, J = 8.3 Hz	4”’, 7”’, 8”’a
6”’	126.3	7.50, 1H, td, J = 6.9, 1.4 Hz, overlapped	4”’a, 7”’, 8”’
7”’	126.7	7.45, 1H, ddd, J = 8.3, 6.9, 1.4 Hz	5”’, 8”’a
8”’	126.0	8.17, 1H, brd, J = 8.3 Hz	1”’, 4”’a, 6”’, 8”’a
8”’a	130.8	–	–

Recorded in $CDCl_3$ at 600 and 800 MHz (^{1}H) and 150 and 200 MHz (^{13}C), respectively; data in δ ppm

[a] For heteronuclear multiple-bond correlation (HMBC), J = 8 Hz, the proton signal correlated with the indicated carbons

the mass number observed and theoretical mass number of $[M+H]^+$ was –0.10 amu.

The ^{1}H NMR spectrum of **2** showed 23 nonexchangeable protons, including a methyl signal at δ 0.83 (3H, t, J = 7.0 Hz), AB_2-type aromatic proton signals at δ 7.51 (1H, dd, J = 8.3, 7.1 Hz), 7.64 (1H, dd, J = 7.1, 1.3 Hz), and 7.95 (1H, brd, J = 8.3 Hz), and AA’BB’-type aromatic proton signals at δ 7.45 (1H, ddd, J = 8.3, 6.9, 1.4 Hz), 7.50 (1H, td, J = 6.9, 1.4 Hz), 7.90 (1H, brd, J = 8.3 Hz), and 8.17 (1H, brd, J = 8.3 Hz) as shown in Table 1. In addition, the ^{1}H NMR spectrum also showed three methylene proton signals, at δ 1.24 and 1.28 (each 2H, m) and at 1.79 (2H, quint, J = 7.4 Hz), as well as a characteristic methylene signal connected to a nitrogen atom at δ 4.05 (2H, t, J = 7.4 Hz). The ^{13}C NMR spectrum of **2** showed 24 carbon signals, suggesting the

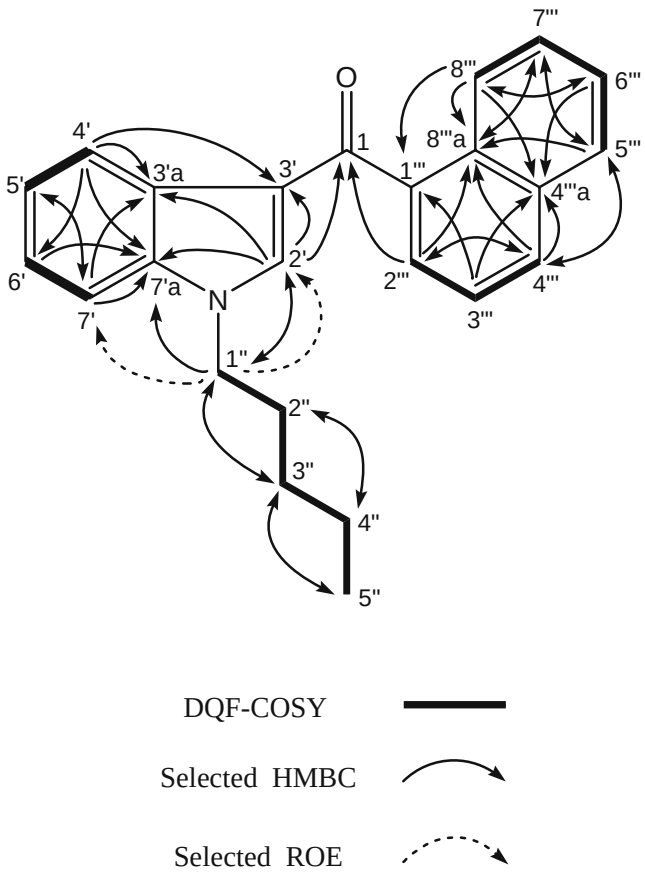

Fig. 4 Selected correlations for compound **2** by two-dimensional nuclear magnetic resonance spectroscopy techniques. *DQF-COSY*, Double quantum filtered correlation spectroscopy; *HMBC*, heteronuclear multiple-bond correlation spectroscopy; *ROE*, rotating frame nuclear overhauser effect spectroscopy

presence of a methyl, 4 methylenes with a nitrogenated carbon (δ 47.2), 12 aromatic carbons (δ 110.0, 122.8, 122.9, 123.6, 124.5, 125.8, 126.0, 126.3, 126.7, 128.1, 129.9, and 137.9), 6 aromatic quaternary carbons (δ 117.5, 127.0, 130.8, 133,7, 137.0, and 139.1), and a carbonyl carbon (δ 192.0). The presence of three partial structures (a 1,3-substituted indole group, a 1-substituted naphthalene group, and an *n*-pentyl group) was suggested from the DQF-COSY, HMQC, and HMBC spectra (Table 1, Fig. 4). The connectivity of these groups and the carbonyl group was deduced from the HMBC spectrum. An aromatic proton at δ 7.33 (H-2') of the indole group correlated to the carbonyl carbon at δ 192.0 (C-1), and the methylene carbon at δ 47.2 (C-1") of the *n*-pentyl group and an aromatic proton at δ 7.64 (H-2"') of the naphthalene group showed correlations to the carbonyl carbons at δ 192.0 (C-1). In addition, irradiation of the methylene protons at δ 4.05 (H-1") of the *n*-pentyl group resulted in ROE responses by the aromatic protons (H-2' and H-7') as shown in Fig. 4.

On the basis of the mass spectra (Figs. 2, 3) and NMR data (Table 1, Fig. 4), the structure of **2** was finally elucidated as 1-pentyl-3-(1-naphthoyl)indole [or naphthalen-1-yl-(1-pentylindol-3-yl)methanone]. The deduced compound had been already synthesized and named JWH-018 by Wiley et al. [2] in 1998. This compound is a potent cannabinoid receptor agonist possessing a pharmacological activity of a cannabinoid in vivo [2–5]. Wiley et al. [2] described that **2** showed a 4.5-fold more potent affinity for the CB_1 receptor ($K_i = 9 \pm 5$ nM) than did Δ^9-tetrahydrocannabinol (Δ^9-THC, Fig. 1), which is psychoactive and a major constituent of *Cannabis sativa* L. (cannabis, hemp, marijuana, marihuana) ($K_i = 41 \pm 2$ nM). Compound **2** produced potent cannabinoid effects of antinociception, hypomobility, hypothermia, and ring immobility in in vivo assays [2,3]. In the present study, we have identified compound **2** as a designer drug and an adulterant together with **1** in a herbal product.

The synthesis of many analogs of **1** and **2** together with pharmacological data has been already described [2–5,8–11]. In the past few decades, a number of analogs of Δ^9-THC have been synthesized based on the partially reduced dibenzopyran structure of THC, and their structure–activity relationships were studied [12,13]. In the 1980s, a group at Pfizer explored the development of analgesics using potent synthetic nontraditional cannabinoids, which lack the dibenzopyran structure present in the traditional cannabinoids but exhibit typical cannabinoid pharmacological effects [14–22]. On the other hand, D'Ambra et al. [23] reported in 1992 that aminoalkylindoles, such as WIN-55212-2, were bound to a cannabinoid brain receptor with high affinity (Fig. 1). A subsequent study by Huffman et al. [24] established that an aminoalkyl portion of the molecule, such as WIN-55212-2, could be replaced by an alkyl group to provide indole derivatives that have higher affinity for the brain receptor and exhibit typical cannabinoid pharmacological effects in vivo. These authors also described the structure–activity relationships of indole-derived, pyrrole-derived, and indene-derived cannabinoids [2,3,11]. After the discovery of cannabinoid receptors, CB_1 (central type) and CB_2 (peripheral type), as well as the discovery of endogenous cannabinoids, their physiological roles were elucidated to some extent [25]. A number of cannabinoid analogs, such as derivatives based on THC, indole, pyrrole, indene, and pyrazole, were then newly synthesized and their pharmacological activities applicable to the treatments of various diseases were studied [26,27]. This situation alerts us that these cannabinoid analogs other than **1** and **2** will be found as designer drugs or adulterants in illegal products as cannabis replacements in the near future. To avoid health problems and abuse caused by new designer

drugs, we must continuously monitor such compounds through surveillance.

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