Forensic Toxicol
DOI 10.1007/s11419-014-0253-6

ORIGINAL ARTICLE

Identification of *N,N*-bis(1-pentylindol-3-yl-carboxy)naphthylamine (BiPICANA) found in an herbal blend product in the Tokyo metropolitan area and its cannabimimetic effects evaluated by in vitro [^{35}S]GTPγS binding assays

Jun'ichi Nakajima · Misako Takahashi · Nozomi Uemura · Takako Seto · Haruhiko Fukaya · Jin Suzuki · Masao Yoshida · Maiko Kusano · Hiroshi Nakayama · Kei Zaitsu · Akira Ishii · Takako Moriyasu · Dai Nakae

Received: 18 September 2014 / Accepted: 30 October 2014

Abstract During our careful survey of unregulated psychotropic drugs in June 2013 in the Tokyo metropolitan area, we found a new compound in a herbal product. It was identified as an analog of NNEI (MN-24) and differed in that its molecule possessed another *N*-pentyl indole-carbonyl group: *N*, *N*-bis(1-pentylindol-3-yl-carboxy)naphtylamine (BiPICANA, compound **2**). Compound **2** was purified by silica and octadecyl group bonded type silica gel (C18, ODS) columns and confirmed by liquid chromatography–mass spectrometry, accurate mass spectrometry, nuclear magnetic resonance spectroscopy, and X-ray crystallography. No pharmacological information on compound **2** has been reported previously; our experiments on the binding ability of compound **2** to cannabinoid receptors revealed that it has affinities for CB_1 and CB_2 receptors (EC_{50} = 3.80 and 8.77 × 10^{-7} M, respectively). This is the first report identifying compound **2** in a dubious herbal product and demonstrating its binding affinities to cannabinoid receptors. Binding affinities of azepane isomers (compounds **3** and **4**) of AM-1220 and AM-2233, also found in commercial products in Japan, are also presented in this report.

Keywords *N,N*-Bis(1-pentylindol-3-yl-carboxy)naphtylamine (BiPICANA) · Azepane isomers of AM-1220 and AM-2233 · [^{35}S]GTPγS binding assay · X-ray crystallographic analysis · Synthetic cannabinoid

Introduction

Recently, various types of illegal drug products having cannabimimetic actions are being made commercially available via the Internet and street shops not only in Tokyo, but also in other areas of Japan. Many drug compounds in herbal products that have been identified so far [1–17] are regulated as scheduled substances (Shitei–Yakubutsu) by the Pharmaceuticals Affairs Law of Japan. Despite these administrative actions, new compounds continue to appear one after another, leading to detrimental accidents caused by their careless use. To counteract such a phenomenon, we strongly believe that identification of new designer drugs is crucial. In 2014, the detection of NNEI (compound **1**), along with its chlorinated and fluorinated analogs were reported by Uchiyama et al. [15]. We newly identified compound **2** as a unique analogue of compound **1** in our June 2013 survey in Tokyo. This article reports the details of the identification of compound **2** in an herbal blend product. The cannabimetic effects of compound **2** as well as azepane isomers of AM-1220 and AM-2233 (compounds **3** and **4,** respectively), which had been identified by Kneisel et al. [18] and by our group [10], respectively, are also described. The chemical structures of compounds **1**–**4** are shown in Fig. 1.

J. Nakajima (✉) · M. Takahashi · N. Uemura · T. Seto · J. Suzuki · M. Yoshida · T. Moriyasu · D. Nakae
Department of Pharmaceutical and Environmental Sciences, Tokyo Metropolitan Institute of Public Health, 3-24-1 Hyakunin-cho, Shinjuku-ku, Tokyo 169-0073, Japan
e-mail: Junichi_Nakajima@member.metro.tokyo.jp

H. Fukaya
Instrumental Analysis Center, Tokyo University of Pharmacy and Life Science, Hachioji, Japan

M. Kusano · H. Nakayama · K. Zaitsu · A. Ishii
Department of Legal Medicine and Bioethics, Nagoya University Graduate School of Medicine, Nagoya, Japan

Published online: 27 November 2014

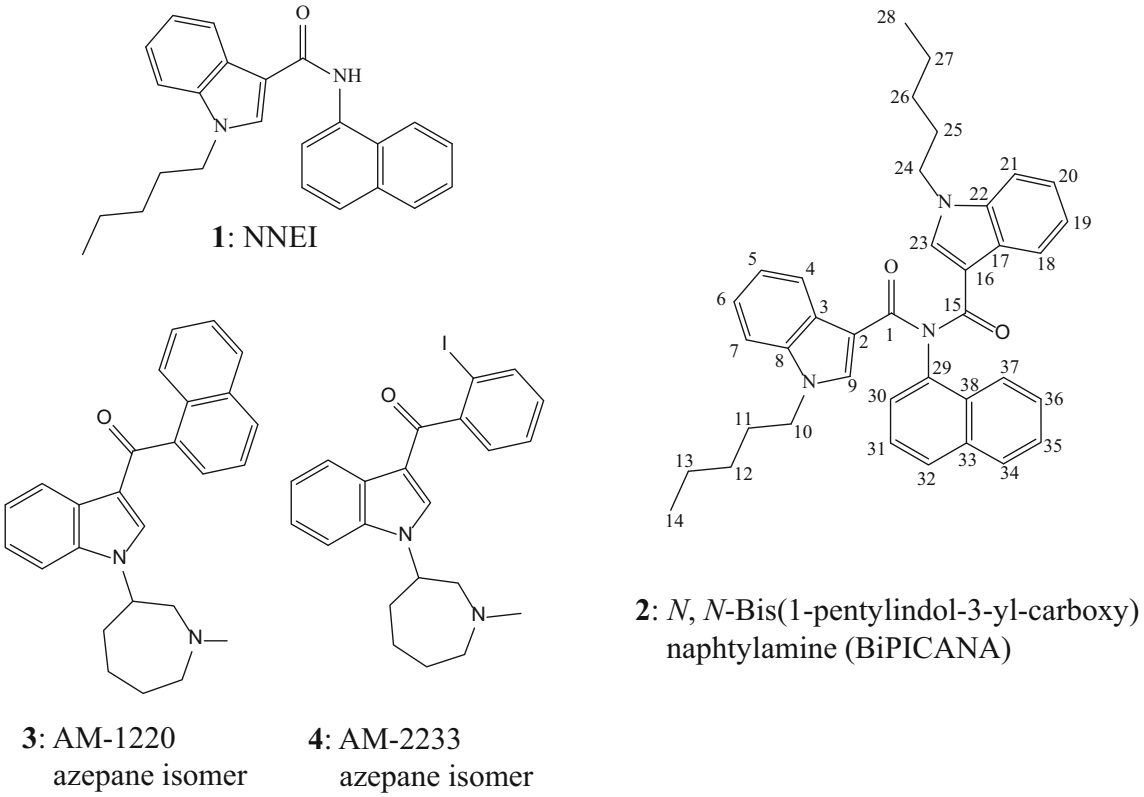

Fig. 1 Chemical structures of compounds **1**–**4**

Materials and methods

Chemicals and reagents

NNEI (**1**), CP55940 and 5F-PB-22 were purchased from Cayman Chemical (Ann Arbor, MI, USA), azepane isomers of AM-1220 and AM-2233 (**3** and **4**) were isolated in our laboratory from the commercial herbal products and their chemical structures were confirmed by comparing with the relevant reference data [10, 18]. Formic acid in acetonitrile [0.1 %, v/v, liquid chromatography–mass spectrometry (LC–MS) grade] and $CDCl_3$ (99.8 %) for nuclear magnetic resonance (NMR) analysis were purchased from Wako (Osaka, Japan); 1*H*-indole-3-carboxylic acid, *N*,*N*-dimethylformamide, sodium hydride, 1-chloropentane, thionyl chloride, α-naphthylamine, triethylamine, and dichloromethane from Tokyo Chemical Ind. (Tokyo, Japan); dimethyl sulfoxide (DMSO) (guaranteed reagent) from Kanto chemical (Tokyo, Japan); [^{35}S]GTPγS (46.25 TBq/mmol) from PerkinElmer (Boston, MA, USA); guanosine 5′-diphosphate sodium salt (GDP) and fatty acid-free bovine serum albumin (BSA) from Sigma–Aldrich (St. Louis, MO, USA). All other common chemicals and solvents were of analytical or high-performance liquid chromatography (HPLC) grade. Silica gel for chromatography (Chromatorex Q-PACK SI 50, particle size 60 μm, 100 × 27 mm i.d. (column I); and Chromatorex Q-PACK C18 SI 50, particle size 60 μm, 50 × 10 mm i.d. (column II) were purchased from Fuji Silysia Chemical (Kasugai, Japan).

Sample and test solution preparation

The commercial product analyzed in this report was purchased at a street shop in Tokyo, June 2013. The test solution for liquid chromatography (LC) and gas chromatography was prepared according to the previously reported procedures [5] except that acetonitrile was used instead of methanol. Where necessary, the test solution was diluted with acetonitrile to an adequate concentration before analysis.

Isolation of compound **2**

Approximately 3 g of the herbal product was extracted in 100 ml of acetonitrile with ultrasonication for 30 min. Acetonitrile solution was then evaporated to dryness, and the residue (350 mg) was reconstituted in 1 ml of chloroform solution and loaded onto column I. Chromatographic separation by semi-preparative LC was performed with the

total flow velocity of the mobile phase at 16 ml/min, using hexane (A) and ethyl acetate (B) gradient elution. The gradient program was 100 % A/0 % B held for 20 min, followed by linear gradient to 50 % A/50 % B over 20–70 min. The fractions eluted at 40–50 min were combined and evaporated to dryness, and this residue (80 mg) was reconstituted in 1 ml of methanol solution and loaded onto column II for final purification. Separation by column II with the total flow velocity of the mobile phase at 3 ml/min was performed using purified water (C) and methanol (D) gradient elution. The gradient program was 50 % C/50 % D held for 10 min followed by a linear gradient to 0 % C/100 % D over 10–110 min. The fractions eluted at 90–105 min were combined (45 ml), 10 ml of which was kept in cold room at 12 °C in 50 % relative humidity for 7 days, then a small single crystal was obtained for X-ray crystallographic analysis. The remaining 35 ml of the solution was evaporated to dryness resulting in pale brown oil (20 mg).

Synthesis of compound **2**

Synthesis of 1-pentyl-1*H*-indole-3-carboxylic acid was performed as follows. 1*H*-Indole-3-carboxylic acid (500 mg) was dissolved in *N*,*N*-dimethylformamide (7 ml) and cooled to 0 °C. The total weight (400 mg) of sodium hydride was added to the solution carefully, and the mixture was warmed to room temperature and stirred for 1 h. The mixture was cooled again to 0 °C, and then a solution of 1-chloropentane (500 mg) in *N*,*N*-dimethylformamide (3 ml) was slowly added dropwise; the mixture was warmed to room temperature and stirred for 2 h. After cooling the reaction mixture to 0 °C, ice was added followed by water (20 ml). Precipitated crystals were collected by filtration and washed with water and *n*-hexane. The product was dried under reduced pressure to obtain 1-pentyl-1*H*-indole-3-carboxylic acid (610 mg, yield 85 %) as yellow crystals. Synthesis of 1-pentyl-1*H*-indole-3-carbonyl chloride was performed as follows. 1-Pentyl-1*H*-indole-3-carboxylic acid (500 mg, 2.2 mmol) was dissolved in thionyl chloride (5 ml) and refluxed for 10 h. Thionyl chloride was removed under reduced pressure. The crude product was used without further purification. Synthesis of *N*, *N*-bis(1-pentylindol-3-yl-carboxy)naphthylamine was performed first by adding α-naphthylamine (135 mg, 0.94 mmol) to triethylamine (286 mg, 2.8 mmol) in dichloromethane (5 ml) and cooling to 0 °C in an ice bath. After 5 min of stirring at the same temperature, 1-pentyl-1*H*-indole-3-carbonyl chloride (540 mg, 2.2 mmol) was added dropwise to the reaction mixture and was allowed to warm to room temperature, and stirred for 20 h. The reaction mixture was poured into a separatory funnel and washed with water (10 ml). The aqueous layer was further extracted with dichloromethane (5 ml, twice). The combined organic extract was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude products were purified by flash chromatography [hexane/ethyl acetate (8:2 v/v)] to produce pure *N*, *N*-bis(1-pentylindol-3-yl-carboxy)naphthylamine in (320 mg) with 60 % overall yield in the three steps as a pale-yellow solid.

Instrumental analyses

Semi-preparative LC was performed on a LC-2000 Plus system with a photodiode array (PDA) detector (Jasco, Hachioji, Japan). LC–MS in the electrospray ionization (ESI) mode was performed on an ACQUITY LC instrument connected to PDA and quadrupole mass detectors (Waters, Milford, MA, USA), using an ACQUITY UPLC HSS T3 column (50 × 2.1 mm i.d., particle size 1.8 μm) (Waters) at 40 °C. The composition of the mobile phase, the mode of gradient elution, PDA condition, and MS conditions were identical to LC condition 1, which was described previously [7].

The accurate mass spectrum of the target compound was measured by LC–quadrupole time-of-flight–mass spectrometry (LC–QTOF–MS) system with an Acquity UPLC and Synapt G2-Si (Waters) in the positive ion mode. The above-described analytical LC conditions [7] were also used, and the TOF–MS conditions were as follows: ion source, positive ESI mode; ion source temperature, 150 °C; desolvation gas, nitrogen with 1,000 l/h flow rate at 500 °C; capillary and cone voltages, 2,000 and 40 V, respectively; collision energy, 20 V; mass range, *m/z* 50–800. Leucine enkephalin at 1 ng/μl (in 50 % acetonitrile solution with 0.1 % formic acid), with its $[M + H]^+$ ion at *m/z* 556.2771, was used as the calibrant. MS data were acquired using MS^E mode and the collision energy was set to 15–35 eV. Gas chromatography–mass spectrometry (GC–MS) was performed on a Hewlett-Packard (Palo Alto, CA, USA) 6890 network GC system with a 5973 mass-selective detector. The GC–MS conditions were exactly the same as described previously [7]. NMR spectra of the isolated target compound were recorded on an ECA-500 spectrometer (JEOL, Tokyo, Japan). Assignments were made by ^{1}H- and ^{13}C-NMR, with distortionless enhanced by polarization transfer (DEPT), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), and total correlation spectroscopy (TOCSY) spectra.

Single crystal X-ray diffraction data were collected using a Bruker Smart Apex II CCD diffractometer (Bruker AXS, Bremen, Germany) equipped with a multilayer confocal mirror and a fine-focus rotating anode (Mo Kα, $\lambda = 0.71073$ Å) in the phi and omega scan modes operating at 90 K. A total of 14,368 reflections were collected

with a scan width of 0.5° and an exposure time of 3 s/frame in the range of $2.18 \leq \theta \leq 25.02°$. The independent reflections were 5,452 ($R_{int} = 0.032$), and the observed reflections with $I > 2\sigma$ (I) were 4,495. The crystal belongs to the monoclinic, and the space group was $P2_1/c$, with cell dimensions of $a = 16.693$ (3) Å, $b = 11.443$ (2) Å, $c = 16.784$ (3) Å, $\beta = 104.980$ (2)°, $V = 3{,}097.0$ (9) Å^3, $D_{calc} = 1.222$ g/cm^3, $Z = 4$ and $F(000) = 1{,}216$. Integration and reduction of all data were carried out with a Bruker Suite software package (Bruker AXS) [19]. A numerical absorption correction was applied with a SADABS program [20]. The structures were solved by direct methods using a SHELXS-97 program [21] and refined by full-matrix least-squares calculations with a SHELXL-97 program [22] based on F^2. The non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were located at the calculated positions. The final R indices were $R_1 = 5.37$ %, $wR_2 = 13.98$ % for reflections with $I > 2\sigma$ (I) and $R_1 = 6.63$ %, $wR_2 = 14.87$ % for all data. The S (goodness-of-fit) value was 1.025, and the largest residual peak and hole in the final difference map were 0.63 and −0.31 e/Å^3, respectively.

[^{35}S]GTPγS binding assays

The assays were entrusted to and performed at ADME & TOX., Research Institute, SEKISUI MEDICAL CO. LTD. (Ibaraki, Japan). The cannabinoid receptors CB_1 (human recombinant, PerkinElmer) and CB_2 (human recombinant, Chemicon, Billerica, MA, USA) were used in the assay buffer consisting of 50 mM Tris–HCl (pH 7.4) containing 1 mg/ml fatty acid free BSA, 100 mM NaCl, 3 mM $MgCl_2$, and 1 mM ethylenediaminetetraacetic acid (EDTA) at 30 °C for 60 min. Test compound solutions were prepared as follows: after weighing, the test compound was dissolved in and diluted with DMSO to prepare solutions at 100-fold higher concentrations of the final concentrations (1×10^{-12} to 1×10^{-5} M). These solutions were further diluted 10-fold with purified water to prepare test article solutions just before use. The stock tracer solution was diluted with the buffer to prepare a tracer solution at a concentration fivefold higher than the final concentration just before use. The assay was performed using the following solutions: buffer containing 10 % (v/v) DMSO (50 µl) for calculation of total binding, maximal response solution (50 µl) for calculation of maximal response, and test article solutions (50 µl) or positive article solutions (50 µl) for calculation of response ratios of the test article or positive article [(final DMSO concentration: 1 % (v/v)]. Buffer and tracer solutions, 100 µl each, were added to each tube; then 50 µl of GDP solution and the receptor solutions containing 4.0 µg CB_1 protein or 5.0 µg of CB_2 protein were further added. The mixtures were incubated for 60 min at 30 °C, and the incubated mixtures were filtered with GF/C [Whatman, Sigma-Aldrich, St. Louis, MO, USA, treated with phosphate buffer solution (PBS)] using a cell harvester (PerkinElmer). The precipitate was rinsed thrice with ice-cold PBS (3 ml). Filter papers were placed in assay vials, mixed with 5 ml of liquid scintillator (PICO-FLUORTM PLUS, PerkinElmer) and the radioactivity was counted using a liquid scintillation counter for 2 min. The assay was performed using duplicate samples and measured twice. Agonistic activities (EC_{50} value: concentration showing 50 % response) of the test compound to cannabinoid receptors CB_1 and CB_2 were assessed.

Results and discussion

Identification of unknown peak **2**

In the herbal product test solution, three peaks appeared at 5.32, 5.78, and 7.03 min by LC-ultraviolet (UV) detection (Fig. 2a). The first and second peaks were easily identified as 5F-PB-22 and NNEI (**1**), respectively [13, 15]. However, the third peak (peak **2**) was a new compound and the PDA-sliced UV spectrum of peak **2** showed maxima at 220.0 and 308.7 nm (Fig. 2b), which seemed to be related to naphthoylindoles when comparing to our PDA-sliced UV spectral library data. The mass spectrum of peak **2** showed the base peak at m/z 570 $[M + H]^+$ in the positive scan mode (Fig. 2c). The total ion chromatogram (TIC) by GC–MS showed 8-quinolinol, which derived from 5F-PB-22, 5F-PB-22 itself, and NNEI appearing at 11.7, 30.6, and 31.4 min, respectively (Fig. 3). On the other hand, the peak corresponding to compound **2** was not observed in the sample solution by GC–MS as shown in Fig. 3. The LC–QTOF–MS analysis showed that the accurate mass of the $[M + H]^+$ ion for isolated peak **2** was m/z 570.3112 in the positive scan mode (Fig. 4), suggesting its protonated molecular formula to be $C_{38}H_{40}N_3O_2$ (Calcd. 570.3121). The error between the observed and the theoretical masses of $[M + H]^+$ ion was −0.9 mmu. Additionally, the ion detected at m/z 214.1229 in the accurate mass spectrum was tentatively thought to be an *n*-pentylindole resulting from the fragmentation of the precursor ion m/z 570.3112. The NMR data of the isolated compound **2** and possible assignments are shown in Table 1 (for location numbers, see Fig. 1). There were 17 aromatic protons and 22 side-chain signals detected. According to the ^{13}C-NMR spectrum, 24 signals were detected, and the existence of the *n*-pentylindole and a naphthalene moiety were suggested from the DEPT, TOCSY, and HMQC spectra. Judging from these instrumental analysis data, it was also strongly

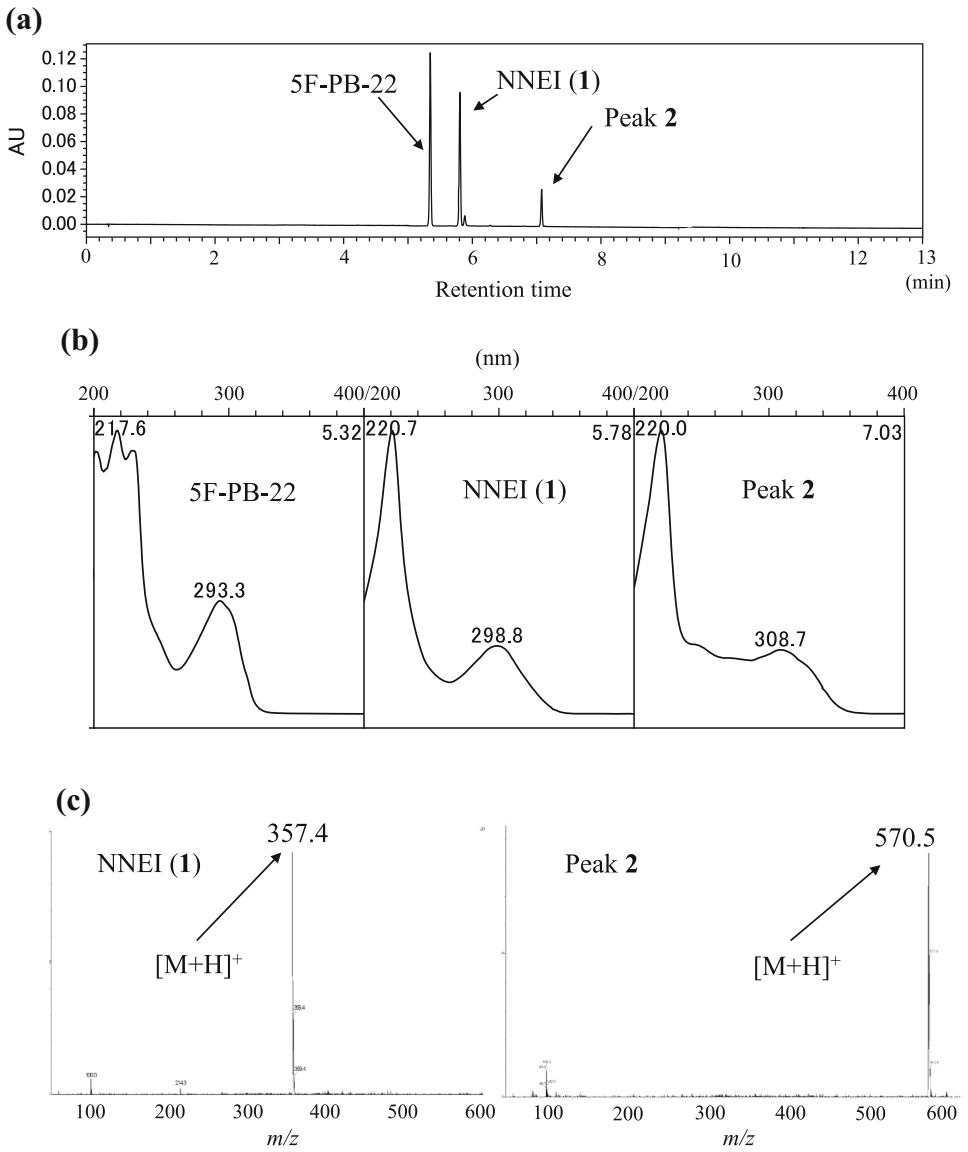

Fig. 2 Liquid chromatography–ultraviolet (UV) detection chromatogram at 275 nm (**a**), photodiode array-UV spectra (**b**), and electrospray ionization mass spectra (**c**) for NNEI (**1**) and peak **2** appearing at 7.03 min for the sample solution

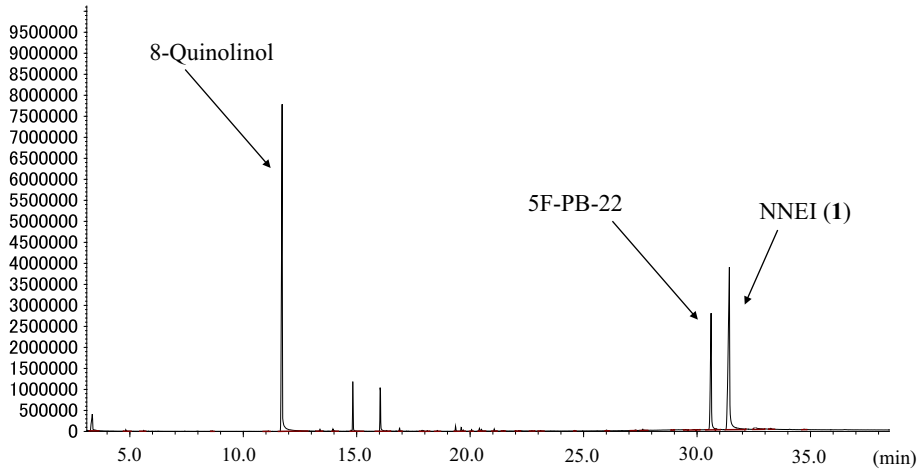

Fig. 3 Total ion chromatogram recorded by gas chromatography–mass spectrometry for the sample solution

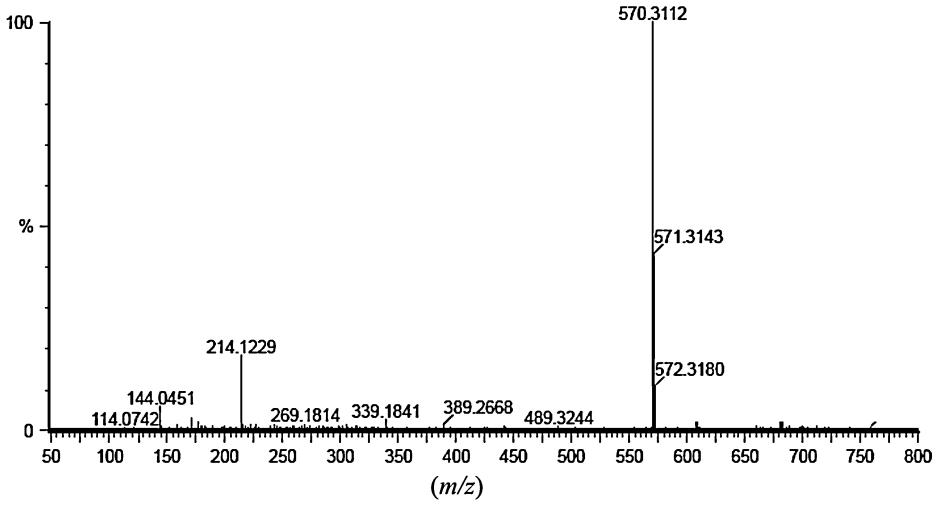

Fig. 4 High-resolution mass spectrum of the isolated compound 2 obtained by liquid chromatography–quadrupole time-of-flight–mass spectrometry

Table 1 Nuclear magnetic resonance (NMR) data for compound 2 in $CDCl_3$ (ppm)

No.	^{13}C	^{1}H
1, 15	168.7	–
9, 23	133.9	7.43 (2H, brs, overlapped)
2, 16	111.5	–
3, 17	127.5	–
4, 18	122.0	8.27 (2H, m)
5, 19	122.2	7.19–7.27 (2H, m, overlapped)
6, 20	123.0	7.19–7.27 (2H, m, overlapped)
7, 21	109.8	7.19–7.27 (2H, m, overlapped)
8, 22	136.2	–
29	138.7	–
30	127.0	7.48–7.51 (1H, m, overlapped with 34, 37)
31	125.7	7.44 (1H, m, overlapped)
32	128.4	7.83 (1H, brd, $J = 8.0$ Hz)
33	134.7	–
34	127.4	7.48–7.51 (1H, m, overlapped with 30, 37)
35	122.8	8.19 (1H, m)
36	128.6	7.89 (1H, m)
37	126.3	7.48–7.51 (1H, m, overlapped with 30, 34)
38	131.0	–
10, 24	46.8	3.91 (4H, m, overlapped)
11, 25	29.1	1.54 (4H, m, overlapped)
12, 26	28.5	0.96 (4H, m, overlapped)
13, 27	22.0	1.14 (4H, m, overlapped)
14, 28	13.8	0.79 (6H, t, $J = 7.5$ Hz)

suggested that compound **2** is a bis-adduct of the *n*-pentylindole. This estimation was difficult to confirm only by NMR experiments, because *n*-pentylindole in the molecule showed the same signals.

We, therefore, confirmed our estimation by the following X-ray crystallographic analysis. A crystal of the target compound was made in the preparative LC fraction solution for recrystallization, and its chemical structure was explored by X-ray crystallography. The size of the crystal was 0.19 × 0.09 × 0.05 mm, and its chemical structure was elucidated as shown in Fig. 5. The detailed data were as follows: $C_{38}H_{39}N_3O_2$, molecular weight = 569.72, monoclinic, $P2_1/c$, $a = 16.693$ (3) Å, $b = 11.443$ (2) Å, $c = 16.784$ (3) Å, $\beta = 104.980$ (2)°, $V = 3{,}097.0$ (9) Å^3, $Z = 4$, $D_{calc} = 1.222$ g/cm^3, μ(Mo Kα) = 0.08 mm^{-1}, $T = 90$ K, 14,368 measured reflections, 5,452 independent reflections ($R_{int} = 0.032$), 4,495 observed reflections with $I > 2\sigma$ (I), $R_1 = 5.37$ %, $wR_2 = 13.98$ % (observed data), $R_1 = 6.63$ %, $wR_2 = 14.87$ % (all data) and $S = 1.025$. These crystallographic data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Centre and were allocated the deposition number CCDC 1023314. Copies of the data and CIF files can be obtained free of charge through application to the CCDC, http://www.ccdc.cam.ac.uk/pages/Home.aspx. By the above X-ray crystallographic analysis, compound **2** was named *N*,*N*-bis(1-pentylindol-3-yl-carboxy)naphtylamine (BiPICANA). We also confirmed the chemical structure of compound **2** by conducting the above-mentioned synthesis of BiPICANA. Chromatographic and spectral results obtained from the isolated and synthesized compounds were exactly the same (data not shown). It was thought that compound **2** might be a byproduct of compound **1**, but the content of compound **2** per package (net weight) was as much as 56.5 mg. We believe that this content amount should not be overlooked. Compound **2** has not been reported to date, and its pharmacological activities are also not available to

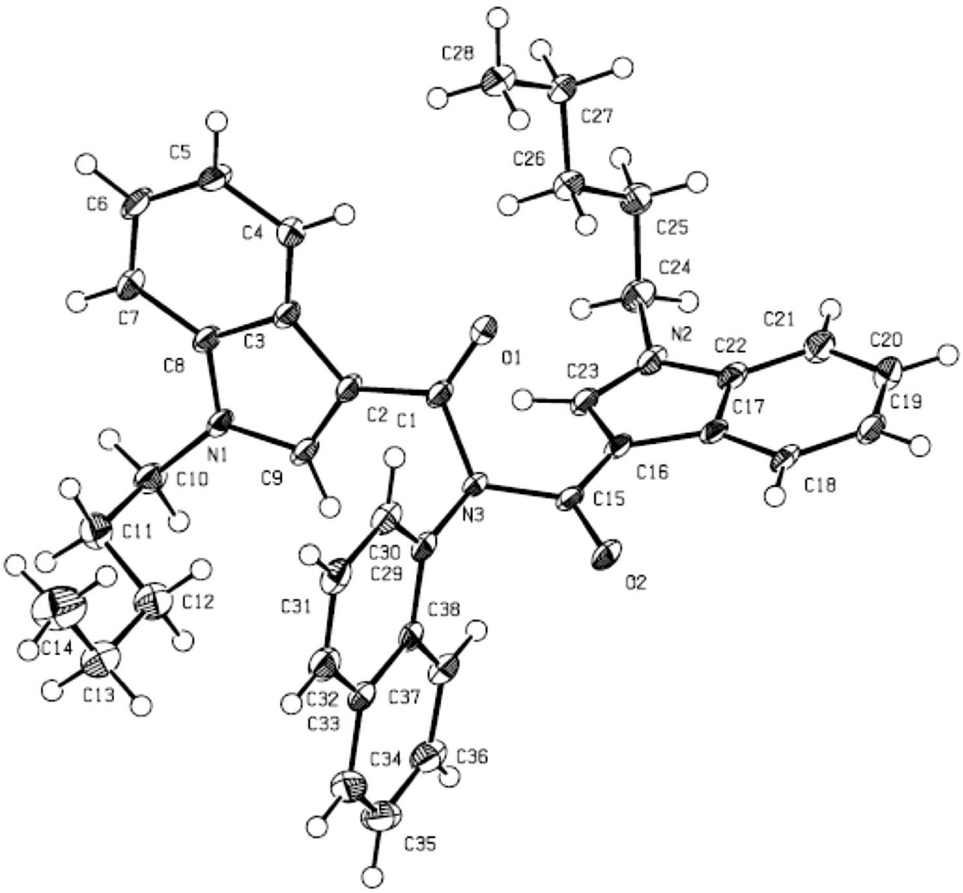

Fig. 5 Structure of the compound *N,N*-bis(1-pentylindol-3-yl-carboxy)naphtylamine (BiPICANA) elucidated by X-ray crystallographic analysis

Table 2 EC_{50} values of compounds **2–4** as a function of the stimulation of [^{35}S]GTPγS binding to human recombinant cannabinoid receptors CB_1 and CB_2

Compound	EC_{50} (M)	
	CB_1	CB_2
N,N-Bis(1-pentylindol-3-yl-carboxy)naphtylamine (BiPICANA, **2**)	3.80×10^{-7}	8.77×10^{-7}
AM-1220 azepane isomer (**3**)	7.13×10^{-6}	2.21×10^{-7}
AM-2233 azepane isomer (**4**)	2.27×10^{-5}	2.32×10^{-7}
CP55940 (positive control)	4.15×10^{-10}	2.78×10^{-10}

EC_{50} 50 % effective concentration

our knowledge; the cannabimimetic activities were hence tested by the following assay.

Cannabimimetic effects of the isolated compounds **2–4** on CB_1 and CB_2 receptors evaluated by [^{35}S]GTPγS binding assays

Three cannabimimetic indoles (**2–4**, Fig. 1) were tested for their ability to stimulate [^{35}S]GTPγS binding to cannabinoid receptors CB_1 and CB_2, and their results are summarized in Table 2. Compounds **2–4** showed binding with EC_{50} (M) values in the range of 3.80×10^{-7}–2.27×10^{-5} for CB_1 receptor, and 2.21–8.77×10^{-7} for CB_2 receptor. Their binding abilities to CB_1 and CB_2 receptors were lower than that of the positive control CP55940, but they certainly showed cannabimimetic activities with this assay. AM-1220, AM-2233, and their azepane isomers have been detected from herbal products [10, 18]; AM-1220 and AM-2233 are already regulated in Japan, and their azepane isomers **3** and **4** are also to be regulated as scheduled substances by the Pharmaceuticals Affairs Law of Japan.

Conclusions

In this study, we could identify a new compound *N,N*-bis(1-pentylindol-3-yl-carboxy)naphtylamine (BiPICANA, compound **2**) in an herbal blend product purchased at a street shop in Tokyo. Its structure was so unique that two NNEI molecules are dimerized having only one naphthylamino moiety in common. We also tested cannabimimetic activities of compound **2** and azepane isomers **3** and **4** using human CB_1 and CB_2 receptors

(human recombinant) by [^{35}S]GTPγS binding assays. The EC_{50} value of BiPICANA was one or two orders of magnitude lower than those of compounds **3** and **4** for CB_1 receptor, showing that BiPICANA possesses sufficient cannabimimetic activities for CB_1 upon its use. Because chemical structures of newly emerging psychotropic drugs are becoming much more complicated, the NMR spectroscopy plus accurate mass spectrometry occasionally cannot give the final elucidation of the chemical structure of an unknown compound. In such a case, alternative analysis by X-ray crystallography was very useful to determine the chemical structure, though X-ray crystallography needs a single crystal of the target compound, which led us to recognize that it is important to develop a general method for producing a single crystal for synthetic cannabinoids. To counteract newly emerging drugs, we must continue the strict surveillance of the street market products not only by the conventional instrumental techniques, but also by incorporating alternative instrumental analysis techniques such as the X-ray crystallographic analysis.

Acknowledgments This work was supported by Pharmaceutical Affairs Section, Tokyo Metropolitan Government, Tokyo, Japan.

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

References

1. Uchiyama N, Kikura-Hanajiri R, Kawahara N, Haishima Y, Goda Y (2009) Identification of a cannabinoid analog as a new type of designer drug in a herbal product. Chem Pharm Bull 57:439–441
2. Uchiyama N, Kikura-Hanajiri R, Kawahara N, Goda Y (2009) Identification of a cannabimimetic indole as a designer drug in a herbal product. Forensic Toxicol 27:61–66
3. Uchiyama N, Kawamura M, Kikura-Hanajiri R, Goda Y (2010) Identification and quantitation of two cannabimimetic phenylacetylindoles JWH-251 and JWH-250, and four cannabimimetic naphthoylindoles JWH-081, JWH-015, JWH-200, and JWH-073 as designer drugs in illegal products. Forensic Toxicol 29:25–37
4. Nakajima J, Takahashi M, Seto T, Suzuki J (2011) Identification and quantitation of cannabimimetic compound JWH-250 as an adulterant in products obtained via the Internet. Forensic Toxicol 29:51–55
5. Nakajima J, Takahashi M, Seto T, Kanai C, Suzuki J, Yoshida M, Hamano T (2011) Identification and quantitation of two benzoylindoles AM-694 and (4-methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone, and three cannabimimetic naphthoylindoles JWH-210, JWH-122, and JWH-019 as adulterants in illegal products obtained via the Internet. Forensic Toxicol 29:95–110
6. Nakajima J, Takahashi M, Nonaka R, Seto T, Suzuki J, Yoshida M, Kanai C, Hamano T (2011) Identification and quantitation of a benzoylindole (2-methoxyphenyl)(1-pentyl-1*H*-indol-3-yl)methanone and a naphthoylindole 1-(5-fluoropentyl-1*H*-indol-3-yl)-(naphthalene-1-yl)methanone (AM-2201) found in illegal products obtained via the Internet and their cannabimimetic effects evaluated by in vitro [^{35}S]GTPγS binding assays. Forensic Toxicol 29:132–141
7. Nakajima J, Takahashi M, Seto T, Yoshida M, Kanai C, Suzuki J, Hamano T (2012) Identification and quantitation of two new naphthoylindole drugs-of-abuse, [1-(5-hydroxypentyl)-1*H*-indol-3-yl](naphthalen-1-yl)methanone (AM-2202) and (1-(4-pentenyl)-1*H*-indol-3-yl)(naphthalen-1-yl)methanone, with other synthetic cannabinoids in unregulated "herbal" products circulated in the Tokyo area. Forensic Toxicol 30:33–44
8. Uchiyama N, Kawamura M, Kikura-Hanajiri R, Goda Y (2012) Identification of two new-type synthetic cannabinoids, *N*-(1-adamantyl)-1-pentyl-1*H*-indole-3-carboxamide (APICA) and *N*-(1-adamantyl)-1-pentyl-1*H*-indazole-3-carboxamide (APINACA), and detection of five synthetic cannabinoids, AM-1220, AM-2233. AM-1241, CB-13 (CRA-13), and AM-1248, as designer drugs in illegal products. Forensic Toxicol 30:114–125
9. Kikura-Hanajiri R, Uchiyama N, Kawamura M, Goda Y (2013) Change in the prevalence of synthetic cannabinoids and cathinone derivatives in Japan until early 2012. Forensic Toxicol 31:44–53
10. Nakajima J, Takahashi M, Seto T, Kanai C, Suzuki J, Yoshida M, Uemura N, Hamano T (2013) Analysis of azepane isomers of AM-2233 and AM-1220, and detection of an inhibitor of fatty acid amide hydrolase [3′-(aminocarbonyl)(1,1′-biphenyl)-3-yl]-cyclohexylcarbamate (URB597) obtained as designer drugs in the Tokyo area. Forensic Toxicol 31:76–85
11. Uchiyama N, Matsuda S, Wakana D, Kikura-Hanajiri R, Goda Y (2013) New cannabimimetic indazole derivatives, *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1*H*-indazole-3-carboxamide (AB-PINACA) and *N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1*H*-indazole-3-carboxamide (AB-FUBINACA) identified as designer drugs in illegal products. Forensic Toxicol 31:93–100
12. Uchiyama N, Matsuda S, Kawamura M, Kikura-Hanajiri R, Goda Y (2013) 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. Forensic Toxicol 31:223–240
13. Uchiyama N, Matsuda S, Kawamura M, Kikura-Hanajiri R, Goda Y (2014) Identification of two new-type designer drugs, piperazine derivative MT-45 (I-C6) and synthetic peptide Noopept (GVS-111), with synthetic cannabinoid A-834735, cathinone derivative 4-methoxy-α-PVP, and phenethylamine derivative 4-methylbuphedrine from illegal products. Forensic Toxicol 32:9–18
14. Uemura N, Fukaya H, Kanai C, Yoshida M, Nakajima J, Takahashi M, Suzuki J, Moriyasu T, Nakae D (2014) Identification of a synthetic cannabinoid A-836339 as a novel compound found in a product. Forensic Toxicol 32:45–50
15. Uchiyama N, Matsuda S, Kawamura M, Shimokawa Y, Kikura-Hanaji R, Aritake K, Urade Y, Goda Y (2014) Characterization of four new designer drugs, 5-chloro-NNEI, NNEI indazole analog, α-PHPP and α-POP, with 11 newly distributed designer drugs in illegal products. Forensic Sci Int 243:1–13
16. Uchiyama N, Shimokawa Y, Kawamura M, Kikura-Hanajiri R, Hakamatsuka T (2014) Chemical analysis of a benzofuran derivative, 2-(2-ethylaminopropyl)benzofuran (2-EAPB), eight synthetic cannabinoids, five cathinone derivatives, and five other designer drugs newly detected in illegal products. Forensic Toxicol 32:266–281
17. Wurita A, Hasegawa K, Minakata K, Watanabe K, Suzuki O (2014) A large amount of new designer drug diphenidine coexisting with a synthetic cannabinoid 5-fluoro-AB-PINACA found in a dubious herbal product. Forensic Toxicol 32:331–337
18. Kneisel S, Bisel P, Brecht V, Broecker S, Müller M, Auwärter V (2012) Identification of the cannabimimetic AM-1220 and its

azepane isomer (*N*-methylazepan-3-yl)-3-(1-naphthoyl)indole in a research chemical and several herbal mixtures. Forensic Toxicol 30:126–134
19. Bruker AXS (2008) Bruker suite. Bruker AXS Inc., Madison
20. Sheldrick GM (2008) SADABS—Bruker AXS area detector scaling and absorption, version 2008/1. University of Göttingen, Göttingen
21. Sheldrick GM (1997) SHELXS-97, program for crystal structure solution. University of Göttingen, Göttingen
22. Sheldrick GM (1997) SHELXL-97, program for crystal structure refinement. University of Göttingen, Göttingen