SHORT COMMUNICATION

Comprehensive analytical and structural characteristics of methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1*H*-indazole-3-carboxamido)butanoate (MDMB-4en-PINACA)

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Received: 3 January 2021 / Accepted: 20 March 2021 / Published online: 1 April 2021
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Abstract

Purpose The purpose of the study was to evaluate a complete analytical and structural characterization of methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1*H*-indazole-3-carboxamido)butanoate (MDMB-4en-PINACA), a novel synthetic cannabinoid being the analogue of 5F-ADB.

Methods The compound was analyzed by gas chromatography–mass spectrometry (GC–MS), high-resolution liquid chromatography–mass spectrometry (LC–MS), X-ray diffraction and spectroscopic methods, such as nuclear magnetic resonance (NMR) and Fourier-transform infrared (FTIR) spectroscopies. To derive MDMB-4en-PINACA molecular geometry and to assign infrared absorption bands, quantum calculations with the employment of density functional theory were also used.

Results We present a wide range of chromatographic and spectroscopic data supported with theoretical calculations allowing to identify MDMB-4en-PINACA.

Conclusions To our knowledge, this is the first report presenting a comprehensive analytical and structural characterization of MDMB-4en-PINACA obtained by 1D and 2D NMR, GC–MS, LC–MS(/MS), attenuated total reflection-FTIR spectroscopy, powder X-ray diffraction and quantum chemical calculations. The presented results not only broaden the knowledge about this psychoactive substance but also are useful for forensic and clinical purposes.

Keyword MDMB-4en-PINACA · Comprehensive analytical characteristics · 1D and 2D NMR · Powder X-ray diffraction · Quantum chemical calculations

Introduction

Among the wide range of psychoactive substances used notoriously in recent years, synthetic cannabinoids deserve special attention [1]. When used for recreational purposes, they often bring about psychophysical states difficult to control and leading to numerous accidents, fatalities and severe social consequences [2–4]. One of the most prevalent subclasses of synthetic cannabinoids is the group of compounds with 3-carboxamide indazole entity—i.e., with the structural motif probably coming from novel compounds with cannabinoids activity patented by Pfizer [5, 6]. Many of

them, e.g., ADB-CHMINACA and AB-CHMINACA, were directly implemented—as illicit substances [7, 8], whereas some others were modified and increased the range of synthetic cannabinoids on the drug market. The most frequently employed structural alternation among several modifications was the replacement of indazole core *N*-substituent with pentyl or 5-halogenated pentyl chain. Such modification is easily explained by well-known QSAR observations of analogous indole-based cannabimimetics indicating that *N*-alkyl chains containing from 4 to 6 carbon atoms cause effective hydrophobic interaction of a synthetic cannabinoid with its binding sites of CB₁ and CB₂ receptors [9]. Besides halogen atoms, different terminal groups were also attached to the alkyl chains of synthetic cannabinoids, e.g., nitrile and vinyl groups [10].

The paper presents the full analytical and structural characterization of MDMB-4en-PINACA, methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1*H*-indazole-3-carboxamido)butanoate (Fig. 1), a novel synthetic cannabinoid

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Fig. 1 Chemical structure of methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1*H*-indazole-3-carboxamido)butanoate (MDMB-4en-PINACA)

being the analogue of 5F-ADB, with pent-4-en-1-yl fragment in place of 5-fluoropentyl chain, found in the material seized by the Polish law authorities. Analytical features of MDMB-4en-PINACA were characterized by gas chromatography hyphenated with mass spectrometry (GC–MS), liquid chromatography (LC) with photodiode array (PDA) and mass spectrometry (MS) detection, attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and powder X-ray diffraction. The density functional theory (DFT) quantum chemical methods were used to derive molecular geometry and to assign the infrared absorption bands. As far as we know, this is the first report showing a comprehensive analytical and structural characterization of MDMB-4en-Pinaca, which has already been examined, but only by some of the enlisted methods [11–16].

Materials and methods

Materials

Acetonitrile and methanol (all LC–MS grade) were supplied by the Polish Chemical Plant POCh (Gliwice, Poland); formic acid from Sigma-Aldrich (Seelze, Germany); deuterated chloroform from Armar AG (Döttingen, Switzerland); the Milli-Q system from Millipore (Millipore, Bedford, MA, USA) to be applied for water purification. MDMB-4en-PINACA sample was donated by the forensic laboratory of the Provincial Police Department in Lublin. Working solutions were stored in stable conditions at $-20\text{ }^{\circ}\text{C}$.

GC–MS analysis

The qualification of MDMB-4en-PINACA in the prepared solution was performed using GC–MS QP2010 (Shimadzu, Kyoto, Japan). GC–MS equipment details and measurement conditions are described in [17]. The retention index of the examined substance was calculated according to [18].

LC analysis with photodiode array detection

The UV–VIS spectrum for the tested compound was obtained using HPLC system equipped with a Thermo Scientific Dionex UltiMate DAD-3000 detector—1024 element diode array working with the frequency of collecting the spectrum equal 100 Hz. The UV–VIS spectrum for the MDMB-4en-PINACA was recorded in the range 190–600 nm. The LC–PDA equipment details and measurements conditions are described in [17].

LC–MS analysis

The experiments were performed on an LC–MS(/MS) system consisting of a UHPLC chromatograph (UltiMate 3000; Dionex, Sunnyvale, CA, USA), a high-resolution (HR) linear ion trap quadrupole-Orbitrap mass spectrometer (LTQ-Orbitrap Velos; Thermo Fisher Scientific, San Jose, CA, USA) and an electrospray ionization (ESI) source. The LC–MS equipment details and measurements conditions are described in [17].

High-resolution-MS/MS analysis with direct injection

The solution of MDMB-4en-PINACA in methanol (5 µg/mL) was directly introduced to the above linear trap quadrupole-Orbitrap mass spectrometer at the rate 50 µL/min. The positive ionization mode with the ESI needle potential at 4.5 kV was used. The function of secondary ion fragmentation (MS^2) was applied for the m/z 358 ion. The collision energy used in the experiment was 10, 15, 20, 25 and 30%.

ATR-FTIR

The infrared (IR) spectrum of the examined compound was registered in the wavenumber range from 4000 to 400 cm^{-1} using a Nicolet™iS50 FTIR spectrometer (Thermo Fisher Scientific) with the ATR crystal.

Powder X-ray diffraction

The powder diffraction data of the seized material were obtained using an Empyrean diffractometer (PANalytical, Malvern, United Kingdom) equipped with Cu anode

(voltage: – 40 kV; current: 25 mA), registering signals in the 2θ range between 4.96° and 90.03° using a step size of 0.026°.

NMR spectroscopy

The examined compound (10 mg dissolved in 0.5 mL of $CDCl_3$) was analyzed by NMR using an ASCEND 600 apparatus (Bruker, Bremen, Germany). The following NMR spectra were acquired:

- 1D: 1H , ^{13}C and
- 2D: 1H – 1H correlation spectroscopy (COSY), 1H – ^{13}C multiplicity-edited heteronuclear single quantum coherence (HSQC), 1H – ^{13}C heteronuclear multiple bond correlation (HMBC), 1H – 1H nuclear Overhauser effect spectroscopy (NOESY)

The chemical shifts were referenced to the tetramethylsilane (TMS) signal.

Quantum chemical calculations

Conformer search for MDMB-4en-PINACA was performed using Pmodel (Serena Software, Bloomington, IN, USA) utilizing MMFF94 force field [19]. The DFT method with B3LYP hybrid exchange correlation functional [20] and 6-31G basis set was employed for the initial geometry optimization of the obtained conformers. The geometries exhibiting the lowest energy were re-optimized on the B3LYP/6-311++G(d,p) level of theory. The final geometries were used for the calculation of vibrational frequencies. Described ab initio computations were performed using the NWChem package [21].

Results

GC–MS

The GC–MS chromatogram of methanolic extract of the seized material and the electron ionization (EI) MS spectrum of MDMB-4en-PINACA are presented in Figs. 2a and 3a, respectively. It can be observed that the obtained EI spectrum is in a good agreement with that from the SWGDRUG database. The estimated retention index equaled 2537. The structure of molecular ion and fragment ions corresponding to the most intense peaks are shown in Fig. 3b. The following ions can be recognized:

- Acylium-indazole-alkyl ion at m/z 241. The structure of this main ion is similar to the structure of the main ions

Fig. 2 **a** Gas chromatography–mass spectrometry (GC–MS) and **b** liquid chromatography–mass spectrometry (LC–MS) total ion chromatograms of MDMB-4en-PINACA

- occurring in the EI-MS spectrum of other synthetic cannabinoids with carboxyindazole core;
- Acylium-indazole ion and methyldene-indazolium ion (m/z 145 and 131, respectively), confirming the presence of indazole-carboxy moiety in the examined molecule, and
- m/z 301 ion. This ion is the product of the McLafferty rearrangement of the molecular ion [22]. The visible m/z 269 fragment ion is formed by the loss of methanol molecule from the m/z 301 ion. The presence of these two ions is characteristic for the fragmentation

Fig. 3 **a** GC–electron ionization mass spectrum of MDMB-4en-PINACA and **b** its probable fragmentation pathways

pathways of synthetic cannabinoids containing *tert*-leucinate residue attached to the carboxamide group.

LC–MS, direct injection ESI–MS² and LC–PDA

The exemplary LC–MS chromatogram of MDMB-4en-PINACA methanolic solution is shown in Fig. 2b, whereas HR–MS data corresponding to this compound, for 30% collision energy (CE), are gathered in Table 1. The obtained results indicate that the molecular weight of the MDMB-4en-PINACA $[M+H]^+$ ion and its elemental composition are as follows: 358.2132 Da and $C_{20}H_{28}N_3O_3$, respectively. The very low difference between the theoretical and the experimental mass (Δ ppm) for MDMB-4en-PINACA ions proved the correctness of the elemental analysis.

Figure 4 shows the influence of the applied fragmentation energy (CE) on the profile of ions forming during the

HR–MS/MS analysis with direct injection. The presented GC–MS and LC–MS data indicate a similarity between ESI and EI fragmentation patterns (see Figs. 3b and 5).

As results from the LC–PDA analysis, two maxima (at 209 nm and at 301 nm) are observed on the UV–VIS spectrum of MDMB-4en-PINACA (see supplementary material Fig. S1).

NMR spectroscopy

1H and ^{13}C NMR spectra of the analyzed material dissolved in $CDCl_3$ are shown in Figs. S2 and S3. The 2D spectra, required for the assignment of resonance signals, are presented in Figs. S4–S7. Table 2 summarizes the data from 1 and 2D spectra. The 1H spectrum contains overlapping signals in the regions of 7.38–7.42, 5.03–5.08 and 2.04–2.13 ppm. Their assignment was mainly conducted with the use of HSQC and HMBC spectra. It is worth mentioning that, on the 1H MDMB-4en-PINACA spectra,

Table 1 MS² high-resolution mass spectrometry data of MDMB-4en-PINACA for 30% collision energy

Theoretical mass (Da)	Experimental mass (Da)	Δ mDa	Δ ppm	Formula
145.04019	145.04012	−0.07	0.48	C ₈ H ₅ N ₂ O
187.12352	187.12355	0.03	0.16	C ₁₂ H ₁₅ N ₂
213.10279	213.10271	−0.08	0.38	C ₁₃ H ₁₃ N ₂ O
231.11335	231.11339	0.04	0.17	C ₁₃ H ₁₅ N ₂ O ₂
233.15281	233.15279	−0.02	0.09	C ₁₃ H ₁₉ N ₃ O
236.10352	236.10356	0.04	0.17	C ₁₁ H ₇ N ₃ O ₃
245.15281	245.15287	0.06	0.24	C ₁₄ H ₁₉ N ₃ O
298.19194	298.19192	−0.02	0.07	C ₁₈ H ₂₄ N ₃ O
358.21307	358.21309	0.02	0.06	C ₂₀ H ₂₈ N ₃ O ₃

For structures of MS² product ions, see Fig. 5

signals corresponding to dimethyl formamide (¹H signals: 2.88 ppm, 2.95 ppm and 8.01 ppm) were also appearing, which indicated a probability of its usage as a solvent or a catalyst during the drug synthesis.

Powder X-ray diffraction

Powder X-ray diffraction was employed for the structural characterization of MDMB-4en-PINACA (see Fig. S8). All of the major peaks were observed in the range of 2 θ below 35°, with the most intensive ones at the positions of 6.4, 16.4 and 19.1°. The calculated parameters of the crystal structure are as follows:

- Crystal system—monoclinic;
- Space group—P21; a [Å]=8.719(2); b [Å]=8.552(3); c [Å]=14.284(3); β [°]=103.62(2); V [Å³]=1035.1(5); Z =2
- Density: 1.147 g cm^{−3}

Quantum chemical calculation

The ab initio characterization of MDMB-4en-PINACA was performed on DFT [23] B3LYP level in gas phase, where only *R*-enantiomer was considered. Conformational analysis was performed to obtain stable compound geometries. During the rotamers searching process, the molecular mechanics with MMFF94 force field revealed 73 conformers that were subsequently used for further ab initio computation. Initial geometry optimization was performed with the B3LYP functional and 6-31G basis set. To avoid high computational costs, the frequency calculation was not performed. The obtained results indicated the presence of 5 stable lowest-energy conformers. To prove that the minima of energy were reached, the obtained stable geometries were re-optimized and subjected to vibrational analysis using the B3LYP functional and 6-311++G(d,p) basis set. The structures of the re-optimized conformers and their relative populations are presented in Fig. S9. As results from the figure, the final conformers contained amide protons with hydrogen bonds towards the closest hydrogen acceptors: indazole 2*H* nitrogen and carbonyl oxygen. The H bond length of 2.3 and 2.5 Å for N–H and H–O pairs, respectively, indicated strong character of these interactions. Intramolecular hydrogen bonding seemed to be the interaction stabilizing the lowest-energy conformers. Moreover, the rigid position of the ester group, forced by intramolecular bonding, did not allow for conformational flexibility of methyl and *tert*-butyl fragments. These groups were similarly oriented in all the stable conformers. The alkyl chain was the only MDMB-4en-PINACA fragment with position differing between the obtained rotamers. The conformers d and e are the most stable energetically (conformer e had only slightly higher

Fig. 4 Product ion mass spectra of MDMB-4en-PINACA as a function collision energy (CE) obtained by high-resolution-tandem mass spectrometry

energy), which leads to their similar abundances—38 and 36%, respectively. The other conformations of pent-4-enyl chain only slightly increased the energy of the molecule—the differences in free energy between all of the conformers did not exceed 2 kcal/mol.

IR spectroscopy

The IR spectrum of the examined material is presented in Fig. S10. Its interpretation was as follows:

- Sharp band at 3412 cm^{-1} represented N–H stretching of amide group;
- Alkyl C–H stretching vibrations gave rise to the signal in the $3000\text{--}2800\text{ cm}^{-1}$ region;
- Weak aromatic and alkene C–H stretching bands were present in the region from 3100 to 3000 cm^{-1}
- Ester and amide carbonyl group modes appeared at 1727 and 1666 cm^{-1} , respectively.

To conduct the assignment of the most important absorption bands below 1600 cm^{-1} , the analysis of theoretically obtained harmonic frequencies was performed, using conformer d with highest abundance. The theoretical harmonic frequencies of normal modes computed on the B3LYP/6–311++G(d,p) level of theory were scaled using the least square methods on the basis of experimental wavenumbers of the most prevalent absorption bands below 1600 cm^{-1} . The obtained scaling factor was 0.980. The scaled frequencies and calculated intensities of rotamer d were subsequently used for the assignment of normal modes of the experimental signals. Table 3 presents the assigned bands. Several bands of the considered spectral range were assigned to the normal modes of indazole and vinyl groups. The region of $1600\text{--}1300\text{ cm}^{-1}$ contains multiple singals from the stretching of indazole ring with aromatic C–H rocking. The out-of-plane modes of indazole were assigned to the signals from the range of $1000\text{--}700\text{ cm}^{-1}$. For the vinyl moiety, 1007 and 925 cm^{-1} were correlated with the out-of-plane bending modes of alkene C–H bonds. Moreover, the contribution of in-plane bending of vinyl was observed for the modes assigned to the positions of 1214 and 1328 cm^{-1} .

Discussion

The social problems related to the uncontrolled use of psychoactive compounds, including new synthetic cannabinoids, motivate researchers to learn about their properties and to develop methods for their identification. MDMB-4en-PINACA is an example of an illegally traded synthetic cannabinoid that requires precise detection procedures. Some fractions of analytical data concerning this compound can

Fig. 5 Fragmentation pathways of MDMB-4en-PINACA by electrospray ionization mass spectrometry

Table 2 Nuclear magnetic resonance spectroscopic data of MDMB-4en-PINACA in CDCl_3

No	^1H	^{13}C	COSY	HMBC	NOESY
3	—	136.7	—	—	—
3'	—	122.9	—	—	—
4	8.35 (brd, $J=8.2$ Hz, 1H)	122.8	H5; H6 or H7	C3, C6, C7', C7	H5
5	7.26 (ddd, $J=8.2, 5.7, 2.1$ Hz, 1H)	122.6	Overlapped H6 and H7	C3', C7	H6
6	7.38–7.42 (m, 1H)	126.7	—	C7'	—
7	7.38–7.42 (m, 1H)	109.2	—	C7'	H1a, H2a, H3a
7'	—	140.9	—	—	—
1a	4.41 (t, $J=6.9$ Hz, 2H)	48.6	H2a	C2a, C3a, C7'	H2a, H3a
2a	2.04–2.09 (m, 2H)	28.7	—	C1a, C3a, C4a	—
3a	2.09–2.13 (m, 2H)	30.7	H4a, H5a, H5a'	C1a, C2a, C4a, C5a	H4a, H5a
4a	5.82 (ddt, $J=16.9, 10.5, 6.4$ Hz, 1H)	137.0	H5a', H5a	C2a, C3a	—
5a	5.06 (dd, $J=16.8, 1.6$ Hz, 1H)	115.9	—	C3a, C4a	—
5a'	5.04 (dd, $J=10.2, 1.4$ Hz, 1H)	—	—	C3a	—
1b	—	162.3	—	—	—
2b	7.56 (d, $J=9.8$ Hz, 1H)	—	H3b	C1b	H3b, H5b
3b	4.74 (d, $J=9.7$ Hz, 1H)	59.5	—	C1b, C4b, C5b, C6b	H5b
4b	—	35.0	—	—	—
5b	1.10 (s, 9H)	26.7	—	C3b, C4b	—
6b	—	172.2	—	—	—
7b	3.76 (s, 3H)	51.8	—	C6b	—

For carbon skeleton numbers of MDMB-4en-PINACA, see Fig. 1

COSY ^1H – ^1H correlation spectroscopy, *HMBC* ^1H – ^{13}C heteronuclear multiple bond correlation spectroscopy, *NOESY* ^1H – ^1H nuclear Overhauser effect spectroscopy

Table 3 Theoretical assignments of the most important absorption bands of infrared spectrum of MDMB-4en-PINACA in the region below 1600 cm⁻¹

Experimental frequency [cm ⁻¹]	Theoretical frequency [cm ⁻¹]	Scaled theoretical frequency [cm ⁻¹]	Description of normal modes ^a	Calculated intensity [km/mol]	Experimental intensity
1575	1608	1575	Indazole C–C, C–N and N–N stretch; in-plane bend of indazole C–H	8.5	vw
1519	1551	1519	In-plane bend of amide N–H and methine C–H; indazole C–C stretch with C–H in-plane bend	495.3	vs
1490	1521	1490	Asymmetric in-plane bend of <i>tert</i> -butyl C–H; in-plane bend of amide N–H; indazole C–C and C–N stretch with C–H in-plane bend	65.8	s
1474	1502	1471	Symmetric in-plane bend of methylene C–H; indazole C–C and C–N stretch with C–H in-plane bend	18.6	m
1438	1469	1439	Symmetric in-plane C–H bend of ester methyl; ester C–C–O stretch	17.8	m
1405	1437	1407	Symmetric in-plane bend of <i>tert</i> -butyl C–H; in-plane bend of methine C–H; indazole C–C and C–N stretch with C–H in-plane bend	7.0	w
1367	1387	1359	Indazole C–C, C–N and N–N stretch with C–H in-plane bend; in-plane bend of methine C–H	7.2	m
1328	1359	1331	Out-of-plane bend of methine C–H and in-plane bend of amide N–H; indazole C–C stretch with C–H in-plane bend; ester C–C–O stretch	205.8	s
1312	1343	1316	Out-of-plane bend of methylene C–H; indazole N–N and C–C stretch with C–H in-plane bend	55.3	s
1264	1309	1282	Out-of-plane bend of methylene C–H; indazole C–H in-plane bend with N–N and C–C stretch	5.5	w
1215	1238	1213	<i>tert</i> -Butyl in-plane bend of C–H and C–C stretch; out-of-plane bend of methylene C–H; in-plane bend of amide N–H	48.5	s
1165	1182	1158	Ester C–C–O stretch; in-plane bend of methyl C–H; indazole C–H in-plane bend	128.5	vs
1137	1150	1126	Amide C–N–C stretch; indazole C–H in-plane bend with C–C, C–N and N–N stretch	58.8	m
1030	1048	1026	<i>tert</i> -Butyl C–H in-plane bend; indazole N–N and C–N stretch	5.5	w
1006	1038	1017	Out-of-plane bend of vinyl C–H; C–C stretch	11.3	m
987	1006	986	Ester O–C stretch; C–C stretch; in-plane bend of <i>tert</i> -butyl C–H	22.9	m
925	949	930	Vinyl = CH ₂ out-of-plane bend	52.9	m
909	935	916	Delocalized vibrations of <i>tert</i> -leucinate moiety	16.9	s
858	865	847	Out-of-plane bend of indazole C–H; C–C stretch; out-of-plane bend of methylene C–H	0.3	vw
833	843	826	Delocalized vibrations of <i>tert</i> -leucinate and amide groups	44.8	m
787	800	783	Out-of-plane bend of indazole C–C, C–N and C–H; out-of-plane bend of amide C–C–N	3.9	m
774	796	780	In-plane bend of indazole C–C, C–N and N–N	23.0	m
755	762	746	Out-of-plane bend of indazole C–H, C–C and C–N; out-of-plane bend of amide C–C–N	47.1	vs
637	647	633	Delocalized vibrations of indazole, amide and pentenyl groups	7.0	m
602	615	602	In-plane bend of indazole C–C, C–N and N–N; out-of-plane bend of amide N–H	28.3	m
554	593	581	Out-of-plane bend of amide N–H	81.2	s
469	455	446	Delocalized vibrations	19.6	m
436	448	439	Delocalized vibrations	2.6	m

The term “delocalized” was employed for the description of normal modes consisting of vibrations of all functional groups in whole molecule or its fragments

vw very weak, w weak, m medium, s strong, vs very strong

^aThe vibrations involved in every mode are listed according to the decreasing contribution in the normal mode

be found in the literature [11–16]. They are consistent with those we obtained using some basic analytical techniques, such as GC–EI–MS, FTIR spectroscopy, LC–MS and ¹H NMR spectroscopy. The present paper reports additional

information from product ion spectra at different CEs, 2D NMR, UV–VIS spectroscopy and Powder X-ray diffraction measurements that significantly broadens the analytical knowledge of MDMB-4en-PINACA.

Crystallographic analysis

The obtained sample of MDMB-4en-PINACA had well-developed crystal line. The material was used for the powder X-ray diffraction. Unfortunately, the sample did not contain monocrystals suitable for the single crystal diffraction. Attempts for the recrystallization did not result in the growth of monocrystals, and it was decided to conduct only powder diffraction measurements. For the acquired powder diffractogram (Fig. S8), the parameters of elementary cell were determined, but the determination of crystal lattice structure was not possible. Although the crystallographic data for synthetic cannabinoids are scarce, it can be seen that most of these compounds form crystals of monoclinic system with density in the range of 1.1–1.4 g cm⁻³ [17, 24]. Analogous structural features were observed for the MDMB-4en-PINACA sample. For the powder diffraction measurements of organic compounds, majority of the peaks appear in the 2θ range below 50°. In the case of MDMB-4en-PINACA, most of the significant signals were observed within the mentioned region, with the most intense and characteristic peaks placed below 20°.

NMR analysis

Most of MDMB-4en-PINACA proton signals were clearly assigned to the corresponding nuclei. The differentiation of the overlapping aromatic and pent-4-en-1-yl chain resonances was conducted using heteronuclear 2D techniques. These could be done due to the greater dispersion of ¹³C signals for mentioned fragments leading to the well-separated correlation peaks. This was especially useful for 7.38–7.42 ppm signals corresponding to proton H6 and H7 (Table 2, Fig. 1).

The acquired 2D NOESY spectrum contained multiple cross-peaks (see Table 2) corresponding to the through-space correlations between protons. In the case of *tert*-leucinate and indazole groups, observed nuclear Overhauser effect (NOE) signals led to the connectivities similar to those from other 2D spectra. The only novel conformational information was obtained from cross-peaks of pent-4-en-1-yl protons. Signals correlating nuclei H1a, H2a and H3a to the aromatic protons of 7.38–7.42 ppm (Table 2) indicated the presence of small intramolecular distances between considered atoms pairs. Although the 7.38–7.42 ppm region contained overlapping signals from protons H6 and H7, the NOE typically occurs, when the internuclei distance does not exceed 4.5 Å, which excludes the possibility of NOE for pairs of H6-aliphatic proton. Hence, the aromatic—pent-4-en-1-yl cross-peaks were assigned to the correlations H7-H1a, H7-H2a and H7-H3a (see Table 2). Considering the possible conformations of MDMB-4en-PINACA aliphatic

chain, it could be noticed that protons H2a and H3a may have differing positions and distances with respect to nuclei H7 in particular rotamers. The steric hindrance between considered aliphatic protons may lead to the conformations with H2a being in close proximity to aromatic proton, and H3a with distance to H7 greater than 4 Å, and opposite situation with H3a oriented near H7 nucleus. The former conformation would lead to the NOE cross-peak occurring only between H7 and H2a, while the latter would give correlation signal for pair H7-H3a. As those correlations were observed in 2D NOESY, it indicates that MDMB-4en-PINACA rotamers occurring in solution contain both of the described H2a and H3a orientations. Moreover, very weak cross-peaks between vinyl and aromatic protons were also observed indicating long intramolecular distances between nuclei or small abundance of rotamers with these structural feature.

Conformational analysis of MDMB-4en-PINACA

Although multitude of physicochemical techniques can be employed for structural characterization of synthetic cannabinoids, only the single crystal X-ray diffraction is able to determine precisely the atom positions and the resulting conformations. The other way is to perform the geometry optimization of the molecule by quantum chemical methods. The results obtained using B3LYP DFT [23] functional for MDMB-4en-PINACA indicate the presence of five stable conformers of the molecule (see Fig. S9 in supplementary material). For each of the obtained rotamers, the conformation of carboxamide moiety is fixed by intramolecular hydrogen bonding. The amide group, which is one of the synthetic cannabinoid parts responsible for the bonding to the cannabinoid receptors, should have a substituent generating steric hindrance required for effective binding [25]. The position of the *tert*-leucinate group, a steric substituent in MDMB-4en-PINACA, does not change during conformational transitions. The only fragment of MDMB-4en-PINACA with conformational flexibility is the pentenyl chain. Considering the structures of the obtained rotamers and their populations, none of the determined chain orientations is highly favored over others (see abundances of rotamers at Fig. S9 caption). Although the presence of the alkene double bond may suggest the possibility of π - π stacking with the indazole ring, such interaction did not occur in the stable conformers. This fact is also confirmed by the experimental results. In the case of π - π stacking, the centers of interacting groups are in close proximity, which leads to multiple NOE signals between the protons of the interacting groups. 2D NOESY spectrum showed only a very small correlation signal of protons 5a and 7 (see Table 2, Fig. 1), which is consistent with the edge-to-edge orientation of the vinyl and indazole ring in conformers a and b (see Fig. S9). The lack of π - π interactions is related to the polarized electrostatic

nature of the indazole ring. The nitrogen atoms of the indazole and carbonyl group attached to the ring cause a significant polarization of aromatic moiety leading to the change in its quadrupole moment. A significant negative partial charge, being a component of the quadrupole moment, is distributed above both aromatic ring faces in the aromatic rings without electron-withdrawing motifs [25]. For the indazole part of MDMB-4en-PINACA, the negative partial charge is distributed in the regions close to the nitrogen atoms. On the other hand, in the subsequent region of the heteroaromatic group, the deficit of electron density is observed. This distribution of electrostatic potential favors π – π interactions to the π fragments with electron realizing groups. No such effect was observed in MDMB-4en-PINACA due to fact that only a simple vinyl group is present in the described analyte.

IR spectroscopy

GC–MS and ATR-FTIR are most frequently used in routine identification of synthetic cannabinoids, including MDMB-4en-PINACA. In these cases, the substance identification is based on the comparison of EI mass spectra or infrared spectra with those in the database (e.g., NIST database or SWGDRUG mass spectral library). Although it ensures fast routine workflow, a more elaborate analysis of the EI and IR spectra should also be conducted. It is especially apparent for the vibrational spectra of carboxamid-indazole with regions below 1600 cm^{-1} being the place of multiple signals (Fig. S10). Most of the bands appearing in these range cannot be simply assigned with the “rule of thumb” as little is known about the character of their normal modes, e.g. for MDMB-4en-PINACA. For this compound, it is difficult to differentiate between the bands of indazole and vinyl vibrations and those of alkyl and carbonyl fragments in the range of low wavenumbers. The theoretical vibrational analysis of MDMB-4en-PINACA allowed us to assign the positions of the multiple bands to the stretching and the out-of-plane bending modes of the indazole ring in the regions of 1600 – 1300 cm^{-1} and of 1000 – 700 cm^{-1} , respectively. Yet, the indazole normal modes are influenced by the vibrations of the rest of the molecule. The normal modes of the ring stretching (1600 – 1300 cm^{-1}) involve the aromatic C–H bond rocking. Moreover, a small contribution of C–(C=O)–N bending is observed in the out-of-plane bending modes (1000 – 700 cm^{-1}). For the vinyl group, the out-of-plane vibrations were assigned to the signals in 1007 cm^{-1} and 925 cm^{-1} positions. Contrary to the enlisted modes of indazole, the normal modes of the vinyl group include insignificant contributions from other parts of the molecule.

Conclusions

MDMB-4en-PINACA, the methyl 3,3-dimethyl-2-(1-(pent-4-en-1-yl)-1*H*-indazole-3-carboxamido)butanoate, is a novel synthetic cannabinoid being the analogue of 5F-ADB with pent-4-en-1-yl fragment in place of 5F-ADB 5-fluoropentyl chain. The present paper is the first comprehensive analytical and structural characterization of MDMB-4en-PINACA obtained by 1D and 2D NMR, GC–MS, LC–PDA-MS, ATR-FTIR spectroscopy and powder X-ray diffraction. To derive molecular geometry of MDMB-4en-PINACA and to assign individual band of its FTIR spectrum to individual fragments of the molecule, the quantum calculations with the employment of B3LYP functional were additionally used.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11419-021-00573-y>.

Acknowledgements We gratefully acknowledge the permission of the Ecotech-Complex in Lublin to use their facilities for the research Project No. UDA-POIG.02.01.00-06-212/09-03.

Declarations

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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