Cumyl-CBMICA: A new synthetic cannabinoid receptor agonist containing a cyclobutyl methyl side chain

Sebastian Halter1,2 | Benedikt Pulver1,2,3 | Maurice Wilde1,2 | Belal Haschimi1,2 | Folker Westphal3 | Jan Riedel4 | Michael Pütz4 | Torsten Schönberger4 | Stefanie Stoll4 | Jan Schäper5 | Volker Auwärter4

1Institute of Forensic Medicine, Forensic Toxicology, Medical Center—University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany
2Hermann Staudinger Graduate School, University of Freiburg, Freiburg, Germany
3State Bureau of Criminal Investigation Schleswig-Holstein, Forensic Science Institute, Kiel, Germany
4Federal Criminal Police Office, Forensic Science Institute, Wiesbaden, Germany
5State Bureau of Criminal Investigation Bavaria, Forensic Science Institute, Munich, Germany

Correspondence
Volker Auwärter, Institute of Forensic Medicine, Forensic Toxicology, Medical Center—University of Freiburg, Faculty of Medicine, University of Freiburg, Albertstr. 9, Freiburg 79104, Germany.
Email: volker.auwaerter@uniklinik-freiburg.de

Funding information
Internal Security Fund of the European Union, Grant/Award Number: IZ25-5793-2019-33

Abstract
Since the beginning of the phenomenon of new psychoactive substances (NPS), synthetic cannabinoid receptor agonists (SCRAs) have been the largest and most prevalent subclass of these drugs in Europe. Many countries implemented specific legislation scheduling classes of substances defined on the basis of their chemical structure to reduce supply. We describe the identification and analytical characterization within the EU project ADEBAR plus of 1-(cyclobutylmethyl)-N-(2-phenylpropan-2-yl)-1H-indole-3-carboxamide which resulted in the formal notification through the Early Warning System of the European Monitoring Centre for Drug and Drug Addiction (EMCDDA). This is the first identification of this new SCRA worldwide and the analytical data was distributed (inter-)nationally right after identification in 2019. First, the substance was isolated from the herbal material using preparative high-performance liquid chromatography (HPLC). Structure elucidation and analytical characterization were performed using gas chromatography–mass spectrometry (GC–MS), gas chromatography–solid state infrared spectroscopy (GC-sIR), liquid chromatography–electrospray ionization–quadrupole time of flight–mass spectrometry (LC-ESI-qToF-MS), Raman spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy. The new compound contains a cyclobutyl methyl group as a side chain and has not been described in any patent to our knowledge. Based on the semisystematic nomenclature of SCRA, we propose Cumyl-CBMICA as a short name for the compound.

KEYWORDS
generic laws, NPS, NpSG, structure elucidation

1 | INTRODUCTION

Synthetic cannabinoid receptor agonists (SCRAs) were originally synthesized for medical purposes but have become the largest group of new psychoactive substances (NPS) monitored by the European Monitoring Centre for Drug and Drug Addiction (EMCDDA) since their first emergence in 2008.1,2 The structural diversity of SCRA is huge, although in recent years, the number of new SCRA hitting the market decreased. Among these new compounds, only a small number gained higher margins. One example is 4F-MDMB-BINACA (Figure 1), which gained international relevance after its first appearance in...
In Germany, however, this SCRA was already controlled by a specific law on NPS, the NpSG, enacted in November 2016. The NpSG is a generic law that controls groups of substances based on a modular system defined by chemical structures. Concerning SCRAs, the law defines four structural elements: A core structure, which is connected to a linked group by a linker with a defined position, and a side chain attached to a designated nitrogen atom of the core structure. This modular structure was previously proposed by Kikura-Hanajiri et al. A few online shops selling herbal blends containing SCRAs have a particular interest in and emphasize the legality of their products. Only a few weeks after the introduction of the NpSG, these online shops reacted by selling herbal blends with the SCRA Cumyl-PEGACLONE, which carries a γ-carbolinone structure not covered by the law at that time. In addition, substances with core structures like carbazoles and azaindoles appeared on the market. To keep up with these developments, the NpSG was amended in July 2019 by adding several core structures and substitution patterns. Due to this regulation, all SCRAs with considerable prevalence on the drug market at that time were covered by the law.

Similar to Cumyl-PEGACLONE (Figure 1), the new synthetic cannabinoid reported in this work is structurally related to Cumyl-PICA (Figure 1). However, instead of modifying the core structure, this time, the side chain was modified to bypass the NpSG. Although there are no data so far on the pharmacology of such compounds, cannabinimetic activity can be anticipated due to the high variability of the side chain reaching from aliphatic butyl (e.g., JWH-073), pentyl (5F-Cumyl-PINACA), or cyclohexyl methyl (e.g., MDMB-the side chain reaching from aliphatic butyl (e.g., JWH-073), pentyl nabinimetic activity can be anticipated due to the high variability of their products. Only a few weeks after the introduction of the NpSG, these online shops reacted by selling herbal blends with the SCRA Cumyl-PEGACLONE, which carries a γ-carbolinone structure not covered by the law at that time. In addition, substances with core structures like carbazoles and azaindoles appeared on the market. To keep up with these developments, the NpSG was amended in July 2019 by adding several core structures and substitution patterns. Due to this regulation, all SCRAs with considerable prevalence on the drug market at that time were covered by the law.

![Figure 1](image-url)  Structures of 4F-MDMB-BINACA (left), Cumyl-PICA (middle) and Cumyl-PEGACLONE (right)
2.2.4 | GC–MS method

For gas chromatography–mass spectrometry (GC–MS) analysis of the unknown compound, 10 μl of the extract were transferred into a GC-vial and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 100 μl of dried ethyl acetate. One microliter was injected into the GC–MS system. The GC–MS system consisted of a 6890N-series gas chromatograph combined with a 5973-series mass selective detector and a 7683 B series injector (Agilent, Waldbronn, Germany). The software used was Chemstation (Waldbronn, Germany). The detailed method is described elsewhere.6

Briefly, carrier gas was helium, injection port temperature was set to 270°C, the flow rate was 1 ml/min; oven temperature was 100°C for 3 min, then ramped to 310°C at 30 K/min, 310°C were kept for 10 min. Electron ionization (EI, 70 eV) was used, and the MS was operated in scan mode (m/z 50 to 550 amu). The obtained mass spectra were compared with commonly used EI-MS spectra libraries (NIST, Wiley, MPW) and to an in-house library of previously identified synthetic cannabinoids.

2.2.5 | LC-qToF-MS method

Liquid chromatography-electrospray ionization-quadrupole time of flight-mass spectrometry (LC-qToF-MS) analysis was performed on an impact II qToF instrument coupled with an Elute high-performance liquid chromatography (HPLC) system (both from Bruker Daltonik, Bremen, Germany). Chromatographic separation was performed on a Bruker Intensity solo 1.8 C18-2 2.1 × 100 mm and a VanGuard® BEH C18 1.7 μm guard-column. A changing flow rate of 0.200–0.480 ml/min and the following gradient elution were applied: starting with 4% of solvent B, held for 0.1 min, increased to 18% within 0.9 min, and to 50% within another 1.5 min. Flow increased to 0.223 ml/min within this 1.5 min. Solvent B increased to 99% within 11.5 min, whereas the flow increased to 0.400 ml/min. B was held for 2 min, and flow increased to 0.480 ml/min. The initial conditions were then restored within 0.1 min and held for 2.9 min. The flow decreased to 0.20 ml/min within 0.1 min and was held for 0.9 min to re-equilibrate the system. The total runtime was 20 min.

2.2.6 | Preparative HPLC

Two grams of the herbal blend were extracted with 14 ml ACN. After 10 min of ultrasonication, the extract was filtered with 0.45 μm syringe filters. The separation was performed on a preparative HPLC system from Waters, consisting of a 515 HPLC Pump, a B2525 Binary Gradient Pump, a 2767 Sample Manager, a 2996 Photo-Diode-Array Detector and a ZQ 2000 quadrupole mass detector. The software used was MassLynx 4.0. Eluents were A: water with 0.5% formic acid, B: ACN. The elution mode was isocratic with a composition of the eluents 45/55 (A/B−v/v) using a flow rate of 22 ml/min and a runtime of 13 min.

The preparative column was a Waters XBridge Prep C18, 5.0 μm OBD, 19 × 150 mm with an XBridge Prep C18, 5.0 μm, 19 × 10 mm precolumn. After the separation, ACN was evaporated, and water eliminated by freeze-drying. Afterwards, the target substance was diluted in a few microliter ACN and dried in nitrogen current.

2.2.7 | Attenuated total reflection-infrared spectroscopy

A Nicolet 380 Fourier-transform infrared (FT-IR) spectrometer with Smart Golden Gate Diamond attenuated total reflection (ATR) and the software OMNIC, Ver. 7.4.127 (Thermo Electron Corporation, Dreieich, Germany) were used. Wavelength resolution was 4 cm⁻¹, scan range 650–4000 cm⁻¹, and 32 scans/spectrum were done. IR spectra were recorded from the solid and as neat film. The neat film was prepared by the following sample preparation procedure: The compound was dissolved in diethyl ether, and the solvent was evaporated under a stream of nitrogen at room temperature until the volume reached approximately 100 μl. The remaining fluid was aspirated with a glass pipette and transferred directly to the ATR crystal, where the remaining diethyl ether was evaporated.

2.2.8 | Gas chromatography solid-state infrared spectroscopy

A GC-solid phase-IR-system consisting of an Agilent GC 7890B (Waldbronn, Germany) with an Agilent G4567A probe sampler and a DiscovIR-GCTM (Spectra Analysis, Marlborough, MA, USA) was used. Approximately 2 mg of the compound were dissolved in 2 ml CHCl₃. The column eluent was cryogenically accumulated on a spirally rotating ZnSe disk cooled by liquid nitrogen. IR spectra were recorded through the IR-transparent ZnSe disk using a nitrogen-cooled mercury-cadmium-telluride (MCT) detector.

GC parameters were injection: 1 μl, splitless mode; injection port temperature 240°C; carrier gas helium; flow rate 2.5 ml/min. Chromatographic conditions: fused silica capillary DB-1 column (30 m × 0.32 mm i.d., 0.25 μm film thickness); oven temperature program: 80°C for 2 min, ramped to 290°C at 20 K/min, and held at for 20 min; transfer line: 280°C. Infrared conditions: oven temperature 280°C; restrictor temperature 280°C; disc temperature −40°C; dewar cap temperature 35°C; vacuum 0.2 mTorr; disc speed 3 mm/min; spiral separation 1 mm; wavelength resolution 4 cm⁻¹; IR range 650–4000 cm⁻¹; acquisition time: 0.6 s per file; 64 scans per spectrum.
Data were processed using GRAMS/Alr Ver. 9.1 (Gramps Spectroscopy Software Suite, Thermo Fischer Scientific, Dreieich, Germany) followed by OMNIC Software, Ver. 7.4.127 (Thermo Electron Corporation, Dreieich, Germany).

2.2.9 Raman spectroscopy at 785 nm

A B&W TEK Inc. i-Raman® Plus system was used with a laser wavelength of 785 nm and a BWS465-785S spectrometer using a scan range of 174–3200 cm⁻¹; the resolution was <4.5 cm⁻¹ at 912 nm. A BAC151B Raman Video Microsampling System was applied with an objective lens magnification of 20x (camera: active pixels 1280 × 1024). The applied software was BWSpec® 4.10.4.

Integration time was chosen to reach a relative intensity above 45000 arbitrary units for the most intense peak. Additional information on parameters can be found in Supporting Information S17 and S16.

2.2.10 Raman spectroscopy at 1064 nm

A B&W TEK Inc. i-Raman® EX system was used with a laser wavelength of 1064 nm and a BWS485-1064S-05 spectrometer using a scan range of 174–3200 cm⁻¹; the resolution was ~9.5 cm⁻¹ at 1296 nm. A BAC151B Raman Video Microsampling System was applied with an objective lens magnification of 20x (camera: active pixels 1280 × 1024). The applied software was BWSpec® 4.10.4.

Integration time was chosen to reach a relative intensity above 45000 arbitrary units for the most intense peak. Additional information on parameters can be found in Supporting Information S17 and S18.

2.2.11 NMR method

Approx. 1 mg of the isolated unknown substance was dissolved in CDCl₃ and acetone-d₆. The NMR spectra were recorded at room temperature with an AVANCE III HD 500 spectrometer and a 5 mm BBO Prodigy Cryo probe from BRUKER BioSpin (Karlsruhe, Germany). Chemical shifts are reported in ppm relative to TMS (1H/13C: δ = 0.00) as reference. The compound was fully characterized by 1D- and 2D-NMR spectra. The following spectra were recorded: 1D-¹H (single pulse experiment with 90° pulse, four scans, relaxation delay 63 s, exponential multiplication with line broadening 0.2 Hz), 1D-¹³C (2000 scans, exponential multiplication with line broadening 1.0 Hz), ¹H,¹³C-HOSY (pulse sequence: cosygpqf.3, acquired size [2028, 256], four scans, spectral size [4096, 2048]), ¹H,¹³C-HSQC (pulse sequence: hscedetgssp.3, acquired size [1024, 128], nonuniform sampling [sampling amount 25%], eight scans, spectral size [1024, 1024]), ¹H,¹³C-HMBC (pulse sequence: hmbcetgpl3nd, acquired size [1024, 128], nonuniform sampling [sampling amount 25%], 64 scans, spectral size [1024, 1024]), ¹H,¹⁵N-HMBC (pulse sequence: hmbcgpdqf, acquired size [1024, 128], nonuniform sampling [sampling amount 25%], 32 scans, spectral size [2048, 1024]).

The spectra evaluation was performed by using the software MNova V. 14.1 Suite Expert (Mestrelab, Santiago de Compostela, Spain). The integrals of the 1D-¹H spectrum were defined by the line fitting mode “qGSD.” The ¹H and ¹³C spectra predictions were performed by using the MNova “NMR Predict” plugin with the “Modgraph NMRPredict Desktop” model with “fast increments” algorithm. The model has been additionally trained by the implementation of approx. 1500 own, fully assigned spectra of drugs and related substances.

3 RESULTS AND DISCUSSION

The unknown substances in the 14 purchased herbal blends, and the seized material of the State Bureau of Criminal Investigation Bavaria showed identical mass spectra. The GC–MS chromatogram of the herbal blend extract showed a dominant peak at 13.03 min (Supporting Information S1). Figure 2 shows the respective mass spectrum including the proposed structures of the compound and its most relevant fragments (relative intensity given in brackets): m/z 346 (35%), 228 (74%), 212 (100%), 144 (49%), 119 (6%), 116 (10%). Compared with the SCRA Cumyl-PICA, also known as SGT-56, the obtained mass spectrum differs by Δm/z = 2 for the fragments containing the intact side chain. The fragments without the side chain are identical. Meanwhile, GC–MS data of Cumyl-CBMICA were made available by Cayman Chemical Company, showing good agreement with the spectrum shown in Figure 2. To confirm the molecular formula of the single fragments, an LC-qToF-MS analysis was conducted. Figure 2 also shows the LC-qToF-MS spectrum including the monoisotopic masses of the detected fragments, the suggested ion formula, and the calculated monoisotopic masses of the suggested ion. Due to the measured monoisotopic mass of m/z 347.2120 for the [M + H⁺] ion, the molecular formula of the unknown compound was suggested to be C₂₃H₂₇N₂O (calculated monoisotopic mass of the predicted molecular formula: m/z 347.2118). The fragment ion with the m/z 144.0447 suggested an indole nucleus or an alternative structure with the same sum formula. The other measured and calculated masses of predicted fragments also matched very well. The proposed molecular formulas of the unknown substance and its fragments could thus be tentatively confirmed.

The structure of 1-(cyclobutylmethyl)-N-(2-phenylpropan-2-yl)-1H-indol-3-carboxamide was clearly confirmed by the NMR spectra. In a first experiment, the herbal mixture was extracted directly in the NMR tube with CDCl₃. This very simple preparation technique is often sufficient because the sprayed-on active ingredient is dissolved very well while only small portions of the plant matrix are transferred into the solution. However, in this sample, the signals of the alkyl part were strongly overlaid by signals of the plant material. For the second experiment, the active ingredient was isolated by preparative HPLC. The NMR spectrum of the isolate was then almost free of impurity signals (Supporting Information S2). This enabled unambiguous structure verification.
The 1D-\textsuperscript{1}H-NMR spectrum with expanded regions and the 1D-\textsuperscript{13}C-NMR-APT spectrum of the isolated substance in CDCl\textsubscript{3} are shown in the supplementary material (Supporting Information S3 and S4). All signals could be assigned to their expected shifts. The correlation signals, especially of the HMBC spectrum (Supporting Information S5), clearly confirm the suggested chemical structure. While the partial structure of cumyl-1H-indole-3-carboxamide was already well known from other SCRAs, the cyclobutyl methyl group had to be examined more closely.

The position of the methylene group could be clearly determined due to the typical chemical shifts and correlation signals in the HMBC spectrum to the indole core and the aliphatic ring. For the less trivial determination of the cyclobutyl ring, the combined evaluation of information from all 1D and 2D NMR spectra was necessary. In the \textsuperscript{1}H spectrum, for example, the signal of the methine group C\textsubscript{22} splits into a septet, which clearly indicates the six neighboring protons. Four of them are assigned to the CH\textsubscript{2} signal from C\textsubscript{23} and C\textsubscript{25}, whereas the other two protons belong to the C\textsubscript{21} signal.

The aromatic signal group at 7.25 ppm is overlaid by the solvent signal. This signal pattern can be observed in the spectrum of the sample dissolved in acetone-d\textsubscript{6} (Supporting Information S6). The acetone spectrum was also used for the comparison with spectra of similar structures (Supporting Information S7). The acetone spectra do not provide full separation of the signals in the cyclobutyl ring. For that reason, the full structure assignment provided here are primarily on the basis of the chloroform spectra.

In the \textsuperscript{13}C spectrum, the two isochronous (symmetric) carbon atoms C\textsubscript{23} and C\textsubscript{25} result in a single signal with approximately twice the intensity of the remaining methylene group of the ring (C\textsubscript{24}).

The correlation signals of the 2D spectra (Supporting Information S5 and S8–S10) also clearly prove the constitution of the indole ring system. Following the approach of the established semisystematic nomenclature used for synthetic cannabinoids, we proposed the name Cumyl-CBMICA following its identification (Cumyl-CycloButyl-Methyl-IndoleCarboxAmide). Table 1 shows the NMR assignments and correlations within H,H-COSY and HMBC spectra. Furthermore, we provide all measured data obtained during structure elucidation: the H,H-COSY NMR spectrum (Supporting Information S8), the H,C-HSQC NMR spectrum (Supporting Information S9), and the H,N-HMBC NMR spectrum (Supporting Information S10).

Analysis of a test-purchased herbal blend laced with Cumyl-CBMICA by IR spectroscopy was complicated by the coextracted constituents from the plant matrix. Albeit, extraction of the active ingredient using an organic solvent like acetone or CHCl\textsubscript{3}, and subsequent acquisition of neat film ATR-IR spectra can be sufficient to generate a spectrum for the identification of the substance contained in the herbal matrix, if impurities greater than approx. 10\% are present, serious interferences can be present and hamper the

\textbf{FIGURE 2} A, gas chromatography-electrospray ionization-mass spectrometry (GC-EI-MS) spectrum including proposed fragmentation of the new compound. B, liquid chromatography-quadrupole time of flight-mass spectrometry (LC-qToF-MS) spectrum. Measured and calculated m/z, suggested ion formulas of the fragments and mass errors are given in Supporting Information S20.
| Atom no. | \( \delta \) (ppm) determined | \( \delta \) (ppm) predicted | \( \delta \) (ppm) difference | Multiplicity | Coupling constant | COSY (coupling atoms) | HMBC (coupling atoms) |
|---------|--------------------------------|-----------------------------|-------------------------------|-------------|-----------------|---------------------|-----------------------|
| 1 N     | 142.81                         | 131.52                      | 0.33                          |             |                 |                     |                       |
| 2 C     | 131.85                         | 131.52                      | 0.33                          |             |                 |                     | 2                     |
| H       | 7.69                           | 7.47                        | 0.22                          | s           |                 |                     |                       |
| 3 C     | 111.61                         | 111.89                      | –0.28                         |             |                 |                     | 2                     |
| 4 C     | 119.61                         | 121.09                      | –1.48                         |             |                 |                     | 5                     |
| H       | 7.88                           | 8.03                        | –0.15                         | dd          | 6.9, 1.3        |                     | 6                     |
| 5 C     | 121.29                         | 121.45                      | –0.16                         |             |                 |                     | 7                     |
| H       | 7.25                           | 7.00                        | 0.25                          | m           | n.d.            | 4, 6                | 4, 7                  |
| 6 C     | 122.23                         | 123.15                      | –0.92                         |             |                 |                     | 4                     |
| H       | 7.28                           | 7.12                        | 0.16                          | m           | n.d.            | 5, 7                | 4, 7, 8               |
| 7 C     | 110.48                         | 111.10                      | –0.62                         |             |                 |                     | 5, 6                  |
| H       | 7.40                           | 7.35                        | 0.05                          | dd          | 7.0, 1.3        |                     | 6                     |
| 8 C     | 136.86                         | 137.26                      | –0.40                         |             |                 |                     | 2, 4, 6, 21          |
| 9 C     | 124.97                         | 126.51                      | –1.54                         |             |                 |                     | 2                     |
| 10 C    | 164.34                         | 164.54                      | –0.20                         |             |                 |                     |                       |
| 11 N    | 132.01                         |                             |                               |             |                 |                     |                       |
| H       | 6.27                           | 7.37                        | –1.10                         | s           |                 |                     |                       |
| 12 C    | 56.06                          | 56.21                       | –0.15                         |             |                 |                     | 13, 14, 16            |
| 13 C    | 29.71                          | 30.17                       | –0.46                         |             |                 |                     |                       |
| H\(_2\) | 1.87                           | 1.78                        | 0.09                          | s           |                 |                     | 11, 12, 15            |
| 14 C    | 29.71                          | 30.17                       | –0.46                         |             |                 |                     |                       |
| H\(_2\) | 1.87                           | 1.78                        | 0.09                          | S           |                 |                     | 11, 12, 15            |
| 15 C    | 147.37                         | 149.07                      | –1.70                         |             |                 |                     | 13, 14, 17, 19       |
| 16 C    | 124.83                         | 125.77                      | –0.94                         |             |                 |                     | 18                    |
| H       | 7.51                           | 7.53                        | –0.02                         | dd          | 8.4, 1.2        | 17                  | 12, 17, 18            |
| 17 C    | 128.45                         | 128.82                      | –0.37                         |             |                 |                     | 16, 19                |
| H       | 7.35                           | 7.32                        | 0.03                          | t           | 8.4, 1.2        | 16, 18              | 15, 19                |
| 18 C    | 126.56                         | 126.70                      | –0.14                         |             |                 |                     | 16                    |
| H       | 7.25                           | 7.17                        | 0.08                          | m           | n.d.            | 17, 19              | 16, 20                |
| 19 C    | 128.45                         | 128.82                      | –0.37                         |             |                 |                     | 17, 20                |
| H       | 7.35                           | 7.32                        | 0.03                          | t           | 8.4, 7.1        | 18, 20              | 15, 17                |
| 20 C    | 124.83                         | 125.77                      | –0.94                         |             |                 |                     | 18                    |
| H       | 7.51                           | 7.53                        | –0.02                         | dd          | 8.4, 1.2        | 19                  | 19                    |
| 21 C    | 51.83                          | 51.94                       | –0.11                         |             |                 |                     | 22, 22', 23', 23'', 25', 25'' |
| H\(_2\) | 4.12                           | 4.10                        | 0.02                          | d           | 7.4             | 22                  | 1, 2, 8, 22, 23, 25   |
| 22 C    | 35.48                          | 36.11                       | –0.63                         |             |                 |                     | 21, 24                |
| H       | 2.85                           | 2.90                        | –0.05                         | sept        | 7.4             | 21, 23', 23'', 25', 25'' | 21, 23, 25          |
| 23 C    | 26.28                          | 26.44                       | –0.16                         |             |                 |                     | 21, 22                |
| H'      | 1.81                           | 1.90, 2.02                  | –0.09                         | m           | n.d.            | 22, 24              | 21, 24                |
| H''     | 2.08                           | 1.90, 2.02                  | 0.06                          | m           | n.d.            | 22, 24              | 21                    |
| 24 C    | 18.16                          | 18.40                       | –0.24                         |             |                 |                     | 23', 25'              |
| H\(_2\) | 1.91                           | 1.89                        | 0.02                          | m           | n.d.            | 23', 23'', 25', 25'' | 22                    |
| 25 C    | 26.28                          | 26.44                       | –0.16                         |             |                 |                     | 21, 22                |
| H'      | 1.81                           | 1.90, 2.02                  | –0.09                         | m           | n.d.            | 22, 24              | 21, 24                |
| H''     | 2.08                           | 1.90, 2.02                  | 0.06                          | m           | n.d.            | 22, 24              | 21                    |

*Note: Predicted values for \( \delta \) were generated using the MNova "NMR predict" plugin of the software MNova V. 14.1.*
successful identification. The protocol utilized here is steered towards reducing the presence of compounds from the plant matrix in the solution. In addition, the hyphenated method of a gas chromatograph interfaced with an IR detection system was used due to its capability to elucidate molecular structures and differentiate isomers in complex mixtures. The IR spectrum of a herbal blend extract recorded after chromatographic separation is shown in Figure 3.

The gas chromatography-solid state infrared spectroscopy (GC-sIR) spectrum is the product of the IR measurement of the cryogenically frozen eluent from the GC column. The bands for the stretching vibrations of the N–H bond of the amide group are evident at 3444 and 3324 cm$^{-1}$. The N–H stretching can be found in either one of these wavenumber regions depending on the formation of hydrogen bonds which decrease the wavenumber. In this case, both forms of the amide are present resulting in the detection of two broad absorption bands. The solid IR spectrum exhibits only a signal for the stretching mode at 3261 cm$^{-1}$ (Supporting Information S11 and S12), indicating a more compact structural form in contrast to a partially amorphous structure in the GC-sIR-spectrum. The methyl moieties of the cumyl group and the C–H bonds of the cyclobutyl group are represented in the GC-sIR spectra as well. The band at 2975 cm$^{-1}$ can be attributed to the asymmetric stretching of the C–H bond in the methyl groups, 2937 cm$^{-1}$ derives from the asymmetric stretching of C–H bonds in the side-chain, and the signal at 2867 cm$^{-1}$ can be assigned to the symmetric stretching mode of both aforementioned moieties in Cumyl-CBMICA. Together with the two bands at 3058 and 3027 cm$^{-1}$, characteristic for the C–H stretching vibrations of a benzene ring, the signals generated by vibration modes from these three groups of Cumyl-CBMICA are congruent to the signals in the solid IR spectrum. Additionally, an IR spectrum of the neat film was recorded (Supporting Information S13 and S14). All IR spectra attached in the supplement show the complete measurement range as well as an enlarged range facilitating isomer identification.

In Figure 4, the fingerprint regions of the GC-sIR and IR solid spectra acquired for the isolated substance are shown. Absorption bands at 768, 746, and 698 cm$^{-1}$ were mainly due to C–H bending modes of the indole system observed in both spectra. The two prominent fingers at 746 and 698 cm$^{-1}$ point towards the mono-substituted benzene ring in the cumyl moiety with five adjacent hydrogen atoms as a result of out-of-plane C–H bending vibrations. The symmetric C–H bending modes of the –C(CH$_3$)$_2$ result in an almost symmetrical doublet around 1380 cm$^{-1}$ (1387 and 1364 cm$^{-1}$ in the solid IR and 1399 and 1383 cm$^{-1}$ in the GC-sIR) visible in both spectra with varying peak shape. On the other hand, the asymmetric CH$_3$ bending modes are evident in both spectra at 1466 cm$^{-1}$.

At a wavenumber of 1540 cm$^{-1}$, the C–N stretch and C–N–H in-plane bend (amide II) mode which is highly characteristic for non-cyclic monosubstituted amides can be observed in both spectra. Furthermore, the prominent absorption bands at 1650 and 1617 cm$^{-1}$ can be attributed to C=O stretching and complex deformation modes in the carbonyl group. Notably, the absorption band in the GC-sIR spectra is shifted to a higher wavenumber compared with the solid-state spectrum. The hydrogen bonds formed in conjunction with the crystalline structure in the solid-state lead to a decreased observed frequency for the C=O stretching mode in the solid IR spectrum. The vibration modes of the amide function are sensitive to the degree of hydrogen bond association. Coherent with the observation of a decreased wavenumber of the bending mode is the decreased wavenumber of the stretching mode of the N–H bond in the range of 3500 to 3300 cm$^{-1}$ (see Figure 3). From the observed bands for the stretching modes, a partially amorphous structure of the cryogenically deposited Cumyl-CBMICA was deduced. The minor shoulder peak at 1616 cm$^{-1}$ in the GC-sIR spectrum, where the C=O stretching mode of a solid crystalline state is observed, substantiates this theory further.

**FIGURE 3** Gas chromatography-solid state infrared spectroscopy (GC-sIR) spectrum of Cumyl-CBMICA chromatographically isolated from the herbal plant extract [Colour figure can be viewed at wileyonlinelibrary.com]
We expect two absorption bands for the stretching vibrations of the N–H bond of the amide group as well as a shift to a higher wavenumber for the absorption band of the C=O stretching mode in the GC-sIR spectrum compared with the solid IR spectrum to be present in SCs of similar structure. The IR spectra of 5F-Cumyl-PICA and 5F-Cumyl-P7AICA are compared in Supporting Information S19. The GC-sIR spectra of both compounds each exhibit two peaks for the N–H stretching mode in line with the comparison of solid IR and GC-sIR spectrum of Cumyl-CBMICA. Also, the comparison of both spectra for the two substances shows an increased wavenumber of the C=O stretching mode for the absorption band of the amide structure. The absorption band in the spectrum of 5F-Cumyl-P7AICA and 5F-Cumyl-PICA are shifted from 1620 to 1649 cm$^{-1}$ and 1621 to 1650 cm$^{-1}$ respectively, reflecting the observations made for Cumyl-CBMICA.

The case samples 17/ADB-025.2 and 17/ADB-036 analyzed here were analytically confirmed within ADEBAR and fully characterized including NMR spectroscopy (data not shown).

In GC-sIR, the compound is deposited cryogenically onto a ZnSe disc. The cryogenic deposition of the eluting compound occurs rapidly which could decrease the chance to form these hydrogen bonds. The deposited analyte is in the free base form and is believed to be of a partially amorphous crystal structure. The resulting GC-sIR spectrum is in good agreement with the solid ATR-IR spectrum. Because spectra acquired using GC-sIR are always from compounds in the free base form, the congruence of both spectra leads to the conclusion that the solid ATR-IR spectrum is also generated from the free base form of the compound.

The comparison of the GC-sIR and IR solid spectra reveals similarities but also some dissimilarities in the absorption bands. We suspect this is due to crystalline structures forming in the process of condensation after GC separation. In contrast, the solid isolated substance was slowly dried down from a solution in ACN. The difference in the measurement geometry for GC-sIR and the solid IR itself neither changes the relative intensity nor the location of the absorption bands as the physical principle behind the measurement stays the same. With greater sample thickness of a transmission IR measurement, overall intensities of the bands are expected to increase. This effect does not hinder the qualitative analysis of the analyte. Differences in the location, shape and relative intensity of the absorption bands in IR spectra are a result of the covalent bonds and of bonds between molecules in the crystal lattice. These differences, when measuring the same compound, result from the varying degree of long-range order present in amorphous and solid states.

Overall, GC-sIR provides a powerful analytical method for the identification of analytes in mixtures or on herbal matrices. The detection method IR is considered an A-method in analytical chemistry for the unambiguous identification of a compound and differentiation from its isomers.

Additionally, Raman spectra at wavelengths of 785 nm (Supporting Information S15 and S16) and 1064 nm (Supporting Information S17 and S18) were recorded. In the case of the Raman spectra, the Raman shifts and an enlarged area of the Raman shifts are shown. The spectra can also be used for the rapid identification of substances.

4 | CONCLUSION

A new SCRA with a cyclobutyl methyl side chain was detected in herbal blends from a seizure and test-purchase conducted in August 2019 and July 2019, respectively, and identified employing GC-EI-MS, LC-qToF-MS and NMR. Based on the semi-systematic nomenclature for synthetic cannabinoids, the short name Cumyl-CBMICA is proposed. Apart from the side chain, the substance shows high similarity to the already known SCRA Cumyl-PICA. Unlike a variety of other cumyl derivatives described in a patent of Bowden and Williamson on “Cannabinoid Compounds,”$^{18}$ compounds carrying the side chain identified in Cumyl-CBMICA have not yet been described.
in the literature. The new substance initially bypassed the latest definitions of SCRA s of the amended German generic law on NPS (NpSG). Meanwhile, the generic definitions of the law have been updated and cover these derivatives. It seems like some retailers of products containing SCRA s are well prepared for legislative changes, despite the introduction of a law based on generic definitions. Consequently, further attempts to circumvent structural definitions of such laws can be expected, requiring continuous monitoring activities of forensic laboratories and legislative action.

ACKNOWLEDGEMENT

The project ADEBAR plus is cofunded by the Internal Security Fund of the European Union (grant no.: IZ25-5793-2019-33). Open access funding enabled and organized by Projekt DEAL.

ORCID

Benedikt Pulver https://orcid.org/0000-0002-7772-2111
Belal Haschimi https://orcid.org/0000-0003-2954-7539
Volker Auwärter https://orcid.org/0000-0002-1883-2804

REFERENCES

1. de Luca MA, Fattore L. Therapeutic use of synthetic cannabinoids: still an open issue? Clin Ther. 2018;40(9):1457-1466. https://doi.org/10.1016/j.clinthera.2018.08.002
2. European Monitoring Centre for Drugs and Drug Addiction. European Drug Report 2019: Trends and Developments. Luxembourg: Publications Office of the European Union; 2019.
3. Haschimi B, Mogler L, Halter S, et al. Detection of the recently emerged synthetic cannabinoid 4F-MDMB-BINACA in “legal high” products and human urine specimens. Drug Test Anal. 2019;11(9):1377-1386. https://doi.org/10.1002/dta.2666
4. Norman C, Walker G, McKirdy B, et al. Detection and quantitation of synthetic cannabinoid receptor agonists in infused papers from prisons in a constantly evolving illicit market. Drug Test Anal. 2020;12(4):538-554. https://doi.org/10.1002/dta.2767
5. Krotulski AJ, Mohr ALA, Kacinko SL, et al. 4F-MDMB-BINACA: a new synthetic cannabinoid widely implicated in forensic casework. J Forensic Sci. 2019;64(5):1451-1461. https://doi.org/10.1111/1556-4029.14101
6. Neue-psychoaktive-Stoffe-Gesetz: NpSG; 2016, BGBl. 2016 Teil I Nr. 55:2615-2622 (www.gesetze-im-internet.de/npsg/NpSG.pdf, last accessed 02/09/2020)
7. Kikura-Hanajiri R, Kawamura NUM, Goda Y. Changes in the prevalence of new psychoactive substances before and after the introduction of the generic scheduling of synthetic cannabinoids in Japan. Drug Test Anal. 2014;6(7-8):832-839. https://doi.org/10.1002/dta.1584
8. Angerer V, Mogler L, Steitz J-P, et al. Structural characterization and pharmacological evaluation of the new synthetic cannabinoid CUMYL-PEGACLONE. Drug Test Anal. 2018;10(3):597-603. https://doi.org/10.1002/dta.2237
9. Verordnung zur Änderung der Anlage des Neue-psychoaktive-Stoffe-Gesetzes und von Anlagen des Betäubungsmittelgesetzes; 2019, BGBl. 2019 Teil I Nr. 27:1083-1094 (Last access: 16/07/2020).
10. Angerer V, Franz F, Moosmann B, Bisel P, Auwärter V. 5F-Cumyl-PINACA in “e-liquids” for electronic cigarettes: comprehensive characterization of a new type of synthetic cannabinoid in a trendy product including investigations on the in vitro and in vivo phase I metabolism of 5F-Cumyl-PINACA and its non-fluorinated analog Cumyl-PINACA. Forensic Toxicol. 2019;37(1):186-196. https://doi.org/10.1002/fotx.11419-018-0451-8
11. Banister SD, Adams A, Kevin RC, et al. Synthesis and pharmacology of new psychoactive substance 5F-CUMYL-P7AICA, a scaffold-hopping analog of synthetic cannabinoid receptor agonists 5F-CUMYL-PICA and 5F-CUMYL-PINACA. Drug Test Anal. 2019;11(2):279-291. https://doi.org/10.1002/dta.2491
12. Franz F, Angerer V, Moosmann B, Auwärter V. Phase I metabolism of the highly potent synthetic cannabinoid MDMB-CHMICA and detection in human urine samples. Drug Test Anal. 2017;9(5):744-753. https://doi.org/10.1002/dta.2049
13. Banister SD, Longworth M, Kevin R, et al. Pharmacology of Valinate and tert-Leucinate synthetic cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and their analogues. ACS Chem Neurosci. 2016;7(9):1241-1254. https://doi.org/10.1021/acschemneuro.6b00137
14. de Brabanter N, Esposito S, Tudela E, et al. In vivo and in vitro metabolism of the synthetic cannabinoid JWH-200. Rapid Commun Mass Spectrom. 2013;27(18):2115-2126. https://doi.org/10.1002/rcm.6673
15. EDND. Case Report: EDND-CR-2020-567. Netherlands: Drug Information & Monitoring System/Trimbos Institute, February 7, 2020. https://ednd2.emcdda.europa.eu/ednd/report/view/6158/dataset/seizure/view/4816
16. Moosmann B, Kneisel S, Girreser U, Brecht V, Westphal F, Auwärter V. Separation and structural characterization of the synthetic cannabinoids JWH-412 and 1-(5-fluoropentyl)-1H-indol-3-yl-(4-methylnaphthalen-1-yl)methanone using GC-MS, NMR analysis and a flash chromatography system. Forensic Sci Int. 2012;220(1-3):e17-e22. https://doi.org/10.1016/j.forsciint.2011.12.010
17. Cayman spectral library. GC-MS data. CUMYL-CBMICA. Cayman Chemical Company. Ann Arbor, MI, USA. Available at: https://www.caymanchem.com/gcms/30207-0586261GCM5.pdf [last accessed 29 Sep 2020]
18. Bowden MJ, Williamson JPB, inventors. Cannabinoid compounds. WO2014167530A1, 2014, Auckland, New Zealand.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Halter S, Pulver B, Wilde M, et al. Cumyl-CBMICA: A new synthetic cannabinoid receptor agonist containing a cyclobutyl methyl side chain. Drug Test Anal. 2021;13:208–216. https://doi.org/10.1002/dta.2942