Etazene (*N,N*-diethyl-2-[[*(4*-ethoxyphenyl)methyl]-1*H*-benzimidazol-1-yl]-ethan-1-amine (dihydrochloride)): a novel benzimidazole opioid NPS identified in seized material: crystal structure and spectroscopic characterization

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Received: 17 July 2020 / Accepted: 31 August 2020 / Published online: 22 September 2020
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Abstract

Purpose The aim of the study was to present the spectroscopic characteristics and crystal structure of the etazene—a benzimidazole opioid, which appeared on the illegal drug market in Poland in the last weeks.

Methods The title compound was analyzed by X-ray crystallography as well as gas and liquid chromatography combined with mass spectrometry. Spectroscopic techniques have also been used, such as nuclear magnetic resonance, infrared and ultraviolet-visible spectroscopies.

Results We presented the identification and the broad chemical characterization of etazene, a synthetic opioid that has recently been introduced on the illegal drug market.

Conclusions In this paper, we described single-crystal X-ray, chromatographic and spectroscopic characterization of a synthetic opioid that emerged on the new psychoactive substance (NPS) market in Poland. To the best of our knowledge, this is the first full characterization of etazene. Analytical data presented in the work can be helpful in identification and detection of the NPS in forensic and clinical laboratories.

Keywords Etazene · New psychoactive substance · Synthetic opioid · X-ray crystallography · NMR spectroscopy · Mass spectrometry

Introduction

In recent years, an increase in the popularity of synthetic opioids on the illegal drug market has been observed, which is reflected in the annual reports of European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [1–5].

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11419-020-00552-9>) contains supplementary material, which is available to authorized users.

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Particularly many cases of intoxication have been reported in connection with the use of fentanyl derivatives, such as butyrfentanyl, 4-fluorobutyrfentanyl, furanylfentanyl, and 4-fluoroisobutyrfentanyl [6–11]. Recent months have, however, brought a new trend that can be attributed to the legislative changes introduced in China [12] in May 2019. The use of non-fentanyl synthetic opioids [13], including benzimidazole derivatives [14, 15], has started to appear on the European and American markets.

The first papers on the synthesis and properties of benzimidazole opioids (Fig. 1) were published in 1957 and 1960 [16–23]. Already then it was signaled that these compounds could be used as painkillers. It was estimated that their analgesic properties are many times stronger than morphine's and comparable with fentanyl's, and etonitazene (**1**) was considered the strongest analgesic among benzimidazole opioids [24, 25]. However, despite the fact that the following years brought simplification of synthesis methods [26], these compounds have never been widely applied in medicine.

Fig. 1 Structures of etonitazene (1), isotonitazene (2) and etazene (3)

With the exception of 1996, when the illegal sale of etonitazene in Moscow was described, there were no literature reports regarding the use of benzimidazole opioids as a new psychoactive substances (NPS) [27]. However, this situation has recently changed. In 2019, a paper was published on the detection of isotonitazene (2) on the drug market in Belgium [14], and poisoning cases associated with its use in Canada and the USA were reported [15]. The described cases allow to expect a dynamic increase in the number of this type of NPS on the drug market in the coming months.

Growing popularity of synthetic opioids observed in recent years is particularly worrying because their use is associated with a real threat to the lives of those who take them. The most serious adverse reaction associated with opioid is respiratory depression leading to sudden respiratory arrest and death. Synthetic opioids can have hallucinogenic effects (they cause visual and tactile hallucinations) and induce spatial perception disorders. Other symptoms of these compounds include: coma, convulsions (rare), myoclonus, drop in blood pressure causing shock (hypoxic effect), non-cardiogenic pulmonary edema, flatulence, cramping abdominal pain, and acute renal failure as a result of rhabdomyolysis. The particular danger associated with opioid NPS is due to the low effective dose (causing narcotic effects), and hence there is the high risk of its overdose.

Herein we describe the full characteristics of etazene (3)—a benzimidazole opioid, which has just appeared on the illegal drug market. To the best of our knowledge, this compound has not yet been described or reported as an NPS, although its strong analgesic activity (70-fold stronger than morphine) had been noted many years ago [18]. Some information regarding etazene are available only in databases like TG Chemicals or Reddit [28, 29]. We present the results of the analyses carried out using various instrumental methods [X-ray crystallography, ¹H nuclear magnetic resonance (NMR) and ¹³C NMR spectroscopies, liquid chromatography–tandem mass spectrometry (LC–MS/MS), gas

chromatography–mass spectrometry (GC–MS), ultraviolet-visible (UV–VIS) spectroscopy, and Fourier transform infrared spectroscopy (FTIR)]; the analyzed samples were products seized by the police.

Materials and methods

Chemicals

Chemicals and reagents used in analyses were acetonitrile, methanol, and water (Chem Solve LC–MS; Witko, Łódź, Poland); ammonium formate (Fluka, Seelze, Germany); formic acid, 4-methylmethcathinone (4-MMC)-*d*₃ and dimethyl sulfoxide (DMSO)-*d*₆ (Sigma-Aldrich, Darmstadt, Germany).

Samples

Analyzed compound has been seized by the police during the search of parcels at the post office in May 2020. The secured evidence in the form of grey crystalline powder (Fig. 2) was packed in sachets of two different sizes: one of them contained 0.9 g, and the other 25.0 g. Performed analyses showed that each of the evidence contained only one psychoactive substance—etazene.

X-ray crystallography

X-ray quality crystals were obtained by recrystallization of the crude sample (sachet content) from methanol. Suitable crystal was mounted on a thin quartz fiber attached to a copper pin and immediately transferred to the diffractometer. Crystallographic data were collected at 100 (2) K on a KUMA X-ray diffractometer equipped with an Oxford Cryosystems open-flow nitrogen cryostat, using ω -scan and graphite-monochromated Mo K α ($\lambda = 0.71073$ Å) radiation

Fig. 2 Evidence material seized by the police

(Agilent, Yarnton, Oxfordshire, UK). Cell refinement, data reduction and analysis were carried out with CRYSLISPro software (Agilent).

Structure was solved with direct methods using with SHELXS [30] and refined with full-matrix least-squares techniques on F^2 with SHELXL [30]. The H atoms were included in idealized geometry riding on their parent atoms with C–H = 0.95–0.99 Å, N–H = 0.88–1.00 Å, and with $U_{\text{iso}}(\text{H}) = 1.2U_{\text{eq}}(\text{CH}, \text{CH}_2, \text{NH})$ or $1.5U_{\text{eq}}(\text{CH}_3)$ except for water H atoms, which were located in the Fourier maps, refined with O–H distances restrained to 0.860(1) Å and then constrained parent atoms (AFIX 3 instruction). The molecular structure plots were prepared using Diamond [31]. Full details can be found in the crystallographic information file (CIF) file CCDC 2016899.

Powder X-ray diffraction of the crude sample (sachet content) was performed using a Bruker D8 Advance X-ray diffractometer operating with Cu K α radiation ($\lambda = 1.5418$ Å), equipped with a VANTEC-1 PSD (Bruker AXS GmbH, Karlsruhe, Germany). Data were recorded on a silicon low background sample holder in the range 5–60° 2 θ with an angular step size of 0.016° and a counting time of 1 s per step.

NMR spectra

NMR spectra were recorded in DMSO- d_6 at room temperature on a Bruker 500 MHz Avance II spectrometer (Bruker,

Billerica, MA, USA) (^1H frequency 500.13 MHz, ^{13}C 125.7 MHz). Spectra were referenced to the residual solvent signals (2.50 and 39.5 ppm). 2D experiments (correlation spectroscopy, heteronuclear single-quantum correlation spectroscopy, and heteronuclear multiple-bond correlation spectroscopy) were performed by means of standard Bruker software, recorded with 2048 data points in the t_2 domain and up to 1024 points in the t_1 domain, with a 0.5–1 s recovery delay. Analyzed sample was dissolved in DMSO- d_6 with addition of gas HCl.

LC–MS/MS analysis

A total of 100 mg of the sample was dissolved in 5 mL of methanol and diluted 1000-fold with methanol. A total of 10 μL of diluted solution was transferred to a 150 μL vial with a cap containing 80 μL of methanol and 10 μL of internal standard (IS) (4-MMC- d_3 , 1 $\mu\text{g}/\text{mL}$), mixed on a vortex and analyzed by LC–MS/MS as below.

Analysis was performed using an ultra-high-performance liquid chromatograph (Nexera X2; Shimadzu, Kyoto, Japan). The separation was done using a Kinetex XB-C18 (150 $\times$ 2.1 mm i.d., 2.6 μm particle size column; Phenomenex, Torrance, CA, USA) with the thermostat set at 40 °C. The mobile phase consisted of a mixture of 10 mM ammonium formate and 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution was carried out at a constant flow of 0.4 mL/min. The gradient

applied was as follows: 0 min, 5% B; 12 min, 98% B; 14 min, 98% B; and 15 min, 5% B. The return to the initial gradient compositions (95% A/5% B) was held for 5 min.

Detection of the investigated compound was achieved using a triple-quadrupole mass spectrometer (LCMS-8050; Shimadzu). The spectrometer was equipped with an electrospray ionization source; determination of the investigated substance was carried out in the scan mode with positive ionization, and mass range of m/z 50–1000. The following MS parameters were fixed: nebulizing gas flow, 3 L/min; heating gas flow, 10 L/min; interface temperature, 250 °C; desolvation line temperature, 200 °C; heat block temperature, 350 °C; and drying gas flow, 10 L/min.

GC–MS/MS analysis

A 100 mg sample was dissolved in 5 mL of methanol and analyzed by GC–MS/MS. The GC–MS/MS analysis was performed using a gas chromatograph coupled with a triple quadrupole mass spectrometer (GC–MS–TQ8040; Shimadzu). The injector was maintained at 250 °C. Sample injection (2 μ L) was in the splitless mode. Separation was conducted using the SH-5MSi column (30 m length, 0.25 mm inner diameter and 0.25 μ m film thickness; Shimadzu). Helium was used as a carrier gas at the flow rate of 0.94 mL/min. The mass detector was set to positive electron ionization (EI) mode and the electron beam energy was 70 eV. The mass detector was operating in a full scan mode in the 40–500 amu range.

UV-VIS and FTIR spectroscopies

The UV-VIS absorption spectrum was recorded in the methanol solution using the UV-1900i UV/VIS spectrophotometer (Shimadzu), and the spectrum was collected in the range of wavelength 190–1100 nm at room temperature. The UV-VIS absorption spectrum was recorded in 1 cm quartz cuvette. The FTIR spectrum was recorded in the KBr tablet using the VERTEX 70 FTIR spectrometer (Bruker). Spectrum was collected in range of 4000–400 cm^{-1} . Sample analysis was conducted at room temperature.

Results

X-ray studies

Molecular structure of $[\text{etazeneH}_2]\text{Cl}_2 \cdot 1.5(\text{H}_2\text{O})$ is presented in Figs. 3 and 4, while selected interatomic distances and angles are listed in Table S1 (supplementary material). Crystal data and structure refinement details are presented in Table 1.

Fig. 3 Molecular structure of $[\text{etazeneH}_2]\text{Cl}_2 \cdot 1.5(\text{H}_2\text{O})$, showing the atom-labelling scheme

Fig. 4 Part of the crystal structure of $[\text{etazeneH}_2]\text{Cl}_2 \cdot 1.5(\text{H}_2\text{O})$ with shown H-bonding

The analyzed compound crystallized in the triclinic *P*-1 space group. Its crystal structure was built up from one $[\text{etazeneH}_2]^{2+}$ dication, chloride anion and water molecules. All distances and angles were similar to those previously reported for a nitro analog [32, 33]; however, in the present study, organic cations showed different conformation of *N,N*-diethylethanamine chain. All components were held together by N–H...Cl, O–H...Cl and O–H...O hydrogen bonding (Table 2, Fig. 4). More specifically, organic dications

Table 1 Crystal data and structure refinement parameters

	[etazeneH ₂]Cl ₂ ·1.5(H ₂ O)
Chemical formula	[C ₂₂ H ₃₁ N ₃ O]Cl ₂ ·1.5(H ₂ O)
Formula mass	451.42
Crystal system	Triclinic
a[Å]	9.595 (5)
b[Å]	11.247 (6)
c[Å]	11.476 (5)
α[°]	72.70 (4)
β[°]	86.99 (3)
γ[°]	86.82 (4)
Unit cell volume[Å ³]	1179.7 (10)
Temperature[K]	100 (2)
Space group	<i>P</i> -1
Z	2
Radiation type	MoKα
No. of reflections measured	10211
No. of independent reflections	5151
<i>R</i> _{int}	0.058
Final <i>R</i> _i values (<i>I</i> > 2σ(<i>I</i>))	0.070
Final w <i>R</i> (<i>F</i> ²) values (<i>I</i> > 2σ(<i>I</i>))	0.198
Goodness of fit on <i>F</i> ²	1.03

Table 2 Hydrogen-bond geometry (Å, °)

<i>D</i> —H... <i>A</i>	<i>D</i> —H	H... <i>A</i>	<i>D</i> ... <i>A</i>	<i>D</i> —H... <i>A</i>
N2—H2...Cl 2	0.88	2.18	3.041 (3)	165
N3—H3...Cl 1	1.00	2.16	3.146 (3)	171
O1W—H1WB...Cl 2	0.86	2.32	3.174 (14)	169
O1W—H1WA...Cl 3	0.86	2.31	3.142 (14)	164
O2W—H2WB...Cl 2	0.86	2.23	3.076 (14)	169
O2W—H2WA...Cl 3 ⁱ	0.86	2.22	3.063 (16)	166
O3W—H3WB...O2W	0.86	2.47	2.89 (2)	111
O3W—H3WA...O2W ⁱ	0.86	2.57	3.06 (3)	118

Symmetry code: (i) $-x+2, -y+1, -z+2$

[etazeneH₂]²⁺ were hydrogen-bonded via one Cl[−] anion to form a dimer.

The powder diffraction studies was performed on the crude sample before recrystallization. The comparison of diffractogram from the crude sample and the simulated diffractogram (obtained from single crystal data), showed the similarities and a good correspondence of the crystalline phases (Fig. 5), which indicated the crystalline phase purity of the sample.

NMR spectra

Conclusion and findings regarding the structure of etazene based on X-ray analysis results were confirmed by

interpretation of NMR spectroscopy data. ¹H NMR spectrum for analyzed sample (Fig. 6) displayed one set of signals. They were unambiguously assigned due to 2D NMR and ¹³C NMR experiments (see Figs. S1–S4).

Protons of both phenyl and benzimidazole rings gave signals in a range characteristic for aromatic systems (6.9–8.3 ppm): aromatic protons of ethoxyphenyl unit gave two doublets at 7.51 and 6.94 ppm, whereas signals from benzimidazole ring could be observed at 8.21, 7.81 and 7.60 ppm. The broad peak with the highest chemical shift (11.97 ppm) could be assigned to the N–H proton of diethylamine moiety. Signal attributed to N–H proton incorporated in benzimidazole ring could not be observed, due to the fast (on the NMR time scale) chemical exchange.

Two equivalent ethyl substituents localized on nitrogen atom yielded triplet at 1.24 ppm (–CH₃) and multiplet at 3.21 ppm (–CH₂–). In case of ethoxyl group, peaks could be observed at 1.30 (–CH₃) and 4.00 ppm (–CH₂–). Singlet derived from methylene bridge between phenyl and benzimidazole ring appeared in the spectrum at 4.72 ppm. Multiplets attributed to –CH₂–CH₂– were located at 3.37 and 5.11 ppm.

All resonances (with atoms assignment, according to the numbering in Fig. 6) for the analyzed compound are listed in Table 3.

LC–MS/MS and GC–MS/MS analyses

The liquid chromatography showed that analyzed sample contained only one substance—etazene [retention time (RT) = 4.95 min]. Additional peak visible in the total ion chromatogram (Fig. 7a) corresponded to the IS (4-MMC-*d*₃, RT = 3.82 min). A chromatogram in positive ion mode was dominated by ion at *m/z* 352. Thus, it was concluded that a precursor ion [M + H]⁺ of etazene should be chosen for further product ion scan (PIS) (Fig. 7c–e). During the PIS analysis, ions at *m/z* 100, 72 and 44 were observed; all derived from the diethylamine fragment.

The gas chromatography also confirmed the purity of the analyzed material evidence; only one signal, from etazene, was visible in the chromatograph (RT = 25.4 min). In the mass spectrum obtained in the EI-MS mode (Fig. 8), main fragment ions were those at *m/z* 58, 86, 107 and 135. They corresponded to the methylethoxybenzene side chain (*m/z* 135 and 107, depending on the location of the fragmentation) and diethylamine moiety (*m/z* 86, 58). Analyses of PIS with different collision energies (0, 2, 3, 5, 15 and 25 V) showed presence of several consecutive ions, such as *m/z* 72, 100, 216, 265 and 280 (Fig. S5). Fragmentation pattern proposed based on the measured spectra is shown in Fig. 8. Some of observed ions (*m/z* 72, 100, 107,

Fig. 5 Comparison between experimental (lower: black) and simulated (upper: red) diffractograms of [etazeneH₂]Cl₂·1.5(H₂O) (color figure online)

Fig. 6 ¹H nuclear magnetic resonance spectrum of etazene dihydrochloride (500 MHz, DMSO-*d*₆)

Table 3 ^1H and ^{13}C nuclear magnetic resonance data for etazene dihydrochloride (DMSO; 500 and 126 MHz, respectively)

Atom position	Carbon chemical shift (ppm)	Proton chemical shift (ppm)
1	130.1	—
2	113.2	8.21 (dd, $J=6.8, 1.8$ Hz, 1H)
3	126.3	7.66–7.56 (m, 2H)
4	126.0	
5	114.3	7.84–7.78 (m, 1H)
6	131.5	—
7	153.6	—
8	29.9	4.72 (s, 2H)
9	124.5	—
10	130.7	7.51 (d, $J=8.73$ Hz, 2H)
11		
12	115.1	6.94 (d, $J=8.75$ Hz, 2H)
13		
14	158.3	—
15	63.2	4.00 (q, $J=7.0$ Hz, 2H)
16	14.7	1.30 (t, $J=7.0$ Hz, 3H)
17	39.5	5.16–5.06 (m, 2H)
18	48.0	3.37 (dt, $J=10.7, 5.5$ Hz, 2H)
19	—	11.97 (s, 1H)
20	46.2	3.30–3.10 (m, 4H)
21		
22	8.3	1.24 (t, $J=7.2$ Hz, 6H)
23		
24	—	Fast chemical exchange

The numbering of atoms is given in Fig. 6

135) was also recorded in case of isotonitazene [14] and etonitazene [34].

UV-VIS and FTIR spectroscopies

The UV spectrum of etazene dihydrochloride recorded by UV-VIS spectrometry showed absorption maxima at 205, 272 and 278 nm (Fig. S6). The FTIR spectrum recorded in KBr tablet (Fig. S7) displayed bands that could be attributed to the characteristic elements of the etazene structure, such as benzimidazole ring (sharp bands at 762–1609 cm^{-1}), methylene bridges (1466 cm^{-1}), aliphatic-aryl ethers (1259 cm^{-1}), aromatic systems (1600–2000 cm^{-1}) and

amines (2947 and 2974 cm^{-1}). They were very similar to the FTIR spectra recorded for structurally related substances: etonitazene and isotonitazene [14].

Discussion

To the best of our knowledge, the analytical data presented in this publication are the first information regarding etazene since its synthesis was developed. Never before has this substance been described in the context of NPS, and the samples that we analyzed were sent to our laboratory only a few weeks ago.

A careful observation of the changes on the illegal NPS markets both in Europe and all over the world suggests that, in the near future, an increase in the popularity of benzimidazole opioids will occur. These suppositions are confirmed, for example, by the new EMCDDA reports published in recent months regarding isonitazene, a substance with a very similar structure to etazene [35, 36]. Toxicology and forensic laboratories, in which both evidence material and biological samples are analyzed, should be aware of changing trends and be prepared for quick response as well as possible.

It is worth emphasizing that the evidence material analyzed in our laboratory was of high purity, which may be considered as a specially dangerous. Interestingly, the authors of the paper regarding another benzimidazole opioid, isotonitazene, also noticed that the compound they described was sold undiluted [14].

Conclusions

We presented complete chemical identification of a synthetic opioid seized by the police from an illegal drug market in Poland. Analyzed compound was characterized with the use of X-ray crystallography, NMR, UV-VIS and FTIR spectroscopies, and mass spectrometry. Analytical data for etazene were presented for the first time.

In view of the continuous increase in the number of compounds entering the illegal drug market, this is valuable information. It can be relevant not only to the police, but also to customs services and forensic laboratories in which such substances are analyzed and identified. Expanding the existing databases for new psychoactive substances is important, because it can be successfully used in interception of illegal substances on the market and for pursuing clandestine drug synthesis.

Fig. 7 Total ion chromatogram (TIC) of the sample with addition of internal standard—4-methylmethcathinone- d_3 (**a**), mass spectrum of the precursor ion (**b**) and the product ion scan (PIS) with collision

energies at -10 V (**c**), -20 V (**d**) and -35 V (**e**) obtained by liquid chromatography–tandem mass spectrometry

Fig. 8 Gas chromatography–mass spectrometry data of the analyzed sample and etazene molecule with marked fragmentation patterns (main ions marked with bold); for ions recorded in PIS mode (not marked with bold) see supplementary material (Fig. S5)

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

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