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Report on a novel emerging class of highly potent benzimidazole NPS opioids: Chemical and in vitro functional characterization of isotonitazene

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Abstract

This paper reports on the identification and full chemical characterization of isotonitazene (*N*,*N*-diethyl-2-[5-nitro-2-({4-[(propan-2-yl)oxy]phenyl}methyl)-1H-benzimidazol-1-yl]ethan-1-amine), a potent NPS opioid and the first member of the benzimidazole class of compounds to be available on online markets. Interestingly, this compound was sold under the name etonitazene, a structural analog. Identification of isotonitazene was performed by gas chromatography mass spectrometry (GC–MS) and liquid chromatography time-of-flight mass spectrometry (LC-QTOF-MS), the latter identifying an exact-mass m/z value of 411.2398. All chromatographic data indicated the presence of a single, highly pure compound. Confirmation of the specific benzimidazole regio-isomer was performed using ^{1}H and ^{13}C NMR spectroscopy, after which the chemical characterization was finalized by recording Fourier-transform (FT-IR) spectra. A live cell-based reporter assay to assess the in vitro biological activity at the μ-opioid receptor (MOR) revealed that isotonitazene has a high potency (EC_{50} of 11.1 nM) and efficacy (E_{max} 180% of that of hydromorphone), thus confirming that this substance is a strong opioid. Isotonitazene has not been previously detected, either in powder form, or in biological fluids. The high potency and efficacy of isotonitazene, combined with the fact that this compound was being sold undiluted, represents an imminent danger to anyone aiming to use this powder.

KEYWORDS

new psychoactive substances, synthetic opioids, isotonitazene, NPS, NPS opioids

1 | INTRODUCTION

For about a decade large and increasing numbers of new psychoactive substances (NPS) with similar effects to classic illicit drugs have started to appear in Europe, escaping legislation by using chemical structures that are similar to, yet differ from, those of known illicit drugs. Well-known examples include synthetic cannabinoids and cathinones. Substances with opioid activity remained absent from the market for a relatively long time. The first synthetic opioids appearing for sale online included AH-7921 and MT-45 in 2012 and 2013, respectively.[1-3] From around 2014 increasing numbers of novel fentanyl analogs started to appear for sale, both in clear web online markets as well as on darknet markets. Also fentanyl derivatives already used in human or veterinary medicine were increasingly being sold illegally, including alfentanil, sufentanil, remifentanil, and carfentanil.[4,5] The arrival of these fentanyl analogs was accompanied by an increasing and alarming number of deaths, with the USA especially being hit hard by this opioid epidemic.[6-9] While the death toll in Europe

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remained relatively limited, compared with the USA, also here, an increasing number of fatalities linked to the use of synthetic opioids has been observed.[4,10,11]

Because of the relatively simple synthesis, in addition to excellent online precursor availability, the large majority of NPS opioids appearing until the end of 2018 were derivatives of fentanyl (marked in blue and with horizontal stripes in Figure 1).[12] From 2018 onwards, these numbers dropped and only one novel fentanyl analog was identified in 2019. This strong reduction was most probably a result of the introduction of new legislation in China in 2018.[13] Similarly, the number of newly detected U-compounds (U-47700 is the best known example[10,14]) increased until 2018, and started to decrease from then on. In contrast, from the end of 2018 onwards, there has been a prominent increase in the general "others" category (examples include 2F-viminol, 2-Me-AP-237, and the molecule discussed in this paper, isotonitazene) (Figure 1), suggesting the resilience of Asian suppliers after the recent tightening of domestic and international regulations of fentanyl-type opioids.

In this paper we report on the identification and full characterization of a novel NPS opioid sourced online: isotonitazene (*N*,*N*-diethyl-2-[5-nitro-2-({4-[(propan-2-yl)oxy]phenyl}methyl)-1H-benzimidazol-1-yl]ethan-1-amine; chemical structure can be found in Figure 2), which is the first detected member of a new benzimidazole class of NPS opioids. This type of compound is not new – the synthesis of benzimidazole derivatives with analgesic activity was first reported in 1957.[15,16] Since then, other, simplified synthesis pathways have been described, including a relatively simple one-pot, three-component synthesis at high yield.[17] Although several derivatives were patented,[18-21] no clinically approved therapeutics have made it to the market. This benzimidazole class of compounds (Figure 2) differs radically in structure from other potent analgesics.[22] The most potent compound in this class, with an estimated reported potency of a hundred to a thousand times that of morphine, is etonitazene.[23,24] Interestingly, the compound that was obtained online was wrongly sold under this name. In addition to the full chemical characterization and identification, we also report here the in vitro functional characterization of this compound, using a μ-opioid receptor activation assay.

2 | MATERIALS AND METHODS

The sample was obtained during routine online monitoring of drug markets, performed continuously in the framework of the functioning of the Belgian Early Warning System Drugs (BEWSD), located at Sciensano, previously known as the Scientific Institute of Public Health.

2.1 | Materials

All reagents used during the analyses were at least of HPLC grade. For NMR analysis, deuterated dimethyl sulfoxide (DMSO-d_6, 99.8%) was purchased from Eurisotop (Saint-Aubin, FR). Hydromorphone was purchased as hydromorphone HCl from Fagron (Nazareth, Belgium). Fentanyl and isotonitazene (1 mg) were obtained as a free base from LGC Chemicals (Wesel, Germany) and Cayman Chemicals (Ann Arbor, Michigan, US), respectively. Dulbecco's modified Eagle's medium (DMEM; GlutaMAX™), Opti-MEM® I reduced serum medium, penicillin–streptomycin (5.000 U/mL), and amphotericin B (250 μg/mL) were purchased from Thermo Fisher Scientific (Pittsburg, PA, USA). Fetal bovine serum (FBS) and poly-D-lysine were supplied by Sigma Aldrich (Overijse, Belgium). The Nano-Glo® Live Cell reagent, which was used for the readout of the bioassay, was procured from Promega (Madison, WI, USA).

2.2 | Sample preparation

The sample was obtained from an online supplier in June 2019 as a white homogenous powder, sold as etonitazene. It was provided in a small sealed plastic bag (Supplementary Data, Figure S1), which itself was again sealed inside an aluminum pouch. It was used as provided, after short-term storage in the freezer (range −20°C to −30°C) for analysis. For all the chromatographic analyses and the determination of the biological activity, 5.44 mg of the powder was dissolved in 0.544 mL of methanol as a stock solution. For NMR analysis, a sample (5.1 mg) was dissolved in DMSO-d_6 (~ 0.75 mL).

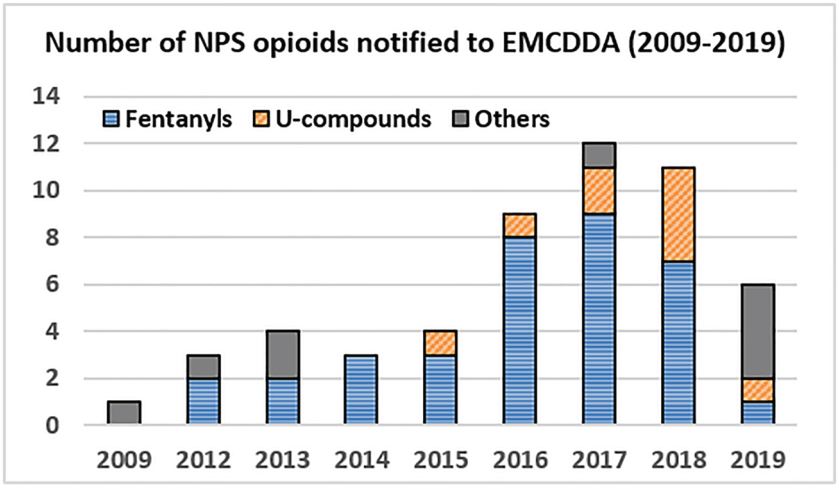

FIGURE 1 NPS opioids formally notified to the European monitoring Centre for Drugs and Drug Addiction (EMCDDA) 2009 - June 2019

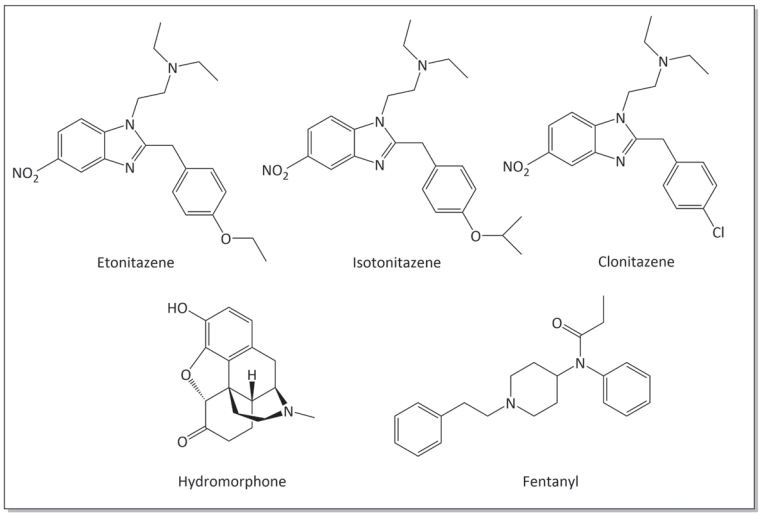

FIGURE 2 Chemical structures of analgesic nitrobenzimidazole derivatives (etonitazene, isotonitazene, and clonitazene), hydromorphone and fentanyl

2.3 | Instrumentation

2.3.1 | Liquid chromatography time-of-flight mass spectrometry (LC-QTOF-MS)

Chromatographic separation was accomplished with an Agilent 1290 Infinity LC system and a Phenomenex Kinetex C18-column (2.6 μm, 3 × 50 mm), maintained at 30°C. The high resolution mass spectrometry (HRMS) system was a 5600+ QTOF from Sciex, with an electrospray ionization (ESI) source and Analyst TF 1.7.1 software, from the same provider. The LC-HRMS analysis was performed using the same settings to those described,[25] obtaining a TOF-MS full scan combined with a data dependent acquisition of product ion spectra. Ten μL of a 1/10 000 dilution of the stock solution in 0.05/50/25/25 formic acid/water/methanol/acetonitrile containing 2.5 mM ammonium formate was injected (resulting in an absolute amount of 10 ng). This solution was also directly infused into the QTOF-MS to obtain additional mass spectra (infusion rate 5 μL/min, scanning in TOF-MS mode from 100–500 Da, and in product ion mode for products of 411.2398 Da from 5–450 Da; other MS settings were ion source gas 1: 20 psi; ion source gas 2: 30 psi, curtain gas: 20 psi; temperature: 325°C, ion spray voltage: 5500 V, declustering potential: 100 V; collision energy: 35 V).

2.3.2 | Gas chromatography mass-spectrometry (GC–MS)

One μL of a 1/10 dilution of the stock solution was injected on an Agilent 7890A GC system coupled to a 5975 XL mass-selective detector operated by MSD Chemstation software. Splitless injections were performed automatically at an injection temperature of 250°C and a purge time of 1 min, with helium as a carrier gas at a constant flow rate of 1 mL/min. A 30 m × 0.25 mm i.d. × 0.25 μm Agilent HP-5-MS column was used. The temperature program started at 80°C for 1 min, was ramped at 20°C/min to 200°C, then raised by 4°C/min to 260°C and by 30°C/min to 300°C, which was held for 8 min more. The transfer line temperature and ion source temperature were set at 300 and 230°C, respectively. The MS quadrupole temperature was set at 150°C and an ionization energy of 70 eV was used. The mass spectrometer operated in SCAN-mode, scanning the range of 50–700 Da.

2.3.3 | High-performance liquid chromatography diode array detector (HPLC-DAD)

Reversed-phase separation of the extract was performed on a LaChrom HPLC system from Merck-Hitachi (Tokyo, Japan), using a Merck Purospher® Star RP-8 endcapped column (5 μm, 125 mm × 4.6 mm) fitted with a Merck Purospher® Star RP-8 endcapped guard column (5 μm, 4 mm × 4 mm), using a methanol–water (containing 150 mM phosphate buffer pH 2.3) gradient of 5:95 to 95:5 (v/v) within 30 min. Detection was done by a diode array detector (DAD), monitoring a wavelength from 220–350 nm with a slit of 1 nm, a spectral bandwidth of 1 nm, and a spectral interval of 200 ms. A two-step dilution of the stock solution was made (1/10 in methanol, followed by 1/50 in 88/12 water/methanol), 50 μL of the final dilution was injected.

2.3.4 | Nuclear magnetic resonance spectroscopy (NMR)

The NMR measurements were performed on a Varian Mercury 300 MHz spectrometer, operated at room temperature (25°C). Chemical shifts (δ) are reported in ppm and spectra are referenced to the residual solvent peak signal. Coupling constants are given in Hz. Figures of the relevant ^{1}H- and ^{13}C-NMR spectra are presented in this report. Copies of 2D NMR spectra are included in the Supporting Information.

2.3.5 | Determination of powder sample melting point

The melting point (mp) was determined on a Büchi-545 apparatus, and is uncorrected.[26]

2.3.6 | Fourier-transform infrared spectroscopy (FT-IR) analysis

A Nicolet iS10 FT-IR (ThermoFisher Scientific, Waltham, USA) equipped with a Smart iTR accessory and a deuterated triglycine sulfate (DTGS) detector was used to record the FT-IR spectra. The Smart iTR accessory is equipped with a single bounce diamond crystal and is calibrated weekly by means of a polystyrene film. A small amount of the sample powder was deposited directly on the crystal without any preliminary sample preparation. To ensure spectral uniformity, the optimal pressure, which is part of the basic qualifications of the instrument set by the provider, was applied to the sample. The infrared spectrum was subsequently recorded from 4000–400 cm^{-1} and each spectrum was measured at a spectral resolution of 4 cm^{-1} and consisted of 32 co-added scans. Spectral data were obtained using the OMNIC Software version 8.3 (Thermo Scientific, Madison, USA). Before the measurement, the crystal was cleaned using a soft tissue soaked with methanol and left to dry in ambient air. Before recording the sample spectrum, a blank measurement was performed to check the crystal for contamination and to carry over using the absorbance limits for contamination defined by the European Directorate for the Quality of Medicines and HealthCare (EDQM).[27] A background spectrum was measured against air using identical instrumental conditions as for the sample.

The analysis was also performed in transmittance mode, using the Smart Omni Transmission accessory (Thermo Scientific, Madison, WI, USA). The sample powder was blended with potassium bromide for IR spectroscopy (Uvasol®, Merck Millipore, Darmstadt, Germany) in a concentration of 0.5 w/w% using a pestle and mortar. This mixture was put under a pressure of 20 tons for 5 min, resulting in a clear potassium bromide tablet which was analyzed in the range of 4000 to 400 cm^{-1} with the same resolution and number of co-added scans as described before. The small amount of isotonitazene reference standard that was purchased (only 1 mg) did not allow us to perform the FT-IR analysis on that material.

2.3.7 | Determination of biological activity at the μ-opioid receptor (MOR)

A live cell-based reporter assay that monitors protein–protein interactions via the NanoLuc Binary Technology was used to assess the biological activity of isotonitazene. This bioassay evaluates MOR activation via its interaction with β-arrestin 2 (βarr2), a cytosolic protein. Both βarr2 and MOR are fused to an inactive part of a split nanoluciferase. Upon MOR activation, βarr2 is recruited to the receptor, allowing interaction of the complementary nanoluciferase subunits, yielding a functional enzyme that generates a bioluminescent signal in the presence of the substrate furimazine.[28] The original human embryonic kidney (HEK)293 T cell line was provided by Prof. O. De Wever (Laboratory of Experimental Cancer Research, Ghent University Hospital, Belgium) and was modified to stably express the opioid reporter system, similar to that done for the cannabinoid receptor bioassays.[29] The stability of the cell line (i.e. the expression levels of fusion proteins) was monitored by flow cytometric analysis of co-expressed markers.[30] The cells were routinely maintained at 37°C, 5% CO_2, under a humidified atmosphere in DMEM (GlutaMAX™) supplemented with 10% heat-inactivated FBS, 100 IU/mL penicillin, 100 mg/L streptomycin, and 0.25 mg/L amphotericin B. On the day prior to the experiments, the cells were seeded on poly-D-lysine coated 96-well plates at 5×10^4 cells/well and incubated overnight. The cells were washed twice with Opti-MEM® I reduced serum medium to remove any remaining FBS, and 90 μL Opti-MEM® I was added. The Nano-Glo® Live Cell reagent, a non-lytic detection reagent containing the cell permeable furimazine substrate, was prepared by 20-fold dilution of the Nano-Glo® live cell substrate using Nano-Glo® LCS dilution buffer, and 25 μL was added to each well. Subsequently, the plate was placed into a TriStar^2 LB 942 multimode microplate reader (Berthold Technologies GmbH & Co., Germany). Luminescence was monitored during the equilibration period until the signal stabilized (15 min). Next, 20 μL per well of test compounds, present as concentrated (6.75-fold, as 20 μL was added to generate a final volume of 135 μL) stock solutions in Opti-MEM® I was added. The luminescence was continuously monitored for 120 min. Solvent controls were included in all experiments. Curve fitting and statistical analyses were performed using GraphPad Prism software (San Diego, CA, USA). The results are represented as the mean area under the curve (AUC) ± standard deviation (SD), with at least five replicates for each data point (obtained in three independent experiments). All results were normalized to the E_{max} of hydromorphone (= 100%), our reference compound. Curve fitting of the concentration–response curves via non-linear regression (four parameters logistic fit) was employed to determine EC_{50} (a measure of potency) and the E_{max} (a measure of efficacy).

3 | RESULTS AND DISCUSSION

3.1 | LC-QTOF-MS, GC–MS, and HPLC-DAD analysis

Using QTOF-MS in the full scan mode, a peak with an exact-mass m/z value of 411.2398 was found. Calculating the elemental composition, assuming a single protonation of the molecule, resulted in a 1.78 ppm error for the molecular formula of isotonitazene. The obtained product ion spectrum is shown in Figure 3A. The main product ions observed were those with an m/z value of 72, 100, and 107, corresponding to either the amine side chain (72 and 100) or the methylethoxybenzene side chain (107). These fragments, as well as

the less abundant fragments, match with those obtained for the reference standard and with those proposed for benzimidazole derivatives as found in the literature.[31,32] Using the LC-QTOF-MS method, the compound had a retention time of 5.86 min (the LC-QTOF chromatogram is shown in Supplementary Data, Figure S2). Both from the infusion and the chromatographic experiment, a high purity of the compound can be estimated, as no other peaks different from the blank were observed. GC–MS analysis revealed only one peak (retention time 28.5 min). The GC–MS mass spectrum (Figure 3B) matches with the one found online.[33] The spectrum of the only peak (retention time 20.73 min) obtained with the HPLC-DAD method is shown in Figure 3C. The compound had two absorption maxima, at 238.1 and 305.0 nm. No impurities were found via any of the applied methodologies.

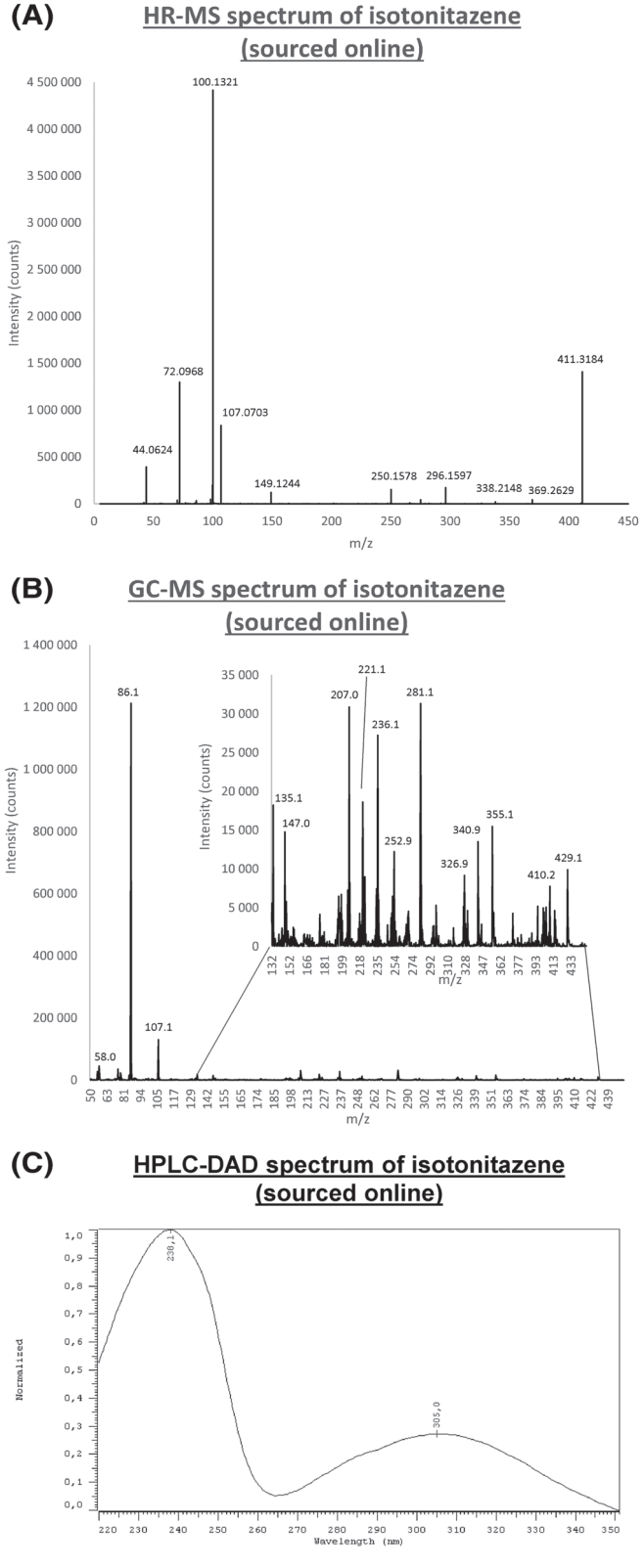

FIGURE 3 (A) HR-MS fragment ion spectrum after infusion; (B) EI spectrum obtained via GC–MS; (C) HPLC-DAD chromatogram spectrum

3.2 | NMR and melting point analysis

The QTOF-MS data indicated that the exact mass corresponded to the proposed isotonitazene structure (see Figure 2). In order to fully confirm the molecule's identity and to exclude the possibility of the presence of regio-isomeric compounds as well as to confirm the position of the nitro-group in the benzimidazole portion, a detailed NMR analysis was performed.[34] A suite of 1D and 2D NMR experiments was employed to establish the connectivity pattern of the molecule. The ^{1}H-NMR and ^{13}C-NMR spectra (Figure 4A, B) demonstrate the presence of only one compound, with no indication of the presence of impurities. A full structural assignment was achieved by employing 2D NMR techniques (gCOSY, ^{1}H-^{13}C gHSQC, ^{1}H-^{13}C gHMBC, and ^{1}H-^{1}H 2D NOESY) (Supplementary Data, Figure S4–S6). This confirmed the proposed structure and ascertained the position of the nitro functional group to be C5, based on the presence of a cross-peak between NCH_2 and H7 (^{1}H-^{1}H 2D NOESY, Supplementary Data, Figure S4–S6). The presence of a broad signal at δ = 11.17 ppm was noted, which does not appear to be part of a spin system present in the molecule. We therefore hypothesized that the obtained powder sample was a salt, most likely of inorganic origin, given the lack of additional signals in the ^{1}H and ^{13}C spectrum. A melting point determination gave a mp of 174°C, indicating that the obtained compound is a HCl salt, as this is in line with the reported mp of 172–173°C.[35] From all the above-mentioned analyses, we concluded that the powder sample that was obtained online is of high purity.

3.3 | FT-IR analysis

To finalize the chemical characterization of the sample, the FT-IR spectrum of the sample was recorded using attenuated total reflectance (Figure 5A). The presence of the nitro group on the benzimidazole structure could clearly be demonstrated with characteristic peaks at wavenumbers 1507–1523 and 1335–1347 (Figure 5A). The obtained spectrum was matched against an in-house library and resulted in a best match (55.97%) with the spectrum of etonitazene. Since this reference spectrum is an old spectrum recorded in absorption mode, the analysis was repeated in absorption mode, preparing a KBr tablet, as had been done for recording the reference spectrum of

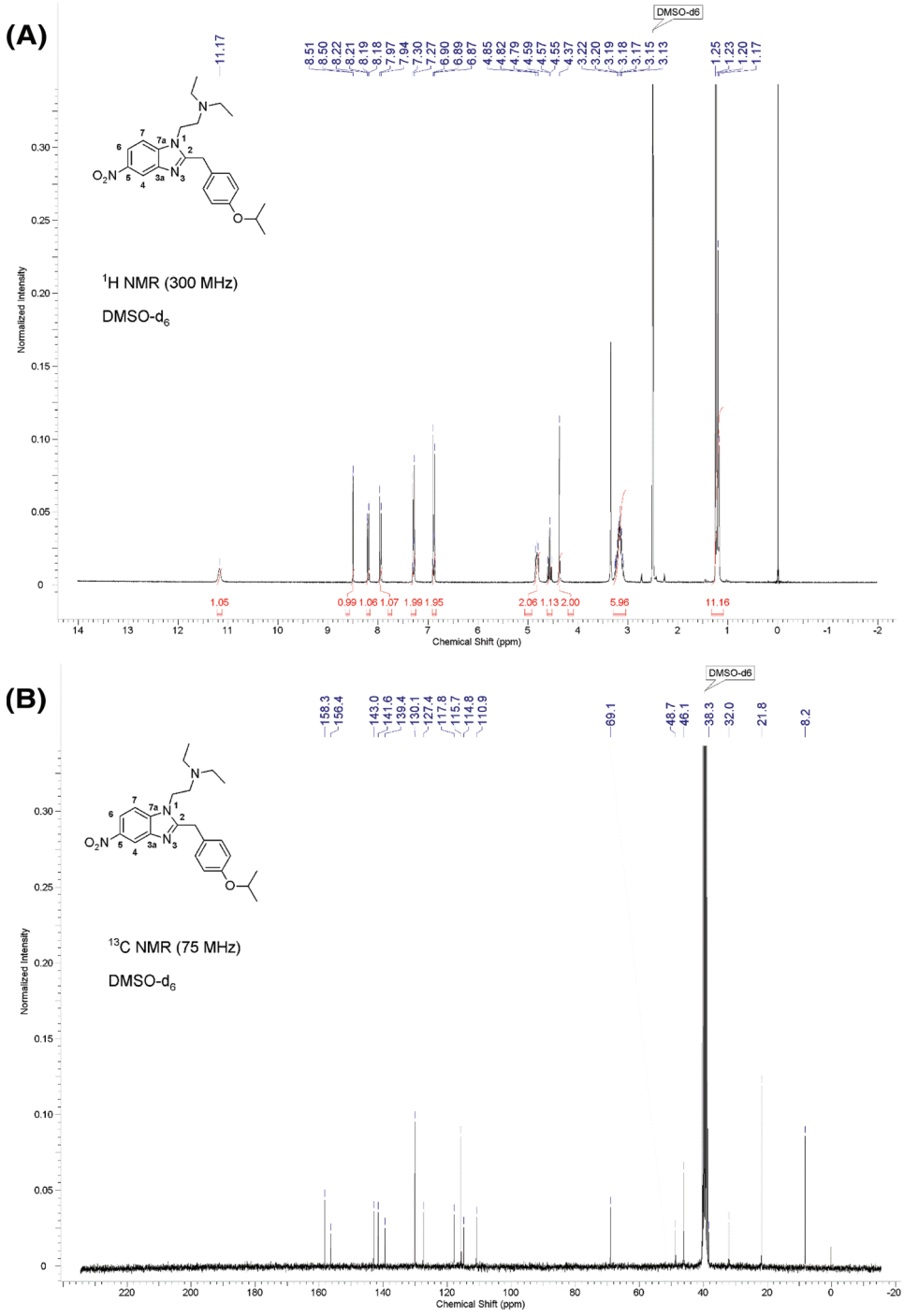

FIGURE 4 (A) ^{1}H NMR spectrum (300 MHz, DMSO-d_6) δ: 1.20 (t, *J* = 7.3 Hz, 2x 6H, N ($CH_2\underline{CH_3}$)$_2$, 1.24 (d, *J* = 5.9 Hz, 6H, OCH ($\underline{CH_3}$)$_2$), 3.08–3.26 (m, 6H, (CH_3CH_2)$_2$N$\underline{CH_2}$, N ($\underline{CH_2}CH_3$)$_2$), 4.37 (s, 2H, CH_2Phe), 4.57 (septet, *J* = 5.9 Hz, 1H, OCH), 4.79–4.85 (m, 2H, NCH_2), 6.86–6.91 (m, 2H, $H3_{Phe}$, $H5_{Phe}$), 7.26–7.31 (m, 2H, $H2_{Phe}$, $H6_{Phe}$), 7.95 (d, *J* = 9.1 Hz, 1H, H7), 8.20 (dd, *J* = 9.1, 2.3 Hz, 1H, H6), 8.50 (d, *J* = 2.1 Hz, 1H, H-4), 11.17 (broad s, 1H); (B) ^{13}C NMR spectrum (75 MHz, DMSO-d_6) δ: 8.2 (2C, N ($CH_2\underline{CH_3}$)$_2$, 21.8 (2C, OCH ($\underline{CH_3}$)$_2$), 32.0 (Phe-$\underline{CH_2}$), 38.3 ($N\underline{CH_2}CH_2N(CH_2CH_3)_2$), 46.1 (2C, N ($\underline{CH_2}CH_3$)$_2$), 48.7 ($NCH_2\underline{CH_2}N(CH_2CH_3)_2$), 69.1 (O$\underline{CH}$ (CH_3)$_2$), 110.9 (C7), 114.8 (C4), 115.7 (2C, $C3_{Phe}$, $C5_{Phe}$), 117.8 (C6), 127.4 ($C1_{Phe}$), 130.1 (2C, $C2_{Phe}$, $C6_{Phe}$), 139.4 (C7a), 141.6 (C-3a), 143.0 (C5), 156.4 ($C4_{Phe}$), 158.3 (C-2)

etonitazene. The resulting spectrum (Figure 5B) was then again compared with the library, resulting in a match with etonitazene of 75.95%, confirming the structural analogy between the two compounds.

3.4 | Pharmacological evaluation of isotonitazene in vitro

For the in vitro pharmacological evaluation, the online purchased product was compared with the reference standard of isotonitazene. The isotonitazene reference standard yielded a concentration-dependent response in the MOR activation assay (Figure 6), which could be antagonized by the mu opioid receptor antagonist naloxone (data not shown). The EC_{50} and E_{max} values were determined as a measure of potency and relative efficacy, respectively (see Table 1). The obtained data revealed that isotonitazene has a high potency (EC_{50} of 11.1 nM) and efficacy (E_{max} 180% of that of the reference compound used in this assay, hydromorphone), indicating that this compound is a strong opioid (which is also in good correspondence with data previously reported in the literature[16,34,35]). These values are even slightly higher than those we obtained for the known potent

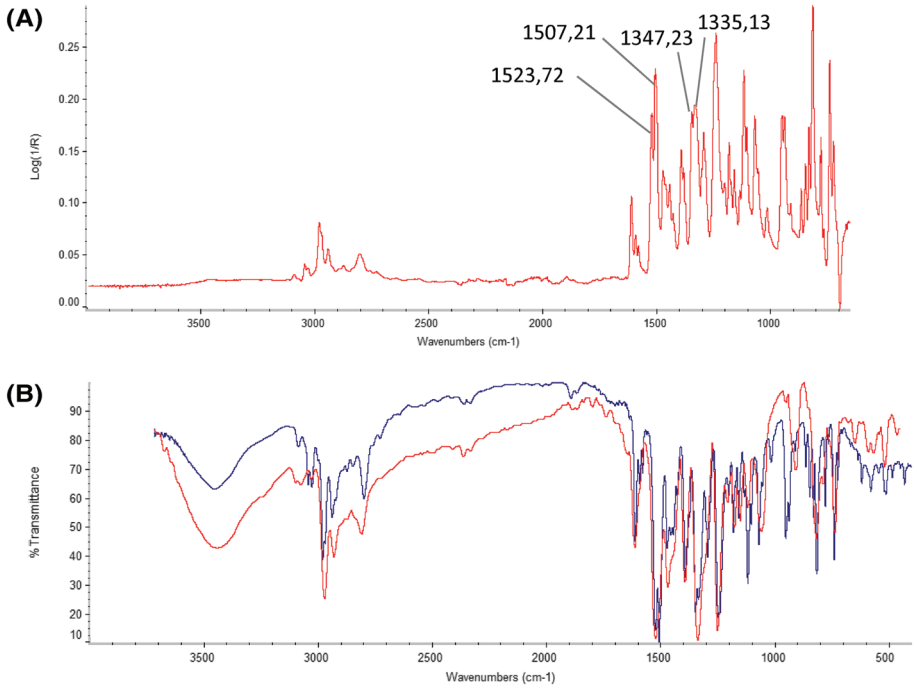

FIGURE 5 (A) IR spectrum of the sample, recorded in ATR mode with indication of the characteristic bands for the nitro group; (B) IR spectrum of the sample (blue), recorded in absorbance mode compared with the library spectrum of etonitazene (red)

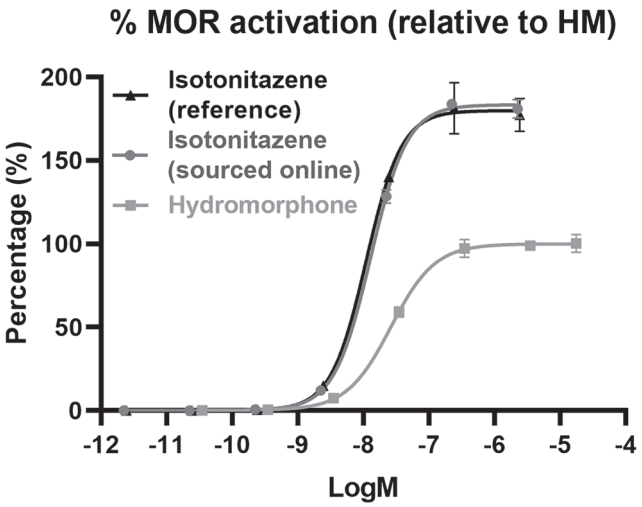

FIGURE 6 Concentration-dependent interaction of MOR with βarr2 upon stimulation with hydromorphone (HM), isotonitazene (reference material), and isotonitazene (sourced online). Data are given as mean receptor activation ± SD (n = 3), normalized to the E_{max} of HM (= 100%)

opioid fentanyl in this bioassay (EC_{50} = 18.7 nM (95%CI: 15.1–23.3); E_{max} = 155% (95%CI: 149–161), unpublished results). It is also relevant to note that the concentration–response curve of the powder that was sourced online essentially overlapped with that of the reference material, once more indicating the high purity of the material. Chemically, the nitro-group at the 5-position of the benzimidazole ring appears to be optimal for high analgesic activity. For etonitazene, the removal of this nitro group (des-nitro analog) or repositioning of this nitro group to the 6-position was reported to result in a reduction of antinociceptive activity, although the resulting compounds were respectively still 70- and 20-fold more potent than morphine in vivo.[35] Since multiple substances in the benzimidazole class trump morphine's potency by at least an order of magnitude, the number of future possible benzimidazole opioids cannot be underestimated.

3.5 | A future for the benzimidazole opioids?

Although this is the first report of isotonitazene being sold via an online vendor, it should be noted that this compound, as well as other related compounds, are not "new kids on the block". The first synthesis was already reported over 60 years ago and already in 1975, Alexander Shulgin, well known for his work on stimulants and hallucinogens, referred to the benzimidazole opioids as "a fertile field for the search for heroin substitutes that can be domestically synthesized and are potent at levels that would encourage illicit investigation".[36] Two

TABLE 1 The EC_{50} and E_{max} values are presented as a measure of potency and efficacy, respectively. Data are given as EC_{50}/E_{max} values (95% CI profile likelihood)

Substrance	EC_{5D}(nM)	E_{max}(%)
Hydromorphone (HM)	26.3 (22.0-30.7)	100 (97.3-103
Isotonitazene (reference material)	11.1 (9.10-13.6)	180 (174-186)
Isotonitazene (sourced online)	12.9 (11.7-14.3)	183 (180-187)

members, etonitazene and clonitazene (Figure 2), have since long been listed as schedule I drugs in the US Controlled Substances Act and there are historic reports about (mis)use of etonitazene in Russia, Germany and the USA.[37,38] Noteworthy in the context of the powder reported here is that it was being sold as the even more potent etonitazene. Hence, this is yet another example demonstrating the well-known problematic issue of a mismatch between adverted identity and true identity. Although in this case, isotonitazene is reportedly less potent than etonitazene, the identity, potency or purity of potential future preparations, containing (previously reported or entirely new) benzimidazole derivatives, can only be guessed.

4 | CONCLUSION

The isotonitazene in this study was obtained from an online NPS marketplace and its identity was confirmed using several analytical techniques. High-resolution mass spectrometry confirmed the expected molecular mass together with the expected observed mass fragmentation. The specific 5-nitrobenzimidazole regio-isomer was identified and confirmed using ^{1}H- and ^{13}C-NMR and FT-IR, and both the GC–MS and HPLC-DAD revealed no impurities. Pharmacological evaluation of isotonitazene using a MOR activation assay confirmed that this substance is a strong opioid. This, combined with the fact that our data indicate that this compound was being sold undiluted, represents an imminent danger to anyone aiming to use this powder.

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SUPPORTING INFORMATION

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

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