World Health Organization

Critical review report:

Protonitazene

Expert Committee on Drug Dependence

Forty-fifth Meeting

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This report contains the views of an international group of experts, and does not necessarily represent the decisions or the stated policy of the World Health Organization.

Contents

Executive summary

Protonitazene and several other "nitazenes" or benzimidazole-subclass opioids were first synthesized in the late 1950s by a Swiss chemical company as novel opioid alternatives to morphine *(1–3)*. Although they were found to have potent analgesic effects, clinical development of benzimidazole opioids was abandoned because of an increased risk of adverse events. Currently, no drugs in this class are approved for medicinal use.

In-vitro studies the efficacy of protonitazene at the μ-opioid receptor indicate that it is slightly greater than that of fentanyl (107–129%) and significantly greater than that of hydromorphone (174–365%). Protonitazene is pharmacodynamically a typical, robust μ-opioid receptor agonist. Robust antinociception with a potency many times greater than that of morphine has been observed in preclinical studies. Preclinical studies also indicate significant morphine-like affective properties, which suggest similar abuse potential. The data suggest that the pharmacodynamics of protonitazene can be reversed by an opioid antagonist, indicating that many of its primary pharmacodynamics are mediated through the μ-opioid receptor, although activity at the other opioid receptor subtypes has been reported. No clinical studies were found of the abuse potential of protonitazene; however, given its chemical properties and its availability as a water-soluble powder, protonitazene could be used recreationally by intranasal and intravenous administration, and, given its opioid-like pharmacodynamics, protonitazene has significant abuse potential in humans.

Protonitazene was detected during forensic sampling in Canada and the USA in 2020 and more recently in Australia. Protonitazene is currently under the most restrictive scheduling in the USA but does not appear to be subject to restrictive measures in the Member States of the European Union. Protonitazene has no approved therapeutic applications and has never been granted marketing authorization as a medicinal product for human or veterinary use. Protonitazene can be purchased legitimately from pharmaceutical retailers for research but not for human or veterinary use. Various non-pharmaceutical Internet retailers openly advertise protonitazene for sale internationally.

1. Substance identification

A. International nonproprietary name

Not available.

B. Chemical Abstracts Service registry number

95958-84-2 (free base)
119276-01-6 (hydrochloride salt)

C. Other chemical names

Free base:

Benzimidazole, 1-[2-(diethylamino)ethyl]-5-nitro-2-(*p*-propoxybenzyl)- (6CI, 7CI)
N,*N*-Diethyl-5-nitro-2-[(4-propoxyphenyl)methyl]-1*H*-benzimidazole-1-ethanamine (ACI)
Protonitazene

Hydrochloride salt:

Benzimidazole, 1-(2-diethylaminoethyl)-5-nitro-2-p-propoxybenzyl-, hydrochloride (6CI)

D. Trade names

Protonitazene is sold as hydrochloride salt under its own name, protonitazene (hydrochloride) *(4)*

E. Street names

Protonitazene is known under its own name or as pronitazene or propoxynitazene (e.g., *5*).

F. Physical appearance

Synthetic protonitazene hydrochloride is sold as a standard is a white powder *(6)* or as a crystalline solid *(4)*.

It is described as a "crystallin solid" (no colour specified) by a chemical supply company that sells protonitazene hydrochloride "for research use only, not for human or veterinary use" *(4)*.

A chemical manufacturer based in China *(7)* describes the protonitazene HCl it sells as a yellow or "brown/yellow" powder. The website provides little other information (e.g., no safety data sheet or drug information sheet), which may put into question its legitimacy. Another company based in China *(8)* also offers direct sale of protonitazene HCl, as both a white and a brown powder.

In a health alert issued by the Victoria State Department of Health in June 2022, a "yellow powder" protonitazene was reported as being sold as ketamine in Melbourne *(9)*.

Generally, benzimidazole opioids such as protonitazene lack the bitter taste of other opioid subclasses *(10)*.

G. WHO review history

Protonitazene has not been formally reviewed by WHO and is not currently under international control. Several detections of this drug in the USA in 2021 *(11)* brought this drug to the attention of WHO. As it has no recognized therapeutic use, these detections suggest that protonitazene is manufactured illicitly and poses a risk to public health.

2. Chemistry

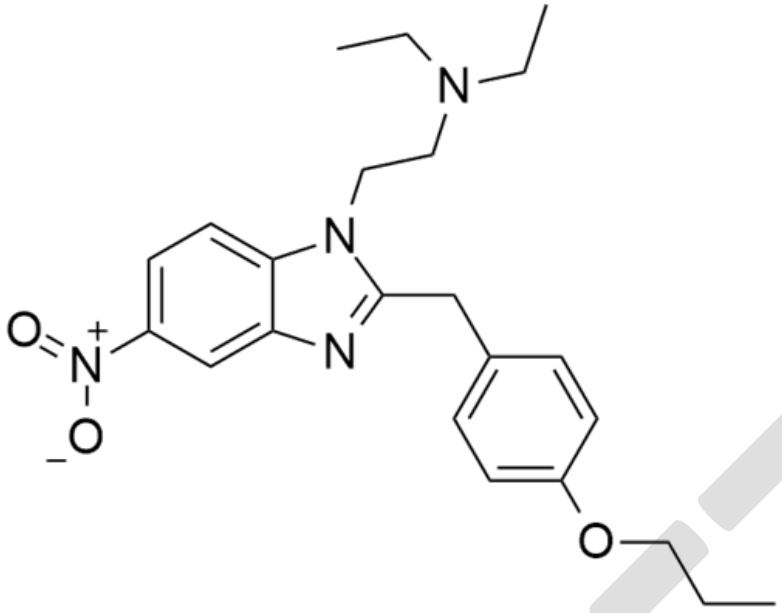
A. Chemical name

IUPAC name: *N*,*N*-Diethyl-5-nitro-2-[(4-propoxyphenyl)methyl]-1*H*-benzimidazole-1-ethanamine

Chemical Abstracts Service index name: 1*H*-Benzimidazole-1-ethanamine, *N*,*N*-diethyl-5-nitro-2-[(4-propoxyphenyl)methyl]- (ACI)

B. Chemical structure

Free base:



Molecular formula: $C_{23}H_{30}N_4O_3$

Molecular weight: 410.51 g/mol

C. Stereoisomers

No information was found.

D. Methods and ease of illicit manufacture

Protonitazene is a 5-nitro-2-benzylbenzimidazole belonging to the series of 2-benzylbenzimidazole compounds developed in the late 1950s as opioid analgesics *(10)*. It is a metonitazene and etonitazene homologue in which the C4 position of the benzyl moiety is substituted by a methoxy and an ethoxy group, respectively. Protonitazene is an isomer of isotonitazene, as they can be distinguished by the substitution at C4 position of the benzyl moiety with an *n*-propoxy and an isopropoxy group, respectively.

Synthesis of protonitazene was reported by Hunger et al. *(2)* and more recently by Vandeputte et al. *(12)*. The activated chloro atom of 1-chloro-2,4-dinitrobenzene can easily be substituted by 2-diethylaminoethylamine. Then, a regioselective reduction of the nitro group in the ortho position to the resulting amino function and condensation of the ortho-phenylenediamine species with an *n*-propoxyphenyl imidate (obtained from *n*-propoxyphenylacetonitrile derivative) affords the 5-nitro-substituted product protonitazene.

Protonitazene can also be obtained through synthetic routes reported for the synthesis of its 5-nitro-2-benzylbenzimidazole homologues and isomers (metonitazene, etonitazene and isonitazene) *(12–16)*.

Although no information was found on the actual method and scale of manufacture of protonitazene, the synthetic methods are simple and cost-efficient and do not require regulated precursors *(10)*.

E. Chemical properties

Melting-point

115–116 °C (hydrochloride salt) *(2)*

Boiling-point

No information was found.

Solubility

Protonitazene hydrochloride salt is soluble in dimethylformamide at 25 mg/mL and in dimethyl sulfoxide at 20 mg/mL. It was soluble at 0.5 mg/mL in a 1:1 mixture of dimethylformamide and phosphate-buffered saline (pH 7.2) and at 10 mg/mL in ethanol *(4)*. No definitive data on the solubility of protonitazene free base or its hydrochloride salt were found.

F. Identification and analysis

Synthetic protonitazene was characterized by nuclear magnetic resonance spectroscopy, high-performance liquid chromatography (LC) coupled to diode-array detection, gas chromatography coupled to mass spectrometry and LC coupled to high-resolution mass spectrometry (MS) *(10)*.

Protonitazene hydrochloride is available as a reference material from commercial suppliers for routine analysis in forensic and clinical investigations *(4)*. A method with LC coupled to tandem MS has been published for identification and quantification of protonitazene in biological sample matrices, such as human blood and urine *(17)*.

Analysis of protonitazene and of its isopropoxy isomer isotonitazene is critical, as they have the same molecular weight and similar MS fragmentation patterns. Distinction between the two isomers requires chromatography with analytical reference standards *(18)*.

3. Ease of conversion into controlled substances

No information was found.

4. General pharmacology

A. Routes of administration and dosage

In seizures, protonitazene has been found in tablet form, presumably for oral use *(19)*.

On an online discussion forum *(20)*, it was reported that protonitazene, although readily soluble in water, could not be vaped through a methamphetamine pipe, as it would "pop and explode around", and a weak effect was observed. Online forums included descriptions of "nodding" and euphoric effects after insufflation of the powder and "skin popping" (i.e., subcutaneous [s.c.] injection). Another user reported that "Inhaling [protonitazene] felt like suffocating on sand and sawdust" *(21)*. In another post, non-tolerant users were advised to try 1 mg of protonitazene per 10 mL of water or ethanol *(22)*.

Low heat resistance is consistent with a hydrochloride formulation that cannot be smoked or vaped (as noted by users). A water- or ethanol-soluble powder would, however, lend itself to intranasal, intravenous, intramuscular or s.c. use, which are routes of opioid administration with significant abuse potential *(23–25)*. Although water-solubility has been reported, empirical data suggest low solubility in water (see section 2). Benzimidazole opioids can be synthesized in either salt or base forms *(2, 3, 13, 26)*. Thus, a free-base formulation of protonitazene can be formulated that can be smoked or vaped, although no evidence was found of the existence of a base formulation.

B. Pharmacokinetics

No information was found *(10, 17)*.

C. Pharmacodynamics

Opioid receptor activity

Investigations of the pharmacodynamics of protonitazene have mainly addressed its affinity for the μ-opioid receptor; however, activity has also been characterized at the other opioid receptor subtypes. Table 1 shows the affinity and efficacy of protonitazene for the μ-, δ- and κ-opioid receptor subtypes (MOR, DOR, KOR, respectively) in comparison with the prototypical opiate, morphine, and the potent synthetic opioid, fentanyl *(27)*.

Table 1. Opioid affinity and efficacy of receptor subtypes

Opoiod	MOR Ki (nM)	DOR Ki (nM)	KOR Ki (nM)	EC50 (nM)	Efficacy (% DAMGO)
Protonitazene	21.5	1796	579	0.14	109
Fentanyl	4.8	356	204	0.10	98
Morphine	2.9	294	74	1.21	99

Source: reference *27*
DAMGO, D-Ala2, *N*-MePhe4, Gly5-ol

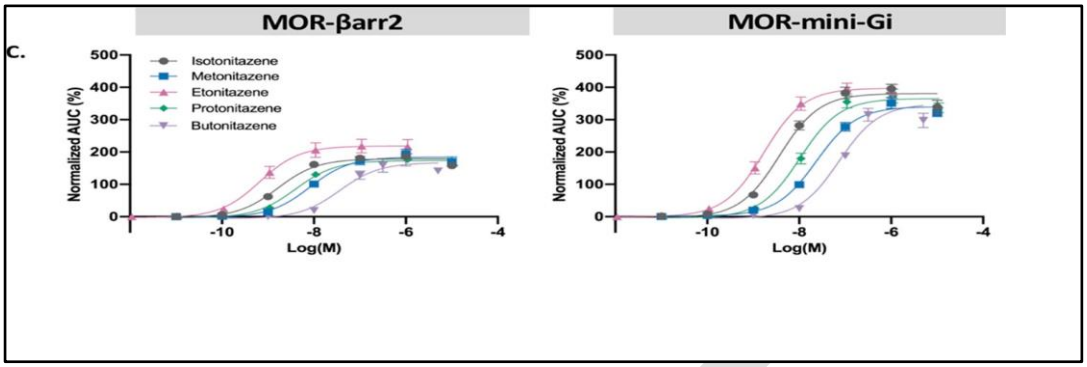
Volz and Moosmann *(28)* characterized the binding affinity (K_i) of six benzimidazoles and 17 non-benzimidazole opiates or opioids at human MOR (n = 3) in a competitive binding assay against 2 nM of [D-Ala2, N-MePhe4, Gly5-ol]-enkephalin, DAMGO). Table 2 shows that the binding affinity of protonitazene is weaker than those of fentanyl, morphine and hydromorphone (a potent opioid analgesic with robust abuse potential) *(29)*.

Table 2. Binding affinity (K_i) of opiates at the human μ-opioid receptor

Opiate	K_i ± SEM (nM)
Protonitazene	1.09 ± 0.17
Fentanyl	2.17 ± 0.27
Morphine	3.04 ± 0.28
Hydromorphone	0.448 ± 0.048

Vandeputte and colleagues *(12)* characterized the μ-opioid receptor activation profiles of five benzimidazole opioids in in-vitro recruitment assays (MOR-βarr2 and MOR-mini-Gi). Fig. 1 shows the mean receptor activation (± standard error), normalized to the maximum response of hydromorphone.

Fig. 1. Mean receptor activation of MOR-βarr2 and MOR-mini-Gi normalized to that of hydromorphine



Reproduced from reference *12* with permission

As shown in Table 3, the investigators calculated the potency (EC_{50}) and efficacy (E_{max}) of protonitazene relative to those of fentanyl and hydromorphone. In both assays, protonitazene was highly active in MOR activation, with a potency and efficacy slightly greater than those of fentanyl (107, 129%) and significantly greater than those of hydromorphone (174, 365%).

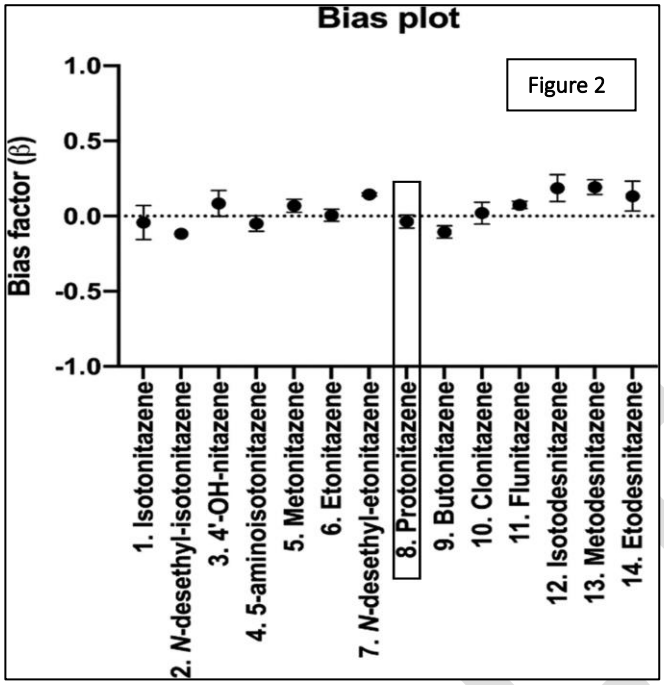
Table 3. Potency (with 95% confidence intervals) of protonitazene in comparison with those of fentanyl and hydromorphone

	MOR-βarr2			MOR-mini-Gi		
	EC_{50}	% Fentanyl (E_{max})	% HM (E_{max})	EC_{50}	% Fentanyl (E_{max})	% HM (E_{max})
Protonitazene	3.95 nM (2.78 ; 5.60)	107 (102 ; 111)	174 (165 ; 182)	10.4 nM (7.79 ; 14.7)	129 (123 ; 136)	365 (347 ; 384)

From reference *12*

The authors (*12)* found no evidence of significantly biased agonism (i.e., a preference for βarr2 or mini-Gi recruitment) in the effects of protonitazene at the μ-opioid receptor, in contrast to hydromorphone (Fig. 2).

Fig. 2. Mu-opioid receptor bias plot for protonitazene and other 2-Benzylbenzimidazole opioids.



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Analgesia and antinociception

In mice, the relative potency of the antinociceptive activity of protonitazene was estimated to be 200 times that of 5 mg/kg morphine (s.c.) (*1–3*; see reference *10* for a review). In a recent preclinical investigation *(30)*, the analgesic effects of s.c. protonitazene (0.001–0.1 mg/kg) were tested in the rodent tail-withdrawal test with fentanyl (0.0032–0.1 mg/kg) and morphine (1–32 mg/kg). All three opioids dose-dependently increased tail withdrawal latency (i.e., induced antinociception). The calculated ED_{50} values were 0.035 mg/kg for protonitazene, 0.035 mg/kg for fentanyl and 4.9 mg/kg for morphine. These data suggest that the antinociceptive potency of protonitazene is equivalent to that of fentanyl and more than 130 times greater than that of morphine. In an antagonism study, the opioid receptor antagonist naltrexone (0.1 mg/kg) caused a 12-fold rightward shift in the effects of protonitazene and a 7-fold shift for fentanyl.

5. Toxicology

Paronis *(30)* administered protonitazene (0.001–0.1 mg/kg) to rats and found that it produced notable adverse motor effects in several animals, including righting reflex, twitching and other involuntary motor movements. The investigator also reported that seven or eight rats died after antagonism with naltrexone. In another study, Paronis *(31)* (described in detail below) administered protonitazene at 0.0032–0.32 mg/kg s.c. to rats and observed no unusual behaviour.

6. Adverse reactions in humans

The Health Canada Drug Analysis Service has reported detection of protonitazene, but no information was provided on whether the samples were found in cases of fatal drug poisoning *(19, 32)*.

Protonitazene was analytically confirmed in nine fatal poisonings or deaths in the USA *(33–35)*. The average blood concentration was 286 (± 556) ng/mL.

Although data on humans are limited, a review of original research on benzimidazole opioids noted that, when administered intravenously, all the drugs caused respiratory depression, with a narrow therapeutic ratio between analgesia and respiratory depression *(36)*.

7. Dependence potential

A. Studies in experimental animals

No information was found.

B. Studies in humans

No information was found. Given its pharmacological profile, however, protonitazene is likely to induce physiological dependence, like other opioids *(12, 37)*.

8. Abuse potential

A. Studies in experimental animals

Drug discrimination

Paronis *(31)* tested the ability of protonitazene (0.0032–0.32 mg/kg s.c.), morphine (0.1–3.2 mg/kg s.c.) and fentanyl (0.001–0.032 mg/kg s.c.) to substitute for morphine in a drug discrimination behavioural paradigm. Like morphine and fentanyl, protonitazene fully substituted for the discriminative stimulus effects of morphine, indicating traditional opioid-like affective properties. The ED_{50} values were 0.008 mg/kg for protonitazene, 0.004 mg/kg for fentanyl and 0.8 mg/kg for morphine.

B. Studies in humans

No information was found. Protonitazene appears to be commonly available online as a hydrochloride salt powder and could thus be administered by routes with faster pharmacokinetics, associated with greater abuse potential, such as insufflation and injection. Anecdotal reports on user forums such as Reddit (see section 4A) support the hypothesis that protonitazene has a robust opioid-like effect, particularly when administered via these routes. Forensic examination of substances found in syringes obtained from a syringe exchange programme in Washington DC (USA) also indicated that protonitazene may be injected *(38)*.

9. Therapeutic applications and extent of therapeutic use and epidemiology of medical use

Protonitazene has no approved therapeutic application.

10. Listing on the WHO Model Lists of Essential Medicines

Protonitazene is not listed on the 22nd WHO Model List of Essential Medicines or the 8th WHO list of Essential Medicines for Children.

11. Marketing authorizations (as a medicinal product)

Protonitazene has no approved therapeutic applications and has never been granted marketing authorization as a medicinal product for human or veterinary use.

12. Industrial use

Protonitazene has no reported industrial uses.

13. Non-medical use, abuse and dependence

No information was found from population surveys; however, data from post-mortem reports suggest that, because of its potency, protonitazene may be used to increase the potency of heroin (similarly to fentanyl) *(37)*. Internet forums for people who use drugs suggest interest in protonitazene among people who are experienced with opioid use (queries about, e.g., its potency and subjective pharmacodynamic profile).

Protonitazene has been detected in drug seizures and toxicology samples in Australia, Canada and the USA, but it does not appear to be present in Europe *(34, 39)*, although this may be due to lack of testing. Furthermore, some data suggest that commonly used analytical methods cannot distinguish protonitazene from its isomer isotonitazene *(17, 28, 34)*.

The frequency of protonitazene use could not be estimated from the available data; however, an investigation of online surveillance of novel psychoactive substances as a predictor of their use found no mention of protonitazene *(40)*. Similarly, a recent systematic review on acute intoxications and fatalities associated with benzimidazole opioids did not mention reports related to protonitazene *(35)*.

Fewer detections of protonitazene were made than for other synthetic opioids such as fentanyl and other benzimidazole opioids such as isotonitazene and etonitazene. Data for 2020–2022, however, indicate an increasing presence on the illicit opioid market *(34, 35)*. In Canada, there were no detections in 2019, one in 2020, 63 in 2021 and 64 in 2022 *(19).*

14. Nature and magnitude of public health problems related to misuse, abuse and dependence

Protonitazene is offered for sale by numerous Internet retailers. As people who use drugs are likely to obtain protonitazene from unregulated sources, its purity and quantity are not assured, posing an additional risk of adverse reactions. Currently, protonitazene has a small impact on public health, as its presence on the drug market is minimal; however, given its pharmacodynamics, protonitazene has a high risk for recreational use, physiological dependence, adverse side-effects and overdose *(10).*

15. Licit production, consumption and international trade

Protonitazene is available for sale from pharmaceutical retailers for research and forensic applications only.

16. Illicit manufacture and traffic and related information

Protonitazene is offered for sale on numerous Internet sites that do not appear to be reputable pharmaceutical retailers. Some are reported to be based in China and openly advertise sale of protonitazene to other countries.

17. Current international controls and their impact

Protonitazene is not currently under international control.

18. Current and past national controls

Protonitazene does not appear to be subject to restrictive measures in the Member States of the European Union *(18)*. In the USA, protonitazene has been placed under the most restrictive controls (i.e., Schedule 1) *(41)*.

19. Other medical and scientific matters relevant for a recommendation on scheduling of the substance

No other matters were identified.

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