The Newest Wave of Nitazene Analogues

What’s Trending: NPS Discovery Webinar Series – Friday July 7, 2023

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Disclosures

▪ I have no conflicts of interest to disclose.

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History
Types of Novel Synthetic Opioids

- **Fentanyl Analogues**
- **Benzimidazolones** (Brorphine)
- **Cyclohexylbenzamides** (AP-Series)
- **Cinnamylpiperazines** (U-Series)
- **2-Benzylbenzimidazoles** (Nitazene Analogues)
- **Benzimidazolones** (Brorphine)
2-Benzylbenzimidazoles

- Synthesized in 1957
  - Hunger, Rossi, and Hoffman (Switzerland)
  - Intended for opioid analgesic use

- Increasing polydrug use
  - Benzo-dope: causes increased effects on breathing and sedation

- Produce analgesia, euphoria, and sedation by activating the µ-opioid receptor (MOR)
  - Easily pass through the blood-brain barrier

- Adverse effects:
  - Respiratory depression*
  - GI effects (nausea, vomiting), reduced blood pressure and heart rate
  - Dependence/tolerance from repeated use
“Nitazene” Core Structure

- Benzimidazole core
- R1:
  - Nitro group
  - No modification (desnitazenes)
- R2:
  - Benzyl side chain
- R3:
  - N,N-Diethylamine
  - N-Pyrrolidino ring
  - N-Piperidinyl ring
Butonitazene | Etodesnitazene | Flunitazene | Isotonitazene | Metodesnitazene
---|---|---|---|---
Metonitazene | N-Desethyl Isotonitazene | N-Piperidinyl Etonitazene | N-Pyrrolidino Etonitazene | N-Pyrrolidino Metonitazene

Protonitazene | N-Pyrrolidino Protonitazene

Nitazene Analogues Reported by NPS Discovery
General Concentration Ranges

- AP-Series
- U-Series
- Fentanyl Analogues*
- Brorphine
- Nitazene Analogues

Increased Potency → Increased Conc
### Published Potency Data – Vandeputte et al. (2020)

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>E&lt;sub&gt;max&lt;/sub&gt; (% of fentanyl)</th>
<th>E&lt;sub&gt;max&lt;/sub&gt; (% of HM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>isotonitazene</td>
<td>1.63 (1.17–2.28)</td>
<td>110 (105–115)</td>
<td>179 (171–187)</td>
</tr>
<tr>
<td>N-desethylisotonitazene</td>
<td>0.614 (0.377–0.985)</td>
<td>140 (131–149)</td>
<td>229 (214–243)</td>
</tr>
<tr>
<td>4’-OH-nitazene</td>
<td>176 (124–250)</td>
<td>81.9 (76.4–87.5)</td>
<td>133 (125–143)</td>
</tr>
<tr>
<td>S-aminoisotonitazene</td>
<td>383 (263–554)</td>
<td>115 (108–123)</td>
<td>188 (176–201)</td>
</tr>
<tr>
<td>metonitazene</td>
<td>8.14 (5.12–12.8)</td>
<td>113 (106–121)</td>
<td>184 (172–197)</td>
</tr>
<tr>
<td>etonitazene</td>
<td>0.661 (0.338–1.26)</td>
<td>134 (122–146)</td>
<td>219 (199–238)</td>
</tr>
<tr>
<td>N-desethyltonitazene</td>
<td>1.81 (1.14–2.94)</td>
<td>101 (94.7–107)</td>
<td>164 (154–175)</td>
</tr>
<tr>
<td>protonitazene</td>
<td>3.95 (2.78–5.60)</td>
<td>107 (102–111)</td>
<td>174 (165–182)</td>
</tr>
<tr>
<td>butonitazene</td>
<td>36.2 (20.2–63.9)</td>
<td>103 (92.8–113)</td>
<td>167 (151–184)</td>
</tr>
<tr>
<td>clonitazene</td>
<td>140 (93.6–210)</td>
<td>106 (98.0–114)</td>
<td>173 (160–187)</td>
</tr>
<tr>
<td>flunitazene</td>
<td>377 (295–481)</td>
<td>118 (113–124)</td>
<td>192 (183–202)</td>
</tr>
<tr>
<td>isotodesnitazene</td>
<td>34.8 (22.1–54.4)</td>
<td>94.9 (88.1–102)</td>
<td>155 (144–166)</td>
</tr>
<tr>
<td>metodesnitazene</td>
<td>548 (365–811)</td>
<td>91.2 (85.1–97.5)</td>
<td>149 (139–159)</td>
</tr>
<tr>
<td>etodesnitazene</td>
<td>54.9 (36.1–82.0)</td>
<td>96.8 (90.2–103)</td>
<td>158 (147–169)</td>
</tr>
<tr>
<td>morphine</td>
<td>338 (239–478)</td>
<td>71.9 (68.3–75.4)</td>
<td>117 (111–123)</td>
</tr>
<tr>
<td>fentanyl</td>
<td>14.4 (11.5–18.0)</td>
<td>100 (96.5–103)</td>
<td>163 (157–169)</td>
</tr>
<tr>
<td>hydromorphone</td>
<td>36.2 (27.9–47.0)</td>
<td>61.3 (58.9–63.8)</td>
<td>100 (95.9–104)</td>
</tr>
</tbody>
</table>
Waves of Nitazene Analogues
CFSRE First Identifications

2019
Isotonitazene

2020
Metonitazene

2021
Butonitazene
Etodesnitazene
Metodesnitazene
Flunitazene
N-Pyrrolidino
Etonitazene
Protonitazene
N-Piperidinyl
Etonitazene

2022
N-Desethyl Isotonitazene

2023
N-Pyrrolidino Metonitazene
N-Pyrrolidino Protonitazene
Nitazene Analogue Trends: 2021 – 2023

Data to Q1 2023

Isotonitazene
Metonitazene
N-Pyrrolidino Etonitazene
Protonitazene
Etodesnitazene
N-Desethyl Isotonitazene
N-Pyrrolidino Protonitazene
N-Pyrrolidino Metonitazene
Nitazene Analogues Prevalent vs Infrequent

- **Prevalent**
  - Metonitazene: 74
  - Isotonitazene: 63
  - N-Pyrrolidino Etonitazene: 27
  - Protonitazene: 21
  - N-Desethyl Isotonitazene: 18
  - Etodesnitazene: 13

- **Infrequent**
  - Flunitazene: 1
  - Butonitazene: 2
  - N-Piperidinyl Etonitazene: 2
  - Metodesnitazene: 1

**Up-and-coming**

- N-Pyrrolidino Protonitazene: 7
- N-Pyrrolidino Metonitazene: 3
Nitazene Analogues Legal Actions in the U.S.

- Etonitazene & clonitazene placed under **Schedule I**
  - 1961

- Isotonitazene placed under **Schedule I**
  - August 2020

- Butonitazene, etodesnitazene, flunitazene, metodesnitazene, metonitazene, *N*-pyrrolidino etonitazene, protonitazene placed under **Schedule I**
  - December 2021
Newest Additions to the Drug Market
“Pyrrolidino” or “Ring” Nitazenes

N-Pyrrolidino Metonitazene

N-Pyrrolidino Protonitazene
“Pyrrolidino” or “Ring” Nitazenes

\[
\text{N-Pyrrolidino Metonitazene} \quad \text{N-Pyrrolidino Protonitazene}
\]
Methods
CFSRE Postmortem Workflow

Case Submitted or Inquiry Made

Screening by LC-QTOF-MS

In Scope

Out of Scope

Few Cases?

Many Cases?

Develop Standard Addition Method

Develop Fully Validated Method

Attempt to Identify

Purchase (or request synthesis) of Standard

Add to Scope & Confirm Standard is Unknown
Standard Addition Method

Sample Preparation

- **Standard Addition**
  - Four total aliquots
  - Up-spikes at 0.2, 2, 20 ng/mL
- **Internal Standard**
  - Isotonitazene-D7
- **Basic Liquid-Liquid Extraction**
  - 0.1 M Borax Buffer, pH 10.4
  - 70:30 N-butyl chloride:ethyl acetate

Instrumental Analysis

- **Waters Xevo TQ-S Micro LC-QQQ-MS**
- **Mobile phase compositions:**
  - 0.1% Formic Acid in Water
  - 0.1% Formic Acid in Methanol
- **Analytical Column**
  - Agilent InfinityLab Poroshell 120 EC-C18 3.0 x 100mm, 2.7 µm

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow (mL/min)</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.4</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>3.5</td>
<td>0.4</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>4.0</td>
<td>0.4</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>4.5</td>
<td>0.4</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>4.6</td>
<td>0.4</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>5.0</td>
<td>0.4</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>
Case Study & Concentrations
Postmortem Case

- 43 y/o male
- West Virginia
- Case history: history of substance abuse
  - Received femoral blood
- Polydrug use:
  - Synthetic opioids
  - Designer benzodiazepines
  - Stimulants
- Found without other nitazene analogues, fentanyl, or any other opioids

**Results:**

- \(N\text{-Pyrrolidino protonitazene}: 1.1 \text{ ng/mL}\)
- Other results:
  - Bromazolam: 36 ng/mL
  - Desalkylflurazepam: 11 ng/mL
  - Methamphetamine: 480 ng/mL
  - Amphetamine: 59 ng/mL
### Comparing Concentrations in Death Cases

*Order similar to reported in vitro potency*

<table>
<thead>
<tr>
<th>Drug</th>
<th>N</th>
<th>Mean (±SD) (ng/mL)</th>
<th>Median (ng/mL)</th>
<th>Range (ng/mL)</th>
<th>Potency Compared to Fentanyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Pyrrolidino Etonitazene*</td>
<td>15</td>
<td>3.9 ± 5.9</td>
<td>2.4</td>
<td>0.3 - 25</td>
<td>43x more</td>
</tr>
<tr>
<td>N-Pyrrolidino Protonitazene*</td>
<td>9</td>
<td>0.90 ± 0.43</td>
<td>1.0</td>
<td>0.1-1.5</td>
<td>25x more</td>
</tr>
<tr>
<td>Isotonitazene*</td>
<td>69</td>
<td>1.59 ± 1.81</td>
<td>1.0</td>
<td>0.5 - 9</td>
<td>9x more</td>
</tr>
<tr>
<td>Protonitazene*</td>
<td>3</td>
<td>11 ± 9.9</td>
<td>5</td>
<td>3.1 – 25</td>
<td>4x more</td>
</tr>
<tr>
<td>Metonitazene</td>
<td>18</td>
<td>6.3 ± 7.5</td>
<td>3.8</td>
<td>0.5 - 33</td>
<td>2x more</td>
</tr>
<tr>
<td>N-Pyrrolidino Metonitazene</td>
<td>5</td>
<td>0.46 ± 0.14</td>
<td>0.49</td>
<td>0.25-0.63</td>
<td>2x more</td>
</tr>
<tr>
<td>Butonitazene</td>
<td>1</td>
<td>3.2</td>
<td>N/A</td>
<td>N/A</td>
<td>2x less</td>
</tr>
<tr>
<td>Etodesnitzazene</td>
<td>15</td>
<td>40 ± 61</td>
<td>5.2</td>
<td>0.53 - 230</td>
<td>4x less</td>
</tr>
</tbody>
</table>
### Qualitative Trends (*N*-Pyrro. Meto and *N*-Pyrro. Proto)

<table>
<thead>
<tr>
<th>Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl</td>
<td>89%</td>
</tr>
<tr>
<td>Quinine</td>
<td>89%</td>
</tr>
<tr>
<td>Other Nitazene Analogues</td>
<td>78%</td>
</tr>
<tr>
<td>Xylazine</td>
<td>67%</td>
</tr>
<tr>
<td>Other NPS</td>
<td>56%</td>
</tr>
<tr>
<td>Stimulants</td>
<td>56%</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>33%</td>
</tr>
</tbody>
</table>
Distribution of “Ring” Nitazenes in U.S.
Discussion & Conclusion
Nitazene analogues continue to proliferate in the United States
- Scheduled nitazene analogues still appear in casework

- New “pyrrolidino” nitazene analogues are identified in samples at sub-ng/mL concentrations
  - Sensitive instrumentation is needed

- “Pyrrolidino” nitazenes are most often found with
  - Other nitazene analogues
    - Example: N-pyrrolidino metonitazene with metonitazene
  - Designer benzodiazepines
  - Fentanyl

- Importance of up-to-date scope of testing
  - NPS Scope Recommendations (SOFT & CFSRE)
Acknowledgements

- Our many collaborations
  - Medical examiners & coroners
  - Toxicology laboratories
  - Hospitals
  - Police departments
  - Public health & safety

- CFSRE Staff

- NMS Labs
  - Donna Papsun
Thank you! Questions?

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