SOFT 2022
Abstract Submission Form

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*****Do not exceed 600 words including tables and charts.*****

TITLE: Pharmacology and Toxicology of *N*-Pyrrolidino Etonitazene – A New Nitazene Synthetic Opioid Increasingly Observed in Forensic Cases

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ABSTRACT:

Background/Introduction:

The rise of non-fentanyl synthetic opioids in forensic casework has increased in the recent years, with the fastest growing subclass being the 2-benzylbenzimidazoles (or the "nitazenes"). Isotonitazene was the first drug in this subclass to be identified in forensic samples in 2019; however, since the emergence of isotonitazene, eight additional nitazenes have been reported with little knowledge of their pharmacological activity and toxicity. The most recent proliferation of *N*-pyrrolidino etonitazene (etonitazepyne), an opioid much more potent than fentanyl, has been an increasing public health concern demonstrated by the need for continuous detection and pharmacological evaluation of this opioid subclass.

Objectives:

The objective of this study was to pair *in vitro* and *in vivo* methodologies to characterize the new synthetic opioid, *N*-pyrrolidino etonitazene. This was achieved by completing radioligand binding assays and activation potential assays, pharmacodynamic studies (i.e., catalepsy, body temperature), as well as developing a validated method to quantify by standard addition the concentrations of *N*-pyrrolidino etonitazene in authentic biological samples using liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS).

Methods:

For the *in vitro* and *in vivo* analyses, radioligand binding assays in rat brain tissue using three different radioligands ([^{3}H]DAMGO, [^{3}H]DADLE, [^{3}H]U69593) and NanoBiT® MOR-β-arrestin-2 cell-based recruitment assays to determine MOR activation potential were performed. Pharmacodynamic studies were performed using Male Sprague-Dawley rats, and procedures for catalepsy, body temperature, and hot plate

latency were carried out after administration of differing doses of *N*-pyrrolidino etonitazene (0.0003-0.010 mg/kg). For toxicological analysis of *N*-pyrrolidino etonitazene, initial drug screening was completed using a Sciex TripleTOF 5600+ liquid chromatograph time-of-flight mass spectrometer (LC-QTOF-MS). For confirmation, a method employing standard addition was developed and validated. A basic liquid-liquid extraction was used consisting of borax buffer (1 mL, 10 mM, pH 10.4) and extraction solvent (3 mL, 70:30 *N*-butyl chloride/ethyl acetate). Quantitative analysis was performed using a Waters Xevo TQ-S Micro LC-QQQ-MS. Chromatographic separation was achieved using an Agilent InfinityLab Poroshell C-18 (2.7 µm, 3.0 x 100 mm) column using gradient elution. The flow rate was 0.4 mL/min (mobile phase compositions: 0.1% formic acid in water and 0.1% formic acid in methanol). The injection volume was 5 µL and the column temperature was 30°C. For standard addition the peak area ratios were plotted against the up-spike values to determine the concentration of *N*-pyrrolidino etonitazene. This workflow has been applied to numerous authentic samples since its implementation.

Results:

N-Pyrrolidino etonitazene was determined to have a Ki of 4.09±0.63 nM at the MOR, showing greater affinity to MOR than fentanyl. The *in vitro* activation potential of *N*-pyrrolidino etonitazene was similar to etonitazene and the potency (EC_{50}: 0.348 nM) was much greater than both morphine and fentanyl; 800x and 40x, respectively. The analgesic effect of *N*-pyrrolidino etonitazene was ~10x greater than that of fentanyl, depicting significant effects on all pharmacodynamic studies completed. *N*-Pyrrolidino etonitazene was confirmed in 21 postmortem cases collected from January-October 2021. This opioid was found in combination with other opioids and stimulants, but also with a wide variety of novel benzodiazepines (suggesting potential benzo-dope drug materials). The range of concentration for *N*-pyrrolidino etonitazene in blood was 0.3-25 ng/mL (mean: 2.5±2.0 ng/mL, n=13), which is comparable with the low concentrations observed with other 2-benzylbenzimidazole analogues.

Conclusion/Discussion:

Using this paired approach of *in vivo* and *in vitro* characterization of *N*-pyrrolidino etonitazene, it was determined that this new synthetic opioid is an extremely potent MOR agonist with high MOR activation potential and produces significant analgesic effects. *N*-Pyrrolidino etonitazene was discovered in 21 postmortem cases and quantitated at low concentrations, showing the need for sensitive methodology. The continued proliferation of *N*-pyrrolidino etonitazene depicts the risks to public health and safety.