Synthesis, chemical characterization, and μ-opioid receptor activity assessment of the emerging group of "nitazene" 2-benzylbenzimidazole synthetic opioids

Marthe M. Vandeputte[1], Katleen Van Uytfanghe[1], Nathan K. Layle[2], Danielle M. St. Germaine[2], Donna M. Iula[2], Christophe P. Stove[1]

[1] Laboratory of Toxicology, Department of Bioanalysis, Ghent University, Belgium; [2] Forensic Chemistry Division, Cayman Chemical Company, Ann Arbor, Michigan, USA. The authors declare no conflict of interest.

Background

Several **2-benzylbenzimidazoles/"nitazenes"** recently emerged on the illicit drug market. The most frequently encountered member, **isotonitazene**, has been identified in multiple **fatalities** since its appearance in 2019. Although isotonitazene was recently put under international control, many other analogues remain **unregulated**. Apart from a series of research articles exploring their analgesic potential in the 1950-60s, little is known about the harm potential of these opioids. Here, **10 nitazenes and 4 metabolites** were **synthesized** and **characterized** to lay out an analytical framework for their **detection** as well as provide insights into their *in vitro* **μ-opioid receptor (MOR) activation potential.**

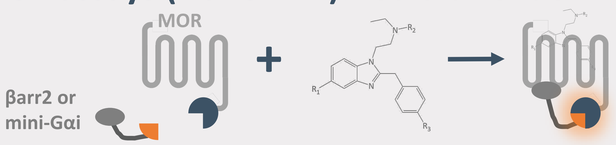
Methods

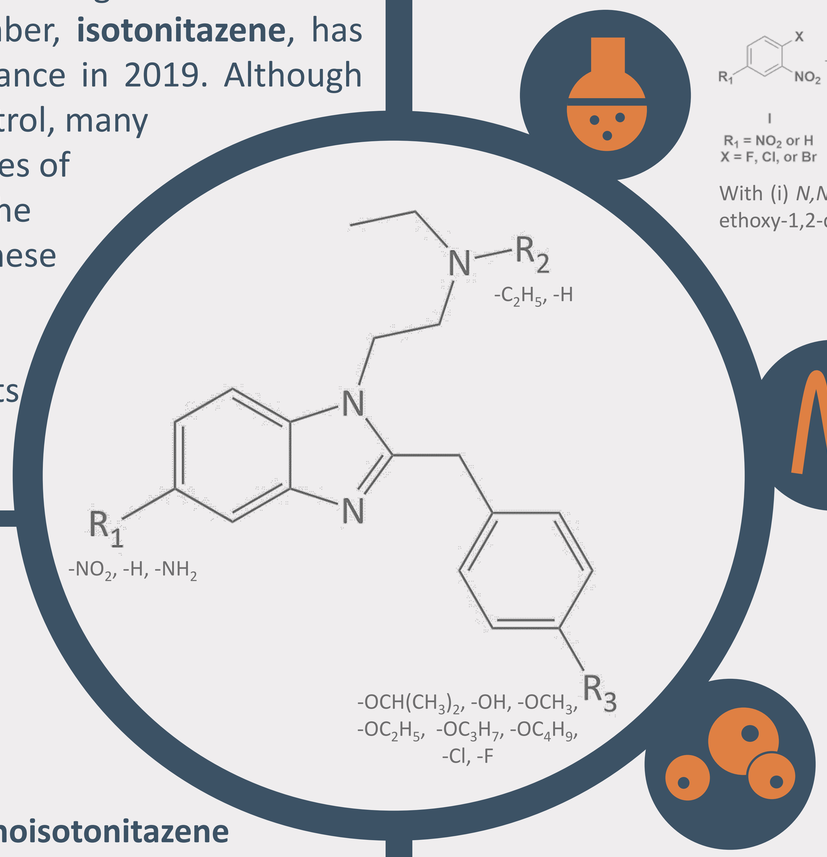
Generic synthesis based on published routes:

- **Isoto-, meto-, eto-, proto-, butonitazene**
- **Clo-, flunitazene**
- **Isoto-, meto-, etodesnitazene**
- ***N*-desethylisoto-/eto-, 4'-hydroxy-, 5-aminoisotonitazene**

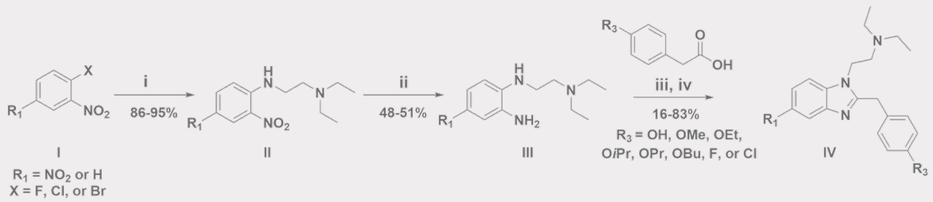
Analytical characterization via **^{1}H-NMR, HPLC-DAD, GC-MS, LC-QTOF-MS**.

2 cell-based **MOR activation assays (NanoBiT®)**: recruitment of β-arrestin 2 or mini-$G_{\alpha i}$

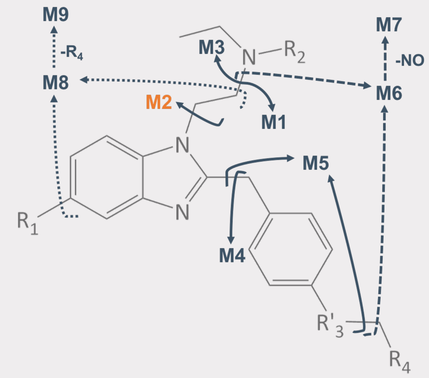




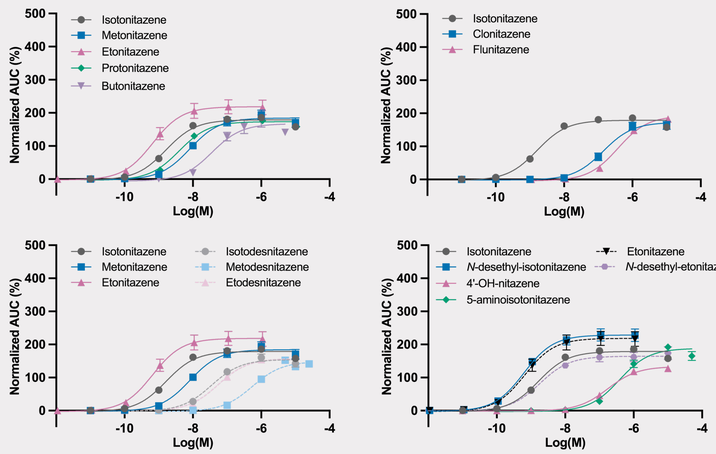
Results



With (i) *N,N*-diethylethylenediamine, EtOH, reflux; (ii) $(NH_4)_2S$, EtOH, reflux; (iii) *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, CH_2Cl_2; (iv) HCl or citric acid. Percentages indicate the yield ranges.



m/z 100.11 (M2) found for 12/14 compounds → Basis for MS-based nitazene screening



Conclusions

As the presence of **non-fentanyl synthetic opioids** on the illicit drug market continues to rise, it will become increasingly important to rapidly identify and characterize new compounds as they emerge. Nitazenes are among the newest to appear and, given their **high potential to activate MOR**, their use may pose an **imminent threat to any user**. Particularly relevant is the **unexpected very high potency of the *N*-desethyl metabolite**, rivalling the potency of etonitazene and exceeding that of isotonitazene itself. Supported by its identification in fatalities, this likely has *in vivo* consequences. The extensive characterization performed in this study may contribute to **increased awareness and detection** of this dangerous class of emerging synthetic opioids.