Structural Characterization of Nitazene Analogs Using Electron Ionization-Mass Spectrometry (EI-MS)

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ABSTRACT

Nitazene analogs are among the most recent and potent additions to the novel synthetic opioid (NSO) market, and new analogs continue to emerge. Seized drug analysis commonly utilizes gas chromatography-electron ionization-mass spectrometry (GC-EI-MS), so it is therefore imperative to understand how nitazene analogs behave under EI-MS conditions, and how substitution at various locations on the molecule may impact the resulting EI mass spectra. This study explores the EI fragmentation behavior of nitazene analogs with differing substitutions, and identifies rational mechanisms to explain this behavior, with the goal of identifying conserved fragmentation patterns and diagnostic fragment ions to help identify novel nitazene analogs.

INTRODUCTION

The presence of NSOs is growing worldwide and constitutes a major problem for public health as well as forensic practitioners[1]. Specifically, nitazene analogs have become prevalent on the illicit drug market since 2018, when the United States Drug Enforcement Administration (DEA) passed an act enabling blanket scheduling of fentanyl and its analogs[2]. There has since been a continual surge of novel nitazene analogs, likely due to their potency and initial lack of scheduling[3]. The continuous modification to the core nitazene structure poses a challenge for forensic scientists and creates the need for further analytical characterization to aid in the identification of novel nitazene analogs.

GC-EI-MS is a widely accepted technique used for analyzing seized drugs that can be used to gain detailed structural information about a compound through the formation of fragment ions. However, due to their novelty, there is a lack of fundamental understanding regarding the fragmentation behavior of nitazene analogs under EI-MS conditions. This knowledge has become a necessity due to the role of GC-EI-MS in the identification of novel compounds in seized drug casework.

This study aims to explore the effect of substitution at four regions of the core nitazene structure on the resulting EI mass spectra, with the goal of being able to identify conserved fragment ions as well as unique ions that can be used to differentiate similar analogs. Twenty nitazene analogs were analyzed, with varying functional groups at common locations of substitution **(Figure 1)**.

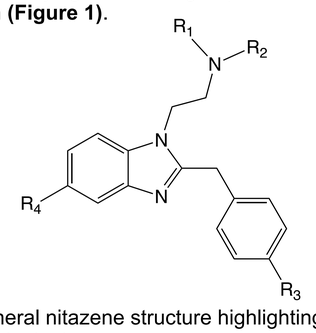

Figure 1. General nitazene structure highlighting common locations of substitution.

RESULTS & DISCUSSION

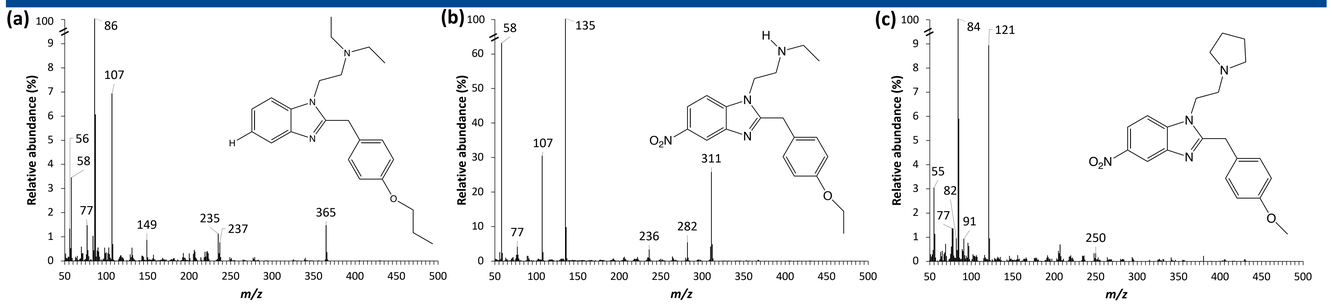

Figure 2. Average (n=12) EI mass spectra of a) protodesnitazene, b) N-desethyl etonitazene, and c) N-pyrrolidino metonitazene.

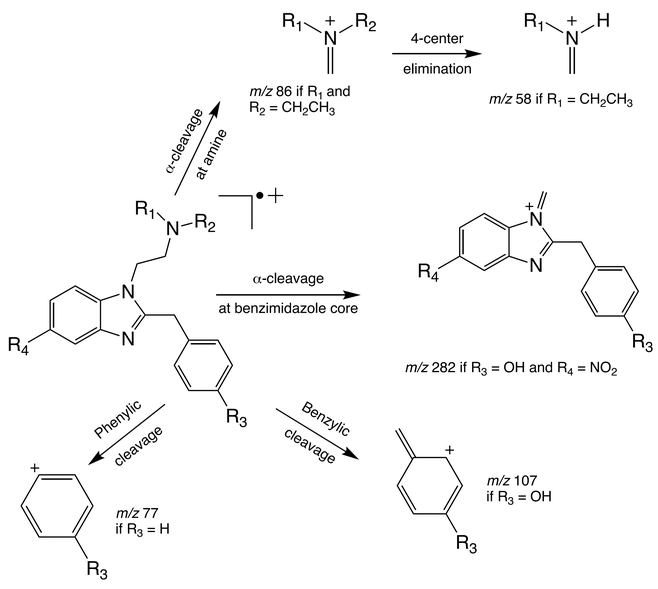

Figure 3. Proposed fragmentation pathways of nitazene analogs.

- The most common fragment ions for nitazene analogs are *m/z* 86, *m/z* 107, *m/z* 58, and *m/z* 77.
- Compounds with a substitution other than NO_2 at R_4 tend to show a molecular ion, which can be used for differentiation.
- N-desethyl compounds undergo a loss of 57 Da, which is a loss unique to that substitution.
- Ring compounds (N-pyrrolidino and N-piperidinyl) show *m/z* 55 as well as *m/z* 84 and *m/z* 96, respectively.

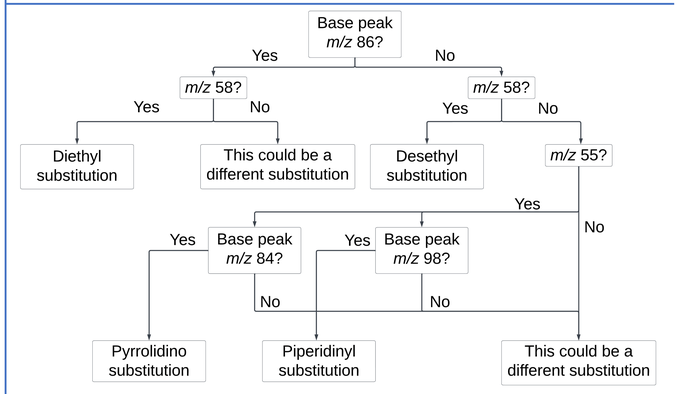

Figure 4. R_1/R_2 identification based on observed EI-MS fragment ions.

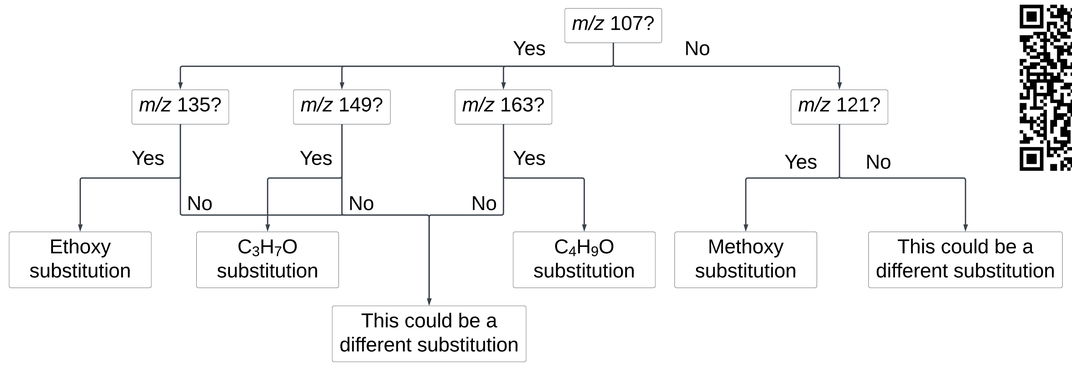

Figure 5. R_3 identification based on observed EI-MS fragment ions.

MATERIALS & METHODS

Sample Preparation

All nitazene analogs were prepared as 100 ppm solutions in methanol. The twenty compounds analyzed were 3'-methoxy metodesnitazene, 4'-hydroxy nitazene, 5-aminoisotonitazene, 5-methyl etodesnitazene, ethyleneoxynitazene, isobutonitazene, isotodesnitazene, menitazene, metodesnitazene, N-desethyl etonitazene, N-desethyl isotonitazene, N-piperidinyl 4'-hydroxy nitazene, N-piperidinyl etonitazene, N-piperidinyl protonitazene, N-pyrrolidino 4'-hydroxy nitazene, N-pyrrolidino isotonitazene, N-pyrrolidino metonitazene, nitazene, propylnitazene, and protodesnitazene.

Instrumentation and Data Analysis

An Agilent 8890 GC-5977B with a 30 m × 0.250 mm × 0.25 µm HP-5MS column was utilized for this study. 1 µL was injected into a 250 ºC inlet with a 10:1 split ratio. The initial oven temperature was 150 ºC (1 min), followed by a ramp of 20 ºC /min to 250 ºC (1 min), and then a ramp of 5 ºC /min to 300 ºC (15 min) with a 1.5 mL/min helium flow rate. The total run time was 32 min. The 70-eV ionization energy used to collect from *m/z* 50-500 began after a 2-minute solvent delay. Twelve replicate analyses were completed over the course of two months for each compound. Mass spectral data was exported from ChemStation to Microsoft Excel and plotted using the average of all twelve analyses.

CONCLUSIONS

- Unique EI-MS spectra were obtained for all twenty analogs.
- Substitution to the core nitazene structure affects the resulting EI mass spectra.
- Common patterns were observed that can be used to differentiate nitazene analogs.
- Specific fragment ions are diagnostic for functional groups or substitution location.
- The combination of common and unique fragment ions enables the identification of the specific substitution, assisting with novel nitazene analog identification.

REFERENCES

[1]World Drug Report 2022, UNODC, 2022.
[2]H.R.2617 - Consolidated Appropriations Act, 2023, 117th Congress.
[3]M.M. Vandeputte, K. Van Uytfanghe, N.K. Layle, D.M. St Germaine, D.M. Iula, C.P. Stove, Synthesis, Chemical Characterization, and mu-Opioid Receptor Activity Assessment of the Emerging Group of "Nitazene" 2-Benzylbenzimidazole Synthetic Opioids, *ACS Chem Neurosci* 12(7) (2021) 1241-1251.

ACKNOWLEDGEMENTS

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