Structural Characterization of Nitazene Analogs Using Electrospray Ionization-Tandem Mass Spectrometry (ESI-MS/MS)

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ABSTRACT

Nitazene analogs are a class of novel synthetic opioids (NSOs) that have become prevalent on the illicit drug market in recent years due to increasing legislation of fentanyl analogs in both the United States and China. Although several nitazene analogs have been categorized under Schedule I of the U.S. Controlled Substances Act, novel analogs continue to emerge to circumvent legislation. This study provides a structural characterization of 38 nitazene analogs using electrospray ionization-tandem mass spectrometry (ESI-MS/MS), with an emphasis on how different substitutions to the core nitazene structure affect the resulting product ion spectra.

INTRODUCTION

With the increased legislation surrounding fentanyl and its analogs in 2018, NSOs structurally unrelated to fentanyl have emerged on the illicit drug market [1]. Nitazene analogs are among the most recent and deadly compounds to appear and have been the dominant class of NSOs since 2018 [2,3]. In response, the United States has placed several nitazene analogs into Schedule I of the U.S. Controlled Substances Act; however, novel nitazene analogs continue to emerge. These novel analogs contain various substitutions to the core nitazene structure **(Figure 1)** making their identification in forensic laboratories challenging.

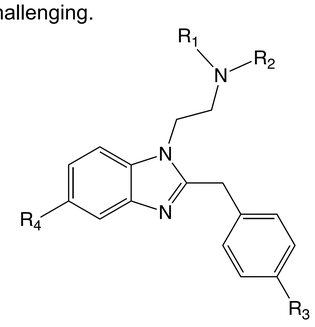

Figure 1. Core nitazene structure with four R group locations highlighting the areas of common substitution.

Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) instrumentation is routinely utilized in toxicology laboratories and is beginning to be applied to seized drug analysis for compounds not well suited to gas chromatography. Given the potency of nitazene analogs and their relatively low volatility, LC-ESI-MS/MS instrumentation is appropriate for the analysis of nitazene analogs. This study provides a structural characterization of 38 nitazene analogs using ESI-MS/MS to identify conserved fragmentation pathways and diagnostic product ions to assist with identifying novel nitazene analogs.

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RESULTS & DISCUSSION

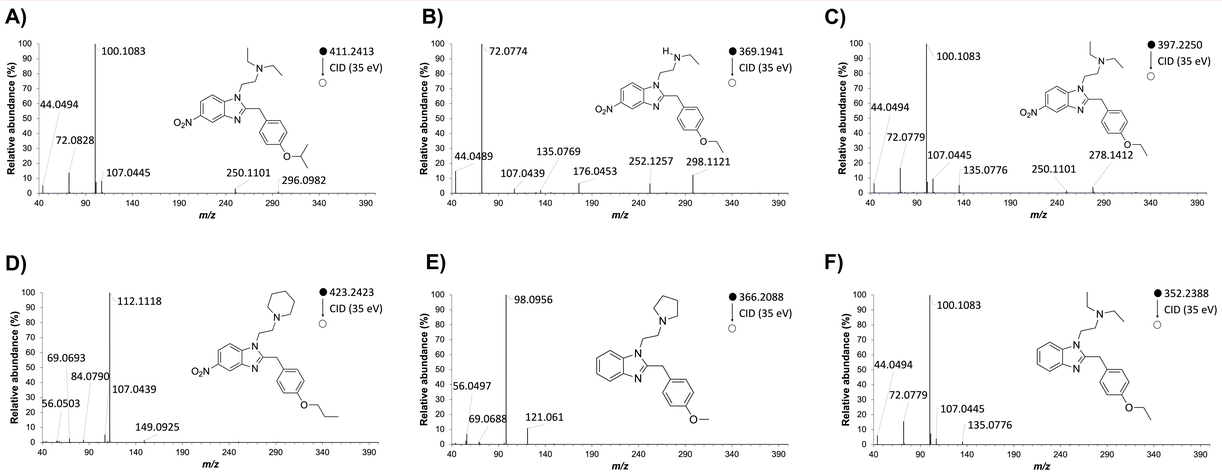

Figure 2. Product ion spectra collected at 35 eV for A) isotonitazene, B) N-desethyl etonitazene, C) etonitazene, D) N-piperidinyl protonitazene, E) N-pyrrolidino metodesnitazene, and F) etodesnitazene.

- The base peak of nitazene analog product ion spectra is diagnostic of the R_1/R_2 substitution.
- The desnitazene compounds (i.e., no substitution at R_4) typically have fragment-poor mass spectra compared to their nitro group-containing counterparts (Figure 2F vs. Figure 2C).

Table 1. Diagnostic ions by substitution groups.

Substitution	Diagnostic Ions
Diethyl (R_1/R_2)	*m/z* 100, 86, 72, 44
Desethyl (R_1/R_2)	No *m/z* 100
Piperidinyl (R_1/R_2)	*m/z* 112, 84, 69, 56
Pyrrolidine (R_1/R_2)	*m/z* 98, 69, 56
Methoxy (R_3)	*m/z* 121
Ethoxy (R_3)	*m/z* 135
Propoxy (R_3)	*m/z* 149
Desnitazene (R_4)	Doubly charged ion in full scan

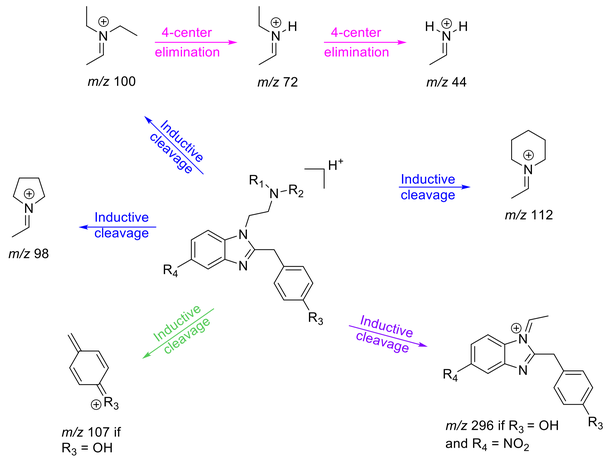

Figure 3. General fragmentation pathways for nitazene analogs.

- The general nitazene fragmentation pathways include inductive cleavages and rearrangements.
- Nitazenes with a diethyl substitution produce a product ion characterized by a 73 Da loss from the protonated molecule, whereas desethyl compounds produce a product ion with a 71 Da loss from the protonated molecule.
- All nitazene analogs with a desnitazene substitution contained a doubly charged molecule in their full scan mass spectrum.

MATERIALS & METHODS

Chemicals & Sample Preparation

In total, 41 nitazene analogs were analyzed in this study, including three isotopically labeled analogs. All analogs were prepared at 10 ppm in a 49.9:49.9:0.2% methanol:deionized water:formic acid solvent.

Instrumentation & Data Analysis

An Agilent 6530 Q-TOF mass spectrometer was used with a dual Agilent Jet Stream (AJS) ESI source. The samples were introduced directly into the source via a syringe pump with an 18 µL/min flow rate. The AJS source was operated in positive ionization mode, with a 300 °C drying gas and 350 °C sheath gas, both at a flow rate of 8 L/min. The capillary voltage was 3500 V, the nozzle voltage was 1500 V, and the nebulizer pressure was 40 psi. Three replicates of each sample were collected with a scan range of *m/z* 40-500 and CID activation energies of 15, 25, 35, and 45 eV.

All data analysis was performed using Agilent MassHunter Qualitative Analysis. Product ion spectra were normalized to the base peak before being exported and plotted using Microsoft Excel.

CONCLUSIONS

- Overall, although there are differences in the product ion spectra due to substitution, nitazene analogs tend to fragment similarly under ESI-MS/MS conditions.
- However, most nitazene analogs can be distinguished by diagnostic product ions.
- The most common product ions are *m/z* 100, 72, 44, and 107.
- Several substitution groups, such as pyrrolidine and piperidine rings, produce diagnostic product ions that can be used for identification.
- Diagnostic ions provide the framework for future multiple reaction monitoring (MRM) methods for toxicology and seized drug analysis.
- Identifying novel nitazene analogs can be expedited by identifying the provided diagnostic ions in product ion spectra.

REFERENCES

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