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TOXICOLOGY OBSERVATION

2-(4-Iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25I-NBOMe): Clinical Case with Unique Confirmatory Testing

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Abstract
Introduction 2C designer drugs have been in use since the 1970s, but new drugs continue to develop from substitutions to the base phenethylamine structure. This creates new clinical profiles and difficulty with laboratory confirmation. 2-(4-Iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25I-NBOMe) is a relatively new 2C drug that is more potent than structural 2C analogs; exposure reports are rare. Testing for 2C drugs is developing; specific testing for new analogs such as 25I-NBOMe is a challenge. These drugs do not reliably trigger a positive result on rapid drug immunoassays. Additionally, most facilities with confirmatory testing capabilities will not identify 25I-NBOMe; methods for detecting 25I-NBOMe in biological samples have not been clearly described nor have optimal metabolic targets for detecting 25I-NBOMe ingestion.
Case Report An 18-year-old female presented following use of 25I-NBOMe. She had an isolated brief seizure, tachycardia, hypertension, agitation, and confusion. She improved with intravenously administered fluids and benzodiazepines and was discharged 7 h postingestion. Urine was analyzed using quantitative LC-MS/MS methodology for 25I-NBOMe, 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)-methyl] ethanamine (25C-NBOMe), and 2-(2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine (25H-NBOMe). 25I-NBOMe was found at a concentration of 7.5 ng/mL, and 25H-NBOMe was detected as well. Additional testing was pursued to characterize the metabolism of 25I-NBOMe; the sample was reanalyzed with UPLC–time-of-flight mass spectrometry to identify excreted metabolites. The sample was additionally analyzed for the presence of 2,5-dimethoxy-4-iodophenethylamine (2C-I), 4-bromo-2,5-dimethoxyphenethylamine (2C-B), and 1-(2,5-dimethoxy-4-ethylphenyl)-2-aminoethane (2C-E).
Discussion This is a report of a patient presenting following exposure to 25I-NBOMe, a dangerous member of the evolving 2C drug class. The exposure was confirmed in a unique manner that could prove helpful in guiding further patient analysis and laboratory studies.

Keywords 25-I · 25i · Phenethylamine · Hallucinogen · Shulgin

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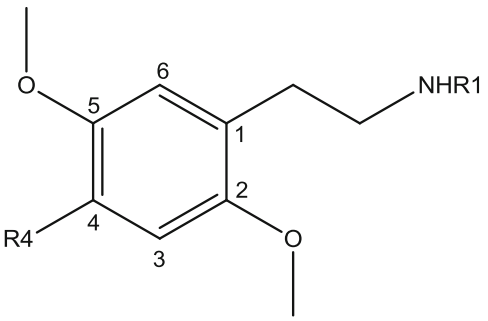
Introduction

The 2C class of designer phenethylamine drugs has gained popularity recently, joining synthetic cannabinoids and "bath salts" in the group of drugs having been abused in increasing amounts in part because of the easy access and perceived technical legality [1]. The phenethylamine-based structure of the 2C drugs is shared among amphetamines, catecholamines, cathinones, and many other drugs. The terminology "2C" is in reference to an acronym created by Alexander Shulgin to describe the two carbons between the amino group and the benzene ring in the chemical structure [2]. In this manuscript, we use 2C to refer to substituted designer hallucinogens with methoxy groups at positions 2 and 5 on the ring (Fig. 1), rather than the larger group of phenethylamine-based compounds

that would include epinephrine, dopamine, bupropion, MDMA, methamphetamine, cathinones, and a multitude of other commonly known similarly structured agents. Designer substitution to the 2C structure can result in altered hallucinogenic and stimulant activity. For example, substitution of iodine or bromine at position 4 results in increased hallucinogenic effects, as does changing the carbon branch chain attached to the amine group (Figs. 1 and 2).

One of the newer 2C drugs that has developed into a drug of abuse is 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2 methoxyphenyl)methyl]ethanamine, also recognized as 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25I-NBOMe) or 25i. Synthesized in the 1990s, this drug is a powerful analog of 2,5-dimethoxy-4-iodophenethylamine (2C-I) (Fig. 2). Like 2C-I and most of the rest of the 2C class, 25I-NBOMe is a hallucinogenic stimulant inducing euphoria, hallucinations, mydriasis, agitation, tachycardia, and hypertension, among other effects [3]. Unlike many of the other 2C drugs however, the high potency of 25I-NBOMe is likely linked to a higher likelihood of causing seizures and has even been linked to multiple deaths in users [4–6].

Testing for the 2C drugs in general is evolving, and specific testing for newly developed analogs such as 25I-NBOMe has proved to be a challenge. These drugs, and specifically 25I-NBOMe, will not be discovered on standard rapid drug screen urine immunoassays, and even facilities that have advanced confirmatory testing capabilities do not have clearly described methods for discovering presence of the parent compound or metabolites.



R1	R4	Compound
H	H	2C-H
H	I	2C-I
H	Br	2C-B
H	Cl	2C-C
H	$-C_2H_5$	2C-E
$-CH_2C_6H_4OCH_3$	H	25H-NBOMe
$-CH_2C_6H_4OCH_3$	I	25I-NBOMe
$-CH_2C_6H_4OCH_3$	Br	25B-NBOMe
$-CH_2C_6H_4OCH_3$	Cl	25C-NBOMe

Fig. 1 Base 2C structure with examples of substitutions

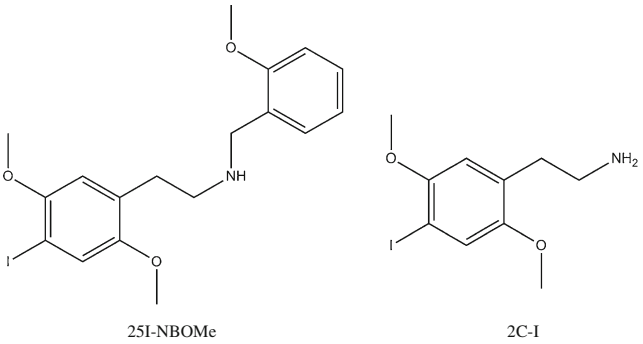


Fig. 2 Chemical structures of 25I-NBOMe and 2C-I

Given that 25I-NBOMe has increasing presence in intoxicated patients presenting to the hospital and possesses increased danger as compared with the other 2C agents, providers need to be aware of the background, clinical presentation, and testing aspects of 25I-NBOMe intoxication. The testing methods themselves need to be described clearly as well. We present a patient with 25I-NBOMe intoxication followed by a description and discussion of the unique laboratory testing that was done to confirm 25I-NBOMe as the offending agent.

Clinical Case

An 18-year-old female presented to the emergency department (ED) by ambulance after a witnessed grand mal seizure at a party. The seizure reportedly occurred roughly 90 min after exposure to 25I-NBOMe and had stopped when the paramedics arrived to transport her. She was disoriented but interactive enough to confirm the 25I-NBOMe exposure, which was one sublingual dose of unspecified exact amount. She had used no other substances during the day or evening, including ethanol, although she admitted to being a moderate alcohol drinker and regular marijuana user. She had no past medical problems, history of seizures, or psychiatric history. Her prehospital vital signs included a blood pressure (BP) of 145/100 mmHg, a heart rate (HR) of 145 beats per minute (BPM), a respiratory rate (RR) of 18 breaths per minute, and an oxygen saturation level of 98 %. Her pupils were 7–8 mm and minimally reactive bilaterally, Glasgow coma scale score was 14, and fingerstick blood glucose level was 11.82 mmol/L. Upon arrival to the ED roughly 120 min postexposure, the patient was slightly confused, anxious-appearing, and agitated and had pressured inappropriate speech and hypersexual interaction. Her initial ED vital signs were similar, with the addition of a temperature of 36.9 °C, and further exam revealed cutaneous flushing and hyperreflexia. The patient was observed for 5 h in the ED and was intravenously given 2 mg of lorazepam and 1 L of 0.9 % sodium chloride solution. She had no witnessed seizure activity and was ultimately discharged with

vital signs that had slowly normalized along with normalized mental status and interaction. Discharge vitals included a BP of 108/51 mmHg, a HR of 87 BPM, a RR of 15, and an oxygen saturation level of 99 %. No laboratory analysis was done immediately in the ED, including a serum basic metabolic panel looking for electrolyte abnormalities. The providers caring for the patient reasoned that there was a definite causal agent for the seizure and that serious electrolyte disturbance was highly unlikely given the self-limited nature of the seizure along with the normalization of the neurologic exam with just supportive care. Urine was obtained for further drug analysis (below) with the patient's verbal consent.

Laboratory Analysis

Methods

The urine sample was analyzed using a validated quantitative LC–MS/MS methodology monitoring for 25I-NBOMe along with 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe) and 2-(2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl)ethanamine (25H-NBOMe). The assay quantifies the three targets over matching concentration ranges of 1 to 500 ng/mL using a concurrently analyzed six-point calibration curve and D_6-2C-I as an internal standard. Reference standards for the three targeted analytes were obtained from Cayman Chemical (Ann Arbor, MI). Sample aliquots were first hydrolyzed, removing phase II conjugates using β-glucuronidase (from *Escherichia coli*, Roche Diagnostics, Indianapolis, IN) and a 3-h incubation at 38 °C. Posthydrolysis, samples were mixed with 0.5 mL of methanol, precipitating the enzyme and clarifying the sample with the aid of centrifugation. An aliquot of the supernatant was then analyzed via a UPLC ESI + MS/MS procedure on a system comprised of a Waters® ACQUITY UPLC® system (Waters Corporation, Milford, MA) coupled to a 5500 QTRAP® mass spectrometer (AB Sciex®, Framingham, MA). Chromatographic analysis was performed using a gradient elution of 10 mM ammonium acetate with 0.1 % formic acid and methanol at a flow rate of 700 μL/min through an ACQUITY UPLC HSS T3 column (50×2.1, 1.8 μm). The targeted analytes and internal standard were monitored using compound-specific transitions; 25I-NBOMe was monitored using the multiple reaction monitoring (MRM) transitions 428.1 to 121.2*m/z* and 428.1 to 91.2*m/z*. Two transitions were monitored for the purpose of confirming the identity of detected components through MRM ratio comparisons. The other two targeted analytes and internal standard were monitored using transitions specific for these molecules. A sample chromatogram displaying the target analytes and the internal standard can be found in Fig. 3. The assay had been previously validated in a manner compliant with SWGTOX and FDA Bioanalytical guidance [7, 8]. The assay possessed excellent selectivity, specificity, and accuracy with an inter-run precision and accuracy of 2.3 and 91.3 %, respectively.

After the initial result, additional testing was pursued to characterize and understand the metabolism and markers of 25I-NBOMe. The processed sample was reanalyzed on a UPLC–high-resolution time-of-flight mass spectrometer (Waters® ACQUITY UPLC® and Waters® Xevo® TOF mass spectrometer) using a slow gradient elution and collecting full-spectrum data with and without collision-induced fragmentation to identify excreted metabolites. The data were then evaluated with a simple metabolite identification strategy, searching for intact precursor molecules of likely metabolic transformations and confirming that the collision-induced fragmentation of any identified species was consistent with the structure of the metabolically transformed molecule. The sample was then reanalyzed without utilizing a hydrolysis procedure; this evaluated whether the identified 25I-NBOMe metabolites were excreted as free metabolites or as phase II glucuronide conjugates.

The sample was additionally analyzed using a separate UPLC–MS/MS methodology designed to detect and quantify the presence of 2C-I, 4-bromo-2,5-dimethoxyphenethylamine (2C-B), and 1-(2,5-dimethoxy-4-ethylphenyl)-2-aminoethane (2C-E). The utilized methodology is conceptually similar to that described above for 25I-NBOMe but uses a normal-phase chromatographic separation and monitors for MRM transitions

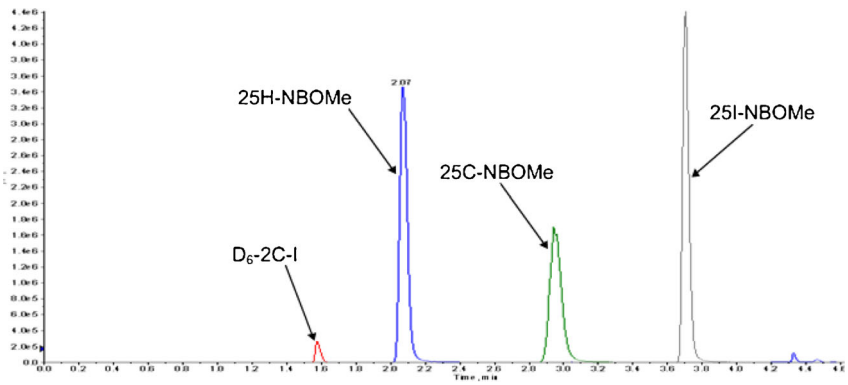


Fig. 3 Overlay chromatogram of the targeted NBOMe compounds

specific to these three targeted analytes. This methodology had been previously validated over a dynamic range of 1 to 500-ng/mL for all analytes in an analogous manner to that of the 25I-NBOMe procedure. Assay performance is additionally similar to that of the 25I-NBOMe assay, generating inter-run precision and accuracy of 12.2 and 99.6 %, respectively, for the measurement of 2C-I.

Results

Initial testing using the validated quantitative assay revealed that the urine sample contained 25I-NBOMe at a concentration of 7.5 ng/mL. 25H-NBOMe was also detected at a level below the lower quantitation limit estimated at 0.9 ng/mL. 2C-I was detected in the sample using the independent quantitative method at a concentration of 1.8 ng/mL.

Untargeted time-of-flight analysis of the sample revealed a dominant peak with a mass corresponding to the mass of 25I-NBOMe minus a single methyl group. The full fragmentation spectrum of the identified peak was then compared to a theoretical fragmentation pattern of demethylated 25I-NBOMe. The theoretical and observed fragmentation patterns closely agreed when utilizing a starting structure of 25I-NBOMe lacking a single methyl group from one of the methoxy positions of the dimethoxy iodophenyl ring. Close examination of the data revealed two other demethylated metabolites chromatographically resolved from the primary metabolite but present at levels dramatically lower than the primary metabolite. These correspond to the demethylation of the other methoxy groups present in 25I-NBOMe. No other metabolite of 25I-NBOMe was identified in the sample. The urinary concentration of the primary metabolite was then estimated based on the concentration response ratio of the parent species as listed in Table 1. Complete reanalysis of the urine sample without the aid of the enzymatic hydrolysis step did not alter the amount of 25I-NBOMe in the sample. However, all three demethylated metabolites were no longer detectable in the sample.

It should be noted that no other proconvulsant agents were discovered during the testing, and although the methods performed would not have exhausted all possible exogenous proconvulsant drugs, it would have revealed most of the common available agents.

Table 1 Measured analyte levels

Analyte	Concentration (ng/mL)
25I-NBOMe	7.5
25C-NBOMe	None detected
25H-NBOMe	0.9[a]
Desmethyl-25I-NBOMe	600 ng/mL[b,c]
2C-I	1.8
2C-B	None detected
2C-E	None detected

[a] Extrapolated below LLOQ
[b] Estimated based on response of demethylated analyte in comparison to response of 25I-NBOMe
[c] Present as the glucuronide conjugate

Discussion

There are relatively new 2C drugs, such as 25I-NBOMe, but 2Cs as a class are not new. Shulgin developed 2C-B in 1974, and it was subsequently sold through the 1980s and 1990s under such names as Nexus, Erox, Performax, Toonies, Bromo, Spectrum, and Venus [2]. In 1995, 2C-B was placed on Schedule I of the Controlled Substances Act by the Drug Enforcement Agency [9]. However, following the scheduling of 2C-B, other 2C analogs were made available by suppliers as legal alternatives. In 2012, nine other 2C agents were added to the Schedule 1 restricted list, and under the Federal Analogue Act amending the Controlled Substances Act, any compounds substantially similar in structure and activity to these or other controlled substances also are subject to the Controlled Substances Act and its prohibitions [10]. This Federal Analogue Act, however, does not necessarily preclude the possession and use of unscheduled 2Cs, depending on the nature of the structural modification and intention of possession; manufacturers often attempt to exploit this potential *loophole* by stating an "intended use" precluding consumption on the deceptive packaging characteristic of these products. As a result of this, some drugs were developed for abuse purposes specifically, and some developed for other reasons were ultimately used for abuse purposes primarily. 25I-NBOMe was developed as a 5-HT_{2A}-specific tag for PET-scanning brain research [11]. A radiolabeled form of 25I-NBOMe was developed for mapping the distribution of 5-HT_{2A} receptors in the brain. It is a highly potent agonist at 5-HT_{2A} receptors, with a K_i of 0.087 nM, making it some 16 times as potent as 2C-I itself [11–14]. This high potency at 5-HT_{2A} is likely the reason 25I-NBOMe was hijacked for recreational use.

The patient described above admitted to intentional recreational use, and the clinical course is congruent with the description of other cases of 2C intoxication and specifically with the limited information regarding 25I-NBOMe intoxication. There are some limited data in abstract form, along with one more clearly described case, demonstrating some of the same case features found in the patient here: tachycardia, hypertension, altered mental status, and seizure [15–17].

As mentioned above, 25I-NBOMe will not trigger a positive result on any currently available rapid drug screen urine

immunoassay. Additionally, most facilities would not specifically identify 25I-NBOMe even if they have confirmatory drug-testing capabilities. Methods for detecting 25I-NBOMe in urine have not been well described nor have the optimal metabolic targets for detecting 25I-NBOMe ingestion been clearly detailed in the literature; there is one case description in the literature accompanied by serum testing [17]. Additionally, the high potency of 25I-NBOMe challenges sophisticated drug analysis techniques where the relatively small signal of 25I-NBOMe is easily lost in the background noise of a sample. This urine sample provided an opportunity to investigate the human metabolism and excretion of the drug. The metabolic profile elucidated in this one sample may not be representative of the typical 25I-NBOMe metabolism; it does however offer an opportunity to detail likely metabolites and urinary markers of 25I-NBOMe use. The urinary sample was collected approximately 3 h postingestion of the drug. At that point, unchanged 25I-NBOMe was detectable in the urine sample. Additionally, a single demethylated metabolite was present at a level approximately 80-fold higher than the unchanged drug. The exact structure of this primary metabolite could not be established with the available methodologies; however, the identity of the metabolite was deduced to being demethylated at either position 2 or 5 of the dimethoxyphenyl ring. Other demethylation from the other two methoxy groups of the drug was also detected, but at levels similar to that of the parent compound. All of the demethylated metabolites were found to be excreted exclusively as glucuronide conjugates.

Additionally, the urine sample was found to contain 25H-NBOMe and 2C-I. The reason for the presence of 25H-NBOMe in the sample is unclear. 25H-NBOMe may have been uniquely consumed by the patient, but use of 25H-NBOMe was not reported. 25H-NBOMe could potentially be a metabolite of 25I-NBOMe, but we are unsure of what metabolic path would accomplish the removal of iodine from an aromatic ring of a xenobiotic. More likely, 25H-NBOMe was present in the consumed drug formulation as a contaminant. Internet discussions of clandestine chemists indicate that 25I-NBOMe is most commonly synthesized by reductive coupling of methoxybenzaldehyde with 2C-I. Within the synthesis of 2C-I is an iodination step of a precursor common to both 2C-I and 2C-H [2]. If the iodination step was incomplete and the unreacted material was not adequately removed by purification, both 2C-I and 2C-H would be produced as intermediates and subsequently reacted to form both 25I-NBOMe and 25H-NBOMe in the final drug product. The presence of 2C-I in the sample is similarly unclear. 2C-I may exist in the drug product due to an incomplete linking of methoxybenzaldehyde with 2C-1 during synthesis. Alternatively, 2C-I may result from metabolic cleavage of 25I-NBOMe at the amine within the chain linking the two ring structures.

Conclusion

The patient in this case presented following an exposure to 25I-NBOMe, a particularly dangerous member of the evolving 2C drug class. The exposure was confirmed in the patient's urine in a unique manner that could prove helpful in guiding further patient analysis and laboratory studies.

Conflict of Interest The authors have no disclosures to declare.

References

1. Rosenbaum CD, Carreiro SP, Babu KM (2012) Here today, gone tomorrow…and back again? A review of herbal marijuana alternatives (K2, Spice), synthetic cathinones (bath salts), kratom, *Salvia divinorum*, methoxetamine, and piperazines. J Med Toxicol 8:15–32
2. Shulgin AT, Shulgin A (1991) PIHKAL: a chemical love story. Transform, Berkeley
3. Dean BV, Stellpflug SJ, Burnett AB, Engebretsen KM (2013) 2C or not 2C: phenethylamine designer drug review. J Med Toxicol 9:172–178
4. Rupar A (2012) Adam Budge, 18, charged with murder following friend's synthetic drug overdose. Minneapolis: City Pages [updated 21 Jun 2012]. http://blogs.citypages.com/blotter/2012/06/adam_budge_18_charged_with_murder_following_friends_synthetic_drug_overdose.php. Accessed 16 Feb 2013
5. Walsh P (2012) U.S. indicts four in synthetic drug deaths. Minneapolis: Star Tribune [updated 27 Dec 2012]. http://www.startribune.com/printarticle/?id=184934801. Accessed 16 Feb 2013
6. Erowid.com (2012) 25I-NBOMe (2C-I-NBOMe) fatalities/deaths [updated 12 Sep 2012]. http://www.erowid.org/chemicals/2ci_nbome/2ci_nbome_death.shtml. Accessed 16 Feb 2013
7. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM) (2001) Guide for industry: bioanalytical validation. http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf. Accessed 16 Feb 2013
8. Scientific Working Group for Forensic Toxicology (SWGTOX) (2012) Standard practices for method validation in forensic toxicology. http://www.swgtox.org/documents/Validation_public_comment.pdf. Accessed 16 Feb 2013
9. Drug Enforcement Administration (2011) 4-Bromo-2,5-dimethoxyphenethylamine information sheet. Washington, DC: Drug Enforcement Administration [updated 16 Feb 2011]. http://www.deadiversion.usdoj.gov/drugs_concern/bromo_dmp/bromo_dmp.pdf. Accessed 12 Feb 2013
10. 112th Congress (2012) S. 3187: Food and Drug Administration Safety and Innovation Act. Section 1152. Washington, DC: Library of Congress [updated 10 Jul 2012]. http://beta.congress.gov/bill/112th-congress/senate-bill/3187. Accessed 12 Feb 2013
11. Nichols DE (2004) Hallucinogens. Pharmacol Ther 101:131–181
12. Nichols DE, Frescas SP, Chemel BR, Rehder KS, Zhong D, Lewin AH (2008) High specific activity tritium-labeled *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine (INBMeO): a high affinity 5-HT2A receptor-selective agonist radioligand. Bioorg Med Chem 16:6116–6123
13. Marek GJ, Wright RA, Schoepp DD, Monn JA, Aghajanian GK (2000) Physiological antagonism between 5-hydroxytryptamine$_{2A}$ and group II metabotropic glutamate receptors in prefrontal cortex. J Pharmacol Exp Ther 292:76–87

14. Braden MR, Parrish JC, Naylor JC, Nichols DE (2007) Molecular interaction of serotonin 5-HT2A receptor residues Phe339$^{(6.51)}$ and Phe340$^{(6.52)}$ with superpotent *N*-benzyl phenethylamine agonists. Mol Pharmacol 70:1956–1964
15. Kelly A, Eisenga B, Riley B, Judge B (2012) Case series of 25I-NBOMe exposures with laboratory confirmation. Clin Toxicol 50:70
16. Rose SR, Cumpston KL, Stromberg PE, Wills BK (2012) Severe poisoning following self-reported use of 25-I, a novel substituted amphetamine. Clin Toxicol 50:70
17. Rose SR, Poklis JL, Poklis A (2013) A case of 25I-NBOMe (25-I) intoxication: a new potent 5-HT2A agonist designer drug. Clin Toxicol 51(3):174–177