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2-(4-Iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25I-NBOME): A Harmful Hallucinogen Review

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Abstract

NBOMes are *N*-benzylmethoxy derivatives of the 2C family compounds with *N*-2-methoxybenzyl moiety substituted by the methoxy group at the 2- and 5-position and the halogen group at the 4-position of the phenyl ring. These substances are a new class of potent serotonin 5-HT2A receptor agonist hallucinogens with potential harmful effects. The substitution with halogen of the already psychoactive phenethylamine produces a derivative (2C-I) with increased hallucinogenic effects. This class of hallucinogens has chemical structures very similar to natural hallucinogenic alkaloid mescaline and these are sold mainly via internet as a 'legal' alternative to other hallucinogenic drug-lysergic acid diethylamide (LSD). 25I-NBOMe is the first synthesized and one of the most common compound from NBOMes. Knowledge of pharmacological properties of 25I-NBOMe is very limited so far. There are only a few *in vivo* and *in vitro* so far published studies. The behavioral experiments are mainly related with the hallucinogenic effect of 25I-NBOMe while the *in vitro* studies concerning mainly the affinity for 5-HT2A receptors. The 25I-NBOMe Critical Review 2016 reported 51 non-fatal intoxications and 21 deaths associated with 25I-NBOMe across Europe. Case reports describe various toxic effects of 25I-NBOMe usage including tachycardia, hypertension, hallucinations, rhabdomyolysis, acute kidney injury and death. The growing number of fatal and non-fatal intoxication cases indicates that 25I-NBOMe should be considered as a serious danger to public health. This review aims to present the current state of knowledge on pharmacological effects and chemical properties of 25I-NBOMe and to describe reported clinical cases and analytical methods available for identification of this agent in biological material.

Introduction

The novel psychoactive substances (NPSs) commonly known as legal highs, designer drugs or research chemicals are analogues of well-known illicit drugs designed to mimic the euphoric and hallucinogenic effects. NPSs have become very popular especially among young people due to their legality and easy access mostly via internet and low price (1). Confirmation on this fact is the increase in availability of NSP on the European drug market in recent years as evidenced by increasing numbers of seizures reported by the European Drug Reports (2–6). NBOMes are *N*-benzylmethoxy derivatives of the 2C family compounds with the *N*-2-methoxybenzyl moiety substituted by the methoxy group at the 2- and 5-position and the halogen atom at the 4-position of the phenyl ring (Figure 1) (7). The substitution with halogen of the already psychoactive phenethylamine produces a derivative 2,5-dimethoxy-4-iodophenethylamine (2C-I) with increased hallucinogenic effects (8). Some authors hypothesized that this substitution also increases these drugs lipophilicity and thus absorption at the blood–brain barrier (9). The chemical structures

Table I. Case Reports Concerning 25I-NBOMe-Induced Intoxication

Gender/age	The effect	Analyzed material/technique	Identified substances	Reference
Male/42	Strong sweating, disorientation, agitation, cenesthesia and complex hallucinations	Blood serum/LC–ESI-MS-MS	25I-NBOMe: 34 ng/mL; 2C-I: 12 ng/mL; and 25I-NBOH: <1 ng/mL	(49)
Eight males and two females/mean age of 17 years (14–20 years)	Tachycardia (90%), hypertension (90%), hyperglycaemia (90%), agitation (70%), hallucinations (50%), two patients were found in status epilepticus, one patient was unresponsive, one patient with seizures and multiple discrete intraparenchymal cerebral hemorrhages and acute kidney injury	Blood serum from one agitated and one tachycardic patient/LC–MS-MS	25I-NBOMe: 0.76 ng/mL	(50)
Male/18	Heart rate of 90 bpm, blood pressure of 140/84 mmHg, respirations of 20/min and oxygen saturation of 99% on room air	Blood serum and urine/not mentioned	25I-NBOMe: 34 pg/mL	(7)
Male/18	Agitation, hallucinations after jumping out of a moving car, tachycardia (150–160 bpm), and hypertensive (150–170 mm Hg systolic and 110 mg Hg diastolic)	Blood serum/LC–MS-MS	25I-NBOMe: 0.76 ng/mL	(20)

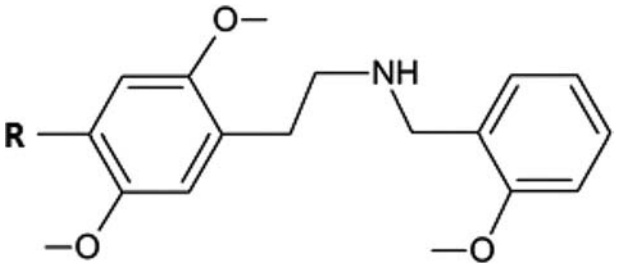

Figure 1. The chemical structure of NBOMes substituted by halogen atom at the fourth position of the phenyl ring. R = I: 25I-NBOMe, R = Br: 25B-NBOMe, R = Cl: 25C-NBOMe.

of NBOMes are very similar to natural hallucinogenic alkaloid mescaline that occurs in Peyote cactus (*Lophophora williamsii*) (10). Mescaline hallucinogenic effects are comparable to those of lysergic acid diethylamide (LSD).

NBOMes were originally synthesized for research purposes as potent agonists of serotonin 5-HT_{2A} receptor subtype (11). Low-affinity binding was observed for α1 adrenergic and H_1 histaminergic receptors (11). Juncosa et al. and Rickli et al. (12, 13) demonstrated that NBOMes have high affinity for both 5-HT_{2A} and 5-HT_{2C} receptors and much lower potencies for other serotonin receptors (e.g., NBOMes are 3000–7100-fold more selective for 5-HT_{2A} than to 5-HT_{1A}) (12, 13). Thus, the most frequent reasons for NBOMes intoxication are associated with the activation of the serotoninergic pathway and the sympathetic nervous system, which leads to hyper stimulation of cardiopulmonary and nervous system. Hence, the clinical effects of the NBOMe usage are frequently characterized by tachycardia, hypertension, seizures, hyperpyrexia, agitation, aggression, hyperventilation, increased physical strength or visual and oral hallucinations (14–18). Other recognized clinical effects of NBOMe usage are clonus, metabolic acidosis, rhabdomyolysis and acute kidney injury (8, 15, 19, 20). The name 'NBOMes' is informal and comes from the '*N*-benzylmethoxy' substituent (–methoxy being written in chemical shorthand as 'OMe'). NBOMes are commonly known under various names like N-bomb, Cimbi, Smiles or Solaris. The compounds are distributed as uncut powders or diluted to sub-milligram doses laced into perforated blotter paper (21) and are sold mainly via internet as a 'legal' alternative to LSD. This class of phenylethylamine derivatives is active at very low doses (50–250 µg) and is highly potent when taken sublingually, buccally or in inhalation form but have very low oral bioavailability. Activity time length depends on the route of administration and lasts from 3 to 13 h (22). The most common compounds from NBOMes class are 25B-NBOMe, 25C-NBOMe and 25I-NBOMe. These substances have been detected in Europe since 2011 (18). The first synthesized NBOMe is 25I-NBOMe (2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine). Global Drugs Survey reported in 2013 that 25I-NBOMe was the most commonly used drug form this group. These data were consistent with trends found in databases (23). Usage of 25I-NBOMe was associated with some deaths and high number of non-fatal intoxications reported in many countries (2–6). Therefore, this study aims to review the current state of knowledge on the pharmacological effects and chemical properties of one of the most popular NPS, NBOMes–25I-NBOMe. We included an analysis of reported clinical cases and analytical methods available for detection of this agent in biological material as well as in emerging street drugs.

Chemical structure and properties

Information about chemical properties of 25I-NBOMe in the literature is very limited. Its IUPAC name is 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamines) and chemically is a *N*-benzyl derivative of the iodo-substituted dimethoxyphenethylamine (2C-I) (21). 25I-NBOMe occurs under various names:

(i) 2C-I-NBOMe, (ii) 25I, 2CINBOMe, (iii) 2C-I-NBOMe, (iv) NBOMe-2C-I, (v) NBOMe-2CI, (vi) 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl)ethanamine, (vii) 2-(4-Iodo-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl)ethan-1-amine, (viii) 4-iodo-2,5-dimethoxy-*N*-(2-methoxybenzyl)phenethylamine, (ix) 4-iodo-2,5-

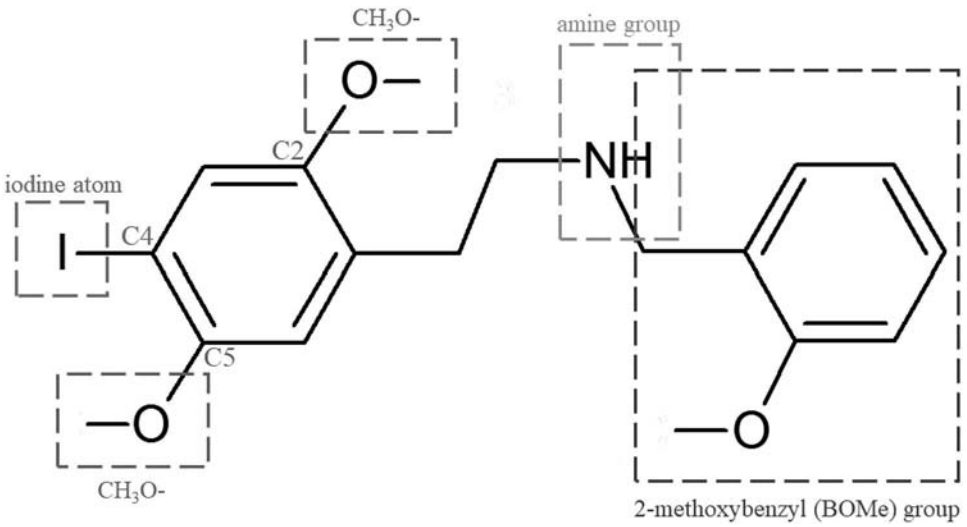

Figure 2. Structure of 25I-NBOMe with marked chemical groups.

dimethoxy-*N*-(*o*-methoxybenzyl)phenethylamine, (x) 4-iodo-2,5-dimethoxy-*N*-[(2-methoxyphenyl)methyl]-benzeneethanamine, (xi) *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine, (xii) *N*-(2-methoxybenzyl)-4-iodo-2,5-dimethoxyphenethylamine and (xiii) Cimbi-5 (11C radiolabelled for positron-emission tomography [PET] scanning): Center for Integrated Molecular Brain Imaging (CIMBI).

25I-NBOMe is a *N*-benzyl derivative of the substituted phenethylamine with methoxy groups CH_3O-attached to carbons C2 and C5 as well as an iodine atom attached to carbon C4. It differs from 2C-I structurally by the substitution on the amine group (NH_2) by the 2-methoxybenzyl group (BOMe; Figure 2). The molecular formula of 25I-NBOMe is $C_{18}H_{22}INO_3$ and its molecular weight 427.28 g/mol. Attributable to the absence of chiral centers, it does not have stereoisomers.

Solid 25I-NBOMe exists in the forms of hydrochloride salt and free base as white and colorless, respectively (24). It is a water soluble powder that has melting point equal to 162–166°C (Et_2O/EtOH·HCl) (25, 26) and 166°C (Et_2O/iPrOH·HCl) (24). In recent years, da Cunha et al. (27, 28) studied long-term stability of 25I-NBOMe, and observed 20% decomposition in dried blood spots after 180 days in room temperature. At lower temperature (4 and −20°C) degradation did not occur. Poor stability was also observed in liquid blood. After 15 days 25I-NBOMe in low concentration (0.3 ng/mL) was reduced by 40 and 20% at 25 and 4°C, respectively, but at −20°C 25I-NBOMe was stable for 6 months. In the case of high concentration (8 ng/mL), 20% of decomposition of the compound was determined after 15 days at room temperature and after 180 days. No effect was observed for T < −20°C.

25I-NBOMe was first synthesized in 2003 by Heim at the Free University of Berlin via reductive alkylation of 2C-I with 2-methoxybenzaldehyde. The product is hydrochloride salt while reaction is stepwise as follows (24): (i) Generation of the imine intermediate from aldehyde (2-methoxybenzaldehyde) and the primary amine and (ii) reduction of imine by sodium borohydride ($NaBH_4$) or sodium triacetoxyborohydride ($Na(CH_3COO)_3BH$).

This two-step reaction is used to synthesize various of these compounds (21, 24, 26).

Metabolism

Caspar and co-workers (29) identified 37 phase I and 31 phase II metabolites of 25I-NBOMe in *in vivo* stud as on rats and humans study. Most of identified metabolites from both, animal and human sample were generated by *O*-demethylation, *O*-di-demethylation and hydroxylation. In turn, Wolfarth et al. (30) reported metabolic studies on 25I-NBOMe with the use of cryopreserved human hepatocytes and mouse urine. The samples were analyzed by liquid chromatography–high-resolution mass spectrometry (LC–HR-MS) method. Both, *in vitro* and *in vivo* results confirmed that 25I-NBOMe were predominantly metabolized by *O*-demethylation, followed by *O*-di-demethylation and hydroxylation (30). In a study by Poklis et al. (31), 15 metabolites were identified in two urine samples from human and in fresh liver microsomes from mouse (31). After confirmation with synthesized standards, the 2′ and 5′-desmethyl metabolites were proposed as potential biomarkers of the 25I-NBOMe usage (31, 32). In 2014, Walterscheid and Stellpflug reported one demethylated and three possible demethylated metabolites in two independent studies. Transformation to 2′- and 5′-desmethyl 25I-NBOMe and 4-hydroxy 25I-NBOMe was confirmed in human liver microsomes after *in vitro* incubation with 25I-NBOMe (33). Finally, Temporal and co-workers (34) presented schematically a metabolite profile of 25I-NBOMe as shown in Figure 3.

Pharmacological characterization of 25I-NBOMe

Despite frequent recreational use of 25I-NBOMe, pharmacological properties and mechanism of action of this substance has not been described in detail so far. There are only a few behavioral and neurochemical (*in vitro* and *in vivo*) studies published nowadays. *In vitro* experiments were mainly focused on the determination of the 25I-NBOMe affinity with 5-HT_{2A} receptors.

In vitro studies

Receptors affinity

Like other hallucinogens, NBOMes are fully efficacious 5-HT_{2A} agonists relatively nonselective for 5-HT_{2A} vs. 5-HT_{2C} sites (35).

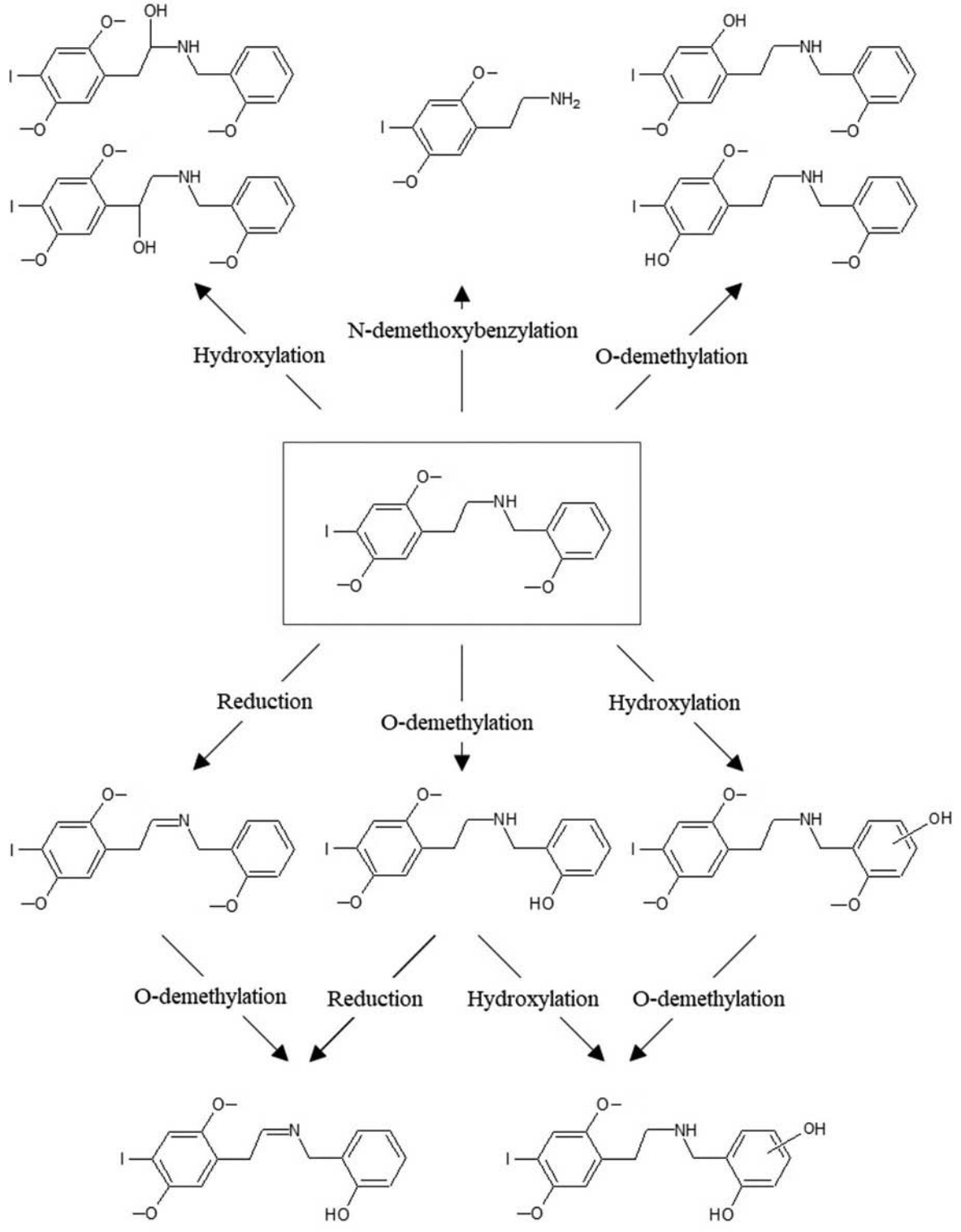

Figure 3. A 25I-NBOMe metabolic profile according to Temporal and colleagues (34).

25I-NBOMes exhibit high affinity for serotonin 5-HT_{1A} (Ki = 1800 nM), 5-HT_{2A} (Ki = 0.6 nM) and 5-HT_{2C} receptors (Ki = 4.6 nM) as well as for adrenergic α_{1A} (Ki = 370 nM) and α_{2A} (Ki = 320 nM) receptors (13). Moreover, NBOMes show a greater potency to inhibit binding at 5-HT_{2A} receptors (IC50 range 4–9 nM) than the 2C compounds (IC50 range 125–307 nM) (36). For instance, 25I-NBOMe displayed a 32-fold higher affinity than 2C-I at this site. According to data obtained by Elmore et al. (36), the 2C compounds showed greater potency in the 5-HT_{2A} functional assay than NBOMes. As shown, 2C-C was 13-fold more potent

in the 5-HT_{2A}-mediated calcium mobilization assay than 25C-NBOMe, whereas 2C-I was 8-fold more potent than 25I-NBOMe. All compounds acted as the full agonist of 5-HT_{2A} receptors. In the 5-HT_{2C}-mediated calcium mobilization assay, NBOMes were more efficacious (60–70% E_{max}) than the 2C compounds (~30% E_{max}).

Ettrup et al. (37) studied the 5-HT_{2A} receptor binding affinity of carbon-11-labeled, substituted phenethylamines with the PET. It was found that 25I-NBOMe showed an *in vitro* 5-HT_{2A} receptor agonistic binding affinity of 1.49 ± 0.35 nM.

Neurogenesis studies

Surprisingly, there is only one report in the literature investigating this aspect of the NBOMes action. 25I-NBOMe was used in a study on hippocampal neurogenesis in mice at doses of 0.1, 0.3 and 1 mg/kg administrated subcutaneous (sc) (38). It resulted in a significant decrease in the number of surviving BrdU+ cells and BrdU/NeuN+ neurons in hippocampus compared to control. Doses lower than 0.1 mg/kg did not induce neurogenesis (38).

In vivo studies

In vivo microdialysis

In vivo microdialysis allows for sampling of small-molecular-weight substances like neurotransmitters from the interstitial brain structure. In microdialysis experiment in freely moving Wistar male rats, Herian et al. (39) observed an enhanced extracellular level of dopamine (DA), serotonin (5-HT) and glutamate (GLU) in the frontal cortex after administration of 25I-NBOMe in doses of 0.3, 1.0, 3.0 and 10.0 mg/kg. In the same study, a tissue content of cortical 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) was increased after treatment with single administration of 25I-NBOMe in the same doses as mentioned before (39). The changes in rat cortical extracellular levels of DA, 5-HT, and GLU may be explained by 25I-NBOMe agonist action on 5-HT_{2A} receptors (13). In addition, Herian and co-workers (39) suggested that hallucinogenic activity of 25I-NBOMe may be related with the increase of a cortical extracellular GLU level mediated via 5-HT_{2A} receptors.

Behavioral studies

Behavioral studies are often used as a tool to predict the expected effects produced by psychoactive substances in humans. Since now, only certain acute behavioral effects in animals after 25I-NBOMe administration were observed. There is no data about any chronical behavioral experiment with the use of 25I-NBOMe.

Hallucinogenic properties

Head twitch response (HTR) is one of few behaviors that can reliably distinguish hallucinogenic and non-hallucinogenic 5-HT_{2A} agonists (40). Thus, it is used as a behavioral marker for the hallucinogenic effect of 5-HT_{2A} receptor activation in animal models. Halberstadt and Geyer investigated head twitches response in C57BL/6 J male mice after single administration of 2CI, 25I-NBOMe and 25I-NBMD at doses of 1–10 mg/kg (sc), 0.1–1 mg/kg (sc) and 1–10 mg/kg (sc), respectively (41). All doses of these drugs induced HTR while 25I-NBOMe displayed a 14-fold higher potency than 2C-I (41). This effect was dose dependently blocked by pretreatment with M100,907—a 5-HT_{2A} receptor antagonist what suggested that HTR was induced through 5-HT_{2A} receptor activation by the studied compounds including 25I-NBOMe (41). In the study of Herian et al., 25I-NBOMe showed hallucinogenic activity in the HTR test 4 h after drug administration. Interestingly, the dose of 3 mg/kg (*iv*) produced a weak effect whereas the effect of 1 and 10 mg/kg doses was significantly stronger (39).

The effects of 25I-NBOMe on hallucinogenic behavior in male Sprague-Dawley rats was also reported by Elmore and co-workers (36) by using wet dog shakes response (WDS—a paroxysmal shaking of the head, neck and trunk). The WDS was investigated in the rats as a possible animal model for central serotonin (5-HT) activity (42–44). 25I-NBOMe at doses of 0.1 and 0.3 mg/kg (sc) induced the WDS compared to vehicle-treated rats and generated a hyperbolic dose–response curve. This effect was blocked by 0.1 mg/kg M100907 administrated 30 min before treatment (36).

Locomotor activity

Various compounds found in 'legal highs' increase locomotor activity. In a Halberstadt study, intraperitoneal (ip) administration of 25I-NBOMe in doses of 0.03–3 mg/kg had no effect on locomotor activity in mice. But when administered sc, 0.1 mg/kg 25I-NBOMe increased the locomotor activity, whereas the 3 mg/kg dose reduced it (45). These findings suggested that the route of administration is important for 25I-NBOMe action. Gatch et al. (46) found that administration of 25I-NBOMe produced both time and dose-dependent (2.5, 5,10 and 25 mg/kg) depression of the locomotor activity in male Swiss-Webster mice. The depressant effect on locomotor effects occurred within 10 min after drug administration and lasted 30–90 min (46).

Dependence and abuse potential

There are not many data concerning the abuse or dependence potential of 25I-NBOMe. Jeon et al. (47) showed that administration of 25I-NBOMe might produce rewarding and reinforcing effects, indicating its dependence liability in mice. In conditioned place, a preference test that can evaluate a hedonic value (rewarding or aversive) of 25I-NBOMe at a dose of 0.3 mg/kg, produced place preference similar to methamphetamine used as a positive control. Moreover, 25I-NBOMe at dose of 0.03 mg/kg lead to a lower level of overall self-administration test (measurement of motivational/reinforcing effects) compared to that of conditioned place preference (47,48).

Toxic effects of 25I-NBOMe in humans

Adverse effects of 25I-NBOMe

Adverse effects of 25I-NBOMe in humans, according to the scientific data, can be divided into following groups: (i) Cardiovascular: hypertension, tachycardia and palpitations (7, 49, 50); (ii) psychiatric: (iii) agitation, euphoria, paranoia, fear, panic, 'out of control thinking', paranoid delusion, visual and auditory hallucinations (50); (iv) neurological: insomnia, tremors, seizures, headache, dizziness, epileptic, cerebral hemorrhages and tonic-clonic seizure (49); and (v) others: sweeting, hyperglycaemia, nausea, swelling of feet, hands, face, increased muscle tone in upper and lower extremities, hypoactive bowel sound and muscle rigidity (49, 50).

Clinical cases reported so far are included in Table 1.

These scientifically confirmed effects of the 25I-NBOMe usage are not the only known effects. There are many stories about 25I-NBOMe action on websites, blogs, message boards, forums etc. Knowledge of such reports is important for clinical reasons. Treatment of intoxication occurs after toxicological tests and in connection with the observed symptoms. The following list of effect received after 25I-NBOMe intake is based upon subjective and personal experiences of its users. The list of symptoms was not created as a result of scientific research, thus we cannot be sure of the taken doses

Table II. Case reports concerning 25I-NBOMe: Death related

Gender/age	Analyzed material/technique	Identified substances	Reference
Male/15	Blood serum/LC–MS-MS with reporting limit of 0.50 ng/mL and urine/LC–MS-MS with reporting limit of 10 and 20 ng/mL for NBOMes and psilocin, respectively	Blood positive for 25I-NBOMe, urine positive for 25C- NBOMe, 25H-NBOMe, 25I-NBOMe, psilocin and antemortem blood confirmed positive for 25I-NBOMe: 0.76 ng/mL	(51)
Male/teenagers	Heart blood and urine/UHPLC–MS-MS	First case: postmortem heart blood positive for 5B-NBOMe: 1.59 ng/mL; second case: postmortem heart blood positive for 25I-NBOMe: 19.8 ng/mL	(52)
Female/23	Postmortem aortic non-preserved blood/LC–MS QTOF and LC–MS QQQ	Postmortem aortic non-preserved blood positive for 25I-NBOMe: 28 µg/L	(53)
Male/21 Female/15	LC–MS-TOF	Unknown data	(54)
Female/19	Blood serum and urine/brak techniki	Peripheral blood, 405 pg/mL; heart blood, 410 pg/mL; urine, 2.86 ng/mL; and vitreous humor, 99 pg/mL	(55)

or exclude the presence of other harmful substances. Therefore, these effects should be considered carefully. High doses (about 1000 µg) are able to increase possibility of inducing a full range of these effects, but it is unlikely that all of them will occur at once (48).

- Physical effects: Stimulation, perception of bodily lightness, spontaneous physical sensations, mouth numbing, nausea, temperature regulation suppression, increased bodily temperature, abnormal heartbeat, increased heart rate, increased blood pressure, muscle contractions, muscle cramps, muscle tension, gustatory hallucination, vasoconstriction, appetite suppression, stomach cramps, dehydration, dry mouth, difficulty urinating or frequent urination, restless legs, pupil dilation, headaches and seizures (7, 49, 50).
- Cognitive effects: Analysis enhancement, thought acceleration and thought deceleration, conceptual thinking, anxiety and paranoia, feelings of impending doom, empathy, affection and sociability enhancement, memory suppression, ego death, amnesia, novelty enhancement, immersion enhancement, emotion enhancement, increased sense of humor, laughter fits, increased music appreciation, personal bias suppression, increased libido, time distortion and wakefulness.
- Auditory effects: Enhancements, distortions and hallucinations (20, 50).
- Visual effects: Enhancements (visual acuity enhancement, color enhancement, pattern recognition enhancement and magnification), suppressions (frame rate suppression and peripheral information misinterpretation), distortions (drifting, color shifting, color replacement, color tinting, depth perception distortions, perspective distortions, tracers, diffraction, brightness alteration, after images, symmetrical texture repetition, recursion and scenery slicing) and hallucinatory states (transformations, machinescapes and internal hallucination) (20, 49).
- Multi-sensory effects: Dosage independent intensity, synaesthesia (7).
- Transpersonal effects: Existential self-realization, unity and interconnectedness (20).

The observed effects are summarized in Table I.

Death cases related to the recreational use of 25I-NBOMe

There are many examples of case reports related to death in the literature, which are summarized in Table II.

International Agencies like Advisory Council on the Misuse of Drugs (ACMD), Drug Enforcement Administration (DEA) and United Nations Office on Drugs and Crime (UNODC) also indicated that 25I-NBOMe is linked to several deaths across the world (56, 57, 58). It is important to know that many other cases with likely use of 25I-NBOMe have not been analytically confirmed. Therefore, it is possible that in result of 25I-NBOMe use, more people have died than was reported.

Analytical techniques

Various analytical techniques such as gas chromatography (GC), high performance liquid chromatography (HPLC), mass spectrometry (MS) and infrared (IR) spectroscopy are employed in investigations of drugs content in biological material. The listed methods were proven to be effective in the toxicological/forensic identification of the 25I-NBOMe usage in humans as well as in laboratory animals (8, 15, 20, 27–31, 49, 51–55, 59–61, 63–72, 79, 80).

Based on the literature, the most commonly used method to determine the concentration of the drug are high- and ultra-performance liquid chromatography (HPLC and UPLC, respectively) in combination with MS or tandem mass spectrometry (MS-MS), including high-resolution MS. These combinations of LC and MS exhibited limit of detection and limit of quantification at a level of pg-ng/mL and were able to distinguish the psychoactive substance and its metabolites. This analytical technology requires the use of internal standard [e.g., 2-(2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl) ethanamine (59), 4-iodo-2,5-dimethoxy-*N*-[(2-methoxyphenyl)methyl]-benzeneethanamine-d_3 (52), 4-bromo-2,5-dimethoxy-*N*-[(2-methoxyphenyl)methyl]-benzeneethanamine-d_3 (61, 62, 79), 3,4-methylenedioxypyrovalerone-d_8 (80), mephedrone-d_3 (69)] and solid-phase extraction (49, 55, 59–61, 80). In that way, 25I-NBOMe and its metabolites were identified and quantified in human serum, plasma, peripheral and heart blood, dried blood spots, hair, hepatocytes, urine from intoxicated patients, acute poisoning and death cases. Several reports showed the applicability of the GC–MS method for ante- and postmortem 25I-NBOMe analysis of human blood and urine, as well as for the identification of NBOMe metabolites in mouse hepatic microsomes (31, 71).

In the case of studies on characterization of 25I-NBOMe metabolites, various options of chromatography coupled to MS (GC–MS, LC/HPLC/UPLC–MS, LC–QTOF-MS, UPLC–QTOF-MS and LC–HRMS-MS) and nuclear magnetic resonance (NMR) were

Table III. Analytical techniques involved in detection of 25I-NBOMe and its metabolites content in biological material and in emerging street drugs

Method	Sample	Reference
HPLC–MS-MS	Postmortem blood, body fluids and tissues	(55)
	Human serum and urine specimens	(61)
LC–MS-MS	Human peripheral blood, human heart blood, human plasma after solid-phase extraction, human urine and blotter papers	(15, 20, 49, 51, 54, 60, 62)
	Human whole blood to evaluate instability	(27)
	Human dried blood spots to examine of long-term stability	(28)
	Pocrine and human liver microsomes and blood samples to identify metabolites	(63)
	Human serum and urine samples to identify metabolites	(67)
	Human urine and hair samples in rats	(79)
	Human heart blood and urine specimens	(8)
	Human whole blood	(69)
UPLC–MS-MS	Human hair	(64)
	Human plasma	(65)
	Metabolite biomarkers in mouse hepatic microsomal preparations and human urine samples	(31)
UHPLC with positive electrospray ionization	Human blood, plasma and urine	(80)
TOF-MS with UHPLC	Blotter papers	(73)
PSI-MS	Blotter papers	(75)
LC–MS QTOF	Human aortic blood and urine	(53)
UPLC–TOF-MS	Metabolites of 25I-NBOMe	(8)
LC–HRMS	Metabolites studies in human hepatocytes, human urine, mouse urine	(30)
	Metabolites	(67)
	Identification of 25I-NBOMe in human blood plasma	(68)
	Postmortem peripheral human blood, human urine	(66)
GC–MS	Postmortem analysis of human blood and urine	(52)
	Urine samples	(71)
	Identification of metabolites in mouse hepatic microsomal preparations and human urine specimens	(31)
	Blotter papers with detection based on mass ion trap detector	(70)
LC–HRMS-MS	Human and rat urine	(29)
HPLC–MS	Human urine, metabolites	(72)
LC–QTOF-MS	Human urine, metabolites	(72)
NMR	Human urine	(72,74)
UPLC–QTOF-MS	Human blood and urine	(34)
DART-TOF	Human liver microsomes, blotter papers	(62)
ATR-FTIR	Blotter papers	(76)
FTIR with chemometric analysis	Blotter papers	(77)
NIR with chemometric analysis	Detection in emerging street drugs	(78)
Cyclic and differential pulse voltammetry	Street drugs	(81)

employed to analyze human and rat urine and blood samples and human liver microsomes (29, 34, 68, 72). The same set of instrumental techniques was successfully applied to recognize molecular structures of NBOMe in 22 samples intended for use as ingredients in the recreational drug product before being sold on the drug market (74).

Time-of-flight-mass spectrometry (TOF-MS) was proven to be a useful tool to detect NBOMe on blotter papers (73). The authors compared results achieved from a TOF-MS system itself with a support of direct sample analysis (DSA) with conventional UPLC–TOF-MS technique. Analysis time of the designed DSA TOF-MS method was only ~15 s per sample and did not required NBOMe from the blotter paper as needed for UPLC–TOF-MS. A fast and powerful qualitative method to analyze designer drugs directly on the surface of blotters was proposed by Carralho and co-workers (75) who employed paper spray ionization-mass spectrometry (PSI-MS) (75). As open-air ionization technique is easy to perform and does not require complex MS instrumentation and expensive materials, this method can be employed in portable mass spectrometers allowing in situ analysis when designer drugs are seized. An interesting approach is the use of spectroscopic techniques based on absorption of IR light, as they are able to detect the drugs in situ and without sample preparation. The main application of this method is the in situ detection of NBOMes on blotter papers by attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and hand-held near IR spectrometry (76, 77). Although the final outcome of this analysis requires the use of chemometric tools, their results were confirmed GC and LC–MS.

All the analytical techniques used so far to identify and quantify NBOMe are summarized in Table III. A plethora of techniques and

investigated samples indicate that the efficient detection methods of 25I-NBOMe for clinical and forensic purposes are already well established.

Conclusions

25I-NBOMe, a *N*-benzylmethoxy derivative of the 2C family, is a novel potent hallucinogenic agent. Recent studies showed that this drug produces a variety of adverse physiological and psychiatric effects including visual and auditory hallucinations, aggressiveness, panic attacks and psychotic behavior. *In vitro* studies showed that 25I-NBOMe is a potent and full agonist of the serotonin 5-HT_{2A} receptor, but the mechanism of action of the drug is still not well understood; thus, its impact on human nervous system remains poorly characterized. Moreover, there is a lack of systematic studies on animal models, especially in the field of psychopharmacology. There is a limited number of *in vivo* research and data already published showed only effects of this agent after chronical administration. Most information about 25I-NBOMe still comes from clinical cases studies. The Critical Review Report from the Expert Committee on 36th Meeting of Drug Dependence in 2016 reported 51 non-fatal intoxications and 21 deaths associated with 25I-NBOMe across Europe (82). The growing number of fatal and non-fatal intoxication cases indicates that 25I-NBOMe should be considered as a serious danger to public health. The toxicity, pharmacological mechanism of action and the long-term effects are needed to be explored.

Abbrevations

ATR-FTIR, attenuated total reflectance-Fourier transform infrared spectroscopy; DA, dopamine; DART-TOF, direct analysis in real time-time of flight; DSA, direct sample analysis; FTIR, Fourier transform infrared spectroscopy; GLU, glutamate; GS, gas chromatography; GS–MS, gas chromatography–mass spectrometry; HPLC, high performance liquid chromatography; HPLC–MS, high-performance liquid chromatography–mass spectrometry; HPLC–MS-MS, high-performance liquid chromatography–tandem mass spectrometry; HTR, head twitch response; H_1, histamine receptor; ip, intraperitoneal injection; iv, intravenous injection; Ki, binding affinity; LC–HRMS, liquid chromatography–high-resolution mass spectrometry; LC–HRMS-MS, liquid chromatography–high-resolution tandem mass spectrometry; LC–MS-MS, liquid chromatography–tandem mass spectrometry; LC–MS-QTOF, liquid chromatography–mass spectrometry-quadrupole time of flight; M100907, selective 5-HT_{2A} receptor antagonist; NBOMes, *N*-benzylphenethylamines; NIR, near infrared spectroscopy; NMR, nuclear magnetic resonance; NSP, novel psychoactive substances; PSI-MS, paper spray ionization-mass spectrometry; sc, subcutaneous injection; TOF-MS, time-of-flight-mass spectrometry; UPLC–QTOF-MS, ultra-high performance liquid chromatography–quadrupole time-of-flight-mass spectrometry; UPLC–MS-MS, ultra-high performance liquid chromatography–tandem mass spectrometry; UPLC–QTOF-MS, ultra-high performance liquid chromatography–mass spectrometry quadrupole time of flight; 25I-NBOMe, 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine; 25B-NBOMe, 2-(4-bromo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine; 25C-NBOMe, 2-(4-chlor-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl) methyl]ethanamine; 25I-NBOH, 2-((2-(4-iodo-2,5-dimethoxyphenyl) ethylamino)methyl)phenol; 2C, 2-phenylethylamine; 2C-I, 2,5-dimethoxy-4-iodophenethylamine; 5-HT, serotonin; 5-HT_{1A}, serotonin receptor; 5-HT_{2A}, serotonin receptor; 5-HT_{2C}, serotonin receptor; 5-HIAA, 5-hydroxyindoleacetic acid; α_{1A}, adrenaline receptor; α_{2A}, adrenaline receptor.

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