



## SHORT COMMUNICATION

## 25C-NBOMe short characterisation

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### Abstract

**Purpose** *N*-Methoxybenzyls, a group of toxic phenylethylamine derivatives of the 2C family compounds, are a new class of potent serotonin 5-HT<sub>2A</sub> receptor agonist hallucinogens with potential harmful effects. This study summarizes current state of knowledge of one of the most dangerous representative of this group—*N*-(2-methoxybenzyl)-2,5-dimethoxy-4-chlorophenethylamine (25C-NBOMe). Due to hallucinogenic properties similar to those observed after lysergic acid diethylamide (LSD) usage (altered thoughts, feelings, and awareness of one's surroundings), this compound is very attractive to hallucinogenic substances users.

**Methods** An exhaustive literature search was carried out in PubMed, Google Scholar and other biomedical data bases without limiting period, to identify relevant articles.

**Results** Despite frequent recreational use, knowledge about the 25C-NBOMe action and toxic and fatal consequences is still very limited. Most data on this drug come from clinical reports, from cases of acute fatal and non-fatal intoxications. Some animal and in vitro studies indicated a route of metabolism of the drug in the body. The drug and its metabolites were also detected in human blood and urine using combinations of chromatographic separation and mass spectrometry detection.

**Conclusions** Overall, findings show that 25C-NBOMe is a powerful hallucinogen. Easy online availability, low price and the lack of knowledge of 25C-NBOMe makes this substance potentially very dangerous to its users. Thus, further investigation on the mechanism of action, chemical, pharmacological and toxicological properties is needed to evaluate 25C-NBOMe potential harmful effects.

**Keywords** 25C-NBOMe · Hallucinogen · Novel psychoactive substance · Legal highs · Designer drug

### Introduction

According to the European Drug Reports from last years, the use of novel psychoactive substances (NPS) popularly known as legal highs, designer drugs or research chemicals, rapidly increased among young people [1]. *N*-Methoxybenzyls (NBOMes) are a new group of toxic phenylethylamine derivatives of the 2C family compounds with *N*-2-methoxy-benzyl substituted by the methoxy group at the 2- and 5-positions and a halogen atom attached to C4 of the phenyl ring. Low price on the market and an easy access

via the internet caused that NBOMes are potentially very dangerous agents; however, their pharmacological properties, mechanism of action, metabolism, and toxicity have not been yet fully recognised. It is known that NBOMes potentially interact with serotonin 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub> receptors, adrenergic  $\alpha_1$  receptors and dopaminergic D<sub>1</sub> receptors, but have lower affinity at 5-HT<sub>1A</sub> receptor [2]. All agents of the NBOMes group exhibit low nanomolar affinity for 5-HT<sub>2A</sub> receptors which is higher in comparison to other 2C compounds [3]. This affinity correlates with NBOMes hallucinogenic potency in humans [4]. Moreover, 5-HT<sub>1A</sub> receptor stimulation has been hypothesized to counteract hallucinogenic activity and in consequence a lower 5-HT<sub>1A</sub> receptor stimulation for the NBOMes compounds may further enhance their hallucinogenic properties [5].

25C-NBOMe [*N*-(2-methoxybenzyl)-2, 5-dimethoxy-4-chlorophenethylamine] known also as NBOMe-2C-C, 2C-C-NBOMe C-Boom, 25C, legal acid, NBomb, NEBOME, Pandora, Dime, NBOMe-2C-C, BOM, 2-C-Cor,

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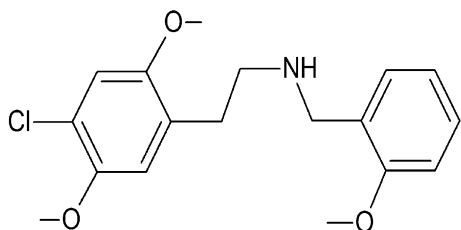
Cimbi-82 began to be sold via online sites in 2010 but its use was not reported in the scientific literature until 2011 [6]. The effect of 25C-NBOMe usage is characterized by various psychiatric and physiological effects like hallucination [7], violent agitation, euphoria, insomnia, rhabdomyolysis and kidney injury. 25C-NBOMe has effects similar to those of lysergic acid diethylamide (LSD) and according to some media reports LSD users may often unwittingly ingest 25C-NBOMe, instead of LSD [8].

The present study provides a brief review on available data about 25C-NBOMe chemical structure and properties, widely understood biological effects of its intake and analytical methods used for identification of this compounds in humans.

## Chemical characterisation

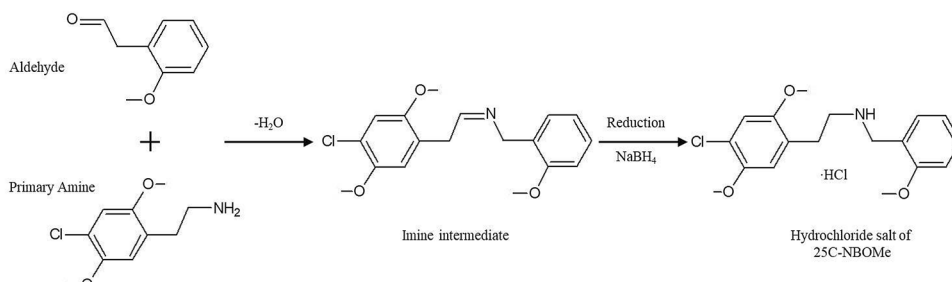
Published data about chemical characterisation of 25C-NBOMe is very limited so far. 25C-NBOMe contains the substructure of 4-chloro-2, 5-dimethoxyphenethylamine (2C-C), substituted with the *N*-(2-methoxy) benzyl group (Fig. 1). The molecular formula of 25C-NBOMe is  $C_{18}H_{22}ClNO_3$  and the molecular weight of free base is 335.8. 25C-NBOMe does not have chiral centres and thus does not form stereoisomers [9].

There are several chemical names for 25C-NBOMe, the most commonly use are 2-(4-chloro-2, 5-dimethoxyphenyl)-*N*-(2-methoxybenzyl) ethanamine, 2-(4-chloro-2, 5-dimethoxyphenyl)-*N*-(2-methoxybenzyl) ethan-1-amine, Cimbi-82 (11C radiolabelled for PET



**Fig. 1** Chemical structure of 25C-NBOMe

**Fig. 2** Schematic of the 25I-NBOMe synthesis process



scanning)—Center for Integrated Molecular Brain Imaging (CIMBI).

The synthesis of 25C-NBOMe, schematically illustrated in Fig. 2, follows the process described by Heim in 2003 [10].

## Pharmacological characterisation

25C-NBOMe acts as a potent partial agonist for the 5-HT<sub>2A</sub> serotonin receptor with binding affinity of  $2.89 \pm 1.05$  nM in vitro [11]. Stimulation of the 5-HT<sub>2A</sub> receptors is essential for the hallucinogenic effects of drugs [12, 13]. Limited studies using animal experiments have shown that 25C-NBOMe induced a head twitch response of a behavioural marker of hallucinogenic effects induced by activation of the 5-HT<sub>2A</sub> receptor [14]. In the work of Xu et al. 25C-NBOMe was tested in vitro against neuroblastoma human cell line SH-SY5Y, PC12-cell line derived from a pheochromocytoma of the rat adrenal medulla, and a mouse neuron-like dopaminergic cell line SN4741 to evaluate neurotoxic effects of the drug. The cells were treated with 25C-NBOMe (25–400  $\mu$ M) and measured after 24 h by the in vitro cytotoxicity assay with MTT dye (MTT) a colorimetric assay for assessing cell metabolic activity [15]. It was found that concentrations above 100  $\mu$ M of 25C-NBOMe significantly decreased cell viability in all three cell lines. An increased apoptotic process was observed only for SN4741 cells suggesting preferentially potent neurotoxicity of 25C-NBOMe in the dopamine cells. Xu and co-workers also showed that 25C-NBOMe concentration of 50  $\mu$ M inhibited activities of phosphorylated Akt-kinase (pAkt) and pSer9 (GSK3b Antibody)-Glycogen synthase kinase 3 beta (GSK3 $\beta$ ) and enhanced expression of pERK. Thus 25C-NBOMe may produce in vitro neurotoxicity via a PI3-K/Akt pathway inhibition and the activation of the extracellular signal-regulated kinase (ERK) signaling pathway cascade by agonist action on 5-HT<sub>2A</sub> ERK [15, 16]. Gatch and co-workers have reported in their work that administration of 25C-NBOMe (0.5, 1, 2.5, and 5 mg/kg) resulted in time- and dose-dependent locomotor activity depression with depressant effects occurred within 10 min after injection which lasted

30–60 min [17]. Moreover, 25C-NBOMe (0.5, 1, 2.5, and 5 mg/kg) generated substantial fluctuations in drug-appropriate responding with maximum effects of approaching 80% drug-appropriate responding. To our best knowledge there is no data about the abuse potential of 25C-NBOMe neither in animals or in humans. Importantly, these novel hallucinogen is extremely potent and psychoactive at microgram doses when taken buccally, administrated sublingually and insufflated.

Wohlfarth et al. have demonstrated in in vitro and in vivo studies that 25C-NBOMe is metabolized by *O*-demethylation, *O*-di-demethylation and hydroxylation. According to authors, all methoxy groups in 25C-NBOMe could be demethylated with hydroxylation preferably occurred at the NBOMe ring [18]. Furthermore, phase I metabolites were extensively conjugated with glucuronic acid and sulfate in human urine [18]. Caspara et al., have investigated the metabolism of 25C-NBOMe in rats and humans using liquid chromatography coupled to mass spectrometry high-resolution [19]. 25C-NBOMe was metabolized by *O*-demethylation, *O*, *O*-bis-demethylation and hydroxylation. Sixty nine phase I metabolites were identified. Most metabolites were common for all investigated species but *N*-dealkylated, *O*-demethylated metabolites and various isomers of *O*, *O*-bis-demethyl-hydroxy metabolites were detected in rat urine only [19].

## Effects of 25C-NBOMe intake

According to internet web sites dedicated to the hallucinogenic substances users—Erowid, 25-NBOMe acts as an active hallucinogen agent at a dose of 200–500 µg when insufflated. The users described effects as light for 50–200 µg, mild for 200–350 µg, strong for 350–700 µg, and very strong after intake of higher doses. Three hundred to six hundred µg doses taken buccally are only one third potency of LSD (2C-C-NBOMe Dose—Erowid). When administered sublingually, the threshold for the onset of hallucinogenic effects reportedly is about 100–250 µg, with mild, strong, and very strong effects after 250–450, 450–800, and over 800 µg, respectively [20, 21]. Overdose of 25C-NBOMe have been linked to hospitalizations due to multi-organ failures and deaths [22, 23].

## Applied analytical methods

Non-fatal case studies have reported various psychiatric and physiological effects of 25C-NBOMe intake, like hallucination [7], violent agitation, euphoria, insomnia, rhabdomyolysis and kidney injury, tachycardia, hypertension, seizures, hyperpyrexia [24]. Zygowiec et al. have described

a case of a 27-year-old male with confirmed ingestion of a psychoactive substance. High-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) detected 25C-NBOMe, 25H-NBOMe and 25B-NBOMe in blood samples with concentrations above the 0.50 ng/mL reporting limits [25]. Rajotte et al. have described toxicological analyses including 25C-NBOMe in a drug-impaired driver. Gas chromatography-mass spectrometry (GC–MS) showed a mass spectral identification of this agent [26]. First the driver urine sample was analysed by two immunoassay panels screened for low concentration of psychoactive substances. GC–MS was performed for beta-hydroxybutyrate (20 mg/L) and gamma-hydroxybutyrate (2.5 mg/L) analysis. The only positive drug finding from these analyses was an NBOMe compound. Retrospectively, the detected compound was identified as 25C-NBOMe [26]. A young male died in a hospital approximately 12 h after the use of the psychoactive substance. The death was preceded by hallucinations and convulsions. A hospital examination showed a wild spectrum of physiological effects. 25C-NBOMe and its demethylated and glucuronidated metabolites were identified in urine and whole blood using ultra-performance liquid chromatography with high-resolution time-of-flight mass spectrometry (UPLC–HRTOF–MS) and ultra-performance liquid chromatography with tandem mass spectrometry (UPLC–MS/MS) [27]. The limit of detection and lower limit of quantification were 0.02 mg/kg and 0.08 mg/kg, respectively [27]. In turn Murini and co-workers have reported a case of a teenager male who was found dead in a waterway after jumping off into water stream [28]. The death occurred by drowning but to evaluate the potential role of psychoactive substances the toxicological exams were performed. Peripheral and central blood as well urine samples were collected and analysed by liquid chromatography tandem mass spectrometric (LC–MS/MS). The method was linear over the range from 0.1 to 5.0 ng/mL. Accuracy and imprecision were measured, calibration curve used in this at two different quality controls (0.2 and 1.0 ng/mL) and were found to be within the 15%. Five 25-NBOMes, including 25C-NBOMe, were identified in the samples [28]. An accidental death of a 23-year-old male has been reported by Kristofic et al. A fast liquid chromatography quadrupole time-of-flight mass spectrometry (LC–QTOF–MS) with a basic solid-phase extraction was employed to isolate 25C-NBOMe, 25C-NBOH and 2C–C from blood and urine specimens. An LC–MS/MS analysis exhibited the presence of 25C-NBOMe and 2C–C in blood and urine samples The QTOF mass spectrometer was operated in positive electrospray ionization mode utilizing MSE acquisition, which permits simultaneous acquisition under low collision energy and high collision energy functions [29]. Similarly, Soh and Ellion have showed two fatal cases in which a possible metabolite of 25C-NBOMe was detected in blood and urine [30]. Detection and identification

**Table 1** Effect of 25C-NBOMe intake, analytical methods and the result of the real sample analysis

## The effect of 25-NBOMe intake

## Psychiatric effects

Positive effects: mental stimulation, physical stimulation, creative thinking, mood lift, open and closed eye visuals, increased awareness, life-changing spiritual experiences, euphoria [8]

Negative effects: general change in consciousness, pupil dilation, difficulty focusing, unusual body sensations, change in perception of time, slight increase in heart rate, hot flushes and/or cold chills [8]

Neutral effects: nausea, insomnia, paranoia, fear, panic, unwanted and overwhelming feelings, unwanted life-changing spiritual experiences [8]

## Physiological effects

Organ injuries: rhabdomyolysis and kidney injury [24]

Cardiological effects: tachycardia, hypertension, seizures, hyperpyrexia [24]

Other: dry mucous membranes and skin [25], bleeding from all mucosa, respiratory and metabolic acidosis, high lactic acid, anuria, hyperthermia, hyperkalaemia [27]

## Analytical methods

High performance liquid chromatography: tandem mass spectrometry (HPLC–MS/MS)

Gas chromatography: mass spectrometry (GC–MS)

Ultra: performance liquid chromatography with high-resolution time-of-flight mass spectrometry (UPLC–HRTOF–MS)

Ultra-performance liquid chromatography with tandem mass spectrometry (UPLC–MS/MS)

Liquid chromatography tandem mass spectrometric (LC–MS/MS)

Fast liquid chromatography quadrupole time-of-flight mass spectrometry (LC–QTOF–MS)

Ultra high performance liquid chromatography with high mass accuracy quadrupole time-of-flight mass spectrometry (UHPLC–Q–TOF–MS)

High performance liquid chromatography with diode-array detection (HPLC–DAD)

Liquid chromatography with mass spectrometry (LC–MS)

Fourier-transform infrared spectrometry (FTIR)

Nuclear magnetic resonance spectroscopy (NMR)

## Result of the real sample analysis (25-NBOMe examples concentration in biological samples by different analytical methods)

## Blood samples

Peripheral blood: 2.80 ng/mL by LC–MS/MS [28]

Central blood: 1.43 ng/mL by LC–MS/MS [28]

Post-mortem peripheral blood: 0.60 mg/kg by UPLC–HRTOF–MS and UPLC–MS/MS [27]

Ante-mortem whole blood: 0.81 mg/kg by UPLC–HRTOF–MS and UPLC–MS/MS [27]

## Urine samples

Urine: 0.94 ng/mL by LC–MS/MS [28]

## Liver

Liver: 0.82 mg/kg by UPLC–HRTOF–MS and UPLC–MS/MS [27]

Liver: 15.2 ng/g by LC–QTOF–MS and LC–MS/MS [29]

## Gastric content

Gastric sample: 0.32 mg by UPLC–HRTOF–MS and UPLC–MS/MS [27]

Gastric contents: 30.2 µg total in 100 mL by LC–QTOF–MS and LC–MS/MS [29]

## Vitreous humour samples

Vitreous humor: 0.33 mg/kg UPLC–HRTOF–MS and UPLC–MS/MS [27]

## Brain

Brain: 19.1 ng/g by LC–QTOF–MS and LC–MS/MS [29]

## Spleen

Spleen: 27.1 ng/g by LC–QTOF–MS and LC–MS/MS [29]

## Lungs

Lung: 25.2 ng/g by LC–QTOF–MS and LC–MS/MS [29]

## Kidneys

Kidney: 25.1 ng/g by LC–QTOF–MS and LC–MS/MS [29]

of 25C-NBOMe were carried out using high-performance liquid chromatography with diode-array detection (HPLC-DAD), LC-MS/MS and ultra high performance liquid chromatography with high mass accuracy quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS) [30].

Till now 25C-NBOMe was identified in ante-mortem and post-mortem samples of whole blood, liver, urine, gastric content, and vitreous humour samples [27]. Zuba and co-workers have proposed analytical procedures for the identification of 25C-NBOMe in blotter papers originating from the drug market [20]. Several analytical techniques were applied to detect this drug such as GC-MS with and without derivatization with trifluoroacetic anhydride, liquid chromatography with mass spectrometry (LC-MS), Fourier-transform infrared spectrometry (FTIR) and nuclear magnetic resonance (NMR) spectroscopy [31]. The dominant ions, representatives of 25-NBOMe series in GC-MS spectrum of 25C-NBOMe were observed at  $m/z = 121, 150$  and  $91$  [31]. The FTIR spectrum of the sample was recorded in the  $600\text{--}4000\text{ cm}^{-1}$  rang. The strong peaks assigned to C–O–C (25-NBOMe series, are characterized by the asymmetric C–O–C stretch vibrations near) vibrations were observed at  $1036\text{ cm}^{-1}$ ,  $1215\text{ cm}^{-1}$  and  $1252\text{ cm}^{-1}$  [31]. In case of NMR spectroscopy, the signals in the  $^1\text{H}$  and  $^{13}\text{C}$  spectra were assigned on the basis of one- and two-dimensional homo- and heteronuclear experiments [20].

The table below shows the effect of 25C-NBOMe intake, analytical methods and the result of the real sample analysis (Table 1).

## Conclusions

The case studies clearly indicated that the exposure on 25C-NBOMe leads to fatal and non-fatal intoxication of its users and it can be unwittingly ingest instead of LSD. However, a number of NBOMe-related intoxications and deaths could be underestimated due to the lack of proper and sensitive analytical methods. Despite the fact of recognised fatal cases there is still a lack of experimental studies explaining the mechanism of its action and providing information about pharmacological properties and toxicity. Although adverse effects of 25C-NBOMe are known, long-lasting and chronic usage effects have not been recognised so far. To overcome these issues and to develop analytical procedures of identification of this toxic agent further extensive studies on the 25C-NBOMe action are urgently needed.

## Compliance with ethical standards

**Conflict of interest** The authors declare that he has no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants performed by the authors.

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