

# Pharmacology and Toxicology of *N*-Benzylphenethylamine (“NBOMe”) Hallucinogens

Adam L. Halberstadt

**Abstract** Serotonergic hallucinogens induce profound changes in perception and cognition. The characteristic effects of hallucinogens are mediated by 5-HT<sub>2A</sub> receptor activation. One class of hallucinogens are 2,5-dimethoxy-substituted phenethylamines, such as the so-called 2C-X compounds 2,5-dimethoxy-4-bromophenethylamine (2C-B) and 2,5-dimethoxy-4-iodophenethylamine (2C-I). Addition of an *N*-benzyl group to phenethylamine hallucinogens produces a marked increase in 5-HT<sub>2A</sub>-binding affinity and hallucinogenic potency. *N*-benzylphenethylamines (“NBOMes”) such as *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine (25I-NBOMe) show subnanomolar affinity for the 5-HT<sub>2A</sub> receptor and are reportedly highly potent in humans. Several NBOMes have been available from online vendors since 2010, resulting in numerous cases of toxicity and multiple fatalities. This chapter reviews the structure–activity relationships, behavioral pharmacology, metabolism, and toxicity of members of the NBOMe hallucinogen class. Based on a review of 51 cases of NBOMe toxicity reported in the literature, it appears that rhabdomyolysis is a relatively common complication of severe NBOMe toxicity, an effect that may be linked to NBOMe-induced seizures, hyperthermia, and vasoconstriction.

**Keywords** Head twitch response • Locomotor activity • Psychedelic • Research chemical • Serotonin syndrome

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## 1 Introduction

Serotonergic hallucinogens, also known as classical hallucinogens or psychedelics, produce marked alterations of perception, mood, and cognition (reviewed by Halberstadt [1] and Nichols [2]). Indoleamines and phenylalkylamines are the two main structural classes of hallucinogens. The phenylalkylamines can be divided into two categories: *phenethylamines*, including mescaline and the 2C-X compounds 2,5-dimethoxy-4-bromophenethylamine (2C-B) and 2,5-dimethoxy-4-iodophenethylamine (2C-I), and *phenylisopropylamines* (“amphetamines”) such as 2,5-dimethoxy-4-bromoamphetamine (DOB) and 2,5-dimethoxy-4-methylamphetamine (DOM). In contrast to indoleamine hallucinogens, which are relatively nonselective for serotonin (5-HT) receptors, phenylalkylamine hallucinogens are highly selective for 5-HT<sub>2</sub> sites.

The characteristic effects of serotonergic hallucinogens are believed to be mediated by activation of 5-HT<sub>2A</sub> receptors. Pretreatment with the 5-HT<sub>2A</sub> antagonist ketanserin blocks the hallucinogenic effects of psilocybin and the botanical hallucinogen *ayahuasca* in human volunteers [3–5]. Similarly, most of the behavioral effects of hallucinogens in laboratory animals are linked to 5-HT<sub>2A</sub> activation [1]. There is also a significant correlation between 5-HT<sub>2A</sub> affinity and hallucinogen potency [6, 7].

Although designer drugs are not a new phenomenon, the number and availability of new psychoactive substances (NPS) are unprecedented and have increased dramatically over the last 5 years. At least 299 different NPS were available across Europe in 2013, with an additional 101 new drugs appearing in 2014 [8]. The four main classes of NPS are cannabinoids, psychostimulants, opioids, and hallucinogens. Cannabinoids and psychostimulants are the most commonly abused NPS, but hallucinogenic NPS are also very popular and their proliferation is causing a significant public health problem.

Internet vendors have been marketing a class of hallucinogens known as *N*-benzylphenethylamines (NBOMes) as NPS since 2010 [9, 10]. *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine (25I-NBOMe) was the first NBOMe to appear on the illicit market, followed by *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-bromophenethylamine (25B-NBOMe) and *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-chlorophenethylamine (25C-NBOMe) [11, 12]. Numerous other NBOMes have now been detected [13–15]. The most common method of NBOMe distribution is on blotter paper, but powdered material, solutions, and pills are also available. 25I-NBOMe is reportedly a highly potent hallucinogen in humans, with typical doses ranging between 0.5 and

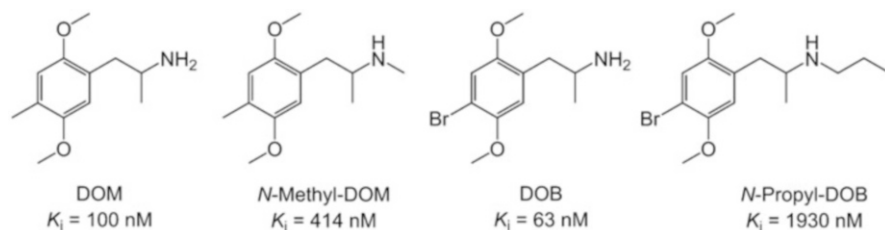
1 mg. NBOMes are reportedly not active orally and are usually taken sublingually or buccally.

## 2 Structure–Activity Relationships of NBOMe Hallucinogens

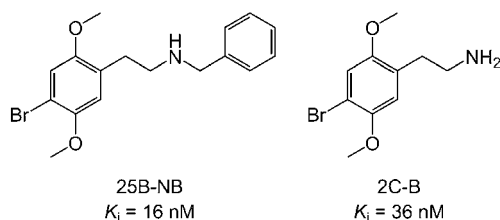
For phenylalkylamine hallucinogens, *N*-alkyl substitution results in a marked reduction of 5-HT<sub>2A</sub> affinity and behavioral potency (Fig. 1). For example, 2,5-dimethoxy-4-methylamphetamine (DOM;  $K_i = 100$  nM) has higher affinity than *N*-methyl-DOM ( $K_i = 414$  nM) for 5-HT<sub>2A</sub> sites labeled by [<sup>3</sup>H]ketanserin in rat frontal cortex homogenates [17]. When tested in rats trained to discriminate DOM (1.0 mg/kg, IP) from saline [18], *N*-methyl-DOM (ED<sub>50</sub> = 3.99 mg/kg, IP) was found to be ninefold less potent than DOM (ED<sub>50</sub> = 0.44 mg/kg, IP). It has also been reported that addition of an *N*-methyl group to DOM produces a tenfold reduction of hallucinogenic potency in humans [19]. The presence of a longer alkyl group is apparently even more detrimental; compared to 2,5-dimethoxy-4-bromoamphetamine (DOB;  $K_i = 63$  nM; [17]), *N*-propyl-DOB has much lower affinity for 5-HT<sub>2A</sub> sites ( $K_i = 1,930$  nM [16]).

Surprisingly, however, the presence of an *N*-benzyl group can actually *increase* 5-HT<sub>2A</sub> affinity. Glennon first reported in 1994 [20] that *N*-benzyl-2,5-dimethoxy-4-bromophenethylamine (25B-NB;  $K_i = 16$  nM vs. [<sup>3</sup>H]ketanserin) has higher binding affinity than the unsubstituted parent compound 2,5-dimethoxy-4-bromophenethylamine (2C-B;  $K_i = 36$  nM) for 5-HT<sub>2A</sub> receptors labeled with [<sup>3</sup>H]ketanserin (see Fig. 2). Although Glennon et al. did not report functional data, further work, published in abstract format, confirmed that *N*-benzylphenethylamines act as potent 5-HT<sub>2A</sub> agonists [21, 22].

Nichols and colleagues subsequently conducted a detailed investigation of the effects of *N*-benzyl substitution on the 5-HT<sub>2A</sub> receptor-binding affinity and efficacy of phenylalkylamine hallucinogens [23]. These investigations revealed several important findings. First, although *N*-benzyl substitution consistently increases the 5-HT<sub>2A</sub> affinity of phenethylamine hallucinogens, compounds having low-to-moderate affinity tend to be the most sensitive to the substitution. For example, for



**Fig. 1** Effect of *N*-alkyl substitution of the binding affinity of 2,5-dimethoxy-4-methylamphetamine (DOM) and 2,5-dimethoxy-4-bromoamphetamine (DOB) for 5-HT<sub>2A</sub> receptors labeled with [<sup>3</sup>H]ketanserin in rat brain homogenates [16, 17]



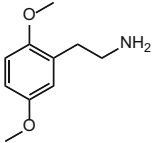
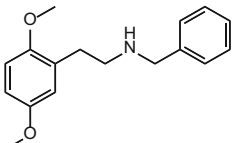
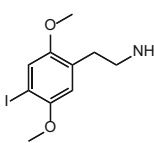
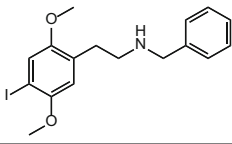
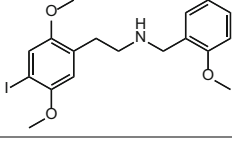
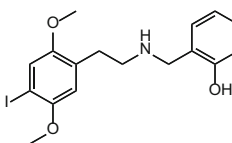
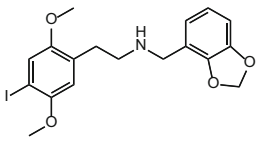
**Fig. 2** Comparison of the affinities of *N*-benzyl-2,5-dimethoxy-4-bromophenethylamine (25B-NB) and 2,5-dimethoxy-4-bromophenethylamine (2C-B) for 5-HT<sub>2A</sub> receptors labeled with [<sup>3</sup>H]ketanserin in rat frontal cortex homogenates [20]

2,5-dimethoxyphenethylamine (2C-H), which binds to 5-HT<sub>2A</sub> sites with moderate affinity, the addition of an *N*-benzyl group increased affinity five- to tenfold (see Table 1). By contrast, the effect of *N*-benzylation on the affinity of 2,5-dimethoxy-4-iodophenethylamine (2C-I), which has relatively high affinity for 5-HT<sub>2A</sub> sites, is comparatively modest. Second, addition of an *N*-benzyl group with an oxygenated substituent at the ortho position results in an even greater increase in 5-HT<sub>2A</sub> affinity. As shown in Table 1, 25I-NBOMe, 25I-NBOH, and 25I-NBMD have higher 5-HT<sub>2A</sub> affinity than 25I-NB.

Homology modelling [23] indicates that the presence of an *N*-benzyl moiety increases 5-HT<sub>2A</sub> receptor affinity because the benzyl ring is stabilized by aromatic stacking with Phe<sup>339</sup> in transmembrane domain 6 (TM6). Indeed, mutagenesis of Phe<sup>339</sup> does not normally alter the binding affinity of 5-HT<sub>2A</sub> agonists [24] but does detrimentally affect the affinity and agonist activity of 25I-NBOMe and other *N*-benzyl-substituted phenethylamines [23]. Replacement of the *N*-benzyl ring in 25B-NBOMe with an electron-deficient heterocyclic ring (e.g., *N*-pyridinyl) reportedly produces a marked reduction in 5-HT<sub>2A</sub> affinity (see Fig. 3), which is consistent with the evidence that electron-deficient ring systems produce relatively weak aromatic stacking interactions [27]. One explanation proposed to account for the effect of an oxygenated *N*-benzyl ring on affinity is that it may serve as a hydrogen-bond (H-bond) acceptor. Indeed, in silico homology models predict that the 2-position oxygen can form a H-bond with Tyr<sup>370</sup> in TM7 [28, 29]. Some attempts to investigate the predicted interaction, however, have yielded conflicting findings. For example, according to an unpublished study, mutation of Tyr<sup>370</sup> to Phe (which cannot form a H-bond) does not significantly alter the affinity of 25I-NBOMe [30]. Other studies indicate that Tyr<sup>370</sup> plays a general role in 5-HT<sub>2A</sub> signal transduction [24] and is unlikely to interact directly with the *N*-benzyl moiety. As an alternative to Tyr<sup>370</sup>, the 2-position oxygen may interact with a different H-bond donor, e.g., with an amine moiety in the protein backbone.

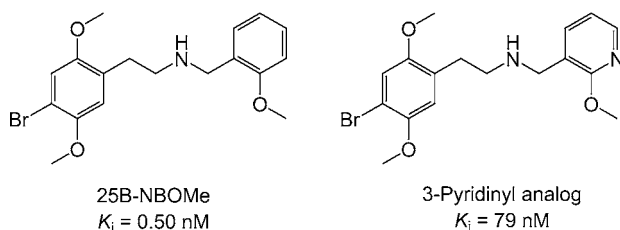
In rodents, 5-HT<sub>2A</sub> receptor activation induces the head twitch response (HTR), a rapid paroxysmal head rotation [31–33]. The HTR is widely used as a behavioral proxy in rodents for hallucinogen effects in humans and is one of the few behaviors that can reliably be used to distinguish hallucinogenic and non-hallucinogenic 5-HT<sub>2A</sub> agonists [34]. Although the HTR is usually assessed by direct observation, we have

**Table 1** Effect of *N*-benzyl substitution of the 5-HT<sub>2A</sub> affinity (*K*<sub>i</sub>, nM) of phenethylamine hallucinogens at 5-HT<sub>2A</sub> receptors labeled with [<sup>125</sup>I]DOI or [<sup>3</sup>H]ketanserin

| Ligand name | Structure                                                                           | Rat 5-HT <sub>2A</sub> | Human 5-HT <sub>2A</sub> | Human 5-HT <sub>2A</sub>    |
|-------------|-------------------------------------------------------------------------------------|------------------------|--------------------------|-----------------------------|
|             |                                                                                     | [ <sup>125</sup> I]DOI | [ <sup>125</sup> I]DOI   | [ <sup>3</sup> H]ketanserin |
| 2C-H        |    | 227                    | 377                      | 1,999                       |
| 25H-NB      |    | 17.5                   | 68.1                     | 184                         |
| 2C-I        |    | 0.62                   | 0.73                     | 4.52                        |
| 25I-NB      |    | 0.31                   | 0.25                     | 0.28                        |
| 25I-NBOMe   |   | 0.087                  | 0.044                    | 0.15                        |
| 25I-NBOH    |  | 0.12                   | 0.061                    | 0.068                       |
| 25I-NBMD    |  | 0.19                   | 0.049                    | 0.21                        |

Data from Braden et al. [23]

developed a magnetometer coil-based system for recording head movements that can detect the behavior with high sensitivity and reliability [33]. The HTR detection



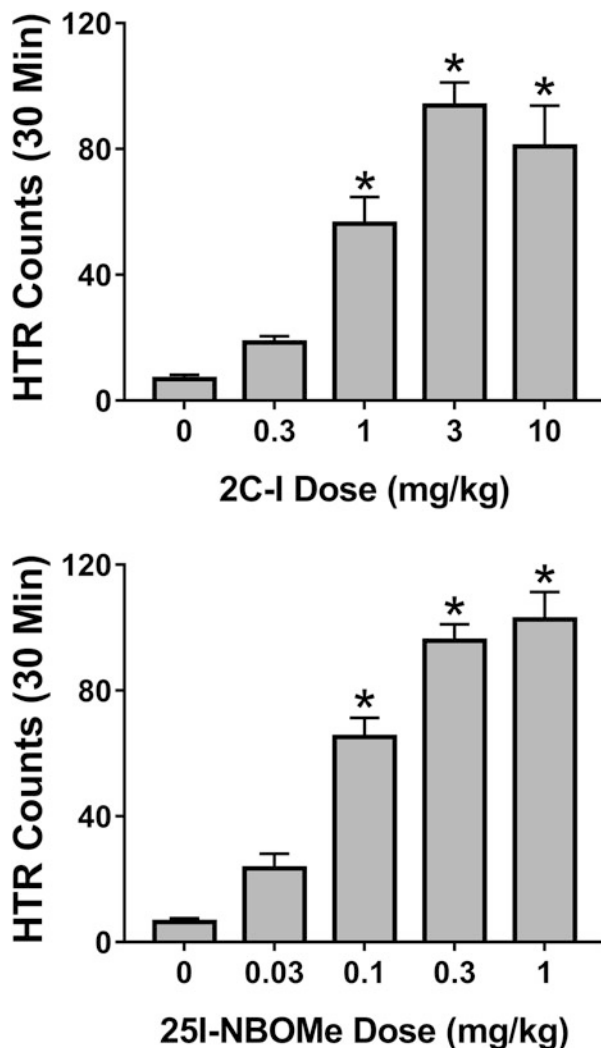
**Fig. 3** Comparison of the 5-HT<sub>2A</sub> affinities of *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-bromophenethylamine (25B-NBOMe) [25] and its 3-pyridinyl analog [26]. Affinity was assessed at human 5-HT<sub>2A</sub> receptors labeled with [<sup>3</sup>H]ketanserin

system has been used to assess the HTR induced by a variety of hallucinogens and analogs [35–39].

We have used the HTR to assess whether *N*-benzyl substitution alters the behavioral pharmacology of phenethylamines. Our experiments demonstrated that 25I-NBOMe produces a robust HTR in C57BL/6J mice [37]. After subcutaneous (SC) administration, 25I-NBOMe induced the HTR with an ED<sub>50</sub> of 78 µg/kg (0.17 µmol/kg), making it slightly less potent than LSD, which induces the HTR with an ED<sub>50</sub> of 53 µg/kg (0.13 µmol/kg; [33]). Additionally, as shown in Fig. 4, 25I-NBOMe has tenfold higher potency than 2C-I (ED<sub>50</sub> = 830 µg/kg; 2.42 µmol/kg), which is consistent with their relative binding affinities at 5-HT<sub>2A</sub> receptor sites (Table 1). According to another study, 25B-NBOMe induces the HTR in mice with similar potency to that of 25I-NBOMe [40]. Pretreatment with M100907, a 5-HT<sub>2A</sub> receptor antagonist that is highly selective versus 5-HT<sub>2C</sub> sites, produced a dose-dependent blockade of the HTR induced by 25I-NBOMe [37]. Hence, the results of our studies are consistent with anecdotal evidence that 25I-NBOMe acts as a hallucinogen in humans with potency approaching that of LSD. 25I-NBMD also induces the HTR but its potency (ED<sub>50</sub> = 1.13 mg/kg; 2.36 µmol/kg) is not as high as would be anticipated based on its 5-HT<sub>2A</sub> affinity.

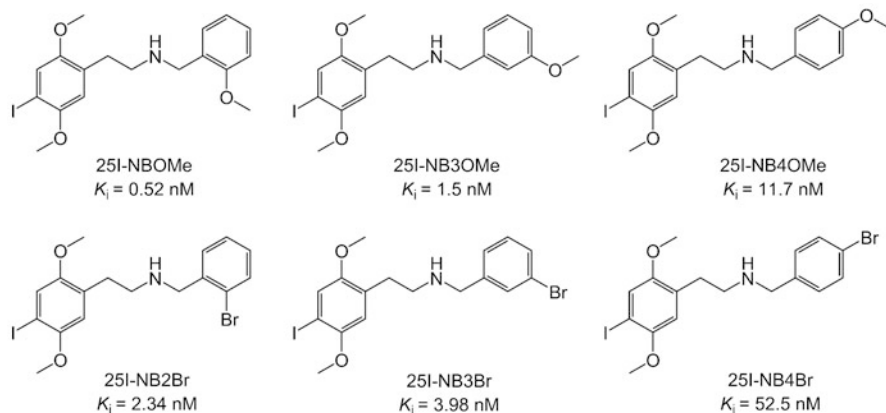
There appear to be strict steric and positional constraints on the *N*-benzyl moiety in NBOMes. As shown in Fig. 5, the effect of the *N*-benzyl ring H-bond acceptor on 5-HT<sub>2A</sub> affinity depends on its position. Moving the *ortho*-methoxy group in 25I-NBOMe to the meta position (25I-NB3OMe) or the para position (25I-NB4OMe) progressively reduces 5-HT<sub>2A</sub> affinity [39]. H-bond formation is dependent on the distance between acceptor and donor; hence, the diminished 5-HT<sub>2A</sub> affinities of the meta and para isomers of 25I-NBOMe are consistent with the methoxy group on the *N*-benzyl ring being moved away from a H-bond donor in the binding pocket. However, steric factors may also contribute to the reduction of 5-HT<sub>2A</sub> affinity that occurs with *para*-methoxy substitution. Substitution of bromine in the para position in the *N*-benzyl ring (25I-NB4Br) produces a >tenfold reduction in affinity compared with substitution in the *ortho* (25I-NB2Br) or meta (25I-NB3Br) positions (see Fig. 5) [39]. These findings indicate that the region of the binding pocket proximal to the para position of the *N*-benzyl ring may be sterically constrained. The existence of such steric constraints may

**Fig. 4** Head twitch response (HTR) induced by 2C-I (*top*) and 25I-NBOMe (*bottom*) in male C57BL/6J mice. Data are mean  $\pm$  SEM. \* $p < 0.01$  vs. vehicle control group. Redrawn from Halberstadt and Geyer [37]



explain why replacement of the *N*-benzyl group in 25I-NB with a bulky *N*-naphthyl group reduces 5-HT<sub>2A</sub> affinity by a factor of ten- to 20-fold [23].

We have compared the behavioral potencies of the compounds shown in Fig. 5 using the HTR assay [39]. For the methoxy-substituted regioisomers, moving the methoxy group from the ortho position (25I-NBOMe) to the meta position (25I-NB3OMe) produced a significant drop in potency ( $ED_{50} = 4.34$  mg/kg;  $9.36$   $\mu$ mol/kg), whereas the para isomer (25I-NB4OMe) was inactive at doses up to 30 mg/kg SC. Although it is not clear why the potency of the meta regioisomer 25I-NB3OMe is so low compared to 25I-NBOMe, 25I-NB3OMe was observed to have a relatively brief duration of action in mice (data not shown), meaning the clearance rate of 25I-NB3OMe in mice may limit



**Fig. 5** Comparison of the binding affinities of the regoisomers of 25I-NBOMe (*top row*) and 25I-NB2Br (*bottom row*) at human 5-HT<sub>2A</sub> receptors labeled with [<sup>3</sup>H]ketanserin [39]

the magnitude of its behavioral response. Nevertheless, the relative potencies of the methoxy-substituted regoisomers (*ortho* > *meta* > *para*) are consistent with their relative 5-HT<sub>2A</sub> affinities. According to studies with the bromine-substituted regoisomers, the *ortho*-bromo isomer 25I-NB2Br is active ( $ED_{50} = 2.31$  mg/kg;  $4.50$   $\mu$ mol/kg) but no HTR was observed with the *meta*- or *para*-bromo isomers (25I-NB3Br and 25I-NB4Br, respectively) at doses up to 30 mg/kg.

Differences exist between the structure–activity relationships (SAR) of hallucinogens in the NBOMe and phenylalkylamine classes. First, there is a difference in the effect of  $\alpha$ -methyl substitution. Compared to their  $\alpha$ -desmethyl congeners, phenylisopropylamine hallucinogens have higher intrinsic activities at 5-HT<sub>2A</sub>, which is thought to be the reason why the phenylisopropylamines have higher potency *in vivo* [41, 42]. With NBOMes, however, the presence of an  $\alpha$ -methyl group reduces intrinsic activity and 5-HT<sub>2A</sub> affinity [23]. According to Braden et al., adding an  $\alpha$ -methyl group to 25I-NBOMe reduced its efficacy ( $E_{max}$ ) from 78% to 43% and produced a 12-fold reduction of affinity for rat 5-HT<sub>2A</sub> receptors labeled with [<sup>125</sup>I]DOI.

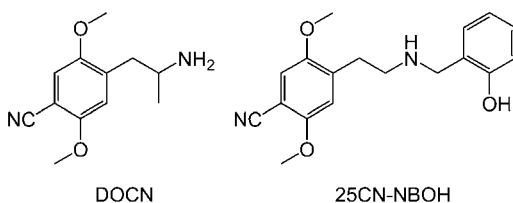
Second, compared to phenylalkylamine hallucinogens, NBOMes are less sensitive to the loss of an oxygenated five-position substituent. It is well established that 2,5-dimethoxy substitution is optimal for activity in phenylalkylamine hallucinogens. The 5-methoxy group is believed to interact with Ser<sup>239</sup> in TM5 of the 5-HT<sub>2A</sub> receptor [43]. Based on the results of mutagenesis studies performed by Braden and Nichols, it appears that Ser<sup>239</sup> donates a H-bond to the 5-methoxy in DOM. Indeed, removal of the 5-methoxy group in DOM produces a 28-fold reduction in 5-HT<sub>2A</sub> affinity and a 130-fold reduction in agonist potency [43]. Interestingly, however, removal of the 5-methoxy group from 25B-NBOMe produces only a tenfold reduction in 5-HT<sub>2A</sub> affinity and does not appreciably alter agonist potency [44]. Hence, the 5-methoxy group in NBOMes may not play an essential role in 5-HT<sub>2A</sub> binding and activation.

### 3 Discovery of NBOMes That Are Selective for 5-HT<sub>2A</sub> Vs. 5-HT<sub>2C</sub> Receptors

5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors display parallel structure–affinity relationships for ligand binding [20, 45, 46]. Hallucinogens display nonselective agonist activity at 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors [46, 47]. The cloning of rat and human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors [48, 49] revealed that these 5-HT receptor subtypes exhibit significant sequence homology, especially in the  $\alpha$ -helical regions where the ligand-binding sites are located. Depending upon the species examined, the seven trans-membrane domains of 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors display between 79 and 80% sequence conservation. Because of the high degree of structural homology shared by 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors, it is not surprising that most ligands that bind to 5-HT<sub>2A</sub> sites are also capable of interacting with 5-HT<sub>2C</sub> sites.

Similar to other classes of hallucinogens, NBOMes act as 5-HT<sub>2C</sub> receptor agonists and are relatively nonselective for 5-HT<sub>2A</sub> vs. 5-HT<sub>2C</sub> sites [39]. Given their high affinity and efficacy at 5-HT<sub>2A</sub> receptors, NBOMes have been developed as 5-HT<sub>2A</sub> agonist radioligands [50] and as PET tracers [51, 52]. Such work has also encouraged development of NBOMes exhibiting selectivity for 5-HT<sub>2A</sub> receptors compared with 5-HT<sub>2C</sub> sites. In contrast to other phenylisopropylamines, 2,5-dimethoxy-4-cyanoamphetamine (DOCN, Fig. 6) exhibits moderate (22-fold) selectivity for human 5-HT<sub>2A</sub> ( $K_i = 45.7$  nM) vs. 5-HT<sub>2C</sub> ( $K_i = 1,011$  nM) sites labeled with [<sup>125</sup>I]DOI [46], indicating that 4-cyano substitution represents a potential strategy to augment 5-HT<sub>2A</sub> selectivity. Applying this strategy to the NBOME class led to the discovery of *N*-(2-hydroxybenzyl)-2,5-dimethoxy-4-cyanophenethylamine (25CN-NBOH), which is reportedly 100-fold selective for 5-HT<sub>2A</sub> receptors ( $K_i = 1.3$  nM vs. [<sup>3</sup>H]ketanserin) compared with 5-HT<sub>2C</sub> receptors ( $K_i = 132$  nM vs. [<sup>3</sup>H]mesulergine) [25]. However, according to a more recent investigation using the same antagonist radioligands, 25CN-NBOH is only 23-fold selective for 5-HT<sub>2A</sub> receptors ( $K_i = 2.2$  nM) relative to 5-HT<sub>2C</sub> sites ( $K_i = 49.8$  nM) [38]. Although the selectivity of 25CN-NBOH may be less than was initially believed, it still exhibits moderate 5-HT<sub>2A</sub> selectivity. Importantly, we have confirmed that SC administration of 25CN-NBOH induces the HTR in mice with moderate potency ( $ED_{50} = 0.36$  mg/kg;  $1.03$   $\mu$ mol/kg [38]). Another group has confirmed that the HTR induced by 25CN-NBOH is blocked by pretreatment with  $0.01$  mg/kg M100907 [53]. The latter study also showed that pretreatment with 25CN-NBOH produces a dose-dependent blockade of the HTR induced by *R*-(-)-2,5-

**Fig. 6** Structures of the 4-cyano-substituted phenylalkylamines DOCN and 25CN-NBOH

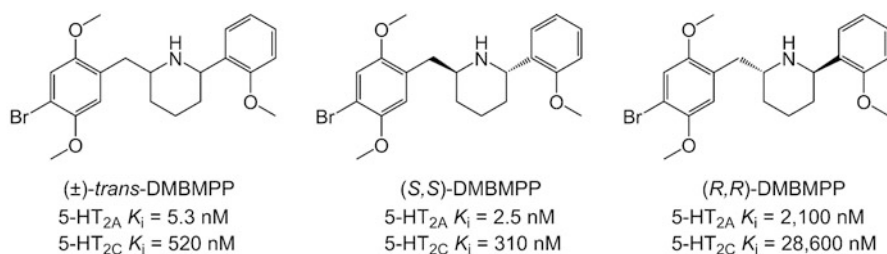


dimethoxy-4-iodoamphetamine (*R*-(–)-DOI), indicating that 25CN-NBOH acts as a partial agonist.

NBOMes exhibit a high degree of conformational flexibility and could potentially adopt a range of active binding poses. In order to identify the active conformation, Nichols and colleagues synthesized a series of rigid analogues of 25B-NBOMe [54]. Of the nine structurally constrained compounds tested, ( $\pm$ )-*trans*-DMBMPP (Fig. 7) was the most potent, binding to human 5-HT<sub>2A</sub> receptors with a  $K_i$  of 5.3 nM. Interestingly, the affinity of ( $\pm$ )-*trans*-DMBMPP for human 5-HT<sub>2C</sub> sites is significantly lower in comparison, making it 98-fold selective for 5-HT<sub>2A</sub> receptors. The (*S,S*) enantiomer of DMBMPP, resolved by derivatization with a chiral auxiliary, has even higher 5-HT<sub>2A</sub> affinity ( $K_i$  = 2.5 nM) and is reportedly 124-fold selective for 5-HT<sub>2A</sub> vs. 5-HT<sub>2C</sub> receptors. By contrast, (*R,R*)-DMBMPP has  $\mu$ M affinity for 5-HT<sub>2A</sub> receptors (Fig. 7). It appears that the structural configuration of (*S,S*)-DMBMPP closely mirrors the active binding conformation of NBOMes.

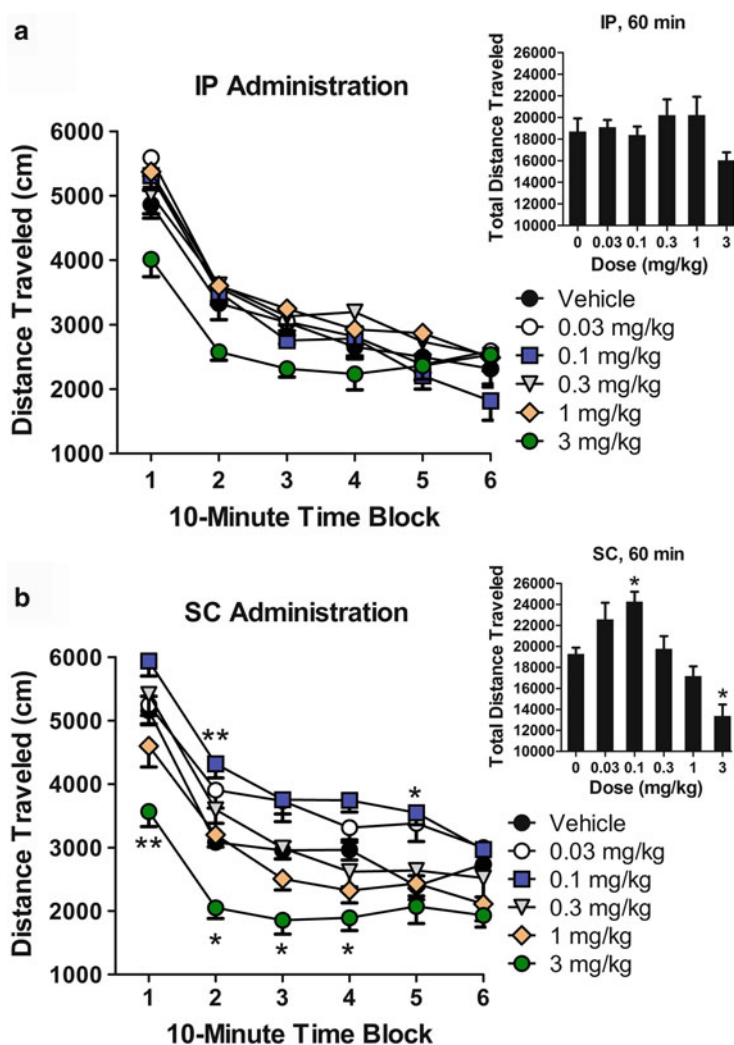
## 4 Other Behavioral Studies with NBOMes

Our previous studies have shown that phenylalkylamine hallucinogens produce dose-dependent effects on locomotor activity in C57BL/6J mice, increasing activity at low-to-moderate doses and reducing activity at higher doses [55, 56]. For example, DOI and DOM increase locomotor activity at 1 mg/kg and reduce activity at 10 mg/kg [32, 56]. The hyperactivity produced by DOI and other phenylalkylamines is blocked by M100907 and is absent in 5-HT<sub>2A</sub> knockout mice, indicating mediation by 5-HT<sub>2A</sub> receptors [55, 56]. To determine whether 25I-NBOMe produces similar effects on locomotor activity, we conducted dose-response studies in C57BL/6J mice after IP and SC administration. Administration of 25I-NBOMe by the IP route had no effect on locomotor activity (Fig. 8a), although there was a trend toward a main effect of drug treatment ( $F(5,54) = 2.08$ ,  $p < 0.09$ ) and a significant interaction between drug treatment and time block ( $F(25,270) = 2.22$ ,  $p < 0.001$ ). By contrast, when



**Fig. 7** Structures of racemic *trans*-2-(2,5-dimethoxy-4-bromobenzyl)-6-(2-methoxyphenyl)piperidine ( $\pm$ -*trans*-DMBMPP) and its *S,S* and *R,R* enantiomers. Binding affinities were assessed at human 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors labeled with [<sup>3</sup>H]ketanserin and [<sup>3</sup>H]mesulergine, respectively [54]

administered SC as in all the HTR experiments, 25I-NBOMe produced effects on locomotor activity that mimicked those induced by phenylalkylamine hallucinogens (drug effect:  $F(5,52) = 5.16$ ,  $p < 0.001$ ; drug  $\times$  time:  $F(25,260) = 2.26$ ,  $p < 0.001$ ). As shown in Fig. 8b, 0.1 mg/kg 25I-NBOMe increased locomotor activity during



**Fig. 8** Effect of 25I-NBOMe on locomotor activity after intraperitoneal (IP) (a) or subcutaneous (SC) (b) administration. Male C57BL/6J mice ( $n = 9-11$ , 60 total in experiment a;  $n = 8-10$ , 58 total in experiment b) were treated with vehicle or 25I-NBOMe (0.03, 0.1, 0.3, 1, and 3 mg/kg) and activity recorded in the mouse behavioral pattern monitor for 60 min. Data are presented as group means  $\pm$  SEM for successive 10-min intervals, or group means  $\pm$  SEM for the entire 60-min test session (inset histograms). \* $p < 0.05$ , \*\* $p < 0.01$ , significant difference from vehicle control group

time blocks 2 and 5 ( $p < 0.01$ , 0.05, Tukey's test), and 3 mg/kg 25I-NBOMe reduced locomotor activity during blocks 1, 2, 3, and 4 ( $p < 0.01$ , 0.05, Tukey's test). In summary, the effects of 25I-NBOMe on locomotor activity in mice mirror those produced by phenylalkylamine hallucinogens, but 25I-NBOMe appears to be unusually potent in comparison.

## 5 Toxicity of NBOMe Hallucinogens

Since their appearance in 2010, NBOMe hallucinogens have caused numerous cases of toxicity, sometimes with fatal consequences [11, 57]. The first incidents of toxicity linked to NBOMe use occurred in Richmond, Virginia [58], and in Grand Rapids, Michigan [59] in 2012. One hundred and forty-eight cases of NBOMe toxicity were reported to the National Poison Data System between September 2012 and September 2014 [60]. According to the US Drug Enforcement Administration (DEA), 19 fatalities were linked to 25I-NBOMe between March 2012 and August 2013 [61]. Incidents of NBOMe use and toxicity have been reported worldwide, including cases from Europe [57], the UK [62], Australia [63], New Zealand [64], Hong Kong [65], and Japan [66]. The features of 51 NBOMe toxicity cases reported in the literature are described below.

**Cases 1–4** As noted above, the first reports of 25I-NBOMe exposure appeared in 2012 [59]. Four adult males presented to an ED with tachycardia, hypertension, agitation, and hyperglycemia. Three (**Nos. 2–4**) experienced protracted seizures, necessitating intubation, mechanical ventilation, and pharmacological therapy. One of the cases (**No. 4**) developed rhabdomyolysis (creatinine kinase (CK) level 30,000 U/L) and renal failure, necessitating hemodialysis. The presence of 25I-NBOMe in biological specimens collected from the patients was confirmed by LC-MS analysis.

**Cases 5–14** Hieger and colleagues described ten other cases of 25I-NBOMe exposure from 2012 [67]. Effects included tachycardia (9/10), hypertension (9/10), hyperglycemia (9/10), leukocytosis (7/10), agitation (7/10), hallucinations (5/10), and seizures (2/10). The most severely affected case (**No. 5**) experienced status epilepticus, multiple intracerebral hemorrhages, and acute renal injury. The presence of 25I-NBOMe was only confirmed in one of the cases (**No. 6**) – an 18-year-old male who was admitted to the ED after jumping out of a moving car [68]. The initial examination showed severe agitation, tachycardia ( $>150$  bpm), and hypertension (150–170/100 mmHg). Treatment included physical restraint and continuous infusion of lorazepam. Symptoms improved over 48 h although lorazepam and dexmedetomidine had to be administered on the second day of hospitalization due to continuing episodes of agitation. 25I-NBOMe was detected (LC-MS/MS) in serum at a concentration of 0.76  $\mu\text{g/L}$ .

**Cases 15–21** Hill described seven cases of 25I-NBOMe toxicity that occurred in the UK in January 2013 [62]. The first case (**No. 15**) was a 29-year-old male who injected an unknown quantity of 25I-NBOMe intravenously. The initial examination showed

agitation, aggression, self-harm, seizures, tachycardia (160 bpm), hypertension (187/171 mmHg), tachypnea (58 breaths/min), low oxygen saturation (94%), and hyperthermia (39.0°C). He also presented with leukocytosis (WBC  $23.5 \times 10^9/L$ ), respiratory and metabolic acidosis (pH 7.20, PaCO<sub>2</sub> 66 mmHg), rhabdomyolysis (CK 15,424 U/L), and impaired renal function. Treatment included intubation, mechanical ventilation, sedation/neuromuscular blockade, and administration of fluids and antibiotics. He subsequently developed anuria and renal impairment, and there was evidence of pulmonary injury. Normalization of renal and pulmonary function required 43 days of hospitalization, including ICU admission for 38 days where he received hemodialysis. In the second case (**No. 16**), a 20-year-old-male collapsed and had convulsions after ingesting a powder containing 25I-NBOMe. In addition to agitation, he exhibited tachycardia (126 bpm), hypertension (170/90 mmHg), hyperthermia (38.8°C), tachypnea (24 breaths/min), low oxygen saturation (92%), and urinary retention. He was anesthetized, intubated, and artificially ventilated. Sustained clonus and ocular clonus were noted 5 h after ED admission, necessitating treatment with cyproheptadine. His CK level was initially elevated (peak value of 550 U/L) but renal function was restored by fluid replacement. He was extubated 3 days after admission and discharged on the fifth day. The clonus was likely due to serotonin syndrome; the patient had a history of depression and was being treated with fluoxetine. The third case (**No. 17**, a 19-year-old male) insufflated a powder containing 25I-NBOMe. He became agitated and violent, with tachycardia (110 bpm) and hypertension (138/100 mmHg). He also exhibited leukocytosis (WBC  $18.9 \times 10^9/L$ ). Recovery occurred after treatment with diazepam. The fourth case (**No. 18**) was a 22-year-old male who insufflated 25I-NBOMe and then had a tonic-clonic seizure. When he arrived at the ED he was agitated and aggressive and had to be sedated with diazepam. He presented with mild tachycardia (104 bpm) and elevated CK levels, peaking at 633 U/L. Recovery occurred without further intervention and he was discharged from the hospital on the same day he was admitted. The other three cases (**Nos. 19–21**) were young adult males who experienced more moderate symptoms after insufflating or ingesting 25I-NBOMe. In addition to visual and auditory hallucinations, all three cases exhibited tachycardia and hypertension. One of those cases (**No. 19**) exhibited agitation and aggressive behavior severe enough to require sedation; he also showed hyperthermia (38.4°C) and elevated CK levels (598 U/L). Another case (**No. 20**) had inducible ankle clonus. LC-MS/MS analysis of plasma samples confirmed that all the individuals had taken 25I-NBOMe.

**Case 22** In another reported case of 25I-NBOMe overdose, an 18-year-old female became confused and agitated and had a grand mal seizure after taking an unknown amount of the drug sublingually at a party [69]. The initial examination revealed tachycardia (145 bpm) and hypertension (145/100 mmHg). Hyperreflexia was also present. Her condition normalized after administration of intravenous fluids and lorazepam. LS-MS/MS analysis of a urine sample confirmed the presence of 25I-NBOMe and suspected *O*-desmethyl metabolites, as well as small amounts of 25H-NBOMe and 2C-I.

**Case 23** Umemura described the case of a 17-year-old female who died after ingesting blotter paper containing 25I-NBOMe [70]. She was hospitalized with status epilepticus and subsequently developed hyperthermia, metabolic acidosis, rhabdomyolysis, and kidney injury. The patient survived for 7 days, but brain death occurred due to cerebral edema. The presence of 25I-NBOMe in whole blood collected antemortem was confirmed by LC-TOF-MS. Therapeutic levels of lithium were also present in the blood sample.

**Cases 24–25** Another two deaths linked to 25I-NBOMe were reported by Walterscheid and colleagues [71]. In both cases, the decedents began to “flail about” before becoming unresponsive. The first victim (**No. 24**, a 21-year-old male) became violent while driving, damaging the interior of the vehicle. Cardiopulmonary resuscitation was attempted but was unsuccessful. The second victim (**No. 25**, a 15-year-old female) had ingested a liquid containing 25I-NBOMe; death occurred due to cardiac failure soon after arriving at the ED. Hyperthermia was noted in the second victim (39.9°C). 25I-NBOMe was detected in heart blood and urine collected from both decedents. Analysis also revealed evidence of marijuana use in both individuals.

**Case 26** A behavioral fatality linked to 25I-NBOMe has been reported [72]. In that case, a 19-year-old male ingested blotter paper containing “acid.” The man exhibited bizarre behavior prior to falling multiple stories from an apartment balcony. 25I-NBOMe was detected (LC-MS/MS) in samples of heart blood (410 ng/L), peripheral blood (405 ng/L), and brain tissue (2,780 pg/g) collected 7 h postmortem.

**Case 27** A 15-year-old male had multiple seizures and lost consciousness after ingesting hallucinogenic mushrooms in combination with a liquid containing 25I-NBOMe [73]. He developed renal and liver failure. Death ultimately occurred following cardiopulmonary arrest. An antemortem blood sample contained 0.76 µg/L 25I-NBOMe.

**Case 28** A 16-year-old male was found dead after consuming 25I-NBOMe on a piece of blotter paper [74]. There was evidence that his death was preceded by violent behavior (broken glass was found at the scene and the decedent had multiple contusions and abrasions). Analysis of heart blood collected postmortem revealed 19.8 µg/L 25I-NBOMe.

**Case 29** Another death linked to 25I-NBOMe was described by Keuppers and Cooke [63]. In that case, a 23-year-old female insufflated a powder purported to be “synthetic LSD.” She soon became severely agitated and aggressive, and then had a seizure before collapsing. Cardiopulmonary resuscitation (CPR) was attempted but was unsuccessful. 25I-NBOMe (28 µg/L), 25H-NBOMe (1 µg/L), and 25C-NBOMe (0.7 µg/L) were detected in aortic blood postmortem. Methamphetamine (0.39 mg/L) and THC (3.4 µg/L) were also present.

**Case 30** An unsuccessful suicide attempt following 25I-NBOMe ingestion has been reported [75]. After taking “two hits of acid” sublingually, the 18-year-old male had a panic attack, and stabbed himself in the throat and chest. A serum sample, collected ~11 h post-ingestion, contained 34 ng/L 25I-NBOMe (LC-MS/MS).

**Case 31** Tang described one case [65] where a 17-year-old male was hospitalized in a confused and agitated state. He had a seizure after being admitted to the ED. Other effects included hypertension (215/94 mmHg), tachycardia (140 bpm), hyperthermia (38.4°C), and diaphoresis. Examination also showed sinus tachycardia. He was initially treated with intravenous fluids and diazepam. Later, he was intubated and admitted to the pediatric ICU, where he was given sedatives/neuromuscular blockade and treated with cyproheptadine. After the patient regained consciousness, he admitted ingesting a pill containing an “NBOMe.” LC-MS/MS analysis of the patient’s urine confirmed the presence of 25B-NBOMe.

**Case 32** Another case reported by Tang involved a 31-year-old male who presented with confusion, agitation, hypertension (160/123 mmHg), sinus tachycardia (162 bpm), hyperthermia (39.6°C), and diaphoresis [65]. Troponin I (0.38 ng/L) and lactate (8.6 mmol/L) were also elevated. Treatment included administration of fluids and lorazepam, as well as cooling measures. He subsequently developed rhabdomyolysis (CK levels peaked at 11,066 U/L), impaired renal function, and altered liver function. Rhabdomyolysis was treated with fluids and bicarbonate. The patient left the hospital on day 3 against medical advice. Prior to release, he admitted sublingual use of a drug called “Holland film.” His urine was positive for 25B-NBOMe and 25C-NBOMe.

**Case 33** A 19-year-old male who had taken 25B-NBOMe subsequently had generalized grand mal seizures and became unresponsive [76]. Examination showed hyperthermia (40°C), tachycardia (152 bpm), hypertension (145/90 mmHg), agitation, diaphoresis, and respiratory distress. He was intubated and mechanically ventilated; sedatives and a neuromuscular blocking agent were administered to control agitation and seizures. Laboratory values indicated hyperglycemia (286 mg/L), leukocytosis ( $\text{WBC } 26.1 \times 10^9/\text{L}$ ), and respiratory and metabolic acidosis (pH 6.9,  $\text{pCO}_2$  89 mmHg,  $\text{HCO}_3^-$  19.3 mEq/L, base deficit 13 mmol/L). The patient subsequently developed signs of rhabdomyolysis, with CK levels peaking at 11,645 U/L. Recovery required 6 days of ICU treatment. Serum from the patient contained 180 ng/L 25B-NBOMe.

**Case 34** Isbister described the case of a 15-year-old male who ingested blotter paper purportedly containing LSD [77]. He had three seizures before arriving at the ED and one following admission. He presented with acute respiratory acidosis (venous pH 6.93,  $\text{PvCO}_2$  120 mmHg, base excess  $-7$  mEq/L). Seizures were treated with midazolam; he was intubated, ventilated, sedated and paralyzed, and transferred to the ICU. Examination in the ICU showed leukocytosis ( $\text{WBC } 16.3 \times 10^9/\text{L}$ ). On day 2, he began to show signs of rhabdomyolysis; his CK level peaked at 34,778 U/L on day 3. He recovered and was discharged on day 5. Analysis of blood collected 22 h post-dosing confirmed the presence of 25B-NBOMe at 0.089  $\mu\text{g/L}$ .

**Case 35** Yoshida recounted the case of a male, approximately 20 years old, who exhibited violent behavior and convulsions after ingesting blotter paper containing 25B-NBOMe [66]. He was hospitalized in a comatose state; other effects included tachycardia (156 bpm), tachypnea (48 breaths/min), hyperthermia (41.5°C), and systolic hypotension (90 mmHg). He also presented with thrombocytopenia, rhabdomyolysis,

acidosis, and multi-organ failure. Myoclonus and tendon hyperreflexia were also observed. Despite supportive therapy, the patient died 3 days later. A plasma sample collected when the man was admitted to the hospital (2–3 h after drug intake) contained 3.15 µg/L 25B-NBOMe and 0.433 µg/L 25C-NBOMe, as well as benzodiazepines.

**Case 36** An 18-year-old man died after consuming two squares of blotter paper containing 25B-NBOMe [74]. His death was preceded by destructive behavior and loss of consciousness. Autopsy revealed pulmonary edema and aspiration of gastric contents. 25B-NBOMe (1.59 µg/L) and cannabinoids were present in postmortem heart blood.

**Case 37** Laskowski described a 15-year-old male who became agitated ~6 h after sublingual administration of blotter paper impregnated with 25B-NBOMe [78]. After ED admission, the patient had multiple tonic-clonic seizures, which were treated with IV lorazepam. He also exhibited hypertension (177/93 mmHg), tachycardia (111 bpm), diaphoresis, and mild hyperthermia (37.7°C). Laboratory tests showed hyperglycemia (224 mg/dL), leukocytosis (WBC  $17 \times 10^9$ /L), and acidosis ( $\text{HCO}_3^-$  13 mEq/L, lactate 7.3 mmol/L). Additionally, CK levels were elevated (429 U/L). He recovered after being transferred to the pediatric ICU and was discharged <48 h after drug intake. Serum collected from the patient when he arrived at the ED contained 1.2 µg/L 25B-NBOMe (LC-MS/MS).

**Cases 38–47** Gee reported ten cases where recreational use of 25B-NBOMe resulted in adverse effects [64]. Agitation, hallucinations, tachycardia, and hypertension were present in all of the cases. Diaphoresis occurred in eight cases and hyperthermia in four cases. The toxicity was not severe in nine of the cases; two of those patients recovered with limited medical intervention and the other seven required only physical restraint and/or sedation with benzodiazepines. The toxicity was more severe in the remaining case – a 24-year-old male (**No. 47**) who snorted an unknown amount of 25B-NBOMe. He was hospitalized due to agitation and self-injurious behavior. The patient continued to struggle despite physical restraint and administration of haloperidol and a large dose of midazolam. Heart rate, blood pressure, and temperature peaked at 175 bpm, 200/90 mmHg, and 38.5°C, respectively. He was eventually anesthetized in the ED, intubated, and transferred to the ICU. CK and troponin I levels peaked at 18,361 U/L and 399 ng/L, respectively. The patient gradually improved after being anesthetized; he was discharged from the hospital 60 h after admission. Three of the patients, including **No. 47**, reportedly exhibited inducible clonus; one of the other cases exhibited tremor and hyperreflexia. According to LC-MS/MS analysis, plasma levels of 25B-NBOMe in the ten patients ranged from 0.7 to 10.7 µg/L.

**Case 48** In one case reported by Grautoff and Kähler [79], a 19-year-old male had a generalized seizure and lost consciousness 2 h after snorting 2 mg of 25C-NBOMe (identity confirmed by GC-MS). Other presenting features included tachycardia (120 bpm) and hypertension, as well as low oxygen saturation (50%), which necessitated intubation and mechanical ventilation. Despite supportive care, the patient developed rhabdomyolysis (CK levels peaked at 5,533 U/L), renal failure, pulmonary

hypertension, and evidence of lung injury. He required hemodialysis and administration of multiple antihypertensive agents. The patient remained in the ICU for 13 days before making a full recovery.

**Case 49** A 24-year-old woman became confused and agitated 30 min after consuming three squares of blotter paper impregnated with 25C-NBOMe [80]. Examination revealed tachycardia (140 bpm), tachypnea (32 breaths/min), and skin that was "moist and hot to the touch." After being physically restrained, she was given intravenous fluids and lorazepam. Full recovery occurred within 10 h. The identity of the drug was confirmed by LC-TOF/MS.

**Case 50** A fatality due to 25C-NBOMe has also been described [81]. A 22-year-old man snorted an unknown amount of 25C-NBOMe. Over the next few hours he acted agitated and incoherent. He had lost consciousness by the time an ambulance arrived. The initial examination showed low oxygen saturation (80%) and generalized seizures, so he was intubated and treated with multiple sedatives and neuromuscular blockade. When he arrived at the ED 30 min later he exhibited hyperthermia (40°C), tachycardia (140 bpm), bleeding from mucous membranes, rhabdomyolysis, respiratory and metabolic acidosis (pH 6.69, PaCO<sub>2</sub> 78 mmHg, lactate 28 mmol/L), hyperkalemia, and low BP. His CK and troponin I levels eventually peaked at >42,670 U/L and 3,513 ng/L, respectively. Although the patient was placed in a medically induced coma and cooling measures were attempted, he died of multi-organ failure approximately 12 h after drug intake. A blood sample collected antemortem contained 0.81 µg/kg 25C-NBOMe (LS-MS/MS).

**Case 51** A 16-year-old female was hospitalized due to tonic-clonic seizures and altered mental status after sublingual use of blotter paper purportedly containing 25I-NBOMe [78]. She presented with hypertension (130/73 mmHg) and tachycardia (146 bpm). There was also evidence of hyperglycemia (glucose 170 mg/dL), hypernatremia (serum Na<sup>+</sup> 149 mEq/L), leukocytosis (WBC 19 × 10<sup>9</sup>/L), and acidosis (HCO<sub>3</sub><sup>-</sup> 11 mEq/L, anion gap 21 mmol/L). Seizures were treated with intravenous lorazepam. Ankle clonus was observed when the patient was examined in the pediatric ICU. CK levels were elevated, peaking at 47,906 U/L on the third day in the hospital. Her mental status normalized 24 h after drug intake and she was discharged 7 days later. Analysis of serum confirmed the presence of 25C-NBOMe; no 25I-NBOMe was detected.

As noted elsewhere, cases of NBOMe toxicity can be divided into two general categories based on their severity [57, 82]. Hallucinations, agitation, confusion, diaphoresis, hypertension, and tachycardia are common features of NBOMe toxicity. These symptoms usually resolve spontaneously or with minimal medical intervention. Twenty-four of the cases described above fall into the less severe category. By contrast, the features associated with severe cases of NBOMe toxicity include seizures, rhabdomyolysis, metabolic acidosis, renal failure, multi-organ failure, and coma. Death may occur, especially in the absence of supportive care. Twenty-three of the cases were of the latter type (see Table 2).

**Table 2** Clinical features observed in moderate-to-severe cases of NBOME toxicity.<sup>a</sup>

| Case | Seizures | Hyperthermia | Rhabdomyolysis | Renal injury | Acidosis | Death | Pharmacological therapies                                            |
|------|----------|--------------|----------------|--------------|----------|-------|----------------------------------------------------------------------|
| 2    | YES      |              |                |              |          |       | "Aggressive pharmacologic therapy"                                   |
| 3    | YES      |              |                |              |          |       | "Aggressive pharmacologic therapy"                                   |
| 4    | YES      |              | YES            | YES          |          |       | "Aggressive pharmacologic therapy"                                   |
| 5    | YES      |              |                | YES          |          |       | Benzodiazepines                                                      |
| 7    | YES      |              |                |              |          |       | Benzodiazepines                                                      |
| 15   | YES      | YES          | YES            | YES          | YES      |       | Propofol, midazolam, atracurium                                      |
| 16   | YES      | YES          | CK elevated    |              |          |       | Diazepam, propofol, midazolam, atracurium, lorazepam, cyproheptadine |
| 18   | YES      |              | CK elevated    |              |          |       | Diazepam                                                             |
| 19   |          | YES          | CK elevated    |              |          |       | Diazepam, lorazepam, haloperidol                                     |
| 22   | YES      |              |                |              |          |       | Lorazepam                                                            |
| 23   | YES      | YES          | YES            | YES          | YES      | YES   | UNKNOWN                                                              |
| 25   |          | YES          |                |              |          | YES   | UNKNOWN                                                              |
| 27   | YES      |              |                | YES          |          | YES   | UNKNOWN                                                              |
| 29   | YES      |              |                |              |          | YES   | NONE                                                                 |
| 31   | YES      | YES          |                |              |          |       | Diazepam, midazolam, rocuronium, cyproheptadine                      |
| 32   |          | YES          | YES            | YES          | YES      |       | Lorazepam                                                            |
| 33   | YES      | YES          | YES            |              | YES      |       | Lorazepam, propofol, pancuronium, midazolam, vecuronium              |
| 34   | YES      |              | YES            |              |          |       | Rocuronium, morphine, midazolam                                      |
| 35   | YES      | YES          | YES            | YES          | YES      | YES   | Diazepam, fosphenytoin, vecuronium                                   |
| 37   | YES      | YES          | YES            |              | YES      |       | Lorazepam                                                            |
| 47   |          | YES          | YES            |              |          |       | Midazolam, haloperidol, propofol                                     |
| 48   | YES      |              | YES            | YES          |          |       | Sedatives, antihypertensives, vasodilators, diuretics                |
| 50   | YES      | YES          | YES            | YES          | YES      | YES   | Diazepam, midazolam, suxamethonium, ketamine, fentanyl               |
| 51   | YES      |              | YES            | YES          | YES      |       | Lorazepam                                                            |

<sup>a</sup>Cases were included if there was evidence of rhabdomyolysis or if seizures, renal failure, or metabolic acidosis occurred

## 5.1 Mild Cases of NBOMe Toxicity

The features of the less severe cases are not unique to NBOMes and are also induced by other serotonergic hallucinogens. Serotonergic hallucinogens can cause confusion, agitation, and panic attacks, especially during “bad trips” or after administration of high doses [83, 84]. LSD, DOM, mescaline, DMT, and psilocybin produce hypertension, tachycardia, and diaphoresis in humans [85–91]. The hypertension induced by DOI is mediated in the periphery by activation of vascular 5-HT<sub>2A</sub> receptors, which produces vasoconstriction [92–95].

## 5.2 Moderate-to-Severe Cases of NBOMe Toxicity

The progression from rhabdomyolysis to metabolic acidosis and renal failure is a common complication of severe NBOMe toxicity. Of the 23 moderate-to-severe cases listed in Table 2, 14 had at least one of these features, and three others showed subclinical features such as elevated creatinine kinase (CK) levels (indicating that rhabdomyolysis may have developed in the absence of medical treatment). Rhabdomyolysis is caused by skeletal muscle damage, for example by prolonged muscle activity or compression of muscles. Ischemic muscle tissue produces lactic acid, resulting in metabolic acidosis. The disintegration of striated myocytes releases their intracellular contents, including CK, myoglobin, and potassium, into plasma. Myoglobin release can produce acute renal failure due to tubule obstruction, whereas hyperkalemia increases the risk of cardiac failure.

Increased muscle activity due to seizures appears to be the primary cause of rhabdomyolysis in cases of NBOMe toxicity, although agitation, hyperthermia, and ischemia due to peripheral vasoconstriction may also contribute. A similar constellation of factors (muscle hyperactivity due to agitation or seizures, hyperthermia, and vasoconstriction) are thought to underlie cocaine-induced rhabdomyolysis [96]. Seizures can induce rhabdomyolysis [97–101]. Seizure activity was present in 12/14 (85.7%) of the NBOMe toxicity cases featuring rhabdomyolysis (CK levels  $\geq$  1,000 U/L), metabolic acidosis, or renal failure (Table 2). Seizures did not always result in rhabdomyolysis but in many of the non-progressing cases the patients received medications that would minimize muscle tissue damage (e.g., benzodiazepines, anesthetics, or paralytic agents).

Similar to NBOMes, phenylalkylamines such as 2C-I, 2C-T-7, 2C-T-21, DOB, DOC, and bromo-drugonfly (1-(8-bromobenzo[1,2-*b*;4,5-*b'*]difuran-4-yl)-2-aminopropane) can also induce seizures [102–108]. Srisuma et al. [60] compared the clinical features of 148 cases of NBOMe exposure and 193 cases of 2C-X hallucinogen exposure reported to the National Poison Data System, a database of poison exposures. The features of NBOMe and 2C-X toxicity were virtually identical, with the exception of single-episode seizures, which were significantly more likely to occur with NBOMes (8.8% of cases) than with phenethylamines (3.1% of cases). Although it is not clear

why NBOMes are more likely to induce seizures compared with other phenethylamines, this propensity may increase the likelihood of severe NBOMe toxicity. In contrast to phenylalkylamines and NBOMes, it is uncommon for LSD to induce seizures, even following massive overdoses [109], but reports of LSD-induced seizures have appeared in the literature [110–112].

Hyperthermia may contribute to the development of severe NBOMe toxicity. The hyperthermia produced by serotonergic hallucinogens is thought to reflect sympathetically mediated cutaneous vasoconstriction, which reduces heat dissipation [113]. Activation of 5-HT<sub>2A</sub> receptors in the CNS increases sympathetic outflow by exciting bulbospinal neurons [114]. More than half of the severe cases (12/23) – including the four cases that did not feature seizure activity – showed evidence of hyperthermia (Table 2). Elevated body temperature is known to exacerbate the muscle tissue damage underlying rhabdomyolysis [115, 116]. However, hyperthermia was also reported to occur in many of the less severe cases that resolved spontaneously. Therefore, in contrast to seizures, the presence of hyperthermia does not reliably predict that NBOMe toxicity will result in serious physiological sequelae or death.

Direct effects on muscle tissue may also play a role in NBOMe-induced damage to myocytes. 5-HT<sub>2A</sub> activation releases Ca<sup>2+</sup> from the endoplasmic reticulum in cells including myocytes, increasing the intracellular concentration of Ca<sup>2+</sup>. Additionally, 5-HT<sub>2A</sub> receptor activation causes vascular smooth muscle contraction and reduces peripheral blood flow [117–120]. Phenylalkylamine hallucinogens can cause significant peripheral vasoconstriction and vasospasm, occasionally resulting in limb ischemia [121–123].

In summary, NBOMes produce several direct and indirect effects (hyperthermia, ischemia due to vasoconstriction, hyperactivity) that would likely exacerbate muscle tissue injury. Such effects would increase the likelihood that seizure activity would produce severe muscle damage. Hence, the high incidence of seizures with NBOMes compared to other hallucinogens may translate into an elevated risk for rhabdomyolysis.

The features of serotonin syndrome were not present in most cases of NBOMe toxicity. The most important diagnostic criteria for serotonin toxicity is the presence of clonus [124]. In the absence of clonus, the co-occurrence of hyperreflexia and tremor is also evidence of serotonin toxicity. Clonus, or hyperreflexia and tremor, was rarely noted in cases of NBOMe toxicity (9/51 total cases), even in the most severe cases (5/23 cases). In at least one case of NBOMe toxicity where clonus occurred (**No. 16**), the patient had also taken the selective serotonin reuptake inhibitor (SSRI) fluoxetine. SSRIs are a known risk factor for serotonin toxicity and it is possible that combined use of NBOMes and SSRIs increases the risk for serotonin excess. Co-abuse of multiple substances is extremely common and it is possible that use of other recreational substances with serotonergic effects was a contributing factor in some of the cases featuring clonus or hyperreflexia. Although it is possible that symptoms of serotonin toxicity were present but undetected in other cases, the generally low rate at which such features occurred indicates that NBOMe toxicity is not due to serotonin excess. Indeed, classical serotonergic hallucinogens rarely produce serotonin toxicity.

## 6 Biotransformation of NBOMe Hallucinogens

NBOMes are extensively metabolized. Caspar et al. tentatively identified 37 phase I and 31 phase II metabolites of 25I-NBOMe in rat and human urine [125]. The primary metabolites of 25I-NBOMe are 2-*O*-desmethyl-25I-NBOMe, 5-*O*-desmethyl-25I-NBOMe, 25I-NBOH, and their glucuronic acid conjugates [125–128]. Similar findings have been reported for 25B-NBOMe and 25C-NBOMe [128–130]. CYP3A4 is the major cytochrome P450 isoenzyme responsible for the biotransformation of 25I-NBOMe, with CYP2C9 and CYP2C19 potentially also contributing [125, 126].

As noted above, NBOMes are reportedly inactive after oral administration. Leth-Petersen et al. assessed the microsomal stability of NBOMes and found that they have much higher clearance rates than the corresponding 2C-X phenethylamine hallucinogens [131]. For example, 2C-I has an intrinsic clearance rate of 0.20 L/kg/h, whereas the clearance rate for 25I-NBOMe is 4.1 L/kg/h. Because 25I-NBOMe clearance exceeds the hepatic blood flow rate (1.2 L/h/kg [132]), Leth-Petersen concluded that it is subject to extensive first-pass metabolism, potentially explaining why NBOMes are not active orally. In our locomotor studies, 25I-NBOMe altered activity in mice when administered SC but not IP, which is consistent with first-pass metabolism. CYP3A4, the CYP isoenzyme primarily responsible for metabolizing 25I-NBOMe, is expressed heavily in the gut and liver.

It is also possible that oral administration increases the *N*-dealkylation of NBOMes to their parent phenethylamines, which are generally an order of magnitude less potent. *N*-dealkylation of 25I-NBOMe to 2C-I is normally a relatively minor route of biotransformation [69, 127, 128]. For example, a urine specimen collected in a case of sublingual 25I-NBOMe exposure (case No. 22) contained 7.5 ng/mL of 25I-NBOMe and 1.8 ng/mL 2C-I [69]. The *N*-dealkylation route, however, may be more prominent after oral administration. Grumann reported a clinical case where a man inadvertently ingested a “sip” of a liquid containing 2.8 mg/mL 25I-NBOMe [133]. Analysis of serum from the patient showed that the level of 2C-I (290 ng/mL) greatly exceeded that of 25I-NBOMe (2.6 ng/mL). Because 2C-I is significantly less potent than 25I-NBOMe, *N*-dealkylation after oral administration would produce a marked reduction of hallucinogenic potency, potentially contributing to the perceived inactivity of NBOMes when administered by that route.

Another unresolved question is whether metabolites contribute to the toxicity of NBOMes. The major metabolites of NBOMes are their 2- and 5-*O*-desmethyl derivatives. The *O*-desmethyl derivatives of 2,5-dimethoxy-substituted phenylalkylamine hallucinogens are known to be pharmacologically active [134, 135]. However, the 2- and 5-*O*-desmethyl derivatives of 25I-NBOMe, 25B-NBOMe, and 25C-NBOMe are rapidly conjugated with glucuronic acid [130], limiting their systemic exposure. A plasma sample collected 30 min after IV administration of 2 mg 25B-NBOMe to a Danish landrace pig contained 87 nM 5-*O*-desmethyl-25B-NBOMe glucuronide but only 0.63 nM 5-*O*-desmethyl-25B-NBOMe [130]. There may be a region of bulk tolerance associated with the five-position of 2,5-dimethoxy-substituted phenylalkylamines. The affinity of the 5-benzyloxy analog of DOB for the rat 5-HT<sub>2A</sub> receptor

( $K_i = 140$  nM [136]) is only slightly lower than the affinity of DOB (see Fig. 1). Although substitution of bulky isopropoxy or 2-methoxyethoxy groups in the 5-position of 25B-NBOMe significantly reduces 5-HT<sub>2A</sub> affinity, those ligands still bind to the receptor with affinities in the 10<sup>-9</sup> M range [44]. If the 5-*O*-desmethyl NBOMe glucuronide conjugates bind the 5-HT<sub>2A</sub> receptor with nM affinity then they would likely contribute to the in vivo response.

## 7 Conclusions

The hallucinogenic effects induced by LSD, mescaline, and related substances are mediated by activation of 5-HT<sub>2A</sub> receptors. The 5-HT<sub>2A</sub> receptor affinities of phenethylamine hallucinogens from the 2C-X class are markedly increased by addition of an *N*-benzyl group. Likewise, the presence of an *N*-benzyl group increases the behavioral potency of phenethylamine hallucinogens in laboratory animals. Anecdotal reports from recreational users confirm that *N*-benzylphenethylamines such as 25I-NBOMe and 25B-NBOMe are potent hallucinogens, active at sub-milligram doses. Unfortunately, use of NBOMe hallucinogens has been linked to cases of severe toxicity. Potential complications of NBOMe use include hyperthermia, seizures, metabolic acidosis, rhabdomyolysis, organ failure, and death. Prompt treatment is required to manage cases of NBOMe toxicity.

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