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Synthesis and identification of metabolite biomarkers of 25C-NBOMe and 25I-NBOMe

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ABSTRACT

Synthetic routes have been developed for synthesis of potential metabolites of 25C-NBOMe and 25I-NBOMe. Nine potential metabolites have been synthesized, among which compounds **8** and **20a** could be used as metabolite biomarkers of 25C-NBOMe and **20b** of 25I-NBOMe in urinary detection at forensic laboratories to prove intake.

1. Introduction

It is estimated that a total of 246 million people, or 1 out of 20 people between the ages of 15 and 64 years, used an illicit drug in 2014 according to the *World Drug Report 2016*. Although drug abuse may lead to many health and social problems,[1] new psychoactive substances (NPS) that are produced by clandestine laboratories and purchased *via* the internet head shops, keep being pumped into the market with 66 new psychoactive substances firstly reported to the EU early warning system in 2017.[2]

The NBOMes (*N*-Benzyl-oxy-methyl derivatives of 2C phenylethylamines), a new group of NPS, have strong hallucinogenic effects and have been reportedly sold as a legal alternative to lysergic acid diethylamide (LSD). More than 39 NBOMes and analogues have been reported.[3,4] The most commonly abused NBOMes are 25I-NBOMe, 25C-NBOMe and 25B-NBOMe (Fig. 1), which are now scheduled as controlled substances in many countries. Ingestion of these substances can cause tachycardia, agitation, hallucination, hypertension, confusion and mydriasis.[5,6] Cases of fatal intoxication associated with the use of NBOMes have been reported around the world, e.g. in the US, Europe, and Australia.[6–8]

It can be challenging to detect NPS because the parent drugs are not always found in urine specimens. Metabolites can be a more suitable target and can also extend the time window of detection.

Therefore, metabolite identification studies to determine NPS biomarkers are important. There are only a few reports on NBOMe metabolism although fatal intoxication cases caused by using NBOMes have been reported in several countries. Studies on the metabolism of 25I-NBOMe and 25B-NBOMe using LC-HR-MS etc. analytical methods have been carried out by Caspar and Boumrah in 2015.[9,10] In the same year, Poklis et al. reported the identification of metabolite biomarkers of 25I-NBOMe as well as the synthesis of two major metabolites with 8 and 9 steps respectively.[11] With synthetic reference standards, the metabolites with monodemethylation of the 2,5-dimethoxyphenyl ring can now be distinguished. Since the synthetic route is long, there is need for optimization or identification of other metabolite biomarkers, which could be synthesized in fewer steps.

25C-NBOMe is one of the most abused NBOMes; to the best of our knowledge, there are no reports on the synthesis of its metabolite biomarkers. In collaboration with the National Board of Forensic Medicine of Sweden we synthesized several potential

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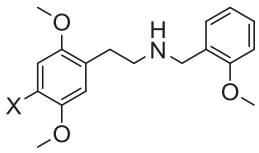


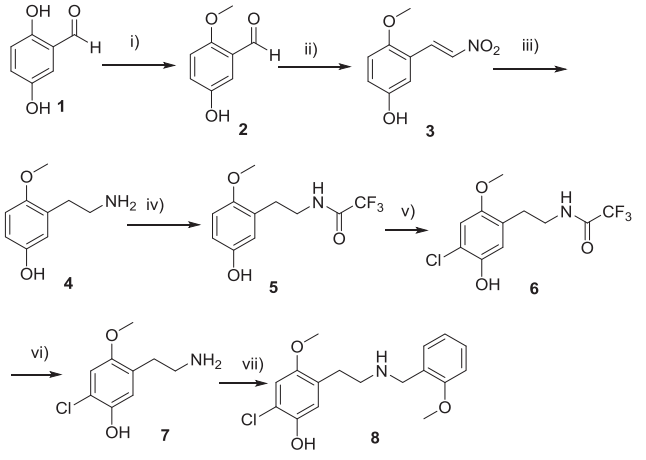
Fig. 1. Structures of 25I-NBOMe, 25B-NBOMe and 25C-NBOMe.

metabolites of 25C-NBOMe and 25I-NBOMe for comparison.

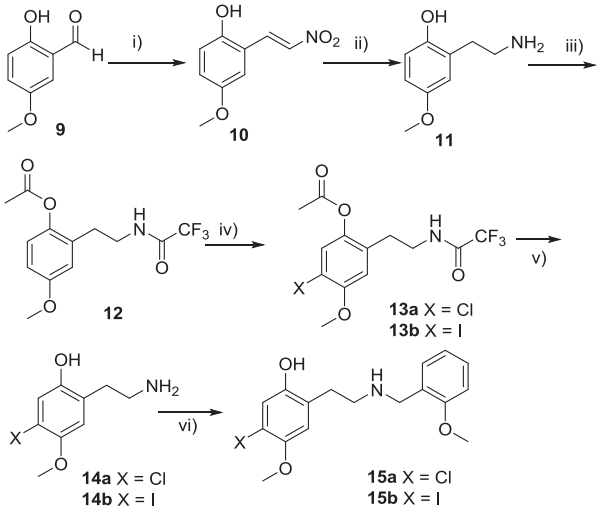
2. Results and discussion

Aldehyde **2** is commercially available. It can also be synthesized from compound **1** using MeI under basic conditions.[12] The methylation is selective, which might be due to the acidic difference of the two phenol groups. The yield was moderate due to incomplete conversion. Henry reaction between compound **2** and nitromethane gave **3** in high yield. When a higher amount of nitromethane was used with starting material of **9**, a lower yield was obtained and dialkylation side product was increased. The reduction of **3** to **4** with $LiAlH_4$ was straightforward, but the work-up was problematic and led to lower yield than expected. Protection of **4** using trifluoroacetic anhydride gave **5**, which could undergo chlorination with NCS without protection of the phenol. The chlorination selectively occurred at the ortho position of phenol to give compound **6** as the major product. After deprotection and reductive amination, compound **8** was obtained. The yield was low. Full conversion was not achieved with $NaCNBH_3$, although an excess amount of reagent was added. $NaBH_4$ was then added. The lower yield might be caused by the poor quality of $NaCNBH_3$. The synthetic route of **8** was two steps shorter than its metabolite analogue of 25I-NBOMe, which was reported by Poklis et al.[11] The overall yield might be increased with reagents of better quality (Scheme 1).

Following a similar procedure **15a**, another potential metabolite of 25C-NBOMe, was synthesized (Scheme 2). The difference was that the phenol had to be protected with an acyl group to increase the selectivity of chlorination at the ortho position of the methoxy



Scheme 1. i) MeI, K_2CO_3, DMF, 57%; ii) CH_3NO_2 (14 equiv.), NH_4OAc (0.65 equiv.), AcOH, 94%; iii) $LiAlH_4$, THF, 59%; iv) TFAA, DCM, 52%; v) NCS, acetonitrile, 75 °C, 30 min, 73%; vi) 5% NaOH, EtOH, rt, vii) 2-methoxybenzaldehyde, $NaCNBH_3$ and $NaBH_4$, EtOH, rt, 26%.



Scheme 2. i) CH_3NO_2 (30 equiv.), NH_4OAc (1 equiv.), AcOH, 120 °C, 70 min, 68%; ii) $LiAlH_4$, THF, 65 °C, 4 h, 71%; iii) a) TFAA, TEA, THF, rt; b) acetyl chloride, TEA, $CHCl_3$, rt, 51%; iv) **13a**: NCS, acetonitrile, rt, 65%; **13b**: NIS, conc. H_2SO_4, acetonitrile, rt, 65%; v) 5% NaOH, EtOH, rt, 100% for both **14a** and **14b**; vi) 2-methoxybenzaldehyde, $NaCNBH_3$, DCE, rt, 36% and 35% for **15a** and **15b**, respectively.

group. Instead of protecting with an acyl group, excess amount of trifluoroacetic anhydride was also tested to give a di-trifluoroacyl protected intermediate to reduce one step, unfortunately it failed. Both protective groups could be removed under basic conditions in the same procedure. A similar metabolite of 25I-NBOMe, compound **15b**, was synthesized using a similar procedure (Scheme 2). Compared to the synthesis reported in the literature, our procedure required one step less and replaced 2-bromo propane for protection and BCl_3 for deprotection by more common reagents.

Both metabolites of 25C-NBOMe, compound **8** and **15a,** were used as reference standards in the analysis of two authentic forensic urine specimens (Fig. 2A). It was found that compound **8**, 5′-desmethyl-25C-NBOMe, was more abundant than **15a**, 2′-desmethyl-25C-NBOMe, suggesting that compound **8** is a good metabolite biomarker of 25C-NBOMe. Moreover, it was found that **15b,** 2′-desmethyl-25I-NBOMe, was not a good metabolite biomarker for analysis of intake of 25I-NBOMe either (Fig. 2C).[13] Although the synthesis of **8** and **15** was carried out successfully with improvement, the synthetic route was still long. However other metabolites that can be synthesized with shorter synthetic routes could be identified and used as biomarkers too. 25B/I/C-NBOMes were metabolized by O-demethylation, O-di-demethylation and hydroxylation.[11,13] Metabolites with a hydroxyl group on the benzyl ring of 25C/I-NBOMe were then synthesized.

The position of the hydroxyl group of a major metabolite of NBOMes at the benzyl ring was controversial in literature.[13,15–17] Therefore, it is imperative to have all four possible metabolites to address the problem. Here we have synthesized all of them, **20a**, **21a**, **22** and **23** (Scheme 3).

Compound **17** was synthesized using a Curtius rearrangement from **16** with Boc_2O/NaN_3 under catalysis of $Zn(TfO)_2$.[14] The amino group was protected at the same time. The resulting product **17** was then halogenated, deprotected using TFA, followed by reductive amination to give compounds **20**–**23**, with hydroxylation at 6, 5, 4 or 3 position of the benzyl ring of NBOMe, respectively. Compound **19** was also synthesized for comparison with metabolite **15b**. It was found that it is not a proper metabolite biomarker (Fig. 2B). Therefore, we refrained from synthesizing its chloro analogue. The synthesis of **20**–**23** was straightforward (Scheme 3). The