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Synthesis and Identification of Metabolite Biomarkers of 25C-NBOMe and 25I-NBOMe

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ARTICLE INFO

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, 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.

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. 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. With synthetic reference standards, the metabolites with mono-demethylation 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.

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. 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. With synthetic reference standards, the metabolites with mono-demethylation 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.

ARTICLE INFO

1. Introduction

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.

Keywords: Biomarker, 25C-NBOMe, 25I-NBOMe, Metabolite, Urinary detection

25I-NBOMe: X = I
25B-NBOMe: X = Br
25C-NBOMe: X = Cl

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

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 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 Mel under basic conditions. 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 LiAlH₄ 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 the methoxy group. Instead of protecting with an acyl group, excess amount of trifluoroacetic anhydride was also tested to give a di-trifluoroacyl group. Instead of protecting with an acyl group, excess amount of trifluoroacetic anhydride was also tested to give a di-trifluoroacyl group. Protection of 5 with an acyl group to increase the selectivity of chlorination at the ortho position of the methoxy group.

A similar metabolite of 25I-NBOMe, 2'-desmethyl-25I-NBOMe, was not a good metabolite biomarker for analysis of intake of 25I-NBOMe either (Fig. 2C). 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. Metabolites with a hydroxyl group on the benzyl ring of 25C/I-NBOMe were then synthesized.

15b, 2'-desmethyl-25I-NBOMe, was not a good metabolite biomarker for analysis of intake of 25I-NBOMe either (Fig. 2C). 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. Metabolites with a hydroxyl group on the benzyl ring of 25C/I-NBOMe were then synthesized.
Compound 17 was synthesized using a Curtius rearrangement from 16 with Boc₂O/NaN₃ under catalysis of Zn(TfO)₂. 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 rearrangement worked but was sensitive. The yield of 17 varied from 45-72% under similar conditions by different chemists. Chlorination of 17 gave full conversion at 75 °C using either MW irradiation or conventional heating. The selectivity was good, but product with chloro group at position 6 was still found. Compound 21a was not stable and decomposed during concentration under heating at 50 °C after LC purification. The fractions from preparative LC turned red and the bond between the amine group and the benzyl ring was broken to give 2-(4-chloro-2,5-dimethoxyphenyl)ethan-1-amine according to LC-MS. Its stability was much better at room temperature. Freeze-drying was then used for removing solvent. It was found that compound 21b was not stable either and gave a similar side product together with another N,N-dibenzylation side product under heating after LC preparation. The stabilities of 20a, 22 and 23 were better.

After analysis of the human urine samples and comparison with these references, it was found that compound 20a was the correct mono-hydroxy metabolite of 25C-NBOMe and can be used as another suitable metabolite biomarker (Fig. 2C). The result was consistent with the metabolite study of 25B-NBOMe by Kristersen et al. Interestingly, Nielsen et al. reported that the 4-hydroxy metabolite, compound 21b, was identified as one of the major metabolites of 25I-NBOMe using human liver microsomes. In the same paper, the authors also reported that, instead of the 4-hydroxy metabolite, the 5-hydroxy metabolite at the benzyl ring was the major mono-hydroxy metabolite of 25I-NBOH, which is an analogue of 25I-NBOMe. During our study it was found that the four metabolites of 25C-NBOMe had very close retention times and required optimized conditions to improve separation. This may be also true for 25I-NBOMe. Compound 20b was then synthesized using a similar method (Scheme 3). It was found that instead of 21b, compound 20b was more likely to be the major mono-hydroxy metabolite of 25I-NBOMe in authentic human urine sample in our analysis (Fig. 2D).

3. Conclusion

Straightforward synthetic routes have been developed for the synthesis of potential metabolites of 25C-NBOMe and 25I-NBOMe. The yield might be improved if further optimization is carried out. Analysis of authentic urine species and human hepatocyte samples showed that 5'-desmethyl-25C-NBOMe (compound 8) and 5-OH-25C-NBOMe (compound 20a) are two metabolite biomarkers, which can be used as targets in urine analysis for 25C-NBOMe. Further study on the metabolites of 25I-NBOMe suggested that 5-OH-25I-NBOMe (20b), not 4-OH-25I-NBOMe (21b), was the major mono-hydroxy metabolite of 25I-NBOMe in our human urine specimen. All the three most commonly abused NBOMes, 25C/C/B-NBOMe, showed similar metabolites suggesting that other NBOMes might follow a similar metabolic pattern. Therefore, after considering the synthetic route, we found that the 5-OH-NBOMe metabolite has a potential to be developed as a general metabolite biomarker for detection of NBOMe intake from human urine.

4. Experimental section

4.1 General information

TLC was performed using 0.25 mm precoated silica-gel plates (Merck 60 F₂₅₄), detection by UV-abs at 254 nm. H¹ and ¹³C-NMR spectra were recorded on a Varian Mercury 300 MHz instrument (25 °C in CDCl₃ or methanol-d₄). HPLC-MS was performed on a Waters system (Column: XSELECT Phenyl-Hexyl, 5 μm, 250 x 19 mm and Waters X-Bridge C-18 3.5 μm, 50 x 4.6 mm) for preparative and analytical experiments respectively; Mobile
phase: organic phase: acetonitrile:water 90:10, with 10 mM
NH₄OAc; water phase: acetonitrile:water 5:95, with 10 mM
NH₄OAc. Flash chromatography was performed using the following silica gel: High purity grade (Merck Grade 9385), pore
size 60 Å, 230-240 mesh particle size. The results of the high-
resolution mass measurements using LC-QTOF-MS by National
Board of Forensic Medicine, Sweden, can be found in the
experimental.

4.2 Synthesis

4.2.1 5-hydroxy-2-methoxybenzaldehyde (2)

A mixture of 2,5-dihydroxybenzaldehyde (1 g, 7.24 mmol) and
K₂CO₃ (1 g, 7.24 mmol) was dissolved in DMF (10 mL) and
stirred at room temperature for 30 min before methyl iodide (0.679
mL, 10.9 mmol) was added. The reaction mixture was stirred at
room temperature for 30 min before methyl iodide (0.679
mL, 10.9 mmol) was added. The reaction mixture was left to stir under nitrogen
gas flow at 65 ˚C for 70 min. The reaction mixture was cooled
to room temperature overnight. The solvent was evaporated
in vacuum and the crude was purified using flash-column chromatography (n-heptane/EtOAc 2:1) to gain compound 2 (621 mg, 57% yield) as a yellow solid. ¹H-NMR (300 MHz, CDCl₃) δ 7.46 (broad s, 1H), 6.78-6.69 (m, 2H), 6.65 (d, J = 2.2 Hz, 1H),

4.2.2 (E)-4-methoxy-3-(2-nitrovinyl)phenol (3)

4.2.3 3-(2-aminoethyl)-4-methoxyphenol phenol (4)

Compound 4 (194 mg, 1.16 mmol) was dissolved in THF (4 mL) and
and sodium cyanoborohydride (31.4 mg, 0.499 mmol) was added
and sodium cyanoborohydride (31.4 mg, 0.499 mmol) was added
to the solution that was left to stir at room temperature overnight. Finally sodium borohydride (9.44 mg, 0.25 mmol) was added
to the solution and left to stir for about 1 h. The solvent was evaporated in vacuum and the crude purified using preparative LC to gain product 8 (20.5 mg, 26% yield) as colorless syrup. ¹H-NMR (300 MHz, CDCl₃) δ 7.21 (td, J = 8.0, 1.7 Hz, 1H), 7.14 (dd, J = 8.0, 1.7 Hz, 1H), 6.85 (td, J = 8.0, 1.0 Hz, 1H), 6.77 (dd, J = 8.0, 1.0 Hz, 1H), 6.73 (s, 1H), 6.68 (s, 1H), 3.80 (s, 2H), 3.65 (s, 3H), 3.58 (s, 3H), 2.85 (t, J = 5.9 Hz, 2H), 2.75 (t, J = 5.9 Hz, 2H).

4.2.6 5-(2-aminoethyl)-2-chloro-4-methoxyphenol (7) and 2-chloro-4-methoxy-5-(2-(2-methoxybenzyl)amino)phenyl) (8)

Tetrahedron

N-(4-chloro-5-hydroxy-2-methoxyphenethyl)-2,2,2-
trifluoroacetamide (6)

Compound 5 (89.5 mg, 0.34 mmol) was dissolved in ACN (2
mL) and NCS (45.5 mg, 0.34 mmol) was added. The reaction mixture was stirred at 75 ˚C for 30 min and left to cool to room
temperature. The solvent was evaporated in vacuum and the crude was purified using preparative LC to gain compound 6 (74.3 mg, 73% yield) as a white solid. ¹H-NMR (300 MHz, CDCl₃) δ 6.84 (s, 1H), 6.82 (s, 1H), 3.79 (s, 3H), 3.59-3.49 (m, 2H), 2.86-2.81 (m, 2H).

4.2.7 (E)-4-methoxy-2-(2-nitrovinyl)phenol (10)

A mixture of 2-hydroxy-5-methoxybenzaldehyde 9 (76.1 mg, 0.5 mmol), nitromethane (0.82 mL, 15.3 mmol) and ammonium acetate (38.5 mg, 0.5 mmol) in a microwave vial was irradiated under MW at 120 ˚C for 70 min. The reaction mixture was cooled
to room temperature, water was added and extracted using EtOAc (3 x 10 mL). The solvent was evaporated in vacuum and the crude was purified using flash-column chromatography (n-heptane/EtOAc 1:1) to gain compound 10 (67.4 mg, 68% yield). ¹H-NMR (300 MHz, CDCl₃) δ 8.11 (d, J = 13.6 Hz, 1H), 7.90 (d, J = 13.6 Hz, 1H), 6.96-6.88 (m, 2H), 6.82-6.74 (m, 1H), 5.33 (broad s, 1H), 3.80 (s, 3H). ¹C-NMR (75 MHz, MeOD) δ 154.4, 129.1, 127.1, 125.6, 120.5, 118.5, 118.1, 112.2, 110.2, 56.0, 55.2, 49.1, 46.6, 30.2. ESI-HRMS positive mode (m/z): calcld. for C₁₇H₁₄Cl₂N₂O₃ ([M+H]+): 324.1180. Found: 324.1182. For C₁₇H₁₄Cl₂N₂O₃ ([M+H]+): 324.1180. Found: 324.1182.
reaction along with 15% NaOH (aq.) in excess and some additional THF. The solution was vacuum filtered and the filtrate was evaporated in vacuum and pre-absorbed on to silica gel before purification using flash-column chromatography (DCM/MeOH/EtOH 90:10:1) to gain compound 11b (150 mg, 71% yield). \(^{1}H\)-NMR (300 MHz, CDCl\(_3\)) \(\delta 6.84\) (d, \(J = 8.7\) Hz, 1H), 6.67 (dd, \(J = 8.7, 3.0\) Hz, 1H), 6.58 (d, \(J = 3\) Hz, 1H), 3.73 (s, 3H), 3.10-3.03 (m, 2H), 2.80-2.72 (m, 2H). \(^{13}C\)-NMR (75 MHz, CDCl\(_3\)) \(\delta 152.8, 150.6, 128.8, 118\). Reaction mixture was then left to reach room temperature and evaporated in vacuum. The crude was purified using flash-column chromatography (n-heptane/EtOAc 2:1) to gain compound 12 (70.6 mg, 51% yield). \(^{1}H\)-NMR (300 MHz, CDCl\(_3\)) \(\delta 7.29\) (td, \(J = 7.7, 1.1\) Hz, 1H), 7.21 (dd, \(J = 7.7, 1.7\) Hz, 1H), 6.98 (s, 1H), 6.96-6.85 (m, 2H), 6.57 (s, 1H), 3.90 (s, 2H), 3.86 (s, 3H), 3.81 (s, 3H), 2.97-2.87 (m, 2H), 2.85-2.72 (m, 2H). \(^{13}C\)-NMR (75 MHz, CDCl\(_3\)) \(\delta 157.7, 151.5, 147.9, 130.8, 129.6, 126.4, 121.5, 120.8, 120.4, 119.4, 115.9, 110.6, 57.2, 55.5, 49.0, 48.9, 33.3. ESI-HRMS positive mode (m/z): calcd. for C\(_{17}\)H\(_{21}\)ClNO\(_3\) ([M+H]+): 324.1180. Found: 324.1180.

4.2.8 5-chloro-4-methoxy-2-(2,2,2-trifluoroacetamido)ethyl phenyl acetate (13a)

Compound 12 (53.3 mg, 0.175 mmol) was dissolved in ACN (1 mL) and NCS (25.7 mg, 0.192 mmol) was added at 0°C. The reaction mixture was then left to reach room temperature and stirred overnight. The solvent was evaporated in vacuum and the crude was purified using flash-column chromatography (n-heptane/EtOAc 2:1) to gain compound 13a (38.4 mg, 65% yield). \(^{1}H\)-NMR (300 MHz, CDCl\(_3\)) \(\delta 7.46\) (s, 1H), 6.63 (s, 1H), 6.56 (s, 1H), 3.85 (s, 3H), 3.62-3.52 (m, 2H), 2.77 (t, \(J = 6.8, 2H\)), 2.32 (s, 3H).

4.2.10 2-(2-aminomethyl)-5-chloro-4-methoxyphenol (14a) and 5-chloro-4-methoxy-2-(2-(2-methoxybenzyl)amino)ethylphenol (15a)

NaOH (aq.) (40 μL, 0.223 mmol, 5 M) was added to a solution of compound 13a (38.4 mg, 0.113 mmol) in EtOH (1 mL) and the reaction mixture was stirred at room temperature overnight. The pH was adjusted to around 8-9 using 1 N HCl (aq.) and the solution was extracted using DCM (3 x 10 mL). The solvent was evaporated and part of the resulting compound 14a (11.7 mg, 0.0582 mmol) was re-dissolved in DCE (1 mL). 2-methoxy benzaldehyde (7.7 μL, 0.0640 mmol), sodium cyanoborohydride (7.3 mg, 0.116 mmol) and one drop of AcOH were added to the solution, which was stirred at room temperature overnight. The solvent was evaporated in vacuum and the crude purified using flash-column chromatography (DCM/MeOH/Et\(_2\)N 96:4:1) and preparative LC to gain product 15a (6.8 mg, 36% yield) as colorless syrup. \(^{1}H\)-NMR (300 MHz, CDCl\(_3\)) \(\delta 7.29\) (td, \(J = 7.7, 1.1\) Hz, 1H), 7.21 (dd, \(J = 7.7, 1.7\) Hz, 1H), 6.98 (s, 1H), 6.96-6.85 (m, 2H), 6.57 (s, 1H), 3.90 (s, 2H), 3.86 (s, 3H), 3.81 (s, 3H), 2.97-2.87 (m, 2H), 2.85-2.72 (m, 2H). \(^{13}C\)-NMR (75 MHz, CDCl\(_3\)) \(\delta 157.7, 151.5, 147.9, 130.8, 129.6, 126.4, 121.5, 120.8, 120.4, 119.4, 115.6, 110.6, 57.2, 55.5, 49.0, 48.9, 33.3. ESI-HRMS positive mode (m/z): calcd. for C\(_{17}\)H\(_{21}\)ClNO\(_3\) ([M+H]+)\(^{+}\): 322.1210. Found: 322.1211; calcd. for C\(_{17}\)H\(_{21}\)ClNO\(_3\) ([M+H]+)\(^{+}\): 324.1180. Found: 324.1180.

4.2.11 2-(2-aminomethyl)-5-iodo-4-methoxyphenol (14b) and 5-iodo-4-methoxy-2-(2-(2-methoxybenzyl)amino)ethylphenol (15b)

NaOH (aq.) (20 μL, 0.111 mmol, 5 M) was added to a solution of compound 13b (24 mg, 0.0557 mmol) in EtOH (0.5 mL) and water (0.5 mL) and the reaction mixture was stirred at room temperature overnight. The pH was adjusted to around 8-9 using 1 N HCl (aq.) and the solution was extracted using DCM (3 x 10 mL). The solvent was evaporated in vacuum and the crude purified using flash-column chromatography (DCM/MeOH/Et\(_2\)N 98:2:1) and preparative LC to gain product 15b (5.8 mg, 25% yield) as pale yellow syrup. \(^{1}H\)-NMR (300 MHz, CDCl\(_3\)) \(\delta 7.32-7.28\) (m, 2H), 7.18 (dd, \(J = 7.4, 1.7\) Hz, 1H), 6.96-6.86 (m, 2H), 6.48 (s, 1H), 3.85 (s, 5H), 3.78 (s, 3H), 2.91-2.82 (m, 2H), 2.79-2.71 (m, 2H). \(^{13}C\)-NMR (75 MHz, CDCl\(_3\)) \(\delta 157.9, 152.4, 151.3, 130.7, 129.4, 128.7, 128.2, 125.8, 120.8, 114.1, 110.5, 84.1, 57.4, 55.4, 49.1, 49.0, 34.2. ESI-HRMS positive mode (m/z): calcd. for C\(_{17}\)H\(_{21}\)I\(_2\)NO\(_3\) ([M+H]+)\(^{+}\): 414.0566. Found: 414.0569.

4.2.12 Tert-butyl 2,5-dimethoxyphenethylcarbamate (17)

A mixture of 3-(2,3-dimethoxyphenyl) propionic acid 16 (200 mg, 0.950 mmol), sodium azide (216 mg, 3.33 mmol), tetrabutylammonium bromide (45.6 mg, 0.143 mmol), zinc triflate (11.4 mg, 31.4 μmol) and di-tert-butylcarbonate (228 mg, 1.05 mmol) in dry THF (9.5 mL) was stirred at 40 °C under nitrogen overnight. When the reaction was completed according to LC-MS,
a 10 % NaNO₂ aq. solution (6 mL) was added along with EtOAc (6 mL) and the reaction mixture was left stir at room temperature for 20 min. The water phase was extracted using additional EtOAc (3 x 6 mL) and the organic phases were combined and washed with saturated aq. NH₄Cl (6 mL), saturated Na₂SO₄ (6 mL) and saturated NaHCO₃ (6 mL). The solvent was evaporated in vacuum and the crude product purified using flash-column chromatography (n-heptane/EtOAc 3:1). The fractions containing the product were concentrated in vacuum to afford compound 17 21 (192 mg, 72% yield) as a white solid. ¹H-NMR (300 MHz, CDCl₃) δ 6.79-6.68 (m, 3H), 4.68 (broad s, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 3.39-3.26 (m, 2H), 2.77 (t, J = 6.8 Hz, 2H), 1.42 (s, 9H). ¹³C-NMR (75 MHz, CDCl₃) δ 156.0, 152.6, 152.5, 131.2, 129.1, 121.9, 114.1, 107.6, 99.6, 82.9, 57.2, 56.3, 53.2, 49.2, 48.4, 30.6. ESI-HRMS positive mode (m/z): calcd. for C₁₇H₂₃INO₄ ([M+H]+): 444.0566. Found: 444.0576.

4.2.13 2-(2-(((tert-butoxycarbonyl)amino)ethyl)-5-chloro-4-methoxyphenyl acetate (18a) The mixture of 17 (50.2 mg, 0.178 mmol) and NCS (25.0, 0.187 mmol) in acetonitrile (1 mL) was irradiated using microwave at 75 °C for 30 min. The mixture was purified directly using preparative LC to give compound 18a (47 mg, 84% yield) as a white solid. ¹H-NMR (300 MHz, CDCl₃) δ 6.86 (s, 1H), 6.74 (s, 1H), 3.83 (s, 3H), 3.76 (s, 3H), 3.38-3.25 (m, 2H), 2.76 (d, J = 6.6 Hz, 2H), 1.42 (s, 9H). ¹³C-NMR (75 Hz, CDCl₃) δ 156.1, 151.8, 149.1, 127.0, 120.5, 115.4, 113.1, 79.2, 57.0, 56.1, 40.6, 30.8, 28.5.

4.2.14 2-(((tert-butoxycarbonyl)amino)ethyl)-5-iodo-4-methoxyphenyl acetate (18b) A mixture of compound 17 (434 mg, 1.54 mmol) and N-iodosuccinimide (434 mg, 1.93 mmol) in AcOH (12 mL) was stirred at room temperature for 4 h. The reaction was completed according to LC-MS an excess amount of saturated Na₂SO₄ (aq.) was added to the reaction mixture and the water phase was then extracted using DCM (3 x 10 mL). The solvent was evaporated in vacuum and the crude product was purified using flash-column chromatography twice i) n-heptane/EtOAc 2:1; ii) DCM/MeOH (98:4:0.6) to give compound 18b (411 mg, 65% yield) as a white solid. ¹H-NMR (300 MHz, CDCl₃) δ 7.21 (s, 1H), 6.64 (s, 1H), 4.61 (broad s, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 3.36-3.25 (m, 2H), 2.76 (t, J = 6.9 Hz, 2H), 1.41 (s, 9H). ¹³C-NMR (75 MHz, CDCl₃) δ 156.0, 152.6, 152.5, 128.9, 128.8, 128.4, 122.6, 121.8, 119.1, 116.5, 113.8, 83.1, 57.3, 56.2, 52.6, 48.3, 30.9. ESI-HRMS positive mode (m/z): calcd. for C₁₇H₂₃INO₄ ([M+H]+): 444.0566. Found: 444.0576.

4.2.15 2-(4-(4-iodo-2,5-dimethoxyphenethyl)amino)methyl)phenol (19) Deprotection of 18b: TFA (1.35 mL) was added dropwise to a solution of compound 18b (367 mg, 0.902 mmol) in DCM (1.5 mL). The reaction mixture was left stir at room temperature for about 1 h. When the reaction was completed according to LC-MS the solvent was evaporated in vacuum and saturated NaHCO₃ (aq.) was added to adjust the pH to around 8-9. The product was then extracted using DCM (3 x 10 mL) and the solvent was removed in vacuum to give the amine (275 mg, 99% yield). The amine was used further for the synthesis of compound 19 and 21b.

Reductive amination: Et₃N (28.4 μL, 0.204 mmol) was added to a solution of resulting amine (62.6 mg, 0.204 mmol) in EtOH (2 mL). The reaction was monitored by TLC and after two hours a full conversion of the starting material had occurred and an imine had formed. NaBH₄ (15.4 mg, 0.407 mmol) was added and the mixture was stirred for 30 min. The solvent was evaporated and the pH of the crude mixture was adjusted to around 8-9 using K₂CO₃ (aq.). The mixture was extracted using DCM (3 x 10 mL) and the crude was then purified using flash-column chromatography twice i) DCM/MeOH/Et₃N 92:8:1; ii) n-heptane/EtOAc/Et₃N 99:1:1 and preparative LC to gain the final product 4 (16.3 mg, 19 % yield) as pale yellow syrup. The low yield was due to the problem of purification. ¹H-NMR (300 MHz, CDCl₃) δ 7.22 (s, 1H), 7.16 (d, J = 7.8, 1.5 Hz, 1H), 6.97 (dd, J = 7.8 Hz, 1.5 Hz, 1H), 6.82 (br d, J = 7.8 Hz, 1H), 6.76 (br t, J = 7.8 Hz, 1H), 6.65 (s, 1H), 3.98 (s, 2H), 3.82 (s, 3H), 3.76 (s, 3H), 2.91 (t, J = 6.1 Hz, 2H), 2.82 (t, J = 6.1 Hz, 2H). ¹³C-NMR (75 MHz, CDCl₃) δ 158.4, 152.7, 152.5, 128.9, 128.8, 128.4, 122.6, 121.8, 119.1, 116.5, 113.8, 83.1, 57.3, 56.2, 52.6, 48.3, 30.9. ESI-HRMS positive mode (m/z): calcd. for C₁₇H₂₃INO₄ ([M+H]+): 444.0566. Found: 444.0566.

4.3 General procedure for synthesis of 20, 21a, 22 and 23

4.3.1 Chlorination The mixture of 17 (102 mg, 0.363 mmol) and NCS (50.9 mg, 0.381 mmol) in acetonitrile (2 mL) was irradiated using microwave at 75 °C for 30 min or under conventional heating at 75 °C for 1 h. The reaction was analyzed using LC-MS. The mixture was concentrated followed by adding 3 mL water. The mixture was extracted with DCM (3 x 3 mL) and concentrated to give the crude product for next step without further purification.

4.3.2 Deprotection To the crude product (0.363 mmol) in DCM (2 mL), TFA (277.8 μL, 3.63 mmol) was added. The mixture was stirred overnight at
rt. The mixture was concentrated, co-evaporated with DCM three times to remove excess amount of TFA. The crude product was about 140 mg, suggesting the amount of TFA is 0.526 mmol, 1.5 equiv. of the free amine because the reaction gave full conversion. The mixture was dissolved in 5 mL 1,2-dichloroethane (DCE) and divided into two parts for reductive amination.

4.3.3 Reductive amination

To the crude product (0.182 mmol) in DCE (2.5 mL) from last step, TEA (38.1 μL, 0.273 mmol) was added and the mixture was stirred at rt for about 15 min. Aldehyde (30.4 mg, 0.200 mmol) was then added to the mixture and stirred at rt. The reaction was monitored using LC-MS. The imine formation was not completed after 3 h for 20, 22 and 23. Reducing reagent (0.363 mmol, NaNBH₃ for 20b, 22 and 23, or Na(OAc)₂BH for 20a) and EtOH (1.5 mL) were then added to the mixture and stirred at rt for 2 h (20a) or overnight (20b, 22 and 23). The imine formation was completed after 3 h for 21a, NaBH₄ (1.09 mmol) and 1.5 mL EtOH were added and stirred at rt for 2 h. The reaction mixture was then concentrated at 30 °C, re-dissolved in MeOH and purified using preparative LC and freeze-drying to give the desired product as white solid. The solid became sticky under air atmosphere. Chemical shifts of NMR can be slightly different for the same compound but containing different amount of solvent residue from the preparative LC. Compound 20a was used as an example to show the difference. Its chemical shifts were different when the solvent was removed using freeze-drying or extraction using DCM followed by concentration at about 25 °C.

4.3.4 3-(((4-chloro-2,5-dimethoxyphenethyl)amino)methyl)-4-methoxyphenol (20a)

The synthesis was started from 18a (30 mg, 0.0950 mmol), following the general procedure from the step of deprotection. The reaction mixture was purified using preparative LC. The fractions containing desired product were dried using freeze-drying to give compound 21a, NaBH₄ (1.09 mmol) and 1.5 mL EtOH were added and stirred at rt for 2 h. The reaction mixture was then concentrated at 30 °C, re-dissolved in MeOH and purified using preparative LC and freeze-drying to give the desired product as white solid. Its chemical shifts were different when the solvent was removed using freeze-drying or extraction using DCM followed by concentration at about 25 °C.

4.3.5 3-(((4-iodo-2,5-dimethoxyphenethyl)amino)(methyl)-4-methoxyphenol (20b)

The synthesis was started from 18b (40 mg, 0.098 mmol), following the general procedure for the synthesis of 20-23 from the step of deprotection, the reaction mixture was purified using preparative LC and the fractions containing 20b were dried using freeze-drying to give compound 20b (21.8 mg, 50% yield) as a white solid.

1H-NMR (CDCl₃, 300 MHz) δ 7.19 (s, 1H), 6.77-6.61 (m, 4H), 3.84 (s, 2H), 3.78 (s, 3H), 3.69 (s, 3H), 3.60 (s, 3H), 2.99 (t, J = 6.2 Hz, 2H), 2.90 (t, J = 6.2 Hz, 2H). 13C-NMR (CDCl₃, 75 MHz) δ 152.7, 152.4, 151.03, 150.97, 127.7, 123.8, 121.9, 118.3, 116.3, 114.1, 111.6, 83.5, 57.2, 56.2, 55.8, 48.3, 47.0, 29.6. ESI-HRMS positive mode (m/z): calcd. for C₁₈H₂₃NO₄ ([M+H]+): 344.0672. Found: 344.0660.

4.3.6 4-(((4-chloro-2,5-dimethoxyphenethyl)amino)(methyl)-3-methoxyphenol (21a)

The synthesis was started from compound 17 (51.0 mg, 0.182 mmol), following the general procedure step from chlorination. The reaction mixture was purified using preparative LC. The LC fractions containing desired product were dried using freeze-drying to give compound 21a (38.4 mg, 60% yield) as a white solid.

1H-NMR (CDCl₃, 300 MHz) δ 6.90-6.84 (m, 3H), 6.15-6.02 (m, 2H), 3.83 (s, 3H), 3.77 (s, 2H), 3.76 (s, 3H), 3.56 (s, 3H), 3.04 (t, J = 1.6 Hz, 2H), 2.94 (t, J = 1.6 Hz, 2H). 13C-NMR (CDCl₃, 75 MHz) δ 159.0, 158.5, 151.8, 149.2, 131.3, 126.1, 121.0, 115.8, 114.4, 113.3, 107.8, 99.4, 57.0, 56.2, 55.2, 48.7, 47.8, 29.5. ESI-HRMS positive mode (m/z): calcd. for C₁₈H₂₃NO₄ ([M+H]+): 352.1316. Found: 352.1315; calcd. for C₁₈H₂₃ClNO₄ ([M+H]+): 354.1286. Found: 354.1287.

4.3.7 3-(((4-chloro-2,5-dimethoxyphenethyl)amino)(methyl)-2-methoxyphenol (22)

The synthesis was started from compound 17 (51.0 mg, 0.182 mmol), following the general procedure step from chlorination. The reaction mixture was purified using preparative LC. The LC fractions containing desired product were dried using freeze-drying to give compound 22 (17.3 mg, 27% yield) as a white solid.

1H-NMR (CDCl₃, 300 MHz) δ 6.91 (dd, J = 8.1, 7.5 Hz, 1H), 6.68 (s, 1H), 6.82 (dd, J = 8.1, 1.8 Hz, 1H), 6.77 (s, 1H), 6.75 (dd, J = 7.5, 1.8 Hz, 1H), 3.85 (s, 2H), 3.83 (s, 3H), 3.76 (s, 3H), 3.74 (s, 3H), 2.96-2.81 (m, 4H). 13C-NMR (CDCl₃, 75 MHz) δ 151.8, 149.3, 149.1, 145.8, 131.2, 127.0, 125.0, 121.4, 120.6, 116.2, 115.3, 113.2, 61.3, 57.0, 56.2, 48.6, 48.3, 30.3. ESI-HRMS positive mode (m/z): calcd. for C₁₈H₂₃ClNO₄ ([M+H]+): 352.1316. Found: 352.1329; calcd. for C₁₈H₂₃ClNO₄ ([M+H]+): 354.1286. Found: 354.1294.

4.3.8 2-(((4-chloro-2,5-dimethoxyphenethyl)amino)(methyl)-3-methoxyphenol (23)

The synthesis was started from compound 17 (51.0 mg, 0.182 mmol), following the general procedure step from chlorination. The reaction mixture was purified using preparative LC. The LC fractions containing desired product were dried using freeze-drying to give compound 23 (35.9 mg, 56% yield) as a white solid.

1H-NMR (CDCl₃, 300 MHz) δ 7.09 (t, J = 8.4Hz, 1H), 6.87 (s, 1H), 6.75 (s, 1H), 6.50 (dd, J = 8.4, 0.9 Hz, 1H), 6.36 (dd, J = 8.4, 0.9 Hz, 1H), 4.09 (s, 2H), 3.83 (s, 3H), 3.75 (s, 3H), 3.74 (s, 3H), 3.01-2.80 (m, 4H). 13C-NMR (CDCl₃, 75 MHz) δ 159.3, 158.0, 151.7, 149.1, 129.3, 126.0, 120.9, 115.3, 113.3, 109.9, 109.0, 75.4, 47.8, 29.5. ESI-HRMS positive mode (m/z): calcd. for C₁₈H₂₃ClNO₄ ([M+H]+): 352.1316. Found: 352.1316; calcd. for C₁₈H₂₃ClNO₄ ([M+H]+): 354.1286. Found: 354.1294.
Tetrahedron

101.6, 57.0, 56.1, 55.7, 47.8, 43.9, 29.8. ESI-HRMS positive mode (m/z): calcd. for C_{18}H_{23}{^{35}}ClNO_{4} ([M+H]^{+}): 352.1316. Found: 352.1322; calcd. for C_{18}H_{23}{^{37}}ClNO_{4} ([M+H]^{+}): 354.1286. Found: 354.1288.

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References:

2. EMCDDA highlights growing threads posed by new and established substances, European Drug Report 2017-Highlights.

Supplementary Material

Supplementary data associated with this article can be found, in the online version, at
Synthesis and Identification of Metabolite Biomarkers of 25C-NBOMe and 25I-NBOMe
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Supporting information

Synthesis and Identification of Metabolite Biomarkers of 25C-NBOMe and 25I-NBOMe

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Copies of NMR spectra of the prepared compounds.

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5-hydroxy-2-methoxybenzaldehyde (2)  
Solvent: CDCl₃
(E)-4-methoxy-3-(2-nitrovinyl)phenol (3)  Solvent: MeOD
3-(2-aminoethyl)-4-methoxyphenol (4)  Solvent: MeOD
2,2,2-trifluoro-N-(5-hydroxy-2-methoxyphenethyl)acetamide (5)

Solvent: CDCl$_3$
N-(4-chloro-5-hydroxy-2-methoxyphenethyl)-2,2,2-trifluoroacetamide (6)  
Solvent: CDCl$_3$
(E)-4-methoxy-2-(2-nitrovinyl)phenol (10)  
Solvent: CDCl₃ (¹H-NMR), MeOD (¹³C-NMR)
2-(2-aminoethyl)-4-methoxyphenol (11)

Solvent: CDCl₃
4-methoxy-2-(2-(2,2,2-trifluoroacetamido)ethyl)phenyl acetate (12)  
Solvent: CDCl₃
5-chloro-4-methoxy-2-(2-(2,2,2-trifluoroacetamido)ethyl)phenyl acetate (13a)  Solvent: CDCl₃
5-iodo-4-methoxy-2-(2-(2,2,2-trifluoroacetamido)ethyl)phenyl acetate (13b) Solvent: CDCl$_3$
5-chloro-4-methoxy-2-(2-((2-methoxybenzyl)amino)ethyl)phenol (15a)  Solvent: CDCl₃

5-iodo-4-methoxy-2-(2-((2-methoxybenzyl)amino)ethyl)phenol (15b)  Solvent CDCl₃
Tert-butyl 2,5-dimethoxyphenethylcarbamate (17)  
Solvent: CDCl₃
2-(2-((tert-butoxycarbonyl)amino)ethyl)-5-chloro-4-methoxyphenyl acetate (18a)  Solvent: CDCl₃
2-(2-((tert-butoxycarbonyl)amino)ethyl)-5-iodo-4-methoxyphenyl acetate (18b)  Solvent: CDCl₃
2-(((4-ido-2,5-dimethoxyphenethyl)amino)methyl)phenol (19)  
Solvent: CDCl$_3$
3-(((4-chloro-2,5-dimethoxyphenethyl)amino)methyl)-4-methoxyphenol (20a)  
Solvent: CDCl$_3$
3-(((4-chloro-2,5-dimethoxyphenethyl)amino)methyl)-4-methoxyphenol (20a)  Solvent: CDCl₃
3-(((4-iodo-2,5-dimethoxyphenethyl)amino)methyl)-4-methoxyphenol (20b)  Solvent: CDCl$_3$
4-(((4-chloro-2,5-dimethoxyphenethyl)amino)methyl)-3-methoxyphenol (21a)  Solvent: CDCl₃
4-(((4-iodo-2,5-dimethoxyphenethyl)amino)methyl)-3-methoxyphenol (21b)  
Solvent: CDCl₃
3-(((4-chloro-2,5-dimethoxyphenethyl)amino)methyl)-2-methoxyphenol (22)  
Solvent: CDCl$_3$
2-(((4-chloro-2,5-dimethoxyphenethyl)amino)methyl)-3-methoxyphenol (23)  Solvent: CDCl₃