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Analytical characterization of bioactive *N*-benzyl-substituted phenethylamines and 5-methoxytryptamines

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RATIONALE: Substances based on the *N*-(2-methoxybenzyl)phenethylamine template ('NBOMe' derivatives) play an important role in medicinal research but some of these derivatives have also appeared as 'research chemicals' for recreational use which has attracted attention worldwide. A major challenge associated with newly emerging substances includes the lack of analytical data and the ability to correctly identify positional isomers.
METHODS: Six *N*-benzylphenethylamines based on the 2,5-dimethoxy-4-iodophenethylamine structure ('25I') and twelve substituted *N*-benzyl-5-methoxytryptamines ('5MT') have been prepared and extensively characterized. Techniques used for characterization were gas chromatography/ion trap mass spectrometry in electron and chemical ionization mode, liquid chromatography/diode array detection (DAD), infrared spectroscopy, electrospray high mass accuracy quadrupole time-of-flight tandem mass spectrometry, and triple quadrupole tandem mass spectrometry.
RESULTS: The characterization of 18 'NBOMe' compounds provided a comprehensive collection of chromatographic and spectral data. Four groups of three positional isomers, i.e. 25I-NB2OMe, 25I-NB3OMe, 25I-NB4OMe, 25I-NB2B, 25I-NB3B, 25I-NB4B and their 5-methoxytryptamine counterparts, were included and assessed for ability to obtain differentiation. Six *meta*-substituted *N*-benzyl derivatives of 5-methoxytryptamine (CF_3, F, CH_3, Cl, I, SCH_3) were also studied.
CONCLUSIONS: The implementation of mass spectral techniques was helpful for the differentiation between isomers, for example, when considering the difference in a number of ion ratios. This was considered beneficial in cases where chromatographic separation was only partially achieved under liquid chromatography (LC) conditions. The use of LC/DAD analysis was also found to be valuable for this particular purpose, which confirmed the integrative value of complementary techniques used in areas related to forensic toxicology. Copyright © 2015 John Wiley & Sons, Ltd.

The diverse nature of serotonin (5-HT) receptor activation forms a fundamental basis of the functioning of biological systems including human physiology and the regulation and modulation of psychological operation.[1] In humans, profound alterations in mood, thought, and cognition can be induced by a range of substances that activate the 5-HT_{2A} receptor, which is believed to play a central role in the mediation of psychoactive effects associated with so-called 'classic' hallucinogens or psychedelics, such as lysergic acid diethylamide (*d*-LSD), psilocybin and a number of ring-substituted phenethylamines and amphetamines.[2–5] Although many of the highly selective 5-HT_{2A} receptor agonists remain in the pre-clinical space, a diverse range of these derivatives has been encountered as commercially available 'research chemicals' used for recreational purposes.[6,7] 5-HT_{2A} receptor agonist activity appears to be a necessary requirement for a compound to display psychedelic effects although this might not always be sufficient.[8] It is this particular feature, however, that has led to the recreational and commercial exploration, including manufacturing and sale via various outlets, such as online retailers or 'head shops'.

The translation of laboratory-based research into a wider commodified open market has attracted concern worldwide. A number of *N*-(2-methoxybenzyl)phenethylamines became known as 'NBOMe' derivatives and common examples include 25I-NBOMe (**1a**) 25B-NBOMe and 25C-NBOMe (Fig. 1(A)), although other substituted *N*-benzyl groups also have been encountered. Between 2011 and 2013, the European Monitoring Centre for Drugs and Drug Addiction

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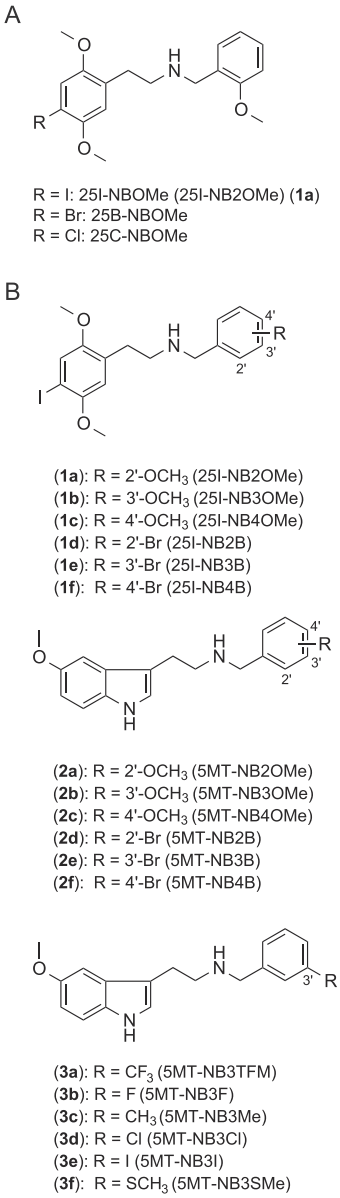

Figure 1. (A) Three representative 5-HT_{2A} receptor agonists and *N*-benzylphenethylamine derivatives 25I- (**1a**), 25B-, and 25C-NBOMe that are available as recreational psychoactive substances. (B) Chemical structures of 'NBOMe' derivatives (**1a**)–(**3f**) subjected to analytical characterization reported in the present study.

(EMCDDA) received notifications from partners of the EU Early Warning System on the detection of 12 'NBOMe' derivatives.[9–11] The United Nations Office on Drugs and Crime (UNODC) also confirmed the detection of 'NBOMe' compounds in various UN Member States.[12] The World Health Organization, supported by the Expert Committee on Drug Dependence (ECDD), has recently held the 36th ECDD meeting to consider the available data on 25I-NBOMe (**1a**), 25B-NBOMe, and 25C-NBOMe for options regarding potential international control[13] and recommendations made are expected to be announced in due course. In Europe, the Scientific Committee of the EMCDDA convened a risk assessment on 25I-NBOMe,[14] which was later followed by a European Council decision to subject this substance to EU-wide control.[15] In the United States, 25I-NBOMe, 25B-NBOMe, and 25C-NBOMe have been temporarily scheduled into the Controlled Substances Act as Schedule 1 substances.[16]

A common challenge associated with the ability to identify newly emerging psychoactive substances includes the lack of analytical data, which can reflect the fact that many of them may have only been described in the patent literature or medicinal and pharmacological literature, where comprehensive analytical characterization is often not carried out.[6] Once a particular analog of interest has been identified, further questions commonly arise when considering the presence of positional isomers, because reference standards for these isomers are often not available.

The present study presents the analytical characterization of six *N*-benzylphenethylamines (**1a**)–(**1f**) and twelve *N*-benzyl-5-methoxytryptamines (**2a**)–(**3f**) (Fig. 1(B)). Four groups of three positional isomers that differed in the position of substituents on the *N*-benzyl group, e.g. 25I-NBOMe (**1a**) and its 3- and 4-methoxybenzyl analogues (**1b**) and (**1c**), were included to determine the potential for differentiation using techniques commonly employed in forensic toxicology. The analytical characterizations were carried out by gas chromatography/ion trap mass spectrometry in electron and chemical ionization mode, liquid chromatography/diode-array detection, infrared spectroscopy, electrospray high mass accuracy quadrupole time-of-flight tandem mass spectrometry and triple quadrupole tandem mass spectrometry.

EXPERIMENTAL

HPLC-grade acetonitrile was supplied by Rathburn Chemicals Ltd (Walkerburn, UK) and triethylammonium phosphate (TEAP, 1.0 M) (pH 3.0) was obtained from Fluka (Gillingham, UK). All other solvents and reagents were from Aldrich (Gillingham, UK). All 18 substituted *N*-benzyl derivatives ((**1a**)–(**3f**)) were synthesized following established procedures.[5,17,18]

Gas chromatography (GC)/ion trap mass spectrometry

GC/MS data for all 18 compounds (0.5 mg/mL in methanol) were obtained in electron (EI) and chemical ionization (CI) mode (scan range *m/z* 41–500) using a Varian 450-GC gas chromatograph (Walnut Creek, CA, USA) coupled to a Varian 220-MS ion trap mass spectrometer. A Varian 8400 autosampler was employed with a Varian CP-1177 injector (275 °C) in split mode (1:50). Data acquisition was performed with the Varian MS Data Review function of the Workstation software (version 6.91). Transfer line, manifold and ion trap temperatures were set at 310, 80 and 220 °C, respectively. The carrier gas was helium at a flow rate of 1 mL/min using the EFC constant flow mode. The liquid CI reagent was HPLC grade methanol. The default settings for CI ionization parameters (0.4 s/scan) were used: CI storage level *m/z* 19.0; ejection amplitude *m/z* 15.0; background mass *m/z* 55; maximum ionization time 2000 µs; maximum reaction time 40 ms; target TIC 5000 counts. A J&W VF-5ms GC column (30 m × 0.25 mm, 0.25 µm film thickness; Agilent, Cheadle,

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UK) was employed for separation. The starting temperature was set at 130 °C and held for 1 min. The temperature was then increased at 20 °C/min to 280 °C and held constant for 11.50 min to give a total run time of 20.00 min.

Electrospray triple quadrupole mass spectrometry

Electrospray triple quadrupole tandem mass spectrometry experiments were carried out by direct infusion (10 µL/min at 0.01 mg/mL) of compounds using a Waters Micromass Quattro Premier triple quadrupole MS/MS system (Waters Micromass, Manchester, UK) operated under Masslynx version 4.1 software. MS optimizations were performed in MS scan and in product ion scan modes. Product ion scans were obtained in positive ion mode and the optimized source condition settings were as follows: capillary voltage 3.12 kV; cone voltage 28 V; rf lens voltage 0.1 V; source temperature 100 °C; desolvation temperature 200 °C; and multiplier voltage 650 V. Nitrogen was used as the cone gas (50 L/h flow rate) and desolvation gas (200 L/h flow rate) while the collision gas was argon (0.3 mL/min flow rate). The $[M + H]^+$ ions obtained for all 18 compounds were selected for MS/MS experiments. Product ions were collected over the range *m/z* 45–500. The scan time for each channel was 0.5 s and the interscan delay was 0.1 s. The cone voltage was set at 28 V in all cases. The collision energy used for (**1d**)–(**1f**) was 20 eV while a value of 10 eV was chosen for the tryptamine-based compounds (**2a**)–(**3f**).

High-resolution electrospray mass spectrometry

High-resolution electrospray mass spectra were obtained using an Agilent 6540 quadrupole time-of-flight mass spectrometer, with the compounds being characterized by UHPLC/QTOF-MS/MS as described previously.[19,20] Briefly,

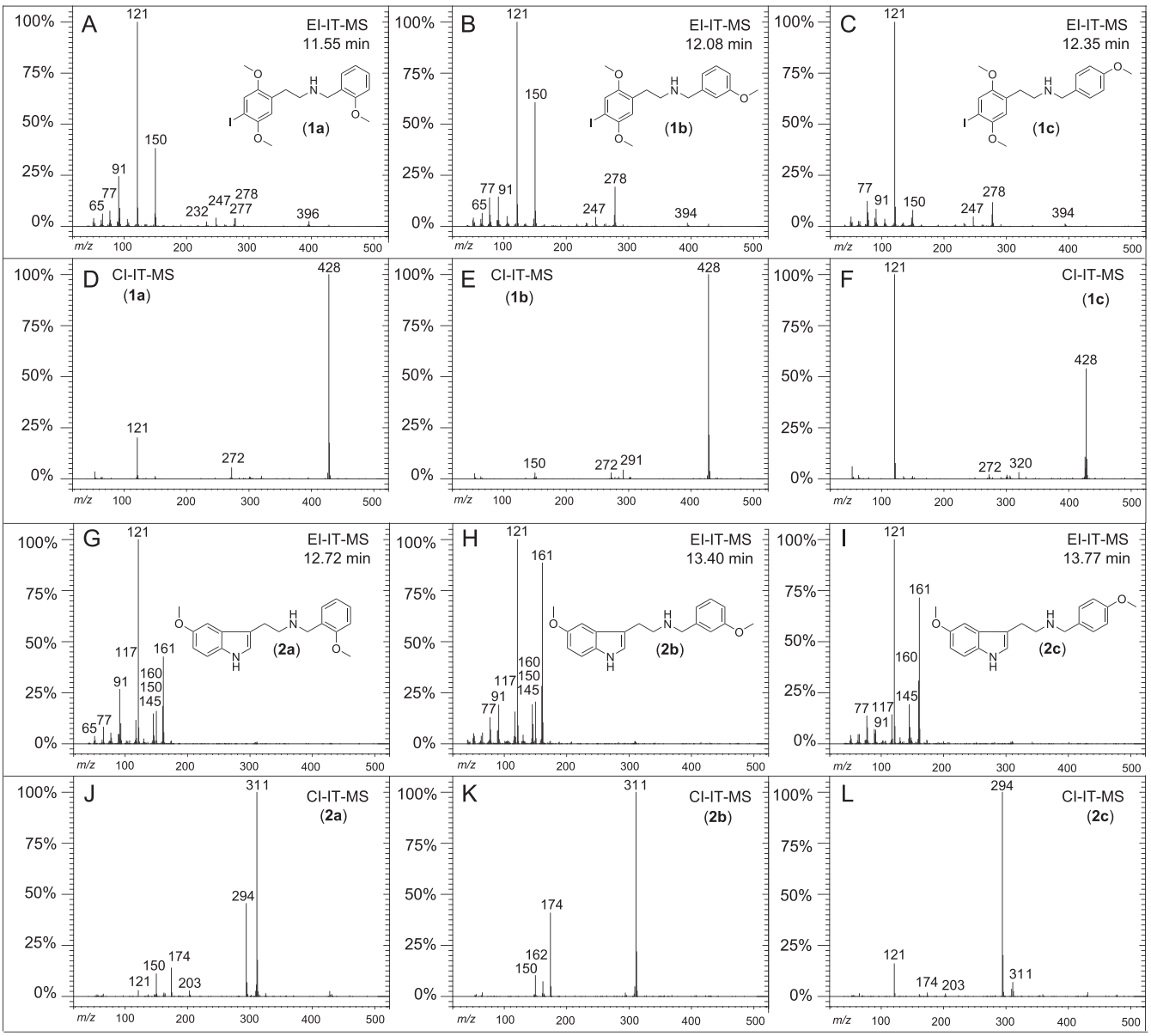


Figure 2. Gas chromatography retention times, electron (EI)- and chemical (CI) ionization ion trap (IT) mass spectra of *N*-(methoxybenzyl)-substituted isomers (**1a**)–(**1c**) and (**2a**)–(**2c**).

the mobile phases used for UHPLC separation consisted of 100% acetonitrile (1% formic acid) and an aqueous solution of 1% formic acid. The column temperature was set at 40 °C (0.6 mL/min flow rate) and data were acquired for 5.5 min. The elution gradient was a 5–70% acetonitrile ramp over 3.5 min, then increased to 95% acetonitrile in 1 min and held for 0.5 min before returning to 5% acetonitrile in 0.5 min. QTOF-MS data were acquired in positive mode scanning from *m/z* 100 to 1000 with and without auto MS/MS fragmentation. Ionization was achieved with an Agilent JetStream electrospray source and infused internal reference masses. The Agilent 6540 QTOF-MS parameters were: gas temperature 325 °C; drying gas (N_2) flow rate 10 L/min; and sheath gas (N_2) temperature 400 °C. Internal ions at *m/z* 121.05087 and 922.00979 were used as reference ions.

High-performance liquid chromatography/diode array detection

HPLC/DAD analyses[19,20] were carried out on a Dionex 3000 Ultimate system coupled to a Dionex UV diode-array detector (Thermo Fisher, St Albans, UK), using a Synergi Fusion column (150 mm × 2 mm, 4 µm) that was protected by a Synergi Fusion guard column (4 mm x 3 mm; both Phenomenex, Macclesfield, UK). The mobile phases were 70% acetonitrile with 25 mM TEAP buffer and an aqueous solution of 25 mM TEAP buffer. Elution was achieved with a gradient that started with 4% acetonitrile and ramped to 70% acetonitrile in 15 min and held for 3 min. The total acquisition time was 18 min at a flow rate of 0.6 mL/min. The diode-array detection window was set at 200 to 595 nm (collection rate 2 Hz).

RESULTS AND DISCUSSION

The high potency associated with substances such as 25I-NBOMe (**1a**),[21,22] and the possibility that some of these 5-HT_{2A} receptor agonists may not be orally active,[23] might reflect the fact that these substances are often encountered in the form of absorbent paper (blotters), which are ingested via sublingual and buccal routes. However, the presence of powdered forms, capsules and liquids has also been reported.[14] Although the availability of bulk drugs may make them amenable to a large range of analytical

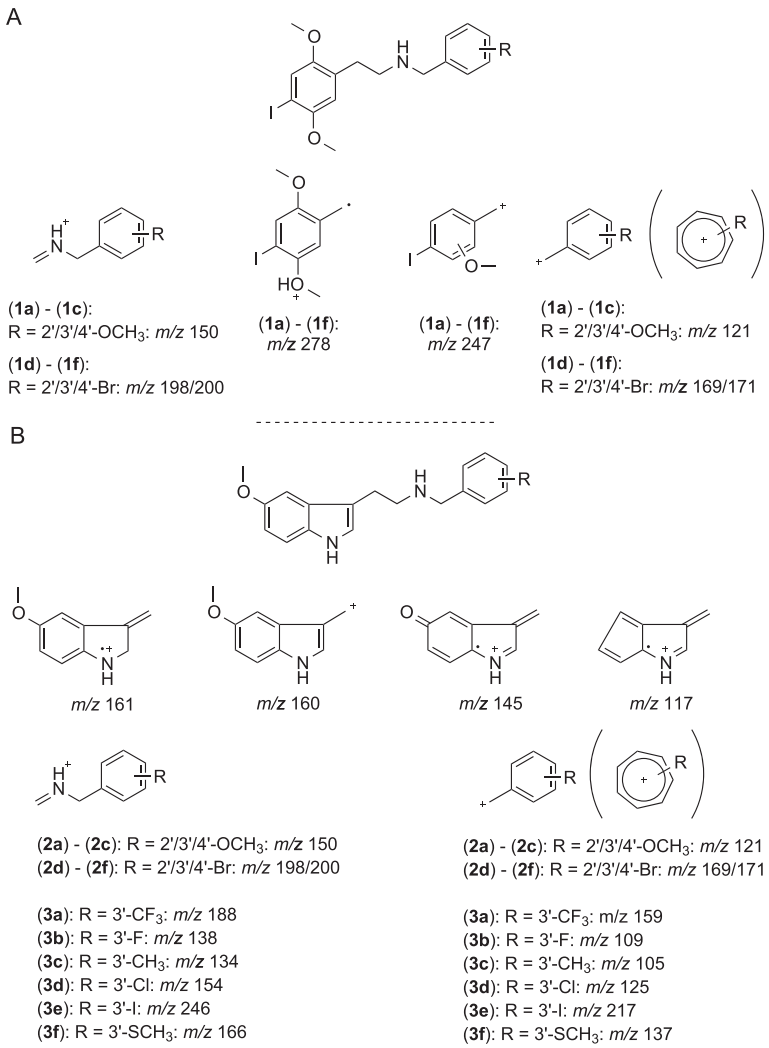


Figure 3. Structural representations of suggested key ions formed during analysis by electron ionization ion trap mass spectrometry: (A) 25I-NBOMes (**1a**)–(**1f**) and (B) 5MT-NBOMes (**2a**)–(**3f**).

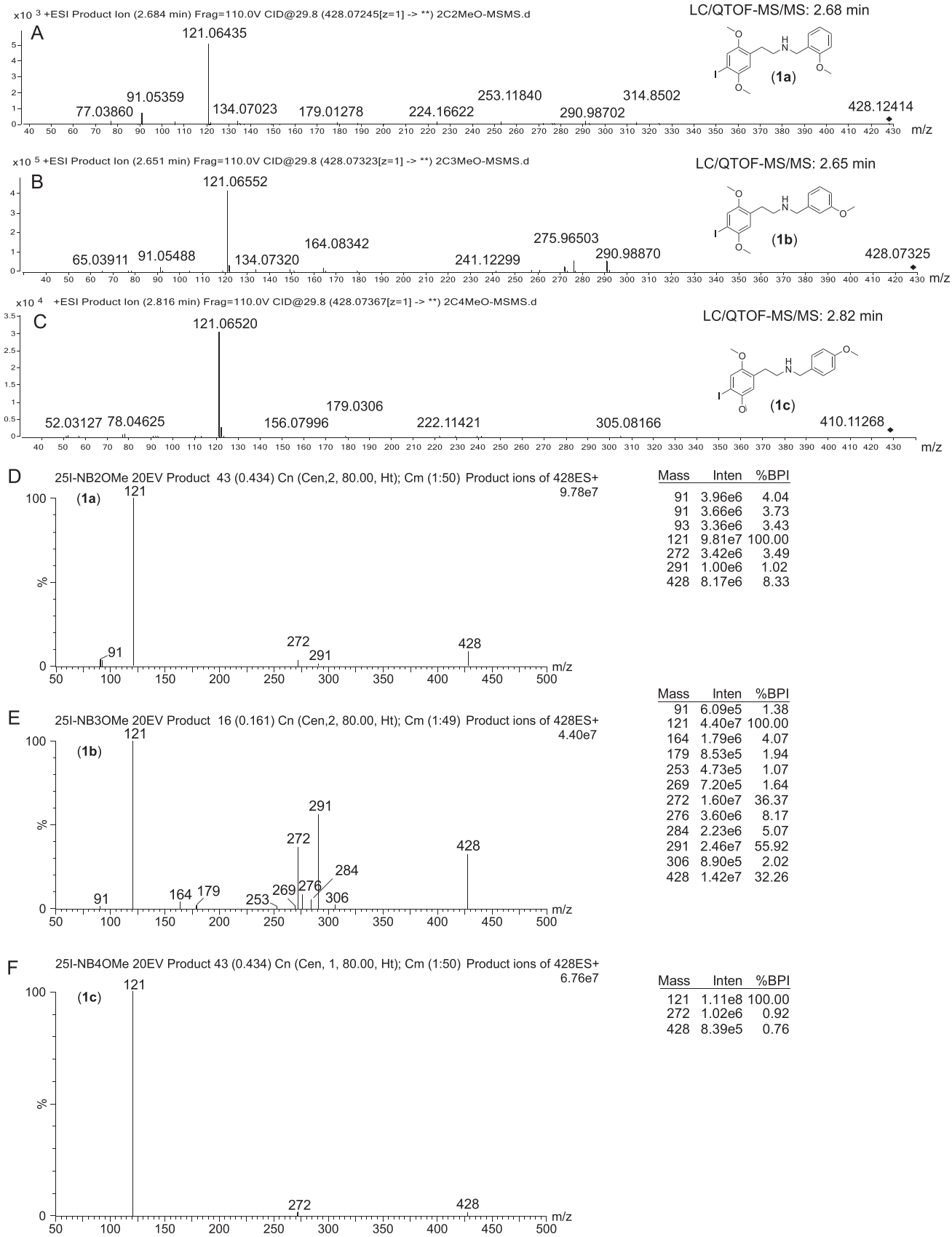


Figure 4. Electrospray ionization mass spectra obtained from positional 25I-NBOMe isomers (**1a**)–(**1c**). (A–C) UHPLC/QTOF-MS/MS. The retention time of (**1c**) in trace (C), i.e. associated with the peak top, was 2.66 min. The given retention time of 2.82 min represented the attempt to obtain a less saturated signal response for the base peak ion. (D–F) Direct infusion triple quadrupole MS/MS and corresponding ion ratios.

investigations, detection and identification in biofluids often requires the implementation of mass spectrometry based techniques due to the high potency associated with some of these derivatives.[24] Whilst knowledge about the formation of fragment and product ions form an important basis for the development of suitable screening techniques, further complexities might arise from the need to differentiate between isomers.[19]

In addition to the study of bioactive *N*-benzylphenethylamines, a number of their 5-methoxytryptamine counterparts were investigated. For example, in the rat tail artery assay, *N*-(2-methoxybenzyl)-5-methoxytryptamine (**2a**) was shown to have appreciable partial 5-HT_{2A} receptor agonist activity (pEC_{50} = 7.08; E_{max} = 54% relative to serotonin),[25] which indicated potential for further exploration. Indeed, all the substituted *N*-benzylphenethylamines and 5-methoxytryptamines (**1a**)–(**3f**) (Fig. 1(B)) characterized in the present study have recently undergone a comprehensive investigation into serotonin receptor affinity, functional activity and mouse head-twitch response in order to acquire insights into their structure-activity relationships. One of the observations made was that *meta*-substitution in the *N*-benzyl derivatives of 5-methoxytryptamine was important for their affinity to the 5-HT_2 receptor family and functional activity *in vitro* and *in vivo*.[26]

In the interest of clarity and brevity, a more detailed discussion of analytical data obtained for the *N*-(methoxybenzyl)-phenethylamine isomers (**1a**)–(**1c**) and *N*-(methoxybenzyl)-5-methoxytryptamine isomers (**2a**)–(**2c**) is included here to serve as representative examples. The data for all the remaining derivatives, including infrared spectroscopy of all 18 substances, are shown as Supporting Information.

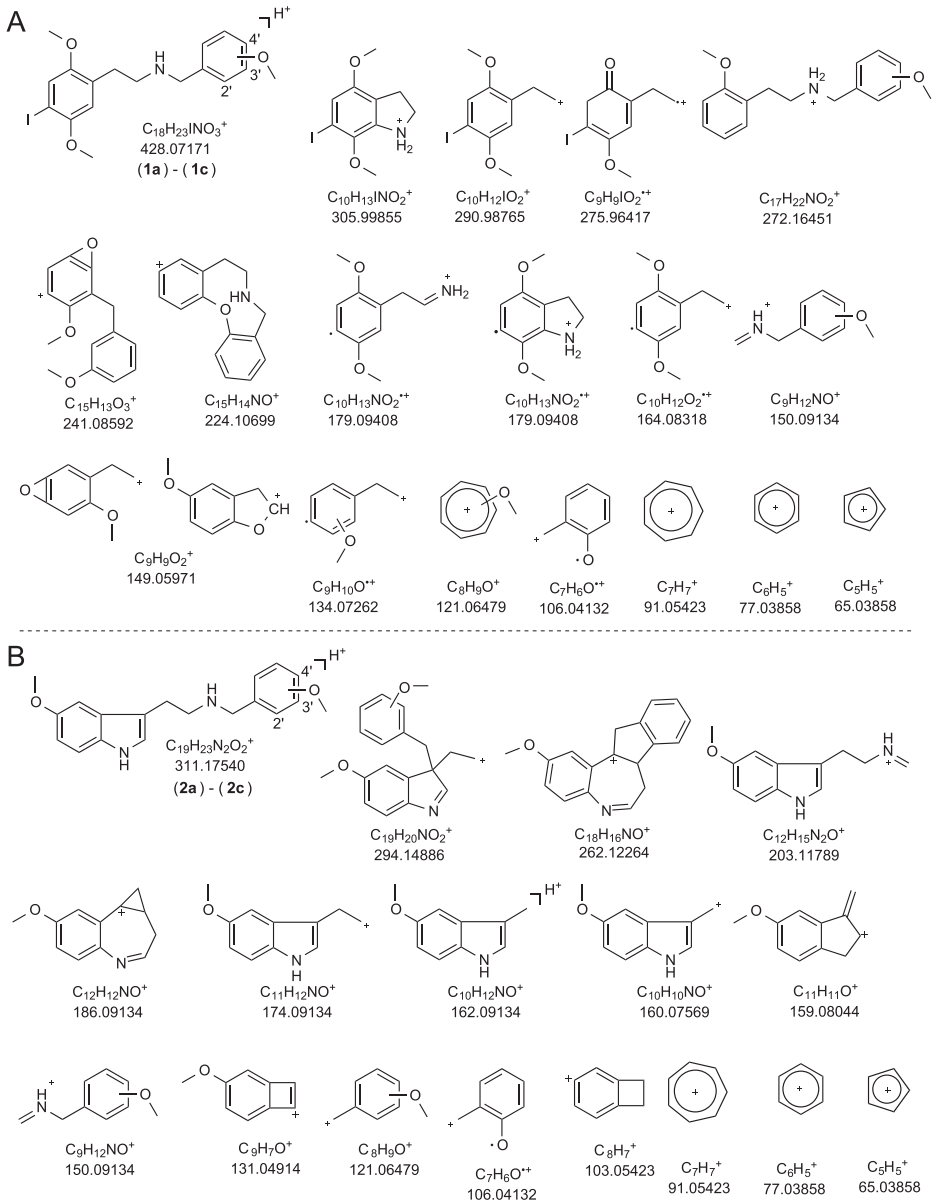


Figure 5. Structural representations of suggested key ions formed during analysis by QTOF-MS/MS and their calculated theoretical *m/z* values: (A) 25I-NBOMes (**1a**)–(**1c**) and (B) 5-MT-NBOMes (**2a**)–(**2c**).

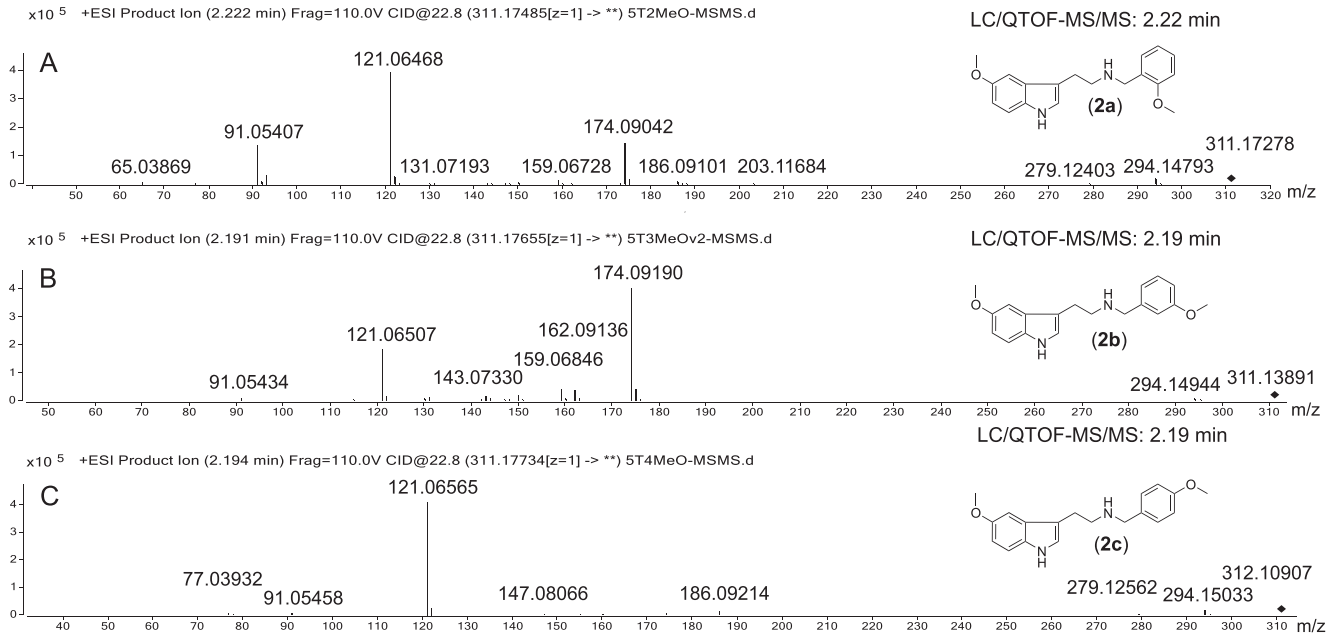
Gas chromatography/electron and chemical ionization ion trap mass spectrometry

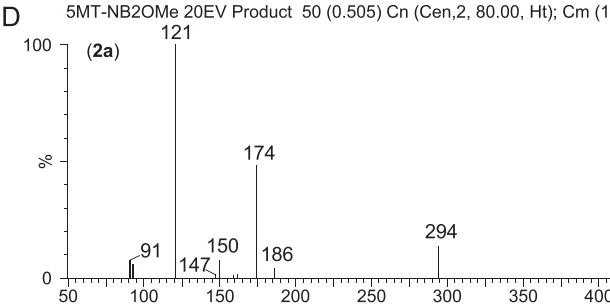
Gas chromatography (GC) retention times and ion trap (IT) electron ionization and chemical ionization (EI/CI) mass spectra for *N*-(methoxybenzyl)phenethylamine (**1a**)–(**1c**) are shown in Figs. 2(A)–2(C) as representative examples while the remaining mass spectra including all GC chromatograms are available as Supporting Information. All three sets of positional isomers, i.e. (**1a**)–(**1f**) and (**2a**)–(**2f**), could be separated under GC conditions and the elution order followed a similar pattern with increasing retention times in the order of *ortho*-, *meta*- and *para*-substitution, as was also observed with a range of other isomeric *N*-(methoxybenzyl)-phenethylamine analogs. Indeed, the approach taken here when attempting to use the abbreviations (e.g. Fig. 1) for the corresponding 'NBOMe' isomers was adopted from the same authors.[27] The EI-IT mass spectra obtained for the three 25I-NBOMe isomers (**1a**)–(**1c**) were largely consistent with the EI mass spectra reported previously using a quadrupole mass analyzer and included key fragment ions at *m/z* 121 (methoxylated tropylium ion) and 150 (iminium ion formation following *α*-cleavage).[27] Correspondingly, the mass spectra associated with the three *N*-(bromobenzyl)-substituted phenethylamine isomers (**1d**)–(**1f**) showed the tropylium and iminium counterparts at *m/z* 169/171 and 198/200, respectively (Supporting Information). In contrast to the *N*-(methoxybenzyl) isomers (**1a**)–(**1c**) that gave the same base peak at *m/z* 121, the brominated counterparts (**1d**)–(**1f**) revealed increasing relative abundance values for the equivalent ions at *m/z* 169/171 following the order of *ortho*- (**1d**), *meta*- (**1e**), and *para*-substitution (**1f**) (Supporting Information). Possible EI-MS key fragment ions are proposed in Fig. 3(A), which includes suggestions incorporated from Zuba and colleagues on structurally related substances.[28–30] Representative GC/CI-IT mass spectra for *N*-(methoxybenzyl)phenethylamine analogs (**1a**)–(**1c**) are depicted in Figs. 2(D)–2(F) and revealed facile detection of the $[M + H]^+$ ion at *m/z* 428 to confirm the mass of the molecule, which was more challenging under EI-IT-MS conditions due to extensive fragmentation of the molecular ion. Some variations in the relative abundance of key EI fragment ions, e.g. *m/z* 91, 121 and 150, have been reported previously for (**1a**)–(**1c**) when using a single quadrupole mass analyzer.[27] Similar variations have been observed in the present study under three-dimensional ion-trap conditions (Figs. 2(A)–2(C)), which might point towards the ability to differentiate between isomers. A more pronounced difference that might allow for differentiation between the *N*-(methoxybenzyl) phenethylamine isomers on mass spectral grounds was observed under CI-IT-MS conditions (Figs. 2(D)–2(F)) for the relative abundance of the *m/z* 121 ion. In the case of 25I-NB4OMe (**1c**), this ion formed the base peak whereas it was absent in the mass spectrum of the *N*-(3-methoxybenzyl) analog (**1b**). By contrast, 25I-NB2OMe (**1a**) yielded a relative abundance value of 20% for *m/z* 121. Interestingly, in the case of the three *N*-(bromobenzyl) isomers (**1d**)–(**1f**) the stability

Table 1. Product ions obtained from *N*-(methoxybenzyl)-substituted phenethylamines (**1a**)–(**1c**). Suggested elemental formulae of proposed product ions (Fig. 5(A)) that could be detected by QTOF-MS/MS analysis with acceptable mass accuracy

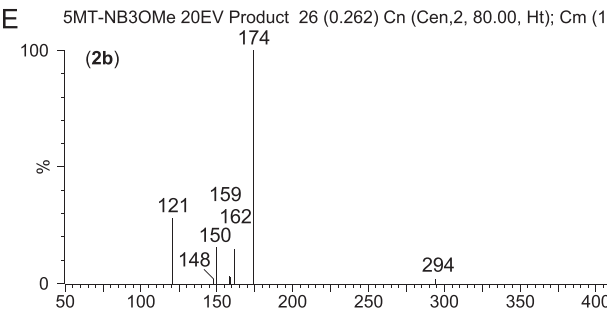
Compound	Measured *m/z* value	Suggested formula	Calculated *m/z* value	Δm [ppm]
1a 25I-NB2OMe	428.07239 (PM)	$C_{18}H_{23}INO_3^+$	428.07171	−1.59
	314.08502	*No formula*	–	–
	290.98702	$C_{10}H_{12}IO_2^+$	290.98765	+2.17
	179.01278	$C_{12}H_{13}O_2^{\bullet+}$	179.01276	−0.11
	134.07023	$C_9H_{10}O^{\bullet+}$	134.07262	−17.8
	121.06435 (BP)	$C_8H_9O^+$	121.06479	+3.68
	106.04068	$C_7H_6O^{\bullet+}$	106.04068	−6.04
	91.05359	$C_7H_7^+$	91.05423	+7.07
1b 25I-NB3OMe	428.07319 (PM)	$C_{18}H_{23}INO_3^+$	428.07171	−3.46
	306.99932	$C_{10}H_{13}INO_2^+$	305.99855	−2.53
	290.98870	$C_{10}H_{12}IO_2^+$	290.98765	−3.62
	275.96503	$C_9H_9IO_2^{\bullet+}$	275.96417	+3.12
	179.09422	$C_{10}H_{13}NO_2^{\bullet+}$	179.09408	+0.78
	164.08342	$C_{10}H_{12}O_2^{\bullet+}$	164.08318	+1.46
	149.05990	$C_9H_9O_2^+$	149.05971	−1.31
	134.07320	$C_9H_{10}O^{\bullet+}$	134.07262	+4.33
	121.06552 (BP)	$C_8H_9O^+$	121.06479	−6.07
	91.05488	$C_7H_7^+$	91.05423	−6.26
	65.03911	$C_5H_5^+$	65.03858	−8.33
1c 25I-NB4OMe	428.07302 (PM)	$C_{18}H_{23}INO_3^+$	428.07171	+3.06
	121.06520 (BP)	$C_8H_9O^+$	121.06479	−3.40
	106.04158	$C_7H_6O^{\bullet+}$	106.04132	+2.45
	91.05437	$C_7H_7^+$	91.05423	−1.59
	77.03923	$C_6H_5^+$	77.03858	−8.59

PM: $[M + H]^+$ ion; BP: base peak.

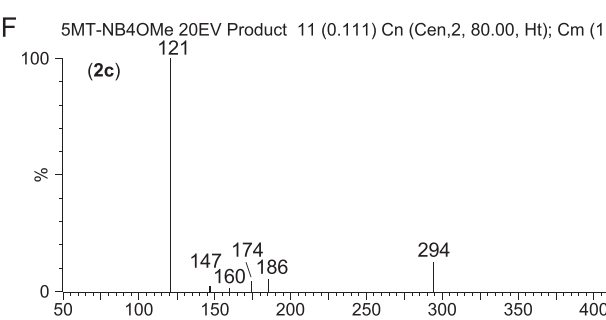




Mass	Inten	%BPI
91	6.13e6	7.32
121	8.37e7	100.00
147	1.02e6	1.22
150	6.21e6	7.43
159	9.25e5	1.11
162	1.21e6	1.44
174	4.03e7	48.20
186	3.23e6	3.86
294	1.14e7	13.63



Mass	Inten	%BPI
121	2.64e7	28.04
148	1.40e6	1.49
150	1.44e7	15.32
159	2.85e6	3.03
162	1.39e7	14.76
174	9.40e7	100.00
294	1.42e6	1.51



Mass	Inten	%BPI
121	9.16e7	100.00
147	1.96e6	2.14
160	1.22e6	1.34
174	4.07e6	4.44
186	4.60e6	5.02
294	1.14e7	12.43

Figure 6. Electrospray ionization mass spectra obtained from positional 5MT-NBOMe isomers (**2a**)–(**2c**): (A–C) UHPLC/QTOF-MS/MS and (D–F) direct infusion triple quadrupole MS/MS and corresponding ion ratios.

of the $[M + H]^+$ ion at *m/z* 476/478 dominated throughout the three CI mass spectra with negligible relative abundance for the brominated tropylium counterpart at *m/z* 169/171 (Supporting Information).

GC retention times and EI/CI-IT-MS data for the *N*-(methoxybenzyl)-5-methoxytryptamine isomers (**2a**)–(**2c**) are provided in Figs. 2(G)–2(L). Similar to the corresponding phenethylamine 25I-NB4OMe (**1c**) (Fig. 2 (C)), the relative abundance in the EI spectra of the *m/z* 150 iminium ion appeared to be significantly lower in the tryptamine counterpart (**2c**) (Fig. 2(I)) than in the remaining isomers. The tropylium ion at *m/z* 121 also formed the base peak in all three isomeric cases, similar to the phenethylamines (**1a**)–(**1c**). The main difference found in the EI-IT mass spectra, however, which facilitated the identification of the 5-methoxytryptamine moieties, was seen in key ions at *m/z* 160, 145 and 117. In addition, the key indicator that differentiated between a potentially existing *N*,*N*-dialkyltryptamine from a *N*-mono-substituted compound, as is the case with the *N*-benzyl species, was the occurrence of a strong *m/z* 161 ion which, for example, would not be observed with *N*,*N*-dialkylated compounds (Figs. 2(G)–2(I) and 3 (B)).[31] Interestingly, apart from 5MT-NB3Me (**3c**), which yielded the methyltropylium base peak at *m/z* 105, all remaining *N*-benzyltryptamines, (**2d**)–(**2f**), (**3a**), (**3b**) and (**3d**)–(**3f**), formed a base peak at *m/z* 161 (Supporting Information). The CI-IT-MS data for *N*-benzyltryptamines (**2a**)–(**2c**) (Figs. 2(J)–2(L)) provided $[M + H]^+$ ions as expected although, as observed with the phenethylamines, distinct differences were observed due to further dissociation, which might be useful for differentiating these three isomers.

Some moderate differences were noted in the abundance values associated with the *N*-(methoxybenzyl) group, i.e. *m/z* 121 and 150. One feature that did not appear to be detected in the CI mass spectra of the *N*-(methoxybenzyl) phenethylamine counterparts (**1a**)–(**1c**) (Figs. 2(D)–2(F)) was the ion at *m/z* 294 formed following the loss of ammonia from $[M + H]^+$ (Figs. 2(J)–2(L)). As shown in Fig. 6, such a loss of ammonia was also observed under electrospray ionization (ESI) tandem mass spectrometry conditions. It also appeared that the *m/z* 174 ion (also described below under ESI conditions), which has only been detected to a minor extent in *N*,*N*-dialkylated tryptamines,[32] showed a comparatively higher abundance that differed between isomers (**2a**)–(**2c**) under CI conditions. Another ion of interest was at *m/z* 162 (Fig. 2 and Supporting Information), which may be characteristic for *N*-mono-substituted tryptamines when using GC/CI-IT-MS, i.e. also absent in *N*,*N*-dialkyltryptamines.[31,32]

Table 2. Product ions obtained from *N*-(methoxybenzyl)-substituted 5-methoxytryptamines (**2a**)–(**2c**). Suggested elemental formulae of proposed product ions (Fig. 5(B)) that could be detected by QTOF-MS/MS analysis with acceptable mass accuracy

Compound	Measured *m/z* value	Suggested formula	Calculated *m/z* value	Δm [ppm]
2a 5MT-NB2OMe	311.17548 (PM)	$C_{19}H_{23}N_2O_2^+$	311.17540	−0.24
	294.14793	$C_{19}H_{20}NO_2^+$	294.14886	+3.16
	262.11985	$C_{18}H_{16}NO^+$	261.11536	+10.69
	203.11684	$C_{12}H_{15}N_2O^+$	203.11789	+5.19
	186.09101	$C_{12}H_{12}NO^+$	186.09134	+1.79
	174.09042 (BP)	$C_{11}H_{12}NO^+$	174.09134	+5.32
	162.09067	$C_{10}H_{12}NO^+$	162.09134	+4.16
	121.06468	$C_8H_9O^+$	121.06479	+0.93
	103.05381	$C_8H_7^+$	103.05423	+4.08
	91.05407	$C_7H_7^+$	91.05423	+1.74
	65.03869	$C_5H_5^+$	65.03858	−1.77
2b 5MT-NB3OMe	311.17650 (PM)	$C_{19}H_{23}N_2O_2^+$	311.17540	−3.53
	294.14838	$C_{19}H_{20}NO_2^+$	294.14886	+1.62
	174.09190 (BP)	$C_{11}H_{12}NO^+$	174.09134	−3.23
	162.09136	$C_{10}H_{12}NO^+$;	162.09134	−1.8
	150.09104	$C_9H_{12}NO^+$	150.09134	+2.02
	131.07282	$C_9H_9N^{\bullet+}$	131.0735	−5.19
	121.06507 (BP)	$C_8H_9O^+$	121.06479	−2.32
	91.05434	$C_7H_7^+$	91.05423	−1.26
	65.03869	$C_5H_5^+$	65.03858	−1.77
	77.03878	$C_6H_5^+$	77.03858	−2.68
2c 5MT-NB4OMe	311.17632 (PM)	$C_{19}H_{23}N_2O_2^+$	311.17540	−2.95
	294.15033	$C_{19}H_{20}NO_2^+$;	294.14886	−5.03
	174.09207	$C_{11}H_{12}NO^+$	174.09134	−4.22
	160.07655	$C_{10}H_{10}NO^+$	160.07569	−5.40
	147.08066	$C_{10}H_{11}O^+$	147.08044	−1.50
	121.06565 (BP)	$C_8H_9O^+$	121.06479	−7.15
	106.04105	$C_7H_6O^{\bullet+}$	106.04186	−7.64
	91.05458	$C_7H_7^+$	91.05423	−3.92
	77.03932	$C_6H_5^+$	77.03858	−9.78

PM: $[M + H]^+$ ion; BP: base peak.

Electrospray ionization mass spectrometry

All *N*-benzyl-substituted phenethylamines (**1a**)–(**1f**) and 5-methoxytryptamines (**2a**)–(**2f**) and (**3a**)–(**3f**) were characterized by electrospray ionization (ESI) high mass accuracy quadrupole time-of-flight tandem mass spectrometry (QTOF-MS/MS) and ESI triple quadrupole (QqQ) tandem mass spectrometry. The mass spectral data for the *N*-(methoxybenzyl) isomers (**1a**)–(**1c**) are presented in Fig. 4. A comparison between the QTOF-MS/MS (Figs. 4(A)–4(C)) and QqQ-MS/MS (Figs. 4(D)–4(F)) data showed an essentially similar trend with regard to the extent of product ion formation. The most extensive dissociation of the $[M + H]^+$ ion to yield the largest number of product ions was observed in the spectra of 25-NB3OMe (**1b**), whereas 25I-NB4OMe (**1c**) appeared to give the lowest level of product ion diversity. Figs. 4(D)–4(F) also show a summary of the ion ratios determined under QqQ-MS/MS conditions, which displayed the extent of differential dissociation. All three isomers gave the expected *m/z* 121 ion as a base peak. A selection of proposed structural representations of product ions that might have formed during MS/MS analysis is shown in Fig. 5(A), adapted in part from previously published work on related substances.[28–30] Figure 5(A) also lists the calculated exact masses, and Table 1 lists the masses and suggested elemental formulae that could be detected by QTOF-MS/MS analysis with acceptable mass accuracy. In some cases, however, the product ion counts observed under QTOF-MS/MS conditions did not yield sufficient signal intensity to confirm the suggested elemental composition with acceptable accuracy although some product ions were more abundant when employing QqQ-MS/MS detection.

The ESI-MS/MS data for the *N*-(methoxybenzyl)-tryptamines (**2a**)–(**2c**) can be seen in Fig. 6 and the mass spectral information of the remaining twelve substances are available as Supporting Information. As observed with the phenethylamine counterparts, the relative abundance values observed with QTOF-MS/MS and QqQ-MS/MS showed marked differences, which translated into distinct ion ratios (e.g. Figs. 6(D)–6(F)). Interestingly, the *m/z* 121 product ion formed the base peak in two rather than all three isomers, with 5MT-NB3OMe (**2b**) producing a base peak at *m/z* 174. Visual inspection of the QTOF and QqQ spectra indicated comparable relative abundance values for some of the ion ratios encountered. For example, the distinct ratios observed for the *m/z* 121 and 174 species (e.g. Figs. 6(D)–6(F)) might serve as a helpful feature when dealing with these isomers in the absence of reference material. A summary of suggested product ions, in part adapted from previously published work on *N*,*N*-dialkylated tryptamines,[31] is shown in Fig. 5(B) and Table 2.

The UHPLC/QTOF-MS method did not result in sufficient separation of the *N*-(methoxybenzyl) isomers (**1a**)–(**1c**) and (**2a**)–(**2c**), and this led to an implementation of an HPLC/DAD method that has been successfully applied previously as a screening method in forensic case-work.[19,20,33,34] Figure 7 shows the retention times and overlaid DAD spectra following separation by HPLC. Both 25I-NB2OMe (**1a**) and 5MT-NB2OMe (**2a**) were separated from the remaining two NB3OMe and NB4OMe isomers. Although the NB3OMe and NB4OMe isomers (**1b**)/(**1c**) and (**2b**)/(**2c**) could not be separated, it was found that they could

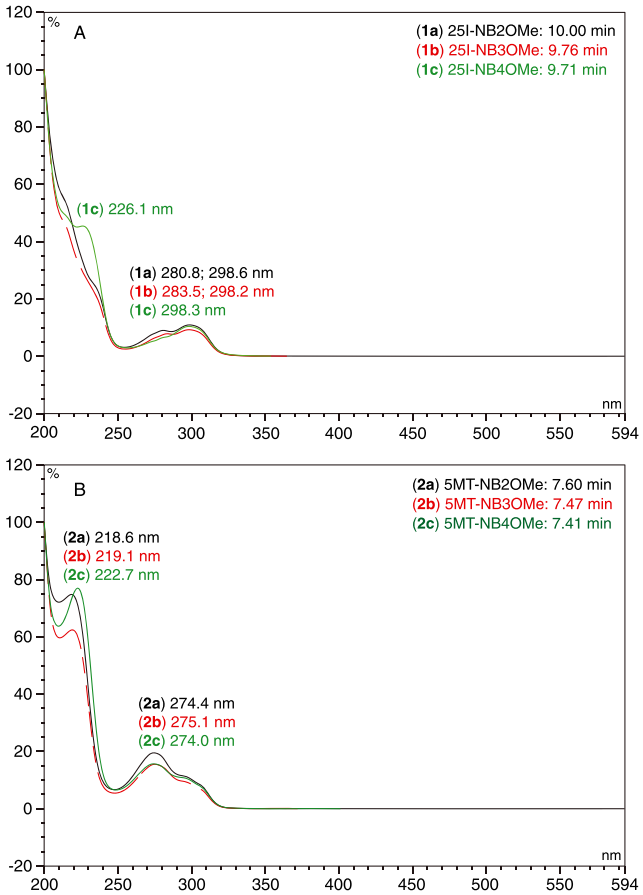


Figure 7. HPLC retention times and full scan UV spectra (DAD) for the *N*-(methoxybenzyl)-substituted isomers: (A) (**1a**)–(**1c**) and (B) (**2a**)–(**2c**). Differentiation was possible by the combination of UV full scan and retention time data.

be distinguished by their full scan UV spectra (Fig. 7(A)). The *N*-(methoxybenzyl)phenethylamines (**1a**)–(**1c**) showed a slight shift of increasing wavelength for the secondary UV maximum. The difference was especially pronounced in the case of the two co-eluting isomers (**1b**) and (**1c**) where the latter displayed a distinct shoulder at 226.1 nm that was absent in the *ortho*- and *meta*-NBOMe isomers ((**1a**) and (**1b**)). Whilst some of the spectral differences were seemingly small, experiences in casework has previously shown how valuable such differences can be when dealing with isomers and other closely related compounds.[19,20] Similarly, although the 5MT-based *N*-(methoxybenzyl) isomer (**2a**) could be separated from the *meta*- and *para*-substituted isomers (**2b**) and (**2c**), inspection of their UV full scan spectra confirmed the presence of distinctive differences to facilitate their differentiation (Fig. 7(B)). By contrast, all three 5MT-based *N*-(bromobenzyl) isomers (**2d**)–(**2f**) were fully separated under identical conditions (Supporting Information).

CONCLUSIONS

As is the case with most new psychoactive substances, the appearance of potent 5-HT_2 receptor agonists on the 'research chemicals' market highlighted the need to provide analytical

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data. The present study involved a comprehensive analytical characterization of six *N*-benzylphenethylamines and twelve *N*-benzyl-5-methoxytryptamines and the majority of these substances have not yet been described in the analytical literature. Differentiation between positional isomers may be obtained by mass spectral investigations to some extent but the implementation of chromatographic techniques with additional methods such as diode-array detection was found to offer additional value. The collection of spectral data provided in the present study was aimed to support those researchers in the field who have to deal with exposure to these particular substances, such as clinicians, forensic providers and law enforcement.

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