Drug Testing and Analysis

Preparation and characterization of the 'research chemical' diphenidine, its pyrrolidine analogue, and their 2,2-diphenylethyl isomers

Journal:	*Drug Testing and Analysis*
Manuscript ID:	DTA-14-0117.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	n/a
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Keywords:	research chemicals, psychoactive, Internet, diarylethylamines, isomers, NMDA receptor, PCP, arylcyclohexylamines

Abstract:	Substances with the diphenylethylamine nucleus represent a recent addition to the product catalog of dissociative agents sold as research chemicals on the Internet. Diphenidine, i.e. 1-(1,2-diphenylethyl)piperidine (1,2-DEP), is such an example but detailed analytical data are less abundant. The present study describes the synthesis of diphenidine and its most obvious isomer, 1-(2,2-diphenylethyl)piperidine (2,2-DEP), in order to assess the ability to differentiate between them. Preparation and characterization were also extended to the two corresponding pyrrolidine analogues 1-(1,2-diphenylethyl)- and 1-(2,2-diphenylethyl)pyrrolidine, respectively. Analytical characterizations included high-resolution electrospray mass spectrometry (HR-ESI-MS), liquid chromatography ESI-MS/MS, gas chromatography ion trap electron and chemical ionization MS,

	nuclear magnetic resonance spectroscopy (NMR) and infrared spectroscopy. Differentiation between the two isomeric pairs was possible under GC-(EI/ CI)-MS conditions and included the formation of distinct iminium ions, such as m/z 174 for 1,2-DEP and m/z 98 for 2,2-DEP, respectively. The pyrrolidine counterparts demonstrated similar phenomena including the expected mass difference of 14 Da due to the lack of one methylene unit in the ring. Two samples obtained from an Internet vendor provided confirmation that diphenidine was present in both samples, concurring with the product label. Finally, it was confirmed that diphenidine (30 μM) reduced NMDA-mediated field excitatory postsynaptic potentials (NMDA-fEPSPs) to a similar extent to that of ketamine (30 μM) when using rat hippocampal slices. The appearance of 1,2-diphenylethylamines appears to reflect the exploration of alternatives to arylcyclohexylamine-type substances, such as methoxetamine, PCP and PCPy-based analogues that also show N-methyl-D-aspartate (NMDA) receptor activity as demonstrated here for diphenidine.

Preparation and characterization of the 'research chemical' diphenidine, its pyrrolidine analogue, and their 2,2-diphenylethyl isomers

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Running title: Characterization of diphenylethylamines

Abstract

Substances with the diphenylethylamine nucleus represent a recent addition to the product catalog of dissociative agents sold as research chemicals on the Internet. Diphenidine, i.e. 1-(1,2-diphenylethyl)piperidine (1,2-DEP), is such an example but detailed analytical data are less abundant. The present study describes the synthesis of diphenidine and its most obvious isomer, 1-(2,2-diphenylethyl)piperidine (2,2-DEP), in

order to assess the ability to differentiate between them. Preparation and characterization were also extended to the two corresponding pyrrolidine analogues 1-(1,2-diphenylethyl)- and 1-(2,2-diphenylethyl)pyrrolidine, respectively. Analytical characterizations included high-resolution electrospray mass spectrometry (HR-ESI-MS), liquid chromatography ESI-MS/MS, gas chromatography ion trap electron and chemical ionization MS, nuclear magnetic resonance spectroscopy (NMR) and infrared spectroscopy. Differentiation between the two isomeric pairs was possible under GC-(EI/CI)-MS conditions and included the formation of distinct iminium ions, such as *m/z* 174 for 1,2-DEP and *m/z* 98 for 2,2-DEP, respectively. The pyrrolidine counterparts demonstrated similar phenomena including the expected mass difference of 14 Da due to the lack of one methylene unit in the ring. Two samples obtained from an Internet vendor provided confirmation that diphenidine was present in both samples, concurring with the product label. Finally, it was confirmed that diphenidine (30 µM) reduced NMDA-mediated field excitatory postsynaptic potentials (NMDA-fEPSPs) to a similar extent to that of ketamine (30 µM) when using rat hippocampal slices. The appearance of 1,2-diphenylethylamines appears to reflect the exploration of alternatives to arylcyclohexylamine-type substances, such as methoxetamine, PCP and PCPy-based analogues that also show *N*-methyl-D-aspartate (NMDA) receptor activity as demonstrated here for diphenidine.

Keywords: diphenidine; research chemicals; psychoactive; Internet; diarylethylamines; isomers; NMDA receptor; PCP; arylcyclohexylamines

Introduction

The history of medicinal research and recreational use of so-called dissociative agents is closely linked with the two prototypical substances phencyclidine (PCP) and ketamine (Figure 1). A central pharmacological feature associated with dissociative agents includes the ability to act as a non-competitive antagonist at the *N*-methyl-D-aspartate (NMDA) receptor[1] which also provides access to important leads for drug discovery.[2,3] From the perspective of recreational explorations, and what was traditionally thought of as clandestine approaches to the preparation of psychoactive material, a close structural relationship with known arylcyclohexylamine-type substances, such as PCP, formed a key basis.[4-6] While it appeared that PCP-type substances did not attract much attention for decades, renewed interest was sparked since the mid-2000s within the recreational substance community, which resulted in a re-emergence of a number of PCP-based substances predominantly encountered as research chemicals available from online vendors.[7]

Methoxetamine (2-(ethylamino)-2-(3-methoxyphenyl)cyclohexan-1-one, MXE, Figure 1), which may be considered as an invention conceived and developed from within the recreational drug community, began to appear more widely in 2010 and attracted some

form of popularity amongst users who showed interest in ketamine and arylcyclohexylamine-type substances.[7] In the UK, methoxetamine and a range of associated substances based on the 1-phenylcyclohexanamine and 2-amino-2-phenylcyclohexanone template became subject to legislative control in early 2013 using a set of generic definitions, thus, impacting on commercial availability of these substances.[8]

A feature increasingly observed with a range of research chemicals and so-called new psychoactive substances is the expansion from the structurally-based search to the exploration of novel structures with equivalent pharmacological targets as banned substances.[9] This approach, which commonly includes an evaluation of medicinal chemistry and patent literature, has lead to the discovery of structurally diverse templates that may be explored for manufacturing and sale on the Internet.

A more recent example of this approach may be found in the 1,2-diarylethylamine class which gives rise to a range of substances with diverse properties and may include examples such as MT-45 (analgesic activity in mice and also sold as a research chemical)[10,11] and AZD6765 (potential antidepressant properties in humans)[12] (Figure 1). A relatively new addition to the product catalog of online retailers is diphenidine (**1**) (1-(1,2-diphenylethyl)piperidine, 1,2-DEP) (Figure 1). Racemic (**1**), but also (*S*)- and (*R*)-diphenidine, have been shown to bind to NMDA receptors[13,14] and it has also been evaluated for its ability to protect hippocampal neurons from hypoxia-induced cell death,[15] although its effect on NMDA receptor-mediated synaptic transmission has not been demonstrated. The appearance of diphenidine as a psychoactive research chemical was first noticed in 2013.[7] Since analytical details appear to be currently absent in the scientific literature, it was decided to synthesize this substance for analytical characterization and to confirm its action as an NMDA antagonist at a brain synapse. An obvious isomer of diphenidine is the 2,2-diphenylethylamine counterpart 1-(2,2-diphenylethyl)piperidine (**2**) (2,2-DEP, Figure 1) and it was also aimed to prepare and characterize this candidate in order to assess the ability to differentiate between the two substances. In recent years, the unprecedented growth of newly emerging psychoactive substances has placed increasing demands on the capability to identify and differentiate between closely related analytes (e.g. isomers), thus eliminating the possibility of misinterpretation when encountering these substances. Finally, two diphenidine samples were also obtained from an Internet vendor to investigate consistency with the product label.

A range of arylcylohexylamines has appeared in recent years on the market and while some have been based on the phencyclidine (PCP) template, seen for example in 3-MeO- or 4-MeO-PCP[16-18], others have included the exploration of pyrrolidine versions, such as 3-MeO-PCPy (Figure 1). Although these analogues have been introduced to the market, the availability of analytical data has been less abundant.[7,19] For this reason, it was decided to prepare the two corresponding pyrrolidine derivatives of these diphenylethylamines, i.e. 1-(1,2-diphenylethyl)pyrrolidine (1,2-DEPy) (**3**) and 1-(2,2-

diphenylethyl)pyrrolidine (2,2-DEPy) (**4**), respectively. While detailed investigations about the psychoactive properties of all these four substances are currently absent, it has been shown that both 1,2-DEP (**1**) and 1,2-DEPy (**3**) have been explored for their antitussive properties in dogs, which might serve as a typical example for the origin of research chemicals within the medicinal chemistry context.[20]

((Please insert Figure 1 about here))

Experimental

Materials

All starting materials, reagents and solvents used for synthesis (≥ 96%) were obtained from Sigma-Aldrich (St. Louis, MO, USA) Column chromatography was conducted using Merck silica gel, grade 9385, 230-400 mesh, 60 Å. Melting point ranges were obtained using a DigiMelt A160 SRS melting point apparatus (Stanford Research Systems, USA) at a ramp rate of 2 °C/min and are uncorrected. Two samples representing two distinct batches of diphenidine (**1**) were obtained from an online vendor on two different occasions. A representative photograph of the purchased product is shown in the supplemental information.

Synthesis procedures

*1-(1,2-Diphenylethyl)piperidine (1,2-DEP, diphenidine) (**1**)*

A suspension of 1,2- diphenylethanamine (1.2 g, 6.08 mmol), K_2CO_3 (18.2 mmol) and 1 mL triethylamine (TEA) in 30 mL dry acetonitrile (4Å molecular sieves) was stirred under nitrogen. 1,5-Dibromopentane (1.05 mL, 7.9 mmol) was added and the solution was stirred at room temperature under nitrogen over several days. Following completion (GC), the reaction was quenched by the addition distilled H_2O (dH2O) (300 mL). A few mL 2N KOH were added to ensure a basic solution and the solution was extracted with ethyl acetate (EtOAc) (3 x 100 mL). The organic phases were pooled and extracted with 3 x 200 mL 2N aqueous HCl. Aqueous phases were pooled and made basic with KOH pellets (pH 12) and extracted with EtOAc (3 × 70 mL). The pooled organic phases were dried with anhydrous sodium sulfate and concentrated under vacuum to give amber oil. The crude base was purified by flash column chromatography on silica gel with hexanes/ethyl acetate (3:1) as mobile phase. The desired fractions were pooled and evaporated to give (**1**) as a colorless oil (1.4 g, 5.28 mmol) in 86.8% yield. This oil spontaneously crystallized to a white solid during storage at 0 °C.

The freebase was dissolved in acetone and titrated to pH 2 with concentrated HCl. Solvent was evaporated under warm air to give an oily residue. Additional acetone was added and evaporated until all residual HCl was driven off to give a white waxy solid. As the salt was semi-soluble in acetone, the crystals were washed three times with a 50:50 mixture of EtOAc and diethyl ether (Et_2O). The crystals were dried and crystallized at

0 °C from methanol and Et_2O. Melting point: 209–210.5 °C with some sublimation (lit.: 120 °C,[21] 158 °C,[22] 207–208.5 °C,[22] 207–209 °C,[22] 207–209 °C (HCl × H_2O,[14] 207 °C[20]). ^{1}H NMR (400 MHz, $CDCl_3$, free base) δ 7.37–7.03 (8H, m, Ar-H), 7.04–6.95 (2H, m, Ar-H), 3.58 (1H, dd, *J* = 9.4, 5.2 Hz, C_1H), 3.30 (1H, dd, *J* = 13.3, 5.2 Hz, C_2H), 2.99 (1H, dd, *J* = 13.4, 9.4 Hz, C_2H), 2.55–2.29 (4H, m, 2 x $C_\alpha H_2$), 1.64□1.45 (4H, m, 2 x $C_\beta H_2$), 1.36 (2H, quintet, *J* = 5.9 Hz, $C_\gamma H_2$). ^{13}C NMR (100 MHz, $CDCl_3$, free base) δ 139.98 (quat. Ar-C), 139.44 (quat. Ar-C), 129.35 (2 × Ar-CH), 128.89 (2 × Ar-CH), 127.81 (2 × Ar-CH), 127.62 (2 × Ar-CH), 126.77 (Ar-CH), 125.59 (Ar-CH), 72.32 (CH, C_1), 51.40 (2 × CH_2, C_α), 39.18 (CH_2, C_2), 26.37 (2 × CH_2, C_β), 24.66 (CH_2, C_γ). HR-ESI-MS: 266.1896 (theory [M+H$^+$], $C_{19}H_{24}N^+$, 266.1903).

^{1}H NMR (400 MHz, $CDCl_3$, hydrochloride) δ 12.37 (1H, s, NHCl), 7.52□7.29 (5H, m, Ar-H), 7.18□6.97 (5H, m, Ar-H), 4.23 (1H, d, *J* = 11.6 Hz, C_1H), 4.04 (1H, dd, *J* = 12.8, 3.1 Hz, C_2H), 3.64 (1H, d, *J* = 10.3 Hz, $C_\alpha H$), 3.54 (1H, d, *J* = 11.6 Hz, $C_\alpha H$), 3.46 (1H, t, *J* = 12.2 Hz, C_2H), 2.68□2.39 (3H, m, $C_\alpha H_2$, $C_\beta H$), 2.31 (1H, q, *J* = 13.2, 12.2 Hz, $C_\gamma H$) 1.93-1.76 (3H, m, $C_\beta H$), 1.27 (1H, q, *J* = 13.4, 12.4 Hz, $C_\gamma H$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.77 (quat. Ar-C), 131.01 (quat. Ar-C), 130.23 (2 x Ar-CH), 129.94 (Ar-CH), 129.30 (2 x Ar-CH), 129.11 (2 x Ar-CH), 128.35 (2 x Ar-CH), 126.75 (Ar-CH), 72.90 (CH, C_1), 53.39 (CH_2, C_α), 48.81 (CH_2, C_α), 36.75 (CH_2, C_2), 22.71 (CH_2, C_β), 22.65 (CH_2, C_β), 22.24 (CH_2, C_γ).

*1-(2,2-Diphenylethyl)piperidine (2,2-DEP, diphenidine isomer) (**2**)*

This diphenidine isomer was prepared following the same procedure using 2,2-diphenylethanamine (1.2 g, 6.08 mmol). The free base product (80.7% yield) was converted into the hydrochloride salt as described above. The crystals were recrystallized twice from methanol and Et_2O at 0 °C to give a hygroscopic white solid (1.3 g, 4.31 mmol). Melting point: 144.5–145.5 °C (lit.: 140 °C,[23] 145–147 °C,[24] 130–132 °C[25]). ^{1}H NMR (400 MHz, $CDCl_3$, free base) δ 7.30–7.21 (8H, m, Ar-H), 7.19–7.13 (2H, m, Ar-H), 4.21 (1H, t, *J* = 7.3 Hz, C_2H), 2.93 (2H, d, *J* = 7.3 Hz, C_1H_2), 2.39 (4H, t, *J* = 5.4 Hz, 2 × $C_\alpha H_2$), 1.46 (4H, quintet, *J* = 5.5 Hz, 2 × $C_\beta H_2$), 1.37 (2H, quintet, *J* = 5.4 Hz, $C_\gamma H_2$). ^{13}C NMR (100 MHz, $CDCl_3$, free base) δ 144.40 (2 × quat. Ar-C), 128.22 (6 × Ar-CH), 126.01 (2 × Ar-CH), 64.53 (CH_2, C_1), 54.85 (2 × CH_2, C_α), 48.89 (CH, C_2), 25.99 (2 × CH_2, C_β), 24.43 (CH_2, C_γ) HR-ESI-MS: 266.1895 (theory [M+H$^+$]: $C_{19}H_{24}N^+$, 266.1903).

*1-(1,2-Diphenylethyl)pyrrolidine (1,2-DEPy) (**3**)*

This diphenidine isomer was prepared following the same procedure using 1,2-diphenylethanamine (1.2 g, 6.08 mmol) and 1,5-dibrombutane (1 mL, 7.9 mmol) in 58.9% yield. The freebase product was converted into the hydrochloride salt as described above to give a white solid. The hydrochloride salt gave a melting point of 205.5–207.8 °C (lit.: 212 °C[20,26]). ^{1}H NMR (400 MHz, $CDCl_3$, free base) δ 7.24–7.08 (8H,

m, Ar-H), 6.91–6.83 (2H, m, Ar-H), 3.36 (1H, dd, *J* = 13.3, 4.3 Hz, C_2H), 3.30 (1H, dd, *J* = 9.9, 4.3 Hz, C_1H), 2.96 (1H, dd, *J* = 13.3, 9.9 Hz, C_2H), 2.64 (2H, m, $C_\alpha H_2$), 2.45 (2H, m, $C_\alpha H_2$), 1.77 (4H, quintet, *J* = 3.3 Hz, 2 × $C_\beta H_2$). ^{13}C NMR (100 MHz, $CDCl_3$, free base) δ 142.34 (quat. Ar-C), 139.13 (quat. Ar-C), 129.48 (2 × Ar-CH), 128.29 (2 × Ar-CH), 127.80 (2 × Ar-CH), 127.75 (2 × Ar-CH), 126.80 (Ar-CH), 125.66 (Ar-CH), 73.31 (CH, C_1), 53.0 (2 × CH_2, C_α), 42.96 (CH_2, C_2), 23.35 (2 × CH_2, C_β). HR-ESI-MS: 252.1740 (theory [M+H$^+$]: $C_{18}H_{22}N^+$, 252.1747).

*1-(2,2-Diphenylethyl)pyrrolidine (2,2-DEPy (**4**)*

This isomer was prepared as described above using 2,2-diphenylethanamine (1.2 g, 6.08 mmol), which gave a yellow oil (85.0% yield). The hydrochloride salt was recrystallized three times at 0 °C from methanol and Et_2O which provided a melting point of 174.3–176 °C (lit.: 174–175 °C[27]). ^{1}H NMR (400 MHz, $CDCl_3$, free base) δ 7.33–7.25 (8H, m, Ar-H), 7.24–7.16 (2H, m, Ar-H), 4.22 (1H, t, *J* = 7.1 Hz, C_2H), 3.14 (2H, d, *J* = 7.4 Hz, C_1H_2), 2.51 (4H, t, *J* = 6.1 Hz, 2 × $C_\alpha H_2$), 1.72 (2H, quintet, *J* = 3.1 Hz, $C_\beta H_2$). ^{13}C NMR (100 MHz, $CDCl_3$, free base) δ 144.04 (2 × quat. Ar-C), 128.36 (4 × Ar-CH), 128.03 (4 × Ar-CH), 126.20 (2 × Ar-CH), 61.74 (CH_2, C_1), 54.55 (2 × CH_2, C_α), 50.87 (CH, C_2), 23.50 (2 × CH_2, C_β). HR-ESI-MS: 252.1740 (theory [M+H$^+$]: $C_{18}H_{22}N^+$, 252.1747).

Instrumentation

Gas chromatography ion trap mass spectrometry

GC ion trap MS data were obtained in electron (EI) and chemical ionization (CI) mode (scan range *m/z* 41–*m/z* 500) using a Varian 450-GC gas chromatograph coupled to a Varian 220-MS ion trap mass spectrometer. A Varian 8400 autosampler was employed with a CP-1177 injector (275 ºC) in split mode (1:50). Data acquisition was performed with the 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 ºC, respectively. The liquid CI reagent was HPLC grade methanol. CI ionization parameters (0.4 s/scan): CI storage level 19.0 *m/z*; ejection amplitude 15.0 *m/z*; background mass 55 *m/z*; maximum ionization time 2000 µs; maximum reaction time 40 ms; target TIC 5000 counts. A 30 m × 0.25 mm (0.25 µm film thickness) Agilent J&W VF-5ms GC column (Stockport, UK) was employed for separation. The starting temperature was set at 50 ºC and held for 1 min. The temperature then increased at 20 ºC/min to 300 ºC and held constant for 5.00 minutes to give a total run time of 18.50 min. Samples were prepared by dissolving the HCl salt in methanol at a concentration of 0.5 mg/mL.

Liquid chromatography electrospray triple quadrupole mass spectrometry

LC-MS/MS experiments were performed on a Waters (Micromass) Quattro Premier (Waters Ltd., Manchester, UK) with an ESI source and interfaced to a Waters Alliance 2695 HPLC system operated under Masslynx v 4.1 software. Separation was obtained using a Waters Sunfire™ C8 column (3.5 µm; 4.6 mm × 50 mm) held at 30 °C and water: methanol gradient elution with a flow rate of 0.8 mL/min. Solvent A consisted of 10 mM aqueous ammonium formate, 0.1% formic acid and Solvent B consisted of methanol with 10 mM ammonium formate, 0.1% formic acid. The gradient elution method was: 0–2 min: 98% A and 2% B; 2–18 min: gradient to 32% A and 68% B; 18-19 min: returned to 98% A and 2% B then equilibrated for 11 min. Samples were dissolved in mobile phase (50% A: 50% B) at a concentration of 10 µg/mL and the injection volume was 5 µL.

Tandem mass spectra (MS/MS) were collected in positive ion mode by multiple reaction monitoring (MRM). The optimized source conditions were as follows: capillary 3.12 kV, cone 28 V, rf lens 0.1 V, source temperature 100 °C, desolvation temperature 400 °C, cone gas flow 50 L/hr, desolvation gas flow 500 L/hr. The collision gas was argon (0.3 mL/min flow). The protonated precursor ion for piperidines (**1**) and (**2**) was set at *m/z* 266 and four product ions were collected, i.e. at *m/z* 181, *m/z* 166, *m/z* 103 and *m/z* 86 using collision energies of 20 eV, 28 eV, 35 eV and 48 eV respectively. The protonated precursor ion for pyrrolidines (**3**) and (**4**) was at *m/z* 252 and four product ions were collected, i.e. at *m/z* 181, *m/z* 166, *m/z* 103 and *m/z* 72 using collision energy values of 20 eV, 28 eV, 35 eV and 48 eV respectively. Dwell time for each channel was 0.05 s. Interchannel delay was 0.02 s. Product ion spectra were obtained from direct infusion (10 µL/mL; concentration 1 µg/mL). The standard MS/MS settings were applied, however, masses were collected between *m/z* 45 and *m/z* 200; collision voltage 28 eV; desolvation temperature 200°C; desolvation gas flow 200 L/hr.

High-resolution electrospray mass spectrometry

HR-ESI mass spectra for the substances obtained from synthesis and test purchases were recorded by direct injection into a LTQ Orbitrap Discovery (Thermo Fisher, Bremen, Germany). Samples were dissolved in acetonitrile/water (1:1, containing 0.1% formic acid) and infused at a rate of 5 µL/min. Full accurate high-resolution (30000) mass scans were performed in positive electrospray mode. Measured accurate masses were within ± 5 ppm of the theoretical masses. The following conditions were used: drying gas (N_2) 10 L/min, capillary temperature 310 °C, spray voltage 4 V, capillary voltage 22 V and tube lens 77 V.

Nuclear magnetic resonance spectroscopy

^{1}H (400 MHz) and ^{13}C NMR spectra (100 MHz) were obtained in $CDCl_3$ solutions (100% and 99.96% D, 0.03% (v/v) TMS) on a Bruker Ultrashield 400 plus spectrometer with a 5 mm BBO S1 (Z gradient plus) probe at 24 °C. Internal chemical shift references were TMS (δ = 0.00 ppm) and solvent (δ = 77.0 ppm). Samples were run as the free bases at

a concentration of approximately 20 mg/mL. Aliphatic chemical shifts were assigned using 1-D and 2-D heteronuclear experiments.

Electrophysiology

Four to six week old male and female Wistar rats (Charles River, UK) were euthanized by cervical dislocation, in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986. The brains were rapidly removed and placed into artificial cerebrospinal fluid (aCSF: 124 mM NaCl, 3 mM KCl, 26 mM $NaHCO_3$, 1.4 mM NaH_2PO_4, 1 mM $MgSO_4$, 10 mM D-glucose, 2 mM $CaCl_2$), continuously oxygenated with 95% O_2 and 5% CO_2. Parasagittal hippocampal slices (400 µM) were prepared, and experiments carried out in submerged conditions and at a temperature of 28–30°C, as described previously.[28] Briefly, field excitatory postsynaptic potentials (fEPSPs) were evoked at 0.03 Hz using a bipolar stimulating electrode, and recorded using glass micropipettes pulled to a resistance of 3-6 MΩ and filled with 3 M NaCl. Both electrodes were positioned in stratum radiatum of area CA1, and NMDA-fEPSPs were isolated by addition of picrotoxin (50 µM), CGP 55845 hydrochloride (1 µM) and NBQX (10 µM) to the perfusate, in order to block GABA-A, GABA-B and AMPA/kainate receptors, respectively. Responses were measured and recorded online using WinLTP.[29]

Results and discussion

The preparation of diphenidine (**1**), its 2,2-DEP isomer (**2**) and their pyrrolidine counterparts (**3**) and (**4**) was based on the use of the commercially available primary amines 1,2- and 2,2-diphenylethanamine, respectively, followed by reaction with the corresponding 1,5-dibromoalkane (Figure 2). This procedure was also employed by Berger et al. for the preparation of diphenidine and its single enantiomers [14] and it previously provided convenient access to the preparation of PCP and PCPy analogues.[19] The synthesis of diphenidine was reported first in 1924 and was based on a modified Bruylanys reaction between benzylmagnesium bromide and the corresponding α-arylamino nitrile.[21] A number of additional synthetic routes to diphenidine have been described and include modified Mannich-type reactions employing benzyl bromide, piperidine and benzaldehyde[30] and gold-catalyzed hydroamination of diphenylacetylene with piperidine.[31]

((Please insert Figure 2 about here))

Mass spectrometry and chromatography

The electron- and chemical ionization (EI/CI) ion trap (IT) mass spectra and their gas chromatography (GC) retention times for all four diphenylethylamines (**1**) – (**4**) are summarized in Figure 3A–□H. Representative GC traces are shown as supplemental data to indicate separation of isomers. EI-induced fragmentation of all four substances gave rise to a base peak, which was consistent with an iminium species following α-

cleavage (Figure 4). In case of diphenidine (**1**), for example, base peak formation was observed at *m/z* 174 (Figures 3A and 4A) while the pyrrolidine counterpart fragmented to the equivalent ion at *m/z* 160 due to the mass difference of 14 Da (Figure 3C). The significance of this particular base peak was that it would allow for the ability to differentiate between some isomers in cases where one of the phenyl rings showed additional substituents. In other words, substituents present on the phenyl ring attached to the 1-position ethylamine chain would be expected to shift the *m/z* value to larger *m/z* values beyond *m/z* 174. Another main observation made with the 1-(1,2-diphenylethyl) substituted compounds (**1**) and (**3**) was the presence of the tropylium species at *m/z* 91 (Figures 3A and 3C) which, given their difference in structure, were not detected with their 1-(2,2-diphenylethyl) isomers (**2**) and (**4**) (Figures 3E and 3G). The significance of the tropylium ion would, however, become of additional interest if there was a need to verify the presence of a substituted phenyl ring located at the 2-position of the ethylamine chain, thus, giving a tropylium ion at higher *m/z* values.

While implementation of EI resulted in very low abundance of the molecular ions, a protonated molecule was conveniently detected when operating under GC-CI-IT-MS conditions, which aided confirmation of the corresponding molecular mass values (Figures 3B, 3D, 3F and 3H). Additional dissociation of the protonated molecule provided further information, which included the detection of an even-electron species associated with the primary amine, i.e. *m/z* 86 for diphenidine and *m/z* 72 for 1,2-DEPy, respectively (Figures 3B, 3D and 4B). This was consistent with previous CI-IT-MS studies of a range of PCP and PCPy analogues where the piperidine and pyrrolidine fragments have also been detected.[19]

A mass spectral comparison between the 1-(1,2-diphenylethyl) and 1-(2,2-diphenylethyl) compounds (**1**)/(**3**) and (**2**)/(**4**) highlighted that differentiation between isomers was possible under EI- and CI conditions. For example, the diphenidine isomer (**2**) gave an iminium base peak at *m/z* 98 instead of *m/z* 174 observed for diphenidine (Figures 3E and 4). A suggested fragmentation pathway for 2,2-DEP (**2**) is shown in Figures 4C–F and equivalent mechanisms may have been involved in the fragmentation behaviour of the pyrrolidine counterpart 2,2-DEPy (**4**) considering the associated mass difference of 14 Da. GC analysis also indicated that it was possible to distinguish between the two pairs of constitutional isomers.

((Please insert Figures 3 and 4 about here))

Mass spectra and ion ratios (five most abundant species) for compounds (**1**) – (**4**) obtained from liquid chromatography electrospray triple quadrupole mass spectrometry analysis (LC-ESI-MS/MS) are shown in Figure 5. Characteristic fragments observed with all four analytes included *m/z* 181, 179, 166, 165 and 103, respectively, and a suggested fragmentation pathway is shown in Figure 6. The implementation of ESI-MS/MS did not reveal differential fragmentations between isomers (**1**)/(**2**) and (**3**)/(**4**) as observed under EI and CI conditions (Figure 3). However, a difference in relative abundance observed

for the detection of protonated piperidine (*m/z* 86) and protonated pyrrolidine (*m/z* 72) was noted which might be useful when considering the use of distinct ion ratios (Figure 5). Further studies, including the implementation of different ESI-based instrumentation, might be indicated to assess robustness.

((Please insert Figures 5 and 6 about here))

Nuclear magnetic resonance spectroscopy

The C_1 proton ^1H chemical shifts of diphenidine (**1**) and its pyrrolidine analogue (**3**) appeared as a doublet of doublets split by the two non-equivalent C_2 vicinal protons. The C_2 protons are diastereotopic relative to the C_1 proton, leading to two separate doublets of doublets for the C_2 ^1H chemical shifts (Figure 7). This reflected both geminal ($^2J_{HH}$) coupling to each other and vicinal ($^3J_{HH}$) coupling with the C_1 proton. The C_2 ^1H chemical shifts were separated by ~0.2–0.3 ppm. The $^2J_{HH}$ coupling constants were equivalent (~13 Hz) between the upfield and downfield C_2 ^1H chemical shifts although the $^3J_{HH}$ coupling constants were distinct between the upfield and downfield C_2 chemical shifts ~9.5 vs. ~4.5 Hz, respectively. Presumably, this was due to the different dihedral angles between each of the C_2 protons and the C_1 proton about the C_1–C_2 bond.

The ^1H and ^{13}C spectra of (**1**), (*R*)-(**1**) and (*S*)-(**1**) free base and the hydrochloride salt have been previously published[14,31] and the results obtained in the present study were largely consistent. Two "additional" ^{13}C chemical shifts were reported with the hydrochloride salt.[14] When acquiring the NMR spectra for the HCl salt of diphenidine, a similar observation was made and 2-D HMQC analysis carried out here suggested that C_α and C_β carbons appeared as four ^{13}C chemical shifts as opposed to two. The free base, however, did not reveal distinct signals. Conformation alteration resulting from protonation and a resulting non-equivalence of both C_α and C_β carbons may have been involved in the observed effect. The ^1H and ^{13}C spectra of (**3**) free base were consistent with data published previously.[31] For convenience the ^1H and ^{13}C assignments for the assigned aliphatic shifts for compounds (**1**) – (**4**) and the HCl salt of (**1**) are presented in in the supplementary information.

((Please insert Figure 7 about here))

The C_1 ^1H chemical shifts appeared as a doublet due to $^3J_{HH}$ coupling to the single C_2 proton. The C_1 resonance of 1,2-diphenylethylamines (**1**) and (**3**) were observed upfield relative to the 2,2-diphenylethylamines counterparts (**2**) and (**4**). The C_2 ^1H chemical shifts gave a triplet (J ~7.3 Hz) due to $^3J_{HH}$ coupling to the two equivalent C_1 protons.

The C_1 ^{13}C chemical shifts for the 1,2-diphenylethylamines (**1**) and (**3**) were observed further downfield when compared to their 2,2-diphenylethylamine isomers (**2**) and (**4**) due to the electron-withdrawing effect of the phenyl substituent at C_1. This ΔC_1 was 5.6–11.6 ppm. Correspondingly, in the 2,2-diphenylethylamines, the C_2 ^{13}C chemical

shift was further downfield from their 1,2-diphenylethylamine counterparts due to the deshielding effect of the diphenyl rings (ΔC_2 = ~8−10 ppm). The ring-related methylene groups C_α, C_β and C_γ showed similar ^{13}C chemical shifts for all four compounds. In all cases, the C_α chemical shift was furthest downfield due to its proximity to the basic amine nitrogen, followed by the C_β chemical shift and C_γ which appeared to be most upfield in (**1**) and (**3**).

Confirmation of NMDA receptor antagonism

Hippocampal slices were pharmacologically treated to isolate NMDA receptor mediated events, confirmed by blocking with the selective NMDA antagonist, *d*-2-amino-5-phosphonopentanoate (D-AP5; Figure 8). On such slices, diphenidine (30 µM) reduced NMDA-fEPSPs to 32 ± 6% of baseline (Figure 8A). This was similar to the effect of ketamine (30 µM), which reduced NMDA-fEPSPs to 35 ± 2% of baseline (Figure 8B). The onset of antagonism by diphenidine was slower than that of ketamine and reminiscent of the slower kinetics of NMDA antagonism by MK-801 on brain slices.[32] Although beyond the scope of this study, more complex intracellular recordings with varying membrane potentials are required to calculate the on and off kinetics in order to give more accurate biophysical potencies. Further *in vivo* recordings would be needed to provide systemic potencies.

((Please insert Figure 8 about here))

The analytical characterization and synthesis of diphenidine (**1**) and its three analogues (**2**)–(**4**) confirmed that the two products obtained from an online retailer were consistent with diphenidine as indicated on the product label (see supplementary information for additional data). Differentiation between the two pairs of isomers was observed when comparing NMR, EI- and CI-MS spectra and chromatographic retention times. Diphenidine was introduced to the UK market in 2013 and it appears to be psychoactive in man,[7] most likely due to the antagonism of NMDA receptor-mediated synaptic events as demonstrated here. However the overall psychopharmacological and physiological activity of diphenidine in humans may involve additional molecular targets including non-NMDA receptors, transport proteins and enzymes. A shift to diarylethylamines might represent an example where the expected pharmacological rather than structural similarities may have impacted on its exploration as a research chemical. In the UK, recently introduced legislative measures[8] precludes the commercial availability of methoxetamine and related derivatives which might add to the attempt to explore potential alternatives for the research chemicals market that are not controlled. Indeed, 2-methoxyphenidine, i.e. 1-(1-(2-methoxyphenyl)-2-phenylethyl)piperidine, has also recently appeared as a commercially available research chemical and detailed investigations, including their differentiation from their corresponding isomers, are currently underway.

Conclusion

1-(1,2-Diphenylethyl)piperidine (diphenidine) and related substances have recently appeared as research chemicals. Detailed analytical data, which are relevant for forensic and clinical work, appear to be currently absent. The preparation and in-depth characterization of diphenidine, its pyrrolidine analogue, and their 2,2-diphenylethyl isomers were aimed to close this gap and it was also revealed that differentiation between isomers was feasible. Diphenidine reduced NMDA-mediated field excitatory postsynaptic potentials similar to ketamine when using rat hippocampal slices and further studies on the potential similarity to other dissociative agents are warranted.

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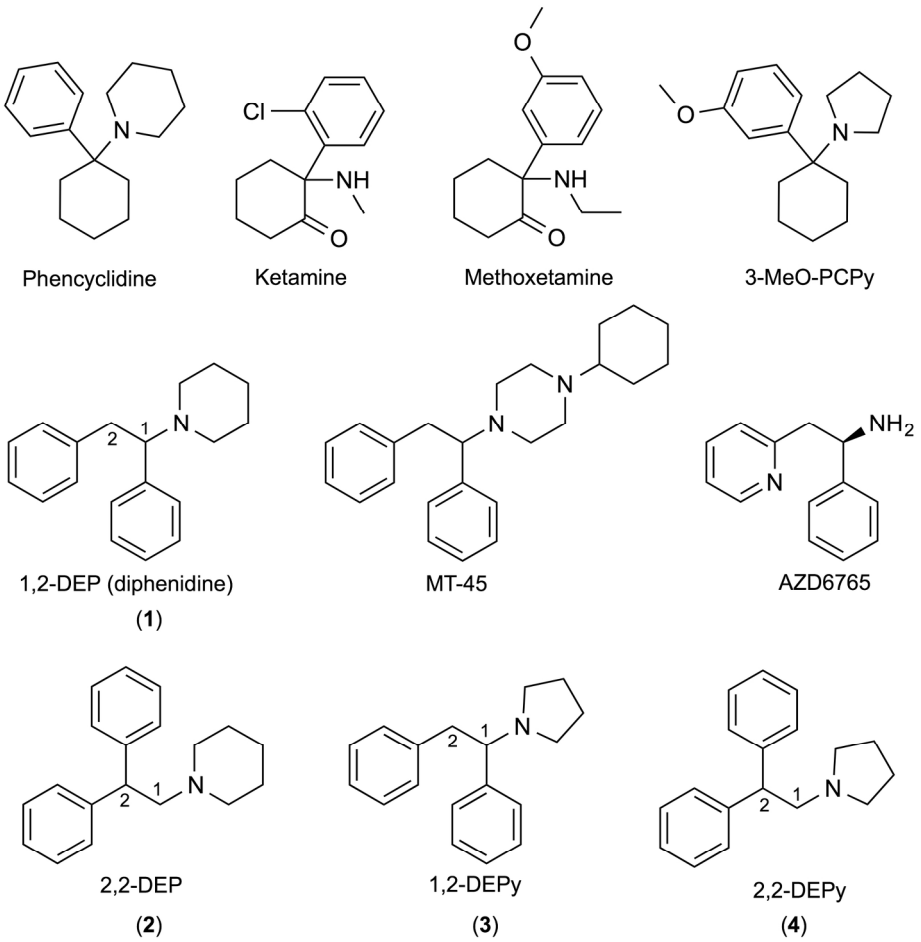

Figure 1. Diphenidine (**1**) has recently appeared as a psychoactive research chemical. Substances (**1**) – (**4**) were subject to synthesis and characterization in the present study.
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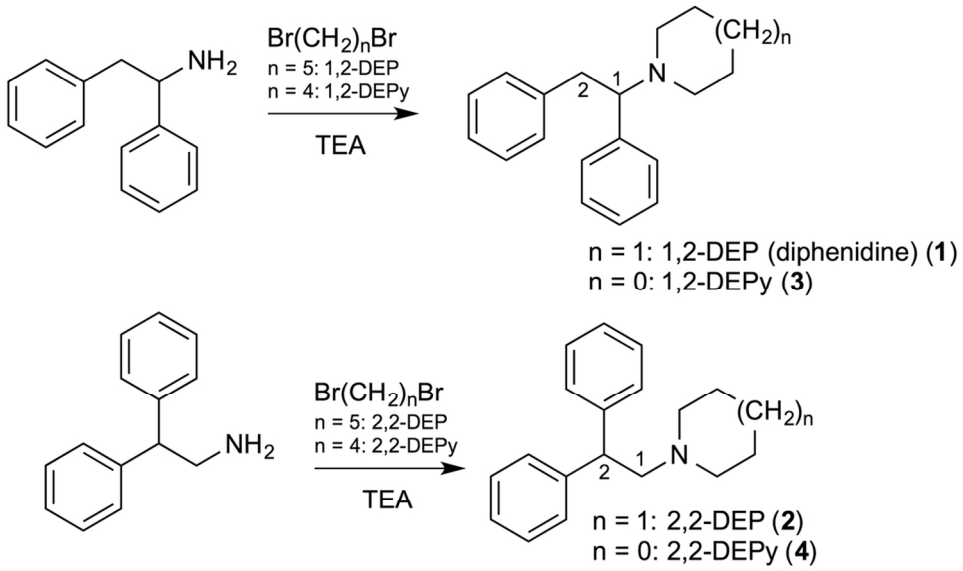

Figure 2. Synthesis scheme used for the preparation of (**1**) – (**4**) in the presence of potassium carbonate and triethylamine (TEA). The primary 1,2- or 2,2-diphenylethanamines were reacted with 1,5-dibromopentane or 1,5-dibromobutane to give the desired products.
110x69mm (300 x 300 DPI)

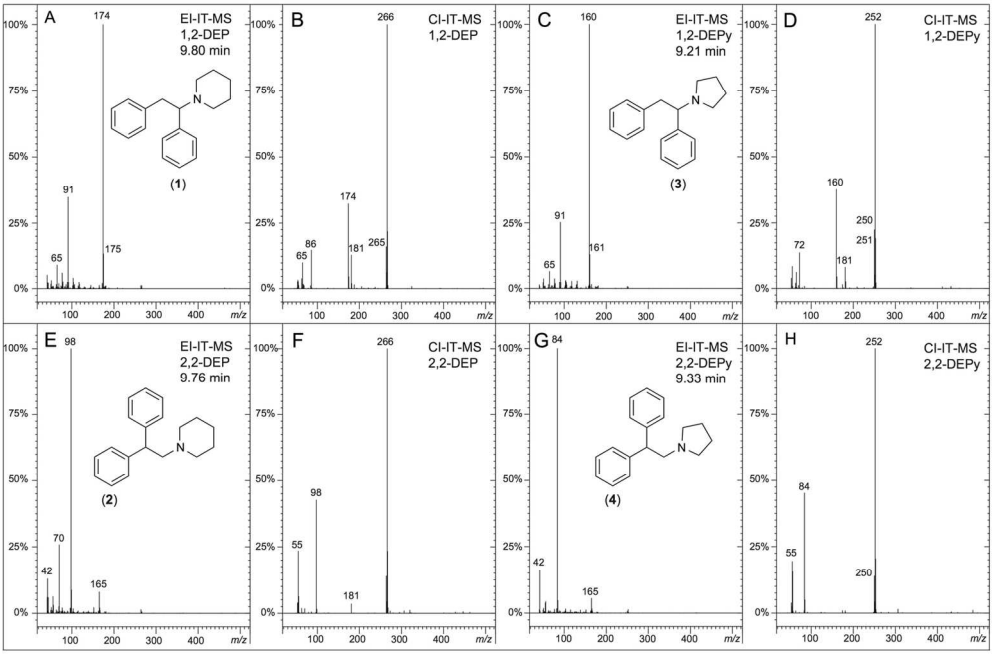

Figure 3. A — H: Gas chromatography retention times, electron ionization and chemical ionization ion trap mass spectra (EI/CI-IT-MS) observed for compounds of (**1**) – (**4**). The differentiation between isomers was possible.
144x96mm (300 x 300 DPI)

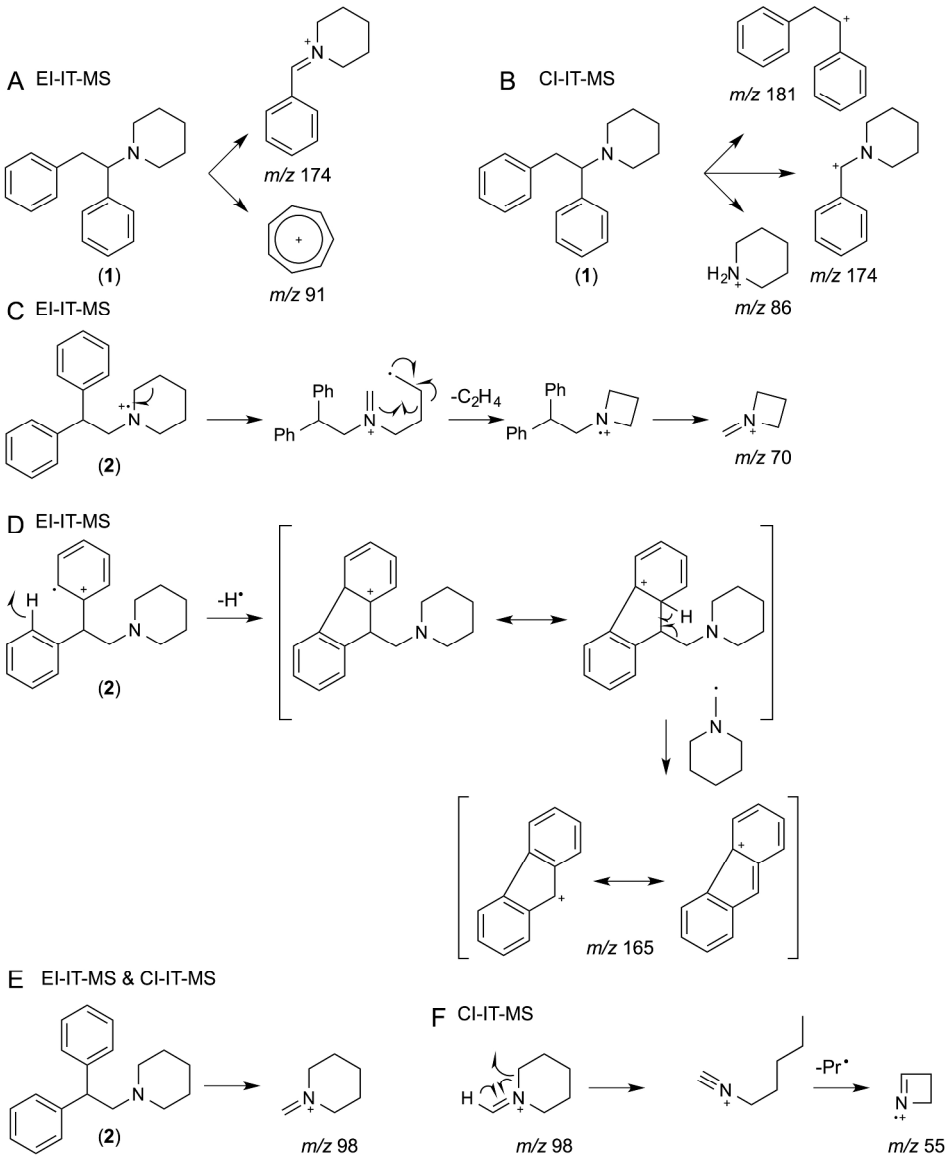

Figure 4. Suggested fragmentation pathways for diphenidine (**1**) as a representative example under electron ionization and chemical ionization ion trap mass spectrometry conditions (see Figure 3 for spectra).
266x328mm (300 x 300 DPI)

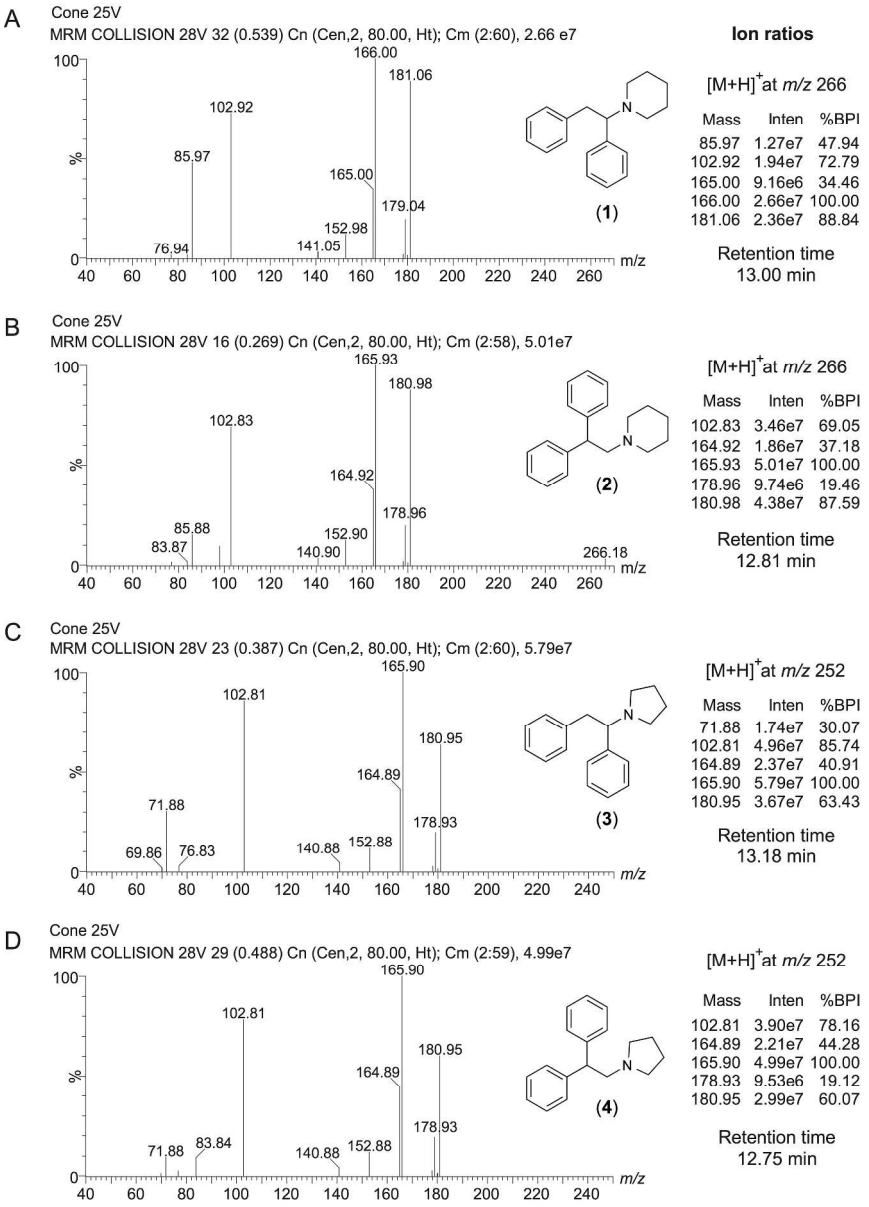

Figure 5. Liquid chromatography retention times, ion ratios and electrospray ionization triple quadrupole mass spectra obtained for substances (**1**) – (**4**).
277x380mm (300 x 300 DPI)

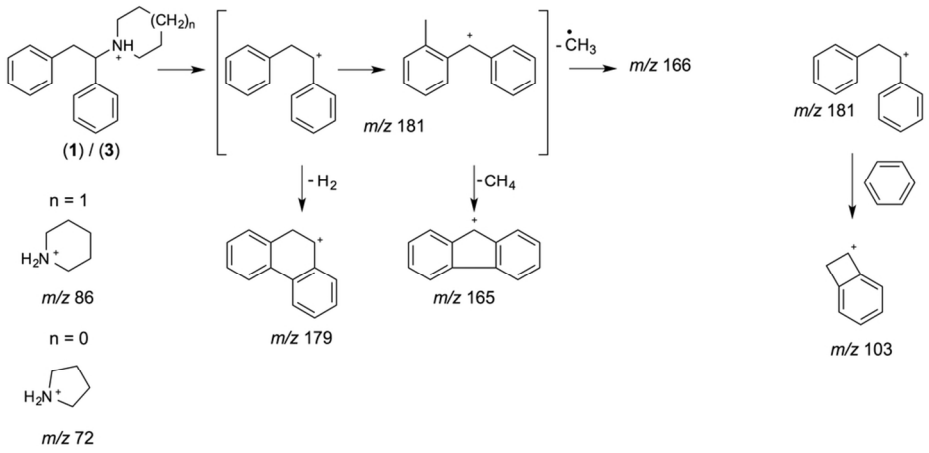

Figure 6. Suggested fragmentation pathways for diphenidine (**1**) and its pyrrolidine analogue (**3**) as a representative example under ESI-MS/MS conditions (see Figure 5 for spectra).
96x46mm (300 x 300 DPI)

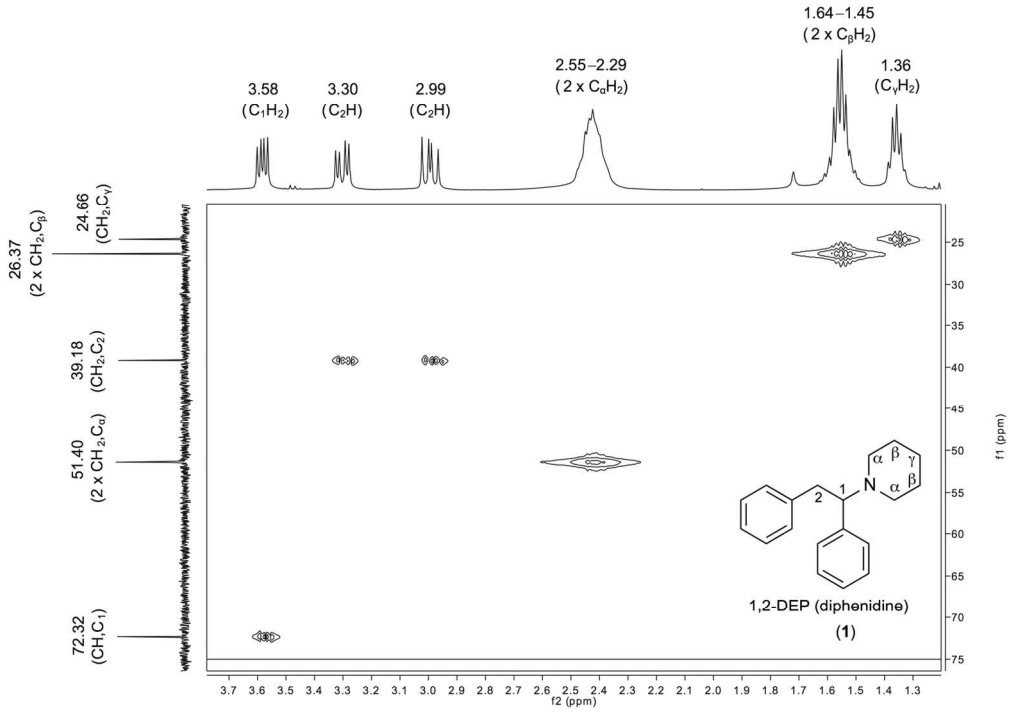

Figure 7. A partial Heteronuclear Multiple Quantum Coherence (HMQC) NMR spectrum of diphenidine (**1**).
146x103mm (300 x 300 DPI)

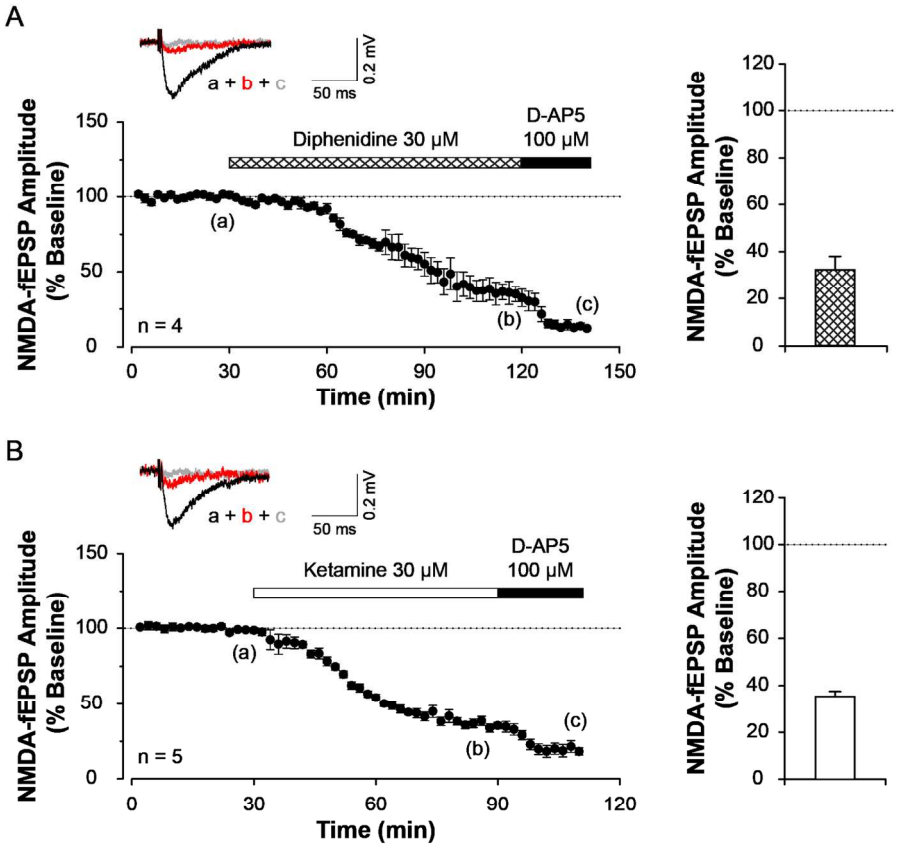

Figure 8. Effects of ketamine and diphenidine (**1**) on NMDA-fEPSPs in rat hippocampal slices. Pooled data and representative traces showing the reduction of NMDA-fEPSPs induced by A: a 90 minute application of diphenidine (30 μM); and B: a 60 minute application of ketamine (30 μM). D-AP5 (100 μM) application at the end of experiments induced a near-complete depression of responses. Drug effects were quantified and results are shown in the bar graphs on the right.
164x152mm (300 x 300 DPI)