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Syntheses, analytical and pharmacological characterizations of the "legal high" 4-[1-(3-methoxyphenyl)cyclohexyl]morpholine (3-MeO-PCMo) and analogues

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Keywords:	New psychoactive substances, Dissociative anesthetics, NMDA receptor, PCP, Arylcyclohexylmorpholines
Abstract:	New psychoactive substances (NPS) are commonly referred to as "research chemicals", "designer drugs" or "legal highs." One NPS class is represented by dissociative anesthetics, which include analogues of the arylcyclohexylamine phencyclidine (PCP), ketamine, and diphenidine. A recent addition to the NPS market was 4-[1-(3-methoxyphenyl)cyclohexyl]morpholine (3-MeO-PCMo), a morpholine analogue of 3-MeO-PCP. Although suspected to have dissociative effects in users, information about its pharmacological profile is not available. From clinical and forensic perspectives, detailed analytical data are needed for identification, especially when facing the presence of positional isomers, as these are frequently unavailable commercially. This study presents the analytical and pharmacological characterization of 3-MeO-PCMo along with five additional analogues including the 2- and 4-MeO- isomers, 3,4-methylenedioxy-PCMo (3,4-MD-PCMo), 3-Me-PCMo and PCMo. All six arylcyclohexylmorpholines were synthesized and characterized by chromatographic, mass spectrometric and spectroscopic techniques. The three positional isomers could be differentiated and the identity of 3-MeO-PCMo obtained from an internet vendor was verified. All six compounds were also evaluated for affinity at 46 central nervous system receptors

	including the N-methyl-D-aspartate receptor (NMDAR), an important target for dissociative anesthetics such as PCP and ketamine. In vitro binding studies using [3H]-MK-801 in rat forebrain preparations revealed moderate affinity for NMDAR in the rank order of 3-Me > 3-MeO >PCMo > 3,4-MD > 2-MeO > 4-MeO-PCMo. 3-MeO-PCMo was found to have moderate affinity for NMDAR comparable to that of ketamine, and had an approximate 12-fold lower affinity than PCP. These results support the anecdotal reports of dissociative effects from 3-MeO-PCMo in humans.

Syntheses, analytical and pharmacological characterizations of the "legal high" 4-[1-(3-methoxyphenyl)cyclohexyl]morpholine (3-MeO-PCMo) and analogues

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Running title: Synthesis, analytical and pharmacological characterization of PCMo analogues

Keywords: New psychoactive substances; dissociative anesthetics; NMDA receptor; PCP; arylcyclohexylmorpholines

Abstract

New psychoactive substances (NPS) are commonly referred to as “research chemicals”, “designer drugs” or “legal highs.” One NPS class is represented by dissociative anesthetics, which include analogues of the arylcyclohexylamine phencyclidine (PCP), ketamine, and diphenidine. A recent addition to the NPS market was 4-[1-(3-methoxyphenyl)cyclohexyl]morpholine (3-MeO-PCMo), a morpholine analogue of 3-MeO-PCP. Although suspected to have dissociative effects in users, information about its pharmacological profile is not available. From clinical and forensic perspectives, detailed analytical data are needed for identification, especially when facing the presence of positional isomers, as these are frequently unavailable commercially. This study presents the analytical and pharmacological characterization of 3-MeO-PCMo along with five additional analogues including the 2- and 4-MeO- isomers, 3,4-methylenedioxy-PCMo (3,4-MD-PCMo), 3-Me-PCMo and PCMo. All six arylcyclohexylmorpholines were synthesized and characterized by chromatographic, mass spectrometric and spectroscopic techniques. The three positional isomers could be differentiated and the identity of 3-MeO-PCMo obtained from an internet vendor was verified. All six compounds were also evaluated for affinity at 46 central nervous system receptors including the *N*-methyl-D-aspartate receptor (NMDAR), an important target for dissociative anesthetics such as PCP and ketamine. *In vitro* binding studies using [^{3}H]-MK-801 in rat forebrain preparations revealed moderate affinity for NMDAR in the rank order of 3-Me > 3-MeO >PCMo > 3,4-MD > 2-MeO > 4-MeO-PCMo. 3-MeO-PCMo was found to have moderate affinity for NMDAR comparable to that of ketamine, and had an approximate 12-fold lower affinity than PCP. These results support the anecdotal reports of dissociative effects from 3-MeO-PCMo in humans.

Introduction

A high number of new psychoactive substances (NPS)[1] continue to be available from online vendors and are sold as “research chemicals”. These chemicals are largely designed to by-pass governmental restrictions on existing psychoactive drugs. Dissociative agents that target the *N*-methyl-D-aspartate receptor (NMDAR) represent one of many available classes of compounds that are encompassed by the NPS term. Substances with dissociative profile (Figure 1A) comprise structural analogues of the arylcyclohexylamines such as phencyclidine (PCP), ketamine and methoxetamine.[2] More recently, 1,2-diarylethylamines such as diphenidine and its 2-methoxy analogue 2-MXP have also appeared.[3,4]

Substances that target the NMDAR are of interest for the development of treatment options for conditions such as depression, neuropathic pain, and a variety of neurodegenerative disorders and dementias.[5-9] At the same time, a number of these substances are used recreationally, outside of a medical setting, and include compounds that have not undergone any substantial pharmacological and toxicological evaluations. A systematic methodology is needed in order to address the chemical, pharmacodynamic, and pharmacokinetic properties of these substances,[3,4,10,11] thus facilitating drug development efforts, and identification of toxicity profiles as well as adverse events associated with recreational drug use.[12-14]

The earliest reported synthesis of 4-(1-phenylcyclohexyl)morpholine (PCMo) was found in a patent submitted in 1954[15] and predates that of PCP,[16] however, its pharmacology, or dissociative profile, was not recognized at that time. PCMo made brief documented appearances as an “analog” of PCP in the recreational market during the 1970’s and again in the early 2000’s.[2] More recently, 4-[1-(3-methoxyphenyl)cyclohexyl]morpholine (3-MeO-PCMo) has become available for purchase as a “research chemical” on a number of websites, which encouraged the authors to explore its chemistry and pharmacology. To gain further insight into this class of compounds, 2-MeO- and 4-MeO positional isomers were synthesized, as well as 3,4-methylenedioxy-PCMo (3,4-MD-PCMo), 3-Me-PCMo, and the unsubstituted PCMo template (Figure 1B). The entire series was subjected to comprehensive analytical characterization including chromatographic, mass spectrometric and spectroscopic methods. In addition, a test purchase of 3-MeO-PCMo was compared to the synthesized reference material confirming its identity.

With the exception of 2-MeO-PCMo and PCMo, pharmacological data on the arylcyclohexylmorpholines investigated in the present study are not available. 2-MeO-PCMo was shown to reduce acute thermal (tail immersion test) and chronic chemical pain (formaldehyde) induced in adult female rats. [17] In the tail immersion test, analgesic effects were found to be more pronounced compared to PCP and PCMo.[17] PCMo was also demonstrated to display lower potencies compared to PCP in a range of *in vitro* and *in vivo* assays targeting a number of different receptors.[18-29] In order to explore whether the six arylcyclohexylmorpholines showed PCP or ketamine-like properties *in vitro*, all test drugs were pharmacologically characterized in the present study for binding affinity at 46 CNS receptors including NMDAR, and monoamine transporters for dopamine, norepinephrine and serotonin.

Experimental

Materials

All starting materials, reagents, and solvents used for syntheses were obtained from Sigma Aldrich (St. Louis, MO, USA). Flash column chromatography was performed using Merck silica gel grade 9385 (230-400 mesh, 60 Å). Melting points were obtained using a DigiMelt A160 SRS digital melting point apparatus (Stanford Research Systems, Sunnyvale, CA, USA) at a ramp rate of 2°C/min. Melting points, spectral analyses, and receptor binding studies were performed on target compounds following flash chromatography purification.

Instrumentation

Nuclear magnetic resonance (NMR) spectroscopy

^{1}H (400 MHz) and ^{13}C NMR spectra (101 MHz) were obtained from the freebase material in $CDCl_3$ solution (100% and 99.96% D, 0.03% (*v/v*) TMS) at a concentration of 20 mg/mL using 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 $CDCl_3$ (δ = 77.0 ppm). Spectra were recorded with the freebases and the test purchase of 3-MeO-PCMo was determined to be the freebase. NMR assignments were made as described previously[10,30,31] using chemical shift position, splitting, ^{13}C PENDANT and 2-D experiments (HMQC, HMBC, and COSY).

Gas chromatography (EI/CI) ion trap mass spectrometry

Data for all six PCMo analogues (0.5 mg/mL in methanol) were recorded under full scan electron (EI) and chemical ionization (CI) conditions using HPLC grade methanol as the liquid CI reagent. A Varian 450-GC gas chromatograph coupled to a Varian 220-MS ion trap mass spectrometer (scan range *m/z* 41– *m/z* 500) and a Varian 8400 autosampler was employed with a Varian CP-1177 injector (275ºC) in split mode (1:50) (Walnut Creek, CA, USA). The Varian MS Data Review function of the Workstation software, version 6.91, was used for data acquisition. Transfer line, manifold and ion trap temperatures were set at 310, 80 and 220ºC, respectively. The carrier gas was helium at a flow rate of 1 mL/min using the EFC constant flow mode. 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. An Agilent J&W VF-5ms GC column (30 m × 0.25 mm, 0.25 µm) was employed for separation. The starting temperature was set at 80ºC and held for 1 min. The temperature then increased at 20ºC/min to 280ºC and held constant for 9.0 min to give a total run time of 20.00 min.

High mass accuracy mass spectrometry using an atmospheric solids analysis probe (ASAP)

ASAP was employed with a Thermo Fisher Scientific Inc., (Waltham, MA, USA) Orbitrap Exactive using an Ion Max source in positive mode. Measured accurate masses were within ± 5 ppm of the theoretical masses. The following parameters were used: resolution was set to ultra-high, sheath gas (N_2) flow 5 (arbitrary units), auxiliary gas flow 6 (arbitrary units), sweep gas

flow 0 (arbitrary units), corona discharge 4 kV, capillary temperature 275 °C, capillary voltage 25.0 V, skimmer voltage 14 V and a tube lens voltage of 85 V. Instrument calibrations were performed using the ProteoMass LTQ/FT-Hybrid ESI Positive Mode Calibration Mix from Supelco Analytical (Bellefonte, PA, USA).

Ultra-high performance liquid chromatography (UHPLC) high mass accuracy electrospray mass spectrometry

Mobile phases used for UHPLC separation consisted of acetonitrile with 1% formic acid (v/v) and an aqueous solution of 1% formic acid. The column temperature was set at 40°C (0.6 mL/min) and data were acquired for 5.5 min. The elution was a 5–70% acetonitrile gradient 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–*m/z* 1000 with and without auto MS/MS fragmentation. Ionization was achieved with an Agilent JetStream electrospray source and infused internal reference masses. Agilent 6540 QTOF-MS parameters: gas temperature 325°C, drying gas 10 L/min and sheath gas temperature 400 °C. Internal reference ions at *m/z* 121.05087 and *m/z* 922.00979 were used.

High performance liquid chromatography diode array detection

HPLC-DAD analyses were carried out on a Dionex 3000 Ultimate system coupled to a UV diode array detector (Thermo Fisher, St Albans, UK), using a Phenomenex Synergi Fusion column (150 mm x 2 mm, 4 µm) that was protected by a 4 mm x 3 mm Phenomenex Synergi Fusion guard column (Phenomenex, Macclesfield, UK). The mobile phases were made from 70% acetonitrile with 25 mM triethylammonium phosphate (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 nm to 595 nm (collection rate 2 Hz).

Infrared spectroscopy

Infrared (IR) spectra were obtained on a Perkin Elmer Spectrum BX FTIR model (Llantrisant, UK) using a Pike MIRacle ATR system. Data were acquired with the Spectrum v5.01 software (scan range 4000–400 cm^{-1}, resolution 4 cm^{-1}, 16 scans). Spectral data can be found in Supporting Information.

Microwave synthesizer

Conversion from primary amine intermediate to morpholine-ring products were performed using a CEM Discover SP microwave synthesizer (CEM Corporation, Matthews NC, USA). Reactions were carried out in 35 mL microwave vessels from CEM. Conditions for the reactions are detailed below.

Syntheses procedures

The syntheses of the primary amine intermediates were performed using a modified Geneste route (Figure 2) as described previously.[5,10,32] Reactions starting from the primary amine

intermediate to yield the morpholine ring products were carried out in a CEM Discover SP microwave synthesizer. The primary amine (PCA) intermediates were available from previous studies.[5,10,30]

Preparation of 4-[1-(2-methoxyphenyl)cyclohexyl]morpholine (2-MeO-PCMo)

1-(2-Methoxyphenyl)cyclohexanamine (2-MeO-PCA) (4.87 mmol, 1.00 g) and triethylamine (14.61 mmol, 2.03 mL) were added to acetonitrile (~15 mL). The solution was dried for 10 minutes with 4Å molecular sieves and then decanted into a 35 mL microwave vessel. 2-Bromoethyl ether (9.74 mmol, 1.22 mL) was added to the solution, the vessel was sealed under inert argon, and then reacted for 1.5 h at 85°C with 50 W power and stirring. Reaction pressures did not exceed 25 psi. Afterwards, the reaction mixture (a red/dark red color) was transferred to an aqueous 0.2 M HCl solution (60 mL) and washed with ethyl acetate (EtOAc) (3 x 60 mL). The aqueous phase was basified to pH >12 with KOH pellets and extracted with EtOAc (3 x 60 mL). The pooled organic extraction was washed once with 10 mL brine, dried with anhydrous magnesium sulfate, and concentrated under reduced pressure to produce a light amber oil. The crude product was collected and purified using flash column chromatography with a mobile phase consisting of hexane/EtOAc (80/20) with TEA (1%, *v/v*). Fractions containing the product were pooled and concentrated to yield light-yellow oil, which solidified upon cooling (3.16 mmol, 0.869 g, 64.7% yield). This solid was recrystallized from boiling hexanes. Upon cooling at 0°C, colorless crystals formed and were collected by decanting, washed with hexanes, and dried at room temperature (m.p. 67.1–68.6°C). HR-ASAP-MS of the freebase observed: *m/z* 276.1949 (theory $[M+H]^+$ $C_{17}H_{26}NO_2^+$, *m/z* 276.1958, Δ = -3.3 ppm).

The HCl salt of 2-MeO-PCMo was prepared by dissolving the solidified freebase in 100% ethanol (EtOH), titrating to pH 1.0 with concentrated HCl, and evaporating under a stream of warm air. EtOH (100%) was added in 10 mL increments and evaporated until all residual moisture and HCl were removed. The resulting solid was dried and washed with EtOAc (2 x 5 mL). The dried solid was then recrystallized by dissolving in a minimal amount of warm EtOH and diluted 3-fold with Et_2O. The solution was stored at 0°C overnight. The resulting crystals were collected by decanting the solvent, washing the solid with EtOAc (2 x 5 mL) and drying. Recrystallization was repeated 2 additional times as described to produce white flakey crystals with m.p. 179.5–181.5°C (lit. 167–169°C[17]).

Preparation of 4-[1-(3-Methoxyphenyl)cyclohexyl]morpholine (3-MeO-PCMo)

3-MeO-PCMo was prepared in 50.9% yield from 3-MeO-PCA as described above and formed a colorless crystalline solid (m.p. 74.4–75.3°C) HR-ASAP-MS of the freebase observed: *m/z* 276.1952 (theory $[M+H]^+$ $C_{17}H_{26}N_1O_2^+$, *m/z* 276.1958, Δ = -2.2 ppm). The HCl salt was a white flakey crystalline powder (m.p. 209.1–209.4°C).

Preparation of 4-[1-(4-methoxyphenyl)cyclohexyl]morpholine (4-MeO-PCMo)

4-MeO-PCMo was prepared in 43% yield from 4-MeO-PCA as described above and formed a colorless crystalline solid (m.p. 79.9–81.5°C). HR-ASAP-MS of the freebase observed: *m/z* 276.1951 (theory $[M+H]^+$ $C_{17}H_{26}N_1O_2^+$, *m/z* 276.1958, Δ = -2.5 ppm). The HCl salt formed translucent amber crystals (m.p. 153.1–156.1°C).

Preparation of 4-[1-(1,3-benzodioxol-5-yl)cyclohexyl]morpholine (3,4-MD-PCMo)

3,4-MD-PCMo was prepared in 44% yield from 3,4-MD-PCA as described above and formed a colorless crystalline solid (m.p. 123.4–124.9°C). HR-ASAP-MS of the freebase observed: *m/z* 290.1752 (theory $[M+H]^+$ $C_{17}H_{26}N_1O_2^+$, found *m/z* 290.1751, Δ = 0.3 ppm). The HCl salt was a white fluffy crystalline powder (m.p. 180.5–181.7°C).

Preparation of 4-[1-(3-methylphenyl)cyclohexyl]morpholine *(3-Me-PCMo)*

3-Me-PCMo was prepared in 46.4% yield from 3-Me-PCA as described above as a colorless oil. The HCl salt was a white fluffy crystalline powder (m.p. 211.2–211.7°C). HR-ASAP-MS of the HCl salt observed: *m/z* 260.2018 (theory $[M+H]^+$ $C_{17}H_{26}N_1O_2^+$, *m/z* 260.2009, Δ = 3.5 ppm).

Preparation of 4-(1-phenylcyclohexyl)morpholine (PCMo)

PCMo was prepared as described in 60% yield from PCA; however, microwave reaction parameters were slightly altered (80°C, 65 W, and monitored by GC-MS for a total reaction time of 2.5 h). HR-ASAP-MS of the freebase observed: m/z 246.1845 (theory $[M+H]^+$ $C_{17}H_{26}N_1O_2^+$, *m/z* 246.1852, Δ = 2.8 ppm). HCl salt was obtained as the hemihydrate (^{1}H NMR) white powder with a melting point of 197.3–198.5°C (lit. 187–188°C[17]; 188–190°C;[33] 187–188°C;[34] 181–182°C;[35] 199–201°C (hemihydrate);[36] 182°C[15]). An alternate route for the synthesis of PCMo was also employed and is provided as Supporting Information.

NMDA receptor binding studies

In vitro binding affinities (K_i) of the target compounds were determined using competitive radioligand binding studies with [^{3}H]-MK-801, a high-affinity ligand for the PCP site within the NMDAR channel, in accordance with established protocols.[37,38] Thoroughly washed rat forebrain homogenate was used as the NMDAR source (whole brain obtained from Pel-Freez Biologicals, Rogers AR, USA) and prepared as described by Reynolds and Sharma[37]. Suspensions of 10 mM HEPES buffer (pH 7.4, 25°C) containing 100 µg/mL protein, 1 nM [^{3}H]-MK-801, 100 µM glutamate, 10 µM glycine, and various concentrations of unlabeled test drugs were incubated in the dark on a mechanical rocker at 25°C for 2 h. (+)-MK-801 (30 µM) was used for nonspecific binding (and positive control). The reaction was terminated by vacuum filtration using a 24-well cell harvester (Brandel, Gaithersburg MD, USA) over presoaked GF/B glass fiber filters (Brandel, Gaithersburg, MD, USA). Filters were washed with room temperature HEPES buffer (3 x 5 mL). Tritium trapped on the filter was measured via liquid scintillation counting, using a Beckman LS 6500 multipurpose scintillation counter (BeckmanCoulter, USA) at 57% efficiency. IC_{50} values were determined with Graphpad Prism 5.0 (GraphPad Software, La Jolla, USA) using non-linear regression with log-concentration plotted against percent specific binding. Percent specific binding for [^{3}H]-MK-801 in a control experiment was ~95%. K_i values were calculated using the equation of Cheng and Prusoff.[39] The K_d for (+)-MK-801 (1.75 nM), was determined via a homologous binding assay as described by Reynolds and Sharma and was consistent with the literature.[38] Protein concentration was determined via the Bradford method[40] using Coomassie protein assay reagent and rat albumin

as standard (Sigma Aldrich, USA). Experiments were performed in duplicate and repeated three times.

Non-NMDA receptor binding studies

Competitive binding studies of PCMo and analogues at 45 additional CNS receptors were performed through the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH PDSP). Briefly, target compounds were dissolved in DMSO and subjected to primary screening at 10,000 nM concentrations. Compounds exhibiting >50 % inhibition underwent secondary assay at varying concentrations to determine K_i values. Additional experimental details are available in the NIMH PDSP assay protocol book.[41]

Results and Discussion

The six morpholine analogues investigated in this study (Figure 1B) were synthesized using the modified Geneste route as reported previously for the preparation of PCP and PCPy analogs.[5,10,32] The conversion from the primary amine (PCA) to the morpholine ring was performed using an S_N2 cyclization reaction between the substituted PCA material and bis(2-bromoethylether) (Figure 2) and gave ~45% yields following purification by column chromatography and recrystallization. The synthesized PCMo HCl was found to be the hemihydrate salt (^{1}H NMR) and was consistent with a literature melting point value reported for the hemihydrate salt.[36] The remaining analogues contained less than a 0.25 molar equivalent of water. A discrepancy with the 2-MeO PCMo HCl melting point exists herein with a previously reported value,[17] which may be due to polymorphism, solvates or purity. The appearance of 3-MeO-PCMo on the “research chemicals” market triggered questions about the ability to differentiate this compound from its positional 2-MeO-PCMo and 4-MeO-PCMo isomers, given that isomeric sets of compounds are frequently unavailable as reference material that can be used for forensic and clinical investigations. With the exception of 2-MeO-PCMo and PCMo, where some, albeit limited analytical data are available, the remaining compounds presented in this study are reported for the first time.

Gas chromatography ion trap mass spectrometry (GC-IT-MS) data obtained from electron ionization (EI) and chemical ionization (CI) methods recorded for the HCl salts are summarized in Figure 3. The positional isomers 2-, 3-, and 4-MeO-PCMo could be separated on the GC column (10.04, 10.30 and 10.52 min). Under EI conditions, both the molecular ion and a $[M\text{-}H^{\bullet}]^+$ species were visible in appreciable relative abundance and implementation of CI facilitated detection of the corresponding protonated molecules. The EI mass spectrum obtained in the present study for PCMo was comparable with a spectrum published 40 years ago[33] although differences were observed in the relative abundance of various fragment, possibly due to implementation of different mass analyzers. CI mass spectra of PCMo, using both methane and isobutane as the reagent gas, appeared 3-4 years later[42,43] which revealed the formation of fragment ions also detected in the present study, such as *m/z* 88, *m/z* 159 and *m/z* 202, respectively. The ions formed under EI and CI ion trap MS conditions appeared to be equivalent to those reported previously for a range of 1-(1-phenylcyclohexyl)piperidine (PCP) and 1-(1-

phenylcyclohexyl)pyrrolidine[10] and *N*-alkyl-arylcyclohexylamines[5] and proposed fragmentation pathways have been described. The implementation of GC-MS analysis also resulted in degradation of the PCMo products that gave rise to a GC-induced degradant consistent with what appeared to be a 1-(1-cyclohexen-1-yl)-ring-substituted benzene species which has been described for other PCP-type substances before[10] (Supporting Information). Conversion of the hydrochloride salts to the freebases and analysis by a different instrument (GC quadrupole EI-MS) revealed a significant reduction in degradation (Supporting Information). The sample advertised as 3-MeO-PCMo by an online vendor was found to be consistent with the information provided on the product label. Implementation of GC-sIR also allows for the analysis of compound mixtures and/or substances that may only be available in small amounts, including the GC-induced degradation products (Supporting Information). As shown in the Supporting Information, the three positional isomers could be differentiated by ATR-IR (HCl salts) and GC-sIR. The purity of the freebase was not determined; however, no impurity peaks were observed with GC-MS, LC-MS, or NMR, and the melting point of the test purchase exactly matched that of the synthesized 3-MeO-PCMo when run side by side. Attempts to separate the three positional isomers using various solvent combinations and two different TLC plates, however, were unsuccessful.

Ultra-high performance liquid chromatography electrospray quadrupole time-of-flight tandem mass spectra for all six PCMo analogues are shown in Figure 4, which illustrated that product ion formations were also comparable to a number of PCP/PCPy[10] and *N*-alkyl-arylcyclohexylamine analogues[5] Examples observed in Figure 4 include a neutral loss of morpholine, formation of the respective tropylium ion or detection of protonated morpholine. Implementation of the HPLC-diode array detection (DAD) procedure showed only partial separation of the three positional isomers due to co-elution of 3-MeO- and 4-MeO-PCMo (Figure 5). However, the ultraviolet spectra scanned between 200 and 594 nm provided distinct differences that allowed for facile differentiation between the isomers. 3-MeO-PCMo gave rise to distinctive peaks at 218 nm and 278 nm whereas 4-MeO-PCMo displayed a slight shift to 230 nm although the 277 nm peak remained indistinguishable. UV spectra recorded for 3,4-MD-PCMo, 3-Me-PCMo, and PCMo and their corresponding HPLC retention times are provided as Supporting Information.

Detailed NMR analyses on PCMo have been reported previously and were consistent with the results presented in this study (Tables 1 and 2).[44,45] PCMo HCl was also characterized by ^{13}C NMR and the recorded spectrum was in agreement with literature values.[36] In general, the chemical shift behavior of the series was consistent with those observed previously with related arylcyclohexylamines and a detailed discussion can be found elsewhere.[5,10,30] One notable distinction unique to the PCMo series worth addressing, however, is with respect to the morpholine ring, as this feature may be useful for the identification of related arylcyclohexylmorpholines. Due to the presence of the O heteroatom in the ring system, the β-chemical shifts were more deshielded and, thus, appeared further downfield than those found in the α-position (NCH) in both the ^{1}H (~3.6 ppm vs. ~2.3 ppm) and ^{13}C spectra (~68 ppm vs. ~46 ppm). In the ^{1}H spectra, the β-protons consistently appeared as a triplet, integrating to four protons, due to vicinal coupling (J ~4.6 Hz) with the two α-protons (magnetically equivalent due to ring flipping). The occurrence of ring flipping appeared to be consistent with the fact that the

^{1}H NMR spectra of the HCl salts (Supporting Information) showed separate axial and equatorial shifts for the beta protons. Protonation is known to prevent ring flipping, and this effect was observed with other compounds including arylcyclohexylamines.[10] Similarly, the α-protons appeared as a triplet due to vicinal coupling with the β-protons (*J* ~4.6 Hz). Furthermore, the 2,6 and α-proton chemical shifts in 2-MeO-PCMo appeared further downfield compared to those deriving from the 3-MeO and 4-MeO counterparts and a similar effect was observed in the ^{13}C spectra. The proton chemical shifts linked to the 3,5 and β-positions on the other hand were equivalent in all three positional isomers. This effect was observed with the corresponding PCP HCl salt series[30] although it was not consistently observed with the *N*-alkyl secondary anisyl-cyclohexylamines.[5]

NMDAR and off-target receptor binding studies

With regards to NMDAR, the results of competitive [^{3}H]-MK-801 displacement assays are provided in Table 3 as IC_{50} and K_i values and shown graphically in Figure 6. Compared to some previously investigated PCP analogs,[30] substitution of piperidine for a morpholine ring reduced NMDAR affinity. Consistent with the present results, PCMo was previously reported to show a ~10 fold reduced affinity to NMDAR using [^{3}H]-PCP in central nervous system tissue.[18,46] Furthermore, PCMo had 10-fold reduced potency relative to PCP in a number of experimental models.[46,47] The affinity rank order determined in this study was comparable to their PCP counterparts with 3-MeO > H > 2-MeO > 4-MeO).[30] Interestingly, the same affinity order was seen with a series of diphenidine analogs,[4] although it was not observed with the methoxylated PCPy series (3-MeO > 4-MeO > 2-MeO).[30]

A heatmap containing the results of the binding experiments on the 46 assessed CNS receptors is presented in Figure 7. Besides NMDAR, all compounds had moderate affinity for the sigma-2 receptor, which is commonly seen with this class of compounds.[4,30,48] 3,4-MD-PCMo was the most selective compound and this selectivity was consistent with other 3,4-MD substituted arylcyclohexylamines.[30] Likewise, 3,4-MD-PCMo and PCMo had moderate NMDAR affinity values comparable to ketamine and memantine.[4,49,50] PCMo was shown to be less potent and toxic than PCP,[24] which may be explained by the moderate NMDAR affinity.[30,49,50]

Arylcyclohexylamines have displayed variable affinities at the monoamine reuptake transporters for serotonin, norepinephrine, and dopamine (SERT, NET, and DAT, respectively).[30,51] Interestingly, the morpholine ring abolished NET activity for all compounds relative to their piperidine counterparts.[30] 3-Me-PCMo was the only compound with affinity for both SERT and DAT. The 2-MeO and 3-MeO analogues displayed selectivity towards SERT over DAT, whereas 4-MeO-PCMo had appreciable affinity for DAT.

Larger 1,4-diaminocyclohexane derivatives containing the PCMo moiety displayed *in vitro* μ-opioid receptor activity in previous cell-based assays.[52] However, the binding experiments in this study revealed no affinity for the δ-, κ- or μ-opioid receptors, which indicate that the anti-nociceptive properties may have been the result of NMDAR antagonism.[53-56] Previous pharmacological experiments with PCMo, 2-MeO-PCMo, 4-Me-PCMo, and 2-Me-4-HO-PCMo found analgesic activity in rats[17] which further suggests analgesic effects being mediated independently from opioid receptor affinity.

Conclusion

4-[1-(3-Methoxyphenyl)cyclohexyl]morpholine (3-MeO-PCMo), a morpholine analogue of 3-MeO-PCP, is available for purchase as a "research chemical" and suspected to share some psychopharmacological properties with ketamine and perhaps phencyclidine (PCP). The present study described the analytical characterization of 3-MeO-PCMo, its two positional isomers and three additional analogues. Differentiation between 2-MeO-, 3-MeO- and 4-MeO-PCMo was detectable by chromatographic and spectroscopic methods. *In vitro* pharmacological investigations also revealed that the compounds displayed moderate affinity toward the *N*-methyl-D-aspartate receptor with off-target activities at sigma-2 and monoamine transporters for dopamine and serotonin. These findings suggest that at least some of the investigated arylcyclohexylmorpholines, including 3-MeO-PCMo, may be psychoactive in humans and thus have abuse potential which may account for some of the purchases of this "research chemical." Clinical and forensic studies would be required to investigate this hypothesis further.

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Figure Captions

Figure 1. A: Examples of psychoactive substances with dissociative profiles. B: Morpholine analogs investigated in the present study. The numbering scheme employed for ^{13}C NMR assignments is shown for the isomers substituted with methoxy groups.

Figure 2. Synthetic scheme used to for the preparation of the investigated PCMo series *via* the modified Geneste route.[5,10,32] TFA: trifluoroacetic acid; TEA: triethylamine. R = 2-, 3-, and 4-MeO, 3,4-OCH_2O, 3-Me or H.

Figure 3. Gas chromatography ion trap mass spectrometry (GC-IT-MS) data obtained from electron ionization (EI) and chemical ionization (CI) methods.

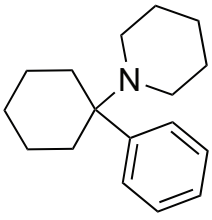
Figure 4. Ultra-high performance liquid chromatography high mass accuracy electrospray tandem mass spectra.

Figure 5. Diode array ultraviolet full scan spectra and high performance liquid chromatography data for 2-, 3- and 4-MeO-PCMo.

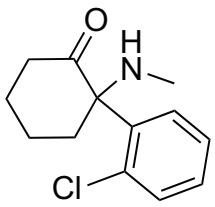
Figure 6. Competitive binding curves for PCP, PCMo, and analogues from [^3H]-MK-801 displacement using rat forebrain homogenate.

Figure 7. Heatmap of compound affinities (K_i) at CNS receptors. Solid green without number indicates IC_{50} was >10,000 nM in primary assay.

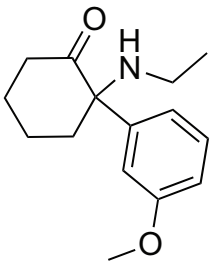
A



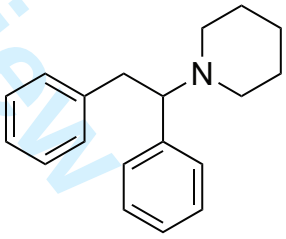
PCP



Ketamine

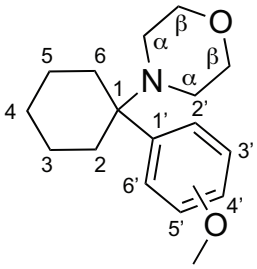


Methoxetamine

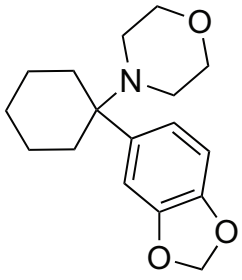


Diphenidine

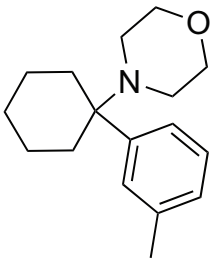
B



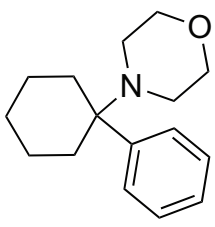
2-MeO-PCMo
3-MeO-PCMo
4-MeO-PCMo



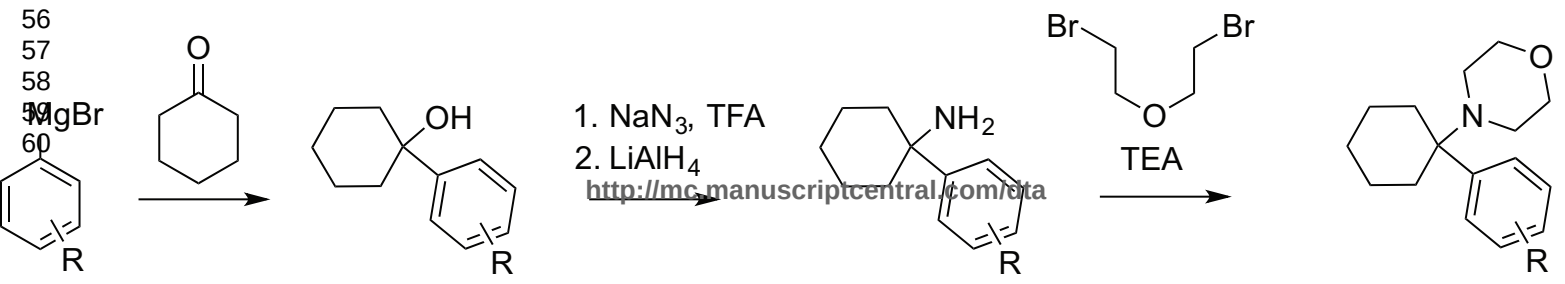
3,4-MD-PCMo

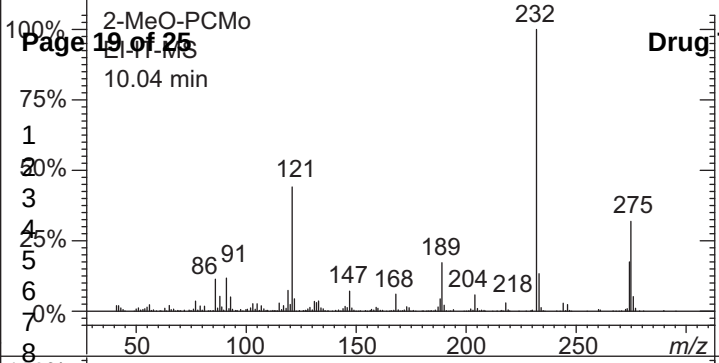


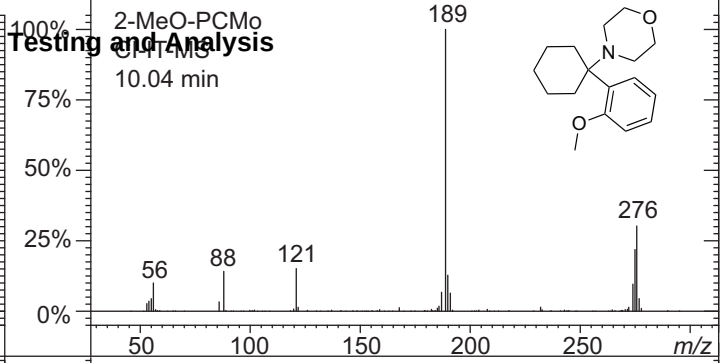
3-Me-PCMo

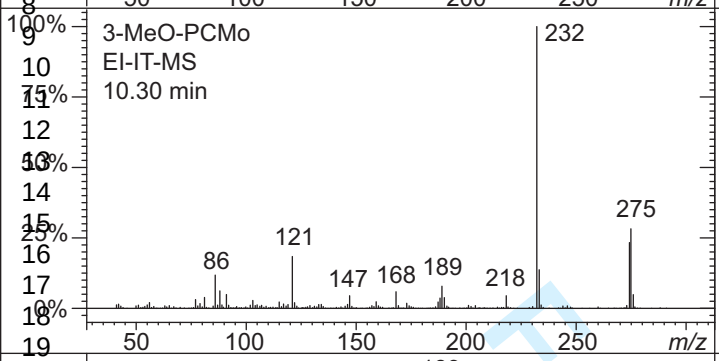


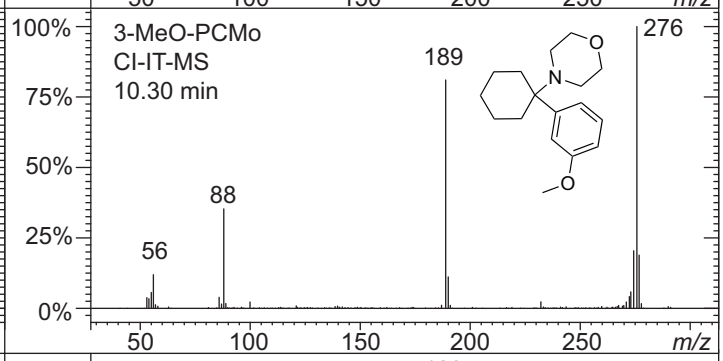
PCMo

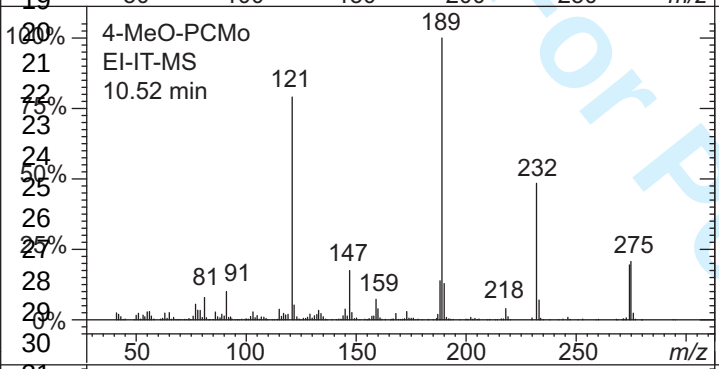


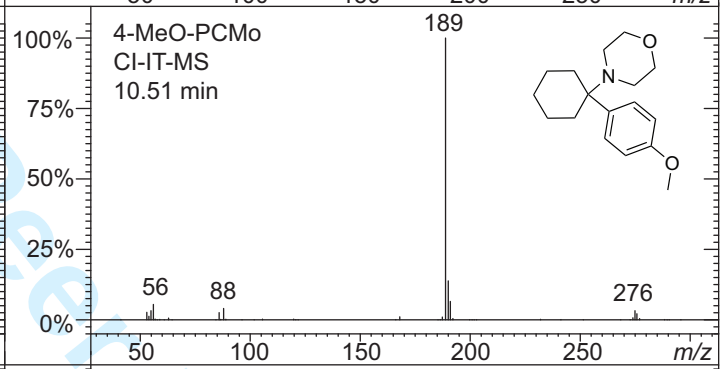


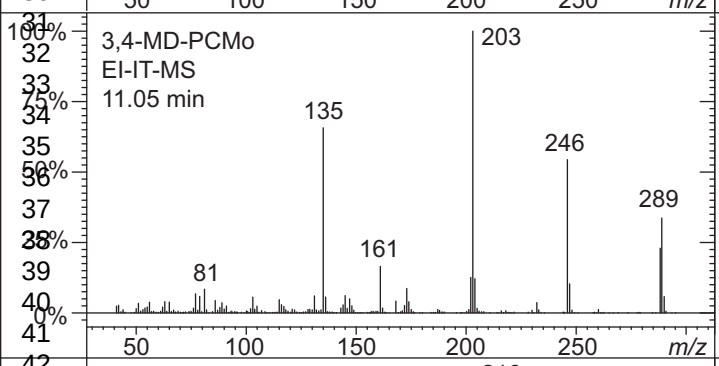


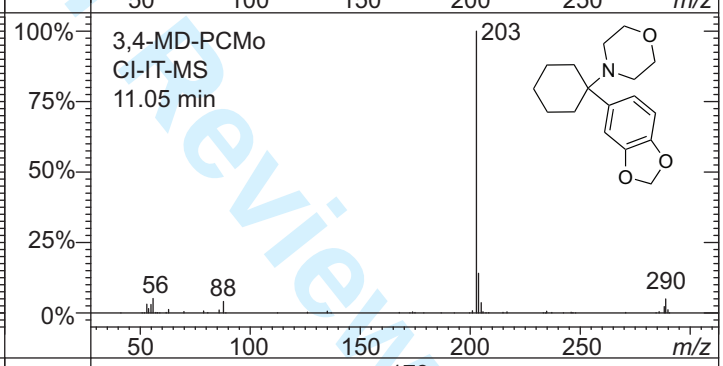


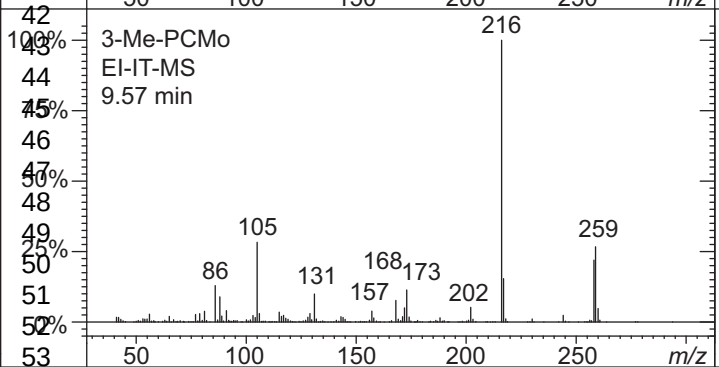


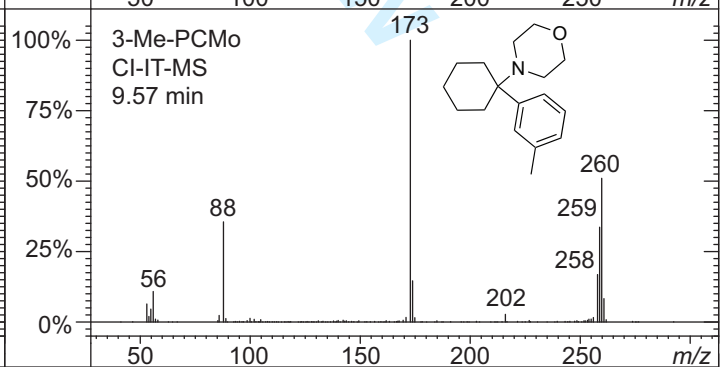


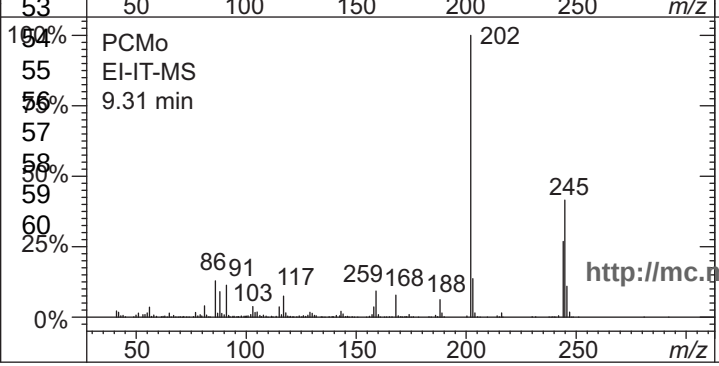


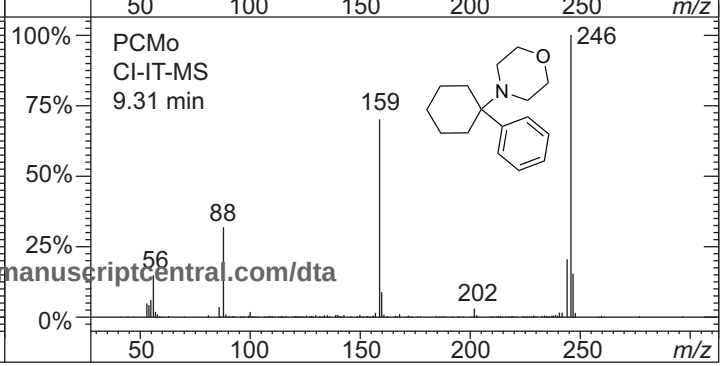


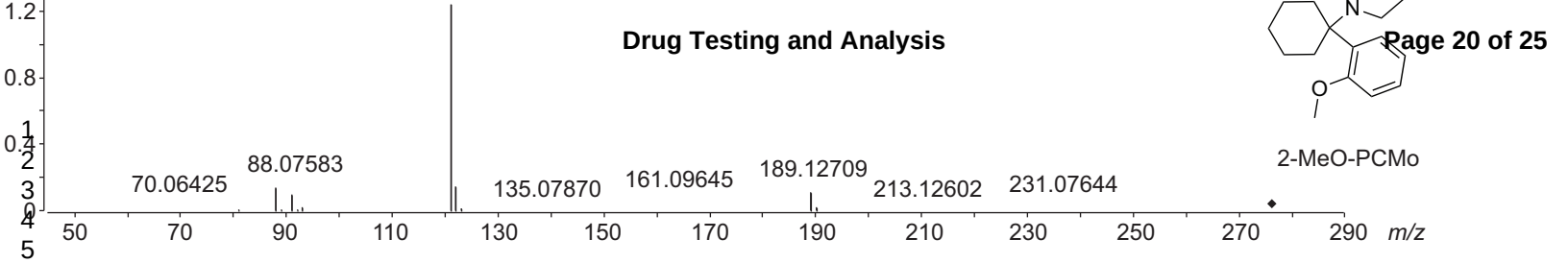




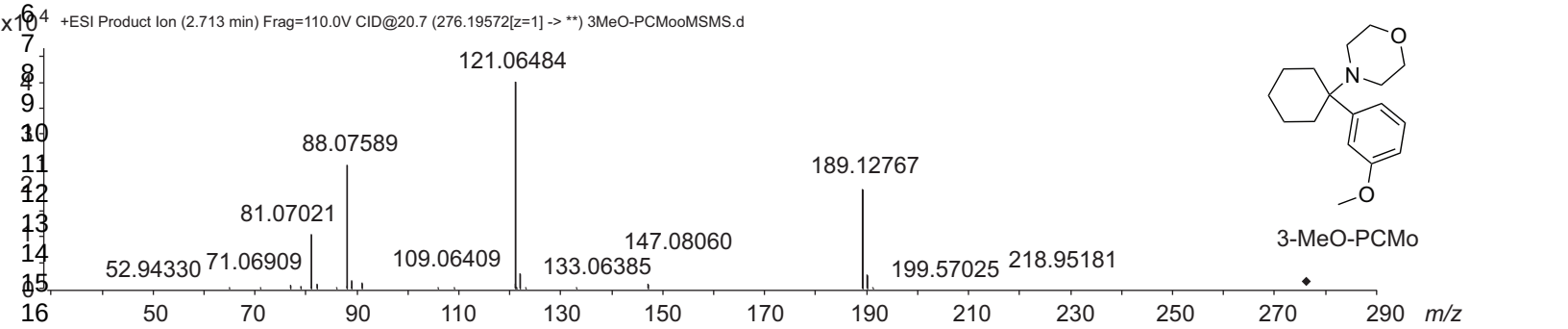




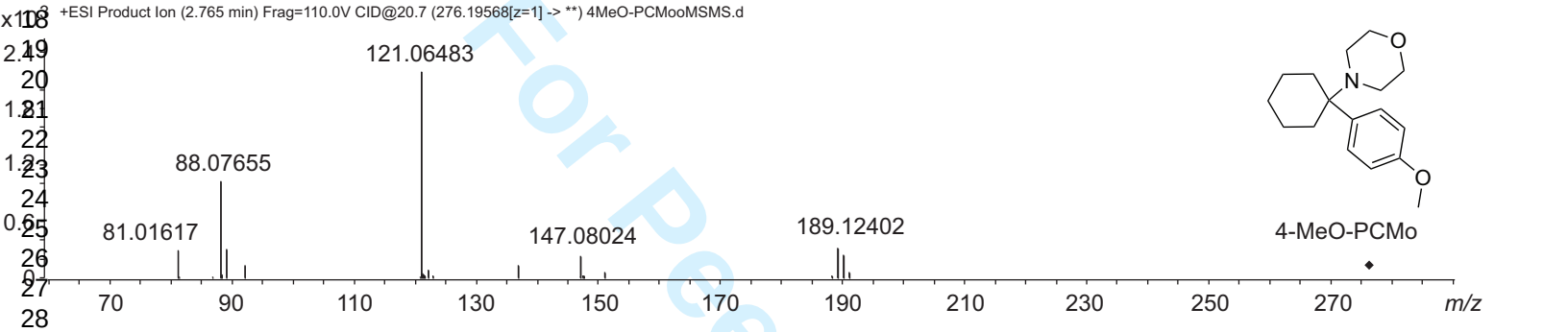




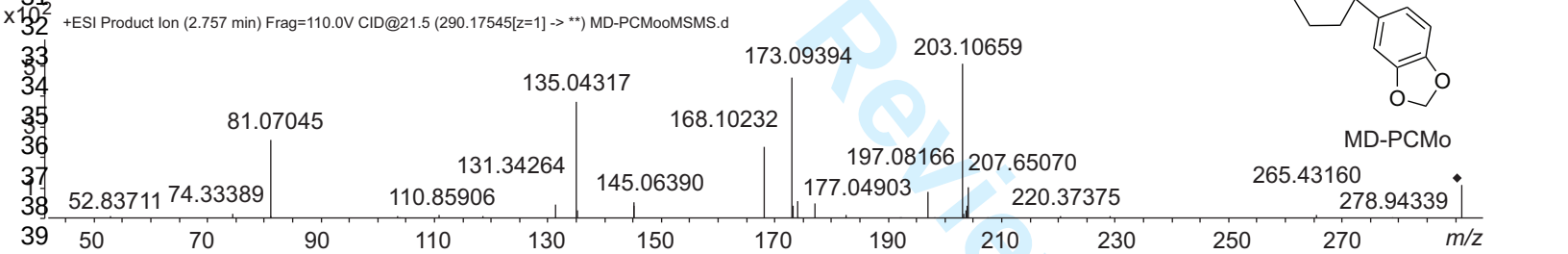
2-MeO-PCMo



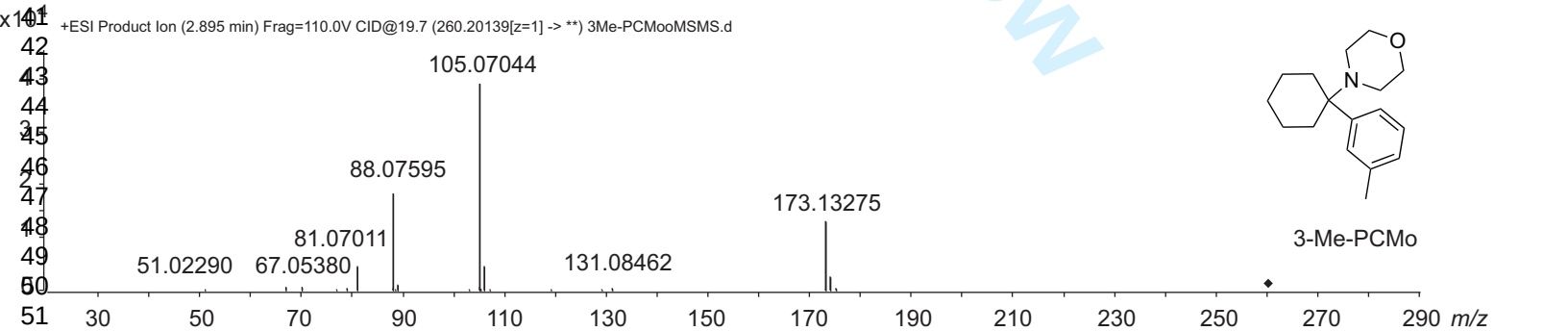
3-MeO-PCMo



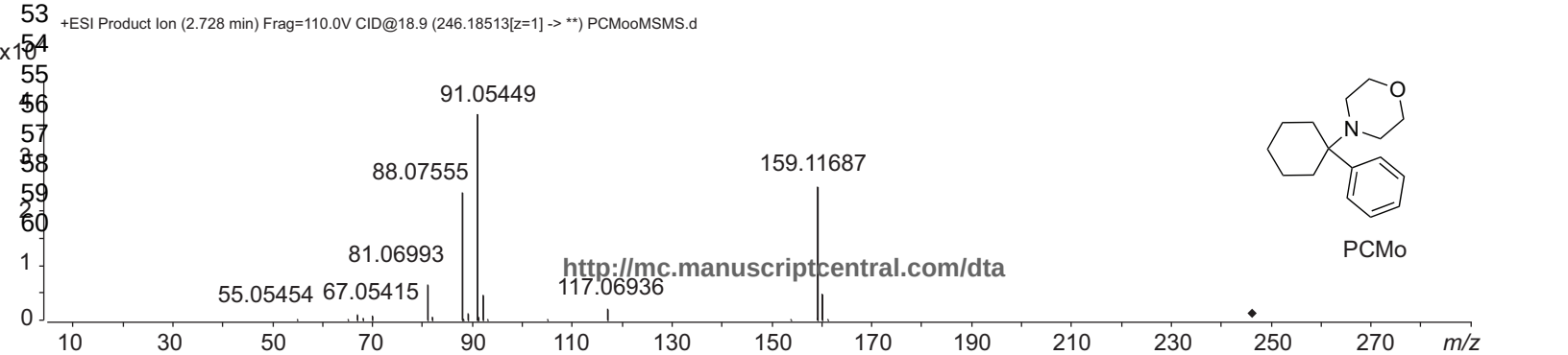
4-MeO-PCMo



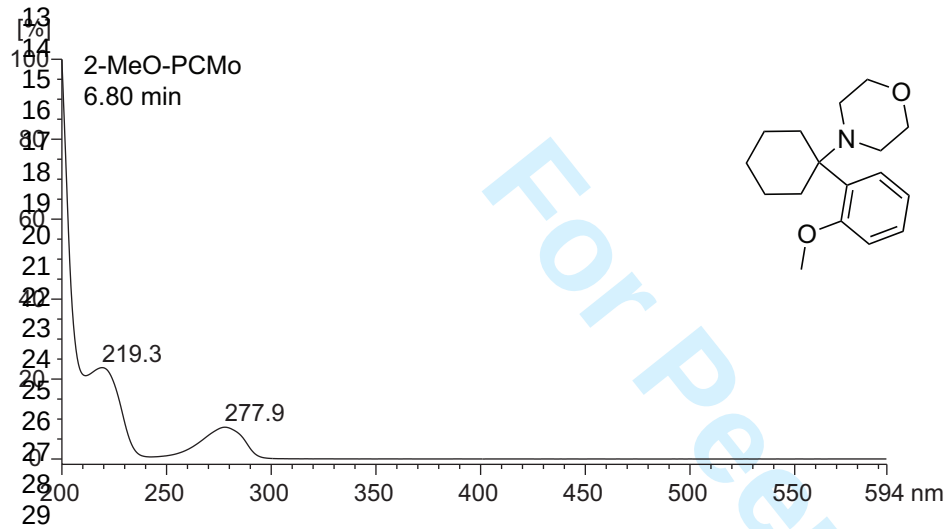
MD-PCMo

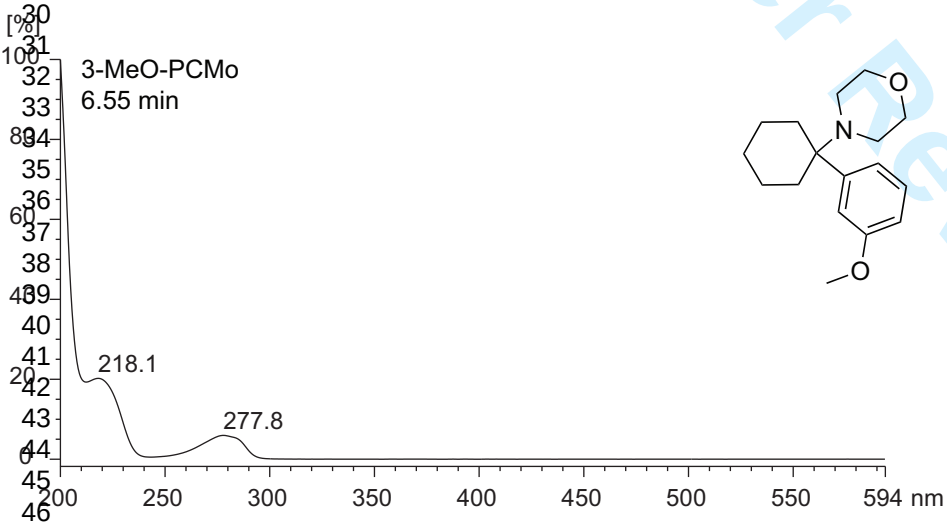


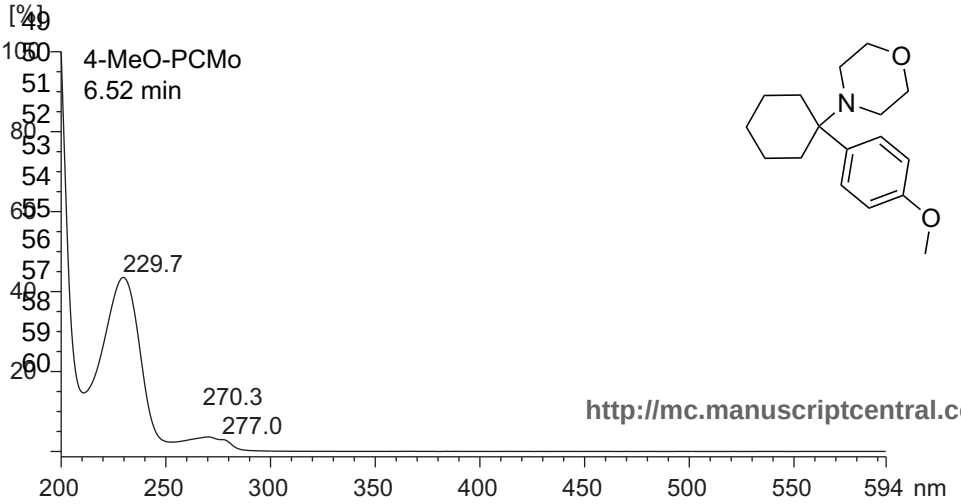
3-Me-PCMo

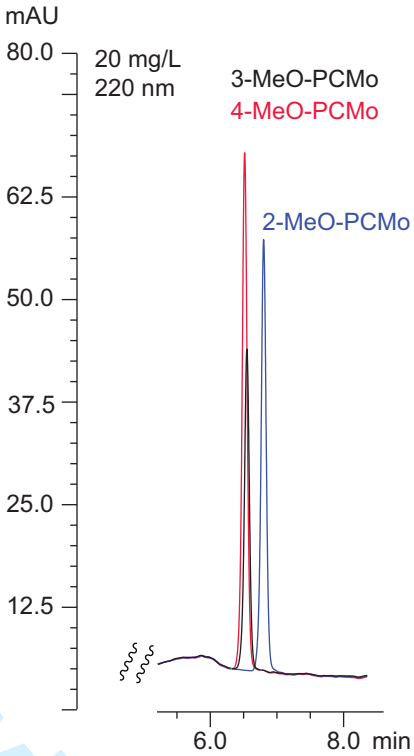


PCMo









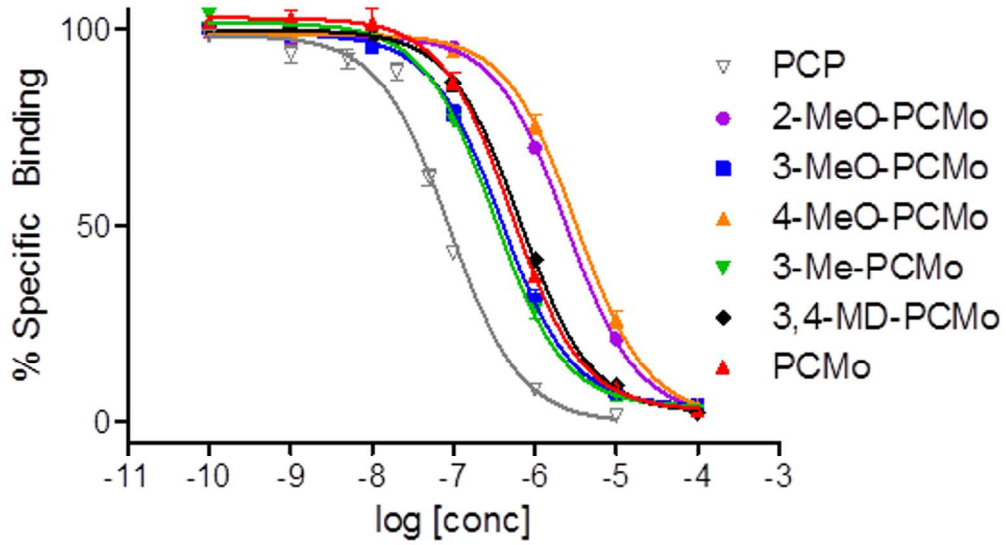


Figure 6. Competitive binding curves for PCP, PCMo, and analogues from [^{3}H]-MK-801 displacement using rat forebrain homogenate.

145x80mm (300 x 300 DPI)

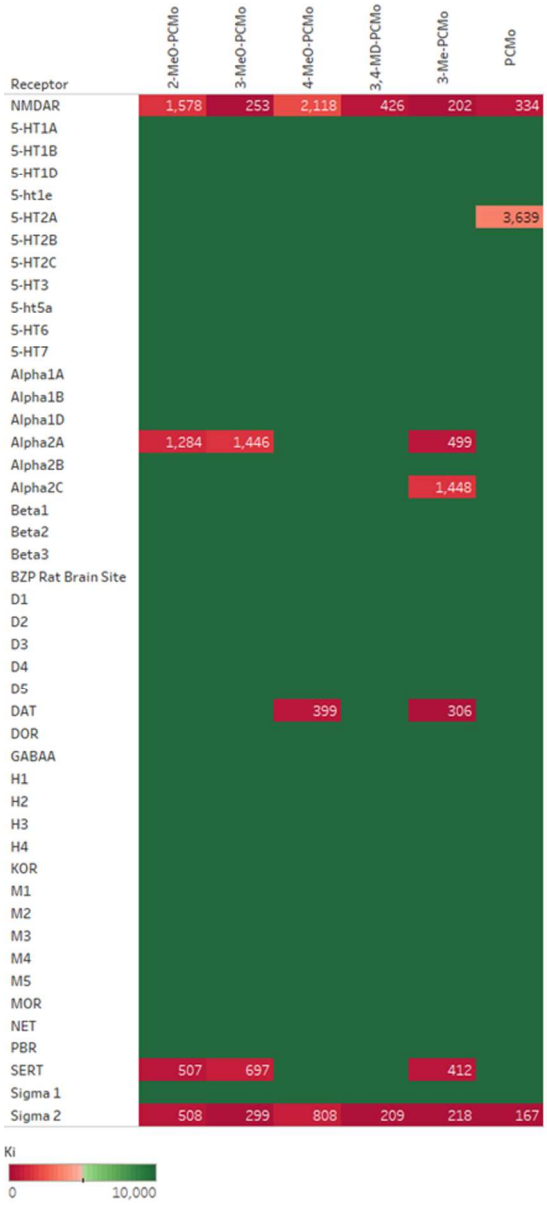


Figure 7. Heatmap of compound affinities (K_i) at CNS receptors. Solid green without number indicates IC_{50} was >10,000 nM in primary assay.

87x190mm (300 x 300 DPI)

Table 1. ^{1}H NMR data for PCMo freebases in $CDCl_3$

Proton	2-MeO- PCMo	3-MeO- PCMo	4-MeO- PCMo	3,4-MD-PCMo	3-Me-PCMo	PCMo
H_1	-	-	-	-	-	-
$H_{2,6}$	2.64–2.55 m ($2H_{eq}$) 1.80 ddd (J = 13.6, 10.5, 3.0 Hz, $2H_{ax}$)	2.13–2.03 m ($2H_{eq}$) 1.93 ddd (J = 13.3, 9.5, 3.3 Hz, $2H_{ax}$)	2.15–2.05 m ($2H_{eq}$) 1.91 ddd (J = 13.4, 9.7, 3.3 Hz, $2H_{ax}$)	2.08–1.97 m ($2H_{eq}$) 1.89 ddd (J = 13.3, 9.6, 3.3 Hz, $2H_{ax}$)	2.15–2.04 m ($2H_{eq}$) 1.94 ddd (J = 13.4, 9.4, 3.3 Hz, $2H_{ax}$)	2.19–2.04 m ($2H_{eq}$) 1.95 ddd (J = 13.5, 9.6, 3.4 Hz, $2H_{ax}$)
$H_{3,5}$	1.74–1.61 m ($2H_{eq}$) 1.32–1.20 m ($2H_{ax}$)	1.74–1.65 m ($2H_{eq}$) 1.38–1.27 m ($2H_{ax}$)	1.74–1.64 m ($2H_{eq}$) 1.35–1.23 m ($2H_{ax}$)	1.74–1.63 m ($2H_{eq}$) 1.39–1.25 m ($2H_{ax}$)	1.76–1.66 m ($2H_{eq}$) 1.37–1.26 m ($2H_{ax}$)	1.76–1.64 m ($2H_{eq}$) 1.39–1.24 m ($2H_{ax}$)
H_4	1.51–1.32 m (2H)	1.47–1.40 m (2H)	1.49–1.39 m (2H)	1.49–1.39 m (2H)	1.48–1.39 m (2H)	1.50–1.39 m (2H)
$H_{1'}$	-	-	-	-	-	-
$H_{2'}$	-	6.85 t (J = 1.9 Hz, 1H)	7.21 dm (J = 8.9 Hz, 1H)	6.79 d (J = 8.3 Hz, 1H)	7.10 s (1H) *overlap with $H_{6'}$	7.32–7.27 m (1H)
$H_{3'}$	6.98–6.88 m (1H)	-	6.88 dm (J = 8.9 Hz, 1H)	-	-	7.38–7.32 m (1H)
$H_{4'}$	7.29–7.18 m (1H)	6.79 dd (J = 8.1, 2.5 Hz, 1H)	-	-	7.10–7.06 m (1H) *overlap with $H_{2'}$	7.27–7.20 m (1H)
$H_{5'}$	6.98–6.88 m (1H)	7.27 t (J = 8.0 Hz, 1H)	6.88 dm (J = 8.9 Hz, 1H)	6.82 d (J = 1.8 Hz, 1H)	7.30–7.19 m (1H)	7.38–7.32 m (1H)
$H_{6'}$	7.29–7.18 m (1H)	6.89 dd (J = 7.8, 1.8 Hz, 1H)	7.21 dm (J = 8.9 Hz, 1H)	6.75 dd (J = 8.3, 1.8 Hz, 1H)	7.05 dm (J = 7.6 Hz, 1H)	7.32–7.27 m (1H)
H_α	2.43 t (J = 4.5 Hz, 4H)	2.34 t (J = 4.6 Hz, 4H)	2.32 t (J = 4.6 Hz, 4H)	2.33 t (J = 4.5 Hz, 4H)	2.33 t (J = 4.6 Hz, 4H)	2.33 t (J = 4.5 Hz, 4H)
H_β	3.63 t (J = 4.6 Hz, 4H)	3.63 t (J = 4.6 Hz, 4H)	3.63 t (J = 4.6 Hz, 4H)	3.63 t (J = 4.6 Hz, 4H)	3.63 t (J = 4.7 Hz, 4H)	3.63 t (J = 4.6 Hz, 4H)
Cc	3.77 s (OCH_3)	3.82 s (OCH_3)	3.81 s (OCH_3)	5.95 s (OCH_2O)	2.37 s (CH_3)	-

Table 2. ^{13}C NMR data for PCMo analogues (freebase, in $CDCl_3$)

Carbon	2-MeO-PCMo	3-MeO-PCMo	4-MeO-PCMo	3,4-MD-PCMo	3-Me-PCMo	PCMo
C_1	63.12	60.69	60.35	60.72	60.62	60.71
$C_{2,6}$	34.49	33.00	33.00	33.21	32.93	32.86
$C_{3,5}$	22.93	22.29	22.28	22.29	22.28	22.25
C_4	26.46	26.26	26.32	26.27	26.33	26.31
$C_{1'}$	126.91	141.18	131.37	133.70	139.16	139.22
$C_{2'}$	159.75	114.34	128.52	107.23	127.99	127.34
$C_{3'}$	112.43	159.19	112.87	147.41	136.97	127.64
$C_{4'}$	130.52	110.51	157.87	145.72	124.47	126.35
$C_{5'}$	119.97	128.45	112.87	107.97	127.48	127.64
$C_{6'}$	127.92	119.95	128.52	120.66	127.06	127.34
C_α	46.64	45.91	45.84	45.88	45.88	45.86
C_β	68.13	67.88	67.84	67.86	67.89	67.87
C_c	55.16 (OCH_3)	55.17 (OCH_3)	55.14 (OCH_3)	100.82 (OCH_2O)	21.86 (CH_3)	-

Table 3. NMDAR binding affinities for PCMo series using [^{3}H]-MK-801 in rat forebrains. Means ± SEM from three separate experiments run in duplicate.

Compound	**IC_{50} ± SEM (nM)**	**K_i ± SEM (nM)**
PCP	34.7 ± 2.5	22.1 ± 1.6
Ketamine [4]	508.5 ± 30.1[4]	323.9 ± 19.2[4]
2-MeO-PCMo	2,477 ± 115	1,578 ± 73.2
3-MeO-PCMo	397.0 ± 45.4	252.9 ± 28.9
4-MeO-PCMo	3,326 ± 343.3	2,118 ± 218.7
3,4-MD-PCMo	668.0 ± 30.5	425.5 ± 19.4
3-Me-PCMo	316.8 ± 29.1	201.8 ± 18.5
PCMo	524.6 ± 13.7	334.1 ± 8.8