Chem Biol Drug Des 2011; **78:** 25–32

Research Article

doi: 10.1111/j.1747-0285.2011.01124.x

Syntheses and Pharmacological Evaluations of Novel N-Substituted Bicyclo-Heptane-2-amines at *N*-Methyl-D-Aspartate Receptors

Zeynep Ates-Alagoz[1,2], Shengguo Sun[1], Jason Wallach[1] and Adeboye Adejare[1,*]

[1]*Department of Pharmaceutical Sciences, Philadelphia College of Pharmacy, University of the Sciences in Philadelphia, Philadelphia, PA 19104, USA*

[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandogan, Ankara, Turkey*

**Corresponding author: Adeboye Adejare, a.adejar@usp.edu*

Several novel norcamphor (bicycloheptane)-based compounds were designed and synthesized as non-competitive *N*-methyl-D-aspartate receptor antagonists at the phencyclidine binding sites. The heterocyclic ring was also varied to examine piperidine, pyrrolidine, and morpholine groups. We examined pharmacological activities of these compounds *in vitro* (binding studies) and *in vivo* (maximal electroshock test). Pharmacological evaluations revealed one of the compounds, 5a, to be a good lead, exhibiting moderate binding at *N*-methyl-D-aspartate receptors (IC_{50} = 7.86 μM; K_i = 5.28 μM), maximal electroshock neuroprotection activity at 100 mg/kg and acceptable toxicity profile.

Key words: anticonvulsant activity, bicycloheptane derivatives, maximal electroshock test, *N*-methyl-D-aspartate receptor antagonist, phencyclidine binding site

Received 22 September 2010, revised 28 February 2011 and accepted for publication 1 April 2011

Glutamate is one of the principal excitatory neurotransmitters in the mammalian central nervous system (CNS). A major function of glutamate is control of ion flow at excitatory synapses. Glutamate receptors are subdivided into two, namely metabotropic and ionotropic. Three ionotropic receptor types have been identified based on ligand selectivity; AMPA, N-methyl-D-aspartate (NMDA), and kainate. In addition to ionotropic receptors, three classes of metabotropic receptors are acknowledged (mGluRs) (1). The ionotropic NMDA receptor (NMDAR) is noteworthy in that it requires binding by agonist glutamate and co-agonist D-serine or glycine for it to be activated (open state). NMDAR is also distinct in that it exhibits slow kinetics, is permeable to Na^+, K^+, and Ca^{2+}(2–4); and is both ligand and voltage gated (5,6). It is a complex made up of distinct binding sites including sites for amino acids L-glutamate, glycine, and D-serine. In addition to these sites, allosteric modulatory sites for Mg^{2+}, phenylcyclidine (PCP), polyamines, and $Zn^{2+}$are known (7). While glutamate, glycine, and polyamine sites are found outside the ion channel; the sites for $Mg^{2+}$and PCP are located within the channel itself (8). The NMDA receptor has been implicated in the pathophysiology of a variety of neurological and neuropsychiatric diseases including Alzheimer's disease (9), epilepsy, chronic pain syndrome, schizophrenia, Parkinson's disease, Huntington's disease (10,11), major depression, addiction, and anxiety (12). Excessive glutamate and subsequent over-stimulation of NMDA receptors leading to excessive Ca^{2+}influx has been implicated in the pathophysiology of these disease states (13,14). Several preclinical paradigms have found that non-competitive NMDA antagonism can effectively reduce NMDAR-mediated neurotoxicity (15).

A major limitation for therapeutically available NMDA antagonists is the essential role of NMDAR in neuro-physiology. While blockade of excessive NMDAR activity is desirable, it must be achieved without complete amelioration of normal glutamate function. As a result of this dichotomy, many competitive antagonists have failed in clinical trials (16). Utilization of non-competitive antagonists working through open-channel blockade has been proposed as an attractive alternative, as this mechanism requires initial activation of the channel for inhibition to occur, possibly leading to a higher likelihood of channel blockade in the presence of excessive levels of glutamate and a lower likelihood of antagonism with normal physiological levels of glutamate (16).

Phenylcyclidine is a non-competitive open-channel antagonist of NMDAR. PCP and it`s derivatives have attracted medicinal chemists for years, as these drugs have exhibited a variety of therapeutically desirable effects including anti-convulsant (9), anxiolytic (11), and neuroprotective effects against neural damage resulting from ischemia, anoxia, hypoglycemia, and endogenous neurotoxins (12–14). Unfortunately, in addition to this array of therapeutically desirable effects, most of these ligands exhibit an undesirable side effect profile, most noteworthy of which are the psychotomimetic effects.

Some authors have speculated that high affinity at the NMDA receptor may contribute to undesirable effects. There has thus been some interest in obtaining low to moderate affinity non-competitive NMDA antagonists (17–20). This hypothesis is supported by the fact that several therapeutically used moderate affinity NMDA antagonists, such as ketamine, DXM, memantine, and adamantine are generally well tolerated (18,20–22).

Our group is involved in the design and syntheses of NMDA receptor inhibitors as possible therapies for neurodegenerative diseases. Several novel norcamphor (bicyloheptane) derivatives were designed and synthesized as non-competitive antagonists of the NMDA receptor. Syntheses of these compounds are displayed in Scheme 1. Pharmacological activities of these compounds were evaluated *in vitro* (binding studies) and *in vivo* (MES test).

Materials and Methods

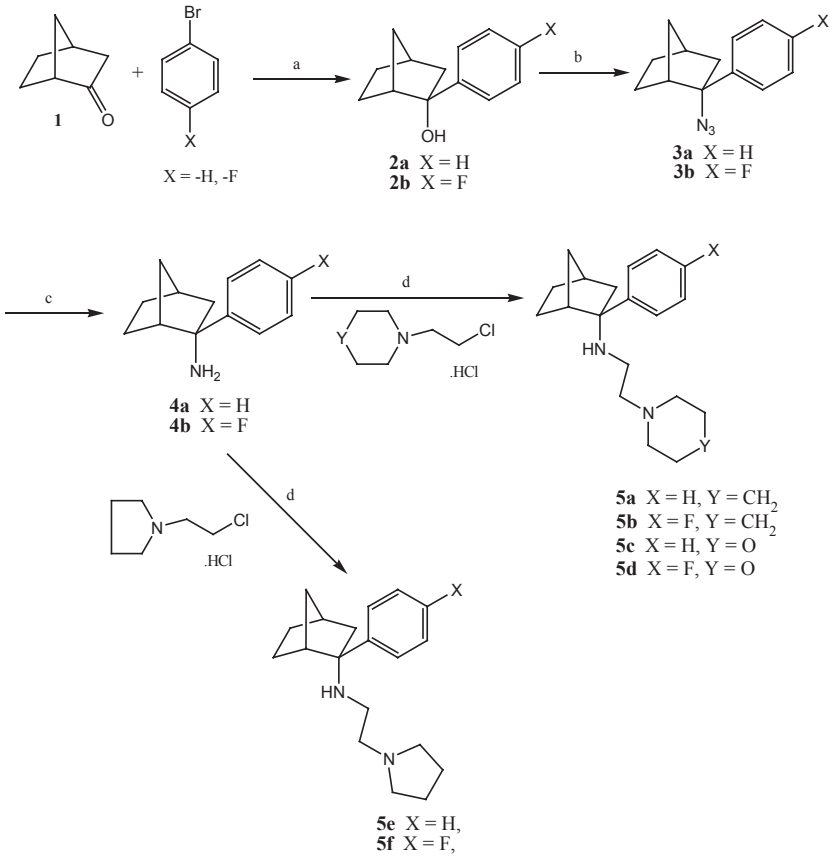
Melting points were determined with a Mel-Temp electrothermal apparatus and are uncorrected. The ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded with a 400-MHz Bruker NMR spectrophotometer with TMS as internal standard and $CDCl_3$ as solvent. The mass spectra were recorded with a Varian 1200 Triple Quadrupole instrument using electrospray ionization (ESI) technique. NMR and MS were obtained on the free base of each amine. Column chromatography was conducted using Merck silica gel, grade 9385, 230–400 mesh, 60 Å. Compound purity was determined by elemental analysis conducted by Galbraith Laboratories, (Knoxville, TN, USA). The chemical reagents used were purchased from Aldrich (St. Louis, MO, USA), Acros (Pittsburgh, PA, USA), and Alfa Aesar (Ward Hill, MA, USA).

Synthesis of intermediate 4a

A mixture of bromobenzene (5.5 mL, 52.2 mmol), magnesium turnings (3.81 g, 157 mmol), and a few iodine crystals was stirred to give Grignard reagent and was added to norcamphor (5.75 g, 52.2 mmol) according to modification of the method of Geneste *et al.* (23–25) to give a crude alcohol as a red oil (**2a**, 9.2 g, 93% yield). Treatment of this alcohol (9.0 g, 47.8 mmol) with TFA (32 mL, 430 mmol) in the presence of sodium azide (9.3 g, 143 mmol) resulted in the tertiary azide as a red oil (**3a**, 9.3 g, 91% yield). This azide (9.3 g, 40 mmol) was reduced to the corresponding amine with $LiAlH_4$ (2.5 g, 67 mmol) to give 2-phenylbicyclo[2.2.1]heptan-2-amine (**4a**) as a clear oil (7.8 g, 90% yield). This oil (0.2 g) was purified by preparative TLC developed using a mixture of chloroform and diethyl ether (9:1, v/v) as mobile phase. A pale yellow oil was obtained which solidified at 0 °C. ^{1}H NMR ($CDCl_3$): δ ppm 7.3–7.4 (m, 5H), 1.0–2.6 (b, 10 H, $4CH_2$ and 2CH). ^{13}C NMR ($CDCl_3$): δ 148.3, 128.6, 127.0, 126.4, 64.1, 48.5, 45.2, 37.1, 36.9, 28.8, 24.8. MS (ESI+) *m/z*: 188 (10%), [M+H], 171 (100%), [M-16]. The amine hydrochloride salt was obtained by bubbling hydrogen chloride gas through the ethyl ether solution. The solvent used for crystallization was the mixture of methanol and ethyl ether. Anal. Calcd. for compound **4a** hydrochloride, $C_{13}H_{18}ClN\cdot0.1H_2O$: C, 69.23; H, 8.13; N, 6.21. Found: C, 69.22; H, 8.11; N, 6.10. m.p. 243–244 °C (26).

Synthesis of intermediate 4b

Crude alcohol **2b** (9.4 g, 99% yield) was obtained from p-bromofluorobenzene (5.00 mL, 45.5 mmol), magnesium turnings (3.32 g, 137 mmol), and norcamphor (5.00 g, 45.5 mmol) as described for synthesis of compound **2a**. ^{1}H NMR ($CDCl_3$): δ ppm 7.63–7.43 (m,



Scheme 1: Syntheses of target compounds **5a–5f** (a) Mg, I_2, THF; (b) NaN_3, TFA, $CHCl_3$; (c) $LiAlH_4$, THF; (d) TEA, Et-OH.

2H), 7.15–6.96 (m, 2H), 2.58 (s, 1H), 2.44–2.11 (m, 3H), 1.81–1.26 (m, 7H); ^{13}C NMR ($CDCl_3$): δ ppm 162.8, 144.89, 127.7, 127.6, 114.9, 114.7, 80.45, 47.6, 46.9, 38.8, 37.6, 29.1, 22.28. Treatment of the alcohol (9.4 g, 46 mmol) with TFA (30.5 mL, 410 mmol) in the presence of sodium azide (8.89 g, 137 mmol) resulted in the tertiary azide as a red oil (**3b**, 10.5 g, 93.6% yield). This azide (10.5 g, 45.4 mmol) was then reduced to the corresponding amine with $LiAlH_4$ (2.60 g, 68 mmol) to give 2-(4-fluorophenyl)bicyclo[2.2.1]heptan-2-amine (**4b**) as a clear oil (6.6 g, 70.8% yield). This oil (0.4 g) was purified by preparative TLC developed using a mixture of chloroform and diethyl ether (9:1, v/v) as mobile phase. A pale yellow oil was obtained which solidified at 0 °C. ^{1}H NMR ($CDCl_3$): δ ppm 7.2 (m, 2H), 6.8 (m, 2H), 1.0–2.6 (b, 10 H, $4CH_2$ and 2CH); ^{13}C NMR ($CDCl_3$): δ 163.0, 160.4, 128.6, 128.5, 115.3, 115.0, 63.6, 48.7, 45.6, 37.1, 36.8, 28.7, 24.7; ^{19}F NMR ($CDCl_3$): δ -117.6 ppm. MS (ESI+) *m/z*: 206 (10%), [M+H], 189 (100%), [M-16]. The amine hydrochloride salt was obtained as described for compound **4a**. Anal. Calcd. for compound **4b** hydrochloride, $C_{13}H_{17}ClFN$: C, 64.59; H, 7.09; F, 7.86; N, 5.79. Found: C, 64.30; H, 7.29; F, 7.56; N, 5.62. m.p. 214–216 °C (26).

General procedure for syntheses of compounds 5a–5f

The crude base of compound **4**, 2-phenylbicyclo[2.2.1] heptan-2-amine or 2-(4-fluoro-phenyl)-bicyclo[2.2.1]hept-2-ylamine (5.4 mmol) and appropriate alkyl halides derivatives (5.6 mmol) dissolved in ethanol (30 mL) and triethylamine (1 mL, 7.1 mmol) was stirred and the mixture heated at 50 °C for 20–48 h. After cooling, the solvent was removed under reduced pressure and the residue was treated with water. The mixture was extracted with ethyl acetate (3 × 20 mL). Organic phase extracts were combined, dried over sodium sulfate, and purified by column chromatography. Hydrochloride salt was obtained as described for compound **4a**. Fumarate salt was obtained using equal molar amounts of fumaric acid and compound in methanol. The solvent used for crystallization was the mixture of methanol and ethyl ether.

2-Phenyl-N-(2-(piperidin-1-yl) ethyl)bicyclo[2.2.1]heptan-2-amine (5a)

The compound was prepared from **4a** (1.25 g, 6.7 mmol) and 1-(2-chloroethyl)piperidine hydrochloride (1.2 g, 8.1 mmol) according to the general procedure and was purified by column chromatography (EtOAc/i-prOH/TEA 8:2:0.2) to give **5a** (0.36 g, 18% yield) as colorless crystals: m.p. 32–34 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.37–7.27 (m, 4H), 7.22–7.16 (m, 1H), 2.54 (d, J = 3.82 Hz, 1H), 2.36 (s, 1H), 2.31–2.02 (m, 9H), 1.80 (m, 3H), 1.53–1.27 (m, 9H), 1.15–1.00 (m, 2H); ^{13}C (400 MHz, $CDCl_3$) δ ppm 144.75, 128.06, 127.63, 125.70, 68.00, 58.48, 54.24, 45.80, 41.78, 38.84, 36.95, 36.84, 29.23, 26.09, 24.55, 24.45. MS (ESI+) *m/z*: 299 (100%), [M+H]. Anal. Calcd. for $C_{20}H_{30}N_2$: C, 80.48; H, 10.13; N, 9.39. Found: C, 80.70; H, 10.20; N, 9.15. HCl salt was prepared, colorless crystals, m.p. 202–204 °C.

2-(4-Fluorophenyl-N-(2-(piperidin-1-yl) ethyl)bicyclo[2.2.1]heptan-2-amine (5b)

The compound was prepared from **4b** (1.11 g, 5.4 mmol) and 1-(2-chloroethyl)piperidine hydrochloride (1.10 g, 5.6 mmol) according to the general procedure and was purified by column chromatography (EtOAc/i-prOH/TEA 8:2:0.1) to give **5b** (0.43 g, 25% yield) as colorless crystals: m.p 66–68 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.03–6.96 (m, 2H), 7.34–7.28 (m, 2H), 2.51 (d, J = 4.06 Hz, 1H), 2.36 (s, 1H), 2.30–2.02 (m, 9H), 1.88–1.67 (m, 3H), 1.55–1.26 (m, 9H), 1.12–0.98 (m, 2H); ^{13}C (400 MHz, $CDCl_3$) δ ppm 162.24, 140.59, 129.56, 129.48, 114.40, 114.19, 67.55, 58.42, 54.28, 46.03, 42.08, 38.81, 37.01, 36.86, 29.15, 26.09, 24.52, 24.40; ^{19}F (400 MHz, $CDCl_3$) δ ppm -117.75; MS (ESI+) *m/z*: 317 (100%), [M+H]. Anal. Calcd. for $C_{20}H_{29}FN_2$: C, 75.91; H, 9.24; N, 8.85. Found: C, 75.80; H, 8.86; N, 8.65. HCl salt was prepared, colorless crystals, m.p. 185–187 °C.

N-(2-morpholinoethyl)-2-phenylbicyclo [2.2.1] heptan-2-amine (5c)

The compound was prepared from **4c** (0.84 g, 4.5 mmol) and 4-(2-chloroethyl) morpholine hydrochloride (0.91 g, 4.91 mmol) according to the general procedure and was purified by column chromatography (EtOAc/i-prOH/TEA 7:3:0.1) to give **5c** (0.22 g, 16% yield) as colorless crystals: m.p. 29–31 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.31 (dq, J = 8.20, 7.90 Hz, 4H), 7.22–7.15 (m, 1H), 3.59 (td, J = 20.60, 4.55 Hz, 4H), 2.56 (d, J = 3.27 Hz, 1H), 2.36 (s, 1H), 2.33–2.17 (m, 4H), 2.15–2.02 (m, 5H), 1.92–1.74 (m, 3H), 1.53–1.34 (m, 2H), 1.30 (d, J = 9.53 Hz, 1H), 1.18–0.98 (m, 2H); ^{13}C (400 MHz, $CDCl_3$) δ ppm 144.55, 128.00, 127.69, 125.89, 68.07, 67.02, 57.90, 53.13, 45.45, 41.71, 38.07, 36.90, 36.83, 29.29, 24.43; MS (ESI+) *m/z*: 301 (80%), [M+H]. Anal. Calcd. for $C_{19}H_{28}N_2O$: C, 75.96; H, 9.39; N, 9.32. Found: C, 75.71; H, 9.29; N, 9.33. HCl salt was prepared, colorless crystals, m.p. 197–199 °C.

2-(4-Fluorophenyl)-N-(2-morpholinoethyl)bicyclo [2.2.1]heptan-2-amine (5d)

The compound was prepared from **4d** (0.75 g, 3.66 mmol) and 4-(2-chloroethyl) morpholine hydrochloride (0.75 g, 4.0 mmol) according to the general procedure and was purified by column chromatography (EtOAc/i-prOH/TEA 7:3:0.2) to give **5d** (0.16 g, 14% yield) as colorless crystals: m.p. 47–49 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.35–7.26 (m, 2H), 7.09–6.95 (m, 2H), 3.70–3.55 (m, 4H), 2.51 (d, J = 3.60 Hz, 1H), 2.40–2.02 (m, 10H), 1.90–1.61 (m, 3H), 1.55–1.27 (m, 3H), 1.15–0.94 (m, 2H); ^{13}C (400 MHz, $CDCl_3$) δ ppm 162.31, 140.56, 129.51, 129.43, 114.46, 114.25, 67.56, 67.00, 58.01, 53.26, 45.81, 42.06, 38.12, 36.94, 36.85, 29.19, 24.38; ^{19}F (400 MHz, $CDCl_3$) δ ppm -117.45; MS (ESI+) *m/z*: 319 (80%), [M+H]. Anal. Calcd. for $C_{19}H_{27}FN_2O$: C, 71.66; F, 5.96; H, 8.53; N, 8.79. Found: C, 71.54; F, 5.81; H, 8.13; N, 8.76. Hydrogen fumarate salt was prepared, white crystal, m.p. 152–154 °C.

2-Phenyl-N-(2-(pyrrolidin-1-yl) ethyl) bicyclo[2.2.1]heptan-2-amine (5e)

The compound was prepared from **4e** (1.83 g, 9.82 mmol) and 1-(2-chloroethyl) pyrrolidine hydrochloride (1.70 g, 10 mmol) according to the general procedure and was purified by column chromatography ($CHCl_3$/i-prOH/TEA 7:3:0.5) to give **5e** (0.64 g, 23% yield) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.43–7.27 (m, 4H), 7.27–7.14 (m, 1H), 2.55 (s, 1H), 2.49–2.18 (m, 9H), 2.10 (dd, J = 9.44 Hz,

1H), 1.94–1.63 (m, 7H), 1.53–1.25 (m, 3H), 1.17–0.99 (m, 2H); MS (ESI+) *m/z*: 285 (100%), [M+H]. Anal. calcd. for $C_{19}H_{28}N_2$-0.1$CHCl_3$: C, 77.40; H, 9.55; N, 9.45. Found: C, 77.43; H, 9.33; N, 9.82. HCl salt was prepared, colorless crystal, m.p. 180–182 °C.

2-(4-Fluorophenyl-N-(2-(pyrrolidin-1-yl) ethyl) bicyclo[2.2.1]heptan-2-amine (5f)

The compound was prepared from **4f** (1.80 g, 8.78 mmol) and 1-(2-chloroethyl) pyrrolidine hydrochloride (1.63 g, 9.58 mmol) according to the general procedure and was purified by column chromatography ($CHCl_3$/i-prOH/TEA 6.5:3:0.5) to give **5f** (0.28 g, 11% yield) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.37–7.26 (m, 2H), 7.08–6.95 (m, 2H), 2.51 (d, J = 3.83 Hz, 1H), 2.47–2.16 (m, 8H), 2.06 (dd, J = 6.07 Hz, 1H), 1.89–1.78 (m, 2H), 1.76–1.60 (m, 5H), 1.53–1.26 (m, 4H), 1.05 (td, J = 12.13, 6.58 Hz, 2H); MS (ESI+) *m/z*: 303 (100%), [M+H]. Hydrogen fumarate salt was prepared, white crystals, m.p. 118–120 °C. Anal. calcd. for fumarate salt; $C_{23}H_{31}FN_2O_4$-H_2O: C, 63.28; F, 4.35; H, 7.61; N, 6.41. Found: C, 62.92; F, 4.29; H, 7.40; N, 6.36.

In vitro *biological studies*

Receptor binding studies

To evaluate *in vitro* affinities of the compounds at the PCP site of the NMDA receptor complex, radioligand binding studies were conducted in accordance with published protocol by Reynolds and Sharma (27). Thoroughly washed rat forebrain homogenate was used as receptor source [whole brain obtained from Pel-Freez Biologicals, forebrain tissue preparations prepared as in Reynolds and Sharma (27)].

Suspensions of 10 mM HEPES (pH 7.4 at room temperature) containing 30 μg/mL protein, 1 nM (+)-[^{3}H]MK-801, 100 μM glutamate, 30 μM glycine, and various concentrations of unlabeled competitor or 30 μM (+)-MK-801 for non-specific binding, were incubated at room temperature for 2 h. Termination of reaction was performed via vacuum filtration using a 24-well cell harvester (Brandel, Gaithersburg, MD, USA) over GF/B glass fiber filters (Brandel). Filters were washed with three 5-mL aliquots of assay buffer. Radioactivity trapped on filters was measured via liquid scintillation counting, using a Beckman LS 6500 multipurpose scintillation counter (Beckman Coulter, Brea, CA, USA) at 64% efficiency.

IC_{50} values were determined with GraphPad (GraphPad Software, La Jolla, CA, USA) using log-concentration plotted against percent specific binding. Percent specific binding for [^{3}H]-MK-801 in control experiment was 70% of total. K_i values were calculated using the equation of Cheng and Prusoff (28). The K_d for (+)-MK-801 binding under the saturation conditions was 1.747 nM and this is consistent with that reported in literature (27). The K_d of (+)-MK-801 was determined via homologous binding assay as described by Reynolds and Sharma. The protein concentration was determined by the method of Bradford (29) using coomassie protein assay reagent.

The compounds were evaluated through the National Institute of Mental Health (NIMH) Psychoactive Drug Screening Program (PDSP), National Institutes of Health (NIH). Compounds evaluated by this program are typically subjected to a 'primary assay' designed to identify receptors, transporters, and ion channels for which the compounds display affinity. Compounds found active in the primary screening (>10 000 nM) are subjected to a secondary screen where affinity (K_i) is calculated. Experimental details and procedure can be found through Assay Protocol Book, National Institute of Mental Health Psychoactive Drug Screening Program, and University of North Carolina at Chapel Hill[a].

In vivo *biological studies*

Maximal electroshock (MES) test

The MES test is a model for generalized tonic–clonic seizures and provides an indication of a compound's ability to prevent seizure spread when all neuronal circuits in the brain are maximally active. These seizures are highly reproducible and are electrophysiologically consistent with human seizures. For this test, 60 Hz of alternating current (50 mA in mice, 150 mA in rats) is delivered for 0.2 seconds by corneal electrodes which have been primed with an electrolyte solution containing an anesthetic agent (0.5% tetracaine HCl). In Test 1, mice are evaluated at various intervals following doses of 30, 100, and 300 mg/kg of test compound given by i.p. injection of a volume of 0.01 mL/g. In Test 2, rats are tested after a dose of 30 mg/kg (p.o.) in a volume of 0.04 mL/10 g. Final test uses varying doses administered via i.p. injection, again in a volume of 0.04 mL/10 g. An animal is considered 'protected' from MES-induced seizures upon abolition of the hindlimb tonic extensor component of the seizure (30–32).

Subcutaneous Metrazol seizure threshold test (scMET)

Subcutaneous injection of the convulsant Metrazol produces clonic seizures in laboratory animals. The scMET test detects the ability of a test compound to raise the seizure threshold of an animal and thus protect it from exhibiting clonic seizure. Animals are pretreated with various doses of the test compound (in a similar manner to MES test, although a dose of 50 mg/kg (p.o.) is the standard for Test 2 scMET). At the previously determined therapeutic plasma exchange (TPE) of the test compound, the dose of Metrazol which will induce convulsions in 97% of animals (CD_{97}: 85 mg/kg mice; 70 mg/kg rats) is injected into a loose fold of skin in the midline of the neck. The animals are placed in isolation cages to minimize stress (33) and observed for the next 30 min for the presence or absence of seizure. An episode of clonic spasms, approximately 3–5 seconds, of the fore and/or hindlimbs, jaws or vibrissae is taken as the end-point. Animals that do not meet this criterion are considered protected.

Acute toxicity-minimal motor impairment (MMI)

To assess a compound's undesirable side effects (toxicity), animals are monitored for overt signs of impaired neurological or muscular function. In mice, the rotorod (34) procedure is used to evaluate such impairment. When a mouse is placed on a rod that rotates at a speed of 6 rpm, the animal can maintain its

equilibrium for long periods of time. The compound is considered toxic if the animal falls off this rotating rod three times during a 1-min period. In rats, minimal motor deficit is indicated by ataxia, which is manifested by an abnormal, uncoordinated gait. Rats used for evaluating toxicity are examined before the test drug is administered, as individual animals may have peculiarities in gait, equilibrium, placing response, among others which might be attributed erroneously to the test substance. In addition to MMI, animals may exhibit a circular or zigzag gait, abnormal body posture and spread the legs, tremors, hyperactivity, lack of exploratory behavior, somnolence, stupor, catalepsy, loss of placing response and changes in muscle tone.

Results and Discussion

Our studies examined design, syntheses, and pharmacological evaluations of novel, non-competitive, NMDAR antagonists as potential therapies for neurodegenerative diseases (35,36). The syntheses of N-substitued bicyclo-heptan-2-amines (**5a–5f**) were carried out starting from commercially available norcamphor and a phenyl Grignard reagent. Phenyl magnesium bromides were added to norcamphor **1** to give alcohols **2a** and **2b**. This addition has been reported to give exo-phenyl tertiary alcohols (37). Treatment of the resulting tertiary alcohol with trifluoroacetic acid (TFA) in the presence of sodium azide resulted in the tertiary azide, which was then reduced to the corresponding amine with lithium aluminum hydride ($LiAlH_4$) to give intermediates **4**. Treatment of these amines with alkyl halides in the presence of ethanol and triethylamine completed syntheses of the target compounds (Scheme 1).

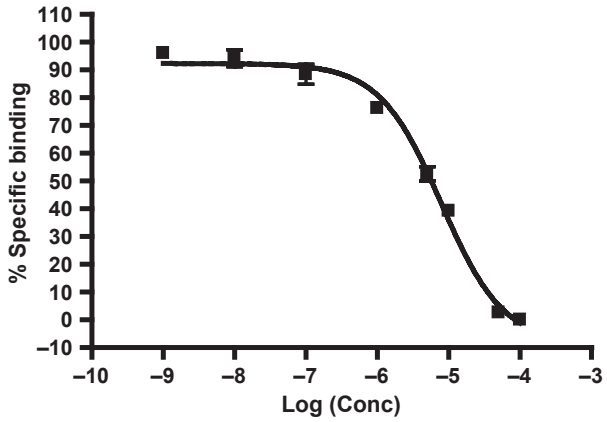


Figure 1: Concentration % specific binding curve for compound **5a**.

Radioligand binding studies were utilized to evaluate *in vitro* affinities of the target compounds at the PCP site of the NMDA receptor complex. A representative plot from which the IC_{50} values were extracted is depicted in Figure 1. All 8 novel amines (**4a**, **4b**, **5a–f**) at 10 000 nM were screened for % inhibition of 1 nM [^{3}H] (+)-MK-801. As compounds with moderate NMDAR activity were desired, only those possessing greater than 90% inhibition at 10 μM were subjected to further analysis. The IC_{50} and K_i values were calculated for the 4 compounds (**4a**, **5a**, **5e**, and **5f**) with greater than 90% inhibition. As depicted in Table 1, the binding affinities of the compounds are comparable, though lower than that of (+)-MK-801.

Each compound was evaluated for affinity at human or rat receptors through the NIMH-PDSP program. *In vitro* binding affinities of these novel NMDAR antagonists at Serotonin $_{2A}$ (5-HT_{2A}), Dopamine 1 (D_1), Dopamine 2 (D_2), the dopamine transporter (DAT), kappa opioid receptor (KOR), μ-opioid receptors (MOR), Norepinephrine transporter (NET), Serotonin transporter (SERT), Sigma-1, and Sigma-2 receptors are shown in Table 2. The receptors evaluated, radiolabeled ligand, and receptor source are depicted in Table 3. The compounds lacked significant affinity for the majority of receptors screened. However, many of the compounds possessed low affinity at KOR and the DAT. In almost all cases these affinities were

Table 1: The IC_{50} and K_i for the four compounds subjected to further analysis and (+)-MK-801 run under experimental condition as reference

Compound	IC_{50}	N-methyl-D-aspartate affinity K_i
(+)-MK-801	2.02 ± 0 .797 nM	1.357 nM
4a	13.27 ± 0.63 μM	8.45 μM
5a	7.86 ± 1.64 μM	5.28 μM
5e	8.48 ± 3.106 μM	5.67 μM
5f	8.657 ± 2.564 μM	5.82 μM

Table 2: *In vitro* binding affinities of novel *N*-methyl-D-aspartate receptor antagonists for 5-HT_{2A}, D_1, D_2, DAT, KOR, MOR, NET, SERT, Sigma-1, and Sigma-2 receptors

Compound	5HT2A (nM)	D1 (nM)	D2 (nM)	DAT (nM)	KOR (nM)	MOR (nM)	NET (nM)	SERT (nM)	Sigma 1 (nM)	Sigma 2 (nM)
4a	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000	2201	995
4b	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000
5a	2643	>10 000	>10 000	>10 000	876 nM	>10 000	>10 000	>10 000	>10 000	>10 000
5b	8822	>10 000	>10 000	1231	3546	>10 000	>10 000	>10 000	>10 000	>10 000
5c	>10 000	>10 000	>10 000	2570	988	>10 000	>10 000	>10 000	>10 000	>10 000
5d	>10 000	>10 000	>10 000	6482	>10 000	>10 000	>10 000	>10 000	>10 000	>10 000
5e	>10 000	>10 000	>10 000	3624	4507	>10 000	>10 000	>10 000	>10 000	>10 000
5f	>10 000	>10 000	>10 000	226	2111	>10 000	>10 000	4607	>10 000	>10 000

Table 3: Novel *N*-methyl-D-aspartate receptor antagonists were screened for the following receptors, and sources

Receptor	Source	Hot ligand
Serotonin$_{2A}$ (5-HT$_{2A}$)	Human cloned	Ketanserin
Dopamine 1 (D1)	Human cloned	SCH23390
Dopamine 1(D2)	Human cloned	N-methylspiperone
Dopamine transporter (DAT)	Human cloned	WIN35428
κ-opioid receptors (KOR)	Rat cloned	U69593 (2007-07-27)
μ-opioid receptors (MOR)	Human cloned	DAMGO (2007-07-27)
Norepinephrine transporter (NET)	Human cloned	Nisoxetine
Serotonin transporter (SERT)	Human cloned	Citalopram
Sigma$_1$	Rat brain	Pentazocine(+)
Sigma$_2$	Rat C12	DTG

moderate at best. A notable exception is compound **5f**, which showed significant affinity (226 nM) at DAT (Table 2).

The novel compounds also were accepted into the Anticonvulsant Screening Program (ASP), National Institute of Neurological Disorders and stroke (NINDS). Their anticonvulsant activities were evaluated by MES test and scMET, and their neurotoxicities were measured by the rotorod test. These tests showcase the ability of the compound to enter the CNS and infer NMDA receptor antagonism. Compound **5a** exhibited the highest affinity for NMDA and greatest degree of protection from MES-induced neural damage and death. However, compounds **5b**, **5c**, and **5d** which did not exhibit significant affinity also displayed MES neuroprotective activity (Tables 4 and 5). This could be explained by several factors one of which may be the high doses tested *in vivo* may have made up for the low affinity. Other possibilities are differences between *in vitro* versus *in vivo* characteristics and/or bioavailabilities of the compounds in the brain. Yet another possibility is that the compounds displaying minimal NMDA affinity and significant MES protection may in fact be prodrugs and thus mediate their NMDA effects through active metabolites. A slightly similar case is known with the antitussive agent dextromethorphan (DXM) which itself has low activity at NMDA, whereas one of its metabolites, dextrophan (DX), has μM affinity (38,39). It has been speculated that some of the therapeutic effects of DXM are mediated by DX (40). DXM has been found to have neuroprotective activity in the MES test.

Toxicity was observed for every compound at ≥100 mg/kg. Severe mortality (either 3/4 or 4/4) was also noted for all these molecules in the TOX evaluation at the higher dose of 300 mg/kg. Other comments on these novel NMDAR antagonists are displayed on Table 5.

Conclusions and Future Directions

All target compounds were synthesized in hundred milligram quantities showing feasibility of the synthetic scheme. They were all protective in the MES test at the dose of 100 mg/kg except for compound **5e.** None of the compounds showed protection in the scMET model. When tested in rats, **5b** and **5d** did not display any protection or toxicity at the dose of 30 mg/kg. None of the target compounds exhibited significant toxicity at 30 mg/kg based on the rotorod TOX assessment. At 100 mg/kg, several test compounds exhibited toxicity and at 300 mg/kg all compounds exhibited toxicity (Table 4).

Non-competitive low-affinity NMDA antagonists have received attention as a means of reducing the intolerable side effects of higher-affinity NMDA antagonists while still retaining the therapeutic profile (17,41,42). Thus, compound **5a** may be worthy of further investigation as well as serve as a good lead for the discovery of more suitable compounds.

Table 4: The MES, scMET and rotorod TOX assessments of novel *N*-methyl-D-aspartate receptor antagonists

Compound	Time (h)	MES (30) N/F	MES (100) N/F	MES (300) N/F	6 Hz (50)	scMET (30) N/F	scMET (100) N/F	scMET (300) N/F	TOX (30) N/F	TOX (65) N/F	TOX (100) N/F	TOX (300) N/F
4a	0.5	0/1	0/0			0/1	0/1		0/4		8/8	4/4
	4.0	0/1	1/1			0/1	0/1		0/2		0/2	/
4b	0.5	0/1	3/3			0/1	0/1		0/4		8/8	4/4
	4.0	0/1	0/3			0/1	0/1		0/2		0/4	/
5a	0.5	0/1	3/3			0/1	0/1		0/4		8/8	4/4
	4.0	0/1	1/3			0/1	0/1		0/2		0/4	/
5b	0.5	0/1	2/3		0/4	0/1	0/1		0/4	2/4	3/8	4/4
	4.0	0/1	0/3		2/4	0/1	0/1		0/2	1/4	0/4	/
5c	0.5	0/1	2/3			0/1	0/1	0/1	0/4		6/8	4/4
	4.0	0/1	0/3			0/1	0/1	0/0	0/2		0/4	1/1
5d	0.5	0/1	2/3	1/1	1/4	0/1	0/1		0/4	0/4	1/8	4/4
	4.0	0/1	0/3	0/0	1/4	0/1	0/1		0/2	0/4	0/4	/
5e	0.5	0/1	0/3			0/1	0/1	0/1	0/4		0/8	4/4
	4.0	0/1	0/3			0/1	0/1	0/0	0/2		0/4	/
5f	0.5	0/1	1/3			0/1	0/1		0/4		5/8	4/4
	4.0	0/1	0/1			0/1	0/1		0/2		0/4	/

Doses are in () and are mg/kg.
N/F, number of the animals active or toxic over the number tested.

Table 5: Other comments on the novel *N*-methyl-D-aspartate receptor antagonists

Compound	Test	Dose (mg/kg)	Time (h)	Comments
4a	TOX	100	0.5	Severe tremors, death following clonic seizure
	TOX	300	0.5	Death
4b	TOX	100	0.5	Severe tremors, clonic seizures
	TOX	300	0.5	Death following clonic seizure
5a	TOX	100	0.5	Clonic seizures
	TOX	300	0.5	Death
5b	TOX	50	0.25	Clonic seizures
	TOX	100	0.5	Clonic seizures
	TOX	300	0.5	Death
5c	TOX	100	0.5	Clonic seizures
	TOX	300	0.5	Death following clonic seizure
	TOX	300	0.5	Clonic seizures
	TOX	300	4	Death
5d	TOX	65	0.25	Clonic seizures
	TOX	100	0.5	Clonic seizures
	TOX	300	0.5	Loss of righting reflex
	TOX	300	0.5	Death
5e	TOX	300	0.5	Diarrhea
	TOX	300	0.5	Unable to grasp rotorod
	TOX	300	0.5	Death
5f	TOX	100	0.5	Clonic seizures
	TOX	300	0.5	Death

Acknowledgments

This work was supported by NIH grant 7R15NS36393-04; Anticonvulsant Screening Program, NINDS; and Psychoactive Drug Screening Program, NIMH.

References

1. Madden D.R. (2002) The structure and function of glutamate receptor ion channels. Nat Rev Neurosci;3:91–101.
2. Dingledine R., Borges K., Bowie D., Traynelis S.F. (1999) The glutamate receptor ion channels. Pharmacol Rev;51:7–61.
3. Liu Y., Zhang J. (2000) Recent development in NMDA receptors. Chin Med J;113:948–956.
4. Cull-Candy S., Brickley S., Farrant M. (2001) NMDA receptor subunits: diversity, development and disease. Curr Opin Neurobiol;11:327–335.
5. Schiller J., Schiller Y., Clapham D.E. (1998) NMDA receptors amplify calcium influx into dendritic spines during associative pre- and postsynaptic activation. Nat Neurosci;1:114–118.
6. Yuste R., Majewska A., Cash S.S., Denk W. (1999) Mechanisms of calcium influx into hippocampal spines: heterogeneity among spines, coincidence detection by NMDA receptors, and optical quantal analysis. J Neurosci;196:1976–1987.
7. Iversen L.L., Iversen S.D., Bloom F.E., Roth R.H. (2009) Introduction to Neuropsychopharmacology. Oxford, UK: Oxford University Press; p. 93.
8. Javitt D.C., Zukin S.R. (1989) Biexponential kinetics of [3H]MK-801 binding: evidence for access to closed and open *N*-methyl-D-aspartate receptor channels. Mol Pharmacol;35:387–393.
9. Cacabelos R., Takeda M., Winblad B. (1999) The glutamatergic system and neurodegeneration in dementia: preventive strategies in Alzheimer's disease. Int J Geriatr Psychiatry;14:3–47.
10. Raymond L.A. (2003) Excitotoxicity in Huntington disease. Clin Neurosci Res;3:121–128.
11. Fan M.M.Y., Raymond L.A. (2007) N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. Prog Neurobiol;81:272–293.
12. Parsons C.G., Danysz W., Quack G. (1998) Glutamate in CNS disorders as a target for drug development: an update. Drug News Perspect;11:523–569.
13. Mody I., MacDonald J.F. (1995) NMDA receptor-dependent excitotoxicity: the role of intracellular Ca^{2+}release. Trends Pharmacol Sci;16:356–359.
14. Sattler R., Tymianski M. (2000) Molecular mechanisms of calcium-dependent excitotoxicity. J Mol Med;78:3–13.
15. Rothman S.M., Olney J.W. (1987) Excitotoxicity and the NMDA receptor. Trends Neurosci;10:299–302.
16. Chen H.-S.V., Lipton S.A. (2006) The chemical biology of clinically tolerated NMDA receptor antagonists. J Neurochem;97:1611–1626.
17. Geter-Douglass B., Witkim J.M. (1999) Behavioral effects and anticonvulsant efficacies of low-affinity, uncompetitive NMDA antagonists in mice. Psychopharmacology (Berl);146:280–289.
18. Rogawski M.A. (2000) Low affinity channel blocking (uncompetitive) NMDA receptor antagonists as therapeutic agents – toward an understanding of their favorable tolerability. Amino Acids;19:133–149.
19. Lees K.R., Dyker A.G., Sharma A., Ford G.A., Ardron M.E., Grosset D.G. (2001) Tolerability of the low-affinity, use-dependent NMDA antagonist AR-R15896AR in stroke patients: a dose-ranging study. Stroke;32:466–472.
20. Lipton S.A. (2006) Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. Nat Rev Drug Discov;5:160–170.
21. Lipton S.A. (2004) Failures and successes of NMDA receptor antagonists: molecular basis for the use of open-channel blockers like memantine in the treatment of acute and chronic neurologic insults. NeuroRX;1:101–110.
22. Parsons C.G., Danysz W., Quack G. (1999) Memantine is a clinically well tolerated *N*-methyl-D-aspartate (NMDA) receptor antagonist – a review of preclinical data. Neuropharmacology;38:735–767.
23. Thurkauf A., de Costa B., Yamaguchi S., Mattson M.V., Jacobson A.E., Rice K.C., Rogawski M.A. (1990) Synthesis and anticonvulsant activity of 1-phenylcyclohexylamine analogues. J Med Chem;33:1452–1458.
24. Geneste P., Herrmann P., Kamenka J.M., Pons A. (1975) New synthesis of isomers of 1-phenylcyclohexylamines substituted on the cyclohexane ring. Bull Soc Chim Fr;7–8:1619–1626.
25. de Costa B.R., Mattson M.V., George C., Linders J.T. (1992) Synthesis, Configuration, and Activity of Isomeric 2-Ph enyl-2-(N-piperidinylb) icycle[3.I.Olhexanes at Phencyclidine and *u* Binding Sites. J Med Chem;35:4704–4712.
26. Adejare A. (2007) Non-competitive NMDA receptor antagonists. PCT Int Appl WO 2007149508 A2 20071227. Language: English, Database: CAPLUS.

27. Reynolds I.J., Sharma T.A. (1999) The use of ligand binding in assays of NMDA receptor function. In: Li M, editor. Methods in Molecular Biology, NMDA receptor protocols. Totowa, NJ, Humana Press; Vol. 128, p. 93–102.
28. Cheng Y., Prusoff W.H. (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol;22:3099–3108.
29. Bradford M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem;72:248–254.
30. Swinyard E.A., Woodhead J.H., White H.S., Franklin M.R. (1989) General principles: experimental selection, quantification, and evaluation of anticonvulsant. In: Levy R.H., Mattson R.H., Melrum B., Penry J.K., Dreifussed F.E., editors. Antiepileptic Drugs. NewYork: Raven Press; p. 85–102.
31. White H.S., Johnson M., Wolf H.H., Kupferberg H.J. (1995) The early identification of anticonvulsant activity: role of the maximal electroshock and subcutaneous entylenetetrazol seizure models. Ital J Neurol Sci;16:73–77.
32. White H.S., Woodhead J.H., Franklin M.R. (1995) General principles: experimental selection, quantification, and evaluation of antiepileptic drugs. In: Levy R.H., Mattson R.H., Melrum B.S., editors. Antiepileptic Drugs. NewYork: Raven Press; p. 99–110.
33. Swinyard E.A., Clark L.D., Miyahara J.T., Wolf H.H. (1961) Studies on the mechanism of amphetamine toxicity in aggregated mice. J Physiol;132:97–102.
34. Dunham M.S., Miya T.A. (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc Am Pharm Assoc (Baltim);46:208–209.
35. Ogunbadeniyi A.M., Adejare A. (2002) Syntheses of fluorinated phencyclidine analogs. J Fluor Chem;114:39.
36. Sun S. (2007) Synthesis and Noncompetitive NMDA Receptor Antagonists A dissertation, University of the Sciences in Philadelphia.
37. Macdonald D., Brideau C., Chan C.C., Falgueyret J.-P., Frenette R., Guay J., Hutchinson J.H., Perrier H., Prasit P., Riendeau D., Tagari P., Thérien M., Young R.N., Girard Y. (2008) Substituted 2,2-bisaryl-bicycloheptanes as novel and potent inhibitors of 5-lipoxygenase activating protein. Bioorg Med Chem Lett;18:2023–2027.
38. Church J., Lodge D., Berry S.C. (1985) Differential effects of dextrorphan and levorphanol on the excitation of rat spinal neurons by amino acids. Eur J Pharmacol;111:185–190.
39. Franklin P.H., Murray T.F. (1992) High affinity [^{3}H]dextrorphan binding in rat brain is localized to a noncompetitive antagonist site of the activated *N*-methyl-D-aspartate receptor-cation channel. Mol Pharmacol;41:134–146.
40. Pechnick R.N., Poland R.E. (2004) Comparison of the effects of dextromethorphan, dextrorphan, and levorphanol on the hypothalamo–pituitary–adrenal axis. J Pharmacol Exp Ther;309:515.
41. Chen H.S., Pellegrini J.W., Aggarwal S.K., Lei S.Z., Warach S., Jensen F.E., Lipton S.A. (1992) Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. J Neurosci;12:4427.
42. Naish H.J., Marsh W.L., Davies J.A. (2002) Effect of low-affinity NMDA receptor antagonists on electrical activity in mouse cortical slices. Eur J Pharmacol;443:79.

Note

[a]Roth B.L. Available at: http://pdsp.med.unc.edu/UNC-CH%20Protocol%20Book.pdf