Articles

Synthesis, Resolution, and SAR of (±)-2-Amino-*N*-methyl-α-(3-methyl-2-thienyl)benzeneethanamine and Related Analogs as Noncompetitive NMDA Antagonists with Neuroprotective Properties†

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Received December 22, 1993®

The preparation and structure–activity relationships of a series of 2-amino-α-thienylbenzeneethanamines are described. From this work, (±)-2-amino-*N*-methyl-α-(3-methyl-2-thienyl)-benzeneethanamine (**3a**) and the homologous *N*-ethyl analog **3b** emerged as novel noncompetitive NMDA antagonists with neuroprotective properties. Optical resolution of **3a** and X-ray crystallography of (+)**3a** were performed. The racemate and enantiomers were evaluated for neuroprotective properties in models of ischemia-induced hippocampal damage (gerbil) and cerebral focal ischemia (rat). Pretreatment with **3a**, (+)**3a**, or (−)**3a** significantly reduced ischemia-induced CA1 hippocampal damage. Posttreatment with **3a** afforded a lower degree of neuroprotection. A highly significant reduction in infarct volume was observed with **3a** in the cerebral focal ischemia model, with only weak positive effects being displayed by (+)**3a**. Dose-limiting side effects were associated with all three compounds in this model. In summary, the results demonstrate the utility of noncompetitive NMDA antagonists as neuroprotective agents for ischemia-induced neurodegeneration.

Introduction

The amino acids glutamic acid and aspartic acid have been characterized as major excitatory neurotransmitters in the mammalian central nervous system.[1] Effects of these amino acids are mediated by glutamate receptors, which have been classified into subtypes on the basis of studies with selective prototypical agonists such as *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainic acid (KA).[2,3] Of these subtypes, the NMDA receptor has been extensively investigated,[4,5] and modulation of NMDA receptor activity may have therapeutic potential in seizure disorders, Parkinson's disease, and memory dysfunction.[6,7] Excessive stimulation of the NMDA receptor results in a cascade of biochemical events leading to neuronal degeneration and cell death. The accumulated evidence has led to the proposal of the "excitotoxic hypothesis" of neuronal degeneration, which suggests there is an intimate association between the overactivity at the NMDA receptor and the brain damage that occurs in situations leading to cerebral ischemia (e.g., heart attack, stroke, drowning, and possibly Alzheimer's disease[8–10]). Competitive and noncompetitive NMDA antagonists display neuroprotective properties in models of hypoxia- or ischemia-induced neuronal cell death and thus may be useful for the treatment or prevention of brain damage associated with cerebral ischemia.[9]

As part of a program to further explore the therapeutic potential of compounds related to the 1,3-benzodiazepine antidepressant dazepinil,[11,12] a series of 2-amino-α-thienylbenzeneethanamines was synthesized.[13] The excellent antiseizure properties of (±)-2-amino-*N*-methyl-α-(3-methyl-2-thienyl)benzeneethanamine (**3a**) prompted broader CNS screening. From this work, **3a** (P7189, formerly 7189) and the homologous *N*-ethyl analog **3b** (P8319, formerly 8319) emerged as novel noncompetitive NMDA antagonists with anticonvulsant, anxiolytic, and neuroprotective properties.[14–16] Compound **3a** was selected for further assessment of neuroprotective properties. The synthesis, optical resolution, and SAR of **3a** and related compounds as noncompetitive NMDA antagonists with neuroprotective properties are the subjects of this paper.

Chemistry

The synthesis of **3a–n** is shown in Schemes 1–3. A convenient, versatile route to most of the desired compounds involved condensing the dianion of pivaloyl amide **1** with alkylimines of substituted 2-thiophenecarboxaldehydes to provide amides **2a–e**, which were smoothly hydrolyzed to target diamines **3a–e** (Scheme 1). Treatment of **3a** with *N*-bromo- or *N*-chlorosuccinimide afforded the 5-halo analogs **3f,g**, respectively. Methylation of **3a** by reductive amination gave the tetra-*N*-methyl analog **3h**. Treatment of **3a** with ethyl formate, or an equivalent of formic anhydride generated *in situ*, provided mixtures of products from which **4a,b**

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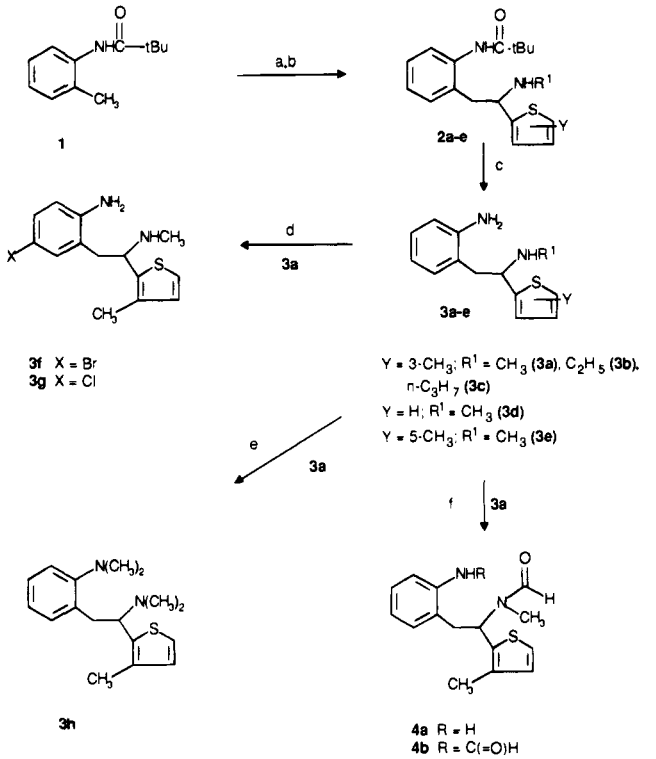
® Abstract published in *Advance ACS Abstracts*, July 15, 1994.

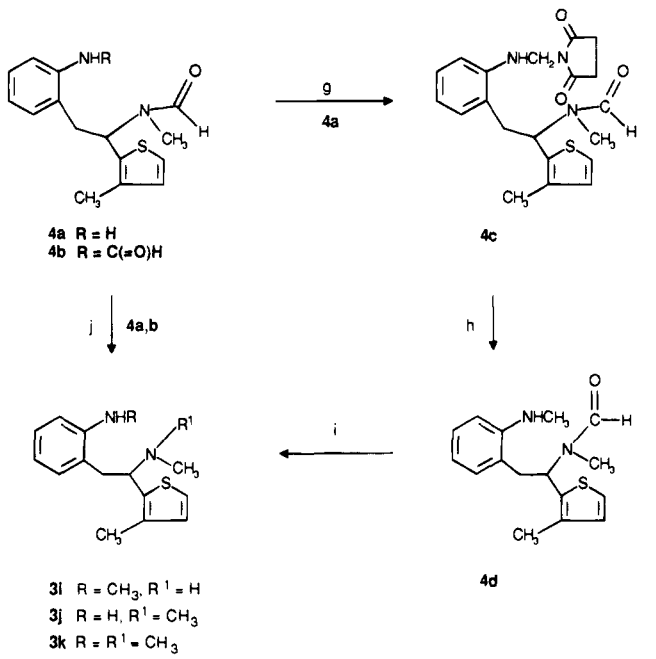
0022-2623/94/1837-3008$04.50/0

Scheme 1[a]



[a] (a) 2 *n*-BuLi; (b) 2-thienyl-CH=NR[1]; (c) 6 N HCl/reflux; (d) NBS or NCS/DMF; (e) H_2CO/$NaBH_3CN$; (f) EtOC(=O)H/reflux or HCO_2H/DCC.

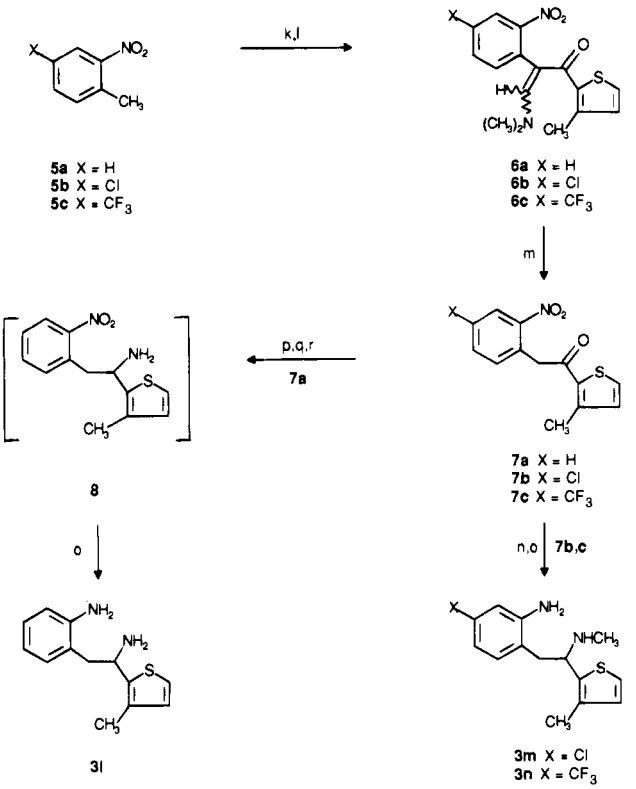
Scheme 2[a]



[a] (g) H_2CO/succinimide; (h) $NaBH_4$/DMSO; (i) 3 N HCl/reflux; (j) BH_3/THF.

were isolated by chromatography. Borane reduction of **4a,b** provided di- and trimethyl analogs **3j,k**, respectively (Scheme 2). Monomethylation of primary aromatic amines involving condensation of the amines with formaldehyde and succinimide followed by solvent specific borohydride reduction of the aminal intermediate was described by Kadin[17] and utilized here to prepare formamide **4d** from **4a**. Hydrolysis of **4d** provided the *N,N'*-dimethyl analog **3i**.

Scheme 3[a]



[a] (k) $(CH_3O)_2CHN(CH_3)_2$/DMF; (l) 3-methyl-2-thiophenecarbonyl chloride/Et_3N; (m) H_2O/dioxane/reflux; (n) CH_3NH_2/$NaBH_3CN$; (o) Fe/HCl; (p) NH_2OH; (q) Ac_2O/pyridine; (r) BH_3/THF.

Other analogs (**3l–n**) were synthesized as shown in Scheme 3. Utilizing methodology described by Garcia and Fryer,[18] 2-nitrotoluene analogs **5a–c** were condensed with *N,N*-dimethylformamide dimethyl acetal and acylated with 3-methyl-2-thiophene carbonyl chloride to afford the corresponding enamino ketones **6a–c**, which were hydrolyzed to give ketones **7a–c**, respectively. Conversion of **7a** to the oxime acetate and borane reduction provided nitro amine **8**, which was reduced chemically with iron and hydrochloric acid to provide the bis-primary amine **3l**. Reductive amination of ketones **7b,c** with methylamine and sodium cyanoborohydride followed by chemical reduction of the nitro group gave the chloro and trifluoromethyl analogs **3m,n**, respectively.

Chromatographic resolution of **3a** free base on a triacetyl cellulose column provided the enantiomers which were converted to dihydrochloride salts. Single-crystal X-ray crystallography of the (+)-enantiomer was performed and permitted assignment of the absolute configuration as *S*(+)**3a** (Figure 1).

Results and Discussion

Biological data for compounds **3a–n** are summarized in Table 1. Affinity for the competitive and noncompetitive sites associated with the NMDA receptor was determined by the ability of the compounds to displace binding by the specific ligands [^{3}H]-(±)-[3-(2-carboxypiperazin-4-yl)propyl]phosphonate ([^{3}H]CPP) and [^{3}H]-*N*-[1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]TCP), respectively. *In vivo* NMDA antagonist activity was assessed in mice by evaluation of the ability of a compound to prevent clonic seizures induced by racemic *N*-methyl-aspartic acid (NMDLA). Most of the compounds sig-

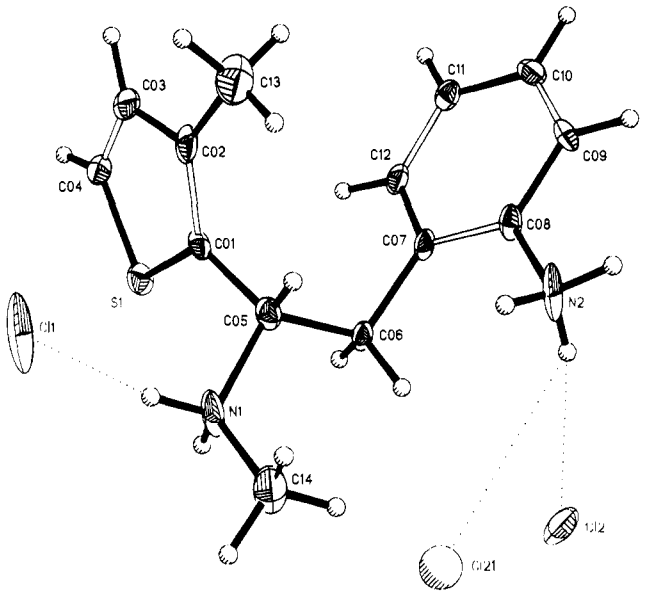


Figure 1. X-ray structure of compound (+)**3a** (thermal ellipsoids).

nificantly inhibited *in vitro* binding to the noncompetitive [^{3}H]TCP site but not to the competitive [^{3}H]CPP site (Table 1, footnote a), within the NMDA receptor complex.[19] Optimal *in vitro* inhibition of [^{3}H]TCP binding is associated with the presence of a small alkyl substituent on the α-amino group (**3a**–**c** versus **3l**) and an ortho substituent on the thiophene ring (**3a** versus **3d**,**e**). *In vivo* antagonism of NMDLA-induced seizures generally followed a similar pattern, with the exception of **3l**. Increasing the number of *N*-methyl groups did not enhance affinity *in vitro* for the [^{3}H]TCP binding site, and antiseizure activity was significantly reduced for all analogs (**3h**–**k** versus **3a**). Halogen substitution at C-5 of the benzene ring enhanced *in vitro* inhibition of [^{3}H]TCP binding (**3f**,**g** versus **3a**), but C-4 substitution significantly reduced binding affinity (**3m**,**n** versus **3a**). Compound **3n** retained *in vivo* activity.[20] Preliminary assessment of neuroprotective properties for **3a**,**b** involved evaluation of compound effects for protection against NMDA-induced hippocampal cell necrosis in rats. As shown in Table 3, pretreatment with **3a** or **3b** significantly reduced NMDA-induced hippocampal damage, as did the reference noncompetitive NMDA antagonist dizocilpine.

Since the stereoisomers of a compound can exhibit significantly different pharmacological and toxicological properties, **3a** was resolved into its enantiomers (+)**3a** and (−)**3a**. Both enantiomers inhibited *in vitro* [^{3}H]TCP binding at the NMDA receptor-associated noncompetitive site, with (+)**3a** being equipotent or slightly more potent than the racemate and 300-fold more potent than (−)**3a** (Table 1). Acute side effect liability studies indicated both **3a** and (+)**3a** were almost equipotent with respect to side effects (including ataxia and impaired motor coordination) and symptom-free in doses up to 10 mg/kg ip, whereas (−)**3a** was symptom-free in doses up to 100 mg/kg ip.[21]

Further neuroprotective assessment of **3a**, (+)**3a**, and (−)**3a** was performed in a model of ischemia-induced hippocampal damage (gerbils), administering the compounds 15 min before or after a 5-min bilateral carotid occlusion and reperfusion. Hippocampal CA1 damage was assessed under blinded conditions, and the results are shown in Table 4. Pretreatment with **3a** or its enantiomers significantly reduced CA1 hippocampal damage, with a lower degree of neuroprotection being afforded by posttreatment. Side effects (ataxia, impaired motor coordination) led to incomplete dose–response curves for the compound.

Neuroprotective effects were also evaluated on the degree of infarct volume in a model of cerebral focal ischemia. Compounds were administered to groups of six to seven rats 30 min following photochemically-induced ischemia, and the results are summarized in Table 5. At the single dose of 1 mg/kg iv, **3a** produced a highly significant reduction in infarct volume, as did dizocilpine at 3 mg/kg iv. Weaker effects were observed with (+)**3a**, while (−)**3a** was not effective at the doses evaluated. Dose-limiting side effects were observed for all three compounds and included motor disturbances and breathing difficulties.

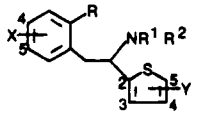
In summary, these studies show that **3a** is a noncompetitive NMDA antagonist *in vitro* with *in vivo* neuroprotective properties as demonstrated by prevention of NMDA-induced hippocampal damage, carotid occlusion-induced necrosis, and photochemically-induced focal cerebral infarction. Although the enantiomers (+)**3a** and (−)**3a** displayed differences in efficacy and side effect liability, the therapeutic window was considered to be smaller than the racemate.

Experimental Section

The structures of all compounds are supported by their IR (Perkin-Elmer 841 and 1420 spectrometers), ^{1}H NMR (Varian Gemini 200 spectrometer, tetramethylsilane as internal standard), and MS (Finnigan 4500 and Kratos MS 80 RFA spectrometers) spectra. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were taken with a Perkin-Elmer 241 polarimeter. Elemental analyses were performed by Midwest Microlab, Indianapolis, IN, or Oneida Research Services, Whitesboro, NY. Results are within ±0.4% of theoretical values unless otherwise noted in the tables. Reactions with moisture-sensitive reagents were maintained under a dry nitrogen atmosphere. Preparative HPLC was performed with a Waters Associates Prep LC/System 500 on silica gel and a Gow Mac Model 80–800 UV detector, with a 200 mL/min flow rate.

(±)-2,2-Dimethyl-*N*-[2-[2-(methylamino)-2-(3-methyl-2-thienyl)ethyl]phenyl]propanamide (2a). Method A. *n*-Butyllithium (90.4 mL, 0.23 mol, 2.5 M in hexanes) was added to a stirred suspension of 2,2-dimethyl-*N*-(2-methylphenyl)-propanamide[22] (1, 21.6 g, 0.11 mol) in dry tetrahydrofuran (THF, 65 mL) at 2–10 °C under nitrogen. After 1 h, 3-methyl-2-thiophenecarboxaldehyde methylimine[22] (17.0 g, 0.124 mol) was added and the mixture was stirred an additional 1 h at 0–10 °C. The reaction was quenched with water (250 mL), the cold mixture was acidified with 12 N HCl (27.4 mL, 0.33 mol), and the THF phase was extracted with 6 N HCl (50 mL). The combined acidic aqueous phases were washed with toluene, basified with 25% NaOH (150 mL), and extracted with toluene. The basic toluene extract was concentrated under reduced pressure and the residue purified by chromatography (silica gel/dichloromethane (DCM)) to give 22.2 g (61%) of **2a** (99.6% HPLC purity). A portion of this material was further purified by chromatography (silica gel/ethyl acetate) to afford 8 g of analytically pure **2a** as an oil: ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 9H), 1.48 (s, 1H, exch), 2.08 (s, 3H), 2.29 (s, 3H), 2.84–2.93 (dd, 1H, J = 14, 5 Hz), 2.99–3.10 (dd, 1H, J = 14, 8 Hz), 4.04–4.11 (dd, 1H, J = 8, 5 Hz), 6.78–6.81 (d, 1H, J = 5 Hz), 7.04–7.30 (m, 4H), 7.80–7.84 (d, 1H, J = 8 Hz), 9.13 (s, 1H, exch); IR (film) 3324, 2929, 1667, 1586, 1519, 1447, 1300, 1165, 752, 709 cm^{-1}; MS (CI, methane) *m/e* (rel intensity) 331 (M + 1, 100), 300 (13). Properties of **2a**, and of **2b**–e prepared in a similar manner, are included in Table 2.

Table 1. (±)-2-Amino-α-thienylbenzeneethanamines[a]



no.	R	R^1	R^2	X	Y	starting material	method	mp[b] (°C)	yield[c] (%)	recrystn solvent[d]	formula	anal.[e]	[^{3}H]TCP[f] IC_{50} (μM)	NMDLA[g] ED_{50} (mg/kg ip)
3a	NH_2	CH_3	H	H	3-CH_3	**2a**	B	203–204	61	A–B	$C_{14}H_{18}N_2S$·HCl	C,H,N	0.35	27.8
								203–208[h]		C–D	$C_{14}H_{18}N_2S$·2HCl	C,H,N	(0.26–0.46)	(17.3–44.8)
(+)**3a**	NH_2	CH_3	H	H	3-CH_3	**3a**		214		H	$C_{14}H_{18}N_2S$·2HCl	C,H,N,Cl	0.18 (0.11–0.29)	
(−)**3a**	NH_2	CH_3	H	H	3-CH_3	**3a**		214		H	$C_{14}H_{18}N_2S$·2HCl	C,H,N,Cl	57 (14–241)	
3b	NH_2	C_2H_5	H	H	3-CH_3	**2b**	B	202–204	71	C–D	$C_{15}H_{20}N_2S$·2HCl	C,H,N	0.043 (0.025–0.075)	6.4 (4.6–9.1)
3c	NH_2	n-C_3H_7	H	H	3-CH_3	**2c**	B	196–198	70	C–D	$C_{16}H_{22}N_2S$·2HCl	C,H,N	0.13 (0.08–2.1)	14.1 (9.8–20.5)
3d	NH_2	CH_3	H	H	H	**2d**	B	210–215[h]	57	E	$C_{13}H_{16}N_2S$·2HCl[i]	C,H,N	2.1 (1.2–3.5)	>60
3e	NH_2	CH_3	H	H	5-CH_3	**2e**	B		64	oil	$C_{14}H_{18}N_2S$	C,H,N	5.2 (2.3–11.5)	>60
3f	NH_2	CH_3	H	5-Br	3-CH_3	**3a**	C	68–70	40	D	$C_{14}H_{17}BrN_2S$	H,N,C[j]	0.045 (0.019–0.10)	>5
3g	NH_2	CH_3	H	5-Cl	3-CH_3	**3a**	C	59–61	12	F–I	$C_{14}H_{17}ClN_2S$	C,H,N	0.025 (0.013–0.048)	≥30
3h	$N(CH_3)_2$	CH_3	CH_3	H	3-CH_3	**3a**	D		49	oil	$C_{17}H_{24}N_2S$	C,H,N	0.33 (0.17–0.64)	>60
3i	$NHCH_3$	CH_3	H	H	3-CH_3	**4d**	G	191–192	62	C–D	$C_{15}H_{20}N_2S$·2HCl	C,H,N	3.8 (2.9–5.1)	>60
3j	NH_2	CH_3	CH_3	H	3-CH_3	**4a**	H	75–77	68	D	$C_{15}H_{20}N_2S$	C,H,N	0.37 (0.21–0.66)	>60
3k	$NHCH_3$	CH_3	CH_3	H	3-CH_3	**4b**	H		68	oil	$C_{16}H_{22}N_2S$	C,H,N	5.1 (3.9–6.7)	>60
3l	NH_2	H	H	H	3-CH_3	**7a**	I	228–229[h]	17	C–D	$C_{13}H_{16}N_2S$·2HCl	C,H,N	4.0 (2.5–6.6)	19 (12–31)
3m	NH_2	CH_3	H	4-Cl	3-CH_3	**7b**	I	209–211[h]	43	C–D	$C_{14}H_{17}ClN_2S$·2HCl	C,H,N	18 (11–30)	≥60
3n	NH_2	CH_3	H	4-CF_3	3-CH_3	**7c**	I	52–54	45	F–I	$C_{15}H_{17}F_3N_2S$	C,H,N	>10	26.4 (14.1–49.3)
dizocilpine (MK-801)													0.0089 (0.0051–0.016)	0.38 (0.32–0.45)
(±)-[3-(2-carboxypiperazin-4-yl)propyl]phosphonate (CPP)													>100	

[a] All compounds exhibited IR, ^{1}H NMR, and MS spectra consistent with the assigned structure. Displacement of [^{3}H]CPP binding: IC_{50} > 100 μM, **3a–j,n**; IC_{50} > 10 μM, **3k,m**; **3l** not determined. [b] Melting points are uncorrected. [c] Yields were calculated from the indicated starting material unless otherwise noted. Yields were not optimized. [d] A = isopropyl alcohol; B = water; C = methanol; D = ether; E = absolute ethanol; F = hexane; G = ethereal hydrogen chloride; H = 95% ethanol; I = triturated with solvent. [e] Analytical results were within ±0.4% of theoretical values unless otherwise noted. [f] Displacement of [^{3}H]TCP binding from rat cortical membranes. For **3a**·2HCl: TCP IC_{50} = 0.35 (0.18–0.66) μM. [g] Prevention of clonic seizures in mice induced by racemic NMDLA. Dosages are corrected for the percentage of base compound. For **3a**·2HCl: NMDLA ED_{50} = 26.7 (16.8–42.2) mg/kg ip. [h] Decomposition point. [i] Hemihydrate. [j] Anal. ($C_{14}H_{17}BrN_2S$) H,N;C: calcd, 51.70; found, 52.11.

(±)-2-Amino-*N*-methyl-α-(3-methyl-2-thienyl)benzeneethanamine Hydrochloride (3a). Method B. A mixture of **2a** (138.4 g, 0.42 mol) and 6 N HCl (244 mL, 7 equiv) was heated to 100 °C for 24 h, cooled to 20 °C, basified with 50% NaOH solution (240 g), and extracted with toluene, and the extract was concentrated under reduced pressure. The crude product was purified by chromatography (silica gel/ethyl acetate) to give **3a** free base. Acidification of the free base (105 g) in 2-propanol (575 mL) with 37% HCl afforded 91.5 g of the hydrochloride salt. Recrystallization from 90% 2-propanol (550 mL) gave 72 g (61%) of analytically pure **3a** as the monohydrochloride salt: mp 203–204 °C; ^{1}H NMR (DMSO-d_6) δ 1.79 (s, 3H), 2.40 (s, 3H), 2.85 (t, 1H, J = 12 Hz), 3.60 (dd, 1H, J = 12, 4 Hz), 4.71 (dd, 1H, J = 12, 4 Hz), 6.27 (t, 1H, J = 7 Hz), 6.44 (d, 1H, J = 7 Hz), 6.61 (d, 1H, J = 7 Hz), 6.78 (d, 1H, J = 5 Hz), 6.88 (t, 1H, J = 7 Hz), 7.45 (br s, 4H), 7.56 (d, 1H, J = 5 Hz); IR (KBr) 3390, 3327, 3231, 2959, 2925, 2760, 2708, 1634, 1586, 1497, 1459, 746, 717 cm^{-1}; MS (CI, methane) m/e (rel intensity) 247 (M + 1, 100), 216 (80), 140 (39).

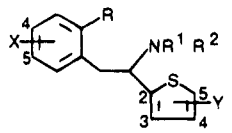
The dihydrochloride salt was prepared by treating a methanol solution of **3a** free base with excess ethereal hydrogen chloride and recrystallization of the precipitate from methanol–ether without heating: mp 203–208 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.81 (s, 3H), 2.51 (s, 3H, coincident with DMSO), 3.04 (dd, 1H, J = 14, 11 Hz), 3.77 (dd, 1H, J = 14, 4 Hz), 5.09 (dd, 1H, J = 11, 4 Hz), 6.65 (d, 1H, J = 8 Hz), 6.82 (d, 1H, J = 5 Hz), 6.95 (t, 1H, J = 7 Hz), 7.20–7.27 (m, 2H), 7.62 (d, 1H, J = 5 Hz); IR (KBr) 3450 (br), 2970, 2820, 2580, 1640, 1580, 1560, 1500, 1470, 1455, 1210, 1185, 1135, 965, 920, 895, 770, 760, 720 cm^{-1}; MS (CI, methane) m/e (rel intensity) 247 (M + 1, 70), 216 (100), 140 (49). Properties of **3a**, and of **3b–e** prepared in a similar manner from **2b–e**, respectively, are included in Table 1.

Chromatographic Resolution of 3a. Resolution of **3a** free base (1 g) was achieved on a 3.6- × 90-cm triacetyl cellulose (E. Merck, Darmstadt) column eluting with ethanol (flow rate 2 mL/min). The (−)-enantiomer eluted first. The enantiomers were converted to their dihydrochloride salts with ethanolic hydrogen chloride and recrystallized from ethanol to provide 0.4 g of each enantiomer, mp 214 °C. For (+)**3a**, $[\alpha]^{30}_D$ 35.936°, and for (−)**3a**, $[\alpha]^{30}_D$ −35.936° (c = 1, methanol).

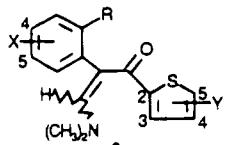
X-ray Determination of (+)3a. Crystal Data: $C_{14}H_{18}N_2S$·2HCl, M_r = 319.3, monoclinic, $P2_12_12_1$, a = 9.440(3) Å, b = 10.491(6) Å, c = 17.164(8) Å, V = 1699.8(14) Å^3, Z = 4, D_x = 1.248 Mg/m^{-3}, λ(Mo Kα) = 0.71073 Å, μ = 0.494 mm^{-1}, F(000) = 672, T = 193 K.

Experimental: Crystals were made by recrystallization from ethanol. A crystal of dimensions 0.55 × 0.15 × 0.10 mm^3 was sealed in a Lindemann glass capillary. Twenty-five reflections with ϑ > 8° were used to determine the cell parameters on a four-circle computer-controlled diffractometer (R3m/V, Siemens). The intensities were measured on the same

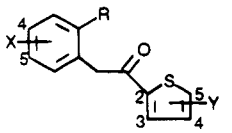
Table 2. Intermediates[a]



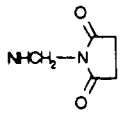
2a-e; 4a-d; 8



6a-c



7a-c

no.	R	R^1	R^2	X	Y	starting material	method	mp[b] (°C)	yield[c] (%)	recrystn solvent[d]	formula	anal.[e]
2a	tBu(C=O)NH	CH_3	H	H	3-CH_3		A		61	oil	$C_{19}H_{26}N_2OS$	C,H,N
2b	tBu(C=O)NH	C_2H_5	H	H	3-CH_3		A	114[f]	71	D–G	$C_{20}H_{28}N_2OS$·HCl	C,H,N
2c	tBu(C=O)NH	n-C_3H_7	H	H	3-CH_3		A		80	oil	$C_{21}H_{30}N_2OS$	C,H,N
2d	tBu(C=O)NH	CH_3	H	H	H		A	78–82[g]	37		$C_{18}H_{24}N_2OS$	C,H,N
2e	tBu(C=O)NH	CH_3	H	H	5-CH_3		A	91–101	30	C–D	$C_{19}H_{26}N_2O$·HCl[h]	C,H,N
4a	NH_2	CH_3	C(=O)H	H	3-CH_3	3a	E, F	111–112	22, 56	C–B	$C_{15}H_{18}N_2OS$	C,H,N
4b	NHC(=O)H	CH_3	C(=O)H	H	3-CH_3	3a	E, F	128–130	15	F–I	$C_{16}H_{18}N_2O_2S$	C,H,N
4c		CH_3	C(=O)H	H	3-CH_3	4a	G	160–164	68	H	$C_{20}H_{23}N_3O_3S$	C,H,N
4d	$NHCH_3$	CH_3	C(=O)H	H	3-CH_3	4c	G	140–145	29	D–G	$C_{16}H_{20}N_2OS$·HCl[i]	C,H,N
6a	NO_2			H	3-CH_3	5a[j]	I	101–103	65	H	$C_{16}H_{16}N_2O_3S$	C,H,N
6b	NO_2			4-Cl	3-CH_3	5b[j]	I	111–113	72	H	$C_{16}H_{15}ClN_2O_3S$	C,H,N
6c	NO_2			4-CF_3	3-CH_3	5c[k]	I	118–119	42	H	$C_{17}H_{15}F_3N_2O_3S$	C,H,N
7a	NO_2			H	3-CH_3	6a	I	85–87	67	H	$C_{13}H_{11}NO_3S$	C,H,N
7b	NO_2			4-Cl	3-CH_3	6b	I	105–107	61	H	$C_{13}H_{10}ClNO_3S$	C,H,N
7c	NO_2			4-CF_3	3-CH_3	6c	I	120–122	35	H	$C_{14}H_{10}F_3NO_3S$	C,H,N
8	NO_2	H	H	H	3-CH_3	7a	I				$C_{13}H_{14}N_2O_2S$	

[a–e] See corresponding footnotes to Table 1. [f] Decomposition point. [g] Obtained as an oil which crystallized. [h] Hemihydrate. [i] Monohydrate. [j] Aldrich Chemical Co. [k] Torssell, K. *Tetrahedron* **1977**, *33*, 2287–2291 (ref 24). [l] Not isolated.

Table 3. Reduction of NMDA-Induced Hippocampal Necrosis (Rat)

compound	dose (mg/kg)	necrosis volume × μM^3 (SEM)	% reduction cell necrosis
3a	100 sc	0.057 (±0.021)	68[a]
3b	30 sc	0.059 (±0.017)	67[a]
	100 sc	0.0098 (±0.0066)	95[a]
dizocilpine	0.7 ip	0.1 (±0.023)	44[a]
	2.0 ip	0.01 (±0.004)	94[a]
	7.0 ip	0.0025 (±0.00065)	98[a]
control[b]		0.18 (±0.011)	

[a] Statistically significant ($p < 0.05$). [b] NMDA (20 nmol).

Table 4. Carotid Artery Occlusion-Induced Hippocampal Damage (Gerbil)

compound	dose (mg/kg ip)	control (mean ± SD)	treated (mean ± SD)	% reduction cell necrosis
		Pretreatment		
3a	30	1.20 ± 1.1	0.30 ± 0.6	75[a]
(+)**3a**	30	3.10 ± 1.3	1.50 ± 1.19	51[a]
(−)**3a**	30	3.40 ± 1.08	2.80 ± 1.09	32[a]
	60	3.50 ± 0.87	0.90 ± 1.11	75[a]
dizocilpine	5	1.05 ± 0.48	0.8 ± 0.49	22[a]
		Posttreatment		
3a	10	2.87 ± 1.13	2.80 ± 1.09	2
	30	2.86 ± 1.41	2.13 ± 1.69	26[a]
	50	2.60 ± 1.30	2.13 ± 1.19	18
(−)**3a**	60	3.10 ± 1.90	3.30 ± 1.19	−9

[a] Statistically significant ($p < 0.05$).

apparatus: Mo Kα radiation, 3317 reflections ($-11 < h < 11$, $-1 < k < 12$, $-3 < l < 20$), of which 3015 were unique (R_{int} = 0.028) and 2806 had structure factors $|F| > 1\sigma(F)$, which were used for the structure analysis. Direct methods was used for solving the phase problem;[23] refinement of the structure parameters was by least-squares methods (minimization of $(|F_o| - |F_s|)^2$); weighting scheme: $w = 1/\sigma^2(F)$ according to the counting statistics, 183 parameters; coordinates of the H atoms were obtained from a difference synthesis, S = 0.97, R_1 = 0.112, R_w = 0.0423, 10 largest peaks in the difference map: 0.67–1.41 electrons/Å^3. All calculations were done by a microVAX II computer with the SHELXTL-PLUS programs.[23] Figure 1 shows the molecular structure of (+)**3a** together with the 20% probability thermal ellipsoids for the non-hydrogen atoms and the atom numbering scheme, which permits assignment of the absolute configuration as *S*(+)**3a**. The chloride atoms show a certain degree of disorder. Cl-2 has an occupancy of 0.844 and Cl-21 of 0.156. Further tables of the results are available as supplementary material.

Table 5. Reduction of Photochemically-Induced Cerebral Focal Ischemia (Rat)

compound	dose (mg/kg iv)	control (mean ± SD)	treated (mean ± SD)	% change
3a	0.3	72 ± 6	72 ± 6	0
	1.0	78 ± 7	57 ± 8	−27[a]
	3.0[b]	75 ± 6	63 ± 6	−16
	10.0[b]	81 ± 8	86 ± 6	6
(+)**3a**	0.1[b]	69 ± 7	59 ± 8	−14
	0.3[b]	83 ± 8	72 ± 8	−13
	1.0[b]	78 ± 7	67 ± 7	−14
(−)**3a**	1.0	71 ± 7	66 ± 6	−7
	3.0	78 ± 7	73 ± 6	−6
	10.0[b]	88 ± 7	93 ± 8	6
	20.0[b]	91 ± 9	79 ± 10	−13
dizocilpine	3.0	68 ± 6	52 ± 5	−24[a]

[a] Statistically significant. [b] Side effects observed.

(±)-2-Amino-5-bromo-*N*-methyl-α-(3-methyl-2-thienyl)-benzeneethanamine (3f). Method C. A stirred solution of **3a** free base (5.02 g, 0.02 mol) and dimethylformamide (DMF, 79 mL) was treated with a solution of *N*-bromosuccinimide (4.71 g, 0.026 mol) in DMF (55 mL). After 8 h, the black solution was concentrated under high vacuum to an oil. The oil was diluted with water (100 mL), treated with 2.5 N sodium hydroxide solution (25 mL), and extracted with DCM (3 × 100 mL). The dried (Na_2SO_4) extract was filtered and concentrated to a dark red oil, which was purified by preparative HPLC (methanol). Concentration of the desired fractions provided an oil which was dissolved in ether and filtered. The crystalline precipitate was collected and dried *in vacuo* (35 °C) to afford 2.6 g (40%) of **3f** as a tan solid: mp 68–70 °C; ^{1}H NMR ($CDCl_3$, D_2O exch) δ 1.99 (s, 3H), 2.33 (s, 3H), 2.75 (dd, 1H, *J*

= 6, 14 Hz), 2.90 (dd, 1H, J = 7, 14 Hz), 4.10 (apparent t, 1H, J = 7 Hz), 6.53 (d, 1H, J = 8 Hz), 6.75 (d, 1H, J = 5 Hz), 7.09–7.12 (m, 2H), 7.16 (d, 1H, J = 5 Hz); IR ($CHCl_3$) 3490, 3460, 3390, 3020, 2960, 2870, 2810, 1628, 1493, 1455, 1415, 1330, 1290, 1280, 1158, 1140, 1100, 890, 840, 820, 718 cm^{-1}; MS (CI, methane) *m/e* (rel intensity) 327 + 325 (M + 1, 96 and 100, respectively), 247 (62), 140 (25). Properties of **3f**, and of **3g** prepared in a similar manner from **3a** and NCS, are included in Table 1.

(±)-***N,N*-Dimethyl-2-(dimethylamino)-α-(3-methyl-2-thienyl)benzeneethanamine (3h). Method D.** A stirred solution of **3a** free base (5.4 g, 0.022 mol) and acetonitrile (50 mL) was treated sequentially with 87% aqueous formaldehyde (11.5 mL) and sodium cyanoborohydride (3.1 g, 0.049 mol). After 2.5 h, the mixture was diluted with ether (80 mL) and washed successively with 1 N potassium hydroxide solution (3 × 75 mL) and saturated brine (150 mL). The dried (K_2CO_3) organic phase was filtered and concentrated to a brown oil, which was purified by preparative HPLC (methanol). The desired fractions were concentrated to an oil which was dissolved in ether and filtered. Concentration of the filtrate provided 3.1 g (49%) of **3h** as a light brown oil: ^1H NMR ($CDCl_3$) δ 1.76 (s, 3H), 2.34 (s, 6H), 2.67 (s, 6H), 2.78 (dd, 1H, J = 10, 13 Hz), 3.55 (dd, 1H, J = 5, 13 Hz), 4.02 (dd, 1H, J = 5, 10 Hz), 6.62 (d, 1H, J = 5 Hz), 6.81–6.85 (m, 2H), 7.08–7.13 (m, 3H); IR ($CHCl_3$) 3080, 3020, 3000, 2959, 2880, 2840, 2800, 1608, 1502, 1460, 1345, 1310, 1260, 1195, 1160, 1103, 1055, 1000, 955, 888, 718 cm^{-1}; MS (CI, methane) *m/e* (rel intensity) 289 (M + 1, 12), 244 (65), 154 (100). Properties of **3h** are included in Table 1.

(±)-**2-Amino-*N*-formyl-*N*-methyl-α-(3-methyl-2-thienyl)benzeneethanamine (4a). Method E.** A stirred solution of **3a** free base (5.0 g, 0.02 mol) and ethyl formate (235 mL) was refluxed for 8 h and then concentrated to afford a mixture of products as an oil, which was purified by preparative HPLC (ethyl acetate). Concentration of the desired fractions and recrystallization from methanol–water afforded 1.2 g (22%) of **4a** as beige crystals: mp 111–112 °C (TLC, silica, EtOAc, R_f 0.49); ^1H NMR (DMSO-d_6) indicated a mixture of rotamers at room temperature which were incompletely coalesced at 90 °C; IR ($CHCl_3$) 3022, 1668, 1635, 1508, 1475, 1410, 1390, 1080 cm^{-1}; MS (CI, methane) *m/e* (rel intensity) 275 (M + 1, 87), 216 (100), 168 (24). Properties of **4a** are included in Table 2.

Method F. Compound **4a** was also synthesized using formic anhydride. A stirred, ice water-chilled solution of dicyclohexylcarbodiimide (1.34 g, 0.0065 mol) and DCM (15 mL) was treated dropwise over 10 min with a solution of 95% formic acid (0.63 g, 0.013 mol) and DCM (20 mL). The suspension was stirred for 10 min and filtered, and the filtrate was added dropwise over 15 min to a stirred, ice water-chilled solution of **3a** free base (1.23 g, 0.005 mol) and KOH-dried pyridine (15 mL). After stirring with cooling for 2 h, the solution was decanted over ice and basified with NaOH solution and the phases were separated. The dried (Na_2SO_4) organic phase was filtered and concentrated to an oil, which was purified by preparative HPLC (ethyl acetate) to afford 0.77 g (56%) of **4a**.

(±)-***N*-[2-[2-(*N*-Formyl-*N*-methylamino)-2-(3-methyl-2-thienyl)ethyl]phenyl]formamide (4b).** This material was obtained as the major byproduct from HPLC purification of crude **4a** prepared from **3a** (0.23 mol) by method F. Concentration of the appropriate HPLC fractions and trituration of the residual oil with hexane provided 10 g (15%) of **4b**: mp 128–130 °C (TLC, silica gel, EtOAc, R_f 0.32); ^1H NMR (DMSO-d_6) indicated a mixture of rotamers at room temperature and 90 °C; IR ($CHCl_3$) 3280, 3018, 2930, 2880, 1695, 1650, 1590, 1540, 1482, 1455, 1405, 1375, 1290, 1075, 710 cm^{-1}; MS (CI, methane) *m/e* (rel intensity) 303 (M + 1, 46), 272 (22), 244 (100), 146 (23). Properties of **4b** are summarized in Table 2.

(±)-***N*-Methyl-2-(methylamino)-α-(3-methyl-2-thienyl)-benzeneethanamine Dihydrochloride (3i). Method G.** Utilizing the method of Kadin,[17] **4a** (8 g, 0.029 mol) was converted to **4c** (7.6 g (68%), mp 160–164 °C (95% ethanol)), and a solution of **4c** (6.39 g) in dimethyl sulfoxide was reduced ($NaBH_4$) to **4d** (1.7 g (29%), hydrochloride monohydrate, mp 140–145 °C). A stirred solution of **4d** (4.1 g, 0.012 mol) and 3 N hydrochloric acid (18 mL) was refluxed for 1.5 h, decanted over ice (100 g), basified with 50% sodium hydroxide solution, and extracted with DCM (3 × 60 mL). The dried (Na_2SO_4) organic phase was filtered and concentrated to an oil, which was purified by preparative HPLC (methanol). The desired fractions were concentrated to an oil which was dissolved in ether, filtered, and concentrated to an oil. The material was converted to the dihydrochloride salt with methanol–ethereal hydrogen chloride to give 2.4 g (62%) of **3i**: mp 191–192 °C; ^1H NMR ($CDCl_3$–DMSO-d_6) 1.76 (s, 3H), 2.50 (s, 3H), 3.05 (s, 3H), 3.37 (dd, 1H, J = 11, 13 Hz), 3.95 (dd, 1H, J = 2, 13 Hz), 4.76 (dd, 1H, J = 2, 11 Hz), 6.66 (d, 1H, J = 8 Hz), 6.78 (d, 1H, J = 5 Hz), 7.05 (t, 1H, J = 7 Hz), 7.33 (t, 1H, J = 7 Hz), 7.42–7.65 (m, 2H); IR (KBr) 3490–3360 (br), 3100, 2930, 2800–2580 (br), 2430, 1585, 1502, 1462, 1425, 1202, 1125, 1020, 1005, 930, 890, 870, 835, 770, 750 cm^{-1}; MS (CI, methane) *m/e* (rel intensity) 261 (M + 1, 52), 230 (100), 140 (54). Properties of **3i** are summarized in Table 1.

(±)-**2-Amino-*N,N*-dimethyl-α-(3-methyl-2-thienyl)benzeneethanamine (3j). Method H.** A stirred solution of **4a** (5.8 g, 0.021 mol) and THF (400 mL) was treated dropwise with a 1 M borane in THF solution (90 mL, 0.09 mol) at room temperature (N_2 atmosphere). The reaction was quenched with 2.5 N sodium hydroxide solution (60 mL) and the mixture extracted with DCM (2 × 100 mL). The dried (Na_2SO_4) organic phase was filtered and concentrated to an oil, which was largely a borane complex of the product. The oil was diluted with glacial acetic acid (15 mL) and treated with concentrated hydrochloric acid (35 mL). After stirring overnight at room temperature, the solution was decanted over ice (200 g), basified with 50% sodium hydroxide solution (20 mL), and extracted with DCM (3 × 100 mL). The dried (Na_2SO_4) organic phase was concentrated to an oil, which was purified by preparative HPLC (methanol). Concentration of the desired fractions afforded a solid which was dissolved in ether (100 mL) and filtered. Concentration of the filtrate provided **3j** (3.7 g, 68%) as a colorless solid: mp 75–77 °C; ^1H NMR ($CDCl_3$, D_2O exch) δ 1.71 (s, 3H), 2.36 (s, 6H), 2.75 (dd, 1H, J = 10, 13 Hz), 3.24 (dd, 1H, J = 4, 13 Hz), 3.90 (dd, 1H, J = 4, 10 Hz), 6.57–6.66 (m, 3H), 6.77 (d, 1H, J = 9 Hz), 6.94 (t, 1H, J = 8 Hz), 7.12 (d, 1H, J = 5 Hz); IR ($CHCl_3$) 3460, 3380, 3078, 3010, 2955, 2870, 2830, 2780, 1625, 1578, 1500, 1460, 1335, 1315, 1270, 1180, 1160, 1100, 1040, 1020, 995, 885, 712 cm^{-1}; MS (CI, methane) *m/e* (rel intensity) 261 (M + 1, 87), 216 (27), 154 (100). Properties of **3j**, and of **3k** prepared in a similar manner from **4b** (0.024 mol) and 1 M borane in tetrahydrofuran (0.144 mol), are included in Table 1.

Preparation of 3l–n. Method I. The multistep conversion of **5a–c** to **3l–n** is depicted in Scheme 3. Starting materials **5a,b** were purchased from Aldrich Chemical Co., and **5c** was prepared from 4-(trifluoromethyl)benzaldehyde (Aldrich) as described by Torssell.[24] The synthesis of **3l–n** is based on the preparation of *trans*-β-(dimethylamino)-2-nitrostyrenes from **5a–c**, acylation of the styrenes to give **6a–c**, and hydrolysis to **7a–c**, using methodology described by Garcia and Fryer[18] and utilized previously in this laboratory to prepare a number of related ketones.[12] Procedures for conversion of **7a–c** to **3l–n** were analogous to methods previously described.[11,12] Properties of **6a–c**, **7a–c**, and **3l–n** are included in the tables. Intermediate **8** was converted to **3l** without isolation. Spectral data for representative examples **6b**, **7b**, and **3m** are included.

6b: ^1H NMR ($CDCl_3$) δ 2.36 (s, 3H), 2.82 (s, 6H), 6.85 (d, 1H, J = 5 Hz), 7.24 (d, 1H, $J \simeq$ 8 Hz), 7.28 (d, 1H, J = 5 Hz), 7.45 (s, 1H), 7.54 (dd, 1H, J = 2, 8 Hz), 7.94 (d, 1H, J = 2 Hz); IR ($CHCl_3$) 3025, 2945, 1640, 1618, 1580, 1540, 1500, 1425, 1402, 1365, 1315, 1120, 1105, 1050, 900, 818 cm^{-1}; MS *m/e* (rel intensity) 352 + 350 (M, 1 and 5, respectively), 211 (10), 209 (27), 166 (18), 125 (100), 44 (39).

7b: ^1H NMR ($CDCl_3$) δ 2.55 (s, 3H), 4.59 (s, 2H), 6.99 (d, 1H, J = 5 Hz), 7.30 (d, 1H, $J \simeq$ 8 Hz), 7.48 (d, 1H, J = 5 Hz), 7.59 (dd, 1H, J = 2, 8 Hz), 8.16 (d, 1H, J = 2 Hz); IR ($CHCl_3$) 3120, 3030, 2935, 1670, 1618, 1570, 1530, 1488, 1420, 1405, 1385, 1375, 1352, 1328, 1200, 1155, 1115, 1035, 980, 892, 848, 815 cm^{-1}; MS *m/e* (rel intensity) 297 + 295 (M, 0.12 and 0.43, respectively), 125 (100), 53 (11).

3m: ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.88 (s, 3H), 2.50 (s, 3H), 3.23 (dd, 1H, J = 11, 14 Hz), 3.92 (dd, 1H, J = 2, 14 Hz), 4.87 (dd, 1H, J = 2, 11 Hz), 6.57 (d, 1H, J = 8 Hz), 6.79 (d, 1H, J = 5 Hz), 6.89 (dd, 1H, J = 2, 7 Hz), 7.36–7.43 (m, 2H); IR (KBr) 3600–3310 (br), 3200–2100 (br), 1595, 1562, 1532, 1500, 1485, 1455, 1420, 1315, 1295, 1148, 1110, 1035, 905, 890, 825, 725 cm^{-1}; MS (CI, methane) *m/e* (rel intensity) 283 + 281 (M + 1, 8 and 33, respectively), 252 (25), 250 (82), 245 (25), 140 (100).

[^{3}H]TCP Binding. [^{3}H]-*N*-[1-(2-thienyl)cyclohexyl]piperidine binding was determined in extensively washed rat cortical membrane preparations as described by Snell et al.[25] Membrane homogenates were incubated for 120 min at 25 °C with 2.5 nM [^{3}H]TCP in 0.1 M Hepes, pH 7.5, in the presence of 100 μM L-glutamate and 10 μM glycine. Binding was terminated by rapid filtration and washing over Whatman GF/B filters presoaked in 0.05% poly(ethyleneimine), and the filters were counted for bound radioligand in Liquiscint. Specific binding was determined by the difference of binding in the absence or presence of 100 μM PCP and was approximately 90% of total binding. IC_{50} values for the competing drug were calculated by log–probit analysis.

[^{3}H]CPP Binding. [^{3}H]-(±)-[3-(2-Carboxypiperazin-4-yl)-propyl]phosphonate binding was measured in Triton-X-treated rat cortical membrane preparations as described by Murphy et al.[26] Membrane homogenates were incubated for 20 min at 25 °C with 10 nM [^{3}H]CPP in 0.05 M Tris, pH 7.6. Binding was terminated by centrifugation. The pellets were gently rinsed with ice-cold buffer, transferred to scintillation vials, and counted for radioactivity in Liquiscint. Specific binding was defined as the difference of binding in the absence or presence of 100 μM L-glutamate and was approximately 60–70% of total binding. IC_{50} values were determined by log–probit analysis.

NMDLA-Induced Convulsions. Groups of 10 Swiss Webster mice were treated with the test compound (60 mg/kg ip) dissolved in distilled water 0.5 h prior to challenge with *N*-methyl-D,L-aspartic acid (350 mg/kg sc). The animals were placed individually in clear plastic cylinders (12 × 5.5 × 0.25 in.^{3}) and then observed over 30 min for clonic seizures (defined as a single episode of clonic spasms of at least a 3-s duration). Mice treated with NMDLA were considered protected when there was a total absence of clonic seizures during the observation period. Control animals received vehicle. For ED_{50} determination, test compounds were run at the time of peak effect using three or more compound doses and 10 mice/group. Data were calculated by the method of Litchfield and Wilcoxon.[27]

NMDA-Induced Hippocampal Damage. Male Sprague–Dawley rats (280–310 g) were anesthetized with chloral hydrate (400 mg/kg ip) and placed in a stereotaxic instrument (David Kopf). A unilateral lesion of the dorsal CA1 region of the hippocampus (coordinates: 3.8 mm posterior, 2.2 mm lateral, and 3.4 mm dorsal from the surface of the skull: Paxinos and Watson[28]) was induced by infusing 20 nmol of NMDA (1 μL over a period of 5 min) via a 30-gauge cannula fitted to a 5-μL Hamilton syringe. Control injections of the phosphate buffer vehicle (0.1 M, pH 7.4) were made in a separate group of subjects. The injected solution was allowed to diffuse away from the injection site for 5 min, after which the cannula was slowly (3–4 min) withdrawn. The burr hole was sealed with bone wax, a topical antibiotic applied to the wound, and the scalp apposed with would clips. Seven days following surgery, the animals were anesthetized with chloral hydrate (400 mg/kg ip) and transcardially perfused with 0.9% NaCl in phosphate buffer (0.1 M, pH 7.4) followed by 4% formaldehyde in phosphate buffer. Perfused brains were removed and placed in buffered fixative for 1 day followed by cryopreservation in 30% sucrose solution for 1–3 days. Cryostat sections (20–40 μm) through the area of the lesion (ca. 1–5 mm posterior to bregma) were mounted on gel-coated slides and stained with cresyl violet.[29]

The stained sections were histologically evaluated via light microscopy and the size of the lesion quantitated with a RAS 1000 Image Analysis System (Amersham). The lateral extent of pyramidal cell necrosis in mm^2 was measured in each of a series of sections through the area of the lesion. The area under the curve, where the x-values represent the consecutive sections with an evident lesion and the y-values the lateral extent of the lesion in mm^2, was calculated with the PHARM/PCS computer program.[30] The volume of the lesion in mm^3 was generated by multiplying the area under the curve by the distance between sections (typically 180 μm). Control and experimental groups (n = 4) were compared by t-test. **3a**,**b** were administered sc and dizocilpine ip 1 h prior to infusion of NMDA. Results are presented in Table 3.

Carotid Artery Occlusion-Induced Hippocampal Damage.[31] Forebrain ischemia was induced in halothane-anesthetized (2.6 vol% initially, 1.2 vol% maintenance in nitrogen: oxygen, 2:1) male Mongolian gerbils (65–75 g, n = 14–15) via bilateral 5-min carotid occlusion. One week following ischemia, the brains were histologically prepared (Carnoy's fixative and hematoxilin-eosin stain, 4-6-μm-thick sections) for light microscopic evaluation. The degree of damage in the dorsal hippocampal CA1 region was rated on a scale of 0–4 (see below) by a pathologist blind to treatment conditions. Group means were compared by t-test. **3a**, **3b**, or dizocilpine was administered ip either 15 min before or 15 min after occlusion. Results are presented in Table 4.

Ischemia-Induced Necrosis Rating Scale

rating	cell necrosis
0	none
1	few cells or cell groups
2	large cell groups
3	most cells
4	all CA1 cells

Focal Ischemia-Induced Cerebral Infarct. This model of thrombotic stroke utilizes a method in which the photosensitive dye Rose Bengal under specific light conditions (wavelength 560 nM) generates oxygen radicals which damage blood vessel endothelial cells leading to platelet aggregation, thrombus formation, and eventually vessel occlusion. The specific procedure used in this study is described in Grome et al.[32]

Male rats (300–350 g) were anesthetized with halothane (3 vol% initially, 1.2 vol% maintenance in oxygen), and the right side of the skull was exposed. Rose Bengal (5 mg in 1 mL) was infused via a catheter in the right femoral vein. An intense green light (570 nm from an xenon lamp) of 3-mm diameter was focused on the skull surface at the level of bregma for 15 min immediately following infusion of the dye. At the end of this time period, the incisions were apposed, a topical anesthetic was applied, and the animals were allowed to recover from anesthesia. The volume of the induced infarct was quantitated (mm^3) with an image analysis system (Stemmer, Munich, Germany) in 20-μm-thick Heidenhain's Susa fixed sections stained with cresyl violet utilizing linear trapezoidal extrapolation similar to that described above for the NMDA-induced lesions. **3a** and dizocilpine were administered iv 30 min following ischemia to groups of five to six rats. Groups were compared using the Scheffe test. Results are presented in Table 5.

Acknowledgment. The authors express their appreciation to Anastasia Linville and Sandra Anselmo for providing spectral data, to June Strupczewski and Bettina Spahl for library research, to Silke Hepok for technical assistance in resolving the enantiomers, and to Dianne Saumsiegle for assistance in preparation of the manuscript.

Supplementary Material Available: Tables of X-ray parameters for *S*(+)**3a** (8 pages); a table of observed and calculated structure factors (11 pages). Ordering information is given on any current masthead page.

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