4-Hydroxy-1-[2-(4-hydroxyphenoxy)ethyl]-4-(4-methylbenzyl)piperidine: A Novel, Potent, and Selective NR1/2B NMDA Receptor Antagonist

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A structure-based search and screen of our compound library identified N-(2-phenoxyethyl)-4-benzylpiperidine (8) as a novel N-methyl-D-aspartate (NMDA) receptor antagonist that has high selectivity for the NR1/2B subunit combination (IC$_{50}$ = 0.63 µM). We report on the optimization of this lead compound in terms of potency, side effect liability, and in vivo activity. Potency was assayed by electrical recordings in Xenopus oocytes expressing cloned rat NMDA receptors. Side effect liability was assessed by measuring affinity for α₁-adrenergic receptors and inhibition of neuronal K$^+$ channels. Central bioavailability was gauged indirectly by determining anticonvulsant activity in a mouse maximal electroshock (MES) assay. Making progressive modifications to 8, a hydroxyl substituent on the phenyl ring para to the oxyethyl tether (10a) resulted in a 25-fold increase in NR1A/2B potency (IC$_{50}$ = 0.025 µM). p-Methyl substitution on the benzyl ring (10b) produced a 3-fold increase in MES activity (ED$_{50}$ = 0.7 mg/kg iv). Introduction of a second hydroxyl group into the C-4 position on the piperidine ring (10e) resulted in a substantial decrease in affinity for α₁ receptors and reduction in inhibition of K$^+$ channels with only a modest decrease in NR1A/2B and MES potencies. Among the compounds described, 10e (4-hydroxy-N-[2-(4-hydroxyphenoxy)ethyl]-4-(4-methylbenzyl)piperidine, Co 101244/PD 174494) had the optimum pharmacological profile and was selected for further biological evaluation.

Introduction

Overactivation of N-methyl-D-aspartate (NMDA) receptors by glutamate is believed to play a role in numerous acute and chronic neurodegenerative disorders.1,2 In the search for clinically effective neuroprotectants, a large variety of NMDA receptor antagonists have been identified and characterized.3 Thus far, however, many NMDA antagonists tested clinically have been compromised by dose-limiting cardiovascular, behavioral, or neurotoxic side effects.4

Native NMDA receptors are heterooligomeric assemblies composed of two different types of subunits termed NMDA receptor 1 (NR1) and NR2.5 NR1 subunits have eight different isoforms generated by alternative RNA splicing, and NR2 subunits have four distinct subtypes.5 NMDA receptor subunits are differentially distributed across brain regions.6 This raises the possibility that subtype-selective NMDA receptor antagonists could have therapeutic potential as neuroprotectants without the cardiovascular, behavioral, and neurotoxic side effects often associated with broad-spectrum antagonists.

Ifenprodil (1) was the first NMDA receptor antagonist shown to have pronounced subtype selectivity. Selectivity for this class of antagonist is directed toward the NR2B subunit.7 A number of structurally related compounds have subsequently been identified as NR1/2B-selective NMDA receptor antagonists. Exemplary compounds (Chart 1) include eliprodil (2),7b,8 haloperidol (3),7b,9 CP-101,606 (4),10 and Ro 25-6981 (5).11 Parallel experiments have investigated the complex mechanism of allosteric inhibition and the actions of this class of antagonists on neuronal NMDA currents in situ.11a,12,13

Herein we report on a novel series of NR2B-selective antagonists. Structure–activity relationship (SAR) studies reveal how three simple structural modifications can...
transform the screening lead into a potent and specific inhibitor.14

**Chemistry**

Compound 8 was prepared by N-alkylation of 4-benzylpiperidine (6b) with 2-phenoxyethyl bromide (7a). Compounds 10a–10e were prepared by N-alkylation of the requisite piperidines 6a–6e with bromide 7b followed by debenzylation of the benzyl analogues 9a–9e as shown in Scheme 1.

Those substituted 4-benzylpiperidines that were not commercially available were prepared from the appropriately substituted benzyl bromides through a Wittig reaction.15 Briefly, reaction of substituted benzyl bromide 11 with triphenylphosphine afforded the corresponding phosphonium salt 12. Treatment of 12 with sodium methylylphosphine carbanion in dimethyl sulfoxide at 80 °C gave the ylide which reacted with 1-benzyl-4-piperidone to form the olefin 13. Reduction of 13 with H2 in the presence of PtO2 gave compound 14. Further debenzylation of 14 by hydrogenation in the presence of 10% Pd/C gave the desired piperidines 6c and 6d in quantitative yield (Scheme 2).

4-Hydroxy-4-(4-methylbenzyl)piperidine (6e) was prepared according to Scheme 3. Reaction of 4-methylbenzylmagnesium chloride, which was made in situ from unsubstituted 2-phenoxyethyl bromide (7a) and 2-(4-benzylxyloxy)ethyl bromide (7b) were synthesized from the reaction of the corresponding phenol with 1,2-dibromoethane.

**Pharmacology**

Compounds were tested for antagonism of cloned NMDA receptors expressed in *Xenopus* oocytes using standard two-electrode voltage clamp techniques.16,17 IC50 values for inhibition of NMDA responses were determined by curve fitting.18 Sample data for compound 10e illustrating selectivity for NR1A/2B are given in Figure 1. Affinity for R1-adrenergic receptors was determined by [3H]prazocin binding assays.19 Inhibition of voltage-gated K+ currents was measured by whole-cell voltage clamp recordings from dissociated rat superior cervical ganglion neurons.20 Mouse MES assays were done as reported previously.21,22

**Results and Discussion**

SARs of N-(2-phenoxyethyl)-4-benzylpiperidine (8) and related analogues are given in Figure 2 and Table 1. The screening lead 8 had an IC50 value of 0.63 μM at NR1A/2B receptors: roughly comparable in potency to the prototype compound eliprodil and 6-fold less active than ifenprodil. Like eliprodil and ifenprodil, 8 was >100-fold selective for NR1A/2B as compared to NR1A/
2A or NR1A/2C. In terms of potential side effects, 8 had submicromolar affinity for $\alpha_1$-adrenergic receptors (IC$_{50}$ = 0.82 $\mu$M), presenting a liability to produce hypotension. In addition, 8 inhibited neuronal voltage-gated $K^+$ channels by 65% at 10 $\mu$M, suggesting a potential for prolongation of cardiac QT interval, a dose-limiting clinical side effect for eliprodil (in our assay eliprodil inhibited neuronal $K^+$ channels by 67% at 10 $\mu$M). Like eliprodil, 8 was a potent anticonvulsant when administered intravenously (iv) to mice (ED$_{50}$ = 2 mg/kg). Our goal was to modify 8 to improve NR2B potency while at the same time reducing $\alpha_1$ affinity and $K^+$ channel inhibition while retaining in vivo activity.

Introduction of a hydroxyl group on the 4-position of the phenyl ring, which is attached to the tether connecting to the nitrogen atom of the piperidine ring, led to compound 10a and increased NR2B potency by 25 times. As described in other series of compounds, this emphasizes the importance of having a hydrogen-bond-donating group at this position for interaction with the NMDA receptor. Though NR2B potency was increased in 10a, the $\alpha_1$ activity remained less than 1 $\mu$M.

Removing the methylene group from the spacer connecting the C-4 phenyl group to the piperidine ring gave compound 10d and unexpectedly resulted in a 200-fold drop in potency. In this instance, the SAR did not follow that described previously for compounds such as CP-101,606 where phenylpiperidines are optimal.

Simple substitution on the benzyl ring of 10a, such as 4-methyl (10b) and 4-fluoro (10c), had little effect on NR1A/2B potency or on the potential side effects profile. However, the in vivo potency of 10b was increased roughly 3 times (MES ED$_{50}$ = 0.7 mg/kg) compared to that of 10a, while the in vivo potency of 10c was slightly reduced (MES ED$_{50}$ = 3 mg/kg). This

![Diagram of compounds 8, 10a, 10b, 10c, 10d, 10e with IC$_{50}$ values]

**Figure 2.** SAR of the substituted benzylpiperidines at the NR1A/2B subtype.
suggests that substitutions on the benzyl ring can have significant effects on in vivo potency.

Introduction of a hydroxyl group into the C-4 position of the piperidine ring of 10b led to 10e and resulted in a modest reduction in NR2B potency (IC$_{50}$ = 0.043 µM). However, this substitution greatly improved the side noxyethyl-4-(4-methylbenzyl)piperidine, Co 101244/PD 1.5 mg/kg) giving a molecule with the desired pharmacological properties.

In conclusion, 10e (4-hydroxy-N-[2-(4-hydroxyphenoxy)ethyl]-4-(4-methylbenzyl)piperidine, Co 101244/PD 174494) has the best overall profile of the compounds described, one that rivals or exceeds that of previously reported NR2B antagonists. In addition, 10e has the advantage of achieving the desired profile without introduction of stereocenters. The SAR leading to 10e illustrates some of the key features necessary for designing potent, highly specific NR2B antagonists. Compound 10e is currently undergoing pharmacological evaluation for a variety of therapeutic applications including stroke and Parkinson’s disease.

Experimental Section

Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. The 'H NMR spectra were recorded at 300 MHz. Chemical shifts are reported in ppm (δ), and J coupling constants are reported in Hz. Elemental analyses were performed by Desert Analytics, Tucson, AZ. Mass spectra (MS) were obtained with a VG 12-250 or VG ZAB-2HF mass spectrometer. Reagent grade solvents were used without further purification unless otherwise specified. TLC was performed using TLC plates GF254. Column chromatography was performed on silica gel (230–400 mesh). Reverse-phase HPLC analyses were obtained at 254 nm on a 4.6 × 250 mm microsorb-MV C18 column, using 0.1% trifluoroacetic acid in water (A) and 0.1% trifluoroacetic acid in acetonitrile (B) as solvents. The linear gradient was 20% B in A to 95% B in A with a flow rate of 1 mL/min.

4-Benzyl-1-[2-(4-hydroxyphenyl)ethoxy]piperidine Hydrochloride (9a). A mixture of 4-benzylpiperidine (35 g, 0.20 mol), 1-bromo-2-phenoxymethane (42 g, 0.21 mol), and potassium carbonate (111 g, 0.80 mol) in 400 mL of methyl ethyl ketone was heated at reflux with vigorous stirring for 12 h. The reaction mixture was filtered, and the filtrate was evaporated. The residue was extracted with water/ether, and the ether layer was washed with 2 N hydrochloric acid. The amine hydrochloride salt separated from the aqueous layer was collected by filtration. The salt was treated with 2 N aqueous NaOH solution (150 mL) and extracted with ether. The extract was dried over MgSO$_4$ and treated with Norite. After filtration, the ether was removed, and the product was vacuum-distilled (45.6 g, bp 159–162 °C at 0.1 mmHg, 75% yield). The hydrochloride salt was formed in 2-propanol/HCl and recrystallized from ethyl acetate to give 8 as colorless crystals: mp 170–172 °C; 'H NMR (300 MHz, CDCl$_3$) δ 1.62 (m, 10 H), 1.82 (m, 2 H), 2.10 (m, 2 H), 2.80 (m, 2 H), 3.39 (m, 2 H), 3.65 (m, 2 H), 4.54 (m, 2 H), 6.85–7.31 (m, 10 H), 12.50 (brs, 1 H). Anal. (C$_{20}$H$_{26}$ClNO) C, H, N.

2-(4-Benzoxoxyphenoxo)ethyl Bromide (7b). A mixture of 4-benzoxoyphenoxy(10 g, 0.05 mol) and potassium carbonate (17.3 g, 0.125 mol) in 50 mL of acetonitrile and 21.6 mL of 1,2-dibromoethane was refluxed with stirring for 24 h. The inorganic salt was removed by filtration through a short column of silica gel which was washed with ethyl acetate (3 × 25 mL). The combined filtrate was evaporated in vacuo, and the residue was further purified by flash chromatography (5% EtOAc in hexane) to give 12 g (79%) of the title product as a white solid: mp 75–77 °C; 'H NMR (300 MHz, CDCl$_3$) δ 3.61 (t, J = 6.2 Hz, 2 H), 4.24 (t, J = 6.2 Hz, 2 H), 5.02 (s, 2 H), 6.87 (m, 4 H), 7.38 (m, 5 H).

4-Benzyl-1-[2-(4-benzoxoxyphenoxo)ethoxy]piperidine Hydrochloride (9a). A mixture of 2-(4-benzoxoxyphenoxy)ethyl bromide (7b) (1.44 g, 4.7 mmol), 4-benzylpiperidine (0.876 g, 5.0 mmol), potassium carbonate (1.725 g, 12.5 mmol), and 50 mL of acetonitrile was refluxed for 24 h. The inorganic salt was removed by filtration through a short column of silica gel which was washed with ethyl acetate (3 × 25 mL). The combined filtrate was evaporated in vacuo, and the residue was further purified by flash chromatography (50% EtOAc in hexane), giving 1.62 g (86%) of the free amine. The amine was dissolved in 50 mL of methanol and treated with 1 N HCl in methanol (6 mL). The solution was evaporated in vacuo and titrated with ether (100 mL). The white solid was collected by filtration and dried in vacuo, giving the title HCl salt in 100% yield: mp 164–166 °C; 'H NMR (300 MHz, CDCl$_3$) δ 1.51 (m, 1 H), 1.68 (d, J = 12.3 Hz, 2 H), 2.46 (m, 4 H), 2.94 (m, 2 H), 3.35 (m, 2 H), 3.45 (d, J = 11.7 Hz, 2 H), 4.26 (s, 2 H), 5.01 (s, 2 H), 6.89 (m, 4 H), 7.18–7.40 (m, 10 H), 10.2 (brs, 2 H); EIMS m/e 311 (M$^+$, 5), 202 (10), 188 (100), 91 (30).

4-Benzyl-1-[2-(4-hydroxyphenyl)ethoxy]piperidine Hydrochloride (9a). A solution of 4-benzyl-1-[2-(4-hydroxyphenyl)ethoxy]piperidine (9a) (401 mg, 1.0 mmol) in 25 mL of ethanol were added 1.0 mL of 1 M HCl in methanol and 100 mg of 10% Pd/C. The resulting mixture was hydroxidated at 30 psi of H$_2$ for 2 h. The catalyst was removed by filtration through a short column of Celite (5 g) and washed with methanol (3 × 15 mL). The combined filtrate was evaporated in vacuo to give an oil, and then ether (30 mL) was added to the residue. The resulting mixture was stirred at room temperature overnight. The white solid was collected by filtration and dried in vacuo, giving 330 mg (100%) of the title compound: mp 212–215 °C; 'H NMR (300 MHz, CDCl$_3$ + DMSO-d$_6$) δ 1.66 (m, 3 H), 1.83 (m, 2 H), 2.43 (s, 2 H), 2.63 (m, 2 H), 3.19 (m, 2 H), 3.40 (brs, 1 H), 4.25 (s, 2 H), 6.55 (m, 4 H), 6.94–7.09 (m, 5 H), 12.0 (brs, 1 H); EIMS m/e 311 (M$^+$, 5), 202 (10), 188 (100), 91 (30). Anal. (C$_{20}$H$_{26}$ClNO) C, H, N.

Table 1. In Vitro and in Vivo Profiles of Substituted Benzylpiperidines

<table>
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<tr>
<th>no.</th>
<th>NR1a/2A</th>
<th>NR1a/2B</th>
<th>NR1a/2C</th>
<th>IC$_{50}$ (µM)</th>
<th>α$<em>{1}$: IC$</em>{50}$ (µM)</th>
<th>% Inhib at 10 µM</th>
<th>MES: ED$_{50}$ (mg/kg)</th>
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<td>1</td>
<td>20 ± 6</td>
<td>0.11 ± 0.01</td>
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<td>0.22</td>
<td>54 ± 7.7</td>
<td>7.0</td>
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<tr>
<td>2</td>
<td>100.0</td>
<td>1.40</td>
<td>100.0</td>
<td>3.30</td>
<td>67 ± 6.2</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>8a</td>
<td>&gt;100</td>
<td>0.63 ± 0.10</td>
<td>&gt;100</td>
<td>0.82</td>
<td>65 ± 4.3</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>10a</td>
<td>&gt;100</td>
<td>0.025 ± 0.0009</td>
<td>&gt;100</td>
<td>0.47</td>
<td>46 ± 2.9</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>10b</td>
<td>&gt;100</td>
<td>0.028 ± 0.0004</td>
<td>&gt;100</td>
<td>1.80</td>
<td>67 ± 5.2</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>10c</td>
<td>21 ± 4</td>
<td>0.05 ± 0.017</td>
<td>&gt;100</td>
<td>1.60</td>
<td>53 ± 8.9</td>
<td>3.0</td>
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<tr>
<td>10d</td>
<td>110 ± 19</td>
<td>7.7 ± 1.5</td>
<td>&gt;100</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>10e</td>
<td>&gt;100</td>
<td>0.043 ± 0.0004</td>
<td>&gt;100</td>
<td>27</td>
<td>23 ± 5.6</td>
<td>1.5</td>
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a IC$_{50}$ values for inhibition of NMDA responses at cloned NMDA receptors expressed in oocytes. IC$_{50}$ values for inhibition of NMDA responses at cloned NMDA receptors expressed in Xenopus oocytes, as illustrated in Figure 1. Data are presented as mean ± SEM. Values were obtained from at least three oocytes for NR1a/2B and at least two oocytes for the other subunits. nd, not determined.
4-Methylbenzyltriphenylphosphonium Bromide (12a).

To a solution of triphenylphosphine (35.45 g, 0.135 mol) in 100 mL of methanol was added 1.02 g of 20% Pd(OH)₂. The resulting mixture was heated at 80 °C overnight. The white solid was collected by filtration and dried in vacuo, giving 650 mg (72%) of the title compound: mp 111–113 °C; 1H NMR (CDCl₃, 300 MHz) δ 1.70 (m, 2 H), 1.88 (m, 2 H), 2.02 (m, 2 H), 2.31 (s, 3 H), 2.57 (d, J = 6.9 Hz, 2 H), 2.72 (m, 2 H), 3.34 (m, 2 H), 3.63 (d, J = 11.4 Hz, 2 H), 4.49 (s, 2 H), 5.01 (s, 2 H), 6.81 (d, J = 8.4 Hz, 2 H), 6.88 (d, J = 8.4 Hz, 2 H), 6.99 (d, J = 7.8 Hz, 2 H), 7.08 (d, J = 7.8 Hz, 2 H), 7.32–7.39 (m, 5 H), 12.51 (brs, 1 H).

1-1[4-(Hydroxyphenyl)ethoxy]-1-(4-methylbenzyl)piperidine Hydrochloride (10b).

To a solution of 1-[2-(4-benzylxoyphenyl)ethoxy]-1-(3-methylbenzyl)piperidine hydrochloride (9b) (250 mg, 0.55 mmol) in 25 mL of ethanol was added 60 mg of 20% Pd(OH)₂. The resulting mixture was hydrogenated at 30 psi of hydrogen for 2 h. The catalyst was removed through a short column of Celite (5 g) and washed with methanol (3 × 15 mL). The combined filtrate was evaporated in vacuo and triturated with 30 mL of ether. The white solid was collected by filtration and dried in vacuo, giving 140 mg (88%) of the title product: mp 198–200 °C; 1H NMR (CDCl₃, 300 MHz) δ 1.60 (m, 2 H), 1.88–1.92 (m, 3 H), 2.29 (s, 2 H), 2.57 (d, J = 6.6 Hz, 2 H), 3.06 (m, 2 H), 3.47 (m, 2 H), 3.61 (m, 2 H), 4.24 (t, J = 5.1 Hz, 2 H), 6.71 (dd, J₁ = 2.4 Hz, J₂ = 6.6 Hz, 2 H), 6.83 (dd, J₁ = 2.4 Hz, J₂ = 6.6 Hz, 2 H), 7.70 (m, 4 H), Anal. C₂₅H₂₀O₂N₂ H₂O.

Fluorobenzyltriphenylphosphonium Bromide (12b).

To a solution of triphenylphosphine (26.2 g, 0.1 mol) in 100 mL of ether was added 4-fluorobenzyl bromide (12b) (18.9 g, 0.1 mol). The resulting solution was stirred at room temperature overnight. The white solid was collected by filtration and dried at 80 °C under vacuum. The resulting mixture was stirred at 80 °C overnight and then poured over ice (400 g) and extracted with ether (3 × 200 mL). The combined extracts were dried over sodium sulfate. The solvent was evaporated in vacuo and further purified by flash chromatography (elucent 5% EtOAc in hexanes), giving 7.0 g (83%) of the title compound: 1H NMR (CDCl₃, 300 MHz) δ 1.60 (m, 1 H), 1.75 (m, 2 H), 2.07 (m, 2 H), 2.29 (s, 3 H), 2.54 (m, 4 H), 3.40 (d, J = 10.5 Hz, 2 H), 4.09 (d, J = 4.8 Hz, 2 H), 6.99 (d, J = 7.8 Hz, 2 H), 7.05 (d, J = 7.8 Hz, 2 H), 7.42 (m, 4 H), 7.60 (m, 2 H), 12.40 (brs, 1 H).

4-(Methylbenzyl)piperidine Hydrochloride (6c).

A mixture of 1-benzyl-4-(4-methylbenzyl)piperidine hydrochloride (14a) (4.1 g, 13 mmol) and 1.02 g of 10% Pd/C in 100 mL of methanol was hydrogenated at 60 psi for 12 h. The catalyst was removed by filtration through a short column of Celite (10 g) and washed with methanol (3 × 20 mL). The filtrate was evaporated in vacuo, redissolved into 20 mL of methanol, and treated with 30 mL of 1 M solution of HCl in methanol. The resulting solution was evaporated in vacuo and triturated with 60 mL of ether. An off-white solid was collected by filtration and dried at 80 °C overnight and then poured over ice (400 g) and extracted with ether (3 × 200 mL). The combined extracts were dried over sodium sulfate. The solvent was evaporated in vacuo and further purified by flash chromatography (elucent 5% EtOAc in hexanes), giving 7.0 g (83%) of the title compound as a pale-yellow oil: 1H NMR (CDCl₃, 300 MHz) δ 2.26 (s, 3 H), 2.32 (m, 4 H), 2.43 (m, 4 H), 3.45 (s, 2 H), 6.16 (s, 1 H), 7.03 (m, 4 H), 7.18–7.26 (m, 5 H).
hydrochloride (6c) (2.6 g, 11.4 mmol), and potassium carbonate (3.91 g, 28 mmol) in 60 mL of acetonitrile was refluxed for 12 h. The inorganic salt was removed by filtration through a short column of silica gel and washed with ethyl acetate (3 × 25 mL). The combined filtrate was evaporated in vacuo to give a crude mixture, which was purified by flash chromatography (20% methanol in ethyl acetate), giving 4.0 g (84%) of the title product: mp 196–198 °C; 1H NMR (300 MHz, CD3OD) δ 1.58 (m, 2 H), 1.89 (m, 3 H), 2.60 (d, J = 6.3 Hz, 2 H), 3.08 (m, 2 H), 3.49 (t, J = 5.1 Hz, 2 H), 3.62 (m, 2 H), 4.25 (t, J = 5.1 Hz, 2 H), 6.77 (d, J = 9.3 Hz, 2 H), 7.02 (m, 2 H), 7.20 (m, 2 H).

4-(4-Fluorobenzyl)-1-[2-(4-hydroxyphenyl)ethoxy]-piperidine Hydrochloride (10c). To a solution of 1-[2-(4-benzylxoxyphenyl)ethoxy]-4-[4-(fluorobenzyl)]piperidine (9c) (4.0 g, 9.5 mmol) in 100 mL of methanol was added 1.0 g of 5% Pd/C. The resulting mixture was hydrogenated at 35 psi of hydrogen for 4 h. The catalyst was removed through a short column of Celite (5 g) and washed with methanol (3 × 15 mL). The combined filtrate was treated with 15 mL of 1 M HCl in methanol. The solution was evaporated in vacuo and triturated with 100 mL of ether. An off-white solid was collected by filtration and dried in vacuo, giving 3.2 g (95%) of the title product: mp 196–198 °C; 1H NMR (300 MHz, CD3OD) δ 0.82; NaHCO3, 2.4; Hepes, 5; pH 7.4, with 0.1 mg/mL gentamicin sulfate. Standard voltage clamp recordings were made using manual micromanipulators and glass capillary microelectrodes as previously.16 Individual oocytes were microinjected with a 1:4 ratio; all other binary subunit combinations were injected 1:1 (1 ng each subunit). P. H. Seeburg (Heidelberg University, Heidelberg, Germany).5e

After the addition was complete, the reaction mixture was stirred at room temperature for 3 h and stood overnight. The reaction mixture was treated with 100 mL of saturated aqueous NH4Cl solution at 0 °C and then extracted with dichloromethane (2 × 50 mL). The combined organic phases were evaporated in vacuo to give an oil, which was redissolved in 200 mL of dichloromethane, washed with saturated aqueous NH4Cl solution (2 × 30 mL) and brine (50 mL), and then dried over anhydrous sodium sulfate (Et2OAc, Rv = 0.25) gave 7.5 g (96%) of the product as a pale-yellow oil: 1H NMR (300 MHz, CDCl3) δ 1.48 (m, 2 H), 1.73 (m, 2 H), 2.05 (s, 1 H), 2.32 (m, 5 H), 2.61 (m, 2 H), 2.71 (s, 2 H), 3.51 (s, 2 H), 7.09 (m, 4 H), 7.30 (m, 5 H).

4-Hydroxy-4-(4-methylbenzyl)piperidine Hydrochloride (6e). A mixture of 1-benzyl-4-hydroxy-4-(4-methylbenzyl)piperidine (16) (2.8 g, 9.5 mmol) and 700 mg of 10% Pd/C in 100 mL of 95% ethanol was hydrogenated at 50 psi for overnight. The catalyst was removed by filtration through a short column of Celite (10 g) and washed with methanol (3 × 15 mL). The filtrate was treated with 12 mL of 1 M HCl in methanol. The resulting solution was evaporated in vacuo and triturated with 30 mL of ether. A white solid was collected by filtration and dried in vacuo, giving 2.1 g (92%) of the title product: mp 183–185 °C; 1H NMR (300 MHz, CDCl3) δ 1.68 (m, 2 H), 2.10 (m, 2 H), 2.34 (3, 3 H), 2.78 (s, 2 H), 3.24 (m, 5 H), 7.05 (d, J = 7.5 Hz, 2 H), 7.14 (d, J = 7.5 Hz, 2 H), 9.30 (brs, 1 H), 9.52 (brs, 1 H).

1-[2-(4-Benzylxoxyphenyl)ethyl]oxy]-4-hydroxy-4-(4-methylbenzyl)piperidine Hydrochloride (9e). A mixture of 2-(4-benzylxoxyphenyl)ethyl bromide (7b) (368 mg, 1.2 mmol), 4-hydroxy-4-(4-methylbenzyl)piperidine hydrochloride (6e) (290 mg, 1.2 mmol), and potassium carbonate (414 mg, 3 mmol) in 30 mL of acetonitrile was refluxed for 12 h. The inorganic salt was removed through a short column of silica gel and washed with ethyl acetate (3 × 25 mL). The combined filtrate was evaporated in vacuo to give a crude mixture, which was purified by flash chromatography (50% methanol in ethyl acetate) to give a residue, a solution of which in 10 mL of methanol was treated with 3 mL of 1 M HCl in methanol. The resulting solution was evaporated in vacuo and triturated with 50 mL of ether. A white solid was collected by filtration and dried in vacuo, giving 420 mg (75%) of the title product: mp 179–181 °C; 1H NMR (300 MHz, CDCl3) δ 1.61 (s, 2 H), 1.73 (d, J = 14.1 Hz, 2 H), 2.33 (s, 3 H), 2.45 (m, 2 H), 2.81 (s, 2 H), 3.22 (m, 2 H), 3.36 (s, 1 H), 3.46 (d, J = 8.4 Hz, 2 H), 4.49 (s, 2 H), 5.01 (s, 2 H), 6.82 (d, J = 9.0 Hz, 2 H), 6.90 (d, J = 7.5 Hz, 2 H), 7.08 (m, 2 H), 7.17 (d, J = 7.5 Hz, 2 H), 7.38 (m, 5 H), 12.40 (brs, 1 H).

4-Hydroxy-1-[2-(4-benzylxoxyphenyl)ethyl]-4-(4-methylbenzyl)piperidine Hydrochloride (10e). To a solution of 1-[2-(4-benzylxoxyphenyl)ethyl]-4-(4-methylbenzyl)piperidine hydrochloride (9e) (0.25 g, 0.53 mmol) in 30 mL of methanol was added 62.5% of 20% Pd(OH)2. The resulting mixture was hydrogenated at 20 psi of hydrogen for 3 h. The catalyst was removed through a short column of Celite (5 g) and washed with methanol (3 × 15 mL). The filtrate was evaporated in vacuo and triturated with 30 mL of ether. A white solid was collected by filtration and dried in vacuo, giving 240 mg (75%) of the title product: mp 180–182 °C; 1H NMR (300 MHz, CDCl3) δ 1.61 (s, 2 H), 1.73 (d, J = 14.1 Hz, 2 H), 2.33 (s, 3 H), 2.45 (m, 2 H), 2.81 (s, 2 H), 3.22 (m, 2 H), 3.36 (s, 1 H), 3.46 (d, J = 8.4 Hz, 2 H), 4.49 (s, 2 H), 5.01 (s, 2 H), 6.82 (d, J = 9.0 Hz, 2 H), 6.90 (d, J = 7.5 Hz, 2 H), 7.08 (m, 2 H), 7.17 (d, J = 7.5 Hz, 2 H), 7.38 (m, 5 H), 12.40 (brs, 1 H).

Oocyte Electrophysiology. Oocytes were obtained from mature female Xenopus laevis and were prepared as described previously.16 Individual oocytes were microinjected with a mixture of NDMA receptor-encoding cDNAs, provided by Dr. P. H. Seeburg (Heidelberg University, Heidelberg, Germany).5b NA11 and NA21 were injected at a 1:4 ratio; all other binary subunit combinations were injected 1:1 (1–10 ng of each subunit). Oocytes were stored in Barth’s medium containing (in mM): NaCl, 88; KCl, 1; CaCl2, 0.41; (Na2SO4)0.33; MgSO4, 0.82; NaHCO3, 2.4; Hepes, 5; pH 7.4, with 0.1 mg/mL gentamicin sulfate. Standard voltage clamp recordings were made using manual micromanipulators and glass capillary microelectrodes as previously.16
at -70 mV in nominally Ca²⁺-free Ringer solution (in mM): NaCl, 115; KCl, 2; BaCl₂, 1.8; Hepes, 5; pH 7.4. Drugs were diluted in Ca²⁺-free Ringer solution and applied by bath perfusion (7-10 mL/min) in a conventional flow-through chamber (volume ~0.2 mL). Test drugs were dissolved in DMSO and diluted into Ringer just prior to application (final [DMSO] = 0.1-1%). IC₅₀ values were obtained from partial (3-5 points) concentration–inhibition curves using the equation:

$$I / I_{\text{control}} = \left[\frac{1}{1 + \text{[antagonist]/IC}_{50}}\right] + \text{min}$$

where Icontrol is the current in the absence of antagonist, min (minimum) is the residual fractional response at a saturating concentration of antagonist, n is the slope factor, and IC₅₀ is the concentration of drug that produces one-half this level of inhibition. To fit the curves for NR1a/2B, ‘min’ was fixed at 0.15. Data in the text are mean ± standard error (SE).

α₂-Adrenergic Receptor Binding. Test compounds were evaluated at nine concentrations in duplicate added in 5-µL aliquots (1% DMSO final) to 96-well, 1.0-mL volume assay plates and incubated in a total volume of 500 µL for 60 min at room temperature as described below. Assays were terminated by filtration through GF/B filter plates (Packard, Meriden, CT), and the filter plates were rinsed three times with ~0.8 mL of assay buffer/well. Microscint-20 scintillation cocktail (50 µL/well; Packard) was added to the dried filter plates, which were then counted on a TopCount (Packard) scintillation counter for 2 min/well. IC₅₀ values were determined by fitting the data to the sigmoidal equation using Prism (GraphPad, San Diego, CA). [1H]Prazosin binding assay was modified from previously described methods. Frozen Sprague-Dawley rat cortices obtained from ABS (Wilmington, DE) were thawed, homogenized in 10 volumes of ice-cold 0.25 M sucrose/10 mM Tris/HCl (pH 7.4) buffer, and centrifuged at 10000 × g for 30 min at 4 °C. The supernatant was centrifuged at 40000 × g for 30 min; the pellet was resuspended in 0.2 mL of assay buffer/well. Microscint-20 scintillation cocktail (200 µL/well; Packard) was added to the dried filter plates, which were then counted on a TopCount (Packard) scintillation counter for 2 min/well. IC₅₀ values were determined by fitting the data to the sigmoidal equation using Prism (GraphPad, San Diego, CA). The [1H]prazosin binding assay was modified to previously described methods.19

K⁺ Channel Electrophysiology. Superior cervical ganglion (SCG) neurons from 1- to 4-day-old rat pups were dissociated and plated into 35-mm dishes using standard techniques. Whole-cell voltage clamp recordings of K⁺ channel currents were made 24-48 h later.20 The external solution contained NaCl (150 mM), KCl (5 mM), MgCl₂ (1.1 mM), CaCl₂ (2.6 mM), Hepes (10 mM), and glucose (10 mM), with pH adjusted to 7.4 with NaOH. The internal solution contained KCl (80 mM), potassium aspartate (50 mM), EGTA (10 mM), and Hepes (10 mM), with pH adjusted to 7.3 with KOH. Test compounds were dissolved in DMSO at a concentration of 10 mM with final concentrations obtained by serial dilution in the external solution. Neurons were voltage-clamped at a potential of -60 mV, and 30-ms steps to +50 mV elicited K⁺ channel currents. Control responses were obtained before application of drug to neurons by local perfusion. Data were expressed as a percent inhibition of sustained K⁺ current.

MES Assays. Procedures for mouse MES assay were as reported previously. Compounds were dissolved in 0.05 M Tris and tested for anticonvulsant effect at the peak of activity which occurred 2 min after iv administration. ED₅₀ values were determined by Litchfield and Wilcoxon analysis.

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