Structure−Activity Relationships for a Series of Bis(phenylalkyl)amines: Potent Subtype-Selective Inhibitors of *N*-Methyl-D-aspartate Receptors

Amir P. Tamiz,[†] Edward R. Whittemore,[‡] Zhang-Lin Zhou,[‡] Jin-Cheng Huang,[‡] John A. Drewe,[‡] Jie-Cheng Chen,[‡] Sui-Xiong Cai,[‡] Eckard Weber,[‡] Richard M. Woodward,*[,‡] and John F. W. Keana*[,†]

Department of Chemistry, University of Oregon, Eugene, Oregon 97403, and CoCensys, Inc., 201 Technology Drive, Irvine, California 92618

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A series of bis(phenylalkyl)amines, structural analogues of ifenprodil and nylidrin, were synthesized and tested for antagonism of *N*-methyl-D-aspartate (NMDA) receptors. Potency and subunit selectivity were assayed by electrical recordings in *Xenopus* oocytes expressing three binary combinations of cloned rat NMDA receptor subunits: NR1A expressed in combination with either NR2A, NR2B, or NR2C. The bis(phenylalkyl)amines were selective antagonists of NR1A/2B receptors. Assayed under steady-state conditions, the most potent of these, *N*-[2-(4-hydroxyphenyl)ethyl]-5-phenylpentylamine hydrochloride **(20)**, has an IC_{50} value of 8 nM and >1000-fold selectivity with respect to NR1A/2A and NR1A/2C receptors. The structure−activity relationship of the bis(phenylalkyl)amine series indicates that the piperidine ring and alkyl chain substitutions common to NR2B-selective antagonists such as ifenprodil, CP 101,606, and Ro 25-6981 are not necessary to generate potent and selective ligands. The primary determinants of potency are the phenolic OH group, acting as a hydrogen bond donor, the distance between the two rings, and an electrostatic interaction between the receptor and the basic nitrogen atom. This study provides a framework for designing structurally novel NR2B-selective antagonists which may be useful for treatment of a variety of neurological disorders.

Introduction

N-Methyl-D-aspartate (NMDA) receptor antagonists have therapeutic potential as neuroprotectants, anticonvulsants, analgesics, and agents that augment the effects of L-DOPA for the treatment of Parkinson's disease.[1] Development of clinically useful drugs, however, has been hampered by a variety of dose-limiting side effects which include neurotoxicity, psychotomimetic behaviors, and a narrow therapeutic index with respect to sedation.[2] Studies at the molecular level suggest that native NMDA receptors are heterooligomeric assemblies of two different types of subunits. The subunits are designated NMDA receptor 1 (NR1), of which there are eight isoforms generated by alternate RNA splicing, and NR2, of which there are four distinct types each transcribed from a separate gene.[3] Subunit composition and distribution of native receptors in adult mammalian brain differ significantly from region to region.[4] Characterization of recombinant receptors of defined subunit composition indicates that NMDA receptor subtypes have different pharmacological properties and thus represent discrete therapeutic targets.[3,4] It follows that by designing subtype-selective antagonists it may be possible to find high-potency drugs that also have more favorable side effect profiles when compared to broad-spectrum inhibitors.

The first subtype-selective NMDA receptor antagonist to be discovered was ifenprodil **(1)**, which has pronounced selectivity for receptors composed of NR1 and NR2B subunits.[5] Using ifenprodil as a starting point, novel series of NR2B-selective antagonists have been designed leading to compounds such as eliprodil **(2)**,[6] CP 101,606 **(3)**,[7] and Ro 25-6981 **(4)** (Figure 1).[8] These drugs are all reported to have neuroprotective effects in animal models of focal cerebral ischemia without themselves inducing neurotoxicity or showing behavioral liability in drug discrimination studies.[6−8] In addition to ifenprodil analogues, recent studies indicate

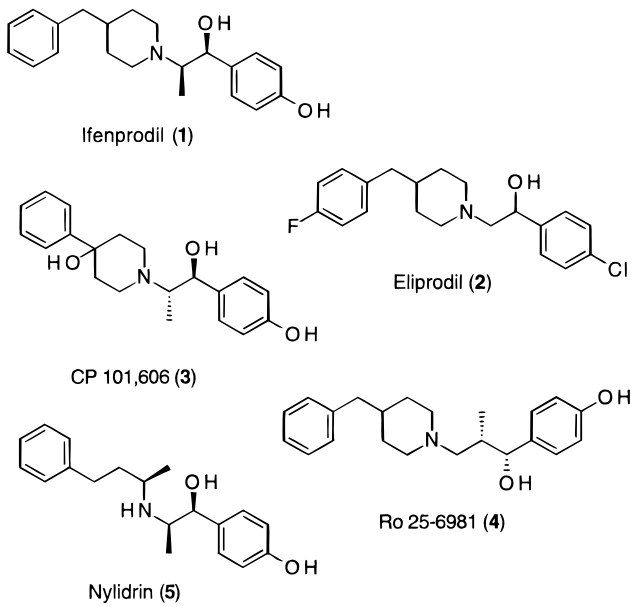

Figure 1. Potent antagonists of the NR2B subtype of the NMDA receptor. Compounds **1**, **2**, and **5** were tested as racemates; only one enantiomer of **1** and **5** is shown.

* To whom correspondence should be addressed.
[†] University of Oregon.
[‡] CoCensys, Inc.

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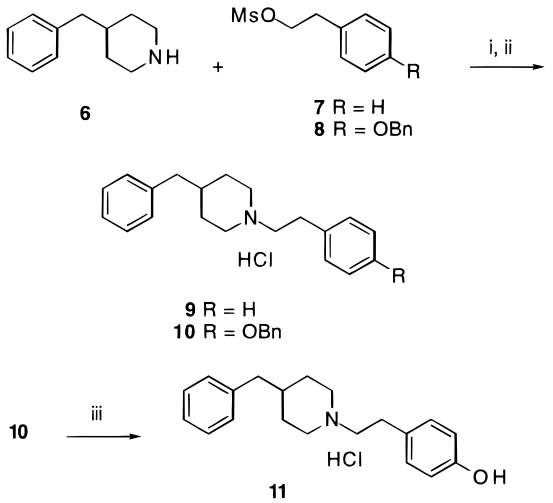
Scheme 1[a]



[a] Reagents: (i) K_2CO_3, CH_3CN; (ii) 1 M HCl/MeOH; (iii) 10% Pd/C, H_2, EtOH.

that haloperidol and related molecules are also NR2B-selective antagonists.[9,10] Whether related to ifenprodil or haloperidol, all these compounds are 1,4-disubstituted piperidines, and all inhibit NMDA receptor function by a noncompetitive, allosteric mechanism at a site, or sites, which are not located in the membrane-spanning region of the channel pore.[11]

In the present study we have designed a series of bis-(phenylalkyl)amines to investigate some of the basic determinants of potency for NR2B-selective inhibitors. Potency and selectivity of ligands were assessed by functional assays in *Xenopus* oocytes expressing recombinant NMDA receptors.[9] The impetus for the series came from the observation that nylidrin (**5**), a nonpiperidine analogue of ifenprodil, is a surprisingly potent NR2B-selective NMDA receptor antagonist.[12] The study led us to the discovery of the amine **20**, one of the most potent and selective inhibitors of NR2B NMDA receptors reported to date. Amine **20** shows low-nanomolar potency for the NR1A/2B subunit combination and >1000-fold selectivity with respect to NR1A/2A and NR1A/2C.

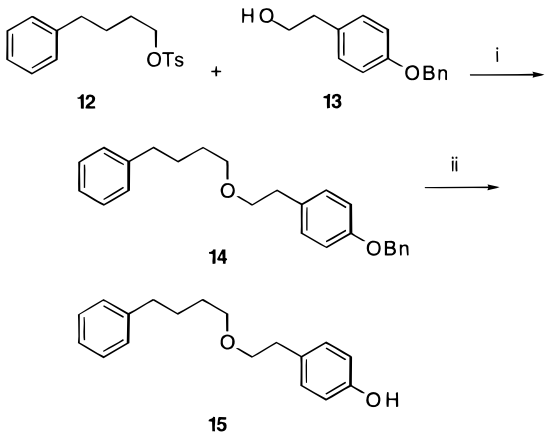
Chemistry

Piperidine **11** was prepared by *N*-alkylation of 4-benzylpiperidine (**6**) with mesylate **8** followed by *O*-debenzylation (Scheme 1). *N*-Alkylation of **6** with mesylate **7** gave piperidine **9**. Ether analogue **15** was prepared by the reaction the sodium salt of alcohol **13** with tosylate **12**, followed by *O*-debenzylation (Scheme 2).

For the synthesis of amines **20** and **21**, commercially available alcohols **16** and **17** were first oxidized to the corresponding aldehydes (**18** and **19**) with pyridinium chlorochromate (PCC) in CH_2Cl_2 in high yield (Scheme 3). Reductive amination with 2-(4-hydroxyphenyl)-ethylamine using $NaCNBH_3$ in MeOH gave the free amines which were isolated as their respective HCl salts **20** and **21**.

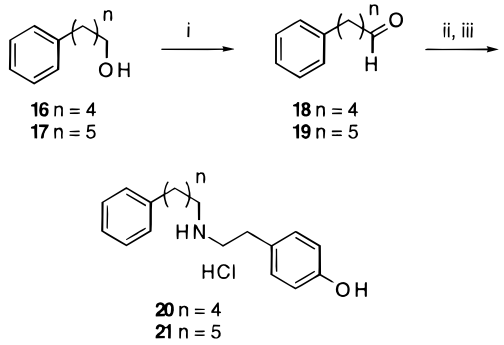
Open-chain secondary amines **29**−**34** were prepared as depicted in Scheme 4. Condensation of acids **22**−**24** with the corresponding amines **25**−**28** in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) followed by $LiAlH_4$ reduction of the intermediate amide gave amines **29**−**34** in good yields. Amines **29**−**33** were isolated as the hydrochloric salts. *N*-Alkylation of **34** with ethyl iodide gave amine **35**.

Scheme 2[a]



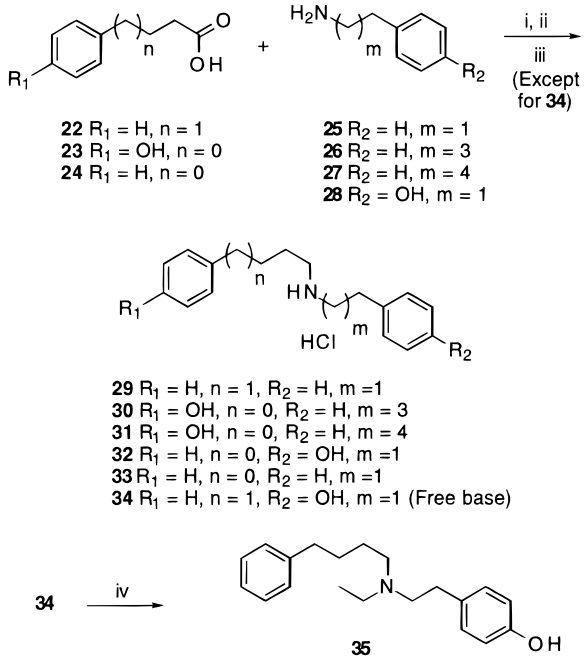
[a] Reagents: (i) NaH, DMF; (ii) 10% Pd/C, H_2, MeOH.

Scheme 3[a]



[a] Reagents: (i) PCC, CH_2Cl_2; (ii) 2-(4-hydroxyphenyl)ethylamine, $NaCNBH_3$, MeOH; (iii) HCl/ether.

Scheme 4[a]



[a] Reagents: (i) DCC, HOBT, DMF, 60 °C; (ii) $LiAlH_4$, THF, reflux; (iii) HCl/MeOH; (iv) CH_3CH_2I, $NaHCO_3$, CH_3CN.

Table 1. Functional Antagonism of Substituted Amines at NMDA Receptor Subtypes

	IC_{50} (μM)[a]		
compd no.	1A/2A	1A/2B	1A/2C
1	20 ± 6	0.11 ± 0.01	>100
5[b]	32 ± 2.0	0.18 ± 0.03	42 ± 1.1
9	>100	1.1 ± 0.2	>100
11	57 ± 8	0.20 ± 0.02	>100
15	>100	3.7 ± 0.3	>100
20	12 ± 2	0.008 ± 0.001	39 ± 4
21	17 ± 4	0.035 ± 0.008	30 ± 2
29	46 ± 7	3.5 ± 0.3	36 ± 4
30	18 ± 2	0.015 ± 0.002	48 ± 3
31	18 ± 3	0.045 ± 0.01	39 ± 4
32	26 ± 13	0.096 ± 0.01	>100
33	>100	7.3 ± 0.8	>100
34	15 ± 3	0.043 ± 0.004	65 ± 5
35	27 ± 5	0.30 ± 0.03	88 ± 13

[a] IC_{50} values (±SEM) were determined by electrical assays in *Xenopus* oocytes expressing the NMDA receptor combinations. Values were obtained from ≥3 oocytes for NR1A/2B and ≥2 oocytes for the other subunit combinations. [b] See ref 12a.

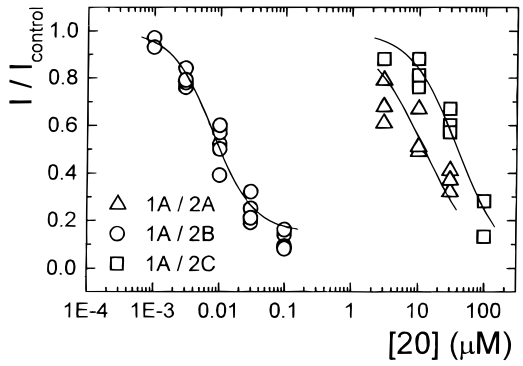


Figure 2. Concentration−inhibition curves for amine **20:** effect of varying the NR2 subunit.

Biological Evaluation

Potencies of antagonism at the three binary NMDA receptor subunit combinations are given in Table 1. Potency and subunit selectivity were assayed by electrical recordings under steady-state conditions in *Xenopus* oocytes expressing three binary combinations (NR1A expressed in combination with either NR2A, NR2B, or NR2C) of cloned rat NMDA receptor subunits. The IC_{50} values were determined by curve fitting to concentration−inhibition data pooled from 2−7 separate experiments (see the Experimental Section for details). Sample data are given in Figure 2. All compounds showed high or moderate potency ($IC_{50} \leq 7.5$ μM) at the NR1A/2B subunit combinations. In the following discussion we consider the high-potency inhibition of NR1A/2B receptors first, before briefly addressing the weak inhibition of NR1A/2A and NR1A/2C receptors.

Structure−Activity Relationship

Structural relationships between ifenprodil, nylidrin, and the related bis(phenylalkyl)amines are outlined in Figure 3. In the current experiments ifenprodil inhibited NR1A/2B receptors with an IC_{50} value of 0.11 μM. This is a slightly higher potency than we had reported in a previous study,[10] but it is comparable with other data from recombinant receptors[5] and native receptors in cultured rat neurons.[10,11] Removal of the methyl and hydroxyl groups from the ethylene linker gives **11** and results in a small but significant decrease in potency. Removal of the phenolic hydroxyl group gave **9**, the unsubstituted analogue of ifenprodil. Potency of piperidine **9** is 10-fold weaker than that of ifenprodil at NR1A/2B receptors. Opening the piperidine ring of **11** to form the tertiary amine **35** causes only a slight decrease in potency. The activity of this non-piperidine amine was portended by the potent inhibitory effects of nylidrin. Interestingly, removing the ethyl group from the nitrogen atom to form the secondary amine **34** results in a 7-fold increase in potency. This simple molecule has an apparent potency for NR1A/2B receptors that is more than twice that of ifenprodil. The increase in potency can be attributed to the less hindered nature of the basic nitrogen atom providing a more effective electrostatic interaction with the receptor pocket. The potency and selectivity of **34** indicate that conformational constraints conferred by the piperidine ring are not important for generating high-potency NR2B-selective antagonists. In the secondary amine series, removing the hydroxyl group from the phenolic ring of **34** gives **29** and results in an 80-fold drop in potency as compared to a 5-fold drop in potency in going from **11** to **9**. The non-phenolic **29** is only ∼10-fold selective for NR1A/2B as compared to the other two subunit combinations. Shortening the chain between the nitrogen atom and the non-phenolic ring in **34** by one methylene gave **32** and results in a 2-fold reduction in potency. Phenol **32** is an analogue of nylidrin with all substitutions on the chain removed. These structural modifications result in a 2-fold increase in potency with respect to the parent structure **5**. It is also noteworthy that **32** can be considered as the secondary amine analogue of amine **3**, by removing the CH_3 and OH substituents on the chain and the OH substitution on the piperidine ring. In our assays amine **3** (synthesized in house as a reference) has an IC_{50} value of ∼0.1 μM, a value similar to that of **32**. Whether comparing **32** with nylidrin or amine **3**, the results indicate that the piperidine ring and substituents on the chain with all the associated stereochemistry are superfluous to generating potent NR2B-selective antagonists. In contrast, removing the phenolic hydroxyl group (**33**) causes a substantial drop in activity.

Extending the chain length of **34** by addition of one methylene between the nitrogen atom and the unsubstituted ring gives **20** and results in a further 5-fold increase in potency. With an IC_{50} of 8 nM, **20** is among the most potent NR2B-selective antagonists yet reported. Levels of selectivity for **20** are >1000-fold with respect to either the NR2A- or NR2C-containing subunit combinations. Similarly, extending the chain of **34** by one methylene between the nitrogen atom and the phenolic ring gives **30** and also increases potency. This molecule can be considered the secondary amine analogue of amine **4** with OH and CH_3 substituents removed from the chain. The two molecules again have comparable IC_{50} values,[8] further suggesting that the substituents on the chain are not important determinants of potency. It is noteworthy that the methylene count between the two phenyl groups in compounds **20** and **30** is the same. Adding one methylene between the piperidine and phenolic ring of ifenprodil results in amine **4** and a ∼10-fold increase in potency on recom-

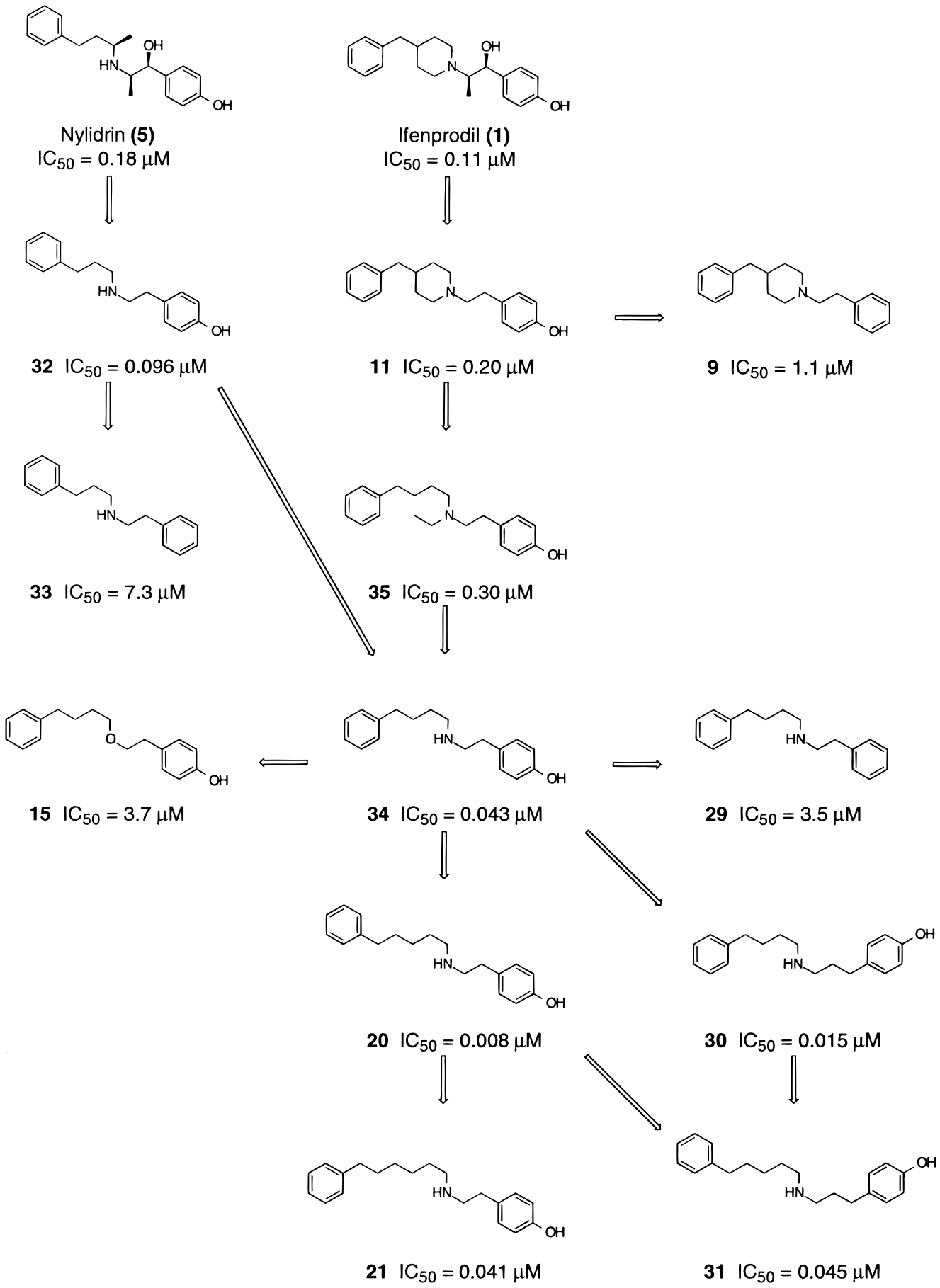
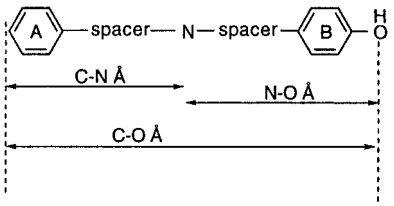


Figure 3. Structure−activity relationship of the substituted amines at the NR1A/2B subtype.

Table 2. Intramolecular Distances (Å) between Atoms Measured on the Fully Extended Conformer Calculated at the Semiempirical (AM1) Level



compd no.	C−O	N−O	C−N	1/2B (μM)
1	13.9	7.7	6.6	0.11
4	15.0	8.8	6.6	0.009[a]
5	13.8	7.8	7.1	0.18
20	17.5	7.8	9.9	0.008
30	17.0	8.8	8.9	0.015

[a] See ref 8.

binant NR1A/2B receptors.[8] In contrast, making the same modification on **20** to give **31** does not produce an increase in potency; instead, potency is reduced ~5-fold. One explanation for this difference is that it is not the distance between the nitrogen atom and the phenolic ring that is being optimized with amine **4** but instead the total distance separating the aromatic rings (Table 2). Thus the increase in potency between **34** and **20** may occur for the same reason as the increase in potency between ifenprodil and amine **4**. Extending the length of **20** by an additional methylene between the nitrogen atom and the unsubstituted ring gives **21** and results in a 5-fold loss of potency. Similarly, extending **30** by one methylene to give **31** reduces potency 3-fold. There is no significant difference in potency between **21** and **31**. In both cases it would appear that the optimum distance between the two phenyl groups has been exceeded and that the additional chain length is now becoming a steric liability. Finally, substituting the nitrogen atom in **34** by oxygen gives **15** and results in a 100-fold reduction in potency. Hence the existence of effective electrostatic interaction between the nitrogen atom and the receptor is very important for high potency.

Intramolecular distances (Table 2) were measured (Å) corresponding to the fully extended minimized conformer calculated using AM1 semiempirical calculations. After geometry optimization, the fully elongated conformer for amine **20** has an overall length of 17.5 Å (distance measured from the para carbon of the A-ring to the hydroxyl oxygen of the B-ring). This length is approximately 3 Å longer than that calculated for ifenprodil or nylidrin. The distance from the nitrogen atom to the phenolic hydroxyl group **20** is ~7.8 Å. This distance is an important component leading to effective binding and is close to the distance observed for nylidrin and ifenprodil. Comparison of the distances between the nitrogen atom and the A-ring (see Table 2) indicates that a hydrophobic pocket presumably interacting at the A-ring side of the inhibitor should be located within 9−10 Å from the nitrogen binding site. This distance is about ~2 Å longer when compared with the piperidine-based molecules (having an out-of-plane equatorial benzylic moiety). Yet amine **4** (a piperdine-based molecule) has comparable potency when directly compared to amine **30**. Thus the bound secondary amine inhibitors possibly adopt a bent conformation on the

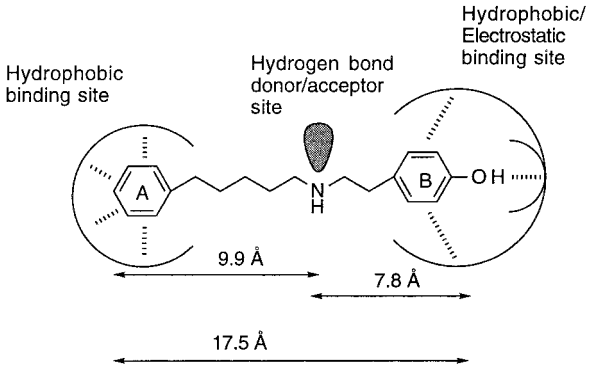


Figure 4. Receptor features presumed important for the binding of inhibitors at the NR1A/2B subtype.

methylene linker toward the A-ring for most effective binding. Extending the total length of the molecule to 19 Å in amine **21** renders the molecule less potent.

Inhibition of NR1A/2A and NR1A/2C receptors by this series of compounds was consistently weaker when compared to inhibition of NR1A/2B receptors, though NR1A/2A receptors generally had higher sensitivity than NR1A/2C. It is difficult to develop a structure−activity relationship (SAR) for inhibition of NR1A/2A because differences in potency between compounds are not great: the most potent inhibitor **20** has an IC_{50} of 12 μM, and the cutoff for measurements was 100 μM. Within this narrow range, there appears to be a trend such that the most potent NR1A/2B antagonists also have the higher potency for inhibition of NR1A/2A. Exceptions include amine **29** that is more active at NR1A/2A than would have been predicted by its NR1A/2B potency. Even given this trend, the most potent NR1A/2B antagonists still have the highest levels of selectivity with respect to the other two subunit combinations. Developing a SAR for inhibition of NR1A/2C receptors, where IC_{50} values only range from 30 to 100 μM, is even more fruitless than for the NR1A/2A receptor. The most potent NR1A/2B antagonists also appear to have higher potency at NR1A/2C. Again the obvious exception is **29**, which has an IC_{50} of 3.5 μM at NR1A/2B but is still among the most potent inhibitors at NR1A/2C.

Separate studies indicate that high-potency NR1A/2B antagonism by this class of drug is largely unaffected by voltage, whereas the low-potency inhibition of NR1A/2A and NR1A/2C is voltage-dependent.[5b,9,10] For the low-potency antagonism this implies that the primary site of inhibition is probably located in the channel pore. Thus, there are no a priori reasons why there should be parallels between SARs for the different subunit combinations. To the extent that such relationships exist, it would suggest that the distinct sites mediating the high-potency and low-potency inhibition share common structural features in their ligand binding pockets.

Conclusion

For the current series of subtype-selective NMDA receptor antagonists, the primary determinants of potency at the 1A/2B NMDA receptors are (1) the phenolic OH group which presumably serves as a H-bond donor; (2) the distance between the two aromatic rings; (3) an electrostatic interaction between the receptor and the nitrogen atom (see Figure 4). Our data

demonstrate that the piperidine ring and alkyl chain substitutions common to many NR2B-selective antagonists such as ifenprodil are not necessary for high potency and selectivity, while the presence of a basic nitrogen atom, whether secondary or tertiary, in the chain does contribute to more potent binding. The potency and subtype selectivity of this series of bis-(phenylalkyl)amines provide a framework for designing other types of novel NR1A/2B-selective antagonists.

Experimental Section

General. Compounds **1** and **5** were purchased from commercial sources. Compound **3** was prepared according to the literature procedures.[7a] Reagents and solvents were purchased from commercial suppliers and used as received. All starting materials were commercially available unless otherwise indicated. Melting points were taken on a Mel-Temp melting point apparatus and are uncorrected. Tetrahydrofuran (THF) was distilled from blue sodium benzophenone ketyl solution. Column chromatography was performed in the flash mode on Davisil silica gel (200−425 mesh), unless otherwise stated. Yields are of purified product and are not optimized. ^{1}H NMR spectra were recorded on a 300-MHz Varian spectrometer; chemical shifts are reported in δ units referenced to residual proton signals of the deuterated solvents (chloroform-d_1, 7.26; dimethyl-d_6 sulfoxide, 2.49; methyl alcohol-d_4, 3.31), and coupling constants are reported in Hz.

4-Benzyl-1-(2-phenylethyl)piperidine Hydrochloride (9). The title compound was prepared from 2-phenylethyl methanesulfonate[13a] **(7)** by the method described for **10**: mp 251−252 °C; ^{1}H NMR ($CDCl_3$) δ 1.3−1.4 (m, 2H), 1.4−1.6 (m, 1H), 1.6−1.7 (m, 3H), 1.9−2.0 (m, 2H), 2.5−2.6 (m, 3H), 2.7−2.8 (m, 2H), 2.98 (m, 2H), 7.1−7.3 (m, 10H). Anal. ($C_{20}H_{26}$NCl) C, H, N.

4-Benzyl-1-[2-(4-(benzyloxy)phenyl)ethyl]piperidine Hydrochloride (10). A mixture of 2-[(methylsulfonyloxy)ethyl]-4-(benzyloxy)benzene[13a] (**8**; 0.96 g, 3.5 mmol), 4-benzylpiperidine (**6**; 0.53 g, 3.0 mmol), and potassium carbonate (1.0 g, 7.5 mmol) in acetonitrile (20 mL) was refluxed for 24 h. The inorganic salts were removed through a short column of silica gel and washed with EtOAc (3 × 25 mL). The combined filtrate was evaporated in vacuo to give a crude mixture. Column chromatography (20−50% EtOAc in hexanes then 20% MeOH in EtOAc) resulted in the free base of the title compound as an oil. A solution of the free base (0.47 g) in methanolic HCl (5 mL, 5 M) was magnetically stirred for 20 min. The solvent was removed in vacuo to give a white solid which was precipitated from EtOAc/MeOH to yield the title compound as a colorless powder, 0.50 g (34%): mp 183−185 °C; ^{1}H NMR ($CDCl_3$) δ 1.72 (m, 1H), 1.80 (d, J = 12.6 Hz, 2H), 2.11 (m, 2H), 2.57 (brs, 2H), 2.62 (d, J = 6.9 Hz, 2H), 3.08 (m, 2H), 3.19 (m, 2H), 3.57 (m, 2H), 5.03 (s, 2H), 6.89 (d, J = 8.4 Hz, 2H), 7.11 (m, 3H), 7.2−7.4 (m, 9H), 12.42 (brs, 1H).

4-Benzyl-1-[2-(4-hydroxyphenyl)ethyl]piperidine Hydrochloride (11). A mixture of piperidine **10** (0.20 g, 0.46 mmol) and Pd/C (10%, 50 mg) in EtOH (95%, 25 mL) was shaken in a Parr flask under 30 psi of hydrogen for 2 h. The catalyst was removed through a short column of Celite and washed with MeOH (3 × 15 mL). The combined filtrate was evaporated in vacuo to give an oil. This oil was titrated in ether (30 mL) overnight. The white solid was collected by filtration and dried in vacuo resulting in 0.16 g (98%) as the title product: mp 222−224 °C; ^{1}H NMR (CD_3OD) δ 1.48 (m, 2H), 1.89 (m, 3H), 2.62 (d, J = 6.6 Hz, 2H), 2.9−3.0 (m, 4H), 3.2−3.3 (m, 2H), 3.57 (m, 2H), 6.73 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.4 Hz, 2H), 7.17 (m, 3H), 7.31 (m, 2H). Anal. ($C_{20}H_{26}$NClO) C, H, N.

2-(4-(Benzyloxy)phenyl)ethyl 4-Phenylbutyl Ether (14). 4-Phenylbutyl *p*-toluenesulfonate[13b] (**12**; 0.80 g, 2.6 mmol) was added to a stirring solution of 2-(4-(benzyloxy)phenyl)ethanol[13c] (**13**; 0.40 g, 1.8 mmol) and NaH (84 mg, 3.5 mmol) in DMF (5 mL), and the mixture was stirred for 12 h. H_2O (30 mL) was added, and the solution was extracted with ether (3 × 30 mL). The combined ether was dried over Na_2SO_4, and the solvent was removed in vacuo. Column chromatography (CH_2Cl_2/hexane) resulted in the title compound as a transparent oil, 0.59 g (93%): ^{1}H NMR ($CDCl_3$) δ 1.6−1.8 (m, 4H), 2.63 (t, J = 6.9 Hz, 2H), 2.83 (t, J = 7.2 Hz, 2H), 3.46 (t, J = 6.0 Hz, 2H), 3.60 (t, J = 6.9 Hz, 2H), 5.04 (s, 2H), 6.93 (d, J = 9.0 Hz, 2H), 7.0−7.5 (m, 12H).

2-(4-Hydroxyphenyl)ethyl 4-Phenylbutyl Ether (15). Ether **14** (0.50 g, 1.4 mmol) and Pd/C (10%, 0.10 g) in MeOH (30 mL) were shaken in a Parr apparatus under 45 psi of H_2 for 12 h. The catalyst was removed by filtration and the solvent removed in vacuo. Column chromatography (CH_2Cl_2/hexane) resulted in the title compound as a transparent oil, 0.32 g (85%): ^{1}H NMR ($CDCl_3$) δ 1.6−1.8 (m, 4H), 2.62 (t, J = 6.9 Hz, 2H), 2.82 (t, J = 7.2 Hz, 2H), 3.46 (t, J = 6.0 Hz, 2H), 3.58 (t, J = 6.9 Hz, 2H), 5.29 (s, 1H), 6.71 (d, J = 8.9 Hz, 2H), 7.0−7.4 (m, 12H). Anal. ($C_{18}H_{22}O_2$) C, H.

5-Phenylpentaldehyde (18). The title compound was prepared from 5-phenyl-1-pentanol **(16)** by the method described for **19**: ^{1}H NMR ($CDCl_3$) δ 1.6−1.8 (4H, m), 2.4−2.8 (4H, m), 7.0−7.4 (5H, m), 9.76 (1H, s).

6-Phenylhexaldehyde (19). A solution of 6-phenyl-1-hexanol (**17**; 1.0 g, 5.6 mmol) in CH_2Cl_2 (10 mL) was added to a solution of PCC (1.8 g, 8.4 mmol) in CH_2Cl_2 (30 mL), and the mixture was stirred at room temperature for 3 h. Ether (30 mL) was added, and the solution was passed through a short path of florosile. The solvent was removed in vacuo to give an oil. Column chromatography (CH_2Cl_2/hexane) resulted in the title compound as a transparent oil, 0.40 g (85%): ^{1}H NMR ($CDCl_3$) δ 1.40 (p, J = 6.3 Hz, 2H), 1.6−1.8 (m, 4H), 2.42 (dt, J = 1.2 Hz, 7.8, 2H), 2.62 (t, J = 7.5 Hz, 2H), 7.0−7.4 (m, 5H), 9.76 (s, 1H).

***N*-[2-(4-Hydroxyphenyl)ethyl]-5-phenylpentylamine Hydrochloride (20).** The title compound was prepared from aldehyde **18** by the method described for **21**: mp 189−190 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (p, J = 7.8 Hz, 2 H), 1.59 (m, 4H), 2.56 (t, J = 7.8 Hz, 2H), 2.7−2.9 (m, 4H), 3.01 (t, J = 7.2 Hz, 2H), 6.71 (d, J = 8.7 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H), 7.1−7.3 (m, 5H), 8.71 (2H, s), 9.33 (s, 1H). Anal. ($C_{19}H_{26}$NClO) C, H, N.

***N*-[2-(4-Hydroxyphenyl)ethyl]-6-phenylhexylamine Hydrochloride (21).** Sodium cyanoborohydride (0.47 g, 7.4 mmol) was added to a stirring solution of aldehyde **19** (0.48 g, 2.7 mmol) and tyramine (0.34 g, 2.5 mmol) in MeOH (20 mL), and the resulting solution was stirred for 24 h. The solvent was removed in vacuo. The residual solid was dissolved in EtOAc (20 mL) and washed with $NaHCO_3$ (saturated in H_2O, 2 × 20 mL) and water (20 mL). The solution was dried over Na_2SO_4 and the solvent was removed in vacuo. Column chromatography (EtOAc) resulted in a transparent oil. This oil was dissolved in CH_2Cl_2 (5 mL) and treated dropwise with a solution of HCl/ether (1.0 M, 10 mL). The solid formed was collected and precipitated from EtOAc/MeOH to yield the title compound as a white powder, 0.50 g (56%): mp 140−141 °C; ^{1}H NMR (DMSO-d_6) δ 1.29 (m, 4H), 1.55 (m, 4H), 2.55 (t, J = 7.5 Hz, 2H), 2.80 (m, 4H), 3.02 (t, J = 8.4 Hz, 2H), 6.68 (d, J = 8.7 Hz, 2H), 7.03 (d, J = 8.1 Hz, 2H), 7.1−7.5 (m, 5H), 8.60 (s, 2H), 9.32 (s, 1H). Anal. ($C_{20}H_{28}$NClO) C, H, N.

***N*-[2-(4-Hydroxyphenyl)ethyl]-4-phenylbutylamine (34).** A solution of 4-phenylbutyric acid (**22**; 1.0 g, 6.1 mmol), 1,3-dicyclohexylcarbodiimide (1.3 g, 6.2 mmol), 1-hydroxybenzotriazole (0.84 g, 6.2 mmol), and tyramine (**28**; 0.85 mg, 6.2 mmol) in DMF (10 mL) was stirred at room temperature for 3 h and then at 60 °C for 24 h. The solid was removed by filtration. The solution was diluted with H_2O (150 mL), and the yellow oil was extracted with CH_2Cl_2 (3 × 20 mL). The combined CH_2Cl_2 portion was washed with H_2O (2 × 50 mL) and dried over Na_2SO_4, and the solvent was removed in vacuo to give a yellow oil. Column chromatography (CH_2Cl_2/MeOH, 5:1) gave the intermediate *N*-[2-(4-hydroxyphenyl)ethyl]-4-phenylbutyramide as a transparent oil, 1.6 g (92%): ^{1}H NMR ($CDCl_3$) δ 1.93 (p, J = 8.1 Hz, 2H), 2.13 (t, J = 7.2 Hz, 2H), 2.60 (t, J = 7.8 Hz, 2H), 2.71 (t, J = 7.2 Hz, 2H), 3.48 (q, J =

6.9 Hz, 2H), 5.57 (bt, 1H), 6.80 (d, J = 6.3 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 7.1−7.4 (m, 5H), 7.99 (s, 1H).

A solution of *N*-[2-(4-hydroxyphenyl)ethyl]-4-phenylbutyramide (2.0 g, 3.7 mmol) in THF (10 mL) was slowly added to a stirred suspension of $LiAlH_4$ (1.0 g, 26 mmol) in THF (100 mL) at room temperature. After addition, the mixture was stirred at room temperature for 3 h and then at reflux for 24 h. H_2O (10 mL) was slowly added to the mixture. The resulting precipitate was removed by filtration. The solvent was removed in vacuo. The resulting yellow oil was partitioned between H_2O (25 mL) and CH_2Cl_2 (25 mL). The aqueous phase was separated and extracted with CH_2Cl_2 (2 × 25 mL). The combined CH_2Cl_2 extract was washed with H_2O (50 mL), dried over Na_2SO_4, and concentrated in vacuo to give a white powder. Precipitation from EtOAc yielded the title compound **34** as a white solid, 0.95 g (50%): mp 113−114 °C; ^{1}H NMR ($CDCl_3$) δ 1.56 (m, 4H), 2.61 (m, 4H), 2.73 (t, J = 7.2 Hz, 2H), 2.85 (t, J = 6.9 Hz, 2H), 6.25 (d, J = 8.4 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 7.1−7.3 (m, 5H). Anal. ($C_{18}H_{23}NO$) C, H, N.

The following examples (**29**−**33**) were prepared by the method described for **34** using the appropriate combination of the carboxylic acid and the amine. All the compounds were isolated as their respective HCl salts as described for **21**.

***N*-(4-Phenylbutyl)-2-phenylethylamine hydrochloride (29):** mp 192−194 °C; ^{1}H NMR ($CDCl_3$) δ 1.4−1.8 (m, 4H), 2.59 (t, J = 6.3 Hz, 2H), 2.92 (m, 4H), 3.09 (t, J = 6.3 Hz, 2H), 7.0−7.4 (m, 10H), 8.95 (s, 2H). Anal. ($C_{18}H_{24}NClO$) C, H, N.

***N*-[3-(4-Hydroxyphenyl)propyl]-4-phenylbutylamine hydrochloride (30):** mp 177−179 °C; ^{1}H NMR ($CDCl_3$) δ 1.81 (p, J = 7.5 Hz, 2H), 2.54 (t, J = 6.9 Hz, 2H), 2.66 (t, J = 8.1 Hz, 2H), 2.86 (m, 4H), 6.63 (d, J = 7.2 Hz, 2H), 6.92 (d, J = 8.4 Hz, 2H), 7.3 (m, 5H). Anal. ($C_{19}H_{26}NClO$) C, H, N.

***N*-[3-(4-Hydroxyphenyl)propyl]-5-phenylpentylamine hydrochloride (31):** mp 115−116 °C; ^{1}H NMR (DMSO) δ 1.29 (p, J = 6.9 Hz, 2H), 1.4−1.6 (m, 4H), 1.81 (p, J = 7.5 Hz, 2H), 2.4−2.6 (m, 4H), 2.8−3.0 (bm, 4 H), 6.65 (d, J = 8.1 Hz, 2H), 6.96 (d, J = 8.1 Hz, 2H), 7.0−7.4 (m, 5H), 8.59 (bs, 2H), 9.21 (s, 1H). Anal. ($C_{20}H_{28}NClO$) C, H, N.

***N*-[2-(4-Hydroxyphenyl)ethyl]-3-phenylpropylamine hydrochloride (32):** mp 161−162 °C; ^{1}H NMR ($CDCl_3$) δ 1.84 (p, J = 7.4 Hz, 2H), 2.59 (t, J = 7.8 Hz, 2H), 2.69 (t, J = 7.2 Hz, 2H), 2.76 (t, J = 6.3 Hz, 2H), 2.89 (t, J = 7.2 Hz, 2H), 6.74 (d, J = 8.4 Hz, 2H), 6.9−7.3 (m, 6H). Anal. ($C_{17}H_{22}NClO$) C, H, N.

***N*-(2-Phenylethyl)-3-phenylpropylamine hydrochloride (33):** mp 270−272 °C; ^{1}H NMR ($CDCl_3$) δ 1.32−1.40 (m, 2H), 1.46 (bs, 1H), 1.6−1.8 (m, 2H), 2.6−2.6 (m, 4H), 2.7−2.9 (m, 2H), 7.1−7.3 (m, 10H). Anal. ($C_{17}H_{22}NCl$) C, N; H: calcd, 8.04; found, 7.53.

***N*-Ethyl-*N*-[2-(4-hydroxyphenyl)ethyl]-4-phenylbutylamine (35).** A mixture of amine **34** (0.20 g, 0.74 mol), iodoethane (0.13 g, 0.81 mmol), and $NaHCO_3$ (68 mg, mmol) in CH_3CN was refluxed for 4 h. The solvent was removed in vacuo, and the resulting oil was dissolved in H_2O/CH_2Cl_2 mixture (1:1, 20 mL). The CH_2Cl_2 portion was separated, and the water layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined CH_2Cl_2 portions was dried over Na_2SO_4, and the solvent was removed in vacuo to give a yellow oil. Column chromatography (EtOAc) resulted in the title compound as a transparent oil, 70 mg (32%): ^{1}H NMR ($CDCl_3$) δ 1.08 (t, J = 7.2 Hz, 2H), 1.60 (m, 4H), 2.5−2.8 (m, 10H), 5.5 (s, 1H), 6.73 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.7 Hz, 2H), 7.1−7.4 (m, 5H). Anal. ($C_{20}H_{27}NO$) C, H, N.

Pharmacology. cDNAs encoding the rat NR1A, NR2A, NR2B, and NR2C subunits were provided cloned into the pRSSP6 vector (a modified version of the pSP64T vector created by Dr. Rolf Schoepfer) in which the coding region for each subunit is attached to a 150-base poly-A tail. The cDNA template was linearized with *Mlu*I and cRNAs transcribed using the SP6 mMessage mMachine system (Ambion, Austin, TX). *Xenopus* oocytes were prepared as described previously.[9b] Oocytes were injected with 1:1 mixtures of NR1- and NR2-encoding cRNA: 0.5−1.5 pg of NR1A/2A; 50−150 pg of NR1A/2B; 100−300 pg of NR1A/2C. Membrane current responses were recorded 3−14 days after injection in a nominally Ca^{2+}-free Ringer's solution containing (in mM): NaCl, 115; KCl, 2; $BaCl_2$, 1.8; HEPES, 5 (pH = 7.4).[12] Levels of expression were as reported previously.[9b,10,12a] In general, the aim was to limit antagonist-evoked current responses to ∼100 nA in Ba^{2+} Ringer (−70 mV): i.e., sufficient current to do accurate pharmacology without causing appreciable activation of secondary Ca^{2+}-gated Cl^- currents.[5b]

Potency of antagonism was estimated by measuring reductions in currents elicited by saturating, or near-saturating, concentrations of agonists: 10 μM glycine plus 100 μM glutamate for NR1A/2A; 1 μM glycine plus 100 μM glutamate for NR1A/2B and NR1A/2C. In all cases the glutamate concentration was sufficient to give an optimum measure of potency.[11b] Inhibition was measured under conditions approximating steady state on desensitized receptors. Potent antagonists had slower onset and washout kinetics than weak antagonists, in some cases requiring >5 min to equilibrate. Compounds were assayed at multiple concentrations over a range that spanned the IC_{50} value. The number of separate experiments for each compound was taken as the number of different oocytes examined. Values from concentration−inhibition experiments were then pooled to avoid issues of weighting from unequal data sets and fit with logistic equations. For NR1A/2A and NR1A/2C, where inhibition was complete and had a single component, data was fit with eq 1:

$$I/I_{control} = 1/\{1 + ([antagonist]/IC_{50})^n\} \quad (1)$$

where I is the measured current, $I_{control}$ is the current in the absence of antagonist, IC_{50} is the concentration of drug that causes 50% inhibition of the control response, and n is the slope factor of the inhibition curve. For NR1A/2B, where inhibition had two components, data was fit with eq 2:

$$I/I_{control} = min + \{(1 - min)/(1 + ([antagonist]/IC_{50})^n)\} \quad (2)$$

where min (minimum) is the residual fractional response at concentrations of antagonist that are saturating for the first component of inhibition and IC_{50} is the concentration that causes half this level. For reasons that remain unclear, residual fractional responses could vary from about 0.05 to 0.25 between different oocytes. Given the variability between oocytes it was impractical to try to define individual min values for each antagonist. Therefore, to make the analysis more uniform, min was set at 0.15 for all experiments on NR1A/2B. This value was chosen as the typical mean level of residual current in the current study and is also consistent with previous reports for this class of antagonist.[5,10,12a] Slope values for the concentration−inhibition curves were varied between −1.5 and −0.8. The degree to which differences in potency measured between two antagonists were significant was assessed by comparing mean IC_{50} values, $<X_1>$ and $<X_2>$, each with an associated variance, S_1^2 and S_2^2, respectively. First, the population variance, S_p^2, was calculated using eq 3:[14]

$$S_p^2 = \{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2\}/(n_1 + n_2 - 2) \quad (3)$$

where n_1 and n_2 are the corresponding numbers of sample points and $n_1 + n_2 - 2$ stands for the degrees of freedom. The analysis assumes that both means come from populations with normal distributions and equal variances. To test the two-tailed hypothesis a t value was then calculated using eq 4:

$$t = (<X_1> - <X_2>)/\{S_p^2(1/n_1 + 1/n_2)\}^{1/2} \quad (4)$$

Values for p were estimated using a built-in function in Microsoft Excel Software (v. 5.0) to calculate the corresponding critical values of t. A p value ≤0.05 was taken as significant.

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