NR2B-Selective *N*-Methyl-D-aspartate Antagonists: Synthesis and Evaluation of 5-Substituted Benzimidazoles

John A. McCauley,*,† Cory R. Theberge,† Joseph J. Romano,† Susan B. Billings,† Kenneth D. Anderson,† David A. Claremon,† Roger M. Freidinger,† Rodney A. Bednar,‡ Scott D. Mosser,‡ Stanley L. Gaul,‡ Thomas M. Connolly,‡ Cindra L. Condra,‡ Menghang Xia,‡ Michael E. Cunningham,‡ Bohumil Bednar,‡ Gary L. Stump,|| Joseph J. Lynch,|| Alison Macaulay,# Keith A. Wafford,# Kenneth S. Koblan,‡ and Nigel J. Liverton†

Departments of Medicinal Chemistry, Molecular Pharmacology, and Pharmacology, Merck Research Laboratories, West Point, Pennsylvania 19486

Received September 25, 2003

Two classes of 5-substituted benzimidazoles were identified as potent antagonists of the NR2B subtype of the *N*-methyl-D-aspartate (NMDA) receptor. Selected compounds show very good selectivity versus the NR2A, NR2C, and NR2D subtypes of the NMDA receptor as well as versus hERG-channel activity and α_1-adrenergic binding. Benzimidazole **37a** shows excellent activity in the carrageenan-induced mechanical hyperalgesia assay in rats as well as good pharmacokinetic behavior in dogs.

Introduction

The *N*-methyl-D-aspartate (NMDA) receptor is a gated ion channel that is present throughout the mammalian central nervous system (CNS) and is activated by the excitatory amino acid, glutamate. This receptor plays a fundamental role in a number of physiological processes and may provide an avenue for the treatment of such disease states as stroke, Parkinson's disease, and neuropathic pain.[1] Agents that block the NMDA channel, such as ketamine, have been shown to alleviate pain in human clinical trials; however, the small therapeutic window relative to side effects such as hallucinations, dysphoria, and loss of coordination makes such compounds unattractive for chronic pain therapy.[2,3] The NMDA receptor is a hetero-oligomeric ion channel made up of a combination of NR1 subunits (a−f) and at least one of the four NR2 subunits (A−D).[4,5] While the NR2A subunit is ubiquitously expressed in brain, the NR2B subunit is largely confined to structures in the forebrain including the cerebral cortex, hippocampus, and olfactory bulb.[6] The reduced level of NR2B subunits in the cerebellum suggests that NR2B-selective agents may show an enhanced therapeutic window vs locomotor side effects,[7] and this hypothesis has been borne out in several animal studies with NR2B selective compounds such as **1** (ifenprodil)[8,9] and **2** (CP-101,606).[10] Recently, a variety of structurally diverse compounds, such as aminoquinoline **3**,[11] sulfoxide **4** (CI-1041),[12] and benzamidine **5**[13,14] have also shown selectivity for NR2B.[15,16] We report here a series of structurally novel benzimidazole NR2B-selective NMDA antagonists that demonstrate excellent activity in the carrageenan-induced mechanical hyperalgesia assay in rats as well as good pharmacokinetic properties in dogs.

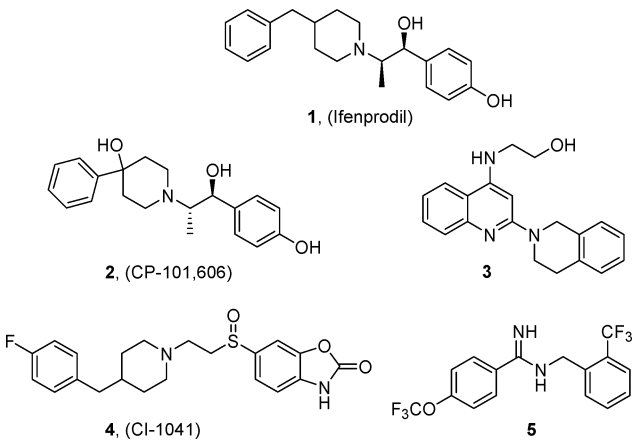


Figure 1. Structures of NR2B selective NMDA receptor antagonists.

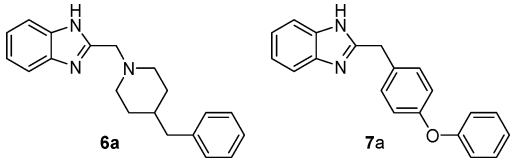


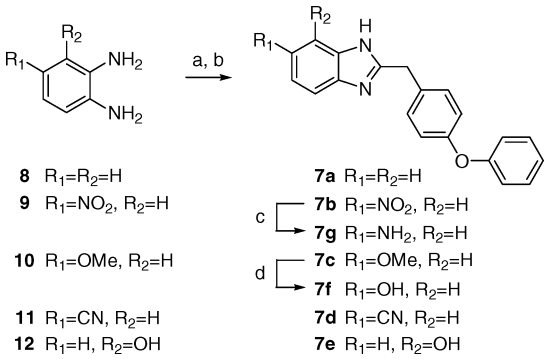
Figure 2. NR2B active benzimidazoles.

Screening of the Merck sample collection led to the identification of a benzimidazole derivative **6a** (Figure 2) with modest NR2B activity (Table 2). This lead shares a benzylpiperidine substituent in common with a number of published NR2B-selective compounds (see Figure 1). Issues with compound **6a** included comparable binding at the hERG potassium channel (see Table 2) and α-adrenergic receptors. On the basis of this lead, a rapid analogue synthesis was carried out to prepare a series of 2-substituted benzimidazoles from *o*-phenylenediamine and a variety of acids. Of the approximately 100 benzimidazoles prepared (not shown), biphenyl ether **7a** (Figure 2 and Scheme 1), derived from

* To whom correspondence should be addressed. Phone: 215-652-4133. Fax: 215-652-3971. E-mail: john_mccauley@merck.com.
† Department of Medicinal Chemistry.
‡ Department of Molecular Pharmacology.
|| Department of Pharmacology.
Department of Pharmacology, Merck Sharp & Dohme Research Laboratories, The Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR, UK.

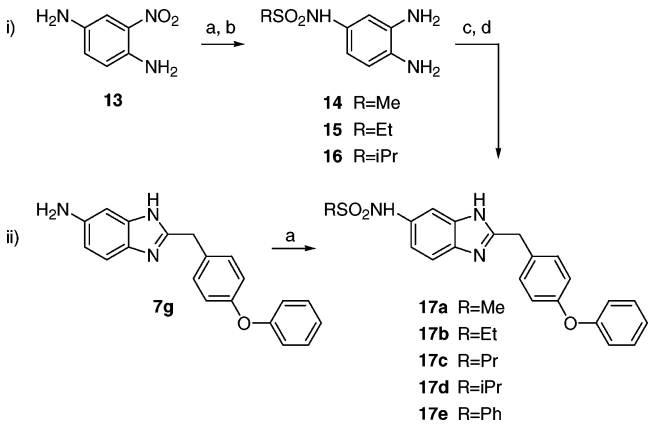
10.1021/jm030483s CCC: $27.50
Published on Web 03/11/2004

Scheme 1[a]



[a] Reagents and conditions: (a) EDC, HOBt, (4-phenoxyphenyl)-acetic acid, DMF, room temp, 1 h; (b) acetic acid, 140 °C, 1 h; (c) H_2, 10% Pd/C, ethanol, room temp, 30 min; (d) 48% HBr/H_2O, 100 °C, 15 h.

Scheme 2[a]



[a] Reagents and conditions: (a) RSO_2Cl, pyridine, reflux, 18 h; (b) H_2, 10% Pd/C, ethanol/ethyl acetate, room temp, 15 h; (c) EDC, HOBt, (4-phenoxyphenyl)acetic acid, DMF, room temp, 1 h; (d) acetic acid, 140 °C, 1 h.

(4-phenoxyphenyl)acetic acid and *o*-phenylenediamine (**8**), was the only significantly active compound (K_i < 2 μM) observed. With this structurally novel lead in hand, we undertook an effort to explore benzimidazole substituent effects.

Chemistry

Compounds **7a**−**e** were prepared from the appropriately substituted phenylenediamines (**8**−**12**), which were coupled to (4-phenoxyphenyl)acetic acid using a standard amide coupling reaction,[17] followed by dehydration to the benzimidazole in acetic acid at reflux.[18] Reduction of the nitro group of **7b** gave **7g**, and hydrolysis of methyl ether **7c** gave **7f**.

A related series of sulfonamides (**17a**−**e**) were prepared via the routes outlined in Scheme 2. The required phenylenediamines (**14**−**16**) could be prepared from nitro aromatic **13** via selective sulfonylation followed by reduction of the nitro group (path i).[19] These compounds then were used as above in preparation of benzimidazoles **17a**, **17b**, and **17d**. Alternatively, benzimidazole **7g** could be sulfonylated to give **17c** and **17e** (path ii).

The syntheses of the piperidine derivatives followed the general procedure shown in Scheme 3. The appropriate benzyl phosphonates[20] were prepared from benzyl chlorides **20**−**24** and subjected to the Horner−Emmons reaction[21] with 4-benzylpiperidone. The resultant olefins were reduced under hydrogenation conditions with concomitant *N*-benzyl loss providing 4-benzylpiperidines **26**−**30**. Alkylation with ethyl bromoacetate and ester hydrolysis gave acids **31**−**36**, which could be converted to benzimidazoles **6a**, **6b**, and **6d**−**e** employing the appropriate phenylenediamine (**8**, **10**, **14**, **16**). The 5-hydroxyl group of **6c** was then unmasked by heating **6b** in a solution of 48% HBr in H_2O. Phenyl-substituted derivatives **37a**−**e** were prepared in a similar fashion from 4-methoxy-1,2-phenylenediamine (**10**) and acids **32**−**36** followed by demethylation.

Results and Discussion

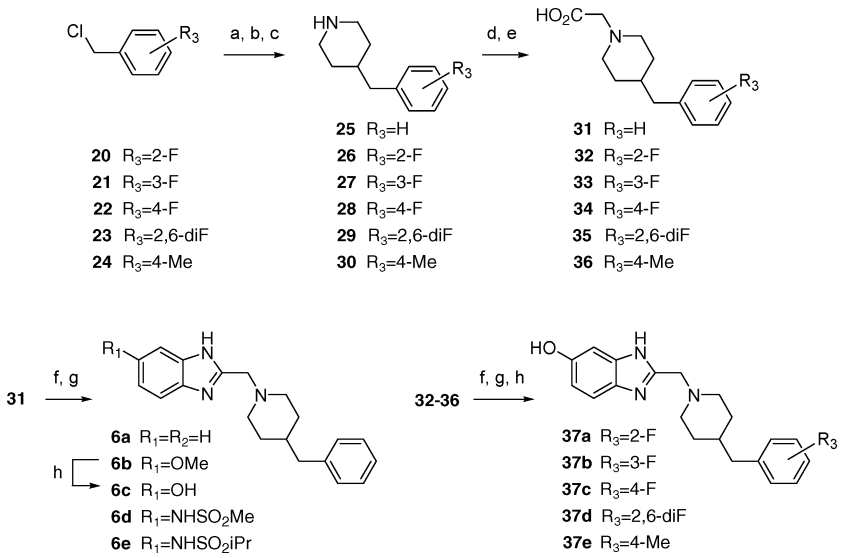
Compounds shown in Table 1 were evaluated in an NR2B-selective binding assay (NR2B binding) and a cell-based assay measuring Ca^{2+} flux in cells expressing the NR2B subunit (NR2B Ca^{2+} influx).[22] Selected compounds were screened for hERG-channel activity using an MK-499 binding assay[23] and for α-adrenergic activity using a prazosin binding assay.[24] Compounds were also counterscreened using a Ca^{2+} influx assay in cells expressing the NR2A subtype. All compounds described showed IC_{50} values greater than 10 μM. The potency of the initial phenoxyphenylbenzimidazole compound (**7a**) was improved by hydroxylation of the 4-position on the benzimidazole ring (**7e**); however, a more significant 80-fold enhancement was observed when the hydroxy group was installed at the 5-position (**7f**). Even though absolute hERG activity was higher, compound **7f** shows a 30-fold increase in selectivity over hERG. We further hypothesized, on the basis of the lack of activity of methylated derivative **7c** and similarity to other NR2B ligands, that a hydrogen bond donor was necessary for good NR2B binding. This led to the synthesis of methanesulfonamide **17a**, which had a K_i for binding to the NR2B receptor of 0.68 nM. This compound also had excellent potency in the cell-based assay, >150-fold selectivity over hERG and very low α-adrenergic activity.

As shown in entries **17a**−**e** in Table 1, binding activity generally decreased with increasing steric bulk of the sulfonamide substituent. These changes seem to have little effect on activity in the counterscreens. Methylation of the sulfonamide nitrogen led to substantial loss of activity (**19**), providing further evidence for the need of a hydrogen-bond-donating substituent.

As a result of the enhanced potency and selectivity seen by introducing hydrogen-bond-donating substituents on the benzimidazole ring of compound **7a**, we proceeded to apply the same strategy to the initial screening lead **6a**. By installing the 5-hydroxy group (**6c**, Table 2), as in the phenoxyphenyl series, we achieved a 280-fold increase in NR2B potency as well as a 2-fold reduction in hERG binding. As shown in Table 2, these derivatives (**6c**−**e**) were selective, showed similar SAR profiles to the phenoxyphenyl derivatives described above, and also benefited from increased aqueous solubility.

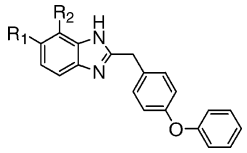
We then examined the SAR on the pendent phenyl ring. As shown in Table 3, installation of a 2-fluoro group on the phenyl ring gave **37a**, a compound with a 0.85 nM K_i for NR2B. This modification further improved the selectivity over hERG binding and α-adrenergic activity to >3000-fold and >800-fold, respectively. To evaluate NR2 subtype selectivity more fully, com-

Scheme 3[a]



[a] Reagents and conditions: (a) triethyl phosphite, 150 °C, 15 h; (b) sodium hydride, *N*-benzyl-4-piperidone, 1,3-dimethyl-2-imidazoline, room temp, 20 min; (c) H_2, 50 psi, $Pd(OH)_2$/C, ethanol, room temp, 15 h; (d) ethyl bromoacetate, diisopropylethylamine, DMF, room temp, 1 h; (e) 6 N HCl; 100 °C, 1 h; (f) EDC, HOAt, triethylamine, phenylenediamine (**8**, **10**, **14**, **16**), DMF, room temp, 20 min; (g) acetic acid, 140 °C, 15 min; (h) 48% HBr/H_2O, 100 °C, 3 h.

Table 1. In Vitro Binding Data for Phenoxyphenyl Benzimidazoles[a]



compd	R_1	R_2	NR2B binding K_i (nM)[b]	NR2B Ca^{2+} influx IC_{50} (nM)[c]	hERG IP (nM)[d]	α_1-adrenergic IC_{50} (nM)[e]
7a	H	H	260 */1.4	200 */1.2	2000 */1.8	4200 */1.3
7b	NO_2	H	5400 */1.6	6600 */2.1	770 */1.7	1200 */1.4
7c	OMe	H	8400 */1.1	9200 */1.7	590 */2.0	530 */1.3
7d	CN	H	790 */1.5	800 */1.4	1100 */2.4	3400 */1.0
7e	H	OH	140 */1.2	150 */1.5	860 */2.6	5500 */1.1
7f	OH	H	3.2 */1.3	9.2 */1.7	720 */1.6	200 */1.0
7g	NH_2	H	180 */1.2	78 */1.3	120 */1.2	2600 */1.3
17a	$NHSO_2Me$	H	0.68 */1.4	0.72 */1.2	120 */1.1	4000 */1.2
17b	$NHSO_2Et$	H	3.0 */1.1	2.1 */1.5	1600 */1.1	2800 */1.4
17c	$NHSO_2Pr$	H	21 */1.5	19 */1.4	400 */1.3	4800 */1.5
17d	$NHSO_2{}^iPr$	H	15 */1.1	14 */1.1	1900 */1.2	3100 */1.5
17e	$NHSO_2Ph$	H	17 */1.3	49 */1.4	680 */1.1	14000 */2.0
18	NHAc	H	93 */1.2	42 */2.8	2600 */1.7	20000 */1.6
19	$N(Me)SO_2Me$	H	470 */1.4	25 */3.0	640 */2.1	2000 */1.2

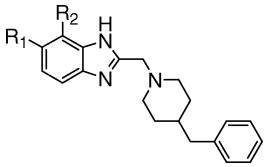
[a] All values are the geometric mean of at least $n = 4$ measurements. The value shown in the table after the "*/" symbol is twice the standard error of the geometric mean [2(SEGM)]. The confidence limits (95%) can be calculated by multiplying (∗) and dividing (/) the geometric mean by this value. For example, in the NR2B binding assay, compound **7a** has a mean K_i of 260 nM with a 95% confidence limit range of 186−364 nM. [b] Inhibition of ^{3}H-[(*E*)-*N*^{1}-(2-methoxybenzyl)cinnamamidine] binding to hNR1a/NR2B receptors expressed in Ltk− cells.[22] [c] Fluorescent measurement of inhibition of Glu/Gly stimulated Ca^{2+} flux in Ltk− cells expressing the hNR1a/NR2B receptor.[22] [d] Inhibition of MK-499 binding to hERG in HEK293 cells.[23] [e] Inhibition of α_1-receptor binding on membranes prepared from rat brain.[24]

pound **37a** was tested against the four known NR2B subtypes in a whole-cell patch−clamp assay and was shown to be selective for NR2B (Figure 3).

Compounds from each series described above were evaluated in the carrageenan-induced mechanical hyperalgesia assay in rats[7] and for pharmacokinetic properties (Table 4).[25] Methanesulfonamidobenzimidazole **17a** had excellent oral bioavailabilty in rats; however, it lacked any activity in the carrageenan-induced mechanical hyperalgesia in rats at the highest oral dose tested (30 mg/kg). Plasma and brain concentrations were measured following this experiment, and these data showed a brain-to-plasma ratio of 0.038, thus suggesting that the lack of pharmacologic activity was due to poor brain penetration.

The benzylpiperidines (**6c** and **37a**) showed poor oral bioavailability in rat but, in contrast, demonstrated good pharmacokinetic profiles in dog, with low clearance and iv half-lives in the 6−7 h range. On the basis of the pharmacokinetic properties in dogs, we were interested in evaluating compounds **6c** and **37a** in the rat carrageenan-induced mechanical hyperalgesia assay, with an iv dosing protocol to overcome the poor plasma levels generated after oral dosing in rats. Both compounds showed good activity with compound **37a** having an ED_{50} of <3 mg/kg iv.

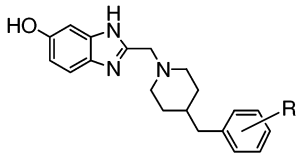
Table 2. In Vitro Binding Data for Benzylpiperidine Benzimidazoles[a]



compd	R_1	NR2B binding K_i (nM)[b]	NR2B Ca^{2+} influx IC_{50} (nM)[c]	hERG IP (nM)[d]	α_1-adrenergic IC_{50} (nM)[e]
6a	H	420 */1.1	710 */1.3	1400 */1.2	2800 */1.2
6c	OH	1.5 */1.1	8.2 */1.2	2600 */1.3	670 */1.1
6e	$NHSO_2Me$	0.99 */1.5	1.9 */1.3	340 */1.7	1500 */1.2
6f	$NHSO_2{}^iPr$	240 */1.1	300 */1.2	3300 */1.1	570 */1.1

[a] All values are the geometric mean of at least $n = 4$ measurements. The value shown in the table after the "*/" symbol is twice the standard error of the geometric mean [2(SEGM)]. The confidence limits (95%) can be calculated by multiplying (*) and dividing (/) the geometric mean by this value. For example, in the NR2B binding assay, compound **6a** has a mean K_i of 420 nM with a 95% confidence limit range of 382−462 nM. [b] Inhibition of ^{3}H-[(*E*)-*N*^{1}-(2-methoxybenzyl)cinnamamidine] binding to hNR1a/NR2B receptors expressed in Ltk− cells.[22] [c] Fluorescent measurement of inhibition of Glu/Gly stimulated Ca^{2+} flux in Ltk− cells expressing the hNR1a/NR2B receptor.[22] [d] Inhibition of MK-499 binding to hERG in HEK293 cells.[23] [e] Inhibition of α_1-receptor binding on membranes prepared from rat brain.[24]

Table 3. In Vitro Binding Data for Benzylpiperidine Benzimidazoles[a]



compd	R	NR2B binding K_i (nM)[b]	NR2B Ca^{2+} influx IC_{50} (nM)[c]	hERG IP (nM)[d]	α_1-adrenergic IC_{50} (nM)[e]
37a	2-F	0.85 */1.1	9.7 */1.5	2900 */1.3	730 */1.1
37b	3-F	4.2 */1.3	44 */1.3	2100 */1.3	520 */2.0
37c	4-F	1.1 */1.1	12 */1.4	500 */1.4	620 */1.4
37d	2,6-diF	0.82 */1.3	21 */1.3	3000 */1.3	2600 */1.2
37e	4-Me	0.4 */1.8	5.7 */1.8	390 */1.5	830 */1.0

[a] All values are the geometric mean of at least $n = 4$ measurements. The value shown in the table after the "*/" symbol is twice the standard error of the geometric mean [2(SEGM)]. The confidence limits (95%) can be calculated by multiplying (*) and dividing (/) the geometric mean by this value. For example, in the NR2B binding assay, compound **37a** has a mean K_i of 0.85 nM with a 95% confidence limit range of 0.77−0.94 nM. [b] Inhibition of ^{3}H-[(*E*)-*N*^{1}-(2-methoxybenzyl)cinnamamidine] binding to hNR1a/NR2B receptors expressed in Ltk− cells.[22] [c] Fluorescent measurement of inhibition of Glu/Gly stimulated Ca^{2+} flux in Ltk− cells expressing the hNR1a/NR2B receptor.[22] [d] Inhibition of MK-499 binding to hERG in HEK293 cells.[23] [e] Inhibition of α_1-receptor binding on membranes prepared from rat brain.[24]

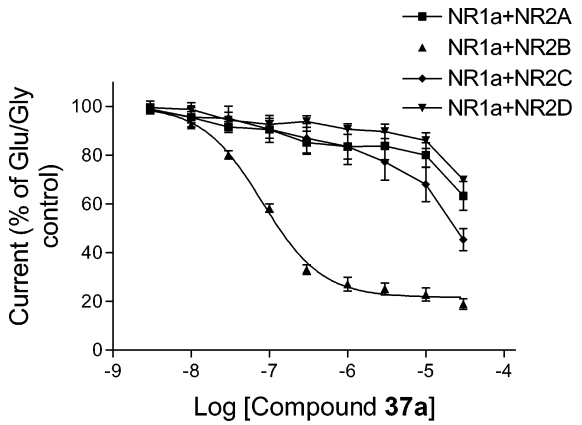


Figure 3. Inhibition of NMDA receptor activated currents at recombinant human NMDA receptor subtypes. NR1a + NR2A, NR2B, and NR2D were stably expressed in L(tk−) cells, while NR1a + NR2C were expressed transiently in HEK cells.[26] Data represent the inhibition of maximally evoked currents in response to a glutamate/glycine application and are the mean ± SEM from at least five individual cells. Mean data from NR1 + NR2B were fitted using the Hill equation.

In conclusion, we have developed two novel classes of NR2B-selective NMDA receptor antagonists. The piperidine class demonstrates efficacy in a rodent hyperalgesia model and possesses excellent pharmacokinetic properties in dogs.

Experimental Section

General. All reagents and solvents were of commercial quality and used without further purification unless indicated otherwise. All reactions were carried out under an inert atmosphere of nitrogen. ^{1}H NMR spectra were obtained on a Varian VXR-300S spectrometer or a Varian Unity Inova 400 spectrometer. Chemical shifts are reported in parts per million relative to TMS as internal standard. Samples provided for accurate mass measurement were taken up in acetonitrile/water/glacial acetic acid (50:50:0.1% v/v). The solutions were analyzed by use of electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) on either a Bruker Daltonics 3T or 7T Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. External calibration was accomplished with polypropylene glycol (425 or 750). Melting points were determined in open glass capillaries using a Thomas-Hoover UniMelt melting point apparatus and are uncorrected. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. Silica gel chromatography was carried out with an ISCO CombiFlash Sg 100c purification system using ISCO silica gel RediSep cartridges. Preparative reverse-phase HPLC was performed using a Gilson 215 liquid handler and a YMC CombiPrep Pro C18 column (50 mm × 20 mm i.d.) with a linear gradient over 15 min (95:5 to 5:95 H_2O/acetonitrile, containing 0.1% trifluoroacetic acid).

Table 4. In Vivo Evaluation of Selected Compounds

compd	carrageenan-induced mechanical hyperalgesia in rats[a] (mg/kg)	pharmacokinetic profile in rats[e]			pharmacokinetic profile in dogs[f]		
		% *F*	$T_{1/2}$ (min)	Cl ((mL/min)/kg)	% *F*	$T_{1/2}$ (min)	Cl ((mL/min)/kg)
17a	>30, po[b]	97	118	8.3			
6c	3.3 ± 0.3, iv[c]	20	>480	22.8	90	402	0.6
37a	<3, iv[d]	6.4	60	12.3	87	390	0.6

[a] Carrageenan-induced mechanical hyperalgesia is defined as the difference in vocalization threshold between vehicle-treated rats receiving ipl injections of carrageenan vs saline. Paw pressure scores for test-agent-treated rats are expressed as a percentage of the hyperalgesia response induced by carrageenan. The ED_{50} value for test agents is defined as the dose of drug at which the paw pressure score is 50% of the hyperalgesia response induced by carrageenan. [b] Compound **17a** showed no reduction in paw pressure score at the highest oral dose tested (30 mg/kg); one determination. [c] Three ED_{50} determinations. Study doses = 1, 3, and 10 mg/kg iv. [d] One ED_{50} determination. Study doses = 3 and 10 mg/kg iv. [e] Sprague-Dawley rats (n = 3−4). Oral dose = 10 mg/kg. Intravenous dose = 2 mg/kg. Interanimal variability was less than 20% for all values. Abbreviations: % *F*, oral bioavailability; Cl, clearance. [f] Mongrel dogs (n = 2). Oral dose = 3 mg/kg. Intravenous dose = 1 mg/kg. Interanimal variability was less than 20% for all values. Abbreviations: % *F*, oral bioavailability; Cl, clearance.

Carrageenan-Induced Mechanical Hyperalgesia in Rats. The ability of the agents to reverse carrageenan-induced hyperalgesia was determined using the method described by Boyce et al.[7] In brief, mechanical paw pressure thresholds were determined in male Sprague-Dawley rats (100−120 g) using a modified Ugo Basile algesiometer by positioning the animal's hind paw over a convex surface (radius 2.5 mm) and gradually increasing pressure applied to the dorsal surface until the animals vocalized. Following a 1 h equilibration in the lab, mechanical thresholds were determined for both hind paws to provide a baseline for subsequent comparison following injection of carrageenan into one paw. Rats then received an intraplantar (ipl) injection of either carrageenan (Sigma; 4.5 mg in 0.15 mL of saline) or saline (0.15 mL) into one hind paw. Post-treatment mechanical paw pressure thresholds of both hind paws were determined 3 h after the ipl injections of carrageenan or saline, with validation studies having established 3 h as the peak time for development of carrageenan-induced inflammation. Carrageenan-induced hyperalgesia was defined as the difference in vocalization threshold between vehicle-treated rats receiving ipl injections of carrageenan vs saline. Paw pressure scores for test-agent-treated rats were expressed as a percentage inhibition of the hyperalgesia induced by carrageenan. Test agents were administered orally by gavage in a vehicle of 1% methylcellulose aqueous suspension (n = 8/dose) at 1 h prior to post-treatment paw pressure thresholds determinations or intravenously in a vehicle of 50% PEG-200/D5W (n = 6/dose) at 15 min prior to post-treatment paw pressure thresholds determinations.

Determination of Plasma and Brain Concentrations. In some carrageenan-induced mechanical hyperalgesia studies, after the determination of post-treatment mechanical paw pressure thresholds, blood and brain samples were obtained for the determination of test agent tissue concentration. A 1 mL blood sample was removed by cardiac puncture (25 gauge needle containing 0.1 mL 3.8% citrate attached to a 3 mL syringe) and spun to obtain plasma, which was frozen (−70 °C). The animals were then euthanized in a carbon dioxide chamber, and the forebrain was removed rapidly and frozen.

Mobile phase A was water/methanol (90:10) with 0.1% formic acid. Mobile phase B was 100% acetonitrile. Plasma samples were prepared using a semiautomated protein precipitation method assisted by a robotic sample processor (Tecan Genesis RSP 150, Tecan, Durham, NC) by mixing 100 μL of plasma, 50 μL of the internal standard solution (10 000 ng/mL in mobile phase A), and 400 μL of acetonitrile in a 96-well plate. The 96-well plate was capped, vortex, and centrifuged at 3740*g* for 15 min. After centrifugation, a 450 μL aliquot of the supernatant was removed, transferred into a second 96-well plate, and evaporated. Samples were reconstituted in 200 μL of mobile phase A using the Tomtec Quadra 96 (Tomtec, Hamden, CT) and transferred into glass inserts for analysis.

Brain samples were weighed exactly, placed into a 20 mL plastic scintillation, and homogenized in mobile phase A (1/5, w/v), yielding a solution of 0.166 g of brain tissue per mL of brain homogenate. Brain samples were prepared by pipetting 1 mL of brain homogenate into a microcentrifuge tube and centrifuging at 13500*g* for 15 min. After centrifugation, the microcentrifuge tubes were transferred to the Tecan Genesis RSP for additional liquid transfers, in which 200 μL of brain homogenate supernatant was mixed with 50 μL of the internal standard solution and 700 μL of acetonitrile in a 96-well plate. The 96-well plate was capped, vortex, and centrifuged at 3740*g* for 15 min. After centrifugation, a 450 μL aliquot of the supernatant was removed, transferred into a second 96-well plate, and evaporated. Samples were reconstituted in 200 μL of mobile phase A using the Tomtec Quadra 96 and transferred into glass inserts for analysis.

Samples were separated chromatographically using an Agilent 1100 series HPLC instrument (Agilent Technologies, Palo Alto, CA) and a CTC PAL autosampler (LEAP Technologies, Carboro, NC). A Prodigy 5 1 ODS3 (50 mm × 2 mm) analytical column (Phenomenex, Torrance, CA) was used for the analysis. Samples were analyzed on a PE/Sciex API 2000 triple quadrupole mass spectrometer (PE Sciex, Toronto, Canada).

Pharmacokinetics. Pharmacokinetic characterization of test agents was conducted in conscious male Sprague-Dawley rats (300−500 g; n = 3−4/study) or male and female beagle dogs (13−15 kg; n = 2/study). In both species, single doses of test agents were administered either intravenously in a vehicle of 100% DMSO or orally by gavage in a vehicle of 1% methylcellulose aqueous suspension. Typical test doses were 2 mg/kg iv and 10 mg/kg po to rats and 1 mg/kg iv and 1−3 mg/kg po to dogs. Blood samples for the determination of test agent plasma concentration were obtained at multiple time points up to 24 h after single-dose test-agent administration.

Inhibition of NMDA Receptor-Activated Currents at Recombinant Human NMDA Receptor Subtypes. Whole-cell patch−clamp experiments were performed using an Axoclamp 200B on coverslip-plated L(tk−) cells stably transfected with NR1a/NR2A, NR1a/NR2B, or NR1a/NR2D receptors, and HEK cells were transiently transfected with NR1a/NR2C receptors. The external recording solution consisted of the following (in mM): NaCl (149), KCl (3.25), $CaCl_2$ (2), HEPES (10), glucose (11), sucrose (21.9); pH 7.4. This buffer solution was superfused at a rate of 0.7−2.0 mL/min. The recording pipet solution was composed of the following (in mM): CsF (120), CsCl (10), EGTA (10), $CaCl_2$ (0.5); pH 7.25. Agonists and antagonist were applied locally by fast perfusion from a double-barreled pipet, and experiments were performed at room temperature. Inhibition curves for NMDA antagonists were obtained in the presence of 10 μM glutamate and 1 μM glycine (10 μM for NR1a/NR2A). Increasing concentrations of antagonist were preapplied for 30 s followed by two coapplications of Glu/Gly for 5 s. Inhibition of the second Glu/Gly response in the presence of antagonist was determined.

2-(4-Phenoxybenzyl)benzimidazole (7a). To a solution of (4-phenoxyphenyl)acetic acid (250 mg, 1.10 mmol) in dimethylformamide (5 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (252 mg, 1.31 mmol), 1-hydroxybenzotriazole hydrate (HOBt) (178 mg, 1.31

mmol), and phenylenediamine **8** (124 mg, 1.15 mmol). The reaction mixture was stirred at room temperature for 1 h followed by quenching with aqueous $NaHCO_3$ and ethyl acetate. The layers were separated, and the organic layer was washed twice with water, dried over Na_2SO_4, filtered, and concentrated.

The crude product was dissolved in acetic acid (10 mL) and heated to 140 °C for 1 h. The reaction mixture was cooled, concentrated, and partitioned between aqueous 1 M NaOH and ethyl acetate, and the organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by silica gel chromatography (gradient elution, 1:1 hexanes/ethyl acetate to ethyl acetate) to give **7a** (240 mg, 73% yield) as a white amorphous solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.55−7.40 (m, 2 H), 7.33 (m, 4 H), 7.19 (dd, 2 H), 7.07 (t, 1 H), 6.92 (m, 4 H), 4.21 (s, 2 H) ppm; HRMS (ESI) *m/z* 301.1327 [$(M + H)^+$, calcd for $C_{20}H_{17}N_2O$, 301.1335]. Anal. ($C_{20}H_{16}N_2O$) C, H, N.

5-Nitro-2-(4-phenoxybenzyl)benzimidazole (7b). Using the above procedure for **7a** with 4-nitro-1,2-phenylenediamine (**9**) followed by silica gel chromatography (gradient elution, 10:1 hexanes/ethyl acetate to ethyl acetate) provided **7b** as a yellow amorphous solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (d, 1 H), 8.20 (d, 1 H), 7.39 (t, 2 H), 7.31 (m, 3 H), 7.12 (t, 1 H), 7.04 (m, 4 H), 4.34 (s, 2 H) ppm; HRMS (ESI) *m/z* 346.1183 [$(M + H)^+$, calcd for $C_{20}H_{16}N_3O_3$, 346.1186]. Anal. ($C_{20}H_{15}N_3O_3 \cdot 1.1H_2O$) C, H, N.

5-Methoxy-2-(4-phenoxybenzyl)benzimidazole (7c). Using the above procedure for **7a** with 4-methoxy-1,2-phenylenediamine (**10**) followed by silica gel chromatography (gradient elution, 10:1 hexanes/ethyl acetate to ethyl acetate) provided **7c** as a white amorphous solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.60 (d, 1 H), 7.39 m, 4 H), 7.17 (m, 3 H), 7.00 (m, 4 H), 4.49 (s, 2 H), 3.89 (s, 3 H) ppm; HRMS (ESI) *m/z* 331.1437 [$(M + H)^+$, calcd for $C_{21}H_{19}N_2O_2$, 331.1441]. Anal. ($C_{21}H_{18}N_2O_2 \cdot 0.1H_2O$) C, H, N.

2-(4-Phenoxybenzyl)benzimidazole-5-carbonitrile (7d). Using the above procedure for **7a** with 3,4-diaminobenzonitrile (**11**) followed by silica gel chromatography (gradient elution, 9:1 hexanes/ethyl acetate to ethyl acetate) provided **7d** as a white amorphous solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.04 (s, 1 H), 7.62 (d, 2 H), 7.58 (d, 2 H), 7.37 (m, 4 H), 7.11 (t, 1 H), 6.96 (m, 4 H), 4.11 (s, 2 H) ppm; HRMS (ESI) *m/z* 326.1288 [$(M + H)^+$, calcd for $C_{21}H_{16}N_3O$, 326.1288]. Anal. ($C_{21}H_{15}N_3O \cdot 0.1H_2O$) C, H, N.

2-(4-Phenoxybenzyl)benzimidazol-4-ol (7e). Using the above procedure for **7a** with 2,3-diaminophenol (**12**) followed by silica gel chromatography (gradient elution, 10:1 hexanes/ethyl acetate to ethyl acetate) provided **7e** as a white amorphous solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (t, 2 H), 7.28 (m, 2 H), 7.12 (t, 2 H), 7.00 (m, 4 H), 6.88 (d, 1 H), 6.79 (d, 1 H), 4.31 (s, 2 H), 2.18 (s, 1 H) ppm; HRMS (ESI) *m/z* 317.1284 [$(M + H)^+$, calcd for $C_{20}H_{17}N_2O_2$, 317.1285]. Anal. ($C_{20}H_{16}N_2O_2 \cdot 0.7MeOH$) C, H, N.

2-(4-Phenoxybenzyl)benzimidazol-5-ol (7f). A solution of **7c** (100 mg, 0.30 mmol) in HBr/H_2O (48%, 2 mL) was heated to 100 °C for 15 h. The reaction mixture was cooled, concentrated, and purified by preparative reverse-phase HPLC to give the trifluoroacetate salt of **7f**. The free base was prepared by dissolving the residue in ethyl acetate, washing with saturated aqueous $NaHCO_3$, drying the organic over Na_2SO_4, filtering, and concentrating to give **7f** as a white amorphous solid (85 mg, 90% yield): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.99 (s, 1 H), 7.38 (m, 5 H), 7.22 (d, 1 H), 7.09 (t, 1 H), 6.95 (m, 3 H), 6.77 (s, 1 H), 6.59 (dd, 1 H), 4.05 (s, 2 H) ppm; HRMS (ESI) *m/z* 317.1285 [$(M + H)^+$, calcd for $C_{20}H_{17}N_2O_2$, 317.1291]. Anal. ($C_{20}H_{16}N_2O_2$) C, H, N.

2-(4-Phenoxybenzyl)benzimidazol-5-amine (7g). To a solution of **7b** (1.09 g, 3.16 mmol) in ethanol (15 mL) was added 10% Pd/C (200 mg). The mixture was stirred at room temperature under a balloon of hydrogen gas for 30 min. The reaction mixture was filtered through Celite, and the catalyst washed with ethanol. The filtrate was concentrated to give **7g** as a yellow amorphous solid (965 mg, 97% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (m, 6 H), 7.03 (t, 1 H), 6.87 (d, 2 H), 6.73 (d, 2 H), 6.63 (s, 1 H), 6.57 (d, 1 H), 4.19 (s, 2 H) ppm; HRMS (ESI) *m/z* 316.1444 [$(M + H)^+$, calcd for $C_{20}H_{18}N_3O$, 316.1445]. Anal. ($C_{20}H_{17}N_3O \cdot 0.4H_2O$) C, H, N.

***N*-[2-(4-Phenoxybenzyl)benzimidazol-5-yl]methanesulfonamide (17a).** Using the above procedure for **7a** with *N*-(3,4-diaminophenyl)methanesulfonamide (**14**) followed by silica gel chromatography (gradient elution, dichloromethane to 90:10:1 dichloromethane/methanol/NH_4OH) provided **17a**. The HCl salt was then prepared via treatment with HCl/ether and concentration in vacuo. Recrystallization from 2-propanol gave the HCl salt of **17a** as a white crystalline solid: mp = 221−222 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.71 (m, 2 H), 7.39 (m, 5 H), 7.16 (t, 1 H), 7.01 (m, 4 H), 4.53 (s, 2 H), 2.98 (s, 3 H) ppm; HRMS (ESI) *m/z* 394.1231 [$(M + H)^+$, calcd for $C_{21}H_{20}N_3O_3S$, 394.1220]. Anal. ($C_{21}H_{19}N_3O_3S \cdot HCl$) C, H, N.

***N*-[2-(4-Phenoxybenzyl)benzimidazol-5-yl]ethanesulfonamide (17b).** Using the above procedure for **7a** with *N*-(3,4-diaminophenyl)ethanesulfonamide (**15**) followed by silica gel chromatography (gradient elution, dichloromethane to 90:10:1 dichloromethane/methanol/NH_4OH) provided **17b**. The HCl salt was then prepared via treatment with HCl/ether and concentration in vacuo. Recrystallization from 2-propanol gave the HCl salt of **17b** as a white crystalline solid: mp = 193−195 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.68 (m, 2 H), 7.38 (m, 5 H), 7.13 (t, 1 H), 7.01 (m, 4 H), 4.50 (s, 2 H), 3.12 (q, 2 H), 1.30 (t, 3 H) ppm; HRMS (ESI) *m/z* 408.1382 [$(M + H)^+$, calcd for $C_{22}H_{22}N_3O_3S$, 408.1377]. Anal. ($C_{22}H_{21}N_3O_3S \cdot HCl$) C, H, N.

***N*-[2-(4-Phenoxybenzyl)benzimidazol-5-yl]propane-1-sulfonamide (17c).** To a solution of **7g** (300 mg, 0.95 mmol) in pyridine (5 mL) was added *n*-propylsufonyl chloride (136 mg, 0.95 mmol). The reaction mixture was heated and stirred at reflux for 18 h. Water was then added, and the mixture was extracted with dichloromethane. The organic layer was dried over Na_2SO_4, filtered, concentrated, and purified by reverse-phase HPLC to give the trifluoroacetate salt of **17c** as a white amorphous solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.81 (d, 2 H), 7.40−7.35 (m, 5 H), 7.16 (t, 1 H), 7.05 (m, 4 H), 4.52 (s, 2 H), 3.08 (dd, 2 H), 1.79 (m, 2 H), 0.98 (t, 3 H) ppm; HRMS (ESI) *m/z* 422.1526 [$(M + H)^+$, calcd for $C_{23}H_{24}N_3O_3S$, 422.1533]. Anal. ($C_{23}H_{23}N_3O_3S \cdot TFA$) C, H, N.

***N*-[2-(4-Phenoxybenzyl)benzimidazol-5-yl]propane-2-sulfonamide (17d).** Using the above procedure for **7a** with *N*-(3,4-diaminophenyl)propane-2-sulfonamide (**16**) followed by silica gel chromatography (gradient elution, dichloromethane to 90:10:1 dichloromethane/methanol/NH_4OH) provided **17d**. The HCl salt was then prepared via treatment with HCl/ether and concentration in vacuo. Recrystallization from 2-propanol gave the HCl salt of **17d** as a white crystalline solid: mp = 206−207 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.62 (m, 2 H), 7.35 (m, 5 H), 7.10 (t, 1 H), 6.97 (m, 4 H), 4.46 (s, 2 H), 3.25 (m, 1 H), 1.30 (d, 6 H) ppm; HRMS (ESI) *m/z* 422.1538 [$(M + H)^+$, calcd for $C_{23}H_{24}N_3O_3S$, 422.1533]. Anal. ($C_{23}H_{23}N_3O_3S \cdot HCl$) C, H, N.

***N*-[2-(4-Phenoxybenzyl)benzimidazol-5-yl]benzenesulfonamide (17e).** Using the above procedure for **17c** with phenylsulfonyl chloride followed by silica gel chromatography (gradient elution, 2:1 hexanes/ethyl acetate to 95:5:0.5 ethyl acetate/methanol/NH_4OH) provided **17e** as a white amorphous solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1 H), 7.72 (d, 2 H), 7.51 (t, 1 H), 7.29−7.43 (m, 5 H), 7.28 (s, 1 H), 7.13 (t, 1 H), 7.05 (d, 2 H), 6.98 (d, 2 H), 6.66 (br s, 1 H), 6.55 (br s, 1 H), 4.22 (s, 2 H) ppm; HRMS (ESI) *m/z* 456.1359 [$(M + H)^+$, calcd for $C_{26}H_{22}N_3O_3S$, 456.1298]. Anal. ($C_{26}H_{21}N_3O_3S \cdot 0.5MeOH$) C, H, N.

***N*-[2-(4-Phenoxybenzyl)benzimidazol-5-yl]acetamide (18).** Using the above procedure for **17c** with acetic anhydride followed by silica gel chromatography (gradient elution, dichloromethane to 85:15:1.5 dichloromethane/methanol/NH_4OH) provided **18** as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (br s, 1 H), 7.51 (br s, 1 H), 7.34 (m, 3 H), 7.24 (m, 2 H), 7.11 (t, 2 H), 6.98 (m, 5 H), 4.27 (s, 3 H), 3.50 (s, 2 H) ppm; HRMS (ESI) *m/z* 358.1550 [$(M + H)^+$, calcd for $C_{22}H_{20}N_3O_2$, 358.1545]. Anal. ($C_{22}H_{19}N_3O_2 \cdot 0.75H_2O$) C, H, N.

***N*-Methyl-*N*-[2-(4-phenoxybenzyl)benzimidazol-5-yl]-methanesulfonamide (19).** To a solution of **17a** (15 mg, 0.04 mmol) in dimethylformamide (1 mL) was added cesium carbonate (25 mg, 0.08 mmol) and methyl iodide (3 μL, 0.04 mmol). The reaction mixture was stirred at room temperature for 2 h followed by quenching with aqueous $NaHCO_3$ and ethyl acetate. The layers were separated, and the organic was washed twice with water, dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography (gradient elution, dichloromethane to 98:2:0.2 dichloromethane/methanol/NH_4OH) gave **19** as a clear oil (8.1 mg, 52% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (m, 2 H), 7.35 (d, 1 H), 7.32 (d, 1 H), 7.24 (m, 2 H), 7.11 (t, 1 H), 6.99 (m, 5 H), 4.25 (s, 2 H), 3.35 (s, 3 H), 2.86 (s, 3 H) ppm; HRMS (ESI) *m*/*z* 408.1377 [(M + H)$^+$, calcd for $C_{22}H_{22}N_3O_3S$, 408.1374]. Anal. ($C_{22}H_{21}N_3O_3S$·0.8MeOH) C, H, N.

4-(2-Fluorobenzyl)piperidine (26). A neat mixture of 2-fluorobenzyl chloride (**20**) (10.0 g, 69.0 mmol) and triethyl phosphite (11.5 g, 69.0 mmol) was heated to 150 °C and stirred for 15 h. The reaction mixture was concentrated twice from toluene and then concentrated and purified by silica gel chromatography (gradient elution, 1:1 hexanes/ethyl acetate to 10% methanol/ethyl acetate) to give diethyl 2-fluorobenzylphosphonate.

To a solution of diethyl 2-fluorobenzylphosphonate (16 g, 69 mmol) in 1,3-dimethyl-2-imidazolidinone (25 mL) was added sodium hydride (4.4 g, 110 mmol) followed by slow addition of a solution of *N*-benzyl-4-piperdinone (13 g, 69 mmol). The reaction mixture was stirred for 20 min, cooled to 0 °C, and carefully quenched with water. Dichloromethane was added, and the layers were separated. The organic layer was dried over Na_2SO_4, filtered, and concentrated to give 1-benzyl-4-(2-fluorobenzyl)piperidine, which was used without further purification.

To a solution of 1-benzyl-4-(2-fluorobenzyl)piperidine (7 g, 25 mmol) in ethanol (150 mL) was added 10% palladium hydroxide (500 mg). The reaction mixture was placed on a Parr shaker with 50 psi of hydrogen and shaken for 15 h. The reaction mixture was filtered through Celite, concentrated, and purified by silica gel chromatography (gradient elution, 95:5:0.5 to 90:10:1 dichloromethane/methanol/NH_4OH) to give 4-(2-fluorobenzyl)piperidine (**26**): mass spectrum *m*/*z* 321 [(M + H)$^+$, calcd for $C_{20}H_{24}N_4$, 321].

(4-Benzylpiperidin-1-yl)acetic Acid (31). To a solution of 4-benzylpiperidine (**25**) (1.0 g, 5.7 mmol) in dimethylformamide (20 mL) was added diisopropylethylamine (990 μL, 5.7 mmol) and ethyl bromoacetate (637 μL, 5.7 mmol), and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was partitioned between ethyl acetate and aqueous $NaHCO_3$, and the organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by silica gel chromatography (gradient elution, 4:1 hexanes/ethyl acetate to ethyl acetate) to give ethyl (4-benzylpiperidin-1-yl)-acetate.

Ethyl (4-benzylpiperidin-1-yl)acetate (700 mg, 2.6 mmol) was dissolved in 6 N HCl (5 mL), and the mixture was heated to 100 °C for 1 h. The reaction mixture was cooled and concentrated to give the HCl salt of **31** as a white solid that was used without further purification.

2-[(4-Benzylpiperidin-1-yl)methyl]-5-methoxybenzimidazole (6b). To a solution of (4-benzylpiperidin-1-yl)acetic acid hydrochloride salt (**31**) (2.0 g, 7.41 mmol) in dimethylformamide (20 mL) was added EDC (1.56 g, 8.16 mmol), 1-hydroxy-7-azabenzotriazole (HOAt) (1.11 g, 8.16 mmol), 4-methoxy-1,2-phenylenediamine (**10**) (1.02 g, 7.41 mmol), and triethylamine (2.06 mL, 14.8 mmol). The reaction mixture was stirred at room temperature for 20 min followed by quenching with aqueous $NaHCO_3$ and ethyl acetate. The layers were separated, and the organic portion was washed twice with water, dried over Na_2SO_4, filtered, and concentrated.

The crude oil was dissolved in acetic acid (20 mL) and heated to 140 °C for 15 min. The reaction mixture was cooled, concentrated twice from toluene, and used without further purification. An aliquot of the reaction mixture was purified by silica gel chromatography (gradient elution, 95:5:0.5 to 80:20:2 dichloromethane/methanol/NH_4OH) to give methoxybenzimidazole **6b** (1.86 g, 75% yield, two steps) as a white amorphous solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.46 (d, 1 H), 7.28 (m, 2 H), 7.20 (t, 1 H), 7.13 (d, 2 H), 7.05 (br.s, 1 H), 6.87 (dd, 1 H), 3.87 (s, 2 H), 3.84 (s, 3 H), 2.94 (d, 2 H), 2.57 (d, 2 H), 2.23 (t, 2 H), 1.68 (d, 2 H), 1.59 (m, 1 H), 1.42 (q, 2 H) ppm; HRMS (ESI) *m*/*z* 336.2070 [(M + H)$^+$, calcd for $C_{21}H_{26}N_3O$, 336.2058]. Anal. ($C_{21}H_{25}N_3O$·0.65H_2O) C, H, N.

2-[(4-Benzylpiperidin-1-yl)methyl]benzimidazol-5-ol (6c). Benzimidazole **6b** (1.86 g, 5.55 mmol) was dissolved in HBr/H_2O (48%, 10 mL), and the mixture was heated to 100 °C for 3 h. The reaction mixture was cooled, concentrated, and purified by silica gel chromatography (gradient elution, dichloromethane to 80:20:2 dichloromethane/methanol/NH_4OH). The HCl salt was then prepared via treatment with HCl/ether and concentration in vacuo. Trituration in methanol/ether (1:2) gave the HCl salt of **6c** as a white crystalline solid (1.0 g, 56% yield): mp = 235 °C dec; ^{1}H NMR (400 MHz, CD_3OD) δ 7.61 (d, 1 H), 7.24 (t, 2 H), 7.17 (m, 5 H), 4.75 (s, 2 H), 3.60 (d, 2 H), 3.15 (t, 2 H), 2.60 (d, 2 H), 1.91 (m, 3 H), 1.61 (m, 2 H) ppm; HRMS (ESI) *m*/*z* 322.1914 [(M + H)$^+$, calcd for $C_{20}H_{24}N_3O$, 322.1914]. Anal. ($C_{20}H_{23}N_3O$·2HCl·0.9^{i}PrOH) C, H, N.

***N*-{2-[(4-Benzylpiperidin-1-yl)methyl]benzimidazol-5-yl}methanesulfonamide (6d).** Using the above procedure for **6b** with *N*-(3,4-diaminophenyl)methanesulfonamide (**14**) provided **6d**. The HCl salt was then prepared via treatment with HCl/ether and concentration in vacuo. Recrystallization from 4:1 methanol/ethyl acetate gave the HCl salt of **6d** as a white crystalline solid: mp = 230 °C dec; ^{1}H NMR (300 MHz, CD_3OD) δ 7.79 (d, 1 H), 7.75 (s, 1 H), 7.42 (d, 1 H), 7.32−7.17 (m, 5 H), 4.85 (s, 2 H), 3.65 (d, 2 H), 3.20 (t, 2 H), 3.00 (s, 3 H), 2.62 (d, 2 H), 2.00−1.88 (m, 3 H), 1.67 (m, 2 H) ppm; HRMS (ESI) *m*/*z* 399.1851 [(M + H)$^+$, calcd for $C_{21}H_{27}N_4O_2S$, 399.1849]. Anal. ($C_{21}H_{26}N_4O_2S$·2HCl) C, H, N.

***N*-{2-[(4-Benzylpiperidin-1-yl)methyl]benzimidazol-5-yl}propane-2-sulfonamide (6e).** Using the above procedure for **6b** with *N*-(3,4-diaminophenyl)propane-2-sulfonamide (**16**) followed by purification by preparative reverse-phase HPLC provided **6e** as a white amorphous solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1 H), 7.47 (d, 1 H), 7.22 (m, 2 H), 7.11 (m, 4 H), 3.75 (s, 2 H), 3.20 (m, 1 H), 2.90 (m, 2 H), 2.51 (d, 2 H), 2.10 (t, 2 H), 1.61 (m, 2 H), 1.54 (m, 1 H), 1.36 (m, 2 H), 1.31 (d, 6 H) ppm; HRMS (ESI) *m*/*z* 427.2161 [(M + H)$^+$, calcd for $C_{23}H_{31}N_4O_2S$, 427.2162]. Anal. ($C_{23}H_{30}N_4O_2S$·0.3TFA) C, H, N.

2-{[4-(2-Fluorobenzyl)piperidin-1-yl]methyl}benzimidazol-5-ol (37a). Using the above procedure for **6b** with [4-(2-fluorobenzyl)piperidinyl]acetic acid (**32**) followed by silica gel chromatography (gradient elution, 95:5:0.5 to 80:20:2 dichloromethane/methanol/NH_4OH) provided **37a**. The HCl salt was then prepared via treatment with HCl/ether and concentration in vacuo. Recrystallization from 2-propanol gave the HCl salt of **37a** as a white crystalline solid: mp = 240 °C dec; ^{1}H NMR (400 MHz, CD_3OD) δ 7.61 (d, 1 H), 7.23 (m, 2 H), 7.07 (m, 4 H), 4.65 (s, 2 H), 3.59 (d, 2 H), 3.10 (t, 2 H), 2.67 (d, 2 H), 1.93 (d, 3 H), 1.62 (m, 2 H) ppm; HRMS (ESI) *m*/*z* 340.1821 [(M + H)$^+$, calcd for $C_{20}H_{23}FN_3O$, 340.1820]. Anal. ($C_{20}H_{22}FN_3O$·2HCl) C, H, N.

2-{[4-(3-Fluorobenzyl)piperidin-1-yl]methyl}benzimidazol-5-ol (37b). Using the above procedure for **6b** with [4-(3-fluorobenzyl)piperidinyl]acetic acid (**33**) followed by silica gel chromatography (gradient elution, 95:5:0.5 to 80:20:2 dichloromethane/methanol/NH_4OH) provided **37b**. The HCl salt was then prepared via treatment with HCl/ether and concentration in vacuo. Recrystallization from 2-propanol gave the HCl salt of **37b** as a white crystalline solid: mp = 215 °C dec; ^{1}H NMR (300 MHz, CD_3OD) δ 7.70 (d, 1 H), 7.30 (m, 1 H), 7.17 (m, 2 H), 6.93 (m, 3 H), 4.90 (s, 2 H), 3.63 (d, 2 H), 3.22 (t, 2 H), 2.62 (d, 2 H), 1.92 (d, 3 H), 1.63 (m, 2 H) ppm; HRMS (ESI) *m*/*z* 340.1821 [(M + H)$^+$, calcd for $C_{20}H_{23}FN_3O$, 340.1820]. Anal. ($C_{20}H_{22}FN_3O$·2HCl·0.65^{i}PrOH) C, H, N.

2-{[4-(4-Fluorobenzyl)piperidin-1-yl]methyl}benzimidazol-5-ol (37c). Using the above procedure for **6b** with [4-(4-fluorobenzyl)piperidinyl]acetic acid (**34**) followed by silica gel

chromatography (90:10:1 dichloromethane/methanol/NH_4OH) provided **37c** as a white amorphous solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (br s, 1 H), 7.28 (s, 1 H), 7.05 (m, 2 H), 6.92 (m, 2 H), 6.81 (d, 1 H), 3.75 (s, 2 H), 2.91 (d, 2 H), 2.47 (d, 2 H), 2.12 (t, 2 H), 1.58 (m, 2 H), 1.50 (m, 1 H), 1.28 (m, 2 H) ppm; HRMS (ESI) *m/z* 340.1808 [$(M + H)^+$, calcd for $C_{20}H_{23}FN_3O$, 340.1820]. Anal. ($C_{20}H_{22}FN_3O \cdot 0.4$ dichloromethane) C, H, N.

2-{[4-(2,6-Difluorobenzyl)piperidin-1-yl]methyl}benzimidazol-5-ol (37d). Using the above procedure for **6b** with [4-(2,6-difluorobenzyl)piperidinyl]acetic acid (**35**) followed by silica gel chromatography (gradient elution, 95:5:0.5 to 80:20:2 dichloromethane/methanol/NH_4OH) provided **37d**. The HCl salt was then prepared via treatment with HCl/ether and concentration in vacuo. Recrystallization from 2-propanol gave the HCl salt of **37d** as a white crystalline solid: mp = 230 °C dec; ^{1}H NMR (300 MHz, CD_3OD) δ 7.65 (d, 1 H), 7.29 (m, 1 H), 7.15 (m, 2 H), 6.95 (t, 2 H), 4.72 (s, 2 H), 3.61 (d, 2 H), 3.14 (t, 2 H), 2.72 (d, 2 H), 1.92 (d, 3 H), 1.70 (m, 2 H) ppm; HRMS (ESI) *m/z* 358.1725 [$(M + H)^+$, calcd for $C_{20}H_{22}F_2N_3O$, 358.1726]. Anal. ($C_{20}H_{21}F_2N_3O \cdot 3HCl \cdot 0.6H_2O$) C, H, N.

2-{[4-(4-Methylbenzyl)piperidin-1-yl]methyl}benzimidazol-5-ol (37e). Using the above procedure for **6b** with [4-(4-methylbenzyl)piperidinyl]acetic acid (**36**) followed by silica gel chromatography (gradient elution, 90:10:1 to 80:20:2 dichloromethane/methanol/NH_4OH) provided **37e** as a white amorphous solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (br s, 1 H), 7.28 (s, 1 H), 7.10 (d, 2 H), 6.95 (d, 2 H), 6.81 (d, 1 H), 3.70 (s, 2 H), 2.90 (d, 2 H), 2.40 (d, 2 H), 2.29 (s, 3 H), 2.10 (t, 2 H), 1.57 (m, 2 H), 1.48 (m, 1 H), 1.23 (m, 2 H) ppm; HRMS (ESI) *m/z* 336.2082 [$(M + H)^+$, calcd for $C_{21}H_{26}N_3O$, 336.2071]. Anal. ($C_{21}H_{25}N_3O \cdot 0.95H_2O$) C, H, N.

Acknowledgment. The authors thank Dr. Charles W. Ross, III and Ms. Joan S. Murphy for determination of high-resolution mass spectra.

References

(1) Le, D. A.; Lipton, S. A. Potential and Current Use of *N*-Methyl-D-aspartate (NMDA) Receptor Antagonists in Diseases of Aging. *Drugs Aging* **2001**, *18* (10), 717−724.

(2) Max, M. B.; Byas-Smith, M. G.; Gracely, R. H. Bennet, G. J. Intravenous infusion of the NMDA antagonist, ketamine, in chronic posttraumatic pain with allodynia: a double-blind comparison to alfentanil and placebo. *Clin. Neuropharmacol.* **1995**, *18*, 360−368.

(3) Muir, K. W.; Lees, K. R. Clinical Experience with Excitatory Amino Acid Antagonist Drugs. *Stroke* **1995**, *26*, 503−513.

(4) Sugihara, H.; Moriyoshi, K.; Ishii, T.; Masu, M.; Nakanishi, S. Structures and Properties of Seven Isoforms of NMDA Receptor Generated by Alternative Splicing. *Biochem. Biophys. Res. Commun.* **1992**, *185*, 826−832.

(5) Cull-Candy, S.; Brickley, S.; Farrant, M. NMDA receptor subunits: diversity, development and disease. *Curr. Opin. Neurobiol.* **2001**, *11*, 327−335.

(6) Monyer, H.; Burnashev, N.; Laurie, D. J.; Sakmann, B.; Seeburg, P. H. Developmental and Regional Expression in the Rat Brain and Functional Properties of Four NMDA Receptors. *Neuron* **1994**, *12*, 529.

(7) Boyce, S.; Wyatt, J. K.; Webb, R.; O'Donnell, R.; Mason, G.; Rigby, M.; Sirinathsinghji, D.; Hill, R. G.; Rupniak, N. M. J. Selective NMDA NR2B antagonists induce antinociception without motor dysfunction: correlation with restricted localisation of NR2B subunit in dorsal horn. *Neuropharmacology* **1999**, *38*, 611−623.

(8) Williams, K. Ifenprodil discriminates subtypes of the *N*-methyl-D-aspartate receptor: selectivity and mechanisms at recombinant heteromeric receptors. *Mol. Pharmacol.* **1993**, *44*, 851−859.

(9) Kew, J. N. C.; Trube, G.; Kemp, J. A. A novel mechanism of activity-dependent NMDA receptor antagonism describes the effect of ifenprodil in rat cultured cortical neurones. *J. Physiol. (London)* **1996**, *497* (3), 761−772.

(10) Chenard, B. L.; Bordner, J.; Butler, T. W.; Chambers, L. K.; Collins, M. A.; DeCosta, D. L.; Ducat, M. F.; Dumont, M. L.; Fox, C. B.; Mena, E. E.; Menniti, F. S.; Nielsen, J.; Pagnozzi, M. J.; Richter, K. E. G.; Ronau, R. T.; Shalaby, I. A.; Stemple, J. Z.; White, W. F. (1*S*,2*S*)-1-(4-Hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol: A Potent New Neuroprotectant Which Blocks *N*-Methyl-D-aspartate Responses. *J. Med. Chem.* **1995**, *38* (16), 3138−3145.

(11) Pinard, E.; Alanine, A.; Bourson, A.; Buttelmann, B.; Heitz, M.-P.; Mutel, V.; Gill, R.; Trube, G.; Wyler, R. 4-Aminoquinolines as a Novel Class of NR1/2B Subtype Selective NMDA Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2615−2619.

(12) Wright, J. L.; Kesten, S. R.; Upasani, R. B.; Lan, N. C. 4-Benzylpiperidinylalkylsulfinyl-substituted heterocycles and their use as subtype-selective NMDA receptor antagonists. PCT Int. Appl. WO 2000000197, January 6, 2000.

(13) Curtis, N. R.; Diggle, H. J.; Kulagowski, J. J.; London, C.; Grimwood, S.; Hutson, P. H.; Murray, F.; Richards, P.; Macaulay, A.; Wafford, K. A. Novel N^1-(Benzyl)cinnamamidine Derived NR2B Subtype-Selective NMDA Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 693−696.

(14) Claiborne, C. F.; McCauley, J. A.; Libby, B. E.; Curtis, N. R.; Diggle, H. J.; Kulagowski, J. J.; Michelson, S. R.; Anderson, K. D.; Claremon, D. A.; Freidinger, R. M.; Bednar, R. A.; Mosser, S. D.; Gaul, S. L.; Connolly, T. M.; Condra, C. L.; Bednar, B.; Stump, G. L.; Lynch, J. J.; Macaulay, A.; Wafford, K. A.; Koblan, K. S.; Liverton, N. J. Orally Efficacious NR2B-Selective NMDA Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 697−700.

(15) Chizh, B. A.; Headley, P. M.; Tzschentke, T. M. NMDA Receptor Antagonists as Analgesics: Focus on the NR2B Subtype. *Trends Pharmacol. Sci.* **2001**, *22* (12), 636−642.

(16) (a) Nikam, S. S.; Meltzer, L. T. NR2B Selective NMDA Receptor Antagonists. *Curr. Pharm. Des.* **2002**, *8*, 845−855. (b) Chenard, B. L.; Menniti, F. S. Antagonists Selective for NMDA Receptors Containing the NR2B Subunit. *Curr. Pharm. Des.* **1999**, *5*, 381−404.

(17) (a) Konig, W.; Geiger, R. A New Method for the Synthesis of Peptides. *Chem. Ber.* **1970**, *103*, 788−798. (b) Sheehan, J. C.; Cruickshank, P. A.; Boshart, G. L. A Convenient Synthesis of Water-Soluble Carbodiimides. *J. Org. Chem.* **1961**, *26*, 2525−2528.

(18) Preston, P. N. Synthesis, Reactions and Spectroscopic Properties of Benzimidazoles. *Chem. Rev.* **1974**, *74*, 279−314.

(19) Rajappa, S.; Sreenivasan, R. Selective Functionalizaiton in 2-Nitro-*p*-phenylenediamine. *Indian J. Chem., Sect. B* **1980**, *19B* (7), 533−535.

(20) Bhattacharya, A. K.; Thyagarajan, T. The Michaellis−Arbuzov Rearrangement. *Chem. Rev.* **1981**, *81*, 415−430.

(21) Wadsworth, W. S. Synthetic Applications of Phosphoryl-Stabilized Anions. *Org. React.* **1977**, *25*, 73−253.

(22) For NR2B binding and Ca^{2+} influx assay protcols, see the following. McCauley, J. A.; Theberge, C. R.; Liverton, N. J.; Claremon, D. A.; Claiborne, C. F. 2-Benzyl and 2-Heteroaryl Benzimidazole NMDA/NR2B Antagonists. U.S. Patent 6,316,474 B1, November 13, 2001.

(23) For hERG binding assay protocol, see the following. Butcher, J. W.; Claremon, D. A.; Connolly, T. M.; Dean, D. C.; Karczewski, J.; Koblan, K. S.; Kostura, M. J.; Liverton, N. J.; Melillo, D. G. Radioligand and Binding Assay. PCT Int. Appl. WO 0205860 A1, 2002.

(24) For prazosin binding assay protocol, see the following. Greengrass, P.; Bremner, R. Binding characteristics of 3H-prazosin to rat brain alpha-adrenergic receptors. *Eur. J. Pharmacol.* **1979**, *55*, 323−326.

(25) All animal studies described in this report were approved by the Merck Research Laboratories Institutional Animal Care and Use Committee.

(26) Priestley, T.; Laughton, P.; Myers, J.; Le Bourdellès, B.; Kerby, J.; Whiting, P. Pharmacological properties of recombinant human *N*-methyl-D-aspartate receptors comprising NR1a/NR2A and NR1a/NR2B subunit assemblies expressed in permanently transfected mouse fibroblast cells. *Mol. Pharmacol.* **1995**, *48*, 841−848.

JM030483S