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Orally Efficacious NR2B-Selective NMDA Receptor Antagonists

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Abstract—A novel series of benzamidines was synthesized and shown to exhibit NR2B-subtype selective NMDA antagonist activity. Compound **31** is orally active in a carrageenan-induced rat hyperalgesia model of pain and shows no motor coordination side effects.

N-Methyl-D-aspartate (NMDA) receptor antagonists have considerable therapeutic potential in the treatment of several disease states including stroke, neuropathic pain, and Parkinson's disease.[1] The clinical development of NMDA antagonists however, has been encumbered by their small therapeutic index, since they have unacceptable side effects including, but not limited to, motor deficit, sedation, and psychotomimetic states.[2,3]

The discovery that the NMDA channel is a hetero-oligomeric assembly composed of a minimum of two different subunits, assigned NR1 and NR2, with at least eight isoforms (NR1a–h) for the former and four distinct subtypes (NR2A–D) for the latter, afforded the possibility of mapping the regional distributions of these polypeptides in the brain.[4,5] mRNA mapping studies indicate that whereas 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 absence of NR2B message in the cerebellum would suggest that NR2B-selective antagonists might not adversely affect locomotor function.[7] Ifenprodil (**1**, Fig. 1), a ligand which binds selectively to the NR2B subtype, effectively modulates ion flux[8,9] and demonstrates a separation of side effects from antinociceptive properties in animal models.[7] Thus, the ifenprodil binding site has proven an attractive target to enhance the pharmacological profile relative to non-selective NMDA antagonists.[10,11] A variety of compounds have since shown NR2B subtype selectivity, including CP-101,606 (**2**),[12] CI-1041 (**3**)[13] and aminoquinoline **4**.[14]

Screening efforts at Merck for compounds binding at the ifenprodil site resulted in the identification of styryl amidine **5** (Fig. 2) which displayed significant affinity for the NR2B receptor ($K_i = 9$ nM vs [^{3}H]-ifenprodil). The potency of this compound could be enhanced by addition of a methoxy substituent on the benzyl ring (**6**, $K_i = 0.7$ nM versus [^{3}H]-ifenprodil). The NR2B binding data reported in this communication utilizes radiolabeled amidine **6** ($K_D = 1.0$ nM) as the ligand rather than ifenprodil ($K_D = 94$ nM) due to its superior signal-to-noise ratio.[15]

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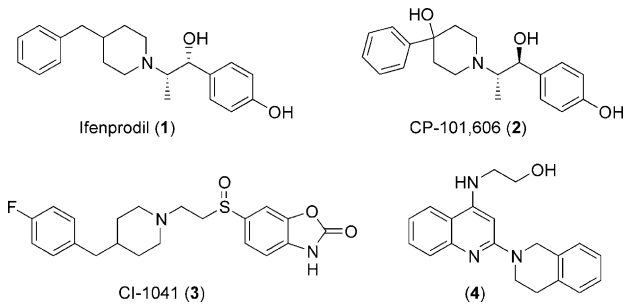


Figure 1. Structures of NR2B-selective NMDA receptor antagonists.

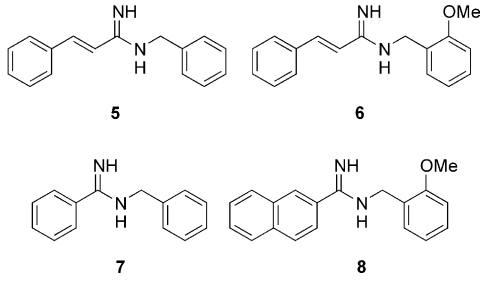
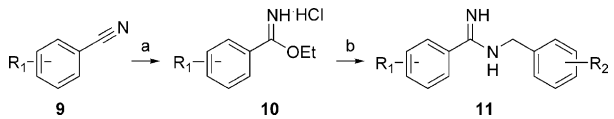


Figure 2. Amidine derived NR2B/NMDA receptor antagonists.

Recognizing the possibility that styryl amidine **5** may act as a Michael acceptor, we sought to develop a non-styryl series compounds,[16] into a novel class of selective NR2B receptor antagonists with the goal of demonstrating oral activity in a rodent hyperalgesia model.

It had been shown that a naphthamidine (**8**), combined with appropriate substitution on the benzyl ring, gave compounds of comparable activity to the styryl amidines.[16] However, benzamidine **7** had weak affinity for the NR2B subtype ($K_i = 10{,}000$ nM vs [^{3}H]-Ifenprodil). Therefore, initial efforts focused on developing the benzamidine series into a potent and selective series of NR2B antagonists by exploring substituent effects on the phenyl ring. Toward this end, commercially available reagents, in addition to a few individually prepared nitriles, were utilized to rapidly diversify the lead structure (Scheme 1).

Approximately 150 compounds were prepared from 10 aryl nitriles and 35 benzyl amines via the Pinner synthesis.[18] The selection criteria for the nitriles was mostly determined by our desire to temper the basicity of the amidine functionality with electron withdrawing groups. The results of the binding and functional assays (FLIPR) for selected compounds are summarized in Table 1.[15] As compared to the parent structure (**7**), significant effects on affinity were observed for the substitutions made around the *N*-benzylbenzamidine core.



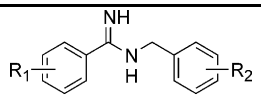
Scheme 1. Synthesis of amidine **11**: (a) HCl, EtOH; (b) R_2BnNH_2, EtOH.[17]

Simply substituting the benzyl ring of compound **7** with chloro at the 3-position (**12**) partially restored the activity observed in the styryl series. Holding R_2 constant as 3-Cl, it was clear that R_1 substituents in the 4-position led to compounds with the greatest activity in the NR2B binding assay (**13**–**18**). Furthermore, a variety of 4-R_1 substitutions were tolerated (**19**–**23**). Reexamination of benzyl substituent effects in the presence of a potency enhancing R_1 group demonstrated that both the 2- and 3-positions were accommodating of an array of R_2 substituents (**24**–**30**). Substitution of the benzylic amine fragment at the 4-position (**26**) generally led to diminished activity.

Using the above SAR data, we designed a number of high affinity compounds (Table 2), including amidine **35** which shows a remarkable >25,000-fold improvement over the parent benzamidine **7** in the NR2B binding assay. Selected compounds were screened against the closely related NR2A subtype in the FLIPR assay. The results demonstrated no measurable NR2A activity ($IC_{50} > 10$ μM) for compounds **31**–**35**. Furthermore, compound **31** was tested against the four known NR2B subtypes in a whole cell patch-clamp assay and was shown to be selective for NR2B (Fig. 3).

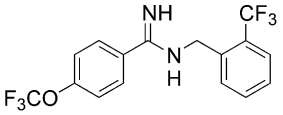
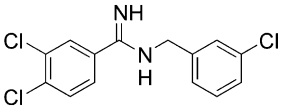
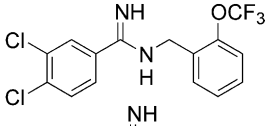
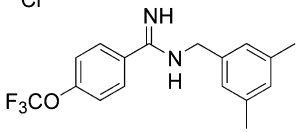
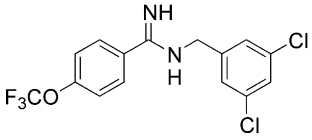
We proceeded to evaluate the pharmacokinetic properties and oral activity of selected compounds (results summarized in Table 2). The PK experiments used rats dosed at 2 mpk iv and 10 mpk po. With the exception of compound **34** (the most potent compound in the functional assay), compounds in the benzamidine series demonstrated good oral bioavailability and plasma half-lives with compound **35** having a remarkably long 7-h half-life. Efficacy was measured by scoring behavioral responses to noxious stimuli in a carageenan-induced

Table 1. Structure, binding affinity and functional activity of amidine derived NR2B/NMDA receptor antagonists



Entry	R_1	R_2	NR2B K_i (nM)	NR2B FLIPR IC_{50} (nM)
7	H	H	>15,000	—
12	H	3-Cl	4200	—
13	2-F	3-Cl	>15,000	—
14	3-F	3-Cl	2000	—
15	4-F	3-Cl	600	450
16	2-Cl	3-Cl	3900	—
17	3-Cl	3-Cl	800	420
18	4-Cl	3-Cl	63	160
19	4-Ph	H	1800	—
20	4-OCF_3	H	120	46
21	4-Cl	H	970	720
22	4-CF_3	H	480	360
23	4-SO_2Me	H	3500	—
24	4-OCF_3	2-OMe	5.7	4.1
25	4-OCF_3	3-OMe	23	9.7
26	4-OCF_3	4-OMe	1100	—
27	4-CF_3	2-Cl	56	48
28	4-CF_3	3-Cl	20	32
29	4-CF_3	3-CF_3	700	660
30	4-CF_3	3,5-diMe	3.9	8.7

Table 2. Binding affinity, functional activity, rat PK and oral efficacy of compounds **31**–**35**

Entry	Structure	NR2B K_i (nM)	NR2B FLIPR IC_{50} (nM)	NR2A FLIPR IC_{50} (nM)	%F	$T_{1/2}$ (min)	ED_{50} (mpk)
31		72	47	>10,000	100	147	5.5
32		16	110	>10,000	44	90	14
33		12	57	>10,000	31	183	7.5
34		1.2	4.2	>10,000	4	92	>30
35		0.6	24	>10,000	68	420	16

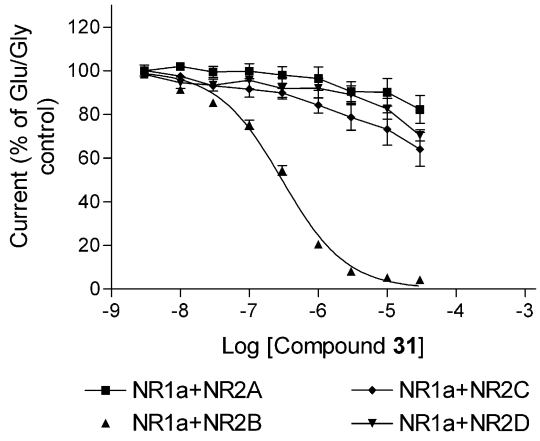


Figure 3. Inhibition of NMDA receptor-activated currents at recombinant human NMDA receptor subtypes. NR1a + NR2A, NR2B and NR2D were stably expressed in L(tk-) cells while NR1a + NR2C were expressed transiently in HEK cells.[19] Data represents the inhibition of maximally evoked currents in response to a glutamate/glycine application, and are mean ± SEM from at least five individual cells. Mean data from NR1 + NR2B was fitted using the Hill Equation.[20]

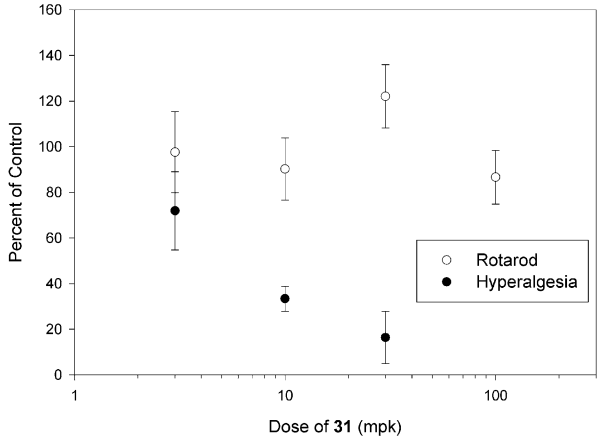


Figure 4. Effect of oral administration of **31** in methocel to rats in carrageenan-induced hyperalgesia and rotarod assays. Data are mean + SEM with $n = 8$ for hyperalgesia, $n = 6$ for rotarod.

hyperalgesia assay in the rat.[7] Compounds were dosed orally, and results are reported as the dose (mpk) required to achieve a 50% reduction in hyperalgesic response as compared to control. All agents having good oral pharmacokinetics provided ED_{50} values ranging from 5.5 to 16 mpk.

Compound **31** was further evaluated in a rat rotarod assay[7] to assess any effect on locomotor function (Fig. 4). Non-selective NMDA antagonists, such as MK-801, produce significant locomotor effects in the rotarod assay, and virtually no separation exists between efficacy and loss of motor coordination.[7] In contrast, dosed orally at 10 and 30 mpk, compound **31** exhibits significant block of the hyperalgesic response with no measurable effect on motor function. At 100 mpk, compound **31** continues to be free from locomotor side effects.

In conclusion, we have developed a novel class of NR2B-selective NMDA receptor antagonists which demonstrate oral efficacy in a rodent hyperalgesia model with no untoward effect on locomotor function associated with non-selective NMDA antagonists.

References and Notes

1. Le, D. A.; Lipton, S. A. *Drugs & Aging* **2001**, *18*, 717.
2. Max, M. B.; Byas-Smith, M. G.; Gracely, R. H.; Bennet, G. J. *Clin. Neuropharmacol.* **1995**, *18*, 360.
3. Muir, K. W.; Lees, K. R. *Stroke* **1995**, *26*, 503.
4. Sugihara, H.; Moriyoshi, K.; Ishii, T.; Masu, M.; Nakanishi, S. *Biochem. Biophys. Res. Commun.* **1992**, *185*, 826.
5. Cull-Candy, S.; Brickley, S.; Farrant, M. *Curr. Opin. Neurobiol.* **2001**, *11*, 327.
6. Monyer, H.; Burnashev, N.; Laurie, D. J.; Sakmann, B.; Seeburg, P. H. *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. *Neuropharmacology* **1999**, *38*, 611.
8. Williams, K. *Mol. Pharmacol.* **1993**, *44*, 851.
9. Kew, J. N. C.; Trube, G.; Kemp, J. A. *J. Physiol.* **1996**, *497.3*, 761.
10. Chizh, B. A.; Headley, P. M.; Tzschentke, T. M. *Trends Pharmacol. Sci.* **2001**, *22*, 636.
11. (a) Nikam, S. S.; Meltzer, L. T. *Curr. Pharm. Des.* **2002**, *8*, 845. (b) Chenard, B. L.; Menniti, F. S. *Curr. Pharm. Des.* **1999**, *5*, 381.
12. 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. *J. Med. Chem.* **1995**, *38*, 3138.
13. Wright, J. L.; Kesten, S. R.; Upasani, R. B.; Lan, N. C. PCT Int. Appl., WO 2000000197, 2000. *Chem. Abstr.* **2000**, *132*, 64255.
14. Pinard, E.; Alanine, A.; Bourson, A.; Buttelmann, B.; Heitz, M.-P.; Mutel, V.; Gill, R.; Trube, G.; Wyler, R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2615.
15. For NR2B Binding and FLIPR assay protcols see: McCauley, J. A.; Theberge, C. R.; Liverton, N. J.; Claremon, D. A.; Claiborne, C. F. US Patent 6,316,474 B1, 2001. *Chem. Abstr.* **2001**, *134*, 340508.
16. 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. *Bioorg. Med. Chem. Lett.* See preceeding communication. doi:10.1016/S0960-894X(02)01060-0.
17. Experimental procedure for the preparation of compound **31**: A solution of 4-(trifluoromethoxy)benzonitrile (3.0 g, 16.0 mmol) in EtOH (40 mL) under N_2 was cooled to 0 °C. HCl gas was bubbled through the solution for 10 min and the solution was warmed to rt and stirred for 1 h. The reaction mixture was concentrated, diluted with Et_2O, sonicated for 5 min and filtered. The imidate salt (2.8g, 65% yield) was recovered as a white solid.

To a solution of the imidate salt (1.0 g, 3.7 mmol) in EtOH (15 mL) at rt was added 2-(trifluoromethyl)benzyl amine (0.52 mL, 3.7 mmol) and triethylamine (1.54 mL, 11.1 mmol). After 4 h, the reaction mixture was concentrated and purified by reverse-phase HPLC. The TFA salt of **31** was partitioned between EtOAc/$NaHCO_3$, dried and evaporated to give the free base. HCl/Et_2O (1 equiv) was then added to a CH_2Cl_2 solution of the free base. The mixture was sonicated and filtered to give the HCl salt of **31** as a white solid (650 mg, 44% yield): ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.87 (d, 2H), 7.83 (d, 1H), 7.73 (dd, 1H), 7.66 (d, 1H), 7.60 (dd, 1H), 7.52 (d, 2H), 4.85 (s, 2H) ppm; HRMS (FT/ICR) *m*/*z* 363.0896 [$(M+H)^+$; calcd for $C_{16}H_{13}F_6N_2O$: 363.0927].
18. Dox, A. W. *Org. Synth.* **1932**, *CV1*, 5.
19. Priestley, T.; Laughton, P.; Myers, J.; Le Bourdellès, B.; Kerby, J.; Whiting, P. J. *Mol. Pharmacol.* **1995**, *48*, 841.
20. 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 transiently transfected with NR1a/NR2C receptors. The external recording solution consisted of (in mM): NaCl (149), KCl (3.25), $CaCl_2$ (2), HEPES (10), glucose (11), sucrose (21.9); pH 7.4. Ringer was superfused at a rate of 0.7–2.0 mL/min. The recording pipette solution was composed of (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 pipette and experiments were performed at room temperature. Inhibition curves for NMDA antagonists were obtained in the presence of 10μM glutamate and 1 μM glycine (10 μM for NR1a/NR2A). Increasing concentrations of antagonist was pre-applied for 30 s followed by two co-applications of glu/gly for 5 s. Inhibition of the second glu/gly response in the presence of antagonist was determined.