Bioorganic & Medicinal Chemistry Letters, Vol.3, No.1, pp. 43-48, 1993
Printed in Great Britain

0960-894X/93 $5.00 + .00

TETRAZOLE AMINO ACIDS AS COMPETITIVE NMDA ANTAGONISTS

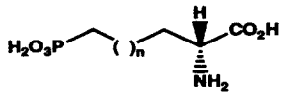
Paul L. Ornstein*, M. Brian Arnold, Debbie Evrard, J. David Leander, David Lodge and Darryle D. Schoepp, Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana, 46285

Abstract. We describe here the synthesis and pharmacological characterization of two novel series of acidic amino acids (tetrazole-substituted acyclic amino acids and piperazine-2-carboxylic acids) as potential NMDA receptor antagonists. Potent, systemically active NMDA antagonists were discovered in the series of tetrazole-substituted piperazine-2-carboxylic acids.

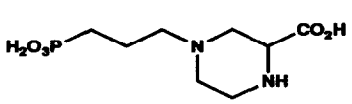
The synthesis of novel agents to antagonize neurotransmission at the *N*-methyl-D-aspartate (NMDA) subclass[1] of excitatory amino acid (EAA) receptors[2] is an exciting challenge for medicinal chemists.[3] Excitotoxicity,[4] resulting from inappropriate stimulation by glutamic acid (the putative endogenous EAA neurotransmitter) may be a component of the pathophysiology of a number of acute (e.g., cerebral ischemia[5]) and chronic (e.g., Alzheimer's disease[6] and Parkinson's disease[7]) neurodegenerative disorders in the central nervous system (CNS). Ample evidence supports the potential therapeutic use of an NMDA receptor antagonist for the treatment of such disorders.

The NMDA receptor is a macromolecular complex,[1] consisting of a number of neurotransmitter and modulatory sites that gate an ion channel permeable to calcium and sodium ions. One approach to antagonizing NMDA receptor-mediated neurotransmission is to develop compounds that act at the glutamic acid binding site (e.g., **1** and **2**; competitive NMDA antagonists).[3] Another approach is to develop compounds that act at a site inside the ion channel (e.g., phencyclidine (PCP); non-competitive PCP-like antagonists).[3] We decided to focus our efforts on the synthesis of competitive NMDA antagonists, based on observed differences in the behavioral pharmacological profile of competitive versus non-competitive PCP-like NMDA antagonists, which might indicate a more favorable side effect profile for the former class of compounds.[9]

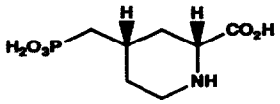
The first potent and selective NMDA antagonists were the phosphonic acid substituted acyclic amino acids **1** (2*R*-AP5) and **2** (2*R*-AP7).[10] Their utility was limited, however, by their relatively poor activity following systemic administration in animals.[11] Our goal was the synthesis of novel high affinity NMDA antagonists with potent systemic activity, which we sought to accomplish by incorporating the required acidic amino acid substructure into cyclic frameworks and to use the tetrazole group as a bioisosteric replacement for the phosphonic acid moiety. The new NMDA antagonists, **3** ((±)-CPP),[12] **4** ((±)-CGS 19755),[13] **5** ((±)-LY233053),[14] **6** ((±)-LY274614)[15,16] and **7** ((±)-LY233536),[16] that we and others have prepared, exemplify these concepts. These are potent NMDA antagonists that are active following systemic administration in mice, rats and pigeons at relatively low doses when compared to **1** and **2**.[16] In this communication we describe the synthesis and pharmacological characterization of two novel series of acidic amino acids (ω-tetrazole-substituted acyclic α-amino acids and 4-(tetrazolylalkyl)piperazine-2-carboxylic acids) as potential NMDA antagonists.



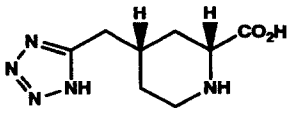
1 (n = 1); 2 (n = 3)



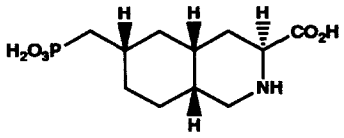
3



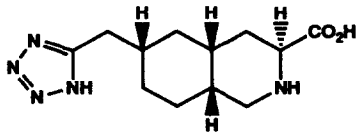
4



5



6



7

The ω-tetrazole-substituted acyclic α-amino acids, or acyclic tetrazoles, were prepared as shown in Scheme 1.[20] The aspartate and glutamate tetrazoles were prepared as single optical antipodes, while the higher homologues were prepared as racemates. All the tetrazoles in both series were prepared by treatment of the requisite nitrile with azido tri-*n*-butylstannane neat at 80 °C for three days.[14,17] *N*-BOC-*S*-asparagine methyl ester was dehydrated to the nitrile **8**,[18] and the nitrile converted to the tetrazole, followed by hydrolysis and cation exchange chromatography to afford the inner salt of the amino acid **12**.[18] By the same route, the nitriles **9-11**[18,19] were prepared and then converted to **13**,[18] **14**[19] and **15**[19] (isolated as their inner salts). Treatment of the sodium salt of **16**[21] with a cyanoalkyl bromide afforded the nitriles **17-20**, which were converted as above to the desired amino acids **21-24**. Amino acids **21**, **22** and **24** were isolated as their hydrochloride salts, while amino acid **23** was isolated as the inner salt following treatment with propylene oxide.

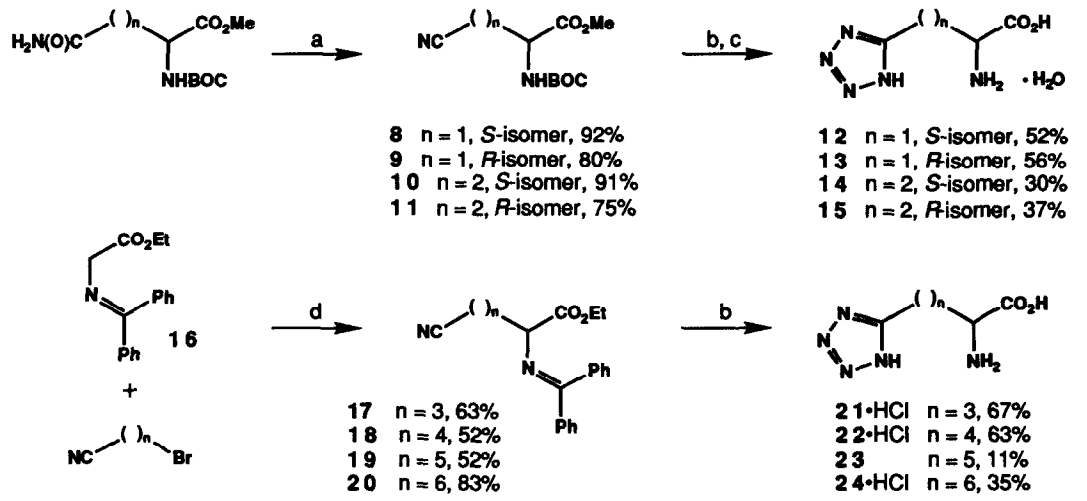
The series of tetrazole-substituted piperazines[22] were prepared as shown in Scheme 2.[20] 2-Pyrazinamide was reduced to piperazine-2-carboxamide, which was regioselectively alkylated with a cyanoalkyl bromide in ethanol in the presence of *N*,*N*-diisopropyl-*N*-ethylamine to afford the corresponding nitriles **25-28** after conversion to their *N*-t-butoxycarbonyl derivatives. Formation of the tetrazole, hydrolysis and cation exchange chromatography afforded the inner salts of the desired amino acids **29-32**, respectively.

The tetrazole-substituted amino acids that we prepared were evaluated for affinity at the NMDA receptor by examining their ability to inhibit [^{3}H]CGS-19755 binding,[23] and in a cortical slice assay[24] for both intrinsic agonist activity and antagonist activity versus 40 μM NMDA-induced depolarization. As a measure of in vivo antagonist activity, these amino acids were examined for their ability to block NMDA-induced lethality in mice[25] following intraperitoneal (i.p.) administration, and this data is also reported in the Table.

The acyclic tetrazoles **12**, **13**, **14** and **15** showed moderate affinity for the NMDA receptor. Compounds **12** and **13** were weakly active as antagonists in the cortical wedge, with IC_{50} values >100 μM; **14** was an agonist[26] that was about as half as potent as NMDA; and **15** was an antagonist, with an IC_{50} of about 31.6 μM. Of the higher homologues **21**, **22**, **23** and **24** (which are structurally similar to AP5-AP8) **21** and **22** showed moderate affinity for the NMDA receptor and weak antagonist activity in the cortical wedge assay; **23** and **24** were inactive. None of these amin

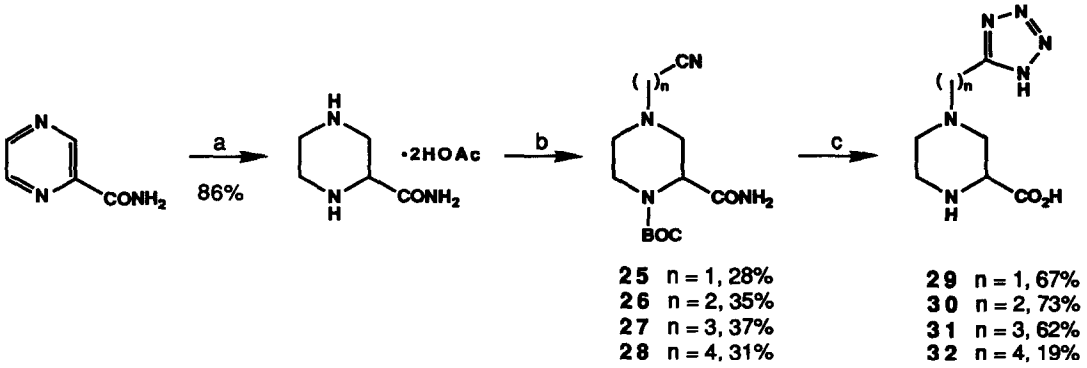
acids protected mice from NMDA-induced lethality, and none of the agonists showed convulsant effects when administered alone.

Scheme 1. Synthesis of acyclic tetrazole-substituted amino acids



a. $PhP(O)Cl_2$, pyridine, CH_2Cl_2, 0 °C. b. n-Bu_3SnN_3, 80 °C; MeOH, HCl; 6 N HCl, reflux. c. Dowex 50X-8, 10% pyridine/H_2O. d. $NaN(SiMe_3)_2$, THF, -78 °C to RT.

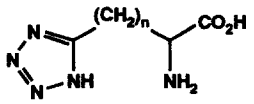
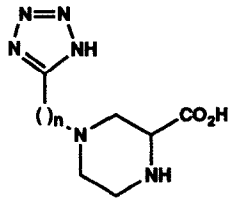
Scheme 2. Synthesis of tetrazole-substituted piperazine-2-carboxylic acids



a. H_2, PtO_2, 6/1 EtOH/acetic acid, 60 °C, 60 psi. b. $Br(CH_2)_nCN$, i-Pr_2NEt, EtOH, 80 °C; $(BOC)_2O$. c. n-Bu_3SnN_3, 80 °C; HCl, MeOH; 6N HCl, reflux (n = 1,3, 4) or 2N NaOH, MeOH, reflux (n = 2); Dowex 50X-8, 10% pyridine/H_2O.

The piperazine tetrazoles **29**, **30** and **31** showed moderate affinity for the NMDA receptor, and were active as NMDA antagonists in the cortical wedge assay. However, only the tetrazolylethyl and tetrazolylpropyl compounds **30** and **31** were able to block NMDA-induced lethality in mice, with **31** (MED = 40 mg/kg) being more potent in vivo than **30** (MED = 160 mg/kg). The tetrazolylbutyl analog **32** was inactive.

Table: In Vitro and In Vivo Activity of Tetrazole-Substituted Amino Acids

Compound[a]	n	[^{3}H]CGS19755[b] Binding IC_{50} (nM)	Cortical Wedge vs. NMDA[c] IC_{50} (μM)	MED[d] (mg/kg, i.p.) To Prevent NMDA-Induced Lethality in Mice[e]	Structure
12 (*S*-isomer)	1	639 ± 186	>100	>160	
13 (*R*-isomer)	1	685 ± 239	>100	>160	
14 (*S*-isomer)	2	1601 ± 701	agonist	>160	
15 (*R*-isomer)	2	925 ± 110	31.6	>160	
21	3	1055 ± 478	antagonist	>160	
22	4	10,030 ± 600	antagonist	>160	
23	5	>10,000	>100	>160	
24	6	>10,000	>100	>160	
29	1	1670 ±620	8.2 ± 2.2	>80	
30	2	781 ± 62	4.7 ± 0.7	160	
31	3	3150 ± 1210	16.3 ± 4.2	40	
32	4	>10,000	---	>80	
1	--	177 ± 34	3.7 ± 0.3	160[g]	
2	--	461 ± 34	11.1 ± 2.1[g]	160[g]	
3	--	220	0.6 ± 0.06	2.5	
4	--	54 ± 13	1.6 ± 0.13	1.25	
5	--	107 ± 7	4.2 ± 0.4	5	

[a]All compounds are racemic unless otherwise indicated. [b]See reference 20. [c]See reference 21. [d]MED = minimum effective dose. This is the lowest dose where at least three of the five mice tested survived. [e]See reference 22. Animals were given the test compound intraperitoneally (i.p.) 30 minutes before a dose of 200 mg/kg of NMDA. [f]NT = not tested. [g]Data for this compound in this assay is for the racemate.

We have prepared and evaluated two novel series of compounds as NMDA receptor antagonists. While a number of the acyclic tetrazoles that we prepared had moderate affinity and weak antagonist activity, none of these compounds were active following systemic administration in mice. In the piperazine tetrazole series, the tetrazolylethyl analog **30** is the most potent in vitro while the tetrazolylpropyl analog **31** is the most potent in vivo. The tetrazolylmethyl analog **29** is less potent in vitro (and inactive in vivo) than the structurally analogous piperidine tetrazole **5** (which is the most potent compound in vitro and in vivo from that series).[14] Similar results were observed for a series of piperazine phosphonates[12,27] (e.g., **3**); 4-phosphonomethylpiperazine-2-carboxylic acid was less potent as an NMDA antagonist than the corresponding piperidine phosphonate **4**,[13] and the phosphonopropyl analog **3** is the most potent in vitro and in vivo. The effects of tetrazole substitution in the piperazine-2-carboxylic acid series are quite different from our earlier results with amino acids **5** and **7**, which are only two- to fourfold less potent in vitro and in vivo than their phosphonic acid counterparts **4** and **6**, respectively.

NMDA antagonist activity is observed with tetrazole-substituted amino acids, when the two acid moieties are separated by either four, five or six atoms,[14] whereas phosphonic acid substituted amino acids show NMDA antagonist activity only when the two acid moieties are separated by either four or six atoms. We are currently trying to understand the nature of the differences between the tetrazole and phosphonic acid in their interaction with the NMDA receptor protein. In certain cases (e.g., **5** and **7**), however, the tetrazole group is an effective acid isostere providing potent, systemically active NMDA antagonists.

References and Notes

1. Wong, E.H.F.; Kemp, J.A. *Annu. Rev. Pharmacol. Toxicol.* **1991**, *31*, 401.
2. Collingridge, G.L.; Lester, R.A. *Pharmacol. Rev.* **1989**, *40*, 143.
3. Johnson, G.; Bigge, C.F. *Ann. Rep. Med. Chem.* **1991**, *26*, 11.
4. Meldrum, B.; Garthwaite, J. *Trends Pharmacol Sci.* **1990**, *11*, 379.
5. Meldrum, B. *Cerebrovasc. Brain Metab. Rev.* **1990**, *2*, 27.
6. Greenamyre, J.T.; Young , A.B. *Neurobiol. Aging* **1989**, *10*, 593.
7. Greenamyre, J.T.; O'Brien, C.F. *Arch. Neurol.* **1991**, *48*, 977.
8. Ornstein, P.L.; Schoepp, D.D. *Curr. CNS Patents* **1990**, *1*, 413.
9. Willetts, J.; Balster, R.L.; Leander, J.D. *Trends Pharmacol. Sci.* **1990**, *11*, 423.
10. Evans, R.H.; Francis, A.A.; Jones, A.W.; Smith, D.A.S.; Watkins, J.C. *Br. J. Pharmac.* **1982**, *75*, 65.
11. Meldrum, B.S.; Croucher, M.J.; Czuczwar, S.J.; Collins, J.F.; Curry, K.; Joseph, M.; Stone, T. *Neuroscience* **1983**, *4*, 925.
12. Aebischer, B.; Frey, P.; Haerter, H.P.; Herrling, P.L.; Mueller, W.; Olverman, H.J.; Watkins, J.C. *Helv. Chim. Acta* **1989**, *72*, 1043.
13. Hutchinson, A. J.; Williams, M.; Angst, C.; de Jesus, R.; Blanchard, L.; Jackson, R. H.; Wilusz, E. J.; Murphy, D. E.; Bernard, P. S.; Schneider, J.; Campbell, T.; Guida, W.; Sills, M. A. *J. Med. Chem.* **1989**, *32*, 2171.

14. Ornstein P.L.; Schoepp D.D.; Arnold, M.B.; Leander, J.D.; Lodge, D.; Paschal, J.W.; Elzey, T. *J. Med. Chem.* **1991**, *34*, 90.
15. Schoepp, D.D.; Ornstein, P.L.; Salhoff, C.R.; Leander, J.D. *J. Neural Transm [Gen. Sect.]* **1991**, *85*, 131.
16. Ornstein, P.L.; Schoepp, D.D.; Leander, J.D.; Lodge, D. In *Excitatory Amino Acids 1990*; Meldrum, B., Moroni, F., Simon, R., Woods J, Eds.; Raven Press: New York, 1991; pp 415-423.
17. Sisido, K.; Nabika, K.; Isida, T.; Kozima, S. *J. Organometal. Chem.* **1971**, *33*, 337.
18. Grzonka, Z. *Rocz. Chem.* **1972**, *46*, 1265.
19. Van, T.T.; Kojro, E.; Grzonka, Z. *Tetrahedron* **1977**, *33*, 2299.
20. All new compounds gave satisfactory IR, MS, ^{1}H NMR and elemental analyses (C, H and N). The following optical rotations (c = 1, 6N HCl) were obtained for the amino acids **12-15**: **12**, $[\alpha]_D$ = +17.8°; **13**, $[\alpha]_D$ = -17.8°; **14**, $[\alpha]_D$ = +37.0°; **15**, $[\alpha]_D$ = -36.1°.
21. O'Donnel, M.J.; Polt, R. L. *J. Org. Chem.* **1982**, *47*, 2663.
22. Ornstein, P.L.; Arnold, M.B.; Schoepp, D.D.; Leander, J.D.; Lodge, D. *Abstracts of Papers 200th National Meeting of the ACS*; American Chemical Society, Washington, D.C., 1990; Medi. 20.
23. Murphy, D.E.; Hutchinson, A.J.; Hurt, S.D.; Williams, M.; Sills, M.A. *Br. J. Pharmac.* **1988**, *95*, 932.
24. Harrison, N.L.; Simmonds, M.A. *Br. J. Pharmac.* **1985**, *84*, 381.
25. Leander, J.D.; Lawson, R.R.; Ornstein, P.L.; Zimmerman, D.M. *Brain Res.* **1988**, *448*, 115.
26. Johnson, R.L.; Koerner, J.F. *J. Med. Chem.* **1988**, *31*, 2057.
27. Hays, S.J.; Bigge, C.F.; Novak, P.M.; Drummond, J.T.; Bobovski, T.P.; Rice, M.J.; Johnson, G.; Brahce, L.J.; Coughenour, L.L. *J. Med. Chem.* **1990**, *33*, 2916.