Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 389–394

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

HETEROATOM-SUBSTITUTION AS A STRATEGY FOR INCREASING THE POTENCY OF COMPETITIVE NMDA ANTAGONISTS

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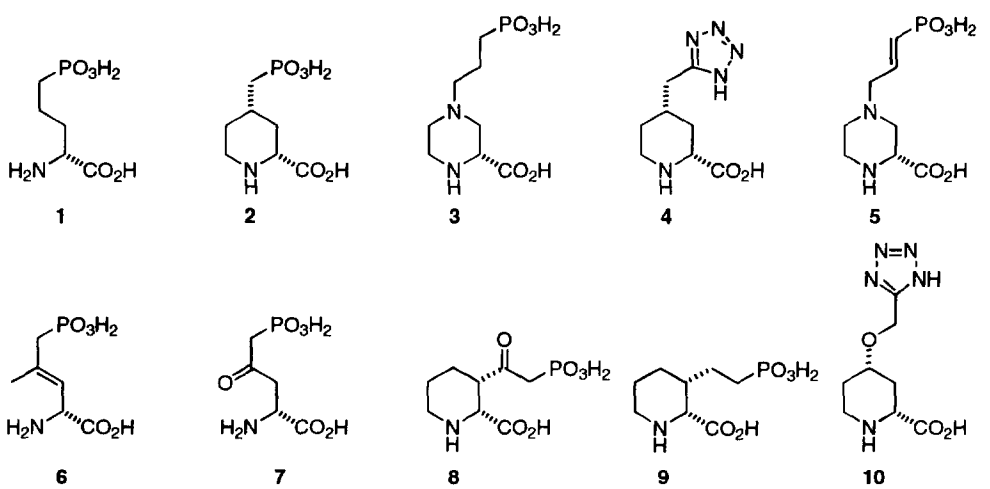
Received 8 December 1997; accepted 14 January 1998

Abstract. We report the synthesis and characterization of compounds that are competitive NMDA receptor antagonists. Significant increases in affinity and potency were obtained by incorporation of a heteroatom into the substructure of the tetrazole-substituted amino acid LY233053.

Competitive antagonists of neurotransmission mediated through the *N*-methyl-D-aspartate (NMDA) subclass of excitatory amino acid (EAA) receptors are important targets for the development of novel therapeutic agents.[1] It is now well documented that these compounds are effective anticonvulsant agents in animal models of epilepsy.[2] They are also efficacious in animal models of focal cerebral ischemia and head trauma.[3] Thus, these compounds may find utility in the treatment of a variety of central nervous system disorders.[4]



1 2 3 4 5

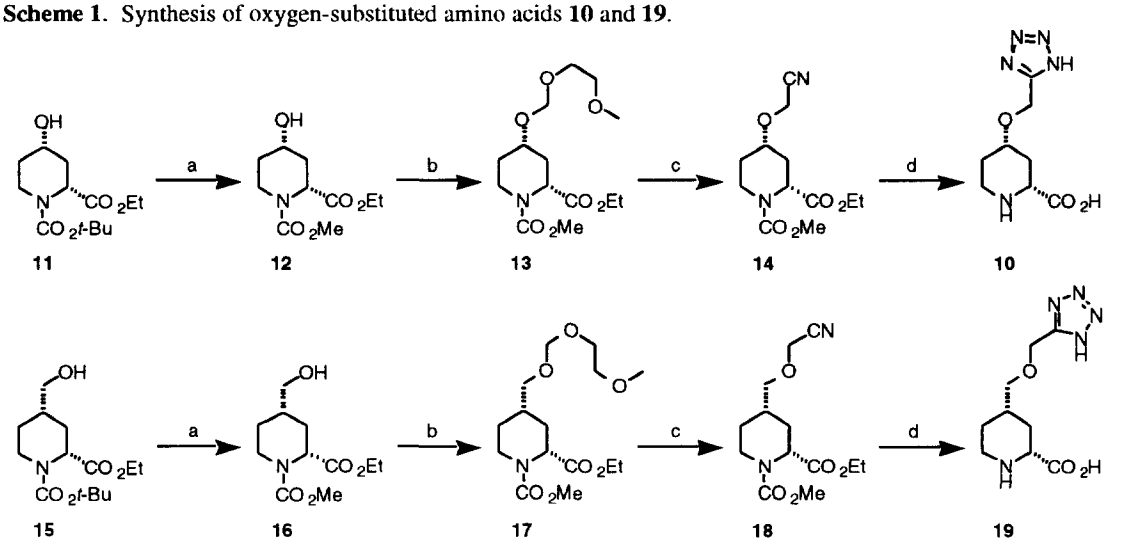
6 7 8 9 10

Structure activity studies on competitive NMDA antagonists revealed that an α-amino acid substituted with another acidic group was required for activity, and that the spacing between the two acid groups was critical for potency.[5] The prototypical 2*R*-AP5 (**1**) best exemplifies these requirements.[6] One strategy that we and others have used to enhance the potency and CNS availability of these compounds was to incorporate this acidic

0960-894X/98/$19.00
PII: S0960-894X(98)00038-9

amino acid substructure into a cyclic array, and compounds such as **2** (CGS 19755)[7] and **3** (2*R*-CPP)[8] represent potent NMDA antagonists with good systemic bioavailability. Another strategy has been to explore bioisosteric replacement for the distal acidic moiety, and the tetrazole analog **4** (2*R*,4*S*-LY233053)[9] is an example of this concept. Yet another strategy has been to introduce functionality such as an olefin into the backbone of **1** which constrains conformational mobility and therefore enhances potency. This latter idea is best exemplified by **5** (2*R*-CPP-ene)[10] and **6** (2*R*-CGP37849).[11] Alternatively, a ketone functionality was incorporated into the backbone of **1** to yield **7** (MDL-100,453);[12] and it was proposed that the increase in affinity of this compound may result from internal hydrogen bonding between the ketone and the amine or phosphono functionalities. This beneficial effect was also observed for **8**, which was significantly more potent than its counterpart **9** which lacks the ketone on the side chain at C-3.[13] The exact reason for the increase in potency from incorporation of heteroatom functionality is as yet unknown, and may result from a fortuitous hydrogen bonding interaction (between, e.g., a ketone and the receptor protein). We report here the synthesis of compounds such as **10**, in which a heteroatom such as oxygen or sulfur has been incorporated into the off-ring side chain in order to probe the potential for enhanced potency due to such an interaction.

Scheme 1. Synthesis of oxygen-substituted amino acids **10** and **19**.

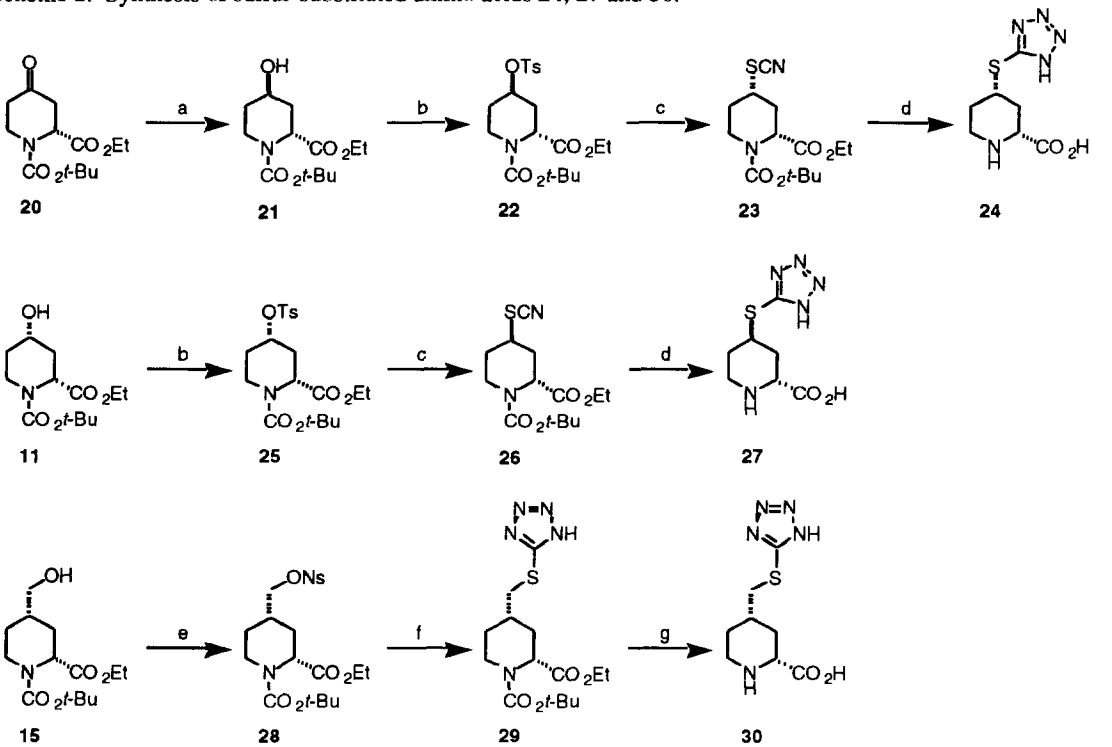


All compounds are racemic; one isomer is shown for clarity. a. TFA, CH_2Cl_2, room temperature; $ClCO_2Me$, iPr_2NEt, CH_2Cl_2, 0 °C; 57% for **12**, 55% for **16**. b. MEMCl, *i*-Pr_2NEt, DMAP, CH_2Cl_2, room temperature. c. TMSCN, BF_3•Et_2O, CH_2Cl_2, 0 °C to room temperature; 70% for **14**, 72% for **18**, two steps. d. n-Bu_3SnN_3, 90 °C; 6 N HCl, reflux; Dowex 50-X8, 10% pyridine/water; 33% for **10**; 71% for **19**.

Scheme 1 shows the preparation of amino acids **10** and **19**, which contain an oxygen atom in the chain that connects the piperidine ring to the tetrazole.[14] As we reported earlier, direct *O*-cyanomethylation of a compound such as **11**[15] was not possible.[16] Therefore, a two step procedure was necessary. Because this process involves treament with a strong Lewis acid, the *N*-BOC of **11** was converted to the more stable *N*-

methylcarbamate, **12**. Formation of the methoxyethoxymethyl (MEM) ether **13** was followed by treatment with cyanotrimethylsilane in the presence of 25 mol% of boron trifluoride etherate to afford ether **14**. Reaction of **14** with 2 equivalents of azido tri-*n*-butylstannane, followed by exhaustive hydrolysis with 6 N hydrochloric acid and then ion exchange chromatography yielded the desired amino acid **10**. Conversion of the known homologous alcohol **15**[15] to amino acid **16** was accomplished using an identical sequence of steps.

Scheme 2. Synthesis of sulfur-substituted amino acids **24**, **27** and **30**.



All compounds are racemic; one isomer is shown for clarity; Ts = *p*-toluenesulfonyl; Ns = *p*-nitrobenzenesulfonyl. a. $NaBH_4$, $CeCl_3$, MeOH, -35 °C to room temperature; 83% of a 10:1 mixture. b. TsCl, Et_3N, DMAP, CH_2Cl_2, room temperature; 71% for **22**; 78% for **25**. c. KSCN, DMF, 78 °C to room temperature; 43% for **23**; 39% for **26**. d. n-Bu_3SnN_3, 90 °C; 5 N HCl, reflux; Dowex 50-X8, 10% pyridine/water; 69% for **24**; 20% for **27**. e. NsCl, Et_3N, DMAP, CH_2Cl_2, CH_3CN, 0 °C to room temperature; 75%. f. 5-mercapto-1*H*-tetrzole, Et_3N, CH_3CN, 58 °C to reflux; 30%. g. 5 N HCl, reflux; Dowex 50-X8, 10% pyridine/water; 14%.

Compounds with a sulfur in the connecting chain were prepared as shown in Scheme 2.[14] Ketone **20** was reduced with sodium borohydride and cerium trichloride in methanol to afford the trans-alcohol **21**. Tosylation gave **22**, and displacement with potassium thiocyanate gave compound **23**. This was converted to the tetrazole

as usual and exhaustively hydrolyzed to afford amino acid **24**. The same sequence of reactions with the cis-alcohol **11** gave the epimeric amino acid **27**, through tosylate **25** and thiocyanate **26**. The homologous amino acid **30** was prepared by conversion of alcohol **15** to the corresponding nosylate **28** followed by displacement with 5-mercaptotetrzole to afford compound **29**. Exhasutive hydrolysis then afforded amino acid **30**.

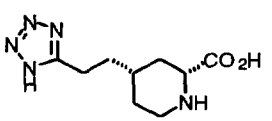
These compounds were evaluated for their ability to inhibit the binding of [^{3}H]CGS 19755 in rat brain membranes as a measure of their affinity at the NMDA receptor;[17] and for their ability to inhibit depolarizations induced by 40 μM NMDA in a cortical slice preparation to determine their relative potency as NMDA antagonists.[18] Data for these assays are shown in the Table[19] (where compounds are grouped according to the number of atoms in the chain that connects the tetrazole to the piperidine nucleus). For comparison, the table also contains data for the 4-tetrazolylmethyl (**4**), -ethyl (**31**) and -propyl (**32**) analogs that we previously reported.[15] The *cis*- and *trans*-amino acids **24** and **27**, respectively, where the tetrazole is connected to the piperidine ring through a sulfur atom, were about the same potency as **4** in terms of their affinity for NMDA receptors. In the cortical slice preparation, **27** was somewhat less potent than **4**. Substitution with an oxygen atom in the two-atom connecting chain adjacent to the piperidine ring (e.g., **10**) gave a compound that had about 2.5-fold lower affinity than **4**, but 8-fold higher than the directly analogous amino acid **31**, which has an ethylene connector. In the cortical slice preparation, **10** was equipotent to **4** and twice as potent as the structurally analogous **31**. Thus, the increase in affinity for **10** was also manifest in an increase in functional antagonist activity in vitro. Compound **30**, where a sulfur atom is adjacent to the tetrazole ring in a two-atom tether, was only slightly more potent than **31**, and less potent than **10**. And amino acid **19**, with an oxygen atom in the middle of a three-atom connecting chain, was lower in affinity and antagonist potency than **32**, which has a propylene connector.

Amino acid **27** was examined for effects on increases in firing rate evoked by iontophoretic ejection of NMDA and AMPA on rat spinal cord neurons in vivo.[20] At ejection currents of 3-10 nA, a 5mM solution of **27** in 200 mM aqueous sodium chloride gave a 70.5 ± 8.0% reduction of responses to NMDA with no effect on AMPA-evoked excitation. A 10 mg/kg intravenous dose of **27** also significantly reduced (73 ± 3%) excitations due to iontophoretic NMDA on rat spinal neurons with no effect on responses to AMPA or kainic acid.

We determined NMDA antagonist activity in mice (Table) for amino acids **10** and **19** by measuring the minimum effective dose (MED, in mg/kg following intraperitoneal (ip) administration 30 min prior to NMDA) of these compounds required to block lethality induced by a 200 mg/kg, ip, dose of NMDA in mice.[21] Amino acid **10** was very effective in this assay, blocking NMDA lethality at a MED of 2.5 mg/kg. This compound was four-times more potent in vivo than **31**, and twice as potent as **4**. The homologous amino acid **19** was significantly less potent in vivo than its all-carbon counterpart, with an MED in this assay of 80 mg/kg.

One explanation that was offered for the increase in affinity seen for compounds such as **7** or **8** was that an intramolecular hydrogen bond between the ketone and either the amine or the phosphonic acid might orient these compounds in a way that favors a bioactive conformation. While such an interaction is possible for amino acid **10**, it is less likely for the recently reported[16] oxygen substituted compound **33**. Although **33** was prepared amongst a series of AMPA antagonists, it was found that the inclusion of oxygen in the side chain enhanced NMDA affinity at the expense of AMPA affinity; and in this amino acid, an intramolecular hydrogen bond is impossible between the heteroatom and the amine. We propose that the increase in affinity for these compounds at NMDA receptors results from a fortuitous hydrogen bonding interaction between these ligands

and the NMDA receptor protein. However, no evidence is available to conclusively determine whether this increase results from intramolecular or intermolecular hydrogen bonding.



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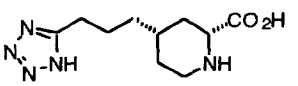

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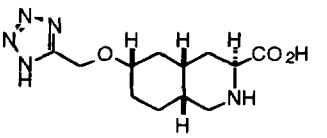

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We have prepared piperidine amino acids substituted with oxygen and sulfur atoms in the chain that connects a tetrazole to the piperidine ring, and have characterized these compounds as NMDA receptor antagonists. An appropriately placed heteroatom does appear to improve affinity and functional antagonist activity at NMDA receptors, and in certain cases also leads to an increase in potency in vivo.

Table. In Vitro and In Vivo Data for Oxygen and Sulfur Substituted Amino Acids

Compound[a]	IC_{50} (μM) for Inhibition of [^{3}H]CGS19755 Binding[b]	IC_{50} (μM) for Antagonism of 40 μM NMDA-Induced Depolarizations in a Cortical Slice[c]	MED[d] (mg/kg, i.p.) to Block NMDA-Induced Lethality in Mice[e]
4[f]	0.107 ± 0.007	4.2 ± 0.4	5
24	0.11[g]	Not Tested	Not Tested
27	0.075[g]	75% inhibition at 10 μM; 20% inhibiton at 3.2 μM	Not Tested
10	0.28 ± 0.02	4.7 ± 0.4	2.5
30	1.3 ± 0.2	Not Tested	Not Tested
31[f]	2.3 ± 0.4	8.1 ± 0.7	20
19	8.3[h]	34.5 ± 5.7	80
32[f]	5.8 ± 0.9	12.4 ± 1.0	10

[a]All compounds are racemic. [b]Unless otherwise indicated, IC_{50}s were the average of three determinations. See ref 17. [c]IC_{50}s were the average of three determinations. See ref 18. [d]MED = minimum effective dose. [e]See ref 21. [f]Data for these compounds is from ref 15. [g]Average of two determinations. [h]One determination.

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