Bioisosteric Replacement of the α-Amino Carboxylic Acid Functionality in 2-Amino-5-phosphonopentanoic Acid Yields Unique 3,4-Diamino-3-cyclobutene-1,2-dione Containing NMDA Antagonists

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In this report, a novel bioisostere of the α-amino acid, 3,4-diamino-3-cyclobutene-1,2-dione, has been incorporated into a series of compounds which are NMDA antagonists. These compounds, which are achiral and easily prepared, demonstrated good affinity at the NMDA receptor by their ability to displace [^{3}H]CPP binding in vitro. In particular, the phosphonic acid **24** provided protection against NMDA-induced lethality in mice equivalent to 2-amino-7-phosphonoheptanoic acid (**5**). This was considered an encouraging result in lieu of the fact that **24**, like **5**, lacks the conformational rigidity of the more potent NMDA antagonists. In addition, analogs that incorporate the 1,2,4-oxadiazolidine-3,5-dione heterocycle of quisqualic acid and the unsaturation of kainic acid were prepared to explore selectivity at the non-NMDA receptor subtypes.

Excitatory amino acids (EEA) such as glutamic acid (**1**) have been proposed to be important neurotransmitters in the CNS,[1] which are linked to a sequence of events that ultimately leads to neuronal cell death.[2] The excitatory amino acids exert their effects upon four major subtypes of receptors, the *N*-methyl-D-aspartic acid (NMDA, **2**), the kainic acid (**3**), the DL-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and the metabotropic receptors;[3] important agonists which have characterized these receptors are shown in Chart I. The utility of selective antagonists of EAA receptors was first demonstrated by competitive NMDA-receptor antagonists such as 4-(3-phosphonopropyl)-2-piperazinecarboxylic acid (**6**, Chart II), which has been shown to prevent ischemic brain damage in gerbils,[4] to prevent NMDA-induced convulsions in mice,[5] and to prevent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonian-like symptoms in rats.[6] For these reasons, NMDA-receptor antagonists have been considered potential therapeutic agents for the treatment of epilepsy, stroke,[7] and neurodegenerative disorders such as Alzheimer's disease[8] and Parkinson's disease.

Chart I

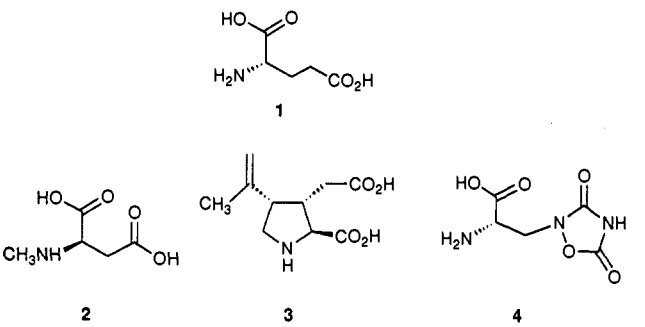
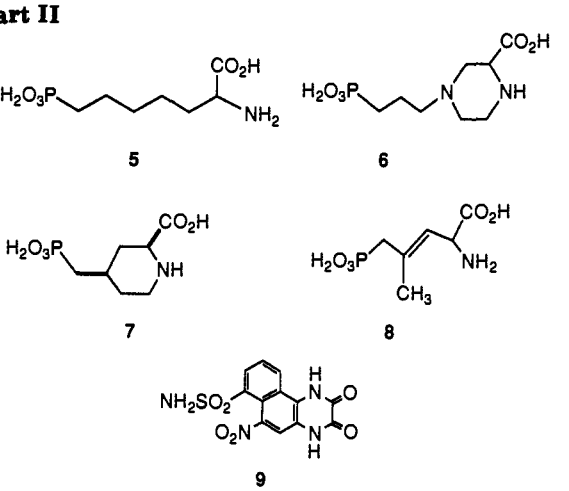


Chart II



The selective AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline (**9**) has also demonstrated potential therapeutic utiliy as an anticonvulsant[9] and neuroprotectant in animal models of global[10] and focal[11] cerebral ischemia. Therefore, there has been considerable interest in finding other selective antagonists at these and other EAA receptors.

(1) Johnson, R. L.; Koerner, J. F. Excitatory Amino Acid Neurotransmission. *J. Med. Chem.* **1988**, *31*, 2057-2066.
(2) Choi, D. W. Calcium-Mediated Neurotoxicity: Relationship to Specific Channel Types and Role in Ischemic Damage. *Trends Neurosci.* **1988**, *11*, 465-469.
(3) Watkins, J. C.; Krogsgaard-Larsen, P.; Honoré, T. Structure-Activity Relationships in the Development of Excitatory Amino Acid Receptor Agonists and Competitive Antagonists. *Trends Pharm. Sci.* **1990**, *11*, 25-33.
(4) Boast, C. A.; Gerhardt, S. C.; Pastor, G.; Lehmann, J.; Etienne, P. E.; Liebman, J. M. The *N*-methyl-D-Aspartate Antagonists CGS 19755 and CPP Reduce Ischemic Brain Damage in Gerbils. *Brain Res.* **1988**, *442*, 345-348.
(5) Lehmann, J.; Schneider, J.; McPherson, S.; Murphy, D. E.; Bernard, P.; Tsai, C.; Bennett, D. A.; Pastor, G.; Steel, D. J.; Boehm, C.; Cheney, D. L.; Liebman, J. M.; Williams, M.; Wood, P. L. CPP, a Selective *N*-Methyl-D-Aspartate-Type Receptor Antagonist: Characterization *in Vitro* and *in Vivo*. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 737-746.
(6) Turski, L.; Bressler, K.; Rettig, K-J.; Löschmann, P-A.; Wachtel, H. Protection of Substantia Nigra From MPP$^+$ Neurotoxicity by *N*-Methyl-D-Aspartate Antagonists. *Nature* **1991**, *349*, 414-418.
(7) Engelsen, B. Neurotransmitter Glutamate: Its Clinical Importance. *Acta. Neurol. Scand.* **1986**, *74*, 337-355.
(8) Maragos, W. F.; Greenamyre, J. T.; Penney, J. B., Jr.; Young, A. B. Glutamate Dysfunction in Alzheimer's Disease: an Hypothesis. *Trends Neurosci.* **1987**, *10*, 64-67.
(9) Chapman, A. G.; Smith, S. E.; Meldrum, B. S. The Anticonvulsant Effect of the Non-NMDA Antagonists, NBQX and GYKI-52466, in Mice. *Epilepsy Res.* **1991**, *9*, 92-96.
(10) Sheardown, M. J.; Nielsen, E. Ø.; Hansen, A. J.; Jacobsen, Pl; Honoré, T. 2,3-Dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline: a Neuroprotectant for Cerebral Ischemia. *Science* **1990**, *247*, 571-574.

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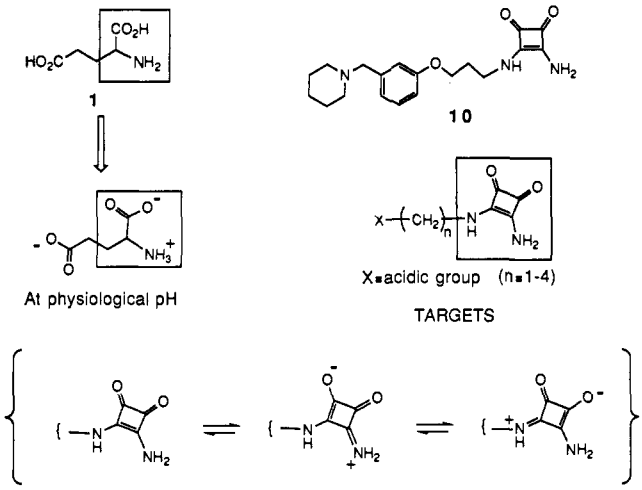
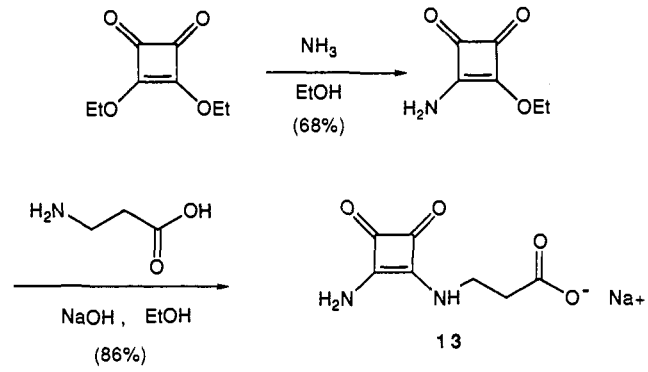
Chart III



Chemical entities known to be competitive NMDA-receptor antagonists contain the α-amino carboxylic acid and phosphonic acid functionalities separated by a variety of spacer units containing three or five carbon atoms (Chart II). An unembellished example containing a five-atom spacer between the glycine and the phosphonic acid groups is 2-amino-7-phosphonoheptanoic acid (**5**).[12] More potent examples, which contain elements enhancing structural rigidity, include compound **6**, *cis*-4-(phosphonomethyl)-2-piperidinecarboxylic acid (**7**),[13] and (*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (**8**).[14]

Although there have been attempts to find groups which are bioisosteric with the phosphonic acid group,[15,16] no examples of NMDA-receptor antagonists have appeared in the literature which demonstrate a bioisosteric replacement of the α-amino carboxylic acid functionality. It was thought that a mimic of this group might provide greater bioavailability or brain penetration for these agents relative to the α-amino acid class of EAA antagonists. At physiological pH the α-amino carboxylic acid group is present as an ammonium carboxylate. Therefore, the 3,4-diamino-3-cyclobutene-1,2-dione moiety was chosen as an appropriate electronic mimic, which contains a dipole possessing a partial negative charge on the carbonyls and a partial positive charge on the nitrogens; one can predict the dipole from the contributing canonical forms (Chart III). This functionality has been used as a thiourea

Scheme I



bioisostere, for example, in the potent H_2-receptor antagonist **10**;[17,18] in this agent a strong dipole is thought to be important for binding to the receptor. Though the 3,4-diamino-3-cyclobutene-1,2-dione group resembles the ammonium carboxylate functionality electronically, it should be cautioned that it lacks other properties of an α-amino carboxylic acid. For instance, the group is not basic or acidic at physiological pH and the amino group is not nucleophilic. Therefore, a receptor which requires such features for molecular recognition of its amino acid substrates would not be expected to accept this group as an α-amino acid bioisostere.

Targets, chosen to test this functionality as an α-amino carboxylic acid bioisostere at the NMDA receptor, contained the 3,4-diamino-3-cyclobutene-1,2-dione group connected by a variable chain length to an acidic functionality. A potential advantage of this mimic over an α-amino acid is it lack of chirality, making these derivatives more readily accessible.

Chemistry

The synthesis of targets **11–17** is illustrated by the synthesis of **13** in Scheme I. A saturated solution of ammonia in ethanol was added to 3,4-diethoxy-3-cyclobutene-1,2-dione in ethanol at room temperature until disappearance of starting material by TLC. The purified monoadduct was treated with β-alanine and 1 equiv of base to yield **13** as the sodium salt. The synthesis of **17** required the (aminoethyl)-1,2,4-oxadiazolidine-3,5-dione which was prepared by the literature method.[19] The synthesis of compound **16** differed only in the first step, in which 1 equiv of benzylamine was used instead of a saturated solution of the gaseous amine.

The N-alkylated derivatives **18–20** were prepared as illustrated (Scheme II) for the synthesis of **18**. One equivalent of allylamine in ethanol was added to 3,4-diethoxy-3-cyclobutene-1,2-dione in ethanol. Alkylation was achieved with sodium hydride in dimethylformamide using *tert*-butyl bromoacetate to yield **21**. The second displacement of ethanol was accomplished with ammonia at room temperature to afford **22**. The ester was deprotected with formic acid to yield **18**.

(11) Gill, R.; Nordholm, L.; Lodge, D. The Neuroprotective Actions of 2,3-Dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline (NBQX) in a Rat Focal Ischemia Model. *Brain Res.* **1992**, *580*, 35–43.

(12) Watkins, J. C.; Olverman, H. J. Agonists and Antagonists for Excitatory Amino Acid Receptors. *Trends Neurosci.* **1987**, *10*, 265–272.

(13) Lehman, J.; Hutchison, A. J.; McPherson, S. E.; Mondadori, C.; Schmutz, M.; Sinton, C. M.; Tsai, C.; Murphy, D. E.; Steel, D. J.; Williams, M.; Cheney, D. L.; Wood, P. L. CGS 19755, a Selective and Competitive *N*-Methyl-D-Aspartate-Type Excitatory Amino Acid Receptor Antagonist. *J. Pharmacol. Exp. Ther.* **1988**, *246*, 65–75.

(14) Fagg, G. E.; Olpe, H. R.; Pozza, M. F.; Baud, J.; Steinmann, M.; Schmutz, M.; Portet, C.; Baumann, P.; Thedinga, K.; Bittiger, H.; Allgeier, H.; Heckendorn, R.; Angst, C.; Brundish, D.; Dingnall, J. G. CGP 37849 and CGP 39551: Novel and Potent Competitive *N*-Methyl-D-aspartate Receptor Antagonists with Oral Activity. *Br. J. Pharmacol.* **1990**, *99*, 791–797.

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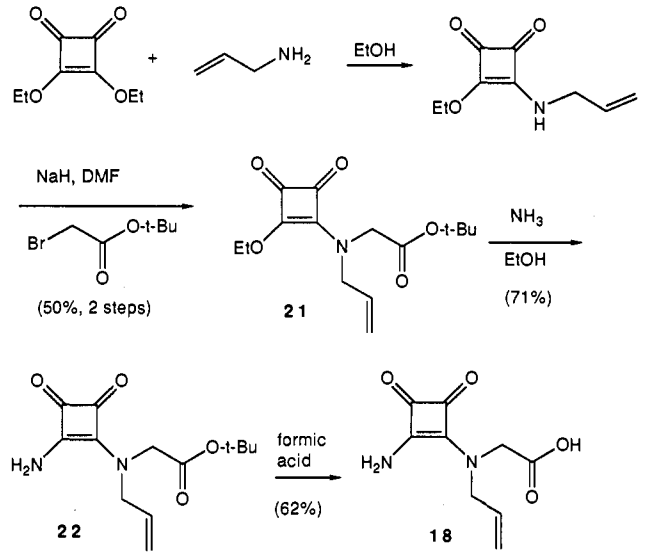
(16) Ornstein, P. L.; Schoepp, D. D.; Arnold, M. B.; Leander, J. D.; Lodge, D.; Paschal, J. W.; Elzey, T. 4-(Tetrazolylalkyl)piperidine-2-carboxylic Acids. Potent and Selective *N*-Methyl-D-Aspartic Acid Receptor Antagonists with a Short Duration of Action. *J. Med. Chem.* **1991**, *34*, 90–97.

(17) Ganellin, C. R. Medicinal Chemistry and Dynamic Structure–Activity Analysis in the Discovery of Drugs Acting at Histamine H_2 Receptors. *J. Med. Chem.* **1981**, *24*, 913–920.

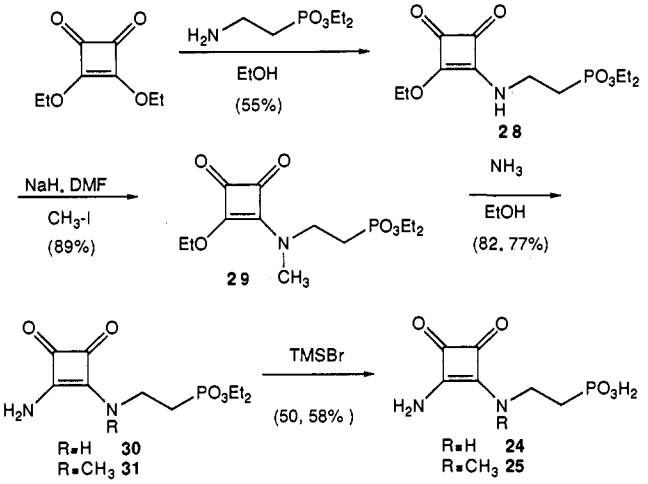
(18) Young, R. C.; Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Graham, M. J.; Mitchell, R. C.; Prain, H. D.; Roantree, M. L. Dipole Moment in Relation to H_2 Receptor Histamine Antagonist Activity for Cimetidine Analogues. *J. Med. Chem.* **1986**, *29*, 44–49.

(19) Dugenet, P.; Yaouanc, J. J.; Sturtz, G. A Selective and Efficient Synthesis of Quisqualamine, a Novel GABA-Related Depressant Amino Acid. *Synthesis* **1982**, 781–782.

Scheme II



Scheme III

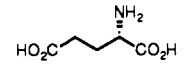
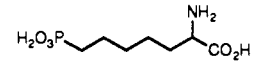
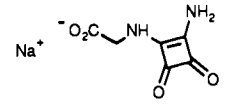
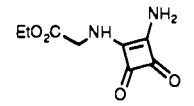
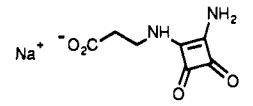
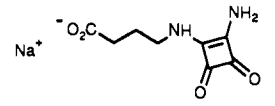
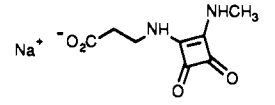
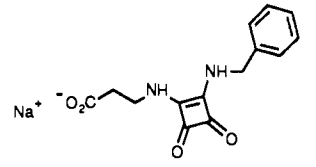


The phosphonic acid derivatives **23–27** were prepared similarly as shown in the synthesis of **24** and **25** (Scheme III). Diethyl (2-aminoethyl)phosphonate[20] was added to 3,4-diethoxy-3-cyclobutene-1,2-dione in ethanol to afford **28**, which can be alkylated with iodomethane to yield **29**. Esters **28** or **29** were treated with ethanolic ammonia delivering diamides **30** and **31**, respectively. Deprotection of the phosphonate esters was achieved in refluxing 1,2-dichloroethane with 6 equiv of bromotrimethylsilane to yield **24** or **25**.

Biological Results and Discussion

The first compound used to investigate the utility of the 3,4-diamino-3-cyclobutene-1,2-dione group (**11**, Table I) was found to have affinity for the NMDA receptor as measured by its ability to displace [^{3}H]CPP binding. However, compound **11** was 6 times less potent than 2-amino-7-phosphonoheptanoic acid (**5**) and 30 times less potent than glutamic acid (**1**). The ability of **11** to act as a ligand at this glutamic acid receptor suggests that the 3,4-diamino-3-cyclobutene-1,2-dione group is a bioisostere for the α-amino carboxylic acid functionality. The corresponding ester **12** was much less potent. The homolog **13** was equipotent with **11**, whereas the higher homolog **14** had reduced affinity. Substitution on nitrogen, as in **15** and **16**, led to reduced affinity. None of these cyclobutenediones had affinity for the AMPA or kainic acid receptors at a concentration of 100 μM. To assess its functional activity, compound **13** was initially evaluated in the stimulated [^{3}H]TCP binding assay. The incorporation of glutamic acid and/or glycine in this assay has been demonstrated to increase the association rate of [^{3}H]-TCP binding to rat brain tissue, when examined within 2 h of incubation.[21] The further addition of a competitive NMDA receptor antagonist has been demonstrated to preclude the increased association rate of [^{3}H]TCP binding and, thereby, appears to inhibit stimulated [^{3}H]TCP binding.[22] Compound **13** was found to block TCP binding with an IC_{50} of 941 μM (844–1048 μM, 95% CI) versus 45 μM (39–52 μM, 95% CI) for **6**, suggesting that it was a weak functional NMDA antagonist. Therefore, the 3,4-diamino-3-cyclobutene-1,2-dione group mimics the α-amino acid functionality in binding to the NMDA receptor, but converts these derivatives to antagonists relative to glutamic acid. Compound **13** was unable to prevent NMDA-induced lethality at 100 mg/kg.

Table I. NMDA Receptor Affinity of 3,4-Diamino-3-cyclobutene-1,2-dione Derivatives

entry	structure	NMDA receptor affinity IC_{50} (μM)[a]
1		0.070 (0.064–0.078)
5		0.39 (0.28–0.51)
11		2.3 (1.9–2.8)
12		190 (160–220)
13		1.6 (1.3–2.1)
14		10 (9–12)
15		20 (17–23)
16		>100

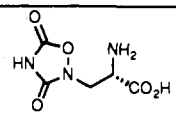
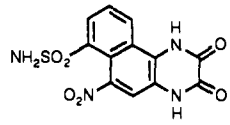
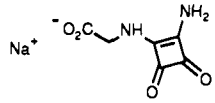
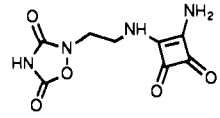
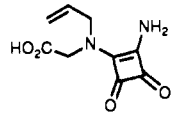
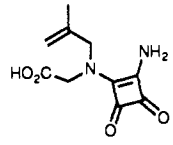
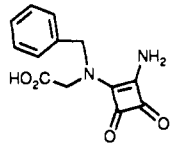
[a] 95% Confidence interval in brackets.

(20) Berges, D. A. 7β-Acylamino-3-(phosphonoalkyl and Esterified Phosphonoalkyl Substituted Tetrazolylthiomethyl) Cephalosporins. *U.S. Patent* 4,112,086, Sept 5, 1978.

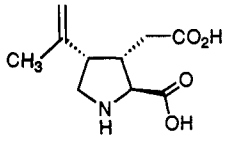
(21) Kloog, Y.; Haring, R.; Sokolovsky, M. Kinetic Characterization of the Phencyclidine-*N*-Methyl-D-aspartate Receptor Interaction: Evidence for a Steric Blockade to the Channel. *Biochemistry* **1988**, *27* (3), 843–848.

(22) Ransom, R. W.; Stec, N. L. Cooperative Modulation of [^{3}H]MK801 Binding to the *N*-Methyl-D-Aspartate Receptor-Ion Channel Complex by L-Glutamate, Glycine, and Polyamines. *J. Neurochem.*, **1988**, *51*, 830–836.

Table II. Affinity of 3,4-Diamino-3-cyclobutene-1,2-dione Derivatives at Three Receptor Subtypes

entry	structure	NMDA IC_{50} (μM)[a]	AMPA IC_{50} (μM)[a]	kainate IC_{50} (μM)
4		>100	1.3 (1.0–1.7) × 10^{-3}	0.15 (0.079–0.47)
9		>10	0.10 (0.07–0.15)	56% (100)[b]
11		2.3 (1.9–2.8)	>100	>100
17		5.2 (4.0–6.8)	>100	>100
18		10 (8–13)	>100	>100
19		46% (30)[b]	>100	>100
20		50% (100)[b]	>100	>100

[a] 95% Confidence interval in brackets. [b] Percent inhibition at concentration in brackets.



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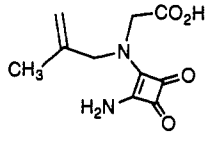

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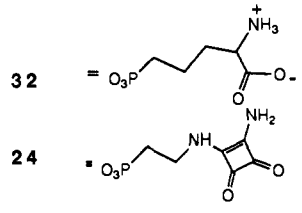
Figure 1.



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24

Figure 2.

Structural features were then incorporated, which might introduce selectivity for the AMPA or kainic acid receptors (Table II). Compound **17** contains the 1,2,4-oxadiazolidine-3,5-dione heterocycle of quisqualic acid (**4**) in place of the carboxylic acid in **13**. This analogous change from aspartic acid affords quisqualic acid with selectivity for the AMPA receptor, while it retains some affinity for the kainic acid receptor (Table II). Surprisingly, no affinity was observed for **17** at the AMPA or kainic acid receptors, but NMDA affinity was retained. It has been shown that unsaturated groups such as isopropenyl or phenyl at the C-4 position of the pyrrolidine residue of kainoids are important for activity at the kainic acid receptor.[23,24] Therefore, unsaturation was incorporated in compounds **18–20** in the same area of the molecule as kainic acid. In Figure 1, compound **19** and kainic acid are shown in an orientation emphasizing their similarities. However, these derivatives did not bind to the kainic acid receptor. NMDA affinity decreased in this series with increasing bulk of the *N*-alkyl group.

The phosphonic acid derivatives **23–27** (Table III) were then prepared to optimize NMDA receptor potency. Compound **23**, in which the phosphonic acid replaces the carboxylic acid of **11**, showed no NMDA receptor binding as was the case for 2-amino-4-phosphonobutanoic acid relative to glutamic acid. However the homolog **24**, corresponding to a 2-amino-5-phosphonopentanoic acid length, was found to have improved potency relative to **11** and **13** with an IC_{50} of 0.47 μM. Also, compound **24** was found to prevent NMDA-induced lethality at a dose of 29 mg/kg, making **24** equipotent with 2-amino-7-phosphonoheptanoic acid (**5**). In this example an equal bioequivalence of 3,4-diamino-3-cyclobutene-1,2-dione to an α-amino acid was demonstrated. In overlapping models of **24** and 2-amino-5-phosphonopentanoic acid (**32**, Figure 2), the 3-oxo and the 2-amino substituents on the cyclobutene ring overlap with one of the carboxylate oxygens and the α-amino group of **32**, respectively. Since **24** and **32** have approximately the same length, one can consider 3,4-

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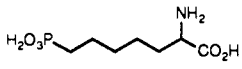
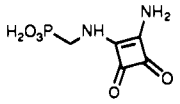
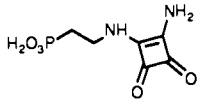
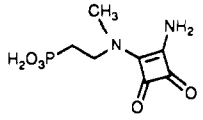
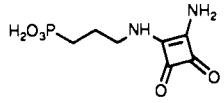
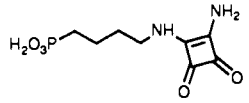
(24) Kozikowski, A. P.; Fauq, A. H. Probing the Topography of Kainate Recognition Sites: Synthesis of a Novel Oxetane Containing Kainic Acid Analogue. *Tetrahedron Lett.* **1990**, *31*, 2967–2970.

Table III. Phosphonoalkyl 3,4-Diamino-3-cyclobutene-1,2-dione Derivatives

entry	structure	NMDA affinity IC_{50} (μM)[a]	NMDA-induced lethality ED_{50} (mg/kg)[a]
5		0.39 (0.28–0.51)	38 (31–45)
23		>10	>100
24		0.47 (0.36–0.61)	29 (16–51)
25		1.0 (0.7–1.5)	50% (50)[b]
26		2.6 (2.0–3.4)	>100
27		68% (10)[b]	>30

[a] 95% Confidence interval in brackets. [b] Percent inhibition at concentration in brackets.

Table IV. Physical Properties of 3,4-Diamino-3-cyclobutene-1,2-dione Derivatives

entry	mp, °C	formula[a]	yield, %[b]	purification[c]
11	210–215 dec	$C_6H_5NaN_2O_4 \cdot {}^4/_3H_2O$	56	none
12	231–233	$C_8H_{10}N_2O_4$	54	none
13	280–282	$C_7H_7NaN_2O_4 \cdot 1/2H_2O$	58	none
14	240–243 dec	$C_8H_9NaN_2O_4 \cdot 0.58H_2O$	48	none
15	310 dec	$C_8H_9NaN_2O_4 \cdot 1/4H_2O$	56	none
16	298–302 dec	$C_{14}H_{13}NaN_2O_4 \cdot 1/2H_2O$	27	none
17	225 dec	$C_8H_8N_4O_5 \cdot 0.45H_2O$	31	A
18	172–175	$C_9H_{10}N_2O_4 \cdot 1/4H_2O$	22	B
19	184–186	$C_{10}H_{12}N_2O_4 \cdot 0.1H_2O$	6	B
20	177–179	$C_{13}H_{12}N_2O_4$	15	F,C
23	220–250 dec	$C_5H_7N_2O_5P \cdot 3/4H_2O$	63	E
24	230–239 dec	$C_6H_9N_2O_5P \cdot 1/5H_2O$	22	D
25	230–260 dec	$C_7H_{11}N_2O_5P$	22	B
26	220–230 dec	$C_7H_{11}N_2O_5P$	15	B
27	220–242 dec	$C_8H_{13}N_2O_5P \cdot 1/4H_2O$	31	D

[a] All compounds exhibited satisfactory (±0.4%) elemental analyses. [b] Unoptimized yields from 3,4-diethoxy-3-cyclobutene-1,2-dione. [c] (A) Ion-exchange using AG 50W-X2 resin (H^+ form) with water elution. Recrystallization using (B) methanol in ethyl acetate, (C) acetone/ethyl acetate/petroleum ether, (D) wet methanol in ethyl acetate. (E) Trituration with ethanol/ethyl acetate (3:1). (F) Chromatography on H_3PO_4-treated silica gel (1% in methanol) using 2.5–5% methanol in dichloromethane elution.

diamino-3-cyclobutene-1,2-dione to be an alanine bioisostere, i.e. **32** is alanine connected to ethylphosphonic acid as **24** is 3,4-diamino-3-cyclobutene-1,2-dione connected to ethylphosphonic acid. The *N*-methyl derivative **25** was less potent as was the case for example **15**, which had methyl substitution on the other nitrogen. The three- and four-methylene derivatives **26** and **27** showed decreased potency, unlike the α-amino acid series in which the two-carbon homolog of **32** (**5**) was also active. This result is consistant with Whitten's single binding site model,[25] in which both **32** and **5** bind through a similar three-point pharmacophore, attainable from low-energy conformers of the two molecules. Perhaps because of different geometrical constraints within the sp^2-hybridized cyclobutenedione moiety of compound **27**, relative to the sp^3-hybridized 2-amino-7-phosphonoheptanoic acid (**5**), it can not achieve an orientation resembling the bioactive form of **24**.

In conclusion, a new achiral bioisostere for the α-amino carboxylic acid functionality has been identified: 3,4-diamino-3-cyclobutene-1,2-dione. This group was easily incorporated into a novel structural type of NMDA antagonist. The phosphonic acid derivatives were the most potent, and most of the activity resided in the two-carbon spaced example **24**; in the [^{3}H]CPP binding and NMDA-induced lethality models this compound was equipotent to **5**, which also lacks the conformational rigidity of the more potent NMDA antagonists.

Experimental Section

Melting points were obtained on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 781 spectrophotometer. ^{1}H NMR spectra were obtained at either 200 or 400 MHz on a Varian XL-200 or Bruker AM-400 spectrometer, respectively. Mass spectra were measured on either a Finnigan 8230 or Hewlett-Packard 5995A mass spectrometer. Elemental analyses were obtained on a Control Equipment 240-XA elemental analyzer. Flash chromatography refers to the technique described by Still.[26] The diameter of the column used is noted, but the height of silica gel 60 (230–400 mesh) was 20 cm in all cases. 3,4-Diethoxy-3-cyclobutene-1,2-dione was obtainable from the Aldrich Chemical Co.

***N*-(2-Amino-3,4-dioxo-1-cyclobuten-1-yl)-β-alanine (13).** A solution of 3-amino-4-ethoxy-3-cyclobutene-1,2-dione[27] (2.0 g, 14 mmol) in ethanol (100 mL) was treated with β-alanine (1.26 g, 14 mmol) dissolved in 1 N sodium hydroxide solution (14 mL, 14 mmol). After 5.5 h at room temperature, the resulting yellow solid was filtered, washed with ethanol, and concentrated under high vacuum to yield **13** as the sodium salt, hemihydrate (2.6 g, 86% of theory, mp 280–282 °C): IR (KBr, cm^{-1}) 1810; MS (-FAB) 205 (M - H, 13) 183 (M - Na, 44), 175 (17), 148 (100); ^{1}H NMR (D_2O, 400 MHz) δ 3.59 (br s, 2 H), 2.31 (t, J = 6 Hz, 2 H); ^{13}C NMR (D_2O, 400 MHz) ppm 182.01, 181.61, 179.30, 168.94, 168.54, 41.18, 37.97. Anal. ($C_7H_7NaN_2O_4 \cdot {}^1/_2H_2O$) C, H, N.

2-[2-[(2-Amino-3,4-dioxo-1-cyclobuten-1-yl)amino]ethyl]-1,2,4-oxadiazolidine-3,5-dione (17). A solution of 3-amino-4-ethoxy-3-cyclobutene-1,2-dione (0.56 g, 4.0 mmol) in ethanol (20 mL) was added to 2-(2-aminoethyl)-1,2,4-oxadiazolidine-3,5-dione hydrobromide (0.90 g, 4.0 mmol) in ethanol (100 mL). The reaction mixture was treated with 1 N sodium hydroxide solution (8 mL, 8 mmol) and allowed to stir for 24 h at room temperature. The resulting precipitate was filtered, dissolved in water, and passed through an ion-exchange column (AG 50W-X2, 100–200 mesh, H^+ form), eluting with water. The eluent was freeze-dried yielding **17** as a cream-colored solid, partial hydrate (0.45 g, 45%, mp 225 °C dec: IR (KBr, cm^{-1}) 3300, 3140, 1820, 1740, 1720, 1640; MS (+FAB) 241 (MH^+); ^{1}H NMR (DMSO, 400 MHz) δ 12.4 (br s, NH), 7.5 (br s, 3 NH), 4.0–3.5 (m, 4 H); ^{13}C NMR (DMSO, 400 MHz) ppm 183.72, 183.63, 170.06, 168.96, 158.17, 152.72, 50.41, 41.68. Anal. ($C_8H_8N_4O_5 \cdot 0.45H_2O$) C, H, N.

***N*-(2-Ethoxy-3,4-dioxo-1-cyclobuten-1-yl)-*N*-(2-propenyl)-glycine 1,1-Dimethylethyl Ester (21).** A solution of 3,4-diethoxy-3-cyclobutene-1,2-dione (5.2 g, 31 mmol) in ethanol (80 mL) was treated at room temperature with allylamine (2.3 mL, 31 mmol), which was dissolved in ethanol (40 mL), over a 2-h period. The reaction mixture was concentrated in vacuo to afford

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crude 1-(2-propenylamino)-2-ethoxy-3,4-dioxo-1-cyclobutene as a light yellow solid (5.6 g). The crude intermediate was dissolved in anhydrous dimethylformamide (50 mL) and added dropwise to a suspension of 60% sodium hydride (1.5 g, 37 mmol) in anhydrous dimethylformamide (50 mL) under nitrogen. The anion was quenched with *tert*-butyl bromoacetate (6.0 mL, 37 mmol), and the reaction mixture was stirred for 1.5 h, poured into water (500 mL), extracted with ethyl acetate (2 × 200 mL), and dried ($MgSO_4$) to yield **21**, which was purified by flash chromatography (10-cm diameter, elution with 20% ethyl acetate in petroleum ether) affording a yellow oil (4.56 g, 50%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.88–5.72 (m, 1 H), 5.35–5.22 (m, 2 H), 4.80–4.68 (m, 2 H), 4.35, 4.08 (d, J = 7 Hz, 2 H), 4.28, 3.95 (s, 2 H), 1.48 (s, 9 H), 1.45 (t, J = 7 Hz, 3 H).

***N*-(2-Amino-3,4-dioxo-1-cyclobuten-1-yl)-*N*-(2-propenyl)-glycine 1,1-Dimethylethyl Ester (22).** Ethanolic ammonia (25 mL) was added to a flask containing **21** (2.5 g, 8.5 mmol) at room temperature. After 5 h the reaction mixture was concentrated and purified by flash chromatography (5-cm diameter, elution with 5% methanol in dichloromethane) to yield **22** as a white solid (1.6 g, 71%, mp 175–176 °C): IR (KBr, cm^{-1}) 3300, 3140, 1810, 1740, 1670, 1650; MS (EI) 266 (M^+, 34), 210 (24), 165 (100), 109 (54), 95 (89), 68 (68); ^{1}H NMR (DMSO, 400 MHz) δ 7.70 (br s, NH_2), 5.84–5.77 (m, 1 H), 5.26 (d, J = 17 Hz, 1 H), 5.19 (d, J = 10 Hz, 1 H), 4.3–4.0 (br m, 4 H), 1.39 (s, 9 H).

***N*-(2-Amino-3,4-dioxo-1-cyclobuten-1-yl)-*N*-(2-propenyl)-glycine (18).** Deprotection of **22** (1.6, 6.0 mmol) was performed by stirring in formic acid (20 mL) for 24 h. The reaction mixture was concentrated, azeotroped with dichloromethane, and recrystallized from methanol in ethyl acetate with difficulty (oiled out several times) to afford **18** as an off-white solid (0.80 g, 62%, mp 172–175 °C): IR (KBr, cm^{-1}) 3330, 3180, 1810, 1720, 1640; MS (EI) 210 (M^+, 75), 165 (34), 109 (41), 95 (100), 68 (63); ^{1}H NMR (DMSO, 400 MHz) δ 12.94 (br s, OH), 7.70 (s, NH_2), 5.86–5.77 (m, 1 H), 5.26 (d, J = 17 Hz, 1 H), 5.19 (d, J = 10 Hz, 1 H), 4.3–4.0 (br m, 4 H). Anal. ($C_9H_{10}N_2O_4 \cdot {}^1/_4H_2O$) C, H, N.

[2-[(2-Ethoxy-3,4-dioxo-1-cyclobuten-1-yl)amino]ethyl]-phosphonic Acid Diethyl Ester (28). To a solution of 3,4-diethoxy-3-cyclobutene-1,2-dione (4.00 g, 23.5 mmol) in ethanol (100 mL) was added (2-aminoethyl)phosphonic acid diethyl ester (5.43 g, 30.0 mmol) in ethanol (100 mL) over a 1-h period. After leaving overnight the reaction mixture was preadsorbed onto silica gel and purified by flash chromatography (5.5-cm diameter, gradient elution with 2.5–10% 2-propanol in dichloromethane) to yield **28** as an oil which solidifies on standing (3.98 g, 55%, mp 66–68 °C): IR (KBr, cm^{-1}) 3400, 3180, 1800, 1700, 1600; MS (+FAB) 306 (MH^+, 100), 278 (14), 137 (14), 109 (35); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.58, 6.46 (br s, NH), 4.75 (br m, 2 H), 4.21–4.07 (m, 4 H), 4.00, 3.75 (br m, 2 H), 2.08 (d of t, J = 17.5 and 6.5 Hz, 2 H), 1.46 (br m, 3 H), 1.35 (t, J = 7 Hz, 6 H).

[2-[(2-Amino-3,4-dioxo-1-cyclobuten-1-yl)amino]ethyl]-phosphonic Acid Diethyl Ester (30). A solution of **28** (1.69 g, 5.5 mmol) in 100% ethanol (40 mL) was placed in flask and treated with saturated ethanolic ammonia (190 mL). The reaction mixture was stirred at room temperature for a total of 24 h and then concentrated in vacuo. The resulting solid was recrystallized from methanol in ethyl acetate to afford **30** as a yellow solid (1.27 g, 82%, mp 150–152 °C dec): IR (KBr, cm^{-1}) 1805, 1650; MS (+FAB) 277 (MH^+, 100), 182 (20), 109 (15); ^{1}H NMR (DMSO, 400 MHz) δ 7.5 (br s, 3 NH), 4.1–3.9 (m, 4 H), 3.7–3.6 (m, 2 H), 2.11 (d of t, J = 17.5 and 7.5 Hz, 2 H), 1.22 (t, 6 H). Anal. ($C_{10}H_{17}N_2O_5P \cdot {}^1/_5H_2O$) C, H, N.

[2-[(2-Amino-3,4-dioxo-1-cyclobuten-1-yl)amino]ethyl]-phosphonic Acid (24). A suspension of **30** (0.90 g, 3.2 mmol) in dry 1,2-dichloroethane (47 mL) was placed in a flask which was equipped with a reflux condenser and which had previously been evacuated and placed under nitrogen. Bromotrimethylsilane (2.6 mL, 19.8 mmol) was added to the flask via syringe, and the reaction mixture was refluxed for 10 min. The mixture was then concentrated in vacuo to produce a rust-colored solid which was dissolved in water (80 mL). The water was washed with diethyl ether (2 × 100 mL) and concentrated in vacuo. The resulting rust-colored solid was recrystallized from wet methanol in ethyl acetate to produce **24** as a dark yellow solid (0.360 g, 50%, mp 230–239 °C dec): IR (KBr, cm^{-1}) 1790; ^{1}H NMR (DMSO, 400 MHz) δ 7.5 (br s, 3 NH), 3.67 (br s, 2 H), 1.85 (d of t, J = 17.5 and 7.5 Hz, 2 H). Anal. ($C_6H_9N_2O_5P \cdot {}^1/_5H_2O$) C, H, N.

[2-[(2-Ethoxy-3,4-dioxo-1-cyclobuten-1-yl)methylamino]-ethyl]phosphonic Acid Diethyl Ester (29). A cold (0 °C) suspension of 60% sodium hydride (500 mg, 12.5 mmol) in anhydrous dimethylformamide (15 mL) under nitrogen was treated with a solution of **28** (3.23 g, 10.6 mmol) in dimethylformamide (20 mL) over 30 min. Iodomethane (0.78 mL, 12.5 mmol) was introduced and the ice bath was removed for 30 min and then reapplied for introduction of 1 N hydrochloric acid solution (20 mL). The reaction mixture was poured into water (200 mL), extracted with dichloromethane (2 × 200 mL), dried ($MgSO_4$), and concentrated under high vacuum (1 mm) at 40 °C. The crude material was purified by flash chromatography (7.5-cm diameter, elution with 2.5% methanol in dichloromethane) to afford **29** as an oil (3.00 g, 89%): IR (neat, cm^{-1}) 1805, 1715, 1620; MS (+FAB): 320 (MH^+, 100), 109 (20); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.78–4.74 (m, 2 H), 4.16–4.09 (m, 4 H), 3.94, 3.68 (m, 2 H), 3.35, 3.19 (s, 3 H), 2.15–2.09 (m, 2 H), 1.48–1.44 (m, 3 H), 1.34 (t, J = 7 Hz, 6 H).

[2-[(2-Amino-3,4-dioxo-1-cyclobuten-1-yl)methylamino]-ethyl]phosphonic Acid Diethyl Ester (31). An ethanolic solution (40 mL) of **29** (3.00 g, 9.40 mmol) was combined with ethanolic ammonia solution (70 mL) and left for 18 h. After concentrating in vacuo, the solid was recrystallized twice from methanol in ethyl acetate (final volume = 50 mL) to yield **31** as a solid (2.10 g, 77%, mp 130–132 °C): IR (KBr, cm^{-1}) 3320, 3160, 1800, 1670, 1650, 1640; MS (+FAB) 291 (MH^+, 100), 196 (22), 109 (20); ^{1}H NMR (DMSO, 400 MHz) δ 7.61 (br s, NH_2), 4.02–3.94 (m, 4 H), 3.74 (br s, 2 H), 3.13 (br s, 3 H), 2.13 (d of t, J = 18 and 7.5 Hz, 2 H), 1.22 (t, J = 7 Hz, 6 H). Anal. ($C_{11}H_{19}N_2O_5P$) C, H, N.

[2-[(2-Amino-3,4-dioxo-1-cyclobuten-1-yl)methylamino]-ethyl]phosphonic Acid (25). A suspension of **31** (660 mg, 2.3 mmol) in anhydrous 1,2-dichloroethane (20 mL) under nitrogen was treated with bromotrimethylsilane (2.0 mL, 15 mmol) and heated to reflux for 10 min. The yellow solution was concentrated, and the resulting solid was dissolved in water (75 mL), washed with diethyl ether (2 × 50 mL), and evaporated. The solid was dissolved in boiling methanol, filtered, and concentrated with the addition of ethyl acetate to a final volume of 75 mL to afford **25** as a yellow solid (310 mg, 58%, mp 230–260 °C dec): IR (KBr, cm^{-1}) 3340, 1800; MS (–FAB) 233 (M – H, 32), 148 (100); ^{1}H NMR (DMSO, 400 MHz) δ 7.62 (br s, NH_2), 3.68 (br s, 2 H), 3.16 (br s, 3 H), 1.90 (d of t, J = 18 and 7.5 Hz, 2 H). Anal. ($C_7H_{11}N_2O_5P$) C, H, N.

Binding Assays. Crude synaptic membrane preparations (CSMs) were prepared from rat brains as described by Murphy[28] and subsequently treated with 0.04% Triton X-100 (Eastman Kodak, Rochester, NY) as described by Enna and Snyder;[29] the CSM pellets were then frozen at –70 °C for storage. Prior to use in the NMDA receptor binding assay and the stimulated [^{3}H]-TCP binding assay, the CSMs were thawed, washed once in Tris HCl buffer, and resuspended in buffer to a final concentration of 0.3–0.5 mg protein/mL as determined by the method of Lowry.[30] Displacement of [^{3}H]CPP binding, as described by Murphy,[31] to CSMs was utilized to determine NMDA receptor affinity. The methods of London and Coyle[32] and Murphy[28] were adopted to assess kainic acid and AMPA receptor affinities, respectively.

The stimulated [^{3}H]TCP binding assay was a modification of the [^{3}H]MK801 binding assay of Ransom[22] using the CSMs

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described above. In triplicate, 1000-μL samples of the CSMs were incubated at 25 °C for 60 min in the presence of 2.5 nM [^{3}H]TCP (specific activity 45–50 Ci/mmol; DuPont NEN, Boston, MA), 3 μM L-glutamic acid, 1 μM glycine, one of various test drugs or concentrations thereof, and an appropriate volume of buffer for a final incubation volume of 2 mL. Tris buffer and a 100 μM solution of MK-801 were substituted for the test solution in separate triplicates to define total and nonspecific binding, respectively. The tissue homogenates were then filtered under vacuum, using 0.1% polyethylenimine pretreated filters, and rinsed with three 2-mL rinses of ice-cold buffer. The filters were placed into individual 20-mL glass scintillation vials and prepared for counting using conventional liquid spectroscopy. The concentration of test compound which displaced 50% of [^{3}H]TCP binding and its 95% confidence limits were determined from concentration–response (5 to 10 concentrations) curves derived using a nonlinear logistic regression of counts vs the log of the test drug concentration.[33]

NMDA-Induced Lethality.[34] The compounds were also evaluated for NMDA antagonist properties in male Swiss-albino mice (CD-1 strain, 18–22 g, Charles River, Wilmington, DE). The mice were acclimated for 30 min prior to treatment (ip) with the representative test compounds or vehicle (control), followed 30 min later with NMDA (195 mg/kg, ip, the LD_{90} dose). The mice were then observed for 30 min, noting the latency of onset of generalized myoclonus and death. From the latter, percentage survival was determined. Animals were tested in groups of 10 mice/dose level and dose–response data was analyzed using the probit analysis program PS NONLIN (Natural Response Rate Version) to determine the dose (ED_{50}) which provides 50% protection and the 95% confidence interval.

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