Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 8, pp 1569–1575, 1997

PII: S0968-0896(97)00110-7
0968-0896/97 $17.00 + 0.00

Bioisosterically Modified Dipeptide Excitatory Amino Acid Receptor Antagonists Containing 3-Oxygenated Isothiazole Ring Systems

Lisa Matzen, Bjarke Ebert, Tine B. Stensbøl, Bente Frølund, Jerzy W. Jaroszewski and Povl Krogsgaard-Larsen*

Department of Medicinal Chemistry, The Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen, Denmark

Abstract—The AMPA receptor agonist Thio-AMPA, the 3-isothiazolol analogue of AMPA was converted into the selective NMDA antagonist, **2**, in which a 3-isothiazolone unit is a bioisosteric analogue of the peptide bond of the NMDA antagonist, γ-(*R*)-Glu-Gly. The isomeric 3-oxygenated isothiazole amino acid, **3**, and the corresponding isothiazole phosphono amino acid **4** were also synthesized, and were shown to be selective AMPA receptor antagonists. Compound **1**, in which the peptide bond of γ-(*R*)-Glu-Gly is replaced by an ester group, was synthesized and shown to be unstable in the test buffer system. © 1997 Elsevier Science Ltd.

Introduction

(*S*)-Glutamic acid (Glu) is the major excitatory amino acid (EAA) neurotransmitter in the central nervous system and operates through multiple receptors, including the *N*-methyl-D-aspartic (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), and kainic acid receptors.[1–4] The NMDA and AMPA subtypes of EAA receptors are the subject of extensive exploration as potential targets for drug intervention in different neurodegenerative diseases.[4–7]

A number of highly selective phosphono amino acid NMDA antagonists, including (*RS*)-[3-(2-carboxypiperazin-4-yl)propyl]phosphonic acid (CPP), have been described.[8] In addition, certain dipeptides, notably γ-D-glutamylglycine [γ-(*R*)-Glu-Gly] and γ-D-glutamylaminomethylphosphonic acid [γ-(*R*)-Glu-AMP] (Fig. 1), have been shown to antagonize NMDA-induced responses with some selectivity[2] (Table 1). 6-Nitro-7-sulfamoylbenzo[*f*]quinoxaline-2,3-dione (NBQX) is a very potent and relatively selective AMPA receptor antagonist.[9] Furthermore, the AMPA-derived isoxazole amino acids, (*RS*)-2-amino-3-[3-(carboxymethoxy)-5-methylisoxazol-4-yl]propionic acid (AMOA)[10] and, more recently, (*RS*)-2-amino-3-[5-methyl-3-(phosphonomethoxy)isoxazol-4-yl]propionic acid (AMPO)[11] (Fig. 1) have been shown to block excitatory responses by AMPA, AMPO being the more potent and selective AMPA receptor antagonist.[11] Previous attempts to synthesize compound **A**, the 3-isoxazolone bioisostere of γ-(*R*)-Glu-Gly (Fig. 1) were unsuccessful,[10] probably

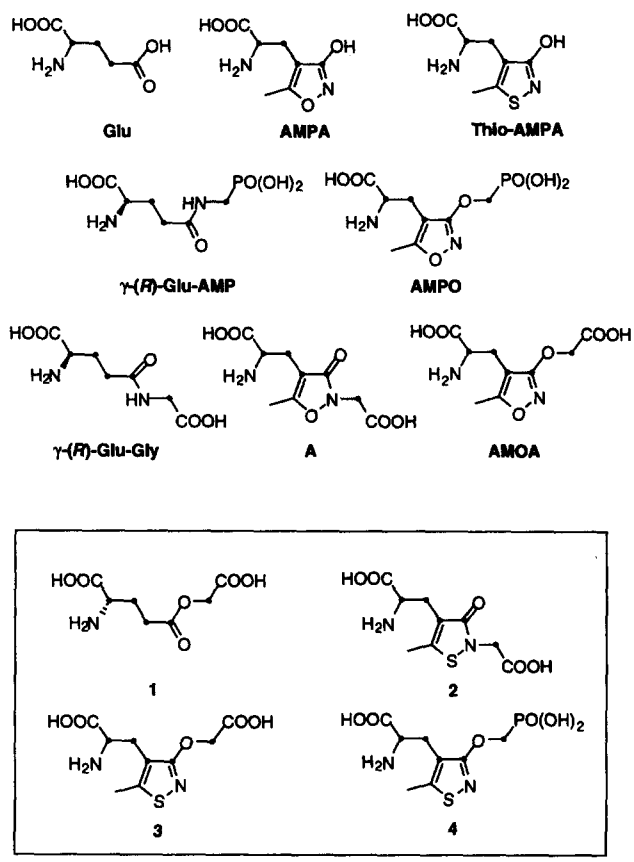


Figure 1. Structures of glutamic acid (Glu), the previously described EAA receptor agonists and antagonists AMPA, Thio-AMPA, AMOA, AMPO, compound **A**, γ-(*R*)-Glu-Gly and γ-(*R*)-Glu-AMP, and the four new compounds **1–4**.

*Author to whom correspondence should be addressed: Povl Krogsgaard-Larsen, Department of Medicinal Chemistry,The Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen Ø, Denmark.

Table 1. Receptor binding and electropharmacological data (values ± SEM, n = 3–6)

Compound	Receptor binding			Electropharmacology		
	[^{3}H]AMPA	[^{3}H]Kainic acid IC_{50} (μM)	[^{3}H]CPP	AMPA	Kainic acid K_i (μM)	NMDA
γ-(*R*)-Glu-Gly	>100	>100	0.9±2.3	410±48	>1000	27±2
γ-(*S*)-Glu-Gly	>100	>100	25±5	>1000	>1000	290±2
γ-(*R*)-Glu-AMP	>100	>100	3.4±0.9	220±9	>1000	24±1
2	>100	>100	>100	>1000	>1000	51±3
AMOA	90±14[a]	>100[a]	>100[a]	250±40	>1000	>1000[a]
3	38±4.2	>100	>100	160±28	>1000	>1000
AMPO	31±3[a]	>100[a]	>100[a]	50±8	>1000	>1000[a]
4	19±2.0	>100	>100	26±3	>1000	>1000

[a]Ref. 11.

due to rearrangement into a 1,3-oxazin-4-one isomer[12] and subsequent decomposition.

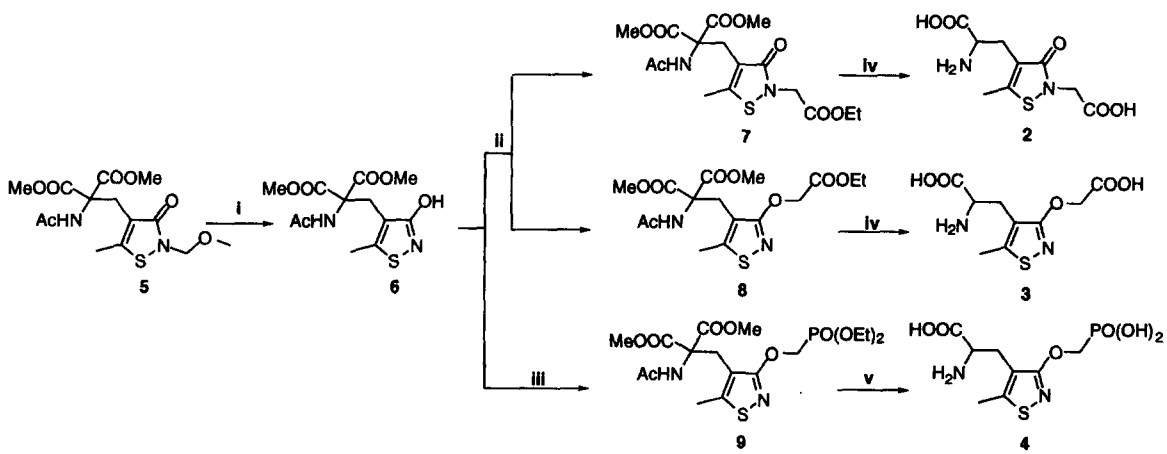
(*RS*)-2-Amino-3-(3-hydroxy-5-methylisothiazol-4-yl)propionic acid (Thio-AMPA) (Fig. 1) has recently been shown to be an AMPA receptor agonist equiselective with but somewhat less potent than AMPA.[13] We here report the conversion of Thio-AMPA into 2-amino-3-[2-(carboxymethyl)-5-methyl-3-oxoisothiazolin-4-yl]propionic acid (**2**), a 3-isothiazolone bioisostere of γ-(*R*)-Glu-Gly, and into the thio analogues of AMOA and AMPO, compound **3** and **4**, respectively (Fig. 1). The pharmacology of **2**–**4** at NMDA, AMPA, and kainic acid receptors is described. We also describe the synthesis of compound **1**, the acyclic ester analogue of AMOA and compounds **2**–**4** (Fig. 1).

Synthesis

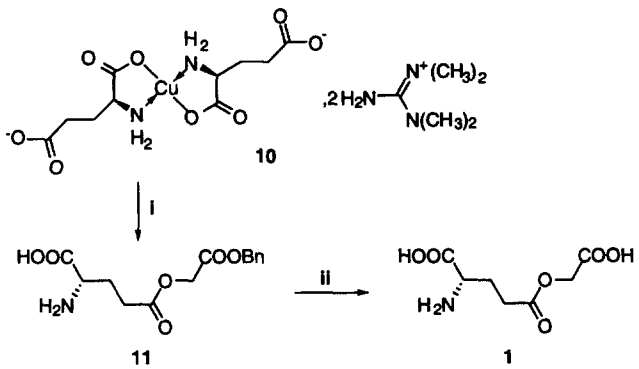
The isothiazole amino acids **2**–**4** were synthesized as outlined in Scheme 1 using methyl 2-acetamido-2-methoxycarbonyl-3-(2-methoxymethyl-5-methyl-3-oxoisothiazolin-4-yl)propionate (**5**)[13] as starting compound. Treatment of **5** with boron trifluoride etherate in the presence of acetic anhydride and subsequent heating under reflux of the intermediate product in methanolic sodium methoxide (0.1 M) provided the 3-isothiazolol **6** in 60% yield. Alkylation of **6** with ethyl chloroacetate gave a reaction product containing two main components, **7** (26%) and **8** (31%), which were separated and purified using column chromatography. Compounds **7** and **8** were deprotected using 1 M trifluoroacetic acid to give **2** (14%) and **3** (31%), respectively. Both reactions, in particular the conversion of **7** into **2**, were accompanied by the formation of decomposition products and both of the target compounds, **2** and **3**, were isolated via cation exchange chromatography (IRA-400).

The synthesis of **9** by alkylation of compound **6** with diethyl 4-toluenesulfonyloxymethylphosphonate under basic conditions was accompanied by extensive decomposition reactions. Compound **9** was thus obtained in a low yield (15%). Attempts to detect the isomeric 2-alkylated 3-isothiazolone analogue of compound **7** in the complex reaction mixture failed. Compound **9** was deprotected to give the target phosphono amino acid **4** with a 32% yield.

γ-Carboxymethyl (*S*)-glutamate (**1**), the acyclic analogue of AMOA, was synthesized by alkylation of the *N*,*N*,*N*′,*N*′-tetramethylguanidinium salt of the copper(II)



Scheme 1. (i) 1. $BF_3.OEt_2$, Ac_2O 2. 0.1 M NaOMe; (ii) $ClCH_2COOEt$, K_2CO_3; (iii) $CH_3C_6H_4SO_3CH_2PO(OEt)_2$, K_2CO_3; (iv) 1 M TFA; (v) 1 M HCl.



Scheme 2. (i) $BrCH_2COOBn$, EDTA disodium salt; (ii) H_2, Pd–C 10%.

complex, **10**,[14] with benzyl 2-bromoacetate (Scheme 2). The free γ-benzyl ester, **11**, was obtained in 35% yield by treatment of the copper(II) ester complex with an aqueous solution of EDTA. Hydrogenolysis of the benzyl ester group of **11** gave the target amino acid, **1**, in 54% yield.

Dissolution of compound **1** in test buffer solutions of physiological pH (25 °C) led to the formation of decomposition products, one of which co-chromatographed with pyroglutamic acid in several TLC and HPLC systems.

In Vitro Pharmacology

With the exception of **1**, which was unstable in test buffer solutions, the new acidic amino acids as well as the known dipeptides, γ-(*R*)- and γ-(*S*)-Glu-Gly and γ-(*R*)-Glu-AMP, were characterized pharmacologically using receptor binding techniques and the rat cortical wedge preparation[15] for electropharmacological studies. NMDA, AMPA and kainic acid receptor affinities were determined using [^{3}H]CPP,[16] [^{3}H]AMPA[17] and [^{3}H]-kainic acid,[18] respectively as radioligands.

None of the compounds under study showed detectable agonist effects at any of the target EAA receptors, and in no case was significant affinity for or antagonist effect at kainic acid receptors observed (Table 1 and Fig. 2). Like the dipeptides γ-(*R*)- and γ-(*S*)-Glu-Gly and γ-(*R*)-Glu-AMP, compound **2**, which contains a 3-isothiazolone peptide isosteric group, showed NMDA antagonist effect. In contrast to the slightly more potent NMDA antagonists γ-(*R*)-Glu-Gly and γ-(*R*)-Glu-AMP, compound **2** did not affect AMPA-induced excitation, and, quite surprisingly, compound **2** did not displace [^{3}H]CPP from NMDA receptor sites (Table 1). Like the isoxazole amino acids, AMOA and AMPO,[11] the corresponding isothiazoles **3** and **4** affected neither [^{3}H]CPP binding nor NMDA-induced depolarization significantly.

Within the group of heterocyclic amino acids, a reasonable correlation between potencies as inhibitors of [^{3}H]AMPA binding and as antagonists of AMPA-induced depolarization was observed. Whereas the 3-isothiazolone NMDA antagonist **2** did not interact with AMPA receptors, the isomeric isothiazole amino acid, **3**, was a selective AMPA antagonist, twice as potent as AMOA (Table 1 and Fig. 2). Similarly, the isothiazole amino acid **4** was shown to be twice as potent as the corresponding isoxazole phosphono amino acid, AMPO (Fig. 1), as an inhibitor of [^{3}H]AMPA binding and as an AMPA receptor antagonist.

Discussion

The dipeptides γ-(*R*)-Glu-Gly and γ-(*R*)-Glu-AMP (Fig. 1) are relatively selective NMDA receptor antagonists[2] (Table 1), whereas AMPA is a highly selective agonist at the AMPA subtype of EAA receptors.[19] In order to shed more light on the structural requirements for blockade of EAA receptor subtypes, we decided to synthesize and to characterize pharmacologically structural hybrids between agonist and antagonist ligands showing different receptor selectivities.[10,11] AMPA contains the non-symmetrical 3-isoxazolol carboxyl group bioisostere, which opens up the prospect of synthesizing 'ester-like' as well as 'amide-like' hybrid molecules. The former type of structural hybrids between AMPA and γ-(*R*)-Glu-Gly or γ-(*R*)-Glu-AMP, AMOA[10] and AMPO[11] respectively, have been synthesized and shown to be selective AMPA receptor antagonists (Table 1). However, attempts to synthesize the 'amide-like' 3-isoxazolone isomer of AMOA, i.e. compound **A**,[10] (Fig. 1) and the corresponding isomer of AMPO[11] were unsuccessful. Based on studies of the reaction between ethyl chloroacetate and 5-*tert*-butyl-4-

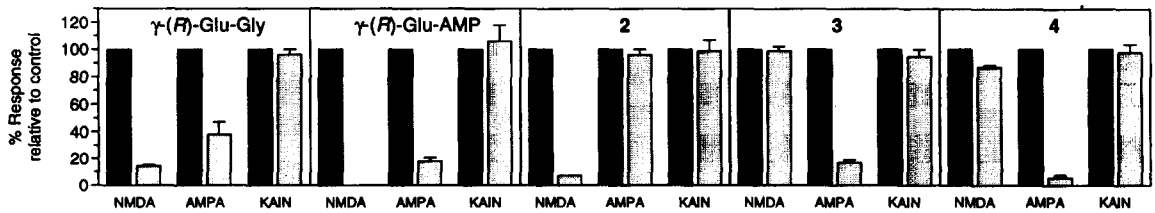


Figure 2. Pharmacological profile of a series of dipeptide EAA antagonists in the rat cortical wedge preparation. Percentage inhibition of responses to NMDA (10 μM), AMPA (3.5 μM) and kainic acid (KAIN) (5 μM) in the presence of the antagonists were determined relative to control responses. Concentrations of the antagonists used were: γ-(*R*)-Glu-Gly (1 mM) and γ-(*R*)-Glu-AMP (0.5 mM), **2** (0.5 mM), **3** (1 mM), **4** (0.25 mM). Values are mean±SEM of at least three individual experiments.

methyl-3-isoxazolol it was concluded that 2-carboxymethyl-3-isoxazolones re-arrange under basic conditions to 1,3-oxazin-4-ones.[12] It was suggested that the unsuccessful attempts to synthesize **A**[10] and the 3-isoxazolone analogue of AMPO[11] are the results of such re-arrangement reactions and subsequent decomposition of intermediate 1,3-oxazin-4-ones.

The 3-isothiazolol analogue of AMPA, Thio-AMPA, has recently been synthesized and shown to be a highly selective AMPA receptor agonist, though about an order of magnitude weaker than AMPA.[13] We report here the synthesis and pharmacological characterization of the 'amide-like' compound **2**, as well as the 'ester-like' compound **3**, which are structural hybrids between Thio-AMPA and γ-(*R*)-Glu-Gly (Fig. 1). Furthermore, the 'ester-like', compound **4**, but not the 'amide-like' Thio-AMPA/γ-(*R*)-Glu-AMP hybrid was synthesized. Moreover, the ester analogue of γ-(*R*)-Glu-Gly, compound **1** (Fig. 1), was prepared. Since the AMPA receptor antagonist of AMOA resides in the (*S*)-enantiomer, the (*S*)-form of **1** was synthesized, but, unfortunately, instability of **1** in test buffer solutions prevented pharmacological characterization of this compound.

Like the isoxazole amino acids AMOA[10] and AMPO,[11] the corresponding isothiazoles, **3** and **4**, turned out to be selective AMPA receptor antagonists (Table 1 and Fig. 2). Interestingly, whereas Thio-AMPA is substantially weaker than AMPA as an AMPA agonist,[13] substitution of sulfur for oxygen in AMOA and AMPO, to give **3** and **4** respectively (Fig. 1), resulted in a twofold increase in antagonist potency (Table 1). Several physicochemical parameters may have to be considered in an analysis of these structure–activity relationships, which seem to reflect that, within this class of cyclic AMPA receptor ligands, the heteroaromatic rings of agonist and antagonist molecules interact with the receptor in a dissimilar fashion or, alternatively, that the AMPA receptor exists in pharmacologically distinct agonist and antagonist preferring conformations. In any case, the increased volume and lipophilicity of the sulfur atom, as compared with the oxygen atom, obviously favours the interaction of this type of antagonists, but not agonists, with the AMPA receptor complex.

Whereas compounds **3** and **4** selectively block AMPA receptors, compound **2** shows a similar degree of selectivity for the NMDA receptor (Table 1 and Fig. 2). Although **2** and **3** have isomeric structures, only the 3-isothiazolone structural unit can bioisosterically substitute for the peptide bond γ-(*R*)-Glu-Gly. It looks as if the carbonyl group rather than the hydrogen atom of the peptide bond of γ-(*R*)-Glu-Gly is essential for NMDA receptor antagonism, and that the imine groups of **3** and **4** are unable to mediate this function. It is noteworthy that **2**, which is a moderately potent NMDA antagonist (K_i = 51±3 μM), does not significantly affect the binding of the NMDA antagonist radioligand, [^{3}H]CPP (Table 1). A similar observation has, however, recently been made for (*R*)-2-amino-2-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)acetic acid [(*R*)-ATAA], which also is an antagonist of NMDA-induced depolarization (K_i = 75±5 μM) showing no detectable effect on the binding of [^{3}H]CPP.[19] The structurally related compound, (*R*)-2-amino-2-(3-hydroxy-5-methylisoxazol-4-yl)acetic acid [(*R*)-AMAA] is a very potent NMDA agonist, but (*R*)-AMAA (IC_{50} = 3.7 μM)[20] as well as NMDA itself (IC_{50} = 35 μM)[2] are rather weak inhibitors of [^{3}H]CPP binding, and attempts to use [^{3}H]AMAA[21] or [^{3}H]NMDA[22] as radioligands for NMDA receptors have been unsuccessful. Thus, **2** as well as (*R*)-ATAA, (*R*)-AMAA and NMDA may interact with a low-affinity site at the NMDA receptor complex, which is different from that, which potently binds CPP and other competitive phosphono amino acid antagonists. Alternatively, these compounds may interact with a receptor conformation different from that, with which compounds like CPP interact.

Thio-AMPA has been shown to be systemically active at markedly lower concentrations than AMPA,[13] but comparative behavioral pharmacological studies on AMOA and AMPO and the isothiazoles **3** and **4** have not yet been performed. Furthermore, it remains to be studied whether the 3-isothiazolone unit is a generally useful bioisostere of peptide bonds.

Experimental

Chemistry

Melting points were determined in capillary tubes and are uncorrected. Elemental analyses were performed by Mrs K. Linthoe, Department of General and Organic Chemistry, University of Copenhagen or Analytical Research Department, H. Lundbeck A/S, Denmark, and are within ±0.4% of the calculated values, unless otherwise stated (see Table 2). ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker AC 200 spectrometer, using $CDCl_3$ or D_2O (with 1,4-dixoane, δ = 3.69, as an internal standard) as solvent. Drying of organic phases was performed using $MgSO_4$. Column chromatography and TLC were performed on silica gel 60 (70–230 mesh, Merck) and silica gel F_{254} (Merck), respectively. The amino acids were visualized using a ninhydrin spraying reagent, whereas compounds containing the 3-isothiazolol unit were visualized using a $FeCl_3$ spraying reagent. Optical rotations were determined on a Perkin–Elmer 141 polarimeter.

Methyl-2-acetamido-3-(3-hydroxy-5-methylisothiazol-4-yl)-2-(methoxycarbonyl)propionate (6). Boron trifluoride etherate (1.2 mL, 9.2 mmol) was added to a mixture of **5**[13] (2.5 g, 6.9 mmol), acetic anhydride (35 mL) and dichloromethane (50 mL). The reaction mixture was stirred for 16 h at room temperature and evaporated. The residue was dissolved in water (100 mL) and stirred for 1 h at room temperature. Extraction with dichloromethane (4 × 75 mL), drying and evaporation gave a residue, which was added to a

Table 2. Elemental analyses

	Calcd (%)				Found (%)			
	C	H	N	S	C	H	N	S
(1) $C_7H_{11}NO_6 \cdot 0.75H_2O$	38.45	5.76	6.41	—	38.56	5.04	6.14	—
(3) $C_9H_{12}N_2O_5S \cdot 0.25H_2O$	40.86	4.76	10.58	12.11	40.86	4.64	10.72	12.13
(4) $C_8H_{13}N_2O_6SP \cdot 1H_2O$	30.58	4.81	8.91	10.20	30.72	4.43	8.74	ND
(6) $C_{12}H_{16}N_2O_6S \cdot 0.5H_2O$	44.33	5.27	8.61	9.83	44.45	5.11	8.62	10.37
(7) $C_{16}H_{22}N_2O_8S$	47.75	5.51	6.96	7.95	47.73	5.31	6.69	7.73
(8) $C_{16}H_{22}N_2O_8S$	47.75	5.51	6.96	7.95	47.69	5.43	6.69	8.21
(9) $C_{17}H_{27}N_2O_9PS$	43.77	5.84	6.01	6.86	43.57	5.84	5.93	6.55
(11) $C_{14}H_{17}NO_6$	56.95	5.80	4.74	—	57.37	5.75	4.70	—

ND, not determined.

solution of sodium methoxide (from 0.26 g of sodium, 11.5 mmol) in methanol (115 mL). The mixture was refluxed for 5 h and the pH of the solution was adjusted to 7 with acetic acid. After evaporation, the residue was dissolved in water (50 mL) and the solution extracted with dichloromethane (5 × 50 mL). The combined extracts were dried and evaporated and the residue was dissolved in methanol–ethanol (1:1, 100 mL), boiled with charcoal for 2 min, filtered and evaporated. Recrystallization from ethanol/ether gave **6** (1.3 g; 60%), mp 184–185 °C. ^{1}H NMR ($CDCl_3$) δ 6.95 (1H, s), 3.81 (6H, s), 3.49 (2H, s), 2.32 (3H, s), 2.00 (3H, s). Anal. ($C_{12}H_{16}N_2O_6S \cdot 0.5\ H_2O$) C, H, N; S: calcd 9.83, found 10.37.

Methyl-2-acetamido-3-{2-[(ethoxycarbonyl)methyl]-5-methyl-3(2*H*)-oxoisothiazol-4-yl}-2-(methoxycarbonyl)propionate (7) and methyl 2-acetamido-3-{3-[(ethoxycarbonyl)methoxy]-5-methylisothiazol-4-yl}-2-(methoxycarbonyl)propionate (8). Ethyl chloroacetate (1.0 mL, 9.6 mmol) was added to a suspension of **6** (1.0 g, 3.2 mmol) and K_2CO_3 (1.1 g, 8.0 mmol) in acetone (25 mL), and the mixture was stirred at 50 °C for 20 h. The reaction mixture was filtered and evaporated, and the residue subjected to flash chromatography [ethyl acetate–toluene (3:1)]. Recrystallization from ethyl acetate/light petroleum gave **7** (330 mg, 26%), mp 105–106 °C, and **8** (393 mg, 31%), mp 121–122 °C. ^{1}H NMR ($CDCl_3$) (**7**) δ 7.33 (1H, s), 4.44 (2H, s), 4.21 (2H, q, J = 7.4 Hz), 3.80 (6H, s), 3.43 (2H, s), 2.30 (3H, s), 2.00 (3H, s), 1.28 (3H, t, J = 7.0 Hz). ^{1}H NMR ($CDCl_3$) (**8**): δ 7.15 (1H, s), 4.83 (2H, s), 4.19 (2H, q, J = 7.2 Hz), 3.78 (6H, s), 3.24 (2H, s), 2.32 (3H, s), 1.93 (3H, s), 1.36 (3H, t, J = 7.0 Hz). Anal. (**7**) ($C_{16}H_{22}N_2O_8S$) C, H, N, S. Anal. (**8**) ($C_{16}H_{22}N_2O_8S$) C, H, N, S.

Methyl-2-acetamido-3-{3-[(diethoxyphosphoryl)-methoxy]-5-methylisothiazol-4-yl}-2-(methoxycarbonyl)propionate (9). A solution of diethyl 4-toluene sulfonyloxymethylphosphonate (3.6 g, 11.2 mmol) in acetone (20 mL) was added to a suspension of **6** (2.0 g, 6.4 mmol) and K_2CO_3 (880 mg, 6.4 mmol) in acetone (140 mL). The reaction mixture was stirred at 50 °C for 96 h, filtered and evaporated. The residue was dissolved in ethyl acetate (75 mL), and the organic phase was washed with water (75 mL), dried and evaporated. Column chromatography [ethyl acetate–toluene (4:1) containing 1% of glacial acetic acid] followed by recrystallization from ethyl acetate/light petroleum gave **9** (447 mg, 15%), mp 85–87 °C. ^{1}H NMR ($CDCl_3$) δ 6.96 (1H, s), 4.69 (2H, d, J = 7.8 Hz), 4.17 (4H, m), 3.80 (6H, s), 3.52 (2H, s), 2.34 (3H, s), 1.99 (3H, s), 1.29 (6H, m). Anal. ($C_{17}H_{27}N_2O_9PS$) C, H, N, S.

(*RS*)-2-Amino-3-[2-(carboxymethyl)-5-methyl-3(2*H*)-oxoisothiazol-4-yl]propionic acid (2). A solution of **7** (320 mg, 0.8 mmol) in 1 M trifluoroacetic acid (TFA) (20 mL, 20 mmol) was refluxed for 12 h and then evaporated. The residue was dissolved in water (5 mL) and subjected to cation exchange chromatography (IRA-400) using 1 M acetic acid as an eluent. Evaporation of the ninhydrin-reactive fractions and recrystallization from ethanol/ether gave **2** (30 mg, 14%). ^{1}H NMR (D_2O) δ 4.33 (1H, t, J = 6.0 Hz), 3.60 (2H, s), 2.99 (2H, d, J = 6.2 Hz), 2.32 (3H, s). ^{13}C NMR (D_2O) δ 173.6 (CO_2H), 173.0 (CO_2H), 171.0 (C3), 157.2 (C5), 117.7 (C4), 54.0 (CH), 46.8 ($N\underline{C}H_2CO_2H$), 27.9 (CH_2), 14.0 (CH_3).

(*RS*)-2-Amino-3-[3-(carboxymethoxy)-5-methylisothiazol-4-yl]propionic acid (3). A solution of **8** (407 mg, 1.0 mmol) in 1 M TFA (25 mL, 25 mmol) was refluxed for 48 h and then evaporated. The residue was dissolved in water (5 mL) and subjected to cation exchange chromatography (IRA-400) using 1 M acetic acid as an eluent. Evaporation of the ninhydrin-reactive fractions and recrystallization from ethanol/ether gave **3** (80 mg, 31%), mp 250 °C (decomp). ^{1}H NMR (D_2O) δ 4.94 (2H, d, J = 4.8 Hz), 4.21 (1H, t, J = 6.1 Hz), 3.13 (2H, d, J = 6.0 Hz), 2.40 (3H, s). Anal. ($C_9H_{12}N_2O_5S \cdot 0.25H_2O$) C, H, N.

(*RS*)-2-Amino-3-[5-methyl-3-(phosphonomethoxy)-isothiazol-4-yl]propionic acid monohydrate (4). A solution of **9** (257 mg, 0.6 mmol) in 1 M hydrochloric acid (25 mL, 25 mmol) was refluxed for 43 h and evaporated to dryness. Recrystallization from ethanol/ether gave **4** (56 mg, 32%), mp 248–250 °C (decomp). ^{1}H NMR (D_2O) δ 4.33 (2H, d, J = 8.0 Hz),

4.12 (1H, t, J = 6.7 Hz), 2.98 (2H, d, J = 6.1 Hz), 2.22 (3H, s). Anal. ($C_8H_{13}N_2O_6SP \cdot 1H_2O$) C, H, N, S.

γ-Benzyloxyoxomethyl (*S*)-glutamate (11). Benzyl 2-bromoacetate (1.9 mL, 11.8 mmol) was added to a solution of **10**[14] (2.0 g, 3.4 mmol) in DMF/water (9:1) (15 mL) and the reaction mixture was stirred for 24 h at room temperature. After addition of a solution of EDTA disodium salt (2.0 g, 5.9 mmol) in water (50 mL) the solution was stirred at room temperature for 1 h followed by cooling. The precipitate formed was filtered, washed with cold water and recrystallized from water to give **11** (700 mg, 35%), mp 149 °C. $[\alpha]^{20}_D$ +20.6° (*c* 0.2 in glacial acetic acid). ^{1}H NMR (D_2O) δ 7.39 (5H, s), 5.20 (2H, s), 4.73 (2H, s), 3.69 (1H, m), 2.62 (2H, m), 2.12 (2H, m). Anal. ($C_{14}H_{17}NO_6$) H, N; C: calcd 56.95, found 57.37.

γ-Carboxymethyl (*S*)-glutamate (1). A solution of **11** (50 mg, 0.2 mmol) in methanol (35 mL) was hydrogenated at ambient pressure over Pd–C 10% (5 mg) for 2 h. Filtration, evaporation and recrystallization from water/isopropanol afforded **1** (22 mg, 54%), mp 159–161 °C. $[\alpha]^{20}_D$ +5.6° (*c* 0.2 in water). ^{1}H NMR (D_2O) δ 4.50 (2H, s), 3.80 (1H, t, J = 7.8 Hz), 2.63 (2H, t, J = 8.0 Hz), 2.16 (2H, m). Anal. ($C_7H_{11}NO_6 \cdot 0.75H_2O$) C, N; H: calcd 5.76, found 5.04.

In vitro pharmacology

Receptor binding assays. Affinity for AMPA receptors was determined using the ligand [^{3}H]AMPA[17] and for determination of NMDA and kainic acid receptor affinities, [^{3}H]CPP[16] and [^{3}H]kainic acid,[18] respectively, were used. The membrane preparations used in all the receptor binding experiments were prepared according to the method of Ransom and Stec.[25]

In vitro electrophysiology. A rat cortical wedge preparation for determination of excitatory amino acid-evoked depolarizations described by Harrison and Simmonds[15] was used in a slightly modified version. Wedges (500 μm thick) of rat brain, containing cerebral cortex and corpus callosum, were placed through a grease barrier for electrical isolation with each part in contact with an Ag/AgCl pellet electrode. The cortex and corpus callosum parts were constantly superfused with a Mg^{2+}-free oxygenated Krebs buffer at room temperature. The test compounds were added to the cortex superfusion medium containing 2.5 mM $CaCl_2$, and the induced potential difference between the electrodes was recorded on a chart recorder.

The compounds were initially screened as inhibitors of responses to NMDA (10 μM), AMPA (3.5 μM), and kainic acid (5 μM). Percentage inhibition of the responses in the presence of the antagonist relative to the control responses were calculated. In the case of more than 50% inhibition of a response, a K_i value for the antagonist versus the agonist was calculated. By using a fixed concentration of the antagonist, the dose–response curve for the agonist was parallel shifted rightwards, the dose ratio (D_r calculated as the ratio between EC_{50} value in the presence and absence, respectively, of the antagonist) determined, and the K_i value calculated ($\log(D_r - 1) = \log[\text{Antagonist}] - \log(K_i)$). Application of agonists were done for 90 s, and for antagonist experiments, the antagonists were applied alone for 90 s followed by coapplication of the agonist and antagonist for another 90 s.

Acknowledgements

This work was supported by grants from the Danish State Biotechnology Program (1991–1995) and the Lundbeck Foundation. The secretarial assistance of Mrs Anne Nordly is gratefully acknowledged.

References

1. Wheal, H. V.; Thomson, A. M. *Excitatory Amino Acids and Synaptic Transmission*, Academic: London, 1995.

2. Collingridge, G. L.; Watkins, J. C. *The NMDA Receptor*, Oxford University: Oxford, 1994.

3. Conn, P. J.; Patel, J. *The Metabotropic Glutamate Receptors*, Humana: New Jersey, 1994.

4. Krogsgaard-Larsen, P.; Hansen, J. J. *Excitatory Amino Acid Receptors: Design of Agonists and Antagonists*, Ellis Horwood: Chichester, 1992.

5. Lodge, D. *Excitatory Amino Acids in Health and Disease*, John Wiley: Chichester, 1988.

6. Meldrum, B. S. *Excitatory Amino Acid Antagonists*, Blackwell: Oxford, 1991.

7. Krogsgaard-Larsen, P.; Ebert, B.; Lund, T. M.; Bräuner-Osborne, H.; Sløk, F. A.; Johansen, T. N.; Brehm, L.; Madsen, U. *Eur. J. Med. Chem.* **1996**, *31*, 515.

8. Watkins, J. C.; Olverman, H. J. *Trends Neurosci.* **1987**, *10*, 265.

9. Sheardown, M. J.; Nielsen, E. Ø.; Hansen, A. J.; Jacobsen, P.; Honoré, T. *Science* **1990**, *247*, 571.

10. Krogsgaard-Larsen, P.; Ferkany, J. W.; Nielsen, E. Ø.; Madsen, U.; Ebert, B.; Johansen, J. S.; Diemer, N. H.; Bruhn, T.; Beattie, D. T.; Curtis, D. R. *J. Med. Chem.* **1991**, *34*, 123.

11. Madsen, U.; Bang-Andersen, B.; Brehm, L.; Christensen, I. T.; Ebert, B.; Kristoffersen, I. T. S.; Lang, Y.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1996**, *39*, 1682.

12. Brehm, L.; Madsen, U.; Johansen, J. S.; Krogsgaard-Larsen, P. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2009.

13. Matzen, L.; Engesgaard, A.; Ebert, B.; Didriksen, M.; Frølund, B.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *J. Med. Chem.* **1997**, *40*, 520.

14. Van Heeswijk, W. A. R.; Eenink, M. J. D.; Feijen, J. *Synthesis* **1982**, 744.

15. Harrison, N. L.; Simmonds, M. A. *Br. J. Pharmacol.* **1985**, *84*, 381.

16. Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, K. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 778.

17. Honoré, T.; Nielsen, M. *Neurosci. Lett.* **1985**, *54*, 27.

18. Braitman, D. J.; Coyle, J. T. *Neuropharmacology* **1987**, *26*, 1247.

19. Johansen, T. N.; Frydenvang, K.; Ebert, B.; Madsen, U.; Krogsgaard-Larsen, P. *Chirality* **1997** (in press).

20. Madsen, U.; Frydenvang, K.; Ebert, B.; Johansen, T. N.; Brehm, L.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1996**, *39*, 183.

21. Johansen, T. N.; Balasubramanian, V.; Madsen, U.; Ferkany, J. W.; Krogsgaard-Larsen, P. *J. Labelled Compd. Radiopharm.* **1996**, *38*, 915.

22. Foster, A. C.; Fagg, G. E. *Eur. J. Pharmacol.* **1987**, *133*, 291.

23. Ransom, R. W.; Stec, N. L. *J. Neurochem.* **1988**, *51*, 830.

(*Received in U.S.A.* 19 *February* 1997; *accepted* 31 *March* 1997)