Synthesis and Single Cell Pharmacology of Potential Heterocyclic Biososteres of the Excitatory Amino Acid Antagonist Glutamic Acid Diethyl Ester

Ulf Madsen,* Elsebet Ø. Nielsen, David R. Curtis, David T. Beattie and Povl Krogsgaard-Larsena

aPharmaBiotech, Department of Organic Chemistry, The Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark and bDivision of Neuroscience, John Curtin School of Medicinal Research, Canberra, A.C.T. 2601, Australia


A series of heterocyclic analogues of glutamic acid diethyl ester (GDEE), an antagonist at central excitatory amino acid receptors, have been synthesized and tested biologically. (RS)-Ethyl α-amino-α-(3-ethoxyisoxazol-5-yl)acetate (7), (RS)-ethyl 2-amino-3-(3-ethoxy-5-methylisoxazol-4-yl)propionate (16) and closely related analogues were synthesized. Compound 7, a diethyl derivative of the naturally occurring excitatory amino acid ibotenic acid (IBO), was synthesized from 3-hydroxy-5-methylisoxazole (1) via 3-ethoxyisoxazol-5-ylacetic acid (5) and its ethyl ester. Nitrosation of this ester followed by catalytic reduction gave 7. The ethyl ester of IBO, 9, was synthesized in a similar manner from 3-benzyloxyisoxazol-5-ylacetic acid (8). Ethyl derivatives of the synthetic excitatory amino acid 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) were synthesized from 3-hydroxy-4,5-dimethylisoxazole (10) through a diethyl acrylamidomalonate derivative, which upon deprotection gave the 3-ethoxy derivative of AMPA (15). Esterification of 15 gave the diethyl derivative 16 and the ethyl ester of AMPA (18) as well as N-ethylated derivatives of AMPA, 21 and 22 were synthesized. The final products were tested microelectrophoretically. The derivatives 7, 9, 15, 16 and 18 were weak and non-selective excitatory amino acid antagonists, whereas 21 and 22 were found to be inactive.

Glutamic acid (GLU) is the major excitatory amino acid (EAA) neurotransmitter in the central nervous system.1–3 Much pharmacological and therapeutic interest is focused on antagonists for central EAA synapses in particular, due to the possible involvement of hyperactivity at these synapses in the development of certain neurodegenerative diseases.4–6 At present, receptors for the EAA’s are subdivided into three main classes.7–9 (1) NMDA receptors, at which N-methyl-d-aspartic acid (NMDA) is a selective agonist; (2) QUIS/AMPA receptors, at which quisqualic acid (QUIS) is a potent but non-selective agonist, whereas 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) is a potent and selective agonist; and (3) KA receptors, at which kainic acid (KA) is a potent agonist (Fig. 1).

The pharmacology of the NMDA receptor complex has been quite extensively elucidated,10 largely due to the availability of potent and selective antagonists such as 2-amino-5-phosphonovaleric acid and (RS)-3-(2-carboxypiperazin-4-yl)propylphosphonic acid.10–12 In contrast, selective antagonists for AMPA or KA receptors have not yet been described. Glutamic acid diethyl ester (GDEE) is an antagonist for QUIS/AMPA receptors,7–9,14 but low potency, lack of specificity and chemical instability limit the utility of GDEE. During the past years GDEE has, however, been used to characterize the in vivo pharmacology of a number of QUIS/AMPA receptor agonists.15–17 Using GDEE as a lead structure a series of compounds has been designed and synthesized as potential antagonists at EAA receptors. Thus, two GLU analogues, ibotenic acid (IBO) and AMPA have been converted into mono- and di-ethyl derivatives (Fig. 1). IBO is a naturally occurring amino acid, which primarily acts as an NMDA agonist and to a lesser extent activates QUIS/AMPA and KA receptors.13–19 As mentioned above, AMPA is a highly selective synthetic agonist for the QUIS/AMPA receptors.5,20–22 IBO and AMPA are GLU biososteres, in which the ω-carboxy group of GLU has been replaced by 3-hydroxyisoxazole moieties. An analogous ester biososteric approach has now been designed, and we have synthesized isoxazole ester analogues of GDEE, namely (RS)-ethyl α-amino-α-(3-ethoxyisoxazol-5-yl)acetate (7), (RS)-ethyl 2-amino-3-(3-ethoxy-5-methylisoxazol-4-yl)propionate (16), (RS)-ethyl 2-amino-3-(2-ethyl-5-methyl-3-oxoisoxazolin-4-yl)propionate (22) and other closely related analogues. Pharmacological investigations of these compounds using microelectrophoretic techniques are also described.
**Results and discussion**

3-Hydroxy-5-methylisoxazole\(^1\) (1) was ethylated with ethyl bromide, which gave an easily separable mixture of 2 and 3, the O-ethylated compound 2 being the major product (Scheme 1). Lithiation of 2 followed by reaction with carbon dioxide gave the two carboxylic acids 4 and 5 in 4.4 and 42% yield, respectively. Esterification of 5 was accomplished by reaction with ethyl chloroformate and triethylamine dissolved in dichloromethane to give the desired ester in practically quantitative yield. This mixed anhydride synthesis has been described as requiring \(N,N\)-dimethylaminopyridine (DMAP) as a catalyst,\(^2\) but this and other syntheses\(^3\) have shown no need for DMAP as a catalyst. Nitrosation of the obtained ester by reaction with NaH and immediate addition of butyl nitrite gave the oxime 6, which could be reduced to the final product 7 by low-pressure hydrogenation with Pd-on-C as a catalyst. The ethyl ester of IBO (9) was synthesized by analogy with 7 except for the protecting group of the 3-hydroxyisoxazole. Thus, 3-benzylisoxazol-5-ylacetic acid\(^3\) (8) was esterified and converted into an oxime by mixed anhydride esterification and

![Scheme 1](image)

(i) \(C_2H_5Br, K_2CO_3\)  (ii) \(1)\) BaLi 2) \(CO_2\)  (iii) \(Cl\text{-COOC}_2H_5, TEA\)  (iv) \(1)\) NaH 2) \(BuONO\)  (v) \(H_2/Pd, HCl\)
Scheme 2. 

(i) C₂H₂Br, K₂CO₃ (ii) NBS (iii) C₂H₅ONa, AAME (iv) 1 M HCl (v) C₂H₅OH/H⁺ (vi) HBr/H₂O

subsequent nitrosation. The final compound 9 was obtained by simultaneous reduction of the oxime and reductive cleavage of the benzyloxy group.

The preparation of ethylated derivatives of AMPA are shown in Scheme 2. Ethylation of 3-hydroxy-4,5-dimethylisoxazole₂₉ (10) gave 11 as the major product, easily separable from the N-ethylated product 12. NBS bromination of 11 and subsequent reaction with the sodium salt of diethyl acetamidomalonate (AAME) gave 13 and 14. Deprotection of compound 14 with 1 M HCl gave 15, which, by conventional esterification with ethanolic HCl, led to 16. Reflux of 14 in aqueous HBr (48%) gave AMPA hydrobromide (17). This synthetic route to AMPA is slightly different from previously published procedures for the

Scheme 3. 

(i) NBS (ii) C₂H₅ONa, AAME (iii) HBr/AcOH (iv) C₂H₂Br, K₂CO₃ (v) 1 M HCl (vi) C₂H₅OH/H⁺
preparation of AMPA. Conventional esterification of AMPA gave
18.

The syntheses of N-ethylated derivatives of AMPA are shown in Scheme 3. NBS bromination of 12 and subsequent reaction with the sodium salt of AAME gave 19 and probably also a 5-methyl substituted isomer, which was not isolated. The structure of 19 was established by its preparation by an alternative route. Compound 14 was selectively de-ethylated to 20 by treatment with HBr in glacial acetic acid (33%) and re-ethylated to give a mixture of 14 and 19. Compound 19 prepared from 12 was identical with the sample of 19 synthesized from 14 via 20, and the structure of 14 was confirmed by its conversion into AMPA hydrobromide (17) (Scheme 2). Deprotection of 19 with 1 M HCl gave 21, which by conventional esterification gave 22.

The pharmacological effects of compounds 7, 9, 15, 16, 18, 21 and 22 on cat spinal neurones were tested using microelectrophoretic techniques. The 3-ethyloxazoloisosteres of GDEE, compounds 7 and 16, were shown to antagonize reversibly excitations induced by NMDA, QUIS or KA (results not shown). On most cells studied these EAA antagonist effects were weaker than those of GDEE. Thus, although the 3-ethyloxazoloisazole nucleus has been shown to act as a biosostere for the ω ester group of GDEE, this biososteric substitution has not led to EAA antagonists superior to GDEE in terms of potency or receptor selectivity.

The mono-ethyl derivatives 9, 15 and 18 showed EAA antagonist profiles similar to those of 7 and 16, but with most cells studied these three compounds were generally weaker than GDEE and the GDEE isosteres 7 and 16. The N-ethylated compounds 21 and 22 did not significantly reduce excitations induced by NMDA, QUIS or KA on cat spinal neurones. Thus, although the 2-ethyloxazolizin-3-one nucleus in 21 and 22 can be considered an isostere of the ethyl ester group, this heterocyclic unit does not act as an effective ester biosostere at the EAA receptors. This lack of effect may reflect the structural and electronic differences between the ethyl ester group and the 2-ethyloxazolizin-3-one group or, alternatively, that this latter group in 21 or 22, cannot easily adopt a conformation that reflects the active conformation of the ω ester ester group of GDEE at EAA receptors, notably the QUIS/AMPA receptor subtype.

Experimental

Melting points are corrected and were determined in capillary tubes. Elemental analysis were performed by Mr. G. Cornali, Microanalytical Laboratories, Leo Pharmaceutical Products, Denmark or Mr. P. Hansen, Department of General and Organic Chemistry, University of Copenhagen. The 60 MHz 1H NMR spectra were recorded on a Varian EM 360L spectrometer and the 90 MHz 1H NMR spectra on a Jeol FX 90Q spectrometer with compounds dissolved in CDC3 using TMS as a reference unless otherwise stated.

IR spectra, obtained on a Perkin-Elmer 781 Infrared spectrophotometer, were recorded in KBr pellets or as liquid films between NaCl discs. A Waters PrepLC system 500A instrument was used for the preparative high-pressure liquid chromatography (HPLC) using silica gel columns (PrepPAK®-500/Silica). Thin layer chromatography (TLC) and gravity column chromatography were performed using silica gel F254 plates (Merck) and silica gel (Woelm, 0.063–0.200 mm), respectively. Compounds containing the isoxazol-3-ol unit were visualized on TLC plates using UV light and an FeCl3 spraying reagent (yellow color). Compounds containing amino groups were visualized using a ninhydrin spraying reagent, and all compounds under study were detected on TLC plates using a KMnO4 spraying reagent. All evaporation were performed at ca. 15 mmHg using a rotary evaporator.

3-Ethoxy-5-methylisoxazole (2) and 2-ethyl-5-methylisoxazolizin-3-one (3). To a solution of 1 (20 g, 0.2 mol) in acetone (500 ml) was added K2CO3 (56 g, 0.4 mol) and the mixture was stirred at 60°C for 0.5 h. Ethyl bromide (23 ml, 0.3 mol) was added dropwise and the mixture was left to stir at 60°C overnight. After cooling, filtration and evaporation, preparative HPLC (ethyl acetate–dichloromethane 5:1) of the product gave 2 (16.7 g, 65%) as a colourless oil. Anal. C9H8NO: C, H, N. 1H NMR: δ 5.5 (s, 1 H), 4.2 (q, J 7 Hz, 2 H), 2.3 (s, 3 H), 1.35 (t, J 7 Hz, 3 H). IR: 2975 (m), 2925 (w), 1620 (s), 1505 (s), 1455 (cm⁻¹).

Further elution (ethyl acetate) gave 3 (6.5 g, 25%) as a colourless oil. Anal. C9H8NO: C, H, N; Found, C 56.06; Calc., C 56.68. 1H NMR: δ 5.35 (s, 1 H), 3.8 (q, J 7 Hz, 2 H), 2.2 (s, 3 H), 1.25 (t, J 7 Hz, 3 H). IR: 2975 (m), 2925 (w), 1775 (s), 1625 (m), 1440 (cm⁻¹).

3-Ethoxy-5-methylisoxazol-4-ylcarboxylic acid (4) and 3-ethyloxazolizin-5-ylacetic acid (5). To a solution of butyl lithium (50 ml, 1.1 M in hexane; 55 mmol) in dry tetrahydrofuran (THF) (100 ml) cooled to −78°C was added slowly a solution of 2 in dry THF (50 ml). After being stirred for 15 min at −78°C the brown–black solution was poured into a slurry of crushed carbon dioxide (ca. 250 g) in dry ether (200 ml). The excess of carbon dioxide evaporated overnight and the reaction mixture was evaporated, and treated with water (75 ml), which was then acidified with 4 M HCl and extracted with dichloromethane (3×100 ml). The combined dichloromethane extracts were dried (MgSO4) and evaporated and the residue gave, by preparative HPLC (toluene–ethyl acetate 9:1 with 1% glacial acetic acid), compound 4 (385 mg, 4.4%) after recrystallization (ethyl acetate–light petroleum), m.p. 162–164°C. Anal. C9H8NO: C, H, N. 1H NMR: δ 11.3 (s, 1 H), 4.1 (q, J 7 Hz, 2 H), 2.2 (s, 3 H), 1.2 (t, J 7 Hz, 3 H). IR: 3300–2350 (w–m, several bands), 1685 (s), 1620 (s), 1515 (s), 1470 (cm⁻¹). Further elution gave 5 (3.7 g, 42%) after recrystallization (ethyl acetate–light petroleum), m.p. 69–70°C. Anal. C9H8NO: C, H, N. 1H NMR: δ 10.8 (s, 1 H), 5.7 (s, 1 H), 3.85 (q, J 7 Hz, 2 H), 3.2 (s, 2 H), 1.3 (t, J 7 Hz)
Hz, 3 H). IR: 3300–2350 (w–m, several bands), 1730 (s), 1625 (s), 1515 (s), 1465 (s) cm\(^{-1}\).

(EZ)-Ethyl \(\alpha\)-hydroxyiminoo-\(\alpha\)-(3-ethoxyisoxazol-5-yl)acetate (6). To a solution of 5 (3.4 g, 20 mmol) and triethylamine (3 ml, 22 mmol) in dichloromethane (50 ml) cooled to 0°C, was added slowly ethyl chloroformate (2.3 ml, 24 mmol). After being stirred for 5 min the reaction mixture was extracted with 1 M HCl (50 ml) and semi-saturated NaHCO\(_3\) (50 ml). The dried (MgSO\(_4\)) dichloromethane phase was evaporated and subjected to Kugelrohr distillation (0.2 mmHg, 150°C), which gave ethyl 3-ethoxyisoxazol-5-ylacetate (3.7 g, 93%) as a colourless oil. Anal. C\(_6\)H\(_9\)NO\(_2\): C, H, N. \(\text{\(^1\)H NMR: } \delta 5.8\) (s, 1 H), 4.25 (q, J 7 Hz, 2 H), 4.15 (q, J 7 Hz, 2 H), 3.65 (s, 2 H), 1.35 (t, J 7 Hz, 3 H), 1.25 (t, J 7 Hz, 3 H). IR: 3140 (w), 2980 (s), 2935 (m), 1740 (s), 1620 (s), 1510 (s) cm\(^{-1}\). Na\(\text{HT}(100 \text{ mg, } 80\% \text{ dispersion in water; } 3.3 \text{ mmol})\) and followed immediately by butyl nitrite (500 \(\mu\)l, 5 mmol). After being stirred for 5 min the reaction was quenched with glacial acetic acid (200 \(\mu\)l) and treated with water (15 ml). Extraction with dichloromethane (3 \(\times\) 10 ml), drying and evaporation of the combined dichloromethane extracts, and column chromatography (dichloromethane containing 5–11% ethyl acetate) of the residue gave (EZ)-ethyl \(\alpha\)-hydroxyiminoo-\(\alpha\)-(3-benzoxlyisoxazol-5-yl)acetate (455 mg, 52%). An analytical sample was recrystallized (toluene–light petroleum), m.p. 107–109°C. Anal. C\(_9\)H\(_{10}\)N\(_2\).O\(_2\): C, H, N. \(\text{\(^1\)H NMR: } \delta 10.6–10.0\) (broad, 1 H), 7.4 (s, 5 H), 6.8 (s, 1 H), 5.3 (s, 2 H), 4.4 (q, J 7 Hz, 2 H), 1.35 (t, J 7 Hz, 3 H). IR: 3280 (s), 3030 (w), 2975 (w), 1735 (s), 1570 (s) cm\(^{-1}\).

A stream of hydrogen was passed through a solution of (EZ)-ethyl \(\alpha\)-hydroxyiminoo-\(\alpha\)-(3-benzoxlyisoxazol-5-yl) acetate (100 mg, 0.34 mmol) and acetyl chloride (100 \(\mu\)l; ca. 1.5 mmol) in ethanol (10 ml) containing Pd-on-C (40 mg, 10%) for 3 h. After filtration of the reaction mixture through Celite and evaporation of the filtrate, recrystallization (ethanol–ether) gave 9 (60 mg, 78%), m.p. 184–186°C (decomp.). Anal. C\(_8\)H\(_9\)ClIN\(_2\).O\(_2\): C, H, Cl, Found: C 36.12, N 11.93; Calc., C 37.75, N 12.63. \(\text{\(^1\)H NMR (D}_2\text{O): } \delta 6.35\) (s, 1 H), 5.55 (s, ca. 1/4 H, partially exchanged), 4.3 (q, J 7 Hz, 2 H), 1.25 (t, J 7 Hz, 3 H). IR: 3300–2400 (m–s, several bands), 2050 (w), 1745 (s), 1625 (s), 1535 (s) cm\(^{-1}\).

3-Ethoxy-4,5-dimethylisoxazole (11) and 2-ethyl-4,5-di-methylisoxazolin-3-one (12). To a solution of 10 (7.7 g, 68 mmol) in acetone (300 ml) was added K\(_2\)CO\(_3\) (18.8 g, 136 mmol), and the mixture was stirred at 60°C for 1 h. Ethyl bromide (10.4 ml, 136 mmol) was slowly added and the reaction mixture was stirred overnight at 60°C. After cooling and filtration of the reaction mixture and evaporation of the filtrate, column chromatography (dichloromethane) gave 11 (4.7 g, 48%) as a colourless oil. Anal. C\(_7\)H\(_9\)NO\(_2\): C, H, N. \(\text{\(^1\)H NMR: } \delta 4.1\) (q, J 7 Hz, 2 H), 2.2 (s, 3 H), 1.75 (s, 3 H), 1.4 (t, J 7 Hz, 3 H). IR: 2980 (m), 2930 (m), 1665 (m), 1515 (s), 1470 (s) cm\(^{-1}\). Further elution (dichloromethane–ethyl acetate 3:1) gave 12 (2.4 g, 25%) as a yellow oil. Anal. C\(_7\)H\(_9\)NO\(_2\): C, H, N; Found: C 58.99; Calc., C 59.55. \(\text{\(^1\)H NMR: } \delta 3.6\) (q, J 7 Hz, 2 H), 2.1 (s, 3 H), 1.65 (s, 3 H), 1.2 (t, J 7 Hz, 3 H). IR: 2980 (m), 2930 (m), 1660 (s, broad), 1440 (s), 1415 (s) cm\(^{-1}\).

Ethyl 2-acetamido-2-ethoxyfarnonyl-3-(3-ethoxy-4-methyl-isoxazol-5-yl)propionate (13) and ethyl 2-acetamido-2-ethoxyfarnonyl-3-(3-ethoxy-5-methylisoxazol-4-yl)propionate (14). A mixture of 11 (1.3 g, 9.2 mmol), N-bromosuccinimide (5 mmol), and pyridine (5 mL) was stirred at room temperature for 20 min, and the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic layers were dried over Na\(_2\)SO\(_4\) and evaporated to dryness. The residue was recrystallized from ethanol to give 13 (0.7 g, 86%) and 14 (0.5 g, 85%) as colorless oils.
cinimide (NBS) (1.64 g, 9.2 mmol) and benzoyl peroxide (40 mg) in carbon tetrachloride was refluxed for 3 h. The NBS and benzoyl peroxide were added in four equal portions at intervals of 45 min. After being cooled and filtered, the reaction mixture was evaporated to give an oil, which was dissolved in ethanol (5 ml) and added to a solution of Na (212 mg, 9.2 mmol) and AAME (2 g, 9.2 mmol) in ethanol (15 ml). The reaction was refluxed for 4 h, cooled, filtered and evaporated. Water (25 ml) was added and the mixture was extracted with dichloromethane (3×75 ml). The combined organic phases were washed with ice-cold 1 M NaOH (50 ml), dried (MgSO4) and evaporated and the residue was subjected to column chromatography (dichloromethane containing 10–50% ethyl acetate), which gave 13 (23 mg, 0.7%) and 14 (436 mg, 13.3%) both after recrystallization (ethyl acetate–light petroleum).

13: m.p. 132–132.5°C. Anal. C16H16N2O2; H, N; Found, C 52.46; Calc., C 53.92. 1H NMR: δ 6.5 (s, 1 H), 4.15 (q, J 7 Hz, 2×2 H), 4.1 (q, J 7 Hz, 2 H), 3.6 (s, 2 H), 1.95 (s, 3 H), 1.7 (s, 3 H), 1.2 (t, J 7 Hz, 3×3 H). IR: 3420 (m, broad), 3250 (s), 2985 (s), 2935 (m), 1750 (s), 1640 (s), 1515 (s) cm⁻¹.

14: m.p. 125.5–126.0°C. Anal. C16H16N2O2; C, H, N. 1H NMR: δ 6.5 (s, 1 H), 4.15 (3×3 overlapping, J 7 Hz, 3×2 H), 3.25 (s, 2 H), 2.15 (s, 3 H), 1.95 (s, 3 H), 1.25 (t, J 7 Hz, 3×3 H), 1.72 (s, 3 H), 1.56 (s, 3 H), 1.25 (t, J 7 Hz, 3×3 H). IR: 3420 (m, broad), 3250 (s), 2985 (s), 2935 (m), 1740 (s), 1640 (s), 1515 (s) cm⁻¹.

(RS)-2-Amino-3-(3-ethoxy-5-methylisoxazol-4-yl)propionic acid hydrochloride (15). A solution of 14 (200 mg, 0.56 mmol) and 1 M HCl (5 ml) was refluxed for 20 h. The reaction mixture was extracted with ether (2×10 ml) and the aqueous phase was evaporated. Recrystallization (ethanol–water) of the residue gave 15 (90 mg, 64%), m.p. 199.5–200.5°C (decomp.). Anal. C16H16ClIN2O2; C, H, N; Cl; Found, C 42.65; Calc., C 43.40. 1H NMR (D2O): 6.4.8 (q, J 7 Hz, 2 H + 1 H), 2.9 (d, J 6 Hz, 2 H), 2.3 (s, 3 H), 1.35 (t, J 7 Hz, 3 H). IR: 3420 (m, broad), 3300–2400 (m–s, several bands), 1725 (s), 1640 (s), 1590 (s) cm⁻¹.

(RS)-Ethyl 2-amino-3-(3-ethoxy-5-methylisoxazol-4-yl)propionate hydrochloride (16). Compound 15 (105 mg, 0.42 mmol) was refluxed for 20 h in ethanol (10 ml) containing acetyl chloride (2 ml, ca. 25 mmol). The reaction mixture was evaporated, treated with 2 M ice-cold Na2CO3 (10 ml) and extracted with ether (3×10 ml). The combined organic phases were dried (K2CO3) and evaporated and the residue was dissolved in ethanol, to which acetyl chloride (2 ml) had been added and re-evaporated. The residue was recrystallized (ethanol–ether), which gave 16 (83 mg, 71%), m.p. 199–200°C (decomp.). Anal. C18H18ClIN2O2; C, H, N; Found, Cl 13.21; Calc., Cl 12.59. 1H NMR (D2O): 6.4.2 (q, J 7 Hz, 2×2 H + 1 H), 2.9 (d, J 6 Hz, 2 H), 2.25 (s, 3 H), 1.25 (t, J 7 Hz, 2×3 H). IR: 3450 (m), 2985 (s), 2850 (s), 2720 (m), 2640 (m), 1735 (s), 1645 (s) cm⁻¹.

Ethyl 2-acetamido-2-ethoxycarbonyl-3-(2-ethyl-5-methylisoxazol-4-yl)propanoate (20). A mixture of 14 (200 mg, 0.56 mmol) and a solution of HBr in glacial acetic acid (10 ml, 33%) was refluxed for 1 h and evaporated. After re-evaporation from water, recrystallization (ethyl acetate–light petroleum) gave 20 (127 mg, 69%), m.p. 155.5–156.5°C. Anal. C18H18N2O2; H; Found, C 49.70, N 8.08; Calc., C 51.21, N 8.53. 1H NMR: 6.8.35 (s, 1 H), 6.6 (s, 1 H), 4.15 (q, J 7 Hz, 2×2 H), 3.3 (s, 2 H), 2.1 (s, 3 H), 1.95 (s, 3 H), 1.25 (t, J 7 Hz, 2×3 H). IR: 3320 (m, broad), 2980 (m), 2930 (m), 1740 (s), 1650 (s), 1510 (s) cm⁻¹.

(RS)-2-Amino-3-(2-ethyl-5-methyl-3-oxoisoxazol-4-yl) propionic acid zwitterion (21). A mixture of 19 (300 mg,
0.84 mmol) and 1 M HCl (30 ml) was refluxed for 16 h. After evaporation and re-evaporation from water, the residue was dissolved in water (2 ml) and loaded onto a basic ion-exchange column (IRA-400). Elution with 1 M acetic acid gave, after recrystallization (water–ethanol), 21 (98 mg, 54%), m.p. 211.5–212°C (decomp.). Anal. C_{9}H_{8}N_{2}O_{4}: H, N: Found, C 49.42; Calc., C 50.46. 1H NMR (90 MHz, D_{2}O): δ 3.92 (q, J 7.0 Hz, 2 H), 3.86 (t, J 5.5 Hz, 1 H), 2.81 (d, J 5.5 Hz, 2 H), 2.21 (s, 3 H), 1.24 (t, J 7.0 Hz, 3 H). IR: 3400 (w, broad), 3300–2300 (m–s, several bands), 1640 (s), 1610 (s), 1435 (s) cm⁻¹.

(RS)-Ethyl 2-amino-3-(2-ethyl-5-methyl-3-oxoisoxazolin-4-yl)propanoate · fumaric acid (22). A solution of 21 (50 mg, 0.23 mmol) in ethanol (5 ml) and acetyl chloride (1 ml, ca. 12 mmol) was refluxed for 24 h. The reaction mixture was evaporated, treated with 3 M NaHCO₃ (5 ml) and extracted with ether (3×10 ml). The combined organic phases were dried (MgSO₄) and evaporated. The residue was dissolved in ether (ca. 2 ml) and a solution of fumaric acid (27 mg, 0.23 mmol) in isopropyl alcohol (500 µl) was added. The precipitated product was recrystallized (ethanol–ether) to give 22 (41 mg, 49%), m.p. 147–147.5°C. Anal. C_{9}H_{8}N_{2}O_{4} · C_{2}H_{4}O_{2}: C, H, N. 1H NMR (90 MHz, D_{2}O): δ 6.60 (s, 2 H), 4.30 (t, J 6.4 Hz, 1 H), 4.19 (q, J 7.0 Hz, 2 H), 3.90 (q, J 7.3 Hz, 2 H), 2.90 (d, J 6.4 Hz, 2 H), 2.21 (s, 3 H), 1.21 (t, J 7.0 Hz, 3 H), 1.17 (t, J 7.3 Hz, 3 H). IR: 3300–2300 (m–s, several bands), 1745 (s), 1640 (s), 1610 (s), 1560 (s), 1510 (s) cm⁻¹.

Microelectrophoretic studies. Experiments were performed on lumbar dorsal horn interneurones or Renshaw cells of cats anaesthetized with pentobarbitone sodium (35 mg kg⁻¹ intraperitoneally initially, supplemented intermittently when required). Extracellular action potentials were recorded by means of the central barrel of seven-barrel micropipettes, which contained 3.6 M NaCl. The compounds were administered electrophoretically from the outer barrels of the micropipettes, which contained aqueous solutions of NMDA (0.05 M in 0.15 M NaCl, pH 7.6), QUIS (0.005 M in 0.15 M NaCl, pH 7.5), KA (0.005 M in 0.15 M NaCl, pH 7.5) and the test compounds 0.1 M, pH 3–3.4, ejected as cations). The excitatory amino acids were administered for periods of time sufficient to obtain maximal effects at the particular rate of ejection.

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References


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