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Experimental Section

Material and Methods. Ethyl 2-chloro-2-(hydroxyimino)acetate[1] and racemic 3,4-dehydroproline[2] were prepared according to literature procedures. The synthesis of the ester moiety and the protection of the secondary amine of racemic 3,4-dehydroproline were accomplished along standard methodologies. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker AC-E 300 (300 Mhz) spectrometer in toluene d_8 at 80 °C or as $CDCl_3$ (or CF_3COOD) solution at 20 °C; assignments by a combination of 1D and 2D COSY;[3] chemical shifts (δ) are expressed in ppm and coupling constants (J) in hertz. TLC were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution. Melting points were determined on a Büchi apparatus and are uncorrected. Microanalyses of new compounds agreed with theoretical value ± 0.3%.

1,3-Dipolar cycloaddition of ethoxycarbonylformonitrile oxide to (±)-3,4-dehydroproline. To a solution of N-BOC 3,4-dehydroproline methyl ester (3.1 g, 13.7 mmol) in ethyl acetate (50 mL) was added ethyl chlorooximinoacetate (6.2 g, 41.1 mmol) and $NaHCO_3$ (15 g). The mixture was vigorously stirred for 3 days, than other 3 equivalents (6.2 g, 41.1 mmol) of ethyl chlorooximinoacetate were added and the mixture was stirred for additional 3 days. The progress of the reaction was monitored by TLC (petroleum ether/ethyl acetate 7:3). Water was added to the reaction mixture and the organic layer was separated and dried over anhydrous sodium sulfate. The crude material, obtained after evaporation of the solvent, was chromatographed on silica gel (eluant: petroleum ether/ethyl acetate 7:3) to give 1.30 g of unreacted olefin, 0.90 g of **7** as a yellowish solid and 1.71 g of a mixture of cycloadducts **8** and **9**. Overall yield: 56%.

Compound **7** crystallized from diisopropyl ether as colorless prisms, mp 78-80 °C; R_F (petroleum ether/ ethyl acetate 7:3) 0.30; ^{1}H NMR (C_7D_8) 1.00 (t, 3, CH_2*CH_3*; J = 7.1); 1.35 (s, 9, t.Bu); 3.37 (s, 3, OCH_3); 3.47 (dddd, 1, H-4; J = 0.8, 2.1, 7.6 and 9.5); 3.63 (dd, 1, H-8a; J = 8.0 and 11.6); 3.98 (m, 2, *CH_2*CH_3); 4.09 (bd, 1, H-8b; J = 11.6); 4.80 (bs, 1, H-6); 4.88 (bd, 1, H-5; J = 9.5).

Mixture **8** and **9**: R_F (petroleum ether/ethyl acetate 7:3) 0.23.

Synthesis of derivatives 10 and 11. The mixture of **8** and **9** (1.71 g, 5.0 mmol) was treated with a 30% dichloromethane solution of trifluoroacetic acid (12.7 mL) at 0°C. The reaction mixture was stirred at room temperature until disappearance of the starting material (2 h). The volatiles were removed under vacuum and the residue was treated with a 10% potassium carbonate solution (30 mL) and extracted with ethyl acetate (3 x 10 mL). The pooled organic extracts were dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel column chromatography to give 0.33 g of **10** and 0.66 g of **11** as yellowish oils in 82% overall yield.

Compound **10**: R_F (petroleum ether/ ethyl acetate 1:4) 0.15; ^{1}H NMR ($CDCl_3$) 1.36 (t, 3, CH_2CH_3; J = 6.9); 2.10 (bs, 1, NH); 3.08 (dd, 1, H-8a; J = 7.3, and 12.9); 3.49 (bd, 1, H-8b; J = 12.9); 3.81 (s, 3, OCH_3); 3.87 (d, 1, H-6; J = 4.7); 3.97 (m, 2, CH_2CH_3); 4.07 (dd, 1, H-4; J = 7.3 and 7.5); 5.48 (dd, 1, H-5; J = 4.7 and 7.5).

Compound **11**: R_F (petroleum ether/ ethyl acetate 1:4) 0.33; ^{1}H NMR ($CDCl_3$) 1.21 (t, 3, CH_2CH_3, J = 6.9); 2.35 (bs, 1, NH); 3.01 (dd, 1; H-6a; J = 4.1, and 13.1); 3.26 (bd, 1, H-6b; J = 13.1); 3.60 (s, 3, OCH_3); 3.98 (bs, 1, H-8); 4.16 (m, 3, CH_2CH_3 and H-4); 5.21 (bdd, 1, H-5; J = 4.1 and 8.9).

Synthesis of 9. To a solution of **11** (0.66 g, 2.73 mmol) in dichloromethane (6.5 mL) was added triethylamine (0.57 mL, 4.1 mmol) at 0 °C followed by a solution of BOC_2O (0.895 g, 4.1 mmol) in dichloromethane (6.5 mL). The reaction mixture was magnetically stirred at room temperature until disappearance of the starting material then treated with 3N HCl (5 mL) and washed with water. The organic layer was separated, dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel column chromatography (eluant: petroleum ether/ ethyl acetate 7:3) to give 0.90 g of **9** as a colorless viscous oil in 96% yield.

The same treatment carried out on amine **10** gave pure cycloadduct **8** in comparable yield.

Compound **8**: ^{1}H NMR (C_7D_8) 0.98 (t, 3, CH_2CH_3; J = 6.9); 1.37 (s, 9, t.Bu); 3.46 (s, 3, OCH_3); 3.48 (ddd, 1; H-4; J = 5.2, 9.0 and 10.5); 3.70 (dd, 1, H-8a; J = 9.0 and 11.5); 3.82 (dd, 1, H-8b; J = 5.2 and 11.5); 3.95 (m, 2, CH_2CH_3); 4.48 (d, 1, H-6; J = 8.0); 4.80 (dd, 1, H-5; J = 8.0 and 10.5).

Compound **9**: ^{1}H NMR (C_7D_8) 1.02 (t, 3, CH_2*CH_3*; J = 6.9); 1.35 (s, 9, t.Bu); 3.39 (s, 3, OCH_3); 3.56 (dd, 1; H-6a; J = 6.0, and 12.6); 3.68 (bd, 1, H-4; J = 10.0); 3.84 (dd, 1, H-6b; J = 0.7 and 12.6); 3.97 (m, 2, *CH_2*CH_3); 4.69 (ddd, 1, H-5; J = 0.7, 6.0 and 10.0); 4.90 (bs, 1, H-8).

Synthesis of 3a,5,6,6a-Tetrahydro-4H-pyrrolo[3,4:d]isoxazole-3,4-dicarboxylic acid (±)-5. To a solution of **9** (0.90 g, 2.63 mmol) in methanol (7.9 mL) was added a 1N NaOH solution (7.9 mL) and the mixture was stirred at room temperature for 12 h. Methanol was evaporated under vacuum and the aqueous layer was extracted with ethyl acetate (2 x 5 mL), acidified with 3N HCl and extracted with ethyl acetate (3 x 5 mL). The pooled organic extracts were dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was taken up with a 30% dichloromethane solution of trifluoroacetic acid (5.6 mL) at 0 °C. The reaction mixture was stirred at room temperature until disappearance of the starting material (2 h). The volatiles were removed under vacuum and the residue was taken up with methanol and filtered under vacuum to give 0.330 g (40 % overall yield) of **5** as colorless prisms.

Compound **5**: R_F (butanol/H_2O/acetic acid 60:25:15) 0.11; mp 190-222°C dec; ^{1}H NMR (CF_3COOD): 4.03 (dd, 1; H-6a; J = 4.3, and 13.7); 4.20 (bd, 1, H-6b; J = 13.7); 4.88 (bd, 1, H-4; J = 9.7); 5.26 (bs, 1, H-8); 5.74 (bdd, 1, H-5; J = 4.3 and 9.7); ^{13}C NMR (CF_3COOD): 56.3 (C-4); 56.7 (C-6); 66.6 (C-8); 89.5 (C-5); 152.6 (C-3); 171.9 (*C*OOH).

Synthesis of 3a,5,6,6a-Tetrahydro-4H-pyrrolo[3,4:d]isoxazole-3,6-dicarboxylic acid (±)-6. The above-reported treatment carried out both on cycloadducts **7** and **8** gave final derivative **6** in 47% yield.

Compound **6**: H: R_F (butanol/H_2O/acetic acid 60:25:15) 0.11; mp 155-160 °C dec.; ^{1}H NMR (CF_3COOD): 4.36 (dd, 1; H-8a; J = 7.8, and 12.5); 4.62 (bd, 1, H-8b; J = 12.5); 4.93 (bdd, 1, H-4; J = 7.8 and 9.7); 5.40 (bs, 1, H-6); 6.29 (bd, 1, H-5; J = 9.7); ^{13}C NMR (CF_3COOD): 52.9 (C-4); 53.2 (C-8); 70.8 (C-6); 91.6 (C-5); 153.7 (C-3); 170.6 (*C*OOH).

Biological testing

Receptor binding. Affinity for NMDA, AMPA and kainic acid receptors were determined using the ligands [^{3}H]CPP, [^{3}H]AMPA and [^{3}H]kainic acid, respectively. The membrane preparations used in all the receptor-binding experiments were prepared according to the method of Ransom and Stec.[4]

***In vitro* electrophysiology.** A rat cortical slice preparation for determination of EAA-evoked depolarizations described by Harrison and Simmonds[5] 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 DriRef-5SH (World Precision Instruments) electrode. The cortex and corpus callosum parts were constantly superfused with a Mg^{++} free (and Ca^{++} free for the corpus callosum) oxygenated Krebs buffer at room temperature. The test compounds were added to the cortex superfusion medium and the potential difference between the electrodes recorded on a chart recorder. Applications of agonists were made for 90 s at each concentration tested, typically at 15 min. intervals. The sensitivity of agonist effects to CPP (10 μM) or NBQX (5 or 20 μM) was tested at agonist concentrations producing at least 50 % of maximal responses. In experiments designed to detect antagonist effects the potential antagonist were applied alone for 90 s followed by co-application of agonists (NMDA, AMPA or kainic acid) and the potential antagonist for another 90 s.

Metabotropic testing. Three metabotropic subtypes $mGluR_{1\alpha}$, $mGluR_2$ or $mGluR_{4a}$ were expressed in chinese hamster ovary cell lines and used as representatives for group I, II and III metabotropic receptors.

***In vivo* pharmacology.** Male DBA/2 mice (12-22 g; 4-6 weeks old) were used. The animals were housed in groups of 10 in PVC cages (260 x 440 mm long x 120 mm high) with a temperature of 20-22°C and a relative humidity of 57 ± 2%; a 12 h light/dark cycle was applied (light on in the interval 07:00 a.m. to 07:00 p.m.). Food and water were available ad libitum.

Apparatus: A 50 μL Hamilton microsyringe was adapted for constant depth icv injections using a Butterfly-25 short winged needle infusion set (Abbott, Rome, Italy). A needle of 0.5 mm external diameter was inserted into a polyethylene cannula leaving 3 mm of the needle exposed. A new infusion set was employed for each compound and for the different dosages studied.

Procedure: For icv injection, groups of at least 10 mice were anesthetized with diethyl ether and the drug was injected as a 67 mM phosphate buffer solution. The following amounts were used: KAIN 0.01-5.0 nmol, (RS)-AMPA 0.25-15.0 nmol, CIP-A 0.01-10.0 nmol, CIP-B 5-200 nmol. The injection site was 1 mm anterior to bregma, 1 mm lateral to the midline and 3 mm below the surface of the cranium. The animals were than observed for 60 min. and the induced seizures detected and characterized.

The anticonvulsant effects of CPP (0.32 μmol/mouse), ip administered 60 min. before the icv injection of KAIN, (RS)-AMPA, CIP-A, or CIP-B, were evaluated. The anticonvulsant activity of GYKI 52466 (1.6 μmol/mouse ip, 15 min. in advance) and NBQX (1.4 μmol/mouse ip, 30 min. in advance) was also evaluated. The incidence of a clonic and tonic seizure response for 50% of mice (CD_{50} values) with 95% confidence limits was estimated by using the method of Litchfield and Wilcoxon.[6] The relative potency ratios are the ratio between the CD_{50} value of the drug in the presence of an antagonist i.e. CPP, GYKI 52466, or NBQX versus its CD_{50} value.

Statistical analysis. The data of the convulsant tests were statistically analyzed according to the method of Litchfield and Wilcoxon. In Table II the 95% confidence limits of the CD_{50} values are shown.

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