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The first AMPA receptor negative modulators based on the tetrahydroquinazoline scaffold

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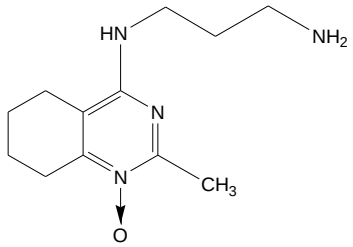
Experimental

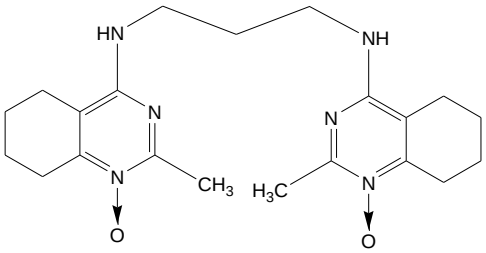
General. ^{1}H and ^{13}C NMR spectra were recorded on a spectrometer Agilent 400MR (400.0 MHz for ^{1}H and 100.6 MHz for ^{13}C) at room temperature; chemical shifts δ were referenced to the solvent for ^{1}H ($CDCl_3$, δ = 7.26 ppm; CD_3OD, δ = 3.31 ppm) and ^{13}C ($CDCl_3$, δ = 77.1 ppm; CD_3OD, δ = 49.0 ppm). When necessary, assignments of signals in NMR spectra were made using 2D techniques. Accurate mass measurements (HRMS) were measured on Jeol GCMate II mass spectrometer (70 eV). Analytical thin layer chromatography was carried out with silica gel plates (supported on aluminum); the detection was done by UV lamp (254 nm). Column chromatography was performed on silica gel (0.015-0.04 mm). Pyrimidine *N*-oxides **4a,b** were obtained *via* literature procedure [S1]. All other starting materials were commercially available. All reagents except commercial ones of satisfactory quality were purified by literature procedures prior to use.

***N*-(2-Methyl-1-oxido-5,6,7,8-tetrahydroquinazolin-4-yl)propane-1,3-diamine (5b)**



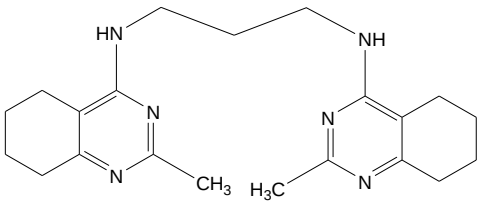
Yield 93 mg (79%); colourless oil; R_f = 0.1 (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.71–1.87 (m, 6H, $2CH_2$, cy-Hex + CH_2, diamine), 2.31 (br t, 2H, $^{3}J_{HH}$ 5.9, CH_2, cy-Hex), 2.63 (s, 3H, CH_3), 2.92 (br t, 2H, $^{3}J_{HH}$ 5.9, CH_2, cyclohexane), 2.87–2.95 (m, 2H, C**H**$_2$NH$_2$), 3.60 (dt, 2H, $^{3}J_{HH}$ 5.9, $^{3}J_{HH}$ 5.5, C**H**$_2$NH), 6.35 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.2 (CH_3), 20.9 (CH_2, cyclohexane), 21.1 (CH_2, cyclohexane), 22.3 (CH_2, cy-Hex), 24.5 (CH_2, cyclohexane), 31.2 (CH_2), 40.9 ($2CH_2N$), 111.8 (C4a), 152.1 (C4), 152.9 (C8a), 154.6 (C2); HRMS (ESI^+, 70 eV, m/z): calcd. for $C_{12}H_{20}N_4O$ [M+H]: 237.1710, found: 237.1735.

***N,N'*-Bis(2-methyl-1-oxido-5,6,7,8-tetrahydroquinazolin-4-yl)propane-1,3-diamine (6b)**



Yield 92 mg (38%); yellowish oil; R_f = 0.1 (MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.74–1.88 (m, 8H, $4CH_2$, cy-Hex), 1.94 (quint, 2H, $^{3}J_{HH}$ 6.8, CH_2, diamine), 2.37 (br t, 4H, $^{3}J_{HH}$ 5.8, $2CH_2$, cy-Hex), 2.51 (s, 6H, $2CH_3$), 2.81 (br t, 4H, $^{3}J_{HH}$ 5.7, $4CH_2$, cyclohexane), 3.57 (t, 4H, $^{3}J_{HH}$ 6.8, 2C**H**$_2$NH); ^{13}C NMR (100 MHz, CD_3OD) δ: 18.9 ($2CH_3$), 20.3 ($2CH_2$, cyclohexane), 20.5 ($2CH_2$, cyclohexane), 21.8 ($2CH_2$, cyclohexane), 24.0 ($2CH_2$, cyclohexane), 28.3 (CH_2), 38.2 ($2CH_2N$), 112.8 (2C4a), 152.4 (2C8a), 155.1 (2C4), 155.6 (2C2); HRMS (ESI^+, 70 eV, m/z): calcd. for $C_{21}H_{30}N_6O_2$ [M+H]: 399.2494, found: 399.2503.

***N,N'*-Bis(2-methyl-5,6,7,8-tetrahydroquinazolin-4-yl)propane-1,3-diamine (7b)**



Yield 79 mg (72%); white crystals; m.p. 148°C; ^{1}H NMR (400 MHz, CD_3OD) δ: 1.75–1.85 (m, 8H, $4CH_2$, cy-Hex), 1.89 (quint, 2H, $^{3}J_{HH}$ 6.5, CH_2, diamine), 2.29 (br t, 4H, $^{3}J_{HH}$ 5.4, $2CH_2$, cy-Hex), 2.34 (s, 6H, $2CH_3$), 2.58 (br t, 4H, $^{3}J_{HH}$ 5.5, $4CH_2$, cy-Hex), 3.55 (t, 4H, $^{3}J_{HH}$ 6.5, C**H**$_2$NH); ^{13}C NMR (100 MHz, CD_3OD) δ: 21.9 ($2CH_3$), 22.2 ($2CH_2$, cyclohexane), 22.4 ($2CH_2$, cy-Hex), 24.1 ($2CH_2$, cyclohexane), 28.3 (CH_2, diamine), 30.9 ($2CH_2$, cyclohexane), 37.9 ($2CH_2N$), 112.8 (2C4a), 159.9 (2C8a), 161.3 (2C4), 163.6 (2C2); HRMS (ESI^+, 70 eV, m/z): calcd. for $C_{21}H_{30}N_6$ [M+H]: 367.2605, found: 367.2598.

Electrophysiological experiments

All compounds were dissolved in a mixture of dimethyl sulfoxide (90%) with ethanol (10%) to make 10 mM solutions, which were then diluted into extracellular solution to attain the final concentrations desired.

Freshly isolated neurons from 9-to-16-day-old rat pups were used for the patch-clamp technique; AMPA-receptor-mediated currents were studied in Purkinje neurons of the cerebellum, as described elsewhere [S2]. Briefly, for cell isolation, a selected region of the brain was cut into slices 0.4-0.6 mm wide followed by incubation in buffer (150 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid), 10 mM glucose, pH 7.4) for one hour. The slices were transferred to fresh buffer solution with 2 mg ml^{-1} of Protease (Sigma-Aldrich, St. Louis, MO, US) and 1 mg ml^{-1} of Collagenase (Sigma-Aldrich, St. Louis, MO, US) and incubated for 30 – 60 min. Then, the slices were transferred to the fresh buffer solution and incubated about 20 min. The slices were incubated at 34°C and pre-gassed with 100% O_2. Finally, the slices were mechanically dissociated into individual cells by means of Pasteur pipettes. The composition of extracellular saline was 150 mM NaCl, 5 mM KCl, 2.6 mM $CaCl_2$, 2.0 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, pH 7.32. The composition of the intracellular saline was 140 mM KCl, 10 mM HEPES, 5 mM EGTA [ethylene glycol bis(2-aminoethyl) ether]-*N*,*N*,*N′*,*N′*-tetraacetic acid, 1 mM $MgCl_2$, 1 mM ATP. The transmembrane currents were registered in the configuration of the ‘whole cell’ using the electrophysiological EPC-9 set-up (HEKA, Lambrecht, Germany); data were processed with HEKA software (Pulsefit/HEKA, Lambrecht, Germany). Tested compounds were exposed to neurons by the fast perfusion method [S3].

The effects of tested compounds on the stimulation of AMPA receptors were investigated on isolated Purkinje neurons using partial receptor agonist kainic acid (KA), which induces AMPA-receptor mediated currents while evoking relatively low receptor desensitization. Baseline recordings of AMPA-receptor mediated transmembrane currents were carried out three times after each application of KA (20 μM) that were spaced from each other in this and any other applications during recordings by 2 min. Thereafter, the physiological solution in the recording chamber was replaced with increasing concentrations of test compounds. The application of each tested concentration was accompanied by a concomitant triple application of KA at above-indicated concentration. After each tested compound application, a 3-min wash-out with physiological solution was carried out and responses to three applications of KA were recorded

for a control. The next concentration was then applied followed by a wash-out session and triple application of KA. The mean of amplitude of the AMPA-mediated currents measured during all applications of KA was taken as a control value (100%), means of measurements of this parameter during applications were normalized to control for each concentration and expressed as a percentage. Each concentration was tested on 4-6 Purkinje neurons; five to eight concentrations in a range from 0.01 μM to 100 μM of each drug were tested.

Quantum Chemical Calculations

Quantum chemical calculations were performed using program system ORCA 4.0.1. [S4]. Geometry optimization was carried out on MP2 theory level with cc-pVTZ (correlation-consistent polarized valence-only triple-zeta) basis set.

References

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[S3] V. V. Grigoriev, L. N. Petrova, T. A. Ivanova, A. V. Gabreliyan and T. P. Serkova, *Bull. Exp. Biol. Med.*, 2009, **147**, 319.

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