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Ifenprodil-like NMDA receptor modulator based on the biphenyl scaffold

Nadezhda S. Temnyakova, Dmitry A. Vasilenko, Oleg I. Barygin, Mikhail Y. Dron, Elena B. Averina, Yuri K. Grishin, Vladimir V. Grigoriev, Vladimir A. Palyulin, Maxim V. Fedorov and Dmitry S. Karlov

Synthetic details

NMR spectra were recorded on spectrometer Agilent 400-MR (400.0 MHz for ^{1}H; 100.6 MHz for ^{13}C) at room temperature; chemical shifts (δ) were measured with reference to the solvents (^{1}H: $CDCl_3$ δ = 7.26 ppm, CD_3OD, δ = 3.31 ppm; ^{13}C: $CDCl_3$, δ = 77.16 ppm, CD_3OD, δ = 49.0 ppm). Chemical shifts (δ) are given in ppm; *J* values are given in Hz. Accurate mass measurements (HRMS) were performed on a Bruker micrOTOF II instrument using electrospray ionization (ESI). The measurements were done in a positive ion mode (interface capillary voltage 4500V) or in a negative ion mode (3200V). Analytical thin layer chromatography was carried out with Silufol silica gel plates (supported on aluminum); the detection was done by UV lamp (254 and 365nm) and chemical staining (5% aqueous solution of $KMnO_4$). Column chromatography was performed on silica gel (230–400 mesh, Merck).

All other starting materials were commercially available.

All reagents except commercial products of satisfactory quality were purified by literature procedures prior to use.

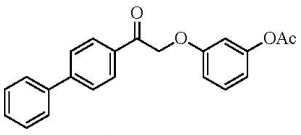
3-[2-(1,1'-Biphenyl-4-yl)-2-oxoethoxy]phenyl acetate. 3-Hydroxyphenyl acetate (1.28 g, 8.43 mmol) and anhydrous K_2CO_3 (1.17 g, 8.43 mmol) were stirred in dry DMF (8 ml) at room temperature for 30 min. Then 1-(1,1'-biphenyl]4-yl)-2-bromoethanone **2** (2.32 g, 8.43 mmol) in DMF (4 ml) was added and the resulting mixture was stirred at room temperature for 24 h (TLC monitoring). The mixture was quenched with cold water (15 ml) and extracted with CH_2Cl_2 (3×25 ml). The combined organic layers were washed with water (3×25 ml) and brine (2×25 ml), and dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo*, and the residue was purified by preparative column chromatography on silica gel (hexane/EtOAc, 8:1). Yield 1.8 g (62%); light yellow solid, mp 107-110 °C; R_f =0.48 (hexane/EtOAc, 8:1). ^{1}H NMR δ_H (400 MHz, $CDCl_3$): 2.28 (s, 3H, CH_3), 5.28 (s, 2H, CH_2), 6.71-6.78 (m, 2H, 2CH(Ar)), 6.82-6.88 (m, 1H, CH(Ar)), 7.26-7.32 (m, 1H, CH(Ar)), 7.39-7.45 (m, 1H, CH(Ar)), 7.45-7.52 (m, 2H, 2CH(Ar)), 7.61-7.66 (m, 2H, 2CH(Ar)), 7.69-7.75 (m, 2H, 2CH(Ar)), 8.04-8.10 (m, 2H, 2CH(Ar)); ^{13}C NMR δ_C(101 MHz, $CDCl_3$): 21.2 (CH_3), 71.0 (CH_2), 108.7 (CH(Ar)), 112.4 (CH(Ar)), 114.9 (CH(Ar)), 127.3 (2CH(Ar)), 127.5 (2CH(Ar)), 128.5 (CH(Ar)), 128.8 (2CH(Ar)), 129.0 (2CH(Ar)), 130.0 (CH(Ar)), 133.1 (C(Ar)), 139.6 (C(Ar)), 146.7 (C(Ar)), 151.6 (C(Ar)), 158.9 (C(Ar)), 169.4 (C=O), 193.8 (C=O). HRMS $[M + H]^+$: calcd. for $C_{22}H_{19}O_4$ 347.1278, found 347.1274.

1-(1,1'-Biphenyl-4-yl)-2-(3-hydroxyphenoxy)ethanone. To a solution of 3-[2-(1,1'-biphenyl-4-yl)-2-oxoethoxy]phenyl acetate (0.25 g, 0.72 mmol) in MeOH (16 ml), anhydrous K_2CO_3 (0.5g, 3.6 mmol) was added and the resulting mixture was stirred at room temperature for 6 h (TLC monitoring). Then the solvent was evaporated *in vacuo*, the residue was dissolved in CH_2Cl_2 (50 ml) and washed with water (20 ml). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography ($CHCl_3$/MeOH, 50/1). Yield 103 mg (47%); light yellow solid, mp 180-182 °C; R_f = 0.55 (CH_2Cl_2/MeOH, 20/1). ^{1}H NMR δ_H (400 MHz, CD_3OD + $CDCl_3$): 5.27 (s, 2H, CH_2), 6.41-6.47 (m, 3H, 3CH(Ar)), 7.04-7.10 (m, 1H, CH(Ar)), 7.35-7.40 (m, 1H, CH(Ar)), 7.41-7.48 (m, 2H, 2CH(Ar)), 7.57-7.63 (m, 2H, 2CH(Ar)), 7.67-7.73 (m, 2H, 2CH(Ar)), 8.01-8.07 (m, 2H, 2CH(Ar)); ^{13}C NMR δ_C(101 MHz, CD_3OD + $CDCl_3$): 70.9 (CH_2), 102.8 (CH(Ar)), 106.0 (CH(Ar)), 109.3 (CH(Ar)), 127.5 (2CH(Ar)), 127.7 (2CH(Ar)), 128.7 (CH(Ar)), 128.9 (2CH(Ar)), 129.3 (2CH(Ar)), 130.3 (CH(Ar)), 133.3 (C(Ar)), 139.8 (C(Ar)), 147.1 (C(Ar)), 158.4 (C(Ar)), 159.4 (C(Ar)), 195.2 (C=O). HRMS $[M + H]^+$: calcd. for $C_{20}H_{17}O_3$ 305.1172, found 305.1182.

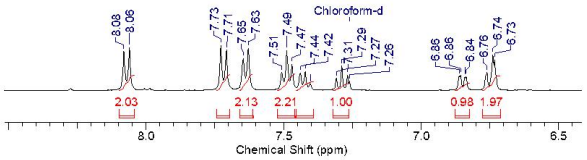
3-[2-(1,1'-Biphenyl-4-yl]-2-hydroxyethoxy)phenol (1). To a suspension of $LiAlH_4$ (0.03 g, 0.75 mmol) in dry diethyl ether (6 ml), a solution of 3-[2-(1,1'-biphenyl-4-yl)-2-oxoethoxy]phenyl acetate (0.13 g, 0.38 mmol) in a mixture of diethyl ether (9 ml) and THF (5 ml) was added dropwise at 0°C under argon. The resulting mixture was stirred at reflux for 2 h, then was cooled to 0 °C and quenched with water (20 ml), and then 10% H_2SO_4 was added to adjust pH value to 3. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Yield 0.07 g (79%); colorless solid, mp 138-139 °C; R_f = 0.19 (CH_2Cl_2/MeOH, 20:1). ^{1}H NMR δ_H (400 MHz, CD_3OD + $CDCl_3$): 4.00 (dd, 1H, CH_2, *J* 9.8, 8.3 Hz), 4.05 (dd, 1H, CH_2, *J* 9.8, 3.6 Hz), 5.06 (dd, 1H, CH, *J* 9.8, 3.6 Hz), 6.38-6.44 (m, 3H, 3CH(Ar)), 7.02-7.09 (m, 1H, CH(Ar)), 7.28-7.34 (m, 1H, CH(Ar)), 7.37-7.43 (m, 2H, 2CH(Ar)), 7.44-7.50 (m, 2H, 2CH(Ar)), 7.52-7.60 (m, 4H, 4CH(Ar)); ^{13}C NMR δ_C(101 MHz, CD_3OD): 71.4 (CH), 72.3 (CH_2), 101.4 (CH(Ar)), 105.1 (CH(Ar)), 107.4 (CH(Ar)), 126.16 (2CH(Ar)), 126.19 (2CH(Ar)), 126.22 (2CH(Ar)), 126.6 (CH(Ar)), 128.1 (2CH(Ar)), 129.2 (CH(Ar)), 139.8 (C(Ar)), 140.2 (C(Ar)), 140.3 (C(Ar)), 157.8 (C(Ar)), 159.7 (C(Ar)). HRMS $[M + K]^+$: calcd. for $C_{20}H_{18}O_3K$ 345.0888, found 345.0894.

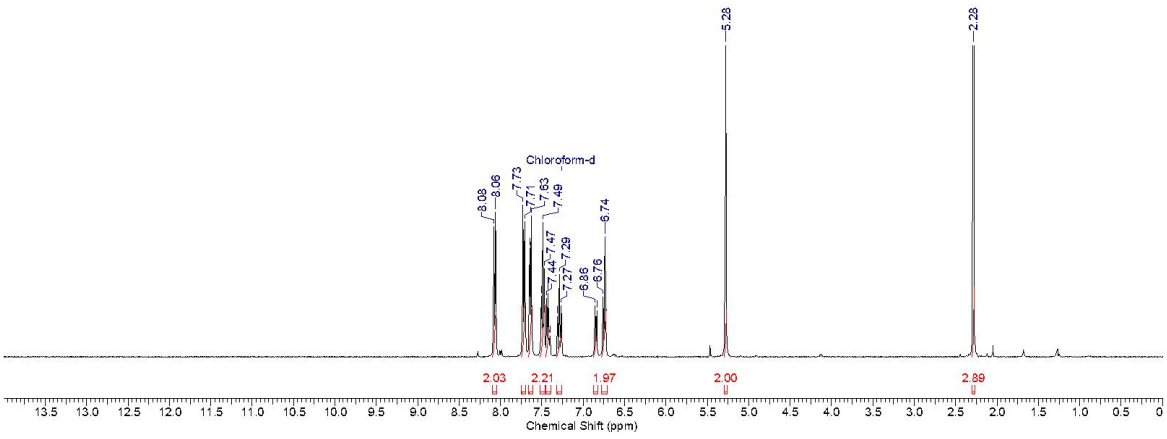
NMR Spectra

3-(2-([1,1'-biphenyl]-4-yl)-2-oxoethoxy)phenyl acetate (2)



^{1}H NMR





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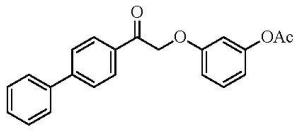
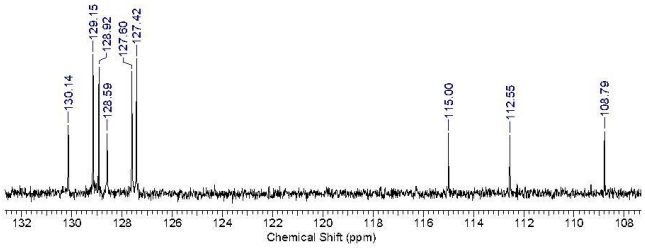
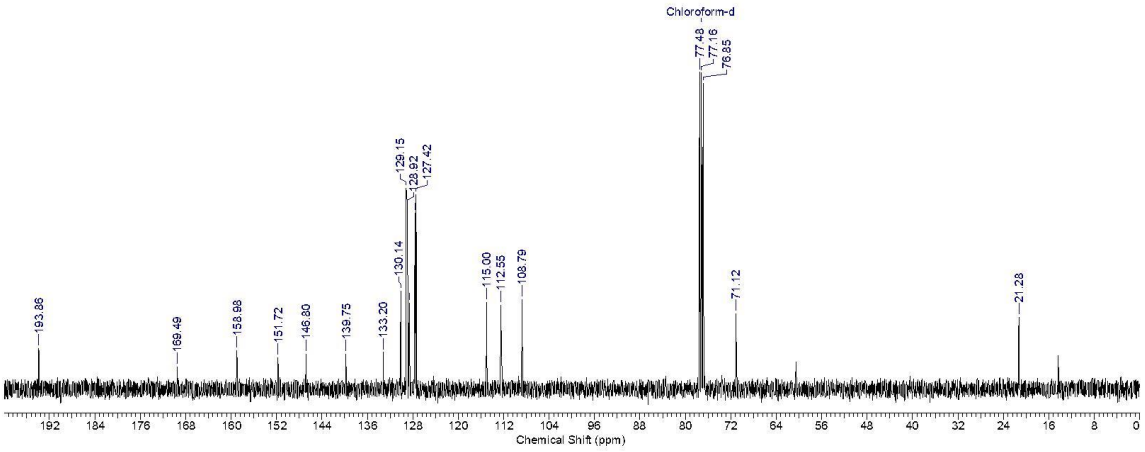
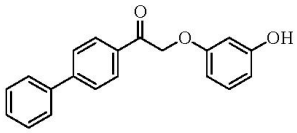


^{13}C NMR



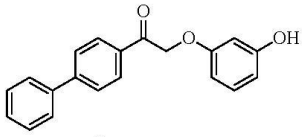


1-([1,1'-biphenyl]-4-yl)-2-(3-hydroxyphenoxy)ethanone (3)



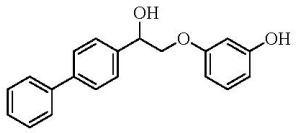
^{1}H NMR

1-([1,1'-biphenyl]-4-yl)-2-(3-hydroxyphenoxy)ethanone (3)

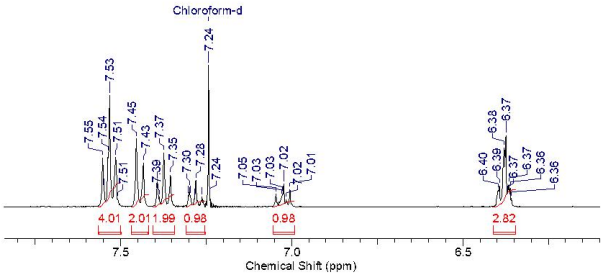


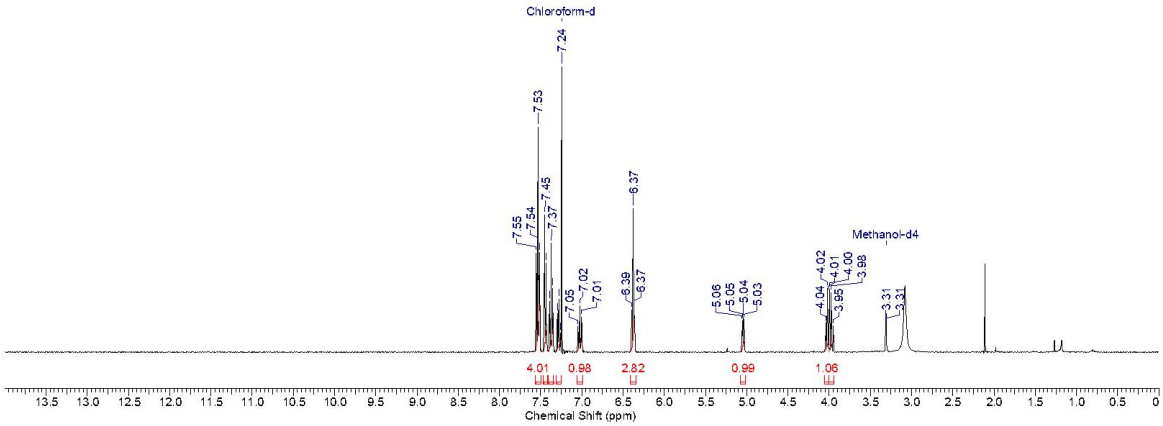
^{13}C NMR

3-(2-([1,1'-biphenyl]-4-yl)-2-hydroxyethoxy)phenol (4)



^{1}H NMR





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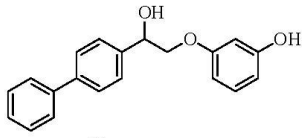
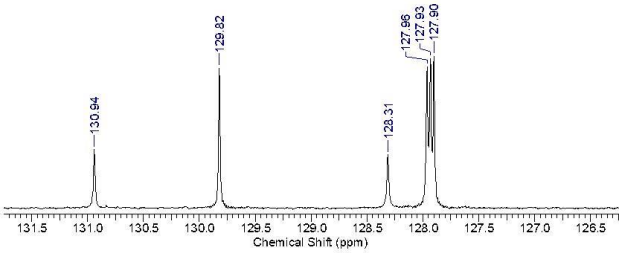
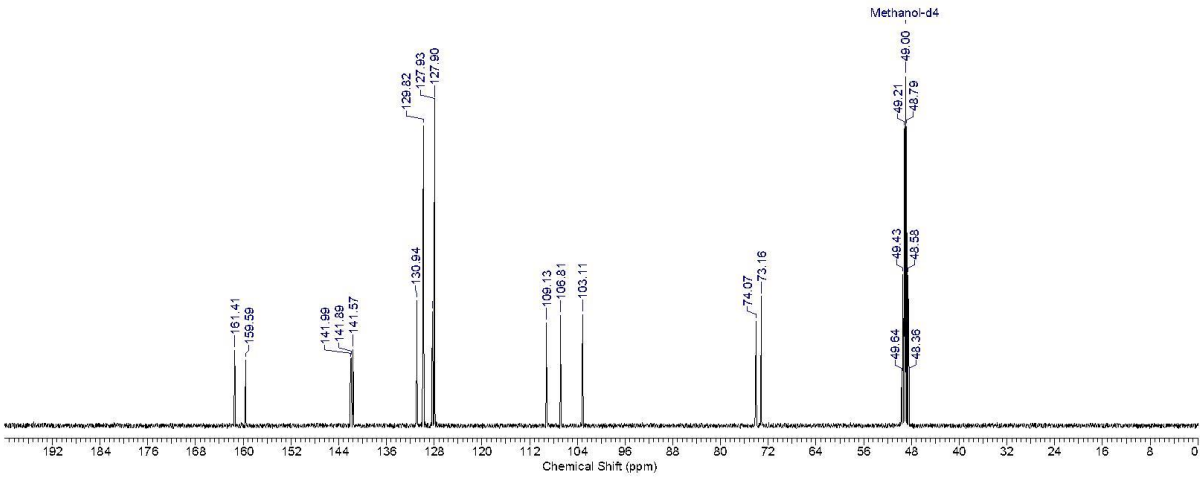


^{13}C NMR





Radioligand binding assay

The preparation of rat brain membranes was described previously [S1]. The hippocampal tissue was homogenized with a Potter homogenizer (Teflon pestle and glass vessel), using a tissue grinder at half maximal setting, in 20 vol of buffer No. 1 (5 mM HEPES / 4.5 mM Tris buffer, pH 7.6, sucrose 0.32 M). The homogenate is diluted with a buffer for study No. 2 (5 mM HEPES / 4.5 mM Tris buffer, pH 7.6) in a ratio of 1:50, then it was centrifuged at 1000 *g* for 10 min. The supernatant was collected and then centrifuged at 25000 *g* for 20 min. The precipitate is homogenized in buffer No. 2 at a ratio of 1:50 and centrifuged for 20 min at 8000 *g*. The precipitate is suspended in buffer No. 3 (5 mM HEPES / 4.5 mM Tris buffer, (pH 7.6), 5 mM Na_4EDTA), and the suspension is centrifuged again. This washing procedure is performed four times, and at the last wash, Na_4EDTA is excluded from the buffer composition. The final precipitate is re-suspended in 5 vol of buffer No. 2 and stored in liquid nitrogen. On the day of use the membrane fraction is thawed. The working solution (final volume 0.5 ml) contains 200 µl of buffer No. 2, 50 µl of [H^3]-ifenprodil (40 nM) with specific activity 179 Ci/mM and 250 µl of the membrane suspension. Non-specific binding is determined in the presence of 50 µL of unlabeled ifenprodil (10 µM). The mixture is incubated at room temperature for 2 hours. At the end of the incubation, the samples are filtered through GF/B glass fiber filters (Whatman), pre-moistened in 0.3% polyethyleneimine for 2 hours at 4 °C. Each tube is washed once with cold buffer No. 2, then the filters are washed three times with the same buffer volume. Filters are air-dried until completely dry and transferred to scintillation vials, into which scintillation fluid (5 ml) [prepared from diphenyloxazole (PPO, 4 g), diphenyloxazoylbenzene (POPOP, 0.2 g) and toluene (1 dm^{-3})] are added. Radioactivity is determined on a TriCarb2800 TR scintillation counter (PerkinElmer, Packard, USA) with a counting efficiency of about 65%. The study of the effect of the investigated compounds on the binding of [H^3]-ifenprodil with rat hippocampal membranes is carried out with the addition of 50 µl of the solution of the studied compounds to the incubation medium in the concentration range 10^{-8}-10^{-3} M. Each concentration was measured 3 times and the average value is reported.

Electrophysiological experiments

All experimental procedures were approved by Animal Care and Use Committee of the Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences. Outbred male Wistar rats of 13–18 d old and 25–35 g were obtained from the local (IEPHB) facility. Maximum efforts were made to minimize the number of animals used and to minimize discomfort. Rats were anesthetized with urethane and then decapitated. Brains were removed quickly and cooled to 2–4 C. Transverse hippocampal slices were prepared using a vibratome (Campden Instr.) and single neurons were freed from slices by vibrodissociation [S2]. All experiments were performed at room temperature. The whole-cell patch-clamp technique was used for recording membrane currents in response to applications of an agonist. The series resistance of about 20 MΩ was compensated by 70–80% and monitored during experiments. Currents were recorded using an EPC-8 amplifier (HEKA Electronics, Lambrecht, Germany), filtered at 5 kHz, sampled and stored on a personal computer. Drugs were applied using RSC-200 (BioLogic) perfusion system under computer control. The extracellular solution contained (in mM): NaCl 143, KCl 5, $CaCl_2$ 2.5, D-glucose 18, HEPES 10 (pH was adjusted to 7.4 with NaOH). The pipette solution contained (in mM): CsF 100, CsCl 40, NaCl 5, $CaCl_2$ 0.5, EGTA 5, HEPES 10 (pH was adjusted to 7.2 with CsOH). Drugs were purchased from Tocris Bioscience (Bristol, UK) and Sigma (St Louis, MO, USA). Experiments were conducted on hippocampal pyramidal neurons (CA1 area). NMDA receptors were activated by 100 µM NMDA plus 10 µM glycine. The percentage of the block of the steady-state current by different drug concentrations was measured at –80 mV holding potential and IC50 values were obtained from fits

by the Hill equation of concentration-inhibition relationships. All data are presented as means ± SD estimated from at least four experiments.

Docking

The docking study was performed using Autodock Vina [S3]. Structure of the receptor taken from Protein Data Bank (PDB code: 6e7r [S4]) was prepared with MGL Tools [S4].

References

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S2 V. S. Vorobjev, *J. Neurosci. Methods,* 1991, **38**, 145.
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S4 M. C. Regan, Z. Zhu, H. Yuan, S. J. Myers, D. S. Menaldino, Y. A. Tahirovic, D. C. Liotta, S. F. Traynelis and H. Furukawa, *Nat. Commun.*, 2019, **10**, 321.