Supporting Information

Synthesis and Pharmacological Evaluation of Novel *cis* and *trans* 3-Supstitued Anilidopiperidines

Ivana I. Jevtić[a], Katarina Savić Vujović[b], Dragana Srebro[b], Sonja Vučković[b], Milovan D. Ivanović[c], Slađana V. Kostić-Rajačić[a]*

[a]*ICTM-Department of Chemistry, University of Belgrade, Njegoševa 12, Belgrade, Serbia*

[b]*Department of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Serbia*

[c]*Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, Belgrade, Serbia*

*e-mail: sladjana.kostic@ihtm.bg.ac.rs

Chemistry

General

Unless stated otherwise all solvents and chemicals were used as supplied from Alfa Aesar. ^{1}H and ^{13}C NMR spectra were recorded at 200 or 500 MHz for the proton (^{1}H) and at 50 or 126 MHz for the carbon (^{13}C). Chemical shifts are given in parts per million from tetramethylsilane (TMS) as internal standard in $CDCl_3$ or referenced to the residual solvent signal of DMSO for DMSO-d6. 2D NMR spectra (HSQC, NOESY and COSY) were recorded at 500 MHz. Coupling constants (*J*) are reported in Hz. Unless stated otherwise all spectra were recorded at 25 °C. High resolution mass spectra (HRMS) were obtained with an ESI-ToF or Heated ESI (HESI)-orbitrap spectrometer. All reactions were monitored by thin layer chromatography (TLC). Flash and dry-column flash chromatography were carried out using silica gel (10−18 or 18-32 μm, ICN-Woelm). Melting points were obtained at a heating rate of 4 °C/min, and are uncorrected. IR spectra were recorded by using Fourier-transform spectrometer operated in the ATR mode. All solvents were freshly distilled under argon prior to being used. All reagents were purchased from a commercial vendor except NBA which was synthesized according to literature procedure.[1] *Cis*/*trans* diastereomers were designated using 2D NMR techniques, namely HSQC and NOESY.

*(±) cis 5-phenethyl-1-phenyloctahydro-2H-imidazo[4,5-c]pyridin-2-one (cis-**2**)*

Obtained from *cis*-**1**. Yield:0.24 g (80%); yellow, viscous, oil. *Rf* = 0.53 (SiO_2; CH_2Cl_2/MeOH=9:1). IR (ATR): 3281.1, 3027.0, 2947.0, 2812.2, 1705.9, 1599.6, 1499.0, 1403.8, 1309.9, 1253.8, 753.2, 698.2 cm-1. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.39–7.12 (m, 10H), 6.10 (s, 1H), 4.21 (dt, J_1 = 6.5, J_2 = 4.6 Hz, 1H), 3.82 (dd, J_1 = 12.6, J_2 = 7.1 Hz, 1H), 2.87 (ddd, J_1 = 11.7, J_2 = 5.3, J_2 = 1.0 Hz, 1H), 2.81-2.71 (m, 2H), 2.67-2.54 (m, 2H), 2.45 (dd, J_1 = 11.8, J_2 = 7.7 Hz, 2H), 2.39-2.25 (m, 1H), 2.05-1.88 (m, 2H) ppm.^{13}C NMR (126 MHz, $CDCl_3$): δ = 161.1, 139.9, 137.8, 128.8, 128.6, 128.3, 126.0, 124.7, 123.2, 60.0, 55.8, 54.0, 49.2, 48.6, 33.4, 25.3 ppm. HRMS-ESI-ToF: calculated for $C_{20}H_{24}N_3O$: $[M+H]^+$ 322.19139; found 322.18990. (HRMS was performed for a mixture of *cis*-**2**/*trans*-**2** diastereomers)

*(±) trans 5-phenethyl-1-phenyloctahydro-2H-imidazo[4,5-c]pyridin-2-one (trans-**2**)*

Obtained from *trans*-**1**. Yield: 0.25 g (85 %); yellow, viscous oil. *Rf* = 0.58 (SiO_2; CH_2Cl_2/MeOH=9:1). IR (ATR): 3228.7, 3105.0, 3027.8, 2953.4, 2808.2, 1702.6, 1601.3, 1498.3, 1351.9, 1236.1, 1165.7, 1139.3, 753.9, 698.0 cm-1. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37 (t, *J* = 7.9 Hz, 2H), 7.32 – 7.22 (m, 4H), 7.22 – 7.14 (m, 4H), 5.74 (s, 1H), 3.52 – 3.46 (m, 1H), 3.42 (td, J_1 = 11.1, J_2 = 3.1 Hz, 1H), 3.30 (dd, J_1 = 9.9, J_2 = 2.8 Hz, 1H), 3.08 (d, *J* = 11.9 Hz, 1H), 2.84-2.67 (m, 4H), 2.37-2.29 (m, 2H), 2.08 (dd, J_1 = 12.2, J_2 = 2.6 Hz, 1H), 1.73 (qd, J_1 = 11.8, J_2 = 4.0 Hz, 1H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 162.6, 139.9, 138.3, 128.8, 128.6, 128.3, 126.0, 125.0, 123.2, 63.6, 59.6, 56.6, 55.5, 51.8, 33.6, 28.1 ppm. HRMS-ESI-ToF: calculated for $C_{20}H_{24}N_3O$: $[M+H]^+$ 322.19139; found 322.18990. (HRMS was performed for a mixture of *cis*-**2**/*trans*-**2** diastereomers)

*(±) cis Methyl (1-phenethyl-4-(N-phenylpropionamido)piperidin-3-yl)carbamate (cis-**4**)*

Obtained from *cis*-**3**. Yield: 0.24 g (64%); yellow, viscous oil. *Rf* = 0.50 (SiO_2; n-hexane/EtOAc = 1:1). IR (ATR): 3417, 2942, 2809, 1724, 1654, 1594, 1498, 1456, 1376, 1249, 1087 cm-1. ^{1}H NMR (500 MHz, DMSO-d6, 75 °C) δ = 7.41 – 7.35 (m, 3H), 7.26 – 7.21 (m, 2H), 7.20 – 7.15 (m, 3H, partially overlapped), 7.12 (d, *J* = 7.0 Hz, 2H, partially overlapped), 5.76 (br. s, 1H), 4.40 (d, *J* = 9.5 Hz, 1H), 4.24 (dt, J_1 = 13.2, J_2 = 4.0 Hz, 1H), 3.61 (s, 3H), 2.93 – 2.78 (m, 2H), 2.69 (t, *J* = 7.4 Hz, 2H), 2.56 (br. s, 2H), 2.32 (dd, J_1 = 15.1, J_2 = 7.6 Hz, 1H), 2.12 (br. s, 1H), 1.80 (q, *J* = 7.4 Hz, 2H), 1.39 (d, *J* = 11.3 Hz, 1H, partially overlapped), 1.33 – 1.26 (m,

1H, partially overlapped), 0.89 (t, *J* = 7.4 Hz, 3H) ppm. ^{13}C NMR (126 MHz, DMSO-d6, 348 K) δ = 172.4, 155.8, 139.9, 139.8, 129.7, 128.4, 128.1, 127.7, 127.4, 125.3, 58.2, 56.8, 55.1, 51.9, 51.0, 47.7, 32.3, 27.5, 25.3, 8.9 ppm. HRMS-ESI-ToF: calculated for $C_{24}H_{31}N_3O_3$ $[M+H]^+$ 410.24382; found 410.24231.

*(±) trans Methyl (1-phenethyl-4-(N-phenylpropionamido)piperidin-3-yl)carbamate (trans-**4**)*

Obtained from *trans*-**3**. Yields: 0.27 g (74 %); off-white, crystal solid; T.t. 65-66 °C. *Rf* = 0.45 (SiO_2; n-hexane/EtOAc = 1:1). IR (ATR): 3307, 3027, 2942, 2807, 1720, 1645, 1594, 1523, 1496, 1456, 1378, 1267, 1039, 703 cm1. ^{1}H NMR (500 MHz, DMSO-d6, 75 °C) δ = 7.47 – 7.39 (m, 3H), 7.26 – 7.19 (m, 4H), 7.18 – 7.12 (m, 3H), 6.47 (br. s, 1H), 4.46 (br. s, 1H), 3.57 (s, 3H), 3.41 (br. s, 1H), 3.01 (ddd, J_1 = 10.9, J_2 = 4.5, J_3 = 2.0 Hz, 1H), 2.86 (dd, J_1 = 9.5, J_2 = 2.0 Hz, 1H), 2.69 – 2.62 (m, 2H), 2.52 – 2.49 (m, 2H, overlapped with DMSO), 2.03 (td, J_1 = 12.0, J_2 = 2.3 Hz, 1H), 1.97 (t, *J* = 10.6 Hz, 1H), 1.86 (br. s, 2H), 1.76 (d, *J* = 10.1 Hz, 1H), 1.30 (br. d, *J* = 10.4 Hz, 1H), 0.90 (t, *J* = 7.4 Hz, 3H) ppm. ^{13}C NMR (126 MHz, DMSO-d6, 75 °C) δ = 173.1, 155.8, 139.9, 138.6, 129.6, 128.8, 128.0, 127.8, 127.7, 125.3, 58.3, 57.7, 55.1, 51.4, 50.9, 50.1, 32.6, 28.8, 27.4, 9.0 ppm. HRMS-ESI-ToF: calculated for $C_{24}H_{31}N_3O3$ $[M+H]^+$ 410.24382; obtained 410.24267.

*(±) cis N-(3-cyano-1-phenethylpiperidin-4-yl)-N-phenylpropionamide (cis-**5**)*

Obtained from *cis*-**3**. Yield: 0.056 g (66 %); yellow, viscous oil. *Rf* = 0.52 (SiO_2; n-hexane/EtOAc = 6:4). IR (ATR):2937, 2240, 1663, 1585, 1494, 1455, 1378, 1268, 1093, 751, 704 cm-1. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.73 – 7.66 (m, 1H), 7.47 – 7.44 (m, 1H), 7.41 – 7.35 (m, 2H), 7.29 – 7.23 (m, 2H), 7.18 (d, *J* = 7.4 Hz, 3H), 7.06 – 7.01 (m, 1H), 4.32 (dt, J_1 = 13.0, J_2 = 4.3 Hz, 1H), 3.91 (td, *J* = 4.3, 2.5 Hz, 1H), 3.20 (dt, *J* = 11.8, 2.5 Hz, 1H), 3.00 – 2.90 (m, 1H), 2.79 – 2.70 (m, 2H), 2.66 – 2.57 (m, 2H), 2.40 (dd, J_1 = 11.9, J_2 = 2.6 Hz, 1H), 2.13 (td, J_1 = 11.6, J_2 = 2.8 Hz, 1H), 2.02 – 1.93 (m, 2H), 1.57 – 1.46 (m, 1H), 1.46 – 1.37 (m, 1H), 1.02 (t, *J* = 7.4 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 174.9, 139.9, 138.3, 131.3, 130.1, 129.5, 128.8, 128.6, 128.6, 128.2, 125.96, 120.5, 59.2, 54.5, 53.8, 52.6, 33.5, 32.6, 28.7, 27.8, 9.3 ppm. HRMS-ESI-ToF: m/z calculated for $C_{23}H_{28}N_3O$ $[M+H]^+$ 362.2227; found 362.2227.

*(±) trans N-(3-cyano-1-phenethylpiperidin-4-yl)-N-phenylpropionamide (trans-**5**)*

Obtained from *trans*-**3**. Yield: 0.052 g (61 %); yellow, viscous oil. *Rf* = 0.46 (SiO_2; n-hexane/EtOAc = 6:4). IR (ATR): 2939, 2241, 1663, 1595, 1494, 1454, 1379, 1256, 1091, 704 cm-1. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.54 – 7.35 (m, 4H), 7.32 – 7.24 (m, 2H), 7.25 – 7.10 (m, 4H), 4.87 (s, 1H), 3.26 (ddd, J_1 = 11.2, J_2 = 3.7, J_3 = 2.1 Hz, 1H), 3.04 – 2.97 (m, 1H), 2.78 (br. s, 1H, partially overlapped), 2.72 (dd, J_1 = 10.1, J_2 = 5.7 Hz, 2H, partially overlapped), 2.65 – 2.58 (m, 2H), 2.43 (t, *J* = 11.3 Hz, 1H), 2.24 (td, J_1 = 11.9, J_2 = 2.4 Hz, 1H), 2.09 – 1.93 (m, 2H, partially overlapped), 1.90 (ddd, J_1 = 12.6, J_2 = 6.6, J_3 = 2.6 Hz, 1H, partially overlapped), 1.69 (br. s, 1H), 1.06 (t, *J* = 7.4 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 174.1, 139.5, 138.4, 129.8, 128.9, 128.5, 128.4, 126.2, 119.2, 59.2, 54.8, 54.2, 52.0, 33.5, 32.1, 29.1, 28.4, 9.4 ppm. HRMS-ESI-ToF: calculated for $C_{23}H_{28}N_3O[M+H]^+$ 362.2227; found 362.2225.

*(±) cis N-(3-amino-1-phenethylpiperidin-4-yl)-N-phenylpropionamide (cis-**6**)*

Obtained from *cis*-**4**. Yield: 0.071 g (83%); yellow, viscous oil. *Rf* = 0.23 (SiO_2; CH_2Cl_2/MeOH = 9:1). IR (ATR): 3278, 3027, 2939, 2812, 1660, 1594, 1496, 1456, 1399, 1246, 700 cm-1. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.43 (t, *J* = 7.7 Hz, 2H), 7.35 – 7.25 (m, 4H), 7.19 (dd, J_1 = 13.9, J_2 = 7.1 Hz, 4H), 7.11 (d, *J* = 7.5 Hz, 2H), 4.26 – 4.15 (m, 2H), 3.13 (dd, J_1 = 11.8, J_2 = 3.7 Hz, 1H), 2.87 – 2.76 (m, 2H), 2.71 – 2.58 (m, 3H), 2.33 – 2.19 (m, 3H), 2.14 (dd, J_1 = 11.6, J_2 = 7.0 Hz, 1H), 1.90 (ddd, J_1 = 15.6, J_2 = 9.5, J_3 = 4.6 Hz, 1H), 1.79 (d, *J* = 15.3 Hz, 1H), 1.14 (t, *J* = 7.5 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 169.9, 139.9, 137.8, 129.6, 128.5, 128.2, 127.4, 126.4, 125.8, 61.5, 59.9, 58.4, 55.6, 48.4, 33.3, 23.8, 21.4, 10.4 ppm. HRMS-ESI-ToF: calculated for $C_{22}H_{30}N_3O$ $[M+H]^+$ 352.2383; found 352.2386.

*(±) trans N-(3-amino-1-phenethylpiperidin-4-yl)-N-phenylpropionamide (trans-**6**)*

Obtained from *trans*-**4**. Yield: 0.077 g (90%); pale-yellow oil. *Rf* = 0.27 (SiO_2; CH_2Cl_2/MeOH = 9:1). IR (ATR): 3327, 3147, 3062, 2940, 2807, 1645, 1592, 1495, 1455, 1400, 1268, 703 cm-1. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.45 – 7.33 (m, 4H), 7.24 (t, *J* = 7.4 Hz, 2H), 7.18 – 7.11 (m, 3H), 7.07 (d, *J* = 6.7 Hz, 1H), 4.63 (td, J_1 = 12.2, J_2 = 4.1 Hz, 1H), 3.24 – 3.10 (m, 1H), 2.93 (d, *J* = 11.4 Hz, 1H), 2.78 – 2.66 (m, 3H), 2.63 – 2.51 (m, 2H, partially overlapped), 2.44 (br. s, 2H, partially overlapped), 2.15 (td, J_1 = 12.0, J_2 = 2.3 Hz, 1H), 2.07 – 1.92 (m, 3H), 1.78 – 1.70 (m, 1H), 1.42 (qd, J_1 = 12.4, J_2 = 4.1 Hz, 1H), 1.03 (t, *J* = 7.4 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 175.2, 140.1, 138.5, 130.7, 130.2, 129.7, 129.2, 128.8, 128.5, 128.4, 126.1, 61.5, 60.0, 57.9, 52.8, 50.5, 33.7, 29.6, 28.7, 9.7 ppm. HRMS-ESI-ToF: calculated for $C_{22}H_{30}N_3O[M+H]^+$ 352.2383; found 352.2387.

In vivo determination of antinociceptive activity

General

The study was carried out on 186 adult male Wistar rats (200-250 g) obtained from the Military Medical Academy (Belgrade, Serbia). The animals were housed in groups of three per cage (42.5×27×19 cm) under standard conditions of temperature (22±1°C), relative humidity (60%) and a 12 h light/dark cycle, with lights on at 8:00 a.m. Food and water were freely available, except during the experimental procedures. The animals were fed standard rat pellets obtained from the Veterinary Institute Subotica, Serbia. The experiments were conducted by the same experimenter on consecutive days, always at the same time of the day, between 8:00 a.m. and 2:00 p.m., to avoid diurnal variation in the behavioral tests. The animals were unrestrained during testing. Each animal was used only once and was killed at the end of the experiments by an intraperitoneal (i.p.) injection of sodium thiopental (200 mg/kg). Only animals that have been used as control animals (24 animals who received a single injection of saline) were transferred later to a protocol with naloxone and they were reused.

Prior to each experiment, the animals were habituated to the handling and experimental procedure for three consecutive days. The antinociceptive activity was determined by the tail-immersion[2] and the formalin test.[3]

All compounds were dissolved in saline and injected i.p. in a final volume of 2 ml/kg.[2] Doses of the test compounds were calculated for the free base. Naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) was also dissolved in saline and injected subcutaneously (s.c.) in the back (0. 1 mg/kg s.c.) 15 min before the i.p. injection of $4ED_{50}$ of *cis*-**4**, *trans*-**4**, *cis*-**5** and *trans*-**5** in the same volume. Control animals received the corresponding volume of saline (s.c.) instead of naloxone plus test compound. To test whether naloxone reverse the antinociceptive effects of drugs tested, the t-test for unpaired values was used.

Tail-immersion test

The rat was placed in a cylindrical rat holder with its tail hanging freely outside the cage. The distal 5 cm of the tail was immersed in a warm water bath (55 ± 0.5cC) and the time for tail-withdrawal was measured as a response latency. To minimize tissue damage by repeated testing, a 10-s cutoff time was imposed for all animals that failed to respond to the stimulus. This means that the maximal duration of a single exposure of rat tail to hot water was 10 s.

Predrug response latency was obtained 5 min before i.p. drug administration. Post-drug response latency was measured after i.p. administration of test compound at 5, 10, 15, 20, etc. min. Response latency is expressed as a percent maximum possible effect (%MPE) and calculated according to the following formula: %MPE = (postdrug latency — predrug latency)/(cutoff time - predrug latency) x 100. Dose-response curves were analyzed by linear regression. The ED_{50} and 95% confidence limits were estimated from the dose-response curve according to Tallarida and Murray.[4] Relative potency estimates were considered statistically significant when 95% confidence limits did not overlap 1.0.

The duration of analgesic activity was determined by using equianalgesic doses of the compound tested (e.g. ED_{99}, the least dose that produce the maximum analgesic effect). To compare time courses of the effects produced by the several drugs, data were expressed as the area under the curve (AUC); e.g., the area of a series of trapezoids in which the height was post-drug response latency minus the pre-drug response latency (s) and the base, the interval (min) between measurements. This provided an index with the dimension of second-minute.[4] The slopes of the regression lines were compared by using a test for parallelism.[4]

To test whether saline injection in control rats has any effect on the tail-immersion latency, the t-test for paired values was used.[4] A P value of less than 0.05 was considered statistically significant.

Formalin test

The formalin test is a chemically induced inflammatory pain.[3,5] Animals were injected with 100 μL of 2.5% formalin into the plantar surface of the right hind paw using a microliter syringe and a 29-gauge needle. Drugs were given by i.p. route 10 min before formalin. After formalin injection, the animals were individually placed in Plexiglas observation chambers. Data were recorded as the total time (in sec) spent in pain related behavior (flinching and licking or biting) after the injection of formalin. The recordings were performed in 9 intervals of 5 min. At each time interval (eg. 0-5, 5-10, 10-15 min, etc) the mean ±SEM of the nociceptive time in seconds (s) was obtained in 6–8 rats. First phase responses were seen in the 0–10 min interval whereas the second phase responses were evident in the 10–45 minute intervals.

The time-course of the antinociceptive responses of individual drugs were constructed by plotting the mean time that the animal spent in pain-related behavior as a function of time. The areas delineated by the pain-related behavior and the time curves (AUC) were calculated using

the trapezoidal rule.[4] AUC was calculated for the second phase (10-45 min) of the assay and the percentage of antinociception was calculated according to the following equation.[3] Percent of antinociception (AA%)=[(AUCvehicle-AUCpost compound)/AUCvehicle]×100.

References:

[1] E. P. Oliveto, C. Gerold, Org. Synth. 1951, 31, 17

[2] Vučković, S.; Prostran, M.; Ivanović, M.; Ristović, Z.; Stojanović, R. Jpn. J. Pharmacol. 2000, 84, 188-195

[3] K. S. Vujović, S. Vučković, D. Vasović, B. Medić, N. Knežević, M. Prostran, Acta Neurobiol. Exp. (Wars). 2017, 77, 137-146

[4] R. J. Tallarida, R. B Murray in The Manual of pharmacologic calculations with computer programs, 2nd Ed. Springer-Verlag: New York, 1986 (Ref. 13 in the manuscript)

[5] D. Dubuisson, S. G. Dennis, Pain 1977, 4, 161-174