Supporting Information

Determination of lipophilicity and ionization of fentanyl and its 3-substituted analogs by reversed-phase thin-layer chromatography

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Chemistry

General

Unless stated otherwise all solvents and chemicals were used as supplied. 1H and 13C NMR spectra were recorded at 200 or 500 MHz for the proton (1H) and at 50 or 126 MHz for the carbon (13C). Chemical shifts are given in parts per million from tetramethylsilane (TMS) as internal standard in CDCl3 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. Cis/trans diastereomers were designated using 2D NMR techniques, namely HSQC and NOESY.
Synthesis of (±) trans methyl 3-((1-phenethyl-4-(N-phenylpropionamido)piperidin-3-yl)amino)propanoate (T7)

To a magnetically stirred solution of anilido amine T6 (0.055 g, 1.0 eq.) in MeOH (3 mL), methyl acrylate (9.0 eq.) was added. The reaction was monitored using TLC on SiO2 plates with a mixture of CH2Cl2/MeOH = 95:5, as an eluent. After stirring for 6 hours at 60 ºC the mixture was concentrated on a rotary evaporator. The crude product is purified by dry-column flash chromatography (SiO2; CH2Cl2/MeOH =100:0 to 95:5). Yield: 0.063 g (93%); yellow, viscous oil. 

IR (ATR): 3451, 2945, 1734, 1648, 1494, 1456, 1375, 1263, 704 cm⁻¹. ¹H NMR (500 MHz, CDCl3) δ = 7.45 – 7.32 (m, 4H), 7.30 – 7.22 (m, 2H), 7.21 – 7.12 (m, 3H), 7.07 (d, J = 5.6 Hz, 1H), 4.69 – 4.59 (m, 1H), 3.68 (s, 3H), 3.40 – 3.27 (m, 1H), 3.10 – 2.90 (m, 2H), 2.87 – 2.70 (m, 3H), 2.68 – 2.57 (m, 2H), 2.53 (t, J = 6.3 Hz, 3H), 2.19 (t, J = 11.2 Hz, 1H), 2.06 – 1.87 (m, 3H), 1.80 (dt, J = 10.0, 3.9 Hz, 1H), 1.64 – 1.47 (m, 1H), 1.02 (t, J = 7.5 Hz, 3H) ppm.

13C NMR (126 MHz, CDCl3) δ = 175.0, 173.3, 139.9, 138.7, 130.7, 130.4, 129.7, 129.2, 128.7, 128.5, 128.4, 126.2, 60.2, 58.7, 56.2, 55.8, 52.7, 51.6, 41.9, 35.1, 33.5, 29.6, 28.5, 9.8 ppm. HRMS-Heated ESI-Orbitrap: calculated for C26H36N3O3 [M+H]+ 438.27512; found 438.27468.

Synthesis of (±) trans N-(3-formamido-1-phenethylpiperidin-4-yl)-N-phenylpropionamide (T8)

To a magnetically stirred solution of anilido amine T6 (0.080 g, 1.0 equiv.) in DMF (1.5 mL), formamide (4.0 equiv.) and LiH (2.0 equiv.) were added. The mixture was stirred at 150 ºC. and the reaction was monitored using TLC on SiO2 plates with a mixture of CH2Cl2/MeOH = 95:5, as an eluent. After 4 h, H2O (10 mL) was added and the mixture was extracted with CH2Cl2 (3x5 mL). The organic layers are combined and concentrated on a rotary evaporator. The crude product is purified by dry-column flash chromatography (SiO2; CH2Cl2/MeOH =100:0 to 95:5). Yield: 0.070 g (81%); pale-yellow, amorphous solid. 

IR (ATR): 3306, 3057, 2936, 2808, 1647, 1599, 1496, 1379, 1266, 701 cm⁻¹. ¹H NMR (500 MHz, CDCl3) δ = 8.22 (s, 1H), 7.54 – 7.34 (m, 3H), 7.33 – 7.19 (m, 2H), 7.22 – 7.09 (m, 4H), 7.09 – 7.03 (m, 1H), 6.56 (d, J = 7.9 Hz, 1H), 4.72 (td, J = 11.9, 4.3 Hz, 1H), 4.20 – 3.93 (m, 1H), 3.42 – 3.28 (m, 1H), 3.00 – 2.87 (m, 1H), 2.76 – 2.65 (m, 2H), 2.60 – 2.49 (m, 2H), 2.21 – 2.08 (m, 1H), 2.07 – 1.87 (m, 3H), 1.68 (ddt, J = 12.8, 5.2, 2.7 Hz, 1H), 1.39 – 1.20 (m, 1H), 1.00 (t, J = 7.5 Hz, 3H) ppm.

13C NMR (126 MHz, CDCl3) δ = 176.5, 161.2, 140.1, 137.9, 130.4, 130.3, 129.9, 129.5, 129.3,
128.9, 128.8, 128.7, 128.5, 128.6, 126.1, 59.7, 57.8, 54.6, 52.5, 49.7, 33.8, 29.6, 28.6, 9.8 ppm. HRMS-Heated ESI-Orbitrap: calculated for C_{23}H_{30}N_{3}O_{2}\ [M+H]^+ 380.23325; found 380.23271.

Synthesis of (±) trans N-(3-acetamido-1-phenethylpiperidin-4-yl)-N-phenylpropionamide (T9)

To a magnetically stirred solution of anilido amine T6 (0.100 g, 1.0 equiv.) in CH_{2}Cl_{2} (5 mL), Et_{3}N (1.0 equiv.) and acetic anhydride (4.0 equiv.) were added. The mixture was stirred at 25 °C, and the reaction was monitored using TLC on SiO_{2} plates with a mixture of CH_{2}Cl_{2}/MeOH = 95:5, as an eluent. After 4 h, MeOH was added and the mixture concentrated on a rotary evaporator. A solution of K_{2}CO_{3} (5 M) was added (pH ~ 11) and the mixture was extracted with CH_{2}Cl_{2} (2x5 mL). The organic layers are combined and concentrated on a rotary evaporator. The crude product is purified by dry-column flash chromatography (SiO_{2}; CH_{2}Cl_{2}/MeOH =100:0 to 95:5). Yield: 0.108 g (96%); pale-yellow, amorphous solid. R_{f} = 0.50 (SiO_{2}; CH_{2}Cl_{2}/MeOH = 95:5). IR (ATR): 3245, 3066, 2941, 2794, 1645, 1554, 1492, 1376, 1626, 703 cm\(^{-1}\). \(^{1}\)H NMR (500 MHz, CDCl_{3}) δ = 7.39 – 7.27 (m, 3H), 7.20 – 7.13 (m, 2H), 7.13 – 7.01 (m, 4H), 6.98 (d, J = 6.4 Hz, 1H), 6.44 (d, J = 6.2 Hz, 1H), 4.62 (tdd, J = 12.0, 4.1 Hz, 1H), 4.11 – 3.91 (m, 1H), 3.36 – 3.22 (m, 1H), 2.95 – 2.78 (m, 1H), 2.74 – 2.59 (m, 2H), 2.58 – 2.43 (m, 2H), 2.10 (t, J = 11.2 Hz, 1H), 1.99 – 1.75 (m, 6H), 1.60 (dd, J = 12.8, 3.7 Hz, 1H), 1.32 – 1.13 (m, 1H), 0.93 (t, J = 7.5 Hz, 3H) ppm. \(^{13}\)C NMR (126 MHz, CDCl_{3}) δ = 176.5, 170.2, 139.9, 138.0, 130.3, 129.8, 129.2, 128.8, 128.7, 128.4, 126.1, 59.6, 57.7, 54.8, 52.4, 50.6, 33.5, 29.3, 28.5, 23.4, 10.0 ppm. HRMS-Heated ESI-Orbitrap: calculated for C_{24}H_{32}N_{3}O_{2}\ [M+H]^+ 394.24890; found 394.24848.

Synthesis of (±) trans N-(1-phenethyl-4-(N-phenylpropionamido)piperidin-3-yl)acrylamide, (T10)

To a magnetically stirred solution of anilido amine T6 (0.100 g, 1.0 eq.) in CH_{2}Cl_{2} (2 mL), Et_{3}N (1.0 equiv.) and acryloyl chloride (1.5 equiv.) at -20 °C were. The mixture was stirred at 25 °C, and the reaction was monitored using TLC on SiO_{2} plates with a mixture of CH_{2}Cl_{2}/MeOH = 95:5, as an eluent. After 30 min, MeOH was added and the mixture concentrated on a rotary evaporator. A solution of K_{2}CO_{3} (5 M) was added (pH ~ 11) and the mixture was extracted with CH_{2}Cl_{2} (2x25 mL). The organic layers are combined and concentrated on a rotary evaporator. The crude product is purified by dry-column flash chromatography (SiO_{2}; CH_{2}Cl_{2}/MeOH =100:0 to 95:5). Yield: 0.105 g (91%); pale-yellow, amorphous solid. R_{f} = 0.50 (SiO_{2}; CH_{2}Cl_{2}/MeOH = 95:5). IR (ATR): 3322, 2925, 2809, 1670, 1635, 1529, 1492, 1405, 1266, 705 cm\(^{-1}\). \(^{1}\)H NMR (500
MHz, CDCl₃) δ = 7.47 – 7.32 (m, 3H), 7.28 – 7.19 (m, 2H), 7.19 – 7.07 (m, 4H), 7.09 – 6.93 (m, 1H), 6.66 (d, J = 7.8 Hz, 1H), 6.28 (d, J = 17.6 Hz, 1H), 6.11 (dd, J = 17.1, 10.3 Hz, 1H), 5.65 (d, J = 9.9 Hz, 1H), 4.73 (td, J = 12.0, 4.2 Hz, 1H), 4.19 – 4.04 (m, 1H), 3.48 – 3.30 (m, 1H, 2.98 – 2.83 (m, 1H), 2.77 – 2.64 (m, 2H), 2.64 – 2.47 (m, 2H), 2.23 – 2.09 (m, 1H), 2.06 – 1.84 (m, 3H), 1.67 (dq, J = 12.7, 3.0 Hz, 1H), 1.33 – 1.17 (m, 1H), 0.98 (t, J = 7.5 Hz, 3H) ppm. ¹³C NMR (126 MHz, CDCl₃) δ 176.8, 165.7, 140.2, 138.0, 131.3, 130.4, 130.3, 130.0, 129.3, 128.9, 128.7, 128.5, 126.4, 126.1, 59.8, 57.9, 55.0, 52.6, 51.1, 33.8, 29.6, 28.6, 10.0 ppm. HRMS-Heated ESI-Orbitrap: calculated for C₂₅H₃₂N₃O₂ [M+H]^+ 406.24890; found 406.24859.

**Synthesis of (±) trans N-[1-phenethyl-3-(2,2,2-trifluoroacetamido)piperidin-4-yl]-N-phenylpropionamide (T11)**

To a magnetically stirred solution of anilido amine T6 (0.100 g, 1.0 equiv.) in CH₂Cl₂ (3 mL), Et₃N (1.0 equiv.) and trifluoroacethanhydride (4.0 equiv.) were added. The mixture was stirred at 25 ºC, and the reaction was monitored using TLC on SiO₂ plates with a mixture of CH₂Cl₂/MeOH = 95:5, as an eluent. After 1 h, MeOH was added and the mixture concentrated on a rotary evaporator. A solution of K₂CO₃ (5 M) was added (pH ~ 11) and the mixture was extracted with CH₂Cl₂ (2x 25 mL). The organic layers are combined and concentrated on a rotary evaporator. The crude product was purified by dry-column flash chromatography (SiO₂; CH₂Cl₂/MeOH =100:0 to 95:5). Yield: 0.0120 g (94%); yellow, amorphous solid. Rf = 0.45 (SiO₂; CH₂Cl₂/MeOH = 95:5). IR (ATR): 3276, 3095, 2929, 2796, 1713, 1610, 1592, 1399, 1183, 704 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ = 7.75 (d, J = 7.4 Hz, 1H), 7.53 – 7.38 (m, 3H), 7.32 – 7.21 (m, 2H), 7.15 (dd, J = 23.8, 7.4 Hz, 3H), 7.07 (d, J = 4.9 Hz, 1H), 7.00 (d, J = 7.5 Hz, 1H), 4.76 (td, J = 11.9, 4.2 Hz, 1H), 4.02 (dt, J = 10.9, 3.7 Hz, 1H), 3.43 – 3.26 (m, 1H), 3.00 – 2.86 (m, 1H), 2.76 – 2.67 (m, 2H), 2.58 (t, J = 8.1 Hz, 2H), 2.24 – 2.12 (m, 1H), 2.07 (t, J = 10.6 Hz, 1H, partially overlapped), 2.05 – 1.81 (m, 2H, partially overlapped), 1.77 – 1.63 (m, 1H), 1.36 – 1.19 (m, 1H), 1.00 (t, J = 7.4 Hz, 3H) ppm. ¹³C NMR (126 MHz, CDCl₃) δ = 177.2, 157.8, 157.5, 139.9, 137.7, 130.4, 130.4, 129.5, 128.9, 128.7, 128.6, 128.5, 126.2, 117.1, 59.7, 56.8, 54.7, 52.3, 52.2, 33.7, 29.6, 28.5, 9.8 ppm. HRMS-Heated ESI-Orbitrap: calculated for C₂₅H₂₉F₃N₃O₂ [M+H]^+ 448.22064; found 448.21973.

**In vivo determination of antinociceptive activity**

The antinociceptive activity was determined by the tail immersion test [2]. The experiments were approved by the Local Ethical Committee of the Faculty of Medicine, University of Belgrade.
(permit No. 5784/1) and the Ethical Council of the Ministry of Agriculture, Forestry and Water Management, which are in compliance with the European Community Council Directive of November 24th, 1986 (86/609/EEC) and the International Association for the Study of Pain (IASP) Guidelines for the Use of Animals in Research.

References
