

Supporting Information

Novel indole-based sigma-2 receptor ligands: synthesis, structure-affinity relationship and antiproliferative activity

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1. Chemistry: synthesis of siramesine

1'-{4-[1-(4-fluorophenyl)-1H-indol-3-yl]butyl}-3H-spiro[2-benzofuran-1,4'-piperidine] (siramesine). Siramesine was synthesized according to the method reported previously.¹ The detailed synthetic procedure was similar to that of **1a–1g**. Compound **6** (150 mg, 0.44 mmol), 3H-spiro[2-benzofuran-1,4'-piperidine] (67 mg, 0.36 mmol) and K₂CO₃ (60 mg, 0.44 mmol) in CH₃CN (15 mL) afforded siramesine (106 mg, 66%) as yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 7.65 (d, *J* = 7.4 Hz, 1H), 7.49–7.41 (m, 3H), 7.28–7.26 (m, 2H), 7.23–7.13 (m, 6H), 7.09 (s, 1H), 5.07 (s, 2H), 3.09–2.80 (m, 4H), 2.65–2.43 (m, 4H), 2.11 (s, 2H), 1.86–1.69 (m, 6H). ¹³C-NMR (400 MHz, CDCl₃) δ 160.7, 145.7, 138.9, 136.3, 136.0, 128.9, 127.5, 127.3, 125.8, 125.7, 125.1, 122.4, 120.9 (2C), 119.8, 119.4, 117.8, 116.3 (2C), 110.1, 84.7, 70.7, 58.9, 50.3 (2C), 36.6 (2C), 28.1, 27.1, 25.0. Purity: >99% (HPLC). Anal. Calcd. for C₃₀H₃₁FN₂O·HCl·H₂O (509.05): C 70.78, H 6.73, N 5.50; Found: C 71.24, H 6.62, N 5.43.

2. *In vitro* binding studies

Competition binding assay of σ_1 and σ_2 receptors were performed as previously reported.^{2,3} Rat brain homogenates and rat liver homogenates were used as receptor materials. (+)-[³H]Pentazocine and [³H]-1,3-dio-tolylguanidine (DTG) with 10 μ M dextrallorphan for the selective masking of σ_1 receptors were employed as the radioligand for σ_1 and σ_2 receptors, respectively. Nonspecific binding was determined with 10 μ M haloperidol. *K_i* values were calculated by the Cheng–Prusoff equation and represent as mean \pm standard deviation (SD) from at least two independent experiments, each performed in triplicate.

3. Cell culture

The human cancer cell lines MCF7 (human mamma carcinoma), DU145 (human prostate carcinoma) and C6 (rat glioma) were obtained from the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM;

MCF-7), Eagle's Minimal Essential Medium (EMEM; DU145) or RPMI-1640 medium (C6) supplemented with 10% fetal bovine serum and 1% penicillin at 37°C in a humidified atmosphere with 5% CO₂.

4. Antiproliferative assay

The antiproliferative effect was evaluated using the MTT assay as reported in the literature.^{4,5} The MCF7, DU145 or C6 cells (5 000 cells/well) were seeded in 96-well plates in the absence or presence of known concentrations of compound **1a**, **1b** and siramesine ranging from 100 nM to 100 µM for 24 h. After cultured by 24 h, MTT solution (5 mg/mL, 10 mL/well) was added, and the cells were incubated for another 4 h. After cell lysates (100 mL/well) was added and incubated overnight, cell viability was determined by monitoring the conversion of tetrazoliumbromide (absorption at 570/650 nm) on an automated microplate spectrophotometer (Multiskan Go 1510-00433C, ThermoFisher SCIENTIFIC). Two to three independent experiments, each in triplicate, were performed.

5. Flow cytometry cell cycle analysis

The cell cycle inhibition was determined by flow cytometry analysis method as previously reported with minor modification.⁶ DU145 cells were plated in 24 well plates and incubated with different concentration of **1a**, **1b** and siramesine for 24 h. Then cells were harvested, washed with PBS and fixed in cold ethanol (70% v/v) for more than 24 h. After being washed with PBS twice and resuspended in 0.4 mL of PBS, RNaseA (40 µL, 1 mg/mL) was added into the harvested cells at 37 °C for 30 min, and PI (40 µL, 500 ug/mL) was added at 4 °C under darkness for 30 min to stain the cellular DNA. The DNA content of the stained cells was analyzed by flow cytometry (BD Biosciences, California, USA) and DNA distributions were analyzed by Modfit LT MacIntel (Verity Software House, Topsham, ME, USA). All the experiments were carried out in triplicate.

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