Supporting information

(3-(1H-indol-3-yl)-2-(7,8,12,13-tetraoxa-10-azaspiro[5.7]tridecan-10-yl)propanoic acid), which has cytotoxic activity

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A. General Information

General Remarks. The reaction were performed at room temperature in air in round-bottom flasks equipped with a magnetic stir bar. The NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500.17 MHz for ^{1}H and 125.78 MHz for ^{13}C according to standard Bruker procedures. $CDCl_3$ was used as the solvent, and tetramethylsilane, as the internal standard. Mass spectra were recorded on a Bruker Autoflex III MALDI TOF/TOF instrument with α-cyano-4-hydroxycinnamic acid as a matrix. Samples were prepared by the dried droplet method. The C, H, and N were quantified by a Carlo Erba 1108 analyzer. The progress of reactions was monitored by TLC on Sorbfil (PTSKh-AF-A) plates, with a 5:1 hexane : EtOAc mixture as the eluent and visualization with I2 vapor. For column chromatography, silica gel MACHEREY-NAGEL (0.063-0.2 mm) was used. The synthesis of the 7,8,10,12,13-pentaoxaspiro[5.7]tridecane **2** was as reported in the literature [1]. THF was freshly distilled over LiAlH4.

Ring transformation reaction of 7,8,10,12,13-pentaoxaspiro[5.7]tridecane with tryptofane catalyzed by $Sm(NO_3)_3 \cdot 6H_2O$. General procedure. A round-botton flask mounted on a magnetic stirrer was charged with THF (5 mL), $Sm(NO_3)_3 \cdot 6H_2O$ (0.5 mmol), tryptofane (10 mmol), and 7,8,10,12,13-pentaoxaspiro[5.7]tridecane (10 mmol). The reaction mixture was stirred at room temperature (~25 °C) for 6 h, the solvent was evaporated, and the residue was chromatographed on a SiO_2 column to give 33-(1H-indol-

3-yl)-2-(7,8,12,13-tetraoxa-10-azaspiro[5.7]tridecan-10-yl)propanoic acid) **3** as a pure compound.

33-(1H-indol-3-yl)-2-(7,8,12,13-tetraoxa-10-azaspiro[5.7]tridecan-10-yl)propanoic acid) (**3**).

Colorless oil; 0.31 g (84% yield), R_f 0.76 (PE/Et_2O = 10/1). ^1H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 1.40 (m, 1H, CH_2, *J* = 5 Hz), 1.58 (m, 2H, CH_2, *J* = 5 Hz), 1.68 (m, 1H, CH_2, *J* = 5 Hz), 1.80 (m, 6H, CH_2), 3.25 (dd, *J* = 10 Hz, 1H, CH_2), 3.36 (dd, *J* = 10 Hz, 1H, CH_2), 3.91 (t, *J* = 10 Hz, 1H, CH), 5.11-5.31 (m, 4H, CH_2), 6.96 (s, 1H, CH), 7.23 (m, 4H, CH), 9.54 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 22.4, 22.5, 22.6, 22.7, 25.2, 25.3, 25.6, 29.5, 29.8, 30.1, 30.5, 67.9, 85.6, 86.0, 109.3, 109.8, 112.2, 117.3, 119.2, 120.6, 121.5, 124.2, 127.2, 134.8, 173.5. MALDI TOF/TOF, m/z: 375 [M-H]+. Anal.calcd. For $C_{19}H_{24}N_2O_6$: C, 60.63; H, 6.43; N, 7.44%. Found C, 60.61; H, 6.42; N, 7.43%.

Biological screening

Cell culturing

Cells (Jurkat, K562, U937, HL60) were purchased from the HPA Culture Collection (UK) and cultured according to standard human cell culture protocols. All cell lines used in this work were suspension cultures, and they were cultured in medium (RPMI, Gibco BRL) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution at 37°C in a humid medium containing 5% CO_2. The cells were then seeded into 24-well plates at 10 x 10^4 cells per well and incubated for 24 hours with test compounds.

Cytotoxicity assay

Cell viability was assessed by staining cells with 7-AAD (7-aminoactinomycin D) (Biolegend). After incubation with the test compound, the cells were harvested, washed 2 times with PBS buffer, and centrifuged at 380g for 6 minutes. The cell pellet was resuspended in 200 µl of flow cytometry staining buffer (PBS without Ca^{2+} and Mg^{2+}, 2.5% FBS) and stained with 6 µl of 7-AAD solution for 20 minutes at 37° C. in the dark. Samples were detected using the NovoCyte Penteon Flow Cytometer (ACEA) flow cytometry system.

Apoptosis assay

Presented here is an apoptosis assay that allowed us to assess two markers of cell health: cell surface expression of phosphatidylserine and membrane permeabilization. Using reagents from the Millipore FlowCellect™ Apoptosis Assay Kit provides information on early, mid and late apoptosis with one simple assay. Cells were treated with synthesized compounds and incubated at 37°C for 24 h. After this time, cells were dissociated with acutase solution, stained and analyzed using flow cytometry (NovoCyte Penteon Flow Cytometer Systems (ACEA)) according to the protocols of the FlowCellect™ Apoptosis Assay Kit kit manufacturer.

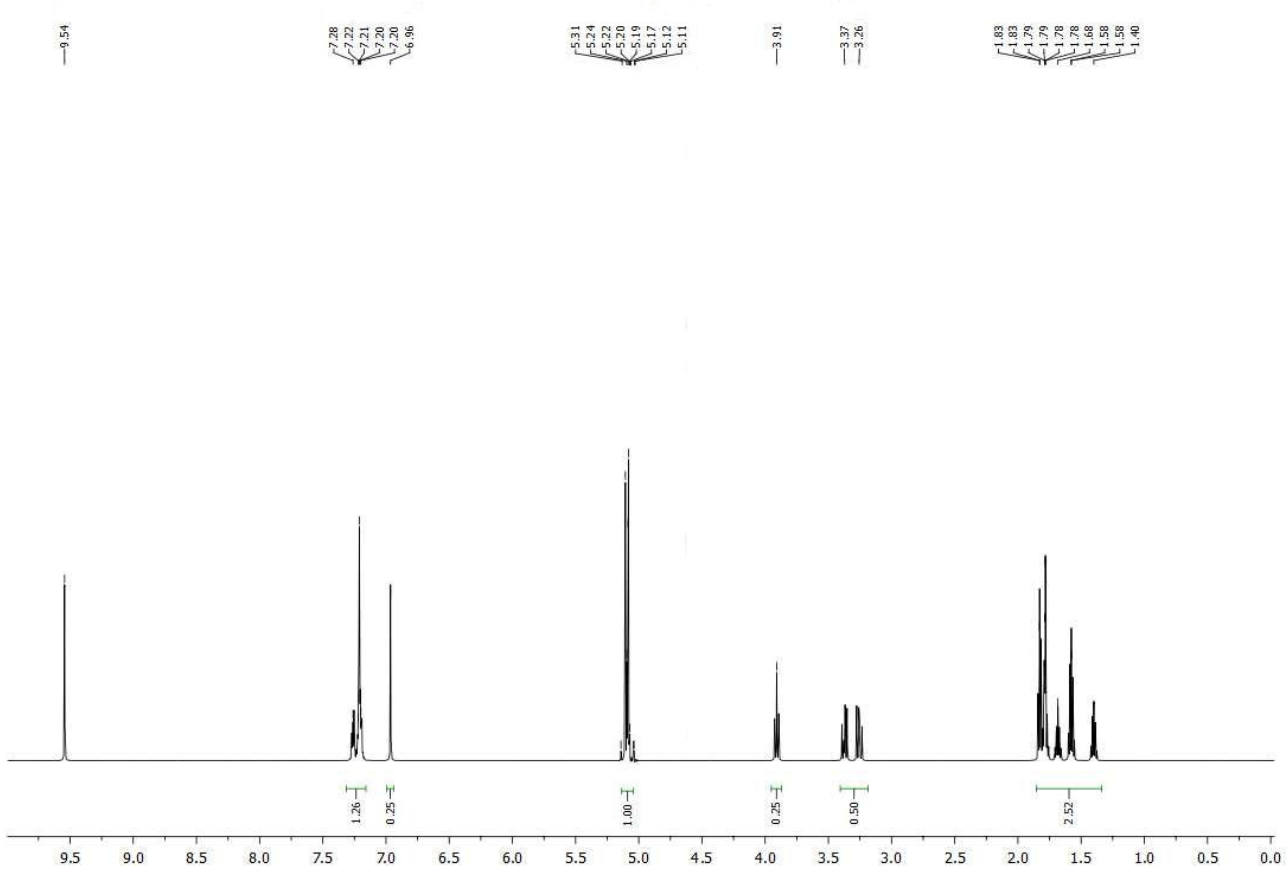
Cell Cycle assay

The cell cycle was analyzed with propidium iodide staining. After the cells were incubated with the test compound for 24 h, they were collected, washed 1–2 times with phosphate

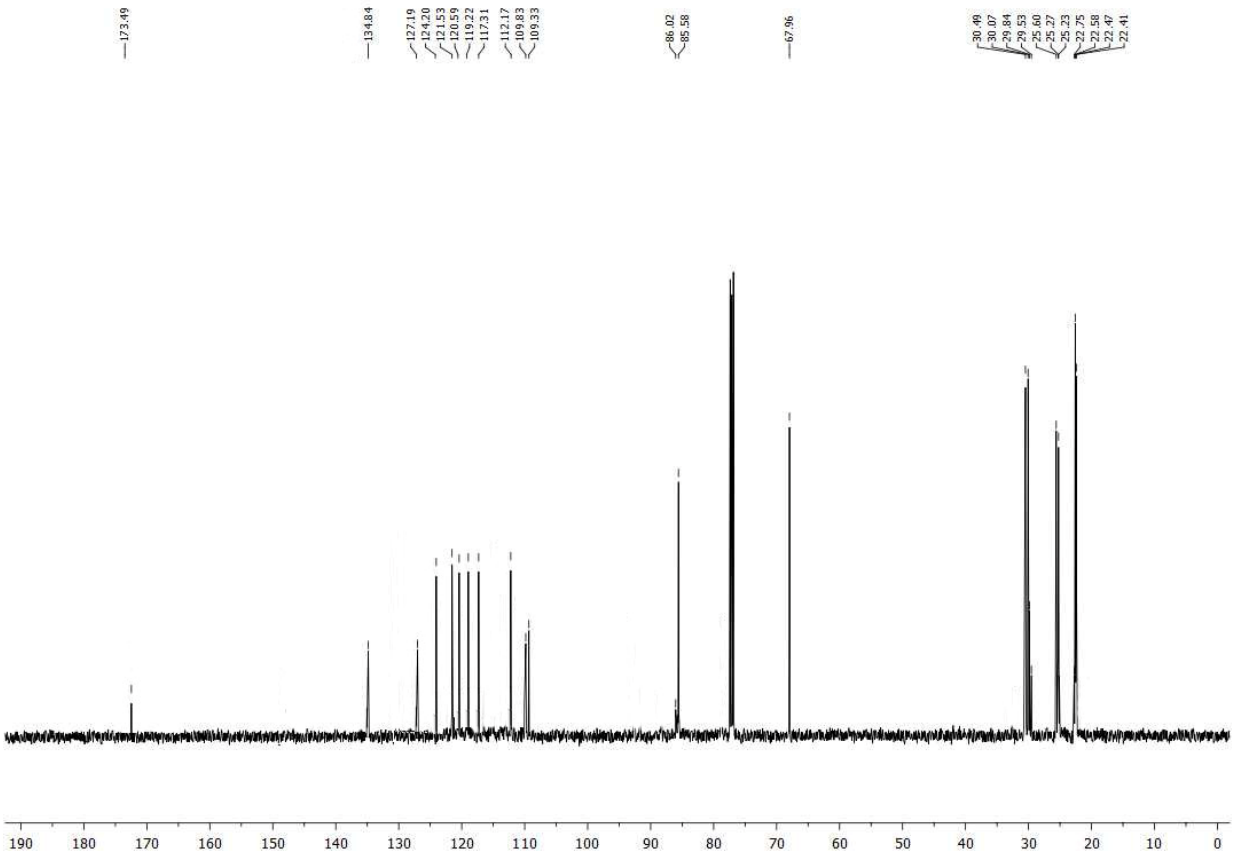
buffered saline (PBS), and centrifuged at 450 g for 5 min. The cells were then resuspended in 200 µl of flow cytometry staining buffer (PBS without Ca2+ and Mg2+, 2.5% FBS). At the next stage, the cells were transferred to 24-well plates with a density of 10 × 105 cells per well, then centrifuged at 450 g for 5 min, then fixed with cold ethanol 70% at 0°C for 24 h. Then the cells were washed from ethanol PBS buffer and incubated with 250 µl cell cycle detection reagent (Millipore) for 40 min at 22° C. in the dark. The prepared samples were analyzed on the NovoCyte Penteon Flow Cytometer Systems (ACEA) flow cytometry system.

B. Copy of NMR spectra

^{1}H-NMR spectrum (500 MHz, $CDCl_3$, 25 °C) of **33-(1H-indol-3-yl)-2-(7,8,12,13-tetraoxa-10-azaspiro[5.7]tridecan-10-yl)propanoic acid) (3)**



^{13}C-NMR spectrum (100 MHz, $CDCl_3$, 25 °C) of **33-(1H-indol-3-yl)-2-(7,8,12,13-tetraoxa-10-azaspiro[5.7]tridecan-10-yl)propanoic acid) (3)**



C. References

1. Makhmudiyarova, N. N.; Khatmullina, G. M.; Rakhimov, R. Sh.; Ibragimov, A. G.; and Dzhemilev, U. M. Synthesis of pentaoxaspiroalkanes and pentaoxocanes catalyzed by lanthanide compounds. *Arkivoc*, **2016**, *5*, 427-433.