

**A novel microtubule-targeting estradiol dimer as a cell division inhibitor
with minimal impact on interphase cells**

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1. Experimental Section

1.1. Synthesis of Compound 2

(17 β ,17' β)-17,17'-[1,5-Dihydroxycyclooctane-1,5-diylbis(methanediyl-1*H*-1,2,3-triazole-1,4-diyl)]-bis[estra-1,3,5(10)-triene-3,17-diol] (**2**) was obtained *via* described procedure.^{S1} To a solution of (1*s*,5*s*)-1,5-bis(azidomethyl)cyclooctane-1,5-diol (25 mg, 0.1 mmol) in dry DMF (5 mL) CuSO₄·5H₂O (5 mg, 0.02 mmol), Et₃N (4 mg, 0.04 mmol), ethinylestradiol (71 mg, 0.24 mmol) and sodium ascorbate (16 mg, 0.08 mmol) were added. The mixture was stirred at 80 °C for 24 hours and quenched with a saturated aqueous solution of Trilon B (3 mL). The organic layer was separated; the aqueous layer was extracted using EtOAc (3 × 3 mL). Combined organic layers were washed with a saturated aqueous solution of Trilon B (3 × 3 mL) and water (3 × 3 mL). The solvent was evaporated under reduced pressure; the product was isolated via preparative column chromatography (SiO₂). Yield 52% (44 mg). Brown solid, m.p. 187–189 °C, R_f = 0.5 (CHCl₃–CH₃CN 5:1).

¹H NMR (400 MHz, CD₃OD) δ , ppm: 0.61-0.78 (m, 2H, 2CH₂, estradiol), 1.02 (s, 6H, 2CH₃), 1.20-1.68 (m, 18H, 6CH₂, cyclooctane; 8CH₂, estradiol; 4CH, estradiol), 1.69-2.18 (m, 16H, 6CH₂, cyclooctane; 8CH₂, estradiol; 2CH, estradiol), 2.38-2.51 (m, 2H, 2CH₂, estradiol), 2.64-2.84 (m, 4H, 2CH₂, estradiol), 4.21-4.38 (m, 4H, 2CH₂N), 6.37-6.55 (m, 4H, 4CH, estradiol), 6.97 (d, 2H, ³*J* = 8.4 Hz, 2CH, estradiol), 7.78 (s, 1H, CH, triazole), 7.79 (s, 1H, CH, triazole).

¹³C NMR (400 MHz, CD₃OD) δ , ppm: 15.0 (2CH₃), 18.6 (2CH₂, cyclooctane), 24.8 (2CH₂, estradiol), 27.7 (2CH₂, estradiol), 28.9 (2CH₂, estradiol), 30.9 (2CH₂, estradiol), 34.5 (2CH₂, estradiol), 36.8 (CH₂, cyclooctane), 36.9 (CH₂, cyclooctane), 36.96 (CH₂, cyclooctane), 37.02 (CH₂, cyclooctane), 38.5 (2CH₂, estradiol), 41.2 (2CH, estradiol), 45.1 (2CH, estradiol), 61.4 (CH₂N), 61.5 (CH₂N), 74.87 (C-OH), 74.90 (C-OH), 83.3 (2C, estradiol), 113.8 (2CH, estradiol), 116.2 (2CH, estradiol), 125.7 (2CH, triazole), 127.3 (2CH, estradiol), 132.6 (2C, estradiol), 138.9 (2C, estradiol), 154.9 (2C, triazole), 156.0 (2C, estradiol). Two signals in δ_c 48.5-50.0 ppm region were not observed due to overlapping with CD₃OD signal. Analytical data were in accordance to those previously reported.^{S1}

1.2. Cell Cultures

This study utilized the human breast adenocarcinoma cell line (MCF7), obtained from the European collection of authenticated cell cultures (ECACC), and the venous endothelial cells culture (EA.hy926), obtained from Clonetics BioWhittaker Inc. company (USA). Cells were cultured in a cell culture incubator (CellXpert C170, Eppendorf, Germany) at 37 °C in a humidified atmosphere containing 5% CO₂. The hybrid endothelial cell line (EA.hy926) was generated by fusing human umbilical vein endotheliocytes (HUVEC) with the human thioguanine-resistant A549 lung carcinoma clone. According to the ATCC and the manufacturer's database, EA.hy926 cells beyond the 100th population doubling retain the key properties and functions characteristic of differentiated endothelium. Cells were maintained in DMEM medium (Gibco, USA) supplemented with the 10% fetal bovine serum (FBS, Hyclone, USA), 2 mM L-glutamine (Hyclone, USA), 50 μ M penicillin–streptomycin (PanEco, Russia). Subculturing was performed twice a week using 0.25% trypsin (Hyclone, USA). Experiments were conducted with EA.hy926 cells between passages 6 and 10.

The compound **2** was initially dissolved in dimethyl sulfoxide (DMSO) to prepare a 20 mM stock solution, which was then added to the culture medium to achieve the required working concentrations. The final DMSO concentration did not exceed 0.5% (v/v), a level that does not affect cell viability. For immunofluorescence assays, cells were treated with compound **2** (0.5, 1.5, and 25 μ M) for 24 h at 37 °C.

1.3. Immunofluorescence

For immunostaining, cells were grown on glass coverslips. All samples were fixed in ice-cold 100% methanol (−20°C) for 6 min. After washing with phosphate-buffered saline (PBS), cells were blocked by incubation with 3% bovine serum albumin (BSA, PanEco, Russia) in PBS for 30 min at room temperature. Cells were then incubated for 1 h at room temperature with a mouse monoclonal anti- α -tubulin antibodies (Clone DM1A, mab50379, Covalab, France), diluted 1 : 100 in PBS containing 1% BSA. Following three washes with PBS containing 1% BSA, bound primary antibodies were detected by incubation for 1 hour at room temperature with an Alexa Fluor 488-conjugated goat anti-mouse immunoglobulin G (IgG) antibody (Jackson ImmunoResearch, USA), diluted 1 : 1000 in PBS containing 1% BSA. After staining, coverslips were mounted onto glass slides using Mowiol mounting medium (Sigma-Aldrich Corp., USA) containing 0.6 mg/mL 4',6-diamidino-2-phenylindole (DAPI, Thermo Fisher Scientific, USA). Samples were analyzed using using a Zeiss LSM 900 confocal microscope equipped with an Airyscan 2 detector and a Plan-Apochromat 63 $\times$ /1.4 Oil DIC objective (Zeiss, Germany).

1.4. Live-Cell Imaging and Video Microscopy

For live-cell imaging, cells were plated on Petri dishes with a 170 μ m glass bottom (SPL Lifesciences, Korea). During imaging, dishes were maintained at 37 °C using a heated stage (Warner Instruments, USA) on an Eclipse Ti-E inverted microscope (Nikon Instruments Inc., Japan) equipped with a PerfectFocus system. After the addition of compound **2** at the specified concentration, single-plane time-lapse images were acquired every 10 minutes for a total duration of 10 hours.

1.5. Image and Video Analysis

All images were processed using ImageJ (open-source image processing software), Nikon ViewNX-i (Nikon Instruments Inc., Japan), and NIS-Elements D (Nikon Instruments Inc., Japan) software. Statistical analysis was performed using Microsoft Excel (Microsoft Corp., USA) and GraphPad Prism (GraphPad Software Inc., USA). Mean and standard deviation values were calculated from three independent experiments for each concentration. Data distribution was assessed for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Subsequent comparisons were made using an unpaired Student's t-test.

2. References

- S1 O. V. Ryzhikova, A. S. Churkina, K. N. Sedenkova, D. V. Savchenkova, A. S. Shakhov, S. V. Lavrushkina, Y. K. Grishin, N. A. Zefirov, O. N. Zefirova, Y. A. Gracheva, E. R. Milaeva, I. B. Alieva and E. B. Averina, *Arch. Pharm.*, 2024, **357**, e2400483; <https://doi.org/10.1002/ardp.202400483>.

3. Copies of NMR spectra

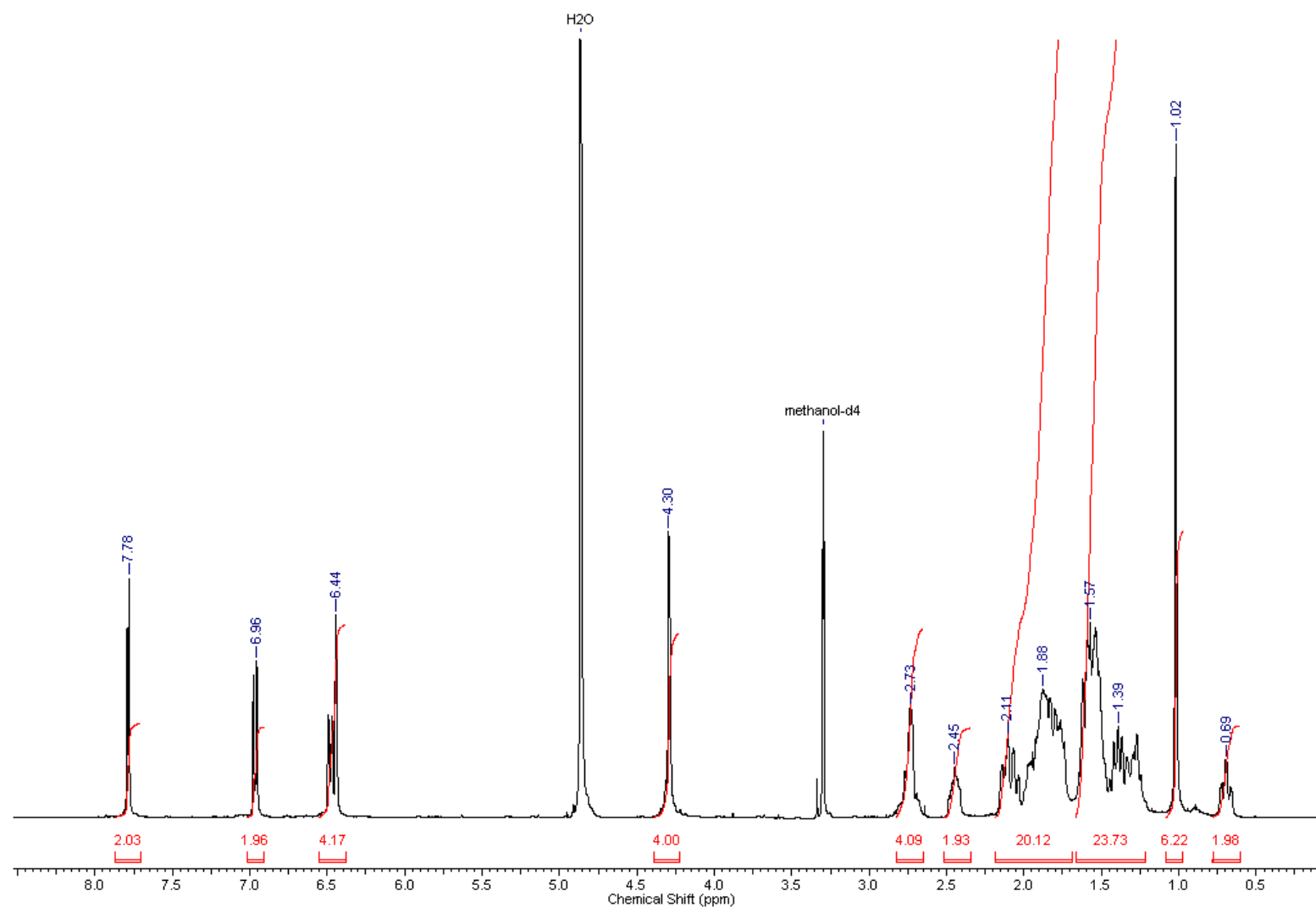


Figure S1 ¹H NMR spectrum (CD₃OD) of compound 2

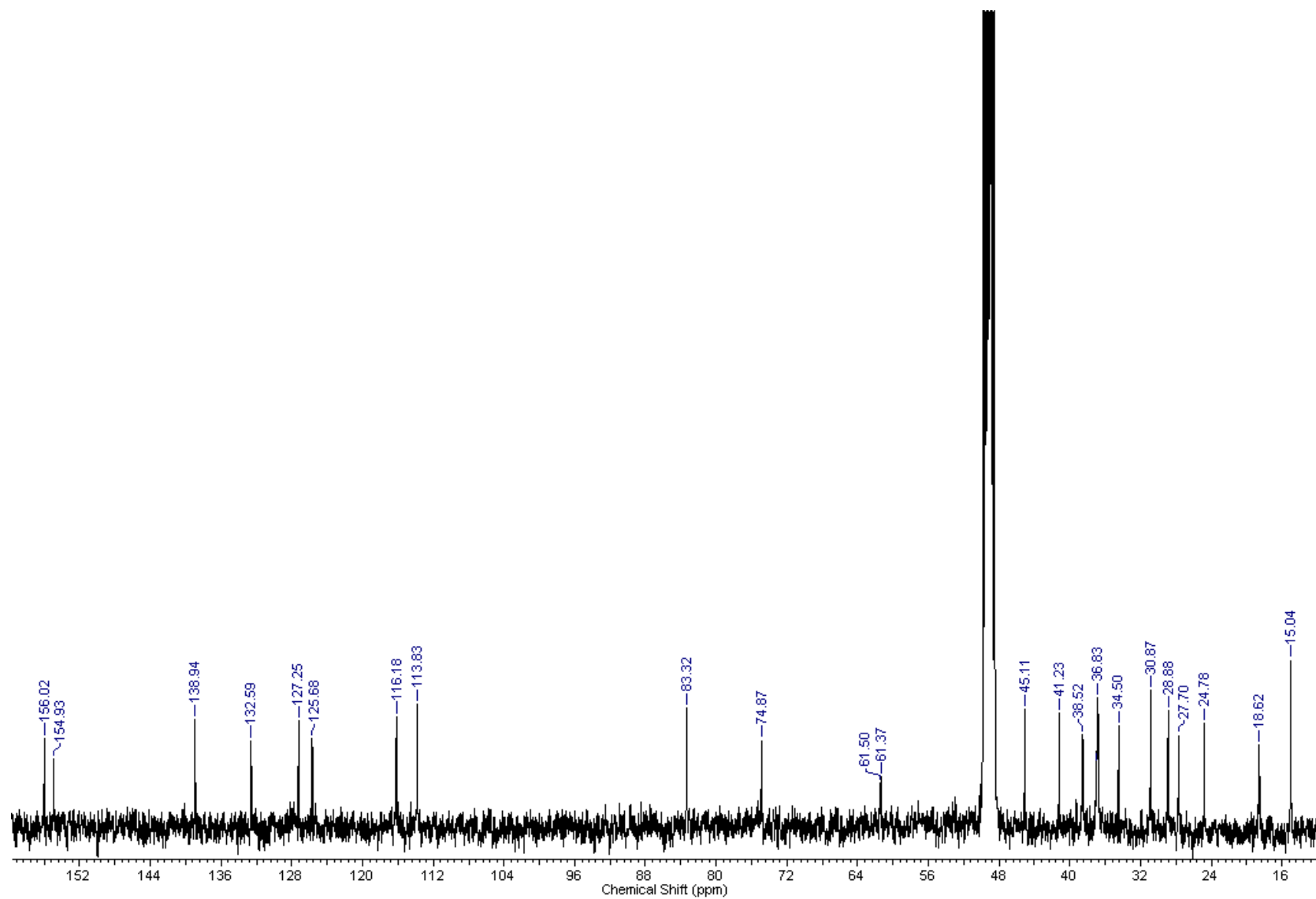


Figure S2 ^{13}C NMR spectrum (CD_3OD) of compound 2