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Synthesis of 1,4,2,6-dithiadiazinane 1,1-dioxide and study of its cytotoxic activity

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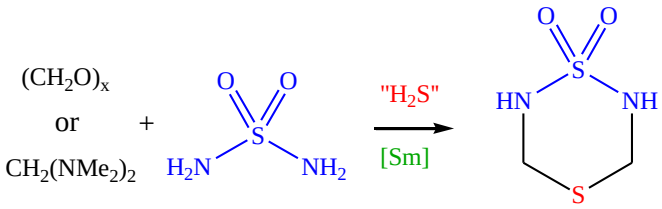
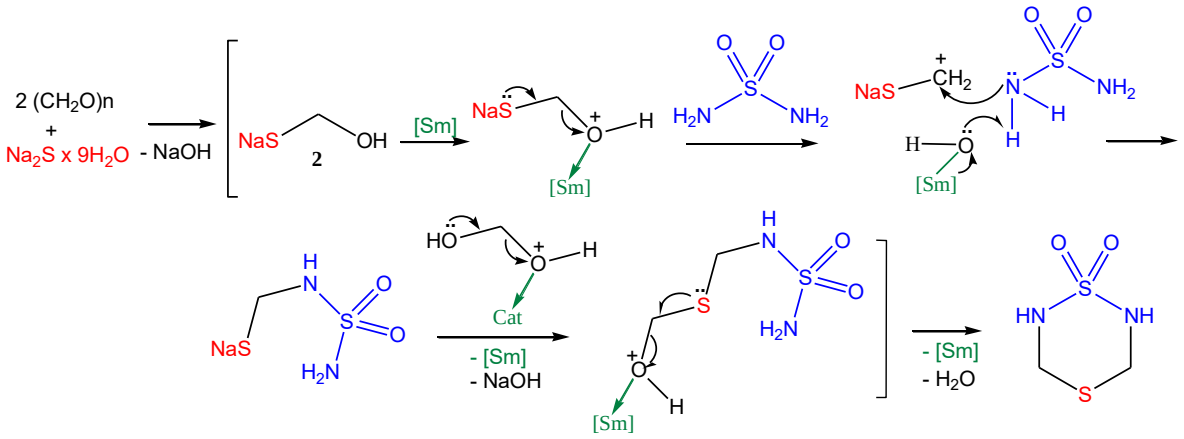


cytotoxic activity on tumor cell lines
Jurkat, K562, U937, HL60

Table of contents

Probable mechanism of the cyclothiomethylation reaction of sulfamide

As for the probable mechanism of sulfamide cyclothiomethylation with formaldehyde and $Na_2S•9H_2O$, one can assume that intermediate **2** is initially formed *in situ*. Then, the coordination of the hydroxyl oxygen in species **2** with the central atom of the catalyst [S1-S3] leads to the formation of a carbocation. Subsequent nucleophilic addition of sulfamide to the latter favors the formation of the final product **1** (Scheme S1).



Scheme S1 Probable mechanism of the cyclothiomethylation reaction of sulfamide with formaldehyde and $Na_2S•9H_2O$

References

[S1] M. Tramontini, L. Angiolini, *Tetrahedron*, 1990, **46**, 1791.
[S2] R. G. Pearson, *J. Chem. Edu.*, 1968, **45**, 643.
[S3] V. R. Akhmetova, G. R. Nadyrgulova, Z. T. Niatshina, U. M. Dzhemilev, *Chem. Heterocycl. Compd.*, 2009, **45** (10), 1155.

General information

The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance-400 spectrometer (400.13 and 100.62 MHz, respectively) in DMSO-d_6, internal standard was TMS. Two-dimensional homonuclear (COSY) and heteronuclear (HSQC, HMBC) experiments were carried out under Bruker standard procedures at the same operating frequencies. Infrared spectra (IR) were recorded using FT-IR spectrometer Bruker Vertex 70 v (Nujol mulls). Mass spectrum of compound **1** was recorded on a Bruker Autoflex III MALDI TOF/TOF instrument with sinapic acids as a matrix. Samples of the compound were prepared by the 'dried droplet method'. Mass spectrum of compound **2** was obtained on an LCMS-2010 EV quadrupole liquid chromatograph-mass spectrometer (Shimadzu) using atmospheric pressure chemical ionization (solutions of sample in MeOH were syringed at a rate of 100 μl min^{-1}, eluent acetonitrile—water (80 : 20)) in the positive ion mode (capillary poten tial 4.5 kV). Capillary temperature was 250°C, capillary

voltage was 25 V. Flow rate of nebulizer gas (nitrogen) was 1.5 dm min^{-1}. The voltage across the high-frequency lenses (Q-array) was 5.–5 V. Melting points were determined on a PHMK 80/2617 apparatus. Monitoring of the progress of reactions was effected by TLC on Sorbfil (PTSKh-AF-A) plates, eluent was acetone/ethyl acetate, 2:1, visualization with I_2 vapor.

Cyclothiomethylation of sulfamide with paraformaldehyde or bis(dimethylamino)methane and H_2S. A glass reactor equipped with a magnetic stirrer and an adapter for gas input, was charged with paraformaldehyde (2.5 mmol, 0.06 g, method *a*) or bis-(dimethylamino)methane (method *b*, 2.5 mmol, 0.255 g), EtOH (5 ml), and with constant stirring for 1 hour, the mixture was saturated with hydrogen sulfide. Then sulfamide (1 mmol, 0.096 g), EtOH (5 ml) and $SmCl_3 \cdot 6H_2O$ (0.2 mmol) were added, and this was stirred at 60 ºC for 3 h. Compound **1** was purified by column chromatography on SiO_2.

Cyclothiomethylation of sulfamide with paraformaldehyde and $Na_2S \cdot 9H_2O$ or NaHS was carried out similarly to method *a*. Hydrogen sulfide was replaced by $Na_2S \cdot 9H_2O$ (1.5 mmol, 0.360 g, method *c*) or NaHS (1.5 mmol, 0.09 g, method *d*).

1,4,2,6-Dithiadiazinane 1,1-dioxide (1). Yield 0.105 g (68%, method *a*), 0.014 g (9%, method *b*), 0.100 g (65%, method *c*), 0.034 g (23%, method *d*), white powder, mp 190-192 °C. IR ν cm^{-1}: 3265 (N—H); 2923-2854 (CH); 1615, 1414 (NH); 1385 (CH); 1330 (S=O), 1300, 1207 (C—S); 1149 (S=O); 722 (C—S). ^{1}H NMR (400.13 MHz, DMSO-d_6): 4.62 (4 H, d, C<u>H</u>$_2$, ^3J 8.0 Hz); 7.01 (2 H, t, N<u>H</u>, ^3J 8.0 Hz). ^{13}C NMR (100.62 MHz, DMSO-d_6): 49.02 (N-<u>C</u>H_2-S). MALDI TOF, *m/z*: 189.0027 $[M + 2H_2O - H]^+$ (189.0004 calculated for $C_2H_9N_2O_4S_2^+$).

Reaction of paraformaldehyde with $Na_2S \cdot 9H_2O$ (*in situ*). Paraformaldehyde (2.5 mmol, 0.06 g), $Na_2S \cdot 9H_2O$ (1.5 mmol, 0.360 g), EtOH (10 ml) were loaded into a glass reactor, and the mixture was stirred for 2 h at 60 ºC.

Sodium (hydroxymethyl)thiolate (2) *(in situ).* ^{1}H NMR (400.13 MHz, DMSO-d_6): 4.63 (2 H, s, OC<u>H</u>$_2$S). ^{13}C NMR (100.62 MHz, DMSO-d_6): 89.03 (O<u>C</u>H_2S). MS (APCI, 200 eV), *m*/*z* (I_{rel} (%)): 105 $[M + H + H_2O]^+$ (37); 64 $[M - Na]^+$ (36).

NMR and mass spectra of compounds 1 and 2

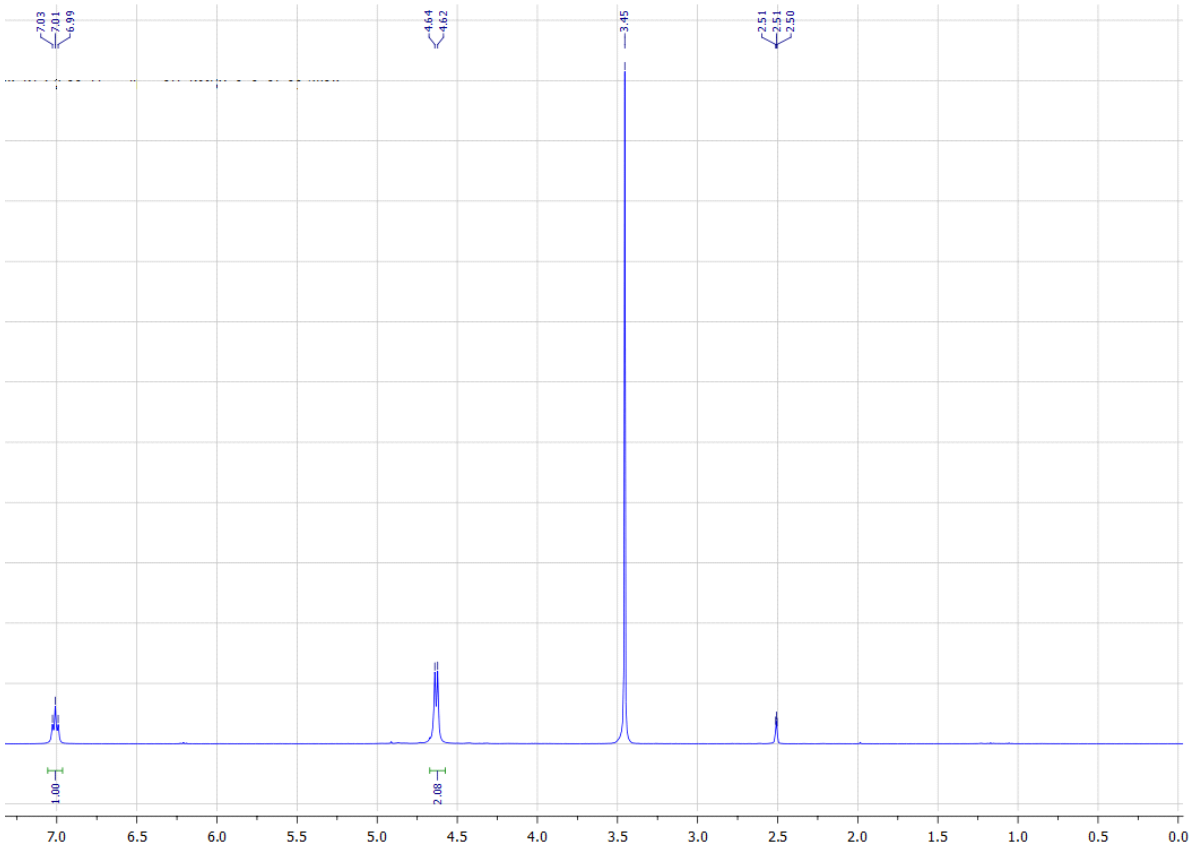


Figure S1. ^{1}H NMR spectrum of **1** in DMSO-d_6.

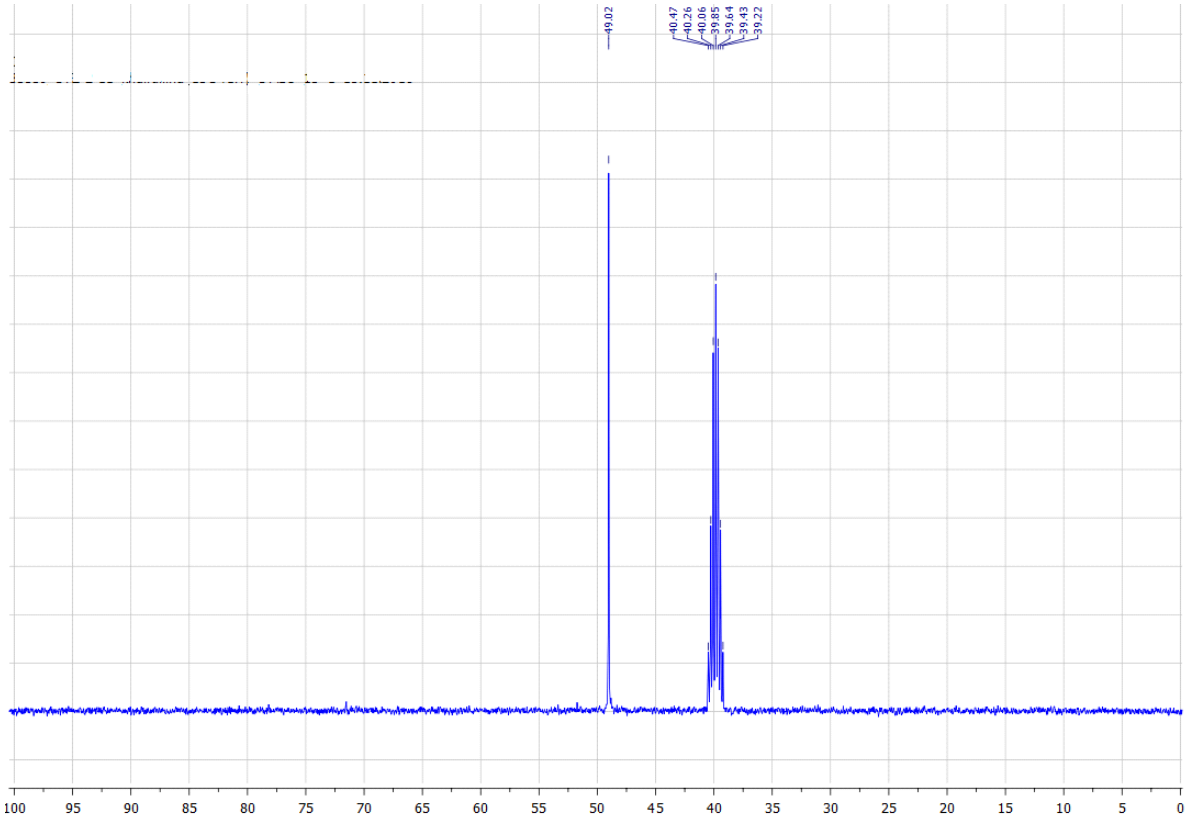


Figure S2. ^{13}C NMR spectrum of **1** in DMSO-d_6.

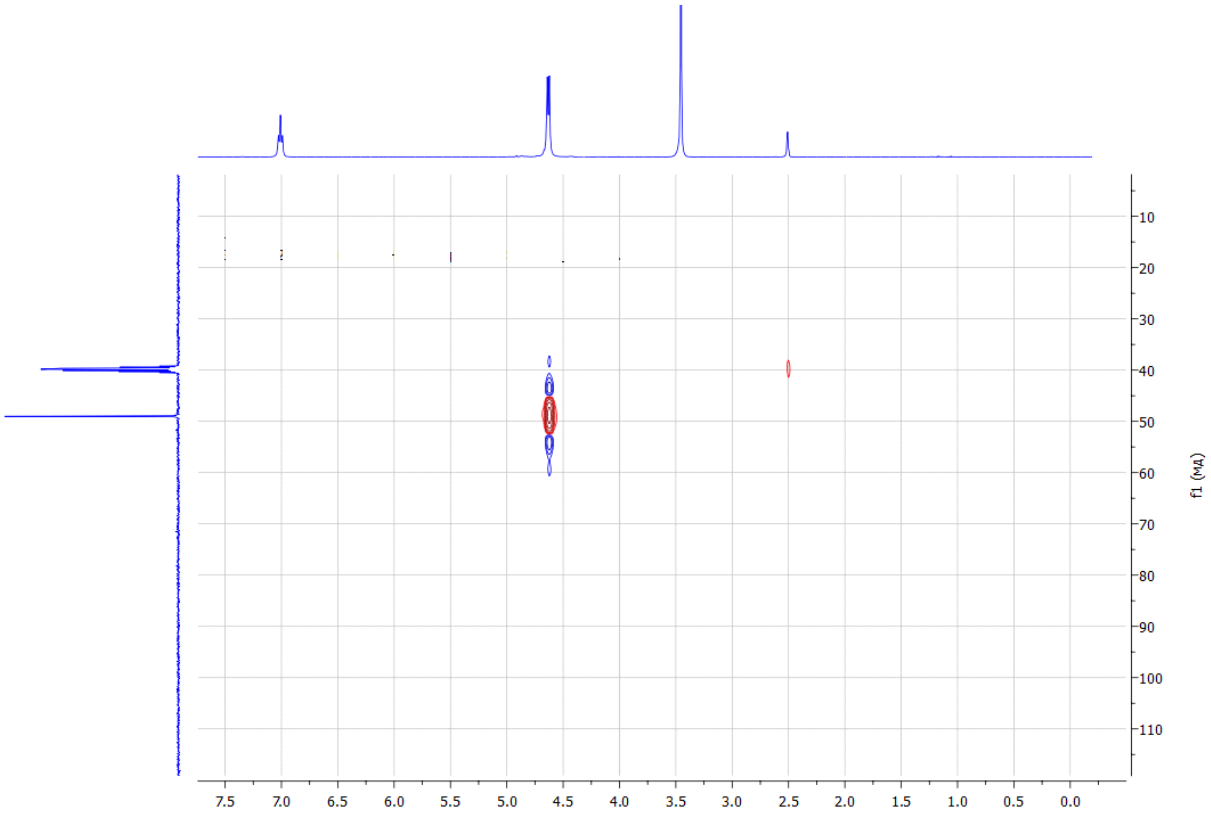


Figure S3. HSQC spectrum of **1** in DMSO-d_6.

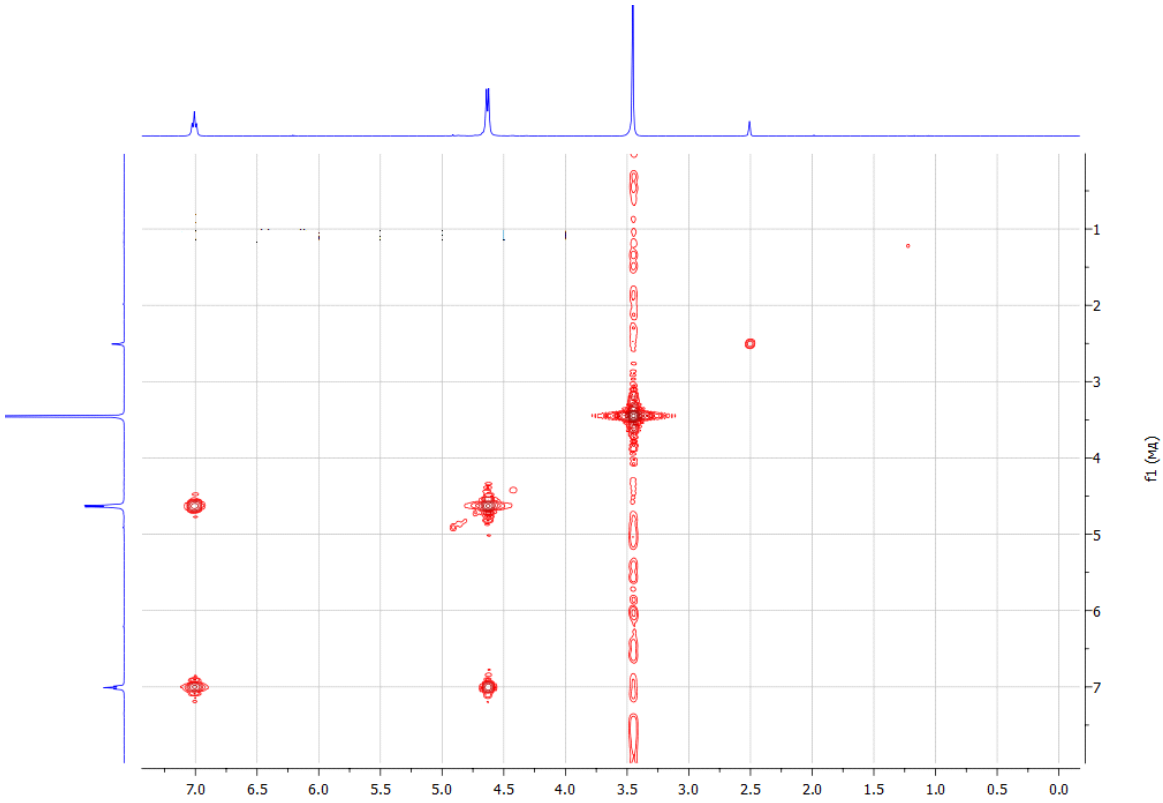


Figure S4. COSY spectrum of **1** in DMSO-d_6.

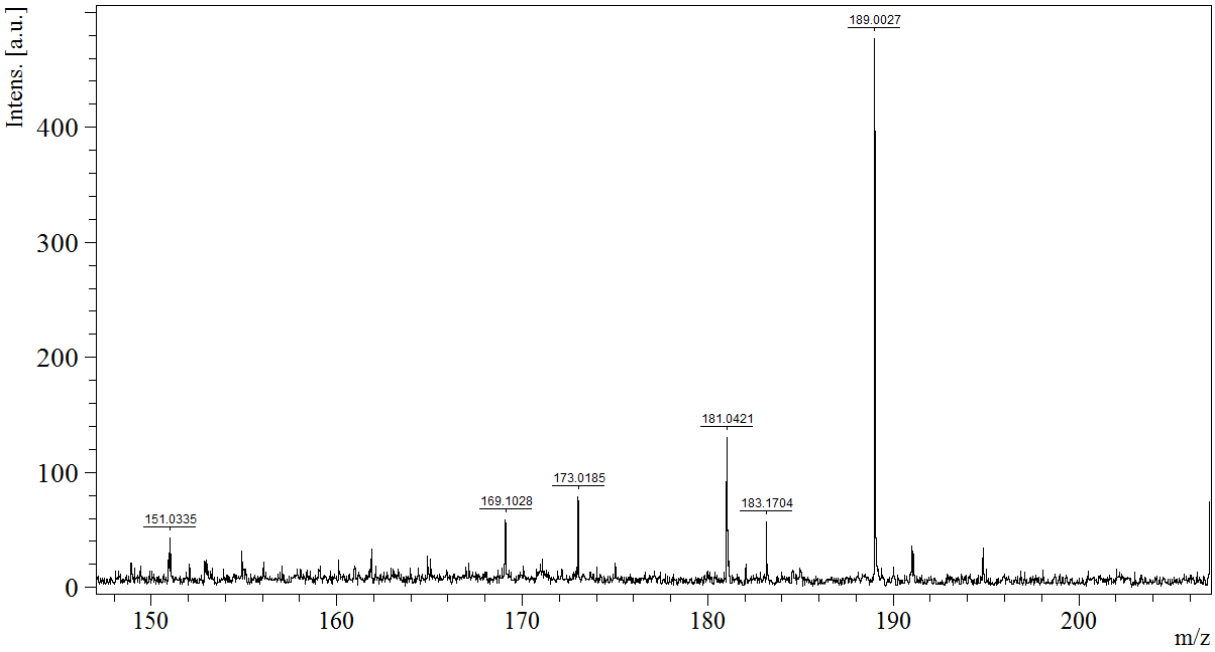


Figure S5. MALDI TOF mass spectrum of **1** (Sinapic acid matrix)

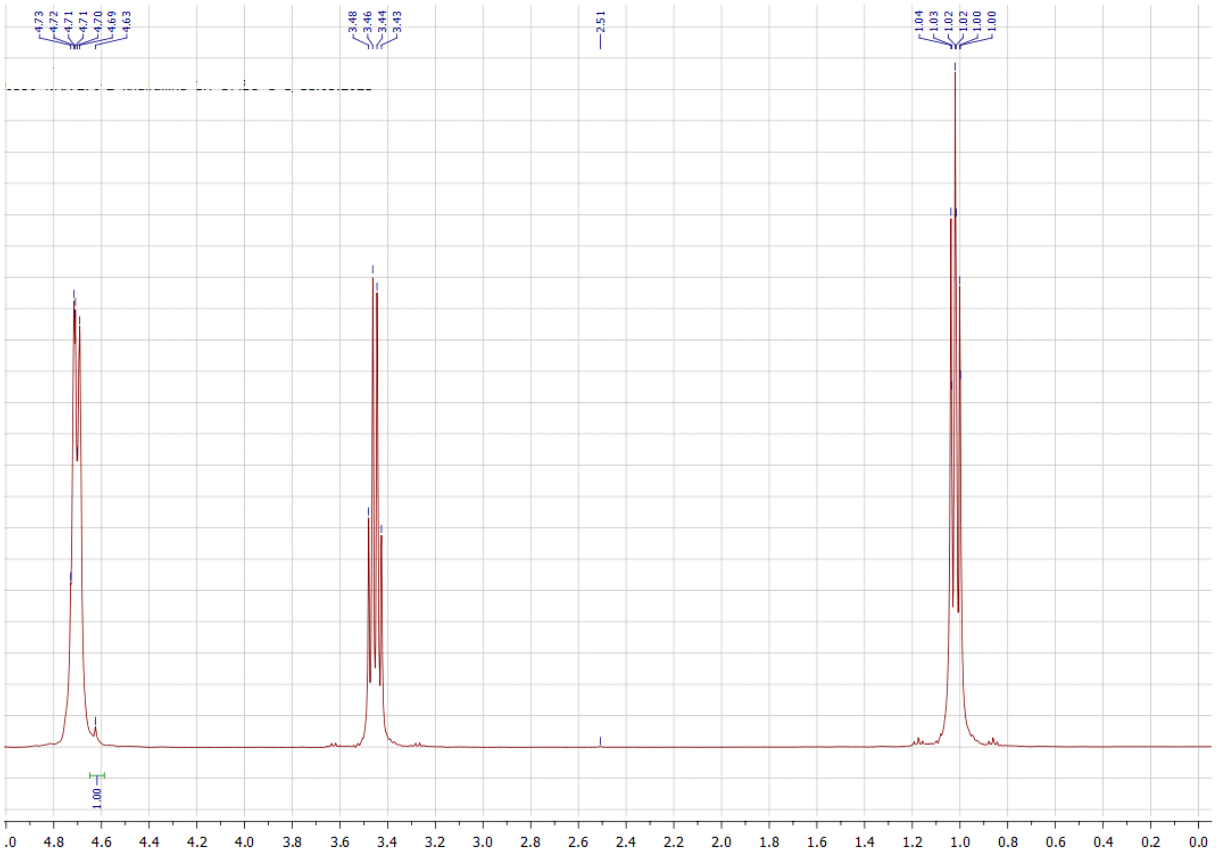


Figure S6. ^{1}H NMR spectrum of **2** in DMSO-d_6.

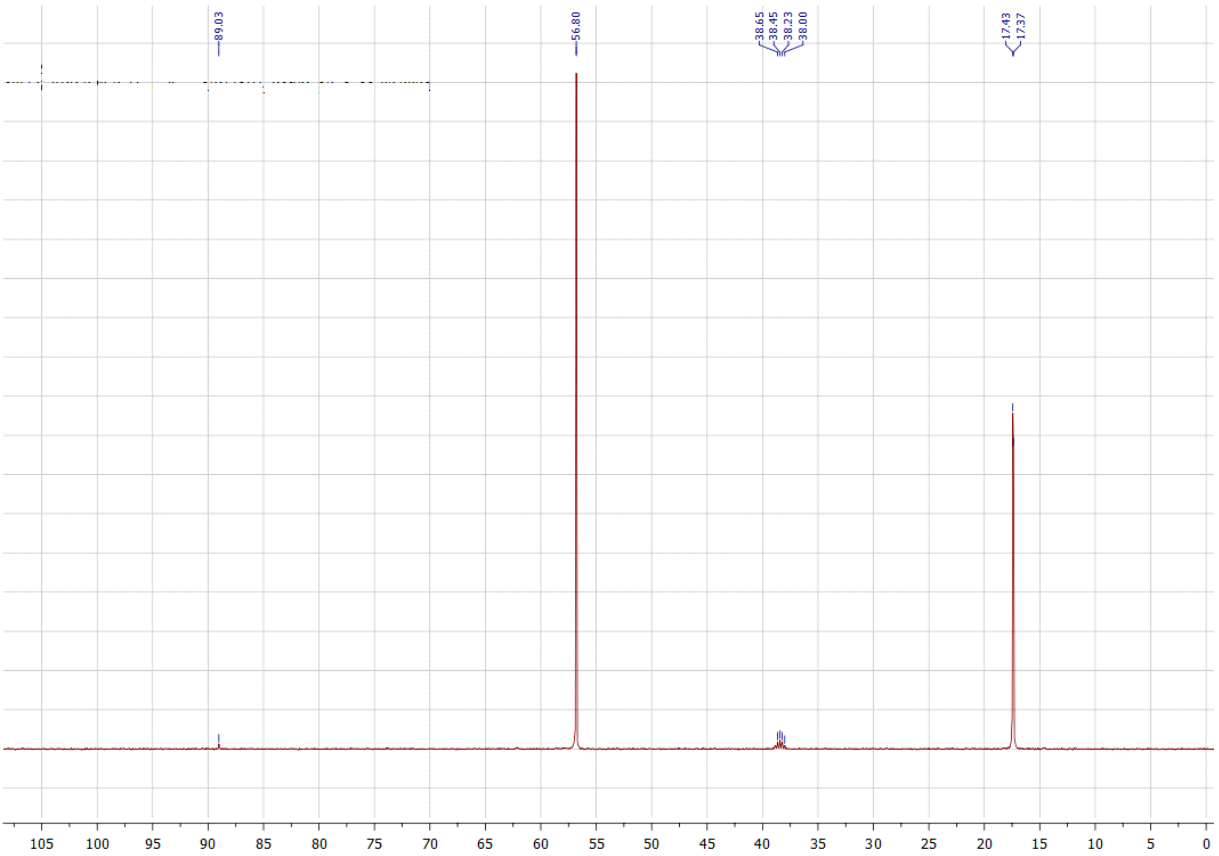


Figure S7. ^{13}C NMR spectrum of **2** in DMSO-d_6.

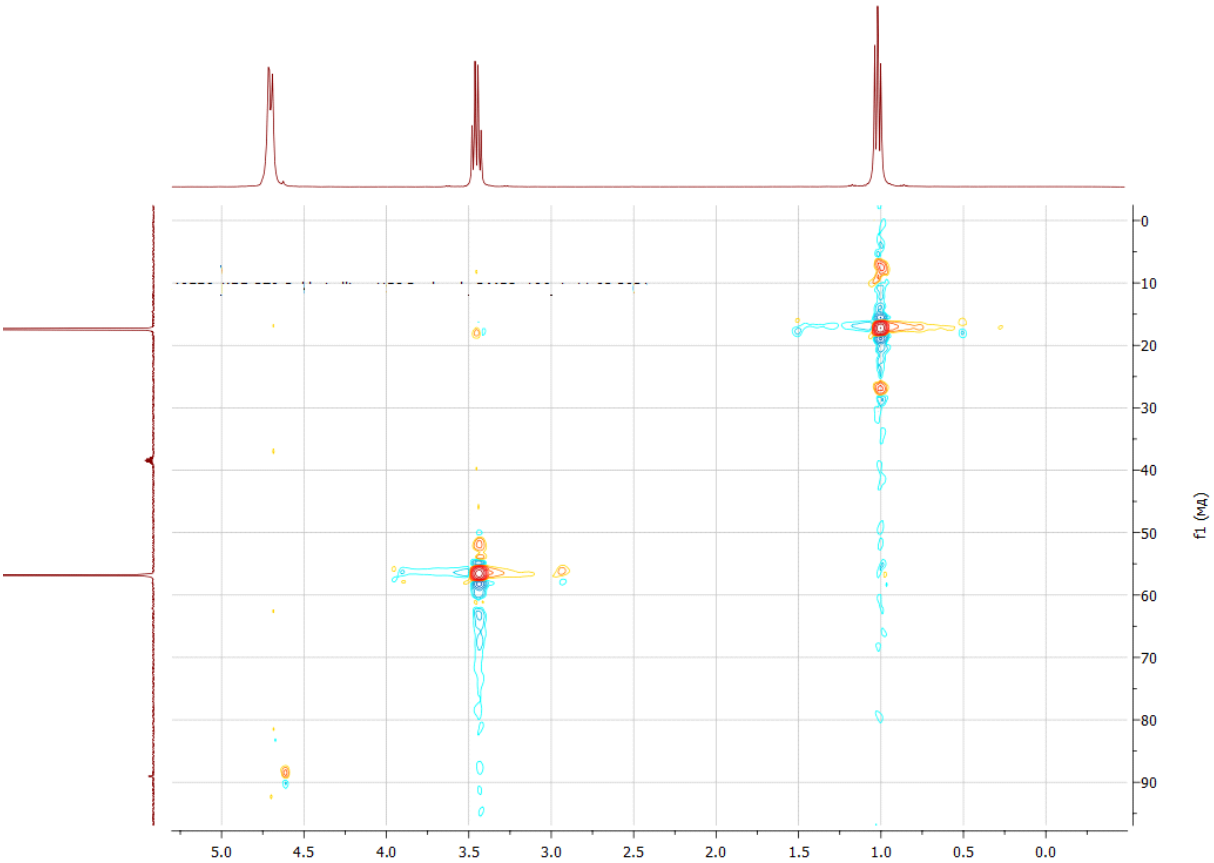


Figure S8. HSQC spectrum of **2** in DMSO-d_6

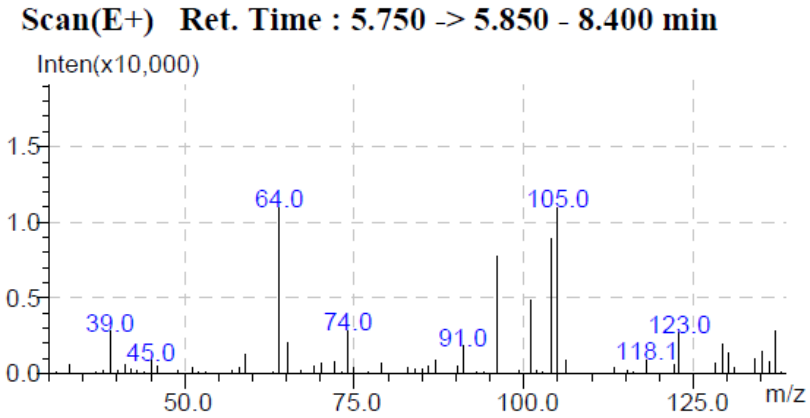


Figure S9. ESI mass spectrum (MeOH/HOH 100/0, 0.1 mL/min, CH_3OH) of **2**

Biology studies

Cell culturing

Cells (Jurkat, K562, U937, HL-60) were purchased from Russian Cell Culture Collection (Institute of Cytology of the Russian Academy of Sciences) and cultured according to standard mammalian tissue culture protocols and sterile technique. All cell lines used in the study were tested and shown to be free of mycoplasma and viral contamination.

Cells were maintained in RPMI 1640 (Jurkat, K562, U937) (Gibco) supplemented with 4 mM glutamine, 10% FBS (Sigma) and 100 units per ml penicillin-streptomycin (Sigma). All types of cells were grown in an atmosphere of 5 % CO_2 at 37 °C. The cells were subcultured at 2-3 days intervals. Cells were then seeded in 24 well plates at $5x10^4$ cells per well and incubated overnight. Jurkat, K562, U937, HL-60 cells were subcultured at 2 day intervals with a seeding density of 1×10^5cells per 24 well plates in RPMI with 10% FBS.

Cytotoxicity assay

Viability (live/dead) assessment was performed by staining cells with 7-AAD (7-Aminoactinomycin D) (Biolegend). After treatment cells were harvested, washed 1-2 times with phosphate-buffered saline (PBS) and centrifuged at 400g for 5 minutes. Cell pellets were resuspended in 200 uL of flow cytometry staining buffer (PBS without $Ca^{2+}$and Mg^{2+}, 2.5% FBS) and stained with 5 uL of 7-AAD staining solution for 15 minutes at room temperature in the dark. Samples were acquired on NovoCyteTM 2000 FlowCytometry System (ACEA) equipped with 488 nm argon laser. Detection of 7-AAD emission was collected through a 675/30 nm filter in FL4 channel.