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A naphthalimide-based fluorescent and colorimetric probe for the detection of mercury(II) ions in aqueous solutions and in living cells

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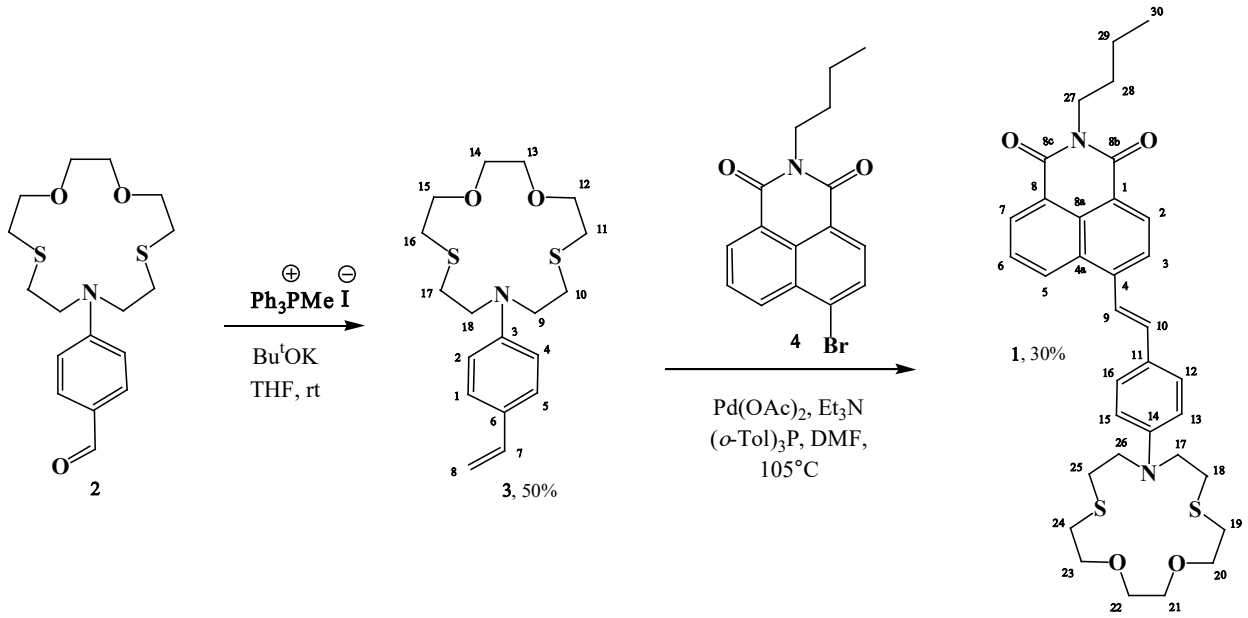
CONTENTS

1. Experimental Section

Synthesis of compounds

Compound **1** was synthesized by the Heck reaction between 4-bromo-*N*-butyl-1,8-naphthalimide **4** and crown-containing styrene **3** (Scheme S1). Styrene **3** was synthesized from aldehyde **2** using the Wittig reaction (see experimental protocol for the styrene derivative with similar to **3** structure in *Angew. Chemie*, 2018, **130**, 3784 - 3788). Melting point of **1** was measured on Melt-temp melting point electrothermal apparatus and was uncorrected. The reaction course and purity of the final product was followed by TLC on silica gel (DC-Alufolien Kieselgel 60 F254, Merck). Column chromatography was conducted on silica gel (Kieselgel 60, particle size 0.063-0.200 mm, Merck). Flash chromatography was performed using a Biotage IsoleraTM Prime system. ^{1}H and ^{13}C NMR spectra were recorded on an Avance 300 and Avance 600 spectrometers (Bruker). The measurements were performed in DMSO-d_6 and CD_2Cl_2 solutions. The chemical shifts (given as δ) were determined with an accuracy of 0.01 ppm relative to the signals corresponding to the residual solvents and recalculated to the internal standard (TMS); the spin-spin coupling constants (J) were measured with an accuracy of 0.1 Hz. The assignment of ^{1}H and ^{13}C signals is based on 2D NMR experiments (HMBC, HSQC, ^{1}H COSY), which were performed using standard pulse sequences from the Bruker library. LC-ESI-MS analyses were performed on a Finnigan LCQ Advantage mass spectrometer equipped with octopole ion-trap mass-analyzer, MS Surveyor pump, Surveyor auto sampler, Schmidlin-Lab nitrogen generator (Germany) and Finnigan X-Calibur 1.3 software for data collecting and processing.



Scheme S1

10-(4-Vinylphenyl)-1,4-dioxa-7,13-dithia-10-azacyclopentadecane (**3**). In argon atmosphere Bu^tOK (168 mg, 1.5 mmol) was added to $Ph_3P^+MeI^-$ (606 mg, 1.5 mmol) in dry THF (10 ml), then aldehyde **2** (264 mg, 0.75 mmol) in dry THF (5 ml) was quickly added dropwise. The reaction mass was stirred for 15 hours at ambient temperature. At the end of the reaction, hexane (5 ml) was added to the mixture. The precipitate was filtered off and the filtrate was evaporated to yield 133 mg (50%) of crude product **3** as a yellow oil which was immediately used at the next step without further purification. ^{1}H NMR (300.13 MHz, CD_2Cl_2, 21 ºC, δ / ppm, J / Hz): 2.70-2.80 (m,4H, H(10), H(17)), 2.83-2.94 (m, 4H, H(11), H(16)); 3.58-3.65 (m, 8H, H(12), H(13), H(14), H(15)); 3.75-3.81 (m, 4H, H(9), H(18)); 5.52 (d, 1H, H(8), ^3J = 17.3), 6.55-6.62 (m, 2H, H(7)); 6.69 (d,2H, H(2), H(4) ((H(1), H(5)), ^3J = 8.4); 7.26(d, 2H, (d,2H, H(1), H(5) ((H(2), H(4))), ^3J = 8.4). ^{1}H NMR spectrum of compound **3** in DMSO-d_6 is shown in Fig.S5.

(E)-6-[4-(1,4-dioxa-7,13-dithia-10-azacyclopentadecan-10-yl)styryl]-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (**1**). A solution of 4-bromo-*N*-butyl-1,8-naphthalimide **4** (55 mg, 0.17 mmol), $Pd(OAc)_2$ (19 mg, 0.08 mmol), tris(*o*-tolyl)phosphine (25 mg, 0.08 mmol), triethylamine (300 μL) and styrene **3** (118 mg, 0.33 mmol) in dry DMF (5 ml) was stirred at 105°C for 16 h under argon atmosphere. The reaction mass was evaporated and subjected to column flash-chromatography (silica gel, gradient elution with CH_2Cl_2 - MeOH solvent mixture) to give pure product **1** (30 mg, 29%). M. p. 244 – 246 ºC. ^{1}H NMR (400.02 MHz, DMSO-d_6, 21 ºC, δ / ppm, J / Hz): 0.88 - 0.97 (m, 3H, H (30)), 1.27 - 1.47 (m, 2H, H (29)), 1.55 - 1.69 (m, 2H, H (28)), 2.70 – 2.80 (m, 4H, H(18), H(25), 2.81 – 2.90 (m, 4H, H(19), H(24), 3.54 – 3.61 (m, 4H, H(21), H(22), 3.62 – 3.77 (m, 8H, CH_2(17), H(26), H(20), H(23)), 6.68 (d, 2H, H(13), H(15), ^3J = 7.5), 7.52 (d, 1H, H(10), ^3J = 16.5), 7.69 (d, 2H, H(12), H(16), ^3J = 7.5), 7.83 – 7.98 (m, 2H, H(9), H(6)), 8.18 (д, 1H, H(3), ^3J = 7.9), 8.43 (d, 1H, H(2), ^3J = 7.9), 8.52 (d, 1H, H(7), ^3J = 6.6), 8.97 (d, 1H, H(5), ^3J = 8.3). ^{13}C NMR (150.93 MHz, DMSO-d_6, 22 ºC, δ / ppm): 154.79 (C(30)), 20.59 (C(29)), 30.46 (C(19), C(24)), 30.79 (C(28)), 31.21 (C(18), C(25)), 40.77 (C(27)), 51.64 (C(17), C(26)), 69.72 (C(21), C(22)), 73.82 (C(23), C(20)), 111.85 (C(13), C(15)), 118.13 (C(9)), 121.81 (C(1)), 122.66 (C(3)), 122.73 (C(8)), 126.24 (C(6)), 128.64 (C(8a)), 128.75 (C(12), C(16)), 129.44 (C(4a)), 129.55 (C(11)), 129.91 (C(5)), 130.66 (C(7)), 130.82 (C(2)), 135.29 (C(10)), 142.20 (C(4)), 147.72 (C(14)), 163.91 (C(8b)), 164.23 (C(8c)). ESI-mass spectrum, calculated ($[M+H]^+$), m/z: 605.25; found: 605.45. ^{1}H and ^{13}C NMR spectrum of compound **1** in DMSO-d_6 are shown in Figures S1 S6.

Optical studies in solution

The absorption spectra of **1** in the presence and absence of metal cations were taken on a Cary 300 spectrophotometer (Agilent Technologies). The fluorescence quantum yield measurement for compound **1** was performed using a Cary 300 spectrophotometer and a Fluorolog3-221 spectrofluorimeter (Horiba Jobin Yvon). Spectral measurements were carried out in air-saturated dilute solutions at ambient temperature. All measured fluorescence spectra were corrected for the nonuniformity of detector spectral sensitivity. Coumarin 481 in acetonitrile (φ^{fl} = 0.08) [*J. Phys. Chem. A*, 2003, **107**, 4808] was used as a reference for the fluorescence quantum yield measurements. The fluorescence quantum yield was calculated by the Eq. (S1) [*Anal. Chem.*, 1983, **55**, 798],

$$\varphi^{fl} = \varphi_R^{fl} \frac{S}{S_R} \cdot \frac{(1-10^{-A_R})n^2}{(1-10^{-A})n_R^2} \quad (S1)$$

wherein φ^{fl} and φ_R^{fl} are the fluorescence quantum yields of the studied solution and the standard compound respectively; A and A_R are the absorptions of the studied solution and the standard respectively; S and S_R are the areas underneath the curves of the fluorescence spectra of the studied solution and the standard respectively; and n and n_R are the refraction indices of the solvents for the substance under study and the standard compound.

Acetate buffer solution was prepared with deionized water (18.2 MΩ·cm). Perchlorates of Ag^+, Zn^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, Cd^{2+}, Fe^{2+}, Mg^{2+} and Ca^{2+} were dissolved in deionized water (18.2 MΩ·cm) and then used in spectroscopic studies. Salt $Hg(ClO_4)_2$ was dissolved in water and stabilized by the addition of 0.5 equiv. $HClO_4$. The exact concentration of $Hg(ClO_4)_2$ was determined by complexometric titration using EDTA and xylenol orange as an indicator. All other reagents were purchased from commercial sources. DMSO and acetonitrile used in spectroscopic studies was of HPLC grade. Compound **1** was weighted and dissolved in DMSO. Then, the obtained stock solution (2·mM) was added to a deionized water to get diluted 5 μM solution containing 0.2 vol.% of DMSO for optical measurements.

Biological studies

Human embryonic kidney cells HEK 293 were grown in DMEM/F12 medium (PanEco, Russia) with the addition of 10% fetal calf serum HyClone defined (GE Healthcare Life Sciences, USA) and 2 mM glutamine (PanEco, Russia). Reseeding was carried out 2 times a week. For the experiment, cells in the logarithmic growth phase were planted on coverslips in 24-well plates (2×10^5 cells per well) and grown for 24 hours at 37°C, 5% CO_2. To conduct the studies, we used 1 and 5 mM stock solutions of compound **1** in DMSO and a 0.5 mM solution of **1** in 10% Cremophor EL emulsion (CrEL, provides stabilization of the monomeric form of hydrophobic compounds in aqueous solutions and is approved for use in clinics). To prepare stock solutions in emulsions, dry samples of the compounds were rubbed into CrEL and further adjusted with phosphate buffer (pH=7.0) to 10% CrEL. The concentration of compound **1** in 10% CrEL emulsion was determined spectrophotometrically using the parameters obtained for **1** in solutions of DMSO and 1% CrEL emulsion. For confocal laser scanning microscopy (CLSM), an LSM-710 microscope (Carl Zeiss AG, Germany, 100×/1.4 oil-immersion objective, resolution 0.3 and 1.5 μm in the lateral and axial planes) was used.

2. Figures S1 – S6

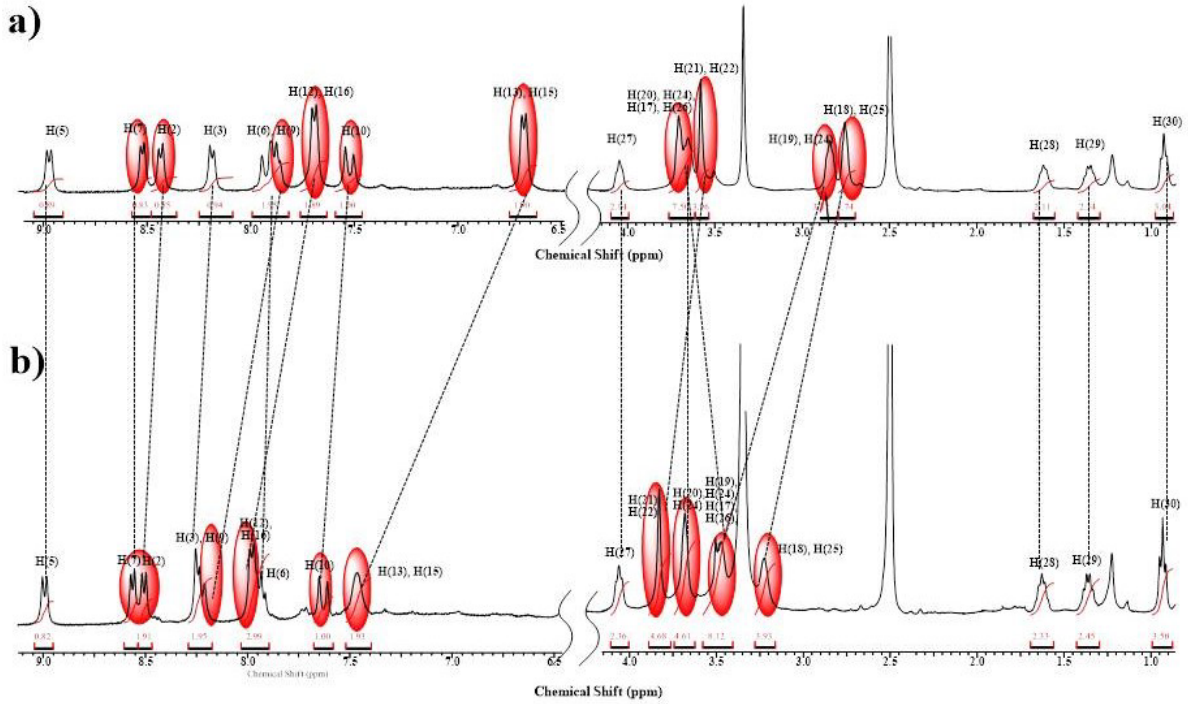


Figure S1. ^{1}H NMR spectra of compound **1** (10 mmol dm^{-3}) in DMSO-d_6 (*a*) before and (*b*) after addition of 5 equiv. $Hg(ClO_4)_2$. Spectrometer frequency 400.02 MHz.
The numbering of atoms in the **1** used to assign signals is shown in Scheme S1. Dash lines connect proton aignals of the complex and ligand having the same number. Proton signals undergoing a strong signal shift are marked with the red color.

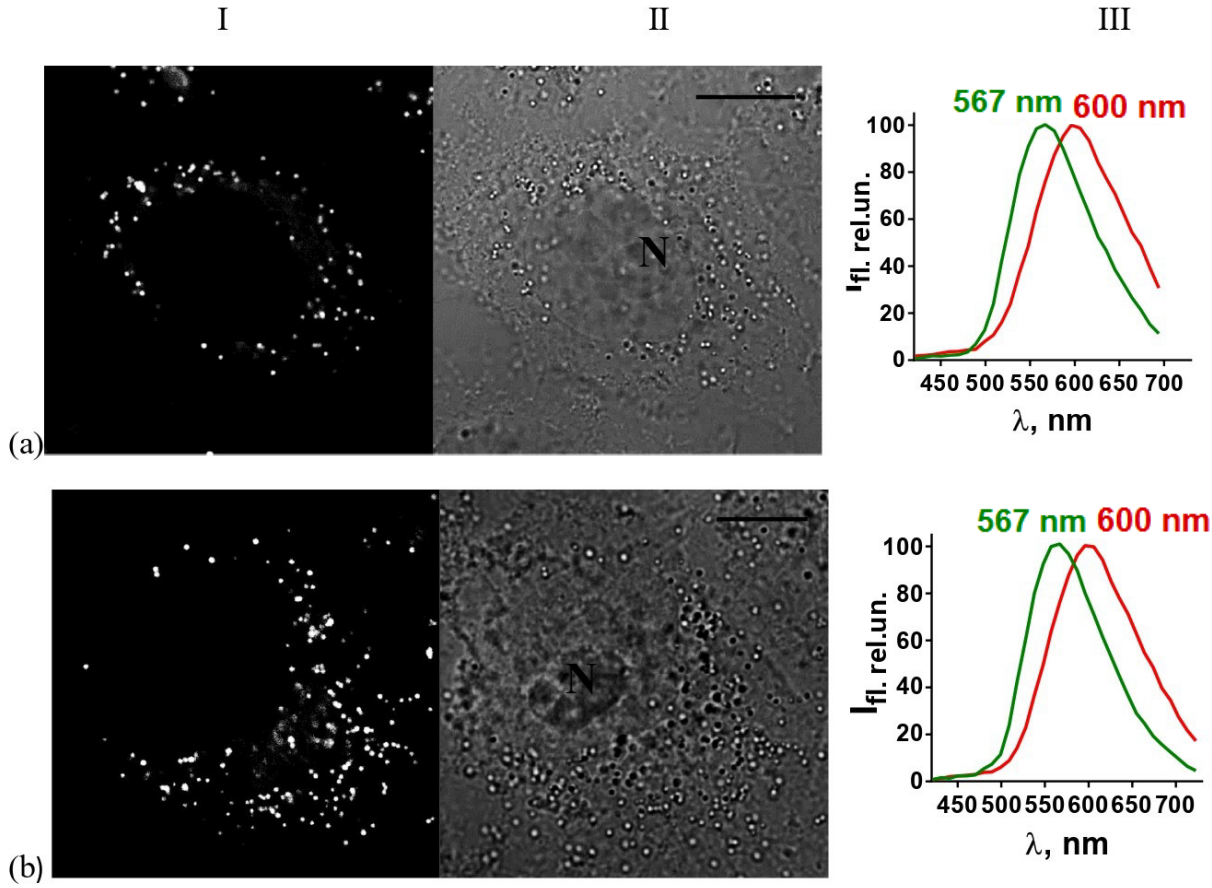


Figure S2. Confocal fluorescence images and fluorescence spectra of **1** in HEK293 cells. Column I (a, b) – typical intracellular distributions of compound **1** from stock in DMSO (a) and 10% CrEL emulsion (b) in the range of 510 – 730 nm and corresponding images of cells in transmitted light (column II). The scale mark corresponds to 5 μm. N – nucleus of cell. Cells were incubated with **1** (5 μM) for 30 min. Column III - typical intracellular normalized fluorescence spectra of **1** in vesicular (green line) structures and cytoplasm (red line) in HEK293 cells (λ_{ex} = 405 nm).

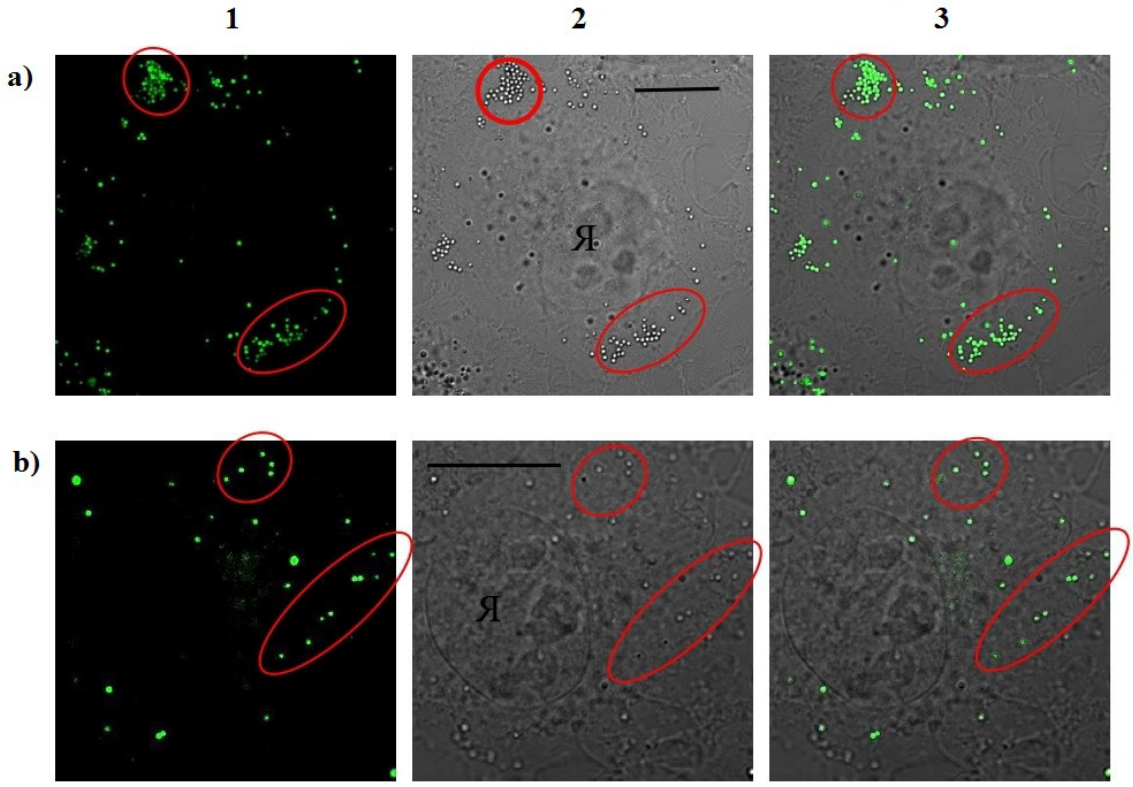


Figure S3. Cellular organelles in which **1** is localized in HEK293 cells. Column 1 is a confocal fluorescence image showing the intracellular distribution of compound **1** (λ_{ex} = 405 nm). The scale mark represents 10 μm. Column 2 is an image of cells in transmitted light. The oval outlines a group of lipid droplets located on the periphery of the cell and visible as bright white or black granules. Column 3 is an overlay of columns 1 and 2 showing the accumulation of **1** in lipid droplets. Cells were incubated with 5 μM **1** from stock in 10% CrEL (a) or from stock in DMSO (b) for 30 min.

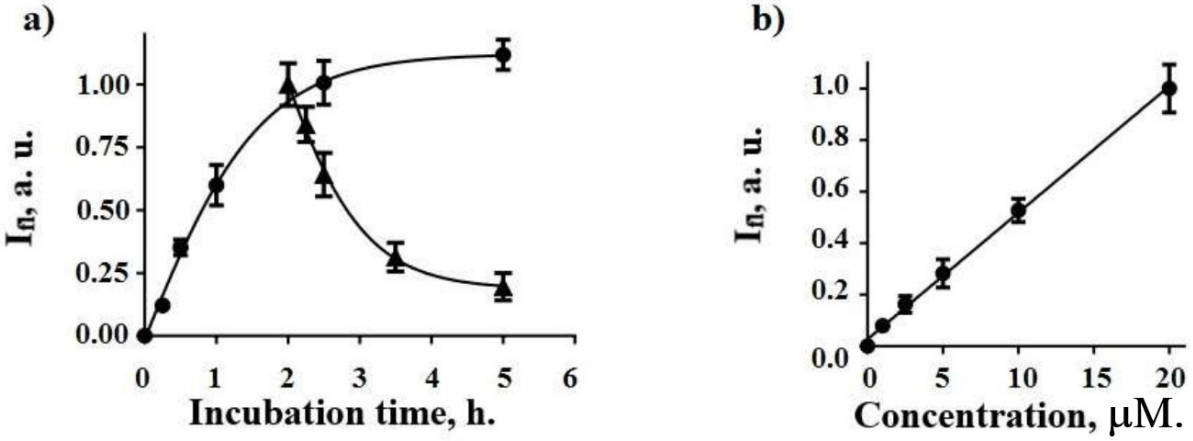


Figure S4. Kinetics of accumulation (a (●)), excretion (a (▲)) and concentration dependence (b) of **1** in HEK293 cells. (a, ●) - Cells were incubated with **1** (5 μM, DMSO stock) for different time intervals and recorded using CLSM under the same measurement conditions. (a, ▲) – Cells were incubated with **1** (5 μM) for 2 hours, washed twice with Hanks' solution and placed in fresh medium (without **1**) for different (0 – 3 hours) periods of time and recorded using CLSM under the same conditions measurements. (b) – cells were incubated with 1 – 20 μM compound **1** for 30 min. Data are averaged over 30 – 40 cells in each measurement and presented as mean fluorescence intensity (Ifl) per cell ± SD.

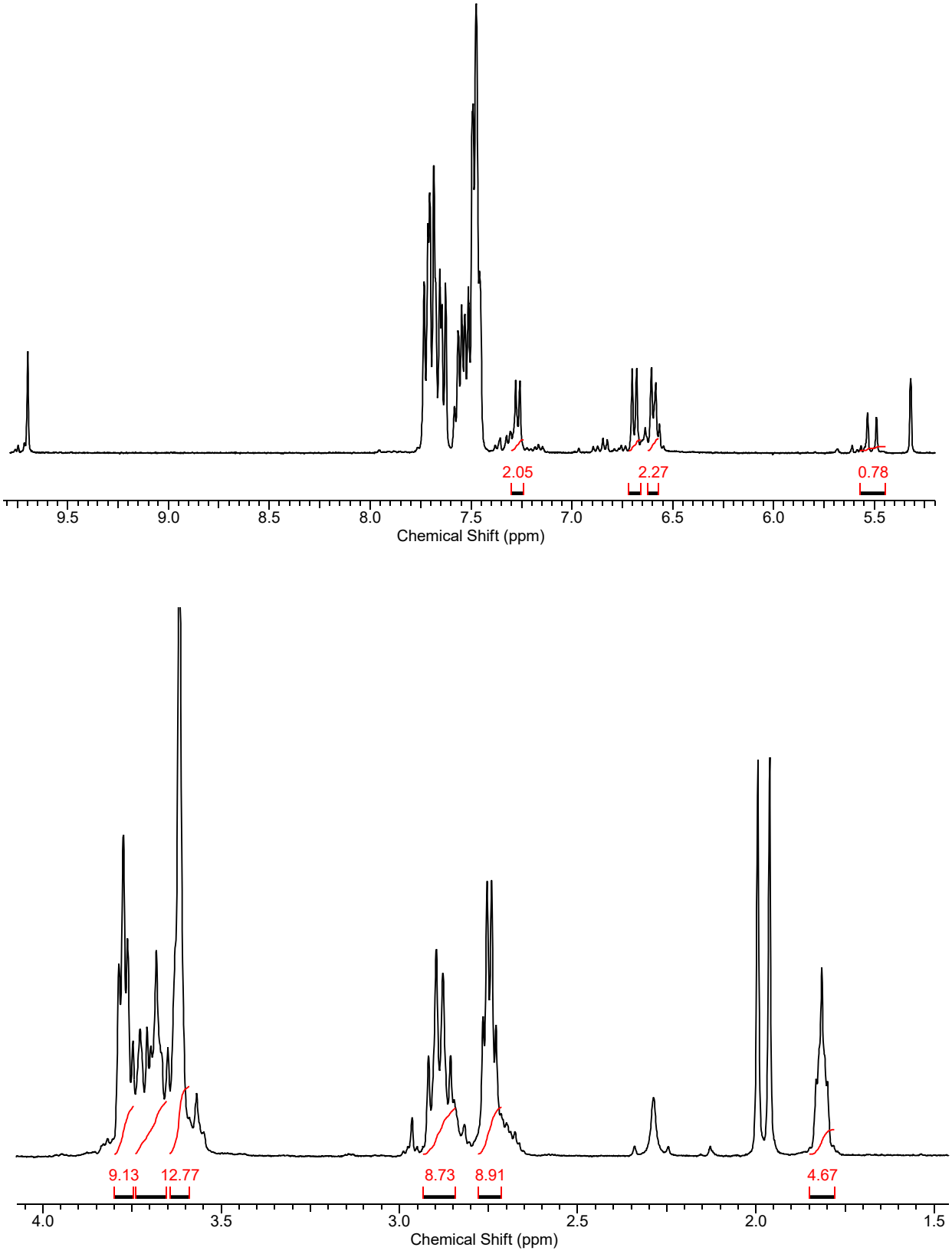


Figure S5. ^{1}H NMR spectrum of compound **3** in DMSO-d_6.

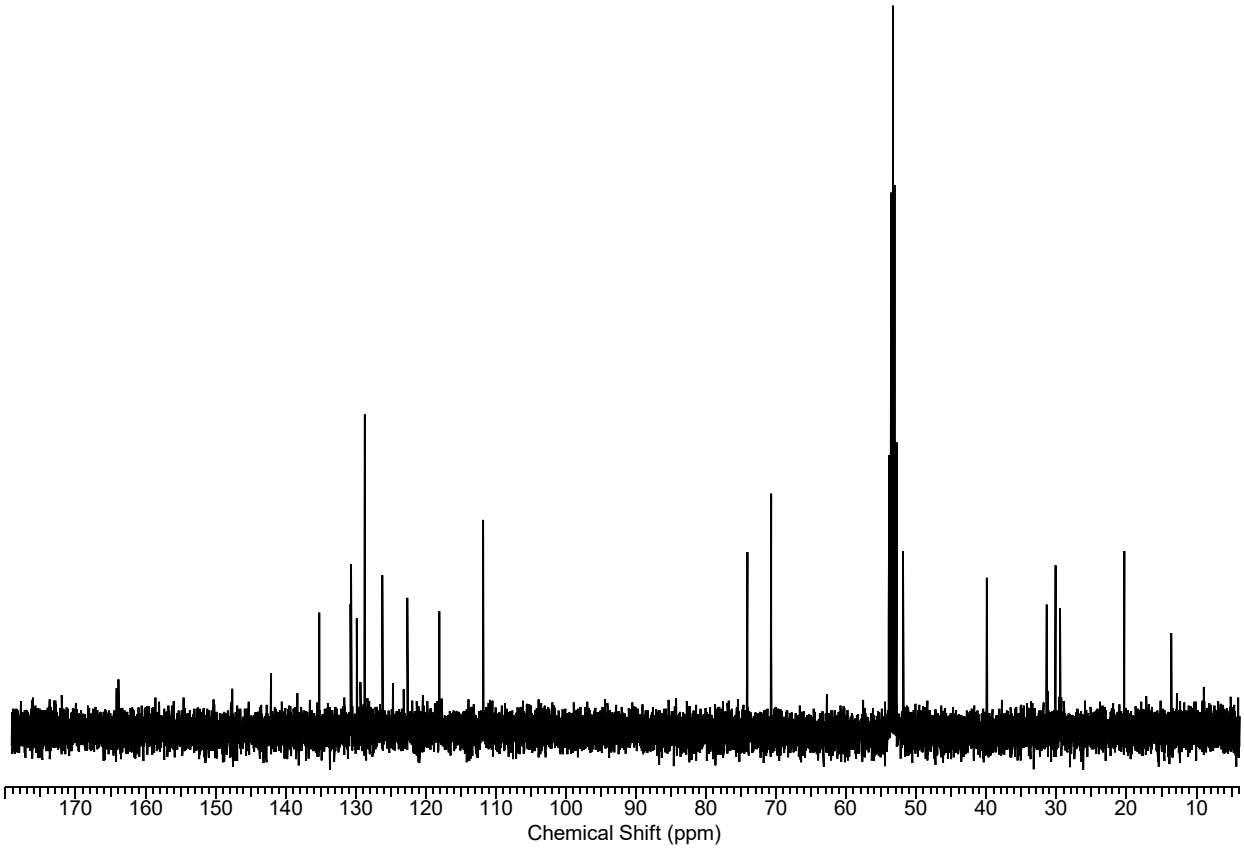


Figure S6. ^{13}C NMR spectrum of compound **1** in DMSO-d_6.