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Synthesis and antiproliferative activity of new chlorin e_6 glycoconjugates

Natalia S. Kuzmina, Vasilii F. Otvagin, Lubov V. Krylova, Alexander V. Nyuchev, Yuliya V. Romanenko, Oscar I. Koifman, Irina V. Balalaeva and Alexey Yu. Fedorov

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General

^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent DD2 400 MHz spectrometer. Chemical shifts (δ) are reported in ppm for the solution of compound in $CDCl_3$, DMSO-d_6 or CD_3OD, with the residual peak of solvent as an internal reference, *J* values in Hertz. Mass spectra were recorded using the MALDI method on a time-of-flight Bruker Microflex LT mass-spectrometer. TLC analyses were carried out on Merck TLC Silica gel 60 F_{254}. Column chromatography separation was performed using Macherey-Nagel Kieselgel 60 (70-230 mesh). Commercially available reagents (Aldrich, Alfa Aesar) were used without additional purification. Solvents were purified according to the standard procedures. Petroleum ether used was of bp 40-70 $^{\circ}$C.

Compound **3a**. ^{1}H NMR (400 MHz, CD_3OD): δ 9.58 (s, 1H), 9.55 (s, 1H), 8.65 (s, 1H), 8.13 (dd, *J* = 17.8, 11.5 Hz, 1H), 7.91 (s, 1H), 6.19 (d, *J* = 17.0 Hz, 1H), 6.01 (d, *J* = 11.4 Hz, 1H), 5.49 – 5.38 (m, 2H), 5.20 (d, *J* = 18.8 Hz, 1H), 4.45 (d, *J* = 7.3 Hz, 1H), 4.39 – 4.26 (m, 3H), 4.02 (t, *J* = 9.4 Hz, 1H), 3.93 – 3.80 (m, 4H), 3.76 (s, 3H), 3.70 – 3.66 (m, 1H), 3.65 – 3.55 (m, 6H), 3.41 (s, 3H), 3.38 (s, 3H), 3.35 – 3.33 (m, 3H), 2.64 (s, 2H), 2.50 – 2.38 (m, 1H), 2.12 – 2.02 (m, 1H), 1.98 – 1.86 (m, 1H), 1.74 (t, *J* = 7.5 Hz, 3H), 1.67 (d, *J* = 7.1 Hz, 3H).^{13}C NMR (101 MHz, CD_3OD): 176.50, 175.55, 167.13, 163.40, 154.23, 153.36, 148.77, 146.64, 146.35, 145.03, 142.77, 142.68, 140.32, 139.55, 134.42, 134.08, 131.86, 122.86, 119.85, 103.68, 102.79, 101.31, 94.15, 90.13, 79.80, 75.23, 71.31, 70.26, 62.27, 54.02, 52.79, 45.54, 35.57, 33.55, 31.65, 23.47, 20.28, 18.18, 12.39, 12.01, 11.14.

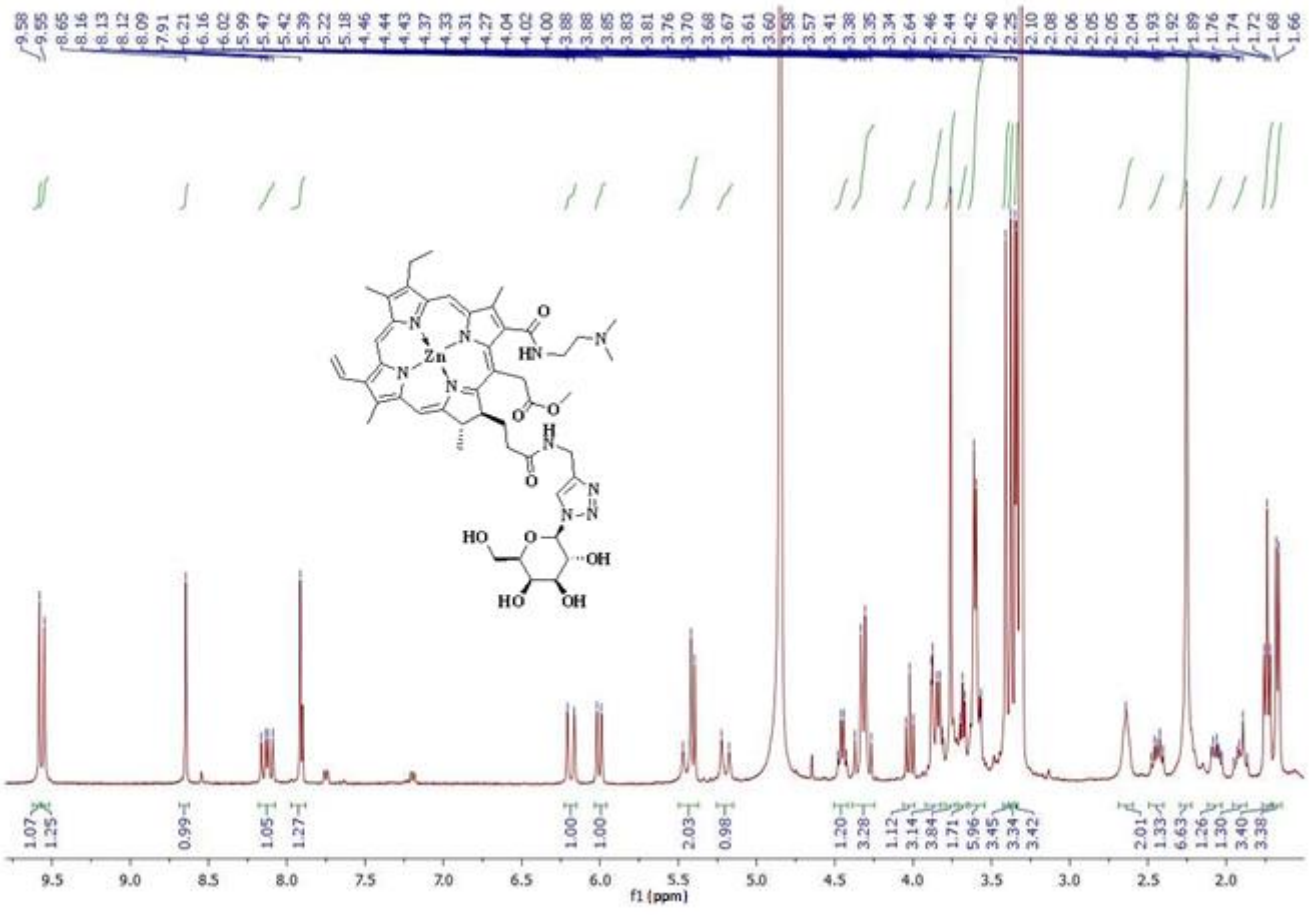

Figure S1 ^{1}H NMR of Compound **3a** in CD_3OD at 400 MHz.

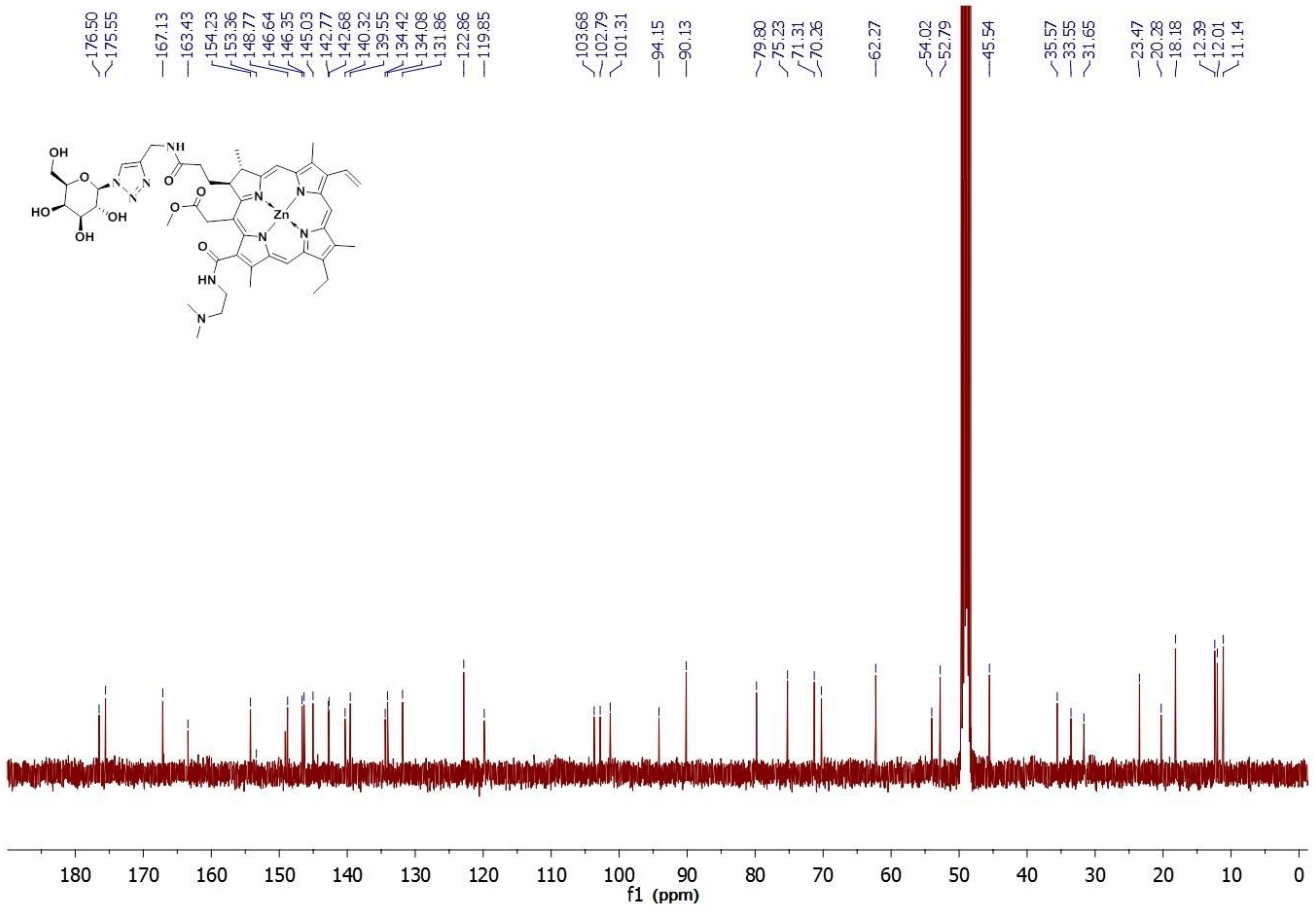

Figure S2 ^{13}C NMR of Compound **3a** in CD_3OD at 101 MHz.

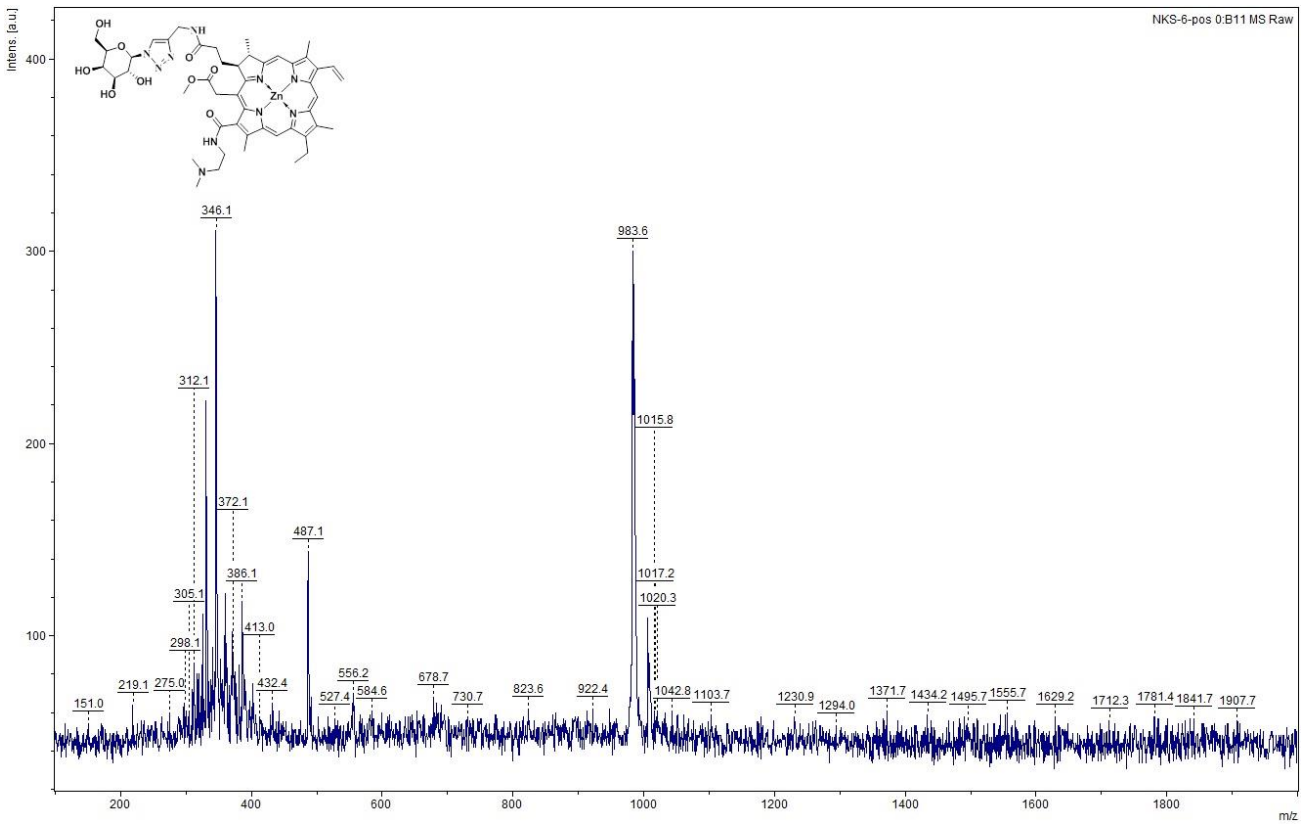

Figure S3 MS (MALDI) spectra of Compound **3a**.

Compound **3b**. ^{1}H NMR (400 MHz, CD_3OD): δ 9.58 (s, 1H), 9.55 (s, 1H), 8.65 (s, 1H), 8.13 (dd, J = 17.9, 11.5 Hz, 1H), 7.84 (s, 1H), 6.18 (d, J = 17.9 Hz, 1H), 6.00 (d, *J* = 10.5 Hz, 1H), 5.51 – 5.38 (m, 2H), 5.20 (d, *J* = 18.7 Hz, 1H), 4.45 (q, *J* = 7.6 Hz, 1H), 4.36 – 4.28 (m, 3H), 3.84 (q, *J* = 7.7 Hz, 3H), 3.80 – 3.72 (m, 6H), 3.62 – 3.55 (m, 2H), 3.49 – 3.42 (m, 2H), 3.41 (s, 3H), 3.35 (s, 1H), 3.34 (s, 2H), 2.71 (s, 2H), 2.50 – 2.38 (m, 1H), 2.32 (s, 6H), 2.29 – 2.19 (m, 1H), 2.12 – 2.02 (m, 1H), 1.96 – 1.85 (m, 1H), 1.74 (t, *J* = 7.5 Hz, 3H), 1.67 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD): 176.49, 175.54, 167.13, 163.44, 154.23, 146.65, 146.16, 145.04, 142.77, 142.69, 140.33, 139.55, 134.43, 134.08, 133.21, 131.86, 123.34, 119.86, 103.69, 102.80, 101.32, 94.16, 89.48, 81.02, 78.40, 73.90, 70.81, 62.30, 58.99, 54.00, 52.78, 45.49, 35.51, 33.51, 31.63, 23.49, 20.27, 18.18, 12.40, 12.02, 11.15.

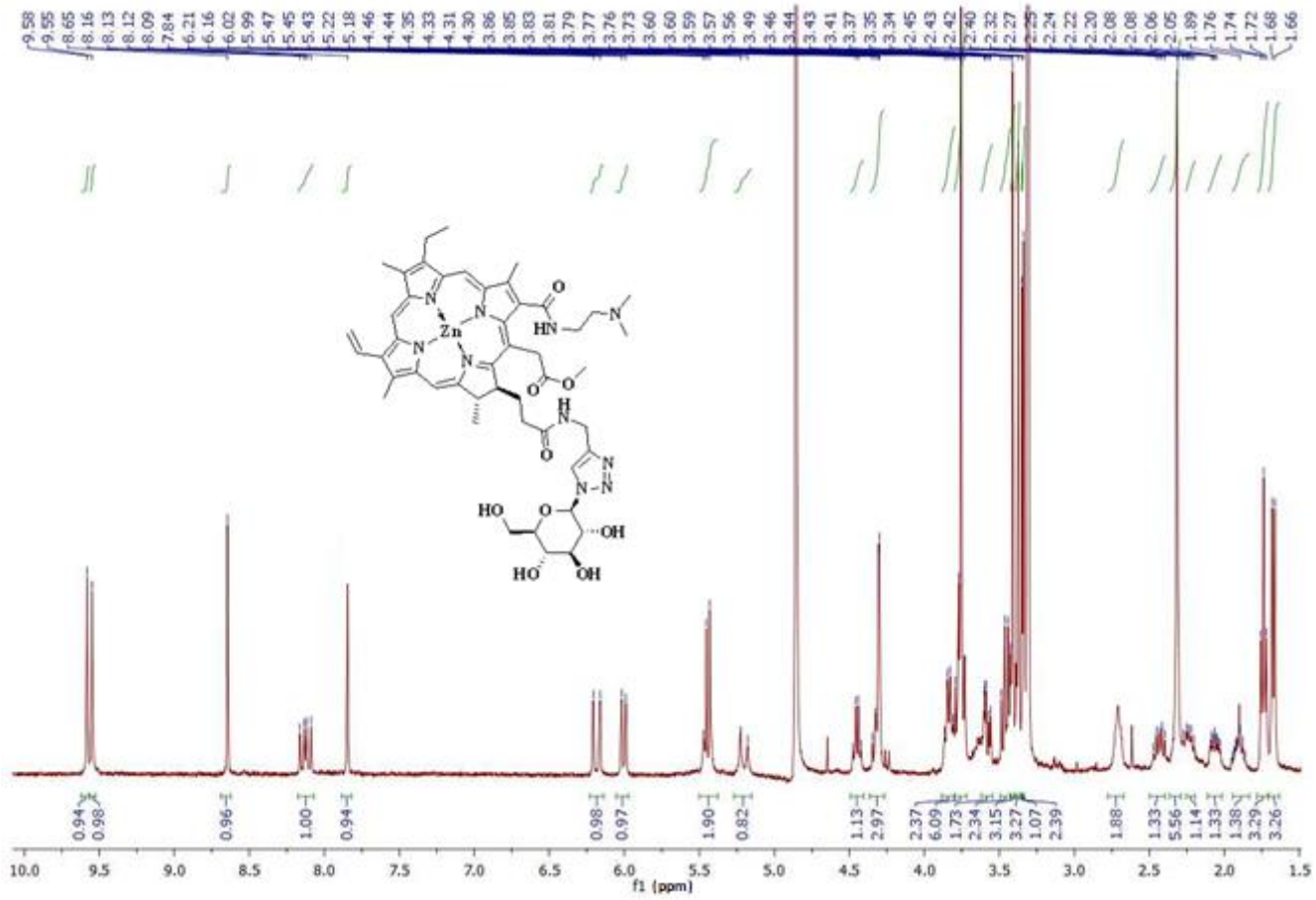

Figure S4 ^{1}H NMR of Compound **3b** in CD_3OD at 400 MHz.

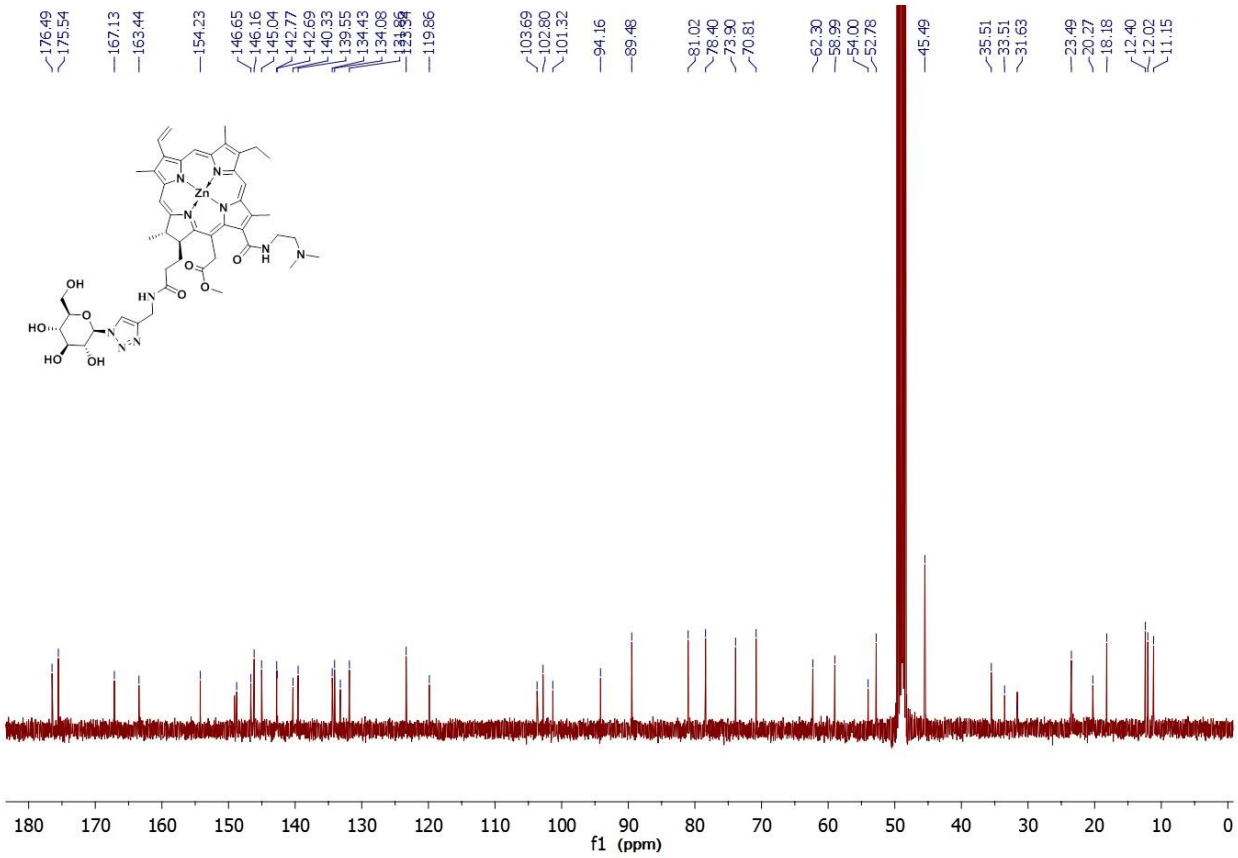

Figure S5 ^{13}C NMR of Compound **3b** in CD_3OD at 101 MHz.

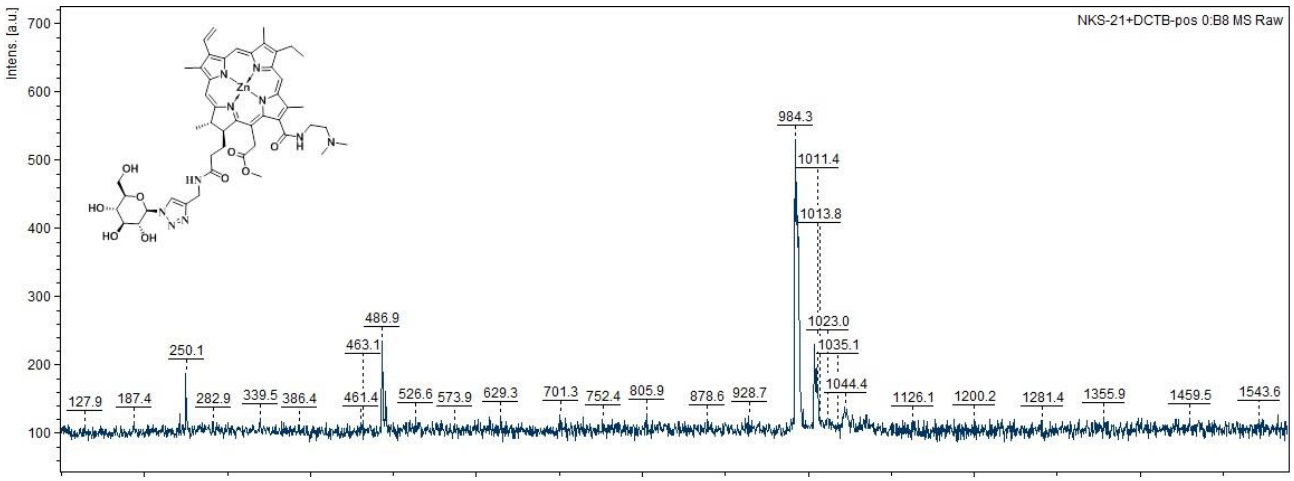

Figure S6 MS (MALDI) spectra of Compound **3b** in DCTB matrix.

Compound **4a.** ^{1}H NMR (400 MHz, CD_3OD): δ 9.61 (s, 1H), 9.55 (s, 1H), 8.66 (s, 1H), 8.11 (dd, *J* = 17.7, 11.9 Hz, 1H), 7.95 (s, 1H), 6.18 (d, *J* = 17.6 Hz, 1H), 6.01 (d, *J* = 11.5 Hz, 1H), 5.44 (d, *J* = 9.2 Hz, 1H), 5.41 – 5.09 (m, 2H), 4.52 – 4.41 (m, 1H), 4.41 – 4.25 (m, 3H), 4.05 (t, *J* = 9.3 Hz, 1H), 3.95 – 3.79 (m, 4H), 3.74 – 3.67 (m, 5H), 3.66 – 3.58 (m, 4H), 3.43 – 3.33 (m, 9H), 3.08 – 2.90 (m, 6H), 2.58 – 2.45 (m, 1H), 2.30 – 2.19 (m, 1H), 2.19 – 2.07 (m, 1H), 1.95 – 1.83 (m, 1H), 1.75 (t, *J* = 7.2 Hz, 3H), 1.68 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD): 175.52, 167.43, 154.57, 149.15, 146.95, 146.37, 142.45, 140.31, 139.75, 134.39, 131.74, 122.89, 103.98, 102.59, 101.34, 90.12, 79.81, 75.21, 71.33, 70.29, 62.31, 53.90, 52.88, 51.01, 44.49, 35.61, 33.50, 31.49, 23.44, 20.86, 18.20, 14.46, 12.38, 12.19, 11.14, 8.38.

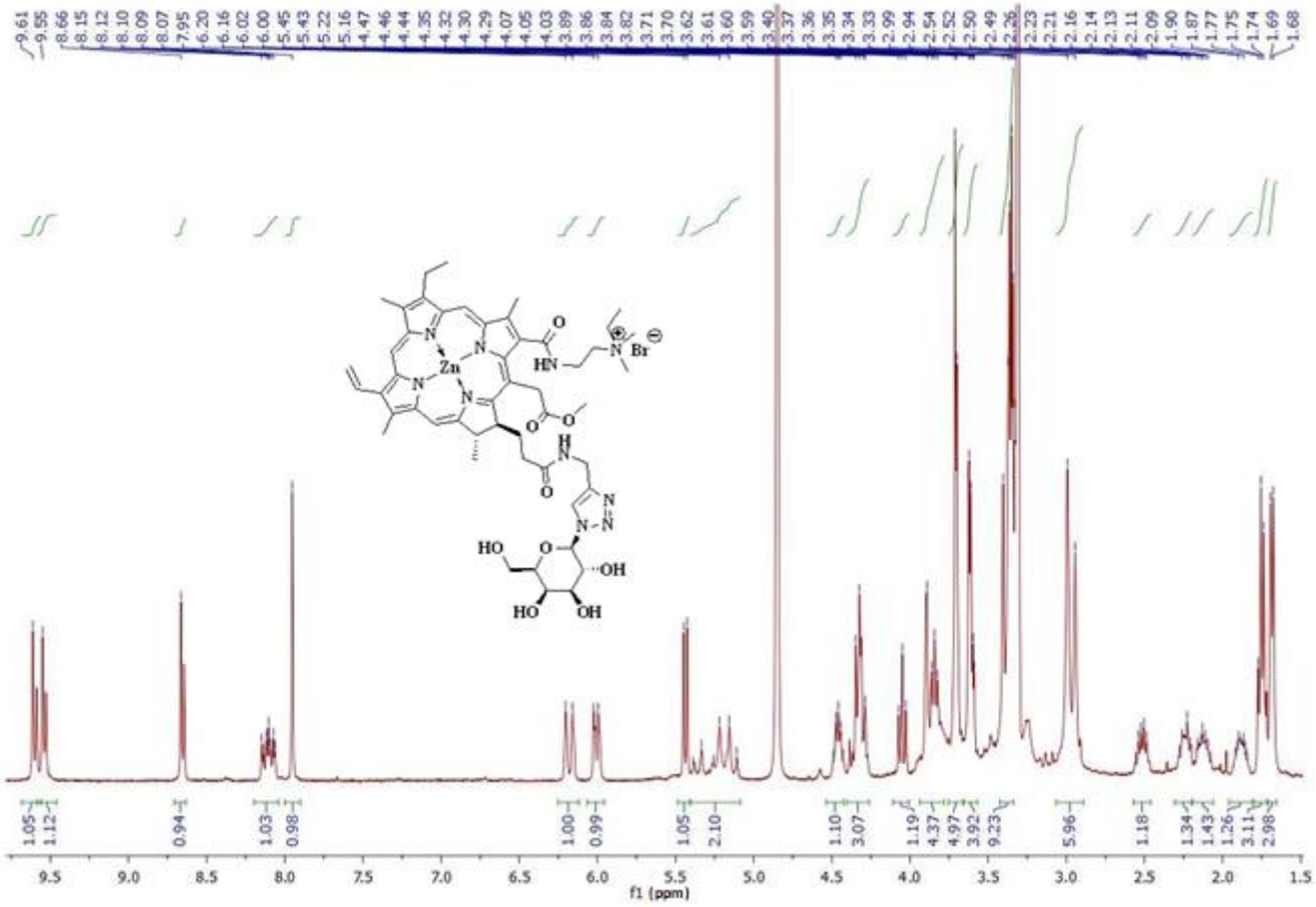

Figure S7 ^{1}H NMR of Compound **4a** in CD_3OD at 400 MHz.

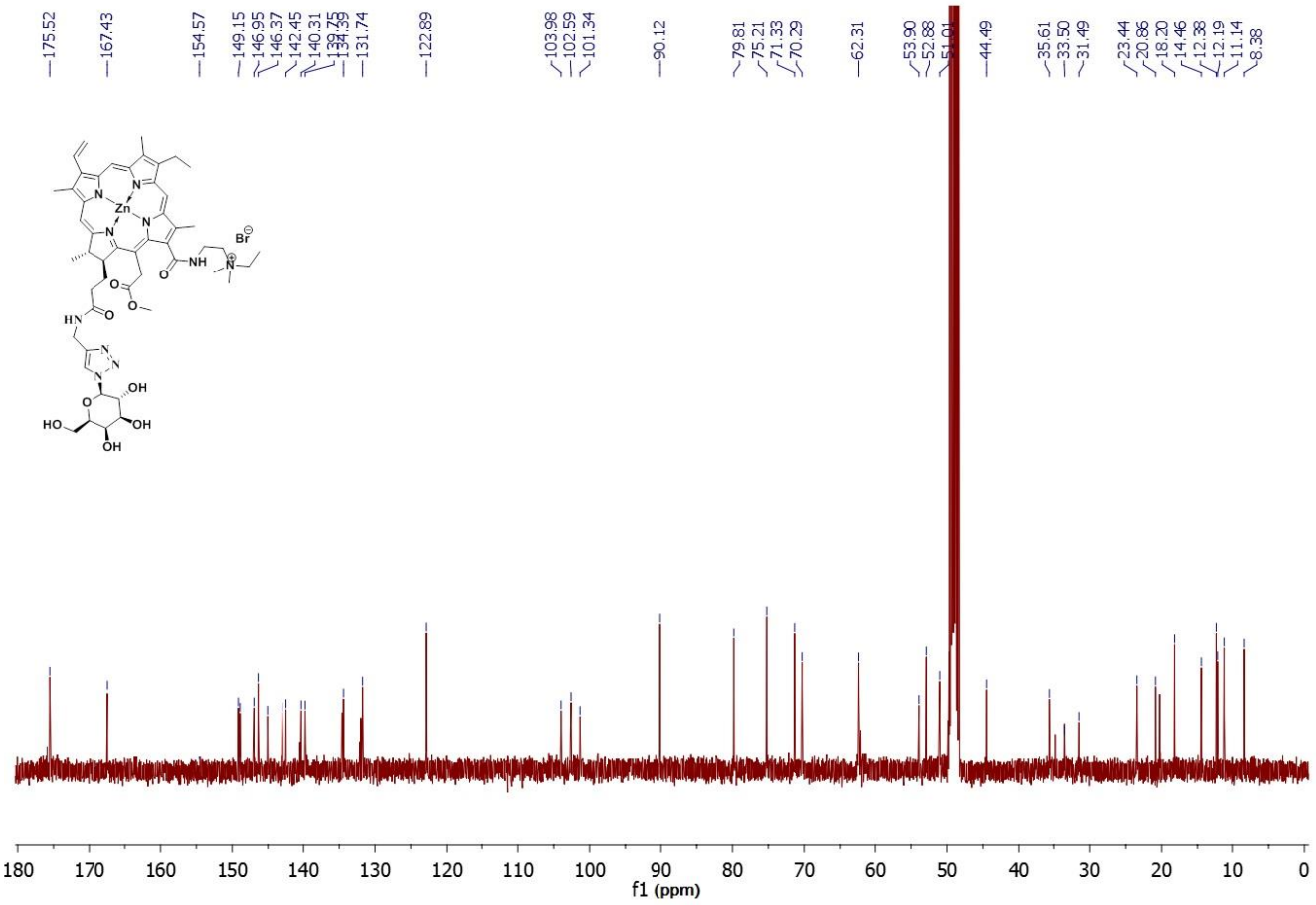

Figure S8 ^{13}C NMR of Compound **4a** in CD_3OD at 101 MHz.

Compound **4b**. ^{1}H NMR (400 MHz, CD_3OD): δ 9.60 (s, 1H), 9.55 (s, 1H), 8.66 (s, 1H), 8.11 (dd, *J* = 17.2, 12.0 Hz, 1H), 7.90 (s, 1H), 6.18 (d, *J* = 17.7 Hz, 1H), 6.01 (d, *J* = 11.4 Hz, 1H), 5.48 (d, *J* = 9.1 Hz, 1H), 5.43 – 5.10 (m, 2H), 3.90 – 3.69 (m, 10H), 3.64 – 3.57 (m, 3H), 3.52 – 3.45 (m, 3H), 3.44 – 3.33 (m, 9H), 3.12 (s, 6H), 2.57 – 2.46 (m, 1H), 2.31 – 2.19 (m, 1H), 2.19 – 2.08 (m, 1H), 1.95 – 1.83 (m, 1H), 1.75 (t, *J* = 7.4 Hz, 3H), 1.69 (d, *J* = 7.1 Hz, 3H).^{13}C NMR (101 MHz, CD_3OD): 175.54, 167.40, 154.50, 149.14, 146.95, 146.20, 145.05, 142.45, 140.31, 134.36, 131.76, 123.34, 103.98, 102.59, 101.32, 89.49, 81.05, 78.41, 73.93, 70.86, 62.31, 53.89, 52.89, 51.16, 44.46, 35.56, 33.48, 30.68, 23.47, 20.27, 18.18, 12.38, 12.18, 11.14, 8.49.

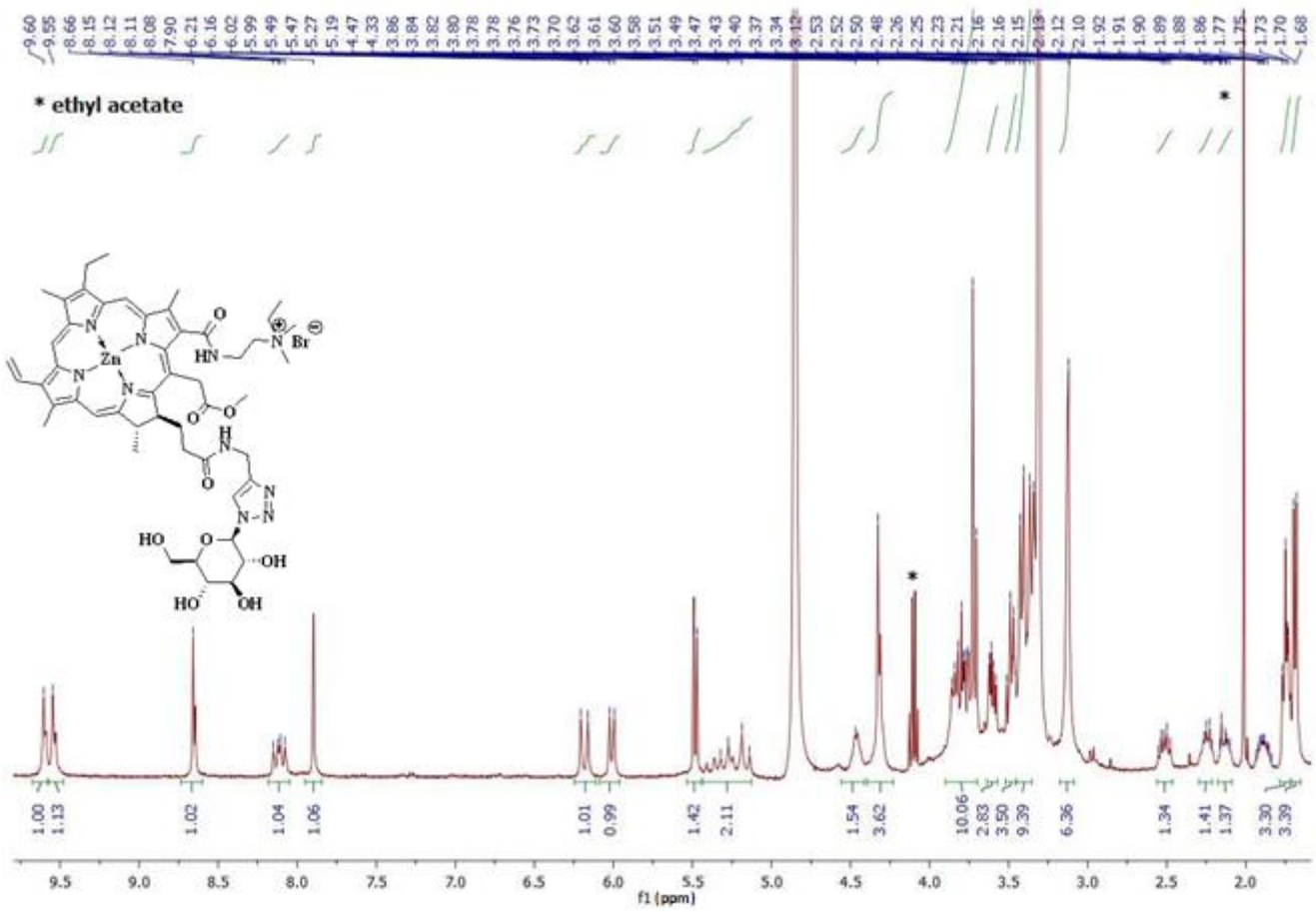

Figure S9 ^{1}H NMR of Compound **4b** in CD_3OD at 400 MHz.

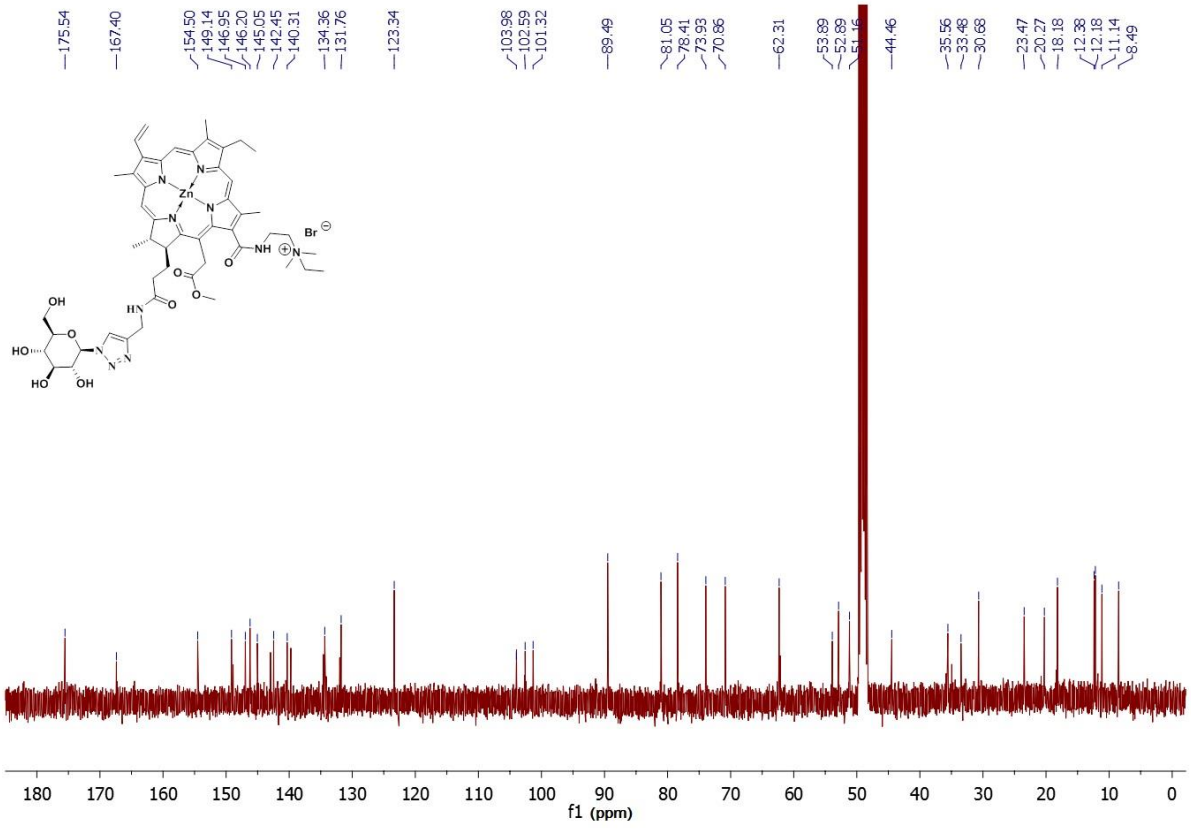

Figure S10 ^{13}C NMR of Compound **4b** in CD_3OD at 101 MHz.

Photophysical measurements

Photophysical properties of compounds **4a**,**b** were measured in deionized water at 5 mM solutions. Absorption and fluorescence spectra were registered using a Synergy MX spectrophotometer-spectrofluorometer (BioTek, USA). Fluorescence was excited at 410 nm. The molar extinction coefficient ε was determined using the following equation:

$$\varepsilon = {}^{D}/_{cl} \ ,$$

where *D* - optical density; *l* - path length; *c* - concentration. The fluorescence quantum yield ϕ_1 was calculated using the equation:

$$\varphi_1 = \frac{\varphi_2 F_1 D_2}{F_2 D_1} \ ,$$

where F_1 and D_1 - integral fluorescence intensity and optical density of 6 (or 7), respectively; ϕ_2 - quantum yield of Rhodamine B (Sigma, USA) in water (0.31); F_2 and D_2 - integral fluorescence intensity and optical density of Rhodamine B, respectively.

The fluorescence was excited at 410 nm, the optical density was measured at the same wavelength. The fluorescence signal was detected at 550-850 nm.

Cell lines and culturing conditions

Cell line of human epidermoid carcinoma A431 was obtained from the Russian cell culture collection of vertebrates. The cells were cultured in Eagle MEM medium (PanEco, Russia) with 10% (v/v) fetal calf serum (HyClone, USA) and 2mM L-glutamine in 5% CO_2 at 37°C. At each passaging stage the cells were treated with Versene solution (PanEco, Russia).

Study of cellular uptake

Cells were seeded in glass bottom 96-well plates (Corning, USA) at the density of 9×10^3 cells per cell with 24 h incubation in 5% CO_2 at 37 °C. The medium was then exchanged with fresh serum-free growth medium containing 5 μM of tested compound (200 μM per well). Cells were incubated for 4 hours, washed thrice with PBS, fixed with 4% formaldehyde for 30 min and washed again.

Cells were imaged using laser scanning confocal microscope Axio Observer Z1 LSM 710 NLO/Duo (Carl Zeiss, Germany) equipped with C-Apochromat 63×water immersion objective lens with numerical aperture 1.2. Fluorescence was excited at 405 nm and registered in the range of 600-740 nm.

Cytotoxicity study

The effect of tested compounds on cell viability was estimated using the microculture tetrazoline test (MTT) [1]. Cells were seeded in 96-well plates at the density of $4\cdot10^3$ cells per well and allowed to attach overnight. The medium was then exchanged with fresh serum-free growth medium containing tested compound in different concentration. After 4 h of incubation, the medium was exchanged with full fresh growth medium.

To estimate the photoinduced toxicity of the tested compound, the cells were exposed to light irradiation (615-635 nm, 20 mW/cm^2, 20 J/cm^2) using LED light source providing a homogeneous light distribution in 96-well plates [2]. Irradiated cells were then incubated for 24 h before cell viability was measured. To this aim, the cells were incubated with serum-free medium containing 0.5 mg/mL MTT-reagent (3-(4,5-dimethyl-2-thiazolyl)-2,5- diphenyl-2H-tetrazole bromide, Alfa Aesar, USA) for 4 h. The formazan formed from the reduction of MTT by cells' dehydrogenases was dissolved in DMSO, and the absorbance was measured at 570 nm with a Synergy MX plate reader (BioTeck, USA).

A similar procedure was used for the estimation of the dark toxicity. The cells, grown overnight, were incubated in the medium with the compound being tested, washed with full fresh growth medium, then incubated for 24 h and undergo MTT assay. Cell viability was expressed as the ratio of the optical density of treated and untreated cells (in percentage). Three independent experiments (all in triplicate) were performed. Experimental data were fitted using four parameters model for lognormal distribution to obtain dose-effect relationship and calculate inhibition concentration IC_{50}:

$$Y = Y_{min} + \frac{Y_{max} - Y_{min}}{1 + 10^{(\lg(IC_{50}) - X)\cdot SF}},$$

where Y_{max} and Y_{min} - maximal and minimal Y values, respectively; SF-slope factor.

Data analysis was performed using the GraphPad Prism 6 software.

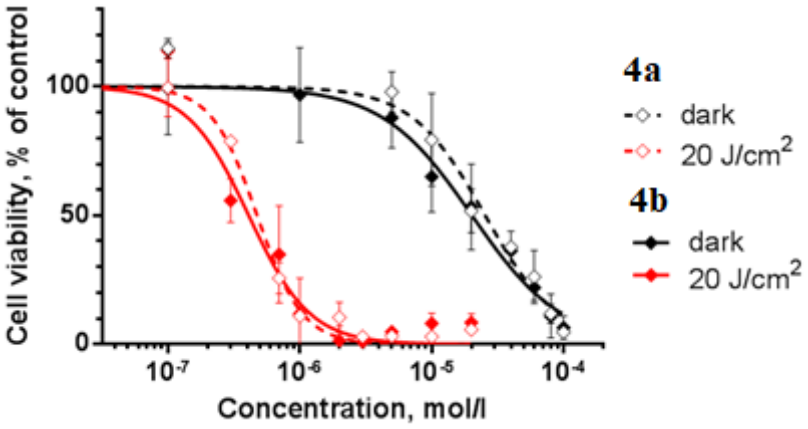

Figure S11 Relative viability of A431 cells treated with compounds **4a**,**b** in dark or under light exposure.

References

[S1] T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55.

[S2] N. Y. Shilyagina, V. I. Plekhanov, I. V. Shkunov, P. A. Shilyagin, L. V. Dubasova, A. A. Brilkina, E. A. Sokolova, I. V. Turchin and I. V. Balalaeva, *Sovremennye Tekhnologii v Meditsine*, 2014, **6**, 15 (in Russian).