L-cysteine/$AgNO_2$ low molecular weight gelators: self-assembly and suppression of MCF-7 breast cancer cells

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1. Materials

L-cysteine (>99 %) was obtained from Acros. Silver nitrite (>99 %) was obtained from Lancaster. All chemicals were used as received. All solutions were prepared on de-ionized water.

Preparation of gels

The solutions (2 ml.) were prepared by the following scheme: the empty vessel 0.8 (0.65) ml of de-ionized water was filled, then 0,6 ml of L-cysteine (L-cys, 0.01 M) was added, finally 0.6 (0.75) ml of silver nitrite ($AgNO_2$, 0.01 M) was added. The ratio of the components L-cys:$AgNO_2$ was 1:1 (CS-1) and 1:1.25 (CS-2) respectively. The resulting yellowish white-opalescent mixtures were stirred at room temperature (25°C) for 1 minute and solutions were stayed in dark place for 3 hours. As a result, the yellowish transparent hydrogels were obtained.

2. Methods

Viscosity measurements

The viscosity of solutions and hydrogels was measured with a vibratory viscometer SV-10 (A&D, https://www.aandd.jp/products/test_measuring/sv10/sv10.html). The vibration of the sensor plates was carried out with a frequency of 30 Hz and constant amplitude of about 1 mm. For viscosity measuring, 10 ml of CS-1 (or CS-2) solutions were prepared in the special polycarbonate cups (A&D). In the kinetic study, these solutions were immediately transferred to the viscometer. After complete gelation, the samples in the cups were transferred to the viscometer and measurements were recorded. All measurements were performed at room temperature (25°C).

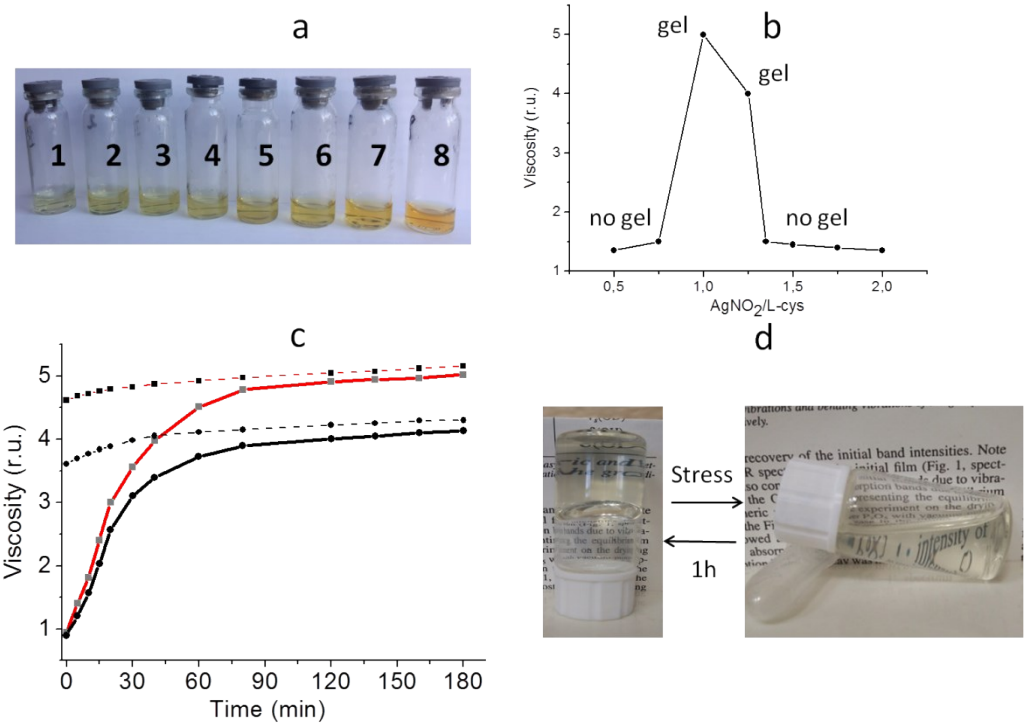


Figure S1. a – the systems obtained after 24 h at mixing of L-cys/$AgNO_2$ of the various ratio: 0.5:1, 0.75:1, 1:1, 1:1.25, 1:1.5, 1:1.75, 1:2 for 1-8 systems respectively. **b** – the dependence of the viscosity of the 1-8 systems on the composition. **c** - the kinetic curves of the gelation process for CS-1 (red bold curve) and CS-2 (black bold curve), the viscosity of completely formed CS-1 (red dash curve) and CS-2 (black dash curve) gels. **d** – the mechanical destruction – restoration of hydrogels.

TEM analysis

Sample micrographs were obtained using a LEO 912 ABOMEGA (Carl-Zeiss) transmission electron microscope. The samples were preliminarily placed on a standard copper grid with a 100 nm thick Formvar (polyvinylformal) polymer support, dried, and placed in the microscope.

SEM and elemental analysis

The microstructure and chemical composition of the samples were studied using a raster JEOL 6610 LV electron microscope (JEOL Ltd.) with x-ray system energy dispersive microanalysis Oxford INCA Energy 350. The micromorphology of gels was investigated in a high vacuum mode with accelerating voltage of 15 kV. To get an image signals of low-energy secondary electrons were used, providing topographical contrast, and high-energy back-up scattered (reflected) electrons that determine the composition and phase contrast. The elemental chemical composition of the samples was determined via x-ray spectral microanalysis based on registration and analysis of the energy spectra of the characteristic x-ray radiation excited by electrons passing through the sample. The qualitative and quantitative elemental composition was determined with using an energy-dispersive spectrometer (EDS) that sorts photons by their energy. Sample preparation consisted of spraying of the samples on the surface of a thin conductive layer of platinum and drying in a vacuum (10^{-4} Pa).

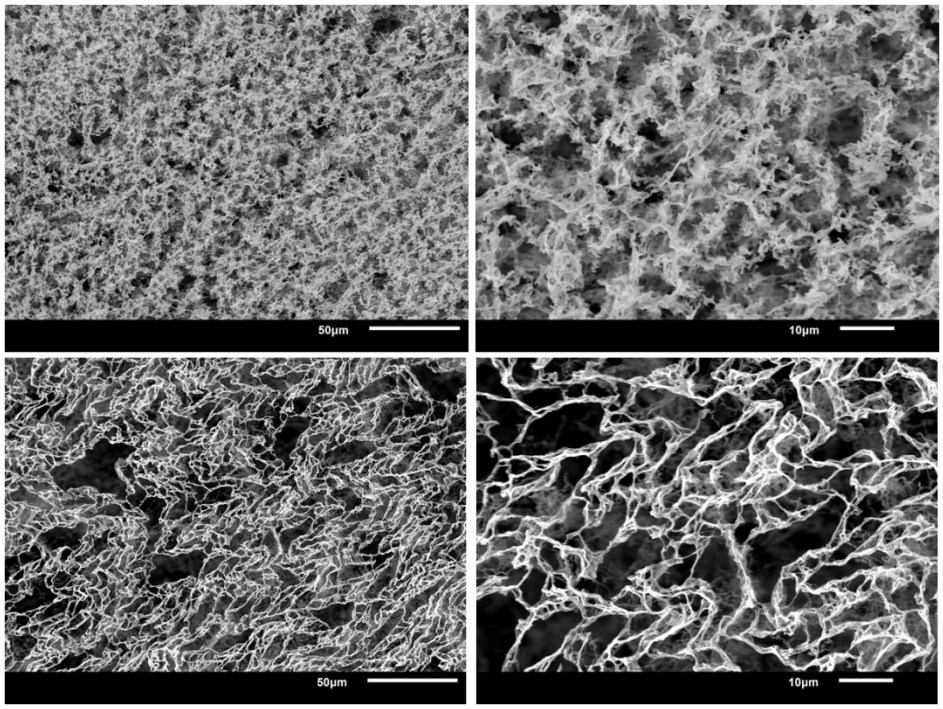


Figure S2. SEM micrographs of CS-1 (Top) and CS-2 (Bottom) hydrogels at different scale.

UV analysis

Electronic spectra of the samples were recorded on the UV spectrophotometer Evolution Array (Thermo Scientific) in a quartz cell with a 1 mm path length.

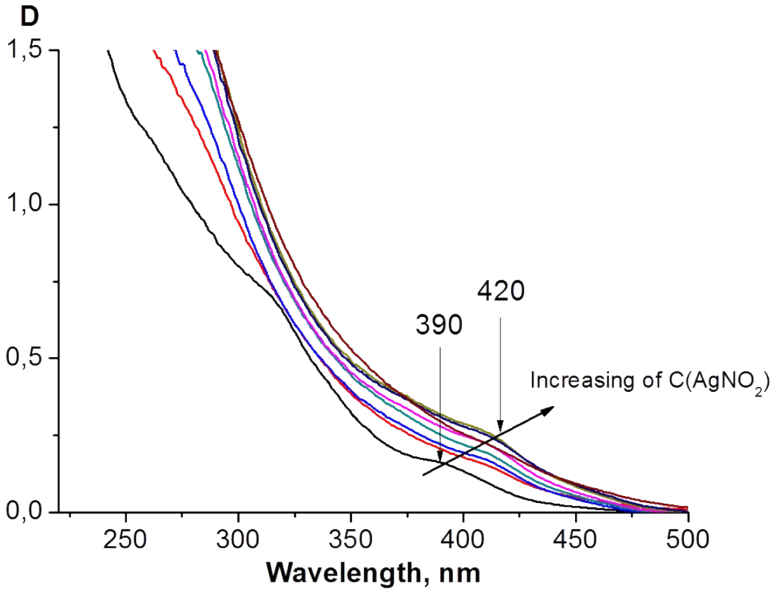


Figure S3. UV-spectra of the 1-8 systems after 24 h.

DLS analysis and zeta-potential measurements

Measurement of intensity of light scattering in the studied samples was carried out using analyzer Zetasizer Nano ZS (Malvern) with He-Ne laser (633 nm), power of 4 mW. For the correct analysis of the particle sizes and zeta-potential, after complete gelation, the samples were mechanically destroyed and diluted two, four and eight times. All measurements were carried out at 25°C in the backscattering configuration (173°), providing the highest sensitivity of the device. Mathematical processing of the results of the obtained cross-correlation functions of the diffuse light intensity fluctuations g2 was carried out in the program Zetasizer Software, where the solution of the obtained equation of the g2 dependence on the diffusion coefficient was performed by the cumulant method. The result of the solution was the function z(D). The hydrodynamic radii of the scattering particles were calculated from the diffusion coefficients by the Stokes-Einstein formula: $D= kT/6\pi\eta R$, where D is the diffusion coefficient, k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the medium, R is the radius of the scattering particles. Measurement of the electrophoretic mobility of aggregates in the samples was carried out in U-shaped capillary cuvettes. Zeta potential distributions were calculated using the Henry equation: UE = 2ezf(Ka)/3Z, where UE – electrophoretic mobility, z – zeta potential, e – dielectric constant, Z – viscosity, and f (Ka) – Henry's function, f (Ka) = 1.5 for aqueous media.

Table S1. The electrochemical measurements of CS-1 and CS-2 diluted systems.

Sample	zeta-potential, mV	Mobility, μmcm/Vs	Conductivity, mS/cm
CS-1, 2 times diluted	+9,80±1,35	0,82±0,04	0,088
CS-1, 4 times diluted	+10,10±1,42	0,83±0,07	0,042
CS-1, 8 times diluted	+10,20±1,39	0,85±0,05	0,027
CS-2, 2 times diluted	+14,70±1,44	1,22±0,12	1,055
CS-2, 4 times diluted	+15,20±1,45	1,25±0,11	0,062
CS-2, 8 times diluted	+15,30±1,52	1,27±0,15	0,034

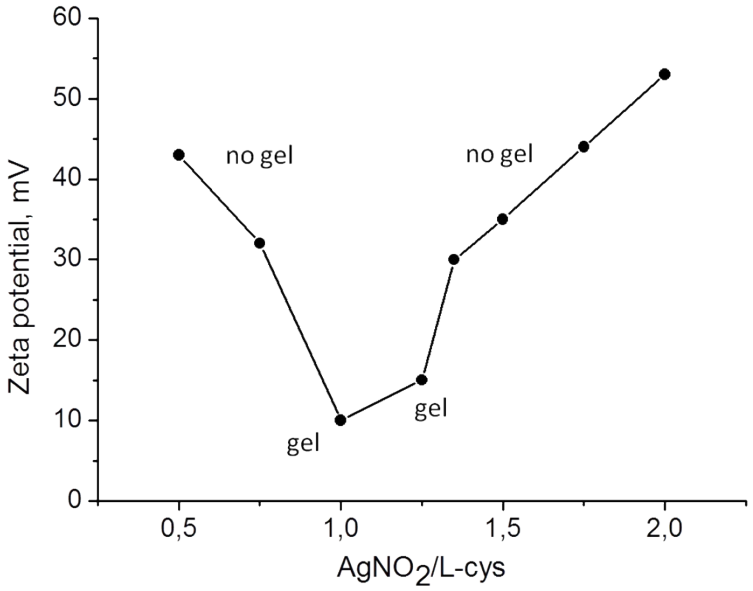


Figure S4. The zeta-potential of the 1-8 systems.

pH measurements

The pH of the solutions was measured using a Seven Multi S70 (Mettler Toledo) pH meter.

Elemental analysis

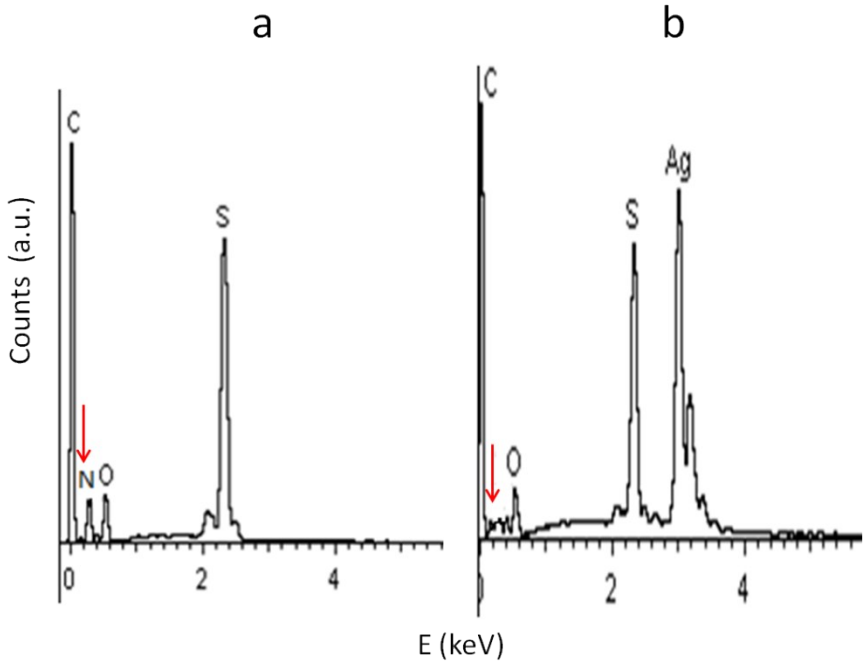


Figure S5. Elemental analysis of L-cysteine (a) and hydrogels (b).

FTIR analysis

FTIR spectra of the samples were recorded on a Vertex 70 spectrometer (Bruker) in the range of 7000–400 cm^{-1} at a resolution of 4 cm^{-1}. The number of scans was 32. The studied samples (CS-1 and CS-2 hydrogels) were preliminarily frozen in liquid nitrogen; the obtained yellowish precipitates were carefully washed with de-ionized water and vacuum dried at 25°C. 22 mg of the

sample was mixed with 700 mg of potassium bromide and pressed into a pellet.

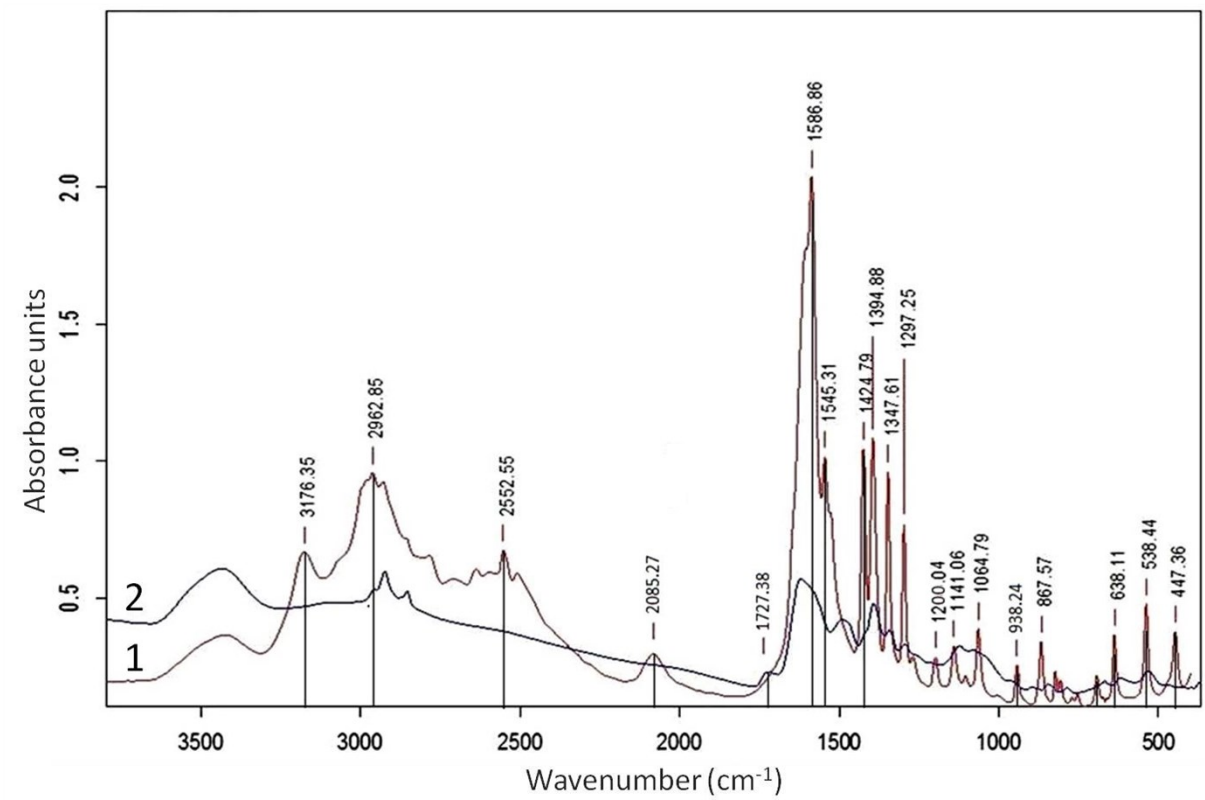


Figure S6. FTIR spectra of L-cysteine (1) and hydrogels (2).

Cytotoxicity of hydrogels in MCF-7 cells

MCF-7 human breast cancer cells obtained from the American Tissues and Cells Collection (ATCC) were cultivated in 96-well plates at 37°C in atmosphere of 5% CO_2 in a DMEM medium with the addition of L-glutamine (2 mM), antibiotics (100 units per mL of penicillin and 100 μg/mL of streptomycin), and 10% of FBS. The cells were incubated in a serum medium with the tested compounds at various concentrations for 48 h. PBS (10μL) containing MTT (5 mg/mL) was added to each well, and the cells were incubated at 37°C for 4 h. The culture medium was removed, DMSO (100 μL) was added to each well, a plate was vortex for 20 minutes, and then the optical absorbance in each well was measured at 570 nm in a Multiskan Spectrum Microplate Reader instrument (Thermo Scientific, United States). The MTT test readings were averaged for three independent determinations Readings of MTT test, in the absence of the tested compounds were taken as 100%.

Cytotoxicity of hydrogels in Wi-38 cells

WI-38 human normal embryonic lung cells obtained from the American Tissues and Cells Collection (ATCC) were cultivated in 96-well plates at 37°C in atmosphere of 5% NI2 in a DMEM medium with the addition of L-glutamine (2 mM), antibiotics (100 units per mL of penicillin and 100 μg/mL of streptomycin), and 10% of FBS. The cells were incubated in a serum medium with the tested compounds at various concentrations for 24 and 72 h. PBS (10 μL) containing MTT (5 mg/mL) was added to each well, and the cells were incubated at 37°N for 4 h. The culture medium was removed, DMSO (100 μL) was added to each well, a plate was vortex for 20 minutes, and then the optical absorbance in each well was measured at 570 nm in a Multiskan Spectrum Microplate Reader instrument (Thermo Scientific, United States). The MTT test readings were averaged for three independent determinations Readings of MTT test, in the absence of the tested compounds were taken as 100%.

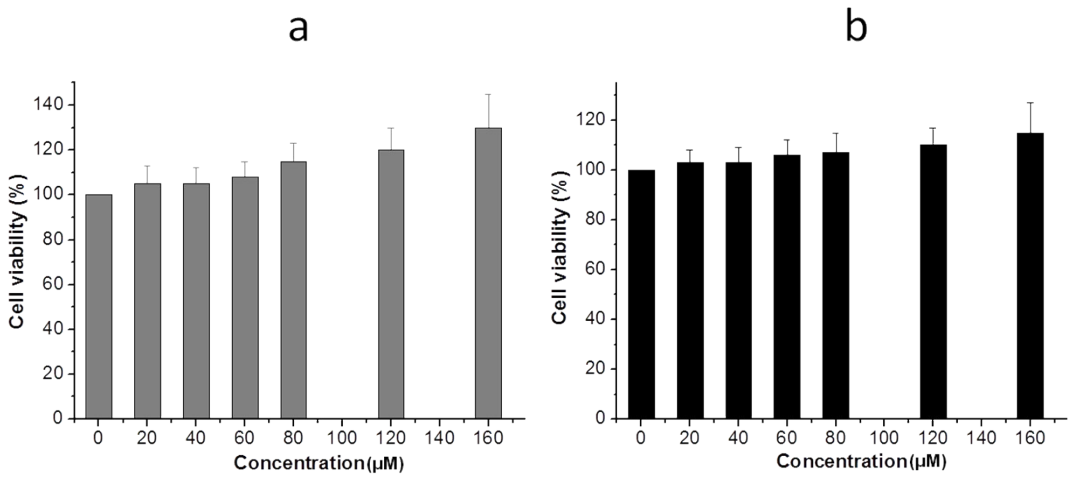
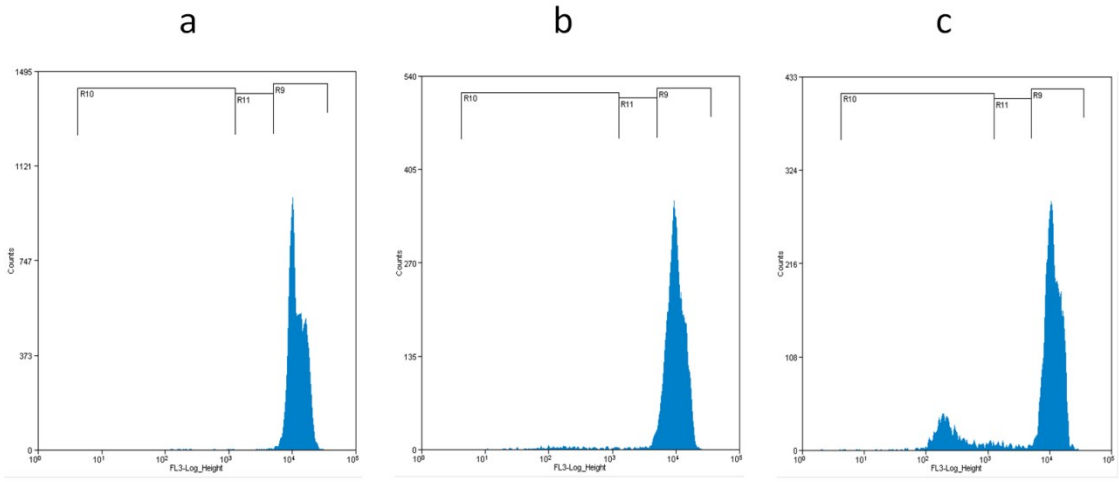


Figure S7. Cytotoxicity of CS-1 (a) and CS-2 (b) hydrogels to Wi-38 cells. The cell incubation time with hydrogels is 72 h.

Analysis of the cell cycle of MCF-7 cells incubated with hydrogels

MCF-7 cells were incubated in 6well plates for 24 h in a medium with the addition of 10% FBS and the tested compounds at various concentrations, then treated successively with Versen solution (2 mL) and 0.25% trypsin solution, after exposure at 37°C for 1 min they were suspended in 2 mL of a medium. The cells were centrifuged at 1500 rpm for 3 min, washed with PBS(2× 3 mL), treated with 70% EtOH (0.5 mL) and stored at –20°C. Before an experiment the cells were washed with PBS (2× 3 mL) and suspended in 1 mL of PBS, then 200μL of RNase A (200 μg/mL), and after incubation at 37°C for 30 min 100 μL of propidium iodide (1 mg/mL) were added, after which the cells were kept at 20°C for 10 min. Analysis was performed on a Beckmann MoFLO XDP flow cytofluorometer in FL3 mode.



Sample	G1/G0_phase, %	S_phase, %	G2/M_phase, %
a	0,42	0,33	99,29
b	2,71	2,44	95,35
c	17,61	2,92	79,72

Figure S8. Results of the flow cytofluorometric analysis of intact MCF-7 cells incubated for 24 h with CS-1 (b) and CS-2 (c) in a medium containing 10% FBS. a – control. CS-1 and CS-2 concentration is 80 μM. The X axis, fluorescence PI (propidium iodide); fluorescence intensity in the FL-3 channel 620/29 nm; the Y axis, number of cells; R10 - G0/G1- phase, R11 – S- phase and R9 - G2/M - phase of cell division.