Electronic supplementary materials *Mendeleev Commun.*, 2022, **32**, 591–593

Synthesis and evaluation of the anticancer activity of the water-dispersible complexes of 4-acylaminoisoxazole derivative with biocompatible nanocontainers based on Ca^{2+} (Mg^{2+}) cross-linked alginate

Vasily V. Spiridonov, Kirill S. Sadovnikov, Dmitry A. Vasilenko, Kseniya N. Sedenkova, Alina R. Lukmanova, Alina A. Markova, Anna V. Shibaeva, Anastasia V. Bolshakova, Sergey S. Karlov, Elena B. Averina and Alexander A. Yaroslavov

General information

The synthesis of isoxazole **1** was carried out using previously described method [D. V. Tsyganov *et al*, *Mendeleev Commun.*, 2019, **29**, 163]. The preparation and TEM-visualization, DLS and electro-kinetic measurements of specimens **I–IV** were carried out according to the procedures described earlier [(*a*) N. B. Chernysheva *et al*, *Eur. J. Med. Chem.*, 2018, **146**, 511; (*b*) K. S. Sadovnikov *et al*, *Arch. Pharm*., 2022, **335**, 2100425].

Table S1 Nanocontainers studied in the work

Nanocontainer			
(I)	**(II)**	**(III)**	**(IV)**
(Alg)-Ca^{2+}	(Alg)-Mg^{2+}	(Alg)-Ca^{2+}-Izoxazole	(Alg)-Mg^{2+}-Izoxazole

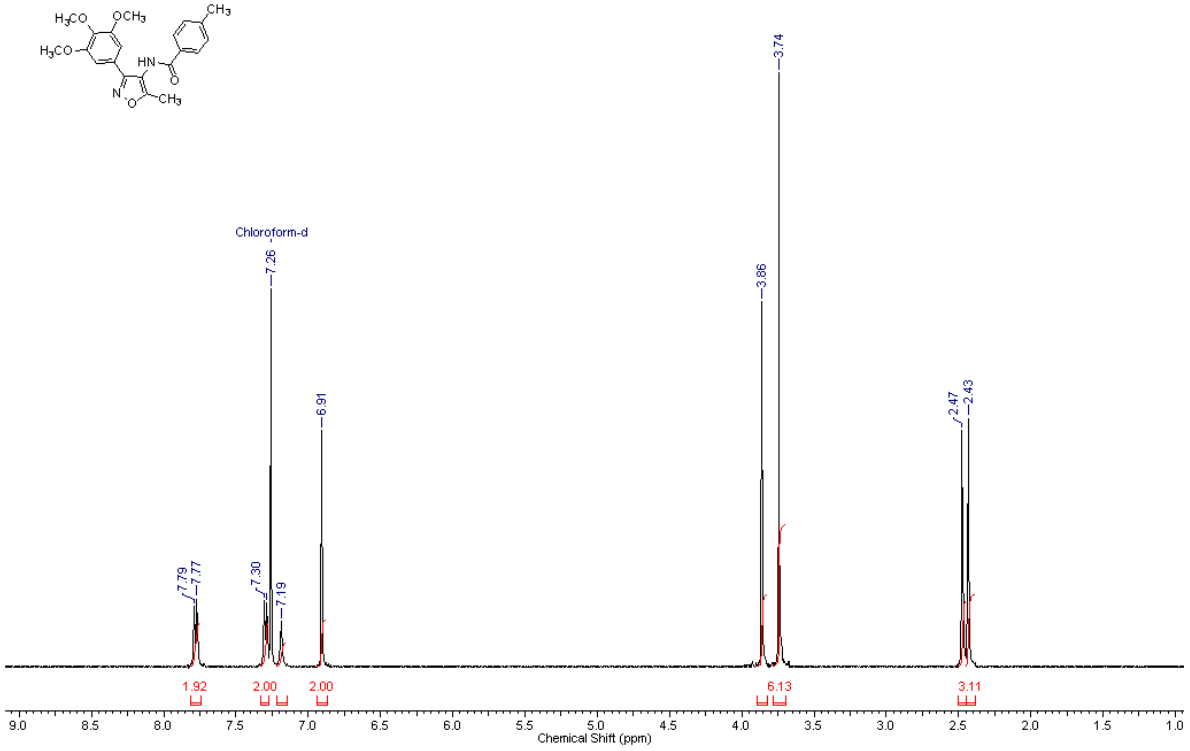
General experiment

Protonation of isoxazole **1** was carried out as follows. Sulfuric acid (50%, 300 μl) was added to isoxazole derivative **1** (2.5 mg, 6.5 mmol). The resulting suspension was heated at 50°C for 15 minutes until complete dissolution. Immobilization of the protonated form of isoxazole **1'** was carried out as described below. The solution containing 2.5 mg of isoxazole in protonated form **1'** was added dropwise to an aqueous solution (10 ml) containing 5 mg of **I** or **II** with vigorous stirring. The resulting mixture was adjusted to pH=7, then purified by dialysis due to 24 hours.

Control experiment

Control experiment to assess the stability of isoxazole **1** under protonation conditions was carried out as follows. Sulfuric acid (50%, 3.0 ml) was added in portions to isoxazole **1** (10.0 mg, 26 mmol). The resulting suspension was heated to 40°C and kept at this temperature until the precipitate dissolved and a yellow color appeared. The resulting yellow solution was cooled to room temperature, neutralized with NaOH solution (40%, 3.6–4 ml) to pH=7–8, water (20 ml)

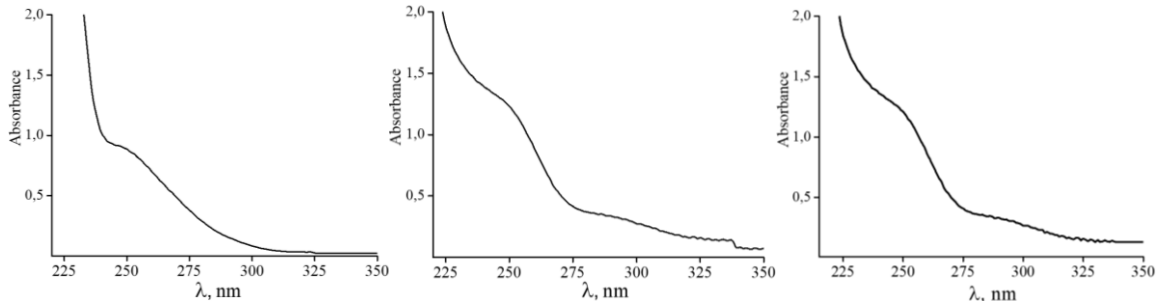
was added, and the mixture was extracted with CH_2Cl_2 (4×25 ml). The combined organic fractions were dried over $MgSO_4$, the solvent was distilled off under reduced pressure to recover 9.5 mg (95%) of compound **1**.



^{1}H NMR spectrum (400 MHz, $CDCl_3$): δ 2.43 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.74 (s, 6H, $2CH_3O$), 3.86 (s, 3H, CH_3O), 6.91 (s, 2H, 2CH(Ar)), 7.19 (br.s, 1H, NH), 7.27-7.32 (m, 2H, 2CH(Ar)), 7.76-7.80 (m, 2H, 2CH(Ar)).

Quantitative determination of isoxazole **1'** in the composition of **III** and **IV** was carried out by UV spectroscopy. The calibration curve method was used for this purpose. A series of isoxazole **1** solutions was preliminarily obtained in the DMSO–water medium. UV spectra in the wavelength range from 200 to 350 nm were recorded on Ultrospec 500/1100 pro (Amersham, UK) spectrophotometer using quartz cuvettes at room temperature.

A typical UV absorption spectrum of isoxazole **1** is presented. This spectrum contains a broad absorption peak with a maximum at 250 nm, which obviously corresponds to the superposition of n→π*, as well as π→π* transitions in the isoxazole molecule containing conjugated aromatic substituent. The intensity of the absorption peak at 250 nm changes with varying the concentration of the heterocycle in solution ratably. Based on this fact a reliable calibration curve was made.



A) B) C)

Figure S1 UV spectra of DMSO/H_2O solutions of isoxazole **1** (A) concentration c=1.03×10^{-4} M; isoxazole **1'** immobilized in nanocontainer **III**, total concentration of the ternary system c = 0.25 mg ml^{-1} (B); isoxazole **1'** immobilized in nanocontainer **IV**, total concentration of the ternary system c = 0.25 mg ml^{-1} (C).

UV spectra of samples **III** and **IV**, consist of peak at 250 nm and broad peak at 295 nm (Figure S1, parts B,C). Peak at 295 nm can reflect realization of the n→π* transition, which takes place in C=O groups, as well as the π→π* transition in the substituted aromatic ring of compound **1'**. Its appearance in the spectrum of the nanocontainers–heterocycle is due to the electrostatic interaction between the COO^--groups of nanocontainers **I** and **II** and protonated moiety in heterocycle **1'**. It should be noted that this peak is absent both in the spectrum of the polysaccharide and in the spectrum of isoxazole **1**. The protonation of isoxazole **1** can be accompanied by a violation in the electronic conjugation between the heterocyclic and aromatic fragments of **1**. This phenomenon leads to the appearance of additional peak in UV-spectrum. In addition, the electrostatic interaction leads to the partial transformation of anionic residues of **I** and **II** from carboxylate to carboxylic one.

The procedure of MTT-assay

The human colon adenocarcinoma cell line HCT-116 and non-tumor fibroblasts were obtained from American Type Culture Collection, USA. Cells were propagated in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (PanEco, Russia) at 37°C in a humidified 5% CO_2-controlled atmosphere. Cells in logarithmic phase of growth were used in the experiments. The cytotoxic activity of compounds was assessed *in vitro* using the MTT assay. Cells were plated in 96 well plates (NUNC, USA, 5·103 cells in 190 μL culture medium per well). After 24 hours of incubation at 37 °C (5% CO_2, in a humidified atmosphere), 10 μL cell media solutions of compounds for final concentrations 0.1-50 μmol/L were added. The cells

without substances served as an intact control. Doxorubicin (Teva, Netherlands) was used as a positive control. The cytotoxicity of compounds was assessed in a formazan conversion assay (MTT-test) after a 72 h drug exposure. After the completion of drug exposure, the 0.5 mg ml^{-1} 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reagent) was added to cells. Cells were incubated with MTT for 2 h, the culture medium was removed, the cells were resuspended in DMSO (100 μl), and the optical density of the solution was measured on a Multiscan FC plate spectrophotometer (Thermo Scientific, USA) at a wavelength of 571 nm. The percentage of survived cells for each dose was calculated as the quotient of the average optical density in the wells after incubation with this dose to the average optical density of the control wells (the values of the latter are taken as 100%). Four independent experiments were performed for each concentration; the results are statistically processed. Standard deviations did not exceed 10%.