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Non-covalent conjugate based on graphene oxide and a cytotoxic agent containing 1,3,5-triazine derivative and (5-phenyl-2*H*-tetrazol-2-yl)acetoxy moiety: synthesis, characterization, properties and cytotoxic activity

Olga V. Mikolaichuk, Olga S. Shemchuk, Alexandra V. Protas, Elena A. Popova, Vladimir A. Ostrovskii, Dmitriy N. Maystrenko, Oleg E. Molchanov, Vladimir V. Sharoyko and Konstantin N. Semenov

Content

Experimental part

Synthesis of GO

Diffractometer Rigaku "MiniFlex II" (Japan) was used to perform the X-ray phase analysis of GO-**3**. Raman spectra were recorded using a Horiba spectrometer Jobin-Yvon LabRam HR800 (Japan); IR spectra were recorded on a Nicolet 8700 IR-Fourier spectrometer (USA). The morphology of the obtained GO-**3** nanoparticles was determined using a JSM-7001F scanning electron microscope (Japan). Quantitative and quantitative analysis of GO-**3** was carried out using X-ray photoelectron spectroscopy (XPS) on a Thermo spectrometer Fisher Scientific ESCAlab 250Xi (USA) using monochromatic AlKα radiation (photon energy 1486.6 eV).

Registration of ^{13}C NMR spectra was carried out on a Bruker spectrometer Avance III 400 WB (USA); operating frequency 100.64 MHz.

Study of the size distribution of GO-**3** nanoparticles in aqueous dispersions and their ζ-potentials was carried out on the Malvern instrument Zetasizer 3000 (Great Britain). The values of the polydispersity index were 0.24–0.27.

GO has been synthesized from graphite via a modified Hummers and Offeman oxidation reaction. Graphite powder (3 g) was dispersed in sulfuric acid (200 ml) in an ice bath with stirring for 30 minutes. Then solid $KMnO_4$ (8 g) was added slowly with continuous stirring for 45 min, after which $NaNO_3$ (2 g) was added with stirring for 2 h at 5°C. Then the reaction mixture was stirred for 90 min at 96°C, gradually adding deionized water (200 ml). Additional portions of deionized water (350 ml) and 30% H_2O_2 (10 ml) were added to complete the oxidation process and remove excess $KMnO_4$. The GO precipitate was separated by filtration and washed repeatedly in 5% HCl solution and deionized water until a neutral pH was reached. The precipitate was dried at 65°C for 5 h, then redispersed in deionized water under sonication for 1 h and then centrifuged (20 min at 4000 rpm). As a result, a red-brown GO precipitate was obtained in 90% yield.[S1,S2]

$$\%DLE = \frac{m_{GO-3} - m_{GO}}{m_{compound\,3}} \cdot 100\%, \quad \text{(S1)}$$

where $m_{GO\text{-}3}$, m_{GO} and $m_{compound\ 3}$ are the mass of the conjugate, GO and compound **3**, respectively.

MTT analysis

MTT analysis (colorimetric test using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was performed on HeLa and A549 cell lines. Cells at a concentration of $5 \cdot 10^4$ per well were seeded in a 96-well plate and incubated for 12 h in DMEM-F12 medium supplemented with 10% thermally inactivated fetal bovine serum, 1% L-glutamine, 50 $U \cdot ml^{-1}$ penicillin and 50 $\mu g \cdot ml^{-1}$ streptomycin. After cultivation, fresh DMEM-F12 medium

containing various concentrations of GO-**3** was added to the wells. Next, the plate was then incubated at 37°C in a humidified atmosphere CO_2 -incubator in the presence of 20% O_2, 5% CO_2. After 48 h, 0.1 ml of DMEM-F12 and 0.03 ml of the MTT reagent (0.5 $mg \cdot ml^{-1}$) were added to the wells and the incubation continued for 1 h, after which the supernatant was removed. The formazan crystals formed during MTT reduction by viable cells were dissolved in 0.1 ml of DMSO and the optical density was measured on a BioRad x Marx plate spectrophotometer (USA) at λ = 540 nm, subtracting the background optical density at λ = 690 nm.

For each cell line, the half-maximal inhibition concentration (IC_{50}) of GO-**3** was determined. The obtained values were compared with IC_{50} substance **3**.[S3,S4]

NMR spectra of compounds

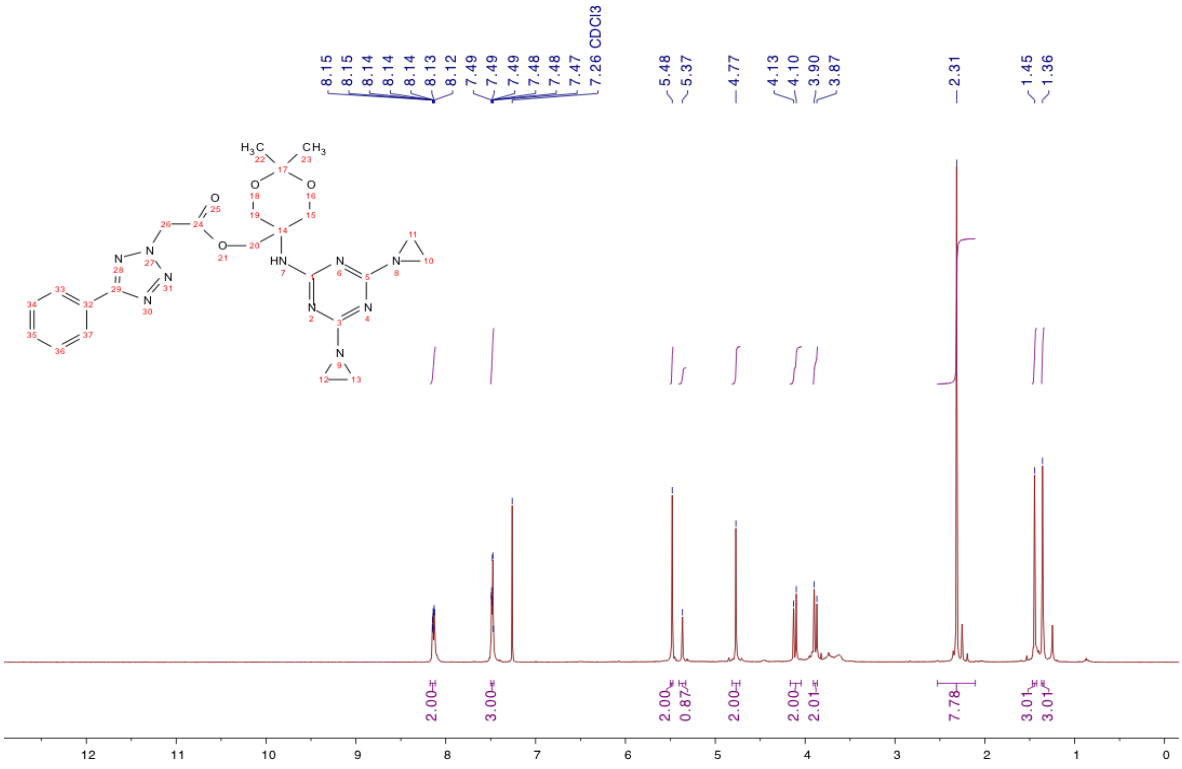

Figure S1. ^{1}H NMR spectrum of (5-{[4,6-di(aziridin-1-yl)-1,3,5-triazin-2-yl]amino}-2,2-dimethyl-1,3-dioxan-5-yl)methyl (5-phenyl-2*H*-tetrazol-2-yl)acetate (compound **3**).

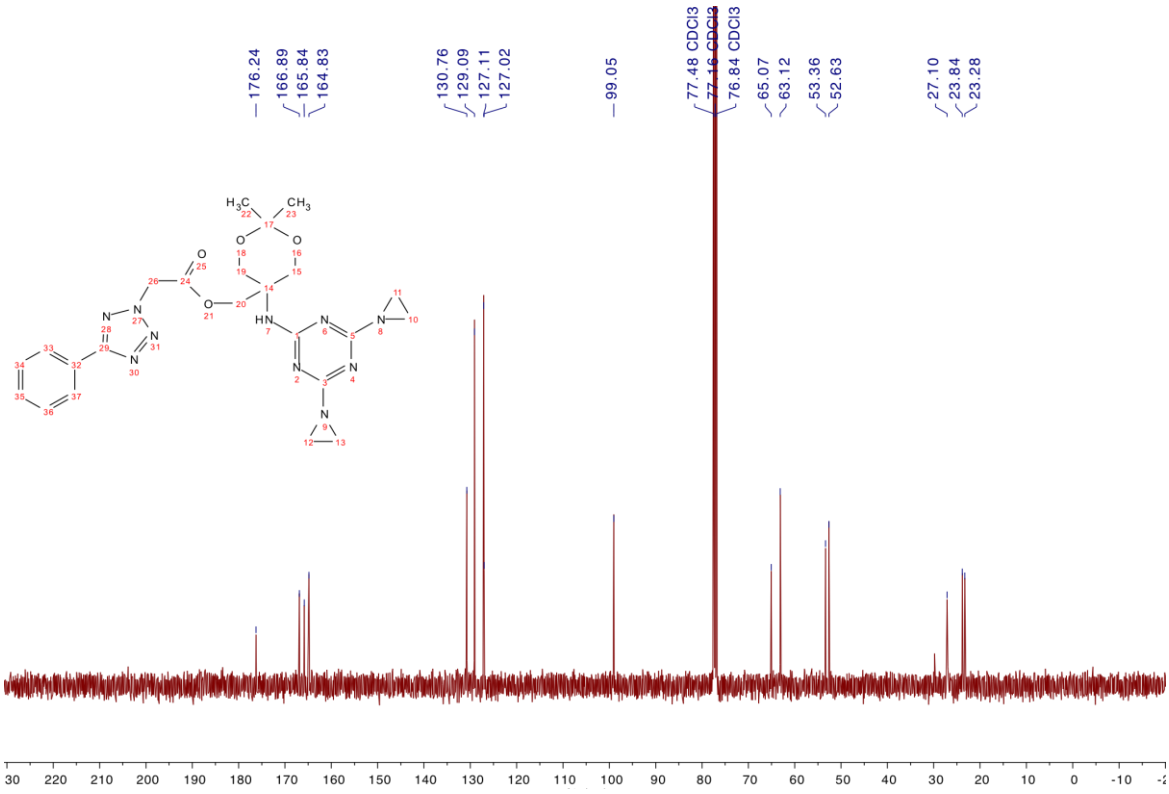

Figure S2. ^{13}C NMR spectrum of (5-{[4,6-di(aziridin-1-yl)-1,3,5-triazin-2-yl]amino}-2,2-dimethyl-1,3-dioxan-5-yl)methyl (5-phenyl-2*H*-tetrazol-2-yl)acetate (compound **3**).

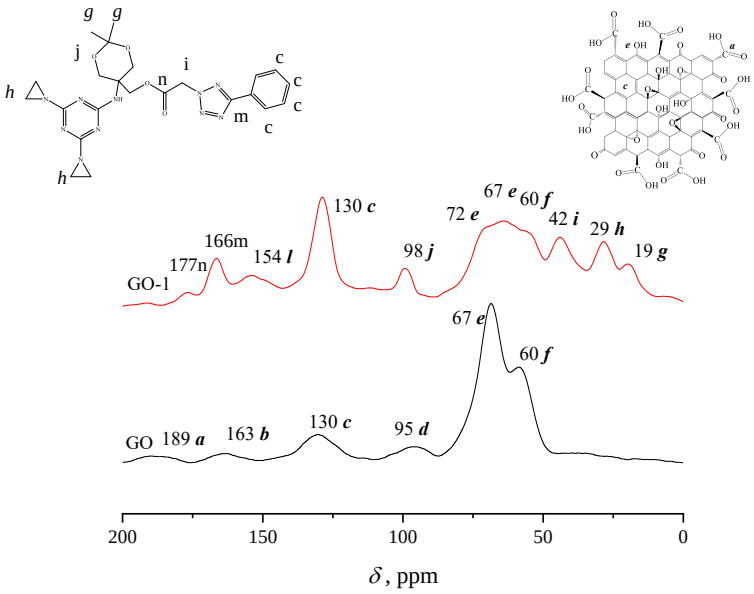

Figure S3. Solid state ^{13}C NMR spectra of GO и GO-**3**.

Mass spectra of compounds

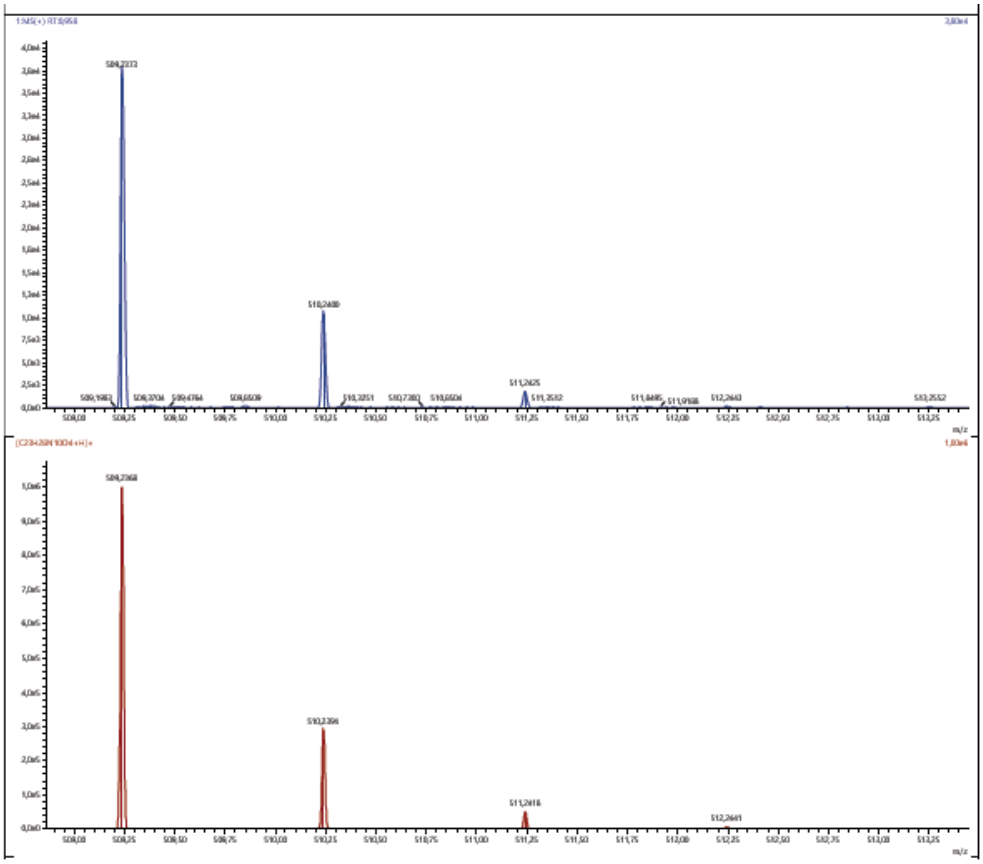

Figure S4. Mass spectrum (HRESI$^+$-MS) of (5-{[4,6-di(aziridin-1-yl)-1,3,5-triazin-2-yl]amino}-2,2-dimethyl-1,3-dioxan-5-yl)methyl (5-phenyl-2*H*-tetrazol-2-yl)acetate (compound **3**).

IR spectra of compounds

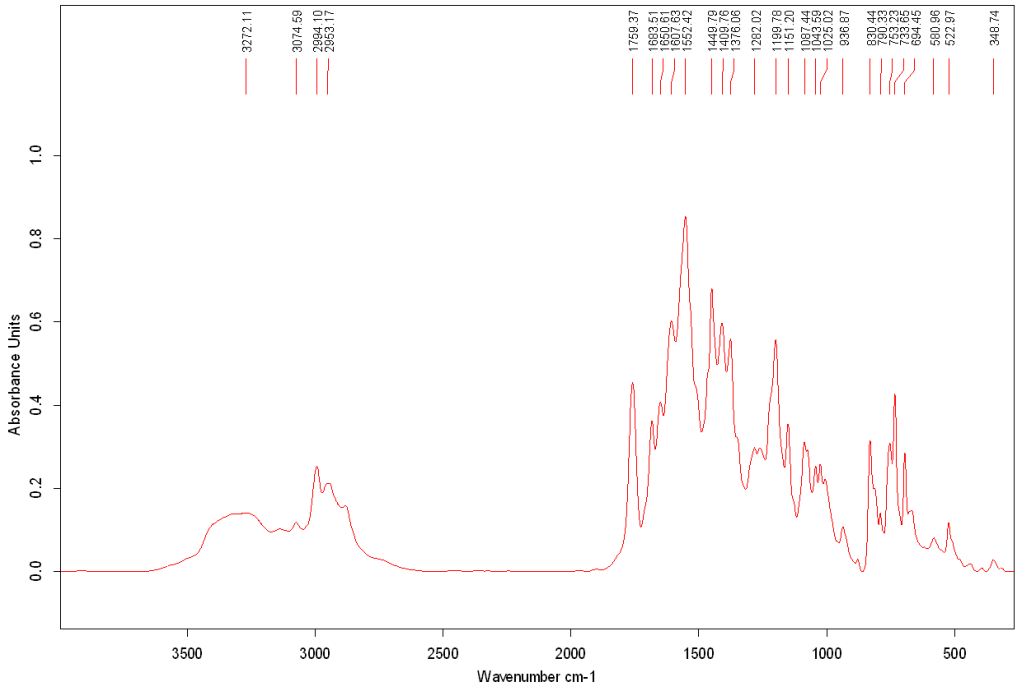

Figure S5. IR spectrum of (5-{[4,6-di(aziridin-1-yl)-1,3,5-triazin-2-yl]amino}-2,2-dimethyl-1,3-dioxan-5-yl)methyl (5-phenyl-2*H*-tetrazol-2-yl)acetate (compound **3**).

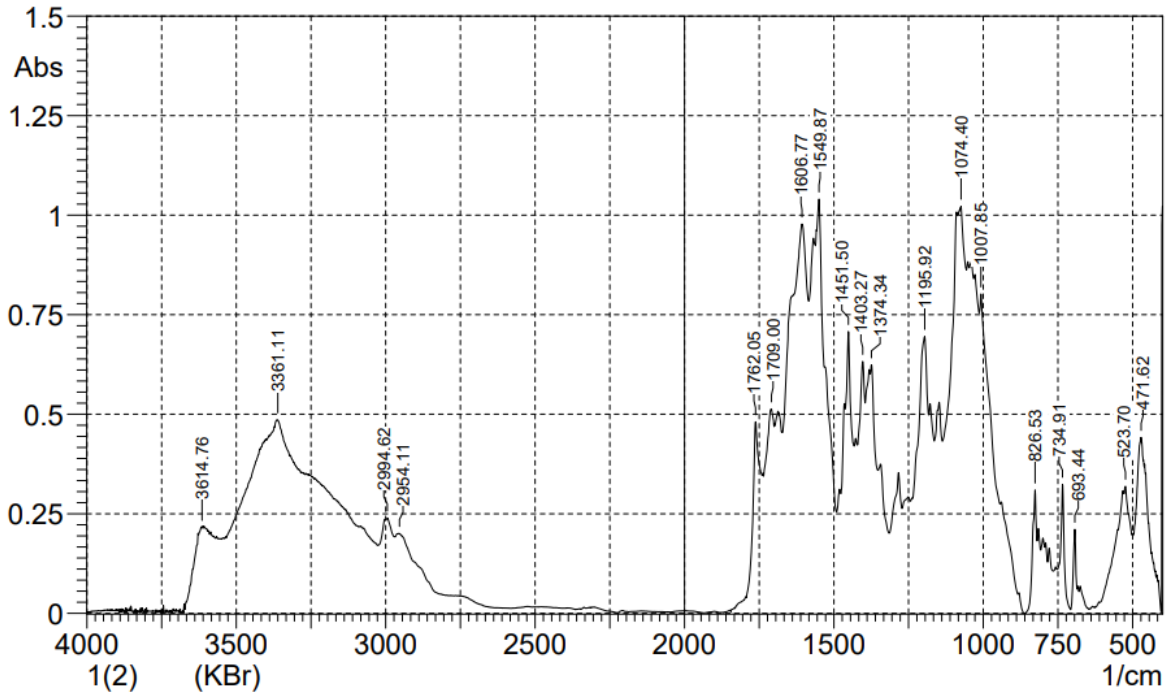

Figure S6. IR spectra of GO-**3**.

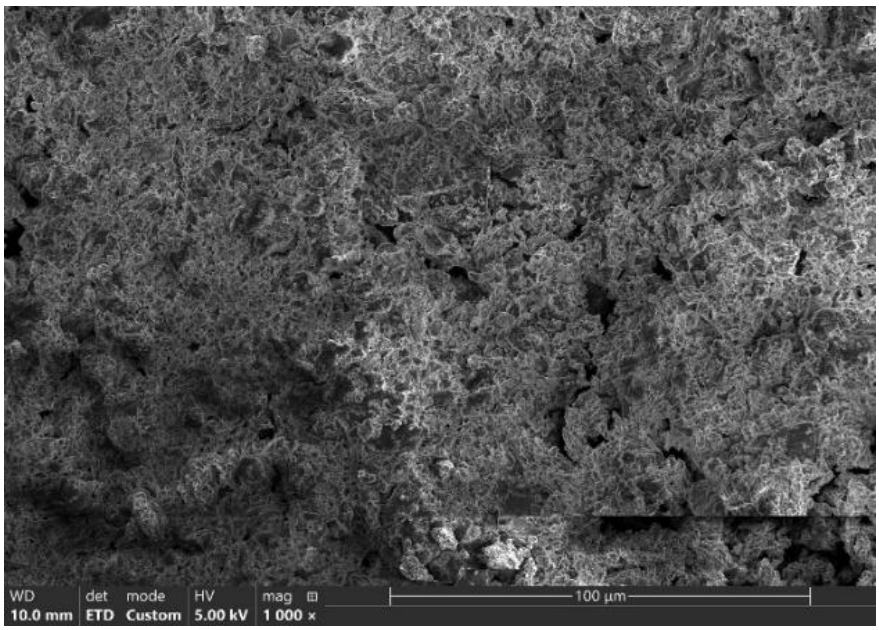

Figure S7. The SEM photograph of GO-**3**.

Elemental analysis

Table S1. Data of the elemental analysis.

Sample	[C], % *wt*.	[H], % *wt*.	[N], % *wt*.
GO	43.0±0.1	2.4±0.2	-
GO-**3**	59.7±0.7	25.1±0.4	15.1±0.2

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