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Synthesis, physicochemical properties and *in vitro* cytotoxic activity of aziridine-containing derivatives of 1,3,5-triazine

Olga V. Mikolaichuk,*[a,b] Alexandra V. Protas,[a,b] Elena A. Popova,[a,b] Olga S. Shemchuk,[a] Mikhail D. Luttsev,[a] Daler R. Dadadzhanov,[c] Tigran A. Vartanyan,[c] Andrey V. Petrov,[d] Vladimir V. Sharoyko,[a,b,d] Konstantin N. Semenov,[a,b,d] Oleg E. Molchanov[b] and Dmitrii N. Maistrenko[b]

[a] *I. P. Pavlov First St. Petersburg State Medical University, 197022 St. Petersburg, Russian Federation*
[b] *A. M. Granov Russian Research Center for Radiology and Surgical Technologies, 197758 St. Petersburg, Russian Federation. E-mail: olga19940519@mail.ru*
[c] *International Research and Education Centre for Physics of Nanostructures, ITMO University, 197101 St. Petersburg, Russian Federation*
[d] *Institute of Chemistry, St. Petersburg State University, 198504 St. Petersburg, Russian Federation*

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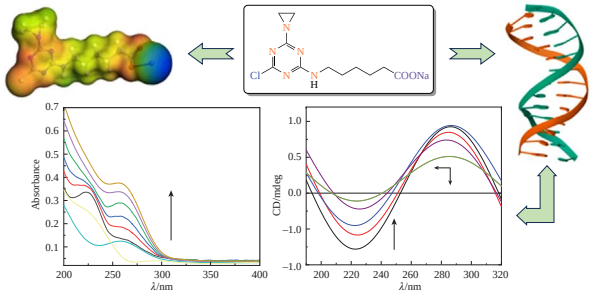
6-{[4-(Aziridin-1-yl)-6-chloro-1,3,5-triazin-2-yl]amino}-hexanoic acid methyl ester and sodium salt were synthesized from cyanuric chloride. The sodium salt would interact with free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and inhibit hemolysis induced by Radachlorin. Both compounds interact with DNA with K_{bin} values of 1.09×10^7 and 0.99×10^7 dm^3 mol^{-1}, respectively; they also demonstrate cytotoxic activity against human cancer cell lines.



Keywords: 1,3,5-triazine, aziridine, cyanuric chloride, imidazo[1,2-*a*][1,3,5]triazine, physicochemical properties, biocompatibility, interaction with DNA, cytotoxic activity.

Some 1,3,5-triazines are acknowledged as cytostatic agents due to their capacity to impede cell proliferation and division. The ZSTK474 that contains morpholine moieties and AMG511 with piperazine cycle are currently being evaluated in clinical trials as pan inhibitors of class I PI3K (Figure 1).[1–14] 1,3,5-Triazine derivatives containing aziridine moieties can act as alkylating agents, and these interactions can cause DNA damage, inhibition of the division of cancer cells, which finally result in cell death.[15,16] 2,4,6-Tri(aziridin-1-yl)-1,3,5-triazine (tretamine) was introduced into therapeutic practice for adjuvant chemotherapy back in the 1950s.[17]

In this study, the synthesis and structural characterization of two new 1,3,5-triazine derivatives containing 6-aminohexanoic acid and aziridine moieties have been undertaken. Their antioxidant activity has been tested by photoinduced hemolysis and by antiradical activity towards NO-radicals. Binding of these compounds with DNA was evaluated using UV- and CD-spectroscopies. Their antiproliferative properties were determined *in vitro* by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays against A549 and PANC-1 tumor cells lines.

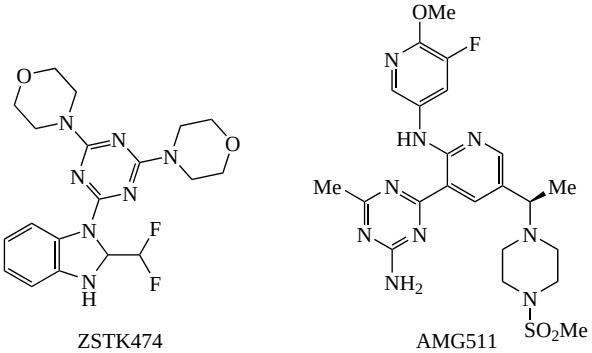
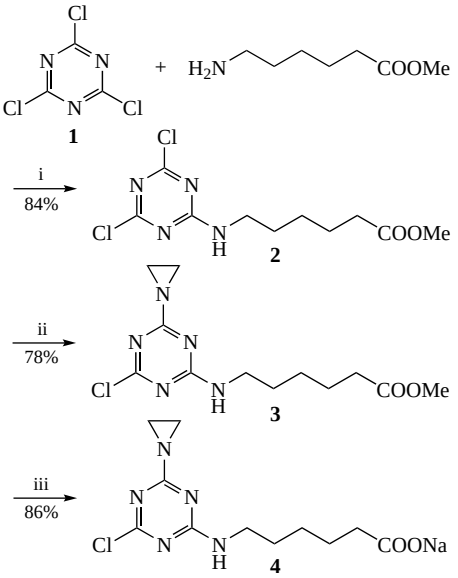


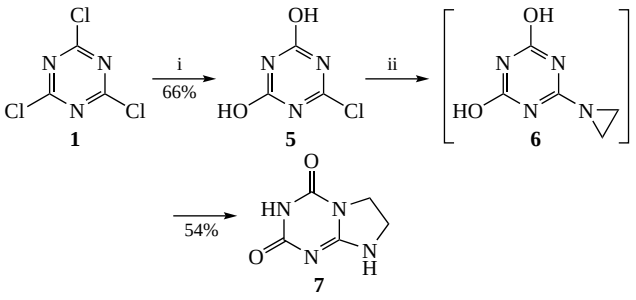
Figure 1 1,3,5-Triazine derivatives that demonstrated significant antitumor effect.

Herein, cyanuric chloride **1** was used as a starting reagent in the synthesis of methyl 6-[(4,6-dichloro-1,3,5-triazin-2-yl)-amino]hexanoate **2** (Scheme 1). The next chlorine atom in intermediate **2** was replaced with aziridine to afford 6-{[4-(aziridin-1-yl)-6-chloro-1,3,5-triazin-2-yl]amino}-hexanoic acid methyl ester **3**. The saponification of ester **3** in methanol–water solution with NaOH gave sodium salt **4** of the corresponding acid.

An attempt to obtain the very 6-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]hexanoic acid by reacting cyanuric chloride **1** with 6-aminohexanoic acid was not successful. Thus, when an aqueous solution of 6-aminohexanoic acid was added to a solution of cyanuric chloride **1** in acetone in the presence of triethylamine, the formation of 6-chloro-1,3,5-triazine-2,4-diol **5** was observed (Scheme 2). The similar processes have been previously described by K. Venkataraman.[18] At the next stage, by treatment of chloride **5** with an aqueous solution of aziridine (preliminarily obtained *in situ*), 7,8-dihydroimidazo[1,2-*a*]-[1,3,5]triazine-2,4(3*H*,6*H*)-dione **7** was obtained. Apparently, product **7** resulted from the rearrangement of compound **6**. The structure of compound **7** was additionally confirmed by XRD (Figure 2).†



Scheme 1 *Reagents and conditions*: i, Et_3N, H_2O, acetone (pH 8–9), 0–5 °C, 40 min, then room temperature, 2 h; ii, aziridine, H_2O, $CHCl_3$, NaOH, Na_2CO_3, 45 °C, 20 h; iii, NaOH, H_2O, MeOH, room temperature, 24 h.



Scheme 2 *Reagents and conditions*: i, $H_2N(CH_2)_5CO_2H$, Et_3N, H_2O, acetone (pH 8–9), 0–5 °C, 40 min, then room temperature, 2 h; ii, aziridine, H_2O, $CHCl_3$, Na_2CO_3.

The values of density and viscosity as functions of the concentration of compounds **3** (C = 2–20 μM) and **4** (C = 2–40 μM) at the temperature range from 25 to 50 °C are presented in Figure S1 and Table S1 (see Online Supplementary Materials). Data of Table S1 reveal that the values of the studied parameters are insignificantly changed in all the considered concentration and temperature ranges. Salt formation is one of the general methods employed to enhance the aqueous solubility of poorly water-soluble substances.[19] The solubility of compounds **3** and **4** in water was investigated and presented in Figure S2 (T = 293.15–318.15 K). The solubility values were varied in the range 5.8–13.0 g dm^{-3} (for compound **3**) and 16.5–24.1 g dm^{-3} (for compound **4**) in the studied temperature range. Also, it was shown that there were no significant changes in the spectral characteristics in the ^{1}H NMR (D_2O) spectra for 7 days, which indicated the stability of compound **4** in water medium (Figure S3).

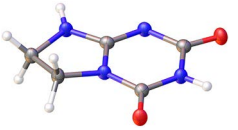


Figure 2 Solid-state structure of compound **7** shown from the front. Ellipsoids are shown at the 50% probability level.

The electron density distribution in the molecules of compounds **3** and **4** was analyzed using the DFT with the inclusion of COSMO model, MD (molecular dynamics) was carried out using the Forcite module within Materials Studio software package. The energy values (Ha) HOMO and LUMO for compounds **3** and **4** were as follows: HOMO3 –0.2231, LUMO3 –0.0755 and HOMO4 –0.1999, LUMO4 –0.0795, respectively. The values of the optimal distance between water molecules and the nitrogen, chlorine, sodium and oxygen atoms in compounds **3** and **4** are given in Table S2.

The data obtained indicate that 1,3,5-triazines **3** and **4** do not react with NO radicals (Figure S4). The photodynamic effect is primarily associated with the generation of singlet oxygen, and then other reactive oxygen species. The binding of porphyrins to cell membranes leads to a decrease in the photostability of membranes.[20] It was found that compound **4** showed antiradical activity against free radicals of DPPH [(Figure 3(*a*)]. Furthermore, it is shown that kinetic dependences of DPPH reduction by compound **4** are linear and depend on temperature. The activation energy and pre-exponential factor for the reduction reaction of DPPH with substance **4** are E_a = 125.1 ± 0.4 kJ mol^{-1} and ln$A$ = 46.3 ± 0.2, respectively. As a result of the studies, a concentration dependence of the degree of photoinduced hemolysis in the presence of compounds **3** and **4** was measured (Figure S5). The data obtained reveal that compound **3** does not cause hemolysis, and compound **4** suppresses hemolysis induced by Radachlorin, which manifests itself in an increase in the time of hemolysis [see Figure 3(*a*)].

In the UV spectra of aqueous DNA solutions in the range from 200 to 350 nm, a wide absorption band with maximum at 260 nm is observed. The absorption spectra of DNA in aqueous

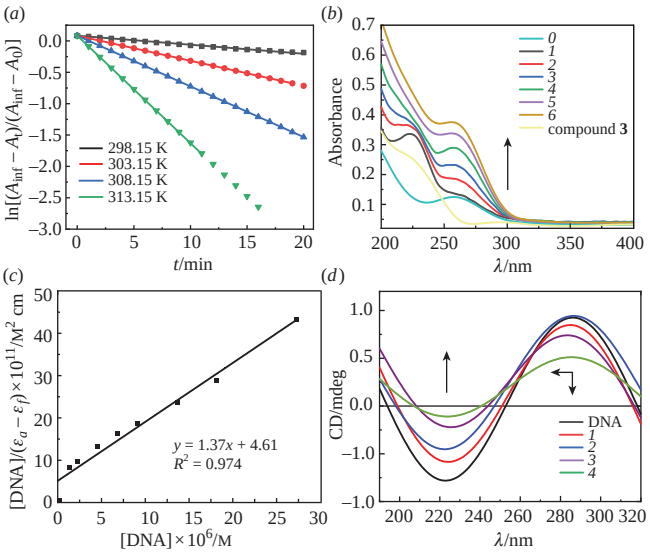


Figure 3 (*a*) Kinetic dependence of DPPH reduction by compound **4** in the temperature range 298.15–313.15 K, in the time interval 0–20 min. (*b*) UV spectra of aqueous solutions (0.9% NaCl) DNA in the presence of compound **3** (5.7 μM) at various concentrations of DNA: (*0*) 0.57 μM (without compound **3**), (*1*) 0.57 μM, (*2*) 1.14 μM, (*3*) 2.28 μM, (*4*) 4.56 μM, (*5*) 9.12 μM, and (*6*) 14.25 μM. (*c*) Dependence in Wolfe–Shimmer coordinates for compound **3**. (*d*) CD spectra of aqueous solutions of DNA (5.7 μM) in NaCl (0.9%) and 3.5 mM Tris–HCl/50 mM NaCl buffer solution (pH 7.2) in the presence of compound **3** at various concentrations: (*1*) 0.57 μM, (*2*) 1.14 μM, (*3*) 4.56 μM, and (*4*) 14.25 μM.

† *Crystal data for* **7**. $C_5H_6N_4O_2$ (M = 154.14 g mol^{-1}), triclinic, space group $P\bar{1}$ (no. 2), a = 4.2285(3), b = 8.2532(8) and c = 9.5635(5) Å, α = 105.974(7)°, β = 97.567(6)°, γ = 104.502(7)°, V = 303.34(4) Å^3, Z = 2, T = 100(2) K, μ(CuKα) = 1.156 mm^{-1}, d_{calc} = 1.688 g cm^{-3}, 2855 reflections measured (9.852° ⩽ 2θ ⩽ 144.984°), 1195 unique (R_{int} = 0.0340, R_{sigma} = 0.0396). The final R_1 was 0.0495 [$I > 2\sigma(I)$] and wR_2 was 0.1485 (all data).

CCDC 2243309 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* https://www.ccdc.cam.ac.uk.

solutions of 0.9% NaCl at a constant DNA concentration (5.7 μM) and various concentrations of compounds **3**, **4** are presented in Figure 3(*b)* (for compound **3**) and Figure S6 (for compound **4**).

Since substances **3** and **4** in the range of 260–300 nm are practically not absorbed, the hyperchromic effect in this area may be attributed to the formation of associations between DNA and compounds **3** and **4**. As shown in Figure 3(*c*), [DNA]/($\varepsilon_a - \varepsilon_f$) *vs.* [DNA] dependence is linear. The obtained K_{bin} values are 1.09×10^7 dm^3 mol^{-1} (for compound **3**) and 0.99×10^7 dm^3 mol^{-1} (for compound **4**). Previously,[21] the binding constant for analogous 5-{[4,6-di(aziridin-1-yl)-1,3,5-triazin-2-yl]amino}-2,2-dimethyl-1,3-dioxan-5-yl)methanol (dioxadet) was calculated to be 3.44×10^7 dm^3 mol^{-1}. The value of the change in the Gibbs energy (ΔG) was calculated and turned out to be −37.3 kJ mol^{-1} (for compound **3**) and −32.3 kJ mol^{-1} (for compound **4**). As can be seen from CD spectra [Figure 3(*d*)], an increase in the concentration of compounds **3** and **4** leads to an increase in the $\Delta\varepsilon$ value of the negative DNA band and the shape of the spectrum practically does not change. However, increasing concentration of compounds **3** and **4** leads to a decrease in the absorption intensity of the positive band. The observed changes in the CD spectra may propose that compounds **3** and **4** are able to disrupt the stacking interaction between nucleotides in the DNA duplex (positive band), and lead to minor changes in the secondary DNA strand (negative band).

It was observed that the 1,3,5-triazine derivatives demonstrate cytotoxic activity against human cancer cell lines, PANC-1 with IC_{50} = 12.4 ± 0.6 μM for compound **3** and IC_{50} = 3.1 ± 0.2 μM for compound **4**; A549 with IC_{50} = 66.8 ± 3.3 μM for compound **3** and IC_{50} = 10.3 ± 0.5 μM for compound **4**. The obtained data for A549 cell line are comparable with IC_{50} values for {5-[(4,6-di(aziridin-1-yl)-1,3,5-triazin-2-yl)amino]-2,2-dimethyl-1,3-dioxan-5-yl}methyl 2-(5-phenyl-2*H*-tetrazol-2-yl)-acetate with IC_{50} = 41.3 ± 1.3 μM and for doxorubicin with IC_{50} = 46.9 ± 1.5 μM.[21]

Cytotoxic activity studies for compound **7** showed that it did not exhibit cytotoxic activity against A549 and PANC-1 cancer cell lines.

In summary, methyl ester and sodium salt of 6-{[4-(aziridin-1-yl)-6-chloro-1,3,5-triazin-2-yl]amino}hexanoic acid were readily obtained from available reagents. The biological studies show them to be promising compounds, which may be the basis for the preparation of their new analogs.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.10.028.

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