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SHORT COMMUNICATION

Aziridine-Functionalized 1,3,5-Triazine Derivatives as Promising Anticancer Agents: Synthesis, DFT Study, DNA Binding Investigations and In Vitro Cytotoxic Activity

Aleksandra V. Protas[1,2] | Olga V. Mikolaichuk[1,2] | Elena A. Popova[1,2] | Kirill V. Timoshchuk[1,3] | Ilya V. Kornyakov[4,5] | Dmitrii N. Maistrenko[2] | Oleg E. Molchanov[2] | Vladimir V. Sharoyko[1,2,4] | Konstantin N. Semenov[1,2,3,4]

[1]General Chemistry Department, Pavlov First Saint-Petersburg State Medical University, Petersburg, Russia | [2]A. M. Granov Russian Research Centre for Radiology and Surgical Technologies, Saint Petersburg, Russia | [3]Saint Petersburg State Institute of Technology (Technical University), Saint Petersburg, Russia | [4]Institute of Chemistry, Saint-Petersburg State University, Saint Petersburg, Russia | [5]Laboratory of Nature-Inspired Technologies and Environmental Safety of the Arctic, Kola Science Centre, Russian Academy of Sciences, Apatity, Russia

Correspondence: Olga V. Mikolaichuk (olgamedchem@gmail.com)

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Keywords: 1,3,5-triazine | aziridine | cytotoxic activity | dioxane | interaction with DNA | MTT test

ABSTRACT

Herein, we report a synthesis and characterization of aziridine-functionalized 1,3,5-triazine derivatives. Electronic structure and the most preferable geometry of substances were calculated by DFT method. DNA binding investigations were conducted as part of the biomedicinal research as well as the cytotoxic activity of these compounds was evaluated using in vitro assays toward Huh-7 and A549 cancer cell lines and HEK-293 normal cell line. The results demonstrate that some of the synthesized compounds exhibit potent cytotoxic activity ([5-[[4,6-*bis*(aziridin-1-yl)-1,3,5-triazin-2-yl]amino]-2,2-dimethyl-1,3-dioxan-5-yl]methanol (**1**) and 4,6-*di*(aziridine -1-yl)-*N*-(2,2,5-trimethyl-1,3-dioxane-5-yl)-1,3,5-triazine-2-amine (**9**)), making them potential candidates for further development as anticancer agents.

1 | Introduction

One of the promising areas of application of 1,3,5-triazines in medicinal chemistry is the development of highly effective cytostatics. A significant antitumor effect was observed in some 2,4,6-substituted 1,3,5-triazine derivatives, which can act on different mechanisms and effect on various biological targets of tumor cells (DNA, enzymes, etc.) [1]. Trisubstituted 1,3,5-triazines with morpholine rings have been identified as ATP-competitive inhibitors of kinases, including phosphoinositide 3-kinase (PI3K) pan-class I [2]. A significant anticancer effect is shown by 2,4,6-substituted derivatives of 1,3,5-triazine containing aziridine cycles as substitutes. These compounds belong to the group of alkylating agents of the ethylenimine class, for which the main target is a DNA, including tumor cells [3, 4]. Thus, in the 1950s, 2,4,6-tri(aziridin-1-yl)-1,3,5-triazine (tretamine, triethylenemelamine, TEM) was introduced into therapeutic practice for adjuvant chemotherapy (Figure 1) [5, 6]. In 1996, [5-[[4,6-bis(aziridine-1-yl)-1,3,5-triazine-2-yl]-amino]-2,2-dimethyl-1,3-dioxane-5-yl]methanol **1** with high antitumor activity was synthesized (Figure 1) [7].

Compound **1** demonstrates a notable localized anticancer effect, with significantly lower local and systemic toxicity compared with platinum-based coordination compounds. When administered intraperitoneally and intrapleurally, compound **1** does not induce sclerosing effects, in contrast to the complications associated with adhesions resulting from cisplatin administration. Furthermore, compound **1** exhibits amphiphilicity, enabling versatile administration [7]. Previously, we synthesized (5-((4,6-di(aziridin-1-yl)-1,3,5-triazin-2-yl)amino)-2,2-dimethyl-1,3-dioxan-5-yl)methyl

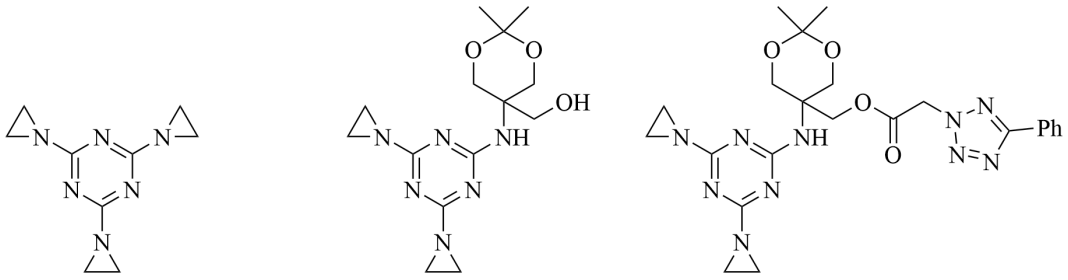
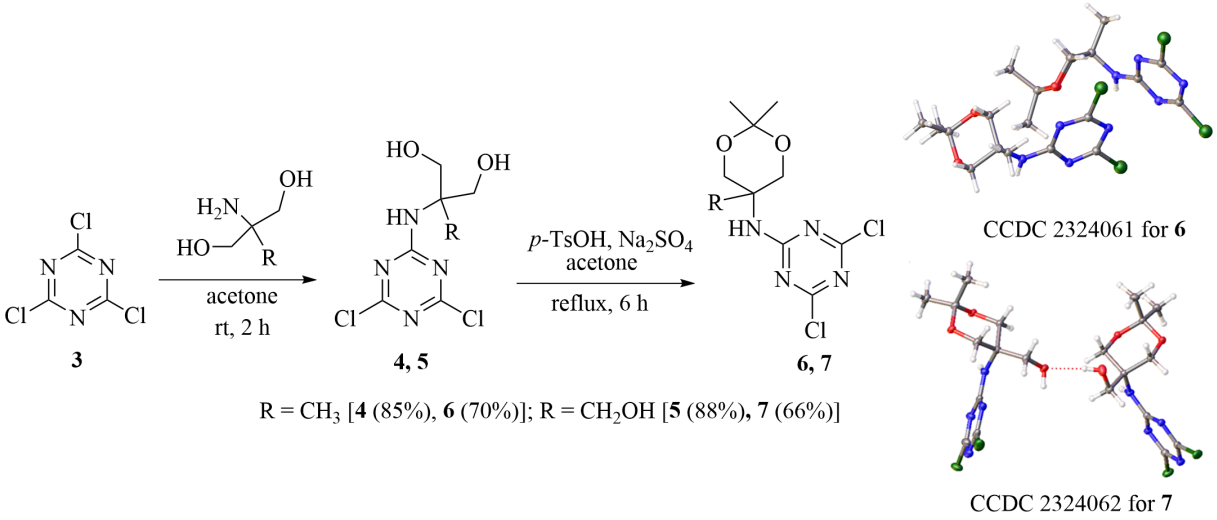


FIGURE 1 | Alkylating agents of the ethylenimine class based on 1,3,5-triazine.



R = CH_3 [**4** (85%), **6** (70%)]; R = CH_2OH [**5** (88%), **7** (66%)]

SCHEME 1 | Synthesis of 4,6-dichloro-N-(2,2,5-trimethyl-1,3-dioxan-5-yl)-1,3,5-triazin-2-amine (**6**) and (5-((4,6-dichloro-1,3,5-triazin-2-yl)amino)-2,2-dimethyl-1,3-dioxan-5-yl)methanol (**7**).

2-(5-phenyl-2*H*-tetrazol-2-yl)acetate (**2**) (Figure 1) and showed that the compound **2** demonstrates higher antitumor activity against the SK-HEP-1 tumor cell line than compound **1** [8].

In this study, novel derivatives of 1,3,5-triazine containing one or two aziridine cycles and a 1,3-dioxane moiety as substituents were synthesized and characterized by means of mass spectrometry (HRESI$^+$-MS), elemental analysis, as well as ^{1}H, ^{13}C{^{1}H} NMR spectroscopy. Their interaction with DNA was investigated using UV spectroscopy. The cytotoxic effect on tumor cell lines Huh-7 and A549 and normal control cells HEK-293 was evaluated by the MTT assay. A comparative analysis of the cytotoxic properties of the new synthesized compounds with compound **1** was conducted.

2 | Results and Discussion

2.1 | Experimental Procedure and Characterization Data

It is known that 2-substituted 1,3-dioxanes can be obtained by acetalization or ketalization of triols [9, 10]. Aforementioned, using azeotropic distillation can be quite labor-intensive and can be accompanied by the formation of a mixture of 1,3-dioxolanes and 1,3-dioxanes. It has been shown that using of 2-amino-2-hydroxymethylpropane-1,3-diol leads to the exclusive formation of dioxane and not dioxolane [10]. In this regard, 2-amino-2-methylpropane-1,3-diol and 2-amino-2-hydroxymethylpropane-1,3-diol were initially involved in the reaction with 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) (**3**) (Scheme 1). We have found that an effective method for obtaining compounds **6**, **7** is the reaction of cyanuric chloride **3** with corresponding alcohol without preliminary isolation of compounds **4**, **5**. First, the cyanuric chloride **3** reacted with the corresponding amino alcohol in acetone at rt. until 2,4,6-trichloro-1,3,5-triazine **3** has been completely consumed (control by TLC). Thereafter *p*-toluenesulfonic acid and sodium sulfate were added to the reaction mixture and refluxing for 6 h. Compounds **4**, **5** were also isolated, characterized and used to monitor the above reaction and biological studies.

The aziridine was synthesized following a previously established method, involving the reaction of 2-bromoethanamine hydrobromide in an aqueous solution of NaOH and Na_2CO_3 at 50°C for 2 h [11]. The resulting aqueous solution of aziridine was then combined with a solution of compound **6** or **7** in chloroform (Scheme 2). The heterophase reaction system was maintained at 45°C with vigorous stirring for 20 h. Following separation and evaporation of the organic phase in both cases, a mixture of 1,3,5-triazine derivatives containing one **8**, **9** and two **9**, **1** aziridine cycles was isolated and purified by column chromatography using chloroform as an eluent.

The obtained compounds were characterized by mass spectrometry (HRESI$^+$-MS), elemental analysis, as well as ^{1}H, ^{13}C{^{1}H} NMR spectroscopy. The structures of compounds **6** and **7** were also confirmed by XRD data (Scheme 1). In the crystal lattice, a pair of

compounds **7** molecules form a dimer through two intermolecular hydrogen bonds between H3···O1 atoms. Additionally, the fragments at the 2,4,6 positions of compounds **6** and **7** are rotated at an angle of 60°–90° relative to the 1,3,5-triazine cycle. Importantly, these findings are consistent with XRD results previously obtained for compound **1** [12].

In the ^{1}H NMR spectra for compounds **1**, **6–10**, signals appeared at 1.21–1.23 ppm corresponding to the chemical shifts of the protons of CH_3 groups located in the dioxane ring. The signals of the methylene groups protons of the aziridine rings located in the 2- and 4-positions of the substituted 1,3,5-triazines is observed at 2.31 ppm. In the ^{13}C NMR spectra there are characteristic signals of the aziridine ring carbons in the range of 24.0–29.2 ppm for compounds **1, 8–10**. The signals of the quaternary carbon atoms of the dioxane ring ($\underline{C}(CH_3)_2$) appear in the range 98.5–99.9 ppm.

X-ray crystallographic analysis of compounds **6** and **7** (Scheme 1) showed a Z-configuration and monoclinic structure. An asymmetric unit cell contains one molecule of compound. Triazine ring is planar and the chlorines are located in the plane of the ring. It is worth noting that the triazine ring is flat. The chlorine is coplanar with the triazine ring: the C1—N1—Cl1 an C2—N1—Cl2 torsion angle is 181.6(2)° and 179.9(2)° No intramolecular hydrogen bonds are observed. On the other hand, highly directed intermolecular O1—H1 hydrogen bond (O…H 2.751(3) Å, H…O 1.90 Å) is formed between molecules related by the screw axis.

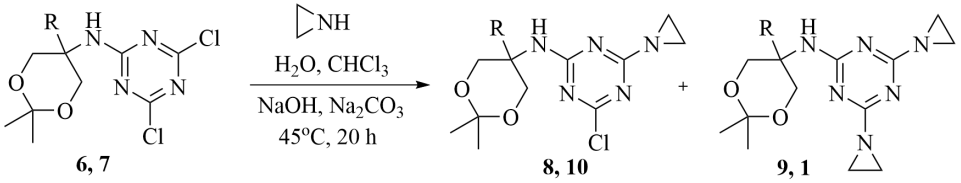
2.2 | DFT

The electron density distribution in the molecules of compounds **8, 9,** and **10** was analyzed using the DFT with the inclusion of COSMO model. The energy values (Ha) HOMO and LUMO for compounds **8, 9,** and **10** were: HOMO3: −6.507, LUMO3: −1.139, HOMO4: −6.245, LUMO4: −0.575, and HOMO: −6.649, LUMO: −1.179, respectively. The Figure 2 illustrates the results of the spatial distribution of the charge density surface. The charges on the atoms in accordance with Figure 1a–c are given in Table 1.

2.3 | Biological Study

2.3.1 | Effect of Compounds 1, 4–10 on the Survival of Tumor Cell Lines A549, Huh-7 and Normal Cell Line HEK 293

In this work, the cytotoxic activity of compounds **1**, **4–10** in relation to human adenocarcinoma cell line A549, to human liver carcinoma cell line Huh-7 and to human embryonic kidney 293 cell line HEK 293 were investigated using the MTT assay. Studies have shown that dichlorosubstituted 1,3,5-triazines **4–7** do not have a pronounced cytotoxic effect on the studied tumor cell lines. The introduction of aziridine moiety into the triazine cycle leads to an increase in the cytotoxic effect. Compounds **1** and **9** containing two aziridine rings expectedly showed a more significant cytotoxic effect on the tumor cell



R = CH_3 [**8** (31%), **9** (18%)]; R = CH_2OH [**10** (46%), **1** (21%)].

SCHEME 2 | Synthesis of aziridine-functionalized 1,3,5-triazine derivatives **1**, **8–10**.

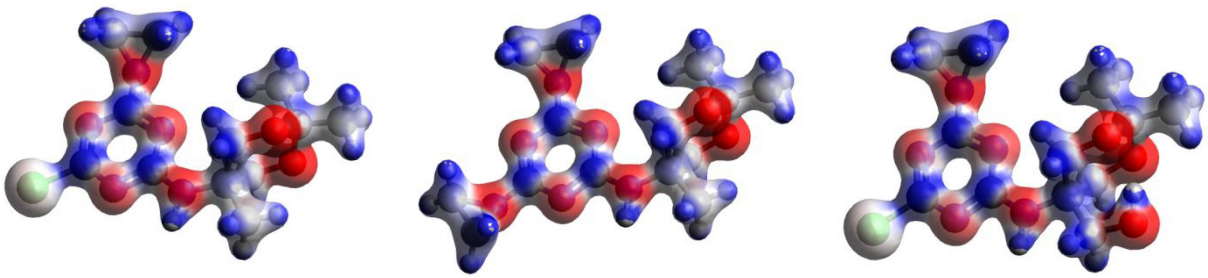


FIGURE 2 | Molecules of compounds **8** (*left*), **9** (*middle*), and **10** (*right*). Red color is an area of increased negative charge, blue color is positive charge.

TABLE 1 | The charges on the atoms.

Compound	N1	N2	N3	N4	N5	N6	O1	O2	O3	Cl
8	−0.305	−0.440	−0.380	−0.337	−0.673	—	−0.515	−0.514	—	0.130
9	−0.432	−0.378	−0.433	−0.413	−0.686	−0.414	−0.518	−0.517	—	—
10	−0.304	−0.442	−0.383	−0.685	−0.336	—	−0.530	−0.504	−0.622	0.137

lines compared to compounds **8**, **10** (Table 2). Compounds **1, 8–10** are reported to be consistently cytotoxic to the normal HEK 293 cell line.

2.3.2 | Study of the Interaction of Compounds 8 and 9 With DNA by UV Spectroscopy

As noted above, most likely mechanism of the cytotoxic action of aziridine-containing 1,3,5-triazine derivatives is based on the interaction of these compounds or their metabolites with DNA, causing DNA damage that prevents DNA replication [1, 2]. The mechanism of interaction of small molecules with biopolymer can be established by studying the binding of biologically active compounds to DNA, in particular by spectral methods [13]. Herein, UV spectroscopy was employed to study the interaction of aziridine-containing 1,3,5-triazine derivatives **1, 8–10** with DNA. Nitrogenous heterocyclic bases in the DNA molecule are chromophore groups and responsible for the appearance in the 200–350 nm range of the UV spectra of the aqueous DNA solutions of broad absorption maximum at 260 nm. The interaction of a bioactive small molecules with a DNA in aqueous solutions of 0.9% NaCl at pH 7.4 typically causes characteristic changes in the electronic absorption spectra of biopolymer, depending on the binding mechanism [13–16].

Figure 3 shows the absorption spectra of DNA in aqueous solutions of NaCl (0.9%) at variable concentration of DNA and constant concentration of compounds **8** (Figure 3A), **9** (Figure 3B), and **10** (Figure S19). The absorption spectra of DNA in aqueous solutions of NaCl (0.9%) at a constant DNA concentration (5.7 μmol L^{-1}).

As shown in Figure 3, the dependence of [DNA]/($\varepsilon_a - \varepsilon_f$) on [DNA] is linear. Based on the Wolf-Schimmer equation, the binding constants K_{bin} obtained for compounds **8**, **9,** and **10** are 9.97×10^6 M^{-1}; 2.76×10^7 M^{-1}, and 9.10×10^6 M^{-1}, previously the binding constant for compound **1** was calculated to be 3.44×10^7 M^{-1}, the data obtained indicate that compound **1** has the highest ability to form bonds with DNA. So, all studied compounds may form complexes with biopolymer.

3 | Conclusion

To conclude, novel derivatives of 1,3,5-triazine containing one or two aziridine cycles and a 1,3-dioxane moiety as substituents were synthesized. The obtained compounds were characterized by spectral methods. The structures of 4,6-dichloro-N-(2,2,5-trimethyl-1, 3-dioxan-5-yl)-1,3,5-triazin-2-amine and (5-((4,6-dichloro-1,3,5-tri azin-2-yl)amino)-2,2-dimethyl-1,3-dioxan-5-yl)methanol also were proved by the X-ray structural analysis technique.

TABLE 2 | Cytotoxic activity of compounds **1**, **8–10**.

Compound	IC_{50}, μM		
	A549	Huh-7	HEK-293
1	15.5 ± 0.9	13.9 ± 0.9	46.2 ± 1.7
8	24.5 ± 1.4	24.0 ± 1.1	34.6 ± 1.2
9	18.4 ± 0.7	16.1 ± 0.8	41.1 ± 2.6
10	27.9 ± 1.5	N/D	38.6 ± 1.5

Obtained binding constants K_{bin} values are in the range $0.91–3.44\times10^7$ M^{-1} and clearly indicates effective binding and formation of stable complexes with the biopolymer. However, K_{bin} values some higher for compounds with two aziridine rings **1**, **9**. In general, derivatives of 1,3,5-triazine containing two aziridine cycles **1** and **9** showed a more pronounced cytotoxic effect against studied tumor lines. The compounds **1**, **9** exhibited cytotoxic activity against Huh7 cells (compound **1** [IC_{50} = 13.9 μM] **9** [IC_{50} = 16.1 μM]) and A549 cells (compound **1** [IC_{50} = 15.5], compound **9** [IC_{50} = 18.4]), at the same time compounds **1**, **9** showed comparable effect against normal cell line HEK 293 (compound **1** [IC_{50} = 46.2 μM] **9** [IC_{50} = 41.1 μM]). The findings of this study may be useful insights into the design and development of novel 1,3,5-triazine derivatives with potential anticancer properties.

4 | Experimental

4.1 | General Information

^{1}H and ^{13}C{^{1}H} NMR spectra were obtained on a Bruker Avance III 400 (400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C) in $CDCl_3$, DMSO-d_6 at 298.15 K. Mass spectral analysis was performed on a Bruker GmbH "MaXis" (Germany). Elemental analysis (C, H, N) was performed on a Euro EA 3028 HT CHNSO analyzer.

Single crystals of compounds **6** and **7** were visually identified under an optical microscope, coated in an oil-based cryoprotectant and mounted on cryoloops. The diffraction data were collected using a Rigaku XtaLAB Synergy S X-ray diffractometer operated with monochromated microfocus CuKα tube PhotonJet-S (λ = 1.54184 Å) at 50 kV and 1.0 mA and equipped with a HyPix 6000HE hybrid photon-counting detector. CrysAlisPro software [14] was used for integration and correction of obtained diffraction data for polarization, for background and Lorentz effects as well as for an empirical absorption correction based on spherical harmonics implemented in the SCALE3 ABSPACK algorithm. The unit-cell parameters were refined using the least-square technique. Both structures were solved by a dual-space algorithm and refined using the SHELX programs incorporated into the *OLEX*2 program package [8–10]. The carbon-, nitrogen-, and oxygen-bound hydrogen atoms were placed in calculated positions and were included in the refinement in the "riding" model approximation with U_{iso}(H) set to $1.2U_{eq}$(C), $1.2U_{eq}$(N), and $1.2U_{eq}$(O). The U_{iso}(H) for CH_3 groups were fixed as $1.5U_{eq}$(C).

4.2 | General Procedures for the Synthesis of Compounds 4, 5

A solution of 2.0 g (10.8 mmol) cyanuric chloride (**3**) in 100 mL of acetone was added to 1.14 g (10.8 mmol) 2-amino-2-methylpr opane-1,3-diol (for compound **4**) or 1.3 g (10.8 mmol) 2-amino-2 -hydroxymethylpropane-1,3-diol (for compound **5**). The reaction mixture was kept for 2 h at rt. After completion of the reaction (control by TLC), the solvent was removed under reduced pressure, target product (compound **4** or **5**) was washed with acetone and air dried.

2-((4,6-dichloro-1,3,5-triazin-2-yl)amino)-2-methyl propane-1,3-diol (**4**): Yield 2.33 g (85%), white powder. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.55 (s, 4H, $2CH_2$), 1.29 (s, 3H,

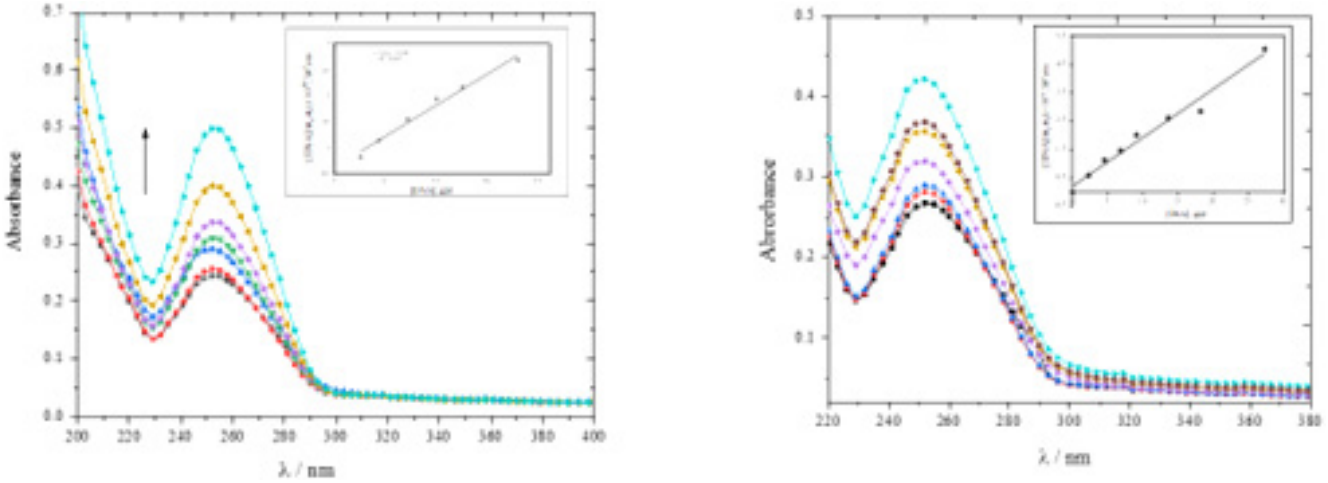


FIGURE 3 | UV spectra of solutions of DNA (C = 2.7–27.3 μM) with the constant concentration of compound **8** (C = 5.37 μM) and DNA (C = 3.6–24.4 μM) at a constant concentration of compound **9** (C = 5.42 μM), as well as the dependence in coordinates [DNA]/($\varepsilon_a - \varepsilon_f$) on [DNA] ($R^2$ = 0.9774) at C [DNA] = 2.7, 4.5, 6.8, 9.1, 13.6, 18.2, and 27.3 and C [DNA] = 3.6, 5.4, 8.2, 11.1, 13.5, and 24.4 μM.

CH_3). ^{13}C NMR (101 MHz, DMSO-d_6), δ: 167.4 ($C_{triazine}$), 163.4 ($C_{triazine}$), 67.1 (CH_2), 66.0 (CH_2), 60.8 (C).

2-((4,6-dichloro-1,3,5-triazin-2-yl)amino)-2-(hydroxymethyl)propane-1,3-diol (5): Yield 2.57 g (88%), white powder, m.p. 189–191°C. ^1H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.47 (s, 4H, $2CH_2$), 1.23 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6), δ, ppm: 164.7 ($C_{triazine}$), 162.0 ($C_{triazine}$), 61.9 (CH_2), 60.0 (CH_2), 59.6 (C), 18.1 (CH_3).

4.3 | General Procedure for the Synthesis of Compounds 6, 7

A solution of 2.0 g (10.8 mmol) cyanuric chloride (**3**) in 100 mL of acetone was added to 1.14 g (10.8 mmol) 2-amino-2-methylpropane-1,3-diol (for compound **6**) or 1.31 g (10.8 mmol) 2-amino-2-hydroxymethylpropane-1,3-diol (for compound **7**). The reaction mixture was kept for 2 h at rt. Subsequently, 0.4 g (4.65 mmol) *p*-toluenesulfonic acid and 3.1 g (21.6 mmol) Na_2SO_4 were added to the solution. The resulting suspension was refluxed for 6 h. After cooling, the precipitate was filtered, and the product was extracted with chloroform. Following air-drying, the product was purified by column chromatography ($CHCl_3$:MeOH 9.5:0.5).

4,6-dichloro-N-(2,2,5-trimethyl-1,3-dioxan-5-yl)-1,3,5-triazin-2-amine (6): Yield 1.63 g (70%), white crystalline product. m.p. 191–193°C. ^1H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.40 (dd, J = 11.4, 11.2 Hz, 4H, $2CH_2$), 1.21 (s, 6H, $2CH_3$), 1.10 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6), δ, ppm: 161.4 ($C_{triazine}$), 150.1 ($C_{triazine}$), 99.9 ($C(CH_3)_2$), 62.9 (CH_2), 58.2 (CH_2), 58.1 (CH_2), 18.5 (CH_3), 18.0 (CH_3), 17.9 (CH_3). HRESI$^+$-MS, *m/z*: 293.0573 $[M]^+$.

Crystal Data for $C_{10}H_{14}Cl_2N_4O_2$ (M = 586.30 g·mol^{-1}): monoclinic, space group $P2_1/c$ (no.14), a = 14.1989(2) Å, b = 16.7693(2) Å, c = 11.8876(2) Å, β = 110.301(2)°, V = 2654.68(7) Å^3, Z = 8, T = 100(2) K, μ(CuKα) = 4.426 mm^{-1}, D_{calc} = 1.467 g/cm^3, 23,063 reflections measured (6.638° ≤ 2Θ ≤ 139.986°), 5036 unique (R_{int} = 0.0299, R_{sigma} = 0.0256) which were used in all calculations. The final R_1 was 0.0255 ($I > 2\sigma(I)$) and wR_2 was 0.0648 (all data).

(5-((4,6-dichloro-1,3,5-triazin-2-yl)amino)-2,2-dimethyl-1,3-dioxan-5-yl)methanol (7): Yield 1.69 g (66%), white crystalline product. m.p. 193–195°C. ^1H NMR (400 MHz, DMSO-d_6), δ, ppm: 8.86 (s, 1H), 4.03 (d, J = 11.9 Hz, 2H), 3.84 (d, J = 11.9 Hz, 2H), 3.36 (s, 2H), 1.35 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6), δ, ppm: 169.0 ($C_{triazine}$), 165.7 ($C_{triazine}$), 98.5 ($C(CH_3)_2$), 62.2 (CH_2), 59.5 (CH_2), 56.2 (CH_2), 24.3 (CH_3), 23.7 (CH_3). Found, %: C, 37.22; H, 5.10; N, 18.91. $C_6H_{11}N_5O_2$ Calculated, %: C 38.85; H 4.56; N 18.12. HRESI$^+$-MS, *m/z*: 307.0366 $[M]^-$.

Crystal Data for $C_{10}H_{14}Cl_2N_4O_3$ (M = 309.02 g/mol): monoclinic, space group $P2_1/n$ (no. 14), a = 11.7710(1) Å, b = 12.7205(1) Å, c = 18.5584(2) Å, β = 93.573(1), V = 2773.40(4) Å^3, Z = 8, T = 100(2) K, μ(CuKα) = 4.318 mm^{-1}, D_{calc} = 1.480 g/cm^3, 23,256 reflections measured (8.432° ≤ 2Θ ≤ 139.994°), 5260 unique (R_{int} = 0.0411, R_{sigma} = 0.0315) which were used in all calculations. The final R_1 was 0.0376 ($I > 2\sigma(I)$) and wR_2 was 0.1021 (all data).

4.4 | General Procedure for the Synthesis of Compounds 1, 8–10

A total of 1.94 g (9.5 mmol) of bromethanamine hydrobromide was added to a solution of 0.4 g (10 mmol) of sodium hydroxide and 2.22 g (21 mmol) of sodium carbonate in 50 mL of water. The reaction mass was stirred at 50°C for 2 h. Solution of 4.0 mmol compound **6** (for compounds **8**, **9**) or compound **7** (for compounds **1**, **10**) in chloroform (50 mL) were added to the resulting aziridine solution and stirred at 45°C for 20 h. At the end of the holding time, the reaction mass was evaporated at reduced pressure. The products were separated by column chromatography on silica gel (Silica gel 60 [0.040–0.063 mm] for column chromatography [230–400 mesh ASTM]) using chloroform as an eluent.

The spectral characteristics of compound **1** are fully consistent with previously published data [7, 17].

4-(aziridin-1-yl)-6-chloro-N-(2,2,5-trimethyl-1,3-dioxan-5-yl)-1,3,5-triazin-2-amine (8): Yield 0.19 g (31%), white powder product. m.p. 198–200°C. ^1H NMR (400 MHz, DMSO-d_6) δ,

ppm: 3.71 (dt, $J = 17.8$, 5.2 Hz, 2H, CH_2), 3.60 (ddd, $J = 15.0$, 10.5, 4.5 Hz, 2H, CH_2), 2.35 (s, 2H, $CH_{2aziridine}$), 2.32 (s, 2H, $CH_{2aziridine}$), 1.37 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.28 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6), δ, ppm: 176.7 ($C_{triazine}$), 170.3 ($C_{triazine}$), 166.6 ($C_{triazine}$), 108.1 ($C(CH_3)_2$), 59.7 (CH_2), 58.3 (CH_2), 27.4 ($CH_{2aziridine}$), 27.3 (CH_3), 27.2 (CH_3). HRESI$^+$-MS, *m/z*: 300.1225 $[M]^+$.

4,6-di(aziridin-1-yl)-N-(2,2,5-trimethyl-1,3-dioxan-5-yl) -1,3,5-triazin-2-amine (9): Yield 0.52 g (18%), white powder product, m.p. 197–199°C. ^{1}H NMR (400 MHz, DMSO- d_6) δ, ppm: 4.12 (dd, $J = 22.2$, 11.6 Hz, 2H, CH_2), 3.65 (d, $J = 11.6$ Hz, 2H, CH_2), 2.32 (s, 4H, $2CH_{2aziridine}$), 2.31 (s, 4H, $CH_{2aziridine}$), 1.34 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.26 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ, ppm: 175.6 ($C_{triazine}$), 169.1 ($C_{triazine}$), 166.0 ($C_{triazine}$), 98.1 ($C(CH_3)_2$), 79.5 (CH_2), 65.8 (CH_2), 65.7 (CH_2), 50.8 (CH_2), 27.3 ($CH_{2aziridine}$), 27.2 ($CH_{2aziridine}$), 25.1 (CH_3), 22.9 (CH_3), 19.6 (CH_3).

(5-((4-(aziridin-1-yl)-6-chloro-1,3,5-triazine-2-yl)amino)-2,2-dimethyl-1,3-dioxan-5-yl) methanol (10): Yield 1.63 g (46%), white crustal product, m.p. 194–196°C. ^{1}H NMR (400 MHz, DMSO- d_6) δ, ppm: 3.85 (s, 4H), 3.62 (s, 4H), 2.27 (s, 4H, $2CH_{2aziridine}$), 1.33 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ, ppm: 169.0 ($C_{triazine}$), 168.8 ($C_{triazine}$), 165.6 ($C_{triazine}$), 98.5 ($C(CH_3)_2$), 63.9 (CH_2), 62.1 (CH_2), 59.4 (CH_2), 56.0 (CH_2), 31.1 ($CH_{2aziridine}$), 24.0 (CH_3), 23.8 (CH_3). Found, %: C 45.65; H 5.75; N 22.18 $C_{12}H_{18}ClN_5O_3$ Calculated, %: C 47.13; H 4.99; N 21.08. HRESI$^+$-MS, *m/z*: 316.1178 $[M]^+$.

Supporting Information provides study methods of DNA binding study and cytotoxicity, computational approach and can be found in earlier published materials [18].

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.