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Synthesis and biological activities of quinazoline derivatives with *ortho*-phenol-quaternary ammonium salt groups

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Abstract—One phenol-quaternary ammonium salt derivative with a flexible linker and three derivatives with a quinazoline moiety are present. Their binding affinities for DNA are discussed and it is clearly demonstrated that this class of phenol-quaternary ammonium salt derivatives could inhibit DNA transcription effectively.

1. Introduction

DNA is an important target for drug design in the research for various anti-tumor drugs.[1] Several clinically useful DNA reactive agents have been found, such as cisplatin, mitomycin C, and cytoxan.[2] Meanwhile, transcription is a critical cellular process in protein synthesis and mRNA is assembled from one DNA template.[3] Therefore, inhibition of transcription could be a target for drug design since it could induce coding of RNA and proteins incomplete and finally leads to cell death. Based on these ideas, some agents have been found to be potentially useful in the treatment of various cancers.[4]

The quinazoline ring system along with many alkaloids is a widely recognized moiety in organic syntheses and medicinal application.[5,6] It has been reported that modification of quinazoline structure could be applied in many biological studies,[7] such as anticonvulsant, antibacterial, antidiabetic, and anticancer.[8] Many groups have reported that *o*-quinone methide (*o*-QM) with electrophilic properties is a quinone methide derivative and it has been found to play important roles in organic syntheses both in chemical and biological processes.[9,10] However, as the fact that *o*-QM could react with DNA not only in normal cells but also cancer cells,[11] how to get more agents which can selectively cross-link or alkylate DNA is key for drug design. The Freccero group once reported that phenols or a phenol possessing a quaternary ammonium group could form *o*-quinone methide by photoactivation in aqueous solution.[12] Based on his results, our group found a biphenol diquaternary ammonium salt, both a potent photo inducible DNA cross-linking agent and a good DNA transcription inhibitor.[13,14] Therefore, in this paper, we shall report our new design molecule based upon three kinds: key functionality, a quinazoline moiety, and a phenol group possessing an *ortho*-substituted quaternary alkylammonium group which is a good DNA alkylating or DNA cross-linking moiety (Scheme 1). Their activities of alkylating DNA and inhibition on transcription are investigated.

2. Results and discussion

2.1. Chemistry

In order to synthesize phenol derivatives with quaternary ammonium salts, we introduced the quaternary ammonium group by a Mannich reaction followed by alkylation of the intermediate *tert*-amine with iodomethane. Compound **1** was synthesized in three steps. In the first step, after mixing 3-(4-hydroxyphenyl) propionic acid with formaldehyde and dimethylamine, the product was purified by silica gel chromatography using methanol as the eluent to afford compound **5** as a white

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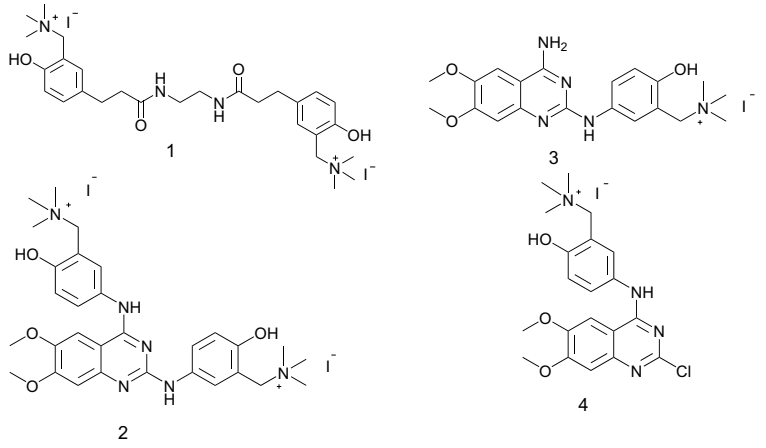


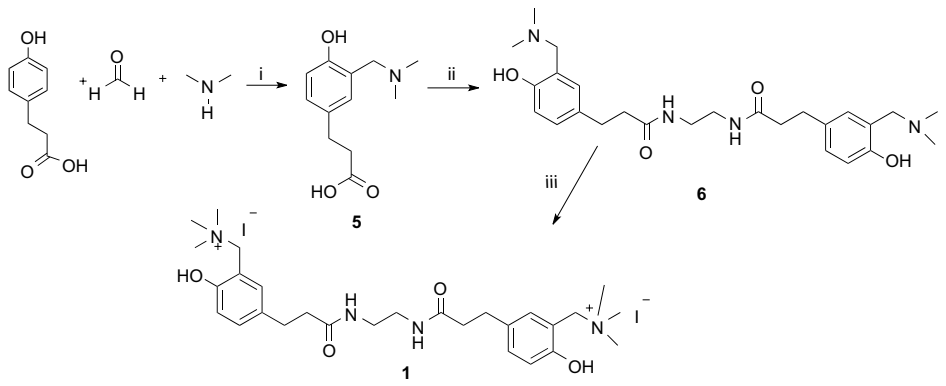
Scheme 1. Chemical formulae of the molecules studied.

powder. The active succinimidyl esters were prepared directly from the non-protected hydroxymandelic acid (e.g., **5**) by the NHOSu/DCC method in THF. The reaction mixtures were filtered to remove the precipitated *N*,*N*′-dicyclohexylurea (DCU) and then the amine was added to the solution (Scheme 2).[15] Compound **9** was synthesized by two consecutive nucleophilic displacement reactions. At first, we tried one-pot method to get compound **9**, but all our observation was substitution at C-4 and the reaction could not be finished using either thermal or microwave condition. Therefore, we split the synthesis of compound **9** into two individual steps. Firstly, 2,4-dichloro-6,7-dimethoxyquinazoline was mixed with the hydrolysis product of compound **7** and reaction mixture was stirred at room temperature to get compound **8**. Secondly, excessive hydrolysis product of compound **7** was mixed with compound **8** and reaction mixture was refluxed in ethanol for 24 h to get compound **9**. Compound **10** was synthesized by a displacement reaction using similar conditions for the synthesis of compound **9**. Methylation of compounds **6**, **8**, **9**, and **10** was performed in CH_3CN and the compounds **1**–**4** were obtained with very good yields (Schemes 2 and 3).

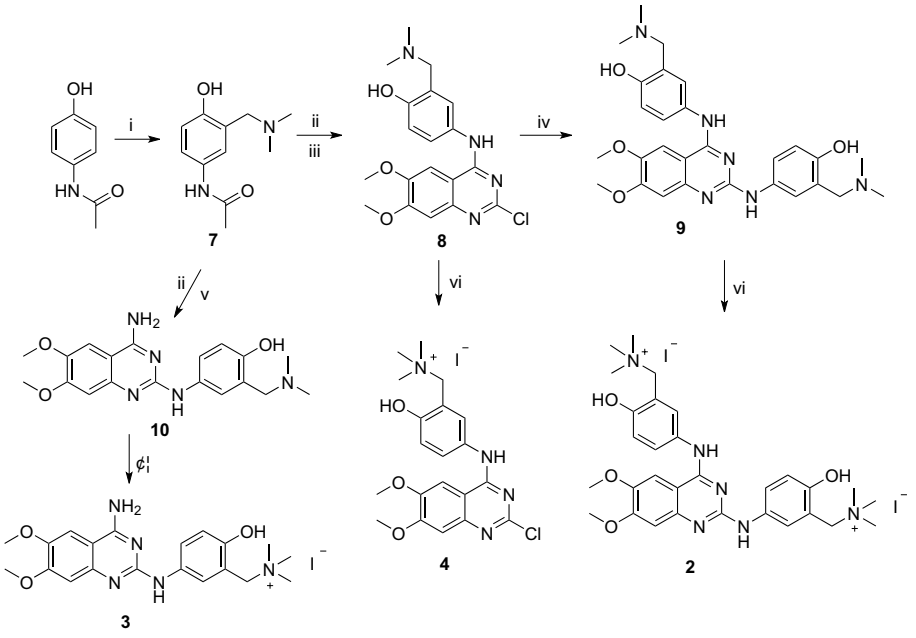
All the new compounds were fully characterized by NMR and HRMS.

2.2. Biology

2.2.1. DNA cross-linking. The abilities of compounds **1** and **2** to induce DNA–DNA cross-linking was investigated by linearized plasmid DNA by denaturing alkaline agarose gel electrophoresis.[16,17] The duplex DNA was linearized using the EcoRI restriction endonuclease digestion and DNA cross-linking experiments were carried out in phosphate buffer (pH 7.7). All samples were exposed to a 50-W high mercury lamp placed 20 cm away at 37 °C. The crude reaction mixtures were loaded onto a denaturing 0.9% alkaline agarose gel. Lambda HindIII DNA was used as a molecular weight marker.[13] We found that DNA cross-linking by compound **2** was affected by pH variation (Fig. 1A). These results indicated that the most intense cross-linking occurred at pH 7.7. On the contrary, no DNA cross-linking occurred at all at pH 2.0, 4.0, and 6.0. The ability of cross-linking DNA decreases at pH 10 and 12 compared with 7.7. Results of concentration-dependent cross-linking of DNA by compound **2** are illustrated in Figure 1B. DNA cross-linking by compound **2** was observed at a concentration as low as 1.0 μM. More DNA became cross-linked as the concentration of the compound increased. Compound **2** could cross-link linear DNA 28% at the concentration of 1 μM and cross-link linear DNA 46% at the concentration of 5 μM. Compared



Scheme 2. Synthetic pathway for the synthesis of compound **1**. Reagents and conditions: (i) CH_3CH_2OH, rt, 12 h; (ii) NHOSu, DCC, DMF, THF, rt, 48 h then added ethylenediamine, rt, 16 h; (iii) CH_3I, rt, CH_3CN, 12 h.



Scheme 3. Synthetic pathway for the synthesis of compounds **2**, **3**, and **4**. Reagents and conditions: (i) dimethylamine, formaldehyde, CH_3CH_2OH, rt, 15 h; (ii) 20% HCl, reflux 10 h; (iii) 2,4-dichloride-6,7-dimethoxyquinazoline, CH_3CH_2OH, rt, 10 h; (iv)*N*-(3-((dimethylamino)methyl)-4-hydroxyphenyl) acetamide, 20% HCl, reflux 10 h; then added compound **8**, CH_3CH_2OH, reflux 24 h; (v) 2-chloro-4-amino-6,7-dimethoxyquinazoline, CH_3CH_2OH; (vi) CH_3I, CH_3CN, rt 12 h.

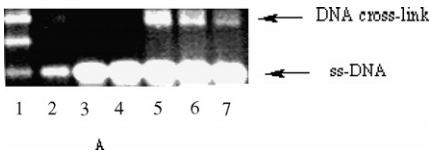


Figure 1A. pH dependence of DNA cross-link by compound **2**. Lane 1, 1.5 μg lambda DNA/HindIII (molecular weight standard); lane 2, 0.7 μg pBR322 + 5.0 μM **2** (pH 2.0) + hv (2 h); lane 3, 0.7 μg pBR322 + 5.0 μM **2** (pH 4.0) + hv (2 h); lane 4, 0.7 μg pBR322 + 5.0 μM **2** (pH 6.0) + hv (2 h); lane 5, 0.7 μg pBR322 + 5.0 μM **2** (pH 7.7) + hv (2 h); lane 6, 0.7 μg pBR322 + 5.0 μM **2** (pH 10) + hv (2 h); lane 7, 0.7 μg pBR322 + 5.0 μM **2** (pH 12) + hv (2 h).

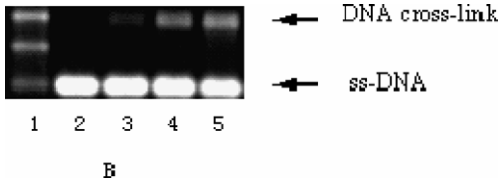


Figure 1B. Concentration dependence of compounds **2** for DNA cross-linking. Lane 1, 1.5 μg lambda DNA/HindIII (molecular weight standard); lane 2, 0.7 μg pBR322 + hv (2 h); lane 3, 0.7 μg pBR322 + 1.0 μM **2** + hv (2 h); lane 4, 0.7 μg pBR322 + 2.5 μM **2** + hv (2 h); lane 5, 0.7 μg pBR322 + 5.0 μM **2** + hv (2 h).

with compound **2**, compound **1** cannot cross-link DNA even if the concentration reached to 100 μM. For our *o*-QM trapping experiment by ethyl vinyl, we could not find the formation of *o*-QM for compound **1**.[18] It suggests that compound **1** might have an amide linker between two phenol-quaternary ammonium and not lead to form *o*-QM for compound **1**. It implied that the quinazoline group might be very helpful for compound **2** to cross-link DNA compared to compound **1**. The good binding ability of compound **2** with quinazoline group might increase its ability to cross-link DNA.[19] And our later results also confirm this point.

2.2.2. Inhibition of transcription. Inhibition of transcription by compounds **1**, **2**, **3**, and **4** was tested by selecting target DNA consisting of T7 promoter that was completely controlled in vitro transcription system by T7 RNA polymerase.[20] Prior to transcription, the target DNA was mixed at room temperature with a given concentration of DNA cross-linking agents **1**, **2**, **3**, and **4**, respectively, and then the mixtures were irradiated for 1 or 2 h. Finally, transcription with T7 RNA polymerase was performed according to the protocol recommended by Toyobo TSK-101. These results are shown in Figures 2A and 2B.

The alkylation of DNA template by the agents might be a key factor to inhibit transcription. The inhibition of transcription by compounds **1**, **2**, **3**, and **4** is shown in Figure 2. Compound **1** cannot inhibit the transcription of the DNA even at 20 μM, while compounds **2**, **3**, **4** could inhibit the transcription almost completely even at the concentration of 10 μM. The comparison of compounds **2**, **3** and **4** shows that at a concentration of 5 μM, compounds **2** and **3** had significant abilities to inhibit transcription, whereas compound **4** had little ability of inhibition. Bad interaction between compound **1** and DNA (ΔTm = 0.5 °C) might lead compound **1** to not cross-link DNA and then not inhibit transcription.

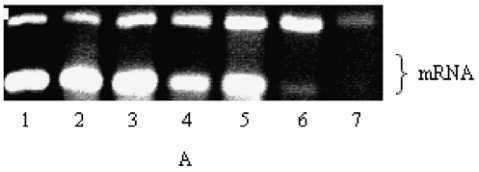


Figure 2A. Concentration dependence of compounds **1** and **2** for transcribed mRNA (ethidium bromide-stained agarose gel (1.5%)). Lane 1, from DNA template (25 ng) + hv (2 h); lane 2, from DNA template (25 ng) + **1** (5 μM) + hv (2 h); lane 3, from DNA template (25 ng) + **1** (10 μM) + hv (2 h); lane 4, from DNA template (25 ng) + **1** (20 μM) + hv (2 h); lane 5, from DNA template (25 ng) + **2** (1 μm) + hv (2 h); lane 6, from DNA template (25 ng) + **2** (5 μM) + hv (2 h); lane 7, from DNA template (25 ng) + **2** (10 μM) + hv (2 h).

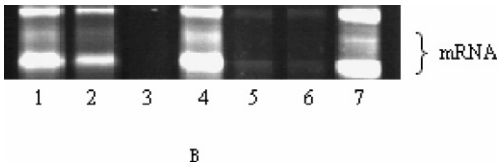


Figure 2B. Concentration dependence of compound **3** and **4** for transcribed mRNA (ethidium bromide-stained agarose gel (1.5%)). Lane 1, from DNA template (25 ng) + **4** (1 μM) + hv (1 h); lane 2, from DNA template (25 ng) + **4** (5 μM) + hv (1 h); lane 3, from DNA template (25 ng) + **4** (10 μM) + hv (1 h); lane 4, from DNA template (25 ng) + **3** (1 μM) + hv (1 h); lane 5, from DNA template (25 ng) + **3** (5μM) + hv (1 h); lane 6, from DNA template (25 ng) + **3** (10 μM)+hv (1 h); lane 7, from DNA template (25 ng) + hv (1 h).

Table 1. Thermal denaturizing data of compounds **1**, **2**, **3**, and **4**

Compound	**1**	**2**	**3**	**4**
*Δ*Tm (°C)	0.5	8.6	7.4	5.8

CT-DNA alone at pH 7.40 ± 0.01 (Tris·HCl buffer), Tm = 70.1, for a 1:5 molar ratio of [drug]:[DNA], where CT-DNA concentration = 10 μM and drug concentration = 2 μM in aqueous Tris·HCl buffer 10 μM.

2.2.3. Thermal denaturizing study. The DNA binding abilities for these quaternary ammonium salt derivatives have been determined by thermal denaturation studies using calf thymus DNA (CT-DNA).[14] The results (Table 1) indicated that compounds **2**, **3**, and **4** could interact with DNA strongly. The interaction between compound **1** and DNA is weak and compound **3**, having a free amine group present, may form hydrogen bonds with DNA explaining its stronger interaction ability than that of compound **4**. It is probably partly attributed to electrostatic force caused by two cations. And also the two phenyl groups in compound **2** may have good shape orientation to adopt a helical conformation which could fit well to DNA helical.[13]

3. Conclusion

Four compounds with quaternary ammonium salt derivatives were designed and synthesized, and their interactions with DNA and inhibition activity on transcription have been studied. We found a new DNA cross-linking agent which could not only cross-link DNA at low concentration but also have good inhibiting activity on transcription. UV melting experiments suggested that it can bind strongly to DNA. Our results also demonstrated that quinazoline moiety is very helpful for these good biology activities. The quinazoline moiety may be good intercalator as well and insert in DNA to increase their interactions with DNA. It suggested that compound containing moieties of *o*-QM precursor and quinazoline agents has good biology activity in our study. Current results might raise new opportunities in design and search of more potent anti-tumor drugs in future.

4. Experiments

3-(4-Hydroxyphenyl) propionic acid was purchased from Acros. Super coiled pBR322 and CT-DNA was purchased from Toyobo (Japan). ScriptMAX™ Thermo T7 Transcription Kit was purchased from Toyobo. All other chemicals and solvents were commercially available. NMR spectra were recorded on a Varian Mercury-VX300 spectrometer at 300 MHz. MS were recorded on a Brucker Daltonics APE XII 47e and VG-707VHF mass spectrometer. UV–vis spectra were carried out on a Scinco S-3100 Spectrophotometer.

4.1. Chemistry

4.1.1. 3-(3-((Dimethylamino) methyl)-4-hydroxyphenyl) propanoic acid (5). 3-(4-Hydroxyphenyl) propionic acid (500 mg, 3 mmol) was dissolved in ethanol first, then aqueous solutions of dimethylamine (3.0 equiv, 33%) and formaldehyde (4.0 equiv, 37%) were added to the reaction mixture. After the reaction mixture was stirred overnight at room temperature, the solvents were then evaporated under reduced pressure and the crude product was subjected to silica gel chromatography with ethanol as the eluent. After evaporation of the solvent, the product was obtained. Yield: 210 mg (33%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.05–7.02 (dd, J_1 = 1.8 Hz, J_2 = 9.9 Hz, 1H), 7.02 (s, 1H), 6.70 (d, J = 8.4, 1H), 4.06 (s, 2H), 2.65 (s, 6H), 2.62 (t, J_1 = 6.3 Hz, J_{12} = 7.2 Hz, 2H), 2.25 (t, J_1 = 7.5 Hz, J_{12} = 7.2 Hz, 2H); HRMS (ESI$^+$) MH$^+$: 224.1284 (Calcd mass for $C_{12}H_{17}NO_3MH^+$: 224.1281).

4.1.2. *N*,*N*-(Ethane-1,2-diyl)bis(3(3((dimethylamino) methyl)-4-hydroxyphenyl) propanamide) (6). 2-Dimethylaminemethylene-3-(4-hydroxyphenyl) propionic acid (209 mg, 1 mmol) and *N*-hydroxy succinimide (137 mg, 1.2 mmol) were dissolved in THF (25 mL) under nitrogen. *N*,*N*-Dicyclohexylcarbodiimide (247 mg, 1.2 mmol) was added to the mixture at room temperature, and the mixture was stirred at room temperature for 48 h. Then ethyldiamine (30 mg, 1 mmol) was added under nitrogen and the mixture was stirred at 45 °C for 16 h. After removing the by-product, the filtrate was evaporated under reduced pressure. Purification was carried out by chromatography on silica gel using the eluent methyl alcohol. After evaporation of the solvent, the product was obtained. Yield: 70 mg (30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.98–6.95 (dd, J_1 = 1.8 Hz,

J_2 = 8.1 Hz, 2H), 6.80 (s, 2H), 6.74 (d, J = 8.1 Hz, 2H), 3.60 (s, 4H), 3.17 (s, 4H), 3.17 (br s, 4H), 2.82 (t, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 4H), 2.38 (t, J_1 = 7.8 Hz, J_2 = 7.2 Hz, 4H), 2.32 (s, 12H); HRMS (ESI^+) MH^+: 471.2962 (Calcd mass for $C_{26}H_{38}N_4O_4MH^+$: 471.2966).

4.1.3. *N*-(3-((Dimethylamino)methyl)-4-hydroxyphenyl) acetamide (7). *N*-(3-((Dimethylamino)methyl)-4-hydroxyphenyl) acetamide (500 mg, 3.3 mmol) was dissolved in ethanol and aqueous solutions of dimethylamine (3.0 equiv, 33%) and formaldehyde (4.0 equiv, 37%) were added. After the reaction mixture was stirred overnight at room temperature, the solvents were then evaporated under reduced pressure and the crude product was subjected to silica gel chromatography eluting with a mixture of $CHCl_3$/CH_3OH (5:1). After evaporation of the solvent, the product was obtained. Yield: 240 mg (35%). ^1H NMR (300 MHz, $CDCl_3$) δ = 7.29 (d, J = 2.1 Hz, 1H), 7.06 (dd, J_1 = 2.7 Hz, J_2 = 9 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 3.58 (s, 2H), 2.30 (s, 6H), 2.11 (s, 3H); HRMS (ESI^+) MH^+: 209.1284 (Calcd mass for $C_{11}H_{16}N_2O_2MH^+$: 209.1285).

4.1.4. 4-(2-Chloro-6,7-dimethoxyquinazolin-4-ylamino)2-((dimethyl amino)methyl)phenol (8). Compound **7** (300 mg, 1.44 mmol) was dissolved in 20 mL 20% HCl (W/W) and refluxed for 10 h under a nitrogen atmosphere. After reaction, the solvent was evaporated under reduced pressure. The solid was dissolved in ethanol and 2,4-dichloro-6,7-dimethoxyquinazoline (200 mg, 0.77 mmol) was added to the mixture. The mixture was stirred for 10 h at room temperature and the solvent was evaporated under reduced pressure. The crude product was neutralized using the solution of 10% sodium hydrogen carbonate (20 mL). The mixture was extracted three times with $CHCl_3$ (a total of 150 mL). The organic phase was washed with saturated NaCl solution, dried over Na_2SO_4, filtered, and the filtrate was evaporated under reduced pressure. The product was purified by chromatography on silica gel using the eluent methyl alcohol. Yield: 224 mg (75%). ^1H NMR (300 MHz, $CDCl_3$) δ = 7.34 (s, 2H), 7.16 (s, 1H), 6.92 (s, 1H), 6.84 (d, J = 9 Hz, 1H), 3.99 (s, 3H), 3.94 (s, 3H), 3.67 (s, 2H), 2.36 (s, 6H); HRMS (ESI^+) MH^+: 389.1368 (Calcd mass for $C_{19}H_{21}N_4O_3Cl$ MH^+: 389.1365).

4.1.5. 4,4′-(6,7-Dimethoxyquinazoline-2,4-diyl)bis(azanediyl)bis(2-((dimethyl-amino)methyl)phenol) (9). Compound **7** (300 mg, 1.44 mmol) was dissolved in 20 mL of 20% HCl (W/W) and mixture was refluxed for 10 h under a nitrogen atmosphere. After reaction, the solvent was evaporated under reduced pressure. The solid was dissolved in ethanol and compound **8** (272 mg, 0.7 mmol) was added to the mixture. The mixture was refluxed for 24 h and the solvent was evaporated under reduced pressure. The crude product was neutralized using the solution of 10% sodium hydrogen carbonate (20 mL). The mixture was extracted three times with $CHCl_3$ (a total of 150 mL). The organic phase was washed with saturated NaCl solution, dried over $NaSO_4$, filtered, and the filtrate was evaporated under reduced pressure. The product was purified by chromatography on silica gel using the eluent methyl alcohol. Yield: 80 mg (60%). ^1H NMR (300 MHz, $CDCl_3$) δ = 7.36 (d, J = 8.1 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 7.22 (s, 1H), 7.11 (s, 2H), 6.86 (s, 1H), 6.78 (d, J = 8.4 Hz, 1H), 6.70 (d, J = 8.7 Hz, 1H), 3.92 (s, 3H), 3.88 (s, 3H), 3.54 (s, 2H), 3.52 (s, 2H), 2.30 (s, 12H); HRMS (ESI^+) MH^+: 519.2705 (Calcd mass for $C_{28}H_{34}N_6O_4MH^+$: 519.2714).

4.1.6. 4-(4-Amino-6,7-dimethoxyquinazolin-2-ylamino)-2-((dimethylamino)methyl)phenol (10). Compound **7** (104 mg, 0.5 mmol) was dissolved in 15 mL of 20% HCl (W/W) and refluxed for 10 h under nitrogen. After reaction, the solvent was evaporated under reduced pressure. The solid was dissolved in ethanol and 2-chloro-4-amino-6,7-dimethoxyquinazoline (390 mg, 1.5 mmol) was added to the mixture. The mixture was refluxed for 10 h and the solvent was evaporated under reduced pressure. The crude product was neutralized using the solution of 10% sodium hydrogen carbonate (20 mL). The mixture was extracted three times with $CHCl_3$ (a total of 120 mL). The organic phase was washed with saturated NaCl solution, dried over Na_2SO_4, filtered, and the filtrate was evaporated under reduced pressure. The product was purified by chromatography on silica gel using the eluent methyl alcohol. Yield: 258 mg (70%). ^1H NMR (300 MHz, $CDCl_3$) δ = 7.40 (d, J = 8.7 Hz, 1H), 7.19 (s, 1H), 6.92 (s, 1H), 6.85 (s, 1H), 6.79 (d, J = 8.4 Hz, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.60 (s, 2H), 2.30 (s, 6H); HRMS (ESI^+) MH^+: 370.1880 (Calcd mass for $C_{19}H_{23}N_5O_3MH^+$: 370.1874).

4.1.7. (5,5′-(3,3′-(ethane-1,2-diylbis(azanediyl))bis(3-oxopropane-3,1-diyl))bis(2-hydroxy-5,1-phenylene))bis(*N*,*N*,*N*-trimethanaminium)iodine (1). Compound **6** (25 mg, 0.053 mmol) was dissolved in CH_3CN (20 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was stirred at room temperature for 12 h. After finishing the reaction, the solution was concentrated to about 3 mL and then 30 mL of ether was poured into the mixture. A precipitate was obtained which was collected by filtration and dried under vacuum. Yield: 37 mg (92%).^1H NMR (300 MHz, DMSO-d_6) δ = 7.01 (dd, J_1 = 8.4 Hz, J_2 = 2.1 Hz, 2H), 6.98 (s, 2H), 6.71 (d, J = 8.1 Hz, 2H), 4.19 (s, 4H), 2.86 (s, 18H), 2.62 (t, J_1 = 6.3 Hz, J_2 = 6.6 Hz, 4H), 2.52 (s, 4H), 2.74 (t, J_1 = 7.2 Hz, J_2 = 6.9 Hz, 4H); HRMS (ESI^+) $(M-I)^+$: 627.2402 (Calcd mass for $C_{28}H_{44}N_4O_4(M-I)^+$: 627.2402).

4.1.8. (5,5′-(6,7-Dimethoxyquinazoline2,4diyl)bis(azanediyl)bis(2-hydroxy-5,1phenylene))bis(*N*,*N*,*N*-trimethylmethanaminium) iodide (2). Compound **9** (40 mg, 0.077 mmol) was dissolved in CH_3CN (20 mL), and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was stirred at room temperature for 12 h. After finishing the reaction, the solution was concentrated to about 3 mL and then 30 mL of ether was poured into the mixture. The precipitate was obtained by filtering and dried under vacuum. Yield: 59 mg (94%). ^1H NMR (300 MHz, DMSO-d_6) δ = 7.94 (br, 1H), 7.63 (br, 2H), 7.40 (br, 2H), 7.16 (br, 1H), 7.04 (br, 2H), 4.47 (s, 3H), 4.42 (s, 3H), 3.92 (s, 2H),

3.89 (s, 2H), 2.96 (s, 18H); HRMS (ESI^+) $(M-I)^+$: 675.2148 (Calcd mass for $C_{30}H_{40}N_6O_4I_2$ $(M-I)^+$: 675.2148).

4.1.9. (5-(4-Amino-6,7-dimethoxyquinazolin-2-ylamino)-2-hydroxyphenyl)-*N*,*N*,*N*-trimethylmethanaminium iodide (3). Compound **10** (30 mg, 0.08 mmol) was dissolved in CH_3CN (20 mL) and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was stirred at room temperature for 12 h. After finishing the reaction, the solution was concentrated to about 3 mL and then 30 mL of ether was poured into the mixture. The precipitate was obtained by filtering and dried under vacuum. Yield: 338 mg (93%). ^1H NMR (300 MHz, DMSO-d_6) $\delta = 9.76$ (br, 1H), 8.58 (br, 1H), 7.94 (s, 1H), 7.64 (d, $J = 8.7$ Hz, 1H), 7.46 (s, 1H), 7.10 (br, 2H), 6.86 (d, $J = 7.8$ Hz, 1H), 6.70 (s, 1H), 4.41 (s, 2H), 3.82 (s, 3H), 3.78 (s, 3H), 3.07 (s, 9H); HRMS (ESI^+) $(M-I)^+$: 384.2031 (Calcd mass for $C_{20}H_{26}N_5O_3I$ $(M-I)^+$: 384.2030).

4.1.10. (5-(2-Chloro-6,7-dimethoxyquinazolin-4-ylamino)-2-hydroxyphenyl)-*N*,*N*,*N*-trimethylmethan-aminium iodide (4). Compound **8** (30 mg, 0.077 mmol) was dissolved in CH_3CN (20 mL) and methyl iodide (0.15 g, 1.059 mmol) was added to this solution. The mixture was stirred at room temperature for 12 h. After finishing the reaction, the solution was concentrated to about 3 mL and then 30 mL of ether was poured into the mixture. The precipitate was obtained by filtering and dried under vacuum. Yield: 38 mg (95%). ^1H NMR (300 MHz, DMSO-d_6) $\delta = 10.35$ (s, 1H), 9.93 (s, 1H), 7.82 (s, 1H), 7.75 (s, 1H), 7.25 (d, $J = 8.7$ Hz, 1H), 7.14 (s, 1H), 7.02 (d, $J = 8.7$ Hz, 1H), 4.46 (s, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.11 (s, 9H); HRMS (ESI^+) $(M-I)^+$: 403.1553 (Calcd mass for $C_{20}H_{24}N_4O_3ClI$ $(M-I)^+$: 403.1557).

4.2. Biology

4.2.1. DNA cross-linking. The duplex DNA was linearized by restriction endonuclease digestion with EcoRI. DNA cross-linking experiments were carried out in phosphate buffer (pH 7.7). All samples were exposed to a 50-W high-pressure mercury lamp placed 20 cm away at 37 °C. The crude reaction mixtures were loaded onto a denaturing 0.9% alkaline agarose gel. Lambda HindIII was employed as a molecular weight marker and the gel was stained in an ethidium bromide solution (ethidium bromide solution in 1 L of 1 M Tris–1.5 M NaCl buffer at pH 7.5) for 1 h. Gels were visualized by UV and photographed using Vilber Lourmat video system.

4.2.2. Study of transcription inhibition activity. General protocol for transcription inhibition in vitro: the transcription experiments were finished by control DNA template containing T7 promoter and ScriptMAX™ Thermo T7 Transcription Kit (Toyobo TSK-101). The cross-linking reaction system that was mixed target DNA together with DNA cross-linking agents (compound **1** or **2**). Then the mixtures were irradiated for 2 h by 50-W high-pressure mercury lamp placed 20 cm away at room temperature. The alkylating reaction system mixed target DNA together with DNA alkylating agents (compound **3** or **4**) was irradiated for 1 h by 50-W high-pressure mercury lamp placed 20 cm away at room temperature. Then transcription with T7 RNA polymerase was performed according to the general procedure recommended by Toyobo TSK-101. The transcription system consisted of basal reaction buffer, 5 × accelerator solution, 5 mM each ATP, CTP, GTP, and UTP, RNase inhibitor, template DNA 25 ng, thermo T7 RNA polymerase and nuclease-free water, was incubated at 37 °C for 2 h. The products were analyzed by 1.5% agarose gel electrophoresis in 1× TBE buffer and stained with 0.1 mg/mL EB. The gels were observed and photographed under UV light.

4.2.3. Thermal denaturation study. DNA melting experiments were carried out using a UV/vis spectrophotometer (Scinco S-3100) equipped with a temp-control cell. The concentrations of CT-DNA were determined spectrophotometrically from appropriate molar absorptivity values and calculated using a molar absorptivity of 6600 mol L^{-1} cm^{-1} at 260 nm. In order to test the interaction between drug molecules with DNA, the melting curves were recorded at different compounds to CT-DNA by following the absorption change at 260 nm as a function of temperature with a heating rate of 1.0 °C/min. Tm values were determined from the maximum of the first derivative or from the graphs at the mid point of the transition curves. ΔTm values were calculated as usual by subtracting the Tm of the free nucleic acid from the Tm of the complex.

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