Supplementary Information

An assay for human telomeric DNA binding drugs

Derrick Watkins[b] , Nihar Ranjan[a] , Sunil Kumar[a,†], Changjun Gong[a] and Dev P. Arya[a,b,*]

[a] *Laboratory of Medicinal Chemistry, Department of Chemistry, Clemson University, Clemson, South Carolina (U.S.A) 29634, United States*

[b] *NUBAD LLC, 900 B West Faris Road, Greenville, SC 29630, United States*

Materials and methods.

Chemicals.

All chemicals used in this synthesis were purchased from commercial suppliers (Aldrich, Acros, VWR, Sorbtech). Neomycin was purchased from MP Biomedicals (solon, OH, USA). NMR solvents were purchased from Cambridge Isotopes Limited (Andover, MA, USA).

Nucleic acids.

Human telomeric DNA oligonucleotide d(AGGGTTAGGGTTAGGGTTAGGG) was purchased from MWG Operon (Huntsville, AL) in a standard desalted form. No further purification of the DNA sample was done. The human telomeric quadruplex was prepared by dissolving the lyophilized solid in buffer 10 mM sodium cacodylate, 0.5 mM EDTA and 100 mM NaCl at pH 7.0 or 10 mM HEPES and 100 mM KCl at pH 7.0. The DNA quadruplex solution in the appropriate buffer was heated at 90 °C for 30 minutes. The DNA solution was then allowed to cool back slowly to room temperature followed by incubation for at least a week at 4 °C. The stock solution (typically ~1 mM/strand) was then diluted as desired.

Circular dichroism (CD) spectroscopy.

CD experiments were performed at 20 °C using a Jasco J-810 spectropolarimeter with a thermo-electrically controlled cell holder. The CD spectra were recorded as an average of three scans. The concentration of human telomeric quadruplex DNA used in each experiment was 10μM/strand. The buffer used in the experiments was sodium 10 mM sodium cacodylate, 0.5 mM EDTA and 100 mM NaCl at pH 7.0.

HPLC analysis. HPLC analysis of compounds **3, 5a** and **5** were performed on HP1100 series analytical HPLC instrument. The experiments were performed on a Supelcosil LC-18S column using the following gradient conditions- 100% H_2O containing 0.1% TFA to 10% methanol in 10 minutes.

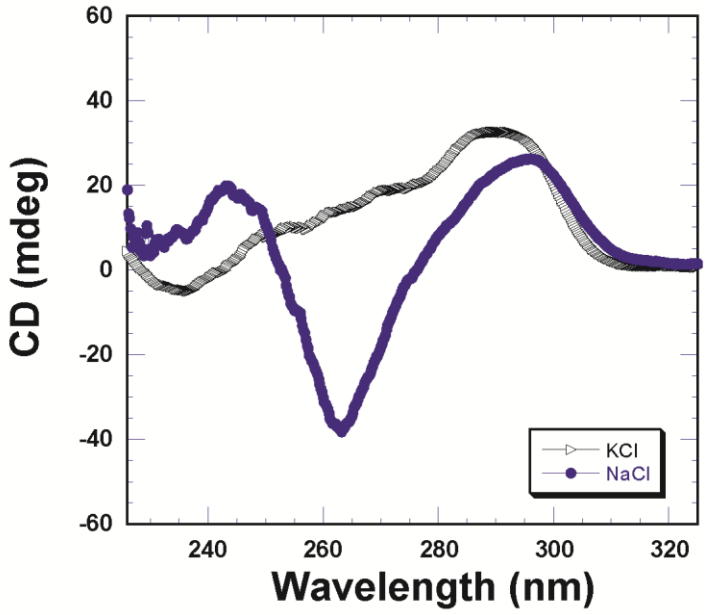
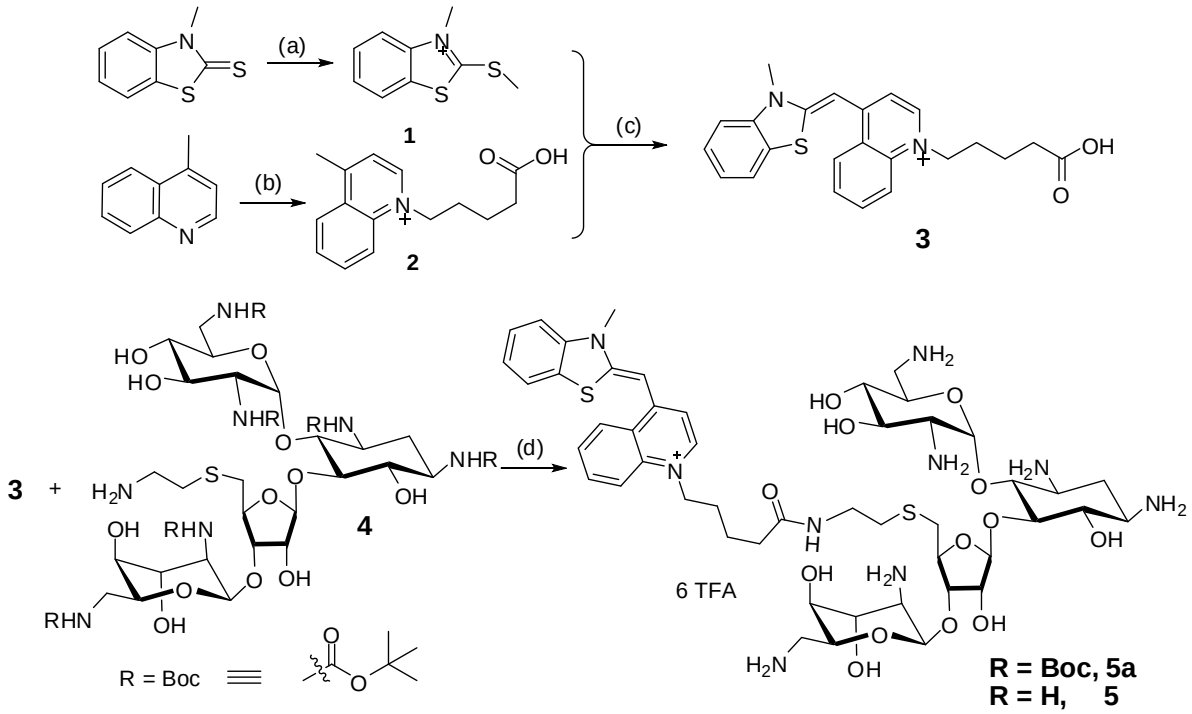


Figure S1. CD spectra of human telomeric DNA quadruplex (10 μM) in the presence of sodium ions (gray) and potassium ions (blue). For CD spectrum in the presence of sodium ions, the buffer consisted of 10 mM Sodium cacodylate, 0.5 mM EDTA, 100 mM NaCl at pH 7.0 while the CD spectrum in the presence of potassium ions consisted of buffer 10 mM HEPES, 100 mM KCl at pH 7.0. Each spectrum represents an average of three scans. The experiments were performed at 20 °C.

Synthesis of thiazole orange-neomycin conjugate (5).



Scheme 1. Reagents and conditions (a) CH_3I, 4 h, 50 °C, 84% (b) 5-bromovaleric acid, 3 h, 110 °C, 38% (c) Et_3N, 50 °C for 2 h, then 1 h at room temperature, 24% (d) (i) DMF, TBTU, DIPEA, room temperature , 65%; (ii) TFA, DCM, 3 h, r.t., quant.

Thiazole orange carboxylic acid (3). To a dry 0.1 L round bottom flask was added "**1**" (1.0 g, 2.4 mmol) and "**2**" (0.8 g, 2.4 mmol) in dry EtOH (24.0 mL). Dry Et_3N (0.7 mL, 5.2 mmol, 2.2 equiv.) was added to the mixture which resulted in a red solution immediately. The reaction mixture was stirred at 55 °C for 2 h then at room temperature for 1 h under an argon atmosphere. The mixture was allowed to cool to room temperature then precipitation was induced by addition of Et_2O (50.0 mL). The crude solid was suspended in acetone (70 mL)/Et_2O (100 mL) for 1 h then collected via vacuum filtration followed by washing with Et_2O (3 × 20 mL). The mixture was dried under reduced pressure to give a red solid (250 mg, 24%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.62 (p, J = 7.0 Hz, 2H, CH_2), 1.90 (p, J = 7.3 Hz, 2H, CH_2), 2.29 (t, J = 6.9 Hz, 2H, CH_2), 4.03 (s, 3H, CH_3), 4.64 (t, J = 6.9 Hz, 2H, NCH_2), 6.97 (s, 1H, CH), 7.38-7.49 (m, 2H, Ar), 7.62 (t, J = 6.9 Hz, 1H, Ar), 7.70-7.90 (m, 2H, Ar), 7.97-

8.03 (m, 1H, Ar), 8.06 (d, *J* = 7.5 Hz, 1H, Ar), 8.17 (d, *J* = 6.9 Hz, 1H, Ar), 8.67 (d, *J* = 7.2 Hz, 1H, Ar), 8.81 (d, *J* = 8.4 Hz,1H, Ar), 8.85-9.15 (br, 1 H, COOH); UV (H_2O) λ_{max} = 503 nm; ε_{503} = 8833.8 $M^{-1}cm^{-1}$; MALDI-TOF *m/z* calcd for $C_{23}H_{23}N_2O_2S^+$ 391.14, found 391.61; ESI-HRMS calcd 391.1480, found 391.1483; HPLC purity 97.8% (t_R = 0.97 min)

Synthesis of TO-neo conjugate (5).

Synthesis of Boc protected TO-neo conjugate (5a). To a solution of Boc protected neomycin amine (**4**) (15.3 mg, 12.0 µmol) in dry DMF (3.0 mL), TBTU (15.4 mg, 48.0 µmol, 4.0 mol equivalents) were added followed by addition of DIPEA (12.4 mg, 96.0 µmol, 8.0 mol equival.) and TO-COOH (7.0 mg, 24.0 µmol) under the atmosphere of argon at room temperature. The progress of the reaction was monitored by TLC. A new deep red colored spot was observed on TLC which gives positive stain with ninhydin staining solution. The volatiles were removed by rotary evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel (32-60 micron mesh size) using dichloromethane-ethanol as eluent (0 to 15 % ethanol in dicholoromethane) to afford the desired compound **5a** as a deep red solid (17 mg, 82 %): [R_f 0.38, 10% MeOH in DCM (*v/v*)];^{1}H NMR (500 MHz, CD_3COCD_3) δ 8.85-8.94 (d, *J* = 7.09 Hz, 1H, Ar), 8.70-8.77 (d, *J* = 8.51 Hz, 1H, Ar), 8.56-8.66 (br, s, 1H), 8.24 (d, *J* = 8.52 Hz, 1H, Ar), 8.11 (d, *J* = 7.57 Hz, 1H, Ar), 8.05 (t, *J* = 7.41 Hz, 1H, Ar), 7.71-7.81 (m, 2H, Ar), 7.61-7.68 (m, 2H, Ar), 7.55 (m, *J* = 6.46 Hz, 1H, Ar), 7.01 (m, 2H), 6.45-6.55 (br, s, 1H, NH), 6.37 (d, *J* = 8.83 Hz, 1H, NH), 6.30 (d, *J* = 8.20 Hz, 1H, NH), 6.22 (d, *J* = 9.62 Hz, 1H, NH), 5.86-5.94 (br, s, 1H), 5.11 (d, *J* =5.68 Hz, 1H), 5.06 (br, s, 1H), 5.03 (br, s, 1H), 4.74-4.84 (m, 2H), 4.26-4.32 (m, 1H), 4.18-4.23 (m, 1H), 4.09-4.18 (m, 4H), 4.01-4.08 (m, 2H), 3.97 (t, *J* = 9.46 Hz, 1H), 3.76-3.91 (m, 4H), 3.47-3.72 (m, 12H), 3.27-3.45 (m, 4H), 3.16-3.44 (m, 2H), 3.05-3.14 (m, 4H), 2.57-2.66 (m, 2H), 2.31-2.50 (m, 3H), 1.93 (p, *J* = 2.21 Hz, 1H), 1.83 (p, *J* = 6.94 Hz, 1H), 1.36-1.61 (m, 54H); MS (MALDI-TOF) calcd. for $C_{78}H_{120}N_9O_{25}S_2$ 1646.78, found 1646.67 $[M]^+$; UV (DCM) λ_{max} = 508 nm; HPLC purity 91.1% (t_R = 1.84 min)

Synthesis of To-neo conjugate (5). To a solution of **5a** (8.2 mg, 5.0 µmol) in dichloromethane (1.0 mL), TFA (0.1 mL) was added and the reaction mixture was stirred at room temperature for 3h under darkness. The progress of the reaction was monitored by TLC which showed completion of reaction in 3h. Water (2.0 mL) was added to the reaction mixture and then washed with dichloromethane (3 × 3.0 mL). The water layer was lyophilized to give the desired product **5** as dark red colored compound (4.9 mg, 80%): ^{1}H NMR (500 MHz, D_2O) δ 9.22 (d, *J* = 5.99 Hz,

1H), 8.44 (d, *J* = 8.99 Hz, 1H), 8.37 (d, *J* = 8.51 Hz, 1H), 8.21 (d, *J* = 8.04 Hz, 1H), 8.11 (d, *J* = 8.67 Hz, 1H), 8.03 (d, *J* = 8.20 Hz, 1H), 7.96 (t, *J* = 8.04 Hz, 1H), 7.81-7.88 (m, 3H), 7.06-7.16 (m, 1H), 6.99 (t, *J* = 6.78 Hz, 1H), 6.82 (d, *J* = 5.84 Hz, 1H), 6.60-6.69 (m, 1H), 5.92-6.03 (m, 1H), 5.29-5.35 (m, 1H), 5.14-5.23 (m, 1H), 5.01 (t, *J* = 7.14 Hz, 2H), 4.93 (t, *J* = 7.26 Hz, 1H), 4.26-4.41 (m, 2H), 4.18-4.26 (m, 2H), 4.01-4.15 (m, 2H), 3.90-3.99 (m, 1H), 3.78-3.90 (m, 2H), 3.67-3.76 (m, 2H), 3.57-3.67 (m, 2H), 3.33-3.42 (m, 4H), 3.20-3.30 (m, 3H), 3.10-3.19 (m, 2H), 2.99-3.09 (m, 2H), 2.91-2.98 (m, 2H), 2.32-2.38 (m, 1H), 2.57-2.67 (m, 1H), 2.33-2.43 (m, 1H, H_{I2eq}), 2.15-2.29 (m, 2H), 1.96-2.01 (m, 1H), 8.04 (d, *J* = 12.7 Hz, 1H, H_{I2ax}), 1.58-1.74 (m, 1H); MS (MALDI-TOF) calcd. for $C_{48}H_{72}N_9O_{13}S_2$ 1046.47, found 1047.59$[M+H]^+$; UV (H_2O) λ_{max} = 508 nm. ε_{503} = 13835 $M^{-1}cm^{-1}$; HPLC purity 94.1% (t_R = 3.00 min)

S6. NMR, IR, and MALDI spectra of the synthesized compounds.

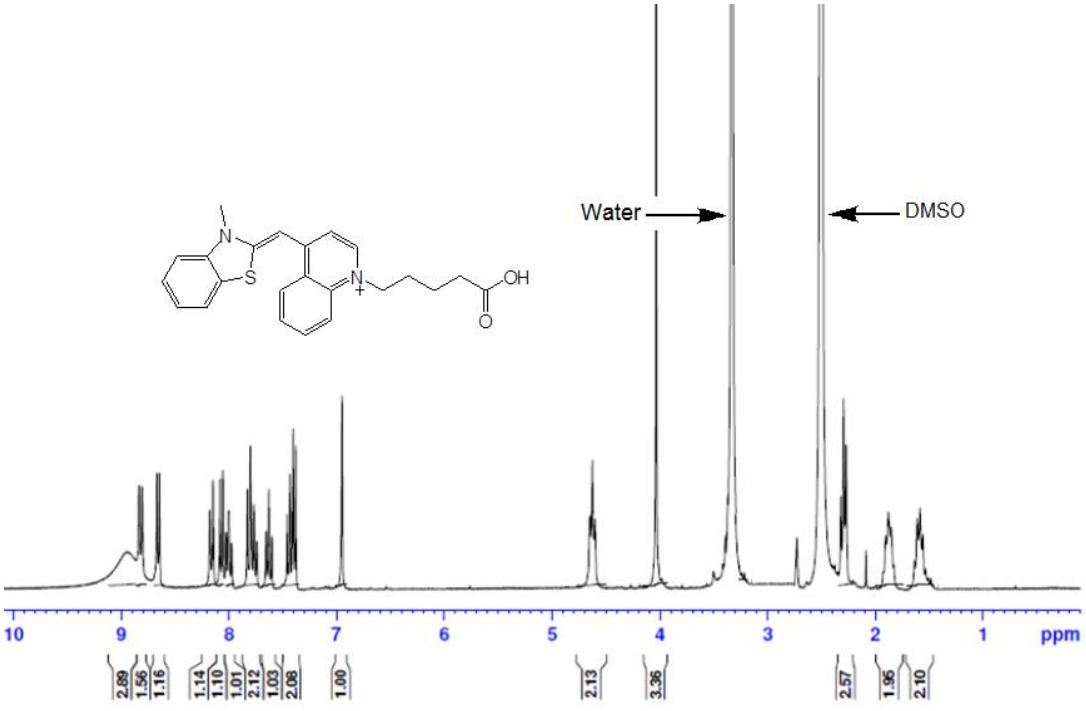


Figure S2a. ^{1}H-NMR of thiazole orange carboxylic acid (**3**).

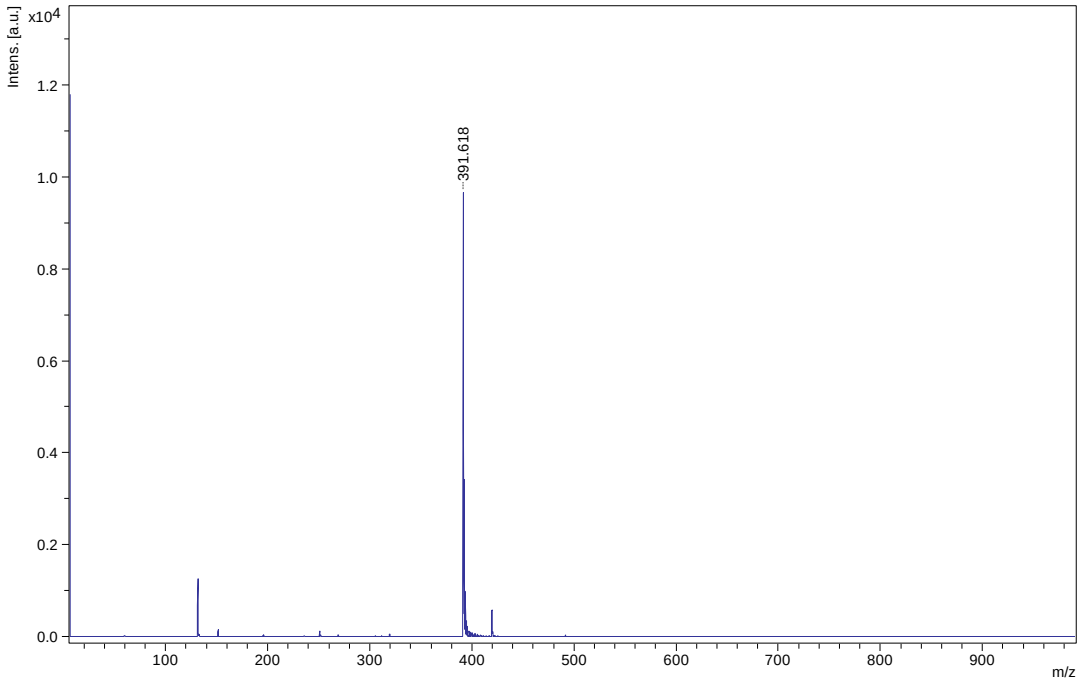


Figure S2b. MALDI-TOF spectrum of thiazole orange carboxylic acid (**3**).

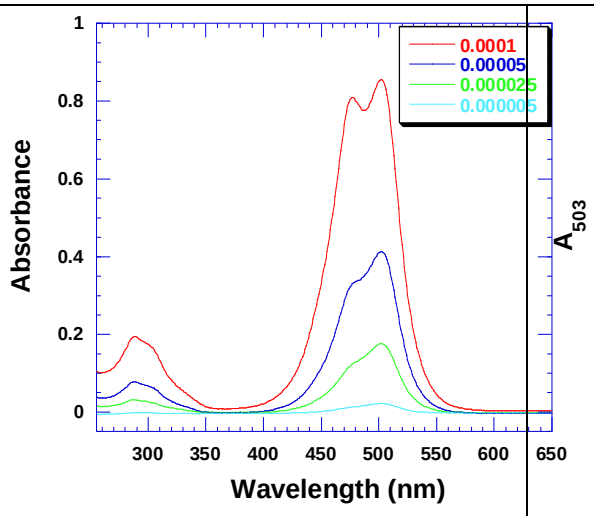


Figure S3. (A) Absorbance scans of **3** in water at 5µM, 25µM, 50µM, and 100µM. **(B)** Determination of extinction coefficient of **3** in water at 503.

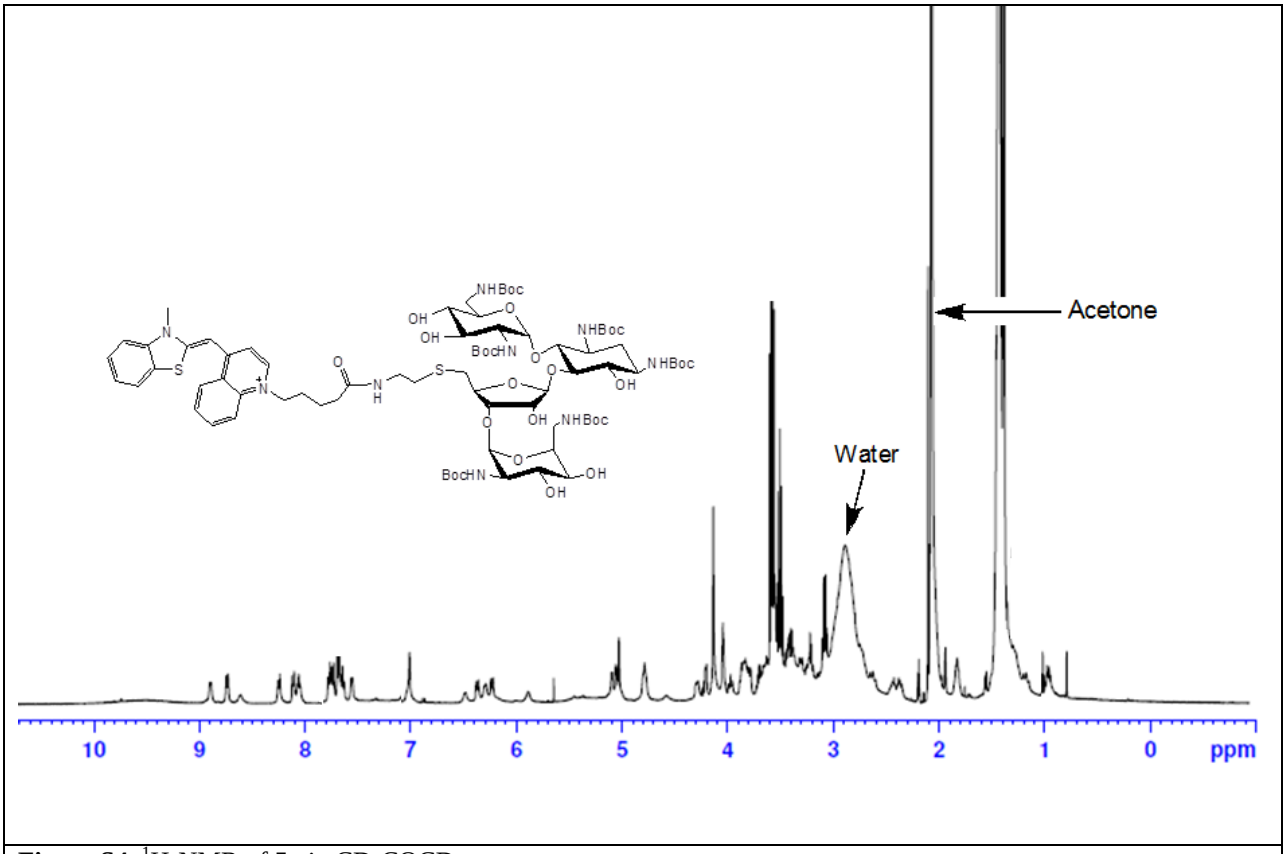


Figure S4. ^{1}H-NMR of **5a** in CD_3COCD_3.

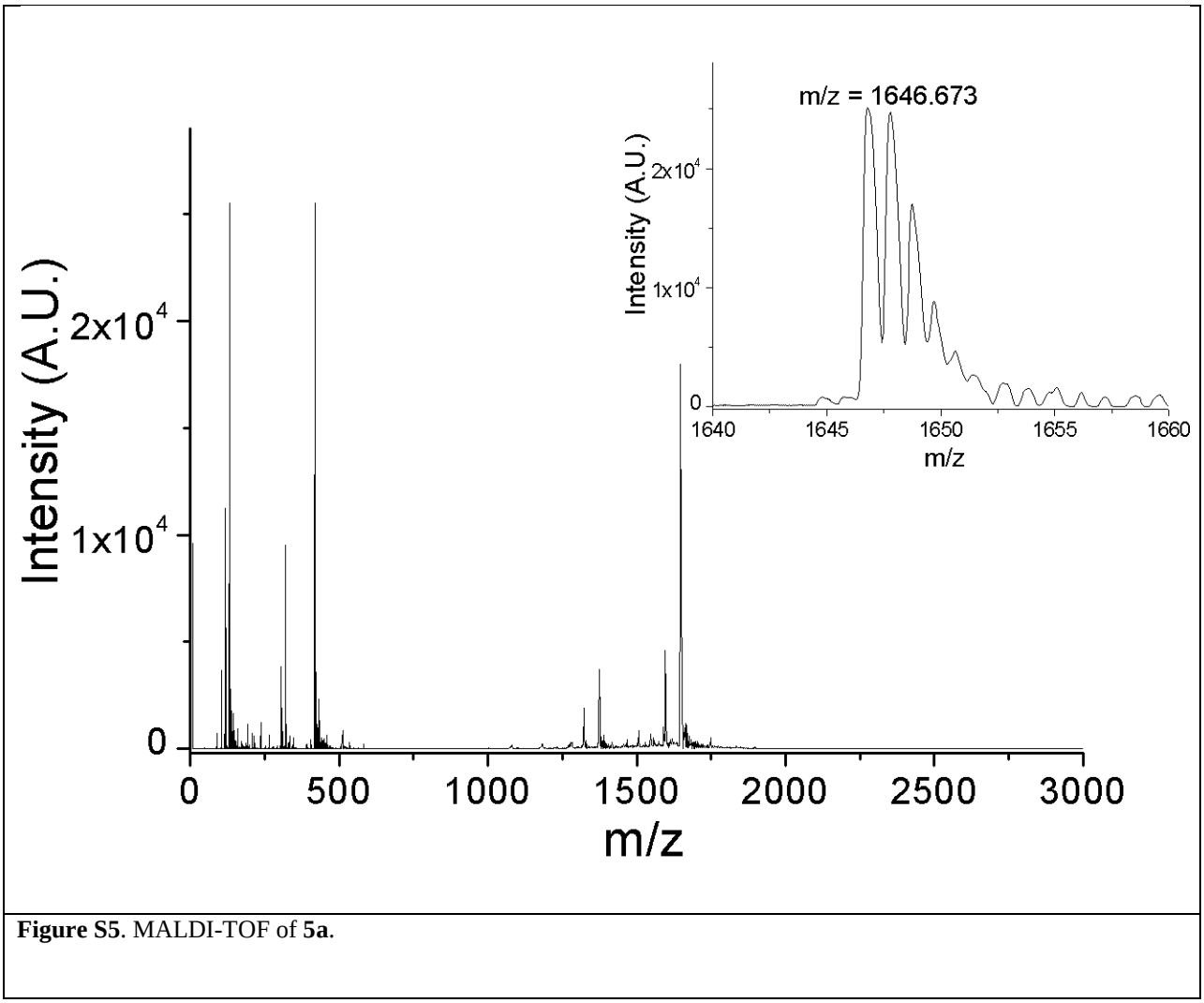


Figure S5. MALDI-TOF of **5a**.

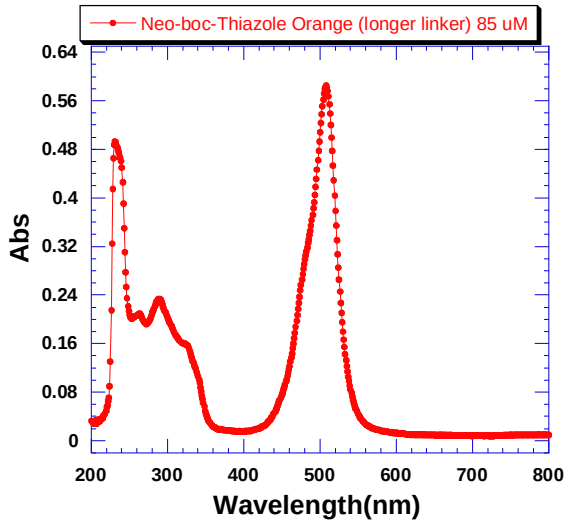


Figure S6. UV-absorbance spectrum of **5a** in DCM at 20°C.

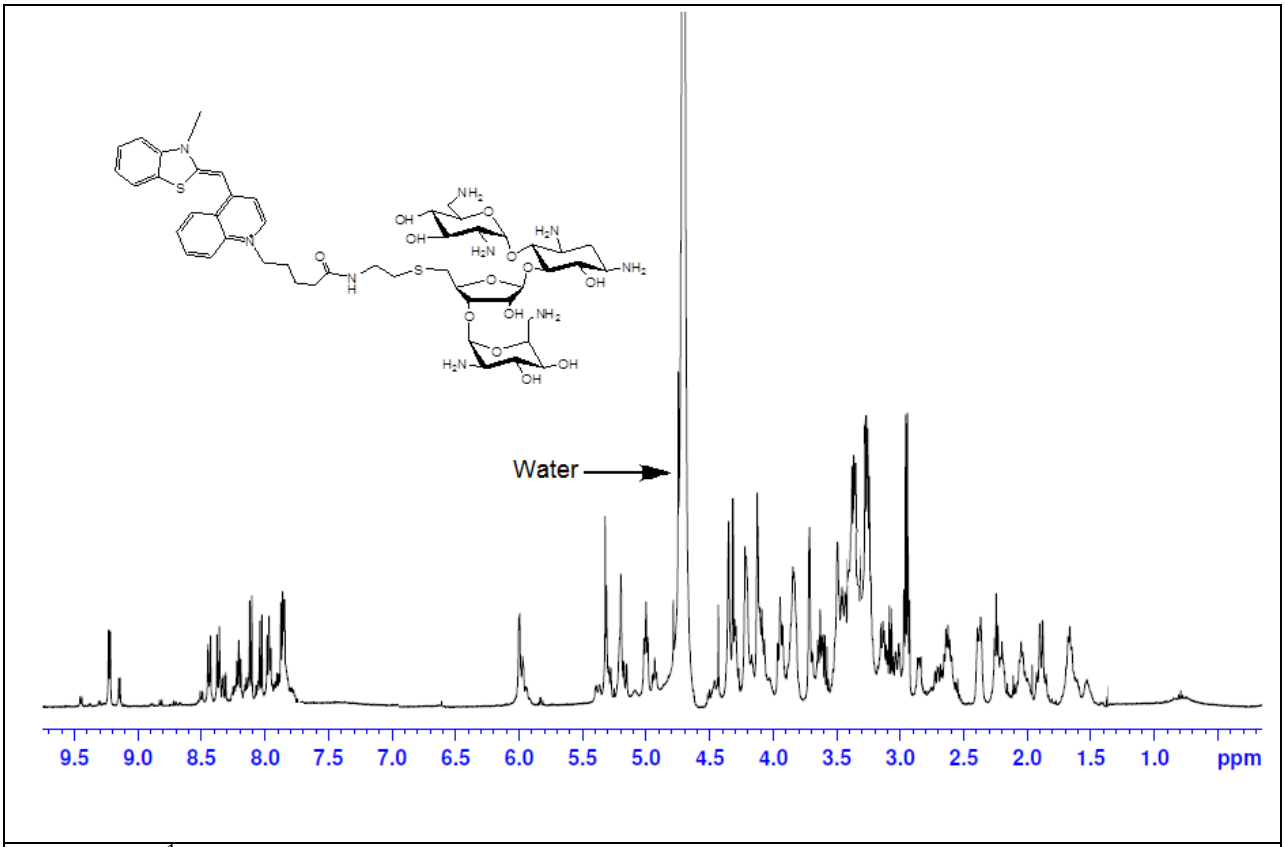


Figure S7. ^{1}H-NMR of **5** in D_2O.

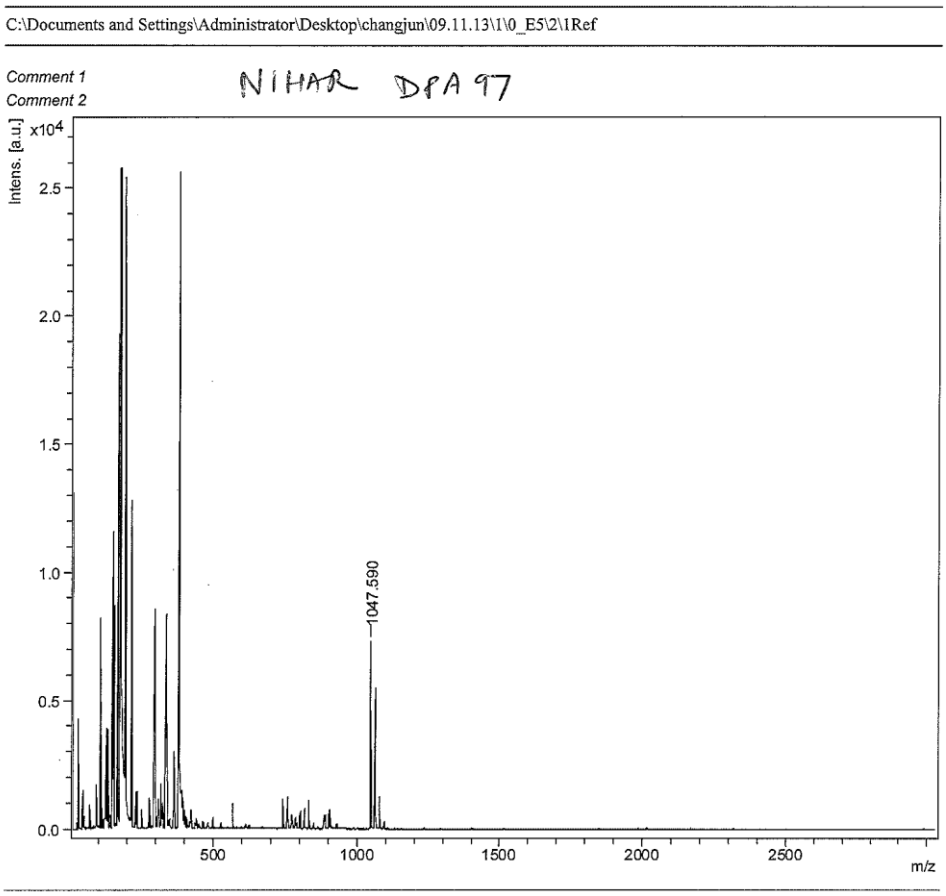


Figure S8. MALDI-TOF of **5**.

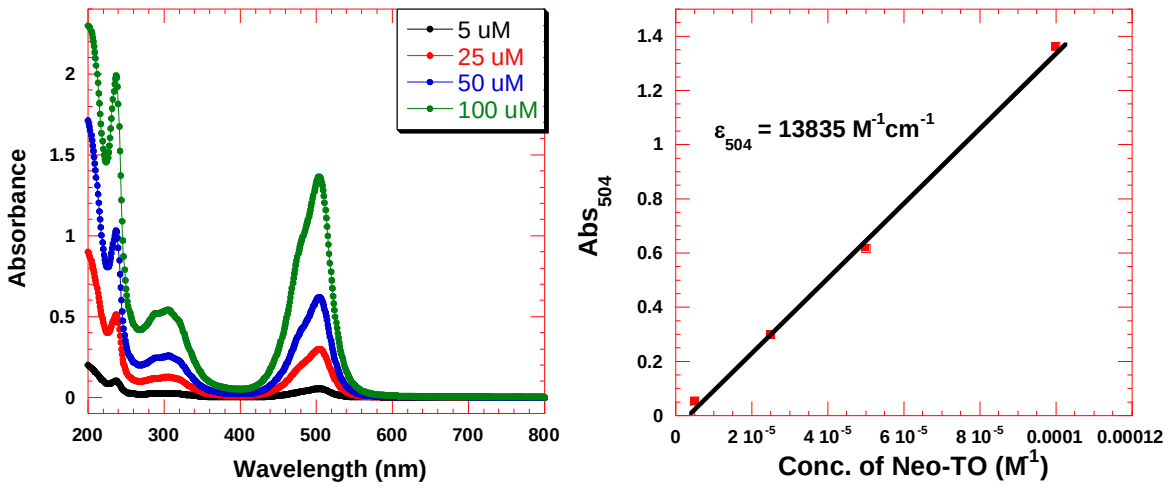


Figure S9. UV- absorbance spectrum of **5** in H_2O at 20°C.

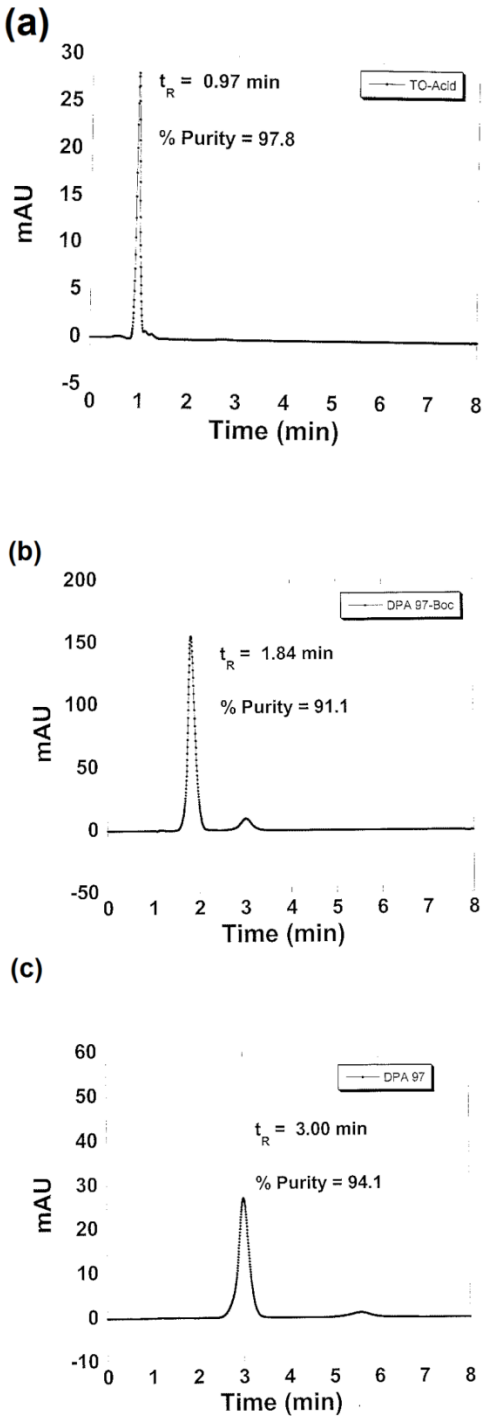


Figure S10. HPLC profiles of compounds **3**, **5a** and **5**.

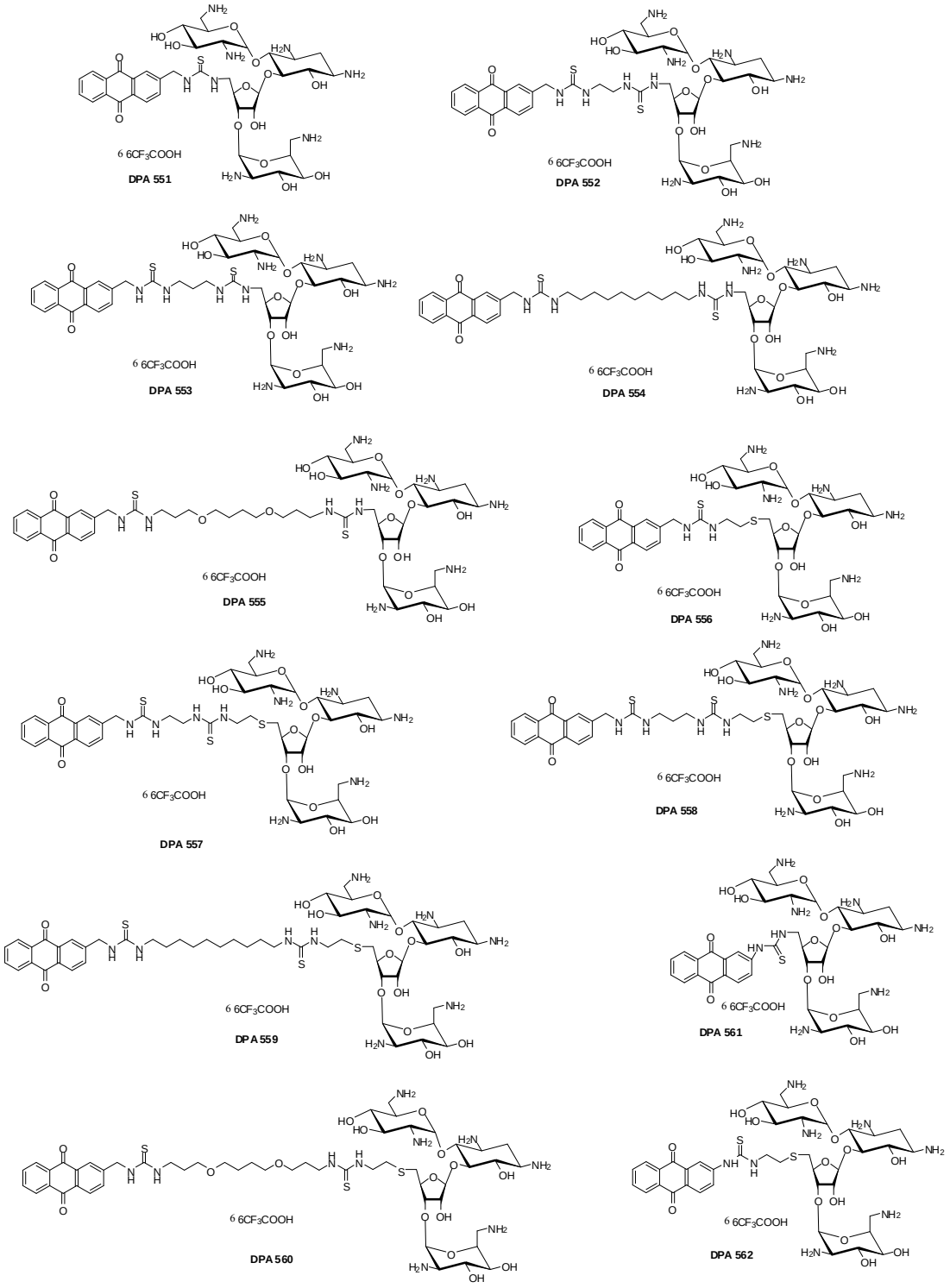


Figure S11. Structures of neomycin anthraquinone conjugates (DPA 551-562) used in the screening.