SYNTHESIS OF ACRIDINE BASED THREADING INTERCALATORS

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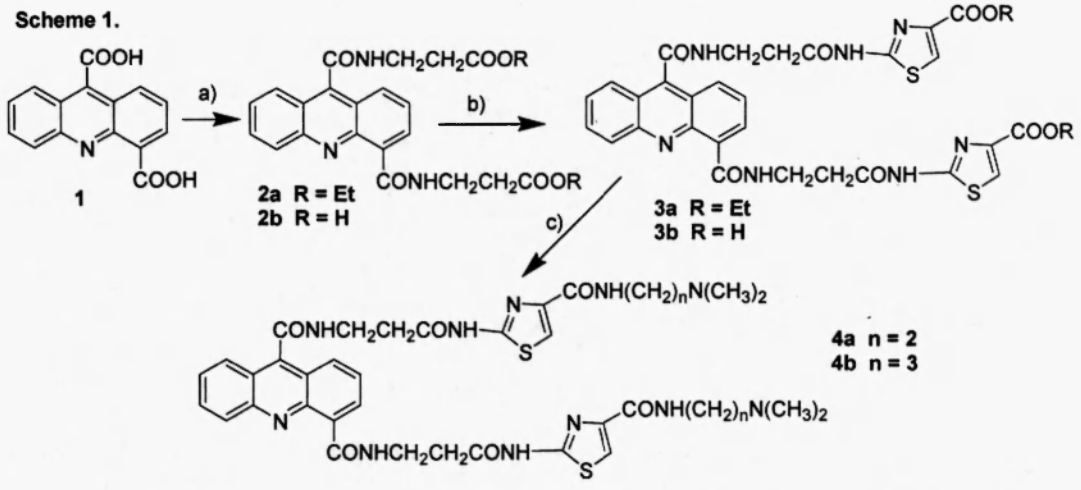
Abstract: The multistep synthesis of two *N,N*-Bis[2[[4-[[*N*-(dimethylamino)alkyl]carbamoyl]thiazol-2-yl]carbamoyl]ethyl]acridine-4,9-dicarboxamides as novel threading intercalators is reported. Preliminary studies show that the molecules exhibit moderately strong DNA affinity.

Introduction

DNA threading intercalators offer the potential for the development of sequence specific binding agents for a number of purposes including therapeutics and diagnostics. We have previously reported the synthesis of planer aromatic systems based upon a naphthothiophene system with two cationic side chains which are thought to bind to DNA by a threading intercalation mode. [1] Threading intercalators are molecules which have two side chains on opposite sides of a planer aromatic ring and binding requires that one of the side chains slide through the stacked base pairs in order to achieve stacking interactions between the base pairs and the planar aromatic ring. A number of threading intercalators have been investigated due to their ability to selectively interact with both DNA [2,3] and RNA[4,5]. Several acridine systems with two cationic side chains have been described that bind to DNA by a threading mechanism including 9-anilinoacridine-4-carboxamide derived compounds which are of therapeutic interest.[6-9] Other di- and tri-substituted acridine and acridone DNA binders have been found to induce G-quadruplex formation and thereby indirectly inhibit telomerase function and thus provide an anticancer drug development strategy which is receiving considerable attention.[10] Acridine bis-carboxamides with extended side chains which have the potential for multiple interactions with the DNA grooves do not appear to have been previously reported. This communication describes the synthesis and preliminary DNA binding studies, including quadruplex binding, of acridine-4,9-dicarboxamide based threading intercalation systems.

Results and Discussion

The synthetic approach employed for the preparation of the target threading intercalators is outlined in Scheme 1. Acridine-4,9-dicarboxlic acid (**1**) on coupling with β-alanine ethyl ester mediated by BOP in the mixed solvent 1:1 DMF/MeCN at room temperature gave the diester **2a** in 43% yield. Hydrolysis of the diester 2a was achieved



Reagents and conditions: a) i) alanine ethyl ester hydrochloride, Et_3N, BOP, DMF/MeCN ii) NaOH, MeOH, rt
b) i) 2-amino-4-ethoxycarbonylthiazole, DCC, HOBt, DCM/DMF ii) NaOH, EtOH, rt
c) CDI, DMF, $(CH_3)_2N(CH_2)_nNH_2$

Scheme-1

by the use of sodium hydroxide in methanol at room temperature to yield the the diacid **2b** in 61% yield. The reaction of **2b** with 2-amino-4-ethoxycarbonylthiazole was first attempted by a BOP mediated process in DMF, however despite the fact that NMR evidence suggested the presence of the desired product **3a** we were unable to obtain it pure. An alternate approach employing CDI/HOBt in DCM/DMF yielded **3a** in a 42% yield. The diester 3a was readily converted the diacid **3b** in a yield of 98% by the action of sodium hydroxide in ethanol at room temperature. The target dimethylaminoalkyl analogues **4a** and **4b** were obtained by CDI promoted coupling between 3b with the appropriate dimethylaminoalkylamine in yields of 70 and 73 % , respectively.
The interaction of **4a** and **4b** with calf thymus DNA was evaluated and the ΔTm values observed were 12.3 and 13.2 °C, respectively. Comparison of ΔTm values as an assessment of DNA affinity for different molecular frameworks, even when obtained under similar experimental conditions, can only be of qualitative significance. With this in mind, we note that the acridines **4a** and **4b** show moderately strong DNA affinity. The acridines appear to bind with significantly lower affinity than the symmetrical threading intercalator *N,N* -bis[2-(dimethylamino)ethyl]1,4,5,8-naphthaleneteracarboxylic-1,8,4,5-dimide which gave a ΔTm value of 24 °C under

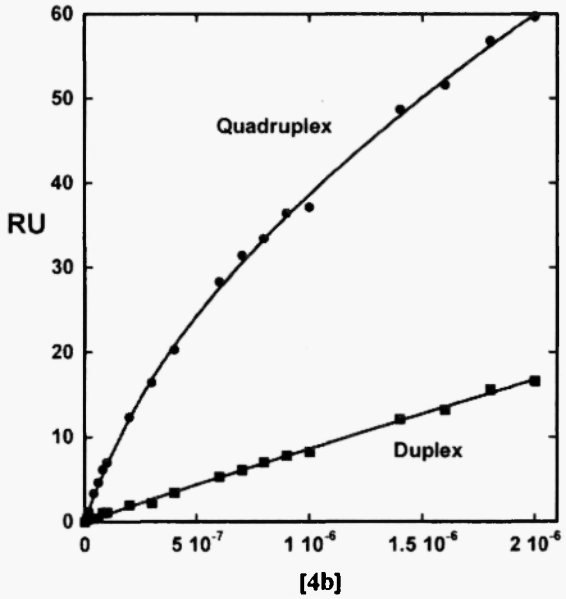


Figure-1: Sensorgrams for the interaction of **4b** with the 5'biotin-labeled HuTelo and AATT duplex were collected in 0.1 M KCl. 0.01 M Tris. 0.003 M EDTA. pH 7.3 over a **4b** concentration range from 10 nM to 2.0 μM (flow rate of 40 μl/min at 25C). The response (RU) in the plateau region of the sensorgrams at each **4b** concentration were determined and binding isotherms for **4b** complexes with the HuTelo and AATT were obtained by plotting RU versus the compound concentration. The lines in the Figures were obtained by non-linear least-squares fits of the data to one and two site binding equations as described in the Methods Section.

these conditions.[2] The acridines show comparable ΔTm values to that observed for *N,N* –bis[2-[[2-(dimethylamino) ethyl]carbamoyl]ethyl]naphtho[2,1-b]thiophene-4,8-dicarboxamide which gave, under the same conditions, a ΔTm value of 11.4 °C.[1] Given the rather long side chains for **4a** and **4b** that must slide through the base pairs and then be accommodated in the DNA grooves the moderately strong affinity of these acridine molecules is surprising. Quantitative analysis of **4b** interactions was determined by biosensor-SPR methods with immobilized DNA quadruplex and duplex model systems. Sensorgrams (not shown) for binding to a human telomere model quadruplex and an AATT duplex were obtained as described in the Methods Section. Analysis of the sensorgrams revealed that the compound binds rapidly to both DNAs and reaches a steady state response value (RU) in less than 20 s. Over the same concentration range there was a much larger response with the quadruplex than with the duplex DNA on adding **4b**, indicating more extensive binding of acridine to the quadruplex DNA. The differences in binding constants may easily be seen in the binding plots of Figure 1 and fitting of the curves,

as described in Methods, gives an equilibrium binding constant, Ka = 2 x 10^6 M^{-1}, for binding to the human telomere quadruplex and Ka = 3 x 10^4 M^{-1} for the AATT DNA duplex under the same conditions. Similar SPR experiments with **4b** binding to the cMyc quadruplex under the same conditions give a Ka = 8 x 10^5 M^{-1}, slightly lower than with the human telomere quadruplex DNA. The compound also has a second binding site on the telomere and cMyc sequences that binds the compound approximately ten times more weakly than the strong site. The SPR results show strong binding selectivity of this compound to quadruplex DNA over duplex, 67 fold for binding to HuTelo and 27 fold for binding to cMyc over the duplex DNA. The acridine derivative thus presents a new direction for development of compounds that have strong binding and selectivity for quadruplex DNA structures.

Experimental

Tm Measurements. Thermal melting experiments were conducted with a Cary 300 spectrophotometer. For these measurements cuvettes are mounted in a thermal block and the solution temperatures are monitored by a thermistor in a reference cuvette. Temperatures are maintained under computer control and are increased at 0.5 °C/min. The thermal melting studies with calf thymus DNA(Worthington Bichemicals) were performed in PIPES00 buffer (PIPES 10mM, EDTA 1mM) are conducted in 1 cm path length quartz cuvettes . The concentrations of DNA were determined by measuring the absorbance at 260nm. A ratio of 0.1 compound per base was used for the complex and DNA with no compound was used as a control.

Surface Plasmon Resonance Studies. The oligonucleotides: G4 human telomere (HuTelo); 5'-Biotin-d[$AG_3(T_2AG_3)_3$], G4-cMyc; 5'-Biotin-d[AGGGTGGGGAGGGTGGGGA] , and the hairpin duplex (AATT duplex); 5'-Biotin-d[CGAATTCGTCTCCGAATTCG] were purchased from Midland Certified Reagent Company or IDT (Integrated DNA Technologies, Inc.) with HPLC purification and mass spectrometry characterization. The concentration of each DNA sample was determined spectrophotometrically at 260 nm using the nearest neighbor extinction coefficient. A 1mM stock solution of each compound was prepared in H_2O and diluted to working concentrations with buffer. Surface plasmon resonance measurements were performed with a four-channel BIAcore 2000 optical biosensor system (BIAcore Inc.). 5'-biotin labeled DNA was immobilized onto streptavidin-coated sensor chips (BIAcore SA) as previously described.[11] Three flow cells were used to immobilize the DNA oligomer samples, while a fourth cell was left blank as a control. The SPR experiments were performed at 25°C in filtered, degassed, 10 mM Tris buffer (pH 7.0) containing 100 mM KCl, 3 mM EDTA and 0.005% surfactant P20. Compound solutions were prepared by serial dilutions from stock solution and injected from 7 mm plastic vials with pierceable plastic crimp caps. Solutions of known ligand concentration were injected through the flow cells until a constant steady-state response was obtained. Compound solution flow was then replaced by buffer flow resulting in dissociation of the complex. The reference response from the blank cell was subtracted from the response in each cell containing DNA to give a signal (RU, response units) that is directly proportional to the amount of bound compound. A set of sensorgrams at different concentrations for binding of the compound to DNA was obtained. The instrument response (RU) in the steady-state region was determined by linear averaging over a selected time span. The predicted maximum response per bound compound in the steady-state region (RU_{max}) was determined from the DNA molecular weight, the amount of DNA on the flow cell, the compound molecular weight, and the refractive index gradient ratio of the compound and DNA, as previously described.[12] The number of binding sites and the equilibrium constant were obtained from fitting plots of RU versus C_{free}. The data were fitted to a two-site equilibrium model using Kaleidagraph for nonlinear least squares optimization of the binding parameters:

$$RU = RU_{max}*(K_1*C_{free} + 2*K_1*K_2*C_{free}^2)/(1 + K_1*C_{free} + K_1*K_2*K_{Cfree}^2)$$

where RU_{max} is the maximum response per bound compound and K_1 and K_2 are the macroscopic binding constants for a two-site binding model. For a single binding site model K_2 is equal to zero.

Chemistry. Melting points were determined in open capillary tubes with a Mel-Temp 3.0 melting point apparatus and are uncorrected. ^1H- and ^{13}C -NMR spectra were recorded on Varian Unity 300 and Varian VRX 400 instruments and chemical shifts are reported in ppm relative to TMS. Mass spectra (MS) were performed by the Georgia Tech Mass Spectra Laboratory at Georgia Institute of Technology in Atlanta, GA. Elemental analyses were performed by Atlantic Microlab in Norcross, GA. All chemicals and solvents were purchased from Aldrich Chemical Co. or Fisher Scientific.

Synthesis of *N,N*-Bis[2-(ethoxycarbonyl)ethyl]acridine-4,9-dicarboxamide (2a). To a solution of acridine 4,9-dicarboxylic acid (**1**, 0.267 g, 0.001 mole), β-alanine ethyl ester hydrochloride 0.307 g (0.002 mole) and BOP (0.884 g, 0.002 mole) in a mixture of anhydrous dimethyl formamide/anhydrous acetonitrile (1:1), triethylamine (0.556 ml, 0.004 mole) was added. The mixture was stirred at room temperature, with exclusion of moisture, for 8 h. Water was added to precipitate the crude product which was extracted three times with ethyl acetate. The organic phase washed successively with 2N hydrochloric acid, water, 5% sodium hydrogen carbonate, water, and dried over magnesium sulfate. The solvent was removed under reduced pressure to give 0.2 g (43 % yield) of **2a** which crystallized from EtOH/H_2O to give pure yellow crystalline compound, mp 115-117 °C. ^{1}H NMR (DMSO-d_6): δ 1.21 (t, 3H, J = 7Hz); 1.23 (t, 3H, J = 7Hz); 2.78 (t, 4H, J = 6Hz); 3.80 (t, 4H, J = 6Hz); 4.16 (q, 4H J = 7Hz); 7.78 (m, 2H,); 8.02 (m, 2H); 8.18 (d, 1H, J = 8Hz); 8.39 (d, 1H, J = 8Hz); 8.75 (d, 1H, J = 7Hz); 9.21(brs, 1H); 11.61 (brs, 1H). ^{13}C NMR (DMSO-d_6): δ 14.1, 33.7, 34.0, 35.0, 35.5, 60.0, 60.1, 121.3, 122.0, 125.6, 126.0, 127.4, 128.2, 128.8, 129.8, 131.8, 134.7, 144.2, 145.2, 146.8, 164.5, 165.6, 171.3, 172.1.Calcd. mass: 466.5(M^+ + H), observed mass: 466.2. Anal. Calcd. for $C_{25}H_{27}N_3O_6$: C, 64.50; H, 5.84; N, 9.02; Found: C, 64.39; H, 5.87; N, 8.90.

Synthesis of *N,N*-Bis[2-carboxyethyl]acridine-4,9-dicarboxamide (2b). A solution of 0.93 g (0.002 mole) of **2a** and 4 ml (0.016 mole) of 4N aqueous sodium hydroxide in 15 ml of methanol was stirred at room temperature. The progress of the reaction was monitored by TLC and was judged to be complete after 6 h. The solvent was evaporated under reduced pressure and the residue was taken up in water and impurities were extracted with ether and ethyl acetate. The acid was then precipitated by cautious acidification to pH 5-6 with cold 1N hydrochloric acid. The obtained compound was crystallized from EtOH/H_2O to give 0.5 g (61 % yield) of a yellow crystalline compound, mp 237-40 °C. ^{1}H NMR(DMSO-d_6): δ 2.70 (t, 4H, J = 6Hz); 3.76 (m, 4H); 7.77 (m, 2H); 8.01 (m, 1H); 8.08 (d, 1H, J = 8Hz); 8.20 (d, 1H, J = 7Hz); 8.43 (d, 1H, J = 8Hz); 8.78 (d, 1H, J = 7Hz); 9.20 (brs, 1H); 11.61 (brs, 1H); 12.40 (brs, 2H,). ^{13}C NMR (DMSO-d_6): δ 33.8, 34.2, 35.1, 35.7, 121.5, 122.2, 125.8, 126.2, 127.5, 128.2, 129.1, 130.0, 131.9, 134.9, 144.3, 145.4, 147.0, 164.6, 165.8, 173.0, 173.9. Calcd. mass: 410.48(M^+ + H), observed mass: 410.2. Anal. Calcd. for $C_{21}H_{19}N_3O_6$•0.5H_2O: C, 60.28; H, 4.81; found: C, 60.38; H, 4.82.

Synthesis of *N,N*-Bis[2[[4- ethoxycarbonyl] thiazol-2-yl]carbamoyl]ethyl]acridine-4,9-dicarboxamide (3a). A cold solution of DCC (5.28 g, 0.0254 mole) and HOBt (3.9 g, 0.0254 mole) in anhydrous dichloromethane/dimethylformamide (1:1, 40 ml) was added to a solution of **2b** (4.74 g, 0.0116 mole) in 15 ml of DMF portionwise at 0°C for 1 h under stirring. Stirring was continued for 3 h at 0°C and a cold solution of 2-amino-4-ethoxy carbonylthiazole[13] (3.995 g, 0.0232 mole) in anhydrous dichloromethane/dimethylformamide (1:1, 40 ml) was added and stirring was continued for an additional 3 h at 0°C. The solution was allowed to warm to room temperature and stirring was continued for 48 h. The precipitated compound was filtered. The by-product dicyclohexylurea (DCU) was removed using soxlet extraction with methanol. The material was crystallized from DMF to yield 3.5 g (42 % yield) of the desired compound, mp 295-7 °C. ^{1}H NMR (DMSO-d_6): δ 1.28 (t, 6H, J = 7Hz); 2.93 (t, 4H, J = 6Hz); 3.90 (t, 4H, J = 6Hz), 4.28 (q, 4H, J = 7Hz); 7.68 (m, 3H); 7.87 (d, 1H, J = 8Hz); 8.03 (s, 1H); 8.04 (s, 1H); 8.18 (d, 1H, J = 7 Hz); 8.36(d, 1H, J = 8 Hz); 8.72 (d, 1H, J = 7Hz); 9.09 (brs, 1H); 11.58 (brs, 1H); 12.58 (brs, 2H). ^{13}C NMR (DMSO-d_6): δ14.4, 35.0, 35.2, 35.5, 35.7, 61.1, 121.7, 122.4, 123.0, 125.8, 126.3, 127.7, 128.4, 129.2, 130.2, 132.0, 135.0. 141.3, 144.2, 145.5, 147.2, 158.1, 158.2, 161.5, 165.3, 166.2, 170.6, 171.4. Calcd. mass: 718.7(M^+ + H), observed mass: 718.0. Anal. Calcd. for $C_{33}H_{31}N_7O_8S_2$: C, 55.21; H, 4.35. Found: C, 55.29, H, 4.41.

Synthesis of *N,N*-Bis[2[[4-carboxythiazol-2-yl]carbamoyl[ethyl]acridine-4,9-dicarboxamide (3b). A solution of 1.435 g (0.002 mole) of **3a** and 4 ml (0.016 mole) of 4N aqueous sodium hydroxide in 20 ml of ethanol was stirred at room temperature. The progress of the reaction was monitored by TLC and was judged to be complete after 7 h. The solvent was evaporated under reduced pressure, the residue was taken up in water and impurities were extracted with ether and ethyl acetate. The product was then precipitated by cautious acidification to pH 3-4 with cold 1N hydrochloric acid. The compound obtained was collected, washed with ether, ethanol and dried to give 1.3 g (98 % yield) of a yellow solid, mp 242-5 °C (dec). ^{1}H NMR (DMSO-d_6): δ 2.96 (t, 4H, J = 6Hz); 3.92 (t, 4H, J = 6Hz); 7.63 (m, 1H) 7.71 (m, 1H); 7.88 (m, 1H); 7.97 (s, 2H,); 8.06 (d, 1H, J =

8Hz); 8.22 (d, 1H, J = 7Hz); 8.41 (d, 1H, J = 8Hz); 8.76 (d, 1H, J = 7Hz); 9.12 (brs, 1H); 11.61 (brs, 1H); 12.44 (brs, 4H). ^{13}C NMR (DMSO-d_6): δ 34.7, 34.9, 35.3, 35.4, 121.3, 121.8, 122.0, 125.5, 125.8, 127.1, 128.3, 128.9, 129.8, 131.4, 134.5, 142.2, 144.1, 145.2, 146.8, 157.5, 157.6, 162.3, 164.5, 165.5, 170.0, 170.8. Calcd. mass: 662.6 (M^+ + H), observed mass: 662.1. Anal. Calcd. for $C_{29}H_{23}N_7O_8S_2 \cdot 1H_2O$: C, 51.20; H, 3.70; N, 14.40. Found: C, 51.03; H, 3.80; N, 14.23.

Synthesis of *N,N*-Bis[2[[4-[[*N*-(dimethylamino)alkyl]carbamoyl]thiazol-2-yl]carbamoyl]ethyl]acridine-4,9-dicarboxamides 4a and 4b. A mixture of **3b** (0.661 g, 0.001 mole), 1,1 - carbonyl diimidazole (0.486 g, 0.003 mole) in 4 ml of anhydrous *N,N*-dimethylformamide was stirred at 20 °C, with exclusion of moisture, for 2 h. The mixture was then cooled to 0 °C, the *N,N*-dialkylamino compound (0.05 mole) was added, the mixture was stirred for another 3 h at 20 °C. Volatiles were removed under reduced pressure. The residue was partitioned between chloroform and 0.2M sodium carbonate, and the organic layer was washed with water, brine and the solvent removed under vacuum to yield a brown oil. The oil was dissolved in 30 ml of methanol, treated with charcoal, filtered and the methanol removed under reduced pressure to yield a brown oil. The oil was added to 10 ml of a boiling benzene, the benzene was decanted, then the solid was washed with petroleum ether, the petroleum ether was decanted and the solid was washed with ethyl acetate by stirring in an ice bath for 2 h, the flask was kept overnight in refrigerator to yield yellow colored flakes, which were washed with cold ether and crystallized from ethanol/water to yield pure free base. The solid was dissolved in absolute ethanol, cooled in an ice bath and concentrated HCl was added dropwise to pH 2. The contents were stirred at room temperature for 2.5 h. Dilution of the solution with anhydrous ethyl acetate gave the hydrochloride salt as yellow crystals which were washed with anhydrous ether and dried under vacuum to give pure compound.

***N,N*-Bis[2[[4-[[*N*-(dimethylamino)ethyl]carbamoyl]thiazol-2-yl]carbamoyl]ethyl]acridine-4,9-dicarboxamide (4a):** Yield 70 %, mp 167-70 °C (dec). ^{1}H NMR (DMSO-d_6): δ 2.12 (s, 6H); 2.13 (s, 6H); 2.39 (t, 4H, J = 5Hz); 2.96 (t, 4H, J = 6Hz); 3.19 (m, 4H); 3.92 (t, 4H, J = 6Hz); 7.63 (m, 1H); 7.72 (m, 1H); 7.79 (s, 1H); 7.80 (s, 1H); 7.88 (m, 1H); 8.08 (d, 1H, J = 8Hz); 8.21 (d, 1H, J = 7Hz); 8.29 (d, 1H, J = 8Hz); 8.88 (d, 1H J = 7Hz); 9.10 (brs, 1H); 11.58 (brs, 1H). ^{13}C NMR (DMSO-d_6): δ 34.7, 34.8, 35.2, 35.4, 36.2, 44.8, 44.9, 57.6, 117.1, 121.2, 122.1, 125.6, 126.0, 127.0, 128.0, 129.0, 130.0, 131.2, 134.5, 144.1, 144.3, 145.2, 146.9, 157.7, 157.8, 160.2, 160.3, 164.4, 165.5, 170.1, 170.9. Calcd. mass: 802.9 (M^+ + H), observed mass: 802.5. Anal. Calcd. for $C_{37}H_{43}N_{11}O_6S_2$: C, 55.41; H, 5.40; N, 19.21, Found: C, 55.44; H, 5.51; N, 19.02.

Dihydrochloride of 4a: Yield 67 %, mp 202-4 °C (dec). ^{1}H NMR (DMSO-d_6): δ 2.79 (t, 4H, J = 6Hz); 3.22 (t, 4H, J = 6 Hz); 3.65 (m, 4H); 3.88 (t, 4H, J = 6Hz); 4.19 (s, 12H); 7.62 (m, 1H,); 7.75 (m, 1H); 7.81 (s, 1H); 7.82 (s, 1H); 8.01 (d, 1H, J = 7Hz); 8.20 (d, 1H, J = 8Hz); 8.30 (d, 1H, J = 7Hz); 8.41 (d, 1H, J = 8Hz); 8.69 (d, 1H, J = 7Hz); 9.11 (brs, 1H); 11.62 (brs, 1H); 12.44 (brs, 2H). ^{13}C NMR (DMSO-d_6): δ 34.1, 34.7, 34.8, 35.2, 35.4, 42.3, 55.8, 117.8, 121.3, 122.0, 125.6, 125.9, 127.2, 128.1, 128.7, 129.9, 131.6, 134.6, 144.1, 144.3, 145.0, 146.6, 157.4, 157.6, 161.2, 164.5, 165.5, 170.2, 171.0. Anal. Calcd. for $C_{37}H_{43}N_{11}O_6S_2 \cdot 2HCl \cdot 2H_2O$: C, 48.78; H, 5.42; N, 16.91. Found: C, 49.11; H, 5.54; N, 16.47.

***N,N*-Bis[2[[4-[[*N*-(dimethylamino)propyl]carbamoyl]thiazol-2-yl]carbamoyl]ethyl]acridine-4,9-dicarboxamide (4b):** Yield 73 %, mp 152-5 °C (dec.). ^{1}H NMR (DMSO-d_6): δ 1.62 (m, 4H,); 2.11 (s, 6H); 2.12 (s, 6H); 2.32 (t, 4H, J = 6Hz); 2.98 (t, 4H, J = 6Hz); 3.31 (m, 4H,); 3.91(t, 4H, J = 6Hz); 7.62 (m, 1H); 7.66 (m, 1H); 7.78 (s, 1H); 7.81 (s, 1H), 7.85 (m, 1H); 8.06 (d, 1H, J = 7Hz); 8.20 (d, 1H, J = 8Hz); 8.40 (d, 1H, J= 7Hz); 8.68 (d, 1H, J = 8Hz); 9.08 (brs, 1H); 11.62 (brs, 1H). ^{13}C NMR (DMSO-d_6): δ 26.8, 34.6, 34.8, 35.2, 35.3, 37.2, 37.3, 44.9, 45.0, 56.8, 56.9, 117.0, 121.2, 122.0, 125.4, 125.8, 127.1, 128.2, 128.9, 129.7, 131.3, 134.4, 144.1, 144.6, 145.1, 146.5, 157.4, 157.6, 160.4, 160.5, 164.5, 165.5, 170.8. Calcd. mass: 831.0 (M^+ + H), observed mass: 830.2. Anal. Calcd. for $C_{39}H_{47}N_{11}O_6S_2$: C, 56.43; H, 5.70; N, 18.56. Found: C, 56.45; H, 5.80; N, 18.34

Trihydrochloride of 4b: Yield 68 %, mp 205-7 °C (dec.). ^{1}H NMR (DMSO-d_6): δ 1.88 (m, 4H); 2.72 (t, 4H, J = 7Hz); 3.01 (t, 4H, J = 6Hz); 3.35 (m, 4H); 3.92 (t, 4H, J = 6Hz); 4.68 (s, 12H); 7.64 (m, 1H); 7.74 (m, 1H); 7.82 (s, 1H); 7.83 (s, 1H); 7.85 (m, 1H); 8.02 (d, 1H, J = 8Hz); 8.15 (brs, 2H); 8.22 (d, 1H, J = 7Hz); 8.42 (d, 1H, J = 8Hz); 8.75 (d, 1H, J = 7 Hz); 9.25 (brs, 1H); 11.63 (brs, 1H); 12.45 (brs, 2H). ^{13}C NMR (DMSO-d_6): δ 34.7 , 34.8, 35.2, 35.4. 36.2, 44.8, 44.9, 57.7, 117.1, 121.3, 122.0, 125.1, 126.2, 127.2, 128.2,

129.0,130.0,131.2,134.9,144.2,144.3, 145.2, 147.0, 157.7, 157.8, 160.2, 160.3, 164.4, 165.5, 170.9. Anal. Calcd.for $C_{39}H_{47}N_{11}O_6S_2 \cdot 3HCl \cdot H_2O$: C, 48.92; H, 5.47; N, 16.09. Found: C, 49.03; H, 5.79; N, 16.00.

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