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BISELECTROSTATIC INTERACTION WITH DNA AS POTENTIAL ANTI-TUMOR AGENTS: NOVEL 2, 11- DIAMINO- 5, 8- DITHIAUNDECANE DERIVATIVES.

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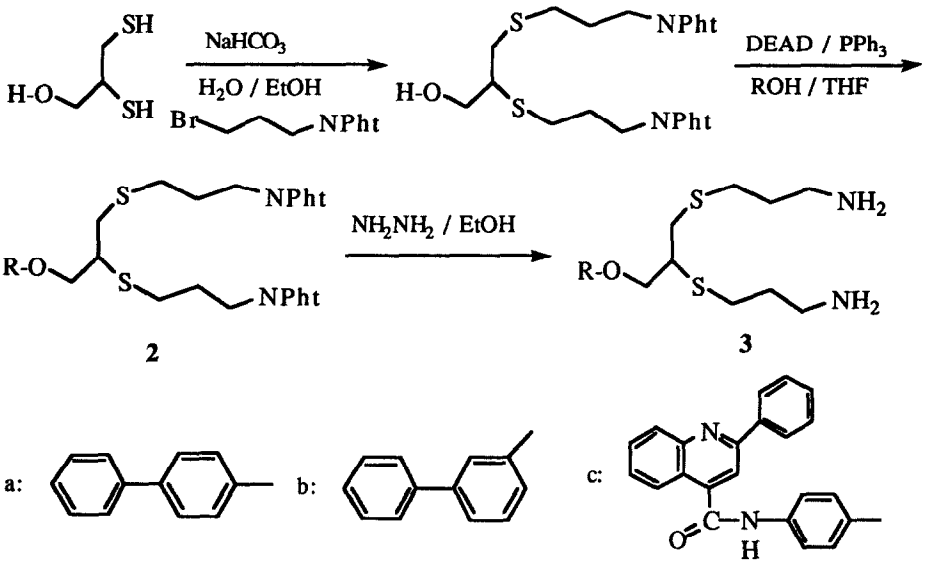
Abstract: The prototype biselectrostatic chain, 6-(hydroxymethyl)-2,11-diamino-5,8-dithiaundecane linked to the 4-phenylphenol, 3-phenylphenol and 2-phenyl-4-quinoline derivatives bind strongly to double helical DNA with binding constants of 1.5 x 10^5, 3.9 x 10^5, and 5.0 x 10^5, respectively. This new class of biselectrostatic models showed potent cytotoxicity against a series of cell line *in vitro*.

Compounds which bind to DNA interact with the duplex through four primany models[1]: (1) electrostatic interactions with the anionic sugar phosphate backbone of DNA; (2) interactions to the DNA major groove; (3) interaction in the DNA minor groove; and (4) intercalation. Recently, the use of DNA interacting compounds as potentiators for the enchancement of the activity of antitumor drugs has been reported[2].The stimulation of bleomycin-induced fragmentation of DNA by DNA interacting agents was noticed for the first time by Bearden[3]. These DNA interacting agents do not necessarily have biological activity of their own and are unfused polyaromatic cations having a single eletrostatic interacting side chain, having relatively weak DNA binding. An intriguing question that has be left unanswered is that whether by increasing the number of electrostatic interaction will enchances binding with DNA and thus biological activity. This work is part of our systematic research effort directed toward the understanding of the benefits of biseletrostatic interaction with the anionic sugar phosphate backbone in DNA.

The synthesis of the ether linked 2,11-diamino-5,8-dithiaundecane DNA interacting models **3a**, **3b**, **3c** is outlined in scheme 1. The diazadithia ligand **1** was synthesized in high yield by the selective bis-S-alkylation of 2,3-dimercaptopropanol and 3-bromopropylphthalimide with sodium bicarbonate as base in a water-ethanol mixture. The use of alternative base such as sodium ethoxide gave only a low yield of **1**. Our initial aim was the transformation of the hydroxy group in **1** to its chloro derivative which was readily achieved by reacting with triphenylphosphine in carbon tetrachloride. Displacement of the chloride with alkoxy or amino compounds tends to form a large number of side products and no attempt was made to isolate and characterized them. In order to accomplish the selective coupling of the hydroxy group in **1** to the DNA interacting compounds, we chose to use their phenolic derivatives. The coupling of alcohol with phenol under Mitsunobu conditions is the most widespread method for the preparation of site selective ether bond. 4-Phenylphenol and 3-phenylphenol[2] were chosen as the simplest DNA interacting

moieties for coupling with **1** under the Mitsunobu reaction conditions[4] to give a good yield of the ether linked product **2a, 2b** respectively. The phenyl-quinoline moiety has recently been reported to be the minimal DNA intercalating ligand[5] and we choose to examine the commercially available 2-phenyl-4-quinoline carboxylic acid. We first needed to introduce a phenolic group in the molecule and this was achieved by coupling with 4-aminophenol in the presence of carbonyl diimidazole to give 4-[(2-phenyl-4-quinoline)carboxyamido]-phenol, **4.** Compound **4** was again successfully coupled to **1** under Mitsunobu reaction to give a good yield of **2c.** The phthalimide protecting group in **2a, 2b** and **2c** was readily removed with hydrazine in refluxing ethanol to give the 2,11-diamino-5,8-dithiaundecane DNA interacting models containing biselectrostatic group **3a**[6], **3b**[7], **3c**[8] respectively.

Scheme 1



The association constant for the intercalative binding of the compounds were determined by the ethidium displacement of Morgan et. al..[9] Calf thymns DNA (2.5 µl, 5.0 $O.D_{unit}$) was added to an ethidium assay solution which contain 0.5 µg/ml ethidium bromide and 0.5 mM EDTA in 20 mM K_3PO_4 at pH 11.8. (The drug was added until 50% of the ethidium was displaced.) The binding constant of ethidium was taken as 10^7 M^{-1} at a concentration of 1 µM. Compounds **3a, 3b** and **3c** were found to bind to double helical calf thymus DNA with an approximate binding constant of 1.5 x 10^5, 3.9 x 10^5 and 5.0 x 10^5 respectively. These values are much higher as compare with those of the individual non classical intercalator, 4-phenylphenol (3 x 10^3), 3-phenylphenol (3 x 10^3) having only a monoelectrostatic chain and the minimal DNA intercalating ligand 2-phenyl-4-quinoline which binds weakly.[2] These data suggest interaction with DNA having biselectrostatic binding have been greatly enchanced by the use of 2,11-diamino-5,8-dithiaundecane.

These models were tested for its *in vitro* cytotoxicity[10] against KB, HEP-2, Hela and Colon-205 cells. Compounds **3a, 3b, 3c** were found to possess potent cytotoxicity against the cell line tested as shown in Table 1. No significant cytotoxicity were observed for the individual DNA interacting compounds alone. Thus a DNA interacting moiety associated with 2,11-diamino-5,8-dithiaundecane is required for the expression of cytotoxic activity.

Table 1: In Vitro Cytotoxicity of Compounds

Compounds	LD_{50} (μg / ml)			
	HEP-2	KB	Hela	Colo-205
3 R=H	—	—	—	—
m-phenyl-phenol	—	—	—	—
p-phenyl-phenol	—	—	—	—
4	—	—	—	—
3 a	1.45	0.92	0.68	1.05
3 b	1.36	1.55	1.20	1.41
3 c	1.92	—	6.18	5.20

Another possible mechanisms of action for these compounds may be the chelation of ferrous ion by the N_2S_2 ligand which should cause DNA cleavage[11]. Herein, it is of special interest to examine the DNA cleavage reaction of our compounds in the presence of ferrous ion. An agarose gel electrophoretic experiments of ethidium bromide stained Φ 174 DNA after treatment with and without ferrous ion of **3a, 3b** and **3c** with bleomycin as control in the presence of dithiothretiol or NADPH as reducing agents were performed. In this studies, bleomycin in the presence of ferrous ion showed very distinct DNA cleavage (form II DNA), whereas **3a, 3b** and **3c** in the presence of ferrous ion did not cleave DNA. Prolong incubation periods also did not show any DNA cleaving activity for our compounds. This further substantiate the fact that the *in vitro* cytotoxicity was due to drug binding with DNA, which was enchanced with biselectrostatic interaction.

In conclusion, the results of this investigation are sufficiently encouranging to warrant further studies of this family of biselectrostatic DNA interacting compounds with a view that this class of compound itself binds strongly to DNA and showed potent cytotoxicity. Their role as new bleomycin amplifiers are currently under investigation.

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6. 6-[(4-phenylphenoxy)methyl]-2,11-diaza-5,8-dithiaundecane, **3a**.
   IR ($CHCl_3$) 3400, 3650 (NH), 1600 (arom.), 1485 (C-O-C) cm^{-1}; ^1H NMR ($CDCl_3$) 1.22 (br., s, 4H, 2 x NH_2, exchangable with D_2O), 1.60-1.71 (m, 4H), 2.52-2.74 (m, 8H), 2.81-3.11 (m, 2H), 3.09-3.13 (m, 1H), 4.01-4.20 (m, 2H), 6.91-7.36 (m, 5H), 6.93 (d, 2H), 7.25 (m, 1H), 7.34 (d, 2H), 7.47 (dd, 4H); mass spectrum (FAB) 391 (M^++2), 360, 334, 300, 289. Anal. Calcd for $C_{12}H_{30}N_2S_2O$: C: 64.59; H: 7.74; N: 7.17. Found: C: 64.40; H: 7.72; N: 7.01.
7. 6[3-(phenylphenoxy)methyl]-2,11-diaza-5,8-dithiaundecane, **3b**.
   IR ($CHCl_3$) 3400, 3650 (NH), 1600 (arom.), 1475 (C-O-C) cm^{-1}; ^1H NMR ($CDCl_3$) 1.65-1.69 (m, 4H), 1.77 (br., s, 4H, 2 x NH_2, exchangable with D_2O), 2.48-2.71 (m, 8H), 2.77-2.94 (m, 2H), 3.06-3.10 (m, 1H), 4.12-4.18 (m, 2H), 6.79-6.90 (m, 1H), 7.07-7.11 (m, 2H), 7.24-7.51 (m, 6H); mass spectrum (FAB) 391 (M^++2), 360, 300, 221. Anal. Calcd for $C_{21}H_{30}N_2S_2O$. 0.5 H_2O: C: 64.59; H: 7.74; N: 7.17. Found: C: 64.54; H: 7.69; N: 7.14.
8. 6{[[3-(2-phenyl-4-quinoline)carboxyamido]phenoxy]methyl}-2,11-diaza-5,8-dithiaundecane, **3c**.
   IR (nujol) 3500, 3600 (NH), 1650 (NHCO), 1380 (C-O-C) cm^{-1}; ^1H NMR (D^6-DMSO) 1.59 (m, 4H), 2.50-2.70 (m, 8H), 2.88 (m, 2H), 2.96 (br., s, 4H, 2 x NH_2, exchangable with D_2O), 3.10 (m, 1H), 4.12 (m, 2H), 7.01 (d, 2H), 7.58 (m, 4H), 7.68 (d, 2H), 7.85 (m, 1H), 8.15 (d, 2H), 8.23 (s, 1H), 8.29 (d, 2H); mass spectrum (FAB) 560 (M^+), 470, 380, 340. Anal. Calcd. for $C_{31}H_{36}N_4S_2O_2$: C: 66.40; H: 6.47; N: 9.99. Found: C: 66.20; H: 6.25; N: 9.26.
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