

SYNTHESIS AND ANTITUMOR EVALUATIONS OF 1, 8-BIS[(2-AMINOETHYL)AMINO]ANTHRACENE-9,10-DIONES

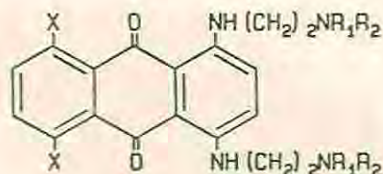
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ABSTRACT. Several 1,8-bis[(2-aminoethyl)amino]anthracene-9,10-diones have been synthesized by S_NAr -type displacements of fluoride from 1,8-difluoroanthracene-9,10-dione by various diamines. These synthetics have been evaluated in an L-1210 assay for their antitumor activities both *in vitro* and *in vivo*. Although three of these analogs exhibit moderate *in vitro* activity, they have been found to be inactive *in vivo*. These results emphasize the importance of the side-chain positioning on the anthracene-9,10-dione at 1 and 4 to maximize the antitumor activity.

INTRODUCTION

Substituted anthracene-9,10-diones (anthraquinones) are widely found in nature (1), find use as important dyestuffs (2,3), and have been used medicinally since ancient times (4-7). More recently, a number of 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones have been synthesized and evaluated for their antitumor activities (4-10). Among the numerous synthetics, 1a (ametantrone) and 1b (mitoxantrone) have shown outstanding antitumor activities against many tumor cell lines. Mitoxantrone (1b) is currently under extensive clinical evaluation (4-12) and has recently been approved by the FDA as a drug for leukemia treatment. The analogs 1c and 1d are also quite active against several tumor lines (4,5).

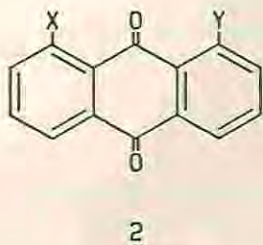


- a) X = H, $R_1 = H$, $R_2 = NH(CH_2)_2OH$
b) X = OH, $R_1 = H$, $R_2 = NH(CH_2)_2OH$
c) X = H, $R_1 = R_2 = H$
d) X = H, $R_1 = R_2 = CH_3$

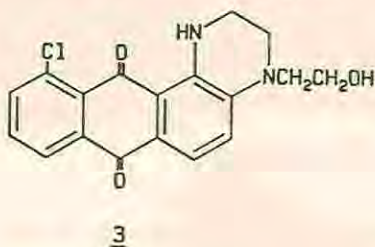
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In order to establish the relative positioning of the substituted side-chains on the anthracene-9,10-dione backbone for maximum antitumor activity, we wished to prepare analogs related to 1a, 1b and 1c but which have these side-chains at positions 1 and 8. An Italian group has shown that the reaction of 1,8-dichloroanthracene-9,10-dione (2a) with 2-[(2-aminoethyl)amino]ethanol in refluxing pyridine leads to the quinoxaline analog 3 (13,14). On the other hand, Cheng and co-workers report that if this reaction is performed in refluxing dioxane the

mono-substituted product 2b is formed (6). The type of cyclization reported by the Italians is quite commonly observed because of the high temperature necessary to effect displacements of chloride from anthracene-9,10-diones (15-17). Only one bis-1,8-analog, namely 2c has been previously prepared and found to be inactive (7).



- a) X = Y = Cl
 b) X = Cl, Y = NH(CH₂)₂NH(CH₂)₂OH
 c) X = Y = NH(CH₂)₂N(CH₃)₂
 d) X = Y = F
 e) X = Y = NH(CH₂)₂NH₂
 f) X = Y = NH(CH₂)₂N(CH₃)₂
 g) X = Y = NH(CH₂)₂NH(CH₂)₂OH
 h) X = Y = NH(CH₂)₂OH



RESULTS AND DISCUSSION

Since it is known that fluoride is much more readily displaced than chloride in S_NAr-type substitutions (18-21), we wish to report the synthesis of 1,8-difluoroanthracene-9,10-dione (2d) and its facile stepwise displacement of fluoride by amines to lead to 1,8-bis[(2-aminoethyl)amino]anthracene-9,10-diones.

Treatment of 2a with KF (sealed tube, 245°C, 44 h) leads to 2d in good yield (71%) (22). In pyridine solution at room temperature, 2d readily undergoes nucleophilic substitution by 1,2-diaminoethane, 2-dimethylaminoethylamine, and 2-[(2-aminoethyl)amino]ethanol to yield the bis-substituted analogs 2e, 2f and 2g, respectively, in good yields. Treatment of 2d with 2-aminoethanol readily yields 2h.

These 1,8-analogs were evaluated as inhibitors of the growth of L1210 cells *in vitro*. The ID₅₀ values (µg/ml) for 2e, 2f and 2g, respectively, were 0.43, 0.29 and 1.3. Although these compounds are active, under the same tumor assay screen, 1a, 1c and 1d exhibit ID₅₀ values of < 0.01, <0.01 and 0.08 µg/ml, respectively.

The compounds 2e, 2f and 2g were evaluated as inhibitors of the growth of L1210 inoculated mice. No significant antineoplastic activity was observed in any of these compounds in the 12.5-50 mg/kg dose range using several dose regimens. On the other hand, the standard 1a shows a % T/C of 160 (50 mg/kg on days 1, 5 and 9).

On the basis of our biological assessment it is clear that the substitution patterns we have studied at the 1,8-positions are not active as antitumor agents in the L1210 line.

EXPERIMENTAL

Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. ^1H NMR spectra were run on a Bruker WM-250 pulsed Fourier transform spectrometer. TLC precoated silica plates (Eastman chromatogram sheets) with fluorescent indicator were used to monitor products. For column chromatography Baker analyzed 80-200 mesh silica gel was utilized. Microanalyses were performed by Robertson laboratories, Madison, NJ. Mass spectra were run on a Finnigan MAT 4610 spectrometer. The *in vitro* and *in vivo* protocols are reported in a previous publication (10).

1,8-Difluoroanthracene-9,10-Dione (2d). A pulverized mixture of 2a (2.0 g, 7.2 mmol) and anhydrous KF (2.5 g, 43.2 mmol) was placed in a thick-walled Carius tube. The open tube was dried under *vacuo* (80 °C, 12 h) and the tube was sealed and heated in a sand bath to 245 °C over a period of 1 h. The reaction was held at this temperature for 44 h and the dark yellow solid was removed from the tube and pulverized. The product 2d (1.25 g, 71%) sublimed as a bright yellow solid when the crude solid was heated under *vacuo* in a Kugelrohr apparatus. TLC analysis (silica gel, hexane/methylene chloride 4/1) showed only one spot. The product was recrystallized from chloroform/ethanol or THF/ethanol; mp 227-228 °C, lit. mp 227-228 °C (22); ^1H NMR (CDCl_3) δ 8.09 (m, 2H), 7.72 (m, 2H) and 7.48 (m, 2H).

General Displacement Procedure. Excess diamine (7 molar equivalents) and 2d were dissolved in pyridine (2.4 ml/mmol of 2d) and the solution stirred at room temperature for 17-24 h. Petroleum ether (bp 60-80 °C) was added (2 ml/1 ml pyridine) and the precipitate was filtered. The pure product was obtained by column chromatography over silica gel or by crystallization.

1,8-Bis[[2-(dimethylamino)ethyl]amino]anthracene-9,10-dione (2f). Purification was effected by chromatography using chloroform/methanol 4/1 as eluant. Compound 2f was isolated in a 89% yield, mp 100-101 °C; ^1H NMR (CDCl_3) δ 9.71 (b, 2H) 7.51 (m, 4H), 7.01 (d, 2H), 3.45 (m, 4H), 2.62 (t, 4H), and 2.39 (s, 12H); MS, m/z (rel. int.) 380 M^+ (32), 352 (18), 322 (20), 264 (12), 58 (100); Anal. Calcd for $\text{C}_{22}\text{H}_{28}\text{N}_4\text{O}_2$: % C, 69.47, % H 7.37, % N 14.74; found % C 69.18, % H 7.08, % N 14.44.

1,8-Bis[[2-(amino)ethyl]amino]anthracene-9,10-dione (2e). Analysis of the crude product (56% yield) by TLC (silica gel) using three solvent systems (chloroform/methanol 4/1; methanol or ethyl acetate) showed one purple spot. Attempts at crystallization from chloroform/petroleum ether or ethanol/hexane were unsuccessful. ^1H NMR (CDCl_3) δ 9.75 (t, 2H), 7.54 (m, 2H), 7.47 (m, 2H), 7.04 (d, 2H), 3.43 (m, 4H), 3.07 (m, 4H), 1.34 (b, undefined integral); MS, m/z (rel. int.) 324 M^+ (100), 296 (12), 294 (14), 264 (7). For analysis 2e was converted into its dihydrochloride salt by treatment with a solution of methanolic HCl. Removal of the methanol led to 2e.2HCl, 90%, mp 141-145 °C (dec). The analytical sample was prepared by crystallization from acetone/water. Anal. Calcd for $\text{C}_{18}\text{H}_{22}\text{N}_4\text{O}_2\text{Cl}_2$: % C 54.41, % H 5.54, % N 14.11; found % C 54.62, % H 5.46, % N 14.20.

1,8-Bis[[2-(2-hydroxyethyl)amino]ethyl]amino]anthracene-9,10-dione (2g). The crude product was obtained in a 43% yield, mp 90-92 °C, TLC (silica gel, chloroform/methanol 4/1) showed one purple spot. The analytical sample was crystallized from chloroform/petroleum ether; ^1H NMR (CDCl_3) δ 9.74 (b, 2H), 7.5 (m, 2H), 7.42 (m, 2H), 6.95 (d, 2H), 3.69 (m, 4H), 3.39 (m, 4H), 3.01 (m, 4H), 2.85 (m, 4H), 1.4 (br s, undefined integral); Anal. Calcd for $\text{C}_{22}\text{H}_{28}\text{N}_4\text{O}_4$, % C 64.08, % H 6.80, % N 13.59; Found, % C 63.70, % H 6.51, % N 13.92.

1,8-Bis[[2-(2-hydroxyethyl)amino]ethyl]amino]anthracene-9,10-dione (2h). The crude product was precipitated from the pyridine solution by the addition of 1 N HCl (67%), mp 264-266 °C, lit. mp 260-265 °C (13,14); ^1H NMR ($\text{DMSO}-d_6$) δ 10.04 (b, 2H), 8.00-7.38 (m, 6H), 5.09 (t, 2H), 3.80 (m, 4H), 3.48 (m, 4H).

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