Antineoplastic Anthraquinones. II [1]. Design and Synthesis of 1,2-Heteroannelated Anthraquinones [2]

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Received July 31, 1995

Based on the "2-phenyinaphthalene-type" structural pattern hypothesis, a number of heterocycle-fused anthraquinones were designed by taking morindaparvin-A (**2a**) as the lead structure. The compounds we synthesized and tested for antineoplastic activity include 1,2-alkylenedioxyanthraquinone, naphtho [2,3-*f*]-quinoxaline-7,12-dione, anthra[1,2-*d*]imidazole-6,11-dione and naphtho[2,3-*f*]quinoxaline-7,12-dione derivatives. Most of the synthesized anthraquinones possessed various degrees of anticancer activity. One of these compounds, 2-chloromethyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (**4b**), exhibited cytotoxic activity against all tested human carcinoma cell lines.

J. Heterocyclic Chem., **33**, 367 (1996).

Recently a structural pattern hypothesis for drug design was proposed from the finding that a coplanar "2-phenyl-naphthalene-type" structure is the common structure feature among many biologically and pharmacologically active compounds [3]. This hypothesis coincides with the requirement of the minimal DNA-intercalating ligand [4]. Experimental verification further supports the proposed hypothesis [5-7]. Among compounds in this structural category, we noticed that many of the antineoplastic agents concomitantly contain anthraquinone skeletons, with examples depicted in Scheme 1. On the basis of this observation the design and synthesis of novel anthraquinones as potential anticancer drugs was conducted.

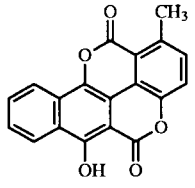
Our previous work suggested that a naturally occurring anthraquinone, morindaparvin-A (**2a**) [8], would be a promising lead compound for further drug development. Because of the antitumor activity exhibited by **2a** and the structural resemblance between the 2-dioxolenaphthalene skeleton in **2a** and the "2-phenylnaphthalene-type" structure, a series of 1,2-heteroannelated anthraquinones were designed and rationalized by bioisosterism. Now, we wish to report the results of synthesis and anticancer evaluations.

Results and Discussion.

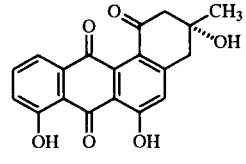
The designed 1,2-heteroannelated anthraquinones consist of a five- or six-membered heterocycle fused to the C1-C2 side of an anthraquinone. Thus, the lead compound **2a** was synthesized by refluxing alizarin (**1a**) with dibromomethane in DMF for 9 hours (Scheme 2). It gave **2a** in 40% yield. By substitution of dibromomethane with 1,2-dibromoethane, the dioxene **2b** was prepared in 21% yield.

Cyclocondensation of 1,2-diaminoanthraquinone (**1b**) with appropriate carbonyl-containing reagents gave anthra[1,2-*d*]imidazole-6,11-dione and naphtho[2,3-*f*]-quinoxaline-7,12-dione derivatives (Scheme 2). For

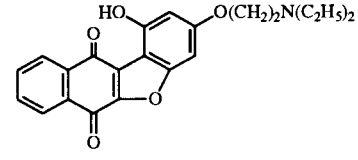
Scheme 1

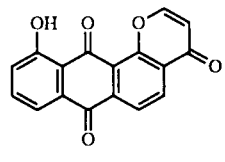


Chartreusin
(aglycone)

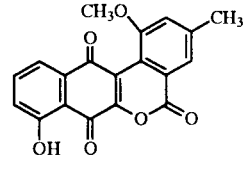


Rabelomycin

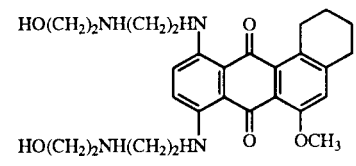




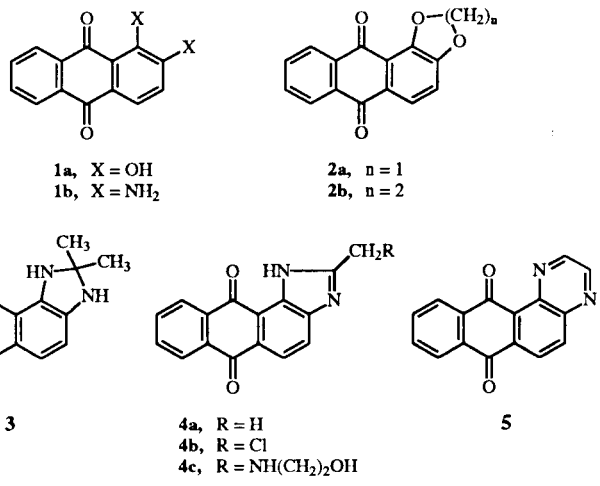
Kindamycin
(chromophore)



WS-5995A



Scheme 2



1a, X = OH
1b, X = NH_2

2a, n = 1
2b, n = 2

3

4a, R = H
4b, R = Cl
4c, R = $NH(CH_2)_2OH$

5

Table 1

Antileukemic Activities of 1,2-Heteroannelated Anthraquinones

Compounds	T/C (optimum dose, mg/Kg) P-388	
2a	129	(10)
2b	130	(10)
6a	145	(200) [20]
6b	>300	(2)
8a	—	
8b	150	(8)
8c	—	

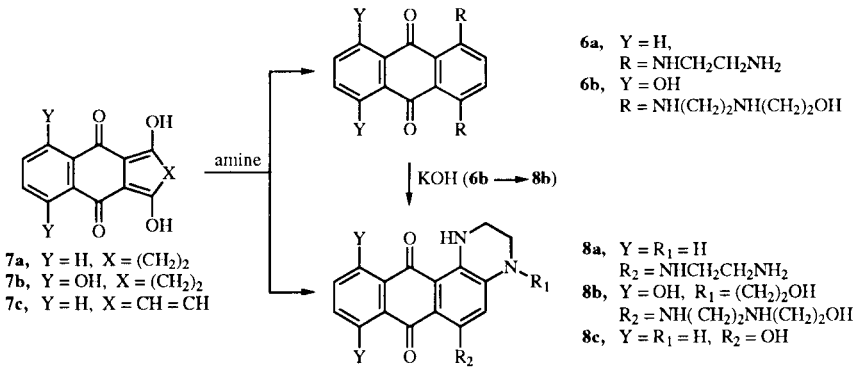
"—" indicates inactive in all tested doses.

example, the imidazoline **3** was prepared in 60% yield by stirring **1b** with acetone in the presence of sulfuric acid at room temperature for 2 days. The reaction of **1b** with acetylacetone was accomplished in ethanolic hydrochloride solution by heating under reflux for 2 hours. The cyclized product **4a** was obtained in 43% yield and its structure was confirmed by the NH singlet appeared at δ 13.05 ppm in the ^1H nmr spectrum. When chloroacetyl chloride and glyoxal were used in the reaction, the corresponding heteroanthraquinones **4b** and **5** were produced in 39 and 54% yields, respectively. Though **5** was purified by repeated chromatography and recrystallization, its melting point is not consistent with the known data [9,10]. The purity of this compound was confirmed by analytical data. Synthesis of **4c** was performed by refluxing **4b** with 2-aminoethanol in benzene for 3 hours. It gave the crystalline product in 83% yield. Attempted condensation of **1b** with carbon disulfide was unsuccessful.

The tetrahydropyrazine **8a** was described as a side product when the reaction for preparing 1,4-bis-(aminoethylamino)anthraquinone (**6a**) was proceeded at 65° or higher temperature [11]. Therefore the preparation of **8a** was carried out by heating leucoquinizarin (**7a**) with 1,2-ethylenediamine at 100°, followed by chromatographic purification which gave **8a** in 56% yield (Scheme 3). The analog **8b** has been reported to be both an oxidative metabolite of mitoxantrone (**6b**) [12-14] and a possible side product [15]. However, no spectral data were provided to confirm the assigned structure. In our recent study the impurity containing in mitoxantrone was isolated and its structure was confirmed to be **8b** [1]. Preparation of **8b** was first performed by heating leuco-1,4,5,8-tetrahydroxyanthraquinone **7b** [16] with 2-(2-aminoethylamino)ethanol at 100° for 12 hours. A tedious isolation process was needed because of the limited yield of **8b**. An alternative practical method for preparing **8b** was found and achieved by heating dihydroanthraquinone **6b** with potassium hydroxide in methanol at reflux for one hour. This reaction gave **8b** in 85% yield, whereas **8a** was not convertible from **6a** by the same method. Treatment of quinizarin **7c** with 1,2-ethylenediamine in the presence of cupric chloride at room temperature afforded **8c** in 33% yield [17].

Compounds **2a,b** and **8b** were found to possess significant activity against P388 leukemia in mice [18], whereas they were inactive against KB cells *in vitro* (Table 1). The antileukemic activities of **8a-c**, were much diminished or nil in comparison with their parent compounds. This effect might be attributed to interference in the DNA-intercalation process by the flapped heterocycle.

Scheme 3



6a, Y = H, R = $NHCH_2CH_2NH_2$
6b, Y = OH R = $NH(CH_2)_2NH(CH_2)_2OH$

7a, Y = H, X = $(CH_2)_2$
7b, Y = OH, X = $(CH_2)_2$
7c, Y = H, X = CH = CH

8a, Y = R_1 = H, R_2 = $NHCH_2CH_2NH_2$
8b, Y = OH, R_1 = $(CH_2)_2OH$, R_2 = $NH(CH_2)_2NH(CH_2)_2OH$
8c, Y = R_1 = H, R_2 = OH

Imidazole-containing anthraquinones **3** and **4a-c** were inactive in the P388 test. They exhibited, however, varied cytotoxic spectrum against cancer cell lines including KB, Colo 205, HeLa and Hep-2 [19]. Compound **4b** demonstrated potent activity throughout the entire series of tumor cells with ED_{50} down to 1 μg/ml (Table 2). Planar compound **5** was inactive both *in vivo* and *in vitro* assays.

Table 2

Cytotoxic Activities of 1,2-Heteroannelated Anthraquinones

Compounds	ED_{50} (μg/ml)			
	KB	Hep-2	CoLo	Hela
3	4.9	—	—	—
4a	—	16.7	—	5.7
4b	3.6	2.4	3.9	< 1
4c	20	—	—	20
5	—	—	—	—

"—" indicates that the ED_{50} was not within the tested concentration range.

In conclusion, the heteroanthraquinones containing dioxole, dioxene or tetrahydroxypyrazine expressed inhibitory action against acute lymphocytic leukemia *in vivo*, and the corresponding imidazole-anthraquinones possessed cytotoxic activity against human carcinomas *in vitro*. The designed structures presented in this report do not exactly fulfill the requirement of the "2-phenylnaphthalene-type" structural pattern. Nevertheless, from the standpoint of bioisosterism, these synthesized 1,2-heteroannelated anthraquinones further support the structural pattern hypothesis, and provide a new direction for the study of drug design. It is believed that with proper attachment of groups or substituents to specific positions on both ring units, 1,2-heteroannelated anthraquinones possessing potent biological activity can be designed. Biological evaluation and synthesis of related compounds based on the characteristic structural pattern are in progress and will be reported elsewhere.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. Infrared spectra (ir) were recorded on a Perkin-Elmer 983G grating spectrophotometer. The ^1H nuclear magnetic resonance (nmr) spectra were recorded on a Jeol FT-100 at National Taiwan Normal University, or on a Bruker AM-300WB spectrometer at National Taiwan University, Taipei, and are reported in parts per million (δ) downfield from an internal tetramethylsilane standard, or otherwise specified. The mass spectra (ei) were determined on a Jeol JMS D-300 Instrument at National Taiwan University. Elemental analyses were carried out on a Perkin-Elmer 240C elemental analyzer at National Taiwan University. Silica gel 60 was used in column and preparative layer chromatography. CDF_1 mice were supplied by the Experimental Animal Center, National Defense Medical Center, Taipei. The human tumor cells were generously supplied by Dr. C.-M. Chang, National Yang-Ming Medical College, Taipei, Republic of China.

1,2-Methylenedioxyanthraquinone (**2a**, Morindaparvin-A).

Potassium carbonate (0.6 g, 4.3 mmoles), cuprous oxide (0.48 g, 3.3 mmoles) and dibromomethane (10.4 g, 60 mmoles) was added to a stirred mixture of alizarin (**1a**) (4.8 g, 20 mmoles) in DMF (44.5 ml), followed by heating with reflux. After 9 hours, the reaction mixture was poured into sodium hydroxide aqueous solution (1*N*, 200 ml) and extracted with chloroform. The extract was concentrated, purified by column chromatography and recrystallized from methanol to give **2a** (2 g, 40%) as yellow needles. The melting point and nmr supported the structure of **2a** [8], mp 255-257°; ^1H nmr (deuteriochloroform, 100 MHz): δ 6.32 (s, 2H, CH_2), 7.15 (d, 1H, 3-H, J = 9.0 Hz), 7.80 (m, 2H, 6- and 7-H), 7.98 (d,1H, 4-H, J = 9.0 Hz), 8.31 (m, 2H, 5- and 8-H).

Anal. Calcd. for $C_{15}H_8O_4$: C, 71.43; H, 3.20. Found: C, 71.14; H, 3.34.

1,2-Ethylenedioxyanthraquinone (**2b**).

Compound **2b** was prepared from **1a** (1.6 g, 6.6 mmoles) and 1,2-dibromoethane (3.7 g, 19 mmoles) according to the procedure described for **2a**. The resulting product was recrystallized from chloroform-ether (3:1) to afford **2b** (0.36 g, 21%) as yellow needles, mp 233-235°; ir (neat): 1670, 1570, 1474, 1428, 1335, 1288, 1275, 1082, 896, 710 cm^{-1}; ^1H nmr (deuteriochloroform, 100 MHz): δ 4.48 (s, 4H, CH_2), 7.23 (d, 1H, 3-H, J = 8.4 Hz), 7.75 (m, 2H, 6- and 7-H), 7.93 (d, 1H, 4-H, J = 8.4 Hz), 8.22 (m, 2H, 5- and 8-H).

Anal. Calcd. for $C_{16}H_{10}O_4$: C, 72.18; H, 3.79. Found: C, 72.21; H, 3.73.

2,3-Dihydro-2,2-dimethyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (**3**).

Sulfuric acid (98%, 0.1 ml) was added to a stirred mixture of 1,2-diaminoanthraquinone (**1b**) (1 g, 4 mmoles) in dry acetone (250 ml) for two days at room temperature. The reaction mixture was passed through a potassium carbonate column, followed by evaporation under reduced pressure to dryness. The resulting product was purified by column chromatography and recrystallized from methanol to give **3** (0.7 g, 60%) as dark blue needles, mp 234-237°; ir (potassium bromide): 3420, 3240, 1629, 1618, 1451, 1283 cm^{-1}; ^1H nmr (deuteriochloroform, 100 MHz): δ 1.65 (s, 6H, CH_3), 4.78 (b, 1H, 3-NH), 6.47 (d, 1H, 4-H, J = 8.0 Hz), 7.68 (m, 4H, 5-, 8-, 9-H and 1-NH), 8.24 (m, 2H, 7- and 10-H).

Anal. Calcd. for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.47; H; 5.25; N, 9.83.

2-Methyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (**4a**).

To a mixture of 1,2-diaminoanthraquinone (**1b**) (1.2 g, 5 mmoles) and acetylacetone (0.9 g, 5.8 mmoles) in ethanol (60 ml) was added ethanolic hydrochloride solution (2.4 *N*, 3 ml), then heated under reflux for 2 hours. The reaction mixture was poured into water (200 ml) and neutralized with sodium carbonate solution. The resulting precipitate was collected by filtration and purified by column chromatography. The crude product was recrystallized from methanol, and further purified by recrystallization from THF to give **4a** (0.56 g, 43%) as light brown

scales, mp 307° dec; ^{1}H nmr (DMSO-d_6, 300 MHz, DMSO peak at δ 2.48 was taking as the reference): δ 2.62 (s, 3H, CH_3), 7.91 (m, 2H, 8- and 9-H), 7.93 (d, 1H, 4-H, J = 8.4 Hz), 7.99 (d, 1H, 5-H, J = 8.4 Hz), 8.20 (m, 2H, 7- and 10-H), 13.09 (s, 1H, NH); ir (nujol): 1660, 1575, 1520, 1325, 1290, 1150, 1065, 1050, 970, 855, 845, 720 cm^{-1}; ms: m/z 262 (M$^+$, 100), 234 (26), 205 (12),164 (30),138 (16), 117 (10), 76 (25), 62 (10), 50 (13).

Anal. Calcd. for $C_{16}H_{10}N_2O_2$: C, 73.27; H, 3.84; N, 10.68. Found: C, 73.62; H, 4.05; N, 10.47.

2-Chloromethyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (**4b**).

To a solution of 1,2-diaminoanthraquinone (**1b**, 2.8 g, 12 mmoles) in DMF (30 ml) was added chloroacetyl chloride (2.8 g, 25 mmoles) dropwise with stirring. The reaction mixture was then heated on a steam bath for 2 hours, cooled, and poured into ice water (100 ml). The precipitate was collected on a filter, washed with water and dried in an oven. The crude product was purified by column chromatography, chromatotrone and recrystallization twice from chloroform-THF (1:1) to give **4b** (1.4 g, 39%) as yellow-brown needles, mp 227-230° dec; ^{1}H nmr (deuteriochloroform, 300 MHz): δ 4.93 (s, 2H, CH_2Cl), 7.82 (m, 2H, 8- and 9-H), 8.10 (d, 1H, 4-H, J = 8.6 Hz), 8.24 (d, 1H, 5-H, J = 8.6 Hz), 8.33 (m, 2H, 7- and 10-H), 11.23 (s, 1H, 1-NH); ir (nujol): 3320, 3304,1658, 1646, 1578, 1559,1521, 1326, 1299, 1280, 1160, 1000, 862, 840, 715, 660, 640 cm^{-1}; ms: m/z 298 (12), 296 (M$^+$, 43), 262 (17), 261 (100), 233 (10), 177 (11), 164 (20), 151 (19), 102 (10), 77 (10), 76 (23), 75 (14), 50 (10).

Anal. Calcd. for $C_{16}H_9N_2O_2Cl$: C, 64.77; H, 3.06; N, 9.44. Found: C, 64.98; H, 3.17; N, 9.10.

2-[(2-Hydroxyethyl)amino]methyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (**4c**).

Ethanolamine (1 g, 1.6 mmoles) and pyridine (2 ml) was added to mixture of compound **4b** (0.5 g, 0.17 mmole) in benzene (150 ml) with stirring. DMF was then added dropwise until the solution become clear and the reaction was heated under reflux. After 3 hours, the mixture was cooled and the yellow crystals were collected, washed with methanol and dried to give **4c** (0.45 g, 83%), mp 193-195° dec; ^{1}H nmr (DMSO-d_6, 300 MHz, DMSO peak at δ 2.48 was taking as the reference): δ 2.60 (t, 2H, J = 5.6 Hz, NCH_2), 3.35 (b, 2H, NH and OH, deuterium oxide exchangeable), 3.47 (t, 2H, J = 5.6 Hz, CH_2O), 4.03 (s, 2H, $ArCH_2$), 7.92 (m, 2H, 8- and 9-H), 8.03 (s, 2H, 4- and 5-H), 8.20 (m, 2H, 7- and 10-H), 11.23 (s, 1H, NH); ir (nujol): 3410, 1662, 1326, 1296, 1152, 1045, 844, 718, 478 cm^{-1}; ms: m/z 322 (M$^+$ +1, 10), 291 (21), 290 (22), 263 (18), 262 (100), 261 (91), 205 (11), 164 (17), 151 (12).

Anal. Calcd. for $C_{18}H_{15}N_3O_3$: C, 67.28; H, 4.70; N, 13.08. Found: C, 66.97; H, 4.61; N, 12.87.

Naphtho[2,3-*f*]quinoxaline-7,12-dione (**5**).

Compound **5** was prepared according to the method described by Ohta, *et al.* [9]. The resulting product was purified by repeated chromatography and recrystallized twice with chloroform-methanol (1:1) to give **5** (54%) as orange needles, mp 236-237° (lit [9] 241-242°, red-violet needles from 1,2-dichloroethane, lit [10] 277-278°, from chlorobenzene). The nmr, ir and ms spectra supported the structure of **5**; ^{1}H nmr (deuteriochloroform, 300 MHz): δ 7.83 (m, 2H, 9- and 10-H), 8.32 (m, 2H, 8- and 11-H), 8.49 (d, 1H, 5-H, J = 8.8 Hz), 8.72 (d, 1H, 6-H, J = 8.8 Hz), 9.00 (d, 1H, 3-H, J = 1.7 Hz), 9.25 (d, 1H, 2-H, J = 1.7 Hz); ir (nujol): 1669, 1586, 1324, 1310, 1292, 1270, 1154, 986, 917, 854, 799, 714, 475 cm^{-1}; ms: m/z: 261 (22), 260 (M$^+$, 100), 233 (49), 232 (52), 204 (50), 203 (23), 178 (35), 177 (23), 150 (77), 149 (20), 102 (19), 76 (45), 7S (51), 74 (56), 50 (34).

Anal. Calcd. for $C_{16}H_8N_2O_2$: C, 73.84; H, 3.10; N, 10.76. Found: C, 74.14; H, 3.15; N, 10.34.

6-(2-Aminoethylamino)-1,2,3,4-tetrahydronaphtho[2,3-*f*]quinoxaline-7,12-dione (**8a**).

Compound **8a** was prepared according to the method described by Greenhalgh and Hughes [11]. The melting point and nmr support the structure of **8a**, mp 215-217°; ^{1}H nmr (deuteriochloroform, 100 MHz): δ 1.39 (b, 2H, NH_2), 2.82 (m, 2H, -CH_2-), 3.34 (m, 6H, -CH_2-), 6.25 (s, 1H, 5-H), 7.14 (b, 1H, NH), 7.67 (m, 2H, 9- and 10-H), 8.13 (m, 2H, 8- and 11-H), 10.81 (b, 1H, Ar-NH), 11.20 (b, 1H, Ar-NH).

1,2,3,4-Tetrahydro-8,11-dihydroxy-4-(2-hydroxyethyl)-6-{[2-[(2-hydroxyethyl)amino]ethyl]amino}naphtho[2,3-*f*]quinoxaline-7,12-dione (**8b**).

To a mixture of mitoxantrone (**6b**, 3 g, 5.8 mmoles) in methanol (300 ml) was added methanolic potassium hydroxide solution (10%, 2 ml) with stirring. The reaction mixture was refluxed for 5 hours and was then evaporated under reduced pressure to dryness. The resulting product was recrystallized in acetone-DMF to afford **8b** as dark blue powder (2.5 g, 85%). The melting point, nmr, and ir were consistent with the report [1], mp 181-183°; ^{1}H nmr (Acetone-d_6, 300 MHz, acetone peak at δ 2.03 was taking as the reference): δ 3.01 (t, 2H, CH_2N, J = 6.44 Hz), 3.53 (m, 2H, CH_2N), 3.75 (m, 8H, $ArNCH_2$), 3.83 (t, 2H, CH_2O, J = 6.44 Hz), 3.94 (m, 2H, CH_2O), 6.29 (s, 1H, s, 5-H), 6.97 (d, 1H, 9- or 10-H, J = 8.79 Hz), 7.01 (d, 1H, 9- or 10-H, J = 8.79 Hz), 11.02 (b, 1H, ArNH), 11.30 (b, 1H, ArNH), 13.42 (s, 1H, ArOH), 14.32 (s, 1H, ArOH); ir (potassium bromide): 3376, 1624, 1594, 1549,1520, 1451, 1409, 1382, 1330, 1223, 1206, 1163, 1122, 1052, 973, 952, 873, 819, 715 cm^{-1}.

Anal. Calcd. for $C_{22}H_{26}N_4O_6 \cdot 0.7H_2O$: C, 58.06; H, 6.07; N, 12.31. Found: C, 58.17; H, 5.87; N, 12.43.

6-Hydroxy-1,2,3,4-tetrahydroxynaphtho[2,3-*f*]quinoxaline-7,12-dione (**8c**).

Compound **8c** was prepared according to the method described by Matsuoka *et al.* [17]. The melting point and nmr spectrum support the structure of **8c**, mp 273-275°; ^{1}H nmr (DMSO-d_6,100 MHz): δ 3.50 (s, 4H, CH_2), 6.06 (s, 1H, H-5), 7.71 (m, 2H, 9- and 10-H), 7.95 (m, 1H, 4-NH), 8.12 (m, 2H, 8-and 11-H), 10.50 (b, 1H, NH), 15.41 (s, 1H, OH).

Anticancer Activity Tests.

In vivo P388 Assay: The average mass of CDF_1 mice on the day of tumor implantation (day 0) was 20 ±2 g. This test was conducted according to the previously reported method using murine leukemia cells [18]. Mitoxantrone **6b** was used as a positive control.

Anticancer assay *in vitro* (a modified MTT method): Cell lines used in this test included human oral epidermoid carcinoma (KB), laryngeal epidermoid carcinoma (Hep-2), adenocarcinoma of the colon (CoLo 205) and human cervical uteri carcinoma (Hela). Cancer cells in an exponential phase were trypsinized, disaggregated and counted with a homocytometer. Cells (3 x 10^3) were inoculated into each well of a 96-well culture plate in RPMI-1640 (0.18 ml) medium supplemented with

fetal bovine serum (5%), glutamine (1 nM), penicillin and streptomycin. The drug (0.02 ml) was added and cells were cultured 4 days at 37° in a carbon dioxide atmosphere (5%). To each well 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.1 mg) was added and the plates were incubated for 4 hours. The medium was removed, DMSO (0.2 ml) was added to each well and the plates were agitated for 10 minutes. The optical density of each well was measured at 545 nm with a 690 nm reference wavelength using a Titertek Multiskan plate reader. Absorbance readings of drug treated cells were compared with untreated cell absorbance values.

Acknowledgments.

We thank the National Science Council, R. O. C., for research grants (No. NSC 83-0412-B016-104 & NSC 84-2331-B016-019 to PC).

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