Structural Modification Study of Anthracyclinones: Synthesis and Biological Activity of Several Derivatives of η-Pyrromycinone

Dun-Fu Liu,[1,2] Hui Xiong,[1] and Chia-Chung Cheng[2,3]

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On the basis of the N–O–O triangular pharmacophore hypothesis postulated earlier in our laboratory, selected side chains with or without the nitrogen atom at the strategic position were incorporated to η-pyrromycinone, one of the anthracyclinones derived from the antibiotic cinerubins. Since none of the anthracyclinones (the aglycones of anthracyclines) were reported to have antineoplastic activity, the validity of the proposed hypothesis could be tested. Results indicated that a compound designed in this manner, 1,4-bis[2-(2,2-dimethyloxazolidin-3-yl)ethylamino]-1,4-didehydroxy-η-pyrromycinone (**9c**) possessed both *in vitro* and *in vivo* antineoplastic activity comparable to that of mitoxantrone. The structure–activity relationship of this class of compounds is discussed.

KEY WORDS: N–O–O triangular pharmacophore; anthracyclinones; anthracyclines; antineoplastic activity; structure–activity relationship.

INTRODUCTION

Since the discovery of clinical effectiveness and cardiotoxicity of the anthracycline anticancer drugs daunomycin (daunorubicin) and adriamycin (doxorubicin), numerous investigators have searched for more effective and less toxic congeners. However, cardiotoxicity still remains as a serious side effect among the existing and newer anthracyclines. The glycosides present in these anthracyclines may be responsible for the unwanted cardiac action in analogy to the case of cardiotonic digitalis (1), but most anthracyclinones were found to possess little, if any, antineoplastic activity. Since the majority of glycosides contain an amino group at a distinct position, in accord with a working hypothesis developed in our laboratory earlier (2,3), elimination of the amino group in the sugar moiety may also void the antineoplastic activity. Certain de-L-nogalose analogues and derivatives of the antineoplastic antibiotic nogalamycin (**1**), such as nogarol (**2a**) and menogaril (7-con-*O*-methylnogarol, 7-OMEN; **2b**), not only retained the activity but are actually more potent than the original anthracycline (4–6) (Scheme I). This may be due to the fact that L-nogalose is not an amino-containing sugar and the portion which contains the pertinent nitrogen atom is located at the dimethylamino-oxocin moiety; thus, the proposed N–O–O triangular pharmacophore (3) remains intact. This concept also led to the development of the potent anticancer drug mitoxantrone (DHAQ; 3) from our laboratory (7,8).

In order to substantiate further the usefulness of the N–O–O triangulation hypothesis, we decided to attach selected N-containing side chains to the "biologically inert" anthracyclinones and examine the antineoplastic activity of the resulting derivatives. In this regard, η-pyrromycinone (**5**) was selected as the starting chemical for our study. η-Pyrromycinone (**5**) is an aromatized anthracyclinone originally isolated in 1959 from a *Streptomyces* strain (9–11) along with ε-pyrromycinone (**4**). We obtained an operative quantity of **5** from a *Streptomyces* sp. SIIA 8593 strain through **4** at our institute.

MATERIALS AND METHODS

The anthracycline cinerubins (11–13), isolated from the fermentation broth of *Streptomyces* sp. SIIA 8593 at our institute, were hydrolyzed in dilute hydrochloric acid to yield ε-pyrromycinone (10,11) (**4**) (Scheme II). Treatment of **4** with hydrobromic acid in acetic acid gave the dark red dehydrated derivative η-pyrromycinone (9) (**5**). The latter was reduced by sodium hydrosulfite to give the corresponding yellow *leuco*-η-pyrromycinone (**6**). Without excessive purification, compound **6** was condensed with several primary amines to form either the diimino or the monoimino derivative, **7** or **8**, respectively. Conditions for the production of the di- and the mono derivatives would depend on the temperature, the reaction time, and the amount of amine used during the condensation. The structure of the monoimino-substituted compound **8** was assigned on the basis of an analogous reaction reported earlier by Morris *et al.* (14). Compounds **7** and **8** can then be oxidized to the corresponding quinones **9** and **10**, respectively, by a number of oxidizing agents such as air or chloroanil, as in the case of DHAQ (mitoxantrone) preparation (7,8). We have now found that manganese dioxide can be used to serve as an excellent oxidizing agent for our purpose.

The amino-substituted side chains used in **9** and **10** consist of the benzylamino (series **a**) and the 2-(diethylamino)ethylamino (series **b**) groups. Substitution on **9** with the "DHAQ side chain," the 2-[(2-hydroxyethyl)aminoethyl]amino group, was also attempted. The resulting derivative, however, was difficult to purify. Treatment of the crude product with acetone yielded a dimethyloxazolidinyl derivative, **9c**, as black crystalline needles. For comparison, the corresponding DHAQ–acetone condensation product **11** was also prepared and its cytotoxicity was evaluated along with the parent compound.

EXPERIMENTAL

Melting points were determined on a PHMK microscope melting-point apparatus. The ultraviolet data were recorded on a Philips Pye Unican PU 8800 uv/vis spectrophotometer. The elemental analysis values were determined by an Italy Carlo Erba 1106 analyzer. The mass spectra were determined with a Hitachi 900 mass spectrophotometer.

[1] Sichuan Industrial Institute of Antibiotics, Chengdu 610051, China.

[2] Drug Development Laboratory, University of Kansas Cancer Center, and Department of Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, Kansas 66103.

[3] To whom correspondence should be addressed.

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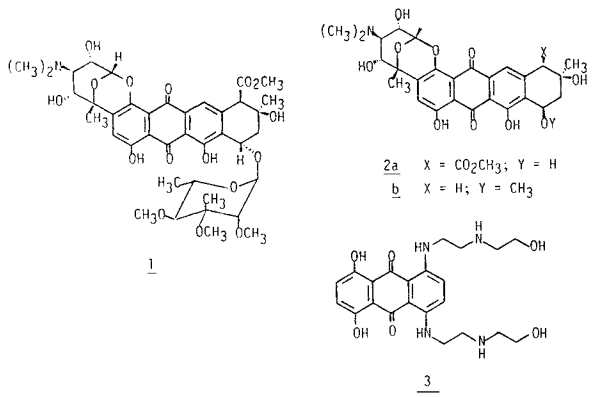
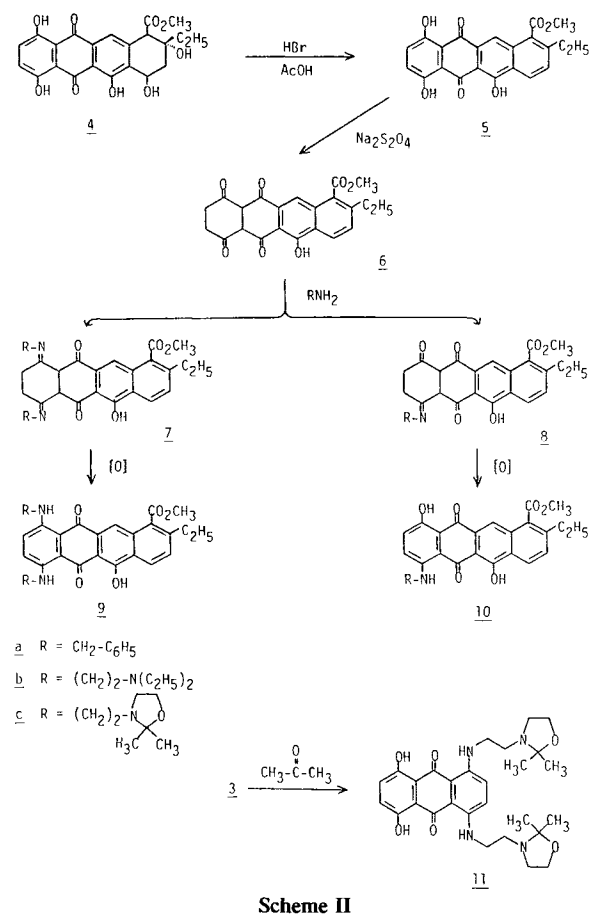


Scheme I

ϵ-Pyrromycinone (9–11) (4). The mycelium portion of the fermentation broth of *Streptomyces* sp. SIIA 8593 was extracted with acetone. The extract, which contained the cinerubins (10–13), was concentrated and acidified with dilute hydrochloric acid, the oily substance was separated, and the aqueous portion was neutralized with dilute sodium hydroxide and extracted with ethyl acetate. The extract was concentrated and hydrolyzed with dilute hydrochloric acid to give crude **4**. About 4.8–11.7 g of crude **4** could be isolated from 100 liters of the fermentation broth. Purification of **4** was carried out first through silica gel column chromatography followed by rotating chromatotron on silica gel G plates eluted with a mixture of toluene–methanol–benzene (6:0.4:1). The solvent was evaporated under reduced pressure and the residue recrystallized from benzene to give orange needles, m.p. 196–197°C.



Scheme II

η-Pyrromycinone (9–11) (5). This compound was prepared by the reported procedure (9–11) from a mixture of hydrobromic acid and acetic acid as dark red needles, m.p. 243.5–244.5°C.

Methyl 4-Benzylimino-9-ethyl-1,2,3,4,4a,12a-hexahydro-6-hydroxy-1,5,12-naphthacenetrione-10-carboxylate (8a). A mixture of 1.02 g of **5**, 3.12 g of sodium hydrosulfite, 3 ml of benzylamine, 100 ml of ethanol, and 0.5 ml of water was placed in a 200-ml round-bottom flask. Under nitrogen, the mixture was heated at 50°C with stirring for 5 hr. The reaction mixture was cooled and the solid product collected by filtration, washed with ethanol and ether, and dried. The crude product was pulverized and the impurities were removed successively with benzene–ether (26:1) and methylene chloride over a silica gel column (4 × 80 cm). The desired product was eluted with benzene–methanol (10:1). The yellow eluate was evaporated and the residue chromatographed again with hexane–tetrahydrofuran–chloroform (4:1:2). The eluate was evaporated and the yellow solid recrystallized from benzene–hexane to give 0.35 g (28% yield) of **8a** as yellow rods, m.p. 124–125°C. *Anal.* ($C_{29}H_{25}NO_6$). Calcd.: C, 72.04; H, 5.21; N, 2.89. Found: C, 72.18; H, 5.00; N, 2.80. UV: λ_{max} ($CHCl_3$) 270, 290 (sh), 315 (sh), 450, and 482 nm. Mass spec., *m/e* 483 (M^+).

4-Benzylamino-4-dehydroxy-η-pyrromycinone (10a). To 50 ml of chloroform containing 0.2 g of **8a** was added 2 g of chloroanil. The mixture was stirred at 50°C for 10 hr. It was then cooled and filtered. The filtrate was concentrated and eluted through a column with benzene initially, then with benzene–ether (3:5). The purple eluate was evaporated to dryness and crystallized from benzene to give 50 mg (25% yield) of **10a** as purple silky crystals, m.p. 246.7–247.8°C. *Anal.* ($C_{29}H_{23}NO_6$). Calcd.: C, 72.34; H, 4.81; N, 2.91. Found: C, 72.34; H, 4.81; N, 2.86. UV λ_{max} ($CHCl_3$) 255, 282, 559, and 592 nm. Mass spec., *m/e* 481 (M^+).

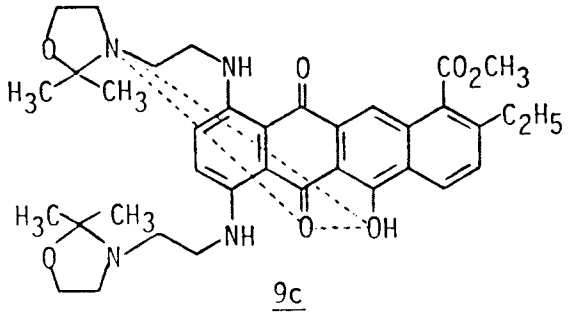
1,4-Bisbenzylamino-1,4-didehydroxy-η-pyrromycinone (9a). A mixture of 0.52 g of **5**, 2.6 g of sodium hydrosulfite, 3 ml of benzylamine, and 150 ml of ethanol was heated with stirring at 50–60°C under nitrogen for 8 hr. To the cooled reaction mixture was added an additional 1 ml of benzylamine and heating was continued at 75°C for 3 hr. Thin-layer chromatography indicated that the major product was the disubstituted derivative. The reaction mixture was evaporated to dryness and the residue was triturated with chloroform. To the chloroform solution was added chloroanil for the conversion of **7a** to **9a**. The resulting product was chromatographed through a silica gel column (4 × 30 cm) initially using hexane–ethylacetate–chloroform (5:1:2) as the eluant. It was chromatographed again by eluting with hexane–ether–chloroform (10:10:1). The yellow-colored impurity was removed and the blue zone was washed down with benzene. The product was further purified through a silica gel column with benzene–ether (6:1). The eluate was evaporated and the residue crystallized from benzene to give 94 mg (12% yield) of **9a** as blue silky crystals, m.p. 230.4°C. *Anal.* ($C_{36}H_{30}N_2O_5$). Calcd.: C, 75.77; H, 5.30; N, 4.91. Found: C, 75.89; H, 5.44; N, 4.83. UV: λ_{max} ($CHCl_3$) 255, 282, 599, and 647 nm. Mass spec., *m/e* 570 (M^+).

4-[(2-Diethylamino)ethylamino]-4-dehydroxy-η-pyrromycinone (10b). A mixture of 0.36 g of **5**, 1 g of sodium hydrosulfite, 0.8 ml of 2-(diethylamino)ethylamine, and 50 ml of 90% ethanol was heated with stirring at 40°C under nitrogen for 40 min. The reaction mixture was evaporated under reduced pressure and the resulting black solid was dissolved in a mixture of ethanol and benzene. To the solution was added 4 g of manganese dioxide. The mixture was stirred at room temperature for 30 min and filtered. The filtrate was evaporated to dryness under reduced pressure and the residue purified through a silica gel column using benzene as the eluant. The eluted solvent was evaporated and the residue was column chromatographed with toluene–methanol–chloroform (5:1:1). The eluate was evaporated and the residue crystallized from benzene–hexane to give 90 mg (20% yield) of **10b** as black needles, m.p. 190.5°C. *Anal.* ($C_{28}H_{30}N_2O_6$). Calcd.: C, 68.55; H, 6.16; N, 5.71. Found: C, 68.44; H, 6.17; N, 5.59. UV: λ_{max} ($CHCl_3$) 254, 272, 569, and 608 nm. Mass spec. 490 (M^+).

1,4-Bis[(2-diethylamino)ethylamino]-1,4-didehydroxy-η-pyrromycinone (9b). A mixture of 0.27 g of **5**, 0.46 g of sodium hydrosulfite, 1 ml of 2-(diethylamino)ethylamine, and 20 ml of 90% ethanol was heated with stirring at 55°C under nitrogen for 1.5 hr. The reaction mixture was evaporated, dissolved in chloroform, and oxidized with manganese dioxide as described previously. Purification was effected with silica gel plates and developed in a mixture of methanol–toluene–chloroform (5:2:1), and the desired colored zone collected. This was chromatographed with the above-mentioned eluants followed by a rapid washing with the addition of 20% (by volume) chloroform–methanol (20:1) saturated with ammonia. The solution was evaporated and the residue crystallized from ether and a small amount of petroleum ether (b.p. 30–60°C) to give 80 mg (20% yield) of **9b** as black needles, m.p. 131°C. *Anal.* ($C_{34}H_{44}N_4O_5$). Calcd.: C, 69.36; H, 7.53; N, 9.51. Found: C, 69.73; H, 7.59; N, 9.47. UV: λ_{max} ($CHCl_3$) 258, 290, 605, and 655 nm. Mass spec., *m/e* 588 (M^+).

1,4-Bis[2-(2,2-dimethyloxazolidin-3-yl)ethylamino]-1,4-didehydroxy-η-pyrromycinone (9c). A mixture of 0.70 g of **5**, 2.6 g of sodium hydrosulfite, 1.5 ml of 2-[(2-hydroxyethyl)aminoethyl]amine, and 85 ml of ethanol was heated with stirring at 50°C under nitrogen for 6 hr. The reaction mixture was evaporated under reduced pressure to dryness and the residue was dissolved in 100 ml of acetone. To the solution was added 4 g of manganese dioxide and the mixture was stirred for 30 min. After filtration, the volume of the filtrate was reduced to about 10 ml, and after standing overnight, the solid which had separated was crystallized from ethanol to give 210 mg (18% yield) of **9c** as black needles, m.p. 176–177°C. *Anal.* ($C_{36}H_{44}N_4O_7$): Calcd.: C, 67.06; H, 6.87; N, 8.68. Found: C, 66.75; H, 6.83; N, 8.53. UV: λ_{max} (CH_3OH) 203, 253, 285, 560 (sh), 600, and 649 nm. Mass spec., *m/e* 644 (M^+).

1,4-Bis[2-(2,2-dimethyloxazolidin-3-yl)]ethylamino-5,8-dihydroxy-9,10-anthracenedione (11). A mixture of 0.51 g of DHAQ (**7**) and 50 ml of acetone was stirred at room temperature for 3 days. The solvent was evaporated and the residue crystallized from 2-propanol to give 0.5 g (83% yield) of a solid. An additional recrystallization from acetone gave **11** as black crystals, m.p. 196–198°C. *Anal.* ($C_{28}H_{36}N_4O_6$): Calcd.: C, 64.10; H, 6.91; N, 10.67. Found: C, 63.63; H, 6.65; N, 10.64. UV: λ_{max} ($CHCl_3$) 240, 280, 580 (sh), 626, and 682 nm. Mass spec., *m/e* 525 ($M^+ + 1$).



9c

Scheme III

RESULTS AND DISCUSSION

The inhibitory effects of the aforementioned anthracyclinone derivatives on the incorporation of ^{3}H-thymidine into DNA of leukemia P388 tumor cells *in vitro* were studied (15). At concentrations of 10 and 100 μg/ml, the respective percentages of inhibition were found to be as follows: Compound **5** (0, 0%), **9a** (0, 0%), **10a** (0, 0%), **9b** (71, 94%), **10b** (0, 44%), **9c** (82, 97%), **11** (80, 97%), and DHAQ (95, 97%).

Information from the above *in vitro* experiments indicated that (a) DHAQ (**3**) and its corresponding oxazolidine derivative **11** possessed similar inhibitory activity; (b) compounds which do not contain the postulated N–O–O triangular pharmacophore (**3**), such as **5**, **9a**, and **10a**, did not exhibit inhibitory activity *in vitro;* (c) compound **10b**, which contains an incomplete N–O–O triangle, possessed some inhibitory activity only at the higher concentration; and (d) compound **9c**, which contains a complete N–O–O triangular structural feature (Scheme III), exhibited *in vitro* inhibitory activity as good as that of DHAQ (mitoxantrone). The interesting *in vitro* activity displayed by **9c** prompted its antineoplastic evaluation *in vivo,* and the following experiments were carried out. For the purpose of comparison, DHAQ was simultaneously tested in every study.

Compound **9c** (as its hydrochloride salt) was evaluated against sarcoma S180, leukemia L1210, and Ehrlich ascites systems in mice following conventional protocols (16–18). The percentage of inhibition against S180 at 500, 250, and 125 μg/ml was 100, 97, and 70%, respectively (for DHAQ, 100% inhibition was observed at 250 μg/ml). Against L1210 in DBA/2 mice, the test/control (T/C) ratio of mean survival time for **9c** at 8 mg/kg (ip × 5) was 171 (for DHAQ, T/C of 177 was observed at 1 mg/kg, ip × 5). Against Ehrlich ascites, the T/C ratio of mean survival time for **9c** at 2 mg/kg (ip × 8) was 275 (for DHAQ, T/C of 325 was observed at 1 mg/kg, ip × 4). It thus appears that the inhibitory activity of compound **9c**, although slightly inferior, is comparable to that of DHAQ.

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