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NEW HYDROXY-AMIDO-ANTHRAQUINONES AS POTENTIAL ANTINEOPLASTIC DRUGS.

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Abstract. A series of new dihydroxy- and trihydroxy- 9,10-anthracenedione derivatives having N,N-diethylaminopropionamido or N,N-diethylaminoacetamido side chains have been synthesized. The propionamido derivatives inhibit very efficiently tumor cell growth and deserve further testing as potential anticancer drugs.

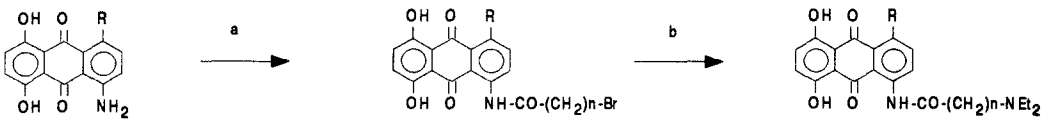
The anthracenedione pharmacophore is very important in cancer chemotherapy. In fact it belongs to anthracyclines whose outstanding antineoplastic activity is very well documented [1]. Even if they still represent the drug of choice for a number of liquid and solid tumors, their use is somewhat limited by the concurrent development of undesired side effects such as bone-marrow depression and cardiac toxicity. In addition resistance phenomena usually appear following repeated treatment with anthracyclines.

To approach a solution for such problems, synthetic anthracenediones were developed, among which Mitoxantrone is clinically used today. Notwithstanding its usefulness, it still exhibits some of the above limitations, including partial cross-resistance with anthracyclines [2,3].

We and others have recently developed a related class of anthracenediones, with an amido moiety replacing the amino group linking the side chain to the planar tricyclic system [4-9]. The new compounds exhibited interesting activity in vitro and some of them were also marginally effective in vivo [10]. Besides the presence of two positively charged side chains, the active derivatives were characterized by 1,8 dihydroxy substitution. Toghether with the nature of the side chain, number and position of OH groups could also play a relevant role in modulating drug activity [11]. Thus we deemed it useful to extend our investigation to amido-anthraquinones having hydroxy substituents at different positions of the fused ring system. In particular in the present paper we report on the synthesis and preliminary biological properties of a number of new 1,4-dihydroxy-5,8-dialkyamido- or 1,4,5-trihydroxy-8-alkylamido- anthracenediones, having N,N-diethylaminopropionamido or N,N-diethylaminoacetamido substituents at position 5,8 or 8 of the anthraquinone structure (Scheme 1).

The new derivatives allow a direct structural comparison with anthracyclines and Mitoxantrone. In addition, the number and length of side chains substituents were varied to gain information on the requirements for activity in this series of compounds.

Scheme 1



1 R = OH
2 R = NH_2

3 n = 1, R = OH
4 n = 2, R = OH
5 n = 1, R = NH-CO-$(CH_2)_n$-Br
6 n = 2, R = NH-CO-$(CH_2)_n$-Br

7 n = 1, R = OH
8 n = 2, R = OH
9 n = 1, R = NH-CO-$(CH_2)_n$-NEt_2
10 n = 2, R = NH-CO-$(CH_2)_n$-NEt_2

a) Br-$(CH_2)_n$-COCl, Et_3N, Toluene. b) Et_2NH, Ethanol

The synthesis of the final compounds **7** - **10** was carried out in two steps starting from the aminohydroxyanthracenediones **1** and **2** previously reported[12] Treatment of the latter compounds with the corresponding bromoacyl chloride in the presence of triethylamine in toluene for 1-2 h at 80 °C led to the bromoacyl derivatives **3** - **6** in 70-84 % yield. Subsequent substitution on these bromo derivatives with diethylamine in ethanol by heating 15 min. at reflux provided the desired diethylaminoalkylamido derivatives **7** - **10**[13] in 71-82 % yield.

The redox properties of derivatives **7** - **10** were investigated in aqueous solution at physiological pH and salt concentration by cyclic voltammetry measurements. The reduction potentials are reported in Table 1.

Table 1. Redox properties of the test amidoanthracenediones.

Compound	$E_{1/2}$ (V against SCE)
7	-0.67
8	-0.69
9	-0.70
10	-0.67
Daunorubicin[14]	-0.62

All of them occurred at values more negative than those observed for Daunorubicin in similar conditions [14]. Thus the test compounds are less readily reduced as compared to anthracyclines and should therefore exhibit a decreased cardiotoxicity.

Tumor cell growth inhibition properties are reported in Table 2, in which Mitoxantrone is included for comparison .

Table 2. Cell growth inhibition by the test hydroxy-anthracenediones [ID_{50} (μm)]

Compound	Cell line	
	HeLa	HL60
7	26.1±0.7	1.64 ±0.12
8	8.05±0.15	0.11 ±0.01
9	>38	4.90 ±0.71
10	3.14±0.26	0.10 ±0.04
Mitoxantrone	2.77±0.80	0.09 ±0.02

Interestingly, while compounds **8** and **10** exhibit cytotoxic properties very close to Mitoxantrone, compounds **7** and **9** appear to be much less effective in cell growth inhibition. Thus the length of the side chain group plays a major role in biological activity. In fact, shortening the distance of the two nitrogens by just one methylene group generates almost inactive compounds. Interestingly the "inactive" distance corresponds to two carbon atoms between nitrogens which represents the "most active" distance in Mitoxantrone analogues. The change from amino to amido moiety is therefore quite substantial in eliciting drug activity. On the other hand activity appears completely restored upon addition of a methylene group in the amido derivatives The reason for this behaviour possibly rests on the fact that the mechanism of action of anthraquinone derivatives envisages side-chain groups involved in specific interactions with DNA-processing enzymes, in particular Topoisomerase II, in a ternary drug-DNA-enzyme complex[15]. Appropriate recognition in the amido congeners should then occur when three carbon atoms separate the nitrogens in the side chain. The similarity in the results obtained with compounds **8** and **10** suggests that one aminoalkylamido side chain is sufficient to obtain active derivatives. If the same findings hold true for Mitoxantrone, the synthesis and investigation of the corresponding di- or trihydroxy monoalkylaminoethylamino congeners could lead to new interesting drugs.

Preliminary in vivo data on the propionylamido compounds are quite promising and warrant further investigation to fully assess their potential as anticancer agents.

Studies are also in progress on the hydroxyethyl substituted compounds of intermediates **3 - 6**.

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13 Compound **7**: ^{1}H NMR ($CDCl_3$) 13.18, 13.07, 12.90, and 12.37 (4s, 1 + 3 H, (NH), 3(OH)); 9 29 (d, 1H, J=9.6, H7); 7.38(d, 1H, J=9 6, H6); 7.33 (s, 2H, H2 + H3); 3.27 (s, 2H, -CH_2-CO); 2.72 (q, 4H, J=7 1, (-$\underline{CH_2}$-$CH_3)_2$); 1.14 (t, 6H, J=7.1, (-$CH_3)_2$).- ^{1}H NMR ($CDCl_3$) (as the hydrobromide salt): 12.84, 12 71, 12 65 and 12.39 (4s, 1 + 3 H, (NH) + 3(OH)); 8.98 (d, 1H, J=9 3, H7); 7.40 (d, 1H, J=9 3, H6); 7.38 (s, 2H, H2 + H3); 4.09 (s, 2H, -CH_2-CO); 3 47(m, 4H, (-$\underline{CH_2}$-$CH_3)_2$); 1.52 (t,, 6H, J=7.1, (-$CH_3)_2$)

Compound **8**: ^{1}H NMR ($CDCl_3$) (as the hydrobromide salt)· 12.82 and 12 31 (2s, 1 + 3 H, (NH) + 3(OH)); 9.01 (d, 1H, J=9.6, H7); 7.40 (d, 1H, J=9.6, H6); 7.31 (s, 2H, H2 + H3); 3.41 (t, 2H, J=7.1, -CH_2-N); 3.31 (t, 2H, J=7.1, -CH_2-CO); 3.17 (q, 4H, J=7.3, (-$\underline{CH_2}$-$CH_3)_2$); 1 44 (t, 6H, J=7.3, (-$CH_3)_2$).

Compound **9**: ^{1}H NMR (DMSO-d_6) (as the dihydrobromide salt). 12.57 and 11.95 (2s, 2 + 2 H, 2(NH) + 2(OH)); 8.75 (s, 2H H6 +H7); 7 51 (s, 2H, H2 + H3); 4.45 (s, 4H, -CH_2-CO); 3.27 (m, 8H, (-$\underline{CH_2}$-$CHH_3)_4$), 1.26 (t, 12H, J=7.0, (-$CH_3)_4$).

Compound **10**: ^{1}H NMR (DMSO-d_6) (as the dihydrochloride salt) 12.64 and 11.97 (2s, 2 + 2 H, 2(NH) + 2(OH)); 8.90 (s, 2H, H6 + H7); 7.52 (s, 2H, H2 + H3); 3.43 (t, 4H, J=7.1, (-CH_2-N)$_2$), 3.21 (q, 8H, J=7.3, (-$\underline{CH_2}$-$CH_3)_4$), 3.10 (t, 4H, J=7.1, (-CH_2-CO)$_2$); 1.24 (q, 12 H, J=7.3, (-$CH_3)_4$).

An additional broad, exchanging peak, corresponding to the resonance of the proton on the amino nitrogen can be sometimes observed in the range 9.2 - 9.6 ppm.

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