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Esters of 2-(1-Hydroxyalkyl)-1,4-dihydroxy-9,10-anthraquinones with Melphalan as Multifunctional Anticancer Agents

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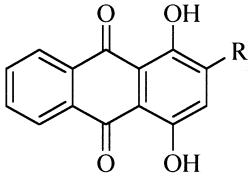
Abstract—Eight esters of 2-(1-hydroxyalkyl)-1,4-dihydroxy-9,10-anthraquinone with melphalan were prepared and tested for their antitumor activity (S-180) and cytotoxicity. 2-{1-[4-(*p*-Bis(2-chloroethyl)-aminophenyl)-butanoyloxy]methyl}-1,4-dihydroxy-9,10-anthraquinone and 2-{1-[4-(*p*-bis(2-chloroethyl)-aminophenyl)-butanoyloxy]ethyl}-1,4-dihydroxy-9,10-anthraquinone showed remarkable antitumor activity (T/C, 265 and 272%).

1,4-Dihydroxy-9,10-anthraquinone (**DHAQ**) is the common structural moiety of anthracycline anticancer agents[1,2] and mitoxanthron.[3] The mechanisms involving DNA intercalation and sequential inhibition of DNA topoisomerase II are well known.[4,5] Anthraquinones generally bind to DNA by insertion and stacking between the base pairs of the DNA double helix.[6] Our recent study showed that derivatives of 2-(1-hydroxyalkyl)-**DHAQ** showed antitumor activity.[7]



R=H; DHAQ
R=1'-hydroxyalkyl; 2- (1-hydroxyalkyl)DHAQ

In contrast, the tumor cell cytotoxicity of melphalan is believed to be the result of DNA alkylation.[8] Similar to other alkylating agents, the dose limiting factor of melphalan is hematologic suppression.[9] Thus, some derivatives of melphalan were synthesized to enhance the selective accumulation to cancer cell or to increase the antitumor activity.[10,11]

In our recent study, esters of 2-(1-hydroxyalkyl)-**DHAQ**s with chlorambucil showed enhanced cytotoxicity against L1210 cells and increased antitumor activity in mice bearing S-180 ascitic cells.[12] The enhanced activity could be attributed to the synergistic action of **DHAQ** and chlorambucil: facilitated transportation of chlorambucil to cell nucleus and subsequent alkylation of the DNA nucleophiles by chlorambucil moiety. These results prompted us to prepare esters of 2-(1-hydroxyalkyl)-**DHAQ**s with melphalan and investigate their antitumor activity (Scheme 1).

Melphalan did not undergo esterification with the 1′-OH of 2-(1-hydroxyalkyl)-**DHAQ** derivatives under the presence of DCC (dicyclohexylcarbodiimide) and DMAP (4-dimethylaminopyridine) due to the presence of the amino group. Therefore, the amino group was formylated to produce formylmelphalan according to the general *N*-formylation method.[13] The **DHAQ** derivative (0.66 mmol) was added to a solution of formylmelphalan (0.55 mmol), DCC (0.605 mmol) and DMAP (0.275 mmol) in CH_2Cl_2 (30 mL) in an ice bath. After stirring for 3 h at room temperature, hexane (30 mL) was added to the reaction mixture. The filtrate was purified over a silica gel column (hexane/ethyl acetate, 3:1). The esters were obtained in 30–50% overall yield. The chemical shift of 1.0 ppm of 1′-H in the ^{1}H NMR spectrum verified the esterification. The multiplet at 3.62 ppm, equivalent to the eight protons of the bis(2-chloroethyl)amino group in the ^{1}H NMR spectra of the synthesized esters confirmed the presence of the chloroethyl moieties of the esters, which are capable of alkylating the DNA nucleophiles. The racemic 2-(1-hydroxyalkyl)-**DHAQ** derivatives were used without resolution for the esterification.

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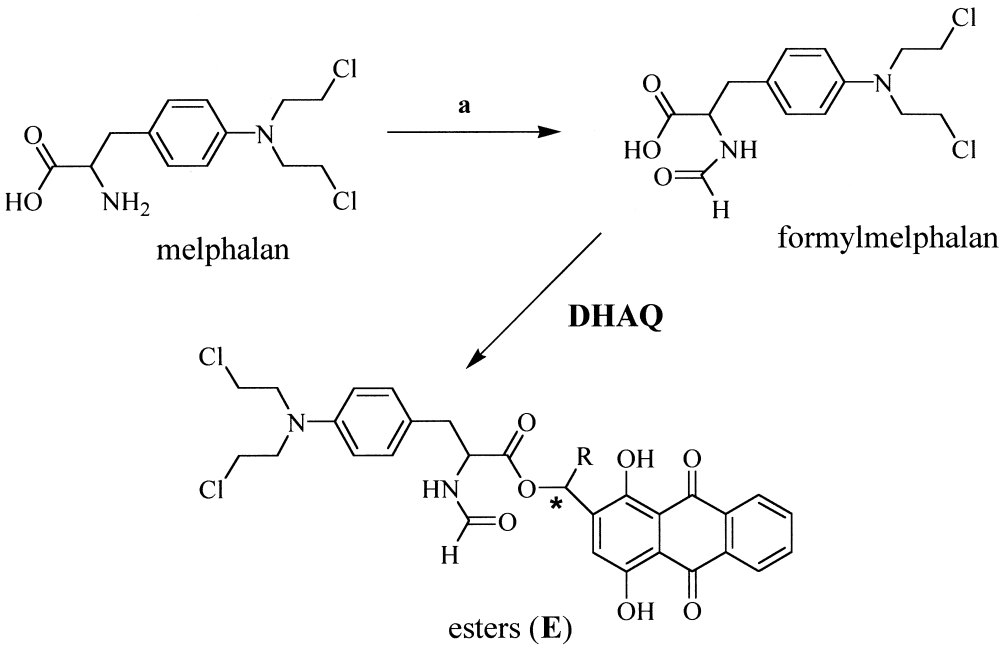

Scheme 1. (a) HCOOH, $CO(COCH_3)_2$; (b) DCC, DMAP, 0 °C.

Table 1. Antitumor effect of 2-{1-[4-(*p*-bis(2-chloroethyl)-aminophenyl)-butanoyloxy]alkyl}-**DHAQ**s[a]

	R	R_1 = mpl			R_1 = H	
		ED_{50} (μg/mL)	T/C[b] (%)	SR[c]	ED_{50}[d] (μg/mL)	T/C[d] (%)
Ea	H	0.086	265	3/8	15	125
Eb	Methyl	0.12	272	6/8	1.9	139
Ec	Ethyl	0.2	245	3/8	7.2	135
Ed	Propyl	0.97	240	3/8	10.2	125
Ee	Butyl	0.4	230	2/8	23.7	110
Ef	Pentyl	3.1	230	1/8	58.0	108
Eg	Hexyl	4.5	210	0/8	60	115
Eh	Heptyl	5.9	205	0/8	>80	103
	Melphalan	0.89	225	3/8		

[a]R = alkyl group; mpl = (*p*-bis(2-chloroethyl)-aminophenyl)-butanoyloxy group of melphalan.
[b]Mice were injected via ip with 1×10^7 S-180 cells on day 0. Compounds were administered ip on days 1, 2, 3, 4, 5, 6 and 7 with a dose of 16 μmol/kg. Melphalan was administered at 16 μmol/kg.
[c]SR = survival ratio, no. of mice that survived after 50 days among eight mice.
[d]The data was adopted from ref 7.

The cytotoxicity of the esters against L1210 cells and the antitumor activity in mice bearing S-180 cells are shown in Table 1. All of the esters showed an enhanced cytotoxic activity when compared to those of 2-(1-hydroxyalkyl)-**DHAQ**s. Furthermore, four of them (**Ea**–**c**,**e**, R′ = H, methyl, ethyl, butyl) showed an even higher cytotoxic activity than melphalan (ED_{50} = 0.89 μg/mL). The derivatives, of which the alkyl chain was H (**Ea**, ED_{50} = 0.086 μg/mL) or methyl (**Eb**, ED_{50} = 0.12 μg/mL), exhibited increased cytotoxic activity by factors of 174 and 16 times that of their 2-(1-hydroxyalkyl)-**DHAQ**s, respectively. The hydrophilicity of the molecule might be one factor responsible for the enhancement of cytotoxicity. As shown in Table 1, the esters with a shorter alkyl chain, expected to offer a relatively higher hydrophilicity, possessed higher cytotoxicity.

Consistent with cytotoxicity, the antitumor activity of the esters tended to be dependent on the size of the alkyl chains. The esters with shorter alkyl chains displayed better antitumor activity than the ones with longer chains. With the exception of **Eg** and **Eh**, the other esters expressed greater antitumor activity than either 2-(1-hydroxyalkyl)-**DHAQ**s or melphalan itself, suggesting that the anthraquinone and melphalan moieties of the esters exerted the antitumor action in a synergistic manner. In other words, in addition to the alkylating capability of the melphalan component, the **DHAQ** moiety should contribute to prolonging the life span of the mice with their own mechanisms. Survival ratio (SR) expressing the number of mice that survived after 50 days among the experimental mice must be important for evaluation of the antitumor activity. Among the esters, **Ea**, **Eb** and **Ec**, all of which showed high T/C values, displayed high SR values. Six of the eight experimental mice survived more than 50 days after administration of **Eb** (R = methyl).

The intercalating capability of the **DHAQ** moiety[4,5] could be expected to speed up alkylation of the melphalan moiety as an intramolecular reaction. The bioreductive alkylation[12] and the oxidative stress[14,15] may be also important action mechanisms for the antitumor activity and were summarized in Figure 1. According to the proposed mechanism of bioreductive alkylation, the ester accepts an electron from the redox system of the cell and converts to an anion radical (**II**), which transforms to a quinone methide (**IV**) and melphalan molecule (**VI**). The reaction **II**→**IV** and **VI** could be accelerated since the melphalan moiety in **II** is a good leaving group. Both **IV** and **VI** are capable of alkylating

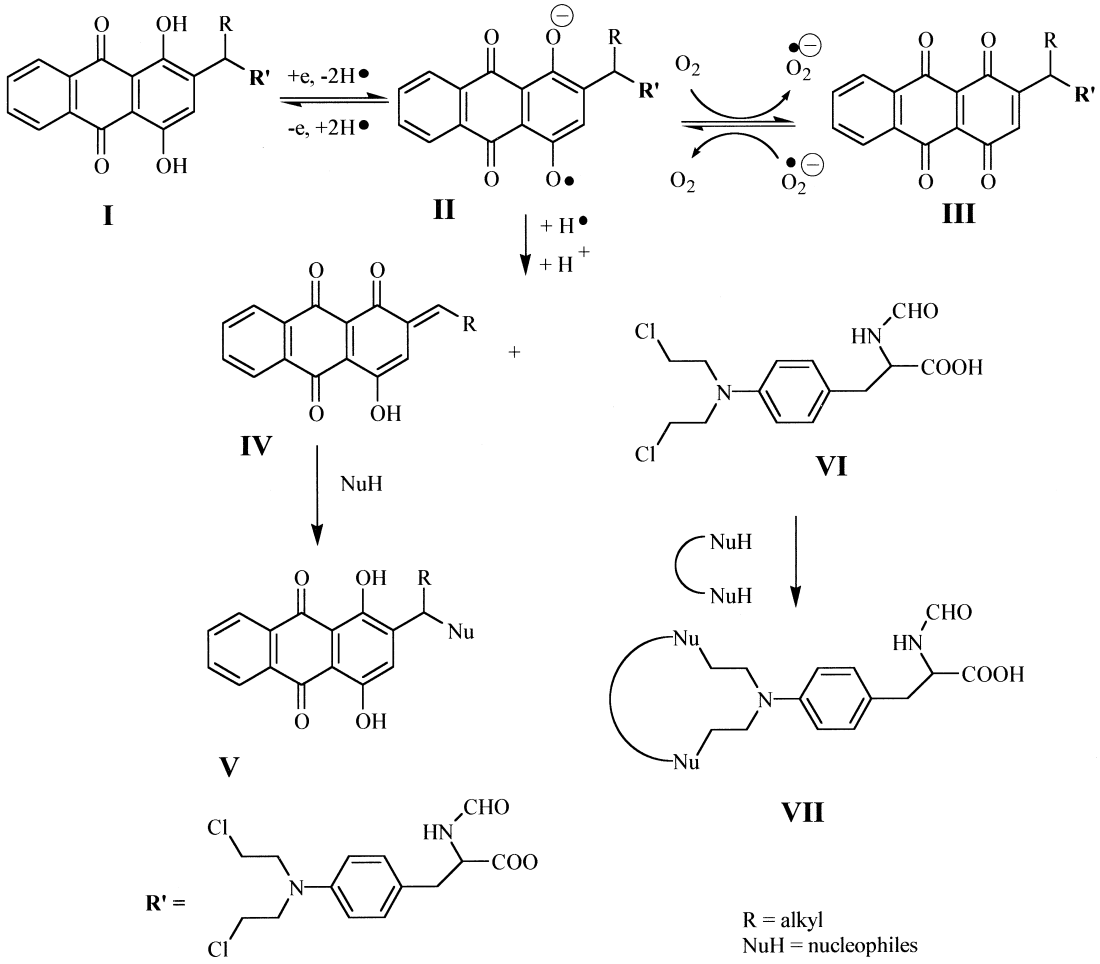

Figure 1. Bioreductive alkylation as a proposed action mechanism of the esters.

nucleophiles in DNA, enzymes, and other molecules.[16,17] Furthermore, the radical anion transforms molecular oxygen to a superoxide, which triggers an oxidative stress.[18]

In conclusion, we report that esters of melphalan with 2-substituted-**DHAQ**s show increased antitumor activity as well as increased cytotoxicity, with both 2-{1-[4-(*p*-bis(2-chloroethyl)-aminophenyl)-butanoyloxy]methyl}-**DHAQ** (**Ea**) and 2-{1-[4-(*p*-bis(2-chloroethyl)-aminophenyl)-butanoyloxy]ethyl}-**DHAQ** (**Eb**) displaying the greatest activity. The results of this study support our belief that **DHAQ** can play a multifunctional role.

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