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Potential bioreductive alkylating agents—VI. Determination of the relationship between oxidation–reduction potential and antineoplastic activity

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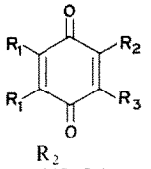
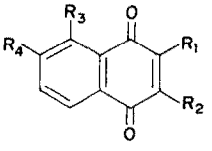
The design, synthesis and antitumor activity of a number of naphthoquinone and benzoquinone derivatives containing a side chain capable of alkylation after reduction have been reported in a series of experiments [1–5]. The naphthoquinone derivatives of this series appear to have a broader antineoplastic spectrum of activity, producing significant prolongation of the survival time of mice bearing either Adenocarcinoma 755 or Sarcoma 180, while benzoquinone derivatives are, in general, only active against the more sensitive neoplasm, Adenocarcinoma 755.

Table 1. Oxidation–reduction potentials and antineoplastic activities of bioreductive alkylating agents

Compound				$E_{1/2}$ (volt)	Antitumor activity*
					
R_1	R_2	R_3			
CH_3	CH_2OAc	CH_2OAc		−0·11	−
OCH_3	CH_2OAc	CH_2OAc		−0·05	−
CH_3	CH_2OAc	H		−0·11	−
OCH_3	CH_2OAc	H		−0·07	−
CH_3	CH_2Cl	CH_2Cl		−0·03	+†
					
R_1	R_2	R_3	R_4		
CH_2Cl	H	H	H	−0·24	+ +
CH_2Br	H	H	H	−0·23	+ +
CH_2OAc	H	H	H	−0·22	+
CH_2Cl	CH_3	H	H	−0·32	+
CH_2Br	CH_3	H	H	−0·31	+ +
CH_2OAc	CH_3	H	H	−0·28	+ +
CH_2Cl	CH_2Cl	H	H	−0·29	+ +
CH_2Br	CH_2Br	H	H	−0·27	+ +
CH_2OAc	CH_2OAc	H	H	−0·25	+ +
CH_2Cl	$NHCC_6H_5$ (O)	H	H	−0·23	−
CH_2Cl	C_6H_5	H	H	−0·28	+ +
CH_2Cl	$-SCH_2CH_3$	H	H	−0·27	+ +
CH_2Cl	$-S-C_6H_5$	H	H	−0·23	+ +
CH_2Br	Br	H	H	−0·24	+ +
CH_2Br	Cl	H	H	−0·25	+ +
CH_2Cl	CH_2Cl	H	CH_3	−0·31	+
CH_2Cl	CH_2Cl	CH_3	H	−0·28	+ +
CH_2Cl	CH_2Cl	Cl	H	−0·26	+ +
CH_2Cl	CH_2Cl	H	Cl	−0·28	+
Mitomycin C				−0·44	+ + +

* The relative potency of the various agents against Sarcoma 180 ascites cells is expressed by symbols −, +, + + and + + +. The + represents a T/C value within the range of 1·5 to 1·9, + + is equivalent to a T/C of 2·0 to 2·5 and + + + a T/C of 3·0; − indicates a compound with a T/C of 1·2 which is considered to be inactive. T/C represents the ratio of the survival time of treated to control animals.

† Unpublished data.

This class of compounds has been hypothesized to require reductive activation *in vivo* [6], in a manner analogous to that described for mitomycin C [7–9]. Thus, an NADPH-dependent reductase enzyme system(s) is envisioned to convert the quinones to their corresponding dihydroquinones which are relatively unstable and decompose to form *o*-quinone methides. These reactive intermediates presumably then act to alkylate DNA and other critical cellular components. Chemical evidence has been obtained to substantiate the formation of an *o*-quinone methide intermediate in the reductive ($NaBH_4$) amination of 2,3-dimethyl-5,6-bis(acetoxymethyl)-1,4-benzoquinone by aniline and morpholine [10].

The hypothesis of bioreductive activation of these materials necessarily requires strict structural properties to allow the generation of an *o*-quinone methide, as well as a redox potential that is compatible with biological activation. Earlier studies [1, 2] on structure–activity relationships demonstrated that essentially all compounds possessing the quinone ring and an appropriate side chain(s) exhibited antitumor activity, whereas compounds with a side chain(s) ultimately capable of alkylation, but without the quinone nucleus or vice versa, were totally devoid of antineoplastic potency. These findings were interpreted to indicate that these molecules were unable to generate the required *o*-quinone methide. In this communication the oxidation–reduction potentials of a variety of derivatives of this class were measured; evidence is presented to indicate that a reasonable correlation exists between the redox potentials of the quinones of this class and their activities as antitumor agents.

The reduced forms (i.e. the dihydroquinones) of the quinones of this class are unstable; therefore, redox potentials were determined by measurement of the half-wave potential ($E_{1/2}$) of the quinones using a polarographic method [11, 12]. A Heath polarographic system (EUW-401) was employed using a saturated calomel electrode as the reference standard. The potential was ascertained in a solvent mixture containing 0·05 M phosphate buffer (pH 7) and isopropyl alcohol (1:1, v/v) plus 0·2 M KCl as the supporting electrolyte. Since the solubility of these quinones in this solvent system is relatively low, a saturated solution of each of these compounds was used for the measurement of $E_{1/2}$. Each solution was saturated with nitrogen for 15 min prior to analysis and maintained under nitrogen during the polarographic determinations.

The antitumor effects of these quinones on Adenocarcinoma 755 and Sarcoma 180 have been reported previously [1–5]. The former tumor line was especially sensitive to the members of this series of compounds (i.e. both benzo- and naphthoquinone derivatives were extremely active), a situation which prevented effective discrimination between the various agents; therefore, the growth inhibitory activities of the optimal levels of these compounds against Sarcoma 180 were used to relate the magnitude of the redox potential to anticancer potency.

The results shown in Table 1 indicate that benzoquinone derivatives possessed higher (less negative) redox potentials than naphthoquinones and in general were inactive against the Sarcoma 180 test system. The oxidation–reduction potentials of all of the naphthoquinones examined fell into a relatively narrow range $\pm 0{\cdot}1$ volt) and the differences between these materials with respect to antitumor activity against Sarcoma 180 ascites cells were only slight. Mitomycin C, which is a more potent inhibitor of the growth of Sarcoma 180 than the benzo- and naphthoquinones of this series [13], possessed a more negative redox potential ($-0{\cdot}44$ volt) than the benzo- or naphthoquinones tested.

The findings indicated that, with this class of molecules, the compounds with the lower (more negative) redox potentials generally possessed the most potent antitumor properties. Exceptions appeared to be 2-benzamido-3-chloromethyl-1,4-naphthoquinone, which was inactive against this neoplasm, yet possessed a redox potential similar to those of the other active naphthoquinones, and 2,3-bis(chloromethyl)-5,6-dimethyl-1,4-benzoquinone which was a weak inhibitor of the growth of Sarcoma 180, even though its redox potential was relatively high. Since antineoplastic efficacy was determined by measurement of the prolongation of the life span of tumor-bearing mice caused by these agents, other pharmacological and biological parameters, in addition to bioreductive activation (i.e. drug uptake, distribution, metabolism and toxicity), obviously contribute to the ultimate expression of cancer inhibitory capabilities. Quinones with higher redox potentials would be expected to be reduced more readily and thereby be more reactive than those with lower potentials. Thus, benzoquinone derivatives may be activated before reaching susceptible cellular target site(s) in neoplastic cells, thereby resulting in more random alkylations. Evidence for an analogous phenomenon was reported for nitrogen mustards containing various halogenated leaving groups [14]. In this instance, chloro or bromo analogs of nitrogen mustards possessed better antitumor activities than fluoro or iodo analogs, a finding that was interpreted as indicating that the chloro and bromo groups possessed relatively optimal leaving properties as compared to fluoro and iodo groups, which were poorer and better leaving groups, respectively, a property influencing the extent to which various biological molecules would be alkylated.

Since it is probable that an optimal redox potential exists for maximum antitumor potency of bioreductive alkylating agents, the findings encourage the design and preparation as antineoplastic agents of new quinones with even lower redox potentials.

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