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Correlation between reduction potentials and inhibitions of Epstein–Barr virus activation by anthraquinone derivatives

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ABSTRACT

As a continuation of studies using natural and synthetic products as cancer chemopreventive agents, we used cyclic voltammetry to examine the reduction–oxidation potentials of methylated emodin derivatives prepared from emodin in phosphate buffer at pH 7.2. A good correlation was found between the inhibitory effects on Epstein–Barr virus early antigen (EBV-EA) activation and the reduction potential of methylated emodin derivatives. Furthermore, there was significant correlation between EBV-EA activation and the reduction potential of 35 anthraquinone derivatives including methylated emodin derivatives. It was further shown that the correlation could be enhanced by including LUMO energy and the number of hydroxy groups as additional parameters.

Naturally occurring quinones are known to be widely distributed in both animals and plants. They function as pigments and as intermediates in cellular respiration and photosynthesis. A number of them possess anti-cancer[1,2] and anti-microbial activities.[3,4] In previous studies, we reported the in vitro anti-tumor promoting activity of mono-, di-, and poly-substituted anthraquinones by determining the inhibitory effects on Epstein–Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells.[5–8] In studies relating the structures and activities of drugs, their standard redox potential is important in determining physiological activity.[9] We employed cyclic voltammetry to determine the standard redox potentials or the first reduction potentials of anthraquinones, bianthraquinones, naphthoquinones, and azaanthraquinones at a physiological pH of 7.2, and found significant correlations between the standard redox or the first reduction potentials and the inhibitory effects ($\log IC_{50}$) on EBV-EA activation.[10–15]

In the present study, we have reported the inhibitory effects on EBV-EA activation of 9 methylated emodin derivatives on EBV-EA activation, and the structure–activity relationships between the inhibitory effects ($\log IC_{50}$) and the first reduction potentials of methylated emodin derivatives and other anthraquinones. Furthermore, we have calculated certain molecular properties of anthraquinones with the CAChe MOPAC program and the PM3 method,[16] and have correlated them with their inhibitory effects on EBV-EA activation. The number of the hydroxy groups, LUMO, HOMO, and steric energies was found to be useful parameters for predicting structure–activity relationships.

Emodin (**1**) and 2,8-dihydroxy-1-methoxy-3-methylanthraquinone (regioisomer of *O*-methylemodin) (**10**) were isolated from the root bark of *Cassia siamea*.[17] 1-*O*-Methylemodin (**2**), 3-*O*-methylemodin (**3**), 8-*O*-methylemodin (**4**), 1,3-di-*O*-methylemodin (**5**), 1,8-di-*O*-methylemodin (**6**), 3,8-di-*O*-methylemodin (**7**), 1,3,8-tri-*O*-methylemodin (**8**), and 2-methyl-3-*O*-methylemodin (**9**) were obtained by methylation of **1**. For the chemical structures of these compounds, see Figure 1.

Although **1** and **2** were previously reported,[14] their inhibitory effects and electrochemical properties have been reexamined in this study for rigorous comparison with those of the other compounds.

Tissue culture reagents TPA and *n*-butyric acid were obtained from Nacalai Tesque (Kyoto, Japan). The EBV-genome-carrying lymphoblastoid cells Raji cells (derived from Burkitt's lymphoma) were cultured in RPMI 1640 medium (Sigma, R8758), as described elsewhere.[18] Spontaneous activation of EBV-EA in our subline of Raji cells was less than 0.1%.

Cyclic voltammetric measurements were performed with a conventional three-electrode system using a laboratory-constructed microcomputer-controlled system, in which a potentiostat (Hokuto Denko, HA-301) controlled the working electrode potential. A plastic-formed-carbon (PFC) electrode with a surface area of

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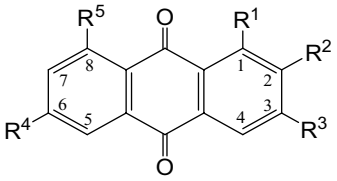

Compound	R^1	R^2	R^3	R^4	R^5	Number of OH
1	OH	H	OH	CH_3	OH	3
2	OCH_3	H	OH	CH_3	OH	2
3	OH	H	OCH_3	CH_3	OH	2
4	OH	H	OH	CH_3	OCH_3	2
5	OCH_3	H	OCH_3	CH_3	OH	1
6	OCH_3	H	OH	CH_3	OCH_3	1
7	OH	H	OCH_3	CH_3	OCH_3	1
8	OCH_3	H	OCH_3	CH_3	OCH_3	0
9	OH	CH_3	OCH_3	CH_3	OH	2
10	OCH_3	OH	CH_3	H	OH	2

Figure 1. Structures of emodin derivatives.

0.071 cm^2 (BAS, PFCE-3), an Ag/AgCl (saturated NaCl) electrode, and a platinum coil electrode were used as the working, reference, and counter electrodes, respectively. Before recording each voltammogram, pretreatment of the working electrode was done, as described.[10,11] Aliquots of a 0.05 mM emodin derivative solution in 2:1 (v/v) 0.1 M phosphate buffer containing 0.1 M KCl (pH 7.2)–ethanol were degassed with purified N_2 gas prior to voltammetric measurements. The electrolytic cell was water-jacketed to maintain temperature at 25 ± 0.1 °C.

The correlation of electrochemical and electronic parameters upon EBV-EA activation of the anthraquinones was determined using Pearson's correlation coefficient.

Ten emodin derivatives were tested for their ability to inhibit in vitro EBV-EA activation induced by TPA in Raji cells; the results are summarized in Table 1. In the previous study, we found that acetylation of the phenolic hydroxy group of emodin (**1**) resulted in a decrease of potency, while oxidized emodin derivatives possessed greater inhibitory ability on EBV-EA activation, as compared to emodin.[15] Monomethylation of emodin led to a decrease of potency (**1** → **2**–**4**). Emodin derivatives with more than two methoxy groups tended to increase the inhibitory effects (**1** → **6**–**8**). The inhibitory effects also tended to decrease when methoxy groups were distributed on one ring (**6–8** → **5, 6, 7** → **4**).

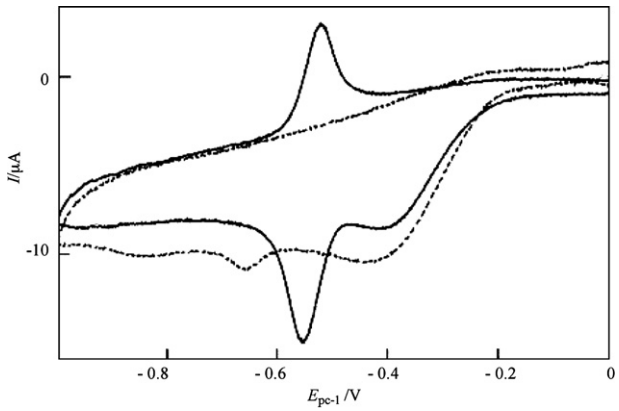

Figure 2. Cyclic voltammograms of compounds **3** and **6** at a PFC electrode in 2:1 (v/v) 0.1 M phosphate buffer (pH 7.2)–ethanol. Voltage scan rate: 20 mV s^{-1}. **3**: ----, **6**:—-.

Table 1
Inhibitory effects of emodin derivatives on EBV-EA activation

Compound	% To control (% viability) Molar ratio (to 32 pmol TPA)				log IC_{50}[a]
	1000	500	100	10	
1	1.5 (70)	24.8	51.6	91.0	2.48
2	1.1 (60)	24.1	49.8	94.1	2.49
3	1.5 (60)	24.7	51.1	94.2	2.50
4	3.7 (60)	25.6	52.8	95.6	2.52
5	2.9 (60)	24.2	51.7	94.7	2.50
6	0 (60)	22.0	47.3	92.6	2.45
7	0 (60)	21.3	45.3	91.8	2.43
8	0 (60)	21.1	44.0	90.1	2.41
9	1.2 (60)	23.2	50.0	93.0	2.48
10	4.3 (60)	27.8	54.9	98.7	2.55

[a] The molar ratio of test compound to TPA giving 50% inhibition against a positive control (100%) was defined as IC_{50}.

Table 2
First and second cathodic peak potentials (E_{pc-1} and E_{pc-2}) and the anodic peak potential (E_{pa}) versus Ag/AgCl (saturated NaCl) obtained 20 mV s^{-1} for emodin derivatives

Compound	E_{pc-1} (V)	E_{pc-2} (V)	E_{pa} (V)
1	−0.460	−0.640	−0.267
2	−0.395	−0.615	−0.580
3	−0.420	−0.655	ND
4	−0.405	−0.615	−0.235
5	−0.415	−0.623	−0.583
6	−0.415	−0.557	−0.518
7	−0.460	−0.605	−0.580
8	−0.460	−0.537	−0.496
9	−0.450	−0.810	ND
10	−0.400	−0.644	−0.620

ND, not detected.

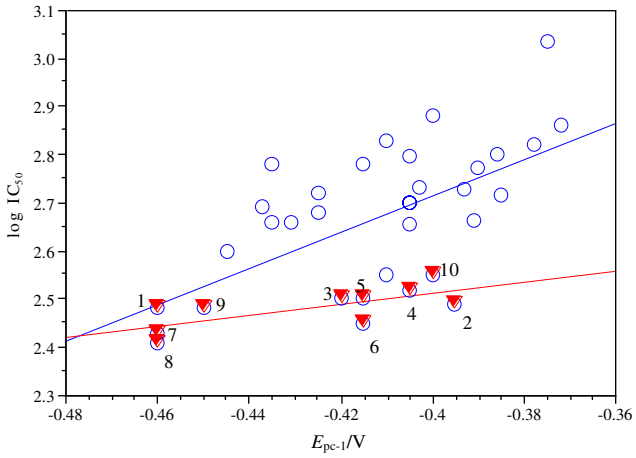

Figure 3. Regression plot of $\log IC_{50}$ and the first reduction potential at pH 7.2 of anthraquinone derivatives with their EBV-EA activation. Plot of $\log IC_{50}$ against E_{pc-1}. Red line: Eq. 1, Blue line: Eq. 4.

Cyclic voltammograms of **3** and **6** obtained at 20 mV s^{-1} are shown in Figure 2. As represented by the voltammogram for compound **6**, emodin derivatives produced two cathodic (reduction) peaks (cathodic and anodic peak potentials are summarized in Table 2 at 20 mV s^{-1}). When the voltage scan rate was increased (e.g., up to 200 mV s^{-1}), the peak current of the second sharp reduction peak (accompanied by an anodic peak) became larger than that of the first reduction peak; therefore, this peak pair could be considered as an adsorption wave due to the redox reaction of the emodin derivative adsorbed at the electrode surface. In contrast, the peak current of the first reduction peak was usually proportional to the square root of the scan rate, indicating that the first reduction peak was limited mainly by diffusion of the emodin derivative. However, the corresponding anodic peak was not detected for all compounds tested, which was probably due to the instability of the reduction product. Accordingly, the first reduction peak potential at 20 mV s^{-1} (E_{pc-1}) was employed to examine any connections with EBV-EA activation. In Figure 3, the values of $\log IC_{50}$ versus first reduction potential (E_{pc-1} in V) of compounds **1**–**10** are plotted. The plot shows a certain correlation between the values of $\log IC_{50}$ and E_{pc-1}. The $\log IC_{50}$ is represented by a regression equation:

$$\log IC_{50} = 2.957 + 1.113E_{pc-1} \quad (n = 10,\ r = 0.709) \qquad (1)$$

where n and r are the number of test compounds and the correlation coefficient, respectively. A large number of biological active quinones contain acidic hydrogens (i.e., hydroxy groups) and are more active than quinones lacking acidic hydrogens. Methylation of the α-hydroxy groups of the quinone carbonyl groups in the anti-cancer agent results in a reduction in anti-tumor effects.[19] This could be due to greater difficulty in semiquinone formation resulting from obstruction of the hydroxy groups by methylation. Regarding the inhibition of EBV-EA activation for emodin derivatives, the number of the hydroxy groups involved in their structure showed a certain correlation to the activity.[15] As a result, it was found that

Table 3
Inhibitory effects, E_{pc-1} values, and electronic properties of anthraquinones

Compound	$\log IC_{50}$	E_{pc-1} (V)	HOMO (eV)	LUMO (eV)	Steric energy (kcal/mol)	Number of OH*1
1	2.48	−0.460	−9.390	−1.643	−23.47	3
2	2.49	−0.395	−9.259	−1.307	−20.42	2
3	2.50	−0.420	−9.356	−1.570	−20.22	2
4	2.52	−0.405	−9.265	−1.320	−20.50	2
5	2.50	−0.415	−9.323	−1.361	−14.36	1
6	2.45	−0.415	−9.215	−0.960	−17.11	1
7	2.43	−0.460	−9.208	−1.290	−16.71	1
8	2.41	−0.460	−9.194	−0.941	−13.37	0
9	2.48	−0.450	−9.375	−1.574	−20.49	2
10	2.55	−0.400	−9.454	−1.463	−20.32	2
Anthraquinone (AQ)	3.04	−0.375	−10.172	−1.381	−21.82	0
1-HydroxyAQ	2.77	−0.390	−9.466	−1.548	−26.17	1
1,8-DihydroxyAQ	2.78	−0.435	−9.491	−1.704	−30.25	2
2,6-DihydroxyAQ	2.88	−0.400	−9.628	−1.396	−26.43	2
2-MethylAQ	2.66	−0.435	−9.916	−1.350	−22.37	0
2-EthylAQ	2.70	−0.405	−9.985	−1.356	−21.63	0
1-Hydroxy-2-methylAQ	2.72	−0.425	−9.329	−1.510	−26.82	1
Chrysophanol	2.68	−0.425	−9.444	−1.673	−21.65	2
1,7-Dihydroxy-6,8-di-methoxy-2-methylAQ	2.55	−0.410	−9.296	−1.458	−18.55	2
Aloe-emodin	2.66	−0.405	−9.574	−1.792	−25.30	3
Obtusifolin	2.60	−0.445	−9.185	−1.382	−18.31	2
Chryso-obtusin	2.73	−0.403	−9.041	−1.164	−7.77	1
Obtusin	2.73	−0.393	−8.881	−1.416	−11.08	2
Aurantio-obtusin	2.72	−0.385	−8.882	−1.336	−14.98	3
1-Hydroxy-8-methoxy-6-methylAQ	2.82	−0.378	−9.322	−1.335	−18.14	1
Cassiamin C	2.86	−0.372	−9.440	−1.703	−54.74	2
Cassiamin A	2.80	−0.386	−9.441	−1.700	−62.27	2.5
Cassiamin B	2.70	−0.405	−9.404	−1.687	−62.35	3
A*	2.83	−0.410	−9.320	−1.700	−60.19	2.5
B*	2.78	−0.415	−9.660	−1.948	−63.83	3
C*	2.80	−0.405	−9.483	−1.879	−70.44	3
1-Acetylemodin	2.69	−0.437	−9.391	−1.473	−19.47	2
1,8-Diacetylemodin	2.70	−0.405	−9.592	−1.342	−15.23	1
1,3,8-Triacetylemodin	2.67	−0.391	−9.811	−1.222	−10.58	0
1,3,8-Triacetylemodic acid	2.66	−0.431	−9.731	−1.424	−19.40	1

A*: 1,1′,3,8,8′-Pentahydroxy-3′,6-dimethyl(2,2′-bianthracene)-9,9′,10,10′-tetrone. B*: 1,1′,8,8′-Tetrahydroxy-3,3′-dihydroxymethyl(2,2′-bianthracene)-9,9′,10,10′-tetrone.
C*: 1,1′,3,8,8′-Pentahydroxy-3-hydroxymethyl-6′-methyl(2,2′-bianthracene)-9,9′,10,10′-tetrone. *1: Number of the hydroxy groups for one anthraquinone skelton.
Data were calculated by the PM3 method using the CAChe MOPAC program.

the $\log IC_{50}$ could be expressed much better than Eq. 1 using the following equation:

$$\log IC_{50} = 2.849 + 0.957E_{pc-1} + 0.026m \quad (n = 10, r = 0.879) \quad (2)$$

where m is the number of hydroxy groups (Fig. 1).

In studies of the structure–activity relationships, electronic properties have so far been used as useful parameters. So we examined the correlation of $\log IC_{50}$ with the electronic properties of emodin derivatives (**1**–**10**), including HOMO, LUMO energies, steric energy, total energy, and solvent accessible surface area (SASA). It was then shown that LUMO energy is the most effective as an additional parameter:

$$\log IC_{50} = 2.824 + 1.137E_{pc-1} - 0.107 \text{ LUMO} \quad (n = 10,\ r = 0.940) \quad (3)$$

Furthermore, we summed up the structure–activity relationship between $\log IC_{50}$ and the first reduction potential for 35 anthraquinones including the methylated emodin derivatives (Table 3).[14,15] A certain correlation between $\log IC_{50}$ and E_{pc-1} was obtained:

$$\log IC_{50} = 4.228 + 3.784E_{pc-1} \quad (n = 35,\ r = 0.618) \quad (4)$$

We also performed multiple regression analyses for the $\log IC_{50}$ values using the following electronic and molecular properties of the anthraquinone derivatives: HOMO, LUMO energies, steric energy, total energy, SASA, ionization potential, and log*P*. Among these properties, HOMO, LUMO, and steric energies improved the multiple regression analyses, altering the regression equation to

$$\log IC_{50} = 3.819 + 3.596E_{pc-1} - 0.226 \text{ LUMO} \quad (n = 35,\ r = 0.709) \quad (5)$$

$$\log IC_{50} = 3.987 + 3.379E_{pc-1} - 0.003 \text{ Steric energy} \quad (n = 35,\ r = 0.694) \quad (6)$$

$$\log IC_{50} = 2.857 + 3.476E_{pc-1} - 0.132 \text{ HOMO} \quad (n = 35,\ r = 0.670) \quad (7)$$

Furthermore, the number of the hydroxy groups was used as an additional parameter to the analysis with E_{pc-1} and a multiple regression analysis was performed. Further improvement of the correlation was achieved, the regression equation being

$$\log IC_{50} = 3.622 + 3.531E_{pc-1} - 0.415 \text{ LUMO} - 0.064\, m \quad (n = 35,\ r = 0.771) \quad (8)$$

Thus, E_{pc-1}, LUMO, and the number of the hydroxy groups (m) seem to be promising parameters for predicting IC_{50}. However, it is still not clear why these parameters play significant roles in determining the IC_{50} of anthraquinone derivatives.

In conclusion, the first reduction potentials determined at a physiological pH (7.2) correlated with the inhibitory effects of anthraquinone derivatives on EBV-EA activation. In addition, the number of the hydroxy groups and LUMO is useful parameters for predicting the inhibitory activity. Based on these results, a reliable evaluation of the potential anti-tumor promoting effects of quinone derivatives can be made by measuring their reduction potentials, without in vitro screening.

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