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STUDIES ON INHIBITORS OF SKIN TUMOR PROMOTION, VI.[1] INHIBITORY EFFECTS OF QUINONES ON EPSTEIN-BARR VIRUS ACTIVATION

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ABSTRACT.—To search for possible antitumor promoters, we carried out a primary screening of fifty-one quinones (anthraquinones, naphthoquinones, azaanthraquinones, and azafluorenones) and related compounds, using their possible inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells. Some of these quinones, notably 5-hydroxy-1,2-methylenedioxy-anthraquinone [**9**], shikonin [**29**], 2-acetylfuranonaphthoquinone [**32**], 5,8-dihydroxycleistopholine [**37**], and 5,8-dihydroxy-2-methyl-1-azaanthraquinone [**45**], were observed to significantly inhibit the EBV-EA activation at low doses. The position and number of hydroxyl groups on phenyl rings of these quinones affected the inhibitory activity on EBV-EA activation. The investigation indicates that **9**, **29**, **32**, **37**, and **45** might be valuable anti-tumor promoters.

Previously, we have reported that several natural products, triterpenoids (1), triterpenoid saponins (2) and flavonoids (3–5), showed strong inhibitory activities on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Tokuda *et al.* (6) and other research groups (7–9) also reported that many compounds having the inhibitory effects on EBV-EA induced by tumor promoters such as TPA or teleocidin B have been shown to act as inhibitors of tumor promotion in vivo.

Anthraquinones and naphthoquinones occur widely in the plant kingdom and in crude drugs, and these quinones may have an important role in the biological activities of many plants and crude drugs. Koyama and co-workers (10,11) have reported new general synthetic methods for azaanthraquinones and azafluorenones that had been isolated from plants in the family Annonaceae (12,13).

As a continuation of our biological studies on the potential antitumor promoting activities of natural products, we have now carried out an evaluation of the inhibitory activities of these fifty-one quinones on EBV-EA.

In this paper, we report the results of the assay of anthraquinones, naphthoquinones, azaanthraquinones, azafluorenones, and their related compounds on inhibitory effects of EBV-EA activation in Raji cells using the synergetic method (14).

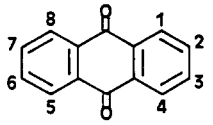
The cytotoxicity (15–18) and the mutagenesis (19) of quinones have been reported, but studies on their inhibitory effects on EBV-EA for antitumor promoting activity have not been published thus far.

RESULTS AND DISCUSSIONS

Among the anthraquinones **1–18** (Tables 1,2), **9**, **10**, and **16** exhibited very strong inhibitory activities on EBV-EA activation induced by TPA even at low doses (1×10^2 mol ratio/TPA) and preserved high viabilities even at high doses (1×10^3 mol

[1]For Part V, see Konoshima *et al.* (5).

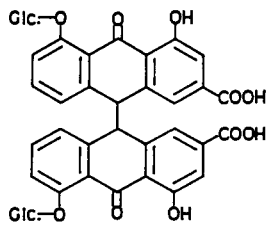
TABLE 1. Structures of Anthraquinones and Related Compounds.



Compound	Substituents						
	C-1	C-2	C-3	C-4	C-5	C-6	C-8
1 (quinizarin)	OH	—	—	OH	—	—	—
2 (alizarin)	OH	OH	—	—	—	—	—
3	OH	—	—	—	—	—	OH
4 (anthrarufin)	OH	—	—	—	OH	—	—
5 (anthraflavicacid)	—	OH	—	—	—	OH	—
6 (purpurin)	OH	OH	—	OH	—	—	—
7 (quinalizarin)	OH	OH	—	—	OH	—	OH
8 (emodin)	OH	—	OH	—	—	Me	OH
9	O-CH_2-O	—	—	—	OH	—	—
10	O-CH_2-O	—	—	—	OMe	—	—
11	O-CH_2-O	—	—	—	—	—	OMe
12	O-CH_2-O	—	—	—	—	OMe	—
13 (damnacanthol)	OMe	CH_2OH	OH	—	—	—	—
14	OH	CH_2OMe	OH	—	—	—	—
15 (juzunol)	OMe	CH_2OH	OH	—	OH	—	—
16	OH	CH_2OMe	OH	—	OH	—	—
17 (damnacanthal)	OMe	CHO	OH	—	—	—	—
18 (sennoside B)							

[a]Each value represents the average of three determinations ± SD.

18



ratio/TPA). On the other hand, emodin [**8**], damnacanthol [**13**], 1,3-dihydroxy-2-methoxymethyl-anthraquinone [**14**], and damnacanthal [**17**] showed the inhibitory effects only at high doses (1×10^3 mol ratio/TPA), and quinizarin [**1**], alizarin [**2**], and sennoside B [**18**] showed no activity at all. Furthermore, those anthraquinones in which hydroxyl groups were evenly distributed on phenyl rings, 1,8-dihydroxyanthraquinone [**3**], anthrarufin [**4**], anthraflavic acid [**5**], and quinalizarin [**7**] exhibited

TABLE 2. Inhibitory Effects of Anthraquinones and Related Compounds on Epstein-Barr Virus Early Antigen (EBV-EA) Activation.

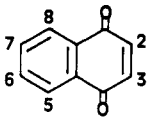
Compound	% to control (% viability)[a]			
	Concentration (mol/TPA)			
	×1000	×500	×100	×10
	TPA (20 ng = 32 pmol/ml) 100 = positive control			
1	89.5 ± 3.1 (70.0)	100.0 ± 2.0 (>80)	100.0 ± 0.0 (>80)	100.0 ± 0.0 (>80)
2	71.4 ± 3.4 (60.0)	92.4 ± 1.7 (>80)	100.0 ± 0.9 (>80)	100.0 ± 0.0 (>80)
3	23.8 ± 3.0 (>80)	61.9 ± 3.3 (>80)	71.2 ± 2.4 (>80)	94.2 ± 4.0 (>80)
4	27.1 ± 3.6 (70.0)	70.1 ± 2.4 (>80)	92.3 ± 1.5 (>80)	100.0 ± 0.0 (>80)
5	28.5 ± 4.1 (>80)	78.6 ± 2.4 (>80)	89.9 ± 2.5 (>80)	100.0 ± 0.0 (>80)
6	34.6 ± 2.6 (70.0)	54.3 ± 3.1 (>80)	100.0 ± 1.3 (>80)	100.0 ± 0.0 (>80)
7	0.0 ± 0.0 (40.0)	0.0 ± 0.4 (60.0)	88.4 ± 3.0 (>80)	100.0 ± 0.0 (>80)
8	18.2 ± 4.1 (>80)	54.2 ± 5.0 (>80)	91.1 ± 0.8 (>80)	100.0 ± 0.0 (>80)
9	28.0 ± 2.6 (>80)	36.5 ± 2.2 (>80)	53.8 ± 4.7 (>80)	79.5 ± 6.2 (>80)
10	0.0 ± 0.0 (>80)	32.7 ± 2.4 (>80)	33.0 ± 3.6 (>80)	100.0 ± 0.0 (>80)
11	35.1 ± 2.2 (60.0)	57.6 ± 4.8 (>80)	82.5 ± 2.0 (>80)	100.0 ± 0.0 (>80)
12	40.5 ± 2.1 (70.0)	56.8 ± 3.4 (>80)	78.9 ± 2.2 (>80)	100.0 ± 0.0 (>80)
13	0.0 ± 0.5 (70.0)	91.2 ± 2.1 (>80)	100.0 ± 1.1 (>80)	100.0 ± 0.0 (>80)
14	0.0 ± 1.4 (60.0)	77.0 ± 4.7 (>80)	80.4 ± 2.6 (>80)	100.0 ± 0.0 (>80)
15	51.1 ± 4.4 (>80)	100.0 ± 1.3 (>80)	100.0 ± 0.0 (>80)	100.0 ± 0.0 (>80)
16	5.7 ± 1.2 (>80)	21.0 ± 3.1 (>80)	51.8 ± 4.6 (>80)	100.0 ± 0.0 (>80)
17	21.4 ± 3.8 (60.0)	51.6 ± 2.4 (>80)	92.8 ± 1.6 (>80)	100.0 ± 0.0 (>80)
18	88.0 ± 3.0 (70.0)	100.0 ± 1.8 (>80)	100.0 ± 0.0 (>80)	100.0 ± 0.0 (>80)

greater inhibitory effects than **1**, **2**, and **6**, in which hydroxyl groups were unevenly distributed on phenyl rings.

In the naphthoquinone series **19–34** (Tables 3,4), naphthazalin [**21**], shikonin [**29**], and the furanonaphthoquinones **31–34** exhibited very strong inhibitory activities even at low doses (1 × 10 mol ratio/TPA), and these activities were more than ten times higher than those of the active anthraquinones and azaanthraquinones. Also these inhibitory activities are more than 100 times higher than those of glycyrrhetinic acid and retinoic acid, which are known as inhibitors of EBV-EA activation and tumor promotion (20). In general, naphthoquinones showed higher cytotoxicities on Raji cells than anthraquinones, whereas *N*,*N*-dimethyl derivatives of naphthoquinones **24–28** preserved high viabilities even at high doses (1×10^3 mol ratio/TPA). Thiophenonaphthoquinone [**34**] exhibited lower inhibitory effect than furanonaphthoquinones **31–33**, and vitamin K_1 [**30**] showed no activity at all even at high dose (1×10^3). From the comparisons of the inhibitory effects of **21**, **29**, and **27** with those of **19**, **20**, **22**, **23**, and **24**, it was also deduced that the hydroxyl groups at C-5 and C-8 on the naphthoquinone skeleton enhanced these inhibitory effects.

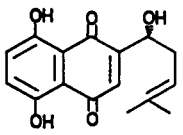
In the 1-azaanthraquinone series **35–45** (Tables 5,6), compounds **36**, **37**, **41**, **43**, **44**, and **45** exhibited significant inhibitory activities at 5×10^2 mol ratio, whereas these significant inhibitory effects were not found with cleistopholine [**35**], **38**, **39**, **40**, and **42**. From these facts, it was concluded that the hydroxyl groups at C-5 and/or C-8 of 1-azaanthraquinones enhanced the inhibitory effects, and the methylation of these hydroxyl groups led to a decrease of the inhibitory effects. The position of methyl group substitution on the pyridine ring of **1**-azaanthraquinones did not markedly affect the potency.

TABLE 3. Structures of Naphthoquinones and Related Compounds.



Compound	Substituents			
	C-2	C-5	C-6	C-8
19	—	—	—	—
20 (juglone)	—	OH	—	—
21 (naphthazalin)	—	OH	—	OH
22	—	—	OH	—
23 (vitamin-K_3)	Me	—	—	—
24	NMe_2	—	—	—
25	NMe_2	—	—	OH
26	NMe_2	OH	—	—
27	NMe_2	OH	—	OH
28	NMe_2	—	OH	—
29 (shikonin)				
30 (vitamin-K_1)				
31				
32				
33				
34				

[a]Each value represents the average of three determinations ± SD.



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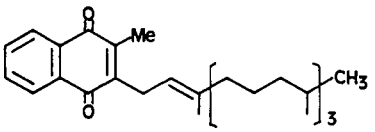

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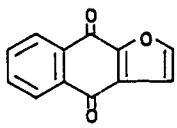

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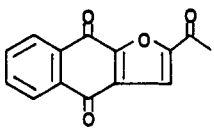

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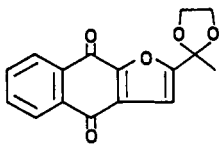

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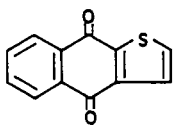


34

For the three azafluorenones **49–51** (Table 6), significant inhibitory effects on EBV-EA activation were not shown even at 5×10^2 mol ratio.

Based on the studies described above, the following structure-activity relationships can be postulated: 1. The position and the number of hydroxyl groups affect the inhibitory activity on EBV-EA activation. 2. Methylation of phenolic hydroxyl groups leads to a decreasing of potency in the azaanthraquinones investigated. 3. The presence of a furan ring enhances the inhibitory effect of EBV-EA activation and the cytotoxicity

TABLE 4. Inhibitory Effects of Naphthoquinones and Related Compounds on Epstein Barr Virus (EBV) Activation.

Compound	% to control (% viability)[a]			
	Concentration (mol/TPA)			
	×1000	×500	×100	×10
	TPA (20 ng = 32 pmol/ml) 100 = positive control			
19	0.0 ± 0.0 (20.0)	0.0 ± 1.1 (50.0)	37.5 ± 3.6 (50.0)	100.0 ± 0.0 (>80)
20	0.0 ± 0.0 (40.0)	13.1 ± 1.6 (60.0)	97.8 ± 2.4 (>80)	100.0 ± 0.0 (>80)
21	0.0 ± 0.0 (20.0)	0.0 ± 0.0 (40.0)	0.0 ± 3.4 (50.0)	68.9 ± 5.6 (>80)
22	0.0 ± 0.0 (10.0)	0.0 ± 4.8 (60.0)	67.2 ± 3.8 (60.0)	92.3 ± 2.1 (>80)
23	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	65.7 ± 3.9 (70.0)	89.4 ± 2.4 (>80)
24	45.8 ± 3.2 (>80)	89.2 ± 2.9 (>80)	92.5 ± 1.0 (>80)	100.0 ± 0.0 (>80)
25	0.0 ± 0.0 (>80)	53.8 ± 3.6 (>80)	89.7 ± 4.0 (>80)	100.0 ± 1.1 (>80)
26	0.0 ± 1.6 (>80)	60.5 ± 2.6 (>80)	61.5 ± 3.2 (>80)	89.2 ± 6.1 (>80)
27	11.1 ± 2.2 (50.0)	28.2 ± 4.1 (>80)	87.2 ± 3.8 (>80)	100.0 ± 0.0 (>80)
28	55.3 ± 2.0 (60.0)	68.5 ± 3.9 (>80)	100.0 ± 2.4 (>80)	100.0 ± 0.0 (>80)
29	0.0 ± 0.0 (0.0)	0.0 ± 1.3 (10.0)	15.8 ± 5.0 (50.0)	31.5 ± 3.4 (>80)
30	92.1 ± 3.4 (70.0)	100.0 ± 1.4 (>80)	100.0 ± 0.0 (>80)	100.0 ± 0.0 (>80)
31	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (10.0)	0.0 ± 2.4 (10.0)	59.4 ± 4.3 (70.0)
32	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	0.0 ± 1.2 (70.0)	32.7 ± 3.0 (>80)
33	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (10.0)	0.0 ± 3.6 (10.0)	32.4 ± 2.4 (60.0)
34	0.0 ± 0.0 (0.0)	0.0 ± 0.0 (0.0)	52.8 ± 2.6 (70.0)	81.4 ± 1.8 (>80)

on Raji cells. 4. Hydroxylated azaanthraquinones exhibit remarkable inhibitory activity and high viability for Raji cells.

These results suggested that the anthraquinones **9** and **10**, shikonin [**29**], 2-acetylfuranonaphthoquinone [**32**] (16), 5,8-dihydroxycleistopholine [**37**], and **45** might be valuable anti-promoters in carcinogenesis.

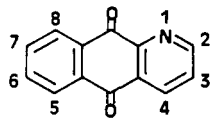
From the results of a binding assay, it was deduced that these active quinones **9**, **10**, **29**, **32**, **37**, and **45** showed no effect on [^{3}H]-TPA binding to the TPA receptor, while TPA significantly inhibited it. These results indicated that quinones may act at some point after the binding of the tumor promoters to the receptors.

The details of the inhibitory mechanisms of quinones, the relationship between the activity and the molecular models including the uneven distribution of electrons on quinone molecule, and the initiation-promotion tests using ICR mice (6) of these quinones **9**, **10**, **29**, **32**, **37**, and **45** are now in progress.

EXPERIMENTAL

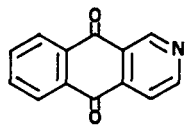
MATERIALS.—Samples tested were obtained from the following methods: Azaanthraquinones **35–45** were synthesized using Diels-Alder reaction, by treatment of the *N*-dimethylhydrazone (1 equiv) derived from crotonaldehyde with naphthoquinone (2 equiv) corresponding to each azaanthraquinone in C_6H_6 for 48 h followed by the addition of DDQ (1 equiv) to afford the aromatized adduct, azaanthraquinones, in about 50% yield (**47** and **48** are intermediates of **39** and **33**, respectively). Naphthoquinones **24–28** were obtained as the by-products of the syntheses of azaanthraquinones **35**, **36**, **37** and **39**. Azafluorenones **49–51** were also synthesized by the new method using the thermolysis of oxime *O*-allyl ether of 1-indanone (11). The synthesis of anthraquinones **10–12** was accomplished according to Snieckus's method (21) by using metalation of methylenedioxylated *N*,*N*-diethylbenzamide as the key step. Sequential treatment of *N*,*N*-diethylbenzamide with *sec*-BuLi and methoxybenzaldehyde gave the hydroxylamine, which was subjected to *p*-toluenesulfonic acid to provide the phthalide. Hydrogenolysis of the phthalide followed by Friedel-Crafts cyclization and oxidation yielded anthraquinones. Furanonaphthoquinones **31–34** were synthesized by the application of tandem-directed metalation reac-

TABLE 5. Structures of Azaanthraquinones and Azafluorenones.



Compound	Substituents					
	C-2	C-3	C-4	C-5	C-6	C-8
35 (cleistopholine)	—	—	Me	—	—	—
36	—	—	Me	—	—	OH
37	—	—	Me	OH	—	OH
38	—	—	Me	OMe	—	OMe
39	—	—	Me	—	OH	—
40	—	Me	—	—	—	—
41	—	Me	—	—	—	OH
42	—	Me	—	—	—	OMe
43	—	Me	—	OH	—	OH
44	Me	—	—	—	—	OH
45	Me	—	—	OH	—	OH
46						
47						
48						
49 (onychine)						
50						
51						

[a]Each value represents the average of three determinations ± SD.



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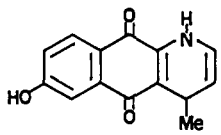

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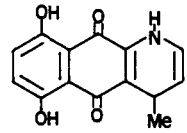

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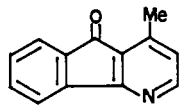

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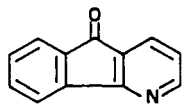

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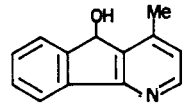

51

tion of *N*,*N*-dimethylbenzamide and 3-furaldehyde (22). Compound **22** was synthesized from 1,6-dihydroxy-naphthoquinone (23). The structures of all synthetic compounds were confirmed by ir, ^{1}H-nmr, and ms spectra as shown in Table 7.

Anthraquinones **9** and **13–17** were isolated from *Damnacanthus indicus* (Rubiaceae), which is used in folk medicine for the treatment of lumbago in China (24). Herbarium specimens are deposited in the herbarium of Kobe Women's College of Pharmacy. Compounds **1**, **2**, **6**, **7**, **20**, **23**, and **30** were purchased from Nakarai Chemical Co., Japan. Compounds **3**, **4**, **8**, **18**, **19**, and **29** were purchased from Wako Pure Chemical Ind., Japan, and **5**, **21**, and **46** were purchased from Aldrich Chemical Company, USA.

BIOLOGICAL ACTIVITIES.—*The inhibition of* EBV-EA activation was assayed using Raji cells (virus non-producer), the EBV genome-carrying human lymphoblastoid cells, which were cultivated in 8% FBS RPMI 1640 medium (Nissui). The indicator cells (Raji) (1×10^6 ml) were incubated at 37° for 48 h in 1 ml of the medium containing *n*-butyric acid (4 mM, inducer) 2 μl of TPA (20 ng/ml in DMSO), and a known amount of test compound of DMSO. Smears were made from the cell suspension. The activated cells were

TABLE 6. Inhibitory Effects of Azaanthraquinones and Azafluorenones on Epstein Barr Virus (EBV) Activation.

Compound	% to control (% viability)[a]			
	Concentration (mol/TPA)			
	×1000	×500	×100	×10
	TPA (20 ng = 32 pmol/ml) 100 = positive control			
35	40.6 ± 2.1 (70.0)	48.7 ± 2.4 (>80)	77.5 ± 1.8 (>80)	100.0 ± 0.0 (>80)
36	0.0 ± 0.0 (40.0)	15.4 ± 2.0 (60.0)	67.4 ± 4.1 (>80)	89.1 ± 1.1 (>80)
37	10.4 ± 1.4 (60.0)	23.1 ± 2.6 (60.0)	52.1 ± 5.0 (>80)	88.6 ± 3.3 (>80)
38	88.6 ± 3.6 (70.0)	100.0 ± 1.0 (>80)	100.0 ± 0.0 (>80)	100.0 ± 0.0 (>80)
39	63.9 ± 5.3 (60.0)	73.9 ± 3.1 (60.0)	74.4 ± 4.8 (>80)	100.0 ± 0.0 (>80)
40	0.0 ± 0.0 (70.0)	48.7 ± 3.1 (>80)	76.5 ± 2.6 (>80)	100.0 ± 0.0 (>80)
41	0.0 ± 0.0 (30.0)	15.8 ± 3.4 (>80)	72.4 ± 2.8 (60.0)	100.0 ± 1.6 (>80)
42	40.8 ± 4.1 (>80)	83.0 ± 2.9 (>80)	100.0 ± 1.1 (>80)	100.0 ± 0.0 (>80)
43	6.1 ± 5.8 (40.0)	16.8 ± 3.4 (50.0)	82.6 ± 3.0 (>80)	100.0 ± 0.0 (>80)
44	8.7 ± 4.9 (10.0)	17.3 ± 2.0 (50.0)	56.8 ± 2.3 (>80)	92.4 ± 0.8 (>80)
45	0.0 ± 0.0 (10.0)	13.5 ± 3.4 (60.0)	43.8 ± 6.2 (>80)	86.0 ± 2.4 (>80)
46	0.0 ± 1.4 (10.0)	13.0 ± 2.3 (10.0)	69.5 ± 5.1 (>80)	100.0 ± 0.4 (>80)
47	47.3 ± 2.8 (70.0)	62.1 ± 3.0 (>80)	66.7 ± 4.8 (>80)	100.0 ± 3.1 (>80)
48	0.0 ± 1.4 (10.0)	20.4 ± 3.6 (70.0)	100.0 ± 2.3 (>80)	100.0 ± 0.0 (>80)
49	55.5 ± 5.1 (70.0)	94.1 ± 2.6 (>80)	100.0 ± 0.6 (>80)	100.0 ± 0.0 (>80)
50	38.0 ± 4.4 (70.0)	97.6 ± 1.8 (>80)	100.0 ± 1.4 (>80)	100.0 ± 0.0 (>80)
51	39.7 ± 5.3 (>80)	100.0 ± 0.5 (>80)	100.0 ± 0.0 (>80)	100.0 ± 0.0 (>80)

TABLE 7. ^{1}H-Nmr Chemical Shift Values, ir, and hrms of Synthetic Quinones.

Compound	Chemical shift values (δ)[a]	hrms $[M]^{+}$[b]	ir (cm^{-1})[c]
10	4.05 (3H, s, OMe), 6.32 (2H, s, OCH_2O), 7.16 (1H, d, H-3), 7.36 (1H, dd, H-6), 7.73 (1H, dd, H-7), 7.95 (1H, d, H-4), 7.98 (1H, dd, H-8)	282.0519 ($C_{16}H_{10}O_5$, 282.0526)	1670
11	4.04 (3H, s, OMe), 6.31 (2H, s, OCH_2O), 7.12 (1H, d, H-3), 7.36 (1H, dd, H-7), 7.74 (1H, t-like, H-6), 7.93 (1H, d, H-4), 7.98 (1H, dd, H-5)	282.0564 ($C_{16}H_{10}O_5$, 282.0526)	1675
12	4.00 (3H, s, OMe), 6.34 (2H, s, OCH_2O), 7.15 (1H, d, H-3), 7.29 (1H, dd, H-7), 7.76 (1H, d, H-5), 8.00 (1H, d, H-4), 8.28 (1H, d, H-8)	282.0520 ($C_{16}H_{10}O_5$, 282.0526)	1670
24	3.24 (6H, s, Me × 2), 5.87 (1H, s, CH=CN), 7.68 (2H, m, H-6,7), 8.05 (2H, m, H-5,8)	201.0798 ($C_{12}H_{11}NO_2$, 201.0789)	1680
25	3.26 (6H, s, Me × 2), 5.74 (1H, s, CH=CN), 7.22 (1H, dd, H-6), 7.53 (2H, m, H-5,7)	217.0735 ($C_{12}H_{11}NO_3$, 217.0738	1625
26	3.25 (3H, s, Me), 3.26 (3H, s, Me), 5.86 (1H, s, CH=CN), 7.18 (1H, m, H-7), 7.63 (2H, m, H-6,8)	217.0741 ($C_{12}H_{11}NO_3$, 217.0738	1620, 1680
27	3.28 (6H, s, Me × 2), 5.80 (1H, s, CH=CN), 7.11 (1H, d, H-6 or H-7), 7.24 (1H, d, H-7 or H-6)	233.0682 ($C_{12}H_{11}NO_4$, 233.0687)	1630, 1670
28	3.27 (6H, s, Me × 2), 5.82 (1H, s, CH=CN), 7.09 (1H, dd, H-7), 7.73 (1H, d, H-5), 7.96 (1H, d, H-8)	217.0732 ($C_{12}H_{11}NO_3$, 217.0738)	1650
35	2.92 (3H, s, Me), 7.52 (1H, d, H-3), 7.86 (2H, m, H-6,7), 8.29 (1H, m, H-5 or H-8), 8.40 (1H, m, H-8 or H-5), 8.93 (1H, d, H-2)	223.0647 ($C_{14}H_9NO_2$, 223.0633)	1680
36	2.93 (3H, s, Me), 7.38 (1H, dd, H-5), 7.56 (1H, d, H-3), 7.77 (1H, t, H-6), 8.86 (1H, dd, H-7), 8.96 (1H, d, H-2)	239.0588 ($C_{14}H_9NO_3$, 239.0582)	1650
37	2.94 (3H, s, Me), 7.36 (2H, s, H-6,7), 7.52 (1H, d, H-3), 8.93 (1H, d, H-2),	255.0504 ($C_{14}H_9NO_4$, 255.0530)	1630
38	2.80 (3H, s, Me), 3.99 (3H, s, OMe), 4.00 (3H, s, OMe), 7.34 (2H, d-like, H-6,7), 7.41 (1H, d, H-3), 8.81 (1H, d, H-2)	283.0845 ($C_{16}H_{13}NO_4$, 283.0844)	1680
39	2.95 (3H, s, Me), 7.29 (1H, dd, H-7), 7.59 (1H, d, H-3), 7.67 (1H, d, H-5), 8.21 (1H, d, H-8), 8.84 (1H, d, H-2)	239.0592 ($C_{14}H_9NO_3$, 239.0582)	1660, 1680
40	2.59 (3H, s, Me), 7.88 (2H, m, H-6,7), 8.35 (2H, m, H-5,8), 8.45 (1H, d, H-4), 8.97 (1H, d, H-2)	223.0640 ($C_{14}H_9NO_2$, 223.0632)	1680
41	2.60 (3H, s, Me), 7.39 (1H, dd, H-5), 7.75 (1H, t, H-6), 7.87 (1H, dd, H-7), 8.42 (1H, d, H-4), 8.96 (1H, d, H-2)	239.0570 ($C_{14}H_9NO_3$, 239.0582)	1650, 1680

TABLE 7. Continued.

Compound	Chemical shift values (δ)[a]	hrms $[M]^{+}$[b]	ir (cm^{-1})[c]
42	2.56 (3H, s, Me), 4.08 (3H, s, OMe), 7.43 (1H, dd, H-5), 7.79 (1H, t, H-6), 8.00 (1H, dd, H-7), 8.37 (1H, dd, H-4), 8.95 (1H, dd, H-2)	253.0721 ($C_{15}H_{11}NO_3$, 253.0737)	1675
43	2.60 (3H, s, Me), 7.37 (2H, s, H-6,7), 8.45 (1H, d, H-4), 8.96 (1H, d, H-2)	255.0500 ($C_{14}H_9NO_4$, 255.0530)	1635
44	2.83 (3H, s, Me), 7.39 (1H, dd, H-5), 7.63 (1H, d, H-3), 7.76 (1H, t, H-6), 7.87 (1H, dd, H-7), 8.53 (1H, d, H-4)	239.0581 ($C_{14}H_9NO_3$, 239.0582)	1680
45	2.85 (3H, s, Me), 7.38 (2H, s, H-6,7), 7.64 (1H, d, H-3), 8.57 (1H, d, H-4)	255.0530 ($C_{14}H_9NO_4$, 255.0530)	1640
47	1.16 (3H, d, Me), 3.72 (1H, m, CH-Me), 4.97 (1H, m, CH=CH-N), 6.19 (1H, d, CH=CH-N), 7.12 (1H, dd, H-7), 7.40 (1H, dd, H-5), 7.96 (1H, d, H-8)	241 (by lrms)[d]	
48	1.18 (3H, d, Me), 3.73 (1H, m, CH-Me), 4.99 (1H, m, CH=CH-N), 6.16 (1H, dd, CH=CH-N), 7.08 (1H, d, H-6 or H-7), 7.24 (1H, d, H-7 or H-6)	257.0988 ($C_{14}H_{11}NO_4$, 257.0688)	3410, 1640
50	7.40 (1H, q, H-3), 8.13 (1H, dd, H-4), 8.85 (1H, dd, H-2)	181.0535 ($C_{12}H_7NO$, 181.0527)	1715

[a]^{1}H-nmr spectra were taken on NEVA NV-21 spectrometer with TMS as an internal standard in $CDCl_3$.
[b]Mass spectra were recorded on a JEOL JMS-01SG spectrometer, and values in parentheses are calculated mol wt.
[c]Ir spectra were recorded on HITACHI 270-30 infrared spectrometer in $CHCl_3$.
[d]This compound was very unstable.

stained by high-titer EBV-EA positive sera from nasopharyngeal carcinoma (NPC) patients and detected by a conventional indirect immunofluorescence technique. In each assay, at least 500 cells were counted, and the experiments were repeated three times. The average EA induction was compared with that of positive control experiments with *n*-butyric acid (4 mM) plus TPA (20 ng/ml) in which EA induction was ordinarily around 30%. In this screening method, the cell viability required for the judgment of inhibitory effects was more than 60% (20).

BINDING ASSAY.—Specific [^{3}H]-TPA binding was assayed by the cold Me_2CO filter method. An epidermal particulate fraction was prepared from dorsal epidermis of female ICR mice. Protein (100 mg) from the particulate fraction was incubated at 4° for 3 h with [^{3}H]-TPA (4 nM) with the test compound in 1 ml of 20 mM Tris-HCl buffer (pH 7.4) containing 2-mercaptoethanol (2 mM). Nonspecific binding was determined by measuring the binding of the [^{3}H]-TPA to the particulate fraction in the presence of a 500-fold excess of unlabeled TPA.

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