Synthesis of Novel Rhinacanthins and Related Anticancer Naphthoquinone Esters

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Rhinacanthin-M, -N and -Q, natural products isolated from the medicinal plant *Rhinacanthus nasutus*, and 39 novel naphthoquinone esters have been synthesized in excellent yield by esterification of naphthoquinone-3-(propan-3′-ols) with benzoic or naphthoic acids. Almost all the naphthoquinone esters that contain a C-3 hydroxy group showed significant cytotoxicities against KB, HeLa, and $HepG_2$ cell lines. In contrast, ester derivatives lacking the C-3 hydroxy group were inactive to the cancer cell lines. Two methyl substituents on the C-2′ of propyl chain conferred more potent cytotoxicity than when there is only one or no methyl group. Naphthoate esters exhibited greater cytotoxicity than benzoate esters. Computer modeling has been done to obtain a first look at the mode of action in connection with these observations.

Introduction

Rhinacanthins[1−3] are naphthoquinone ester derivatives isolated from the methanolic extract of the roots of the medicinal plant *Rhinacanthus nasutus* (Acanthaceae). Some of these compounds have been reported[2] to exhibit cytotoxicity against P388, A-549, HT-29, and HL-60 cell lines. In Thailand, the roots and leaves of *R. nasutus* are used for the treatment of cancer.[4] There has been no reported synthesis of rhinacanthins. We now report the synthesis of rhinacanthin-M, -N, -Q and 39 related naphthoquinone esters (Figure 1) together with their cytotoxicities against human carcinoma cell lines, KB (oral human epidermoid carcinoma), HeLa (human cervical carcinoma), and $HepG_2$ (human hepatocellular carcinoma). We also conducted preliminary studies on the mode of action of some of these naphthoquinone esters by computer modeling.

Chemistry

Rhinacanthins and related naphthoquinone esters were obtained by esterification of naphthoquinone alcohols with benzoic or 2-naphthoic acids based on the disconnection shown in Figure 2. Naphthoquinone alcohols (**51**, **52**) with a *gem*-dimethyl group on the propyl chain were prepared from 1-hydroxy-2-naphthoic acid,[3,5,6] whereas naphthoquinone alcohols (**45**, **46**, **49**, and **50**) were prepared from 2-allyl or 2-methylallyl-1-naphthol[7−9] via hydroboration followed by two successive oxidations using Fremy's salt followed by DDQ. Finally, hydrolysis of **47** and **48** under basic condition provided **49** and **50** in 86% and 94% yield, respectively (Scheme 1).

Preparation of rhinacanthin-Q (**3**) and its analogues (**14**, **15**, **18**, **19**, **21**, **23**, **24**, and **26**) was achieved in 58−

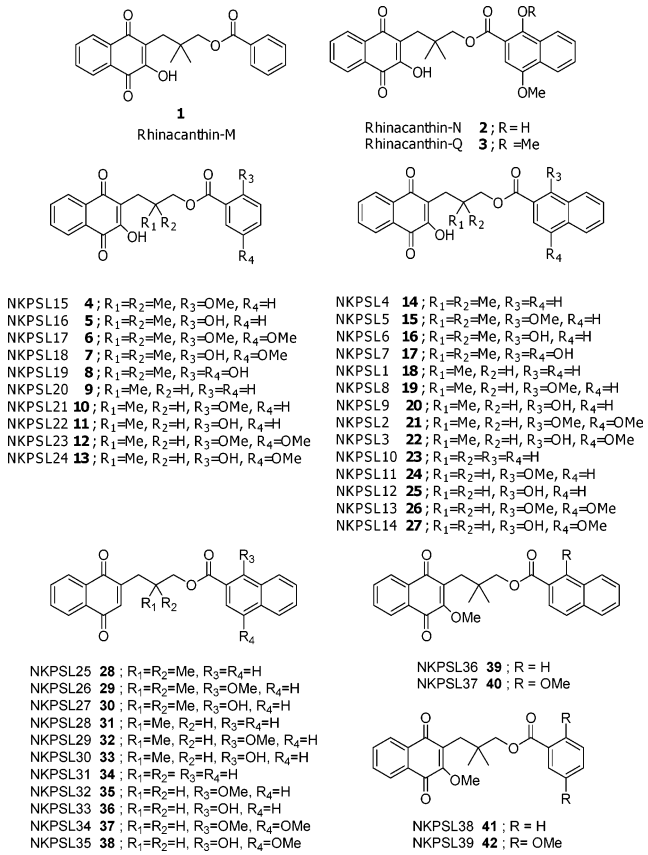

Figure 1. Structures of rhinacanthin-M, -N, -Q and 39 related novel naphthoquinone esters.

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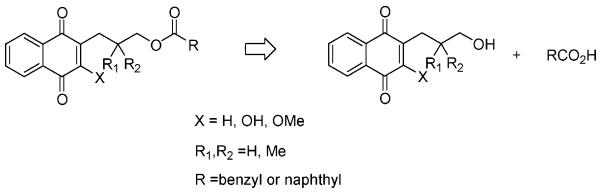


Figure 2. Retrosynthetic analysis of rhinacanthins and their derivatives.

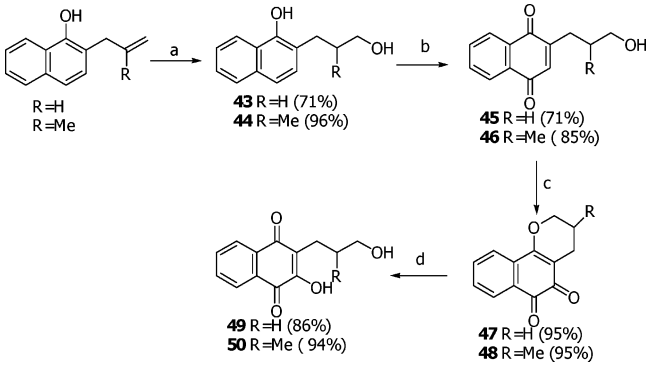
87% yield by esterification of naphthoquinone alcohols (**49**−**51**) with various 2-naphthoic acids in the presence of 1,1′-carbodiimidazole (CDI)[10] (Table 1). The corresponding diester was also produced in trace amounts.

Rhinacanthin-M (**1**) and its analogues (**4**, **6**, **9**, **10**, and **12**) were prepared in 69−98% yield by esterification of naphthoquinone alcohols (**50** and **51**) with a range of benzoic acids in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP)[11] (Table 2). Low yields of esters were encountered when 1,1′-carbodiimidazole was used for coupling.

Naphthoquinone esters (**28**, **29**, **31**, **32**, **34**, **35**, and **37**) lacking a C-3 hydroxy group were prepared according to the procedure used to synthesize rhinacanthin-M and its analogues (Table 2).

Selective demethylation of rhinacanthin-Q (**3**) and naphthoquinone esters (**4**, **6**, **10**, **12**, **15**, **19**, **21**, **24**, **26**, **29**, **32**, **35**, and **37**) by treatment with boron tribromide[12] in dichloromethane at −78 °C for 15 min afforded

Scheme 1. Synthesis of Naphthoquinone-3-(propan-3′-ols) (**45, 46, 49**, and **50**)[a]

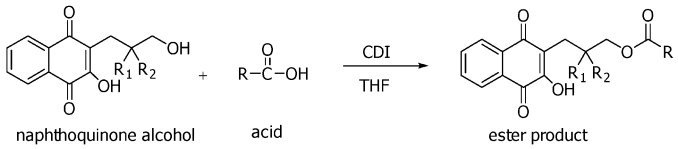


[a] Conditions: (a) BH_3·THF and then H_2O_2/OH^-; (b) Fremy's salt, MeOH−DMF, 1 M NaOAc, room temp, 12 h; (c) DDQ, *p*-TsOH, benzene, reflux, 20 min; (d) 1% aqueous NaOH, reflux, 2 h.

rhinacanthin-N (**2**) in 95% yield and naphthoquinone esters (**5**, **7**, **11**, **13**, **16**, **20**, **22**, **25**, **27**, **30**, **33**, **36**, and **38**) in good yield (Table 3). In the case of naphthoquinone esters (**8** and **17**), yields of 72% and 73%, respectively, were obtained by demethylation at both methoxy groups of naphthoquinone ester (**6**) and rhinacanthin-Q (**3**) with boron tribromide in dichloromethane at room temperature for 2 h (Table 3).

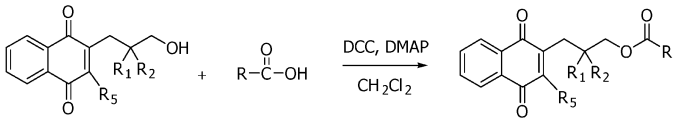
Naphthoquinone esters (**39**−**42**) containing a C-3 methoxy group were synthesized in good yield by

Table 1. Carbodiimidazole (CDI) Mediated Esterification of Naphthoquinone Alcohol



Entry	Naphthoquinone Alcohol	Acid (**R-**)	Ester product	Yield (%)
1	$R_1,R_2 = CH_3$		**14**	87
2	$R_1,R_2 = CH_3$		**15**	82
3	$R_1,R_2 = CH_3$		**3**	71
4	$R_1 = CH_3$, $R_2 = H$		**18**	83
5	$R_1 = CH_3$, $R_2 = H$		**19**	73
6	$R_1 = CH_3$, $R_2 = H$		**21**	73
7	$R_1,R_2 = H$		**23**	73
8	$R_1,R_2 = H$		**24**	77
9	$R_1,R_2 = H$		**26**	58

Table 2. DCC/DMAP Mediated Esterification of Naphthoquinone Alcohol



Entry	Naphthoquinone Alcohol	Acid (**R-**)	Ester product	Yield (%)
1	R_1,R_2=CH_3, R_5=OH		**1**	91
2	R_1,R_2=CH_3, R_5=OH		**4**	98
3	R_1,R_2=CH_3, R_5=OH		**6**	92
4	R_1=CH_3, R_2=H, R_5=OH		**9**	69
5	R_1=CH_3, R_2=H, R_5=OH		**10**	93
6	R_1=CH_3, R_2=H, R_5=OH		**12**	69
7	R_1,R_2=CH_3, R_5=H		**28**	74
8	R_1,R_2,CH_3, R_5=H		**29**	98
9	R_1=CH_3, R_2=H, R_5=H		**31**	83
10	R_1=CH_3, R_2=H, R_5=H		**32**	90
11	R_1,R_2,R_5=H		**34**	86
12	R_1,R_2,R_5=H		**35**	85
13	R_1,R_2,R_5=H		**37**	82

methylation of naphthoquinone esters (**1**, **6**, **14**, and **15**) with methyl iodide (Table 4).

Biological Activity

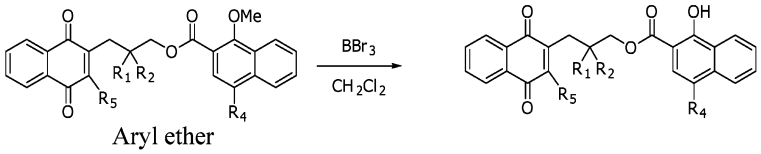
Compounds **1**−**42** (Figure 1) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma), HeLa (human cervical carcinoma), and $HepG_2$ (human hepatocellular carcinoma) cell lines as well as against the normal Vero cell line employing the MTT colorimetric method.[13] Adriamycin, which exhibits cytotoxicity against KB, HeLa, and $HepG_2$ cell lines, was used as reference. The results are summarized in Tables 5 and 6. Parent naphthoquinone alcohols and acids were tested for cytotoxicity against the KB cell line as shown in Table 7.

Results and Discussion

Rhinacanthin-M, -N, -Q and naphthoquinone esters (**4**−**42**) (Figure 1) were tested for cytotoxicity against the cancer cell lines KB, HeLa, and $HepG_2$ as well as against the normal Vero cell line. The results[14] showed that the naphthoate (**2**) (Table 6) inhibits the cancer cell lines more effectively than the benzoate (**7**) (Table 5) with IC_{50} in the range <0.22 to 0.38 ± 0.06 and 1.18 ± 0.26 to 3.33 ± 0.37 μM while they were much less toxic to the Vero cell line (12.65 and 11.49 μM). Other naphthoates (**3**, **14**, **15**, **16**, and **17**) exhibit more potent cytotoxicity (Table 6) against the cancer cell lines than the benzoates having the same substitution (**1**, **4**, **5**, **6**, and **8**). The substituents on the naphthoquinone ring (R_5), propyl chain (R_1 and R_2), and benzene or naphthalene ring (R_3 and R_4) affect the activity. The presence of a C-3 hydroxy group (R_5 = OH) on the naphthoquinone ring resulted in significantly greater cytotoxicity of **3**; KB, HeLa, and $HepG_2$ inhibited with IC_{50} values of 0.35 ± 0.16, 1.09 ± 0.11, and 0.97 ± 0.10 μM, respectively, compared to **37** (R_5 = H), which is inactive.

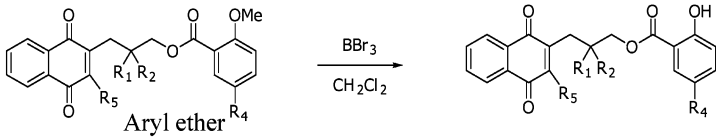
Table 3. Boron Tribromide Demethylation of Aryl Ethers

3a: Boron tribromide demethylation of aryl ethers of naphthoate esters



Entry	Aryl ether	Product	Yield (%)
1	**3:** R_1,R_2=CH_3, R_4=OCH_3, R_5=OH	**2**	95
2	**15:** R_1,R_2=CH_3, R_4=H, R_5=OH	**16**	80
3	**19:** R_1=CH_3, R_2=H, R_4=H, R_5=OH	**20**	81
4	**21:** R_1=CH_3, R_2=H, R_4=OCH_3, R_5=OH	**22**	80
5	**24:** R_1,R_2,R_4=H, R_5=OH	**25**	85
6	**26:** R_1,R_2=H, R_4=OCH_3, R_5=OH	**27**	79
7	**29:** R_1,R_2=CH_3, R_4,R_5=H	**30**	80
8	**32:** R_1=CH_3, R_2=H, R_4,R_5=H	**33**	84
9	**35:** R_1,R_2,R_4,R_5=H	**36**	92
10	**37:** R_1,R_2R_5=H, R_4=OCH_3	**38**	46
11	**3:** R_1,R_2=CH_3, R_4=OCH_3, R_5=OH	**17** (R_4=OH)	73

3b: Boron tribromide demethylation of aryl ethers of benzoate esters

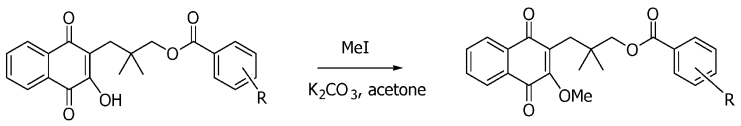


Entry	Aryl ether	Product	Yield (%)
1	**4:** R_1,R_2=CH_3, R_4=H, R_5=OH	**5**	90
2	**6:** R_1,R_2=CH_3, R_4=OCH_3, R_5=OH	**7**	89
3	**10:** R_1=CH_3, R_2=H, R_4=H, R_5=OH	**11**	70
4	**12:** R_1=CH_3, R_2=H, R_4=OCH_3, R_5=OH	**13**	83
5	**6:** R_1,R_2=CH_3, R_4=OCH_3, R_5=OH	**8** (R_4=OH)	72

With regard to the propyl chain of the naphthoquinone ring, methyl substituents at the C-2′ position affected cytotoxicity. Two methyl groups at the C-2′ position of the propyl chain conferred more potent cytotoxicity against cancer cell lines than when there is only one methyl or no methyl group (see compounds **15**, **19**, and **24** in Table 6). For example, compound **15** showed better cytotoxicity against the KB cell line than **19** and **24** with IC_{50} values of <0.22, 0.66 ± 0.19, and 33.66 ± 6.58 μM, respectively. In addition, the R_3 and R_4 substituents on the naphthalene ring affect cytotoxicity. Thus, **14** (NKPSL4, R_3, R_4 = H), **2** (rhinacanthin-N, R_3 = OH, R_4 = OMe), and **3** (rhinacanthin-Q, R_3, R_4 = OMe) inhibit KB, HeLa, and $HepG_2$ cell lines differently. Rhinacanthin-N showed better cytotoxicity against the KB cell line (Table 6) than rhinacanthin-Q and NKPSL4 with IC_{50} values of <0.22, 0.35 ± 0.16, and 1.59 ± 0.37 μM, respectively. Moreover, NKPSL3 (**22**) showed greater cytotoxicity against the cancer cell lines (IC_{50} values in the range 2.16 ± 0.94 to 3.14 ± 0.45 μM) than NKPSL2 (**21**) (IC_{50} values in the range 3.52 ± 0.18 to 3.80 ± 0.38 μM). The presence of a hydroxy group at C-1 and a methoxy group at C-4 of the naphthalene ring resulted in stronger cytotoxicity than the presence of methoxy groups at both of these positions. For example, in Table 6, rhinacanthin-N (**2**) showed greater cytotoxicity against the KB, HeLa, and $HepG_2$ cell lines (IC_{50} values of <0.22, 0.30 ± 0.05, and 0.38 ± 0.06 μM, respectively) than rhinacanthin-Q (**3**) (IC_{50} values of 0.35 ± 0.16, 1.09 ± 0.11, and 0.97 ± 0.10 μM, respectively). This might be due to either the better electron-donating capacity of the hydroxy group or the better hydrogen-bonding ability of the hydroxy group and carbonyl group which facilitates better binding.

The X-ray crystallographic analyses of rhinacanthin-N (**2**) and rhinacanthin-Q (**3**) crystals revealed that rhinacanthin-N and rhinacanthin-Q crystals have folded structures in the crystalline state whereas NKPSL4 (**14**)

Table 4. Methylation of 3-Hydroxynaphthoquinone Esters



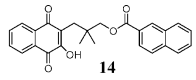
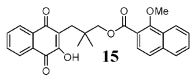
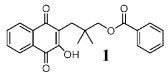
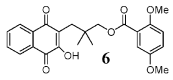
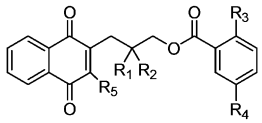
Entry	Substrate	Product	Yield (%)
1	 **14**	**39**	75
2	 **15**	**40**	92
3	 **1**	**41**	91
4	 **6**	**42**	93

Table 5. Cytotoxicities of Rhinacanthin-M (**1**) and Naphthoquinone Esters (**4**−**13**, **41**, and **42**) against Human Carcinoma Cell Lines (KB, HeLa, and $HepG_2$) and Vero Cell Line[a]



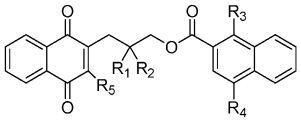
compd	R_1	R_2	R_3	R_4	R_5	cancer cell lines, IC_{50} (μM)[b] KB	HeLa	$HepG_2$	Vero cell IC_{50} (μM)[b]
1	Me	Me	H	H	OH	1.53 ± 0.34	3.02 ± 0.58	4.85 ± 1.14	48.64
4	Me	Me	OMe	H	OH	1.86 ± 0.45	4.04 ± 0.99	4.72 ± 0.43	8.98
5	Me	Me	OH	H	OH	4.65 ± 0.91	4.34 ± 0.22	5.44 ± 0.79	>135.58
6	Me	Me	OMe	OMe	OH	3.38 ± 0.76	3.42 ± 0.46	3.54 ± 0.39	9.11
7	Me	Me	OH	OMe	OH	1.18 ± 0.26	3.33 ± 0.37	1.61 ± 0.31	11.49
8	Me	Me	OH	OH	OH	64.40 ± 8.38	45.48 ± 0.00	47.56 ± 4.90	77.05
9	Me	H	H	H	OH	4.57 ± 1.27	4.76 ± 0.29	8.43 ± 0.16	2.31
10	Me	H	OMe	H	OH	1.64 ± 0.32	4.12 ± 0.32	4.04 ± 0.49	29.79
11	Me	H	OH	H	OH	4.65 ± 1.57	10.29 ± 1.88	11.62 ± 0.62	75.85
12	Me	H	OMe	OMe	OH	37.56 ± 1.34	41.87 ± 3.24	53.05 ± 7.55	4.05
13	Me	H	OH	OMe	OH	4.04 ± 0.27	4.55 ± 0.41	4.96 ± 0.26	14.02
41	Me	Me	H	H	OMe	42.99 ± 2.53	40.56 ± 2.73	46.74 ± 4.32	34.79
42	Me	Me	OMe	OMe	OMe	14.94 ± 2.80	38.81 ± 2.50	40.71 ± 2.25	13.39
adriamycin[c]						0.033	0.33	0.40	23.94

[a] KB = human epidermoid carcinoma. HeLa = human cervical carcinoma. $HepG_2$ = human hepatocellular carcinoma. Vero cell line = African green monkey kidney cell. [b] The results are the mean of six replicate determinations ± SD. [c] Used as reference.

showed an extended structure (Figures 3−5). In **2** and **3**, the R_3 and R_4 substituents are electron-donating groups that enhance the electron density of the naphthalene moiety and provide $\pi-\pi$ interaction with the naphthoquinone moiety (intramolecular charge transfer), leading to the folded conformation with an average distance between the two planes of 3.65 Å. The crystal conformations of the other naphthoates and benzoates in the series are also being investigated by X-ray crystallography.

For studying the mode of action of the naphthoquinone esters, we have done a preliminary test for topoisomerase II (Topo II) inhibitory activity of the two most active compounds (**2** and **3**) by the electrophoresis method, as shown in Figure 11. In this figure, special interest is focused on the relaxed DNA of row a. By comparison with lane 3, lanes 4−7 indicated that rhinacanthin-N and -Q inhibit Topo II activity by relaxing supercoiled DNA. So both compounds inhibited topoisomerase II. Therefore, Topo II was chosen as a target enzyme based on this preliminary result and some reports[15−17] that showed that 1,2-naphthoquinone and 1,4-naphthoquinone inhibit Topo II. Our cytotoxicity results revealed that the naphthoquinone ester containing an −OH group at C-3 of the quinone ring showed high toxicity against the cancer cell lines tested, whereas the naphthoquinone esters with a methoxy group or without an −OH group at that position showed no activity or much less cytotoxicity. So we proposed that the naphthoquinone esters with the C-3 hydroxyl group on the quinone ring could exist in two tautomers that are 1,4-naphthoquinone (A) and 1,2-naphthoquinone (B) as shown in Figure 7. We first tried docking some of our naphthoquinone esters, compared to that of adriamycin[18,19] (doxorubicin) (Figure 6). Adriamycin, a commonly used anthracycline, is of primary importance in the treatment of patients suffering from lymphomas, breast cancer, and sarcomas. Topoisomerase II is the

Table 6. Cytotoxicities of Rhinacanthin-N (**2**), Rhinacanthin-Q (**3**), and Naphthoquinone Esters (**14**−**40**) against Human Carcinoma Cell Lines (KB, HeLa, and $HepG_2$) and Vero Cell Line[a]



compd	R_1	R_2	R_3	R_4	R_5	cancer cell lines, IC_{50} (μM)[b] KB	HeLa	$HepG_2$	Vero cell IC_{50} (μM)[b]
2	Me	Me	OH	OMe	OH	<0.22	0.30 ± 0.05	0.38 ± 0.06	12.65
3	Me	Me	OMe	OMe	OH	0.35 ± 0.16	1.09 ± 0.11	0.97 ± 0.10	32.70
14	Me	Me	H	H	OH	1.59 ± 0.37	1.17 ± 0.21	1.30 ± 0.21	1.50
15	Me	Me	OMe	H	OH	<0.22	0.23 ± 0.02	0.24 ± 0.05	17.60
16	Me	Me	OH	H	OH	<0.23	<0.23	0.32 ± 0.02	1.00
17	Me	Me	OH	OH	OH	4.22 ± 0.10	12.44 ± 0.76	12.93 ± 0.76	15.42
18	Me	H	H	H	OH	3.58 ± 0.20	4.25 ± 0.42	3.70 ± 0.27	1.80
19	Me	H	OMe	H	OH	0.66 ± 0.19	0.38 ± 0.03	1.04 ± 0.10	0.97
20	Me	H	OH	H	OH	1.06 ± 0.23	3.97 ± 0.40	1.00 ± 0.08	0.70
21	Me	H	OMe	OMe	OH	3.52 ± 0.18	3.80 ± 0.38	3.65 ± 0.24	5.91
22	Me	H	OH	OMe	OH	2.16 ± 0.94	2.83 ± 1.15	3.14 ± 0.45	<0.22
23	H	H	H	H	OH	14.40 ± 1.27	15.80 ± 0.52	15.54 ± 0.00	23.96
24	H	H	OMe	H	OH	33.66 ± 6.58	42.87 ± 0.98	45.67 ± 2.15	120.19
25	H	H	OH	H	OH	12.32 ± 0.67	15.09 ± 0.54	16.16 ± 1.36	124.39
26	H	H	OMe	OMe	OH	12.78 ± 1.00	32.28 ± 6.46	35.87 ± 1.83	75.38
27	H	H	OH	OMe	OH	4.09 ± 0.35	4.24 ± 0.54	4.28 ± 0.35	40.89
28	Me	Me	H	H	H	32.04 ± 2.41	41.21 ± 3.41	45.23 ± 3.55	33.29
29	Me	Me	OMe	H	H	12.50 ± 0.98	36.92 ± 4.49	14.41 ± 0.96	30.37
30	Me	Me	OH	H	H	38.24 ± 3.59	45.89 ± 0.00	48.31 ± 1.71	90.82
31	Me	H	H	H	H	28.99 ± 9.83	38.20 ± 2.69	27.30 ± 5.26	43.40
32	Me	H	OMe	H	H	26.45 ± 2.33	39.45 ± 3.64	39.45 ± 2.50	20.24
33	Me	H	OH	H	H	38.56 ± 5.24	46.25 ± 2.09	42.08 ± 1.88	37.92
34	H	H	H	H	H	13.74 ± 1.66	12.92 ± 0.82	10.22 ± 1.67	14.86
35	H	H	OMe	H	H	50.00 ± 0.00	47.50 ± 1.58	54.17 ± 4.08	40.00
36	H	H	OH	H	H	>259.07	>259.07	>259.07	>259.07
37	H	H	OMe	OMe	H	>232.56	>232.56	>232.56	>232.56
38	H	H	OH	OMe	H	93.75 ± 13.60	45.40 ± 5.23	64.42 ± 5.21	48.48
39	Me	Me	H	H	OMe	91.51 ± 10.29	44.86 ± 1.05	127.10 ± 10.13	47.90
40	Me	Me	OMe	H	OMe	10.08 ± 0.76	9.47 ± 0.33	11.50 ± 1.09	5.96
adriamycin[c]						0.033	0.33	0.40	23.94

[a] KB = human epidermoid carcinoma. HeLa = human cervical carcinoma. $HepG_2$ = human hepatocellular carcinoma. Vero cell line = African green monkey kidney cell. [b] The results are the mean of six replicate determinations ± SD. [c] Used as reference.

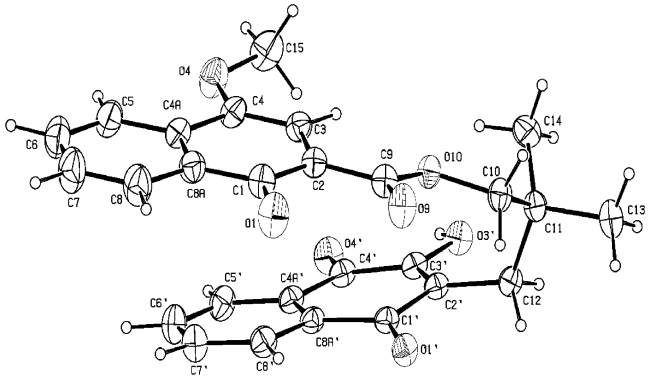


Figure 3. ORTEP plot of the X-ray crystal structure of rhinacanthin-N (**2**).

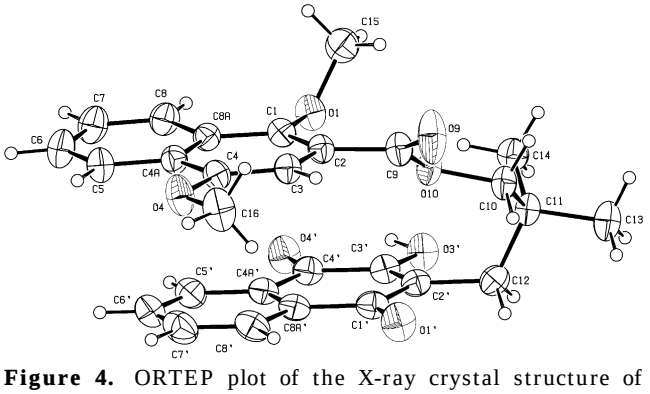


Figure 4. ORTEP plot of the X-ray crystal structure of rhinacanthin-Q (**3**).

primary cellular target for a number of clinically important antitumor agents, including adriamycin. Frydman's group[15] (1997) also reported the mechanism of inhibition of topoisomerase II by β-lapachone (1,2-naphthoquinone) and 1,4-naphthoquinone (α-lapachone) (Figure 6). They reported β-lapachone was 10-fold more reactive than 1,4-naphthoquinone. Therefore, we decided to carry out molecular modeling or docking experiments on *Saccharomyces cereviseae* topoisomerase II as the target enzyme for our cytotoxic naphthoquinone esters. There is no human Topo II crystal to do docking, but the amino acid sequences[20] of Topo II especially in their DNA-binding domains are highly conserved in eukaryotes. The DNA-binding[21] domains show 50.1% homology + 80.4% similarity between *S. cereviseae* (residues 500−990) and human (residues 512−999) (accession numbers M13814 and NP-001058). The program AUTODOCK, version 3.05,[22] was used to dock separately adriamycin (reference drug), four active compounds (**2**, **3**, **26**, and **27**), and three inactive compounds (**37**−**39**) into one of the best binding site according to PASS[23] (Figures 8−10). We have found that all active compounds have the quinone ring lying on the right-hand side and the propyl chain pointing down (Figure 9), whereas the inactive compounds bind in a different direction with the quinone ring lying on the right and the propyl chain pointing up (Figure 10), which supports the cytotoxicity result. The quinone ring of adriamycin lies on the same plane as that of our active compounds but not that of the inactive com-

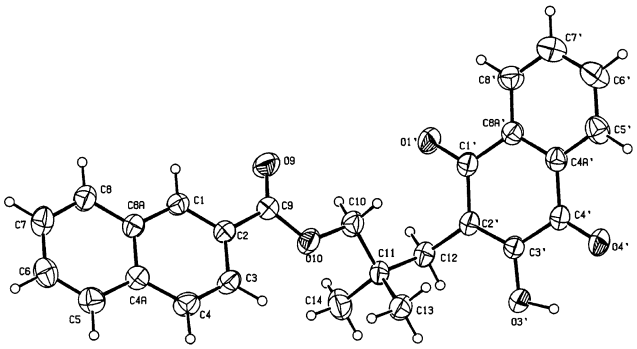
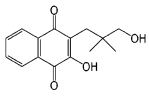
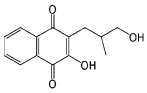
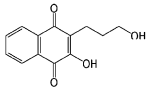
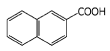
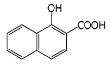
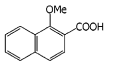
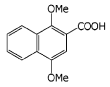


Figure 5. ORTEP plot of the X-ray crystal structure of NKPSL4 (**14**).

Table 7. Cytotoxicity of Parent Naphthoquinone Alcohols and Acids

Samples	IC_{50} values(μM)[a] against KB cell line
	11.15
	59.35
	Inactive
	Inactive
	Inactive
	Inactive
	Inactive
Adriamycin[b]	0.033

[a] The results are the mean of six replicate determinations. KB = human epidermoid carcinoma. [b] Used as reference.

pounds (see Figures 9 and 10). The details of how the naphthoquinone esters interact with topoisomerase II and/or the DNA substrate are being investigated by our group.

In addition, it is cautioned that our naphthoquinone esters might be hydrolyzed in vitro and in vivo by carboxylate esterases to the parent carboxylic acids and alcohols. It was found that the esters were not hydrolyzed in vitro, which were proved by cytotoxicity testing of parent naphthoquinone alcohols and naphthoic acids. The results (Table 7) showed that both the alcohols and acids exhibited less or no toxicity against the KB cell line.

Conclusion

Our report shows that naphthoquinone naphthoate esters exhibit a stronger cytotoxicity against the cancer

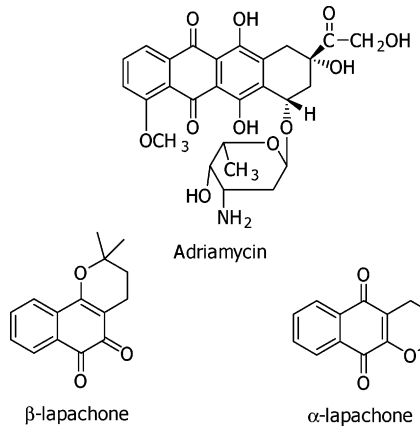


Figure 6. Structures of adriamycin, β-lapachone, and α-lapachone.

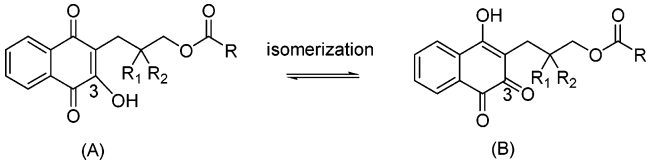


Figure 7. Two isomers of naphthoquinone esters having a C-3 hydroxy group.

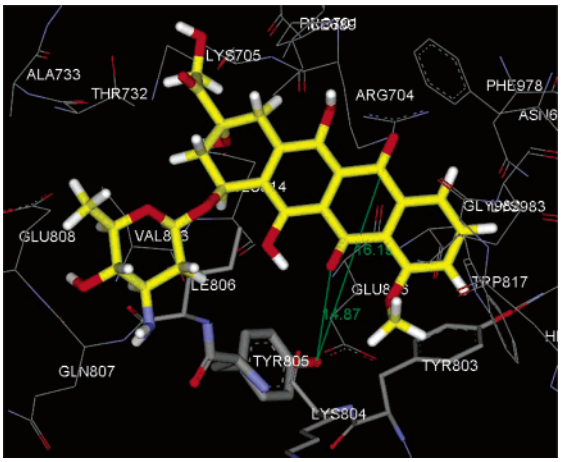


Figure 8. Adriamycin (doxorubicin) in yellow, docked into the proposed binding site.

cell lines KB, HeLa, and $HepG_2$ than the benzoate esters. Some naphthoate esters such as rhinacanthin-N (**2**), rhinacanthin-Q (**3**), and NKPSL5 (**15**) promise to possess great cytotoxicity against the said cancer cell lines and to cause little harm to the normal Vero cell line. Almost all the naphthoquinone esters that contain a C-3 hydroxy group on the naphthoquinone ring showed significant cytotoxicities against KB, HeLa, and $HepG_2$ cell lines, whereas esters with a methoxy group or without a hydroxy group at that position showed no activity or much less toxicity. In addition, two methyl substituents on the C-2′ propyl chain of the esters conferred more potent cytotoxicity against the cancer cell lines than one methyl group or without a methyl group on that position. Substituents on C-1 and C-4 positions of the naphthalene ring showed some effects on the cytotoxicity. Therefore, varying the substituents on the naphthalene moiety of the naphthoate ester supported by molecular modeling would be valuable to our investigation on the synthesis and cytotoxicity testing of rhinacanthins. In addition, we are synthesiz-

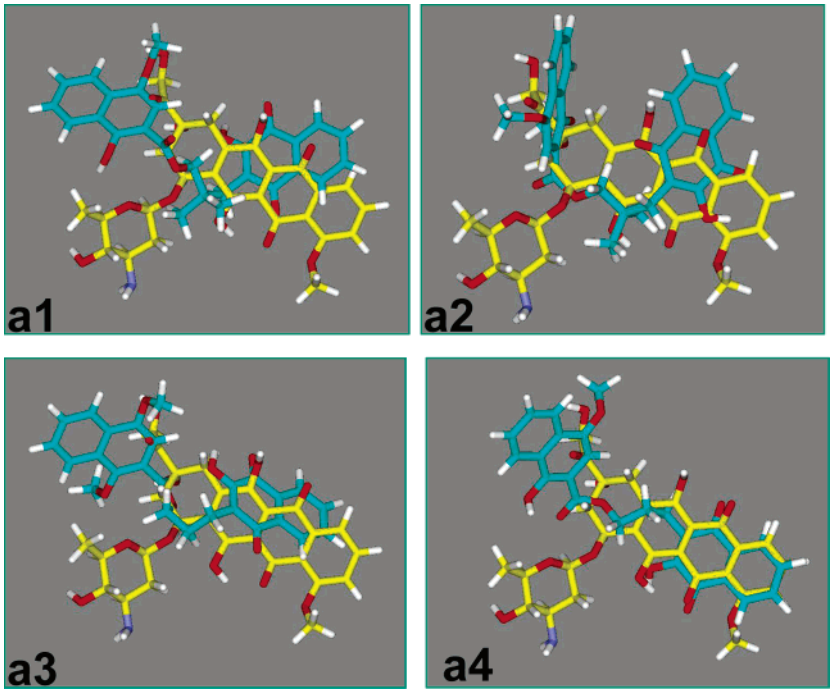


Figure 9. Four active compounds (a1 = compound **2**, a2 = compound **3**, a3 = compound **26**, and a4 = compound **27**) docked at the same site as that of adriamycin (in yellow).

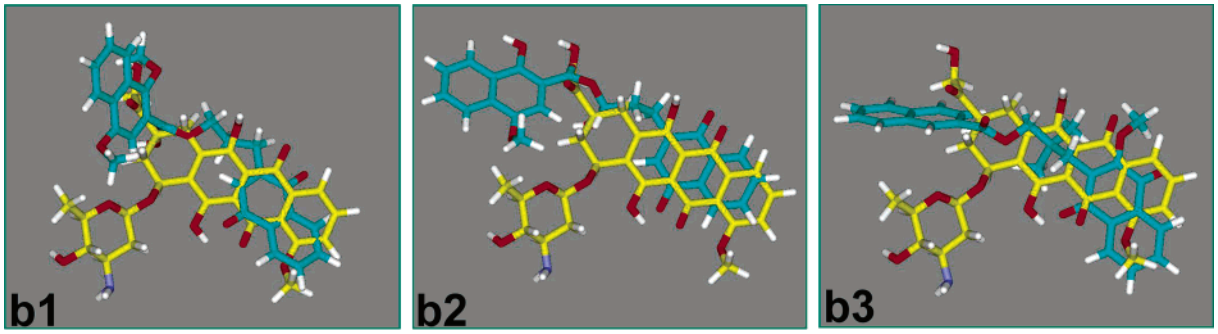


Figure 10. Three inactive compounds (b1 = compound **37**, b2 = compound **38**, and b3 = compound **39**) docked at the same site as that of adriamycin (in yellow).

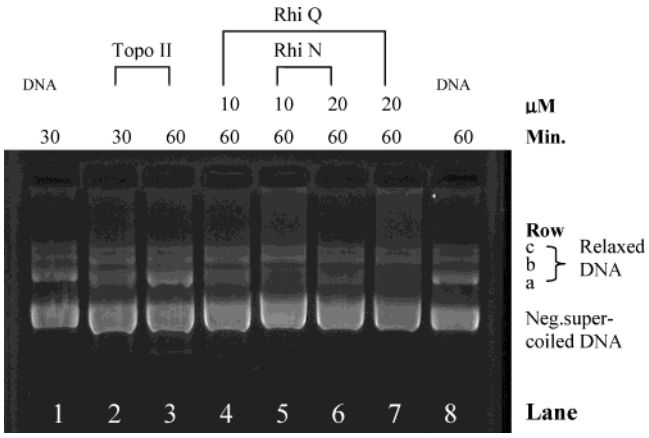


Figure 11. Inhibition of topoisomerase II. Supercoiled *p*-bluescript DNA was relaxed with Topo II in the presence or absence of the inhibitors rhinacanthin-Q (Rhi Q) and rhinacanthin-N (Rhi N) for 30 or 60 min. The figure shows inhibitor-free control (lanes 2 and 3), Rhi Q at 10 μM (lane 4) and at 20 μM (lane 7), and Rhi N at 10 μM (lane 5) and at 20 μM (lane 6).

ing naphthoquinone esters with aliphatic moiety as well as testing their cytotoxicity. Acquiring knowledge on new anticancer naphthoquinone esters including their mechanism of action challenges us to work relentlessly.

Experimental Section

General Remarks. Melting points were determined on a Fisher John apparatus and are uncorrected. The IR spectra were recorded on an FTIR Perkin-Elmer system 2000. Mass spectral data were obtained on the GCMS-QP-5050A. Nuclear magnetic resonance spectra were recorded at 400 MHz on a Brucker Advance DPX-400. Chemical shifts are given in parts per million (δ) downfield from tetramethylsilane (TMS) as internal standard. Coupling constants are given in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, dd = doublet of doublet, dt = doublet of triplet. Column chromatography was performed with flash silica gel (Merck 9385).

2-(3-Hydroxypropyl)naphthalen-1-ol (43). A 1 M solution of borane in tetrahydrofuran (7.4 mL, 7.4 mmol) was added dropwise to a stirred solution of 2-allylnaphthalen-1-ol (1.36 g, 7.4 mmol) in anhydrous tetrahydrofuran (15 mL) at room temperature under nitrogen. After the mixture was stirred at room temperature for 4 h, water (0.74 mL) was added dropwise, followed by 3 M sodium hydroxide (1 mL). Then hydrogen peroxide (40%, 1 mL) was added at such a rate that the temperature of the reaction mixture stayed between 30 and 50 °C. Following the addition, stirring was continued

for 4 h at room temperature. Diethyl ether (15 mL) was added to the reaction mixture, and the mixture was washed with brine and water. After being dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo, the residue was purified by flash column chromatography, eluting with 9:1 v/v hexane−ethyl acetate to afford the product **43** (71%) as a colorless amorphous powder, mp 86−87 °C. IR (KBr) cm^{-1}: 3481, 1513, and 1272.

2-(3-Hydroxy-2-methylpropyl)naphthalen-1-ol (44). The method for preparation of **44** is the same as described for **43**. The product **44** was obtained in 96% yield as a colorless amorphous powder, mp 89−90 °C. IR (KBr) cm^{-1}: 3376, 1384, and 1016.

2-(3-Hydroxypropyl)-[1,4]-naphthoquinone (45). To a stirred solution of compound **43** (0.2 g, 0.99 mmol) in methanol−dimethylformamide (MeOH−DMF) (3:1) (13 mL), a solution of Fremy's salt (2.7 g) in water (52 mL) and 1 M aqueous sodium acetate solution (1.44 mL) was added. After being stirred for 12 h at room temperature, the reaction mixture was extracted with diethyl ether (3 × 20 mL). The combined organic phase was washed with water and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by flash column chromatography, eluting with 4:1 v/v hexane−ethyl acetate to afford the product **45** (71%) as a yellow solid, mp 66−67 °C. IR (KBr) cm^{-1}: 3249, 1663, and 1305.

2-(3-Hydroxy-2-methylpropyl)-[1,4]-naphthoquinone (46). Compound **46** was prepared from compound **44** in the same manner as for compound **45**. The product **46** was obtained in 85% yield as a yellow oil. IR (neat) cm^{-1}: 3403, 1662, 1589, and 1029.

3,4-Dihydro-2*H*-benzo[*h*]chromene-5,6-dione (47). A mixture of compound **45** (0.2 g, 0.9 mmol), dichlorodicyanobenzoquinone (DDQ) (0.3 g, 1.4 mmol), and *p*-toluenesulfonic acid monohydrate (0.02 g, 0.09 mmol) in dry benzene (4 mL) was stirred for 30 min under reflux. Then the reaction mixture was cooled to room temperature, filtered, washed with dichloromethane, and concentrated in vacuo. The residue was filtered through aluminum oxide to afford the product **47** (95%) as an orange amorphous powder, mp 184−185 °C. IR (KBr) cm^{-1}: 1687, 1642, 1564, and 1258.

3-Methyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (48). Compound **48** was prepared from compound **46** in the same manner as for compound **47**. The product **48** was obtained in 95% yield as an orange amorphous powder, mp 148−149 °C. IR (KBr) cm^{-1}: 1688, 1645, 1256, and 1168.

2-Hydroxy-3-(3-hydroxypropyl)-[1,4]-naphthoquinone (49). A solution of compound **47** (0.2 g, 0.93 mmol) in 1% aqueous sodium hydroxide solution (6 mL) was refluxed for 2 h. Then the reaction mixture was cooled to room temperature, acidified with acetic acid, and extracted with dichloromethane (3 × 30 mL). The combined organic phase was washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was recrystallized from hexane−dichloromethane to give the product **49** (86%) as a yellow amorphous powder, mp 106−107 °C. IR (KBr) cm^{-1}: 3342, 1640, 1638, 1365, and 1272.

2-Hydroxy-3-(3-hydroxy-2-methylpropyl)-[1,4]-naphthoquinone (50). Compound **50** was prepared from compound **48** in the same manner as for compound **49**. The product **50** was obtained in 94% yield as a yellow amorphous powder, mp 146−147 °C. IR (KBr) cm^{-1}: 3485, 1656, 1371, and 1215.

^{1}H NMR, ^{13}C NMR, MS, HR-MS, and elemental analysis of compounds **43**−**50** are in Supporting Information.

General Procedure for Synthesis of Naphthoquinone Esters 3, 14, 15, 18, 19, 21, 23, 24, and 26. To a stirred solution of acid (0.3 mmol) in tetrahydrofuran (THF) (3 mL), a solution of 1,1′-carbodiimidazole (CDI) (0.3 mmol) in THF (2 mL) was added. After the mixture was stirred for 3 h at room temperature, a solution of quinone (0.2 mmol) in THF (5 mL) was added. Then it was continuously stirred for 7 h at room temperature. The reaction mixture was quenched with saturated ammonium chloride solution (30 mL) and extracted with dichloromethane (3 × 30 mL). The combined organic phase was washed with brine and water, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography.

Rhinacanthin-Q (3). Flash column chromatography eluting with 17:3 v/v hexanes−ethyl acetate afforded the product rhinacanthin-Q (**3**) (71%) as an orange amorphous powder,[2] mp 117−118 °C. IR (KBr) cm^{-1}: 3374, 1705, 1652, 1595, and 1276.

Naphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (14). Flash column chromatography eluting with 9:1 v/v hexane−ethyl acetate afforded the product **14** (87%) as a yellow amorphous powder, mp 114−115 °C. IR (KBr) cm^{-1}: 3352, 1711, 1665, 1589, 1273, and 1224.

1-Methoxynaphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (15). Flash column chromatography eluting with 17:3 v/v hexane−ethyl acetate afforded the product **15** (82%) as a yellow oil. IR (neat) cm^{-1}: 3363, 1712, 1650, 1593, 1280, and 1084.

Naphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (18). Flash column chromatography eluting with 17:3 v/v hexanes−ethyl acetate provided the product **18** (83%) as a yellow amorphous powder, mp 164−166 °C. IR (KBr) cm^{-1}: 3363, 1715, 1649, 1589, 1275, and 1224.

1-Methoxynaphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (19). Flash column chromatography eluting with 7:1 v/v hexanes−ethyl acetate afforded the product **19** (73%) as a yellow amorphous powder, mp 85−86 °C. IR (KBr) cm^{-1}: 3347, 1728, 1646, 1589, 1276, and 1225.

1,4-Dimethoxynaphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (21). Flash column chromatography eluting with 17:3 v/v hexanes−ethyl acetate afforded the product **21** (73%) as a yellow amorphous powder, mp 113−115 °C. IR (KBr) cm^{-1}: 3295, 1727, 1648, 1592, 1276, and 1223.

Naphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)propyl Ester (23). Flash column chromatography eluting with 17:3 v/v hexanes−ethyl acetate provided the product **23** (73%) as a yellow amorphous powder, mp 120−121 °C. IR (KBr) cm^{-1}: 3362, 1714, 1645, 1590, 1278, and 1226.

1-Methoxynaphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)propyl Ester (24). Flash column chromatography eluting with 9:1 v/v hexanes−ethyl acetate provided the product **24** (77%) as a yellow amorphous powder, mp 125−126 °C. IR (KBr) cm^{-1}: 3356, 1704, 1643, 1589, 1276, and 1132.

1,4-Dimethoxynaphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)propyl Ester (26). Flash column chromatography eluting with 17:3 v/v hexanes−ethyl acetate provided the product **26** (58%) as a yellow amorphous powder, mp 146−147 °C. IR (KBr) cm^{-1}: 3372, 1727, 1650, 1593, 1235, and 1097.

^{1}H NMR, ^{13}C NMR, MS, and elemental analysis of naphthoquinone esters **3**, **14**, **15**, **18**, **19**, **21**, **23**, **24**, and **26** are in Supporting Information.

General Procedure for Synthesis of Naphthoquinone Esters 1, **4**, **6**, **9**, **10**, **12**, **28**, **29**, **31**, **32**, **34**, **35**, **and 37.** To a stirred solution of acid (0.26 mmol) and 4-(dimethylamino)-pyridine (DMAP) (0.06 mmol) in dry dichloromethane (2 mL), a solution of naphthoquinone alcohol (0.2 mmol) in dry dichloromethane (2 mL) was added. The reaction mixture was stirred at room temperature for 5 min, and then a solution of dicyclohexylcarbodiimide (DCC) (0.26 mmol) in dry dichloromethane (3 mL) was added to the reaction mixture. After that, it was continuously stirred overnight at room temperature. Filtered off was the precipitate of dicyclohexyl urea, and then the organic solution was washed twice with saturated ammonium chloride solution and water, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography.

Rhinacanthin-M (1). Flash column chromatography eluting with 9:1 v/v hexanes−ethyl acetate afforded the product rhinacanthin-M (**1**) (91%) as a yellow amorphous powder,[1] mp 100−101 °C. IR (KBr) cm^{-1}: 3240, 1719, 1639, 1586, and 1271.

2-Methoxybenzoic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (4). Flash column chromatography eluting with 22:3 v/v hexane−ethyl acetate afforded the product **4** (98%) as a yellow amorphous powder, mp 122−123 °C. IR (KBr) cm^{-1}: 3243, 1687, 1640, 1590, 1303, and 1215.

2,5-Dimethoxybenzoic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (6). Flash column chromatography eluting with 17:3 v/v hexane−ethyl acetate afforded the product **6** (92%) as a yellow amorphous powder, mp 109−110 °C. IR (KBr) cm^{-1}: 3269, 1673, 1500, 1277, and 1219.

Benzoic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (9). Flash column chromatography eluting with 9:1 v/v hexane−ethyl acetate afforded the product **9** (69%) as a yellow amorphous powder, mp 144−145 °C. IR (KBr) cm^{-1}: 3351, 1719, 1651, 1589, and 1276.

2-Methoxybenzoic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (10). Flash column chromatography eluting with 22:3 v/v hexane−ethyl acetate afforded the product **10** (93%) as a yellow amorphous powder, mp 109−110 °C. IR (KBr) cm^{-1}: 3350, 1730, 1657, 1242, and 1085.

2, 5-Dimethoxybenzoic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (12). Flash column chromatography eluting with 4:1 v/v hexane−ethyl acetate afforded the product **12** (69%) as a yellow amorphous powder, mp 144−145 °C. IR (KBr) cm^{-1}: 3343, 1728, 1643, 1585, 1277, and 1216.

Naphthalene-2-carboxylic Acid 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)-1,2-dimethylpropyl Ester (28). Flash column chromatography eluting with 47:3 v/v hexane−ethyl acetate afforded the product **28** (74%) as a yellow solid, mp 93−94 °C. IR (KBr) cm^{-1}: 1711, 1657, 1591, 1281, and 1224.

1-Methoxynaphthalene-2-carboxylic Acid 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (29). Flash column chromatography eluting with 47:3 v/v hexane−ethyl acetate afforded the product **29** (98%) as a yellow solid, mp 66−68 °C. IR (KBr) cm^{-1}: 1706, 1662, 1597, 1275, and 1133.

Naphthalene-2-carboxylic Acid 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (31). Flash column chromatography eluting with 19:1 v/v hexane−ethyl acetate afforded the product **31** (83%) as a yellow oil. IR (neat) cm^{-1}: 1716, 1663, 1595, 1280, and 1094.

1-Methoxynaphthalene-2-carboxylic Acid 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (32). Flash column chromatography eluting with 47:3 v/v hexane−ethyl acetate afforded the product **32** (90%) as a yellow oil. IR (neat) cm^{-1}: 1721, 1664, 1595, 1274, and 1134.

Naphthalene-2-carboxylic Acid 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)propyl Ester (34). Flash column chromatography eluting with 9:1 v/v hexane−ethyl acetate afforded the product **34** (86%) as a yellow amorphous powder, mp 114−116 °C. IR (KBr) cm^{-1}: 1712, 1660, 1589, and 1289.

1-Methoxynaphthalene-2-carboxylic Acid 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)propyl Ester (35). Flash column chromatography eluting with 23:2 v/v hexane−ethyl acetate afforded the product **35** (85%) as a yellow amorphous powder, mp 95−96 °C. IR (KBr) cm^{-1}: 1718, 1657, 1589, 1276, 1237, and 1141.

1,4-Dimethoxynaphthalene-2-carboxylic Acid 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)propyl Ester (37). Flash column chromatography eluting with 9:1 v/v hexane−ethyl acetate afforded the product **37** (82%) as a yellow amorphous powder, mp 121−122 °C. IR (KBr) cm^{-1}: 1698, 1662, 1594, 1242, and 1104.

^1H NMR, ^{13}C NMR, MS, and elemental analysis of naphthoquinone esters **1**, **4**, **6**, **9**, **10**, **12**, **28**, **29**, **31**, **32**, **34**, **35**, and **37** are in Supporting Information.

General Procedure for Synthesis of Naphthoquinone Esters 2, 5, 7, 11, 13, 16, 20, 22, 25, 27, 30, 33, 36, and 38. To a solution of naphthoquinone ester (0.1 mmol) in dry dichloromethane (5 mL), 1 M boron tribromide in dichloromethane (0.2 mmol) was added dropwise at −78 °C, and the reaction mixture was stirred for 15 min. Then cold water was added, and the reaction mixture was extracted with dichloromethane (3 × 30 mL). The combined organic phase was washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography.

Rhinacanthin-N (2). Flash column chromatography eluting with 23:2 v/v hexanes−ethyl acetate afforded the product rhinacanthin-N (**2**) (95%) as an orange amorphous powder,[1] mp 124−125 °C. IR (KBr) cm^{-1}: 3235, 1663, 1638, 1592, and 1243.

2-Hydroxybenzoic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (5). Flash column chromatography eluting with 47:3 v/v hexane−ethyl acetate afforded the product **5** (90%) as a yellow amorphous powder, mp 110−111 °C. IR (KBr) cm^{-1}: 3358, 1670, 1641, 1589, 1363, and 1275.

2-Hydroxy-5-methoxybenzoic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (7). Flash column chromatography eluting with 9:1 v/v hexane−ethyl acetate afforded the product **7** (89%) as a yellow amorphous powder, mp 92−93 °C. IR (KBr) cm^{-1}: 3319, 1665, 1612, 1492, 1283, and 1232.

2-Hydroxybenzoic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (11). Flash column chromatography eluting with 93:7 v/v hexane−ethyl acetate afforded the product **11** (70%) as a yellow amorphous powder, mp 121−122 °C. IR (KBr) cm^{-1}: 3354, 1674, 1641, 1482, and 1222.

2-Hydroxy-5-methoxybenzoic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (13). Flash column chromatography eluting with 22:3 v/v hexane−ethyl acetate afforded the product **13** (83%) as a yellow solid, mp 142−143 °C. IR (KBr) cm^{-1}: 3357, 1677, 1645, 1492, 1273, and 1224.

1-Hydroxynaphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (16). Flash column chromatography eluting with 9:1 v/v hexane−ethyl acetate afforded the product **16** (80%) as a yellow solid, mp 162−163 °C. IR (KBr) cm^{-1}: 3327, 1661, 1580, 1257, and 1162.

1-Hydroxynaphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (20). Flash column chromatography eluting with 9:1 v/v hexane−ethyl acetate afforded the product **20** (81%) as a yellow amorphous powder, mp 191−192 °C. IR (KBr) cm^{-1}: 3363, 1658, 1593, 1259, and 1166.

1-Hydroxy-4-methoxynaphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (22). Flash column chromatography eluting with 23:2 v/v hexane−ethyl acetate afforded the product **22** (80%) as an orange solid, mp 149−151 °C. IR (KBr) cm^{-1}: 3354, 1660, 1592, 1234, and 1152.

1-Hydroxynaphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)propyl Ester (25). Flash column chromatography eluting with 23:2 v/v hexane−ethyl acetate afforded the product **25** (85%) as a yellow amorphous powder, mp 137−138 °C. IR (KBr) cm^{-1}: 3350, 1658, 1585, 1254, and 1162.

1-Hydroxy-4-methoxynaphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)propyl Ester (27). Flash column chromatography eluting with 9:1 v/v hexane−ethyl acetate afforded the product **27** (79%) as an orange solid, mp 164−165 °C. IR (KBr) cm^{-1}: 3365, 1663, 1590, 1245, and 1092.

1-Hydroxynaphthalene-2-carboxylic Acid 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (30). Flash column chromatography eluting with 24:1 v/v hexane−ethyl acetate afforded the product **30** (80%) as a

yellow amorphous powder, mp 151−152 °C. IR (KBr) cm^{-1}: 1663, 1589, 1251, and 1161.

1-Hydroxynaphthalene-2-carboxylic Acid 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)-2-methylpropyl Ester (33). Flash column chromatography eluting with 24:1 v/v hexane−ethyl acetate afforded the product **33** (84%) as a yellow amorphous powder, mp 130−131 °C. IR (KBr) cm^{-1}: 3492, 1716, 1662, 1461, 1273, and 1133.

1-Hydroxynaphthalene-2-carboxylic Acid 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)propyl Ester (36). Flash column chromatography eluting with 24:1 v/v hexane−ethyl acetate afforded the product **36** (92%) as a yellow amorphous powder, mp 173−175 °C. IR (KBr) cm^{-1}: 1662, 1593, 1254, and 1165.

1-Hydroxy-4-methoxynaphthalene-2-carboxylic Acid 3-(1,4-Dioxo-1,4-dihydronaphthalen-2-yl)propyl Ester (38). Flash column chromatography eulting with 19:1 v/v hexanes−ethyl acetate afforded the product **38** (46%) as an orange amorphous powder, mp 129−130 °C. IR (KBr) cm^{-1}: 3440, 1662, 1593, and 1240.

^{1}H NMR, ^{13}C NMR, MS, and elemental analysis of naphthoquinone esters **2**, **5**, **7**, **11**, **13**, **16**, **20**, **22**, **25**, **27**, **30**, **33**, **36**, and **38** are in Supporting Information.

General Procedure for Synthesis of Naphthoquinone Esters 8 and 17. To a solution of naphthoquinone ester (0.05 g, 0.1 mmol) in dichloromethane (5 mL) at room temperature, boron tribromide (0.05 mL, 0.5 mmol) was added dropwise, and the solution was stirred for 1 h at room temperature. Then water was added and a portion was extracted with dichloromethane (3 × 30 mL). The combined organic phase was washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography.

2,5-Dihydroxybenzoic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (8). Flash column chromatography eluting with 21:4 v/v hexane−ethyl acetate afforded the product **8** (72%) as an orange amorphous powder, mp 147−149 °C. IR (KBr) cm^{-1}: 3365, 1670, 1488, and 1213.

1,4-Dihydroxynaphthalene-2-carboxylic Acid 3-(3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (17). Flash column chromatography eluting with 17:3 v/v hexane−ethyl acetate afforded the product **17** (73%) as an orange amorphous powder, mp 160−162 °C. IR (KBr) cm^{-1}: 3372, 1662, 1634, 1593, and 1250.

^{1}H NMR, ^{13}C NMR, MS, and elemental analysis of compounds **8** and **17** are in Supporting Information.

General Procedure for Synthesis of Naphthoquinone Esters 39−42. A mixture of naphthoquinone ester (0.1 mmol), potassium carbonate (0.2 mmol), acetone (3 mL), and iodomethane (1.0 mmol) was refluxed for 3 h. Then the reaction mixture was cooled to room temperature. Water was added to the reaction mixture, and the sample was then extracted with dichloromethane, washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography.

Naphthalene-2-carboxylic Acid 3-(3-Methoxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (39). Flash column chromatography eluting with 19:1 v/v hexane−ethyl acetate afforded the product **39** (75%) as a yellow oil. IR (neat) cm^{-1}: 1715, 1667, 1597, 1462, 1275, and 1222.

1-Methoxynaphthalene-2-carboxylic Acid 3-(3-Methoxy-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (40). Flash column chromatography eluting with 19:1 v/v hexane−ethyl acetate afforded the product **40** (92%) as a yellow oil. IR (neat) cm^{-1}: 1716, 1666, 1599, 1461, 1271, and 1135.

Benzoic Acid 3-(3-Methoxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (41). Flash column chromatography eluting with 97:3 v/v hexane−ethyl acetate afforded the product **41** (91%) as a yellow oil. IR (neat) cm^{-1}: 1718, 1668, 1599, 1454, 1269, and 1114.

2,5-Dimethoxybenzoic Acid 3-(3-Methoxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-2,2-dimethylpropyl Ester (42). Flash column chromatography eluting with 9:1 v/v hexane−ethyl acetate afforded the product **42** (93%) as a yellow oil. IR (neat) cm^{-1}: 1701, 1667, 1599, 1499, 1217, and 1046.

^{1}H NMR, ^{13}C NMR, MS, and elemental analysis of naphthoquinone esters **39**−**42** are in Supporting Information.

X-ray Crystallography of Compounds 2, 3, and 14. Crystals of compounds **2**, **3**, and **14**, suitable for an X-ray structure analysis, were obtained by slow evaporation from EtOAc−hexane. For each sample, data were collected at room temperature on a Bruker-Nonius κCCD diffractometer with Mo Kα radiation (λ = 0.710 73 Å). The crystal structures were solved by direct methods using SHELXS-97, and then all atoms except hydrogen atoms were refined anisotropically on F^2 using SHELXL-97. Atomic coordinates, bond lengths, bond angles, and thermal parameters have been deposited with the Cambridge Crystallographic Data Center (CCDC 211195−211197). Copies of the data can be obtained free of charge on application to the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, U.K. (e-mail: deposite@ccdc.cam.ac.uk).

Single-Crystal X-ray Diffraction Analysis of Rhinacanthin-N (2). $C_{27}H_{24}O_7$, MW 460.482, monoclinic, $P2_1/c$, a = 9.7885(3) Å, b = 24.3474(7) Å, c = 12.7679(4) Å, β = 130.005-(1)°, V = 2330.82(12)) Å^3, Z = 4, μ = 0.095 mm^{-1}, D_x = 1.313 g·cm^{-3}, R = 0.0446 (R_w = 0.1107 for 2692 observed reflections).

Single-Crystal X-ray Diffraction Analysis of Rhinacanthin-Q (3). $C_{28}H_{26}O_7$, MW 474.509, triclinic, $P\bar{1}$, a = 8.3524(4) Å, b = 9.8595(7) Å, c = 15.2588(13) Å, α = 96.791-(3)°, β = 105.678(4)°, γ = 94.520(5)°, V = 1193.24(1) Å^3, Z = 2, μ = 0.095 mm^{-1}, D_x = 1.321 g·cm^{-3}, R = 0.0507 (R_w = 0.1253 for 2356 observed reflections).

Single-Crystal X-ray Diffraction Analysis of Compound 14. $C_{26}H_{22}O_5$, MW 414.457, monoclinic, $P2_1/c$, a = 18.8100(15) Å, b = 6.1294(3) Å, c = 22.6710(15) Å, β = 126.356-(3)°, V = 2104.9(2) Å^3, Z = 4, μ = 0.090 mm^{-1}, D_x = 1.308 g·cm^{-3}, R = 0.0454 (R_w = 0.1128 for 1937 observed reflections).

Other crystal data and structure refinements of rhinacanthin-N (**2**), rhinacanthin-Q (**3**), and compound **14** are in Supporting Information.

Cytotoxicity Assay. The MTT Colorimetric Method.[13] Compounds **1**−**42** dissolved in dimethyl sulfoxide (DMSO) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma), HeLa (human cervical carcinoma), and $HepG_2$ (human hepatocellular carcinoma) cell lines employing the colorimetric method. Adriamycin was used as the reference drug which exhibits cytotoxicity against KB, HeLa, and $HepG_2$ cell lines.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma Chemical Co.) was dissolved in saline to make a concentration of 5 mg/mL as a stock solution. Cancer cells (3 × 10^3 cells) suspended in 100 μg/well of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY) were seeded onto a 96-well culture plate (Costar, Corning Incorporated, NY 14831). After 24 h of preincubation at 37 °C in a humidified atmosphere of 5% CO_2/95% air to allow cell attachment, various concentrations of test solution (10 μL/well) as listed in Tables 5 and 6 were added and then incubated for 48 h under the above conditions. At the end of the incubation, 10 μL of tetrazolium reagent was added to each well and then incubated at 37 °C for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected by a Microplate reader (Bio-Rad, Benchmark Microplate reader) at 550 nm and for correction at 595 nm. Each determination represents the mean of six replicates. The 50% inhibition concentration (IC_{50}) was determined by curve fitting.

Electrophoresis Method for Topoisomerase II Inhibition. The method is in Supporting Information.

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, MS, HR-MS, and elemental analysis data for compounds **1**−**50**, crystal data, atomic coordinates, equivalent isotropic displacement parameters, anisotropic displacement parameters, bond lengths and angles, torsion angles, hydrogen coordinates, isotropic displacement parameters, and observed hydrogen bonds for **2**, **3**, and **14**, and electrophoresis method for topoisomerase II inhibition. This material is available free of charge via the Internet at http://pubs.acs.org. In addition, tables of coordinates, bond distances, bond angles, and anisotropic thermal parameters have been deposited with the Crystallographic Data Centre, Cambridge, CB2, 1EW, England.

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