Synthesis and Cytotoxicity of 1,2-Disubstituted Naphth[2,3-*d*]imidazole-4,9-diones and Related Compounds

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Received April 3, 1995⊗

As part of our continuing search for potential anticancer drug candidates that are selective against slowly growing solid tumors, we have synthesized several series of 1- and 2-substituted derivatives of the lead structure, 1-ethyl-2-methylnaphth[2,3-*d*]imidazole-4,9-dione (**5**). Their cytotoxic activity in the National Cancer Institute's *in vitro* cancer cell line panel is reported. In general, substitution of various alkyl, phenyl, or benzyl moieties did not improve activity, and compound **5** remains the most active naphth[2,3-*d*]imidazole-4,9-dione derivative. However, high levels of activity and selectivity were found with several related 2-(acylamino)-3-chloro-1,4-naphthoquinones (**2f**−**j**). Compound **2i**, 2-[(2-fluorophenyl)acetamido]-3-chloro-1,4-naphthoquinone, has been selected for further *in vivo* testing and as an additional lead compound for further structural modification.

Introduction

The quinone moiety is involved in a wide variety of biochemical processes[1] including electron transport and oxidative phosphorylation. Biological activities that have been reported for quinones and quinone derivatives include enzyme inhibition[2] and antibacterial,[3] antifungal,[4] and anticancer activities.[5,6] Numerous structurally diverse quinone derivatives have been synthesized and investigated; one class of such compounds is the heterocyclic quinones, including imidazole derivatives of 1,4-naphthoquinones.[7,8] Naphth[2,3-*d*]-imidazole-4,9-dione[9] and its 1-β-D-ribofuranosyl derivative[10] were prepared in a study of naphthoquinone heterocycles and were found to be effective inhibitors of hypoxanthine phosphoribosyltransferase,[10] which is involved in an initial step in purine nucleotide biosynthesis. In addition, the naphthoquinone psychorubrin was isolated as a cytotoxic natural product from *Psychotria rubra* (*Chiou Chie Mu*) (Rubiaceae) by this laboratory. Synthetic modification of this compound led to more cytotoxic derivatives.[11]

As an extension of our synthesis of cytotoxic antitumor analogs related to psychorubrin derivatives and as part of our continuing search for cytotoxic agents that are selective against slowly growing solid tumors,[12] 1-ethyl-2-methylnaphth[2,3-*d*]imidazole-4,9-dione (**5**) was synthesized and exhibited potent cytotoxicity against ovarian cancer cell lines. Therefore, several series of 1- and 2-substituted naphth[2,3-*d*]imidazole-4,9-dione derivatives were synthesized and evaluated for cytotoxic activity. This paper describes both the synthesis and cytotoxicity of these derivatives in the National Cancer Institute's (NCI) *in vitro* cancer cell line panel.

Chemistry

Scheme 1 shows two routes to 1-ethyl-2-methylnaphth[2,3-*d*]imidazole-4,9-dione (**5**) and related compounds; 2-amino-3-chloro-1,4-naphthoquinone (**1**) was used as the common starting material. In route A, **1** underwent N-acylation with acetic anhydride in the presence of H_2SO_4 to give 2-acetamido-3-chloro-1,4-naphthoquinone (**2a**). Amination of intermediate **2a** with gaseous NH_3 gave 2-acetamido-3-amino-1,4-naphthoquinone (**3a**, route A). Cyclization with NaOH or Zn/AcOH afforded 2-methyl-1*H*-[2,3-*d*]imidazole-4,9-dione (**4a**). Reaction of **4a** with NaOH and ethyl iodide gave 1-ethyl-2-methylnaphth[2,3-*d*]imidazole-4,9-dione (**5**). The structure of this known compound was confirmed by IR, ^{1}H- and ^{13}C-NMR, MS, and elemental analysis data.

Compound **5** was also prepared by a literature procedure (route B). First, **2a** was reacted with ethylamine to give 2-acetamido-3-(ethylamino)-1,4-naphthoquinone (**29**), which was then cyclized with NaOH, affording **5**. Route B gives better yields of 1,2-substituted naphth-[2,3-*d*]imidazole-4,9-diones and easier workup procedures than does route A. Also, since we wanted to study the cytotoxicity of the 2-(acylamino)-3-(alkylamino)-1,4-naphthoquinone intermediates, most of the final compounds were synthesized by route B. Accordingly, the 1-substituted-2-methylnaphth[2,3-*d*]imidazole-4,9-diones **6**−**16**, **18**, and **20**−**21** were prepared in two steps from **2a** and the appropriate alkylamine through the corresponding 2-(acylamino)-3-(alkylamino)-1,4-naphthoquinones **30**−**43**. Compounds **17**, **19**, and **20** were prepared using route A from **4a** and the appropriate alkyl halide.

Evaluation of the cytotoxicity of these 1-substituted-2-methylnaphth[2,3-*d*]imidazole-4,9-dione derivatives (**5**−**21**) showed that the *N*-ethyl analog (**5**) displays strong cytotoxicity. Therefore, we prepared a series of 1-ethyl-2-substituted-naphth[2,3-*d*]imidazole-4,9-diones (**22**−**28**) where the N-1 substituent was fixed as

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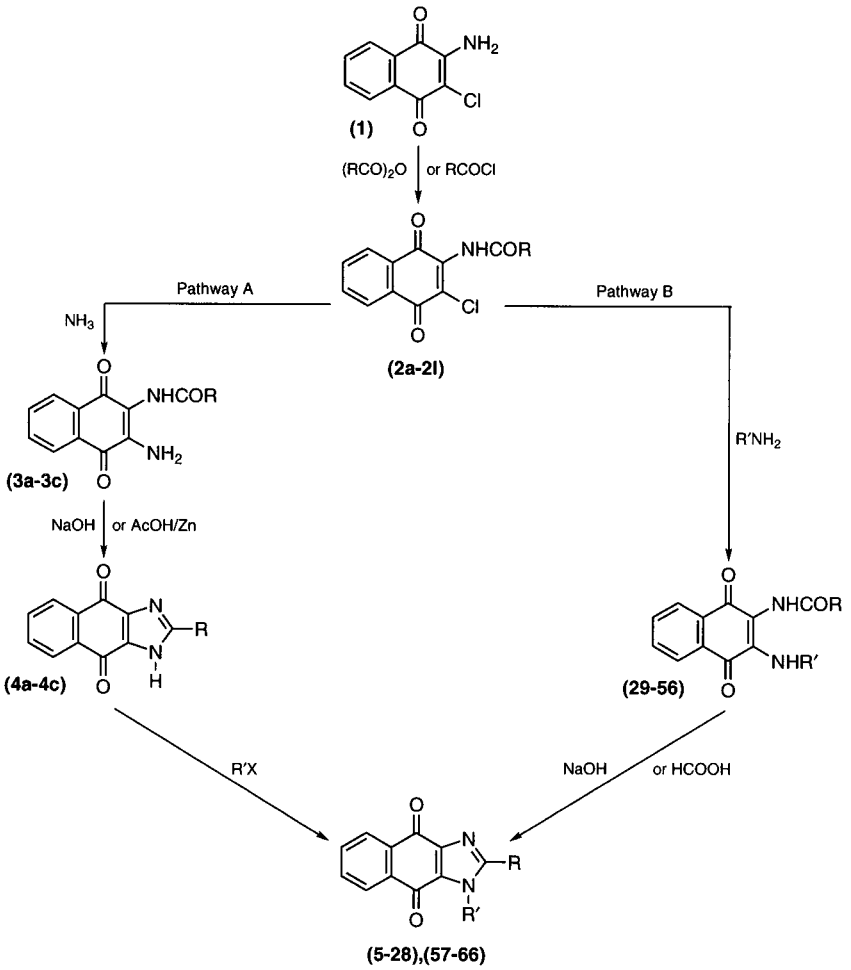
⊗ Abstract published in *Advance ACS Abstracts,* March 1, 1996.

0022-2623/96/1839-1447$12.00/0

Scheme 1



Cmpd	R	R'
2-Acylamino-3-chloro-1,4-naphthoquinones		
2a	CH_3	–
2b	CH_2Cl	–
2c	CH_2CH_3	–
2d	$CH_2CH_2CH_3$	–
2e	C_6H_5	–
2f	C_6H_4-(4-F)	–
2g	C_6H_4-(4-OCH_3)	–
2h	C_6H_3-(3,5-OCH_3)	–
2i	$CH_2C_6H_4$-(2-F)	–
2j	$CH_2C_6H_4$-(4-F)	–
2k	$(CH_2)_3COOCH_3$	–
2l	$(CH_2)_2COOCH_3$	–
2-Acylamino-3-alkyl (or aryl)amino-1,4-naphthoquinones		
3a	CH_3	–
3b	$CH_2C_6H_4$-(2-F)	–
3c	$CH_2C_6H_4$-(4-F)	–
29	CH_3	CH_2CH_3
30	CH_3	$CH(CH_3)CH_2CH_3$
31	CH_3	$CH_2CH(CH_3)_2$
32	CH_3	$C(CH_3)_3$
33	CH_3	$CH(CH_3)CH_2CH_2CH_3$
34	CH_3	$CH(CH_2CH_3)_2$
35	CH_3	$CH_2CH_2CH(CH_3)_2$
36	CH_3	$C(CH_3)_2CH_2CH_3$
37	CH_3	$CH_2CH(CH_3)CH_2CH_3$
38	CH_3	$CH_2C(CH_3)_3$
39	CH_3	$CH_2CH_2N(CH_3)_2$
40	CH_3	CH_2CH_2OH
41	CH_3	$CH_2C_6H_4$-(4-OCH_3)
42	CH_3	$CH_2C_6H_4$-(4-Cl)
43	CH_3	CH_2CH_2Cl
44	CH_2Cl	CH_2CH_3
45	CH_2CH_3	CH_2CH_3
46	$CH_2CH_2CH_3$	CH_2CH_3
47	C_6H_5	CH_2CH_3
48	C_6H_4-(4-F)	CH_2CH_3
49	C_6H_4-(4-OCH_3)	CH_2CH_3
50	C_6H_3-(3,5-OCH_3)	CH_2CH_3
51	$(CH_2)_3COOCH_3$	$CH_2C_6H_5$
52	$(CH_2)_3COOCH_3$	C_6H_5
53	$(CH_2)_3COOCH_3$	C_6H_4-(4-OCH_3)
54	$(CH_2)_2COOCH_3$	C_6H_5
55	$(CH_2)_2COOCH_3$	$CH_2C_6H_5$
56	$(CH_2)_2COOCH_3$	C_6H_4-(4-OCH_3)
1,2-Disubstituted naph[2,3-d]imidazole-4-9-diones		
4a	CH_3	–
4b	$CH_2C_6H_4$-(2-F)	–
4c	$CH_2C_6H_4$-(4-F)	–
5	CH_3	CH_2CH_3
6	CH_3	$CH(CH_3)CH_2CH_3$
7	CH_3	$CH_2CH(CH_3)_2$
8	CH_3	$C(CH_3)_3$
9	CH_3	$CH(CH_3)CH_2CH_2CH_3$
10	CH_3	$CH(CH_2CH_3)_2$
11	CH_3	$CH_2CH_2CH(CH_3)_2$
12	CH_3	$C(CH_2CH_3)_2CH_3$
13	CH_3	$CH_2CH(CH_3)CH_2CH_3$
14	CH_3	$CH_2C(CH_3)_3$
15	CH_3	$CH_2CH_2N(CH_3)_2$
16	CH_3	CH_2CH_2OH
17	CH_3	$CH_2C_6H_4$-(4-CH_3)
18	CH_3	$CH_2C_6H_4$-(4-OCH_3)
19	CH_3	$CH_2C_6H_4$-(4-F)
20	CH_3	$CH_2C_6H_4$-(4-Cl)
21	CH_3	CH_2CH_2Cl
22	CH_2Cl	CH_2CH_3
23	CH_2CH_3	CH_2CH_3
24	$CH_2CH_2CH_3$	CH_2CH_3
25	C_6H_5	CH_2CH_3
26	C_6H_4-(4-F)	CH_2CH_3
27	C_6H_4-(4-OCH_3)	CH_2CH_3
28	C_6H_3-(3,5-OCH_3)	CH_2CH_3
57	$(CH_2)_3COOH$	$CH_2C_6H_5$
58	$(CH_2)_3COOH$	C_6H_5
59	$(CH_2)_3COOH$	C_6H_4-(4-OCH_3)
60	$(CH_2)_2COOH$	C_6H_5
61	$(CH_2)_2COOH$	$CH_2C_6H_5$
62	$(CH_2)_2COOH$	C_6H_4-(4-OCH_3)
63	$CH_2C_6H_4$-(2-F)	CH_2CH_3
64	$CH(CH_2CH_3)C_6H_4$-(2-F)	CH_2CH_3
65	$CH(CH_2CH_3)C_6H_4$-(4-F)	CH_2CH_3
66	$CH_2C_6H_4$-(4-F)	CH_2CH_3

an ethyl group. The 2-alkyl compounds (**22**−**24**) were readily prepared in two steps from **2b**−**d** as shown above in route B. However, the 2-(benzoylamino)-3-chloro-1,4-naphthoquinone intermediates (**2e**−**h**) needed for the synthesis of the 2-aryl derivatives (**25**−**28**) could not be prepared under the strongly acidic conditions used in the synthesis of **2a**−**d**. (The failure of benzoyl chloride and **1** to react under these conditions has been previously reported by Hoover.[3]) Increasing the heating time also failed to give any 2-benzoylamino intermediate (**2e**). We then tried strongly basic conditions; compound **1** was treated first with an equimolar ratio of NaH and then with benzoyl chloride. Under these conditions, N-acylation occurred readily, giving an 80% yield of

2-(benzoylamino)-3-chloro-1,4-naphthoquinone (**2e**). The substituted benzoyl compounds (**2f**−**h**) could also be prepared using the same reaction conditions. Reaction of these 2-benzoylamino intermediates with the appropriate reagents then proceeded normally to give the expected products **25**−**28** using route B.

Naphth[2,3-*d*]imidazole-4,9-diones **4b** and **4c**, which contain fluorinated benzyl groups at the C-2 position and hydrogen at the N-1 position, were prepared from intermediates **2i** and **2j** using route A. Compounds **51**−**62**, which contain a terminal carboxylic acid or ester functionality in the acylamino or C-2 substitution, were prepared from intermediates **2k** and **2l** using route B. Reaction of compounds **4b** and **4c** with an equimolar ratio of both NaH and ethyl iodide resulted in ethylation at N-1 giving compounds **63** and **66**, respectively. The use of 2 molar equiv of NaH and excess ethyl iodide resulted in compounds **64** and **65**, which contain a second ethyl group at the benzyl carbon as confirmed by mass spectral and NMR analysis.

Results and Discussion

The lead compound, 1-ethyl-2-methylnaphth[2,3-*d*]-imidazole-4,9-dione (**5**), was assayed for *in vitro* cytotoxicity against KB cell growth (at the University of North Carolina) and showed good activity with an ED_{50} value of <0.4 μg/mL. This compound was then submitted to NCI for testing against a panel of 53 tumor cell lines including leukemia, non small cell and small cell lung, colon, central nervous system (CNS), ovarian, and renal cancers and melanoma. The assay results are reported both as dose−response curves and pictographically as "mean graphs". Compound **5** showed good activity and high selectivity in four ovarian cancer cell lines (log GI_{50} values ranging from −6.1 to −7.3 in OVCAR-3, −4, −5, and −8 cell lines, GI_{50} = compound concentration that inhibits 50% cell growth). Selectivity was also found in the small cell lung cancer cell line DMS-114 (log GI_{50} = −6.56). Compound **5** has been selected by NCI for testing in an *in vivo* tumor xenograft model.

On the basis of this promising activity, several series of 1- and 2-substituted naphth[2,3-*d*]imidazole-4,9-dione derivatives were synthesized and evaluated for cytotoxic activity. log GI_{50} values were determined for all final products and their parent 2-(acylamino)-3-chloro and 2-(acylamino)-3-amino compounds in ovarian cancer, non small cell lung cancer, CNS cancer, melanoma, colon cancer, leukemia, prostate cancer, small cell lung cancer, breast cancer, and renal cancer cell lines. Compounds were considered active only if their log GI_{50} values were less than −4. Certain cell lines including OVCAR-3 ovarian cancer, NCI-H522 non small cell lung cancer, several melanomas, HCT-116 colon cancer, and HL-60 leukemia showed significant sensitivity to these compound classes. Experimental data for selected compounds from each compound class in representative cell lines are shown in Table 1.

The 1,2 substituted naphth[2,3-*d*]imidazole-4,9-dione derivatives contained various alkyl, benzyl, or phenyl groups at the C-2 or N-1 positions. When the C-2 substituent was fixed as a methyl group, larger and more branched alkyl groups were inserted at the N-1 position. When compared with compound **5** (which showed log GI_{50} values in the range of −6 to −7 in most cell lines), compounds **6**−**14** showed decreased activities with log GI_{50} values generally in the range of −4 to −5 in ovarian, non small cell lung, CNS, colon, and small cell lung cancer cells. Activities for **6**−**14** were similar for melanoma and leukemia cancer cells; however, in these lines, **5** did not show activity in the range of <−4. Compounds **15**, **16**, and **21** contain N-1 ethyl groups substituted at the β position with polar moieties (dimethylamino, hydroxy, or chloro, respectively). The activities of these compounds are on the same order as compounds **6**−**14**. Four N-1 parasubstituted benzyl compounds (**17**, *p*-methylbenzyl; **18**, *p*-methoxybenzyl; **19**, *p*-fluorobenzyl; **20**, *p*-chlorobenzyl) showed slightly higher activity (log GI_{50} values of −5 to −6) in non small cell lung, CNS, colon, leukemia, small cell lung, and renal cancer and melanoma cell lines.

Table 1. Inhibition of *in Vitro* Cancer Cell Lines by Compounds **2i**, **5**, and **50**

	cytotoxicity log GI_{50} (M)[a,b]		
cell line	**2i**	**5**	**50**
ovarian			
IGROV1	−6.17	−5.69	−5.81
OVCAR-3	−6.03	−7.30	−6.28
non small cell lung			
HOP-92	−5.96	−5.79	−5.95
HCI-H522	−6.67	−6.62	−6.43
CNS			
SF-539	−6.62	−5.57	−5.72
U-251	−6.48	−5.72	−6.28
melanoma			
MALME-3M	−5.77	−5.60	−6.11
UACC-62	−6.48	−5.31	−6.45
colon			
COLO-205	−5.72	−6.45	−5.46
HCT-116	−6.37	−5.91	−6.38
leukemia			
HL-60(TB)	−6.55	−4.78	−6.39
K-562	−6.53	−5.78	−6.31
prostate			
PC-3	NT	NT	−6.42
DU-145	NT	NT	−5.57
small cell lung			
DMS 114	−6.76	−6.56	NT
DMS 273	−5.89	−5.78	NT
breast			
MCF-7	NT	−6.17	−6.43
MDA-N	NT	NT	−6.92
renal			
ACHN	−6.32	NT	−6.01
RXF-393	−6.78	−4.56	−6.49
mean value[c]	−6.08	−5.63	−5.80

[a] Data obtained from NCI's *in vitro* disease-oriented tumor cells screen (see refs 17 and 18 for details). [b] NT = not tested. [c] Mean value over all cell lines tested.

Naphth[2,3-*d*]imidazole-4,9-dione derivatives that contain a fixed N-1 ethyl substituent and different C-2 substituents were also synthesized. Compounds with ethyl (**23**), propyl (**24**), phenyl (**25**), *p*-fluorophenyl (**26**), *p*-methoxyphenyl (**27**), and 3,5-dimethoxyphenyl (**28**) groups were, in general, less active (log GI_{50} values around −5) or inactive in the cell line panel. Compound **22**, which contains a chloromethyl group at C-2 and a N-1 ethyl group, was more cytotoxic with log GI_{50} values ranging from −6 to −6.8.

The 2-(acylamino)-3-(alkylamino)-1,4-naphthoquinone intermediates (**29**−**50**) prepared by synthetic route B were also tested for their cytotoxic activity. In this series, compound **50** with a dimethoxybenzoyl group showed significant activity in all cancer cell lines (log

GI_{50} values less than −6) tested except for small cell lung cancer cells.

Biological evaluation of the starting 2-(acylamino)-3-chloro-1,4-naphthoquinones resulted in the discovery of additional promising compounds. While the 2-benzoylamino compound, **2e**, showed low activity in the cancer cell line panel (log GI_{50} values ranging from −4 to −5), addition of a *p*-fluoro (**2f**) or a *p*-methoxy (**2g**) group increased activity (log GI_{50} values generally around −6) in most cell lines. The 3,5-dimethoxy-substituted compound, **2h**, showed similar activity. Compounds **2i** and **2j** contain phenylacetamido groups fluorinated at the ortho and para positions, respectively. Both of these compounds showed high activity in many cell lines with log GI_{50} values ranging from −5.6 to −7.6.

Replacement of the 3-chloro group of **2i** and **2j** with an NH_2 group following synthetic pathway A gave compounds **3b** and **3c**; cyclization then gave the tricyclic imidazole compounds **4b** and **4c**. These compounds showed greatly reduced cytotoxicity (**3b** and **3c** were inactive in all cell lines and **4b** and **4c** had no log GI_{50} values less than −5.3) compared with the parent chloro compounds.

In general, compounds **51**−**62**, which contain terminal carboxylic groups, showed cytotoxicity similar to that of the naphth[2,3-*d*]imidazole-4,9-diones without these polar functionalities.

Compounds **63** and **66**, which contain fluorinated benzyl groups at the C-2 position, and compounds **64** and **65**, which are ethylated at the benzyl carbon, showed activity profiles similar to those of the other naphth[2,3-*d*]imidazole-4,9-diones.

In summary, the promising activity of the lead compound, **5**, in the NCI *in vitro* cancer panel prompted synthesis of 33 1,2-disubstituted naphth[2,3-*d*]imidazole-4,9-diones and 45 related compounds. One 2-(acylamino)-3-(alkylamino)-1,4-naphthoquinone, **50**, showed activity and selectivity, and high levels of activity were found with several 2-(acylamino)-3-chloro-1,4-naphthoquinones, **2f**−**j**. Compound **2i**, 2-[(2-fluorophenyl)acetamido]-3-chloro-1,4-naphthoquinone, has been selected for further *in vivo* testing. Compound **5** with an ethyl group at N-1 and a methyl group at C-2 remains the most active naphth[2,3-*d*]imidazole-4,9-dione derivative. Substitution of other alkyl groups or phenyl or benzyl moieties did not improve activity. Further work will be done to optimize the lead structures found in this data set.

Experimental Section

All melting points are uncorrected. IR spectra were recorded on a Shimadzu IR-440 spectrometer as KBr pellets. NMR spectra were obtained on JEOL FX-90Q and Varian VXR-300 FT NMR spectrometers in $CDCl_3$ (or DMSO-d_6 when so noted in Tables 1−3 in the Supporting Information) with tetramethylsilane (TMS) as an internal standard. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. Mass spectra (MS) were measured with an HP 5995 GC-MS instrument. The UV spectra were recorded on a Shimadzu UV-160A UV−visible recording spectrophotometer in EtOH. Elemental analyses were performed by National Cheng Kung University and National Chung Hsing University, Taiwan. Results obtained were within ±0.4% of the theoretical value.

2-Acetamido-3-chloro-1,4-naphthoquinone (2a). To a suspension of 2-amino-3-chloro-1,4-naphthoquinone (**1**) (52 g, 0.25 mol) in acetic anhydride (75 mL) was added 5 drops of concentrated H_2SO_4. The reaction mixture was stirred at room temperature for 20 min and then filtered. The precipitate was washed with Et_2O and recrystallized from EtOH, giving golden needle crystals (mp 219−220 °C) of **2a** in a 98% yield: ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H, $COCH_3$), 7.71 (br, 1H, NH), 7.73−7.82 (m, 2H, H-6,7), 8.10−8.13 (m, 2H, H-5), 8.18−8.20 (m, 2H, H-8); MS *m*/*z* 249.5 (M^+).

Compounds **2c** and **2d** were also prepared by this method from **1** and the appropriate acid anhydride.

2-(Chloroacetamido)-3-chloro-1,3-naphthoquinone (2b). To a suspension of 2-amino-3-chloro-1,4-naphthoquinone (**1**) (10.4 g, 0.05 mol) in anhydrous xylene (100 mL) were added 50 mL of chloroacetyl chloride and dry HCl. After a 40 min reflux, the reaction mixture cooled to room temperature for 6 h. An equal amount of Et_2O was added, and the solution sat for 1 day. The solution was then filtered, and the precipitate was recrystallized from benzene to give **2b** as light yellow needle crystals (mp 167−169 °C) in an 85% yield: ^{1}H NMR ($CDCl_3$) δ 4.24 (s, 2H, $COCH_2Cl$), 7.24−7.78 (m, 2H, H-6,7), 8.09−8.12 (m, 1H, H-5), 8.13−8.18 (m, 1H, H-8); MS *m*/*z* 284 (M^+).

Compounds **2k** and **2l** were also prepared by this method from **1** and the appropriate acid chloride.

2-(Benzoylamino)-3-chloro-1,4-naphthoquinone (2e). To a solution of 2-amino-3-chloro-1,4-naphthoquinone (**1**) (1.0 g, 4.8 mmol) in THF (50 mL) was added 0.2 g NaH at room temperature, and the reaction mixture was stirred for 30 min. Then, 1 g of benzoyl chloride was added and stirring continued for 5 min. The reaction mixture was then poured into ice water, extracted with $CHCl_3$, and evaporated. The residue was chromatographed on silica gel with benzene as eluent to give **2e** (mp 254−256 °C) in an 80% yield: ^{1}H NMR ($CDCl_3$) δ 7.25−7.45 (m, 5H, aromatic protons), 7.71−7.83 (m, 2H, H-6,7), 8.02−8.20 (m, 2H, H-5,8); MS *m*/*z* 311.5 (M^+).

Compounds **2f**−**h** were prepared in an analogous manner from **1** and the appropriate acid chlorides.

2-[(2-Fluorophenyl)acetamido]-3-chloro-1,4-naphthoquinone (2i). This compound was prepared by modification of the procedure of Hoover and Day.[3] To a stirred mixture of 2-amino-3-chloro-1,4-naphthoquinone (**1**) (16.6 g, 0.080 mol) and 2-fluorophenylacetyl chloride (17.2 g, 0.1 mol) was added 3 mL of $BF_3{\cdot}Et_2O$, and the mixture was heated under reflux for 24 h. The mixture was then concentrated under reduced pressure to give a semisolid. Acetone (50 mL) was added, and stirring was continued for 20 min under reflux. After cooling, the precipitate was collected by filtration and washed with acetone. Recrystallization from DMF:acetone (1:1) gave 18 g (66% yield) of **2i** as yellow crystals (mp 232 °C): ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 2H, $COCH_2$), 7.17 (t, 2H, H-3′,5′), 7.29−7.36 (m, 1H, H-4′), 7.40−7.46 (m, 1H, H-6′), 7.86−7.92 (m, 2H, H-6,7), 8.01−8.10 (m, 2H, H-5,8); MS *m*/*z* 343 (M^+).

Compound **2j** was synthesized using the same method.

2-Acetamido-3-amino-1,4-naphthoquinone (3a). Compound **2a** (8.5 g, 0.34 mol) was dissolved in anhydrous nitrobenzene (300 mL), dry NH_3 gas was added, and the reaction mixture was heated at reflux for 1 h. After cooling, the precipitate was filtered and recrystallized from EtOH to give dark red needle crystals (mp 233−234 °C) of **3a** in a yield of 92%: ^{1}H NMR ($CDCl_3$) δ 2.04 (s, 3H, $COCH_3$), 6.78 (br s, 2H, NH_2), 7.69−7.84 (m, 2H, H-6,7), 7.93−7.99 (m, 2H, H-5,8), 9.58 (br s, 1H, NHCO); MS *m*/*z* 230 (M^+).

Compounds **3b** and **3c** were prepared in an analogous manner from **2i** and **2j**, respectively.

2-Methyl-1*H*-naphth[2,3-*d*]imidazole-4,9-dione (4a). Method 1. To a solution of **3a** (4.0 g, 0.017 mol) in EtOH (50 mL) was added 2 N NaOH (20 mL). The reaction mixture was heated to reflux for 24 h, then cooled, and filtered. The precipitate was recrystallized from EtOH to give **4a** as a dark brown powder (mp 360−370 °C) in a 98% yield: ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3H, CH_3), 7.61−7.64 (m, 2H, H-6,7), 7.89−7.93 (m, 2H, H-5,8); MS *m*/*z* 212 (M^+).

Compounds **4b** and **4c** were synthesized in the same manner from **3b** and **3c**.

Method 2. A solution of **3a** (2.0 g, 0.0009 mol) and Zn (1.0 g) in glacial HOAc (50 mL) was refluxed for 24 h. A small amount of active charcoal was added, and the solution was filtered. The filtrate was poured into a 4-fold excess of H_2O,

the pH was adjusted to 8 with $NaHCO_3$ solution, and the solution was filtered. The precipitate was recrystallized from EtOH to give a dark brown powder (**4a**).

1-Ethyl-2-methylnaphth[2,3-*d*]imidazole-4,9-dione (5). Method 1 (Route A). A solution of **4a** (2.0 g, 9.4 mmol) in a small amount of DMF was heated to 40−50 °C; 1.0 g of NaOH was added, and the heating was continued until the NaOH had dissolved. After cooling to room temperature, an equimolar amount of EtI was added, and stirring was continued for 1 h. The reaction mixture was then poured into ice water, filtered, and purified with column chromatography ($CHCl_3$, silica gel). Recrystallization of the product (**5**) from EtOH gave yellow crystals (mp 185−186 °C, 66% yield): ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H, CH_2CH_3), 2.58 (s, 3H, CH_3), 4.44 (q, 2H, CH_2), 7.69−7.72 (m, 2H, H-6,7), 8.07−8.09 (m, 1H, H-5), 8.17−8.20 (m, 1H, H-8); MS *m*/*z* 240 (M^+).

Compounds **17**, **19**, and **20** were also prepared from **4a** using an analogous procedure.

Method 2 (Route B). To a solution of **29** (4.0 g, 0.017 mol, synthesis given below) in EtOH (50 mL) was added 2 N NaOH. The reaction mixture was heated to reflux for 30 min, then cooled, and filtered. The precipitate was washed with H_2O, dried, and recrystallized from EtOH:$CHCl_3$ to afford the same product (**5**) as obtained in method 1 in a 75% yield.

Compounds **6**−**16**, **18**, and **20** were prepared similarly in two steps from **2a** and the appropriate alkylamine through intermediates **30**−**42**. Compounds **57**−**62** were also prepared similarly from **51**−**56**.

1-(2′-Chloroethyl)-2-methylnaphth[2,3-*d*]imidazole-4,9-dione (21). A solution of **43** (5 g, 0.02 mol, prepared from **2a** and 2-chloroethylamine in an analogous manner to that of **29** given below) in formic acid (50 mL) was refluxed for 1 h and then concentrated. Purification by column chromatography ($CHCl_3$, silica gel) followed by recrystallization from benzene gave **21** as yellow crystals (mp 200−202 °C, 67% yield): ^{1}H NMR ($CDCl_3$) δ 2.64 (s, 3H, CH_3), 3.95 (t, 2H, CH_2Cl), 4.66 (t, 2H, NCH_2), 7.68−7.72 (m, 2H, H-6,7), 8.06−8.09 (m, 1H, H-5), 8.18−8.22 (m, 1H, H-8); MS *m*/*z* 274 (M^+).

Compounds **22**−**28** were synthesized by the same method from **2b**−**h** and ethylamine through intermediates **44**−**50**.

2-Acetamido-3-(ethylamino)-1,4-naphthoquinone (29). To a suspension of **2a** (5.0 g, 0.02 mol) in toluene (100 mL) was added an excess of ethylamine. The reaction mixture was stirred for 30 min at room temperature and then filtered. The precipitate was recrystallized from EtOH giving dark red crystals of **29** (mp 198−200 °C, 88% yield): ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, CH_2CH_3), 2.24 (s, 3H, $COCH_3$), 3.46 (q, 2H, CH_2), 7.58−7.72 (m, 2H, H-6,7), 8.02−8.08 (m, 2H, H-5,8); MS *m*/*z* 258 (M^+).

Compounds **30**−**43** were prepared from **2a** and the appropriate amine using the same method. Compounds **44**−**56** were prepared in an analogous manner from **2b**−**h**, **2k**, and **2l**.

1-Ethyl-2-[(2′-fluorophenyl)methyl]naphth[2,3-*d*]imidazole-4,9-dione (63). To a stirred suspension of 28.8 mg (1.2 mmol) of NaH in 1 mL of DMF was added a solution of 306 mg (1 mmol) of **4b** in 3 mL of DMF at 0 °C, and the mixture was stirred for 15 min. Ethyl iodide (0.3 mL) was added to the above mixture, and the mixture was stirred for 3 h at room temperature. Ice−water (2 mL) was added, and the mixture was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure to leave a brown oil, which was purified by flash chromatography over silica gel with *n*-hexane: EtOAc (4:1) to yield 240 mg (72%) of the title compound. Recrystallization from a mixture of $CHCl_3$:Me_2CO (1:5) gave yellow crystals: mp 176 °C; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 3H, CH_2CH_3), 4.31 (s, 2H, $CH_2C_6H_4F$), 4.47 (q, 2H, CH_2CH_3), 7.18−7.27 (m, 2H, H-3′,5′), 7.34−7.41 (m, 2H, H-4′,6′), 7.81−7.85 (m, 2H, H-6,7), 8.02−8.07 (m, 2H, H-5,8); MS *m*/*z* 334 (M^+).

By a procedure identical with that described for the synthesis of **63**, **4c** was converted to **66**. Compounds **64** and **65** were prepared in a similar manner from **4b** and **4c**, respectively, but using 2 molar equiv of NaH and excess EtI.

Acknowledgment. This work was supported by grants from the National Science Council of the Republic of China (S.C.K.) and the U.S. National Cancer Institute CA 17625 (K.H.L.).

Supporting Information Available: Yields and complete physical and spectral data for compounds **2**−**66** and data tables (log GI_{50} values) from the NCI screen for compounds **2**−**66** (25 pages). Ordering information is given on any current masthead page.

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JM950247K