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Synthesis and Cytotoxicity Evaluation of 2-Amino- and 2-Hydroxy-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole-4,9-dione Derivatives

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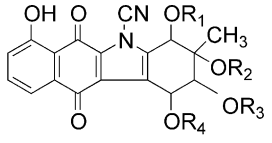
Abstract—Reaction between 2,3-dichloronaphthoquinone (**I**) and ethyl cyanoacetate or diethyl malonate under different conditions gave the starting materials, 2-chloro-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**A**) or 2-chloro-3-(diethoxy-carbonyl-methyl)-1,4-naphthoquinone (**B**). The 2-amino-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole-4,9-dione derivatives [**A-(1–10)**] and 2-hydroxy-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole-4,9-dione derivatives [**B-(1–12)**] were prepared from compounds **A** and **B**, respectively, by using various alkyl-, and arylamines. The cytotoxic activities of the prepared compounds were evaluated by SRB (Sulforhodamine B) assay against the following tumor cell lines: A459 (human lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), XF498 (human CNS), and HCT 15 (human colon). Many of the derivatives mentioned exhibited more potent cytotoxic effects against SK-OV-3 and XF498 than etoposide. Significantly, 2-amino-3-ethoxycarbonyl-*N*-(3-methyl-phenyl)-benzo[*f*]indole-4,9-dione (**A-8**) showed potent activity against all tumor cell lines, and in particular, its cytotoxic effect against SK-OV-3 was much higher than doxorubicin.

Introduction

The aminoquinones are a subject of interest because of their remarkable anticancer activities.[1,2] Moreover, the planar tri- and tetracyclic quinones, such as adriamycin have been found to act as intercalating agents.[3] It is also known that benzo[*f*]indole-4,9-dione derivatives posses versatile pharmacological activities, which include, anticancer, virus-static, antibacterial, tuberculostatic, antinaphylastatic, fungicidic, and anticoagulant effects.[4–12] Kinamycin A, B, C, and D (Fig. 1) are antibiotics that contain the benzo[*f*]indole-4,9-dione structure, a heterocycle with a pyrrole ring attached to 1,4-naphthoquinone.[13] Tolypomycin, mitomycin C, porfiromycin, actinomycin, rifamycin, and geldanamycin are examples of currently used antibiotics of this type.[14–18]

The antitumor activities of quinone derivatives have been thoroughly studied, and it is known that they act as topoisomerase inhibitors via DNA intercalation and by consuming oxido-reductase (DT-diaphorase), due to quinone reduction.[19–22]

According to the theory of Moore[23] and Pindur,[24] the DNA-intercalating molecule must have a planar tricyclic or tetracyclic ring, of length 3–4 Å and width 6–8 Å. It must also have a *p*-conjugated quinone containing nitrogen as this enables hydrogen bonding with DNA. The structure of benzo[*f*]indole-4,9-dione shown in Figure 2 illustrates a planar tri-heterocyclic ring of width 7.161 Å (C2–C7) and a *p*-conjugated ketone group. Therefore, the compounds prepared during the present study are fully consistent with the conditions prescribed for intercalator species.



	R_1	R_2	R_3	R_4
Kinamycin A	$COCH_3$	$COCH_3$	$COCH_3$	H
Kinamycin B	H	$COCH_3$	H	H
Klnamycin C	$COCH_3$	H	$COCH_3$	$COCH_3$
Kinamycin D	$COCH_3$	H	$COCH_3$	H

Figure 1. Structures of kinamycin A, B, C, and D.

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Recently, the synthesis of benzo[*f*]indole-4,9-diones was reported by Suh.[25] The compound **1** was formed when 2,3-dichloro-1,4-naphthoquinone (**I**) was reacted in sodium ethoxide with ethyl acetoacetate. Further reaction with various amines converts compound (**1**) to 2-amiαno-3-(α-aceto-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**2**). In the presence of sodium ethoxide, the nitrogen of the amino group attacks the carbonyl group of the ester, causing compound (**2**) to undergo an intramolecular cyclization to yield compound (**3**), which has a hydroxy group at 2-position of benzo[*f*]indole-4,9-dione (Scheme 1).

In this study, we designed and synthesized a new series of benzo[*f*]indole-4,9-dione derivatives. Reaction between 2,3-dichloronaphthoquinone (**I**) and ethyl cyanoacetate or diethyl malonate under different conditions gave the starting materials, 2-chloro-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**A**)[26] or 2-chloro-3-(diethoxycarbonyl-methyl)-1,4-naphthoquinone (**B**),[27] respectively. Derivatives of 2-amino-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole-4,9-dione [**A-(1–10)**] and 2-hydroxy-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole-4,9-dione [**B-(1–12)**] were then prepared by reacting compounds **A** and **B**, respectively, with various amines (Scheme 2). Prepared compounds were evaluated for cytotoxic activity by using a SRB (Sulforhodamine B) assay against the following cancer cell lines: A459 (human lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), XF498 (human CNS), and HCT 15 (human colon) and their activities compared with clinically available anticancer agents, such as, doxorubicin and etoposide.

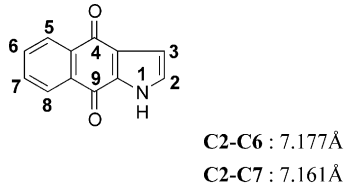
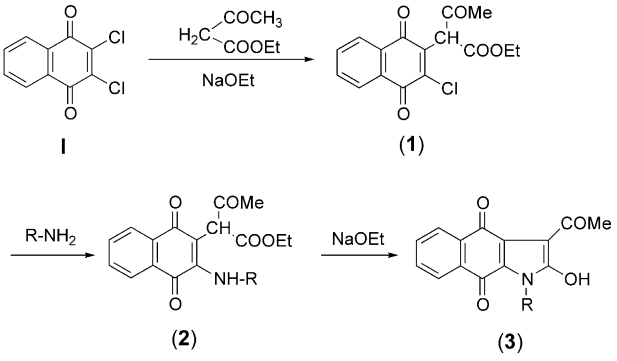


Figure 2. Structure and dimensions (Å) of benzo[*f*]indole-4,9-dione.



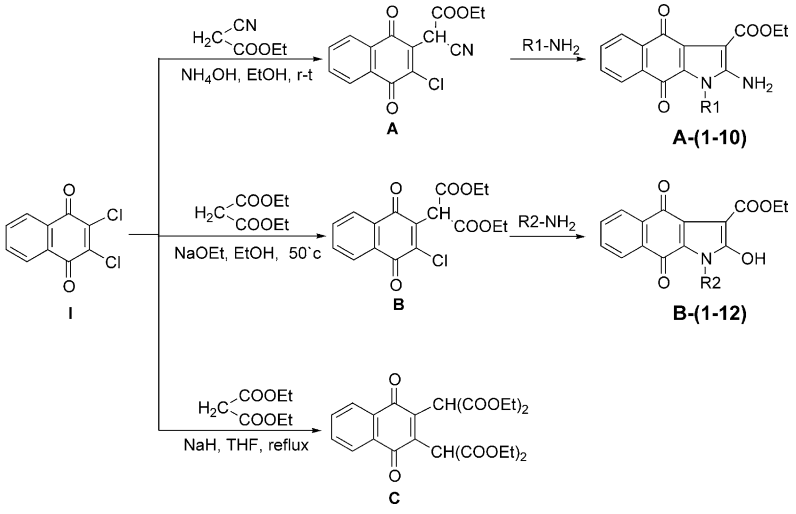
Scheme 1. The synthetic scheme of 2-hydroxy-3-methylcarbonyl-*N*-substituted-benzo[*f*]indole-4,9-dione derivatives (**3**).

Results and Discussion

Synthetic chemistry

2-Chloro-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**A**) and 2-chloro-3-(diethoxycarbonyl-methyl)-naphthoquinone-4,9-dione (**B**) were synthesized as starting materials. Scheme 2 shows that reaction between the active methylene compounds (ethyl cyanoacetate, diethyl malonate) and 2,3-dichloronaphthoquinone (**I**) required different base catalysts, solvents and reaction conditions (i.e., time, temperature, quantity of solvent). Several other approaches were used to synthesize compounds **A** and **B**, but it proved difficult to produce pure end-products. When 2,3-dichloronaphthoquinone (**I**) was reacted with diethyl malonate as an active methylene compound, under different reaction conditions, the products **B** and **C** were obtained.

The structures of compounds **A**, **B**, and **C** were determined by IR and ^{1}H NMR analysis. Compound **A** had an absorption in the region 2240 cm^{-1} (–CN) in the IR spectrum, compound **B** showed resonances at δ 1.2 (t, 6H, 2×–$COOCH_2CH_3$), and δ 4.3 (q, 4H, 2×–$COOCH_2CH_3$) by ^{1}H NMR, and compound **C** showed a double integral of compound **B**. Both compounds **A**



Scheme 2. Synthetic routes of 2-amino-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole and 2-hydroxy-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole.

and **B** were positive by Beilstein's test,[28] and therefore, contained chloride, but compound **C** was negative by the same test because both chlorides had been substituted by the diethyl malonate.

When 2-chloro-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**A**) was reacted with amines in the presence of triethylamine, as a base catalyst, nucleophilic substitution occurred, and 2-alkylamino-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone, the probable intermediate, underwent transient ring-formation to give the compounds **A-1–A-10**. The reaction mechanism seems to involve an intramolecular cyclization as shown in Scheme 3. Chemical structures of the 2-amino-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole-4,9-diones were confirmed by IR and NMR. Doublet absorption at 3400–3500 cm^{-1} in the IR spectrum and at δ 6.5–7.0 (s, 2H, $-NH_2$) in the NMR spectrum indicated the presence of a primary amine, thereby suggesting a closed ring structure. With increasing amine size, yields decreased and reaction time increased, which is believed to be related to the steric effect of the R amino group. When R was aryl, triethylamine had to be employed to form the ring-closed compound. On the other hand, when R was alkyl, the triethylamine was not required to form the closed ring. However, when triethylamine was employed as the catalyst, the reactivity of the amines improved, which in practice meant higher yields and shorter reaction times. Since it appeared that this catalytic effect was basicity related, it seemed reasonable to expect that compound **A** would react with arylamines under basic conditions to give cyclized compounds. The basic catalysts examined were ammonia solution, NaOH, KOH, triethylamine, pyridine, and sodium ethoxide. Triethylamine proved the most efficient basic catalyst examined.

When 2-chloro-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**A**) was reacted with the arylamines (*p*-anisidine or *p*-bromoaniline) without triethylamine in refluxing ethanol, 2-*N*-(4-methoxy-phenylamino)- and 2-*N*-(4-bromo-phenylamino)-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**D**, **E**) were prepared via nucleophilic substitution, and no cyclization occurred (Scheme 5). When pyridine was used as catalyst, the reactivity of the arylamines was enhanced, which does not imply ring-closure. We believe that pyridine accelerates the substitution reaction rate by entrapping the HCl evolved. We took several different approaches to prepare the closed ring products from compounds **D** and **E**, but unfortunately we failed to produce pure forms of 2-amino-3-ethoxycarbonyl-*N*-aryl-benzo[*f*]indole-4,9-naphthoquinones.

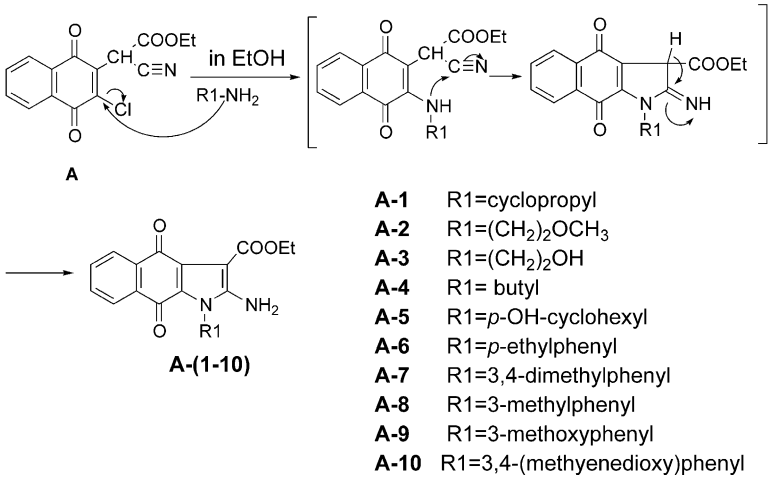
The proposed mechanism of the reaction between 2-chloro-3-(diethoxycarbonyl-methyl)-naphthoquinone-4,9-dione (**B**) with amine is illustrated in Scheme 4. The reactions between **B** and alkylamines produced closed ring compounds independently of the presence of triethylamine. Moreover, reactions of **B** with 3-hydroxypropylamine, cyclopropylamine, furfurylamine, and *trans*-4-amino-cyclohexanol gave closed ring compounds (**B-1**, **B-3**, **B-5**, and **B-7**) at room temperature.

In vitro antitumor activity evaluation by SRB assay

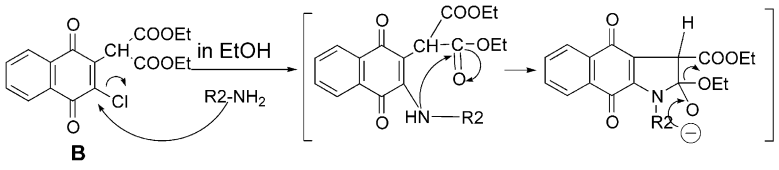
The newly obtained derivatives of benzo[*f*]indole-4,9-dione were evaluated for cytotoxic activity to cancer cell lines at the Korea Research Institute of Chemical Technology by using the sulforhodamine B (SRB) assay.[29,30] This method was developed for measuring cellular culture protein content, and involves tumor cell lines representing five different cancer types, namely, A549 (human lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), HCT 15 (human colon), and XF 498 (human CNS).

Cells were maintained as stocks in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Gibco), and cultures were passaged once or twice a week using trypsin-EDTA to detach the cells from their culture flasks.

Rapidly growing cells were harvested, counted, and incubated at the appropriate concentration ($1–2\times10^4$ cells/well) in 96-well micro plates. After being incubated for 24 h, the compounds, dissolved in culture medium, were applied to the culture wells in triplicate and

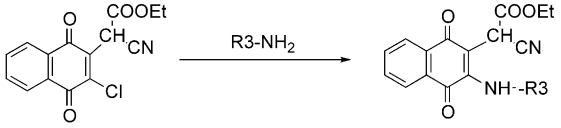


Scheme 3. Proposed mechanism of 2-amino-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole-4,9-dione derivatives.



B-1 R3=$(CH_2)_2OH$
B-2 R3=$(CH_2)_2Cl$
B-3 R3=cyclopropyl
B-4 R3= $(CH_2)_2OCH_3$
B-5 R3=furfuryl
B-6 R3=cyclohexyl
B-7 R3=*p*-OH-cylcohexyl
B-8 R3=*p*-methylphenyl
B-9 R3=*p*-NH_2-phenyl
B-10 R3=3-methylphenyl
B-11 R3=3-methoxyphenyl
B-12 R3=3,4-(methyenedioxy)phenyl

Scheme 4. Proposed mechanism of 2-hydroxy-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole-4,9-dione derivatives.



D R3=*p*-methoxy-phenyl
E R3=*p*-bromo-phenyl

Scheme 5. Structures of 2-*N*-(4-methoxy-phenylamino)- and 2-*N*-(4-bromo-phenylamino)-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**D**, **E**).

incubated for 48 h at 37 °C under a 5% CO_2 atmosphere. The cultures were fixed with cold TCA and stained with 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound stain with 10 mL of unbuffered tris base solution (pH 10.5) using a gyratory shaker, absorbance at 520 nm was measured with a microplate reader (Molecular Devices E-max, Sunnyvale, USA). Cytotoxic activity was evaluated by measuring the concentration of a compound that was required to inhibit protein synthesis by 50% (i.e., ED_{50}) as comparison. Each value shown in Table 1 represents the mean of triplicate experiments.

Many compounds prepared in this study: 2-chloro-3-(α-cyano-α-ethoxy-carbonyl-methyl)-1,4-naphthoquinone (**A**), 2-chloro-3-(diethoxycarbonyl-methyl)-naphthoquinone-4,9-dione (**B**), 2,3-di(diethoxycarbonyl-methyl)-1,4-naphthoquinone (**C**), 2-amino-3-ethoxycarbonyl-*N*-(4-hydroxy-cyclohexyl)-benzo[*f*]indole-4,9-dione (**A-5**), 2-amino-3-ethoxy-carbonyl-*N*-(3-methyl-phenyl)-benzo[*f*]indole-4,9-dione (**A-8**), 2-amino-3-ethoxycarbonyl-*N*-(3-methoxy-phenyl)-benzo[*f*]indole-4,9-dione (**A-10**), 2 - hydroxy - 3 - ethoxycarbonyl - *N* - cyclohexyl - benzo [*f*]indole-4,9-dione (**B-6**), 2-hydroxy-3-ethoxycarbonyl-*N*-(3-methyl-phenyl)-benzo[*f*]indole-4,9-dione (**B-10**), showed more potent cytotoxic activity against human ovarian tumor cells (SK-OV-3) and CNS tumor cells (XF 498) than etoposide. Of the above mentioned compounds, both compounds **A** and **A-8** exhibited inhibited SK-OV-3 more than doxorubicin, and the cytotoxic activities of compounds **A**, **A-8**, and **B-6** to A 549 cell line; **A**, **C**, and **A-8** to SK-MEL-2 cell line; and **A**, **C**, **A-8**, and **B-6** to HCT 15 cell line were greater than that of etoposide.

Summarizing, compounds **A**, **A-8**, and **B-6** exhibited greater cytotoxic activity upon the growth of a wide variety of human tumor cell lines in vitro than those of etoposide. In particular, the ED_{50} values of **A-8** to all tumor cell lines were much lower than those of etoposide and similar to those of doxorubicin. Moreover, the cytotoxicity of **A-8** against human ovarian tumor cells

Table 1. ED_{50} of benzo[*f*]indole-4,9-dione derivatives

Compd	ED_{50} (μg/mL)				
	A549	SK-OV-3	SK-MEL-2	XF 498	HCT 15
Doxorubicin	0.03	0.18	0.03	0.11	0.05
Etoposide	0.65	2.08	0.71	1.48	0.78
A	**0.34**	**0.16**	**0.13**	**0.27**	**0.10**
A-1	>30	>30	>30	>30	>30
A-2	>30	>30	>30	>30	>30
A-4	>30	>30	>30	>30	>30
A-5	1.42	**1.20**	1.00	2.49	2.13
A-6	>30	>30	>30	>30	>30
A-7	>30	>30	>30	>30	>30
A-8	**0.07**	**0.04**	**0.05**	**0.09**	**0.07**
A-9	1.25	**0.68**	0.78	**1.46**	1.74
A-10	>30	>30	>30	>30	>30
B	1.99	**1.07**	1.07	**1.48**	1.01
B-1	>30	>30	>30	>30	>30
B-2	1.00	2.34	1.59	**1.47**	2.22
B-3	6.56	9.26	8.20	8.03	9.27
B-4	5.77	4.63	3.18	8.81	8.72
B-5	2.42	3.19	2.08	2.35	3.46
B-6	**0.61**	**0.78**	0.81	**0.75**	**0.71**
B-7	12.08	9.53	6.41	18.67	9.25
B-8	24.94	16.37	12.69	25.85	23.00
B-10	1.01	**1.21**	1.20	1.67	1.21
B-12	6.79	9.67	6.81	8.88	6.26
C	1.39	**0.59**	**0.68**	**1.02**	**0.83**
D	>30	>30	>30	>30	>30
E	>30	>30	>30	>30	>30

(SK-OV-3) (ED_{50} = 0.04 μg/mL) was 4–5 times greater than that of doxorubicin (ED_{50} = 0.18 μg/mL).

Generally, compounds possessing an amino group at the 2-position of benzo[*f*]indole-4,9-dione showed more cytotoxic activity than those with a hydroxy group at the same position.

Conclusion

Reactions of 2,3-dichloronaphthoquinone (**I**) with ethyl cyanoacetate or diethyl malonate under different conditions gave the starting materials, 2-chloro-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**A**) or 2-chloro-3-(diethoxycarbonyl-methyl)-1,4-naphthoquinone (**B**), respectively. When the starting materials, compound **A** or **B** was reacted with various amines, nucleophilic substitution occurred, followed by intramolecular cyclization to give the 2-amino-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole-4,9-dione derivatives [**A-(1–10)**] or the 2-hydroxy-3-ethoxycarbonyl-*N*-substituted-benzo [*f*]indole-4,9-dione derivatives [**B-(1–12)**], respectively.

The cytotoxicities of the prepared compounds were evaluated by using a SRB assay versus doxorubicin and etoposide. Many of the compounds prepared showed more potent cytotoxic activity to human ovarian cancer cells (SK-OV-3) and CNS cancer cells (XF498) than etoposide. Compounds **A**, **A-8**, and **B-6** proved to be potent cytotoxic agents against all tumor cell lines. In particular, the cytotoxic activity of 2-amino-3-ethoxycarbonyl-*N*-(3-methyl-phenyl)-benzo[f]indole-4,9-dione (**A-8**) against human ovarian tumor cells (SK-OV-3) was 4–5 times higher than that of doxorubicin.

Generally, compounds possessing an amino at the 2-position of benzo[*f*]indole-4,9-dione had greater cytotoxicities than those with a hydroxy group at the same position. We believe that these compounds have the potential to become valuable anticancer agents and that they should be tested for in vivo antitumor activity in human cancer xenograft models.

Experimental

Materials and methods

The reagents and the solvents used in this study were of analytical grade and were used without further purification. Infrared spectra were recorded on a Perkin–Elmer Model 1420 Infrared Spectrophotometer using a pressed KBr pellet. ^{1}H NMR spectra were analyzed using a Varian Unity Innova 400 (9.4 T) Spectrometer in $CDCl_3$ using trimethylsilane as an internal standard. Melting points were determined using an Electrothermal Digital Melting Point Apparatus and are uncorrected. Elementary analysis was performed on a Thermoquest (CE Instruments) EA 1110 elemental analyzer. TLC was conducted on plastic plates precoated with Kieselgel 60 F-254 (0.2 nm, Merck), under an UV 254nm lamp. Most reagents were purchased from Aldrich and Merck.

2-Chloro-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (A). To a solution of 2,3-dichloronaphthoquinone (**I**) (1 g, 4.40 mmol) and ethyl cyanoacetate 0.6 mL (5.52 mmol) in 300 mL of ethanol, ammonia solution (40 mL) was added dropwise. The mixture was stirred at room temperature for 20 min, dil HCl was then added, and the ammonium chloride formed was removed by suction. The mixture was then extracted several times with methylenechloride, and the organic layer was washed with water, dried with anhydrous $MgSO_4$, and concentrated. The residue was recrystallized from methanol twice to give 860 mg (64%) of yellow powder: mp 121 °C; IR (KBr, cm^{-1}) 1680 (C=O), 2240 (CN); ^{1}H NMR ($CDCl_3$, δ) 1.4 (t, 3H, –COOCH$_2$CH$_3$), 4.4 (q, 2H, –COOCH$_2$CH$_3$), 5.5 (s, 1H, C3-CH), 7.9 (m, 2H, CH, C5, C6), 8.2 (m, 2H, CH, C4, C7). Anal. calcd ($C_{15}H_{10}NO_4$): C, 59.32; H, 3.32; N, 4.61. Found: C, 59.26; H, 3.03; N, 4.65.

The general procedure for 2-amino-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole-4,9-dione derivatives (A-1–A-10)

To a suspension of 2-chloro-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**A**) (500 mg, 1.65 mmol) in abs ethanol (50 mL), amine (about 3.3 mmol) was added. The reaction mixture was heated under reflux for 10–22 h, cooled, and extracted several times with methylenechloride. The organic layer was washed with water, dried over anhydrous $MgSO_4$, and concentrated. Finally, the crude product was crystallized from 95% ethanol.

2-Amino-3-ethoxycarbonyl-*N*-cyclopropyl-benzo[*f*]indole-4,9-dione (A-1). The general procedure was followed for a reaction time of 40 min with cyclopropylamine (0.24 mL, 3.38 mmol) as amine to give 130 mg (24%) of purple powder: mp 201 °C; IR (KBr, cm^{-1}): 1730 (C=O), 3400, 3500 (NH_2); ^{1}H NMR ($CDCl_3$, δ) 1.0 (m, 2H, –CHCH$_2$CH$_2$–), 1.3 (m, 2H, –CHCH$_2$CH$_2$–), 1.5 (t, 3H, –COOCH$_2$CH$_3$), 3.2 (m, 1H, –N–CH–), 4.4 (q, 2H, –COOCH$_2$CH$_3$), 6.1 (s, 2H, –NH_2), 7.6 (m, 2H, CH, C5, C6), 8.1 (m, 2H, CH, C4, C7). Anal. calcd ($C_{18}H_{16}N_2O_4$): C, 66.66; H, 4.97; N, 8.64. Found: C, 66.92; H, 5.14; N, 9.02.

2-Amino-3-ethoxycarbonyl-*N*-(2-methoxyethyl)-benzo-[*f*]indole-4,9-dione (A-2). The general procedure was followed for a reaction time of 16 h with 2-methoxyethylamine (0.3 mL, 3.4 mmol) to give 410 mg (73%) of purple powder: mp 148 °C; IR (KBr, cm^{-1}) 1750 (C=O), 3400, 3500 (NH_2); ^{1}H NMR ($CDCl_3$, δ) 1.5 (t, 3H, –COOCH$_2$CH$_3$), 3.4 (s, 3H, –O–CH$_3$), 3.8 (m, 2H, –N–CH$_2$CH$_2$–O–), 4.4 (q, 2H, –COOCH$_2$CH$_3$), 4.6 (t, 2H, –NH$_2$), 6.2 (s, 3H, –NH_2, –OH), 7.6 (m, 2H, CH, C5, C6), 8.0–8.2 (m, 2H, CH, C4, C7). Anal. calcd ($C_{18}H_{18}N_2O_5$): C, 63.15; H, 5.30; N, 8.18. Found: C, 62.99; H, 5.38; N, 8.18.

2-Amino-3-ethoxycarbonyl-*N*-(2-hydroxypropyl)-benzo-[*f*]indole-4,9-dione (A-3). The general procedure was followed for a reaction time of 21 h with triethylamine (0.5 mL) and 3-hydroxypropylamine (0.5 mL, 6.47 mmol), and crystallized from methanol to give 140 mg (25%) of

red-purple powder: mp 111–112 °C; IR (KBr, cm^{-1}) 1680 (C=O), 3400, 3450 (NH_2), 3500 (OH); ^1H NMR ($CDCl_3$, δ) 1.5 (t, 3H, –$COOCH_2\underline{CH_3}$), 2.2 (m, 2H, –N–$CH_2\underline{CH_2}CH_2$–OH), 3.7 (m, 2H, –N–$CH_2CH_2\underline{CH_2}$–OH), 4.4 (m, 2H, –N–$\underline{CH_2}CH_2CH_2$–OH), 4.6 (q, 2H, –$COO\underline{CH_2}CH_3$), 6.4 (s, 3H, –NH_2, –OH), 7.6 (m, 2H, CH, C5, C6), 8.1 (m, 2H, CH, C4, C7). Anal. calcd ($C_{18}H_{18}N_2O_5$): C, 63.15; H, 5.32; N, 8.18. Found: C, 63.14; H, 5.51; N, 7.90.

2-Amino-3-ethoxycarbonyl-*N*-butyl-benzo[*f*]indole-4,9-dione (A-4). The general procedure was followed for a reaction time of 16 h with *n*-butylamine (0.34 mL, 3.44 mmol) to give 260 mg (46%) of red powder: mp 134 °C; IR (KBr, cm^{-1}) 1750 (C=O), 3400, 3500 (NH_2); ^1H NMR ($CDCl_3$, δ) 1.0 (t, 3H, –$CH_2CH_2CH_2\underline{CH_3}$), 1.3 (m, 3H, –$CH_2CH_2\underline{CH_2}CH_3$), 1.5 (t, 3H, –$\underline{COOCH_2}CH_3$), 1.8 (t, 3H, –$\underline{CH_2CH_2}CH_2CH_3$), 4.3–4.5(m, 4H, –$COO\underline{CH_2}CH_3$, –$\underline{CH_2}CH_2CH_2CH_3$), 5.7 (s, 2H, –$NH_2$), 7.6 (m, 2H, CH, C5, C6), 8.0–8.2 (m, 2H, CH, C4, C7). Anal. calcd ($C_{19}H_{20}N_2O_4$): C, 67.05; H, 5.92; N, 8.23. Found: C, 66.38; H, 6.12; N, 8.15.

2-Amino-3-ethoxycarbonyl-*N*-(4-hydroxy-cyclohexyl)-benzo[*f*]indole-4,9-dione (A-5). The general procedure was followed for a reaction time of 5 h with *trans*-4-amino-cyclohexanol (400 mg, 2.59 mmol) and triethylamine (0.4 mL), and crystallized from ethylacetate/*n*-hexane to give 90 mg (14%) of deep-red powder: mp 217 °C; IR (KBr, cm^{-1}) 1650 (C=O), 3300, 3400 (NH_2), 3500 (OH); ^1H NMR ($CDCl_3$, δ) 1.4 (t, 3H, –$COOCH_2\underline{CH_3}$), 1.8–2.2 (m, 8H, CH_2, —N–cyclohexyl), 2.4 (m, 1H, –$\underline{CH}$–OH), 3.9 (m, 1H, –N–$\underline{CH}$–), 4.4 (q, 2H, –$COO\underline{CH_2}CH_3$), 5.8 (s, 3H, –OH, –NH_2), 7.8 (m, 2H, CH, C5, C6), 8.0 (m, 2H, CH, C4, C7). Anal. calcd ($C_{21}H_{22}N_2O_5$): C, 65.96; H, 5.90; N, 7.33. Found: C, 64.51; H, 6.02; N, 6.93.

2-Amino-3-ethoxycarbonyl-*N*-(4-ethyl-phenyl)-benzo[*f*]indole-4,9-dione (A-6). The general procedure was followed for a reaction time of 4 h with 4-ethylaniline (0.5 mL, 3.92 mmol) and triethylamine (0.4 mL) to give 380 mg (59%) of red powder: mp 218 °C; IR (KBr, cm^{-1}) 1650 (C=O), 3300, 3400 (NH_2); ^1H NMR ($CDCl_3$, δ) 1.2 (t, 3H, –$COOCH_2\underline{CH_3}$), 1.4 (t, 3H, –$CH_2\underline{CH_3}$), 2.7 (q, 2H, –$\underline{CH_2}CH_3$), 4.4 (q, 2H, –$COO\underline{CH_2}CH_3$), 5.6 (s, 2H, –NH_2), 7.2–7.4 (m, 4H, –N–phenyl), 7.5 (m, 2H, CH, C5, C6), 7.9 (dd, 2H, CH, C4), 8.1 (dd, 2H, CH, C7). Anal. calcd ($C_{23}H_{20}N_2O_4$): C, 71.12; H, 5.19; N, 7.21. Found: C, 71.22; H, 5.60; N, 7.43.

2-Amino-3-ethoxycarbonyl-*N*-(3,4-dimethyl-phenyl)-benzo[*f*]indole-4,9-dione (A-7). The general procedure was followed for a reaction time of 9 h with 3,4-dimethyl-aniline (0.4 mL, 3.30 mmol) and triethylamine (0.4 mL), and crystallized from methanol to give 110 mg (17%) of red powder: mp 239 °C; IR (KBr, cm^{-1}) 1650 (C=O), 3300, 3400 (NH_2); ^1H NMR ($CDCl_3$, δ) 1.5 (t, 3H, –$COOCH_2\underline{CH_3}$), 2.4 (m, 6H, 2×-CH_3), 4.4 (q, 2H, –$COO\underline{CH_2}CH_3$), 5.6 (s, 2H, –NH_2), 7.1–7.4 (m, 3H, –N–phenyl), 7.6 (m, 2H, CH, C5, C6), 7.9 (dd, 2H, CH, C4), 8.1 (dd, 2H, CH, C7). Anal. calcd ($C_{23}H_{20}N_2O_4$): C, 71.12; H, 5.19; N, 7.21. Found: C, 70.81; H, 5.63; N, 7.11.

2-Amino-3-ethoxycarbonyl-*N*-(3-methyl-phenyl)-benzo-[*f*]indole-4,9-dione (A-8). The general procedure was followed for a reaction time of 14 h with *m*-toluidine (0.5 mL, 3.30 mmol) and triethylamine (0.5 mL) to give 310 mg (48%) of chestnut powder: mp 209 °C; IR (KBr, cm^{-1}) 1750 (C≒O), 3350, 3450 (NH_2); ^1H NMR ($CDCl_3$, δ) 1.5 (t, 3H, –$COOCH_2\underline{CH_3}$), 3.9 (s, 3H, –CH_3), 4.4 (q, 2H, –$COO\underline{CH_2}CH_3$), 5.7 (s, 2H, –NH_2), 6.9–7.5 (m, 3H, –phenyl), 7.6 (m, 2H, CH, C5, C6), 7,9 (dd, 2H, CH, C4), 8.2 (dd, 2H, CH, C7). Anal. calcd ($C_{22}H_{18}N_2O_4$): C, 70.58; H, 4.85; N, 7.48. Found: C, 70.34; H, 4.82; N, 7.32.

2-Amino-3-ethoxycarbonyl-*N*-(3-methoxy-phenyl)-benzo-[*f*]indole-4,9-dione (A-9). The general procedure was followed for a reaction time of 19 h with *m*-anisidine (0.6 mL, 5.26 mmol) and triethylamine (0.5 mL) to give 230 mg (38%) of red powder: mp 232 °C; IR (KBr, cm^{-1}) 1700 (C=O), 3350, 3480 (NH_2); ^1H NMR ($CDCl_3$, δ) 1.4 (t, 3H, –$COOCH_2\underline{CH_3}$), 2.4 (s, 3H, –OCH_3), 4.4 (q, 2H, –$COO\underline{CH_2}CH_3$), 5.6 (s, 2H, –NH_2), 7.1–7.4 (m, 3H,–phenyl), 7.6 (m, 2H, CH, C5, C6), 7,9 (dd, 2H, CH, C4), 8.1 (dd, 2H, CH, C7). Anal. calcd ($C_{22}H_{18}N_2O_5$): C, 67.69; H, 4.65; N, 7.18. Found: C, 67.38; H, 4.70; N, 7.03.

2-Amino-3-ethoxycarbonyl-*N*-(3,4-methyenedioxy-phenyl)-benzo[*f*]indole-4,9-dione (A-10). The general procedure was followed for a reaction time of 24 h with 3,4-(methylenedioxy)aniline (600 mg, 4.33 mmol) and triethylamine (0.5 mL) to give 310 mg (47%) of dark-red powder: mp 261 °C; IR (KBr, cm^{-1}) 1680 (C=O), 3400, 3500 (NH_2); ^1H NMR ($CDCl_3$, δ) 1.4 (t, 3H, –$COOCH_2\underline{CH_3}$), 4.4 (q, 2H, –$COO\underline{CH_2}CH_3$), 5.6 (s, 2H, –NH_2), 6.1 (s, 2H, –O–CH_2–O–), 6.8–7.0 (m, 3H, –N–aromatic), 7.6 (m, 2H, CH, C5, C6), 8.0 (dd, 1H, CH, C4), 8.2 (dd, 1H, CH, C7). Anal. calcd($C_{22}H_{16}N_2O_6$): C, 65.34; H, 3.99; N, 6.93. Found: C, 65.30; H, 4.13; N, 6.65.

2-Chloro-3-(diethoxycarbonyl-methyl)-1,4-naphthoquinone (B). After metal sodium (1.2 g, 0.052 mol) was added to abs ethanol (25 mL) and dissolved, it was warmed and stirred at 50 °C for 1 h. And in this mixture diethylmalonate (12 mL, 0.075 mol) was dropped. The reaction mixture was added very slowly with stirring at 0 °C to a suspension of 2,3-dichloro-naphthoquinone (**I**) (10.0 g, 0.044 mol) in abs ethanol (150 mL). The solution was then stirred at 0 °C in ice bath for an additional 7 min and poured into water (175 mL). The filtered precipitate was removed, acetic acid was added to the solution to pH 6, and the solution allowed to solidify on standing overnight. The precipitate formed was collected by suction and recrystallized twice from ethanol to give 4.14 g (27%) of bright yellow powder: mp 102 °C; IR (KBr, cm^{-1}) 1780 (C=O); ^1H NMR ($CDCl_3$, δ) 1.3 (t, 6H, 2× –$COOCH_2\underline{CH_3}$), 4.5 (q, 4H, 2×–$COO\underline{CH_2}CH_3$), 5.1 (s, 1H, C3-CH), 7.8 (m, 2H, CH, C5, C6), 8.2 (m, 2H, CH, C4, C7). Anal. calcd ($C_{17}H_{15}O_6Cl$): C, 58.21; H, 4.31. Found: C, 58.19; H, 4.21.

The general procedure for 2-hydroxy-3-ethoxycarbonyl-*N*-substituted-benzo[*f*]indole-4,9-dione derivatives (B-1–B-12). Amine (about 2.8 mmol) was added to a suspension of 2-chloro-3-(diethoxycarbonyl-methyl)-naphthoquinone-4,9-dione (**B**) (500 mg, 1.43 mmol) in abs ethanol (50 mL). The reaction mixture was heated under reflux for 5–24 h, cooled, and extracted several times with methylenechloride. The organic layer was washed with water, dried over anhydrous $MgSO_4$, and concentrated. Crude product was crystallized from 95% ethanol.

2-Hydroxy-3-ethoxycarbonyl-*N*-(2-hydroxyethyl)-benzo[*f*]indole-4,9-dione (B-1). The general procedure was followed for a reaction time of 10 h with ethanolamine (0.5 mL, 4.45 mmol) to give 110 mg (22%) of orange powder: mp 186 °C; IR (KBr, cm^{-1}) 1700 (C=O), 3500(OH); ^1H NMR ($CDCl_3$, δ) 1.5 (t, 3H, –$COOCH_2CH_3$), 4.0 (t, 2H, –N–CH_2CH_2OH), 4.4 (q, 2H, –$COOCH_2CH_3$), 4.6 (t, 2H, –N–CH_2CH_2OH), 7.6 (m, 2H, CH, C5, C6), 8.0–8.2 (m, 2H, CH, C4, C7), 11.8 (s, H,-OH). Anal. calcd ($C_{17}H_{15}NO_6$): C, 62.00; H, 4.59; N, 4.25. Found: C, 61.50; H, 4.51; N, 4.25.

2-Hydroxy-3-ethoxycarbonyl-*N*-(2-chloroethyl)-benzo[*f*]indole-4,9-dione (B-2). The general procedure was followed for a reaction time of 31 h with 2-chloroethylamine (500 mg, 4.31 mmol) and triethylamine (0.5 mL) to give 250 mg (51%) of bright yellow powder: mp 246 °C; IR (KBr, cm^{-1}) 1750(C=O), 3450 (OH); ^1H NMR ($CDCl_3$, δ) 2.4 (t, 3H, –$COOCH_2CH_3$), 4.4 (q, 2H, –$COOCH_2CH_3$), 4.8 (m, 2H, –N–CH_2CH_2Cl), 5.2 (m, 2H, –N–CH_2CH_2Cl), 7.6 (m, 2H, CH, C5, C6), 8.0 (dd, H, CH, C4), 8.2 (dd, H, CH, C7), 11.6 (s, H, –OH).

2-Hydroxy-3-ethoxycarbonyl-*N*-cyclopropyl-benzo[*f*]indole-4,9-dione (B-3). The general procedure was followed for a reaction time of 16 h with cycropropylamine (0.4 mL, 5.6 mmol), and the crude product was crystallized from ethylacetate to give 160 mg (18%) of golden-yellow powder: mp 226–227 °C; IR (KBr, cm^{-1}) 1680 (C=O), 3450(OH); ^1H NMR ($CDCl_3$, δ) 1.1 (m, 2H, –$CHCH_2CH_2$–), 1.3 (m, 2H, –$CHCH_2CH_2$–), 1.5 (t, 3H, –$COOCH_2CH_3$), 3.4 (m, 1H, –N–CH–), 4.5 (q, 2H, –$COOCH_2CH_3$), 7.7 (m, 2H, CH, C5, C6), 8.2 (m, 2H, CH, C4, C7), 11.8 (s, 1H,-OH). Anal. calcd ($C_{18}H_{15}NO_5$): C, 66.46; H, 4.65; N, 4.31. Found: C, 66.60; H, 5.02; N, 4.29.

2-Hydroxy-3-ethoxycarbonyl-*N*-(2-methoxyethyl)-benzo[*f*]indole-4,9-dione (B-4). The general procedure was followed for a reaction time of 16 h with 2-methoxyethylamine (0.25 mL, 2.85 mmol) to give 90 mg (19%) of golden-yellow powder: mp 153–154 °C; IR (KBr, cm^{-1}) 1670 (C=O), 3500 (OH); ^1H NMR ($CDCl_3$, δ) 1.5 (t, 3H,–$COOCH_2CH_3$), 3.4 (s, 3H, –O–CH_3), 3.8 (t, 2H, –N–CH_2CH_2–O–), 4.5 (q, 2H, –$COOCH_2CH_3$), 4.7 (t, 2H, –N–CH_2CH_2–O–), 7.6 (m, 2H, CH, C5, C6), 8.0–8.2 (m, 2H, CH, C4, C7), 11.6 (s, 1H, –OH). Anal. calcd ($C_{18}H_{17}NO_6$): C, 62.97; H, 4.99; N, 4.08. Found: C, 63.26; H, 4.93; N, 4.20.

2-Hydroxy-3-ethoxycarbonyl-*N*-furfuryl-benzo[*f*]indole-4,9-dione (B-5). The general procedure was followed for a reaction time of 16 h with furfurylamine (0.4 mL, 4.28 mmol) to give 80 mg (16%) of golden colored powder: mp 164 °C; IR (KBr, cm^{-1}) 1680 (C=O), 3500 (OH); ^1H NMR ($CDCl_3$, δ) : 1.5 (t, 3H, –$COOCH_2CH_3$), 4.5 (q, 2H, –$COOCH_2CH_3$), 5.6 (s, 2H, –N–CH_2–), 6.3 (q, 1H, CH, C3 at furan), 6.5 (d, 1H, CH, C4 at furan), 7.3 (t, 1H, CH, C2 at furan), 7.9 (m, 2H, CH, C5, C6), 8.1 (m, 2H, CH, C4, C7), 11.6 (s, 1H, –OH). Anal. calcd ($C_{20}H_{15}NO_6$): C, 65.75; H, 4.14; N, 3.83. Found: C, 65.16; H, 4.09; N, 3.78.

2-Hydroxy-3-ethoxycarbonyl-*N*-cyclohexyl-benzo[*f*]indole-4,9-dione (B-6). The general procedure was followed for a reaction time of 16 h with cyclohexylamine (0.5 mL, 4.39 mmol) and triethylamine (0.5 mL), and the crude product was crystallized from ethylacetate/*n*-hexane to give 150 mg (28%) of violet powder: mp 219 °C; IR (KBr, cm^{-1}) 1650 (C=O), 3400(OH); ^1H NMR ($CDCl_3$, δ) 1.4 (t, 3H,–$COOCH_2CH_3$), 1.8–2.4 (m, 10H, –N–cyclohexyl), 4.5 (q, 2H, –$COOCH_2CH_3$), 7.8 (m, 2H, CH, C5, C6), 8.1 (m, 2H, CH, C4, C7), 11.9 (s, 1H, –OH)

2-Hydroxy-3-ethoxycarbonyl-*N*-(4-hydroxy-cyclohexyl)-benzo[*f*]indole-4,9-dione (B-7). The general procedure was followed for a reaction time of 25 h with *trans*-4-amino-cycylohexanol (500 mg, 3.20 mmol) and triethylamine (0.4 mL), and crystallized from ethylacetate to give 120 mg (21%) of bright yellow powder: mp 268 °C; IR (KBr, cm^{-1}) 1700 (C=O), 3500(OH); ^1H NMR ($CDCl_3$, δ) 1.5 (t, 3H, –$COOCH_2CH_3$), 2.0–2.6 (m, 9H, CH_2, –N–cyclohexyl, –CH–OH), 3.9 (m, 1H, –N–CH–), 4.5 (q, 2H, –$COOCH_2CH_3$), 7.7 (m, 2H, CH, C5, C6), 8.0–8.2 (m, 2H, CH, C4, C7), 11.9 (s, 1H, –OH). Anal. calcd ($C_{21}H_{21}NO_6$): C, 65.59; H, 5.52; N, 3.65. Found: C, 65.23; H, 5.33; N, 3.67.

2-Hydroxy-3-ethoxycarbonyl-*N*-(4-methyl-phenyl)-benzo[*f*]indole-4,9-dione (B-8). The general procedure was followed for a reaction time of 6 h with *p*-toluidine (300 mg, 2.28 mmol) and triethylamine (0.5 mL) to give 120 mg (11%) of deep yellow powder: mp 213 °C; IR (KBr, cm^{-1}) 1680 (C=O), 3500 (OH); ^1H NMR ($CDCl_3$, δ) 1.5 (t, 3H, –$COOCH_2CH_3$), 2.5 (s, 3H, Ar –CH_3), 4.6 (q, 2H, –$COOCH_2CH_3$), 7.2–7.4 (m, 4H, –N–phenyl), 7.7 (m, 2H, CH, C5, C6), 8.0–8.2 (m, 2H, CH, C4, C7), 11.8 (s, 1H, –OH). Anal. calcd ($C_{22}H_{17}NO_5$): C, 70.39; H, 4.56; N, 3.73. Found: C, 69.60; H, 4.57; N, 3.73.

2-Hydroxy-3-ethoxycarbonyl-*N*-(4-amion-phenyl)-benzo[*f*]indole-4,9-dione (B-9). The general procedure was followed for a reaction time of 10 h with 1,4-phenylenediamine (300 mg, 3.35 mmol) and triethylamine (0.4 mL) to give 480 mg (45%) of blue-violet powder: mp >270 °C; IR (KBr, cm^{-1}) 1700 (C=O), 3300, 3400(NH_2), 3500 (OH); ^1H NMR ($CDCl_3$, δ) 1.2 (t, 3H, –$COOCH_2CH_3$), 3.7 (s, 2H, –NH_2), 4.2 (q, 2H, –$COOCH_2CH_3$), 6.6–7.0 (m, 4H, –N–phenyl), 7.6–8.2 (m, 4H, CH, aromatic), 11.7 (s, 1H, –OH).

2-Hydroxy-3-ethoxycarbonyl-*N*-(3-methyl-phenyl)-benzo[*f*]indole-4,9-dione (B-10). The general procedure was followed for a reaction time of 37 h with *m*-toluidine

(0.7 mL, 6.42 mmol) and triethylamine (0.7 mL), and the product was crystallized from ethylacetate to give 110 mg (21%) of deep yellow powder: mp 264 °C; IR (KBr, cm^{-1}) 1700 (C=O), 3500(OH); ^1H NMR ($CDCl_3$, δ) 1.5 (t, 3H, $-COOCH_2\underline{CH_3}$), 2.4 (s, 3H, $-CH_3$), 4.5 (q, 2H, $-COO\underline{CH_2}CH_3$), 7.1–7.4 (m, 4H, –N–phenyl), 7.6 (m, 2H, CH, C5, C6), 8.0–8.2 (m, 2H, CH, C4, C7), 13.5 (s, 1H,-OH). Anal. calcd ($C_{22}H_{17}NO_5$): C, 70.39; H, 4.56; N, 3.73. Found: C, 70.32; H, 4.72; N, 3.98.

2-Hydroxy-3-ethoxycarbonyl-*N*-(3-methoxy-phenyl)-benzo[*f*]indole-4,9-dione (B-11). The general procedure was followed for a reaction time of 20 h with *m*-anisidine (0.7 mL, 6.13 mmol) and triethylamine (0.7 mL), and the product was crystallized from ethylacetate to give 160 mg (29%) of deep yellow powder: mp 217 °C; IR (KBr, cm^{-1}) 1680 (C=O), 3500 (OH); ^1H NMR ($CDCl_3$, δ) 1.5 (t, 3H, $–COOCH_2\underline{CH_3}$), 3.9 (s, 3H, $–O–CH_3$), 4.5 (q, 2H, $–COO\underline{CH_2}CH_3$), 7.0–8.2 (m, 8H, CH, –N–phenyl, C4, C5, C6, C7), 12.0 (s, 1H, –OH). Anal. calcd ($C_{22}H_{17}NO_6$): C, 67.51; H, 4.38; N, 3.58. Found: C, 66.87; H, 4.38; N, 4.04.

2-Hydroxy-3-ethoxycarbonyl-*N*-(3,4-methylenedioxy-phenyl)-benzo[*f*]-indole-4,9-dione (B-12). The general procedure was followed for a reaction time of 29 h with 3,4-(methylenedioxy)aniline (500 mg, 3.61 mmol) and triethylamine (0.7 mL) to give 70 mg (12%) of deep yellow powder: mp 262 °C; IR (KBr, cm^{-1}) 1700 (C=O), 3500 (OH); ^1H NMR ($CDCl_3$, δ) 1.5 (t, 3H, $–COOCH_2\underline{CH_3}$), 4.5 (q, 2H, $–COO\underline{CH_2}CH_3$), 6.1 (s, 2H, $O–\underline{CH_2}–O–$), 6.8–7.0 (m, 3H, –N–aromatic), 7.8 (m, 2H, CH, C5, C6), 8.0 (dd, 1H, CH, C4), 8.2 (dd, 1H, CH, C7), 11.6 (s, 1H,-OH). Anal. calcd ($C_{22}H_{15}NO_7$): C, 65.19; H, 3.73; N, 3.46. Found: C, 64.74; H, 3.81; N, 3.60.

2,3-i(Diethoxycarbonyl-methyl)-1,4-naphthoquinone (C). To a suspension of sodium amide 8.0 g (0.352 mol) in THF (70 mL) at 0 °C, diethyl malonate (28 mL, 0.176 mol) was added slowly and then refluxed for about 2 h. The reaction mixture was added very slowly with stirring at 0 °C in ice-bath to a suspension of 2,3-dichloro-naphthoquinone (**I**) (20.0 g, 0.088 mol) in THF (50 mL). The solution was then stirred at 0 °C for an additional 10 min, after dil HCl added to pH 2–3, and the solution extracted with methylenechloride. The organic layer was washed with water, dried over anhydrous $MgSO_4$, and concentrated. The residue was crystallized from ethanol. After cooling for 20 min, the solution was purified by filtration and allowed to cool to produce bright yellow crystal. The crystals were recrystallized three times from ethyl acetate and *n*-hexane to give 7.27 g (24%) of yellow powder: mp 96–97deg;C; IR (KBr, cm^{-1}) 1700 (C=O); ^1H NMR ($CDCl_3$, δ) 1.2 (t, 12H, $4\times–COOCH_2\underline{CH_3}$), 4.2 (m, 8H, $4\times–COO\underline{CH_2}CH_3$), 5.2 (s, 2H, $2\times–\underline{CH}$, C2, C3), 7.8 (m, 2H, CH, C5, C6), 8.2 (m, 2H, CH, C4, C7). Anal. calcd ($C_{24}H_{26}O_6$): C, 60.76; H, 5.52. found C, 60.89; H, 5.82.

2-*N*-(4-Methoxy-phenylamino)-3-(α-cyano-α-tethoxycarbonyl-methyl)-1,4-naphthoquinone (D). To a suspension of 2-chloro-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**A**) (1.0 g, 3.29 mmol) in abs ethanol (50 mL), *p*-anisidine (400 mg, 3.35 mmol) was added. The reaction mixture was stirred at room temperature for 18 h, cooled, and the precipitate was filtered and washed with ethanol. The filtered solid was purified by recrystallization from ethylacetate/*n*-hexane to give 580 mg (45%) of dark red powder: mp 187–188 °C; IR (KBr, cm^{-1}) 1740 (C=O), 2250 (CN), 3300 (NH); ^1H NMR ($CDCl_3$, δ) 1.3 (t, 3H, $–COOCH_2\underline{CH_3}$), 3.8 (s, 3H, $–O–CH_3$), 4.1–4.3 (m, 3H, $–COO\underline{CH_2}CH_3$, C3-CH), 7.0–7.3 (m, 4H, –N–phenyl), 7.7–7.9 (m, 2H, CH, C5, C6), 8.0–8.2 (m, 2H, CH, C4, C7). Anal. calcd ($C_{22}H_{18}N_2O_5$): C, 67.69; H, 4.65; N, 7.18. Found: C, 68.27; H, 4.64; N, 7.18.

2-*N*-(4-Methoxy-phenylamino)-3-(α-cyano-α-etethoxy-carbonyl-methyl)-1,4-naphthoquinone (E). To a suspension of 2-chloro-3-(α-cyano-α-ethoxycarbonyl-methyl)-1,4-naphthoquinone (**A**) (1.0 g, 3.29 mmol) in abs ethanol (50 mL), *p*-bromoaniline (800 mg, 4.65 mmoml) was added. The reaction mixture was refluxed for 16 h, concentrated, and cooled. The precipitate was filtered, washed with ethanol and crystallized from dioxane. The crystals so obtained were recrystallized from *n*-hexane to give 280 mg (19%) of orange powder: mp >270 °C; IR (KBr, cm^{-1}) 1750(C=O), 2350(CN), 3350(NH); ^1H NMR ($CDCl_3$, δ) 1.4 (t, 3H, $–COOCH_2\underline{CH_3}$), 4.4 (m, 2H, $–COO\underline{CH_2}CH_3$), 7.0–8.2 (m, 8H, –N–phenyl, aromatic), 9.9 (s, 1H, –NH–). Anal. calcd ($C_{21}H_{15}N_2O_4Br$): C, 57.42; H, 3.44; N, 6.38. Found: C, 57.66; H, 3.08; N, 6.32.

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