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Synthesis and Cytotoxicity Evaluation of Pyridin[2,3-*f*]indole-2,4,9-trione and Benz[*f*]indole-2,4,9-trione Derivatives

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Abstract—3-Ethoxycarbonyl-3-methyl-1*N*-substrituted-2,3-dihydro-pyridin[2,3-*f*]indole-2,4,9-trione [**9(a–d)**] and 3-ethoxycarbonyl-3-methyl-*N*-substituted-2,3-dihydro-benz[*f*]indole-2,4,9-trione [**10(a–i)**] derivatives were synthesized from 7-chloro-6-(1,1-diethoxycarbonyl-ethyl)-5,8-quinolinedione (**7**) and 2-chloro-3-(1,1-diethoxycarbonyl-ethyl)-1,4-naphthoquinone (**8**), respectively, using a variety of alkyl- and arylamines. The cytotoxic activities of the synthesized compounds were evaluated by a Sulforhodamine B (SRB) assay against the following tumor cell lines: A459 (human non-small cell lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), XF498 (human CNS), and HCT 15 (human colon). Almost all the derivatives mentioned above had a more potent cytotoxic effect against SK-OV-3 than etoposide. In particular, 3-ethoxycarbonyl-3-methyl-*N*-(4-aminophenyl)-2,3-dihydro-benz[*f*]indole-2,4,9-trione (**10h**) exhibited greater activity against all the tumor cell lines, and its cytotoxic effect against SK-OV-3 was especially higher than doxorubicin.

Introduction

Streptonigrin (**1**) was first isolated from *Streptomyces flocculs* in 1959 and is known to be an antitumor and antibiotic agent against a variety of transplanted tumors[1–5] and viruses including HIV-1.[6,7] However, its application is limited because of its toxicity.[8,9] The 7-amino-6-methoxy-5,8-quinolinedione (**2**) moiety in streptonigrin is responsible for the antitumor activity (Fig. 1).[10] Studies on the activity of heterocyclic quinones containing nitrogen such as quinolinedione have reported that the number and position of the nitrogen atoms are considerably important for the observed cytotoxicity.[11,12]

According to the Moore and Pindure's theory,[13,14] the DNA-intercalating molecule must have a planar tricyclic or tetracyclic ring with a length of 3–4 Å and a width of 6–8 Å. It must also have a *p*-conjugated quinone containing nitrogen, as this enables hydrogen bonding with the DNA. The structure of pyridin[2,3-*f*]indole-2,4,9-trione (**3**) and benz[*f*]indole-2,4,9-trione (**4**), shown in Figure 2, shows a planar tri-heterocyclic ring and a *p*-conjugated ketone group. Therefore, the compounds prepared in this study were fully consistent with the conditions prescribed for the intercalator species.

Based on the cytotoxic potential of the heterocyclic quinones, 3-ethoxycarbonyl-1*N*-substituted-pyridin[2,3-*f*]indole-4,9-dione and 3-ethoxycarbinyl-*N*-substituted-benz[*f*]indole-4,9-dione derivatives, which possessing an amino, hydroxy, or methyl group at the 2-position,

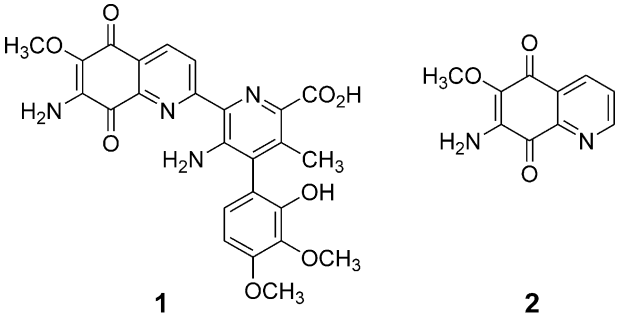

Figure 1. Streptonigrin (**1**) and 7-amino-6-methoxy-5,8-quinolinedione (**2**).

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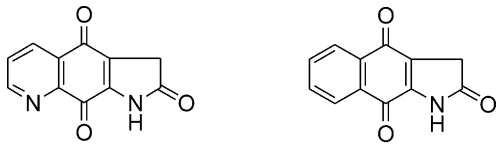


3 **4**

Figure 2. Structures of the pyridino[*f*]indole-2,4,9-trione (**3**) and benz[*f*]indole-2,4,9-trione (**4**).

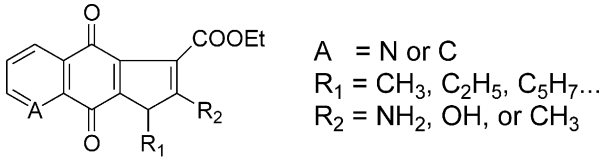


Figure 3. The structure of 3-ethoxycarbonyl-1*N*-substituted-pyridin[2,3-*f*]indole-4,9-dione and benz[*f*]indole-4,9-dione.

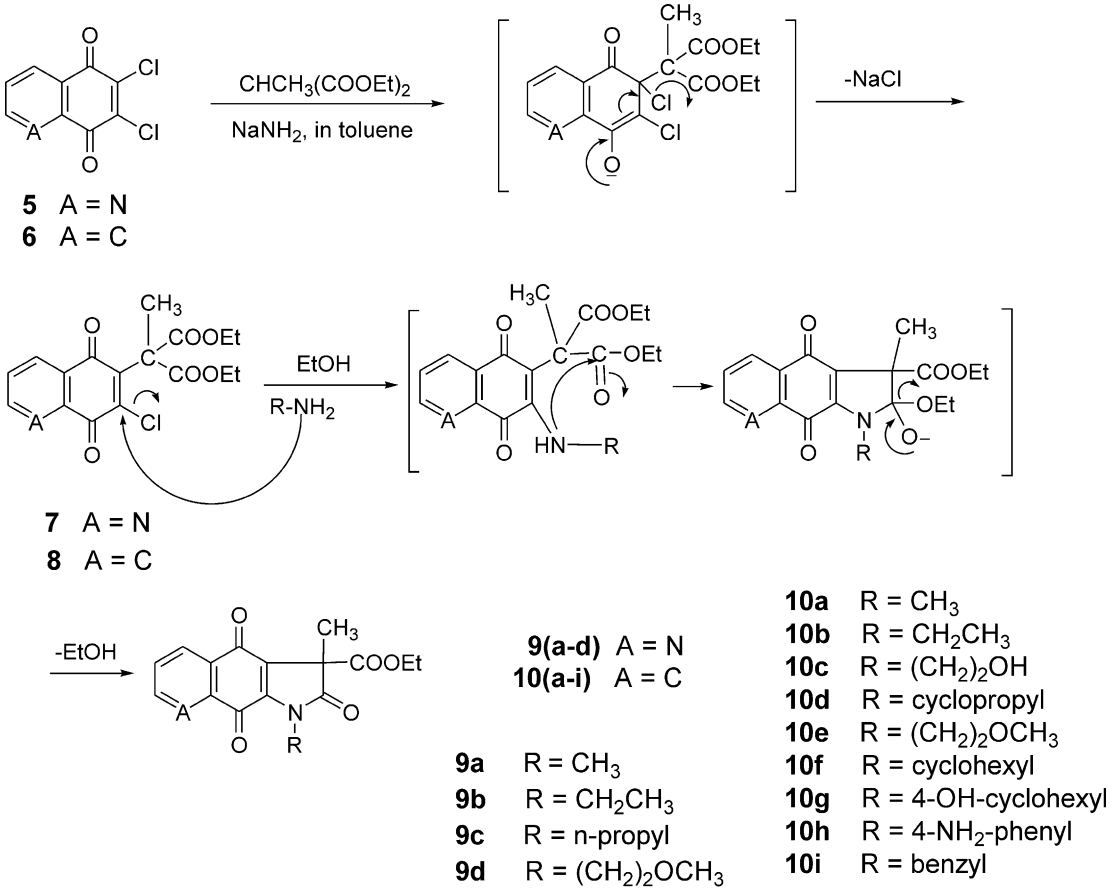
were synthesized and their cytotoxic activities were evaluated[15–19] (Fig. 3). In general, these compounds exhibited potential cytotoxic activities. Furthermore, Lee et al.[20] recently investigated one possible mechanism of their action using the topoisomerase I or II inhibitory activity.

Therefore, in a continuous effort to develop novel anticancer agents, a series of 3-ethoxycarbonyl-3-methyl-1*N*-substituted-2,3-dihydro-pyridin[2,3-*f*]indole-2,4,9-trione [**9(a–d)**] and 3-ethoxycarbonyl-3-methyl-*N*-substituted-2,3-dihydro-benz[*f*]indole-2,4,9-trione[21] [**10(a–i)**] derivatives were designed and synthesized. The prepared compounds were evaluated for their cytotoxic activity using a Sulforhodamine B (SRB) assay against the following cancer cell lines: A459 (human non-small cell lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), XF498 (human CNS), and HCT 15 (human colon). Their activities were compared with clinically available anticancer agents, such as, doxorubicin and etoposide.

Result and Discussion

Synthetic chemistry

When 7-chloro-6-(1,1-diethoxycarbonyl-ethyl)-5,8-quinolinedione (**7**) and 2-chloro-3-(1,1-diethoxycarbonyl-ethyl)-1,4-naphthoquinone[21] (**8**) were reacted with various alkyl- and arylamines, nucleophilic substitution occurred, which was followed by the nucleophilic attack of the ester group by an amine, and the intramolecular cyclization to yield the 3-ethoxycarbonyl-3-methyl-1*N*-substituted-2,3-dihydro-pyridin[2,3-*f*]indole-2,4,9-trione [**9(a–d)**] and 3-ethoxycarbonyl-3-methyl-*N*-substituted-2,3 - dihydro - benz[*f*]indole - 2,4,9 - trione derivatives [**10(a–i)**] as shown in Scheme 1. However, this study



Scheme 1. The proposed mechanism of 3-ethoxycarbonyl-3-methyl-2,3-dihydro-3-methyl-1*N*-substituted-pyridin[*f*]indole-2,4,9-trione [**9(a–d)**] and benz[*f*]indole-2,4,9-trione [**10(a–i)**].

could not identify the intermediates, which must have been formed prior to cyclization. The reactions of compound **7** or **8** with the various arylamines were examined, but it was very difficult to obtain compounds in a reasonable yield. The yield decreased and the reaction time increased with increasing amine size. This was attributed to the steric effect of the R amino group.

The structure of compounds **7** and **8** was determined by IR and ^{1}H NMR analysis and both were tested positive by the Beilstein's test,[22] which confirmed the presence of chloride.

In vitro antitumor activity evaluation by SRB assay

The prepared pyridin[2,3-*f*]indole-2,4,9-trione [**9(a–d)**] and benz[*f*]indole-2,4,9-trione [**10(a–i)**] derivatives were evaluated for their cytotoxic activity against cancer cell lines at the Korea Research Institute of Chemical Technology using a SRB assay.[23,24] This method was developed to measure the cellular culture protein content, and involved the tumor cell lines representing five different cancer types, namely, A549 (human non-small cell lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), HCT 15 (human colon), and XF 498 (human CNS).

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

The rapidly growing cells were harvested, counted, and incubated at the appropriate concentration ($1–2\times10^4$ cells/well) in 96-well micro plates. After incubation for 24 h, the compounds, which were dissolved in the culture medium, were applied to the culture wells in triplicate and 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 disolving the bound stain with 10 mL of the unbuffered Tris base solution (pH 10.5) using a gyratory shaker, the absorbance at 520 nm was measured using a microplate reader (Molecular Devices E-max, Sunnyvale, USA). The cytotoxic activity was evaluated by measuring the concentration required to inhibit protein synthesis by 50% (i.e., ED_{50}) as a comparison. Each value shown in Table 1 represents the mean of triplicate experiments.

All the compounds prepared in this study, except for compound **9d**, showed greater cytotoxic activity against the human ovarian tumor cells (SK-OV-3) than etoposide. In addition, almost all the benz[*f*]indole 2,4,9-trione derivatives [**10(a–i)**] showed greater cytotoxic activity against the growth of a wide variety of human tumor cell lines in vitro than those of etoposide. However, compounds **7**, **8**, **9a** and **9b** showed selective cytotoxicity against human ovarian tumor cells (SK-OV-3) among the tumor cell lines. Of the above mentioned compounds, compound **10f** inhibited XF 498 growth. Compound **10g** inhibited the growth of the SK-OV-3 cell line more than doxorubicin did. Compound **10h** exhibited greater cytotoxic activity against the growth of a wide variety of human tumor cell lines, except for XF 498, than did doxorubicin.

Table 1. ED_{50} of the pyridin[2,3-*f*]indole-4,9-dione and benz[*f*]-indole-4,9-dione derivatives

Compd	ED_{50}(μg/mL)				
	A549	SK-OV-3	SK-MEL-2	XF 498	HCT 15
Doxorubicin	0.02	0.18	0.03	0.09	0.03
Etoposide	0.43	2.08	0.71	1.21	0.47
7	18.84	2.02	1.75	11.56	0.88
8	2.7	2.02	2.03	5.57	1.99
9a	1.2	0.88	0.96	1.25	0.87
9b	9.09	0.95	1	11.69	1.37
9c	0.26	0.09	0.11	0.75	0.3
9d	13.54	9.05	6.55	15.3	20.98
10a	0.2	0.33	0.24	0.62	0.13
10b	2.06	0.72	0.51	>30	0.36
10c	0.19	0.23	0.18	0.28	0.12
10d	0.6	0.21	0.2	0.73	0.46
10e	0.59	0.2	0.18	0.6	0.16
10f	0.23	0.23	0.12	0.05	0.1
10g	0.3	0.11	0.13	0.34	0.08
10h	0.02	0.02	0.02	0.24	0.01
10i	0.4	0.53	0.37	1.79	0.3

In summary, almost all the compounds exhibited greater cytotoxic activity against the growth of a wide variety of human tumor cell lines in vitro than etoposide. In particular, the ED_{50} values of 3-ethoxycarbonyl-3-methy-*N*-(4-aminophenyl)-2,3-dihydro-benz[*f*]indole-2,4,9-trione (**10h**) to all the tumor cell lines, except XF 498, were much lower than those of doxorubicin. Moreover, the cytotoxicity of **10h** against the human ovarian tumor cells (SK-OV-3) ($ED_{50}=0.02$ μg/mL) was 9 times greater than that of doxorubicin ($ED_{50}=0.18$ μg/mL).

In contrast to the concept that there is an increasing cytotoxic effect with more nitrogen atoms,[11,12] the pyridin[2,3-*f*]indole-2,4,9-trione [**9(a–d)**] series showed lower cytotoxic activity when compared to the benz[*f*]indole-2,4,9-trione derivatives [**10(a–i)**]. The cytotoxic activities of the compounds with three ketone groups in the pyridin- or benz[*f*]indole structure gave contrasting results when compared to those of the compounds possessing a amino, hydroxy, or methyl group at the 2-position in the structure.

Conclusion

The reactions of either 6,7-dichloro-5,8-quienolinedione (**5**) ,which was prepared according to the method reported in the literature[2], or 2,3-dichloro-1,4-naphthoquinone (**6**) with diethyl methylmalonate gave the following starting materials, 7-chloro-6-(1,1-diethoxycarbonyl-ethyl)-5,8-quinolinedione (**7**) or 2-chloro-3-(1,1-diethoxycarbonyl-ethyl)-1,4-naphthoquinone[21] (**8**), respectively. Nucleophilic substitution occurred when the starting materials, compound **7** or **8**, were reacted with the various amines, which was followed by intra-

molecular cyclization to give either the 3-ethoxycarbonyl-3-methyl-1*N*-substituted-2,3-dihydro-pyridin[2,3-*f*]indole-2,4,9-trione derivatives [**9(a–d)**] or the 3-ethoxycarbonyl-3-methyl-*N*-substituted-2,3-dihydrobenz[*f*]indole-2,4,9-trione derivatives [**10(a–i)**], respectively.

The cytotoxicity of the prepared compounds was evaluated using a SRB assay and compared with doxorubicin and etoposide. Almost all the compounds prepared showed greater cytotoxic activity to the human ovarian cancer cells (SK-OV-3) than etoposide. Compounds **10a**, **10c**, **10f**, **10g**, and **10h** proved to be more potent cytotoxic agents against all the tumor cell lines than etoposide. In particular, the cytotoxicity of **10h** against the human ovarian tumor cells (SK-OV-3) ($ED_{50}=0.02$ μg/mL) was 9 times greater than that of doxorubicin ($ED_{50}=0.18$ μg/mL).

Generally, in this study, the benz[*f*]indole-2,4,9-trione derivatives [**10(a–i)**], exhibited greater cytotoxic activity when compared to the series of compounds possessing one more nitrogen, pyridin[2,3-*f*]indole-2,4,9-trione [**9(a–d)**]. It is believed that these compounds have the potential to become valuable anticancer agents, and that they should be tested for their 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 254 nm lamp. Most reagents were purchased from Aldrich and Merck.

7-Chloro-6-(1,1-diethoxycarbonyl-ethyl)-5,8-quinolinedione (7). In a two-necked round flask, sodium amide (0.59 g, 0.015 mmol) was suspended in toluene (40 mL) for 0.5 h. It was equipped with reflux condenser and drying $CaCl_2$ guard tube. To the mixture, diethyl methylmaolonate (2.05 mL, 0.012 mol) was slowly added and then heated for 2 h. To 6,7-dichloro-5,8-quinolinedione (**5**) (2.28 g, 0.1 mol) in toluene (20 mL), the prepared solution, the sodium salt of diethyl methylmalonate, was slowly dropwised through dropping funnel in the water bath. It was stirred for 0.5 h at room temperature and then was heated under reflux for additional 0.5 h. The reaction mixture was filtered and the residue was evaporated under reduced pressure. The crude product was crystallized from ethylacetate and hexane to produce 0.82 g (22%) of pale yellow: mp 187–188 °C; IR (KBr, cm^{-1}): 1750 (C=O); ^{1}H NMR ($CDCl_3$, δ) 1.3 (t, 6H, 2×–$COOCH_2\underline{CH_3}$), 1.9 (s, 3H, –$C\underline{CH_3}$), 4.3 (q, 4H, 2×–$COO\underline{CH_2}CH_3$), 7.2 (dd, 1H, C3), 8.4 (d, 1H, C4), 9.1 (dd, 1H, C-2). Anal. calcd ($C_{17}H_{16}ClNO_6$):C, 55.82; H, 4.41; N, 3.83. Found: C, 56.04; H, 4.40; N, 3.83. Beilstein test:[22] Cl positive.

2-Chloro-3-(1,1-diethoxycarbonyl-ethyl)-1,4-naphthoquinone (8).[21] To a mixture of diethyl methylmalonate (6.8 mL, 0.039 mol) and sodium amide (2.0 g, 0.051 mol) in toluene (300 mL), 2,3-dichloro-1,4-naphthoquinone (**6**) (6.0 g, 0.026 mol) was added. The reaction mixture was refluxed for 3 h and cooled. Then the reaction mixture was filtered and the residue was concentrated. The crude product was recrystallized twice from methanol to give 3.42 g (35%) of yellow powder: mp 75 °C; IR (KBr, cm^{-1}) : 1700 (C=O); ^{1}H NMR ($CDCl_3$, δ) 1.2 (t, 6H, 2×–$COOCH_2\underline{CH_3}$), 1.8 (q, 3H, –$C\underline{CH_3}$), 4.3 (q, 4H, 2×–$COO\underline{CH_2}CH_3$), 7.5–8.0 (m, 4H, aromatic). Anal. calcd ($C_{18}H_{17}O_6Cl$): C, 59.27; H, 4.70. Found: C, 59.65; H, 4.83. Beilstein test:[22] Cl positive.

General procedure of 3-ethoxycarbonyl-3-methyl-1*N*-substituted-2,3-dihydro-pyridin[2,3-*f*]indole-2,4,9-trione [9(a–d)]. To the suspension of 7-chloro-6-(1,1-diethoxycarbonyl-ethyl)-5,8-quinolinedione (**7**) (0.5 g, 1.34 mmol) in abs ethanol (20 mL), alkylamine as reagents and base was added and the mixture was refluxed for 12 h. The reaction mixture was concentrated, cooled and then filtered. The filtered precipitate was purified by recrystallization from 95% ethanol.

3-Ethoxycarbonyl-3-methyl-1*N*-methyl-2,3-dihydro-pyridin[2,3-*f*]indole-2,4,9-trione (9a). The general procedure was followed for 12 h using methylamine (0.11 mL, 2.94 mmol) to give 0.20 g (47%) of orange powder: mp 189–190 °C; IR (KBr, cm^{-1}): 1758 (C=O); ^{1}H NMR ($CDCl_3$, δ) 1.1 (t, 3H, –$COOCH_2\underline{CH_3}$), 1.7 (s, 3H, C3), 4.2 (m, 2H, –$COO\underline{CH_2}CH_3$), 7.9 (dd, 1H, C6), 8.4 (d, 1H, C5), 9.1 (d, 1H, C7). Anal. calcd ($C_{16}H_{14}N_2O_5$): C, 61.14; H, 4.49; N, 8.91. Found: C, 60.91; H, 4.30; N, 8.49.

3-Ethoxycarbonyl-3-methyl-1*N*-ethyl-2,3-dihydro-pyridin[2,3-*f*]indole-2,4,9-trione (9b). The general procedure was followed for 15 h using ethylamine (0.17 mL, 2.94 mmol) to give 0.11 g (24%) of pale yellow powder: mp 190–192 °C; IR (KBr, cm^{-1}): 1760 (C=O); ^{1}H NMR ($CDCl_3$, δ) 1.1 (t, 6H, –$COOCH_2\underline{CH_3}$, –N–$CH_2\underline{CH_3}$), 1.7 (s, 3H, C-3), 3.3 (s, 3H, –N–$\underline{CH_3}$), 4.1 (m, 4H, –$COO\underline{CH_2}CH_3$, –N–$\underline{CH_2}CH_3$), 7.9 (dd, 1H, C6), 8.4 (d, 1H, C5), 9.0 (d, 1H, C7). Anal. calcd ($C_{17}H_{16}N_2O_5$): C, 62.19; H, 4.91; N, 8.53. Found: C, 59.97; H, 4.59; N, 8.23.

3-Ethoxycarbonyl-3-methyl-1*N*-propyl-2,3-dihydro-pyridin[2,3-*f*]indole-2,4,9-trione (9c). The general procedure was followed for 5 h using *n*-propylamine (0.29 mL, 3.34 mmol) and the mixture was purified by column chromatography (hexane/ethylacetate, 1:2) and crystallized from hexane and ethylacetate to afford 0.14 g

(30%) of yellow powder: mp 136 °C; IR (KBr, cm^{-1}) : 1756 (C=O); ^1H NMR ($CDCl_3$, δ) 1.0 (t, 3H, –N–$CH_2\underline{CH_3}$), 1.2 (t, 3H, –$COOCH_2\underline{CH_3}$), 1.7 (q, –N–$CH_2\underline{CH_2}CH_3$), 1.8 (s, 3H, C3), 4.1 (q, 2H, –N–$\underline{CH_2}CH_2CH_3$), 4.2 (q, 2H, –$COO\underline{CH_2}CH_3$), 7.7 (dd, 1H, C6), 8.4 (d, 1H, C5), 9.0 (d, 1H, C7). Anal. calcd ($C_{18}H_{18}N_2O_5$): C, 63.15; H, 5.30; N, 8.18. Found: C, 62.86; H, 5.62; N, 8.50.

3-Ethoxycarbonyl-3-methyl-1*N*-(2-methoxyethyl)-2,3-dihydro-pyridin[2,3-*f*]indole-2,4,9-trione (9d). The general procedure was followed for 10 h using 2-methoxyethylamine (0.5 mL, 5.70 mmol) and the mixture was purified by column chromatography (hexane/ethylacetate, 1:1) and recrystallized from hexane and ethylacetate to afford 0.14 g (29%) of orange powder: mp 131 °C; IR (KBr, cm^{-1}): 1756 (C=O); (KBr, cm^{-1}) 1750 (C=O); ^1H NMR ($CDCl_3$, δ) 1.1 (t, 3H, –$COOCH_2\underline{CH_3}$), 1.9 (s, 3H, –CH_3, C3), 3.3 (s, 3H, –*O*–CH_3), 3.5 (t, 2H, –N–$CH_2\underline{CH_2}$–), 4.2 (t, 2H, –N–$\underline{CH_2}CH_2$–), 4.4 (q, 2H, –$COO\underline{CH_2}CH_3$), 7.9 (dd, 1H, C6), 8.6 (d, 1H, C5), 9.1 (d, 1H, C7). Anal. calcd ($C_{18}H_{18}N_2O_6$): C, 60.33; H, 5.06; N, 7.82. Found: C, 60.21; H, 5.30; N, 7.49.

General procedure for 3-ethoxycarbonyl-3-methyl-*N*-substituted-2,3-dihydro-benz[*f*]indole-2,4,9-trione derivatives [10(a-i)][21]

To a suspension of 2-chloro-3-(1,1-diethoxycarbonylethyl)-1,4-naphthoquinone (**8**) (1.0 g, 2.73 mmol) in abs ethanol (30 mL), amine was added. The reaction mixture was heated under reflux, concentrated, and cooled. The filtered precipitate was purified by recrystallization with 95% ethanol.

3-Ethoxycarbonyl-3-methyl-*N*-methyl-2,3-dihydro-benz[*f*]-indole-2,4,9-trione (10a). The general procedure was followed with methylamine (0.6 mL, 7.58 mmol) to give 0.32 g (38%) of yellow powder: mp 140–141 °C; IR (KBr, cm^{-1}) 1750 (C=O); ^1H NMR (DMSO-d_6, δ) 1.2 (t, 3H, –$COOCH_2\underline{CH_3}$), 1.8 (s, 3H, –C–CH_3, C3), 3.5 (s, 3H, –N–CH_3), 4.1 (q, 2H, –$COO\underline{CH_2}CH_3$), 7.7 (m, 2H, CH, C5, C6), 8.1 (m, 2H, CH, C4, C7). Anal. calcd ($C_{17}H_{15}NO_5$): C, 65.17; H, 4.87; N, 4.47. Found: C, 64.97; H, 4.79; N, 4.95.

3-Ethoxycarbonyl-3-methyl-*N*-ethyl-2,3-dihydro-benz[*f*]-indole-2,4,9-trione (10b). The general procedure was followed with ethylamine (0.4 mL, 6.25 mmol) was added to give 0.31 g (35%) of greenish yellow powder: mp 157–158 °C; IR (KBr, cm^{-1}) 1750 (C=O); ^1H NMR (DMSO-d_6, δ) 1.2 (t, 6H, –$COOCH_2\underline{CH_3}$, –N–$CH_2\underline{CH_3}$), 1.6 (s, 3H, –CH_3, C3), 4.1 (q, 4H, –$COO\underline{CH_2}CH_3$, –N–$\underline{CH_2}CH_3$), 7.7 (m, 2H, CH, C5, C6), 8.1 (m, 2H, CH, C4, C7). Anal. calcd ($C_{18}H_{17}NO_5$): C, 66.05; H, 5.23; N, 4.28. Found: C, 65.94; H, 5.38; N, 4.07.

3-Ethoxycarbonyl-3-methyl-*N*-(2-hydroxyethyl)-2,3-dihydro-benz[*f*]indole-2,4,9-trione (10c),[21] 3-ethoxycarbonyl-3-methyl-*N*-cyclopropyl-2,3-dihydro-benz[*f*]indole-2,4,9-trione (10d). The general procedure was followed with cyclopropylamine (0.4 mL, 5.63 mmol). The reaction mixture was stirred at room temperature for 2 h and cooled. The filtered precipitate was recrystallized from ethylacetate and hexane to give 0.13 g (28%) of bright yellow powder: mp 101 °C; IR (KBr, cm^{-1}) 1800 (C=O); ^1H NMR ($CDCl_3$, δ) 0.9 (m, 2H, –$CH\underline{CH_2}CH_2$–), 1.1 (m, 2H, –$CHCH_2\underline{CH_2}$–), 1.2 (t, 3H, –$COOCH_2\underline{CH_3}$), 1.8 (s, 3H, –CH_3), 3.0 (m, 1H, –N–CH–), 4.2 (q, 2H, –$COO\underline{CH_2}CH_3$), 7.8 (m, 2H, CH, C5, C6), 8.0–8.2 (m, 2H, CH, C4, C7). Anal. calcd ($C_{19}H_{17}NO_5$): C, 67.25; H, 5.05; N, 4.13. Found: C, 67.11; H, 5.04; N, 4.09.

3-Ethoxycarbonyl-3-methyl-*N*-(2-methoxyethyl)-2,3-dihydro-benz[*f*]indole-2,4,9-trione (10e). The general procedure was followed with 2-methoxyethylamine (0.5 mL, 5.70 mmol) for 20 h. The filtered precipitate was recrystallized from ethanol twice to give 0.19 g (20%) of greenish yellow powder: mp 109 °C; IR (KBr, cm^{-1}) 1750 (C=O); ^1H NMR ($CDCl_3$, δ) 1.2 (t, 3H, –$COOCH_2\underline{CH_3}$), 1.8 (s, 3H, –CH_3, C3), 3.3 (s, 3H, –*O*–CH_3), 3.6 (t, 2H, –N–$CH_2\underline{CH_2}$–), 4.2 (t, 2H, –N–$\underline{CH_2}CH_2$–), 4.4 (q, 2H, –$COO\underline{CH_2}CH_3$), 7.7 (m, 2H, CH, C5, C6), 8.1 (m, 2H, CH, C4, C7). Anal. calcd ($C_{19}H_{19}NO_6$): C, 63.86; H, 5.36; N, 3.92. Found: C, 63.87; H, 5.40; N, 3.91.

3-Ethoxycarbonyl-3-methyl-*N*-cyclohexyl-2,3-dihydro-benz[*f*]indole-2,4,9-trione (10f). The general procedure was followed with cyclohexylamine (0.7 mL, 6.00 mmol) to give 0.40 g (40%) of yellow powder: mp 190–191 °C; IR (KBr, cm^{-1}) 1760 (C=O); ^1H NMR (DMSO-d_6, δ) 1.1–1.4(m, 17H, –$COOCH_2\underline{CH_3}$, –CH_3, C3, –N-cylcohexyl), 4.1 (q, 2H, –$COO\underline{CH_2}CH_3$), 7.8–8.1 (m, 4H, aromatic). Anal. calcd ($C_{22}H_{23}NO_5$): C, 69.28; H, 6.08; N, 3.67. Found: C, 68.97; H, 6.19; N, 3.27.

3-Ethoxycarbonyl-3-methyl-*N*-(4-hydroxycyclohexyl)-2,3-dihydro-benz[*f*]indole-2,4,9-trione (10g). The general procedure was followed with trans-4-amino-cycylohexanol (1.0 g, 6.40 mmol) and triethylamine (0.8 mL). The reaction mixture was stirred at room temperature for 23 h and extracted several times with methylene chloride. The organic layer was washed with water, dried over anhydrous $MgSO_4$, and concentrated. The residue was recrystallized from ethylacetate and hexane to give 0.42 g (39%) of real yellow powder: mp 127 °C; IR (KBr, cm^{-1}) 1700 (C=O), 3400(OH); ^1H NMR ($CDCl_3$, δ) 1.2 (t, 3H, –$COOCH_2\underline{CH_3}$), 1.7 (s, 3H, –CH_3, C3), 2.0–2.4 (m, 8H, –N-cyclohexyl), 3.8 (m, 1H, –$\underline{CH}$–OH), 4.2 (q, 2H, –$COO\underline{CH_2}CH_3$), 4.8 (m, 1H, –N–CH–), 7.8 (m, 2H, CH, C5, C6), 8.0–8.2 (m, 2H, CH, C4, C7). Anal. calcd ($C_{22}H_{23}NO_6$): C, 66.49; H, 5.83; N, 3.52. Found: C, 66.46; H, 6.05; N, 3.49.

3-Ethoxycarbonyl-3-methyl-*N*-(4-aminophenyl)-2,3-dihydroxy-benz[*f*]indole-2,4,9-trione (10h). The general procedure was followed with 1,4-phenylenediamine (0.4 g, 3.70 mmol) and triethylamine (0.8 mL). The reaction mixture was refluxed for 26 h, cooled and extracted several times with methylene chloride. The organic layer was washed with water, dried over anhydrous $MgSO_4$, and concentrated. The residue was recrystallized from

ethylacetate and hexane to give 0.18 g (17%) of dark-brown powder: mp 161–162 °C; IR (KBr, cm^{-1}) 1700 (C=O), 3300, 3400(NH_2); ^1H NMR ($CDCl_3$, δ) 1.2 (t, 3H, –$COOCH_2\underline{CH_3}$), 1.8 (s, 3H, –CH_3, C3), 3.8 (s, 2H, –NH_2), 4.2 (q, 2H, –$COO\underline{CH_2}CH_3$), 6.7–7.0 (m, 4H, CH, N-phenyl), 7.6–7.8 (m, 2H, CH, C5, C6), 8.0–8.2 (m, 2H, CH, C4, C7). Anal. calcd ($C_{22}H_{14}N_2O_5$): C, 67.69; H, 4.65; N, 7.18. Found: C, 67.54; H, 5.07; N, 7.05.

3-Ethoxycarbonyl-3-methyl-*N*-benzyl-2,3-dihydroxybenz[*f*]indole-2,4,9-trione (10i). The general procedure was followed with benzylamine (0.6 mL, 5.39 mmol) to give 0.35 g (33%) of greenish yellow powder: mp 214 °C; IR (KBr, cm^{-1}) 1760 (C=O); ^1H NMR (DMSO-d_6, δ) 1.1 (t, 3H, –$COOCH_2\underline{CH_3}$), 1.7 (s, 3H, –CH_3, C3), 4.1 (q, 2H, –$COO\underline{CH_2}CH_3$), 5.2 (s, 2H, N–CH_2), 7.1–8.0 (m, 9H, aromatic). Anal. calcd ($C_{23}H_{19}NO_5$): C, 70.94; H, 4.92; N, 3.60. Found: C, 70.74; H, 5.04; N, 3.35.

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