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Synthesis of Pyridino[2,3-*f*]indole-4,9-dione 6,7-Disubstituted Quinoline-5,8-dione Derivatives and Evaluation on their Cytotoxic Activity

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Abstract—We report upon the synthesis of the following derivatives: *N*-substituted-pyridino[2,3-*f*]indole-4,9-dione, and 6-(α-diethoxycarbonyl-methyl)-7-substituted-amino-quinoline-5,8-dione, which contain the active quinoline-5,8-dione (**VII**) moiety. The cytotoxic activities of these compounds have been tested in SRB (SulfoRhodamine B) assays against the cancer cell lines of A-549 (human lung cancer), SK-MEL-2 (human melanoma cancer), SK-OV-3 (human ovarian cancer), XF-498 (human brain cancer) and HCT 15 (human colon cancer). The compound, *N*-benzyl-3-ethoxycarbonyl-2-hydroxy-pyridino[2,3-*f*]indole-4,9-dione (**A-9**), also showed higher activity than *cis*-platin. The highest level of cytotoxic activity in these human tumor cell lines was observed in the compound 6-(α-diethoxycarbonyl-methyl)-7-(2-methyl-phenylamino)-quinoline-5,8-dione (**B-3**).

Introduction

Streptonigrin was isolated from *Streptomyces flocculs* in 1959[1] and is a known antitumor and antibiotic agent in a variety of transplanted tumors,[2–4] and viruses including HIV-1.[5,6] In the presence of species, such as, O_2, and NADH and a divalent cation, for example Cu^{2+}, Fe^{2+} and so forth, streptonigrin generates either a semiquinone or a hydroxy radical, which induce single-strand DNA breaks.[7,8] Streptonigrin is also known to possess topoisomerase II[7] and reverse transcriptase inhibition properties.[9] It readily forms complexes with some divalent transition metal cations and topoisomerase II, and these complexes are known to binds to DNA.[10] Streptonigrin is a member of a group of aminoquinone containing antitumor agents, which includes mitomycin C,[11] actinomycin,[12] rifamycin[13] and geldanamycin[14] and was found to be one of the most potent inhibitors of avian myeloblastosis virus reverse transcriptase (AMV-RT)[15] known. However, the use of streptonigrin has been limited because of its bone marrow toxicity and adverse reactions.[16–18]

According to the study made by Johnson,[19] quinoline-5,8-dione, the active group of streptonigrin, has stronger antitumor activity and lower toxicity than streptonigrin. In the study of structure–activity relationships (SAR), the antitumor activity of streptonigrin (**I**) was completely lost when the aminoquinone moiety (**II**) was blocked, as in azastreptonigrin (**III**). The methoxy group of quinone ring, the pyridyl group, and its phenyl ring were found to be not essential for the activity of murine tumors, although they enhanced activities against human tumor cells. Synthetic analogues lacking the 7-aminoquinolinequinone-moiety (**II**) proved inactive as antitumor agents (Fig. 1). The electron withdrawing groups at the 6- and 7-positions of the quinolinediones also contributed to the activity[20] and more condensed heterocyclic quinones were also reported to enhance antitumor activity.[21]

Streptonigrin and most of other quinone derivatives examined inhibit topoisomerase II via DNA-intercalators[22] into the DNA minor groove.[23,24] According to Moore's theory,[25] the properties required of DNA-intercalator are as follows:

i. A planar tri- or tetracyclic aromatic ring structure;
ii. A surface area exceeding 28 Å^2;
iii. A *p*-conjugated quinone containing a nitrogen atom, which enables hydrogen bonding with DNA.

In 1900, Liebermann[26] synthesized 3-bromo-2-(α-diethoxycarbonyl-methyl)-1,4-naphthoquinone (**V**) with

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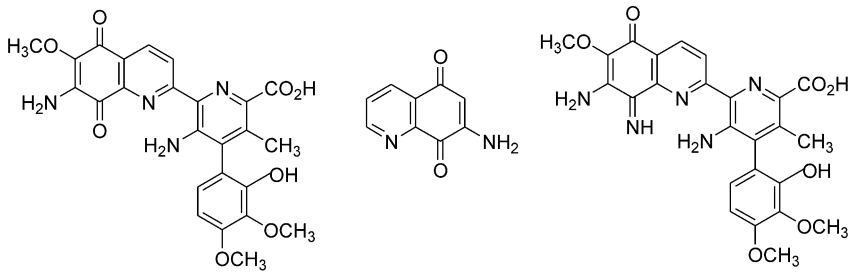


I II III

Figure 1. Streptonigrin (**I**), aminoquinone (**II**) and azastreptonigrin (**III**). The antitumor activity of streptonigrin (**I**) was completely lost when the aminoquinone moiety (**II**) was blocked, as in azastreptonigrin (**III**). In the structure of streptonigrin (**I**), aminoquinone (**II**) is essential as anticancer.

2,3-dibromo-1,4-naphthoquinone (**IV**) and sodium diethyl malonate. Subsequently, Kitasato[27] synthesized 2-hydroxy-3-ethoxycarbonyl-1*N*-methyl-benz[*f*]indole-4,9-dione (**VI**) from 3-bromo-2-(α-diethoxycarbonyl-methyl)-1,4-naphthoquinone (**V**) and methyl amine in 1930 (Scheme 1).

We synthesized 3-ethoxycarbonyl-2-hydroxy-*N*-substituted-pyridino[2,3-*f*]indole-4,9-dione derivatives (**A**), and 6-(α-diethoxycarbonyl-methyl)-7-substituted-amino-quinoline-5,8-dione derivatives (**B**), which were predicted to have antitumor and antibiotic activities from 6,7-dichloro-5,8-quinolinedione (**VII**) as a starting material (Scheme 2). The cytotoxicity of these compounds was evaluated to develop new antitumor agents against human cancer cell lines, including, lung (A 549), ovarian (SK-OV-3), melanoma (SK-MEL-2), brain (XF-498), and colon (HCT 15) for comparisons with clinically viable anticancer agents, such as, *cis*-platin and doxorubicin.

Results and Discussion

Chemistry

The starting material, 6,7-dichloroquinoline-5,8-dione was synthesized as described in the literature.[28]

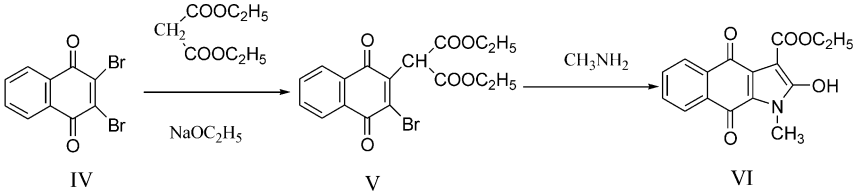
6-(α-Diethoxycarbonyl-methyl)-7-chloroquinolin-5,8-dione (**I-A**) was synthesized by reacting 6,7-dichloroquinoline-5,8-dione (**VII**) with sodium diethylmalonate, which was obtained by reacting excess sodium amide with diethylmalonate in toluene (99.9%). When hexamethylphosphoramide was used as a solvent instead of toluene, bis-(7-chloro-5,8-dioxo-quinolino)-6,6′-diehtylmalonate(**F**) was produced. The compound was predicted to be a *trans*-formed structure by computer-aided molecular energy optimization (SYBYL, version 6.5, Tripos) (Scheme 3).

The compounds, **A-1** to **A-11** were obtained by treating **1-A** with alkylamines or aniline, using either potassium carbonate or triethylamine as base in absolute ethanol. The reaction mechanism seems to involve an intramolecular cyclization (Scheme 4).

Although the synthetic method was similar in the case of all derivatives of **A**, when **I-A** was reacted with substituted aryl amines using triethylamine as a catalyst in refluxing ethanol, it did not give the respective 3-ethoxycarbonyl-2-hydroxy-*N*-substituted-pyridino[2,3-*f*]-indole-4,9-dione derivatives (**A**), but instead yielded the 6-(α-diethoxycarbonyl-methyl)-7-substituted-amino-quinoline-5,8-dione derivatives (**B**). This was probably, caused by the steric hindrance of the majority of the arylamines when the triethylamine or potassium carbonate was used as base (Scheme 5).

Cytotoxicity assay by SRB assay

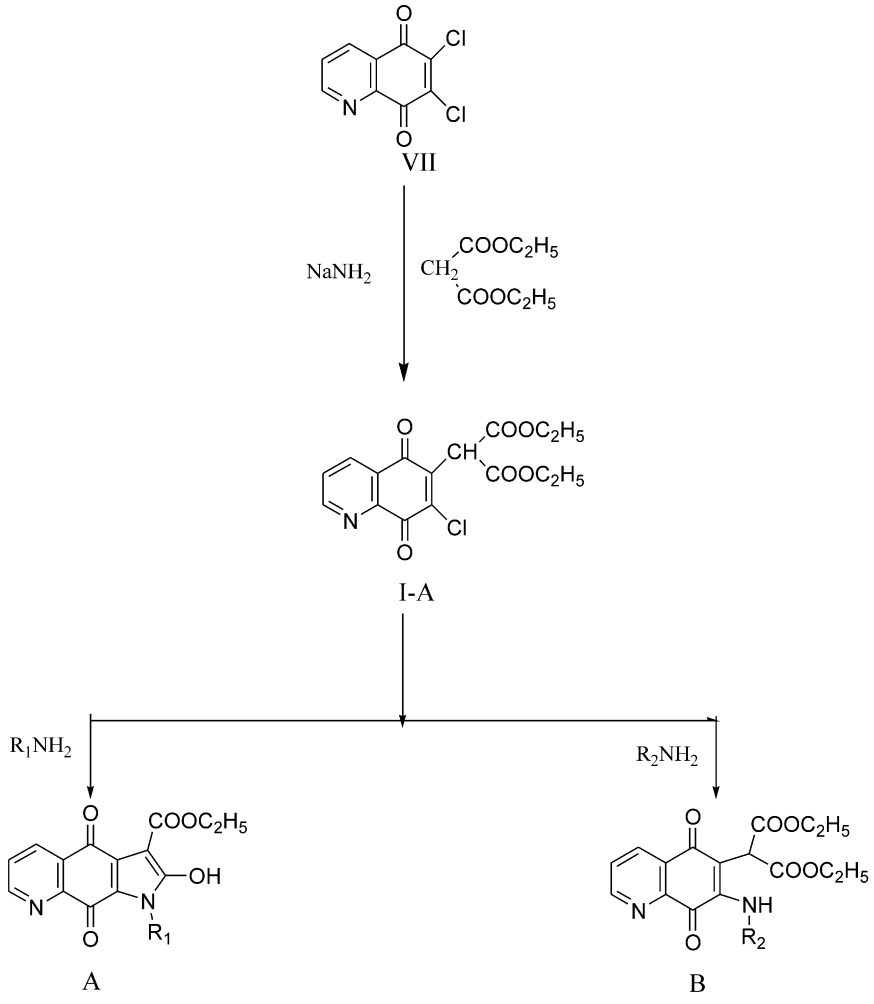
Human cancer cell lines of lung (A 549), ovarian (SK-OV-3), melanoma (SK-MEL-2), brain (XF 498) and colon (HCT 15) cancers were used for cytotoxicity testing in vitro using the SRB (Sulforhodamine B) assay.[29,30] Cells were maintained as stocks in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Gibco). Cell cultures were passaged once or twice weekly using trypsin–EDTA to detach the cells from their culture flasks.



IV V VI

Scheme 1. The synthetic scheme of 2-hydroxy-3-ethoxycarbonyl-1*N*-methyl-benz[*f*]indole-4,9-dione (**VI**). 2,3-Dibromo-1,4-naphthoquinone (**IV**) and sodium diethyl malonate was reacted in absolute ethanol to yield 3-bromo-2-(α-diethoxycarbonyl-methyl)-1,4-naphthoquinone (**V**) and then 2-hydroxy-3-ethoxycarbonyl-1*N*-methyl-benz[*f*]indole-4,9-dione (**VI**) were synthesized from **V** and methyl amine in 1930.

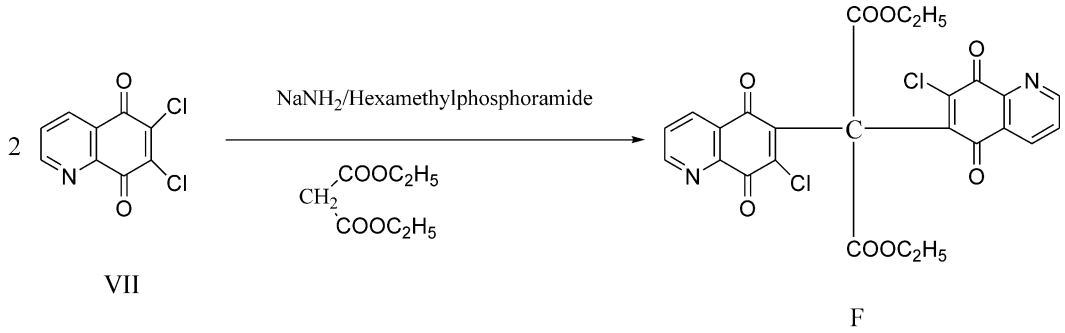
The rapidly growing cells were harvested, counted, and incubated under the appropriate concentrations (1–2×10^4 cells/well) in 96-well microtiter plates. After incubation for 24 h, the compounds dissolved in culture medium were applied to the culture wells in triplicate and incubated for 48 h at 37 °C in a 5% CO_2 atmosphere. Cultures fixed with cold TCA were stained by 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound stain with 10 mL unbuffered tris base, the absorbance at 520 nm was measured with a microplate



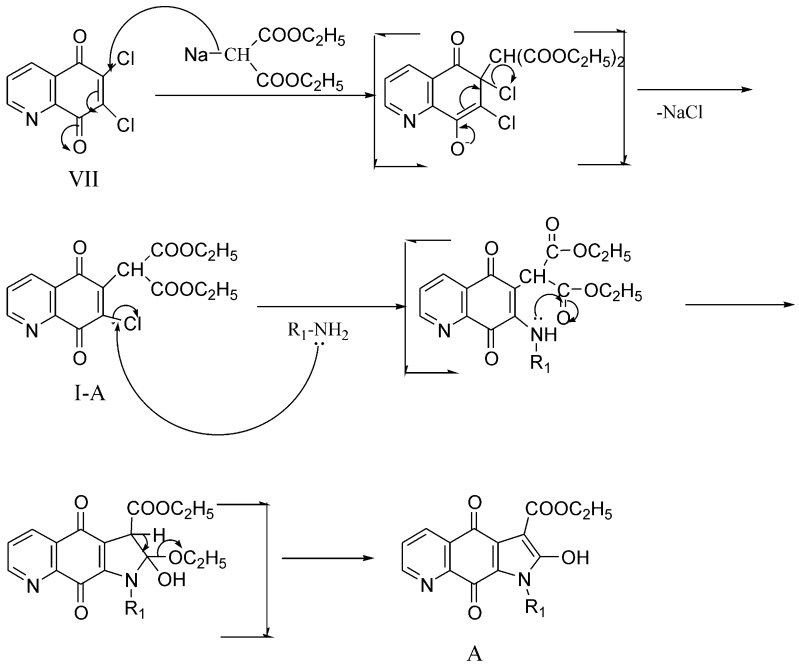
A: R_1=-CH_3, -C_2H_5, -C_3H_7, -C_4H_9, -isopropyl, -CH_2CH_2OH, -$CH_2CH_2OCH_3$, -cyclopropyl, -benzyl, -cyclohexyl, -phenyl

B: R_2=-4-methyl-phenyl, -3-methyl-phenyl, -2-methyl-phenyl, -4-methoxy-phenyl, -4-ethoxy-phenyl

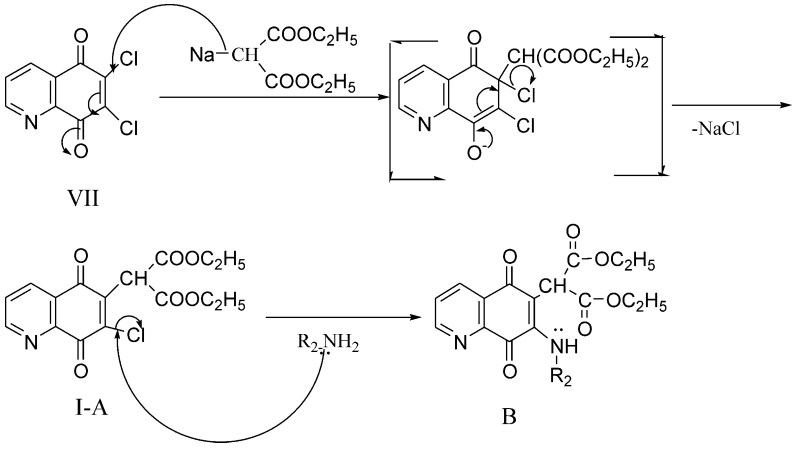
Scheme 2. The synthesis of pyridino[2,3-*f*]indole-4,9-dione derivatives (**A**), and 6-(diethoxy-carbonyl-methyl)-7-*N*-substituted-amino-quinoline-5,8-dione derivatives (**B**), from 6,7-dichloroquinoline-5,8-dione (**VII**).



Scheme 3. The synthesis of bis-(7-chlroro-5,8-dioxo-quinolino)-6,6′-diehtylmalonate (**F**) which was produced by reacting one molecular equivalent of diethyl malonate with two molecular equivalents of **VII** in hexamethyl phosphoramide.



Scheme 4. The predicted reaction mechanism of 3-ethoxycarbonyl-2-hydroxy-*N*-substituted-pyridino[2,3-*f*]indole-4,9-dione (**A**).



Scheme 5. The predicted reaction mechanism of 6-(α-diethoxycarbonyl-methyl)-7-substituted-amino-quinoline-5,8-dione (**B**).

reader (Dynatech Model MR 700). The cytotoxic activity was evaluated by measuring the concentration of a compound required to inhibit protein synthesis by 50% (IC_{50}). The IC_{50} values evaluated was compared with those of doxorubicin and *cis*-platin (Table 1). Each quoted value represents the mean of triplicate experiments.

The compounds, 3-ethoxycarbonyl-2-hydroxy-*N*-(β-hydroxyethyl)-pyridino[2,3-f]indole-4,9-dione (**A-6**), 6-(α-diethoxycarbonyl-methyl)-7-(3-methyl-phenylamino)-quinoline-5,8-dione (**B-2**) and 6-(α-diethoxycarbonyl-methyl)-7-(4-ethoxy-phenyl-amino)-quinoline-5,8-dione (**B-5**) exhibited more potent cytotoxicity than *cis*-platin to human ovarian tumor cell line (SK-OV-3). The activities of the componds, *N*-butyl-3-ethoxycarbonyl-2-hydroxy-pyridino [2,3-*f*] indole-4, 9-dione (**A-4**) and **A-6** were also lower than doxorubicin but higher than *cis*-platin to human melanoma tumor cell line (SK-MEL-2). Compound, **A-6**, demonstrated more potent cytotoxic activities than *cis*-platin to human colon tumor cell line (HCT 15). The IC_{50} values of the compound 6-(α-diethoxycarbonyl-methyl)-7-chloroquinolin-5,8-dione (**I-A**) were lower than these of *cis*-platin or similar to these of *cis*-platin, respectively, to all human tumor cell lines tested. The cytotoxicity, of *N*-benzyl 3-ethoxycarbonyl-2-hydroxy-pyridino[2,3-*f*]indole-4,9-dione (**A-9**), was significantly higher than that of *cis*-platin. The most potent compound found was 6-(α-diethoxycarbonyl-methyl)-7-(2-methyl-phenylamino)-quinoline-5,8-dione (**B-3**). Its cytotoxic activity was similar to that of doxorubicin and was much higher than *cis*-platin to all of the human tumor cell line tested.

Conclusion

6-(α-Diethoxycarbonyl-methyl)-7-chloroquinolin-5,8-dione(**I-A**) was synthesized from 6,7-dichloroquinoline-5,8-dione (**VII**).

Table 1. Cytotoxicity data on human lung tumor cell lines (A 549), human ovarian tumor cell lines (SK-OV-3), human melanoma tumor cell lines (SK-MEL-2), human brain tumor cell lines (XF 498) and human colon tumor cell lines (HCT 15)

Compounds	IC_{50} (μg/mL)				
	A549	SK-OV-3	SK-MEL-2	XF 498	HCT 15
Cisplatin	0.83	1.39	1.74	1.59	0.93
Doxorubicin	0.01	0.08	0.02	0.07	0.03
I-A	1.06	0.79	0.89	1.50	0.80
A-1	7.95	6.15	5.79	8.84	13.18
A-2	4.47	12.42	3.51	9.92	14.39
A-3	1.17	3.59	2.13	4.78	1.65
A-4	0.95	1.71	1.45	2.22	0.94
A-5	2.31	5.37	5.38	5.57	5.32
A-6	1.00	0.90	1.73	8.56	0.80
A-7	>30	>30	7.05	>30	>30
A-8	14.97	12.90	3.56	9.16	15.36
A-9	0.44	0.34	0.37	0.13	0.30
A-10	>30	>30	>30	>30	>30
A-11	>30	>30	>30	>30	>30
B-1	1.96	1.65	7.69	1.94	5.08
B-2	1.14	1.15	4.46	2.16	2.64
B-3	0.08	0.05	0.05	0.06	0.05
B-4	19.18	7.65	13.95	12.66	18.87
B-5	0.92	1.29	2.94	1.76	2.75

The *N*-substituted-pyridino[2,3-*f*]indole-4,9-dione (**A**) derivatives, and 6-(α-diethoxy-carbonyl-methyl)-7-substituted-amino-quinoline-5,8-dione (**B**) derivatives were obtained. The majority of these were tested for cytotoxicity in vitro and compared with *cis*-platin and doxorubicin, a number of these compounds showed cytotoxic effects against various human tumor cell lines. We believe that these are valuable compounds and should be tested for in vivo antitumor activity in human cancer xenograft models.

Experimental

Materials and methods

All melting points were taken in Pyrex capillaries using electrothermal digital melting point apparatus (Büchi). The IR spectra were recorded on a FT-Infrared spectrometer (Bio-Rad. Co., USA) using KBr pellet. ^{1}H NMR spectra were recorded on a 400 MHz Varian FT-NMR spectrometer facility using trimethylsilane as an internal standard. The abbreviation of multiplicity was s, singlet, d, doublet, t, triplet, q, quartet and m, multiplet. Samples were dissolved in DMSO-d_6 and $CDCl_3$. Elemental analyses were performed using ThermoQuest (CE Instruments) EA 1110. For column chromatography, Kieselgel 60, particles size 70–230 mesh (ASTM, Merck. Co.) was used. Precoated plates (Kieselgel 60) were used for TLC analysis. Most reagents were purchased from Aldrich Chemical Company and Merck Company. 6,7-Dichloro-5,8-quinolinedione (**VII**) was prepared according to the literature.[28]

7-Chloro-6-(α-diethoxycarbonyl-methyl)-quinoline-5,8-dione (I-A). A suspension of 0.59 g (0.015 mol) of sodium amide in 40 mL of toluene was stirred in two-necked round flasks equipped with a reflux condenser, a drying calcium chloride guard tube for 30 min. To the mixture, 1.82 mL (0.012 mol) of diethylmalonate was slowly dropped and then was heated for 2 h. To 2.28 g (0.01 mol) of 6,7-dichloro-quinoilin-5,8-dione (**VII**) in 20 mL of toluene, the solution, the sodium salt of diethylmalonate, was slowly dropped using dropping funnel in water bath. The mixture immediately became blue. It was stirred for 30 min at room temperature and then was heated under reflux for 30 min. The reaction mixture was filtered and the residue solution was evaporated under the reduced pressure. The crude product was crystallized from ethylacetate and hexane to give 1.517 g (43%) of yellow **I-A**. Mp 113 °C, IR (KBr, cm^{-1}): 1674 (C=O). ^{1}H NMR ($CDCl_3$): 1.3 (t, 6H, –$COOCH_2CH_3$), 4.3 (m, 4H, –$COOCH_2CH_3$), 5.1 (s, 1H,–*CH*), 7.7 (dd, 1H, C-3), 8.5 (d, 1H, C-4), 9.1 (d, 1H, C-2). Anal. calcd for $C_{16}H_{14}ClNO_6$ C, 54.63; H, 4.01; N, 3.98. Found: C, 54.64; H, 4.01; N, 4.05, Beilstein test: Cl positive.

The general procedure of 3-ethoxycarbonyl-2-hydroxy-*N*-substituted pyridino[2,3-*f*]indole-4,9-dione (A). A solution of 0.5 g (1.422 mmol) of **I-A** in 20 mL of absolute ethanol was heated under reflux. To the solution, alkylamine and 0.49 g (3.555 mmol) of anhydrous potassium carbonate as a base was added and the mixture was refluxed. The reaction mixture was cooled and then filtered. The residue solution was diluted with water, acidified with dilute HCl and extracted with methylene chloride. The extract was washed with water, dried over magnesium sulfate and then evaporated. The crude product was crystallized from 95% ethanol.

3-Ethoxycarbonyl-2-hydroxy-*N*-methyl-pyridino [2,3-*f*] indole-4, 9-dione (A-1). A solution of 0.5 g (1.422 mmol) of **I-A** in 40 mL of absolute ethanol was heated under reflux. To the solution, 0.11 mL (3.124 mmol) of methylamine was added and the mixture was refluxed for 12 h. The reaction mixture was cooled and then filtered. The filtered precipitation was purified by column chromatography (ethylacetate/hexane = 2:1) and crystallized from ethylacetate and hexane to afford 0.132 g (31%) of green precipitation. Mp 216 °C, IR (KBr, cm^{-1}): 3400 (–OH), 1674 (–C=O), ^{1}H NMR ($CDCl_3$, δ) 1.5 (t, 3H, –$COOCH_2CH_3$), 3.9 (s, 3H, N–CH_3), 4.5 (q, 2H, –$COOCH_2CH_3$), 7.7 (dd, 1H, C-6), 8.4 (d, 1H, C-5), 8.9 (d, 1H, C-7),11.4 (s, 1H, –OH, C-2) –D_2O exchange. Anal. calcd for $C_{15}H_{12}N_2O_5$: C, 60.00; H, 4.03; N, 9.33. Found: C, 59.90; H, 4.17; N, 9.23.

3-Ethoxycarbonyl-2-hydroxy-*N*-ethyl-pyridino [2,3-*f*] indole-4, 9-dione (A-2). The general procedure was followed for 12 h using 0.16 mL (2.844 mmol) of ethylamine and crystallized from ethanol to give 0.188 g (42%) of golden yellow needle. Mp 206 °C, IR (KBr, cm^{-1}): 3427 (–OH), 1693 (–C=O). ^{1}H NMR ($CDCl_3$) δ 1.5 (t, 3H, –$COOCH_2CH_3$), 1.5 (t, 3H, –N–CH_2CH_3), 4.5 (q, 2H, –$COOCH_2CH_3$), 4.6 (q, 3H, –N–CH_2CH_3),7.6 (dd, 1H, C-6), 8.5 (d, 1H, C-5), 9.0 (d, 1H, C-7), 11.5 (s, 1H, –OH, C-2)–D_2O exchange. Anal. calcd for $C_{16}H_{14}N_2O_5$: C, 61.14; H, 4.49; N, 8.91. Found: C, 61.14; H, 4.65; N, 8.80.

3-Ethoxycarbonyl-2-hydroxy-*N*-propyl-pyridino [2,3-*f*] indole-4, 9-dione (A-3). The general procedure was followed for 12 h using 0.16 mL (2.844 mmol) of *n*-propylamine and 0.98 g (7.1075 mmol) of anhydrous potassium carbonate and chromatographed on a silica gel column using ethylacetate/hexane (2:1) to produce 0.099 g (21%) of green precipitation. Mp 180 °C, IR (KBr, cm^{-1}): 3415 (–OH), 1669 (–C=O), ^1H NMR ($CDCl_3$) δ 1.0 (t, 3H, –$NCH_2CH_2CH_3$), 1.5 (t, 3H, –$COOCH_2CH_3$), 1.9 (m, 2H, –$NCH_2CH_2CH_3$), 4.5 (m, 4H, –$COOCH_2CH_3$, –$NCH_2CH_2CH_3$), 7.6 (dd, 1H, C-6), 8.5 (d, 1H, C-5), 9.0 (d, 1H, C-7),11.5 (s, 1H, –OH, C-2)–D_2O exchange. Anal. calcd for $C_{17}H_{16}N_2O_5$: C, 62.19; H, 4.91; N, 8.53, Found: C, 62.00; H, 5.08; N, 8.56.

***N*-Butyl-3-ethoxycarbonyl-2-hydroxy-pyridino [2,3-*f*] indole-4, 9-dione (A-4).** The general procedure was followed for 12 h using 0.21 mL (2.133 mmol) of *n*-butylamine and 0.98 g (7.1075 mmol) of anhydrous potassium carbonate to give 0.133 g (27%) of greenish yellow powder. Mp 159 °C, IR (KBr, cm^{-1}): 3410 (–OH),1680 (–C=O), ^1H NMR ($CDCl_3$) δ 1.0 (t, 3H, –$NCH_2CH_2CH_2CH_3$), 1.4 (m, 2H, –$NCH_2CH_2CH_2CH_3$), 1.5 (t, 3H, –$COOCH_2CH_3$), 1.8 (m, 2H, –$NCH_2CH_2CH_2CH_3$), 4.5 (m, 4H, –$COOCH_2CH_3$, –$NCH_2CH_2CH_2CH_3$), 7.6 (dd, 1H, C-6), 8.5 (d, 1H, C-5), 8.9 (d, 1H, C-7), 11.5 (s, 1H, –OH, C-2)–D_2O exchange. Anal. calcd for $C_{18}H_{18}N_2O_5$: C, 63.15; H, 5.30; N, 8.18. Found: C, 62.78; H, 5.27; N, 8.12.

3-Ethoxycarbonyl-2-hydroxy-*N*-isopropyl-pyridino [2,3-*f*] indole-4, 9-dione (A-5). The general procedure was followed for 12 h using 0.243 mL (2.844 mmol) of isopropylamine, chromatographed on a silica gel column using ethylacetate/hexane (2:1) and crystallized from ethanol to give 0.049 g (11%) of yellow needle. Mp 261 °C, IR (KBr, cm^{-1}): 3433 (–OH), 1697 (–C=O), ^1H NMR ($CDCl_3$) δ 1.5 (t, 3H, –$COOCH_2CH_3$), 1.6 (d, 6H, –$CH(CH_3)_2$), 4.5 (q, 2H, –$COOCH_2CH_3$), 5.9 (m, 1H, –$CH(CH_3)_2$), 7.6 (dd, 1H, C-6), 8.5 (d, 1H, C-5), 9.0 (d, 1H, C-7), 11.4 (s, 1H, –OH, C-2)–D_2O exchange. Anal. calcd for $C_{17}H_{16}N_2O_5$: C, 62.19; H, 4.91; N, 8.53, Found: C, 61.74; H, 5.25; N, 8.50.

3-Ethoxycarbonyl-2-hydroxy-*N*-(β-hydroxyethyl)-pyridino[2,3-*f*]indole-4,9-dione (A-6). A solution of 0.5 g (1.422 mmol) of **I-A** in 50 mL of absolute ethanol was heated under reflux. To the solution, 0.17 mL (2.844 mmol) of ethanolamine and 0.49 g (3.555 mmol) of potassium carbonate were added and the mixture was refluxed for 12 h. The reaction mixture was cooled and then filtered. The filtered precipitate was solved in water, acidified with dilute HCl and extracted with methylene chloride. The extract was washed with water, dried over magnesium sulfate and then evaporated under reduced pressure. The crude product was crystallized from 95% ethanol to afford 0.163 g (35%) of green powder. Mp over 250 °C, IR (KBr, cm^{-1}): 3440 (–OH), 1662 (–C=O). ^1H NMR (DMSO-d_6) δ 1.3 (t, 3H, –$COOCH_2CH_3$), 3.7 (t, 2H, –NCH_2CH_2OH), 4.2 (m, 2H, –$COOCH_2CH_3$), 4.3 (t, 2H, –NCH_2CH_2OH), 7.7 (dd, 1H, C-6), 8.3 (d, 1H, C-5), 8.9 (d, 1H, C-7), 9.2 (s, 1H, –NCH_2CH_2OH)–D_2O exchange, 9.6 (s, 1H, –OH, C-2)–D_2O exchange. Anal. calcd for $C_{16}H_{14}N_2O_6$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.26; H, 4.26; N, 8.76.

3-Ethoxycarbonyl-2-hydroxy-*N*-(β-methoxyethyl)-pyridino[2,3-*f*]indole-4,9-dione (A-7). A solution of 0.5 g (1.422 mmol) of **I-A** in 20 mL of absolute ethanol was heated under reflux. To the solution, 0.244 mL (2.844 mmol) of β-methoxyethylamine and 0.98 g (7.1075 mmol) of anhydrous potassium carbonate were added and the mixture was refluxed for 2 h. The reaction mixture was cooled and then filtered. The residue solution was evaporated and crystallized from 95% ethanol to give 0.176 g (36%) of yellow precipitation. Mp 196 °C, IR (KBr, cm^{-1}): 3412 (–OH), 1670 (–C=O). ^1H NMR ($CDCl_3$) δ 1.5 (t, 3H, –$COOCH_2CH_3$), 3.4 (s, 3H, –$NCH_2CH_2OCH_3$), 3.8 (t, 2H, –$NCH_2CH_2OCH_3$), 4.5 (q, 2H, –$COOCH_2CH_3$), 4.7 (t, 2H,-$NCH_2CH_2OCH_3$), 7.6 (dd, 1H, C-6), 8.5 (d, 1H, C-5), 8.9 (d, 1H, C-7), 11.6 (s, 1H, –OH, C-2)–D_2O exchange. Anal. calcd for $C_{17}H_{16}N_2O_6$: C, 59.30; H, 4.68; N, 8.14. Found: C, 59.22; H, 5.00; N, 8.12.

***N*-Cyclopropyl-3-ethoxycarbonyl-2-hydroxy–pyridino [2,3-*f*] indole-4, 9-dione (A-8).** The general procedure was followed for 12 h using 0.2 mL (2.844 mmol) of cyclopropylamine and chromatographed on a silica gel column using ethylacetate/hexane/methanol (2:1:1) and crystallized from ethanol to give 0.158 g (34%) of brownish yellow needle. Mp 226 °C, IR (KBr, cm^{-1}): 3425 (–OH), 1677 (–C=O) ^1H NMR ($CDCl_3$) δ 1.1, 1.3 (m, m, 4H, –cyclopropyl), 1.5 (t, 3H, –$COOCH_2CH_3$), 3.5 (m, 1H, –cyclopropyl), 4.5 (q, 2H, –$COOCH_2CH_3$), 7.6 (dd, 1H, C-6), 8.5 (d, 1H, C-5), 9.0 (d, 1H, C-7), 11.7 (s, 1H, –OH, C-2)–D_2O exchange. Anal. calcd for $C_{17}H_{14}N_2O_5$: C, 62.57; H, 4.32; N, 8.59, Found: C, 62.50; H, 4.52; N, 8.65.

***N*-Benzyl 3-ethoxycarbonyl-2-hydroxy-pyridino[2,3-*f*] indole-4,9-dione (A-9).** The general procedure was followed for 12 h using 0.25 mL (1.707 mmol) of benzylamine and 0.3 mL (2.133 mmol) of triethylamine as a base and then crystallized from ethanol to give 0.171 g (32%) of green precipitation. Mp 169.5 °C, IR (KBr, cm^{-1}): 3438 (–OH), 1658 (–C=O), ^1H NMR (DMSO-d_6) δ 1.3 (t, 3H, –$COOCH_2CH_3$), 4.3 (q, 2H, –$COOCH_2CH_3$), 5.5 (s, 2H, –CH_2Ar), 7.3 (m, 6H, –CH_2Ar), 7.8 (dd, 1H, C-6), 8.4 (d, 1H, C-5), 8.9 (d, 1H, C-7), Anal. calcd for $C_{21}H_{16}N_2O_5$: C, 67.02; H, 4.28; N, 7.44, Found: C, 66.70; H, 4.38; N, 7.79.

***N*-Cyclohexyl 3-ethoxycarbonyl-2-hydroxy-pyridino[2,3-*f*] indole-4,9-dione (A-10).** The general procedure was followed for 48 h using 0.2 mL (1.707 mmol) of cyclohexylamine and 0.3 mL (2.133 mmol) of triethylamine as a base and then crystallized from ethanol to produce 0.212 g (40.5%) of yellow precipitation. Mp 258 °C, IR (KBr, cm^{-1}): 3400 (–OH), 1688 (–C=O), ^1H NMR ($CDCl_3$) δ 1.5 (t, 3H, –$COOCH_2CH_3$), 2.3, 1.9, 1.5, 0.9 (m, m, m, m, 10H, –cyclohexyl), 4.5 (q, 2H, –$COOCH_2CH_3$), 7.6 (dd, 1H, C-6), 8.4 (d, 1H, C-5), 8.9 (d, 1H, C-7), 11.8 (s, 1H, –OH, C-2)–D_2O exchange. Anal. calcd for $C_{20}H_{20}N_2O_5$: C, 65.21; H, 5.47; N, 7.60. Found: C, 65.06; H, 5.21; N, 7.20.

3-Ethoxycarbonyl-2-hydroxy-*N*-phenyl-pyridino[2,3-*f*] indole-4,9-dione (A-11). A solution of 0.5 g (1.422 mmol) of **I-A** in 20 mL of absolute ethanol was heated under reflux. To the solution, 0.234 mL (2.560 mmol) of aniline was added and the mixture was refluxed for 15 h. The reaction mixture was concentrated, cooled and then filtered. The filtered precipitation was purified by column chromatography (ethylacetate/hexane = 2:1) and crystallized from ethylacetate and hexane to give 0.167 g (32%) of green precipitation. Mp over 250 °C, IR (KBr, cm^{-1}): 3419 (–OH), 1682 (–C=O), ^1H NMR ($CDCl_3$) δ 1.5 (t, 3H, –$COOCH_2CH_3$), 4.5 (q, 2H, –$COOCH_2CH_3$), 7.4, 7.5 (m, m, 5H, –phenyl), 7.6 (dd, 1H, C-6), 8.5 (d, 1H, C-5), 8.9 (d, 1H, C-7), Anal. calcd for $C_{20}H_{14}N_2O_5$: C, 66.30; H, 3.89; N, 7.73. Found: C, 65.96; H, 4.44; N, 7.39.

The general procedure of 6-(α-diethoxycarbonyl-methyl)-7-*N*-substituted-amino-quinoline-5,8-dione (B). A solution of 0.5 g (1.422 mmol) of **I-A** in 20 mL of absolute ethanol was heated under reflux. To the solution, arylamine and 0.4955 mL (3.555 mmol) of triethylamine as a base was added and the mixture was refluxed. The reaction mixture was cooled and then filtered. The filtered precipitaion was purified by column chromatography (ethylacetate/hexane = 1:2) and crystallized from ehthylacetate and hexane.

6-(α-Diethoxycarbonyl-methyl)-7-(4-methyl-phenylamino)-quinoline-5,8-dione (B-1). The general procedure was followed for 12 h using 0.313 mL (2.844 mmol) of ρ-toluidine to give 0.201 g (34%) of violet needle. Mp 263 °C, IR (KBr, cm^{-1}): 3328 (–OH), 1683 (–C=O), ^1H NMR ($CDCl_3$) δ 1.2 (t, 6H, –$COOCH_2CH_3$), 2.3 (s, 3H, –NH–$C_6H_4CH_3$), 4.2 (q, 4H, –$COOCH_2CH_3$), 6.9, 7.1 (d, d, 2H, 2H, –NH–$C_6H_4CH_3$), 7.7 (dd, 1H, C-6), 7.9(s, 1H, –NH)–D_2O exchange, 8.5 (d, 1H, C-5), 9.0 (d, 1H, C-7). Anal. calcd for $C_{23}H_{22}N_2O_6$: C, 65.39; H, 5.25; N, 6.63, Found: C, 65.29; H, 5.21; N, 6.59.

6-(α-Diethoxycarbonyl-methyl)-7-(3-methyl-phenylamino)-quinoline-5,8-dione (B-2). The general procedure was followed for 12 h using 0.313 mL (2.844 mmol) of m-toluidine affording 0.184 g (31%) of violet precipitation. Mp 262 °C, IR (KBr, cm^{-1}): 3436 (–OH), 1695 (–C=O). ^1H NMR ($CDCl_3$) δ 1.2 (t, 6H, –$COOCH_2CH_3$), 2.4 (s, 3H, –NH–$C_6H_4CH_3$), 4.2 (q, 4H, –$COOCH_2CH_3$), 6.8–7.4 (m, 4H, –NH-$C_6H_4CH_3$), 7.7 (dd, 1H, C-6), 7.9 (s, 1H, –NH)–D_2O exchange, 8.5 (d, 1H, C-5), 9.0 (d, 1H, C-7). Anal. calcd for $C_{23}H_{22}N_2O_6$: C, 65.40; H, 5.25; N, 6.63. Found: C, 65.61; H, 5.07; N, 6.49.

6-(α-Diethoxycarbonyl-methyl)-7-(2-methyl-phenylamino)-quinoline-5,8-dione (B-3). The general procedure was followed for 12 h using 0.18 mL (1.7064 mmol) of o-toluidine and 0.4 mL (2.844 mmol) of triethylamine to give 0.153 g (26%) of orange precipitation. Mp over 250 °C, IR (KBr, cm^{-1}): 3259 (–OH), 1690 (–C=O). ^1H NMR ($CDCl_3$) δ 1.6 (t, 6H, –$COOCH_2CH_3$), 2.2 (s, 3H, –NH–$C_6H_4CH_3$), 4.2 (q, 4H, –$COOCH_2CH_3$), 4.7 (s, 1H, –$CH(COOC_2H_5)_2$)–D_2O exchange, 7.1, 7.1, 7.2 (d, d, t, 3H, –NH-$C_6H_4CH_3$), 7.5 (s, 1H, –NH)–D_2O exchange, 7.6 (dd, 1H, C-6), 8.4 (d, 1H, C-5), 9.1 (d, 1H, C-7), Anal. calcd for $C_{23}H_{22}N_2O_6$: C, 65.40; H, 5.25; N, 6.63. Found: C, 65.33; H, 5.11; N, 6.42.

6-(α-Diethoxycarbonyl-methyl)-7-(4-methoxy-phenylamino)-quinoline-5,8-dione (B-4). The general procedure was followed for 12 h using 0.21 g (1.7064 mmol) of ρ-methoxyaniline to give 0.213 g (34%) of violet precipitation. Mp over 250 °C, IR (KBr, cm^{-1}): 3284 (–OH), 1659 (–C=O), ^1H NMR ($CDCl_3$, δ): 1.2 (t, 6H, –$COOCH_2CH_3$), 3.8 (s, 3H, –NH–$C_6H_4OCH_3$), 4.2 (q, 4H, –$COOCH_2CH_3$), 4.7 (s, 1H, –$CH(COOC_2H_5)_2$) –D_2O exchange, 6.9, 7.0, 7.1 (d, d, dd, 4H, –NH–$C_6H_4OCH_3$), 7.7 (dd, 1H, C-6), 7.9(s, 1H, –NH)–D_2O exchange, 8.5 (d, 1H, C-5), 9.0 (d, 1H, C-7). Anal. calcd for $C_{23}H_{22}N_2O_7.H_2O$: C, 63.01; H, 5.06; N, 6.39. Found: C, 62.03; H, 4.68; N, 5.94.

6-(α-Diethoxycarbonyl-methyl)-7-(4-ethoxy-phenylamino)-quinoline-5,8-dione (B-5). The general procedure was followed for 12 h using 0.37 mL (2.843 mmol) of ρ-ethoxyaniline to produce 0.183 g (29%) of violet precipitation. Mp 228–230 °C, IR (KBr, cm^{-1}): 3300 (–OH), 1748 (–C=O), ^1H NMR ($CDCl_3$) δ 1.2 (t, 6H, –$COOCH_2CH_3$), 1.4 (t, 3H, –NH–$C_6H_4OCH_2CH_3$), 4.0 (q, 2H, –NH–$C_6H_4OCH_2CH_3$), 4.2 (q, 4H, –$COOCH_2CH_3$), 4.8 (s, 1H, –$CH(COOC_2H_5)_2$)–D_2O exchange, 6.9, 7.0, 7.1 (d, d, dd, 4H, –NH–$C_6H_4OCH_2CH_3$), 7.7 (dd, 1H, C-6), 7.9(s, 1H, –NH)–D_2O exchange, 8.5 (d, 1H, C-5), 9.0 (d, 1H, C-7). Anal. calcd for $C_{24}H_{24}N_2O_7$: C, 63.71; H, 5.35; N, 6.19. Found: C, 63.78; H, 5.38; N, 6.21.

***Bis*-(7-chloro-5,8-dioxo-quinolino)-6,6′-diethylmalonate (F).** A suspension of 1.17 g (0.03 mol) of sodium amide in 15 mL of hexamethyl phosphoramide was stirred in two-necked round flask equipped with a reflux condenser, a drying calcium chloride guard tube, at 0 °C, for 30 min. To the mixture, 4.555 mL (0.03 mol) of diethylmalonate was slowly dropped in ice bath for 20 min, stirred at room temperature for 30 min and was heated for 1 h. To 2.28 g (0.01 mol) of 6,7-dichloro-quinoilin-5,8-dione (**VII**) in 20 mL of absolute ethanol, the solution, the sodium salt of diethylmalonate, was slowly dropped using dropping funnel in an ice bath. The mixture was stirred for 1 h at 0 °C and then was heated under reflux for 45 min. To the reaction mixture, 250 mL of water was added and the mixture was filtered under the reduced pressure. The crude product was crystallized from ethanol to give 0.937 g (9%) of dark green scale precipitation. Mp 164 °C, IR (KBr, cm^{-1}): 1675 (–C=O), ^1H NMR ($CDCl_3$) δ 1.5 (t, 6H, –$COOCH_2CH_3$), 4.7 (q, 4H, –$COOCH_2CH_3$), 7.7 (dd, 2H, C-3, C-3′), 8.4 (d, 2H, C-4, C-4′), 9.1 (d, 2H, C-2, C-2′), Anal. calcd for $C_{21}H_{11}Cl_2N_2O_6$: C, 55.04; H, 2.42; N, 6.11. Found: C, 55.40; H, 3.05; N, 5.81, Beilstein test: Cl positive.

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