Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 7370–7376
Bioorganic & Medicinal Chemistry

Synthesis and antiproliferative evaluation of certain pyrido[3,2-*g*]quinoline derivatives

Shu-Yu Li,[a,c] Yeh-Long Chen,[a] Chihuei Wang[b,*] and Cherng-Chyi Tzeng[a,*]

[a]*Faculty of Medicinal and Applied Chemistry, College of Life Science, Kaohsiung Medical University, Kaohsiung City 807, Taiwan*
[b]*Faculty of Biotechnology, College of Life Science, Kaohsiung Medical University, Kaohsiung City 807, Taiwan*
[c]*Graduate Institute of Pharmaceutical Sciences, College of Pharmacy, Kaohsiung Medical University, Kaohsiung City 807, Taiwan*

Received 24 May 2006; revised 7 July 2006; accepted 11 July 2006
Available online 2 August 2006

Abstract—The present report describes the synthesis and evaluation of tricyclic pyrido[3,2-*g*]quinoline derivatives in which an additional pyridine ring is linearly fused on the antibacterial quinoline-3-carboxylic acid. Among them, only diethyl 4,6-diamino-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (**9a**) and diethyl 4,6-bis-(3-dimethylaminopropylamino)-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (**9d**) were able to completely inhibit cell proliferation of MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) implying either amino or dimethylaminopropyl moiety at C-4 and C-6 positions is crucial for the antiproliferative activity of pyrido[3,2-*g*]quinoline derivatives. Compounds **9a**–**9d** were further evaluated for their activity against the growth of MCF-7 and two prostate cancer cell lines, LNCaP and PC-3. Results indicated the antiproliferative activity decreased in an order **9d** > **9a** ≫ **9b** and **9c**. Compound **9d** was the most cytotoxic with an IC_{50} value of 5.63 and 3.96 μM, respectively, against LNCaP and MCF-7. Flow cytometric analyses revealed that growth inhibition of LNCaP by **9d** was due to cell cycle arrest in G1 phase, and followed by apoptosis.
© 2006 Elsevier Ltd. All rights reserved.

1. Introduction

Tricyclic 4,6-dihydroxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylic acid (**3**) and its diethyl ester **2** have been discovered to be useful for the treatment of disease or syndrome which is initiated by an antigen–antibody reaction, for example, allergic asthma, hay fever, urticaria, or an auto-immune disease.[1] The skeleton of these compounds belongs to DNA-intercalating coplanar aromatic chromophores and therefore, cytotoxic evaluation of pyrido[3,2-*g*]quinoline derivatives becomes crucial because highly cytotoxic agents will hardly be developed as anti-allergic drug candidates. For the past few years, we were interested in the synthesis and biological evaluation of quinolone-3-carboxylic acids.[2–5] These compounds are potent antibacterial agents that target the bacterial type II DNA topoisomerases (DNA gyrase and topoisomerase IV).[6] Due to structural and functional similarities between bacterial DNA gyrase and mammalian topoisomerase II, a series of modified tricyclic benzoquinolones have been synthesized in an attempt to shift the activity from antibacterial to DNA-intercalating.[7,8] Extensive SAR studies with DNA-intercalating chromophores reveal a positive correlation between the strength of reversible DNA binding and the cytotoxic potency.[9–11] For example, benzoacronycine (IC_{50} = 1.9 μM), the acronycine analogue with an additional aromatic ring linearly fused on its A-ring to enhance DNA-intercalating capability, exhibits 10-fold more potent than the parent acronycine (IC_{50} = 19.9 μM) in inhibiting L1210 cell proliferation.[12]

Due to DNA-intercalating capability exhibited by coplanar tricyclic benzoquinolones, the present report describes the synthesis and antiproliferative evaluation of potential anti-allergic pyrido[3,2-*g*]quinolin-4(1*H*)-ones **2** and **3**. Their substituted amino- and anilino-derivatives have also been synthesized for evaluation because a number of 4-anilinoquinoline derivatives were found to possess potent antiproliferative activity.[13–15]

2. Chemistry

Thermal condensation of 2,6-diaminotoluene with diethyl ethoxymethylenemalonate (EMME) by the

Keywords: Pyrido[3,2-*g*]quinoline; Antiproliferative.
* Corresponding authors. Tel.: +886 7 3121101x6985; fax: +886 7 3125339; e-mail: tzengch@kmu.edu.tw

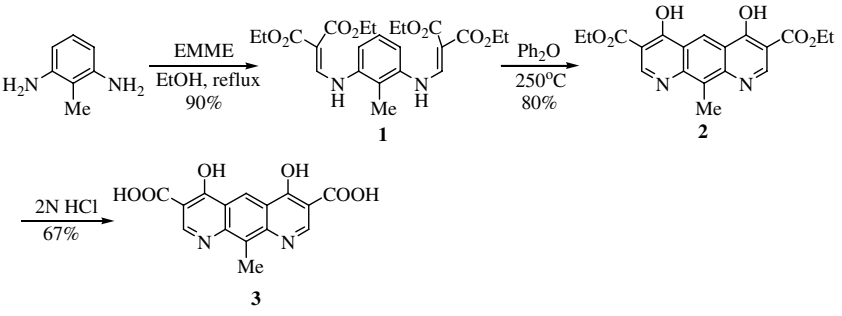
0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.07.030

Marcos procedure[16] gave diethyl 4,6-dihydroxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (**2**) which was hydrolyzed with 2 N HCl to give dicarboxylic acid **3** in a fairly good overall yield (Scheme 1).

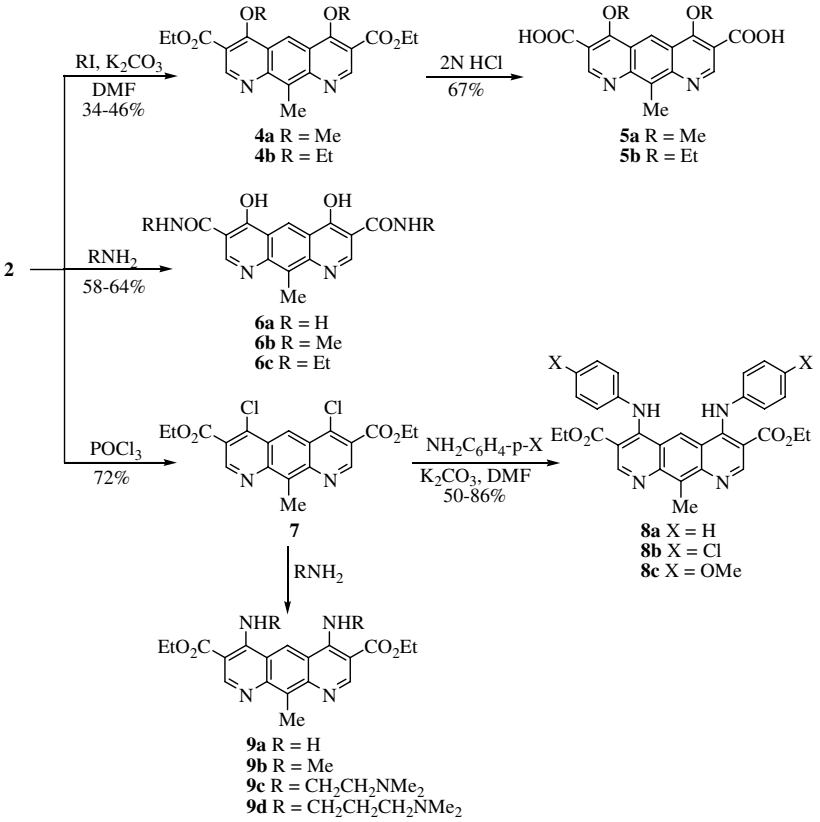
Alkylation of **2** with methyl iodide or ethyl iodide gave diethyl 4,6-dimethoxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (**4a**) or its 4,6-diethoxy counterpart **4b**. Hydrolysis of either **4a** or **4b** with 2 N HCl afforded their respective dicarboxylic acids **5a** or **5b** as depicted in Scheme 2. Treatment of **2** with ammonia afforded 4,6-dihydroxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxamide (**6a**). Accordingly, **6b** and **6c** were synthesized from **2** by reaction with methylamine and ethylamine, respectively. Chlorination of **2** with $POCl_3$ gave diethyl 4,6-dichloro-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (**7**) which was treated with aniline or 4-substituted anilines to afford **8a** and its substituted derivatives **8b** and **8c**. Accordingly, reaction of **7** with amine or substituted amines gave **9a** and its substituted derivatives **9b**–**9d** (see Scheme 2).

3. Pharmacological results and discussion

All the pyrido[3,2-*g*]quinoline derivatives **2–9** were evaluated in vitro against a 3-cell line panel consisting of MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) in US National Cancer Institute (NCI). In this protocol, each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration (100 μM) and the culture was incubated for 48 h. End-point determinations were made with alamar blue.[17] Results for each test agent were reported as



Scheme 1.



Scheme 2.

the percent of growth of the treated cells in comparison with the untreated control cells. Compounds which reduced the growth of any one of the cell lines to 32% or less are considered active. The results indicated most of them were inactive against the primary 3-cell line panel except diethyl 4,6-diamino-10-methylpyrido[3,2-*g*]-quinoline-3,7-dicarboxylate (**9a**) and diethyl 4,6-bis-(3-dimethylaminopropylamino)-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (**9d**) that were able to completely inhibit cell proliferation of all three cells (Table 1). Either amino or dimethylaminopropyl moiety at C-4 and C-6 positions is crucial for the antiproliferative activity of pyrido[3,2-*g*]quinoline derivatives. Compounds **9a**–**9d** were further evaluated for their activity against the growth of two prostate cancer cells, LNCaP and PC-3, and a breast cancer cell, MCF-7. For each compound, dose–response curves for each cell line were measured with 10 different drug concentrations, and the concentration for 50% of cell growth inhibition (IC_{50}) was calculated by SigmaPlot. Results from Table 2 indicated the antiproliferative activity decreased in an order **9d** > **9a** ≫ **9b** and **9c**. Compound **9d** was the most cytotoxic with an IC_{50} value of 5.63 and 3.96 μM, respectively, against LNCaP and MCF-7, and therefore was selected for further evaluation of its effect on cell cycle distribution of LNCaP cells. As shown in Figure 1, the proportion of cells was slightly accumulated in G1 phase, however, was decreased in the S phase of the cell cycle after 24 h treatment. After 48 h, the accumulation of G1 DNA content significantly decreased, while the hypodiploid (sub G1 phase) cells increased as shown in Figure 2. Compound **9d** inhibited proliferation of LNCaP by the alteration of cell division, accumulation of cells in G1 phase at early 24 h, and it was then decreased followed by the increase of apoptotic cells (sub G1 phase) after 48 h treatment. This suggested that compound **9d** induces cell cycle arrest in G1 phase followed by apoptosis.

Table 1. Antiproliferative activity of pyrido[3,2-*g*]quinoline derivatives[a]

Compound	Growth percentages		
	NCI-H460 (Lung)	MCF7 (Breast)	SF-268 (CNS)
2	118	115	93
3	107	97	81
4a	122	104	99
4b	95	105	119
5a	111	108	97
5b	120	106	114
6a	96	85	107
6b	76	75	84
6c	104	114	110
8a	111	117	108
8b	108	111	108
8c	115	120	110
9a	0	0	0
9c	74	101	99
9d	0	0	0

[a] Compounds were evaluated in vitro against a 3-cell line panel consisting of MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS). In this protocol, each cell line is inoculated and preincubated on a microtiter plate. Test agents are then added at a single concentration (100 μM) and the culture is incubated for 48 h. End-point determinations are made with alamar blue.[17] Results for each test agent are reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds which reduced the growth of any one of the cell lines to 32% or less are active.

Table 2. IC_{50} (μM) values of selective pyrido[3,2-*g*]quinoline derivatives against LNCaP, PC-3 (Prostate cancer), and MCF-7 (Breast cancer) cells

Compound	LNCaP	PC-3	MCF-7
9a	16.91	14.73	14.54
9b	51.04	71.98	76.38
9c	62.49	59.57	69.52
9d	5.63	6.42	3.96

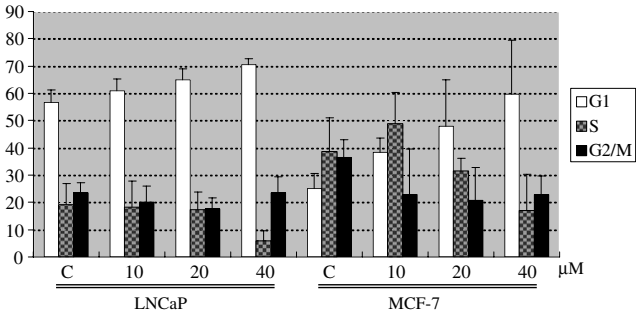


Figure 1. Dose–response effects of compound **9d** on cell cycle in LNCaP and MCF-7 cancer cells for 24 h.

4. Conclusion

A number of pyrido[3,2-*g*]quinoline derivatives were synthesized and evaluated for their antiproliferative activity. The results indicated most of them, with exception of **9a** and **9d**, are non-cytotoxic against MCF7, NCI-H460, and SF-268 cells at a concentration of 100 μM although their skeleton belongs to DNA-intercalating coplanar aromatic chromophores. These results are valuable because **9a** and **9d** are new leads for further design and synthesis of potential anticancer agents, while others (**2–8** and **9c**) can be developed as anti-allergic drug candidates.

5. Experimental

5.1. General

TLC: precoated (0.2 mm) silica gel 60 F_{254} plates from EM Laboratories, Inc.; detection by UV light (254 nm). All chromatographic separations were performed using silica gel (Merck 60 230–400 mesh). Mp: Electrothermal IA9100 melting point apparatus; uncorrected. ^{1}H and ^{13}C NMR spectra: Varian-Unity-400 spectrometer at 400 and 100 MHz or Varian-Gemini-200 spectrometer at 200 and 50 MHz chemical shifts δ in ppm with $SiMe_4$ as an internal standard (=0 ppm), coupling constants J in Hz. Mass spectra (HRMS) were recorded on Finnigan/Thermo Quest MAT 95XL.

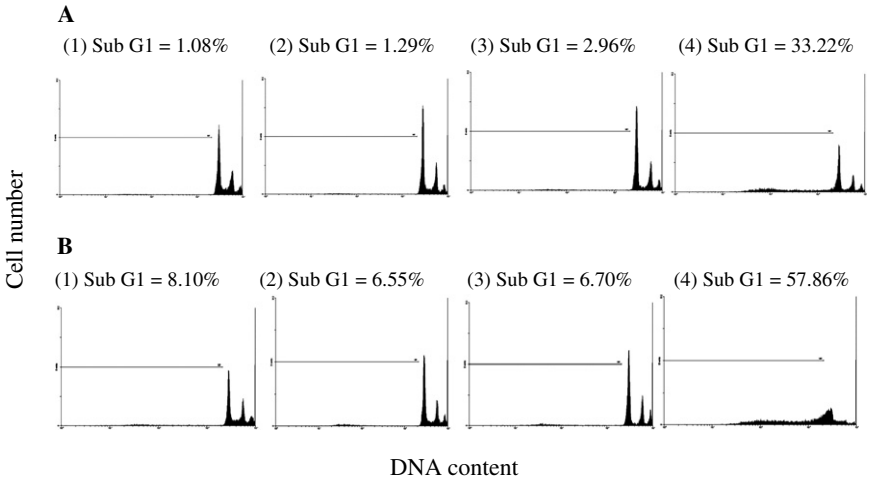


Figure 2. Apoptosis is induced by **9d**. LNCaP cells were treated in (A) 24 h and (B) 48 h culture with: (1) 0.1% DMSO control, (2) 5 μM, (3) 10 μM, or (4) 20 μM of **9d** prior to PI staining and analysis of DNA content. Apoptosis is apparent by the large population of cells with increased DNA content in sub G1.

Elemental analyses were carried out on a Heraeus CHN-O-Rapid elemental analyzer, and results were within ±0.4% of calculated values.

5.1.1. Diethyl 2-{[3-(2,2-bisethoxycarbonylvinylamino)-2-methylphenylamino]-methylene}malonate (1). A mixture of 2,6-diaminotoluene (0.61 g, 5 mmol) and diethyl ethoxymethylenemalonate (2.16 g, 10 mmol) in EtOH (15 mL) was heated at reflux for 5 h. The mixture was evaporated under reduced pressure and then *n*-hexane (100 mL) was added. The resulting precipitate was collected and crystallized from EtOH to afford **1** (2.08 g, 90%). Mp 138–140 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 1.30–1.43 (m, 12H, OCH_2CH_3), 2.34 (s, 3H, 2-CH_3), 4.20–4.39 (m, 8H, OCH_2CH_3), 7.07 (d, J = 8.2 Hz, 2H–C(4, 6)), 7.32 (t, J = 8.2 Hz, 1H–C(5)), 8.49 (d, J = 13.2 Hz, 2H, NHC*H*═), 11.20 (d, J = 13.2 Hz, 2H, N*H*CH═). ^{13}C NMR (50 MHz, $CDCl_3$): 11.27 (2-*C*H_3), 14.26 (2C), 14.36 (2C), 60.13 (2C), 60.51 (2C), 94.51 (2C), 112.96 (2C), 117.42, 128.04, 139.39 (2C), 152.68 (2C), 165.57 (2C), 169.19 (2C). Anal. Calcd for $C_{23}H_{30}N_2O_8$·0.2 EtOH: C, 59.57; H, 6.68; N, 5.94. Found: C, 59.25; H, 6.49; N, 5.98.

5.1.2. Diethyl 4,6-dihydroxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (2). A solution of **1** (0.93 g, 2 mmol) in Ph_2O (30 mL) was heated at 250–260 °C for 2 h (TLC monitoring). The reaction mixture was cooled and then *n*-hexane (100 mL) was added. The resulting precipitate was filtered, washed with *n*-hexane, CH_2Cl_2, MeOH, and dried under vacuum to afford **2** (0.59 g, 80%). Mp > 300 °C. ^{1}H NMR (200 MHz, TFA-*d*): 1.61 (t, J = 7.2 Hz, 6H, OCH_2CH_3), 3.31 (s, 3H, 10-CH_3), 4.80 (q, J = 7.2 Hz, 4H, OCH_2CH_3), 9.68 (s, 2H–C(2, 8)), 10.09 (s, 1H–C(5)). ^{13}C NMR (50 MHz, TFA-*d*): 12.31 (10-*C*H_3), 14.40 (2C), 67.86 (2C), 107.71 (2C), 122.04 (2C), 124.25, 127.37, 141.49 (2C), 153.26 (2C), 169.31 (2C), 178.41 (2C). Anal. Calcd for $C_{19}H_{18}N_2O_6$: C, 61.62; H, 4.90; N, 7.56. Found: C, 61.63; H, 4.98; N, 7.69.

5.1.3. 4,6-Dihydroxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylic acid (3). To a suspension of **2** (0.37 g, 1 mmol) in EtOH (5 mL) was added a solution of 2 N HCl (20 mL) and the mixture heated at reflux for 2 h. The mixture was evaporated under reduced pressure and the residual solid was crystallized from MeOH to give **3** (0.21 g, 67%). Mp > 300 °C [lit.[1] mp 358–360 °C]. ^{1}H NMR (200 MHz, TFA-*d*): 3.23 (s, 3H, 10-CH_3), 9.64 (s, 2H–C(2, 8)), 9.96 (s, 1H–C(5)). ^{13}C NMR (50 MHz, TFA-*d*): 11.75 (10-*C*H_3), 107.84 (2C), 122.24 (2C), 123.10, 127.69, 142.18 (2C), 152.88 (2C), 171.80 (2C), 179.89 (2C). Anal. Calcd for $C_{15}H_{10}N_2O_6$·0.8 H_2O: C, 54.81; H, 3.56; N, 8.52. Found: C, 54.78; H, 3.95; N, 8.19.

5.1.4. Diethyl 4,6-dimethoxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (4a). A mixture of **2** (0.37 g, 1 mmol), K_2CO_3 (1.10 g, 8 mmol), and CH_3I (0.85 g, 6 mmol) in DMF (50 mL) was heated at 40–45 °C for 22 h (TLC monitoring). The mixture was evaporated under reduced pressure and then H_2O (200 mL) was added. The resulting precipitate was collected, purified by flash column chromatography (FC, silica gel; *n*-hexane/EtOAc = 1:1), and crystallized from EtOH to afford **4a** (0.14 g, 34%). Mp > 300 °C. ^{1}H NMR (200 MHz, TFA-*d*): 1.66 (t, J = 7.0 Hz, 6H, OCH_2CH_3), 3.34 (s, 3H, 10-CH_3), 4.87 (m, 10H, 4,6-O*C*H_3, OCH_2CH_3), 9.70 (s, 2H–C(2, 8)), 10.06 (s, 1H–C(5)). ^{13}C NMR (50 MHz, TFA-*d*): 12.69 (10-*C*H_3), 14.42 (2C), 52.14 (2C), 67.83 (2C), 108.57 (2C), 123.56 (2C), 126.44, 127.89, 149.15 (2C), 160.38 (2C), 168.92 (2C), 176.85 (2C). Anal. Calcd for $C_{21}H_{22}N_2O_6$·H_2O: C, 60.56; H, 5.82; N, 6.73. Found: C, 60.30; H, 5.80; N, 6.87.

5.1.5. Diethyl 4,6-diethoxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (4b). Prepared from **2** by the same procedure as described for **4a**. Compound **4b** was purified by FC (*n*-hexane/EtOAc = 3:1) and crystallized from EtOH in a 46% yield. Mp 156–157 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 1.48 and 1.62 (two t, J = 7.0 Hz, 12H, OCH_2CH_3), 3.32 (s, 3H, 10-CH_3), 4.48 (m, 8H,

OCH_2CH$_3$), 9.25 (s, 1H–C(5)), 9.34 (s, 2H–C(2, 8)). ^{13}C NMR (50 MHz, $CDCl_3$): 12.36 (10-CH$_3$), 14.24 (2C), 15.79 (2C), 61.54 (2C), 72.54 (2C), 112.40 (2C), 118.15 (2C), 122.52, 136.06, 148.19 (2C), 153.00 (2C), 165.08 (2C), 165.22 (2C). Anal. Calcd for $C_{23}H_{26}N_2O_6$: C, 64.78; H, 6.15; N, 6.57. Found: C, 64.79; H, 6.17; N, 6.54.

5.1.6. 4,6-Dimethoxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylic acid (5a). Prepared from **4a** by the same procedure as described for **3**. Compound **5a** was crystallized from EtOH in a 67% yield. Mp > 300 °C. ^{1}H NMR (200 MHz, TFA-*d*): 3.15 (s, 3H, 10-CH$_3$), 4.64 (s, 6H, OCH$_3$), 9.51 (s, 2H–C(2, 8)), 9.81 (s, 1H–C(5)). ^{13}C NMR (50 MHz, TFA-*d*): 14.49 (10-CH$_3$), 51.26 (2C), 108.35 (2C), 124.60 (2C), 124.77, 128.19, 149.96 (2C), 160.22 (2C), 171.49 (2C), 178.46 (2C). Anal. Calcd for $C_{17}H_{14}N_2O_6{\cdot}H_2O$: C, 56.66; H, 4.48; N, 7.78. Found: C, 56.47; H, 4.52; N, 7.52.

5.1.7. 4,6-Diethoxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylic acid (5b). Prepared from **4b** by the same procedure as described for **3**. Compound **5b** was crystallized from EtOH in a 77% yield. Mp > 300 °C. ^{1}H NMR (200 MHz, TFA-*d*): 1.60 (t, J = 7.0 Hz, 6H, OCH$_2$CH_3), 3.30 (s, 3H, 10-CH$_3$), 4.78 (q, J = 7.0 Hz, 4H, OCH_2CH$_3$), 9.67 (s, 2H–C(2, 8)), 10.07 (s, 1H–C(5)). ^{13}C NMR (50 MHz, TFA-*d*): 12.26 (10-CH$_3$), 14.35 (2C), 67.79 (2C), 107.61 (2C), 122.93 (2C), 124.20, 127.32, 141.40 (2C), 153.21 (2C), 169.26 (2C), 178.35 (2C). Anal. Calcd for $C_{19}H_{18}N_2O_6{\cdot}H_2O$: C, 58.75; H, 5.20; N, 7.21. Found: C, 58.40; H, 5.30; N, 7.12.

5.1.8. 4,6-Dihydroxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxamide (6a). A mixture of **2** (0.50 g, 1.35 mmol) and 30% NH_4OH (50 mL) in a sealed vessel was heated at 70 °C for 8 h (TLC monitoring). The resulting mixture was evaporated under reduced pressure and then H_2O (100 mL) was added. The precipitate was collected, washed with H_2O, and then crystallized from EtOH to give **6a** (0.25 g, 60%)as a yellow powder. Mp > 300 °C. ^{1}H NMR (200 MHz, TFA-*d*): 3.32 (s, 3H, 10-CH$_3$), 9.77 (s, 2H–C(2, 8)), 10.00 (s, 1H–C(5)). ^{13}C NMR (50 MHz, TFA-*d*): 11.35 (10-CH$_3$), 107.57 (2C), 120.49 (2C), 124.34, 126.69, 142.00 (2C), 150.75 (2C), 175.33 (2C), 180.28 (2C). HRMS (ESI) Calcd for $C_{15}H_{13}FN_4O_4$(M+1): 313.0937, found: 313.0940.

5.1.9. 4,6-Dihydroxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylic acid bis-methylamide (6b). Prepared from **2** and 40% CH_3NH_2 by the same procedure as described for **6a**. Compound **6b** was crystallized from EtOH in a 64% yield. Mp > 300 °C. ^{1}H NMR (200 MHz, TFA-*d*): 3.11 (s, 3H, 10-CH$_3$), 3.32 (s, 6H, NCH$_3$), 9.57 (s, 2H–C(2, 8)), 9.78 (s, 1H–C(5)). ^{13}C NMR (50 MHz, TFA-*d*): 11.26 (10-CH$_3$), 28.56 (2C), 108.08 (2C), 120.28 (2C), 124.14, 127.61, 141.69 (2C), 149.61 (2C), 170.69 (2C), 180.79 (2C). Anal. Calcd for $C_{17}H_{16}N_4O_4{\cdot}2.0H_2O$: C, 54.24; H, 5.37; N, 14.89. Found: C, 54.46; H, 5.46; N, 14.46. HRMS (ESI) Calcd for $C_{17}H_{17}N_4O_4$(M+1): 341.1250, found: 341.1253.

5.1.10. 4,6-Dihydroxy-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylic acid bis-ethylamide (6c). Prepared from **2** and 70% $EtNH_2$ by the same procedure as described for **6a**. Compound **6c** was crystallized from EtOH in a 58% yield. Mp > 300 °C. ^{1}H NMR (200 MHz, TFA-*d*): 1.47 (t, J = 7.4 Hz, 6H, NCH$_2$CH_3), 3.08 (s, 3H, 10-CH$_3$), 3.79 (q, J = 7.4 Hz, 4H, NCH_2CH$_3$), 9.55 (s, 2H–C(2, 8)), 9.77 (s, 1H–C(5)). ^{13}C NMR (50 MHz, TFA-*d*): 11.27 (2C), 14.23 (10-CH$_3$), 38.66 (2C), 107.73 (2C), 120.32 (2C), 124.18, 127.61, 141.66 (2C), 149.56 (2C), 170.02 (2C), 181.06 (2C). Anal. Calcd for $C_{19}H_{20}N_4O_4{\cdot}1.3\ H_2O$: C, 58.23; H, 5.82; N, 14.30. Found: C, 58.27; H, 5.85; N, 14.17.

5.1.11. Diethyl 4,6-dichloro-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (7). A mixture of **2** (0.37 g, 1 mmol) in $POCl_3$ (10 mL) was heated at 90 °C for 4 h (TLC monitoring). After cooling, the mixture was poured into ice-water (50 mL) and neutralized with saturated Na_2CO_3until pH 7 resulted. This aqueous mixture was extracted with CH_2Cl_2 (50 mL 3×) dried ($MgSO_4$), and concentrated to yield a brown solid. The crude product was purified by FC (using *n*-hexane/EtOAc = 9:1 as the eluent) to give a yellow solid, which was recrystallized with MeOH to give **7** (0.29 g, 72%). Mp 125–126 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 1.51 (t, J = 7.0 Hz, 6H, OCH$_2$CH_3), 3.33 (s, 3H, 10-CH$_3$), 4.55 (q, J = 7.0 Hz, 4H, OCH_2CH$_3$), 9.33 (s, 2H–C(2, 8)), 9.37 (s, 1H–C(5)). ^{13}C NMR (50 MHz, $CDCl_3$): 12.85 (10-CH$_3$), 14.23 (2C), 62.30 (2C), 122.46 (2C), 122.87 (2C), 124.92, 138.42, 144.87 (2C), 146.42 (2C), 150.94 (2C), 164.13 (2C). Anal. Calcd for $C_{19}H_{16}Cl_2N_2O_4$: C, 56.03; H, 3.97; N, 6.88. Found: C, 56.32; H, 3.97; N, 7.07.

5.1.12. Diethyl 10-methyl-4,6-bisphenylaminopyrido[3,2-*g*]quinoline-3,7-dicarboxylate (8a). A mixture of **7** (0.20 g, 0.5 mmol), aniline (0.28 g, 3 mmol), and dry DMF (20 mL) was heated in 100 °C for 6 h (TLC monitoring). The mixture was then cooled and evaporated in vacuo to yield a yellow residue, treated with H_2O (50 mL), and the resulting precipitate was filtered and washed with H_2O. The crude product was purified by FC (using *n*-hexane/EtOAc = 3:1 as the eluent) to give a yellow solid, which was recrystallized with EtOH to give **8a** (0.15 g, 56%). Mp 251–253 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 1.43 (t, J = 7.2 Hz, 6H, OCH$_2$CH_3), 3.27 (s, 3H, 10-CH$_3$), 4.40 (q, J = 7.2 Hz, 4H, OCH_2CH$_3$), 6.59 (m, 4H, Ar–H), 7.72 (m, 6H, Ar–H), 8.38 (s, 1H–C(5)), 9.33 (s, 2H–C(2, 8)), 10.52 (br s, 2H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): 12.62 (10-CH$_3$), 14.23 (2C), 61.36 (2C), 104.56 (2C), 115.30 (2C), 119.72, 122.52 (4C), 124.90 (2C), 125.30, 129.47 (4C), 141.26 (2C), 146.22 (2C), 151.96 (2C), 154.22 (2C), 168.15 (2C). Anal. Calcd for $C_{31}H_{28}N_4O_4$: C, 71.51; H, 5.43; N, 10.76. Found: C, 71.31; H, 5.50; N, 10.76.

5.1.13. Diethyl 4,6-bis-(4-chlorophenylamino)-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (8b). Prepared from **7** and *p*-chloroaniline by the same procedure as described for **8a**. Compound **8b** was crystallized from EtOH in a 68% yield. Mp 308–309 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 1.44 (t, J = 7.2 Hz, 6H, OCH$_2$CH_3),

3.29 (s, 3H, 10-CH_3), 4.43 (q, J = 7.2 Hz, 4H, OCH_2CH_3), 6.60 (m, 4H, Ar–H), 7.16 (m, 4H, Ar–H), 8.42 (s, 1H–C(5)), 9.38 (s, 2H–C(2, 8)), 10.71 (br s, 2H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): 12.70 (10-CH_3), 14.24 (2C), 61.37 (2C), 105.10 (2C), 115.52 (2C), 120.74, 123.14 (4C), 124.11 (2C), 129.38 (4C), 129.61 (2C), 135.72, 140.23 (2C), 147.81, 152.06 (2C), 153.12 (2C), 168.37 (2C). Anal. Calcd for $C_{31}H_{26}Cl_2N_4O_4 \cdot 0.5\ H_2O$: C, 62.20; H, 4.56; N, 9.36. Found: C, 62.13; H, 4.47; N, 9.27.

5.1.14. Diethyl 4,6-bis-(4-methoxyphenylamino)-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (8c). Prepared from **7** and *p*-anisidine by the same procedure as described for **8a**. Compound **8c** was crystallized from EtOH in a 50% yield. Mp 290–292 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 1.43 (t, J = 7.2 Hz, 6H, OCH_2CH_3), 3.25 (s, 3H, 10-CH_3), 3.84 (s, 6H, OCH_3), 4.41 (q, J = 7.2 Hz, 4H, OCH_2CH_3), 6.57 (m, 4H, Ar–H), 6.73 (m, 4H, Ar–H), 8.46 (s, 1H–C(5)), 9.34 (s, 2H–C(2, 8)), 10.71 (br s, 2H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 12.620 (10-CH_3), 14.23 (2C), 55.62 (2C), 61.35 (2C), 103.79 (2C), 114.73 (4C), 115.03 (2C), 122.92, 123.99 (4C), 125.12 (2C), 134.12 (2C), 135.16, 151.90 (2C), 154.71 (2C), 157.13 (2C), 168.18 (2C). Anal. Calcd for $C_{33}H_{32}N_4O_6 \cdot 0.2\ H_2O$: C, 67.83; H, 5.60; N, 9.59. Found: C, 67.94; H, 5.56; N, 9.58

5.1.15. Diethyl 4,6-diamino-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (9a). Prepared from **7** and 30% NH_4OH by the same procedure as described for **6a**. Compound **9a** was crystallized from EtOH in a 56% yield. Mp > 300 °C. ^{1}H NMR (200 MHz, TFA-*d*): 1.57 (t, J = 7.0 Hz, 6H, OCH_2CH_3), 3.08 (s, 3H, 10-CH_3), 4.66 (q, J = 7.0 Hz, 4H, OCH_2CH_3), 9.33 (s, 2H–C(2, 8)), 9.97 (s, 1H–C(5)). ^{13}C NMR (50 MHz, TFA-*d*): 12.10 (10-CH_3), 14.47 (2C), 66.36 (2C), 103.94 (2C), 118.28 (2C), 121.44, 124.72, 140.22 (2C), 150.97 (2C), 161.97 (2C), 167.72 (2C). Anal. Calcd for $C_{19}H_{20}N_4O_4 \cdot 1.5$ HCl: C, 53.92; H, 5.13; N, 13.24. Found: C, 53.68; H, 5.28; N, 13.33.

5.1.16. Diethyl 4,6-bismethylamino-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (9b). Prepared from **7** and 40% CH_3NH_2 by the same procedure as described for **6a**. Compound **9b** was crystallized from EtOH in a 41% yield. Mp 219–221 °C. ^{1}H NMR (200 MHz, TFA-*d*): 1.54 (t, J = 7.2 Hz, 6H, OCH_2CH_3), 2.98 (s, 3H, 10-CH_3), 3.94 (s, 6H, NCH_3), 4.60 (q, J = 7.2 Hz, 4H, OCH_2CH_3), 9.15 (s, 2H–C(2, 8)), 9.70 (s, 1H–C(5)). ^{13}C NMR (50 MHz, TFA-*d*): 11.99 (10-CH_3), 14.42 (2C), 37.37 (2C), 66.32 (2C), 104.93 (2C), 116.69 (2C), 119.39, 131.16, 141.62 (2C), 148.60 (2C), 162.65 (2C), 168.58 (2C). Anal. Calcd for $C_{21}H_{24}N_4O_4 \cdot 0.2$ HCl: C, 62.46; H, 6.05; N, 13.87. Found: C, 62.74; H, 5.96; N, 13.53.

5.1.17. Diethyl 4,6-bis-(2-dimethylaminoethylamino)-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (9c). Prepared from **7** and *N*,*N*-dimethylethylenediamine by the same procedure as described for **6a**. Compound **9c** was crystallized from EtOH in a 63% yield. Mp 186–188 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 1.43 (t, J = 7.2 Hz, 6H, OCH_2CH_3), 2.35 (s, 12H, $N(CH_3)_2$), 2.62 (t, J = 6.0 Hz, 4H, $NCH_2CH_2N(CH_3)_2$), 3.17 (s, 3H, 10-CH_3), 3.89 (q, J = 6.0 Hz, 4H, $NCH_2CH_2N(CH_3)_2$), 4.40 (q, J = 7.2 Hz, 4H, OCH_2CH_3), 8.98 (s, 1H–C(5)), 9.21 (s, 2H–C(2, 8)), 9.59 (br s, 2H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): 12.62 (10-CH_3), 14.29 (2C), 45.35 (4C), 47.02 (2C), 59.43 (2C), 60.51 (2C), 101.60 (2C), 116.28 (2C), 122.58 (2C), 133.78, 148.51, 152.46 (2C), 158.00 (2C), 168.37 (2C). Anal. Calcd for $C_{27}H_{38}N_6O_4$: C, 63.49; H, 7.52; N, 16.46. Found: C, 63.10; H, 7.49; N, 16.43.

5.1.18. Diethyl 4,6-bis-(3-dimethylaminopropylamino)-10-methylpyrido[3,2-*g*]quinoline-3,7-dicarboxylate (9d). Prepared from **7** and *N*,*N*-dimethylpropane-1,3-diamine by the same procedure as described for **6a**. Compound **9d** was crystallized from EtOH in a 46% yield. Mp 184–186 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 1.43 (t, J = 7.2 Hz, 6H, OCH_2CH_3), 1.94 (q, J = 6.6 Hz, 4H, $NCH_2CH_2CH_2N(CH_3)_2$), 2.22 (s, 12H, $N(CH_3)_2$), 2.44 (t, J = 6.6 Hz, 4H, $NCH_2CH_2CH_2N(CH_3)_2$), 3.16 (s, 3H, 10-CH_3), 3.91 (q, J = 6.6 Hz, 4H, $NCH_2CH_2CH_2N(CH_3)_2$), 4.39 (q, J = 7.2 Hz, 4H, OCH_2CH_3), 9.02 (s, 1H–C(5)), 9.20 (s, 2H–C(2, 8)), 9.58 (br s, 2H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): 12.66 (10-CH_3), 14.29 (2C), 29.10 (2C), 45.44 (4C), 47.46 (2C), 56.64 (2C), 60.55 (2C), 100.97 (2C), 116.21 (2C), 123.38 (2C), 133.76, 148.68, 152.40 (2C), 158.34 (2C), 168.92 (2C). Anal. Calcd for $C_{29}H_{42}N_6O_4 \cdot 0.5$ HCl: C, 62.53; H, 7.71; N, 15.09. Found: C, 62.46; H, 7.89; N, 15.02.

5.2. Flow cytometric analysis

5.2.1. Chemicals. RPMI 1640 medium, fetal bovine serum (FBS), trypsin-EDTA, phosphate-buffered saline (PBS), penicillin G, and streptomycin were obtained from GIBCO BRL (Gaithersburg, MD). Trypan blue, dimethylsulfoxide (DMSO), ribonuclease A (RNase A), Triton X-100, and propidium iodide (PI) were purchased from Sigma Chemical (St. Louis, MD).

5.2.2. Cell culture. LNCaP, MCF-7, and PC-3 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) at 37 °C in a humidified atmosphere of 5% CO_2.

5.2.3. Cell viability and cytotoxicity. The viability and cytotoxicity of cells were determined by the ATPLite assay system (Perkin-Elmer). Exponentially growing cells (5×10^3 cells) were plated in 96-well plates and treated with a series of concentrations of compounds dissolved in RPMI 1640 medium. Incubation was carried out at 37 °C for 48 h. ATPLite assay reagents (Perkin-Elmer) were added and luminescence measured by TopCounter (Perkin-Elmer). The concentration that killed 50% of cells (IC_{50}) was determined from the curve by calculating the concentration of agent that reduced the readout of luciferase activity in treated cells, compared to control cells by SigmaPlot (SYSTAT).

5.2.4. Flow cytometric analysis. Cells were seeded onto 10-cm dishes and treated with or without compounds for 24 and 48 h. Cells were harvested, washed twice with ice-cold PBS, and collected by centrifugation at 2000*g* for 5 min at 4 °C. The harvested cells were fixed in 70% (v/v) ethanol at 4 °C for 30 min. After fixation, cells were centrifuged and the solution was discarded, and then the fixed cells were incubated with 500 μl PBS containing 5% of Triton X-100 and 2 μg/mL RNase A at 37 °C for 1 h. Then, 50 μl of propidium iodide (500 μg/mL propidium iodide in PBS) was added and shaked in dark for 30 min at room temperature. Cells were detected using a cytometer (Coulter EpicsXL, Beckman) and analyzed by Win cycle and Win MDI 2.8 programs.

Acknowledgments

Financial support to this work by the National Science Council of the Republic of China is gratefully acknowledged. We also thank National Cancer Institute (NCI) of the United States for the anticancer screenings, and the National Center for High-Performance Computing for providing computer resources and chemical database services.

References and notes

1. Imperial Chemical Industries Ltd, Germany Patent No. DE2220294, 1972.
2. Fang, K. C.; Chen, Y. L.; Sheu, J. Y.; Wang, T. C.; Tzeng, C. C. *J. Med. Chem.* **2000**, *43*, 3809.
3. Chen, Y. L.; Fang, K. C.; Sheu, J. Y.; Hsu, S. L.; Tzeng, C. C. *J. Med. Chem.* **2001**, *44*, 2374.
4. Tzeng, C. C.; Chen, Y. L. *Chin. Pharm. J.* **2002**, *54*, 229.
5. Sheu, J. Y.; Chen, Y. L.; Tzeng, C. C.; Hsu, S. L.; Fang, K. C.; Wang, T. C. *Helv. Chim. Acta* **2003**, *86*, 2481.
6. Marians, K. J.; Hiasa, H. *J. Biol. Chem.* **1997**, *272*, 9401.
7. Tabarrini, O.; Cecchetti, V.; Fravolini, A.; Nocentini, G.; Barzi, A.; Sabatini, S.; Miao, H.; Sissi, C. *J. Med. Chem* **1999**, *42*, 2136.
8. Hsu, S. L.; Chen, Y. L.; Tzeng, C. C. *Heterocycles* **2004**, *63*, 529.
9. Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem* **1981**, *24*, 520.
10. Hartley, J. A.; Reszka, K.; Zuo, E. T.; Wilson, W. D.; Morgan, A. R.; Lown, J. W. *Mol. Pharmacol.* **1988**, *33*, 265.
11. Denny, W. A. *Anti-Cancer Drug Des.* **1989**, *4*, 241.
12. Costes, N.; Deit, H. L.; Michel, S.; Tillequin, F.; Koch, M.; Pfeiffer, B.; Renard, P.; Leonce, S.; Guilbaud, N.; Kraus-Berthier, L.; Pierre, A.; Atassi, G. *J. Med. Chem* **2000**, *43*, 2395.
13. Zhao, Y. L.; Chen, Y. L.; Chang, F. S.; Tzeng, C. C. *Eur. J. Med. Chem* **2005**, *40*, 792.
14. Chen, Y. L.; Chen, I. L.; Wang, T. C.; Han, C. H.; Tzeng, C. C. *Eur. J. Med. Chem* **2005**, *40*, 928.
15. Chen, Y. L.; Huang, C. J.; Huang, Z. Y.; Tseng, C. H.; Chang, F. S.; Yang, S. H.; Lin, S. R.; Tzeng, C. C. *Bioorg. Med. Chem* **2006**, *14*, 3098.
16. Marcos, A.; Pedregal, C.; Avendano, C. *Tetrahedron* **1994**, *50*, 12941.
17. Gray, G. D.; Wickstrom, E. *Biotechniques* **1996**, *21*, 780.