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A C-Ring Regioisomer of the Marine Alkaloid Meridine Exhibits Selective In Vitro Cytotoxicity for Solid Tumours

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Abstract—9-Hydroxybenzo[*b*]pyrido[4,3,2-*de*](1,10)-phenantrolin-8-one (**1**), a regioisomer of the marine alkaloid meridine, was synthesized from 5,8-dimethoxy-6-nitro-4(1*H*)-quinolinone in eight steps and 23% overall yield. A shorter route was also investigated, based on the hetero Diels–Alder reaction between *o*-nitrocinnamaldehyde dimethylhydrazone and 4-halogen-6-bromo-5,8-quinolinequinones followed by reductive cyclization onto the C-5 carbonyl of the quinone. Compound **1** showed a remarkable in vitro cytotoxicity, with a pattern of selectivity towards solid tumours that is not found in the reference alkaloid, the activity against the human lung carcinoma (A-549) being particularly noteworthy. The activities of meridine and compound **1** as inhibitors of topoisomerase II were also significantly different.

Introduction

Marine organisms are increasingly important as sources of new natural products with interesting biological properties, which include excellent antitumour activities.[1,2] One of the most interesting groups of compounds of marine origin is the family of polycyclic aromatic alkaloids derived from the pyrido[*kl*]acridine skeleton,[3] such as meridine[4] and amphimedine.[5,6] These compounds have been normally isolated from sponges or tunicates,[7] although they might be synthesized by associated microorganisms.[8] The marine alkaloids are normally isolated in minute amounts and this factor has precluded the systematic study of the structural requirements for their biological properties; hence the need for synthetic routes to the natural products themselves and to their analogues. We describe here two efficient syntheses of compound **1**, a regioisomer of meridine.[9] Compound **1** is also a derivative of isoascididemin,[10] which is not a natural product but can be regarded as a structural fragment of several complex marine alkaloids, such as eilatine[11] and eudistone A[12] (Fig. 1).

We have developed two routes to compound **1**, which are summarized in Scheme 1. The first approach is based on the stepwise disconnection of rings C and D and in the introduction of ring E through a Stille cross-coupling reaction. In the second approach, rings A and E are introduced simultaneously by means of a hetero Diels–Alder reaction between a 4-aryl-1-dimethylamino-1-azadiene and a quinolinequinone.

Results and Discussion

Our first route is depicted in Scheme 2, and can be related to previous work on the synthesis of isoascididemin[10] and meridine[9a] in that all of these routes use a Stille coupling as the key step. Bromide **4** was obtained in 76% overall yield by treatment of the known[10] 5,8-dimethoxy-6-nitro-4(1*H*)-quinolinone **2** with trifluoromethanesulfonic anhydride to give triflate **3**, followed by reaction with lithium bromide. Stille coupling of **4** with tributyl (2-*tert*butoxycarbonylaminophenyl) stannane[10] catalyzed by $PdCl_2(CH_3CN)_2$ afforded compound **5** in quantitative yield. Hydrogenation of **5** with cyclohexene in the presence of palladium gave amine **6**, which was transformed into compound **7** by treatment with Meldrum's acid and triethyl orthoformate. All attempts to oxidize **7** to a quinone led to complex mixtures; fortunately, a simple change in the *N*-protecting group from BOC to trifluoroacetyl (**8**) allowed the oxidative demethylation and afforded the

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rather unstable quinone **9**, which by thermolysis gave **1** in 42% yield from **8**. This one-pot double cyclization from **9** to **1** is remarkable. We assume that cyclization of the trifluoroacetamido group onto the quinone carbonyl must involve its prior hydrolysis under the harsh reaction conditions, catalyzed by the presence of traces of acid (from cerium ammonium nitrate) in compound **9**.[13] The overall yield of the eight-step route to **1** was 23%.

In search of a shorter route, we decided to investigate an alternative approach based on the hetero Diels–Alder reaction[14] between a 4-aryl-1-dimethylamino-1-azadiene bearing a nitrogenated function at the *o*-position and a 4-substituted derivative of 6-bromo-5,8-quinolinequinone, where the presence of the bromine atom at C-6 was expected to revert the 'natural' regiochemistry of the Diels–Alder reactions of quinolinequinones.[15] This strategy has been previously employed by the Kubo group in the synthesis of meridine (see ref 20). In order to increase the reactivity of the quinones as dienophiles, we decided to use a second halogen atom rather than a hydroxy group at C-4 of the quinoline ring. The known 6-bromo-4-chloro-5,8-quinolinequinone **12** was prepared by a literature method[16] involving oxidative demethylation of the corresponding 5,8-dimethoxyquinoline **10** with cerium ammonium nitrate.[17] 4,6-Dibromoquinolinequinone **13** was similarly obtained from the known[10] dibromoquinoline **11**.

The Diels–Alder reactions of quinones **12** and **13** with *o*-nitrocinnamaldehyde dimethylhydrazone[18] gave the results summarized in Scheme 3. Owing to the low reactivity of 4-aryl-1-dimethylamino-1-azadienes, we employed ultrasound irradiation[19] using the neat azadiene as the reaction medium. Under these conditions, quinone **12** gave the aromatic adduct **16** in 21% yield

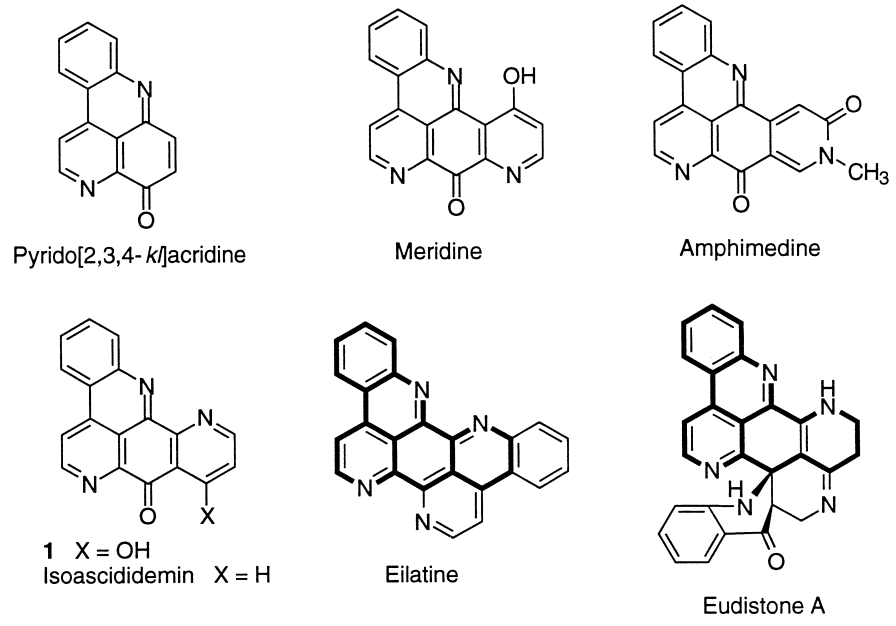
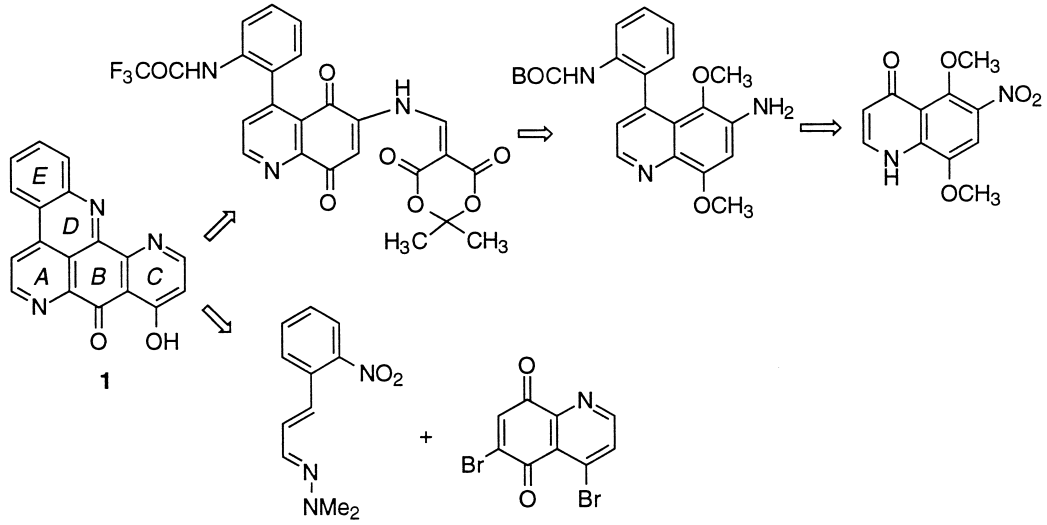


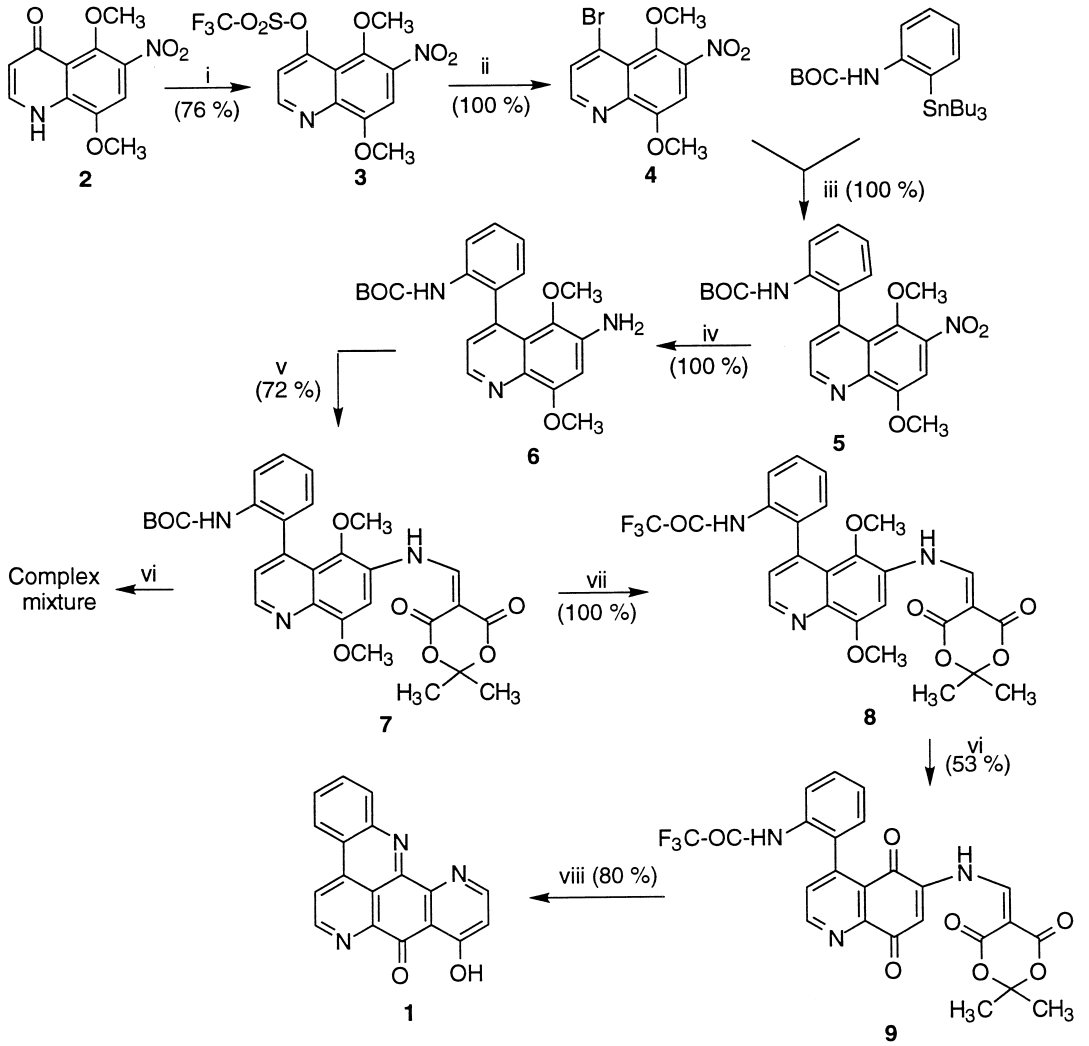
Figure 1.



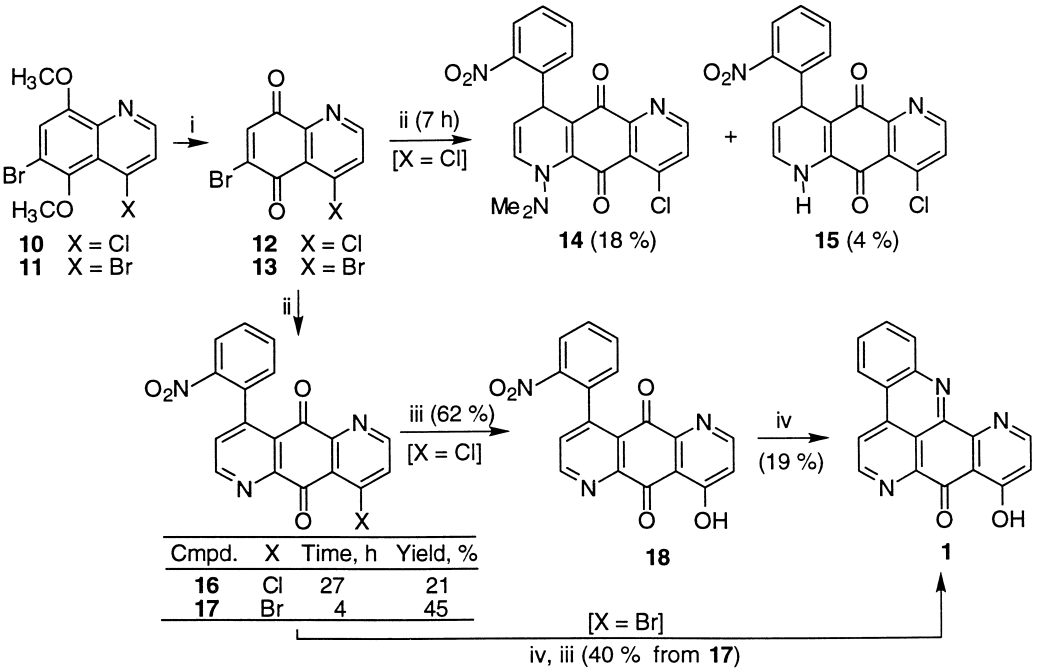
Scheme 1.

after 27 h;[20] interruption of the reaction after 7 h allowed detection of intermediates **14**, formed by elimination of HBr from the primary Diels–Alder adduct, and **15**, formed by acid-catalyzed elimination of dimethylamine from **14**. Better results were obtained with the more reactive dibromoquinone **13**, which gave a 45% yield of the aromatic compound **17** after 4 h of irradiation. Two methods were then assayed for the transformation of **16** or **17** into the target compound **1**. In the initial approach, compound **16** was transformed into the hydroxy derivative **18** in 62% yield by brief exposure to wet trifluoroacetic acid; subsequent catalytic hydrogenation of **18** afforded **1** in 19% yield. The order of these steps was inverted when applied to the bromo derivative **17**, which afforded **1** in 40% yield after catalytic hydrogenation followed by addition of wet trifluoroacetic acid (Scheme 3). The overall yield of **1** in this route (four steps from **11**) was 11%.

Table 1 shows the cytotoxic activities of compound **1** and some selected precursors, compared with those of the related natural product meridine and also with adriamycin, one of the most commonly used anticancer drugs worldwide. The compounds under assay were dissolved in DMSO/MeOH (1:9) and tested following the methods described by Bergeron et al.[21] and Schroeder et al.[22] The activity of quinone **9** in comparison with that of its precursor **8** (inactive) is noteworthy. The remarkable antitumour properties of compound **17**, which is more active (but less selective) than **1**, are in agreement with the good antitumour activity found in a series of dialkyl 1,5-diazaanthraquinones;[23] comparison of the activities of **17** and **18** indicates the importance of the substitution pattern in these tricyclic compounds. Finally, the target compound **1** shows a high selectivity for solid tumours that is not present in its regioisomer meridine, proving that the C-ring has a significant relationship with the activity in these pyridoacridine skeletons, although the establishment of detailed structure–activity relationships in the pyridoacridine family of alkaloids is still not possible due to the lack of a sufficient number of analogues. Particularly noteworthy are the high activity and selectivity of **1** towards the human lung carcinoma A-549. This selectivity is a very important characteristic of compound **1** and constitutes a distinct advantage over meridine, since a high activity



Scheme 2. Reagents and conditions: (i) Tf_2O, 2,6-lutidine, DMAP, CH_2Cl_2, rt, 20 h; (ii) LiBr, 1,4-dioxane, reflux, 2 h; (iii) $PdCl_2(CH_3CN)_2$, 1,4-dioxane, 55 °C, 14 h; (iv) Cyclohexene, Pd-C (10%), EtOH, reflux, 45 min; (v) Meldrum's acid, $HC(OEt)_3$, reflux, 2 h, then add **6**, reflux, 4 h; (vi) $(NH_4)_2Ce(NO_3)_6$, CH_3CN, 2 M H_2SO_4, rt, 3 h; (vii) TFA, TFAA, rt, 90 min; viii. Ph_2O, 200–220 °C, 15 min.



Scheme 3. Reagents and conditions: (i) $(NH_4)_2Ce(NO_3)_6$, CH_3CN, H_2O, 0 °C, 3 h; (ii) *o*-Nitrocinnamaldehyde dimethylhydrazone (neat), ultrasound, 50 °C; (iii) CF_3CO_2H-$CHCl_3$ (3:1), rt, 5 min; (iv) H_2, Pd-C, 1 atm, 1 h.

Table 1. Cytotoxic activity in cultured cell lines (IC_{50} μM)

Cell line[a]	Adriamycin	Meridine	1	8	9	13	17	18
P-388	0.03	0.08	4.18	>18	0.97	0.3	0.27	7.20
A-549	0.01	0.08	0.03	>18	0.10	>3	0.03	7.20
HT-29	0.09	0.84	0.40	>18	0.97	>3	0.27	7.20
MEL-28	0.03	0.08	0.17	>18	0.10	NM[b]	0.03	7.20

[a]P-388D_1; mouse lymphoma (ATCC CCL-46); A-549, human lung carcinoma (ATCC CCL-185); HT-29, human colon carcinoma (ATCC HTB-38); MEL-28, human melanoma (ATCC HTB-72).
[b]NM, not researched.

towards all types of tumours is normally considered as a sign of indiscriminate cytotoxicity, and therefore is an undesirable feature for a drug candidate.

The antitumour activity of the pyridoacridine class of alkaloids is normally associated with their interaction with DNA and subsequent inhibition of the activity of toposiomerase II (TOPO II).[3b,e] Therefore, we have measured the in vitro relative ability of meridine and compound **1** to inhibit TOPO II activity. Meridine showed some activity in this test, (IC_{50} = 3 μM) but compound **1** was inactive at the highest concentration assayed (33 μM). This different inhibitory activity agrees with the different cytotoxicity patterns of both compounds and again gives an indication of the importance of the effects of structural variations in the C-ring within this class of pyridoacridines. It must be remarked that the precise mechanism of action of the pyridoacridine alkaloids is far from being fully understood, and that several mechanisms different from TOPO II inhibition have been described in the case of ascididemin.[24,25]

Experimental

All reagents were of commercial quality (Aldrich, Fluka, SDS, Probus) and were used as received. Solvents (SDS) were dried and purified using standard techniques. "Petroleum ether" refers to the fraction boiling at 40–60 °C. Reactions under ultrasound irradiation were performed with a Branson 450 ultrasound probe, using the pulsed mode (pulse duration, 0.3 s), with output values between 30 and 40 W. Reactions were monitored by thin layer chromatography, on aluminium plates coated with silica gel with fluorescent indicator (Macherey-Nagel Alugram Sil G/UV_{254}). Separations by flash chromatography were performed on silica gel (SDS 60 ACC, 230–40 mesh). Melting points were measured with a Reichert 723 hot stage microscope, and are uncorrected. Infra-red spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer, with all compounds examined in KBr pellets or as films on a NaCl disk. NMR spectra were obtained on Varian Unity-300 (300 MHz for ^1H, 75 MHz for ^{13}C), Bruker AC-250 (250 MHz for ^1H, 63 MHz for ^{13}C) and Bruker AC-500 (500 MHz for ^1H) spectrometers (Servicio de RMN, Universidad Complutense), with $CDCl_3$, CD_3OD or CF_3CO_2D as solvents. Mass spectra were obtained by the Servicio de Espectroscopía, Universidad Complutense. Combustion elemental analyses were determined by the Servicio de Microanálisis Elemental, Universidad Complutense.

5,8-Dimethoxy-5-nitro-4-(trifluoromethanesulfonyloxy)-quinoline (3). To a solution of the quinolone **2**[11] (0.2 g, 0.8 mmol), 4-dimethylaminopyridine (10 mg, 0.08 mmol) and 2,6-lutidine (0.14 mL, 1.22 mmol) in CH_2Cl_2 (6 mL), was slowly added trifluoromethanesulfonic anhydride (0.15 mL, 0.88 mmol), at 0 °C and under

argon. After the addition, the mixture was warmed to room temperature and stirred for 20 h. The solution was diluted with CH_2Cl_2 and washed with a saturated NH_4Cl aqueous solution, and the organic layer was dried over Na_2SO_4. Evaporation of the solvent afforded a crude which was chromatographed (hexane–AcOEt 4:6) to yield the triflate **3** (232 mg, 76%) as pale yellow crystals. (Found: C, 37.51; H, 2.60; N, 7.37; S, 8.27. $C_{12}H_9F_3SN_2O_7$ requires: C, 37.70; H, 2.37; N, 7.33; S, 8.39); mp 146–148 °C; ν_{max} (KBr): 1600, 1427, 1239, 1033 cm^{-1}; δ_H ($CDCl_3$, 300 MHz) 9.09 (d, $J=4.6$ Hz, 1H), 7.46 (s, 1H), 7.45 (d, $J=4.6$ Hz, 1H), 4.13 (s, 3H), 4.01 (s, 3H). δ_C ($CDCl_3$, 75 MHz) 153.5, 152.3 (two signals), 144.4, 142.0, 141.5, 118.6 (q, $J=321$ Hz), 118.2, 116.2, 103.9, 64.5, 56.9.

4-Bromo-5,8-dimethoxy-5-nitroquinoline (4). A suspension of the triflate **3** (400 mg, 1.05 mmol) and lithium bromide (273 mg, 3.15 mmol) in 1,4-dioxane (4 mL) was refluxed for 2 h under argon. After cooling to room temperature, the mixture was diluted with AcOEt and washed with H_2O and the organic layer was dried over Na_2SO_4. Evaporation of the solvent gave the bromo derivative **4** (328 mg, 100%), as pale yellow crystals. (Found: C, 42.43; H, 2.93; N, 8.93. $C_{11}H_9BrN_2O_4$ requires: C, 42.20; H, 2.90; N, 8.95); mp 180–182 °C; ν_{max} (KBr): 1602; 1492; 1360; 1242; 1005 cm^{-1}; δ_H ($CDCl_3$, 300 MHz) 8.69 (d, $J=4.6$ Hz, 1H), 7.85 (d, $J=4.6$ Hz, 1H), 7.35 (s, 1H), 4.08 (s, 3H), 3.92 (s, 3H). δ_C ($CDCl_3$, 75 MHz) 152.2, 150.6, 143.6, 143.5, 141.3, 130.1, 123.1, 103.0, 100.5, 64.5, 56.7.

4-(2-*tert*Butyloxycarbonylaminophenyl)-5,8-dimethoxy-6-nitroquinoline (5). A suspension of compound **4** (498 mg, 1.6 mmol), tributyl (*o-tert*butyloxycarbonylaminophenyl) stannane[10] (1.02 g, 2.86 mmol) and bis(acetonitrile)dichloropalladium (II) (54 mg, 0.21 mmol) in 1,4-dioxane (13 mL) was heated at 55 °C for 14 h, under an argon atmosphere. The cooled reaction mixture was diluted with AcOEt and washed with H_2O, and the organic layer was dried over Na_2SO_4. The resulting crude was chromatographed (CH_2Cl_2/AcOEt 8:2) to afford compound **5** (680 mg, 100%), as pale yellow crystals. (Found: C, 62.11; H, 5.46; N, 9.75. $C_{22}H_{23}N_3O_6$ requires C, 62.11; H, 5.45; N, 9.88); mp 194–196 °C; ν_{max} (KBr): 3222; 1724; 1532; 1508; 1236; 1162 cm^{-1}; δ_H ($CDCl_3$, 300 MHz) 9.04 (d, $J=4.1$ Hz, 1H), 7.88 (d, $J=7.8$ Hz, 1H), 7.41 (m, 3H), 7.19 (m, 2H), 5.95 (s, 1H), 4.13 (s, 3H), 3.17 (s, 3H), 1.30 (s, 9H). δ_C ($CDCl_3$, 75 MHz) 152.7, 152.4, 151.5, 145.8, 145.6, 143.0, 140.3, 135.6, 131.9, 129.0, 128.9, 126.7, 126.3, 123.1, 121.6, 102.7, 80.6, 63.1, 56.6, 28.0.

6-Amino-4-(2-*tert*butyloxycarbonylaminophenyl)-5,8-dimethoxyquinoline (6). To a solution of the nitro derivative **5** (67 mg, 0.16 mmol) in ethanol (5 mL) was added 10% Pd/C (100 mg) and cyclohexene (0.081 mL, 0.8 mmol). After being refluxed for 45 min, the solution was cooled to room temperature, filtered and evaporated to yield the amine **6** (62 mg, 100%), as red crystals. (Found: C, 66.54; H, 6.09; N, 10.42. $C_{22}H_{25}N_3O_4$ requires C, 66.82; H, 6.37; N, 10.63); mp 170–172 °C; ν_{max} (KBr): 3388, 1707, 1618, 1241, 1161 cm^{-1}. δ_H ($CDCl_3$, 300 MHz) 8.86 (br. s, 1H), 7.79 (d, $J=7.5$ Hz, 1H), 7.46 (m, 3H), 7.27 (m, 1H), 7.19 (t, $J=6.7$ Hz, 1H), 6.01 (s, 1H), 4.10 (s, 3H), 2.95 (s, 3H), 1.29 (s, 9H). δ_C ($CDCl_3$, 75 MHz) 152.8, 148.9, 147.1, 142.1, 138.2, 135.9, 131.5, 130.2, 129.4, 128.9, 126.4, 124.9, 123.3, 123.2, 121.7, 104.3, 80.5, 59.7, 56.6, 27.9.

4-(2-*tert*Butyloxycarbonylaminophenyl)-6-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-ylidene-methylamino)-5,8-dimethoxyquinoline (7). A solution of Meldrum's acid (519 mg, 3.6 mmol) in triethyl ortoformate (8 mL) was refluxed for 2 h under argon. The reaction mixture was added to compound **6** (1.18 g, 3 mmol) and the solution thus obtained was refluxed for 4 h. After cooling to 0 °C, the precipitated crystals were filtered and washed with methanol to yield compound **7** as a yellow solid (750 mg, 72%). (Found: C, 63.14; H, 5.68; N, 7.58. $C_{29}H_{31}N_3O_8$ requires: C, 63.38; H, 5.69; N, 7.65); mp 292–294 °C; ν_{max} (KBr): 3114, 1702, 1676, 1618, 1271 cm^{-1}; δ_H ($CDCl_3$, 300 MHz) 8.95 (d, $J=4.2$ Hz, 1H), 8.74 (d, $J=14.4$ Hz, 1H), 7.90 (d, $J=7.6$ Hz, 1H), 7.44 (t, $J=7.3$ Hz, 1H), 7.37 (d, $J=4.2$ Hz, 1H), 7.26 (m, 1H), 7.17 (t, $J=7.3$ Hz, 1H), 7.07 (s, 1H), 5.91 (s, 1H), 4.19 (s, 3H), 3.09 (s, 3H), 1.75 (s, 6H), 1.30 (s, 9H); δ_C ($CDCl_3$, 75 MHz): 165.1, 163.6, 154.3, 152.8, 150.3, 148.8, 142.8, 139.6, 137.8, 135.9, 131.6, 129.4, 129.1, 128.7, 126.4, 123.2, 122.8, 121.5, 105.3, 95.7, 88.2, 80.5, 61.9, 56.8, 28.1, 27.1, 27.0.

4-(2-Trifluoracetamidophenyl)-6-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-ylidenemethyl-amino)-5,8-dimethoxyquinoline (8). To a solution of compound **7** (487 mg, 1.2 mmol) in trifluoroacetic acid (8 mL) was added trifluoroacetic anhydride (4 mL) at 0 °C and under argon. After the addition, the reaction mixture was allowed to warm to room temperature, stirred for 90 min and evaporated. The crude was dissolved in CH_2Cl_2, washed with 0.1 M $HNaCO_3$ and a saturated aqueous NaCl solution, and dried over Na_2SO_4. Evaporation of the solvent gave compound **8** (484 mg, 100%), as pale yellow crystals. (Found: C, 57.18; H, 4.06; N, 7.41. $C_{26}H_{22}N_3O_7F_3$ requires: C, 57.25; H, 4.07; N, 7.70); mp 229–231 °C; ν_{max} (KBr): 3418, 1734, 1684, 1618, 1275 cm^{-1}; δ_H ($CDCl_3$, 300 MHz) 8.92 (d, $J=4.3$ Hz, 1H), 8.75 (d, $J=14.0$ Hz, 1H), 7.83 (d, $J=7.5$ Hz, 1H), 7.72 (s, 1H), 7.53 (td, $J=6.7$ and 2.0 Hz, 1H), 7.44 (m, 2H), 7.37 (d, $J=4.3$ Hz, 1H), 7.10 (s, 1H), 4.16 (s, 3H), 3.06 (s, 3H), 1.73 (s, 3H), 1.72 (s, 3H). δ_C ($CDCl_3$, 75 MHz): 165.2, 163.4, 155.0, 154.5, 150.3, 148.7, 141.4, 139.1, 136.6, 134.4, 132.9, 129.9, 129.5, 129.0, 126.7, 126.5, 123.6, 121.9, 115.3 (q, $J=289$ Hz), 105.3, 95.8, 88.5, 62.0, 56.8, 27.1, 26.9.

4-(2-Trifluoracetamidophenyl)-6-(2,2-dimethyl-4,6-dioxo-1,3-dioxan-5-ylidenemethylamino)-5,8-quinolinequinone (9). To a solution of compound **8** (150 mg, 0.27 mmol) in CH_3CN (2.1 mL) was added a solution of ammonium cerium (IV) nitrate (636 mg, 1.16 mmol) in 2M H_2SO_4 (3 mL). After stirring for 3 h, the reaction mixture was partitioned between AcOEt and saturated aqueous NH_4Cl solution. The organic layer was dried over Na_2SO_4, and evaporation of the solvent gave a residue which was purified by flash column chromatography

(AcOEt) to afford **9** as a red solid (75 mg, 53%). δ_H (CD_3OD, 300 MHz): 8.96 (d, $J=4.9$ Hz, 1H), 8.78 (s, 1H), 7.58 (m, 2H), 7.50 (td, $J=7.2$ and 1.4 Hz, 1H), 7.43 (dd, $J=7.5$ and 2.2 Hz, 1H), 7.32 (dd, $J=7.5$ and 1.4 Hz, 1H), 7.22 (s, 1H), 1.72 (s, 6H); δ_C ($CDCl_3$, 75 MHz): 180.9, 179.3, 164.0, 161.9, 154.8, 154.6 (d, $J=111.5$ Hz), 148.3, 148.1, 141.2, 134.1, 131.2, 130.5, 130.1, 129.0, 128.8, 128.2, 126.1, 124.5, 115.5 (q, $J=288$ Hz), 113.1, 106.1, 105.1, 94.3, 27.3. MS (FAB, CH_3CN/NaOAc): 538.1 ($M^+ + 23$).

9-Hydroxybenzo[*b*]pyrido[4,3,2-*de*](1,10)-phenantrolin-8-one (1). A solution of compound **9** in diphenyl ether (22 mL) was heated between 200 and 220 °C for 15 min. The reaction mixture was cooled to room temperature and added to hexane at 0 °C. Filtration of the precipitated solid afforded compound **1**, as orange crystals (35 mg, 80%). (Found: C, 71.96; H, 2.92; N, 13.85. $C_{18}H_9N_3O_2$ requires: C, 72.24; H, 3.03; N, 14.04); mp 245 °C; ν_{max} 3426, 1683 cm^{-1}; δ_H (CF_3CO_2D, 300 MHz): 9.53 (d,$J=6.3$ Hz, 1H), 9.35 (d, $J=6.3$ Hz, 1H), 8.91 (m, 2H), 8.54 (d, $J=8.1$ Hz, 1H), 8.29 (t, $J=8.1$ Hz, 1H), 8.18 (t, $J=8.1$ Hz, 1H), 7.65 (d, $J=7.2$ Hz, 1H); δ_C (CF_3CO_2D, 75 MHz): 177.9, 174.1, 146.5, 146.5, 145.8, 144.7, 140.7, 138.6, 137.0, 136.2, 134.5, 133.1, 126.2, 125.3, 120.5, 117.6, 116.9, 113.4. MS (CI, methane): 300 ($M^+ + 1$). MS (FAB, CH_3CN/NaOAc): 322 ($M^+ + 23$).

4,6-Dibromo-5,8-quinolinequinone (13). To a cooled (0 °C) solution of the dibromoquinoline **11**[10] (110 mg, 0.32 mmol) in acetonitrile (5 mL) was added a solution of ammonium cerium (IV) nitrate (348 mg, 0.63 mmol) in water (5 mL). The solution was stirred at 0 °C for 3 h, diluted with water (10 mL) and extracted with $CHCl_3$ (3×30 mL). The residue was chromatographed on silica gel, eluting with 1:1 petroleum ether/ethyl acetate, yielding quinone 13 (40 mg, 63%) as a yellow solid. (Found: C, 33.96; H, 1.12; N, 4.26. $C_9H_3Br_2NO_2$ requires: C, 34.11; H, 0.95; N, 4.42); mp 147 °C; ν_{max} (KBr): 3056, 2918, 1681, 1595, 1545, 1298, 1092, 641 cm^{-1}; δ_H ($CDCl_3$, 250 MHz): 8.73 (d, 1H, $J=5.2$ Hz), 7.94 (d, 1H, $J=5.2$ Hz), 7.65 (s, 1H); δ_C ($CDCl_3$, 63 MHz): 179.4, 176.0, 153.6, 149.2, 141.0, 139.2, 134.6, 134.4, 126.6.

4-Chloro-8-(2-nitrophenyl)-1,5-diazaanthracene-9,10-dione (16). A mixture of quinone **12** (185 mg, 0.68 mmol) and *o*-nitrocinnamaldehyde dimethylhydrazone[10] (438 mg, 2 mmol) was heated at 50 °C until dissolution. The solution was irradiated with ultrasound at 50 °C for 27 h and then it was chromatographed on silica gel, eluting with ethyl acetate, to yield 52 mg (21%) of **16**[9b] as a brown solid. When the reaction was interrupted after 7 h, two intermediates were isolated, namely compounds **14**[24] (18%)[26] and **15**[27] (4%).

4-Bromo-8-(2-nitrophenyl)-1,5-diazaanthracene-9,10-dione (17). A mixture of quinone **13** (90 mg, 0.28 mmol) and *o*-nitrocinnamaldehyde dimethylhydrazone (415 mg, 1.9 mmol) was heated at 50 °C until dissolution and was irradiated with ultrasound at 50 °C for 4 h. The dark red solution was chromatographed on silica gel, eluting with a gradient from 2:1 petroleum ether/ethyl acetate to neat ethyl acetate, yielding 50 mg (45%) of compound **17**, as a brown solid. (Found: C, 52.45; H, 2.21; N, 10.30. $C_{18}H_8BrN_3O_4$ requires: C, 52.71; H, 1.97; N, 10.24); mp 98-101 °C; ν_{max} (KBr): 1674, 1523, 1346, 757 cm^{-1}; δ_H ($CDCl_3$, 250 MHz): 9.15 (d, 1H, $J=4.9$ Hz), 8.68 (d, 1H, $J=5.0$ Hz); 8.33 (dd, 1H, $J=8.0$ and 1.5 Hz), 7.95 (d, 1H, $J=5.0$ Hz), 7.76–7.62 (m, 2H), 7.49 (d, 1H, $J=4.9$ Hz), 7.23 (m, 1H).

4-Hydroxy-8-(2-nitrophenyl)-1,5-diazaanthracene-9,10-dione (18). A solution of **17** (80 mg, 0.21 mmol) in 10 mL of a 3:1 mixture of $CF_3CO_2H/CHCl_3$ containing water (0.1 mL) was stirred at room temperature and evaporated. The residue was chromatographed on silica gel eluting with 9:1 ethyl acetate/methanol, yielding 45 mg (62%) of compound **18**. (Found: C, 62.02; H, 2.49; N, 12.10. $C_{18}H_9N_3O_5$ requires: C, 62.25; H, 2.61; N, 12.10); mp >250 °C; ν_{max} (KBr): 3422, 1684, 1522, 1350, 1310; δ_H ($CDCl_3$, 250 MHz): 12.36 (s, 1H); 9.16 (d, 1H, $J=4.8$ Hz); 8.73 (d, 1H, $J=5.7$ Hz); 8.34 (dd, 1H, $J=7.5$ and 1.4 Hz); 7.74 (td, 1H, $J=7.5$ and 1.4); 7.66 (td, 1H, $J=7.5$ and 1.4 Hz); 7.53 (d, 1H, $J=4.8$ Hz); 7.21 (dd, 1H, $J=7.5$ and 1.4 Hz); 7.19 (d, 1H, $J=5.7$ Hz); δ_C ($CDCl_3$, 63 MHz): 181.4, 179.4, 159.9, 153.8, 151.6, 149.3, 134.0, 133.7, 129.8, 129.6, 129.4, 128.4, 128.2, 124.8, 124.2; owing to low solubility, the signals due to C-2′, C-9a and C-10a could not be detected.

Reductive cyclization of 18. To a solution of compound **18** (25 mg, 0.7 mmol) in methanol (20 mL) was added 10% Pd-C (8 mg). The suspension was stirred under a hydrogen atmosphere for 60 min, filtered through Celite and evaporated. The residue was chromatographed on silica gel, eluting with 9:1 ethyl acetate–methanol, yielding 6 mg (19%) of compound **1**.

Reductive cyclization-hydrolysis of 17. To a solution of compound **17** (30 mg, 0.073 mmol) in methanol (7 mL) was added 10% Pd-C (10 mg). The suspension was stirred under a hydrogen atmosphere for 1 h at room temperature and filtered through Celite, which was washed with 15 mL of a 3:1 mixture of $CF_3CO_2H/CHCl_3$ containing water (0.1 mL). The combined washings were evaporated under reduced pressure and washed repeatedly with ethyl ether, yielding 8.5 mg (40%) of compound **1**.

Cytotoxicity assays

The compounds were tested for cytotoxic activity against the following cell lines: P-388 (ATCC CCL-46, suspension culture of a lymphoid neoplasm from a DBA/2 mouse); A-549 (ATCC CCL-185, monolayer culture of a human lung carcinoma); HT-29 (ATCC HTB-38, monolayer culture of a human colon carcinoma); MEL-28 (ATCC HTB-72, monolayer culture of a human melanoma). Cells were maintained in logarithmic growth in Eagle's Minimum Essential Medium, with Earle's Balanced Salts, with 2.0 mM L-glutamine, with non-essential amino acids, without sodium bicarbonate (EMEM/neaa), supplemented with 10% Fetal Calf Serum (FCS), 10^{-2} M sodium bicarbonate, 0.1 g/L

penicillin G and 0.1 g/L streptomycin sulfate. The compounds under assay were dissolved in DMSO/MeOH (1:9).

P-388 cells were seeded into 16 mm wells at 1×10^{-4} cells per well in 1 mL aliquots of MEM 5FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells remained in the exponential phase of growth. All determinations were carried out in duplicate. After 3 days of incubation at 37 °C under a 98% humidity atmosphere containing 10% CO_2, IC_{50} values were determined by comparing the growth in wells with drug to the growth in control wells.

A-549, HT-29 and MEL-28 were seeded into 16 mm wells at 2×10^{-4} cells per well in 1 mL aliquots of MEM 10FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells remained in the exponential phase of growth. All determinations were carried out in duplicate. After 3 days of incubation at 37 °C under a 98% humidity atmosphere containing 10% CO_2, the wells were stained with 0.1% crystal violet. IC_{50} values were determined by comparing the growth in wells with drug to the growth in control wells.

Assay for DNA TOPO II catalytic activity. The in vitro inhibition of TOPO II decatenation assay was carried out as described by Hsiang and et al.,[28] with small modifications. Topoisomerase II reaction mixtures (20 μL) containing 10 mM Tris–ClH, pH 7.9, 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/mL bovine serum albumin, 1 mM ATP, 150 ng supercoiled pBR322 DNA (Gibco) and 2 U of calf thymus topoisomerase II (TopoGen) were prepared. Compounds under assay were added in DMSO (1% final DMSO concentration) at 10–0.01 μg/mL. After 30 min at 37 °C, the reaction was stopped with 2 μL 10% SDS. Then 1 μL of 1 mg/mL proteinase K was added and incubated another h at 37 °C. Then 2 μL of 10× gel loading buffer (0.25% bromophenol blue, 50% glycerol) were added to each sample. The samples were submitted to electrophoresis using 1% agarose gel in 40 mM Tris base, 1 mM EDTA and acetic acid to pH 8.0. Gels were run at 1.5–2 V/cm, stained with 0.5 μg/mL ethidium bromide, destained with water and photographed under UV light.

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