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Synthesis and In Vitro Antitumor Activity of an Isomer of the Marine Pyridoacridine Alkaloid Ascididemin and Related Compounds

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Abstract—The isomer (9*H*-quino[4,3,2-*de*][1,7]phenanthroline-9-one) (**2**) of the marine alkaloid ascididemin (9*H*-quino[4,3,2-*de*][1,10]phenanthroline-9-one) (**1**) has been synthesized in six steps from 1,4-dimethoxyacridine (**10**) with an overall yield of 12%. Different related compounds were prepared and tested in vitro at six different concentrations on 12 different human cancer cell lines of various histopathological types (glioblastomas and breast, colon, lung, prostate and bladder cancers). Almost all the compounds present cytotoxic activity of micromolar order.

Introduction

Pyridoacridine alkaloids constitute an important class of natural products isolated from marine organisms, many of which possess cytotoxic activity.[1] Ascididemin **1**, identified by Kobayashi et al. in 1988,[2] was one of the first example of these compounds. From this date, different related compounds have been synthesized in order to discover new potential anticancer drugs or to understand the mechanisms involved in their antitumor activities.[3] The synthesis of an isomer of ascididemin, compound **2**, was previously reported both by Cuerva et al.[4] and Alvarez et al.,[5] this compound was described as having a cytotoxic activity closely related to that of the natural product. In a recent paper, Cuerva et al. corrected their synthesis to yield ascididemin instead of its isomer **2** (Fig. 1).[6]

As part of our work taking aim at designing new antitumor compounds derived from marine pyridoacridines,[7] we are now interested in compound **2** and analogues. We report herein our quite different approach to prepare these compounds along with their in vitro antitumor activity.

Chemistry

The synthesis of **2** (Scheme 1) started with the known anthranilic compound **8**, readily prepared by condensation of 2-chlorobenzoic acid and 2,5-dimethoxyaniline.[8]

Acid **8** was converted into its corresponding methylketone **9** in high yield (92%) with methyllithium. The cyclisation of **9** in polyphosphoric acid, resulted quantitatively into the acridine derivative **10**, which was oxidized by CAN to give in 93% yield the acridinedione **11**. Diels–Alder cycloaddition of propenal-*N*,*N*-dimethylhydrazone to this last compound gave selectively in 31% yield, the cycloadduct of which structure **12** was assigned by comparison with the other tetracyclic regioisomer obtained by Bracher as an intermediate in the synthesis of ascididemin.[9] The formation of the last cycle was achieved using Bracher's methodology[9] involving, in a first step, dimethylformamide-diethylacetal in DMF under nitrogen to form the intermediate enamine **13**, the second step corresponding to cyclization of this

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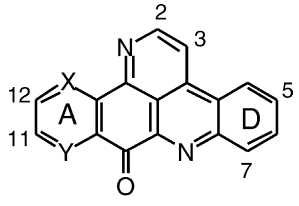
enamine. Attempts to perform this enamine cyclization with ammonium chloride did not work in the solvents usually used (methanol or acetic acid) but proceeded correctly in water, the yield of the two reactions being 47%. In summary, the synthesis of compound **2** was realized in six steps with an overall yield of 12%.

Our efforts in substitution of compound **2** focused to obtain analogues substituted on ring D which have shown the better cytotoxicities in the ascididemin series. The different compounds **3**–**7** were obtained using compound **2** as starting material (Scheme 2).

In this way, compound **3** was prepared in 24% yield by nitration of **2** with fuming HNO_3 whereas direct bromination of **2** gave compound **5** (32%), substituted in position 5. Catalytic hydrogenation of **3** led in 49% yield to the amino-derivative **4**. The treatment of **5** by sodium azide in DMF directly yielded the corresponding amino-derivative **6** (48%). This last compound was transformed in 17% into the dimethylamino compound **7** by reductive formylation.



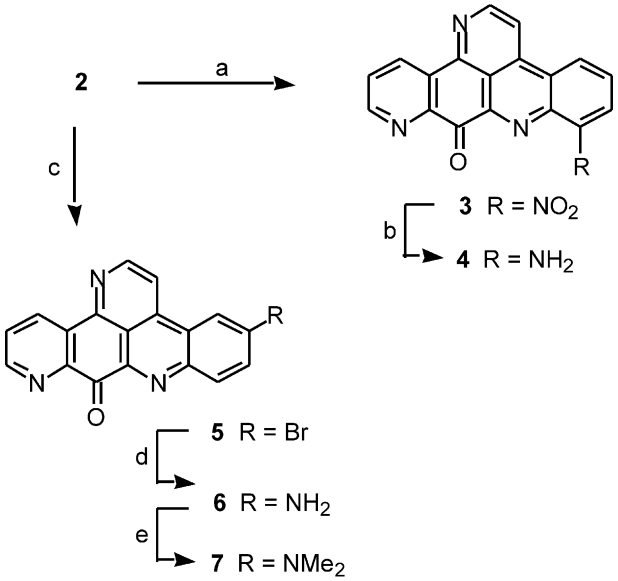
1 X = N, Y =CH

2 X = CH, Y = N

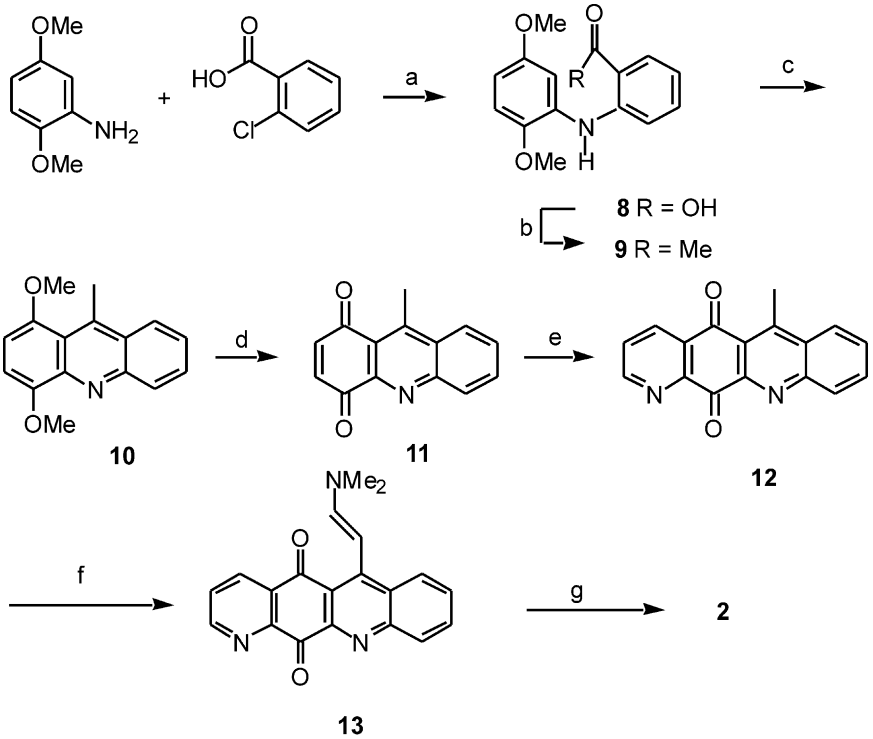
Figure 1.

In vitro determination of the drug-induced inhibition of human cancer cell line growth

For each of the different compounds under study, six concentrations were tested on 12 different human cancer



Scheme 2. (a) Fuming HNO_3, 100 °C, 1.5 h, 24%; (b) H_2, 10% Pd/C, MeOH, 2 h, 49%; (c) Br_2 (16 equiv), CH_3COOH, 100 °C, 4 h, 32%; (d) NaN_3, DMF, 105 °C, 6 h, 48%; (e) formaldehyde, $NaBH_3CN$, CF_3COOH, 0 °C to rt, 17%.



Scheme 1. (a) Cu_2O, Cu, K_2CO_3, diglyme, reflux, overnight, 89%; (b) MeLi, THF, reflux, 4.5 h, 92%; (c) polyphosphoric acid, 100 °C, 1 h, 100%; (d) CAN, CH_2Cl_2/MeOH, 0 °C, 20 min, 93%; (e) acrolein-*N*,*N*-dimethylhydrazone, Ac_2O, toluene, 95 °C, 30 min, 31%; (f) DMF–DEA, DMF, 120 °C, 30 min; (g) NH_4Cl, H_2O, reflux, 25 min, overall yield (f + g) 47%.

Table 1. Characterization of the in vitro cytotoxic-related antitumor effects (IC_{50} value in μM)

Compd	Cell lines											
	U-87MG	U-373MG	SW 1088	T24	J82	HCT-15	LoVo	MCF7	T-47D	A-549	A-427	PC-3
1	0.07	0.5	0.6	0.8	0.3	0.06	0.9	0.07	0.6	0.2	0.06	0.008
2	0.8	0.8	3	0.1	1	0.4	0.7	0.9	0.7	7	0.08	0.09
3	>10	9.0	4	2	6	5	10	1	9	9	0.9	7
4	4	6	0.7	0.09	4	4	1	0.5	5	9	0.3	0.2
5	3	0.8	4	0.8	10	0.9	>10	0.7	2	>10	0.7	0.5
6	0.6	0.4	0.6	0.5	9	9	>10	4	7	>10	0.5	0.7
7	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

The IC_{50} value constitutes the concentration of the compound which inhibits the growth of the human cancer cells by 50% as compared to the control value. Six concentrations ranging from 10 mM to 0.1 nM were assayed on 12 different human cancer cell lines for each compound under study. The drug-induced effects at cell line growth level were determined by means of the MTT colorimetric assay.

cell lines including various histopathological types (glioblastomas, and breast, colon, lung, prostate and bladder cancers). We made use of the colorimetric MTT assay, which indirectly assesses the effect of potentially anticancer compounds on the overall growth of adherent cell lines.[10] The IC_{50} values, that is the concentration which reduced the mean growth value of the 12 cell lines by 50%, was determined for each drug, in comparison with the mean control growth value. Table 1 illustrates the individual IC_{50} values of the different compounds obtained for each of the 12 cell lines under study.

Discussion

The isomer of ascididemin was found as potent or slightly less potent (with 1 log difference) than the natural product ascididemin on the panel of human cell lines: human glioblastoma (U-87MG, U-373MG and SW1088) and human breast (T-47D and CF-7), colon (Lovo and HCT-15), non small-cell lung (A549 and A-427), prostate (PC-3) and bladder (T24 and J82) carcinoma cell lines, tested depending on the strain sensitivity. On the non small-cell lung A 549 cell line, the sole cell line which data can be compared with other groups, although similar results were reported for isomer of ascididemin **2** with IC_{50} equal to 0.02 μM,[5] 0.3 μM[11] and 0.2 μM (present study), quite divergent results from ours (IC_{50} = 7 μM) were reported by Alvarez et al.[5] (IC_{50} = 0.004 μM) for the isomer of ascididemin **2**. Assays experimental conditions (solubilisation, purity…) or strain sensitivity may possibly explain the observed difference.

Ring D-modified analogues with substitutions in position 5 or 7 of compound **2** skeleton exhibit similar cytotoxic effects than the parent compound. Therefore, both positions of the ascididemin isomer (compound **2**) series could be modulated without losing the cytotoxic activity.

Within the limits of the two studies, the ascididemin isomer (compound **2**) analogues were found slightly less potent when compared to their counterparts in the ascididemin series, IC_{50} of which were already reported by our group.[6b] As an example, compounds **5** and **6** substituted in position 5 by a bromo or an amino group exhibited IC_{50} values higher, with one or 2 log difference depending on the cell line assayed, than their bromo and amino analogues of the ascididemin series. Similar observations were also made for the nitro (compound **3**) and the amino (compound **4**) derivatives in position 7 of ring D as compared to their counterpart in the ascididemin series.

Conclusion

In conclusion, this work was aimed to propose a novel synthetic route for the isomer (9H-quino[4,3,2-*de*][1,7]phenanthroline-9-one (**2**) of the natural marine alkaloid ascididemin (9H-quino[4,3,2-*de*][1,10]phenanthroline-9-one (**1**) that showed cytotoxic properties. Ring D-modified ascididemin isomer (**2**)-analogues were evaluated for selective cytotoxicity on a panel of human solid tumor cells in an attempt to determine their pharmaceutical utility. In vitro, within the limits of the study, compounds exhibited cytotoxic effects of the same order than the parent compound suggesting that ring D-positions 5 and 7 of the ascididemin isomer series could be modulated without losing the cytotoxic activity.

Experimental

Chemical synthesis

^1H and ^{13}C NMR spectra were obtained with a JEOL 400 MHz spectrometer, with the chemical shifts in the remaining protons of the deuterated solvents serving as internal standards. IR spectra were obtained with a Perkin-Elmer (1600 series FTIR) spectrometer. Mass spectra (MS) were recorded on an automass Unicam spectrometer. Reagents were purchased from commercial sources and used as received. Chromatography was performed on silicagel (15–40 μm) by means of the solvent systems indicated below. The purity of the different compounds was evaluated on chromatographic systems consisting either of a Kromasil Si, 5-μm column (250 mm × 4.6 mm), isooctane/EtOH/MeOH 80:10:10 at 2 mL/min flow rate, 260 nm (system I), or Zorbax NH_2, 5 μm column (150 mm × 4.6 mm), isooctane/EtOH/MeOH 80:10:10 at 1 mL/min flow rate, 260 nm (system II).

***N*-(2,5-Dimethoxyphenyl)anthranilic acid (8).** A mixture of 2-chlorobenzoic acid (9.2 g, 60 mmol), dimethoxyaniline (10 g, 65 mmol), Cu (0.96 g), Cu_2O (0.96 g) and K_2CO_3 (10.4 g) in diglyme (120 mL) was refluxed overnight. The reaction media was concentrated, made alkaline by addition of 1 N NaOH and diethyl ether was added. After filtration, the alkaline solution was washed with ether (3 × 300 mL) and acidified with concentrated HCl, and extracted with AcOEt (3 × 300 mL). The combined extracts were washed with H_2O, dried over $MgSO_4$ and evaporated to dryness in vacuo. The residue was purified by flash-chromatography (CH_2Cl_2) to give the expected product as a yellow solid (14.5 g, 89%), mp 138 °C. ^{1}H NMR ($CDCl_3$) 3.77 (s, 3H); 3.85 (s, 3H); 6.57 (dd, 1H, J = 8.8 and 2.9 Hz); 6.77 (ddd, 1H, J = 1.9, 8.1 and 7.5 Hz); 6.87 (d, 1H, J = 9.2 Hz); 7.04 (d, 1H, J = 2.9 Hz); 7.3–7.4 (m, 2H); 8.05 (dd, 1H, J = 7.7 and 1.1 Hz); 9.35 (br.s, 1H). ^{13}C NMR ($CDCl_3$) 55.76; 56.45; 107.30; 107.71; 112.00; 112.26; 114.70; 117.53; 130.78; 132.60; 134.09; 145.98; 147.71; 153.75; 172.95. IR ($CHCl_3$) 3327; 1685 cm^{-1}.

2-(2′,5′-Dimethoxyphenylamino)acetophenone (9). MeLi (1.4 M/Et_2O, 140 mL, 196 mmol) was added to a mixture of compound **8** (17.6 g, 64.5 mmol) in THF (140 mL), at 0 °C under nitrogen atmosphere. The reaction mixture was refluxed for 4 h 30 min, H_2O (400 mL) was added and the mixture was extracted with ether (3 × 400 mL). The combined extracts were dried over $MgSO_4$, and the solvant was removed in vacuo. The crude product was purified by flash-chromatography (CH_2Cl_2) to give the expected product as a yellow solid (16.1 g, 92%), mp 79 °C. ^{1}H NMR ($CDCl_3$) 2.64 (s, 3H); 3.76 (s, 3H); 3.84 (s, 3H); 6.55 (dd, 1H, J = 8.8 and 2.9 Hz); 6.73 (dd, 1H, J = 1.4 and 7.5 Hz); 6.85 (d, 1H, J = 8.8 Hz); 7.04 (d, 1H, J = 2.9 Hz); 7.3–7.4 (m, 2H); 7.81 (dd, 1H, J = 1.5 and 8.0 Hz); 10.5 (br.s, 1H). ^{13}C NMR ($CDCl_3$) 28.14; 55.68; 56.32; 107.03; 107.66; 111.99; 114.75; 116.83; 120.06; 130.65; 132.39; 134.32; 145.92; 146.62; 153.59; 200.97. IR ($CHCl_3$) 3350; 1642 cm^{-1}.

1,4-Dimethoxy-9-methylacridine (10). A mixture of compound **9** (11.2 g, 40 mmol) and polyphosphoric acid (102 g) was warmed at 100 °C for 1 h. H_2O (400 mL) was added and the mixture was neutralyzed with 5 M NaOH (370 mL), and extracted with $CHCl_3$ (3 × 300 mL). The combined extracts were dried on $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash-chromatography (CH_2Cl_2) to give quantitatively the tricyclic derivative as a orange-brown solid, mp 136 °C. ^{1}H NMR ($CDCl_3$) 3.36 (s, 3H); 3.96 (s, 3H); 4.09 (s, 3H); 6.68 (d, 1H, J = 8.0 Hz); 6.89 (d, 1H, J = 8.4 Hz); 7.54 (m, 1H); 7.73 (m, 1H); 8.32 (d, 1H, J = 8.4); 8.36 (d, 1H, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$) 17.78; 55.66; 56.13; 102.43; 105.18; 120.25; 124.28; 125.62; 126.59; 129.44; 130.81; 142.45; 144.23; 147.21; 149.46; 151.45. IR ($CHCl_3$) 1685; 1661 cm^{-1}.

9-Methylacridine-1,4-dione (11). A solution of compound **10** (1 g, 3.95 mmol) and cerium ammonium nitrate (7 g, 17.6 mmol) in a mixture CH_2Cl_2/H_2O (25 mL/12 mL) was stirred at 0 °C for 20 min. H_2O (20 mL) and $NaHCO_3$ saturated solution (20 mL) were added and stirring was continued. The mixture was extracted with CH_2Cl_2 (3 × 100 mL), the combined extracts were dried over $MgSO_4$ and concentrated in vacuo to give the expected quinone as a brown powder (0.87 g, 93%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 3.22 (s, 3H); 7.09 (d, 1H, J = 10.3 Hz); 7.18 (d, 1H, J = 10.3 Hz); 7.78 (dd, 1H, J = 8.5 and 8.5 Hz); 7.91 (dd, 1H, J = 8.5 and 8.5 Hz); 8.32 (d, 1H, J = 8.5 Hz); 8.43 (d, 1H, J = 8.5 Hz). ^{13}C NMR ($CDCl_3$) 15.87; 124.40; 125.41; 126.30; 129.61; 132.32; 132.52; 137.88; 141.61; 147.05; 148.23; 151.23; 183.43; 186.69. IR ($CHCl_3$) 1701; 1661 cm^{-1}.

6-Methyl-1,11-diazanaphtacene-5,12-dione (12). A solution of quinone **11** (5.02 g, 22.5 mmol), acrolein-*N*,*N*-dimethylhydrazone (3.3 g, 33.8 mmol) acetic anhydride (24.5 mL) in toluene (300 mL) was stirred at 95 °C, in the dark, and under nitrogen atmosphere for 30 min. 10% Pd/C (2.6 g) was added and the reaction mixture was warmed at 95 °C for 35 min. After concentration in vacuo, the crude product was purified by flash-chromatography (CH_2Cl_2/MeOH 98:2, and 99:1) to give the tetracyclic compound which was washed with ether: beige solid (1.9 g, 31%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 3.32 (s, 3H); 7.78–7.83 (m, 2H); 7.95 (ddd, 1H, J = 8.4, 7.7 and 1.5 Hz); 8.39 (dd, 1H, J = 8.8 and 1.5 Hz); 8.51 (dd, 1H, J = 7.7 and 1.5 Hz); 8.68 (dd, 1H, J = 8.1 and 1.9 Hz); 9.16 (dd, 1H, J = 4.8 and 1.9 Hz). ^{13}C NMR ($CDCl_3$) 16.67; 124.59; 125.44; 128.39; 129.76; 129.89; 132.25; 132.54; 132.88; 135.93; 148.00; 148.59; 148.73; 152.48; 155.31; 180.81; 184.37. IR ($CHCl_3$) 1703; 1663 cm^{-1}.

9-*H*-Quino[4,3,2-*de*][1,7]phenanthrolin-9-one (2). Dimethylformamide diethylacetal (0.18 mL, 1.05 mmol) was added dropwise under nitrogen atmosphere to a suspension of tetracyclic compound **12** (83 mg, 0.3 mmol) in anhydrous DMF (2 mL). The reaction mixture was warmed at 120 °C for 30 min. After concentration in vacuo, H_2O (90 mL) and NH_4Cl (0.49 g) were added and the mixture was refluxed for 25 min. The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by flash-chromatography (CH_2Cl_2/MeOH 98:2) to give the pentacyclic compound as a yellow solid (40 mg, 47%), mp > 260 °C. ^{1}H NMR ($CDCl_3$) 7.78 (dd, 1H, J = 8.1 and 4.8 Hz); 7.97 (ddd, 1H, J = 8.0, 7.4 and 1.2 Hz); 8.04 (ddd, 1H, J = 8.0, 7.4 and 1.2 Hz); 8.51 (d, 1H, J = 5.9 Hz); 8.69 (dd, 2H, J = 8.0 and 1.5 Hz); 9.08 (dd, 1H, J = 4.8 and 1.9 Hz); 9.13 (d, 1H, J = 5.9 Hz); 9.27 (d, 1H, J = 1.9 and 8.1 Hz). ^{13}C NMR ($CDCl_3$) 115.15; 121.76; 122.28; 127.13; 127.16; 129.65; 130.82; 132.21; 132.60; 132.99; 136.88; 139.91; 144.85; 148.00; 148.03; 151.75; 151.84; 179.94. IR ($CHCl_3$) 1692 cm^{-1}. HRMS calcd for $C_{18}H_9N_3O$ $(M+H)^+$: 283.0745. Found 283.0749. tr: 7.44 min (100% purity) using system II.

7-Nitro-9-H-quino[4,3,2-*de*][1,7]phenanthrolin-9-one (3). A mixture of compound **2** (200 mg, 0.7 mmol) and fuming HNO_3 (13 mL) was warmed at 100 °C for 1 h 30 min. The reaction mixture was poured into ice (40 g), made

alkaline with NH_4OH (30 mL) and extracted by a mixture $CHCl_3$/MeOH 95:5 (3 × 300 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash-chromatography ($CHCl_3$/MeOH 95:5) to give the nitro compound as a brown solid (55 mg, 24%), Pf >260 °C. ^{1}H NMR (DMSO-d_6) 7.93 (dd, 1H, J = 8.1 and 4.4 Hz); 8.18 (dd, 1H, J = 8.4 and 7.7 Hz); 8.56 (dd, 1H, J = 7.7 and 1.1 Hz); 8.96 (d, 1H, J = 5.9 Hz); 9.0 (dd, 1H, J = 4.4 and 1.5 Hz); 9.15 (dd, 1H, J = 8.1 and 1.4 Hz); 9.25 (d, 1H, J = 5.9 Hz); 9.29 (dd, 1H, J = 8.4 and 1.1 Hz). IR ($CHCl_3$) 1700 cm^{-1}. HRMS calcd for $C_{18}H_8N_4O_3$ $(M+H)^+$: 328.0596. Found 328.0590; tr: 12.58 min (96.4% purity) using system I with isooctane/EtOH 80:20 and flow rate 1 mL/min.

7-Amino-9-H-quino[4,3,2-*de*][1,7]phenanthrolin-9-one (4). A mixture of nitro-derivative **3** (160 mg, 0.54 mmol) and 10% Pd/C (107 mg) in MeOH (80 mL) was stirred under a hydrogen atmosphere for 2 h. After filtration through Celite and evaporation in vacuo, the crude product was purified by flash-chromatography ($CHCl_3$/MeOH 95:5) to give the amino compound as a violine solid (71 mg, 49%), mp >260 °C. ^{1}H NMR (DMSO-d_6) 6.59 (br. s, 2H); 7.20 (d, 1H, J = 7.7 Hz); 7.70 (dd, 1H, J = 7.7 and 7.7 Hz); 7.91 (dd, 1H, J = 8.1 and 4.4 Hz); 8.02 (d, 1H, J = 7.7 Hz); 8.72 (d, 1H, J = 5.7 Hz); 9.00 (dd, 1H, J = 4.4 and 1.8 Hz); 9.07 (d, 1H, J = 5.7 Hz); 9.16 (dd, 1H, J = 8.1 and 1.5 Hz). ^{13}C NMR (DMSO-d_6) 110.01; 113.35; 117.61; 117.74; 124.66; 128.69; 133.01; 133.61; 133.63; 133.88; 137.93; 143.09; 148.39; 148.63; 149.01; 149.41; 152.63; 180.03. IR ($CHCl_3$) 3420; 3315; 1672 cm^{-1}. HRMS calcd for $C_{18}H_{10}N_4O$ $(M+H)^+$: 298.0855. Found 298.0862; tr: 4.52 min (100% purity) using system II with isooctane/EtOH/MeOH 70:20:10.

5-Bromo-9-H-quino[4,3,2-*de*][1,7]phenanthrolin-9-one (5). A mixture of compound **2** (166 mg, 0.59 mmol) and bromine (0.5 mL, 9.7 mmol) in acetic acid (8.3 mL) was warmed at 100 °C for 4 h. After concentration in vacuo, the crude product was washed with $CHCl_3$, made alkaline with $NaHCO_3$ saturated solution and extracted with $CHCl_3$ (3 × 30 mL). The combined extracts were dried over $MgSO_4$ and concentrated in vacuo. After recristallization in $CHCl_3$, the bromo-compound was obtained as a yellow solid (69 mg, 32%), mp >260 °C. ^{1}H NMR ($CDCl_3$) 7.78 (dd, 1H, J = 8.1 and 4.8 Hz); 8.11 (dd, 1H, J = 8.8 and 2.0 Hz); 8.41 (d, 1H, J = 5.6 Hz); 8.53 (d, 1H, J = 8.8 Hz); 8.81 (d, 1H, J = 2.0 Hz); 9.07 (dd, 1H, J = 4.8 and 1.5 Hz); 9.14 (d, 1H, J = 5.6 Hz); 9.25 (dd, 1H, J = 8.1 and 1.5 Hz). ^{13}C NMR ($CDCl_3$) 116.10; 116.94; 124.64; 125.79; 125.84; 128.37; 133.53; 134.16; 134.62; 135.41; 136.95; 144.61; 146.78; 147.64; 149.03; 149.47; 153.02; 180.71. IR ($CHCl_3$) 1694 cm^{-1}. HRMS calcd for $C_{18}H_8N_3OBr$ $(M+H)^+$: 360.9851. Found 360.9852; tr: 10.02 min (98.2% purity) using system I with flow rate 1 mL/min.

5-Amino-9-H-quino[4,3,2-*de*][1,7]phenanthrolin-9-one (6). A mixture of compound **5** (150 mg, 0.42 mmol) and NaN_3 (210 mg, 3.2 mmol) in DMF (4.5 mL) was warmed at 105 °C for 6 h. After concentration in vacuo, H_2O (6 mL) was added and the mixture was filtered. The precipitate was washed with $CHCl_3$/MeOH 98:2 (4 × 10 mL) to give the amino-compound as a violine solid (60 mg, 48%), mp >260 °C. ^{1}H NMR ($CDCl_3$) 6.69 (2H); 7.40 (dd, 1H, J = 9.0 and 2.2 Hz); 7.76 (d, 1H, J = 2.2 Hz); 7.87 (dd, 1H, J = 8.1 and 4.4 Hz); 8.11 (d, 1H, J = 9.0 Hz); 8.45 (d, 1H, J = 5.9 Hz); 8.97 (dd, 1H, J = 4.4 and 1.8 Hz); 9.04 (d, 1H, J = 5.9 Hz); 9.16 (dd, 1H, J = 8.1 and 1.8 Hz); tr: 9.73 min (100% purity) using system I.

5-Dimethylamino-9-H-quino[4,3,2-*de*][1,7]phenanthrolin-9-one (7). To a solution of compound **6** (4.18 g, 28 mmol) in trifluoroacetic acid (1 mL) was added at 0 °C, formaldehyde (37% aqueous, 0.113 mL, 1.52 mmol) and $NaBH_3CN$ (26 mg, 0.41 mmol). The mixture was allowed to warm up to room temperature, made alkaline with 5 N NaOH, and extracted with CH_2Cl_2 (2 × 20 mL). The combined extracts were dried over $MgSO_4$, concentrated in vacuo and the crude product was purified by flash-chromatography (CH_2Cl_2/MeOH 95:5) to give the dimethylamino-compound as a violine solid (4 mg, 17%), mp >260 °C. ^{1}H NMR ($CDCl_3$) 3.28 (s, 6H); 7.45 (dd, 1H, J = 2.8 and 9.2 Hz); 7.56 (d, 1H, J = 2.8 Hz); 7.71 (dd, 1H, J = 8.1 and 4.4 Hz); 8.35 (d, 1H, J = 5.6 Hz); 8.45 (d, 1H, J = 9.2 Hz); 8.99 (d, 1H, J = 5.6 Hz); 9.03 (dd, 1H, J = 4.4 and 1.9 Hz); 9.23 (dd, 1H, J = 8.1 and 1.9 Hz). IR ($CHCl_3$) 1755, 1715 cm^{-1}. HRMS calcd for $C_{20}H_{14}N_4O$ $(M+H)^+$: 326.1167. Found 326.1161; tr: 12.48 min (100% purity) using system I.

In vitro characterization of the drug-induced effects on human cancer cell line growth

Twelve human tumor cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). These included three glioblastomas (SW1088, U-373 MG and U-87 MG), two colon (HCT-15 and LoVo), two non small-cell-lung (A549 and A-427), two bladder (J82 and T24), one prostate (PC-3) and two breast (T-47D and MCF7) cancer models. The ATCC numbers of these cell lines are HTB 12 (SW1088), HTB 14 (U-87 MG), HTB 17 (U-373 MG), CCL225 (HCT-15), CCL229 (LoVo), CCL 185 (A549), HBT 53 (A-427), HTB1 (J82), HTB4 (T24), HTB133 (T-47D), HTB22 (MCF7) and CRL1435 (PC-3). The cells were cultured at 37 °C in sealed (airtight) Falcon plastic dishes (Nunc, Gibco, Belgium) containing Eagle's minimal essential medium (MEM, Gibco) supplemented with 5% fetal calf serum (FCS). All the media were supplemented with a mixture of 0.6 mg/mL glutamine (Gibco), 200 IU/mL penicillin (Gibco), 200 IU/mL streptomycin (Gibco) and 0.1 mg/mL gentamycin (Gibco). The FCS was heat-inactivated for 1 h at 56 °C.

The 12 cell lines were incubated for 24 h in 96-microwell plates (at a concentration of 40,000 cells/mL culture medium) to ensure adequate plating prior to the determination of the cell growth. This process was carried out by means of the colorimetric MTT assay, as detailed previously.[12,13] This assessment of cell population growth is based on the capability of living cells to

reduce the yellow product MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma, St Louis, MO, USA) to a blue product, formazan, by a reduction reaction occurring in the mitochondria. The number of living cells is directly proportional to the intensity of the blue, which is quantitatively measured by spectrophotometry on a DIAS microplate reader (Dynatech Laboratories, Guyancourt, France) at a 570-nm wavelength (with a reference of 630 nm). Each experiment was carried out in sextuplicate. We validated the MTT-related data using two alternative techniques, namely direct cell counting and the genomic incorporation of tritiated thymidine (data not shown).

Six concentrations ranging from 10^{-5} to 10^{-9} M were assayed for each of the compounds under study (see Table 1).

References and Notes

1. (a) Molinski, T. F. *Chem. Rev*. **1993**, *93*, 1825. (b) Ding, Q.; Chichak, K.; Lown, J. W. *Curr. Med. Chem*. **1999**, *6*, 1. (c) Delfourne, E.; Bastide, J. *Med. Res. Rev*. **2003**, *23*, 234.
2. Kobayashi, J.; Cheng, J. F.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoa, S. *Tetrahedron Lett*. **1988**, *29*, 1177.
3. (a) Matsumoto, S. S.; Sidford, M. H.; Holden, J. A.; Barrows, L. R.; Copp, B. R. *Tetrahedron Lett*. **2000**, *41*, 1667. (b) Lindsay, B. S.; Christiansen, H. C.; Copp, B. R. *Tetrahedron* **2000**, *56*, 497.
4. Cuerva, J. M.; Cardenas, D. J.; Echavarren, A. M. *Chem. Commun*. **1999**, 1721.
5. Alvarez, M.; Feliu, L.; Ajana, W.; Joule, J. A.; Fernandez-Puentes, J. L. *Eur. J. Chem*. **2000**, 849.
6. Cuerva, J. M.; Cardenas, D. J.; Echavarren, A. M. *J. Chem. Soc., Perkin Trans. 1* **2002**, *11*, 1360.
7. (a) Delfourne, E.; Darro, F.; Bontemps-Subielos, N.; Decaestecker, C.; Bastide, J.; Frydman, A.; Kiss, R. *J. Med. Chem*. **2001**, *44*, 3275. (b) Delfourne, E.; Darro, F.; Portefaix, P.; Galaup, C.; Bayssade, S.; Bouteille, A.; Le Corre, L.; Bastide, J.; Collignon, F.; Lesur, B.; Frydman, A.; Kiss, R. *J. Med. Chem*. **2002**, *45*, 3765.
8. Ionescu, M.; Mester, I. *Rev. Roum. Chim*. **1969**, *14*, 789.
9. Bracher, F. *Heterocycles* **1989**, *29*, 2093.
10. Weinstein, J. N.; Kohn, K. W.; Grever, M. R.; Viswanadhan, V. N.; Rubinstein, L. V.; Monks, A. P.; Scudiero, D. A.; Welch, L.; Koutsoukos, A. D.; Chiausa, A. J.; Paull, K. D. *Science* **1992**, *258*, 447.
11. Mean graphs depicting the dose–response data for compounds submitted to the NCI can be viewed, by searching for the NSC number of the compound at the NCI web site: http://dtp.nci.nih.gov/docs/cancer/searches/cancer_open_compounds.html.
12. Pauwels, O.; Kiss, R.; Pasteels, J. L.; Atassi, G. *Pharm. Res*. **1995**, *12*, 1011.
13. Camby, I.; Salmon, I.; Danguy, A.; Pasteels, J. L.; Brotchi, J.; Martinez, J.; Kiss, R. *J. Natl. Cancer Inst*. **1996**, *88*, 594.