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Synthesis and biological evaluation of pyrroloiminoquinone derivatives

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Abstract—Synthesis of 10 pyrroloiminoquinone derivatives is presented. The strategy is based around the elaboration of a common intermediate by reaction with primary amines. All the compounds obtained have been subjected to antiproliferative activity with three different cell lines (NCI-H460, HeLa, and HL-60). The capacity of 4 selected compounds to affect the enzymatic activity of the nuclear enzyme DNA topoisomerase II and to form the typical DNA fragmentation which occurs in the apoptotic process is discussed here.

1. Introduction

Drugs that inhibit or poison the function of topoisomerase enzymes are one of the mainstays of cancer chemotherapy. Two classes of inhibitors are known that include some of the most widely used anticancer drugs, topo I, exemplified by the camptothecins, and topo II, exemplified by etoposide and anthracyclines.[1,2] A major effort has been directed toward the synthesis of analogues designed to improve on the deficiencies of these established agents. At the same time, new types of structures are also being explored and developed. Topoisomerase II modulates the topological state of DNA by generating transient double-stranded breaks in the backbone of the genetic material.[3–8] To maintain genomic integrity during this cleavage event, the enzyme forms covalent bonds between active site tyrosyl residues and the 5′-DNA termini created by scission of the double helix.[9–11] These covalent topoisomerase II cleaved DNA intermediates are known as cleavable complexes. Under normal conditions, they are present at low equilibrium levels and are tolerated by the cell. However, conditions that significantly increase the concentration of cleavage complexes generate permanent DNA strand breaks that trigger illegitimate recombination, chromosomal aberrations, sister chromatid exchange, and cell death pathways.[12–16] All topoisomerase II-directed agents are able to interfere with at least one step of the catalytic cycle. Agents able to stabilize the covalent DNA topoisomerase II complex are traditionally called topoisomerase II poisons, while agents acting on any of the other steps in the catalytic cycle are called catalytic inhibitors. Recently a class of marine compounds characterized by the presence of the pyrroloiminoquinone[17] structural motif was discovered to be cytotoxic, potentially by multiple mechanisms but with a proven activity toward the catalytic cycle of topoisomerase II (Fig. 1).[18] As a consequence several syntheses of pyirroloiminoquinone derivatives with interesting pharmacological results have been reported in the literature.[19–23]

In this paper, we report experimental results concerning the synthesis and the biological evaluation of some new compounds characterized by the presence of the pyrroloiminoquinone framework with a methyl group at position 1.

Keywords: Pyrroloiminoquinone derivatives; Antiproliferative activity; Topoisomerase II; DNA fragmentation.
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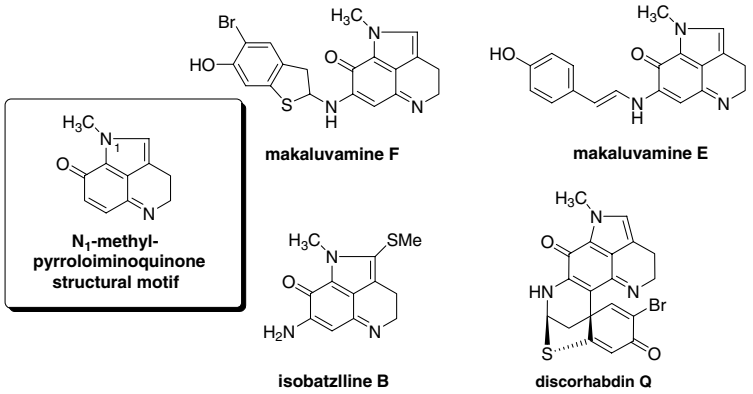


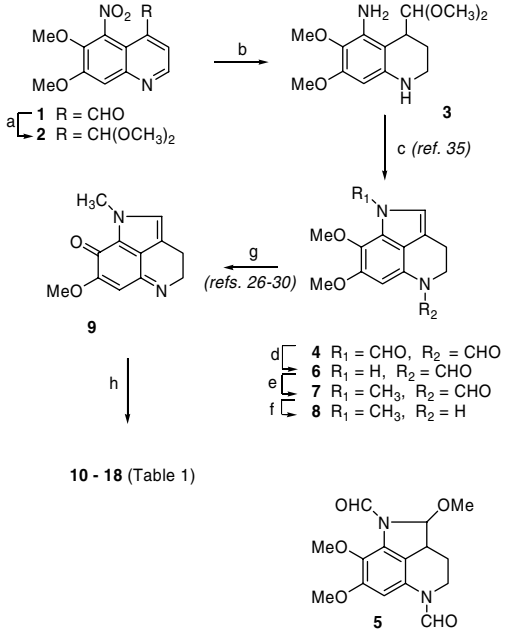
Figure 1. Representative structures of marine compounds characterized by the presence of the N^1-methyl-pyrroloiminoquinone structural motif.

2. Results and discussion

2.1. Synthesis

The preparation of the reported derivatives was realized according to the synthetic Scheme 1 taking advantage from the possibility to substitute the methoxyl group of the intermediate **9** by a conventional addition-elimination reaction.

The preparation of **9** was achieved using nitro-aldehyde **1**[24,25] as described by Alvarez and Joule[24] (Scheme 1). The subsequent formation of the dimethoxyacetal and reduction to obtain tetrahydroisoquinoline **3** was studied in order to simplify the purification. To this end the formation of the acetal was conducted in MeOH in the presence of acidic Amberlite IR-120 resin in order to overcome the problem of the removal of HCl. When the reaction was complete the resin was removed and the solution was directly treated with a large excess of $NiCl_2/NaBH_4$ to give **3** (yield from **1**: 79%). Treatment of compound **3** with a 1:1 Ac_2O/HCOOH mixture for 60 h resulted in the formation of **4** (yield 36%) accompanied by compound **5** (yield 10%) as an inseparable mixture of diastereoisomers. We studied the possibility to convert byproduct **5** to main compound **4** by a formal elimination of methanol. To our surprise compound **5** proved to be unreactive even by treatment with HCOOH at reflux. The formyl groups of compound **4** could be manipulated selectively on the basis of the different reactivity of the indole and aniline nitrogens. Treatment of **4** in $MeOH/CH_2Cl_2$ (1:1) with a saturated solution of Na_2CO_3 for 8 h at room temperature gave selectively compound **6** and this immediately converted to **7** by reaction with NaH and MeI (yield from **4**: 97%). Treatment of **7** with 20% NaOH permitted the removal of the formyl group from the quinoline nitrogen. Finally the oxidation to quinine-imine **9**[26–30] (yield from **7**: 57%) was realized with CAN as described by Alvarez and Joule. The reaction of **9** with different primary amines in EtOH at reflux allowed us to prepare 9 derivatives (**10**–**17**, yields 18–91%) (Table 1).[23] Reaction of **9** with mercaptoethylamine resulted in the formation of compound **18** (yield 40%) by a formal thiol substitution of the methoxy group, a subsequent addition reaction of the primary amine, and aromatization.

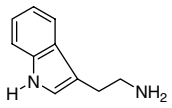
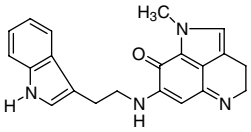
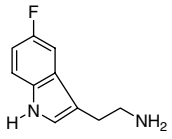
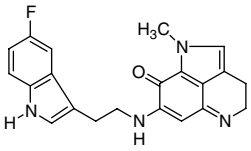
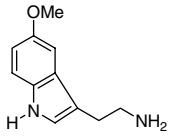
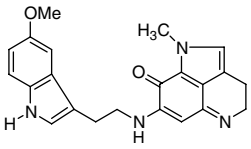
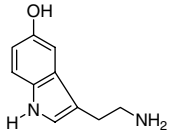
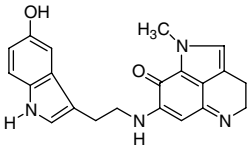
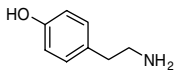
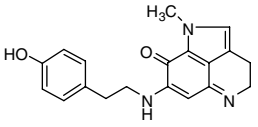
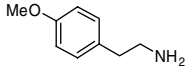
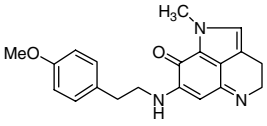
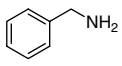
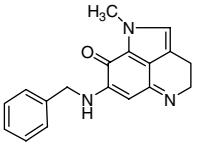
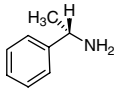
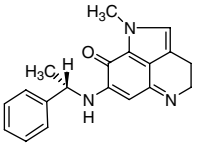
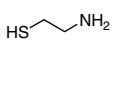
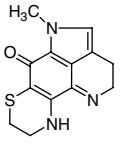


Scheme 1. General synthetic procedure for the preparation of compounds **10**–**18**. Reagents: (a) Amberlite IR-120/MeOH; (b) $NiCl_4$, $NaBH_4$; (c) Ac_2O, HCOOH; (d) Na_2CO_3; (e) NaH, MeI; (f) NaOH 20%; (g) CAN (from **8**); (h) R-NH_2.

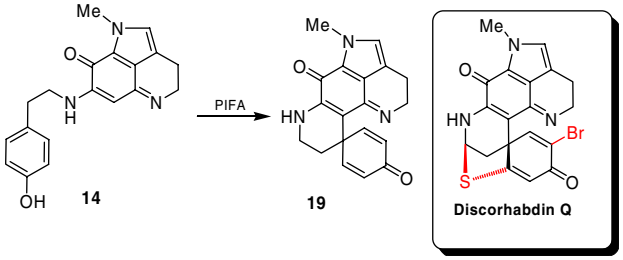
2.2. Biological activity

The biological activity of the compounds was evaluated on three different types of human tumor cell lines representing solid and hematological malignancies. A preliminary evaluation of the antiproliferative activity was performed against the NCI-H460 nonsmall cell lung carcinoma cell line and then, for the most active compounds, against the HeLa cervix adenocarcinoma and HL-60 promyelocytic leukemia cell lines. In order to investigate the mechanism of action responsible for the

Table 1. Structures of compounds **10–18**

R-NH_2	Product	Product number
		**10**
		**11**
		**12**
		**13**
		**14**[31–35,9]
		**15**
		**16**
		**17**
		**18**

antiproliferative activity, we assayed the capacity of the new compounds to affect the enzymatic activity of the nuclear enzyme DNA topoisomerase II. Moreover, the ability of the compounds to induce apoptosis was investigated in the HeLa cells, by DNA fragmentation assay.



Scheme 2. Preparation of compound **19**. Comparison with discorhabdin Q.

2.3. Antiproliferative activity

The in vitro cytotoxic potency of the newly synthesized pyrroloiminoquinones **4-19** was preliminarily evaluated by a growth inhibition assay against the NCI-H460 human nonsmall cell lung carcinoma cell line. The results, expressed as IC_{50} values, are shown in Table 2 and indicated that many compounds of the series possessed a good antiproliferative activity, with a cytotoxic potency lower than 11 μM. Compound **18**, that is characterized by the thiomorpholine subunit, showed the highest potency of the series. The comparison of the IC_{50} values of compounds **4–9** with those of compounds **10–19** highlights the importance of the iminoquinone system. The synthetic intermediate **5**, that is characterized by a benzopyrrolidine framework, is an exception in this behavior. For the most active derivatives **5**, **9**, **10**, **11**, **14**, **18**, and **19**, the antiproliferative function was further investigated on HeLa human cervix adenocarcinoma and HL-60 human promyelocytic leukemia cell lines (Table 2). The majority of derivatives, **10**, **14**, **18**, and **19**, maintained a significant antiproliferative effect and, as for in the NCI-H460 cell line, compound **18** exerted the highest capability to inhibit cell growth, showing the lowest

Table 2. Antiproliferative activity of derivatives, **4–19**, after 72 h of drug exposure

Compound	IC_{50}[a] (μM)		
	NCI-H460	HeLa	HL-60
4	>60	—	—
5	7.7	>20	>20
6	>60	—	—
7	>60	—	—
8	>60	—	—
9	9	>20	>20
10	2.12	4.1 ± 0.3	7.8 ± 0.4
11	5.50	>20	>20
12	8.8	—	—
13	>40	—	—
14	10.8	4.9 ± 0.4	6.9 ± 0.5
15	>40	—	—
16	>40	—	—
17	23	—	—
18	0.96	1.9 ± 0.1	2.7 ± 0.2
19	6.2	5.5 ± 0.4	3.1 ± 0.3
Doxorubicin	0.028	—	—
m-Amsacrine	—	0.012 ± 0.002	0.0051 ± 0.0011

[a] IC_{50}: concentration required to reduce cell number to 50% of control culture.

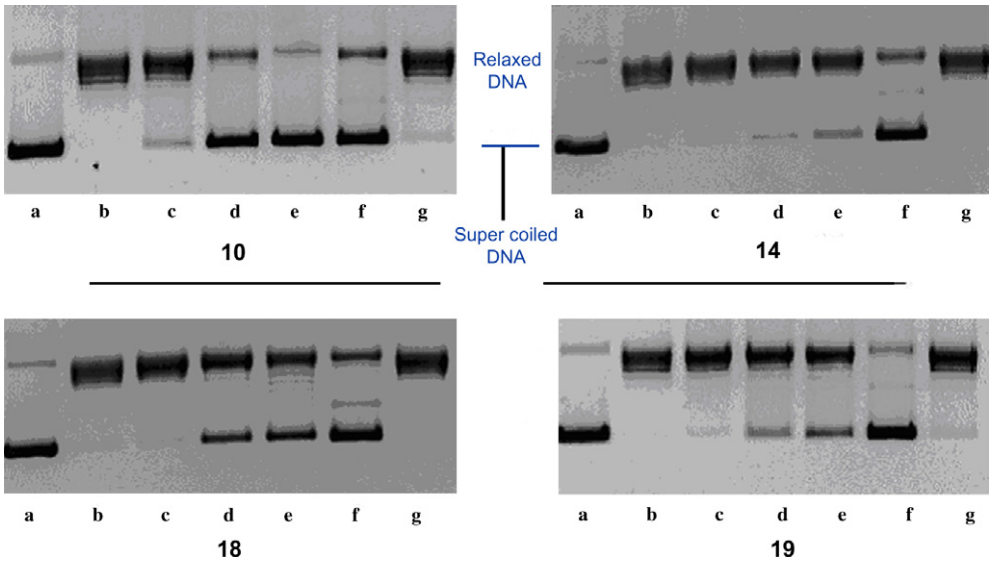


Figure 2. Effect of derivatives **10**, **14**, **18**, and **19** on the relaxation of supercoiled plasmid DNA by human recombinant topoisomerase II. Lane a: pBR322 DNA, lane b: same as lane a in the presence of 1U topoisomerase II, lanes c–e: same as lane b in the presence of 10, 25, and 50 μM test compound, respectively; lane f: same as lane b in the presence of 8 μM *m*-amsacrine; lane g: same as lane b in the presence of solvent alone.

IC_{50} value in the series. The other derivatives were unable to induce 50% growth inhibition up to 20 μM concentration.

2.3.1. Inhibition of topoisomerase II enzymatic activity. In order to investigate the mechanism of action accountable for the antiproliferative activity exerted by **10**, **14**, **18**, and **19**, we assayed their capability to inhibit the activity of the nuclear enzyme DNA topoisomerase II. This enzyme catalyzes the conversion of pBR322 plasmid DNA from the supercoiled to the fully relaxed conformation and Figure 2 shows the effect of the new derivative on topoisomerase II relaxation ability. In detail, compound **10** shows the strongest capability to inhibit the enzyme, whose activity is completely abolished at 50 μM concentration. Derivatives **14** and **19** exerted a quite weak and similar inhibitory effect at test concentrations, while compound **18** displayed a significant level of inhibition, although lower than that of **10**.

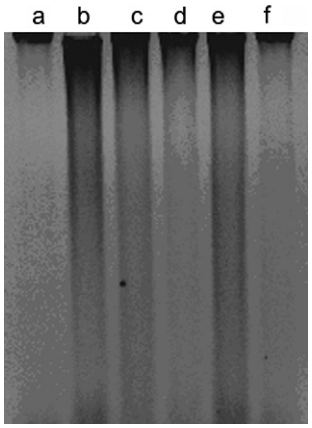


Figure 3. Agarose gel electrophoresis of DNA extracted from HeLa cells. Lane a: untreated control, lane b: 50 μM cisplatin, lanes c–e: 100 μM of compounds **10**, **14**, and **19**, respectively; lane f: solvent alone.

2.3.2. DNA fragmentation. During the apoptotic process, activated endonucleases degrade the high order chromatin of DNA into mono- and oligonucleosomal DNA-fragments, giving rise to a 'ladder' of nucleosomal-sized multimers. To establish the cell death modality that follows the treatment, DNA was extracted from HeLa cells and treated with the test derivatives or with cisplatin (taken as positive control). Figure 3 shows the gel electrophoresis of the DNA extracted from control cells and from cells after treatment with cisplatin or compounds **10**, **14**, and **19**. The results indicate the ability of the compounds to form the typical DNA fragmentation which occurs in the apoptotic process.

3. Conclusions

The discovery that natural marine compounds characterized by the presence of pyrroloiminoquinone system are able to interfere with the catalytic cycle of DNA topoisomerase II has stimulated the interest for new compounds presenting such a structural motif. In this paper, we report an improvement in the synthesis of compound **9** which is pivotal for the preparation of pyrroloiminoquinone derivatives. A quality collection of compounds was synthesized and evaluated for growth inhibitory properties in a small panel of human tumor cell lines. The results of the present study indicated that the insertion of arylamino side chains to the pyrroloiminoquinone system leads to new derivatives which are able to induce a good antiproliferative effect on human tumor cells. In particular, both indole and phenol terminal groups were demonstrated to be the most effective chemical moieties (compounds **10** and **14**). Also, the condensation of one (**18**) or two (**19**) rings to the tricyclic pyrroloiminoquinone moiety yielded derivatives en-

dowed with an interesting antiproliferative activity. Preliminary studies of the mechanism of action of the most active compounds suggested an inhibitory effect on DNA topoisomerase II activity, in accord with results from the natural compounds. The analysis of structure-activity relationships obtained in the series of compounds investigated may represent the basis for the design of more active molecules.

4. Experimental

4.1. General

Thin-layer chromatography (TLC) was performed on Merck precoated 60F254 plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with sulfuric acid. Flash chromatography was performed using Silica gel (240–400 mesh, Merck). ^{1}H NMR spectra were recorded with Brucker 200, 300, and 400 MHz spectrometers using chloroform-d ($CDCl_3$) and methanol-d_4 (CD_3OD). Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS) as internal standard. EI mass spectra were recorded at an ionizing voltage of 6 keV on a VG 70-70 EQ. ESI mass spectra were recorded on FT-ICR APEX^{II} (Bruker Daltonics). All reactions were carried out in dry solvents.

4.1.1. 4-(Dimethoxymethyl)-6,7-dimethoxy-5-nitroquinoline (2). *In HCl/MeOH:* A solution of 4-formyl-6,7-dimethoxy-5-nitroquinoline (**1**, 2.5 g, 9.73 mmol) in MeOH (300 mL) saturated with HCl was refluxed for 24 h, then diluted with EtOAc and washed with small portions of $NaHCO_3$ (5%). Compound **2** (2.8 g, 94%) was obtained as an amorphous solid. *With resin:* To a solution of 4-formyl-6,7-dimethoxy-5-nitroquinoline (2.5 g, 9.73 mmol) in MeOH (40 mL) Amberlite IR-120 (1.02 g) was added. After stirring under reflux for 72 h, the resin was separated by filtration and the solvent was evaporated. Compound **2** (2.5 g, 84%) was obtained as an amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.40 (s, 6H), 4.12 (s, 3H), 4.15 (s, 3H), 5.60 (s, 1H), 7.65 (s, 1H), 7.80 (d, J = 4.9 Hz), 8.80 (d, J = 4.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 54.3 (q); 56.8 (q);63.2 (q); 100.5 (d); 112.9 (d); 113.5 (s); 119.6(d); 140.9 (s); 144.4(s); 146.7 (s); 150.5 (d); 153.9 (s).

4.1.2. 5-Amino-1,2,3,4-tetrahydro-6,7-dimethoxy-4-(dimethoxymethyl)quinoline (3). To a solution of **2** (1.32 g, 4.3 mmol) in MeOH (135 mL) was added $NiCl_2{\cdot}6H_2O$ (12.24 g, 51.5 mmol). After stirring for 5 min. at room temperature the solution was cooled to −20 °C and $NaBH_4$ (11.4 g, 300.5 mmol) was added in small portions. The reaction mixture was then diluted with H_2O (200 mL), filtered, and the water-phase was extracted with EtOAc. Compound **3** (1.34 g, 94%) was obtained as a reddish oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55–1.60 (m, 1H), 2.09–2.20 (m, 1H), 3.00–3.16 (m, 1H), 3.37 (s, 3H), 3.20–3.50 (m,, 2H), 3.67 (s, 3H), 3.77 (s, 3H), 3.82 (s, 3H), 4.51 (d, J = 8.6 Hz, 1H), 5.659 (s, 1H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.0 (q), 26.2 (d), 28.0 (t), 36.8 (t), 55.5 (q), 61.2 (q), 89.2 (d), 105.7 (s), 129.1 (s), 138.9 (s), 140.0 (s), 153.2 (s).

4.1.3. 1,5-Diformyl-1,3,4,5-tetrahydro-7,8-dimethoxy-2,3-dihydropyrrolo[4,3,2-*de*]quinoline (4). Compound **3** was added to a mixture of HCOOH (2.2 ml) and Ac_2O (4.4 ml) at 0 °C and the solution was stirred for 60 h at room temperature. The solvent was then evaporated under reduced pressure to give a crude product, which was purified by column chromatography (EtOAc/Hexane 7:3) to give the **4** (245.6 mg, 36%) as a white amorphous solid and compound **5** (68.2 mg, 10%) as a yellow amorphous solid. **4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52 (ddd, J = 5.5, 12.7 25.4 Hz, 1H), 2.38–2.53 (m, 1H), 3.44 (dt, J = 4.3, 12.7 Hz, 1H), 3.85 (s, 3H), 3.90 (s, 3H), 4.05 (dd, J = 5.5, 13.8 Hz, 1H), 5.76 (s, 1H), 6.92 e 7.85 (2s, 1H), 8.43 e 9.12 (2s, 1H), 9.27 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.1 e 23.2 (2t), 40.4 e 47.4 (2t); 57.6 e 58.2 (2q); 60.9 (q); 94.1 (d); 99.7 (s); 108.8 e 109.3 (2s); 116.8 e 117.5 (2d); 125.8 e 126.8 (2s); 127.8 e 128.3 (2s); 131.7 e 132.4 (2s); 147.9 e 148.5 (2s); 160.2 e 161.7 (2d); EI-MS: 274; *Compound* **5**: ^{1}H NMR (300 MHz, $CDCl_3$, 50 °C) δ 2.95 (m, 2H), 3.5 (s, 3H,), 3.90 (s, 3H), 3.93 (s, 3H), 3.96 (m, 1H), 4.09 (m, 2H), 5.9 (d, J = 6.1 Hz, 1H), 7.45 and 7.90 (2s, 1H), 8.45 and 9.71 (2s, 1H), 8.9 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) 22.1 and 23.2 (2t), 40.4 and 47.4 (2t); 57.6 and 58.2 (2q); 60.9 (q); 94.1 (d); 99.7 (s); 108.8 and 109.3 (2s); 116.8 and 117.5 (2d); 125.8 and 126.8 (2s); 127.8 and 128.3 (2s); 131.7 and 132.4 (2s); 147.9 and 148.5 (2s); 160.2 and 161.7 (2d); EI-MS: 306. Anal. Calcd for $C_{15}H_{18}N_2O_5$: C, 58.82; H, 5.92; N, 9.15; Found: C, 58.78; H, 5.96; N, 9.19.

4.1.4. 5-Formyl-1,3,4,5-tetrahydro-7,8-dimethoxy-2,3-dihydropyrrolo[4,3,2-*de*]quinoline (6). A mixture of **4** (114.8 mg, 0.42 mmoli), MeOH (2 mL), CH_2Cl_2 (2 mL), and Na_2CO_3 (5%, 1 mL) was stirred for 8 h at room temperature. The reaction mixture was then diluted with H_2O and extracted with CH_2Cl_2. Compound **6** (101.8 mg, 99%) was obtained as a yellowish solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.94 e 3.12 (2t, J = 5.7 Hz, 2H), 3.93 e 4.00 (2t, J = 5.8 Hz, 2H), 3.90 (s, 3H), 3.97 (s, 3H), 6.60 e 7.60 (2s, 1H), 6.76 (s, 1H), 8.12 (br s, 1H), 8.35 e 8.68 (2s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.2 e 23.6 (2t), 39.2 e 46.4 (2t), 57.8 e 58.6 (2q), 60.2 (q,), 94.5 (d), 99.1 (s), 107.7 e 108.9 (2s), 116.3 e 117.0 (2d), 125.1 e 126.1 (2s), 127.4 e 128.0 (2s), 131.0 e 132.1 (2s), 147.5 e 148.6 (2s), 160.5 e 161.6 (2d).

4.1.5. 5-Formyl-1,3,4,5-tetrahydro-7,8-dimethoxy-1-methylpyrrolo[4,3,2-*de*]-quinoline (7). A solution of **6** (101.8 mg, 0.42 mmol) in dry THF (4 mL) was added to a suspension of NaH (19.9 mg, 0.83 mmol) in dry THF (4 mL) at room temperature. The mixture was stirred for 30 min. at room temperature, MeI (0.9 mL, 12.6 mmol) was added, and the resulting mixture was stirred for 20 h. Thereafter, H_2O was added, the solution was concentrated under reduced pressure, and extracted with CH_2Cl_2. Compound **7** (105.6 mg, 98%) was obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.96 e 3.05 (2t, J = 5.7 Hz, 2H), 3.90 (s, 3H), 3.94 (s, 3H), 3.95 (s, 3H), 3.85 e 4.08 (2t, J = 5.7 Hz, 2H), 6.60 (s, 1H), 8.38 e 8.85 (2s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3 e 22.9 (2 t), 34.8 (q), 40.1 e 46.6 (2 t); 57.1 e 57.8 (2q), 61.9 (q); 94.5 e 99.4 (2d); 106.8 e

107.9 (2s); 118.0 (s); 122.0 e 122.7 (2d); 124.8 e 127.0 (2s); 127.8 e 128.2 (2s); 132.2 e 133.1 (2s) 148.1 e 148.9 (2s), 158.0 e 161.0 (2d).

4.1.6. 1,3,4,5-Tetrahydro-7,8-dimethoxy-1-methylpyrrolo[4,3,2-*de*]-quinoline (8). A solution of **7** (105.6 mg, 0.41 mmol) in NaOH (20%, 8 mL) was stirred under reflux for 24 h. The solution was extracted with CH_2Cl_2. Compound **8** (73.1 mg, 78%) was obtained as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.90 (t, J = 5.5 Hz, 2H), 3.40 (t, J = 5.5 Hz, 2H), 3.80 (s, 3H), 3.89 (s, 3H), 3.91 (s, 3H), 5.90 (s, 1H), 6.40 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.0 (t), 34.8 (q), 43.4(t); 57.7 (q), 62.1 (q); 89.0 (d); 109.8 (s); 114.6 (s); 120.0 (d),128.6 (s),128.8(s), 136.5(s), 149.0 (s).

4.1.7. 1,3,4,8-Tetrahydro-7-methoxy-1-methyl-8-oxopyrrolo[4,3,2-*de*]-quinoline (9). A mixture of **8** (73.1 mg, 0.32 mmol), MeCN (5 mL), CAN (ammonium ceriu-m(IV) nitrate) (349 mg, 0.64 mmol), and H_2O (9 mL) was stirred for 10 min. The solution was then diluted with H_2O and extracted with CH_2Cl_2. Compound **9** (49.2 mg, 73%) was obtained as an amorphous orange solid, ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (t, J = 8.1 Hz, 2H), 3.99 (s, 3H), 4.00 (s, 3H), 4.14 (t, J = 8.1 Hz, 2H), 6.60 (s, 1H), 6.85 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.6 (t), 36.6 (q), 39.8 (t); 58.5 (q), 97.9 (d); 117.8 (s); 119.3 (s); 124.7 (s), 128.2 (d), 159.9 (s), 162.6 (s), 168.0 (s).

4.2. General procedure for the preparation of compounds 10–18

A solution of **9** (23 mg, 0.11 mmol) and primary amine (0.28 mmol) in EtOH (10 mL) was refluxed for 4 h. The solvent was removed to give a crude product, which was purified by column chromatography.

4.2.1. 1,3,4,8-Tetrahydro-7-(3-(2-aminoethyl)indolyl)-1-methyl-8-oxopyrrolo[4,3,2-*de*]-quinoline (10). Red oil (11 mg, 30%) (EtOAc/MeOH 8:2 with Et_3N 3%); ^{1}H NMR (400 MHz, CD_3OD) δ 2.93 (t, J = 8.0 Hz, 2H), 3.12 (t, J = 6.9 Hz, 2H), 3.60 (t, J = 7.0, 2H), 3.73 (t, J = 8.0 Hz, 2H), 3.85 (s, 3H), 5.36 (s, 1H), 7.00–7.18 (m, 4H), 7.30–7.40 (m, 1H), 7.58 (d, J = 8 Hz, 1H), 8.0 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) (APT) δ 18.6, 24.2, 35.6, 43.0, 44.8, 84.5, 110.7, 111.3, 118.3, 119.1, 123.9, 127.7, 129.2, 131.1, 137.4, 139.5, 153.9, 157.9,168.1, 174.4; ESI positive MS Anal. Calcd for $C_{21}H_{20}N_4O+H^+$ 345.1710. Found: 345.1709 $[M+H]^+$.

4.2.2. 1,3,4,8-Tetrahydro-7-(5-fluoro-3-(2-aminoethyl)indolyl)-1-methyl-8-oxopyrrolo[4,3,2-*de*]-quinoline (11). red oil (17 mg, 47%) (EtOAc/MeOH, 7:3 with Et_3N 3%) ^{1}H NMR (400 MHz, CD_3OD) δ 2.83 (t, J = 7.6 Hz, 2H), 3.08 (t, J = 7.2 Hz, 2H), 3.60 (t, J = 7.2 Hz, 2H), 3.80 (t, J = 7.6 Hz, 2H), 3.90 (s, 3H), 5.35 (s, 1H), 6.85 (ddd, J = 11.6, 9.2, 2.4 Hz, 1H), 7.00 (s, 1H), 7.18 (s,1H), 7.22 (dd, J = 10.0, 2.4 Hz, 1H), 7.30 (dd, J = 8.8, 4.4 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 19.4, 25.0, 36.3, 44.6, 45.5, 86.4, 103.9, 110.86, 111.5, 113.3, 118.0, 119.0, 131.7, 126.1, 129.0, 135.0, 143.0, 154.0, 157.8, 159.0, 160.1; ESI positive MS Anal. Calcd for $C_{21}H_{19}N_4OF+H^+$ 363.16157. Found: 363.16160.

4.2.3. 1,3,4,8-Tetrahydro-7-(5-methoxy-3-(2-aminoethyl)indolyl)-1-methyl-8-oxopyrrolo [4,3,2-*de*]-quinoline (12). Red oil (15 mg, 57%) (EtOAc/MeOH, 7:3 with Et_3N 3%); ^{1}H NMR (400 MHz, CD_3OD) δ 2.78 (t, J = 7.6 Hz, 2H), 3.08 (t, J = 7.0 Hz, 2H), 3.60 (t, J = 7.0 Hz, 2H), 3.75 (t, J = 7.6 Hz, 2H), 3.78 (s, 3H), 3.80(s, 3H), 5.25 (s, 1H), 6.65–6.80 (m), 6.92 (s,1H), 6.95–7.05 (m), 7.08 (s, 1H), 7.15–7.28 (m); ^{13}C NMR (100 MHz, CD_3OD) (APT) δ 20.4, 26.1, 37.2, 40.5, 45.4; 46.4, 87.1, 104.4, 113.6, 112.4, 113.8, 120.5, 125.1, 125.7, 132.7, 134.1, 136.0, 146.0, 152.3, 155.0, 160.0, 171.0; ESI positive MS Anal. Calcd for $C_{22}H_{22}N_4O_2+H^+$ 375.1815. Found: 375.1818.

4.2.4. 1,3,4,8-Tetrahydro-7-(5-hydroxy-3-(2-aminoethyl)indolyl)-1-methyl-8-oxopyrrolo-[4,3,2-*de*]-quinoline (13). Red oil (14 mg, 39%) (EtOAc/MeOH, 8:2 with Et_3N 3%); ^{1}H NMR (400 MHz, CD_3OD) δ 2.86 (t, J = 7.6 Hz, 2 H), 3.08 (t, J = 7.0 Hz, 2H), 3.60 (t, J = 7.0 Hz, 2H), 3.78 (t, J = 7.6 Hz, 2H), 3.92 (s, 3H), 5.33 (s, 1H), 6.68 (dd, J = 8.7, 2.3 Hz, 1H), 6.94 (d, J = 2.3 Hz, 1H), 7.00 (s, 1H), 7.08 (s, 1H), 7.18 (d, J = 8.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) (APT) δ 20.4, 26.1, 37.2, 45.4, 46.4, 87.1, 104.4, 113.6, 112.4, 113.8, 120.5, 125.1, 125.7, 132.7, 134.1, 136.0, 146.0, 152.3, 155.0, 160.0, 171.0; ESI positive MS Anal. Calcd for $C_{21}H_{20}N_4O_2+H^+$ 361.1659. Found: 361.1660.

4.2.5. 1,3,4,8-Tetrahydro-7-(4-hydroxyphenethylamino)-1-methyl-8-oxopyrrolo[4,3,2-*de*]-quinoline (14). Red oil (73 mg, 52%) (EtOAc/MeOH, 8:2 with Et_3N 3%); ^{1}H NMR (400 MHz, CD_3OD) δ_H 2.70 (t, J = 7.6 Hz, 2H), 2.90 (t, J = 7.6 Hz, 2H), 3.45 (t, J = 7.6 Hz, 2H), 3.85(t, J = 7.6 Hz, 2H), 3.95 (s, 3H), 5.40 (s, 1H), 6.75 (d, J = 8.5 Hz, 2H), 7.05 (d, J = 8.5 Hz, 2H), 7.10 (s,1H); ^{13}C NMR (100 MHz, CD_3OD) δ 18.0 (t), 33.0 (t), 34.7 (q), 44.7 (t), 45.2 (t), 88.2 (d), 115.0 (d), 120.2 (s), 123.9 (s), 125.4 (s), 127.2 (s), 129.4 (d), 129.6 (d), 154.9 (s), 157.4 (s), 159.6 (s), 168.4 (s). MS (APCI); m/z = 322 $(M+H)^+$. ESI positive MS Anal. Calcd for $C_{19}H_{19}N_3O_2+H^+$ 322.1550. Found: 322.1553.

4.2.6. 1,3,4,8-Tetrahydro-7-(3-methoxyphenethylamino)-1-methyl-8-oxopyrrolo[4,3,2-*de*]-quinoline (15). Red oil (3 mg, 18%) (EtOAc/MeOH, 8:2 with Et_3N 2%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.65–2.80 (m, 2H), 2.85–3.05 (m, 2H), 3.35–3.45 (m, 2H), 3.80 (s, 3H), 3.95 (s, 3H), 4.00–4.15 (m, 2H), 5.68 (s, 1H), 6.65–6.85 (m, 3H), 7.15–7.25 (m, 1H), 7.28 (s, 1H); ESI MS: 336.4 (M+1); ESI positive MS Anal. Calcd for $C_{20}H_{21}N_3O_2+H^+$ 336.17065. Found: 336.17071.

4.2.7. 1,3,4,8-Tetrahydro-7-(benzylamino)-1-methyl-8-oxopyrrolo[4,3,2-*de*]-quinoline (16). Red oil (25 mg, 86%) (EtOAc/MeOH, 8:2 with Et_3N 2%); ^{1}H NMR (400 MHz, DMSO) δ 2.15–2.25 (m, 2H), 3.78–3.88 (m, 2H), 3.90 (s, 3H), 4.45 (s, 2H), 5.78 (s, 1H), 7.20 (s, 1H), 7.20–7.50 (m, 5H); ESI positive MS Anal. Calcd for $C_{18}H_{17}N_3O+H^+$ 292.1444. Found: 292.1449.

4.2.8. 1,3,4,8-Tetrahydro-7-S(-)-(1-phenylethylamino)-1-methyl-8-oxopyrrolo[4,3,2-*de*]-quinoline (17). Red oil (25 mg, 91%) (EtOAc/MeOH, 8:2 with Et_3N 2%); $[\alpha]_D$11.2 (c 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 2.70 (t, $J = 7.6$ Hz, 2 H), 3.95 (t, $J = 7.6$ Hz, 2H), 4.10 (s, 3H), 4.15 (s, 3H), 4.55–4.65 (m, 1H), 6.65 (s, 1H), 7.20–7.40 (m, 7H). MS (FAB); $m/z = 306$ $(M+H)^+$; ESI positive MS Anal. Calcd for $C_{19}H_{19}N_3O+H^+$ 306.1601. Found: 306.1600.

4.2.9. 5-Methyl-3,5,9,8,10-tetrahydro-2H,8H-7-thia-1,5,10-triaza-acephenantrylen-6-one (18). Red amorphous solid (22 mg, 40%), polarity gradient from (EtOAc/Hexane 1.1) to (EtOAc/MeOH, 8:2 with Et_3N 2%); ^1H NMR (400 MHz, CD_3OD) δ 2.70 (t, $J = 7.7$ Hz, 2H), 2.92 (t, $J = 4.7$ Hz, 2H); 3.70 (t, 4.7 Hz, 2H),3.98 (s, 3H), 4.10 (t, 2H), 5.50 (s, 1H), 6.51 (s, 1H); ESI positive MS Anal. Calcd for $C_{13}H_{13}N_3OS+H^+$ 260.0852. Found: 260.0847.

4.2.10. 1,3,4,8-Tetrahydro-2′H-spiro[cyclohexa[2.5]diene-1-methyl-8-oxopyrrolo[4,3,2-*de*][1,7]phen-antrolyne-12,3′-dione (19). To a solution of **14** (73 mg, 0.23 mmol) in CF_3CH_2OH (4 mL) a solution of PIFA (phenyliodine bis(trifluoroacetate) (147 mg, 0.34 mmol) in CF_3CH_2OH (4 mL) was added at 0 °C. The mixture was stirred for 2 h and then treated with Na_2CO_3 (10%). The mixture was extracted with CH_2Cl_2. Purification by flash chromatography (AcOEt/MeOH 8:2 with Et_3N 3%) gave **19** (35 mg, 47%) as an amorphous brown solid; ^1H NMR (400 MHz, CD_3OD) δ 1.92–1.98 (m, 2 H), 2.73 (t, $J = 7.3$ Hz, 2H), 3.60–3.70 (m, 2H), 3.67–3.73 (m, 2H), 3.95 (s, 3H), 6.25 (d, $J = 10.0$ Hz, 2H), 7.10 (s, 1H), 7.20 (d, $J = 10.0$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) (APT): δ 20.0, 36.5, 36.9, 40.5, 48.0, 48.3, 97.1, 120.3, 122.9, 123.5, 130.1, 130.2, 131.1, 131.2, 133.0, 151.0, 154.1, 165.1, 181.0; MS (APCI): m/z: 320.2 $[M+H]^+$; ESI positive MS Anal. Calcd for $C_{19}H_{17}N_3O_2+H^+$ 320.13935. Found: 320.13940.

4.3. Cell cultures

The NCI-H460 human large cell lung carcinoma cell line (ATCC HTB 177) was cultured in RPMI-1640 (Sigma Chemical Co.) containing 10% fetal calf serum (FCS, Biological Industries). The HeLa human cervical carcinoma (ATCC CCL2) cells were grown in Nutrient Mixture F-12 Ham's (Sigma Chemical Co.) with 10% heat-inactivated FCS. HL-60 human promyelocytic leukemia cells were cultured in RPMI 1640 with 15% heat-inactivated FCS. 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (all Sigma Chemical Co.) were added to media for HeLa and HL-60 cell lines.

4.4. Inhibition growth assays

For cell proliferation assay, cells in the logarithmic phase of growth were harvested and seeded in duplicate into six-well plates. Twenty-four hours after seeding, cells were exposed to the test compound and harvested 72 h after exposure and counted. For NCI-H460, 8×10^4 cells/well were seeded, and the final counting was performed with a Coulter counter model ZM. For HeLa and HL-60, 4×10^4 cells/well were seeded in duplicate into 24-well plates and a Trypan blue assay was performed to determine cell viability. Cytotoxicity data are expressed as IC_{50} values, that is, the concentration (in μM) of the test agent inducing 50% reduction in cell number compared with untreated control cultures. All compounds are insoluble in water and were dissolved in DMSO prior to dilution into the biological assay, being the final concentration of DMSO at a maximum of 1%.

4.4.1. DNA topoisomerase II relaxation assay. DNA topoisomerase II activity was assayed by the relaxation of a supercoiled DNA substrate. In detail, 0.25 μg pBR322 plasmid DNA (Fermentas Life Sciences) was incubated with 1U topoisomerase II (USB) and test compound as indicated for 60 min at 37 °C in 20 μL reaction buffer consisting of 10 mM Tris–HCl (pH = 7.9), 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/mL BSA and 1 mM ATP.

Reaction was stopped by adding 4 μL stop buffer (5% SDS, 0.125% bromophenol blue, and 25% glycerol), 50 μg/mL proteinase K (Sigma Chemical) and incubating for further 30 min at 37 °C. The samples were separated by electrophoresis on a 1% agarose gel. The gel was stained with ethidium bromide 1 μg/ml in TAE buffer, transilluminated by UV light and fluorescence emission visualized using a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.

4.4.2. DNA isolation and electrophoresis. HeLa (5×10^5) cells were incubated in standard conditions for 24 h at 37 °C, then the medium was replaced with an equal volume of fresh medium, and the test agent was added at the indicated concentration. Control cells were grown under the same conditions with the addition of the solvent alone. After 24 h of incubation, DNA from control and treated cells was extracted according to the procedure described by Sambrook et al.[38] The isolated DNA was dissolved in TE buffer (Tris 10 mM, pH = 8, EDTA 1 mM) and analysed by agarose (1%) gel electrophoresis. The gel was stained with ethidium bromide solution (1 μg/mL), transilluminated by UV light, and fluorescence emission visualized using a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.11.063.

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