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New water soluble pyrroloquinoline derivatives as new potential anticancer agents

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Abstract—A new class of water soluble 3*H*-pyrrolo[3,2-*f*]quinoline derivatives has been synthesized and investigated as potential anticancer drugs. Water solubility profiles have been used to select the most promising derivatives. The novel compound **10**, having two (2-diethylamino-ethyl) side chains linked through positions 3N and 9O, presents a suitable water solubility profile, and it was shown to exhibit cell growth inhibitory properties when tested against the in-house panel of cell lines, in particular those obtained from melanoma.

1. Introduction

Medicinal chemistry is paying increasing attention to the development of methods for the synthesis of condensed heterocyclic structures containing indole and pyridine fragments (pyrrole and quinoline). The interest in such compounds is due to the prospect of seeking new biologically active substances, since their molecules contain two widely known pharmacophoric fragments. In this connection, a new direction has been initiated in the synthesis and study of pyrroloquinolines and the structural analogs of such well-known natural compounds as the alkaloid vomipyrine and the coenzyme of certain bacterial and animal dehydrogenases—methoxanthine (PQQ). Many of the recently obtained pyrroloquinolines exhibit a clearly defined physiological activity.[1–6]

In our ongoing search for new potential anticancer agents, we recently reported a new ring-forming method for the synthesis of cytotoxic pyrroloquinoline derivatives. In particular, we synthesized and investigated new derivatives, characterized by an angular 3*H*-pyrrolo[3,2-*f*]quinoline planar nucleus connected to the methanesulfon-anisidide residue characteristic of the known anticancer drug *m*-AMSA[7,8] or connected to the aniline bifunctional mustard from known therapeutic alkylating chlorambucil and mephalan.[9] Interestingly, they were remarkably active against cell lines deriving from solid tumors like CNS-, melanoma-, and prostate-derived cells.[7–9] However, besides their potential interest as antiproliferative agents, the low water solubility of these pyrroloquinolines limits their further development as potential drug candidates. To further investigate the antiproliferative potential of the pyrroloquinoline family and simultaneously improve their solubility, in this paper we describe the synthesis and cell growth inhibitory activity of pyrrolo[3,2-*f*]quinoline derivatives to which the (2-diethylamino-ethyl) side chain is linked through positions N3 and O9 (Scheme 1). Finally, water solubility profiles have been used to select the most promising derivatives.

2. Results and discussion

2.1. Chemistry

Conventional organic reaction schemes were utilized for the synthesis of 4-alkyloxy functionalized tricyclic target compounds. On the basis of our previous experience on pyrroloquinoline synthesis,[7–9] the synthetic strategy was first to construct the indole nucleus, then to complete the structure by cyclization of the pyridine ring, and finally

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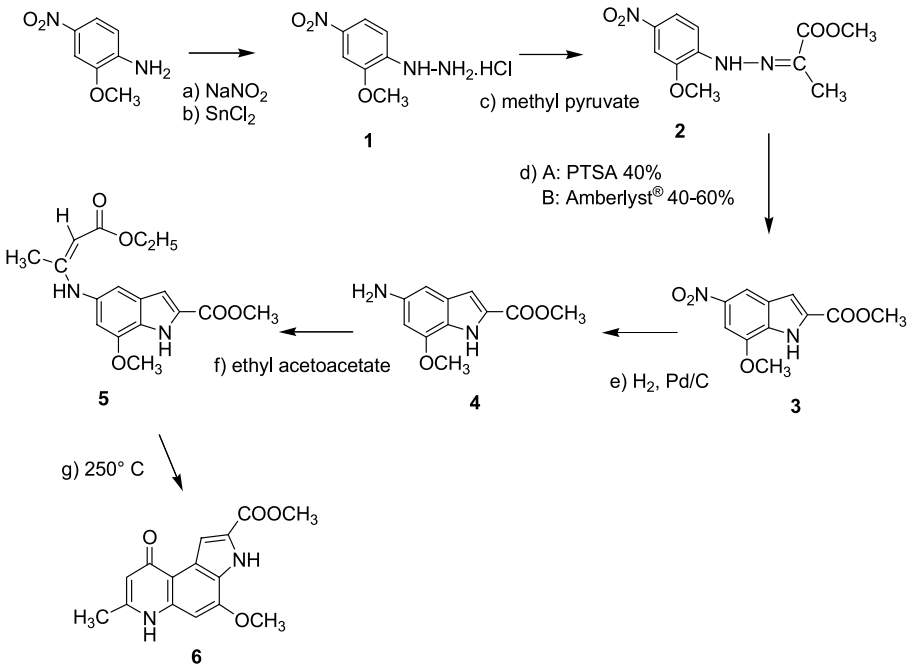
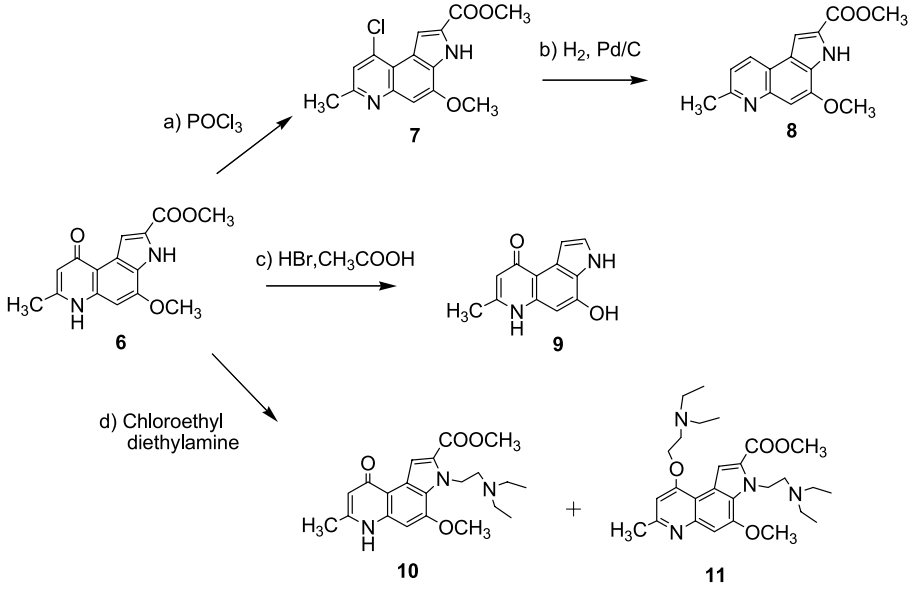


Scheme 1. Synthetic route to synthetize pyrroloquinolin-9-one derivative **6**.

to functionalize the obtained pyrroloquinolinone derivative with appropriate side chain (Schemes 1 and 2).

As shown in Scheme 1, we started from commercial 2-methoxy-4-nitroaniline, which was transformed into the corresponding hydrazine chloride **1** by formation of the diazonium salt and successive reduction by $SnCl_2{\cdot}2H_2O$ (Scheme 1).[10] Hydrazine derivative was condensed with methyl pyruvate to give hydrazone **2** and the latter was cyclized to indole compound **3** by modified Fischer indolization conditions following two previously reported methods:[11] method A consisting of cyclization by PTSA (*p*-toluenesulfonic acid) and method B by ion-exchange resin, Amberlyst®, in aprotic media (toluene). Both methods were much more advantageous (yield of 40 and 60%, respectively) than the one described by Zhang et al.[10] for the similar nitro-indole, $ZnCl_2$ in nitrobenzene, 8%. Particularly, method B was the easiest to work up (see Section 4). The herein described synthetic pathway to indole **3** gave a global yield similar to the one obtained by a recently reported very efficient synthesis of 5-nitro-7-methoxy-indole (60%).[12]

With the aim to avoid the poly-alkylation of the final pyrroloquinoline nucleus and the formation of a com-



Scheme 2. Synthetic route to synthetize the new water soluble pyrroloquinolines.

plex mixture difficult to separate, we had planned to functionalize the nitro-indole **3** at the 4 position after demethoxylation by HBr 48%, but this reaction did not give any result (the attempts are not reported). After catalytic reduction of the nitro-indole **3** to the amino-derivative **4**, this was condensed with ethylacetoacetate to furnish the enamine compound **5**, which was cyclized to 2-carboxylic acid methylester-4-methoxy-7-methyl-pyrrolo[3,2-*f*]quinolin-9-one (**6**). The angular structure of **6** was confirmed by 1-D and 2-D NMR experiments: the ^{1}H NMR showed three aromatic singlets at δ 8.10, 6.80, and 5.91 (each integrating for 1H) that were assigned to protons at positions 1, 5, and 8, respectively. Three more singlets were observed at δ 3.99, 3.85, and 2.31 (three protons each) suggesting the presence of two methoxy groups and a methyl group. Complete assignments were deduced on the basis of the ^{13}C NMR data and both HMQC and HMBC spectra. Diagnostic HMBC correlations were observed between the proton signal at δ 5.91 (H-8) and the carbon resonance at δ 112.0 (C-9a), 146.0 (C-7), and 19.2 (7-CH_3), and between the proton signal at δ 6.80 (H-5) and the carbons at δ 112.0 (C-9a), 138.8 (C-5a), 150.1 (C-4), and 127.5 (C-3a). Further data were obtained from a NOESY experiment. NOESY correlations were observed between the methyl signal in position 7 (δ 2.31) and the proton signal at δ 5.91 (H-8), and between the signal at δ 6.80 (H-5) and the methoxy signal at δ 3.99 (4-OCH_3) (Fig. 1).

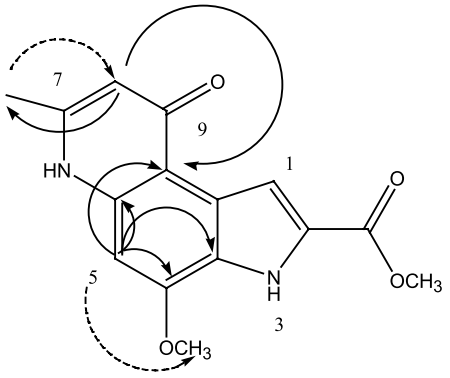


Figure 1. Diagnostic HMBC (solid line) and NOESY (dashed line) correlations for compound **5**.

In Scheme 2, treatment of **6** with $POCl_3$ producing the 9-chloro-derivative **7** was followed by hydrogenolysis yielding the pyrroloquinoline **8**, which was reacted with HBr 48% and acetic acid at refluxing with the aim to obtain the demethoxylation of 4-methoxy-group but without any result.

The same demethoxylation conditions were applied to compound **6**, obtaining the 4-hydroxy-7-methyl-9-oxo-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinoline (**9**) in very small yield. Compound **8** was lacking in the carboxylate group at the 2 position as shown by spectral data and HR MS analysis. Compound **9**, when submitted to alkylation reaction by chloroethyldiethylamine, did not give any isolable product. Finally, the treatment of **6** with (2-chloro-ethyl)-diethyl-amine gave a mixture of substituted pyrroloquinolines from which compounds **10** and **11** were isolated by Flash Chromatography and identified, respectively, as 3N-mono- and 3N,9-bis-functionalized derivatives by means of HR MS, ^{1}H, and ^{13}C NMR spectroscopy.

2.2. Cytotoxicity

The newly synthesized pyrroloquinolines were examined for their cytotoxic properties in a panel of six human tumor cell lines containing examples of ovarian (2008), leukemia (HL60), cervix (A431), lung (A549), breast (MCF-7) cancer, and melanoma (A375). For comparison purpose, the cytotoxicity of amsacrine and ellipticine was evaluated under the same experimental conditions. IC_{50} values, calculated from the dose–survival curves obtained after 48 h drug treatment from MTT test, are shown in Table 1. Compounds **5**, **6**, **7**, and **9** were quite ineffective in all tumor cell lines. However, derivative **10** showed an evident growth inhibitory potency that was concentration-dependent (data not shown) and with IC_{50} values ranging from 4.78 to 28.28 μM. Noticeably, in HL60 cells, the cytotoxicity of the new derivative exceeded those of the parent drugs by a factor of about 2. Moreover, it is interesting to underline that derivative **10** exhibits cell growth inhibitory properties (in the low micromolar range, see Table 1) against the melanoma cell line. We can consider derivative **10** as a potential lead compound to develop new potential drugs against melanoma and other cutaneous malignancies. Chemically speaking, we can speculate

Table 1. Cell growth inhibition in the presence of newly synthesized pyrroloquinolines

Compound	IC_{50} (μM) ± SD					
	2008	HL60	A431	A549	A375	MCF-7
5	>100	>100	>100	>100	>100	>100
6	>100	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100	>100
8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9	>100	>100	>100	>100	>100	>100
10	15.88 ± 1.92	4.78 ± 1.06	28.28 ± 1.96	10.12 ± 1.17	6.23 ± 1.03	22.38 ± 2.76
Ellipticine	2.70 ± 0.69	8.79 ± 1.23	5.67 ± 1.16	1.33 ± 0.50	0.90 ± 0.12	1.81 ± 0.12
Amsacrine	8.21 ± 0.74	8.09 ± 0.55	7.67 ± 1.63	8.27 ± 0.51	2.90 ± 0.13	7.80 ± 0.15

SD = standard deviation; IC_{50} values were calculated by probit analysis ($P < 0.05$, χ^2 test). Cells ($5–3 \times 10^4$ ml^{-1}) were treated for 48 h with increasing concentrations (3125–100 μM) of tested compounds. Cytotoxicity was assessed by MTT test; n.d., not detectable.

that the increased basicity due to the presence of the two protonable (2-diethylamino-ethyl) side chains at physiological pH, coupled with the possibility of positional differences in proton exchange ability, might affect a number of factors such as membrane penetration ability and/or target-binding potential.

2.3. Water solubility profile

As already anticipated, the low water solubility of all previously synthesized pyrroloquinolines limits their further development as potential drug candidates. In the present study, we decided to analyze the water solubility profile of all new pyrroloquinolines selecting those with the best compromise between antiproliferative activity and appropriate water solubility profile. In principle, the aqueous solubility of a chemical compound may be defined as the maximum amount of this chemical that dissolves in water at a specified temperature and pH. Water solubility profiles have been calculated by using ACD/Solubility DB suite at 25 °C for pH values from 0.0 to 14.0.[13]

Interestingly, we found that pyrroloquinoline **10** is the compound that had shown the highest antiproliferative activities against our cell line panel and the best water solubility profile, as reported in Figure 2. In Table 2, we collect the calculated water solubility at 25 °C and pH 7.4 for all newly synthesized pyrroloquinolines.

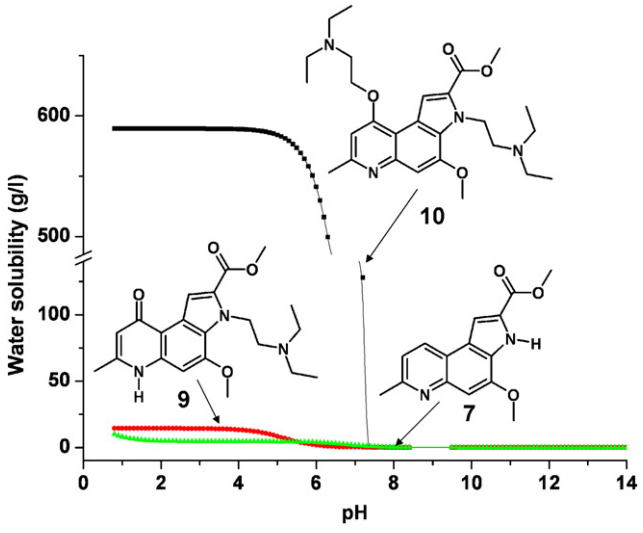


Figure 2. Water solubility profile calculated for the most active pyrroloquinolines.

Table 2. Calculated water solubility at pH 7.4 for the newly synthesized pyrroloquinolines

Compound	Solubility (g/l)
5	0.009
6	0.011
7	0.060
8	0.020
9	0.870
10	57.4

From data reported in Table 2, it is clear that the presence of only one (2-diethylamino-ethyl) side chain is not enough to guarantee an adequate water solubility at physiological pH 7.4.

3. Conclusion

A convenient route has been developed for the synthesis of new pyrroloquinoline derivative **10** which should enhance solubility to heterocycles of this type. The cytotoxicity results for the new derivative **10** appear to confirm that cytotoxicity is enhanced when the water solubility profile is optimized. Considering its cytotoxicity profile, we can also consider derivative **10** as a potential lead compound to develop new potential drugs against melanoma and other cutaneous malignancies. Further investigations are running in our laboratory to improve the antiproliferative activity of this new class of pyrroloquinolines and to clarify the possible mechanism of action.

4. Materials and methods

4.1. Chemistry

Melting points were determined on a Gallenkamp MFB 595 010M/B capillary melting point apparatus, and are not corrected. Infrared spectra were recorded on a Perkin-Elmer 1760 FTIR spectrometer as potassium bromide pressed disks; values are expressed in cm^{-1}. UV–vis spectra were recorded on a Perkin-Elmer Lambda UV/VIS spectrometer. ^1H NMR spectra were recorded on Varian Gemini (200 MHz) and Bruker (300 MHz) spectrometers, using the indicated solvents; chemical shifts are reported in δ (ppm) downfield from tetramethylsilane as internal reference. Coupling constants are given in Hertz. In the case of multiplets, the chemical shift quoted was measured from the approximate center. Integrals corresponded satisfactorily to those expected on the basis of compound structure. Elemental analyses were performed in the Microanalytical Laboratory of the Department of Pharmaceutical Sciences of the University of Padova, using a Perkin-Elmer Elemental Analyzer Model 240B; results fell in the range ±0.4% with respect to calculated values. Mass spectra were obtained with a Mat 112 Varian Mat Bremen (70Ev) mass spectrometer and Applied Biosystems Mariner System 5220 LC/MS (nozzle potential 250.00). Column Flash Chromatography was carried out on Merck silica gel (250–400 mesh ASTM); reactions were monitored by analytical thin-layer chromatography (TLC) using Merck silica gel 60 F-254 glass plates and using dichloromethane/methanol 9:1 mixture as eluant; differences are indicated in the text.

Starting materials were purchased from Aldrich Chimica; solvents were from Carlo Erba, Fluka, and Lab-scan.

4.1.1. 2-Methoxy-4-nitro-phenylhydrazine hydrochloride (1). This compound was prepared from 2-methoxy-4-nitro-aniline according to the procedure of Bergmann

and Hoffmann.[14] 2-Methoxy-4-nitro-aniline (2.000 g, 11.90 mmol) was dissolved in HCl 37% (175 ml) by stirring and heating. To the yellow solution, water (100 ml) was added until it became about 4–5 N in HCl and then it was cooled to 0–4 °C in an ice bath. Cold $NaNO_2$ (0.057 g, 0.82 mmol) water solution was slowly dropped into the stirred mixture. $SnCl_2{\cdot}2H_2O$ (13.425 g, 59.50 mmol) in a minimum of HCl concd was added dropwise avoiding any increase of temperature over 4 °C. A light pink foamy precipitate of hydrazine chloride formed which, after a night at 0 °C, was collected, dried, and used without any purification for preparing hydrazones **2**. Yield 85%; mp 198–201 °C; rf 0.83 (*n*-hexane/ethyl acetate 1:1); for the corresponding hydrazine base ^{1}H NMR (DMSO-d_6) δ: 4.41 (br s, 2H, NH_2), 7.02 (d, 1H, J = 8.9 Hz, HC-6), 7.53 (d, 1H, J = 2.3 Hz, HC-3), 7.74 (br s, 1H, NH), 7.87 (dd, 1H, J = 8.9 and 2.3 Hz, HC-5).

4.1.2. 2-[(2-Methoxy-4-nitro-phenyl)-hydrazono]-propionic acid methyl ester (2). 2-Methoxy-4-nitro-phenylhydrazine chloride (1.296 g, 5.91 mmol) was dissolved in water, added of CH_3COONa (0.484 g, 5.91 mmol) and methyl pyruvate (0.603 g, 5.91 mmol) producing a color change from orange to light yellow. After 1 h of continuous stirring, the precipitate was collected by filtration and washed with water to obtain a yellow-brown solid, which was crystallized from ethanol obtaining the trans isomer. Yield 56%, mp 160–164 °C; rf 0.65 (*n*-hexane/ethyl acetate 1:1). ^{1}H NMR (DMSO-d_6) δ: 2.16 (s, 3H, CH_3), 3.77 (s, 3H, ester OCH_3), 4.00 (s, 3H, ether OCH_3), 8.58 (d, 1H, J = 8.5 Hz, HC-6), 7.80 (d, 1H, J = 2.5 Hz, HC-3), 7.85 (dd, 1H, J = 2.5 Hz, J = 8.5 Hz, HC-5), 9.08 (br s, 1H, NH). The mixture of both isomers is used for the following synthetic step.

4.1.3. 7-Methoxy-5-nitro-1*H*-indole-2-carboxylic acid methyl ester (3).
Method A: In a three-necked flask, a mixture of toluene and PTSA monohydrate (8.000 g, 42.06 mmol) was dehydrated using a Marcusson apparatus and then a solution of the hydrazone **2** (4.071 g, 15.23 mmol) in anhydrous toluene was added. The mixture was refluxed for 3 h, the end of reaction being checked by TLC (*n*-hexane/ethyl acetate 1:1). The solvent was evaporated, the residue was added of water, and the suspension was extracted with ethyl acetate. The organic layer was washed with $NaHCO_3$ 5% and saturated aqueous NaCl, dried over Na_2SO_4, and the solvent was evaporated. The residue was chromatographed twice on silica gel eluting at first with *n*-hexane/ethyl acetate 1:1 and then with chloroform/methanol 99:1 mixture, obtaining pure 7-methoxy-5-nitro-1*H*-indole-2-carboxylic acid methyl ester (**3**). Yield 40%; mp 205–210 °C (from ethanol); rf 0.74 (*n*-hexane/ethyl acetate 1:1). Spectral data were consistent with the literature for the ethyl ester:[10] ^{1}H NMR (DMSO-d_6) δ: 3.89 (s, 3H, ester OCH_3), 4.04 (s, 3H, ether OCH_3), 7.43 (d, 1H, J = 2.1 Hz, HC-3), 7.56 (d, 1H, J = 1.9 Hz, HC-6), 8.37 (d, 1H, J = 1.9 Hz, HC-4), and 12.75 (br s, 1H, NH).

Method B: A mixture of hydrazone **2** (1.000 g, 3.74 mmol) in dry toluene and Amberlyst® (10.000 g) was heated at 115 °C for the time needed for disappearance of the starting material (2–4 h, TLC). The resin was filtered off and washed repeatedly with ethyl acetate. The solvent was evaporated and the residue, 7-methoxy-5-nitro-1*H*-indole-2-carboxylic acid methyl ester (**3**), was pure enough to proceed with synthesis. Yield 60%.

4.1.4. 5-(2-Ethoxycarbonyl-1-methyl-vinylamino)-7-methoxy-1*H*-indole-2-carboxylic acid methyl ester (5). Into a suspension of 10% Pd/C (0.125 g) saturated with H_2 in ethanol (200 ml), a solution of nitro-indole **3** (1.469 g, 5.87 mmol) in ethanol (400 ml) was dropped. The mixture was stirred at room temperature and in the presence of hydrogen at atmospheric pressure for 3 h. The catalyst was filtered off and the solvent was evaporated under reduced pressure to give the corresponding amino-indole (**4**) as greenish solid (96%). This product (0.400 g, 1.42 mmol) was directly condensed with ethyl acetocetate (0.360 g, 2.73 mmol) in absolute ethanol in the presence of catalytic amount of acetic acid and Drierite (0.200 g). The mixture was refluxed for 5–6 h, checking the end of reaction by TLC (*n*-hexane/ethyl acetate 1:1). The solid was filtered off and the solvent was evaporated to dryness to yield a brown residue, which was crystallized from MeOH. Yield 74%; mp 128–131 °C; rf 0.75 (*n*-hexane/ethyl acetate 1:1); IR (KBr) 3437, 1625 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.19 (t, 3H, J = 7.3 Hz, CH_3), 1.96 (br s, 3H, CH_3), 3.75 (q, 2H, J = 7.3 Hz, CH_2), 3.84 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 4.63 (q, 1H, J = 0.8 Hz, HC=), 6.63 (d, 1H, J = 1.9 Hz, HC-6), 7.04 (d, 1H, J = 1.5 Hz, HC-4), 7.10 (d, 1H, J = 2.1 Hz, HC-3), 10.35 (s, 1H, NH), 12.01 (br s, 1H, NH); HR MS [MH^+] 333.1481.

4.1.5. 4-Methoxy-7-methyl-9-oxo-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinoline-2-carboxylic acid methyl ester (6). In a two-necked round-bottomed flask, 50 ml of diphenyl ether was heated at boiling temperature and then 5-(2-ethoxycarbonyl-1-methyl-vinylamino)-7-methoxy-1*H*-indole-2-carboxylic acid methyl ester (**5**) (0.332 g, 1.00 mmol) was added portionwise and the mixture was maintained at reflux for 30 min. After cooling to 60 °C, the separated solid was collected by filtration and washed many times with diethyl ether. The product was purified by flash chromatography (ethyl acetate/methanol 9:1). Yield 54%; mp 287–290 °C; rf 0.61 (chloroform/methanol 8:2); IR (KBr): 3425, 3217, 1717, 1633 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 2.31 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 5.91 (s, 1H, HC-8), 6.80 (s, 1H, HC-5), 8.10 (d, 1H, J = 2.1 Hz, HC-1), 11.37 (br s, 1H, pyrrole NH), 12.16 (br s, 1H, quinoline NH); ^{13}C NMR (DMSO-d_6) δ: 19.2 (CH_3), 51.8 (OCH_3), 55.1 (OCH_3), 94.4 (C-5), 109.8 (C-8), 111.1 (C-1), 112.0 (C-9a), 121.8 (C-9b), 127.5 (C-3a), 128.6 (C-2), 138.8 (C-5a), 146.0 (C-7), 150.1 (C-4), 161.6 (C=O), 202.7 (C-9); HR MS [MH^+] 287.2281. Anal. Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found: C, 62.75; H, 5.05; N, 9.60.

4.1.6. 9-Chloro-7-methyl-4-methoxy-3*H*-pyrrolo[3,2-*f*]-quinoline-2-carboxylic acid methyl ester (7). A mixture of **6** (0.500 g, 2.32 mmol) and $POCl_3$ (1.5 ml) was refluxed for 1 h (TLC), and after cooling it was poured into an ice/water under stirring. The solution was made

alkaline with 28% NH_4OH and the precipitate formed was collected by filtration, washed with water, and dried, obtaining 9-chloro-7-methyl-4-methoxy-3*H*-pyrrolo[3,2-*f*]quinoline-2-carboxylic acid methyl ester (**7**); yield 87%; mp >300 °C; IR (KBr): 3394, 1712 cm^{-1}; rf 0.95 (chloroform/methanol 8:2); ^1H NMR (DMSO-d_6) δ: 2.61 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3), 7.24 (s, 1H, HC-8), 7.50 (s, 1H, HC-5), 8.09 (d, 1H, $J = 2.1$ Hz, HC-1), 12.84 (br s, 1H, indolic NH); HR MS [MH^+] 305.0712. Anal. Calcd for $C_{15}H_{13}N_2O_3Cl$: C, 59.12; H, 4.30; N, 9.19; Cl, 11.63. Found: C, 59.41; H, 4.55; N, 8.98, Cl, 11.68.

4.1.7. 7-Methyl-4-methoxy-3*H*-pyrrolo[3,2-*f*]quinoline-2-carboxylic acid methyl ester (8). Into a suspension in ethanol (200 ml) of 10% Pd/C (100 mg) saturated with H_2, the chloro-derivative **7** (0.200 g, 0.66 mmol) in ethanol (100 ml) was dropped. The mixture was stirred at room temperature and in the presence of hydrogen at atmospheric pressure for 10 h. The catalyst was removed and the solvent was evaporated at reduced pressure and room temperature to give a brown row material, which was crystallized from chloroform/methanol mixture (7:3) to give 7-methyl-4-methoxy-3*H*-pyrrolo[3,2-*f*]quinoline-2-carboxylic acid methyl ester (**8**); yield 76%; mp 230–232 °C; IR (KBr): 3392, 1711 cm^{-1}; rf 0.72 (chloroform/methanol 8:2); ^1H NMR (DMSO-d_6) δ: 2.87 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.15 (s, 3H, OCH_3), 7.43 (s, 1H, HC-5), 7.80 (d, 1H, $J = 8.2$ Hz, HC-8), 8.06 (d, 1H, $J = 2.1$ Hz, HC-1), 9.29 (d, 1H, $J = 8.3$ Hz, HC-9), 13.20 (b s, 1H, indolic NH); HR MS [MH^+] 271.0654. Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.50; H, 5.35; N, 10.01.

4.1.8. 4-Hydroxy-7-methyl-9-oxo-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinoline (9). A solution of **6** (0.330 g, 1.21 mmol) in HBr 48% (45 ml) and acetic acid (21 ml) was heated at 70 °C for 3 h and then at refluxing for 12 h. After evaporation of the solvent, the residue was taken up with water (30 ml) and made neutral with NH_4OH 28%. By cooling at 0 °C a yellow precipitate formed, which was collected by filtration and crystallized from $CHCl_3$/MeOH mixture (8:2) obtaining 4-hydroxy-7-methyl-9-oxo-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinoline (**9**); yield 12%; mp >300 °C; rf 0.41 (chloroform/methanol 8:2); IR (KBr) 3402, 3196, 1619 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.33 (s, 3H, CH_3), 5.79 (s, 1H, HC-8), 6.61 (s, 1H, HC-5), 7.25 (dd, 1H, $J = 2.1$ and 2.6 Hz, HC-1), 7.40 (dd, 1H, $J = 2.6$ and 2.9 Hz, HC-2), 11.15 (br s, 1H, pyrrole NH), 11.28 (br s, 1H, quinoline NH); ^{13}C NMR (DMSO-d_6): 19.29 (CH_3), 93.4 (C-5), 105.8 (C-1), 107.3 (C-8), 107.7 (C-9a), 112.3 (C-3a), 121.8 (C-9b), 125.0 (C-2), 137.1 (C-5a), 147.3 (C-7), 148.9 (C-4), 202.3 (C-9); HR MS [MH^+] 215.0802. Anal. Calcd for $C_{12}H_{10}N_2O_2$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.15; H, 4.91; N, 12.79.

4.1.9. 3-(2-Diethylamino-ethyl)-pyrroloquinoline derivatives 10 and 11. To a solution of pyrroloquinolinone **6** (0.200 g, 0.69 mmol) in 10 ml DMF, K_2CO_3 (0.480 g, 3.47 mmol) was added and the mixture was stirred for 15 min at room temperature and then heated at 70 °C for 3 h, until the starting material disappeared. Ice was added and the mixture was extracted with chloroform (20 ml for three times). The solvent was evaporated from the pooled extracts and the residue was chromatographed by flash chromatography (chloroform/methanol 9:1).

4.1.10. 3*N*-(2-Diethylamino-ethyl)-4-methoxy-7-methyl-9-oxo-6,9-dihydro-3*H*-pyrrolo[3,2-*f*]quinoline (10). Yield 76 mg (28%); rf 0.15; mp 298–300 °C; IR (KBr) 3420, 1718, 1210 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.00 (t, $J = 7.0$ Hz, 2× CH_3), 2.35 (s, 3H, CH_3), 2.63 (q, 4H, $J = 7.1$ Hz, 2× CH_2), 2.8 (t, 2H, $J = 7.0$ Hz, N–CH_2), 3.88 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 5.04 (t, 2H, $J = 7.0$ Hz, N–CH_2), 6.02 (s, 1H, HC-8), 6.57 (s, 1H, HC-5), 8.56 (s, 1H, HC-1); ^{13}C NMR ($CDCl_3$) δ: 162 (C═O), 202.5 (C-9); HR MS [MH^+] 387.2054. Anal. Calcd for $C_{21}H_{27}N_3O_4$: C, 65.44; H, 7.06; N, 10.90. Found: C, 65.28; H, 7.24; N, 10.77.

4.1.11. Diethyl-[3*N*-(2-diethylamino-ethyl)-4-methoxy-7-methyl-3*H*-pyrrolo[3,2-*f*]quinolin-9-yloxy-ethyl]-amine (11). Yield 16 mg (4%); rf 0.35; mp >300 °C; IR (KBr) 1716, 1210 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.03 (t, 6H, $J = 7.3$ Hz, 2× CH_3), 1.12 (t, 6H, $J = 7.3$ Hz, 2× CH_3), 2.63 (q, 4H, $J = 7.5$ Hz, 2× CH_2), 2.66 (s, 3H, CH_3), 2.71 (q, 4H, $J = 7.2$ Hz, 2× CH_2), 2.82 (t, 2H, $J = 7.3$ Hz, N–CH_2), 3.11 (t, 2H, $J = 7.3$ Hz, N–CH_2), 3.9 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 4.32 (t, 2H, OCH_2), 5.07 (t, 2H, $J = 7.8$ Hz, N–CH_2), 6.69 (s, 1H, HC-5), 7.17 (s, 1H, HC-8), 8.13 (s, HC-1); ^{13}C NMR ($CDCl_3$) δ: 202.5 (C-9); HR MS [MH^+] 485.59. Anal. Calcd for $C_{27}H_{40}N_4O_4$: C, 66.91; H, 8.32; N, 11.56. Found: C, 66.69; H, 8.56; N, 11.83.

4.2. Biological data

4.2.1. Chemicals. Compounds were dissolved in dimethylsulfoxide just before the experiments; calculated amounts of drug solution were added to the growth medium containing cells to a final solvent concentration of 0.5%, which had no discernible effect on cell killing.

MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, amsacrine, and ellipticine were obtained from Sigma Chemical Co., St. Louis, USA.

4.2.2. Cell cultures. A549 and MCF7 are human lung and breast carcinoma cell lines, respectively, which were obtained from ATCC (RocKville, MD) along with the melanoma (A375) and human promyelocytic leukemia (HL60) cell lines. The 2008 human ovarian cancer cell line was kindly provided by Prof. G. Marverti (Department of Biomedical Science of Modena University, Italy) and A431 human cervix carcinoma was kindly provided by Professor Zunino (Division of Experimental Oncology B, Istituto Nazionale dei Tumori, Milan, Italy).

Cell lines were maintained in logarithmic phase at 37 °C in a 5% carbon dioxide atmosphere using the following culture media: (i) RPMI-1640 medium (Euroclone, Celbio, Milan, Italy) containing 10% fetal calf serum (Biochrom-Seromed GmbH&Co., Berlin, Germany) and sup-

plemented with 25 mM Hepes buffer, L-glutamine, and with antibiotics penicillin (50 U ml^{-1}) and streptomycin (50 μg ml^{-1}) for HL60, 2008, A431, and MCF7 cells; (ii) F-12 Ham's (Sigma Chemical Co.) containing 15% fetal calf serum, penicillin (50 U ml^{-1}), and streptomycin (50 μg ml^{-1}) for A549 cells; (iii) DMEM (Dulbecco's modified Eagle's) medium (Euroclone) supplemented with 10% fetal calf serum (Euroclone), penicillin (50 U ml^{-1}), and streptomycin (50 μg ml^{-1}) for A375 cells.

4.2.3. Cytotoxicity assay. The growth inhibitory effect on tumor cell lines was evaluated by means of MTT (tetrazolium salt reduction) assay.[15] Briefly, between 3 and 8×10^{-3} cells, dependent upon the growth characteristics of the cell line, were seeded in 96-well microplates in growth medium (100 μl) and then incubated at 37 °C in a 5% carbon dioxide atmosphere. After 24 h, the medium was removed and replaced with a fresh one containing the compound to be studied at the appropriate concentrations (3125–100 μM). Quadruplicate cultures were established for each treatment. Forty-eight hours later, each well was treated with 10 μl of a 5 mg ml^{-1} MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) saline solution, and after 5 h of incubation, 100 μl of a sodium dodecyl sulfate (SDS) solution in HCl 0.01 M was added. After overnight incubation, the inhibition of cell growth induced by the tested complexes was detected by measuring the absorbance of each well at 540 nm using a Canberra-Packard microplate reader. Mean absorbance for each drug dose was expressed as a percentage of the control untreated well absorbance and plotted versus drug concentration. IC_{50} values represent the drug concentrations that reduced the mean absorbance at 540 nm to 50% of those in the untreated control wells.

4.3. Solubility profile

Water solubility profiles have been determined by using ACD/Solubility DB suite (ver 5.08).[13] All aqueous solubility has been calculated at 25 °C and referred at physiological pH (7.4).

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