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Pyrrolo-Quinoline Derivatives as Potential Antineoplastic Drugs

M. G. Ferlin, B. Gatto, G. Chiarelotto and M. Palumbo*

Department of Pharmaceutical Sciences, University of Padova, via Marzolo 5, 35131 Padova, Italy

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Abstract—Some novel pyrrolo-quinoline derivatives have been synthesized as potential antineoplastic agents. They contain an angular aromatic tricyclic or tetracyclic system, to which the methanesulfon-anisidide side chain typical of amsacrine as such, or lacking the *m*-methoxy substituent, is connected. A methyl group can be present at position 7 of the pyrrolo-quinoline ring. The novel compounds exhibit interesting cell growth inhibitory properties when tested against the NCI panel of cell lines, in particular those obtained from solid tumors like CNS-, melanoma- and prostate-derived cells. The mechanism of cytotoxic action does not seem to be related to topoisomerase II poisoning ability. Most active proved to be compound **4a**, which lacks both methyl and methoxy substituents, followed by **5a**, having the methoxy group only. Biological activity is less pronounced in the tetracyclic family of derivatives **6** and **7**.

Introduction

Well known cancer chemotherapeutic agents, such as anthracyclines, camptothecin and amsacrine, are characterized by planar polycyclic systems.[1] They are able to interfere with DNA-processing enzymes (topoisomerases I and II) by forming a ternary (cleavable) complex involving the drug, the DNA and the enzyme.[2,3] Although the molecular details of the ternary complex are still not fully characterized, a number of models are available for both topo I and topo II inhibition.[4–6] It is proposed that an active drug consists of two pharmacophoric domains, one forming appropriate contacts with the nucleic acid and the other binding to the enzyme.[7] Examining the structural determinants for optimal topoisomerase poisoning, we recently demonstrated that the pharmacophores must occupy well defined reciprocal positions within the drug. Indeed, moving one domain with reference to the other gave structural isomers completely differing in their ability of recognizing the cleavable complex and producing different biological and pharmacological responses.[8]

Continuing an investigation aimed at defining the basic rules that govern structure–activity relationships, we synthesized and investigated a number of new derivatives, characterized by an angular 9-anilino-3H-pyrrolo[3,2-f]quinoline planar nucleus connected to the methanesulfon-anisidide residue characteristic of the known anticancer drug *m*-AMSA, or its analogue lacking the *m*-methoxy substituent. The novel structures are presented in Figure 1.

*Corresponding author. Tel.: +39-049-827-5711; fax: +39-049-827-5366; e-mail: mpalumbo@purple.dsfarm.unipd.it

Results and Discussion

Chemistry

The preparation of the desired compounds was accomplished according to Schemes 1 and 2. Intermediates **1a** and **1b** (Scheme 1) were prepared adopting known methods for the synthesis of the quinoline ring such as the Conrad–Limpach[9] and the Gould–Jacobs reactions,[10] respectively. For both, the starting material 5-nitroindole was catalytically reduced to the corresponding amino derivative. In order to obtain the 7-methyl-9-hydroxy-pyrrolo[3,2-f]quinoline **1a**, the acid-catalyzed condensation with ethyl acetoacetate in refluxing ethanol yielded the enamine-derivative crotonate, which then underwent thermal cyclization in boiling phenyl ether. Likewise, condensation of equimolar amounts of 5-aminoindole with diethyl ethoxymethylene malonate yielded the corresponding enamine acrylate which, without requiring purification, was cyclized in boiling phenyl ether to produce exclusively 9-hydroxy-pyrrolo[3,2-f]quinoline ethyl ester as the result of a regioselective reaction.[11] Alkaline hydrolysis, followed by thermal decarboxylation afforded intermediate **1b**. Treatment of **1a,b** with phosphorus oxychloride allowed us to replace the hydroxyl group at C-9 with chlorine to yield **2a,b**, as shown in Scheme 2. Compounds **4a,b** and **5a,b** were obtained as the hydrochlorides after nucleophilic substitution of the chlorine at C-9 in **2a,b** with

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p-methanesulfonamido-aniline or *o*-methoxy-*p*-methanesulfonamido-aniline in refluxing ethanol–methanol mixture. Moreover, hydroxy derivatives **1a,b** were subjected to the Vilsmeier–Haach reaction with dimethylformamide and phosphorus oxychloride to provide the corresponding 9-chloro-1-formylate compounds **3a,b**. The final compounds **6a,b** and **7a,b**, in which the amino-group of the aniline moiety is incorporated into an indole-naphthyridine tetracyclic structure, were obtained as the mono-hydrochlorides by a one-step reaction consisting of a reductive alkylation of methanesulfonamido-anilines; at the same time due to a nucleophilic substitution of chlorine at C-9 the cyclization reaction occurred for probable favorable steric factors. Because the required *p*-methanesulfonamido-anilines were not available commercially, we prepared them by means of known strategies,[12] with just one significant modification. In fact, the carbamoyl ethyl ester protecting group was used instead of the acetyl function, which allowed milder reaction conditions and gave higher global yields.

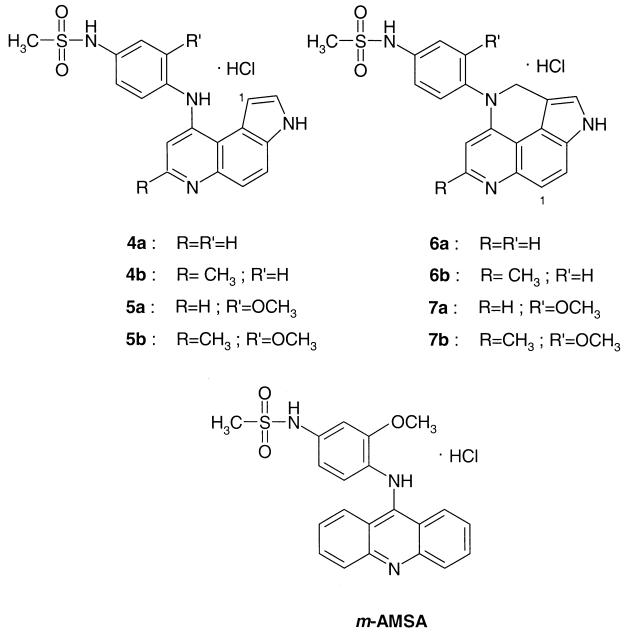
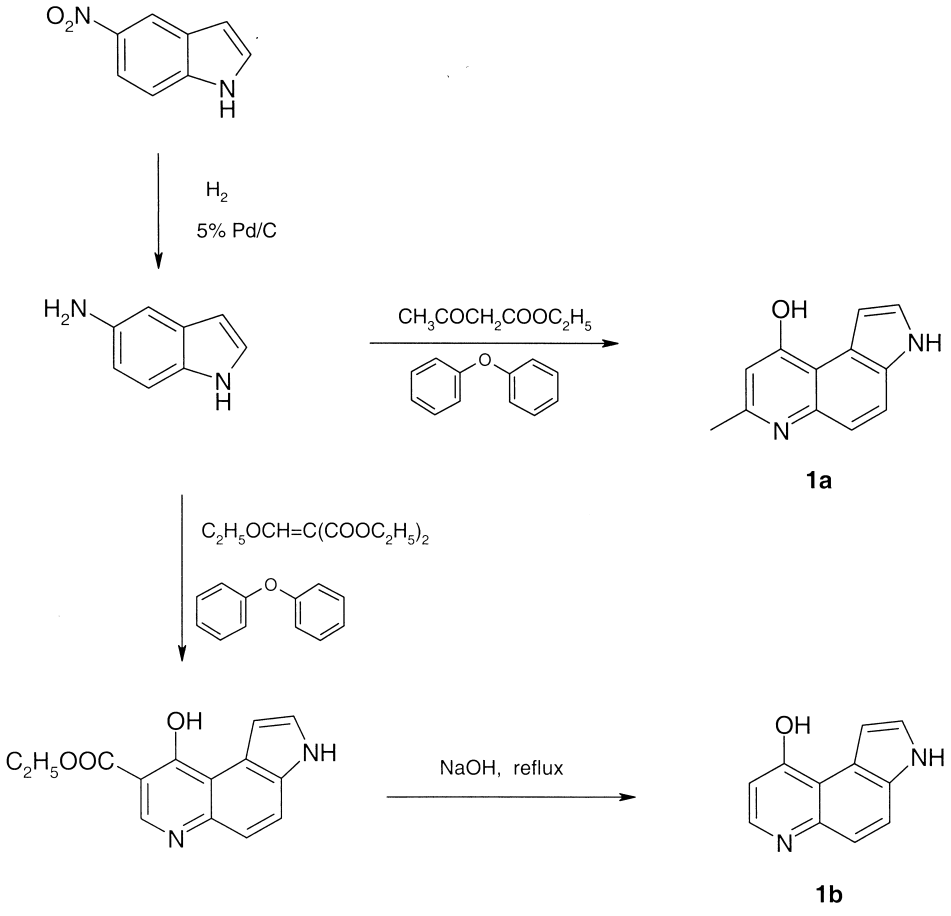


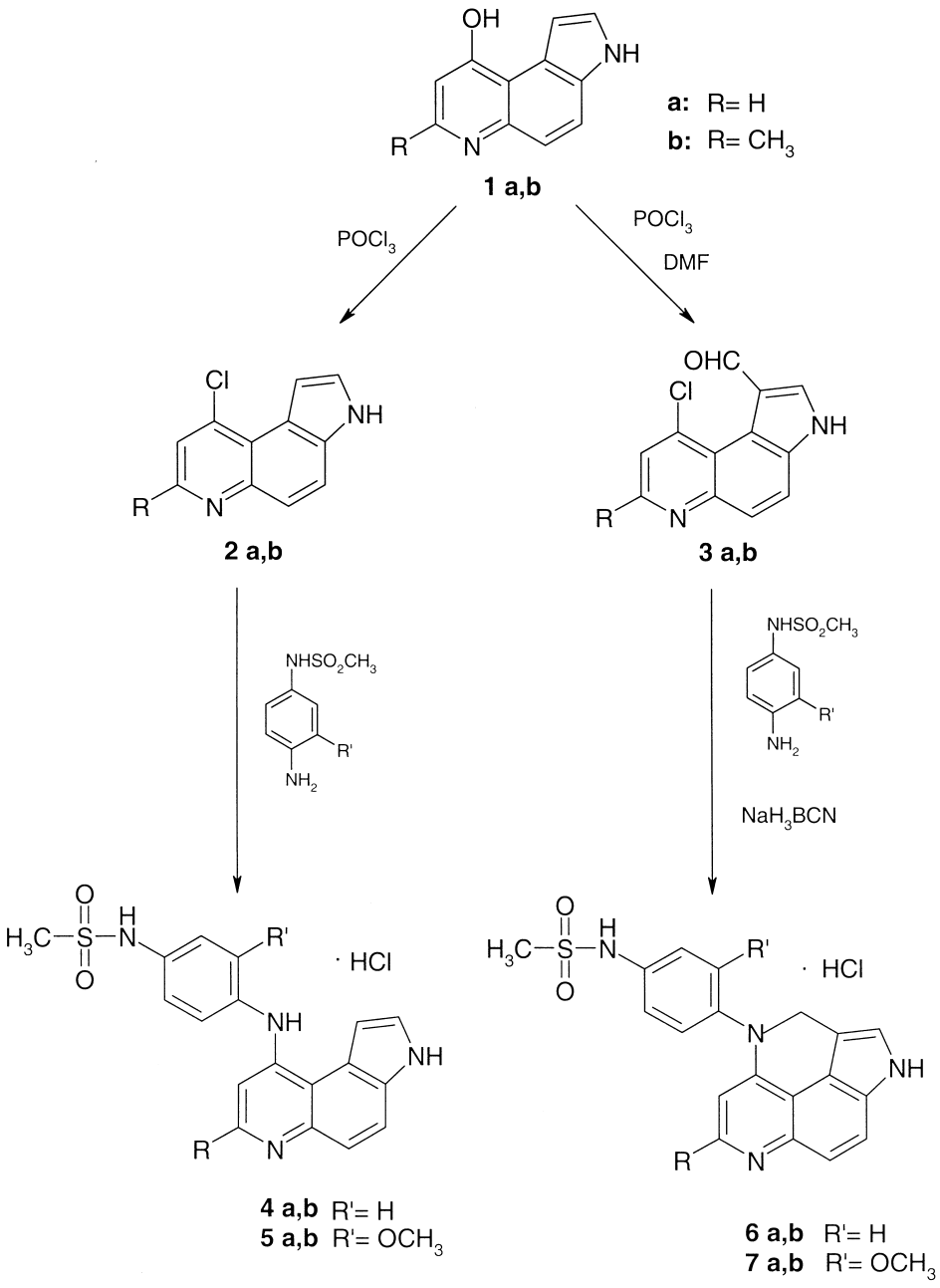
Figure 1. Chemical structure of the test pyrrolo-quinoline derivatives and *m*-AMSA.

Topoisomerase poisoning

The extent of DNA damage induced by the test drugs in the presence of human topoisomerase II α was evaluated by gel electrophoresis techniques.[13] While the well known drug *m*-AMSA produced the expected cleavage of the nucleic acid in a concentration dependent manner, the test compounds failed to show appreciable effects up to 100 μM, with the only exception of **4a**. This compound, as shown in Figure 2, exhibits a slight increase of topo II-mediated DNA cleavage at 10 μM



Scheme 1.



Scheme 2.

concentration (compare with lane showing the background cleavage of topoisomerase II alone on pBR322 plasmid), while, at higher concentration, the drug, as well as all tested derivatives, inhibited topo II activity. This effect is clearly due to the DNA-binding properties of the tested compounds. Moreover, to check whether these amsacrine derivatives could exert their activity through topoisomerase I, as other DNA-targeted agents with dual activity do,[7] we tested the drugs' ability to inhibit the human enzyme. None of them could inhibit topoisomerase I-mediated relaxation of the negatively supercoiled plasmid, or, similarly to CPT, induce nicking of DNA in the presence of the enzyme. Rather, similarly to the intercalator *m*-AMSA, at doses higher than 1 μM they could unwind DNA and influence the migration rate of the plasmid in ethidium bromide-containing agarose gels. The results for representative compounds and appropriate controls are shown in Figure 3. From these data we can conclude that the topoisomerase-dependent mechanism of DNA damage does not appear to be appreciably operating in this case.

Cell cytotoxicity

The novel pyrrolo-quinoline derivatives were tested as cytotoxic agents in the in vitro primary NCI antitumor screen.[14]

The results are reported in Table 1 as the average of the data obtained with cell lines corresponding to specific types of cancer and as the global average.

The overall potency is in the range 10–100 μM for all compounds except **5b**, which proved to be almost inactive. Also, compound **7b** is the least active in the series of the condensed tetracyclic system. Hence the double 7-methyl-4′-methoxy substitution is clearly detrimental to drug activity. Compounds bearing the 7-methyl substituent but not the methoxy group (**4b** and **6b**) are more potent than the **5b** and **7b** congeners, but 2-fold less potent than 7-unsubstituted derivatives **4a** and **6a**. The latter are indeed the most active in the tricyclic and tetracyclic series. Recalling that the planar portion of important anticancer compounds is often a DNA-intercalator, the above findings are consistent with the fact that methyl substitution renders drug intercalation into DNA base-pairs less efficient. Methoxy substitution also appears to reduce the cytotoxic response, in particular when comparing **6a** with **7a**. Finally, the closure of an extra ring in compounds **6** and **7** lowers potency. The lower conformational freedom experienced by the *p*-amino methanesulfonyl anilino moiety when the *p*-amino group is linked to the pyrrole function possibly accounts for these findings.

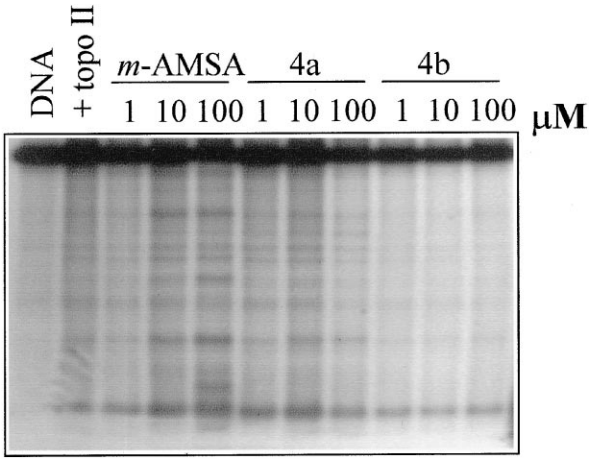


Figure 2. Topoisomerase II-stimulated DNA cleavage. The compounds tested were examined for their ability to stimulate human topoisomerase II-mediated DNA cleavage. A representative gel showing the activity of the most cytotoxic compound **4a** (see Table 1) alongside **4b** and the parent compound *m*-AMSA is reported in the figure.

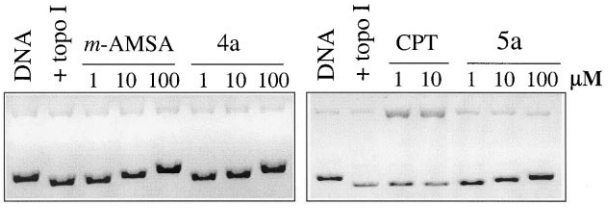


Figure 3. Pyrrolo-quinoline derivatives do not inhibit human topoisomerase I. Representative compounds were tested for their ability to inhibit the relaxation activity of human topoisomerase I toward negatively supercoiled pBR322. 250 ng of plasmid were treated with 1 unit of human topoisomerase I and the indicated concentrations of drugs as described in Experimental, and run on ethidium bromide-containing gels.

As far as the mechanism of action is concerned, topoisomerase II-mediated DNA damage does not seem to be the major cause of cell death. In fact, even considering the relatively low potency exhibited by the novel compounds, very little enzyme-mediated DNA cleavage could be detected in the range of drug concentrations at which remarkable cytotoxic effects are observed. Hence, despite the structural similarity with *m*-AMSA shown by compounds **4** and **5**, the changes occurring in the planar, DNA-interactive region cause a major loss of topoisomerase poisoning ability. Given their remarkable biological response, interference with other important biological target(s) should be operating in the novel compounds.

A COMPARE[15] analysis was performed with compounds **4a** and **6a** to check whether they resemble previously identified anticancer drugs, including *m*-AMSA. The highest correlation coefficients are of the order of 0.7, indicating a modest level of similarity with the test drugs. The 20 compounds most highly correlated to **4a** and **6a** in the COMPARE analysis belong to different structural families and do not exhibit a common pharmacophore. In addition, *m*-AMSA was not found in the above list, which confirms a different mechanism of cytotoxic action.

Table 1. Cell growth inhibition properties of the test pyrrolo-quinolines

Type of cancer cell line[a]	IG_{50} (μM)[b]							
	4a	**4b**	**5a**	**5b**	**6a**	**6b**	**7a**	**7b**
Leukemia (5)	7.41	8.91	14.2	> 100	10.7	24.5	23.4	45.7
Non-small cell lung (9)	10.7	19.9	17.8	> 100	16.6	22.4	37.2	47.9
Colon (7)	10.9	23.4	19.9	> 100	20.9	61.6	37.2	93.3
Central nervous system (5)	8.13	14.8	15.8	> 100	17.8	10.5	31.6	26.9
Melanoma (7)	10.0	17.8	16.6	> 100	13.5	89.1	32.3	79.4
Ovary (6)	15.8	20.9	15.9	> 100	15.8	46.8	38.0	43.6
Kidney (7)	18.2	31.6	17.8	> 100	26.9	26.3	52.5	56.2
Prostate (2)	10.0	19.5	14.1	> 100	17.4	70.8	28.2	100
Breast (7)	16.6	17.4	19.9	> 100	17.8	66.1	38.0	46.8
Average	12.0	18.2	16.0	> 100	17.4	36.3	35.5	53.7

[a]Number of tested lines in brackets.
[b]Drug concentration inhibiting tumor cell growth by 50%.

Conclusion

In conclusion, among all tested compounds, **4a** proved to be the most valuable. Besides being effective against leukemia cell lines, it appears to be remarkably active against other usually poorly sensitive lines from solid tumors like CNS-, melanoma- and prostate-derived cells. To further assess its potential as a therapeutic agent, compound **4a** is presently undergoing in vivo testing at the National Cancer Institute.

Experimental

Chemistry

Melting points were determined with a Gallenkamp MFB-595–010M Melting Point Apparatus, and are uncorrected.

^{1}H NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer using the indicated solvents and TMS as internal reference. The ^{1}H NMR signals are reported in parts per million (δ ppm), and are characterized as singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m). In the case of multiplets, the quoted chemical shift corresponds to the multiplet center. Integrals corresponded satisfactorily to those expected on the basis of compound structure. Coupling constants are expressed in Hertz (Hz). Mass spectra were obtained with a mass spectrometer Mat 112 Varian Mat Bremen.

Elemental analyses were performed at the microanalysis laboratory, Department of Pharmaceutical Sciences, University of Padova, Italy, using a Perkin–Elmer elemental analysis apparatus model 240B; results for C, H, N, Cl and S were within ±0.4% of the theoretical values.

IR spectra were recorded in cm^{-1} with a Perkin–Elmer 1760FTIR spectrophotometer as potassium bromide pressed disk.

Thin layer chromatography (TLC) was performed with Merck silica gel F254 polystyrene-backed plates. Eluents used in TLC analysis: (a) (ethyl acetate:*n*-hexane 7:3 v/v), (b) (methanol:*n*-hexane 9:1 v/v). Column flash chromatography was performed with Merck silica gel (250–400 mesh ASTM).

3H-7-Methyl-9-hydroxy-pyrrolo[3,2-f]quinoline (1a). A solution of 5-nitroindole (Aldrich) in absolute ethanol was slowly added to a 5% palladium on charcoal ethanol suspension saturated with hydrogen and the mixture was hydrogenated at atmospheric pressure at the temperature of 40 °C until the starting material disappeared according to TLC analysis (eluent a). After 3–6 h the mixture was filtered and the filtrate evaporated to dryness. The residue (yield 98%) was used in the next condensation step without purification.

Equimolar amounts of 5-aminoindole (11–12 mM) and ethyl acetacetate were dissolved in 70 mL absolute ethanol. After adding 3–4 g of $CaSO_4$ (drierite) and AcOH as the catalyst, the mixture was refluxed for 24–36 h to drive the reaction to completion. $CaSO_4$ was filtered off and the filtrate was evaporated to dryness affording a yellow–brown solid condensation product. A pure sample of the enamine compound was obtained by crystallization from ethanol:ethyl acetate 1:1 (v/v) mixture. Yield 94%; mp 114 °C; R_f 0.8 (eluent a); ^{1}H NMR (acetone-d_6): δ 1.23 (t, 3H, J = 7.1 Hz, ethyl CH_3), 1.91 (s, 3H, CH_3), 4.09 (q, 2H, J = 7.1 Hz, CH_2), 4.62 (s, 1H, =CH-), 6.48 (m, 1H, HC-3), 6.95 (dd, $J_{6,4}$ = 2.1 Hz and $J_{6,7}$ = 8.6 Hz, HC-6), 7.39 (m, 2H, HC-2 and HC-4), 7.44 (d, 1H, $J_{7,6}$ = 8.6 Hz, HC-7), 10.38 (bs, 1H, NH).

The enamine crotonate derivative was suspended in boiling phenyl ether (50 mL), heated to reflux for 30 min to produce the pyrrolo-quinoline, cooled to room temperature and diluted with petroleum ether. The resulting precipitate was collected and washed several times with ethyl acetate and desiccated. Recrystallization from 70% ethanol afforded pure **1a**. Yield 93%; mp 300 °C dec.; R_f 0.54 (eluent b); ^{1}H NMR (DMSO-d_6): 2.35 (s, 3H, CH_3), 5.95 (s, 1H, HC-8), 7.25 (d, 1H, $J_{5,4}$ = 8.7 Hz, HC-5), 7.41 (m, 1H, HC-3), 7.50 (m, 1H, HC-2), 7.69 (d, 1H, $J_{4,5}$ = 8.7 Hz, HC-4), 11.43 (bs, 1H, OH), 11.50 (bs, 1H, NH). Anal. calcd for $C_{12}H_{10}N_2O$: 72.71 C, 5.09 H, 14.14 N; found: 71.97 C, 5.14 H, 14.52 N.

3H-9-Hydroxy-pyrrolo[3,2-f]quinoline (1b). According to a previously published procedure,[9] 5-aminoindole, obtained as described above, and diethylethoxy methylene malonate were mixed together and heated slowly to 130 °C. After 2–3 h, the reaction product ethanol was evaporated under reduced pressure.

Cooling to room temperature converted the semisolid crude malonate into a compact solid. This was used without further purification in the following condensation reaction, accomplished adding portions of enamine malonate derivative to boiling phenyl ether, heating to reflux for 30 min to produce the pyrrolo-quinoline ethyl ester, cooling to room temperature and filtering. The collected precipitate was washed with ethyl acetate and recrystallized from 70% ethanol. Yield 77%; mp 288–290 °C; R_f 0.68 (eluent b); ^{1}H NMR (DMSO-d_6): δ 1.30 (t, 3H, J = 7.1 Hz, CH_3), 4.23 (q, 2H, J = 7.1 Hz, CH_2), 7.33 (d, 1H, $J_{5,4}$ = 8.8 Hz, HC-5), 7.52 (m, 1H, HC-2), 7.58 (m, 1H, HC-1), 7.78 (d, 1H, $J_{4,5}$ = 8.8 Hz, HC-4), 8.45 (s, 1H, HC-7), 11.58 (bs, 1H, OH), 12.2 (bs, 1H, NH).

A mixture of crude pyrroloquinoline ester was refluxed in 2 N NaOH with stirring. After 2 h, the reaction mixture was acidified with acetic acid (AcOH), and the resulting precipitate was filtered off, washed with water, and dried. The solid was recrystallized from dimethylformamide (DMF) to yield 9-hydroxy-pyrrolo[3,2-f]-quinoline-8-carboxylic acid. Yield 88%; mp 282–284 °C dec. (lit.[7] mp 307–308 °C); R_f 0.55 (eluent b); ^{1}H NMR (DMSO-d_6): δ 7.54 (m, 2H, HC-1 and HC-4), 7.66 (m, 1H, HC-2), 7.99 (d, 1H, $J_{5,4}$ = 8.8 Hz, HC-5), 8.81 (s, 1H, HC-7), 11.91 (bs, 1H, OH), 12.3 (bs, 1H, NH).

Crude pyrrolo-quinoline acid was suspended in boiling phenyl ether and allowed to reflux for 20 min. After

cooling to room temperature, the crystalline product was separated, filtered, washed with ethyl ether, and recrystallized twice from ethanol:methanol mixture (1:1) to produce **1b**. Yield 87%; mp 320 °C, dec.; R_f 0.83 (eluent b); ^{1}H NMR (DMSO-d_6): δ 6.8 (d, 1H, $J_{8,7}$ = 7.4 Hz, HC-8), 7.27 (d, 1H, $J_{5,4}$ = 8.8 Hz, HC-5), 7.45 (t, 1H, J = 2.7 Hz, HC-2), 7.55 (bs, 1H, HC-1), 7.73 (d, 1H, $J_{4,5}$ = 8.8 Hz, HC-4), 7.85 (d, 1H, $J_{7,8}$ = 7.4 Hz, HC-7), 11.5 (bs, 1H, OH), 11.7 (bs, 1H, NH). Anal. calcd for $C_{11}H_8N_2O$: 71.73 C, 4.38 H, 15.21 N; found: 71.58 C, 4.30 H, 15.30 N.

9-Chloro-pyrrolo-quinolines (2a,b). 9-Hydroxy-pyrrolo-quinolines **1a,b** (6–7 mmol) were added to phosphorus oxychloride (5 mL) and heated to 140 °C for 1 h. Upon completion of the reaction, as monitored by silica gel thin layer chromatography, excess of phosphorus oxychloride was evaporated and the mixture cooled. Then, a cold 25% (w/v) NH_4OH water solution was added (pH 8) and the solid precipitate was filtered, washed with water and dried.

3H-9-Chloro-pyrrolo[3,2-f]quinoline (2a). Recrystallization from ethanol:ethyl acetate (1:1 v/v) afforded pure compound. Yield 83%; mp 235 °C; R_f 0.86 (eluent b); ^{1}H NMR (DMSO-d_6): δ 7.75 (m, 2H, HC-1 abd HC-2), 7.70 (d, 1H, $J_{7,8}$ = 4.7 Hz, HC-7), 7.76 (d, 1H, $J_{5,4}$ = 9 Hz, HC-5), 7.96 (d, 1H, $J_{4,5}$ = 9 Hz, HC-4), 8.67 (d, 1H, $J_{8,7}$ = 4.7 Hz, HC-8), 12 (bs, 1H, NH). Anal. calcd for $C_{13}H_9ON_2Cl$: 63.82 C, 3.71 H, 11.45 N, 14.49 Cl; found: 63.94 C, 3.68 H, 11.57 N, 14.54 Cl.

3H-7-Methyl-9-chloro-pyrrolo[3,2-f]quinoline (2b). Double recrystallization from ethanol afforded the pure compound. Yield 80%; mp 225 °C dec.; R_f 0.48 (eluent a); ^{1}H NMR (deuterio DMSO): δ 2.38 (s, 3H, CH_3), 7.36 (s, 1H, HC-1), 7.42 (sa, 1H, HC-2), 7.47 (s, 1H, HC-8), 7.53 (1H, d, $J_{5,4}$ = 8.4 Hz, HC-5), 7.78 (d, 1H, $J_{4,5}$ = 8.4 Hz, HC-5), 11.76 (bs, 1H, NH). Anal. calcd for $C_{12}H_7ON_2Cl$: 62.49 C, 3.06 H, 12.15 N, 15.37 Cl; found 61.35 C, 3.25 H, 12.02 N, 15.24 Cl.

9-Anilino-pyrrolo-quinoline hydrochlorides (4a,b and 5a,b). A solution of 9-chloro-pyrrolo-quinolines **2a,b** (1.5–2 mmol) and an equimolar amount of *p*-methanesulfonamido-aniline or *o*-methoxy-*p*-methanesulfonamido-aniline in methanol:water mixture (9:1 v/v) and in the presence of 36% HCl as the catalyst, was refluxed until completion as monitored by TLC (24–48 h). After concentration of methanolic solution and cooling to 4 °C, a yellow crystalline precipitate was formed, which was filtered, washed with water and cold ethanol and dried.

3H-9-(*p*-Methanesulfonamido-aniline)-pyrrolo[3,2-f]quinoline hydrochloride (4a). Double recrystallization from methanol yielded the pure compound. Yield 78%; mp 300 °C; R_f 0.46 (eluent b); ^{1}H NMR (DMSO-d_6): δ 3.08 (s, 1H, CH_3), 6.85 (d, 1H, $J_{8,7}$ = 6.8 Hz, HC-8), 7.39 (d, 2H, J = 8.9 Hz, HC-2′ and HC-6′), 7.50 (d, 2H, J = 8.5 Hz, HC-3′and HC-5′), 7.66 (bs, 1H, HC-1), 7.81 (d, 1H, $J_{5,4}$ = 9 Hz, HC-5), 7.84 (m, 1H, HC-2), 8.20 (d, 1H, $J_{4,5}$ = 9 Hz, HC-4), 8.43 (d, 1H, $J_{7,8}$ = 7 Hz, HC-7), 9.39 (s, 1H, sulfonamide NH), 10.03 (s, 1H, amino NH), 12.47 (s, 1H, indole NH). Anal. calcd for $C_{18}H_{16}N_4O_2S$·HCl: 55.66 C, 4.41 H, 14.43 N, 9.01 Cl, 8.24 S; found: 55.25 C, 4.32 H, 14.10 N, 9.29 Cl, 7.93 S.

3H-7-Methyl-9-(*p*-methanesulfonamido-aniline)-pyrrolo-[3,2-f]quinoline hydrochloride (4b). The crude hydrochloride was dissolved in water and the solution was made alkaline with 5% (w/v) NH_4OH in water. The organic product was extracted with ethyl acetate, washed with water and dried over anhydrous sodium sulfate. After the solution was evaporated in vacuo, the resulting yellowish solid was purified by flash-chromatography using silica gel as the solid support and eluted with methanol:*n*-hexane mixture (9:1 v/v). A crystalline yellow product was obtained, which, after a single crystallization from methanol, gave analytically pure 3H-7-methyl-9-(*p*-methanesulfonamido-aniline)-pyrrolo[3,2-f]-quinoline. Mp 215 °C; R_f 0.27 (eluent b); ^{1}H NMR (DMSO-d_6): δ 2.48 (s, 3H, CH_3), 3.38 (s, 3H, CH_3), 6.97 (s, 1H, HC-8), 7.15 (s, 1H, HC-1), 7.19 (d, 2H, J = 9.1 Hz HC-3′ and HC-5′), 7.27 (d, 2H, HC-2′ and HC-6′), 7.46 (t, 1H, J = 2.6 Hz, HC-2), 7.45 (d, 1H, $J_{5,4}$ = 8.9 Hz, HC-5), 7.77 (d, 1H, $J_{4,5}$ = 8.9 Hz, HC-4), 8.03 (bs, 1H, sulfonamide NH), 11.66 (bs, 1H, indole NH). Anal. calcd for $C_{19}H_{18}N_4O_2S$: 62.28 C, 4.95 H, 15.30 N, 8.73 S; found: 62,26 C, 4.86 H, 15.67 N, 8.61 S.

By treatment of an absolute ethanol solution of the above compound with dry HCl, the desired hydrochloride **4b** was obtained, which was recrystallized from methanol. Yield 86%; mp 304 °C. Anal. calcd for $C_{19}H_{18}N_4O_2ClS$·0.5H_2O: 55.54 C, 4.66 H, 13.64 N, 8.43 Cl, 7.80 S; found: 55.63 C, 4.71 H, 13.41 N, 8.83 Cl, 8.03 S.

3H-9-(*o*-Methoxy-*p*-methanesulfonamido-aniline)-pyrrolo-[3,2-f]quinoline (5a). Pure **5a** was obtained as the hydrochloride following the same procedure described for **4b**. Yield 75%; mp 280 °C dec. R_f 0.29 (eluent b); ^{1}H NMR (DMSO-d_6): δ 3.12 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 6.55 (d, 1H, $J_{8,7}$ = 6.8 Hz, HC-8), 7.00 (dd, 1H, $J_{5',6'}$ = 8.3 Hz and $J_{5',3'}$ = 2.1 Hz, HC-5′), 7.10 (d, 1H, $J_{3',5'}$ = 2.1 Hz, HC-3′), 7.45 (d, 1H, $J_{6',5}$ = 8.3 Hz, HC-6′), 7.72 (m, 1H, HC-1), 7.77 (d, 1H, $J_{5,4}$ = 8.9 Hz, HC-5), 7.85 (t, 1H, $J_{2,1}$ = 2.7 Hz, HC-2), 8.20 (d, 1H, $J_{4,5}$ = 8.9 Hz, HC-4), 8.40 (d, 1H, $J_{7,8}$ = 6.9 Hz, HC-7), 8.96 (d, 1H, sulfonamide NH), 10.04 (d, 1H, anilino NH), 12.45 (bs, 1H, indole NH).). Anal. calcd for $C_{19}H_{19}N_4O_3ClS$: 53.33 C, 4.71 H, 13.09 N, 8.29 Cl, 7.49 S; found: 53.63 C, 4.41 H, 12.96 N, 8.64 Cl, 7.77 S.

3H-7-Methyl-9-(*o*-methoxy-*p*-methanesulfonamido-aniline)-pyrrolo[3,2-f]quinoline hydrochloride (5b). Pure **5b** was obtained following the same procedure described for **4b**. Yield 78%; mp 290 °C dec.; R_f 0.25 (eluent b); ^{1}H NMR (DMSO-d_6): δ 2.63 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 6.44 (s, 1H, HC-8), 7.00 (dd, 1H, $J_{5',6'}$ = 8.3 Hz and $J_{5',3'}$ = 2 Hz, HC-5′), 7.10 (d, 1H, $J_{3',5'}$ = 2 Hz, HC-3′), 7.45 (d, 1H, $J_{6',5'}$ = 8.3 Hz, HC-6′), 7.67 (bs, 1H, HC-1), 7.82 (s, 1H, HC-2), 7.83 (d, 1H, $J_{5,4}$ = 8.7 Hz, HC-5), 8.16 (d, 1H, $J_{4,5}$ = 8.7 Hz, HC-4), 8.79 (s, 1H, sulfonamide NH), 10.08 (s, 1H, aniline NH), 12.45 (s, 1H, indole NH). Anal. calcd for $C_{20}H_{21}N_4O_3ClS$·0.5H_2O: C 53.27, H 5.14, N 12.42, Cl

7.86, S 7.11; found: C 53.09, H 4.85, N 12.36, Cl 8.13, S 7.32.

1-Formyl-pyrrolo-quinolines (3a,b). A solution of 9-hydroxy-pyrrolo-quinolines **1a,b** (3–4 mmol) in DMF was dropped into a mixture of 2 mL $POCl_3$ and 3 mL DMF cooled at 10 °C. Then heating was raised to 35 °C and continued with stirring until the starting material was no longer detectable by TLC. The volatile products were removed in vacuo and the residue diluted with water/ice before alkalinization with 25% NH_4OH (v/v). The precipitate was filtered, washed with water and dried.

3H-9-Chloro-1-formyl-pyrrolo[3,2-f]quinoline (3a). This was obtained in almost quantitative yield; mp 255 °C (from ethanol); R_f 0.35 (eluent a); IR (KBr): 3480, 3126, 2885, 1655 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.75 (d, 1H, $J_{7,8}=4.7$ Hz, HC-7), 7.88 (d, 1H, $J_{5,4}=9.0$ Hz, HC-5), 8.00 (d, 1H, $J_{4,5}=9.0$ Hz, HC-4), 8.21 (s, 1H, HC-2), 8.75 (d, 1H, $J_{8,7}=4.7$ Hz, HC-8), 10.50 (s, 1H, CHO).

3H-9-Chloro-7-methyl-1-formyl-pyrrolo[3,2-f]quinoline (3b). Yield 76%; mp 165–168 °C; R_f 0.33 (eluent b); IR (KBr): 3420, 2915, 2875, 1645 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.69 (s, 1H, CH_3), 7.59 (s, 1H, HC-8), 7.83 (d, 1H, $J_{5,4}=9$ Hz, HC-5), 8.00 (d, 1H, $J_{4,5}=9$ Hz, HC-4), 8.11 (d, 1H, $J_{2,NH}=3.1$ Hz, HC-2), 10.63 (s, 1H, CHO).

Indolo-naphthyridine chlorides 6a,b and 7a,b. A methanolic solution of equimolar amounts of 1-formyl-pyrrolo-quinolines **3a,b** (1–1.5 mmol) and of *p*-methane-sulfonamido-aniline or *o*-methoxy-*p*-methanesulfonamido-aniline was acidified to pH = 6 with glacial acetic acid. Stirring was continued at room temperature until the starting compounds were no longer detectable by TLC, while the iminium salt (R_f 0.18, eluent b) appeared as a new fluorescent band. Then, $NaBH_3CN$ (slight molar excess) dissolved in 3 mL methanol was added to the solution, and the reduction reaction was carried out at room temperature with stirring until completion (30–48 h, TLC monitoring). On partial evaporation of the solvent and cooling at 4 °C, a crystalline product precipitated from the reaction mixture. The precipitate was filtered, washed with methanol and dried yielding a pure product.

3H-5,6-Dihydro-6-(4′-methanesulfonamido-benzen)-indolo-[3,3a,4,5-c,d,e][1,6]naphthyridine hydrochloride (6a). Double recrystallization from ethanol:methanol mixture (1:1 v/v) afforded the pure compound. Yield 50%; R_f 0.18 (eluent b); IR (KBr): 3350, 3026, 2875, 1639, 1328–1152 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.11 (s, 3H, CH_3), 5.44 (s, 2H, CH-5a and CH-5b), 6.16 (d, 1H, $J_{7,8}=6.5$ Hz, HC-7), 7.31 (s, 1H, HC-4), 7.42 (d, 2H, $J_{2',3''}=8.8$ Hz and $J_{6',5'}=8.8$ Hz, HC-2′, HC-6′), 7.86 (d, 1H, $J_{2,1}=8.9$ Hz, HC-2), 7.46 (d, 1H, $J_{1,2}=8.9$ Hz, HC-1), 7.58 (d, 2H, $J_{3',2''}=8.8$ Hz and $J_{5',6'}=8.8$ Hz, HC-3′, HC-5′), 8.25 (d, 1H, $J_{8,7}=6.5$ Hz, HC-8), 10.1 (bs, 1H, sulfonamide NH), 11.65 (bs, 1H, indole NH); MS: 364 (M+), 285, 166,79,76. Anal. calcd for $C_{19}H_{16}N_4O_2S{\cdot}HCl$: 56.93 C, 4.27 H, 13.98 N, 8.84 Cl, 8.00 S; found: 56.57 C, 4.36 H, 13.96 N, 8.60 Cl, 7.79 S.

3H-5,6-Dihydro-6-(4′-methanesulfonamido-benzen)-8-methyl-indolo[3,3a,4,5-c,d,e][1,6]naphthyridine chloride (6b). This compound was recrystallized several times from an ethanol:methanol mixture (1:1 v/v). Yield 30%; R_f 0.21 (eluent b); IR (KBr): 3377, 2875, 1323–1152 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.53 (s, 3H, CH_3-8), 3.13 (s, 3H, CH_3SO_2), 5.45 (s, 2H, HC-5a and HC-5b), 6.07 (s, 1H, HC-7), 7.36 (s, 1H, HC-4), 7.43 (d, 2H, $J_{2',3' \text{ and } 6',5'}=8.9$ Hz, HC-2′, HC-6′), 7.44 (d, 1H, $J_{2,1}=8.9$ Hz, HC-2), 7.58 (d, 2H, $J_{3',2' \text{ and } 5',6'}=8.9$ Hz, HC-3′ and HC-5′), 7.90 (d, 1H, $J_{1,2}=8.9$ Hz, HC-1), 10.14 (bs, 1H, sulfonamide NH), 11.76 (bs, 1H, indole NH); MS: 382 (M+), 301, 208, 180, 108, 92, 65. Anal. calcd for $C_{20}H_{18}N_4O_2S{\cdot}0.5H_2O$: 56.67 C, 4.76 H, 13.22 N, 8.36 Cl, 7.56 S; found: 57.00 C, 4.82 H, 13.20 N, 8.36 Cl, 7.73 S.

3H-5,6-Dihydro-6-(2′-methoxy-4′-methanesulfonamido-benzen)-indolo[3,3a,4,5-c,d,e][1,6]naphthyridine hydrochloride (7a). The compound was recrystallized from ethanol; yield 40%; R_f 0.22 (eluent b); IR (KBr): 3300, 3227, 1639, 1333, 1278, 1142, 1031, 806 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.15 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 5.31 (d, 1H, $J_{5b,5a}=5.6$ Hz, HC-5b), 5.38 (d, 1H, $J_{5a,5b}=5.6$ Hz, HC-5a), 6.08 (d, 1H, $J_{7,8}=6.9$ Hz, HC-7), 7.02 (dd, 1H, $J_{5',3'}=2.2$ Hz and $J_{5',6'}=8.4$ Hz, HC-5′), 7.1 (d, 1H, $J_{3',5'}=2.2$ Hz, HC-3′), 7.38 (d, 1H, $J_{4,3}=1.6$ Hz, HC-4), 7.48 (d, 1H, $J_{1,2}=8.9$ Hz, HC-1), 7.55 (d, 1H, $J_{6',5'}=8.4$ Hz, HC-6′), 7.95 (d, 1H, $J_{2,1}=8.9$ Hz, HC-2), 8.26 (d, 1H, $J_{8,7}=6.9$ Hz, HC-8), 10.18 (bs, 1H, sulfonamide NH), 11.80 (bs, 1H, indole NH); MS: 394 (M+), 364, 315, 285, 194, 186, 79.64. Anal. calcd for $C_{20}H_{19}N_4O_3ClS{\cdot}H_2O$: 53.51 C, 4.71 H, 12.48 N, 7.90 Cl, 7.14 S; found: 53.36 C, 4.91 H, 12.67 N, 7.60 Cl, 7.32 S.

3H-5,6-Dihydro-6-(2′-methoxy-4′-methanesulfonamido-benzen)-8-methyl-indolo[3,3a,4,5-c,d,e][1,6]naphthyridine chloride (7b). This compound was recrystallized from absolute ethanol; yield 45%; R_f 0.2 (eluent b); IR (KBr): 3400, 3267, 2925, 2855, 1639, 1323, 1147, 1027, 800 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.51 (s, 3H, CH_3), 3.15 (s, 3H, CH_3SO_2), 3.78 (s, 3H, OCH_3), 5.29 (d, 1H, $J_{5b,5a}=3.96$ Hz, HC-5b), 5.32 (d, 1H, $J_{5a,5b}=3.96$ Hz, HC-5a), 5.95 (s, 1H, HC-8), 6.99 (dd, 1H, $J_{5',6'}=8.68$ Hz and $J_{5',3'}=2.12$ Hz, HC-5′), 7.10 (d, 1H, $J_{3',5'}=2.12$ Hz, HC-3′), 7.33 (ss, 1H, $J_{4,3}=1.54$ Hz, HC-4), 7.41 (d, 1H, $J_{1,2}=8.67$ Hz, HC-1), 7.50 (d, 1H, $J_{2,1}=8.67$ Hz, HC-2), 7.88 (1H, d, $J_{6',5'}=8.68$ Hz, HC-6′), 10.2 (bs, 1H, sulfonamide NH), 11.7 (bs, 1H, indole NH); MS: 408 (M+), 329, 208, 200, 179, 121, 79, 64. Anal. calcd for $C_{21}H_{21}N_4O_3ClS{\cdot}H_2O$: 54.48 C, 5.01 H, 12.10 N, 7.66 Cl, S 6.93; found: 54.76 C, 4.91 H, 11.97 N, 7.50 Cl, 7.32 S.

***N*-(2-Methoxy-4-nitro-benzen)-ethylcarbamate.** To a solution of commercial 2-methoxy-4-nitro-aniline (2 g, 12 mM) in 50 mL dry THF, 1.4 mL (12 mmol) of ethylchloroformate was added. The mixture was refluxed until the starting compound disappeared according to TLC analysis (eluent a). The yellow solution was evaporated to dryness giving a crystalline yellow product. Yield 95%; mp 154–156 °C. R_f 0.3 (eluent

a). ^{1}H NMR (acetone-d_6): δ 1.29 (t, 3H, J = 7.1 Hz, CH_3), 4.06 (s, 3H, OCH_3), 4.23 (q, 2H, J = 7.1 Hz, CH_2), 7.82 (d, 1H, $J_{3,5}$ = 2.6 Hz, HC-3), 7.92 (dd, 1H, $J_{5,3}$ = 2.6 and $J_{5,6}$ = 9.2 Hz, HC-5), 8.21 (sa, 1H, NH), 8.31 (d, 1H, $J_{6,5}$ = 9.2 Hz, HC-6).

***N*-(2-Methoxy-4-amino-benzen)-ethylcarbamate.** Easily air-oxidable intermediate. Yield 90%. R_f 0.6 (*n*-hexane: ethyl acetate 1:1 v/v)

***N*-(2-Methoxy-4-methanesulfonamido-benzen)-ethylcarbamate.** Yield 84%; mp 139–141 °C; R_f 0.75 (eluent a). ^{1}H NMR (acetone-d_6): δ 1.26 (t, 3H, J = 7 Hz, CH_3), 2.96 (s, 3H, SO_2CH_3), 3.88 (s, 3H, OCH_3), 4.16 (q, 2H, J = 7 Hz, CH_2), 6.9 (dd, 1H, $J_{5,3}$ = 2.5 Hz and $J_{5,6}$ = 8.7 Hz, HC-5), 7.04 (d, 1H, $J_{3,5}$ = 2.5 Hz, HC-3), 7.58 (sa, 1H, urethane NH), 7.94 (d, 1H, $J_{6,5}$ = 8.6 Hz, HC-6), 8.4 (sa, 1H, amide NH).

2-Methoxy-4-methanesulfonamido-aniline.[12] A solution of the urethane derivative in aqueous 20% NaOH was refluxed as appropriate (TLC monitoring). After cooling at room temperature, the reaction mixture was extracted with ethyl ether and the organic layer was separated, dried over Na_2SO_4, and evaporated in vacuo to dryness to give the desired aniline.

Topoisomerase II cleavage assay

The plasmid pBR322 was cut at the EcoR1 site, and labeled by fill-in with the Large (Klenow) Fragment of DNA Polymerase I and [α-^{32}P]dATP. 0.1 μg of DNA were reacted with the indicated concentrations of drugs and 2 units of human topoisomerase II-α (TopoGEN, Inc., Columbus, OH) at 37 °C in 50 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 120 mM KCl, 0.5 mM DTT, 30 μg/mL BSA and 1 mM ATP. Following 30 min of incubation at 37 °C, samples were stopped by incubation with 0.5 mg/mL proteinase K and 1% SDS at 60 °C for 3 h, and then loaded on 1% agarose gel (0.09 M Tris–borate, pH 8.3, 2.5 mM EDTA) and run for 16 h at 40 Volts. The gel was then dried and autoradiographed. The effects of drugs were examined up to 100 μM concentration.

Topoisomerase I relaxation assay

Topoisomerase I catalytic activity assay was performed by incubating 0.25 μg of negative supercoiled pBR322 at 37 °C in 10 mM Tris–HCl, pH 7.5, NaCl 100 mM, β-mercaptoethanol 1 mM and 1 mM PMSF, with 1 unit of human topoisomerase I (TopoGEN, Inc., Columbus, OH) in the presence of the indicated amount of drugs. After 30 min the reaction (final volume 20 μL) was stopped by 1% SDS, and directly loaded on a 0.8% agarose gel in Tris–borate 50 mM, EDTA 1 mM and ethidium bromide 0.5 μg/mL, and run at 5 V/cm for 4 h. The effects of drugs were examined up to 100 μM concentration.

Antitumor screen

The test derivatives were submitted to an in vitro anticancer testing at NCI.[14] The cell panel consists of 60 lines against which compounds are tested at a minimum of five concentrations at 10-fold dilutions. A 48 h continuous drug exposure protocol is used, and a sulforhodamine protein assay is used to estimate cell viability or growth. Plot studies provide the basis for the detection of patterns of interest.

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