Brief Articles

3-Substituted 7-Phenyl-Pyrroloquinolinones Show Potent Cytotoxic Activity in Human Cancer Cell Lines

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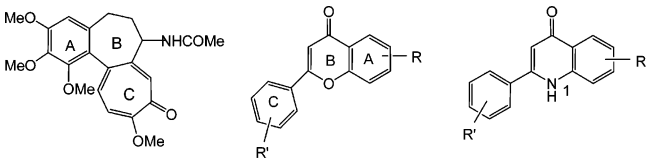
A novel series of 3-alkyl-substituted 7-phenyl-3*H*-pyrrolo[3,2-*f*]quinolin-9-ones (7-PPyQs) was synthesized with the aim to optimize the cytotoxic activity of recently identified PPyQs, promising inhibitors of tubulin polymerization. All compounds inhibited the growth of 11 human tumor cell lines at submicromolar concentrations as well as two human resistant cancer sublines, A549-T12 and A549-T24. FACS analysis indicated that all compounds caused significant arrest of the A549 cell cycle in G_2/M phase at 0.1 and 1 μM and a good correlation between the cytotoxicity IC_{50} and their ability to block the cell cycle was observed.

Introduction

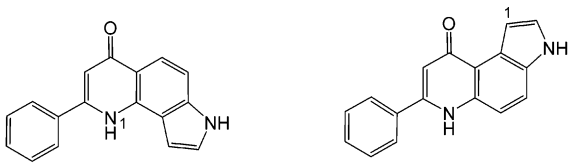
Antimitotics are a class of compounds, mainly of natural origin, which have long been used to treat a variety of malignancies. Although they are sometimes considered "old chemotherapeutics" with respect to modern anticancer approaches,[1] at present they are valuable anticancer molecules in which scientific interest is still very high.[2−6] Their impressive success in clinical use is due to their potent antiproliferative effect (the strongest of commercial anticancer drugs) and to their particular mechanism of action, because they effect microtubule dynamics by targeting either the tubulin polymerization or the microtubule depolymerization processes. The mechanism of action of these antimitotic agents has an important advantage over other antitumor compounds, as they avoid interfering with or damaging the DNA macromolecule and, therefore, do not cause any cell genome mutations.[4] However, taxanes and Vinca alkaloids, the most clinically effective antimitotics, suffer from two major drawbacks: selection of clones within tumor cells[7−9] and neurological toxicity.[5] Therefore, during the past few decades, efforts to identify novel microtubule inhibitors able to overcome the multidrug resistance (MDR[a]) phenotype as well as other modes of resistance[9,10] and, to present improved pharmacology profiles, have produced dozens of microtubule targeting compounds, some of which are in clinical and preclinical trials.[11,12]

2-Phenylquinolin-4-one derivatives (2-PQs; Figure 1) are known for their high antimitotic activity. They are either natural or synthetic small molecules, structurally derived from the flavone nucleus by isosteric substitution of the pyran oxygen with a nitrogen atom. In comparison with flavone derivatives, 2-PQs show higher antiproliferative activity and a more specific mechanism of action, consisting of inhibition of tubulin polimerization by interaction with the colchicine binding site on β-tubulin.[13−16] Their ability to bind to this site has been explained by the presence of a three-ring A−B−C system in the molecular structures of both flavone/azaflavone and colchicine (Figure 1), of which the A and C rings appear to be responsible for useful interactions with the molecular target.



colchicine flavones 2-phenyl-quinolinones

Figure 1. Structural similarities of colchicine, flavones, and 2-phenyl-quinolinones.



2-phenyl-pyrrolo[2,3-*h*]quinolin-4-one 7-phenyl-pyrrolo[3,2-*f*]quinolin 9-one

Figure 2. Structures of the two series of 2-phenyl-pyrroloquinolinone derivatives previously studied.[17,18]

Recently, as a further development of 2-PQs, we synthesized and studied two series of phenylpyrroloquinolinone (PPyQ) derivatives showing different angular geometry, 2-phenyl- and 7-phenyl-PPyQs, in which the pyrrole ring is fused to the *h*- and *f*-side of the quinoline nucleus, respectively[17,18] (Figure 2). The fused pyrrole ring may have rendered the new compounds with mechanism specificity and improved cytotoxicity. In addition, the azole moiety is suitable for furnishing several substituted derivatives to modulate pharmacodynamics and pharmacokinetics.

In vitro and in vivo, all PPyQs showed significant antiproliferative activity by antimitotic effects, with 7-PPyQs being broadly and slightly more active than 2-PPyQs. Interestingly, 2-PPyQs were selective for estrogen-sensitive tumor cell lines.[17]

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[a] Abbreviations: PPyQ, phenyl-pyrroloquinolinone; FACS, fluorescence-activated cell-sorting analysis; MDR, multidrug resistance; PQ, phenylquinolinone; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.

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Recently, using a structure-based approach, Nguyen et al.[19] described a common pharmacophore for a diverse set of small molecules representative of some classes of colchicine site inhibitors. In that study, the authors proposed that the various classes were linked by seven pharmacophore points (three hydrogen bond acceptors, one hydrogen bond donor, two hydrophobic centers, and one planar group). None of the studied inhibitors showed all the points, although three of them turned out to be essential for inhibitory activity, a hydrogen bond acceptor, a hydrophobic center and a planar group. In the light of this report, we analyzed and compared PQ and our PPyQ structures: they share a hydrogen acceptor (carbonyl group), a hydrogen donor (quinolinone NH), two hydrophobic centers (aromatic carbons), and two planar groups (phenyl and benzyl rings). In addition, PPyQs show the hydrogen donor pyrrole NH. However, this extra point does not significantly contribute to increased cytotoxic activity in comparison with PQs.[17,18]

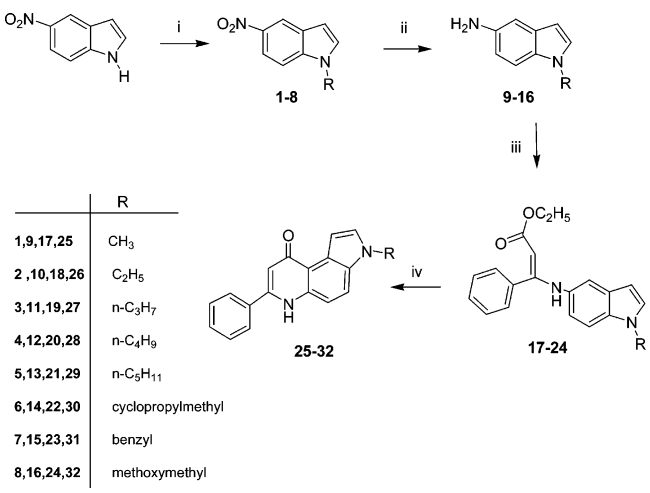
In this paper, we report how we optimized the cytotoxic activity of previously studied 7-PPyQ series,[18] which were the most cytotoxic derivatives of the two series (general IC_{50} 0.4−8 μM for 7-PPyQs, versus selective IC_{50} 0.7−10 μM for 2-PPyQs). To improve cytotoxic activity, we took account of the pharmacophore model of Nguyen et al.[19] and the important role of hydrophobic interactions surrounding the colchicine binding site on β-tubulin at the α,β-tubulin interface.[20] So, considering that further hydrophobic interactions at the colchicine site may have led to higher inhibitory activity and, therefore, to a greater antimitotic effect, as a first approach we modified the 7-PPyQ nucleus by linking a lipophilic group, consisting of an alkyl side-chain, to the pyrrole N. To gain some SARs, we synthesized and investigated the biological activity of eight 3-alkylated 7-PPyQ derivatives with side-chains of increasing length and hindrance and carrying varying polar features (from methyl to *n*-penthyl, cyclopropylmethyl, methoxymethyl, and benzyl). Last, all compounds were assayed for their in vitro antiproliferative activity on a panel of 11 human tumor cell lines. We also examined whether there was any cross-resistance in two selected cancer cell sublines (A549 T12 and A549 T24) to taxol.[21] To elucidate the mechanism(s) of action, the effect on the cell cycle was studied by fluorescence-activated cell-sorting analysis (FACS).

Results and Discussion

Chemistry. Scheme 1 describes the straightforward synthesis of new 3-alkylated PPyQs **25**−**32**, accomplished following a pathway previously described.[18] Preliminarily, 1-subsituted 5-nitro-indole derivatives **1**−**8** were obtained by two different alkylating methods to have higher yields and cleaner products: (a) NaH in DMF was better for compounds **1**−**3** and (b) KOH in acetone for compounds **4**−**8**. Then 5-amino-indole derivatives **9**−**16**, quantitatively obtained by catalytic reduction (H_2, Pd/C 10%) at atmospheric pressure of the corresponding nitro-compounds **1**−**8**, were condensed with ethyl benzoyl-acetate, in ethanol and with acetic acid as catalyst, to give ethyl 3-indole-amino-3-phenyl-acrylates **17**−**24** in good yields. These were thermally cyclized to final 3-substituted 7-PPyQs **25**−**32** in boiling diphenyl ether (250 °C) with high yields. In all cases, the final compounds showed [3,2-*f*] angular geometry (^{1}H NMR *ortho*-couplings of 4-H and 5-H protons) and a quinolinone structure, as confirmed by ^{1}H NMR (N−H at about δ 11.5), ^{13}C NMR (C═O ranging from 184 to 194 δ) and IR (C═O near 1610 cm^{-1}) spectroscopic data.

Biology. In Vitro Cytotoxic Activity. The in vitro cytotoxic activity of the newly synthesized 3-substituted 7-PPyQs **25**−**32** was studied on a panel of 11 human tumor cell lines by applying the MTT colorimetric assay.[22] Compounds were tested over a range of concentrations from 5 to 0.005 μM, and the calculated IC_{50} values, that is, the concentration (μM) of compound able to cause 50% of cell death with respect to the control culture, are reported in Table 1. The results show that all PPyQs inhibited the growth of all human cancer cell lines from various tissues and organs, with IC_{50} values in the submicromolar range. By comparing the cytotoxic activity of the new 3-alkylated 7-PPyQs **25**−**32** with that of the unsubstituted 7-PPyQ (Table 1) and analogs recently described by us,[18] we note that the chemical modification, consisting of inserting a lipophilic group at the *N*-pyrrole ring, generated very potent compounds, with IC_{50} values up to 100-fold lower than the previous compounds and comparable to those of the reference compounds, taxol and vincristine.

Scheme 1[a]



[a] Reagents and conditions (i) (a) CH_3I, C_2H_5Br, *n*-C_3H_7Br, NaH, anhydrous DMF; (b) *n*-C_4H_9Cl, *n*-$C_5H_{11}Cl$, cyclopropylmethylbromide, $BrCH_2OCH_3$, benzylchloride, KOH, acetone; (ii) H_2, Pd/C 10%, absolute ethanol, 30−40 °C; (iii) ethyl benzoylacetate, CH_3COOH, absolute ethanol, refluxing; (iv) diphenyl ether, 250 °C.

Compound **25**, bearing a methyl group, was less active (IC_{50} 0.09−1 μM) than **26**−**30** with longer side-chains (C_2−C_5, IC_{50} 0.005−0.2 μM), with the bulky **30** (cyclopropylmethyl) being highly cytotoxic as well. This indicates that the increase in cytotoxic activity may depend on longer side chains that induce the establishment of specific hydrophobic interactions at the colchicine binding site into β- or α-tubulin. Indeed, it is known that colchicine binds to the β-subunit at its interface with α-, and interacting with both of them, it forms a stable reversible complex. Tubulin conformation changes follow, which prevent or slow polymerization.[20,23]

Compounds **31** and **32**, with less apolar methoxy-, methyl-, and more bulky benzyl- groups, respectively, were less active (IC_{50} about 10-fold higher) than **26**−**30**, again confirming that the more hydrophobic 3−5 C linear and cyclopropylmethyl chains fit and interact better with the hydrophobic cavities. This is further confirmed by much higher IC_{50} values (5−20 μM) found for **33** (Figure 3), a previously described 7-PPyQ,[18] which bears a diethylaminoethyl side-chain at *N*-pyrrole. This construct was generated to increase the water solubility of this class of compounds. As this chain possesses two hindering ethyl groups and a ternary N, which may be protonated at internal cellular pH, becoming positively charged, it is less suitable to interact with hydrophobic cavities. The above qualitative structure−cytotoxic activity relationship, referring to the nature and bulk of 7-PPyQ N-substituents, despite the low number of compounds

Table 1. Cytotoxic Activity of 3-Substituted 7-PPyQs **25**−**32**, **33**,[18] and Unsubstituted 7-PPyQ[18]

	cytotoxic activity IC_{50} (μM)										
cmpd	Hela	Hep G2	H295R	Ovcar-3	MCF-7	Aro	A549	HT-29	PT-45	OE19	OE33
7-PPyQ[a]	0.7 ± 0.2	8 ± 1	0.4 ± 0.2	6 ± 2	0.8 ± 0.3	0.7 ± 0.1	1± 0.2	2 ± 0.5	0.5 ± 0.1	1 ± 0.3	2 ± 0.5
25	0.1 ± 0.05	1 ± 0.2	0.09 ± 0.01	0.7 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.1 ± 0.05	0.1 ± 0.02	0.8 ± 0.3
26	0.01 ± 0.008	0.05 ± 0.01	0.03 ± 0.009	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.08 ± 0.01	0.2 ± 0.05
27	0.03 ± 0.006	0.04 ± 0.01	0.02 ± 0.005	0.02 ± 0.01	0.07 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	0.2 ± 0.05	0.05 ± 0.01	0.05 ± 0.01	0.1 ± 0.05
28	0.02 ± 0.01	0.02 ± 0.005	0.01 ± 0.005	0.01 ± 0.002	0.06 ± 0.01	0.03 ± 0.01	0.03 ± 0.005	0.03 ± 0.01	0.04 ± 0.01	0.007 ± 0.002	0.06 ± 0.01
29	0.02 ± 0.005	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.04 ± 0.02	0.2 ± 0.05	0.04 ± 0.01	0.005 ± 0.002	0.02 ± 0.01
30	0.02 ± 0.01	0.02 ± 0.005	0.01 ± 0.005	0.01 ± 0.008	0.04 ± 0.01	0.03 ± 0.005	0.02 ± 0.002	0.06 ± 0.001	0.02 ± 0.001	0.1 ± 0.05	0.02 ± 0.01
31	0.1 ± 0.07	0.7 ± 0.2	0.4 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.05	0.4 ± 0.2	0.08 ± 0.04	0.5 ± 0.2	0.1 ± 0.05
32	0.3 ± 0.1	0.8 ± 0.1	0.2 ± 0.05	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.3 ± 0.02	1.0 ± 0.5	0.5 ± 0.1
33[a]	5 ± 0.5	7 ± 1	6 ± 0.5	18 ± 2	8 ± 1	20 ± 2.5	12 ± 1.5	6 ± 0.5	5 ± 1	10 ± 2.0	16 ± 1.5
taxol	0.008 ± 0.001	0.3 ± 0.05	0.08 ± 0.01	0.01 ± 0.002	0.007 ± 0.001	0.0002 ± 0.0001	0.006 ± 0.003	0.05 ± 0.02	0.005 ± 0.001	0.4 ± 0.1	0.2 ± 0.05
vincr.	0.005 ± 0.002	0.2 ± 0.05	0.09 ± 0.01	0.02 ± 0.005	0.02 ± 0.01	0.0003 ± 0.0001	0.03 ± 0.01	0.01 ± 0.005	0.004 ± 0.001	0.05 ± 0.002	0.03 ± 0.01

[a] Ref 18, Figure 3.

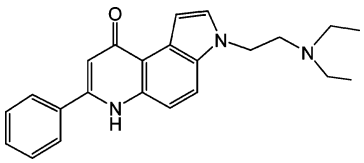


Figure 3. Structure of 3-(2-(diethylamino)-7-phenyl-3*H*-pyrrolo[3,2-*f*]quinolin-9-one, **33**.[18]

Table 2. Cytotoxic Activity of 7-PPyQs **25**−**32** Against Human Lung Carcinoma Taxol-Resistant Cell Sublines

	cytotoxic activity IC_{50} (μM)		
cmpd	A549	A549-T12 (RI)[a]	A549-T24 (RI)[a]
25	0.3 ± 0.1	0.3 ± 0.2 (1)	0.2 ± 0.05 (0.6)
26	0.03 ± 0.01	0.03 ± 0.01 (1)	0.03 ± 0.02 (1)
27	0.05 ± 0.02	0.04 ± 0.007 (1.2)	0.04 ± 0.01 (1)
28	0.03 ± 0.01	0.02 ± 0.005 (0.6)	0.03 ± 0.01 (1)
29	0.04 ± 0.02	0.05 ± 0.01 (0.8)	0.04 ± 0.02 (1)
30	0.02 ± 0.01	0.02 ± 0.01 (1)	0.02 ± 0.005 (1)
31	0.1 ± 0.05	0.1 ± 0.05 (1)	0.2 ± 0.05 (2)
32	0.4 ± 0.1	0.4 ± 0.02 (1)	0.5 ± 0.2 (1.25)
taxol	0.006 ± 0.003	0.06 ± 0.03 (10.2)	0.102 ± 0.6 (17)

[a] RI = resistance index.

examined, allowed us to hypothesize the presence of a deep hydrophobic pocket near the colchicine site, able to accept hindering atom groups like linear aliphatic chains up to five carbons, cyclopropylmethyl and a benzyl group without a loss of potent inhibitory capacity.

In Vitro Cytotoxic Activity against Taxol-Resistant Human Tumor A549 Sublines. The very encouraging results obtained against the in-house panel of cell lines prompted us to test the in vitro cytotoxic activity of compounds **25**−**32** on two additional human cell sublines characterized by acquired resistance to taxol: human lung carcinoma A549-T12 and A549-T24 cell lines.[21] Cytotoxicity was assessed after a 72 h exposure by the MTT test. The results showed that the new 7-PPyQ derivatives are equally potent toward the parental A549 cells and the two taxol-resistant sublines, A549-T12 and A549-T24 (IC_{50} 0.02−0.5 μM; Table 2). Cross-resistance profiles were evaluated by the resistance index (RI), and defined as the ratio between IC_{50} values calculated for cells and those arising from chemosensitive ones. Comparable sensitivity between parental and resistant cell lines was observed (RI = 0.6−1.2 and 0.6−1 for **25**−**32**), as opposed to the RI values of 10.2 and 17 for taxol, on A549-T12 and A549-T24, respectively (Table 2). Results indicate that 7-PPyQs escape the mechanism of taxol resistance and further support the existence of differing mechanisms of action.

Table 3. Flow Cytometry: Percentage of A549 Cells in Each Phase of the Cell Cycle at Concentrations of 1 and 0.1 μM

cmpd	% cells					
	G1		S		G2/M	
concentration (μM)	1	0.1	1	0.1	1	0.1
25	15.6	66.9	9.2	8.8	74.9	18.2
26	14.1	11.7	8.1	8.1	77.3	79.8
27	13.5	13.6	9.6	8.7	76.9	76.2
28	12.9	11.8	9.2	7.7	77.6	80.4
29	15.5	12.4	8.8	7.2	75.5	79.6
30	16.3	16.5	8.6	7.0	74.6	75.7
31	14.6	78.9	9.4	9.7	75.8	10.4
32	13.6	81.7	8.4	7.9	77.9	10.2
control	74.0		11.8		13.3	
vincristine	46.8	nd	7.9	nd	45.2	nd

Fluorescence-Activated Cell-Sorting Analysis. To examine the effect of the new derivatives **25**−**32** on cell cycle progression, FACS was performed. This method highlights the effects of drugs on the distribution of cells in specific phases of the cell cycle. Indeed, antimitotic drugs block cells in the G_2/M phase, causing an increment of the relative peak in the DNA histogram.[24,3] All compounds were tested on the A549 cell line at concentrations of 0.1 and 1 μM. Table 3 shows the results following after 24 h of treatment. All compounds tested at 1 μM caused a significant arrest of the cell cycle in G_2/M phase, raising the G_2/M peak from 13.3% (control) to 77.9% (**32**). At 0.1 μM, compounds **26**−**30** again increased the G_2/M peak from 13.3% (control) to 80.4% (**28**), whereas compounds **25**, **31**, and **32** did not. It is worth noting that, at 0.1 μM, there is a good correlation between the cytotoxicity of 3-substituted 7-PPyQs

and the ability to arrest the A549 cell cycle in G_2/M, as compounds **25**, **31**, and **32** turned out to be less potent in the MTT proliferation assay, too (Table 1).

Conclusions

In the area of antimitotic agents interfering with the colchicine binding site on β-tubulin, a series of 3-substituted 7-PPyQs were synthesized and tested for cytotoxicity on 11 human tumor cell lines and for their effects on cell cycle progression. These agents show an high cytotoxic activity with IC_{50} values in the nanomolar range, up to 100-fold lower than the 3-unsubstituted analogs previously studied[18] and, in some cases, lower than those of reference compounds, taxol and vincristine (Table 1). When assayed against taxol-resistant human lung carcinoma A549-T12 and A549-T24 cell lines, the new 7-PPyQ derivatives were equally potent (IC_{50} 0.02−0.5 μM) toward the parental A549 cells (IC_{50} 0,03−2 μM; Table 2). Furthermore, preliminary experiments showed that the most active compound (**30**) exerted significant cytotoxic activity (>30% inhibition) on multidrug-resistant breast cancer MCF-7 MDR cells at concentrations up to 1 μM, whereas vincristine was ineffective at this concentrations. All these compounds caused G_2/M phase arrest in the cell cycle after a 24 h incubation (Table 2). Results obtained in this study and the previous one on PPyQs contribute to knowledge about structure−activity relationship, referring to the pyrroloquinolinone nucleus. It is clear that two structural features, that is, the phenyl in position 7 and a hydrophobic group at *N*-pyrrole, are essential for potent cytotoxic activity and efficient mitotic arrest. As the most active compounds turned out to be those carrying more hindering side chains at *N*-pyrrole, we also suggest the probable presence of a large, deep hydrophobic pocket near the colchicine binding site, in which useful hydrophobic interactions occur. Besides, preliminary data from tubulin polymerization assays revealed relevant antimicrotubule effects as all new compounds caused significant tubulin depolymerisation (data not shown). The most active compound **30** displayed a behavior similar to colchicine. Indeed, colchicin completely inhibited tubulin polymerization at 3 μM and **30** exhibited an IC_{50} of 0.312 μM. In conclusion, these overall results shown by 3-substituted 7-PPyQs further increase our interest in PPyQs and prompts us to carry on mechanicistic studies to better characterize biological targets at the molecular level.

Materials and Methods

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 using potassium bromide pressed disks; all 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 a Bruker (300 MHz) spectrometer, 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, chemical shift was measured starting from the approximate center. Integrals were satisfactorily in line with those expected on the basis of compound structure. Elemental analyses were performed in the Microanalytical Laboratory, Department of Pharmaceutical Sciences, University of Padova, on a model 240B Perkin-Elmer elemental analyzer model; results fell in the range of calculated values ±0.4%. Analytical data are presented in detail for each final compound. Mass spectra were obtained on a Applied Biosystems Mariner System 5220 LC/Ms (nozzle potential 250.00). Column flash chromatography was performed on Merck silica gels (250−400 mesh ASTM). Chemical reactions were monitored by analytical thin-layer chromatography (TLC) with Merck silica gel 60 F-254 glass plates with a 9:1 dichloromethane/methanol mixture as eluant, unless otherwise specified. Solutions were concentrated in a rotary evaporator under reduced pressure. Starting materials were purchased from Aldrich Chimica and Fluka Riedel-de Haen ($TiCl_3$, 15% HCl solution), and solvents were purchased from Carlo Erba, Fluka, and Lab-Scan. DMSO was made anhydrous by distillation under vacuum and stored on molecular sieves. Colchicine, vincristine, and taxol were purchased from Sigma.

General Procedure for the Synthesis of 1-Substituted 5-Nitroindoles 1−3. In a three-necked, 100 mL, round-bottomed flask, an amount corresponding to 37−55.5 mmol of NaH (60% dispersion in mineral oil) was placed and washed with toluene (3 × 10 mL). A solution of commercial 5-nitro-indole (12.3−18.5 mmol) in 20−30 mL of dry DMF was dropped into the flask, and the starting yellow solution changed to red, with the formation of H_2 gas. After 30 min at room temperature, a solution of halo-derivative in dry DMF was added, and the reaction mixture was left under stirring for 20−48 h, monitoring ongoing reactions by TLC analysis (eluent: toluene/*n*-hexane/ethyl acetate 1:1:0.3). At the end, the solvent was evaporated and the residue was extracted with ethyl acetate; the organic phase, washed with water and dried over anhydrous Na_2SO_4, was concentrated until dryness under vacuum. The raw products were purified by flash chromatography.

General Procedure for the Synthesis of 1-Substituted 5-Nitroindoles 4−8. To a stirring solution of 1 g (6.2 mmol) of commercial 5-nitro-indole in 30 mL of acetone, 1.728 g powdered KOH at 0 °C was added, giving a dark-red color, and then an excess of halo-derivative (12.3 mmol) was added. The stirred mixture was heated at 40 °C for 3 h, until the starting material disappeared at TLC analysis (toluene/*n*-hexane/ethyl acetate 1:1:0.3). After cooling to room temperature, 200 mL of toluene was added and the formed suspension was left under stirring for 30 min. The insoluble solid was then filtered off and the filtrate was washed several times with NaCl−saturated water, dried over anhydrous Na_2SO_4, and evaporated to dryness, yielding oily or solid reddish-orange products. These were used in the following step without any purification.

General Procedure for the Synthesis of 1-Substituted 5-Aminoindoles 9−16. A solution of a nitro-indole **1−8** (5.87 mmol) in 400 mL of ethanol was dropped into a suspension of 10% Pd/C (125 mg) saturated with H_2 in ethanol (200 mL). The mixture was stirred at room temperature with hydrogen at atmospheric pressure for 3 h, according to TLC analysis (ethyl acetate/*n*-hexane 1:3). The catalyst was then filtered off, and the filtrate was evaporated under reduced pressure to dryness to give the corresponding amino-indole. The almost pure residue (90% yield) was used in the next reaction without purification.

General Procedure for the Synthesis of Ethyl 3-(1-Substituted-indole-amino)-3-phenyl-acrylates 17−24. In a 50 mL round-bottomed flask, 3−4 mmol of 3-substituted-aminoindole **9−16** in 10−20 mL of absolute ethanol was condensed with an equimolar quantity of commercial ethyl benzoylacetate and with 1 mL of glacial acetic acid and 100 mg drierite. The mixture was refluxed for about 24 h, with the reaction being monitored by TLC analysis (dichloromethane/ethyl acetate 9:1). As the reaction did not come to completion, after 24 h, the mixture was cooled and filtered to remove the drierite; the resulting solution was evaporated to dryness under vacuum, and the residue was purified by flash chromatography and then recrystallized from a suitable solvent.

General Procedure for the Synthesis of 3-Substituted 7-Phenyl-3*H*-pyrrolo[3,2-*f*]quinolin-9-(6*H*)ones 25−32. In a two-necked, round-bottomed flask, 50 mL of diphenyl ether was heated to boiling temperature; 1−2 mmol of acrylates **17−24** were then added portionwise, and the mixture was refluxed for 30 min. After cooling to 60 °C, the separated precipitate was collected by filtration and washed many times with diethyl ether. In all cases, the collected products were purified by flash chromatography (ethyl acetate/methanol 9:1).

In Vitro Cytotoxicity Assay. The cytotoxic activity of 3-alkylated 7-PPyQs **25−32** was determined using a standard (MTT)-based colorimetric assay (Sigma), using vincristine and taxol as

reference drugs. Briefly, cell lines were seeded at a density of 7×10^3 cells/well in 96-well microtiter plates (Costar). After 24 h, exponentially growing cells were exposed to the indicated compounds at final concentrations ranging from 0.005 to 100 μM. After 72 h, cell survival was determined by the addition of an MTT solution (10 μL of 5 mg/mL MTT in PBS). After 4 h, 100 μL of 10% SDS in 0.01 N HCl was added, and the plates were incubated at 37 °C for a further 18 h; optical absorbance was measured at 550 nm on an LX300 Epson Diagnostic microplate reader. Survival ratios are expressed in percentages with respect to untreated cells. IC_{50} values were determined from replicates of 6−8 wells from at least two independent experiments.

In Vitro Cytotoxic Activity against Taxol Human Tumor A549 Sublines. Exponentially growing cells were resuspended in either drug-free medium (A549) or in presence of 12 nM (A549-T12) or 24 nM (A549-T24) taxol. The resistant cells require taxol to maintain normal cell proliferation. Cells were seeded at a density of 10^4 cells/mL (A549) or 3×10^4 cells/mL (A549-T12 and A549-T24) in triplicate six-well plates and allowed to attach for 24 h before the addition of the indicate drug concentration. After a 72 h incubation, cells were trypsinized and counted.

Fluorescence-Activated Cell-Sorting Analysis. A549 cells were cultured for 24 h in a drug-free medium or supplemented with compounds **25**−**32** (0.1−1 μM) and vincristine (0.1 μM). As previously described,[37,38] cells were trypsinized with a cell scraper, washed twice with PBS, and fixed in 70% cold ethanol (30 min at −20 °C). Cells (10^6) were then washed once in citrate phosphate buffer (0.2 N Na_2HPO_4 and 0.1 M citric acid, 24:1), followed by PBS, and finally incubated in a RNAse solution (100 μg/mL in PBS). After 30 min at 37 °C, the cells were incubated in a propidium iodide solution (PI, Sigma, 100 μg/mL in PBS) at room temperature for a further 30 min. To determine the effects of compounds on cell cycle dynamics, DNA fluorescence was measured by flow cytometry, examining at least 15 000 events with Lysis II software (Becton Dickinson, Franklin Lakes, NJ) at 488/525 nm (excitation/emission wavelengths). All experiments were repeated 3−4 times and DNA content analysis was carried out on both logarithmic and linear scales. Results were comparable, irrespective of the scale used, and are shown on a logarithmic scale.

Statistical Analysis. Results are reported as means ± standard error (M ± S.E.). Statistical analysis was performed by one-way analysis of variance or Student's *t*-test, as appropriate. A *P* value of less than 0.05 was considered statistically significant.

Supporting Information Available: Yields, mps, rfs, spectral data, cell lines, culture conditions, and FACS analysis histograms for all compounds **1**−**32**. This material is available free of charge via the Internet at http:// pubs.acs.org.

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