# New C5-Alkylated Indolobenzazepinones Acting as Inhibitors of Tubulin Polymerization: Cytotoxic and Antitumor Activities

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A series of 5-alkylindolobenzazepin-7-ones was synthesized by Suzuki coupling between 3-iodoindole-2-carboxylates and the appropriate α-alkylbenzylamino *o*-boronic acids followed by cyclization to the lactam. Derivatives having a linear alkyl chain at C5 were found to be highly cytotoxic to KB cells with $IC_{50}$ values in the 30−80 nM range. These compounds also inhibited the polymerization of tubulin with $IC_{50}$'s of 1−2 μM. Compound **4f** ((*S*)-5-ethyl) showed comparable antiproliferative activities ($IC_{50}$'s of 30−70 nM) in a variety of cancer cell lines, cell growth being arrested at the G2/M phase. Compound **4f** induced apoptosis in a dose-dependent manner in three different cancer cell lines and was shown to affect cell morphology in a manner consistent with its inhibitory action on tubulin polymerization. Using the experimental model of glioma grafted on the chick chorio-allantoic membrane, local treatment with compound **4f** markedly reduced tumor progression.

## Introduction

Microtubules are important for the formation of the mitotic spindle during the process of mitosis. They have a major role to play in maintaining the growth, division, and functioning of both normal and cancer cells as well as being implicated in motility, shape maintenance, and intracellular transport.[1] Microtubules are formed by polymerization of two closely related heterodimers composed of α- and β-tubulin subunits. Interference with the dynamic equilibrium between the assembly of tubulin into microtubules or, inversely, the depolymerization of microtubules into tubulin leads to arrested cell division and eventually to apoptosis. Compounds that can inhibit either of these important processes are thus referred to as spindle poisons or antimitotics.[2,3]

The search for selective inhibitors of tubulin assembly or disassembly has led to the development of some of the most clinically useful antitumor drugs currently in use.[4,5] In the first group are the naturally occurring *Vinca* alkaloids vincristin and vinblastin as well as their synthetic analogues (e.g., navelbine).[6a] The taxoids (taxol, taxotere), which are inhibitors of microtubule depolymerization, are widely used for the treatment of breast, ovarian, and nonsmall cell lung carcinomas.[6b] Epothilone[6c] and discodermolide[6d] are also two potent microtubule-stabilizing agents that have undergone intensive clinical evaluation. However, these complex molecules are generally difficult to synthesize, are toxic, and become rapidly prone to resistance phenomena.[2–5]

The antitumor action of colchicine (**1**, Chart 1), a natural product isolated from *Colchicum autumnale L.*, has been known for a long time.[7] This compound, which acts as an inhibitor of tubulin polymerization,[8] binds to β-tubulin at the interface with α-tubulin, a site different from that of the *Vinca* alkaloids.[9,10] However, its narrow therapeutic index precludes its clinical use.[11] A number of other compounds are known to bind to the colchicine site of tubulin and consequently display cytotoxic and antitumor properties.[11b] These include synthetic *N*-acetylcolchinol (NAC,[a] **2a**) and its water-soluble phosphate prodrug form **2b** (ZD6126),[12] which are structurally simpler analogues of colchicine, their recently described heterocyclic analogues **2c**,[13] as well as the natural products combretastatin A-4 (**3**) and podophyllotoxin.[14] Interestingly, while a wide variety of analogues of these compounds can be accessed relatively easily, no compounds binding to the colchicine site have yet found their way into the clinic as anticancer agents.[5]

The indole nucleus is a structural component of a wide variety of antimitotic compounds.[15] We thus decided to investigate the effect of replacing one of the phenyl rings of the antimitotic compounds of type **2** by an indole moiety by synthesizing 5-substituted indolobenzazepinones of general structure **4**. We describe herein the preparation of these compounds and report their potent cytotoxic and antimitotic properties, which allow us to conclude that 5-alkylindolobenzazepinones represent a new class of antimitotic agents demonstrating antitumor activity.

## Chemistry

The general strategy for the synthesis of the indolobenzazepinones of type **4** consisted of Suzuki coupling of the *o*-boronic acid of an appropriately substituted benzylamine with a 3-iodoindole-2-carboxylate, followed by intramolecular cyclization after amine deprotection. Thus, the aminobenzyl *o*-boronic acids **7a**−**i** were first prepared as shown in Scheme 1. Benzylamine (**5a**) and the commercially available racemic or optically pure α-methyl and α-ethyl benzylamines (**5b**−**5f**) were protected as their *N*-Boc derivatives **6a**−**6f**. The noncommercial, racemic α-*n*-propyl-, α-*n*-butyl-, and α-*i*-propylbenzylamines **5g**−**5i**, respectively, were prepared by Leuckart reductive amination[16] of the precursor ketones, which were subsequently *N*-Boc protected to give **6g**−**6i**. These *N*-Boc-benzylamines were then ortho-lithiated using *t*-butyllithium in THF and reacted with

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[a] Abbreviations: CAM, chorio-allantoic membrane; DMEM, Dulbecco minimal essential medium; EGTA, [ethylene bis(oxyethylenenitrilo)]tetraacetic acid; EMEM, Eagle's minimal essential medium; FCS, fetal calf serum; GTP, guanosine triphosphate; MES, 2-(*N*-morpholino)ethanesulfonic acid; NAC, *N*-acetylcolchinol; PBS, phosphate buffered saline.

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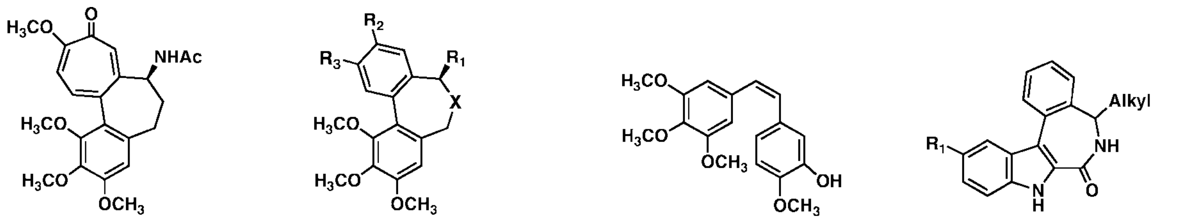
**Chart 1.** Structures of Various Known Antimitotic Agents and of the 5-Alkyl Indolobenzazepinones



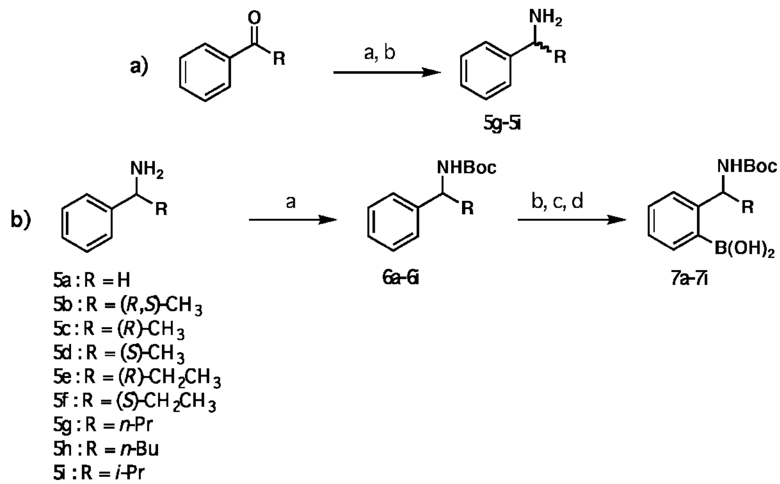
1: Colchicine

2a: X = $CH_2$; $R_1$ = NHAc; $R_2$ = OH; $R_3$ = H: N-Acetylcolchinol (NAC)
2b: X = $CH_2$; $R_1$ = NHAc; $R_2$ = OP(=O)$OH_2$, $R_3$ = H: ZD6126
2c: X = O; $R_1$ = Et; $R_2$, $R_3$ = $OCH_2O$

3: Combretastatin A-4

4: 5-Alkyl indolobenzazepinones

**Scheme 1**[a]




[a] Reagents and conditions (for reaction (a)): (a) $HCO_2H$, $HCONH_2$, 180 °C, 16 h; (b) aq EtOH, HCl, reflux, 8 h. Reagents and conditions (for reaction (b)): (a) $Boc_2O$, $Et_3N$, DCM, room temperature, 24 h; (b) *t*-BuLi (2.8 eq), THF, −78 °C to −20 °C, 2.5 h; (c) $B(OMe)_3$ (5 eq), −20 °C to room temperature, 2 h; (d) 5% aq HCl, 0 °C.

trimethylborate to give the corresponding *o*-boronates.[17] Hydrolysis of these products with aqueous HCl afforded the desired boronic acids **7a**−**7i**, generally mixed with unreacted starting material, which were then used for the subsequent coupling reaction without purification.

The required 3-iodoindole-2-carboxylates **9** were prepared as shown in Scheme 2. Commercial indole 2-carboxylic acids **8a**−**8c** were first transformed into the corresponding ethyl esters. These were reacted with iodine in DMF in the presence of potassium hydroxide, affording the 3-iodoindole-2-carboxylate derivatives in good yields.[18] The indole nitrogen of each compound was then protected with a benzenesulfonyl group to give **9a**−**9c**. The latter were subjected to a palladium-catalyzed Suzuki coupling reaction[19] with the benzylboronic acids **7a**−**7i** to give the corresponding C3 coupled products **10a**−**10k** in yields ranging from 42 to 81%. The benzylamino group of compounds **10** was then deprotected with trifluoroacetic acid in dichloromethane. Treatment of the resulting amines with sodium in ethanol led to cyclization with concomitant deprotection of the indole nitrogen, providing the desired indolobenzazepinones **4a**−**4k**.

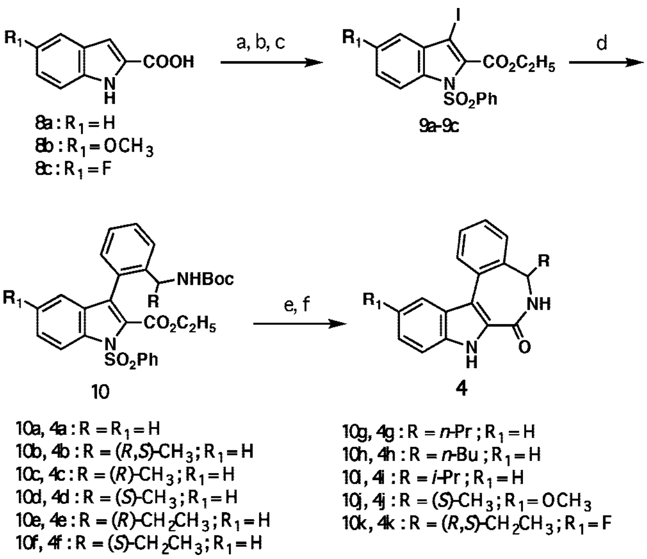
To study the importance of the carbonyl group of the benzazepinone ring for the activity of these compounds, compound **4b** was transformed into its thiocarboxamide analogue **11** using Lawesson's reagent (Scheme 3), while reduction of the carbonyl group of **4b** with lithium aluminum hydride/aluminum chloride afforded the benzazepine derivative **12.**

The effect on activity of eliminating one or both of the NH groups of the indolobenzazepinones was evaluated by preparing first the di-*N*-methylated derivative **13** via reaction of **4b** with sodium hydride and excess methyl iodide. Selective methylation of the indole nitrogen to give **14** was achieved using methyl iodide under phase transfer conditions.

## Results and Discussion

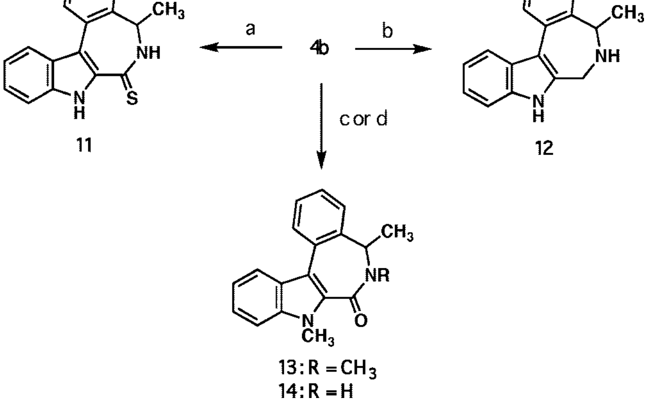
**In Vitro Cell Growth Inhibition.** The cytotoxic properties of the various indolobenzazepinone derivatives synthesized (**4a**−**4k**, **11**−**14**) were first evaluated on KB cells (Table 1). While the completely unsubstituted compound **4a** displayed only modest activity ($IC_{50}$ = 0.550 $\mu$M),[20] introduction of a racemic methyl group at C5 (compound **4b**) led to a doubling of cytotoxicity ($IC_{50}$ = 0.280 $\mu$M). When the two 5-methyl enantiomers were tested separately, it was found that the (*S*)-isomer **4d** was considerably more active than the (*R*)-isomer **4c** ($IC_{50}$ = 0.080 and 0.340 $\mu$M, respectively). Replacement of the methyl group by an ethyl group also resulted in higher cytotoxicity, although this time there was not a considerable difference between the (*R*)- and the (*S*)-isomers **4e** ($IC_{50}$ = 0.035 $\mu$M) and **4f** ($IC_{50}$ = 0.037 $\mu$M), respectively. Elongation of the alkyl chain at C5 had little incidence on activity. Thus, both

**Scheme 2**[a]




[a] Reagents and conditions: (a) EtOH, cat. $H_2SO_4$, reflux, 16 h; (b) KOH, $I_2$, DMF, room temperature, 45 min; (c) $PhSO_2Cl$, NaH, THF, room temperature, 16 h; (d) **7a**−**7i** (1.2 eq), $Pd(PPh_3)_4$ (10 mol%), $Na_2CO_3$ (2 eq), toluene−EtOH (1:1), reflux, 20 h; (e) TFA, DCM, room temperature, 16 h; (f) Na/EtOH, reflux, 10 h.

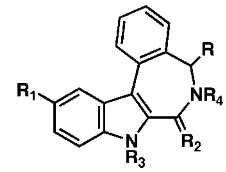
**Scheme 3**[a]




[a] Reagents and conditions: (a) Lawesson's reagent, toluene, reflux, 30 min; (b) $AlCl_3$ (0.5 eq), $LiAlH_4$ (1 eq), THF, 4 h at 60 °C then 12 h at reflux; (c) NaH (4 eq), $CH_3I$ (6 eq), THF, room temperature, 2 h; (d) $CH_3I$ (10 eq), cat. TBAB, toluene, $H_2O$, room temperature, 2 h.

the racemic *n*-propyl (**4g**) and *n*-butyl (**4h**) homologues were as cytotoxic ($IC_{50}$ = 0.032 and 0.035 $\mu$M, respectively) as the C5 ethyl compounds **4e**,**f**. These activities are comparable to those of colchicine (**1**, $IC_{50}$ = 0.020 $\mu$M) and NAC (**2a**, $IC_{50}$ = 0.075 $\mu$M) in the same cell line. However, a more sterically demanding alkyl group at this position was less active, as demonstrated by the isopropyl analogue (**4i**) ($IC_{50}$ = 0.280 $\mu$M). Small substituents at the C11 position of the more active indolobenzazepinone derivatives did not enhance the biological activity. Thus, both the 5-methyl-11-methoxy and the 5-ethyl-11-fluoro analogues (**4j** and **4k**, respectively) were considerably less cytotoxic ($IC_{50}$ = 3.50 and 0.500 $\mu$M) than their C11 nonsubstituted counterparts **4d** and **4e**,**f** ($IC_{50}$ = 0.080 and 0.035 $\mu$M). With regard to the carboxamide function, its replacement by a thiocarboxamide had a small detrimental effect on activity (**11**, $IC_{50}$ = 0.360 $\mu$M compared to **4b**, $IC_{50}$ = 0.280 $\mu$M), while its reduction to an amine practically abolished the cytotoxicity (**12**, $IC_{50}$ = 12 $\mu$M). Finally, methylation of both nitrogen atoms

**Table 1.** Cytotoxic Effects of Compounds **4a**−**4k** and Derivatives with Respect to KB cells[a] and Inhibition of Tubulin Polymerization by Selected Compounds




| compd | R | $R_1$ | $R_2$ | $R_3$ | $R_4$ | cytotoxicity $IC_{50}$ [$\mu$M][b] | ITP[c] $IC_{50}$ [$\mu$M][d] |
|---|---|---|---|---|---|---|---|
| **4a** | H | H | O | H | H | 0.550 | nd[e] |
| **4b** | Me | H | O | H | H | 0.280 | nd |
| **4c** | (*R*)-Me | H | O | H | H | 0.340 | nd |
| **4d** | (*S*)-Me | H | O | H | H | 0.080 | 1.9 |
| **4e** | (*R*)-Et | H | O | H | H | 0.035 | 1.8 |
| **4f** | (*S*)-Et | H | O | H | H | 0.037 | 1.9 |
| **4g** | *n*-Pr | H | O | H | H | 0.032 | 2.0 |
| **4h** | *n*-Bu | H | O | H | H | 0.035 | 1.0 |
| **4i** | *i*-Pr | H | O | H | H | 0.280 | 1.7 |
| **4j** | (*S*)-Me | OMe | O | H | H | 3.50 | 51 |
| **4k** | Et | F | O | H | H | 0.500 | 3.6 |
| **11** | Me | H | S | H | H | 0.360 | nd |
| **12** | Me | H | 2H | H | H | 12.0 | nd |
| **13** | Me | H | O | Me | Me | 28.0 | nd |
| **14** | Me | H | O | Me | H | 7.0 | nd |
| **colchicine (1)** | | | | | | 0.020 | 2.2 |
| **NAC (2a)** | | | | | | 0.075 | 3.0 |

[a] KB = cervical carcinoma cells. [b] $IC_{50}$ is the concentration of compound inducing 50% cell growth inhibition after 72 h incubation. [c] Inhibition of tubulin polymerization. [d] $IC_{50}$ is the concentration of compound required to inhibit 50% of the rate of microtubule assembly; values representative of three experiments. [e] Not determined.

(**13**) or only the indole nitrogen (**14**) led to severe loss of activity ($IC_{50}$ = 28 and 7 $\mu$M, respectively).

While compounds **4e**−**4h** all present essentially the same cytotoxic activities, compound **4f** was selected for further study because of its availability in enantiomerically pure form from commercially available **5f**. The cytotoxic activity of **4f** in a variety of cancer cell lines was first determined and in some cases compared to that of colchicine (**1**) and NAC (**2a**) (Table 2). Thus, the cytotoxicity of **4f** in all cell lines was of the same order of magnitude as in KB cells, $IC_{50}$'s ranging from 0.030 $\mu$M in MDA-MB231 cells to 0.070 $\mu$M in MDA-MB435 and K562 cells, thus clearly indicating an antiproliferative activity regardless of the origin of the tumor cells. Compound **4f** was as active as colchicine in A549 and HCT116 cells but about twice less active in B16F10 and MDA-MB435 cells. Inversely, **4f** was over twice as cytotoxic to MDA-MB231 than colchicine. On the other hand, **4f** was 2-fold more active than NAC in MDA-MB435 and HCT116 cells, 5-fold more active in A549 cells and over 20 times more active in MDA-MB231 cells, while the cytotoxicities of these two compounds were identical in B16F10 cells ($IC_{50} \approx 0.070$ $\mu$M).

**Effect on Cell Cycle Progression.** The effect of compound **4f** on the cell cycle of K562, MCF7, and MDA-MB231 cells was then investigated by flow cytometry at various concentrations (Table 3). After 24 h treatment with 0.05 $\mu$M of **4f**, a net increase in the number of cells arrested at the G2/M growth phase was observed. Increasing the concentration of **4f** to 0.1 $\mu$M led to essentially the same result in K562 and MCF7 cells (20.3% and 62.6% in G2/M phase, respectively), while practically all MDA-MB231 cells were arrested in the G2/M phase at this concentration. A concentration of 0.5 $\mu$M of compound **4f** was required to arrest 44.7% of K562 cells in the G2/M phase. In K562 cells, the percentage of cells arrested in the G2/M phase tended to increase with concentration of **4f** (0.05, 0.1, 0.5 $\mu$M) regardless of length of treatment (6, 16, 24 h) (see Supporting

**Table 2.** Antiproliferative Activities of **4f**, **1**, and **2a** against Various Cancer Cell Lines after 72 h Treatment ($IC_{50}$'s, $\mu$M)[a]

| compound | B16F10 | A549 | MDA-MB435 | MDA-MB231 | MCF7 | L1210 | K562 | HT29 | HCT116 |
|---|---|---|---|---|---|---|---|---|---|
| **4f** | 0.068 | 0.050 | 0.070 | 0.030 | 0.040 | 0.034 | 0.070 | 0.042 | 0.042 |
| colchicine (**1**) | 0.029 | 0.045 | 0.040 | 0.070 | nd[b] | nd | nd | nd | 0.040 |
| NAC (**2a**) | 0.072 | 0.250 | 0.140 | 0.700 | nd | nd | nd | nd | 0.100 |

[a] $IC_{50}$ is the concentration of test compound inducing 50% cell growth inhibition after 72 h incubation; cell lines: B16F10 = skin melanoma, A549 = human nonsmall cell lung cancer, MDA-MB435, MDA-MB231, MCF7 = breast cancer, L1210, K562 = leukemia, HT29, HCT116 = human colon cancer. [b] Not determined.

**Table 3.** Percentage of K562, MCF7, and MDA-MB231 Cancer Cells in the G2/M Phase after 24 h of Treatment with Compound **4f**[a]

| concentration of **4f** ($\mu$M) | K562 (% G2/M) | MCF7 (% G2/M) | MDA-MB231 (% G2/M) |
|---|---|---|---|
| control | 3.9 | 10.7 | 25.9 |
| 0.05 | 24.9 | 65.6 | 44.5 |
| 0.1 | 20.3 | 62.6 | ~100 |
| 0.5 | 44.7 | nd[b] | nd |

[a] Data are representative of three independent experiments. [b] Not determined.

Information). In all cells treated with **4f** for 24 h, a subdiploid DNA content was observed (data not shown), indicating that cells are undergoing apoptosis, probably as a result of the cell cycle being arrested in the G2/M phase.[21]

**Effect on Apoptosis.** The ability of compound **4f** to induce apoptosis was further characterized by a specific apoptosis assay. Cleavage of pro-caspases to active caspases is one of the hallmarks of apoptosis. K562, HCT116, and MDA-MB231 cell lines were thus treated with 0.01, 0.1, and 1 $\mu$M of compound **4f** and caspase 3 and 7 activities were evaluated using the standard caspase cleavage assays (Figure 1). It was observed that, after 24 h of treatment, **4f** induced apoptosis in all three investigated cell lines. In particular, a 6-fold increase in apoptosis occurred in K562 leukemia cells previously described as being resistant to apoptosis induction by a variety of agents including diphtheria toxin, camptothecin, cytarabine, etoposide, paclitaxel, staurosporine, and antifas antibodies.[22–27] These results show that treatment of cancer cells with compound **4f** activates caspases leading to cellular apoptosis.

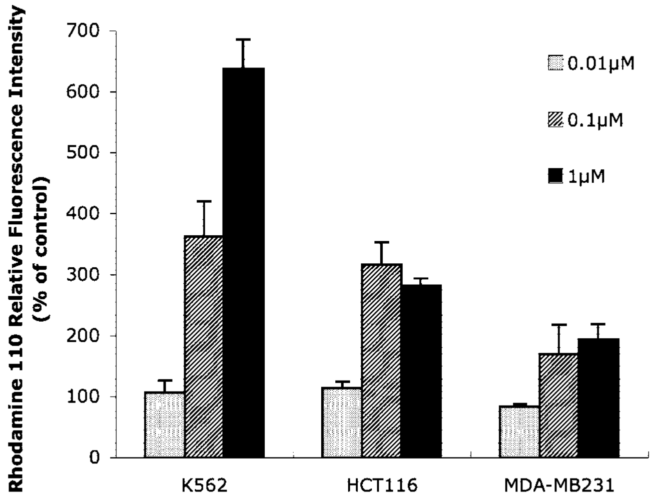



**Figure 1.** Induction of apoptosis in three different cancer cell lines by 24 h treatment with **4f** as a function of concentration.

**In Vitro Tubulin Polymerization Inhibition.** The structural resemblance of the indolobenzazepinones with colchicine (**1**) and NAC (**2a**) as well as the observation that **4f** arrests the cell cycle at the G2/M phase[21] encouraged us to investigate the ability of the more cytotoxic compounds of type **4** to inhibit tubulin polymerization. Indeed, the high cytotoxicities of compounds **4d**−**4h** are well correlated with their ability to effectively inhibit tubulin polymerization, their $IC_{50}$'s all being in the 1−2 $\mu$M range and thus comparable to the inhibitory activity of colchicine and NAC ($IC_{50}$ = 2.2 and 3.0 $\mu$M, respectively, Table 1). On the other hand, compound **4i** displayed cytotoxicity, which was 10-fold less than the most active compounds yet inhibited tubulin polymerization with the same order of potency ($IC_{50}$ = 1.7 $\mu$M). This may reflect the fact that compound **4i**, while effectively binding to tubulin in vitro, has difficulty penetrating into cells, a prerequisite for cytotoxicity. The methoxy derivative **4j** displays very weak cytotoxicity with respect to KB cells ($IC_{50}$ = 3.5 $\mu$M) and a correspondingly high $IC_{50}$ for inhibition of tubulin polymerization (51 $\mu$M). This is compatible with tubulin being the principal target of these C5-alkylated indolobenzazepinones. Interestingly, tubulin polymerization inhibition and, to a lesser extent, cytotoxicity, are restored by replacing the methoxy group of **4j** by a fluoride (**4k**, $IC_{50}$ = 3.6 and 0.500 $\mu$M with respect to tubulin activity and cytotoxicity, respectively).

**Effect on Cell Morphology.** Because microtubules as well as microfilaments are essential for cell division and their disruption can induce G2/M arrest and apoptosis, triple fluorescence labeling was employed to analyze cell morphology. As shown in Figure 2A (60× magnification), 24 h after treatment with **4f** at 0.050 $\mu$M, MDA-MB231 cells differed in size and shape, the cell mass became smaller, and the cell body was shrunken. Some of the exposed cells were binucleated, multinucleated, or micronucleated, indicating dividing failure and mitotic catastrophe (Figure 2B, 100× magnification). Notably, the cells had altered fibrillar array of actin filaments, and it was clearly seen that **4f** caused abnormal cellular microtubule organization and arrangement in these cells, further evidence that the cytotoxic effects of this compound are the result of its interaction with tubulin. Similar observations have been made by others using tubulin-disrupting agents.[28,29]

**Antitumor Effect.** Finally, the effect of compound **4f** on tumor growth was determined in vivo and compared to the effects of colchicine and NAC. Human glioma growth was assessed using an avian embryo model. The experimental glioma grafted on the chick chorio-allantoic membrane (CAM) replicates characteristics of the human disease and allows rapid testing of new anticancer drugs.[30] Local treatment of size-matched glioma from day 3 to day 6 with single daily doses (10 $\mu$M) of **4f** markedly inhibited tumor progression (Figure 3). After 4 days of treatment, tumor volume decreased by 76% while the control tumor volume increased by 20%. By comparison, colchicine (10 $\mu$M) and NAC (100 $\mu$M) decreased tumor volume by only 50% and 42%, respectively, after 4 days of treatment.

## Conclusion

We have shown that 5-alkylindolobenzazepin-7-ones of general structure **4** are potent antiproliferative agents across a wide variety of cancer cell lines. The observed cytotoxic effects are most probably due to interaction with tubulin. This is indicated by equipotency of **4f** with the antimitotics colchicine

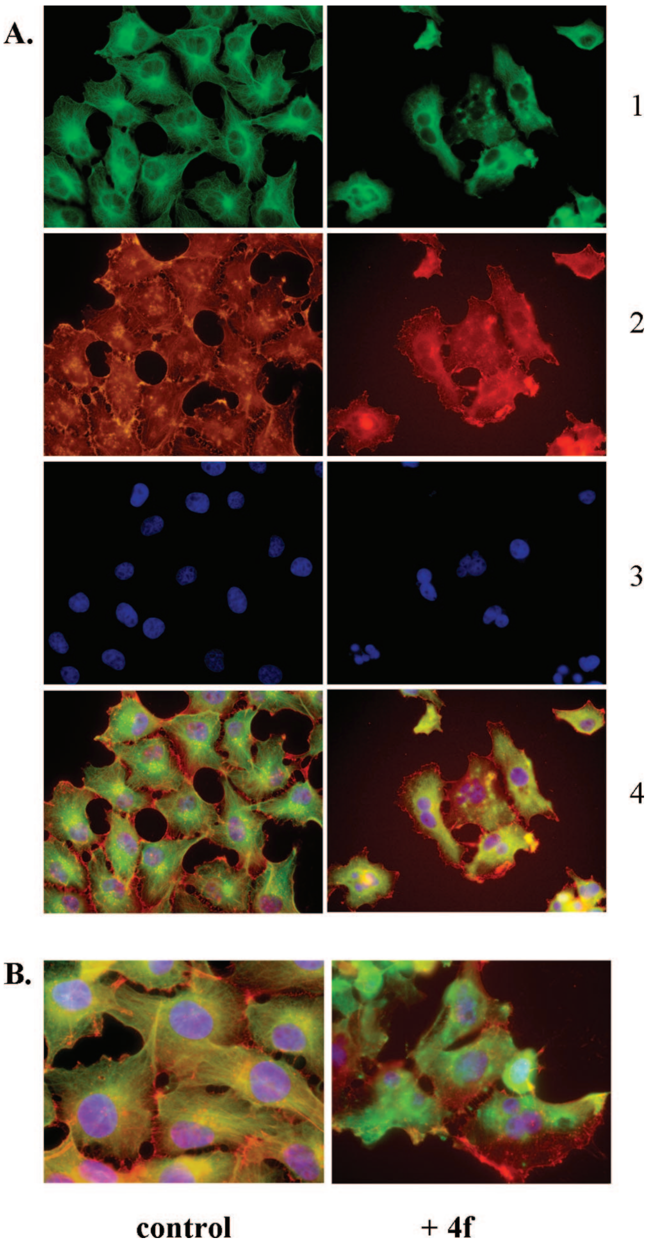



**Figure 2.** Effect of compound **4f** (50 nM) on the morphology of MDA-MB231 cells after 24 h of treatment. (1) tubulin, (2) actin, (3) nucleus, (4) merged image; (A) 60× magnification; (B) merged image of MDA-MB231 cells (100× magnification).

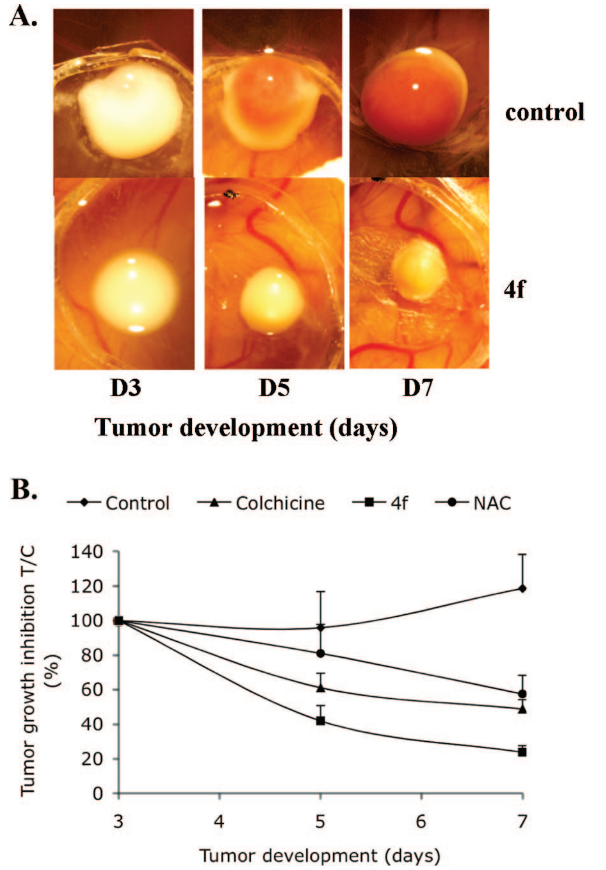



**Figure 3.** In vivo effect of compound **4f** on the size of a human glioma (U87) tumor transplanted in the chick chorio-allantoic membrane (CAM). (A) Microscopic images of tumor size regression produced by treatment with 10 $\mu$M of compound **4f** 3 days (D3), 5 days (D5), and 7 days (D7) after tumor transplant (bottom) compared to control (top) which received no treatment. (B) Percentage of tumor regression caused by local treatment with compound **4f** (10 $\mu$M, ■), colchicine (10 $\mu$M, ▲), and NAC (100 $\mu$M, ●) compared to control (◆).

and NAC in inhibiting tubulin polymerization, by the interruption of cancer cell growth at the G2/M stage in the presence of **4f**, and by the morphological changes seen in cancer cells treated with this compound typical of antimitotic agents. The ability of **4f** to promote apoptosis in the cancer cells studied was also clearly demonstrated. Finally, these in vitro observations were confirmed in vivo whereby compound **4f**, used in a CAM model of tumor growth at concentrations inhibiting tubulin polymerization, was significantly more effective than colchicine and NAC in causing tumor volume regression after only several days of treatment. Further structure–activity studies in this series are in progress as are in vivo studies in xenografted mice.

## Experimental Section

**Chemistry.** Melting points, measured in capillary tubes and recorded using a Büchi B-540 melting point apparatus, are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrometer. Optical rotations were determined with a JASCO P-1010 polarimeter. Proton ($^1$H) and carbon ($^{13}$C) NMR spectra were recorded on Bruker spectrometers: AC 250 (250 MHz), Aspect 3000 (300 MHz), Avance 300 NMR or 500 NMR (respectively 300 and 500 MHz). Chemical shifts ($\delta$) are reported in parts per million (ppm) with reference to tetramethylsilane (TMS) as internal standard. NMR experiments were carried out in deuterochloroform ($CDCl_3$) or in deuterobenzene ($C_6D_6$). The following abbreviations are used for the proton spectra multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; qu, quintet; m, multiplet; td, triplet of doublets; tt, triplet of triplets; sept, septet. Coupling constants ($J$) are reported in Hertz (Hz). Mass spectra were obtained either with an AEI MS-9 instrument using electron spray (ESI-MS) or with a MALDI-TOF instrument for high resolution mass spectra (HREIMS). Thin-layer chromatography was performed on silica gel 60 plates with a fluorescent indicator and visualized under a UVP Mineralight UVGL-58 lamp (254 nm) and with a 7% solution of phosphomolybdic acid in ethanol. Flash chromatography was performed using silica gel 60 (40–63 $\mu$m, 230–400 mesh ASTM) at medium pressure (200 mbar). All solvents were distilled and stored over 4 Å molecular sieves before use. All reagents were obtained from commercial suppliers unless otherwise stated. Organic extracts were, in general, dried over magnesium sulfate ($MgSO_4$) or sodium sulfate ($Na_2SO_4$). Elemental analyses were performed at the ICSN, CNRS, Gif-sur-Yvette, France.

Complete procedures for the preparation of compounds **4f**, **11**–**14** are given below. For general procedures and characterization of the other compounds, see Supporting Information.

**(*S*)-*N*-(*tert*-Butyloxycarbonyl)-α-ethylbenzylamine (6f).** A solution of di-*tert*-butyl dicarbonate (4.58 g, 21 mmol) in dry DCM (11 mL) was added under argon at 0 °C to a solution of (*S*)-α-ethylbenzylamine **5f** (2.32 g, 15 mmol) and triethylamine (2.09 mL, 15 mmol) in dry DCM (33 mL). The reaction mixture was stirred at room temperature for 24 h. The solvent was removed in vacuo, and the residue was dissolved with DCM (60 mL) and water (40 mL). The aqueous layer was extracted with DCM (3 × 50 mL). The combined organic extracts were washed with water (25 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting white solid was purified by flash chromatography on silica gel (heptane/AcOEt 9/1) to give compound **6f** in 73% yield as a white solid; mp 68 °C ($Et_2O$). $^1H$ NMR (250 MHz, $CDCl_3$) δ: 7.33 (m, 5H), 4.92 (m, 1H), 4.65 (m, 1H), 1.83 (m, 2H), 1.47 (s, 9H), 0.95 (t, $J = 7.5$ Hz, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 155.3, 142.9, 128.4, 127.0, 126.3, 79.3, 56.3, 29.8, 28.3, 10.6. FTIR ($\nu$/cm$^{-1}$) 3384, 1682, 1517. $[\alpha]_D = -44.6$ ($CHCl_3$, $c = 6.06$). Anal. ($C_{14}H_{21}NO_2$) C, H, N.

**(*S*)-*N*-(*tert*-Butyloxycarbonyl)-α-ethylbenzylamino-2-boronic acid (7f).** *tert*-Butyllithium (4.94 mL, 8.4 mmol, 1.7 M in pentane) was added under argon at −78 °C to a solution of **6f** (3 mmol) in dry THF (15 mL). The reaction mixture was allowed to warm to −20 °C for 2.5 h. Trimethylborate (2 mL, 15 mmol) was added at −20 °C, and the reaction was allowed to warm to rt for 2 h. A 5% aqueous solution of HCl was added at 0 °C with vigorous stirring until a pH = 5−6 was attained (≈ 3 mL). The aqueous layer was extracted with DCM (3 × 25 mL). The combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to give an approximately 1:1 mixture of boronic acid **7f** and starting material **6f** (as estimated by NMR). The crude boronic acid was used in the following step without further purification.

**Ethyl 1-(Benzenesulfonyl)-1*H*-3-iodoindole-2-carboxylate (9a).** Sulfuric acid (2 mL) was added to a solution of indole-2-carboxylic acid **8a** (3.22 g, 20 mmol) in absolute ethanol (60 mL). The reaction mixture was refluxed overnight. After cooling to rt, the solvent was removed under vacuum, the residue was dissolved in DCM (50 mL), and the resulting solution was washed with a 1 M aqueous solution of $NaHCO_3$ (3 × 20 mL) and water (2 × 20 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to give the corresponding ethyl ester as a solid. The ethyl ester (3.59 g, 19.0 mmol) was dissolved in DMF (11 mL) and potassium hydroxide (4.05 g, 72.4 mmol) was added portionwise. The reaction mixture was stirred at rt for 15 min, and a solution of iodine (4.84 g, 19.0 mmol) in DMF (11 mL) was added. The reaction mixture was stirred at rt for a further 45 min and then poured into a mixture of a 25% aqueous solution of $NaHSO_3$ (8 mL), 33% aqueous solution of $NH_4OH$ (16 mL), and water (320 mL). The resulting precipitate was filtered and dried in vacuo to give a yellow solid, which was dissolved in hot EtOH (60 mL) and crystallized at 0 °C to give the iodoindole as needles. A solution of the iodoindole (14.8 mmol) in THF (70 mL) was added at 0 °C under argon to a suspension of sodium hydride (0.89 g, 22.3 mmol, 60% in oil) in dry THF (70 mL). The reaction mixture was stirred for 45 min, and benzenesulfonyl chloride (6.0 mL, 29.8 mmol) was added. The reaction mixture was warmed to rt and left overnight. The solvent was evaporated in vacuo, and the residue was dissolved with DCM (100 mL) and water (20 mL). The aqueous layer was extracted with DCM (3 × 20 mL). The combined organic extracts were washed with water (40 mL) and a 1 M aqueous solution of $NaHCO_3$ (2 × 20 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The resulting brown oil was dissolved in hot hexane/$Et_2O$ (1/1) and crystallized at 0 °C to give compound **9a** in 64% yield as a white solid; mp 139 °C (hexane/$Et_2O$). $^1H$ NMR (250 MHz, $CDCl_3$) δ: 8.00 (d, $J = 7.5$ Hz, 3H), 7.60−7.30 (m, 6H), 4.54 (q, $J = 7.1$ Hz, 2H), 1.48 (t, $J = 7.1$ Hz, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 161.7, 137.3, 135.5, 134.2, 133.4, 131.4, 129.1, 127.4, 127.3, 124.8, 123.1, 114.7, 73.5, 62.8, 14.0. FTIR ($\nu$/cm$^{-1}$) 3065, 1729, 1192, 726. Anal. ($C_{17}H_{14}INO_4S$) C, H, N.

**(*S*)-Ethyl 1-(Benzenesulfonyl)-3-{2-[1-(*tert*-butyloxycarbonylamino)propyl]phenyl}-1*H*-indole-2-carboxylate (10f).** Under argon, a 1.5 M aqueous solution of $Na_2CO_3$ (0.75 mL, 1.1 mmol) was added to a solution of iodoindole **9a** (0.260 g, 0.57 mmol), *o*-boronic acid **7f** (0.207 g, 0.74 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.062 g, 0.04 mmol) in toluene/EtOH 1/1 (10 mL). The reaction mixture was stirred at reflux for 20 h. After cooling, ice was added and the mixture was extracted with AcOEt (3 × 20 mL). The combined organic extracts were washed with water (10 mL), dried ($MgSO_4$), filtered, and evaporated. The resulting oil was purified by flash chromatography on silica gel (heptane/AcOEt 95/5 then 75/25) to give compound **10f** as a mixture of atropoisomers in 67% yield; white foam. $^1H$ NMR (250 MHz, $CDCl_3$) δ: 8.10 (m, 3H), 7.74 (d, $J = 7.4$ Hz, 1H), 7.63−7.10 (m, 8H), 4.85 (m, 1H), 4.39 (m, 1H), 4.22 (q, $J = 7.1$ Hz, 2H), 2.25 (m, 1H), 1.93 (m, 1H), 1.57 (s, 9H), 1.09 (t, $J = 7.1$ Hz, 3H), 0.51 (t, $J = 7.4$ Hz, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 162.1, 152.8, 141.9, 137.7, 135.8, 133.9, 131.4, 130.5, 129.6, 129.5, 129.0, 127.7, 127.4, 127.3, 126.8, 125.3, 124.5, 124.3, 122.2, 114.8, 79.4, 61.9, 53.8, 30.2, 28.2, 13.7, 10.2. FTIR ($\nu$/cm$^{-1}$) 3347, 1724, 1681, 1173. HRMS (ESI+) calcd for $[C_{31}H_{34}N_2O_6SNa]^+$ 585.2035, found 585.2040. Anal. ($C_{31}H_{34}N_2O_6S \cdot 0.85C_2H_5OH \cdot 0.85H_2O$) C, H, N.

**(*S*)-5-Ethyl-5,8-dihydroindolo[2,3-*d*][2]benzazepin-7(6*H*)-one (4f).** Trifluoroacetic acid (0.18 mL, 2.3 mmol) was added at 0 °C to a solution of *N*-Boc protected derivatives **10f** (0.186 g, 0.33 mmol) in dry DCM (2.4 mL). The reaction mixture was stirred at rt overnight. Solvent and excess TFA were evaporated in vacuo and the residue was dissolved in DCM and made basic with a 1 M aqueous solution of NaOH. The aqueous layer was extracted with DCM (3 × 10 mL). The combined organic extracts were washed with brine (5 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to give the free amine. This crude product (74 mg, 0.16 mmol) was dissolved in absolute ethanol (2 mL) and a solution of sodium (29 mg, 1.3 mmol) in absolute ethanol (2 mL) was added. The reaction mixture was stirred at reflux for 10 h. Silica was added and the solvent was evaporated in vacuo. The resulting residue was purified by flash chromatography on silica gel (heptane/AcOEt 9/1 then 3/7) to give lactams **4f** in 72% yield as a white solid; mp 157 °C (heptane/AcOEt). $^1H$ NMR (250 MHz, $CDCl_3$) δ: 11.61 (br s, 1H), 8.07 (m, 2H), 7.56 (m, 4H), 7.33 (m, 4H), 4.12 (m, 1H), 2.20 (m, 2H), 1.15 (m, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 164.9, 139.9, 138.4, 137.1, 133.9, 128.7, 128.5, 127.7, 126.9, 125.1, 121.5, 123.8, 120.9, 119.0, 112.8, 54.7, 30.3, 11.6. FTIR ($\nu$/cm$^{-1}$) 3179, 1635. HRMS (ESI) calcd for $[C_{18}H_{16}N_2ONa]$ 299.1160, found 299.1175. $[\alpha]_D = -77$ ($CHCl_3$, $c = 0.09$). Anal. ($C_{18}H_{16}N_2O \cdot 0.2CH_3CO_2C_2H_5 \cdot 0.2C_7H_{16}$) C, H, N.

**(*R*,*S*)-5-Methyl-5,8-dihydroindolo[2,3-*d*][2]benzazepin-7(6*H*)-thione (11).** Lawesson's reagent (15 mg, 0.038 mmol) was added under argon to a suspension of lactam **4b** (10 mg, 0.038 mmol) in dry toluene (0.5 mL). The reaction mixture was stirred at reflux for 30 min. Silica was added and solvent was evaporated in vacuo. The residue was purified by flash chromatography on silica gel ($CH_2Cl_2$/AcOEt 98/2) to give thiolactam **11** as a pale-yellow solid (7 mg, 65% yield). $^1H$ NMR (250 MHz, $CDCl_3$) δ: 9.50 (br s, 1H), 8.06 (m, 1H), 7.75 (br s, 1H), 7.53−7.12 (m, 7H), 4.40 (m, 1H), 1.88 (m, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ: 185.2, 137.6, 137.2, 133.4, 133.2, 128.6, 128.2, 127.9, 126.2, 125.9, 123.5, 122.5, 121.6, 112.3, 76.2, 52.7, 16.7. FTIR ($\nu$/cm$^{-1}$) 3416, 1556. HRMS (ESI+) calcd for $[C_{17}H_{15}N_2S]^+$ 279.0956, found 279.0968.

**(*R*,*S*)-5-Methyl-5,6,7,8-tetrahydro-6*H*-benzo[5,6]azepino[3,4-*b*]indole (12).** A solution of aluminum chloride (45 mg, 0.34 mmol) in dry THF (1 mL) was added under argon to a suspension of lithium aluminum hydride (26 mg, 0.68 mmol) in dry THF (1 mL). The reaction mixture was stirred for 30 min at rt and a solution of lactam **4b** (90 mg, 0.34 mmol) in dry THF (1 mL) was added. The reaction mixture was stirred for 4 h at 60 °C. After cooling, additional lithium aluminum hydride (26 mg, 0.68 mmol) was added and the mixture was stirred overnight at reflux. After cooling, a THF/$H_2O$ (9/1) mixture was slowly added until gas evolution ceased followed by addition of a 1 M aqueous solution of NaOH until basic pH was attained. The mixture was extracted with AcOEt (3 × 10 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated in vacuo. The resulting oil was purified by flash chromatography on silica gel (AcOEt/MeOH 100/0 to 80/20) to give benzazepine **12** as a white solid (70 mg, 83% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 8.35 (br s, 1H), 7.93 (m, 2H), 7.43 (m, 3H), 7.18 (m, 3H), 4.43 (d, $J$ = 16.5 Hz, 1H), 4.24 (d, $J$ = 16.5 Hz, 1H), 4.06 (q, $J$ = 6.6 Hz, 1H), 1.59 (d, $J$ = 6.6 Hz, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 142.1, 136.1, 135.9, 134.7, 128.1, 127.2, 126.8, 125.2, 125.0, 122.1, 120.2, 119.5, 113.0, 111.0, 53.2, 45.5, 19.0. FTIR ($\nu$/cm$^{-1}$) 3395, 739. MS (ESI+): 249 [M + H$^+$]. HRMS (ESI+) calcd for $[C_{17}H_{17}N_2]^+$ 249.1392, found 249.1422.

**(*R,S*)-5,6,8-Trimethyl-5,8-dihydroindolo[2,3-*d*][2]benzazepin-7(6*H*)-one (13).** A solution of lactam **4b** (13 mg, 0.05 mmol) in dry THF (0.5 mL) was added at 0 °C under argon to a suspension of sodium hydride (9 mg, 0.2 mmol, 60% in oil) in dry THF (0.5 mL). The reaction mixture was stirred for 30 min and methyl iodide (20 $\mu$L, 0.3 mmol) was added. The reaction mixture was stirred at rt for 2 h. Methanol and silica were added and the solvent and excess methyl iodide were evaporated in vacuo. The residue was purified by flash chromatography on silica gel (heptane/AcOEt 70/30) to give **13** as a white solid (9 mg, 70% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 8.03 (m, 2H), 7.51 (m, 5H), 7.25 (m, 1H), 4.68 (q, $J$ = 7.3 Hz, 1H), 4.12 (s, 3H), 3.03 (s, 3H),1.81 (d, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 162.4, 138.8, 138.1, 133.4, 131.4, 128.4, 127.8, 126.4, 124.7, 124.1, 123.3, 121.3, 120.9, 117.4, 110.3, 52.1, 32.0, 26.8, 15.0. FTIR ($\nu$/cm$^{-1}$) 1627. HRMS (ESI+) calcd for $[C_{19}H_{18}N_2ONa]^+$ 313.1317, found 313.1280. Anal. ($C_{19}H_{18}N_2O \cdot 0.4CH_3CO_2C_2H_5 \cdot 0.4H_2O$) C, H, N.

**(*R,S*)-5,8-Dimethyl-5,8-dihydroindolo[2,3-*d*][2]benzazepin-7(6*H*)-one (14).** Methyl iodide (31 $\mu$L, 0.5 mmol) was added to a solution of lactam **4b** (13 mg, 0.05 mmol) in toluene (0.5 mL) and water (0.25 mL) containing a catalytic amount of TBAB. The reaction mixture was stirred at rt for 2 h and extracted with DCM (3 × 5 mL). The combined organic layers were dried ($MgSO_4$), filtered, and evaporated in vacuo. The resulting residue was purified by flash chromatography on silica gel (heptane/AcOEt 90/10 to 50/50) to give compound **14** as a white solid (6.5 mg, 47% yield). $^1$H NMR (250 MHz, $CDCl_3$) $\delta$: 8.06 (d, $J$ = 8.0 Hz, 1H), 8.00 (d, $J$ = 7.3 Hz, 1H), 7.49 (m, 5H), 7.25 (m, 1H), 6.14 (br s, 1H), 4.37 (qu, $J$ = 7.3 Hz, 1H), 4.15 (s, 3H), 1.79 (d, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 163.0, 140.1, 139.0, 133.4, 129.7, 128.9, 127.7, 126.8, 125.0, 124.3, 123.1, 121.6, 121.6, 118.8, 110.4, 48.0, 31.8, 17.2. FTIR ($\nu$/cm$^{-1}$) 3196, 1657. HRMS (ESI+) calcd for $[C_{18}H_{17}N_2O]^+$ 277.1341, found 277.1338.

**Cell Culture and Proliferation Assay.** Cancer cell lines were obtained from the American type Culture Collection (Rockville, MD) and were cultured according to the supplier's instructions. Briefly, human KB epidermal carcinoma cells were grown in Eagle's minimal essential medium (EMEM) containing 4.5 g/L glucose supplemented with 10% fetal calf serum (FCS) and 1% glutamine. A549 lung carcinoma, MDA-MB231, MDA-MB435, and MCF7 breast carcinomas and mouse B16F10 melanoma cells were grown in Dulbecco minimal essential medium (DMEM) containing 4.5 g/L glucose supplemented with 10% FCS and 1% glutamine. Human K562 leukemia and HCT116 colorectal carcinoma cells were grown in RPMI 1640 containing 10% FCS and 1% glutamine. All cell lines were maintained at 37 °C in a humidified atmosphere containing 5% $CO_2$. Cell viability was assessed using Promega CellTiter-Blue TM reagent according to the manufacturer's instructions. Briefly, cells were seeded in 96-well plates (5 × 10$^3$ cells/well) containing 50 $\mu$L growth medium. After 24 h of culture, the cells were supplemented with 50 $\mu$L of the tested compound dissolved in DMSO (less than 0.1% in each preparation). After 72 h of incubation, 20 $\mu$L of resazurin was added for 2 h before recording fluorescence ($\lambda_{ex}$ = 560 nm, $\lambda_{em}$ = 590 nm) using a Victor microtiter plate fluorimeter (Perkin-Elmer, USA). The $IC_{50}$ corresponds to the concentration of the tested compound that caused a decrease of 50% in fluorescence of drug-treated cells compared with untreated cells. Experiments were performed in triplicate.

**Cell Cycle Analysis.** Exponentially growing cancer cells (K562, MCF7, MDA-MB231) were incubated with tested compound or DMSO for 6, 16, and/or 24 h. Cell-cycle profiles were determined by flow cytometry on a FC500 flow cytometer (Beckman-Coulter, France) as described previously.[31]

**Apoptosis Assay.** Apoptosis was measured by the Apo-one homogeneous caspase-3/7 assay (Promega Co, WI) according to the manufacturer's recommendations. Briefly, cells were subcultured on a 96-well plate with 5 × 10$^4$ cells/well in 100 $\mu$L medium. After 24 h of incubation, the medium in the 96-well plate was discarded and replaced with medium containing different concentrations of compound **4f** (1, 0.1, and 0.01 $\mu$M) or 0.1% DMSO (as negative control). The treated cells were incubated for 24 h, each well then received 100 $\mu$L of a mixture of caspase substrate and Apo-one caspase 3/7 buffer. After 1 h of incubation, the fluorescence of sample was measured using a Victor microtiter plate fluorimeter (Perkin-Elmer, USA) at 527 nm.

**Immunocytochemistry.** MDA-MB231 cells cultured on a Laboratory-Tek chamber slide (VWR International, Strasbourg, France) were treated with 50 nM of compound **4f** for 24 h. At the end of treatment, cells were fixed with 3.7% formaldehyde and then permeabilized with 0.1% Triton X-100 in PBS for 3 min at room temperature. The cells were first incubated for 1 h in a solution of PBS containing 1% BSA and 10% goat serum to block nonspecific antibody binding and next for 12 h at 4 °C with the mouse anti-$\beta$ tubulin antibody (1:100) (Zymed, Invitrogen, Cergy Pontoise, France). Cells incubated with preimmune mouse serum instead of primary antibody were used as negative control. At the end of incubation, cells were washed 3 times in PBS containing 1% BSA and incubated for 2 h at room temperature with secondary goat antimouse antibody Alexa 488 labeled (1:200) (Molecular Probes, Invitrogen, Cergy Pontoise, France) for tubulin staining, Alexa Fluor 546 phalloidin labeled (1:40) (Molecular Probes, Invitrogen, Cergy Pontoise, France) for actin staining, and Hoechst 33342 labeled (1:5000) (Molecular Probes, Invitrogen, Cergy Pontoise, France) for nuclear staining. At the end of incubation, cells were washed once in PBS and mounted in fluorescent mounting medium (Dako, Trappes, France). Images were acquired using a Nikon TE2000E fluorescent microscope (Nikon, Champigny-sur-Marne, France) equipped with a Nikon DXM1200F digital camera.

**Tubulin Binding Assay.** Sheep brain tubulin was purified according to the method of Shelanski et al.[32] by two cycles of assembly—disassembly and then dissolved in the assembly buffer containing 0.1 M MES, 0.5 mM $MgCl_2$, 1 mM EGTA, and 1 mM GTP, pH 6.6 (the concentration of tubulin was about 2−3 mg/mL). Tubulin assembly was monitored and recorded continuously by turbidimetry at 350 nm in a UV spectrophotometer equipped with a thermostatted cell at 37 °C. The $IC_{50}$ value of each compound was determined as the concentration which decreased the maximum assembly rate of tubulin by 50% compared to the rate in the absence of compound. The $IC_{50}$ values for all compounds were compared to the $IC_{50}$ of colchicine, measured the same day under the same conditions.

**Experimental Glioma Assay.** U87 human glioma cells (American type Culture Collection) were maintained in DMEM with 10% FBS, antibiotics, and L-glutamine. Fertilized chicken eggs (Earl Morizeau, Dangers, France) were handled as described.[33] On embryonic day 10, 5 × 10$^6$ U87 cells in 20 $\mu$L of medium were deposited on chick chorio-allantoic membrane (CAM). On day 2 of tumor development, size matched tumors were divided into control and treatment groups. Twenty $\mu$L of each tested compound (**4f** and colchicine at 10 $\mu$M and NAC at 100 $\mu$M) were deposited locally on the tumor once a day (from day 3 to day 6 of tumor growth). Controls received solvent of the corresponding drug. Tumor size was calculated from the following formula: $V = 4/3\pi r^3$, with $r = 1/2\sqrt{(\text{length} \times \text{width})}$. Quantification of drug effect on tumor size was determined in six representative tumors per group. Digital photos were taken under a stereomicroscope (Nikon SMZ1500).

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**Supporting Information Available:** Physical and spectroscopic data for new compounds. Cytometric flow results with compound **4f**. Elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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