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Indolobenzazepin-7-ones and 6-, 8-, and 9-Membered Ring Derivatives as Tubulin Polymerization Inhibitors: Synthesis and Structure−Activity Relationship Studies

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Several small weight indole derivatives (D-64131, D-24851, BPR0L075, BLF 61−3, and ATI derivatives) are potent tubulin polymerization inhibitors and show nanomolar antiproliferative activity. Among them, indolobenzazepin-7-ones were recently disclosed as potent antimitotic agents. In an effort to improve this structure, we prepared new derivatives in order to evaluate their antiproliferative activity. 5,6,7,9-Tetrahydro-8*H*-indolo[2,3-*e*][3]benzazocin-8-one (**1m**) was found to be the most potent derivative inhibiting the cell growth of several cancer cell lines in the lower nanomolar range.

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Introduction

Present in all eukaryotic cells, microtubules are primordial components of cell structure, which play a pivotal role in a wide number of essential cellular functions, such as morphogenesis, motility, cell division, and intracellular transport.[1] Microtubules are composed of α- and β-tubulin heterodimers characterized by a highly dynamic behavior (fluctuations between phases of elongation and shortening).[2] Drugs interfering with microtubules/tubulin dynamics or other proteins of the mitotic spindle (kinases, kinesins) are called antimitotic agents.[3] The tubulin-targeting agents are important in cancer therapy and have been grouped into two main classes:[4] (1) tubulin stabilizing derivatives such as paclitaxel, docetaxel, and epothilone B,[5] and (2) tubulin polymerization inhibitors with different mechanisms of action[6,7] (t-BCEU, T138067, macrocyclic polyethers, depsipeptides, maytansine, rhizozine, colchicine, and combretastatin A4[8]) (Figure 1).

In the past decade, an increasing number of small molecular weight indole derivatives have been described as potent tubulin polymerization inhibitors through binding to the colchicine site.[9] Among them, D-64131 (IC_{50} U373 = 72 nM, IC_{50} inhibition of tubulin polymerization (ITP[a]) = 0.53 μM),[10] D-24851 (IC_{50} HT29 = 72 nM, IC_{50} MDA-MB231 = 74 nM, and IC_{50} ITP = 1.0 μM),[11] BPR0L075 (IC_{50} KB = 4 nM, IC_{50} H460 = 7 nM, and IC_{50} ITP = 2.8 μM),[12] BLF 61−3 (IC_{50} MCF7 = 45 nM and IC_{50} ITP = 1.6 μM),[13] and 3-arylthioindole (ATI) derivative (IC_{50} MCF7 = 13 nM and IC_{50} ITP = 2.0 μM)[14] displayed nanomolar cell growth inhibition (Figure 1). The indole scaffold has also been investigated intensively due to the advantages of easier access and lower cost. These initial results highlighted the indole nucleus as a valuable pharmacophore for potent tubulin polymerization inhibitors.

Previously, we reported the synthesis of indolobenzazepin-7-ones **1a**−**1f** and derivatives **2a**−**3a** via an original C-3 indole direct arylation mediated by palladium (Figure 1).[15] An initial screening showed antiproliferative properties for this scaffold.[15b] Similarly, R. Dodd et al. have prepared the same heterocyclic core by a Suzuki coupling reaction between ethyl 3-iodoindole-6-carboxylates and appropriate α-alkylbenzylamino *o*-boronic acids followed by final lactamization. These compounds displayed potent inhibition of tubulin polymerization and high antiproliferative activities *in vitro* and in cell-based assays (i.e., (*S*)-5-ethyl indolobenzazepin-7-one: IC_{50} MCF7 = 40 nM, IC_{50} HCT116 = 42 nM, and IC_{50} ITP = 1.9 μM) (Figure 1).[16] These data prompted us to evaluate indolobenzazepinones **1a**−**1f** and derivatives **2a**−**3a**, and we initiated a structure−activity relationship study to provide more derivatives in order to compare their antiproliferative and tubulin inhibitory activities with those obtained for (*S*)-5-ethyl indolobenzazepinone and **1a**−**1f**.

Results and Discussion

Chemistry. The syntheses of 6-alkyl- or 6-alkenylindolobenzazepin-7-ones **1g**−**1k** are depicted in Scheme 1. The starting material **5** was prepared from 1-(ethoxymethyl)-1*H*-indole-2-carboxylic acid **4** as previously described.[15]

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[a] Abbreviations: U373, human glioblastoma-astrocytoma cell line; ITP, inhibition of tubulin polymerization; HT29, human colon cancer cell line; MDA-MDB231, human breast carcinoma cell line; KB, vincristine-resistant human cancer cell line; H460, human lung cancer cell line; ATI, 3-arylthioindole; MCF7, human breast cancer cell line; DMAP, 4-(dimethylamino)pyridine; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMF, *N*,*N*-dimethylformamide; 5-FU, 5-fluorouracil; PDB, protein data bank; PE, petroleum ether; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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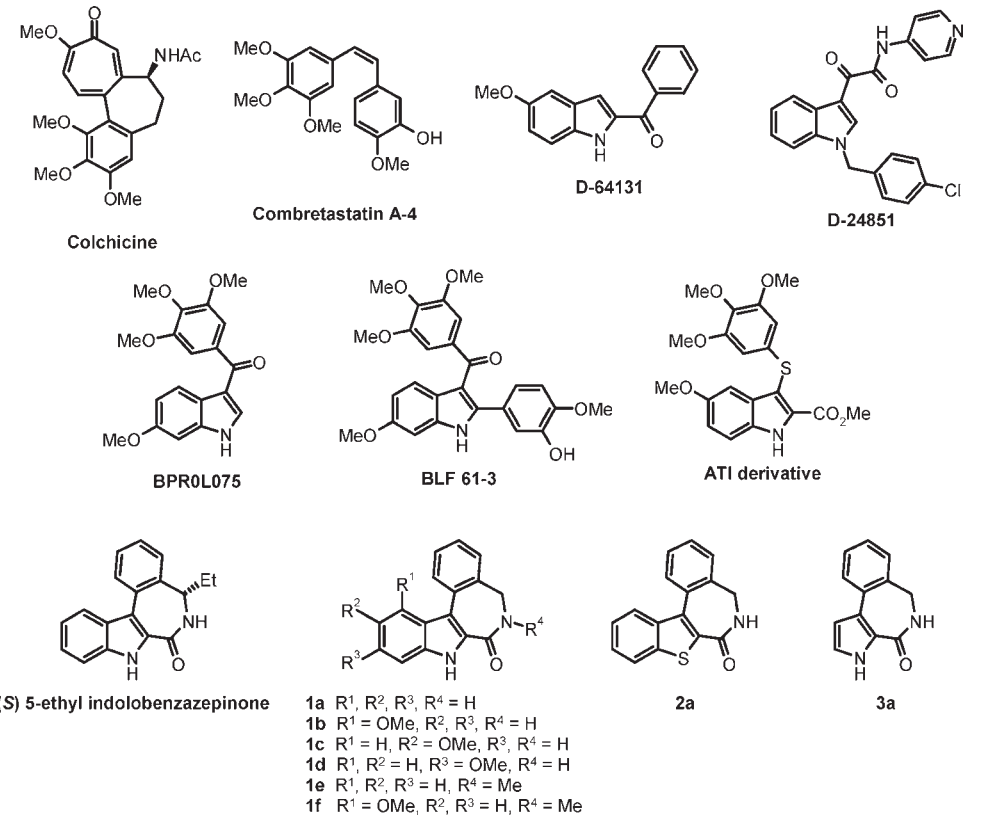
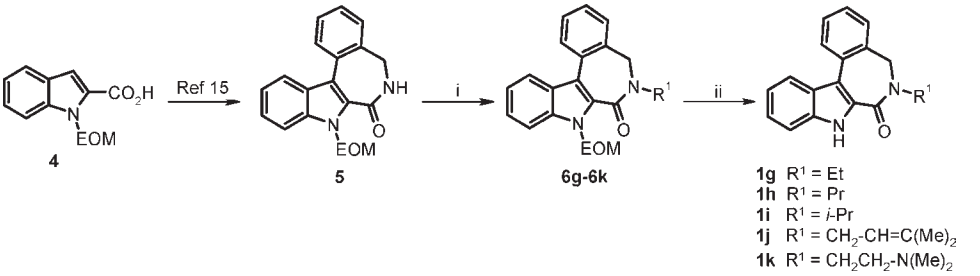


Figure 1. Structures of various known antimitotic agents and indolobenzazepin-7-ones **1a**−**1f** and derivatives **2a** and **3a**.

Scheme 1[a]



[a] Reagents and conditions: (i) NaH, R^1I or R^1Br, THF, rt, 4−60 h, **6g** (EtI, 20 h) = 87%, **6h** (PrI, 60 h) = 90%, **6i** (*i*-PrI, 80 °C, sealed tube, 20 h) = 77%, **6j** ($BrCH_2$-CH=$C(Me)_2$, 4 h) = 91%, **6k** ($ClCH_2$-CH_2-$N(Me)_2$, 4 h) = 74%; (ii) 1 N HCl, dioxane, 80 °C, 2 h, **1g** (Et) = 93%, **1h** (Pr) = 95%, **1i** (*i*-Pr) = 57%, **1j** (CH_2-CH=$C(Me)_2$) = 83%, **1k** (CH_2-CH_2-$N(Me)_2$) = 44%.

N-Alkylation of **5** was performed in the presence of sodium hydride and halogen halides (iodoethane, iodopropane, 2-iodopropane, 1-bromo-3-methylbut-2-ene, and 1-chloro-2-(dimethylamino)ethane) to afford derivatives **6g**−**6k** in fair yields. Final deprotection of **6** in acidic medium gave **1g**−**1k** in 83−95% yield. Lower or higher membered ring analogues **1l**−**1o** were then prepared in four steps from **4** through the key intramolecular direct arylation reaction (Scheme 2).[17] Acid **4** was first treated with various amines **7l**−**7n**,[18] DMAP, and EDCI to give amides **8l**−**8n**. Boc protection (Boc_2O, DMAP) of **8** was carried out to afford **9l**−**9n** in near-quantitative yields. N-Methylation of **8m** was also performed to obtain compound **9o** in 99% yield. The cyclization mediated by Pd(0) of **9l** was performed in the presence of 20 mol % of $Pd(OAc)_2$, PPh_3 (0.4 equivalent), and Ag_2CO_3 (2 equivalents) in DMF at 100 °C for 1 h to give indoloquinolin-6-one **10l** in 71% yield. Lower amounts of $Pd(OAc)_2$ led to a decrease of product yield. For **9m**−**9o**, the direct arylation on C-3 position of the indole occurred in the presence of 5 mol % of $Pd(OAc)_2$ at 140 °C for 1−3.5 h to give **10m**−**10o** in 47−97% yield. In all cases except for **9o**, the Boc protecting group on amide is required to get the cyclized compound. HCl hydrolysis of **10** eventually led to final compounds **1l**−**1o**. Low deprotection yields were observed with 8-membered ring compounds **1m** and **1o** because of the opening of the lactam ring under acidic conditions. Finally, the same synthetic strategy was applied to obtain compounds **1p**−**1s** with the methoxy substituent on the benzene moiety (Scheme 3). Amides **12p**−**12s** were prepared from **4** and 2-iodobenzylamines[18b,19] **11p**−**11s**, then Boc derivatives **13p**−**13s** were obtained quantitatively. Intramolecular arylation of **13** was effective in the presence of 20 mol % of $Pd(OAc)_2$ to give **14** in 40−83% yield. Cyclization assays on **13** with lower quantities of $Pd(OAc)_2$ led to low yields. Removal of both protecting groups on **14** was achieved in acidic conditions (1 N HCl, 1,4-dioxane) to afford **1p**−**1s**.

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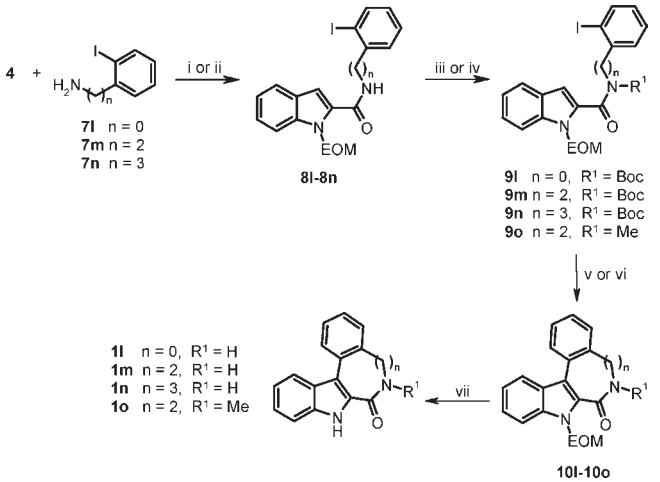
Biological Evaluation: *In Vitro* Cell Growth Inhibition. All of the reported compounds were first evaluated for cytotoxicity in the human breast cancer cell line MCF7. Paclitaxel, vinorelbine, and 5-fluorouracil (5-FU) were included in the assays as examples of antitumor agents currently used in the clinic. IC_{50} values for cell growth inhibition are shown in Tables 1 and 2. The core indolobenzazepin-7-one compound **1a** displayed submicromolar activity ($IC_{50} = 0.3\ \mu M$) as already observed by Dodd et al.[16] When a methoxy group was introduced at the C-4 position of the indole group (**1b**), the resulting compound presented a cell growth inhibition activity very similar to that of **1a** ($IC_{50} = 0.4\ \mu M$). The presence of the same group at the C-5 or C-6 position of the indole group (**1c** and **1d**) led to decreasing antiproliferative activity ($IC_{50} = 3.1-3.2\ \mu M$). Methylation of the amide nitrogen atom leads to compounds as potent as **1a** (**1e** $IC_{50} = 0.3\ \mu M$), and a slight loss of activity was observed for **1f**. Homologation of the alkyl chain in this position (**1g**−**1i**) resulted in a drastic reduction of cytotoxic activity ($IC_{50} > 30\ \mu M$). However, the presence of 3-methylbut-2-enyl chain or *N*,*N*-dimethylaminoethyl chain at the same position (**1j** and **1k**) led to modest antiproliferative activity ($IC_{50} = 6.9-18.6\ \mu M$). Seven-membered ring compound **1a** is approximately 22 times more active than the quinolin-6-one derivative **1l** ($IC_{50} = 6.5\ \mu M$). Eight-membered ring compound **1m** produced similar cytotoxic activity in MCF7 cells ($IC_{50} = 0.2\ \mu M$) compared to that of **1a** and **1e** ($IC_{50} = 0.3\ \mu M$). The nine-membered heterocyclic derivative **1n** showed micromolar cell growth inhibition and was the least active compound of all homologues tested. N-Methylation of **1m** induced a loss of antiproliferative activity in MCF7 cells (**1o**, $IC_{50} = 2\ \mu M$). Methoxy groups on the benzene cycle led to decreasing antiproliferative activity. Compounds **1p**−**1r** were more than 100-fold less active than **1a**. It should be noted that the presence of three methoxy groups in **1s** induced a higher cell growth inhibitory activity with IC_{50} values at the low micromolar range and only 17-fold higher than those obtained with **1a**. The indole moiety is crucial for the retention of the antiproliferative activity. Benzo[*b*]thieno[2,3-*d*][2]benzazepin-7-one **2a** or pyrrolo[2,3-*d*][2]benzazepin-7-one **3a** were considerably less active ($IC_{50} > 30\ \mu M$). As already mentioned by Dodd et al., N-alkylation of the indole nitrogen had a detrimental effect on activity ($IC_{50} > 30\ \mu M$).[16]

The most potent agents (**1a**, **1b**, **1e**, and **1m**), **1c**, **1d**, and **1o** were next tested for their antiproliferative activity on a panel of eight human cancer cell lines: sarcoma Mes-sa cells, breast cancer T47D and UACC812 cells, lymphoma CEM cells, lymphoma RL cells and colorectal cancer HCT116, SW48 and SW480 cells. As shown in Table 3, all seven compounds exhibited similar cell growth inhibitory activities against all cell lines as in MCF7 cells. Again, compound **1m** showed the highest IC_{50} values for all cancer cell lines tested except for sarcoma Mes-sa cells.

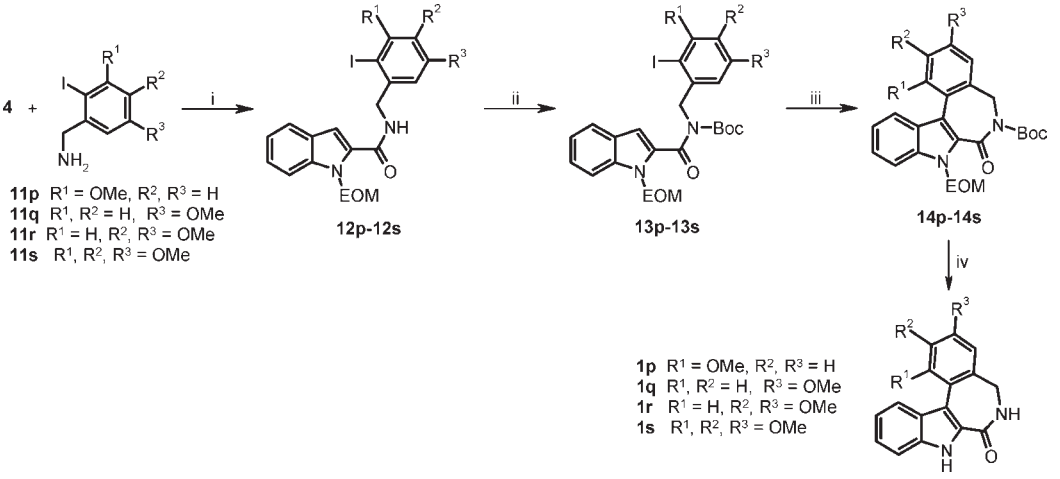
Effect on Cell Cycle Progression. Compounds **1a**, **1b**, **1e**, and **1m** were selected for cell cycle studies in MCF7, Mes-sa,

Scheme 2[a]



[a] Reagents and conditions: (i) 2 M $AlMe_3$ in toluene, CH_2Cl_2, −20 °C to rt, 19 h, **8l** = 98%; (ii) DMAP, EDCI, CH_2Cl_2, 0 °C to rt, 24 h, **8m** = 98%, **8n** = 97%; (iii) Boc_2O, DMAP, MeCN, rt, 15 h, **9l**−**9n** = 99%; (iv) NaH, MeI, THF, rt, 4 h, **9o** = 99%; (v) $Pd(OAc)_2$ (20 mol %), PPh_3 (0.4 equiv), Ag_2CO_3 (2 equiv), DMF, 100 °C, 1 h, **10l** = 71%; (vi) $Pd(OAc)_2$ (5 mol %), PPh_3 (0.1 equiv), Ag_2CO_3 (2 equiv), DMF, 140 °C, **10m** = 97% (1 h), **10n** = 47% (1 h), **10o** = 87% (3.5 h); (vii) 1 N HCl, dioxane, 80 °C, **1l** = 70% (3 h), **1m** = 28% (5 h), **1n** = 67% (2 h), **1o** = 51% (1 h).

Scheme 3[a]



[a] Reagents and conditions: (i) DMAP, EDCI, CH_2Cl_2, 0 °C to rt, 24 h, **12p** = 85%, **12q** = 97%, **12r** = 85%, **12s** = 95%; (ii) Boc_2O, DMAP, MeCN, rt, 15 h, **13p**−**13s** = 99%; (iii) $Pd(OAc)_2$ (20 mol %), PPh_3 (0.4 equiv), Ag_2CO_3 (2 equiv), DMF, 100 °C, **14p** = 73% (1 h), **14q** = 53% (23 h), **14r** = 83% (1 h), **14s** = 69% (2 h); (iv) 1 N HCl, dioxane, 80 °C, **1p** = 20% (3 h), **1q** = 91% (3 h), **1r** = 92% (1 h), **1s** = 73% (1 h).

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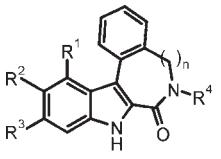
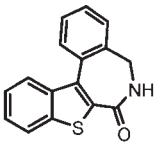
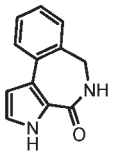
Table 1. Cytotoxic Effects of Compounds **1a**−**1o**, **2a**, **3a**, and **5** on MCF7 Cancer Cell Lines and Inhibition of Tubulin Polymerization (ITP)



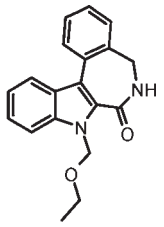
1a-1o



2a



3a

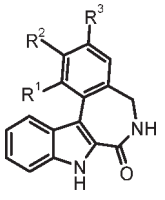


5

compd	R^1	R^2	R^3	R^4	n	MCF7[a] IC_{50} ± SD (μM)[b]	ITP[c] ± SD (μM)[d]
5-FU						0.3 ± 0.1	
vinorelbine						0.07 ± 0.01	
paclitaxel						0.003 ± 0.002	
1a	H	H	H	H	1	0.3 ± 0.1	5.3 ± 1.3
1b	OMe	H	H	H	1	0.4 ± 0.2	6.1 ± 0.4
1c	H	OMe	H	H	1	3.1 ± 1.2	ni[e]
1d	H	H	OMe	H	1	3.2 ± 0.6	6.2 ± 1.2
1e	H	H	H	Me	1	0.3 ± 0.1	4.4 ± 0.9
1f	OMe	H	H	Me	1	3.1 ± 1.1	3.7 ± 1.6
1g	H	H	H	Et	1	> 30	nd[f]
1h	H	H	H	i-Pr	1	> 30	ni
1i	H	H	H	Pr	1	> 30	nd
1j	H	H	H	$-CH_2-CH=C(Me)_2$	1	6.9 ± 2	9.1 ± 2.3
1k	H	H	H	$-CH_2-CH_2-N(Me)_2$	1	18.6 ± 2.3	ni
1l	H	H	H	H	0	6.5 ± 0.7	ni
1m	H	H	H	H	2	0.2 ± 0.1	7.7 ± 1.2
1n	H	H	H	H	3	8 ± 1.4	13.0 ± 4.4
1o	H	H	H	Me	2	2 ± 0.07	5.4 ± 1.3
2a						> 30	14.9 ± 1.3
3a						> 30	ni
5						> 30	ni

[a] MCF7 = breast cancer. [b] IC_{50} is the concentration of the test compound inducing 50% cell growth inhibition after 72 h of incubation. [c] ITP = inhibition of tubulin polymerization. [d] IC_{50} is the concentration of compound required to inhibit 50% of the rate of microtubule assembly. [e] ni = no inhibition. [f] nd = not determined.

Table 2. Cytotoxic Effects of Compounds **1p**−**1s** on MCF7 Cancer Cell Lines and Inhibition of Tubulin Polymerization



1p-1s

compd	R^1	R^2	R^3	MCF7[a] IC_{50} ± SD (μM)[b]	ITP[c] ± SD (μM)[d]
1p	OMe	H	H	> 30	ni[e]
1q	H	H	OMe	> 30	ni
1r	H	OMe	OMe	> 30	ni
1s	OMe	OMe	OMe	5 ± 4	9 ± 0.6

[a] MCF7 = breast cancer. [b] IC_{50} is the concentration of the test compound inducing 50% cell growth inhibition after 72 h of incubation. [c] ITP = inhibition of tubulin polymerization. [d] IC_{50} is the concentration of compound required to inhibit 50% of the rate of microtubule assembly. [e] ni = no inhibition.

and HCT116 tumor cell lines. Cell cycle analysis (Table 4) following treatment with 10 μM of each compound showed that most of the cells were arrested in the G2/M phase after 24 h of treatment. Similar features were observed after paclitaxel or vinorelbine treatment (data not shown). In these cancer cell lines, compounds **1a**, **1b**, **1e**, and **1m** induced similar levels of G2/M arrest. In order to detect whether the G2/M arrest was specific to G2 or the mitotic phase, the morphological features of compound-treated cells were then analyzed by Hoechst33258/PI staining of cellular DNA. The nuclei of untreated MCF7, Mes-sa, and HCT116 cells showed the typical diffuse pattern of chromatin distribution (data not shown), whereas cells treated with compound derivatives became arrested in prometaphase as an effect of perturbation of tubulin function by these compounds (Figure 2).

***In Vitro* Tubulin Polymerization.** We investigated the inhibition of tubulin polymerization of all compounds (except for **1g** and **1i**) using *in vitro* turbidimetric measurements of microtubule assembly (Tables 1 and 2). Out of 20 compounds tested, 9 did not significantly influence tubulin polymerization. The other 11 compounds inhibited assembly into microtubules with estimates of IC_{50} values in the range from 3.7 μM to 15.9 μM. The rate as well as the final extent of assembly were reduced by most of these compounds. In agreement with other indole-based inhibitors, inhibition of different tumor cell lines in the nanomolar range corresponds to IC_{50} values in the lower micromolar range in *in vitro* assays.[5−10] The *in vitro* data are in good agreement with the data obtained from cell-based assays: compounds that do not inhibit the MCF7 tumor cell line (**1h**, **1k**, **2a**, **3a**, **5**, **1p**, **1q**,

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Table 3. Antiproliferative Activities of **1a**−**e**, **1m**, and **1o** against Various Cancer Cell Lines

	$IC_{50} \pm SD$ (μM)[a,b]							
compd	Mes-sa	T47D	UACC812	RL	CEM	HCT116	SW48	SW480
5-FU	1.1 ± 0.4	0.6 ± 0.1	3.1 ± 1	3.2 ± 1	125 ± 35	2.6 ± 1	4.4 ± 1	2.1 ± 1
paclitaxel	< 0.001	0.004 ± 0.002	0.7 ± 0.1	0.003 ± 0.001	0.002 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.002 ± 0.001
1a	0.3 ± 0.08	0.6 ± 0.1	0.76 ± 0.19	0.22 ± 0.005	0.2 ± 0.06	0.4 ± 0.09	0.2 ± 0.06	0.4 ± 0.1
1b	0.5 ± 0.1	0.6 ± 0.1	2.7 ± 1	0.5 ± 0.007	0.2 ± 0.03	0.5 ± 0.09	0.5 ± 0.04	0.7 ± 0.1
1c	5 ± 0.7	nd[c]	nd	nd	1.4 ± 0.1	1.9 ± 0.1	2.1 ± 0.4	3.1 ± 1.2
1d	3.5 ± 0.6	nd	nd	nd	2.6 ± 0.8	110 ± 21	2.3 ± 0.3	5.4 ± 3.3
1e	0.4 ± 0.2	0.6 ± 0.3	0.81 ± 0.3	0.21 ± 0.007	0.1 ± 0.02	0.2 ± 0.09	0.2 ± 0.05	0.3 ± 0.2
1m	21.5 ± 4.9	0.1 ± 0.005	0.26 ± 0.05	0.07 ± 0.03	0.05 ± 0.03	0.08 ± 0.03	0.05 ± 0.03	0.18 ± 0.02
1o	1.4 ± 0.3	nd	nd	nd	1.5 ± 0.5	1 ± 0.03	0.9 ± 0.2	19 ± 1.4

[a] IC_{50} is the concentration of the test compound including 50% cell growth inhibition after 72 h of incubation; Mes-sa = uterine sarcoma; T47D, UACC812 = breast cancer; RL = lymphoma; CEM = T-cell acute lymphoblastic leukemia; HCT116, SW48, SW480 = human colon cancer. [b] Values are the means of three different experiments ± SD. [c] nd = not determined.

Table 4. Percentage of MCF7, Mes-sa and HCT116 Cancer Cell Lines in the Different Phases after 24 h of Treatment with Compounds **1a**, **1b**, **1e**, and **1m**

	MCF7			Mes-sa			HCT116		
compd[a]	G1	S	G2/M	G1	S	G2/M	G1	S	G2/M
control	47.6 ± 7	37.9 ± 6	14.4 ± 3.2	61 ± 5	30 ± 4	10 ± 2	62 ± 6	25 ± 8	12 ± 3
1a	29.6 ± 8.8 ±	4.2 ± 4.7	66.6 ± 4.8	7 ± 2	5 ± 1	87 ± 4	5 ± 1	2 ± 0.2	94 ± 1
1b	27.5 ± 2.8	9 ± 0.3	66 ± 0.9	9 ± 3	10 ± 5	82 ± 2	5 ± 0.4	3 ± 0.4	92 ± 1
1e	27.5 ± 2.8	5.3 ± 1	67.1 ± 1.8	9 ± 1	2 ± 1	89 ± 2	2 ± 1	2 ± 2	96 ± 2
1m	2.3 ± 1.7	16 ± 4.4	81 ± 6.1	9 ± 2	18 ± 7	72 ± 9	3.3 ± 0.5	13.7 ± 0.4	95.3 ± 4

[a] Compounds were tested at 10 μM.

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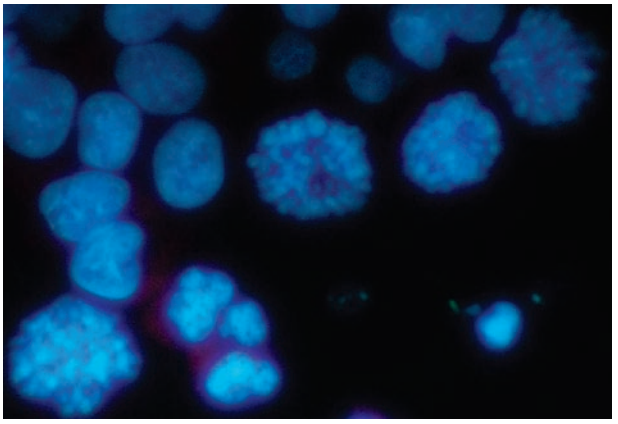

Figure 2. Prometaphase arrest induced by treatment of HCT116 cells with **1a** at 10 μM.

1r, and **1s**) do not inhibit or only weakly inhibit tubulin polymerization. Compounds that inhibit MCF7 in the nanomolar range (**1a**, **1b**, **1e**, and **1m**) all inhibit tubulin polymerization in the low μmolar range indicating that the *in vitro* assay can predict whether or not a compound will be active in cell-based assays.

Colchicine-Binding Site. To investigate whether our compounds bind in or close to the colchicine-binding domain, we performed competition assays in the presence of fluorescent colchicine using compounds **1e**, **1m**, and **1p**. Compound **1e** is one of the potent compounds in inhibiting tubulin polymerization, whereas **1m** is the most potent compound in cell-based assays. Compound **1p** has the same scaffold as the other two compounds but did not inhibit tubulin polymerization. The results of the competition assays are presented in Figure 3. As expected, vinblastine, which binds to the vinca domain does not compete with colchicine, indicated by the same fluorescence intensity as that for fluorescent colchicine alone. Colchicine competes with its fluorescent analogue

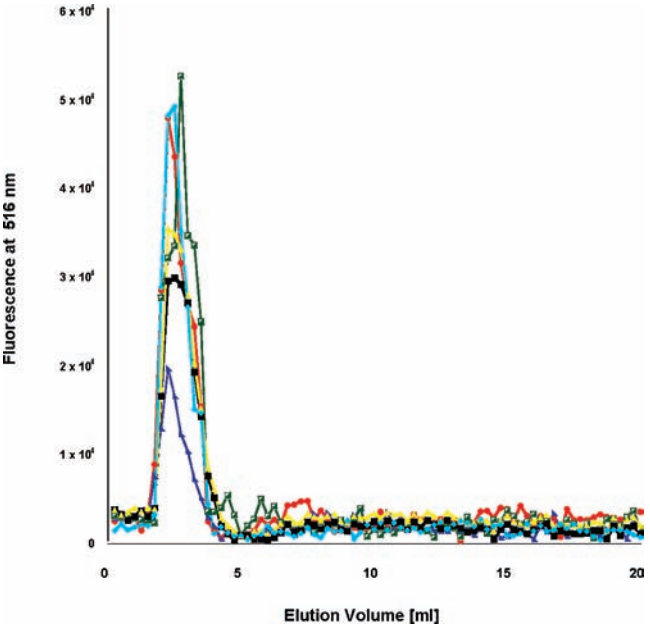

Figure 3. Competition assays of different tubulin-binding drugs with fluorescent colchicine. The fluorescence intensity at 516 nm is plotted against the elution volume. Fluorescent colchicine in the absence of any inhibitor (red), colchicine (positive control, dark blue), vinblastine (negative control, green), and compounds **1e** (black), **1m** (yellow), and **1p** (light blue).

indicated by the disappearance of the intensity peak. Compound **1p**, which does not inhibit tubulin polymerization does not compete with fluorescent colchicine. Compounds **1e** and **1m** do compete with fluorescent colchicine as indicated by the loss of fluorescence intensity, and interestingly, the extent of competition of the two compounds correlates with their respective IC_{50} values obtained from inhibition of tubulin polymerization assays. We can conclude that our

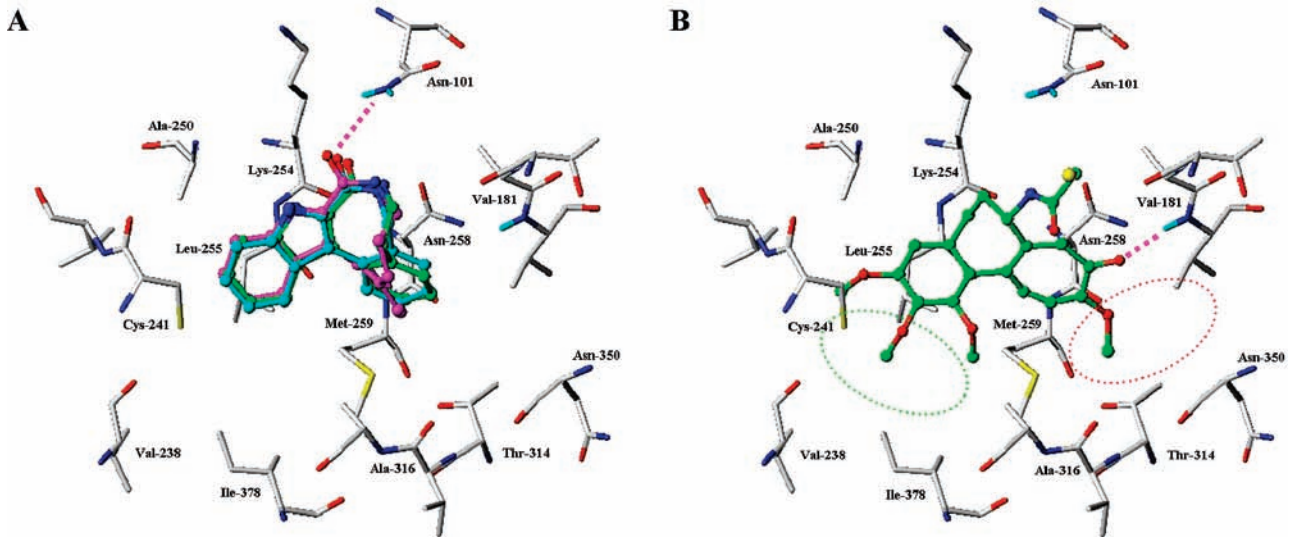

Figure 4. (A) Proposed binding mode for **1a** (cyan), **lm** (green), and **1n** (magenta). (B) Binding mode of cocrystallized colchicine (green). The green dotted ellipse highlights the zone of the binding site where substitutions are possible, while the red one locates the steric hindrance part of the cavity. Pink dotted lines represent possible hydrogen bond interactions between the protein and the different ligands. Residue numbers are those used by Ravelli et al.[7]

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active compounds bind in or close to the colchicine binding domain.

Docking and Molecular Modeling. To validate this hypothesis, compounds **1a**, **1m**, and **1n** were docked into the colchicine binding site of tubulin. The tubulin crystal structure was retrieved from the Protein Data Bank (PDB). The accession code 1SA0 corresponds to the α,β-tubulin heterodimer with bound colchicine.[7] Docking studies were undertaken with FlexX v2.2 with default settings in order to predict the binding mode of our ligands. It was found that the indole part of **1m** is probably overlaid with the trimethoxyphenyl group of the colchicine derivative cocrystallized with tubulin as shown in Figure 4. The carboxyl group of the amide may form an H-bond with the nitrogen of the Asn-101 side chain. There is no interaction of the nitrogen amide of indolobenzazepinone derivatives with the protein. Alkylation at that position decreased the activity of most compounds in the MCF7 assays, while its influence on tubulin polymerization is not obvious (Table 1). Methoxy groups on R^1, R^2, and R^3 did not give benefits in terms of biological activities. From the results in Table 2, the added methoxy group of the other phenyl group is damaging to the compound's activities even if the trimethoxy derivative **1s** displayed micromolar antiproliferative property.

According to molecular modeling predictions, there may be two possible H-bond donors from the protein: the NH of the Asn-101 side chain (Figure 4A) and the NH of the Val-181 main chain (Figure 4B). This observation raises the possibility of an additionnal H-bond formation with the protein (NH Val-181) and the 5,6,7,9-tetrahydro-8*H*-indolo-[2,3-*e*][3]benzazocin-8-one scaffold.

Conclusions

Synthesis and structure−activity relationship studies for indolobenzazepinone as anticancer agents were carried out. The SAR information indicated that the introduction of the methoxy group on benzene or indole moieties, or alkylation of the amide nitrogen did not improve the antiproliferative activity compared with that of the unsubstituted compound. Compared to six-, seven- and nine-membered ring derivatives, 5,6,7,9-tetrahydro-8*H*-indolo[2,3-*e*][3]benzazocin-8-one **1m** is the most potent antiproliferative agent across a wide variety of cancer cell lines. As mentioned by Dodd et al., these compounds are antimitotic agents acting through the colchicine binding site of the tubulin. The docking studies suggest that the indole part of **1m** is likely overlaid with the aryl moiety of the colchicine, and the carbonyl group of the amide may form the single H-bond with the nitrogen of the Asn-101 side chain of the colchicine binding site. Structural modifications of the basic 5,6,7,9-tetrahydro-8*H*-indolo[2,3-*e*][3]benzazocin-8-one core are under investigation to improve the inhibitory potency, and will be reported in due course.

Experimental Section

Chemistry. General Methods. Commercial reagents (Fluka, Aldrich) were used without purification. Solvents were distillated prior to use. The light petroleum ether (PE) refers to the fraction boiling at 40−60 °C. Melting points were determined using a Buchi capillary instrument and are uncorrected. IR spectra were recorded on Perkin-Elmer Spectrum One. NMR spectra were recorded at 300 K on a Bruker Avance spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane as an internal standard. Mass spectra were recorded with a Perkin-Elmer SCIEX API spectrometer. Elemental analyses were performed on a Thermoquest Flash 1112 series EA analyzer. Elemental analyses were found to be within ± 0.4% of the theoretical values. Purity of tested compounds was > 95%. Thin layer chromatography (TLC) analyses were conducted on aluminum sheet silica gel Merck $60F_{254}$. The spots were visualized using ultraviolet light. Flash chromatography was carried out on silica gel 60 (40−63 μm, Merck) using the indicated solvents. Compounds **1a**−**1f**, **2a**, **3a**, **4** and **5** were prepared as previously described.[15]

1-(Ethoxymethyl)-*N*-(2-iodophenyl)-1*H*-indole-2-carboxamide (8l). At −20 °C and under inert atmosphere, a 2.0 M $AlMe_3$ solution in toluene (5.05 mL, 10.11 mmol) diluted in CH_2Cl_2 (16 mL) was added dropwise to a solution of 2-iodoaniline **7l** (1.77 g, 8.08 mmol) in CH_2Cl_2 (5 mL). The solution was stirred at −20 °C for 45 min then abandoned to reach 0 °C. A solution of ethyl 1-(ethoxymethyl)-1*H*-indole-2-carboxylate **5** (500 mg, 2.02 mmol) in CH_2Cl_2 (4 mL) was added dropwise. The final solution was stirred at room temperature for 19 h. The medium

was carefully acidified at 0 °C by the addition of 1 N HCl (45 mL) then stirred for 10 min. The medium was diluted by the addition of H_2O (10 mL) and extracted by CH_2Cl_2 (3 × 50 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The crude solid was recrystallized from MeOH to give **8l** (836 mg, 98%). Mp 147−148 °C (MeOH). IR (KBr): ν 3278, 3026, 2978, 1648, 1522, 1433, 1312, 1292, 1086, 749 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.15 (t, 3H, J = 7.0 Hz, CH_3), 3.58 (q, 2H, J = 7.0 Hz, CH_2), 6.05 (s, 2H, CH_2), 6.91 (dt, 1H, J = 1.3, 8.0 Hz, H_{arom}), 7.21−7.26 (m, 2H, H_{arom}), 7.37−7.44 (m, 2H, H_{arom}), 7.62 (d, 1H, J = 8.5 Hz, H_{arom}), 7.72 (d, 1H, J = 7.9 Hz, H_{arom}), 7.84 (dd, 1H, J = 1.3, 8.0 Hz, H_{arom}), 8.37 (dd, 1H, J = 1.3, 8.3 Hz, H_{arom}), 8.41 (broad s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 15.2 (CH_3), 64.2 (CH_2), 73.7 (CH_2), 90.5 (C), 107.4 (CH), 111.5 (CH), 121.7 (CH), 122.1 (CH), 122.3 (CH), 125.4 (CH), 126.3 (CH), 126.5 (C), 129.5 (CH), 131.8 (C), 138.4 (C), 139.1 (CH), 139.7 (C), 160.2 (CO). MS (IS): m/z 421 ($M + H^+$). Anal. ($C_{18}H_{17}IN_2O_2$) C, H, N.

1-(Ethoxymethyl)-*N*-[2-(2-iodophenyl)ethyl]-1*H*-indole-2-carboxamide (8m). Under argon atmosphere, indole **4** (940 mg, 4.29 mmol), DMAP (0.52 g, 4.29 mmol), and EDCI (0.91 g, 4.72 mmol) were added to a solution of amine **7m** (4.72 mmol) in CH_2Cl_2 (45 mL) at 0 °C. The mixture was stirred for 4 h at 0 °C then for 20 h at room temperature. The mixture was acidified by the addition of a 6 N HCl solution (pH 1−2) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried over Na_2SO_4, then filtered, and concentrated *in vacuo*. The crude solid was washed with EtOAc/PE 2:8 to afford **8m** (1.88 g, 98%) as a solid. Mp 105−106 °C (EtOAc/PE). IR (KBr): ν 3276, 3049, 2962, 1627, 1542, 1344, 1313, 1229, 1092, 747 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.98 (t, 3H, J = 7.2 Hz, CH_3), 2.97 (t, 2H, J = 7.0 Hz, CH_2), 3.31 (q, 2H, J = 7.2 Hz, CH_2), 3.50 (broad q, 2H, J = 7.0 Hz, CH_2), 5.96 (s, 2H, CH_2), 6.95−7.00 (m, 1H, H_{arom}), 7.08 (s, 1H, H_{arom}), 7.13 (t, 1H, J = 7.1 Hz, H_{arom}), 7.26−7.34 (m, 3H, H_{arom}), 7.61 (d, 1H, J = 8.3 Hz, H_{arom}), 7.64 (d, 1H, J = 7.9 Hz, H_{arom}), 7.85 (d, 1H, J = 7.9 Hz, H_{arom}), 8.70 (broad t, 1H, J = 5.3 Hz, NH). ^{13}C NMR (DMSO-d_6): δ 14.9 (CH_3), 38.9 (CH_2), 39.6 (CH_2), 63.0 (CH_2), 72.5 (CH_2), 101.0 (C), 106.1 (CH), 111.2 (CH), 120.8 (CH), 121.6 (CH), 123.9 (CH), 126.0 (C), 128.4 (2 CH), 130.1 (CH), 132.3 (C), 138.3 (C), 139.1 (CH), 141.8 (C), 161.7 (CO). MS (IS): m/z 449 ($M + H^+$). Anal. ($C_{20}H_{21}IN_2O_2$) C, H, N.

1-(Ethoxymethyl)-*N*-[3-(2-iodophenyl)propyl]-1*H*-indole-2-carboxamide (8n). According to the procedure described for the synthesis of **8m**, compound **8n** was prepared from **4** and **7n** in 97% yield. Mp 99−100 °C (EtOAc/PE). IR (KBr): ν 3321, 3045, 2965, 1638, 1548, 1448, 1310, 1226, 1093, 1009, 751 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.99 (t, 3H, J = 7.2 Hz, CH_3), 1.75−1.85 (m, 2H, CH_2), 2.71−2.77 (m, 2H, CH_2), 3.28−3.40 (m, 4H, 2 CH_2), 5.98 (s, 2H, CH_2), 6.93−6.99 (m, 1H, H_{arom}), 7.11 (s, 1H, H_{arom}), 7.13 (t, 1H, J = 7.7 Hz, H_{arom}), 7.28 (t, 1H, J = 8.1 Hz, H_{arom}), 7.30−7.36 (m, 2H, H_{arom}), 7.61 (d, 1H, J = 8.3 Hz, H_{arom}), 7.65 (d, 1H, J = 8.3 Hz, H_{arom}), 7.83 (d, 1H, J = 7.7 Hz, H_{arom}), 8.65 (t, 1H, J = 5.5 Hz, NH). ^{13}C NMR (DMSO-d_6): δ 14.9 (CH_3), 29.8 (CH_2), 37.6 (CH_2), 38.3 (CH_2), 63.0 (CH_2), 72.4 (CH_2), 100.7 (C), 106.0 (CH), 111.2 (CH), 120.8 (CH), 121.6 (CH), 123.9 (CH), 126.0 (C), 128.1 (CH), 128.6 (CH), 129.7 (CH), 132.5 (C), 138.3 (C), 139.1 (CH), 144.1 (C), 161.7 (CO). MS (IS): m/z 463 ($M + H^+$). Anal. ($C_{21}H_{23}IN_2O_2$) C, H, N.

***tert*-Butyl-{[1-(ethoxymethyl)-1*H*-indol-2-yl]carbonyl}(2-iodophenyl)carbamate (9l).** A solution of amide **8l** (800 mg, 1.9 mmol), Boc_2O (663 mg, 3.04 mmol), and a catalytic amount of DMAP (46 mg, 0.38 mmol) in MeCN (30 mL) was stirred overnight at room temperature. After evaporation of the solvent, the residue was partitioned between EtOAc (10 mL) and H_2O (10 mL). The two phases were separated, and the aqueous phase was extracted with EtOAc (2 × 10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by flash chromatography (eluent: PE/EtOAc 95:5 to 9:1) to give **9l** (980 mg, 99%) as an oil. IR (film): ν 3054, 2983, 1737, 1678, 1469, 1265, 1151, 1096, 741 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.15 (t, 3H, J = 7.2 Hz, CH_3), 1.31 (s, 9H, 3 CH_3), 3.50−3.59 (m, 2H, CH_2), 5.76−5.87 (m, 2H, CH_2), 7.08 (dt, 1H, J = 1.3, 7.9 Hz, H_{arom}), 7.17−7.23 (m, 2H, H_{arom}), 7.33−7.48 (m, 3H, H_{arom}), 7.59 (d, 1H, J = 8.5 Hz, H_{arom}), 7.66 (d, 1H, J = 7.9 Hz, H_{arom}), 7.94 (dd, 1H, J = 1.3, 7.9 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 15.1 (CH_3), 27.8 ($3CH_3$), 64.0 (CH_2), 73.6 (CH_2), 83.9 (C), 99.6 (C), 110.6 (CH), 111.3 (CH), 121.4 (CH), 122.5 (CH), 125.4 (CH), 126.3 (C), 129.3 (CH), 129.8 (CH), 129.9 (CH), 132.5 (C), 139.2 (C), 140.0 (CH), 141.8 (C), 152.3 (CO), 164.2 (CO). MS (IS): m/z 521 ($M + H^+$). Anal. ($C_{23}H_{25}IN_2O_4$) C, H, N.

***tert*-Butyl-{[1-(ethoxymethyl)-1*H*-indol-2-yl]carbonyl}[2-(2-iodophenyl)ethyl]carbamate (9m).** According to the procedure described for the synthesis of **9l**, compound **9m** was prepared from **8m** in 99% yield as an oil (chromatography eluent: PE/EtOAc 9:1). IR (film): ν 3056, 2977, 1731, 1667, 1522, 1368, 1318, 1144, 743 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.15 (t, 3H, J = 7.2 Hz, CH_3), 1.16 (s, 9H, $3CH_3$), 3.20 (t, 2H, J = 7.1 Hz, CH_2), 3.54 (q, 2H, J = 7.2 Hz, CH_2), 4.05 (t, 2H, J = 7.1 Hz, CH_2), 5.77 (s, 2H, CH_2), 6.73 (d, 1H, J = 0.5 Hz, H_{arom}), 6.91 (td, 1H, J = 1.9, 7.2 Hz, H_{arom}), 7.17 (t, 1H, J = 7.0 Hz, H_{arom}), 7.27−7.36 (m, 3H, H_{arom}), 7.57 (d, 1H, J = 8.3 Hz, H_{arom}), 7.61 (d, 1H, J = 7.9 Hz, H_{arom}), 7.83 (dd, 1H, J = 1.1, 7.9 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 15.0 (CH_3), 27.5 (3 CH_3), 39.9 (CH_2), 45.5 (CH_2), 63.9 (CH_2), 73.5 (CH_2), 83.0 (C), 100.7 (C), 108.1 (CH), 111.0 (CH), 121.2 (CH), 122.0 (CH), 124.8 (CH), 126.3 (C), 128.3 (CH), 128.4 (CH), 130.5 (CH), 133.6 (C), 138.7 (C), 139.5 (CH), 141.4 (C), 153.5 (C), 165.8 (CO). MS (IS): m/z 549 ($M + H^+$). Anal. ($C_{25}H_{29}IN_2O_4$) C, H, N.

***tert*-Butyl-{[1-(ethoxymethyl)-1*H*-indol-2-yl]carbonyl}[3-(2-iodophenyl)propyl]carbamate (9n).** According to the procedure described for the synthesis of **9l**, compound **9n** was prepared from **8n** in 99% yield as an oil (chromatography eluent: PE/EtOAc 95:5). IR (film): ν 3063, 2977, 2919, 1732, 1667, 1524, 1317, 1144, 1010, 744 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.15 (t, 3H, J = 7.2 Hz, CH_3), 1.20 (s, 9H, 3 CH_3), 1.97−2.07 (m, 2H, CH_2), 2.83 (broad t, 2H, J = 7.1 Hz, CH_2), 3.56 (q, 2H, J = 7.2 Hz, CH_2), 3.90 (t, 2H, J = 7.1 Hz, CH_2), 5.77 (s, 2H, CH_2), 6.82 (s, 1H, H_{arom}), 6.86−6.91 (m, 1H, H_{arom}), 7.18 (t, 1H, J = 7.1 Hz, H_{arom}), 7.26−7.28 (m, 2H, H_{arom}), 7.34 (t, 1H, J = 7.9 Hz, H_{arom}), 7.57 (d, 1H, J = 8.5 Hz, H_{arom}), 7.62 (d, 1H, J = 7.9 Hz, H_{arom}), 7.81 (d, 1H, J = 8.1 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 14.9 (CH_3), 27.5 ($3CH_3$), 29.3 (CH_2), 38.2 (CH_2), 45.3 (CH_2), 63.8 (CH_2), 73.4 (CH_2), 82.8 (C), 100.4 (C), 108.0 (CH), 110.9 (CH), 121.2 (CH), 121.9 (CH), 124.8 (CH), 126.2 (C), 127.8 (CH), 128.3 (CH), 129.2 (CH), 133.7 (C), 138.6 (C), 139.3 (CH), 143.9 (C), 153.6 (C), 165.7 (CO). MS (IS): m/z 563 ($M + H^+$). Anal. ($C_{26}H_{31}IN_2O_4$) C, H, N.

1-(Ethoxymethyl)-*N*-[2-(2-iodophenyl)ethyl]-*N*-methyl-1*H*-indole-2-carboxamide (9o). At 0 °C, sodium hydride (27 mg, 0.67 mmol, 60% dispersed in oil) was added to a solution of **9m** (200 mg, 0.45 mmol) in THF (1.5 mL). The reaction mixture was stirred for 10 min at room temperature, and iodomethane (34 μL, 0.53 mmol) was added dropwise. The mixture was stirred for 4 h at room temperature. The solvent was evaporated *in vacuo*. The residue was taken up in H_2O (5 mL) and extracted with CH_2Cl_2 (2 × 5 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by flash chromatography (PE/EtOAc 65:35) to give **9o** (204 mg, 99%) as an oil. IR (film): ν 3056, 2975, 2936, 1633, 1538, 1455, 1400, 1312, 1180, 1095, 1013, 742 cm^{-1}. ^1H NMR (DMSO-d_6 at 75 °C): δ 1.02 (t, 3H, J = 7.0 Hz, CH_3), 3.06 (q, 2H, J = 7.0 Hz, CH_2), 3.09 (s, 3H, CH_3), 3.37 (q, 2H, J = 7.0 Hz, CH_2), 3.70 (broad t, 2H, J = 7.4 Hz, CH_2), 5.55 (broad s, 2H, CH_2), 6.58 (broad s, 1H, H_{arom}), 6.96−7.01 (m, 1H, H_{arom}), 7.12 (t, 1H, J = 7.5 Hz, H_{arom}), 7.22−7.37 (m, 3H, H_{arom}), 7.58 (broad d, 2H, J = 9.0 Hz, H_{arom}), 7.83 (d, 1H, J = 7.8 Hz, H_{arom}). MS (IS): m/z 463 ($M + H^+$). Anal. ($C_{21}H_{23}IN_2O_2$) C, H, N.

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***tert*-Butyl-7-(ethoxymethyl)-6-oxo-6,7-dihydroindolo[2,3-*c*]quinoline-5-carboxylate (10l).** A mixture of **9l** (650 mg, 1.25 mmol), $Pd(OAc)_2$ (56 mg, 0.25 mmol), PPh_3 (131 mg, 0.5 mmol), and Ag_2CO_3 (688 mg, 2.50 mmol) in DMF (25 mL) was vigorously stirred at 100 °C for 1 h. After cooling, the solvent was removed *in vacuo*. The residue was taken up in $MeOH/CH_2Cl_2$ (9:1), filtered over Celite, and rinsed with CH_2Cl_2. The solvent was evaporated *in vacuo* and the crude residue was purified by flash chromatography (PE/EtOAc 85:15 to EtOAc) to give **10l** (347 mg, 71%) as an oil. IR (film): ν 3070, 2977, 1768, 1654, 1466, 1231, 1146, 740 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.12 (t, 3H, J = 7.0 Hz, CH_3), 1.75 (s, 9H, 3 CH_3), 3.59 (q, 2H, J = 7.0 Hz, CH_2), 6.37 (s, 2H, CH_2), 7.26−7.28 (m, 1H, H_{arom}), 7.41−7.48 (m, 3H, H_{arom}), 7.58 (t, 1H, J = 7.8 Hz, H_{arom}), 7.80 (d, 1H, J = 8.3 Hz, H_{arom}), 8.43 (d, 1H, J = 8.1 Hz, H_{arom}), 8.51 (dd, 1H, J = 2.1, 7.5 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 15.2 (CH_3), 27.8 (3 CH_3), 63.9 (CH_2), 73.8 (CH_2), 86.8 (C), 112.3 (CH), 114.4 (CH), 118.7 (C), 121.1 (C), 122.2 (CH), 122.5 (C), 122.9 (CH), 123.5 (CH), 124.1 (CH), 125.1 (C), 127.0 (2 CH), 133.4 (C), 140.8 (C), 151.4 (CO), 154.7 (CO). MS (IS): m/z 415 ($M + Na^+$). Anal. ($C_{23}H_{24}N_2O_4$) C, H, N.

***tert*-Butyl-9-(ethoxymethyl)-8-oxo-5,6-dihydro-9*H*-indolo[2,3-*e*][3]benzazocine-7-carboxylate (10m).** According to the procedure described for the synthesis of **10l** ($Pd(OAc)_2$ = 5 mol %, temperature = 140 °C, reaction time = 1 h), compound **10m** was prepared from **9m** in 97% yield as an oil (chromatography eluent: PE/EtOAc 85:15). IR (film): ν 3057, 2978, 2936, 1758, 1716, 1682, 1455, 1368, 1148, 1095, 740 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.17 (t, 3H, J = 7.2 Hz, CH_3), 1.23 (s, 9H, 3 CH_3), 2.89−2.96 (m, 2H, CH_2), 3.51−3.71 (m, 3H, CH_2), 4.19−4.29 (m, 1H, CH_2), 5.87 (d, 1H, J = 10.7 Hz, CH_2), 6.00 (d, 1H, J = 10.7 Hz, CH_2), 7.20 (t, 1H, J = 7.4 Hz, H_{arom}), 7.33−7.36 (m, 3H, H_{arom}), 7.41−7.46 (m, 2H, H_{arom}), 7.60−7.63 (m, 2H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 15.0 (CH_3), 27.8 (3 CH_3), 33.1 (CH_2), 47.7 (CH_2), 64.1 (CH_2), 73.5 (CH_2), 81.7 (C), 111.1 (CH), 121.7 (CH), 122.2 (CH), 124.8 (C), 126.5 (C + 2 CH), 128.0 (CH), 129.5 (C), 130.9 (CH), 131.3 (CH), 133.0 (C), 136.5 (C), 139.8 (C), 151.8 (C), 166.1 (CO). MS (IS): m/z 421 ($M + H^+$). Anal. ($C_{25}H_{28}N_2O_4$) C, H, N.

***tert*-Butyl-10-(ethoxymethyl)-9-oxo-5,6,7,10-tetrahydroindolo[2,3-*f*][4]benzazonine-8-carboxylate (10n).** According to the procedure described for the synthesis of **10l**, ($Pd(OAc)_2$ = 5 mol %, temperature = 140 °C, reaction time = 1 h), compound **10n** was prepared from **9n** in 47% yield as an oil (chromatography eluent: PE/EtOAc 85:15). IR (film): ν 3047, 2969, 2930, 1716, 1693, 1465, 1337, 1297, 1149, 1092, 732 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.16 (s, 9H, 3 CH_3), 1.19 (t, 3H, J = 7.2 Hz, CH_3), 1.90−1.95 (m, 1H, CH_2), 2.11−2.23 (m, 1H, CH_2), 2.47−2.57 (m, 1H, CH_2), 2.74−2.80 (m, 1H, CH_2), 3.48−3.63 (m, 3H, CH_2), 3.87 (dd, 1H, J = 4.0, 14.5 Hz, CH_2), 5.74 (d, 1H, J = 10.7 Hz, CH_2), 5.83 (d, 1H, J = 10.7 Hz, CH_2), 7.14−7.47 (m, 7H, H_{arom}), 7.59 (d, 1H, J = 8.5 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 15.0 (CH_3), 27.5 (CH_2), 27.7 (3 CH_3), 31.2 (CH_2), 46.1 (CH_2), 64.1 (CH_2), 73.4 (CH_2), 81.5 (C), 110.5 (CH), 121.3 (CH), 121.8 (CH), 122.9 (C), 125.2 (CH), 125.6 (CH), 127.1 (C), 128.0 (CH), 129.2 (CH), 130.9 (C), 131.2 (CH), 131.9 (C), 138.5 (C), 141.9 (C), 152.1 (C), 167.2 (CO). MS (IS): m/z 435 ($M + H^+$). Anal. ($C_{26}H_{30}N_2O_4$) C, H, N.

9-(Ethoxymethyl)-7-methyl-5,6,7,9-tetrahydro-8*H*-indolo[2,3-*e*][3]benzazocin-8-one (10o). According to the procedure described for the synthesis of **10l**, ($Pd(OAc)_2$ = 5 mol %, temperature = 140 °C, reaction time = 3.5 h), compound **10o** was prepared from **9o** in 87% yield as an oil (chromatography eluent: PE/EtOAc 3:2). IR (film): ν 3056, 2971, 1640, 1542, 1454, 1329, 1219, 1099, 755 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.15 (t, 3H. J = 7.0 Hz, CH_3), 3.10 (s, 3H, CH_3), 3.18−3.34 (m, 3H, CH_2), 3.42−3.57 (m, 2H, CH_2), 4.26−4.37 (m, 1H, CH_2), 5.63 (d, 1H, J = 10.9 Hz, CH_2), 5.84 (d, 1H, J = 10.9 Hz, CH_2), 7.18−7.38 (m, 6H, H_{arom}), 7.59 (d, 1H, J = 8.3 Hz, H_{arom}), 7.64 (d, 1H, J = 7.9 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 15.0 (CH_3), 32.7 (CH_3), 34.0 (CH_2), 50.8 (CH_2), 64.0 (CH_2), 73.5 (CH_2), 110.6 (CH), 118.2 (C), 120.8 (CH), 121.2 (CH), 124.0 (CH), 126.4 (CH), 127.0 (C), 127.3 (CH), 129.4 (C), 131.9 (CH), 132.5 (C), 133.1 (CH), 134.6 (C), 138.0 (C), 164.9 (CO). MS (IS): m/z 335 ($M + H^+$). Anal. ($C_{21}H_{22}N_2O_2$) C, H, N.

5,7-Dihydroindolo[2,3-*c*]quinolin-6-one (11). A solution of **10l** (300 mg, 0.76 mmol) and 1 N HCl (6.2 mL) in 1,4-dioxane (13 mL) was stirred at 80 °C for 3 h. After cooling, the solution was neutralized by a saturated aqueous $NaHCO_3$ solution (pH 10) and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The crude solid was washed with cold MeOH to afford **11** (126 mg, 70%). Mp > 210 °C (washing cold MeOH). IR (KBr): ν 3319, 3156, 3007, 2971, 1660, 1613, 1539, 1329, 1043, 732 cm^{-1}. ^1H NMR (DMSO-d_6): δ 7.29−7.52 (m, 5H, H_{arom}), 7.64 (d, 1H, J = 8.1 Hz, H_{arom}), 8.44−8.50 (m, 2H, H_{arom}), 11.86 (s, 1H, NH), 12.35 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 113.1 (CH), 116.2 (CH), 118.1 (C), 118.3 (C), 120.7 (CH), 122.3 (C), 122.4 (2 CH), 123.1 (CH), 125.7 (CH), 126.0 (CH), 127.7 (C), 135.0 (C), 138.9 (C), 155.8 (CO). MS (IS): m/z 235 ($M+H^+$). Anal. ($C_{15}H_{10}N_2O$) C, H, N.

5,6,7,9-Tetrahydroindolo[2,3-*e*][3]benzazocin-8-one (1m). According to the procedure described for the synthesis of **1l** (reaction time = 5 h), compound **1m** was prepared from **10m** in 28% yield as a solid. Compound **1m** was purified by flash chromatography (chromatography eluent: CH_2Cl_2/MeOH 98.5:1.5). Mp > 210 °C (washing cold CH_2Cl_2). IR (KBr): ν 3255, 3168, 3046, 2922, 1623, 1542, 1460, 1336, 1294, 746 cm^{-1}. ^1H NMR (DMSO-d_6 + D_2O at 90 °C): δ 2.97 (t, 2H, J = 6.9 Hz, CH_2), 3.51 (t, 2H, J = 6.9 Hz, CH_2), 7.08 (t, 1H, J = 7.0 Hz, H_{arom}), 7.24 (t, 1H, J = 7.7 Hz, H_{arom}), 7.28−7.52 (m, 6H, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 32.8 (CH_2), 45.4 (CH_2), 112.2 (CH), 114.9 (C), 120.0 (CH), 120.1 (CH), 123.5 (CH), 125.8 (C), 126.3 (CH), 127.0 (CH), 129.9 (C), 130.0 (CH), 130.1 (CH), 133.7 (C), 136,6 (C), 137.5 (C), 166.2 (CO). MS (IS): m/z 263 ($M + H^+$). Anal. ($C_{17}H_{14}N_2O$) C, H, N.

5,6,7,10-Tetrahydroindolo[2,3-*f*][4]benzazonin-9(8*H*)-one (1n). According to the procedure described for the synthesis of **1l** (reaction time = 1 h), compound **1n** was prepared from **10n** in 51% yield as a solid. Compound **1n** was purified by flash chromatography (chromatography eluent: CH_2Cl_2/MeOH 98.5:1.5). Mp > 210 °C (washing cold MeOH). IR (KBr): ν 3279, 3227, 3057, 2930, 1658, 1634, 1561, 1408, 1332, 747 cm^{-1}. ^1H NMR (DMSO-d_6 + D_2O): δ 1.58 (broad s, 1H, CH_2), 1.83 (broad s, 1H, CH_2), 2.13 (broad s, 1H, CH_2), 2.73 (broad s, 1H, CH_2), 3.00 (broad s, 1H, CH_2), 3.25 (broad s, 1H, CH_2), 7.00−7.05 (m, 2H, H_{arom}), 7.17−7.22 (m, 3H, H_{arom}), 7.30−7.31 (m, 2H, H_{arom}), 7.46 (d, 1H, J = 8.1 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 32.7 (CH_2), 34.0 (CH_2), 44.2 (CH_2), 111.8 (CH), 114.5 (C), 119.3 (CH), 119.7 (CH), 122.5 (CH), 125.3 (CH), 127.1 (CH), 127.8 (C), 129.6 (CH), 131.3 (CH), 131.7 (CH), 132.9 (C), 135.4 (C), 143.1 (C), 166.5 (CO). MS (IS) m/z 277 ($M + H^+$). Anal. ($C_{18}H_{16}N_2O$) C, H, N.

7-Methyl-5,6,7,9-tetrahydroindolo[2,3-*e*][3]benzazocin-8-one (1o). According to the procedure described for the synthesis of **1l** (reaction time = 2 h), compound **1o** was prepared from **10o** in 67% yield as a solid. Compound **1o** was purified by flash chromatography (chromatography eluent: PE/EtOAc 1:1). Mp > 210 °C (washing cold CH_2Cl_2). IR (KBr): ν 3215, 3070, 2992, 1613, 1548, 1505, 1404, 1326, 1259, 1085, 745 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.96 (s, 3H, CH_3), 3.18 (s, 2H, CH_2), 3.72 (broad s, 2H, CH_2), 7.09 (t, 1H, J = 7.4 Hz, H_{arom}), 7.20−7.31 (m, 5H, H_{arom}), 7.45 (d, 1H, J = 8.1 Hz, H_{arom}), 7.52 (d, 1H, J = 7.9 Hz, H_{arom}), 11.78 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 33.2 (CH_3), 33.3 (CH_2), 51.3 (CH_2), 112.2 (CH), 114.9 (C), 119.9 (CH), 120.1 (CH), 123.1 (CH), 126.1 (C), 126.2 (CH), 126.9 (CH), 128.9 (C), 131.8 (CH), 131.9 (CH), 132.6 (C), 135.5 (C), 136.4 (C), 164.8 (CO). MS (IS): m/z 277 ($M + H^+$). Anal. ($C_{18}H_{16}N_2O$) C, H, N.

Biology. Reagents. Paclitaxel was obtained from Bristol-Myers Squibb (Paris, France). 5-FU, propidium iodide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT),

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Hoescht 33258, vinblastine, vinorelbine, and colchicine (Biochemika, ≥97.0%, HPLC) were purchased from Sigma.

Cell Lines. All cell lines were grown on 25 cm^2 flasks and were placed at 37 °C in a humidified atmosphere containing 5% CO_2. The MCF7 and the UACC812 breast cancer cell lines and the HCT116, SW48, and SW480 colorectal cancer cell lines were cultured in DMEM culture medium containing 10% fetal calf serum, 1% L-glutamine, and 2% penicillin-streptomycin. The Mes-sa sarcoma cell line and the CEM and RL lymphoma cell lines were cultured in RPMI1640 culture medium containing 10% fetal calf serum, 1% L-glutamine, and 1% penicillin−streptomycin.

Cell Growth Inhibition Assay. Antiproliferative activity was determined in three separate experiments, each of which was performed in triplicate as previously described.[20] Briefly, asynchronously growing cells were transferred into 96-well culture plates (Costar, Corning Inc., New York) in 100 μL of medium at a final concentration of 5×10^3 cells/well and incubated for 24 h. Corresponding drug concentrations were then added to each plate. After 72 h of drug exposure, 20 μL of MTT reagent (5 mg/mL) was added to each well. Cell growth was expressed as the percent of absorbance of treated wells relative to the untreated control wells. IC_{50} values were defined as drug doses resulting in 50% cell growth inhibition relative to untreated cells.

Cell Cycle Assay. For analysis of DNA content and cell cycle distribution, colorectal cancer cell lines were treated with compounds **1a**, **1b**, **1e**, and **1m** for 24 h. On the basis of the cytotoxicity assay, a concentration of 30 μM (aprox. IC_{80} values for these cell lines) was chosen for drug exposure experiments. After drug exposure, 10^6 cells/mL were resuspended in 2 mL of propidium iodide solution (50 μL/mL), incubated at 4 °C overnight, and then analyzed by flow cytometry. Flow cytometry was performed on FACScalibur (Becton Dickinson, San Jose, California). Cell cycle distribution and DNA ploidy status were calculated after exclusion of cell doublets and aggregates on a FL2-area/FL2-width dot plot using Modfit LT 2.0 software (Verity Software Inc. Topsham. ME).

Immunofluorescence and Microscopy. Exponentially growing cells were plated on 18-mm microscope glass slides and incubated with **1a**, **1b**, **1e**, and **1m** at 10 μM for 24 h. Then, cells were fixed in 100% methanol. DNA was counterstained with 5 μg/mL Hoescht 33258. Coverslips were examined with a Zeiss axioplasm microscope using a Zeiss x100 1.3 oil-immersion objective.

Purification of Tubulin and MT Polymerization Assays. Tubulin was purified from bovine brain from freshly slaughtered animals as described earlier.[21] In total, we obtained 244 mg of pure tubulin (Figure S1, Supporting Information) from two brains. The mixing of tubulin samples with different inhibitor concentrations was carried out on ice, and only freshly thawed tubulin was used. Unused tubulin was discarded after 20 min. Tubulin polymerization experiments were carried out at 37 °C in a 96-well Sunrise spectrophotometer (TECAN) in 96-well microclear plates (Greiner BIO-One) at a wavelength of 340 nm. First, we determined the optimal polymerization conditions by varying GTP, DMSO, and tubulin concentrations, and the best conditions were found to be 40 μM tubulin (based on the concentration of the α,β-tubulin heterodimer), 1.25 mM GTP, and 2.5% DMSO. Subsequently, tubulin polymerization assays under these conditions were performed in the absence of inhibitors (polymerization control) and in the presence of increasing inhibitor concentrations (1 μM to 75 μM). All compounds were measured at least in triplicate. Colchicine as an inhibitor of tubulin polymerization served as additional control. An example of the inhibition of tubulin polymerization by compound **1e**, a potent inhibitor analogue *in vitro* and in cell-based assays, and **1h**, as an example for an inhibitor that does not inhibit tubulin polymerization, and their corresponding concentration−response plots are shown in Supporting Information (Figure S2).

Colchicine-Binding Site Assay. Competition assays were performed as described previously with some modifications.[22,23] Five microliters of 2 mM stock solution of different tubulin-binding drugs to a final concentration of 50 μM were incubated with 100 μL of tubulin (1.1 mg/mL, 10 μM) at 37 °C for 30 min. Unlabeled colchicine and vinblastine (not-competitive) were used as positive and negative controls, respectively. The mixture was then incubated with fluorescent colchicine at 0.5 μM (Genolite Biotek, USA) under the same conditions. After incubation, each reaction was applied to a 5 mL HiTrap desalting column (GE Healthcare) previously calibrated with competition binding assay buffer (0.25 mM PIPES, pH 6.9, 0.05 mM GTP, and 0.25 mM $MgCl_2$) and eluted using 0.25 mL fractions. The peak position for tubulin was determined with an AKTA purifier (GE Healthcare) using UV absorbance at 280 nm. Since GTP also absorbs at this wavelength, the protein peak was further validated by measuring the protein concentration of the peak fractions using the Bradford reagent.[24] From each fraction, 50 μL were transferred into a 96-well flat bottom polystyrene NBS microplate (Product N°3650, Corning), and the fluorescence intensity was measured at an excitation of 493 nm and an emission wavelength of 516 nm using a TECAN Safire plate reader. The fluorescence intensity was plotted against the elution volume. All experiments were at least performed in triplicate.

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Supporting Information Available: Elemental analyses, experimental procedures, and spectral data of **6**, **7**, **11**, **12**, **13**, **14**, **1g**−**1s**, SDS−PAGE to estimate the purity of the protein, and polymerization of tubulin in the presence of inhibitors. This material is available free of charge via the Internet at http://pubs.acs.org.

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