Methoxy-Substituted 3-Formyl-2-phenylindoles Inhibit Tubulin Polymerization

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The aim of this study was the identification of the essential structural elements in the 12-formyl-5,6-dihydroindolo[2,1-*a*]isoquinoline system required for the inhibition of tubulin polymerization which is understood to be the predominant mode of action of this class of cytostatics. Since 2-phenylindole forms the main fragment of this tetracycle, it was used as the basic structure and modified with respect to the number and positions of the oxygen functions in the aromatic rings. Further modifications related to the nitrogen, which was both replaced by oxygen and sulfur and alkylated. All derivatives were tested for cytostatic activity in human breast cancer cells (MDA-MB 231, MCF-7) and inhibition of tubulin polymerization. The spectrum of activity ranged from inactive to IC_{50} values of 35 nM (cell growth inhibition) and 1.5 μM (tubulin polymerization), respectively, for the most active derivative **3e** (3-formyl-6-methoxy-2-(4-methoxyphenyl)indole). Although the correlation between antiproliferative activity and inhibition of tubulin polymerization was not very pronounced, all of the potent cytostatic agents in this study disrupted microtubule assembly completely at the standard concentration of 40 μM. By fluorescence microscopy it was demonstrated that the derivative **3e** degrades the cytoskeleton in a similar fashion as colchicine does leading to the condensation of the microtubules around the nucleus after treatment. The comparison between hydroxy and methoxy derivatives revealed a striking difference between the 2-phenylindole derivatives and the indoloisoquinolines. In the 2-phenylindole series, the methoxy compounds were much more effective than the free phenols, whereas in the tetracyclic system the effect of the hydroxy derivatives exceeded that of the methylated compounds by 1 order of magnitude. Preliminary studies on the binding mode showed that both the 2-phenylindole derivatives and the indoloisoquinolines bind to the colchicine site on tubulin.

The discovery of a variety of natural products that interact with the tubulin system either by preventing the polymerization of the α-/*β*-tubulin heterodimers to microtubules or by stabilizing the polymeric structures greatly stimulated the interest in this cellular system as a target for anticancer drugs. The assembly of microtubules is understood to be a dynamic process of polymerization and depolymerization. One of the important functions of the microtubules is the formation of the mitotic spindle necessary for the correct distribution of the chromosomes in dividing cells. Therefore, compounds acting on this system have become a valuable alternative to drugs that interact with the DNA as has been demonstrated by the therapeutic use of the vinca alkaloids vincristine and vinblastine. These alkaloids and some other substances of natural origin bind to the dimeric form of tubulin at distinct sites and prevent the formation of functional microtubules.[1] Since the function of the microtubules depends on the dynamic equilibrium between dimeric and polymeric structures, the stabilization of the microtubules also inhibits cell growth as had been demonstrated for paclitaxel (Taxol). After the discovery of this drug a variety of other natural products with structures of similar complexity and high biological activity were identified including epothilone B,[2,3] discodermolide,[4−6] and eleutherobin.[7]

From a synthetic point of view, compounds of low molecular weight are more attractive as a lead. The chemical structure of colchicine,[8] the first antimitotic agent identified, gave hints toward important structural elements required for the interference with the microtubule assembly. Some of these elements were found in podophyllotoxin[9] and combretastatin A-4,[10] two other inhibitors of tubulin polymerization. Over the past few years the spectrum of compounds which inhibit the growth of tumor cells by disrupting the dynamic equilibrium between the dimers and polymeric structures has steadily expanded. Among these substances are synthetic analogues of colchicine[11,12] and combretastatin A,[13,14] various heterocyclic systems such as 2-phenylquinolinone,[15] 2-styrylquinazolin-4-one,[16] 2-phenyl-1,8-naphthyridin-4-ones,[17,18] steroidal structures derived from 2-methoxyestradiol,[19] 2,3-diaryl-1,1-dichlorocyclopropanes,[20] and the sulfonamide E 7010[21] (Chart 1). The experimental data accumulated over the past decade have recently been analyzed by advanced quantitative structure−activity relationship (QSAR) techniques.[22]

In a recent paper we showed that the cytostatic effect of hydroxylated 12-formyl-5,6-dihydroindolo[2,1-*a*]isoquinolines can be attributed to their inhibitory effects on tubulin polymerization.[23] The aim of the present study was the identification of the essential structural elements in this class of heterocycles required for the disruption of microtubule assembly. Since the indolo[2,1-*a*]isoquinoline skeleton can be considered as a

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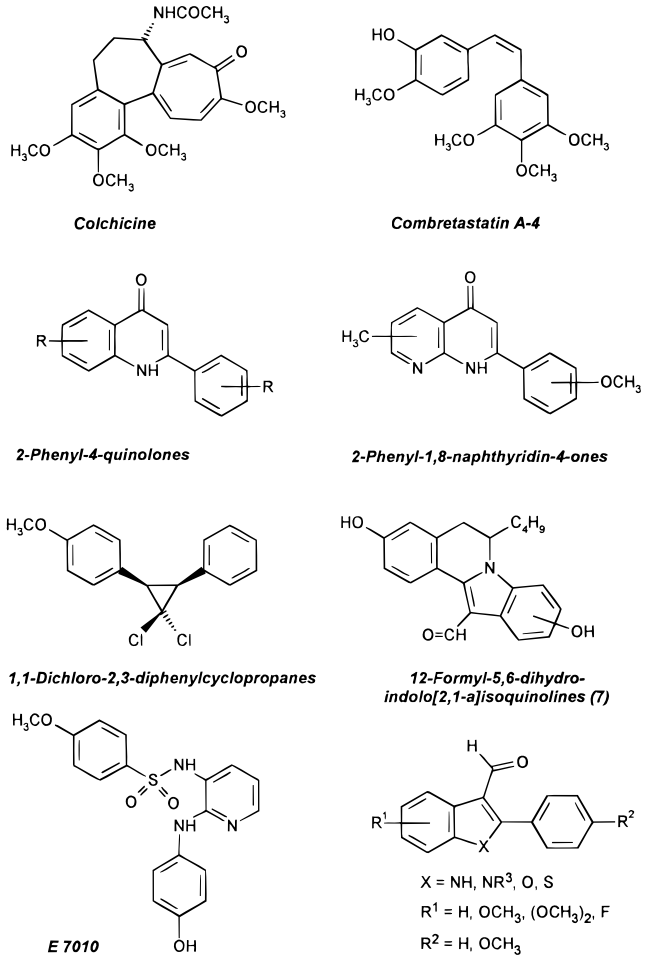
Chart 1. Inhibitors of Tubulin Polymerization



bridged 2-phenylindole, a number of 3-formyl-2-phenylindoles with oxygen functions at one or both aromatic rings were synthesized and evaluated for inhibitory activity on tubulin polymerization and cell proliferation. Additional structural variations concerned the indole nitrogen which was both alkylated and replaced by oxygen and sulfur.

Chemistry

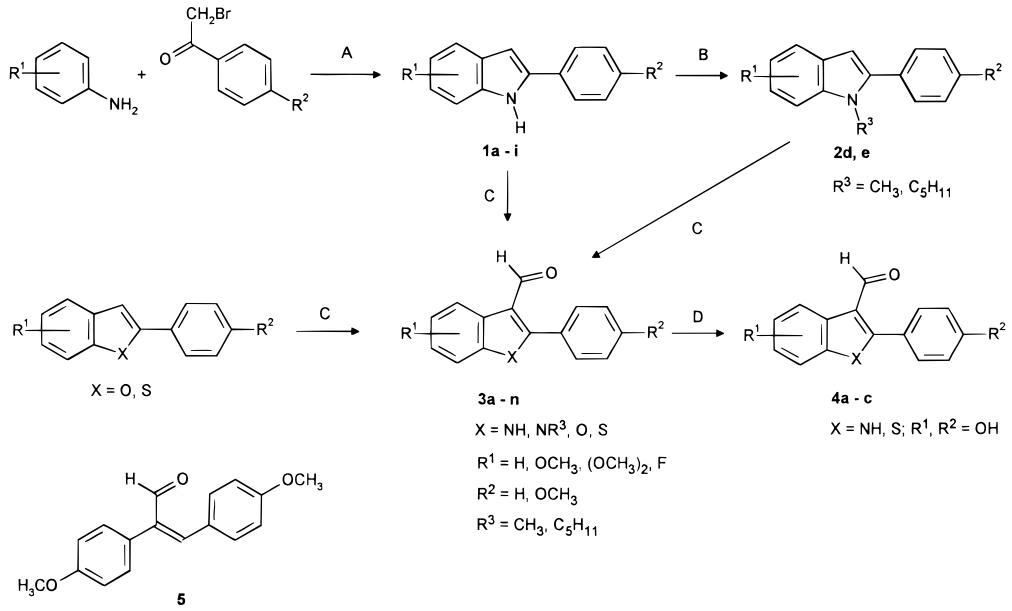
As a general approach to the synthesis of the 2-phenylindole system, the Bischler method was applied (Scheme 1). Ring-substituted aniline derivatives were reacted with ω-bromoacetophenone and its 4-methoxy congener to give the corresponding 2-phenylindole **1**.[24] N-Alkylation was readily achieved by deprotonation of **1** with sodium hydride followed by the reaction with the appropriate alkyl halide.[25] The preparation of the analogous 2-phenylbenzo[*b*]furan[26] and 2-phenylbenzo[*b*]thiophene[27] has been described previously. The formyl group was introduced into the 3-position of the heterocycles by the Vilsmeier reaction using dimethylformamide and $POCl_3$. In some cases, the ether functions were cleaved with boron tribromide to afford the phenolic compounds **4**.

Biological Results and Discussion

Antiproliferative activity was determined in two human breast cancer cell lines, MDA-MB 231 and MCF-7, that are often used for the evaluation of antimitotic compounds.[20,28−30] The latter cell line is estrogen receptor-positive and responds to estrogens and antiestrogens. Since hydroxylated 2-phenylindoles[24] and analogues[27] have been shown to exert their cytostatic action via the estrogen receptor, MCF-7 cells were included in order to detect potential (anti)hormonal effects.

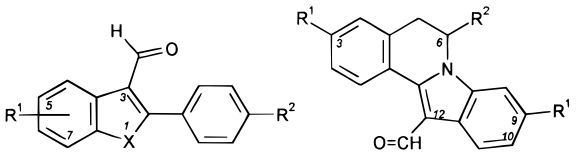
Preliminary experiments showed that contrary to the 5,6-dihydroindolo[2,1-*a*]isoquinoline series the methoxy derivatives of 3-formyl-2-phenylindole were more active than the corresponding hydroxy compounds. For the identification of the structural requirements for cytostatic activity, one or both methoxy groups were omitted. Cytostatic activity decreased only slightly upon this modification (Table 1). Stronger effects were noted when the phenyl ring (3-formylindole) or the indole nitrogen (**5**) was removed or the NH group had been

Scheme 1[a]



[a] (A) *N*,*N*-Dimethylaniline/170 °C; (B) NaH/R^3-Br; (C) DMF/$POCl_3$; (D) BBr_3.

Table 1. Inhibitory Effect of 3-Formyl-2-phenylindoles and Analogues on the Growth of Breast Cancer Cells and Tubulin Polymerization



compd	R^1	R^2	X	inhibn of cell growth MDA[a]	inhibn of cell growth MCF-7[b]	inhibn of tubulin polymerization, %[c] (IC_{50})[d]
3a	H	H	NH	0.42 ± 0.07	0.65 ± 0.08	2 ± 7
3b	6-OCH_3	H	NH	0.24 ± 0.02	0.19 ± 0.08	1 ± 6
3c	H	OCH_3	NH	0.47 ± 0.02	0.18 ± 0.04	97 ± 4 (3.3 μM)
3d	5-OCH_3	OCH_3	NH	0.26 ± 0.03	0.18 ± 0.07	93 ± 4 (4.0 μM)
3e	6-OCH_3	OCH_3	NH	0.035 ± 0.004	0.16 ± 0.03	98 ± 3 (1.5 μM)
3f	4,7-$(OCH_3)_2$	OCH_3	NH	>10	6.6 ± 0.3	14 ± 5
3g	5,6-$(OCH_3)_2$	OCH_3	NH	0.22 ± 0.05	0.23 ± 0.01	5 ± 6
3h	5,7-$(OCH_3)_2$	OCH_3	NH	2.8 ± 0.2	1.7 ± 0.1	48 ± 8
3i	6-F	OCH_3	NH	0.049 ± 0.006	0.043 ± 0.009	92 ± 5 (1.8 μM)
3j	5-OCH_3	OCH_3	NCH_3	>10	>10	4 ± 4
3k	6-OCH_3	OCH_3	NCH_3	2.4 ± 0.2	5.4 ± 0.1	48 ± 3
3l	6-OCH_3	OCH_3	NC_5H_{11}	3.3 ± 0.2	2.4 ± 0.2	4 ± 1
3m	5-OCH_3	OCH_3	O	8.4 ± 1.6	>10	35 ± 9
3n	6-OCH_3	OCH_3	S	0.99 ± 0.12	0.23 ± 0.03	100 ± 3 (1.8 μM)
4a	5-OH	OH	NH	2.5 ± 0.4	6.7 ± 0.2	28 ± 9
4b	6-OH	H	NH	7.9 ± 0.4	3.3 ± 0.1	8 ± 4
4c	6-OH	OH	S	9.3 ± 0.2	>10	21 ± 7
3-formylindole				>10	>10	0 ± 5
5				7.9 ± 0.4	7.0 ± 0.1	11 ± 7
colchicine				0.03 ± 0.01	nd[e]	100 ± 5 (1.9 μM)
7a[f]	OCH_3	C_3H_7	(NR)	>10	nd	17
7b[f]	OCH_3	C_4H_9	(NR)	8.7	nd	11
7c[f]	OH	C_3H_7	(NR)	0.3	0.29	93 (19 μM)
7d[f]	OH	C_4H_9	(NR)	1.4	0.22	100 (8.9 μM)

[a] Inhibition of the growth of MDA-MB 231 human breast cancer cells after incubation for 4 days, determined by measuring optical densities after crystal violet staining of vital cells. Mean of eight replicates ± SD. [b] Inhibition of the growth of estrogen-sensitive MCF-7 human breast cancer cells after incubation for 4 days, determined by measuring optical densities after crystal violet staining of vital cells. Mean of eight replicates ± SD. [c] Inhibition of tubulin polymerization by a 40 μM solution of test compounds, measured after 20 min at 37 °C. Mean values of two independent experiments. Full details are given in the text. [d] IC_{50} values were determined for all compounds that showed inhibition >50% at 40 μM. [e] nd, not determined. [f] Data from ref 23.

replaced by oxygen (**3m**). N-Alkyation also diminished the antiproliferative effect, whereas replacement of the indole nitrogen by sulfur (**3n**) had only a minor effect. The most favorable conditions were provided by a methoxy group (**3e**) or a fluorine atom (**3i**) in position 6 of the indole with IC_{50} values of 0.035 μM (MCF-7: 0.16 μM) and 0.049 μM (MCF-7: 0.043 μM), respectively. Additional methoxy groups did not improve the antiproliferative effect. The equivalence of fluoro and methoxy substituents was also noticed in the 2-phenylquinolone series.[31] The comparison of the data from the estrogen receptor-positive (MCF-7) and -negative (MDA-MB 231) cell lines revealed no significant differences of the IC_{50} values. This result was not unexpected because most of these compounds do not meet the structural requirements for high binding affinity for the estrogen receptor such as free hydroxy groups and lipophilic substituents in the central part of the molecule.[1] Since both elements interfere with the tubulin-directed action (vide infra), no attempt was made to design molecules that act via both the tubulin system and the estrogen receptor.

All of the compounds were tested for their ability to interfere with the tubulin polymerization. In the routine experiments a standard dose of 40 μM was used and the degree of polymerization was determined turbidimetrically at 350 nm (Table 1). At this concentration inhibition ranged from 0% to 100% depending on the molecular structure. The correlation between these data and those from the antiproliferative assays was rather poor. However, all of compounds which completely suppressed microtubule assembly at this concentration also strongly inhibited cellular growth. Similar deviations between these two different biological assays were also observed in other classes of antimitotic agents, e.g., in the diaryldichlorocyclopropane series[20] and the curacin A analogues.[32]

The most potent derivatives (**3c**−**e**,**i**) were tested at various concentrations. The dose−response curves depicted in Figure 1 displayed inhibitory activities similar to that of colchicine. The IC_{50} values were approximately 1 order of magnitude lower than those of the most potent indoloisoquinolines (Table 1). The main difference between the 2-phenylindoles and isosters and the indoloisoquinoline system is the discrepancy between the hydroxy and methoxy forms as the more active structures. This finding can possibly be rationalized by different binding modes or binding sites of these compounds. Since the indolo[2,1-*a*]isoquinolines were shown to bind at the colchicine binding site,[23] two different 3-formyl-2-indoles (**3e**,**k**) and the thiophene analogue of **3e** (**3n**), as well as two representative examples of the indoloisoquinoline series (**7a**,**c**), were tested for their capability of competing with

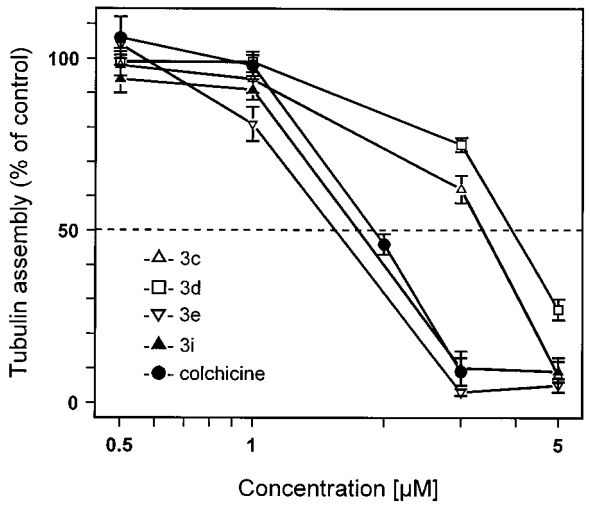


Figure 1. Inhibitory effect of various 3-formyl-2-phenylindoles and of colchicine on the polymerization of calf brain tubulin. Assembly of microtubules was assessed turbidimetrically at 350 nm 20 min after the temperature had been switched from 2 to 37 °C. Polymerization in the absence of inhibitor gave the control readings. Values are means of three independent experiments ± SEM.

Table 2. Inhibition of Colchicine Binding by 3-Formyl-2-phenylindoles and Analogues

compd	inhibn of colchicine binding[a] (%)
3e	23
3k	30
3n	56
7a	<10
7c	40

[a] Reaction mixtures contained tubulin, 1 μM [^{3}H]colchicine, and 10 μM inhibitor and were incubated for 30 min at 37 °C prior to analysis. Mean of two independent experiments.

colchicine for binding to tubulin. In preliminary experiments with a standard concentration of 10 μM, we found that all of these agents are capable of displacing [^{3}H]colchicine from its binding site (Table 2). Since none of the compounds stabilized the colchicine binding as the vinca site ligands do,[21,23,33−35] an interaction with the binding of vinblastine is not very likely.

The degradation of the cytoskeleton as the result of the interference with the microtubule assembly can be demonstrated by fluorescence microscopy. Representative cells treated with **3e** and colchicine in concentrations of 0.1, 1, and 10 μmol/L are shown in Figure 2. Microtubules were visualized by the double-antibody technique with Cy3 as the fluorescence label. Both indole **3e** and colchicine distroyed the cellular network of the microtubules at 0.1 μM and led to a marked tubulin condensation around the nucleus when higher concentrations were applied.

The results from the various assays suggest that the antitumor activity of the 12-formyl-5,6-dihydroindolo[2,1-*a*]isoquinolines is associated with the 2-phenylindole structure incorporated in the tetracyclic system. The removal of the alkane bridge that links carbon 3 of the indole with carbon 2 of the 2-phenyl moiety increased the potency substantially. In both series, 2-phenylindoles and indoloisoquinolines, methoxy and hydroxy derivatives were tested. The results revealed a striking difference between these two classes of compounds. In the 2-phenylindole series, the inhibitory effect of the methoxy compounds exceeded that of the phenols by 1 order of magnitude, whereas the situation in the tetracyclic system was exactly reversed. From the data available it appears that the formyl group and the aromatic rings are the most important structural elements for the interaction with tubulin. Despite the fact that the active forms of the two classes of inhibitors differ in the nature of the aromatic oxygen functions, they are both capable of displacing cochicine from its binding site. Obviously, the heterocyclic 3-formyl group is the dominant structural element, and the oxygen functions can possibly bind to different sites depending on their chemical structure.

The formyl group can be considered as a novel structural element in this class of antimitotic agents. There is only a vague analogy to the 2-phenylquinolone system which contains a six-membered heterocycle with an incorporated carbonyl function (Chart 1). When the latter system was substituted with methoxy or fluorine, its activity on tubulin polymerization and cellular growth was rather similar to that of the 2-phenylindole structure.[31] The strong inhibitory effects of some of these 2-phenylindole derivatives on tumor cell growth and tubulin polymerization make this system attractive for further structural variations which might increase the cytostatic potency of this heterocyclic structure.

Experimental Section

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, University of Regensburg, and were within ±0.40% of the calculated values except where noted. IR spectra were recorded on an Acculab 7 infrared spectrometer (Beckman), and peak positions are expressed in cm^{-1}. NMR spectra were obtained on a Bruker AC-250 spectrometer with TMS as internal standard. They were recorded in DMSO-d_6 as solvent except where noted and are consistent with the assigned structures; values are given in δ units (ppm). The syntheses of the 2-phenylindoles **1a**,[36] **1b**,[37] **1c**,[38] **1d**,[24] **1e**,[24] **1f**,[39] **2a**,[24] and **2d**,[24] 5-methoxy-2-(4-methoxyphenyl)benzo[*b*]furan,[40] 6-methoxy-2-(4-methoxyphenyl)benzo[*b*]thiophene,[41] and (*Z*)-2,3-bis(4-methoxyphenyl)-propenal (**5**)[42] have been described previously.

General Procedure for the Synthesis of 2-Phenylindoles. The respective aniline (60 mmol) was dissolved in 15 mL of *N*,*N*-dimethylaniline and heated to 170 °C. A solution of 20 mmol of the ω-bromoacetophenone in 30 mL of xylene was added dropwise. After addition, the reaction mixture was heated under reflux for 3 h. After the mixture had cooled, 150 mL of EtOAc and 70 mL of 2 N HCl were added and the layers separated. The aqueous layer was extracted with EtOAc several times. The combined organic layers were washed with water and saline and dried ($MgSO_4$). After evaporation of the solvents the crude product was purified by chromatography (SiO_2) and/or crystallization. The following compounds were obtained by this method.

5,6-Dimethoxy-2-(4-methoxyphenyl)indole (1g): yield 16%; colorless crystals; mp 207−209 °C (EtOH); ^{1}H NMR 3.72 (s, 3H, −OCH$_3$), 3.77 (s, 6H, −OCH$_3$), 6.50−7.00 (m, 1H, Ar−H), 6.54 (d, ^4J = 2 Hz, 1H, H-3), 6.93/7.63 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 6.97 (s, 2H, H-4, H-7). Anal. ($C_{17}H_{17}NO_3$) C, H, N.

5,7-Dimethoxy-2-(4-methoxyphenyl)indole (1h): yield 19%; colorless crystals; mp 164−166 °C (EtOH); ^{1}H NMR 3.72 (s, 3H, −OCH$_3$), 3.80 (s, 3H, −OCH$_3$), 3.89 (s, 3H, −OCH$_3$), 6.20−7.80 (m, 1H, Ar−H), 6.23 (d, ^4J = 2 Hz, 1H, H-6), 6.50 (d, ^4J = 2 Hz, 1H, H-4), 6.56 (s, 1H, H-3), 6.89/7.77 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H). Anal. ($C_{17}H_{17}NO_3$) C, H, N.

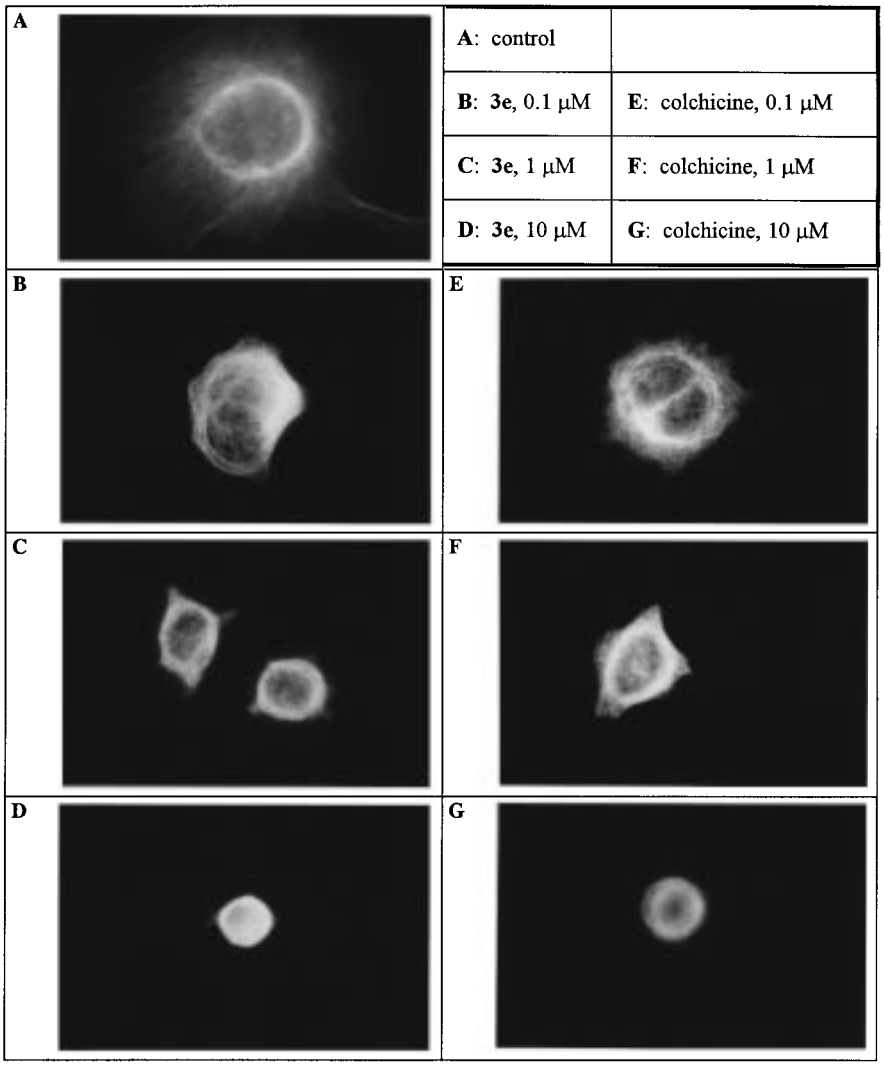


Figure 2. Effect of 3-formyl-6-methoxy-2-(4-methoxyphenyl)indole (**3e**) and colchicine on the cellular distribution of tubulin, determined by fluorescence microscopy. MCF-7 cells were grown on glass slides overnight at 37 °C and subsequently treated with the drugs at various concentrations for 60 min. After fixation, microtubules were visualized by sequential treatment with a murine monoclonal antibody to α-tubulin and a Cy3-labeled goat antibody to mouse IgG: (A) untreated cells; (B) cells treated with **3e**, 0.1 μM; (C) cells treated with **3e**, 1 μM; (D) cells treated with **3e**, 10 μM; (E) cells treated with colchicine, 0.1 μM; (F) cells treated with colchicine, 1 μM; (G) cells treated with colchicine, 10 μM. For the photographs presented, the magnification was 1200-fold (figure reproduced at 65% of original).

6-Fluoro-2-(4-methoxyphenyl)indole (1i): yield 20%; white crystals; mp 208−210 °C (EtOH); ^{1}H NMR 3.92 (s, 3H, −OCH_3), 6.74−7.99 (m, 5H, Ar−H), 7.13/7.88 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H). Anal. ($C_{15}H_{12}FNO$) C, H, N.

6-Methoxy-2-(4-methoxyphenyl)-1-pentylindole (2e). Under N_2, a solution of **1e** (4.3 mmol) in 25 mL of dry DMF was added slowly to an ice-cold suspension of sodium hydride (6.0 mmol) in 10 mL of dry DMF. Stirring was continued until the gas evolution ceased. 1-Bromopentane (4.3 mmol) in 30 mL of dry DMF was added dropwise. After the mixture stirred for 2 h at room temperature, the excess of sodium hydride was carefully destroyed by addition of water. After addition of an additional volume of 80 mL of water, the mixture was extracted with EtOAc several times. The combined organic layers were washed with water and dried ($MgSO_4$). After evaporation of the solvent, the product was purified by chromatography (SiO_2, CH_2Cl_2) and crystallized from EtOH to afford colorless crystals (62%): mp 62−64 °C; ^{1}H NMR 0.58−1.71 (m, 9H, alkyl-H), 3.81 (s, 6H, −OCH_3), 4.31 (t, ^3J = 7 Hz, 2H, N−CH_2-), 6.30−7.43 (m, 2H, Ar−H), 6.30 (s, 1H, H-7), 6.67 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1H, H-5), 7.00/7.37 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H). Anal. ($C_{21}H_{25}NO_2$) C, H, N.

General Procedure for the Formylation Reaction. Under N_2, $POCl_3$ (20 mmol) was added to 15 mmol of dry DMF at a temperature of 15 °C. After the mixture stirred for 10 min, 2.0 mmol of the respective aromatic compound in 10 mL of dry DMF was added to the viscous mixture while the temperature was kept between 10 and 20 °C. The reaction was completed by stirring for 1 h at 40 °C. The mixture was poured into 100 mL of ice−water. The pH was adjusted to 6 by adding NaOH solution (40%). After extraction with $CHCl_3$, the combined organic layers were washed with water and saline and dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by chromatography (SiO_2) and/or crystallization. The following compounds were synthesized by this method.

3-Formyl-2-phenylindole (3a): 31% yield; light-brown powder; mp 236−240 °C; ^{1}H NMR 7.09−7.93 (m, 9H, Ar−H), 8.08−8.33 (m, 1H, H-4), 9.97 (s, 1H, formyl-H). Anal. ($C_{15}H_{11}NO$) C, H, N.

3-Formyl-6-methoxy-2-phenylindole (3b): 23% yield; yellow crystals; mp 255−258 °C; ^{1}H NMR 3.78 (s, 3H, −OCH_3), 6.82 (d, ^3J = 9 Hz, 1H, H-5), 6.90 (s, 1H, H-7), 7.34−7.78 (m, 6H, Ar−H), 7.98 (d, ^3J = 9 Hz, 1H, H-4), 9.78 (s, 1H, formyl-H). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

3-Formyl-2-(4-methoxyphenyl)indole (3c): 20% yield; white crystals; mp 206−208 °C; ^{1}H NMR 3.88 (s, 3H, −OCH_3), 7.10−7.92 (m, 4H, Ar−H), 7.19/7.75 (AA′BB′, ^3J = 9 Hz, 4H,

Ar−H), 8.12−8.36 (m, 1H, H-4), 10.03 (s, 1H, formyl-H). Anal. ($C_{16}H_{13}NO_2$) C, H, N.

3-Formyl-5-methoxy-2-(4-methoxyphenyl)indole (3d): yield 46%; light-pink crystals; mp 230−232 °C (MeOH); IR (KBr) 1630; ^{1}H NMR 3.79 (s, 3H, −OCH_3), 3.87 (s, 3H, −OCH_3), 6.70−7.75 (m, 1H, Ar−H), 6.78 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1H, H-6), 7.13/7.69 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 7.37 (d, ^3J = 9 Hz, 1H, H-7), 7.70 (d, ^4J = 2 Hz, 1H, H-4), 10.09 (s, 1H, formyl-H); ^{13}C NMR (DMSO) 55.40 (2C), 103.05, 112.56, 113.00, 113.12, 114.47 (2C), 122.22, 126.79, 130.67, 131.07 (2C), 149.16, 155.82, 160.53, 185.18. Anal. ($C_{17}H_{15}NO_3$) C, H, N.

3-Formyl-6-methoxy-2-(4-methoxyphenyl)indole (3e): yield 48%; light-yellow crystals; mp 245−249 °C (MeOH); ^{1}H NMR 3.79 (s, 3H, −OCH_3), 3.84 (s, 3H, −OCH_3), 6.60−8.00 (m, 1H, Ar−H), 6.64 (d, ^4J = 2 Hz, 1H, H-7), 6.97 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1H, H-5), 7.02/7.58 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 7.95 (d, ^3J = 8 Hz, 1H, H-4), 9.96 (s, 1H, formyl-H). Anal. ($C_{17}H_{15}NO_3$) C, H, N.

3-Formyl-4,7-dimethoxy-2-(4-methoxyphenyl)indole (3f): yield 33%; light-yellow crystals; mp 79−81 °C; ^{1}H NMR 3.84 (s, 3H, −OCH_3), 3.91 (s, 6H, −OCH_3), 6.60−7.80 (m, 1H, Ar−H), 6.63 (s, 2H, H-5, H-6), 6.97/7.75 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 10.62 (s, 1H, formyl-H). Anal. ($C_{18}H_{17}NO_4$) C, H, N.

3-Formyl-5,6-dimethoxy-2-(4-methoxyphenyl)indole (3g): yield 12%; light-yellow crystals; mp 268−272 °C; ^{1}H NMR 3.89 (s, 3H, −OCH_3), 3.92 (s, 6H, −OCH_3), 6.90−7.80 (m, 1H, Ar−H), 6.98 (s, 1H, H-7), 7.71 (s, 1H, H-4), 7.17/7.69 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 9.94 (s, 1H, formyl-H). Anal. ($C_{18}H_{17}NO_4$) C, H, N.

3,4-Diformyl-5,6-dimethoxy-2-(4-methoxyphenyl)indole was formed as a byproduct in 8% yield and separated from the main product by chromatography (SiO_2; CH_2Cl_2/EtOH, 1:1); white crystals; mp 131−134 °C (MeOH); ^{1}H NMR 3.75 (s, 3H, −OCH_3), 3.82 (s, 6H, −OCH_3), 7.00−7.35 (m, 1H, Ar−H), 7.03/7.43 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 7.25 (s, 1H, H-7), 7.43 (s, 1H, formyl-H), 8.77 (s, 1H, formyl-H). Anal. ($C_{19}H_{17}NO_5$) C, H, N.

3-Formyl-5,7-dimethoxy-2-(4-methoxyphenyl)indole (3h): yield 10%; white crystals; mp 170−172 °C (EtOH); ^{1}H NMR 3.75 (s, 3H, −OCH_3), 3.79 (s, 3H, −OCH_3), 3.89 (s, 3H, −OCH_3), 6.40−7.65 (m, 1H, Ar−H), 6.42 (d, ^4J = 2 Hz, 1H, H-6), 7.23 (d, ^4J = 2 Hz, 1H, H-4), 7.04/7.56 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 9.81 (s, 1H, formyl-H). Anal. ($C_{18}H_{17}NO_4$) H, N; C: calcd, 69.44; found, 68.89.

3,4-Diformyl-5,7-dimethoxy-2-(4-methoxyphenyl)indole was formed as a byproduct in 7% yield and separated from the main product by chromatography (SiO_2; CH_2Cl_2/EtOH, 5:1): yellow crystals; mp 223−225 °C (EtOH); ^{1}H NMR 3.42 (s, 3H, −OCH_3), 3.56 (s, 3H, −OCH_3), 3.68 (s, 3H, −OCH_3), 6.25−7.30 (m, 1H, Ar−H), 6.30 (s, 1H, H-6), 6.61/7.21 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 9.80 (s, 1H, formyl-H), 10.27 (s, 1H, formyl-H). Anal. ($C_{19}H_{17}NO_5$) C, H, N.

6-Fluoro-3-formyl-2-(4-methoxyphenyl)indole (3i): 55% yield; light-brown crystals; mp 238−240 °C (MeOH); ^{1}H NMR 4.06 (s, 3H, −OCH_3), 7.08−7.32 (m, 3H, Ar−H), 7.32/7.90 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 7.96−8.25 (m, 1H, H-4), 10.11 (s, 1H, formyl-H). Anal. ($C_{16}H_{12}NO_2F$) C, H, N.

3-Formyl-5-methoxy-2-(4-methoxyphenyl)-1-methylindole (3j): 45% yield; white crystals; mp 158−160 °C (MeOH); ^{1}H NMR 3.60 (s, 3H, N−CH_3), 3.78 (s, 3H, −OCH_3), 3.83 (s, 3H, −OCH_3), 6.56−7.68 (m, 7H, Ar−H), 9.33 (s, 1H, formyl-H). Anal. ($C_{18}H_{17}NO_3$) C, H, N.

3-Formyl-6-methoxy-2-(4-methoxyphenyl)-1-methylindole (3k): 25% yield; white crystals; mp 143−145 °C (MeOH); IR (KBr) 1665; ^{1}H NMR 3.70 (s, 3H, N−CH_3), 3.91 (s, 6H, −OCH_3), 6.85 (d, ^4J = 2 Hz, 1H, H-7), 7.10 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1H, H-5), 8.04 (d, ^3J = 9 Hz, 1H, H-4), 7.17/7.54 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 9.50 (s, 1H, formyl-H). Anal. ($C_{18}H_{17}NO_3$) C, H, N.

3-Formyl-6-methoxy-2-(4-methoxyphenyl)-1-pentylindole (3l): 44% yield; red crystals; mp 92−94 °C; ^{1}H NMR 0.53−1.79 (m, 9H, CH_3−CH_2−CH_2−CH_2-), 3.83 (s, 6H, −OCH_3), 4.12 (t, ^3J = 8 Hz, 2H, N−CH_2-), 6.67−8.18 (m, 2H, Ar−H), 7.11/7.54 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 8.07 (d, ^3J = 9 Hz, 1H, H-4), 9.53 (s, 1H, formyl-H). Anal. ($C_{22}H_{25}NO_3$) C, H, N.

3-Formyl-5-methoxy-2-(4-methoxyphenyl)benzo[*b*]furan (3m): 64% yield; white crystals; mp 122−124 °C (EtOH); ^{1}H NMR 3.82 (s, 3H, −OCH_3), 3.86 (s, 3H, −OCH_3), 6.99 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1H, H-6), 7.17/7.93 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 7.58 (d, ^3J = 9 Hz, 1H, H-7), 7.60 (d, ^4J = 2 Hz, 1H, H-4), 9.28 (s, 1H, formyl-H). Anal. ($C_{17}H_{14}O_4$) C, H.

3-Formyl-6-methoxy-2-(4-methoxyphenyl)benzo[*b*]thiophene (3n): 58% yield; light-yellow powder; mp 122−124 °C; IR (KBr) 1680; ^{1}H NMR ($CDCl_3$) 3.71 (s, 3H, −OCH_3), 3.81 (s, 3H, −OCH_3), 6.75−7.43 (m, 2H, Ar−H), 6.84/7.34 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 8.50 (d, ^3J = 9 Hz, 1H, H-4), 9.88 (s, 1H, formyl-H); ^{13}C NMR ($CDCl_3$) 55.47 (2C), 104.54, 114.43 (2C), 115.50, 124.10, 125.77, 129.53, 131,18, 131.79 (2C), 139.24, 158.26, 158.54, 161.13, 186.61. Anal. ($C_{17}H_{14}O_3S$) C, H.

3-Formyl-5-hydroxy-2-(4-hydroxyphenyl)indole (4a). Under N_2, a suspension of 3-formyl-5-methoxy-2-(4-methoxyphenyl)indole (**3d**) (1.0 mmol) in 150 mL of dry CH_2Cl_2 was added slowly to a solution of 1.0 mL (10.8 mmol) of BBr_3 in 10 mL of dry CH_2Cl_2 at a temperature of −10 °C. After stirring for 1 h at this temperature, the mixture was allowed to warm to room temperature, and stirring was continued for 3 days. With cooling a saturated solution of $NaHCO_3$ was added until the vigorous reaction ceased. After addition of 150 mL of EtOAc the mixture was stirred vigorously for 30 min. The layers were separated, and the aqueous layer was extracted three times with EtOAc. After washing with water and drying (Na_2SO_4), the solvent was removed in vacuo and the residue purified by chromatography (SiO_2; EtOAc/CH_2Cl_2, 3:1) to give a gray amorphous solid: mp 252−256 °C; ^{1}H NMR (CD_3OD) 6.64−7.74 (m, 4H, H-7, N−H, O−H), 6.78 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1H, H-6), 6.94/7.52 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 7.66 (d, ^4J = 2 Hz, 1H, H-4), 9.82 (s, 1H, formyl-H). Anal. ($C_{16}H_{11}NO_3$) C; H: calcd, 4.38; found, 5.12. N, calcd, 5.53; found, 4.78.

3-Formyl-6-hydroxy-2-phenylindole (4b) was prepared from **3b** in 95% yield by a method similar to that described for **4a**: off-white powder; mp 235−237 °C; ^{1}H NMR (acetone-d_6) 6.83 (d, ^3J = 9 Hz, 1H, H-5), 8.21 (d, ^3J = 9 Hz, 1H, H-4), 7.32−8.11 (m, 7H, Ar−H), 9.95 (s, 1H, formyl-H). Anal. ($C_{15}H_{11}NO_2$) H, N; C: calcd, 75.94; found, 75.51.

3-Formyl-6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]thiophene (4c) was prepared from **3n** in 89% yield by a method similar to that described for **4a**: white powder; mp 242−246 °C; ^{1}H NMR 7.05/7.65 (AA′BB′, ^3J = 9 Hz, 4H, Ar−H), 7.10−7.75 (m, 3H, Ar−H), 8.41 (s, 1H, −OH), 9.82 (s, 1H, −OH), 9.75 (s, 1H, formyl-H). Anal. ($C_{15}H_{10}SO_3$) H; C: calcd, 66.64; found, 66.02.

Materials and Reagents for Bioassays. Drugs and biochemicals were obtained from Sigma (Deisenhofen, Germany). [^{3}H]Colchicine was purchased from New England Nuclear (Dreieich, Germany). The monoclonal antibody anti-α-tubulin (clone DM1A, IgG1 isotype) was obtained from Sigma and the Cy3-linked anti-mouse-Ig goat antibody from Jackson Immuno Research Laboratories Inc. Buffer solutions: PEM, 0.1 M PIPES-NaOH, 1 mM EGTA, 1 mM $MgSO_4$, pH 6.6−6.7; PEMG, 0.1 M PIPES−NaOH, 0.8 M monosodium L-glutamate, 1 mM EGTA, 1 mM $MgSO_4$, pH 6.6−6.7; PBS, 8.0 g/L NaCl, 0.2 g/L KCl, 1.0 g/L $Na_2HPO_4 \cdot H_2O$, 0.2 g/L KH_2PO_4. Scintillation liquid: Rotiszint Eco Plus (Roth, Karlsruhe, Germany).

Isolation and Purification of Calf Brain Tubulin. The cortex of one or two fresh calf brains in ice-cold PEM buffer (1 mL/g of tissue, + 16 mg of DTE/100 mL of buffer solution) was homogenized in portions. After centrifugation (90 min; 20000*g*) at 2−4 °C, the supernatant was carefully decanted. The concentrations of GTP and ATP were adjusted to 0.1 and 2.5 mM, respectively. After stirring gently at 37 °C for 30 min, the solution was transferred to centrifugation tubes and carefully underlayered with a prewarmed (37 °C) sucrose solution (10% in PEM buffer solution containing 1 mM GTP, approximately 10% of the transferred volume). After centrifugation at 37 °C for 45 min (20000*g*) the pellets were weighed

and suspended in ice-cold PEM buffer solution (3 mL/g) and homogenized in a Teflon-in-glass potter. After standing in ice for 30 min, the suspension was centrifuged at 2 °C for 30 min (40000*g*). The supernatant was separated and adjusted to 1 mM GTP. By incubation at 37 °C for 15 min tubulin was polymerized once again. After centrifugation at 37 °C for 30 min microtubules were obtained as shiny gellike pellet. The yields ranged from 2 to 6 g/brain. Aliquots were frozen in liquid nitrogen and stored at −70 °C. Purity was checked by polyacrylamide gel electrophoresis.

Tubulin Polymerization Assay. The pellet of frozen microtubules was warmed to 37 °C in a water bath. After addition of the 20-fold volume of ice-cold PEMG buffer, it was homogenized. Depolymerization was completed by keeping the mixture at 0 °C for 30 min, followed by centrifugation at 2 °C (30 min; 30000*g*) to remove insoluble protein. Each reaction tube contained 0.46 mL of the supernatant and 20 μL of the DMSO solution of the test compound in varying concentrations. Reaction mixtures were preincubated at 37 °C for 15 min and chilled on ice followed by addition of 20 μL of a 25 mM GTP solution in PEMG buffer to each tube. Reaction mixtures were transferred to cuvettes of a UV spectrophotometer connected to two different temperature controllers. First, the temperature inside the cuvettes was held at 2 °C. The cuvette holder was then switched to the second temperature contoller at 37 °C, and the absorption was measured over a period of 20 min at 350 nm. Absorption at the start of the reaction was used as baseline. Two independent experiments were performed for the standard concentration (40 M) and three for determination of IC_{50} values. Each experiment had two control reaction mixtures; their mean value was defined as 100%, and their turbidity readings were generally within 10% of each other.

Determination of Cytostatic Activity: 1. MDA-MB 231 Human Breast Cancer Cells. Hormone-independent human MDA-MB 231 breast cancer cells were obtained from American Type Culture Collection (ATCC, Rockville, MD). Cells were grown in McCoy-5a medium, supplemented with L-glutamine (73 mg/L), gentamycin sulfate (50 mg/L), $NaHCO_3$ (2.2 g/L), and 5% sterilized fetal calf serum (FCS). At the start of the experiment, the cell suspension was tranferred to 96-well microplates (100 μL/well). After the cells grew for 2−3 days in a humidified incubator with 5% CO_2 at 37 °C, medium was replaced by one containing the test compounds (200 μL/well). Control wells (16/plate) contained 0.1% DMF that was used for the preparation of stock solutions. Initial cell density was determined by addition of glutaric dialdehyde (1% in PBS; 100 μL/well) instead of test compound. After incubation for about 4 days, medium was removed and 100 μL of glutaric dialdehyde in PBS (1%) was added for fixation. After 15 min, the solution of aldehyde was decanted. Cells were stained by treating them for 25 min with 100 μL of an aqueous solution of crystal violet (0.02%). After decanting, cells were washed several times with water to remove adherent dye. After addition of 100 μL of EtOH (70%) plates were gently shaken for 2 h. Optical density of each well was measured in a microplate autoreader EL 309 (Bio-tek) at 578 nm.

2. MCF-7 Human Breast Cancer Cells. A similar procedure to that described for MDA-MB 231 cells was applied with alterations: Cells were grown in EMEM supplemented with sodium pyruvate (110 mg/L), gentamycin sulfate (50 mg/L), $NaHCO_3$ (2.2 g/L), and 10% FCS. Medium that contained test compounds was supplemented with dextran-charcoal (DCC)-treated FCS to avoid interference with steroidal hormones in the serum. Incubation with inhibitor lasted ca. 8 days.

Colchicine Binding Assay. The tubulin solution was prepared as described above and diluted 1:10 with ice-cold PEM buffer. [^{3}H]Colchicine and test compounds were dissolved in DMSO. Each 0.1-mL reaction mixture contained 98 μL of the tubulin solution, 1 μM tritium-labeled colchicine (0.5 μCi), 10 μM inhibitor, and 2 μL of DMSO. Incubation was for 30 min at 37 °C. After the reaction mixture cooled on ice, 10 μL of a 11 mM GTP solution in PEM buffer was added, and the mixture was kept on ice. Each reaction mixture (0.1 mL) was filtered under reduced vacuum through a stack of three DEAE-cellulose paper filters and washed four times with 10 mL of PEM buffer. Radioactivity adsorbed on the filter representing tubulin-bound [^{3}H]colchicine was quantified in a liquid scintillation counter. Reaction mixtures without tubulin gave the background values.

Fluorescence Microscopy. MCF-7 breast cancer cells in high dilution were plated on sterile glass slides. After incubation at 37 °C overnight the medium (EMEM with 10% FCS) was replaced by one containing the drug in appropriate concentrations. Incubation was continued for another 60 min. Cells were fixed by consecutive treatments with MeOH and acetone for 5 min at −20 °C. The fixed cells were washed thoroughly with PBS. Nonspecific binding sites were blocked by addition of 90 μL of goat serum onto each slide which was covered with a coverslip and incubated for 30 min at 37 °C. After addition of 100 μL of anti-α-tubulin solution (1:500 in PBS containing 1% BSA) and removal of the coverslips, incubation was continued for another 60 min. After several washings with PBS cells were stained with anti-mouse IgG-Cy3 (1:150 in PBS/1% BSA) for 1 h at 37 °C and treated with 5% propyl gallate in glycerol to reduce fading. Finally, the coverslips were mounted on the slides and sealed with nail varnish. The slides were examined with a fluorescence microscope (Axioskop with Plan Neofluar 100x/1.3 oil; Zeiss) equipped with a fluorescence filter BP546/12LP590. Photographs were taken with a MC100 camera (Zeiss) using Agfa 100 ASA film.

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