Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 5122–5136
Bioorganic & Medicinal Chemistry

Antimitotic activities of 2-phenylindole-3-carbaldehydes in human breast cancer cells

Doris Kaufmann, Michaela Pojarová, Susanne Vogel, Renate Liebl, Robert Gastpar, Dietmar Gross, Tsuyuki Nishino, Tobias Pfaller and Erwin von Angerer*

Institut für Pharmazie, Universität Regensburg, D-93040 Regensburg, Germany

Received 23 January 2007; revised 7 May 2007; accepted 11 May 2007
Available online 17 May 2007

Abstract—Small molecules such as indoles are attractive as inhibitors of tubulin polymerization. Thus a number of 2-phenylindole-3-carbaldehydes with lipophilic substituents in both aromatic rings was synthesized and evaluated for antitumor activity in MDA-MB 231 and MCF-7 breast cancer cells. Some 5-alkylindole derivatives with a 4-methoxy group in the 2-phenyl ring strongly inhibit the growth of breast cancer cells with IC_{50} values of 5–20 nM. Their action can be rationalized by the cell cycle arrest in G_2/M phase due to the inhibition of tubulin polymerization.

1. Introduction

The microtubule system is essential for a number of cellular functions including mitosis and cell replication, maintenance of cell shape, cellular transport, and motility. Microtubules are hollow fibers formed by the polymerization of α- and β-tubulin heterodimers. The formation of microtubules and their depolymerization is a dynamic process which can be interrupted by both stabilization of microtubules and inhibition of polymerization. A large number of natural products are known to shift the dynamic equilibrium of the microtubule system to one or the other side and abrogate the biological functions of microtubules thereby. The taxanes and some other natural products such as epothilones stabilize the microtubule structures, whereas other agents such as colchicine, combretastatin A-4, and the vinca alkaloids inhibit the polymerization of α-/β-tubulin dimers.[1] Some of the natural products are characterized by complex chemical structures which makes synthesis and chemical modifications difficult. Others such as combretastatin A-4 are based on rather simple scaffolds which can easily be modified.[2]

The biological importance of microtubules makes them an interesting target for the development of anticancer drugs. For systematic studies on the use of antimitotic agents in cancer therapy molecules with simple structures are very attractive because they allow extensive chemical modifications. Examples are stilbenes,[2] aryl-substituted heterocycles,[3] anthracenones,[4] benzophenones,[5], and analogues[6] to name only a few. A variety of synthetic antimitotic compounds are based on the indole structure.[7–10] In Figure 1, some aryl-substituted indoles such as **1**,[11–13] **2**,[14] **3**,[15] and **4**[16] are presented. Three of these examples carry a trimethoxyphenyl ring which is considered as important for binding to the colchicine site on tubulin.

An interesting aspect in the application of combretastatin A-4 and related agents in cancer chemotherapy is their antivascular effect.[17] Since microtubules of the cytoskeleton play a major role in maintaining cell shape, the elongated endothelial cells of the tumor neovasculature are particularly sensitive to drugs that depolymerize microtubules and degrade the cytoskeleton. In contrast to drugs that target tumor angiogenesis compounds such as combretastatin A-4 phosphate disrupt the already formed tumor vasculature.[18]

In a previous study we discovered that some methoxy-substituted 2-phenylindole-3-carbaldehydes strongly inhibit the growth of human breast cancer cells.[19] Investigations on the mode of action revealed that the microtubules are the primary target of these agents. In an

Keywords: Phenylindoles; Breast cancer; Tubulin polymerization; Cell cycle arrest.
* Corresponding author. Tel.: +49 941 9434821; fax: +49 941 9434820; e-mail: erwin.von-angerer@chemie.uni-regensburg.de

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.05.030

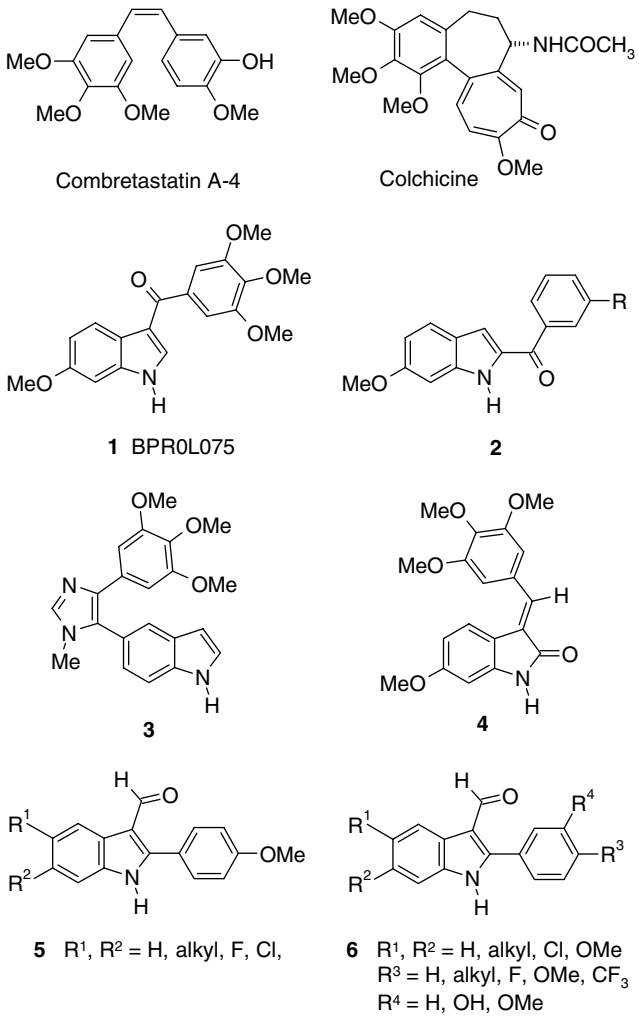


Figure 1. Chemical structures of combretastatin A-4, colchicine, and of some indole-based synthetic inhibitors of tubulin polymerization.

assay with bovine tubulin a marked inhibition on tubulin polymerization was observed. These indole derivatives were also shown to inhibit the binding of colchicine to tubulin. These findings prompted us to study this class of compounds in more detail. Since these derivatives of 2-phenylindole lack structural features considered typical for inhibitors of tubulin polymerization, for example, the 3,4,5-trimethoxyphenyl group which is characteristic for colchicine, combretastatin A-4, and podophyllotoxin, the structural requirements for activity had to be elaborated by a systematic variation of the substituents in both aromatic rings. Since the first investigations showed that these substituents should be lipophilic in nature, alkyl groups of variable length were introduced. Halogen atoms and the trifluoromethyl group completed the spectrum of substituents. The 1-position of the five-membered ring was kept unsubstituted because the N–H element of the indole is essential for the anti-proliferative activity.[19] All derivatives were tested for cytostatic activity. Since the interaction with the tubulin system usually results in an arrest of the cell cycle in the G_2/M-phase, the most potent compounds were submitted to a FACS analysis to record the cell cycle distribution. A small number of derivatives were studied for their inhibitory effects on tubulin polymerization to confirm previous findings. A representative example was used to investigate morphological changes of the cells by confocal laser microscopy.

2. Results and discussion

2.1. Chemistry

For the systematic variation of the substitution pattern in both aromatic rings a versatile route of synthesis was required. An appropriate procedure for the synthesis of the substituted 2-phenylindoles **11** and **12** was the Bischler method which involved the condensation of a substituted aniline **9** with an α-bromophenacyl derivative **10** (Fig. 2). Subsequently, the formyl group was introduced in position 3 of the indole by a Vilsmeier reaction with DMF and $POCl_3$ to give the 2-phenylindole-3-carbaldehydes **5** and **6**. The reaction of the aldehydes with methylamine and hydroxylamine, respectively, afforded the corresponding imines **7** and **8**.

2.2. Anti-proliferative activity

All of the compounds synthesized were first evaluated for cytostatic activity using hormone-independent human MDA-MB 231 breast cancer cells in a microplate assay. Based on previous investigations with methoxy-substituted 2-phenylindole-3-carbaldehydes, first, a series of compounds with a methoxy group in the *para*-position of the phenyl ring and a variety of lipophilic substituents in positions 5 and/or 6 of the indole including halogens and alkyl groups of variable length were tested. Vincristine was used as reference drug in most of the assays. All 2-(4-methoxyphenyl)indole derivatives strongly inhibited the growth of MDA-MB 231 cells with IC_{50} values below 1 μM (Table 1). The presence of fluorine in the indole moiety gave only rise to a positive effect when located in 6-position (**5c**). The cytostatic potency is strongly influenced by the length and the structure of the alkyl substituent in 5-position. It increases with the length up to 5 carbon atoms and decreases with ramification as demonstrated for the butyl derivatives **5j–l**. The lowest IC_{50} values (5.5–7.4 nM) were found for the *n*-butyl (**5j**), *n*-pentyl (**5m**), and *n*-hexyl (**5n**) derivatives. These values are close to the one of vincristine (4.5 nM).

Subsequently the substituent in the phenyl ring was modified (Table 2). Shifting of the methoxy group to the *meta*-position (**6a**) or introduction of an additional methoxy (**6b**) or hydroxyl (**6c**) group into this position strongly reduced the activity. A similar decrease in activity was observed when methoxy was replaced by a *n*-butyl (**6i**) or a fluoro (**6j**) substituent. Replacement of the 4-methoxy group by smaller groups such as methyl, ethyl or trifluoromethyl had only a minor effect on the potency. The lowest IC_{50} value (7.8 nM) was recorded for the 4-methyl derivative **6e**. The comparison of derivatives with *n*-butyl and ethyl as substituents (**6h** and **6i**) revealed that the indole moiety tolerates larger lipophilic substituents than the phenyl ring.

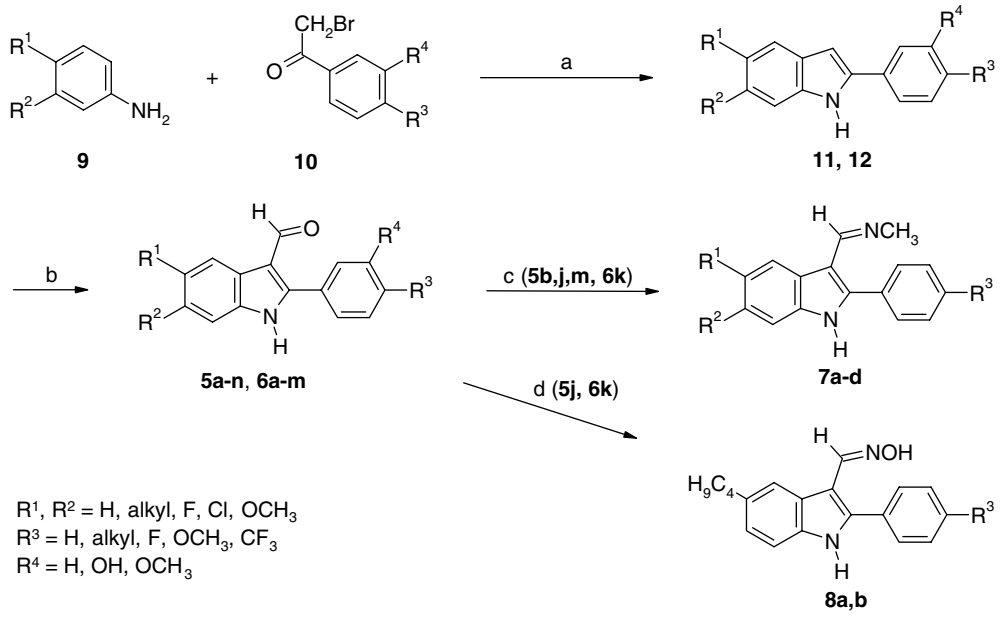
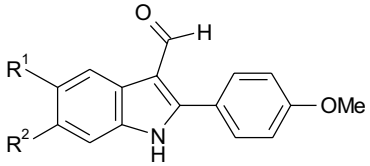


Figure 2. Syntheses of 2-phenylindole-3-carbaldehydes **5** and **6**, and of derivatives **7** and **8**. Reagents and conditions: (a) xylene, *N*,*N*-dimethylaniline, 170 °C; (b) $POCl_3$, DMF, 15 → 70 °C, 3 h; (c) $MeNH_2$, EtOH, 40 °C, 16 h; (d) $H_3NOH^+Cl^-$, H_2O/EtOH, NaOAc, reflux, 3 h.

Table 1. Anti-proliferative activities of 2-(4-methoxyphenyl)indole-3-carbaldehydes **5a**–**n** on human MDA-MB 231 and MCF-7 breast cancer cells



Compound	R^1	R^2	MDA-MB 231[a] IC_{50} (nM)	MCF-7[b] IC_{50} (nM)
5a	OMe	H	260	180
5b	H	OMe	35	160
5c	H	F	59	43
5d	F	H	540	240
5e	H	Cl	27	65
5f	Me	Cl	26	62
5g	Me	H	86	140
5h	Pr	H	20	54
5i	*i*-Pr	H	29	97
5j	*n*-Bu	H	6.7	22
5k	*sec*-Bu	H	72	180
5l	*t*-Bu	H	280	580
5m	*n*-Pent	H	5.5	20
5n	*n*-Hex	H	7.4	6.0
Vincristine			4.5	n.d.[c]

[a] Inhibition of cell growth determined after incubation for 4 days and subsequent crystal violet staining of viable cells. Mean values of two-independent experiments with 16–24 replicates, SD are less than 20%.
[b] Analogous experiment as described for MDA-MB 231 cells with one exception: the incubation period was 5 days.
[c] Not determined.

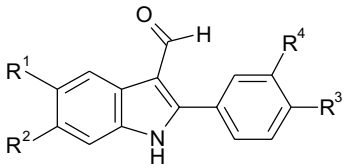
Additional modifications concerned the aldehyde function. Conversion to the methylamines (**7a**–**d**) did not affect the activity in comparison to the free aldehyde, whereas the formation of oximes (**8a** and **8b**) reduced the anti-proliferative activity by one order of magnitude (Table 3). This difference in bioactivity can be rationalized by the higher rate of hydrolysis of the methyl imines in comparison to the oximes. Hydrolysis experiments with **7b** and **8a** followed by HPLC analysis revealed that **7b** undergoes significant hydrolysis to the aldehyde **5j** at 37 °C, whereas **8a** remains stable under these conditions.

All 2-phenylindole derivatives were also tested for anti-proliferative activity in MCF-7 breast cancer cells (Tables 1–3). Though similar activities were observed in both cell lines a tendency to higher IC_{50} values in MCF-7 cells was noticed especially for the two oximes **8a** and **8b** (Table 3).

2.3. Cell cycle arrest in G_2/M-phase

Previous studies with 2-phenylindole-3-carbaldehydes suggested that these aldehydes exert their cytotoxic effects via tubulin as the intracellular target. Both the inhibition of tubulin polymerization and stabilization of microtubules can lead to an arrest of the cell cycle in the G_2/M-phase. Thus, a selection of representative derivatives including aldehydes, methyl imines, and oximes was examined for their effects on the cell cycle progression with MDA-MB 231 cells. The cell cycle-dependent DNA content was determined by flow cytometry using propidium iodide in permeabilized cells. All compounds were tested in different concentrations and compared with vincristine as reference drug. Figure 3 shows the typical decrease of the peak for cells in the G_1/G_0 phase and the parallel increase of the number of cells in G_2/M phase for compound **5n**. This derivative and vincristine are rather similar in potency. Only at the lowest concentration (10 nM) the vinca alkaloid proved to be somewhat more active (Fig. 4a).

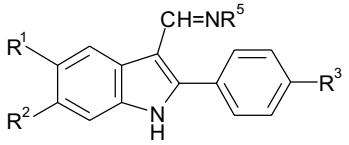
Table 2. Anti-proliferative activities of 2-phenylindole-3-carbaldehydes **6a–m** on human MDA-MB 231 and MCF-7 breast cancer cells



Compound	R^1	R^2	R^3	R^4	MDA-MB 231[a] IC_{50} (nM)	MCF-7[b] IC_{50} (nM)
5b	H	OMe	OMe	H	35	160
6a	H	OMe	H	OMe	1030	390
6b	H	OMe	OMe	OMe	270	20
6c	H	OMe	OMe	OH	800	1650
6d	H	OMe	Me	H	31	100
6e	H	Cl	Me	H	7.8	37
6f	Me	H	Me	H	48	165
6g	*n*-Bu	H	Me	H	34	54
6h	*n*-Bu	H	Et	H	27	58
6i	Et	H	*n*-Bu	H	300	200
6j	*n*-Bu	H	F	H	350	200
6k	*n*-Bu	H	CF_3	H	33	66
6l	*n*-Pent	H	CF_3	H	42	67
6m	*n*-Hex	H	CF_3	H	43	22
Vincristine					4.5	n.d.[c]

[a] Inhibition of cell growth determined after incubation for 4 days and subsequent crystal violet staining of viable cells. Mean values of two-independent experiments with 16–24 replicates, SD are less than 20%.
[b] Analogous experiment as described for MDA-MB 231 cells with one exception: the incubation period was 5 days.
[c] Not determined.

Table 3. Anti-proliferative activities of 3-iminomethyl-2-phenylindoles **7** and **8** on human MDA-MB 231 and MCF-7 breast cancer cells



Compound	R^1	R^2	R^3	R^5	MDA-MB 231[a] IC_{50} (nM)	MCF-7[b] IC_{50} (nM)
7a	H	OMe	OMe	Me	34	220
7b	*n*-Bu	H	OMe	Me	6	27
7c	*n*-Pent	H	OMe	Me	6	21
7d	*n*-Bu	H	CF_3	Me	32	140
8a	*n*-Bu	H	OMe	OH	40	212
8b	*n*-Bu	H	CF_3	OH	497	1660

[a] Inhibition of cell growth determined after incubation for 4 days and subsequent crystal violet staining of viable cells. Mean values of two-independent experiments with 16–24 replicates, SD are less than 20%.
[b] Analogous experiment as described for MDA-MB 231 cells with one exception: the incubation period was 5 days.

All indole derivatives tested were able to block the cell cycle in G_2/M phase (Figs. 4 and 5). Generally, this blockade was accompanied by the appearance of a significant sub-G_1 peak ('debris') probably due to apoptotic processes. The cell cycle arrest occurs at concentrations which were similar to those observed for the inhibition of cell growth except for the oxime **8a** which required a 10-fold higher concentration (Fig. 5b).

2.4. Inhibition of tubulin polymerization

A rational explanation for the cell cycle arrest is the inhibition of tubulin polymerization as it is also observed for colchicine, combretastatin A-4, and many other antimitotic agents. In order to prove this assumption two aldehydes **5j** and **6k**, the imine **7b**, and the oxime **8a** were tested in a microplate assay to obtain preliminary data on their interaction with tubulin. The progression of tubulin polymerization was measured turbidimetrically at 350 nm over 28 min after the temperature had been raised from 2 to 37 °C. At a concentration of 5 μM, the aldehyde **6k** and the oxime were inactive, whereas **5j** exerted a minor inhibition (23%) of tubulin polymerization. However, the imine **7b**, derived from the aldehyde **5j**, strongly inhibited tubulin polymerization. From the curves recorded for various concentrations (Fig. 6) an IC_{50} value of 1.2 μM was calculated which was lower than that of colchicine (5 μM) in this assay.

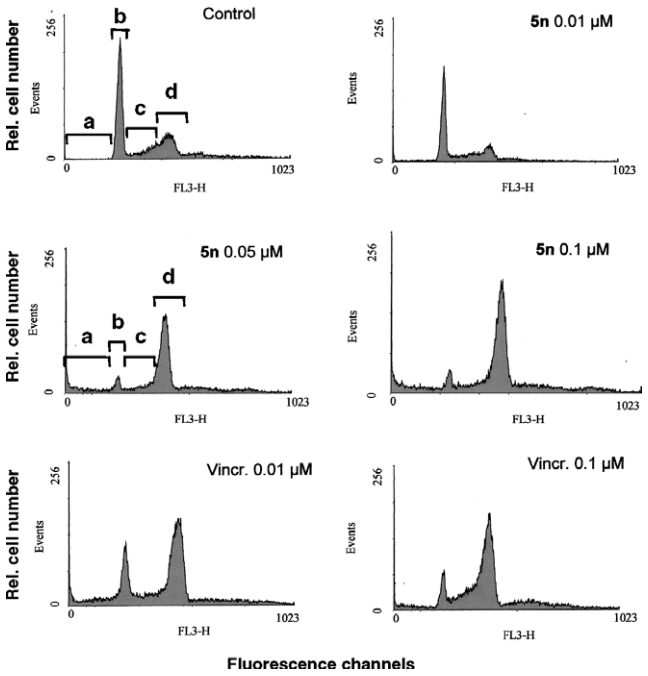


Figure 3. Flow cytometry analysis of cell cycle. MDA-MB 231 breast cancer cells were exposed to compound **5n** and vincristine in various concentrations for 24 h. The DNA content was quantified by the standard propidium iodide procedure, as described in Section 4. Cells are assigned to those in G_0/G_1- (label b), S- (label c), and G_2/M-phase (label d), and to sub-G_1 cells (label a), respectively, according to their DNA content.

A similar assay was performed with a tubulin preparation from calf brains using temperature-controlled cuvettes instead of microplates. This assay is more suitable for the determination of IC_{50} values than the microplate assay because it works with larger volumes and with a better temperature control. However, it requires the multi-step isolation of tubulin from fresh calf brains prior to the experiment. In this assay, the aldehydes **5a–c** and the imine **7a** were tested for inhibitory activity on tubulin polymerization in various concentrations. All four compounds showed a dose-dependent inhibition of polymerization with IC_{50} values of 4.0 (**5a**), 1.5 (**5b**), and 1.8 μM (**5c** and **7a**) (Fig. 7). The figures for the latter three compounds are similar to that of colchicine (1.9 μM). These concentrations are nearly two orders of magnitude higher than those observed for the anti-proliferative effect and the blockade of cell cycle progression. However, this discrepancy in the effective concentrations is generally noticed for antimitotic agents such as combretastatin A-4 and related structures.[12,13,16,20]

2.5. Change of cell morphology

Another method to identify the intracellular target of the 2-phenylindole-3-carbaldehydes was the histological examination of cells by fluorescence microscopy. Confocal laser microscopy allows the simultaneous visualization of chromatin and microtubules and the analysis of their spatial arrangement. Untreated U-87 MG human glioma cells display the normal distribution of microtubules (Fig. 8, panel A). In dividing cells their functional role can be demonstrated (Fig. 8A, panels a–c). Treatment of cells with 10 nM vincristine gave rise to a disruption of the microtubule network and a loss of

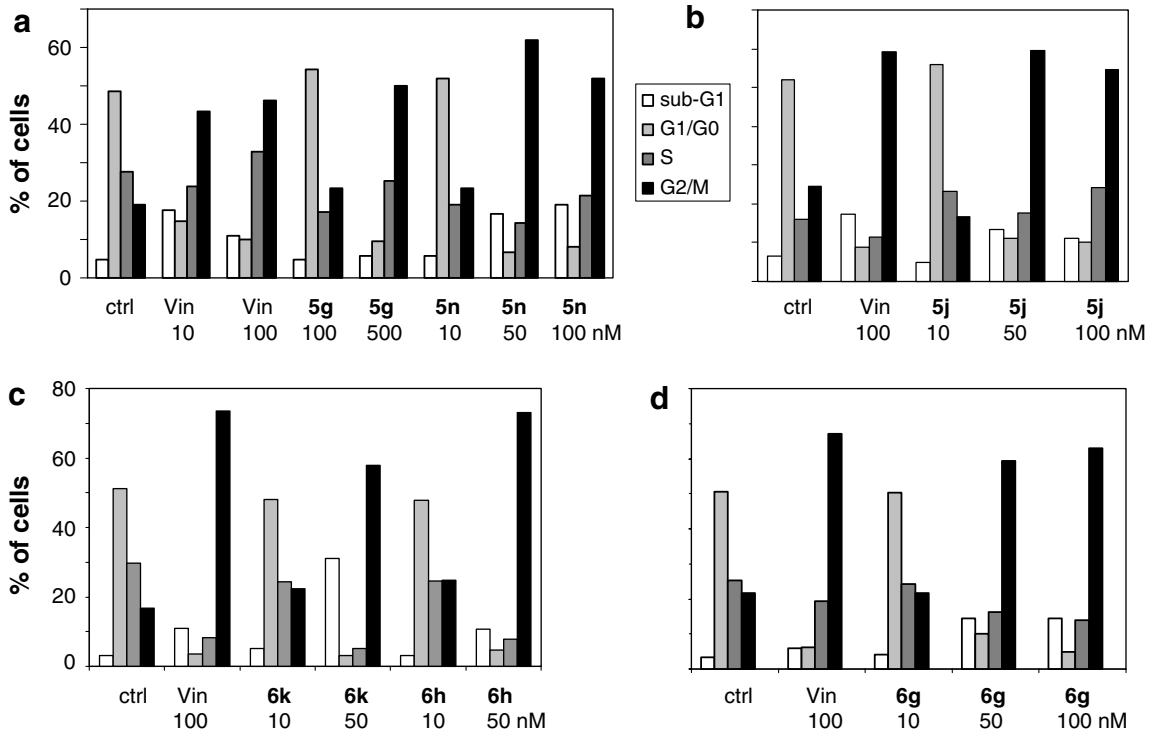


Figure 4. Cell cycle distribution of MDA-MB 231 cells treated for 24 h with 2-phenylindole-3-carbaldehydes **5 g**, **5n** (a), **5j** (b), **6k**, **6h** (c), and **6g** (d) in various concentrations. Vincristine (Vin) was used as reference drug. Percentages of sub-G_1 cells and cells in G_1/G_0-phase, S-phase, and G_2/M-phase are shown. Data refer to a representative experiment out of two.

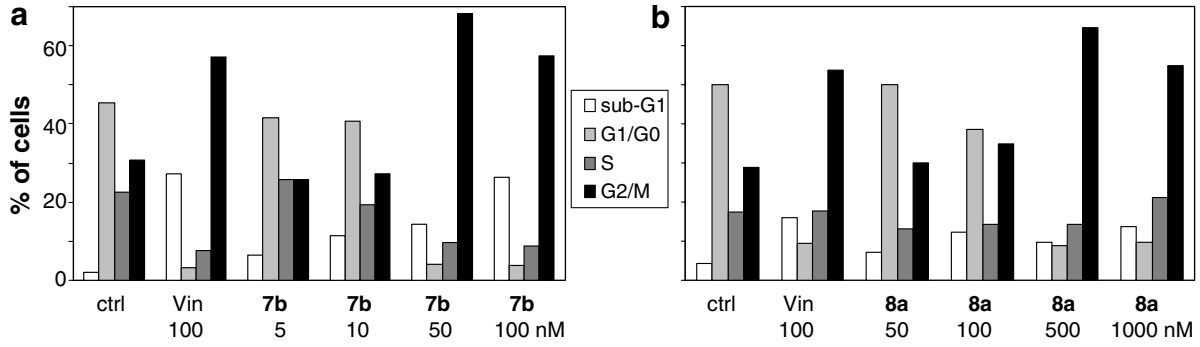


Figure 5. Cell cycle distribution of MDA-MB 231 cells treated for 24 h with the methylimine **7b** (a) and the oxime **8a** (b) in various concentrations. Vincristine (Vin) was used as reference drug. Percentages of sub-G_1 cells and cells in G_1/G_0-phase, S-phase, and G_2/M-phase are shown. Data refer to a representative experiment out of two.

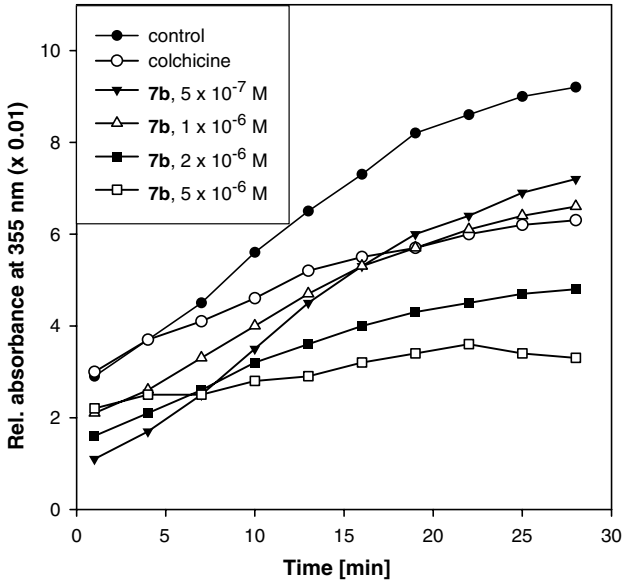


Figure 6. Effect of methylimine **7b** on tubulin assembly. Optical density at 355 nm was measured every minute over a period of 28 min simultaneously for all compounds and concentrations after temperature had been switched from 2 to 37 °C. Control wells contained tubulin and the solvent (0.1% DMSO). The concentration of colchicine was 5 μM.

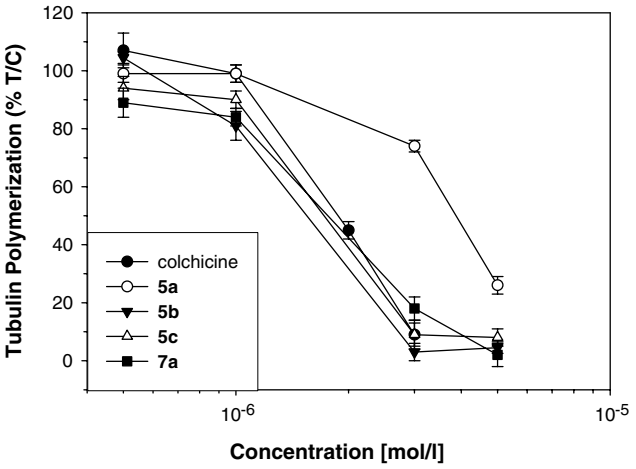


Figure 7. Inhibitory effects of various 2-phenylindole derivatives and of colchicine on the polymerization of calf brain tubulin. Assembly of microtubules was assessed turbidimetrically at 350 nm 20 min after the temperature had been switched from 2 to 37 °C. Polymerization in the absence of inhibitor gave the control readings. Values are means of three-independent experiments ±SEM.

its function in the mitotic process (Fig. 8, panel B). When the cells were treated with the aldehyde **5j** (50 nM) the microtubules condensed around the nucleus and were no longer capable of separating the chromosomes for mitosis (Fig. 8, panel C). The nucleus showed chromatin condensation and disintegration of the nuclear matrix probably due to an apoptotic cell death (Fig. 8C, panel b).[21]

2.6. Discussion

The aim of this study was to identify the most favorable substitution pattern of the 2-phenylindole-3-carbaldehydes for antimitotic activity. Since hydrophilic substituents such as hydroxy functions have been shown to reduce the antitumor effect dramatically[19] only lipophilic groups were considered. Preliminary investigations have shown that substituents in positions 1, 4, and 7 of the indole and 3 in the phenyl ring had a detrimental effect on the anti-proliferative activity. Thus, only substituents in 5- and 6-position of the indole and in the *para*-position of the phenyl ring were modified. The most favorable substitution pattern comprises an alkyl chain of 4 to 6 carbon atoms and a methoxy or a methyl group in the phenyl ring. Though a number of synthetic indole-based inhibitors of tubulin polymerization are known (Fig. 1)[7] the structure of the 2-phenylindole-3-carbaldehydes is unique because it possesses an aldehyde function and lacks the 3,4,5-trimethoxyphenyl ring which is typical for the majority of indole-based inhibitors of tubulin polymerization.[11–13,15,16,22,23] Other indoles with a carbonyl function are the 2-aroylindoles.[14]

Though the 2-phenylindole-3-carbaldehydes can be considered as stilbene analogues they differ from combretastatin A-4 by the trans arrangement of the phenyl rings. This may explain the lack of cytotoxicity of the 4,5,6-trimethoxy-2-(4-methoxyphenyl)indole-3-carbal-

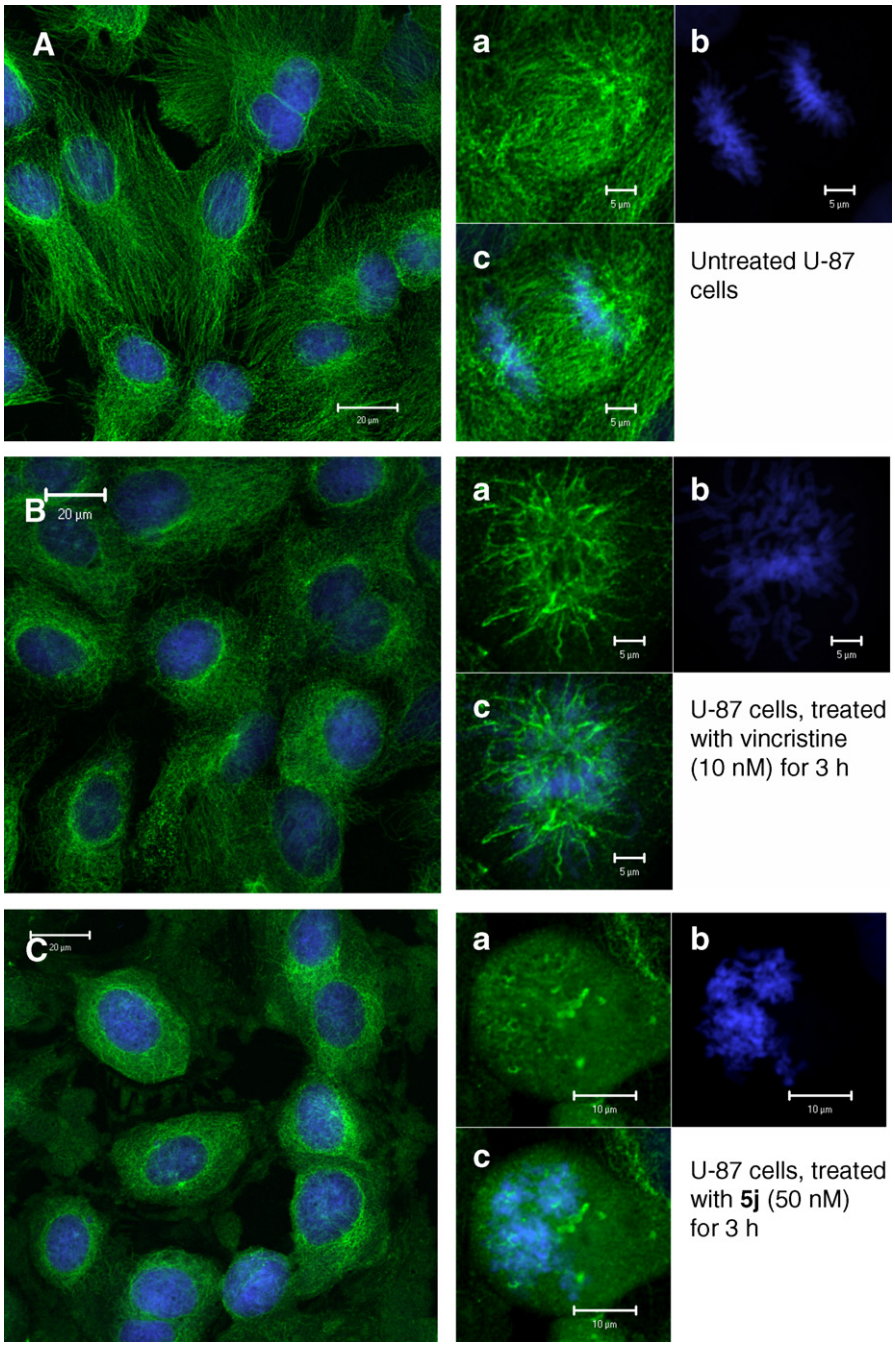


Figure 8. Effects of vincristine and 2-phenylindole-3-carbaldehyde **5j** on the organization of cellular microtubule network and nuclear structure of U-87 MG human glioblastoma cells, visualized by confocal laser fluorescence microscopy. (Panel A) Untreated control cells which undergo normal mitoses as shown in panels a, b, and c (a, stained tubulin; b, stained nucleus; c, merged images). (Panel B) Vincristine (10 nM) leads to a disturbed and partially degraded tubulin network that has lost its function (a, b, c). (Panel C) Indole **5j** (50 nM) causes a condensation of microtubules around the nucleus and a loss of function (a, b, c). Some of the nuclei desintegrate upon treatment (b).

dehyde which is furnished with the 3,4,5-trimethoxyphenyl group (data not shown). Also the low activity found with **6c** which possesses the same substitution pattern as that of the second phenyl ring of combretastatin A-4 is in agreement with these considerations. The results of this and other studies suggest that the colchicine binding site tolerates a large variety of different chemical structures as long as they contain two aromatic moieties with appropriate substituents.[2,3]

From the data presented the primary site of action appears to be the α/β-tubulin heterodimer whose polymerization is strongly inhibited by the aldehydes tested. This action results in the destruction of the microtubule network and abrogates its function. This action can be visualized by fluorescence microscopy as demonstrated for the aldehyde **5j**. Already at a concentration of 50 nM the microtubules condense around the nucleus and the filament structure is lost. At higher concentra-

tions most of the microtubules have disappeared.[19] This change is accompanied by a structural alteration of the nucleus which desintegrates probably due to apoptotic processes.

Interesting results were obtained from the comparison of the aldehydes with the derivatives that possess an imine structure. In the cytotoxicity assays all of the methyl imines showed activities identical to those of the corresponding aldehydes which can be rationalized by the hydrolysis of the imines. The hydrolytic conversion of the imine to the parent aldehyde was confirmed by HPLC analysis. A significant difference was observed when experiments such as the inhibition of tubulin polymerization last only for a short period of time. The imine **7b** proved to be much more potent as inhibitor of tubulin polymerization than the corresponding aldehyde **5j**. This difference may be due to a higher binding affinity of the imine for tubulin compared to the aldehyde, but this assumption has not yet been proved. The oximes which are shown to be resistant to hydrolysis at 37 °C are less active than the corresponding aldehydes by one order of magnitude. This difference is reflected by their inactivity in the tubulin polymerization assay (data not shown).

3. Conclusion

This study revealed that the 2-phenylindole-3-carbaldehydes are an interesting class of compounds with high anti-proliferative activity in two breast cancer cell lines. We were able to show that tubulin is the primary target of these agents which inhibit the polymerization of tubulin to functional microtubules by binding to the colchicine binding site. This interaction with tubulin leads to cell cycle arrest in the G_2/M phase and probably leads to an apoptotic cell death. The in vitro potencies of some of the aldehydes are in same range as those of vincristine and combretastatin A-4. Preliminary investigations on the in vivo activity, however, showed that these aldehydes do not inhibit the growth of transplanted murine tumors. One of the possible reasons might be the instability of the aldehyde function toward metabolic reactions. Insufficient bioavailability could be another reason. In order to overcome this problem we are going to modify the carbonyl function to improve the metabolic stability of this essential structural element. Two of these modifications, conversion of the aldehydes to methyl imines and oximes, respectively, are included in this study. Though both types of imines are active, their mode of action seems to be different. Results from other modifications will be reported in due time.

4. Experimental

4.1. General methods

Melting points were determined on a Büchi 510 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC-250 spectrometer with TMS as internal standard and were in accord with the assigned structures. Mass spectra (PI-EI MS) were measured on a Varian MAT-311A spectrometer at 70 eV. Purity of all compounds was checked by TLC. Elemental analyses of crystalline compounds were performed by the Mikroanalytisches Laboratorium, University of Regensburg. The syntheses of 5-methoxy-2-(4-methoxyphenyl)indole-3-carbaldehyde (**5a**), 6-methoxy-2-(4-methoxyphenyl)indole-3-carbaldehyde (**5b**), and 6-fluoro-2-(4-methoxyphenyl)-indole-3-carbaldehyde (**5c**) have been described previously.[19]

4.2. Preparation of 2-phenylindoles 11 and 12

A solution of 4′(3′)-substituted 2-bromoacetophenone (26 mmol) in xylene (150 mL) was added dropwise to a solution of the substituted aniline (66 mmol) in *N*,*N*-dimethylaniline (15 mL) at 170 °C over a period of 1 h. The reaction mixture was stirred at this temperature for 3–24 h. After cooling, the dark brown solution was poured into 400 mL of a 2 N HCl/EtOAc mixture (1:1). The aqueous phase was extracted twice with EtOAc (200 mL). The organic phase was washed with sat. NaCl solution and dried over Na_2SO_4. The solvent was removed under vacuum and the product was purified by column chromatography (SiO_2) with different mobile phases on the basis of dichloromethane and subsequent crystallization.

4.2.1. 5-Fluoro-2-(4-methoxyphenyl)indole (11d). Colorless crystals (21% yield), mp 215 °C. ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H, –OCH_3); 6.81 (d, ^4J = 2.0 Hz, 1H, indole-H^3); 6.88 (dd, ^4J = 2.2 Hz, ^4J = 2.0 Hz, 1H, indole-H^4); 7.03, 7.77 (AA′BB′, ^3J = 8.8 Hz, 4H, phenyl-H); 7.23 (d, ^3J = 8.0 Hz, 1H, indole-H^7); 7.33 (dd, ^3J = 8.0 Hz, ^4J = 2.2 Hz, 1H, indole-H^6); 11.50 (s, 1H, N–H). Anal. for $C_{15}H_{12}FNO$; calcd C, 74.68; H, 5.01; N, 5.81; found C, 74.39; H, 5.08; N, 5.77.

4.2.2. 6-Chloro-2-(4-methoxyphenyl)indole (11e). Greenish solid (71% yield), mp 198 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H, –OCH_3); 6.64 (s, 1H, indole-H^3); 7.07, 7.49 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.25 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, indole-H^5); 7.49 (d, ^4J = 2 Hz, 1H, indole-H^7); 7.59 (d, ^3J = 8 Hz, 1H, indole-H^4); 8.32 (s, br, 1H, N–H). Anal. for $C_{15}H_{12}ClNO$; calcd C, 69.91; H, 4.69; N, 5.46; found C, 69.12; H, 4.48; N, 5.54.

4.2.3. 6-Chloro-2-(4-methoxyphenyl)-5-methylindole (11f). The preparation afforded two products, **11f** (22% yield) and isomeric 4-chloro-2-(4-methoxyphenyl)-5-methylindole (14% yield), which were separated by column chromatography. The main product (**11f**) was obtained as colorless solid, mp 198 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 3H, –CH_3); 3.81 (s, 3H, –OCH_3); 6.70 (s, 1H, indole-H^3); 7.03, 7.76 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.36 (s, 1H, indole-H^4); 7.43 (s, 1H, indole-H^7); 11.43 (s, 1H, N–H). Anal. for $C_{16}H_{14}ClNO$; calcd C, 70.72; H, 5.19; N, 5.15; found C, 70.49; H, 5.21; N, 5.10.

4.2.4. 2-(4-Methoxyphenyl)-5-methylindole (11g). Colorless crystals (30% yield), mp 237 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.46 (s, 3H, –CH_3); 3.88 (s, 3H, –OCH_3); 6.66 (s, 1H, indole-H^3); 7.00–7.61 (m, 3H, indole-

H^{4,6,7}); 7.00, 7.61 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 8.19 (s, br, 1H, N–H). Anal. for $C_{16}H_{15}NO$; calcd C, 80.98; H, 6.37; N, 5.90; found C, 80.46; H, 6.11; N, 5.80.

4.2.5. 2-(4-Methoxyphenyl)-5-*n*-propylindole (11h). Colorless crystals (20% yield), mp 195–196 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 0.96 (t, ^3J = 7 Hz, 3H, –CH$_2$–CH_3); 1.68 (sext, ^3J = 7 Hz, 3H, –CH$_2$–CH_2–CH$_3$); 2.67 (t, ^3J = 7 Hz, 3H, –CH_2–CH$_2$–); 3.84 (s, 3H, –OCH$_3$); 6.64 (d, ^4J = 1 Hz, 1H, indole-H^3); 6.94–7.01 (m, 1H, indole-H^6); 6.96, 7.26 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.26 (d, ^3J = 7 Hz, 1H, indole-H^7); 7.39 (s, 1H, indole-H^4); 8.13 (s, br, 1H, N–H). Anal. for $C_{18}H_{19}NO$; calcd C, 81.48; H, 7.22, N, 5.28; found C, 81.23; H, 7.32; N, 5.18.

4.2.6. 5-Isopropyl-2-(4-methoxyphenyl)indole (11i). Colorless crystals (14% yield), mp 203 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 1.24 (d, ^3J = 7 Hz, 6H, –CH–(CH_3)$_2$); 2.93 (sept, ^3J = 7 Hz, 1H, –CH–(CH$_3$)$_2$); 3.80 (s, 3H, –OCH$_3$); 6.68 (d, 1H, ^4J = 2 Hz, indole-H^3); 6.96 (dd, ^4J = 2 Hz, ^3J = 8 Hz, 1H, indole-H^6); 7.01, 7.76 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.26 (d, ^4J = 2 Hz, 1H, indole-H^4); 7.30 (d, ^3J = 8 Hz, 1H, indole-H^7); 11.26 (s, br, 1H, N–H). Anal. for $C_{19}H_{21}NO$; calcd C, 81.48; H, 7.22; N, 5.28; found C, 80.34; H, 7.07; N, 5.18.

4.2.7. 5-*n*-Butyl-2-(4-methoxyphenyl)indole (11j). Colorless crystals (76% yield), mp 228 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 0.94 (t, ^3J = 7 Hz, 3H, –CH$_2$–CH_3); 1.38 (sext, ^3J = 7 Hz, 2H, –CH$_2$–CH_2–CH$_3$); 1.65 (quin, ^3J = 7 Hz, 2H, –CH$_2$–CH_2–CH$_2$–); 2.70 (t, ^3J = 7 Hz, 2H, –CH_2–CH$_2$–); 3.85 (s, 3H, –OCH$_3$); 6.63 (d, 1H, ^4J = 2 Hz, indole-H^3), 6.94–7.01 (m, 1H, indole-H^6); 6.96, 7.57 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.26 (d, ^3J = 8 Hz, 1H, indole-H^7); 7.38 (s, 1H, indole-H^4); 8.14 (s, br, 1H, –NH). Anal. for $C_{19}H_{21}NO$; calcd C, 81.68; H, 7.58; N, 5.01; found C, 81.51; H, 7.61; N, 4.97.

4.2.8. 5-*sec*-Butyl-2-(4-methoxyphenyl)indole (11k). Colorless crystals (38% yield), mp 191 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 0.85 (t, ^3J = 7 Hz, 3H, –CH$_2$–CH_3); 1.29 (d, ^3J = 7 Hz, 3H, –CH–CH_3); 1.65 (quin, ^3J = 7 Hz, 2H, –CH_2–CH$_3$); 2.67 (t, ^3J = 7 Hz, 1H, –CH–CH$_3$); 3.85 (s, 3H, –OCH$_3$); 6.65 (s, 1H, indole-H^3); 6.95-7.02 (m, 1H, indole-H^6); 6.97, 7.57 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.30 (d, ^3J = 8 Hz, 1H, indole-H^7); 7.39 (s, 1H, indole-H^4); 8.15 (s, br, 1H, –NH). Anal. for $C_{19}H_{21}NO$; calcd C, 81.68; H, 7.58; N, 5.01; found C, 81.51; H, 7.60; N, 4.94.

4.2.9. 5-*tert*-Butyl-2-(4-methoxyphenyl)indole (11l). White crystals (36% yield), mp 224 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 1.24 (s, 9H, –(CH$_3$)$_3$); 3.80 (s, 3H, –OCH$_3$); 6.70 (d, ^4J = 2 Hz, 1H, indole-H^3); 7.02, 7.76 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.14 (dd, ^5J = 2 Hz, ^3J = 8 Hz, 1H, indole-H^6); 7.29 (d, ^3J = 8 Hz, 1H, indole-H^7); 7.45 (d, ^4J = 2 Hz, 1H, indole-H^4); 11.25 (s, 1H, –NH). Anal. for $C_{19}H_{21}NO$; calcd C, 81.68; H, 7.58; N, 5.01; found C, 81.31; H, 7.57; N, 4.91.

4.2.10. 2-(4-Methoxyphenyl)-5-*n*-pentylindole (11m). White solid (21% yield), mp 197–198 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 0.89 (t, ^3J = 7 Hz, 3H, –CH$_2$–CH_3); 1.34 (m, 2H, –CH$_2$–CH_2–CH$_3$); 1.66 (m, 2H, –CH$_2$–CH_2–CH$_2$–); 2.69 (t, ^3J = 7 Hz, 2H, –CH_2–CH$_2$–); 3.85 (s, 3H, –OCH$_3$); 6.64 (d, ^4J = 1 Hz, 1H, indole-H^3); 6.94–7.01 (m, 1H, indole-H^6); 6.96, 7.57 (AA′BB′, ^3J = 9 Hz, 4H, Phenyl-H); 7.26 (d, ^3J = 8 Hz, 1H, indole-H^7); 7.39 (s, 1H, indole-H^4); 8.13 (s, br, 1H, –NH). Anal. for $C_{20}H_{23}NO$; calcd C, 81.87; H, 7.90; N, 4.77; found C, 82.05; H, 7.79, N, 5.33.

4.2.11. 5-*n*-Hexyl-2-(4-methoxyphenyl)indole (11n). White solid (14% yield), mp 197–198 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 0.88 (t, ^3J = 7 Hz, 3H, –CH$_2$–CH_3); 1.32 (m, 2H, –CH$_2$–CH_2–CH$_3$); 1.65 (m, 2H, –CH$_2$–CH_2–CH$_2$–); 2.17 (m, 4H, –CH$_2$–(CH_2)$_2$–CH$_2$–); 2.71 (t, ^3J = 7 Hz, 2H, –CH_2–CH$_2$–); 3.85 (s, 3H, –OCH$_3$), 6.64 (d, ^5J = 1 Hz, 1H, indole-H^3); 6.94–7.01 (m, 1H, indole-H^6); 6.97, 7.57 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.27 (d, ^3J = 8 Hz, 1H, indole-H^7); 7.39 (s, 1H, indole-H^4); 8.20 (s, br, 1H, –NH). Anal. for $C_{21}H_{25}NO$; calcd C, 82.05; H, 8.20; N, 4.56; found C, 82.04; H, 7.85; N, 4.42.

4.2.12. 2-(3,4-Dimethoxyphenyl)-6-methoxyindole (12b). Colorless crystals (15% yield), mp 161 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H, –OCH$_3$); 3.92 (s, 3H, –OCH$_3$); 3.96 (s, 3H, –OCH$_3$); 6.65 (s, 1H, indole-H^3); 6.79 (dd, ^3J = 7 Hz, ^5J = 2 Hz, 1H, ArH); 6.88–7.15 (m, 4H, indole-H^{4,5,7}, ArH); 7.47 (d, ^3J = 7 Hz, 1H, ArH); 8.20 (s, br, 1H, N–H). Anal. for $C_{17}H_{17}NO_3$; calcd C, 72.07; H, 6.05; N, 4.94; found C, 71.84; H, 5.99; N, 4.90.

4.2.13. 2-(3-Hydroxy-4-methoxyphenyl)-6-methoxyindole (12c). The title compound was not isolated, but used immediately for the next step of synthesis.

4.2.14. 6-Methoxy-2-(4-methylphenyl)indole (12d). White solid (42% yield), mp 162 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H, –CH$_3$); 3.88 (s, 3H, –OCH$_3$); 6.72 (s, br, 1H, indole-H^3); 6.81 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, indole-H^5); 6.92 (s, 1H, indole-H^7); 7.26, 7.50 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.24–7.32 (m, 1H, indol-H^4), 8.24 (s, br, 1H, N–H); MS *m*/*z* (%) 238 (16, [MH]$^{\cdot+}$), 237 (90, M$^{\cdot+}$), 222 (100, [M−$^{\cdot}$CH$_3$]$^+$), 194 (12, [M−$^{\cdot+}$CO–CH$_3$]$^+$).

4.2.15. 6-Chloro-2-(4-methylphenyl)indole (12e). Orange solid (40% yield), mp 145 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 2.34 (s, 3H, –CH$_3$); 6.87 (d, ^4J = 1 Hz, 1H, indole-H^3); 7.00 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, indole-H^5); 7.28, 7.52 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.38 (d, ^4J = 2 Hz, 1H, indole-H^7); 7.51 (d, ^3J = 8 Hz, 1H, indole-H^4); 11.64 (s, 1H, N–H). Anal. for $C_{15}H_{12}ClN$; calcd C, 74.53; H, 5.00; N, 7.79; found C, 74.46; H, 5.02; N, 5.61.

4.2.16. 5-Methyl-2-(4-methylphenyl)indole (12f). White solid (36% yield), mp 227 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H, –CH$_3$); 2.44 (s, 3H, –CH$_3$); 6.69 (s, 1H, indole-H^3); 6.99 (dd, ^3J = 8 Hz, ^4J = 1 Hz, 1H, indole-H^6); 7.24, 7.53 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.20–7.27 (m, 2H, indole-H^{4,7}); 8.19 (s, br, 1H, N–H). Anal. for $C_{16}H_{15}N$; calcd C, 86.82; H, 6.83; N, 6.35; C, 85.94; H, 6.68; N, 6.21.

4.2.17. 5-Butyl-2-(4-methylphenyl)indole (12g). Orange powder (15% yield), mp 181–182 °C. ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.39 (m, 2H, –CH_2–CH_2–CH_3); 1.65 (m, 2H, –CH_2–CH_2–CH_2–); 2.37 (s, 3H, –CH_3); 2.70 (t, 2H, ^3J = 7 Hz, –CH_2–CH_2–); 6.70 (dd, 1H, ^4J = 2 Hz, ^5J = 1 Hz, indole-H^4); 7.01 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.22 (m, 3H, indole-H^7 and phenyl-H); 7.40 (s, 1H, indole-H^3); 7.51 (d, 2H, ^3J = 8 Hz, phenyl-H); 8.16 (s, br, 1H, N–H). Anal. for $C_{19}H_{21}N$; calcd C, 86.64; H, 8.04; N, 5.32; found C, 86.31; H, 7.65; N, 5.34.

4.2.18. 5-Butyl-2-(4-ethylphenyl)indole (12h). Yellow powder (18% yield), mp 167–168 °C. ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.26 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.38 (m, 2H, –CH_2–CH_2–CH_3); 1.65 (m, 2H, –CH_2–CH_2–CH_2–); 2.68 (m, 4H, –CH_2–CH_3 and –CH_2–CH_2–); 6.71 (d, 1H, ^4J = 2 Hz, indole-H^4); 7.01 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.27 (m, 3H, phenyl-H and indole-H^7); 7.40 (s, 1H, indole-H^3); 7.57 (d, 2H, ^3J = 8 Hz, phenyl-H); 8.21 (s, br, 1H, N–H). Anal. for $C_{20}H_{23}N$; calcd C, 86.59; H, 8.36; N, 5.05; found C, 86.55; H, 8.07; N, 5.03.

4.2.19. 2-(4-Butylphenyl)-5-ethylindole (12i). Orange powder (20% yield), mp 183–184 °C. ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.21 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.32 (m, 2H, –CH_2–CH_2–CH_3); 1.58 (m, 2H, –CH_2–CH_2–CH_2); 2.63 (m, 4H, –CH_2–CH_3 and –CH_2–CH_2–); 6.74 (d, 1H, ^4J = 2 Hz, indole-H^4); 7.04 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.31 (d, 1H, ^3J = 8 Hz, indole-H^7); 7.43 (s, 1H, indole-H^3); 7.56, 7.24 (AA′BB′, 4H, ^3J = 8 Hz, phenyl-H); 8.22 (s, br, 1H, N–H). Anal. For $C_{20}H_{23}N$; calcd C, 86.59; H, 8.36; N, 5.05; found C, 86.10; H, 8.33; N, 4.93.

4.2.20. 5-Butyl-2-(4-fluorophenyl)indole (12j). Orange powder (48% yield), mp 140–142 °C. ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.45 (m, 2H, –CH_2–CH_2–CH_3); 1.65 (m, 2H, –CH_2–CH_2–CH_2); 2.70 (t, 2H, ^3J = 7 Hz, –CH_2–CH_2–); 6.69 (1, 1H, indole-H^4); 7.03 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.29 (d, 1H, ^3J = 8 Hz, indole-H^7); 7.41 (s, 1H, indole-H^3); 7.65, 7.13 (m, 4H, phenyl-H); 8.16 (s, br, 1H, –NH–). Anal. for $C_{18}H_{18}FN$; calcd C, 80.96; H, 6.78; N, 5.24; found C, 80.12; H, 6.65; N, 5.11.

4.2.21. 5-Butyl-2-[4-(trifluoromethyl)phenyl]indole (12k). Yellow powder (31% yield), mp 120–121 °C. ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.38 (m, 2H, –CH_2–CH_2–CH_3); 1.70 (m, 2H, –CH_2–CH_2–CH_2–); 2.71 (t, 2H, ^3J = 7 Hz, –CH_2–CH_2–); 6.85 (d, 1H, ^4J = 2 Hz, indole-H^4); 7.07 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.33 (d, 1H, ^3J = 8 Hz, indole-H^7); 7.43 (s, 1H, indole-H^3); 7.74, 7.67 (AA′BB′, 4H, ^3J = 8 Hz, phenyl-H); 8.29 (s, br, 1H, N–H). Anal. for $C_{19}H_{18}F_3N$; calcd C, 71.91; H, 5.72; N, 4.41; found C, 71.56; H, 5.85; N, 4.15.

4.2.22. 5-Pentyl-2-[4-(trifluoromethyl)phenyl]indole (12l). Orange powder (32% yield). The compound was directly used in the following reaction step. ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.34 (m, 4H, –CH_2–(CH_2)$_2$–CH_3); 1.66 (m, 2H, –CH_2–CH_2–CH_2–); 2.70 (t, 2H, ^3J = 7 Hz, –CH_2–CH_2–); 6.68 (d, 1H, ^4J = 2 Hz, indole-H^4); 7.07 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.32 (d, 1H, ^3J = 8 Hz, indole-H^7); 7.43 (s, 1H, indole-H^3); 7.74, 7.68 (AA′BB′, 4H, ^3J = 8 Hz, phenyl-H); 8.29 (s, br, 1H, N–H).

4.2.23. 5-Hexyl-2-[4-(trifluoromethyl)phenyl]indole (12m). Orange powder (31% yield). The compound was directly used in the following reaction step. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.36 (m, 4H, –CH_2–(CH_2)$_2$–CH_3); 1.65 (m, 4H, –CH_2–(CH_2)$_2$–CH_2–); 2.70 (t, 2H, ^3J = 7 Hz, –CH_2–CH_2–); 6.87 (d, 1H, ^4J = 2 Hz, indole-H^4); 7.07 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.33 (d, 1H, ^3J = 8 Hz, indole-H^7); 7.43 (s, 1H, indole-H^3); 7.74, 7.67 (AA′BB′, 4H, ^3J = 8 Hz, phenyl-H); 8.30 (s, br, 1H, N–H).

4.3. Preparation of 2-phenylindole-3-carbaldehydes 5 and 6

Under nitrogen, $POCl_3$ (10 mmol) was added dropwise to DMF (10 mmol) at 15 °C. The mixture was stirred for 15 min, then the solution of the respective 2-phenylindole (1 mmol) in DMF (50 mL) was added dropwise to the mixture, which was then heated to 70 °C. After 3 h of heating, the reaction mixture was diluted with ice water (200 mL), neutralized with 40% NaOH, and extracted with chloroform. The chloroform extract was washed with water and dried over $NaSO_4$. The solvent was removed under vacuum. Crystallization of the residue from methanol gave the pure product.

4.3.1. 5-Fluoro-2-(4-methoxyphenyl)indole-3-carbaldehyde (5d). Beige crystals (85% yield), mp 250 °C. ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H, –OCH_3); 7.05–7.13 (m, 1H, indole-H); 7.15, 7.72 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H); 7.44–7.89 (m, 1H, indole-H); 7.81–7.89 (m, 1H, indole-H); 9.92 (s, 1H, –CHO); 12.39 (s, 1H, N–H). Anal. for $C_{16}H_{12}FNO$; calcd C, 71.37; H, 4.49; N, 5.20; found C, 71.05; H, 4.39; N, 5.08.

4.3.2. 6-Chloro-2-(4-methoxyphenyl)indole-3-carbaldehyde (5e). Gray solid (28% yield), mp 260 °C. ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H, –OCH_3); 7.16, 7.73 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.25 (dd, ^3J = 7 Hz, ^4J = 2 Hz, 1H, indole-H^5); 7.49 (d, ^4J = 2 Hz, 1H, indole-H^7); 8.16 (d, ^3J = 7 Hz, 1H, indole-H^4); 9.95 (s, 1H, –CHO); 12.40 (s, 1H, N–H). Anal. for $C_{16}H_{12}ClNO_2$: calcd C, 67.26; H, 4.23; N, 4.90; found C, 67.48; H, 4.44; N, 4.76.

4.3.3. 6-Chloro-2-(4-methoxyphenyl)-5-methylindole-3-carbaldehyde (5f). Gray solid (28% yield), mp 287 °C. ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3H, –CH_3); 3.33 (s, 3H, –OCH_3); 7.16, 7.73 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.49 (s, 1H, indole-H^4); 8.14 (s, 1H, indole-H^7); 9.92 (s, 1H, –CHO); 12.31 (s, br, 1H, N–H). Anal. for $C_{17}H_{14}ClNO_2$; calcd C, 68.12; H, 4.71; N, 4.67; found C, 67.82; H, 4.85; N, 4.68.

4.3.4. 2-(4-Methoxyphenyl)-5-methylindole-3-carbaldehyde (5g). Gray solid (79% yield), mp 242 °C. ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H, –CH_3); 3.86 (s, 3H, –OCH_3);

7.09 (dd, ^3J = 9 Hz, ^4J = 1 Hz, 1H, indole-H^6); 7.15, 7.71 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.36 (d, ^3J = 8 Hz, 1H, indole-H^7); 8.01 (s, 1H, indole-H^4); 9.92 (s, 1H, –CHO); 12.17 (s, br, 1H, N–H). Anal. for $C_{17}H_{15}NO_2$; calcd C, 76.96; H, 5.70; N, 5.28; found C, 76.57; H, 5.65; N, 5.17.

4.3.5. 2-(4-Methoxyphenyl)-5-*n*-propylindole-3-carbaldehyde (5h). Colorless solid (35% yield), mp 209 °C. ^{1}H NMR (DMSO-d_6) δ 0.91 (t, ^3J = 7 Hz, 3H, –CH$_2$–CH_3); 1.63 (sext, ^3J = 7 Hz, 2 H, –CH$_2$–CH_2–CH$_3$); 2.67 (t, ^3J = 7 Hz, 3H, –CH_2–CH$_2$–); 3.86 (s, 3H, –OCH$_3$), 7.10 (dd, ^4J = 2 Hz, ^3J = 8 Hz, 1H, indole-H^6), 7.15 und 7.70 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H), 7.38 (d, ^3J = 8 Hz, 1H, indole-H^7), 8.01 (d, ^4J = 2 Hz, 1H, indole-H^4), 9.93 (s, 1H, –CHO), 12.17 (s, 1H, N–H). Anal. for $C_{19}H_{19}NO_2$; calcd C, 77.96; H, 6.53; N, 4.77; found C, 77.55; H, 6.47; N, 4.80.

4.3.6. 5-Isopropyl-2-(4-methoxyphenyl)indole-3-carbaldehyde (5i). Colorless solid (54% yield), mp 217 °C. ^{1}H NMR (DMSO-d_6) δ 1.25 (d, ^3J = 7 Hz, 6H, –CH–(CH_3)$_2$); 2.99 (sept, ^3J = 7 Hz, 1H, –CH–(CH$_3$)$_2$); 3.85 (s, 3H, –OCH$_3$); 7.15, 7.69 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.16 (d, ^3J = 8 Hz, 1H, indole-H^6); 7.38 (d, ^3J = 8 Hz, 1H, indole-H^7); 8.05 (s, 1H, indole-H^4); 9.92 (s, 1H, –CHO); 12.16 (s, 1H, N–H). Anal. for $C_{19}H_{19}NO_2$; calcd C, 77.96; H, 6.53; N, 4.77; found C, 77.57; H, 6.45; N, 4.81.

4.3.7. 5-*n*-Butyl-2-(4-methoxyphenyl)indole-3-carbaldehyde (5j). Orange crystals (44% yield), mp 210 °C. ^{1}H NMR (DMSO-d_6) δ 0.92 (t, ^3J = 7 Hz, 3H, –CH$_2$–CH_3); 1.34 (sext, ^3J = 7 Hz, 2H, –CH$_2$–CH_2–CH$_3$); 1.63 (quin, ^3J = 7 Hz, 2H, –CH$_2$–CH_2–CH$_2$); 2.73 (t, ^3J = 7 Hz, 2H, –CH_2–CH$_2$–); 3.89 (s, 3H, –OCH$_3$); 7.22-7.24 (m, 1H, indole-H^6); 7.21, 7.67 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.22–7.24 (m, 1H, indole-H^6); 7.52 (d, ^3J = 8 Hz, 1H, indole-H^7); 7.57 (s, 1H, indole-H^4), 8.71 (s, 1H, –CHO); 13.19 (s, 1H, N–H). Anal. for $C_{20}H_{21}NO_2$; calcd C, 78.15; H, 6.89; N, 4.56; found C, 78.08; H, 6.83; N, 4.66.

4.3.8. 5-*sec*-Butyl-2-(4-methoxyphenyl)indole-3-carbaldehyde (5k). Slightly violet solid (68% yield), mp 179 °C. ^{1}H NMR (DMSO-d_6) δ 0.80 (t, ^3J = 7 Hz, 3H, –CH$_2$–CH_3); 1.25 (d, ^3J = 7 Hz, 3H, –CH–CH_3); 1.61 (quin, ^3J = 7 Hz, 2H, –CH_2–CH$_3$); 2.69 (t, ^3J = 7 Hz, 1H, –CH–CH$_3$); 3.86 (s, 3H, –OCH$_3$); 7.10–7.17 (m, 1H, indole-H^6), 7.15, 7.70 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.39 (d, ^3J = 8 Hz, 1H, indole-H^7); 8.02 (s, 1H, indole-H^4); 9.92 (s, 1H, –CHO); 12.14 (s, 1H, N–H). Anal. for $C_{20}H_{21}NO_2$; calcd C, 78.15; H, 6.89; N, 4.56; found C, 77.81; H, 6.86; N, 4.51.

4.3.9. 5-*tert*-Butyl-2-(4-methoxyphenyl)indole-3-carbaldehyde (5l). Colorless solid (67% yield), mp 223 °C. ^{1}H NMR (DMSO-d_6) δ 1.36 (s, 9H, –CH$_3$); 3.86 (s, 3H, –OCH$_3$); 7.15, 7.69 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.36–7.42 (m, 2H, indole-H^{6,7}); 8.23 (s, 1H, indole-H^4); 9.93 (s, 1H, –CHO); 12.13 (s, 1H, N–H); MS: *m*/*z* (%) = 307 (61, M $^{\cdot+}$), 292 (100, [M−$^{\cdot}$CH$_3$]$^+$).

4.3.10. 2-(4-Methoxyphenyl)-5-*n*-pentylindole-3-carbaldehyde (5m). Orange crystals (60% yield), mp 182 °C. ^{1}H NMR (DMSO-d_6) δ 0.87 (t, ^3J = 7 Hz, 3H, –CH$_2$–CH_3); 1.30 (m, 4H, –CH$_2$–(CH_2)$_2$–CH$_3$); 1.62 (m, 2H, –CH$_2$–CH_2–CH$_2$); 2.68 (t, ^3J = 7 Hz, 2H, –CH_2–CH$_2$–); 3.86 (s, 3H, –OCH$_3$); 7.10 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, indole-H^6); 7.16, 7.70 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.38 (d, ^3J = 8 Hz, 1H, indole-H^7); 8.00 (s, 1H, indole-H^4); 9.92 (s, 1H, –CHO); 12.18 (s, 1H, N–H); Anal. for $C_{21}H_{23}NO_2$; calcd C, 78.47; H, 7.21; N, 4.36; found C, 77.97; H, 6.70; N, 4.25.

4.3.11. 5-*n*-Hexyl-2-(4-methoxyphenyl)indole-3-carbaldehyde (5n). Orange crystals (60% yield), mp 176 °C. ^{1}H NMR (DMSO-d_6) δ 0.88 (t, ^3J = 7 Hz, 3H, –CH$_2$–CH_3); 1.29 (m, 6H, –CH$_2$–(CH_2)$_3$–CH$_3$); 1.61 (m, 2H, –CH$_2$–CH_2–CH$_2$–); 2.68 (t, ^3J = 7 Hz, 2H, –CH_2–CH$_2$–); 3.86 (s, 3H, –OCH$_3$); 7.10 (d, ^3J = 8 Hz, 1H, indole-H^6); 7.15, 7.70 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.37 (d, ^3J = 8 Hz, 1H, indole-H^7); 8.00 (s, 1H, indole-H^4); 9.92 (s, 1H, –CHO); 12.17 (s, 1H, N–H). Anal. for $C_{22}H_{25}NO_2$; calcd C, 78.77; H, 7.51; N, 4.18; found C, 77.95; H, 7.57; N, 4.25.

4.3.12. 6-Methoxy-2-(3-methoxyphenyl)indole-3-carbaldehyde (6a). White crystals (80% yield), mp 190 °C. ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H, –OCH$_3$); 3.85 (s, 3H, –OCH$_3$); 6.80-8.10 (m, 7H, ArH); 9.94 (s, 1H, –CHO); 12.19 (s, 1H, N–H). Anal. for $C_{17}H_{15}NO_3$; calcd C, 72.58; H, 5.37; N, 4.98; found C, 71.97; H, 5.60; N, 4.61.

4.3.13. 2-(3,4-Dimethoxyphenyl)-6-methoxyindole-3-carbaldehyde (6b). Yellow crystals (55% yield), mp 210 °C. ^{1}H NMR (DMSO-d_6) δ 3.81 (s, 3H, –OCH$_3$); 3.85 (s, 3H, –OCH$_3$); 3.87 (s, 3H, –OCH$_3$); 6.87 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1H, phenyl-H^6); 6.95 (d, ^4J = 2 Hz, 1H, indole-H^7); 7.15 (d, ^3J = 8 Hz, 1H, indole-H^5); 7.28 (d, ^4J = 2 Hz, 1H, phenyl-H^2); 7.27-7.32 (m, 1H, phenyl-H^5); 8.05 (d, ^3J = 8 Hz, 1H, indole-H^4); 9.95 (s, 1H, –CHO); 12.06 (s, br, 1H, N–H). Anal. for $C_{18}H_{17}NO_4$; calcd C, 69.44; H, 5.50; N, 4.50; found C, 69.23; H, 5.58; N, 4.22.

4.3.14. 2-(3-Hydroxy-4-methoxyphenyl)-6-methoxyindole-3-carbaldehyde (6c). Colorless crystals (57% yield), mp 253 °C. ^{1}H NMR (DMSO-d_6) δ 3.81 (s, 3H, –OCH$_3$); 3.88 (s, 3H, –OCH$_3$); 6.85 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1H, indole-H^5); 6.96–6.97 (m, 2H, phenyl-H^{2,5}); 7.16 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, phenyl-H^6); 7.28 (dd, ^4J = 2 Hz, 1H, indole-H^7); 8.03 (d, ^3J = 9 Hz, 1H, indole-H^4); 9.54 (s, 1H, –OH); 9.93 (s, 1H, –CHO); 11.99 (s, 1H, N–H). Anal. for $C_{17}H_{15}NO_4$; calcd C, 68.68; H, 5.08, N, 4.71; found C, 68.53; H, 5.02; N, 4.65.

4.3.15. 6-Methoxy-2-(4-methylphenyl)indole-3-carbaldehyde (6d). Colorless crystals (60% yield), mp 250 °C. ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H, –CH$_3$); 3.81 (s, 3H, –OCH$_3$); 6.87 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, indole-H^5); 6.95 (d, ^4J = 2 Hz, 1H, indole-H^7); 7.40, 7.63 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 8.05 (d, ^3J = 8 Hz, 1H, indole-H^4); 9.91 (s, 1H, –CHO); 12.17 (s, 1H, N–H). Anal. for $C_{17}H_{15}NO_2$; calcd C, 76.96; H, 5.70; N, 5.28; found C, 76.24; H, 5.89; N, 4.91.

4.3.16. 6-Chloro-2-(4-methylphenyl)indole-3-carbaldehyde (6e). Colorless crystals (57% yield), mp 268 °C.

^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H, –CH_3); 7.26 (dd, ^3J = 8 Hz, ^4J = 2 Hz, 1H, indole-H^5); 7.42, 7.68 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.50 (d, ^4J = 2 Hz, 1H, indole-H^7); 8.18 (d, ^3J = 9 Hz, 1H, indole-H^4); 9.96 (s, 1H, –CHO); 12.46 (s, 1H, N–H). Anal. for $C_{16}H_{12}ClNO$; calcd C, 71.25; H, 4.48; N, 5.19; found C, 71.45; H, 4.63; N, 4.86.

4.3.17. 5-Methyl-2-(4-methylphenyl)indole-3-carbaldehyde (6f). Colorless crystals (48% yield), mp 226 °C. ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H, –CH_3); 2.43 (s, 3H, –CH_3); 7.10 (dd, ^3J = 8 Hz, ^4J = 1 Hz, 1H, indole-H^6); 7.40, 7.65 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 7.39 (d, ^3J = 8 Hz, 1H, indole-H^7); 8.02 (d, ^4J = 1 Hz, 1H, indole-H^4); 9.94 (s, 1H, –CHO); 12.20 (s, 1H, N–H). Anal. for $C_{17}H_{15}NO$; calcd C, 81.90; H, 6.06; N, 5.62; found C, 81.64; H, 5.95; N, 5.38.

4.3.18. 5-Butyl-2-(4-methylphenyl)indole-3-carbaldehyde (6g). Orange solid (68% yield), mp 175–178 °C. ^{1}H NMR (DMSO-d_6) δ 0.92 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.34 (m, 2H, –CH_2–CH_2–CH_3); 1.61 (m, 2H, –CH_2–CH_2–CH_2–); 2.41 (s, 3H, –CH_3); 2.68 (t, 2H, ^3J = 7 Hz, –CH_2–CH_2–); 7.13 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.41 (m, 3H, indole-H^7, phenyl-H); 7.66 (d, 2H, ^3J = 8 Hz, phenyl-H); 8.03 (s, 1H, indole-H^4); 9.94 (s, 1H, –CHO); 12.24 (s, br, 1H, N–H); MS: *m*/*z* (%) = 291 (67, $[M]^+O$); 248 (100, $[M]^+O$–OC_3H_7). Anal. for $C_{20}H_{21}NO$; calcd C, 82.44; H, 7.26; N, 4.81; found C, 81.22; H, 7.07; N, 4.12.

4.3.19. 5-Butyl-2-(4-ethylphenyl)indole-3-carbaldehyde (6h). Yellow solid (46% yield), mp 178–180 °C. ^{1}H NMR (DMSO-d_6) δ 0.91 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.30 (m, 5H, –CH_2–CH_3, –CH_2–CH_2–CH_3); 1.59 (m, 2H, –CH_2–CH_2–CH_2–); 2.70 (m, 4H, –CH_2–CH_3, –CH_2–CH_2–); 7.11 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.39 (d, 1H, ^3J = 8 Hz, indole-H^7); 7.67, 7.43 (AA′BB′, 4H, ^3J = 8 Hz, phenyl-H); 8.01 (s, 1H, indole-H^4); 9.93 (s, 1H, –CHO); 12.22 (s, br, 1H, N–H); MS: *m*/*z* (%) = 305 (90, $[M]^+O$); 262 (100, $[M]^+O$–OC_3H_7). Anal. for $C_{21}H_{23}NO$; calcd C, 82.59; H, 7.59; N, 4.59; found C, 81.38; H, 7.43; N, 4.21.

4.3.20. 2-(4-Butylphenyl)-5-ethylindole-3-carbaldehyde (6i). Light brown solid (30% yield), mp 175–176 °C. ^{1}H NMR (DMSO-d_6) δ 0.91 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.23 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.35 (m, 2H, –CH_2–CH_2–CH_3); 1.61 (m, 2H, –CH_2–CH_2–CH_2–); 2.70 (m, 4H, –CH_2–CH_3, –CH_2–CH_2–); 7.17 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.40 (m, 3H, indole-H^7, phenyl-H); 7.66 (d, 2H, ^3J = 8 Hz, phenyl-H); 8.03 (s, 1H, indole-H^4); 9.94 (s, 1H, –CHO); 12.22 (s, br, 1H, N–H). Anal. for $C_{21}H_{23}NO$; calcd C, 82.59; H, 7.59; N, 4.59; found C, 81.55; 7.25; N, 4.16.

4.3.21. 5-Butyl-2-(4-fluorophenyl)indole-3-carbaldehyde (6j). Orange solid (40% yield), mp 192–194 °C. ^{1}H NMR (DMSO-d_6) δ 0.91 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.32 (m, 2H, –CH_2–CH_2–CH_3); 1.60 (m, 2H, –CH_2–CH_2–CH_2); 2.69 (t, 2H, ^3J = 7 Hz, –CH_2–CH_2–); 7.13 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.43 (m, 3H, indole-H7, phenyl-H); 7.82 (d, 2H, phenyl-H); 8.02 (s, 1H, indole-H^4); 9.87 (s, 1H, –CHO); 12.30 (s, br, 1H, N–H); MS: *m*/*z* (%) = 295 (64, $[M]^+O$); 252 (100, $[M]^+O$–OC_3H_7). Anal. for $C_{19}H_{18}FNO$; calcd C, 77.27; H, 6.14; N, 4.74; found C, 76.40; H, 6.10; N, 4.60.

4.3.22. 5-*n*-Butyl-2-[4-(trifluoromethyl)phenyl]indole-3-carbaldehyde (6k). Orange powder (45% yield), mp 230–231 °C. ^{1}H NMR (DMSO-d_6) δ 0.91 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.33 (m, 2H, –CH_2–CH_2–CH_3); 1.60 (m, 2H, –CH_2–CH_2–CH_2); 2.70 (t, 2H, ^3J = 7 Hz, –CH_2–CH_2–); 7.17 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.44 (d, 1H, ^3J = 8 Hz, indole-H^7); 7.95, 8.00 (AA′BB′, 4H, ^3J = 8 Hz, phenyl-H); 8.05 (s, 1H, indole-H^4); 9.97 (s, 1H, –CHO); 12.48 (s, br, 1H, N–H). Anal. for $C_{20}H_{18}F_3NO$; calcd C, 69.55; H, 5.25; N, 4.05; found C, 69.14; H, 5.18; N, 4.09.

4.3.23. 5-Pentyl-2-[4-(trifluoromethyl)phenyl]indole-3-carbaldehyde (6l). Yellow powder (32% yield), mp 226–227 °C. ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.31 (m, 4H, –CH_2–(CH_2)$_2$–CH_3); 1.64 (m, 2H, –CH_2–CH_2–CH_2–); 2.71 (t, 2H, ^3J = 7 Hz, –CH_2–CH_2–); 7.19 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.46 (d, 1H, ^3J = 8 Hz, indole-H^7); 7.97, 8.02 (AA′BB′, 4H, ^3J = 8 Hz, phenyl-H); 8.07 (s, 1H, indole-H^4); 9.97 (s, 1H, –CHO); 12.51 (s, br, 1H, N–H). Anal. for $C_{21}H_{20}F_3NO$; calcd C, 70.18; H, 5.61; N, 3.89; found C, 70.00; H, 5.49; N, 3.62.

4.3.24. 5-Hexyl-2-[4-(trifluoromethyl)phenyl]indole-3-carbaldehyde (6m). Yellow solid (34% yield), mp 224–225 °C. ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, ^3J = 7 Hz, –CH_2–CH_3); 1.28 (m, 6H, –CH_2–(CH_2)$_3$–CH_3); 1.61 (m, 2H, –CH_2–CH_2–CH_2–); 2.69 (t, 2H, ^3J = 7 Hz, –CH_2–CH_2–); 7.16 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, indole-H^6); 7.44 (d, 1H, ^3J = 8 Hz, indole-H^7); 7.95, 8.00 (AA′BB′, 4H, ^3J = 8 Hz, phenyl-H); 8.04 (s, 1H, indole-H^4); 9.97 (s, 1H, –CHO); 12.48 (s, br, 1H, N–H). Anal. for $C_{22}H_{22}F_3NO$; calcd C, 70.76; H, 5.94; N, 3.75; found C, 70.98; H, 6.01; N, 3.51.

4.4. Preparation of the carbaldehyde methyl imines 7

To a solution of the 2-phenylindole-3-carbaldehyde **5** or **6** (2 mmol) in CH_2Cl_2 (100 mL) was added under nitrogen methylamine (0.01 mol) as 33% solution in EtOH. The mixture was stirred at 40 °C overnight. Then, H_2O (100 mL) was added and the aqueous mixture was extracted several times with 50-mL portions of $CHCl_3$. The combined organic layers were washed with H_2O and brine, and dried over Na_2SO_4. After evaporation of the solvent in vacuo, the residue was purified by crystallization from EtOH.

4.4.1. 6-Methoxy-2-(4-methoxyphenyl)-3-[(methylimino)-methyl]indole (7a). Yellow resin (85% yield). ^{1}H NMR (d_6-acetone) δ 3.35 (d, ^4J = 1 Hz, 3H, =N–CH_3); 3.73 (s, 3H, –OCH_3); 3.82 (s, 3H, –OCH_3); 6.70 (dd, ^3J = 9 Hz, ^4J = 2 Hz, 1H, indole-H^5); 6.86 (d, ^4J = 2 Hz, 1H, indole-H^7); 6.99, 7.49 (AA′BB′, ^3J = 9 Hz, 4H, phenyl-H); 8.24 (d, ^3J = 9 Hz, 1H, indole-H^4); 8.42 (q, ^4J = 1 Hz, 1H, –CH =N–CH_3).

4.4.2. 5-*n*-Butyl-2-(4-methoxyphenyl)-3-[(methylimino)-methyl]indole (7b). Colorless crystals (67% yield), mp 207–209 °C. ^{1}H NMR (DMSO-d_6) δ 0.91 (t, ^3J = 7.3 Hz, 3H, –CH_2 –CH_3); 1.33 (sext, ^3J = 7.4 Hz, 2H, –CH_2–CH_2–CH_3); 1.58 (quin, ^3J = 7.5 Hz, 2H, –CH_2–CH_2–CH_2–); 2.65 (t, ^3J = 7.6 Hz, 2H, –CH_2–CH_2–CH_2–); 3.40 (d, ^4J = 1.3 Hz, 3H, =N–CH_3); 3.84 (s, 3H, –OCH_3); 7.01 (dd, ^3J = 8.3 Hz, ^4J = 1.6 Hz, 1H, indole-H^6); 7.13, 7.55 (AA′BB′, ^3J = 8.8 Hz, 4H, phenyl-H); 7.29 (d, ^3J = 8.2 Hz, 1H, indole-H^7); 8.09 (d, ^4J = 0.9 Hz, 1H, indole-H^4); 8.43 (d, ^4J = 1.4 Hz, 1H, –CH =NCH_3); 11.56 (s, 1H, N–H). Anal. for $C_{21}H_{24}N_2O$; calcd C, 78.71; H, 7.55; N, 8.74; found C, 78.72; H, 7.55; N, 8.53.

4.4.3. 5-*n*-Pentyl-2-(4-methoxyphenyl)-indol-3-[(methylimino)methyl]indole (7c). Colorless crystals (77% yield), mp 203 °C. ^{1}H NMR (DMSO-d_6) δ 0.86 (t, ^3J = 6.8 Hz, 3H, –CH_2–CH_3); 1.29 (m, 4H, –CH_2–(CH_2)$_2$–CH_3); 1.59 (m, 2H, –CH_2–CH_2–CH_2); 2.64 (t, ^3J = 7.5 Hz, 2H, –CH_2–CH_2–); 3.40 (d, ^4J = 1.1 Hz, 3H, =N–CH_3); 3.84 (s, 3H, –OMe); 7.01 (dd, ^3J = 8.3 Hz, ^4J = 1.6 Hz, 1H, indole-H^6); 7.13, 7.55 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H); 7.29 (d, ^3J = 8.2 Hz, 1H, indole-H^7); 8.09 (s, 1H, indole-H^4); 8.43 (s, 1H, –CH =NCH_3); 11.56 (s, 1H, N–H). Anal. for $C_{22}H_{26}N_2O$; calcd C, 79.01; H, 7.84; N, 8.38; found C, 78.65; H, 7.51; N, 8.27.

4.4.4. 5-*n*-Butyl-2-[4-(trifluoromethyl)phenyl]-3-[(methylimino)methyl]indole (7d). Colorless solid (63% yield), mp 186 °C. ^{1}H NMR (DMSO-d_6) δ 0.91 (t, ^3J = 7.3 Hz, 3H, –CH_2–CH_3); 1.33 (sext, ^3J = 7.4 Hz, 2H, –CH_2–CH_2–CH_3);1.59 (quin, ^3J = 7.5 Hz, 2H, –CH_2–CH_2–CH_2–); 2.67 (t, ^3J = 7.6 Hz, 2H, –CH_2–CH_2–), 3.43 (d, ^4J = 1.2 Hz, 3H, =N–CH_3); 7.08 (dd, ^3J = 8.3 Hz, ^4J = 1.6 Hz, 1H, indole-H^6); 7.36 (d, ^3J = 8.3 Hz, 1H, indole-H^7); 7.85, 7.93 (AA′BB′, ^3J = 8.3 Hz, 4H, phenyl-H); 8.14 (s, 1H, indole-H^4); 8.49 (s, 1H, –CH =NCH_3); 11.85 (s, 1H, N–H). Anal. for $C_{21}H_{21}F_3N_2$; calcd C, 70.37; H, 5.91; N, 7.82; found C, 70.17; H, 5.69; N, 7.04.

4.5. Preparation of the oximes 8

To a solution of hydroxylammonium chloride (1.2 mmol) in H_2O (8 mL), a solution of the 2-phenylindole-3-carbaldehyde (1 mmol) in EtOH (8 mL) and NaOAc (1.6 mmol) were added. The mixture was heated under reflux for 3 h and stirred at room temperature overnight. After repeated extraction with CH_2Cl_2 the combined organic layers were washed with water and dried (Na_2SO_4). The solvent was removed in vacuo and the residue recrystallized from EtOH.

4.5.1. 5-*n*-Butyl-3-[(hydroxyimino)methyl]-2-(4-methoxyphenyl)indole (8a). Colorless crystals (67% yield), mp 138 °C. ^{1}H NMR (DMSO-d_6) δ 0.90 (t, ^3J = 7.3 Hz, 3H, –CH_2–CH_3); 1.32 (sext, ^3J = 7.3 Hz, 2H, –CH_2–CH_2–CH_3); 1.58 (quin, ^3J = 7.5 Hz, 2H, –CH_2–CH_2–CH_2–); 2.65 (t, ^3J = 7.5 Hz, 2H, –CH_2–CH_2–); 3.84 (s, 3H, –OCH_3); 7.02 (dd, ^3J = 8.3 Hz, ^4J = 1.5 Hz, 1H, indole-H^6); 7.13, 7.51 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H); 7.30 (d, ^3J = 8.2 Hz, 1H, indole-H^7); 7.88 (s, 1H, indole-H^4); 8.22 (s, 1H, –CH=N–); 10.63 (s, 1H, –OH), 11.53 (s, 1H, N–H). Anal. for $C_{20}H_{22}N_2O_2$; calcd C, 74.51; H, 6.88; N, 8.69; found C, 74.67; H, 6.84; N, 8.56.

4.5.2. 5-*n*-Butyl-3-[(hydroxyimino)methyl]-2-[4-(trifluoromethyl)phenyl]indole (8b). Colorless crystals (86% yield), mp 166 °C. ^{1}H NMR (DMSO-d_6) δ 0.91 (t, ^3J = 7.3 Hz, 3H, –CH_2–CH_3); 1.33 (sext, ^3J = 7.4 Hz, 2H, –CH_2–CH_2–CH_3); 1.59 (quin, ^3J = 7.5 Hz, 2H, –CH_2–CH_2–CH_2–); 2.67 (t, ^3J = 7.5 Hz, 2H, –CH_2–CH_2–); 7.10 (dd, ^3J = 8.3 Hz, ^4J = 1.6 Hz, 1H, indole-H^6); 7.37 (d, ^3J = 8.3 Hz, 1H, indole-H^7); 7.81, 7.92 (AA′BB′, ^3J = 7.9 Hz, 4H, phenyl-H); 7.94 (s, 1H, indole-H^4); 8.29 (s, 1H, –CH=N–); 10.84 (s, 1H, –OH); 11.81 (s, 1H, N–H). Anal. for $C_{20}H_{19}F_3N_2O$; calcd C, 66.66; H, 5.31; N, 7.77; found C, 66.71; H, 5.32; N, 7.71.

4.6. Materials and reagents for bioassays

Drugs and biochemicals were obtained from Sigma (Deisenhofen, Germany). The microplate-based tubulin polymerization assay (Cytoskeleton®) was purchased from Tebu-bio (Offenbach, Germany). MDA-MB 231 and MCF-7 breast cancer cells were obtained from the American Type Culture collection (ATCC, Rockville, MD, USA). Buffer solutions used: 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 $Na_2HPO_4 \times H_2O$, 0.2 g/L KH_2PO_4.

4.7. Determination of anti-proliferative activity

Hormone-independent human MDA-MB 231 breast cancer 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 transferred to 96-well microplates (100 μL/well). After the cells grew for 4 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% of 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 (Biotek) at 578 nm.

For hormone-sensitive MCF-7 human breast cancer cells a similar procedure to that described for MDA-MB 231 cells was applied with modifications: Cells were grown in EMEM supplemented with sodium pyruvate

(110 mg/L), gentamycin sulfate (50 mg/L), $NaHCO_3$ (2.2 g/L), phenol red, and 10% FCS for 5 days.

4.8. Flow cytometry

MDA-MB 231 cells were grown to 70–80% confluence on the bottom of a 75 cm^2 culture flask. Test substances were dissolved in DMF and diluted to the required concentrations. The content of DMF in medium and in controls after 1000 times dilution was set to 0.1%. For each concentration one culture flask was used. Cells were exposed to test substances for 24 h. This incubation time was necessary to obtain a sufficient number of cells (5×10^6 cells). After incubation cells were trypsinized, centrifuged (250*g*) in an excess of serum containing medium for 10 min, and washed with PBS.

The pellets of cells were resuspended in 1 mL of PBS and 9 mL of 70% EtOH (ice-cold). EtOH and PBS were removed by centrifugation and the cells were washed with PBS. After the addition of 0.5 mL of PBS and 0.5 mL of DNA extraction buffer the cells were transferred into Eppendorf cups (2 mL), incubated for 5 min at room temperature, and centrifuged again. The cells were stained with 1 mL of propidium iodide solution for at least 30 min at room temperature and then analyzed by flow cytometry using a FACS-Calibur™. For analysis, data files for four parameters were collected for 8000–15,000 events from each sample. The flow rate was adjusted to ca. 300 cells/s for sufficient accuracy. Data were analyzed by the WinMDI 2.8 software.

4.9. Tubulin polymerization assay on microplates

This assay was performed according to the vendor's protocol. Stock solutions of test compounds in DMSO were diluted 1:10 with G-PEM buffer. Ten microliters of this solution was added to the corresponding well of a microplate which was warmed in a microplate reader (Tecan) to 37 °C. Ice-cold tubulin was suspended in cold G-PEM buffer + glycerol (5%) and depolymerized at 0 °C within 1 min. Within 30 s, 100 μL of this solution was added to the warm solutions of the test compounds on the microplate and the plate returned to the reader. Absorbance at 355 nm was recorded at 37 °C for 30 min. Reference drugs were taxoterre and colchicine; control wells contained only the solvent (1%).

4.10. Tubulin polymerization assay in cuvettes

4.10.1. Isolation and purification of calf brain tubulin. The cortex of one or two fresh calf brains in ice-cold PEM buffer (1 mL/g tissue, +16 mg DTE/100 mL buffer solution) was homogenized in portions. After centrifugation (90 min; 20,000*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 pre-warmed (37 °C) sucrose solution (10% in PEM buffer solution containing 1 mM GTP, approx 10% of the transferred volume). After centrifugation at 37 °C for 45 min (20,000*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 (40,000*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 gel-like pellet. The yields ranged from 2 to 6 g per brain. Aliquots were frozen in liquid nitrogen and stored at −70 °C. Purity was checked by polyacrylamide gel electrophoresis.

4.10.2. Temperature-induced tubulin polymerization. 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; 30,000*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 controller. First, the temperature inside the cuvettes was held at 2 °C. The cuvette holder was then switched to the second temperature controller 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. Three-independent experiments were performed for the 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.

4.11. Confocal laser scanning microscopy

U-87 MG cells were seeded into 8-well Lab-Tek Chamber Slides (Nunc, Wiesbaden, Germany). At 75% confluence the culture medium was replaced with medium containing vincristine (10 nM), 2-phenylindole-3-carbaldehyde **5j** (50 nM), or corresponding vehicle (EtOH). The cells were incubated at 37 °C for 3 h. After removal of the culture medium the cells were fixed with 4% paraformaldehyde solution in PBS for 20 min at room temperature. Thereafter each well was washed three times with PBS supplemented by 0.5% BSA. For permeabilization cells were incubated with PBS (+0.5% BSA) containing 1% Triton X-100 (Serva, Heidelberg, Germany) for 10 min at room temperature and washed three times with PBS (+0.5% BSA).

Nuclei and chromosomes were stained with SYTOX-Green® nucleic acid stain (Molecular Probes, Eugene, OR, USA). Microtubules were stained using mouse anti-human α-tubulin primary antibody (Dianova, Hamburg, Germany) and Cy5-conjugated goat anti-mouse secondary antibody (Molecular Probes). All

antibodies were used in a 1:200 dilution in PBS, containing 0.5% BSA. A Carl Zeiss Axiovert 200M LSM510 confocal laser scanning microscope was employed for acquisition of fluorescence images.

References and notes

1. von Angerer, E. *Curr. Opin. Drug Discovery Dev.* **2000**, *3*, 575–584.
2. Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, S.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 3033–3044.
3. von Angerer, E. *Exp. Opin. Ther. Patents* **1999**, *9*, 1069–1081.
4. Prinz, H.; Ishii, Y.; Hirano, T.; Stoiber, T.; Camacho Gomez, J. A.; Schmidt, P.; Dussmann, H.; Burger, A. M.; Prehn, J. H.; Gunther, E. G.; Unger, E.; Umezawa, K. *J. Med. Chem.* **2003**, *46*, 3382–3394.
5. Liou, J. P.; Chang, J. Y.; Chang, C. W.; Chang, C. Y.; Mahindroo, N.; Kuo, F. M.; Hsieh, H. P. *J. Med. Chem.* **2004**, *47*, 2897–2905.
6. Romagnoli, R.; Baraldi, P. G.; Pavani, M. G.; Tabrizi, M. A.; Preti, D.; Fruttarolo, F.; Piccagli, L.; Jung, M. K.; Hamel, E.; Borgatti, M.; Gambari, R. *J. Med. Chem.* **2006**, *49*, 3906–3915.
7. Brancale, A.; Silvestri, R. *Med. Res. Rev.* **2007**, *27*, 209–238.
8. De Martino, G.; Edler, M. C.; La Regina, G.; Coluccia, A.; Barbera, M. C.; Barrow, D.; Nicholson, R. I.; Chiosis, G.; Brancale, A.; Hamel, E.; Artico, M.; Silvestri, R. *J. Med. Chem.* **2006**, *49*, 947–954.
9. Dupeyre, G.; Chabot, G. G.; Thoret, S.; Cachet, X.; Seguin, J.; Guenard, D.; Tillequin, F.; Scherman, D.; Koch, M.; Michel, S. *Bioorg. Med. Chem.* **2006**, *14*, 4410–4426.
10. Mahboobi, S.; Sellmer, A.; Eichhorn, E.; Beckers, T.; Fiebig, H. H.; Kelter, G. *Eur. J. Med. Chem.* **2005**, *40*, 85–92.
11. Kuo, C. C.; Hsieh, H. P.; Pan, W. Y.; Chen, C. P.; Liou, J. P.; Lee, S. J.; Chang, Y. L.; Chen, L. T.; Chen, C. T.; Chang, J. Y. *Cancer Res.* **2004**, *64*, 4621–4628.
12. Liou, J. P.; Chang, Y. L.; Kuo, F. M.; Chang, C. W.; Tseng, H. Y.; Wang, C. C.; Yang, Y. N.; Chang, J. Y.; Lee, S. J.; Hsieh, H. P. *J. Med. Chem.* **2004**, *47*, 4247–4257.
13. Liou, J. P.; Mahindroo, N.; Chang, C. W.; Guo, F. M.; Lee, S. W.; Tan, U. K.; Yeh, T. K.; Kuo, C. C.; Chang, Y. W.; Lu, P. H.; Tung, Y. S.; Lin, K. T.; Chang, J. Y.; Hsieh, H. P. *ChemMedChem* **2006**, *1*, 1106–1118.
14. Mahboobi, S.; Pongratz, H.; Hufsky, H.; Hockemeyer, J.; Frieser, M.; Lyssenko, A.; Paper, D. H.; Bürgermeister, J.; Böhmer, F. D.; Fiebig, H. H.; Burger, A. M.; Baasner, S.; Beckers, T. *J. Med. Chem.* **2001**, *44*, 4535–4553.
15. Wang, L.; Woods, K. W.; Li, Q.; Barr, K. J.; McCroskey, R. W.; Hannick, S. M.; Gherke, L.; Credo, R. B.; Hui, Y. H.; Marsh, K.; Warner, R.; Lee, J. Y.; Zielinski-Mozng, N.; Frost, D.; Rosenberg, S. H.; Sham, H. L. *J. Med. Chem.* **2002**, *45*, 1697–1711.
16. Pandit, B.; Sun, Y.; Chen, P.; Sackett, D. L.; Hu, Z.; Rich, W.; Li, C.; Lewis, A.; Schaefer, K.; Li, P. K. *Bioorg. Med. Chem.* **2006**, *14*, 6492–6501.
17. Thorpe, P. E. *Clin. Cancer Res.* **2004**, *10*, 415–427.
18. Chaplin, D. J.; Horsman, M. R.; Siemann, D. W. *Curr. Opin. Investig. Drugs* **2006**, *7*, 522–528.
19. Gastpar, R.; Goldbrunner, M.; Marko, D.; von Angerer, E. *J. Med. Chem.* **1998**, *41*, 4965–4972.
20. Romagnoli, R.; Baraldi, P. G.; Remusat, V.; Carrion, M. D.; Cara, C. L.; Preti, D.; Fruttarolo, F.; Pavani, M. G.; Tabrizi, M. A.; Tolomeo, M.; Grimaudo, S.; Balzarini, J.; Jordan, M. A.; Hamel, E. *J. Med. Chem.* **2006**, *49*, 6425–6428.
21. Watanabe, J.; Natsume, T.; Fujio, N.; Miyasaka, K.; Kobayashi, M. *Apoptosis* **2000**, *5*, 345–353.
22. De Martino, G.; La Regina, G.; Coluccia, A.; Edler, M. C.; Barbera, M. C.; Brancale, A.; Wilcox, E.; Hamel, E.; Artico, M.; Silvestri, R. *J. Med. Chem.* **2004**, *47*, 6120–6123.
23. Flynn, B. L.; Hamel, E.; Jung, M. K. *J. Med. Chem.* **2002**, *45*, 2670–2673.