Synthesis and Biological Evaluation of 1-Methyl-2-(3′,4′,5′-trimethoxybenzoyl)-3-aminoindoles as a New Class of Antimitotic Agents and Tubulin Inhibitors

Romeo Romagnoli,*[,†] Pier Giovanni Baraldi,*[,†] Taradas Sarkar,[‡] Maria Dora Carrion,[†] Carlota Lopez Cara,[†] Olga Cruz-Lopez,[†] Delia Preti,[†] Mojgan Aghazadeh Tabrizi,[†] Manlio Tolomeo,[#] Stefania Grimaudo,[#] Antonella Di Cristina,[#] Nicola Zonta,[§] Jan Balzarini,[⊥] Andrea Brancale,[§] Hsing-Pang Hsieh,[∥] and Ernest Hamel[‡]

Dipartimento di Scienze Farmaceutiche, Università di Ferrara, 44100 Ferrara, Italy, Dipartimento di Oncologia, Policlinico "P. Giaccone", Divisione di Ematologia e Servizio AIDS, Università di Palermo, Palermo, Italy, Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium, The Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff, CF10 3XF, U.K., Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli, Taiwan, Republic of China, and Developmental Therapeutics Program, Toxicology and Pharmacology Branch, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702

Received September 15, 2007

The 2-(3,4,5-trimethoxybenzoyl)-2-aminoindole nucleus was used as the fundamental structure for the synthesis of compounds modified with respect to positions C-4 to C-7 with different moieties (chloro, methyl, or methoxy). Additional structural variations concerned the indole nitrogen, which was alkylated with small alkyl groups such as methyl or ethyl. We have identified 1-methyl-2-(3,4,5-trimethoxybenzoyl)-3-amino-7-methoxyindole as a new highly potent antiproliferative agent that targets tubulin at the colchicine binding site and leads to apoptotic cell death.

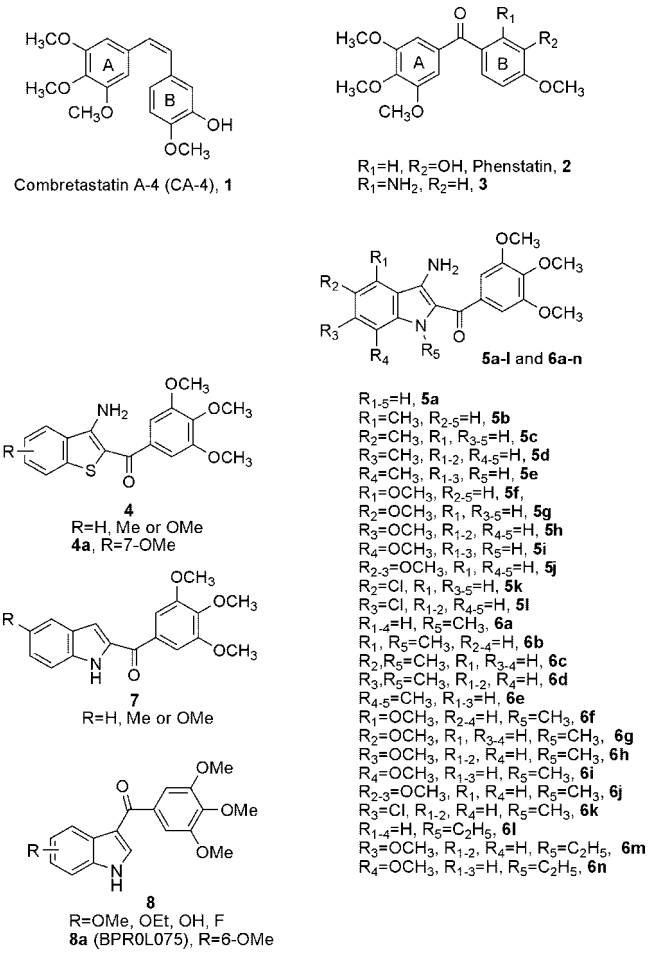
Introduction

The discovery of natural and synthetic compounds targeting the mitotic spindle apparatus has attracted much attention within the past 2 decades, and microtubules are recognized as an important target for the development of potential new chemotherapeutic agents.[1] More recently, it has been established that some tubulin-binding agents selectively target tumor vasculature, and thus, these compounds can also be considered vascular disrupting agents.[2]

One of the most important tubulin-binding agents is combretastatin A-4 (CA-4, **1**; Chart 1). CA-4, isolated from the bark of the South African tree *Combretum caffrum*,[3] is one of the well-known natural tubulin-binding molecules affecting microtubule dynamics. CA-4 strongly inhibits the polymerization of tubulin by binding to the colchicine site.[4] As a result of its simple chemical structure, many analogues that mimic CA-4 activity have been developed and evaluated in SAR studies.[5] Among synthetic small-molecule tubulin inhibitors, replacement of the double bond of **1** by a carbonyl group furnished a benzophenone-type CA-4 analogue named phenstatin (**2**). This compound demonstrated interesting efficacy in a variety of tumor models while retaining the characteristics of **1**.[6] The 2-aminobenzophenone derivative **3** also strongly inhibites cancer cell growth and tubulin polymerization, and it causes mitotic arrest, as does **2**.[7]

In a recent study, we reported the discovery of a series of molecules with general structure **4**, characterized by the presence of a 2-(3,4,5-trimethoxybenzoyl)-3-aminobenzo[*b*]thiophene skeleton, which was designed by bioisosteric replacement of the 2-aminobenzene moiety of **3** with a 3-aminobenzo[*b*]thiophene. This new class of compounds exerts strong inhibition of tubulin

Chart 1. Inhibitors of Tubulin Polymerization



* To whom correspondence should be addressed. For R.R.: phone, 39-(0)532-291290; fax, 39-(0)532-291296; e-mail, rmr@unife.it. For P.G.B.: phone, 39-(0)532-291293; fax, 39-(0)532-291296; e-mail: baraldi@unife.it.

† Università di Ferrara.

‡ National Cancer Institute at Frederick.

Università di Palermo.

§ Cardiff University.

⊥ Rega Institute for Medical Research.

∥ National Health Research Institutes.

10.1021/jm7011547 CCC: $40.75
Published on Web 02/09/2008

polymerization by binding to the colchicine binding site of tubulin, and they cause G2/M phase arrest of the cell cycle.[8]

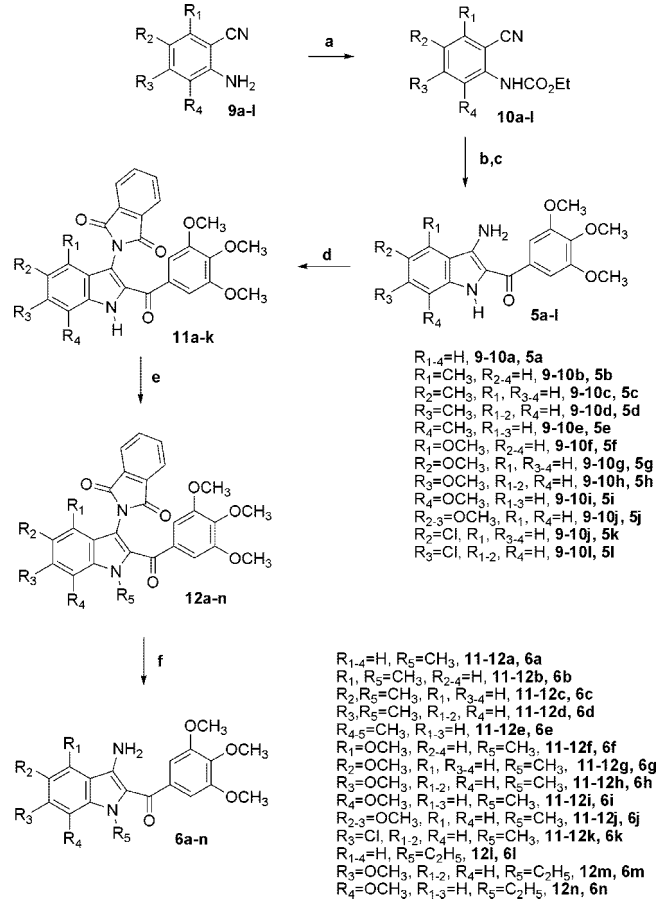
In this article we are interested in determining if the classical divalent ring-equivalent bioisosterism,[9] exemplified by replacement of the sulfur of the benzo[*b*]thiophene nucleus of **4** with a nonsterically hindered NH group, would lead to a novel and active series of 2-(3,4,5-trimethoxybenzoyl)-3-aminoindole derivatives (compounds **5a**−**l**). By the synthesis of compounds **5b**−**l**, we were able to analyze the effect on biological activity of electron-donating (methyl and methoxy) and electron-withdrawing (chloro) substituents at positions 4−7 of the indole ring. Finally, through the synthesis of compounds **6a**−**k** and **6l**−**n**, we investigated whether the introduction of a methyl and ethyl moiety, respectively, at the indolic nitrogen of derivatives **5a**−**l** would lead to loss of activity at the colchicine site through a steric effect.

Antitubulin agents having an indole as their core nucleus have been recently reviewed,[10] and in the past few years an ever increasing number of synthetic indoles as potent tubulin polymerization inhibitors have been reported.[11] The 2- and 3-aroylindole moiety constituted the core structure of two classes of antimitotic compounds, with general structures **7** and **8**, respectively.[12]

Chemistry

The *N*-1H- and *N*-1-methyl- or ethyl-3-aminoindoles (compounds **5a**−**l** and **6a**−**n**, respectively) were prepared following the reaction sequence reported in Scheme 1. 2-Aminobenzonitriles **9a**−**l** were converted to the corresponding *N*-ethoxycarbonylanilines **10a**−**l** by treatment with neat ethyl chloroformate ($ClCO_2Et$) at reflux. The subsequent condensation, followed by intramolecular cyclization, with (3,4,5-trimethoxyphenyl)-2-bromoethanone[8] using NaH as base in DMF provided the *N*-1-ethoxycarbonyl-3-aminoindoles intermediates in good yield. These were subsequently N-deprotected by alkaline hydrolysis using NaOH in aqueous ethanol to furnish the corresponding *N*-1H-2-(3′,4′,5′-trimethoxybenzoyl)-3-aminoindoles **5a**−**l**. With the exception of **5k**, these latter compounds were transformed almost quantitatively into the corresponding *N*-phthalimido derivatives **11a**−**k** using phthalic anhydride in refluxing acetic acid. N-1 alkylation of the indole nucleus was easily achieved by treatment of **11a**−**k** with NaH in DMF, followed by condensation with methyl or ethyl iodide, to furnish intermediates **12a**−**k** or **12l**−**n**, respectively. The removal of the N-protected phthaloyl group was accomplished with hydrazine in refluxing ethanol to afford final N-1-alkylated 2-(3′,4′,5′-trimethoxybenzoyl)-3-aminoindoles **6a**−**n**.

Scheme 1[a]



[a] Reagents: (a) $ClCO_2Et$, reflux, 6 h; (b) (3,4,5-trimethoxyphenyl)-2-bromoethanone, NaH, DMF, room temp, 24 h; (c) NaOH, EtOH/H_2O, reflux, 1 h; (d) phthalic anhydride, AcOH; (e) MeI or EtI, NaH, DMF, room temp; (f) NH_2NH_2, EtOH.

Results and Discussion

Table 1 summarizes the growth inhibitory effects of indole derivatives **5a**−**l** and **6a**−**n** against murine leukemia (L1210), murine mammary carcinoma (FM3A), and human T-lymphoblastoid (Molt/4 and CEM) cells, with CA-4 (**1**), **4a**, and **8a** as reference compounds. In general, the antiproliferative activities of the compounds were greater against the Molt/4 and CEM cells compared with the two murine cell lines. The most active compound identified in this study was **6i**, which inhibited the growth of L1210, FM3A, Molt/4, and CEM cancer cell lines with IC_{50} values at 15, 15, 3.8, and 8.8 nM, respectively, The antiproliferative activity of **6i** was in the same range as those of reference compounds **1**, **4a**, and **8a**.

While the unsubstituted indole **5a** and its *N*-1-ethyl derivative **6l** proved inactive, a modest improvement in activity (IC_{50} = 2–10 μM) was observed for the *N*-1-methyl analogue **6a**. The *N*-1 position of the indole nucleus, along with its C4–C6 substituent pattern, plays an essential role in the antiproliferative activity of derivatives. With the exception of C-4 methyl and methoxy derivatives (compounds **5**–**6b** and **5**–**6f**, respectively), which proved to be inactive, the *N*-methylindole derivatives **6a**−**n** showed increased antiproliferative activity in comparison with their parent N-unsubstituted counterparts **5a**−**l**.

In the *N*-methylindole series, a comparison of substituent effects revealed that the antiproliferative effects of the C-6 and C-7 methoxy compounds **6h** and **6i** exceeded that of their methyl counterparts **6d** and **6e**, respectively, by 2 orders of magnitude. We observed a different effect at the C-5 position, where the substitution of a methoxy with a methyl group (compounds **6g** and **6c**, respectively) caused only a modest 2- to 3-fold increase in activity.

In the series of *N*-H indole derivatives **5b-e**, the C-4 (**5b**), C-5 (**5c**), and C-7 (**5e**) methyl isomers were inactive compounds (IC_{50} > 10 μM), while the C-6 methyl substituted derivative **5d** showed low antiproliferative activity (IC_{50} = 1.4–10 μM). In the corresponding *N*-methyl series **6b**−**e**, the C-4 methyl derivative (**6b**) was inactive, while the C-5 (**6c**), C-6 (**6d**), and C-7 (**6e**) isomers had similar activities against L1210 and FM3A cells, while **6d** was more potent than **6c** and **6e** against Molt4 and CEM cells.

Concerning methoxy substituents, the differences between C-4/5 versus C-6/7 were even more dramatic with the *N*-1-H and *N*-1-methyl series (**5f**−**i** and **6f**−**i**, respectively). For the former series, the C-4 (**5f**) and C-5 (**5g**) methoxy derivatives

Table 1. In Vitro Inhibitory Effects of Compounds **4a**, **5a**−**l, 6a**−**n, 8a**, and CA-4 (**1**) on the Proliferation of Murine Leukemia (L1210), Murine Mammary Carcinoma (FM3A), and Human T-Lymphocyte (Molt/4 and CEM) Cells

	IC_{50} (nM)[a]			
compd	L1210	FM3A	Molt4/C8	CEM
5a	>10000	>10000	>10000	>10000
5b	>10000	>10000	>10000	>10000
5c	>10000	>10000	>10000	>10000
5d	3500 ± 150	>10000	1400 ± 80	2600 ± 0
5e	>10000	>10000	>10000	>10000
5f	>10000	>10000	>10000	>10000
5g	9700 ± 0	9900 ± 30	6900 ± 190	8600 ± 30
5h	970 ± 24	1600 ± 30	630 ± 10	320 ± 90
5i	>10000	>10000	640 ± 260	630 ± 10
5j	>10000	>10000	8600 ± 110	9500 ± 0
5k	>10000	>10000	>10000	>10000
5l	>10000	>10000	9200 ± 60	>10000
6a	8100 ± 37	>10000	2100 ± 0.0	6800 ± 80
6b	>10000	>10000	>10000	>10000
6c	1900 ± 300	1600 ± 200	1700 ± 100	1700 ± 200
6d	1900 ± 150	1500 ± 90	110 ± 30	400 ± 110
6e	1800 ± 120	1400 ± 110	270 ± 90	1200 ± 400
6f	>10000	>10000	>10000	>10000
6g	4200 ± 110	5200 ± 90	2100 ± 0	3900 ± 100
6h	69 ± 37	97 ± 3	57 ± 7	71 ± 5
6i	15 ± 4	15 ± 0	3.8 ± 0.6	8.8 ± 1
6j	5400 ± 270	5000 ± 430	5000 ± 440	5000 ± 260
6k	1100 ± 60	970 ± 75	480 ± 0	1000 ± 60
6l	>10000	>10000	>10000	>10000
6m	>10000	>10000	>10000	>10000
6n	>10000	>10000	9100 ± 210	>10000
4a	33 ± 29	27 ± 13	8.5 ± 1.4	8.9 ± 2.0
8a	3.5 ± 1	3.7 ± 0.2	3.4 ± 0.0	3.6 ± 1
CA-4(**1**)	2.8 ± 1.1	42 ± 6	1.6 ± 1.4	1.9 ± 1.6

[a] IC_{50}: compound concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean ± SE from the dose–response curves of at least three independent experiments.

were inactive, while a 6- to 20-fold increase in potency was observed by moving the methoxy from the C-5 (**5g**) to the C-6 (**5h**) position. A loss of activity against L1210 and FM3A cells was observed for the C-7 derivative **5i**.

In *N*-1-methyl compounds **6f**−**i**, the greatest activity occurred when the methoxy group was introduced at the C-6 or C-7 position, the least when located at the C-4 or C-5 position. For the compounds **6f**−**i**, the C-6 (**6h**) and C-7 (**6i**) derivatives were the most active compounds of the whole series and displayed inhibitory activities comparable to that of CA-4 against four cancer cell lines. The potency improvement by adding a methyl group at the *N*-1-position intrigued us and caused us to explore whether the *N*-1-ethyl series exhibited a similar effect.

From a comparison of the data for compounds **6h**,**i** with those of **6m**,**n**, with an ethyl group on the indolic nitrogen, it is clear that extension of the alkyl chain by an extra methylene unit results in a drastic reduction in activity. There thus appears to be a substantial steric effect resulting from substitution at the N-1 position of the indole. A considerable loss of cytostatic activity was observed with methoxy substituents at C-5 and C-6 (**5**–**6j**).

By comparing the effect of substituents with opposite electronic properties at the C-6 position of the indole nucleus, we found that replacement of the electron-donating methoxy (**5**–**6h**) group with an electron-withdrawing chlorine atom (**5**–**6k**) strongly decreased growth inhibitory properties.

To investigate whether the antiproliferative activities of these compounds were related to an interaction with the microtubule system, **5h** and **6c**−**e**,**g**−**k** were evaluated for their inhibitory effects on tubulin polymerization and on the binding of [^{3}H]colchicine to tubulin (Table 2).[13,14] With the exception of **5h**, which did not inhibit tubulin assembly at a concentration as high as 40 μM, there was a positive correlation between the inhibition of tubulin polymerization and antiproliferative activity. The most potent compound in this series was **6i** (IC_{50} = 1.4 μM), ~2 times more active than **4a** and **8a** (2.1 and 2.9 μM, respectively) and about as active as **1**.

Table 2. Inhibition of Tubulin Polymerization and Colchicine Binding by Compounds **4a**, **5h**, **6c**−**e**,**g**−**k**, **8a**, and CA-4

compd	tubulin assembly,[a] IC_{50} ± SD (μM)	colchicine binding,[b] % inhibition ± SD
5h	>40	nd
6c	10 ± 0.2	nd
6d	6.4 ± 0.07	nd
6e	8.4 ± 0.1	nd
6g	15 ± 2	nd
6h	4.6 ± 0.07	38 ± 5
6i	1.4 ± 0.07	76 ± 5
6j	18 ± 1	nd
6k	7.1 ± 0.3	nd
4a	2.1 ± 0.3	39 ± 1
8a	2.9 ± 0.1	84 ± 0.7
CA-4(**1**)	1.2 ± 0.1	86 ± 3

[a] Inhibition of tubulin polymerization. Tubulin was at 10 μM. [b] Inhibition of [^{3}H]colchicine binding. Tubulin, colchicine, and tested compound were at 1, 5, and 5 μM, respectively. nd: not determined.

Colchicine binding studies were performed on compounds (**6d** and **6i**) with tubulin assembly IC_{50} of <5 μM. Compound **6i** potently inhibited the binding of [^{3}H]colchicine to tubulin, with 76% inhibition occurring when **6i** and radiolabeled colchicine were equimolar (5 μM each) in the reaction mixture. This derivative was slightly less potent than CA-4 and **8a**, which in these experiments inhibited colchicine binding by 86% and 84%, respectively, but **6i** was 2-fold more potent than its benzo[*b*]thiophene counterpart **4a**.

Because molecules exhibiting effects on tubulin assembly should cause alteration of cell cycle parameters, with preferential G2-M blockade, flow cytometry analysis was performed to determine the effect of the most active compounds on K562 (human chronic myelogenous leukemia) cells. Cells were cultured for 24 h in the presence of each compound at the IC_{50} determined after 24 h of growth (**5h**, 3 μM; **6h**, 200 nM; **6i**, 30 nM).

Compounds **5h** and **6h**−**i** caused a marked increase in the percentage of cells blocked in the G2-M phase of the cell cycle, with a simultaneous decrease of cells in the S and G0-G1 phases (Figure 1). Of interest, 24 h exposure of K562 cells with **5h** and **6h**,**i** used at the above concentrations induced about 25% apoptosis (sub-G0-G1 peak). After 48 h of treatment, **6i** showed an AC_{50} (concentration able to induce apoptosis in 50% of cells) of 30 nM. When used at 60 nM, **6i** was able to kill by apoptosis 100% of the K562 cells. In terms of the AC_{50} values, **6i** was 5 times more active than **6h** (AC_{50} = 150 nM) and 100 times more active than **5h** (AC_{50} = 3 μM).

Molecular docking experiments were performed on the series of compounds reported to identify a possible binding mode for these compounds, using MOE[15] and following the same procedure reported before.[8] Compound **6i** appears to bind in a very similar manner to colchicine (Figure 2). The trimethoxyphenyl moiety lies in proximity to βCys241 (the residue numbering is based on the crystal structure used[16]), and the methoxy group occupies the same position as the corresponding group on ring C of colchicine. Furthermore, the *N*-methyl moiety forms a tight hydrophobic contact with βLeu255 and βMet259. This could explain the observed experimental results for **6i** and the loss of biological activity for compounds **5i** and **6n**. The ethyl group of **6n** is too big to be accommodated in the same

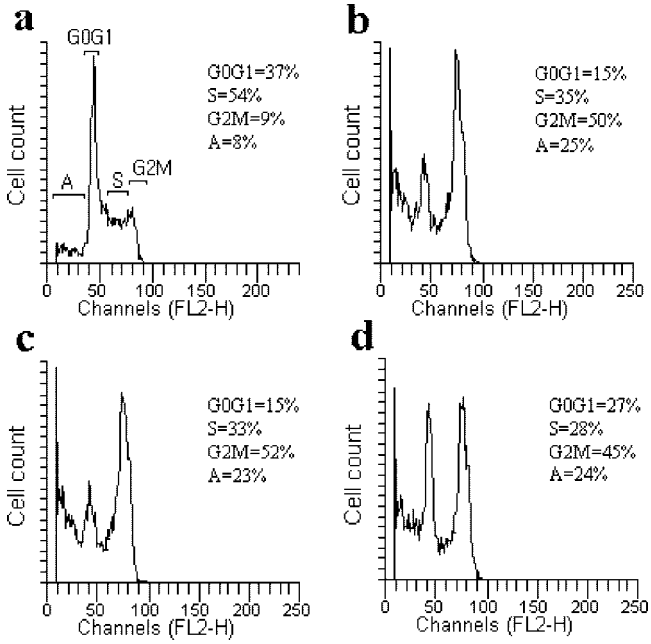


Figure 1. Effects of compounds **5h** (b), **6h** (c), and **6i** (d) on DNA content/cell following treatment of K562 cells for 24 h. The cells were cultured without any compound (a) or with compound used at the concentration leading to 50% cell growth inhibition after 24 h of treatment. Cell cycle distribution was analyzed by the standard propidium iodide procedure as described in the Experimental Section of Supporting Information. Sub-G0-G1 (A), G0-G1, S, and G2-M cells without treatment are indicated in panel a.

position as the methyl group, while the loss of the hydrophobic interaction would reduce the binding affinity for the N unsubstituted **5i**. It should be noted that **6n** and **5i** did not dock in a similar fashion as **6i**. Docking of **6n** did not generate any pose with the trimethoxy ring positioned within 3 Å of the corresponding ring A of colchicine, while the best scored pose for **5i**, despite a good overlap between the trimethoxyphenyl ring and the colchicine ring A, placed the indole ring almost outside the binding pocket, indicating less efficient binding (see Supporting Information).

Conclusions

In conclusion, the synthesis and biological evaluation of a new class of synthetic antitubulin compounds based on the 2-(3′,4′,5′-trimethoxybenzoyl)-3-aminoindole skeleton are described. We have demonstrated that the presence of a hydrogen on the indole nitrogen was detrimental for antiproliferative activity, while *N*-methyl alkylation plays a decisive role increasing antiproliferative potency.

Many active compounds were found among the 1-methyl-2-(3,4,5-trimethoxybenzoyl)-3-aminoindole series, with the C-6 and C-7 methoxy derivatives **6h** and **6i** being the most active, with antiproliferative IC_{50} values ranging from 57 to 97 and 3.8–15 nM, respectively, against the four cancer cell lines. Compound **6i** was both the most active antiproliferative agent and the most effective inhibitor of tubulin polymerization among the newly synthesized compounds. Its activities closely parallel those of reference compounds CA-4 and **8a**. **6i** was more active than the corresponding benzo[*b*]thiophene analogue **4a** as an inhibitor of cancer cell growth and tubulin polymerization.

We also showed by flow cytometry that **6i** had cellular effects typical of agents that bind to tubulin, causing accumulation of cells in the G2-M phase of the cell cycle and extensive apoptosis. Molecular modeling studies were also performed, and the proposed binding mode for **6i** in the colchicine site of tubulin is consistent with the experimental data.

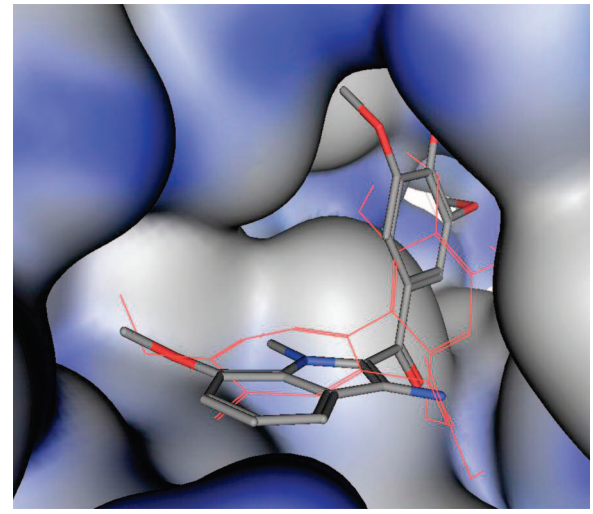


Figure 2. Proposed binding mode of **6i**. DAMA-colchicine is represented in red.

Experimental Section

General Procedure A for the Synthesis of Compounds 10a−l. A vigorously stirred suspension of 2-aminoarylnitriles **8a−l** (5 mmol) in ethyl chloroformate (2.5 mL, 25 mmol) was heated to reflux for 6 h. After complete removal of excess ethyl chloroformate in vacuo, the residue recrystallized from petroleum ether furnished the title compound, which was used without purification for the next reaction.

General Procedure B for the Synthesis of 2-(3′,4′,5′-Trimethoxybenzoyl)-*N*-ethoxycarbonylindoles. Sodium hydride (96 mg, 50% dispersion in mineral oil, 2 mmol) was added to a solution of 2-*N*-ethoxycarbonylarylnitriles **9a−l** (2 mmol) in DMF (5 mL), and the mixture was stirred for 1 h. Then a solution of 2-bromo-1-(3,4,5-trimethoxyphenyl)ethanone (580 mg, 2 mmol) was added in small portions, and the mixture was stirred at room temperature for 18 h. The mixture was poured into water (10 mL) and extracted with dichloromethane (3 × 20 mL). The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel to afford the title compounds.

General Procedure C for the Synthesis of Compounds 5a−l. A solution of NaOH (400 mg, 10 mmol) in water (2 mL) was added to a stirred solution of 2-(3′,4′,5′-trimethoxybenzoyl)-*N*-ethoxycarbonylindoles (1 mmol) in EtOH (3 mL), and the mixture was refluxed for 15 min. The mixture was evaporated to dryness, and water (5 mL) was added to the residue. After acidification of the resulting solution with 0.1 N aqueous solution HCl (pH 3–4) and extraction with dichloromethane (3 × 10 mL), the combined extracts were washed with brine and dried over Na_2SO_4. The residue was purified by flash column chromatography on silica gel to afford the title compounds (EtOAc−petroleum ether, 3:7, for **5b−d**, EtOAc−petroleum ether, 1:1, for **5a**,**e−l**).

General Procedure D for the Synthesis of Compounds 11a−k. To a suspension of **5a−j** or **5l** (6 mmol) in acetic acid (20 mL) was added phthalic anhydride (7.2 mmol, 1.07 g). After the mixture was stirred for 18 h at reflux, the solvent was evaporated and the residue dissolved in EtOAc (30 mL). The organic solution was washed with a saturated solution of $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL), dried, and concentrated. The crude product was purified by crystallization from petroleum ether.

General Procedure E for the Synthesis of Compounds 12a−n. Sodium hydride (60% oil dispersion, 40 mg, 1 mmol, 1 equiv) was carefully added to a solution of alkyl iodide (3 mmol, 3 equiv) and indole derivative **11a−k** (1 mmol) in 5 mL of dry

DMF at 0 °C. The mixture was allowed to warm to room temperature and was stirred for 2 h. The mixture was poured into water (10 mL) and extracted with dichloromethane (3 × 10 mL). The combined organic extracts were washed with water (3 × 10 mL) and brine, dried, and concentrated in vacuo. The residue was purified by column chromatography.

General Procedure F for the Synthesis of Compounds 6a−n. A stirred suspension of indole derivative (**12a−n**, 0.5 mmol) and hydrazine monohydrate (29 μL, 0.6 mmol, 1.2 equiv) in absolute EtOH (10 mL) was refluxed for 3 h. The solvent was evaporated, and the residue was partitioned between EtOAc (10 mL) and water (5 mL). The separated organic phase, washed with brine (2 mL) and dried, was concentrated under vacuo to obtain a residue that was purified by column chromatography to afford the title compounds (EtOAc−petroleum ether, 6:4, for **6a**,**b**,**d**,**e**, **h−j**,**l−n**; EtOAc−petroleum ether, 1:1, for **6c**,**f**,**g**,**k**).

Supporting Information Available: Detailed biological protocols, physical and spectroscopic data for compounds **5a−l**, **6a−n**, **10a−l**, **11a−k**, **12a−n**, and elemental analysis results of **5a−l** and **6a−n**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Mahindroo, N.; Liou, J. P.; Chang, J. Y.; Hsieh, H. P. Antitubulin agents for the treatment of cancer—a medicinal chemistry update. *Expert. Opin. Ther. Pat.* **2006**, *16*, 647–691.

(2) (a) Chaplin, D. J.; Horsman, M. R.; Siemann, D. W. Current development status of small molecules vascular disrupting agents. *Curr. Opin. Invest. Drugs* **2006**, *7*, 522–528. (b) Lippert, J. W., III. Vascular disrupting agents. *Bioorg. Med. Chem.* **2007**, *15*, 605–615. (c) Siemann, D. W. *Vascular-Targeted Therapies in Oncology*; John Wiley and Sons: New York, 2006.

(3) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4. *Experientia* **1989**, *45*, 209–211.

(4) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. Antimitotic natural products combretastatin A-4 and combretastatin A-2: studies on the mechanism of their inhibition of the binding of colchicine to tubulin. *Biochemistry* **1989**, *28*, 6984–6991.

(5) Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, S.; Genazzani, A. A. Medicinal chemistry of combretastatin A4: present and future directions. *J. Med. Chem.* **2006**, *49*, 3033–3044.

(6) Pettit, G. R.; Toki, B.; Herald, D. L.; Verdier-Pinard, P.; Boyd, M. R.; Hamel, E.; Pettit, R. K. Antineoplastic agents. 379. Synthesis of phenstatin phosphate. *J. Med. Chem.* **1998**, *41*, 1688–1695.

(7) Liou, J. P.; Chang, C. W.; Song, J. W.; Yang, Y. N.; Yeh, C. F.; Tseng, H. Y.; Lo, Y. K.; Chang, Y. L.; Chang, C. M.; Hsieh, H. P. Synthesis and struture−activity relationship of 2-aminobenzophenone derivatives as antimitotic agents. *J. Med. Chem.* **2002**, *45*, 2556–2572.

(8) Romagnoli, R.; Baraldi, P. G.; Carrion, M. D.; Lopez Cara, C.; Preti, D.; Fruttarolo, F.; Pavani, M. G.; Tabrizi, M. A.; Tolomeo, M.; Grimaudo, S.; Di Antonella, C.; Balzarini, J.; Hadfield, J. A.; Brancale, A.; Hamel, E. Synthesis and biological evaluation of 2- and 3-aminobenzo[*b*]thiophene derivatives as antimitotic agents and inhibitors of tubulin polymerization. *J. Med. Chem.* **2007**, *50*, 2273–2277.

(9) Lima, L. M.; Barreiro, E. J. Bioisosterism: a useful strategy for molecular modification and drug design. *Curr. Med. Chem.* **2005**, *12* (1), 23–49.

(10) Brancale, A.; Silvestri, R. Indole, a core nucleus for potent inhibitors of tubulin polymerization. *Med. Res. Rev.* **2006**, *27*, 209–238, and references therein.

(11) (a) Liou, J. P.; Wu, C.-Y.; Hsieh, H.-P.; Chang, C.-Y.; Chen, C.-M.; Kuo, C.-C.; Chang, J.-Y. 4- and 5-Aroylindoles as novel classes of potent antitubulin agents. *J. Med. Chem.* **2007**, *50*, 4548–4552. (b) Hu, L.; Jiang, J.-D.; Qu, J.; Li, Y.; Jin, J.; Li, Z.-R.; Boykin, D. W. Novel potent antimitotic heterocylic ketones: synthesis, antiproliferative activity, and structure−activity relationships. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3613–3617. (c) La Regina, G.; Edler, M. C.; Brancale, A.; Kandil, S.; Clouccia, A.; Piscitelli, F.; Hamel, E.; De Martino, G.; Matesanz, R.; Diaz, J. F.; Scovassi, A. I.; Prosperi, E.; Lavecchia, A.; Novellino, E.; Artico, M.; Silvestri, R. Arylthioindole inhibitors of tubulin polymerization. 3. Biological evaluation, structure−activity relationships and molecular modeling studies. *J. Med. Chem.* **2007**, *50*, 922–931.

(12) (a) For 2-aroylindole derivatives, see the following: Mahboobi, S.; Pongratz, H.; Hufsky, H.; Hockemeyer, J.; Frieser, M.; Lyssenko, A.; Paper, D. H.; Burgermeister, J.; Bohmer, F. D.; Fiebig, H. H.; Burger, A. M.; Baasner, S.; Beckers, T. Synthetic 2-aroylindole derivatives as a new class of potent tubulin-inhibitory, antimitotic agents. *J. Med. Chem.* **2001**, *44*, 4535–4553. (b) For 3-aroylindole derivatives see the following: 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. Concise synthesis and structure−activity relationships of combretastatin A-4 analogues, 1-aroylindoles and 3-aroylindoles, as novel classes of potent antitubulin Agents. *J. Med. Chem.* **2004**, *47*, 4247–4257.

(13) Hamel, E. Evaluation of antimitotic agents by quantitative comparisons of their effects on the polymerization of purified tubulin. *Cell Biochem. Biophys.* **2003**, *38*, 1–21.

(14) Verdier-Pinard, P.; Lai, J.-Y.; Yoo, H.-D.; Yu, J.; Marquez, B.; Nagle, D. G.; Nambu, M.; White, J. D.; Falck, J. R.; Gerwick, W. H.; Day, B. W.; Hamel, E. Structure−activity analysis of the interaction of curacin A, the potent colchicine site antimitotic agent, with tubulin and effects of analogs on the growth of MCF-7 breast cancer cells. *Mol. Pharmacol.* **1998**, *53*, 62–67.

(15) *Molecular Operating Environment (MOE 2008)*; Chemical Computing Group, Inc.: Montreal, Quebec, Canada, 2008; http://www.chemcomp.com.

(16) Ravelli, R. B. G.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M. Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. *Nature* **2004**, *428*, 198–202.

JM7011547