Synthesis and Biological Evaluation of 2-(3′,4′,5′-Trimethoxybenzoyl)-3-Amino 5-Aryl Thiophenes as a New Class of Tubulin Inhibitors

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2-(3′,4′,5′-Trimethoxybenzoyl)-3-amino-5-aryl/heteroaryl thiophene derivatives were synthesized and evaluated for antiproliferative activity, inhibition of tubulin polymerization, and cell cycle effects. SARs were elucidated with various substitutions on the aryl moiety 5-position of the thienyl ring. Substituents at the *para*-position of the 5-phenyl group showed antiproliferative activity in the order of F═CH_3 > OCH_3═Br═NO_2 > CF_3═I > OEt. Several of these compounds led to arrest of HL-60 cells in the G2/M phase of the cell cycle and induction of apoptosis.

There is considerable interest in the discovery and development of novel small molecules able to affect tubulin polymerization. Such compounds impair dynamic microtubule elements of the cell cytoskeleton responsible for the formation of the mitotic spindle and required for proper chromosomal separation during cell division.[1−4]

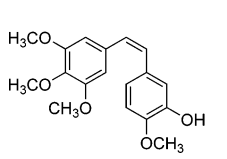
More recently, it has been established that some tubulin-binding agents selectively target the vascular system of tumors. These compounds induce morphological changes in the endothelial cells of the tumor's blood vessels, resulting in their occlusion and interruption of blood flow.[5−6]

Combretastatin A-4 (CA-4, **1**; Chart 1), isolated from the bark of the South African tree *Combretum caffrum*,[7] 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.[8] CA-4 inhibits cell growth even at low nanomolar concentrations and exhibits inhibitory effects on multidrug resistant cancer cell lines. Because of its simple structure, a wide number of CA-4 analogues have been synthesized and evaluated in SAR studies.[9,10]

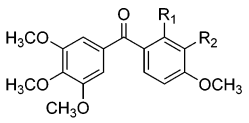
Among synthetic small molecule tubulin inhibitors, replacement of the double bond of CA-4 with a carbonyl group furnished a benzophenone-type CA-4 analogue named phenstatin (**2**). This compound demonstrated interesting efficacy in a variety of tumor models, while retaining the characteristics of CA-4.[11] The 2-aminobenzophenone derivative **3** also strongly inhibited cancer cell growth and tubulin polymerization and caused mitotic arrest, as did **2**.[12]

The classical bioisosteric equivalence between benzene and thiophene prompted us recently to synthesize a series of thiophene derivatives with general formula **4**, in which a 2-aminothiophene system replaced the 2-aminobenzene moiety in the 2-amino phenstatin analogue **3**. Many of the 2-amino-3-(3′,4′,5′-trimethoxybenzoyl)-5-phenyl thiophene molecules with general structure **4** are potent inhibitors of tubulin polymerization and show strong antiproliferative activities against two leukemic cell lines, L1210 and K562, with ac-

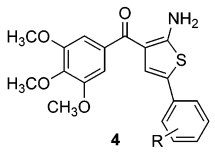
Chart 1



Combretastatin A-4 (CA-4), **1**



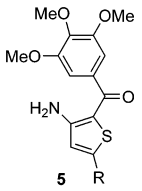
R_1=H, R_2=OH, Phenstatin, **2**
R_1=NH_2, R_2=H, **3**



4

R=H, halogen, CH_3, CF_3, MeO

4a, R=H
4b, R=p-F
4c, R=p-Me
4d, R=p-MeO
4e, R=m-MeO



5

5a, R=C_6H_5
5b, R=2-thienyl
5c, R=3-thienyl
5d, R=pyridin^{-4}-yl
5e, R=p-F-C_6H_4
5f, R=p-Cl-C_6H_4
5g, R=o-Cl-C_6H_4
5h, R=m,p-2Cl-C_6H_4
5i, R=p-Br-C_6H_4
5j, R=p-I-C_6H_4
5k, R=p-CH_3-C_6H_4
5l, R=p-OCH_3-C_6H_4
5m, R=m-OCH_3-C_6H_4
5n, R=m,p-2(OCH_3)-C_6H_3
5o, R=m,m',p-3(OCH_3)-C_6H_2
5p, R=p-OCH_2CH_3-C_6H_4
5q, R=p-CF_3-C_6H_4
5r, R=p-NO_2-C_6H_4
5s, R=m-NO_2-C_6H_4
5t, R=p-C_6H_5-C_6H_4

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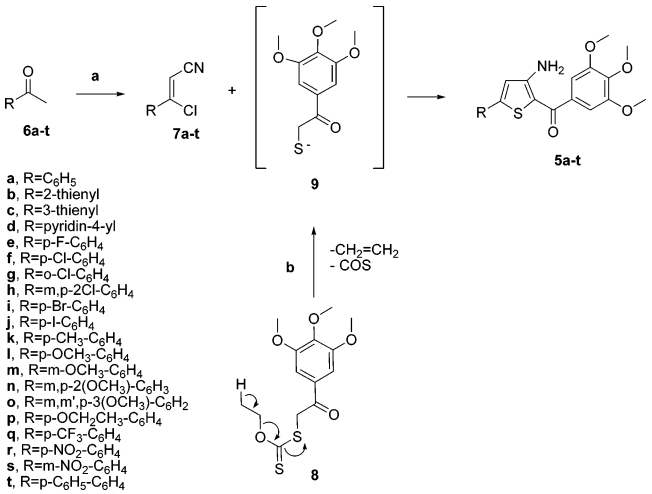
Scheme 1[a]



[a] Reagents and conditions: (a) $POCl_3$, DMF then $NH_2OH{\cdot}HCl$; (b) piperidine, EtOH, reflux, 2h.

cumulation of cells in the G2/M phase of the cell cycle. The most active compounds had activities comparable with those of CA-4.[13]

We have continued to explore the potential of molecules of this type to interact with tubulin by synthesis of a novel series of analogues containing the 2-(3′,4′,5′-trimethoxybenzoyl)-3-amino-5-aryl/heteroaryl thiophene nucleus, with general structure **5**. These new molecules can be viewed as positional isomers of compounds with formula **4**. Further, the synthesis of compounds **5e**−**t** allowed us to investigate SAR effects of electron-withdrawing (F, Cl, Br, I, NO_2, and CF_3) and electron-releasing (Me, MeO, and C_6H_5), as well as bulky, substituents on the phenyl at the 5-position of the thiophene ring.

2-(3′,4′,5′-Trimethoxybenzoyl)-3-amino-thiophene derivatives with general structure **5** were synthesized by a two-step procedure, as shown in Scheme 1. The first step consisted of the synthesis of *β*-chloro aroylcinnamonitriles **7a**−**t**, obtained in moderate yields by a modified Vilsmeier reaction applied to commercially available acetophenones **6a**−**t**, which were treated with $POCl_3$ in DMF, followed by $NH_2OH{\cdot}HCl$.[14,15] The "one-pot" cyclization in refluxing ethanol of **7a**−**t** with the α-mercapto ketone anion **9**, generated in situ by treating *O*-ethyl-*S*-[2-oxo-2-(3,4,5-trimethoxyphenyl)-ethyl] dithiocarbonate **8** with piperidine,[16] furnished the 2-(3′,4′,5′-trimethoxybenzoyl)-3-amino-5-aryl/heteroaryl thiophene derivatives **5a**−**t** in good yields. Dithiocarbonate ester **8** was synthesized from potassium ethyl dithiocarbonate and 2-bromo-1-(3,4,5-trimethoxyphenyl)-ethanone.

Table 1 summarizes the antiproliferative effects of thiophene derivatives **5a**−**t** against a panel of tumor cell lines using CA-4 (**1**) as the reference compound. Several derivatives demonstrated substantial growth inhibitory effects against murine leukemia (L1210), murine mammary carcinoma (FM3A), and human T-lymphoblastoid (Molt/4 and CEM) cells. In general, the antiproliferative activities of the tested compounds were more pronounced against Molt/4 and CEM cells as compared with the two murine cell lines. Moreover, with the FM3A cells, many derivatives (**5a**−**c**, **5e**, **5g** **5k**−**m**, and **5s**) were more active than CA-4. Of the tested compounds, derivatives **5a**−**c** and **5s** possessed the highest overall potency with IC_{50} values of 10−12, 2−17, 5−9, and 5−10 nM against the L1210, FM3A, Molt4, and CEM cell lines, respectively.

Replacement of the phenyl ring of compound **5a** by the bioisosteric 2-thienyl or 3-thienyl groups (compounds **5b** and **5c**, respectively) had generally minor effects on antiproliferative activity against the four cell lines. Replacement of the phenyl group with a more polar pyridine (**5d**) resulted in over a 10-fold reduction of cytostatic activity, which was eliminated by the introduction of a second phenyl ring on the 4-position of the phenyl ring (*p*-biphenyl derivative **5t**).

Table 1. In Vitro Inhibitory Effects of Compounds **5a**−**t** and CA-4 (**1**) against the Proliferation of Murine Leukemia (L1210), Murine Mammary Carcinoma (FM3A), and Human T-lymphocyte (Molt/4 and CEM) Cells

	IC_{50}[a] (nM)			
compd	L1210	FM3A	Molt4/C8	CEM/0
5a	11 ± 6	13 ± 3	5.3 ± 1.2	4.5 ± 1.3
5b	9.8 ± 0.4	1.7 ± 0.1	8.9 ± 1	9.4 ± 0.9
5c	12 ± 0	3.7 ± 0.2	8.8 ± 0.3	9.6 ± 0.1
5d	180 ± 150	150 ± 50	88 ± 4	110 ± 20
5e	20 ± 2	16 ± 2	12 ± 5	15 ± 2
5f	93 ± 26	90 ± 9	77 ± 7	70 ± 20
5g	20 ± 1	34 ± 3	17 ± 1	16 ± 1
5h	260 ± 150	400 ± 90	270 ± 30	290 ± 20
5i	100 ± 00	150 ± 60	92 ± 2	86 ± 6
5j	400 ± 20	420 ± 20	190 ± 60	150 ± 10
5k	18 ± 1	16 ± 3	16 ± 2	15 ± 3
5l	45 ± 21	31 ± 5	18 ± 2	19 ± 7
5m	22 ± 1	36 ± 3	17 ± 0	16 ± 1
5n	40 ± 6	72 ± 15	20 ± 2	20 ± 5
5o	2300 ± 100	3500 ± 1200	1200 ± 100	840 ± 150
5p	590 ± 40	510 ± 80	240 ± 10	270 ± 20
5q	370 ± 10	540 ± 40	250 ± 70	270 ± 20
5r	100±00	64 ± 2	110 ± 10	99 ± 17
5s	11 ± 5	17 ± 3	7.5 ± 0.2	5.9 ± 0.3
5t	>10 000	>10 000	>10 000	>10 000
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.

Para-substituted phenyl derivatives showed variable potencies. Introduction of a weak electron-withdrawing group (EWG), a fluorine atom (**5e**), had little overall effect on antiproliferative activity, but increasing the size of the halogen from chlorine (**5f**) to bromine (**5i**) to iodine (**5j**) caused progressive loss of antiproliferative activity. Moving the chlorine from the *para* (**5f**) to the *ortho* position (**5g**) increased antiproliferative activity, but, in contrast, when a second chlorine atom was inserted to yield the *m*,*p*-dichloro derivative **5h**, activity was substantially reduced. Another EWG, the nitro moiety, intermediate in size between chlorine and bromine, when placed in the *para* position, yielded a compound (**5r**) with antiproliferative activity similar to that of the two halogenated compounds. Placing the nitro group in the *meta* position (compound **5s**) significantly enhanced activity relative to **5r**, yielding a compound with activity little different from that of the unsubstituted **5a**. Thus, selected, single EWGs can be placed at all three positions in the phenyl ring with only minor effects on antiproliferative activity, but no modification improved activity relative to the unsubstituted **5a**.

Turning to the effects of an electron-releasing group (ERG) on the phenyl moiety,we found that *p*-methyl (**5k**) and *p*-methoxy (**5l**) groups caused only minor changes in antiproliferative activity relative to unsubstituted compound **5a**. Moreover, shifting the methoxy group from the *para* to the *meta* position (**5m**) did not affect antiproliferative activity. Potency was also little changed with the *m*,*p*-dimethoxy derivative (**5n**; in contrast to the reduced activity, described above, of **5h**, the *m*,*p*-dichloro derivative), but adding a third methoxy group in the *m*,*m*′,*p*-trimethoxy compound **5o** almost eliminated antiproliferative activity. In an effort to further understand the steric effect of the alkoxy substituent at the *para* position, the ethoxy

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

compd	tubulin assembly[a] $IC_{50} \pm SD$ (μM)	colchicine binding[b] % ± SD
4a[13]	1.3 ± 0.01	77 ± 5
4b[13]	0.86 ± 0.1	81 ± 4
4c[13]	0.80 ± 0.01	90 ± 2
4d[13]	1.1 ± 0.01	66 ± 0.2
4e[13]	1.2 ± 0.06	57 ± 2
5a	1.2 ± 0.08	81 ± 4
5b	1.2 ± 0.06	80 ± 1
5c	1.3 ± 0.1	81 ± 0.6
5e	1.5 ± 0.1	70 ± 2
5g	1.2 ± 0.1	76 ± 1
5k	1.1 ± 0.1	80 ± 1
5l	1.1 ± 0.02	60 ± 2
5m	0.64 ± 0.04	81 ± 0.5
5n	1.1 ± 0.06	53 ± 1
5s	0.97 ± 0.1	74 ± 0.9
CA-4 (1)	1.4 ± 0.1	87 ± 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 1 μM, respectively.

compound **5p** was prepared, resulting in over a 10-fold loss of activity in all cell lines relative to the methoxy derivative **5l**. Replacement of the ERG methoxy group with the EWG and also bulkier trifluoromethyl moiety (compound **5q**) produced a dramatic loss of activity. Because **5p** and **5q** had almost identical activities, the loss of activity in the latter is presumably due primarily to steric limitations around the *para* position of the phenyl ring rather than the electronic properties of the ethoxy and trifluoromethyl moieties.

By comparing the effects of ERGs and EWGs on the phenyl at the C5-thiophene position, no clear influence on antiproliferative activity was observed. In fact, several compounds characterized by the presence of substituents with opposite electronic effects showed the same potency. For example, compound **5e** containing the electron-withdrawing fluoro group showed the same antiproliferative potency as compound **5l** containing the electron-donating methoxy group.

The more active compounds (**5a**−**c**, **5e**, **5g**, **5k**−**n**, and **5s**) were evaluated for their in vitro inhibition of tubulin polymerization and for their inhibitory effects on the binding of [^{3}H]-colchicine to tubulin (in the latter assay, the compounds and tubulin were examined at a concentration of 1 μM with the colchicine at 5 μM; Table 2).[17,18] For comparison, CA-4 was examined in contemporaneous experiments. Keeping the substituent in the 5-position of the thiophene ring constant, the previously prepared[13] positional isomers **4a**−**e** were also re-examined for comparison with derivatives **5a**, **5e**, and **5k**−**m**, respectively. All new compounds were comparable or superior to CA-4 as inhibitors of tubulin assembly, with the order of activity **5m** > **5s** > **5k** = **5l** = **5n** > **5a** = **5b** = **5g** > **5c** > **CA-4** > **5e**. Compound **5m** was the most active (IC_{50}, 0.64 μM), having twice the potency of CA-4 (IC_{50}, 1.4 μM), while the others had IC_{50} values ranging from 1 to 1.5 μM.

In the colchicine binding studies, compounds **5a**−**c**, **5e**, **5g**, **5k**−**n**, and **5s** potently inhibited the binding of [^{3}H]colchicine to tubulin, because 53−82% inhibition occurred with these agents at 1 μM and the colchicine at 5 μM. None, however, was quite as potent as CA-4, which in these experiments inhibited colchicine binding by 87%. While this group of compounds was highly potent in the biological assays (inhibition of cell growth, tubulin assembly, and colchicine binding), correlations between the three assay types were imperfect. Thus, while compound **5m** was the best inhibitor of tubulin assembly, its effect on colchicine binding was matched by compounds

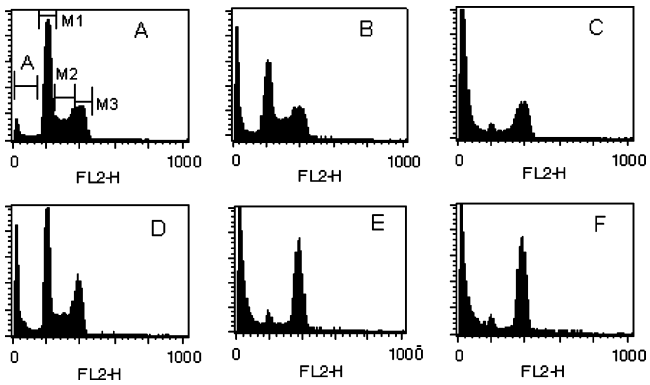


Figure 1. Flow cytometry analysis of the cell cycle. HL60 cells were exposed for 24 h to 8 nM (panel B) or 40 nM (panel C) compound **5a**, 15 nM compound **5s** (panel D), 20 nM compound **5b** (panel E), and 20 nM compound **5c** (panel F). The cell cycle was analyzed by the standard propidium iodide procedure, as described in the methods section. (A) Control cells at the sub-G_0-G_1 (labeled A), G_0-G_1 (labeled M1), S (labeled M2), and G_2-M (labeled M3) phases of the cell cycle are indicated.

5a−**c** and **5k**, all of which were half as active as assembly inhibitors. Compound **5m** was, in general, somewhat less effective as an inhibitor of cell growth than the other four compounds.

We also re-examined five of the active compounds (**4a**−**e**), prepared previously,[13] to compare these 2-amino-5-aryl thiophenes with the newly synthesized isomeric 3-amino-5-aryl thiophenes (**5a**, **5e**, **5k**, **5l**, and **5m** corresponding to **4a**, **4b**, **4c**, **4d**, and **4e**, respectively). Although, as before, **4a**−**e** were potent inhibitors of both tubulin assembly and colchicine binding, there was no consistency in the order of activity of the five sets of compounds in either assay. Also, like the 3-amino-5-aryl data described above, the two tubulin assays with the 2-amino-5-aryl compounds did not correlate perfectly. Significantly, however, the *m*-methoxy analogues (**4e** and **5m**) were the most active compounds in both assays in each series.

The effects on the cell cycle of a selected group of compounds (**5a**−**c** and **5s**) were examined by flow cytometry after staining of the cells with propidium iodide. HL60 cells (acute myeloblastic leukemia) were exposed for 24 h to 8 nM compound **5a**, 20 nM compound **5b**, 20 nM compound **5c**, and 15 nM compound **5s**. As shown in Figure 1B, compound **5a** induced a modest increase of cells in the S and G2-M phases of the cell cycle relative to the control (Figure 1A), while compound **5s** caused an evident increase of cells in G2-M (Figure 1D). Both of these drugs induced apoptosis as shown by the appearance of a sub-G0-G1 peak. Using a higher concentration of **5a** (40 nM), blockage of the cells in G2-M and the percentage of apoptotic cells were more evident (Figure 1C). In contrast to **5a** and **5s**, compounds **5b** and **5c** (Figure 1E,F) caused much more extensive accumulation of cells in both the G2-M phase and the sub-Go-G1 apoptotic peak.

In conclusion, we have discovered a new class of simple synthetic inhibitors of tubulin polymerization, based on a 2-(3′,4′,5′-trimethoxybenzoyl)-3-amino-thiophene molecular skeleton. These derivatives are as effective as their 2-amino-3-(3′,4′,5′-trimethoxybenzoyl)-thiophene isomeric analogues with general structure **4**. The best results for inhibition of antiproliferative activity were obtained with the *p*-F-phenyl (**5e**), the *o*-Cl-phenyl (**5g**), the *p*-Me-phenyl (**5k**), the *m*-OMe-phenyl (**5m**), and the *m*-NO_2-phenyl (**5s**) derivatives at the 5-position of the thiophene ring. Order of activity for *p*-halogenated derivatives was F > Cl > Br > I, with activity thus varying

inversely with the size of the halogen. The inactivity of the *p*-diphenyl derivative **5t** indicates that a bulky substituent on the same position is detrimental for activity, a conclusion supported by the finding with the halides by a comparison of the reduced activity of a *p*-ethoxy substituent relative to the *p*-methoxy analogue and by the effect of a *p*-NO_2 substituent. There was also no clear difference in effect on activity between an EWG versus an ERG, once substituent size was taken into account. We identified tubulin as the molecular target of the compounds, because those with the greatest inhibitory effects on cell growth strongly inhibited tubulin assembly and binding of colchicine to tubulin. These activities differed little from those of CA-4, and all active compounds had quantitatively similar effects in the tubulin assays, varying within a narrow range (IC_{50}s for assembly, 0.64 to 1.5 μM with 10 μM tubulin; 53−81% inhibition of the binding of 5 μM colchicine, with the inhibitor and tubulin both at 1 μM). We also showed by flow cytometry that four of the active compounds had cellular effects typical of agents that bind to tubulin, causing accumulation of cells in the G2/M phase of the cell cycle and a substantial increase in the number of apoptotic cells. These compounds constitute a new class of potent antitubulin agents with the potential to be developed clinically for anticancer chemotherapy.

Experimental Section

General Procedure A for the Synthesis of Compounds 7a−t. Neat $POCl_3$ (12.27 g, 80 mmol) was added dropwise over 30 min, maintaining the temperature below 25 °C, to a stirred solution cooled on ice, of the appropriate acetophenone derivative (20 mmol) dissolved in DMF (60 mL). The reaction mixture was stirred for 2 h at room temperature, and NH_2OH·HCl (1.4 g, 20 mmol) was added portionwise, resulting in an exothermic reaction. The mixture was stirred overnight, cold water (100 mL) was added, and the aqueous phase was extracted with ethyl acetate (3 × 50 mL). The organic phase was washed with brine (50 mL), dried over Na_2SO_4, and concentrated in vacuo. The resulting precipitate was purified by silica gel column chromatography to yield 3-chlorocinnamonitriles **7a−t**.

Synthesis of *O*-Ethyl Ester of *S*-[2-Oxo-2-(3,4,5-trimethoxyphenyl)-ethyl] Dithiocarbonic Acid (8). A solution of 2-bromo-1-(3,4,5-trimethoxyphenyl)-ethanone (5.78 g, 20 mmol) in 20 mL of acetone was added dropwise over 30 min to a solution of potassium ethyl xanthate (4 g, 25 mmol) dissolved in 50 mL of acetone. The reaction mixture was stirred for 1 h at room temperature. Acetone was removed in vacuo, and the residue was suspended in dichloromethane (40 mL). The resulting mixture was washed with water (3 × 10 mL) and brine (10 mL), dried over sodium sulfate, and concentrated in vacuo. The resulting precipitate was purified by column chromatography eluting with petroleum ether−ethyl acetate (8:2, v/v). Compound **8** was isolated as a white solid. Yield 74%; mp 58−60 °C. ^{1}H NMR ($CDCl_3$): δ 1.41 (t, J = 7.2 Hz, 3H), 3.93 (s, 9H), 4.65 (m, 4H), 7.28 (s, 2H).

General Procedure B for the Synthesis of 5-Aryl/heteroaryl-3-amino-thiophen-2-yl-(3,4,5-trimethoxyphenyl)-methanones 5a−t. Piperidine (0.31 mL, 3 mmol) was added to a stirred solution of dithiocarbonic acid *O*-ethyl ester *S*-[2-oxo-2-(3,4,5-trimethoxyphenyl)-ethyl] ester **8** (1.5 mmol) dissolved in ethanol (30 mL). The reaction mixture was stirred for 30 min at room temperature. The corresponding 3-chloroacrylonitrile (1.5 mmol) was added, and the solution was stirred at reflux for 3 h, after which ethanol was removed under reduced pressure and the residue dissolved in dichloromethane (20 mL). The organic phase was washed with a 5% v/v solution of HCl (2 × 5 mL), a saturated solution of sodium carbonate (2 × 5 mL), and brine (5 mL) and dried over sodium sulfate. After concentration under reduced pressure, the residue was purified by silica gel column chromatography (petroleum ether−EtOAc, 6:4) to furnish the corresponding final compounds **5a−t**.

Supporting Information Available: Detailed biological protocols, physical and spectroscopic data for compounds **5a−t** and **7a−t**, and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hamel, E. Antimitotic natural products and their interactions with tubulin. *Med. Chem. Rev.* **1996**, *16*, 207−231.
(2) Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGown, A. T. Tubulin as a target for anticancer drugs: agents which interact with the mitotic spindle. *Med. Res. Rev.* **1998**, *18*, 259−296.
(3) Li, Q.; Sham, H. L. Discovery and development of antimitotic agents that inhibit tubulin polymerization for the treatment of cancer. *Expert Opin. Ther. Pat.* **2002**, *12*, 1663−1702.
(4) Jordan, M. A. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. *Curr. Med. Chem.* **2002**, *2*, 1−17.
(5) Siemann, D. W.; Chaplin, D. J.; Horsman, M. R. Vascular-targeting therapies for treatment of malignant disease. *Cancer* **2004**, *100*, 2491−2499.
(6) Feron, O. Targeting the tumor vascular compartment to improve conventional cancer therapy. *Trends Pharmacol. Sci.* **2004**, *25*, 536−542.
(7) (a) Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavanijaya, P. Isolation and structure of combretastatin. *Can. J. Chem*. **1982**, *60*, 1374−1376. (b) 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.
(8) 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.
(9) Nam, N. H. Combretastatin A-4 analogues as antimitotic antitumor agents. *Curr. Med. Chem.* **2003**, *10*, 1697−1722.
(10) 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.
(11) 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.
(12) 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−reactivity relationship of 2-aminobenzophenone derivatives as antimitotic agents. *J. Med. Chem.* **2002**, *45*, 2566−2572.
(13) 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. Synthesis and biological evaluation of 2-amino-3-(3′,4′,5′-trimethoxybenzoyl)-5-aryl thiophenes as a new class of potent antitubulin agents. *J. Med. Chem*. **2006**, *49*, 3906−3915.
(14) Hartmann, H.; Liebscher, J. A simple method for the synthesis of 5-aryl-3-amino-2-alkoxycarbonylthiophenes. *Synthesis* **1984**, *3*, 275−276.
(15) Hartmann, H.; Liebscher, J. A facile synthesis of 5-aminopyrazoles by reaction of β-chloro cinnamonitriles with hydrazine hydrate. *Synthesis* **1984**, *3*, 276−277.
(16) Brown, M. D.; Gillon, D. W.; Meakins, G. D.; Whitham, G. H. A new general synthesis of 2-(*N*-mono-and *N*-disubstituted amino) thiazoles. *J. Chem. Soc., Perkin. Trans. 1* **1985**, 1623−1626.
(17) 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.
(18) 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 analogues on the growth of MCF-7 breast cancer cells. *Mol. Pharmacol.* **1998**, *53*, 62−67.

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