

Synthesis and Biological Evaluation of 2-and 3-Amino Benzo[b]Thiophene Derivatives as Antimitotic Agents and Inhibitors of Tubulin Polymerization

Romeo Romagnoli*[†], Pier Giovanni Baraldi*[†], Maria Dora Carrion[†], Carlota Lopez Cara[†], Delia Preti[†], Francesca Fruttarolo[†], Maria Giovanna Pavani[†], Mojgan Aghazadeh Tabrizi[†], Manlio Tolomeo^{††}, Stefania Grimaudo^{††}, Antonella Di Cristina^{††}, Jan Balzarini[‡], John A. Hadfield[§], Andrea Brancale^{§§} and Ernest Hamel[‡]

Dipartimento di Scienze Farmaceutiche, Università di Ferrara, 44100 Ferrara, Italy; Divisione di Ematologia e Servizio AIDS, Dipartimento di Oncologia, Policlinico “P. Giaccone”, Università di Palermo, Palermo, Italy; Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Minderbroedersstraat 10, B-3000 Leuven, Belgium; Centre for Molecular Drug Design, Cockcroft Building, University of Salford, Manchester, M5 4W5, UK; The Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff, CF10 3XF, UK; Toxicology and Pharmacology Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702, USA

SUPPORTING INFORMATION

Characterization of compounds **6a-e**, **7a-l**, **9a-e**, **10a-e**, **11a-e**, **20a-e**, **22f-j**, **23a-e**, **23f-j**, elemental analyses of compounds **6a-e** and **7a-l**, biological assays.

Molecular modeling studies of compounds **6d** and **7d**

Molecular Modeling. A series of molecular modeling simulations were performed to investigate possible binding conformation for these new series of compounds. Compounds **6d** and **7d** were docked in the colchicine binding site of tubulin (PDB entry: 1SA0)¹ using MOE 2006.08 (MOE).² To validate our docking protocol, we performed an initial run on DAMA-colchicine, the ligand co-crystallised with the protein, and MOE was able to position DAMA-colchicine in a very similar fashion to the crystallized conformation (RMSD 1.1 Å). We then docked compounds **6d** and **7d**, obtaining two substantially different binding modes (Figure 1) for the two compounds. In both cases, the trimethoxyphenyl group overlaps well with the corresponding colchicinoid A ring, but the benzothiophene moiety is placed in two very distinct pockets within the active site. In the case of **7d**, this group lies on the edge of the colchicine binding site, pointing towards the GTP bound to α -tubulin. This result is in accordance with the observation that compound **7d** is not as effective as compound **6d** as an inhibitor of colchicine binding to tubulin. It is also important to notice that the amino group of **7d** does not appear to have any contact with the protein, consistent with the antiproliferative activity observed with the dimethyl-substituted compound **7k**. Furthermore, the docking results obtained for **7d** also correlate well with the experimental data obtained with the different methyl-substituted benzothiophene analogues **7b-e**, showing that positions 4, 5 and 7 of the heterocycle are more sterically hindered than position 6.

In the case of compound **6d**, the docked conformation overlaps well with the colchicine analogue in the crystallized protein complex. Moreover, although in this case, too, the amino group does not establish any direct interaction with the protein, after energy minimization of the protein/ligand complex, the ligand amino nitrogen atom is just 3.5 Å from the side chain of Ser178 (residue numbering is as in the PDB for the crystal structure used¹) (Figure 2).

Therefore, considering that the docking protocol does not take into account protein flexibility, the formation of an hydrogen bond could be possible between the 2-amino group of compound **6d** and the hydroxyl group of Ser178, a residue that could also be involved in the binding of colchicine, as previously reported.³

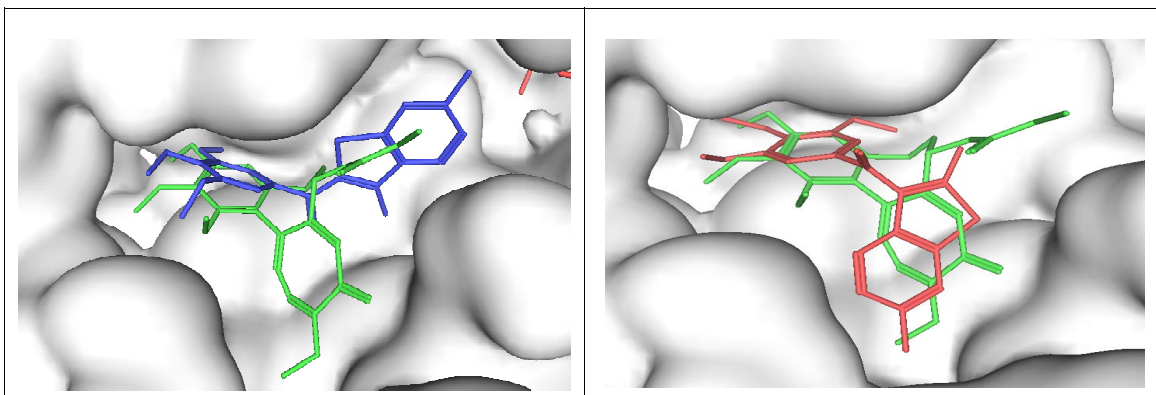


Figure 2: Proposed binding mode for compound **7d** (in blue) and **6d** (in red). DAMA-colchicine is represented in green.

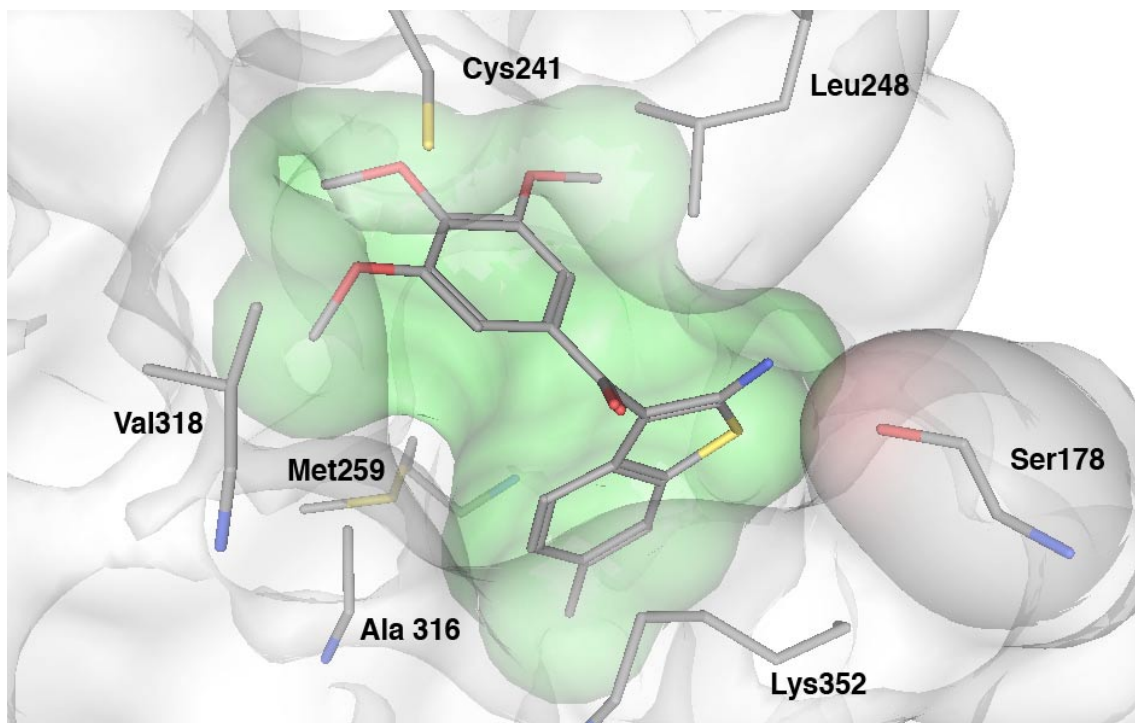


Figure 3: Binding of **6d** after energy minimization. Residue numbering based on that described with the crystal structure used in the modelling studies.

Experimental Section

Chemistry. Materials and Methods. Cyclohexanone, 4-methylcyclohexanone, 2-nitrobenzotrile, 4-methoxybenzoylacetonitrile, 2-methyl-6-nitrobenzotrile and 4-methyl-6-nitrobenzotrile are commercially available and were used as received. For the synthesis of **13** see Seneci, P.; Nicola, M.; Inglesi, M.; Vanotti, E.; Resnati, G. Synthesis of mono- and disubstituted 1H-imidazo[1,2-b] pyrazoles. *Synth. Commun.* **1999**, *2*, 311-341; compound **14** was synthesized following Baba, A.; Mori, A.; Yasuma, T.; Unno, S., Makino, H.; Sohda, T. Studies on disease-modifying antirheumatic drugs. IV. Synthesis of novel thieno[2,3-b:5,4-c']dipyridine derivatives and their anti-inflammatory effect. *Chem. Pharm. Bull.* **1999**, *47*, 993-999. 4-Methoxycyclohexanone was synthesized following Kaiho, T.; Sannohe, K.; Kajiya, S.; Suzuki, T.; Otsuka, K.; Ito, T.; Kamiya, J.; Maruyama, M. Cardiotonic agents. 1-Methyl-7-(4-pyridyl)-5,6,7,8-tetrahydro-3(2H)-isoquinolinones and related compounds. Synthesis and activity. *J. Med. Chem.* **1989**, *32*, 351-357. 2-Bromo-1-(3,4,5-trimethoxyphenyl)-ethanone was synthesized following the procedure: Horton, W. J.; Thompson, G. Seven-membered ring compounds. VII. 7-Acetamidobenzosuberone and α -amino- γ -3,4,5-trimethoxyphenylbutyric acid. *J. Am. Chem. Soc.* **1954**, *76*, 1909-1911. **19c** was synthesized following Werbel, L. M.; Elslager, E. F.; Newton, L. S. Antimalarial agents. Part 62. Synthesis and antimalarial activity of a series of 2,4-diamino-6-[(N-alkylanilino)methyl]quinazolines. *J. Heterocycl. Chem.* **1987**, *24*, 345-349; **19e** was synthesized following Musso, H.; Schroeder, H. Mechanism of the oxygen transfer in the hydrogenation of 2-nitrobenzotrile to 2-aminobenzamide. *Chem. Ber.* **1965**, *98*, 1562-1576. The intermediates **21f-j** were synthesized following **21f**: Klaubert, D.H.; Sellstedt, J.H.; Guinosso, C.J.; Capetola, R.J.; Stanley, C. N-(Aminophenyl)oxamic acids and esters as

potent, orally active antiallergy agents. *J. Med. Chem.* **1981**, *24*, 742-748; **21g**: Tabarrini, O.; Cecchetti, V.; Temperini, A.; Filipponi, E.; Lamperti, M. G.; Fravolini, A.. Velnacrine thiaanalogues as potential agents for treating Alzheimer's disease. *Bioorg. Med. Chem.* **2001**, *9*, 2921-2928; **21h** and **21j**: Bolli, M. H.; Marfurt, J.; Grisostomi, C.; Boss, C.; Binkert, C.; Hess, P.; Treiber, A.; Thorin, E.; Morrison, K.; Buchmann, S.; Bur, D.; Ramuz, H.; Clozel, M.; Fischli, W.; Weller, T.. Novel benzo[1,4]diazepin-2-one derivatives as endothelin receptor antagonists. *J. Med. Chem.* **2004**, *47*, 2776-2795; **21i**: Goto, K.; Nakamura, S.; Morioka, Y.; Kondo, M.; Naito, S.; Tsutsumi, K.. Synthesis and biological activity of the metabolites of diethyl 4-[(4-bromo-2-cyanophenyl)carbamoyl]benzylphosphonate (NO-1886). *Chem. Pharm. Bull.* **1996**, *44*, 547-551.

¹H NMR spectra were recorded on a Bruker AC 200 spectrometer. Chemical shifts (δ) are given in ppm upfield from tetramethylsilane as internal standard, and the spectra were recorded in appropriate deuterated solvents, as indicated. Melting points (mp) were determined on a Buchi-Tottoli apparatus and are uncorrected. All products reported showed ¹H NMR spectra in agreement with the assigned structures. Elemental analyses were conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara. All reactions were carried out under an inert atmosphere of dry nitrogen, unless otherwise described. Standard syringe techniques were applied for transferring dry solvents. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F254 Merck plates) and visualized with aqueous KMnO₄. Flash chromatography was performed using 230-400 mesh silica gel and the indicated solvent system. Organic solutions were dried over anhydrous Na₂SO₄. Calcium chloride was used in the distillation of DMF, and the distilled solvent was stored over molecular sieves (3 Å).

(2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)-(3,4,5-trimethoxyphenyl)-methanone (9a). The crude residue was purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent and furnished the compound **9a** as a yellow oil (83% yield). ¹H-NMR (CDCl₃) δ: 1.52 (m, 2H), 1.72 (m, 2H), 1.95 (t, *J*=6.2 Hz, 2H), 2.53 (t, *J*=6.2 Hz, 2H), 3.86 (s, 3H), 3.88 (s, 6H), 6.54 (bs, 2H), 6.74 (s, 2H).

(2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)-(3,4-dimethoxyphenyl)-methanone (9b). The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent and furnished **9b** as a yellow solid (67% yield), mp 160-161 °C. ¹H-NMR (CDCl₃) δ: 1.52 (m, 2H), 1.72 (m, 2H), 1.95 (m, 2H), 2.53 (m, 2H), 3.92 (s, 3H), 3.94 (s, 3H), 6.30 (bs, 2H), 6.86 (d, *J*=9.0 Hz, 1H), 7.14 (d, *J*=2.0 Hz, 1H), 7.15 (dd, *J*=9.0 and 2.0 Hz, 1H).

(2-Amino-4,5,6,7-tetrahydro-benzo[b]thiophen-3-yl)-(4-methoxy-phenyl)-methanone (9c). The crude residue was purified by flash chromatography using ethyl acetate: petroleum ether 2:8 (v:v) as eluent and furnished the compound **9c** as a yellow solid (65% yield), mp 120-122 °C. ¹H-NMR (CDCl₃): δ 1.5 (m, 2H), 1.7 (m, 2H), 1.9 (t, *J*=6.2 Hz, 2H), 2.5 (t, *J*=6.4 Hz, 2H), 3.8 (s, 3H), 6.3 (bs, 2H), 6.8 (d, *J*=8.8 Hz, 2H), 7.5 (d, *J*=8.4 Hz, 2H).

(2-Amino-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)-(3,4,5-trimethoxyphenyl)-methanone (9d). The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent and furnished **9d** as a yellow solid (64% yield), mp 73-75 °C. ¹H-NMR (CDCl₃): δ 1.01 (d, *J*=6.6 Hz, 3H), 1.16 (m, 1H), 1.74 (m, 2H), 1.97 (m, 2H), 2.84 (m, 2H), 3.86 (s, 3H), 3.89 (s, 6H), 6.52 (bs, 2H), 6.74 (s, 2H).

(2-Amino-6-methoxy-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)-(3,4,5-trimethoxyphenyl)-methanone (9e). The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 4:6 (v:v) as eluent and furnished **9e** as a yellow solid (64% yield), mp 111-113 °C. ¹H-NMR (CDCl₃): δ 1.68 (m, 2H), 2.01 (m, 2H), 2.63 (m, 1H), 2.82 (m, 1H), 3.36 (s, 3H), 3.63 (m, 1H), 3.86 (s, 6H), 3.88 (s, 3H), 6.62 (bs, 2H), 6.74 (s, 2H).

N-[3-(3,4,5-trimethoxybenzoyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl]-acetamide (10a). The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 2:8 (v:v) as eluent and furnished **10a** as a yellow solid (56% yield), mp 178-180 °C. ¹H-NMR (CDCl₃): δ 1.55 (m, 2H), 1.78 (m, 2H), 2.07 (t, *J*=6.2 Hz, 2H), 2.24 (s, 3H), 2.68 (t, *J*=6.2 Hz, 2H), 3.87 (m, 3H), 3.92 (m, 6H), 6.81 (bs, 2H), 11.1 (bs, 1H).

N-[3-(3,4-dimethoxybenzoyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl]-acetamide (10b). The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 2:8 (v:v) as eluent and furnished **10b** as a yellow solid (53% yield), mp 134-136 °C. ¹H-NMR (CDCl₃): δ 1.55 (m, 2H), 1.78 (m, 2H), 2.07 (t, *J*=6.0 Hz, 2H), 2.24 (s, 3H), 2.68 (t, *J*=6.0 Hz, 2H), 3.87 (s, 3H), 3.92 (s, 3H), 6.85 (d, *J*=8.8 Hz, 1H), 7.19 (d, *J*=1.6 Hz, 1H), 7.26 (dd, *J*=8.8 and 1.6 Hz, 1H), 11.1 (bs, 1H).

N-[3-(4-methoxybenzoyl)-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl]-acetamide (10c). The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 1:9 (v:v) as eluent and furnished **10c** as a yellow solid (65% yield), mp 113-115 °C. ¹H-NMR (CDCl₃): δ 1.6 (m, 2H), 1.8 (m, 2H), 2.1 (t, *J*=7.2 Hz, 2H), 2.3 (s, 3H), 2.8 (t, *J*=7.2 Hz, 2H), 3.8 (s, 3H), 6.8 (d, *J*=8.8 Hz, 2H), 7.8 (d, *J*=8.8 Hz, 2H), 11.1 (bs, 1H).

***N*-[6-methyl-3-(3,4,5-trimethoxybenzoyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl]-acetamide (10d).** The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 2:8 (v:v) as eluent and furnished **10d** as a yellow oil (58% yield). ¹H-NMR (CDCl₃): δ 1.06 (d, *J*=6.4 Hz, 3H), 1.17 (m, 1H), 1.68 (m, 2H), 2.29 (s, 3H), 2.34 (m, 2H), 2.88 (m, 2H), 3.86 (s, 3H), 3.91 (s, 6H), 7.00 (s, 2H), 11.3 (bs, 1H).

***N*-[6-methoxy-3-(3,4,5-trimethoxybenzoyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl]-acetamide (10e).** The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 4:6 (v:v) as eluent and furnished **10e** as a yellow oil (57% yield). ¹H-NMR (CDCl₃): δ 1.72 (m, 4H), 2.25 (s, 3H), 2.67 (m, 1H), 3.02 (m, 1H), 3.37 (s, 3H), 3.62 (s, 1H), 3.87 (s, 3H), 3.92 (s, 6H), 6.81 (s, 2H), 11.4 (bs, 1H).

***N*-[3-(3,4,5-trimethoxybenzoyl)-benzo[*b*]thiophen-2-yl]-acetamide (11a).** The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 2:8 (v:v) as eluent and furnished **11a** as a yellow solid (72% yield), mp 166-168 °C. ¹H-NMR (CDCl₃): δ 2.25 (s, 3H), 3.79 (m, 3H), 3.83 (m, 6H), 6.94 (s, 2H), 7.12 (t, *J*=8.4 Hz, 1H), 7.16 (t, *J*=8.8 Hz, 1H), 7.19 (d, *J*=8.4 Hz, 1H), 7.75 (t, *J*=8.8 Hz, 1H), 11.9 (bs, 1H).

***N*-[3-(3,4-dimethoxybenzoyl)-benzo[*b*]thiophen-2-yl]-acetamide (11b).** The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 2:8 (v:v) as eluent and furnished **11b** as a yellow solid (73% yield), mp 147-149 °C. ¹H-NMR (CDCl₃): δ 2.35 (s, 3H), 3.87 (s, 3H), 3.99 (s, 3H), 6.90 (s, *J*=8.4 Hz, 1H), 7.16-7.37 (m, 5H), 7.75 (t, *J*=8.8 Hz, 1H), 11.9 (bs, 1H).

***N*-[3-(4-methoxybenzoyl)-benzo[*b*]thiophen-2-yl]-acetamide (11c).** The crude residue purified by flash chromatography using ethyl acetate:petroleum ether 1:9 (v:v) as eluent and

furnished **11c** as a yellow solid (61% yield), mp 165-167 °C. ¹H-NMR (CDCl₃): δ 2.34 (s, 3H), 3.90 (s, 3H), 6.96 (t, 1H), 7.12 (m, 1H), 7.13 (t, 1H), 7.15 (m, 1H), 7.70 (d, *J*=8.8 Hz, 2H), 7.75 (d, *J*=8 Hz, 2H), 11.8 (bs, 1H).

***N*-[6-methyl-3-(3,4,5-trimethoxybenzoyl)-benzo[*b*]thiophen-2-yl]-acetamide (11d)**. The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 2:8 (v:v) as eluent and furnished **11d** as a yellow solid (62% yield), mp 81-83 °C. ¹H-NMR (CDCl₃): δ 2.34 (s, 3H), 3.90 (s, 3H), 6.96 (t, 1H), 7.12 (m, 1H), 7.13 (t, 1H), 7.15 (m, 1H), 7.70 (d, *J*=8.8 Hz, 2H), 7.75 (d, *J*=8.8 Hz, 2H), 11.8 (bs, 1H).

***N*-[6-methoxy-3-(3,4,5-trimethoxybenzoyl)-benzo[*b*]thiophen-2-yl]-acetamide (11e)**. The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent and furnished **11e** as a yellow solid (51% yield), mp 142-144 °C. ¹H-NMR (CDCl₃): δ 2.34 (s, 3H), 3.79 (m, 6H), 3.84 (m, 3H), 3.95 (s, 3H), 6.80 (d, *J*=9.2 Hz, 1H), 6.92(s, 2H), 6.99 (d, *J*=9.2 Hz, 1H), 7.22 (s, 1H), 11.8 (bs, 1H).

(2-Amino-benzo[*b*]thiophen-3-yl)-(3,4,5-trimethoxyphenyl)-methanone (6a). The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent and furnished **6a** as a yellow solid (63% yield), mp 110-112 °C. ¹H-NMR (CDCl₃): δ 3.80 (s, 3H), 3.86 (s, 6H), 6.84 (s, 2H), 6.86 (bs, 2H), 7.01 (t, *J*=8.4 Hz, 1H), 7.04 (t, *J*=8.8 Hz, 1H), 7.06 (d, *J*=8.4 Hz, 1H), 7.47 (t, *J*=8.8 Hz, 1H). Anal. (C₁₈H₁₇NO₄S): C, H, N.

(2-Amino-benzo[*b*]thiophen-3-yl)-(3,4-dimethoxyphenyl)-methanone (6b). The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 2:8 (v:v) as eluent and furnished **6b** as a yellow solid (89% yield), mp 121-123 °C. ¹H-NMR (CDCl₃): δ

3.86 (s, 3H), 3.97 (s, 3H), 6.90 (m, 1H), 7.12 (m, 1H), 7.16 (t, $J=8.8$ Hz, 1H), 7.19 (d, $J=8.4$ Hz, 1H), 7.26 (m, 2H), 7.75 (t, $J=8.8$ Hz, 1H), 6.53 (bs, 2H). Anal. ($C_{17}H_{15}NO_3S$): C, H, N.

(2-Amino-benzo[b]thiophen-3-yl)-(4-methoxyphenyl)-methanone (6c). The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent and furnished **6c** as a yellow solid (56% yield), mp 177-179 °C. 1H -NMR ($CDCl_3$): δ 3.88 (s, 3H), 6.89 (d, $J=7.6$ Hz, 1H), 6.93 (m, 2H), 6.96 (bs, 2H), 7.0 (m, 2H), 7.5 (d, $J=2$ Hz, 1H), 7.6 (d, $J=8$ Hz, 2H). Anal. ($C_{16}H_{13}NO_2S$): C, H, N.

(2-Amino-6-methylbenzo[b]thiophen-3-yl)-(3,4,5-trimethoxyphenyl)-methanone (6d). The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent and furnished **6d** as a yellow solid (56% yield), mp 150-152 °C. 1H -NMR ($CDCl_3$): δ 2.33 (s, 3H), 3.81 (s, 6H), 3.93 (s, 3H), 6.75 (d, $J=8.8$ Hz, 1H), 6.83 (d, $J=8.8$ Hz, 1H), 6.88 (s, 2H), 7.21 (bs, 2H), 7.33 (s, 1H). Anal. ($C_{19}H_{19}NO_4S$): C, H, N.

(2-Amino-6-methoxybenzo[b]thiophen-3-yl)-(3,4,5-trimethoxyphenyl)-methanone (6e). The crude residue was purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent and furnished **6e** as a yellow solid (57% yield), mp 50-52 °C. 1H -NMR ($CDCl_3$): δ 3.78 (s, 3H), 3.80 (s, 6H), 3.92 (s, 3H), 6.62 (d, $J=9$ Hz, 1H), 6.74 (d, $J=9.0$ Hz, 1H), 6.85 (s, 2H), 7.02 (s, 1H), 7.20 (bs, 2H). Anal. ($C_{19}H_{19}NO_5S$): C, H, N.

General procedure E for synthesis of 2-benzylthioarylnitriles (20a-e). To a cold solution (ice bath) containing o-nitroarylnitriles **19a-e** (20 mmol) and benzylmercaptan (2.48 g., 20 mmol) in 20 mL of DMF was added dropwise a solution of KOH (2 g., 36 mmol) in 5 mL of water. The mixture was stirred at 4 °C for 2 h and then poured into ice water. The solid was

collected, washed with cold water (50 mL), dried in vacuo over P_2O_5 and recrystallised from petroleum ether.

2-Benzylsulfanyl-6-methylbenzonitrile (20b). Following general procedure E, compound **20b** was obtained as a yellow solid, yield 70%; mp 93-95 °C. 1H -NMR ($CDCl_3$) δ : 2.51 (s, 3H), 4.19 (s, 2H), 7.10 (d, $J=8.0$ Hz, 1H), 7.14 (d, $J=8.0$ Hz, 1H), 7.28 (m, 6H).

2-Benzylsulfanyl-5-methylbenzonitrile (20c). Following general procedure E, compound **20c** was obtained as a yellow solid, yield 57%; mp 133-135 °C. 1H -NMR ($CDCl_3$) δ : 2.32 (s, 3H), 4.16 (s, 2H), 7.24 (m, 7H), 7.42 (s, 1H).

2-Benzylsulfanyl-4-methylbenzonitrile (20d). Following general procedure E, compound **20d** was obtained as a yellow solid, yield 72%; mp 88-90 °C. 1H -NMR ($CDCl_3$) δ : 2.33 (s, 3H), 4.20 (s, 2H), 7.05 (d, $J=7.6$ Hz, 1H), 7.16 (s, 1H), 7.28 (m, 5H), 7.49 (d, $J=8.0$ Hz, 1H).

2-Benzylsulfanyl-3-methylbenzonitrile (20e). Following general procedure E, compound **20e** was obtained as a brown solid, yield 89%; mp 101-103°C. 1H -NMR ($CDCl_3$) δ : 2.28 (s, 3H), 4.07 (s, 2H), 7.10 (m, 2H), 7.20 (m, 4H), 7.35 (t, $J=8.0$ Hz, 1H), 7.49 (d, $J=8.0$ Hz, 1H).

General procedure F for synthesis of *O*-ethyl esters of *S*-(2-cyanoaryl) dithiocarbonic acids (22f-j). A solution of $NaNO_2$ (0.604 g., 8.75 mmol) in water (2 mL) was added dropwise over 5 min, with stirring, to a cold (0 °C) solution of compound **21f-j** (8 mmol) in MeOH (4 mL), water (2.4 mL), AcOH (0.8 mL) and 37% aqueous HCl. The resulting mixture was stirred at 0 °C for an additional 10 min, at which time the cold solution was added to a solution of potassium ethyl xanthate (2.45 g., 15.3 mmol) in water (7 mL) maintained at 65 °C. After the mixture was stirred for 15 min, the reaction was cooled to 25 °C. The resulting mixture was extract with EtOAc (3 x 15 mL), and the combined organic extracted were

washed with brine, dried and concentrated *in vacuo*. Purification by silica gel flash column chromatography (EtOAc-petroleum ether) afforded the products **22f-j**.

O-Ethyl ester of 2-cyano-3-methoxyphenyl dithiocarbonic acid (22f). Following general procedure F, compound **22f** was purified by flash column chromatography (elution with 20% EtOAc in petroleum ether). Yellow oil, yield 63%. ¹H-NMR (CDCl₃) δ: 1.36 (t, *J*=7.2 Hz, 3H), 3.90 (s, 3H), 4.64 (t, *J*=7.2 Hz, 2H), 7.12 (m, 1H), 7.36 (d, *J*=8.2 Hz, 1H), 7.52 (d, *J*=8.2 Hz, 1H).

O-Ethyl ester of 2-cyano-4-methoxyphenyl dithiocarbonic acid (22g). Following general procedure F, compound **22g** was purified by flash column chromatography (elution with 20% EtOAc in petroleum ether). Yellow oil, yield 56%. ¹H-NMR (CDCl₃) δ: 1.37 (t, *J*=7.4 Hz, 3H), 3.89 (s, 3H), 4.62 (t, *J*=7.4 Hz, 2H), 7.16 (d, *J*=8.2 Hz, 1H), 7.29 (s, 1H), 7.53 (d, *J*=8.2 Hz, 1H).

O-Ethyl ester of 2-cyano-5-methoxyphenyl dithiocarbonic acid (22h). Following general procedure F, compound **22h** was purified by flash column chromatography (elution with 15% EtOAc in petroleum ether). Yellow oil, yield 53%. ¹H-NMR (CDCl₃) δ: 1.36 (t, *J*=7.2 Hz, 3H), 3.88 (s, 3H), 4.65 (t, *J*=7.2 Hz, 2H), 7.06 (d, *J*=8.6 Hz, 1H), 7.16 (s, 1H), 7.71 (d, *J*=8.6 Hz, 1H).

O-Ethyl ester of 2-cyano-6-methoxyphenyl dithiocarbonic acid (22i). Following general procedure F, compound **22i** was purified by flash column chromatography (elution with 20% EtOAc in petroleum ether). Yellow oil, yield 56%. ¹H-NMR (CDCl₃) δ: 1.33 (t, *J*=7.2 Hz, 3H), 3.92 (s, 3H), 4.67 (t, *J*=7.2 Hz, 2H), 7.24 (m, 1H), 7.35 (d, *J*=8.4 Hz, 1H), 7.54 (d, *J*=8.4 Hz, 1H).

O-Ethyl ester of 2-cyano-4,5-dimethoxyphenyl dithiocarbonic acid (22j). Following general procedure F, compound **22j** was obtained as an orange oil, yield 56%. ¹H-NMR (CDCl₃) δ: 1.37 (t, *J*=7.2 Hz, 3H), 3.90 (s, 3H), 3.94 (s, 3H), 4.64 (t, *J*=7.2 Hz, 2H), 7.06 (s, 1H), 7.19 (s, 1H).

General procedure G for synthesis of 2-mercaptoarylnitriles (23a-e). A solution of *S*-benzylarylnitriles **20a-e** (20 mmol) in dry benzene (40 mL) was added dropwise over 30 min to a suspension of finely powdered anhydrous aluminium chloride (4.5 g., 33 mmol) in dry benzene (40 mL), and the mixture was stirred under nitrogen at room temperature for 48 h. The green-brown mixture was decomposed by the cautious addition of ice-water (100 mL), and the organic layer was separated and washed successively with water (50 mL) and 5% aqueous sodium hydroxide (2 x 50 mL). The alkaline extracts were acidified and extracted with dichloromethane (3 x 50 mL). The organic extracts were washed with brine, dried and evaporated to yield the final products **23a-e**.

2-Mercapto-6-methylbenzonitrile (23b). Following general procedure G, compound **23b** was obtained as a yellow solid, yield 65%; mp 77-79 °C. ¹H-NMR (DMSO-*d*₆) δ: 2.52 (s, 3H), 4.06 (s, 1H), 7.04 (d, *J*=7.2 Hz, 1H), 7.22 (d, *J*=7.2 Hz, 1H), 7.30 (m, 1H).

2-Mercapto-5-methylbenzonitrile (23c). Following general procedure G, compound **23c** was obtained as a yellow solid, yield 67%; mp 45-47 °C. ¹H-NMR (DMSO-*d*₆) δ: 2.32 (s, 3H), 3.97 (s, 1H), 7.23 (d, *J*=8.4 Hz, 1H), 7.33 (d, *J*=8.4 Hz, 1H), 7.41 (s, 1H).

2-Mercapto-4-methylbenzonitrile (23d). Following general procedure G, compound **23d** was obtained as a yellow solid, yield 68%; mp 38-40 °C. ¹H-NMR (DMSO-*d*₆) δ: 2.36 (s, 3H), 4.02 (s, 1H), 7.00 (d, *J*=8.8 Hz, 1H), 7.22 (s, 1H), 7.49 (d, *J*=8.0 Hz, 1H).

2-Mercapto-3-methylbenzotrile (23e). Following general procedure G, compound **23e** was obtained as a yellow solid, yield 74%; mp 50-51 °C. ¹H-NMR (CDCl₃) δ: 2.36 (s, 3H), 4.19 (s, 1H), 7.15 (t, *J*=7.8 Hz, 1H), 7.32 (d, *J*=7.8 Hz, 1H), 7.49 (d, *J*=7.8 Hz, 1H).

General procedure H for the synthesis of 2-mercaptoarylnitriles (23f-j). Xanthate **22f-j** (3 mmol) was dissolved in EtOH (10 mL). A 3 N aqueous solution of NaOH (12 mL) was added, and the reaction mixture was heated to 65 °C for 2 h. The reaction mixture was cooled to 25 °C and acidified to pH 5 by the addition of 10% aqueous HCl. The resulting mixture was extracted with EtOAc (3 x 15 mL), and the combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated *in vacuo* to furnish the corresponding 2-mercaptoarylnitrile **23f-j**, which was used without further purification for the next reaction.

2-Mercapto-6-methoxybenzotrile (23f). Following general procedure H, compound **23f** was obtained as a yellow solid, yield 64%; m.p. 72-74 °C. ¹H NMR (CDCl₃) δ: 3.88 (s, 3H), 4.20 (s, 1H), 7.21 (d, *J*=8.4 Hz, 1H), 7.36 (d, *J*=8.4 Hz, 1H), 7.62 (d, *J*=8.4 Hz, 1H).

2-Mercapto-5-methoxybenzotrile (23g). Following general procedure H, compound **23g** was obtained as a yellow oil, yield 63%. ¹H-NMR (DMSO-*d*₆) δ: 3.86 (s, 3H), 4.02 (s, 1H), 7.34 (d, *J*=8.6 Hz, 1H), 7.42 (d, *J*=8.6 Hz, 1H), 7.52 (s, 1H).

2-Mercapto-4-methoxybenzotrile (23h). Following general procedure H, compound **23h** was obtained as a yellow oil, yield 72%. ¹H-NMR (DMSO-*d*₆) δ: 3.84 (s, 3H), 4.04 (s, 1H), 7.04 (d, *J*=9.0 Hz, 1H), 7.42 (s, 1H), 7.56 (d, *J*=9.0 Hz, 1H).

2-Mercapto-3-methoxybenzotrile (23i). Following general procedure H, compound **23i** was obtained as a yellow solid, yield 57%; mp 44-46 °C. ¹H-NMR (CDCl₃) δ: 3.90 (s, 3H), 4.22 (s, 1H), 7.17 (d, *J*=8.6 Hz, 1H), 7.30 (d, *J*=8.6 Hz, 1H), 7.49 (d, *J*=8.6 Hz, 1H).

2-Mercapto-4,5-dimethoxybenzotrile (23j). Following general procedure H, compound **23j** was obtained as a yellow solid, yield 68%, mp 53-55 °C. ¹H-NMR (DMSO-*d*₆) δ: 3.88 (s, 3H), 3.93 (s, 3H), 4.02 (s, 1H), 7.10 (s, 1H), 7.22 (s, 1H).

General procedure I for the synthesis of 2-(3',4',5'-trimethoxybenzoyl)-3-amino benzo[b]thiophenes (7a-j). To a solution of **23a-j** (1 mmol) in dry acetone (15 mL) was added 2-bromo-1-(3,4,5-trimethoxyphenyl)-ethanone (289 mg, 1 mmol) and anhydrous potassium carbonate (276 mg, 2 mmol) while stirring, and the reaction mixture was refluxed for 18 h. After cooling, the solvent was evaporated, and the residue was dissolved in a mixture of dichloromethane (15 mL) and water (5 mL). The organic layer was washed with brine, dried and evaporated to obtain a residue, which was purified by flash column chromatography. The final product was precipitated from petroleum ether.

2-(3,4,5-Trimethoxybenzoyl)-3-aminobenzo[b]thiophene (7a). The crude residue purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent furnished **7a** as a yellow solid (93% yield); mp 148-150 °C. ¹H-NMR (CDCl₃) δ: 3.93 (s, 3H), 3.94 (s, 6H), 7.02 (bs, 2H), 7.21 (s, 2H), 7.42 (d, *J*=7.6 Hz, 1H), 7.51 (d, *J*=7.2 Hz, 1H), 7.76 (dd, *J*=7.2 and 7.6 Hz, 2H). Anal. (C₁₈H₁₇NO₄S): C, H, N.

2-(3,4,5-Trimethoxybenzoyl)-3-amino-4-methylbenzo[b]thiophene (7b). The crude residue purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent furnished **7b** as a yellow solid (90% yield); mp 197-199 °C. ¹H-NMR (CDCl₃) δ: 2.87 (s, 3H), 3.92 (s, 3H), 3.93 (s, 6H), 6.88 (bs, 2H), 7.10 (d, *J*=8.0 Hz, 1H), 7.16 (s, 2H), 7.34 (t, *J*=7.0 Hz, 1H), 7.53 (d, *J*=8.0 Hz, 1H). Anal. (C₁₉H₁₉NO₄S): C, H, N.

2-(3,4,5-Trimethoxybenzoyl)-3-amino-5-methylbenzo[b]thiophene (7c). The crude residue purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent furnished **7c** as a yellow solid (54% yield); mp 148-150 °C. ¹H-NMR (CDCl₃) δ: 2.44 (s, 3H), 3.89 (s, 3H), 3.91 (s, 6H), 7.07 (bs, 2H), 7.19 (s, 2H), 7.16 (d, *J*=8.2 Hz, 1H), 7.51 (s, 1H), 7.59 (d, *J*=8.2 Hz, 1H). Anal. (C₁₉H₁₉NO₄S): C, H, N.

2-(3,4,5-Trimethoxybenzoyl)-3-amino-6-methylbenzo[b]thiophene (7d). The crude residue purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent furnished **7d** as a yellow solid (78% yield); mp 128-130 °C. ¹H-NMR (CDCl₃) δ: 2.49 (s, 3H), 3.93 (s, 3H), 3.94 (s, 6H), 7.01 (bs, 2H), 7.20 (s, 2H), 7.22 (d, *J*=8.0 Hz, 1H), 7.51 (s, 1H), 7.62 (d, *J*=8.0 Hz, 1H). Anal. (C₁₉H₁₉NO₄S): C, H, N.

2-(3,4,5-Trimethoxybenzoyl)-3-amino-7-methylbenzo[b]thiophene (7e). The crude residue purified by flash chromatography using ethyl acetate:petroleum ether 3:7 (v:v) as eluent furnished **7e** as a yellow solid (88% yield); mp 166-167 °C. ¹H-NMR (CDCl₃) δ: 2.48 (s, 3H), 3.92 (s, 3H), 3.94 (s, 6H), 7.02 (bs, 2H), 7.21 (s, 2H), 7.32 (d, *J*=7.8 Hz, 1H), 7.36 (t, *J*=7.8 Hz, 1H), 7.64 (d, *J*=7.8 Hz, 1H). Anal. (C₁₉H₁₉NO₄S): C, H, N.

2-(3,4,5-Trimethoxybenzoyl)-3-amino-4-methoxybenzo[b]thiophene (7f). The crude residue purified by flash chromatography using ethyl acetate:petroleum ether 4:6 (v:v) as eluent furnished **7f** as a yellow solid (58% yield); mp 163-165 °C. ¹H-NMR (CDCl₃) δ: 3.88 (s, 3H), 3.94 (s, 6H), 3.98 (s, 3H), 7.02 (m, 3H), 7.220 (s, 2H), 7.40 (m, 2H). Anal. (C₁₉H₁₉NO₅S): C, H, N.

2-(3,4,5-Trimethoxybenzoyl)-3-amino-5-methoxybenzo[b]thiophene (7g). The crude residue purified by flash chromatography using ethyl acetate:petroleum ether 4:6 (v:v) as

eluent furnished **7g** as a yellow solid (54% yield); mp 142-144 °C. ¹H-NMR (CDCl₃) δ: 3.91 (s, 3H), 3.92 (s, 6H), 3.94 (s, 3H), 6.88 (bs, 2H), 7.11 (d, *J*=8.8 Hz, 1H), 7.20 (s, 2H), 7.26 (s, 1H), 7.59 (d, *J*=8.8 Hz, 1H). Anal. (C₁₉H₁₉NO₅S): C, H, N.

2-(3,4,5-Trimethoxybenzoyl)-3-amino-6-methoxybenzo[b]thiophene (7h). The crude residue purified by flash chromatography using ethyl acetate:petroleum ether 4:6 (v:v) as eluent furnished **7h** as a yellow solid (64% yield); mp 82-84 °C. ¹H-NMR (CDCl₃) δ: 3.86 (s, 3H), 3.88 (s, 3H), 3.91 (s, 6H), 6.98 (d, *J*=9.0 Hz, 1H), 7.07 (bs, 2H), 7.10 (s, 1H), 7.17 (s, 2H), 7.61 (d, *J*=9.0 Hz, 1H). Anal. (C₁₉H₁₉NO₅S): C, H, N.

2-(3,4,5-Trimethoxybenzoyl)-3-amino-7-methoxybenzo[b]thiophene (7i). The crude residue purified by flash chromatography using ethyl acetate:petroleum ether 4:6 (v:v) as eluent furnished **7i** as a yellow solid (63% yield); mp 152-154 °C. ¹H-NMR (CDCl₃) δ: 3.92 (s, 3H), 3.93 (s, 6H), 3.98 (s, 3H), 7.00 (m, 3H), 7.20 (s, 2H), 7.37 (m, 2H). Anal. (C₁₉H₁₉NO₅S): C, H, N.

2-(3,4,5-Trimethoxybenzoyl)-3-amino-5,6-dimethoxybenzo[b]thiophene (7j). The crude residue purified by flash chromatography using ethyl acetate:petroleum ether 6:4 (v:v) as eluent furnished **7j** as a yellow solid (61% yield); mp 194-196 °C. ¹H-NMR (CDCl₃) δ: 3.92 (s, 3H), 3.93 (s, 6H), 3.97 (s, 3H), 3.98 (s, 3H), 6.96 (bs, 2H), 7.04 (s, 1H), 7.10 (s, 1H), 7.19 (s, 2H). Anal. (C₂₀H₂₁NO₆S): C, H, N.

Synthesis of 2-(3,4,5-trimethoxybenzoyl)-3-dimethylamino-6-methylbenzo[b]thiophene (7k). Sodium hydride (60% oil dispersion, 48 mg, 1 mmol) was carefully added to an ice-cooled solution of CH₃I (62 μL, 1 mmol) and **7d** (100 mg, 0.28 mmol) in 1 mL of DMF. The reaction vessel was sealed, and the mixture was stirred at 40 °C for 72 h, after which time it

was diluted with cold water (1 mL) and extracted with CH₂Cl₂ (3 x 5 mL). The combined organic extracts were washed with water (2 mL) and brine, dried and concentrated *in vacuo*. The resulting residue, purified by flash chromatography using ethyl acetate:petroleum ether 2:8 (v:v), provided **7k** as an orange powder (88% yield), mp 107-108 °C. ¹H-NMR (CDCl₃) δ: 2.47 (s, 3H), 3.04 (s, 6H), 3.89 (s, 6H), 3.93 (s, 3H), 7.19 (s, 2H), 7.22 (d, *J*=8.4 Hz, 1H), 7.51 (s, 1H), 7.87 (d, *J*=8.4 Hz, 1H). Anal. (C₂₁H₂₃NO₄S): C, H, N.

Synthesis of 2-(3,4,5-trimethoxybenzoyl)-3-diacetylamino-6-methylbenzo[b]thiophene (7l). A solution of **7d** (1 mmol) in 3 mL of pyridine and 2.5 mL of Ac₂O was heated under reflux for 1 h. Excess reagents were removed *in vacuo*. The residue was triturated with water (3 mL) and extracted with dichloromethane (3 x 5 mL). The combined organic extracts were washed with brine (5 mL), dried, filtered and evaporated. Flash chromatography on silica gel, eluting with 30% EtOAc in petroleum ether, gave **7l** as a yellow powder, yield 84%, mp 151-153 °C. ¹H-NMR (CDCl₃) δ: 2.30 (s, 6H), 2.53 (s, 3H), 3.89 (s, 6H), 3.94 (s, 3H), 7.19 (s, 2H), 7.32 (d, *J*=8.0 Hz, 1H), 7.52 (d, *J*=8.0 Hz, 1H), 7.69 (s, 1H). Anal. (C₂₃H₂₃NO₆S): C, H, N.

Cell growth inhibitory activity. Murine leukemia L1210, murine mammary carcinoma FM3A and human T-lymphocyte Molt 4 and CEM cells were suspended at 300,000-500,000 cells/mL of culture medium, and 100 μL of a cell suspension was added to 100 μL of an appropriate dilution of the test compounds in wells of 96-well microtiter plates. After incubation at 37 °C for two (L1210 and FM3A) or three (Molt 4 and CEM) days, cell number was determined using a Coulter counter. The IC₅₀ was defined as the compound concentration required to inhibit cell proliferation by 50%.

Effects on tubulin polymerization and on colchicine binding to tubulin. Bovine brain tubulin was purified as described previously.⁴ To evaluate the effect of the compounds on tubulin assembly *in vitro*,⁵ varying concentrations were preincubated with 10 μ M tubulin in glutamate buffer at 30 °C and then cooled to 0 °C. After addition of GTP, the mixtures were transferred to 0 °C cuvettes in a recording spectrophotometer and warmed to 30 °C, and the assembly of tubulin was observed turbidimetrically. The IC₅₀ was defined as the compound concentration that inhibited the extent of assembly by 50% after a 20 min incubation. The ability of the test compounds to inhibit colchicine binding to tubulin was measured as described,⁶ except that the reaction mixtures contained 1 μ M tubulin, 5 μ M [³H]colchicine and 1 μ M test compound.

Flow cytometric analysis of cell cycle distribution. The effects of the most active compounds of the series on cell cycle distribution were studied on K562 cells (myeloblastic leukemia) by flow cytometric analysis after staining with propidium iodide. Cells were exposed 24 h to each compound used at a concentration corresponding to the IC₅₀ determined after a 24 h incubation. After treatment, the cells were washed once in ice-cold PBS and resuspended at 1×10^6 per mL in a hypotonic fluorochrome solution containing propidium iodide (Sigma) at 50 μ g/mL in 0.1% sodium citrate plus 0.03% (v/v) nonidet P-40 (Sigma). After a 30 min incubation, the fluorescence of each sample was analyzed as single-parameter frequency histograms by using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). The distribution of cells in the cell cycle was analyzed with the ModFit LT3 program (Verity Software House, Inc.).

Molecular Modeling. All molecular modeling studies were performed on a RM Innovator with Pentium IV 3 GHz processor, running Linux Fedora Core 4 using Molecular Operating

Environment (MOE) 2006.08.² The tubulin structure was downloaded from the PDB data bank (<http://www.rcsb.org/> - PDB code: 1SA0).¹ Hydrogen atoms were added to the protein with MOE and minimized keeping all the heavy atoms fixed until a RMSD gradient of 0.05 kcal mol⁻¹ Å⁻¹ was reached. Ligand structures were built with MOE and minimized using the MMFF94x forcefield until a RMSD gradient of 0.05 kcal mol⁻¹ Å⁻¹ was reached. The docking simulations were performed using the Alpha Triangle placement method and the London dG scoring method. 300 results for each ligand were generated, and the position of the trimethoxyphenyl moiety was compared with ring A of the colchicine analogue co-crystallized with the protein, discarding the results with a RMSD value > 3Å.⁷ The best scored result of the remaining conformations for each ligand was then further analyzed. The protein/ligand complexes were minimized using the MMFF94x force field, until a RMSD gradient of 0.1 kcal mol⁻¹ Å⁻¹ was reached.

References

- (1). 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.
- (2). Molecular Operating Environment (MOE 2006.08). Chemical Computing Group, Inc. Montreal, Quebec, Canada. <http://www.chemcomp.com>.
- (3). 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. Arythioindoles, potent inhibitors of tubulin polymerization. 2. Structure activity relationships and molecular modeling studies. *J. Med. Chem.* **2006**, *49*, 947-954.

- (4) Hamel, E.; Lin, C.M. Separation of active tubulin and microtubule-associated proteins by ultracentrifugation and isolation of a component causing the formation of microtubule bundles. *Biochemistry* **1984**, *23*, 4173-4184.
- (5) Hamel, E. Evaluation of antimetabolic agents by quantitative comparisons of their effects on the polymerization of purified tubulin. *Cell Biochem. Biophys.* **2003**, *38*, 1-21.
- (6) 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 antimetabolic agent, with tubulin and effects of analogs on the growth of MCF-7 breast cancer cells. *Mol. Pharmacol.* **1998**, *53*, 62-67.
- (7). Code "fragment_rmsd.svl" obtained from SLV Exchange website <http://svl.chemcomp.com.>, Chemical Computing Group, Inc. Montreal, Canada

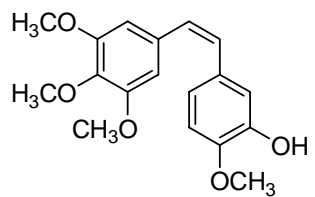
SUPPLEMENTARY

Elemental analyses of derivatives **6a-e** and **7a-l**.

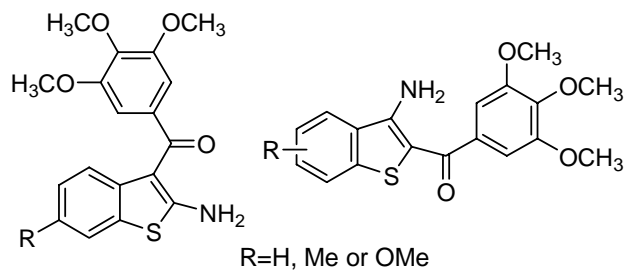
compd	formula	MW	Calcd			Found		
			% C	% H	% N	% C	% H	% N
6a	C ₁₈ H ₁₇ NO ₄ S	343.40	62.96	4.99	4.08	62.78	4.74	3.97
6b	C ₁₇ H ₁₅ NO ₃ S	313.37	65.16	4.82	4.47	65.03	4.67	4.25

6c	$C_{16}H_{13}NO_2S$	283.34	67.82	6.62	4.94	67.58	6.48	4.76
6d	$C_{19}H_{19}NO_4S$	357.42	63.85	5.36	3.92	63.77	5.27	3.78
6e	$C_{19}H_{19}NO_5S$	373.42	61.11	5.13	3.75	60.92	5.02	3.58
7a	$C_{18}H_{17}NO_4S$	343.40	62.96	4.99	4.08	62.88	4.78	3.99
7b	$C_{19}H_{19}NO_4S$	357.42	63.85	5.36	3.92	63.68	5.17	3.80
7c	$C_{19}H_{19}NO_4S$	357.42	63.85	5.36	3.92	63.70	5.22	3.68
7d	$C_{19}H_{19}NO_4S$	357.42	63.85	5.36	3.92	63.58	5.17	3.66
7e	$C_{19}H_{19}NO_4S$	357.42	63.85	5.36	3.92	63.61	5.19	3.70
7f	$C_{19}H_{19}NO_5S$	373.42	61.11	5.13	3.75	60.94	5.01	3.48
7g	$C_{19}H_{19}NO_5S$	373.42	61.11	5.13	3.75	61.01	4.89	3.61
7h	$C_{19}H_{19}NO_5S$	373.42	61.11	5.13	3.75	60.97	4.99	3.62
7i	$C_{19}H_{19}NO_5S$	373.42	61.11	5.13	3.75	60.92	4.86	3.55
7j	$C_{20}H_{21}NO_6S$	403.45	59.54	5.25	3.47	59.25	5.11	3.34
7k	$C_{21}H_{23}NO_4S$	385.48	65.43	6.01	3.63	65.22	5.85	3.51
7l	$C_{23}H_{23}NO_6S$	441.5	62.57	5.25	3.17	62.38	5.02	3.01

Table of Contents Graphic



Combretastatin A-4 (CA-4)



R=H, Me or OMe