Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2341–2343

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Synthesis and Tubulin Binding Activity of Thiophene-Based Analogues of Combretastatin A-4

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Received 21 March 2001; accepted 18 June 2001

Abstract—A number of analogues of combretastatin A-4 (**1**), containing a thiophene ring interposed between the two phenyl groups, have been prepared. The synthesis of these compounds employed a combination of palladium-mediated coupling and iodocyclization techniques. The thiophene compounds **11**, **14**, **18**, and **19** also represent non-benzofused analogues of some recently described tubulin binding benzo[*b*]thiophenes **3**–**5**. The most active thiophene compounds identified in this study were **11**, **14**, and **18**. Overall they are less active than **1** but exhibit comparable activity to the most active of the benzo[*b*]thiophenes **3**–**5**. A structure–activity relationship of these compounds is considered.

Compounds that bind to tubulin and prevent its polymerization into microtubules are effective anti-mitotic agents.[1] The naturally occurring *cis*-stilbene combretastatin A-4 (**1**) is particularly effective in this regard (Fig. 1).[2] It displays exceptional cytotoxicity towards a variety of cancer cell lines.[2]

Tubulin binding agents have also proven to be effective in targeting the tumor vasculature system.[3] The water soluble prodrug form of **1**, combretastatin A-4 disodium phosphate (**2**), is currently undergoing clinical trials as a tumor vascular targeting agent.

Recently, Pinney and co-workers described a new tubulin binding agent **3**, containing a benzo[*b*]thiophene core.[4] It was much less active than **1** and only reduced the rate but not the extent of tubulin assembly (Table 1).[4] It was postulated that this is due to the poor solubility of **3**. Pinney and co-workers also reported an X-ray crystal structure of **3**, which revealed a pseudo-π-stacking arrangement of the D and C rings.[4a,c] This suggested the possibility that the D and C rings in **3** may correspond to the two phenyl rings in the *cis*-stilbene **1**.

Using a novel approach to benzo[*b*]thiophenes, we prepared **3** and a number of analogues.[5] Two of these analogues, compounds **4** and **5**, exhibited greater activity than **3** (Table 1).[5] These compounds inhibited both the rate and extent of tubulin assembly but again were less active than **1**.

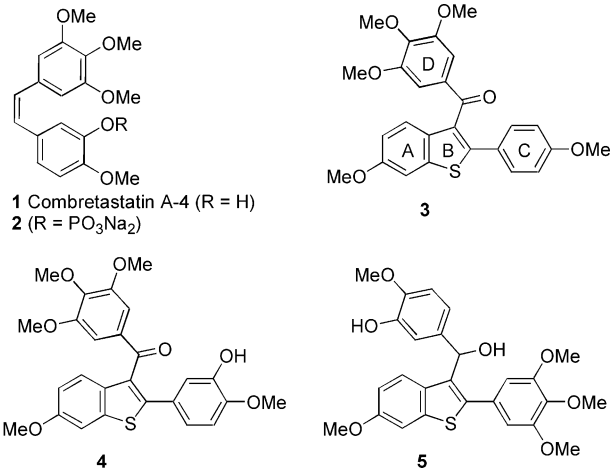

Figure 1. Tubulin binders.

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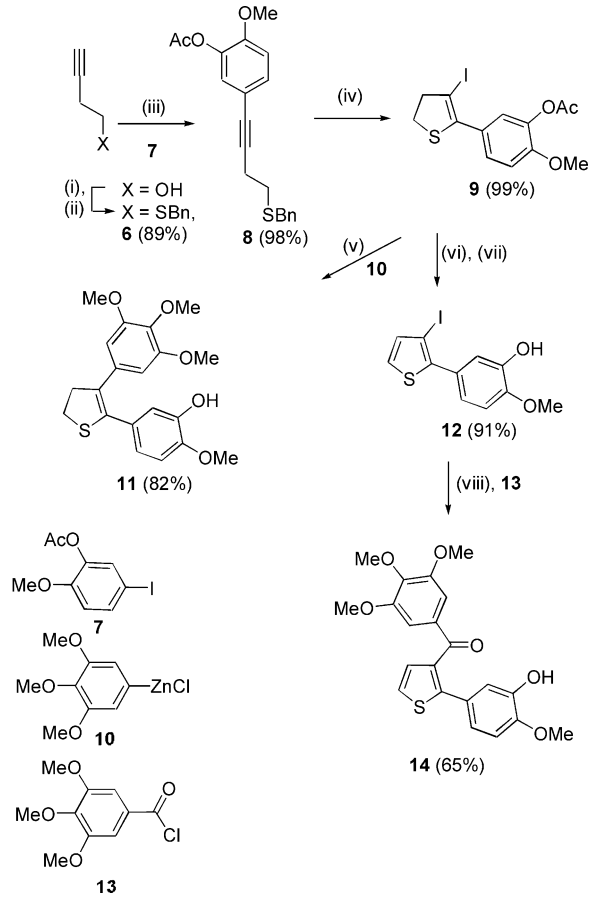
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00436-X

In order to further investigate the structural elements within **3**–**5** that are necessary for activity, we prepared the simple (non-benzofused) thiophene analogues **11**, **14**, **18**, and **19**. The synthetic approach used was similar to the palladium-mediated coupling/iodocyclization approach we developed for gaining access to benzo[*b*]thiophenes **3**–**5**.[5,6]

The synthesis of thiophenes **11** and **14** began with 3-butynol, which was easily converted to the benzyl 3-butynyl sulfide **6** (Scheme 1). Sonogashira coupling of **6** with aryliodide **7** afforded **8** in high yield. Treatment of **8** with iodine resulted in a rapid and efficient *5-endo*-dig-iodocyclization to give **9**.[6] Cross-coupling of vinyliodide **9** with arylzinc **10** and in situ hydrolysis of the acetate group produced **11**. Aromatization of **9** with DDQ and acetate hydrolysis afforded **12**. Treatment of **12** with 3 equivalents of *t*-BuLi, lithiated the phenol and the C-3 postion of the thiophene ring.[7] Reaction of this dilithio species with 3,4,5-trimethoxybenzoyl chloride **13** afforded **14** upon protic workup. All reactions proceeded in good yield, giving **11** and **14** in a 71 and 51% overall yield, respectively, from 3-butynol.

Preparation of **18** and **19** began with Sonogashira coupling of **6** and **15** followed by iodocyclization and DDQ oxidation to give **16**. Lithiation of **16** and reaction with benzaldehyde **17**, followed by in situ methanolysis of the acetate, gave the diol **18** in high yield. Oxidation of **18** to ketone **19** using DDQ also proceeded smoothly. Compounds **18** and **19** were obtained in a 63 and 62% overall yield, respectively, from 3-butynol (Scheme 2).

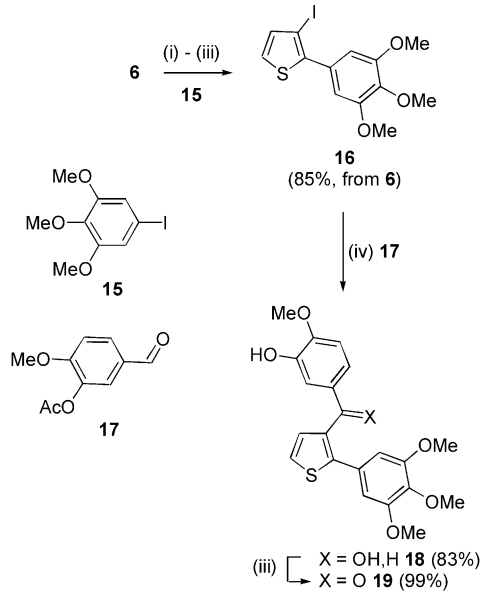


Scheme 1. Reagents and conditions: (i) KOH, TosCl, CH_2Cl_2; (ii) NaH, BnSH, THF, 18 °C; (iii) **7**, $Pd(PPh_3)_2Cl_2$ 2.0 mol%, CuI 4.0 mol%, DMF/Et_3N 3:1, 18 °C; (iv) I_2, CH_2Cl_2; (v) **10** (from 3,4,5-trimethoxyiodobenzene, 2 equiv *t*-BuLi, 1 equiv $ZnCl_2$), $Pd(PPh_3)_2Cl_2$ 5.0 mol%, THF, 18 °C 4 h followed by MeOH, K_2CO_3; (vi) DDQ, CH_2Cl_2 (vii) MeOH, K_2CO_3; (viii) 3 equiv *t*-BuLi, −78 °C then **13**.

Compounds **11**, **14**, **18**, and **19** were first evaluated for inhibition of tubulin assembly (Table 1). Those that displayed an inhibitory effect were also examined for an inhibitory effect on the binding of [^{3}H]colchicine to tubulin and for cytotoxicity against MCF-7 human breast carcinoma cells (Table 1).

Compound **19** did not inhibit tubulin assembly at concentrations as high as 40 μM and was not further examined. Compounds **11**, **14**, and **18** all inhibited tubulin assembly. Compound **14** showed greater potency than combretastatin A-4 **1** in this regard. In the competitive binding studies all compounds were less active than **1** at inhibiting the binding of [^{3}H]colchicine to tubulin. They were also much less cytotoxic towards MCF-7 human carcinoma cells as compared to combretastatin A-4. Interestingly, both **11** and **14** were significantly more potent inhibitors of [^{3}H]colchicine binding to tubulin than the benzo[*b*]thipohene compounds **3**–**5**.

In terms of the structure–activity relationships (SARs), the activity associated with compound **14** supports the notion that the activity of **3** and **4** results from the correspondence of their C and D rings, to the two phenyl rings in **1**. However, our previous studies have shown that the activity of **3** and **4** appears also to be highly dependent upon the presence of a 6-methoxy substituted A ring and a one-carbon linker between the B and D rings since **20** to **22** are inactive (Fig. 2).[5] Interestingly, these two features suggest a correspondence between the



Scheme 2. Reagents and conditions: (i) **15**, $Pd(PPh_3)_2Cl_2$ 2.0 mol%, CuI 4.0 mol%, DMF/Et_3N 3:1, 18 °C; (ii) I_2, CH_2Cl_2; (iii) DDQ, CH_2Cl_2; (iv) *n*-BuLi, −78 °C then **17** followed by MeOH, K_2CO_3.

Table 1. Effects of thiophenes and benzo[*b*]thiophenes on tubulin polymerization, colchicine binding and growth of MCF-7 human breast carcinoma cells[8]

Compound	Inhibition of tubulin polymerization[a] IC_{50} (μM)	Inhibition of colchicine binding (% inhibition)[b]		Inhibition of cell growth IC_{50} (nM)
		5 μM inhibitor	50 μM inhibitor	
1	2.1±0.1[c]	98±3	—	11±4
3	>40*[d,e]	—	28[e]	640±10
4	3.4±0.2	21±10	—	520±400
5[c]	6.1±0.8	5	73	—[f]
11	3.6±1.0	64±2	88	390±100
14	1.0±0.1	67±10	—	300±400
18	8.8±0.9	26±3	74	500±300
19	>40	—	—	—

[a]The tubulin concentration was 10 μM. Inhibition of extent of assembly was the parameter measured.
[b]The tubulin concentration was 1.0 μM and the [^{3}H]colchicine concentration was 5.0 μM.
[c]Data from ref 5.
[d]The asterisk indicates that the rate but not the extent of assembly was reduced by compound concentrations as high as 40 μM.
[e]Data from ref 4a.
[f]Compound **5** was not tested against the MCF-7 cell line but was tested against the Burkitt lymphoma CA46 cell line (IC_{50} > 1000 nM); see ref 5.

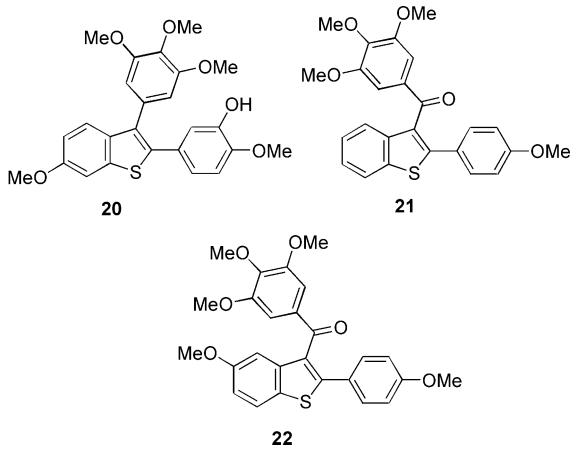


Figure 2.

A and D rings in **3** and **4** to the two phenyl rings in **1**. A more definitive understanding of the relationship between the A, C, and D rings in **3** and **4** and the phenyl rings in **1** is being pursued through the synthesis of additional analogues.

Acknowledgements

The authors thank the Australian Research Council for financial support including an Australian Research Fellowship to BLF. This work was supported in part by NCI, National Institutes of Health Contract N01-CO-56000.

References and Notes

1. Hamel, E. *Med. Res. Rev.* **1996**, *16*, 207.
2. (b) Sackett, D. L. *Pharmacol. Ther.* **1993**, *59*, 163. (a) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666. (b) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. *Biochemistry* **1989**, *28*, 6984. (c) Pettit, G. R.; Cragg, G. M.; Singh, S. B. *J. Nat. Prod.* **1987**, *60*, 1374. (d) Pettit, G. R.; Singh, S. B.; Cragg, G. M. *J. Org. Chem.* **1985**, *50*, 3404. (e) Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavanijaya, P. *Can. J. Chem.* **1982**, *60*, 1374.
3. (a) Chaplin, D. J.; Pettit, G. R.; Parkins, C. S.; Hill, S. A. *Br. J. Cancer* **1996**, *74*, S86. (b) Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. *Cancer Res.* **1997**, *57*, 1829. (c) Tozer, G. M.; Prise, V. E.; Wilson, J.; Locke, R. J.; Vojnovic, B.; Stratford, M. R. L.; Dennis, M. F.; Chaplin, D. J. *Cancer Res.* **1999**, *59*, 1626. (d) Iyer, S.; Chaplin, D. J.; Rosenthal, D. S.; Boulares, A. H.; Li, Lu-Y.; Smulson, M. E. *Cancer Res.* **1998**, *58*, 4510. (e) Grosios, K.; Holwell, S. E.; McGown, A. T.; Pettit, G. R.; Bibby, M. C. *Br. J. Cancer* **1999**, *81*, 1318. (f) Pettit, G. R.; Rhodes, M. R. *Anti-Cancer Drug Des.* **1998**, *13*, 183. (g) Pettit, G. R.; Rhodes, M. R.; Herald, D. L.; Chaplin, D. J.; Stratford, M. R. L.; Hamel, E.; Pettit, R. K.; Chapuis, J.-C.; Oliva, D. *Anti-Cancer Drug Des.* **1998**, *13*, 981.
4. (a) Pinney, K. G.; Bounds, A. D.; Dingeman, K. M.; Mocharla, V. P.; Pettit, G. R.; Bai, R.; Hamel, E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1081. (b) Pinney, K. G.; Pettit, G. R.; Mocharla, V. P.; Del Pilar Majia, M.; Shirali, A. PCT Int. Appl. WO 9839323, 1998. *Chem. Abstr.* **1998**, *129*, 245037. (c) Mullica, D. F.; Pinney, K. G.; Mocharla, V. P.; Dingeman, K. M.; Bounds, A. D.; Sappenfield, E. L. *J. Chem. Cryst.* **1998**, *28*, 289.
5. Flynn, B. L.; Verdier-Pinard, P.; Hamel, E. *Org. Lett.* **2001**, *3*, 651.
6. (a) For some other examples of iodocyclization involving alkynyl benzyl sulfides, see: Ren, X.-F.; Turos, E. *Tetrahedron Lett.* **1993**, *34*, 1575. (b) Ren, X.-F.; Turos, E.; Lake, C. H.; Churchill, M. R. *J. Org. Chem.* **1995**, *60*, 6468. (c) Ren, X.-F.; Konaklieva, M. I.; Shi, H.; Dickey, S.; Lim, D. V.; Gonzalez, J.; Turos, E. *J. Org. Chem.* **1998**, *63*, 8898.
7. We found that attempted metalation of the 3-iodo-4,5-dihydrothiophenes results in ring opening to give lithium sulfides. This ring opening has also been observed by Ren, X.-F. et al.; see ref 6c.
8. For experimental procedures, see: 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. *Mol. Pharmacol.* **1998**, *53*, 62.