One-Pot Synthesis of Benzo[*b*]furan and Indole Inhibitors of Tubulin Polymerization

Bernard L. Flynn,*,† Ernest Hamel,‡ and M. Katherine Jung§

Department of Chemistry, The Faculties, Australian National University, Canberra, ACT, 0200, Australia, Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702, and Science Applications International Corporation−Frederick, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702

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Benzo[*b*]furan and indole analogues of some recently identified benzo[*b*]thiophene inhibitors of tubulin polymerization have been prepared, and their biological activity has been assessed. Several very potent analogues were identified.

Compounds that interfere with the microtubule−tubulin equilibria in cells are useful in the treatment of human disease.[1] Examples are the antiinflammatory activity of colchicine,[1a] the anthelmintic properties of the benzimidazole carbamates,[1b] and the antineoplastic use of the *Catharanthus* (formerly *Vinca*) alkaloids[1c,d] and the taxoids.[1e,f] More recently, it has been established that some tubulin binders selectively target the vascular system of tumors.[2] These agents induce morphological changes in the endothelial cells of the tumor's blood vessels so as to occlude flow.[2i] As a result, virtually complete vascular shutdown is achieved within the tumor in a matter of minutes. Combretastatin A-4 (CA4, Figure 1) is a powerful inhibitor of tubulin polymerization and a potent cytotoxin (Table 1).[3] The prodrug form of CA4, combretastatin A-4 disodium phosphate (CA4P), is undergoing clinical trials as a tumor vascular targeting agent.[2] The prodrug, CA4P, is itself inactive with purified tubulin and is therefore believed to require in vivo dephosphorylation to CA4 before it can act.[2] This process may contribute to its selectivity, with greater rates of dephosphorylation occurring in the tumor vascular systems than in normal vasculature.[2c,j]

The success of tubulin polymerization inhibitors as anticancer agents has stimulated significant interest in the identification of new compounds that may be more potent or more selective in targeted tissues or tumors.

In 1999, Pinney and co-workers[4] reported a benzo[*b*]-thiophene, compound **1** (Figure 1), that exhibited some activity as a tubulin polymerization inhibitor and appeared to bind weakly to the colchicine binding domain of tubulin. However, **1** only inhibited the rate and not the extent of tubulin polymerization. The limited activity of **1** was hypothesized to come from poor solubility.[4a] Using our novel method for the flexible and concise synthesis of benzo[*b*]thiophenes, we prepared analogues of **1**.[5] The most potent of these was **2** (Figure 1, Table 1).[5a] More recently, we have developed a one-pot, multicomponent coupling approach to benzo[*b*]furans and indoles.[6] Here, we report on the application of this

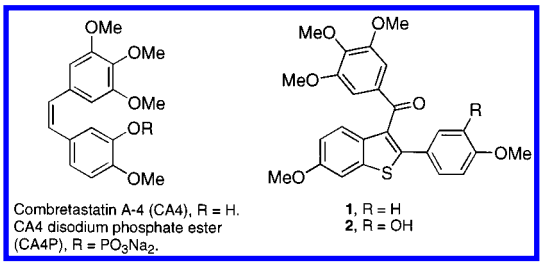
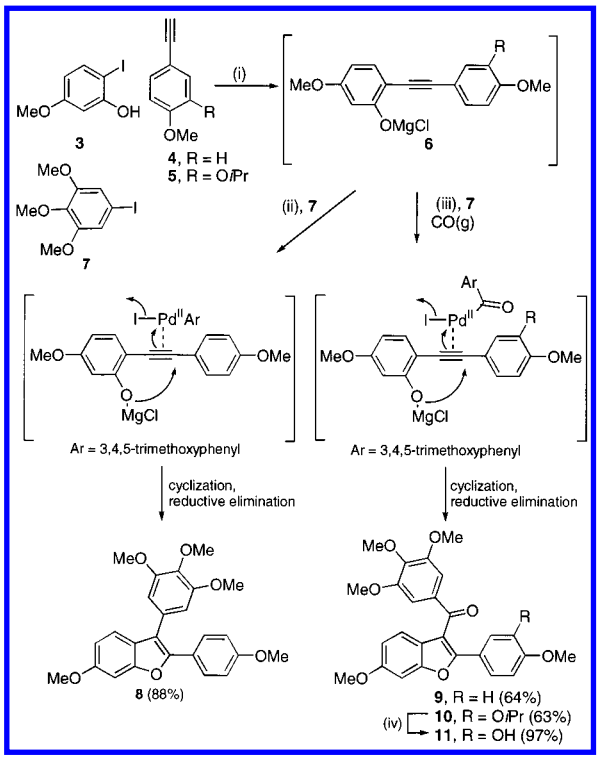


Figure 1.

method to the preparation of various benzo[*b*]furan and indole analogues of **1** and **2** and their effects on tubulin polymerization.

Benzo[*b*]furans **8** and **9** were prepared by multicomponent coupling of 2-iodo-5-methoxyphenol **3**, 4-meth-

Scheme 1[a]



[a] Reagents and conditions: (i) MeMgCl 2 equiv, $Pd(PPh_3)_2Cl_2$ 3 mol %, THF, 65 °C, 1.5 h under N_2(g); (ii) cool to room temp, add **7** and DMSO, then heat to 80 °C, 16−18 h; (iii) same as (ii) except exchange N_2(g) atmosphere for CO(g) atmosphere (balloon); (iv) $AlCl_3$ 3 equiv, CH_2Cl_2.

* To whom correspondence should be addressed. Phone: 61 2 6125 2017. Fax: ++61 2 6125 0760. E-mail: flynn@rsc.anu.edu.au.
† Australian National University.
‡ Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health.
§ Science Applications International Corporation−Frederick, National Cancer Institute at Frederick, National Institutes of Health.

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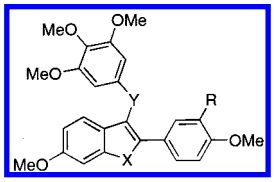
Table 1. Effects of Indole and Benzo[*b*]furan Compounds on Tubulin Polymerization, Colchicine Binding, and Growth of MCF-7 Human Breast Carcinoma Cells[8]

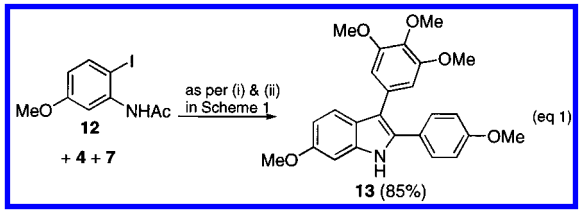


compound	X	Y	R	inhibition of tubulin polymerization,[a] IC_{50} ± SD (μM)	inhibition of colchicine binding[b] (% inhibition ± SD)	inhibition of cell growth,[c] IC_{50} ± SD (nM)
CA4				2.1 ± 0.1[d]	91 ± 2	11 ± 4[d]
1	S	C=O	H	>40[e,f]		640 ± 10[d]
2	S	C=O	OH	3.4 ± 0.2[d]	21 ± 10[d]	520 ± 400[d]
8	O	nothing	H	>40		>1000
9	O	C=O	H	0.41 ± 0.1	77 ± 3	34 ± 10
11	O	C=O	OH	1.3 ± 0.2	80 ± 2	42 ± 10
13	NH	nothing	H	>40[e]		460 ± 5
17	NH	nothing	OH	4.1 ± 0.6	28 ± 8	370 ± 2
19	NH	C=O	OH	1.6[g]	54 ± 0.7	45 ± 5
20	O	CH(OH)	H	>40[e]		>1000
21	O	CH(OH)	OH	8.8 ± 1.4		560 ± 60
22	O	CH_2	H	2.5 ± 0.3	17 ± 10	400 ± 100

[a] The tubulin concentration was 10 μM. Inhibition of extent of assembly, after a 20 min incubation at 30 °C, was the parameter measured. [b] The tubulin concentration was 1.0 μM, and the [^{3}H]colchicine and inhibitor concentrations were both 5.0 μM. Incubation was for 10 min at 37 °C. At least two independent experiments were performed with each of the compounds evaluated. [c] Cells were grown for 48 h at 37 °C in a humidified 5% CO_2 atmosphere. Cell protein was the parameter measured. At least two independent experiments were performed with each compound. [d] Data from ref 5. [e] The rate, but not the extent of assembly, was reduced by compound concentrations as high as 40 μM. [f] Data from ref 4a. [g] Same value in two experiments.

oxyphenylethyne **4**, and 3,4,5-trimethoxyiodobenzene **7** (Scheme 1).[6] This process involved initial deprotonation of **3** and **4** with 2 equiv of methylmagnesium chloride to give the corresponding magnesium phenolate and acetylide, respectively (not shown). Coupling occurred upon addition of a catalytic amount of $Pd(PPh_3)_2Cl_2$ and heating to 65 °C for 1.5 h to give the intermediate *o*-alkynylphenolate **6**. The solution was diluted with dimethyl sulfoxide (DMSO), aryl iodide **7** was added, and the reaction was heated to 80 °C for 16 h under N_2(g). Under these conditions, the palladium(0) present undergoes oxidative addition to **7**, followed by palladium(II)-induced cyclization of **6** and reductive elimination to afford **8** in an excellent yield (88%). When the N_2(g) atmosphere was exchanged for one of CO(g) at the point of introduction of **7**, a carbonyl is inserted between palladium(II) and the trimethoxyphenyl group, leading to the carbonylated product **9** (64%).[6] Using alkyne **5** in place of **4** under these carbonylative conditions gave **10** (63%). The isopropyl ether in **10** was cleaved using $AlCl_3$ to give the desired phenol **11** (97%).[7]

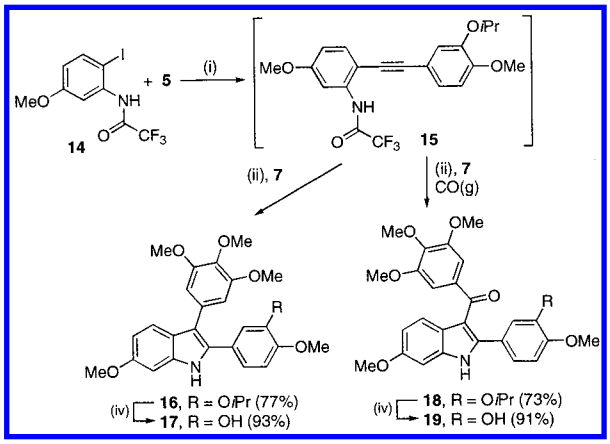
Indoles could be prepared by using *o*-iodoacetanilides in place of the *o*-iodophenols in the multicomponent coupling reaction.[6] Accordingly, reaction of **12** with **4** and **7**, under the multicomponent coupling conditions described above, gave **13** (85%) (eq 1).[6] The acyl group



was lost during the course of the reaction.[6]

Alternatively, indoles could be obtained at room temperature in a one-pot reaction using a variant of the stepwise sequence described by Cacchi.[9] Accordingly,

Scheme 2[a]

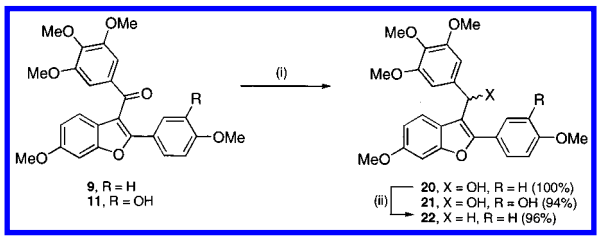


[a] Reagents and conditions: (i) $Pd(PPh_3)_2Cl_2$ 3 mol %, Et_3N 2 equiv, CuI 6 mol %, CH_3CN, 18 °C, 1 h under N_2(g); (ii) K_2CO_3 5 equiv, **7**, 18 °C, 18 h; (iii) same as (ii) except exchange N_2(g) atmosphere for CO(g) atmosphere (balloon); (iv) $AlCl_3$ 4 equiv, CH_2Cl_2.

the *o*-iodotrifluoroacetanilide **14** was coupled to alkyne **5** under Sonogashira conditions in acetonitrile to give **15** (Scheme 2). When this coupling was complete (approximately 2 h), the aryl iodide **7** and 5 equiv of K_2CO_3 were added and the solution was left stirring for a further 18 h to give **16** (77%). As before, when the N_2(g) atmosphere was exchanged for one of CO(g) at the point of introduction of **7**, the product obtained was the carbonylated product **18** (73%). Both **16** and **18** were deprotected to the corresponding phenols **17** and **19**, respectively, using $AlCl_3$.[7,10,11] These alternative multicomponent coupling conditions are not applicable to the synthesis of 2,3-disubstituted benzo[*b*]furans because, under the Sonogashira conditions of coupling, the initially formed *o*-alkynylphenols rapidly cyclize to the simple C3-unsubstituted benzo[*b*]furans.[6]

Additional analogues were generated by reduction of ketones **9** and **11** to give alcohols **20** and **21**, respec-

Scheme 3[a]



[a] Reagents and conditions: (i) $NaBH_4$ 2 equiv, EtOH; (ii) CF_3CO_2H 10 equiv, Et_3SiH 5 equiv, CH_2Cl_2.

tively, using sodium borohydride (Scheme 3). Alcohol **20** was further reduced to the simple methylene system **22** by reaction with trifluoroacetic acid and triethylsilane in excellent yield.

Compounds **8**, **9**, **11**, **13**, **17**, **19**, and **20**−**22** were evaluated for inhibition of tubulin assembly (Table 1). Those that displayed a significant inhibitory effect (defined as $IC_{50} < 5.0$ μM) were also examined for an inhibitory effect on the binding of [^{3}H]colchicine to tubulin (the tubulin used in these studies was purified to electrophoretic homogeneity from bovine brain). These compounds were also evaluated for cytotoxicity against MCF-7 human breast carcinoma cells (Table 1).

Compound **8** did not inhibit tubulin assembly at concentrations as high as 40 μM or cell growth at 1 μM and was not further examined, while compounds **13**, **17**, **20**, and **21** showed moderate to poor activity both as tubulin polymerization inhibitors and as cytotoxins. Compounds **9**, **11**, **19**, and **22** were all similar to or more potent than CA4 as inhibitors of tubulin polymerization. Compound **9** was the most active, exhibiting 5 times the level of potency of CA4. However, all of these compounds (**9**, **11**, **19**, and **22**) were less potent than CA4 as inhibitors of [^{3}H]colchicine binding to tubulin and as cytotoxins (against MCF-7 human breast carcinoma cells). It is nonetheless noteworthy that the cytotoxicity of **9**, **11**, and **19** was only slightly less than that of CA4.

In terms of a structure−activity relationship (SAR) for benzofused heterocyclic compounds, those containing either a nitrogen or an oxygen in the heterocyclic ring are much more effective than their sulfur analogues in all three biological assays (compare **1** and **2** with **9**, **11**, and **19**). As had been previously demonstrated for the sulfur series, the absence of a one-carbon linker between C3 of the benzofused heterocycle and the trimethoxyphenyl ring leads to a complete or near-complete loss in activity (compare **8**, **13**, and **17** with **9**, **11**, and **19**).[5] This carbon linker is much more effective as a carbonyl group than as a carbinol or simple methylene group (compare **9** and **11** with **20**−**22**).

Conclusion

Multicomponent coupling of *o*-iodophenols (or *o*-iodoacetanilides) with terminal alkynes and aryl iodides provides rapid access to potent benzo[*b*]furan- and indole-based tubulin polymerization inhibitors. These systems represent valuable new leads in the pursuit of anticancer chemotherapies.

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Supporting Information Available: Procedures for the synthesis of all new compounds in Table 1, including spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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