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THE SYNTHESIS AND EVALUATION OF TEMPERATURE SENSITIVE TUBULIN TOXINS

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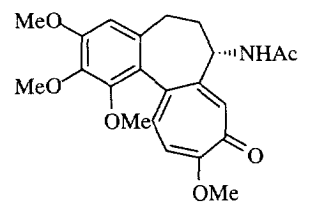
Abstract: The synthesis of several potent inhibitors of tubulin polymerization that exert their activities through interaction at the colchicine binding site is described. These agents were evaluated for their abilities to inhibit the polymerization of tubulin and the growth of neoplastic cell cultures. Additionally, the inhibition of tubulin polymerization activity of these agents was assessed over a temperature range of 30-45°C to ascertain the effect of temperature on this activity. Several of the compounds possess significant inhibition of tubulin polymerization activity, and select compounds exhibit this activity in a temperature dependent manner.

Compounds that inhibit tubulin polymerization through interaction with the colchicine binding site on the tubulin monomer have been of great interest for many years, with colchicine (Figure 1) itself representing one of the oldest known therapeutic agents in the pharmacopeia.[1] Many of these agents exhibit potent antimitotic activity and hold clinical potential, however, clinical efficacy is often attenuated by high general cytotoxicity. Therefore, imparting greater selectivity onto agents that possess this activity may hold significant clinical value.

Figure 1. (-)-Colchicine.



Although prodrug approaches have proven useful for increasing the effectiveness of a variety of therapeutic agents, these methods typically preclude derivatization of the parent compound. One intriguing possibility for overcoming the complications associated with conventional prodrug approaches is the use of hyperthermia induced toxin activation, herein termed HITA. Hyperthermia[2,3] has been studied in combination with chemotherapy to increase toxin effectiveness, yielding both additive and synergistic effects that can be attributed to increased cell stress. Hyperthermia has not to our knowledge, however, been utilized for the temperature mediated control of activity for cytotoxic or cytostatic agents. Ideally, agents of interest would not induce a cytotoxic response at or below systemic body temperature but would elicit their activities at temperatures higher than systemic body temperatures (>37 °C). Herein we disclose our preliminary studies of the synthesis and evaluation of inhibitors of tubulin polymerization that may possess utility in the further investigation of temperature mediated induction and attenuation of cytotoxin activity.

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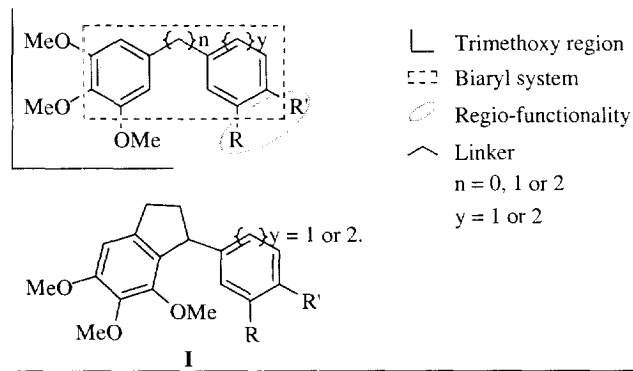
Design

Several compounds containing the biaryl functional arrangement found in colchicine are strong binders of tubulin.[4] Despite a large body of data, an all inclusive minimum pharmacophore has not been defined, owing credence to high steric tolerance in the binding site. One pharmacophore (Figure 2), previously proposed from these laboratories, provides a template for tubulin binder design based on the biaryl paradigm, which includes most inhibitors of tubulin polymerization that act at the colchicine binding site.[5] We were particularly interested in further refining this model by evaluating systems that possess restricted conformational mobility. Rigid ring systems that are capable of obtaining optimal binding geometries should show an increase in binding site affinity and hence an increase in the inhibition of tubulin polymerization activity. With this in mind we sought to evaluate compounds characterized by the core structure **I** (Figure 2).

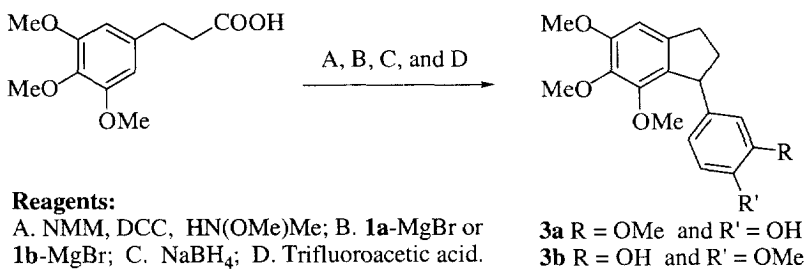
Figure 2. Proposed composite pharmacophore for biaryl tubulin binders and target core structures **I**.



Chemistry[6]

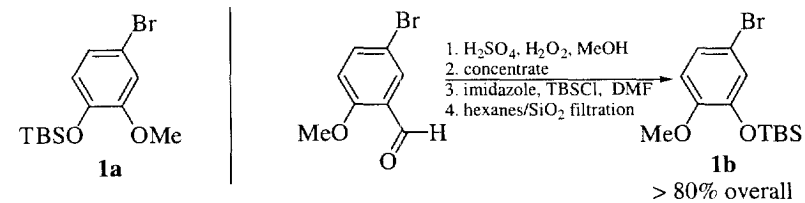
Efficient entries into initial target compounds **3a** and **3b** proceeded via a four-step sequence outlined in Figure 3. Thus, treatment of the Weinreb amide of trimethoxy(phenyl)propionic acid with aryl grinard reagents

Figure 3. The synthesis of **3a** and **3b** (upper), structure **1a** (lower left), and the "one-pot" preparation of **1b** (lower right).



Reagents:
A. NMM, DCC, HN(OMe)Me; B. **1a**-MgBr or **1b**-MgBr; C. $NaBH_4$; D. Trifluoroacetic acid.

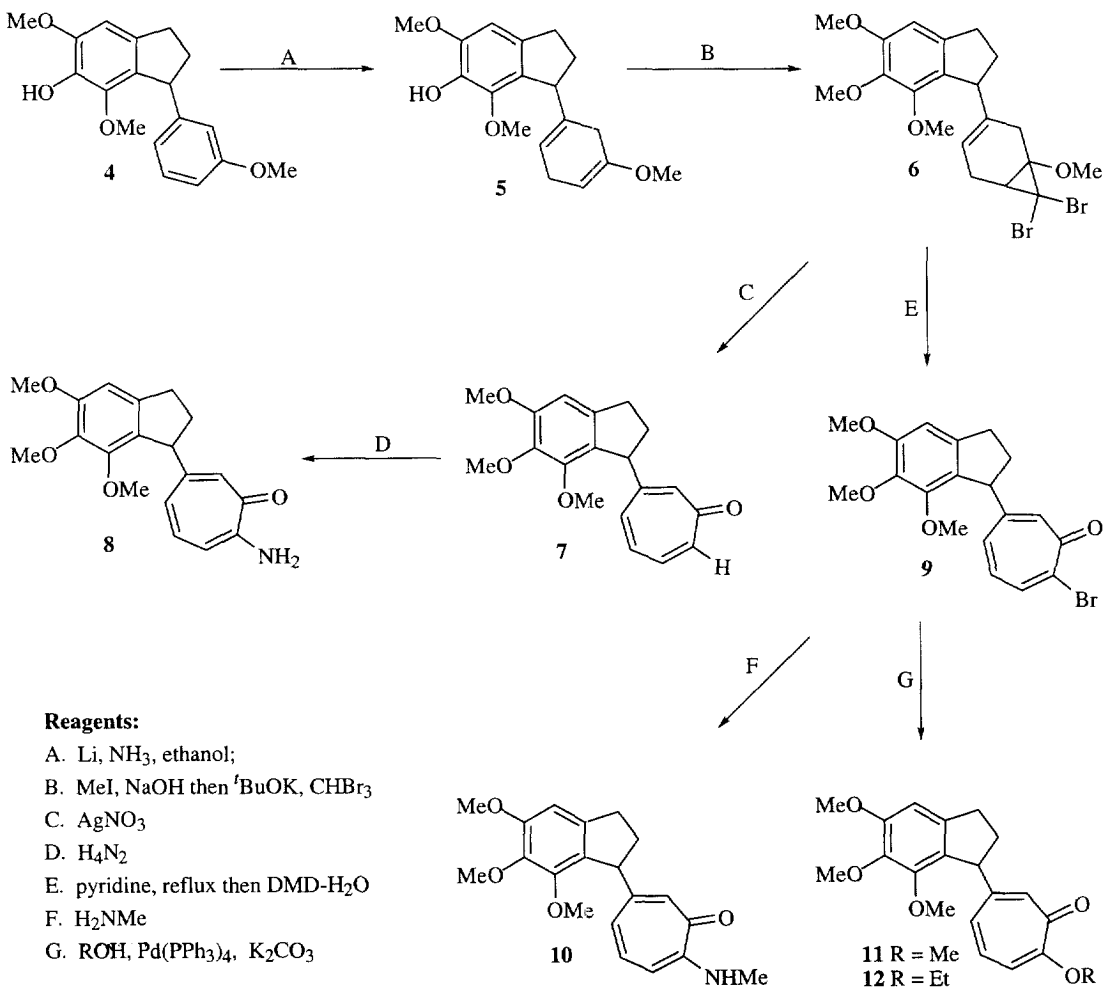
3a R = OMe and R' = OH
3b R = OH and R' = OMe



prepared from **1a**[7] and **1b** (Figure 3) provided the corresponding alkylated aryl-ketones. Treatment of the resultant ketone functionality with sodium borohydride followed by cyclization and concomitant deprotection with trifluoroacetic acid in CH_2Cl_2 afforded methoxyphenols **3a** and **3b** in >65% overall yield.

The synthesis of tropone substituted indanes was realized through the application of methodology developed in these laboratories which allows the conversion of anisole functionalities into troponoids (Figure 4).[8] Starting indane **4** was synthesized from sinapic acid in high overall yield utilizing the method outlined in Figure 3. Selective Birch reduction of **4** afforded the desired dihydro-adduct **5** along with minor quantities of over reduced material. Methylation followed by exposure to dibromocarbene afforded **6**. The conversion of **6** to tropone **7** was accomplished by reaction with $AgNO_3$ in aqueous acetone.[8,9] Further functionalization of **7** with hydrazine afforded **8** as a single product.[10,11] Pyridine catalyzed ring expansion of **6** followed by oxidation with

Figure 4. The synthesis of troponoid-indanes.



dimethyldioxirane afforded **9**, which was elaborated with methylamine to **10**.[11] The conversion of **9** to alkoxytropones **11** and **12** was carried out using previously described methods.[8]

Discussion

All of the compounds synthesized were screened for inhibition of tubulin polymerization activity using homogenous bovine brain tubulin and for growth inhibition (GI) activity using neoplastic cell cultures. Inhibition of tubulin polymerization data for the racemic target compounds are summarized in Table 1. Of the congeners tested, several displayed significant inhibition activity relative to that of colchicine. Additionally, given the chirality requirements for ligands that bind at the colchicine binding site, only one enantiomer is expected to bind effectively.[12] Therefore, it is likely that these agents will elicit greater activity in their optically pure forms and studies are currently underway to address this issue experimentally. The activities of compounds **3a** (IC_{50} = 46.4 μM) and **3b** (IC_{50} = 11.8 μM) demonstrate the importance of functionality position in the pendant aromatic domain. These agents possess a relationship analogous to that of isocolchicine (IC_{50} > 1 mM) and

Table 1. Polymerization Inhibition Data.

Compound	Polym. IC_{50} (μm)[a] 30 °C	37 °C	45 °C	y value[b]	R[c]	R'[c]	Temp. Dependence[d]
colchicine	11.2(±5.0)	8.7(±3.4)	8.7(±4.2)	2	O	OMe	1.3
3a	46.4(±6.0)	46.4(±4.8)	46.4(±6.1)	1	OMe	OH	0
3b	11.8(±2.3)	10.9(±1.8)	10.9(±2.0)	1	OH	OMe	1.1
7	?	?	?	2	O	H	?
8	29.5(±3.0)	23.6(±2.7)	23.6(±3.6)	2	O	NH_2	1.25
9	32(±4)	28.1(±6.3)	22(±5)	2	O	Br	1.45
10	30.6(±4.1)	23.5(±2.4)	-	2	O	NHMe	1.3
11	108(±7)	19(±3)	19(±4)	2	O	OMe	5.7
12	26.2(±2.4)	18.2(±1.5)	18(±2)	2	O	OEt	1.7

[a]IC_{50} for three data points were obtained and averaged. Conditions were as follows: Purified bovine brain tubulin (120 μl, 4 mg/mL), 240 μL PME (1 mM $MgSO_4$, 2 mM EGTA, 100 mM PIPES, pH = 6.9), compound (32 μL, DMSO), and GTP (8 μL, 50 mM) were allowed to polymerize for 10 minutes. Absorbencies were recorded with a Varian DMS 90 UV-VIS spectrophotometer at 351 nm in a temperature controlled curvet holder at the stated temperature. [b]See **I**, Figure 2. [c]Substituent at R or R' on **I** in Figure 2. [d]Temperature Dependence = (IC_{50} μM @ 45°C/IC_{50} μM @ 30°C) for averaged IC_{50} values.

colchicine (IC_{50} = 11.2 μM) with regard to structure and activity.[13] The remaining agents displayed IC_{50} values over a range of 26-108 μM at 30 °C. The relationship between agent structure and inhibition of tubulin polymerization activity may correlate in a manner similar to that found in our previous studies of troponoid inhibitors of tubulin polymerization, although the range of activities is appears too narrow for unambiguous interpretation.[11] Compound **7** yielded spurious data, which is in contrast to previous studies of agents possessing tropone ring systems.[11] Interaction with the colchicine binding site was corroborated for compounds **9** and **11** by assay for the competitive inhibition of colchicine binding using ^3H colchicine displacement experiments. Compound **11** displayed a 61% inhibition of colchicine binding to tubulin, while **9** displayed 55 % inhibition of binding.[8]

Notably, some of the tested compounds displayed inhibition of tubulin polymerization activities that are temperature dependent. Colchicine does not exhibit statistically significant temperature dependence for the inhibition of tubulin polymerization over the range tested (30-45 °C). Methoxytropone **11**, which is essentially devoid of activity at 30 °C, displayed a nearly six fold increase in activity over the temperature range of 30 °C to >37 °C. Compounds **9** and **12** show activity increases of 1.45-fold and 1.7-fold, respectively, for the averaged IC_{50} values over the tested temperature range. Compound **9** also displayed a minor increase in activity over the temperature range of 30-45 °C, however, none of the other compounds included in this study exhibited a marked temperature dependence over the more desirable range of 37-45 °C. Increases in the inhibition of tubulin polymerization activities of these agents are possibly a consequence of restricted conformational mobility and limited conformational tolerance in the binding site.

The effect of structure on temperature dependence appears to be arbitrary on the basis of this limited study, as is evidenced by the difference in temperature dependence for compounds **11** and **12**. Despite this, these results bring to light the exciting possibility of utilizing hyperthermia for the activation of tubulin toxins, thereby providing an avenue for overcoming the general cytotoxicity associated with many antimitotic agents. This could lead to novel chemotherapeutic regimens, provided further SAR studies of this class of agents yield a compound that is a temperature dependent tubulin binder such as **11**, with higher activation temperatures (e.g., 37 °C to 45 °C).

Data from the *in vivo* cellular assays for the growth inhibition (GI) of immortalized cell cultures are summarized in Table 2. Of the compounds tested, the most potent, **3b**, displayed GI_{50} values ranging from 0.1-

Table 2. Growth Inhibition of Selected Agents.

Compound	GI_{50} (μM)[a] PC3	MCF7	NCI-H520
colchicine	<0.05	<0.05	<0.05
3a	>100	>100	>100
3b	0.5	10	0.1
11	5.2	75	5.4

[a]Growth Inhibition (GI) for stated cell line.

10 μM. Compound **11** provides modest activity, yielding GI_{50} values ranging from 5.2-75 μM, while the inactivity of **3a** was further corroborated by low cellular toxicity.

Conclusion

In summary, we have disclosed herein the synthesis and evaluation of several novel inhibitors of tubulin polymerization. Many of these agents possess potent inhibition activity and a select few of these show activity that is temperature dependent, the most dramatic of which shows a greater than five fold differential in activity for the inhibition of tubulin polymerization over the temperature range of 30-37 °C. The results from our continued efforts to understand and enhance the temperature dependent nature of these and other related agents will be reported in future correspondence.

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