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TUBULIN BINDING OF CONFORMATIONALLY RESTRICTED BIS-ARYL COMPOUNDS

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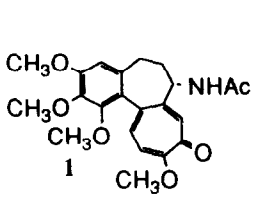
Abstract: A series of bis-aryl ring containing analogs of podophyllotoxin and combretastatin were assayed for their abilities to inhibit tubulin polymerization as part of a program to define the relationship between the aryl ring systems and tubulin binding.

Colchicine **1**, the archetypical tubulin-binding antimitotic drug, has been shown to bind tubulin at a single high affinity site [$K=10^6$-10^7 M^{-1} at 37^oC][1-3]. This binding site has been shown to recognize a number of compounds that contain two aryl rings within a variety of structural frameworks, including the biaryl substructure [e.g. 2-methoxy-5-(2',3',4'-trimethoxyphenyl)tropone **2** and steganacin **3**], the 1,1-diarylmethane substructure [e.g. podophyllotoxin **4**], and the 1,2-diarylethane substructure [e.g. combretastatin **5**]. Although several lines of evidence suggest that these diverse structures bind to the same site on tubulin, their differing effects on tubulin intrinsic GTPase activity and on modes of microtubule depolymerization suggest that the protein exists in different conformations when bound to colchicine versus podophyllotoxin[3].

The binding of colchicine to tubulin is believed to involve three pharmacophore domains, namely the trimethoxyaryl A-ring, the tropone moiety of the C-ring, and the chiral



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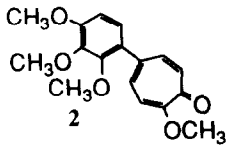

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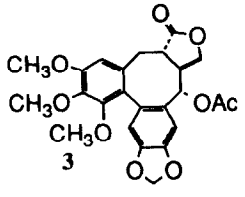

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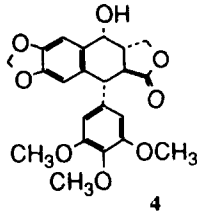

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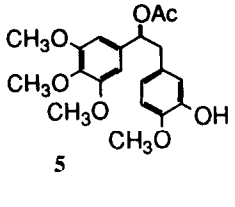

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C-7 acetamide of the B-ring. The primary sites of ligand recognition are the A- and C- ring domains, with apparently minor changes in substitution eliciting large decreases in binding[5-7]. In contrast, the effects of significant structural adjustments in the B ring substituent are rather small[8-10]. In addition, ligand conformation, in particular the dihedral angle between the A- and C-rings, has been suggested to be an important factor in tubulin binding[11].

In connection with our program aimed at elucidation of the structural requirements for ligand binding to this site[7,12], we have prepared a series of conformational analogs of colchicine and podophyllotoxin in which the spatial relationship between the A and C rings has been rationally altered. We describe here our data on the abilities of these compounds to inhibit the in vitro polymerization of purified bovine brain tubulin free from microtubule-associated proteins [MAPs][4,13], a measurement of their abilities to bind to the tubulin monomer. These studies address the importance of the A/C spatial relationship to tubulin binding and define important constraints to further development of analogs directed toward the colchicine binding site.

Our synthetic analogs and their tubulin binding data are summarized in Table 1[12]. Compounds **6-9** and **14** were prepared to probe the tolerance of the enzyme for variation in the biaryl bond. The activities of the biaryl derivatives **6** and **14** were surprisingly low given the high activity of the tropone-containing analog **2** [IC_{50}=7.5x10^{-6} M][5]. This low activity may be explained by the known sensitivity [relative to colchicine] of biaryl AC analogues to variation of substitution on the C-ring[5].

Compounds **7-9** were designed to lock the biaryl moiety into a fixed conformation. Energy minimized structures of these compounds were generated using the Sybyl system with the Maximin2 minimization program[14]. Only **8**, with a biaryl dihedral angle of approximately 60°, exhibited significant activity. Compound **7**, with a dihedral angle of 30-35°, was essentially inactive. This data was consistent with the proposal of Detrich et al. that the 53° biaryl angle of colchicine was necessary for initial binding[7]. Compound **9** is capable of assuming several B-ring conformations; the low energy conformation is a boat-chair, and has a biaryl dihedral angle of 60°. This is in contrast to the boat-boat conformation of the B-ring of steganacin, and may inhibit entry of the analog into the binding site.

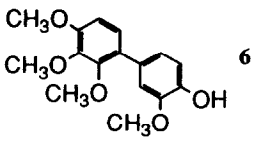
The combretastatin-like compounds **10-13** retained the high degree of conformational freedom of the natural product, and all retained a high level of activity. In contrast, the diarylmethane compound **15**, with podophyllotoxin-like methylenedioxy substitution, exhibited lowered activity. While a methylenedioxy substituent has been shown to be inactive in a 1,2-diarylethane analogue[6], **15** exhibits satisfactory superimposition with the 1,1-diarylmethane substructure and might be expected to bind through a mechanism similar to that of podophyllotoxin. Compounds **10** and **15** were considered to be a point of transition from combretastatin-like analogs to podophyllotoxin-like analogs. The data for tubulin binding of these analogs suggested an increased requirement for conformational restraints in podophyllotoxin-like binding.

The binding data of **16** and **17** might offer further evidence for conformational

Table 1. Binding of conformational analogs to tubulin[a].

Analog	IC_{50} ($x10^{-6}$M)	Analog	IC_{50} ($x10^{-6}$)
1	2.5	**12**	2.4
4	2.5	**13**	5.8
5	5	**14**	150
6	98	**15**	67
7	104	**16**	12
8	5.3	**17**	2.4
9	>125	**18**	>200
10	1.5	**19**	>200
11	1.4		

[a] IC_{50} was determined as described in ref. 4.



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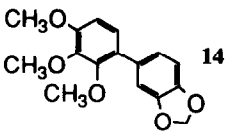

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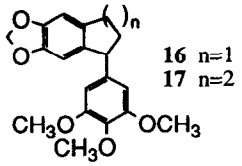
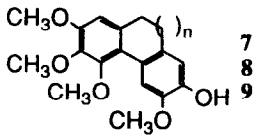
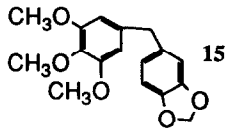


16 n=1
17 n=2



7 n=1
8 n=2
9 n=3



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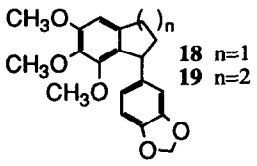
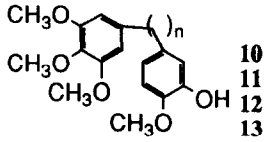


18 n=1
19 n=2



10 n=1
11 n=2
12 n=3
13 n=4

restraints in binding of tubulin by podophyllotoxin-like ligands. In these examples, the rotation of the trimethoxyarylmethylene is restricted so that the analog must assume a more "pendant"-like configuration; that is, the C ring cannot be coplanar with the AB ring system, as is the case with podophyllotoxin. This is especially true in the case of **17**, as the trimethoxyaryl ring is able to assume a pseudoaxial conformation similar to the that of podophyllotoxin.

In conclusion, our data indicate that the conformational relationship of the biaryl moiety is an important component of ligand binding to tubulin. The tubulin binding activity of some analogs [**8**, **10-13**, **19**] equaled or surpassed the activity of the natural products colchicine, combretastatin, and podophyllotoxin. The uniformly high potency of the [1,n]-diarylalkane series **10-13** confirmed that the bis-aryl moiety of colchicinoid microtubule inhibitors is the primary pharmacophore for tubulin binding. The binding data of additional conformational analogs as well as the GTPase activity of tubulin when bound to the above ligands will be reported in due course.

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12. All compounds gave satisfactory analytical data. During the completion of this work, synthesis and biological activity were reported by Lin, et al. for **7**, **11**, **12**, and **13** [Ref. 7]. Our data on the bioassay of these compounds compares with the data of Lin et al., except for **12**, which exhibited tenfold greater activity in our assay.
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