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"COMBRETATROPONES"--HYBRIDS OF COMBRETASTATIN AND COLCHICINE. SYNTHESIS AND BIOCHEMICAL EVALUATION.

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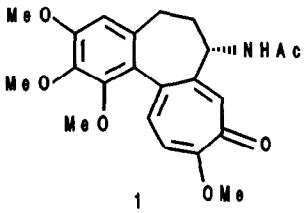
ABSTRACT: The synthesis and biological evaluation is presented for a new class of tubulin-targeting agents, termed "combretatropones," that incorporate the 1,2-diaryl ethane nucleus of combretastatin and the tropone moiety of colchicine.

The tubulin-microtubule system is the target for a large number of drugs which possess a wide range of therapeutic utilities. These drugs include colchicine **1**, combretastatin **2**, the vinca alkaloids, maytansine and taxol.



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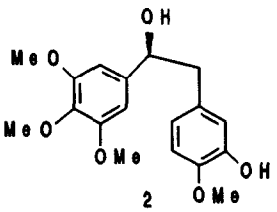

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Figure 1. The structures of colchicine **1** and combretastatin **2**.

Although over 200 analogs of colchicine have been prepared[1], most have been derived through semi-synthetic modification of the parent and, thus, limited in the scope of fundamental molecular alterations. Nonetheless, these analogs have enabled an understanding of the structure-activity relationships for the colchicinoid system,[2] provided a potential avenue for the exploitation of tubulin isotypes in therapy,[3] and assisted in the elucidation of the colchicine binding site on tubulin.[4] Combretastatin has also been shown to associate with the "colchicine binding site" on tubulin. In our design of the "combretatropones", illustrated in Figure 2, several observations derived from the structure-activity evaluations of the combretastatin and colchicine nuclei were utilized. These are summarized as follows:

1. The *bis*-aryl ring system is critical.[5] The two aryl rings can be directly linked (i.e., colchicine **1**) or linked *via* a "bridging carbon spacer", such as a methylene unit (i.e., podophyllotoxin) or a 1,2-disubstituted ethyl unit (i.e, combretastatin **2**).

2. The *bis*-aryl system can consist of two aromatic rings (i.e., combretastatin **2**) or an aromatic ring and a tropone ring (i.e., colchicine **1**).[6]

3. Maximum activity has been observed when a trimethoxy phenyl ring of the appropriate regiochemistry is present.[7]

4. The nature and regiochemistry of the substituents present on the tropone ring are signficant.[8]

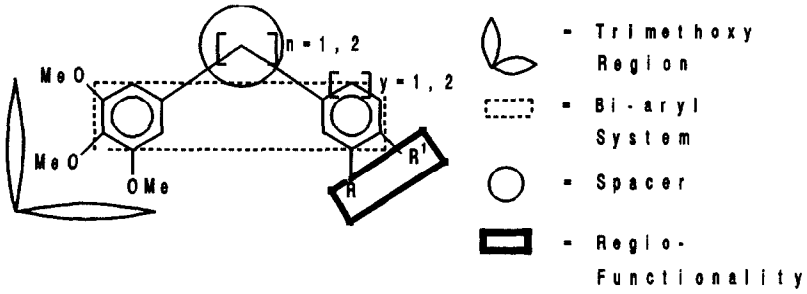
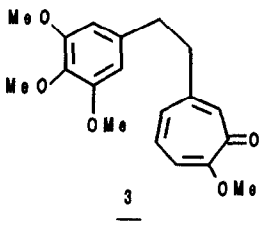


Figure 2. A hypothetical composite pharmacophore for colchicine **1** and combretastatin **2** and their analogs.

Combretatropones **3** and **4** (Figure 3) incorporate the 1,2-diaryl ethane nucleus of combretastatin and the tropone moiety of colchicine in a fashion consistent with the detailed structure-activity relationships. Figures 4 and 5 illustrate the superimposition of combretatropone **3** with colchicine **1** and combretastatin **2**.[9] Figure 4 illustrates the geometric *proximity*, but not *equivalence*, of the aryl substituents in **3** to those found in colchicine **1**. Figure 5 illustrates the geometric *equivalence* of the functional groups in combretatropone **3** to those in combretastatin **2**. Thus, analysis by molecular modelling of the combretatropone system predicts that **3** and **4** should exhibit SAR behavior more "combretastatin-like" than "colchicine-like" in their interactions with tubulin.



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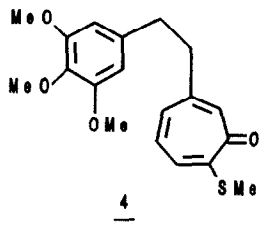

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Figure 3. The structures of combretatropones **3** and **4**, hybrid structures of colchicine and combretastatin.

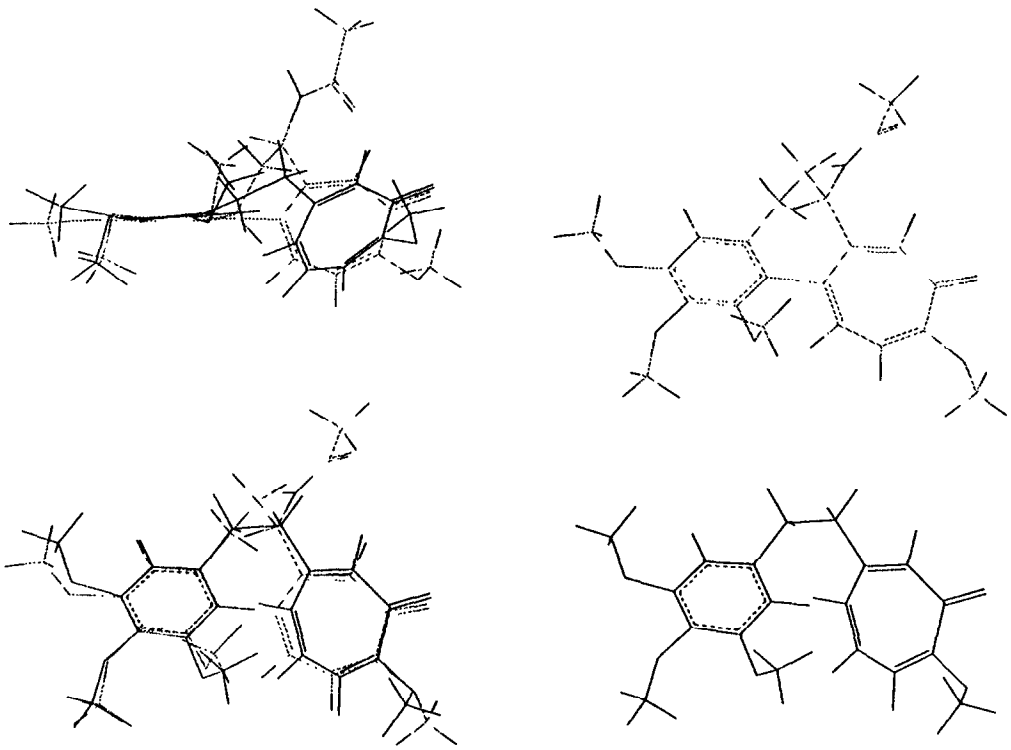


Figure 4. The superimposition of combretatropone **3** and colchicine **1**.

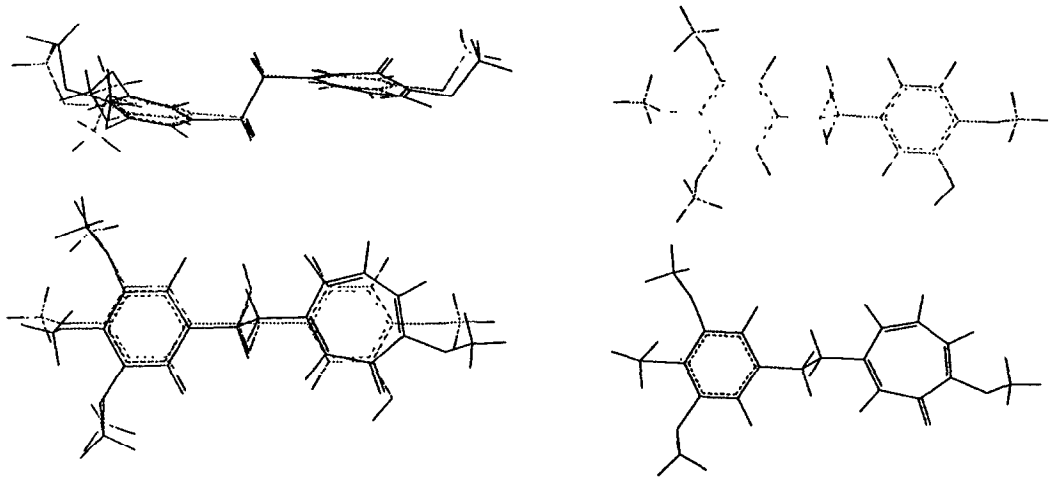


Figure 5. The superimposition of combretatropone **3** and combretastatin **2**.

The synthesis of combretatropones **3** and **4** is illustrated in Figure 6. 3-Hydroxy benzaldehyde was protected, reduced, and trifluoroacetylated to provide 3-siloxybenzyl trifluoroacetate **5** (72%). Trifluoroacetate **5** was converted to the phosphonium salt **6**, which was subsequently deprotonated and subjected to Wittig condensation with 3,5-dimethoxy-4-benzyloxybenzaldehyde **7**, to yield a mixture of *cis*- and *trans*-stilbenes (66%). Catalytic hydrogenation effected both alkene reduction and phenol debenzylation to give 1,2-diarylethane **8** (90%). Regiospecific expansion of the 3-silyloxyphenyl group into an α-chlorotropone was undertaken according to the method of Macdonald.[10] Thus, diarylethane **8** was subjected to Birch conditions and the resulting phenolate moiety methylated to provide the dihydrophenyl derivative **9** (64%). Dichlorocyclopropanation, desilylation, and epoxidation gave **10** (50%). Acid catalyzed rearrangement of **10** gave the moderately sensitive combretatropone **11** (82%). α-Chlorotropone **11** was converted into the stable combretatropone **3** using magnesium methoxide (90%). Combretatropone **3** could be readily transformed into combretatropone **4** using sodium methanethiolate (88%).[11]

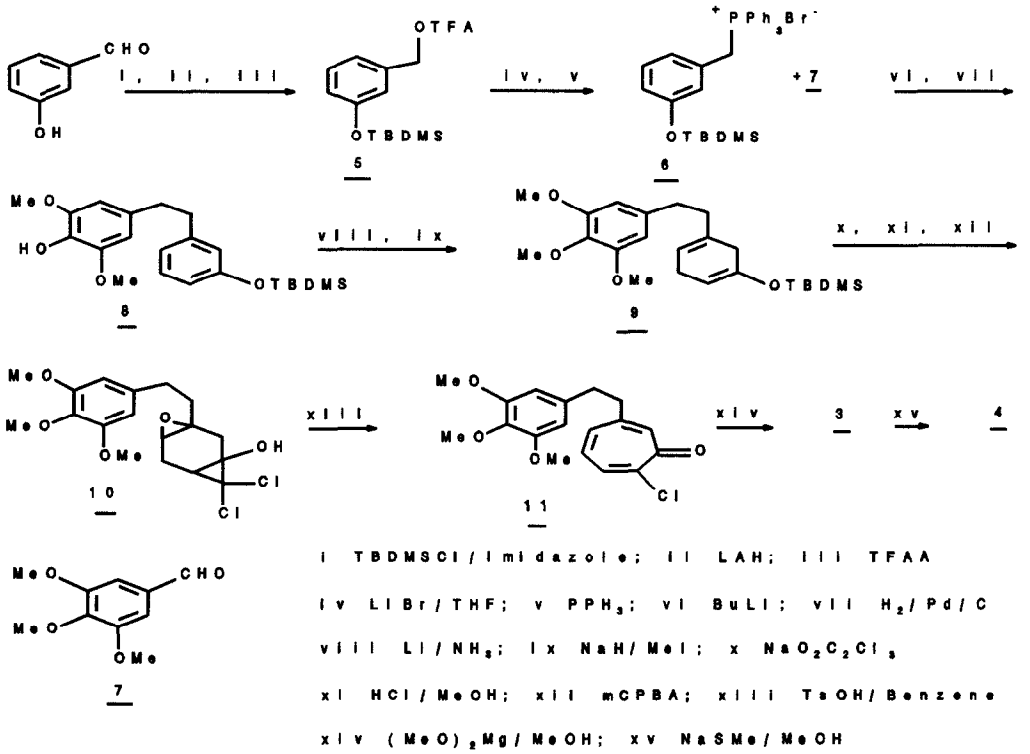


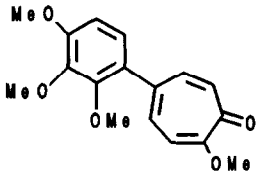
Figure 6. The synthesis of combretatropones **3** and **4**.

Combretatropones **3** and **4** exhibit potent activity in the *in vitro* inhibition of tubulin isolated from bovine brain (Table 1).[12] The observation that **3** and **4** display potent activity in the inhibition of tubulin polymerization assay, while homolog **13** does not,[13] is a significant finding. Both compounds possess the requisite structural and functional features for tubulin binding and the observed dramatic difference in activities may reflect an "entropic binding barrier". Reference IC_{50} values are provided for colchicine **1**, *des*-B ring colchicine analog **12**, and combretastatin A-4 (structure **2** lacking the hydroxyl moiety and possessing a *cis*-substituted ethene spacer; combretastatin A-4 has been shown to be slightly more active than combretastatin). These compounds, along with combretatropones **3** and **4**, were evaluated using the protocol detailed under reference 12.

TABLE 1. The IC_{50} Values for the Combretatropones and Standards

COMPOUND	IC_{50} (μM)
colchicine **1**	3.5
combretastatin A-4	2.9
phenyltropone **12**	2.5
combretatropone **3**	8.6
combretatropone **4**	12.0

IC_{50} values refer to the concentration of agent needed to inhibit the polymerization of tubulin into microtubules by 50% at 1 mg/ml tubulin concentration under standardized polymerization conditions detailed in reference 12.



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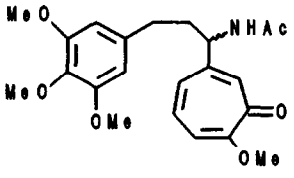

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Figure 7. The structure of *des*-B ring colchicine analog **12** and combretatropone homolog **13**.

Like the phenyltropone analog **12**, combretatropones **3** and **4** were designed to elucidate kinetic/thermodynamic parameters and molecular features of the colchicine-tubulin interaction. The asymmetrically substituted tropone ring, possessed by **3** and **4**, is a unique fluorophore which we will use in the study of a number of binding-associated phenomena. Foremost among these phenomena is the 300 fold increase in colchicine fluorescence upon the binding of colchicine to tubulin[14]. Also, **3** and **4** will be utilized to probe the enthalpy/entropy contributions associated with the B ring of colchicine. Finally,

combretatropones **3** and **4** may hold the answer to a number of proposed structure/spectra associations. Detailed biological and spectral data from **3** and **4**, and the conclusions drawn from this data, will be the subject of a future manuscript.

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    The assay protocol for determination of the IC_{50} values for the agents detailed in the Table is as follows: agents were preincubated with tubulin, at 30 °C, for 20 minutes prior to the initiation of tubulin polymerization. Polymerizations were conducted in a system consisting of: glutamate (1.0M), GTP (0.4 mM), magnesium chloride (0.25 mM), and bovine brain tubulin (1.0 mg/ml) at pH = 6.6 and 30 °C.
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