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SYNTHESIS AND ANTI-TUBULIN ACTIVITY OF *AZA*-COMBRETASTATINS

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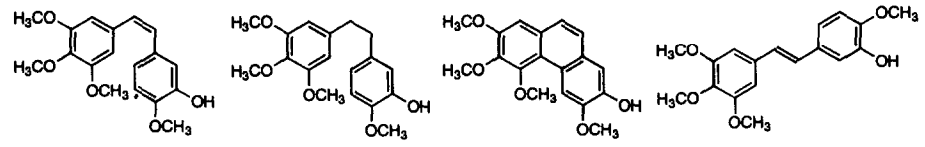
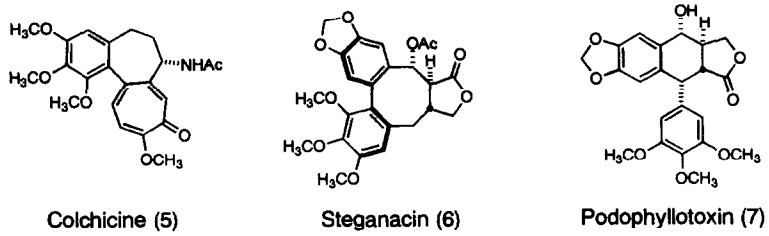
ABSTRACT: A series of *aza*-combretastatin was synthesized and their activity against microtuble assembly was evaluated. *N*-Benzylaniline analogs with a variety of side chains (**10b-10i**) showed moderate to excellent inhibitory activity while benzanilide analogs (**8**, **9** and **12**) had little activity.

Combretastatin A-4 (**1**) isolated from *Combretum caffrum* is reported to be one of the most potent antimitotic agents and strongly inhibits the polymerization of brain tubulin by binding at the colchicine binding site (CLC site).[1] A dihydrogenated analog **2** is also a strong inhibitor of tubulin polymerization, whereas the tricyclic analog **3** and a *trans*-isomer **4** show little activity (**Figure 1**).[2-4]

Figure 1





Common elements can be found in the structures of the active combretastatins congeners and of other well-known CLC site ligands such as colchicine (**5**),[5] steganacin (**6**),[6] and podophyllotoxin (**7**).[7] Inspection of their structural feature suggested that the following factors should be required for the ligand binding at the CLC site:

1. Two aromatic rings of drug molecule can be connected directly or through one or two atoms' bridge spacer of single or double bond.
2. *Cis* orientation of the two aromatic moieties is required.
3. Substitution of hydroxyl and/or alkoxyl groups on the aromatic rings is critical.
4. The appropriate chiral torsion may important in the conformation of the two aromatic rings.

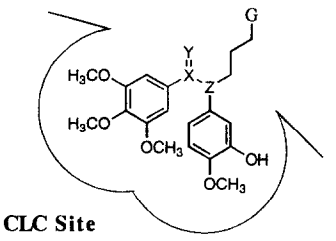
New antimitotic agents have widely been screened for the clinical and agrochemical purposes, but they are desired also as the chemical and biochemical tools for the study of the tubulin function and for the analysis of tubulin-ligand interaction. Because their structures are very simple and have high activity, combretastatins are attractive as the lead compounds to devise new drugs that bind to the CLC site on tubulin. Study on the structure-activity relationships of combretastatins and devicing some analogs have been attempted to some extent from the medicinal chemical point of view.[3, 4]

We planned to prepare a new class of combretastatin analogs, in which the two aromatic rings having the same substitution pattern as **1** and **2** are connected through an amide bond (benzanilides) or an amine bond (*N*-benzylanilines), in place of the C-C bridge in **2**. This design has several advantages as follows:

1. Ease in synthesis
2. Various side arms can be easily introduced on nitrogen atom
3. Creates no chiral and orientational isomers

The design is also suitable to devise new affinity ligands for photolabelling or for immobilized affinity-chromatography, etc as illustrated in **Figure 2**.

Figure 2

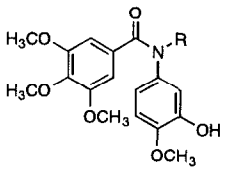


CLC Site

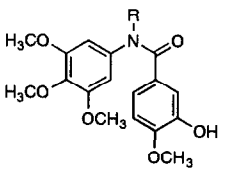
G : Fluorescent Group
Spin Labeled Group
Photoaffinity Label Group
Binding to Chromatography Gel
Hydrophilic Group
etc.

A series of branched and non-branched *aza*-analogs of combretastatins were designed as shown in **Figure 3**. These compounds were synthesized by general procedures as shown in **Scheme 1-4**.

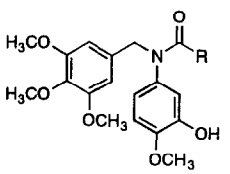
Figure 3



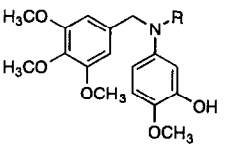
8a R = H
8b R = CH_3



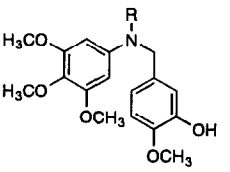
9a R = H
9b R = CH_3



12a R = CH_3
12b R = CH_2CH_3

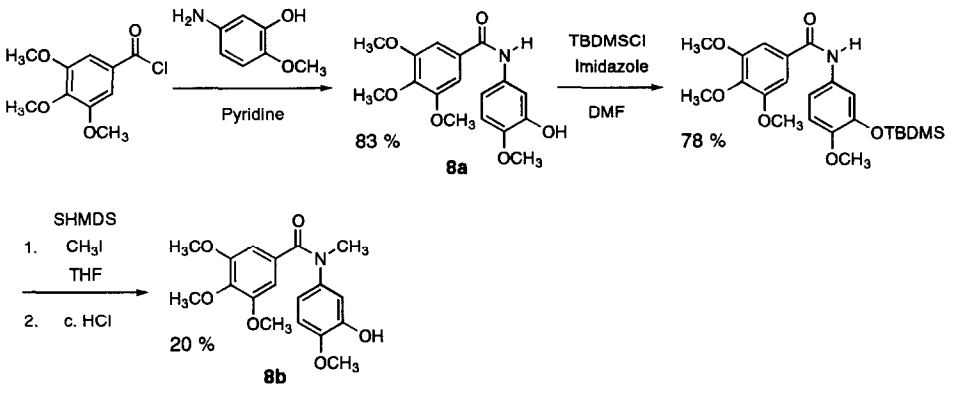


10a R = H
10b R = CH_3
10c R = CH_2CH_3
10d R = $(CH_2)_2CH_3$
10e R = $(CH_2)_3CH_3$
10f R = $(CH_2)_4CH_3$
10g R = $(CH_2)_5CH_3$
10h R = $(CH_2)_3CO_2H$
10i R = $(CH_2)_4CH_2OH$

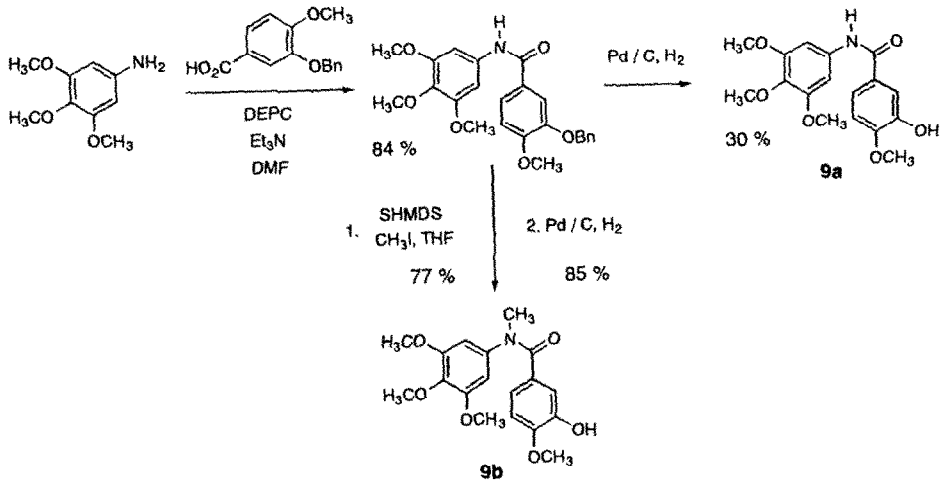


11a R = H
11b R = CH_3

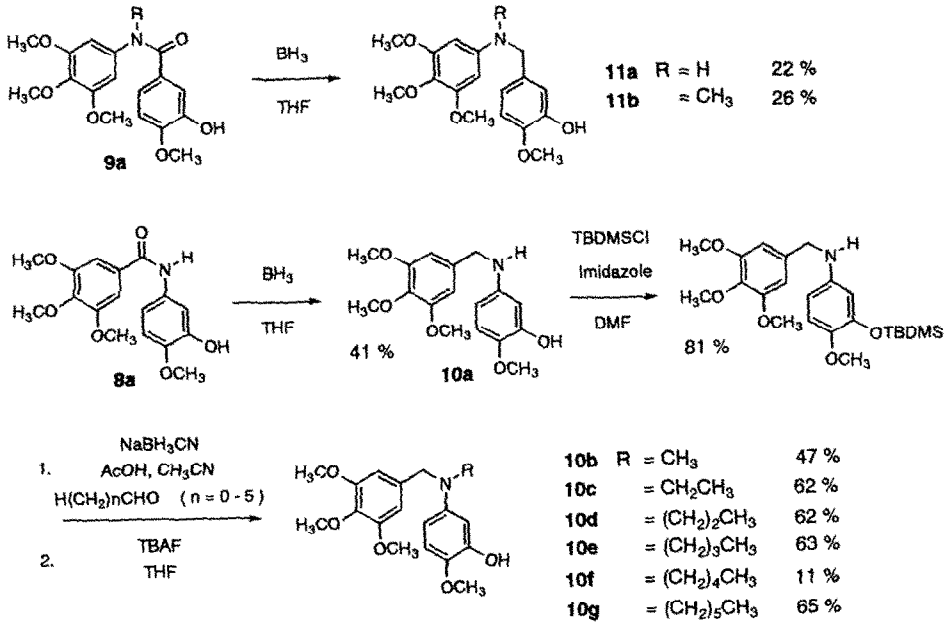
Scheme 1



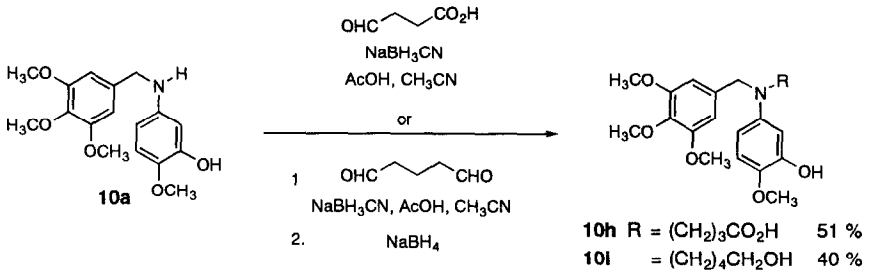
Scheme 2



Scheme 3



Scheme 4



The inhibitory activity of these synthesized combretastatin analogs was tested against porcine brain tubulin polymerization (IC_{50}), and the result is listed in **Table 1**.[8, 9] All amide compounds (**8**, **9** and **12**) did not inhibit the tubulin polymerization. This may due to an electrostatic effect of electron withdrawing amide carbonyl group. On the other hand, amine analogs (**10a**, **10b**, **11a** and **11b**) showed moderate anti-tubulin activity.

Initially, we considered that *N*-substitution of **10a** presumably induce *s-cis* orientation, the active conformer, of the two aromatic moieties to result higher activity. As expected, an introduction of alkyl side chains on nitrogen atom clearly enhanced the anti-tubulin activity as shown in **Table 1**.

Among them, the *N*-*n*-propyl and *N*-*n*-butyl derivatives (**10d** and **10e**) showed such high activity as natural combretastatins. Elongation (**10g** and **10h**) and functionalization (**10h** and **10i**) of the side chain caused slight decrease in their activity, but they still retained moderate activity.[10] This result suggests the possibility of further functionalization on the side chain without loss of activity.

Table 1. An Inhibitory Activity of Porcine Tubulin Polymerization by Drugs

Compound	$IC_{50}(\mu M)$
1a	3.9
8a	>100
8b	>100
9a	>100
9b	>100
10a	100
10b	45
11a	100
11b	100
10c	15
10d	5.4
10e	7.5
10f	20
10g	20
10h	40
10i	20
12a	>100
12b	>100

N-substituted *N*-benzylaniline derivatives are found to be the promising candidate of new ligands that will be widely applicable as the prove for further study of ligand-tubulin interaction at CLC site.[11] Currently, synthesis of functionalized *aza*-combretastatins and their utilization are under investigation in this laboratory.

ACKNOWLEDGMENT:

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9. All new compounds gave satisfactory spectroscopic and analytical data. Representative ^{1}H-NMR data (500MHz, $CDCl_3$) for selected compounds. **10d**: δ 0.92 (3H, t, J=7.6Hz), 1.64 (2H, m), 3.25 (2H, t, J=7.6Hz), 3.80 (6H, s), 3.81 (3H, s), 3.83 (3H, s), 4.38 (2H, s), 5.55 (1H, br), 6.14 (2H, dd, J=3.0 and 8.8 Hz), 6.40 (1H, d, J=3.0Hz), 6.46 (2H, s), 6.72 (1H, d, J=8.8Hz). **10i**: δ 1.35 (2H, m, J=7.7Hz), 1.54 (2H, m, J=7.7Hz), 1.60 (2H, m, J=7.7Hz), 3.24 (2H, t, J=7.7Hz), 3.57 (2H, t, J=7.7Hz), 3.77 (6H, s), 3.78 (3H, s), 3.79 (3H, s), 6.12 (1H, dd, J=3.1, 8.9Hz), 6.37 (1H, d, J=3.1Hz), 6.42 (2H, s), 6.68 (1H, d, J=8.9Hz).
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11. Since we started this project, similar approach to the *aza*-combretastatin was reported. See Reference 3.

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