SYNTHESIS OF CONFORMATIONARY RESTRICTED COMBRETASTATINS[1]

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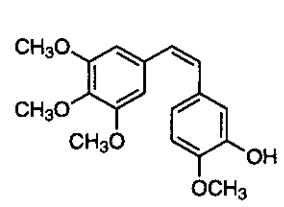
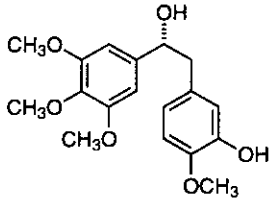
Abstract - A series of conformationary restricted heterocyclic combretastatin analogs have been synthesized and their inhibitory activity of microtubule assembly was evaluated. (4*S*,5*S*)-4-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1,3-dioxolane showed moderate antimitotic activity while its enantiomer and other diastereomers synthesized were inactive.

Combretastatin A-4 (**1**) isolated from *Combretum caffrum* is reported to be one of the most potent antimitotic agents which strongly inhibit the polymerization of brain tubulin by binding to the colchicine site (CLC site).[2-3] Common elements can be found among the structures of the active combretastatins congeners and of other well-known CLC site ligands such as colchicine, steganacin and podophyllotoxin. It has been proposed that CLC site ligands retain optically active conformation at the binding site of tubulin. Although **1** is not a chiral molecule, it is expected to exist as chiral conformer induced by the binding to tubulin.[4-5] Combretastatin (**2**) is one of the combretastatin analogs bearing one asymmetric center on ethylene bridge between two aromatic rings.[6] Optical resolution of racemate[7] and asymmetric synthesis[8] of

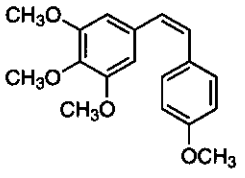
Figure 1



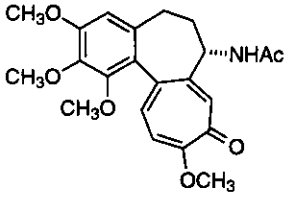
Combretastatin A-4 (**1**)



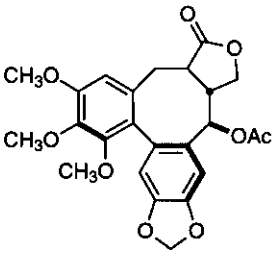
Combretastatin (**2**)



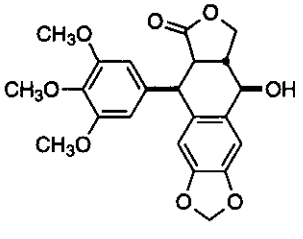
3



Colchicine



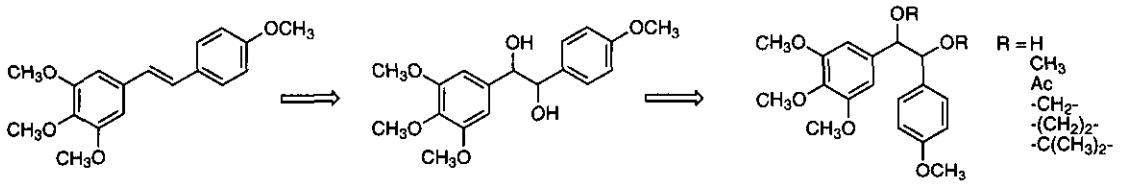
Steganacin



Podophyllotoxin

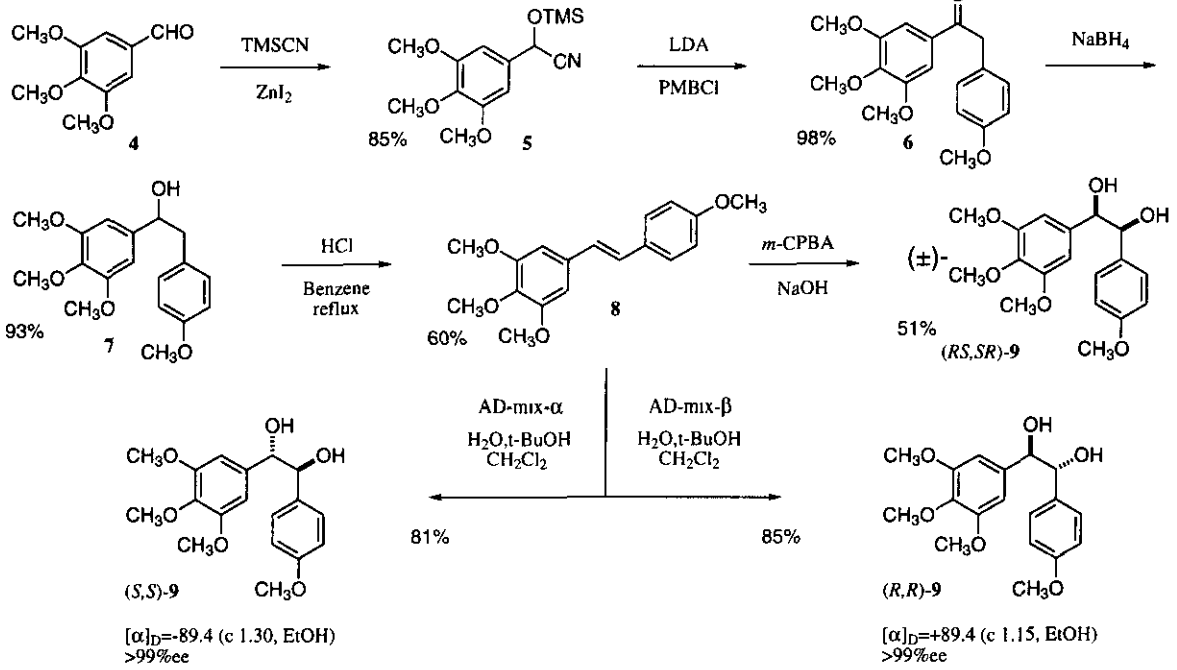
2 have been reported. However, stereochemical differentiation of both enantiomers by tubulin has not been clarified yet. We have been working on the synthesis of novel unnatural analogs of combretastatin.[9] Here, we describe the design and synthesis of conformationally restricted novel heterocyclic combretastatins. For the efficient generation of a variety of restricted conformations regarding to the aromatic ring's orientation, we employed dihydrobenzoins ((*R,R*)-**9**, (*S,S*)-**9**, (*RS,SR*)-**9**) as the key intermediates.[10-11] Since the phenolic hydroxyl group of **1** was not essential for activity,[3] it was omitted because of the ease of synthesis (**Scheme 1**).

Scheme 1

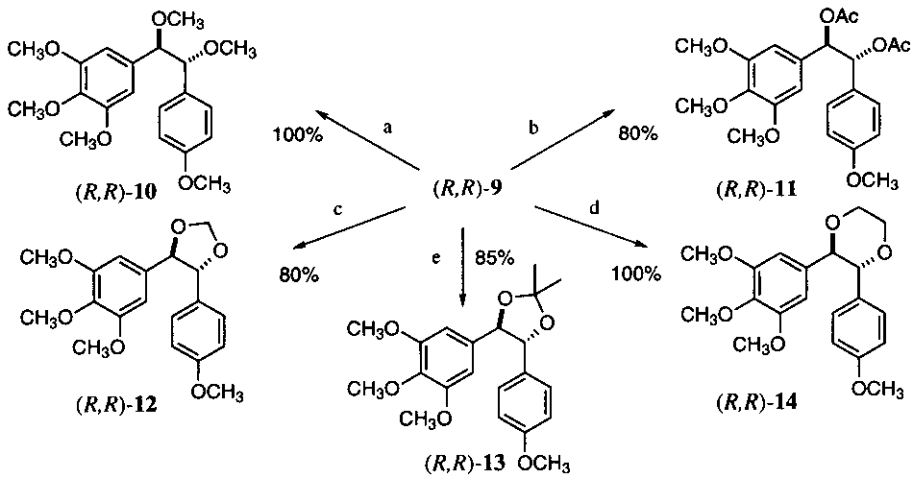


Common precursor *E*-stilbene (**8**) for all compounds was synthesized in 4 steps from 3,4,5-trimethoxybenzaldehyde (**4**) as shown in **Scheme 2**. Chiral (*R,R*)-**9** and (*S,S*)-**9** were synthesized by the asymmetric dihydroxylation of **8** by AD-mix-α and β in high enantiofacial selectivity.[10] Their optical purity was confirmed by ^{1}H-NMR spectra of respective bisMosher esters to be >99%ee. Racemic (*RS,SR*)-**9** isomers were synthesized by the *m*-CPBA oxidation of **8** followed by the ring opening with hydroxide. Derivatization of (*R,R*)-**9**, (*S,S*)-**9** and (*RS,SR*)-**9** was performed as shown in **Scheme 3** to give dimethyl ether (**10**), diacetate

Scheme 2

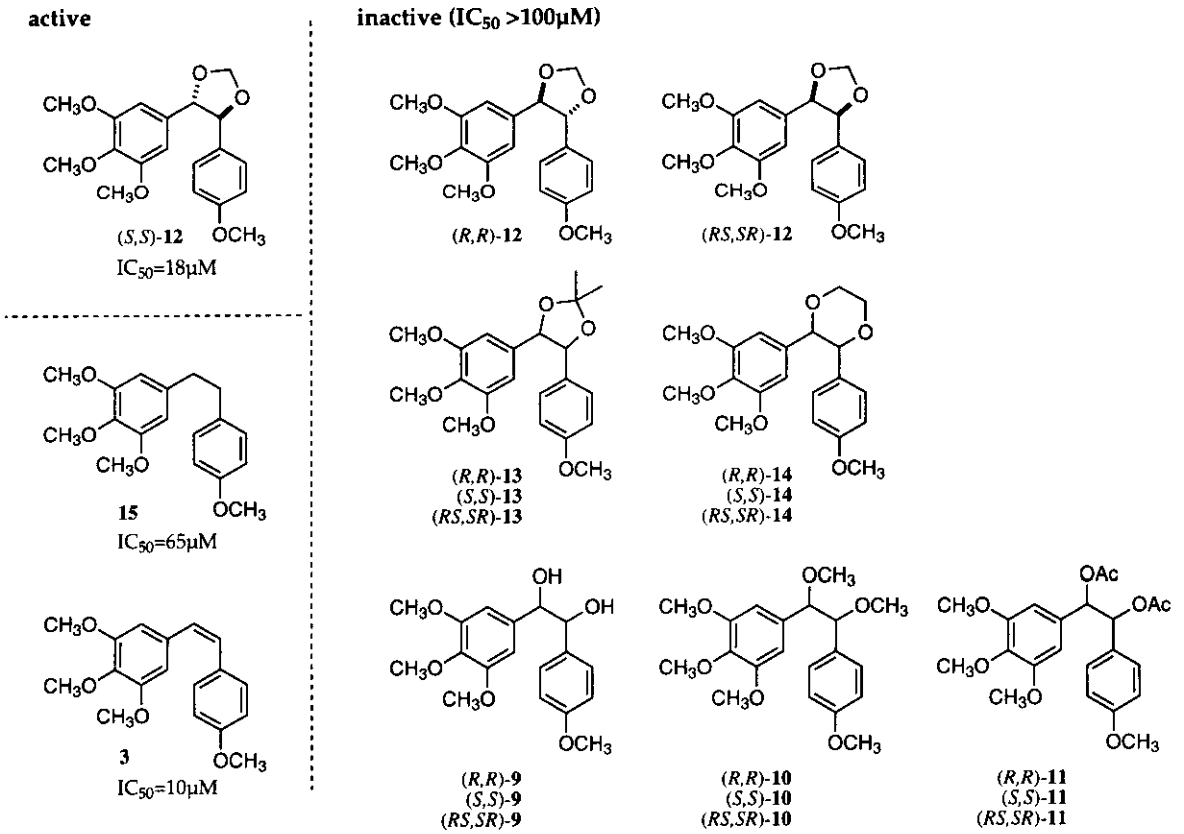


Scheme 3



Reagents and conditions: (a). NaH, CH_3I, THF; (b). Ac_2O, pyridine; (c). CH_2Br_2, aq. 50% NaOH, cetyl$N^+(CH_3)_3Cl^-$; (d). 1,2-dibromoethane, aq. 50% NaOH, cetyl$N^+(CH_3)_3Cl^-$; (e). 2,2-dimethoxypropane, pyridinium *p*-toluenesulfonate.

Figure 2



(**11**), 1,3-dioxolane (**12**), 2,2-dimethyl-1,3-dioxolane (**13**) and 1,3-dioxane (**14**), respectively.
The inhibitory activity of these analogs was examined by the polymerization of porcine brain tubulin assay (IC_{50}, μM) and the result is listed in **Figure 2**. Known synthetic combretastatin analogs (**3**, **15**) reported by Pettit[3] showed strong to moderate activity by our evaluation method.[12] Heterocyclic 1,3-dioxolane analog (*S,S*)-**12** showed relatively strong inhibitory activity. Interestingly, other derivatives and isomers including enantiomer (*R,R*)-**12** and diastereomer (*RS,SR*)-**12** are all inactive. In most cases, restriction of the conformation in chiral states resulted in the disappearance of activity suggesting that these isomers retained disfavored conformation required for tubulin binding. It is reasonable to understand that the conformation of (*S,S*)-**12** is similar to that of both colchicine and **1** bound to tubulin. The stereochemical analysis of these analogs are in progress and will be reported in due course.

ACKNOWLEDGMENT:

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