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DESIGN AND SYNTHESIS OF CURACIN A ANALOGS WITH VARIED SIDE CHAIN STRUCTURES

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Abstract: A series of side chain-modified analogs of curacin A, a powerful antimitotic agent isolated from a Caribbean cyanobacterium was synthesized and the effect of these compounds on *in vitro* microtubule polymerization was examined. The analogs showed weak or no anti-tubulin activity, suggesting that the whole side chain structure of curacin A is required for the interaction with tubulin.
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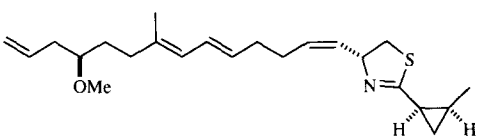
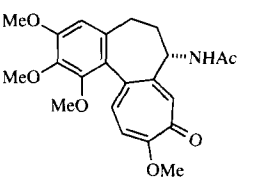
Microtubules are the main component of spindles in the mitotic apparatus of eucaryotic cells, and are also involved in many other essential cell functions, such as axonal transport, motility, and determination of cell shape. The major constituent of the microtubule system is the protein tubulin.[1] There are a number of natural and synthetic compounds that interfere with tubulin function to inhibit the formation of microtubules and to cause the mitotic arrest of eucaryotic cells.[2] Such antimitotic agents show a broad spectrum of biological activities, and have potential applications in the fields of medicine and agriculture. They can also be used as molecular probes for investigating the dynamics of microtubule networks.

Figure 1



Curacin A (**1**)



Colchicine (**2**)

Curacin A (**1**) is a novel antimitotic agent isolated from a Caribbean cyanobacterium, *Lyngbya majuscula*, and consists of a disubstituted thiazoline bearing a chiral cyclopropane ring and an aliphatic side chain.[3] It was reported that curacin A inhibits tubulin assembly by binding to the colchicine-binding site, which is one of two distinct drug-binding sites on tubulin. This is intriguing, because curacin A has little structural similarity to known

natural and synthetic colchicine-site ligands. Thus, elucidation of the nature of curacin A-binding to tubulin should afford further insight into the molecular mechanism of tubulin-ligand interaction at this site, and could lead to the development of new bioactive agents.

In the course of total synthesis of curacin A,[4] we synthesized four stereoisomeric analogs **3a-3b**, comprising a partial structure of curacin A, as an approach to determine the minimal active structure of curacin A (**Figure 2**). However, these compounds did not inhibit tubulin polymerization, and a side chain analog **4** was also inactive. On

Figure 2

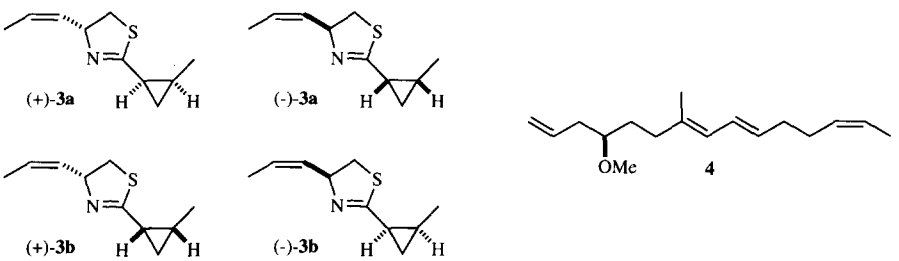
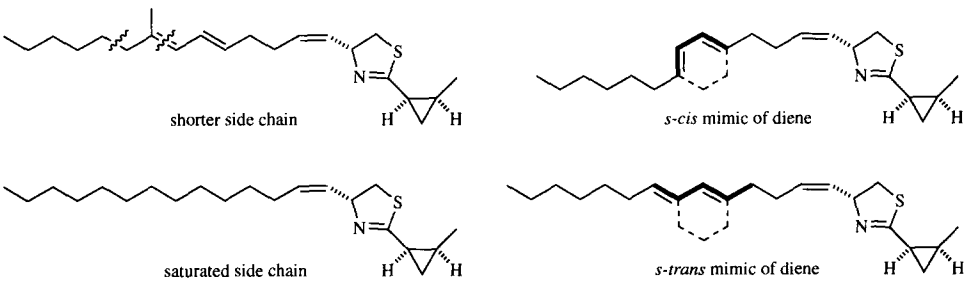
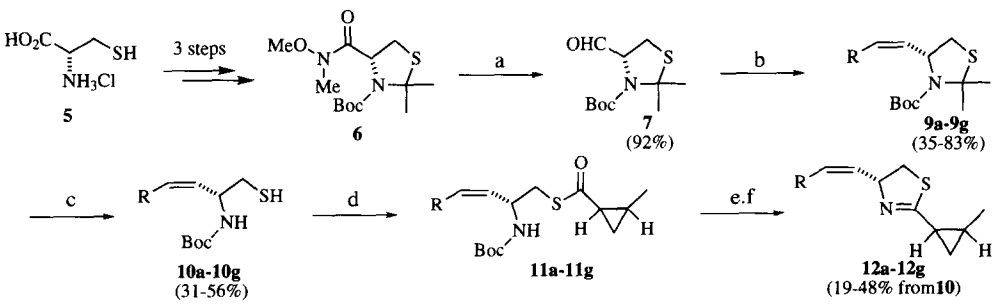


Figure 3



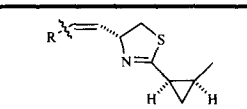
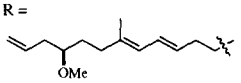
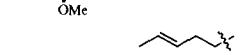
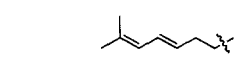
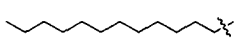
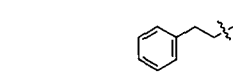
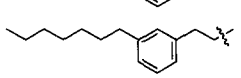
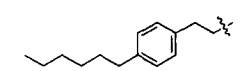
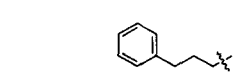
Scheme 1



a). $LiAlH_4$, ether. b). RCH_2PPh_3X (**8a-8g**) (1.2 eq.), NaHMDS (1.1 eq.), THF. c). TFA (15 eq.), sat.H_2O in CH_2Cl_2. d). (±)-2-methylcyclopropane carboxylic acid (1.6 eq.), Et_3N (2.7 eq.), BOPCl (1.9 eq.), CH_2Cl_2. e). TFA (15 eq.), CH_2Cl_2. f). benzene, reflux.

the other hand, Hamel and co-workers reported that several natural and synthetic analogs of curacin A, with diene *E* or *Z* geometry, partially saturated side chain, epimeric 2-methylcyclopropane moiety and ring opening of the cyclopropyl moiety, exhibited strong inhibitory activity towards tubulin polymerization.[5] Their finding is interesting, implying that modification of the side chain or cyclopropane moiety had little effect on microtubule assembly. We thus initiated a structure-activity relationship study of curacin A to clarify the structural requirement for tubulin binding. The variations of the side chain structure, such as shorter, saturated, and fixed conformation of diene geometry through the introduction of aromatic ring frameworks, are shown in **Figure 3**. The general route for the synthesis of these analogs is shown in **Scheme 1**, the same procedures as described for the synthesis of **1** were used.[4a-b, d] Reduction of the amide **6**, prepared from L-cysteine hydrochloride (**5**) in three steps, with $LiAlH_4$ gave the corresponding aldehyde **7** in 92% yield. Wittig reaction of **7** with alkyltriphenylphosphonium halides or alkyltriphenylphosphonium toluenesulfonate (**8a** - **8g**) prepared by conventional methods gave the olefins **9a** - **9g** ($Z : E = > 20 : 1$).[6] Selective deprotection of the *N, S*-acetal group proceeded in diluted TFA in water-saturated CH_2Cl_2 to give the corresponding *N*-Boc amino thiols **10a** - **10g** in moderate yield. Then, thiols **10a** - **10g** were converted to the thiol esters **11a** - **11g** using (±)-cis-2-methylcyclopropanecarboxylic acid and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl). Deprotection of the *tert*-Boc group of **11a** - **11g** with TFA gave the thiol ester ammonium salts, which were refluxed in benzene to yield the thiazolines **12a** - **12g**, respectively.

Table 1. Anti-tubulin Activity and Cytotoxicity of Curacin A Side Chain Analogs

	Compound	Inhibition of tubulin polymerization (IC_{50}: μM)	Tubulin polymerized (%) at 50 μM of drug	Cytotoxicity (IC_{50}: μg/ml)		
				NCI-H69*2	PC-9*2	WiDr*3
R = 	**1**	2.5	-*1	0.89	19.60	<0.20
	**12a**	>50	84	31.67	37.71	33.73
	**12b**	>50	~ 100	-*1	-*1	-*1
	**12c**	>50	~ 100	54.12	77.17	84.91
	**12d**	>50	71	34.06	30.09	19.63
	**12e**	>50	85	64.69	35.53	69.29
	**12f**	>50	95	76.51	50.21	42.49
	**12g**	>50	93	45.16	23.98	19.31

*1 not examined. *2 human lung cancer. *3 human colon cancer.

The effects of the synthesized curacin A and its side chain-modified analogs on microtubule assembly were examined.[7] Curacin A showed strong anti-tubulin activity (IC_{50}=2.5 μM) under the conditions used. Generally, the activity was lost in all the analogs. This result strongly indicates that the side chain of curacin A is strictly recognized by microtubule proteins. Interestingly, analogs with a short side chain **12a** and with an aromatic ring **12d** exhibited weak activity. The activity of these compounds was also examined in three cell lines (NCI-H69, WiDr and PC-9). The cytotoxic activities were not strong, but were in parallel with the respective anti-tubulin activities.

The minimal active structure of the side chain of curacin A is still unclear. Further study is in progress to find the common active structure with colchicine.

Acknowledgment

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