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Antimitotic quinoid triterpenes from *Maytenus chuchuhuasca*

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Abstract—Four cytotoxic quinoid triterpenes, tingenone (**1**), 22β-hydroxytingenone (**2**), pristimerin (**3**), and celastrol (**4**), isolated from *Maytenus chuchuhuasca*, potently inhibit the polymerization of tubulin.
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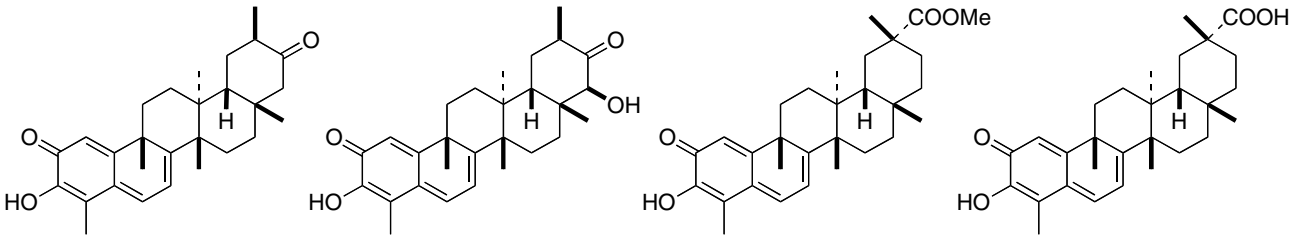
Antimitotic agents that inhibit the microtubule formation and the mitotic arrest of eucaryotic cells, such as paclitaxel and vinblastine, are important components of current anticancer therapy.[1] Paclitaxel is potent inhibitor of cell proliferation and arrests cells in mitosis, but in contrast to vinblastine, promotes the polymerization of purified tubulin, causing stabilization and bundling of microtubules.[1] The antimitotic agents have potential applications in drug development. Recently much effort has been directed to the isolation and synthesis of new antimitotic drugs that target the tubulin/microtubule system and display efficacy against drug-refractory carcinomas.[2]

During our search for bioactive compounds targeting the tubulin/microtubules from medicinal plants,[3] we found that the extract from the bark of *Maytenus chuchuhuasca* (Celastraceae) remarkably inhibits the polymerization of tubulin. Recently we have isolated various triterpenes such as krukovines[4] and 6-oxo-tingenol[5] which showed cytotoxicity, and triterpene dimers such as xuxuarines[6] and isoxuxuarines[7] from *Maytenus krukovii* and *M. chuchuhuasca*. Further investigation on extracts of *M. chuchuhuasca* that target tubulin resulted in the isolation of four quinoid triterpenes, tingenone (**1**), 22β-hydroxytingenone (**2**), pristimerin (**3**), and celastrol (**4**). This paper describes the isolation and inhibitory effects on tubulin assembly of **1**–**4**.

The barks of *M. chuchuhuasca* were extracted with MeOH, and the MeOH extract was partitioned with $CHCl_3$, and then *n*-BuOH, successively. $CHCl_3$-soluble materials inhibiting the polymerization of tubulin were subjected to a silica gel column (*n*-hexane/EtOAc, 1:0 → 0:1) followed by a C_{18} column (CH_3CN/H_2O) to afford four quinoid triterpenes, tingenone (**1**),[8] 22β-



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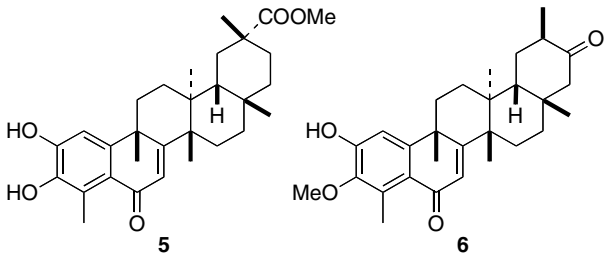
hydroxytingenone (**2**),[9] pristimerin (**3**),[10] and celastrol (**4**),[11] together with two aromatic triterpenes, 6-oxo-pristimerol (**5**)[5] and 3-methyl-6-oxo-tingenol (**6**).[5]

Generally antimitotic agents such as colchicines and vinblastine bind to either the colchicine binding site or the vinca alkaloid binding site. On the other hand, paclitaxel promotes the polymerization of tubulin by binding to and stabilizing the resulting microtubule polymer, which differs from those of colchicines, podophyllotoxin, and the vinca alkaloids.[1]



In this study, it was found that quinoid triterpenes **1**–**4** remarkably inhibited the polymerization of tubulin. Microtubule polymerization and depolymerization were monitored by the increase and the decrease in turbidity. Inhibitory effects of celastrol (**4**) with a carboxylic acid function to tubulin polymerization are shown in Figure 1, in which tubulin polymerization was inhibited in a concentration-dependent manner. On the other hand, pristimerin (**3**) with a methyl carboxylate was found to be 4 times less potent (IC_{50}, 8 μM) than celastrol (**4**), indicating that the carboxylic acid function of quinoid tetracyclic skeleton is favorable for tubulin binding. Tingenone (**1**) without the carboxylic acid function also showed less potent activity than celastrol (**4**). Substitution of the hydroxy group at C-22 did not have much influence on the polymerization of tubulin as shown in Table 1.

We next examined the cytotoxic effects of quinoid triterpenes for human multiple myeloma RPMI8226 cells. The proliferation of RPMI8226 cells was inhibited by each triterpene in a dose-dependent manner. The IC_{50} of each drug against RPMI8226 cells is summarized in Table 2. The degree of cytotoxic activity was reflected by in vitro tubulin polymerization assay. Celastrol (**4**) was more potent than other quinoid triterpenes in the same way, however, pristimerin (**3**) was unexpectedly less effective. It was reported that microtubule agents, such as paclitaxel and vinblastin, induced the formation of reactive oxygen species (ROS) and altered mitochondrial permeability.[12] Therefore, we next investigated whether oxidative stress is involved in the cytotoxicity of celastrol in myeloma cells. Incubation with 5 μM of celastrol resulted in the production of intracellular ROS more effectively than H_2O_2 (data not shown).

Common structural features among these compounds are the presence of diosphenol function in ring A which can be conjugated with ring B. Two triterpenes with an

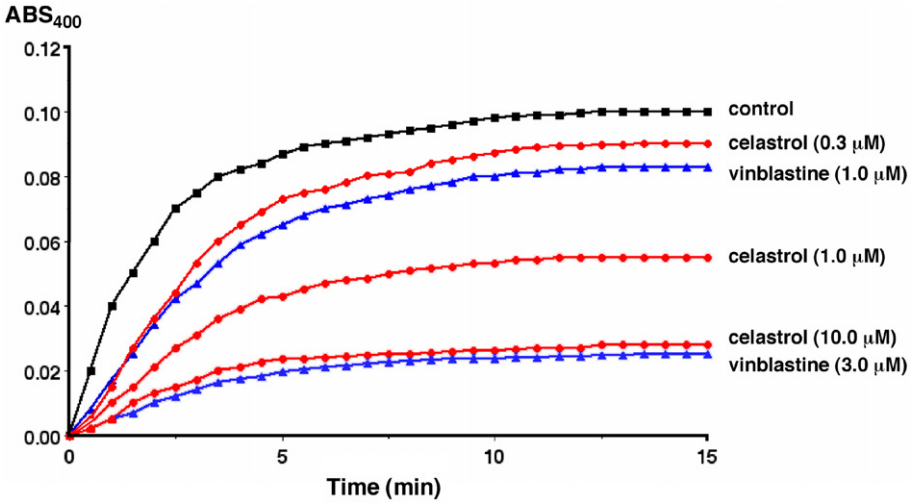


Figure 1. Inhibitory effects of celastrol (**4**) and vinblastine to the polymerization of tubulin protein. Various concentrations of **4** were mixed with tubulin protein (1.0 mg/mL) at 0 °C and incubated at 37 °C. The absorbance at 400 nm was measured.

Table 1. Inhibitory effects of quinoid triterpenes (**1**–**4**) and vinblastine to the polymerization of tubulin

Compound	Tingenone (**1**)	22β-Hydroxytingenone (**2**)	Pristimerin (**3**)	Celastrol (**4**)	Vinblastine
IC_{50} (μM)	8.0	12.0	8.0	2.0	1.0

Table 2. Growth inhibition of human myeloma RPMI8226 cells by quinoid triterpenes (**1**–**4**)

Compound	Tingenone (**1**)	22β-Hydroxytingenone (**2**)	Pristimerin (**3**)	Celastrol (**4**)	Vinblastine
IC_{50} (μM)	7.0	18.3	16.6	3.0	0.028

aromatized A-ring system, 6-oxo-pristimerol (**5**) and 3-methyl-6-oxo-tingenol (**6**), did not show inhibition of polymerization of tubulin at 100 μM. Diosphenol function of these compounds may play a critical role in polymerization of tubulin.

Cytotoxic activity of tingenone related compounds has been well studied so far.[13–15] Investigation of several mechanisms for the cytotoxic action of quinoid triterpenes pointed to the interaction with DNA as the most likely factor involved.[13] However, no evidence could be gained about a relationship between the binding mode and the base composition.[13] Recently, the molecular orbital calculations suggested that a possible mode of cytotoxic action of them involved quasi-intercalative interaction of the compounds with DNA followed by nucleophilic addition of the DNA base to C-6 to the triterpenes.[15]

Conjugated diosphenol function as a common element can be found in the structures of the active triterpenes (**1**–**4**) and this structural feature may show high affinity to the tubulin protein, although the binding site has not been determined yet. Inhibition of tubulin protein, in this experiment, may be one of the important modes of action to show cytotoxic and antitumor activities by quinoid triterpens.

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