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ASYMMETRIC SYNTHESIS OF ANTIMITOTIC COMBRETADIOXOLANE WITH POTENT ANTITUMOR ACTIVITY AGAINST MULTI-DRUG RESISTANT CELLS

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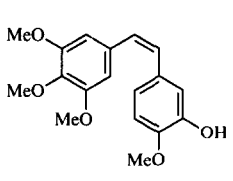
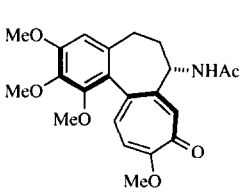
abstract: The (*S,S*)-enantiomer of combretadioxolane (**3**), designed as a chirally preorganized derivative of combretastatin A-4, exhibited quite strong tubulin polymerization-inhibitory activity (IC_{50}: 4-6 μM). (*S,S*)-**3** is 20 times more potent than vincristine as an *in vitro* growth inhibitor (in terms of GI_{50}) of the multi-drug-resistant (MDR) cell line PC-12, which produces P-glycoprotein.

Combretastatin A-4 (**1**), which was isolated from a South African tree, *Combretum caffrum*, exerts its antineoplastic activity by disrupting the polymerization process of tubulin to microtubules. In spite of the structural simplicity of **1**, its antitubulin activity and cytotoxicity to tumor cells are quite strong.[1,2] In the chemotherapy of cancer, the emergence of multi-drug resistant (MDR) cells presents a serious problem, so it is noteworthy that **1** displays strong cytotoxicity toward daunorubicin-resistant P-388 cells.[3] Therefore, considerable attention has been paid in the development of new antineoplastic and antimitotic agents based on **1**.[4] It is well known that the antimitotic agent colchicine (**2**), which shares the same binding site as **1**, has (a*S*,7*S*)-configuration.[5] We have been working on the synthesis of unnatural combretastatins with potent antitubulin activity.[6] In the previous report we demonstrated that the dioxolane based *cis*-(*S,S*) analog was the sole active compound out of twelve chiral stereoisomers.[7] Here, we describe the design and synthesis, by using a dioxolane-based chilaity inducer, of a novel combretastatin (**3**) with strong antitubulin activity and cytotoxic activity towards MDR cells.

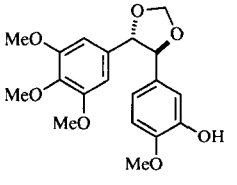
Figure 1



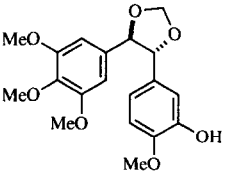
Combretastatin A-4 (**1**)



Colchicine (**2**)



(*S,S*)-**3**



(*R,R*)-**3**

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Asymmetric dihydroxylation of *trans*-stilbene (**6**), synthesized from 3,4,5-trimethoxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehyde, with AD-mix-α gave the chiral diol (*S,S*)-**7**.[8] Its optical purity was confirmed to be >99%ee by examination of the ^{1}H-NMR spectrum of the bisMosher ester. The diol **7** was converted to 1,3-dioxolanes (**8**) by treatment with 50% NaOH and dibromomethane in dichloromethane in the presence of a phase-transfer catalyst. Deprotection of the MOM ether was performed by heating in 80% acetic acid to give the desired (*S,S*)-**3**. (*R,R*)-**3** was synthesized similarly using AD-mix-β (Scheme 1).

The tubulin polymerization-inhibitory activity of (*S,S*)- and (*R,R*)-**3** was examined by measurement of the turbidity of an aqueous solution of porcine brain tubulin in the presence of respective drug (Figure 2).[9] (*S,S*)-**3** exhibited quite strong inhibitory activity (IC_{50}: 4-6 μM), which was comparable to that of combretastatin A-4 (IC_{50}: 3-4 μM) and much stronger than colchicine (IC_{50}: 10 μM) under the experimental conditions. On the other hand, (*R,R*)-**3** was almost inactive (IC_{50}: >50 μM). This result clearly demonstrates that (*S,S*)-**3** takes a favorable conformation for tubulin binding, while (*R,R*)-**3** does not.[7, 10]

Scheme 1

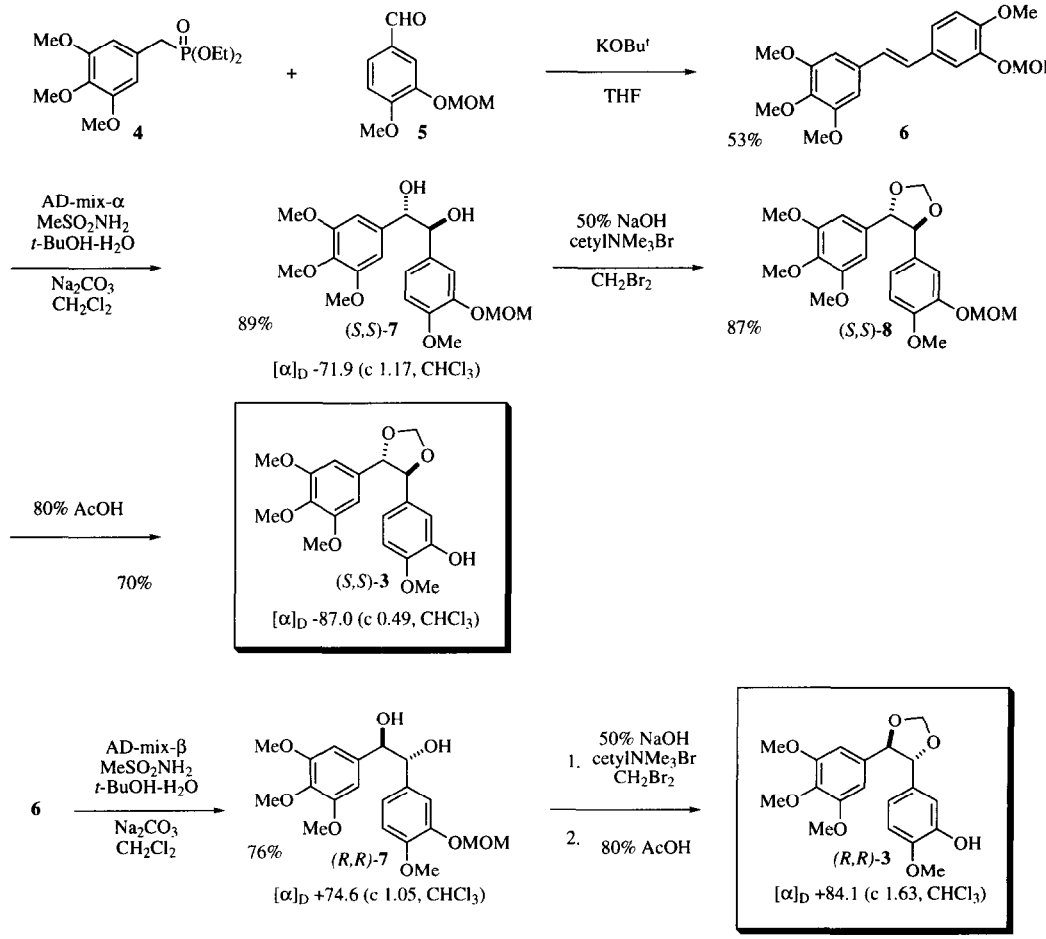
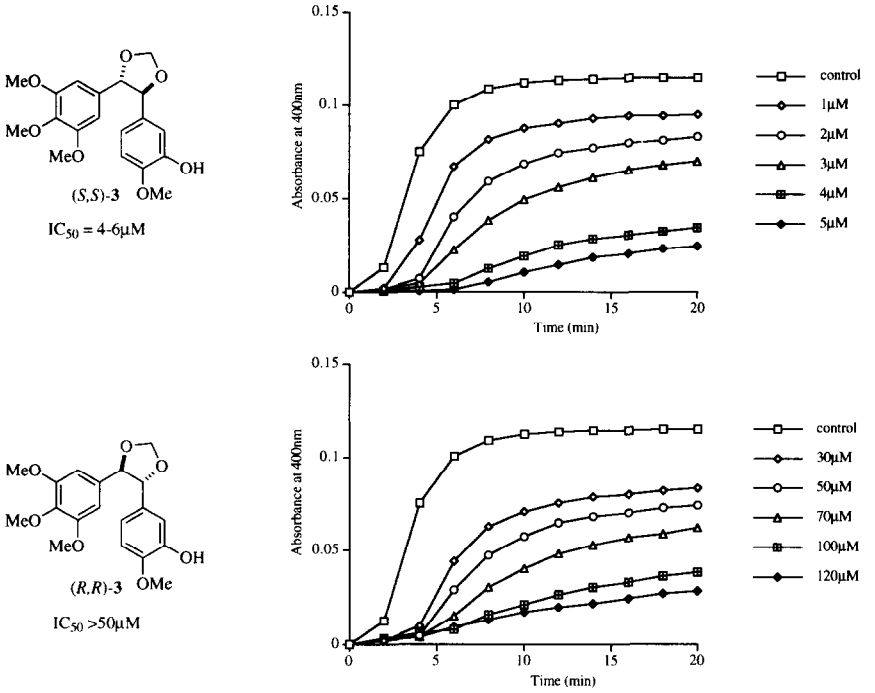


Figure 2. *In vitro* tubulin polymerization in the presence of (*S.S*)- and (*R,R*)-**3**



We also examined the *in vitro* growth inhibition (GI_{50}) of PC-6 and PC-12 cells, of which the latter exhibited multidrug resistance (MDR) (Table 1). (*S,S*)-**3** showed potent activity towards both cell lines with almost the same GI_{50} values, and was 60 to 100 times more potent than cisplatin. In addition, (*S,S*)-**3** exhibited 20 times stronger activity than vincristine towards PC-12, which express P-glycoprotein as a mechanism of MDR resistance, though it was slightly less potent than vincristine against PC-6 cells.

Table 1. *In vitro* growth inhibition (GI_{50}) of sensitive and resistant cells by various drugs

cells	cisplatin	vincristine	(*S,S*)-**3**
PC-6	563 nM	1.14 nM	5.41 nM
PC-12 (MDR)	300 nM	104 nM	5.19 nM

In conclusion, chiral dioxolane-based (*S,S*)-**3** was effectively differentiated by tubulin, displaying strong, chirality-selective inhibition of tubulin polymerization. It also inhibited the *in vitro* growth of PC-12 cells with acquired resistance to vincristine. Since the mode of action of both vincristine and (*S,S*)-**3** is disruption of the polymerization process of tubulin to microtubules, (*S,S*)-**3** is considered to be a new lead compound for the development of MDR-overcoming antineoplastic agents targeting tubulin .

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