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NOVEL B-RING MODIFIED COMBRETASTATIN ANALOGUES : SYNTHESES AND ANTINEOPLASTIC ACTIVITY

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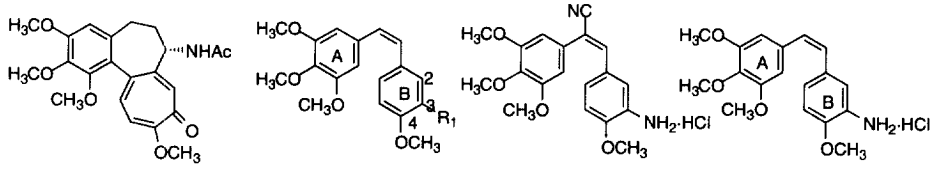
ABSTRACT: A series of B-ring modified combretastatin analogues were synthesized and their inhibitory activity against microtubule assembly, cytotoxic activity against Colon 26 adenocarcinoma cancer cell line were evaluated. Among these, pyridone derivative (**19**) showed strong antimitotic activity and cytotoxicity, along with excellent water-solubility.

INTRODUCTION: Combretastatin A-4 (CA-4) (**2**) is one of the most potent antimitotic agents derived from *Combretum caffrum* and binds to tubulin at the colchicine binding site.[1] This agent shows strong cytotoxicity against variety of human cancer cells including multi-drug resistant cancer cell lines.[2] However, the low water-solubility of CA-4 limits its efficacy *in vivo* and a water-soluble sodium phosphate prodrug of CA-4 (**3**) is being evaluated for clinical application.[3] We recently synthesized a series of CA-4 derivatives to improve water-solubility and obtained new antimitotic agents AC-7728 (**4**) and AC-7739 (**5**) (**Figure 1**).[4] These compounds show marked tumor growth suppression against colon 26 murine tumor model.

Figure 1



The potent *in vivo* efficacy of AC-7739 (**5**) is attributed to the replacement of the phenolic OH of CA-4 with an amino group. This fact prompted us to synthesize other types of nitrogen containing CA-4 analogues in search of more potent compounds *in vivo*. By a series of SAR studies of combretastatins, the cis-orientation of

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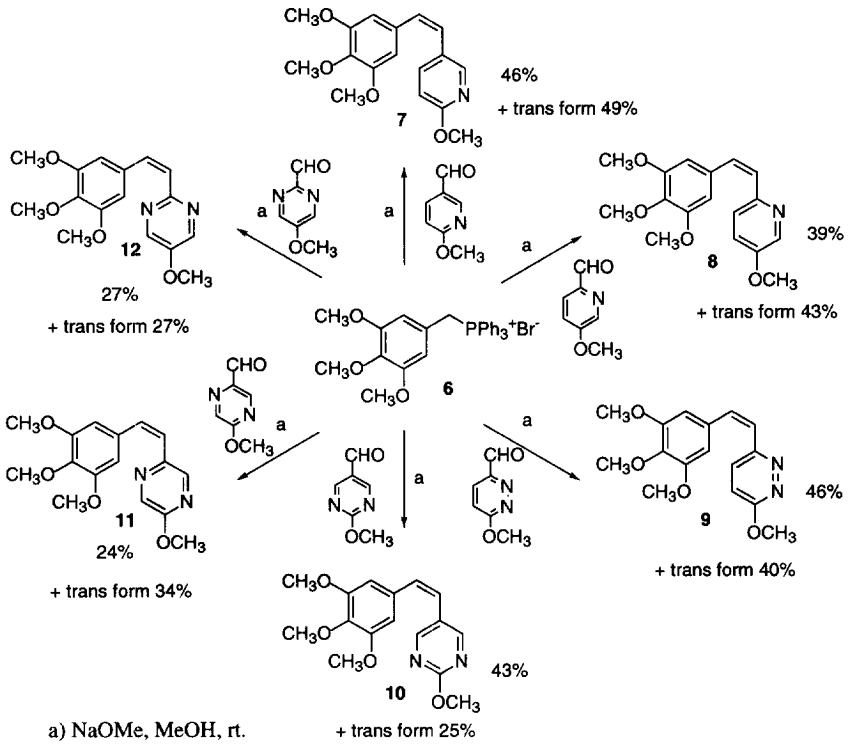
two benzene ring and 3,4,5-trimethoxy group on the A ring were found to be essential to strong cytotoxicity.[5] Substitution on the 4-position of the B ring is also indispensable.[5] To obtain compounds with potent activity and improved physicochemical properties, we designed B-ring modified combretastatins with intact cis olefin.[6] In this paper, we report "heterocombretastatins" in which the B-ring was replaced by a variety of 6-membered heterocycles having substituents on the 4-position.

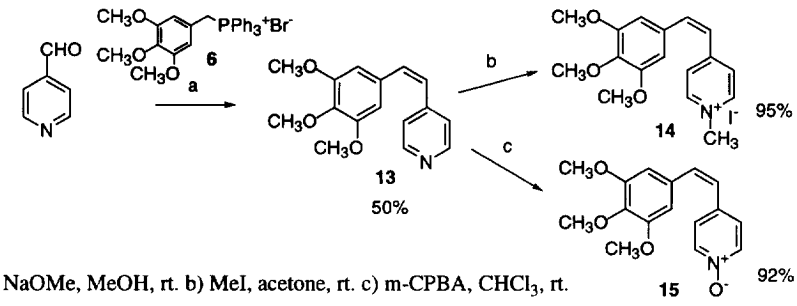
Scheme 1



Synthesis

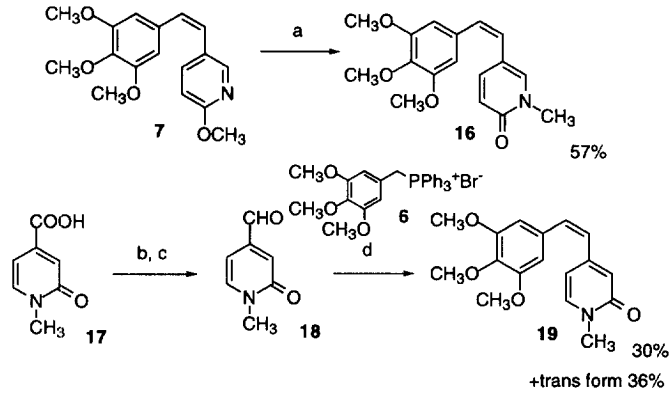
Wittig reaction of heterocyclic aldehydes with phosphonium bromide **6** at room temperature gave ***E***, ***Z***-mixture of stilbenes. Obtained ***E***, ***Z***-mixtures were purified by silica-gel column chromatography to give the desired ***Z***-form derivatives (**7-12**) (**Scheme 1**). The synthesis of pyridinium derivatives (**14**, **15**) is shown in **Scheme 2**. (***Z***)-pyridyl derivative **13** was reacted with MeI to give ***N***-methylpyridinium iodide (**14**) in 95% yields.[7] Derivative **13** was reacted with m-CPBA to give pyridinium ***N***-oxide (**15**) in 92% yields. The synthesis of ***N***-methylpyridone derivatives is shown in **Scheme 3**. Pyridine derivative **7** was isomerized with MeI to give 5-substituted pyridone (**16**) in 57%.[8] The 4-substituted pyridone (**19**) was synthesized by reaction of ***N***-methyl-2-pyridone-4-aldehyde (**18**) and phosphonium bromide **6** in 30% yields. The ***N***-methyl-2-pyridone-4-aldehyde (**18**) was synthesized from **17** which was easily prepared by the previous procedure.[9]

Scheme 2



a) NaOMe, MeOH, rt. b) MeI, acetone, rt. c) m-CPBA, $CHCl_3$, rt.

Scheme 3



a) MeI, MeOH, rt. b) $LiBH_4$, EtOH, rt. c) MnO_2, $CHCl_3$, rt. d) NaOMe, MeOH, rt

Result and Discussion

The biological properties of the synthesized compounds are shown in **Table 1**. Tubulin polymerization inhibitory activity of these compounds was tested against bovine brain tubulin.[10] Cytotoxic activity was tested against Colon 26 adenocarcinoma cancer cell lines.[11]

As a result, 2-methoxypyridine **7**, 3-methoxypyridine **8**, and pyrimidine derivative **10** showed potent antitubulin activity (IC_{50} 2μM, 3μM, and 3μM, respectively), while only compound **7** showed strong cytotoxicity (IC_{50} 29.2nM). Pyridazine derivative **9** and pyrimidine derivative **12** lost their antitubulin activity (IC_{50} >10μM) and cytotoxic activity (IC_{50} >3000nM), while pyrazine derivative (**11**) also showed decreased activity. Introduction of a nitrogen atom at the 2-position of the B ring (**8**, **9**, **11**, **12**) decreased antimitotic activity and cytotoxicity.

N-Substituted pyridinium compounds (**14, 15**), which were synthesized to improve water-solubility, did not show antitubulin or cytotoxic activity. A cationic center on the 4-position is not appropriate. Next, we examined pyridone derivatives, which were neutral and water soluble. 5-Substituted pyridone **16** lost antitubulin activity and cytotoxicity. However, 4-substituted pyridone (**19**) showed strong antitubulin activity (IC_{50} 2 μM) and cytotoxiciy (IC_{50} 19.2nM).

Table 1. Biological Activities of B-ring modified combretastatins

Compd No.	anti-tubulin[a)] IC_{50} (μM)	Cytotoxicity[b)] IC_{50} (nM)
7	2	29.2
8	3	182
9	>10	>3000
10	3	275
11	9	880
12	>10	>3000
14	nt.	>3000
15	>10	>3000
16	>10	>3000
19	2	19.2
5 (AC-7739)	1	2.8
2 (CA-4)	2	8.7

a) Tubulin polymerization was determined as shown in ref 10. b) Drug concentration required to inhibit the growth of Colon 26 cells by 50%, see ref 11.

Plasma solubility of AC-7739 (**5**), **7**, and **19** at physiological pH was examined. Pyridone **19** showed excellent solubility (5200μg/ml in human plasma). Pyridine derivative **7** also showed improved solubility (3400μg/ml) compared to AC-7739 (1520μg/ml).

In conclusion, a number of B-ring modified analogues of CA-4 were prepared and their tubulin inhibitory activity and *in vitro* antineoplastic activity were determined. The fact that pyridine derivative **7** and pyridone derivative **19** exert potent anti-tubulin activity and cytotoxicity indicates that the B-ring of CA-4 can be replaced with heterocycles and still retain biological activity. *In vivo* evaluation of **7** and **19** is now underway in our laboratory.

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