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Antimitotic and antivascular activity of heteroaroyl-2-hydroxy-3,4,5-trimethoxybenzenes

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ABSTRACT

This study reports the synthesis of a series of heteroaroyl-2-hydroxy-3,4,5-trimethoxybenzenes, which are potent antitubulin agents. Compound **13**, (2-hydroxy-3,4,5-trimethoxyphenyl)-(6-methoxy-1*H*-indol-3-yl)-methanone exhibits marked antiproliferative activity against KB and MKN45 cells with IC_{50} values of 8.8 and 10.5 nM, respectively, binds strongly to the colchicine binding site and leads to inhibition of tubulin polymerization. It also behaves as a vascular disrupting agent which suppresses the formation of capillaries. The C2-OH group in the A-ring of this compound not only retains the biological activity but has valuable physicochemical properties.

1. Introduction

Combretastain A4 (**1**, CA-4), a natural component of *Combretum caffrum*, has been identified as a potent antimitotic agent[1] which, after docking into the colchicine binding site of tubulin, interrupts the polymerization of tubulin. Compound **1** is characterized by a *cis*-stilbene containing a 3,4,5-trimethoxyphenyl ring, which is referred to as the A-ring. Recently, prodrugs like compounds **2** and **4**, which possess better solubility than their parent compounds **1** and **3**, have been undergoing clinical trials (see Fig. 1).[2] Since 1995, numerous researchers have invested much effort on this natural chemical due to its remarkable antiproliferative activity and concise structure. Most research has involved modifications to the *cis*-double bond and the B-ring, but work on the A-ring is sparse. To date, we have carried out a good deal of research on the development of potent antimitotic agents, our earlier efforts having been focused on modification of the *cis*-double bond and B-ring regions of compounds such as **1**. We utilized the carbonyl group, which is adopted from phenstatin, as a linker to retain the *Z*-geometry, and then connected it with various aromatic rings as a B-ring thus generating aminobenzophenones,[3,4] 2-amino-3,4,5-trimethoxybenzophenones,[5] and aroylindoles.[6,7] Among these, SCB01A (**5**) which is categorized as a 3-aroylindole derivative, is undergoing clinical trials.[2] In our previous work,[8,9] the trimethoxyindoles were developed to mimic the parts of the A-ring and the *cis*-double bond, and this opened an avenue of A-ring modification in our laboratory. The work reported here on 2-hydroxy-3,4,5-trimethoxy-benzophenones revealed that the 2-hydroxy-3,4,5-trimethoxybenzoyl group possibly mimics the A-ring/*cis*-stilbene region through the formation of a pseudo-ring.[10] This latest finding underlay our efforts to develop promising antitubulin agents.

The strategy of development of prodrugs has been utilized to increase the water solubility of antitubulin agents and some of them such as CA-4P (**2**)[11] and AVE-8062 (**4**)[12] are currently undergoing clinical trials. The phosphate moiety is frequently exploited in the development of prodrugs, and it is commonly connected to the –OH group of B-ring.[13–15] The discovery of the utility of the 2-hydroxy-3,4,5-trimethoxybenzoyl group could provide an opportunity for the development of a prodrug with a phosphate group in the A-ring. To consider the effect on antiproliferative activity of replacing the B-ring by heterocycles, this study uses the 2-hydroxy-3,4,5-trimethoxybenzene moiety as an anchor and connects it with various heterocycles such as quinoline and indole which were used in our previous studies.[16,17] As a result, a series of heteroaroyl-2-hydroxy-3,4,5-trimethoxy-benzenes (**8**–**13**) were

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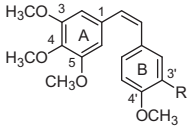


1: Combretastatin A-4 (CA4), R = OH
2: Combretastatin A-4P (CA4P), R = OPO_3Na_2
3: R = NH_2
4: AVE-8062, R = NH-serine

Figure 1. Combretastatin A-4 derivatives.

synthesized and the relevant biological assays were conducted (see Fig. 2).

2. Results and discussion

2.1. Chemistry

The synthesis of heteroaroyl-2-hydroxy-3,4,5-trimethoxybenzenes (**8**–**13**) is illustrated in Scheme 1. The aldehyde group of compound **14** was converted into a hydroxyl by hydrogen peroxide under Dakin oxidation conditions, yielding the corresponding phenol **15**. Treatment of compound **15** with tetrabutylammonium tribromide (TBATB) afforded compound **16** whose hydroxyl group was protected by –MOM to afford compound **17**. The resulting brominated product underwent metal-halogen exchange using a *n*-BuLi, addition reaction with various heterocycle carbaldehydes, followed by oxidation by pyridinium dichromate to furnish compounds **18**–**22**. Reaction of compound **17** with quinoline-4-carbaldehyde under the same reaction conditions afforded compound **10**. The *N*-protected product (**22**) was hydrolyzed under basic conditions to afford compound **23**. Finally, the removal of –MOM group of compounds **18**–**21** and **23** was carried out with 1N HCl, yielding anticipated compounds **8**, **9** and **11**–**13**.

2.2. Biological evaluation

2.2.1. In vitro cell growth inhibitory activity

All synthetic compounds (**8**–**13**) and reference compounds, colchicine and compound **1**, were evaluated for the antiproliferative

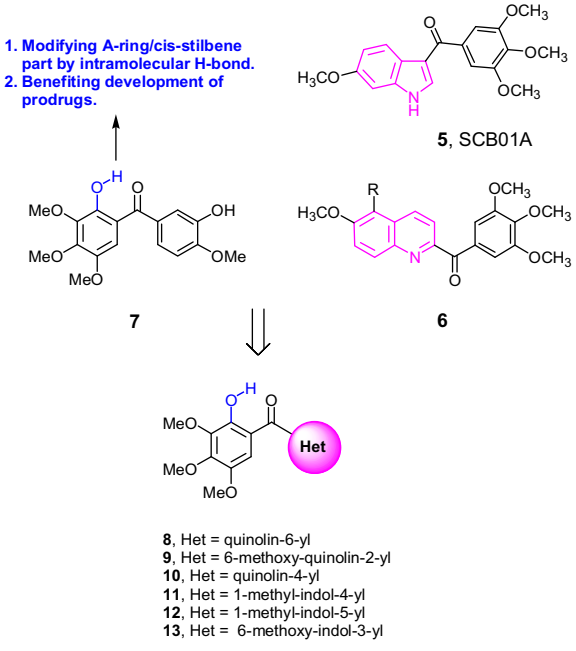
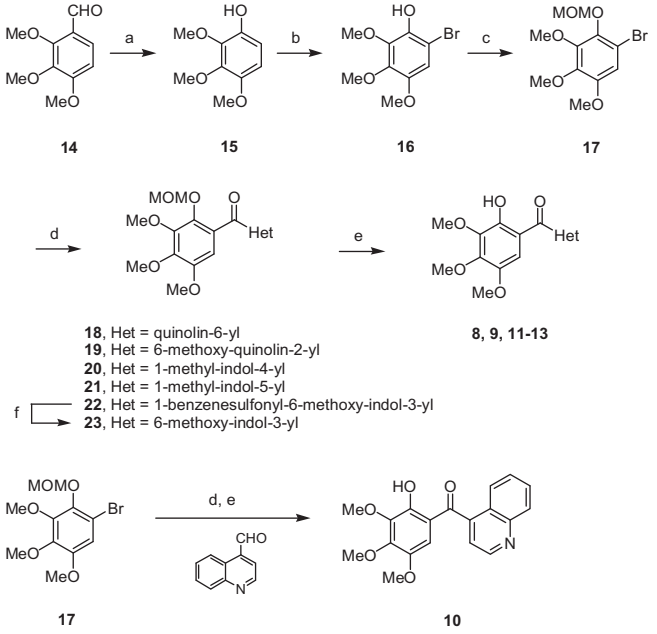


Figure 2. Schematic design of target compounds (**8**–**13**).



Scheme 1. Reagents and conditions: (a) 30% H_2O_2, H_2SO_4, MeOH, rt; (b) tetrabutylammonium tribromide, DCM, rt; (c) MOM-Cl, DIPEA, DCM, 0 °C to rt; (d) (i) *n*-BuLi, THF, −78 °C to 0 °C; (ii) various heterocycle carbaldehydes, THF, 0 °C; (iii) PDC, DCM, rt; (e) 1N HCl, MeOH, rt; (f) 3N NaOH, EtOH, reflux.

activities against two human cancer cell lines, cervical carcinoma KB cells and stomach carcinoma MKN45 cells (Table 1). The results indicated that compounds **11**–**13**, with an indole core exhibited more highly potent antiproliferative activity than quinoline-containing compounds **8**–**10**. All compounds with the exception of colchicine showed mild potency for HT29 cells. Among the compounds with a quinoline core, compound **8** showed moderate inhibition of the growth of KB and MKN45 cells, with IC_{50} values of 22.9 and 116 nM, respectively. Compound **13** showed slightly better antiproliferative activity than that of colchicine, but 3-fold weaker than CA-4. It suppressed the growth of KB and MKN45 cells with IC_{50} values of 8.8 and 10.5 nM, respectively, which is slightly weaker than compound **5** (IC_{50} = 3.6 nM for KB cells;[18] IC_{50} = 4.0 nM for MKN45 cells[16]). This result revealed that introduction of a 2-OH is favorable in the indole-containing compounds. Compound **13** contains the same B-ring unit, 6-methoxyindole, as compound **5**, indicating that the C2-OH group in the A-ring does not significantly affect the biological activity. A literature review revealed that the most phosphate prodrugs of antitubulin agents possess a polar group in the B-ring part rather than in the A-ring. The antiproliferative potency retained after introducing a C2-OH in the A-ring may offer an avenue toward further development

Table 1
Antiproliferative activity of compounds **8**–**13**

Compd	$IC_{50} \pm SD$[a] (nM)	
	KB cells	MKN45 cells
8	22.9 ± 6.7	116 ± 20.8
9	230.4 ± 19.9	1163 ± 375
10	7695 ± 916	5963 ± 1990
11	57.3 ± 6.7	55.4 ± 21.5
12	33 ± 9.3	93.7 ± 20.3
13	8.8 ± 0.7	10.5 ± 2.1
CA4	2.5 ± 0.8	3.2 ± 1.5
Colchicine	12.4 ± 2.5	14.9 ± 2.5

[a] SD: standard deviation, all experiments were independently performed at least three times.