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Application of plant allylpolyalkoxybenzenes in synthesis of antimitotic phenstatin analogues

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ABSTRACT

Phenstatin and its derivatives with the modified ring A have been synthesized, using plant allylpolyalkoxybenzenes as a starting material. The targeted molecules were evaluated in a phenotypic sea urchin embryo assay for antiproliferative activity. It was found that phenstatin ring A modifications yielded antimitotic compounds. The most effective myristicin derivative **7d** (combretastatin A-2 analogue) was determined to be ca. 10 times more potent than phenstatin, displaying antimitotic tubulin-destabilizing activity at the same concentration range as combretastatins. In contrast to combretastatins, **7d** featured the steric stability with potential for further design as anticancer agent.

Combretastatins are natural antimitotic stilbenes derived from the bark of *Combretum caffrum* Kuntze (Combretaceae).[1] The three most active molecules combretastatin A-1, A-2, and A-4 (CA1, CA2, CA4; Fig. 1) have attracted a great attention as potent inhibitors of cancer cell growth and tubulin polymerization.[2–5] The phosphorylated prodrugs Zybrestat and Oxi4503 are currently undergoing clinical evaluation as antitumor vascular targeting agents.[4,6,7] Numerous studies suggest that the olefinic bond plays a significant role in the orientation of benzene rings in stilbene molecule, optimizing the distance and dihedral angle for targeting tubulin.[8,9] The anticancer activity of stilbenes strictly requires for *Z*-geometry, however, the compounds are prone to isomerization during storage and administration and in the course of metabolism in liver microsomes.[10–12] In this regard, benzophenone analogues with biaryl system connected by a carbonyl group have several advantages including no need of controlling the geometric selectivity (*Z*- and *E*-configuration) as well as the ease of synthetic design for increased potency and stability. It was also suggested that the sp^2-hybridized carbonyl group in benzophenones constrains the two aryl rings in a quasi 'cis' orientation necessary for efficient interaction of a molecule with the colchicine binding site of tubulin.[13]

Phenstatin was designed from the CA4 skeleton by replacement of the double bond linker with a carbonyl moiety (Fig. 1).[13,14] It is a potent microtubule destabilizing agent that interacts with colchicine binding site of tubulin and displays significant anticancer and antimitotic activities comparable to those of CA4.[13] Phenstatin and related compounds are currently under preclinical development.[15–18] Among them, the benzophenone derivative of CA1 hydroxyphenstatin (Fig. 1) is a potent inhibitor of tubulin polymerization and displays pronounced cytotoxicity against a panel of cancer cell lines.[15,16] Multiple SAR studies of phenstatin analogues showed that the B-ring (substituted phenyl) could be successfully modified without losing cytotoxicity and antitubulin properties by substitution of *m*-OH for *m*-NH_2 group,[19–21] or by replacement with thienyl,[22] naphthyl,[23,24] benzo[*b*]thiophenyl,[25,26] benzo[*b*]furanyl,[27] indolyl,[28,29] carbazolyl,[30] quinolyl,[31] and indazolyl[32] templates. The carbonyl bridge of phenstatins is not essential for antimitotic behavior, since isocombretastatins featuring a 1,1-diarylethylene scaffold displayed potent inhibition of tubulin assembly and cytotoxicity against cancer cell lines similar to those of the corresponding combretastatins.[33,34] In contrast, the reported modifications of the 3,4,5-trimethoxyphenyl A-ring in phenstatin analogues, namely, changes in distribution or removal of the methoxy groups, dramatically reduced the cytotoxic and antitubulin activity.[23,25,30,33,35,36] Recently it was found that 2,3,4-trimethoxyphenyl A-ring in combination with *N*-methyl-5-indolyl B-ring yielded a potent cytotoxic tubulin destabilizing compound, the only one exception reported

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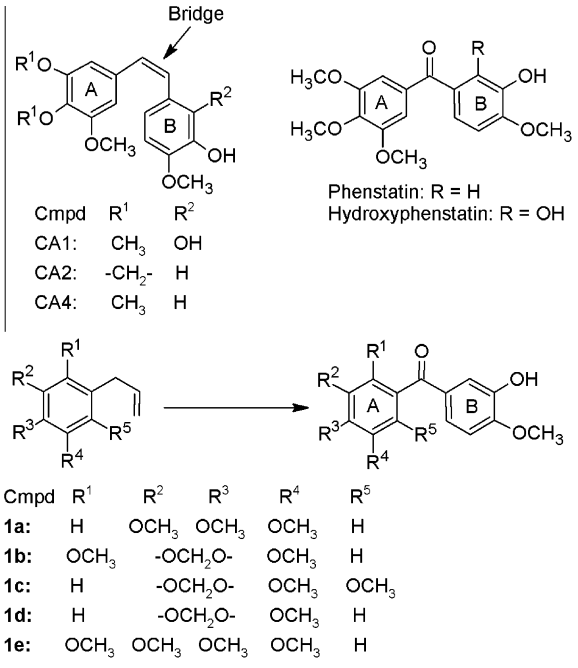


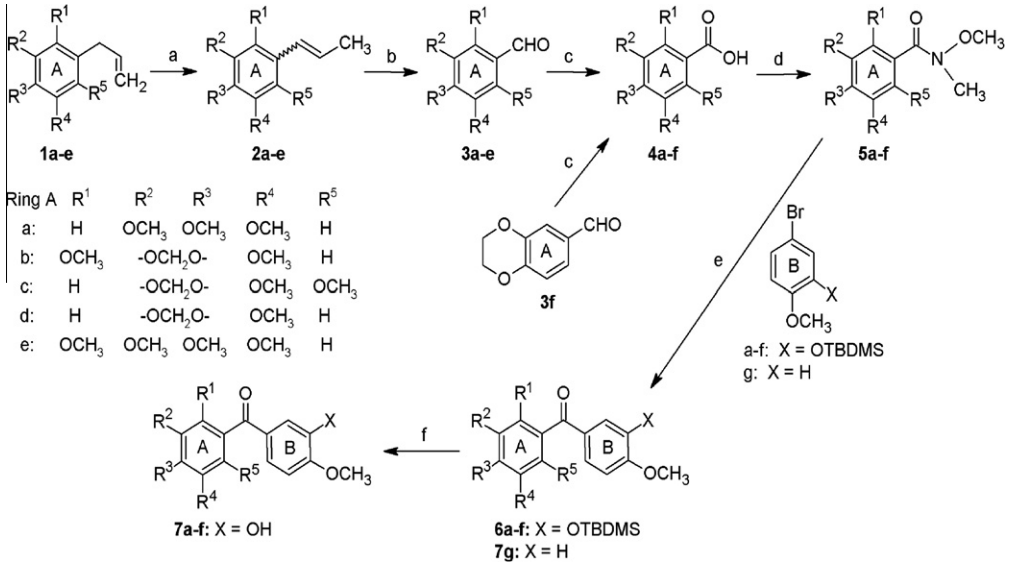
Figure 1.

to date.[37] However, the biological effects of benzophenones derived from CA2 with methylenedioxy moiety in the ring A have never been studied. Moreover, the methylenedioxybenzene pharmacophore is featured frequently in antimitotic natural products interacting with colchicine binding site of tubulin, such as cornigerine, podophyllotoxin, steganacin, and alkaloids isolated from *Papaver* and *Chelidonium*.[38,39]

In searching for a reliable natural source of building blocks containing methylenedioxy group for phenstatine analogues, the Apiaceae family of plants became of interest. Previously it was discovered that seeds of parsley, *Petroselinum sativum* Hoffm., cultivated in Russia, and dill, *Anethum graveolens* L., grown in India, are both versatile sources of allylpolyalkoxybenzenes elemicin (**1a**), apiol (**1b**), dillapiol (**1c**), myristicin (**1d**), and tetramethoxybenzene (**1e**) (Fig. 1) that can be isolated by liquid CO_2 extraction followed by high-efficiency large-scale distillation (up to 40 kg of allylbenzenes).[40] In our recent study compounds **1a–e** have been successfully used in the synthesis of combretastatin analogues with significant antimitotic tubulin destabilizing activity.[41–43]

Previously phenstatin and related compounds were synthesized by the reaction of Ar–Li with amides or aldehydes as a starting material.[13,19,37,44] Another efficient economical route of phenstatin synthesis has been developed, providing overall yield of 60%.[45] The procedure included the reaction of guaiacol, protected by easily cleaved chloroacetyl function, with 3,4,5-trimethoxybenzoic acid, using polyphosphoric acid as condensing agent. Recently a nucleophilic addition of ArMgBr onto the Weinreb amides has been described to produce phenstatin analogues.[46] In the present study a combination of the specialized ozonolysis equipment and optimized synthetic protocol allowed for preparation of the targeted aldehydes **3a–e** from the respective styrene precursors **2a–e** (Scheme 1, step b) in yields of 60–85% on a 100 g scale.[40,42] The polyalkoxybenzoic acids **4a–f** were obtained in high yields (80–90%) from the aldehydes **3a–f** using the urea–hydrogen peroxide complex in water (Scheme 1, step c). Phenstatin (**7a**), as well as its methylenedioxy (**7b–d**), tetramethoxybenzene (**7e**), and ethylenedioxy (**7f**, **g**) analogues were synthesized via transformation of acids **4a–f** to corresponding Weinreb amide precursors **5a–f** (Scheme 1, step d), using a procedure reported previously,[47] with a new modification involving carbonyl diimidazole as condensation agent. Then amides **5a–f** were treated with Li-salt of TBDMS-protected guaiacol followed by the removal of TBDMS-group (Scheme 1, steps e and f). Experimental details regarding syntheses and analytical results are presented in Supplementary data.

Synthetic derivatives of phenstatin (**7a–g**, Scheme 1) were further evaluated for their antiproliferative and tubulin-destabilizing activity using a phenotypic sea urchin embryo assay[48] with CA2 and CA4 as benchmark reference compounds (Table 1). This in vivo assay allows for the robust and reliable identification of compounds targeting tubulin including their antiproliferative, antimitotic, and cytotoxic effects. It features (i) a fertilized egg test for antimitotic activity as displayed by cleavage alteration/arrest and (ii) behavioral monitoring of a free-swimming blastula treated



Scheme 1. Reagents and conditions: (a) powdered KOH, $(n\text{-Bu})_4N^+Br^-$, without solvent, 100 °C, 40 min; (b) O_3, $CHCl_3$–MeOH–pyridine (80:20:3 v/v), −15 °C, 1–2 h; (c) $CO(NH_2)_2{\cdot}H_2O_2$, CH_3OH, reflux, 1.5 h; (d) *N*,*N*′-carbonyldiimidazole, MeNH(OMe)·HCl, CH_3CN–Et_3N, 40 °C, 20 h; (e) Ar–Br, *n*-BuLi–hexane, THF, rt, 10 h; (f) TBAF·$3H_2O$, THF, rt, 1 h.

Table 1
Effects of polyalkoxybenzene analogues of phenstatin on sea urchin embryos

Compound	EC[a] (μM)		
	Cleavage alteration	Cleavage arrest	Embryo spinning
CA4[b]	0.002	0.01	0.05
CA2[c]	0.002	0.01	0.05
7a, phenstatin	0.01	0.05	0.5
7b	2	>4	>5
7c	1	4	>5
7d	0.001	0.01	0.1
7e	2	>4	>5
7f	0.1	0.2	>5
7g	0.5	1	>5

[a] The sea urchin embryo assay was conducted as described in Ref. 48. Duplicate measurements showed no differences in effective threshold concentration (EC) values.
[b] Synthesized according to Ref. 52.
[c] Synthesized according to Ref. 42.

immediately after hatching (8–10 h after fertilization). Lack of forward movement, settlement to the bottom of the culture vessel, and rapid spinning around the animal-vegetal axis of the embryo suggest a tubulin-destabilizing effect caused by a molecule.[49] The specific tuberculate shape of arrested eggs seems to be an additional indicator of the tubulin destabilizing activity. Data generated by the assay for multiple marketed and experimental antimitotics correlated well with the results generated by conventional cell-based and tubulin polymerization procedures.[48,50,51] The details of biological evaluation using the sea urchin embryo assay are presented in Supplementary data.

As evidenced from Table 1, **7a** and **7d** displayed noticeable cleavage alteration, arrest, and embryo spinning, suggesting their tubulin-destabilizing activity. It was assumed that the less potent antimitotics **7c**, **7f**, and **7g** were tubulin destabilizers as well, due to the tuberculate shape of the arrested eggs in the assay, although these molecules failed to induce embryo spinning.[48,50,51] Agents **7b**, **7c**, and **7e–f** were not tested at concentrations of >4–5 μM due to their limited solubility in DMSO and/or seawater.

Literature data suggest that the presence of three methoxy substituents in the A ring of combretastatins and phenstatin analogues is crucial for the antitubulin activity.[3,11,23,25,30,35,36,53] However, in the sea urchin embryo test, CA2-related phenstatin derivative containing myristicin moiety (**7d**) was consistently more potent than phenstatin (**7a**), exhibiting the activity similar to those of CA4 and CA2. Ethylenedioxy derivatives (**7f** and **7g**) were less active. The presence of additional methoxy group in apiol, dillapiol, and tetramethoxybenzene fragments of **7b**, **7c**, and **7e**, respectively, dramatically reduced the antimitotic effect. A similar observation was made for the polyalkoxyphenyl analogues of combretastatins.[42] Biological evaluation of ethylenedioxy derivatives (**7f** and **7g)** showed that the removal of hydroxy group in the ring B decreased antimitotic activity. This was comparable with the reported antimitotic effect of (4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone that was determined to be significantly less potent than phenstatin (IC_{50} values of 0.4–0.6 μM in the sea urchin embryo assay).[18]

In summary, a variety of phenstatin ring A modifications derived from apiol (**1b**), dillapiol (**1c**), myristicin (**1d**), and ethylenedioxybenzaldehyde (**3f**), yielded antimitotic compounds. The myristicin derivative **7d** (CA2 analogue) was more effective than the parent compound phenstatin (**7a**), displaying the antiproliferative tubulin-destabilizing activity at the same concentration range as CA2 and CA4. Compound **7d** was synthesized using the simple starting material extracted from parsley seeds. In contrast to combretastatins, **7d** featured the steric stability with potential for further design as anticancer agent.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.124. These data include MOL files and InChiKeys of the most important compounds described in this article.

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