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Synthesis and Anti-Tumor Activity of Novel Combretastatins: Combretocyclopentenones and Related Analogues

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Abstract—A series of 2-(3,4,5-trimethoxyphenyl)-3-arylcyclopent-2-ene-1-ones (**8a**–**8e**) and their related analogues, including pentenone **9a**, pentenol **10a**, pentene **12a**, and furane **15**, were synthesized and evaluated for cytotoxicity against murine and human tumor cell lines. Compounds **8a**–**c**, **8e** and **9a** showed strong cytotoxicity with IC_{50} values in the range of 8–34 ng/mL. Compound **8e** exhibited significant anti-tumor activity in BDF1 mice bearing Lewis lung carcinoma cells with an inhibition ratio of 59%.

Combretastatin A-4 (CA-4, Fig. 1), isolated from *Combretum caffrum*, is one of the most potent anti-mitotic agents that binds to tubulins on the colchicine binding site.[1] This compound has been shown to exhibit strong cytotoxicity against a variety of human cancer cells, including multi-drug resistant cell lines.[2] Numerous studies on its structure–activity relationships have established that the 3,4,5-trimethoxy substituent in the A ring and the *cis*-orientation between rings A and B are essential for strong cytotoxicity.[3,4] However, during storage and administration *cis*-combretastatin analogues tend to isomerize to *trans*-forms. The *trans*-forms of these compounds show dramatic reduction in both anti-tubulin activity and cytotoxicity. This prompted the design and syntheses of a number of *cis*-restricted analogues of CA-4.[5,6] Among these, an elegant work by Ohsumi et al.[5] reported that five-membered heterocyclic rings flanked between the two benzene rings were tolerable congeners for the bioactivity of combretastatins. We have also recently described several *cis*-restricted analogues of CA-4, including combretoxazolones (**1**, **2**)[7] and furanones (**3**)[8] with very potent cytotoxicity and significant anti-tumor activity. In this paper, we describe the results of our continued works on the other type of combretastatins, analogous to the furanones **3**, namely combretocyclopentenones (Fig. 1), and their related derivatives.

The syntheses of compounds **8** were based on sequential malonate alkylation–acylation followed by ring-closure and decarboxylation (Scheme 1). The syntheses began with the alkylation of diethyl malonate with

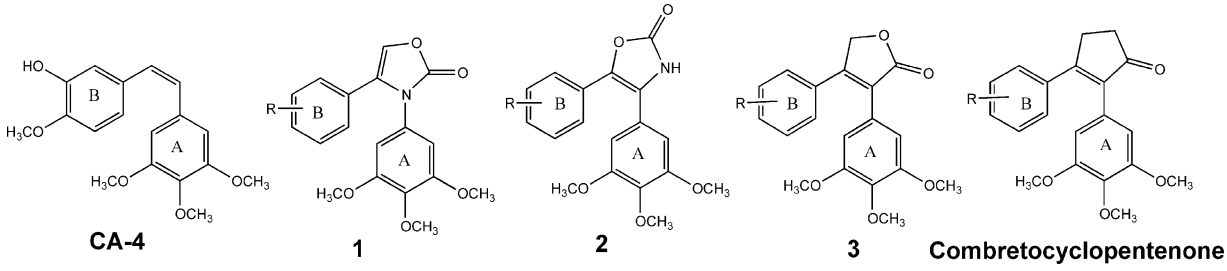

Figure 1.

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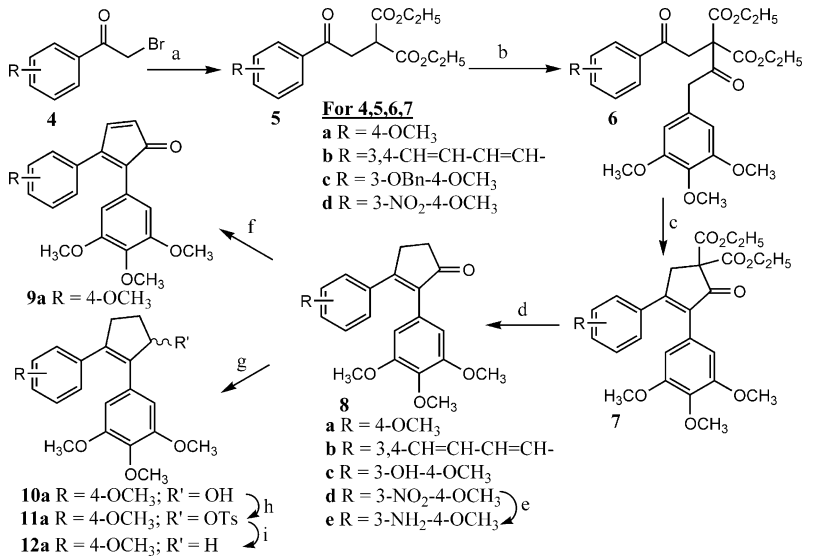
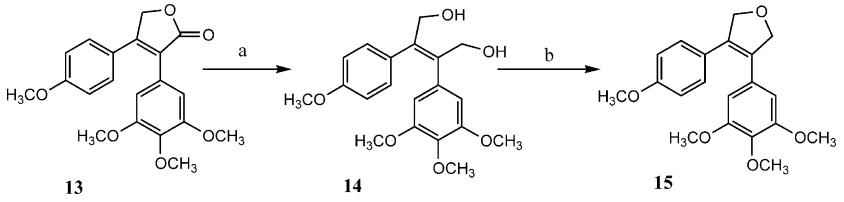


Scheme 1. Reagents and conditions: (a) K_2CO_3, dimethyl malonate, 40 °C, 5 h; (b) (i) $MgBr_2{\cdot}Et_2O$, pyridine, 0 °C, 3 h; (ii) CH_3COCl, −25 °C, 2 h then 1 N HCl; (c) TEA, acetonitrile, 30 min; (d) 3 M H_2SO_4/AcOH, rfx, 3 h; (e) Zn/AcOH, rt, 3 h; (f) (i) PhSeCl, EtOAc, 3 h; (ii) *m*-CPBA, pyridine, 1 h; (g) $NaBH_4$, EtOH, 1 h; (h) TsCl, DMAP, THF; (i) NaH, THF, rfx.

α-bromoacetophenones **4** to give the adducts **5**. The alkylation was found most effective by slow addition of a solution of **4** in acetone to a mixture of diethyl malonate and K_2CO_3 in anhydrous acetone at 45 °C.[9] Nucleophilic addition of **5** to 3,4,5-trimethoxyphenylacetyl chloride to give **6** was mediated by $MgBr_2{\cdot}Et_2O$ using pyridine as base. The procedures for this acetylation involved preparation of the Mg enolate, using $MgBr_2{\cdot}Et_2O$–pyridine in a mixture of MeCN and THF at 0 °C, followed by reaction with 3,4,5-trimethoxyphenylacetyl chloride at −30 °C. THF was used as a co-solvent to prevent acetonitrile from freezing. These procedures gave **6** in good yields. Cyclization of **6** was readily achieved using TEA as base. Decarboxylation of **7** was effected by refluxing in 3 M H_2SO_4–AcOH at 90 °C for 10 h. Conveniently, under these conditions the benzyl group used to protect the phenol hydroxyl in **4c** was removed to afford **8c** in one step. Reduction of **8d** by zinc in acetic acid gave **8e** in 78% yield. The introduction of the second α,β-olefinic moiety into **8a,** resulting in compound **9a**, was achieved by using phenylselenenyl chloride. The alcohol **10a** was obtained as a racemic mixture from **8a** by reduction with $NaBH_4$. Tosylation of **10a** gave **11a** in good yield. Subsequent removal of the tosyl group was effected with NaH to furnish compound **12a**. The synthesis of compound **15** is shown in Scheme 2. Opening of the lactone **13**, which was synthesized as described previously by Kim et al.[8], gave the diol **14**. Intramolecular etherification of **14** afforded **15** in a moderate yield (47%). All compounds were unambiguously characterized by spectroscopic (IR, ^1H NMR) techniques.[10]

Since the 3,4,5-trimethoxy substituent in the A ring has been demonstrated to be essential for the bioactivity of combretastatins[3,4] and furanone **3**,[8] we maintained this substituted pattern of ring A throughout the present investigation and examined several variations of substituents in ring B only (**8a**–**e**). The synthesized compounds were evaluated[11] for the cytotoxicity against three tumor cell lines including B16 (murine melanoma), HCT116 (human colon carcinoma), and A431 (human epidermoid carcinoma). The results are summarized in Table 1. All four compounds **8a**–**c**, **8e** were found to exhibit potent cytotoxicity in three tumor cell lines assayed with IC_{50} values ranging from 8 to 34 ng/mL. Compounds **8c** bearing 3-hydroxy-4-methoxy and compound **8e** with 3-amino-4-methoxy substituent on the 3-aryl ring were most potent among the four compounds in this series, whereas compound **8a**, possessing only 4-methoxy group, or compound **8b**, where the 3-aryl moiety is a 2-naphthyl ring, retained much of the bioactivity. These results indicate that those compounds **8a**–**e** shared similar structure–activity relationships with combretastatins reported previously.[3,4]

Comparison of bioactivity between **8a** and **13** revealed that these two compounds were essentially equipotent in two cell lines B16 and HCT116; the IC_{50} values of **8a** were just slightly higher than that of **13**. Thus, the



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, Et_2O, 3 h, rt; (b) PPh_3, DEAD, THF.

Table 1. Cytotoxicity of synthesized compounds in tumor cell lines[a]

Compd	Cytotoxicity (IC_{50},[b] ng/mL)		
	B16	HCT116	A431
8a	15	21	21
8b	29	31	34
8c	13	12	9
8e	8	8	9
9a	11	13	nt[c]
10a	39	45	nt
12a	>1000	>1000	nt
15	>1000	>1000	nt
13	13	16	nt
Adriamycin	90	110	117

[a]B16, murine melanoma; HCT116, human colon carcinoma; A431, human epidermal carcinoma.
[b]The concentration required to reduce the cell growth by 50%.
[c]Not tested.

lactone oxygen atom in **13** seemed to be not very important for its cytotoxicity. In contrast, removal of the carbonyl group, as seen in compound **15**, proved to be detrimental for the bioactivity of this compound; compound **15** were found inactive up to 1000 ng/mL in both cell lines. Furthermore, reduction of this carbonyl moiety to hydroxyl group led to a racemic mixture **10a** with substantially reduced cytotoxicity. Therefore, no attempt to separate the two enantiomers was further elaborated. Scission of both lactone oxygen and carbonyl group in compound **13** resulted in compound **12a** with lost bioactivity. Thus, it appears that the carbonyl group might play an important role in the binding of these compounds (**8a–e, 13**) with appropriate receptors, likely via hydrogen bonding.

The combretastatins have been widely known as very potent anti-mitotic anti-tumor agents. In this study, compounds **8a–e** have been demonstrated to share common features of the combretastatin class in terms of structure–activity relationships, and it is expected that those compounds in this study also act by binding to tubulins, similar to combretastatins. Though the tubulin binding experiments are pending, we have alternately performed cell cycle analyses to examine the effect of one representative compound, compound **8e**, on the progress of cell cycle events to glean some insights into mechanism(s) mediating the profound cytotoxicity of this series. Cell cycle analysis[12] of CEM (leukemic) cells was performed after 0–12 h incubation with 30 ng/mL of **8e**. It was found that treatment with **8e** for as little as 2 h led to the increase in the number of G_2-M phase cells. It was also observed that G_2-M phase cells accumulated at the expense of G_1 cells and the number of G_1 cells was almost depleted after 8 h. Progressive depletion of S phase then ensued beginning at 10 h because of cessation of new cells entering S phase from G_1 phase. Depletion of S phase cells was almost complete by 12 h, at which time almost all of the liable cells were blocked in G_2-M phase. In addition, increase in the number of hypodiploid events was seen after 8–10 h, indicating apoptosis of cells after a prolonged G_2-M blockage. The fact that both G_1 and S phases became progressively depleted under continuous exposure of **8e** confirms normal movement of cells through these phases even in the presence of a drug. Thus, **8e** appears to be a rapid and effective G_2–M phase blocker that does not affect cell cycle progression through the G_1 or S phases or G_1–S transitional point. These results strongly suggest that **8e** may interfere with a dynamic engine of cellular mitosis, whose critical components include microtubule bundles. Thus, compound **8e** and its close analogues likely act by the same mechanism of combretastatins by binding to tubulins.

In the anti-cancer drugs development arena, it has been widely recognized that those compounds that possess multiple mechanisms of action may be more effective in killing tumor cells and prevention of multi-drug resistance. Dimmock et al.[13] in a recent publication also proposed that interference at multiple points in biological cascades or successive attacks of cellular constituents may be highly deleterious to malignant cells in preference to normal cells. In this regard, we attempted to introduce a second α,β-olefinic moiety in **8a** at the 4,5-position. The resulting **9a** possesses a newly formed conjugation between the 4,5-olefine and 1-one group. This α,β-unsaturated ketone is sterically unhindered and therefore may act as a Michael acceptor, and thus, compound **9a** could be expected to be an alkylating agent also. Indeed, the Michael acceptor is a moiety often employed as a powerful tool in anti-cancer drugs design.[14] Interestingly, compound **9a** was shown to be about 1.5-fold more potent than **8a**. Though this discrepancy was somewhat narrow, it did support the perspectives presented above, and further exploration in this direction in anti-cancer drugs design may prove to be beneficial.

Overall, among eight compounds (**8a–8e**, **9a**, **10a**, **12a**, **15**) of four new types of combretastatin analogues investigated in this work we found six compounds (**8a–8e**, **9a**, **10a**) with potent bioactivities (IC_{50} values of 8–45 ng/mL). However, these compounds seem to be less potent than those combretoxazolones **2** and **3** reported previously.[7] For example, the IC_{50} values of **8a**, **1** (R = 4-OCH_3), and **2** (R = 4-OCH_3) were 15, 2.8 and 5.4 ng/mL, respectively. One possible reason could be that combretoxazolones **1** and **2** possess more flexible conformations, which may allow the correct positioning of the molecules for a facile binding at the active site residues of receptors.

Preliminary in vivo evaluation of a representative compound **8e**, which offered the best water solubility (2.1 mg/mL, the hydrochloride salt form), showed that this compound, when administered at 40 mg/kg/day (maximum injectable dose) into BDF1 mice inoculated with Lewis lung carcinoma cells,[15] inhibited the growth of tumor mass by 59%, compared to 78% of etoposide (36 mg/kg/day, used as a positive control). Of note however, this compound showed little toxicity compared to etoposide, as evidenced by lower body weight loss in mice treated with **8e**. Thus, elevation of dosage is possible given that its water solubility is improved. A series of prodrugs of this compound is being pursued in

our lab and results from this investigation will be reported elsewhere.[16]

In conclusion, we have described here eight compounds of four new types of combretastatin analogues including pentenones (**8a**–**8e**, **9a**), pentenol (**10a**), pentene (**12a**), and furane (**15**). Six compounds among them showed very potent cytotoxicity in three tumor cell lines tested. One representative analogue, compound **8e**, exhibited significant anti-tumor activity.

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10. Spectral data for a representative compound **8e**. IR (KBr, cm^{-1}) 3450, 3380, 1920, 1680, 1620, 1580, 1250. ^1H NMR ($CDCl_3$, 300 MHz) δ 2.59–2.73 (2H, m), 2.88–3.05 (2H, m), 3.84 (3H, s), 3.87 (6H, s), 3.91 (3H, s), 6.52 (2H, s), 6.71 (1H, d, $J=8.11$ Hz), 6.75 (1H, d, $J=2.21$ Hz), 6.80 (1H, dd, $J=2.21$, 8.11 Hz).
11. Cytotoxicity was determined by a method described previously: Skehan, P.; Storeng, R.; Scudiero, D.; Monk, A.; MacMahon, J.; Vistica, D.; Warren, J.; Bokesch, H.; Kenny, S.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1107.
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15. Lewis lung carcinoma cells were subcutaneously inoculated into BDF1 mice on day 0. Compound **8e** was administered ip at 40 mg/kg/day with a total of seven injections every 2 days from day 1. Three days after the final administration, tumor masses were weighed. The inhibition rate was calculated by the equation: inhibition rate = (1−TWt/TWc) × 100 (%). TWt: the mean tumor weight of the treated group. TWc: the mean tumor weight of the control group.
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