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Combretoxazolones: Synthesis, Cytotoxicity and Antitumor Activity

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Abstract—Two series of combretoxazolones including 3,4-diaryloxazolones (**6**) and 4,5-diaryloxazolones (**7**) were synthesized and evaluated for cytotoxicity and antitumor activity. Both series showed strong cytotoxicities against a variety of tumor cell lines. Compound **6g** exhibited a significant antitumor activity in BDF1 mice bearing B16 murine melanoma cells with inhibition rates of 67 and 61% at 100 and 30 mg/kg/day, respectively.

Combretastatin A-4 (**CA-4**, **1**), isolated from *Combretum caffrum*, is one of the most potent antimitotic agents and binds to tubulin on the colchicine binding site.[1] It shows strong cytotoxicity against a variety of human cancer cells, including multi-drug resistant cell lines.[2] However, the low water solubility of CA-4 limits its efficacy in vivo therefore, a water-soluble sodium phosphate prodrug of CA-4 (**2**) was prepared and evaluated for clinical applications.[3] Recently, the first combretastatin A-4 analogues (**3**, **4**) showing potent antitumor activity in vivo were also described.[4] From a series of SAR studies, it was established that the *cis*-orientation of two phenyl rings is essential to strong cytotoxicity. However, *cis*-combretastatin analogues are prone to isomerize to *trans*-forms during storage and administration. The *trans*-forms of these compounds show dramatic reduction in both antitubulin activity and cytotoxicity. This prompted the syntheses of a number of *cis*-restricted five-membered heterocyclic analogues of CA-4[5] (**5**) (Fig. 1). Perusal of these analogues revealed that there was an apparent lack of oxazolone-type compounds (**6**, 3,4-diaryloxazolones; **7**, 4,5-diaryloxazolones) that can be readily synthesized. In this paper we describe the synthesis and evaluation of cytotoxicity of this compound class, hereafter given a trivial name of combretoxazolone.

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The combretoxazolones **6** were synthesized as shown in Scheme 1. Acetophenones **8**[6] were brominated to give α-bromoacetophenones **9** which were converted to α-hydroxyacetophenones **10** with betaine in moderate yields. Reacting of **10** with respective arylisocyanates and subsequent cyclization by refluxing in acetic acid (AcOH) provided **6** in 60–70% yields. Under the cyclization conditions, a 4-methoxybenzyl (PMB) group used to protect a phenol in **8e** was removed to afford **6e** directly. Reduction of a nitro group in **6f** with zinc gave **6g** in a good yield. The combretoxazolones **7** were constructed as shown in Scheme 2. Coupling of **11** with various aryl benzaldehyde Ar_2CHO[7] by a reported procedure[8] gave α-hydroxyketones **12**. Reacting of α-hydroxyketones **12** with PMB-isocyanate and subsequent cyclization provided the intermediates **13**. The N-PMB group was removed by refluxing in trifluoroacetic acid (TFA) for 3 h to give the expected products **7**. Under these conditions, a benzyl group used to protect a phenol in **13e** was removed to afford **7e** directly. Compound **7g** was obtained from **7f** as described for **6g**. All newly synthesized compounds were fully characterized by spectral methods such as IR, ^{1}H NMR, ^{13}C NMR and HRMS.[9]

The synthesized combretoxazolones were evaluated[10] against a small panel of tumor cell lines including murine melanoma (B16), human colon tumor (HCT116), human breast tumor (MCF-7), human lung carcinomas (A549) and prostate tumor (PC-3). The results are summarized in Table 1.

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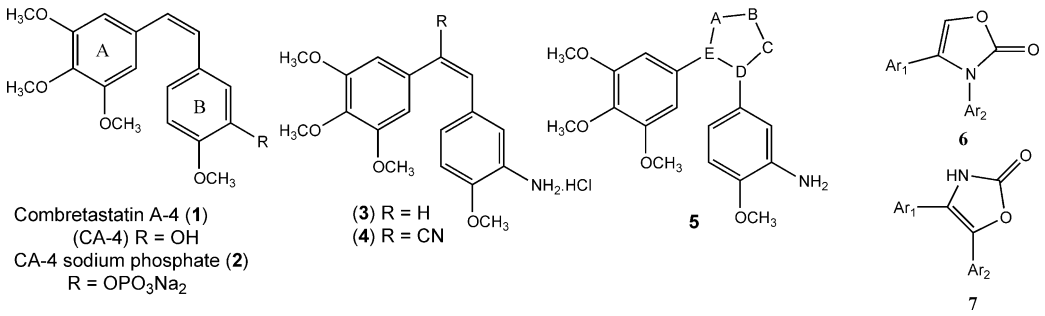
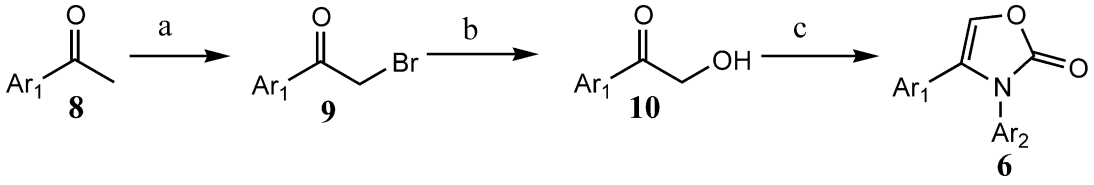


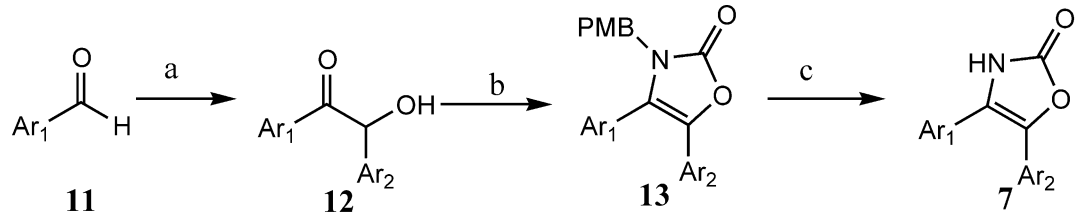
Figure 1.



Ar$_1$ for 8, 9 and 10
a Ar$_1$ = 3,4,5-trimethoxyphenyl
b Ar$_1$ = 4-methoxyphenyl
c Ar$_1$ = 3,4-methylenedioxyphenyl
d Ar$_1$ = 2-naphthyl
e Ar$_1$ = 3-(4-methoxybenzyl)oxy-4-methoxyphenyl
f Ar$_1$ = 3-nitro-4-methoxyphenyl

6a Ar$_1$ = 3,4,5-trimethoxyphenyl
Ar$_2$ = 4-trimethoxyphenyl
6b Ar$_1$ = 4-methoxyphenyl
Ar$_2$ = 3,4,5-methoxyphenyl
6c Ar$_1$ = 3,4-methylenedioxyphenyl
Ar$_2$ = 3,4,5-trimethoxyphenyl
6d Ar$_1$ = 2-naphthyl
Ar$_2$ = 3,4,5-trimethoxyphenyl
6e Ar$_1$ = 3-hydroxy-4-methoxyphenyl
Ar$_2$ = 3,4,5-trimethoxyphenyl
6f Ar$_1$ = 3-nitro-4-methoxyphenyl
d
Ar$_2$ = 3,4,5-trimethoxyphenyl
6g Ar$_1$ = 3-amino-4-methoxyphenyl
e
Ar$_2$ = 3,4,5-trimethoxyphenyl
6g.HCl

Scheme 1. (a) Br_2, $CHCl_3$; (b) betaine, EtOH, 60 °C; (c) (i) Ar_2NCO, toluene, 80 °C, 3 h; (ii) AcOH, rfx, 8 h; (d) Zn, CH_3COOH; (e) 2 N HCl in dioxane, 3 h.



11a Ar$_1$ = 4-methoxyphenyl
11b Ar$_1$ = 3,4,5-trimethoxyphenyl

Ar$_1$, Ar$_2$ for 12 and 13
a Ar$_1$ = 4-methoxyphenyl
Ar$_2$ = 3,4,5-trimethoxyphenyl
b Ar$_1$ = 3,4,5-trimethoxyphenyl
Ar$_2$ = 4-methoxyphenyl
c Ar$_1$ = 3,4,5-trimethoxyphenyl
Ar$_2$ = 3,4-methylenedioxyphenyl
d Ar$_1$ = 3,4,5-trimethoxyphenyl
Ar$_2$ = 2-naphthyl
e Ar$_1$ = 3,4,5-trimethoxyphenyl
Ar$_2$ = 3-benzyloxy-4-methoxyphenyl
f Ar$_1$ = 3,4,5-trimethoxyphenyl
Ar$_2$ = 3-nitro-4-methoxyphenyl

7a Ar$_1$ = 4-methoxyphenyl
Ar$_2$ = 3,4,5-trimethoxyphenyl
7b Ar$_1$ = 3,4,5-trimethoxyphenyl
Ar$_2$ = 4-methoxyphenyl
7c Ar$_1$ = 3,4,5-trimethoxyphenyl
Ar$_2$ = 3,4-methylenedioxyphenyl
7d Ar$_1$ = 3,4,5-trimethoxyphenyl
Ar$_2$ = 2-naphthyl
7e Ar$_1$ = 3,4,5-trimethoxyphenyl
Ar$_2$ = 3-hydroxy-4-methoxyphenyl
7f Ar$_1$ = 3,4,5-trimethoxyphenyl
d
Ar$_2$ = 3-nitro-4-methoxyphenyl
7g Ar$_1$ = 3,4,5-trimethoxyphenyl
Ar$_2$ = 3-amino-4-methoxyphenyl

Scheme 2. (a) (i) TMS-CN, ZnI_2, THF; (ii) LiHMDS. Ar_2CHO, THF, −78 °C; (b) (i) PMB-NCO, toluene, 80 °C, 3 h; (ii) AcOH, rfx, 8 h; (c) TFA, rfx, 3 h; (d) Zn, CH_3COOH.

Table 1. Cytotoxicity of the synthesized combretoxazolones against tumor cell lines[a]

Compd	Cytotoxicity (IC_{50},[b] nM)					Compd	Cytotoxicity (IC_{50}, nM)				
	B16	HCT116	MCF-7	A549	PC-3		B16	HCT116	MCF-7	A549	PC-3
6a	1879	—[c]	—	—	—	**7a**	1390	—	—	—	—
6b	7.9	11.0	10.5	19.9	7.5	**7b**	14.5	21.7	28.4	16.9	20.1
6c	45.0	57.4	39.7	64.5	70.6	**7c**	89.3	101.1	78.5	63.1	112.1
6d	5.8	4.9	3.7	5.9	3.1	**7d**	7.4	11.2	16.7	10.8	7.1
6e	0.9	1.7	2.9	3.1	2.3	**7e**	5.4	6.1	5.7	7.9	6.4
6g	1.1	3.2	1.8	4.3	2.5	**7g**	2.4	3.7	4.9	3.8	2.1
CA-4[d]	1.0	0.9	2.7	2.1	2.7	**CA-4**	1.0	0.9	2.7	2.1	2.7

[a]Cell lines: B16, murine melanoma; HCT116, human colon tumors; MCF-7, human breast tumors; A549, lung carcinomas and PC-3, prostate tumors.
[b]The concentration produces 50% reduction in cell growth.
[c]Reduction in cell growth by 50% was not reached at the highest concentration assayed (10 μM).
[d]CA-4, combretastatin A-4, was synthesized as described previously.[14]

Previously, the Purdue group has studied structure–activity relationships of CA-4 extensively[11,12] and found that a 3,4,5-trimethoxy group on the A ring was essential for strong cytotoxicity. They also showed that the 3-hydroxy group on the B-ring is not necessary for potent activity.[11] Therefore, for ease of preparation, we chose 3,4,5-trimethoxyphenyl and 4-methoxyphenyl as Ar_1 and Ar_2 and synthesized two isomers **6a** and **6b**. An assay determined that **6b** was active against a variety of cancer cell lines with IC_{50} values in a range of 7.5–11 nM while the regio-isomer **6a** was found relatively inactive up to the highest concentration tested (10 μM). From these results, we fixed Ar_2 as 3,4,5-trimethoxyphenyl and examined several variations of Ar_1 including 3,4-methylendioxyphenyl, 3-hydroxy-4-methoxyphenyl, 2-naphthyl and 3-amino-4-methoxyphenyl. Among the synthesized combretoxazolone **6b**–**6g**, it was not surprising that compound **6e** with Ar_1 being 3-hydroxy-4-methoxyphenyl, identical with B ring of CA-4, and compound **6g** with Ar_1 being 3-amino-4-methoxyphenyl, identical with B ring of **3** and **4,** were the most potent ones. These two compounds showed comparable cytotoxicities with CA-4 in all cell lines assayed with IC_{50} values as low as 0.9 nM. Compound **6c** bearing a 3,4-methylenedioxyphenyl group as Ar_1 was the least potent. Compound **6d** with Ar_1 being 2-naphthyl retained much of the activity compared to **6e** and **6g**. This result confirmed a surrogative role of a naphthyl group for B ring of CA-4 reported previously.[13]

In the series of compounds **7**, initially two regio-isomers **7a** and **7b** were synthesized and evaluated for the cytotoxicity. Interestingly, despite the structural similarity between the two isomers, only **7b** showed strong cytotoxicity while **7a** was found to be relatively inactive up to the highest concentration tested (10 μM). The order of cytotoxicity in this series was found to be similar with **6a**–**6g** series; thus, compounds bearing a 3-hydroxy-4-methoxy (**7e**) or a 3-amino-4-methoxy (**7g**) substituted pattern being most potent while compound **7c**, possessing a 3,4-dioxymethylene group on the Ar_2 ring was found to be least cytotoxic. In overall, the compounds in series **7** were less potent than those in series **6**, suggesting that a carbonyl group at position A of the five-membered ring (Structure **5**, Fig. 1) is more favorable for the strong cytotoxicity. This finding is in consistent with the results reported previously.[5] Moreover the compounds **6** could adopt more flexible conformations compared to **7**. This conformational flexibility may allow the correct positioning of the molecules for a facile binding at the active site residues of receptors, e.g., tubulins. Details of molecular modeling studies and tubulin-binding activity of the synthesized compounds will be reported elsewhere.

Although the synthesized combretoxazolones showed very potent cytotoxicity in vitro, they proved to be of limited solubility in aqueous system. Among the synthesized compounds, only **6g** and **7g** were endowed with reasonable water solubility. In vivo evaluation[15] of a representative compound **6g** in the form of hydrochloride salt revealed that, when administered to BDF1 mice bearing B16 murine melanoma cells, **6g** inhibited the growth of tumor mass by 67 and 61% at 100 and 30 mg/kg/day, respectively (Table 2). At a lower dose (10 mg/kg/day), **6g** showed only a marginal activity with the inhibition rate of 34%.

In summary, we have presented here the synthesis and evaluation of cytotoxicity of two series of combretoxazolones, including 3,4-diaryloxazolones (**6**) and 4,5-diaryloxazolones (**7**). These combretoxazolones showed potent cytotoxicity against a variety of tumor cell lines. Structurally, compounds **7** are clearly *cis*-restricted and compounds in series **6** can also be viewed as such in term of position between the two aryl rings, and therefore, these analogues should be stable in term of isomerization. One of the synthesized combretoxazolones, compound **6g**, displayed a significant in vivo antitumor activity.

Table 2. Antitumor activity of compound **6g**

Compd	Dose (mg/kg/day)	IR[a] (%)
6g	100	67
	30	61
	10	34
ADR[b]	3	55

[a]The inhibition rate; see ref 15.
[b]Adriamycin, used as a positive control.

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