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Synthesis and activity of Combretastatin A-4 analogues: 1,2,3-thiadiazoles as potent antitumor agents

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Abstract—A series of 4,5-disubstitute-1,2,3-thiadiazole compounds were designed and synthesized as potent anticancer agents, some of them exhibited excellent in vitro and in vivo inhibitory activity.

Malignant tumor is one of the most serious threats against human health in the world, and the clinical prognosis remains relatively poor. Therefore, it needs to develop the new therapeutic strategies for the improvement of drugs that are currently in use.

There has been more and more interest in the search for antitumor compounds with high efficacy, low toxicity in recent years. For example, Combretastatin A-4 (**CA-4**) is one of the excellent substances for antitumor activity, which was first isolated from the South African bush willow tree *Combretum caffrum*. Because of its structural simplicity and potent cytotoxicity, **CA-4** is a very attractive lead compound, and many synthetic analogues of **CA-4** have been developed.[1–3] The structure–activity relationship demonstrated that the *cis* configuration of double bond and 3,4,5-trimethoxyphenyl group are fundamental.[4] And the restricted rotation of rings A and B of **CA-4** can be maintained by introducing suitable conformationally restricted heterocycles such as dioxolane,[5] thiazole,[6] and imidazole (Fig. 1).[7] According to the SAR, we designed and synthesized a series of *cis* restricted analogues with 1,2,3-thiadiazole instead of the **CA-4**'s olefin group (**4a–t**).

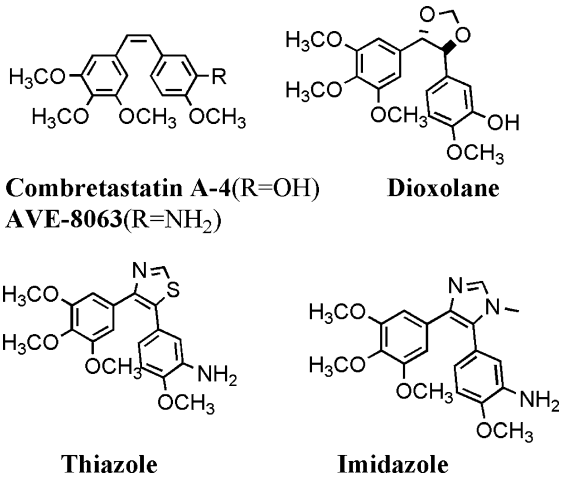

Figure 1. Combretastatin and its derivatives.

Synthetic methods for the preparation of target compounds are summarized as follows.

Deoxybenzoins[8] **3a–i** were prepared as shown in Scheme 1. 3,4,5-Trimethoxybenzaldehyde was converted to phosphonate by Pudovik's reaction with di-*n*-butylamine in ether,[9] which was protected by 3,4-dihydro-2*H*-pyran (THP), and condensed with the appropriate aromatic aldehyde in the presence of EtONa or *t*-BuOK (in case R = NO_2), followed by hydrolysis with diluted acid to afford the deoxybenzoins **3a–i**.

Compounds **3j–l** were easily prepared by Friedel–Crafts reaction of phenylacetic acid with appropriate aryl ether

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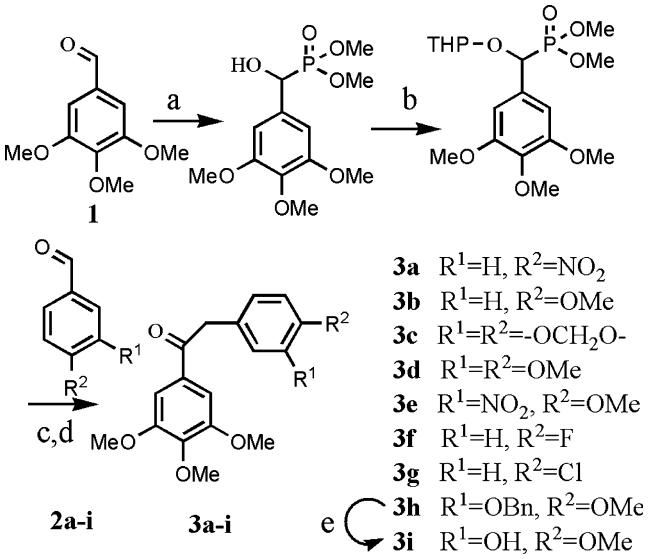


Scheme 1. Reagents and conditions: (a) dimethyl phosphite, *n*-Bu_2NH, ether, rt, 3h; (b) THP, TsOH, benzene, 50 °C, 0.5 h; (c) NaH/EtOH or *t*-BuOK, benzene, rt, 18 h; (d) CH_3OH, HCl aq, rt, 1 h; (e) Pd/C, H_2, rt, 2 h.

in polyphosphoric acid (PPA) at 85–90 °C with mechanical stirring for 2 h (Scheme 2).

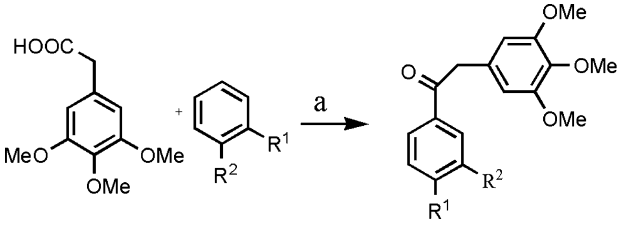
And **3m–r** were synthesized according to Scheme 3.

The trimethoxybenzaldehyde was converted to the corresponding tosylhydrazones and then reacted with appropriate benzaldehydes to afford deoxybenzoins **3m–r**.[10]

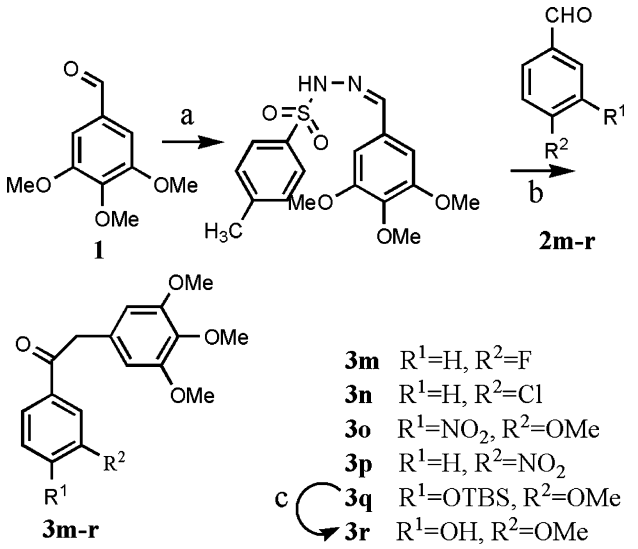
At last, as shown in Scheme 4, substituted deoxybenzoins and TSH (toluenesulfonhydrazide) were condensed in absolute ethanol, and the intermediates were used directly for cyclization with $SOCl_2$ in dry chloroform.[11] The temperature of step b must be kept under 25 °C.[12]

The final compounds **4a–t** were characterized by ^1H NMR and MS.

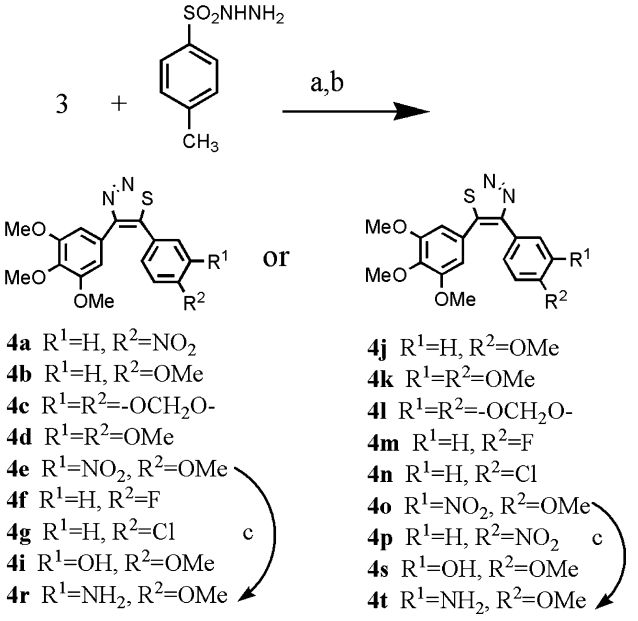
Cytotoxicity assay. Series of the test compounds including compounds **4a–t** and **CA-4** were evaluated for their antiproliferation activities against three types of human cancer cell lines, human myeloid leukemia



Scheme 2. Reagents and conditions: (a) PPA, 90 °C, 2 h.



Scheme 3. Reagents and conditions: (a) TSH, EtOH, rt, 1 h; (b) NaOH, EtOH, reflux, 18 h; (c) TBAF, THF, rt, 1 h.



Scheme 4. Reagents and conditions: (a) EtOH, reflux, 3 h; (b) $SOCl_2$, $CHCl_3$, 0–5 °C, 4 h; (c) AcOH, Zn, rt, 2 h.

cells HL-60, human colon adenocarcinoma cells HCT-116, and human microvascular endothelial cell line (HMEC-1) (Table 1) cells.

As shown in Table 1, there was an interesting phenomenon that if the 3,4,5-trimethoxyphenyl is at 4 position in 1,2,3-thiadiazole, many of the test compounds (**4b–e**, **4i**, and **4r**) have good activities, if at 5 position, only **4s** displays activity. This finding is thought to be beneficial for further SAR studies of **CA-4** analogues.

Effects on tubulin polymerization. To investigate whether the antiproliferation effects of these compounds were related to the interaction with the microtubule system, six of the most active compounds (**4b** and **4d**, **4e** and **4i**, **4r** and **4s**) and control compound **CA-4** were evaluat-

Table 1. IC_{50} Values (nM ± SD) of compounds **4a–t** and **CA-4**

Compound	Cell line		
	HL-60	HCT116	HMEC-1
4a	>1000	>1000	>1000
4b	14.5 ± 6.3	25.0 ± 8.2	24.6 ± 14.9
4c	50.2 ± 29.5	86.6 ± 2.3	73.9 ± 18.0
4d	60.9 ± 30.8	50.9 ± 7.5	75.8 ± 27.8
4e	13.4 ± 1.1	30.2 ± 2.0	26.4 ± 18.5
4f	ND	>1000	>1000
4g	23.6 ± 5.1	18.5 ± 1.7	ND
4i	1.5 ± 0.1	3.0 ± 1.3	3.9 ± 2.8
4j	537 ± 116	616 ± 291	>1000
4k	>1000	>1000	>1000
4l	>1000	>1000	>1000
4m	>1000	>1000	>1000
4n	>1000	>1000	>1000
4o	>1000	>1000	>1000
4p	>1000	>1000	>1000
4r	1.8 ± 1.3	3.7 ± 1.3	2.5 ± 0.1
4s	40.9 ± 20.8	70.4 ± 10.4	41.0 ± 5.1
4t	>1000	>1000	>1000
CA-4	1.9 ± 0.7	3.0 ± 1.2	3.5 ± 0.9

SD, standard deviation. All experiments were independently performed at least three times. ND, not determined.

ed for the inhibition of the polymerization of purified tubulin. The results indicate that tubulin is the intracellular target of these six compounds because they were all potent inhibitors of tubulin polymerization with activities quantitatively similar to those of **CA-4** (Table 2) (Fig. 2).

Table 2. Inhibitory effect of tubulin polymerization by compounds **4b**, **4d**, **4e**, **4i**, **4r**, **4s**, and **CA-4**

Compound	**4b**	**4d**	**4e**	**4i**	**4r**	**4s**	**CA4**
IC_{50} (μM)	0.86	0.72	2.13	0.7	0.68	0.86	0.81

To further confirm the effects of these compounds on tubulin and compared with that of **CA-4**, we evaluated their activities on the alteration of the microtubule network by indirect immunostaining. As shown in the Figure 2, **4r**, **4i**, **4e**, and **4b**, like CA-4, disrupt the cytoskeleton and alter the morphology of HMEC-1 cells significantly.

Cell cycle analysis. That compounds exhibiting activity on tubulin binding should cause the alteration of cell cycle parameters leading to a preferential G2/M arrest,[13] we next investigated whether the compounds **4b** and **4d** influence the cell cycle using flow cytometry assay. Treatment with **4b** and **4d** for 24 h at different concentration, HMEC-1 cells revealed a dose-dependent increase of cells in G2/M and a simultaneous decrease in S and G1 cells compared to control cells. As indicated by the data shown in Table 3, the effects of the com-

Table 3. Effects of **4b**, **4d**, and **CA-4** on mitosis in HMEC-1 cells

	Concn (nM)	G1 (%)	G2/M (%)	S (%)
Control		41	20	39
CA-4	6.25	2	67	31
	12.5	4	88	8
	25	4	83	13
	50	4	80	16
	100	16	80	0
4b	6.25	55	14	31
	12.5	39	28	33
	25	34	40	27
	50	11	78	11
	100	4	87	9
4d	6.25	36	24	40
	12.5	35	25	40
	25	38	33	29
	50	12	61	27
	100	1	78	21

Cell cycle distribution was analyzed by the standard propidium iodide procedure as described in the literature.[13]

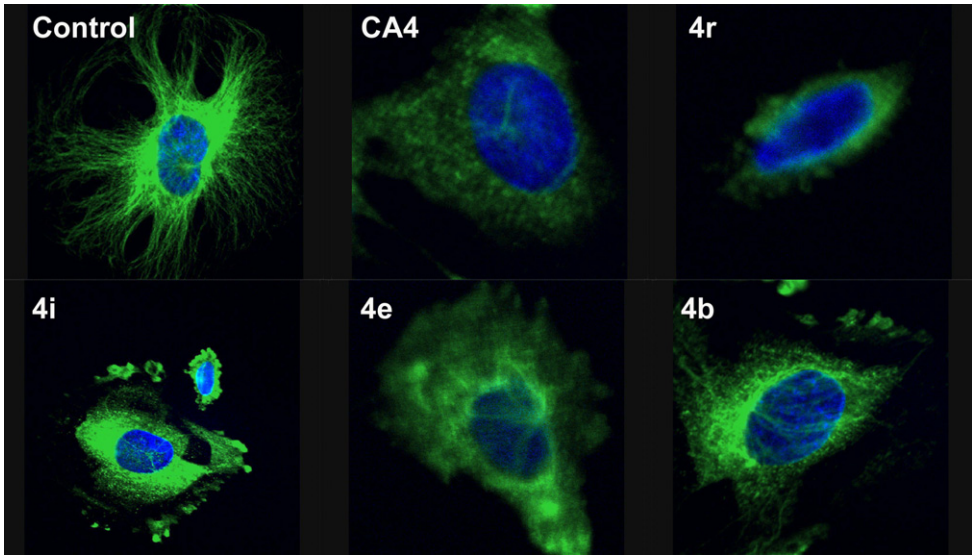


Figure 2. Effects of **4r**, **4i**, **4e**, and **4b** on the cytoskeleton and cell morphology of HMEC-1 cells. Cells cultured on slides were exposed to compounds at 1 μM for 30 min, followed by localization fixed in 3% formaldehyde and permeabilizing with 0.1% Triton X-100, and then incubated with primary antibodies and Alex488-conjugated second antibodies sequentially. Slides were mounted in Vectashield with 4,6-diamidino-2-phenylindole (DAPI) to visualize nuclei. Fluorescent images were acquired with Leica Confocal Scanner.

Table 4. Effect of the compounds on the in vivo growth of mice S-180 sarcoma

Group	Dosage (mg/kg/day)	Mice (*n*) Initial/end	Body weight (g) Initial/end	Tumor weight (g) $x \pm SD$	Inhibition rate (%)
NS	—	20/20	17.4/26.6	1.79 ± 0.56	—
CA-4	40 × 4	10/8	17.0/22.6	0.64 ± 0.33	64.2[a]
4b	40 × 5	10/10	17.2/18.3	0.34 ± 0.28	81.0[a]
	10 × 7	10/10	17.0/23.6	1.25 ± 0.58	30.2
4e	100 × 7	10/9	17.5/21.8	0.64 ± 0.45	64.2[a]
4r	20 × 1	10/0	17.2/—	—	—
	10 × 1	10/0	17.3/—	—	—
4i	20 × 4	10/6	17.1/19.5	0.84 ± 0.59	53.1[a]
	10 × 7	10/9	17.3/23.3	1.46 ± 0.66	18.4
5-Fu	50 × 2	10/10	17.0/23.0	0.35 ± 0.20	80.4[a]

[a] $p < 0.01$ versus NS group

pounds on the cell cycle suggest that this class of molecules arrest the cell cycle at G2/M phase selectively.

In vivo antitumor effect of the test compounds. According to the in vitro cytotoxicities of the test compounds, we chose four compounds **4i**, **4b**, **4e**, **4r**, and **CA-4** to investigate their in vivo antitumor effect. Table 4 and Figure 3 present the results of the experimental therapeutic efficacy of those compounds on mice S180 sarcoma transplant model. After daily i.p. administration with different dosage for 5–7 days, animals were euthanized and the tumors were excised and weighted. As shown in Table 4, the test compounds displayed different anti-cancer efficacy. The compounds shown significant anti-cancer efficacy were marked with '*' ($p < 0.01$).

As shown in Table 4 and Figure 3, when mice were treated with **CA-4** at 40 mg/kg for 4 days, compound **4b** at 40 mg/kg for 5 days, compound **4e** at 100 mg/kg for 7 days, and compound **4i** at 20 mg/kg for 4 days, the growth inhibition rate of sarcoma S180 reached 64.2%, 81.0%, 64.2%, and 53.1%, respectively. When treated with compound **4r** at 20 mg/kg or 10 mg/kg, all mice died after 1 day because of toxicity. It seems that the amino group evidently increases the toxicity of the compound though it would improve the water solubility. The same result was observed by Ohsumi's group.[3]

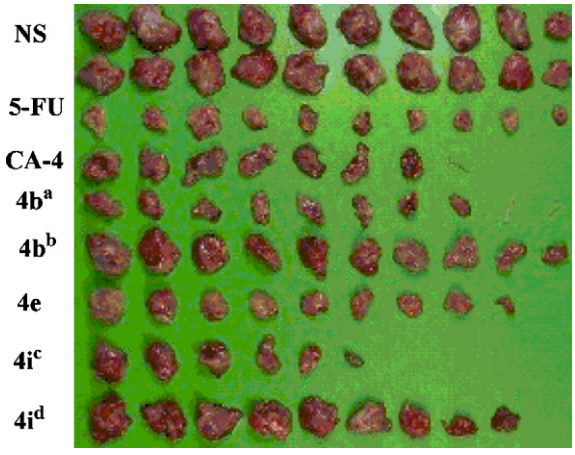


Figure 3. (a) 40 mg/kg, (b) 10 mg/kg, (c) 20 mg/kg, (d) 10 mg/kg. In vivo anti-tumor effect of the test compounds **4i**, **4b**, **4e**, and **CA-4**. The tumors were excised and are shown in the picture.

In conclusion, we have synthesized a series of 1,2,3-thiadiazole derivatives as tubulin polymerization inhibitors. The compounds were evaluated for their antiproliferation activities and some of them displayed potent antitumor effect in vivo. The 3,4,5-trimethoxyphenyl at 5 position in 1,2,3-thiadiazole (**4j–p**, **4t**) led to the loss of antiproliferative activity except for **4s**. Six of the most cytotoxic compounds were evaluated for effects on tubulin assembly and displayed as potent inhibition as that of **CA-4**. Cell-cycle distribution analysis shows that **4b** acts on the G2/M phase of the cell cycle. In vivo antitumor effect indicated that **4b** and **4e** exhibited favorable activity and **4b** is promising for further studies because of its excellent effect and relatively low toxicity.

Acknowledgment

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9. A solution of 3,4,5-trimethoxyaldehyde (4.9 g, 25 mmol), dimethyl phosphite (5 ml), and *n*-Bu_2NH (0.6 ml) in ether (25 ml) was vigorously stirred for 3 h at rt, then the precipitate was collected and washed by ether and then dried. Yield 79%, mp 98–99 °C (Lit.[8]: mp 99–101 °C).
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12. General procedure for the synthesis of **4a–p**: a mixture of 3(1 equiv) and TSH(2.5 equiv) in absolute ethanol (25 ml) was stirred and refluxed for 3 h. (completion of the reaction was monitored by TLC), then the solvent evaporated and dried giving pale yellow solid, which was dissolved in dry $CHCl_3$ (30 ml), violently stirred, and dropped $SOCl_2$ (2 ml) with cool bath at 0–5 °C for 4 h. Then the solvent was evaporated. The residue was extracted with EtOAc (3× 20 ml) then washed with 10% $NaHCO_3$ and brine, dried, and isolated by flash chromatography on silica gel. Yields 35–80%.
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