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Synthesis and anticancer activity of novel 3,4-diarylthiazol-2(3*H*)-ones (imines)

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ABSTRACT

A series of 3,4-diarylthiazol-2(3*H*)-ones and three 3,4-diarylthiazol-2(3*H*)-imines were synthesized and evaluated for their cytotoxicity in a panel of human cancer cell lines. Compounds **21** and **22** showed potential anticancer activity against human CEM cells with IC_{50} values of 0.12 and 0.24 μM, respectively.

Malignant tumors are one of the most serious threats against human health in the world. To target the fundamental machinery driving cellular proliferation remains a mainstay of cancer therapeutic strategies.[1,2] Because microtubules in eukaryotic cells are important in mitosis and cell division, this structure has been an attractive target for development of anticancer drugs for decades. Antimitotic agents lead to cell-cycle arrest and subsequent tumor regression.[3–5]

Combrestatin A4 (**CA-4**), a novel *cis*-stilbene tubulin-binding agent, strongly inhibits tubulin by binding to the colchicine site (Fig. 1). Potent cytotoxicity of **CA-4** against a broad spectrum of human cancer cells including multiple drug-resistant cancer cell lines, has attracted significant attention.[6,7] A water-soluble sodium phosphate prodrug (**CA-4P**) of **CA-4** is currently under clinical evaluation for advanced cancers.[8,9] Structure–activity relationship studies have demonstrated that the *cis* orientation of double bond and the presence of a 3,4,5-trimethoxyphenyl group are fundamental requirements for potent cytotoxicity.[3,5] However, **CA-4** is prone to isomerizes to the *trans* isomer during storage and administration, which significantly reduces its antiproliferative activities.[7,8,10] Accordingly, a number of *cis*-restricted five-membered heterocycles analogues of **CA-4** such as imidazoles, thiazoles, and oxazolones, were prepared to avoid the instability problem.[6,8,11–16] Many of them showed potent cytotoxicity against various cancer cells comparable to **CA-4**. In our efforts to discover active antimitotic agents, we designed and synthesized two series of *cis*-restricted analogues utilized thiazolone and thiazolimine systems (Fig. 1) instead of the olefin group in **CA-4**.

3,4-Diarylthiazolimines **3–5** were prepared as shown in Scheme 1. Acetophenone analogues in anhydrous ether were treated with bromine to give the substituted phenacyl bromides **2**.[17] The reaction of **2** with arylthioureas in ethanol containing concd HCl gave 3,4-diarylthiazolimines **3-5**.[18,19]

3,4-Diarylthiazolones **11–22** were prepared as shown in Scheme 2. The key intermediates (*E*)-*N*-(1-phenyl ethylidene)benzenamines (**7–10**) were obtained in good yield by a 'dehydration' reaction assisted by both heat and molecular sieves.[20,21] Reaction of the ketimine intermediates with chlorocarbonylsulfenyl chloride in the presence of pyridine furnished the corresponding target compounds, 3,4-diarylthiazolones (**11–16, 19–20**), employing the approach reported by Cobas et al.[22] Compounds **17**, **18**, **21**, **22** were obtained from compounds **15**, **16**, **19**, **20**, respectively, by reduction of the nitro group in the presence of $SnCl_2$ dihydrate in refluxing ethanol.[5,23]

The structures of compounds (**3–5**, **11–22**) were confirmed by NMR analysis and elemental analysis. The cytotoxic activities of the synthesized compounds were evaluated against human CEM Leukemia cells as previously described (Table 1).[5]

Previous reports indicated that 3,4,5-trimethoxy phenyl group as one of the two aromatic rings is a fundamental requirement for achieving potent activity. As expected the absence of a 3,4,5-trimethoxyphenyl group, compare results for compound **3** with that of **4** and **5,** resulted in quite low cytotoxicity. It has been reported that 3-hydroxy group on the other ring is not necessary for potent activity.[3] Therefore, for case of preparation, we next chose 3,4,5-trimethoxyphenyl and 4-methoxy phenyl as the two aromatic rings using the thiazolimine linker and synthesized two isomers

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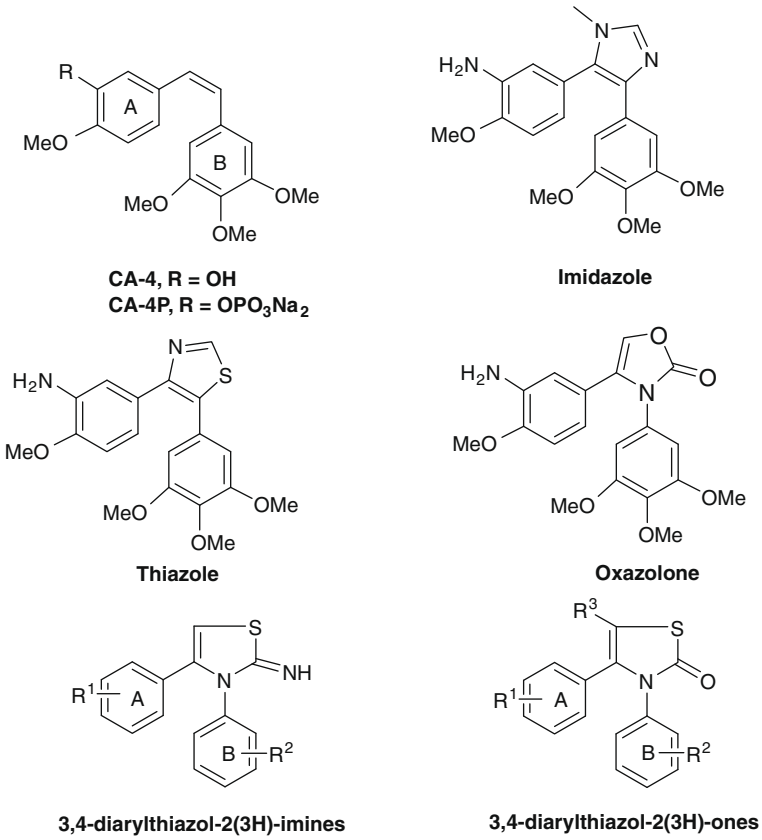
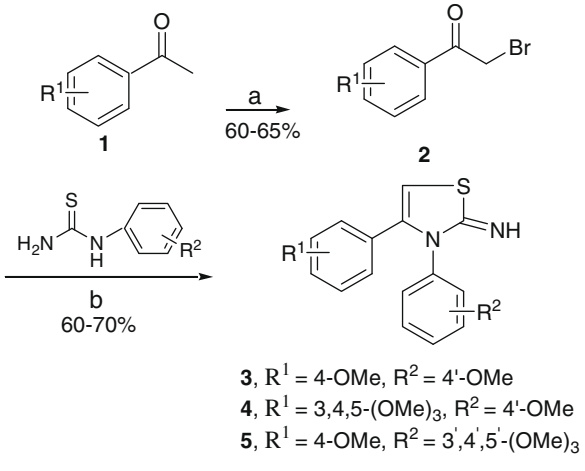


Figure 1. Chemical structures of **CA-4**, its derivatives and 3,4-diaryl thiazolones and thiazolimines.



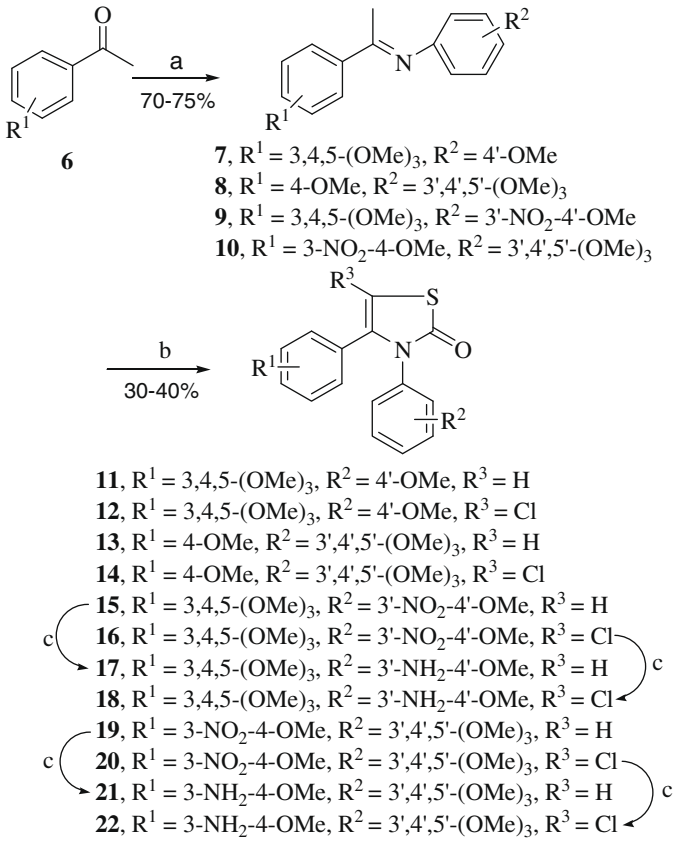
Scheme 1. Reagents and conditions: (a) Br_2, Et_2O, 0 °C, 1 h; (b) concd HCl, EtOH, reflux, 10 h.

4 and **5**. Both compounds **4** and **5** showed only modest cytotoxic activity. The cytotoxicity of compound **5** is ninefold higher than that of compound **4**, which suggests that placing the 3,4,5-trimethoxyphenyl group on the five-membered ring near the imine group is more favorable for cytotoxicity. Due to the only modest activity observed for **3**–**5**, no further modifications were pursued in the thiazolimine system.

Since the 3,4,5-trimethoxyphenyl group is essential and 4-methoxyphenyl group proved to be favorable for cytotoxicity, we maintained this substitution pattern in the series of 3,4-diarylthiazol-2(3*H*)-ones. Initially the two isomers **11** and **13** were synthesized and evaluated for the cytotoxicity. Interestingly, despite the structural similarity between the two pairs of isomers, compound **13** showed eightfold more cytotoxicity than compound **11**. This finding indicated that the 3,4,5-trimethoxy phenyl near the carbonyl group of thiazol-2(3*H*)-one is more favorable for cytotoxicity. This finding is in consistent with the thiazolimine results and with the results reported previously for cyclopentenones.[8]

The analysis of the structure **CA-4** and its derivatives shows that a polar group such as a 3-amino group on the 4-methoxyphenyl ring enhanced activity.[8,15] Therefore, we modified the 4-methoxyphenyl ring of compounds **11**–**14** by the introduction of a 3-amino group, resulting in compounds **17–18** and **21–22**. As shown in Table 1, compounds **21** and **22** with the electron-donating amino group, IC_{50} 0.24 and 0.12 μM, respectively, showed much stronger cytotoxicity compared with compounds **13** and **14**. In contrast, the isomers **17** and **18**, in which the substitution pattern is interchanged between the A- and B-rings did not show an improvement in activity over **11** and **12**. These results demonstrated that introduction of the electron-donating amino group at the C-3 position of the 4-methoxyphenyl ring showed a strong influence on the activity of compounds that have the 3,4,5-trimethoxy phenyl near the carbonyl group of thiazol-2(3*H*)-one.

Replacement of the 4-H of the five-member thiazol-2(3*H*)-one ring with a chlorine atom for the pairs **13**–**14** and **15**–**16** resulted in at least a 10-fold loss of activity. A moderate twofold increase in activity was observed on 4-Cl substitution of **21** (compare results for **21** and **22**). The remaining 4-H and 4-Cl pairs **11**–**12**, **17**–**18**, and **19**–**20** did not show significant differences in their respective cytotoxicity. These results also showed that the activity of compounds that have the 3,4,5-trimethoxyphenyl near the carbonyl group of thiazol-2(3*H*)-one were effected more by introduction of a chlorine atom at the C-4 position of the five-membered ring than their isomers.



Scheme 2. Reagents and conditions: (a) Ar-NH_2, molecular sieves (4 Å), $NaHCO_3$, toluene, reflux, overnight; (b) ClCOSCl, pyridine, CCl_4, 40 °C, 1 h. (c) $SnCl_2{\cdot}2H_2O$, EtOH, reflux, 2 h.

Table 1
Cytotoxicity of synthesized compounds in CEM leukemia cells

Compound	Cytotoxicity IC_{50}[a] (μM)
3	>20
4	13.2
5	1.5
11	14.9
12	13.8
13	1.8
14	>20
15	2.7
16	>20
17	10.1
18	16.2
19	8.2
20	7.5
21	0.24
22	0.12
CA-4	0.002

[a] IC_{50} values, concentration required to inhibit 50% of CEM leukemia cell proliferation, were determined as described in Ref. 5.

The overall biological activity studies of the compounds in these two series indicated that the compound with the B-ring possessing 3,4,5-trimethoxy systems and the A-ring with a 4-methoxy substitution was more potent than their isomers with an interchange of the substitution pattern of rings A and B.

Since **21** and **22** showed good activity against the non-solid human CEM cell line they were further evaluated against a panel of solid human cancer cell lines including human liver cancer cells Bel-7402, HepG2, SMMC-7221, human breast cancer cells MCF-7, human pancreatic cancer cells SW-1990, and human colon adenocarcinoma HCT116. The results are summarized in Table 2 with those of **CA-4** as the positive control. Both compounds **21** and **22** showed only moderate cytotoxicity against the solid human cancer

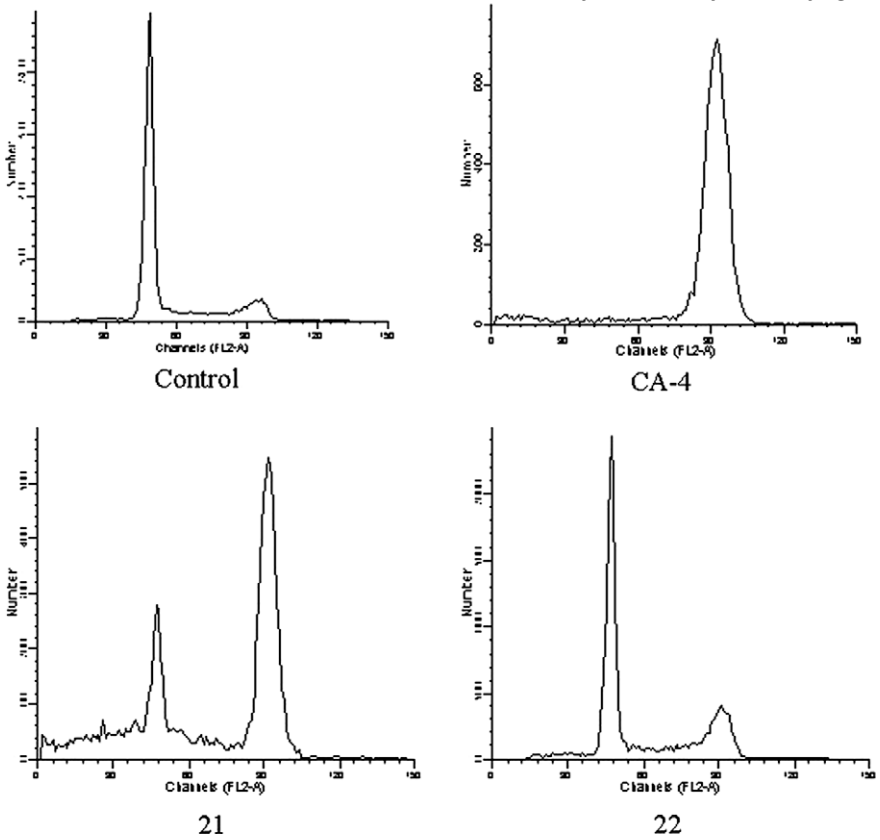


Figure 2. Cell cycle analysis. HepG2 cells were untreated or treated with **CA-4** (0.03 μM), **21** (4.85 μM) and **22** (150 μM), respectively, for 20 h at 37 °C. Cell cycle was analyzed with flow cytometry as described in Ref. 5.

Table 2
Cytotoxicity of synthesized compounds **21**, **22** and **CA-4** in human cancer cell lines

Cell line[a]	IC_{50}[b] (μM)		
	21	**22**	**CA-4**
Bel-7402	3.3	23.4	1.72
HepG2	4.5	96.0	0.006
SMMC-7721	7.4	118.2	0.29
MCF-7	4.4	52.5	0.03
SW-1990	20.3	56.7	0.25
HCT116	3.4	85.1	0.006
CEM	0.24	0.12	0.002

[a] Cell lines: Bel-7402, HepG2, SMMC-7721, human liver cancer; MCF-7, human breast cancer; SW-1990, human pancreatic cancer; HCT116, human colon cancer; CEM, human leukemia.
[b] IC_{50} values, concentration required to inhibit 50% of human tumor cells proliferation, were determined as described in Ref. 5.

cell lines, whereas the activity against the non-solid human CEM cell line was much better. Interestingly compound **21** showed two-fold less cytotoxicity against the non-solid human CEM cell line than compound **22**. However, the cytotoxicity of compound **21** against the solid human cancer cell lines tested are 2–25-fold stronger than compound **22**. Unfortunately, the activity of both **21** and **22** was significantly poorer than that of **CA-4**.

To learn whether, or not, these analogues would interrupt microtubule-tubulin dynamics and cause mitotic arrest, flow cytometric analysis was done for the compounds **21** and **22** (Fig. 2) using **CA-4** as the positive control. Treatment of the HepG2 cells with **21** for 20 h induced a dose-dependent increase of cells at the G2/M and a simultaneous decrease of the S- and G1-phase cells. The EC_{50} (drug concentration that causes 50% of the cancer cells arrested at G2/M) of compound **21** was 3.89 μM, 130-fold higher than that of **CA-4** (EC_{50} <0.03 μM). Treatment of the HepG2 cells with compound **22** under identical conditions caused no arrest at the G2/M-phase. The EC_{50} of compound **22** was over 250 μM.

The results suggest that the reduced activity on mitotic arrest of compound **21** as well as loss of activity for compound **22** appears to contribute to the observed decline in cancericidal activity.

In summary, we have presented the synthesis and evaluation of cytotoxicity of two series of compounds as **CA-4** analogues with thiazol-2(3*H*)-one and thiazol-2(3*H*)-imine as *cis*-restricted five-membered heterocycles. Compounds **21** and **22** showed good cytotoxicity against the non-solid human CEM cell line, however they were much less effective against a panel of solid human cancer cell lines. In addition, the EC_{50} of compounds **21** and **22** is 3.89 μM and over 250 μM, respectively, much less active than that of **CA-4**. The results showed that compounds with the B-ring possessing the 3,4,5-trimethoxy system and the A-ring with a 4-methoxy substitution were more potent than their isomers with an interchange of the substitution pattern of rings A and B.

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