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Synthesis and cytotoxicity of 3,4-disubstituted-5-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4-triazoles and novel 5,6-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives bearing 3,4,5-trimethoxyphenyl moiety

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ABSTRACT

A series of 3,4-disubstituted-5-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4-triazoles and some novel 5,6-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles bearing 3,4,5-trimethoxyphenyl moiety were synthesized and screened for their anticancer activity. The preliminary bioassay results indicated that compounds **14** and **16** showed much stronger cytotoxicity than Doxorubicin against HepG2 cell lines with IC_{50} values of 0.58 and 3.17 μM, respectively. Meanwhile compound **16** also exhibited a broad spectrum of antitumor activity against MCF-7 and MKN45 with IC_{50} values of 10.92 and 13.79 μM, respectively.

In recent years, increasing attentions have been focused on the 1,2,4-triazoles and their fused heterocyclic derivatives owing to their promising biological activities as well as great utility in preparative organic chemistry.[1–5] Especially some sulfur-containing 1,2,4-triazole derivatives always possess broad-spectrum biological activities, such as herbicidal,[6] insecticidal,[7] antifungal,[8] antimicrobial,[9] antiviral,[10] and anti-inflammatory properties.[11] Recently substituted 3-thio-1,2,4-triazoles have emerged as potential antitumor agents inhibiting epidermal growth factor (EGFR) tyrosine kinase.[12]

As a cis-stilbene natural product, combretastatin A-4 (CA-4, **1**, Fig. 1) with simple structure, has been identified as one of the most potent anti-mitotic activity, which make it an attractive lead compound in the development of new antitumor agents. The extensive structure–activity relationship analysis revealed that both the 3,4,5-trimethoxy substitution pattern on the A-ring and the cis-olefin configuration at the bridge were essential for optimal activity, while the B-ring is greater tolerance of structural modifications.[13] However, the cis-stilbenes tend to isomerize to the more stable trans-forms which show dramatic reduction in cytotoxic activities.[14,15] By replacement of the double bond bridge of CA-4 with a rigid triazole ring, compounds **2**, **3** and **4** (Fig. 1) have been found to exhibit potent anticancer activities comparable with CA-4.[16–18]

Therefore, as a part of our research work on the development of novel bioactive nitrogen-containing heterocycle,[19–21] we developed an idea that introducing 3-thio-1,2,4-triazole ring or fused 1,2,4-triazole ring as the locked cis-type bridge might also result in new compounds with high cytotoxic activity (Fig. 1). In this Letter, we described the design, synthesis, and in vitro cytotoxic activities of a series of 3,4-disubstituted-5-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4-triazole **5–13** and some novel 5,6-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives bearing 3,4,5-trimethoxyphenyl moiety **14–16**.

Our synthetic strategy for the target compounds is illustrated in Scheme 1. Starting from 3,4,5-trimethoxybenzoic acid, according to the reported method,[22] the key intermediate in the present study 4-amino-3-(3,4,5-trimethoxy phenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione III was synthesized in four steps employing esterification, hydrazidation, salt formation, and cyclization. Subsequently, the compound III was converted into their corresponding Schiff base IV by condensation reaction with commercially purchased substituted benzaldehyde. Finally, compound IV reacted with various alkylation reagents to afford the target compounds **5–13** in yields of 64–89%. The structures of title compounds **5–13** were elucidated by comprehensive ^{1}H NMR, EI-MS and elemental analysis, and all the analytical data were documented in the Supplementary data. In addition, the crystal structure of **13** was determined by X-ray diffraction analyses (Fig. 2), which revealed that the carbon–nitron double bond in the molecule was of E-configuration.[23] Most interestingly, using phenacyl bromide or ethyl 2-bromoacetate as alkylation reagents, some novel 3,6,6-trisubstituted-5,6-dihydro

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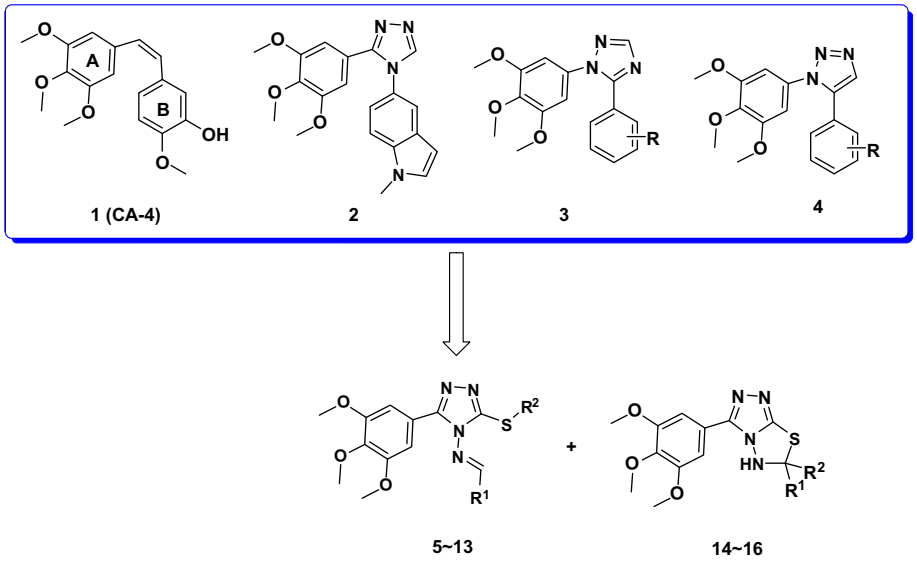
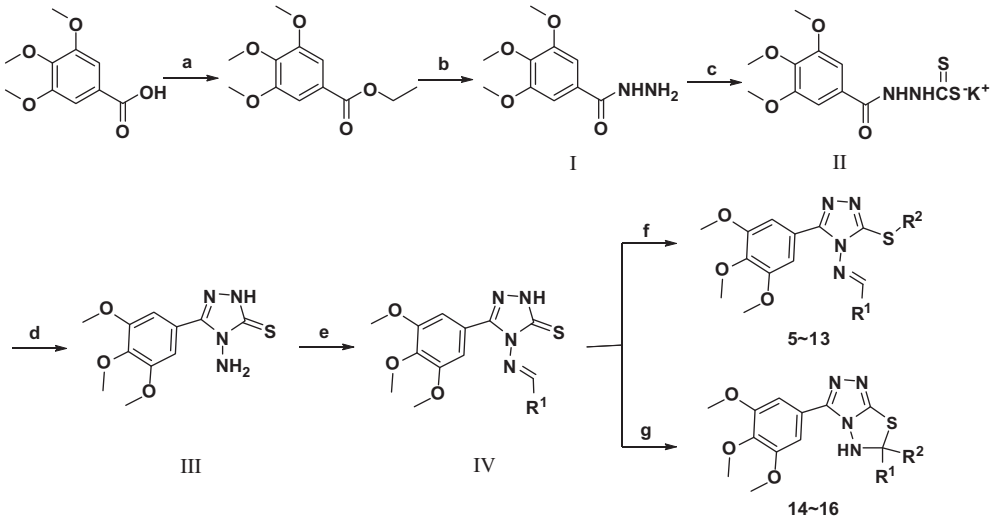


Figure 1. Design strategy of the target compounds **5–16**.



Scheme 1. General synthetic route for compounds **5–16**. Reagents and conditions: (a) concd. H_2SO_4, ethanol, reflux; (b) 60%$NH_2H_2{\cdot}H_2O$, ethanol, reflux; (c) KOH, CS_2, ethanol, rt; (d) 60%$NH_2NH_2{\cdot}H_2O$, H_2O, reflux, HAc; (e) R^1CHO, HAc, reflux; (f) K_2CO_3, acetone, substituted benzyl bromides, ethyl 2-bromoacetate or methyl iodide, reflux; (g) K_2CO_3 acetone, phenacyl bromide or ethyl 2-bromoacetate, reflux.

derivatives of 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole **14–16** were obtained in good yields, and their structures were fully characterized by ^{1}H NMR, ^{13}C NMR, EI-MS and elemental analysis.[24] Further optimization of reaction conditions and study of the reaction mechanism are well under way.

The cytotoxicity of all the title compounds **5–16** against four human cancer cell lines, including HepG2 (hepatocellular carcinoma cell line), MKN45 (gastric carcinoma cell line), MCF-7 (breast cancer cell line) and HT-29 (human colon carcinoma cell line), were evaluated by MTT assay.[25] Doxorubicin was used as the reference drug and the results expressed as IC_{50} (μM) were summarized in Table 1. As the results indicated that some compounds showed excellent antiproliferative activity against selected human tumor cell lines. Among them, two representative compounds **14** and **16** displayed much higher antitumor activity against HepG2 cell lines than positive control Doxorubicin. In particular, the compound **14** showed the most active in inhibiting HepG2 and MKN45 with the IC_{50} values of 0.58 and 7.47 μM, respectively. Interestingly, compound **16** exhibited a broad spectrum of antitumor activity against HepG2, MCF-7 and MKN45 with IC_{50} values of 3.17, 10.92 and 13.79 μM, respectively.

Further analysis on the structure–activity relationship revealed that, in most cases, 1,2,4-triazole derivatives **5–13** did not display high activity against the four tested human cancer cell lines, while most 5,6-dihydro-1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole derivatives showed highly potent cytotoxic activity. These results indicated that iminoacyl moiety, on the 4-position of 1,2,4-triazole ring, is too flexible to keep in cis-orientation between the aryl ring and 3,4,5-trimethoxyphenyl ring, which can be verified by the crystal structure of **13**. Nevertheless, within the series of fused 1,2,4-triazole compounds**,** anticancer activities were influenced by the C-6 substituents on the 5,6-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole ring and phenacyl substitution always gave higher activities (**14** and **16**). Replacement of phenacyl group with ester group led

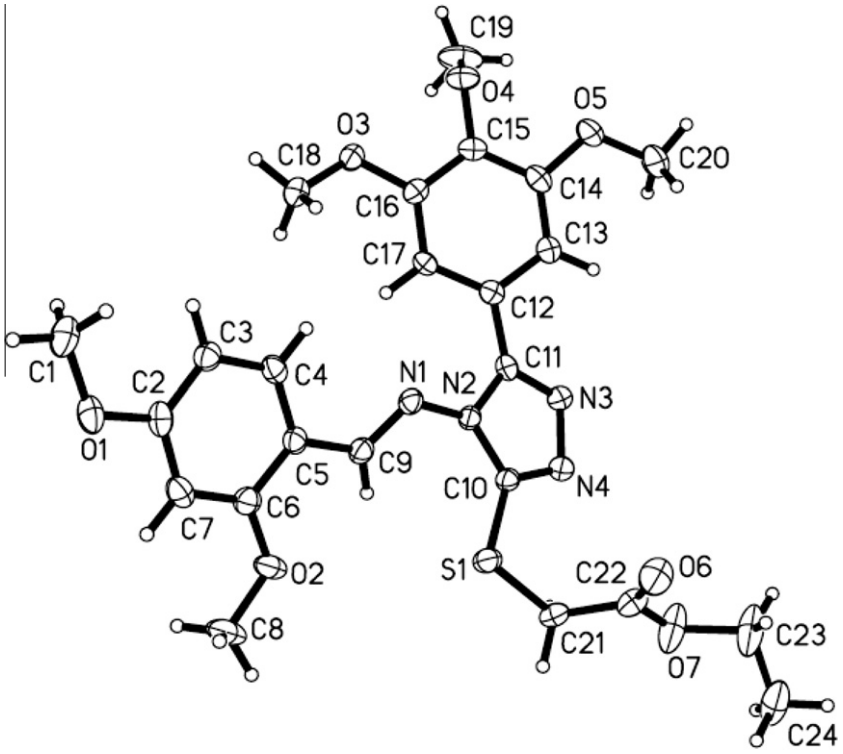
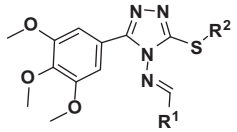
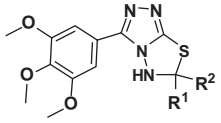


Figure 2. X-ray crystal structures of compound **13** (CCDC 820019).

Table 1
In vitro cytotoxicity (IC_{50}[a], μM) of synthesized compounds **5–13** and **14–16**



5~13



14~16

Compd	R^1	R^2	Cytotoxicity			
			HepG2	MCF-7	MKN45	HT-29
5	C_6H_5	Me	>200	>200	28.50	>200
6	C_6H_5	$C_6H_5CH_2$	>200	>200	>200	>200
7	C_6H_5	4-$FC_6H_4CH_2$	>200	>200	>200	>200
8	C_6H_5	2-$ClC_6H_4CH_2$	31.72	>200	>200	>200
9	2,4-$(MeO)_2C_6H_3$	Me	>200	>200	>200	143.87
10	2,4-$(MeO)_2C_6H_3$	4-$FC_6H_4CH_2$	>200	>200	>200	126.40
11	2,4-$(MeO)_2C_6H_3$	2-$ClC_6H_4CH_2$	>200	63.72	>200	>200
12	2,4-$(MeO)_2C_6H_3$	$C_6H_5CH_2$	60.94	152.59	49.00	>200
13	2,4-$(MeO)_2C_6H_3$	$EtCO_2CH_2$	>200	>200	>200	>200
14	C_6H_5	4-$MeC_6H_4COCH_2$	0.58	>200	7.47	150.79
15	C_6H_5	$EtCO_2CH_2$	>200	>200	>200	>200
16	2,4-$(MeO)_2C_6H_3$	4-$MeC_6H_4COCH_2$	3.17	10.92	13.79	66.81
Doxorubicin			5.57	17.67	0.168	—[b]

[a] IC_{50} values are presented as mean values of three independent experiments done in quadruplicates. Coefficients of variation were <10%.
[b] Not tested.

to a dramatic decrease in antitumor activity (**15**). It can be concluded that phenacyl group at the 6-position of the fused 1,2,4-triazoles play a crucial role in modulating the antitumor activity.

In conclusion, we synthesized a series of 3,4-disubstituted-5-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4-triazoles and three novel 5,6-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives. The preliminary bioassay showed that compound **14** and **16** exhibited much stronger cytotoxicity than Doxorubicin against HepG2 cell lines. Meanwhile compound **16** also displayed potent and broad-spectrum antitumor activity against MCF-7 and MKN45. These results indicated that 5,6-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles could be used as a promising lead for further developing

new antitumor agents. Further structural optimization and the mechanism of reaction in detail will be studied in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.023.

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24. *2-(6-Phenyl-3-(3,4,5-trimethoxyphenyl)-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)-1-p-tolylethanone* (***14***): Following the general procedure described in the Supplementary data, compound **14** was purified by recrystallization from acetone. White solid, yield: 78%. Mp: 191–192 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.41 (s, 3H, SCH_3), 3.80 (s, 6H, 2 × OCH_3), 3.86 (s, 3H, OCH_3), 5.14 (d, J = 4.4 Hz, 1H, SCH), 5.29 (d, J = 2.8 Hz, 1H, SCH), 6.54 (s, 1H, NH), 7.24–7.31 (m, 5H, ArH), 7.36 (s, 2H, ArH), 7.45 (t, J = 4.0 Hz, 2H, ArH), 7.80 (d, J = 8.0 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.8, 41.6, 56.1, 59.1, 60.9, 105.0, 121.3, 127.2, 128.8, 128.9, 129.1, 129.9, 131.6, 136.0, 139.5, 143.0, 145.9, 152.2, 153.1, 194.5. ESI MS: m/z = 525.22 $[M+Na]^+$, 503.14 $[M+1]^+$. Anal. Calcd for $C_{27}H_{26}N_4O_4S$: C, 64.52; H, 5.21; N, 11.15; S, 6.38. Found: C, 64.37; H, 5.43; N, 10.94; S, 6.12.
Ethyl 2-(6-phenyl-3-(3,4,5-trimethoxyphenyl)-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl) acetate (***15***): Following the general procedure described in the Supplementary data, compound **15** was purified by recrystallation from acetone. White solid, yield: 67%. Mp: 145–146 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.27 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 3.83 (s, 6H, 2 × OCH_3), 3.87 (s, 3H, OCH_3), 4.21–4.29 (m, 2H, OCH_2CH_3), 4.38 (d, J = 4.0 Hz, 1H, SCH), 4.96 (t, J = 4.0 Hz, 1H, SCH), 6.38 (s, 1H, NH), 7.29 (d, J = 3.0 Hz, J = 7.0 Hz, 3H, ArH), 7.36 (d, J = 5.6 Hz, 4H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.8, 41.0, 56.1, 58.8, 60.9, 62.7, 104.9, 121.2, 126.9, 128.9, 129.0, 134.9, 139.4, 142.3, 152.0, 153.0, 170.4. ESI MS: m/z = 457.11 [M+1]+. Anal. Calcd for $C_{22}H_{24}N_4O_5S$: C, 57.88; H, 5.30; N, 12.27; S, 7.02. Found: C, 57.61; H, 5.49; N, 11.98; S, 6.86.
2-(6-(2,4-Dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)-1-p-tolylethanone (***16***): Following the general procedure described in the Supplementary data, compound **16** was purified by recrystallation from acetone. White solid, yield: 72%. Mp: 184–185 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.35 (s, 3H, Ar –CH_3), 3.70 (s, 3H, OCH_3), 3.72 (s, 6H, 2 × OCH_3), 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 5.04 (dd, J = 6.4 Hz, J = 8.4 Hz, 1H, SCH), 5.36 (d, J = 6.4 Hz, 1H, SCH), 6.28–6.33 (m, 2H, ArH), 6.41 (d, J = 2.0 Hz, 1H, NH), 7.08 (d, J = 8.4 Hz, 1H, ArH), 7.18 (d, J = 8.0 Hz, 2H, ArH), 7.43 (s, 2H, ArH), 7.71 (d, J = 8.4 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.6, 42.7, 55.3, 55.5, 55.9, 57.9, 60.7, 99.0, 104.5, 104.8, 117.0, 121.5, 128.6, 129.5, 129.7, 131.9, 139.2, 143.9, 145.3, 151.6, 152.9, 157.5, 161.0, 194.0. ESI MS: m/z = 563.23 $[M+1]^+$. Anal. Calcd for $C_{29}H_{30}N_4O_6S$: C, 61.91; H, 5.37; N, 9.96; S, 5.70. Found: C, 61.83; H, 5.14; N, 10.08; S, 5.54.
25. Evaluation of cytotoxicity (MTT): The antitumor activity of compounds **5**–**16** were evaluated with HepG2, MCF-7, MKN45 and HT-29 cell lines by the standard MTT assay in vitro. The cancer cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS). Cells were passaged at 70–80% confluence, about twice a week by trypsinization. Exponentially growing cells were plated in 96-well plates (8×10^4 cells/well for MKN45, HT-29 and MCF-7 cells, 5×10^4 cells/well for HepG2 cells) and incubated at 37 °C for 24 h for attachment. Test compounds were prepared by dissolving in dimethyl sulfoxide (DMSO) at 10 mM and diluted with the medium. Then, culture medium was changed, and cells grew in medium with the test compounds. DMSO (0.1%) was used as negative control. Cells were incubated at 37 °C for 48 h. Then the medium was replaced with MTT solution (5 mg/ml, 100 μL) followed by incubation for another 3 h. The medium was then aspirated and formazan crystals were dissolved in DMSO (100 μL) for about 10 min. The absorbance at 570 nm (Abs) of the suspension was measured by an enzyme-linked immunosorbent assay (ELISA) reader. The inhibition percentage was calculated using the following formula: % inhibition = $(Abs_{control} - Abs_{compound})/Abs_{control} \times 100\%$. The IC_{50} values of the test compounds and Doxorubicin were measured by treating cells with drugs of varying concentrations, and analyzing by use of the prism statistical package (GraphPad Software, San Diego, CA, USA).