Synthesis and evaluation of antitumor activities of novel chiral 1,2,4-triazole Schiff bases bearing γ-butenolide moiety

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Abstract
Background: 1,2,4-Triazole derivatives have received much attention due to their versatile biological properties including antibacterial, antifungal, anticonvulsant, antiinflammatory, anticancer, and antiproliferative properties. 1,2,4-Triazole nucleus has been incorporated into a wide variety of therapeutically interesting molecules to transform them into better drugs. Schiff bases of 1,2,4-triazoles have also been found to possess extensive biological activities. On the other hand, γ-substituted butenolide moiety represents a biological important entity that is present in numerous biologically active natural products.

Results: We have described herein the synthesis of 12 hybrid 1,2,4-triazole Schiff bases bearing γ-substituted butenolide moiety. These compounds were synthesized by utilizing the tandem asymmetric Michael addition/elimination reaction as the key step. All the new compounds were evaluated for their in vitro anticancer activity.

Conclusions: Tandem asymmetric Michael addition/elimination approach has offered an easy access to new chiral 1,2,4-triazole compounds 7a-7l. All these chiral 1,2,4-triazole derivatives exhibited good anticancer activities towards Hela. Of all the tested compounds, the chiral compound 7l with an IC50 of 1.8 μM was found to be the most active.

Keywords: 1,2,4-triazole, Schiff base, γ-butenolide, A activity, HeLa cells

Background
Cancer, a diverse group of diseases characterized by the proliferation and spread of abnormal cells, is a major worldwide problem. Therefore, the discovery and development of new potent and selective anticancer drugs are of high importance in modern cancer research.

1,2,4-Triazole derivatives have received much attention due to their versatile biological properties including antibacterial, antifungal, anticonvulsant, antiinflammatory, anticancer, and antiproliferative properties [1-10]. 1,2,4-Triazole nucleus has been incorporated into a wide variety of therapeutically interesting molecules to transform them into better drugs [11-13]. Schiff bases of 1,2,4-triazoles have also been found to possess extensive biological activities [14-18]. On the other hand, γ-substituted butenolide moiety represents a biological important entity that is present in numerous biologically active natural products [19-24].

Recently, we reported on the synthesis of a series of hybrid 1,3,4-thiadiazoles derivatives possessing γ-substituted butenolide moiety, which exhibited good anticancer activities against cervical cancer cells [25]. In continuation of our studies on the identification of potential active antitumor compounds, herein we report the synthesis and evaluation of a new series of hybrid 1,2,4-triazole Schiff bases bearing γ-substituted butenolide moiety as potential anticancer agents (Figure 1). To the best of authors’ knowledge, the synthesis and anticancer activities of this types of compounds have not been reported so far.

Results and discussion
The enantiomerically pure γ-substituted butenolides 1 were synthesized via acetalization of mucobromic acid by employing (−)-menthol and (+)-borneol as a chiral
The 1,2,4-triazole Schiff bases were synthesized by condensation of 4-amino-5-substituted-4H-1,2,4-triazol-3-thiols with aromatic aldehydes in glacial acetic acid (Scheme 1) [14]. The 4-amino-5-substituted-4H-1,2,4-triazol-3-thiols were prepared according to the previous procedure [28,29]. When $R^1$ is methyl, the compound 5a was prepared by heating a mixture of thiocarbohydrazide with acetic acid [28]. When $R^1$ are aryl, a different procedure was employed as aromatic carboxylic acids are generally solid, have high melting points, and are difficult to react with thiocarbohydrazide fully [29]. Thus, staring from aromatic carboxylic acid esters 2, the aryl hydrazides 3 were obtained by reaction with hydrazine in EtOH. Treatment of the aryl hydrazides 3 with $CS_2$ under a basic condition (KOH/EtOH) gave the corresponding potassium aryl dithiocarbazates 4. Then, the resulting compounds 4 were cyclized with hydrazine to provide the compounds 5b–d in good yields.

The target compounds 7a–l were prepared via tandem Michael addition–elimination reaction of γ-substituted butenolides 1 with 5-substituted 1,2,4-triazole Schiff bases 6 under phase-transfer catalysis conditions (Scheme 2).

The structures of these new compounds 7a–l were characterized with IR, $^1H$, $^{13}C$ NMR, and LC-MS spectra. In addition, the molecular structure of 7a was unambiguously confirmed through X-ray crystallography (Figure 2).a

All newly synthesized compounds 7a–l were initially evaluated for their in vitro anticancer activities against cervical cancer cell lines (HeLa) using the MTT assay, and the results were summarized in Table 1. All the compounds 7a–l displayed good inhibition activities on HeLa cell lines. Of all the studied compounds, the compound 7l exhibited the best inhibitory activity with an $IC_{50}$ of 1.8 μM.

Then, the growth inhibition rates of HeLa cell lines with compounds 7a–l at different concentrations (0.1–20 μM) were evaluated (Table 2). After being treated with 20 μg/mL compound 7l for 24 h, the growth inhibition rate was the highest (90.0%).

Experimental

All the chemicals were used as-received without further purification unless otherwise stated. IR spectra were
recorded on a FTIR-8400S spectrometer as KBr disks. 

$^1$H NMR and $^{13}$C NMR spectra were obtained with a Bruker Avance III 400 MHz spectrometer in chloroform-$d$ (CDCl$_3$) and tetramethylsilane was used as an internal standard. Diffraction measurement was made on a Bruker AXS SMART 1000 CCD diffractometer with graphite-monochromatized Mo K$_{\alpha}$ radiation ($\lambda$ = 0.71073 Å). All the melting points were determined on a WRS-1B digital melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was carried out on silica GF254 plates (Qingdao Haiyang Chemical Co., Ltd., China).

General procedure for the synthesis of compounds 7

To an aqueous solution of dichloromethane was sequentially added the compounds 1 (1.0 mmol), potassium carbonate (1.0 mmol), 18-crown-6 (0.1 mmol), and the compounds 6 (1.1 mmol). The resulting mixture was stirred at room temperature, and the reaction was monitored by TLC. On completion of the reaction (10–20 h), the mixture was exacted and the organic layer was washed with saturated brine. Then the organic layer was dried over anhydrous MgSO$_4$, filtered, and concentrated in vacuo. The purification of the residue by silica gel column chromatography or crystallizations yielded the desired compounds 7a–l in 65–89% yields (For the characterization of compound 7a–7l, please see the Additional file 1: Supporting Information). Compound 7l: white solid, 76% yield, $[\alpha]_{D}^{20} = -37.2$ (c = 0.5 M, CHCl$_3$), mp 131–132°C. IR (KBr) 3210, 1780, 1603, 1523, 1440, 1421, 1319, 1212, 1134, 993 cm$^{-1}$. $^1$HNMR (400 MHz, CDCl$_3$) 10.04 (s, 1H), 8.73 (s, 1H), 7.59–7.04 (m, 2H), 7.14–7.06 (m, 2H), 6.20 (s, 1H), 3.81 (m, 1H), 2.59 (s, 3H), 2.25–2.22 (m, 1H), 1.69–1.09 (m, 6H), 0.78–0.74 (m, 6H), 0.53 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) 170.4, 164.0, 160.3, 152.9, 151.0, 138.2, 136.3, 133.7, 120.6, 118.1, 115.4, 112.8, 103.1, 88.8, 49.3, 47.6, 44.7, 36.7, 27.9, 26.5, 19.5, 18.7, 13.3, 11.2. HRMS calcd. for C$_{24}$H$_{27}$BrN$_4$O$_4$S $[M]^{+}$: 546.0936, found 546.0933.

Pharmacology

Cells (1 × 10$^4$ in 100 µL) were seeded on 96-well plates in triplicate. Following a 24-h culture at 37°C, the medium was replaced with fresh medium at various concentrations (1.25, 2.5, 5, 10, 20 µg/mL) of compounds 7a–l in a final volume of 110 µL. At the same time, set drug-free medium negative control well, and solvent control well of the same volume of dimethyl sulfoxide (DMSO). Cells were incubated at 37°C for 24 h. Then, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (2 mg/mL in a phosphate buffer solution) was added to each well, incubated for an additional 4 h, the plates were centrifuged at 1000 r/min for 10 min, then the medium was removed. MTT formazan precipitates were dissolved in 100 µL of DMSO, shaken mechanically for 10 min and then read immediately at 492 nm in a plate reader (Opsys MR,

![Scheme 2 Synthesis of target compounds 7a–l.](image-url)
Cell inhibition rate = \[
\frac{A_{492}\text{(negative control well)} - A_{492}\text{(dosing well)}}{A_{492}\text{(negative control well)}}\times 100\%.
\]

Conclusions
In summary, a new type of chiral 1,2,4-triazole Schiff bases bearing γ-substituted butenolide moiety have been synthesized and their in vitro anticancer activities against HeLa cell lines have been evaluated. These chiral 1,2,4-triazole derivatives exhibited good anticancer activities towards HeLa. The compound 7l with an IC50 of 1.8 μM was found to be the most active. Further studies of anticancer activities of these compounds are in progress in our group.

Endnote
*The molecular structure of the product 7a was determined by means of X-ray crystallographic studies. CCDC 829447 (7a) contains the supplementary crystallographic data for this article. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Additional file
Additional file 1: Supporting Information Available. Experimental procedures, spectral data of new compounds.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
ZS and XL carried out the design of the project, and drafted the manuscript. XL and HL synthesized target compounds. XZ evaluated in vitro anticancer activities against cervical cancer cell lines (HeLa). All authors read and approved the final manuscript.

Supporting information available
Experimental procedures, spectral data of new compounds.

Acknowledgments
We gratefully acknowledge the financial support from the National Natural Science Foundation of China (20962023, 21062014, 21162034), the Major State Basic Research Development Program of China (2007CB21602), the Program for New Century Excellent Talents in University (NCET-10-0907), the Key Project of Chinese Ministry of Education (210237), and the Natural Science Foundation of Ningxia Province of China (N20606).

Table 1 In vitro anticancer activities against HeLa cell lines with compounds 7a–l (n = 3)

| Compound | IC50 (μM) | Compound | IC50 (μM) |
|----------|-----------|----------|-----------|
| 7a       | 19.7      | 7h       | 7.1       |
| 7b       | 4.4       | 7i       | 3.7       |
| 7c       | 11.6      | 7j       | 4.5       |
| 7d       | 11.2      | 7k       | 6.2       |
| 7e       | 6.8       | 7l       | 1.8       |
| 7f       | 5.1       | DDP (Cisplatin) | 2.6 |
| 7g       | 8.2       |          |           |

The IC50 values represent the compound concentration (μM) required to inhibit tumor cell proliferation by 50%.

Table 2 Growth inhibition rates of HeLa cell lines with compounds 7a–l at different concentrations

| Compounds | Inhibition rates (%) |
|-----------|----------------------|
|           | 1.25 μM | 2.5 μM | 5 μM | 10 μM | 20 μM |
| 7a        | 1.2     | 8.7    | 16.1 | 30.2  | 41.9  |
| 7b        | 26.2    | 30.2   | 53.8 | 65.2  | 85.7  |
| 7c        | 9.4     | 35.3   | 21.3 | 52.3  | 60.2  |
| 7d        | 17.9    | 11.0   | 34.8 | 47.7  | 65.5  |
| 7e        | 10.9    | 11.4   | 24.3 | 76.0  | 85.2  |
| 7f        | 10.3    | 27.4   | 56.9 | 73.4  | 85.1  |
| 7g        | 18.0    | 30.9   | 43.4 | 49.7  | 67.1  |
| 7h        | 8.3     | 14.3   | 40.7 | 77.5  | 71.7  |
| 7i        | 14.3    | 35.6   | 67.8 | 85.4  | 87.7  |
| 7j        | 24.0    | 32.5   | 54.1 | 67.6  | 81.2  |
| 7k        | 14.8    | 36.2   | 60.0 | 56.7  | 67.3  |
| 7l        | 47.5    | 45.4   | 74.1 | 88.6  | 90.0  |

Figure 2 ORTEP view of the crystal structure of compound 7a.
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Received: 9 November 2011 Accepted: 26 February 2012

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Cite this article as: Li et al. Synthesis and evaluation of antitumor activities of novel chiral 1,2,4-triazole Schiff bases bearing γ-butyrolactone moiety. Organic and Medicinal Chemistry Letters 2012 2:26.

doi:10.1186/2191-2858-2-26

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