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Synthesis of aryl dihydrothiazol acyl shikonin ester derivatives as anticancer agents through microtubule stabilization

Hong-Yan Lin [a,b], Zi-Kang Li [a,b], Li-Fei Bai [c], Shahla Karim Baloch [a,b], Fang Wang [a,b], Han-Yue Qiu [a,b], Xue Wang [a,b], Jin-Liang Qi [a,b], Raong-Wu Yang [a,b], Xiao-Ming Wang [a,b,*], Yong-Hua Yang [a,b,*]

[a] State Key Laboratory of Pharmaceutical Biotechnology, NJU-NJFU Joint Institute of Plant Molecular Biology, School of Life Sciences, Nanjing University, Nanjing 210023, China
[b] Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China
[c] Jiangsu Key Laboratory of Biofunction Molecule, Jiangsu Second Normal University, Nanjing 210013, China

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4-Methoxybenzonitrile (PubChem CID: 70129)
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ABSTRACT

The high incidence of cancer and the side effects of traditional anticancer drugs motivate the search for new and more effective anticancer drugs. In this study, we synthesized 17 kinds of aryl dihydrothiazol acyl shikonin ester derivatives and evaluated their anticancer activity through MTT assay. Among them, **C13** showed better antiproliferation activity with $IC_{50} = 3.14 \pm 0.21$ μM against HeLa cells than shikonin ($IC_{50} = 5.75 \pm 0.47$ μM). We then performed PI staining assay, cell cycle distribution, and cell apoptosis analysis for **C13** and found that it can cause cell arrest in G2/M phase, which leads to cell apoptosis. This derivative can also reduce the adhesive ability of HeLa cells. Docking simulation and confocal microscopy assay results further indicated that **C13** could bind well to the tubulin at paclitaxel binding site, leading to tubulin polymerization and mitotic disruption.

1. Introduction

Mitosis is a complicated process that ensures the faithful inheritance of genetic material by cellular progeny while preventing aneuploidy [1]. Mitosis disruption and chromosomal instability are the two established hallmarks of cancer [2]. Microtubules (MTs) are cytoskeletal filaments comprising α- and β-tubulin proteins and an individual MT is a long and hollow cylinder [3]. However, the collective structure of MTs may assume various configurations. For example, MT aster is a star-shaped structure where MTs radiate from a centrally located centrosome [4]. The mitotic spindle that forms during cell division is composed of two asters located at opposite poles of the cell. MTs emanate from asters toward the spindle midzone and exert forces to separate chromosomes between two daughter cells. The formation of MTs is a dynamic process that involves the polymerization and depolymerization of α- and β-tubulin heterodimers [5]. Disruption of the dynamic equilibrium blocks the cell division machinery at mitosis and leads to cells cycle arrest in metaphase, resulting in cell

Abbreviations: MTT, (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide); NMR, nuclear magnetic resonance; TLC, thin layer chromatography; TMS, tetramethylsilane; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; DAPI, 4′,6-diamidino-2-phenylindole; BSA, bovine serum albumin; SAR, structure–activity relationship.

* Corresponding authors. Tel.: +86 25 89681381; fax: +86 25 89681381.
E-mail addresses: Wangxm07@nju.edu.cn (X.-M. Wang), Yangyh@nju.edu.cn (Y.-H. Yang).

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death [5,6]. Given the vital role of MTs in cell growth and function, it has become an important target for the design and development of new anticancer agents.

Tubulin-binding agents, also named "spindle poisons" can bind to tubulin and alter MT polymerization, perturb MT dynamics, disrupt mitotic spindle function, and block cell cycle progression [7]. Historically, taxanes and vinca alkaloids represent tubulin-binding agents [7–10]. The former promotes MT polymerization, whereas the latter inhibits MT polymerization. These agents induce sustained mitotic arrest in metaphase/anaphase transition and subsequent apoptotic cell death [11,12].

Shikonin and its derivatives are active naphthoquinone compounds isolated from the root of the Chinese herbal medicine *Lithospermum erythrorhizon* [13]. Shikonin is attracting considerable attention in the field of natural product chemistry because it has extensive pharmacological activities [14–16], especially good anticancer effects [17–20]. However, the side effects and toxicity of shikonin hinder its development as a new clinical anticancer agent [21]. In our previous study, we found that changing the side chain hydroxyl group into an ester group may reduce the toxicity of shikonin toward non-cancer cells; thus, shikonin ester derivatives may be good anticancer agents [19,20,22–25]. Meanwhile, some researchers have reported aryl thiazole compounds that can disrupt tubulin polymerization, thereby inhibiting the production of functional MTs and cell mitosis [26]. Thiazole also improves the water solubility and pharmacokinetic parameters of the drugs itself [26,27]. Based on the aforementioned results, we proposed in this work that theintroduction of thiazole moiety into shikonin may improve its water solubility and tumor targeting. Based on these studies, we synthesized a series of aryl dihydrothiazol acyl shikonin ester derivatives and evaluated them as tubulin-binding agents. The underlying mechanism was also studied.

2. Results

2.1. Chemistry

The routes to synthesizing the novel aryl dihydrothiazol acyl shikonin ester derivatives **C1–C17** are outlined in Fig. 1. These compounds were obtained in three steps, as elucidated in Section 3. All synthesized compounds were reported and characterized for the first time by ^{1}H NMR, elemental analysis, melting test, and mass spectroscopy, and results were in accordance with their depicted structures.

2.2. Bioactivity

2.2.1. *C13* selectively inhibited cancer-cell proliferation

All synthesized derivatives **C1–C17** were evaluated for their antiproliferation activities against five cancer cell lines [human hepatoma cell line (HepG2), human lung adenocarcinoma epithelial cell line (A549), carcinoma of cervix cell line (HeLa), human breast cancer cell line (MCF-7) and squamous cell carcinoma (SSC-4)], and two non-cancer cell lines [African green monkey kidney cell (VERO) and human normal liver cell (L02)] by MTT assay. Results shown in Table 3 indicated that all compounds had lower toxicity against the non-cancer cells VERO and L02 than shikonin itself. However, the introduction of dihydrothiazol moiety also generally attenuated the antiproliferation activity of shikonin against cancer cells as well. Fortunately, we obtained some good compounds with strong toxicity toward cancer cells while avoiding VERO and L02 cells.

Table 3 shows that most of the target compounds can also effectively inhibit the proliferation of the five tumor cells, but the effects were not better than those of the shikonin. The main reason is dihydrothiazol moiety reduced shikonin cytotoxicity. Interestingly, the introduction of some moieties reduced only the toxicity of shikonin toward the non-cancer cells except for cancer cells, such as **C2** (IC_{50} = 15.6 ± 1.00 μM), **C5** (IC_{50} = 9.91 ± 0.64 μM), and **C7** (IC_{50} = 10.4 ± 0.87 μM) against HepG2 cells (IC_{50} = 9.36 ± 0.57 μM for shikonin); **C1** (IC_{50} = 7.95 ± 0.32 μM), **C12** (IC_{50} = 9.66 ± 0.37 μM), and **C16** (IC_{50} = 7.93 ± 0.47 μM) against HeLa cells (IC_{50} = 5.75 ± 0.47 μM for shikonin); **C13** (IC_{50} = 6.52 ± 0.68 μM), **C7** (IC_{50} = 7.24 ± 0.77 μM), and **C2** (IC_{50} = 6.41 ± 0.38 μM) against MCF-7 cells (IC_{50} = 4.99 ± 0.36 μM for shikonin); and **C1** (IC_{50} = 16.2 ± 1.14 μM), and **C12** (IC_{50} = 11.1 ± 1.00 μM) against SCC-4 cells (IC_{50} = 10.1 ± 0.49 μM for shikonin). We also obtained **C13** (IC_{50} = 3.14 ± 0.21 μM) and **C8** (IC_{50} = 5.69 ± 0.42 μM), which showed better antiproliferation activity against HeLa cells than shikonin (IC_{50} = 5.75 ± 0.47 μM).

To further determine the effect of the compounds on HeLa cell viability, we performed PI staining assay for **C13**. HeLa cells (1×10^5 per well) were seeded in 12-well plates and treated with **C13** of various concentrations (0, 1, 3, and 5 μM) at 37 °C and 5% CO_2 for 0, 12, 24, and 36 h. Cells were then collected and analyzed using PI staining and flow cytometry analysis. From the results shown in Fig. 2, we observed that **C13** induced dose- and time-dependent death of cancer cells. Therefore, **C13** could efficiently inhibit cancer cell growth.

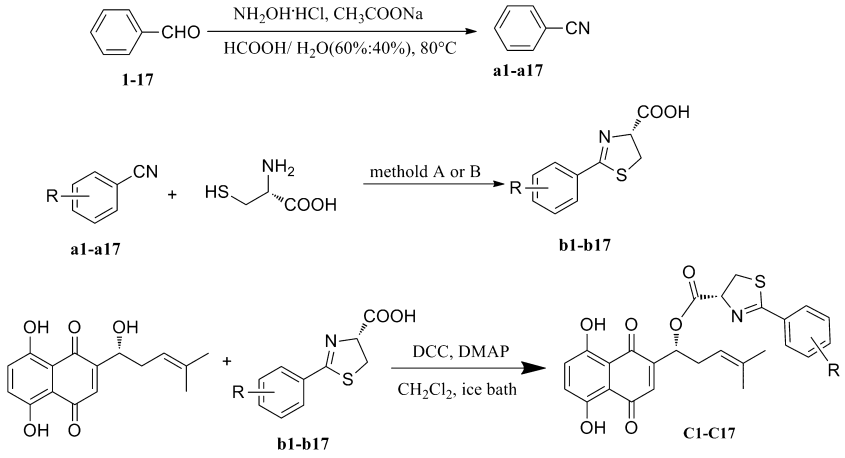

Fig. 1. Synthesis routes of **C1–C17**. The detailed process was described in Section 3.