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Synthesis, cytotoxic evaluation and molecular docking study of 2-alkylthio-4-(2,3,4-trimethoxyphenyl)-5-aryl-thiazoles as tubulin polymerization inhibitors

Marjan Salehi [a], Mohsen Amini [a], Seyed Nasser Ostad [b], Gholam Hossein Riazi [c], Amir Assadieskandar [a], Bentolhoda Shafiei [c], Abbas Shafiee [d,*]

[a] Department of Medicinal Chemistry, Faculty of Pharmacy and Drug Design & Development Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran
[b] Department of Toxicology and Pharmacology, Faculty of Pharmacy and Rational Drug Use Research Center, Tehran University of Medical Sciences, Tehran, Iran
[c] Institute of Biochemistry and Biophysics, University of Tehran, Tehran 13145-1384, Iran
[d] Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran

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ABSTRACT

A series of *cis*-restricted 2-alkylthio-4-(2,3,4-trimethoxyphenyl)-5-aryl-thiazole analogues of combretastatin A-4 were synthesized and investigated for inhibition of cell proliferation against three cancer cell lines, HT-29, MCF-7, and AGS, and a normal mouse fibroblastic cell line, NIH-3T3, using an MTT assay. The biological study showed that 2-(methylthio) substituted compounds showed little cytotoxic activity against the four cell lines. In contrast, the presence of the 2-(benzylthio) group on the thiazole ring resulted in a significant improvement in cytotoxic activity relative to the 2-(methylthio) substituted derivatives. Furthermore, the inhibition of tubulin polymerization by some potent compounds was evaluated. All the compounds studied were moderate tubulin polymerization inhibitors. The flow cytometry analysis confirmed that the synthesized compounds led to cell cycle arrest at the G_2/M phase. Docking simulation was performed to insert these compounds into the crystal structure of tubulin at the colchicine binding site to determine a probable binding model.

1. Introduction

Microtubules are attractive molecular targets for anticancer therapeutics because microtubule polymerization dynamics can greatly affect critical processes, such as cell signaling and mitosis.[1] At the same time, polymerization dynamics can be affected by natural products and synthetic small molecules.[2] There are three major binding sites on tubulin, the taxus, the vinca, and the colchicine binding site.[3] Taxanes are examples of compounds that bind to the taxus binding site and stabilize the microtubule, whereas the majority of compounds that bind to the vinca and colchicine binding sites destabilize the microtubule and promote depolymerization by inhibition of tubulin.[4] The first colchicine site inhibitor, colchicine (Fig. 1), has limited therapeutic application because of its high cytotoxicity.[3] Combretastatin A-4 (**CA-4**) (Fig. 1), isolated from the South African willow tree *Combretum caffrum*, strongly inhibits the polymerization of tubulin by binding to the colchicine site.[5] Although **CA-4** expresses high levels of in vitro activity, it does not show efficacy in vivo because of its low aqueous solubility and the isomerization of its *cis*-double bond into a more thermally stable, but inactive, *trans*-isomer.[6] As **CA-4** has a simple structure, with only two aromatic rings linked by a double bond in the *cis* configuration, a wide number of **CA-4** analogues have been developed and synthesized to date. Among these analogues, compounds in which the olefinic bond is replaced with five-membered rings are promising targets. Imidazole,[7] triazole,[8] thiazole,[9] and pyrazoline[10] are example of five-membered heterocyclic bridges.

In continuation of our research on tubulin inhibitors,[11,12] a series of 2-alkylthio-4-(2,3,4-trimethoxyphenyl)-5-aryl-thiazole (Fig. 1) analogues of **CA-4** were synthesized, and their cytotoxicity and tubulin polymerization inhibitory activity were evaluated. The binding mode of these compounds to tubulin was also studied by molecular docking.

2. Results and discussion

2.1. Chemistry

2-Alkylthio-4-(2,3,4-trimethoxyphenyl)-5-aryl-thiazoles were synthesized by the reaction of different α-bromoketones with methyl

* Corresponding author. Tel.: +98 21 66406757; fax: +98 21 66461178.
E-mail address: ashafiee@ams.ac.ir (A. Shafiee).

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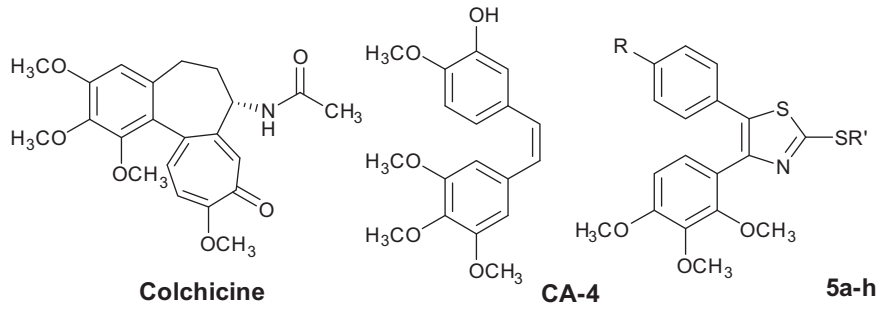


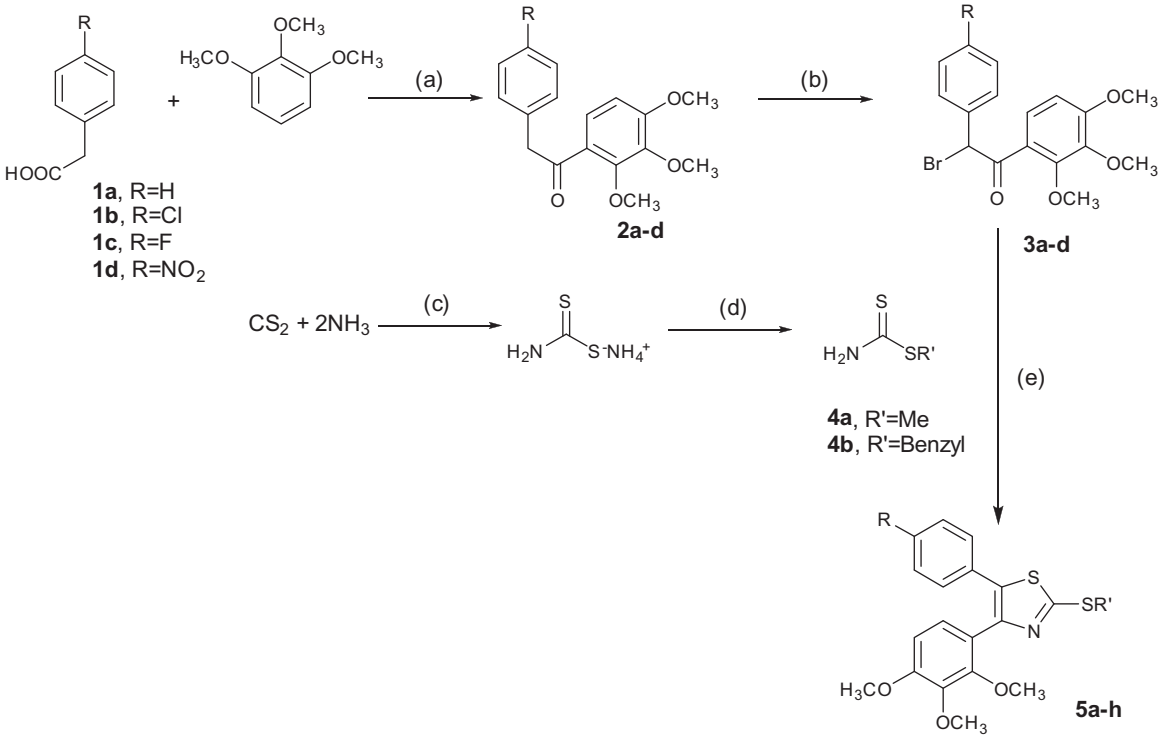
Figure 1. Structure of colchicine, combretastatin A-4, and 2-alkylthio-4-(2,3,4-trimethoxyphenyl)-5-aryl-thiazoles (**5a**–**h**).

and benzyl carbamodithioate **4a**–**b**. In the first step, α-bromoketones **3a**–**d** were prepared from bromination of 1-(2,3,4-trimethoxyphenyl)-2-(4-substitutedphenyl)-1-ethanones **2a**–**d** in glacial acetic acid.[13] These ethanones **2a**–**d** were synthesized by reaction of 1,2,3-trimethoxybenzene and appropriate phenyl acetic acid **1a**–**d** according to the literature.[14] From two expected regio isomers, 2-aryl-1-(2,3,4-trimethoxyphenyl)ethanone and 2-aryl-1-(3,4,5-trimethoxyphenyl)ethanone one of the former was obtained. The structure of **2a**–**d** were confirmed by NMR spectroscopy. In another experiment, ammonium dithiocarbamate was prepared by passing ammonia through a solution of carbon disulfide in tetrahydrofuran.[15] In the next step, methyl and benzyl dithiocarbamate were obtained from reaction of methyl and benzyl iodide with ammonium dithiocarbamate. Finally, treatment of different α-bromoketones with twofold excess of **4a**–**b** in methanol afforded the desired 4-(2,3,4-trimethoxyphenyl)-5-aryl-thiazole-2-thio substituted compounds **5a**–**h** (Scheme 1).

The CA-4 was synthesized as positive control according to the literature.[16] All of synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and CHN analysis.

2.2. Biological study

To test the anticancer activity of the synthesized compounds, the antiproliferative activity of all the derivatives against three cancer cell lines, human colon adenocarcinoma (HT-29), human breast adenocarcinoma (MCF-7), and human stomach adenocarcinoma (AGS), as well as a mouse embryonic fibroblast cell line, NIH-3T3, was evaluated by an MTT colorimetric assay.[17,18] **CA-4** was used as a positive control. The cultured cells were treated with several concentrations of test compounds for 48 h. The ability of these analogues to inhibit cell growth is summarized in Table 1. The results revealed that most of the tested compounds showed moderate to potent cytotoxic activities. Structure-activity relationship (SAR) studies showed that the nature of the benzylthio group on the thiazole ring has a profound influence on the cytotoxicity of the compounds. For example, compounds **5d** and **5f**, both of which contain the 2-(benzylthio) group on thiazole rings, were active (IC_{50} <20 μM) in all three cancer cell lines. In contrast, the replacement of the 2-(benzylthio) group on the thiazole ring with a 2-(methylthio) moiety, namely compounds **5c** and **5e**, led to a



Scheme 1. Reagents and conditions: (a) H_3PO_4, $(CF_3CO)_2O$, 25 °C, 1 min; (b) Br_2, CH_3COOH, 25 °C, 2 h; (c) THF, 25 °C; (d) methanol, benzyl or methyl iodide, 25 °C; (e) methanol, reflux, 24 h.