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Synthesis and biological evaluation of diarylthiazole derivatives as antimitotic and antivascular agents with potent antitumor activity

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ABSTRACT

By switching position of the N and S atom in the thiazole ring which were similar to the previously reported agent 5-(4-ethoxyphenyl)-4-(3′,4′,5′-trimethoxyphenyl)thiazol-2-amine, a series of 4,5-diarylthiazole derivatives were synthesized using Friedel–Crafts reaction based on chemical modification of Combrestatatin A-4 (**CA-4**). Their antiproliferative activities were evaluated and identified as new microtubule destabilizing agents. Structure–activity relationship study indicated that compound **8a** with 3,4,5-trimethoxyphenyl group at the C-4 position and 4-ethoxyphenyl group at the C-5 position of 2-amino substituted thiazole was of the most potent inhibitory activity in this series. **8a** was found to exhibit the IC_{50} values of 8.4–26.4 nM in five human cancer cell lines, with comparable inhibition effects to **CA-4**. Moreover, **8a** showed potency as a tubulin polymerization inhibitor, with colchicine site binding ability and comparable extent of inhibition against the growth of P-glycoprotein over-expressing multidrug resistant cell lines. Mechanism studies revealed that **8a** could block the progression of cell cycle in the G2/M phase and result in cellular apoptosis in cancer cells. As a new tubulin destabilizing agent, **8a** was also found high antivascular activity as it concentration-dependently reduced the cell migration and disrupted capillary like tube formation of HUVEC cells. Furthermore, **8a** significantly suppressed the tumor growth in HCT116 and SK-OV-3 xenograft models with tumor growth inhibitory rate of 55.12% and 72.7%, respectively. Our studies highlighted that **8a** was a promising microtubule targeting antitumor agent.

1. Introduction

Microtubules, the key components of the mitotic spindles of eukaryotic cells composed of α/β-tubulin heterodimers, play a crucial role in many biological processes including cell division,[1] formation, maintenance of cell shape, cell signaling, secretion, and intracellular transport.[2] The primary role of microtubules is to form the mitotic spindle through the polymerization of tubulin, which results in the separation of chromosomal.[3] The dysfunction of microtubules simultaneously results in the cell malfunction and usually leads to cell death.[4] As microtubules present important functions in mitosis and cell division, a large number of anticancer drugs have been developed targeting microtubules.[5,6] These drugs, often regarded as antimitotic agents,[7] are generally classified into two groups: the microtubule-stabilizing agents and the microtubule-destabilizing agents.[8,9]

Combrestatatin A-4 (**CA-4**), a natural product isolated from the African willow that displays potential activity against a broad spectrum of human cancer cells due to its antitubulin activities,[10] has attracted great interest as a microtubule-destabilizing agent that bind to the colchicine-site in β-tubulin, since it represented a class of compounds that possess dual mechanism of anticancer action.[11,12] CA-4 and its derivatives not only inhibit the growth of a wide variety of human cancer cell lines but also show vascular-disrupting effects on tumor endothelial cells. This kind of agents are thus termed vascular-disrupting agents (VDAs).[13] VDAs can cause a significant shutdown in blood flow to solid tumors by selectively targeting established tumor vasculature, leading to tumor shrinkage.[14]

The simple structure and great anticancer potency make **CA-4** a very attractive lead compound for cancer treatment. A large number of derivatives of **CA-4** have been reported. According to previous studies, three important pharmacophore components of **CA-4**

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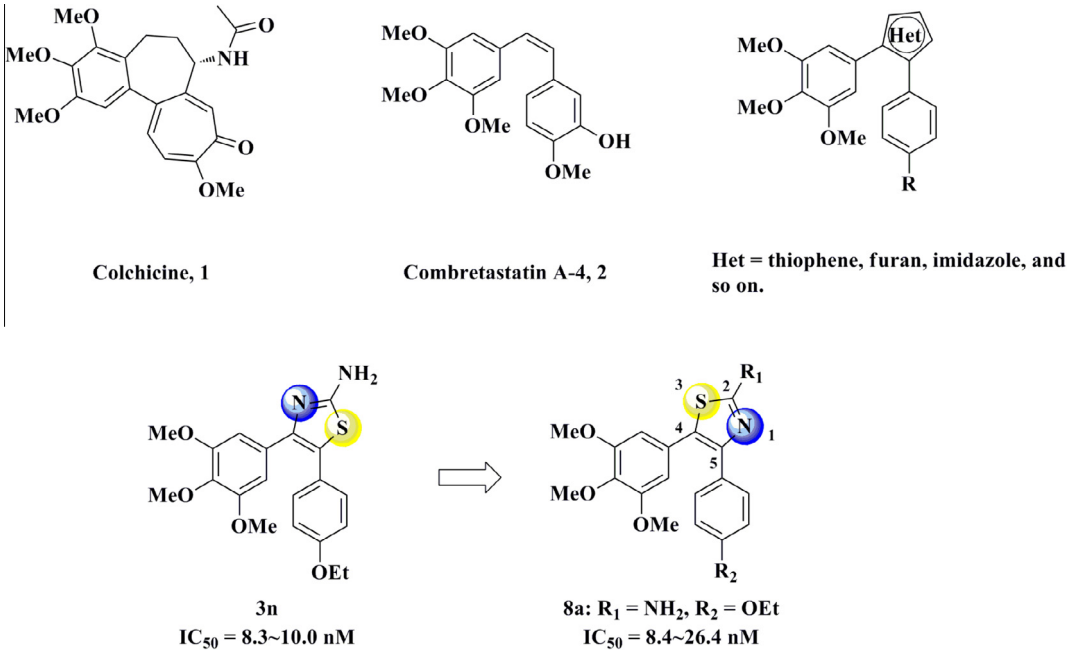

Figure 1. Tubulin interacting agents, **3n** and lead compound (**8a**).

have been reported, which are two hydrophobic phenyl rings and a vinyl in *cis* configuration bridging the two rings.[15] The restricted *cis* configuration structure can also be achieved by introducing the two phenyl ring vicinally on a suitable heterocycle such as dioxolane,[16] pyrazole, imidazole, isoxazole, oxazole, triazole, thiazole and so on.[17,18]

In a previous study, researchers have prepared a series of 2-amino-4-(3′,4′,5′-trimethoxyphenyl)-5-arythiazoles derivatives by using an efficient and versatile convergent synthetic procedure. Among them, the 5-(4-ethoxyphenyl)-4-(3′,4′,5′-trimethoxyphenyl)thiazol-2-amine (**3n**) showed the most attractive IC_{50} values against five cancer cell lines.[18] And their molecular docking studies supported that the substitution pattern on the phenyl moiety at the 5-position of the 2-amino thiazole ring played an important role for antitubulin and antiproliferative activity. Their results encouraged us to further investigate the structure–activity relationship (SAR) of this skeleton. We hypothesized that the N and S atom in the thiazole ring could help retain the correct geometric orientation of the two phenyl rings of **CA-4**, placing them at an appropriate distance for efficient interaction with the colchicine-site of tubulin.[19] In order to further investigate the effects of the position of N and S atom in the thiazole ring on the antitubulin and antiproliferative activity, we prepared a series of 5-(3′,4′,5′-trimethoxyphenyl)thiazole derivatives which were similar to **3n** but had the position of N and S atom switched (Fig. 1). In this series of designed analogues of **CA-4**, we remained the trimethoxyphenyl moiety of the A-ring, which was considered necessary for tubulin binding activity. Our modifications were mainly focused on varying substituents at the 2-position of the thiazole skeleton. Twenty-three compounds were synthesized and evaluated for their antiproliferative activity on several human tumor cell lines. Several compounds were found to show low nanomolar antiproliferative activities. Then these compounds were further evaluated for their microtubule polymerization inhibiting activity, colchicine-site binding ability and drug-resistant overcoming potency in sequence. **8a** showed the most potent activity with IC_{50}s between 8.4 and 26.4 nM in five human cancer cell lines and also showed promising activities in drug-resistant tumor cells. **8a** was further confirmed of vascular disrupting activity and in vivo antitumor activity in both HCT116 and SK-OV-3 xenograft models, suggesting that **8a** is a promising new anticancer agent to be exploited.

2. Chemistry

Starting from 2-(3′,4′,5′-trimethoxyphenyl)acetic acid (**3**), the corresponding acyl chloride **4** was prepared by the chlorination with oxalyl chloride. The following Friedel-Crafts acylation of ethoxybenzene by **4** in the presence of $AlCl_3$ gave **6a**.[20] Subsequently, the bromination of **6a** using bromine at 0 °C afforded the intermediate **7a**.[21] Finally, a series of C-2 substituted thiazole derivatives **8a–g** were obtained by the cyclization of **7a** using thiourea and its derivatives (Scheme 1). For comparison, compound **9** was synthesized using the same synthetic procedure with a *p*-methoxyphenyl substituent at the C-4 position of the thiazole ring (Scheme 1). A variety of alkyl groups were also introduced on the amine group of **8a** using sodium hydride to prepare *N*,*N*-dialkyl substituted derivatives **10a–10d** (Scheme2). Compound **10i** was prepared by the introduction of benzyl group. *N*-Acylated derivatives **10e–h** and *N*-sulfonylated derivatives **10k–o** were prepared by reacting **8a** with the sulfonylchloride. At last, **8a** reacted with ethyl isocyanate to provide **10j** with an ethyl urea at the C-2 position of the thiazole ring.[22]

3. Results and discussion

3.1. In vitro antiproliferative effects

The synthesized derivatives were evaluated for the antiproliferative effects in vitro by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay against HCT116 cell (human colon cancer cell), SMMC-7221 cell (human liver cancer cell), A2780s cell (human ovarian cancer cell), HepG2 cell (human hepatocellular carcinoma cell), and SK-OV-3 cell (human ovarian cancer cell). **3n** was used as positive control.