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Research paper

Synthesis and biological evaluation of novel chalcone derivatives as a new class of microtubule destabilizing agents

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ABSTRACT

A series of novel chalcone derivatives were designed and synthesized as potential antitumor agents. Structures of target molecules were confirmed by ^{1}H NMR, ^{13}C NMR and HR-MS, and evaluated for their *in vitro* anti-proliferative activities using MTT assay. Among them, compound **12k** displayed potent activity against the test tumor cell lines including multidrug resistant human cancer lines, with the IC_{50} values ranged from 3.75 to 8.42 μM. In addition, compound **12k** was found to induce apoptosis in NCI-H460 cells via the mitochondrial pathway, including an increase of the ROS level, loss of mitochondrial membrane potential, release of cytochrome *c*, down-regulation of Bcl-2, up-regulation of Bax, activation of caspase-9 and caspase-3, respectively. Moreover, the cell cycle analysis indicated that **12k** effectively caused cell cycle arrest at G2/M phase. The results of tubulin polymerization assay displayed that **12k** could inhibit tubulin polymerization *in vitro*. Furthermore, molecular docking study indicated that **12k** can be binding to the colchicine site of tubulin.

1. Introduction

Microtubules, as key components of the cytoskeleton, are cytoskeletal filaments consisting of α, β-tubulin heterodimers and involved in a series of cellular processes including regulation of motility, cell signaling, maintenance of cell shape, cell proliferation and intracellular transport [1–4]. Since microtubules play an important role in the life cycle of the cell, they have been considered as a major target for the development of novel anticancer agents in recent years [5–7]. In recent decades, numbers of compounds as anti-mitotic agents derived from natural sources or obtained by chemical synthesis have been reported [8–10]. In general, these compounds were divided into three major types such as the taxane site for microtubule stabilizing agents, the vinca site, and the colchicine site for microtubule polymerization inhibitors [11–13]. Anti-mitotic agents including taxanes and vinca alkaloids have been widely used for the treatment of a variety of cancers in the past decade [14,15]. However, the clinical use of these compounds was always limited by the high toxicity, the development of drug resistance, side effects, poor solubility, low oral bioavailability and complex synthesis [15,16]. Therefore, scientists are eager to develop and discover novel effective anti-mitotic agents for overcoming the above mentioned drawbacks.

Natural products and their derivatives provide a diverse source of new medicinal leads and they play a major role in drug development, especially in the area of cancer therapy [17]. Chalcones are naturally occurring moieties of flavonoid and isoflavonoid compounds, which are an important pharmacophore of many natural products including curcumin, flavokawain, millepachine, and xanthohumol [18–21]. In addition, many researchers reported that chalcone and its analogues also exhibited wide range of biological activities, such as anti-oxidant, anti-filarial, anti-bacterial, anti-fungal, anti-mitotic, anti-tumor, anti-inflammatory and inhibition of enzymes activities [22–29]. Millepachine, as a novel chalcone with a 2,2-dimethylbenzopyran motif (**1a**, Fig. 1) which was first isolated from the *Millettia pachycarpa* by Chen and co-workers in

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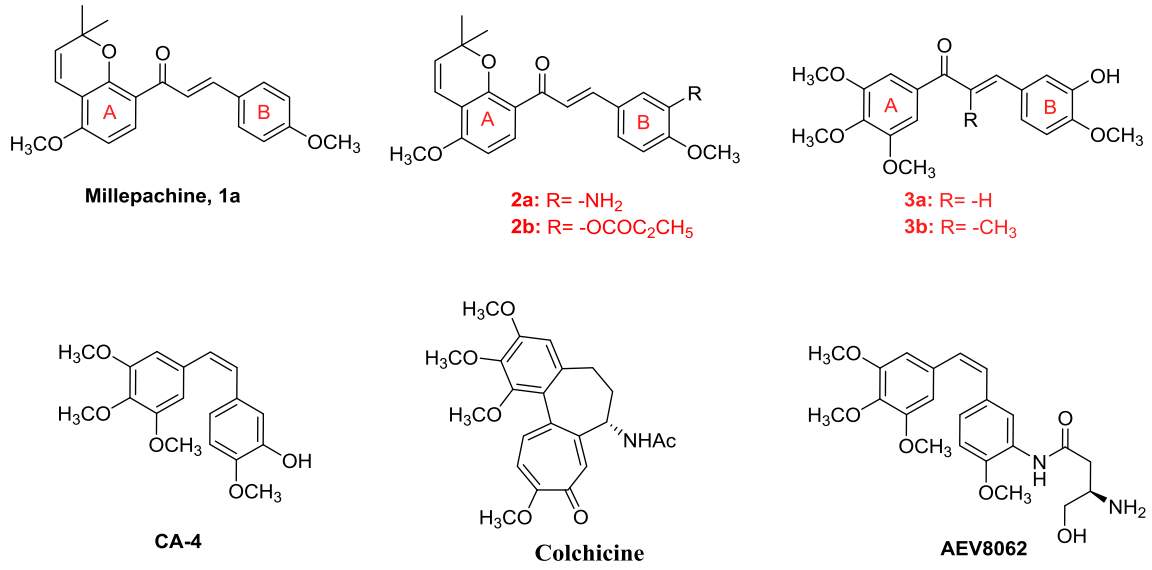

Fig. 1. Structures of natural products and chalcone analogues as potent inhibitors of tubulin polymerization.

2013, has been known to exert potent cytotoxicity *in vitro* against a variety of cancer cells and potent *in vivo* antitumor activity [30]. In order to improve the antitumor activity of millepachine, during the last a few years, Chen and co-workers have explored the B-ring of millepachine with different substituted groups, such as introduction of hydroxyl group on the B-ring of millepachine or replacement of methoxyl with diethyl amine, to result in improvement on their antitumor activity [21,31]. In previous work, a number of millepachine derivatives were designed and synthesized as potential anticancer agents [15,21,30–33]. Among them, compound **2a** (Fig. 1) and **2b** displayed excellent antitumor activities toward various human cancer cell lines including multidrug resistant ones *in vitro* and *in vivo*, and strongly inhibited tubulin polymerization by binding to the colchicine site of tubulin. However, pharmacokinetic studies indicated that low oral bioavailability of these compounds has limited further study. In addition, compounds **3a** and **3b** (Fig. 1), as a combretastatin A-4 analogous chalcone, were reported to have potent antitumor activities against a panel of cancer cell lines and markedly inhibit the polymerization of tubulin [34,35]. These encouraging results prompted us to further design and synthesize a new class of chalcone derivatives as potential anticancer agents.

In an effort to discover more effective compounds that target the tubulin-microtubule system as a tubulin de-polymerization inhibitor, we have designed and synthesized a new series of chalcone derivatives and evaluated their antitumor activities in the present study. Among all the compounds, compound **12k** displayed the most potent antitumor activity against the tested cancer cell lines including multidrug resistant phenotype, and effectively induced cell cycle arrest in G2/M phase. Moreover, molecular docking analysis was made to examine whether compound **12k** could inhibit the polymerization of tubulin by binding to the colchicine binding site.

2. Results and discussion

2.1. Chemistry

The general procedures for the synthesis of chalcone derivatives are described in Schemes 1 and 2. Compounds **1**–**4** and **9** were synthesized according to the reported procedures [15,21,35]. Treatment of compound **1** with 3-chloro-3-methyl-1-butyne in the presence of DBU and catalytic amounts of $CuCl_2 \cdot 2H_2O$ in CH_3CN afforded the intermediate **2** (yield, 76.1%). The resulting compound **2** was cyclized by heating in pyridine to produce the key intermediate **3** (yield, 73.3%). Subsequently, Claisen-Schmidt condensation of **3** or **8** with 3-hydroxy-4-methoxybenzaldehyde in the presence of KOH in CH_3OH led to the production of the intermediate **4** (yield: 65.2%) or **9** (yield, 54.8%), which was upon etherification with α-bromoethyl acetate, K_2CO_3 and KI in the presence of DMF to give ester **5** (yield, 93.3%) or **10** (yield, 90.9%) followed by its hydrolysis with aluminum hydroxide in the presence of THF/H_2O to generate the acid **6** (yield, 95.0%) or **11** (yield, 91.9%). Finally, target compounds **7a-7l** (yields, 52.3%–95.2%) and **12a-12l** (yields, 43.1%–95.6%) were achieved by the formation of amide bond between **6** or **11** and anilines, respectively, in the presence of HOBT/EDCI. The structures of these target compounds were confirmed by ^{1}H NMR, ^{13}C NMR and high resolution mass spectra (HR-MS).

2.2. Biology activity

2.2.1. Cytotoxicity test

The *in vitro* inhibitory effect of these synthesized chalcone derivatives was evaluated by MTT assay against HepG-2, NCI-H460, MGC-803, SK-OV-3 and T-24 cancer cell lines together with HL-7702 normal cell line, and compound **1a** (Millepachine) was chosen as reference. The IC_{50} values obtained in the performed *in vitro* inhibition assays are summarized in Table 1.

As shown in Table 1, the newly synthesized chalcone derivatives are potent anticancer agents, with IC_{50} values mostly in the micromole level. Among all compounds, it was notable that compound **12k** exhibited the best antitumor activity against HepG-2, NCI-H460, SK-OV-3 and T-24 cancer cells and had low cytoxicity on normal human cell line (HL-7702) compared with compound **1a**. The results revealed that the analogues **7b** and **7c**, obtained by inserting a methoxy or methyl moiety in the position 4 of the lateral benzene group, led to significant increase in potency compared with other analogues substituted by halogen. Compound **7b**, with IC_{50} value of 6.73 μM against MGC-803 cells, was about 3-fold more active than derivatives **7d** and **7e**, and undoubtedly emerged as one of the most active compounds within this subset. **7c** containing a methyl group in the 4-position had an IC_{50} of 8.23 μM in the same