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Synthesis and biological evaluation of pyrimidine bridged combretastatin derivatives as potential anticancer agents and mechanistic studies

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ABSTRACT

A number of pyrimidine bridged combretastatin derivatives were designed, synthesized and evaluated for anticancer activities against breast cancer (MCF-7) and lung cancer (A549) cell lines using MTT assays. Most of the synthesized compounds displayed good anticancer activity with IC_{50} values in low micromolar range. Compounds **4a** and **4p** were found most potent in the series with IC_{50} values of 4.67 μM & 3.38 μM and 4.63 μM & 3.71 μM against MCF7 and A549 cancer cell lines, respectively. Biological evaluation of these compounds showed that selective cancer cell toxicity (*in vitro* using human lung and breast cancer cell lines) might be due to the inhibition of antioxidant enzymes instigating elevated ROS levels which triggers intrinsic apoptotic pathways. These compounds were found nontoxic to the normal human primary cells. Compound **4a**, was found to be competitive inhibitor of colchicine and in the tubulin binding assay it showed tubulin polymerization inhibition potential comparable to colchicine. The molecular modeling studies also showed that the synthesized compounds fit well in the colchicine-binding pocket.

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1. Introduction

Cancer is a multifactorial disease and considered as the most serious health problem all over the world. Despite recent advances in our understanding of the biological processes leading to the development of cancer, there is still need for the development of more potent and effective anticancer agents for the complete eradication of the disease [1]. Multi-drug resistance and acute toxicity are the two major issues with most of the currently available chemotherapeutic agents [2,3]. Therefore, novel anticancer agents need to be developed that are more potent, safe and selective. In a quest for the discovery of more effective anticancer drugs, a large number of structurally diverse synthetic and natural products have been screened for their anticancer potential [4,5]. Microtubules have been explored as an important target for the anticancer drug development. Microtubules play crucial role in the spindle formation during cell-division. A highly dynamic equilibrium exists between the microtubules and the free tubulin dimers [4]. Any disruption in the dynamic equilibrium of tubulin-microtubule blocks cell-division at the metaphase–anaphase transition that leads to the induction of the mitochondrial apoptosis [6].

Numerous structurally different natural as well as synthetic compounds have been identified that target microtubule polymerization [7]. Since last few years combretastatins have received special attention due to their simple structure and easy synthesis [8,9]. Combretastatin A-4 (CA-4) binds with the colchicine binding site and disrupts microtubule polymerization and induces rapid vascular disruption which leads to tumor cell death [10,11]. Fosbretabulin, a water-soluble phosphate prodrug of CA-4, is under phase II/III clinical trials alone and/or in combination with other chemotherapeutic agents. Although, numerous CA-4 analogs have been developed with improved antitubulin activities, but clinical application of these agents is not successful till now [12]. Thus, it is a challenge to synthesize CA-4 analogs with improved activity along with therapeutic potency.

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Most of the combretastatin derivatives have been synthesized by carrying out modifications at the ring A and ring B. In addition, olefinic bond of combretastatins have been replaced with number of heterocyclic rings such as, thiazole [13], tetrazole, triazoles [14,15], imidazole [16], pyrazole [17], Oxazole [18], pyrrole [19], indole, furan [20], benzene, pyridine [21], pyrimidine [22], quinoline, isoquinoline, isoxazole, thiazoline, pyrazoline, thiophene [23], benzofuran [24] etc. Many of these compounds displayed cytotoxic and antitubulin activities. In combretastatins, a two carbon olefinic bridge and *cis* conformation was considered crucial for the cytotoxicity and antitubulin activity of the compounds. However, a number of other compounds with varying length of linker chain between the two phenyl rings have been reported with antitubulin activity. For example, phenstatin, with a carbonyl group in place of double bond displayed antitubulin activity [25]. Likewise, chalcone with a three carbon chain, strongly inhibit tubulin polymerization with improved cytotoxicity [26]. In the present study, we have designed, synthesized and evaluated a new series of pyrimidine bridged combretastatin derivatives for their anticancer activities.

Different compounds with a core pyrimidine ring have been reported as inhibitors of cyclin-dependent kinases (CDK) [27], tumor necrosis factor R (TNF-R) [28], ableson protein tyrosine kinase (Abl) [29], 3-phosphatidylinositol kinases (PI-3 K) [30], protein kinase B (Aktkinase), and cytokines [31]. Gangjee et al. synthesized two series of the compounds based on the 6-CH_3 cyclopenta [d]pyrimidine and pyrrolo[2,3-d]pyrimidine scaffolds. These compounds displayed potent antiproliferative activity at nanomolar concentration and target the colchicine binding site of tubulin [32]. Xie et al. synthesized 2,4,5-substituted pyrimidine derivatives and evaluated for antiproliferative and anti-tubulin activities. These compounds arrested cell cycle at G2/M phases of the cell cycle (EC_{50} = 20 nM) and were found as competitive inhibitors of the colchicine binding site [31]. Zhang et al. synthesized a series of 4-substituted 2,6-dimethylfuro[2,3-d]pyrimidines as dual inhibitors of the microtubules and tyrosine kinases (RTKs). These compounds exhibited microtubules de-polymerization activity with EC_{50} values in nano molar range [22]. Zheng et al. evaluated anticancer activities of 3-carbon and 4-carbon linker analogous of combretastatin-A4. 3-Carbon linkers with bridged pyridine ring showed better activities as compared to 4-carbon linkers and a non-heterocyclic bridged system [21].

In the current studies, we have introduced a pyrimidine ring as a 3-carbon linker between the two phenyl rings (Fig. 1) to maintain the *cis* locked conformation of combretastatin-A4. Ring A and ring B were optionally substituted with different substituents having electron releasing and electron withdrawing effects. Molecular modeling studies were also performed in order to understand the binding interactions of the synthesized compounds with tubulin proteins. The mechanistic studies revealed that this series of compounds displayed anticancer activity through inhibition of antioxidant enzymes which may destabilizes the mitochondrial membrane and trigger intrinsic apoptotic pathway. Further, colchicine site binding agents arrest the cell cycle at G2/M phase that lead to mitochondrial apoptosis [6].

2. Results and discussion

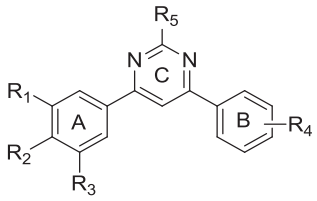
2.1. Chemistry

Target compounds were synthesized as described in Scheme 1. The intermediate chalcones were synthesized through Aldol condensation. Aldehydes **(1)** and acetophenones **(2)** were condensed to give 1,3-Diphenyl-2-propen-1-one **(chalcones, 3)** in varying yields, and recrystallized to obtain pure products. These intermediate chalcones **(3)** were further reacted with acetamidine/formamidine/guanidine to give corresponding pyrimidine bridged analogous of combretastatins **(4a-4t)**. All the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and ESI-MS.

2.2. Biological evaluation of compounds and discussion

2.2.1. Synthesized compounds exhibited significant cytotoxicity and selectively against cancer cell lines

All the synthesized compounds **(4a-4t)** were investigated for their *in vitro* cytotoxicity against human MCF-7 (breast cancer) and A549 (lung cancer) cancer cell lines using standard MTT assays (Table 1 and supplementary Fig. S1a and S1b) while colchicine was used as a reference compound. Three different concentrations (1 μM, 5 μM, and 25 μM in triplicate) of the compounds were used and the results were analyzed after 48 h of drug treatment. It has been found that compound **4a-4c, 4e, 4f, 4j, 4m-4p** showed better antiproliferative activities as compared to the reference colchicine against both the cell lines. In this series of compounds, **4a** and **4p** showed best antiproliferative activity against both the cancer cell lines. Compound **4a** displayed IC_{50} values of 4.67 μM and 3.38 μM while **4p** showed IC_{50} values of 4.63 μM and 3.71 μM against MCF-7 and A549 cell lines, respectively (48 h post treatment). These compounds were also tested on human peripheral blood cells (hPBMCs) which represented the normal cell types. The results indicated that this series of compounds did not display cytotoxicity against the tested cells up to 5 μM concentration of the test drugs. Thus, this series of compounds can selectively target cancer cells and can be used as lead for the development of effective anticancer drug (supplementary information Fig. S1a and S1b).



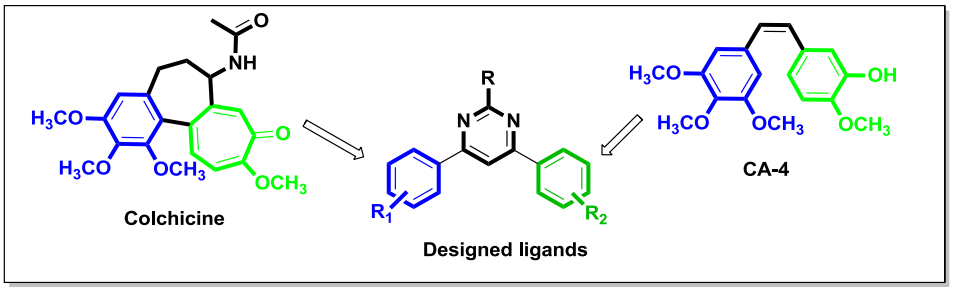


Fig. 1. Target compounds and their structural comparison with colchicine and combretastatin (CA-4).

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