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Research paper

Synthesis and biological evaluation of quinoline analogues of flavones as potential anticancer agents and tubulin polymerization inhibitors

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ABSTRACT

A new series of 2-aryl-trimethoxyquinoline analogues was designed and synthesized as tubulin inhibitors using methoxylated flavones as the lead compounds. The cytotoxic activity of the synthesized compounds was evaluated against four human cancer cell lines including MCF-7, MCF-7/MX, A-2780, and A-2780/RCIS. All the alcoholic derivatives (**6a**–**6e**) showed significant cytotoxic activity with IC_{50} in the range of 7.98–60 μM. The flow cytometry analysis of the four human cancer cell lines treated with **6e** and **5b** showed that **6e** induced cell cycle arrest at G2/M phase and apoptosis as well. The effect of quinolines on tubulin polymerization was also evaluated. Compound **6e** that demonstrated the best antiproliferative activity in the series was identified as the most potent inhibitor of tubulin polymerization as well. Molecular docking studies of **6e** into the colchicine-binding site of tubulin displayed possible mode of interaction between this compound and tubulin.

1. Introduction

Cancer is the cause of one-quarter of all deaths in developed countries. It is now the second leading cause of death in the United States, and is anticipated to surpass heart diseases as the leading cause of death in the futures [1]. Therefore, there is an urgent need to discover and develop novel and more effective drugs. Although, the chemotherapy is the usual method for treatment for different cancer types, it fails to cure most cancer patients with advanced disease due to the occurrence of drug resistance [2,3]. Consequently; increasing interest has been devoted to the design and discovery of more effective anticancer agents in current medicinal chemistry.

Microtubules play essential role in mitosis and have long been considered as an important target for the development of novel anticancer drugs [4].

In general, antitubulin agents exert their effects through binding to one of the three established drug domains on the tubulin heterodimer: the colchicine, the paclitaxel and the vinca alkaloid binding sites. Agents that target the colchicine's domain (e.g., colchicine and podophyllotoxin) or to the vinca alkaloid binding site (e.g., vincristine) are defined as inhibitors of tubulin assembly, that is, microtubule destabilizing agents. On the contrary, agents that bind to the paclitaxel binding site (e.g., paclitaxel) are known to act as tubulin promoters, that is, microtubule-stabilizing agents [5]. Combretastatins which are isolated from the South African tree *Combretum caffrum* are also a group of antimitotic compounds and combretastatin A-4 (CA-4, Fig. 1) is one of the well-known natural antitubulin molecule which exerts its antimitotic effect by binding to colchicine's binding site of tubulin [6].

Centaureidin which is isolated from the tropical plant Potymtifruticosa is the first known example of a flavone with antimitotic activity [7]. Some other synthetic flavones such as compound **1** has been reported as cytotoxic agents and tubulin inhibitors [8]. In the present study some new quinoline derivatives have been designed and synthesized as tubulin inhibitors, as an attempt to check if the replacement of the benzopyrone moiety with the quinoline one is bioisosteric. These quinolines all are possessing trimethoxy phenyl fragment and some of our compounds (**5b** and **6b**) possess also 3-

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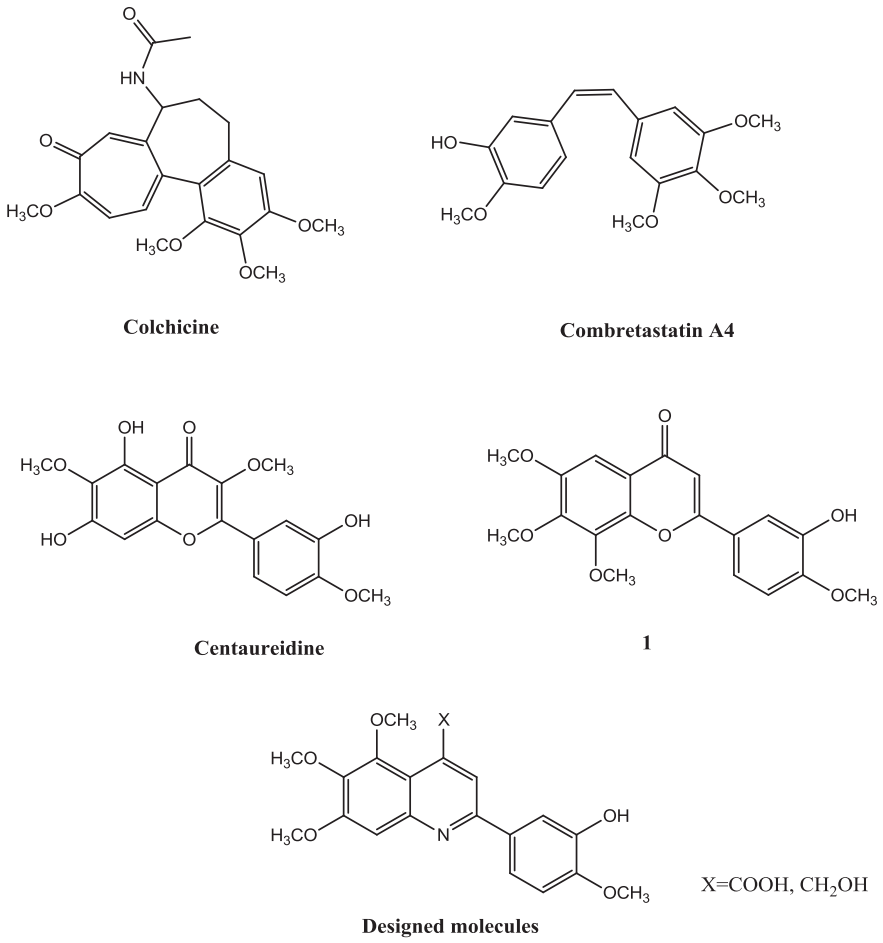

Fig. 1. Chemical structures of known tubulin inhibitors and designed compounds.

hydroxy-4-methoxy phenyl moiety which are present in some potent tubulin inhibitors (Fig. 1). **5b** and **6b** were designed to have similarly substituted aryls positioned at C-2 to the aryl substituent at C-2 of flavones centaureidin and compound **1** (Fig. 1). The synthesized compounds were evaluated for their cytotoxic activity towards four different cancer cell lines including MCF-7 (Human Breast Cancer Cells), MCF-7/MX (Mitoxantrone resistant human Breast Cancer Cells), A-2780 (human ovarian carcinoma), and A2780/RCIS (Cisplatin resistant human ovarian carcinoma). The compounds were also investigated for their activity in a microtubular polymerization assay. Moreover, trying to explain the results of biological experiments docking studies were carried out.

2. Results and discussion

2.1. Synthesis

A one-step Doebner reaction was used to prepare the target 5,6,7-trimethoxy-2-arylquinoline-4-carboxylic acid derivatives **5a**–**5h**. As illustrated in Scheme 1, substituted benzaldehyde **2**, pyruvic acid **3** and 3,4,5-trimethoxyaniline **4** were refluxed in ethanol to afford 4-carboxy quinolines **5a**–**5h** [9,10] and then reduction of carboxyl group to alcoholic substituent was carried out using $LiAlH_4$ in dry THF [11].

However, the well-known Doebner-type synthesis of quinoline did not yield the expected 2-(3,4,5-trimethoxyphenyl)-quinoline-4-carboxylic (**5e**) in ethanol solvent. Therefore, the desire quinoline derivative was prepared in acetic acid. The compounds were characterized by nuclear magnetic resonance, infrared and mass spectrometry.

2.2. Biological evaluation

2.2.1. In vitro anticancer activity

The cytotoxic activity of the synthesized compounds were evaluated against four human cancer cell lines including MCF-7 (Human Breast Cancer Cells), MCF-7/MX (Mitoxantrone resistant human Breast Cancer Cells), A-2780 (human ovarian carcinoma) and A-2780/RCIS (Cisplatin resistant human ovarian carcinoma), employing the MTT assay. As depicted in Table 1, all the alcoholic derivatives showed significant cytotoxic activity with IC_{50} in the range of 7.98–60 μM. However, carboxylic derivatives except **5b** did not display cytotoxic activity at concentrations below 100 μM. This may be due to the poor ability of carboxylic derivatives to penetrate the cell membrane because of their high polarity. The only carboxyl derivative that showed cytotoxicity was **5b**. It caused moderate cytotoxicity in MCF-7 and A-2780 and had no cytotoxic effect on the resistant cell lines at concentrations below 100 μM. Generally the alcoholic derivatives (**6a**–**6e**) showed more cytotoxicity in A-2780 cell line in comparison to other three cell lines. Interestingly, resistant human Breast Cancer Cells (MCF-7/MX) were more sensitive to all the alcoholic derivatives except **6a** in comparison to parental cells (MCF-7). In contrast, they caused more cytotoxicity in A-2780 cell line in comparison to resistant human ovarian carcinoma (A-2780/RCIS), indicating the possibility that our compounds exerted their cytotoxic activity through different mechanism in