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Research paper

Novel myricetin derivatives: Design, synthesis and anticancer activity

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ABSTRACT

Telomere and telomerase were closely related to occurrence and development of some cancers. To enhance ability of myricetin moiety for inhibiting telomerase, we designed a series of novel myricetin derivatives based on reasonable analysis. The telomerase inhibition assay showed that compound **6d** displayed the most potent inhibitory activity with IC_{50} value of 0.91 μM. The anticancer activity assay showed that **6d** exhibited high activity against human breast cells MDA-MB-231. The docking simulation of compound **6d** was performed to get the probable binding model, the results demonstrated that the furan ring inserted into the active site deeply and had hydrophobic interactions with residues of Phe 568, Pro 627, four methoxy groups had hydrophobic interactions with residues of Phe 568, Pro 627, Lys 902, Val 904 and Pro 929. Western blot results showed that expression of p65 and TERT protein was clearly down-regulated by compound **6d**. These data support further studies for the rational design of more efficient p65 and TERT modulators.

1. Introduction

Telomerase is expressed in 80–90% of tumors from all types of cancers [1]. The different telomerase expression levels, as well as the generally longer telomeres in healthy cells versus tumor cells, suggest a high degree of tumor specificity and a low risk of toxicity to normal tissues of compounds that selectively target telomerase [2–5]. Those facts resulted in significant efforts to validate telomerase as an anticancer drug target and to develop effective approaches toward its inhibition [6,7]. Based on searching for telomerase inhibitors, different approaches have been designed: drugs that inhibit telomerase enzymatic activity, active immunotherapy, gene therapy using telomerase promoter-driven expression of a suicide gene, agents that block telomerase biogenesis and G-quadruplex-stabilizing molecules. But, no drugs that inhibit telomerase activity and immunotherapy-based drugs have reached clinical trials so far [1,8]. Therefore, the pursuit of novel telomerase inhibitors with better antitumor effects and more safety profile is still the main issue.

Myricetin, flavonoid compounds (Fig. 1), are present in a wide variety of fruits. Interestingly, those myricetin derivatives are thought to show anticancer activity, which could decrease pancreatic cancer growth via induction of cell apoptosis [9,10]. There is also accumulating evidence to suggest that myricetin can also directly modulate activity of a large number of important signaling molecules, such as: protein kinase (ERK1/2) in lung cancer cells [11] and kinase B (Akt) in cervical and lung cancer cell lines [12–17]. These pathways, through their ability to affect growth and survival of cancer cells, lead to cell cycle progression and proliferation.

It was of our interest to utilize rational chemical approaches to generate and identify novel compounds as potential hTERT (key protein of telomerase) inhibitors for cancer therapy [18]. Steczkiewicz et al. [19] have constructed a three-dimension human telomerase model by systematically utilizing computational methods for distant homology detection, comparative modeling and molecular docking. In this study, above three-dimension human telomerase model and an advanced docking method – IFD (Induced Fit Docking) of Schrödinger were employed to explore the binding mode of BIBR1532, a potent hTERT inhibitor. Based on this,

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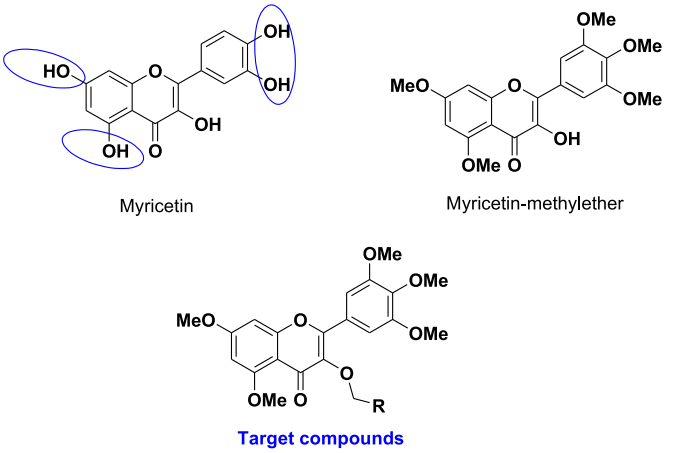

Fig. 1. Idea of design based on myricetin scaffold.

computer-generated molecular model of 5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)-4*H*-chromen moiety docked into the model of hTERT was analyzed, we found four methoxy groups whereas had hydrophobic interactions with the key residues of Phe 568 and Pro 929, the chromen ring also projected into a more stable hydrophobic region. This is motivation provided in the design idea. So, in this design, methoxy (Fig. 1) was introduced.

2. Results and discussion

2.1. Chemistry

Myricetrin was used as the raw material, and hydroxyl groups were protected with methyl iodide, 3-hydroxy-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)-4*H*-chromen-4-one **1** was prepared by removing of glycosides, and then reacted with alkyl halide containing different chains, in this way intermediate **2** was obtained, the next reaction was carried out in the presence of K_2CO_3, DMF was used as solvent at reflux condition (Scheme 1), finally, a series of title myricetin derivatives **3** containing nitrogen heterocycle moiety were synthesized. Myricetin hydrazone derivatives **6** were synthesized by hydrolysis, substitution and condensation reactions, Halogenated hydrocarbons, ethyl bromide, hydrazine hydrate and aromatic aldehyde were used as raw materials (Scheme 2).

2.2. In vitro anticancer activity

To test anticancer activity of the synthesized compounds, we evaluated activity of examined compounds **3** and **6** against Bcap-37 (human breast cancer cell), MDA-MB-231 (human breast adenoma cell), SGC-7901 (human gastric cancer cell) and MGC-803 (human gastric cancer cell) cell lines. The results were summarized in Table 1. From the results of MTT assay, it was found that some examined compounds showed remarkable anticancer effects mainly for MDA-MB-231 cells and Bcap-37 cells. The compounds **3c** and **6d** showed anticancer activities against Bcap-37 cells with IC_{50} values of 2.96, 3.11 μM, respectively, comparable to that of positive control ADM (Doxorubicin, 2.87 μM). Compounds **3c, 3d, 3h, 3j, 6a** and **6f** showed anticancer activities against MDA-MB-231 cells with IC_{50} values of 2.16, 2.90, 3.03, 3.87, 3.40, 3.75 μM, respectively, comparable to that of positive control ADM (2.01 μM). The compound **6a** showed anticancer activity against MGC-803 cells with IC_{50} value 2.78 μM, comparable to that of positive control ADM (3.22 μM). Compounds **3h** and **6d** with the IC_{50} values of 2.62, 1.86 μM, respectively, exhibited promising anticancer activity against Bcap-37 and MDA-MB-231 cell lines.

The SAR indicated that all examined compounds showed good activity against human breast cancer cell lines (MDA-MB-231 and Bcap-37) but poor activity against human gastric cancer cells (SGC-7901 and MGC-803 cell lines). Scanning from Table 1, it is obvious that all the examined compounds exhibited poor inhibitory activity against the SGC-7901 cells. Except compounds **6a** and **6b**, the activity of other compounds against MGC-803 cells was not high.

In the further study, our examined compounds were divided into two series, one was 3-(3-(substituted) propoxy)-5,7-dimethoxy-2-(3,4,5-trimethoxyphenyl)-4*H*-chromen-4-one **3** (compounds **3a~3j**), another was 2-(5,7-dimethoxy-4-oxo-2-(3,4,5-trimethoxy-phenyl)-4*H*-chromen-3-yloxy)-*N*′-(2-substituted) acetylhydrazine **6** (compounds **6a~6e**). Although the majority compounds (**3c**, **3d**, **3h**, **3i** and **3j**) of series **3** exerted the moderate inhibitory activity, the most active agent against the tested cancer cell lines was compound **6d**. Therefore, for this kind of structure moiety, this pointed out the direction for us to further optimize the structure of myricetin derivatives as potent anticancer agents.

2.3. Cells morphology effects

MDA-MB-231 cell was chosen for examination of the cell morphology changes induced by compounds. Results of cells morphology analysis were shown in Figs. 2 and 3. Toxicity of doxorubicin on tumor cells was too serious, so that all cells were broke and cracked. But, damage of target cells caused by compound **6d** was very small, so the inhibitory activity is mainly reflected in the inhibition of cells proliferation, the number of cells decreased obviously compared with the control group. From morphological observation we easily discovered that in reducing the number of cells at the same time, cells were distorted, but injury was not very obvious.

2.4. Cell cycle analysis

To understand whether cell cycle arrest lead to decrease cell proliferation, we used flow cytometric analysis to measure the effect of compound **6d** on induction of cell cycle. As shown in Fig. 4, the cells in G0/G1 phase in the MDA-MB-231 cells control group accounted for about 40.5%, while after MDA-MB-231cells treated with compound **6d** for 48 h, the ratio was approximately 44.3%, this can be described as slight increase of the proportion of cells in G0/G1 phase rather than as the arrest of cell cycle in this phase.

2.5. Telomerase inhibition

Based on the discovery of novel skeleton structure and in continuation to extend our research of telomerase inhibitors, some examined compounds were assayed for telomerase inhibition, using MDA-MB-231 cells extracts, ethidium bromide and BIBR1532 were used as the positive compounds. The results (Table 2) suggested that compounds **3h**, **6a** and **6d** showed strong telomerase inhibitory activity with IC_{50} values of 2.00, 1.82, 0.91 μM, respectively, which surpassing that of the positive control ethidium bromide, and is comparable to that of positive control BIBR1532, Furthermore, there was a good correlation between anti-cancer activity and telomerase activity of compounds (Tables 1 and 2).

2.6. Down-regulated expression of p65 and TERT proteins

hTERT and the protein p65 are essential catalytic core of telomerase. Interaction of the p65 C-terminal domain with TER is