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Preliminary communication

Design and synthesis of celastrol derivatives as anticancer agents

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ABSTRACT

A series of celastrol derivatives as potential telomerase inhibitors were designed and synthesized. The bioassays demonstrated that title compounds displayed potent anticancer activities against SGC-7901, SMMC-7721, MGC-803 and HepG-2 cell lines, among them, compounds **3c** and **3d** which containing hydrophilicity moieties exhibited high anti-proliferative activities (IC_{50} = 0.10–1.22 μM). The preliminary mechanism of antitumor action indicated that title compound **3c** could induce significant SMMC-7721 cells apoptosis. A modified TRAP assay showed that compounds **3c** and **3d** displayed the most potent inhibitory activity with IC_{50} values at 0.11 and 0.34 μM, respectively. And there was a good correlation between telomerase inhibition and anti-proliferative inhibition of SMMC-7721 cells. Moreover, molecular docking indicated that the active compound **3c** was nicely bound into the telomerase hTERT active site, hydrophobic, van der Waals and two hydrogen bond interactions with conserved residues ASP 628 and TYR 949 were found.

1. Introduction

Telomerase plays a pivotal role in bypassing cell senescence and maintaining telomere homeostasis. Telomerase reverse transcriptase (TERT) is a catalytic subunit of the enzyme telomerase [1–3]. TERT has been reported to be over-expressed in more than 90% of cancer cells, while most normal tissues and cells contain inactivated telomerase, thereby, telomerase plays a critical role in sustained proliferation and survival potentials of various cancer cells [4,5]. Numerous evidences have suggested that TERT could modulate the expression of numerous genes including cell cycle regulation and cellular signaling [6,7]. Therefore, targeting TERT is an important strategy for the development of cancer agents [8,9].

It was of our interest to utilize rational chemical approaches and discover novel natural compounds with anticancer as telomerase inhibitors from Traditional Chinese Medicine (TCM). Focused on telomerase TERT (pdb: 3DU6), we disclosed the X-ray crystal structure of some molecule inhibitors bound to active site of TERT [6,10,11], based on this, in our previous study, a compound 18α-GAMG (pentacyclic triterpene scaffold, Fig. 1A) inhibited the expression of TERT and with selectivity activity against tumor cells versus human somatic cells was discovered [12]. But, unfortunately, the activity of this compound against to tumor cell lines is not high enough, herein, in continuation to extend our research on telomerase inhibitors, we began to look for other types of pentacyclic triterpene scaffolds according to above findings.

Among these compounds, celastrol (Fig. 1B) is an active compound isolated from the root extracts of TCM *Tripterygium wilfordii* (Thunder of God Vine). Celastrol is a pentacyclic triterpenoid and belongs in the family of quinone methides. Celastrol has been investigated widely for its anti-inflammatory and anticancer activity [13,14]. This triterpene modulates multiple molecular targets such as Hsp90-Cdc37, TNF-α, NF-κB, VEGF, Akt, CXCR4, pro-inflammatory cytokines and chemokines involved playing a major role in all three steps (initiation, proliferation and progression) of carcinogenesis [15,16]. So far, celastrol has been shown to have beneficial effects on a variety of cancers *in vitro* and *in vivo*, including pancreatic cancer, hepatocellular carcinomas, squamous cancer, and prostate cancer [17–20], suggesting it might be developed as a potential cancer treatment.

Although celastrol has important pharmacological activity, its poor water solubility and high toxicity restricts its application. Thus, some structure modifications were carried out to improve solubility or reduce toxicity, which focus on either the esterification or amidification of 20-carboxylic acid or the reduction products of A/B rings of celastrol [21–26]. Structural modifications at the C-2, 3

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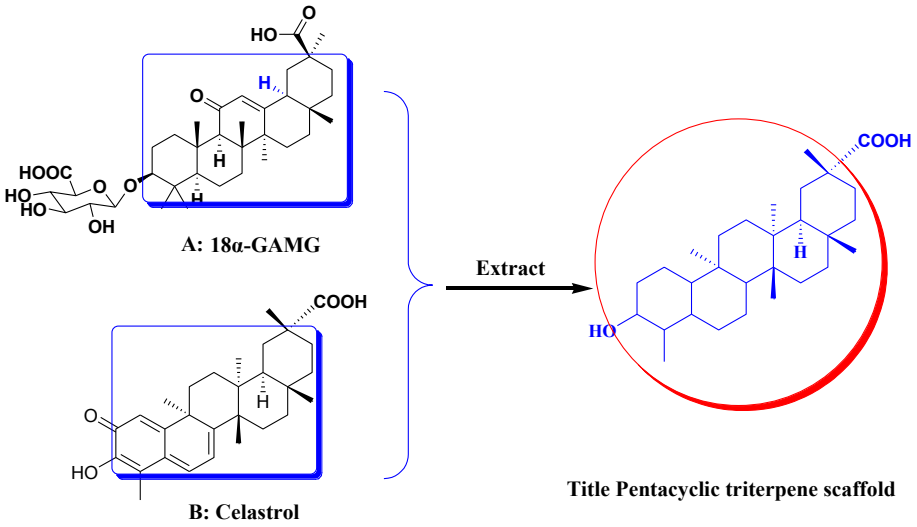

Fig. 1. The general design strategy in this study.

positions were reported as the inducers of heat shock response, while those at C-6 position displayed better anticancer activity. However, previous studies also suggested that the intact quinone methide moiety was crucial for its cytotoxic activity in cancer cell lines and neuroprotective effect [21,23].

In this study, based on improving celastrol water solubility and to further understand the preliminary structure–activity relationship (SAR) against cancer cells, we rationally designed and synthesized a series of celastrol derivatives with the intact quinone methide group. The anti-proliferative activity and telomerase inhibitive activity of these derivatives were evaluated.

2. Results and discussion

2.1. Chemistry

Carboxylic acid can be transferred to ether by alkylation reaction with the catalysis of alkali. Compound **2** was obtained by the esterification at 20-carboxylic acid of celastrol (**1**) in the presence of iodoethane and catalyst $NaHCO_3$ in anhydrous DMF with 72% yield. Amides are commonly formed *via* reactions of an "activated" carboxylic acid with an amine. Compounds **3a**~**3i** were obtained from amide condensation reaction catalyzed by EDC·HCl, HOBt and TEA in anhydrous CH_2Cl_2 conditions with 26%~56% yield (Scheme 1). The products were purified by extraction, column chromatography and recrystallization, and their structures were confirmed by ^1H NMR, ^{13}C NMR and HRMS according to the literature [27]. Purify was evaluated under HPLC conditions and exceeded 96%.

2.2. Crystal structure analysis

The structure of compound **3a** was determined by X-ray crystallography. Crystal data of **3a**: Clear light orange crystals obtained from EtOH/H_2O, yield, 77%; mp 133–135 °C; $C_{30}H_{41}NO_3$, M = 463.64, Monoclinic, space group $P2_1$; a = 11.6031(5), b = 8.1736(2), c = 13.9701(7) (Å); α = 90, β = 106.252(5), γ = 90, V = 1271.96(10) Å^3, T = 294 K, Z = 2, Dc = 1.211 g/cm^3, $F(000)$ = 504, Reflections collected/Independent reflections = 4621/3696, Data/restraints/parameters = 4621/1/315, Goodness of fit on F^2 = 1.045, Fine, R_1 = 0.0480, $wR(F^2)$ = 0.1248.

The molecular structure of compound **3a** was shown in Fig. 2. The absolute configuration of compound **3a** was determined using CuKα radiation (λ = 1.54184 Å), that is C5(*S*), C11(*S*), C15(*R*), C17(*S*), C20(*R*) and C27(*S*). In compound **3a**, rings *A* and *B* showed plane conformations, while the other rings showed mixed forms between envelope and half-chair conformations with atoms in rings *C*, *D* and *E*, respectively. Rings *D* and *E* were *cis*-form, which was similar to that of 18β-GAMG [12]. Crystallographic data (excluding structure factors) for the structure had been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC 1034724 [28].

2.3. In vitro anticancer activity

All title compounds were evaluated for their anticancer activities *in vitro* against SGC-7901 (human gastric cancer cells), SMMC-7721 (human hepatoma cells), MGC-803 (human gastric cancer cells) and HepG-2 (human hepatoma cells) cell lines, also included the activity of references 5-Fluorouracil and doxorubicin (AMD). The cells were allowed to proliferate in presence of tested material for 48 h, and the results are reported in terms of IC_{50} values (Table 1). Celastrol and its derivatives showed remarkable anti-proliferative effects. Among them, compounds **1**, **3c**, **3d** and **3i** displayed potent inhibitory activities ($IC_{50} \leq 1$ μM, as **1**, **3c**, **3d**, IC_{50} = 0.15, 0.16, 0.10 μM for SGC-7901 cells; as **1**, **3c**, **3d**, IC_{50} = 0.58, 0.30, 0.61 μM for SMMC-7721 cells; as **3c**, **3i**, IC_{50} = 0.39, 1.00 μM for MGC-803 cells; as **3c**, **3d**, IC_{50} = 0.61, 0.28 μM for HepG-2 cells, respectively), which were more potent than the positive control 5-Fluorouracil and AMD. Besides, compound **3h** showed potent anticancer activities against SGC-7901 and SMMC-7721 cell lines, surpassing that of the positive control 5-Fluorouracil.

Subsequently preliminary SAR studies were performed to determine how the substituent at 20-carboxylic acid affected the anticancer activity. Celastrol without any substituent group also showed good anti-proliferative activity. Firstly, the esterification of 20-carboxylic acid to form compound **2** showed lowest anticancer activities, whose IC_{50} values (9.28–15.19 μM) was lower than those of other compounds and the positive control. Secondly, the amidification of 20-carboxylic acid to form compounds **3a**~**3i** displayed different anticancer activity (IC_{50} = 0.10–19.97 μM). Compounds **3c**, **3d** with β-hydroxyl-ethylamide substituent exhibited potent anticancer activity against all four cell lines, while compounds **3h**,