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Original article

Enmein-type diterpenoid analogs from natural kaurene-type oridonin: Synthesis and their antitumor biological evaluation

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ABSTRACT

A series of enmein-type diterpenoid analogs (**11**–**20**) derived from natural kaurene-type diterpenoid oridonin were synthesized and biologically evaluated. All target compounds showed improved anti-proliferative activities against four human cancer cell lines compared with natural oridonin and parent compound **10**. Some compounds were more potent than positive control Taxol. Furthermore, mechanistic investigation showed that the representative compound **17** affected cell cycle and induced apoptosis at low micro-molar level in human hepatoma Bel-7402 cells, *via* an oxidative stress triggered mitochondria-related caspase-dependent pathway.

1. Introduction

Diterpenoids have attracted considerable attention because of their unique structural scaffold and interesting biological properties [1–9]. Enmein (**1**), the active *ent*-kaurene diterpenoid, was firstly isolated from Japanese folk medicine '*enmeiso*'. Recently, a lot of enmein-type diterpenoids were isolated and characterized from *Isodon* species, such as isodocarpin (**2**), nodosin (**3**), epinodosin (**4**), carpalasionin (**5**), sculponeatin A (**6**), sculponeatin N (**7**), and macrocalyxoformin B (**8**) (Fig. 1) [10–15]. Most of these diterpenoids were found to exhibit anti-inflammatory, antibacterial, antiviral, and more importantly, selective antitumor activities with very low toxicity to normal cells or tissues [15–17]. Given the intricate ring systems and many chiral centers, total synthesis or isolation and purification from natural plants with low yields could not meet the need for the medicinal and pharmaceutical studies for diterpenoids. In this regard, a practical and convenient synthetic methodology for enmein-type diterpenoid analogs is of great significance. We used a strategy that combined traditional isolation with the *de novo* synthetic method to prepare complex target natural product from commercially available natural products with relevant skeletons. In our previous studies, we disclosed some enmein-type and spirolactone-type diterpenoid analogs synthesized from oridonin (**9**) [18], which exhibited potent antitumor activities against several human cancer cell lines *in vitro* and *in vivo*. The activity of enmein-type diterpenoid compound **10** (Table 1) was less potent than spirolactone-type diterpenoid derivatives, which were firstly selected for further structural modification [19]. Notably, natural enmein-type diterpenoids showed potent anti-proliferative activities against human tumor cells [10–15], which were promising for further investigation. Since the 14-*O*-derivatives of **9** (Scheme 1) exhibited stronger cytotoxicity than their parent compound **9** [20–22], we wonder whether the similar modification on enmein-type diterpenoid could improve the anti-cancer activity of enmein-type diterpenoid. Herein, a series of novel 14-*O*-derivatives of enmein-type diterpenoid were designed and synthesized. The anti-proliferative activities of these compounds were tested by MTT assay. Furthermore, the mechanisms of

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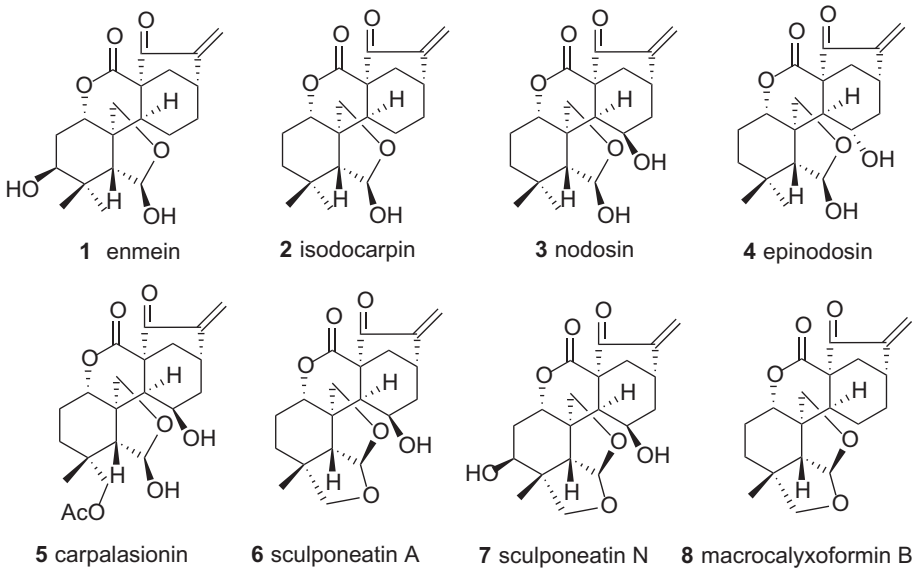

Fig. 1. Several enmein-type diterpenoids (**1**–**8**) isolated from natural plants.

influence on cell cycle progression, induction of apoptosis and effect on apoptosis-related proteins by representative compound **17** were investigated.

2. Results and discussion

2.1. Chemistry

As shown in Scheme 1, starting from commercially available kaurene-type diterpenoid oridonin (**9**), enmein-type diterpenoid analog **10** was obtained in nearly quantitative yield using the method previously well established [23,24] and reported by our research group [18]. Compound **10** could be a good building block to construct libraries of enmein-type diterpenoid analogs. The structure of compound **10** was confirmed by the key HMBC correlations of H-20 with C-6, C-1, C-9, C-10 and C-5 (Fig. 2).

Treatment of compound **10** with corresponding acids in the presence of DMAP/EDCI in DCM at room temperature for 8–12 h gave enmein-type diterpenoid analogs **11**–**20** in moderate to good yields. Flash chromatography was used for purification of target compounds.

Table 1
IC_{50}[a] values (μM) of enmein-type diterpenoid derivatives (**11**–**20**) against human cancer cell lines.[b]

Compd.	K562	MGC-803	CaEs-17	Bel-7402
Taxol	0.41 ± 0.02	0.85 ± 0.06	0.43 ± 0.03	1.89 ± 0.09
Oridonin	4.76 ± 0.32	5.69 ± 0.39	11.03 ± 1.02	7.48 ± 0.53
10	8.11 ± 0.76	14.21 ± 1.22	30.84 ± 2.09	32.96 ± 2.19
11	1.11 ± 0.15	1.99 ± 1.08	1.59 ± 0.43	1.97 ± 0.12
12	1.02 ± 0.26	1.56 ± 0.13	1.21 ± 0.52	1.53 ± 1.00
13	0.21 ± 0.08	0.93 ± 0.09	0.54 ± 0.04	1.42 ± 0.03
14	0.14 ± 0.12	0.61 ± 0.18	0.45 ± 0.13	1.01 ± 0.22
15	0.69 ± 0.08	3.33 ± 0.27	2.71 ± 0.77	1.79 ± 0.15
16	0.26 ± 0.05	0.64 ± 0.02	0.58 ± 0.04	1.01 ± 0.09
17	0.24 ± 0.10	1.22 ± 0.19	0.28 ± 0.06	0.87 ± 0.02
18	0.22 ± 0.02	0.80 ± 0.31	0.60 ± 0.15	0.85 ± 0.04
19	0.37 ± 0.10	0.96 ± 0.24	0.76 ± 0.18	1.27 ± 0.06
20	1.04 ± 0.02	1.24 ± 0.68	1.89 ± 0.44	0.89 ± 0.03

[a] IC_{50}: concentration that inhibits 50% of cell growth.
[b] Results are expressed as the mean ± S.D. of three independent experiments. Cell lines: K562 human leukemia cell line; MGC-803 human gastric cancer cell line; CaEs-17 human esophageal cancer cell line; Bel-7402 human hepatoma cell line.

2.2. MTT assay and SAR study

The anti-proliferative activities of enmein-type diterpenoid analogs **11**–**20** *in vitro* against four different kinds of human cancer cell lines were determined by MTT assay and compared with oridonin, parent compound **10** and positive control Taxol (Table 1). All derivatives exhibited much more potent anti-proliferative activities than oridonin and parent compound **10**; and some were even superior to Taxol *in vitro*. For example, compounds **13**, **14** and **16**–**19** showed stronger anti-proliferative activities in K562 cell line, and compound **14** was the most potent one with IC_{50} value of 0.14 μM. Compounds **14**, **16** and **18** exhibited more promising activities against MGC-803 cancer cells *in vitro* with IC_{50} values of 0.61, 0.64 and 0.80 μM, respectively. For CaEs-17 cell line, compound **17** showed the strongest activity (IC_{50}, 0.28 μM). The IC_{50} values of enmein-type diterpenoid analogs **11**–**20** against Bel-7402 cells ranged from 0.85 to 1.97 μM. The above results demonstrated that the esterification of 14-hydroxy of enmein-type diterpenoid could significantly improve the anti-proliferative activities of the parent compounds. It would be an important SAR in the research of modification of diterpenoids.

Among derivatives **11**–**20**, the SAR showed that when the substitutions R were alkyl groups (compounds **11** and **12**), their IC_{50} values slightly declined with the extension of the length of R in all the four tested cell lines. Stronger anti-proliferative activities were observed in aromatic substituted derivatives (compounds **13**–**20**), suggesting that the lipophilic moiety may be beneficial for the cytotoxicity of derivatives. Different from spirolactone-type diterpenoid derivatives [18,19], no obvious SAR could be concluded from the substituent species and the position on phenyl ring. Compound **17** with chloro at *para*-position of benzene ring showed promising anti-proliferative activities against all the four tested cell lines with IC_{50} values of 0.24 μM against K562 cell line, 0.28 μM against CaEs-17 cell line, 1.22 μM against MGC-803 cell line and 0.87 μM against Bel-7402 cell line. Based on the above results, compound **17** was selected for further pharmacological study.