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Synthesis and antitumor activities of novel dipeptide derivatives derived from dehydroabietic acid

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ABSTRACT

A series of dipeptide derivatives from dehydroabietic acid were designed and synthesized as novel antitumor agents. The antitumor activities screening indicated that many compounds showed moderate to high levels of inhibition activities against NCI-H460, HepG2, SK-OV-3, BEL-7404, HeLa and HCT-116 cancer cell lines and that some displayed more potent inhibitory activities than commercial anticancer drug 5-fluorouracil. The mechanism of representative compound **7b** was studied by AO/EB staining, Hoechst 33258 staining, JC-1 mitochondrial membrane potential staining, TUNEL assay, DNA ladder assay and flow cytometry, which exhibited that the compound could induce apoptosis in HeLa cells. Further investigation showed that compound **7b** induced apoptosis of HeLa cells through a mitochondrial pathway.

Cancer is one of the primary causes of death globally, so searching and developing effective anticancer drugs have greatly attracted bioorganic chemists' interest. After the success of vinblastine, taxol and their related derivatives as antitumor drugs, natural products have been seriously considered as a good traditional source of new antitumor drugs.[1–6]

As a natural occurring diterpenic resin acid, dehydroabietic acid (DHA) and its derivatives has been found to have properties of enhancing the inhibition activity of anticancer drug in various cells, such as hepatocellular carcinoma cells, cervical carcinoma cells, and breast cancer cells.[7] Our previous study has also demonstrated that DHA could be a useful tool in the synthesis of drugs active against some tumour cells.[8] Continuing our research program on the synthesis of antitumor drugs effective, DHA skeleton is chosen in the present work as active structural core and some structural modifications are carried out to explore their antitumor activities. Dipeptide, an important versatile bioactive moiety,[9] is able to improve the antitumor activity by constructing on a pharmacy core.[10–13] Many dipeptide derivatives have exhibited potent inhibition activities against human tumors cells.[9a,10–12] It is thus to expect that introducing of dipeptide group on DHA skeleton may contribute to potential antitumor activity. However, the study on the synthesis, antitumor activities and apoptosis-inducing effects of dipeptide derivatives derived from DHA has not been described. Our present work in this paper is to design and synthesize a series of DHA-dipeptide derivatives, and to evaluate their in vitro antitumor activities. Furthermore, the mechanism of apoptotic effects induced by the representative compound **7b** is also investigated.

DHA-dipeptide derivatives were synthesized as outlined in Scheme 1. Compound **2** was synthesized by the treatment of phenylalanine **1** with phthalic anhydride in the presence of acetic acid according to the literature.[13] Compound **3** was then obtained by the condensation of compound **2** and oxalyl chloride, and it was then treated with series of aromatic primary amines to offer compounds **4**. Compounds **5** were synthesized by the treatment of compounds **4** with hydrazine hydrate in the presence of ethanol at room temperature. DHA was treated with oxalyl chloride to offer compound **6**. Finally, compounds **7** were acquired by the condensation of compound **6** and compounds **5** in the presence of triethylamine at room temperature. The structures of DHA-dipeptide derivatives **7** were confirmed by ^{1}H NMR, ^{13}C NMR and high resolution mass spectrum (HRMS).[14]

The in vitro antitumor activities of DHA-dipeptide derivatives **7a**–**7s** were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)[9,15] assay against NCI-H460, HepG2, SK-OV-3, BEL-7404, HeLa and HCT-116 tumor cell lines, with 5-fluorouracil (5-FU) as the positive control. The tested results were shown in Table 1.

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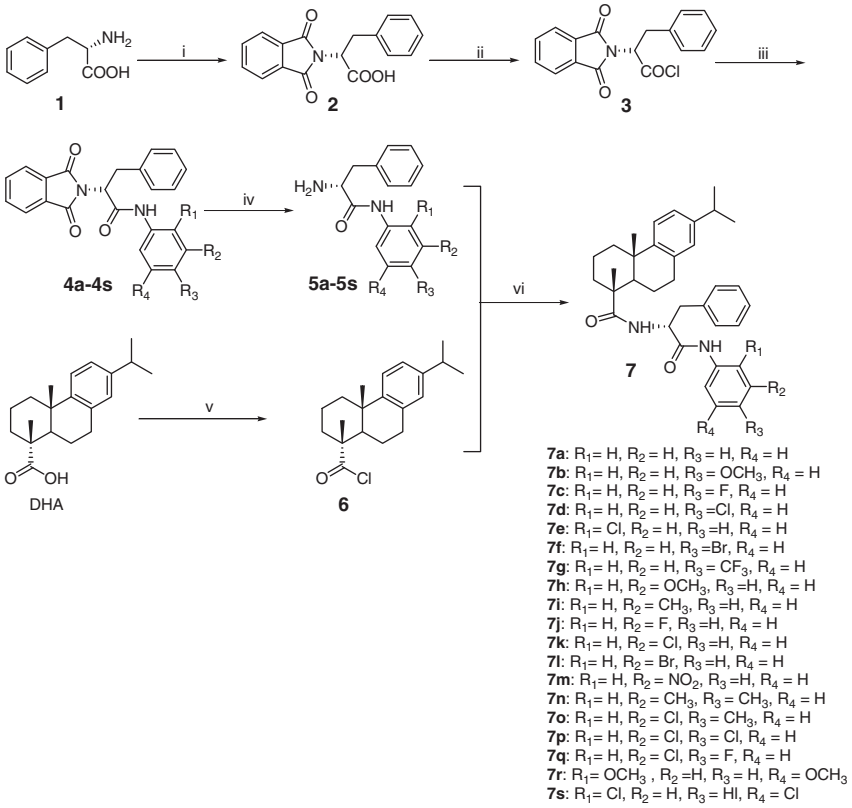

Scheme 1. Synthetic route to target compounds **7a**–**7s**. Reagents and conditions: (i) phthalic anhydride, CH_3COOH, 50 °C; (ii) $(COCl)_2$, CH_2Cl_2, rt; (iii) aromatic primary amines, Et_3N, CH_2Cl_2, rt; (iv) $NH_2NH_2{\cdot}H_2O$, CH_3CH_2OH; (v) $(COCl)_2$, CH_2Cl_2, rt; (vi) Et_3N, CH_2Cl_2, rt.

As shown in Table 1, most of DHA-dipeptide derivatives **7a**–**7s** displayed much higher inhibitory activity than DHA against NCI-H460, HepG2, SK-OV-3, BEL-7404, HeLa and HCT-116 cell lines, indicating the introduction of dipeptide group on DHA should markedly improve the antitumor activity. Moreover, the substituent groups at 4-position of phenyl ring (R_4) have important influence on the cytotoxic inhibition and the introduction of electron donor substituents and halogen groups may result in the increase of cytotoxic inhibition, while substituent groups at 3-position of phenyl ring (R_3) may lead to the decrease of cytotoxic inhibition.

Table 1 also revealed that, in NCI-H460 cell line assay, all the compounds exhibited better inhibition than DHA (IC_{50} = 80.53 μM), and most of compounds (such as **7a**–**7g**, **7i**–**7m** and **7o**–**7s**) even displayed preferable cytotoxic activities than the commercial anticancer drug 5-FU (IC_{50} = 36.04 μM), with IC_{50} in the range of 15.21–33.34 μM, indicating good inhibition activities of

Table 1
Effect of compounds **7a**–**7s** against cell viability of different cell lines

Compounds	IC_{50}(μM)					
	NCI-H460	HepG2	SK-OV-3	BEL-7404	HeLa	HCT-116
7a	28.20 ± 1.42	27.71 ± 1.64	27.36 ± 1.57	16.70 ± 0.56	18.89 ± 1.22	30.33 ± 3.26
7b	17.22 ± 1.04	12.40 ± 0.88	23.75 ± 1.76	15.34 ± 0.78	4.94 ± 0.88	15.67 ± 0.76
7c	22.83 ± 2.45	27.10 ± 3.21	20.07 ± 1.22	21.01 ± 1.27	10.84 ± 1.13	27.43 ± 1.39
7d	15.82 ± 1.43	26.30 ± 2.43	26.34 ± 2.34	33.59 ± 2.52	12.22 ± 1.56	37.79 ± 2.78
7e	15.21 ± 1.86	25.26 ± 2.54	13.52 ± 1.33	15.80 ± 2.43	10.29 ± 0.64	25.48 ± 2.65
7f	28.68 ± 1.25	34.64 ± 1.98	28.68 ± 2.46	16.90 ± 2.36	15.20 ± 1.46	35.33 ± 2.31
7g	28.85 ± 2.36	21.61 ± 1.43	36.34 ± 3.54	36.10 ± 2.38	10.47 ± 0.96	27.38 ± 2.56
7h	40.42 ± 3.45	29.50 ± 2.46	30.52 ± 2.13	38.53 ± 3.33	25.79 ± 1.23	35.77 ± 2.45
7i	32.35 ± 2.48	26.90 ± 3.21	29.84 ± 2.12	34.56 ± 3.21	18.19 ± 1.32	32.52 ± 3.21
7j	26.08 ± 3.21	23.86 ± 1.87	33.92 ± 3.14	27.45 ± 2.87	13.91 ± 0.58	34.76 ± 2.12
7k	26.34 ± 2.41	24.65 ± 1.68	30.62 ± 2.76	23.79 ± 2.34	13.06 ± 1.24	45.85 ± 3.15
7l	33.34 ± 3.45	22.89 ± 1.46	38.80 ± 4.21	19.95 ± 2.61	25.13 ± 0.92	44.10 ± 3.65
7m	32.83 ± 2.65	25.26 ± 2.23	20.07 ± 1.23	27.05 ± 2.15	12.82 ± 1.02	27.43 ± 2.31
7n	36.98 ± 3.25	42.78 ± 4.21	51.79 ± 4.56	48.16 ± 3.11	14.34 ± 1.43	32.14 ± 2.41
7o	26.10 ± 2.23	36.90 ± 2.32	37.82 ± 2.31	41.16 ± 3.45	10.11 ± 0.75	32.30 ± 3.42
7p	26.98 ± 2.46	20.42 ± 1.88	33.15 ± 1.27	44.69 ± 2.46	28.79 ± 1.46	40.85 ± 3.67
7q	26.77 ± 2.54	32.91 ± 2.46	30.54 ± 2.85	26.06 ± 2.12	11.12 ± 0.86	39.27 ± 2.55
7r	27.44 ± 2.48	38.07 ± 2.75	53.75 ± 3.51	56.34 ± 3.31	19.26 ± 2.13	36.84 ± 2.56
7s	15.78 ± 1.75	23.43 ± 2.66	21.58 ± 2.18	30.98 ± 2.88	14.94 ± 1.35	24.10 ± 1.29
DHA	80.53 ± 2.11	76.76 ± 3.68	75.00 ± 2.21	34.70 ± 2.23	29.35 ± 2.32	35.24 ± 3.26
5-FU	36.04 ± 2.54	26.98 ± 2.37	24.43 ± 0.41	26.80 ± 2.23	80.48 ± 2.31	24.43 ± 0.40