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Synthesis of thioether andrographolide derivatives and their inhibitory effect against cancer cells†‡

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A series of novel thioether andrographolide derivatives were synthesized by incorporating various aromatic (or heteroaromatic) substituents into C-12 or 14-OH. A total of 38 andrographolide derivatives were prepared and evaluated for their *in vitro* inhibitory activity against cancer cells. All the derivatives exhibited better activity against prostate cancer cells (PC-3) than the parent compound. Among these, compounds **6a**, **8**, **9**, **17**, **19**, **31**, and **32** demonstrated good activity. These compounds were further evaluated for their anti-cancer activities against other cancer cell lines including MCF-7, MDA-MB-231, and A549. Compounds **31** and **32** showed excellent activity against MCF-7 with an IC₅₀ value of 0.7 and 0.6 μM, respectively. The absolute configuration of **15a** was determined *via* single-crystal X-ray diffraction. The activity of **6a** (12S), which was the precursor of **15a**, was better than that of the diastereoisomer **6b** (12R). Moreover, the preliminary structure–activity relationship has been summarized. The results obtained herein are very important for further optimization of andrographolide.

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Introduction

Andrographolide, a labdane diterpenoid, is the major constituent of *Andrographis paniculata*, which has been widely used in Asia as a herbal medicine to treat a variety of diseases including sore throat, flu, upper respiratory tract infections, bacterial dysentery, malaria, herpes, fever, and the common cold.^{1–3} Modern research reveals that andrographolide is responsible for the above mentioned pharmacological effects.^{2,4,5} As a result, not only the herb, but also pure

andrographolide has already been prepared as tablets or capsules in Eastern Asia to treat bacterial infections and inflammation-related diseases such as bronchitis and pneumonia.⁶ Every year, tons of pure andrographolide are produced from *A. paniculata* through extraction and purification. However, due to the low water solubility of andrographolide, its bioavailability becomes low⁷ and affects the pharmacological effect. Therefore, modification of the chemical structure of andrographolide has been carried out to improve the solubility and activity. A series of andrographolide derivatives have been designed and synthesized.^{2,4,5,8,9} Among these, several compounds (such as Lianbizhi, Chuanhuning, and Yanhuning),¹⁰ which show better activities and drug-like properties, have been further developed and approved for use in Chinese clinics.

In recent years, scientists have revealed that andrographolide and its derivatives possess anticancer activity both *in vitro* and *in vivo*.^{2,11–19} The mechanism of the anti-tumor actions is mainly through induction of cell cycle arrest and promotion of apoptosis in human cancer cells by attenuating various cell signaling pathways.^{12–14} A series of andrographolide derivatives has been previously synthesized to screen their *in vitro* cytotoxicity against cancer cells.^{4–7} Based on previous studies, the γ -lactone moiety, the conjugated double bonds, C=O, and the C-14 hydroxyl group play important roles in cytotoxicity.^{4,5,7,11} Esterification of the C-14 hydroxyl group would result in increased activity, whereas removal of the C-14 hydroxyl group is detrimental

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for the activity. Moreover, C-12 substituted derivatives also significantly improve cytotoxicity against cancer cells.²⁰ Recently, Kandanur *et al.* introduced alkyl amines, benzyl amines, and phenyl thio at C-12 of andrographolide and found that derivatives containing aryl amino, aryl thio, and benzyl amino groups showed a broad range of activity against most of the cancer cell lines.²¹

Natural products are a great source of modern drugs. Among the 1562 new approved drugs during the years 1981 to 2014, 47% of them are from or derived from natural products.²² Therefore, choosing an active natural product as the lead compound may have better chances of displaying desirable biological and pharmacological properties. In continuation of our studies on antitumor natural products, we chose andrographolide as the lead compound to design and synthesize more potent and active antitumor derivatives. Based on previously reported structure–activity relationship studies,^{4–7,11,13–19} we focused our effort on the development of 14-OH and C-12 derivatives of andrographolide. We envisaged that introduction of different aromatic (or heteroaromatic) thioethers into C-12 or 14-OH might increase hydrogen bonding and π – π stacking interactions and thereby have better effects on the hydrophilic and antitumor properties. In the present study, a series of novel andrographolide analogues was synthesized, and these analogues were evaluated for inhibitory effects against cancer cells. Herein, we report the synthesis, cytotoxic activity, and preliminary structure–activity relationship study.

Results and discussion

Chemistry

As the esterification of 14-OH could increase the antitumor activity, we connected the aromatic (or heteroaromatic) thioether moiety to 14-OH using an ester bond (as depicted in Scheme 1). There were three hydroxyl groups in the chemical

structure of andrographolide. To selectively esterify the 14-OH, 3, 19 hydroxyl groups were protected to afford 3,19-isopropylideneandrographolide (**a**), which served as a key intermediate in preparing the following analogues. We adopted Sirion's procedure²³ and obtained the desired product (**a**) with high yield (94%) (ESI†). To prepare the thioether analogues, 2-(tosyloxy)acetyl chloride was used to react with **a** to afford the intermediate 2-(tosyloxy)acetate (**b**), which was expected to undergo a nucleophilic reaction with the aromatic thiolate to form the thioether (**III**) (Scheme 1). However, to our surprise, no desired ester (**III**) was formed after the reaction; instead, the 12-aromatic thioether (**I**) was formed in good yield. The proposed reaction mechanism is depicted in Fig. 1. Thiophene-2-thiolate formed by the reaction of thiophene-2-thiol with CH_3ONa attacked the C-12 of andrographolide to afford **I**. This process, rather than the attack on the carbon connected to the TsO-moiety, was favored by the formation of the stable α,β -unsaturated carbonyl group within the five-membered ring. Thus, thiophene-2-thiolate could attack C-12 from both sides of the plane and therefore generated two possible diastereoisomers. We successfully separated the two diastereoisomers of **6a** (320 mg) and **6b** (38 mg) (Fig. 1). The chemical structures of **6a** and **6b** were unambiguously confirmed *via* MS, ^1H -NMR, ^{13}C -NMR, and H-H-COSY spectra (see ESI†). To determine the configuration of **6a** and **6b**, the main product **6a** of the reaction was further deprotected to afford the product **15a**, which was further recrystallized three times to obtain the single crystal. Then, the single-crystal structure of **15a** was determined *via* X-ray crystallography (its molecular structure is illustrated in Fig. 2, deposition number in the Cambridge Crystallographic Data Centre: CCDC 1508339). As shown in Fig. 2, the absolute configuration of C-12 in the chemical structure of **15a** was determined to be *S*. Therefore, the absolute configuration of C-12 in the precursor **6a** was *S*, whereas that of the diastereoisomer **6b** was *R*. This reaction provided a good

Scheme 1 Reagents and conditions: (i) DMP/PPTS/acetone, rt, 2 h; (ii) 2-(tosyloxy)acetyl chloride, CH_2Cl_2 , Et_3N , 0 °C, 1 h; (iii) $\text{CH}_3\text{ONa}/\text{MeOH}$; reflux; and (iv) 70% HOAc, rt, 45 min.

Fig. 1 Proposed mechanism for the reaction between thiolate and intermediate **b**.

method to prepare the C-12 thioether derivatives. To investigate the influence of the aromatic and heteroaromatic rings, different C-12 thioether andrographolide derivatives (compounds **1**–**18**) substituted with benzene, thiophene, pyridine, pyrimidine, and thiazole were prepared in moderate to good yields (56–80%, see the ESI†). Furthermore, for the purpose of understanding the effects of electron-withdrawing and electron-donating groups, different thioether andrographolide derivatives substituted with fluorobenzene, nitrobenzene, methylbenzene, and methoxybenzene were synthesized using the same strategy. The removal of the isopropylidene group of **I** (**1**–**9**) was carried out by employing AcOH/H₂O to readily furnish the target compounds **II** (**10**–**18**).

As depicted in Scheme 2, for the syntheses of the C-14 thioether andrographolide derivatives, the desired target compound **III** was smoothly achieved *via* treatment of the intermediate **a** with 2-(arythio) acetyl chloride in the presence of Et₃N at room temperature. The preparation of 2-(arythio) acetyl chloride is outlined in Scheme 3. Treatment of ethyl 2-hydroxyacetate with TsCl/Et₃N afforded ethyl 2-(tosyloxy)acetate, which was converted to ethyl 2-(arythio)acetate *via* nucleophilic reaction with RS[−]Na⁺. Hydrolysis of ethyl 2-(arythio)acetate afforded the acid **c**, which was treated with oxalyl chloride to obtain the impor-

tant intermediate 2-(arythio) acetyl chloride. To preliminarily investigate the influence of the length of the linker on the activity, we designed different lengths of linker with two or four carbons (Scheme 2). The syntheses of compounds **V** and **VI**, which had a linker of four carbons, were similar to those of **III** and **IV**.

Study of the andrographolide analogues on cancer cell lines

The andrographolide and the analogues were assayed for their inhibitory activity against human prostate cancer cell line (PC-3). Taxol, which has been used in clinics as an anti-cancer agent, was used as the positive control. The cell viability was assessed according to the MTT assay. The PC-3 cells were exposed to all compounds at certain concentrations in quadruplet. The results of the IC₅₀ values are summarized in Table 1. From the results obtained, it was clear that all the derivatives (**1**–**38**) showed better anticancer activity than the parent compound andrographolide. Among these, compounds **6a**, **8**, **9**, **17**, **19**, **31**, and **32** showed equal or even better activity than the reference compound, Taxol. From the analysis of the data, it was clear that the 3,19-isopropylideneandrographolide analogues exhibited better antitumor activity than the unprotected andrographolide analogues. In general, the heteroaromatic thioether analogues had better activity as compared to the aromatic thioether analogues. The diastereoisomers **6a** (12*S*) and **6b** (12*R*) possessed different activity. The main product **6a** exhibited better activity (IC₅₀ 9 μM) than **6b** (IC₅₀ 20 μM).

For the C-12 andrographolide derivatives, introduction of the aromatic or heteroaromatic thioether to the andrographolide skeleton obviously increased cytotoxicity against prostate cancer cells. Among these, the heteroaromatic thioether andrographolide derivatives showed better activity. Compounds **8** and **17**, bearing a pyrimidine thioether at the 12-position, showed good activity with an IC₅₀ value of 7 μM. To investigate the influence of the electron withdrawing and donating groups, different substituted groups were attached to the aromatic ring. On comparing the activities of compounds **2** and **1** (or **5**) and **11** with **10** (or **14**), it was concluded that activity would decrease when an electron

Fig. 2 The X-ray crystal structure of compound **15a**.

Scheme 2 Reagents and conditions: (i) Et₃N, CH₂Cl₂, 0 °C, 1 h and (ii) 70% HOAc, rt, 45 min.

Scheme 3 Reagents and conditions: (i) TsCl/Et₃N, 0 °C, 2 h; (ii) CH₃ONa/MeOH; reflux; (iii) 5% NaOH/EtOH, 40 °C, 2 h; and (iv) SOCl₂ or (COCl)₂, reflux, 6 h.

donating group is connected to the benzene ring. Data obtained for compounds 3, 4, 12, and 13 indicated that introducing the nitro group and F into the benzene ring had less influence on the activity.

For the 14-OH andrographolide derivatives, connecting the aromatic or the heteroaromatic thioether to the C-14 hydroxyl group through a linker could increase the antitumor activity. Similarly, the heteroaromatic thioether derivatives had better activities. Among these, compounds 31 and 32, bearing a moiety of thiophene and pyridine, respectively, had good activities (IC₅₀ 9 μM). From the data, it was observed that the length of the linker (2 and 4 carbon atoms) had slight influence on the activity. Moreover, the substitution on the benzene ring did not have significant influence on the activity.

Compounds 6a, 8, 9, 17, 19, 31, and 32 were further chosen to evaluate the *in vitro* inhibitory effects against another three different cancer cells. The results are summarized in Table 2. From the data, it was found that most of these compounds also showed good activity against breast cancer cells (MCF-7 and MDA-MB-231). Among these, compounds 31 and 32 showed excellent activity against MCF-7 cells with an IC₅₀ value of 0.7 μM and 0.6 μM, respectively. However, lung cancer cells (A549) were not significantly sensitive to these compounds and only compound 17 showed good activity with an IC₅₀ value of 9 μM. We also tested the toxicity of these compounds against normal cells. No obvious toxicity of these compounds was observed against normal cells (Vero cell) at a concentration of 100 μM. These results indicate that these compounds may be regarded as promising potential anticancer candidates.

Table 1 Evaluation of *in vitro* cell growth inhibitory effects of andrographolide analogues (1–38) in prostate cancer cell (PC-3)^{a,b}

Compound	Analogue	R group	IC ₅₀ (μM)	Compound	Analogue	R ₁ , or R ₂ , or R ₃ group	IC ₅₀ (μM)
1	I-R ₁		23	20	III-R ₂		11
2	I-R ₁		12	21	III-R ₂		13
3	I-R ₁		12	22	III-R ₂		15
4	I-R ₁		13	23	IV-R ₂		17
5	I-R ₁		17	24	IV-R ₂		27
6a ^c	I-R ₁		9	25	IV-R ₂		14
7	I-R ₁		14	26	IV-R ₂		18
8	I-R ₁		7	27	V-R ₃		18
9	I-R ₁		10	28	V-R ₃		17
10	II-R ₁		29	29	V-R ₃		14
11	II-R ₁		14	30	V-R ₃		13
12	II-R ₁		17	31	V-R ₃		9
13	II-R ₁		16	32	V-R ₃		9
14	II-R ₁		15	33	VI-R ₃		19
15a	II-R ₁		15	34	VI-R ₃		17
16	II-R ₁		18	35	VI-R ₃		16
17	II-R ₁		7	36	VI-R ₃		12
18	II-R ₁		14	37	VI-R ₃		16
19	III-R ₂		9	38	VI-R ₃		14

^a Results were expressed as the mean value of four data points. Taxol was used as a positive control (IC₅₀ = 7 μM). ^b IC₅₀ of andrographolide is 30 μM. ^c IC₅₀ of 6b, which is the diastereoisomer of 6a, is 20 μM.

Table 2 Evaluation of *in vitro* cell growth inhibitory effects of andrographolide analogues in different cancer cells (PC-3, MCF-7, MDA-MB-231, and A549)^a

Compound	Cell growth inhibition in IC ₅₀ (μM)			
	PC-3	MCF-7	MDA-MB-231	A549
6a	9	5	8	20
8	7	7	9	20
9	10	3	12	31
17	7	23	16	9
19	9	10	19	11
31	9	0.7	10	25
32	9	0.6	6	23

^a Results were expressed as the mean value of four data points.

Conclusions

In summary, the present study preliminarily investigated the modification of the C-12 and 14-OH of andrographolide by introducing novel aromatic or heteroaromatic thioether into the skeleton. A total of 38 andrographolide derivatives were successfully synthesized and evaluated for their inhibitory effect against cancer cells. All the synthetic compounds exhibited better activity than the parent compound. Among these, compounds 6a, 8, 9, 17, 19, 31, and 32 showed excellent activity against prostate cancer cells (PC-3) and breast cancer cells (MCF-7 and MDA-MB-231). The absolute configuration of 15a was determined to be *S* using single-crystal X-ray crystallography. The activity of 6a (12*S*) was better than that of the diastereoisomer 6b (12*R*).

This study also provided us with a preliminary structure–activity relationship. The 3,19-isopropylideneandrographolide analogues exhibited better antitumor activity than the unprotected andrographolide analogues. The heteroaromatic thioether showed better activity compared to the aromatic thioether. For the C-12 analogues, the substitution of the electron donating group on the benzene ring decreased the activity, whereas the electron withdrawing group on the benzene ring had slight little influence on the activity. For the C-14 analogues, the length of the linker and the substitution pattern on the aromatic ring had little influence on the activity. Inspired by the results, further investigation of the biological mechanism and optimization of the C-12 and 14-OH of andrographolide analogues are ongoing.

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