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Synthesis of Thioether Andrographolide Derivatives and Their Inhibitory Effect Against Cancer Cells‡

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Abstract

A series of novel thioether andrographolide derivatives were synthesized by incorporating various aromatic (or heteroaromatic) substituents into the C-12 or 14-OH. Totally 38 andrographolide derivatives were prepared and evaluated for their *in vitro* inhibitory activity against cancer cells. All of the derivatives exhibited better activity against the prostate cancer cells (PC-3) than that of the parent compound. Among them, compounds **6a**, **8**, **9**, **17**, **19**, **31**, **32** demonstrated good activity. These compounds were further evaluated for their anticancer activities against other cancer cell lines, including MCF-7, MDA-MB-231, A549. Compounds **31** and **32** showed excellent activity against MCF-7 with IC_{50} value of 0.7 and 0.6 μM, respectively. The absolute configuration of **15a** was determined by using the single-crystal X-ray diffraction. The activity of **6a** (12*S*), which is the precursor of **15a**, was better than that of the diastereoisomer **6b** (12*R*). The preliminary structure-activity relationship was summarized. The results are very important for the further optimization of andrographolide.

Keywords: Andrographolide, Antitumor, Thioether, Absolute configuration

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Introduction

Andrographolide, a labdane diterpenoid, is the major constituent of *Andrographis paniculata*, which has been widely used in Asia as 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.[1,2,3] Modern research reveals that andrographolide is responsible for the pharmacological effects mentioned above.[2,4,5] As a result, not only the herb, but also the pure andrographolide has already been prepared as tablets or capsules in Eastern Asia to treat bacterial infections and inflammatory related diseases, such as bronchitis and pneumonia.[6] Every year, tons of pure andrographolide are produced through extraction and purification from *A. paniculata*. However due to the poor water solubility of andrographolide, the bioavailability is low[7] and the pharmacological effect has been affected. Therefore, modification of the chemical structure of andrographolide has been carried out to improve the solubility and the activity. Series of andrographolide derivatives have been designed and synthesized.[2,4,5,8,9] Among them, several compounds (such as Lianbizhi, Chuanhuning and Yanhuning)[10], which show better activities and drug-like properties, have been further developed and approved to be used in Chinese clinic.

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 antitumor actions is mainly through inducing cell cycle arrest and promoting

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apoptosis in human cancer cells by attenuating various cell signaling pathways.[12-14] Series of andrographolide derivatives have been synthesized previously to screen the *in vitro* cytotoxicity against cancer cells[4-7]. Based on the previous studies, γ-lactone moiety, the conjugated double bonds, C=O, and C-14 hydroxyl group play important roles in the cytotoxicity.[4,5,7,11] Esterification of C-14 hydroxyl group would result in the increase of the activity, while removal of C-14 hydroxyl group is detrimental to the activity. Besides, C-12 substituted derivatives also significantly improve the cytotoxicity against cancer cells.[20] Recently, Sai 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 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 year from 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 compounds to design and synthesize more potent and active antitumor derivatives. Based on the previous 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. By introducing different aromatic (or heteroaromatic) thioether into C-12 or 14-OH, we envisaged that it might increase hydrogen bonding and π-π stacking interaction and thereby had

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better effects on the hydrophilic and antitumor properties. In the present study, a series of novel andrographolide analogues were synthesized and the analogues were evaluated for the inhibitory effects against the cancer cells. Herein, we reported 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 by using an ester bond (as depicted in **scheme 1**). There were three hydroxyl groups in the chemical structure of andrographolide. In order to esterify the 14-OH selectively, 3, 19 hydroxyl groups were protected to afford the 3,19-isopropylideneandrographolide (**a**), which served as key intermediate in preparing the following analogues. We adopted Sirion's procedure,[23] and obtained the desired product (**a**) with high yield (94%) (**supplementary data**). In order to prepare the thioether analogues, 2-(tosyloxy)acetyl chloride was used to react with **a** to afford the intermediate 2-(tosyloxy)acetate (**b**), which we envisaged to go through a nucleophilic reaction with the aromatic thiolate to form the thioether (**III**) (**scheme 1**). However, to our surprise, there was no desired ester (**III**) formed after the reaction, instead the 12-aromatic thioether (**I**) was formed in good yield. The proposed reaction mechanism was depicted in **figure 1**. The thiophene-2-thiolate formed by the reaction

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of thiophene-2-thiol with CH_3ONa attacked C-12 of the andrographolide to afford **I**. This process, rather than attacking the carbon that connected to the TsO- moiety, was favored by the formation of the stable α,β-unsaturated carbonyl group within the five-member ring. The thiophene-2-thiolate could attack the C-12 from both side of the plane, and therefore generated two possible diastereoisomers. We successfully separated the two diastereoisomers of **6a** (320 mg) and **6b** (38mg) (**figure 1**). The chemical structures of **6a** and **6b** were unambiguously confirmed by MS, ^{1}H-NMR, ^{13}C-NMR, and H-H-COSY spectra (see **supplementary data**). In order to determine the configuration of **6a** and **6b**, the main product (**6a**) of the reaction was further deprotected to give the product **15a**, which was further recrystallized for three times to get the single crystal. Then the single-crystal structure of **15a** was determined by X-ray crystallography (its molecular structure was illustrated in **figure 2,** deposition number in Cambridge Crystallographic Data Centre: CCDC 1508339). As shown in **figure 2**, the absolute configuration of the C-12 in the chemical structure of **15a** was determined to be *S*. Therefore the absolute configuration of the C-12 in the precursor **6a** was S, while that of the diastereoisomer **6b** was *R*. This reaction provided us with a good method to prepare the C-12 thioether derivatives. In order 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, thiazole were prepared in moderate to good yields (56%-80%, see **supplementary data**). Furthermore, for the purpose of knowing that the effect of electron-withdrawing and electron-donating group, different thioether

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andrographolide derivatives substituted with fluorobenzene, nitrobenzene, methylbenzene, and methoxylbenzene were synthesized using the same strategy. The removal of isopropylidene group of **I** (**1-9**) was carried out by employing AcOH/H_2O to readily furnish the target compounds **II** (**10-18**).

As depicted in **scheme 2**, for the syntheses of C-14 thioether andrographolide derivatives, the desired target compound **III** was smoothly achieved by treatment of intermediate **a** with 2-(arythio) acetyl chloride in the presence of Et_3N at room temperature. The preparation of 2-(arythio) acetyl chloride was outlined in **scheme 3**. Treatment of ethyl 2-hydroxyacetate with TsCl/Et_3N gave the ethyl 2-(tosyloxy)acetate, which was converted to ethyl 2-(arylthio)acetate via the nucleophilic reaction with RS^-Na^+. Hydrolysis of ethyl 2-(arylthio)acetate afforded the acid **c**, which was treated with oxalyl chloride to give the important intermediate 2-(arylthio) acetyl chloride. In order to preliminarily investigate the influence of the length of the linker on the activity, we designed different lengths of the 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 clinic as anti-cancer agent, was used as the positive control. The cell viability was performed according to the MTT assay. The PC-3 cells were exposed to all compounds at certain

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concentrations for four times repeat. The results of IC_{50} values were summarized in **Table 1**. From the results obtained, it was clear that all of the derivatives (**1-38**) showed better anticancer activity than that of the parent compound, andrographolide. Among them, compounds **6a**, **8**, **9**, **17**, **19**, **31**, **32** showed equal or even better activity than that of the reference compound, taxol. From analysis of the data, it was clear that the 3,19-isopropylideneandrographolide analogues exhibited better antitumor activity than that of the C3, C19 hydroxyl groups unprotected andrographolide analogues. In general, the heteroaromatic thioether analogues had better activity compared to those aromatic thioether analogues. The diastereoisomers **6a** (12*S*) and **6b** (12*R*) possessed different activity. The main product (**6a**) exhibited better activity (IC_{50} 9 μM) than that of **6b** (IC_{50} 20 μM).

For the C-12 andrographolide derivatives, introduction of the aromatic or heteroaromatic thioether to the andrographolide skeleton obviously increased the cytotoxity against the prostate cancer cells. Among them, the heteroaromatic thioether andrographolide derivatives showed better activity. Compounds **8** and **17,** bearing a pyrimidine thioether in 12-position, showed good activity with IC_{50} value of 7 μM. In order to investigate the influence of the electron withdrawing and donating groups, different substituted groups were attached to the aromatic ring. Comparing the activities of compounds **2** with **1** (or **5**), **11** with **10** (or **14**), we concluded that it would decrease the activity when connecting an electron donating group to the benzene ring. Data from compounds **3**, **4**, **12**, **13** indicated that introducing the nitro group and F into the benzene ring had less influence on the activity.

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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 them, compounds **31** and **32,** which embraced a moiety of thiophene and pyridine, respectively, had good activities (IC_{50} 9 μM). From the data, the length of the linker (2 and 4 carbon atoms) appeared to have little influence on the activity. Besides, the substitution on the benzene ring didn't have significant influence on the activity.

Compounds **6a**, **8**, **9**, **17**, **19**, **31**, and **32** were further chose to evaluate the *in vitro* inhibitory effects against other three different cancer cells. The results were summarized in **Table 2**. From the data, we could find out that most of these compounds also showed good activity against the breast cancer cells (MCF-7 and MDA-MB-231). Among them, compounds **31** and **32** showed excellent activity against MCF-7 cells with IC_{50} value of 0.7 μM and 0.6 μM, respectively. However, the lung cancer cells (A549) were not significantly sensitive to these compounds. Only compound **17** showed good activity with an IC_{50} 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 indicated that these compounds may be regarded as promising potential anticancer candidates.

Conclusions

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In summary, the present study preliminarily investigated the modification of the C-12 and 14-OH of andrographolide by introducing the novel aromatic or heteroaromatic thioether into the skeleton. Totally 38 andrographolide derivatives were successfully synthesized, and further evaluated for their inhibitory effect against cancer cells. All the synthetic compounds exhibited better activity than that of the parent compound. Among them, compounds **6a**, **8**, **9**, **17**, **19**, **31**, **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* by 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 preliminary structure-activity relationship. The 3,19-isopropylideneandrographolide analogues exhibited better antitumor activity than that of the C3, C19 hydroxyl groups 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 will decrease the activity, while the electron withdrawing group on the benzene ring appeared to have 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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View Article Online
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Figure 1. Proposed mechanism of the reaction between thiolate and intermediate **b**.

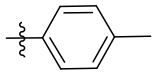
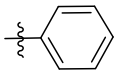
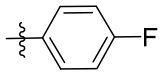
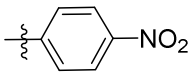
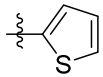
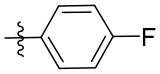
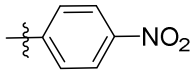
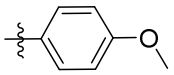
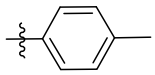
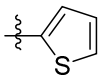
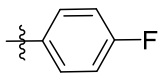
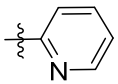
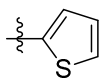
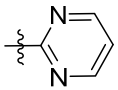
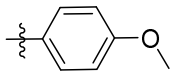
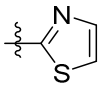
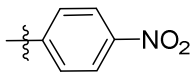
Figure 2. The X-ray crystal structure of compound **15a**.

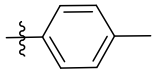
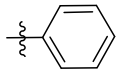
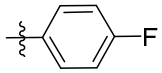
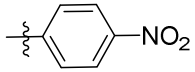
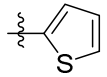
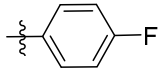
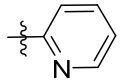
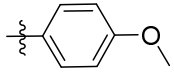
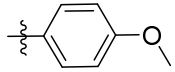
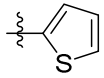
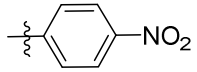
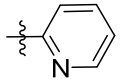
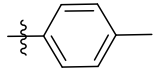
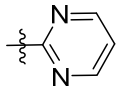
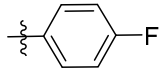
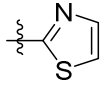
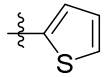
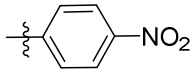
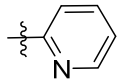
Scheme 1. Reagents and conditions: (i) DMP/PPTS/acetone, rt, 2h; (ii) 2-(tosyloxy)acetyl chloride, CH_2Cl_2, Et_3N, 0˚C, 1h; (iii) CH_3ONa/MeOH; reflux; (iv) 70 % HOAc, rt, 45 min.

Scheme 2. Reagents and conditions: (i) Et_3N, CH_2Cl_2, 0˚C, 1 h; (ii) 70 % HOAc, rt, 45 min.

Scheme 3. Reagents and conditions: (i) TsCl/Et_3N, 0 ˚C, 2 h; (ii) CH_3ONa/MeOH; reflux; (iii) 5 % NaOH/EtOH, 40 ˚C, 2 h; (iv) $SOCl_2$ or $(COCl)_2$, reflux, 6 h.

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 (IC_{50}=7 μM).
[b]. IC_{50} of andrographolide is 30 μM.
[c]. IC_{50} of **6b**, which is the diastereoisomer of **6a**, is 20 μM.

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

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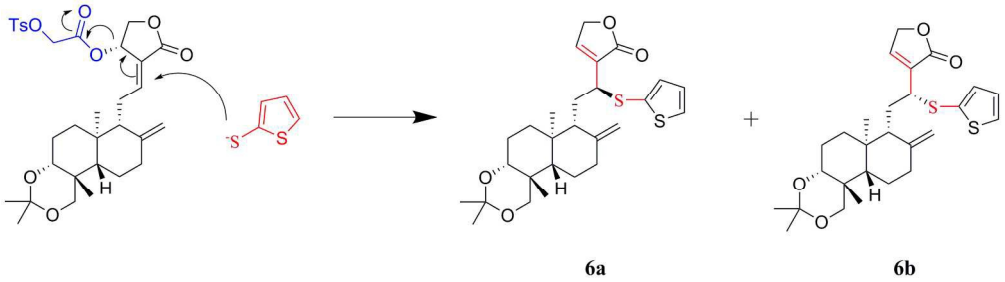

Figure 1. Proposed mechanism of the reaction between thiolate and intermediate b.

197x56mm (300 x 300 DPI)

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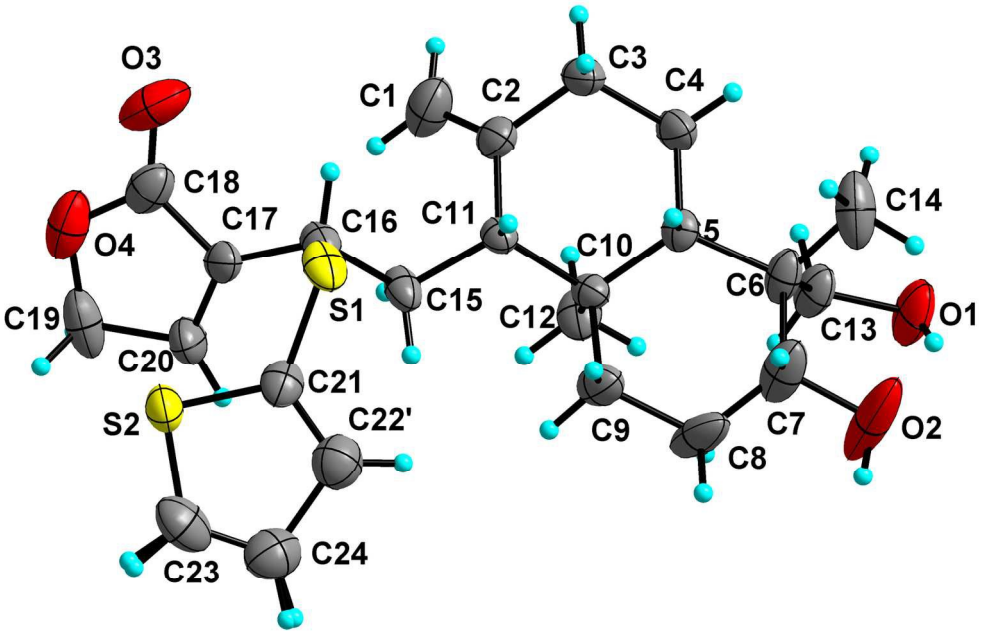

Figure 2. The X-ray crystal structure of compound 15a.

158x119mm (300 x 300 DPI)

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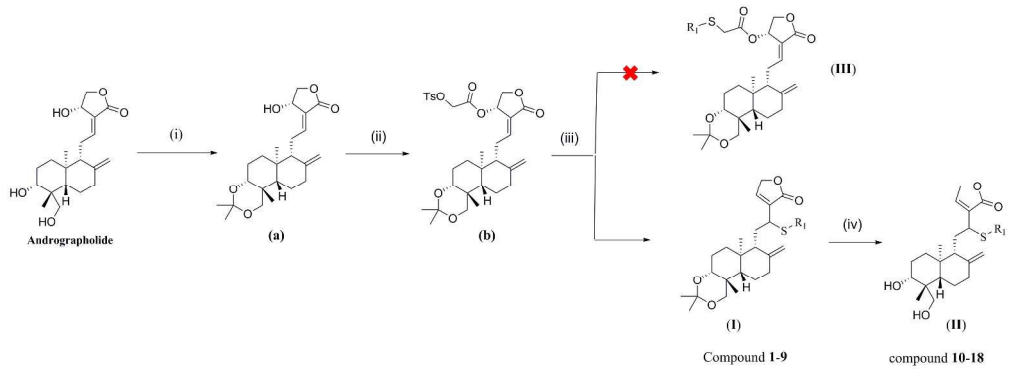

Scheme 1. Reagents and conditions: (i) DMP/PPTS/acetone, rt, 2h; (ii) 2-(tosyloxy)acetyl chloride, CH2Cl2, Et3N, 0°C, 1h; (iii) CH3ONa/MeOH; reflux; (iv) 70 % HOAc, rt, 45 min.

303x110mm (300 x 300 DPI)

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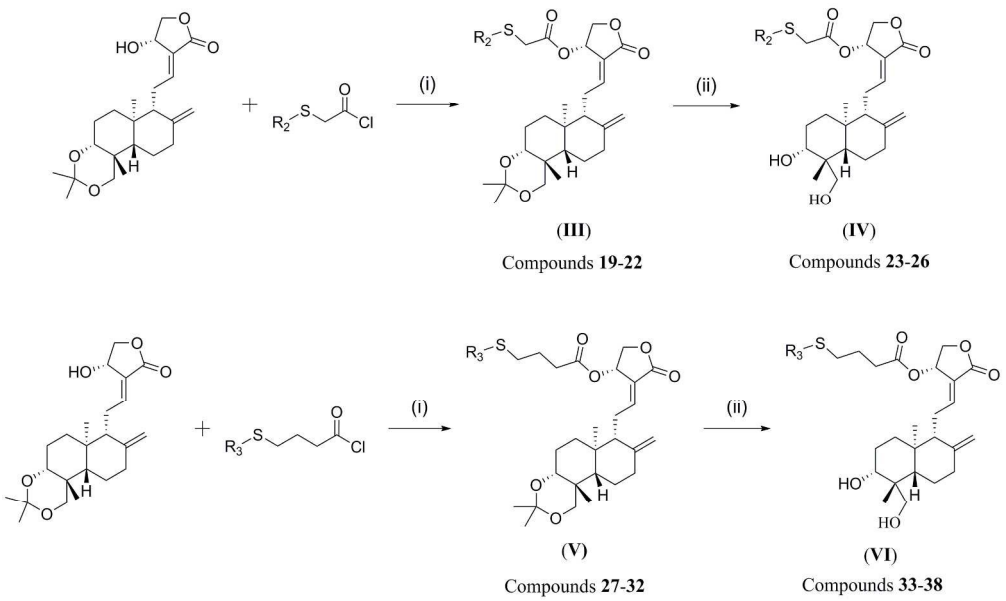

Scheme 2. Reagents and conditions: (i) Et3N, CH2Cl2, 0°C, 1 h; (ii) 70 % HOAc, rt, 45 min.

223x133mm (300 x 300 DPI)

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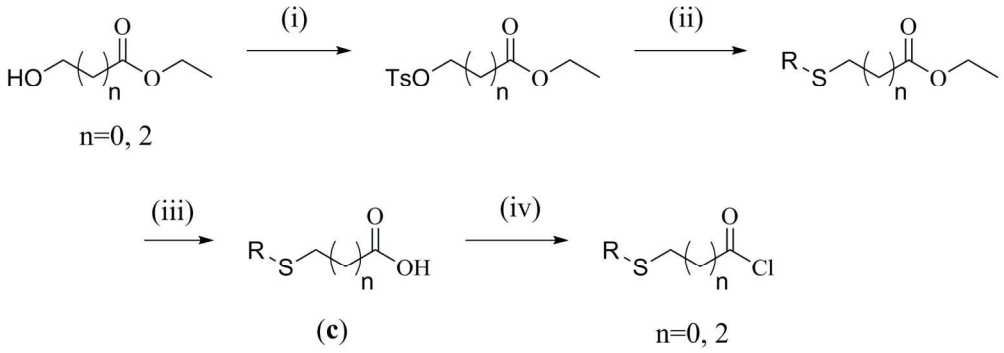

Scheme 3. Reagents and conditions: (i) TsCl/Et3N, 0 °C, 2 h; (ii) CH3ONa/MeOH; reflux; (iii) 5 % NaOH/EtOH, 40 °C, 2 h; (iv) SOCl2 or (COCl)2, reflux, 6 h.

147x52mm (300 x 300 DPI)

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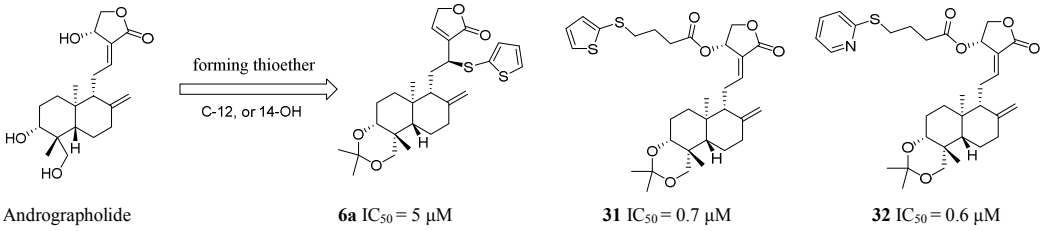
Table of content



Andrographolide **6a** IC_{50} = 5 μM **31** IC_{50} = 0.7 μM **32** IC_{50} = 0.6 μM

Novel thioether andrographolide derivatives were designed and synthesized, and some of them exhibited excellent anticancer activity.