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Synthesis of andrographolide derivatives and their TNF-α and IL-6 expression inhibitory activities

Jing Li,[a] Wenlong Huang,[a] Huibin Zhang,[a,*] Xinyang Wang[b] and Huiping Zhou[c]

[a]*Center of Drug Discovery, College of Pharmacy, China Pharmaceutical University, Nanjing 210009, China*
[b]*Medical College of Nantong University, Nantong 226000, China*
[c]*Department of Microbiology and Immunology, School of Medicine, Virginia Commonwealth University, Richmond, VA 23298, USA*

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Abstract—The synthesis of a series of andrographolide derivatives was described and their inhibitory effects on TNF-α and IL-6 secretion in mouse macrophages were also evaluated. Most of the tested compounds showed inhibitory effects, and the compounds with the structure of 12-hydroxy-14-dehydroandrographolide showed better inhibitory activity than the compounds with the structure of isoandrographolide.

Andrographolide **1** is a bicyclic diterpenoid lactone isolated from leaves of *Andrographis paniculata*,[1] which is used extensively in the traditional systems of Chinese medicine. Andrographolide was reported to possess a wide spectrum of biological activities including antibacterial, antiinflammatory, antimalarial, immunomodulation, antithrombotic, and hepatoprotective effect. It is mainly used to treat pediatric pneumonia and respiratory tract infection in clinic.[2–9]

TNF-α and IL-6 are two major pro-inflammatory mediators secreted by macrophages upon stimulation with microbial infections such as LPS. These two pro-inflammatory cytokines have a wide array of functions. For example, TNF-α can induce apoptosis and the secretion of cytokines such as IL-1, IL-6, and IL-10; it can also activate T cells and other inflammatory cells. However, an overabundance of TNF-α and IL-6 is correlated with the development of various diseases. It is reported that TNF-α inhibitors can be used to treat many diseases such as rheumatoid arthritis, diabetes, sepsis, Alzheimer's disease, tumor, and obesity,[10–13] while the IL-6 inhibitors can be used in Alzheimer's disease, psychiatric disorders, cancer, diabetes, and depression.[14–16]

Recently, it has been reported that andrographolide can decrease the expression of TNF-α, IL-1, IL-6, IL-8, IL-12 at mRNA levels in a concentration-dependent manner in murine macrophages via suppression of the ERK1/2, PI3K-AKt signaling pathway.[17–19] However, the effect of the andrographolide derivatives on LPS-induced TNF-α and IL-6 expression has not been examined so far. Herein, in this paper, we report the synthesis of a series of andrographolide derivatives and their potential inhibitory effects on LPS-induced TNF-α and IL-6 release in mouse macrophages.

The 14-hydroxy of andrographolide is chemically unstable, it can be rearranged to form isoandrographolide, which is much more stable than andrographolide. Isoandrographolide has similar biological activities to andrographolide, such as antiinflammatory and anticancer properties.[20] In addition, the 14-hydroxy can also be rearranged to form 12-hydroxy-14-dehydroandrographolide easily. It has been reported that 12-hydroxy-14-dehydroandrographolide possessed similar biological activity of anticancer as andrographolide.[21] Therefore, isoandrographolide and 12-hydroxy-14-dehydroandrographolide were chosen as the lead generation here, and their derivatives were synthesized. The synthesis of these derivatives is summarized in Schemes 1–3. All the prepared compounds were characterized by spectroscopic tools.[22]

Andrographolide (**1**) was treated with cons. hydrochloric acid at room temperature to afford isoandrographolide (**2**) in 70% yield (Scheme 1), **2** was followed by

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* Corresponding author. Tel.: +86 25 85230904; e-mail: zhanghb80@163.com

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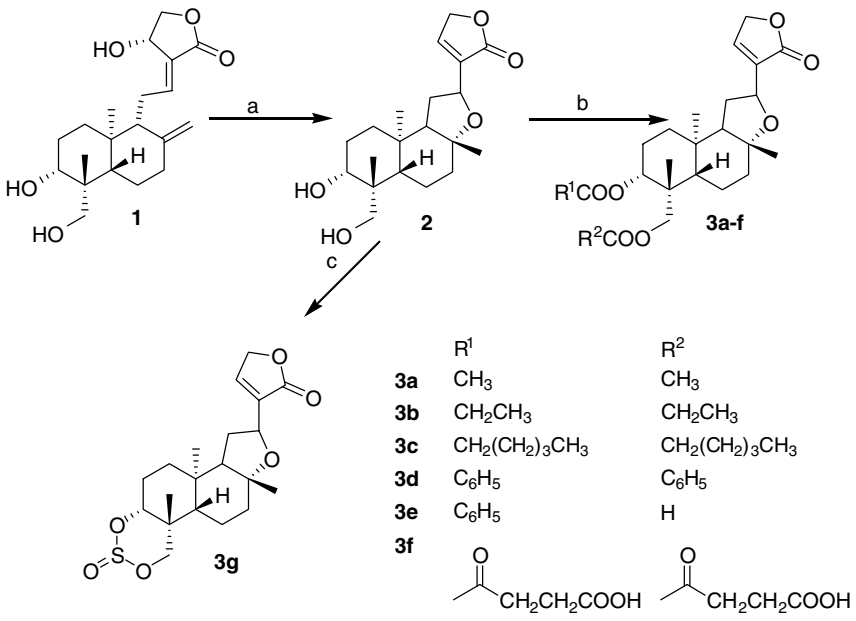
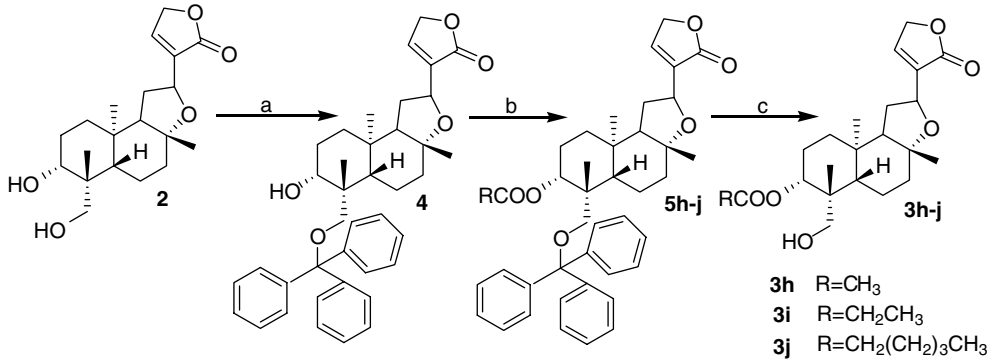
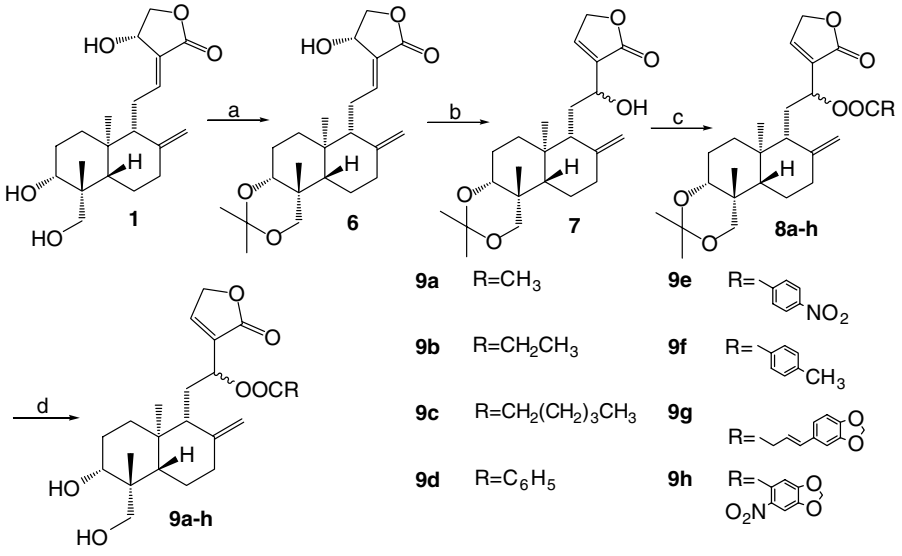


Scheme 1. Reagents and conditions: (a) cons. HCl, rt; (b) for **3a**–**c**, **3f**: $(R^1CO)_2O$, CH_2Cl_2, reflux, for **3d** and **3e**: R^1COCl, CH_2Cl_2, reflux; (c) **3g**: $SOCl_2$, reflux.



Scheme 2. Reagents and conditions: (a) TrCl, *N*-methylmorpholine, CH_2Cl_2, rt; (b) $(R_1CO)_2O$, DMAP, CH_2Cl_2, rt; (c) HCOOH, CH_2Cl_2, rt.



Scheme 3. Reagents and conditions: (a) $(CH_3)_2C(OCH_3)_2$, PPTS, CH_2Cl_2, reflux; (b) PDC, CH_2Cl_2, reflux; (c) for **8a**–**c**: $(RCO)_2O$, CH_2Cl_2, rt, for **8d**: RCOCl, CH_2Cl_2, rt, for **8e**–**h**: RCOOH, DCC, DMAP, CH_2Cl_2, rt; (d) CH_3COOH/H_2O, rt.

esterification with different acid anhydrides, acyl chlorides, or $SOCl_2$ to obtain **3a**–**g** in good yields.

Treatment of **2** with triphenylchloromethane in the presence of *N*-methylmorpholine in CH_2Cl_2 at room temperature afforded trityl ether **4** in 85% yield (Scheme 2). Compound **4** was reacted with acetic anhydride, propionic anhydride, or hexanoic anhydride in the presence of 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 at room temperature to obtain corresponding esters **5h**–**j** in almost quantitative yield. Esters **5h**–**j** were further converted to **3h**–**j** in 75% by hydrolysis of the trityl ether with formic acid in CH_2Cl_2. To prepare **5h**–**j**, it would take much long time if DMAP is not added whether the reaction was carried out at room temperature or at refluxing temperature. The steric hindrance of the trityl ether at C-19 was considered as a major factor.

Reaction of **1** with 2,2-dimethoxypropane in the presence of pyridinium *p*-toluenesulfonate (PPTs) in CH_2Cl_2 at 40 °C gave compound **6** in high yield (Scheme 3). Treatment of **6** with pyridinium dichromate (PDC) in refluxing CH_2Cl_2 obtained **7** in 50% yield. Compound **7** was followed by esterification with acid anhydrides, acyl chlorides or with carboxylic acids in the presence of dicyclohexylcarbodiimide (DCC) and DMAP to give compounds **8a**–**h**. For example, a mixture of **7**, 4-nitrobenzoic acid, DCC, and DMAP in CH_2Cl_2 was stirred overnight at room temperature to obtain **8a** in good yield. Compounds **8a**–**h** were treated with aq acetic acid (acetic acid/water = 7:3) at room temperature for 30 min and afforded **9a**–**h** in almost quantitative yield.

The effects of andrographolide and its derivatives on LPS-induced TNF-α and IL-6 expression in mouse J774A.1 cells were examined using ELISA. Cells were treated with 20 μM of andrographolide, its derivatives, or vehicle control for 24 h. At the end of the treatment, the culture media were collected and centrifuged at 14,000 rpm for 5 min. TNF-α and IL-6 levels in the media were determined by ELISA using mouse TNF-α and mouse IL-6 ELISA Max™ Set Deluxe Kits (Biolegend). The total protein concentrations of the viable cell pellets were determined using Bio-Rad protein assay reagents. Total amounts of the TNF-α and IL-6 in the media were normalized to the total protein amounts of the viable cell pellets.

The results (Tables 1 and 2) showed that andrographolide and its derivatives inhibited LPS-induced TNF-α and IL-6 expression to various degrees in mouse macrophages. Among these compounds, **3c**, **3e**, and **9f** with the % inhibition as 43.75, 36.45, and 41.60, respectively, were more potent than andrographolide (62.54%) in inhibiting LPS-induced TNF-α expression; **9a**, **9d**, **9e**, **9f**, and **9h** with the % inhibition as 44.73, 37.53, 29.21, 32.1, and 44.64, respectively, were more potent than andrographolide (56%) in inhibiting LPS-induced IL-6 expression. For the isoandrographolide derivatives **3a**–**j**, **3c** having hexanoyl moieties at C-3 and C-19 showed better inhibitory activity in both TNF-α and IL-6 expression compared to its mono-hexanoyl derivative **3j**, indicating that both hydroxyls in isoandrographolide need to be converted into esters for potent activity. But for **3d** and **3e**, with di-benzoyl groups at C-3 and C-19 in **3d** and mono-benzoyl group in **3e**, the contradictive results were presented. The hydrophilic analog **3f**, in which carboxyl groups were introduced at C-3 and C-19, displayed almost no inhibitory acitivity in LPS-induced TNF-α and IL-6 expression, indicating that the improvement of aqueous solubility of isoandrographolide would lead to a decline in inhibitory effects, and the introduction of cyclosulfurous substituent (**3g**) is deleterious to the inhibitory activity of isoandrographolide. For the derivatives of 12-hydroxy-14-dehydroandrographolide **9a**–**h**, the compounds having aryl moiety at 12-C (e.g., **9d**, **9e**, **9f**) showed better inhibitory

Table 1. Mouse macrophage TNF-α inhibition assay data for andrographolide and its derivatives

	Normal (%)	LPS-induced TNF-α (%)
Control	100	100
Andrographolide	63.94 ± 2.60	62.54 ± 2.00
3a	80.03 ± 2.13	99.43 ± 3.25
3b	82.78 ± 2.21	114.69 ± 2.33
3c	35.66 ± 2.04**	43.75 ± 2.63**
3d	203.54 ± 2.68	293.69 ± 2.77
3e	28.54 ± 1.92**	36.45 ± 2.23**
3f	81.74 ± 2.09	128.62 ± 2.89
3g	61.82 ± 2.08	230.56 ± 2.96
3j	44.40 ± 3.23**	105.88 ± 2.37
9a	106.65 ± 2.05	62.68 ± 2.55
9b	69.97 ± 2.21	96.87 ± 3.13
9c	79.62 ± 2.51	75.95 ± 2.81
9d	92.88 ± 2.22	60.8 ± 2.34
9e	65.78 ± 2.86	59.96 ± 2.74
9f	152.29 ± 2.52	41.60 ± 2.50**
9g	148.86 ± 2.47	61.03 ± 1.82
9h	115.10 ± 3.90	94.36 ± 2.80

Each value represents mean ± SD of four determinations. $^{**}P < 0.01$ compared to andrographolide.

Table 2. Mouse macrophage IL-6 inhibition assay data for andrographolide and its derivatives

	Normal (%)	LPS-induced IL-6 (%)
Control	100	100
Andrographolide	49.21 ± 3.67	56 ± 2.20
3a	72.87 ± 2.52	150.15 ± 3.13
3b	60.53 ± 2.25	110.25 ± 2.51
3c	31.39 ± 3.67**	85.25 ± 1.57
3d	48.20 ± 1.92	107.19 ± 3.52
3e	27.67 ± 2.38**	85.71 ± 2.81
3f	55.02 ± 2.69	114.12 ± 2.77
3g	79.66 ± 2.12	207.64 ± 3.09
3j	69.80 ± 3.11	101.81 ± 2.50
9a	95.10 ± 2.95	44.73 ± 2.11**
9b	82.56 ± 2.14	74.67 ± 2.54
9c	82.93 ± 2.67	46.61 ± 2.15**
9d	81.97 ± 3.51	37.53 ± 2.65**
9e	58.56 ± 2.80	29.21 ± 2.04**
9f	127.68 ± 2.83	32.1 ± 2.93**
9g	96.05 ± 1.60	56.87 ± 2.38
9h	70.14 ± 2.36	44.64 ± 2.22**

Each value represents mean ± SD of four determinations. $^{**}P < 0.01$ compared to andrographolide.

activities than the compounds having alkyl moiety at 12-C (e.g., **9a**, **9b**, **9c**) in both TNF-α and IL-6 expression, suggesting that the electron-withdrawing group at 12-C might be good for potent activity of 12-hydroxy-14-dehydroandrographolide. However, the underlying mechanisms by which andrographolide and its derivatives inhibited LPS-induced TNF-α and IL-6 expression remain unknown and are the focus of our current research.

In conclusion, a series of andrographolide derivatives have been synthesized and their inhibitory effects on LPS-induced TNF-α and IL-6 expression in mouse macrophages have been evaluated. The data analysis indicated no clear SAR for these compounds, but the compounds with the structure of 12-hydroxyl-14-dehydroandrographolide showed better inhibitory activity than the compounds having the structure of isoandrographolide, suggesting the structure of 12-hydroxy-14-dehydroandrographolide might be a good generation for further optimization.

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22. Analytical data for **3e**: mp: 185–186 °C; IR (KBr, cm^{-1}): 3554, 3105, 2938, 1755, 1708, 1470, 1295, 1124; ^1H NMR($CDCl_3$, 300 MHz) δ: 7.97 (d, 2H, Ph), 7.65–7.45 (t, 3H, Ph), 7.27 (s, 1H, 14-H), 4.83 (s, 2H, 15-H), 4.70–4.66 (m, 3H, 12-H, 3-H, 19-H), 4.44 (d, 1H, 19-H), 3.40 (br, 1H, 3-OH), 1.29 (s, 3H, 17-CH_3), 1.13 (s, 3H, 18-CH_3), 1.04 (s, 3H, 20-CH_3); EIMS: 477.2 $[M+Na]^+$. Analytical data for **9e**: mp: 119–122 °C; IR (KBr, cm^{-1}): 3409, 3111, 2950, 2873, 1729, 1625, 1545, 1344, 1279, 897; ^1H NMR($CDCl_3$, 300 MHz) δ: 9.25–9.14 (m, 4H, Ph), 7.50 (s, 1H, 14-H), 6.00 (t, 1H, J = 6.3 Hz, 12-H), 4.98 (s, 1H, 17-H), 4.91 (s, 2H, 15-H), 4.86 (s, 1H, 17-H), 4.18 (t, 1H, J = 7.2 Hz, 3-H), 3.43 (t, 1H, J = 9.9 Hz, 19-H), 3.30 (t, 1H, J = 9.9 Hz, 19-H), 1.23 (s, 3H, 18-CH_3), 0.68 (s, 3H, 20-CH_3); EIMS: 500.2 $[M+H]^+$. Analytical data for **9f**: mp: 176–178 °C; IR (KBr, cm^{-1}): 3472, 2938, 2866, 1744, 1700, 1636, 1445, 1200, 1037, 828; ^1H NMR($CDCl_3$, 300 MHz) δ: 7.85 (d, 2H, J = 7.2 Hz, Ph), 7.41 (m, 3H, Ph, 14-H), 5.92 (t, 1H, J = 5.7 Hz, 12-H), 4.93 (s, 1H, 17-H), 4.84 (s, 3H, 15-H, 17-H), 4.18 (d, 1H, J = 11.4 Hz, 3-H), 3.49 (t, 1H, J = 7.5 Hz, 19-H), 3.31 (d, 1H, J = 10.8 Hz, 19-H), 2.38 (s, 3H, Ph–CH_3), 1.23 (s, 3H, 18-CH_3), 0.65 (s, 3H, 20-CH_3); EIMS: 491.2 $[M+Na]^+$.