Synthesis and Preliminary Antihyperlipidaemic Activities Evaluation of Andrographolide Derivatives

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Abstracts: Recent studies indicated that andrographolide was a potential antihyperlipidaemic therapeutic agent. In the paper, the synthesis of a series of andrographolide derivatives was described and their antihyperlipidaemic activities were evaluated *in vivo*. As compared with TG, TC, HDL-C and LDL-C concentrations, some of the derivatives exhibited better antihyperlipidaemic effects than positive control atromide. Therein, compound **6i**, which was the most potent compound, could serve as a new lead for further development of antihyperlipidaemic agents.

Keywords: Andrographolide, antihyperlipidaemic activities, derivatives, synthesis.

INTRODUCTION

Cholesterol plays a major role in the assembly of membranes and performs other important biological functions in human heart health. However, excess plasma cholesterol leads to the development of hyperlipidemia and atherosclerotic cardiovascular disease such as coronary heart disease and stroke, which are among the leading causes of death in many countries.The adverse effects on treating hyperlipidemia urged the search of new antihyperlipidaemic agents and targets [1-3]. Natural products are important sources of new drugs and leads with antihyperlipidaemic effects such as Artichoke, Green tea, Guggul, Korean ginseng and Fenugreek, inspiring and motivating researchers to look for new drugs from plants and natural sources [4, 5].

Andrographolide (Andro, **1**) is a bicyclic diterpenoid lactone isolated as the major active constituent from leaves of *Andrographis paniculata*, a traditional herbal medicine used for centuries in China, India for the treatment of various diseases such as respiratory infection, acute gastroenteritis, laryngitis, bacterial dysentery and diarrhea [6]. Andro was known to possess a wide spectrum of bioactivities such as anti-inflammatory, antibacterial, immunomodulatory and etc [7].

Andro exerts its anti-apoptotic potential via activation of the Akt-BAD pathway in HUVECs and thus may represent a candidate of therapeutic agent for atherosclerosis, in which the NF-κB/IκB regulatory system plays a key role [8, 9]. *In vivo* study showed that after being administered orally at 30 to 500 mg/kg·d dose of 3,19-diacetyl-14-(2'-acetyl-3'-acetamido-3'-phenyl)propionyl andrographolide for 6 days, percentage reduction of the plasma triglyceride (TG) of male Swiss albino mice were 42% (100 mg/kg·d) and 62% (500 mg/kg·d), respectively. And for 14-cinnamoyl-3,19-dihydroxy-8,17- epoxy andrographolide, percentage reduction of the plasma triglyceride (TG) of male Swiss albino mice were 42% (100 mg/kg·d), 52% (250 mg/kg·d) and 62% (500 mg/kg·d), respectively [10, 11]. It indicated that Andro derivatives benefit the antihyperlipidaemic treatment and could be regarded as an excellent lead compound.

In our previous studies, isoandrographolide (Isoandro, **2**), which is much more stable than Andro while has similar biological activities to Andro, such as anti-inflammatory, anticancer and anti-HIV properties [12-14], was chosen as the lead generation and the derivatives were synthesized. Isoandro and the derivatives have showed the inhibitory activities on LPS-induced TNF-α, IL-6 and COX-2 expression in mouse macrophages, therein, 19-O-triphenylmethyl isoandrographolide displayed the best activity, suggesting that it could be taken as an optimized lead generation for further biological activities studies [13]. Clofibric acid (4-chlorophenyloxyisobutyric acid) is the active metabolite of the blood lipid regulators clofibrate, etofibrate, and etofyllin-clofibrate, which can lower elevated serum lipids by reducing the very lowdensity lipoprotein fraction [15]. In this study, we disclose the procedures for the synthesis of a series of derivatives by the combination of 19-O-triphenylmethyl isoandrographolide and substituted phenyloxyisobutyric acid with the antihyperlipidaemic activities evaluated *in vivo*.

RESULTS AND DISCUSSION

Synthesis

The synthesis of these derivatives is summarized in Scheme **1**. Substituted phenol **3** was treated with sodium hydroxide in acetone at room temperature and then chloroform was added to afford substituted phenyloxyisobutyric acid **4** in 67% yield. As our previous studies [13], Andro **1** was treated with cons. hydrochloric acid at room temperature to afford Isoandro **2** in 70% yield. Treatment of **2** with

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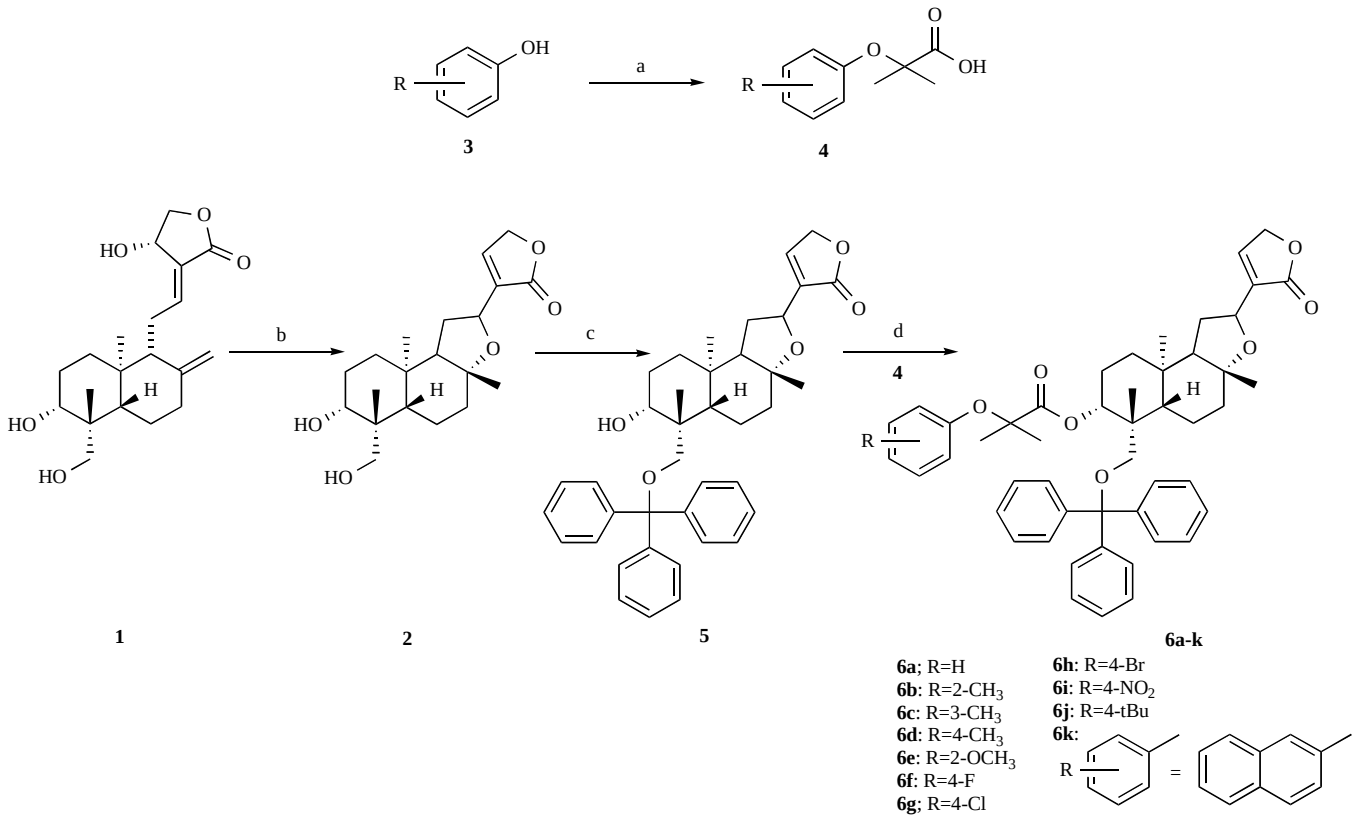

Scheme 1. a) 1) NaOH/acetone; 2) 1 mol/L $CHCl_3$; 3) dilute HCl; b) Cons. HCl; c) TrCl, N-Methylmorpholine, CH_2Cl_2, rt; d) EDC·HCl, DMAP, CH_2Cl_2, rt.

triphenylchloromethane (TrCl) in the presence of *N*-methylmorpholine in CH_2Cl_2 at room temperature afforded trityl ether **5** in 80% yield. Compound **5** was reacted with different substituted phenyloxyisobutyric acid **4** and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) in the presence of DMAP in CH_2Cl_2 at room temperature afforded **6a-k**.

In vivo Activities

Andro and its derivatives were tested for the antihyperlipidaemic activities with Male SD rats which were intragastrically given fat emulsion to afford hyperlipidemia and then given the corresponding drug (160 mg/kg·d) for 7 days. Blood samples were collected via carotid artery, centrifuged and analyzed for serum total triglyceride (TG), cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), respectively, on an Olympus AU 400 automatic biochemistry analyzer. The results of TG, TC, HDL-C and LDL-C of Andro and its derivatives were summarized in Table **1**. Serum TC and LDL-C were significantly lowered by Andro and most of its derivatives compared with the fat control group. The positive control atromide also lowered serum TC and LDL-C significantly. Serum HDL-C was increased by some Andro derivatives.

As it is known, elevated levels of serum TG and LDL-C, accompanied by reduced HDL-C levels, are often associated with an increased risk of coronary heart disease. In particular, research shows that LDL to be the most dangerous among the serum lipids, and the oxidation of LDL leads to its increased penetration of arterial walls [16]. For convenience, the percentage changes in serum TG, TC, HDL-C and LDC-C of andro and its derivatives compared with the fat control group were obtained and summarized in Table **1**. By comparison of **6a**, **6b**, **6c** and **6d**, which significantly lowered the serum TC by 7.85%, 5.24%, -3.14% and 17.28%, and serum LDL-C by 5.36%, 12.50%, 8.93% and 16.07%, respectively, it was very obvious that **6d** exhibited best lipid-lowering activities. It indicates that substitution at position 4 of the benzene ring was better than position 2 and 3. By contrast with **6d**, **6g**, **6h**, **6i** and **6j**, which significantly lowered the serum TC by 17.28%, 2.62%, 6.81%, 14.66% and 5.24%, and serum LDL-C by 16.07%, 0.00%, 8.93%, 28.57% and 10.71%, respectively, It is quite clear that **6i** exhibited best lipid-lowering activities and the preliminary activity sequence decressed in the following order: **6i**>**6d**>**6j**>**6h**>**6g**. It indicates that electron withdrawing substituents (such as nitro group) of position 4 of the benzene ring were beneficial to increasing lipid-lowering activities. As to the halogen substituents of position 4 of the benzene ring, it decressed in the following order: 4-Br>4-Cl>4-F.

It was very obvious that compounds **1**, **6d** and **6i** significantly lowered the serum TC by 19.90%, 17.28%, and 14.66% and serum LDL-C by 28.57%, 16.07% and 28.57%, respectively, which were already close to normal control group and considered a beneficiary effect in the treatment of dyslipidemia condition. These groups were comparable with standard drug atromide at the same dose of 160 mg/kg body weight decreased the levels of serum TC by 17.28% and serum LDL-C by 14.29%. The lipid-lowering effect of other

Table 1. The Assay Data of Antihyperlipidaemic Activities for Andro and its Derivatives

Compd.[a]	TG[e] (%[f])	TC[e](%[f])	HDL-C[e](%[f])	LDL-C[e] (%[f])
1	0.47±0.09(+6.82%)	1.53±0.24**(-19.90%)	1.03±0.14(-13.45%)	0.40±0.12*(-28.57%)
2	0.47±0.04*(+6.82%)	1.74±0.27**(-8.90%)	1.20±0.18(+0.84%)	0.42±0.10(-25.00%)
5	0.49±0.10(+11.36%)	1.67±0.26**(-12.57%)	1.08±0.20(-9.24%)	0.50±0.10**(-10.71%)
6a	0.44±0.08(0)	1.76±0.23**(-7.85%)	1.14±0.14(-4.20%)	0.53±0.12(-5.36%)
6b	0.39±0.05**(-11.36%)	1.81±0.10(-5.24%)	1.19±0.09(0)	0.49±0.09(-12.50%)
6c	0.39±0.07(-11.36%)	1.97±0.09(+3.14%)	1.34±0.10(+12.61%)	0.51±0.09(-8.93%)
6d	0.45±0.07(+2.27%)	1.58±0.22*(-17.28%)	0.97±0.17(-18.49%)	0.47±0.12*(-16.07%)
6e	0.43±0.05*(-2.27%)	1.78±0.25**(-6.81%)	1.20±0.15(+0.84%)	0.47±0.10(-16.07%)
6f	0.47±0.04**(+6.82%)	2.13±0.32**(+11.52%)	1.29±0.21*(+8.40%)	0.68±0.09(+21.43%)
6g	0.52±0.11(+18.18%)	1.86±0.15(-2.62%)	1.19±0.09(0)	0.56±0.08(0)
6h	0.42±0.04**(-4.55%)	1.78±0.14(-6.81%)	1.17±0.08(-1.68%)	0.51±0.08(-8.93%)
6i	0.42±0.05*(-4.55%)	1.63±0.17(-14.66%)	1.08±0.10(-9.24%)	0.40±0.07*(-28.57%)
6j	0.48±0.06(+9.09%)	1.81±0.26**(-5.24%)	1.18±0.17(-0.84%)	0.50±0.10**(-10.71%)
6k	0.44±0.05*(0)	1.87±0.27**(-2.09%)	1.28±0.19(+7.56%)	0.45±0.08(-19.64%)
NC[b]	0.50±0.13* (+13.64%)	1.39±0.09* (-27.23%)	1.01±0.10(-15.13%)	0.37±0.06* (-33.93%)
Atromide[c]	0.54±0.08(+22.73%)	1.58±0.20*(-17.28%)	1.05±0.13(-11.76%)	0.48±0.09(-14.29%)
FC[d]	0.44±0.06	1.91±0.19	1.19±0.16	0.56±0.11

[a]Each value represents means ± SD from 8 rats. [b]Normal control group. [c]Positive control group.[d]Fat control group.
[e]mmol/L serum. [f]The percentage changes in serum TG, TC, HDL-C and LDL-C of andro and its derivatives compared with the fat control group. Calculation formula: $(V_n-V_{FC})/V_{FC}\times100\%$ (V_n stands for TG, TC, HDL-C or LDL-C value of one of the compounds,). $^{*}P<0.05$, $^{**}P<0.01$ vs **FC** (assessed by one-way ANOVA with Bonferroni's Multiple Comparison Test).

Andro derivatives was modest when compared to **1**, **6d** and **6i** derivatives.

CONCLUSION

In summary, a series of Andro derivatives have been synthesized and evaluated for antihyperlipidaemic activities *in vivo*. Some of the derivatives exhibited better antihyperlipidaemic effects than or equal to positive control atromide with the aid of analyzing the concentrations of TG, TC, HDL-C and LDL-C, respectively; especially compound **6i** was the most potent compound, which raised a challenge for lead optimization based on the structure of 19-O-triphenylmethyl isoandrographolide and could serve as a new lead for further development of antihyperlipidaemic agents. A closure look into the structure activity relationship indicates that the substitution at position 4 and with electron withdrawing is benefit to the antihyperlipidaemic activities. Further studies on biological evaluation, structure activity relationship and mechanism of this new class of compounds are currently in progress and the results will be reported in due course.

EXPERIMENTAL SECTION

General

Reactions and the resulted products were monitored by thin-layer chromatography (TLC) on pre-coated silica gel F254 plates with separated compounds visualized by iodine vapour. Melting points (uncorrected) were determined on a RY-1 MP apparatus (Tianjin Analytical Apparatus Corp, Tianjin, China). Mass spectra were obtained on a HP1100LC/MSD mass spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Brucker ACF-300 MHz spectrometer with TMS as internal standard. IR spectra were detected on a Nicolet Impact 410 IR spectrometer.

Chemistry

Andro (**1** >98 %) was purchased from Zelang Medical Technology Co., Ltd (Nanjing, China) and was further purified by recrystallization from ethanol before use. All other reagents were of AR grade and used without any further purification.

Preparation of Substituted Phenyloxyisobutyric Acid (4)

To a solution of sodium hydroxide (4.0 g, 100.0 mmol) in acetone (20 ml) at room temperature was added different substituted phenol **3** (20.0 mmol, dissolved in 20 ml acetone) dropwise. The reaction mixture was stirred under refluxing for 0.5 h, added chloroform (2.0 ml, 24.0 mmol, dissolved in 8 ml acetone) dropwise and stirred under refluxing for 3.5 h. Then, the whole solution was evaporated to obtain a residue, diluted with water (50 ml) and washed with dichloromethane

(25 ml×3). The water layer was neutralized with 5 mol/L HCl and then filtered. The filter cake was washed with water, dried over infrared lamp and purified by recrystallization with ethyl acetate/petroleum ether (EtOAc/PE).

Preparation of Isoandrographolide (2)

To Andro **1** (5.0 g, 14.3 mmol) was added concentrated hydrochloric acid (81 ml, 810 mmol), and the reaction mixture was stirred at room temperature for 24 h. Then, the whole solution was diluted with ice water (200 ml) and then filtered. The filter cake was washed with ice water, dissolved in dichloromethane(200 ml), washed with saturated sodium bicarbonate (75 ml×3) and saturated brine (75 ml×3), dried over Na_2SO_4 and evaporated to obtain a residue purified by recrystallization with ethyl acetate. White powder; 70 % yield; mp: 201-202 ℃ (literature values mp: 198-200 °C) [17].

Preparation of 19-O-tritylisoandrographolide (5)

To a solution of **2** (2.1 g, 6.0 mmol) and N-Methylmorpholine in anhydrous dichloromethane (25 ml) at room temperature was added triphenylmethyl chloride (1.70 g, 7.8 mmol, dissolved in 10 ml dichloromethane) dropwise, and the reaction mixture was stirred for 12 h. Then, the whole solution was diluted with dichloromethane (50 ml), washed with saturated brine (30 ml×3), dried over Na_2SO_4 and evaporated to obtain a residue purified by recrystallization with EtOAc/PE. White powder; 79.5 % yield; mp 208 °C (dec.). MS (ESI) m/z: 615.2 $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3546, 3061, 2927, 2844, 1754, 1741, 1734, 1448, 1207, 933, 705; ^{1}H-NMR ($CDCl_3$, 300 MHz, ppm) δ: 7.51-7.22 (m, 16H, ArH, H-14), 4.78 (s, 2H, H-15), 4.57 (t, 1H, *J*=7.2 Hz, H-12), 4.10 (d, 1H, *J*=8.2 Hz, H-3), 3.46 (d, 1H, *J*=9.6 Hz, H-19a), 3.21 (m, 2H, H-19b, 3-OH), 2.30 (dd, 1H, *J*=7.2 Hz, H-11a), 2.05 (m, 1H, H-1), 1.92 (m, 1H, H-11b), 1.65-1.27 (several protons), 1.14 (s, 3H, H-17), 1.03 (t, 3H, H-18), 0.93 (s, 3H, H-20); ^{13}C-NMR ($CDCl_3$, 75 MHz, ppm) δ: 172.5, 143.4, 143.1, 138.2, 128.4, 128.0, 127.2, 87.7, 82.6, 80.6, 77.5, 77.0, 76.6, 73.0, 70.5, 64.8, 58.4, 52.7, 42.4, 39.3, 35.8, 35.6, 33.0, 31.5, 27.4, 23.3, 18.2, 16.1.

General Procedure for the Preparation of 6a-k

To a solution of **5** (1.19 g, 2.0 mmol), different substituted phenyloxyisobutyric acid **4** (2.4 mmol), DMAP (catalytic amount) in anhydrous dichloromethane (25 ml) at room temperature was added EDC·HCl (0.58 g, 3.0 mmol, dissolved in 15 ml anhydrous dichloromethane) dropwise, and the reaction mixture was stirred until the end of the reaction. Then, the whole solution was diluted with dichloromethane (20 ml), washed with water (30 ml×3) and saturated brine (30 ml×3), dried over Na_2SO_4 and evaporated to obtain a residue purified by column chromatography (EtOAc/PE).

3-O-phenyloxyisobutyryl-19-O-tritylisoandrographolide (6a)

White powder; 58.3 % yield; mp 172-173 °C. MS (ESI) m/z: 777.4 $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3433.56, 3057.61, 2975.31, 2950.98, 2918.40, 1759.97,1728.13, 1598.64, 1491.35, 1448.8, 1156.66, 1074.65, 1001.04, 759.16, 707.09; ^{1}H-NMR ($CDCl_3$, 300 MHz, ppm) δ: 7.43-7.18 (m, 18H, Ph-H, H-14, H-3', 5'), 6.98 (t, 1H, *J*=7.2 Hz, H-4'), 6.70 (d, 2H, *J*=7.5 Hz, H-2', 6'), 4.77 (s, 2H, H-15), 4.57 (m, 1H, H-12), 4.50 (d, 1H, *J*=8.2 Hz, H-3), 3.34 (d, 1H, *J*=9.9 Hz, H-19a), 3.18 (d, 1H, *J*=9.9 Hz, H-19b), 2.30 (dd, 1H, *J*=8.1 Hz, H-11a), 2.08 (dd, 1H, *J*=8.1 Hz, H-11b), 1.98-1.61 (several protons), 1.53 (s, 3H, -OCOCH($C\underline{H}_3$)a), 1.50 (s, 3H, -OCOCH($C\underline{H}_3$)b), 1.46-0.88 (several protons), 1.14 (s, 3H, H-17), 1.04 (s, 3H, H-18), 0.42 (s, 3H, H-20).

3-O-(2-methylphenyloxyisobutyryl)-19-O-tritylisoandrographolide (6b)

White powder; 77.0 % yield; mp 176-177 °C. MS (ESI) m/z: 791.4 $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3496.11, 2922.82, 2868.41, 1757.87, 1739.07, 1637.56, 1599.55, 1491.92, 1449.09, 1227.2,1147.69, 1049.73, 748.99; ^{1}H-NMR ($CDCl_3$, 300 MHz, ppm) δ: 7.40-7.11 (m, 16H, Ph-H, H-14), 7.01 (t, 1H, *J*=7.6 Hz, H-3'), 6.88 (t, 2H, *J*=7.6 Hz, 7.9 Hz, H-4', 6'), 6.61 (d, 1H, *J*=7.9 Hz, H-5'), 4.77 (s, 2H, H-15), 4.58 (s, 1H, H-12), 4.55 (s, 1H, , H-3), 3.38 (d, 1H, *J*=9.7 Hz, H-19a), 3.20 (d, 1H, *J*=9.7 Hz, H-19b), 2.30 (dd, 1H, *J*=8.1 Hz, H-11a), 2.05 (dd, 1H, *J*=8.1 Hz, H-11b), 2.02 (s, 3H, Ph-C$\underline{H}_3$), 1.95-1.60 (several protons), 1.52 (s, 3H, -OCOCH($C\underline{H}_3$)a), 1.49 (s, 3H, -OCOCH($C\underline{H}_3$)b), 1.20-0.89 (several protons), 1.25 (s, 3H, H-17), 1.08 (s, 3H, H-18), 0.43 (s, 3H, H-20).

3-O-(3-methylphenyloxyisobutyryl)-19-O-tritylisoandrographolide (6c)

White powder; 90.4 % yield; mp 177-178°C. MS (ESI) m/z: 791.4 $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3473.08, 3055.57, 3030.02, 1748.91, 1724.51, 1637.99, 1606.92, 1585.87, 1488.73, 1448.44, 1369.64, 1174.67, 1092.83, 1074.21, 831.10, 768.99, 711.98; ^{1}H-NMR ($CDCl_3$, 300 MHz, ppm) δ: 7.40-7.16 (m, 16H, Ph-H, H-14), 7.07 (t, 1H, *J*=7.7 Hz, 7.9 Hz, H-3'), 6.80 (d, 2H, *J*=7.4 Hz, H-2', 4'), 6.49 (d, 1H, *J*=7.4 Hz, H-6'), 4.77 (s, 2H, H-15), 4.56 (m, 1H, H-12), 4.50 (s, 1H, H-3), 3.36 (d, 1H, *J*=9.7 Hz, H-19a), 3.19 (d, 1H, *J*=9.7 Hz, H-19b), 2.26 (s, 3H, Ph-C$\underline{H}_3$), 2.08 (dd, 1H, *J*=7.8 Hz, H-11a), 1.90 (dd, 1H, *J*=7.8 Hz, H-11b), 1.68-1.49 (several protons), 1.51 (s, 3H, -OCOCH($C\underline{H}_3$)a), 1.49 (s, 3H, -OCOCH($C\underline{H}_3$)b), 1.25 (s, 3H, H-17), 1.08 (s, 3H, H-18), 0.42 (s, 3H, H-20).

3-O-(4-methylphenyloxyisobutyryl)-19-O-tritylisoandrographolide (6d)

White powder; 62.4 % yield; mp 180-181 °C. MS (ESI) m/z: 791.4 $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3473.69, 3087.09, 3055.44, 3030.02, 2991.28, 2956.75, 2923.83, 2898.05, 1748.68, 1724.47, 1606.01, 1585.61, 1488.66, 1448.41, 1145.79, 1135.13, 1092.85, 831.13; ^{1}H-NMR ($CDCl_3$, 300 MHz, ppm) δ: 7.42-7.15 (m, 16H, Ph-H, H-14), 6.81 (d, 2H, *J*=7.2 Hz, H-3', 5'), 6.63 (d, 2H, *J*=7.2 Hz, H-2', 6'), 4.80 (s, 2H, H-15), 4.66 (m, 1H, H-12), 4.60 (s, 1H, H-3), 3.35 (d, 1H, *J*=9.6 Hz, H-19a), 3.21 (d, 1H, *J*=9.6 Hz, H-19b), 2.45 (dd, 1H, *J*=7.8 Hz, H-11a), 2.29 (s, 3H, Ph-C$\underline{H}_3$), 2.21 (dd, 1H, *J*=7.8 Hz, H-11b), 2.11-1.03 (several protons), 1.62 (s, 3H, -OCOCH($C\underline{H}_3$)a), 1.60 (s, 3H, -OCOCH($C\underline{H}_3$)b), 1.10 (s, 3H, H-17), 0.96 (s, 3H, H-18), 0.89 (s, 3H, H-20).

3-O-(2-methoxylphenyloxyisobutyryl)-19-O-tritylisoandrographolide (6e)

White powder; 81.3 % yield; mp 186-187 °C. MS (ESI) m/z: 807.4 $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3462.39, 2982.06, 2929.89, 1757.25, 1496.79, 1449.22, 1257.75, 1144.95, 1125.72, 1044.31, 1024.24, 749.74, 709.71; ^{1}H-NMR ($CDCl_3$, 300 MHz, ppm) δ: 7.42-7.17 (m, 16H, Ph-H, H-14), 7.00 (m, 2H, H-4', 5'), 6.88 (m, 2H, H-3', 6'), 4.80 (s, 2H, H-15), 4.60 (m, 1H, H-12), 4.58 (s, 1H, H-3), 3.80 (s, 3H, Ph-OC$\underline{H}_3$), 3.39 (d, 1H, *J*=9.6 Hz, H-19a), 3.28 (d, 1H, *J*=9.6 Hz, H-19b), 2.40 (m, 1H, H-11a), 2.20 (m, 1H, H-11b), 2.05-0.85 (several protons), 1.54 (s, 6H, -OCOCH(C$\underline{H}_3$)$_2$), 1.23 (s, 3H, H-17), 0.85 (s, 3H, H-18), 0.49 (s, 3H, H-20).

3-O-(4-fluorophenyloxyisobutyryl)-19-O-tritylisoandrographolide (6f)

White powder; 85.6% yield; mp 198-199 °C. MS (ESI) m/z: 795.4 $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3455.09, 3078.77, 2995.06, 2983.44, 1755.35, 1741.88, 1637.53, 1504.04, 1449.04, 1205.94, 1126.60, 772.05, 751.17, 710.17; ^{1}H-NMR (DMSO-d_6, 300 MHz, ppm) δ: 7.42-7.22 (m, 16H, Ph-H, H-14), 7.07 (m, 2H, H-3', 5'), 6.71 (m, 2H, H-2', 6'), 4.83 (s, 2H, H-15), 4.48 (d, 1H, *J*=8.7 Hz, H-12), 4.36 (s, 1H, H-3), 3.26 (d, 1H, *J*=9.6 Hz, H-19a), 3.08 (d, 1H, *J*=9.6 Hz, H-19b), 2.09 (dd, 1H, *J*=7.8 Hz, H-11a), 1.98 (dd, 1H, *J*=7.8 Hz, H-11b), 1.90-1.06 (several protons), 1.43 (s, 6H, -OCOCH(C$\underline{H}_3$)$_2$), 1.14 (s, 3H, H-17), 1.01 (s, 3H, H-18), 0.36 (s, 3H, H-20).

3-O-(4-chlorophenyloxyisobutyryl)-19-O-tritylisoandrographolide (6g)

White powder; 76.0 % yield; mp 163-164 °C. MS (ESI) m/z: 811.3 $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3453.96, 2993.70, 1754.22, 1742.05, 1636.64, 1490.89, 1449.14, 1271.41, 1246.05, 1127.69, 771.64, 751.28, 709.66; ^{1}H-NMR ($CDCl_3$, 300 MHz, ppm) δ: 7.41-7.26 (m, 16H, Ph-H, H-14), 7.20 (m, 1H, H-3', 5'), 6.77 (d, 1H, *J*=9.0 Hz, H-2', 6'), 4.81 (s, 2H, H-15), 4.66 (m, 1H, H-12), 4.61 (s, 1H, H-3), 3.48 (d, 1H, *J*=9.6 Hz, H-19a), 3.28 (d, 1H, *J*=9.6 Hz, H-19b), 2.44 (dd, 1H, *J*=7.8 Hz, H-11a), 2.20 (dd, 1H, *J*=7.8 Hz, H-11b), 2.46-1.04 (several protons), 1.61 (s, 6H, -OCOCH(C$\underline{H}_3$)$_2$), 1.24 (s, 3H, H-17), 0.96 (s, 3H, H-18), 0.91 (s, 3H, H-20).

3-O-(4-bromophenyloxyisobutyryl)-19-O-tritylisoandrographolide (6h)

White powder; 79.2 % yield; mp 106-107 °C. MS (ESI) m/z: 855.3 (90%), 857.3 (100%) $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3454.02, 2925.95, 1758.16, 1486.99, 1448.47, 1384.41, 1369.18, 1238.07, 1129.88, 1072.17, 823.92, 764.75, 706.69; ^{1}H-NMR (DMSO-d_6, 300 MHz, ppm) δ: 7.41-7.24 (m, 18H, Ph-H, H-14, H-3', 5'), 6.58 (d, 2H, *J*=8.7 Hz, H-2', 6'), 4.83 (s, 2H, H-15), 4.46 (d, 1H, *J*=7.7 Hz, H-12), 4.35 (s, 1H, H-3), 3.26 (d, 1H, *J*=9.6 Hz, H-19a), 3.05 (d, 1H, *J*=9.6 Hz, H-19b), 2.09 (dd, 1H, *J*=7.8 Hz, H-11a), 1.92 (dd, 1H, *J*=7.8 Hz, H-11b), 1.92-1.04 (several protons), 1.47 (s, 6H, -OCOCH(C$\underline{H}_3$)$_2$), 1.14 (s, 3H, H-17), 1.01 (s, 3H, H-18), 0.38 (s, 3H, H-20); ^{13}C-NMR ($CDCl_3$, 75 MHz, ppm) δ: 173.6, 172.5, 154.6, 143.9, 143.0, 138.4, 131.9, 130.8, 130.6, 130.6, 129.9, 129.8, 129.0, 127.9, 127.9, 126.1, 114.1, 87.2, 82.7, 82.6, 79.4, 77.4, 77.0, 76.6, 73.2, 70.5, 63.0, 58.2, 52.7, 42.1, 39.1, 36.3, 35.8, 32.9, 31.6, 26.4, 24.5, 23.3, 23.0, 19.6, 15.2.

3-O-(4-nitrylphenyloxyisobutyryl)-19-O-tritylisoandrographolide (6i)

White powder; 77.5 % yield; mp 200-201 °C. MS (ESI) m/z: 822.4 $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3454.16, 3089.40, 2933.15, 2852.22, 1750.38, 1736.36, 1719.53, 1630.01, 1589.73, 1549.13, 1458.17, 1285.36, 1164.11, 1091.37, 1052.48, 811.40, 745.36, 720.14; ^{1}H-NMR (DMSO-d_6, 300 MHz, ppm) δ: 8.09 (d, 2H, *J*=9.1 Hz, H-3', 5'), 7.39-7.23 (m, 16H, Ph-H, H-14), 7.07 (t, 1H, *J*=7.7 Hz, 7.9 Hz, H-3'), 6.65 (d, 2H, *J*=9.1 Hz, H-2', 6'), 4.78 (s, 2H, H-15), 4.55 (m, 1H, H-12), 4.48 (s, 1H, H-3), 3.27 (d, 1H, *J*=9.6 Hz, H-19a), 3.14 (d, 1H, *J*=9.6 Hz, H-19b), 2.30 (dd, 1H, *J*=8.0 Hz, H-11a), 2.05 (dd, 1H, *J*=8.0 Hz, H-11b), 1.99-0.97 (several protons), 1.62 (s, 3H, -OCOCH(C$\underline{H}_3$)a), 1.61 (s, 3H, -OCOCH(C$\underline{H}_3$)b), 1.18 (s, 3H, H-17), 1.08 (s, 3H, H-18), 0.42 (s, 3H, H-20); ^{13}C-NMR ($CDCl_3$, 75 MHz, ppm) δ: 173.2, 172.6, 143.9, 143.1, 138.3, 128.9, 127.9, 127.6, 126.9, 125.4, 119.4, 117.2, 87.4, 83.1, 82.7, 80.1, 77.4, 77.0, 76.6, 73.1, 70.5, 62.9, 58.2, 52.7, 42.0, 39.1, 36.2, 35.7, 32.8, 31.5, 26.5, 24.4, 23.2, 23.0, 19.5, 15.2.

3-O-(4-tert-butylphenyloxyisobutyryl)-19-O-tritylisoandrographolide (6j)

White powder; 95.6 % yield; mp 96-97 °C. MS (ESI) m/z: 833.4 $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3448.46, 3058.02, 2961.38, 2869.97, 1758.86, 1608.35, 1510.25, 1490.80, 1448.83, 1383.87, 1365.26, 1281.04, 1129.97, 1060.33, 832.36746.58, 706.39; ^{1}H-NMR ($CDCl_3$, 300 MHz, ppm) δ: 7.45-7.17 (m, 18H, Ph-H, H-14, H-3', 5'), 6.84 (d, 1H, *J*=8.7 Hz, H-2', 6'), 4.80 (s, 2H, H-15), 4.68 (m, 1H, H-12), 4.56 (s, 1H, H-3), 3.40 (d, 1H, *J*=9.6 Hz, H-19a), 3.29 (d, 1H, *J*=9.6 Hz, H-19b), 2.40 (dd, 1H, *J*=8.0 Hz, H-11a), 1.93 (dd, 1H, *J*=8.0 Hz, H-11b), 2.10-0.85 (several protons), 1.51 (s, 3H, -OCOCH(C$\underline{H}_3$)a), 1.48 (s, 3H, -OCOCH(C$\underline{H}_3$)b), 1.27 (s, 9H, Ph-C(C$\underline{H}_3$)$_3$), 1.17 (s, 3H, H-17), 1.08 (s, 3H, H-18), 0.46 (s, 3H, H-20).

3-O-(2-naphthoxyisobutyryl)-19-O-tritylisoandrographolide (6k)

White powder; 93.0 % yield; mp 104-105 °C. MS (ESI) m/z: 827.4 $(M+Na)^+$; IR (KBr, cm^{-1}) ν: 3453.83, 3057.48, 1757.81, 1630.07, 1599.02, 1508.58, 1448.50, 1384.57, 1368.83, 1282.04, 1128.88, 764.78, 746.61, 706.60; ^{1}H-NMR (DMSO-d_6, 300 MHz, ppm) δ: 7.87 (d, 1H, *J*=8.1 Hz, H-5'), 7.79 (d, 1H, *J*=9.0 Hz, H-4'), 7.68 (d, 1H, *J*=7.8 Hz, H-8'), 7.49-7.23 (m, 18H, Ph-H, H-14, H-6', H-7'), 6.30 (s, 1H, H-1'), 6.86 (d, 1H, *J*=9.0 Hz, H-3'), 5.10 (s, 2H, H-15), 4.50 (s, 1H, H-12), 4.31 (s, 1H, H-3), 3.27 (d, 1H, *J*=9.9 Hz, H-19a), 3.01 (d, 1H, *J*=9.9 Hz, H-19b), 2.04 (dd, 1H, *J*=8.0 Hz, H-11a), 2.90 (dd, 1H, *J*=8.0 Hz, H-11b), 1.56 (s, 6H, -OCOCH(C$\underline{H}_3$)$_2$), 1.51-0.88 (several protons), 1.23 (s, 3H, H-17), 0.85 (s, 3H, H-18), 0.27 (s, 3H, H-20); ^{13}C-NMR ($CDCl_3$, 75 MHz, ppm) δ: 173.9, 172.5, 153.3, 143.9, 143.0, 138.4, 134.0, 129.1, 128.9, 127.6, 127.3, 126.8, 126.3, 124.1, 121.0, 112.4, 111.0, 110.6, 87.1, 82.7, 82.4, 79.2, 77.5, 77.1, 76.7, 73.2, 70.5, 63.4, 58.3, 52.7, 42.1, 39.2, 36.3, 35.7, 32.9, 31.6, 26.6, 24.5, 23.3, 22.9, 19.6, 15.1.

IN VIVO ACTIVITIES

Animals

Male Sprague-Dawley rats weighing between 150 g and 180 g at the start of treatment and housed individually in a room with a controlled temperature (23±1°C) and a 12 h/12 h light/dark cycle and a relative humidity of 55 ± 5%, with free access to commercial pellet food and water.

Chemicals and Reagents

Atromide (Xiyue pharm, Shanxi, China), triglyceride reagent kit (BioSino, Beijing, China), cholesterrol reagent kit (BioSino, Beijing, China), high-density lipoprotein cholesterol reagent kit (BioSino, Beijing, China), low-density lipoprotein cholesterol reagent kit (BioSino, Beijing, China).

Standard substances: TG, TC (Randox, Antrim, UK), HDL-C, LDL-C (BioSino, Beijing, China).

Methods

After one-week adaptive breeding, 136 male SD rats were divided into 17 groups randomly: normal control group (1 group), fat group (16 group, including a fat control group), with 8 in each group. All but normal control group was intragastrically given fat emulsion (10 ml/kg·d) in addition to normal feeding for 21 days. Afterward, except the normal black control group and the fat control group, each of the other groups was given the corresponding drug. After given for 7 days, the rats were fasted for 12 h and then anesthetized by intraperitoneal injection of 1.0 g/kg Urethane. Blood was obtained from the arteria cervicalis, the viscera were excised, weighed and tested for tissue section. Blood placed in sterile tubes, serum samples were isolated from the blood by centrifugation (4000 rpm for 30 min) and analyzed for serum total triglyceride (GPO-POD UV method), cholesterol (cholesterol oxidase method), high-density lipoprotein cholesterol (one-step method), and low-density lipoprotein cholesterol (one-step method), respectively, on an Olympus AU 400 automatic biochemistry analyzer (Olympus, Tokyo, Japan) [18-20].

Consisting of fat emulsion: 10% cholesterol, 25% lard oil, 2% sodium cholate, 1% propylthiouracil, 10% Tween-80, 20% propylene glyco and 32% water.

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