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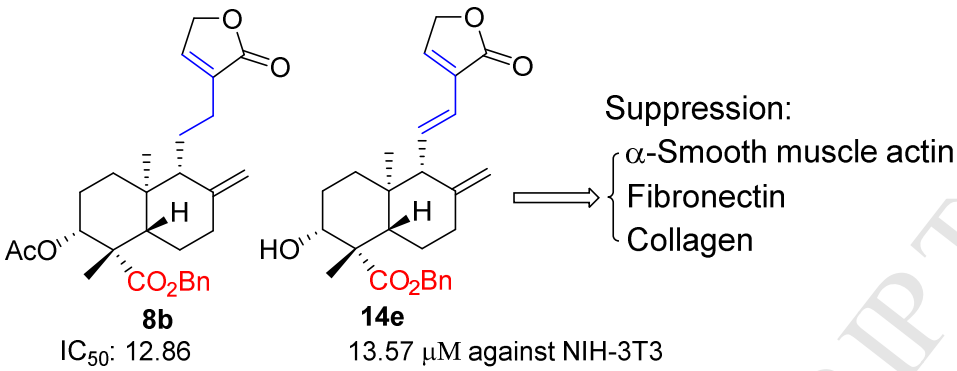
**Graphical Abstract**



# Synthesis and anti-fibrosis activity study of 14-deoxyandrographolide-19-oic acid and 14-deoxydidehydroandrographolide-19-oic acid derivatives

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**Abstract**

A series of 14-deoxyandrographolide-19-oic acid and 14-deoxy-11,12 (or 14,15)-didehydroandrographolide-19-oic acid derivatives were designed, synthesized and screened in vitro against the mouse fibroblast cell lines NIH-3T3. Thirteen compounds **8a-f**, **14a-c**, **14e-f**, and **18a-b** were found to exhibit better anti-fibrotic activities than andrographolide, with compounds **8b** and **14e** displaying best activity with $IC_{50}$ values of 12.86 and 13.57 μM against NIH-3T3 respectively. Further anti-fibrotic investigation was performed in terms of PCR and western bolt analysis. Our study demonstrated that compounds **8b** and **14e** suppressed effectively the expression of α-smooth muscle actin, fibronectin and collagen in NIH-3T3. Preliminary structure-activity analysis revealed that 14-deoxygenation and 19-carboxylation of andrographolide could significantly improve its anti-fibrotic effect, which made 14-deoxyandrographolide-19-oic acid and 14-deoxy-11,12-didehydroandrographolide-19-oic acid promising leads for the development of new anti-fibrotic agents.

*Keywords:*

14-Deoxyandrographolide-19-oic acid; Anti-fibrosis; α-SMA; FN; COL1A1

## 1. Introduction

Fibrosis, defined by the accumulation of excess extracellular matrix (ECM) components such as collagen and fibronectin, is a pathological process of most chronic inflammatory diseases [1]. Fibrosis affects almost every tissue in the body, such as liver [2], lung [3], kidney [4] and skin [5]. The fibrotic process ultimately leads to organ malfunction and even death if highly progressive. Although fibrogenesis is becoming increasingly recognized as a major cause of morbidity and mortality in most chronic inflammatory diseases, there are few treatment strategies available specifically targeting the pathogenesis of fibrosis.

Anti-fibrotic drugs may provide a promising strategy for the treatment of chronic inflammatory diseases associated with pathological fibrosis. Recently nintedanib and pirfenidone have been approved by Food and Drug Administration (FDA) for the treatment of idiopathic pulmonary fibrosis (IPF) [6,7]. Clinical results for these two drugs showed they could reduce the rate of decline of lung function with acceptable adverse effects [8]. However, best to our knowledge, nintedanib and pirfenidone are the only two drugs available now which specifically target the pathogenesis of fibrosis. Therefore, development of new anti-fibrotic drugs is in urgent need.

Natural products have been widely used since ancient time for the treatment of various diseases. Many of them serve successfully as the source of leads for the development of drugs. Andrographolide (**1)**, a naturally occurring labdane diterpenoid isolated from the plant Andrographis paniculata (family Acanthaceae) [9], is used extensively as the traditional medicine in many Asian countries. It has been reported to process a wide spectrum of biological activities such as antibacterial [10], anti-inflammatory [11], anti-hepatitis [12], antimalarial [13], and

anti-tumor activities [14]. Recently, andrographolide was found to exhibit anti-fibrotic activity against pulmonary [15], hepatic [16] or renal fibrosis [17]. Various mechanisms have been suggested for its anti-fibrotic activity, including inactivation of nuclear factor-κB (NF-κB) [15], inhibition of transforming growth factor-β (TGF-β) secretion [17], and inhibition of hepatic stellate cells (HSCs) for hepatic fibrosis [16].

Although andrographolide displays promising anti-fibrotic activity, shortcomings remain such as low potency and poor solubility. In order to increase the anti-fibrotic effects of andrographolide, structural modification appears necessary. The present work describes the structural investigation of andrographolide to improve anti-fibrotic activity.

## 2. Result and discussion

### *2.1. Design concepts*

Structural modification based on andrographolide has been well demonstrated with various biological activities, especially with anti-tumor activity [18]. However, to our surprise, the structural modification for enhancing its anti-fibrotic activity is almost in blank state. Only hit was the findings made by Tzeng that 14-deoxy-11,12-didehydroandrographolide (**2**) showed more potent activity than andrographolide in reducing the production of extracellular molecule fibronectin and cytokine TGF-β [17]. We have recently reported the synthesis and antitumor activity of a series of andrographolide-19-carboxylates, and found that conversion of 19-hydroxyl group of andrographolide into carboxylate group would improve anti-tumor activity [19]. We hypothesized that the similar modification against 19-hydroxyl may be beneficial for improving anti-fibrotic effects. Taking these factors into account, we set to introduce both 14-deoxy-11,12-didehydro unit and 19-caboxylate group into andrographolide. The influences of rearrangement or reduction of the conjugated double bonds on anti-fibrotic activity were also investigated. The corresponding structures of targets designed (**8a-f**, **14a-f** and **18a-d**) are shown in Fig. 1.

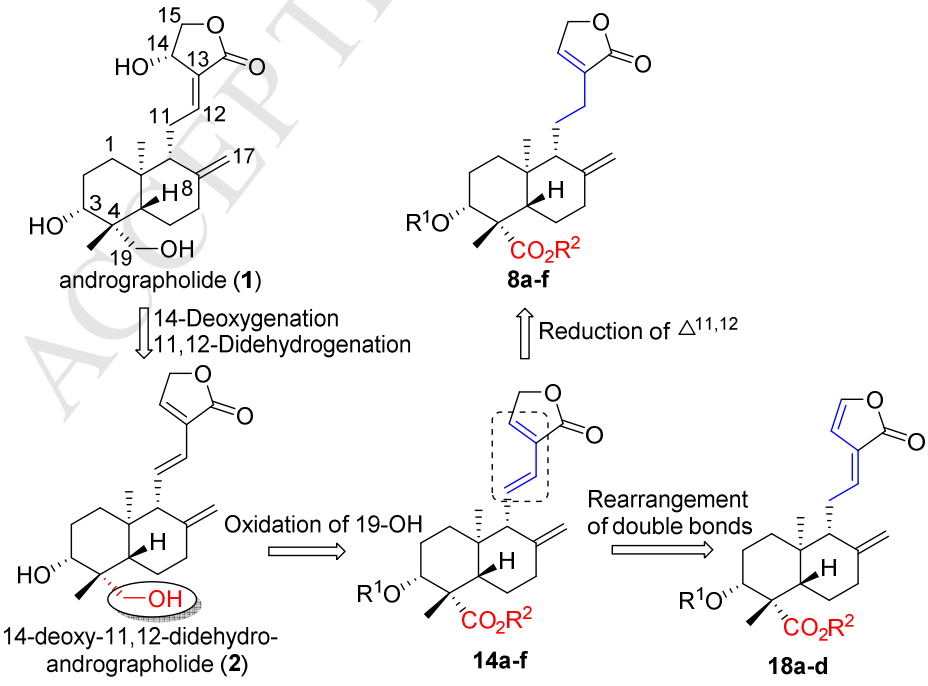
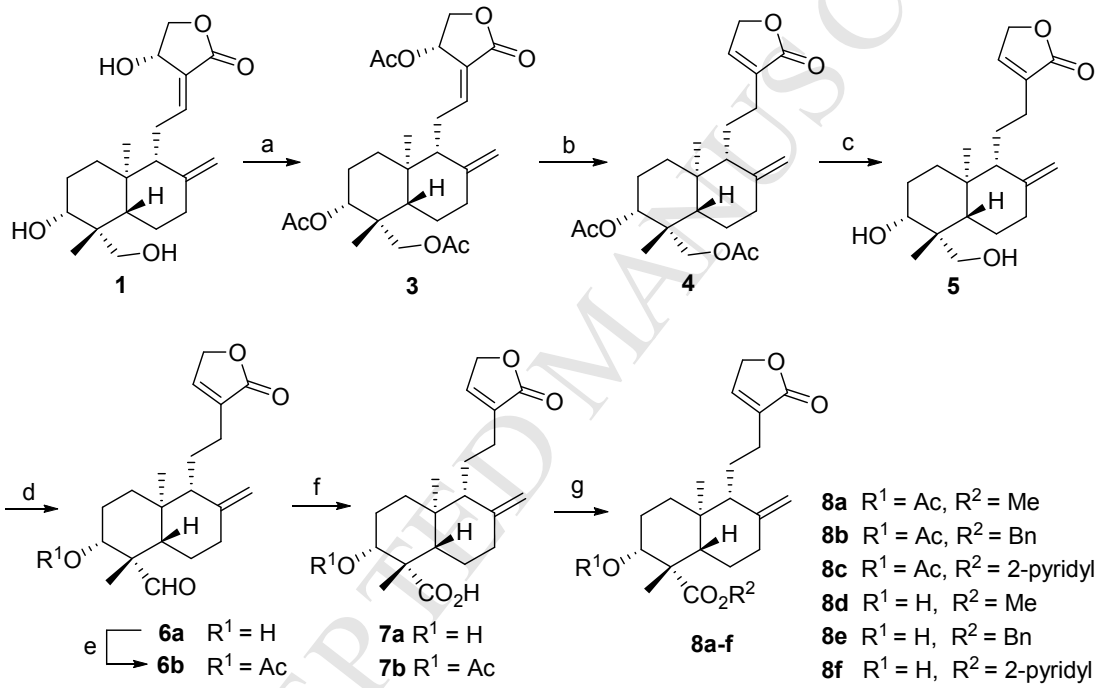



**Fig. 1.** Structural modification of andrographolide to develop new anti-fibrotic andrographolide derivatives.

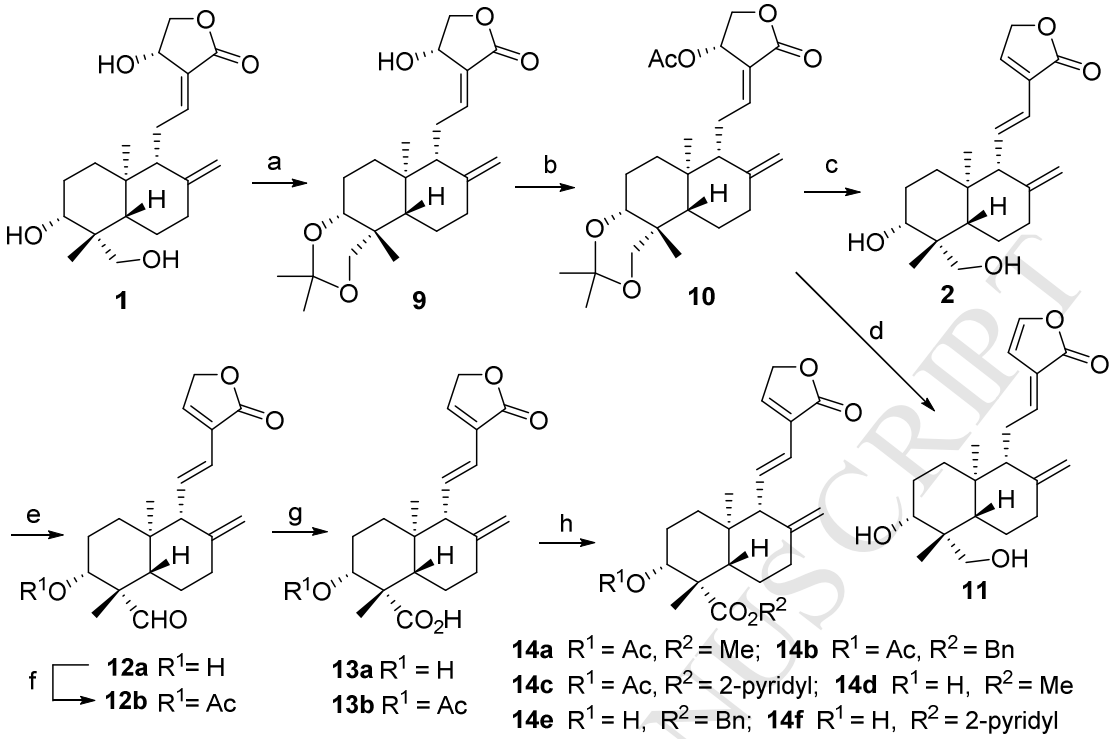
*2.2. Chemistry*

The first series of 14-deoxyandrographolide-19-oic acid derivatives (**8a-f**) were synthesized as shown in Scheme 1. The synthesis began with readily available andrographolide (**1**), which was converted into triacetyl andrographolide **3** by reacting with acetic anhydride under the catalysis of zinc chloride. Reductive rearrangement of **3** with sodium borohydride [20], followed by basic hydrolysis of ester groups at C-3 and C-19 of **4**, furnished 14-deoxyandrographolide **5**. Selective oxidation of primary hydroxyl group at C-19 position of **5** with TEMPO-NCS system [21] to yield corresponding aldehyde **6a**. **6a** was acetylated with acetic anhydride at room temperature to furnish 3-acetylated aldehyde **6b**. The formed aldehyde group of **6a** or **6b** was further oxidized with $NaClO_2$-$NaH_2PO_4$ and isopentene [22], affording corresponding carboxylic acid **7a** or **7b**. The carboxyl group of **7a** and **7b** was esterificated with MeI, BnBr or 2-chloromethylpyridine at the presence of $K_2CO_3$ in DMF at room temperature to provide the first series targets **8a-f**.



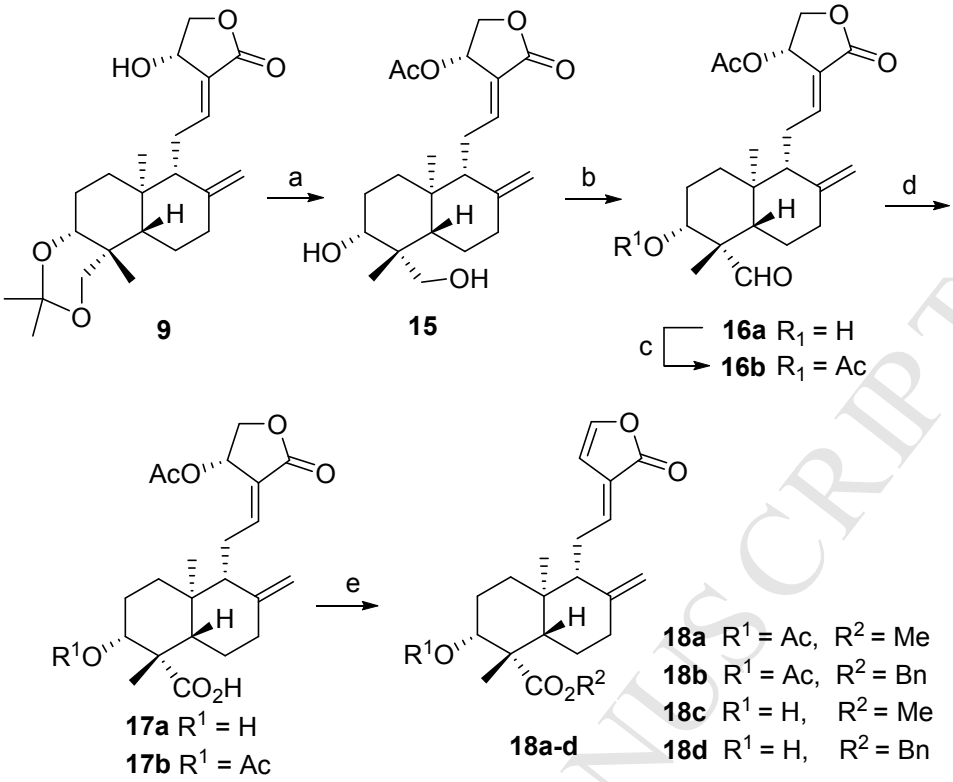

**Scheme 1**. Synthesis of the 14-deoxyandrographolide-19-oic acid derivatives **8a-f**. Reagents and conditions: (a) $Ac_2O$, $ZnCl_2$, 50 °C, 2 h, 97%; (b) $NaBH_4$, MeOH, 0 °C to rt, 2 h, 91%; (c) HCl, MeOH, rt, 16 h, 87%; (d) TEMPO, TBAB, NCS, DCM, $K_2CO_3$-$KHCO_3$ buffer, rt, 9 h, 61%; (e) $Ac_2O$, DMAP, DCM, rt, 5 h, 77%; (f) $NaClO_2$-$NaH_2PO_4$, isopantene, *t*-BuOH, $H_2O$, rt, 30 h, 54% for **7a** from **6a** while 64% for **7b** from **6b**; (g) $R^2X$, $K_2CO_3$, DMF, rt, 0.5 h, 74-91%.

The second series of 14-deoxy-11,12-didehydroandrographolide-19-oic acid derivatives **14a-f** were prepared as depicted in Scheme 2. The hydroxyl groups at C-3 and C-19 of andrographolide were first protected by reacting with 2,2-dimethoxypropane at the presence of catalytic *p*-TsOH, followed by the acetylation of the hydroxyl group at C-14, to produce **10**. Deacetoxylation of **10** under basic conditions was significantly affected by reaction temperature, with 14-deoxy-11,12-didehydroandrographolide (**2**) as deacetoxylation product under reflux while 14-deoxy-14,15-didehydroandrographolide (**11**) at room temperature. Compound **2** underwent a similar series of conversions as described for compound **5** in Scheme 1, to afford the second series targets **14a-f**.




**Scheme 2**. Synthesis of the 14-deoxy-11,12-didehydroandrographolide-19-oic acid derivatives **14a-f**. Reagents and conditions: (a) 2,2-dimethoxypropane, *p*-TsOH, Tol/DMSO, 80 $^{o}$C, 1.5 h, 93%; (b) $Ac_2O$, reflux, 1.5 h, 93%; (c) i. DMAP (1.5 eq), DCM, reflux, 24 h; ii. HCl (dilute), 85% for two steps; (d) i. DMAP (0.2 eq), DCM, rt, 24 h; ii. HCl (dilute), 89% for two steps; (e) TEMPO, TBAB, NCS, DCM, $K_2CO_3$-$KHCO_3$ buffer, rt, 9 h, 91%; (f) $Ac_2O$, DMAP, DCM, rt, 5 h, 76%; (g) $NaClO_2$-$NaH_2PO_4$, isopantene, *t*-BuOH, $H_2O$, rt, 30 h, 64% for **13a** from **12a** while 78% for **13b** from **12b**; (h) $R^2X$, $K_2CO_3$, DMF, rt, 0.5 h, 56-74%.

The third series of 14-deoxy-14,15-didehydroandrographolide-19-oic acid derivatives **18a-d** were prepared as shown in Scheme 3. At first, a similar synthetic strategy as used for the synthesis of the second series compounds was employed with intermediate **11** as starting material. However, the poor solubility of **11** brought big problem for the subsequent oxidation. Considering the fact that poor solubility of compound **11** was caused from the formation of conjugated C-12=C-13 and C-14=C-15 double bonds, a synthetic route with the formation of this conjugated double bonds as the last step was taken to synthesize targets **18a-d**. 3,19-Isopropylidene-andrographolide (**9**) was firstly acetylated by treatment with acetic anhydride, followed by deprotection with aqueous acetic acid to give 14-acetylandrographolide (**15**). Selective oxidation of primary hydroxyl group of **15** with TEMPO-NCS to yield corresponding aldehyde **16a**. **16a** was acetylated with acetic anhydride at room temperature to furnish 3-acetylated aldehyde **16b**. The formed aldehyde group of **16a** or **16b** was further oxidized under Pinnick conditions, providing corresponding carboxylic acid **17a** or **17b**. The carboxyl group of **17a** and **17b** was esterificated with MeI or BnBr at the presence of $K_2CO_3$ in DMF at room temperature, followed by elimination reaction under basic conditions to afford the third series targets **18a-d**. Regretfully, when **17a** and **17b** were esterificated with 2-chloromethylpyridine at the presence of $K_2CO_3$, 2-pyridylmethyl 14-deoxy-11,12-didehydro andrographolide-19-carboxylates **14c** and **14f**, instead of 2-pyridylmethyl 14-deoxy-14,15-didehydroandrographolide-19-carboxylates, were obtained.




**Scheme 3**. Synthesis of the 14-deoxy-14,15-didehydroandrographolide-19-oic acid derivatives **18a-d**. Reagents and conditions: (a) i. $Ac_2O$, reflux, 1.5 h; ii. AcOH, $H_2O$, 85% for two steps; (b) TEMPO, TBAB, NCS, DCM, $K_2CO_3$-$KHCO_3$ buffer, rt, 9 h, 91%; (c) $Ac_2O$, DMAP, DCM, rt, 5 h, 76%; (d) $NaClO_2$-$NaH_2PO_4$, isopantene, *t*-BuOH, $H_2O$, rt, 30 h, 42% for **17a** from **16a** while 76% for **17b** from **16b**; (e) $R^2X$, $K_2CO_3$, DMF, rt, 0.5 h, then cat DMAP, rt, 0.5 h, 38-47%.

The structure of the target compounds was identified using spectroscopic techniques ($^1$H NMR, $^{13}$C NMR, and mass spectroscopic analyses). The compound **8a** was taken to elucidate structure identification as follows. **8a** showed key signals in the $^1$H NMR spectrum for a C-14 alkenyl proton (7.11 ppm, a singlet), a C-15 methylene group (4.79 ppm, singlet), two C-17 alkenyl protons (4.63 and 4.91 ppm, both as a singlet), a methyl carboxylate group (3.65 ppm, singlet), an acetyl group (2.07 ppm, singlet), and two angular methyl groups (1.24 and 0.61 ppm, both as singlet). All other protons of **8a** appeared at the positions similar to those of andrographolide. In addition, the $^{13}$C NMR data clearly showed the presence of three ester carbonyl carbon atoms with chemical shifts at 173.79, 173.54 and 170.53 ppm and four alkenyl carbon atoms with chemical shifts at 145.91, 143.51, 134.12 and 107.13 ppm. Furthermore, the presence of a molecular ion peak at m/z = 422.2544 ($[M+NH_4]^+$) in the mass spectra (calcd. 422.2537) further confirmed the structure of compound **8a**.

For 14-deoxy-11,12-didehydroandrographolide-19-oic acid derivatives **14a-f** and 14-deoxy-14,15-didehydroandrographolide-19-oic acid derivatives **18a-d**, the structure identification was performed very carefully, since the main structural difference between these two series was only the position of conjugated double bonds. Luckily enough, their structures could be distinguished easily by means of the signal of C-15 proton (s) in $^1$H NMR spectra: the signal of C-15 $CH_2$ group for **14** series appeared around 4.8 ppm, while the signal for C-15 alkenyl proton for **18** series appeared around 7.0 ppm.

### *2.3. Biology*

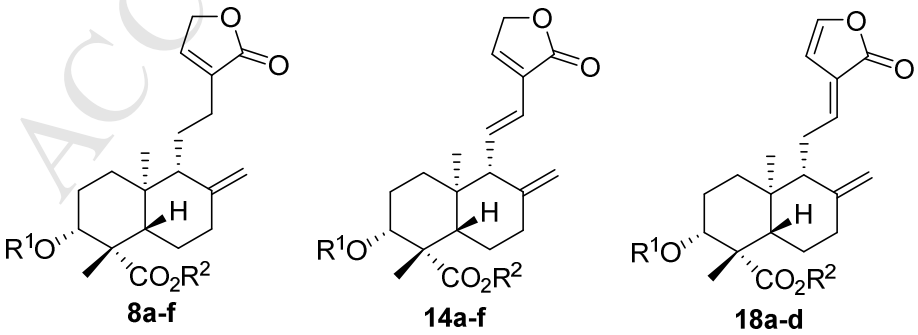
#### *2.3.1 In vitro inhibitory activities against NIH-3T3 cells*

To evaluate the anti-fibrotic effects of synthesized compounds, NIH-3T3 cells (mouse fibroblast cell lines) were employed since they have been recognized as a convenient screening model (*in vitro*) for studies of fibrosis [23]. Firstly, the inhibitory activities of target compounds were tested by MTT assay with andrographolide as control. The biological results, expressed as the compound concentration [μM] that causes a 50% inhibition of cell growth, are expressed as $IC_{50}$ values and are summarized in Table 1. Most compounds tested, except **14d**, **18c** and **18d**, showed better anti-fibrotic activities than andrographolide, with **8b** and **14e** displaying the strongest activities with $IC_{50}$ values of 12.86 and 13.57 μM respectively. The first series of 14-deoxyandrographolide-19-oic acid derivatives **8a-f** and the second series of 14-deoxy-11,12-didehydro andrographolide-19-oic acid derivatives **14a-f** were found to exhibit better activities than the third series of 14-deoxy-14,15-didehydro andrographolide-19-oic acid derivatives **18a-d**, suggesting that molecular rigidity might play a crucial role in biological activities. Significantly, all three series of target compounds showed better anti-fibrotic activities than their counterparts with C-19 hydroxyl unoxidized (**8a-f** vs **5**, **14a-f** vs **2**, and **18a-d** vs **11**), which validated our previous hypothesis that the oxidation of 19-hydroxyl of andrographolide may be of benefit to improving anti-fibrosis effects.

Next, in order to investigate whether the compounds **8b** and **14e** showing the strongest inhibitory effects on NIH-3T3 are also toxic in other normal cell types, HUVEC (human umbilical vein endothelial cells) were chosen for test with MTT assay under the same conditions. As shown in Fig. 2, both **8b** and **14e** showed a certain but much weaker cytotoxicity towards HUVEC compared with NIH-3T3, which meant these two compounds did have anti-fibrotic effects. At low concentrations (10 and 40 μM), **8b** and **14e** both had an inhibition rate almost equivalent to andrographolide against HUVEC (6.6% and 9.7% vs 10.4% at 10 μM, while 41% and 41% vs 38.7% at 40 μM), although they showed a higher inhibition rate (55.7% and 68.7% ) than andrographolide (26.4%) at high concentration (80 μM).

**Table 1**

The structures of 14-deoxyandrographolide-19-oic acid and 14-deoxydidehydroandrographolide-19-oic acid derivatives and their inhibitory activities against NIH-3T3 cell lines



| Entry | Compounds | | | $IC_{50}$ (μM ) |
|---|---|---|---|---|
| | No | $R^1$ | $R^2$ | NIH-3T3 |
| 1 | **8a** | Ac | Me | 80.6 |
| 2 | **8b** | Ac | Bn | 12.86 |
| 3 | **8c** | Ac | 2-pyridyl | 55.45 |
| 4 | **8d** | H | Me | 60.6 |
| 5 | **8e** | H | Bn | 37.86 |
| 6 | **8f** | H | 2-pyridyl | 79.82 |
| 7 | **14a** | Ac | Me | 32.25 |
| 8 | **14b** | Ac | Bn | 52.71 |
| 9 | **14c** | Ac | 2-pyridyl | 34.86 |
| 10 | **14d** | H | Me | 110.7 |
| 11 | **14e** | H | Bn | 13.57 |
| 12 | **14f** | H | 2-pyridyl | 80.16 |
| 13 | **18a** | Ac | Me | 69.66 |
| 14 | **18b** | Ac | Bn | 78.03 |
| 15 | **18c** | H | Me | 177.3 |
| 16 | **18d** | H | Bn | 177.2 |
| 17 | **5** | 14-deoxyandrographolide | | 176.3 |
| 18 | **2** | 14-deoxy-11,12-didehydro-andrographolide | | 64.27 |
| 19 | **11** | 14-deoxy-14,15-didehydro-andrographolide | | not active |
| 20 | **1** | andrographolide | | 95.28. |

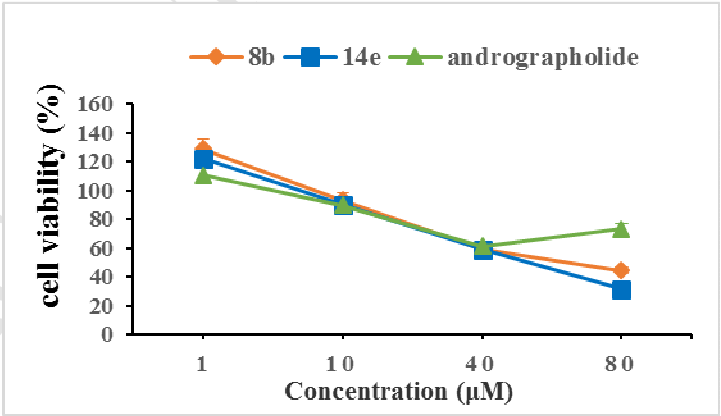



**Fig. 2.** Cell proliferation of human umbilical vein endothelial cells treated with different concentrations of **8b** and **14e** (1, 10, 40, and 80 μM) with andrographolide (**1**) as control. Data are presented as the mean of three repeated experiments.

*2.3.2 **8b** and **14e** suppressed the TGF-β1-induced gene and protein expression of α-SMA, FN and COL1A1 in NIH-3T3*

The hallmark of fibrotic diseases is overexpression of α-smooth muscle actin (α-SMA) and

increased production of matrix proteins, including collagen and fibronectin (FN) [24,25]. Therefore, α-SMA, FN and collagen type I alpha 1 (COL1A1) have been commonly used as fibrotic markers. To further investigate the anti-fibrotic activity of screened compounds (**8b** and **14e**), their abilities to suppress the gene and protein expression of α-SMA, FN and COL1A1 in vitro were investigated. For this purpose, mouse fibroblast cell lines NIH-3T3 were employed again and rat TGF-β1 was used as a fibrosis stimulant in cultured fibroblasts. The effects of **8b** and **14e** on the mRNA expression of α-SMA, FN and COL1A1 were explored with andrographolide as positive control under two conditions: either TGF-β stimulation or not. NIH-3T3 cells were pre-incubated with tested compounds (500 nM) for 1 h alone/and then treated with rat TGF-β1 (5 ng/mL) for 24 h. The results from real-time qPCR were shown in Fig. 3. **8b** and **14e** both displayed better anti-fibrotic effects than andrographolide for all three markers no matter whether they were TGF-β stimulated or not, with **14e** showing better anti-fibrotic activities than **8b**. The data from real-time qPCR also showed that the expression levels of these three fibrotic genes were all significantly up-regulated by rat TGF-β1, but the up-regulated mRNA levels could be suppressed by the tested compounds. Interestedly, **8b** and **14e** showed better anti-fibrotic activities than **5** and **2** respectively for all three fibrotic markers, which further validated our previous hypothesis that the oxidation of 19-hydroxyl of andrographolide would be helpful for improving anti-fibrosis effects.

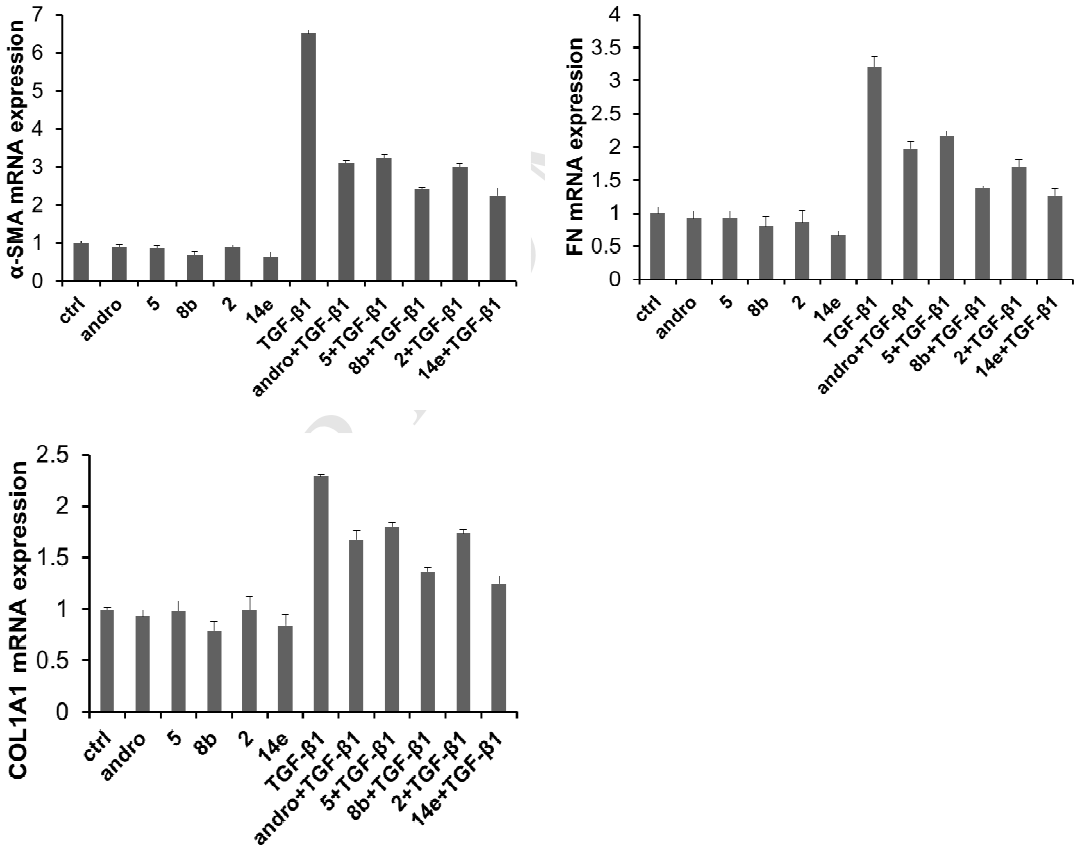



**Fig. 3.** Effects of **8b**, **14e**, **5** and **2** on TGF-β1-induced mRNA expression of α-SMA, FN and COL1A1 in NIH-3T3 by Real Time-PCR with andrographolide (andro) as positive control. NIH-3T3 were treated with tested compounds (500 nM) for 1 h and in the presence or absence of 5 ng/ml TGF-β1 for 24 h. Data were expressed as means ± SD, n = 3. Error bars indicate standard error.

Protein expression revealed by Western Blot (Fig. 4) showed similar results as those in the Real-Time PCR assay. As previously observed, the protein expression levels of α-SMA, FN and COL1A1 were all significantly up-regulated by rat TGF-β1, but the up-regulated protein levels could be suppressed by **8b** and **14e**. **8b** exhibited a little bit better suppression than **14e** on TGF-β1 stimulated COL1A1 protein expression, while **8b** and **14e** displayed almost the same inhibition on TGF-β1 stimulated α-SMA and FN protein expression.

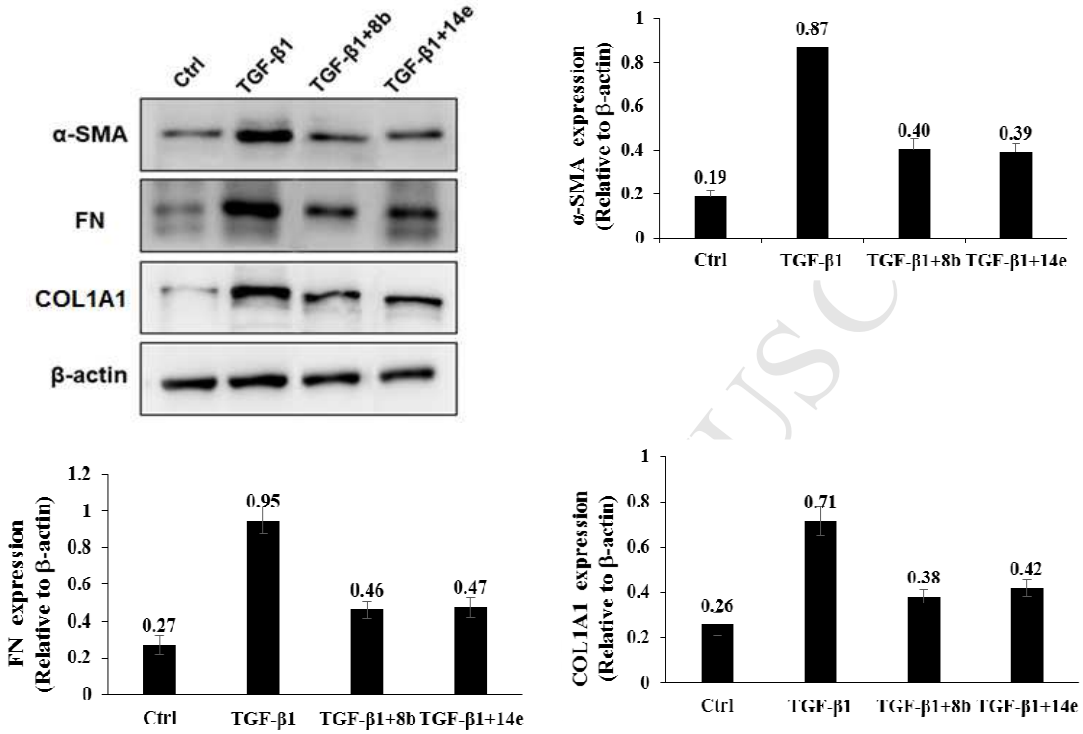



**Fig. 4.** The effects of **8b** and **14e** on TGF-β1-induced α–SMA, FN and COL1A1 expression. NIH-3T3 cells were treated with 500 nM screened compounds in the presence of 5 ng/ml rat TGF-β1 for 24 h. The protein expression levels of α-SMA, FN and COL1A1 were probed through western bloting. β-actin was used as the loading control, and PBS served as the control (Ctl). This experiment was repeated three times under the same conditions and data were expressed as means ± SD. Error bars indicate standard error.

## 3. Conclusion

In summary, a total of sixteen 14-deoxyandrographolide-19-carboxylate compounds were successfully synthesized and characterized. The *in vitro* anti-fibrotic activities of all compounds were primarily evaluated against mouse fibroblast cell lines. To our knowledge, this is the first attempt to modify the structure of andrographolide with the aim of improving its anti-fibrotic effect. The results indicated that thirteen compounds (all except **14d** and **18c-d)** exhibited inhibitory activities stronger than that of parent compound **1**, with **8b** and **14e** displaying the strongest activities with $IC_{50}$ values of 12.86 and 13.57 μM against NIH-3T3 respectively. More important, we demonstrated that the conversion of 19-hydroxyl into 19-carboxylate of andrographolide is of significant benefit for anti-fibrosis. Further PCR and western bolt investigation of **8b** and **14e** exhibited that they both inhibited the proliferation of NIH-3T3 through inhibiting the expression of α-SMA, fibronectin and collagen. The most potent compounds **8b** and **14e** both showed around 7-fold higher inhibitory activities against NIH-3T3

than andrographolide, which made them two promising leads for the development of new anti-fibrotic agents. Studies in this direction are in progress.

## 4. Experimental Section

### *4.1. General*

Reagents were purchased from commercial sources and used without further purification unless otherwise indicated. Melting points were determined with an XT-4 Melting Point apparatus and are uncorrected. $^{1}H$ and $^{13}C$ NMR spectra were recorded on Bruker AV-300 (300 MHz) with $CDCl_3$ or DMSO-$d_6$ as the solvents and chemical shifts are reported as δ values in parts per million (ppm). High-resolution mass spectra (HRMS) were performed on an Agilent ESI TOF (time of flight) mass spectrometer. Anti-alpha smooth muscle actin antibody, anti-fibronectin antibody, and anti-collagen I antibody were purchased from Abcam Co. (Shanghai, China) while β-Actin (8H10D10) Mouse mAb was purchased from Cell Signaling Technology Co. (Shanghai, China).

### *4.2. Chemistry*

#### *4.2.1 Synthesis of the 14-deoxyandrographolide-19-oic acid derivatives **8a-f***

##### *4.2.1.1. 3,14,19-Triacetylandrographolide (**3**)*

To a stirred suspension of andrographolide (**1**) (15.0 g, 42.9 mmol) in $Ac_2O$ (210 mL) were added $ZnCl_2$ (catalytic amount) and $Ac_2O$ (15.0 mL). The mixture was stirred vigorously at 50 $^{o}$C until the suspended solution became clarified and then cooled to r.t. To the reaction mixture was added EtOH (150 mL) $H_2O$ (450 mL), then stirred vigorously at r.t. for 30 min, then filtered to give a white solid (19.3 g, 97%). $^{1}H$ NMR (300 MHz, DMSO-$d_6$) δ: 7.49 (s, 1H), 4.88 (s, 1H), 4.82 (d, *J* = 1.5 Hz, 2H), 4.63 (s, 1H), 4.52 (dd, *J* = 11.2, 4.9 Hz, 1H), 4.35 (d, *J* = 11.7 Hz, 1H), 3.97 (d, *J* = 11.7 Hz, 1H), 2.40–2.15 (m, 2H), 2.00 (d, *J* = 1.0 Hz, 9H), 1.79 (s, 3H), 1.65 (dd, *J* = 13.6, 7.5 Hz, 3H), 1.43 (d, *J* = 13.5 Hz, 2H), 1.23 (s, 3H), 0.94 (s, 3H), 0.65 (s, 3H). The $^{1}H$ NMR spectroscopic data were matched those reported in literature [26].

##### *4.2.1.2. 3,19-Diacetyl-14-deoxyandrographolide (**4**)*

To a solution of **3** (0.4 g, 0.84 mmol) in MeOH (6 mL) was added $NaBH_4$ (0.13 g, 3.36 mmol) at 0 °C, and the resulting solution was stirred at r.t. for 2 h. After the reaction was complete, the reaction mixture was diluted with ethyl acetate (120 mL). The organic layer was separated, washed with water (60 mL×3) and brine (60 mL×3), dried with anhydrous $Na_2SO_4$, concentrated under reduce pressure, and purified by column chromatography (EtOAc/PE = 1/4) to give compound **4** as a white solid (0.32 g, 91%). $^{1}H$ NMR (300 MHz, $CDCl_3$) δ: 7.10 (s, 1H), 4.91 (s, 1H), 4.78 (d, *J* = 1.7 Hz, 2H), 4.63 (s, 1H), 4.61-4.55 (m, 1H), 4.37 (d, *J* = 11.7 Hz, 1H), 4.10 (d, *J* = 11.8 Hz, 1H), 2.53–2.36 (m, 2H), 2.28–2.07 (m, 1H), 2.04 (s, 6H), 1.95-1.80 (m, 3H), 1.74 (dd, *J* = 13.8, 6.5 Hz, 2H), 1.70–1.60 (m, 3H), 1.50 (dd, *J* = 12.8, 3.9 Hz, 1H), 1.36–1.26 (m, 3H), 1.02 (s, 3H), 0.72 (s, 3H). The $^{1}H$ NMR spectroscopic data were matched those reported in literature [20].

##### *4.2.1.3. 14-Deoxyandrographolide (**5**)*

3,19-Diacetyl-14-deoxyandrographolide (**4**) (0.13 g, 0.31 mmol) was dissolved in 6ml of 7% hydrochloric acid methanol solution, then stirred at r.t for 16 h. After the reaction was complete, the reaction mixture was diluted with ethyl acetate (120 mL). The organic layer was separated, washed with water (60 mL×3) and brine (60 mL×3), dried with anhydrous $Na_2SO_4$, concentrated under reduce pressure, and purified by column chromatography (EtOAc/PE = 1/2) to give compound **5** as a white solid (0.09 g, 87%). $^1$H NMR (300 MHz, DMSO-$d_6$) δ: 7.47 (s, 1H), 5.05 (d, *J* = 4.9 Hz, 1H), 4.82 (s, 3H), 4.59 (s, 1H), 4.12 (dd, *J* = 7.5, 2.6 Hz, 1H), 3.83 (dd, *J* = 10.9, 2.7 Hz, 1H), 3.29-3.14 (m, 2H), 2.35–2.22 (m, 2H), 2,03–1.87 (m, 2H), 1.70 (d, *J* = 13.2 Hz, 2H), 1.65–1.50 (m, 3H), 1.32 (dd, *J* = 12.7, 3.9 Hz, 1H), 1.15 (d, *J* = 10.3 Hz, 3H), 1.07 (s, 3H), 0.60 (s, 3H). The 1H NMR spectroscopic data were matched those reported in literature [26].

*4.2.1.4. 14-Deoxyandrographolide-19-al (**6a**)*

To a solution of compound **5** (0.33 g, 0.99 mmol) in 20 ml of methylene chloride, were added TEMPO (0.028 g, 0.18 mmol), 20 ml of $K_2CO_3$-$NaHCO_3$ buffer (pH ~7), TBAB (0.058 g, 0.18 mmol) and NCS (0.48 g, 3.6 mmol) under ice bath. The reaction mixture was then stirred vigorously for 10 h. The organic layer was separated and the aqueous layer was extracted with methylene chloride (60 mL×2). The combined organic layer was washed with brine (30 mL×2), dried over anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/PE = 1/2) to give compound **6a** as a light yellowish solid (0.2 g, 61%). Mp 121-124 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 9.77 (s, 1H), 7.12 (d, *J* = 1.4 Hz, 1H), 4.95 (s, 1H), 4.78 (s, 2H), 4.66 (s, 1H), 3.19 (d, *J* = 24.9 Hz, 2H), 2.53–2.37 (m, 2H), 2.19–1.97 (m, 2H), 1.89 (dd, *J* = 14.8, 2.4 Hz, 3H), 1.78 (dd, *J* = 17.5, 9.3 Hz, 2H), 1.70–1.56 (m, 3H), 1.37 (d, *J* = 13.1 Hz, 1H), 1.29 (s, 3H), 1.26-1.15 (m, 1H), 0.63 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 207.30, 173.78, 145.60, 143.60, 134.03, 107.54, 76.56, 69.63, 55.14, 54.30, 52.32, 39.17, 37.54, 36.44, 28.12, 24.04, 23.61, 21.58, 18.88, 13.23; HRMS (ESI) m/z calcd for $C_{20}H_{28}NaO_4$ $[M+Na]^+$ 355.1885, found 355.1878.

*4.2.1.5. 3-Acetyl-14-deoxyandrographolide-19-al (**6b**)*

To a solution of compound **6a** (0.92 g, 2.77 mmol) in 60 mL of methylene chloride, were added $Ac_2O$ (5.2 mL), DMAP (0.068 g, 0.55 mmol), then stirred at r.t. for 5 h. After the reaction was complete, the reaction mixture was diluted with methylene chloride (120 mL). The organic layer was separated, washed with water (60 mL×3) and brine (60 mL×3), dried with anhydrous $Na_2SO_4$, concentrated under reduce pressure, and purified by column chromatography (EtOAc/PE = 1/3) to give compound **6b** as a white solid (0.8 g, 77%). Mp 142-146 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 9.97 (s, 1H), 7.05 (s, 1H), 4.85 (s, 1H), 4.72 (d, *J* = 1.7 Hz, 2H), 4.69–4.60 (m, 1H), 4.57 (s, 1H), 2.36 (dd, *J* = 9.8, 3.3 Hz, 2H), 2.06 (dd, *J* = 17.1, 8.7 Hz, 1H), 1.99 (s, 3H), 1.95–1.79 (m, 3H), 1.78–1.62 (m, 2H), 1.62–1.48 (m, 2H), 1.40 (d, *J* = 13.4 Hz, 1H), 1.35–1.20 (m, 3H), 1.02 (s, 3H), 0.56 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 203.80, 173.74, 169.98, 145.52, 143.64, 134.00, 107.55, 77.88, 69.61, 56.15, 53.98, 51.65, 38.73, 37.22, 35.69, 23.99, 23.67, 21.59, 20.57, 20.41, 14.76; HRMS (ESI) m/z calcd for $C_{22}H_{30}NaO_5$ $[M+Na]^+$ 397.1985, found 397.1982.

*4.2.1.6. 14-Deoxyandrographolide-19-oic acid (**7a**)*

To a solution of compound **6a** (0.3 g, 0.90 mmol), $H_2O$ (8 mL), isoamylene (1.1 mL) and t-BuOH (20 mL), was added $NaClO_2$ (0.29 g, 3.21 mmol) and $NaH_2PO_4$ (0.5 g, 3.21 mmol), then stirred at r.t. for 30 h. After the reaction was complete, the reaction mixture was diluted with ethyl acetate (120 mL). The organic layer was separated, washed with water (60 mL×3) and brine (60

mL×3), dried with anhydrous $Na_2SO_4$, concentrated under reduce pressure, and purified by column chromatography (EtOAc/PE = 1/2) to give compound **7a** as a light yellowish solid (0.17 g, 54%); Mp 196-200 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.11 (s, 1H), 4.90 (s, 1H), 4.77 (s, 2H), 4.61 (s, 1H), 3.17 (dd, *J* = 11.9, 4.2 Hz, 1H), 2.54–2.27 (m, 2H), 2.09–1.92 (m, 3H), 1.92–1.67 (m, 4H), 1.67–1.51 (m, 2H), 1.43 (d, *J* = 6.5 Hz, 3H), 1.22 (dd, *J* = 19.8, 6.4 Hz, 3H), 0.61 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 181.46, 174.01, 146.14, 143.75, 134.09, 106.81, 77.38, 69.72, 55.14, 54.95, 48.78, 39.50, 37.85, 37.11, 28.03, 25.25, 24.09, 23.51, 21.55, 12.26; HRMS (ESI) m/z calcd for $C_{20}H_{28}NaO_5$ $[M+Na]^+$ 371.1829, found 371.1832.

*4.2.1.7. 3-Acetyl-14-deoxyandrographolide-19-oic acid (**7b**)*

**7b** was synthesized from **6b** using the same method described for the synthesis of **7a** as a light yellowish solid (64%); Mp 166-169 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.11 (s, 1H), 4.91 (s, 1H), 4.78 (s, 2H), 4.64 (s, 1H), 4.58 (dd, *J* = 12.1, 4.3 Hz, 1H), 2.57–2.28 (m, 3H), 2.14 (d, *J* = 8.8 Hz, 1H), 2.07 (s, 3H), 1.95 (dd, *J* = 21.1, 13.0 Hz, 3H), 1.75 (t, *J* = 13.5 Hz, 2H), 1.61 (d, *J* = 7.5 Hz, 3H), 1.47–1.31 (m, 2H), 1.27 (s, 3H), 0.68 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 179.09, 173.81, 170.45, 145.76, 143.58, 134.10, 107.25, 78.41, 69.63, 55.18, 55.02, 47.76, 39.24, 37.56, 36.50, 24.88, 24.02, 23.52, 21.45, 20.77, 11.99; HRMS (ESI) m/z calcd for $C_{22}H_{30}NaO_6$ $[M+Na]^+$ 413.1935, found 413.1935.

*4.2.1.8. General procedure for the synthesis of alkyl 14-deoxyandrographolide-19-oates (**8**)*

To a solution of compound **7a** or **7b** (0.092 mmol) in 2 mL of DMF, were added $K_2CO_2$ (0.026 g, 0.19 mmol), alkyl halide (methyl iodide, benzyl bromide or 2-chloromethylpyridine, 0.14 mmol), then stirred at r.t. for 0.5 h. After the reaction was complete, the reaction mixture was diluted with ethyl acetate (60 mL). The organic layer was separated, washed with water (20 mL×3) and brine (20 mL×3), dried with anhydrous $Na_2SO_4$, concentrated under reduce pressure, and purified by column chromatography (EtOAc/PE = 1/3) to yield corresponding compound **8**.

*4.2.1.8.1. Methyl 3-acetyl-14-deoxyandrographolide-19-oate (**8a**).* A light yellowish solid (0.032 g, 86%); Mp 115-119 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.11 (s, 1H), 4.91 (s, 1H), 4.79 (s, 2H), 4.63 (s, 1H), 4.57 (dd, *J* = 12.2, 4.5 Hz, 1H), 3.65 (s, 3H), 2.44 (dd, *J* = 11.0, 7.4 Hz, 3H), 2.12 (d, *J* = 7.9 Hz, 1H), 2.07 (s, 3H), 2.02–1.84 (m, 3H), 1.84–1.68 (m, 2H), 1.67-1.54 (m, 2H), 1.54–1.43 (m, 1H), 1.43–1.29 (m, 2H), 1.24 (s, 3H), 0.61 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 173.79, 173.54, 170.53, 145.91, 143.51, 134.12, 107.13, 78.73, 69.63, 55.13, 54.96, 50.63, 47.87, 39.06, 37.59, 36.53, 29.18, 25.02, 23.98, 23.48, 21.39, 20.84, 11.95; HRMS (ESI) m/z calcd for $C_{23}H_{36}NO_6$ $[M+NH_4]^+$ 422.2537, found 422.2544.

*4.2.1.8.2. Benzyl 3-acetyl-14-deoxyandrographolide-19-oate (**8b**).* A light yellowish solid (0.040 g, 91%); Mp 125-128 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.37 (m, 5H), 7.11 (s, 1H), 5.17 (d, *J* = 12.6 Hz, 1H), 5.09 (d, *J* = 12.6 Hz, 1H), 4.90 (s, 1H), 4.79 (d, *J* = 1.5 Hz, 2H), 4.62 (s, 1H), 4.58 (d, *J* = 6.0 Hz, 1H), 2.42 (dd, *J* = 18.3, 7.4 Hz, 3H), 2.21–2.08 (m, 1H), 2.06 (s, 3H), 2.04–1.85 (m, 3H), 1.85–1.70 (m, 2H), 1.69–1.55 (m, 3H), 1.55–1.34 (m, 2H), 1.29 (s, 3H), 0.58 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 172.86, 170.48, 145.83, 143.49, 135.34, 134.15, 127.96, 127.71, 127.58, 107.19, 78.77, 69.64, 65.55, 55.07, 48.01, 39.07, 37.58, 36.48, 25.00, 24.13, 23.98, 23.80, 21.38, 20.82, 12.07; HRMS (ESI) m/z calcd for $C_{29}H_{40}NO_6$ $[M+NH_4]^+$ 498.2853, found 498.2857.

*4.2.1.8.3. 2-Pyridylmethyl 3-acetyl-14-deoxyandrographolide-19-oate (**8c**).* A light yellowish solid (0.036 g, 82%); Mp 132-136 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 8.60 (s, 1H), 7.70 (d, *J* = 7.5 Hz,

1H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 9.1 Hz, 1H), 7.11 (s, 1H), 5.24 (q, *J* = 13.8 Hz, 2H), 4.91 (s, 1H), 4.80 (s, 2H), 4.62 (s, 2H), 2.44 (s, 3H), 2.17 (s, 1H), 2.07 (s, 3H), 2.03–1.86 (m, 3H), 1.86–1.67 (m, 3H), 1.57 (d, *J* = 23.5 Hz, 3H), 1.41 (d, *J* = 12.8 Hz, 1H), 1.33 (s, 3H), 0.58 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 172.60, 170.39, 155.27, 148.69, 145.75, 143.50, 136.33, 134.12, 122.38, 121.42, 107.22, 78.80, 69.62, 66.06, 55.08, 48.08, 39.09, 37.56, 36.46, 29.19, 24.99, 24.13, 23.98, 23.77, 21.39, 20.80, 12.05; HRMS (ESI) m/z calcd for $C_{28}H_{36}NO_6$ $[M+H]^+$ 482.2537, found 482.2547.

*4.2.1.8.4. Methyl 14-deoxyandrographolide-19-oate (**8d**).* A light yellowish solid (84%); Mp 76-80 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.12 (d, *J* = 1.5 Hz, 1H), 4.92 (s, 1H), 4.79 (d, *J* = 1.8 Hz, 2H), 4.63 (s, 1H), 3.66 (s, 3H), 3.20–2.90 (m, 1H), 2.61–2.29 (m, 2H), 2.26–1.95 (m, 3H), 1.92–1.85 (m, 3H), 1.82–1.65 (m, 2H), 1.65–1.50 (m, 2H), 1.41 (s, 3H), 1.32–1.11 (m, 3H), 0.54 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 177.49, 173.81, 146.28, 143.52, 134.14, 106.70, 77.69, 69.62, 55.09, 54.94, 50.75, 49.03, 39.33, 37.89, 37.21, 28.14, 25.55, 24.09, 23.23, 21.52, 11.99; HRMS (ESI) m/z calcd for $C_{21}H_{30}NaO_5$ $[M+Na]^+$ 385.1985, found 385.1992.

*4.2.1.8.5. Benzyl 14-deoxyandrographolide-19-oate (**8e**).* A light yellowish solid (74%); Mp 92-95 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.36 (d, *J* = 4.4 Hz, 5H), 7.11 (s, 1H), 5.21 (d, *J* = 12.4 Hz, 1H), 5.01 (d, *J* = 12.4 Hz, 1H), 4.90 (s, 1H), 4.79 (d, *J* = 1.6 Hz, 2H), 4.61 (s, 1H), 3.32 – 3.02 (m, 2H), 2.43 (t, *J* = 13.5 Hz, 2H), 2.23–1.96 (m, 3H), 1.87 (dd, *J* = 15.4, 11.5 Hz, 3H), 1.74 (dd, *J* = 12.7, 3.8 Hz, 2H), 1.61 (d, *J* = 9.7 Hz, 3H), 1.44 (s, 3H), 1.29–1.18 (m, 4H), 0.51 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 176.61, 173.80, 146.20, 143.48, 134.84, 134.17, 128.10, 127.82, 127.76, 106.74, 77.76, 69.62, 65.78, 55.27, 54.93, 49.21, 39.38, 37.89, 37.23, 28.15, 25.62, 24.10, 23.43, 21.53, 12.12; HRMS (ESI) m/z calcd for $C_{27}H_{35}O_5$ $[M+H]^+$ 439.2479, found 439.2493.

*4.2.1.8.6. 2-Pyridylmethyl 14-deoxyandrographolide-19-oate (**8f**).* A light yellowish solid (83%); Mp 105-108 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 8.58 (d, *J* = 4.3 Hz, 1H), 7.71 (td, *J* = 7.7, 1.6 Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.23 (d, *J* = 7.0 Hz, 1H), 7.10 (s, 1H), 5.23 (q, *J* = 13.5 Hz, 2H), 4.89 (s, 1H), 4.77 (d, *J* = 1.7 Hz, 2H), 4.60 (s, 1H), 3.17 (dd, *J* = 12.1, 4.2 Hz, 1H), 2.56–2.33 (m, 2H), 2.23–1.98 (m, 3H), 1.90 (dd, *J* = 15.5, 11.9 Hz, 3H), 1.82–1.64 (m, 2H), 1.60 (d, *J* = 7.8 Hz, 2H), 1.47 (s, 3H), 1.36–1.10 (m, 3H), 0.53 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 175.97, 154.74, 148.93, 146.16, 143.46, 136.36, 134.19, 122.49, 121.29, 106.82, 77.71, 69.62, 65.84, 55.24, 54.96, 49.48, 39.34, 37.84, 37.17, 28.19, 25.51, 24.10, 23.78, 21.52, 12.13; HRMS (ESI) m/z calcd for $C_{26}H_{34}NO_5$ $[M+H]^+$ 440.2431, found 440.2445.

*4.2.2 Synthesis of the 14-deoxy-11,12-didehydroandrographolide-19-oic acid derivatives **14a-f***

*4.2.2.1. 3,19-Isopropylidene-andrographolide (**9**)*

Andrographolide (**1**) (10.0 g, 28.54 mmol) was dissolved in a mixed solution of toluene (200 mL) DMSO (27 mL), and to the formed solution were added 2,2-dimethoxypropane (14 mL) and TsOH (catalytic amount). After the reaction mixture was stirred at 80 ℃ for 1.5 h, and cooled down to room temperature, the reaction was quenched by adding TEA (7 mL). The reaction mixture was diluted with toluene (150 mL) and washed with water. The organic layer was separated, dried with anhydrous $Na_2SO_4$, concentrated under reduced pressure and rinsed with ether to yield **9**. White solid, 93% yield; $^1$H NMR (300 MHz, $CDCl_3$) δ： 6.96 (t, *J* = 6.4 Hz, 1H),

5.03 (d, *J* = 5.9 Hz, 1H), 4.91 (s, 1H), 4.63 (s, 1H), 4.48–4.43 (dd, *J* = 10.4, 6.1 Hz, 1H), 4.28–4.24 (dd, *J* = 1.9, 10.4 Hz, 1H), 3.97 (d, *J* = 5.8 Hz, 1H), 3.52–3.48 (dd, *J* = 3.4, 8.4 Hz, 1H), 3.2 (m, 1H), 2.64–2.40 (m, 4H), 2.05–1.97 (m, 2H), 1.85–1.72 (m, 4H), 1.32–1.25 (m, 3H), 1.42 (s, 3H), 1.37 (s, 3H), 1.20 (s, 3H), 0.96 (s, 3H). The $^1$H NMR spectroscopic data were matched those reported in literature [26].

*4.2.2.2. 3,19-Isopropylidene-14-acetyl-andrographolide (**10**)*

A mixture of 3,19-isopropylidene-andrographolide (**9**) (1.00 g, 2.56 mmol) and acetic anhydride (20 mL) was refluxed for 1.5 h. After cooled down to room temperature, the reaction mixture was neutralized with saturated $NaHCO_3$ until no bubble was formed and then diluted with ethyl acetate (120 mL). The organic layer was separated, washed with water (60 mL×3) and brine (60 mL×3), dried with anhydrous $Na_2SO_4$, concentrated under reduce pressure, and purified by column chromatography (EtOAc/PE = 1/4) to give compound **10** as a white solid (1.06 g, 93%). $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.01 (td, *J* = 6.9, 1.6 Hz, 1H), 5.91 (d, *J* = 6.0 Hz, 1H), 4.88 (s, 1H), 4.52 (d, *J* = 6.0 Hz, 2H), 4.22 (dd, *J* = 11.2, 1.9 Hz, 1H), 3.94 (d, *J* = 11.6 Hz, 1H), 3.48 (dd, *J* = 8.5, 3.7 Hz, 1H), 3.16 (d, *J* = 11.6 Hz, 1H), 2.55–2.30 (m, 3H), 2.11 (s, 3H), 2.05–1.87 (m, 2H), 1.87–1.76 (m, 2H), 1.76–1.61 (m, 3H), 1.39 (s, 3H), 1.35 (s, 3H), 1.26 (td, *J* = 12.6, 7.7 Hz, 2H), 1.18 (s, 3H), 0.92 (s, 3H). The $^1$H NMR spectroscopic data were matched those reported in literature [26].

*4.2.2.3. 14-Deoxy-14,15-didehydroandrographolide (**11**)*

To a solution of compound **10** (0.70 g, 1.62 mmol) in 20 mL of methylene chloride were added DMAP (0.04 g, 0.324 mmol), and the formed mixture was stirred at r.t. for 24 h. After the reaction was complete, the reaction mixture was diluted with methylene chloride (120 mL). The organic layer was separated, washed with dilute hydrochloric acid (60 mL), water (60 mL×3), and brine (60 mL), dried with anhydrous $Na_2SO_4$, concentrated under reduce pressure, and purified by column chromatography (EtOAc/PE = 1/2) to give compound **11** as a white solid (0.48 g, 89.2%). $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.05–6.92 (m, 1H), 6.67 (d, *J* = 7.6 Hz, 1H), 6.17 (d, *J* = 3.7 Hz, 1H), 4.85 (s, 1H), 4.41 (s, 1H), 4.25–4.13 (m, 1H), 3.49 (t, *J* = 8.2 Hz, 1H), 3.32 (d, *J* = 11.2 Hz, 1H), 2.95 (s, 2H), 2.49 (dt, *J* = 7.8, 3.7 Hz, 1H), 2.45–2.30 (m, 2H), 1.96 (t, *J* = 12.3 Hz, 1H), 1.90–1.64 (m, 6H), 1.24 (s, 3H), 0.69 (d, *J* = 2.1 Hz, 3H). The $^1$H NMR spectroscopic data were matched those reported in literature [26].

*4.2.2.4. 14-Deoxy-11,12-didehydroandrographolide (**2**)*

To a solution of compound **10** (0.7 g, 1.62 mmol) in 20 ml of methylene chloride were added DMAP (0.30 g, 2.43 mmol), then stirred at 50 °C for 24 h. After the reaction was complete, the reaction mixture was diluted with methylene chloride (120 mL). The organic layer was separated, washed with dilute hydrochloric acid (60 mL), water (60 mL×2) and brine (60 mL×1), dried with anhydrous $Na_2SO_4$ for 14 h, concentrated under reduce pressure, and purified by column chromatography (EtOAc/PE = 1/2) to give compound **2** as a white solid (0.46 g, 85%). $^1$H NMR (300 MHz, DMSO-$d_6$) δ: 7.65 (s, 1H), 6.75 (dd, *J* = 15.8, 10.1Hz, 1H), 6.13 (d, *J* = 15.8 Hz, 1H), 5.05 (d, *J* = 4.9 Hz, 1H), 4.89 (s, 2H), 4.73 (s, 1H), 4.43 (s, 1H), 4.14 (dd, *J* = 7.4, 2.7 Hz, 1H), 3.85 (dd, *J* = 10.9, 2.7 Hz, 1H), 3.33–3.13 (m, 2H), 2.36 (d, *J* = 10.3 Hz, 2H), 2.03–1.92 (m, 1H), 1.74-1.70 (m, 1H), 1.63–1.54 (m, 2H), 1.47–1.40 (m, 1H), 1.38–1.29 (m, 1H), 1.24–1.13 (m, 2H), 1.09 (s, 3H), 0.76 (s, 3H). The $^1$H NMR spectroscopic data were matched those reported in literature [27].

*4.2.2.5. 14-Deoxy-11,12-didehydroandrographolide-19-al (**12a**)*

**12a** was synthesized from **2** using the same method described for the synthesis of **6a**. A white solid (91%); Mp 65-69 °C; $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ: 9.97 (s, 1H), 7.69 (s, 1H), 6.73 (dd, *J* = 15.9 , 10.0 Hz, 1H), 6.15 (d, *J* = 15.9 Hz, 1H), 5.17 (d, *J* = 4.5 Hz, 1H), 4.9 (s, 2H), 4.75 (s, 1H), 4.43 (s, 1H), 3.32 (s, 1H), 2.36 (d, *J* = 13.1 Hz, 1H), 2.03–1.90 (m, 3H), 1.79–1.74 (m, 2H), 1.47–1.41 (m, 2H), 1.28–1.23 (m, 1H), 1.21–1.13 (m, 1H), 1.09 (s, 3H), 0.65 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 207.20, 171.73, 146.83, 142.73, 135.01, 128.64, 120.96, 109.31, 76.85, 69.14, 59.91, 54.68, 52.32, 38.63, 37.95, 35.98, 27.98, 22.55, 18.87, 13.99; HRMS (ESI) m/z calcd for $C_{20}H_{26}NaO_4$ $[M+Na]^+$ 353.1723, found 353.1722.

*4.2.2.6. 3-Acetyl-14-deoxy-11,12-didehydroandrographolide-19-al (**12b**)*

**12b** was synthesized from **12a** using the same method described for the synthesis of **6b**. A light yellowish solid (76%); Mp 64-68 °C; $^{1}$H NMR (300 MHz, $CDCl_3$) δ: 10.08 (s, 1H), 7.19 (s, 1H), 6.90 (dd, *J* = 15.8, 10.1 Hz, 1H), 6.15 (d, *J* = 15.8 Hz, 1H), 4.83 (s, 3H), 4.78–4.67 (m, 1H), 4.56 (s, 1H), 2.49–2.39 (m, 2H), 2.06 (s, 3H), 1.96–1.88 (m, 1H), 1.69–1.64 (m, 3H), 1.49–1.37 (m, 2H), 1.35–1.33 (m, 1H), 1.11 (s, 3H), 0.88–0.85 (m, 1H), 0.80 (s, 3H) ; $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 203.7, 169.9, 146.8, 142.9, 134.9, 128.6, 121.1, 109.3, 78.0, 69.1, 59.7, 55.6, 51.6, 38.1, 37.1, 35.6, 24.0, 22.7, 20.6, 20.5, 15.5; HRMS (ESI) m/z calcd for $C_{22}H_{29}O_5$ $[M+H]^+$ 373.2010, found 373.2014.

*4.2.2.7. 14-Deoxy-11,12-didehydroandrographolide-19-oic acid (**13a**)*

**13a** was synthesized from **12a** using the same method described for the synthesis of **7a**. A white solid (64%); Mp 170-172 °C; $^{1}$H NMR (300 MHz, $CDCl_3$) δ: 7.20 (s, 1H), 6.89 (dd, *J* = 15.7, 10.2 Hz, 1H), 6.13 (d, *J* = 15.7 Hz, 1H), 4.82 (s, 3H), 4.55 (s, 1H), 3.19 (dd, *J* = 12.1, 4.3 Hz, 1H), 2.47 (d, *J* = 13.1 Hz, 1H), 2.33 (d, *J* = 10.1 Hz, 1H), 2.09-1.95 (m, 3H), 1.92–1.78 (m, 2H), 1.6 (d, *J* = 13.7 Hz, 1H), 1.47 (s, 3H), 1.32–1.13 (m, 3H), 0.80 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 181.09, 172.09, 147.52, 142.90, 135.19, 128.63, 120.71, 108.50, 77.62, 69.32, 60.52, 54.52, 48.75, 38.94, 38.54, 36.32, 27.83, 24.24, 23.52, 13.07; HRMS (ESI) m/z calcd for $C_{20}H_{26}NaO_5$ $[M+Na]^+$ 369.1672, found 369.1681.

*4.2.2.8. 3-Acetyl-14-deoxy-11,12-didehydroandrographolide-19-oic acid (**13b**)*

**13b** was synthesized from **12b** using the same method described for the synthesis of **7a**. A light yellowish solid (78%); Mp 211-214 °C; $^{1}$H NMR (300 MHz, $CDCl_3$) δ: 7.17 (s, 1H), 6.90 (dd, *J* = 15.8, 10.0 Hz, 1H), 6.12 (d, *J* = 15.8 Hz, 1H), 4.81 (s, 3H), 4.61–4.56 (m, 1H), 4.55 (s, 1H), 2.49-2.45 (m, 1H), 2.33 (t, *J* = 9.3 Hz, 1H), 2.06 (s, 3H), 2.04–1.95 (m, 2H), 1.77–1.65 (m, 2H), 1.63–1.58 (m, 2H), 1.39 (d, *J* = 11.6 Hz, 1H), 1.29 (s, 3H), 0.86 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 179.49, 171.81, 170.55, 147.08, 142.88, 135.02, 128.63, 120.90, 109.04, 78.56, 69.18, 60.89, 54.34, 47.80, 38.69, 37.89, 35.95, 23.89, 23.59, 20.83, 12.77; HRMS (ESI) m/z calcd for $C_{22}H_{28}NaO_6$ $[M+Na]^+$ 411.1778, found 411.1784.

*4.2.2.9. Methyl 3-acetyl-14-deoxy-11,12-didehydroandrographolide-19-oate (**14a**)*

**14a** was synthesized from **13b** using the general procedure described for the synthesis of **8**. A light yellowish solid (64%); Mp 52-55 °C; $^{1}$H NMR (300 MHz, $CDCl_3$) δ: 7.16 (s, 1H), 6.88 (dd, *J* = 15.9, 10.1 Hz, 1H), 6.12 (d, *J* = 15.8 Hz, 1H), 4.80 (s, 2H), 4.79 (s, 1H), 4.61–4.55 (m, 1H), 4.54 (s, 1H), 3.66 (s, 3H), 2.49–2.41 (m, 1H), 2.40–2.22 (m, 2H), 2.05 (s, 1H), 2.03–1.91 (m, 2H), 1.74–1.66 (m, 1H), 1.64–1.46 (m, 2H), 1.44–1.27 (m, 2H), 1.24 (s, 3H), 0.77 (s, 3H); $^{13}$C NMR

(75 MHz, $CDCl_3$) δ: 173.49, 171.71, 170.51, 147.28, 142.74, 135.13, 128.66, 120.85, 108.85, 78.90, 69.10, 60.93, 54.32, 50.69, 47.95, 38.50, 37.93, 36.00, 24.02, 23.97, 23.56, 20.81, 12.72; HRMS (ESI) m/z calcd for $C_{23}H_{31}O_6$ $[M+H]^+$ 403.2115, found 403.2121.

*4.2.2.10. Benzyl 3-acetyl-14-deoxy-11,12-didehydroandrographolide-19-oate (**14b**)*

**14b** was synthesized from **13b** using the general procedure described for the synthesis of **8**. A light yellowish solid (62%); Mp 74-78 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.39–7.30 (m, 5H), 7.16 (s, 1H), 6.88 (dd, *J* = 15.9, 10.1 Hz, 1H), 6.11 (d, *J* = 15.8 Hz, 1H), 5.13 (q, *J* = 12.5 Hz, 2H), 4.80 (s, 2H), 4.78 (s, 1H), 4.59 (dd, *J* = 12.1, 4.5 Hz, 1H), 4.53 (s, 1H), 2.47–2.40 (m, 1H), 2.39–2.26 (m, 2H), 2.02 (s, 3H), 1.98–1.95 (m, 2H), 1.74–1.66 (m, 1H), 1.68–1.41 (m, 3H), 1.39–1.33 (m, 1H), 1.29 (s, 3H), 0.73 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 172.82, 171.76, 170.45, 147.21, 142.83, 135.13, 128.61, 127.97, 127.67, 120.84, 108.90, 78.94, 69.13, 65.65, 60.87, 54.41, 48.08, 38.51, 37.88, 35.98, 24.04, 24.00, 23.87, 20.78, 12.84; HRMS (ESI) m/z calcd for $C_{29}H_{34}NaO_6$ $[M+Na]^+$ 501.2248, found 501.2253.

*4.2.2.11. 2-Pyridylmethyl 3-acetyl-14-deoxy-11,12-didehydroandrographolide-19-oate (**14c**)*

**14c** was synthesized from **13b** using the general procedure described for the synthesis of **8**. A light yellowish solid (73%); Mp 57-60 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 8.61 (d, *J* = 4.4 Hz, 1H), 7.72 (td, *J* = 7.7, 1.7 Hz, 1H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.25 (d, *J* = 6.9 Hz, 1H), 7.18 (s, 1H), 6.90 (dd, *J* = 15.8, 10.2 Hz, 1H), 6.14 (d, *J* = 15.8 Hz, 1H), 5.26 (dd, *J* = 26.9, 11.0 Hz, 2H), 4.83 (s, 2H), 4.80 (s, 1H), 4.64 (dd, *J* = 12.2, 4.5 Hz, 1H), 4.55 (s, 1H), 2.55–2.26 (m, 3H), 2.07 (s, 3H), 2.02 (d, *J* = 11.7 Hz, 2H), 1.83–1.68 (m, 1H), 1.67–1.51 (m, 3H), 1.41 (dd, *J* = 15.2, 4.5 Hz, 1H), 1.35 (s, 3H), 0.74 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 172.60, 171.71, 170.38, 155.20, 148.79, 147.13, 142.73, 136.28, 135.11, 128.65, 122.42, 121.57, 120.85, 108.95, 78.95, 69.10, 66.25, 60.87, 54.44, 48.12, 38.53, 37.88, 35.97, 29.19, 24.00, 23.84, 20.80, 12.81; HRMS (ESI) m/z calcd for $C_{28}H_{34}NO_6$ $[M+H]^+$ 480.2381, found 480.2379.

*4.2.2.12. Methyl 14-deoxy-11,12-didehydroandrographolide-19-oate (**14d**)*

**14d** was synthesized from **13a** using the general procedure described for the synthesis of **8**. A light yellowish solid (74%). Mp 53-57 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.17 (s, 1H), 6.90 (dd, *J* = 15.8, 10.0 Hz, 1H), 6.12 (d, *J* = 15.8 Hz, 1H), 4.81 (s, 2H), 4.80–4.75 (m, 1H), 4.54 (s, 1H), 3.67 (s, 3H), 3.12 (dd, *J* = 12.0, 4.3 Hz, 1H), 2.50–2.41 (m, 1H), 2.31 (d, *J* = 10.1 Hz, 1H), 2.04–1.95 (m, 3H), 1.85–1.72 (m, 2H), 1.601–.56 (m, 1H), 1.42 (s, 3H), 1.29–1.13 (m, 3H), 0.70 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 177.29, 171.72, 147.63, 142.55, 135.29, 128.73, 120.74, 108.41, 77.96, 69.10, 60.63, 54.60, 50.82, 49.16, 38.79, 38.69, 36.39, 28.00, 24.53, 23.30, 12.80; HRMS (ESI) m/z calcd for $C_{21}H_{28}NaO_5$ $[M+Na]^+$ 383.1829, found 383.1832.

*4.2.2.13. Benzyl 14-deoxy-11,12-didehydroandrographolide-19-oate (**14e**)*

**14e** was synthesized from **13a** using the general procedure described for the synthesis of **8**. A light yellowish solid (65%); Mp 50-53 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.487–.28 (m, 5H), 7.16 (s, 1H), 6.88 (dd, *J* = 15.9, 10.3 Hz, 1H), 6.11 (d, *J* = 15.8 Hz, 1H), 5.20 (d, *J* = 12.3 Hz, 1H), 5.03 (d, *J* = 12.3 Hz, 1H), 4.81 (s, 2H), 4.77 (s, 1H), 4.52 (s, 1H), 3.13 (dd, *J* = 11.8, 4.3 Hz, 1H), 2.49–2.36 (m, 1H), 2.30 (d, *J* = 10.2 Hz, 1H), 2.05–1.97 (m, 3H), 1.82–1.70 (m, 2H), 1.60–1.55 (m, 1H), 1.45 (s, 3H), 1.29–1.14 (m, 3H), 0.65 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 176.40, 171.72, 147.52, 142.53, 135.34, 128.12, 127.89, 127.85, 120.72, 108.47, 78.03, 69.10, 65.91, 60.62, 54.76, 49.31, 38.81, 38.71, 36.37, 28.00, 24.57, 23.51, 12.93; HRMS (ESI) m/z calcd for

$C_{27}H_{33}O_5$ $[M+H]^+$ 437.2323, found 437.2302.

*4.2.2.14. 2-Pyridylmethyl 14-deoxy-11,12-didehydroandrographolide-19-oate (**14f**)*

**14f** was synthesized from **13a** using the general procedure described for the synthesis of **8**. A light yellowish solid (56%); Mp 48-51 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 8.60 (d, *J* = 4.5 Hz, 1H), 7.72 (td, *J* = 7.7, 1.7 Hz, 1H), 7.34 (d, *J* = 7.8 Hz, 1H), 7.28–7.23 (m, 1H), 7.17 (s, 1H), 6.90 (dd, *J* = 15.8, 10.1 Hz, 1H), 6.13 (d, *J* = 15.8 Hz, 1H), 5.37–5.18 (m, 2H), 4.82 (d, *J* = 1.4 Hz, 2H), 4.80 (s, 1H), 4.54 (s, 1H), 2.53–2.41 (m, 1H), 2.33 (d, *J* = 10.3 Hz, 1H), 2.15–1.95 (m, 4H), 1.80 (ddd, *J* = 15.4, 9.9, 4.3 Hz, 2H), 1.72–1.56 (m, 2H), 1.51 (s, 3H), 1.3–81.29 (m, 2H), 0.70 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 175.80, 154.66, 148.99, 147.52, 142.55, 136.34, 135.36, 128.72, 122.54, 121.45, 120.72, 108.55, 77.97, 69.11, 65.93, 60.65, 54.69, 49.59, 38.77, 38.65, 36.30, 28.05, 24.46, 23.89, 12.93; HRMS (ESI) m/z calcd for $C_{26}H_{32}NO_5$ $[M+H]^+$ 438.2275, found 438.2280.

*4.2.3. Synthesis of the 14-deoxy-14,15-didehydroandrographolide-19-oic acid derivatives **18a-d***

*4.2.3.1. 14-Acetylandrographolide (**15**)*

A mixture of 3,19-isopropylidene-andrographolide (**9**) (7.0 g, 17.93 mmol) and acetic anhydride (60 mL) was refluxed for 1.5 h. After cooled down to room temperature, the reaction mixture was neutralized with saturated $NaHCO_3$ until no bubble was formed and then diluted with methylene chloride (100 mL). The organic layer was separated, washed with saturated $NaHCO_3$ (120 mL×3) and brine (120 mL×3), and concentrated under reduce pressure to provide a crude. The crude was dissolved in aqueous acetic acid solution (AcOH/$H_2O$: 28 mL/12 mL) and stirred at r.t. for 1 h. After the reaction was complete, $NaHCO_3$ powder was added until no bubble was released, and the resulted mixture was diluted with methylene chloride (80 mL). The organic layer was separated, washed with saturated $NaHCO_3$ (100 mL×3) and brine (100 mL×3), dried with anhydrous $Na_2SO_4$, concentrated under reduce pressure, and recrystallized from ethyl acetate-petroleum ether to provide compound **15** as a white needle (5.21 g, 85%). $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.01 (td, *J* = 6.9, 1.5 Hz, 1H), 5.92 (d, *J* = 6.0 Hz, 1H), 4.88 (s, 1H), 4.57–4.51 (m, 2H), 4.24 (dd, *J* = 11.2, 1.9 Hz, 1H), 4.18 (d, *J* = 11.1 Hz, 1H), 3.49 (t, *J* = 16.0 Hz, 1H), 3.33 (d, *J* = 11.1 Hz, 1H), 2.45–2.37 (m, 3H), 2.31 (s, 2H), 2.12 (s, 3H), 2.01–1.92 (m, 1H), 1.831–.72 (m, 5H), 1.25 (s, 3H), 1.33–1.20 (m, 3H), 0.67 (s, 3H). The $^1$H NMR spectroscopic data were matched those reported in literature [26].

*4.2.3.2. 14-Acetylandrographolide-19-al (**16a**)*

**16a** was synthesized from **15** using the same method described for the synthesis of **6a**. A light yellowish solid (91%). $^1$H NMR (300 MHz, $CDCl_3$) δ: 9.74 (d, *J* = 1.9 Hz, 1H), 6.99 (t, *J* = 6.0 Hz, 1H), 5.90 (d, *J* = 5.8 Hz, 1H), 4.92 (s, 1H), 4.62–4.51 (m, 2H), 4.23 (dd, *J* = 11.3, 1.7 Hz, 1H), 3.22 (d, *J* = 9.7 Hz, 1H), 2.57–2.45 (m, 2H), 2.43–2.28 (m, 3H), 2.10 (s, 1H), 2.08–1.97 (m, 2H), 1.96–1.92 (m, 1H), 1.90–1.73 (m, 3H), 1.68–1.50 (m, 2H), 1.44–1.33 (m, 1H), 1.28 (s, 3H), 0.64 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ：207.51, 170.47, 169.01, 150.01, 146.08, 124.06, 109.37, 77.47, 71.57, 67.75, 55.53, 54.62, 52.79, 39.44, 37.60, 37.06, 28.53, 25.30, 23.80, 20.66, 19.39, 13.69; HRMS (ESI) m/z calcd for $C_{22}H_{30}O_6Na$ $[M+Na]^+$ 413.1940, found 413.1932.

*4.2.3.3. 3,14-Diacetylandrographolide-19-al (**16b**)*

**16b** was synthesized from **16a** using the same method described for the synthesis of **6b**. A light yellowish solid (76%). $^1$H NMR (300 MHz, $CDCl_3$) δ 10.04 (s, 1H), 6.99 (t, *J* = 6.0 Hz, 1H), 5.93

(d, *J* = 5.6 Hz, 1H), 4.91 (s, 1H), 4.78–4.66 (m, 1H), 4.52 (s, 2H), 4.25 (dd, *J* = 11.2, 1.7 Hz, 1H), 2.55–2.28 (m, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.02–1.75 (m, 5H), 1.49–1.21 (m, 4H), 1.10 (s, 3H), 0.65 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 203.98, 170.39, 170.31, 168.87, 149.73, 146.06, 124.15, 109.31, 78.10, 71.50, 67.70, 56.38, 54.38, 51.99, 38.99, 37.25, 36.26, 25.36, 24.61, 23.88, 21.0, 20.90, 20.65, 15.13; HRMS (ESI) *m/z* calcd for $C_{24}H_{32}O_7Na$ $[M+Na]^+$ 455.2046, found 455.2052.

*4.2.3.4. 14-Acetylandrographolide-19-oic acid (**17a**)*

**17a** was synthesized from **16a** using the same method described for the synthesis of **7a**. A white solid (42%). $^1$H NMR (300 MHz, $CDCl_3$) δ: 12.31 (s, 1H), 6.83 (t, *J* = 6.3 Hz, 1H), 5.92 (d, *J* = 5.3 Hz, 1H), 4.86 (s, 1H), 4.59–4.52 (m, 2H), 4.38 (bs, 1H), 4.29–4.26 (d, 1H), 3.09 (d, 1H ), 2.50–2.32 (m, 2H), 2.12–1.91 (m, 8H), 1.81–1.59 (m, 3H),1.32–1.20 (m, 5H), 0.63 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 181.42, 170.51, 169.10, 150.32, 146.57, 123.98, 108.69, 77.75, 71.61, 67.76, 55.51, 55.28, 49.38, 39.79, 37.90, 37.74, 28.52, 25.42, 25.30, 24.07, 20.69, 12.78; HRMS (ESI) *m/z* calcd for $C_{22}H_{30}O_7Na$ $[M+Na]^+$ 429.1889, found 429.1898.

*4.2.3.5. 3,14-Diacetylandrographolide-19-oic acid (**17b**)*

**17b** was synthesized from **16b** using the same method described for the synthesis of **7a**. A white solid (76%). $^1$H NMR (300 MHz, $CDCl_3$) δ: 12.34 (s, 1H), 6.84 (t, *J* = 6.5 Hz, 1H), 5.95 (d, *J* = 5.4 Hz, 1H), 4.87(s, 1H), 4.59–4.45 (m, 3H), 4.30–4.27 (d, 1H), 2.42–2.27 (m, 4H), 2.09–1.99 (m, 8H), 1.93–1.76 (m, 2H), 1.631–.60 (m, 1H), 1.52–1.31 (m, 3H), 1.15 (s, 3H), 0.68 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 179.31, 170.82, 170.44, 169.03, 150.06, 146.31, 124.08, 109.02, 78.67, 71.58, 67.76, 55.55, 55.29, 48.26, 39.52, 37.58, 37.06, 25.24, 25.07, 24.56, 24.44, 24.09, 21.23, 20.69, 12.52; HRMS (ESI) *m/z* calcd for $C_{24}H_{32}O_8Na$ $[M+Na]^+$ 471.1995, found 471.1988.

*4.2.3.6. General procedure for the synthesis of alkyl 14-deoxy-14,15-didehydroandrographolide -19-oates (**18**)*

To a solution of compound **17a** or **17b** (0.20 mmol) in 3 ml of DMF, $K_2CO_3$ (0.028 g, 0.20 mmol), alkyl halide (methyl iodide or benzyl bromide, 0.20 mmol), then stirred at r.t. for 0.5 h. Then DMAP (catalytic amount) were added and stirred at r.t. for 0.5 h. After the reaction was complete, the reaction mixture was diluted with ethyl acetate (120 mL). The organic layer was separated, washed with water (60 mL×2) and brine (60 mL×1), dried with anhydrous $Na_2SO_4$, concentrated under reduce pressure, and purified by column chromatography (EtOAc/PE = 1/3) to give corresponding compound **18**.

*4.2.3.6.1. Methyl 3-acetyl-14-deoxy-14,15-didehydro-andrographolide-19-oate (**18a**):* a light yellowish solid (0.030 g, 42%); Mp 118-121°C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.01 (dd, *J* = 3.3, 1.7 Hz, 1H), 6.70 (t, *J* = 7.2 Hz, 1H), 6.17 (dd, *J* = 3.5, 0.7 Hz, 1H), 4.88 (s, 1H), 4.58 (dd, *J* = 12.1, 4.3 Hz, 1H), 4.44 (s, 1H), 3.67 (s, 3H), 2.63–2.28 (m, 4H), 2.08 (s, 3H), 2.05–1.93 (m, 2H), 1.91–1.70 (m, 3H), 1.57–1.31 (m, 3H), 1.25 (s, 3H), 0.67 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 173.45, 170.50, 167.45, 145.87, 144.97, 143.57, 125.34, 108.43, 104.48, 78.57, 55.26, 54.72, 50.74, 47.84, 38.98, 37.12, 36.64, 25.45, 24.73, 23.99, 23.49, 20.85, 12.01; HRMS (ESI) m/z calcd for $C_{23}H_{31}O_6$ $[M+H]^+$ 403.2115, found 403.2115.

*4.2.3.6.2. Benzyl 3-acetyl-14-deoxy-14,15-didehydro-andrographolide-19-oate (**18b**):* light yellowish solid (47%); Mp 123-126 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.35 (s, 5H), 6.99 (d, *J* = 1.4 Hz, 1H), 6.69 (t, *J* = 7.2 Hz, 1H), 6.15 (d, *J* = 3.3 Hz, 1H), 5.13 (q, *J* = 12.5 Hz, 2H), 4.85 (s,

1H), 4.59 (dd, *J* = 12.1, 4.3 Hz, 1H), 4.41 (s, 1H), 2.62–2.24 (m, 4H), 2.05 (s, 3H), 2.00 (d, *J* = 10.2 Hz, 2H), 1.85 (d, *J* = 13.1 Hz, 3H), 1.60–1.33 (m, 3H), 1.29 (s, 3H), 0.62 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 172.76, 170.46, 145.81, 144.97, 143.61, 135.26, 127.98, 127.63, 125.32, 108.47, 104.48, 78.62, 65.64, 55.21, 54.84, 47.99, 38.98, 37.11, 36.59, 29.21, 25.46, 24.71, 24.07, 23.80, 20.80, 12.10; HRMS (ESI) m/z calcd for $C_{29}H_{35}O_6$ $[M+H]^+$ 479.2428, found 479.2432.

*4.2.3.6.3. Methyl 14-deoxy-14,15-didehydro-andrographolide-19-oate (**18c**):* a light yellowish solid (41%); Mp 108-111 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.02 (d, *J* = 1.7 Hz, 1H), 6.71 (t, *J* = 7.2 Hz, 1H), 6.19 (dd, *J* = 3.5, 0.9 Hz, 1H), 4.91 (s, 1H), 4.45 (s, 1H), 3.68 (s, 4H), 3.17 (d, *J* = 7.9 Hz, 1H), 2.65–2.51 (m, 1H), 2.51–2.35 (m, 2H), 2.17–1.99 (m, 2H), 1.98–1.72 (m, 5H), 1.44 (s, 3H), 1.38–1.29 (m, 2H), 0.62 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 177.36, 146.21, 144.94, 143.68, 125.30, 108.03, 104.49, 77.56, 54.95, 50.84, 49.07, 39.25, 37.47, 37.38, 28.09, 25.51, 25.27, 23.25, 12.06; HRMS (ESI) m/z calcd for $C_{21}H_{28}NaO_5$ $[M+Na]^+$ 383.1829, found 383.182.

*4.2.3.6.4. Benzyl 14-deoxy-14,15-didehydro-andrographolide-19-oate (**18d**):* a light yellowish solid (38%); Mp 110-114 °C; $^1$H NMR (300 MHz, $CDCl_3$) δ: 7.35 (d, *J* = 3.8 Hz, 5H), 6.99 (s, 1H), 6.69 (t, *J* = 7.3 Hz, 1H), 6.16 (d, *J* = 3.5 Hz, 1H), 5.20 (d, *J* = 12.4 Hz, 1H), 5.02 (d, *J* = 12.3 Hz, 1H), 4.86 (s, 1H), 4.41 (s, 1H), 3.18 (s, 2H), 2.68–2.27 (m, 3H), 2.04 (td, *J* = 20.9, 9.4 Hz, 3H), 1.94–1.78 (m, 4H), 1.73–1.50 (m, 2H), 1.45 (s, 3H), 0.55 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 172.07, 146.62, 145.43, 144.20, 135.26, 134.22, 128.63, 128.39, 128.31, 108.56, 104.98, 78.12, 66.39, 55.60, 55.44, 49.72, 39.78, 37.96, 37.89, 28.59, 26.01, 25.82, 23.93, 12.66; HRMS (ESI) m/z calcd for $C_{27}H_{32}NaO_5$ $[M+Na]^+$ 459.2142, found 459.2141.

### *4.3. Biology*

#### *4.3.1 Cell culturing*

Mouse fibroblast cell line (NIH-3T3) and human umbilical vein endothelial cell line (HUVEC) were all cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in a humidified atmosphere of 5% $CO_2$ at 37 °C.

#### *4.3.2 MTT assay*

Proliferation of NIH-3T3 and HUVEC cells was evaluated by MTT method. This assay is a well-established colorimetric assay which relies on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to insoluble blue formazan crystals by cellular reductase activities in living cells. Cells were plated on 96-wells with a density of 5000 cells/well. After attachment (24h), the cells were treated for 72h (42h for HUVEC cells) with each compound at different concentrations. After incubation, 20 μL of MTT solution (5 mg/mL in PBS) was added and incubated for an additional 2-4 h. Subsequently, the medium was aspirated carefully, and 150 μL of DMSO was added to dissolve the crystal. The optical density was measured at 490 nm using RT-2100C Micro-plate Reader (Rayto, China). Each experiment was repeated three times, and the data represent the mean of all measurements. Data were recorded and analyzed for the assessment of the effects of the test substances on cell viability and growth inhibition. The $IC_{50}$ values were calculated using regression equation.

#### *4.3.3 Real-time PCR*

NIH-3T3 cells were treated for 1 h with 500 nM screened compounds before the addition of 5 ng/ml TGF-β1. Twenty-four hours after adding TGF-β1, cells were lysed and total RNA was

extracted using an RNAiso Plus (Takara, Dalian) kit. Six microliter of RNA was then reverse transcribed using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Dalian) according to the instruction. The primers for qPCR were listed in Table 2. Subsequent PCR amplification was performed on an ABI StepOne Plus Real-time Detection System (Applied Biosystems, CA, USA) with SYBR® Premix Ex Taq™ (Takara, Dalian). Forty amplification cycles, consisting of 5 s at 95 °C and 60 s at 60 °C, were run on 20 μl reactions. Expression was normalized to GAPDH endogenous control and relative gene expression was calculated by $2^{-\Delta\Delta CT}$ method [28] using cycle time (Ct) values and data for normalization. Data were showed as the mean of three experiments.

**Table 2**

Primers used for real-time qPCR.

| Gene | | Primer sequences | PCR product size (bp) |
|---|---|---|---|
| FN | F | CACCTTCAGTAGAAGGCAGTAG | 122 |
| | R | GTTCTCCTCCACAGCATAGATAG | |
| COL1A1 | F | CCAATGGTGCTCCTGGTATT | 112 |
| | R | GGTTCACCACTGTTACCCTT | |
| α-SMA | F | CCATCATGCGTCTGGACTT | 92 |
| | R | GGCAGTAGTCACGAAGGAATAG | |
| GAPDH | F | GGGTGTGAACCACGAGAAATA | 129 |
| | R | GTCATGAGCCCTTCCACAAT | |

*4.3.4 Western blot*

Whole cell proteins were lysed on ice by RIPA lysis buffer with 1% phenylmethylsulfonyl fluoride (PMSF) for 30 min, centrifuged at 12000g rpm for 5 min and the supernatant was collected. Protein concentration was measured using an enhanced bicinchoninic acid (BCA) protein assay kit. Samples were subsequently loaded into each lane of a 10% SDS–polyacrylamide gel and transferred onto PVDF membranes (Bio-Rad, USA). Membranes were blocked for 1 h in 5% non-fat milk in TBST buffer and then incubated with α-SMA, FN and COL1A1 primary antibodies overnight at 4 °C at a dilution of 1:1000. Having been washed 3 times in TBST, the membranes were incubated with secondary antibody horseradish peroxidase (HRP)-conjugated rabbit IgG (diluted 1:5000) for 2 h at room temperature, and then washed 3 times. Finally, immuno-detection was visualized using the ECL detection system (Tanon-5200) according to the manufacturer's instructions. The load protein was normalized to β-actin. The immunoblotted bands were quantified by Gel-Pro Analyzer 4.0 software. All experiments were performed in triplicate.

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**Appendix A. Supplementary data**

Supplementary data related to this article can be found at

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## Highlights

- 14-Deoxyandrographolide-19-oic acid derivatives were designed and synthesized.
- Anti-fibrotic effects of targets were evaluated by inhibiting the growth of NIH-3T3 cell lines.
- 13 compounds showed better anti-fibrotic activities than andrographolide.
- Compounds **8b** and **14e** displayed best activities with $IC_{50}$ of 12.86 and 13.57 μM against NIH-3T3.
- Compounds **8b** and **14e** suppressed effectively the expression of α-SMA, FN and COL1A1 in NIH-3T3.