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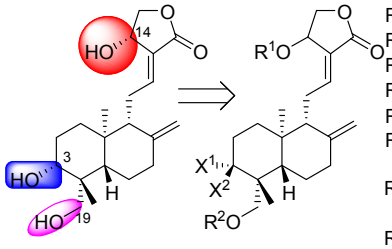

R^1=TBS, R^2=H, X^1=OH, X^2 =H, **5a** (14α)
R^1=TBS, R^2=Ac, X^1=OH, X^2 =H, **6a** (14α)
R^1=TBS, R^2=Ac, X^1=OH, X^2 =H, **6b** (14β)
R^1=TBS, R^2=H, X^1=OH, X^2 =H, **14a** (14α)
R^1=Ac, R^2=H, X^1+ X^2 = O, **17a** (14α)
R^1=Ac, R^2=Ac, X^1=OH, X^2 =H, **18b** (14β)
R^1=TBS, R^2=Ac, X^1+ X^2 = O, **22a** (14α)

5a, 6b, **14a**, **17a**, **18b** and **22a**, selective to cancer cells

6a, selective to zebrafish

R^1 = Ac, R^2 = TBS, X^1 = OH, X^2 = H, **15b** (14β)
R^1 = Ac, R^2 = TBS, X^1 + X^2 = O, **16b** (14β)
R^1 = TBS, R^2 = Ac, X^1 + X^2 = O, **22b** (14β)

The most *in vitro* anti-proliferative active and the most *in vivo* toxic compounds

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ARTICLE TYPE

SAR Studies of 3,14,19-Derivatives of Andrographolide on Anti-Proliferative Activity to Cancer Cells and Toxicity to Zebrafish: An *In Vitro* and *In Vivo* Study

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Andrographolide is bestowed with an interesting pharmacophore and attracting numerous studies on the design and synthesis of andrographolide derivatives. In this study, a small library of 3,14,19-modified derivatives of andrographolide were synthesized and tested for their *in vitro* inhibitory activities to cancer cell growth and proliferation and *in vivo* toxicities against zebrafish embryo development. Structure-anti-proliferative activity and -toxicity relationships in current data revealed that the property of a substituent, substituted position/s, and 14-stereochemistry together determined a compound's *in vitro* anti-proliferative activity of cancer cells, the *in vivo* toxicity to zebrafish, and the selectivity between MDA-MB-231 and A549. Taken together, our SAR studies discovered some potential leads for further anticancer drug development and suggested that the direct and/or indirect toxicity of an active compound with andrographolide pharmacophore should be paid attention.

Introduction

Andrographolide[1] (**1**, Fig. 1), the labdane diterpene, is a representative ingredient of *Andrographis panniculata* (Burm.f.) Nees and plays an important role in "heat-clearing and detoxifying" defined in Chinese Medicine.[2] Even though andrographolide obeys the "Rule of Five" and is bestowed with an interesting pharmacophore that displays various pharmacological activities and has therapeutic potential for a wide range of diseases,[3] its poor water solubility and also relatively low lipo-solubility result in its weak potency and inadequate therapeutic efficacy and restrict its further application. To improve its physiochemical properties and pharmaceutical features, numerous andrographolide derivatives and their pharmacological activities have been reported in recent years, e.g., 14-acyloxy andrographolide derivatives and their antibacterial or/and anticancer activity,[4] 14-phenoxy andrographolide derivatives and their FXR antagonistic activity,[5] dehydroandrographolide derivatives and their antiviral activity,[6] 19-*tert*-butyldimethylsilylated and 19-triphenylmethylated derivatives and their cytotoxic activities.[4e] Our interest was to discover 3,14,19-derivatives of andrographolide as potent anticancer cell proliferative agents and summarize SAR of a small library for drug discovery and development of andrographolide.

Zebrafish (*Danio rerio*) is an excellent *in vivo* model for physiologically relevant whole organism and behavior-based screening,[7] which cannot be achieved with conventional *in vitro* systems. Along with the development of zebrafish embryo, the activity or/and the toxicity of a tested compound reflects its influence degree on the embryo development and integrity, and we have previously used zebrafish model for SAR analysis of seven polymethoxylated flavonoids for the anti-angiogenesis activity and toxicity.[8] Moreover, our previous pilot study proved the concept that zebrafish is equipped with sophisticated drug metabolism system and could address the challenge by predicting both potential toxicity and efficacy with taking consideration of whole content of bioavailability, metabolism and multiple target effect of a hit.[9] Since the pharmaceutical industry frequently encounters a high risk of failure in the development of a new drug candidate, particularly at later stages, owing to intolerable side-effects and/or toxicity in clinical trials, there is a trend in drug discovery strategy to exclude potentially toxic compounds at an early stage. On the basis of the feasibility of screening toxicity of compounds in a zebrafish model, our SAR study of andrographolide derivatives was achieved by the discovery of their *in vitro* anticancer activities to cell proliferation of MDA-MB-231 and A549 and then the comparison of their *in vivo* toxicities against zebrafish embryo development.

In this paper, herein, a series of 3-, or/and 14-, or/and 19-modified derivatives of andrographolide as the small library were designed and synthesized. It was firstly discovered that some derivatives were *in vitro* active against growth and proliferation of two cancer cell lines in an interesting structure-activity relationship. Further studies revealed the *in vivo* structure-toxicity relationship of these compounds against zebrafish embryo development. Interestingly, by contrast, either single or combined modifications at 3-, 14, 19-positions of andrographolide scaffold have resulted in change of the anticancer proliferative activity and the toxicity to zebrafish, the 14-stereochemistry also had a contribution to the anti-proliferative activity of cancer cells and the toxicity to zebrafish to some extent, and the transformation

between hydroxyl and ketone groups sometimes altered the anticancer activity and the toxicity to zebrafish. Importantly, some specific modifications led to the selectivity between MDA-MB-231 and A549.

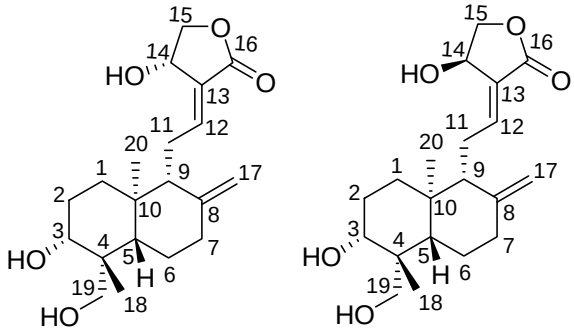


Figure 1. Structures of andrographolide (**1**) and 14β-isomer (**2**) of andrographolide.

Results and discussion

The design of modifications and the preliminary results

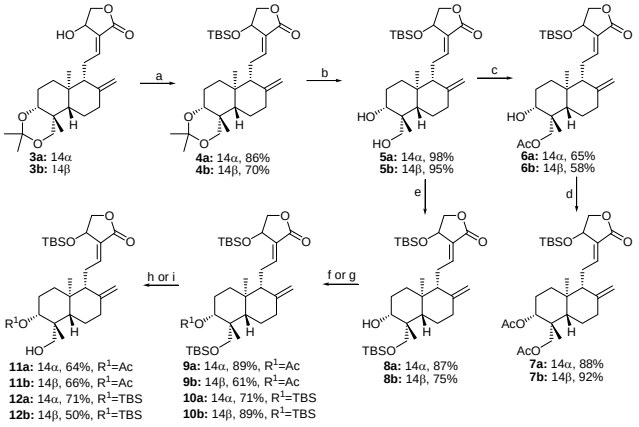
In our initial study, it was discovered that andrographolide (**1**) (Table 1, entry 1) exhibited mild anti-proliferative effects on MDA-MB-231 and A549 and was not toxic to zebrafish; however, one derivative **4a** of andrographolide (Table 1, entry 5) was not active against cancer cell proliferation of MDA-MB231 and A549 but showed a toxicity at 300 μM against zebrafish embryo development. This observation drew our interest in 3,14,19-modifications of **1** that a series of andrographolide derivatives were designed, synthesized and tested for their *in vitro* anti-proliferative activities of cancer cells and their *in vivo* toxicities to zebrafish. In the design of these andrographolide derivatives, the intact core scaffold of **1**[10] was used to explore whether and how the mono- or multi-modifications of **1** affected anti-proliferative effect on cancer cells and the toxicity to zebrafish.

3,14,19-Acylation and silylation are the commonest reported modifications on andrographolide, so, acetylation and silylation were chosen in the paper to be representatives to test *in vitro* anticancer cell proliferative activity and the *in vivo* zebrafish toxicity. Introduction of TBS (Scheme 1) or Ac (Scheme 2) generally increases a compound's lipophilicity and these modifications can provide the correlation between the lipophilicity and the anticancer cell proliferative effect and/or the zebrafish toxicity. In addition, 3-ketone derivatives (Scheme 2) were synthesized and tested since 3-ketone modification increases the lipophilicity and could change possible binding mode due to double bond. In order to know whether the 14-stereochemistry plays a role in exhibiting anti-proliferative activity of cancer cells or/and causing the toxicity to zebrafish, both of 14α- and 14β-isomers were synthesized and assayed.

Synthesis

The transformation procedures of 3,14,19-*O*-substituted groups are depicted in Scheme 1. The preparation of **3a** and stereochemistry transformation of **1** to **2** and **3b** were achieved according to our previous report.[5] 14-*O*-Silylation of 3,19-acetonylidene-protected compounds **3a** and **3b** to give **4a**[11] and **4b**, respectively, was accomplished at 0 °C by TBSOTf and 2,6-lutidine. Removal of 3,19-acetonylidene by *p*-TSA in methanol at 0 °C yielded **5a**[12] and **5b**, which were selectively 19-*O*-acetylated using acetic chloride with TEA at rt to afford **6a** and **6b**.

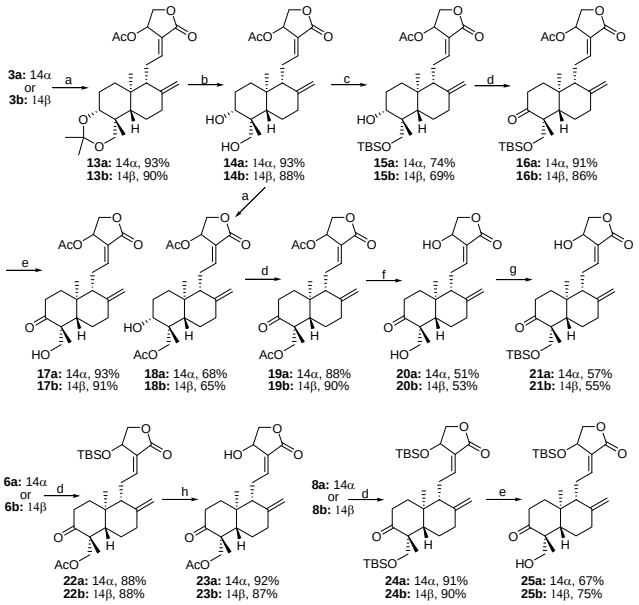
Scheme 1. [a]



[a]Reagents and conditions: (a) TBSOTf, 2,6-lutidine, 0 °C, 0.5 h. (b) *p*-TSA, MeOH, 0 °C, 0.5 h. (c) AcCl, TEA, rt, 2 h. (d) Ac_2O, cat. $ZnCl_2$, 50 °C, 1 h. (e) TBSCl, imidazole, rt, 1 h. (f) to **9a** and **9b**: Ac_2O, cat. DMAP, rt, 24 h. (g) to **10a** and **10b**: TBSOTf, 2,6-lutidine, 0 °C, 1 h. (h) from **9a** and **9b** to **11a** and **11b**: TFA/H_2O(v/v=10/1), -20 °C, 0.5 h. (i) from **10a** and **10b** to **12a** and **12b**: TFA, -20 °C, 5 min.

The reaction of **6a** and **6b** with acetic anhydride catalyzed by anhydrous zinc chloride generated 3,19-diacetylated compounds **7a** and **7b**. Compounds **5a** and **5b** were converted into 14,19-di-TBS compounds **8a** and **8b** by TBSCl and imidazole. The reaction of **8a** and **8b** with acetic anhydride and DMAP (cat.) or with TBSOTf and 2,6-lutidine produced **9a** and **9b** or **10a** and **10b**, respectively, which were selectively hydrolyzed 19-TBS group to afford **11a** and **11b** or **12a** and **12b**, respectively.

Scheme 2. [a]



[a]Reagents and conditions: (a) AcCl, TEA, rt, 1 h. (b) *p*-TSA, MeOH, 0 °C, 1 h. (c) TBSCl, TEA, rt, 1 h. (d) DMP, rt, 1 h. (e) TFA, -20 °C, 0.5 h. (f) *p*-TSA, MeOH, 40 °C, 8 h. (g) TBSCl, imidazole, rt, 1 h. (h) TBAF, THF, 0 °C, 3 h.

The synthetic routes of 3-ketones with 14,19-*O*-substituted groups are shown in Scheme 2. 14-Acetylation of **3a** and **3b** yielded **13a**[13] and **13b**,[5] followed by selective hydrolysis of 3,19-

acetonylidene to provide **14a**[13] and **14b**.[5] 19-*O*-Silylation of **14a** and **14b** produced **15a**[14] and **15b**, which were oxidized into 3-ketones **16a** and **16b** by Dess-Martin oxidation, and then removal of 19-TBS protection of **16a** and **16b** by TFA at -20 °C gave 3-ketones **17a** and **17b**. 19-*O*-Acetylation of **14a** and **14b** produced 14,19-diacetylated 3-alcohol compounds **18a**[15] and **18b**, and then oxidation of **18a** and **18b** by DMP afforded 3-ketones **19a** and **19b**. After **19a** and **19b** were fully hydrolyzed into **20a**[16] and **20b** by *p*-TSA in methanol at 40 °C, **20a** and **20b** were selectively 19-*O*-silylated to afford **21a** and **21b**. Compounds **23a** and **23b** were obtained through oxidation by DMP of **6a** and **6b** into **22a** and **22b** followed by **22a** and **22b**'s deprotection of 14-OTBS using TBAF. Similarly, **10a** and **10b** were oxidized into **24a** and **24b**, and then selective deprotection of 14-OTBS of **24a** and **24b** at -20 °C by TFA produced **25a** and **25b**.

Structure-anti-proliferative activity relationship on cancer cells and structure-toxicity relationship on zebrafish

Results of the *in vitro* anti-proliferative activity of cancer cells and the *in vivo* toxicity to zebrafish and the calculated CLogP values of the synthetic compounds are listed in Table 1. Andrographolide (**1**) and its 14β-epimer (**2**), which conform to the "Rule of Five" with a molecular weight of 350 Da, 3 hydrogen bond donors, 5 hydrogen bond acceptors and 2.1186 of the calculated CLogP, exhibited only weak anti-proliferation to two cancer cell lines (Table 1, entries 1 and 2) and showed no toxicity to zebrafish up to 300 μM (Table 1, entries 1 and 2). 3,19-Acetonylidene-protected compounds **3a** and **3b** (Table 1, entries 3 and 4) became more active against two cancer cell lines than their mother compounds **1** and **2**, and toxic at 300 μM to zebrafish, which could be derived partly from the increased hydrophobicity. Interestingly, **3a** expressed two times more active than **3b** against cell proliferation of two cancer cell lines. After the introduction of TBS at 14-position, both of compound **4a** (Table 1, entry 5) and its 14β-epimer **4b** (Table 1, entry 6) had no anti-cancer cell proliferative effect on MDA-MB-231 and A549 but **4a** was toxic to zebrafish at 300 μM and **4b** was non-toxic to zebrafish development up to 300 μM. Unlike their mother compounds **4a** and **4b**, more hydrophilic 3,19-diol compounds **5a** and **5b** (Table 1, entries 7 and 8), which possess the marginal values of the "Rule of Five", exhibited their anticancer cell proliferative activities with CC_{50} values to MDA-MB-231 and A549 of 4.8 and 17.8 μM, 7.0 and 10.5 μM, respectively. Moreover, **5a** and **5b** showed totally different toxicities to zebrafish from **4a** and **4b** that the 14α–isomer **5a** (Table 1, entry 7) was a safe compound to zebrafish up to 300 μM but the 14β–isomer **5b** (Table 1, entry 8) showed obvious toxicity to zebrafish at 30 μM and made zebrafish dead at 100 μM, suggesting that the stereochemistry of 14-position herein played an important role in the toxicity to zebrafish. The observation, which anti-proliferative activity of cancer cells was increased from **1** and **2** to less hydrophilic **3a** and **3b**, and from **4a** and **4b** to more hydrophilic **5a** and **5b**, but decreased from **3a** and **3b** to less hydrophilic **4a** and **4b**, indicated that the suitable hydrophilicity/lipophilicity of a compound was important to the anti-proliferation of cancer cells. Important finding was that MDA-MB-231 was more sensitive to these series of compounds than A549 and **3a**, **3b** and **5a** were three times more active to MDA-MB-231 than to A549.

Derived from a selective anticancer cell proliferative agent **5a**, 19-acetylated compound **6a** (Table 1, entry 9) had no anti-proliferative effect on two cancer cell lines but became a selective toxic agent to zebrafish, which showed the toxicity to zebrafish at 30 μM, suggesting that the action of **6a** to zebrafish possibly came from its static inhibition. 19-Acetylated compound **6b** (Table 1, entry 10) exhibited its more selective anticancer cell proliferation (>8-fold difference) to MDA-MB-231 than to A549 with CC_{50} values of 6.7 and 56.5 μM, respectively; meanwhile, **6b** showed its toxicity to zebrafish up to 300 μM, which was much less toxic to zebrafish than 19-alcohol compound **5b**. It is obvious that difference of the selectivity and potency of **6a** and **6b** (CLogP > 5 and molecular weight > 500) was due to their distinct 14-stereochemistry. Compared to mono-acetylated compound **6a**, 3,19-diacetylated compound **7a** (Table 1, entry 11) was not active to cancer cell proliferation and also lost the toxicity to zebrafish. 3,19-Diacetylated compound **7b** (Table 1, entry 12) retained the same level toxicity to zebrafish at 300 μM as **6b** but **7b** became a much weaker anti-proliferative agent than **6b** to two cancer cell lines and interestingly, **7b** still possessed the selectivity to MDA-MB-231 over A549 as **6b** did. Further studies showed that among 14,19-di-OTBS compounds, only 3-alcohol **8b** (Table 1, entry 14) showed weak anticancer cell proliferative activity and 3-alcohol **8a** (Table 1, entry 13), 3-acetylated compounds **9a** and **9b** (Table 1, entries 15 and 16) and 3,14,19-tri-OTBS compounds **10a** and **10b** (Table 1, entries 17 and 18) were not active in anticancer cell proliferation and all of 14,19-di-OTBS compounds **8a**, **8b**, **9a**, **9b**, **10a** and **10b** did not exhibit any toxicity to zebrafish.

Contrary to compounds **9a** and **9b**, more hydrophilic compounds **11a** and **11b** (CLogP > 5 and molecular weight > 500) (Table 1, entries 19 and 20) bearing 3-OAc, not only exhibited potent anti-proliferative effect on MDA-MB-231 and A549 with CC_{50} values of 4.7, 9.8 and 4.3, 11.3 μM, respectively, but also showed very strong toxicity to zebrafish. However, the changes of the hydrophilicity from **10a** and **10b** to **12a** and **12b** (Table 1, entries 21 and 22), containing 3-OTBS, did not affect their anti-proliferative effect on MDA-MB-231 and A549 and toxicity to zebrafish that they were not active to two cancer cell lines and not toxic to zebrafish embryo development. By the comparison of **5a** with **11a**, 3-OAc heavily enhanced the toxicity to zebrafish even though 3-OAc did not change too much from **5b** to **11b**. It is concluded from these results that 3,14,19-substituted groups, which contributed the lipophilicity of a compound, together with the stereochemistry of 14-OTBS determined a compound's anti-proliferative activity of cancer cells and its toxicity to zebrafish; and the selectivity of a compound's anticancer cell proliferative activity was MDA-MB-231 over A549 and vice versa. Notably, groups of 14-OTBS and 19-OH provided higher chance to exhibit the toxicity to zebrafish (**5b**, **11a** and **11b**).

How acetylation at 14-position and 3-ketone affected a compound's anti-proliferative effect on cancer cell lines and the toxicity to zebrafish was also explored. Both of 14-actylated analogues **13a** and **13b** (Table 1, entries 23 and 24) were active against the anti-proliferation of two cancer cell lines and toxic to zebrafish development, and **13b** of 14β-isomer was more active to two cancer cell lines and more toxic to zebrafish than **13a** of 14α-isomer. Compared with **13a** and **13b**, removal of 3,19-acetonylidene group made more hydrophilic compounds **14a** and

14b (Table 1, entries 25 and 26) two times less active to two cancer cell lines than **13a** and **13b**, and **14a** showed slightly better activity than **14b** in anticancer cell proliferation. Meanwhile, **14a** exhibited much weaker toxicity to zebrafish than **13a** and **14b** totally lost the toxicity to zebrafish, indicating that herein, the hydrophilicity decreased the toxicity to zebrafish and 14β-OAc isomer of **14b** possessed selective anticancer cell proliferative activities to MDA-MB-231 and A549. Introduction of TBS into 19-position, 3-alcohol compounds **15a** and **15b** (CLogP > 5 and molecular weight > 500) with 14-OAc and 19-OTBS (Table 1, entries 27 and 28) and 3-ketone compounds **16a** and **16b** (CLogP > 5 and molecular weight > 500) with 14-OAc and 19-OTBS (Table 1, entries 29 and 30) showed the strongest anti-proliferative activities of two cancer cell lines and also exhibited the strongest toxicities to zebrafish, suggesting that their anti-proliferative activity of two cancer cell lines and toxicity to zebrafish were correlated and the combination of 14-OAc and 19-OTBS was crucial in playing anti-proliferation activity and the toxicity. It is valuable that the toxicities to zebrafish of 14β-isomers of **15b** and **16b** were stronger than those of 14α-isomers of **15a** and **16a** but the transformation of 3-alcohol **15a** and **15b** to 3-ketone **16a** and **16b** had no influence on the activity and toxicity. Moreover, by the comparison of **15a**/**15b** with **6a**/**6b** and **11a**/**11b**, it was found that different positions of OTBS and OAc afforded different anti-proliferative activity and zebrafish toxicity.

After removal of 19-TBS from 3-ketones **16a** and **16b**, 3-ketones **17a** and **17b** (Table 1, entries 31 and 32) with 14-OAc-19-OH, followed very well the "Rule of Five", were selective agents against cell proliferation of MDA-MB-231 and A549; and importantly, **17a** is the sole compound in the paper which was more active to A549 than to MDA-MB-231. Transformation of 3-alcohol **14a** (Table 1, entry 25) to 3-ketone **17a** did not change the anticancer cell proliferative activity to MDA-MB-231 but increased the anticancer cell proliferative activity to A549 in more than 2 times and **17a** was a safe compound to zebrafish; in the other direction, transformation from alcohol **14b** to ketone **17b** of 14β-isomers (Table 1, entries 26 and 32) almost did not change the activity and toxicity. Compared to 19-OTBS **15a** and **15b**, 19-OAc compounds **18a** and **18b** with 14-OAc and 3-OH (Table 1, entries 33 and 34), which obey the "Rule of Five", selectively inhibited cell proliferation of MDA-MB-231 and A549 but did not exhibit the toxicity to zebrafish; it is interesting that 14β-isomer of **18b** was two times more active to cancer cell proliferation than 14α-isomer of **18a**. 3-Ketone compounds **19a** and **19b** with 14,19-di-OAc (Table 1, entries 35 and 36) showed very close anticancer cell proliferative activities to their corresponding 3-alcohol compounds **18a** and **18b**; however, unlike **18a** and **18b**, **19a** exhibited the toxicity at 100 μM to zebrafish and **19b** exhibited the toxicity at 30 μM to zebrafish and made zebrafish dead at 100 μM, indicating that 3-ketone deeply affected the toxicity to zebrafish and less hydrophilicity increased the toxicity. After hydrolysis of 14,19-di-OAc from **19a** and **19b**, 3-ketones **20a** and **20b** (Table1, entries 37 and 38) bearing 14,19-di-OH became safe to zebrafish as **1** and **2** (Table 1, entries 1 and 2) but lost anticancer cell proliferative activity. Introduction of TBS to provide **21a** and **21b** generated the biggest difference of activity and toxicity between 14α-isomer **21a** and 14β-isomer **21b**: even though 3-ketone **21a** bearing 14α-OH-19-OTBS (Table1, entry 39) was not active against cancer cell proliferation and safe to zebrafish, ketone **21b** bearing 14β-OH-19-OTBS (Table1, entry 40) exhibited very strong anticancer cell proliferative activity and also strong toxicity to zebrafish. These data indicated that 14-stereochemistry, 19-OTBS and the combination of 14-OAc and 19-OTBS exerted important roles in the anti-proliferative activity of two cancer cell lines and the toxicity to zebrafish.

Table 1. *In vitro* anti-proliferative activity to cancer cells after treated with compounds for 24 h and *in vivo* toxicity to zebrafish at 24 hpf.

entry	cmpd	CLogP[a]	CC_{50} (μM)		Observation at concentration (μM)[b,c,d]			
			MDA-MB-231	A549	10	30	100	300
1	**1**	2.1186	109.7±3.1	132	○	○	○	○
2	**2**	2.1186	98.1±3.0	113.6±18.1	○	○	○	○
3	**3a**	4.1596	7.6±0.3	37.2±6.5	○	○	○	T
4	**3b**	4.1596	18.5±3.1	71.7±6.1	○	○	○	T
5	**4a**	7.5782	>300	>300	○	○	○	T
6	**4b**	7.5782	>300	>300	○	○	○	○
7	**5a**	5.5372	4.8±0.5	17.8±3.2	○	○	○	○
8	**5b**	5.5372	7.0±0.3	10.5±0.3	○	T	D	D
9	**6a**	6.4452	>300	>300	○	T	T	T
10	**6b**	6.4452	6.7±0.2	56.5±0.4	○	○	○	T
11	**7a**	7.3532	>300	>300	○	○	○	○
12	**7b**	7.3532	133.2±11.	>300	○	○	○	T
13	**8a**	8.9142	>300	>300	○	○	○	○
14	**8b**	8.9142	40.9±4.8	120.2±5.5	○	○	○	○
15	**9a**	9.8222	>300	>300	○	○	○	○
16	**9b**	9.8222	>300	>300	○	○	○	○
17	**10a**	12.2912	>300	>300	○	○	○	○
18	**10b**	12.2912	>300	>300	○	○	○	○
19	**11a**	6.4452	4.7±0.2	9.8±0.3	○	T	D	D
20	**11b**	6.4452	4.3±0.2	11.3±0.4	○	T	T	D
21	**12a**	8.9142	>300	>300	○	○	○	○
22	**12b**	8.9142	>300	>300	○	○	○	○
23	**13a**	5.0182	7.6±0.3	9.8±0.3	○	T	T	T
24	**13b**	5.0182	4.0±0.2	6.1±0.2	○	D	D	D
25	**14a**	2.9772	10.2±0.3	14.4±0.2	○	○	○	T
26	**14b**	2.9772	8.7±1.4	11.4±2.1	○	○	○	○
27	**15a**	6.3542	2.7±0.1	3.3±0.2	○	T	T	T
28	**15b**	6.3542	2.4±0.2	2.5±0.2	T	D	D	D
29	**16a**	6.1014	2.4±0.1	3.0±0.2	○	T	T	T
30	**16b**	6.1014	2.7±0.1	4.5±0.2	T	D	D	D
31	**17a**	2.7196	10.2±0.1	5.6±1.9	○	○	○	○
32	**17b**	2.7196	6.1±0.6	12.0±2.0	○	○	○	○
33	**18a**	3.8852	9.2±1.4	14.4±2.5	○	○	○	○
34	**18b**	3.8852	3.9±0.6	6.1±1.4	○	○	○	○
35	**19a**	3.6219	9.3±0.3	10.8±0.3	○	○	T	T
36	**19b**	3.6219	3.7±0.8	8.7±1.7	○	T	D	D
37	**20a**	1.861	>300	>300	○	○	○	○
38	**20b**	1.861	226.2±4.7	>300	○	○	○	○
39	**21a**	5.2428	>300	>300	○	○	○	○
40	**21b**	5.2428	2.5±0.5	5.9±0.9	○	D	D	D
41	**22a**	6.1819	3.5±0.2	8.9±0.3	○	○	○	T
42	**22b**	6.1819	3.6±0.6	21.6±6.4	T	D	D	D
43	**23a**	2.7633	27.2±4.3	62.7±9.4	○	○	○	○
44	**23b**	2.7633	101.3±8.4	168.5±23.3	○	○	○	○
45	**24a**	8.6614	>300	>300	○	○	○	○
46	**24b**	8.6614	165.4±6.0	>300	○	○	○	○
47	**25a**	5.2796	10.8±0.3	17.0±0.1	○	○	T	T
48	**25b**	5.2796	4.0±0.5	10.7±1.5	○	T	D	D

[a]calculated from Chem3D; [b,c]"○" represents that no toxicity was observed after zebrafish embryos were treated with the indicated concentration of the compound; [c]"T" means that the indicated concentration of the compound treated to zebrafish embryos showed the toxicity and the toxic effect was not determined; [d]"D" stands that zebrafish embryos treated with the indicated concentration of compound were dead.

Unlike its 3-alcohol mother compound **6a** (Table 1, entry 9) was a selective toxic agent to zebrafish, 3-ketone **22a** (CLogP > 5 and molecular weight > 500) bearing 14-OTBS and 19-OAc (Table 1, entry 41) was much less toxic than **6a** to zebrafish but **22a** was a potent anticancer proliferation agent with IC_{50} values of 3.5 μM and 8.9 μM to MDA-MB-231 and A549, respectively, reflecting 3-alcohol (**6a**) and 3-ketone (**22a**) determined the selectivity towards anti-proliferative activity of two cancer cells and the toxicity to zebrafish. Different from **6b** (Table 1, entry 10), 3-ketone **22b** (Table 1, entry 42) was the most toxic compound to zebrafish; meanwhile, as **6b**, **22b** also showed its

selective anticancer cell proliferative activity (>5-fold difference) to MDA-MB-231 over A549 with CC_{50} values of 3.6 and 21.6 μM, respectively. Compared to **19a**, **19b**, **22a** and **22b**, more polar 3-keto-14-OH-19-OAc **23a** and **23b** (Table 1, entries 43 and 44) reduced the toxicity to zebrafish and also their anticancer cell proliferative effects but 14α-isomer **23a** was more active than 14β-isomer **23b** against cancer cell proliferation, suggesting that the substitution and stereochemistry at 14-position play a key role in the anti-proliferative activity and the toxicity. As its mother 3-alcohol **8a** (Table 1, entry 13), 3-keto-14α,19-di-OTBS **24a** (Table 1, entry 45) was not active against cancer cell proliferation and safe to zebrafish; meanwhile, 3-keto-14β,19-di-OTBS **24b** (Table 1, entry 46) was not toxic to zebrafish as 3-alcohol **8b** (Table 1, entry 14) but **24b** only showed very weak anti-proliferative activity to MDA-MB-231. Furthermore, removal of 19-OTBS from **24a** and **24b** sharply increased the toxicity to zebrafish and also the anticancer cell proliferative activity of **25a** and **25b** (5.2796 of CLogP, Table 1, entries 47 and 48) with 14-OTBS substitution. Contrasted 3-ketone **25a** (Table 1, entry 47) with 3-alcohol **5a** (Table 1, entry 7), 3-keto group did not affect A549 cancer cells but was a cofactor to reduce anti-proliferative activity of MDA-MB-231 and to enhance the toxicity to zebrafish; on the other hand, compared with alcohol **5b**, 3-ketone **25b** (Table 1, entry 48) showed the similar anticancer cell proliferative activity to A549 and the same level toxicity to zebrafish but was more active against cell proliferation of MDA-MB-231, indicating that MDA-MB-231 was more sensitive than A549 to the transformation of 3-OH to 3-ketone and different 14-stereochemistry produced opposite changes of the activity and the toxicity. These data suggest that the selectivity of one compound might be partly derived from the property of the substitution.

Conclusions

The small library in the paper was diversified by 14α- and 14β-isomers, different substituents and mono- or multi-substitutions at 3,14,19-positions, and transformation of 3-alcohol to 3-ketone. As our goal in this study was to discover *in vitro* anticancer cell proliferative compounds of andrographolide derivatives and summarize the SAR of the small library, we also used *in vivo* zebrafish model to analyze the toxicity of these andrographolide derivatives and conclude the structure-toxicity relationship of the small library.

Compared to **1** and **2**, modification/s at 3-,14-,19-positions changed the hydrophilicity of a compound, which was expected to affect activity and the toxicity via its cell permeability to some extent. Compounds of **4a**/**4b**, **7a**/**7b**, **9a**/**9b**, **10a**/**10b**, **13a**/**13b**, **16a**/**16b**, **19a**/**19b**, **22a**/**22b** and **24a**/**24b** have higher lipophilicity among these compounds but most of them were not more active or/and more toxic except **13a**/**13b**, **16a**/**16b** and **22b**. These data suggest that anti-proliferative activity of cancer cells and the toxicity to zebrafish were only in part derived from the permeability and optimal hydrophilicity/lipophilicity was possibly crucial to anti-cancer cell proliferative activity and zebrafish toxicity.

It is interesting and important that some modifications at **1** led to the selectivity to anticancer cell proliferative activity or zebrafish toxicity. Compound **6a** was a selective toxic agent to zebrafish and without anticancer cell proliferative activity up to 300 μM; however, compound **6b** showed selective anti-proliferative effects towards breast cancer MDA-MB-231 cells (>8-fold difference) than non-small cell lung cancer cell A549 but no toxicity to zebrafish up to 100 μM. Compound **17a** was not toxic to zebrafish; importantly and interestingly, **17a** showed more selective anti-proliferative activity to A549 than to MDA-MB-231 even though all of other compounds in the paper were more active against cell proliferation of MDA-MB-231 than A549. It was discovered that **1**, **2**, **5a**, **8b**, **14b**, **17a**, **17b**, **18a**, **18b** and **23a** were selective compounds against cancer cell proliferation of MDA-MB-231 and A549. In addition, **3a**, **3b**, **14a** and **22a** showed low CC_{50} value to both A549 and MDA-MB-231 cells (lower than 20 μM) and they had no toxicity to zebrafish at concentration up to 100 μM.

It was unveiled that the selectivity difference between the anti-proliferative activity of cancer cells and the toxicity to zebrafish of some compounds was only originated from 14α- and 14β-stereochemistry. The 14α-isomers of **5a** and **22a** showed good anticancer cell proliferative effects without toxicity or very low toxicity to zebrafish but their 14β-isomers of **5b** and **22b** not only exhibited good anticancer cell proliferative activity but also expressed very high toxicity to zebrafish. The 14α-isomer of **6a** was very toxic to zebrafish but did not show anticancer cell proliferative activity; however, 14β-isomer of **6b** exhibited good anticancer cell proliferative activity with low toxicity to zebrafish. Although the compound **21a** was safe to zebrafish and not active to cancer cell proliferation, 14β-isomer of **21b** was an excellent anticancer cell proliferation inhibitor while it was also very toxic to zebrafish.

The transformation between 3-alcohol and 3-ketone sometimes changed the activity, the toxicity and the selectivity. 3-Alcohol **6a** exhibited as a selective agent to zebrafish but its corresponding 3-ketone **22a** was a very potent inhibitor to cancer cell proliferation with very low toxicity to zebrafish. On the other hand, 3-ketones **19a** and **19b** showed similar anticancer cell proliferative activities to their corresponding 3-alcohols **18a** and **18b** but ketones **19a** and **19b** were strong toxic to zebrafish development. 3-Ketone **25a** became a toxic compound to zebrafish and less active inhibitor to cell proliferation of MDA-MB-231 while 3-alcohol **5a** was a selective agent to cancer cell proliferation. In addition, the transformation of 3-alcohol **5a** to 3-OAc **11a** made **11a** become a toxic agent to zebrafish; unlike very active and toxic 19-OTBS compounds **16a** and **16b**, 19-OH compounds **17a** and **17b** possessed good anticancer cell proliferative activity but were safe to zebrafish development.

By the analysis of the most active and also most toxic compounds **5b**, **11a**/**11b**, **13a**/**13b**, **15a**/**15b**, **16a**/**16b**, **19b**, **21b**, **22b**, **25b**, it could be concluded that 14β-isomers were more possibly active to cancer cell proliferation and also toxic to zebrafish, and dual substitutions of TBS and Ac at 14 and 19-positions (**16b** and **22b**) were more feasibility to bring about the toxicity to zebrafish development. On the other hand, substitutions of OH, OTBS and OAc at different positions between 3, 14 and 19 afforded different anti-proliferative activity of cancer cells and toxicity to zebrafish by comparison of **6a**/**6b**, **11a**/**11b** and **15a**/**15b**.

Although these compounds in the paper are not to be as drug candidates from the point of drug discovery and development

(calculated CLogP values were listed in Table 1), **5a**, **14a**, **14b**, **17a**, **17b**, **18a**, **18b** and **22a** could be as leads or potential candidates for further anti-cancer drug discovery and development of andrographolide derivatives, and especially, **6b** was a selective hit against MDA-MB-231 proliferation and could be developed into selective anti-proliferative drug candidate to MDA-MB-231; **17a** was to be as a lead to discover selective anti-proliferative inhibitors of A549; **6a** would be studied in the future as a possible selective static inhibitor to zebrafish development; modification of **15b**, **16b, 19b, 21b, 22b** and **25b** by truncating their toxicity ("detoxification") and raising their anticancer cell proliferative activity should be a possible and rational strategy to drug discovery. Targeting on drug discovery and development of anticancer agents, one of our future missions is to focus on replacement of TBS or/and Ac with more stable and suitable hydrophilic groups in view of druggability and Lipinski's "Rule of Five". On the other hand, elucidation of the toxic mechanism/s of these compounds against zebrafish embryo development and drug discovery of excellent static inhibitory agents (possibly like **6a**) to zebrafish embryo development are also promising goals in our future research.

In summary, our SAR data indicated that the stereochemistry of 14-position of **1** and specific single or suitably combined modifications at 3-, 14- and 19-positions of **1** affected not only *in vitro* anticancer cell proliferative activity to MDA-MB-231 and A549, and the *in vivo* toxicity to zebrafish but also the selectivity between the *in vitro* anti-proliferative activity and the *in vivo* toxicity, and different cancer cell lines. Overall, some potential leads or hits for further anti-cancer drug development and a possible static inhibitor to zebrafish embryo development were discovered from this research; moreover, the structure-toxicity relationships to zebrafish suggested that the direct and/or indirect toxicity of an active compound with andrographolide pharmacophore should be paid attention.

Experimental

Materials and equipment

Unless otherwise stated, materials were obtained from commercial suppliers and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 and 101 MHz, respectively, in an indicated deuterated solvent. Coupling constants (J) are expressed in hertz (Hz). Chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to the solvent. The high resolution of MS (HRMS) was recorded on an Applied Biosystems Q-STAR Elite ESI-LC-MS/MS mass spectrometer. Melting points were measured using an YRT-3 melting point apparatus (Shanghai, China) and were uncorrected.

Preparation of the compounds 4a[11] and 4b.

Under N_2 atmosphere, 10.0 g (25.6 mmol) of compound **3a**[5] or **3b**[5] and 6.0 ml (51.3 mmol) of 2,6-lutidine were dissolved in 60.0 ml anhydrous dichloromethane. The solution was cooled to 0 ^{o}C and then 8.8 ml (38.5 mmol) of TBSOTf was added dropwise over 5 min. The reaction mixture was stirred for 0.5 h at 0 ^{o}C and treated with ethyl acetate and sol. sat. $NaHCO_3$ after the reaction was complete. The organic phase was washed with brine for 6 times, dried over anhydrous Na_2SO_4, filtered and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/15) to provide compound **4a** or **4b**. **3,19-Acetonylidene-14*α*-*tert*-butyldimethylsilyloxy-andrographolide (4a):** 86% yield, white solid, m.p. 128.6-129.5 ^{o}C. ^{1}H NMR (400 MHz, C_6D_6) δ 7.00 (1H, ddd, J = 7.3, 5.8, 2.0 Hz, 12-H), 4.86 (1H, s, 17α-H), 4.69 (1H, s, 17β-H), 4.49 – 4.43 (1H, m, 14β-H), 3.85 (1H, d, J = 11.9 Hz, 19α-H), 3.81 (1H, dd, J = 9.7, 6.8 Hz, 15α-H), 3.70 (1H, dd, J = 9.6, 3.5 Hz, 15β-H), 3.50 (1H, dd, J = 7.4, 3.0 Hz, 3β-H), 3.10 (1H, d, J = 11.6 Hz, 19β-H), 2.58 (1H, ddd, J = 17.8, 10.9, 7.0 Hz, 11α-H), 2.25 – 2.14 (2H, m, 9β-H and 11β-H), 1.98 – 1.88 (1H, m, 7α-H), 1.81 – 1.69 (1H, m, 7β-H), 1.69 – 1.57 (2H, m, 1α-H and 2α-H), 1.51 (1H, d, J = 11.8 Hz, 1β-H), 1.44 (3H, s, CCH_3), 1.39 (3H, s, CCH_3), 1.37 – 1.29 (1H, m, 2β-H), 1.11 (3H, s, 18-H), 1.10 – 1.02 (1H, m, 6α-H), 1.02 (3H, s, 20-H), 1.00 – 0.89 (2H, m, 5β-H and 6β-H), 0.87 (9H, s, SiC(CH_3)$_3$), -0.00 (3H, s, SiCH_3), -0.17 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, C_6D_6) δ 169.1 (16-C), 147.5 (12-C), 147.4 (8-C), 128.2 (13-C), 109.9 (17-C), 99.6 (C(CH$_3$)$_2$), 75.4 (3-C), 73.4 (15-C), 67.4 (14-C), 64.4 (19-C), 56.2 (9-C), 51.3 (5-C), 38.4 (4-C), 38.3 (10-C), 37.8 (7-C), 34.4 (1-C), 26.6 (2-C), 26.2 (6-C), 25.8 (SiC(CH$_3$)$_3$), 25.4 (CCH$_3$), 25.0 (CCH$_3$), 24.9 (11-C), 23.3 (18-C), 17.9 (SiC(CH$_3$)$_3$), 17.1 (20-C), -4.3 (SiCH$_3$), -4.8 (SiCH$_3$); HRMS (ESI) *m/z* 527.3190 [M+Na]$^+$, calculated for $C_{29}H_{48}O_5SiNa$, 527.3169. **3,19-Acetonylidene-14β-*tert*-butyldimethylsilyloxy-andrographolide (4b):** 70% yield, white solid, m.p. 158.3-159.2 ^{o}C. ^{1}H NMR (400 MHz, C_6D_6) δ 6.94 (1H, ddd, J = 8.2, 4.7, 2.0 Hz, 12-H), 4.88 (1H, d, J = 1.2 Hz, 17α-H), 4.58 – 4.53 (1H, m, 14α-H), 4.43 (1H, d, J = 0.9 Hz, 17β-H), 3.86 (1H, d, J = 11.5 Hz, 19α-H), 3.76 (1H, dd, J = 9.7, 6.3 Hz, 15α-H), 3.72 (1H, dd, J = 9.7, 3.5 Hz, 15β-H), 3.47 (1H, dd, J = 7.3, 3.1 Hz, 3β-H), 3.11 (1H, d, J = 11.5 Hz, 19β-H), 2.51 – 2.31 (2H, m, 9β-H and 11α-H), 2.21 (1H, ddd, J = 12.8, 3.9, 2.4 Hz, 11β-H), 1.94 – 1.82 (1H, m, 7α-H), 1.74 (1H, td, J = 13.0, 5.0 Hz, 7β-H), 1.63 – 1.52 (2H, m, 1α-H and 2α-H), 1.49 (1H, dd, J = 10.1, 1.1 Hz, 2β-H), 1.43 (3H, s, CCH_3), 1.40 – 1.33 (4H, m, CCH_3 and 1β-H), 1.10 (3H, s, 18-H), 1.08 – 1.02 (2H, m, 6α-H and 5β-H), 1.02 (3H, s, 20-H), 0.90 (1H, dd, J = 12.8, 2.6 Hz, 6β-H), 0.86 (9H, s, SiC(CH_3)$_3$), -0.00 (3H, s, SiCH_3), -0.16 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, C_6D_6) δ 169.1 (16-C), 148.5 (12-C), 147.4 (8-C), 128.6 (13-C), 108.3 (17-C), 99.6 (C(CH$_3$)$_2$), 75.3 (3-C), 73.4 (15-C), 67.7 (14-C), 64.4 (19-C), 56.1 (9-C), 51.1 (5-C), 38.4 (4-C), 38.3 (10-C), 37.8 (7-C), 34.1 (1-C), 26.5 (2-C), 26.1 (6-C), 25.7 (SiC(CH$_3$)$_3$), 25.6 (CCH$_3$), 25.4 (CCH$_3$), 24.8 (11-C), 23.4 (18-C), 18.0 (SiC(CH$_3$)$_3$), 17.1 (20-C), -4.2 (SiCH$_3$), -4.8 (SiCH$_3$); HRMS (ESI) *m/z* 527.3185 [M+Na]$^+$, calculated for $C_{29}H_{48}O_5SiNa$, 527.3169.

Preparation of the compounds 5a[12] and 5b

10.0 g (19.8 mmol) of compound **4a** or **4b** was dissolved in 20.0 ml of methanol. The solution was treated with 0.38 g (2.0 mmol) of *p*-TSA at 0 ^{o}C for 0.5 h. Diluted by ethyl acetate and washed with sol. sat. $NaHCO_3$ and brine in order, the organic phase was dried over anhydrous Na_2SO_4, filtered, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/1) to afford compound **5a** or **5b**. **14*α*-*tert*-Butyldimethylsilyloxy-andrographolide (5a):** 98% yield, white solid, m.p. 116.3-117.8 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.60 (1H, td, J = 6.2, 1.4 Hz, 12-H), 5.19 (1H, d, J = 6.1 Hz, 14β-H), 5.05 (1H, d, J = 4.8

Hz, 3α-O*H*), 4.79 (1H, s, 17α-H), 4.50 (1H, s, 17β-H), 4.46 (1H, dd, *J* = 10.0, 5.9 Hz, 15α-H), 4.11 (1H, dd, *J* = 7.5, 2.8 Hz, 19-O*H*), 4.00 (1H, dd, *J* = 10.0, 2.3 Hz, 15β-H), 3.83 (1H, dd, *J* = 10.9, 2.7 Hz, 19α-H), 3.25 (2H, td, *J* = 9.9, 8.8, 6.5 Hz, 3β-H and 19β-H), 2.44 (2H, t, *J* = 7.0 Hz, 11-H), 2.32 (1H, dt, *J* = 13.0, 3.1 Hz, 9β-H), 1.95 (2H, q, *J* = 8.0, 7.0 Hz, 7-H), 1.78 – 1.69 (1H, m, 2α-H), 1.68 – 1.57 (3H, m, 1-H and 2β-H), 1.35 (1H, qd, *J* = 12.9, 3.9 Hz, 6α-H), 1.28 – 1.17 (2H, m, 5β-H and 6β-H), 1.08 (3H, s, 18-H), 0.86 (9H, s, SiC(CH_3)$_3$), 0.65 (3H, s, 20-H), 0.15 (3H, s, SiCH_3), 0.12 (3H, s, SiCH_3); HRMS (ESI) *m/z* 487.2867 [M+Na]$^+$, calculated for $C_{26}H_{44}O_5SiNa$, 487.2856. **14β-*tert*-Butyl-dimethylsilyloxy-andrographolide (5b):** 95% yield, white solid, m.p. 153.7-154.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.57 (1H, ddd, *J* = 7.9, 4.2, 1.4 Hz, 12-H), 5.23 (1H, d, *J* = 5.7 Hz, 14α-H), 5.06 (1H, d, *J* = 4.3 Hz, 3α-O*H*), 4.79 (1H, s, 17α-H), 4.48 (1H, dd, *J* = 9.9, 6.1 Hz, 15α-H), 4.32 (1H, s, 17β-H), 4.16 – 4.06 (1H, m, 19-O*H*), 3.99 (1H, dd, *J* = 9.9, 2.4 Hz, 15β-H), 3.83 (1H, d, *J* = 10.8 Hz, 19α-H), 3.24 (2H, ddd, *J* = 15.3, 10.0, 5.6 Hz, 3β-H and 19β-H), 2.49 – 2.27 (3H, m, 9β-H and 11-H), 1.96 (2H, qd, *J* = 13.0, 12.0, 3.5 Hz, 7-H), 1.80 – 1.71 (1H, m, 2α-H), 1.70 – 1.54 (3H, m, 1-H and 2β-H), 1.43 – 1.18 (3H, m, 5β-H and 6-H), 1.08 (3H, s, 18-H), 0.87 (9H, s, SiC(CH_3)$_3$), 0.65 (3H, s, 20-H), 0.16 (3H, s, SiCH_3), 0.11 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.3 (16-C), 147.9 (12-C), 147.3 (8-C), 128.1 (13-C), 107.9 (17-C), 78.3 (3-C), 73.8 (15-C), 66.7 (14-C), 62.6 (19-C), 55.1 (9-C), 54.3 (5-C), 42.2 (4-C), 38.4 (10-C), 37.4 (7-C), 36.7 (1-C), 27.8 (2-C), 25.5 (SiC(*C*H$_3$)$_3$), 24.9 (6-C), 24.0 (11-C), 23.0 (18-C), 17.5 (Si*C*(CH$_3$)$_3$), 14.9 (20-C), -4.4 (Si*C*H$_3$), -5.0 (Si*C*H$_3$); HRMS (ESI) *m/z* 487.2868 [M+Na]$^+$, calculated for $C_{26}H_{44}O_5SiNa$, 487.2856.

Preparation of the compounds 6a and 6b

At rt, 3.0 g (6.5 mmol) of compound **5a** or **5b** and 4.04 ml (29.1 mmol) of TEA were dissolved in 20.0 ml ethyl acetate and then 1.61 ml (22.6 mmol) of AcCl was added dropwise over 2 min. The reaction mixture was stirred for 2 h at room temperature and treated with ethyl acetate and sol. sat. $NaHCO_3$ after the reaction was complete. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and then concentrated under reduced pressure. Purification from the residue to give compound **6a** or **6b** was conducted by silica gel column chromatography (ethyl acetate/petroleum ether 1/6). **14α-*tert*-Butyldimethyl-silyloxy-19-acetoxy-andrographolide (6a):** 65% yield, white solid, m.p. 129.7-130.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.64 – 6.57 (1H, m, 12-H), 5.20 (1H, d, *J* = 5.7 Hz, 14β-H), 4.81 (1H, s, 17α-H), 4.72 (1H, d, *J* = 4.7 Hz, 3α-O*H*), 4.51 (1H, s, 17β-H), 4.47 (1H, dd, *J* = 10.0, 5.9 Hz, 15α-H), 4.17 – 4.07 (2H, m, 19-H), 4.01 (1H, dd, *J* = 10.0, 2.3 Hz, 15β-H), 3.18 (1H, dt, *J* = 10.4, 5.0 Hz, 3β-H), 2.46 (2H, t, *J* = 6.5 Hz, 9β-H and 11α-H), 2.33 (1H, dt, *J* = 12.4, 2.4 Hz, 11β-H), 1.99 (1H, s, 7α-H), 1.96 (3H, s, C*H*$_3$CO), 1.96 – 1.89 (1H, m, 7β-H), 1.81 (1H, d, *J* = 11.2 Hz, 2α-H), 1.67 (1H, d, *J* = 13.0 Hz, 2β-H), 1.63 – 1.50 (2H, m, 1-H), 1.44 (1H, td, *J* = 13.3, 4.2 Hz, 6α-H), 1.34 – 1.21 (2H, m, 5β-H and 6β-H), 1.04 (3H, s, 18-H), 0.87 (9H, s, SiC(CH_3)$_3$), 0.68 (3H, s, 20-H), 0.15 (3H, s, SiCH_3), 0.12 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.4 (16-C), 169.4 (CH$_3$*C*O), 147.8 (12-C), 147.4 (8-C), 127.6 (13-C), 108.4 (17-C), 76.5 (3-C), 73.9 (15-C), 66.4 (14-C), 65.1 (19-C), 55.3 (9-C), 53.9 (5-C), 41.6 (4-C), 38.6 (10-C), 37.5 (7-C), 36.8 (1-C), 27.5 (2-C), 25.6 (SiC(*C*H$_3$)$_3$), 24.6 (6-C), 24.2 (11-C), 22.8 (18-C), 20.9 (*C*H$_3$CO), 17.5 (Si*C*(CH$_3$)$_3$), 14.2 (20-C), -4.5 (Si*C*H$_3$), -5.0 (Si*C*H$_3$); HRMS (ESI) *m/z* 529.2972 [M+Na]$^+$, calculated for $C_{28}H_{46}O_6SiNa$, 529.2961. **14β-*tert*-Butyldimethylsilyloxy-19-acetoxy-andrographolide (6b):** 58% yield, white solid, m.p. 149.8-150.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.62 – 6.54 (1H, m, 12-H), 5.24 (1H, d, *J* = 5.5 Hz, 14α-H), 4.80 (1H, s, 17α-H), 4.74 (1H, d, *J* = 4.6 Hz, 3α-O*H*), 4.48 (1H, dd, *J* = 9.9, 6.1 Hz, 15α-H), 4.33 (1H, s, 17β-H), 4.11 (2H, q, *J* = 11.7 Hz, 19-H), 3.99 (1H, dd, *J* = 9.9, 2.4 Hz, 15β-H), 3.17 (1H, dt, *J* = 10.8, 5.1 Hz, 3β-H), 2.49 – 2.28 (3H, m, 9β-H, 11-H), 2.06 – 1.99 (1H, m, 7α-H), 1.96 (3H, s, C*H*$_3$CO), 1.92 (1H, dd, *J* = 13.7, 4.1 Hz, 7β-H), 1.86 – 1.77 (1H, m, 2α-H), 1.65 – 1.50 (3H, m, 1-H and 2β-H), 1.47 (1H, dd, *J* = 13.1, 3.6 Hz, 6α-H), 1.36 – 1.21 (2H, m, 5β-H and 6β-H), 1.03 (3H, s, 18-H), 0.86 (9H, s, SiC(CH_3)$_3$), 0.68 (3H, s, 20-H), 0.16 (3H, s, SiCH_3), 0.11 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.4 (16-C), 169.3 (CH$_3$*C*O), 147.9 (12-C), 147.3 (8-C), 128.1 (13-C), 107.9 (17-C), 76.5 (3-C), 73.9 (15-C), 66.7 (14-C), 65.0 (19-C), 55.2 (9-C), 53.9 (5-C), 41.6 (4-C), 38.6 (10-C), 37.6 (7-C), 36.8 (1-C), 27.5 (2-C), 26.4 (6-C), 25.5 (SiC(*C*H$_3$)$_3$), 24.8 (11-C), 22.8 (18-C), 20.9 (*C*H$_3$CO), 17.5 (Si*C*(CH$_3$)$_3$), 14.2 (20-C), -4.4 (Si*C*H$_3$), -5.0 (Si*C*H$_3$); HRMS (ESI) *m/z* 529.2956 [M+Na]$^+$, calculated for $C_{28}H_{46}O_6SiNa$, 529.2961.

Preparation of the compounds 7a and 7b

0.5 g (1.0 mmol) of compound **6a** or **6b** and 0.013 g (0.1 mmol) of anhydrous $ZnCl_2$ were dissolved in 10.0 ml Ac_2O. The solution was heated to 50 °C and stirred for 1 h to complete the reaction. Treated with ethyl acetate and sol. sat. $NaHCO_3$ after the resulting mixture was cooled to room temperature. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and then concentrated, and silica gel column chromatographed (ethyl acetate/petroleum ether 1/8) to give compound **7a** or **7b**. **14α-*tert*-Butyldimethylsilyloxy-3,19-di-acetoxy-andrographolide (7a):** 88% yield, white solid, m.p. 186.2-186.8 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 6.96 (1H, ddd, *J* = 7.3, 5.2, 2.0 Hz, H-12), 4.83 (1H, s, 17α-H), 4.71 (1H, dd, *J* = 12.0, 4.4 Hz, 3β-H), 4.67 (1H, s, 17β-H), 4.57 (1H, d, *J* = 11.8 Hz, 19α-H), 4.51 – 4.46 (1H, m, 14β-H), 4.09 (1H, d, *J* = 11.8 Hz, 19β-H), 3.84 (1H, dd, *J* = 9.6, 6.6 Hz, 15α-H), 3.71 (1H, dd, *J* = 9.6, 3.6 Hz, 15β-H), 2.48 (1H, ddd, *J* = 17.7, 10.9, 7.7 Hz, 11α-H), 2.19 (1H, ddd, *J* = 12.9, 3.7, 2.3 Hz, 11β-H), 2.10 (1H, d, *J* = 17.2 Hz, 9β-H), 1.78 – 1.71 (2H, m, 7-H), 1.71 (3H, s, C*H*$_3$CO), 1.69 (3H, s, C*H*$_3$CO), 1.67 – 1.55 (2H, m, 2-H), 1.51 – 1.41 (2H, m, 1-H), 1.30 (1H, qd, *J* = 13.1, 4.1 Hz, 6α-H), 1.01 (3H, s, 18-H), 0.99 – 0.89 (2H, m, 5β-H and 6β-H), 0.87 (9H, s, SiC(CH_3)$_3$), 0.66 (3H, s, 20-H), -0.02 (3H, s, SiCH_3), -0.16 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.2 (16-C), 169.9 (CH$_3$*C*O), 169.4 (CH$_3$*C*O), 147.4 (12-C), 147.2 (8-C), 127.7 (13-C), 108.8 (17-C), 79.1 (3-C), 73.9 (15-C), 66.4 (14-C), 63.6 (19-C), 54.8 (9-C), 53.8 (5-C), 40.9 (4-C), 38.4 (10-C), 37.1 (7-C), 36.3 (1-C), 25.6 (SiC(*C*H$_3$)$_3$), 24.2 (2-C), 24.0 (6-C), 23.9 (11-C), 22.3 (18-C), 20.9 (*C*H$_3$CO), 20.8 (*C*H$_3$CO), 17.5 (Si*C*(CH$_3$)$_3$), 14.3 (20-C), -4.5 (Si*C*H$_3$), -5.0 (Si*C*H$_3$); HRMS (ESI) *m/z* 566.3527 [M+NH$_4$]$^+$, calculated for $C_{30}H_{49}NO_7Si$, 566.3513. **14β-*tert*-Butyldimethylsilyloxy-3,19-diacetoxy-andrographolide (7b):** 92% yield, white solid, m.p. 152.7-153.6 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 6.93 (1H, ddd, *J* = 6.7, 4.5, 1.8 Hz, 12-H), 4.84 (1H, s,

17α-H), 4.68 (1H, dd, *J* = 11.9, 4.5 Hz, 3β-H), 4.58 – 4.49 (2H, m, 14α-H and 19α-H), 4.37 (1H, s, 17β-H), 4.11 (1H, d, *J* = 11.8 Hz, 19β-H), 3.80 – 3.73 (1H, m, 15α-H), 3.72 – 3.67 (1H, m, 15β-H), 2.41 – 2.17 (3H, m, 11-H and 9β-H), 1.74 (1H, dd, *J* = 13.3, 4.4 Hz, 7α-H), 1.69 (6H, d, *J* = 3.7 Hz, CH_3CO and CH_3CO), 1.67 – 1.51 (3H, m, 2-H and 7β-H), 1.50 – 1.27 (3H, m, 1-H and 6α-H), 1.00 (3H, s, 18-H), 0.92 (2H, d, *J* = 12.8 Hz, 5β-H and 6β-H), 0.86 (9H, s, SiC(CH_3)$_3$), 0.67 (3H, s, 20-H), -0.02 (3H, s, SiCH_3), -0.17 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, C_6D_6) *δ* 170.0 (16-C), 169.7 (CH$_3$*C*O), 169.0 (CH$_3$*C*O), 147.9 (12-C), 147.0 (8-C), 128.5 (13-C), 108.2 (17-C), 79.4 (3-C), 73.3 (15-C), 67.7 (14-C), 64.6 (19-C), 55.6 (9-C), 54.6 (5-C), 41.5 (4-C), 38.9 (10-C), 38.0 (7-C), 37.0 (1-C), 25.7 (SiC(*C*H$_3$)$_3$), 25.3 (2-C), 24.8 (6-C), 24.5 (11-C), 22.7 (18-C), 20.7[3] (*C*H$_3$CO), 20.6[5] (*C*H$_3$CO), 18.0 (Si*C*(CH$_3$)$_3$), 14.9 (20-C), -4.2 (Si*C*H$_3$), -4.8 (Si*C*H$_3$); HRMS (ESI) *m/z* 571.3062 [M+Na]$^+$, calculated for $C_{30}H_{48}O_7SiNa$, 571.3067.

Preparation of the compounds 8a and 8b

5.0 g (10.8 mmol) of compound **5a** or **5b** and 4.4 g (64.7 mmol) of imidazole were dissolved in 30.00 ml anhydrous dichloromethane and then 8.14 g (52.9 mmol) of TBSCl in 5.0 ml of anhydrous dichloromethane was added dropwise over 2 min. The reaction mixture was stirred for 1 h at room temperature and treated with ethyl acetate and sol. sat. $NaHCO_3$ after the reaction was complete. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/8) to afford compound **8a** or **8b**. **14*α*,19-Di-*tert*-butyldimethylsilyloxy-andrographolide (8a):** 87% yield, white solid, m.p. 122.5-123.6 °C. ^{1}H NMR (400 MHz, C_6D_6) *δ* 6.96 (1H, td, *J* = 5.3, 2.6 Hz, 12-H), 4.84 (1H, s, 17α-H), 4.67 (1H, s, 17β-H), 4.52 – 4.46 (1H, m, 14β-H), 4.22 (1H, d, *J* = 10.0 Hz, 19α-H), 4.01 (1H, d, *J* = 7.3 Hz, 3α-O*H*), 3.83 (1H, dd, *J* = 9.6, 6.6 Hz, 15α-H), 3.71 (1H, dd, *J* = 9.6, 3.6 Hz, 15β-H), 3.47 – 3.37 (2H, m, 3β-H and 19β-H), 2.56 – 2.43 (1H, m, 11α-H), 2.23 – 2.12 (2H, m, 9β-H and 11β-H), 2.04 (1H, dq, *J* = 13.4, 3.7 Hz, 7α-H), 1.78 (2H, qd, *J* = 13.5, 3.5 Hz, 7β-H and 1α-H), 1.61 – 1.46 (3H, m, 1β-H and 2-H), 1.31 (3H, s, 18-H), 1.16 – 1.04 (1H, m, 6α-H), 1.00 (2H, td, *J* = 13.3, 12.8, 3.4 Hz, 5β-H and 6β-H), 0.92 (9H, s, SiC(CH_3)$_3$), 0.87 (9H, s, SiC(CH_3)$_3$), 0.67 (3H, s, 20-H), 0.02 (3H, s, SiCH_3), 0.01 (3H, s, SiCH_3), 0.00 (3H, s, SiCH_3), -0.16 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, C_6D_6) *δ* 169.0 (16-C), 147.3 (12-C), 147.2 (8-C), 128.2 (13-C), 109.7 (17-C), 79.9 (3-C), 73.3 (15-C), 67.5 (14-C), 65.6 (19-C), 56.1 (9-C), 55.1 (5-C), 43.0 (4-C), 39.0 (7-C), 38.0 (10-C), 37.7 (1-C), 29.3 (2-C), 26.0 (SiC(*C*H$_3$)$_3$), 25.8 (SiC(*C*H$_3$)$_3$), 24.7 (6-C), 24.2 (11-C), 23.4 (18-C), 18.3 (Si*C*(CH$_3$)$_3$), 18.0 (Si*C*(CH$_3$)$_3$), 15.8 (20-C), -4.3 (Si*C*H$_3$), -4.7 (Si*C*H$_3$), -5.7[1] (Si*C*H$_3$), -5.7[2] (Si*C*H$_3$); HRMS (ESI) *m/z* 579.3910 [M+H]$^+$, calculated for $C_{32}H_{59}O_5Si_2$, 579.3901. **14β,19-Di-*tert*-butyl-dimethylsilyloxy-andrographolide (8b):** 75% yield, white solid, m.p. 146.5-147.3 °C. ^{1}H NMR (400 MHz, C_6D_6) *δ* 6.92 (1H, ddd, *J* = 8.6, 4.2, 2.0 Hz, 12-H), 4.86 (1H, s, 17α-H), 4.54 (1H, dd, *J* = 4.2, 2.0 Hz, 14α-H), 4.38 (1H, s, 17β-H), 4.20 (1H, d, *J* = 10.0 Hz, 19α-H), 3.90 (1H, d, *J* = 7.2 Hz, 3α-O*H*), 3.76 (1H, dd, *J* = 9.6, 6.4 Hz, 15α-H), 3.70 (1H, dd, *J* = 9.6, 3.6 Hz, 15β-H), 3.47 – 3.33 (2H, m, 3β-H and 19β-H), 2.48 – 2.37 (1H, m, 11α-H), 2.36 – 2.19 (2H, m, 9β-H and 11β-H), 1.98 (1H, dq, *J* = 11.2, 3.7 Hz, 7α-H), 1.83 – 1.68 (2H, m, 7β-H and 2α-H), 1.66 – 1.57 (1H, m, 2β-H), 1.51 (2H, ddd, *J* = 22.3, 11.8, 6.4 Hz, 1-H), 1.31 (3H, s, 18-H), 1.15 (1H, qd, *J* = 13.0, 4.2 Hz, 6α-H), 0.99 – 0.93 (2H, m, 5β-H and 6β-H), 0.92 (9H, s, SiC(CH_3)$_3$), 0.87 (9H, s, SiC(CH_3)$_3$), 0.68 (3H, s, 20-H), 0.05 – -0.05 (9H, m, SiCH_3 and Si(CH_3)$_2$), -0.16 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, C_6D_6) *δ* 169.0 (16-C), 148.3 (12-C), 147.3 (8-C), 128.4 (13-C), 108.0 (17-C), 79.7 (3-C), 73.3 (15-C), 67.8 (14-C), 65.5 (19-C), 55.9 (9-C), 54.9 (5-C), 43.0 (4-C), 39.0 (10-C), 38.0 (7-C), 37.5 (1-C), 29.2 (2-C), 26.0 (SiC(*C*H$_3$)$_3$), 25.7 (SiC(*C*H$_3$)$_3$), 25.4 (6-C), 24.3 (11-C), 23.4 (18-C), 18.3 (Si*C*(CH$_3$)$_3$), 18.0 (Si*C*(CH$_3$)$_3$), 15.7 (20-C), -4.2 (Si*C*H$_3$), -4.8 (Si*C*H$_3$), -5.7 (Si(*C*H$_3$)$_2$); HRMS (ESI) *m/z* 579.3894 [M+H]$^+$, calculated for $C_{32}H_{59}O_5Si_2$, 579.3901.

Preparation of the compounds 9a and 9b

1.5 g (2.6 mmol) of compound **8a** or **8b** and 0.32 g (0.26 mmol) of DMAP were dissolved in 10.0 ml ethyl acetate and then 0.29 ml (3.1 mmol) of Ac_2O was added dropwise in 1 min. The reaction mixture was stirred for 24 h at rt and treated with ethyl acetate and sol. sat. $NaHCO_3$ after the reaction was complete. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in a vaccum. Purification by silica gel column chromatography (ethyl acetate/petroleum ether 1/10) gave compound **9a** or **9b**. **3-Acetoxy-14*α*,19-di-*tert*-butyldimethylsilyloxy-andrographolide (9a):** 89% yield, white solid, m.p. 127.1-127.8 °C. ^{1}H NMR (400 MHz, C_6D_6) *δ* 7.02 (1H, ddd, *J* = 7.3, 5.6, 2.0 Hz, 12-H), 4.85 (1H, s, 17α-H), 4.80 (1H, dd, *J* = 11.5, 4.9 Hz, 3β-H), 4.67 (1H, s, 17β-H), 4.52 (1H, dq, *J* = 4.5, 1.6 Hz, 14β-H), 3.90 (1H, d, *J* = 10.4 Hz, 19α-H), 3.84 (1H, dd, *J* = 9.6, 6.5 Hz, 15α-H), 3.77 – 3.69 (2H, m, 15β-H and 19β-H), 2.53 (1H, ddd, *J* = 18.5, 11.4, 7.3 Hz, 11α-H), 2.28 (1H, dt, *J* = 12.3, 2.5 Hz, 9β-H), 2.20 (1H, dt, *J* = 17.3, 3.7 Hz, 11β-H), 1.83 – 1.78 (1H, m, 7α-H), 1.77 (3H, s, CH_3CO), 1.77 – 1.63 (4H, m, 7β-H, 2-H and 1α-H), 1.60 – 1.51 (2H, m, 1β-H and 6α-H), 1.07 (1H, dd, *J* = 9.8, 3.4 Hz, 5β-H), 1.04 (3H, s, 18-H), 1.02 (1H, d, *J* = 5.9 Hz, 6β-H), 0.97 (9H, s, SiC(CH_3)$_3$), 0.88 (3H, s, 20-H), 0.85 (9H, s, SiC(CH_3)$_3$), 0.06 (6H, d, *J* = 5.6 Hz, Si(CH_3)$_2$), -0.00 (3H, s, SiCH_3), -0.15 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, C_6D_6) *δ* 170.0 (16-C), 169.0 (CH$_3$*C*O), 147.5 (12-C), 147.2 (8-C), 128.2 (13-C), 109.6 (17-C), 79.9 (3-C), 73.3 (15-C), 67.5 (14-C), 64.2 (19-C), 56.2 (9-C), 55.3 (5-C), 42.8 (4-C), 39.1 (10-C), 38.4 (7-C), 37.6 (1-C), 26.1 (SiC(*C*H$_3$)$_3$), 25.8 (SiC(*C*H$_3$)$_3$), 25.6 (2-C), 24.9 (6-C), 24.8 (11-C), 23.4 (18-C), 20.9 (*C*H$_3$CO), 18.5 (Si*C*(CH$_3$)$_3$), 18.0 (Si*C*(CH$_3$)$_3$), 15.0 (20-C), -4.3 (Si*C*H$_3$), -4.7 (Si*C*H$_3$), -5.4[7] (Si*C*H$_3$), -5.5[3] (Si*C*H$_3$); HRMS (ESI) *m/z* 643.3841 [M+Na]$^+$, calculated for $C_{34}H_{60}O_6Si_2Na$, 643.3826. **3-Acetoxy-14β,19-di-*tert*-butyldimethylsilyloxy-andrographolide (9b):** 61% yield, white solid, m.p. 157.8-159.6 °C. ^{1}H NMR (400 MHz, C_6D_6) *δ* 7.00 – 6.94 (1H, m, 12-H), 4.86 (1H, s, 17α-H), 4.76 (1H, dd, *J* = 11.3, 5.2 Hz, 3β-H), 4.58 – 4.49 (1H, m, 14α-H), 4.39 (1H, s, 17β-H), 3.93 (1H, d, *J* = 10.5 Hz, 19α-H), 3.81 – 3.65 (3H, m, 15-H and 19β-H), 2.50 – 2.24 (3H, m, 9β-H and 11-H), 1.82 – 1.74 (3H, m, 2α-H and 7-H), 1.74 (3H, s, CH_3CO), 1.72 – 1.60 (2H, m, 2β-H and 1α-H), 1.58 – 1.49 (2H, m, 1β-H and 6α-H), 1.04 (3H, s, 18-H), 1.02 – 0.99 (2H, m, 5β-H and 6β-H), 0.98 (9H, s, SiC(CH_3)$_3$), 0.90 (3H, s, 20-H), 0.87 (9H, s, SiC(CH_3)$_3$), 0.06 (6H, d, *J* = 4.6 Hz, Si(CH_3)$_2$), -0.00 (3H, s, SiCH_3), -0.16 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, C_6D_6) *δ* 169.8 (16-C), 169.0 (CH$_3$*C*O), 148.6 (12-C), 147.3 (8-C), 128.4 (13-C), 108.0 (17-C),

79.7 (3-C), 73.3 (15-C), 67.8 (14-C), 64.4 (19-C), 56.0 (9-C), 55.1 (5-C), 42.7 (4-C), 39.1 (10-C), 38.5 (7-C), 37.4 (1-C), 26.1 (SiC(CH_3)$_3$), 25.9 (2-C), 25.7 (SiC(CH_3)$_3$), 25.5 (6-C), 24.7 (11-C), 23.3 (18-C), 20.8 (CH_3CO), 18.5 (SiC(CH_3)$_3$), 18.0 (SiC(CH_3)$_3$), 14.9 (20-C), -4.2 (SiCH_3), -4.8 (SiCH_3), -5.4[9] (SiCH_3), -5.5[5] (SiCH_3); HRMS (ESI) *m/z* 643.3822 $[M+Na]^+$, calculated for $C_{34}H_{60}O_6Si_2Na$, 643.3826.

Preparation of the compounds 10a and 10b

Under N_2 atmosphere, 5.0 g (8.6 mmol) of compound **8a** or **8b** and 2.5 ml (21.6 mmol) of 2,6-lutidine were dissolved in 60.0 ml anhydrous dichloromethane. The solution was cooled to 0 °C and then 4.0 ml (17.3 mmol) of TBSOTf was added dropwise over 2 min. The reaction mixture was stirred for 1 h at 0 °C and then treated with ethyl acetate and sol. sat. $NaHCO_3$ after the reaction was complete. The organic phase was washed with brine for 6 times, dried over anhydrous Na_2SO_4, filtered and then concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/30) to yield compound **10a** or **10b**. **3,14*α*,19-Tri-*tert*-butyldimethylsilyloxy-andrographolide (10a):** 71% yield, white solid, m.p. 154.6-155.3 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 7.10 – 7.05 (1H, m, 12-H), 4.87 (1H, s, 17α-H), 4.68 (1H, s, 17β-H), 4.57 – 4.51 (1H, m, 14β-H), 4.07 (1H, d, J = 10.6 Hz, 19α-H), 3.82 (2H, dd, J = 9.7, 6.7 Hz, 15α-H and 19β-H), 3.72 (1H, dd, J = 9.6, 3.5 Hz, 15β-H), 3.29 (1H, dd, J = 11.6, 4.1 Hz, 3β-H), 2.67 – 2.55 (1H, m, 11α-H), 2.42 – 2.23 (2H, m, 9β-H and 11β-H), 1.99 – 1.53 (7H, m, 1-H, 2-H, 6α-H and 7-H), 1.16 (3H, s, 18-H), 1.05 (1H, d, J = 3.5 Hz, 5β-H), 1.03 (9H, s, SiC(CH_3)$_3$), 0.99 (1H, m, 6β-H), 0.97 (12H, d, J = 8.7 Hz, 20-H and SiC(CH_3)$_3$), 0.86 (9H, s, SiC(CH_3)$_3$), 0.15 (3H, s, SiCH_3), 0.10 (6H, d, J = 2.6 Hz, Si(CH_3)$_2$), 0.07 (3H, s, SiCH_3), 0.02 (3H, s, SiCH_3), -0.15 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, C_6D_6) δ 169.0 (16-C), 147.9 (12-C), 147.5 (8-C), 128.4 (13-C), 109.3 (17-C), 79.7 (3-C), 73.3 (15-C), 67.6 (14-C), 64.8 (19-C), 56.6 (9-C), 55.3 (5-C), 44.2 (4-C), 39.2 (10-C), 38.9 (7-C), 38.0 (1-C), 28.8 (2-C), 26.5 (6-C), 26.1[9] (SiC(CH_3)$_3$), 26.1[5] (SiC(CH_3)$_3$), 25.8 (SiC(CH_3)$_3$), 24.9 (11-C), 24.0 (18-C), 18.5 (SiC(CH_3)$_3$), 18.4 (SiC(CH_3)$_3$), 18.0 (SiC(CH_3)$_3$), 14.9 (20-C), -3.7 (SiCH_3), -4.3 (SiCH_3), -4.7 (Si(CH_3)$_2$), -5.4 (SiCH_3), -5.5 (SiCH_3); HRMS (ESI) *m/z* 715.4593 $[M+Na]^+$, calculated for $C_{38}H_{72}O_5Si_3Na$, 715.4585. **3,14β,19-Tri-*tert*-butyldimethylsilyloxy-andrographolide (10b):** 89% yield, white solid, m.p. 159.2-160.5 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 7.01 (1H, ddd, J = 8.6, 4.4, 2.0 Hz, 12-H), 4.88 (1H, s, 17α-H), 4.61 – 4.54 (1H, m, 14α-H), 4.41 (1H, s, 17β-H), 4.06 (1H, d, J = 10.6 Hz, 19α-H), 3.81 (1H, d, J = 10.5 Hz, 19β-H), 3.77 (1H, dd, J = 9.6, 6.4 Hz, 15α-H), 3.71 (1H, dd, J = 9.6, 3.6 Hz, 15β-H), 3.24 (1H, dd, J = 11.7, 4.3 Hz, 3β-H), 2.56 – 2.34 (3H, m, 9β-H and 11-H), 2.06 – 1.74 (3H, m, 2α-H and 7-H), 1.73 – 1.46 (4H, m, 1-H, 2β-H and 6α-H), 1.15 (3H, s, 18-H), 1.02 (9H, s, SiC(CH_3)$_3$), 0.99 (9H, s, SiC(CH_3)$_3$), 0.97 (3H, s, 20-H), 0.93 (2H, dt, J = 10.2, 4.9 Hz, 5β-H and 6β-H), 0.88 (9H, s, SiC(CH_3)$_3$), 0.14 (3H, s, SiCH_3), 0.12 – 0.06 (9H, m, SiCH_3 and Si(CH_3)$_2$), 0.02 (3H, s, SiCH_3), -0.15 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, C_6D_6) δ 169.1 (16-C), 149.0 (12-C), 147.6 (8-C), 128.4 (13-C), 107.7 (17-C), 79.6 (3-C), 73.4 (15-C), 67.8 (14-C), 64.9 (19-C), 56.6 (9-C), 55.1 (5-C), 44.1 (4-C), 39.3 (10-C), 38.9 (7-C), 37.8 (1-C), 28.7 (2-C), 26.7 (6-C), 26.2 (SiC(CH_3)$_3$), 26.1 (SiC(CH_3)$_3$), 25.8 (SiC(CH_3)$_3$), 25.6 (11-C), 23.9 (18-C), 18.5 (SiC(CH_3)$_3$), 18.3 (SiC(CH_3)$_3$), 18.0 (SiC(CH_3)$_3$), 14.9 (20-C), -3.7 (SiCH_3), -4.2 (SiCH_3), -4.7 (SiCH_3), -4.8 (SiCH_3), -5.4 (SiCH_3), -5.5 (SiCH_3); HRMS (ESI) *m/z* 715.4579 $[M+Na]^+$, calculated for $C_{38}H_{72}O_5Si_3Na$, 715.4585.

Preparation of the compounds 11a and 11b

To the solution of 1.0 g (1.6 mmol) of compound **9a** or **9b** in 30.0 ml anhydrous dichloromethane at -20 °C, 10.0 ml (6.7 mmol) of TFA and 1.00 ml (6.7 mmol) of H_2O were added dropwise over 10 min. The reaction mixture was stirred for 0.5 h at -20 °C, and then diluted with ethyl acetate and carefully treated sol. sat. $NaHCO_3$. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and then concentrated. After silica gel column chromatography (ethyl acetate/petroleum ether 1/7), compound **11a** or **11b** was afforded. **3-Acetoxy-14*α*-*tert*-butyldimethylsilyloxy-andrographolide (11a):** 64% yield, white solid, m.p. 113.9-115.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.65 – 6.59 (1H, m, 12-H), 5.20 (1H, d, J = 5.7 Hz, 14β-H), 4.81 (1H, s, 17α-H), 4.52 (1H, s, 17β-H), 4.52 – 4.44 (2H, m, 3β-H and 15α-H), 4.01 (1H, dd, J = 7.3, 2.7 Hz, 15β-H), 3.99 (1H, d, J = 5.3 Hz, 19-OH), 3.64 (1H, dd, J = 11.4, 6.1 Hz, 19α-H), 3.54 (1H, dd, J = 11.4, 4.7 Hz, 19β-H), 2.46 (2H, t, J = 8.0 Hz, 9β-H and 11α-H), 2.31 (1H, dt, J = 12.9, 3.2 Hz, 11β-H), 2.01 (3H, s, CH_3CO), 2.01 – 1.98 (1H, m, 7α-H), 1.93 (1H, td, J = 13.3, 12.8, 4.5 Hz, 7β-H), 1.81 – 1.53 (5H, m, 1-H, 2-H and 6α-H), 1.40 – 1.28 (2H, m, 5β-H and 6β-H), 0.91 (3H, s, 18-H), 0.87 (9H, s, SiC(CH_3)$_3$), 0.75 (3H, s, 20-H), 0.15 (3H, s, SiCH_3), 0.12 (3H, s, SiCH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.1 (16-C), 169.4 ($CH_3$$C$O), 147.9 (12-C), 147.4 (8-C), 127.6 (13-C), 108.4 (17-C), 79.9 (3-C), 73.9 (15-C), 66.4 (14-C), 61.6 (19-C), 55.1 (9-C), 54.4 (5-C), 42.0 (4-C), 38.5 (10-C), 37.5 (7-C), 36.7 (1-C), 25.5 (SiC(CH_3)$_3$), 24.7 (2-C), 24.2 (6-C), 23.9 (11-C), 22.7 (18-C), 20.9 (CH_3CO), 17.5 (SiC(CH_3)$_3$), 14.3 (20-C), -4.5 (SiCH_3), -5.0 (SiCH_3); HRMS (ESI) *m/z* 529.2979 $[M+Na]^+$, calculated for $C_{28}H_{46}O_6SiNa$, 529.2961. **3-Acetoxy-14β-*tert*-butyldimethylsilyloxy-andrographolide (11b):** 66% yield, white solid, m.p. 163.2-164.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.58 (1H, dd, J = 7.2, 3.9 Hz, 12-H), 5.24 (1H, d, J = 5.1 Hz, 14α-H), 4.79 (1H, s, 17α-H), 4.50 – 4.45 (2H, m, 3β-H and 15α-H), 4.32 (1H, s, 17β-H), 4.05 (1H, s, 19-OH), 3.99 (1H, dd, J = 9.9, 2.2 Hz, 15β-H), 3.63 (1H, dd, J = 11.3, 4.6 Hz, 19α-H), 3.57 – 3.48 (1H, m, 19β-H), 2.48 – 2.24 (3H, m, 9β-H and 11-H), 2.05 (1H, d, J = 10.0 Hz, 7α-H), 2.00 (3H, s, CH_3CO), 1.92 (1H, td, J = 12.9, 4.5 Hz, 7β-H), 1.82 – 1.49 (5H, m, 1-H, 2-H and 6α-H), 1.35 (2H, t, J = 13.0 Hz, 5β-H and 6β-H), 0.91 (3H, s, 18-H), 0.86 (9H, s, SiC(CH_3)$_3$), 0.75 (3H, s, 20-H), 0.13 (6H, d, J = 17.8 Hz, Si(CH_3)$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.1 (16-C), 169.4 ($CH_3$$C$O), 148.0 (12-C), 147.4 (8-C), 128.1 (13-C), 107.9 (17-C), 79.8 (3-C), 73.9 (15-C), 66.7 (14-C), 61.6 (19-C), 54.9 (9-C), 54.4 (5-C), 42.0 (4-C), 38.6 (10-C), 37.6 (7-C), 36.7 (1-C), 25.6 (SiC(CH_3)$_3$), 24.9 (2-C), 24.8 (6-C), 24.0 (11-C), 22.7 (18-C), 21.0 (CH_3CO), 17.6 (SiC(CH_3)$_3$), 14.4 (20-C), -4.3 (SiCH_3), -5.0 (SiCH_3); HRMS (ESI) *m/z* 529.2958 $[M+Na]^+$, calculated for $C_{28}H_{46}O_6SiNa$, 529.2961.

Preparation of the compounds 12a and 12b

To the solution of 0.5 g (0.7 mmol) of compound **10a** or **10b** in 10.0 ml anhydrous dichloromethane at -20 °C, 0.1 ml (1.3 mmol) of TFA was added dropwise over 2 min. The reaction mixture was stirred for 5 min at -20 °C and treated with ethyl acetate and

sol. sat. $NaHCO_3$ after the reaction was complete. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/15) to give compound **12a** or **12b**. **3,14α-Di-*tert*-butyldimethylsilyloxy-andrographolide (12a):** 71% yield, white solid, m.p. 176.6-177.7 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 7.00 (1H, ddd, J = 7.3, 5.3, 2.0 Hz, 12-H), 4.82 (1H, s, 17α-H), 4.66 (1H, s, 17β-H), 4.55 – 4.47 (1H, m, 14β-H), 4.24 (1H, d, J = 10.9 Hz, 19α-H), 3.81 (1H, dd, J = 9.6, 6.6 Hz, 15α-H), 3.70 (1H, dd, J = 9.6, 3.6 Hz, 15β-H), 3.42 – 3.30 (2H, m, 3β-H and 19-O*H*), 3.24 (1H, d, J = 10.9 Hz, 19β-H), 2.52 (1H, ddd, J = 17.6, 11.3, 7.6 Hz, 11α-H), 2.23 – 2.11 (2H, m, 9β-H and 11β-H), 1.84 – 1.70 (2H, m, 7-H), 1.66 – 1.43 (4H, m, 1-H and 2-H), 1.24 (3H, s, 18-H), 1.08 (1H, qd, J = 13.1, 4.2 Hz, 6α-H), 0.95 (9H, s, SiC(C*H*$_3$)$_3$), 0.90 (1H, d, J = 2.4 Hz, 6β-H), 0.87 (9H, s, SiC(C*H*$_3$)$_3$), 0.83 (1H, d, J = 3.7 Hz, 5β-H), 0.58 (3H, s, 20-H), 0.10 (3H, s, SiC*H*$_3$), 0.06 (3H, s, SiC*H*$_3$), 0.00 (3H, s, SiC*H*$_3$), -0.16 (3H, s, SiC*H*$_3$); ^{13}C NMR (101 MHz, C_6D_6) δ 169.0 (16-C), 147.3 (12-C), 147.2 (8-C), 128.2 (13-C), 109.8 (17-C), 82.4 (3-C), 73.3 (15-C), 67.6 (14-C), 64.1 (19-C), 56.1 (9-C), 55.0 (5-C), 43.7 (4-C), 38.7 (10-C), 38.0 (7-C), 37.4 (1-C), 28.6 (2-C), 26.0 (SiC(*C*H$_3$)$_3$), 25.8 (SiC(*C*H$_3$)$_3$), 24.9 (6-C), 24.1 (11-C), 23.6 (18-C), 18.1 (Si*C*(CH$_3$)$_3$), 18.0 (Si*C*(CH$_3$)$_3$), 15.5 (20-C), -4.1 (Si*C*H$_3$), -4.3 (Si*C*H$_3$), -4.7 (Si*C*H$_3$), -4.9 (Si*C*H$_3$); HRMS (ESI) (*m/z*): 601.3733 $[M+Na]^+$, calculated for $C_{32}H_{58}O_5Si_2Na$, 601.3720. **3,14β-Di-*tert*-butyldimethylsilyloxy-andrographolide (12b):** 50% yield, white solid, m.p. 175.8-177.0 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 6.96 (1H, ddd, J = 8.4, 4.5, 1.8 Hz, 12-H), 4.83 (1H, s, 17α-H), 4.59 – 4.49 (1H, m, 14α-H), 4.37 (1H, s, 17β-H), 4.23 (1H, d, J = 11.0 Hz, 19α-H), 3.76 (1H, dd, J = 16.0, 1.3 Hz, 15α-H), 3.70 (1H, dd, J = 9.5, 3.4 Hz, 15β-H), 3.37 (1H, t, J = 11.1 Hz, 19-O*H*), 3.30 (1H, dd, J = 11.5, 4.2 Hz, 3β-H), 3.24 (1H, d, J = 10.2 Hz, 19β-H), 2.47 – 2.16 (3H, m, 9β-H and 11-H), 1.74 (2H, tdd, J = 17.2, 13.2, 4.2 Hz, 7-H), 1.65 – 1.37 (4H, m, 1-H and 2-H), 1.25 (3H, s, 18-H), 1.20 – 1.05 (1H, m, 6α-H), 0.94 (9H, s, SiC(C*H*$_3$)$_3$), 0.87 (9H, s, SiC(C*H*$_3$)$_3$), 0.86 – 0.74 (2H, m, 5β-H and 6β-H), 0.59 (3H, s, 20-H), 0.09 (3H, s, SiC*H*$_3$), 0.04 (3H, s, SiC*H*$_3$), -0.00 (3H, s, SiC*H*$_3$), -0.16 (3H, s, SiC*H*$_3$); ^{13}C NMR (101 MHz, C_6D_6) δ 169.0 (16-C), 148.2 (12-C), 147.2 (8-C), 128.5 (13-C), 108.1 (17-C), 82.4 (3-C), 73.4 (15-C), 67.8 (14-C), 64.1 (19-C), 55.9 (9-C), 54.9 (5-C), 43.7 (4-C), 38.7 (10-C), 38.0 (7-C), 37.3 (1-C), 28.5 (2-C), 26.0 (SiC(*C*H$_3$)$_3$), 25.8 (SiC(*C*H$_3$)$_3$), 25.6 (6-C), 24.1 (11-C), 23.6 (18-C), 18.1 (Si*C*(CH$_3$)$_3$), 18.0 (Si*C*(CH$_3$)$_3$), 15.5 (20-C), -4.1 (Si*C*H$_3$), -4.2 (Si*C*H$_3$), -4.8 (Si*C*H$_3$), -4.9 (Si*C*H$_3$); HRMS (ESI) *m/z* 579.3896 $[M+H]^+$, calculated for $C_{32}H_{59}O_5Si_2$, 579.3901.

Preparation of the compounds 13a[13] and 13b[5]

The preparation of compound **13a** or **13b** from **3a** or **3b**, respectively, was used the procedure for the synthesis of **6a** or **6b**. Compound **13a** or **13b** was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/6). **3,19-Acetonylidene-14α-acetoxy-andrographolide (13a):** 93% yield, white solid, m.p. 106.0-107.2 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 7.04 (1H, td, J = 6.9, 1.7 Hz, 12-H), 5.62 (1H, d, J = 6.0 Hz, 14β-H), 4.80 (1H, d, J = 1.1 Hz, 17α-H), 4.48 (1H, s, 17β-H), 3.90 – 3.81 (2H, m, 15α-H and 19α-H), 3.76 (1H, dd, J = 11.1, 2.0 Hz, 15β-H), 3.46 (1H, dd, J = 7.9, 3.5 Hz, 3β-H), 3.09 (1H, d, J = 11.5 Hz, 19β-H), 2.34 – 2.12 (3H, m, 9β-H and 11-H), 1.95 – 1.82 (1H, m, 7α-H), 1.72 (1H, td, J = 13.6, 13.1, 4.7 Hz, 7β-H), 1.65 – 1.58 (1H, m, 2α-H), 1.56 (3H, s, C*H*$_3$CO), 1.55 – 1.52 (1H, m, 2β-H), 1.48 (1H, s, 1α-H), 1.45 (3H, s, CC*H*$_3$), 1.40 (3H, s, CC*H*$_3$), 1.38 – 1.31 (1H, m, 1β-H), 1.11 (3H, s, 18-H), 1.06 – 0.93 (2H, m, 5β-H and 6α-H), 0.90 (1H, dd, J = 12.9, 2.2 Hz, 6β-H), 0.86 (3H, s, 20-H); HRMS (ESI) *m/z* 455.2418 $[M+Na]^+$, calculated for $C_{25}H_{36}O_6Na$, 455.2410. **3,19-Acetonylidene-14β-acetoxy-andrographolide (13b)**: 90% yield, white solid, m.p. 140.0-142.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (1H, td, J = 6.9, 1.3 Hz, 12-H), 5.93 (1H, d, J = 6.0 Hz, 14α-H), 4.87 (1H, s, 17α-H), 4.56 (1H, dd, J = 11.3, 6.2 Hz, 15α-H), 4.41 (1H, s, 17β-H), 4.22 (1H, dd, J = 11.3, 1.8 Hz, 15β-H), 3.94 (1H, d, J = 11.6 Hz, 19α-H), 3.49 (1H, dd, J = 8.3, 3.8 Hz, 3β-H), 3.16 (1H, d, J = 11.6 Hz, 19β-H), 2.56 – 2.31 (3H, m, 9β-H and 11-H), 2.10 (3H, s, C*H*$_3$CO), 2.03 – 1.92 (2H, m, 7-H), 1.88 (1H, d, J = 10.5 Hz, 2α-H), 1.83 – 1.64 (3H, m, 1-H and 2β-H), 1.39 (3H, s, CC*H*$_3$), 1.35 (3H, s, CC*H*$_3$), 1.32 – 1.22 (3H, m, 5β-H and 6-H), 1.18 (3H, s, 18-H), 0.94 (3H, s, 20-H); HRMS (ESI) *m/z* 455.2397 $[M+Na]^+$, calculated for $C_{25}H_{36}O_6Na$, 455.2410.

Preparation of the compounds 14a[13] and 14b[5]

The procedure of the synthesis of **5a** or **5b** was used for the preparation of compound **14a** or **14b** from **13a** or **13b**. Crude product was silica gel column chromatographed (ethyl acetate/petroleum ether 1/2) to give compound **14a** or **14b**. **14α-Acetoxy-andrographolide (14a):** 93% yield, white solid, m.p. 169.8-170.8 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 6.98 – 6.91 (1H, m, 12-H), 6.02 (1H, d, J = 6.0 Hz, 14β-H), 4.89 (1H, s, 17α-H), 4.57 (1H, dd, J = 11.1, 6.1 Hz, 15α-H), 4.55 (1H, s, 17β-H), 4.29 (1H, dd, J = 11.1, 1.8 Hz, 15β-H), 4.11 (1H, d, J = 11.1 Hz, 19α-H), 3.41 (1H, d, J = 7.8 Hz, 3β-H), 3.37 (1H, d, J = 11.2 Hz, 19β-H), 2.57 (1H, ddd, J = 16.8, 6.3, 3.4 Hz, 11α-H), 2.49 – 2.39 (2H, m, 9β-H and 11β-H), 2.10 (3H, s, C*H*$_3$CO), 2.08 – 1.92 (2H, m, 7-H), 1.90 – 1.74 (4H, m, 1-H and 2-H), 1.45 – 1.24 (3H, m, 5β-H and 6-H), 1.22 (3H, s, 18-H), 0.73 (3H, s, 20-H); HRMS (ESI) *m/z* 415.2108 $[M+Na]^+$, calculated for $C_{20}H_{30}O_5Na$, 415.2097. **14β-Acetoxy-andrographolide (14b):** 88% yield, white solid, m.p. 163.0-165.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (1H, td, J = 7.1, 1.6 Hz, 12-H), 5.91 (1H, d, J = 6.0 Hz, 14α-H), 4.85 (1H, s, 17α-H), 4.54 (1H, dd, J = 11.3, 6.1 Hz, 15α-H), 4.36 (1H, s, 17β-H), 4.23 (1H, dd, J = 11.3, 1.8 Hz, 15β-H), 4.16 (1H, d, J = 10.9 Hz, 19α-H), 3.46 (1H, dt, J = 9.4, 4.1 Hz, 3β-H), 3.37 – 3.26 (1H, m, 3α-O*H*), 3.03 – 2.86 (2H, m, 19β-H and 19-O*H*), 2.55 – 2.25 (3H, m, 9β-H and 11-H), 2.11 (3H, s, C*H*$_3$CO), 1.96 (1H, td, J = 12.9, 12.3, 4.6 Hz, 7α-H), 1.89 – 1.75 (4H, m, 1α-H, 2-H and 7β-H), 1.75 – 1.67 (1H, m, 1β-H), 1.35 – 1.25 (1H, m, 6α-H), 1.24 (3H, s, 18-H), 1.23 – 1.13 (2H, m, 5β-H and 6β-H), 0.66 (3H, s, 20-H); HRMS (ESI) *m/z* 415.2067, $[M+Na]^+$, calculated for $C_{22}H_{32}O_6Na$, 415.2097.

Preparation of the compounds 15a[14] and 15b

4.0 g (10.2 mmol) of compound **14a** or **14b** and 8.49 ml (61.2 mmol) of TEA were dissolved in 20.0 ml anhydrous dichloromethane and then 8.47 g (56.1 mmol) of TBSCl in 5.0 ml of anhydrous dichloromethane was added dropwise over 2 min. The reaction mixture was stirred for 1 h at rt and treated with ethyl acetate and sol. sat. $NaHCO_3$ after the reaction was complete. The organic phase was washed with brine, dried over

anhydrous Na_2SO_4, filtered and then concentrated under reduced pressure. Compound **15a** or **15b** was provided by silica gel column chromatography (ethyl acetate/petroleum ether 1/6). **14α-Acetoxy-19-*tert*-butyldimethylsilyloxy-andrographolide (15a):** 74% yield, white solid, m.p. 159.1-159.7 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 7.01 (1H, td, J = 6.8, 1.7 Hz, 12-H), 5.62 (1H, d, J = 6.0 Hz, 14β-H), 4.78 (1H, s, 17α-H), 4.45 (1H, s, 17β-H), 4.17 (1H, d, J = 10.0 Hz, 19α-H), 3.91 – 3.83 (2H, m, 15α-H and 19-O*H*), 3.76 (1H, dd, J = 11.1, 1.9 Hz, 15β-H), 3.43 – 3.34 (2H, m, 3β-H and 19β-H), 2.24 – 2.15 (3H, m, 9β-H and 11-H), 1.97 (1H, dq, J = 13.6, 3.8 Hz, 7α-H), 1.79 – 1.65 (2H, m, 2α-H and 7β-H), 1.60 (1H, dt, J = 5.0, 2.3 Hz, 2β-H), 1.56 (3H, s, CH_3CO), 1.49 – 1.42 (2H, m, 1-H), 1.29 (3H, s, 18-H), 1.11 (1H, qd, J = 12.9, 4.2 Hz, 6α-H), 0.98 – 0.93 (1H, m, 5β-H), 0.92 (9H, s, SiC(CH_3)$_3$), 0.88 (1H, d, J = 3.3 Hz, 6β-H), 0.55 (3H, s, 20-H), 0.00 (6H, d, J = 3.4 Hz, Si(CH_3)$_2$); HRMS (ESI) *m/z* 529.2972 $[M+Na]^+$, calculated for $C_{28}H_{46}O_6SiNa$, 529.2961. **14β-Acetoxy-19-*tert*-butyl-dimethylsilyloxy-andrographolide (15b):** 69% yield, white solid, m.p. 147.9-149.8 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 7.03 (1H, t, J = 6.9 Hz, 12-H), 5.60 (1H, d, J = 5.2 Hz, 14α-H), 4.80 (1H, s, 17α-H), 4.38 (1H, s, 17β-H), 4.17 (1H, d, J = 10.0 Hz, 19α-H), 3.91 (1H, d, J = 6.7 Hz, 19-O*H*), 3.82 (1H, dd, J = 11.4, 6.1 Hz, 15α-H), 3.77 (1H, d, J = 10.9 Hz, 15β-H), 3.40 (1H, d, J = 10.5 Hz, 19β-H), 3.35 (1H, dd, J = 13.0, 5.5 Hz, 3β-H), 2.27 – 2.06 (3H, m, 9β-H and 11-H), 1.92 (1H, dd, J = 13.5, 3.8 Hz, 7α-H), 1.69 (2H, q, J = 14.7, 13.7 Hz, 2α-H and 7β-H), 1.57 (3H, s, CH_3CO), 1.55 (1H, dd, J = 3.4, 2.7 Hz, 2β-H), 1.45 – 1.34 (2H, m, 1-H), 1.28 (3H, s, 18-H), 1.11 (1H, qd, J = 12.5, 12.0, 4.3 Hz, 6α-H), 0.91 (9H, s, SiC(CH_3)$_3$), 0.90 – 0.84 (2H, m, 5β-H and 6β-H), 0.56 (3H, s, 20-H), 0.01 (6H, d, J = 3.5 Hz, Si(CH_3)$_2$); ^{13}C NMR (101 MHz, C_6D_6) δ 169.7 (16-C), 168.5 (CH$_3$*C*O), 149.3 (12-C), 147.6 (8-C), 125.0 (13-C), 108.0 (17-C), 79.8 (3-C), 71.2 (15-C), 68.1 (14-C), 65.5 (19-C), 55.5 (9-C), 55.1 (5-C), 42.9 (4-C), 39.2 (10-C), 38.0 (7-C), 37.1 (1-C), 29.2 (2-C), 26.0 (SiC(*C*H$_3$)$_3$), 25.5 (6-C), 24.2 (11-C), 23.5 (18-C), 20.2 (*C*H$_3$CO), 18.3 (Si*C*(CH$_3$)$_3$), 15.4 (20-C), -5.7[1] (Si*C*H$_3$), -5.7[4] (Si*C*H$_3$); HRMS (ESI) *m/z* 507.3139 $[M+H]^+$, calculated for $C_{28}H_{47}O_6Si$, 507.3142.

Preparation of the compounds 16a and 16b

3.5 g (3.9 mmol) of compound **15a or 15b** and 3.35 g (13.8 mmol) of DMP were dissolved in 30.0 ml anhydrous dichloromethane. The reaction mixture which was protected from light was stirred for 1 h at rt and treated with ethyl acetate and sol. sat. $Na_2S_2O_3$ after the reaction was complete. The organic phase was washed with sat. $NaHCO_3$ and brine sequentially, dried over anhydrous Na_2SO_4, filtered, concentrated, and then silica gel column chromatographed (ethyl acetate/petroleum ether 1/7) to yield compound **16a** or **16b**. **3-Oxo-14α-acetoxy-19-*tert*-butyldi-methylsilyloxy-andrographolide (16a):** 91% yield, white solid, m.p. 122.0-122.7 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 6.98 (1H, ddd, J = 7.6, 6.2, 1.7 Hz, 12-H), 5.63 (1H, d, J = 5.9 Hz, 14β-H), 4.80 (1H, s, 17α-H), 4.48 (1H, s, 17β-H), 3.87 (1H, dd, J = 11.1, 6.0 Hz, 15α-H), 3.79 – 3.71 (2H, m, 15β-H and 19α-H), 3.54 (1H, d, J = 9.9 Hz, 19β-H), 2.45 (1H, ddd, J = 15.1, 14.0, 5.9 Hz, 2α-H), 2.31 (1H, ddd, J = 15.2, 4.9, 3.0 Hz, 11α-H), 2.27 – 2.08 (3H, m, 2β-H, 9β-H and 11β-H), 1.72 (1H, td, J = 13.8, 12.2, 5.3 Hz, 7α-H), 1.56 (4H, m, CH_3CO and 7β-H), 1.51 – 1.29 (4H, m, 1-H, 5β-H and 6α-H), 1.17 (3H, s, 18-H), 1.15 – 1.07 (1H, m, 6β-H), 0.93 (9H, s, SiC(CH_3)$_3$), 0.79 (3H, s, 20-H), 0.01 (6H, d, J = 1.3 Hz, Si(CH_3)$_2$); ^{13}C NMR (101 MHz, C_6D_6) δ 211.2 (3-C), 169.8 (16-C), 168.4 (CH$_3$*C*O), 149.1 (12-C), 147.3 (8-C), 124.9 (13-C), 109.2 (17-C), 71.1 (15-C), 67.8 (14-C), 66.7 (19-C), 56.3 (9-C), 55.1 (5-C), 53.7 (4-C), 38.8 (10-C), 37.9 (7-C), 37.5 (1-C), 36.3 (2-C), 26.0 (SiC(*C*H$_3$)$_3$), 25.6 (6-C), 25.2 (11-C), 22.2 (18-C), 20.1 (*C*H$_3$CO), 18.5 (Si*C*(CH$_3$)$_3$), 14.4 (20-C), -5.5 (Si*C*H$_3$), -5.6 (Si*C*H$_3$); HRMS (ESI) *m/z* 527.2807 $[M+Na]^+$, calculated for $C_{28}H_{44}O_6SiNa$, 527.2805. **3-Oxo-14β-acetoxy-19-*tert*-butyldi-methylsilyloxy-andrographolide (16b):** 86% yield, white solid, m.p. 123.2-124.5 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 7.00 (1H, td, J = 7.0, 1.6 Hz, 12-H), 5.62 – 5.55 (1H, m, 14α-H), 4.82 (1H, s, 17α-H), 4.40 (1H, s, 17β-H), 3.84 – 3.71 (3H, m, 15-H and 19α-H), 3.53 (1H, d, J = 9.9 Hz, 19β-H), 2.41 (1H, td, J = 14.6, 14.2, 5.9 Hz, 2α-H), 2.31 – 2.12 (4H, m, 2β-H, 9β-H and 11-H), 1.66 (1H, td, J = 12.3, 5.0 Hz, 7α-H), 1.55 (3H, s, CH_3CO), 1.53 – 1.24 (5H, m, 1-H, 5β-H, 6α-H and 7β-H), 1.16 (3H, s, 18-H), 1.06 (1H, td, J = 13.5, 4.5 Hz, 6β-H), 0.94 (9H, s, SiC(CH_3)$_3$), 0.79 (3H, s, 20-H), 0.02 (6H, d, J = 1.3 Hz, Si(CH_3)$_2$); ^{13}C NMR (101 MHz, C_6D_6) δ 211.2 (3-C), 169.7 (16-C), 168.5 (CH$_3$*C*O), 149.0 (12-C), 147.3 (8-C), 125.1 (13-C), 108.7 (17-C), 71.2 (15-C), 68.1 (14-C), 66.6 (19-C), 56.2 (9-C), 54.7 (5-C), 53.7 (4-C), 39.0 (10-C), 37.8 (7-C), 37.4 (1-C), 36.2 (2-C), 26.0 (6-C), 25.8 (SiC(*C*H$_3$)$_3$), 25.1 (11-C), 22.2 (18-C), 20.2 (*C*H$_3$CO), 18.5 (Si*C*(CH$_3$)$_3$), 14.4 (20-C), -5.5 (Si*C*H$_3$), -5.6 (Si*C*H$_3$); HRMS (ESI) *m/z* 505.2977 $[M+H]^+$, calculated for $C_{28}H_{45}O_6Si$, 505.2985.

Preparation of the compounds 17a and 17b

The procedure of the preparation of **12a** or **12b** was used for preparation of compound **17a** or **17b** from **16a** or **16b**. Compound **17a** or **17b** was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/3). **3-Oxo-14α-acetoxy-andrographolide (17a):** 93% yield, white solid, m.p. 142.0-142.9 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 6.98 – 6.91 (1H, m, 12-H), 6.03 (1H, d, J = 6.0 Hz, 14β-H), 4.95 (1H, s, 17α-H), 4.62 (1H, s, 17β-H), 4.57 (1H, dd, J = 11.1, 6.1 Hz, 15α-H), 4.30 (1H, dd, J = 11.1, 1.8 Hz, 15β-H), 4.01 (1H, d, J = 11.2 Hz, 19α-H), 3.47 (1H, d, J = 11.2 Hz, 19β-H), 2.82 (1H, td, J = 14.5, 5.8 Hz, 2α-H), 2.61 (1H, ddd, J = 16.5, 6.3, 3.6 Hz, 11α-H), 2.57 – 2.43 (2H, m, 2β-H and 11β-H), 2.32 (1H, ddd, J = 14.7, 4.4, 3.2 Hz, 9β-H), 2.15 – 2.11 (1H, m, 7α-H), 2.11 (3H, s, CH_3CO), 2.09 – 2.03 (2H, m, 1α-H and 7β-H), 1.82 (1H, ddt, J = 12.8, 5.1, 2.3 Hz, 1β-H), 1.75 (1H, dd, J = 12.7, 2.6 Hz, 6α-H), 1.69 – 1.51 (2H, m, 5β-H and 6β-H), 1.15 (3H, s, 18-H), 1.01 (3H, s, 20-H); ^{13}C NMR (101 MHz, CD_3OD) δ 216.8 (3-C), 172.0 (16-C), 171.4 (CH$_3$*C*O), 151.0 (12-C), 148.6 (8-C), 125.9 (13-C), 109.6 (17-C), 73.2 (15-C), 69.3 (14-C), 65.9 (19-C), 58.0 (9-C), 56.5 (5-C), 55.6 (4-C), 40.1 (10-C), 39.3 (7-C), 38.7 (1-C), 36.8 (2-C), 26.5 (6-C), 25.8 (11-C), 20.8 (18-C), 20.6 (*C*H$_3$CO), 15.1 (20-C); HRMS (ESI) *m/z* 413.1939 $[M+Na]^+$, calculated for $C_{22}H_{30}O_6Na$, 413.1940. **3-Oxo-14β-acetoxy-andrographolide (17b):** 91% yield, white solid, m.p. 185.2-185.9 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 6.96 (1H, td, J = 7.0, 1.7 Hz, 12-H), 6.04 (1H, d, J = 6.0 Hz, 14α-H), 4.95 (1H, d, J = 0.8 Hz, 17α-H), 4.63 – 4.53 (2H, m, 15α-H and 17β-H), 4.30 (1H, dd, J = 11.1, 1.8 Hz, 15β-H), 4.01 (1H, d, J = 11.2 Hz, 19α-H), 3.47 (1H, d, J = 11.2 Hz, 19β-H), 2.82 (1H, td, J = 14.5, 5.8 Hz, 2α-H), 2.67 – 2.41 (3H, m, 2β-H and 11-H), 2.31 (1H, ddd, J = 14.7, 4.4, 3.1 Hz, 9β-H), 2.14 – 2.10 (1H, m, 7α-H), 2.10 (3H, s, CH_3CO), 2.08 – 2.02 (2H, m, 1α-H and 7β-H), 1.88 – 1.78 (1H, m, 1β-H), 1.74 (1H, dd, J =

12.7, 2.5 Hz, 6α-H), 1.67 – 1.51 (2H, m, 5β-H and 6β-H), 1.15 (3H, s, 18-H), 1.02 (3H, s, 20-H); ^{13}C NMR (101 MHz, CD_3OD) δ 216.8 (3-C), 171.9 (16-C), 171.4 (CH_3CO), 151.1 (12-C), 148.5 (8-C), 126.1 (13-C), 109.4 (17-C), 73.2 (15-C), 69.6 (14-C), 65.9 (19-C), 58.2 (9-C), 56.5 (5-C), 55.7 (4-C), 40.3 (10-C), 39.4 (7-C), 38.7 (1-C), 36.7 (2-C), 26.8 (6-C), 25.9 (11-C), 20.8 (18-C), 20.7 (CH_3CO), 15.1 (20-C); HRMS (ESI) *m/z* 413.1931 $[M+Na]^+$, calculated for $C_{22}H_{30}O_6Na$, 413.1940.

Preparation of the compounds 18a[15] and 18b

The procedure of the preparation of **6a** or **6b** was used for preparation of compound **18a** or **18b** from **14a** or **14b**. Compound **18a** or **18b** was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/2). **14α,19-Diacetoxy-andrographolide (18a):** 68% yield, white solid, m.p. 145.3-146.7 °C.^{1}H NMR (400 MHz, C_6D_6) δ 7.04 – 6.97 (1H, m, 12-H), 5.61 (1H, d, J = 5.7 Hz, 14β-H), 4.77 (1H, s, 17α-H), 4.44 (1H, s, 17β-H), 4.39 (1H, d, J = 11.8 Hz, 19α-H), 4.10 (1H, d, J = 11.7 Hz, 19β-H), 3.89 – 3.83 (1H, m, 15α-H), 3.75 (1H, d, J = 11.0 Hz, 15β-H), 3.15 – 3.05 (1H, m, 3β-H), 2.24 – 2.05 (3H, m, 9β-H and 11-H), 1.76 – 1.65 (2H, m, 7-H), 1.64 (3H, s, CH_3CO), 1.60 (1H, dd, J = 8.4, 7.5 Hz, 2α-H), 1.56 (3H, s, CH_3CO), 1.56 – 1.34 (4H, m, 1-H, 2β-H and 3α-O*H*), 1.24 (1H, qd, J = 13.0, 3.9 Hz, 6α-H), 1.11 (3H, s, 18-H), 0.90 – 0.77 (2H, m, 5β-H and 6β-H), 0.51 (3H, s, 20-H); HRMS (ESI) *m/z* 457.2215 $[M+Na]^+$, calculated for $C_{24}H_{34}O_7Na$, 457.2202. **14β,19-Diacetoxy-andrographolide (18b):** 65% yield, white solid, m.p. 141.9-142.6 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 7.02 (1H, td, J = 7.0, 1.4 Hz, 12-H), 5.56 (1H, d, J = 2.8 Hz, 14α-H), 4.79 (1H, s, 17α-H), 4.40 (1H, d, J = 11.7 Hz, 19α-H), 4.36 (1H, s, 17β-H), 4.09 (1H, d, J = 11.8 Hz, 19β-H), 3.86 – 3.69 (2H, m, 15-H), 3.16 – 3.01 (1H, m, 3β-H), 2.22 – 2.05 (3H, m, 9β-H and 11-H), 1.83 – 1.64 (2H, m, 3α-O*H* and 7α-H), 1.62 (3H, s, CH_3CO), 1.60 (1H, dd, J = 4.7, 3.0 Hz, 7β-H), 1.56 (3H, s, CH_3CO), 1.55 – 1.41 (2H, m, 2-H), 1.39 – 1.17 (3H, m, 1-H and 6α-H), 1.11 (3H, s, 18-H), 0.78 (2H, dd, J = 31.6, 13.9 Hz, 5β-H and 6β-H), 0.52 (3H, s, 20-H); ^{13}C NMR (101 MHz, C_6D_6) δ 170.5 (16-C), 169.8 (CH_3CO), 168.7 (CH_3CO), 149.4 (12-C), 147.5 (8-C), 124.9 (13-C), 108.1 (17-C), 78.5 (3-C), 71.3 (15-C), 68.2 (14-C), 65.0 (19-C), 55.5 (9-C), 55.0 (5-C), 42.6 (4-C), 39.3 (10-C), 38.0 (7-C), 37.1 (1-C), 28.2 (2-C), 25.5 (6-C), 24.6 (11-C), 22.8 (18-C), 20.6 (CH_3CO), 20.2 (CH_3CO), 14.6 (20-C); HRMS (ESI) *m/z* 457.2197 $[M+Na]^+$, calculated for $C_{24}H_{34}O_7Na$, 457.2202.

Preparation of the compounds 19a and 19b

The oxidation of compound **18a** or **18b** to **19a** or **19b** by DMP was used the procedure of the preparation of **16a** or **16b**. Silica gel column chromatography (ethyl acetate/petroleum ether 1/7) gave compound **19a** or **19b**. **3-Oxo-14α,19-diacetoxy-andrographolide (19a):** 88% yield, white solid, m.p. 105.9-106.7 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 6.95 – 6.89 (1H, m, 12-H), 5.64 – 5.56 (1H, m, 14β-H), 4.76 (1H, s, 17α-H), 4.63 (1H, d, J = 11.2 Hz, 19α-H), 4.44 (1H, s, 17β-H), 3.86 (1H, dt, J = 11.1, 5.3 Hz, 15α-H), 3.75 (1H, dt, J = 11.1, 2.0 Hz, 15β-H), 3.70 (1H, d, J = 11.3 Hz, 19β-H), 2.64 (1H, td, J = 14.6, 5.8 Hz, 2α-H), 2.26 (1H, ddd, J = 14.8, 4.3, 3.0 Hz, 11α-H), 2.22 – 1.98 (3H, m, 2β-H, 9β-H and 11β-H), 1.63 (3H, s, CH_3CO), 1.61 (1H, d, J = 4.8 Hz, 7α-H), 1.56 (3H, s, CH_3CO), 1.53 – 1.44 (1H, m, 7β-H), 1.40 – 1.29 (2H, m, 1-H), 1.28 – 1.20 (1H, m, 6α-H), 1.20 (3H, s, 18-H), 1.12 – 0.98 (2H, m, 5β-H and 6β-H), 0.65 (3H, s, 20-H); ^{13}C NMR (101 MHz, C_6D_6) δ 210.2 (3-C), 170.2 (16-C), 169.8 (CH_3CO), 168.4 (CH_3CO), 148.8 (12-C), 146.7 (8-C), 125.0 (13-C), 109.4 (17-C), 71.1 (15-C), 67.8 (14-C), 66.1 (19-C), 56.6 (9-C), 54.9 (5-C), 52.2 (4-C), 38.9 (10-C), 37.9 (7-C), 37.6 (1-C), 35.5 (2-C), 25.4 (6-C), 24.7 (11-C), 20.9 (18-C), 20.3 (CH_3CO), 20.1 (CH_3CO), 14.4 (20-C); HRMS (ESI) *m/z* 455.2046 $[M+Na]^+$, calculated for $C_{24}H_{32}O_7Na$, 455.2046. **3-Oxo-14β,19-diacetoxy-andrographolide (19b):** 90% yield, white solid, m.p. 135.1-135.8 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 6.95 (1H, td, J = 7.0, 1.6 Hz, 12-H), 5.56 (1H, d, J = 5.5 Hz, 14α-H), 4.78 (1H, d, J = 0.6 Hz, 17α-H), 4.64 (1H, d, J = 11.3 Hz, 19α-H), 4.37 (1H, s, 17β-H), 3.82 (1H, dd, J = 11.1, 5.6 Hz, 15α-H), 3.77 (1H, dd, J = 11.1, 2.1 Hz, 15β-H), 3.70 (1H, d, J = 11.3 Hz, 19β-H), 2.63 (1H, td, J = 14.6, 5.8 Hz, 2α-H), 2.21 (1H, ddd, J = 7.4, 5.8, 3.6 Hz, 11α-H), 2.14 – 2.05 (3H, m, 2β-H, 9β-H and 11β-H), 1.63 (3H, s, CH_3CO), 1.59 (1H, dd, J = 13.2, 5.5 Hz, 7α-H), 1.56 (3H, s, CH_3CO), 1.45 (1H, ddd, J = 13.0, 5.8, 3.1 Hz, 7β-H), 1.33 (2H, dd, J = 9.0, 4.0 Hz, 1-H), 1.23 – 1.16 (4H, m, 6α-H and 18-H), 1.12 – 0.96 (2H, m, 5β-H and 6β-H), 0.68 (3H, s, 20-H); ^{13}C NMR (101 MHz, C_6D_6) δ 210.4 (3-C), 170.3 (16-C), 169.8 (CH_3CO), 168.5 (CH_3CO), 148.8 (12-C), 146.7 (8-C), 125.2 (13-C), 108.8 (17-C), 71.2 (15-C), 68.1 (14-C), 66.1 (19-C), 56.5 (9-C), 54.6 (5-C), 52.2 (4-C), 39.1 (10-C), 37.9 (7-C), 37.5 (1-C), 35.4 (2-C), 25.6 (6-C), 24.7 (11-C), 20.8 (18-C), 20.3 (CH_3CO), 20.2 (CH_3CO), 14.5 (20-C); HRMS (ESI) *m/z* 455.2039 $[M+Na]^+$, calculated for $C_{24}H_{32}O_7Na$, 455.2046.

Preparation of the compounds 20a[16] and 20b

5.0 g (11.6 mmol) of compound **19a** or **19b** was dissolved in 20.0 ml of methanol and then treated with 4.4 g (23.15 mmol) of *p*-TSA at 40 °C for 8 h. Diluted by ethyl acetate and washed with sol. sat. $NaHCO_3$ and brine in-order, the organic phase was dried over anhydrous Na_2SO_4, filtered, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/1) to afford compound **20a** or **20b**. **3-Oxo-andrographolide (20a):** 51% yield, white solid, m.p. 193.4-194.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.63 (1H, td, J = 6.6, 1.5 Hz, 12-H), 5.75 (1H, d, J = 6.0 Hz, 14α-O*H*), 4.93 (1H, t, J = 5.7 Hz, 14β-H), 4.87 (1H, s, 17α-H), 4.70 (1H, s, 17β-H), 4.55 (1H, t, J = 5.4 Hz, 19-O*H*), 4.39 (1H, dd, J = 9.9, 6.1 Hz, 15α-H), 4.04 (1H, dd, J = 9.9, 2.0 Hz, 15β-H), 3.85 (1H, dd, J = 10.9, 5.7 Hz, 19α-H), 3.30 (1H, dd, J = 10.9, 5.2 Hz, 19β-H), 2.75 (1H, td, J = 14.4, 5.5 Hz, 2α-H), 2.53 (2H, t, J = 7.1 Hz, 2β-H and 11α-H), 2.40 – 2.30 (1H, m, 11β-H), 2.14 (1H, dt, J = 14.1, 3.4 Hz, 9β-H), 2.07 – 1.92 (3H, m, 1α-H and 7-H), 1.76 – 1.64 (1H, m, 1β-H), 1.64 – 1.40 (3H, m, 5β-H and 6-H), 1.01 (3H, s, 18-H), 0.93 (3H, s, 20-H); HRMS (ESI)m/z: 371.1846 $[M+Na]^+$, calculated for $C_{20}H_{28}O_5Na$, 371.1834. **3-Oxo-14β-andrographolide (20b):** 53% yield, white solid, m.p. 152.4-153.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.67 – 6.57 (1H, m, 12-H), 5.68 (1H, d, J = 5.9 Hz, 14β-O*H*), 4.96 (1H, t, J = 5.5 Hz, 14α-H), 4.86 (1H, s, 17α-H), 4.56 (1H, t, J = 5.4 Hz, 19-O*H*), 4.49 (1H, s, 17β-H), 4.41 (1H, dd, J = 9.9, 6.2 Hz, 15α-H), 4.02 (1H, dd, J = 9.9, 2.2 Hz, 15β-H), 3.85 (1H, dd, J = 10.9, 5.7 Hz, 19α-H), 3.30 (1H, dd, J = 10.9, 5.2 Hz, 19β-H), 2.75 (1H, td, J = 14.4, 5.6 Hz, 2α-H), 2.60 (1H, ddd, J = 16.1, 5.8, 2.7 Hz, 11α-H), 2.48 – 2.30 (2H, m, 2β-H and 11β-H), 2.14 (1H, dt, J = 14.0, 3.5 Hz, 9β-H), 2.09 – 1.90 (3H, m, 1α-H and 7-

H), 1.77 – 1.65 (1H, m, 1β-H), 1.64 – 1.40 (3H, m, 5β-H and 6-H), 1.01 (3H, s, 18-H), 0.94 (3H, s, 20-H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 213.5 (3-C), 169.9 (16-C), 147.4 (12-C), 146.2 (8-C), 129.3 (13-C), 108.5 (17-C), 74.3 (15-C), 64.9 (14-C), 64.0 (19-C), 56.1 (9-C), 54.7 (5-C), 54.1 (4-C), 38.9 (10-C), 37.8 (7-C), 37.2 (1-C), 35.6 (2-C), 24.7 (6-C), 24.4 (11-C), 20.3 (18-C), 14.6 (20-C); HRMS (ESI)m/z: 371.1829 $[M+Na]^+$, calculated for $C_{20}H_{28}O_5Na$, 371.1834.

Preparation of the compounds 21a and 21b

The 19-silylation of **20a** or **20b** for **21a** or **21b** by TBSCl and imidazole at rt was conducted according to the procedure of the preparation of **8a** or **8b**. Compound **21a** or **21b** was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/3). **3-Oxo-19-*tert*-butyl-dimethylsilyloxy-andrographolide (21a):** 57% yield, white solid, m.p. 174.0-175.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.68 – 6.60 (1H, m, 12-H), 5.72 (1H, d, J = 5.2 Hz, 14α-O*H*), 4.93 (1H, s, 14β-H), 4.89 (1H, s, 17α-H), 4.71 (1H, s, 17β-H), 4.39 (1H, dd, J = 9.9, 6.1 Hz, 15α-H), 4.04 (1H, dd, J = 9.9, 2.0 Hz, 15β-H), 3.85 (1H, d, J = 10.1 Hz, 19α-H), 3.50 (1H, d, J = 10.1 Hz, 19β-H), 2.69 – 2.52 (3H, m, 2α-H and 11-H), 2.41 – 2.32 (1H, m, 9β-H), 2.27 – 2.18 (1H, m, 2β-H), 2.06 – 1.94 (3H, m, 1α-H and 7-H), 1.78 – 1.64 (2H, m, 1β-H and 6α-H), 1.63 – 1.43 (2H, m, 5β-H and 6β-H), 1.01 (3H, s, 18-H), 0.92 (3H, s, 20-H), 0.81 (9H, s, SiC(C*H*$_3$)$_3$), -0.02 (6H, s, Si(C*H*$_3$)$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 212.6 (3-C), 169.9 (16-C), 147.1 (12-C), 146.0 (8-C), 129.1 (13-C), 109.1 (17-C), 74.3 (15-C), 65.6 (14-C), 64.5 (19-C), 55.4 (9-C), 54.5 (5-C), 53.3 (4-C), 38.5 (10-C), 37.1^0 (7-C), 37.0^7 (1-C), 35.5 (2-C), 25.6 (SiC(*C*H$_3$)$_3$), 24.5 (6-C), 24.2 (11-C), 21.0 (18-C), 17.8 (Si*C*(CH$_3$)$_3$), 14.3 (20-C), -5.7 (Si(*C*H$_3$)$_2$); HRMS (ESI) *m/z* 485.2711 $[M+Na]^+$, calculated for $C_{26}H_{42}O_5SiNa$, 485.2699. **3-Oxo-19-*tert*-butyl-dimethyl-silyloxy-14β-andrographolide (21b):** 55% yield, white solid, m.p. 126.1-127.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.67 – 6.59 (1H, m, 12-H), 5.68 (1H, d, J = 4.9 Hz, 14β-O*H*), 4.96 (1H, s, 14α-H), 4.87 (1H, s, 17α-H), 4.50 (1H, s, 17β-H), 4.41 (1H, dd, J = 9.9, 6.2 Hz, 15α-H), 4.02 (1H, dd, J = 9.9, 2.2 Hz, 15β-H), 3.85 (1H, d, J = 10.1 Hz, 19α-H), 3.49 (1H, d, J = 10.1 Hz, 19β-H), 2.69 – 2.55 (2H, m, 2α-H and 11α-H), 2.47 – 2.32 (2H, m, 2β-H and 11β-H), 2.28 – 2.17 (1H, m, 9β-H), 2.10 – 1.93 (3H, m, 1α-H and 7-H), 1.81 – 1.42 (4H, m, 1β-H, 5β-H and 6-H), 1.01 (3H, s, 18-H), 0.92 (3H, s, 20-H), 0.80 (9H, s, SiC(C*H*$_3$)$_3$), -0.03 (6H, s, Si(C*H*$_3$)$_2$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 212.7 (3-C), 169.9 (16-C), 147.4 (12-C), 146.1 (8-C), 129.3 (13-C), 108.5 (17-C), 74.3 (15-C), 65.6 (14-C), 64.9 (19-C), 55.6 (9-C), 54.5 (5-C), 53.4 (4-C), 38.7 (10-C), 37.1^3 (7-C), 37.1^2 (1-C), 35.5 (2-C), 25.7 (SiC(*C*H$_3$)$_3$), 24.7 (6-C), 24.5 (11-C), 21.0 (18-C), 17.9 (Si*C*(CH$_3$)$_3$), 14.4 (20-C), -5.7 (Si(*C*H$_3$)$_2$); HRMS (ESI) *m/z* 485.2672 $[M+Na]^+$, calculated for $C_{26}H_{42}O_5SiNa$, 485.2699.

Preparation of the compounds 22a and 22b

The oxidation of compound **6a** or **6b** to **22a** or **22b** by DMP was used the procedure of the synthesis of **16a** or **16b**. Silica gel column chromatography (ethyl acetate/petroleum ether 1/7) gave compound **22a** or **22b**. **3-Oxo-14α-*tert*-butyldimethylsilyloxy-19-acetoxy-andrographolide (22a):** 88% yield, white solid, m.p. 98.3-99.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.64 – 6.57 (1H, m, 12-H), 5.22 (1H, d, J = 5.7 Hz, 14β-H), 4.88 (1H, s, 17α-H), 4.59 (1H, s, 17β-H), 4.53 – 4.43 (2H, m, 15α-H and 19α-H), 4.01 (1H, dd, J = 10.0, 2.3 Hz, 15β-H), 3.93 (1H, d, J = 11.3 Hz, 19β-H), 2.81 (1H, td, J = 14.6, 6.1 Hz, 2α-H), 2.62 – 2.51 (1H, m, 11α-H), 2.49 – 2.43 (1H, m, 11β-H), 2.41 – 2.34 (1H, m, 2β-H), 2.24 (1H, ddd, J = 14.8, 4.3, 2.8 Hz, 9β-H), 2.11 (1H, dd, J = 10.5, 3.3 Hz, 7α-H), 2.08 – 1.94 (2H, m, 1α-H and 7β-H), 1.92 (3H, s, C*H*$_3$CO), 1.77 (2H, d, J = 10.4 Hz, 6α-H and 7β-H), 1.62 (1H, td, J = 13.5, 4.7 Hz, 6β-H), 1.46 (1H, qd, J = 13.6, 4.6 Hz, 5β-H), 1.06 (3H, s, 18-H), 0.91 (3H, s, 20-H), 0.88 (9H, s, SiC(C*H*$_3$)$_3$), 0.17 (3H, s, SiC*H*$_3$), 0.13 (3H, s, SiC*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 211.9 (3-C), 170.1 (16-C), 169.4 (CH$_3$*C*O), 147.0 (12-C and 8-C), 127.7 (13-C), 109.4 (17-C), 73.9 (15-C), 66.4 (14-C), 65.5 (19-C), 55.4 (9-C), 54.1 (5-C), 51.4 (4-C), 38.4 (10-C), 37.3 (7-C), 36.7 (1-C), 34.9 (2-C), 25.6 (SiC(*C*H$_3$)$_3$), 24.4 (6-C), 24.2 (11-C), 20.5 (18-C), 20.4 (*C*H$_3$CO), 17.5 (Si*C*(CH$_3$)$_3$), 14.3 (20-C), -4.5 (Si*C*H$_3$), -5.0 (Si*C*H$_3$); HRMS (ESI)m/z: 527.2811 $[M+Na]^+$, calculated for $C_{28}H_{44}O_6SiNa$, 527.2805. **3-Oxo-14β-*tert*-butyldimethylsilyloxy-19-acetoxy-andrographolide (22b):** 88% yield, white solid, m.p. 108.3-109.9 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 6.87 – 6.80 (1H, m, 12-H), 4.83 (1H, s, 17α-H), 4.68 (1H, d, J = 11.3 Hz, 19α-H), 4.54 – 4.47 (1H, m, 14α-H), 4.38 (1H, s, 17β-H), 3.76 (1H, dd, J = 9.6, 6.6 Hz, 15α-H), 3.73 – 3.66 (2H, m, 15β-H and 19β-H), 2.66 (1H, td, J = 14.6, 5.9 Hz, 2α-H), 2.28 – 2.19 (3H, m, 2β-H and 11-H), 2.18 – 2.08 (1H, m, 9β-H), 1.67 (1H, m, 7α-H), 1.63 (3H, s, C*H*$_3$CO), 1.57 (1H, ddd, J = 14.5, 7.2, 4.2 Hz, 7β-H), 1.43 – 1.32 (2H, m, 1-H), 1.24 (1H, s, 6α-H), 1.21 (3H, s, 18-H), 1.16 – 0.99 (2H, m, 5β-H and 6β-H), 0.86 (9H, s, SiC(C*H*$_3$)$_3$), 0.78 (3H, s, 20-H), -0.03 (3H, s, SiC*H*$_3$), -0.17 (3H, s, SiC*H*$_3$); ^{13}C NMR (101 MHz, C_6D_6) δ 210.2 (3-C), 170.2 (16-C), 168.9 (CH$_3$*C*O), 147.3 (12-C), 146.6 (8-C), 128.6 (13-C), 108.8 (17-C), 73.3 (15-C), 67.7 (14-C), 66.2 (19-C), 56.2 (9-C), 55.0 (5-C), 52.1 (4-C), 38.8 (10-C), 38.1 (7-C), 37.5 (1-C), 35.3 (2-C), 25.7 (SiC(*C*H$_3$)$_3$), 25.4 (6-C), 24.7 (11-C), 20.9 (18-C), 20.3 (*C*H$_3$CO), 18.0 (Si*C*(CH$_3$)$_3$), 14.8 (20-C), -4.2 (Si*C*H$_3$), -4.8 (Si*C*H$_3$); HRMS (ESI) m/z: 527.2796 $[M+Na]^+$, calculated for $C_{28}H_{44}O_6SiNa$, 527.2805.

Preparation of the compounds 23a and 23b

1.0 g (2.0 mmol) of compound **22a** or **22b** was dissolved in 10.0 ml of tetrahydrofuran and then treated with 0.52 g (2.0 mmol) of TBAF at 0 °C for 3 h. The reaction mixture was treated with ethyl acetate and sol. sat. $NaHCO_3$ quickly after the reaction was complete. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/3) to yield compound **23a** or **23b**. **3-Oxo-19-acetoxy-andrographolide (23a):** 92% yield, white solid, m.p. 126.1-127.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.63 (1H, td, J = 6.6, 1.4 Hz, 12-H), 5.74 (1H, s, 14α-O*H*), 4.93 (1H, d, J = 5.6 Hz, 14β-H), 4.90 (1H, s, 17α-H), 4.72 (1H, s, 17β-H), 4.51 (1H, d, J = 11.3 Hz, 19α-H), 4.40 (1H, dd, J = 9.9, 6.1 Hz, 15α-H), 4.04 (1H, dd, J = 9.9, 2.1 Hz, 15β-H), 3.92 (1H, d, J = 11.3 Hz, 19β-H), 2.81 (1H, td, J = 14.6, 6.0 Hz, 2α-H), 2.55 (2H, t, J = 7.0 Hz, 2β-H and 11α-H), 2.38 (1H, dt, J = 10.5, 2.4 Hz, 11β-H), 2.24 (1H, ddd, J = 14.9, 4.5, 2.9 Hz, 9β-H), 2.09 – 1.96 (3H, m, 1α-H and 7-H), 1.92 (3H, s, C*H*$_3$CO), 1.81 – 1.72 (2H, m, 1-H and 6α-H), 1.60 (1H, td, J = 13.5, 4.7 Hz, 5β-H), 1.46 (1H, qd, J = 13.2, 4.2 Hz, 6β-H), 1.06 (3H, s, 18-H), 0.92 (3H, s, 20-H); ^{13}C NMR (101 MHz, DMSO-

d_6) δ 211.9 (3-C), 170.1 (16-C), 169.9 (CH_3CO), 146.7 (12-C), 145.8 (8-C), 129.1 (13-C), 109.2 (17-C), 74.3 (15-C), 65.5 (14-C), 64.5 (19-C), 55.5 (9-C), 54.3 (5-C), 51.4 (4-C), 38.5 (10-C), 37.1 (7-C), 36.9 (1-C), 34.9 (2-C), 24.3 (6-C), 24.1 (11-C), 20.5 (18-C), 20.4 (CH_3CO), 14.1 (20-C); HRMS (ESI) *m/z* 413.1951 $[M+Na]^+$, calculated for $C_{22}H_{30}O_6Na$, 413.1940. **3-Oxo-19-acetoxy-14β-andrographolide (23b):** 87% yield, white solid, m.p. 144.3-145.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.66 – 6.59 (1H, m, 12-H), 5.69 (1H, d, J = 6.1 Hz, 14β-O*H*), 4.97 (1H, t, J = 6.0 Hz, 14α-H), 4.88 (1H, s, 17α-H), 4.57 – 4.47 (2H, m, 17β-H and 19α-H), 4.42 (1H, dd, J = 9.9, 6.2 Hz, 15α-H), 4.02 (1H, dd, J = 9.7, 2.4 Hz, 15β-H), 3.91 (1H, d, J = 11.3 Hz, 19β-H), 2.81 (1H, td, J = 14.5, 6.0 Hz, 2α-H), 2.62 (1H, ddd, J = 16.2, 5.6, 2.4 Hz, 11α-H), 2.49 – 2.31 (2H, m, 2β-H and 11β-H), 2.23 (1H, ddd, J = 14.7, 4.3, 2.8 Hz, 9β-H), 2.12 – 1.97 (3H, m, 1α-H and 7-H), 1.92 (3H, s, C*H*$_3$CO), 1.81 – 1.69 (2H, m, 1β-H and 6α-H), 1.62 (1H, td, J = 13.5, 4.6 Hz, 5β-H), 1.53 – 1.37 (1H, m, 6β-H), 1.06 (3H, s, 18-H), 0.92 (3H, s, 20-H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 212.1 (3-C), 170.2 (16-C), 169.9 (CH_3CO), 147.0 (12-C), 146.1 (8-C), 129.4 (13-C), 108.7 (17-C), 74.3 (15-C), 65.6 (14-C), 64.9 (19-C), 55.7 (9-C), 54.3 (5-C), 51.5 (4-C), 38.8 (10-C), 37.2 (7-C), 36.9 (1-C), 34.9 (2-C), 24.7 (6-C), 24.3 (11-C), 20.6 (18-C), 20.4 (CH_3CO), 14.2 (20-C); HRMS (ESI) *m/z* 413.1935 $[M+Na]^+$, calculated for $C_{22}H_{30}O_6Na$, 413.1940.

Preparation of the compounds 24a and 24b

The procedure of the synthesis of **16a** or **16b** was used for oxidation of **8a** or **8b** to **24a** or **24b** by DMP. The purification of compound **24a** or **24b** was conducted by silica gel column chromatography (ethyl acetate/petroleum ether 1/8). **3-Oxo-14α,19-di-*tert*-butyldimethylsilyloxy-andrographolide (24a):** 91% yield, white solid, m.p. 94.6-95.2 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 6.94 (1H, ddd, J = 7.3, 5.2, 2.0 Hz, 12-H), 4.86 (1H, s, 17α-H), 4.71 (1H, s, 17β-H), 4.53 – 4.46 (1H, m, 14β-H), 3.88 – 3.78 (2H, m, 15α-H and 19α-H), 3.71 (1H, ddd, J = 9.7, 3.5, 1.6 Hz, 15β-H), 3.55 (1H, d, J = 9.9 Hz, 19β-H), 2.60 – 2.47 (2H, m, 2α-H and 11α-H), 2.40 – 2.31 (1H, m, 2β-H), 2.25 – 2.06 (2H, m, 9β-H and 11β-H), 1.82 – 1.62 (2H, m, 7-H), 1.57 – 1.42 (2H, m, 1-H), 1.41 – 1.30 (2H, m, 5β-H and 6α-H), 1.26 – 1.14 (4H, m, 6β-H and 18-H), 0.93 (9H, s, SiC(C*H*$_3$)$_3$), 0.91 (3H, s, 20-H), 0.87 (9H, s, SiC(C*H*$_3$)$_3$), 0.02 (6H, d, J = 1.6 Hz, Si(C*H*$_3$)$_2$), -0.01 (3H, s, SiC*H*$_3$), -0.16 (3H, s, SiC*H*$_3$); ^{13}C NMR (101 MHz, C_6D_6) δ 211.1 (3-C), 168.9 (16-C), 146.9^4 (12-C), 146.8^7 (8-C), 128.2 (13-C), 110.4 (17-C), 73.3 (15-C), 67.5 (14-C), 66.6 (19-C), 56.3 (9-C), 55.3 (5-C), 53.9 (4-C), 38.8 (10-C), 38.0 (7-C), 37.8 (1-C), 36.2 (2-C), 26.0 (SiC(*C*H$_3$)$_3$), 25.8 (SiC(*C*H$_3$)$_3$), 25.1 (6-C), 24.9 (11-C), 22.1 (18-C), 18.5 (Si*C*(CH$_3$)$_3$), 17.9 (Si*C*(CH$_3$)$_3$), 15.0 (20-C), -4.3 (Si*C*H$_3$), -4.7 (Si*C*H$_3$), -5.4^7 (Si*C*H$_3$), -5.5^3 (Si*C*H$_3$); HRMS (ESI) *m/z* 599.3577 $[M+Na]^+$, calculated for $C_{32}H_{56}O_5Si_2Na$, 599.3564. **3-Oxo-14β,19-di-*tert*-butyl dimethylsilyloxy-andrographolide (24b):** 90% yield, white solid, m.p. 190.3-191.2 °C. ^{1}H NMR (400 MHz, C_6D_6) δ 6.90 (1H, ddd, J = 7.1, 4.9, 2.0 Hz, 12-H), 4.87 (1H, d, J = 1.1 Hz, 17α-H), 4.57 – 4.50 (1H, m, 14α-H), 4.42 (1H, d, J = 0.9 Hz, 17β-H), 3.76 (2H, dd, J = 9.8, 6.7 Hz, 15α-H and 19α-H), 3.70 (1H, dd, J = 9.6, 3.6 Hz, 15β-H), 3.58 (1H, d, J = 9.9 Hz, 19β-H), 2.55 – 2.43 (1H, m, 2α-H), 2.43 – 2.20 (4H, m, 2β-H, 9β-H and 11-H), 1.80 – 1.60 (2H, m, 7-H), 1.56 – 1.28 (4H, m, 1-H, 5β-H and 6α-H), 1.22 – 1.07 (4H, m, 6β-H and 18-H), 0.93 (12H, s, 20-H and SiC(C*H*$_3$)$_3$), 0.86 (9H, s, SiC(C*H*$_3$)$_3$), 0.02 (6H, d, J = 1.3 Hz, Si(C*H*$_3$)$_2$), -0.01 (3H, s, SiC*H*$_3$), -0.17 (3H, s, SiC*H*$_3$); ^{13}C NMR (101 MHz, C_6D_6) δ 211.1 (3-C), 169.0 (16-C), 148.0 (12-C), 146.9 (8-C), 128.5 (13-C), 108.7 (17-C), 73.3 (15-C), 67.7 (14-C), 66.6 (19-C), 55.9 (9-C), 55.1 (5-C), 53.7 (4-C), 38.8 (10-C), 37.9 (7-C), 37.7 (1-C), 36.1 (2-C), 26.0 (SiC(*C*H$_3$)$_3$), 25.7 (SiC(*C*H$_3$)$_3$), 25.6 (6-C), 25.2 (11-C), 22.2 (18-C), 18.5 (Si*C*(CH$_3$)$_3$), 18.0 (Si*C*(CH$_3$)$_3$), 14.8 (20-C), -4.2 (Si*C*H$_3$), -4.8 (Si*C*H$_3$), -5.4^6 (Si*C*H$_3$), -5.5^4 (Si*C*H$_3$); HRMS (ESI) *m/z* 599.3559 $[M+Na]^+$, calculated for $C_{32}H_{56}O_5Si_2Na$, 599.3564.

Preparation of the compounds 25a and 25b

The deprotection of 19-OTBS of **24a** or **24b** used the procedure of the synthesis of **12a** or **12b** for 2 h. Compound **25a** or **25b** was provided by silica gel column chromatography (ethyl acetate/petroleum ether 1/7). **3-Oxo-14α-*tert*-butyldimethylsilyloxy-andrographolide (25a):** 67% yield, white solid, m.p. 138.1-139.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.66 – 6.57 (1H, m, 12-H), 5.21 (1H, d, J = 5.6 Hz, 14β-H), 4.85 (1H, s, 17α-H), 4.57 (1H, s, 17β-H), 4.53 (1H, t, J = 4.6 Hz, 19-O*H*), 4.47 (1H, dd, J = 10.0, 5.9 Hz, 15α-H), 4.01 (1H, dd, J = 10.0, 2.3 Hz, 15β-H), 3.83 (1H, dd, J = 10.8, 4.7 Hz, 19α-H), 3.35 (1H, dd, J = 10.8, 4.7 Hz, 19β-H), 2.74 (1H, td, J = 14.4, 5.5 Hz, 2α-H), 2.59 – 2.51 (1H, m, 11α-H), 2.49 – 2.42 (1H, m, 2β-H), 2.40 – 2.31 (1H, m, 11β-H), 2.22 – 1.90 (4H, m, 1α-H, 7-H and 9β-H), 1.76 – 1.40 (4H, m, 1β-H, 5β-H and 6-H), 1.01 (3H, s, 18-H), 0.93 (3H, s, 20-H), 0.87 (9H, s, SiC(C*H*$_3$)$_3$), 0.17 (3H, s, SiC*H*$_3$), 0.13 (3H, s, SiC*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 213.2 (3-C), 169.4 (16-C), 147.3 (12-C), 147.1 (8-C), 127.7 (13-C), 109.2 (17-C), 73.9 (15-C), 66.4 (14-C), 64.0 (19-C), 55.8 (9-C), 54.5 (5-C), 54.0 (4-C), 38.4 (10-C), 37.9 (7-C), 37.0 (1-C), 35.5 (2-C), 25.5 (SiC(*C*H$_3$)$_3$), 24.4 (6-C), 24.2 (11-C), 20.3 (18-C), 17.4 (Si*C*(CH$_3$)$_3$), 14.6 (20-C), -4.5 (Si*C*H$_3$), -5.0 (Si*C*H$_3$); HRMS (ESI) *m/z* 485.2710 $[M+Na]^+$, calculated for $C_{26}H_{42}O_5SiNa$, 485.2699. **3-Oxo-14β-*tert*-butyldimethylsilyl-oxy-andrographolide (25b):** 75% yield, white solid, m.p. 152.1-152.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.58 (1H, dd, J = 6.6, 4.6 Hz, 12-H), 5.25 (1H, d, J = 5.7 Hz, 14α-H), 4.84 (1H, s, 17α-H), 4.57 (1H, t, J = 5.2 Hz, 19-O*H*), 4.49 (1H, dd, J = 9.9, 6.1 Hz, 15α-H), 4.38 (1H, s, 17β-H), 3.99 (1H, dd, J = 9.9, 2.4 Hz, 15β-H), 3.84 (1H, dd, J = 10.9, 5.4 Hz, 19α-H), 3.30 (1H, dd, J = 10.9, 4.9 Hz, 19β-H), 2.74 (1H, td, J = 14.3, 5.5 Hz, 2α-H), 2.49 – 2.30 (3H, m, 2β-H and 11-H), 2.13 (2H, dd, J = 10.7, 3.8 Hz, 7α-H and 9β-H), 1.99 (1H, td, J = 12.8, 4.7 Hz, 7β-H), 1.88 (1H, ddd, J = 12.6, 5.1, 2.8 Hz, 1α-H), 1.72 (1H, d, J = 4.7 Hz, 1β-H), 1.66 – 1.37 (3H, m, 5β-H and 6-H), 1.01 (3H, s, 18-H), 0.93 (3H, s, 20-H), 0.86 (9H, s, SiC(C*H*$_3$)$_3$), 0.16 (3H, s, SiC*H*$_3$), 0.11 (3H, s, SiC*H*$_3$); ^{13}C NMR (101 MHz, DMSO-d_6) δ 213.2 (3-C), 169.4 (16-C), 147.4 (12-C), 147.1 (8-C), 128.2 (13-C), 108.7 (17-C), 73.9 (15-C), 66.7 (14-C), 64.0 (19-C), 55.8 (9-C), 54.4 (5-C), 54.0 (4-C), 38.5 (10-C), 37.9 (7-C), 37.0 (1-C), 35.5 (2-C), 25.6 (SiC(*C*H$_3$)$_3$), 25.2 (6-C), 24.3 (11-C), 20.3 (18-C), 17.6 (Si*C*(CH$_3$)$_3$), 14.7 (20-C), -4.4 (Si*C*H$_3$), -4.9 (Si*C*H$_3$); HRMS (ESI) *m/z* 485.2696 $[M+Na]^+$, calculated for $C_{26}H_{42}O_5SiNa$, 485.2699.

***In vitro* cancer cell proliferation model**

Cell culture: Cancer cell line A549 was maintained in RPMI 1640 culture medium while cancer cell line MDA-MB-231 was maintained in DMEM culture medium (ATCC, USA), which

were supplemented with 10% heat-inactivated FBS and 1% P/S. Cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2 (v/v).

Cell proliferation assay (MTT assay): A549 or MDA-MB-231 cells were plated into a 96-well plate at a concentration of 0.8-1×10^5 cells/well and incubated overnight at 37°C and 5% CO_2 to allow for cell attachment. Various concentrations of testing compounds were added to the cells and then incubated for another 24 h. Cells treated with DMSO (0.1%) was served as the vehicle control. After treatment, the medium was discarded and cells were incubated for 4 h at 37 ˚C in MTT solution (final concentration 1.0 mg/ml). The solution was then replaced by 100 µl DMSO to dissolve the violet formazan crystals in intact cells. Absorbance was measured by SpectraMax^R M5 Multi-Mode Microplate Readers (Molecular Devices, USA) at 570 nm. CC_{50} value was determined from the curve of cell viability at which the concentration caused 50% cell death. Each experiment was repeated for 3 times independently.

In vivo zebrafish toxicity assay

Maintenance of zebrafish and collection of embryos: All animal experiments were conducted according to the ethical guidelines of Institute of Chinese Medical Science (ICMS), University of Macau and the protocol was approved by ICMS, University of Macau. Transgenic zebrafish *Tg (fli1a- EGFP)y1* were provided by the Zebrafish International Research Center (ZIRC, Oregon) and wild-type zebrafish were purchased from a local pet shop. Both strains were maintained as described in the zebrafish handbook. Stocks were maintained in a controlled environment (28.5 ^oC with a 14 h light/10 h dark cycle) and fed with brine shrimp twice a day. Embryos were collected in the morning and cultured in embryo medium at 28.5 ^oC. At 24 h-post fertilization (hpf), the embryos were dechorionated with tweezers in a Petri dish coated with 1% (w/v) agarose, and then distributed into a 6-well plate with 20–50 embryos/group before drug treatment, depending on the assay.

Morphological observations: 24 hpf embryos were incubated in 3 ml of medium containing different concentrations of the test compounds. Embryos receiving DMSO (0.1–0.3%) were used as a vehicle control. Embryos receiving 300 nM VRI were used as a positive control. After drug treatment for 8, 12, 24 h, the embryos were anesthetized with freshly made 1% (w/v) tricaine (Sigma-Aldrich, St. Louis, MO) and inspected for viability and morphological changes respectively by using an Olympus Microscope System (IX81 Motorized Inverted Microscope [w/ZDC], IX2 universal control box, X-cite series 120, DP71 CCD camera). Photographs were captured at magnifications of 40× and 100×.

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‡ **Abbreviations:** SAR: structure-activity relationship; TBS: *tert*-butyldimethylsilyl; TBSCl: *tert*-butyldimethylsilyl chloride; TBSOTf: *tert*-tutyldimethylsilyl trifluoromethanesulfonate; DMP: Dess-Martin periodinane; DMAP: 4-dimethylaminopyridine; TFA: trifluoroacetic acid; TEA: triethylamine; Ac: acetyl; *p*-TSA: *p*-toluene- sulfonic acid; TBAF: tetrabutylammonium fluoride; rt: room temperature.

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