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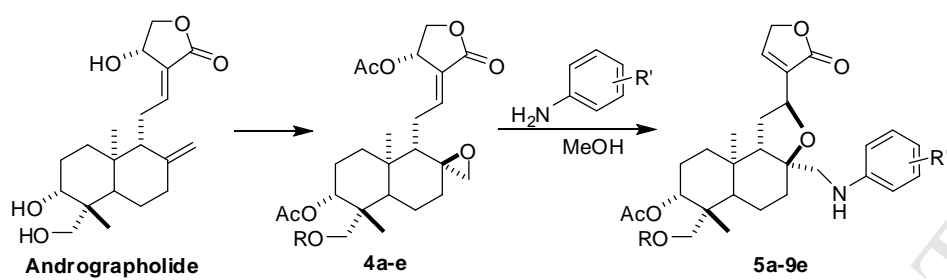
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One-pot three steps cascade synthesis of novel isoandrographolide analogues and their cytotoxic activity

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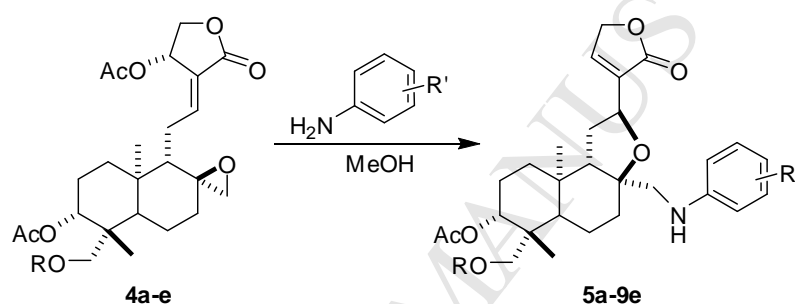
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Abstract

An efficient one-pot synthesis of novel andrographolide analogues is reported from a naturally occurring and abundant andrographolide isolated from aerial parts of *Andrographis paniculata*. Reactions in the one-pot proceed through a cascade epoxide ring opening by aniline derivatives/intramolecular ring closing and oxa-conjugate addition-elimination reactions. This methodology produces a new series of 17-amino-8-*epi*-isoandrographolide analogues in fair to excellent yields with high stereoselectivity using an economic and environmental procedure without base or catalyst at room temperature. Twenty-five analogues were obtained and cytotoxicity of all new analogues were evaluated against six cancer cell lines to search for a new lead compound based on andrographolide structure.



Keywords; epiandrographolide; cytotoxic activity; *Andrographis paniculata*

1. Introduction

Natural products from medicinal herbs have long been the important sources of therapeutic agents in drug discovery process. With diversity in their structures, the lead compound with promising intrinsic therapeutic property has been employed as the template for chemical transformation which is one of the common approaches to improve their therapeutic properties for discovery of new drug in the pharmaceutical industry. Recently, more attention has been focused on improving the biological activity by chemical modification of the labdane diterpenoid andrographolide, a natural product occurring in the aerial parts of *Andrographis paniculata* Nees [1-5]. Andrographolide is of interest because of its potential as a bioactive pharmacophore, capable of energizing a wide range of biological activities including antibacterial, antihepatotoxic, anti-HIV, anticancer, hypoglycemic and hypotensive activities [6-11]. Isolation and purification of andrographolide from *A. paniculata* may be accomplished by simple chromatographic techniques with 2% over all yields [12-13].

Modification of natural andrographolide into a library of new complex analogues with the appropriate structural diversity to improve their bioactivity continues to represent a challenge in drug discovery and drug development. Recently, new andrographolide analogues have been designed, synthesized and evaluated for their biological activity [14-17]. Structural scaffold modification of natural andrographolide has led to improved and diverse biological activities, especially with regard to cytotoxic as well as anticancer activity [18-21].

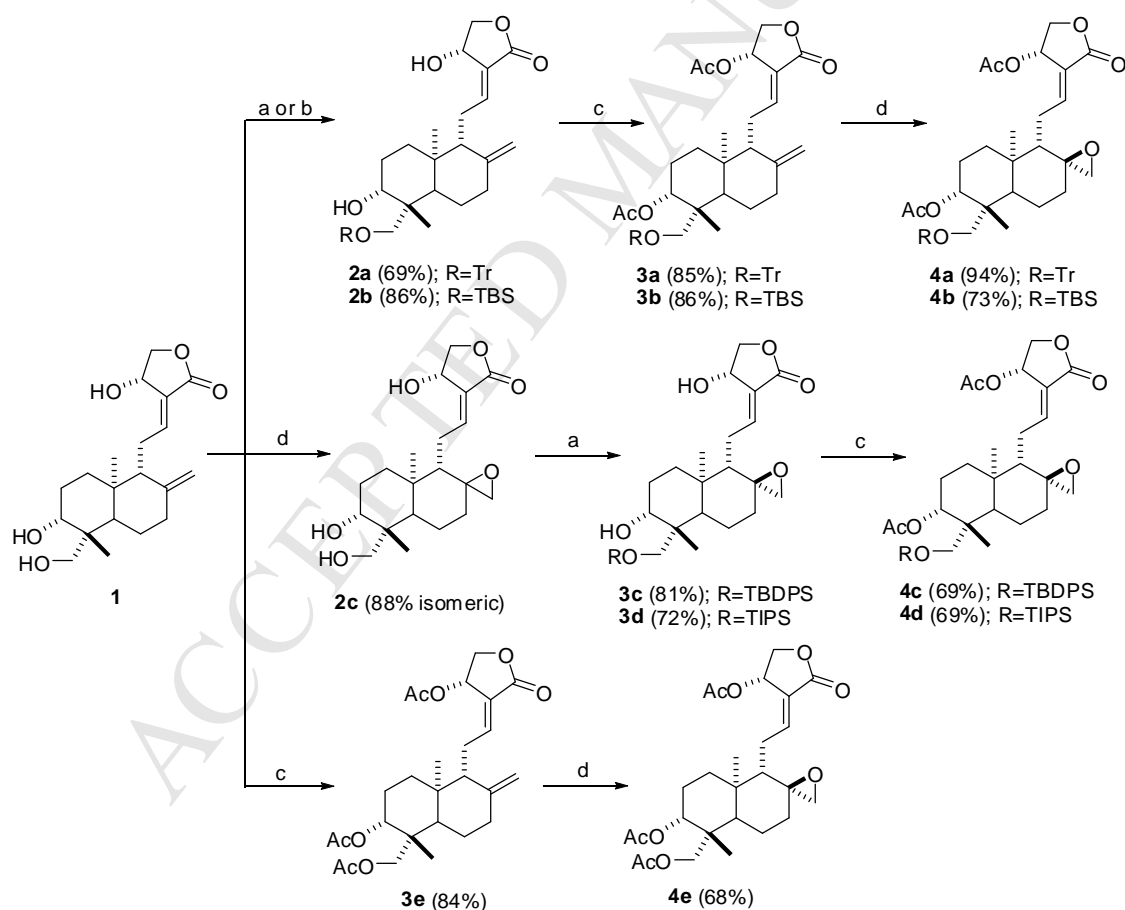
The present study designed and synthesized andrographolide-based derivatives from the perspective of their effectiveness as cytotoxic agents, specifically as anti-cancer agents. In an earlier study we discovered some semi-synthetic compounds derived from the andrographolide framework demonstrated higher bioactivity than the original natural product [22-24]. Andrographolide modification at C-19 of andrographolide has indicated actively and the cytotoxic potential towards cancer [25]. However, the modification of the core structure of *ent*-labdane diterpenoid has been

rarely reported. Therefore, a series of *epi*-isoandrographolide were designed and synthesized by one-pot procedure as novel structural type for searching new potential cytotoxic agents. This one pot method is a suitable procedure for further drug development and an effective approach for scale-up, minimize purification steps and reduce chemical waste are utilized.

In the present study, we explored the modification of andrographolide *via* tandem three steps epoxide ring opening by aniline derivatives followed by intramolecular ring closing and oxa-conjugate addition-elimination reactions leading to a series of 17-amino-8-*epi*-isoandrographolide analogues.

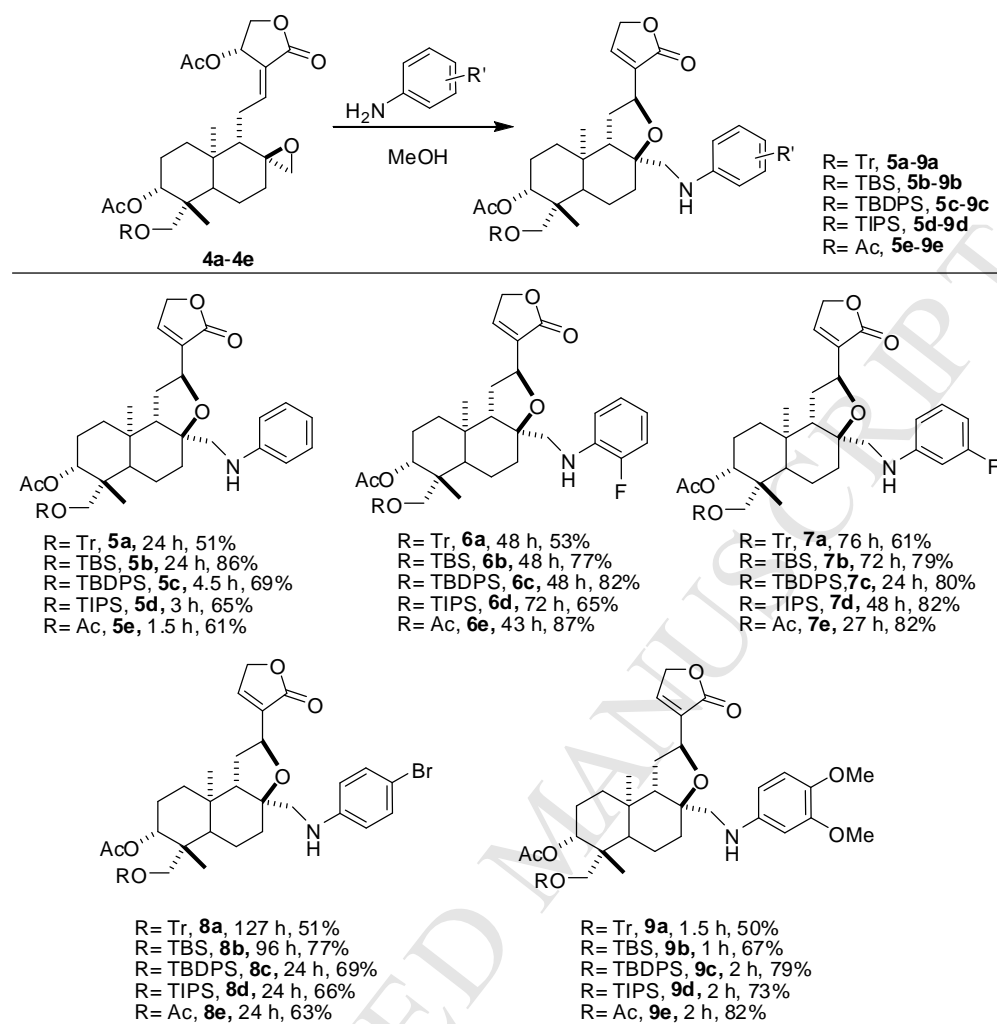
2. Results and discussion

Chemistry



Scheme 1. Reagents and Conditions; a) R_3SiCl , pyridine, rt, b) TrCl , pyridine, reflux, for **2a**; c) Ac_2O , 145°C ; d) m-CPBA , $\text{CH}_2\text{Cl}_2/\text{MeOH}$

C-8-epoxy-andrographolide analogues **4a-4e** were synthesized from natural available andrographolide as starting material for the one-pot synthesis of *epi*-isoandrographolide analogues (Scheme 1). First, introducing of trityl and TBS groups at C-19 were carried out in the presence of triphenylmethylchloride, *tert*-butyldimethylsilyl-chloride and pyridine followed by acetylation at 70°C and epoxidation with MCPBA to afford the desired compounds **4a** and **4b** in high yields. Preparation of C-19 Silyl-precursors **4c** and **4d**, commenced with epoxidation followed by silylation with TBDPSCl and TIPSCl in the presence of pyridine and acetylation to produce the designed products in good yields. C-19 Silyl-precursors **4c** and **4d** can be prepared as per reaction sequence **4a** and **4b**. However, we found that in the preparation of C-19 TBDPS and TIPS-analogues, epoxidation before silyl protection gave higher yield of designed compounds than initial C-19 protection.

Table 1 Synthesis of 17-amino-8-*epi*-isoandrographolide analogues **5-9**

A series of 17-amino-8-*epi*-isoandrographolide analogues were synthesized *via* tandem three steps *N*-alkylation-epoxide ring opening reaction followed by intramolecular ring closing and oxa-conjugate addition-elimination reactions. The reaction was first conducted with C-19-trityl epoxyandrographolide analogue **4a** and aniline in methanol without any base or catalyst to give the cyclized product **5a** in fair yield. In a previous study we found the introduction of halogenated and dimethoxy-aniline to the andrographolide framework led to the enhancing of cytotoxic activity.^{6a} Therefore, anilines bearing halogenated groups (*ortho*-, *meta*-fluoro and *para*-bromo) and 3,4-dimethoxy groups were investigated under the present condition to give the corresponding cyclized product 17-amino-8-*epi*-isoandrographolide **6a-9a** in moderate to good yields as shown in Table 1.

When 2-fluoro-, 3-fluoro- and 4-bromo-aniline were employed as nucleophiles, the reactions were performed more than one days to complete the reactions due to the decreased nucleophilicity of aniline bearing electron withdrawing. Reactions of 3,4-dimethoxyaniline gave the corresponding products **9a-9e** in short reaction time. The preparation of new analogues by one pot reaction

proceeded smoothly, selectively and efficiently at room temperature to give twenty-five analogues in moderate to good yields proving the generality of this method. To our knowledge, this is the first report of one pot cascade reactions of this sequential epoxide ring opening by aniline derivatives/intramolecular ring closing and oxa-conjugate addition-elimination reactions. The *cis*-stereochemistry at C-8 and C-12 of **5e** was determined by NOESY correlations which were supported by the observation between H-17/H-20, H-17/H-19, H-20/ ortho-Ar-H and H-12/ortho-Ar-H as shown in Figure 1.

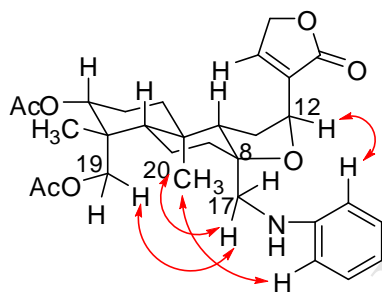


Figure 1. NOESY correlations of compound **5e**.

Biological activity

Table 2 Cytotoxic activities of 17-amino-8-*epi*-isoandrographolide analogues against cancer cell lines.

Compounds	IC ₅₀ (μM) ^a					
	P-388	KB	HT 29	MCF-7	A 549	ASK
Andrographolide (1)	2.25	27.37	9.34	15.40	27.9	16.18
5a	> 50	> 50	> 50	> 50	> 50	29.06±5.59
5b	5.19±0.06	21.25±0.29	8.50±0.15	5.44±0.14	7.80±0.21	5.94±0.05
5c	45.34±1.30	> 50	> 50	> 50	> 50	9.32±0.15
5d	6.54±0.04	18.77±0.24	17.47±1.53	8.33±0.66	9.43±0.26	6.10±0.05
5e	39.96±0.54	> 50	25.72±0.72	> 50	> 50	> 50
6a	> 50	> 50	> 50	> 50	> 50	> 50
6b	4.56±0.08	6.49±0.11	5.59±0.04	4.45±0.07	6.10±0.04	3.33±0.08
6c	> 50	> 50	> 50	> 50	> 50	39.73±0.82
6d	21.42±0.55	30.78±0.63	30.25±0.47	15.31±0.49	21.02±0.23	8.05±0.20
6e	7.50±0.07	26.53±0.33	6.50±0.06	11.81±0.20	8.45±1.65	27.76±0.38
7a	> 50	> 50	> 50	> 50	> 50	> 50
7b	6.44±0.14	27.44±0.18	26.23±0.70	8.64±0.08	21.52±1.81	6.10±0.09
7c	> 50	> 50	> 50	> 50	> 50	44.84±0.09
7d	7.95±0.13	32.86±0.51	30.12±0.84	12.67±0.40	24.48±0.39	6.91±0.03
7e	6.85±0.09	20.75±0.09	5.87±0.07	7.41±0.21	6.52±0.24	22.33±1.24
8a	> 50	> 50	> 50	> 50	> 50	> 50
8b	6.39±0.05	25.71±0.31	27.68±0.99	12.91±1.88	14.98±0.19	7.35±0.02
8c	48.29±2.05	> 50	> 50	> 50	47.77±0.99	> 50
8d	40.54±1.77	> 50	> 50	> 50	> 50	29.73±0.65
8e	7.47±0.77	21.08±0.44	6.09±0.15	6.93±0.12	6.14±0.04	8.40±0.24
9a	30.48±0.59	> 50	> 50	23.14±1.00	> 50	6.51±0.06
9b	5.49±0.08	11.53±0.62	6.16±0.23	5.40±0.27	5.30±0.01	5.13±0.04
9c	27.52±0.71	> 50	> 50	35.80±1.72	48.61±0.36	9.37±0.32
9d	4.98±0.21	17.92±0.65	5.24±0.03	4.70±0.11	6.08±0.05	4.61±0.15
9e	28.82±2.10	> 50	24.12±0.47	> 50	> 50	> 50

^aCell lines used are P-388 (murine leukemia cell line); KB (human epidermoid carcinoma of the mouth); COL-2 (human colon cancer); MCF-7 (human breast cancer); LU-1 (human lung cancer); and ASK (rat glioma). Ellipticine (Ellipt) was used as a positive control. The results were expressed as IC₅₀ values

The cytotoxic activities of andrographolide, the parent compound **1** and a new series of 17-amino-8-*epi*-isoandrographolide analogues were evaluated *in vitro* against seven cancer cell lines; P-388 (mouse lymphoid neoplasma), KB (human epidermoid carcinoma in the mouth), Col-2 (human colon cancer), MCF-7 (human breast cancer), Lu-1 (human lung cancer) and ASK (rat glioma) using sulforhodamine B (SRB) assay. All tested analogues were dissolved in DMSO (0.1%). Ellipticine, a potent anti-cancer agent was used as a positive control. As shown in Table 2, results were expressed as IC₅₀ values (drug concentration causing 50% growth inhibition) in μ M.

As shown in Table 2, an introduction of aniline to C-19 substituted analogues and conversion of andrographolide scaffold to *epi*-isoandrographolide core structure provided some analogues with greater cytotoxicity than that of the parent andrographolide. Comparison of the cytotoxic activities of aniline analogues **5a-5e**, compounds **5b** and **5d** showed increasing cytotoxicity against almost all cancer cells than the natural andrographolide. Interestingly, compound **5c** showed selective cytotoxic activity to ASK cancer cell. Among C-17-*o*-fluoroaniline analogues **6a-6e**, compound **6b** showed the highest activities relative to other analogues on almost all cancer cell lines especially on ASK cancer cells with an IC₅₀ value 3.33 μ M. **6b** was 2 to 5-fold more active on various cancer cells than **1**. Changing the *o*-fluoro aniline to *m*-fluoro aniline derivatives, compound **7a-7e** showed a trend in cytotoxic activity that was similar to that for **6a-6e**. The effects of substituent position of fluoro group on aniline ring did not alter activity. Compound **7e** showed the greatest activity in this group. Compound C-19-acetyl **8e** bearing *p*-bromoaniline at C-17 exhibited greater activities than other C-19 substituted analogues in this group. Introduction of a more electron-donating group such as dimethoxyaniline to C-17 position on the new scaffold led to a dramatic increase in cytotoxic activity of C-19 TBS **9b** and C-19 TIPS **9d**. Moreover, compound **9b** showed the greatest activity on A 549 while compound **9d** on HT 29 cancer cell and compound **9a** showed selective cytotoxic activity to ASK cancer cells.

3. Conclusions

In summary, a convenient and environmentally one pot, three steps cascade reactions has been developed for modification of andrographolide scaffold to provide new 17-amino-8-*epi*-isoandrographolide analogues to screen cytotoxic activity. The reaction proceed *via* epoxide ring opening by aniline derivatives/intramolecular ring closing and oxa-conjugate

addition-elimination reactions without any base or catalyst at room temperature. Twenty five analogues were screened to study the SAR. Among the synthetic *epi*-isoandrographolides, **6b** exhibited the strongest activities relative to other analogues especially on ASK cancer cells with an IC_{50} 3.33 μ M, while **5c** and **9a** showed selective cytotoxic activity to ASK cancer cells.

4. Experimental section

General experimental

All chemicals were purchased from commercial sources and used without further purification. Proton NMR spectra were recorded on a BRUKER AVANC (400 MHz). All spectra were measured in $CDCl_3$ solvent and chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (δ 0.00) or $CDCl_3$ (δ 7.26) as internal standard. Data are reported as follows; chemical shift (multiplicity, integrate intensity or assignment, coupling constants in Hz, assignment). Carbon NMR spectra were recorded on a BRUKER AVANC (100 MHz). All spectra were measured in $CDCl_3$ solvent and chemical shifts are reported as δ values in parts per million (ppm) relative to $CDCl_3$ (δ 77.0) as internal standard. High-resolution mass spectra (HRMS) data were obtained with a Finnigan MAT 95. Infrared spectra were determined on a PERKIN ELMER FT/IR-2000S spectrophotometer and are reported in wave number (cm^{-1}). Analytical thin-layer chromatography (tlc) was conducted on precoated tlc plates; silica gel 60F-254 [E. Merck, Darmstadt, Germany]. Silica gel columns for open-column chromatography utilized silica gel 60 PF254 [E. Merck, Darmstadt, Germany]. Melting points were measured using a Melting point apparatus (Griffin) and are uncorrected.

19-Tr-3,14-diacetyl-8,17-epoxy andrographolide (**4a**)

To a stirred solution of andrographolide (**1**) (2.05 mg, 4.91 mmol) in pyridine (2.5 mL) was added triphenylmethyl chloride (TrCl) (2.28 g, 8.18 mmol), then the reaction mixture was heated to 70 °C. After the stirring was continued at 70 °C for 2.5 h, the reaction mixture was quenched with cold NH_4Cl and extracted with EtOAc (3×50 mL). The combined organic layer was washed with brine (50 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by

column chromatography (50% EtOAc/*n*-hexane) to give 19-Tr-andrographolide (**2a**) in 69% yield (1.47 g) as a white solid, R_f 0.45 (50% EtOAc/*n*-hexane).

To a stirred solution of 19-Tr-andrographolide (**2a**) (1.00 g, 1.69 mmol) in acetic anhydride (10.0 mL) was refluxed to 145 °C. After the stirring was continued at 145 °C for 2.5 h, the reaction mixture was diluted with EtOAc (50 mL) and quenched with saturated NaHCO₃, washed with H₂O, and extracted with EtOAc (3×50 mL). The combined organic layer was washed with brine (50 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to give 19-Tr-3,14-diacetyl-andrographolide (**3a**) in 73% yield (833 mg) as a white solid, R_f 0.54 (30% EtOAc/*n*-hexane).

To a stirred solution of 19-Tr-3,14-diacetyl andrographolide (**3a**) (501 mg, 0.74 mmol) in solution mixture of CH₂Cl₂/methanol (5:1) (20.0 mL) was added *m*-chloroperoxybenzoic acid (*m*-CPBA) (307 mg, 1.78 mmol). After the stirring was continued at room temperature for 24 h, the solvent was removed by evaporator. The reaction mixture was diluted with EtOAc (50 mL) and quenched with saturated NaHCO₃, washed with H₂O, and extracted with EtOAc (3×50 mL). The combined organic layer was washed with brine (50 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to give 19-Tr-3,14-diacetyl-8,17-epoxy andrographolide (**4a**) in 94% yield (481 mg) as a white solid, R_f 0.65 (40% EtOAc/*n*-hexane). (**4a**): Mp. 83-85 °C; IR (Neat): 2946, 1763, 1734, 1678, 1370, 1240, 1028, 750 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.52-7.47 (6H, m, PhH), 7.35-7.24 (6H, m, PhH), 7.23-7.22 (3H, m, PhH), 7.06 (1H, td, J = 7.0, 1.0 Hz, H-12), 5.82 (1H, brd, J = 6.0 Hz, H-14), 4.54 (1H, dd, J = 11.5, 4.0 Hz, H-3), 4.50 (1H, dd, J = 11.0, 6.0 Hz, H-15b), 4.20 (1H, dd, J = 11.0, 1.5 Hz, H-15a), 3.39 (1H, d, J = 10.0 Hz, H-19b), 3.16 (1H, d, J = 10.0 Hz, H-19a), 2.52 (1H, dd, J = 4.0, 2.0 Hz, H-17b), 2.47 (1H, d, J = 4.0 Hz, H-17a), 2.12 (3H, s, COCH₃), 2.03 (3H, s, COCH₃), 2.01-1.77 (4H, m), 1.61-1.10 (8H, m), 1.23 (3H, s, H-18), 0.38 (3H, s, H-20); ¹³C NMR (100 MHz, CDCl₃): δ 170.60 (2×C), 169.00, 150.96, 144.11 (3×C), 128.87 (6×C), 127.67 (6×C), 126.93 (3×C), 123.21, 86.95, 79.87, 71.59, 67.57, 62.22, 58.06, 55.09, 54.11, 49.84, 42.35, 39.46, 37.43, 35.97, 23.56, 23.00, 22.94, 22.45, 21.26, 20.87, 14.25; HRMS (ESI) m/z calcd for C₄₃H₄₈O₈Na [M+Na]⁺ 715.3247, found 715.3275.

19-TBS-3,14-diacetyl-8,17-epoxy andrographolide (4b)

To a stirred solution of andrographolide (**1**) (504 mg, 1.44 mmol) in pyridine (5.0 mL) was added *tert*-butyldimethylsilyl chloride (TBDMSCl) (1.07 g, 7.10 mmol) at room temperature. After the stirring was continued at room temperature for 1 h, the reaction mixture was diluted with EtOAc (50 mL) and quenched with H₂O (50 mL), and extracted with EtOAc (3×50 mL). The combined organic layer was washed with brine (50 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (50% EtOAc/*n*-hexane) to give 19-TBS-andrographolide (**2b**) in 88% yield (591 mg) as a white solid, *R*_f 0.42 (50% EtOAc/*n*-hexane).

To a stirred solution of 19-TBS-andrographolide (**2b**) (591 mg, 1.27 mmol) in acetic anhydride (10.0 mL) was refluxed to 145 °C. After the stirring was continued at 145 °C for 2 h, the reaction mixture was diluted with EtOAc (50 mL) and quenched with saturated NaHCO₃, washed with H₂O, and extracted with EtOAc (3×50 mL). The combined organic layer was washed with brine (50 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to give 19-TBS-3,14-diacetyl andrographolide (**3b**) in 86% yield (598 mg) as a white solid, *R*_f 0.61 (30% EtOAc/*n*-hexane).

To a stirred solution of 19-TBS-3,14-diacetyl andrographolide (**3b**) (598 mg, 1.09 mmol) in solution mixture of CH₂Cl₂/methanol (5:1) (6.0 mL) was added *m*-chloroperoxybenzoic acid (*m*-CPBA) (553 mg, 3.20 mmol). After the stirring was continued at room temperature for 19 h, the solvent was removed by evaporator. The reaction mixture was diluted with EtOAc (50 mL) and quenched with saturated NaHCO₃, washed with H₂O, and extracted with EtOAc (3×50 mL). The combined organic layer was washed with brine (50 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (22% EtOAc/*n*-hexane) to give 19-TBS-3,14-diacetyl-8,17-epoxy andrographolide (**4b**) in 73% yield (451 mg) as a white solid, *R*_f 0.18 (22% EtOAc/*n*-hexane). (**4b**): Mp. 114-116 °C; IR (Neat): 2933, 2870, 1750, 1642, 1449, 1347, 1264, 1206, 1082, 1036, 892, 811 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.10 (1H, t, *J* = 7.0 Hz, H-12), 5.88 (1H, d, *J* = 5.0 Hz, H-14), 4.59-4.47 (1H, m, H-3), 4.52 (1H, d, *J* = 11.0 Hz, H-15b), 4.22 (1H, d, *J* = 11.0 Hz, H-15a), 3.86 (1H, d, *J* =

10.5 Hz, H-19b), 3.58 (1H, d, $J = 10.5$ Hz, H-19a), 2.62 (1H, brs, H-17b), 2.54 (1H, brs, H-17a), 2.12 (3H, s, COCH_3), 2.05 (3H, s, COCH_3), 2.10-1.59 (8H, m), 1.43-1.36 (2H, m), 1.34-1.17 (2H, m), 0.97 (3H, s, H-18), 0.96 (3H, s, H-20), 0.89 (9H, s, $\text{Si}(\text{CH}_3)_3$), 0.03 (6H, s, $\text{Si}(\text{CH}_3)_2$); ^{13}C NMR (100 MHz, CDCl_3): δ 170.68, 170.62, 169.10, 151.17, 123.07, 79.72, 71.61, 67.60, 63.98, 58.37, 55.06, 54.21, 49.90, 42.19, 39.66, 37.81, 36.33, 25.83 (3 \times C), 23.69, 23.30, 23.11, 22.83, 21.11, 20.88, 18.20, 14.51, -5.65, -5.74; HRMS (ESI) m/z calcd for $\text{C}_{30}\text{H}_{48}\text{O}_8\text{SiNa}$ $[\text{M}+\text{Na}]^+$ 587.3016, found 587.3047.

19-TBDPS-3,14-diacetyl-8,17-epoxy andrographolide (**4c**)

A mixture of 8,17-epoxy andrographolide (**2c**) (500 mg, 1.36 mmol) with *tert*-butyldiphenylsilyl chloride (TBDPSCl) (1.12 mL, 4.31 mmol) was stirred at room temperature for 1 h. The reaction mixture was quenched with NH_4Cl , and extracted with EtOAc. The combined organic layer was washed with brine, dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (60% EtOAc/*n*-hexane) to afford the corresponding product **3c** in 81% yield (668 mg) as a white solid, R_f 0.36 (50% EtOAc/*n*-hexane).

To a stirred solution of 19-TBDPS-8,17-epoxy andrographolide (**3c**) (350 mg, 0.58 mmol) in acetic anhydride (2.0 mL) was heated to 145 °C for 1h, the reaction mixture was diluted with EtOAc (30 mL) and quenched with saturated NaHCO_3 , washed with H_2O , and extracted with EtOAc (3 \times 30 mL). The combined organic layer was washed with brine (30 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **4c** in 69% yield (275 mg) as a white solid, R_f 0.49 (30% EtOAc/*n*-hexane). (**4c**): Mp. 92-94 °C; IR (Neat): 2939, 1734, 1241, 1079, 749 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.71-7.63 (4H, m, PhH), 7.47-7.34 (6H, m, PhH), 7.08 (1H, td, $J = 7.0, 1.0$ Hz, H-12), 5.86 (1H, brd, $J = 5.5$ Hz, H-14), 4.53 (1H, dd, $J = 11.0, 4.0$ Hz, H-3), 4.50 (1H, dd, $J = 11.5, 5.5$ Hz, H-15b), 4.22 (1H, dd, $J = 11.5, 1.5$ Hz, H-15a), 3.84 (1H, d, $J = 11.0$ Hz, H-19b), 3.37 (1H, d, $J = 11.0$ Hz, H-19a), 2.60 (1H, d, $J = 4.0$ Hz, H-17b), 2.53 (1H, d, $J = 4.0$ Hz, H-17a), 2.11 (3H, s, COCH_3), 2.15-1.92 (5H, m), 1.90 (3H, s, COCH_3), 1.84-1.39 (5H, m), 1.31-1.17 (2H, m), 1.06 (9H, s, $\text{Si}(\text{CH}_3)_3$), 1.04 (3H, s, H-18), 0.80 (3H, s, H-20); ^{13}C NMR (100 MHz, CDCl_3): δ 170.61, 170.51, 169.01, 150.92, 135.82 (2 \times C), 135.74 (2 \times C), 133.44, 133.39, 129.67, 129.64, 127.62 (2 \times C), 127.59 (2 \times C), 123.19, 79.63,

71.57, 67.57, 63.83, 58.20, 54.99, 54.13, 49.55, 42.82, 39.98, 37.57, 36.18, 26.92 (3×C), 23.58, 23.01, 22.81 (2×C), 21.06, 20.86, 19.25, 14.66; HRMS (ESI) m/z calcd for $C_{40}H_{52}O_8SiNa$ $[M+Na]^+$ 711.3329, found 711.3322.

19-TIPS-3,14-diacetyl-8,17-epoxy andrographolide (**4d**)

To a stirred solution of andrographolide (**1**) (1.50 g, 4.28 mmol) in solution mixture of CH_2Cl_2 /methanol (5:1) (30 mL) was added *m*-chloroperoxybenzoic acid (*m*-CPBA) (1.11 g, 6.43 mmol). After the stirring was continued at room temperature for 24 h, solvent was removed by evaporator. The reaction mixture was diluted with EtOAc (50 mL) and quenched with saturated $NaHCO_3$, washed with H_2O , and extracted with EtOAc (3×50 mL). The combined organic layer was washed with brine (10 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (100% EtOAc) to give mixture of 8,17-epoxy andrographolide isomers (**2c**) in 88% yield (1.38 g) as a white solid (major : minor ~ 18:1), R_f (major) 0.42 (100% EtOAc) and R_f (minor) 0.33 (100% EtOAc).

To a stirred solution of mixture of 8,17-epoxy andrographolide (**2c**) (500 mg, 1.36 mmol) in pyridine 1.0 mL was added triisopropyl chloride (TIPSCl) (0.90 mL, 4.20 mmol) at room temperature. After the stirring was continued at room temperature for 5 h, the reaction mixture was quenched with NH_4Cl , and extracted with EtOAc. The combined organic layer was washed with brine, dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (60% EtOAc/*n*-hexane) to afford the corresponding product **3d** in 72% yield (514 mg) as a white solid, R_f 0.55 (45% EtOAc/*n*-hexane).

To a stirred solution of 19-TIPS-8,17-epoxy andrographolide (**3d**) (350 mg, 0.67 mmol) in acetic anhydride (2.0 mL) was heated to 145 °C for 5.0 h. The reaction mixture was diluted with EtOAc (30 mL) and quenched with saturated $NaHCO_3$, washed with H_2O , and extracted with EtOAc (3×30 mL). The combined organic layer was washed with brine (30 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to afford the corresponding product **4d** in 69% yield (280 mg) as a white solid, R_f 0.39 (20% EtOAc/*n*-hexane). (**4d**): Mp. 104-106 °C; IR (Neat): 2943, 1764, 1735, 1677, 1370, 1242, 1027, 883 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 7.10 (1H, td, J = 7.0, 1.0 Hz, H-12), 5.88

(1H, d, $J = 5.5$ Hz, H-14), 4.60-4.54 (1H, m, H-3), 4.51 (1H, dd, $J = 11.0, 5.5$ Hz, H-15b), 4.22 (1H, dd, $J = 11.0, 2.0$ Hz, H-15a), 3.95 (1H, d, $J = 10.0$ Hz, H-19b), 3.80 (1H, d, $J = 10.0$ Hz, H-19a), 2.63 (1H, d, $J = 4.0$ Hz, H-17b), 2.55 (1H, d, $J = 4.0$ Hz, H-17a), 2.12 (3H, s, COCH₃), 2.04 (3H, s, COCH₃), 2.09-1.57 (9H, m), 1.45-1.05 (6H, m), 1.07 (18H, d, $J = 6.0$ Hz, Si(CH(CH₃)₂)₃), 1.03 (3H, s, H-18), 0.96 (3H, s, H-20); ¹³C NMR (100 MHz, CDCl₃): δ 170.63, 170.56, 169.02, 151.03, 123.19, 79.74, 71.59, 67.59, 63.89, 58.28, 55.08, 54.20, 49.91, 42.78, 39.69, 37.74, 36.26, 23.67, 23.07, 22.98, 22.78, 21.18, 20.88, 18.09 (6 \times C), 14.86, 11.93 (3 \times C); HRMS (ESI) m/z calcd for C₃₃H₅₄O₈SiNa [M+Na]⁺ 629.3486, found 629.3446.

3,14,19-triacetyl-8,17-epoxy andrographolide (4e)

To a stirred solution of andrographolide (**1**) (1.00 g, 2.85 mmol) in acetic anhydride (10.0 mL) was heated to 145 °C. After the stirring was continued at 145 °C for 3.5 h, the reaction mixture was diluted with EtOAc (50 mL) and quenched with saturated NaHCO₃, washed with H₂O, and extracted with EtOAc (3 \times 50 mL). The combined organic layer was washed with brine (50 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (50% EtOAc/*n*-hexane) to give 3,14,19-triacetyl andrographolide (**3e**) in 85% (1.15 g) as a white solid, R_f 0.56 (50% EtOAc/*n*-hexane).

To a stirred solution of 3,14,19-triacetyl andrographolide (**3e**) (909 mg, 1.91 mmol) in solution mixture of CH₂Cl₂/methanol (5:1) (10.0 mL) was added *m*-chloroperoxybenzoic acid (*m*-CPBA) (660 mg, 3.82 mmol). After the stirring was continued at room temperature for 24 h, the solvent was removed by evaporator. The reaction mixture was diluted with EtOAc (50 mL) and quenched with saturated NaHCO₃, washed with H₂O, and extracted with EtOAc (3 \times 50 mL). The combined organic layer was washed with brine (50 mL), dried over sodium sulfate anhydrous, and then concentrated under reduced pressure. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to give 3,14,19-triacetyl-8,17-epoxy andrographolide (**4e**) in 68% yield (641 mg) as a white solid, R_f 0.36 (40% EtOAc/*n*-hexane). (**4e**): Mp. 170-172 °C; IR (Neat): 2951, 1734, 1677, 1372, 1237, 1028, 749 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.08 (1H, td, $J = 7.0, 1.5$ Hz, H-12), 5.87 (1H, brd, $J = 5.0$ Hz, H-14), 4.58 (1H, dd, $J = 11.0, 5.0$ Hz, H-3), 4.51 (1H, dd, $J = 11.0, 6.0$ Hz, H-15b), 4.35 (1H, d, $J = 11.5$ Hz, H-19b), 4.23 (1H, dd, $J = 11.0, 2.0$ Hz, H-15a), 4.16 (1H, d,

$J = 11.5$ Hz, H-19a), 2.62 (1H, dd, $J = 4.0, 1.5$ Hz, H-17b), 2.55 (1H, d, $J = 4.0$ Hz, H-17a), 2.11 (3H, s, COCH_3), 2.06 (3H, s, COCH_3), 2.04 (3H, s, COCH_3), 2.01-1.90 (3H, m), 1.90-1.79 (1H, m), 1.79-1.60 (3H, m), 1.47-1.41 (1H, m), 1.36-1.22 (4H, m), 1.04 (3H, s, H-18), 0.90 (3H, s, H-20); ^{13}C NMR (100 MHz, CDCl_3): δ 170.87, 170.60, 170.38, 169.02, 150.66, 123.35, 79.30, 71.61, 67.57, 64.62, 58.02, 54.78, 54.07, 49.80, 41.13, 39.57, 37.41, 35.95, 23.59, 23.01, 22.67, 22.16, 21.12, 21.08, 20.87, 14.72.

Synthesis of 17-amino-8-*epi*-12-*epi*-isoandrographolide analogues 5-9

General Procedure: To a stirred solution of 19-substituted-3,14-diacetyl-8,17-epoxy andrographolide (**4a-4e**) (0.04-0.10 mmol) in methanol (1.0 mL) was added amine compound (0.06-0.15 mmol) at room temperature. After the stirring was continued at room temperature for 0.5 to 72 h, the reaction mixture was quenched carefully with cooled aq. NH_4Cl (10 mL), extracted with EtOAc (3 \times 10 mL). The combined organic layer was washed with water (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/*n*-hexane) to afford the corresponding products (**5a-9e**).

19-Tr-3-acetyl-17-(phenyl)amino-*epi*-isoandrographolide (**5a**)

According to the general procedure, 19-Tr-3,14-diacetyl-8,17-epoxy andrographolide (**4a**) (51.9 mg, 0.075 mmol) with aniline (9.90 mg, 0.106 mmol) was stirred at room temperature for 24 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **5a** in 51% yield (27.7 mg) as a brown solid, R_f 0.31 (15% EtOAc/*n*-hexane); Mp. 98-100 °C; IR (Neat): 3361, 3093, 1794, 1732, 1603, 1402, 1246, 1067, 886, 708 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.39 (6H, d, $J = 8.0$ Hz, PhH), 7.25-7.13 (9H, m, PhH), 7.10-7.03 (3H, m, H-14, PhH), 6.60 (1H, t, $J = 7.5$ Hz, PhH), 6.40 (2H, d, $J = 7.5$ Hz, PhH), 4.85 (1H, brs, NH), 4.69 (1H, d, $J = 18.0$ Hz, H-15b), 4.61 (1H, d, $J = 18.0$ Hz, H-15a), 4.43 (1H, dm, $J = 11.0$ Hz, H-3), 3.79 (1H, d, $J = 10.0$ Hz, H-12), 3.29 (1H, d, $J = 10.0$ Hz, H-19b), 3.04 (1H, d, $J = 10.0$ Hz, H-19a), 2.56 (1H, brs, H-17b), 2.46 (1H, brs, H-17a), 1.92 (3H, s, COCH_3), 1.90-1.45 (8H, m), 1.10 (3H, s, H-18), 1.40-1.01 (3H, m), 0.60-0.50 (1H, m), 0.22 (3H, s, H-20); ^{13}C NMR (100 MHz, CDCl_3): δ 173.10, 170.61, 146.84, 146.16, 144.12 (3 \times C), 135.68, 129.24 (2 \times C), 128.83 (6 \times C), 127.61 (6 \times C), 126.85 (3 \times C), 117.18, 112.73 (2 \times C), 86.82, 80.20, 70.28, 62.13, 58.83, 54.81, 52.42, 51.85, 50.45, 42.30, 39.99, 35.99 (2 \times C), 26.31, 23.53, 22.88, 22.43, 21.28, 14.00; HRMS (ESI) m/z calcd for $\text{C}_{47}\text{H}_{52}\text{NO}_6$ $[\text{M}+\text{H}]^+$ 726.3795, found 726.3789.

19-Tr-3-acetyl-17-((2-fluoro)phenyl)amino-epi-isoandrographolide (6a)

According to the general procedure, 19-Tr-3,14-diacetyl-8,17-epoxy andrographolide (**4a**) (51.3 mg, 0.074 mmol) with 2-fluoroaniline (10.5 mg, 0.094 mmol) was stirred at room temperature for 48 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **6a** in 53% yield (29.0 mg) as a yellow solid, R_f 0.50 (30% EtOAc/*n*-hexane); Mp. 112-114 °C; IR (Neat): 3367, 3097, 1754, 1621, 1401, 1246, 1192, 1032, 708 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.39 (6H, d, $J = 7.5$ Hz, PhH), 7.25-7.11 (9H, m, PhH), 7.07 (1H, brs, H-14), 6.89 (1H, t, $J = 10.0$ Hz, PhH), 6.83 (1H, t, $J = 7.5$ Hz, PhH), 6.56-6.48 (1H, m, PhH), 6.25 (1H, t, $J = 7.5$ Hz, PhH), 5.10 (1H, brs, NH), 4.69 (1H, d, $J = 18.0$ Hz, H-15b), 4.62 (1H, d, $J = 18.0$ Hz, H-15a), 4.43 (1H, dm, $J = 11.0$ Hz, H-3), 3.80 (1H, d, $J = 10.0$ Hz, H-12), 3.28 (1H, d, $J = 10.0$ Hz, H-19b), 3.04 (1H, d, $J = 10.0$ Hz, H-19a), 2.55 (1H, brs, H-17b), 2.46 (1H, brs, H-17a), 1.92 (3H, s, COCH_3), 1.91-1.02 (11H, m), 1.09 (3H, s, H-18), 0.65-0.55 (1H, m), 0.22 (3H, s, H-20); ^{13}C NMR (100 MHz, CDCl_3): δ 172.94, 170.64, 151.39 (d, $J_{\text{C-F}} = 240$ Hz), 146.04, 144.15 (3×C), 135.49, 135.32 (d, $J_{\text{C-F}} = 12.0$ Hz), 128.86 (6×C), 127.62 (6×C), 126.86 (3×C), 124.47, 116.64 (d, $J_{\text{C-F}} = 7.0$ Hz), 114.59 (d, $J_{\text{C-F}} = 18.0$ Hz), 112.24, 86.88, 80.25, 70.28, 62.17, 58.65, 54.94, 52.17, 51.91, 50.40, 42.36, 39.98, 36.11, 35.90, 26.34, 23.57, 22.88, 22.42, 21.25, 14.07; HRMS (ESI) m/z calcd for $\text{C}_{47}\text{H}_{51}\text{FNO}_6$ $[\text{M}+\text{H}]^+$ 744.3700, found 744.3703.

19-Tr-3-acetyl-17-((3-fluoro)phenyl)amino-epi-isoandrographolide (7a)

According to the general procedure, 19-Tr-3,14-diacetyl-8,17-epoxy andrographolide (**4a**) (52.4 mg, 0.076 mmol) with 3-fluoroaniline (10.45 mg, 0.094 mmol) was stirred at room temperature for 76 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **7a** in 61% yield (34.3 mg) as a white solid, R_f 0.50 (30% EtOAc/*n*-hexane); Mp. 96-98 °C; IR (Neat): 3339, 3119, 1754, 1621, 1404, 1247, 1152, 1032, 708 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.40 (6H, d, $J = 7.5$ Hz, PhH), 7.26-7.12 (9H, m, PhH), 7.08 (1H, brs, H-14), 6.98 (1H, dd, $J = 15.0, 8.0$ Hz, PhH), 6.28 (1H, t, $J = 8.0$ Hz, PhH), 6.17 (1H, d, $J = 8.0$ Hz, PhH), 6.07 (1H, d, $J = 11.5$ Hz, PhH), 5.06 (1H, brs, NH), 4.71 (1H, d, $J = 18.0$ Hz, H-15b), 4.63 (1H, d, $J = 18.0$ Hz, H-15a), 4.44 (1H, dm, $J = 10.0$ Hz, H-3), 3.75 (1H, brd, $J = 7.0$ Hz, H-12), 3.29 (1H, d, $J = 9.5$ Hz, H-19b), 3.05 (1H, d, $J = 9.5$ Hz, H-19a), 2.57 (1H, brs, H-17b), 2.48 (1H, brs, H-17a), 1.93 (3H, s, COCH_3), 1.99-1.03 (11H, m), 1.10 (3H, s, H-18), 0.59-0.48 (1H, m), 0.22 (3H, s, H-20); ^{13}C NMR (100 MHz, CDCl_3): δ 172.90, 170.58, 164.05 (d, $J_{\text{C-F}} = 240$ Hz), 148.72 (d, $J_{\text{C-F}} =$

11.0 Hz), 146.13, 144.14 (3×C), 135.30, 130.33 (d, J_{C-F} = 10.0 Hz), 128.85 (6×C), 127.62 (6×C), 126.87 (3×C), 108.57, 103.73 (d, J_{C-F} = 20.0 Hz), 99.60 (d, J_{C-F} = 25.0 Hz), 86.88, 80.15, 70.27, 62.17, 58.87, 54.88, 52.60, 52.00, 50.49, 42.34, 40.02, 36.04 (2×C), 26.26, 23.56, 22.89, 22.44, 21.26, 14.04; HRMS (ESI) m/z calcd for $C_{47}H_{51}FNO_6$ $[M+H]^+$ 744.3700, found 744.3699.

19-Tr-3-acetyl-17-((4-Bromo)phenyl)amino-epi-isoandrographolide (**8a**)

According to the general procedure, 19-Tr-3,14-diacetyl-8,17-epoxy andrographolide (**4a**) (51.0 mg, 0.074 mmol) with 4-bromoaniline (36.1 mg, 0.210 mmol) was stirred at room temperature for 127 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **8a** in 51% yield (30.2 mg) as a white solid, R_f 0.34 (30% EtOAc/*n*-hexane); Mp. 98-101 °C; IR (Neat): 3372, 2932, 1753, 1595, 1490, 1245, 1070, 708, 632 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 7.40 (6H, d, J = 7.5 Hz, PhH), 7.25-7.10 (11H, m, PhH), 7.06 (1H, s, H-14), 6.28 (2H, d, J = 8.0 Hz, PhH), 4.95 (1H, brs, NH), 4.69 (1H, d, J = 18.0 Hz, H-15b), 4.62 (1H, d, J = 18.0 Hz, H-15a), 4.45 (1H, dm, J = 10.0 Hz, H-3), 3.74 (1H, brd, J = 10.0 Hz, H-12), 3.29 (1H, d, J = 10.0 Hz, H-19b), 3.05 (1H, d, J = 10.0 Hz, H-19a), 2.57 (1H, brs, H-17b), 2.48 (1H, brs, H-17a), 2.05-1.02 (11H, m), 1.93 (3H, s, $COCH_3$), 1.10 (3H, s, H-18), 0.60-0.48 (1H, m), 0.22 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.88, 170.57, 146.11, 145.37, 144.10 (3×C), 135.24, 131.91 (2×C), 128.81 (6×C), 127.58 (6×C), 126.83 (3×C), 114.36 (2×C), 108.80, 86.85, 80.13, 70.23, 62.13, 58.86, 54.84, 52.57, 51.96, 50.46, 42.30, 39.98, 36.02 (2×C), 26.30, 23.51, 22.84, 22.40, 21.21, 14.00; HRMS (ESI) m/z calcd for $C_{47}H_{51}BrNO_6$ $[M+H]^+$ 804.2900, found 804.2894.

19-Tr-3-acetyl-17-((3,4-dimethoxy)phenyl)amino-epi-isoandrographolide (**9a**)

According to the general procedure, 19-Tr-3,14-diacetyl-8,17-epoxy andrographolide (**4a**) (50.7 mg, 0.073 mmol) with 3,4-dimethoxyaniline (20.4 mg, 0.133 mmol) was stirred at room temperature for 1.5 h. The residue was purified by column chromatography (50% EtOAc/*n*-hexane) to afford the corresponding product **9a** in 50% yield (28.6 mg) as a yellow solid, R_f 0.64 (50% EtOAc/*n*-hexane); Mp. 85-87 °C; IR (Neat): 3367, 2924, 1749, 1614, 1513, 1446, 1238, 1027, 708 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 7.40 (6H, d, J = 7.5 Hz, PhH), 7.25-7.12 (9H, m, PhH), 7.10 (1H, s, H-14), 6.61 (1H, d, J = 8.5 Hz, PhH), 6.09 (1H, s, PhH), 5.84 (1H, d, J = 8.5 Hz, PhH), 4.70 (1H, d, J = 18.0 Hz, H-15b), 4.62 (1H, d, J = 18.0 Hz, H-15a), 4.50-4.40 (1H, m, H-3), 4.09-4.01 (1H, m, NH), 3.85-3.75 (1H, m, H-12),

3.74 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 3.29 (1H, d, $J = 9.5$ Hz, H-19b), 3.04 (1H, d, $J = 9.5$ Hz, H-19a), 2.57 (1H, brs, H-17b), 2.46 (1H, brs, H-17a), 1.93 (3H, s, COCH_3), 2.05-1.00 (11H, m), 1.10 (3H, s, H-18), 0.63-0.53 (1H, m), 0.22 (3H, s, H-20); ^{13}C NMR (100 MHz, CDCl_3): δ 173.11, 170.61, 150.10, 146.16, 144.14 ($3\times\text{C}$), 141.80, 141.38, 135.87, 128.85 ($6\times\text{C}$), 127.61 ($6\times\text{C}$), 126.86 ($3\times\text{C}$), 113.30, 103.24, 98.87, 86.87, 80.21, 70.26, 62.17, 58.91, 56.71, 55.82, 54.88, 52.92, 51.70, 50.46, 42.34, 39.97, 36.08 ($2\times\text{C}$), 26.49, 23.56, 22.88, 22.44, 21.25, 14.05; HRMS (ESI) m/z calcd for $\text{C}_{49}\text{H}_{56}\text{NO}_8$ $[\text{M}+\text{H}]^+$ 786.4006, found 786.4004.

19-TBS-3-acetyl-17-(phenyl)amino-epi-isoandrographolide (**5b**)

According to the general procedure, 19-TBS-3,14-diacetyl-8,17-epoxy andrographolide (**4b**) (23.6 mg, 0.042 mmol) with aniline (5.70 mg, 0.061 mmol) was stirred at room temperature for 24 h. The residue was purified by column chromatography (15% EtOAc/*n*-hexane) to afford the corresponding product **5b** in 86% yield (21.6 mg) as a yellow solid, R_f 0.31 (15% EtOAc/*n*-hexane); Mp. 124-126 °C; IR (Neat): 3376, 2954, 2857, 1749, 1732, 1651, 1603, 1513, 1471, 1248, 1060, 1030, 853, 838, 736 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.19 (1H, d, $J = 1.0$ Hz, H-14), 7.14 (2H, t, $J = 7.5$ Hz, PhH), 6.68 (1H, t, $J = 7.5$ Hz, PhH), 6.49 (2H, d, $J = 7.5$ Hz, PhH), 5.01 (1H, brd, $J = 4.5$ Hz, NH), 4.78 (1H, dt, $J = 18.0, 1.0$ Hz, H-15b), 4.71 (1H, brd, $J = 18.0$ Hz, H-15a), 4.53 (1H, dd, $J = 11.0, 5.5$ Hz, H-3), 3.96-3.89 (1H, m, H-12), 3.88 (1H, d, $J = 10.5$ Hz, H-19b), 3.58 (1H, d, $J = 10.5$ Hz, H-19a), 2.76 (1H, brd, $J = 3.0$ Hz, H-17b), 2.63 (1H, d, $J = 3.0$ Hz, H-17a), 2.15 (1H, dt, $J = 13.5, 3.0$ Hz, H-11b), 2.04 (3H, s, COCH_3), 1.94-1.61 (7H, m), 1.45-1.24 (2H, m), 1.18-1.12 (1H, m), 0.94 (3H, s, H-18), 0.90 (3H, s, H-20), 0.89 (9H, s, $\text{Si}(\text{CH}_3)_3$), 0.77-0.68 (1H, m), 0.03 (6H, s, $\text{Si}(\text{CH}_3)_2$); ^{13}C NMR (100 MHz, CDCl_3): δ 173.15, 170.55, 146.94, 146.14, 135.84, 129.26 ($2\times\text{C}$), 117.20, 112.82 ($2\times\text{C}$), 80.12, 70.30, 64.03, 59.16, 54.93, 52.57, 52.10, 50.54, 42.30, 40.33, 36.46, 36.44, 26.59, 25.85 ($3\times\text{C}$), 23.71, 23.11, 23.01, 21.21, 18.19, 14.28, -5.60, -5.70; HRMS (ESI) m/z calcd for $\text{C}_{34}\text{H}_{52}\text{NO}_6\text{Si}$ $[\text{M}+\text{H}]^+$ 598.3564, found 598.3569.

19-TBS-3-acetyl-17-((2-fluoro)phenyl)amino-epi-isoandrographolide (**6b**)

According to the general procedure, 19-TBS-3,14-diacetyl-8,17-epoxy andrographolide (**4b**) (24.0 mg, 0.042 mmol) with 2-fluoroaniline (6.90 mg, 0.062 mmol) was stirred at room temperature for 48 h. The residue was purified by column

chromatography (25% EtOAc/*n*-hexane) to afford the corresponding product **6b** in 77% yield (20.0 mg) as a white solid, R_f 0.59 (25% EtOAc/*n*-hexane); Mp. 66-68 °C; IR (Neat): 3372, 3119, 2956, 2857, 1755, 1620, 1526, 1402, 1249, 1093, 1062, 1030, 855, 837 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.19 (1H, d, $J = 1.5$ Hz, H-14) 6.98 (1H, ddd, $J = 12.0, 8.0, 1.5$ Hz, PhH), 6.92 (1H, t, $J = 8.0$ Hz, PhH), 6.64-6.57 (1H, m, PhH), 6.36 (1H, td, $J = 8.0, 1.5$ Hz, PhH), 5.26 (1H, brs, NH), 4.79 (1H, dt, $J = 18.0, 1.5$ Hz, H-15b), 4.73 (1H, dt, $J = 18.0, 1.5$ Hz, H-15a), 4.54 (1H, dd, $J = 10.0, 6.5$ Hz, H-3), 3.96 (1H, brd, $J = 10.0$ Hz, H-12), 3.88 (1H, d, $J = 10.5$ Hz, H-19b), 3.58 (1H, d, $J = 10.5$ Hz, H-19a), 2.76 (1H, brd, $J = 3.5$ Hz, H-17b), 2.63 (1H, d, $J = 3.5$ Hz, H-17a), 2.14 (1H, dt, $J = 13.5, 3.5$ Hz, H-11b), 2.04 (3H, s, COCH_3), 1.95-1.68 (7H, m), 1.47-1.30 (2H, m), 1.19-1.13 (1H, m), 0.94 (3H, s, H-18), 0.91 (3H, s, H-20), 0.89 (9H, s, $\text{Si}(\text{CH}_3)_3$), 0.84-0.74 (1H, m), 0.03 (6H, s, $\text{Si}(\text{CH}_3)_2$); ^{13}C NMR (100 MHz, CDCl_3): δ 173.03, 170.60, 151.41 (d, $J_{\text{C-F}} = 240$ Hz), 146.09, 135.55, 135.36 (d, $J_{\text{C-F}} = 10.0$ Hz), 124.46 (d, $J_{\text{C-F}} = 3.0$ Hz), 116.60 (d, $J_{\text{C-F}} = 6.0$ Hz), 114.60 (d, $J_{\text{C-F}} = 18.0$ Hz), 112.24 (d, $J_{\text{C-F}} = 3.0$ Hz), 80.12, 70.34, 64.00, 59.00, 54.97, 52.25, 52.14, 50.51, 42.29, 40.29, 36.48, 36.33, 26.56, 25.84 ($3\times\text{C}$), 23.71, 23.10, 22.98, 21.22, 18.19, 14.29, -5.63, -5.73; HRMS (ESI) m/z calcd for $\text{C}_{34}\text{H}_{51}\text{FNO}_6\text{Si}$ $[\text{M}+\text{H}]^+$ 616.3470, found 616.3467.

19-TBS-3-acetyl-17-((3-fluorophenyl)amino)-epi-isoandrographolide (**7b**)

According to the general procedure, 19-TBS-3,14-diacetyl-8,17-epoxy andrographolide (**4b**) (22.3 mg, 0.039 mmol) with 3-fluoroaniline (6.40 mg, 0.058 mmol) was stirred at room temperature for 72 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **7b** in 79% yield (19.0 mg) as a white solid, R_f 0.29 (30% EtOAc/*n*-hexane); Mp. 172-174 °C; IR (Neat): 3371, 2956, 2857, 1755, 1622, 1521, 1403, 1251, 1094, 1031, 837, 775 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.18 (1H, d, $J = 1.5$ Hz, H-14) 7.06 (1H, dd, $J = 15.0, 8.0$ Hz, PhH), 6.36 (1H, td, $J = 8.0, 2.0$ Hz, PhH), 6.26 (1H, dd, $J = 8.0, 2.0$ Hz, PhH), 6.16 (1H, dt, $J = 11.5, 2.0$ Hz, PhH), 5.22 (1H, brs, NH), 4.81 (1H, dt, $J = 18.0, 1.5$ Hz, H-15b), 4.73 (1H, dt, $J = 18.0, 1.5$ Hz, H-15a), 4.55 (1H, dd, $J = 10.5, 6.0$ Hz, H-3), 3.92-3.87 (1H, m, H-12), 3.88 (1H, d, $J = 10.5$ Hz, H-19b), 3.57 (1H, d, $J = 10.5$ Hz, H-19a), 2.77 (1H, dm, $J = 3.5$ Hz, H-17b), 2.63 (1H, d, $J = 3.5$ Hz, H-17a), 2.13 (1H, dt, $J = 13.5, 3.5$ Hz, H-11b), 2.04 (3H, s, COCH_3), 1.95-1.65 (7H, m), 1.45-1.29 (2H, m), 1.19-1.12 (1H, m), 0.94 (3H, s, H-18), 0.90 (3H, s, H-20), 0.88 (9H, s, $\text{Si}(\text{CH}_3)_3$), 0.76-0.66 (1H, m), 0.03 (6H, s, $\text{Si}(\text{CH}_3)_2$);

^{13}C NMR (100 MHz, CDCl_3): δ 173.00, 170.58, 146.17, 135.38, 130.33 (d, $J_{\text{C-F}} = 10.0$ Hz), 108.60, 103.69 (d, $J_{\text{C-F}} = 20.0$ Hz), 99.61 (d, $J_{\text{C-F}} = 25.0$ Hz), 80.04, 70.32, 64.04, 59.22, 54.92, 52.69, 52.20, 50.58, 42.28, 40.33, 36.46, 36.43, 26.48, 25.84 (3 \times C), 23.69, 23.10, 23.00, 21.23, 18.18, 14.26, -5.63, -5.73; HRMS (ESI) m/z calcd for $\text{C}_{34}\text{H}_{51}\text{FNO}_6\text{Si}$ $[\text{M}+\text{H}]^+$ 616.3470, found 616.3463.

19-TBS-3-acetyl-17-((4-Bromo)phenyl)amino-epi-isoandrographolide (8b)

According to the general procedure, 19-TBS-3,14-diacetyl-8,17-epoxy andrographolide (**4b**) (23.4 mg, 0.041 mmol) with 4-bromoaniline (10.0 mg, 0.058 mmol) was stirred at room temperature for 96 h. The residue was purified by column chromatography (15% EtOAc/*n*-hexane) to afford the corresponding product **8b** in 77% yield (21.3 mg) as a yellow solid, R_f 0.33 (15% EtOAc/*n*-hexane); Mp. 52-54 $^\circ\text{C}$; IR (Neat): 3360, 3159, 3098, 2957, 2856, 1755, 1734, 1595, 1400, 1259, 1098, 1030, 837, 813 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.21 (2H, d, $J = 7.5$ Hz, PhH), 7.16 (1H, brs, H-14) 6.37 (2H, d, $J = 7.5$ Hz, PhH), 5.13 (1H, brs, NH), 4.79 (1H, d, $J = 18.5$ Hz, H-15b), 4.72 (1H, d, $J = 18.5$ Hz, H-15a), 4.57-4.51 (1H, m, H-3), 3.87 (1H, d, $J = 10.0$ Hz, H-19b), 3.87-3.82 (1H, m, H-12), 3.57 (1H, d, $J = 10.0$ Hz, H-19a), 2.76 (1H, brs, H-17b), 2.63 (1H, brs, H-17a), 2.13 (1H, dm, $J = 13.5$ Hz, H-11b), 2.04 (3H, s, COCH_3), 1.93-1.67 (7H, m), 1.44-1.10 (3H, m), 0.94 (3H, s, H-18), 0.89-0.88 (12H, m, H-20, $\text{SiC}(\text{CH}_3)_3$), 0.80-0.70 (1H, m), 0.07 (6H, s, $\text{Si}(\text{CH}_3)_2$); ^{13}C NMR (100 MHz, CDCl_3): δ 173.00, 170.57, 146.15, 145.99, 135.42, 131.97 (2 \times C), 114.44 (2 \times C), 108.84, 80.07, 70.31, 64.07, 59.23, 54.96, 52.74, 52.26, 50.60, 42.31, 40.36, 36.51 (2 \times C), 26.59, 25.86 (3 \times C), 23.71, 23.12, 23.02, 21.22, 18.20, 14.29, -5.7 (2 \times C); HRMS (ESI) m/z calcd for $\text{C}_{34}\text{H}_{51}\text{BrNO}_6\text{Si}$ $[\text{M}+\text{H}]^+$ 676.2669, found 676.2664.

19-TBS-3-acetyl-17-((3,4-dimethoxy)phenyl)amino-epi-isoandrographolide

(9b) According to the general procedure, 19-TBS-3,14-diacetyl-8,17-epoxy andrographolide (**4b**) (21.0 mg, 0.037 mmol) with 3,4-dimethoxyaniline (8.30 mg, 0.054 mmol) was stirred at room temperature for 1 h. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to afford the corresponding product **9b** in 67% yield (16.4 mg) as a yellow solid, R_f 0.48 (40% EtOAc/*n*-hexane); Mp. 66-68 $^\circ\text{C}$; IR (Neat): 3373, 3124, 2955, 2857, 1751, 1616, 1516, 1403, 1261, 1248, 1093, 1030, 855, 837 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.21 (1H, brs, H-14) 6.69 (1H, d, $J = 8.0$ Hz, PhH), 6.18 (1H, s, PhH), 5.94 (1H, d, $J = 8.0$ Hz, PhH), 4.79 (1H, d, $J = 18.0$ Hz, H-15b), 4.72 (1H, d, $J = 18.0$ Hz, H-15a), 4.60-4.51 (2H, m, H-3, NH), 3.91 (1H, d, $J = 9.5$ Hz, H-12), 3.87 (1H, d, $J = 10.5$ Hz, H-

19b), 3.82 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.57 (1H, d, $J = 10.5$ Hz, H-19a), 2.77 (1H, brs, H-17b), 2.62 (1H, brs, H-17a), 2.12 (1H, dm, $J = 13.0$ Hz, H-11b), 2.04 (3H, s, COCH_3), 1.93-1.65 (7H, m), 1.45-1.27 (2H, m), 1.19-1.12 (1H, m), 0.93 (3H, s, H-18), 0.89 (3H, s, H-20), 0.88 (9H, s, $\text{SiC}(\text{CH}_3)_3$), 0.80-0.70 (1H, m), 0.03 (6H, s, $\text{Si}(\text{CH}_3)_2$); ^{13}C NMR (100 MHz, CDCl_3): δ 173.17, 170.56, 150.11, 146.19, 141.85, 141.38, 135.94, 113.33, 103.29, 98.91, 80.09, 70.29, 64.01, 59.23, 56.72, 55.82, 54.93, 53.03, 51.92, 50.54, 42.28, 40.27, 36.51, 36.47, 26.71, 25.83 ($3\times\text{C}$), 23.70, 23.09, 22.99, 21.19, 18.17, 14.28, -5.65, -5.75; HRMS (ESI) m/z calcd for $\text{C}_{36}\text{H}_{56}\text{NO}_8\text{Si}$ $[\text{M}+\text{H}]^+$ 658.3775, found 658.3777.

19-TBDPS-3-acetyl-17-(phenyl)amino-epi-isoandrographolide (**5c**)

According to the general procedure, 19-TBDPS-3,14-diacetyl-8,17-epoxy andrographolide (**4c**) (40.4 mg, 0.059 mmol) with aniline (18.2 mg, 0.195 mmol) was stirred at room temperature for 4.5 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **5c** in 69% yield (29.6 mg) as a white solid, R_f 0.35 (30% EtOAc/*n*-hexane); Mp. 97-99 °C; IR (Neat): 3367, 2936, 2857, 1753, 1600, 1508, 1371, 1245, 1077, 1027, 828, 760, 699, 501 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.69-7.63 (4H, m, PhH), 7.45-7.34 (6H, m, PhH), 7.18 (1H, brs, H-14), 7.13 (2H, t, $J = 7.5$ Hz, PhH), 6.67 (1H, t, $J = 7.5$ Hz, PhH), 6.48 (2H, d, $J = 7.5$ Hz, PhH), 4.77 (1H, brd, $J = 18.0$ Hz, H-15b), 4.70 (1H, brd, $J = 18.0$ Hz, H-15a), 4.50 (1H, dd, $J = 12.0, 4.5$ Hz, H-3), 3.90 (1H, brd, $J = 10.0$ Hz, H-12), 3.83 (1H, d, $J = 10.5$ Hz, H-19b), 3.71 (1H, d, $J = 10.5$ Hz, H-19a), 2.72 (1H, brd, $J = 3.0$ Hz, H-17b), 2.61 (1H, brd, $J = 3.0$ Hz, H-17a), 2.11-1.12 (11H, m), 1.88 (3H, s, COCH_3), 1.05 (9H, s, $\text{SiC}(\text{CH}_3)_3$), 1.00 (3H, s, H-18), 0.73 (3H, s, H-20), 0.73-0.64 (1H, m); ^{13}C NMR (100 MHz, CDCl_3): δ 173.08, 170.48, 146.97, 146.09, 135.84 ($4\times\text{C}$), 135.76, 133.57, 133.44, 129.61 ($2\times\text{C}$), 129.27 ($2\times\text{C}$), 127.59 ($2\times\text{C}$), 127.53 ($2\times\text{C}$), 117.26, 112.85 ($2\times\text{C}$), 80.04, 70.26, 63.91, 58.96, 54.86, 52.54, 51.96, 50.51, 42.89, 40.26, 36.29, 36.24, 26.96 ($3\times\text{C}$), 26.51, 23.63, 22.88, 22.74, 21.06, 19.27, 14.49; HRMS (ESI) m/z calcd for $\text{C}_{44}\text{H}_{56}\text{NO}_6\text{Si}$ $[\text{M}+\text{H}]^+$ 722.3877, found 722.3873.

19-TBDPS-3-acetyl-17-((2-fluoro)phenyl)amino-epi-isoandrographolide (**6c**)

According to the general procedure, 19-TBDPS-3,14-diacetyl-8,17-epoxy andrographolide (**4c**) (40.5 mg, 0.059 mmol) with 2-fluoroaniline (19.5 mg, 0.175 mmol) was stirred at room temperature for 48 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **6c** in 82% yield (35.7 mg) as a white solid, R_f 0.39 (30% EtOAc/*n*-hexane); Mp. 83-85 °C; IR (Neat):

3373, 2930, 2857, 1753, 1620, 1519, 1368, 1260, 1081, 1029, 822, 753, 498 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.71-7.63 (4H, m, PhH), 7.46-7.34 (6H, m, PhH), 7.18 (1H, brs, H-14), 6.98 (1H, t, $J = 9.0$ Hz, PhH), 6.91 (1H, t, $J = 7.5$ Hz, PhH), 6.65-6.57 (1H, m, PhH), 6.35 (1H, t, $J = 8.5$ Hz, PhH), 5.23 (1H, brs, NH), 4.78 (1H, brd, $J = 18.0$ Hz, H-15b), 4.72 (1H, brd, $J = 18.0$ Hz, H-15a), 4.52 (1H, dd, $J = 11.5, 3.0$ Hz, H-3), 3.93 (1H, brd, $J = 8.5$ Hz, H-12), 3.84 (1H, d, $J = 10.5$ Hz, H-19b), 3.72 (1H, d, $J = 10.5$ Hz, H-19a), 2.73 (1H, brs, H-17b), 2.62 (1H, brs, H-17a), 2.12-1.95 (2H, m), 1.89 (3H, s, COCH_3), 1.87-1.42 (7H, m), 1.36-1.12 (2H, m), 1.06 (9H, s, $\text{SiC}(\text{CH}_3)_3$), 1.01 (3H, s, H-18), 0.75 (3H, s, H-20), 0.80-0.70 (1H, m); ^{13}C NMR (100 MHz, CDCl_3): δ 172.98, 170.56, 151.42 (d, $J_{\text{C-F}} = 239$ Hz), 146.07, 135.84 (2 \times C), 135.75 (2 \times C), 135.53, 135.34 (d, $J_{\text{C-F}} = 11.0$ Hz), 133.52, 133.39, 129.64, 129.61, 127.60 (2 \times C), 127.53 (2 \times C), 124.46, 116.64 (d, $J_{\text{C-F}} = 7.0$ Hz), 114.61 (d, $J_{\text{C-F}} = 18.0$ Hz), 112.23, 80.02, 70.32, 63.84, 58.81, 54.87, 52.22, 52.00, 50.50, 42.87, 40.20, 36.24, 36.15, 26.94 (3 \times C), 26.46, 23.61, 22.84, 22.73, 21.10, 19.26, 14.49; HRMS (ESI) m/z calcd for $\text{C}_{44}\text{H}_{55}\text{FNO}_6\text{Si}$ $[\text{M}+\text{H}]^+$ 740.3783, found 740.3782.

19-TBDPS-3-acetyl-17-((3-fluoro)phenyl)amino-epi-isoandrographolide (7c)

According to the general procedure, 19-TBDPS-3,14-diacetyl-8,17-epoxy andrographolide (**4c**) (40.7 mg, 0.059 mmol) with 3-fluoroaniline (15.9 mg, 0.143 mmol) was stirred at room temperature for 24 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **7c** in 80% yield (35.0 mg) as a white solid, R_f 0.32 (30% EtOAc/*n*-hexane); Mp. 85-87 $^\circ\text{C}$; IR (Neat): 3356, 2924, 2852, 1753, 1617, 1491, 1371, 1246, 1078, 1029, 825, 761, 702, 498 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.72-7.62 (4H, brs, PhH), 7.46-7.34 (6H, m, PhH), 7.18 (1H, brs, H-14), 7.10-7.02 (1H, m, PhH), 6.36 (1H, td, $J = 7.5, 1.0$ Hz, PhH), 6.26 (1H, dd, $J = 7.5, 1.0$ Hz, PhH), 6.17 (1H, dt, $J = 11.5, 1.0$ Hz, PhH), 4.81 (1H, brd, $J = 18.0$ Hz, H-15b), 4.72 (1H, brd, $J = 18.0$ Hz, H-15a), 4.52 (1H, dd, $J = 12.0, 4.5$ Hz, H-3), 3.89-3.84 (1H, m, H-12), 3.83 (1H, d, $J = 10.5$ Hz, H-19b), 3.71 (1H, d, $J = 10.5$ Hz, H-19a), 2.74 (1H, brd, $J = 3.0$ Hz, H-17b), 2.63 (1H, d, $J = 3.0$ Hz, H-17a), 2.10-1.92 (2H, m), 1.89 (3H, s, COCH_3), 1.87-1.41 (7H, m), 1.36-1.13 (2H, m), 1.06 (9H, s, $\text{SiC}(\text{CH}_3)_3$), 1.01 (3H, s, H-18), 0.74 (3H, s, H-20), 0.73-0.65 (1H, m); ^{13}C NMR (100 MHz, CDCl_3): δ 172.94, 170.53, 164.08 (d, $J_{\text{C-F}} = 241$ Hz), 148.78 (d, $J_{\text{C-F}} = 11.0$ Hz), 146.16, 135.84 (2 \times C), 135.75 (2 \times C), 135.35, 133.52, 133.38, 130.34 (d, $J_{\text{C-F}} = 10.0$ Hz), 129.62 (2 \times C), 127.60 (2 \times C), 127.53 (2 \times C),

108.62, 103.74 (d, $J_{\text{C-F}} = 22.0$ Hz), 99.64 (d, $J = 25.0$ Hz), 79.96, 70.29, 63.88, 59.04, 54.83, 52.65, 52.06, 50.57, 42.87, 40.25, 36.29, 33.22, 26.95 (3×C), 26.40, 23.60, 22.86, 22.73, 21.07, 19.26, 14.47; HRMS (ESI) m/z calcd for $\text{C}_{44}\text{H}_{55}\text{FNO}_6\text{Si}$ $[\text{M}+\text{H}]^+$ 740.3783, found 740.3777.

19-TBDPS-3-acetyl-17-((4-Bromo)phenyl)amino-epi-isoandrographolide (8c)

According to the general procedure, 19-TBDPS-3,14-diacetyl-8,17-epoxy andrographolide (**4c**) (41.4 mg, 0.060 mmol) with 4-bromoaniline (15.0 mg, 0.087 mmol) was stirred at room temperature for 24 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **8c** in 69% yield (33.2 mg) as a yellow solid, R_f 0.35 (30% EtOAc/*n*-hexane); Mp. 87-89 °C; IR (Neat): 3356, 2931, 2852, 1752, 1592, 1491, 1368, 1245, 1075, 1027, 819, 761, 704, 501 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.71-7.63 (4H, brs, PhH), 7.47-7.35 (6H, m, PhH), 7.21 (2H, d, $J = 8.5$ Hz, PhH), 7.16 (1H, brs, H-14), 6.37 (2H, d, $J = 8.5$ Hz, PhH), 4.79 (1H, brd, $J = 18.0$ Hz, H-15b), 4.72 (1H, brd, $J = 18.0$ Hz, H-15a), 4.52 (1H, dd, $J = 12.0, 4.5$ Hz, H-3), 3.88-3.82 (1H, m, H-12), 3.83 (1H, d, $J = 10.5$ Hz, H-19b), 3.71 (1H, d, $J = 10.5$ Hz, H-19a), 2.74 (1H, brd, $J = 3.0$ Hz, H-17b), 2.63 (1H, d, $J = 3.0$ Hz, H-17a), 2.09-1.97 (2H, m), 1.89 (3H, s, COCH_3), 1.86-1.41 (7H, m), 1.35-1.12 (2H, m), 1.06 (9H, s, $\text{SiC}(\text{CH}_3)_3$), 1.00 (3H, s, H-18), 0.73 (3H, s, H-20), 0.72-0.65 (1H, m); ^{13}C NMR (100 MHz, CDCl_3): δ 172.97, 170.53, 146.16, 145.96, 135.84 (2×C), 135.76 (2×C), 135.36, 133.53, 133.38, 131.98 (2×C), 129.65 (2×C), 127.16 (2×C), 127.55 (2×C), 114.44 (2×C), 108.87, 79.96, 70.30, 63.88, 59.06, 54.84, 52.69, 52.11, 50.59, 42.87, 40.25, 36.31, 36.22, 26.96 (3×C), 26.47, 23.60, 22.88, 22.70, 21.10, 19.28, 14.48; HRMS (ESI) m/z calcd for $\text{C}_{44}\text{H}_{55}\text{BrNO}_6\text{Si}$ $[\text{M}+\text{H}]^+$ 800.2982, found 800.2977.

19-TBDPS-3-acetyl-17-((3,4-dimethoxy)phenyl)amino-epi-isoandrographolide (9c)

According to the general procedure, 19-TBDPS-3,14-diacetyl-8,17-epoxy andrographolide (**4c**) (40.2 mg, 0.058 mmol) with 3,4-dimethoxyaniline (13.9 mg, 0.091 mmol) was stirred at room temperature for 2 h. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to afford the corresponding product **9c** in 79% yield (36.1 mg) as brown solid, R_f 0.50 (40% EtOAc/*n*-hexane); Mp. 107-109 °C; IR (Neat): 3366, 2935, 2852, 1751, 1515, 1460, 1365, 1240, 1077, 1029, 822, 752, 705, 502 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.70-7.64 (4H, brs, PhH), 7.46-7.34 (6H, m, PhH), 7.20 (1H,

brs, H-14), 6.69 (1H, d, $J = 8.5$ Hz, PhH), 6.18 (1H, s, PhH), 5.93 (1H, d, $J = 8.5$ Hz, PhH), 4.78 (1H, brd, $J = 18.0$ Hz, H-15b), 4.72 (1H, brd, $J = 18.0$ Hz, H-15a), 4.51 (1H, brd, $J = 11.0$ Hz, H-3), 3.90 (1H, brd, $J = 8.5$ Hz, H-12), 3.83 (1H, d, $J = 11.0$ Hz, H-19b), 3.82 (3H, s, OCH₃), 3.79 (3H, s, OCH₃), 3.71 (1H, d, $J = 11.0$ Hz, H-19a), 2.74 (1H, brs, H-17b), 2.61 (1H, brs, H-17a), 2.09-1.95 (2H, m), 1.89 (3H, s, COCH₃), 1.85-1.12 (9H, m), 1.06 (9H, s, SiC(CH₃)₃), 1.01 (3H, s, H-18), 0.96-0.83 (1H, m), 0.74 (3H, s, H-20); ¹³C NMR (100 MHz, CDCl₃): δ 173.14, 170.53, 150.10, 146.18, 141.82, 141.37, 135.90, 135.82 (2 \times C), 135.73 (2 \times C), 133.50, 133.36, 129.62 (2 \times C), 127.58 (2 \times C), 127.52 (2 \times C), 113.28, 103.24, 98.87, 79.98, 70.29, 63.84, 59.06, 56.70, 55.82, 54.81, 52.97, 51.78, 50.54, 42.84, 40.18, 36.31, 36.22, 26.93 (3 \times C), 26.60, 23.59, 22.86, 22.72, 21.08, 19.25, 14.47; HRMS (ESI) m/z calcd for C₄₆H₆₀NO₈Si [M+H]⁺ 782.4088, found 782.4083.

19-TIPS-3-acetyl-17-(phenyl)amino-epi-isoandrographolide (5d)

According to the general procedure, 19-TIPS-3,14-diacetyl-8,17-epoxy andrographolide (**4d**) (49.9 mg, 0.082 mmol) with aniline (12.0 mg, 0.129 mmol) was stirred at room temperature for 3 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **5d** in 65% yield (34.2 mg) as a white solid, R_f 0.35 (30% EtOAc/*n*-hexane); Mp. 87-89 °C; IR (Neat): 3361, 2945, 2863, 1745, 1600, 1508, 1455, 1253, 1091, 1024, 878, 757, 495 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.19 (1H, brd, $J = 1.0$ Hz, H-14), 7.14 (2H, dd, $J = 8.5, 7.5$ Hz, PhH), 6.68 (1H, t, $J = 7.5$ Hz, PhH), 6.49 (2H, d, $J = 8.5$ Hz, PhH), 5.01 (1H, brs, NH), 4.78 (1H, dt, $J = 18.0, 1.5$ Hz, H-15b), 4.72 (1H, dt, $J = 18.0, 1.5$ Hz, H-15a), 4.53 (1H, dd, $J = 11.5, 4.5$ Hz, H-3), 3.94 (1H, d, $J = 10.5$ Hz, H-19b), 3.95-3.89 (1H, m, H-12), 3.78 (1H, d, $J = 10.5$ Hz, H-19a), 2.76 (1H, d, $J = 3.5$ Hz, H-17b), 2.63 (1H, d, $J = 3.5$ Hz, H-17a), 2.14 (1H, dt, $J = 13.5, 3.0$ Hz, H-11b), 2.03 (3H, s, COCH₃), 1.98-1.69 (8H, m), 1.45-1.29 (2H, m), 1.06 (18H, brd, $J = 4.0$ Hz, 3 \times (SiCH(CH₃)₂)), 1.17-1.02 (3H, m, 3 \times (SiCH(CH₃)₂)), 0.99 (3H, s, H-18), 0.89 (3H, s, H-20), 0.77-0.68 (1H, m); ¹³C NMR (100 MHz, CDCl₃): δ 173.15, 170.58, 146.89, 146.18, 135.75, 129.25 (2 \times C), 117.19, 112.79 (2 \times C), 80.10, 70.30, 63.83, 59.08, 54.83, 52.52, 52.00, 50.55, 42.80, 40.27, 36.30, 36.27, 26.48, 23.64, 22.82 (2 \times C), 21.19, 18.08 (6 \times C), 14.58, 11.90 (3 \times C); HRMS (ESI) m/z calcd for C₃₇H₅₈NO₆Si [M+H]⁺ 640.4033, found 640.4028.

19-TIPS-3-acetyl-17-((2-fluoro)phenyl)amino-epi-isoandrographolide (6d)

According to the general procedure, 19-TIPS-3,14-diacetyl-8,17-epoxy andrographolide (**4d**) (50.2 mg, 0.083 mmol) with 2-fluoroaniline (13.7 mg, 0.123 mmol) was stirred at room temperature for 72 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **6d** in 65% yield (35.4 mg) as a white solid, R_f 0.33 (30% EtOAc/*n*-hexane); Mp. 145-147 °C; IR (Neat): 3378, 2942, 2863, 1753, 1619, 1522, 1455, 1245, 1094, 1027, 853, 838, 758, 495 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.18 (1H, brd, $J = 1.0$ Hz, H-14), 6.98 (1H, ddd, $J = 12.0, 8.0, 1.0$ Hz, PhH), 6.92 (1H, t, $J = 8.0$ Hz, PhH), 6.64-6.57 (1H, m, PhH), 6.36 (1H, td, $J = 8.0, 1.0$ Hz, PhH), 5.25 (1H, brs, NH), 4.80 (1H, dt, $J = 18.0, 1.5$ Hz, H-15b), 4.73 (1H, dt, $J = 18.0, 1.5$ Hz, H-15a), 4.55 (1H, dd, $J = 12.0, 4.5$ Hz, H-3), 3.94 (1H, d, $J = 10.0$ Hz, H-19b), 3.98-3.93 (1H, m, H-12), 3.94 (1H, d, $J = 10.0$ Hz, H-19a), 2.76 (1H, d, $J = 3.5$ Hz, H-17b), 2.64 (1H, d, $J = 3.5$ Hz, H-17a), 2.13 (1H, dt, $J = 13.5, 3.0$ Hz, H-11b), 2.03 (3H, s, COCH_3), 1.98-1.67 (8H, m), 1.48-1.29 (2H, m), 1.06 (18H, brd, $J = 4.5$ Hz, $3 \times (\text{SiCH}(\text{CH}_3)_2)$), 1.12-1.03 (3H, m, $3 \times (\text{SiCH}(\text{CH}_3)_2)$), 1.00 (3H, s, H-18), 0.90 (3H, s, H-20), 0.83-0.73 (1H, m); ^{13}C NMR (100 MHz, CDCl_3): δ 173.01, 170.62, 151.39 (d, $J_{\text{C-F}} = 238$ Hz), 146.12, 135.50, 135.33 (d, $J_{\text{C-F}} = 11.0$ Hz), 124.46 (d, $J_{\text{C-F}} = 3.0$ Hz), 116.60 (d, $J_{\text{C-F}} = 7.0$ Hz), 114.59 (d, $J_{\text{C-F}} = 18.0$ Hz), 112.22 (d, $J_{\text{C-F}} = 3.0$ Hz), 80.11, 70.33, 63.81, 58.92, 54.90, 52.23, 52.07, 50.52, 42.82, 40.25, 36.33, 36.19, 26.47, 23.66, 22.82 ($2 \times \text{C}$), 21.20, 18.08 ($6 \times \text{C}$), 14.61, 11.91 ($3 \times \text{C}$); HRMS (ESI) m/z calcd for $\text{C}_{37}\text{H}_{57}\text{FNO}_6\text{Si}$ $[\text{M}+\text{H}]^+$ 658.3939, found 658.3939.

19-TIPS-3-acetyl-17-((3-fluoro)phenyl)amino-epi-isoandrographolide (**7d**)

According to the general procedure, 19-TIPS-3,14-diacetyl-8,17-epoxy andrographolide (**4d**) (51.7 mg, 0.085 mmol) with 3-fluoroaniline (16.5 mg, 0.148 mmol) was stirred at room temperature for 48 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **7d** in 82% yield (46.0 mg) as a white solid, R_f 0.39 (30% EtOAc/*n*-hexane); Mp. 147-149 °C; IR (Neat): 3361, 2942, 2866, 1753, 1620, 1495, 1370, 1245, 1097, 1030, 884, 837, 761, 495 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.18 (1H, brd, $J = 1.5$ Hz, H-14), 7.10-7.02 (1H, m, PhH), 6.35 (1H, td, $J = 8.0, 1.5$ Hz, PhH), 6.26 (1H, dd, $J = 8.0, 1.5$ Hz, PhH), 6.16 (1H, dt, $J = 11.5, 1.5$ Hz, PhH), 5.21 (1H, brs, NH), 4.81 (1H, dt, $J = 18.0, 1.5$ Hz, H-15b), 4.73 (1H, dt, $J = 18.0, 1.5$ Hz, H-15a), 4.55 (1H, dd, $J = 12.0, 4.5$ Hz, H-3), 3.94 (1H, d, $J = 10.5$ Hz, H-19b), 3.88 (1H, brd, $J = 10.0$ Hz, H-12), 3.78 (1H, d, $J = 10.5$ Hz, H-19a), 2.76 (1H, d, J

= 3.5 Hz, H-17b), 2.64 (1H, d, J = 3.5 Hz, H-17a), 2.12 (1H, dt, J = 13.5, 3.0 Hz, H-11b), 2.03 (3H, s, COCH₃), 1.98-1.59 (8H, m), 1.46-1.27 (2H, m), 1.06 (18H, brd, J = 4.5 Hz, 3×(SiCH(CH₃)₂)), 1.12-1.03 (3H, m, 3×(SiCH(CH₃)₂)), 1.00 (3H, s, H-18), 0.89 (3H, s, H-20), 0.76-0.66 (1H, m); ¹³C NMR (100 MHz, CDCl₃): δ 172.98, 170.58, 164.04 (d, J_{C-F} = 241 Hz), 148.75 (d, J_{C-F} = 10.0 Hz), 146.19, 135.33, 130.32 (d, J_{C-F} = 10.0 Hz), 108.60, 103.70 (d, J_{C-F} = 21.0 Hz), 99.61 (d, J_{C-F} = 25.0 Hz), 80.04, 70.31, 63.85, 59.14, 54.86, 52.66, 52.13, 50.58, 42.81, 40.29, 36.33, 36.29, 26.40, 23.64, 22.83 (2×C), 21.19, 18.08 (6×C), 14.59, 11.91 (3×C); HRMS (ESI) m/z calcd for C₃₇H₅₇FNO₆Si [M+H]⁺ 658.3939, found 658.3934.

19-TIPS-3-acetyl-17-((4-Bromo)phenyl)amino-epi-isoandrographolide (8d)

According to the general procedure, 19-TIPS-3,14-diacetyl-8,17-epoxy andrographolide (**4d**) (50.7 mg, 0.083 mmol) with 4-bromoaniline (21.5 mg, 0.125 mmol) was stirred at room temperature for 24 h. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to afford the corresponding product **8d** in 66% yield (39.6 mg) as a white solid, R_f 0.50 (40% EtOAc/*n*-hexane); Mp. 103-105 °C; IR (Neat): 3361, 2941, 2863, 1752, 1594, 1502, 1245, 1096, 1027, 881, 814, 762, 498 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.21 (2H, d, J = 8.5 Hz, PhH), 7.16 (1H, brs, H-14), 6.37 (2H, d, J = 8.5 Hz, PhH), 5.13 (1H, brs, NH), 4.80 (1H, d, J = 18.0 Hz, H-15b), 4.72 (1H, d, J = 18.0 Hz, H-15a), 4.54 (1H, dd, J = 12.0, 4.5 Hz, H-3), 3.94 (1H, d, J = 10.5 Hz, H-19b), 3.86 (1H, brd, J = 10.0 Hz, H-12), 3.77 (1H, d, J = 10.5 Hz, H-19a), 2.76 (1H, d, J = 3.0 Hz, H-17b), 2.64 (1H, d, J = 3.0 Hz, H-17a), 2.11 (1H, dt, J = 13.5, 3.0 Hz, H-11b), 2.02 (3H, s, COCH₃), 1.99-1.58 (8H, m), 1.45-1.29 (2H, m), 1.06 (18H, brd, J = 4.5 Hz, 3×(SiCH(CH₃)₂)), 1.18-1.03 (3H, m, 3×(SiCH(CH₃)₂)), 1.00 (3H, s, H-18), 0.88 (3H, s, H-20), 0.76-0.66 (1H, m); ¹³C NMR (100 MHz, CDCl₃): δ 172.98, 170.58, 146.19, 145.87, 135.27, 131.91 (2×C), 114.39 (2×C), 108.78, 80.01, 70.30, 63.81, 59.13, 54.81, 52.65, 52.12, 50.58, 42.76, 40.25, 36.30, 36.25, 26.43, 23.60, 22.80 (2×C), 21.17, 18.06 (6×C), 14.56, 11.87 (3×C); HRMS (ESI) m/z calcd for C₃₇H₅₇BrNO₆Si [M+H]⁺ 718.3139, found 718.3133.

19-TIPS-3-acetyl-17-((3,4-dimethoxy)phenyl)amino-epi-isoandrographolide (9d)

According to the general procedure, 19-TIPS-3,14-diacetyl-8,17-epoxy andrographolide (**4d**) (47.8 mg, 0.079 mmol) with 3,4-dimethoxyaniline (19.2 mg, 0.125

mmol) was stirred at room temperature for 2 h. The residue was purified by column chromatography (30% EtOAc/*n*-hexane) to afford the corresponding product **9d** in 73% yield (40.2 mg) as a brown solid, R_f 0.17 (30% EtOAc/*n*-hexane); Mp. 117-119 °C; IR (Neat): 3356, 2936, 2863, 1748, 1513, 1454, 1239, 1096, 1027, 881, 822, 763, 495 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.21 (1H, brs, H-14), 6.69 (1H, d, J = 8.5 Hz, PhH), 6.18 (1H, d, J = 2.5 Hz, PhH), 5.94 (1H, dd, J = 8.5, 2.5 Hz, PhH), 4.80 (1H, d, J = 18.0 Hz, H-15b), 4.72 (1H, d, J = 18.0 Hz, H-15a), 4.54 (1H, dd, J = 11.5, 4.5 Hz, H-3), 3.94 (1H, d, J = 10.5 Hz, H-19b), 3.94-3.88 (1H, m, H-12), 3.81 (1H, d, J = 10.5 Hz, H-19a), 3.82 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 2.77 (1H, d, J = 3.0 Hz, H-17b), 2.63 (1H, d, J = 3.0 Hz, H-17a), 2.11 (1H, dm, J = 13.5 Hz, H-11b), 2.03 (3H, s, COCH_3), 1.98-1.60 (8H, m), 1.45-1.28 (2H, m), 1.06 (18H, brd, J = 4.5 Hz, $3\times(\text{SiCH}(\text{CH}_3)_2)$), 1.12-1.02 (3H, m, $3\times(\text{SiCH}(\text{CH}_3)_2)$), 1.00 (3H, s, H-18), 0.89 (3H, s, H-20), 0.80-0.70 (1H, m); ^{13}C NMR (100 MHz, CDCl_3): δ 173.20, 170.63, 150.10, 146.24, 141.84, 141.37, 135.93, 113.25, 103.23, 98.87, 80.11, 70.33, 63.85, 59.19, 56.71, 55.84, 54.89, 53.01, 51.87, 50.59, 42.83, 40.26, 36.40, 36.35, 26.65, 23.67, 22.88, 22.85, 21.21, 18.10 ($6\times\text{C}$), 14.63, 11.93 ($3\times\text{C}$); HRMS (ESI) m/z calcd for $\text{C}_{39}\text{H}_{62}\text{NO}_8\text{Si}$ $[\text{M}+\text{H}]^+$ 700.4245, found 700.4249.

3,19-Diacetyl-17-(phenyl)amino-epi-isoandrographolide (**5e**)

According to the general procedure, 3,14,19-triacetyl-8,17-epoxy andrographolide (**4e**) (50.0 mg, 0.102 mmol) with aniline (14.1 mg, 0.151 mmol) was stirred at room temperature for 1.5 h. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to afford the corresponding product **5e** in 61% yield (32.5 mg) as a yellow solid, R_f 0.38 (40% EtOAc/*n*-hexane); Mp. 149-151 °C; IR (Neat): 3373, 2941, 2857, 1736, 1600, 1371, 1256, 1029, 752 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.19 (1H, d, J = 1.5 Hz, H-14), 7.14 (2H, dd, J = 8.5, 7.5 Hz, PhH), 6.68 (1H, t, J = 7.5 Hz, PhH), 6.49 (2H, dd, J = 8.5, 1.0 Hz, PhH), 4.97 (1H, brs, NH), 4.78 (1H, dt, J = 18.0, 1.5 Hz, H-15b), 4.71 (1H, d, J = 18.0, 1.5 Hz, H-15a), 4.56 (1H, dd, J = 12.0, 4.5 Hz, H-3), 4.32 (1H, d, J = 11.5 Hz, H-19b), 4.16 (1H, d, J = 11.5 Hz, H-19a), 3.92 (1H, brd, J = 10.0 Hz, H-12), 2.75 (1H, dd, J = 3.5, 1.5 Hz, H-17b), 2.64 (1H, d, J = 3.5 Hz, H-17a), 2.17 (1H, dt, J = 13.5, 2.0 Hz, H-11b), 2.05 (3H, s, COCH_3), 2.04 (3H, s, COCH_3), 1.98-1.77 (4H, m), 1.76-1.60 (4H, m), 1.49-1.31 (2H, m), 1.00 (3H, s, H-18), 0.85 (3H, s, H-20), 0.78-0.67 (1H, m); ^{13}C NMR (100 MHz, CDCl_3): δ 173.13, 170.86, 170.35, 146.87, 146.17, 135.72, 129.27 ($2\times\text{C}$), 117.24, 112.77 ($2\times\text{C}$), 79.61, 70.31, 64.74, 58.72, 54.55, 52.49, 51.90, 50.41, 41.09, 40.14, 36.02,

35.97, 26.50, 23.59, 22.58, 22.20, 21.13, 21.06, 14.49; HRMS (ESI) m/z calcd for $C_{30}H_{40}NO_7$ $[M+H]^+$ 526.2805, found 526.2801.

3,19-Diacetyl-17-((2-fluoro)phenyl)amino-epi-isoandrographolide (6e)

According to the general procedure, 3,14,19-triacetyl-8,17-epoxy andrographolide (**4e**) (51.5 mg, 0.104 mmol) with 2-fluoroaniline (17.0 mg, 0.153 mmol) was stirred at room temperature for 43 h. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to afford the corresponding product **6e** in 87% yield (49.1 mg) as a white solid, R_f 0.49 (40% EtOAc/*n*-hexane); Mp. 91-93 °C; IR (Neat): 3361, 2949, 2863, 1734, 1619, 1374, 1257, 1033, 828, 751 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 7.19 (1H, d, J = 1.0 Hz, H-14), 6.99 (1H, ddd, J = 12.0, 8.0, 1.0 Hz, PhH), 6.92 (1H, t, J = 8.0 Hz, PhH), 6.65-6.58 (1H, m, PhH), 6.36 (1H, td, J = 8.0, 1.0 Hz, PhH), 5.30 (1H, brs, NH), 4.80 (1H, dt, J = 18.0, 1.5 Hz, H-15b), 4.73 (1H, d, J = 18.0, 1.5 Hz, H-15a), 4.57 (1H, dd, J = 12.0, 4.5 Hz, H-3), 4.32 (1H, d, J = 11.5 Hz, H-19b), 4.17 (1H, d, J = 11.5 Hz, H-19a), 3.94 (1H, brd, J = 10.5 Hz, H-12), 2.75 (1H, dd, J = 3.5, 1.0 Hz, H-17b), 2.65 (1H, d, J = 3.5 Hz, H-17a), 2.17 (1H, dt, J = 13.5, 3.0 Hz, H-11b), 2.05 (3H, s, $COCH_3$), 2.04 (3H, s, $COCH_3$), 1.99-1.78 (4H, m), 1.77-1.53 (4H, m), 1.52-1.45 (1H, m), 1.42-1.33 (1H, m), 1.01 (3H, s, H-18), 0.86 (3H, s, H-20), 0.84-0.74 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.99, 170.86, 170.39, 151.41 (d, J_{C-F} = 238 Hz), 146.11, 135.49, 135.28 (d, J_{C-F} = 11.0 Hz), 124.50 (d, J_{C-F} = 3.0 Hz), 116.72 (d, J_{C-F} = 6.0 Hz), 114.64 (d, J_{C-F} = 19.0 Hz), 112.26, 79.64, 70.35, 64.77, 58.59, 54.64, 52.26, 52.01, 50.40, 41.13, 40.14, 36.11, 35.89, 26.50, 23.61, 22.60, 22.23, 21.15, 21.07, 14.53; HRMS (ESI) m/z calcd for $C_{30}H_{38}FNO_7Na$ $[M+Na]^+$ 566.2530, found 566.2532.

3,19-Diacetyl-17-((3-fluoro)phenyl)amino-epi-isoandrographolide (7e)

According to the general procedure, 3,14,19-triacetyl-8,17-epoxy andrographolide (**4e**) (50.9 mg, 0.103 mmol) with 3-fluoroaniline (17.1 mg, 0.154 mmol) was stirred at room temperature for 27 h. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to afford the corresponding product **7e** in 82% yield (45.7 mg) as a white solid, R_f 0.37 (40% EtOAc/*n*-hexane); Mp. 97-99 °C; IR (Neat): 3367, 2935, 2857, 1734, 1512, 1371, 1257, 1033, 822, 750 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$): δ 7.18 (1H, d, J = 1.5 Hz, H-14), 7.10-7.02 (1H, m, PhH), 6.37 (1H, td, J = 8.0, 2.5 Hz, PhH), 6.26 (1H, dd, J = 8.0, 2.5 Hz, PhH), 6.17 (1H, dt, J = 11.5, 2.5 Hz, PhH), 4.81 (1H, dt, J = 18.0, 1.5 Hz, H-15b), 4.73 (1H, dt, J = 18.0, 1.5 Hz, H-15a), 4.58 (1H, dd, J = 12.0, 4.5 Hz, H-3), 4.32 (1H, d, J = 11.5 Hz, H-19b), 4.17 (1H, d, J = 11.5 Hz, H-19a), 3.88 (1H, brd, J = 11.0 Hz, H-12), 2.76 (1H, dd, J

= 3.5, 1.5 Hz, H-17b), 2.65 (1H, d, J = 3.5 Hz, H-17a), 2.16 (1H, dt, J = 13.5, 3.0 Hz, H-11b), 2.05 (3H, s, COCH₃), 2.04 (3H, s, COCH₃), 1.99-1.60 (8H, m), 1.50-1.31 (2H, m), 1.01 (3H, s, H-18), 0.85 (3H, s, H-20), 0.77-0.67 (1H, m); ¹³C NMR (100 MHz, CDCl₃): δ 172.97, 170.89, 170.38, 164.06 (d, J_{C-F} = 240 Hz), 148.68 (d, J_{C-F} = 11.0 Hz), 146.21, 135.29, 130.37 (d, J_{C-F} = 10.0 Hz), 108.62, 103.81 (d, J_{C-F} = 20.0 Hz), 99.64 (d, J_{C-F} = 25.0 Hz), 79.56, 70.34, 64.76, 58.80, 54.58, 52.68, 52.06, 50.47, 41.12, 40.17, 36.06, 36.01, 26.43, 23.60, 22.59, 22.21, 21.15, 21.09, 14.51; HRMS (ESI) m/z calcd for C₃₀H₃₈FNO₇Na [M+Na]⁺ 566.2530, found 566.2525.

3,19-Diacetyl-17-((4-Bromo)phenyl)amino-epi-isoandrographolide (8e)

According to the general procedure, 3,14,19-triacetyl-8,17-epoxy andrographolide (**4e**) (50.3 mg, 0.102 mmol) with 4-bromoaniline (26.6 mg, 0.155 mmol) was stirred at room temperature for 24 h. The residue was purified by column chromatography (40% EtOAc/*n*-hexane) to afford the corresponding product **8e** in 63% yield (38.8 mg) as a yellow solid, R_f 0.47 (40% EtOAc/*n*-hexane); Mp. 153-155 °C; IR (Neat): 3356, 2947, 2863, 1734, 1603, 1371, 1259, 1032, 750 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.21 (2H, d, J = 8.5 Hz, PhH), 7.16 (1H, d, J = 1.2 Hz, H-14), 6.37 (2H, d, J = 8.5 Hz, PhH), 5.12 (1H, brs, NH), 4.79 (1H, dt, J = 18.0, 1.5 Hz, H-15b), 4.72 (1H, d, J = 18.0, 1.5 Hz, H-15a), 4.57 (1H, dd, J = 12.0, 4.5 Hz, H-3), 4.32 (1H, d, J = 12.0 Hz, H-19b), 4.16 (1H, d, J = 12.0 Hz, H-19a), 3.86 (1H, brd, J = 10.0 Hz, H-12), 2.76 (1H, dd, J = 3.5, 1.5 Hz, H-17b), 2.64 (1H, d, J = 3.5 Hz, H-17a), 2.14 (1H, dt, J = 13.5, 3.0 Hz, H-11b), 2.05 (3H, s, COCH₃), 2.04 (3H, s, COCH₃), 1.98-1.77 (4H, m), 1.75-1.59 (4H, m), 1.49-1.43 (1H, m), 1.40-1.30 (1H, m), 1.00 (3H, s, H-18), 0.84 (3H, s, H-20), 0.76-0.66 (1H, m); ¹³C NMR (100 MHz, CDCl₃): δ 172.96, 170.85, 170.35, 146.20, 145.83, 135.25, 131.96 (2×C), 114.41 (2×C), 108.90, 79.54, 70.32, 64.73, 58.79, 54.55, 52.68, 52.07, 50.47, 41.09, 40.14, 36.03, 36.00, 26.48, 23.57, 22.57, 22.19, 21.14, 21.07, 14.49; HRMS (ESI) m/z calcd for C₃₀H₃₉BrNO₇ [M+H]⁺ 604.1910, found 604.1906.

3,19-Diacetyl-17-((3,4-dimethoxy)phenyl)amino-epi-isoandrographolide (9e)

According to the general procedure, 3,14,19-triacetyl-8,17-epoxy andrographolide (**4e**) (53.2 mg, 0.108 mmol) with 3,4-dimethoxyaniline (24.0 mg, 0.157 mmol) was stirred at room temperature for 2 h. The residue was purified by column chromatography (60% EtOAc/*n*-hexane) to afford the corresponding product **9e** in 82% yield (51.8 mg) as a brown solid, R_f 0.67 (60% EtOAc/*n*-hexane); Mp. 109-111 °C; IR (Neat): 3361, 2932, 2852, 1734,

1513, 1370, 1237, 1031, 761 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.21 (1H, brs, H-14), 6.69 (1H, d, J = 8.5 Hz, PhH), 6.19 (1H, d, J = 2.0 Hz, PhH), 5.94 (1H, dd, J = 8.5, 2.0 Hz, PhH), 4.79 (1H, dt, J = 18.0, 1.5 Hz, H-15b), 4.73 (1H, d, J = 18.0 Hz, H-15a), 4.57 (1H, dd, J = 12.0, 4.5 Hz, H-3), 4.32 (1H, d, J = 11.5 Hz, H-19b), 4.16 (1H, d, J = 11.5 Hz, H-19a), 3.94-3.87 (1H, m, H-12), 3.82 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 2.77 (1H, brs, H-17b), 2.64 (1H, d, J = 3.5 Hz, H-17a), 2.14 (1H, dm, J = 13.5 Hz, H-11b), 2.05 (3H, s, COCH_3), 2.04 (3H, s, COCH_3), 1.98-1.30 (10H, m), 1.00 (3H, s, H-18), 0.84 (3H, s, H-20), 0.82-0.72 (1H, m); ^{13}C NMR (100 MHz, CDCl_3): δ 173.16, 170.92, 170.45, 150.10, 146.28, 141.78, 141.39, 135.88, 113.22, 103.17, 98.85, 79.64, 70.38, 64.77, 58.86, 56.70, 55.84, 54.59, 52.98, 51.77, 50.47, 41.12, 40.13, 36.09, 36.05, 26.67, 23.61, 22.60, 22.24, 21.17, 21.10, 14.53; HRMS (ESI) m/z calcd for $\text{C}_{32}\text{H}_{44}\text{NO}_9$ $[\text{M}+\text{H}]^+$ 586.3016, found 586.3011.

Conflicts of interest

We declare that we have no conflict of interest.

Note

In remembrance of His Majesty King Bhumibol Adulyadej (1927-2016), for his life-time dedication to Thailand and people.

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Highlights

- A one pot, three steps reaction was developed for modification of andrographolide.
- New series of amino-isoandrographolides was obtained with high stereoselectivity.
- Twenty five analogues were screened for cytotoxicity against six cancer cell lines.
- Some synthetic analogues exhibited the good activity on ASK cancer cells.