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ORIGINAL RESEARCH

Preparation and α-glucosidase inhibition of andrographolide derivatives

Minh Huy Ly[1,2] · Tuyen Ngoc Truong[1] · Tuoi Thi Hong Do[1]

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Abstract

Series of novel analogs, which were primarily modified on its lactone moiety, was synthesized based on Andrographolide (**1**), a natural product sesquiterpene inhibitor of α-glucosidase. Among new analogs, 14-deoxy-11,12-didehydro-15-(4-ethoxybenzylidene)andrographolide (**3h**) was determined to have the greatest potential of α-glucosidase inhibitor through the calculation of IC_{50} value of 160 ± 5.1 μM, a significant improvement compared to the clinical dose of Acarbose, which showed the IC_{50} value of 390 ± 8.1 μM. In addition, 14-deoxy-11,12-didehydro-3,19-(2′-hydroxybenzylidene)-15-(2-hydroxybenzylidene) andrographolide (**7**), a 15-benzylidene derivative of 14-deoxy-11,12-didehydroandrographolide containing a 1,3-dioxane moiety at C(3) and C(19), also displayed good inhibition with IC_{50} 260 ± 13 μM. These results are promising avenues in the subsequent optimization of antidiabetic drugs.

Keywords Andrographolide derivatives · α-glucosidase inhibitors · Diabetes

Introduction

Diabetes mellitus is one of the most widespread and problematic metabolic disorders globally. Maintaining optimal blood glucose is one of the treatment objectives in type 2 diabetes therapy. Toward this end, α-glucosidases (amylase, sucrase-isomaltase, and maltase-glucoamylase), found on the luminal surface of enterocytes, contribute to increase postprandial blood glucose and have emerged as potential targets to diminish gastrointestinal glucose absorption (Priebe et al. 2018). Therefore, there has been a high interest in the optimization of α-glucosidase inhibitors. Acarbose (Fig. 1) is currently the only α-glucosidase inhibitor clinically used to support type 2 diabetes patients (Rosak and Mertes 2012).

Andrographolide (Fig. 1), the major component in *Andrographis paniculate*, is well known as a complementary medicine to treatments of infections or inflammations (Chao and Lin 2010). Andrographolide and its derivatives possess several attractive biological activities, such as antibacterial (Jiang et al. 2009), antiviral properties (Wang et al. 2010; Uttekar Mayur et al. 2012), tumor growth inhibition (Sirion et al. 2012; Wei et al. 2013), liver protection and immunity booster (Kumar et al. 2004). Regarding α-glucosidase inhibition, some of its derivatives possess improved potency compared to acarbose, making them potential candidates toward novel antidiabetic drugs (Xu et al. 2007).

Previous structure–activity relationship (SAR) studies have shown that the lactone moiety and the conjugated double bond $\Delta^{12(13)}$ played essential roles in biological activity (Xu et al. 2010; Jitendra et al. 2012). Recent reports indicate that introducing a silyl ether or triphenylmethyl ether group into C(19) increased toxicity against cancer cells (Jiang et al. 2011). In other studies, synthetic 14-deoxy-11,12-didehydroandrographolide (**2**, Fig. 1) and its derivatives exhibited high stability in both acidic and basic medium (Wei et al. 2013). In terms of α-glucosidase inhibition, derivatives of C(15)-isopropylidene and benzylidene

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✉ Minh Huy Ly
huyh2002@USherbrooke.ca

✉ Tuyen Ngoc Truong
truongtuyen@ump.edu.vn

[1] Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh city, 41 Dinh Tien Hoang, District 1, Ho Chi Minh city 700000, Vietnam

[2] Université de Sherbrooke, Faculté des Sciences, 2500, boul. de l'Université Sherbrooke, Sherbrooke, QC J1K 2R1, Canada

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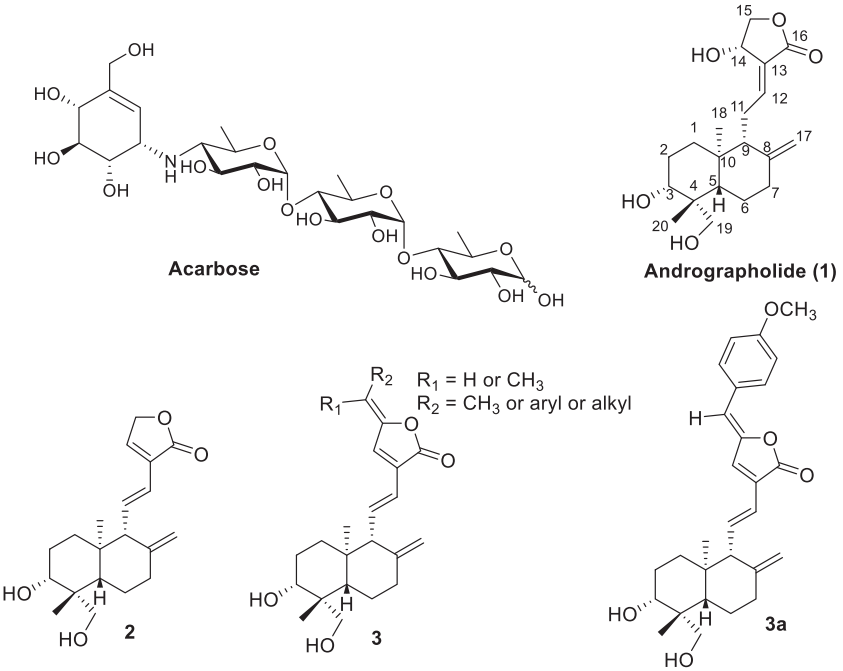
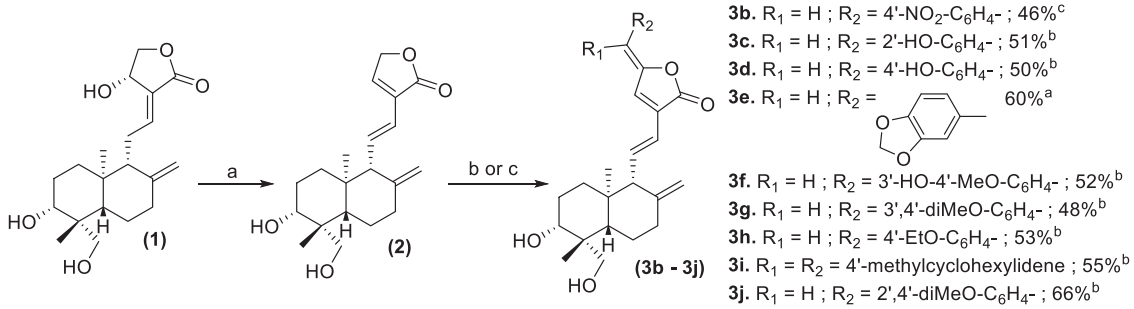


Fig. 1 Structures of acarbose, andrographolide (**1**) with numbering and its derivatives



Scheme 1 Preparation of some derivatives of 15-isopropylidene and benzylidene-14-deoxy-11,12-didehydroandrographolide (**3b–3j**). Reagents and conditions: (**a**) H_2SO_4, pyridine, 90 °C, 91%; (**b**) K_2CO_3 in MeOH, rt; (**c**) CH_3COOH/pyrrolidine in MeOH, 50–60 °C

(**3**, Fig. 1) of 14-deoxy-11,12-didehydroandrographolide (**2**) exhibited more potent inhibition against α-glucosidase than andrographolide, with % inhibition determined at 100 μM up to 100% (for compound **3a**) (Xu et al. 2007). Together, existing studies point to three groups as crucial for biological activity: C(15), C(3), and C(19) (Wei et al. 2013; Xu et al. 2010; Nanduri et al. 2004). Due to their potential inhibition, many efforts have been made to develop procedures for the semisynthesis of these derivatives.

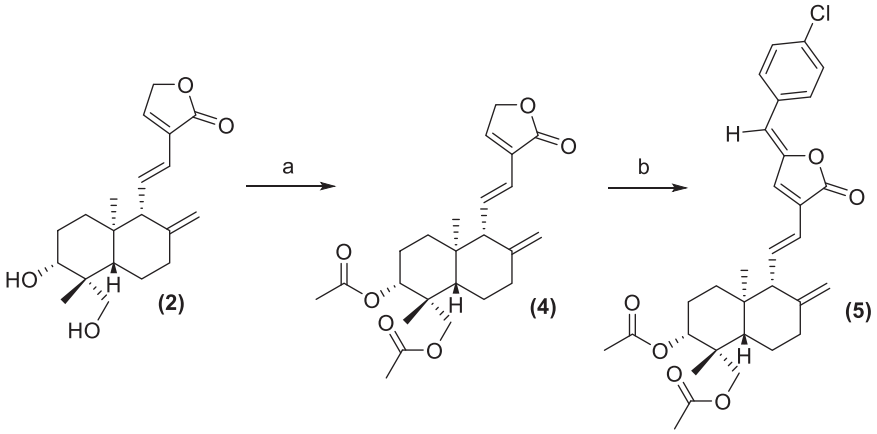
We recently reported a novel approach to the semisynthesis of andrographolide derivatives based on two simple steps: dehydration and vinylogous aldol condensation (Ly Minh et al. 2019). Notably, in the preparation of 14-deoxy-11,12-didehydro-andrographolide (**2**), concentrated sulfuric acid in pyridine was utilized as an alternative condition for Al_2O_3 in xylene (Kumar et al. 2004). In the vinylogous aldol condensation, two new catalysts were successfully implemented: (a) K_2CO_3 in MeOH and (b) a mixture of CH_3COOH/pyrrolidine in MeOH. In the present study, we report new syntheses of andrographolide derivatives that enable further investigation of its SAR on the inhibition of α-glucosidase.

Results and discussion

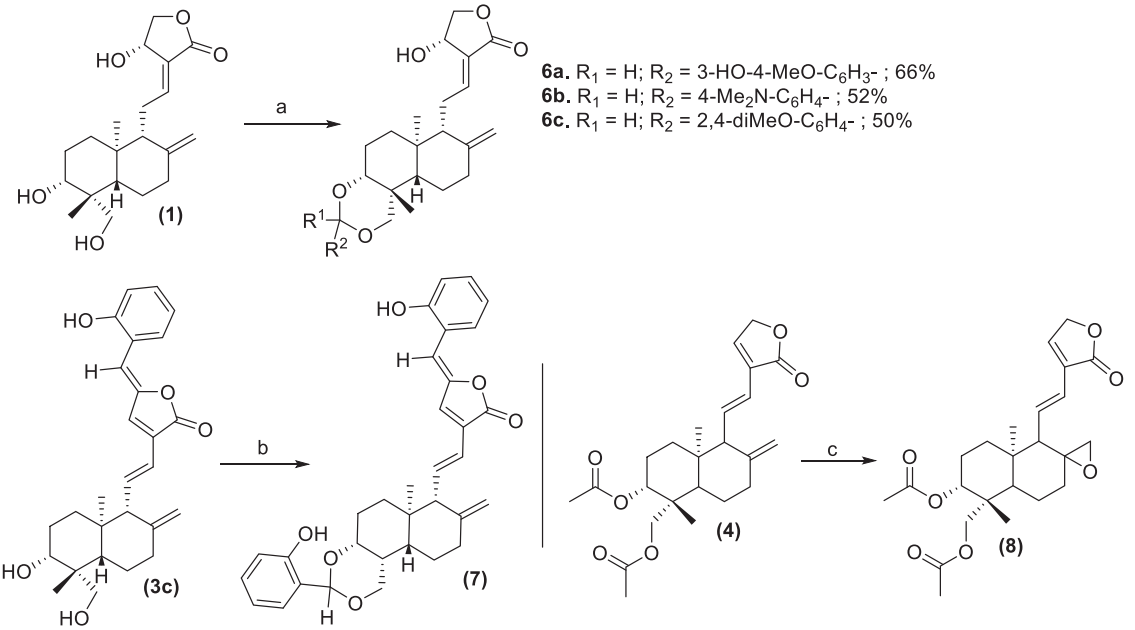
Chemistry

Firstly (Scheme 1), the conjugated dehydration of andrographolide was conducted to prepare the intermediate **2**. Subsequently, synthesis of derivatives of 15-isopropylidene and benzylidene-14-deoxy-11,12-didehydroandrographolide (**3b–3j**) was reached via a reaction of **2** with different benzaldehydes and ketones under suitable conditions.

Overall, nine derivatives of 14-deoxy-11,12-didehydroandrographolide (**3b–3j**) were successfully synthesized



Scheme 2 Preparation of 3,19-diacetyl-15-(4-clorobenzylidene)-14-deoxy-11,12-didehydroandrographolide (**5**). Reagents and conditions: (**a**) Ac_2O, pyridine, $ZnCl_2$, rt, 93%; (**b**) 4-chlorobenzaldehyde, CH_3COOH/pyrrolidine in MeOH, 50–60 °C, 78%



6a. R_1 = H; R_2 = 3-HO-4-MeO-C_6H_3- ; 66%
6b. R_1 = H; R_2 = 4-Me_2N-C_6H_4- ; 52%
6c. R_1 = H; R_2 = 2,4-diMeO-C_6H_4- ; 50%

Scheme 3 Preparation of dioxane and oxirane derivatives (**6a–6c**, **7**, **8**) of andrographolide (**1**) and 14-deoxy-11,12-didehydroandrographolide (**2**). Reagents and conditions: (**a**) R_1COR_2, pyridinium chloride, $CHCl_3$, rt; (**b**) salicylaldehyde, pyridinium chloride, $CHCl_3$, rt, 78%; (**c**) *m*-CBPA, 10 °C, $CHCl_3$, 41%

by aldol-like condensation with different catalysts. As reported in our previous study (Ly Minh et al. 2019), K_2CO_3 in MeOH was favorable for the preparation of **3c–3j**, while the mixture of CH_3COOH and pyrrolidine in MeOH was used to prepare **3b**. In addition, the acetylated form of 15-(4-chlorobenzylidene)-14-deoxy-11,12-didehydroandrographolide (**5**) was prepared following Scheme 2.

The structures of **2**, **3b–3j**, and **5** were elucidated based on their IR, NMR, and HRMS spectra. According to previous studies, the stereoisomer of double bond $\Delta^{11(12)}$ was assumed to be *E* based on the coupling constant $J_{H\text{-}11,H\text{-}12}$ (15.6 Hz) and the relative geometry of the new exocyclic double bond of the lactone has been identified as *Z* isomer due to the interaction between H(14) and the vinylic proton in NOE spectrum (Xu et al. 2007). In order to obtain more insight into the SAR study at C(3), C(19), and the double bond $\Delta^{8(17)}$, dioxane and oxirane derivatives of andrographolide (**1**) and 14-deoxy-11,12-didehydroandrographolide (**2**), respectively were prepared (Scheme 3) in good yields.

Evaluation of *α*-glucosidase inhibition activity

With these derivatives in hand, their inhibition of *α*-glucosidase was investigated as described previously (Xu et al. 2007). Briefly, compounds dissolved in DMSO as stock solutions (1 mM) were diluted in buffer (pH 6.8) to obtain 100-μM samples. These samples were incubated in the presence of *α*-glucosidase for 20 min, then the enzyme substrate *p*-NPG (*p*-nitrophenyl-*α*-D-glucopyranoside) was

Table 1 α-Glucosidase inhibition activity of andrographolide analogs

α-Glucosidase inhibition activity[a] (IC_{50} μM)	
Compound	α-Glucosidase
1	9.47 ± 6.27%
2	N/A[b]
3b	N/A
3c	N/A
3d	N/A
3e	N/A
3f	N/A
3g	N/A
3h	36.6 ± 3.63% (160 ± 5.1)
3i	5.84 ± 0.09%
3j	N/A
4	N/A
5	N/A
6a	N/A
6b	N/A
6c	N/A
7	23.3 ± 0.41% (260 ± 13)
8	N/A

Acarbose was taken as positive control. The inhibition percentage of 1-mM acarbose was 75.3 ± 0.23% (IC_{50} = 390 ± 8.1 μM).

N/A not active at 100 μM.

[a]% Inhibition determined at 100-μM concentration of test compound.

added and incubated for another 20 min, using acarbose as a reference. The reaction was quenched using Na_2CO_3 0.2 M (pH 9.8) and % inhibition was evaluated based on *p*-NPG absorbance at 405 nm. The results are reported in Table 1. Compounds showing more than 20% inhibition at 100 μM were tested in full concentration–response curves to determine their IC_{50}.

In vitro α-glucosidase inhibition of the above derivatives showed that **1**, **3h, 3i** and **7** inhibit α-glucosidase, while the others showed no inhibition at 100 μM (Table 1). In particular, the compounds **3h** and **7** gave the most promising results.

Andrographolide and acarbose inhibited α-glucosidase by 9.47 ± 6.27% at 100 μM and 75.3 ± 0.23% at 1 mM. Among the newly tested derivatives, **3h** and **7** emerge as the most potent inhibitors at 100 μM. Their IC_{50} values were determined using full concentration–response curves. IC_{50} values of **3h** and **7** were found to be 160 ± 5.1 μM and 260 ± 13 μM, respectively. These IC_{50} values are lower than that of acarbose (IC_{50} = 390 ± 8.1 μM) in the same assay.

Moreover, based on the structures of the new active compounds against α-glucosidase at 100 μM (**3h**, **3i**, and **7**), the addition of lipophilic groups and the removal of hydrogen acceptors enhance inhibition of α-glucosidase. Weak inhibition by **3i** suggests that rigid groups, such as conjugated aryl groups, are favorable for enzyme–inhibitor interactions. In particular, **3h**, with one ethoxy group ($-OC_2H_5$) at the *para* position, shows the most potent inhibition; this result is also accordant with a previous 3D SAR analysis which stated that the introducing of the hydrophobic group in particular positions, such as C(3), C (15), and C(19) of phenyl ring could enhance the activity (Jitendra et al. 2012). Interestingly, the transformation from **3c** to **7**, in which the addition of one more hydroxyl group activated the non-active compound (**3c**), indicated that the 1,3-dioxane ring at C(3) and C(19) with proper hydrogen donating groups might contribute to the promotion of inhibition activity.

Conclusion

In summary, 17 derivatives of andrographolide were synthesized in medium-to-good yields. Furthermore, selected compounds were evaluated for in vitro α-glucosidase inhibitions. Compound structures were characterized by IR, NMR, and HRMS spectra. Among newly synthesized compounds, **3h** and **7** exhibit good α-glucosidase inhibition, which will be useful for further development of this class as anti-diabetes drugs. Particularly, 14-deoxy-11,12-didehydro-15-(4-ethoxybenzylidene) andrographolide (**3h**) is the most potent inhibitor of α-glucosidase, with an IC_{50} value of 160 ± 5.1 μM. This is about 2.5 times more potent than the clinically used acarbose (IC_{50} 390 ± 8.1 μM) in the same assay. Moreover, good inhibition of 14-deoxy-11,12-didehydro-3,19-(2′-hydroxybenzylidene)-15-(2-hydroxybenzylidene) andrographolide (**7**, IC_{50} 260 ± 13 μM), shows an interesting SAR.

Materials and methods

Materials

All the reactions were monitored by TLC analysis (precoated silica gel plates with fluorescent indicator UV 254, 0.2 nm) and visualized with 254- and 365-nm UV light. Andrographolide was extracted by the procedure of Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh city. Other chemicals were purchased from Sigma-Aldrich, Merck (Germany), Spain, China. NMR spectra were recorded on Bruker Avance 500 NMR Spectrometer (Ha Noi, Vietnam). High-resolution mass spectrometry was carried out on 1100 series LC/MS/MS Trap Agilent and LC–MS Shimadzu. IR spectra were recorded as ATR method on an IRAFFINITY-1S Shimadzu Spectrometer.

Specific chemical transformation

*Procedure for preparation of 14-deoxy-11,12-didehydroandrographolide (**2**).* Sulfuric acid 98% (0.25 mL) was added into pyridine (10 mL) followed by the addition of andrographolide (3.15 g, 9 mmol). The mixture was stirred at 85–95 °C for 5–6 h. After the completion of reaction by TLC analysis, a yellow-brown crude product was obtained by slowly adding HCl 2-M solution (70 mL) into the reaction mixture and then cooling down. The crude product was purified by silica gel chromatography using an eluent chloroform–acetone = 5:1 to afford colorless corresponding product **2**. Yield 90.4%, mp. 209–210 °C. IR (ATR), ν (cm^{-1}): 3294 ($\nu_{O–H}$), 2933–2850 ($\nu_{C–H}$), 1735 ($\nu_{C=O}$), 1637 ($\nu_{C=C}$), 1350 ($\nu_{C–Oester}$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.63 (*s*, 1H, H-14), 6.74 (*dd*, 1H, J = 15.5, 10.0 Hz, H-11), 6.12 (*d*, 1H, J = 15.5 Hz, H-12), 5.03 (*d*, 1H, J = 5.0 Hz, OH-3), 4.88 (*s*, 2H, H-15), 4.73 (*s*, 1H, H-17a), 4.42 (*s*, 1H, H-17b), 4.14 (*dd*, 1H, J = 7.5, 2.5 Hz, OH-19), 3.86 (*d*, 1H, J = 11.0 Hz, H-19a), 3.28 (*dd*, 1H, J = 11.0, 7.5 Hz, H-19b), 3.22 (*dt*, 1H, J = 10.5, 5.0 Hz, H-3), 2.36 (*d*, 2H, J = 10.0 Hz, H-9, H-7a), 1.98 (*dd*, 1H, J = 13.0, 9.0 Hz, H-7b), 1.72 (*d*, 1H, J = 11.0 Hz, H-1a), 1.58 (*m*, 2H, H-6), 1.42 (*td*, 1H, J = 8.0, 4.0 Hz, H-1b), 1.33 (*d*, 1H, J = 13.5 Hz, H-2a), 1.18 (*d*, 1H, J = 12.5 Hz, H-5), 1.13 (*dd*, 1H, J = 8.0, 4.0 Hz, H-2b), 1.09 (*s*, 3H, H-20), 0.76 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, MeOD, δ ppm): 174.8, 150.1, 146.7, 136.5, 129.6, 122.5, 109.1, 81.2, 71.6, 65.0, 62.8, 55.8, 43.8, 39.6, 39.5, 37.8, 28.9, 24.4, 23.3, 16.3. HRMS *m/z*: $[M + Na]^+$ 355.1876 (calcd 355.1887).

*General procedure for preparation of 15-alkylidene and benzylidene derivatives of 14-deoxy-11,12-didehydroandrographolide (**3b–3j**) and 15-(4-chlorobenzylidene)-3,19-diacetyl-14-deoxy-11,12-didehydroandrographolide (**5**)*

General procedure for catalyst K_2CO_3 in MeOH. To a solution of 14-Deoxy-11,12-didehydro-andrographolide (**2**) (332 mg, 1 mmol) and K_2CO_3 (100 mg, 0.72 mmol) in methanol (10 mL) was slowly added benzaldehyde or ketone derivatives (5 mmol). The mixture was stirred at room temperature for 1–3 h. After the completion of reaction by TLC analysis, the reaction was quenched with distilled water (20 mL) and saturated NaCl solution (20 mL) then cooled down to afford a crude product as a yellow solid, which was then dissolved into ethyl acetate (30 mL). The organic phase was washed with water successively then removed. The crude product was purified by silica chromatography using an eluent chloroform–acetone = 5:1 to afford corresponding products **3**.

General procedure for catalyst acetic acid-pyrrolidine in MeOH. To a 100 mL round bottom flask was added the *catalyst solution** (10 mL) and a benzaldehyde or ketone derivative (5 mmol). The mixture was stirred at room temperature until it turned intense yellow or red. Then, 14-deoxy-11,12-didehydroandrographolide (**2**) (332 mg, 1 mmol) was dissolved and the reaction was slightly heated up to 50–60 °C. After 24 h, the reaction was quenched with distilled water (20 mL) and saturated NaCl solution (20 mL) then cooled down to afford a crude product as a yellow solid. It was then dissolved into ethyl acetate (30 mL). The organic phase was washed with water successively and $NaHCO_3$ 10% solution (20 mL × 2), then removed. The crude product was purified by silica chromatography using an eluent chloroform–acetone = 5:1 to afford corresponding products **3**.

Catalyst solution*: a mixture of MeOH–CH_3COOH–pyrrolidine = 25:4:1 by volume. *14-Deoxy-11,12-didehydro-15-(4-nitrobenzylidene)andrographolide (3b**).* Yield 46%, mp. 199–201 °C. IR (ATR), ν (cm^{-1}): 3366 ($\nu_{O–H}$), 3082 ($\nu_{Ar–H}$), 2926–2849($\nu_{C–H}$), 1751 ($\nu_{C=O}$), 1591 ($\nu_{C=C}$), 1518 ($\nu_{N–O}$), 1337 ($\nu_{C–N}$), 1107 ($\nu_{C–Oester}$), 750 ($\nu_{Ar–H}$). ^{1}H-NMR (500 MHz, MeOD-d_4, δ ppm): 8.28 (*d*, 2H, J = 9.0 Hz, Ar–H-3′, Ar–H-5′), 8.00 (*d*, 1H, J = 9.0 Hz, Ar–H-2′, Ar–H-6′), 7.51 (*s*, 1H, H-14), 7.03 (*dd*, 1H, J = 16.0, 10.0 Hz, H-11), 6.32 (*m*, 2H, H-12, H-21), 4.81 (*s*, 1H, H-17a) 4.53 (*d*, 1H, J = 1.5 Hz, H-17b), 4.16 (*d*, 1H, J = 11.5 Hz, H-3), 3.43 (*d*, 1H, J = 11.5 Hz, H-19a), 3.41 (*d*, 1H, J = 11.0 Hz, H-19b), 1.25 (*s*, 1H, H-20), 0.91 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 168.1, 149.9, 148.7, 146.4, 139.8, 138.4, 136.6, 130.8, 130.2, 127.7, 124.0, 123.9, 121.2, 110.1, 108.23, 78.6, 62.6, 60.8, 53.7, 42.4, 38.5, 38.0, 36.2, 27.6, 23.1, 23.0, 15.4. HRMS *m/z*: $[M - H]^-$ 464.1878 (calcd 464.2073).

*14-Deoxy-11,12-didehydro-15-(2-hydroxybenzylidene) andrographolide (**3c**).* A mixture of *Z* and *E* isomers (7:3), yield 51%, mp. 175–176 °C. IR (ATR), ν (cm^{-1}): 3321 ($\nu_{O–H}$), 2986–2928 ($\nu_{C–H}$), 1732 ($\nu_{C=O}$), 1645–1601 ($\nu_{C=C}$), 1259 ($\nu_{C–Oester}$), 1030 ($\nu_{C–O}$), 750 ($\nu_{Ar–H}$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 10.15 (*s*, 0.7H. Ar–OH), 10.03 (*s*, 0.3H, Ar–OH), 8.03 (*s*, 0.3H, H-14), 7.90 (*dd*, 0.7H, J = 8.5, 2.0 Hz, Ar–H-6′), 7.78 (*s*, 0.7H, H-14), 7.46 (*dd*, 0.3H, J = 8.0, 1.5 Hz, Ar–H-6′), 7.19 (*m*, 1H, Ar–H-4′), 6.90 (*m*, 2H, Ar–H3′, Ar–H-5′), 6.87 (*s*, 0.3H, H-21), 6.80 (*dd*, 1H, J = 15.5, 10.0 Hz, H-11), 6.57 (*s*, 0.7H, H-21), 6.26 (*d*, 0.3H, J = 16.0 Hz, H-12), 6.23 (*d*, 0.7H, J = 16.0 Hz, H-12), 5.03 (*d*, 1H, J = 5.0 Hz, OH-3), 4.75 (*s*, 1H, H-17a), 4.45 (*s*, 1H, H-17b), 4.14 (*dd*, 1H, J = 7.5, 3.0 Hz, OH-19), 3.86 (*d*, 1H, J = 11.0 Hz, H-19a), 3.23 (*dt*, 1H, J = 10.5, 5.0 Hz, H-3), 2.43 (*d*, 1H, J = 10.0 Hz, H-9), 1.10 (*s*, 3H, H-20), 0.79 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 167.9, 156.1, 148.8, 147.9, 146.8, 137.8, 136.3, 132.2, 128.8, 125.1, 121.5, 119.6, 115.6, 110.6, 108.3, 78.9, 62.7, 60.9, 53.7, 42.4, 38.5, 38.0, 36.2, 27.6, 23.2, 23.0, 15.5. HRMS *m/z*: $[M - H]^-$ 435.2173 (calcd 435.2173).

*14-Deoxy-11,12-didehydro-15-(4-hydroxybenzylidene) andrographolide (**3d**).* Yield 50%, mp. 267–268 °C. IR

(ATR), ν (cm^{-1}): 3451 (ν_{O-H}), 3084 (ν_{Ar-H}), 2924–2849 (ν_{C-H}), 1740 ($\nu_{C=O}$), 1607 ($\nu_{C=C}$), 1173 ($\nu_{C-Oester}$), 903 (ν_{Ar-H}). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.67 (*s*, 1H, H-14), 7.61 (*d*, 2H, $J = 8.5$ Hz, Ar–H-2′, Ar–H-6′), 6.82 (*d*, 2H, $J = 9.0$ Hz, Ar–H-3′, Ar–H-5′), 6.78 (*dd*, 1H, $J = 15.5$, 10.0 Hz, H-11), 6.24 (*d*, 1H, $J = 16.0$ Hz, H-12), 6.23 (*s*, 1H, H-21), 5.03 (*d*, 1H, $J = 5.0$ Hz, OH-3), 4.75 (*s*, 1H, H-17a), 4.44 (*s*, 1H, H-17b), 4.14 (*dd*, 1H, $J = 7.5$, 3.0 Hz, OH-19), 3.85 (*dd*, 1H, $J = 10.5$, 2.5 Hz, H-19a), 2.42 (*d*, 1H, $J = 10.5$ Hz, H-9), 1.10 (*s*, 3H, H-20), 0.78 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 168.7, 158.5, 148.9, 145.4, 137.2, 135.8, 132.2, 124.44, 124.37, 121.4, 116.0, 113.6, 108.1, 78.6, 62.6, 60.8, 53.7, 42.4, 38.4, 38.0, 36.2, 27.6, 23.1, 23.0, 15.4. HRMS *m/z*: $[M-H]^-$ 435.2067 (calcd 435.2173).

14-Deoxy-11,12-didehydro-15-(3,4-methylenedioxybenzylidene)andrographolide (***3e***). Yield 60%, mp. 202–203 °C. IR (ATR), ν (cm^{-1}): 3402 (ν_{O-H}), 2943–2845 (ν_{C-H}), 1732 ($\nu_{C=O}$), 1699 ($\nu_{C=C}$), 1263 ($\nu_{C-Oester}$), 1082 (ν_{C-O}), 804 (ν_{Ar-H}). ^{1}H-NMR (500 MHz, MeOD-d_4, δ ppm): 7.46 (*s*, 1H, H-14), 7.14 (*d*, 1H, $J = 8.0$ Hz, Ar–H-6′), 7.08 (s, 1H, Ar-H-2′), 6.88 (dd, 1H, $J = 16.0$, 10.0 Hz, H-11), 6.82 (d, 1H, $J = 8.0$ Hz, Ar-H-5′), 6.19 (d, 1H, $J = 16.0$ Hz, H-12), 6.01 (s, 2H, O–CH_2-O), 5.88 (*s*, 1H, H-21), 4.80 (*s*, 1H, H-17a), 4.54 (*s*, 1H, H-17b), 4.22 (*d*, 1H, $J = 11.0$ Hz, H-19a), 3.49 (*dd*, 1H, $J = 11.5$, 4.5 Hz, H-3), 3.36 (*d*, 1H, $J = 11.0$ Hz, H-19b), 1.27 (*s*, 1H, H-20), 0.84 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 169.0, 149.4, 148.5, 148.3, 146.7, 137.6, 136.9, 128.0, 126.2, 125.7, 121.9, 113.5, 109.6, 109.4, 108.7, 102.1, 79.1, 63.2, 61.3, 54.2, 42.9, 39.0, 38.5, 36.7, 28.1, 23.7, 23.5, 16.0. HRMS *m/z*: $[M+Na]^+$ 487.1732 (calcd 487.2097).

14-Deoxy-11,12-didehydro-15-(3-hydroxy-4-methoxybenzylidene)andrographolide (***3f***). Yield 52%, mp. 159–160 °C. IR (ATR), ν (cm^{-1}): 3362 (ν_{O-H}), 3080 (ν_{Ar-H}), 2918–2849 (ν_{C-H}), 1740 ($\nu_{C=O}$), 1609 ($\nu_{C=C}$), 1258 ($\nu_{C-Oester}$), 1130 (ν_{C-O}), 885 (ν_{Ar-H}). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 9.28 (*s*, 1H, Ar–OH), 7.66 (*s*, 1H, H-14), 7.33 (*d*, 1H, $J = 2.0$ Hz, H-2′), 7.11 (*dd*, 1H, $J = 8.5$, 2.0 Hz, H-6′), 6.98 (*d*, 1H, $J = 8.5$ Hz, H-5′), 6.78 (*dd*, 1H, $J = 16.0$, 10.0 Hz, H-11), 6.25 (*d*, 1H, $J = 16.0$ Hz, H-12), 6.19 (*s*, 1H, H-21), 5.03 (*d*, 1H, $J = 5.0$ Hz, OH-3), 4.75 (*s*, 1H, H-17a), 4.44 (*s*, 1H, H-17b), 4.14 (*dd*, 1H, $J = 7.5$, 3.0 Hz, OH-19), 3.85 (*d*, 1H, $J = 11.0$ Hz, H-19a), 3.81 (*s*, 3H, Ar–OCH_3), 3.28–3.22 (*m*, 2H, H-19b, H-3), 1.10 (*s*, 3H, H-20), 0.78 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 168.7, 148.9, 148.8, 146.6, 145.8, 137.2, 136.0, 126.2, 124.6, 123.1, 121.4, 116.4, 113.6, 112.2, 108.1, 78.6, 62.6, 60.8, 55.5, 53.7, 42.4, 38.4, 38.0, 36.2, 27.6, 23.1, 23.0, 15.4. HRMS *m/z*: $[M-H]^-$ 465.2138 (calcd 465.2279), $[M+H]^+$ 467.1835 (calcd 467.2435), $[M+Na]^+$ 489.2098 (calcd 489.2255), $[M]^-$ 466.2150 (calcd 466.2357).

14-Deoxy-11,12-didehydro-15-(3,4-dimethoxybenzylidene)andrographolide (***3g***). Yield 48%, mp. 133–134 °C. IR (ATR), ν (cm^{-1}): 3377 (ν_{O-H}), 2930–2849 (ν_{C-H}), 1746 ($\nu_{C=O}$), 1643 ($\nu_{C=C}$), 1263 ($\nu_{C-Oester}$), 1022 (ν_{C-O}), 887 (ν_{Ar-H}). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.71 (*s*, 1H, H-14), 7.36 (*m*, 2H, Ar–H-2′, Ar-H-6′), 7.05 (*d*, 1H, $J = 9.0$ Hz, Ar–H-5′), 6.79 (*dd*, 1H, $J = 16.0$, 10.0 Hz, H-11), 6.27 (*s*, 1H, H-21), 6.24 (*d*, 1H, $J = 15.5$ Hz, H-12), 5.04 (*d*, 1H, $J = 5.0$ Hz, OH-3), 4.75 (*s*, 1H, H-17a), 4.45 (*s*, 1H, H-17b), 4.14 (*dd*, 1H, $J = 7.5$, 2.5 Hz, OH-19), 3.85 (*d*, 1H, $J = 11.0$ Hz, H-19a), 3.81 (*s*, 3H, Ar–OCH_3), 3.79 (*s*, 3H, Ar–OCH_3), 3.22 (*m*, 2H, H-3, H-19b), 2.42 (*m*, 2H, H-9, H-7a), 1.10 (*s*, 3H, H-20), 0.79 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 168.7, 149.8, 148.9, 148.7, 146.1, 137.1, 136.2, 126.1, 124.9, 124.1, 121.4, 113.4, 113.3, 112.0, 108.2, 78.6, 62.7, 60.8, 55.6, 55.5, 53.7, 42.4, 38.5, 38.0, 36.2, 27.6, 23.1, 23.0, 15.5. HRMS *m/z*: $[M-H]^-$ 479.2401 (calcd 479.2435), $[M+Na]^+$ 503.2707 (calcd 503.2411).

14-Deoxy-11,12-didehydro-15-(4-ethoxybenzylidene)andrographolide (***3h***). A mixture of *Z* and *E* isomers (2:1), yield 53%, mp. 200–201 °C. IR (ATR), ν (cm^{-1}): 3281 (ν_{O-H}), 3076 (ν_{Ar-H}), 2972–2849 (ν_{C-H}), 1744 ($\nu_{C=O}$), 1603 ($\nu_{C=C}$), 1248 ($\nu_{C-Oester}$), 1034 (ν_{C-O}), 829 (ν_{Ar-H}). ^{1}H-NMR (500 MHz, MeOD-d_4, δ ppm): 7.91 (*s*, 1H, H-14), 7.82 (*s*, 0.5H, H-14), 7.74 (*d*, 2H, $J = 8.5$ Hz, Ar–H-2′, Ar–H-6′), 7.45 (*d*, 1H, $J = 9.0$ Hz, Ar–H-2′, Ar–H-6′), 7.03 (*dd*, 0.5H, $J = 16.0$, 10.0 Hz, H-11), 6.96 (*d*, 3H, $J = 8.5$ Hz, Ar–H-3′, Ar–H-5′), 6.92 (*dd*, 1H, $J = 16.0$, 10.0 Hz, H-11), 6.71 (*s*, 0.5H, H-21), 6.32 (*d*, 0.5H, $J = 16.0$ Hz, H-12), 6.27 (*d*, 1H, $J = 15.5$ Hz, H-12), 6.15 (*s*, 1H, H-21), 4.79 (*s*, 1.5H, H-17a), 4.54 (*s*, 1.5H, H-17b), 4.16 (*d*, 1,5H, $J = 11.0$ Hz, H-3), 4.11 (*m*, 3H, Ar–O–CH_2CH_3), 3.41 (*d*, 3H, $J = 11.5$ Hz, H-19a, H-19b), 1.42 (*t*, 4.5H, $J = 7.0$ Hz, Ar–OCH_2–CH_3), 1.25 (*s*, 4.5H, H-20), 0.89 (*s*, 4.5H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 168.6, 159.1, 148.8, 145.9, 137.1, 136.1, 131.9, 130.9, 124.8, 121.4, 115.0, 113.0, 108.1, 78.6, 63.2, 62.6, 60.8, 53.7, 42.4, 38.5, 38.0, 36.2, 27.6, 23.1, 23.0, 15.4, 14.5. HRMS *m/z*: $[M+Na]^+$ 487.2563 (calcd 487.2462), $[M+K]^+$ 503.2048 (calcd 503.2048).

14-Deoxy-11,12-didehydro-15-(4-methylcyclohexylidene)andrographolide (***3i***). Yield 55%, mp. 175–176 °C. IR (ATR), ν (cm^{-1}): 3285 (ν_{O-H}), 2926–2851 (ν_{C-H}), 1742 ($\nu_{C=O}$), 1643 ($\nu_{C=C}$), 1259 ($\nu_{C-Oester}$). ^{1}H-NMR (500 MHz, MeOD-d_4, δ ppm): 7.67 (*s*, 1H, H-14), 6.88 (*dd*, 1H, $J = 15.5$, 10.0 Hz, H-11), 6.22 (*d*, 1H, $J = 16.0$ Hz, H-12), 4.78 (*s*, 1H, H-17a), 4.53 (*s*, 1H, H-17b), 4.15 (*d*, 1H, $J = 11.0$ Hz, H-3), 3.40 (*d*, 2H, $J = 11.0$ Hz, H-19a,b), 3.03 (*d*, 1H, $J = 14.0$ Hz, cyclo-H), 2.76 (*d*, 1H, $J = 14.0$ Hz, cyclo-H), 2.47 (*d*, 1H, $J = 13.5$ Hz, H-7a), 2.42 (*d*, 1H, $J = 10.0$ Hz, H-9), 2.18 (*td*, 1H, $J = 13.5$, 3.0 Hz, cyclo-H), 2.10 (*td*, 2H, $J = 13.0$, 3.0 Hz, cyclo-H), 1.94 (*t*, 2H, $J =$

15.0 Hz, cyclo-H), 1.71 (*m*, 2H, cyclo-H), 1.25 (*s*, 3H, H-20), 0.97 (*d*, 3H, $J = 6.5$ Hz, cyclo-CH_3), 0.87 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 168.7, 148.9, 142.1, 135.7, 132.2, 129.9, 126.0, 121.3, 108.1, 89.1, 78.6, 62.6, 60.7, 53.7, 42.4, 38.4, 37.9, 36.2, 35.6, 34.9, 34.1, 31.5, 27.6, 23.1, 23.0, 21.5, 15.4. HRMS *m/z*: $[M + Na]^+$ 449.2558 (calcd 449.2670), $[M + K]^+$ 465.2137 (calcd 465.2409).

*14-Deoxy-11,12-didehydro-15-(2,4-dimethoxybenzylidene) andrographolide (**3j**)*. Yield 66%, mp. 144–145 °C. IR (ATR), ν (cm^{-1}): 3360 ($\nu_{O–H}$), 3076 ($\nu_{Ar–H}$), 2928– 2849 ($\nu_{C–H}$), 1744 ($\nu_{C=O}$), 1603 ($\nu_{C=C}$), 1267 ($\nu_{C–Oester}$), 1030 ($\nu_{C–O}$), 889 ($\nu_{Ar–H}$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.92 (*d*, 1H, $J = 8.5$ Hz, Ar–H-6′), 7.74 (*s*, 1H, H-14), 6.77 (*dd*, 1H, $J = 16.0$, 10.0 Hz, H-11), 6.68 (*dd*, 1H, $J = 9.0$, 2.5 Hz, Ar–H-5′), 6.63 (*m*, 1H, Ar–H-3′), 6.48 (*s*, 1H, H-21), 6.23 (*d*, 1H, $J = 16.0$ Hz, H-12), 5.04 (*d*, 1H, $J = 5.0$ Hz, OH-3), 4.75 (*s*, 1H, H-17a), 4.44 (*s*, 1H, H-17b), 4.14 (*dd*, 1H, $J = 7.5$, 2.5 Hz, OH-19), 3.86 (*s*, 3H, Ar–OCH_3), 3.83 (*d*, 1H, $J = 10.0$ Hz, H-19a), 3.82 (*s*, 3H, Ar–OCH_3), 2.40 (*m*, 2H, H-9, H-7a), 1.10 (*s*, 3H, H-20), 0.79 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 168.7, 161.6, 158.7, 148.9, 145.9, 137.5, 131.6, 130.5, 128.3, 124.3, 121.5, 114.7, 108.1, 106.7, 98.2, 79.1, 78.6, 62.7, 55.8, 55.4, 53.7, 42.4, 38.5, 38.0, 36.2, 27.6, 23.1, 23.0, 15.5. HRMS *m/z*: $[M - H]^-$ 479.2358 (calcd 479.2435), $[M + Na]^+$ 503.2497 (calcd 503.2411).

*3,19-Diacetyl-15-(4-clorobenzylidene)-14-deoxy-11,12-didehydroandrographolide (**5**)*. Yield 78%, mp. 158–159 °C. IR (ATR), ν (cm^{-1}): ν (cm^{-1}): 3082 ($\nu_{O–H}$), 2922–2853 ($\nu_{C–H}$), 1734 ($\nu_{C=O}$), 1674 ($\nu_{C=C}$), 1236 ($\nu_{C–Oester}$), 841 ($\nu_{Ar–H}$), 770 ($\nu_{C–Cl}$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.77 (*d*, 2H, $J = 8.5$ Hz, Ar–H-2′, Ar–H-6′), 7.76 (*s*, 1H, H-14), 7.51 (*d*, 2H, $J = 8.5$ Hz, Ar–H-3′, Ar–H-5′), 6.85 (*dd*, 1H, $J = 16.0$, 10.0 Hz, H-11), 6.35 (*s*, 1H, H-21), 6.31 (*d*, 1H, $J = 16.0$ Hz, H-12), 4.79 (*s*, 1H, H-17a), 4.56 (*dd*, 1H, $J = 12.0$, 4.5 Hz, H-3), 4.48 (*s*, 1H, H-17b), 4.36 (*d*, 1H, $J = 11.5$ Hz, H-19a), 4.03 (*d*, 1H, $J = 12.0$ Hz, H-19b), 2.01 (*s*, 3H, $OCOCH_3$), 1.99 (*s*, 3H, $OCOCH_3$), 0.98 (*s*, 3H, H-20), 0.83 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 170.2, 169.8, 168.3, 148.3, 147.8, 137.1, 136.7, 133.3, 132.2, 131.7, 130.9, 129.0, 126.4, 121.6, 111.4, 108.5, 79.1, 63.7, 60.2, 53.2, 41.0, 38.4, 37.5, 36.0, 23.7, 23.3, 22.2, 20.8, 14.9. HRMS *m/z*: $[M - H]^-$ 537.1948 (calcd 537.2045), $[M + Na]^+$ 561.2007 (calcd 561.2021).

*Procedure for preparation of 3,19-diacetyl-14-deoxy-11,12-didehydroandrographolide (**4**)*. To A stirred suspension of zinc chloride (150 mg) in acetic anhydride (20 mL, 0.21 mol) was added 14-deoxy-11,12-didehydroandrographolide (3.32 g, 10 mmol) at 0 °C in 6 h. After the completion of reaction by TLC analysis, the reaction mixture was quenched with cool water (50 mL). To the strongly stirred aqueous solution was slowly added sodium bicarbonate until no longer effervescence and solution pH reached 7–8. The precipitate formed was filtered and rinsed with 20 mL cool ethanol 96% solution. The crude product was purified by silica chromatography using an eluent petroleum ether–chloroform–acetone = 4:5:1 to afford the pure product **4**. Yield 93%, mp. 136–137 °C. IR (ATR), ν (cm^{-1}): 2986–2934 ($\nu_{C–H}$), 1748–1726 ($\nu_{C=O}$), 1643 ($\nu_{C=C}$), 1246 ($\nu_{C–Oester}$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.67 (*s*, 1H, H-14), 6.76 (*dd*, 1H, $J = 16.0$, 10.0 Hz, H-11), 6.16 (*d*, 1H, $J = 16.0$ Hz, H-12), 4.89 (*s*, 2H, H-15), 4.77 (*s*, 1H, H-17a), 4.53 (*dd*, 1H, $J = 12.0$, 4.0 Hz, H-3), 4.46 (*s*, 1H, H-17b), 4.35 (*d*, 1H, $J = 11.5$ Hz, H-19a), 4.02 (*d*, 1H, $J = 12.0$ Hz, H-19b), 2.01 (*s*, 3H, $OCOCH_3$), 1.99 (*s*, 3H, $OCOCH_3$), 1.09 (*s*, 3H, H-20), 0.76 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 172.4, 170.2, 169.8, 148.4, 146.9, 133.7, 127.1, 121.5, 108.3, 79.2, 70.2, 63.7, 60.0, 53.2, 41.0, 38.1, 37.5, 36.0, 23.7, 23.3, 22.2, 20.8, 20.7, 14.8. HRMS *m/z*: $[M - H]^-$ 415.2077 (calcd 415.2122), $[M + Na]^+$ 439.2080 (calcd 439.2098), $[M + K]^+$ 455.1846 (calcd 455.1837).

*General procedure for preparation of 3,19-isopropylideneandrographolide (**6a–6c**) and 14-deoxy-11,12-didehydro-3,19-(2′-hydroxybenzylidene)-15-(2-hydroxybenzylidene) andrographolide (**7**)*. In 100 mL round bottom flask, andrographolide (350 mg, 1 mmol) was dispersed into a solution of chloroform (20 mL) and a benzaldehyde derivative (5 mmol). To the stirred suspension was added pyridinium chloride at 40– 50 °C for 1–3 h. After the completion of reaction by TLC analysis, the solution was dried by sufficient Na_2SO_4 and filtered. The solvent was removed to obtain a crude product which was then purified by silica chromatography using an eluent chloroform–acetone = 5:1.

*3,19-(3-Hydroxy-4-methoxybenzylidene) andrographolide (**6a**)*. Yield 66%, mp. 239–240 °C. IR (ATR), ν (cm^{-1}): 3402 ($\nu_{O–H}$), 3048 ($\nu_{Ar–H}$), 2967–2837 ($\nu_{C–H}$), 1717($\nu_{C=O}$), 1661 ($\nu_{C=C}$), 1279–1231 ($\nu_{C–Oester}$), 1026 ($\nu_{C–O}$), 795 ($\nu_{Ar–H}$). ^{1}H-NMR (500 MHz, MeOD-d_4, δ ppm): 6.97 (*s*, 1H, Ar–H-2′), 6.91 (*m*, 2H, Ar–H-5′, Ar–H-6′), 6.90 (*m*, 1H, H-12), 5.75 (*s*, 1H, H-21), 5.05 (*d*, 1H, $J = 6.0$ Hz, H-14), 4.94 (*s*, 1H, H-17a), 4.73 (*s*, 1H, H-17b), 4.50 (*dd*, 1H, $J = 10.0$, 6.0 Hz, H-15a), 4.34 (*d*, $J = 11.5$, H-19a), 4.19 (*dd*, 1H, $J = 10.0$, 1.5 Hz, H-15b), 3.86 (*s*, 3H, C4′–OCH_3), 3.66 (*dd*, $J = 13.0$, 5.0 Hz, H-3), 3.58 (*d*, 1H, $J = 11.5$ Hz, H-19b), 1.48 (*s*, 3H, H-20), 0.93 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 169.9, 147.7, 147.2, 146.1, 146.0, 132.2, 129.0, 117.2, 113.6, 111.4, 108.7, 94.2, 79.7, 74.3, 68.3, 64.5, 55.6, 55.1, 53.6, 38.4, 37.0, 36.3, 35.2, 25.5, 23.9, 22.3, 21.5, 14.8. HRMS *m/z*: $[M - H]^-$ 483.2166 (calcd 483.2383).

*3,19-(4-Dimethylaminobenzylidene) andrographolide (**6b**)*. Yield 52%, mp. 201–202 °C. IR (ATR), ν (cm^{-1}): 3146 ($\nu_{O–H}$), 2978–2849 ($\nu_{C–H}$), 1724 ($\nu_{C=O}$), 1668 ($\nu_{C=C}$), 1223 ($\nu_{C–Oester}$), 1198 ($\nu_{C–N}$), 1026 ($\nu_{C–O}$), 810 ($\nu_{Ar–H}$). ^{1}H-NMR (500 MHz, MeOD-d_4, δ ppm): 7.32 (*d*, 2H, $J =$ 9.0 Hz, Ar–H-2′, Ar–H-6′), 6.89 (*t*, 1H, $J = 6.5$ Hz, H-12),

6.76 (*d*, 2H, $J = 9.0$ Hz, Ar–H-3′, Ar–H-5′), 5.77 (*s*, 1H, H-21), 5.05 (*d*, 1H, $J = 6.0$ Hz, H-14), 4.94 (*s*, 1H, H-17a), 4.73 (*s*, 1H, H-17b), 4.50 (*dd*, 1H, $J = 6.0$, 10.0 Hz, H-15a), 4.19 (*dd*, 1H, $J = 2.0$, 6.0 Hz, H-15b), 4.34 (*d*, 1H, $J =$ 11.5 Hz, H-19a), 3.66 (*dd*, 1H, $J = 4.5$, 12.5 Hz, H-3), 3.56 (*d*, 1H, $J = 11.5$ Hz, H-19b), 2.95 (*s*, 6H, $-N(CH_3)_2$), 1.49 (*s*, 3H, H-20), 0.93 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 169.9, 150.5, 147.3, 146.1, 129.0, 127.3, 127.0, 111.4, 108.7, 94.6, 79.7, 74.3, 68.3, 64.5, 55.1, 53.6, 38.4, 37.0, 36.3, 35.2, 25.6, 23.9, 22.4, 21.5, 14.8. HRMS *m/z*: $[M - H]^-$ 480.2685 (calcd 480.2750), $[M + H]^+$ 482.2917 (calcd 482.2907), $[M + Na]^+$ 504.2458 (calcd 504.2726), $[M + K]^+$ 520.2520 (calcd 520.2466).

*3,19-(2,4-Dimethoxybenzylidene) andrographolide (**6c**).* Yield 50%, mp. 204–205 °C. IR (ATR), ν (cm^{-1}): 3414 (ν_{O-H}), 2959–2920 (ν_{C-H}), 1759 ($\nu_{C=O}$), 1676 ($\nu_{C=C}$), 1207 ($\nu_{C-Oester}$), 1121 (ν_{C-O}), 793 (ν_{Ar-H}). ^{1}H-NMR (500 MHz, MeOD-d_4, δ ppm): 7.49 (*d*, 1H, $J = 8.5$ Hz, Ar–H-6′), 6.89 (*td*, 1H, $J = 6.5$, 1.5 Hz, H-12), 6.54 (*m*, 2H, Ar–H-3′, Ar–H-5′), 6.13 (*s*, 1H, H-21), 5.05 (*d*, 1H, $J = 6.0$ Hz, H-14), 4.94 (*s*, 1H, H-17a), 4.73 (*s*, 1H, H-17b), 4.50 (*dd*, 1H, $J = 10.0$, 6.0 Hz, H-15), 4.33 (*d*, 1H, $J = 11.0$ Hz, H-19a), 4.19 (*dd*, 1H, $J = 10.0$, 2.0 Hz, H-15), 3.83 (*s*, 3H, C-2′–OCH_3)–3.81 (*s*, 3H, C-4′–OCH_3), 3.65 (*dd*, 1H, $J =$ 12.5, 4.5 Hz, H-3), 3.56 (*d*, 1H, $J = 11.0$ Hz, H-19b), 1.49 (*s*, 3H, H-20), 0.93 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 169.9, 160.6, 157.3, 147.2, 146.1, 129.0, 128.0, 119.9, 108.7, 104.7, 97.9, 89.3, 79.8, 74.3, 68.6, 64.5, 55.5, 55.2, 55.1, 53.6, 38.4, 37.0, 36.3, 35.2, 25.6, 23.9, 22.3, 21.4, 14.9. HRMS *m/z*: $[M + Na]^+$ 521.2508 (calcd 521.2516).

*14-deoxy-11,12-didehydro-3,19-(2′-hydroxybenzylidene)-15-(2-hydroxybenzylidene) andrographolide (**7**).* A mixture of *Z* and *E* isomers (1:1), yield 78%, mp. 158–159 °C. IR (ATR), ν (cm^{-1}): 3354 (ν_{O-H}), 3080 (ν_{Ar-H}), 2918–2851 (ν_{C-H}), 1732 ($\nu_{C=O}$), 1645–1587 ($\nu_{C=C}$), 1254 ($\nu_{C-Oester}$) 1092–1065 (ν_{C-O}), 752 (ν_{Ar-H}). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 10.17 (s, 1H, Ar-OH), 10.05 (s, 1H, Ar-OH), 9.36 (s, 2H, Ar-2′-OH), 8.07 (s, 1H, H-14), 7.91 (dd, 1H, $J = 8.5$, 1.0 Hz, Ar-H-6), 7.81 (s, 1H, H-14), 7.47 (*d*, 1H, $J = 7.0$ Hz, Ar–H-6), 7.40 (*m*, 2H, Hz, Ar–H-6′), 7.19 (*m*, 2H, Ar–H-4′), 7.12 (*td*, 2H, $J = 8.0$, 1.5 Hz, Ar–H-5′), 6.91–6.85 (*m*, 5H, Ar–H-3, Ar–H-4, Ar–H-5, H-3′, H-21), 6.79–6.77 (*m*, 4H, Ar–H-4, Ar–H-3′, H-11), 6.58 (*s*, 1H, H-21), 6.29 (*d*, 1H, J = 15.5 Hz, H-12), 6.28 (*d*, 1H, $J = 15.5$ Hz, H-12), 6.05 (*s*, 2H, H-21′), 4.79 (*s*, 2H, H-17a), 4.50 (*s*, 2H, H-17b), 4.19 (*d*, 2H, $J = 11.0$ Hz, H-19a), 3.56 (*m*, 2H, H-3, H-19b), 1.40 (*s*, 6H, H-20), 0.93 (*s*, 6H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 168.8, 160.7, 156.1, 148.5, 147.9, 146.8, 137.7, 136.4, 132.3, 130.3, 129.7, 128.7, 125.3, 121.7, 120.3, 119.6, 117.2, 115.6, 111.5, 110.7, 108.9, 107.4, 89.9, 80.1, 68.7, 60.6, 52.9, 38.4, 36.5, 29.8, 25.5, 21.6, 21.3, 15.7. HRMS *m/z*: $[M - H]^-$ 539.2268 (calcd 539.2435), $[M + H]^+$ 579.2061 (calcd 579.2151), $[M + Na]^+$ 563.2195 (calcd 563.2411).

*Procedure for preparation of 3,19-diacetyl-14-deoxy-11,12-didehydro-8,17-epoxyandrographolide (**8**).* In an erlenmeyer flask, compound **4** (416 mg, 1 mmol) was dissolved into a solution of *m*-CPBA (525 mg, 3 mmol) in $CHCl_3$ (10 mL) at room temperature. The reaction mixture was stirred at 10–15 °C for 48 h. After the completion of reaction by TLC analysis, the reaction was quenched by adding of Na_2SO_3 and stirring for 10–15 min to completely reduce the remaining *m*-CBPA. The solution was then filtered and rinsed with $NaHCO_3$ 10% solution (3×20 mL). A crude product was obtained by removing the organic solvent and then purified by silica chromatography using an eluent petroleum ether–chloroform–acetone = 4:5:1 to afford the pure product **8**. Yield 41%, mp. 161–162 °C. IR (ATR), ν (cm^{-1}): 2920–2851 (ν_{C-H}), 1753–1726 ($\nu_{C=O}$), 1244 ($\nu_{C-Oester}$), 1034 (ν_{C-O}), 752 (ν_{Ar-H}). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 7.65 (*s*, 1H, H-14), 6.33 (*dd*, 1H, $J = 15.5$, 10.0 Hz, H-11), 6.11 (*d*, 1H, $J = 15.5$ Hz, H-12), 4.87 (*d*, 2H, $J = 1.5$ Hz, H-15), 4.53 (*dd*, 1H, $J =$ 12.0, 4.5 Hz, H-3), 4.36 (*d*, 1H, $J = 12.0$ Hz, H-19a), 4.08 (*d*, 1H, $J = 11.5$ Hz, H-19b), 2.54 (*d*, 1H, $J = 4.5$ Hz, H-17a), 2.52 (*d*, 1H, $J = 4.0$ Hz, H-17b), 2.02 (*s*, 3H, $OCOCH_3$), 2.00 (*s*, 3H, $OCOCH_3$), 0.98 (*s*, 3H, H-20), 0.94 (*s*, 3H, H-18). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 172.3, 170.2, 169.8, 146.9, 129.9, 127.0, 123.6, 79.1, 70.2, 63.7, 57.9, 57.7, 52.7, 49.5, 40.9, 38.5, 37.2, 35.2, 23.3, 23.2, 21.5, 20.8, 20.7, 14.9. HRMS *m/z*: $[M - H]^-$ 493.2382 (calcd 493.1994), $[M + Na]^+$ 477.2200 (calcd 477.2047).

Biological assay

General procedure for α-glucosidase inhibition assay

p-Nitrophenyl-*α*-D-glucopyranoside (*p*-NPG), the substrate, and *α*-glucosidase (*Saccharomyces cerevisiae*) were purchased from Sigma-Aldrich.

The inhibition assays were evaluated at 37 °C in K_2HPO_4/KH_2PO_4 buffer with pH = 6.8. The reaction mixture comprised of an enzyme 0.2 U/mL solution (40 µL), inhibitors or acarbose solution with various concentrations (50 µL) and *p*-NPG 1 mM solution (40 µL). Acarbose was tested as a positive control. Inhibitors were dissolved into aqueous DMSO 10% solution, whereas both enzyme and substrate were dissolved into the buffer solution. After incubation of the enzyme solution in the presence of the inhibitor for 20 min at room temperature, the enzymatic reaction was started by the addition of a substrate (*p*-NPG). The mixture was incubated at room temperature for 20 min, then the reaction was quenched by the addition of Na_2CO_3 0.2 M solution (130 µL). The absorbance at 405 nm

($A_{inhibitor}$) was measured immediately and taken as relative inhibition capacity for an inhibitor. All the experiment was carried out with blank sample (without an inhibitor) to obtain A_{blank} in triplicate.

The inhibition percentage was calculated by the following equation:

$$\%_{Inhibition} = [(A_{blank} - A_{inhibitor})/A_{blank}] \times 100.$$

Data availability

Copies of HRMS, IR, ^{1}H-NMR, and ^{13}C-NMR spectra of **6c** and **7** are available online.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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