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Studies on the novel α-glucosidase inhibitory activity and structure–activity relationships for andrographolide analogues

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Abstract—A series of analogues of andrographolide were synthesized and evaluated as novel α-glucosidase inhibitors. Among them compound **23**, 15-*p*-methoxylbenzylidene 14-deoxy-11,12-didehydroandrographolide, was a potent inhibitor against α-glucosidase whose IC_{50} value was 16 μM. The structure–activity relationships were also discussed.

Glycosidase inhibitors are of particular interest in the development of potential pharmaceuticals such as antitumour,[1–3] antiviral,[4,5] antidiabetics,[6–9] immunoregulatory agents[10] and so forth. The plant, *Andrographis paniculata*,[11,12] is extensively used in the traditional medicines of Chinese, Indian and other Asian countries.[13,14] Extracts of the plant and their isolated constituents are reported possessing a wide spectrum of biological activities including antibacterial,[15,16] antiinflammatory,[17,18] antimalarial, [19,20] immunological,[21,22] hepatoprotective[23] and anticancer[24] properties. In recent years, besides the above bioactivities, the antidiabetic effect of the plant has attracted researchers' attention.[25–29]

The ethanolic extract of *A. paniculata* exhibited antidiabetic property.[26,27] Blood glucose was significantly reduced by 52.9% when hyperglycaemic rats were treated with 50 mg /kg body weight aqueous extract of *A. paniculata*.[28] Therefore, it is very interesting to investigate the inhibitory activities of constituents from *A. paniculata* against glucosidase.

The plant extracts are known containing diterpenes, flavonoids and stigmasterols.[29] Diterpenes from *A. paniculata*, like andrographolide **1**, contain three hydroxyls, an α-alkylidene γ-butyrolactone moiety and two fused six-membered rings. Both the six-membered rings adopt the chair conformation, whereas the five-membered ring is in an envelope conformation.[30] They were structurally similar to some known glycosidase inhibitors to some extent.[31,32] However, there is not any report about activities of the extracts or constituents of *A. paniculata* against glucosidase.

In this study, the α-glucosidase inhibitory activities of various andrographolide analougues were first reported. The structure–activity relationship was also investigated, which would be helpful to screen the bioactive constituents from the plant, design and synthesize novel stronger α-glucosidase inhibitors and explore the molecular mechanisms of extracts from *A. paniculata* as drugs, especially as antidiabetic agents.

Compounds **2**, **3**, **4** and **5** were prepared from andrographolide by condensation reaction with different carbonyl compounds, respectively (Scheme 1).[33,34] Bioactivity evaluation showed that compound **5** has an inhibition of 20.1% at 100 μM against α-glucosidase.[35,36] But no significant activities were observed among compounds **2**–**4**, nor did the mother compound (**1**) (Table 1). That means increasing the lipophilicity of andrographolide by the protection of 3,19-hydroxyls with aliphatic aldehydes with suitable carbochain length is favourable to the inhibitory activity against α-glucosidase. Compounds **6**–**12** were synthesized from andrographolide derivatives and aromatic aldehydes. According to the biological activity evaluation results, all the above aromatic derivatives of andrographolide but **9** showed inhibitory activities against α-glucosidase. The IC_{50} values of **6** and **7** are nearly equal and higher than those of **8**–**12** (Scheme 1, Table 1).

A natural compound from *A. paniculata*, 14-deoxy-11,12-didehydroandrographolide (**13**)[37] which was

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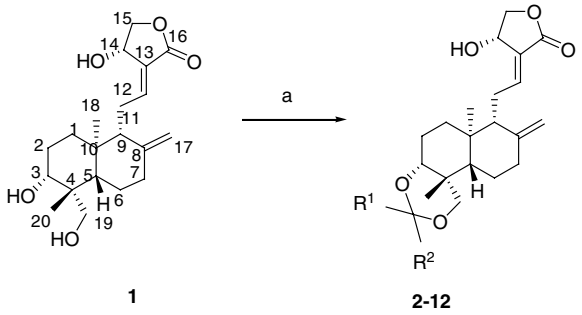


Scheme 1. Reagents and conditions: (a) H_2SO_4(0.1 N), THF, refluxing, 1–20 h, yield > 95%. **2**: $R^1 = R^2 = H$; **3**: $R^1 = R^2 = CH_3$; **4**: $R^1 = H$; $R^2 = (CH_2)_3CH_3$; **5**: $R^1 = H$; $R^2 = (CH_2)_5CH_3$; **6**: $R^1 = H$; $R^2 = C_6H_5$; **7**: $R^1 = H$; $R^2 = 3\text{-Br-}C_6H_4$; **8**: $R^1 = H$; $R^2 = 4\text{-F-}C_6H_4$; **9**: $R^1 = H$; $R^2 = 4\text{-Cl-}C_6H_4$; **10**: $R^1 = H$; $R^2 = 2\text{-MeO}C_6H_4$; **11**: $R^1 = H$; $R^2 = 4\text{-MeO}C_6H_4$; **12**: $R^1 = H$; R^2 =2,4,5-$(MeO)_3C_6H_2$.

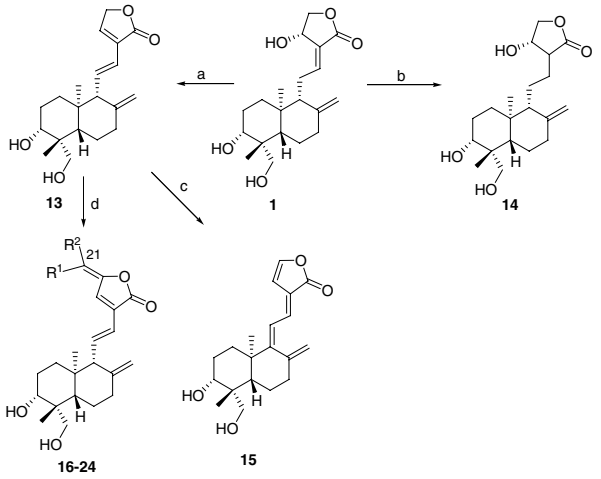
Table 1. Inhibitory activities of andrographolide analogues

Compound	α-Glucosidase	β-Glucosidase
1	ni[c]	ni
2	ni	ni
3	ni	ni
4	ni	ni
5	20.1[a]	ni
6	48.9[a] (101[b])	nd[d]
7	49.6[a] (100[b])	nd
8	26.2[a]	nd
9	ni	ni
10	5.5[a]	nd
11	13.2[a]	nd
12	14.5[a]	nd

[a] Inhibition(%) determined at 100 μM concentration of compound.
[b] IC_{50} (μM).
[c] No inhibition at 100 μM.
[d] Not determined.

synthesized from **1** also, inhibits α-glucosidase 16.5% at 100 μM (Scheme 2, Table 2). Reduction of C-12, 13 olefin bond of andrographolide **1** afforded **14**, which inhibits α-glucosidase 34.2%. But when the number of olefin bonds was increased as compound **15**, the α-glucosidase inhibitory activity was lost. The results indicated that the flexible chain between the γ-butyrolactone moiety and the two six-membered rings is critical to α-glucosidase inhibitory activity. In other words, the large conjugated system between the γ-butyrolactone moiety and the two six-membered rings is unfavourable to the inhibitory activity.

Compounds **16**–**24** are 15-ene-substituted derivatives of compound **13**. The biological activity results showed that increasing the carbochain of C15-substituting group to suitable length (**16**–**19**) could enhance the inhibitory activity against α-glucosidase. But different aromatic substitution derivatives gave inconsistent bioactivity results. 4-Fluoro and 4-chlorobenzylidene substitution (**20**–**21**) makes the inhibitory activity lost, while the activity is retained in 3-bromobenylidene derivative (**22**). The α-glucosidase inhibitory activities of the 4-methoxyphenylidene derivatives (**23**) and the



Scheme 2. Reagents and conditions: (a) pyridine, AL_2O_3, toluene, refluxing, 6 h, yield 90%; (b) $NiCl_2$, methanol, 0–5 °C, 0.5 h, yield 70%; (c) methanol, Et_3N, rt, 48 h, yield 10%; (d) methanol, Na_2CO_3, refluxing, 5–24 h, yield 50–90%. **16**: $R^1 = H$; $R^2 = CH_3$; **17**: $R^1 = CH_3$; $R^2 = CH_3$; **18**: $R^1 = H$; $R^2 = (CH_2)_2CH_3$; **19**: $R^1 = H$; $R^2 = CHC(CH_3)(CH_2)_2CHC(CH_3)_2$; **20**: $R^1 = H$; $R^2 = 4\text{-F-}C_6H_4$; **21**: $R^1 = H$; $R^2 = 4\text{-Cl-}C_6H_4$; **22**: $R^1 = H$; $R^2 = 3\text{-Br-}C_6H_4$; **23**: $R^1 = H$; $R^2 = 4\text{-MeO}C_6H$; **24**: $R^1 = H$; $R^2 = C_6H_5CHCH$.

Table 2. Inhibitory activities of analogues of andrographolide

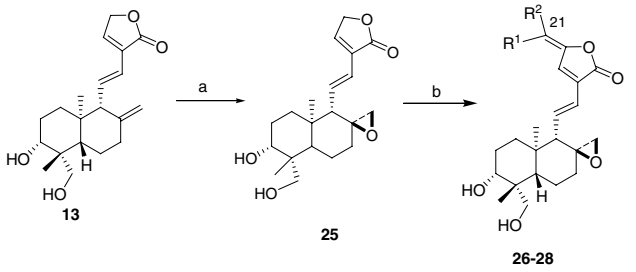
Compound	α-Glucosidase	β-Glucosidase
13	16.5[a]	ni[c]
14	34.2[a]	ni
15	ni	ni
16	15.1[a]	ni
17	17.1[a]	ni
18	43.5[a] (110[b])	ni
19	ni	nd[d]
20	ni	nd
21	ni	nd
22	16.7[a]	nd
23	100[a] (16[b])	nd
24	84.3[a] (58[b])	nd

[a] Inhibition (%)determined at 100 μM concentration of compound.
[b] IC_{50} (μM).
[c] No inhibition at 100 μM.
[d] Not determined.

phenylvinylidene derivative (**24**) were much stronger than that of compound **13**. Their IC_{50} values are 16 μM and 58 μM, respectively.

In order to examine the role of the C-8, C-17 epoxy moiety of the new derivatives in exhibiting the α-glucosidase inhibitory activity, compounds **25**–**28** were designed and synthesized. Both **25** and **26** lose the activity when the exocyclic double bond ($\triangle^{8,17}$) of **13** and **17** (another natural compound)[38] was epoxidized, respectively. Lower activities were observed in **27** and **28** derived by epoxidation of **23** and **24**, respectively (see Scheme 3, Table 3).

In summary, many derivatives of andrographolide exhibited good α-glucosidase inhibitory activities with inhibitory percentage ranging from 5.5% to 100% (**23**: IC_{50} = 16 μM) at 100 μM. But all the compounds



Scheme 3. Reagents and conditions: (a) m-CPBA, $CHCl_3$, refluxing, 2 h, and yield 95%; (b) methanol, Na_2CO_3, aldehyde, refluxing, yield 70–85%. **26**: $R^1 = CH_3$, $R^2 = CH_3$; **27**: $R^1 = H$, $R_2 = 4\text{-}MeOC_6H_4$; **28**: $R^1 = H$, $R^2 = CHCHC_6H_5$.

Table 3. Inhibitory activities of analogues of andrographolide

Compound	α-Glucosidase	β-Glucosidase
25	ni[c]	nd[d]
26	ni	nd
27	37.7[a](120[b])	nd
28	49.2[a] (101[b])	nd

[a] Inhibition(%) determined at 100 μM concentration of compound.
[b] IC_{50} (μM).
[c] No inhibition at 100 μM.
[d] Not determined.

determined for β-glucosidase inhibitory activities showed no inhibitory activities. The results for the structure–activity relationship studies showed that increasing the lipophilicity by protection of 3,19-hydroxyls of andrographolide endows compound **1** with α-glucosidase inhibitory activity. The number and position of olefin bonds between the γ-butyrolactone moiety and the six-membered rings were detrimental to the inhibition effect. In general, 15-ene-substituted derivatives of 14-deoxy-11,12,13,14-tetradehydroandrographolide significantly increased the α-glucosidase inhibitory activities. Epoxidation of the exocyclic double bond makes the inhibitory activity reduced or lost.

Bioactivity studies suggest that probably compounds **13** and **17** with α-glucosidase inhibitory activities may play an important role in the plant extracts exerting antidiabetic effect. Zhang's and Yu's results showed that ethanolic extract of *A. paniculata* or andrographolide can lower plasma glucose.[26,27,39] That means andrographolide without glucosidase inhibitory activity may also exert antidiabetic effect. According to He's result,[40] **13** is one of the andrographolide metabolites isolated from rat urine, faeces, and the contents of the small intestine. So it can be deduced that the andrographolide may exert antidiabetic effect through inhibiting glucosidase after being metabolized to **13**, an α-glucosidase inhibitor in vivo. In other words, α-glucosidase inhibitory activity was the reason or at least one of the reasons that the constituents of *A. paniculata* had antidiabetic effects. The promotion of the glucose metabolism was found when treating diabetic rat with the plant extract or andrographolide.[27,39] So, it can be deduced that extracts of *A. paniculata* and andrographolide lower plasma glucose by inhibiting the disaccharide metabolism and/or promoting the glucose metabolism.

Acknowledgment

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33. *General methods*. IR spectra were recorded as KBr pellets on a Thermo Nicolet (IR200) Spectrometer. Mass spectrometry experiment was performed on a Waters Q-Tof micro mass spectrometer. 1H and 13C spectra were recorded, respectively, at 400 and 100 Hz with a Brüker DPX-400 spectrometer with TMS as internal standard. Melting points were determined on a Beijing Keyi XT5 apparatus. The absorbance at 405 nm was measured by a PowerWaveX Microplate Scanning Spectrophotometer (BIO-TEK INSTRUMENTS, INC).
34. *Materials*. Compound **1** was extracted from *A. paniculata* Compounds **2**–**28** were developed from **1**. Compounds **4**–**12** and **15** are single isomers, but their configurations have not been confirmed yet. Compound **13** is (11*E*) isomer and its structure was confirmed by NMR which was in accordance with Ref. 33. The configuration of compound 14 was confirmed by X-ray crystallographic analysis; Compounds **16**, **18**–**24**, and **26**–**28** are (15*Z*) isomers, which are established from the correlation between H-14 and H-21 in their NOESY spectra. The configuration of epoxide ring in compounds **25**–**28** was confirmed by X-ray crystallographic analysis. The enzymes of α-glucosidase type I from baker's yeast and β-glucosidase from almonds, and the substrates of *p*-nitrophenyl α-D-glucopyranoside and β-D-glucopyranoside were purchased from Sigma Chemical Company. Other chemicals were purchased from native company.
35. *General procedure for α-glucosidase inhibition assay*. Inhibition rate was determined at 37 °C in 0.067 M K_2HPO_4/KH_2PO_4 buffer (pH 6.8). The reaction mixture contained 40 μl of enzyme solution, 40 μl of inhibitor and 20 μl of substrate. The enzymatic reaction was started after incubation of the enzyme (0.04 U/ml) for 30 min in the presence of the inhibitor (0.1 mM) by the addition of substrate(0.5 mM). The mixture was incubated at 37 °C for 5 min, and the reaction was quenched by the addition of 0.1 M Na_2CO_3 (pH 9.8). The absorption at 405 nm was measured immediately and taken as the relative rate for the hydrolysis of substrate. All experiments were carried out in triplicate. The IC_{50} value is the concentration of inhibitor at 50% of enzyme activity.
36. *General procedure for β-glucosidase inhibition assay*. Inhibition rate was determined at 37 °C in 0.08 M citric acid/Na_2HPO_4 buffer (pH 4.2). The enzymatic reaction was started after incubation of the enzyme (0.02 U/ml) for 30 min in the presence of the inhibitor (0.1 mM) by the addition of substrate (5 mM). The mixture was incubated at 37 °C for 5 min, and the reaction was quenched by the addition of 0.25 M borate buffer (pH 9.8). The absorption at 405 nm was measured immediately and taken as the relative rate for the hydrolysis of substrate.
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