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Synthesis and Biological Evaluation of Oleanolic Acid Derivatives-Chalcones Conjugates as α-Glucosidase Inhibitors

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Received (in XXX, XXX) Xth XXXXXXXXX 200X, Accepted Xth XXXXXXXXX 200X
First published on the web Xth XXXXXXXXX 200X
DOI: 10.1039/b000000x

α-Glucosidase is a promising target for treatment of obesity and diabetes mellitus. A series of oleanolic acid derivatives-chalcones conjugates were designed and synthesized as α-glucosidase inhibitors. Their structures were determined by spectroscopic analysis and their α-glucosidase inhibitory activities were investigated in *vitro*. Most of conjugates exhibited moderate inhibitory activity against α-glucosidase, among them, the conjugate 1b (IC_{50} = 3.2±0.2 μM) possessed the strongest α-glucosidase inhibitory activity, and the preliminary structure-activity relationships showed that the furan or thiophene rings in chalcone units of conjugates showed a tendency to enhance the activity. Lineweaver-Burk plots analysis demonstrated competitive inhibition of α-glucosidase activity by 1b, 6b, 5c and 4d, their inhibition constant (K_i) values were 16.6, 29.3, 14.6 and 20.6 μM, respectively. The interaction forces between conjugates and α-glucosidase were hydrogen bonds and van der Waals.

Introduction

Diabetes mellitus (DM) has been one of the most common and serious metabolic disease characterized by high blood-glucose levels and alterative in carbohydrate, protein and lipid metabolism.[1] Hyperglycemia and hyperlipidemia are involved in the development of microvascular and macrovascular complications of diabetes, which are the major causes of morbidity and mortality of diabetes.[2] To date, therapy for type 2 DM is to suppress the postprandial hyperglycemia by reducing the absorption of gut glucose via inhibition of carbohydrate-hydrolyzing enzymes.[3] α-Glucosidase, an enzyme catalyzing the cleavage of glycosidic bonds in oligosaccharides or glycoconjugates and for final step in the digestive process of carbohydrate. Therefore, the inhibition of α-glucosidase is a choice to control elevated glucose level in blood.[4]

Oleanolic acid (**OA**, Fig.**1**), a natural pentacyclic triterpenoid, which has been used as an anti-hepatitis drug in China for over 20 years,[5] exhibits various biological activities including anti-flammation, antitumor, anti-HIV, anti-oxidation activities.[6-8] In previous reports, oleanolic acid and its derivatives have been designed and synthesized to suppress the hyperglycemia as inhibitors of α-glucosidase,[9, 10] and some derivatives showed promising inhibitory activities(**1A**, **1B**, Figure **1**).[11, 12] Although some other pentacyclic triterpenoid compounds like ursolic acid and lupeol have similar structures with OA, ursolic acid displayed weak activity against rat intestinal α-glucosidase,[13] and lupeol derivatives also failed to inhibit α-glucosidase.[14] Therefore, oleanolic acid was used as lead compound. On the other hand, recent investigations have reported that some chalcones also possessed potential anti-diabetic activity.[15, 16] Therefore, on the basis of α-glucosidase inhibition activity of aforementioned OA as well as chalcones, we carried on further structural modifications on OA by incorporating different chalcone units that would allow us to find novel, more potent α-glucosidase inhibitors.

In this work, 26 analogues with oleanolic acid core and different chalcone ligands were synthesized, the α-glucosidase inhibitory activities of these compounds were evaluated and their structure-activity relationships also were discussed.

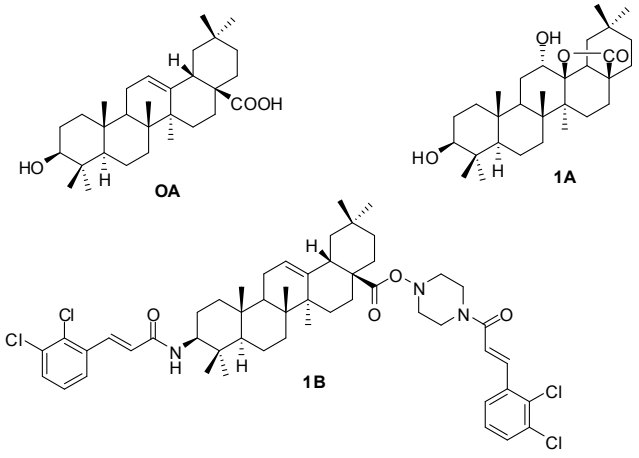


Fig.1. Currently referenced oleanolic acid derivatives as α-glucosidase inhibitors.

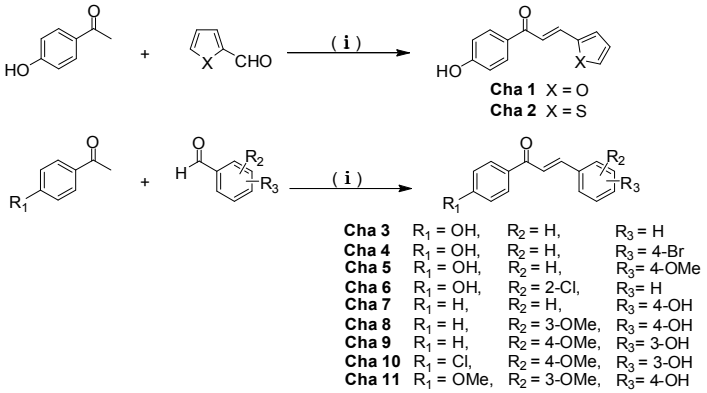
Result and discussion

Chemistry

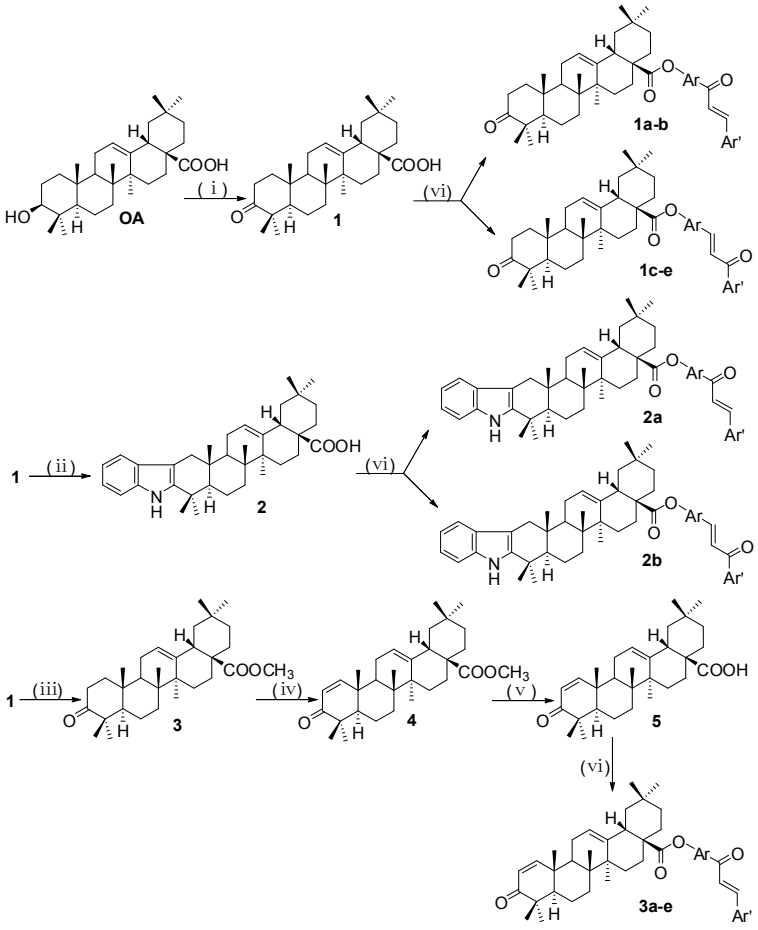
The conjugations (**1a-e**, **2a-b**, **3a-e**, **4a-f**, **5a-c** and **6a-e**) of oleanolic acid derivatives with chalcones were achieved by a well-known esterification procedure using standard EDC/ DMAP conditions (Scheme 1-3).[17] In the initial step, chalcones (**Cha1-11**) were synthesized by condensing the corresponding aldehyde with the corresponding acetophenone by Claisen-Schmidt condensation[18, 19](Scheme 1). Compound **1**, 3-Keto OA, was prepared by Jones oxidation of oleanolic acid in 94.9% yield.[20] Indole compound **2** was prepared by Fischer indolization of compound **1** with the phenylhydrazine in the presence of acetic acid (Scheme 2).[21] Refluxing compound **1** with CH_3I in THF in the presence of KOH generated compound **3**.[19] Thereafter, compound **3** was further treated with phenylselenenyl chloride in the presence of H_2O_2 to yield Methyl 3-oxo-olean-1,12-dien-28-oate **4**,[22] which subsequently was reacted with LiI in dry DMF to

give the target acid **5**.[20]

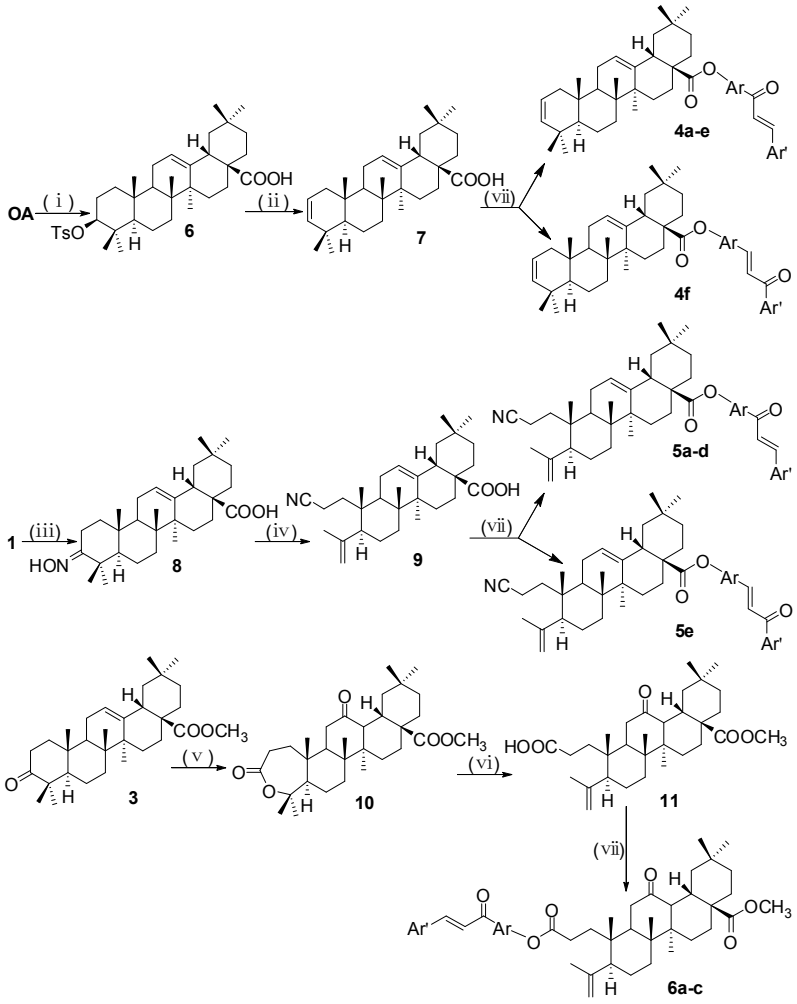
To prepare the compound **7**, we used oleanolic acid as the starting material, and it was deoxygenated via its 3-tosyl OA (Scheme 3). This 3-tosyl compound **6** was deoxygenated by treatment with sodium acetate in DMF at 120 °C for 24 h, giving the compound **7**, which had a double bond between C-2 and C-3.[23] 3-Keto compound **1** reacted with hydroxylamine hydrochloride in pyridine to produce an oxime compound **8**.[21]Refluxing compound **8** with *p*-toluenesulfonyl chloride (*p*-TsCl) in dry pyridine in the presence of 4-*N*,*N*-dimethylamino-pyridine (DMAP) afforded the compound **9**, which was the product of Beckmann fragmentation.[24] The treatment of 28-methy ester derivative **3** with *m*-chloroperbenzoic acid (*m*-CPBA) and $NaHCO_3$ yielded lactone **10**,[25] and the lactone ring was cleaved by treatment of *p*-toluenesulfonic acid (*p*-TSA) in dichloromethane to give product **11**.[26]



Scheme 1. Synthesis of chalcones **Cha1-11**. Reagents and conditions: (i) 5 eq. KOH, EtOH, r.t., 12 h.



Scheme 2. Synthesis of oleanolic acid derivative-chalcone conjugates **1a-e**, **2a-b** and **3a-e**. Reagents and conditions: (i) Jones' reagent, THF, ice-salt bath,1 h; (ii) $PhNHNH_2$, HOAc, reflux, 1.5 h; (iii) CH_3I, KOH, THF, reflux, 3 h; (iv) (a) PhSeCl, AcOEt, r.t., 3 h; (b) py, H_2O_2, r.t.,15 min then 80 °C, 15 min; (v) LiI, DMF, reflux, 3 h; (vi) DMAP, EDC, CH_2Cl_2, Cha1-11, r.t., 24 h.

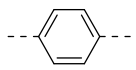
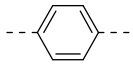
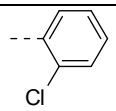
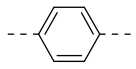
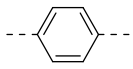
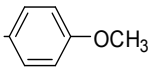
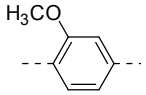
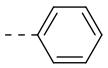
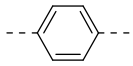
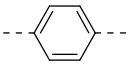
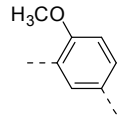
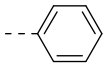
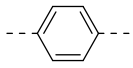
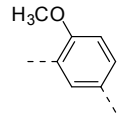
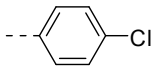
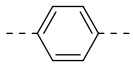
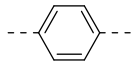
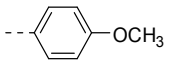
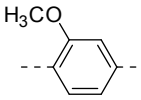
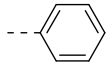
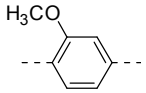
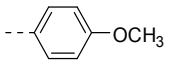
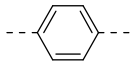
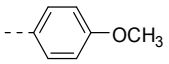
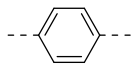
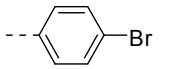
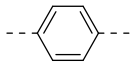
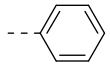
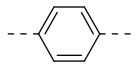
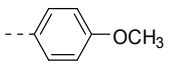
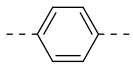
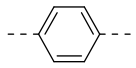
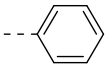
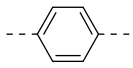
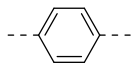


Scheme 3. Synthesis of oleanolic acid derivative-chalcone conjugates **4a-f**, **5a-e** and **6a-c**. Reagents and conditions: (i) *p*-TsCl, py, r.t., 24 h; (ii) NaOAc, DMF, 120 °C, 24 h; (iii) NH_2OH•HCl, py, r.t., 4 h; (iv) *p*-TsCl, DMAP, reflux, 24 h; (v) $NaHCO_3$, *m*-CPBA, CH_2Cl_2, r.t., 24 h; (vi) *p*-TSA, CH_2Cl_2, r.t., 24 h; (vii) DMAP, EDC, CH_2Cl_2, Cha1-11, r.t., 24 h.

α-Glucosidase inhibitory activity

These twenty-six conjugates of oleanolic acid derivatives-chalcones, together with oleanolic acid were evaluated by spectrophotometer for their inhibitory activities against α-glucosidase, and the Acarbose was used as reference. As shown in Table **1**, most of the new conjugates (**1a-d**, **1f**, **2a-b**, **3b-e**, **4a-f**, **5a-c** and **6a-c**) exhibited stronger inhibitory activity against α-glucosidase than Acarbose, except for the compounds **1d** and **3a**. Compared with currently referenced 1A (IC_{50} = 7.97±0.21 μM), **1b** and **5c** displayed stronger inhibitory activity. Interestingly, different chalcone units obviously affected the inhibitory activities of conjugates. Compared with oleanolic acid (IC_{50} = 102.3±2.4 μM), the benzene ring in chalcone units (**1a**, **1c-e**, **2b**, **3a-c**, **4b**, **4f**) did not improve the α-glucosidase inhibitory activity. The Br and Cl atom substation patterns on the chalcone portion (**1a**, **1e** and **3a**) reduced the activity. Among them, the conjugate **1b** (IC_{50} = 3.2±0.2 μM) possessed the strongest α-glucosidase inhibitory activity, which approximately exhibited 34-fold enhanced activities compared with oleanolic acid (IC_{50} = 102.3±2.4 μM), and the furan or thiophene rings in chalcone units of conjugates (**1b**, **3d**, **3e**, **4d**, **4e**) showed a tendency to enhance the activity. This result suggested that furan chalcone unit might be required for strong activity, possibly related to protein binding. This exciting result prompted us to explore additional novel oleanolic acid derivatives-chalcones analogs **5a-5e** and **6a-6c**. These eights conjugates were 3, 4-seco-compounds, conjugates **6a-c** bearing the oleanolic acid derivative ester on the 3-position. In this series, these eight conjugates dramatically enhanced α-glucosidase inhibitory activity than oleanolic acid. Among them, conjugate **5c** (IC_{50} = 4.1±0.2 μM) showed potent α-glucosidase inhibitory activity, being approximately 24-fold higher than oleanolic acid. These results suggested that the inhibitory activity was enhanced by the cleavage of A ring on the oleanolic acid and the C_3 position of chalcone skeletons may be an important factor for the inhibitory activity.

Table 1 α-Glucosidase inhibitory activity (IC_{50}, μM) of oleanolic acid derivatives-chalcones conjugates.

No.	Ar	Ar'	IC_{50}[a](μM)	No.	Ar	Ar'	IC_{50}[a](μM)
1a			159.3±13.6	**4a**			30.8±1.4
1b			3.2±0.2	**4b**			98.9±4.0
1c			259.2±14.1	**4c**			31.8±2.2
1d			NA[b]	**4d**			30.5±1.4
1e			147.7±4.9	**4e**			14.2±0.7
2a			52.7±6.8	**4f**			163.8±8.0
2b			125.6±10.7	**5a**			48.0±3.7
3a			NA	**5b**			20.4±0.8
3b			218.7±1.5	**5c**			4.1±0.2
3c			210.2±1.7	**5d**			11.5±1.0
3d			76.9±4.7	**5e**			33.9±0.4
3e			13.5±1.5	**6a**			10.8±0.9
Oleanolic acid			102.3±2.4	**6b**			8.1±0.7
Acarbose			>300	**6c**			15.5±1.0
Cha 1			362.3±7.6				

[a] standard deviation (n = 3)

[b] not active, the IC_{50} is more than 1000 μM.

Kinetic analysis of α-glucosidase inhibition by compounds 1b, 6b, 5c and 4d

In order to gain further insights into how these conjugates interact with α-glucosidase, the inhibition mode of compounds **1b, 6b, 5c** and **4d** were chosen as typical examples, analyzed by Lineweaver-Burk plots using the data derived from enzyme assays containing various concentrations of *p*-nitrophenyl α-D-glucopyranoside (PNP-glycoside, 0.2-12 mM). Double-reciprocal plots of enzyme kinetics demonstrated competitive inhibition of α-glucosidase activity by **1b**, **6b**, **5c** and **4d**.[27] Lineweaver-Burk plots of α-glucosidase kinetics were shown in Fig.**2**. Increase of

inhibitor concentrations resulted in the growth of slopes of the line, while their y-intercepta was nearly the same. It was indicating that the inhibitor could bind to the active sites of the enzyme. The Michaelis constant (K_m) value of PNP-glycoside for α-glucosidase was 2.14 mM and the K_i value of **1b, 6b, 5c** and **4d** were 16.6, 29.3, 14.6 and 20.6 μM, respectively. The differences of K_i values suggested that the α-glucosidase inhibitory activity of **5c** was higher than that of **1b, 6b** or **4d** due to the differences in affinity to the enzyme inhibitor sites.

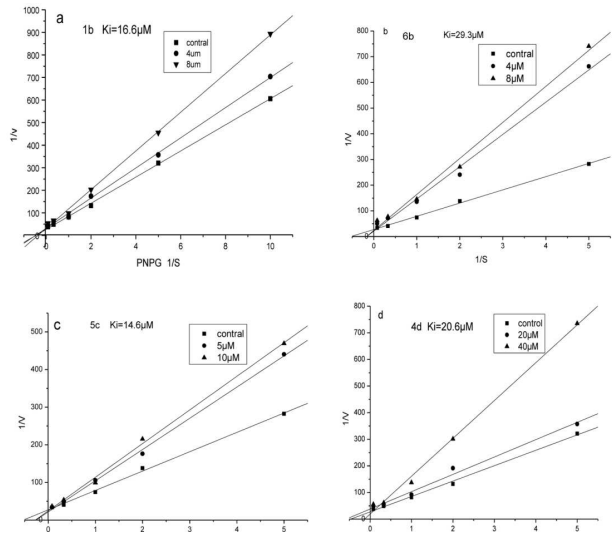


Fig.2. Lineweaver-Burk plots analysis of inhibition kinetics of yeast's α-glucosidase inhibitory effects by compounds **1b**(a), **6b**(b), **5c**(c) and **4d**(d).

Fluorescence quenching spectra of α-glucosidase

The influence of temperature on the quenching

Proteins have intrinsic fluorescence mainly originating from tyrosine (Tyr), tryptophan (Trp) and phenylalanine (Phe) residues. When protein interacts with another compound, its intrinsic fluorescence often changes as the function of ligand concentration.[28] Results demonstrated that conjugates **1b** and **5c** were provided with powerful inhibitory activity towards α-glucosidases, and they could bind to the active sites of the enzyme. In principle, that allowed the employment of fluorescence spectroscopy methods for conducting binding studies. The conjugates **1b** and **5c** were chosen for their lower IC_{50} values. As shown in the Fig.**3**, the fluorescence spectrum of α-glucosidase (2 μM) and conjugates with different concentrations were recorded at 37 °C and 18 °C in phosphate buffer (pH 6.8) for the wavelength range from 300 to 500 nm, showing the characteristic emission singlet at 322 nm. Caused by quenching, an obvious decrease in the fluorescence intensity was observed for the inhibitors in proportion to increasing concentration. The binding of inhibitor **1b** and **5c** to the active sites of the enzyme suppressed the protein fluorescence efficiently. The experimental data was restricted to analysis of quenching constant (K_{SV}) and association constant (K_a). K_{SV} was analyzed using the Stern-Volmer equation as shown in Fig.**4**.[29] Quenching fluorescence spectra of α-glucosidase by conjugates were recorded at two temperatures (18 and 37 °C). As shown in Table **2**, the value of K_{SV} enhanced with the increase of temperature. For dynamic quenching, the relationship between the changes in the fluorescence intensity and the concentration of quencher (Q) for the set of reaction can be described by the equation $\log[(F_0\text{-}F)/F]=\log K_a+n\log[Q]$ (F_0, fluorescence intensity in the absence of quencher; F, fluorescence intensity in the presence of quencher).[30] From the plots of liner obtained by $\log[(F_0\text{-}F)/F]$ vs. $\log[Q]$, the values of K_a could be calculated and the binding sites (n) was shown in Table **3**. For conjugate **5c**, the value of n exhibited a decrease with the increase of temperature, indicating that low temperature was preferred for conjugate/α-glucosidases binding.

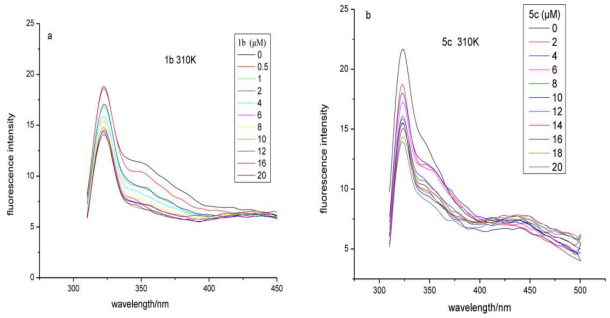


Fig.3. Fluorescence emission spectra of yeast's α-glucosidase (2 μM) in the presence of increasing concentrations of conjugate **1b**(a) and **5c**(b). The band at 322 nm is quenched by inhibitor-enzyme complex formation.

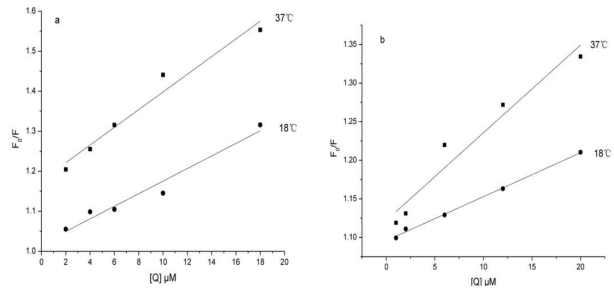


Fig.4. Stern-Volmer plots for the fluorescence quenching of α glucosidase by **1b** (a) and **5c** (b).

Table 2 Binding and quenching constants and binding sites for the tested compounds **1b** and **5c**

Compound	$F_0/F = 1+K_{SV}[Q]$[a]			
	T(K)	K_{SV}(M^{-1})	K_a(L)	n[b]
1b	310	11340	1.49	0.30
	290	5690	6.50	0.206
5c	310	22090	98	0.47
	290	15710	835	0.74

[a] Stern-Volmer equation; [b] the number binding site.

Table 3 Relative thermodynamic parameters on interaction between compound and α-glucosidase at different temperatures

Compound	T(K)	ΔH[a] (KJ/M)	ΔS[b] (KJ/M/K)	ΔG[c](KJ/M)	Interaction types
1b	310	-58.3	-0.18	-1.03	hydrogen bond and van der Waals
	291	-58.3	-0.18	-4.53	
5c	310	-84.8	-0.24	-11.81	hydrogen bond and van der Waals
	290	-84.8	-0.24	-16.28	

[a] enthalpy; [b] entropy; [c] free enthalpy.

Types of interaction force between compound and α-glucosidase

There are four interaction forces between bio-molecules and small molecules, including electrostatic forces, hydrophobic interaction forces, hydrogen bonding and van der waals forces. From the plots of liner obtained by $\log[(F_0-F)/F]$ vs. $\log[Q]$, as shown in Fig.**5**, the thermodynamic parameters were evaluated using Van't Hoff equation and the values were shown in Table **3**. It has reported that the types of interaction forces between bio-molecule and small molecule were associated with the signs of thermodynamic parameters.[31] Only contributions to negative entropy and enthalpy changes arose from hydrogenbond and van der Waals. As presented in Table **3**, the negative ΔH value revealed that the reaction was an exothermic process, and low temperature was helpful for conjugates/α-glucosidase binding. At the same time, the negative value of ΔG demonstrated that the reaction process was spontaneous. In this circumstance, the interaction forces between conjugate and α-glucosidase were hydrogen bond and van der Waals.

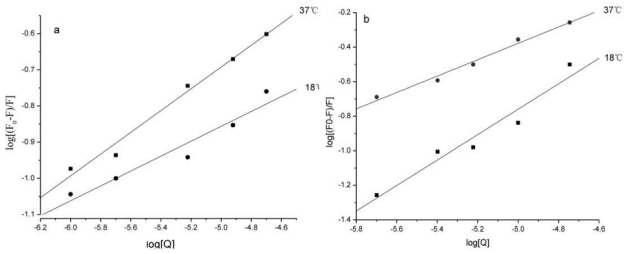


Fig.5. Modified Sten-Volmer plots for the fluorescence quenching of α-glucosidase by **1b** (a) and **5c** (b) at two different temperatures.

Conclusions

In conclusion, a series of novel and potent α-glucosidase inhibitors were synthesized. Most of conjugates exhibited moderate inhibitory activity against α-glucosidase. Among them, the conjugate **1b** (IC_{50} = 3.2±0.2 μM) possessed the strongest α-glucosidase inhibitory activity, the preliminary structure-activity relationships showed that the furan or thiophene rings in chalcone units of conjugates enhanced activities, and these conjugates exhibited inhibitory activities toward yeast α-glucosidase *via* a competitive mechanism. The inhibitor could bind to the active sites of the enzyme and the interaction process was spontaneous. The interaction forces between conjugates and α-glucosidases were hydrogen bond and van der Waals. Thus, oleanolic acid derivatives-chalcones conjugates as a promising new class of α-glucosidase inhibitor leads deserving of further studies.

Experimental Section

General

Melting points were measured on an electro-thermal melting point apparatus and uncorrected. Infrared spectra were taken as KBr disc on a FTIR spectrometer. ^{1}H NMR spectra were recorded in $CDCl_3$ as solvent on a Bruker AVANCE-III-400 (or 500) spectrometer and resonances are in ppm relative to TMS. MS spectra were measured with a Finnigan MS spectrometer. All of the solvents and reagents were purified and dried by standard techniques. All compounds were routinely checked by thin-layer chromatography (TLC) on pre-coated silica gel GF254 plates (Qingdao Haiyang Chemical Co., Ltd., P. R. China). Column chromatography was performed using silica gel (200-300 mesh) from Qingdao Haiyang Chemical Group Co., China.

General Procedure for the synthesis of Chalcones (Cha1-11)

A mixture of the corresponding aldehyde (1.1 equiv) and the corresponding acetophenone (1 equiv) in anhydrous EtOH was stirred at room temperature for 15 min under nitrogen atmosphere, and then KOH (5 equiv) was added. The reaction mixture was stirred at room temperature overnight. After that, 10% HCl was added until pH 3, the aqueous layer was extracted with EtOAc (3×50 mL), washed with water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, Petroleum ether-Acetone, 9:1) to yield chalcone.

Synthesis of oleanolic acid derivatives (1-11)

3-oxo-olean-12-en-28-oic acid (1)

To a solution of OA (10.0 g, 21.9 mmol) in THF (50 mL) in an ice bath was added Jones' reagent (14 mL) and stirred for 1 h, the solvent was removed and water was added. The aqueous mixture was extracted with DCM (3×60 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, Petroleum ether-Acetone, 6:4) to give **1** (9.31 g, 94.9%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.31 (1H, s, H-12), 2.86 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.6 Hz, H-18), 0.82 (s, CH_3), 0.91 (s, CH_3), 0.94 (s, CH_3), 1.05 (s, CH_3), 1.09 (s, CH_3), 1.16 (s, CH_3), 1.26 (s, CH_3).

Indole [3, 2-b] olean-12-en-28-oic acid (2)

A mixture of 3-keto OA (**1**, 2.6 mmol), phenylhydrazine (1.5 equiv) in acetic acid (30 mL) was refluxed for 1.5 h under nitrogen atmosphere. The reaction mixture was pipetted into ice-water (100 mL) and then extracted with DCM (3×25 mL). The extracts were dried (Na_2SO_4) and concentrated under reduced

pressure. The residue was purified by column chromatography (silica gel, Petroleum ether-EtOAc, 1:1) to provide indole derivative **2** (0.71 g, 60.1%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (1H, s, N-H), 7.45 (1H, d, J = 7.2 Hz, A r-H), 7.33-7.28 (1H, m, A r-H), 7.13-7.06 (2H, m, A r-H), 5.42 (1H, s, H-12), 2.8 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.6 Hz, H-18), 0.84 (s, CH_3), 0.89 (s, CH_3), 0.94 (s, CH_3), 1.05 (s, CH_3), 1.10 (s, CH_3), 1.17 (s, CH_3), 1.27 (s, CH_3).

Methyl 3-oxo-olean-12-en-28-oate (3)

To the solution of compound **1** (5.1 mmol) in THF (50 mL), KOH (10 mmol), CH_3I (5.2 mmol) were added and refluxed for 3 h. The reaction mixture was cooled and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography with petroleum ether-EtOAc (7:3) to afford **3** (94% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.31 (1H, s, H-12), 3.67 (3H, s, -$COOCH_3$), 2.89 (1H, dd, J_1 = 3.6 Hz, J_2 = 13.6 Hz, H-18), 0.86 (s, CH_3), 0.93 (s, CH_3), 0.97 (s, CH_3), 1.08 (s, CH_3), 1.11 (s, CH_3), 1.17 (s, CH_3), 1.26 (s, CH_3).

Methyl 3-oxo-olean-1, 12-dien-28-oate (4)

To a solution of **3** (2.40 g, 5.12 mmol) in dry EtOAc (45 mL) was added phenylselenenyl chloride (1.01 g, 5.31 mmol), and the reaction mixture was stirred for 3.5 h at 30 ^{o}C under nitrogen atmosphere. Then pyridine (3.10 mL) was added to the reaction mixture, followed by the addition of H_2O_2 (30%, 2 mL) over a period of 10 min. The reaction mixture was stirred for 15 min at 30 ^{o}C, then refluxed for 15 min, cooled and diluted with EtOAc (50 mL). The organic phase was washed with water (20 mL), saturated aq $NaHCO_3$ (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give **4**. The crude residue (1.24 g, 51.8% yield) was directly used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$): 7.07 (1H, d, J = 10.2 Hz, H-1), 5.83 (1H, d, J = 9.2 Hz, H-2), 5.37 (1H, s, H-12), 3.66 (3H, s, -$COOCH_3$), 2.89 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.6 Hz, H-18), 0.96 (s, CH_3), 1.01 (s, CH_3), 1.02 (s, CH_3), 1.07 (s, CH_3), 1.11 (s, CH_3), 1.18 (s, CH_3), 1.267 (s, CH_3).

3-oxo-olean-1, 12-dien-28-oic acid (5)

To a solution of compound **4** (0.47 g, 1.01 mmol) in dry DCM (50 mL), dried LiI (3.52 g , 26.2 mmol) was added, then refluxed for 2 h under nitrogen atmosphere. The reaction solution was cooled and poured into ice-water (40 mL), acidified by 10% HCl to pH 3, filtered and dried. The residue was purified by column chromatography (silica gel, Petroleum ether-EtOAc, 6:4) to yield the acid **5** as a white solid (0.13 g, 28.4%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.06 (1H, d, J = 10.0 Hz, H-1), 5.84 (1H, d, J = 10.0 Hz, H-2),5.36 (1H, s, H-12), 2.89 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.6 Hz, H-18), 0.86 (s, CH_3), 0.89 (s, CH_3), 0.95 (s, CH_3), 1.04 (s, CH_3), 1.09 (s, CH_3), 1.17 (s, CH_3), 1.27(s, CH_3).

3-tosyl-olean-12-en-28-oic acid (6)

To a solution of oleanolic acid (1.501 g, 3.3 mmol) in Py (30 ml), *p*-toluenesulfonyl chloride (2.196 g, 11.4 mmol) was added. The reaction solution was stirred at room temperature for 24h under nitrogen atmosphere, diluted with water (60 mL) and then extracted with DCM (3×20 mL). The extracts were washed with saturated $KHSO_4$ solution, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, Petroleum ether-EtOAc, 9:1) to provide **6** (1.875g, 95%).

olean-2, 12-dien-28-oic acid (7)

To a solution of compound **6** (0.603 g, 0.8 mmol) in DMF (20mL), sodium acetate (0.302 g, 2.2 mmol) was added and the mixture was heated at 120 ^{o}C for 24h under nitrogen atmosphere. The solvent was removed under reduced pressure and the residue was washed with water (80 mL) and then extracted with DCM (3×20 mL). The extracts were dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (silica gel, Petroleum ether-EtOAc, 9:1), yielding **7** (0.205 g, 42.1%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.40 (3H, m, H-2, H-3, H-12), 3.00 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.6 Hz, H-18), 1.21 (s, CH_3), 1.17 (s, CH_3), 1.09 (s, CH_3), 1.07 (s, CH_3), 0.94 (s, CH_3), 0.93 (s, CH_3), 0.89 (s, CH_3).

3-hydroxyimino-olean-12-en-28-oic acid (8)

A mixture of compound **1** (3.011 g, 6.6 mmol) with hydroxylamine hydrochloride (0.578 mg, 8.32 mmol) and Py (45 mL) was refluxed for 4h, cooled and poured into ice-water (120 mL), acidified by concentrated HCl to pH 3, filtered and dried. The residue was purified by column chromatography (silica gel, Petroleum ether-EtOAc, 8:2), the oxime **8** was obtained as a white solid (2.208g, 71.1%).

3-cyano-3, 4-seco-4-yliden-olean-12-en-28-oic acid (9)

To a solution of compound **8** (1.36 g, 2.9 mmol) in dry Py (50 mL), *p*-toluene sulfonyl chloride (0.732 mg, 3.8 mmol) and 4-*N*,*N*-dimethylamino-pyridine (DMAP) (51 mg, 0.4 mmol) were added, then refluxed for 24 h under nitrogen atmosphere. The reaction solution was cooled and poured into water (80 mL), filtered and dried. The filtrate was concentrated under reduced pressure.The residue was purified by column chromatography (silica gel, Petroleum ether-EtOAc, 9:1) to give **9** (0.548g, 42.4%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34 (1H, s, H-12), 4.90 (1H, s, H_2-24), 4.66 (1H, s, H_2-24), 3.01 (1H, dd, J_1 = 3.6 Hz, J_2 = 14.0 Hz, H-18), 1.73 (3H, s, Me), 1.25 (6H, s, 2×Me), 0.95 (3H, s, Me), 0.92 (3H, s, Me), 0.89 (3H, s, Me).

12-oxo-olean-28-methoxycarbonyl-3-oic acid ε-lactone (10)

A solution of methyl ester **2** (4.216 g, 9.0 mmol), *m*-CPBA (4.654 g, 27.1 mmol), and $NaHCO_3$ (7.552 g, 89.9 mmol) in DCM (50 mL) was stirred at 40^{o}C for 24 h, and the reaction was quenched with Na_2SO_3, diluted with DCM (40 mL), and extracts were washed successively with water (50 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, Petroleum ether-EtOAc, 9:1) to give **10** (2.197 g, 48.8%).

4.1.4.1 3, 4-seco-4-yliden-12-oxo-olean-28-methoxycarbonyl-3-oic acid (11)

To a solution of compound **10** (3.468 g, 6.9 mmol) in DCM (50 mL), *p*-toluenesulfonic acid (*p*-TSA) (3.561 g, 20.7 mmol) was added. The reaction solution was stirred at room temperature for 24 h, diluted with water (100 mL) and then extracted with DCM (3×30 mL). The extracts were dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (silica gel, Petroleum ether-EtOAc, 8:2) to give **11** (1.758 g, 50.7%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.87 (1H, s, H_2-24), 4.67 (1H, s, H_2-24), 3.67 (3H, s, -OMe), 2.80 (1H, dd, J_1 = 3.6 Hz, J_2 = 13.6 Hz, H-18), 2.63 (2H, m, H-2), 1.73 (3H, s, Me), 0.99 (3H, s, Me),

0.96 (6H, s, 2×Me), 0.89 (3H, s, Me), 0.84 (3H, s, Me).

General procedure for esterification (1a-e, 2a-b, 3a-e, 4a-f, 5a-c, 6a-e)

A DCM solution of the same mol ratio of the corresponding OA derivative and chalcone (Cha **1-11**) with a two-fold mol ratio of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (EDC) and 4-*N*, *N*-dimethylamino-pyridine (DMAP) was stirred at room temperature for 24 h under nitrogen atmosphere. The crude mixture was extracted with DCM, and the organic layer was washed with brine, dried (Na_2SO_4). Evaporation of the solvent gave a residue that was purified by column chromatography (silica gel, Petroleum ether-EtOAc, 9:1).

{4-[(E)-3-(4-bromophenyl)acryloyl]phenyl}-3-oxo-olean-12-en-28-oate (1a)

Straw yellow solid, yield 37.4%, m.p. 166.4-167.3 ^{o}C; IR (KBr): 1749 (-COO-), 1666 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (2H, d, J = 8.0 Hz, Ar-H), 7.75 (1H, d, J = 15.6 Hz, H-9'), 7.57 (2H, d, J = 8.0Hz, Ar-H), 7.53 (2H, d, J = 8.0Hz, Ar-H), 7.46 (1H, d, J = 16.0 Hz, H-8'), 7.16 (2H, d, J = 8.0Hz, Ar-H), 5.38 (1H, s, H-12), 3.00 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.6 Hz, H-18), 1.24 (3H, s, Me), 1.14 (3H, s, Me), 1.08 (6H, s, 2×Me), 1.04 (3H, s, Me), 0.97 (3H, s, Me), 0.93 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 217.7, 189.0, 175.7, 154.8, 143.5, 143.2, 135.3, 133.7, 132.2 (C×2 in Ph), 130.0 (C×2 in Ph), 129.8 (C×2 in Ph), 124.9, 122.8, 122.3, 121.9 (C×2 in Ph), 55.3, 47.4, 47.3, 46.8, 45.7, 41.9, 41.5, 39.5, 39.1, 36.7, 34.1, 33.8, 33.0, 32.3 (C×2), 30.7, 27.8 , 26.4, 25.7, 23.6 (C×2), 23.0, 21.5, 19.5, 17.3, 15.0; ESI MS: *m/z* 739 ([M+H]$^+$, 18.8); HRESIMS *m/z* 739.3349 [M+H]$^+$ (calcd. for $C_{45}H_{56}O_4Br$, 739.3361).

{4-[(E)-3-(furan-2-yl)acryloyl]phenyl}-3-oxo-olean-12-en-28-oate (1b)

Yellow solid, yield 45.1%, m.p. 196.6-197.4 ^{o}C; IR (KBr): 1750 (-COO-), 1697 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (2H, d, J = 8.4 Hz, Ar-H), 7.60 (1H, d, J = 15.6 Hz, H-9'), 7.53 (1H, s, H-5"), 7.40 (1H, d, J = 16.4 Hz, H-8'), 7.16 (2H, d, J = 8.4 Hz, Ar-H), 6.72 (1H, d, J = 3.2 Hz, H-3"), 6.51 (1H, t, J = 1.6 Hz, H-4"), 5.41 (1H, s, H-12), 3.00 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.6 Hz, H-18), 1.20 (3H, s, Me), 1.08 (3H, s, Me), 1.04 (6H, s, 2×Me), 0.97 (3H, s, Me), 0.96 (3H, s, Me), 0.94 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 217.7, 188.6, 175.7, 154.7, 151.6, 144.9, 143.2, 135.4, 130.8, 129.9 (C×2 in Ph), 122.8, 121.8 (C×2 in Ph), 118.9, 116.4, 112.7, 55.3, 47.4, 47.3, 46.8, 45.7, 41.9, 41.5, 39.5, 39.1, 36.7, 34.1, 33.8, 33.0, 32.3 (C×2), 30.7, 27.8, 26.4, 25.7, 23.5 (C×2), 23.0, 21.5, 19.5, 17.3, 15.0; ESI MS: *m/z* 651 ([M+H]$^+$, 7.6); HRESIMS *m/z* 651.4054 [M+H]$^+$ (calcd. for $C_{43}H_{55}O_5$, 651.4049).

{2-methoxy-4-[(E)-3-oxo-3-phenylprop-1-enyl]phenyl}-3-oxo-olean-12-en-28-oate (1c)

Straw yellow solid, yield 39.6%, m.p. 143.7-144.6 ^{o}C; IR (KBr): 1749 (-COO-), 1666 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (2H, d, J = 8.4 Hz, Ar-H), 7.80 (1H, d, J = 15.6 Hz, H-9'), 7.61 (1H, m, H- 4"), 7.53 (2H, d, J = 8.4 Hz, Ar-H), 7.48 (1H, d, J = 15.6 Hz, H-8'), 7.28 (1H, m, H-6'), 7.24 (1H, d, J = 2.4 Hz, H-2'), 7.00 (1H, d, J = 8.4 Hz, H-5'), 5.38 (1H, s, H-12), 3.89 (3H, s, -OMe), 3.00 (1H, dd, J_1 = 3.6 Hz, J_2 = 14.0 Hz, H-18), 1.20 (3H, s, Me), 1.06 (3H, s, Me), 1.01 (6H, s, 2×Me), 0.98 (3H, s, Me), 0.97 (3H, s, Me), 0.96 (3H, s, Me); ^{13}C NMR (125 M Hz, $CDCl_3$): δ 217.8, 190.5, 175.5, 151.8, 144.4, 143.4, 142.1, 138.1, 133.5, 132.8, 128.6 (C×2 in Ph), 128.5 (C×2 in Ph), 123.4, 122.5, 122.0, 121.4, 111.7, 55.9, 55.3, 47.4, 47.3, 46.8, 45.7, 41.9, 41.5, 39.4, 39.1, 36.7, 34.1, 33.8, 33.1, 32.3 (C×2), 30.7, 27.8, 26.4, 25.7, 23.6 (C×2), 23.1, 21.5, 19.6, 17.2, 15.1; ESI MS: *m/z* 713.4 ([M+Na]$^+$, 100); HRESIMS *m/z* 713.4204 [M+Na]$^+$ (calcd. for $C_{46}H_{58}O_5Na$, 713.4182).

{2-methoxy-5-[(E)-3-oxo-3-phenylprop-1-enyl]phenyl}-3-oxo-olean-12-en-28-oate (1d)

Straw yellow solid, yield 34.3%, m.p. 174.4-175.3 ^{o}C; (KBr): 1747(-COO-), 1660 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (2H, d, J = 8.4 Hz, Ar-H), 7.78 (1H, d, J = 15.6 Hz, H-9'), 7.59 (1H, m, H-4"), 7.53 (2H, d, J = 8.4 Hz, Ar-H), 7.49 (1H, m, H-6'), 7.39 (1H, d, J = 15.6 Hz, H-8'), 7.28 (1H, m, H-2'), 6.99 (1H, d, J = 8.4 Hz, H-5'), 5.39 (1H, s, H-12), 3.89 (3H, s, -OMe), 3.00 (1H, dd, J_1 = 3.6 Hz, J_2 = 14.0 Hz, H-18), 1.23 (3H, s, Me), 1.12 (3H, s, Me), 1.07 (6H, s, 2×Me), 1.00 (3H, s, Me), 0.98 (3H, s, Me), 0.97 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 217.7, 190.3, 175.6, 153.5, 144.0, 140.4, 143.4, 138.4, 127.9, 132.6, 128.8, 128.5 (C×2 in Ph), 128.4 (C×2 in Ph), 122.5, 122.4, 121.4, 112.3, 55.9, 55.3, 47.4, 47.3, 46.8, 45.8, 41.9, 41.5, 39.5, 39.1, 36.7, 34.1, 33.9, 33.0, 32.3 (C×2), 30.7, 27.7, 26.4, 25.7, 23.6 (C×2), 23.1, 21.5, 19.6, 17.3, 15.1; ESI MS: *m/z* 691 ([M+H]$^+$, 5.6); HRESIMS *m/z* 691.4356 [M+H]$^+$ (calcd. for $C_{46}H_{59}O_5$, 691.4362).

{2-methoxy-5-[(E)-3-(4-chlorophenyl)-3-oxoprop-1-enyl]phenyl}-3-oxo-olean-12- en-28-oate (1e)

Straw yellow solid, yield 33.2%, m.p. 135.6-137.0 ^{o}C; IR (KBr): 1748 (-COO-), 1666 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (2H, d, J = 8.4Hz, Ar-H), 7.75 (1H, d, J = 15.6 Hz, H-9'), 7.47 (1H, m, H-6'), 7.46 (2H, d, J = 8.4 Hz, Ar-H), 7.31 (1H, d, J = 15.6 Hz, H-8'), 7.27 (1H, d, J = 2.4 Hz, H-2'), 6.90 (1H, d, J = 8.8 Hz, H-4'), 5.36 (1H, s, H-12), 3.84 (3H, s, -OMe), 3.00 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.6 Hz, H-18), 1.24 (3H, s, Me), 1.11 (3H, s, Me), 1.07(3H, s, Me), 1.06 (3H, s, Me), 1.05 (3H, s, Me), 0.98 (3H, s, Me), 0.95 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 217.6, 188.9, 175.6, 153.7, 144.4, 143.4, 140.4, 139.0, 136.7, 128.0, 127.7, 129.8 (C×2 in Ph), 128.9 (C×2 in Ph), 122.5, 122.4, 119.9, 112.3, 55.9, 55.3, 47.4, 47.3, 46.8, 45.9, 42.0, 41.5, 39.5, 39.1, 36.7, 34.1, 33.9, 33.0, 32.3 (C×2), 30.7, 27.7, 26.4, 25.6, 23.6 (C×2), 23.2, 21.5, 19.6, 17.3, 15.1; ESI MS: *m/z* 747 ([M+Na]$^+$, 3.7); HRESIMS *m/z* 725.3964 [M+H]$^+$ (calcd. for $C_{46}H_{58}O_5Cl$, 725.3972).

{4-[(E)-3-(4-methoxyphenyl)acryloyl]phenyl}-indole[3,2-b]olean-12-en-28-oate (2a)

Yellow solid, yield 30.8%, m.p. 139.6-141.0 ^{o}C; IR (KBr): 1748 (-COO-), 1661 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (2H, d, J = 8.8Hz, Ar-H), 7.86 (1H, s, -NH), 7.77 (1H, d, J = 16.0 Hz, H-9'), 7.61 (2H, d, J = 8.4Hz, Ar-H), 7.42 (1H, d, J = 7.2 Hz, Ar-H), 7.37 (1H, d, J = 15.6 Hz, H-8'), 7.29 (1H, d, J = 7.6 Hz, Ar-H), 7.19 (2H, d, J = 8.8 Hz, Ar-H), 7.12 (1H, m, Ar-H), 7.09 (1H, m, Ar-H), 6.95 (2H, d, J = 8.4 Hz, Ar-H), 5.50 (1H, s, H-12), 3.86 (3H, s, -OMe), 3.03 (1H, dd, J_1 = 3.6 Hz, J_2 = 13.6 Hz, H-18), 1.31 (3H, s, Me), 1.26 (6H, s, 2×Me), 1.22(3H, s, Me), 1.02 (3H, s, Me), 0.97 (3H, s, Me), 0.96(3H, s, Me); ^{13}C NMR (125

MHz, $CDCl_3$): δ 189.4, 175.8, 161.7, 154.6, 144.8, 142.9, 140.8, 136.1, 135.8, 130.2 (C×2 in Ph), 129.9 (C×2 in Ph), 128.2, 127.5, 123.3, 121.7 (C×2 in Ph), 120.9, 119.5, 118.8, 117.9, 114.4 (C×2 in Ph), 110.4, 106.8, 55.4, 53.2, 47.7, 46.3, 45.8, 42.0, 41.6, 39.6, 38.1, 36.8, 34.0, 33.9, 33.1, 32.3 (C×2), 31.0, 30.7, 27.9, 25.7, 23.6, 23.5, 23.2, 23.1, 19.3, 17.3, 15.6; ESI MS: *m/z* 764 ($[M+H]^+$, 4.2); HRESIMS *m/z* 764.4678 $[M+H]^+$ (calcd. for $C_{52}H_{62}NO_4$, 764.4678).

{2-methoxy-4-[(E)-3-(4-methoxyphenyl)-3-oxoprop-1-enyl]phenyl}-indole [3,2-b]olean-12-en-28-oate (2b)

Straw yellow solid, yield 42.4%, m.p. 173.2-174.1 °C; IR (KBr): 1748 (-COO-), 1661 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.06 (2H, d, J = 8.0Hz, Ar-H), 7.87 (1H, s, -NH), 7.78 (1H, d, J = 15.6 Hz, H-9'), 7.48 (1H, d, J = 15.6 Hz, H-8'), 7.42 (1H, d, J = 7.6 Hz, Ar-H), 7.30 (1H, d, J = 7.6 Hz, Ar-H), 7.27 (1H, m, H-6'), 7.24 (1H, d, J = 2.4 Hz, H-2'), 7.12 (1H, m, Ar-H), 7.09 (1H, m, Ar-H), 7.03 (1H, d, J = 8.8 Hz, H-5'), 6.99 (2H, d, J = 8.0 Hz, Ar-H), 5.47 (1H, s, H-12), 3.89 (3H, s, -OMe), 3.88 (3H, s, -OMe), 3.04 (1H, dd, J_1 = 4.0 Hz, J_2 = 14.0 Hz, H-18), 1.33 (3H, s, Me), 1.27 (3H, s, Me), 1.26(3H, s, Me), 1.24 (3H, s, Me), 1.02 (3H, s, Me), 0.98(6H, s, 2×Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.7, 175.6, 163.4, 151.8, 143.5, 143.0, 142.0, 140.8, 136.1, 133.7, 131.0, 130.8 (C×2 in Ph), 128.2, 123.4, 123.0, 121.8, 121.2, 120.9, 118.8, 117.9, 113.8 (C×2 in Ph), 111.8, 110.3, 106.9, 55.9, 55.5, 53.2, 47.4, 46.3, 45.9, 42.1, 41.6, 39.6, 38.1, 36.8, 34.0, 34.0, 33.1, 32.4 (C×2), 31.0, 30.7, 27.8, 25.6, 23.6, 23.5, 23.3, 23.1, 19.4, 17.2, 15.6; ESI MS: *m/z* 794 ($[M+H]^+$, 6.7); HRESIMS *m/z* 794.4791 $[M+H]^+$ (calcd. for $C_{53}H_{64}NO_5$, 794.4784).

{4-[(E)-3-(4-bromophenyl)acryloyl]phenyl}-3-oxo-olean-1,12-dien-28-oate (3a)

Straw yellow solid, yield 32.8%, m.p. 138.4-139.7 °C; IR (KBr): 1748 (-COO-), 1665 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.04 (2H, d, J = 8.0 Hz, Ar-H), 7.75 (1H, d, J = 15.6 Hz, H-9'), 7.54 (2H, d, J = 8.0Hz, Ar-H), 7.50 (2H, d, J = 8.0Hz, Ar-H), 7.46 (1H, d, J = 16.0 Hz, H-8'), 7.17 (2H, d, J = 8.0Hz, Ar-H), 7.04 (1H, d, J = 10.0 Hz, H-1), 5.82 (1H, d, J = 10.0 Hz, H-2), 5.38 (1H, s, H-12), 3.00 (1H, dd, J_1 = 3.6 Hz, J_2 = 14.0 Hz, H-18), 1.21 (3H, s, Me), 1.17 (3H, s, Me), 1.15 (3H, s, Me), 1.09 (3H, s, Me), 0.99 (3H, s, Me), 0.94 (3H, s, Me), 0.93 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.7, 189.4, 176.1, 159.0, 155.2, 143.9, 143.5, 135.8, 134.1, 132.2 (C×2 in Ph), 130.1 (C×2 in Ph), 129.8 (C×2 in Ph), 125.5, 125.3, 122.3, 122.2, 121.8 (C×2 in Ph), 53.8, 45.0, 47.8, 46.0, 42.6, 42.1 (C×2), 40.7, 39.8, 34.2, 33.5, 33.0, 32.7, 31.2, 28.2 (C×2), 25.7, 23.6, 23.4, 23.0, 22.1, 19.1, 18.7, 17.9; EI MS: *m/z* 738 ($[M+2]^+$, 4), 736 ($[M]^+$, 3), 407 (100), 248 (31), 203 (70), 189 (38), 69 (39), 57 (54); HREIMS *m/z* 736.3125 (calcd. for $C_{45}H_{53}BrO_4$, 736.3105).

{4-[(E)-3-(4-methoxyphenyl)acryloyl]phenyl}-3-oxo-olean-1,12-dien-28-oate (3b)

Straw yellow solid, yield 41.1%, m.p. 151.2-152.1 °C; IR (KBr): 1749 (-COO-), 1667 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.04 (2H, d, J = 8.0 Hz, Ar-H), 7.79 (1H, d, J = 15.6 Hz, H-9'), 7.59 (2H, d, J = 8.4 Hz, Ar-H), 7.39 (1H, d, J = 15.6 Hz, H-8'), 7.16 (2H, d, J = 8.4 Hz, Ar-H), 7.04 (1H, d, J = 10.0 Hz, H-1), 6.94 (2H, d, J = 8.0 Hz, Ar-H), 5.81 (1H, d, J = 10.0 Hz, H-2), 5.43 (1H, s, H-12), 3.84 (3H, s, -OMe), 3.02 (1H, dd, J_1 =3.6 Hz, J_2 = 14.0 Hz, H-18), 1.22 (3H, s, Me), 1.17 (3H, s, Me), 1.16 (3H, s, Me), 1.00 (3H, s, Me), 0.99 (3H, s, Me), 0.95 (3H, s, Me), 0.94 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.3, 189.3, 175.7, 161.7, 159.1, 154.5, 144.9, 143.6, 135.8, 130.8 (C×2 in Ph), 129.9 (C×2 in Ph), 127.5, 125.1, 122.3, 121.7 (C×2 in Ph), 119.4, 114.4 (C×2 in Ph), 55.4, 53.4, 44.5, 47.3, 45.5, 42.2, 41.7, 41.5, 40.2, 39.4, 33.8, 33.0, 32.2 (C×2), 30.7, 27.8, 27.7, 25.7, 23.6, 23.3, 23.0, 21.6, 18.8, 18.7, 17.9; EI MS: *m/z* 692 ($[M+4]^+$, 4), 688 ($[M]^+$, 12), 484 (14), 407 (100), 217 (30), 203 (61), 189 (51), 105 (36), 69 (31); HREIMS *m/z* 688.4112 (calcd. for $C_{46}H_{56}O_5$, 688.4097).

{4-cinnamoylphenyl}-3-oxo-olean-1,12-dien-28-oate (3c)

Straw yellow solid, yield 35.2%, m.p. 139.0-139.7 °C; IR (KBr): 1748 (-COO-), 1667 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.06 (2H, d, J = 8.0 Hz, Ar-H), 7.82 (1H, d, J = 15.6 Hz, H-9'), 7.63 (2H, d, J = 8.4 Hz, Ar-H), 7.51 (1H, d, J = 15.6 Hz, H-8'), 7.47 (3H, m, Ar-H), 7.18 (2H, d, J = 8.0 Hz, Ar-H), 7.04 (1H, d, J = 10.0 Hz, H-1), 5.81 (1H, d, J = 10.0 Hz, H-2), 5.47 (1H, s, H-12), 3.00 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.6 Hz, H-18), 1.22 (3H, s, Me), 1.17 (3H, s, Me), 1.15 (3H, s, Me), 1.10 (3H, s, Me), 0.99 (3H, s, Me), 0.96 (3H, s, Me), 0.94 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.3, 189.3, 175.7, 159.0, 154.7, 144.5, 143.6, 135.5, 134.7, 130.6, 130.1 (C×2 in Ph), 128.9 (C×2 in Ph), 128.4(C×2 in Ph), 125.1, 122.3, 121.8 (C×2 in Ph), 121.7, 53.4, 44.5, 47.3, 45.5, 42.2, 41.7, 41.4, 40.2, 39.4, 33.8, 33.0, 32.6, 32.2, 30.7, 27.8, 27.7, 25.7, 23.6, 23.3, 23.0, 21.6, 18.8, 18.7, 17.9; EI MS: *m/z* 658 ($[M]^+$, 7), 454 (13), 407 (100), 203 (38), 189 (34), 107 (18), 69 (11); HREIMS *m/z* 658.4028 (calcd. for $C_{45}H_{54}O_4$, 658.4034).

{4-[(E)-3-(furan-2-yl)acryloyl]phenyl}-3-oxo-olean-1,12-dien-28-oate (3d)

Yellow solid, yield 34.6%, m.p. 164.7-165.8 °C; IR (KBr): 1749 (-COO-), 1668 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.06 (2H, d, J = 8.0 Hz, Ar-H), 7.60 (1H, d, J = 15.6 Hz, H-9'), 7.52 (1H, s, H-5"), 7.44 (1H, d, J = 15.6 Hz, H-8'), 7.16 (2H, d, J = 8.4 Hz, Ar-H), 7.04 (1H, d, J = 10.0 Hz, H-1), 6.72 (1H, d, J = 3.2 Hz, H-3"), 6.51 (1H, t, J = 1.6 Hz, H-4"), 5.81 (1H, d, J = 10.0 Hz, H-2), 5.43 (1H, s, H-12), 3.02 (1H, dd, J_1 = 4.0 Hz, J_2 = 14.0 Hz, H-18), 1.15 (3H, s, Me), 1.09 (6H, s, 2×Me), 1.04 (3H, s, Me), 0.97 (3H, s, Me), 0.94 (3H, s, Me), 0.92 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.3, 188.6, 175.7, 159.1, 154.6, 151.5, 145.0, 143.6, 135.4, 130.8, 129.9 (C×2 in Ph), 125.0, 122.3, 121.7 (C×2 in Ph), 118.9, 116.4, 112.7, 55.4, 44.5, 47.3, 45.5, 42.2, 41.6, 41.4, 40.2, 39.4, 33.8, 33.0, 32.2 (C×2), 30.7, 27.8, 27.7, 25.7, 23.6, 23.3, 22.9, 21.6, 18.8, 18.7, 17.9; EI MS: *m/z* 648 ($[M]^+$, 10), 444 (14), 407 (100), 215 (24), 203 (44), 187 (32), 107 (16), 69 (29), 55 (15); HREIMS *m/z* 648.3792 (calcd. for $C_{43}H_{52}O_5$, 648.3770).

{4-((E)-3-(thiophen-2-yl)acryloyl)phenyl}-3-oxo-olean-1,12-dien-28-oate (3e)

Yellow solid, yield 37.1%, m.p. 150.8-151.6 °C; IR (KBr): 1748 (-COO-), 1667 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.02 (2H, d, J = 8.0 Hz, Ar-H), 7.90 (1H, d, J = 15.6 Hz, H-9'), 7.41 (1H, d, J = 4.4 Hz, H-5"), 7.34 (1H, d, J = 3.6 Hz, H-3"), 7.30 (1H, d, J = 15.6 Hz, H-8'), 7.16 (2H, d, J = 8.4 Hz, Ar-H), 7.06 (1H, t, J = 3.6 Hz, H-4"), 7.03 (1H, d, J = 10.0 Hz, H-1), 5.80

(1H, d, J = 10.0 Hz, H-2), 5.42 (1H, s, H-12), 3.02 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.6 Hz, H-18), 1.21 (3H, s, Me), 1.17 (3H, s, Me), 1.15 (3H, s, Me), 1.10 (3H, s, Me), 0.99 (3H, s, Me), 0.95 (3H, s, Me), 0.94 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.2, 188.6, 175.6, 159.0, 154.6, 143.6, 140.2, 137.4, 135.4, 132.2, 129.9 (C×2 in Ph), 128.9, 128.4, 125.0, 122.3, 121.8 (C×2 in Ph), 120.4, 53.4, 44.5, 47.3, 45.5, 42.1, 41.7, 41.5, 40.2, 39.4, 33.8, 33.0, 32.6, 32.2, 30.7, 27.8, 27.7, 25.7, 23.6, 23.3, 23.0, 21.6, 18.8, 18.7, 17.9; EI MS: *m/z* 664 ([M]$^+$, 6), 407 (76), 248 (44), 203 (100), 189 (41), 107 (30), 69 (26); HREIMS *m/z* 664.3594 (calcd. for $C_{43}H_{52}SO_4$, 664.3602).

{4-[(E)-3-(2-chlorophenyl)acryloyl]phenyl}-olean-2,12-dien-28-oate (4a)

Straw yellow solid, yield 31.8%, m.p. 132.3-133.2 °C; IR (KBr): 1748 (-COO-), 1660 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (1H, d, J = 15.6 Hz, H-9'), 8.05 (2H, d, J = 8.0 Hz, Ar-H), 7.74 (1H, m, Ar-H), 7.48 (1H, d, J = 16.0 Hz, H-8'), 7.44 (1H, m, Ar-H), 7.19 (2H, d, J = 8.0 Hz, Ar-H), 7.32 (2H, m, Ar-H), 5.41 (3H, m, H-2, H-3, H-12), 3.02 (1H, dd, J_1 = 3.6 Hz, J_2 = 14.4 Hz, H-18), 1.21 (3H, s, Me), 0.98 (3H, s, Me), 0.97 (3H, s, Me), 0.96 (3H, s, Me), 0.95 (3H, s, Me), 0.89 (6H, s, 2×Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 189.6, 176.28, 155.3, 143.4, 141.1, 138.3, 135.9 135.6, 133.6, 131.6, 130.7, 130.6 (C×2 in Ph), 128.2, 127.5, 124.9, 123.3, 121.8, 122.3 (C×2 in Ph), 52.4, 47.8, 46.5, 46.2, 42.4, 42.0, 41.2, 40.1, 36.6, 34.3, 33.5, 33.1, 32.7, 32.2, 31.8, 31.2, 27.8, 25.7, 24.0, 23.8, 23.6, 23.2, 19.9, 17.6, 16.0; EI MS: *m/z* 678 ([M] $^+$, 8), 488 (24), 393 (68), 203 (100), 189 (54), 149 (91), 95 (63), 69 (74) , 57 (94); HREIMS *m/z* 678.3838 (calcd. for $C_{45}H_{55}ClO_3$, 678.3826).

{4-[(E)-3-(4-methoxyphenyl)acryloyl]phenyl}-olean-2,12-dien-28-oate (4b)

Straw yellow solid, yield 38.7%, m.p. 170.8-172.1 °C; IR (KBr): 1749 (-COO-), 1665 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (2H, d, J = 8.0 Hz, Ar-H), 7.79 (1H, d, J = 15.6 Hz, H-9'), 7.60 (2H, d, J = 8.4 Hz, Ar-H), 7.39 (1H, d, J = 15.6 Hz, H-8'), 7.17 (2H, d, J = 8.4 Hz, Ar-H), 6.94 (2H, d, J = 8.0 Hz, Ar-H), 5.40 (3H, m, H-2, H-3, H-12), 3.85 (3H, s, -OMe), 3.02 (1H, dd, J_1 =3.6 Hz, J_2 = 14.0 Hz, H-18), 1.21 (3H, s, Me), 1.00 (3H, s, Me), 0.98 (3H, s, Me), 0.97 (3H, s, Me), 0.96 (3H, s, Me), 0.91 (6H, s, 2×Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 189.4, 175.8, 161.7, 154.6, 144.8, 142.9, 137.9, 135.7, 130.2 (C×2 in Ph), 129.9 (C×2 in Ph), 127.5, 123.2, 121.3, 121.7 (C×2 in Ph), 119.6, 114.4 (C×2 in Ph), 55.4, 52.0, 47.3, 46.1, 45.7, 41.9, 41.6, 40.7, 39.6, 36.2, 34.4, 33.8, 33.0, 32.3 (C×2), 31.8, 30.7, 27.7, 25.6, 23.6, 23.3, 23.1, 22.7, 19.5, 17.2, 15.6; EI MS: *m/z* 674 ([M]$^+$, 4), 484 (10), 393 (42), 203 (100), 189 (44), 95 (28), 69 (22); HREIMS *m/z* 674.4338 (calcd. for $C_{46}H_{58}O_4$, 674.4340).

{4-cinnamoylphenyl}-olean-2,12-dien-28-oate (4c)

Straw yellow solid, yield 37.5%, m.p. 141.1-142.3 °C; IR (KBr): 1749 (-COO-), 1665 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (2H, d, J = 8.0 Hz, Ar-H), 7.82 (1H, d, J = 15.6 Hz, H-9'), 7.64 (2H, d, J = 8.4 Hz, Ar-H), 7.52 (1H, d, J = 16.0 Hz, H-8'), 7.48 (3H, m, Ar-H), 7.18 (2H, d, J = 8.0 Hz, Ar-H), 5.40 (3H, m, H-2, H-3, H-12), 3.00 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.6 Hz, H-18), 1.20 (3H, s, Me), 0.99 (3H, s, Me), 0.97 (3H, s, Me), 0.96 (3H, s, Me), 0.95 (3H, s, Me), 0.90 (6H, s, 2×Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 189.4, 175.8, 154.7, 145.0, 142.9, 137.9, 135.4, 134.8, 130.6, 130.0 (C×2 in Ph), 129.0 (C×2 in Ph), 128.4(C×2 in Ph), 121.4, 123.2, 121.8 (C×2 in Ph), 121.7, 52.0, 47.4, 46.1, 45.7, 42.0, 41.6, 40.7, 39.6, 36.2, 34.4, 33.8, 33.1, 32.3 (C×2), 31.8, 30.7, 27.7, 25.7, 23.6, 23.3, 23.1, 22.8, 19.5, 17.2, 15.6; EI MS: *m/z* 647 ([M+3]$^+$, 4), 644 ([M]$^+$, 9), 454 (38), 393 (78), 203 (100), 189 (54), 107 (23), 81 (16); HREIMS *m/z* 644.4222 (calcd. for $C_{45}H_{56}O_3$, 644.4215).

{4-[(E)-3-(furan-2-yl)acryloyl]phenyl}-olean-2,12-dien-28-oate (4d)

Yellow solid, yield 43.7%, m.p. 172.3-173.5 °C; IR (KBr): 1751 (-COO-), 1668 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (2H, d, J = 8.0 Hz, Ar-H), 7.60 (1H, d, J = 15.6 Hz, H-9'), 7.52 (1H, s, H-5"), 7.44 (1H, d, J = 15.6 Hz, H-8'), 7.17 (2H, d, J = 8.4 Hz, Ar-H), 6.71 (1H, d, J = 3.2 Hz, H-3"), 6.50 (1H, t, J = 1.6 Hz, H-4"), 5.39 (3H, m, H-2, H-3, H-12), 3.00 (1H, dd, J_1 = 3.6 Hz, J_2 = 14.0 Hz, H-18), 1.21 (3H, s, Me), 1.00 (3H, s, Me), 0.98 (3H, s, Me), 0.97 (3H, s, Me), 0.96 (3H, s, Me), 0.91 (3H, s, Me), 0.90 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.6, 175.8, 154.7, 151.6, 144.9, 142.9, 137.9, 135.4, 130.8, 129.9 (C×2 in Ph), 123.2, 121.4, 121.8 (C×2 in Ph), 119.0, 116.4, 112.7, 52.0, 47.4, 46.1, 45.7, 41.9, 41.6, 40.7, 39.6, 36.2, 34.4, 33.8, 33.1, 32.3 (C×2), 31.8, 30.7, 27.7, 25.7, 23.6, 23.3, 23.1, 22.8, 19.5, 17.2, 15.6; EI MS: *m/z* 634 ([M]$^+$, 8), 444 (28), 393 (67), 215 (34), 203 (98), 189 (50), 149 (100), 69 (17), 55 (14); HREIMS *m/z* 634.4037 (calcd. for $C_{43}H_{54}O_4$, 634.4052).

{4-[(E)-3-(thiophen-2-yl)acryloyl]phenyl}-olean-2,12-dien-28-oate (4e)

Yellow solid, yield 36.4%, m.p. 134.2-135.3 °C; IR (KBr): 1749 (-COO-), 1660 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (2H, d, J = 8.0 Hz, Ar-H), 7.95 (1H, d, J = 15.6 Hz, H-9'), 7.41 (1H, d, J = 4.4 Hz, H-5"), 7.35 (1H, d, J = 3.6 Hz, H-3"), 7.31 (1H, d, J = 15.6 Hz, H-8'), 7.17 (2H, d, J = 8.4 Hz, Ar-H), 7.07 (1H, t, J = 3.6 Hz, H-4"), 5.40 (3H, m, H-2, H-3, H-12), 3.02 (1H, dd, J_1 = 3.6 Hz, J_2 = 14.0 Hz, H-18), 1.21 (3H, s, Me), 0.98 (3H, s, Me), 0.97 (3H, s, Me), 0.96 (3H, s, Me), 0.95 (3H, s, Me), 0.89 (6H, s, 2×Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.6, 175.7, 154.7, 142.9, 140.3, 137.9, 137.3, 135.4, 132.2, 129.9 (C×2 in Ph), 128.9, 128.4, 122.3, 121.8 (C×2 in Ph), 121.4, 120.5, 52.0, 47.4, 46.1, 45.1, 42.0, 41.6, 40.7, 39.6, 36.2, 34.4, 33.8, 33.1, 32.3 (C×2), 31.8, 30.7, 27.8, 25.7, 23.6, 23.3, 23.1, 22.8, 19.5, 17.2, 15.6; EI MS: *m/z* 653 ([M+3]$^+$, 3), 650 ([M] $^+$, 8), 460 (40), 393 (80), 203 (100), 189 (50), 95 (33), 69 (13); HREIMS *m/z* 650.3802 (calcd. for $C_{43}H_{54}SO_3$, 650.3810).

{3-methoxy-4-[(E)-3-oxo-3-phenylprop-1-enyl]phenyl}-olean-2,12-dien-28-oate (4f)

Straw yellow solid, yield 31.1%, m.p. 150.7-152.1 °C; IR (KBr): 1749 (-COO-), 1664 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (2H, d, J = 8.4 Hz, Ar-H), 7.79 (1H, d, J = 15.6 Hz, H-9'), 7.61 (1H, m, H- 4"), 7.53 (2H, d, J = 8.0 Hz, Ar-H), 7.48 (H, d, J = 15.6 Hz, H-8'), 7.28 (1H, m, H-6'), 7.24 (1H, d, J = 2.4 Hz, H-2'), 7.01 (1H, d, J = 8.4 Hz, H-5'), 5.40 (3H, m, H-2, H-3, H-12), 3.89 (3H, s, -OMe), 3.00 (1H, dd, J_1 = 3.6 Hz, J_2 = 14.0 Hz, H-18), 1.23 (3H, s, Me), 1.01 (3H, s, Me), 0.99 (3H, s, Me), 0.97 (3H, s, Me), 0.96 (3H, s, Me), 0.93 (3H, s, Me), 0.92 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.5, 175.5, 151.8, 144.4, 143.4,

142.0, 138.2, 137.9, 133.4, 132.7, 128.6 (C×2 in Ph), 128.5 (C×2 in Ph), 123.4, 123.0, 122.0, 121.3, 121.4, 111.8, 55.9, 52.0, 47.4, 46.1, 45.9, 42.0, 41.6, 40.7, 39.6, 36.2, 34.4, 33.9, 33.1, 32.3 (C×2), 31.8, 30.7, 27.7, 25.6, 23.6, 23.3, 23.1, 22.8, 19.5, 17.1, 15.6; EI MS: *m/z* 674 ([M]$^+$, 1), 407 (10), 393 (100), 203 (28), 189 (32), 95 (24), 69 (10); HREIMS *m/z* 674.4310 (calcd. for $C_{46}H_{58}O_4$, 674.4285).

{4-[(E)-3-(4-methoxyphenyl)acryloyl]phenyl}-3-cyano-3,4-seco-4-yliden-olean-12-en-28-oate (5a)

Straw yellow solid, yield 31.4%, m.p. 133.3-134.0 ^{o}C; IR (KBr): 2244 (CN), 1748 (-COO-), 1660 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (2H, d, J = 8.0 Hz, Ar-H), 7.79 (1H, d, J = 16.0 Hz, H-9'), 7.59 (2H, d, J = 8.4 Hz, Ar-H), 7.39 (1H, d, J = 16.0 Hz, H-8'), 7.15 (2H, d, J = 8.4 Hz, Ar-H), 6.94 (2H, d, J = 8.4 Hz, Ar-H), 5.39 (1H, s, H-12), 4.90 (1H, s, H_2-24), 4.66 (1H, s, H_2-24), 3.85 (3H, s, -OMe) 3.02 (1H, dd, J_1 =3.6 Hz, J_2 = 13.6 Hz, H-18), 1.74 (3H, s, Me), 1.21 (3H, s, Me), 0.98 (3H, s, Me), 0.95 (3H, s, Me), 0.93 (3H, s, Me), 0.90 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 189.3, 175.7, 161.7, 154.6, 146.7, 144.9, 143.4, 135.8, 130.2 (C×2 in Ph), 129.9 (C×2 in Ph), 127.5, 122.3, 121.7 (C×2 in Ph), 120.2, 119.5, 114.4 (C×2 in Ph), 114.2, 55.4, 50.6, 47.2, 45.6, 42.3, 41.5, 39.4, 39.2, 37.8, 34.3, 33.8, 33.0, 32.3, 31.4, 30.7, 29.7, 27.7, 25.6, 24.1, 23.7, 23.5, 23.0, 19.1, 17.5, 11.5; EI MS: *m/z* 688 ([M+1]$^+$, 4), 687 ([M]$^+$, 5), 406 (82), 248 (58), 203 (100), 189 (85), 69 (48) , 57 (55); HREIMS *m/z* 687.4271 (calcd. for $C_{46}H_{57}NO_4$, 687.4254).

{4-cinnamoylphenyl}-3-cyano-3,4-seco-4-yliden-olean-12-en-28-oate (5b)

Straw yellow solid, yield 45.6%, m.p. 131.1-132.5 ^{o}C; IR (KBr): 2245 (CN), 1750 (-COO-), 1665 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (2H, d, J = 8.0 Hz, Ar-H), 7.82 (1H, d, J = 15.6 Hz, H-9'), 7.62 (2H, d, J = 8.4 Hz, Ar-H), 7.52 (1H, d, J = 15.6 Hz, H-8'), 7.42 (3H, m, Ar-H), 7.17 (2H, d, J = 8.0 Hz, Ar-H), 5.38 (1H, s, H-12), 4.90 (1H, s, H_2-24), 4.66 (1H, s, H_2-24), 3.01 (1H, dd, J_1 = 3.6 Hz, J_2 = 14.0 Hz, H-18), 1.74 (3H, s, Me), 1.21 (3H, s, Me), 0.98 (3H, s, Me), 0.95 (3H, s, Me), 0.93 (3H, s, Me), 0.90 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 189.7, 176.1, 155.1, 147.1, 145.4, 143.8, 135.9, 135.2, 131.1, 130.5 (C×2 in Ph), 129.4 (C×2 in Ph), 128.9 (C×2 in Ph), 122.8, 122.2 (C×2 in Ph), 121.7, 120.6, 114.6, 51.1, 47.2, 46.0, 42.7, 41.9, 39.9, 39.7, 38.2, 34.7, 34.2, 33.5, 32.7, 31.8, 31.2, 30.1, 27.7, 26.1, 24.5, 24.1, 24.0, 23.4, 19.5, 17.9, 12.0; EI MS: *m/z* 657 ([M]$^+$, 3), 406 (63), 248 (78), 203 (100), 189 (21), 69 (54) , 57 (44); HREIMS *m/z* 657.4190 (calcd. for $C_{45}H_{55}NO_3$, 657.4198).

{4-[(E)-3-(furan-2-yl)acryloyl]phenyl}-3-cyano-3,4-seco-4-yliden-olean-12-en-28-oate (5c)

Yellow solid, yield 29.6%, m.p. 173.6-174.5 ^{o}C; IR (KBr): 2244 (CN), 1748 (-COO-), 1679 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (2H, d, J = 8.0 Hz, Ar-H), 7.61 (1H, d, J = 15.6 Hz, H-9'), 7.53 (1H, s, H-5"), 7.44 (1H, d, J = 15.6 Hz, H-8'), 7.16 (2H, d, J = 8.4 Hz, Ar-H), 6.73 (1H, d, J = 4.0 Hz, H-3"), 6.52 (1H, t, J = 1.6 Hz, H-4"), 5.38 (1H, s, H-12), 4.90 (1H, s, H_2-24), 4.66 (1H, s, H_2-24), 3.01 (1H, dd, J_1 = 4.0 Hz, J_2 = 14.0 Hz, H-18), 1.73 (3H, s, Me), 1.20 (3H, s, Me), 0.98 (3H, s, Me), 0.94 (3H, s, Me), 0.93 (3H, s, Me), 0.89 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.6, 175.7, 154.7, 151.5, 146.7, 145.0, 143.4, 135.4, 130.8, 129.9 (C×2 in Ph), 122.3, 121.8 (C×2 in Ph), 120.2, 118.9, 116.5, 114.2, 112.7, 50.6, 47.2, 45.6, 42.3, 41.5, 39.4, 39.2, 37.8, 34.2, 33.8, 33.0, 32.3, 31.4, 30.7, 29.7, 27.7, 25.6, 24.1, 23.7, 23.5, 23.0, 19.1, 17.5, 11.5; EI MS: *m/z* 647 ([M]$^+$, 3), 406 (100), 248 (16), 203 (38), 189 (13), 69 (11) , 55 (9); HREIMS *m/z* 647.3946 (calcd. for $C_{43}H_{53}NO_4$, 647.3917).

{4-[(E)-3-(thiophen-2-yl)acryloyl]phenyl}-3-cyano-3,4-seco-4-yliden-olean-12-en-28-oate (5d)

Yellow solid, yield 32.3%, m.p. 149.1-150.8 ^{o}C; IR (KBr): 2244 (CN), 1749 (-COO-), 1660 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (2H, d, J = 8.4 Hz, Ar-H), 7.95 (1H, d, J = 16.0 Hz, H-9'), 7.43 (1H, s, H-5"), 7.36 (1H, d, J = 3.6 Hz, H-3"), 7.31 (1H, d, J = 16.4 Hz, H-8'), 7.16 (2H, d, J = 8.4 Hz, Ar-H), 7.08 (1H, t, J = 3.6 Hz, H-4"), 5.38 (1H, s, H-12), 4.90 (1H, s, H_2-24), 4.66 (1H, s, H_2-24), 3.01 (1H, dd, J_1 = 3.6 Hz, J_2 = 13.6 Hz, H-18), 1.73 (3H, s, Me), 1.20 (3H, s, Me), 0.98 (3H, s, Me), 0.94 (3H, s, Me), 0.93 (3H, s, Me), 0.90 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 188.6, 175.7, 154.6, 146.7, 143.4, 140.3, 137.4, 135.4, 132.2, 129.9 (C×2 in Ph), 128.9, 128.4, 122.3, 121.8 (C×2 in Ph), 120.5, 120.2, 114.2, 50.6, 47.2, 45.6, 42.3, 41.5, 39.4, 39.2, 37.8, 34.4, 33.8, 33.0, 32.3, 31.4, 30.7, 29.7, 27.7, 25.6, 24.1, 23.7, 23.5, 23.0, 19.1, 17.5, 11.5; EI MS: *m/z* 663 ([M]$^+$, 8), 406 (100), 248 (34), 203 (43), 189 (11), 69 (9) , 55 (7); HREIMS *m/z* 663.3757 (calcd. for $C_{43}H_{53}NSO_3$, 663.3768).

{4-[(E)-3-oxo-3-phenylprop-1-enyl]phenyl}-3-cyano-3,4-seco-4-yliden-olean-12-en-28-oate (5e)

Straw yellow solid, yield 33.6%, m.p. 147.2-148.1 ^{o}C; IR (KBr): 2245 (CN), 1748 (-COO-), 1667 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (2H, d, J = 8.0 Hz, Ar-H), 7.80 (1H, d, J = 16.0 Hz, H-9'), 7.65 (2H, d, J = 8.0 Hz, Ar-H), 7.57 (1H, d, J = 15.6 Hz, H-8'), 7.48 (3H, m, Ar-H), 7.09 (2H, d, J = 8.0 Hz, Ar-H), 5.37 (1H, s, H-12), 4.90 (1H, s, H_2-24), 4.66 (1H, s, H_2-24), 3.01 (1H, dd, J_1 = 3.6 Hz, J_2 = 14.0 Hz, H-18), 1.74 (3H, s, Me), 1.21 (3H, s, Me), 0.98 (3H, s, Me), 0.95 (3H, s, Me), 0.93 (3H, s, Me), 0.91 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.3, 175.9, 152.8, 146.7, 143.8, 143.4, 138.1, 132.3, 132.8, 139.5 (C×2 in Ph), 128.6 (C×2 in Ph), 128.5 (C×2 in Ph), 122.3, 122.2 (C×2 in Ph), 121.9, 120.2, 114.2, 50.6, 47.2, 45.6, 42.3, 41.5, 39.4, 39.2, 37.8, 34.3, 33.8, 33.0, 32.3, 31.4, 30.7, 29.7, 27.7, 25.6, 24.1, 23.7, 23.6, 23.0, 19.1, 17.5, 11.5; EI MS: *m/z* 657 ([M]$^+$, 1), 406 (100), 248 (8), 203 (17), 189 (8), 69 (7), 55 (6); HREIMS *m/z* 657.4175 (calcd. for $C_{45}H_{55}NO_3$, 657.4168).

{4-[(E)-3-(4-methoxyphenyl)acryloyl]phenyl}-3,4-seco-4-yliden-12-oxo-olean-28-methoxycarbonyl-3-oate (6a)

Straw yellow solid, yield 32.7%, m.p. 142.7-143.5 ^{o}C; IR (KBr): 1761 (-COO-), 1721 (-COO-), 1661 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00 (2H, d, J = 8.5 Hz, Ar-H), 7.74 (1H, d, J = 15.5 Hz, H-9'), 7.55 (2H, d, J = 8.5 Hz, Ar-H), 7.36 (1H, d, J = 16.0 Hz, H-8'), 7.17 (2H, d, J = 8.5 Hz, Ar-H), 6.88 (2H, d, J = 8.5 Hz, Ar-H), 4.87 (1H, s, H_2-24), 4.69 (1H, s, H_2-24), 3.77 (3H, s, -OMe), 3.64 (3H, s, -OMe), 2.76 (1H, dd, J_1 = 4.0 Hz, J_2 = 13.5 Hz, H-18), 2.63 (2H, m, H-2), 1.72 (3H, s, Me), 0.97 (3H, s, Me), 0.95 (3H, s, Me), 0.93 (3H, s, Me), 0.86 (3H, s, Me), 0.84 (3H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 210.8, 188.8, 178.0, 171.1, 161.5, 153.7, 146.3, 135.7, 127.2, 144.6, 130.1 (C×2 in

Ph), 129.7 (C×2 in Ph), 121.4 (C×2 in Ph), 124.2 (C×2 in Ph), 119.1, 113.9, 55.2, 51.6, 51.3, 49.8, 47.0, 42.2, 40.8, 40.5, 38.7, 38.5, 36.0, 34.1, 33.2, 32.9, 32.6, 31.7 , 30.4, 30.1, 28.1, 27.3, 24.1, 23.0 (C×2), 22.5, 20.2, 18.8, 15.6; EI MS: *m/z* 736 ([M]$^+$, 1), 482 (29), 467 (69), 407 (65), 278 (40), 254 (100), 218 (38), 65 (8); HREIMS *m/z* 736.4358 (calcd. for $C_{47}H_{60}O_7$, 736.4377).

{4-[(E)-3-(furan-2-yl)acryloyl]phenyl}-3,4-seco-4-yliden-12-oxo-olean-28-methoxycarbonyl-3-oate (6b)

Yellow solid, yield 43.6%, m.p. 155.9-156.6 ^{o}C; IR (KBr): 1757 (-COO-), 1721 (-COO-), 1664 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.04 (2H, d, J = 8.5 Hz, Ar-H), 7.57 (1H, d, J = 15.5 Hz, H-9'), 7.50 (1H, s, H-5"), 7.42 (1H, d, J = 15.5 Hz, H-8'), 7.19 (2H, d, J = 8.0 Hz, Ar-H), 6.70 (1H, d, J = 3.0 Hz, H-3"), 6.48 (1H, t, J = 1.5 Hz, H-4"), 4.89 (1H, s, H_2-24), 4.71 (1H, s, H_2-24), 3.67 (3H, s, -OMe), 2.80 (1H, dd, J_1 = 3.5 Hz, J_2 = 14.0 Hz, H-18), 2.64 (2H, m, H-2), 1.74 (3H, s, Me), 1.00 (3H, s, Me), 0.98 (3H, s, Me), 0.95 (3H, s, Me), 0.87 (6H, s, 2×Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 211.0, 188.4, 178.2, 171.3, 154.0, 151.4, 146.4, 144.9, 135.5, 130.7, 129.9 (C×2 in Ph), 121.6 (C×2 in Ph), 118.8, 116.3, 114.0, 112.6, 51.7 (C×2), 50.0, 47.1, 42.4, 40.9, 40.7, 38.6, 38.5, 36.1, 34.3, 33.3, 33.0, 32.8, 31.8, 30.5, 30.2, 28.2, 27.5, 24.1, 23.1, 23.0, 22.6, 20.3, 19.0, 15.8; EI MS: *m/z* 696 ([M]$^+$, 12), 485 (24), 407 (28), 214 (55), 149 (100), 69 (13), 57 (19); HREIMS *m/z* 696.4030 (calcd. for $C_{44}H_{56}O_7$, 696.4034).

{4-[(E)-3-(thiophen-2-yl)acryloyl]phenyl}-3,4-seco-4-yliden-12-oxo-olean-28-methoxycarbonyl-3-oate (6c)

Yellow solid, yield 31.8%, m.p. 143.6-144.9 ^{o}C; IR (KBr): 1759 (-COO-), 1721 (-COO-), 1660 (C=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.04 (2H, d, J = 8.5 Hz, Ar-H), 7.92 (1H, d, J = 15.5 Hz, H-9'), 7.43 (1H, d, J = 5.0 Hz, H-5"), 7.36 (1H, d, J = 3.5 Hz, H-3"), 7.31 (1H, d, J = 15.5 Hz, H-8'), 7.22 (2H, d, J = 8.5 Hz, Ar-H), 7.08 (1H, t, J = 3.5 Hz, H-4"), 4.91 (1H, s, H_2-24), 4.73 (1H, s, H_2-24), 3.68 (3H, s, -OMe), 2.82 (1H, dd, J_1 = 3.5 Hz, J_2 = 13.5 Hz, H-18), 2.66 (2H, m, H-2), 1.77 (3H, s, Me), 1.03 (3H, s, Me), 1.00 (3H, s, Me), 0.98 (3H, s, Me), 0.90 (6H, s, 2×Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 211.1, 188.6, 178.3, 171.4, 154.1, 146.5, 140.3, 137.4, 135.5, 132.2, 129.9 (C×2 in Ph), 128.9, 128.4, 121.7 (C×2 in Ph), 120.4, 114.1, 51.9, 51.8, 50.2, 47.3, 42.5, 41.0, 40.8, 38.9, 38.7, 36.2, 34.4, 33.4, 33.1, 32.9, 31.9, 30.6, 30.3, 28.3, 27.6, 24.4, 23.2, 23.1, 22.7, 20.4, 19.1, 15.9; EI MS: *m/z* 712 ([M]$^+$, 13), 482 (32), 467 (65), 407 (70), 278 (47), 230 (100), 218 (48), 149 (41), 69 (12); HREIMS *m/z* 712.3808 (calcd. for $C_{44}H_{56}SO_6$, 712.3818).

α-Glucosidase inhibitory activity determination

The α-glucosidase inhibitory activity of each compound was determined according to the chromogenic method described by Chapdelaine et al. with slight modifications.[32] α-Glucosidase from *Saccharomyces Cerevisias* and substrate solution pNPG were prepared with 0.1 mol/L of Na-phosphate buffer (pH 6.8). The inhibitors were reconstituted in 80 μL phosphate buffer in a 96-well microplate and incubated with 30μL α-glucosidase in 37 ^{o}C for 15 min, and then 30 μL substrate was added. After incubation with substrate for 5 min, release of p-nitrophenol was measured at 405 nm by spectrophotometer. Percentage of enzyme inhibition was calculated according with $\{1-(A_{sample} - A_{blank}) / A_{control}\}\times 100$, where A_{sample} represents absorbance of test samples, $A_{control}$ represents absorbance of solution without sample, and A_{blank} represents absorbance in presence of solution without substrate.

Kinetics of inhibition against α-glucosidase [33]

In order to evaluate the inhibition type of the conjugates against α-glucosidase activities, increasing concentrations of p-nitrophenyl α-D-glucopyranoside were used as substrates in the absence or presence of compounds at two different concentrations around the IC_{50} values. The inhibition types of **1b**, **6b**, **5c** and **4d** were determined by Lineweaver-Burk plots, using the methods that reported in literatures. Inhibition types and K_i values of the inhibitors were determined by Double-reciprocal plots.

Fluorescence quenching measurements [34]

All fluorescence spectra were measured on a fluorescence spectrophotometer (Perkin-Elmer) equipped with a 10.0 mm quartz cell and a thermostat bath. In fluorescence spectrum, 30 μL of α-glucosidase solution (pH 6.8) with the concentration of 2 μM was added accurately to the quartz cell and then titrated by successive additions of inhibitor. The fluorescence emission spectra were measured at 18 and 37 ^{o}C. The excitation wavelength was 290 nm and the emission spectrum was recorded from 300 to 500 nm.

Acknowledgements

The project was supported by National Key Technology R&D Program in the 12th Five Year Plan of China (2012BAI27B06)

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1. S. Shobana, Y. N. Sreerama, N. G. Malleshi, *Food Chem*. **2009**, 15, 1268-1273.
2. A. M. Chang, M. J. Smith, C. J. Bloem, *Am J Physiol Endocrinol Metab*. **2004**, 287, E906-E911.
3. M. R. Bhandari, J. A. Nilubon, H. Gao, J. Kawabata, *Food Chem*. **2008**, 106, 247-252.
4. W. Nie, J. G. Luo, X. B. Wang, H. Yin, H. B. Sun, H. Q. Yao, L. Y. Kong, *Chem Pharm Bull*. **2011**, 59, 1051-1056.
5. J. Liu, Y. Liu, A. Parkinson, C. D. Klaassen, *J Pharmacol Exp Ther*. **1995**, 275, 768-774.
6. E. M. Giner-Larza, S. Manez, M. C. Recio, R. M. Giner, J. M. Prieto, M. Cerda-Nicolas, J. L. Rios, *Eur J Pharmacol*. **2011**,428, 137–143.
7. D. Deeb, X. Gao, S. A. Dulchavsky, S. C. Gautam, *Anticancer Res*. **2007**, 27, 3035–3044.
8. D. L. Yu, Y. Sakurai, C. H. Chen, F. R. Chang, L. Huang, Y.

Kashiwada, K. H. Lee, *J Med Chem.* **2006**, 49, 5462–5469.
9. J. G. Luo, L. Ma, L. Y. Kong, *Bioorg Med Chem*. **2008**, 16, 2912-2920.
10. Y. Liu, L. Ma, W. H. Chen, B. Wang, Z. L. Xu, *Bioorg Med Chem*. **2007**, 15, 2810-2814.
11. W. Nie, J. G. Luo, X. B. Wang, H. Yin, H. B. Sun, H. Q. Yao, L. Y. Kong, *Chem Pharm Bull*. **2011**, 59, 1051-1056.
12. M. S. Ali, M. Jahangir, S. S. Hussan, M.I. Choudhary, *Phytochmistry*. **2002**, 60, 295-299.
13. W. K. Kang, Y. L. Song, L. Zhang, *Med Chem Res.* **2013**, 63, 162-169.
14. T. Narender, G. Madhur, N. Jaiswal, M. Agrawal, C. K. Maurya, N. Rahuja, A. K. Srivastava, A. K. Tamrakar, *Eur J Med Chem.* **2008**, 43, 1865-1877.
15. W. D. Seo, J. H. Kim, J. E. Kang, H. W. Ryu,; M. J. Curtis-Long, H. S. Lee, M. S. Yang, K. H. Park, *Bioorg Med Chem Lett*. **2005**, 15, 5514-5516.
16. S. K. Choi, *Synthetic Multivalent Molecules*; Wiley-Inter-science, New York, **2004**.219-221.
17. K. W. Lin, A. M. Huang, T. C. Hour, S. C. Yang, Y. S. Pu, C. N. Lin, *Bioorg Med Chem*. **2011**, 19, 4274-4285.
18. A. Modzelewska, C. Pettit, G. Achanta, N. E. Davaidson, P. Huang, S. R. Khan, *Bioorg Med Chem*. **2006**, 14, 3491-3495.
19. M. Cabrera, M. Simoens, G. Falchi, M. L. Lavaggi, O. E. Piro, E. E. Castellano, A. Vidal, A. Azqueta, A. Monge, A. L. Cerain, G. Sagrera, H. Cerecetto, M. Gonzalez, *Bioorg Med Chem*. **2007**, 15, 3356-3367.
20. D.Liu, D. D. Song, G. Guo, R.Wang, J. L. Lv, Y. K. Jing, L. X. Zhao, *Bioorg Med Chem*. **2007**, 15, 5432-5439.
21. J. F. Li, Y. Zhao, M. M.Cai, X. F. Li, J. X. Li, *Eur J Med Chem*. **2009**, 44, 2796-2806.
22. Y. J. You, Y. Kim, N. H. Nam, B. Z. Ahn, *Bioorg Med Chem Lett*. **2003**, 13, 3137-3140.
23. A. Parra, P. E. Lopez, G. G. Andres, *Nat Prod Res*. **2010**, 24, 177-196.
24. S. B. Gnoatto, A. Dassonville-Klimpt, S. D. Nascimento, P. Galera, K. Boumediene, G. Gosmann, P. Sonnet, S. Moslemi, *Eur J Med Chem*. **2008**, 43, 1865-1877.
25. S. Qian, H. Li, Y. Chen, W. Zhang, S. Yang, Y. Wu, *J Nat Prod*. 2010, 73, 1743-1750.
26. H. Y. Tu, A. M. Huang, B. L. Wei, K. H. Gan, T. C. Hour, S. C. Ynag, Y. S. Pu, C. N. Lin, *Bioorg Med Chem*. **2009**, 17, 7265-7274.
27. B. K. Suresh, A. K. Tiwari, P. V. Srinivas, A. Z. Ali, B. Raju, J. M. Rao, *Bioorg Med Chem Lett*. **2004**, 14, 3841-3845.
28. A. Papadopoulou, R. J. Green, R. A. Frazier, *J Agric Food Chem.* **2005**, 53, 158-163.
29. J. R. Lakowicz, B. P. Maliwal, *J Biol Chem*. **1983**, 258, 4794-4801.
30. M. X. Xie, X. Y. Xu, Y. D. Wang, *Biochim Biophys Acta*. **2005**, 1724, 215-224.
31. P. D. Ross, S. Subramanian, *Biochemistry*. **1981**, 20, 3096-3102.
32. P. Chapdelaine, R. R.Tremblay, J. Y. Dube, *Clin Chem*. **1978**, 24, 208-211.
33. W. Hou, Y. Li, Q. Zhang, X. Wei, A. Peng, L. Chen, Y. Wei, *Phytother Res.* **2009**, 23, 614-618.
34. M. Aguilar-Moncayo, M. I.García-Moreno, A. E. Stutz, J. M. Garcua Fernandez, T. M. Wrodnigg, C. Ortiz Mellet, *Bioorg Med Chem*. **2010**, 18, 7439-7445.