Synthesis of New α-Glucosidase Inhibitors Based on Oleanolic Acid Incorporating Cinnamic Amides

Wei NIE,[a] Jian-Guang LUO,[a] Xiao-Bing WANG,[a] Hong YIN,[a] Hong-Bin SUN,[b] He-Quan YAO,[b] and Ling-Yi KONG*,[a]

[a] *Department of Natural Medicinal Chemistry, China Pharmaceutical University; and* [b] *Department of Medicinal Chemistry, China Pharmaceutical University; 24 Tongjia Xiang, Nanjing 210009, People's Republic of China.*
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A series of α-glucosidase inhibitors with the oleanolic acid core and different cinnamic amide ligands were designed and synthesized. Their preliminary structure–activity relationships were analyzed. In general, the compounds with 3,28-disubstituted oleanolic acid exhibited stronger activity than those 28-monosubstituted analogues, and variation of cinnamic amide substitution significantly affected α-glucosidase inhibition activities. Most of the compounds showed potent inhibitory activity against α-glucosidase with much greater efficacy than a typical α-glucosidase inhibitor, acarbose.

Key words synthesis; α-glucosidase inhibitor; oleanolic acid; cinnamic amide

Type II diabetes mellitus affects approximately 215 million people worldwide. It is currently clear that aggressive control of hyperglycemia in patients with type II diabetes can attenuate the development of chronic complications such as retinopathy and nephropathy.[1)] To date, therapy for type II diabetes relies mainly on several approaches intended to suppress the hyperglycemia, which include reducing gut glucose absorption. Therefore inhibition of α-glucosidase, an enzyme catalyzing the cleavage of glycosidic bonds in oligosaccharides or glycoconjugates, is a choice to control elevated glucose level in blood.

Oleanolic acid is the hypoglycemic component in many Traditional Chinese Medicines (TCMs). In previous reports, some oleanolic acid derivatives have been designed, synthesized based on inhibitor of glycogen phosphorylase (GP).[2—6)] But it was found that oleanolic acid and its derivates were used to suppress the hyperglycemia for not only inhibitor of glycogen phosphorylase but also inhibitors of α-glucosidase in our previous research.[7,8)] Furthermore, recent investigations have reported that the compounds with cinnamic amide unit have strong α-glucosidase inhibition activities.[9—11)] These motivated us to carry out further structural modifications on oleanolic acid by incorporating different cinnamic amide units.

In the present paper, a series of analogues with the oleanolic acid core and different cinnamic amide ligands were designed, synthesized, and evaluated as α-glucosidase inhibitors (Fig. 1, Table 1). Their structure–activity relationships also were analyzed.

Results and Discussion

With oleanolic acid as a lead compound, we sought to identify a series of oleanolic acid derivatives as novel α-glucosidase inhibitor. Synthetic efforts were focused on structural modifications at C-3 and C-28 positions. The synthetic route of 28-substituted oleanolic acid derivatives is outlined in Chart 1. Piperazine fragment was induced in order to link cinnamic amide unit with oleanolic acid at C-28.

Oleanolic acid unit and cinnamic amide unit were connected at N-1 and N-4 positions of piperazine, respectively. On basis of these, 3,28-disubstituted oleanolic acid deriva-

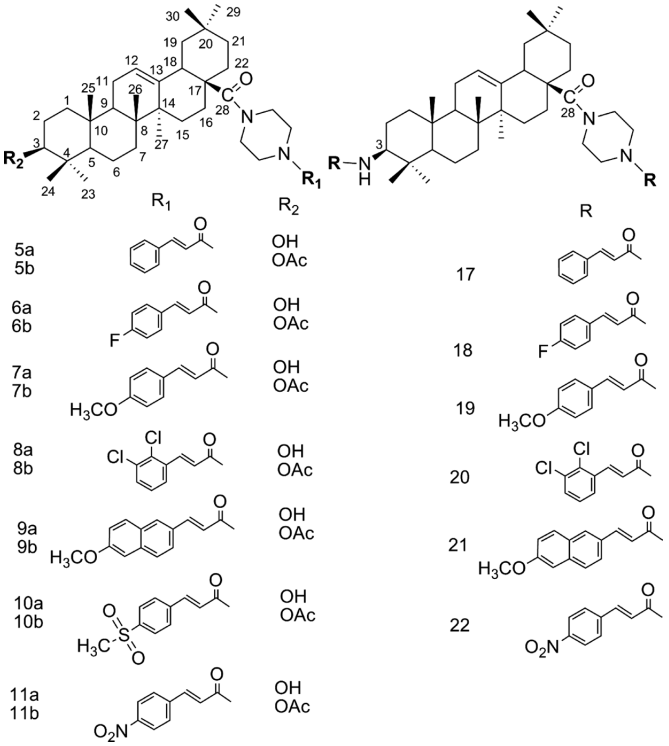

Fig. 1. Structures of Oleanolic Acid Derivatives

Table 1. Inhibitory Activity on α-Glucosidase by Oleanolic Acid Derivatives

Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
5a	92.6±2.0	**10b**	165.2±4.4
5b	96.5±2.1	**11a**	35.5±0.8
6a	149.7±3.4	**11b**	141.8±4.8
6b	165.5±4.3	**17**	5.9±0.3
7a	130.0±2.8	**18**	19.7±0.9
7b	173.4±4.6	**19**	7.9±0.4
8a	78.9±1.8	**20**	1.9±0.2
8b	80.0±1.7	**21**	3.9±0.6
9a	80.8±1.5	**22**	5.9±0.9
9b	108.3±3.1	Oleanolic acid	98.5±1.2
10a	128.0±3.6	Acarbose	388.0±9.6

* To whom correspondence should be addressed. e-mail: cpu_lykong@126.com

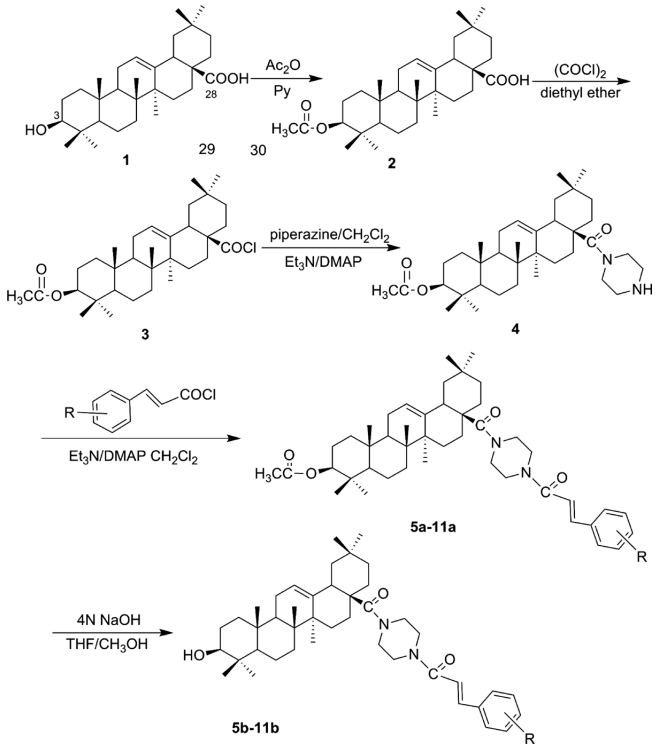

Chart 1. Attempts to Synthesize 28-Substituted Oleanolic Acid Derivatives

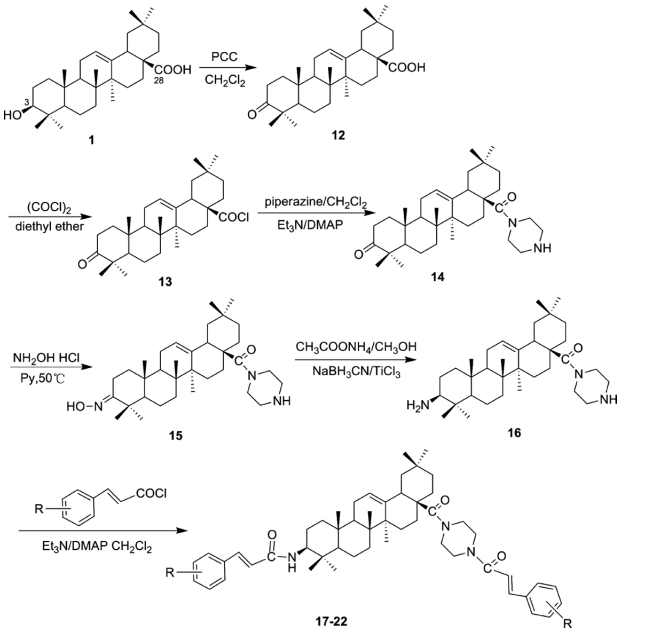

Chart 2. Attempts to Synthesize 3,28-Disubstituted Oleanolic Acid Derivatives

tives were further designed and synthesized, and the synthetic route is outlined in Chart 2. In the reduction reaction, there were two products with different configuration. One was α configuration, and the other was β configuration. 3β-Cinnamamidoolean acid derivatives were many more than 3α-cinnamamidoolean acid derivatives because of their stereospecific blockade. But due to long reaction route, 3β,28-disubtituted oleanolic acid derivatives were obtained merely. 3α,28-Disubtituted oleanolic acid derivatives were not obtained because of their low yield.

Biological Activity The synthesized oleanolic acid derivatives, together with oleanolic acid and acarbose (as positive controls) were biologically evaluated for their inhibitory activities against α-glucosidase. As showed in Table 1, almost all of the newly synthesized compounds exhibited stronger inhibitory activity against α-glucosidase than acarbose (IC_{50}=388.0 μM). Interestingly, different substituted groups on benzene ring of cinnamic amide units were obvious effect on activity. For instance, F atom could reduce activity (**17** *vs.* **18**, **5a** *vs.* **6a**, **5b** *vs.* **6b**), and enlargement of hydrophobic surface could also reinforce α-glucosidase inhibitory activity (**7a** *vs.* **9a**, **7b** *vs.* **9b**, **19** *vs.* **21**).

However, compared with oleanolic acid (IC_{50}=98.5 μM), incorporation of the cinnamic amides at C-28 didn't result in apparent enhancement of the α-glucosidase inhibitory activity (**5a**—**11b**). In this series, the activities of 3-OH derivatives are stronger than 3-OAc derivatives. It may be concluded that the structure size of the hydroxyl was moderate for the size of cavity bonded with enzyme. While 3,28-disubstituted oleanolic acid derivatives (**17**—**22**) dramatically enhance the α-glucosidase inhibitory activity than oleanolic acid. Among them, compound **20** showed potent α-glucosidase inhibitory activity with an IC_{50} value of 1.90 μM, being approximately 50 and 200 fold than oleanolic acid (IC_{50}=98.5 μM) and arcarbose (IC_{50}=388.0 μM)), respectively.

Conclusion

In conclusion, we discovered a series of novel, potent α-glucosidase inhibitors derived from oleanolic acid incorporating various cinnamic amide units. Among them, compound **20** (IC_{50}=1.9 μM) possessed the strongest α-glucosidase inhibitory activity, with 200-fold greater efficacy than a typical α-glucosidase inhibitor, acarbose. These α-glucosidase inhibitors should be useful lead compounds for the development of medicaments to treat diabetic and/or obese patients. Further investigation of the structure–activity relationships and development of superior α-glucosidase inhibitors are in progress.

Experimental

Chemistry NMR spectra were recorded on a Bruker ACF-500 NMR instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz) and chemical shifts are reported as ppm from an internal standard tetramethylsilane (TMS). IR spectra were obtained on a Brucker Tensor 27 spectrophotometer. Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD ion-trap mass spectrometer [electrospray ionization mass spectrum (ESI-MS)]. Elemental analysis (EA) was carried out on a Perkin-Elmer 2400 C, H, N analyzer. All commercially available solvents and reagents were used without further purification. The starting oleanolic acid with 98% purity was purchased from Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, China).

(3-Acetoxyolean-12-en-28-yl)(piperazin-1-yl)methanone (4) 3-Acetoxyolean-12-en-28-oic acid (100 mg) was dissolved in diethyl ether (2 ml) and oxalyl chloride (0.1 ml) was added dropwise slowly, and the solution was stirred at room temperature for 4 h. The solvent was removed under reduced pressure using a rotary evaporator. The residue was 3-acetoxyolean-12-en-28-carbonyl chloride (**3**). Piperazine (40 mg), triethylamine (0.5 ml) and 4-dimethylaminopyridine (DMAP) (20 mg) were dissolved in CH_2Cl_2 (5 ml), then the CH_2Cl_2 solution of **3** was added to mixture and stirred at room temperature for 5 h. The solvent was removed under reduced pressure using a rotary evaporator. The residue was washed with water, and evaporated to dryness. The residue was purified by column chromatography on silica gel to give **4** as a white solid (80 mg, 70.4%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.66 (3H, s), 0.81 (6H, s), 0.88 (9H, s), 1.04 (3H, s) (7×CH_3), 2.95 (1H, br d, J=10.8 Hz, H-18), 4.40 (1H, br d, J=10.8 Hz, H-3), 5.08

(1H, br s, H-12), 2.00 (3H, s, 3-OAc), 3.51 (4H, m, piperazine-H-2, 6), 2.73 (4H, m, piperazine-H-3, 5); ESI-MS: 567 $[M+H]^+$.

(3-Acetoxyolean-12-en-28-yl)(4-cinnamamidopiperazin-1-yl)methanone (5b) Cinnamic acid (60 mg) was dissolved in diethyl ether (2 ml), oxalyl chloride (0.4 ml) and DMF (1 d) was added dropwise slowly, and the solution was stirred at room temperature for 2 h. The solvent was removed under reduced pressure using a rotary evaporator. The residue was cinnamoyl chloride.

4 (100 mg), triethylamine (0.5 ml) and DMAP (20 mg) were dissolved in CH_2Cl_2 (5 ml), then the CH_2Cl_2 solution of cinnamoyl chloride was added to mixture and stirred at room temperature for 5 h. The solvent was removed under reduced pressure using a rotary evaporator. The residue was washed with water, and evaporated to dryness.

The residue was purified by column chromatography on silica gel to give **5b** as white solids (102 mg, 82.9%). ^{1}H-NMR (500 MHz, DMSO-d_6), δ: 0.66 (3H, s), 0.80 (6H, s), 0.88 (9H, s), 1.10 (3H, s) (7×CH_3), 2.98 (1H, br d, J=12.3 Hz, H-18), 4.38 (1H, dd, J=10.8, 3.9 Hz, H-3), 5.10 (1H, br s, H-12), 2.00 (3H, s, 3-OAc), 3.48—3.80 (8H, m, piperazine-H), 7.24 (1H, d, J=15.3 Hz, H-8′), 7.50 (1H, d, J=15.3 Hz, H-7′), 7.71 (2H, m, H-2′, 6′), 7.40 (3H, m, H-3′, 4′, 5′); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.7 (C-28), 170.6 (C-10′), 165.2 (C-7′), 145.2 (C-13), 142.1 (C-5′), 135.6 (C-1′), 130.1 (C-4′), 129.2 (C-2′), 128.5 (C-3′), 121.2 (C-12), 118.6 (C-6′), 80.4 (C-3), 55.1 (C-5), 47.4 (C-9′), 47.2 (C-8′), 46.4 (C-19), 45.5 (C-9), 43.6 (C-17), 42.2 (C-14), 41.9 (C-18), 39.2 (C-8), 38.0 (C-4), 37.7 (C-1), 37.0 (C-10), 33.9 (C-21), 33.3 (C-29), 32.7 (C-7), 30.5 (C-22), 29.6 (C-20), 28.2 (C-15), 28.2 (C-23), 27.9 (C-2), 26.1 (C-27), 24.3 (C-16), 23.7 (C-30), 23.3 (C-11), 21.4 (C-11′), 18.2 (C-6), 17.1 (C-26), 16.9 (C-24), 15.6 (C-25); IR (cm^{-1}): 3457, 2946, 1732, 1649, 1618, 1247, 1005, 763; ESI-MS: 697 $[M+H]^+$; *Anal.* (%) Calcd for $C_{45}H_{64}N_2O_4$: C 77.54, H 9.26, N 4.02. Found: C 77.85, H 9.29, N 4.03.

(3-Hydroxyolean-12-en-28-yl)(4-cinnamamidopiperazin-1-yl)methanone (5a) To a solution of **5b** (50 mg) in CH_3OH/THF (4 : 3, 3 ml) was added 4 N NaOH (0.5 ml). The reaction mixture was allowed to stir at room temperature for 24 h, and the solution was acidified with 10% HCl and filtered. The residue was washed with water, and vacuum dried to give **5a** as a white solid (46.5 mg, 99%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.65 (3H, s), 0.66 (3H, s), 0.84 (3H, s), 0.88 (9H, s), 1.11 (3H, s) (7×CH_3), 2.98 (1H, d, J=11.7 Hz, H-18), 4.28 (1H, dd, J=10.8, 3.9 Hz, H-3), 5.10 (1H, br s, H-12), 3.48—3.80 (8H, m, piperazine-H), 7.24 (1H, d, J=15.6 Hz, H-8′), 7.50 (1H, d, J=15.6 Hz, H-7′), 7.72 (2H, m, H-2′, 6′), 7.41 (3H, m, H-3′, 4′ and 5′); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.7 (C-28), 165.2 (C-7′), 145.1 (C-13), 142.1 (C-5′), 135.6 (C-1′), 130.0 (C-4′), 129.2 (C-2′), 128.5 (C-3′), 121.3 (C-12), 118.6 (C-6′), 77.3 (C-3), 55.4 (C-5), 47.7 (C-9′), 47.2 (C-17), 47.2 (C-8′), 46.4 (C-19), 45.5 (C-9), 43.5 (C-14), 41.9 (C-18), 39.2 (C-8), 38.8 (C-1), 38.5 (C-4), 37.1 (C-10), 33.8 (C-21), 33.3 (C-29), 32.9 (C-7), 30.5 (C-22), 29.6 (C-20), 28.7 (C-23), 27.8 (C-2), 27.4 (C-15), 26.2 (C-27), 25.6 (C-30), 24.4 (C-16), 23.4 (C-11), 18.4 (C-6), 17.0 (C-26), 16.5 (C-24), 15.6 (C-25); IR (cm^{-1}): 3435, 2943, 1646, 1619, 1202, 1003, 764; ESI-MS: 655 $[M+H]^+$; *Anal.* (%) Calcd for $C_{43}H_{62}N_2O_3$: C 78.85, H 9.54, N 4.28. Found: C 79.16, H 9.57, N 4.29.

(3-Acetoxyolean-12-en-28-yl)[4-(4′-fluoro)cinnamamidopiperazin-1-yl]methanone (6b) Following the procedure described for preparation of **5b**, compound **6b** was prepared from **4** and 4-fluorocinnamic acid as white solid **6b** (107.2 mg, 84.9%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.66 (3H, s), 0.80 (6H, s), 0.88 (9H, s), 1.11 (3H, s) (7×CH_3), 2.97 (1H, d, J=11.7 Hz, H-18), 4.38 (1H, dd, J=10.8, 3.9 Hz, H-3), 5.10 (1H, br s, H-12), 2.00 (3H, s, 3-OAc), 3.40—3.80 (8H, m, piperazine-H), 7.16 (1H, d, J=15.6 Hz, H-8′), 7.50 (1H, d, J=15.6 Hz, H-7′), 7.76 (2H, m, H-2′, 6′), 7.25 (2H, m, H-3′, 5′); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.7 (C-28), 170.6 (C-10′), 165.1 (C-7′), 163.3 (C-4′), 145.2 (C-13), 140.8 (C-5′), 132.2 (C-1′), 130.7 (C-2′), 121.2 (C-12), 118.5 (C-6′), 116.1 (C-3′), 80.4 (C-3), 55.1 (C-5), 47.4 (C-9′), 47.3 (C-17), 47.2 (C-8′), 46.4 (C-19), 45.4 (C-9), 43.6 (C-14), 41.9 (C-18), 39.2 (C-8), 38.0 (C-4), 37.7 (C-1), 37.0 (C-10), 33.9 (C-21), 33.3 (C-29), 32.7 (C-7), 30.5 (C-22), 29.7 (C-20), 28.2 (C-15), 28.2 (C-23), 27.9 (C-2), 26.1 (C-27), 24.4 (C-16), 23.7 (C-30), 23.3 (C-11), 21.4 (C-11′), 18.2 (C-6), 17.0 (C-26), 16.9 (C-24), 15.6 (C-25); IR (cm^{-1}): 3450, 2947, 1733, 1650, 1247, 1006, 827; ESI-MS: 715 $[M+H]^+$; *Anal.* (%) Calcd for $C_{45}H_{63}FN_2O_4$: C 75.59, H 8.88, N 3.92. Found: C 75.36, H 8.85, N 3.90.

(3-Hydroxyolean-12-en-28-yl)[4-(4′-fluoro)cinnamamidopiperazin-1-yl]methanone (6a) Following the procedure described for preparation of **5a**, compound **6a** was prepared from **6b** as white solid **6a** (45.3 mg, 95.7%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.65 (3H, s), 0.66 (3H, s), 0.84 (3H, s), 0.88 (9H, s), 1.09 (3H, s) (7×CH_3), 2.97 (1H, d, J=9 Hz, H-18), 4.29 (t, 1H, J=4.8 Hz, H-3), 5.10 (br s, H-12), 3.50—3.70 (m, 8H, piperazine-H), 7.21 (1H, d, J=15.3 Hz, H-8′), 7.50 (1H, d, J=15. Hz, H-7′), 7.79 (2H, m, H-2′, 6′), 7.25 (2H, m, H-3′, 5′); ^{13}C-NMR (125 MHz, DMSO-d_6), δ: 174.0 (C-28), 165.1 (C-7′), 162.3 (C-4′), 145.2 (C-13), 140.9 (C-5′), 132.3 (C-1′), 130.7 (C-2′), 121.3 (C-12), 118.6 (C-6′), 116.2 (C-3′), 77.3 (C-3), 55.3 (C-5), 47.7 (C-9′), 47.3 (C-17), 47.2 (C-8′), 46.4 (C-19), 45.4 (C-9), 43.5 (C-14), 41.9 (C-18), 39.2 (C-8), 38.8 (C-1), 38.6 (C-4), 37.1 (C-10), 33.9 (C-21), 33.3 (C-29), 32.9 (C-7), 30.5 (C-22), 29.6 (C-20), 28.7 (C-23), 28.6 (C-15), 27.9 (C-30), 27.4 (C-2), 26.2 (C-27), 24.3 (C-16), 23.4 (C-11), 18.4 (C-6), 16.9 (C-26), 16.5 (C-24), 15.6 (C-25); IR (cm^{-1}): 3451, 2944, 2865, 1649, 1225, 1003, 827; ESI-MS: 673 $[M+H]^+$; *Anal.* (%) Calcd for $C_{43}H_{61}FN_2O_3$: C 76.75, H 9.14, N 4.16. Found: C 76.59, H 9.12, N 4.15.

(3-Acetoxyolean-12-en-28-yl)[4-(4′-methoxy)cinnamamidopiperazin-1-yl]methanone (7b) Following the procedure described for preparation of **5b**, compound **7b** was prepared from **4** and 4-methoxylcinnamic acid as white solid (102.4 mg, 80.0%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.66 (3H, s), 0.80 (6H, s), 0.88 (9H, s), 1.10 (3H, s) (7×CH_3), 2.97 (1H, d, J=12.0 Hz, H-18), 4.38 (1H, dd, J=10.8 Hz, H-3), 5.10 (1H, br s, H-12), 2.00 (3H, s, 3-OAc), 3.40—3.70 (8H, m, piperazine-H), 7.09 (1H, d, J=15.3 Hz, H-8′), 7.46 (1H, d, J=15.3 Hz, H-7′), 7.66 (2H, d, J=8.7 Hz, H-2′, 6′), 6.96 (2H, d, J=8.7 Hz, H-3′, 5′), 3.79 (3H, s, OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6), δ: 174.7 (C-28), 170.6 (C-10′), 165.4 (C-7′), 160.9 (C-4′), 145.2 (C-13), 142.0 (C-5′), 130.1 (C-2′), 128.2 (C-1′), 121.2 (C-12), 115.9 (C-6′), 114.7 (C-3′), 80.4 (C-3), 55.8 (–OCH_3), 55.1 (C-5), 47.4 (C-9′), 47.2 (C-17), 47.2 (C-8′), 46.4 (C-19), 45.2 (C-9), 43.5 (C-14), 41.9 (C-18), 39.2 (C-8), 38.0 (C-4), 37.7 (C-1), 37.0 (C-10), 33.9 (C-21), 33.3 (C-29), 32.7 (C-7), 30.5 (C-22), 29.6 (C-20), 28.2 (C-23), 28.0 (C-15), 27.8 (C-2), 26.1 (C-27), 24.3 (C-16), 23.6 (C-30), 23.3 (C-11), 21.4 (C-11′), 18.2 (C-6), 17.1 (C-26), 16.9 (C-24), 15.6 (C-25); IR (cm^{-1}): 3450, 2947, 1734, 1650, 1605, 1515, 1247, 1175; ESI-MS: 727 $[M+H]^+$; *Anal.* (%) Calcd for $C_{46}H_{66}N_2O_5$, C 75.99, H 9.15, N 3.85. Found: C 76.10, H 9.16, N 3.86.

(3-Hydroxyolean-12-en-28-yl)[4-(4′-methoxy)cinnamamidopiperazin-1-yl]methanone (7a) Following the procedure described for preparation of **5a**, compound **7a** was prepared from **7b** as white solid (42.4 mg, 90.2%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.65 (3H, s), 0.66 (3H, s), 0.84 (3H, s), 0.88 (9H, s), 1.09 (3H, s) (7×CH_3), 2.97 (1H, d, J=9.3 Hz, H-18), 4.28 (1H, dd, J=5.1 Hz, H-3), 5.10 (1H, br s, H-12), 3.40—3.80 (8H, m, piperazine-H), 7.09 (1H, d, J=15.3 Hz, H-8′), 7.46 (1H, d, J=15.3 Hz, H-7′), 7.66 (2H, d, J=8.7 Hz, H-2′, 6′), 6.96 (2H, d, J=8.7 Hz, H-3′, 5′), 3.79 (3H, s, OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6), δ: 174.7 (C-28), 165.4 (C-7′), 160.9 (C-4′), 145.2 (C-13), 142.0 (C-5′), 130.1 (C-2′), 128.2 (C-1′), 121.3 (C-12), 115.9 (C-6′), 114.7 (C-3′), 77.3 (C-3), 55.7 (-OCH_3), 55.4 (C-5), 47.7 (C-9′), 47.2 (C-17), 47.2 (C-8′), 46.4 (C-19), 45.4 (C-9), 43.6 (C-14), 41.9 (C-18), 39.2 (C-8), 38.8 (C-1), 38.6 (C-4), 37.1 (C-10), 33.9 (C-21), 33.3 (C-29), 32.9 (C-7), 30.5 (C-22), 29.6 (C-20), 28.7 (C-15), 28.7 (C-23), 27.9 (C-30), 27.4 (C-2), 26.0 (C-27), 24.3 (C-16), 23.4 (C-11), 18.4 (C-6), 17.0 (C-26), 16.4 (C-24), 15.6 (C-25); IR (cm^{-1}): 3442, 2941, 1644, 1604, 1513, 1254, 1173; ESI-MS: 685 $[M+H]^+$; *Anal.* (%) Calcd for $C_{44}H_{64}N_2O_4$, C 77.15, H 9.42, N 4.09. Found: C 76.92, H 9.40, N 4.08.

(3-Acetoxyolean-12-en-28-yl)[4-(2′,3′-dichloro)cinnamamidopiperazin-1-yl]methanone (8b) Following the procedure described for preparation of **5b**, compound **8b** was prepared from **4** and 2,3-dichlorocinnamic acid as white solid (116.1 mg, 86.0%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.64 (3H, s), 0.79 (6H, s), 0.87 (9H, s), 1.09 (3H, s) (7×CH_3), 2.97 (1H, d, J=11.4 Hz, H-18), 4.37 (1H, dd, J=10.8, 4.5 Hz, H-3), 5.09 (1H, br s, H-12), 1.99 (3H, s, 3-OAc), 3.40—3.80 (8H, m, piperazine-H), 7.33 (1H, d, J=15.3 Hz, H-8′), 7.82 (1H, d, J=15.3 Hz, H-7′), 7.98 (1H, dd, J=7.8, 1.2 Hz, H-4′), 7.42 (1H, t, J=7.8 Hz, H-5′), 7.67 (1H, dd, J=7.8, 1.2 Hz, H-6′); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.8 (C-28), 170.6 (C-12′), 164.5 (C-9′), 145.2 (C-13), 137.0 (C-7′), 135.9 (C-1′), 132.9 (C-3′), 131.6 (C-2′), 131.6 (C-4′), 128.8 (C-5′), 127.3 (C-6′), 123.2 (C-8′), 121.2 (C-12), 80.4 (C-3), 55.1 (C-5), 47.4 (C-11′), 47.2 (C-17), 47.2 (C-10′), 46.4 (C-19), 45.5 (C-9), 43.6 (C-14), 41.9 (C-18), 39.2 (C-8), 38.0 (C-4), 37.7 (C-1), 37.0 (C-10), 33.9 (C-21), 33.3 (C-29), 32.7 (C-7), 30.5 (C-22), 29.6 (C-20), 28.2 (C-15), 28.2 (C-23), 27.9 (C-2), 26.2 (C-27), 24.3 (C-16), 23.7 (C-30), 23.3 (C-11), 21.4 (C-13′), 18.2 (C-6), 17.1 (C-26), 16.9 (C-24), 15.6 (C-25); IR (cm^{-1}): 3443, 2946, 1731, 1648, 1248; ESI-MS: 765 $[M+H]^+$; *Anal.* (%) Calcd for $C_{45}H_{62}Cl_2N_2O_4$: C 70.57, H 8.16, N 3.66. Found: C 70.85, H 8.19, N 3.67.

(3-Hydroxyolean-12-en-28-yl)[4-(2′,3′-dichloro)cinnamamidopiperazin-1-yl]methanone (8a) Following the procedure described for preparation of **5a**, compound **8a** was prepared from **8b** as white solid (43.9 mg, 92.8%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.65 (6H, s), 0.83 (3H, s), 0.88 (9H, s), 1.09 (3H, s) (7×CH_3), 2.99 (1H, m, H-18), 4.28 (1H, d, J=4.5 Hz, H-3), 5.09 (1H, br s, H-12), 3.40—3.80 (8H, m, piperazine-H), 7.33 (1H, d,

J=15.3 Hz, H-8′), 7.82 (1H, d, J=15.3 Hz, H-7′), 7.98 (1H, d, J=8.1 Hz, H-4′), 7.43 (1H, t, J=7.8 Hz, H-5′), 7.68 (1H, d, J=8.1 Hz, H-6′); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.8 (C-28), 164.5 (C-9′), 145.1 (C-13), 137.0 (C-7′), 135.9 (C-1′), 132.9 (C-3′), 131.6 (C-2′), 131.6 (C-4′), 128.8 (C-5′), 127.3 (C-6′), 123.2 (C-8′), 121.3 (C-12), 77.3 (C-3), 55.4 (C-5), 47.7 (C-11′), 47.2 (C-17), 47.2 (C-10′), 46.4 (C-19), 45.4 (C-9), 43.6 (C-14), 41.9 (C-18), 39.2 (C-8), 38.9 (C-1), 38.5 (C-4), 37.1 (C-10), 33.9 (C-21), 33.3 (C-29), 32.9 (C-7), 30.5 (C-22), 29.6 (C-20), 28.7 (C-15), 28.7 (C-23), 27.8 (C-30), 27.4 (C-2), 26.2 (C-27), 24.3 (C-16), 23.4 (C-11), 18.4 (C-6), 16.9 (C-26), 16.5 (C-24), 15.6 (C-25); IR (cm^{-1}): 3451, 2944, 2864, 1647, 1249; ESI-MS: 723 [M+H]$^+$; *Anal.* (%) Calcd for $C_{43}H_{60}Cl_2N_2O_3$, C 71.35, H 8.35, N 3.87. Found: C 71.06, H 8.31, N 3.85.

(3-Acetoxyolean-12-en-28-yl){4-[3′-(6″-methoxynaphthalen-2′-yl)acrylamido]piperazin-1-yl}methanone (9b) Following the procedure described for preparation of **5b**, compound **9b** was prepared from **4** and 3-(6-methoxynaphthalen-2-yl) acrylic acid as white solid (106.9 mg, 78.0%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.67 (3H, s), 0.79 (6H, s), 0.88 (9H, s), 1.10 (3H, s) (7×CH_3), 2.98 (1H, d, J=12.9 Hz, H-18), 4.38 (1H, dd, J=11.4, 3.6 Hz, H-3), 5.09 (1H, br s, H-12), 1.99 (3H, s, 3-OAc), 3.50—3.80 (8H, m, piperazine-H), 7.30 (1H, d, J=15.3 Hz, H-10′), 7.63 (1H, d, J=15.3 Hz, H-9′), 7.82—7.91 (3H, m, H-3′, 4′ and 8′), 7.19 (1H, d, J=9.0 Hz, H-7′), 7.35 (1H, s, H-5′), 8.08 (1H, s, H-1′), 3.89 (3H, s, 6′-OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.7 (C-28), 170.6 (C-16′), 165.3 (C-13′), 158.6 (C-6′), 145.2 (C-13), 142.4 (C-11′), 135.5 (C-8′), 131.0 (C-1′), 130.0 (C-4′), 129.4 (C-2′), 128.8 (C-3′), 127.7 (C-9′), 125.2 (C-10′), 121.1 (C-12), 119.6 (C-5′), 117.6 (C-12′), 106.7 (C-7′), 80.4 (C-3), 55.8 (-OCH_3), 55.1 (C-5), 47.4 (C-15′), 47.2 (C-17), 47.2 (C-14′), 46.4 (C-19), 45.5 (C-9), 43.6 (C-14), 41.9 (C-18), 39.0 (C-8), 38.0 (C-4), 37.7 (C-1), 37.0 (C-10), 33.9 (C-21), 33.3 (C-29), 32.7 (C-7), 30.5 (C-22), 29.6 (C-20), 28.2 (C-15), 28.2 (C-23), 27.9 (C-2), 26.2 (C-27), 24.4 (C-16), 23.6 (C-30), 23.3 (C-11), 21.4 (C-17′), 18.2 (C-6), 17.1 (C-26), 16.9 (C-24), 15.6 (C-25); IR (cm^{-1}): 3452, 2947, 1734, 1632, 1249, 1030, 849; ESI-MS: 777 [M+H]$^+$; *Anal.* (%) Calcd for $C_{50}H_{68}N_2O_5$, C 77.28, H 8.82, N 3.60. Found: C 77.04, H 8.79, N 3.58.

(3-Hydroxyolean-12-en-28-yl){4-[3′-(6″-methoxynaphthalen-2′-yl)acrylamido]piperazin-1-yl}methanone (9a) Following the procedure described for preparation of **5a**, compound **9a** was prepared from **9b** as white solid (41.4 mg, 88.1%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.65 (6H, s), 0.83 (3H, s), 0.88 (9H, s), 1.09 (3H, s) (7×CH_3), 2.98 (1H, d, J=10.2 Hz, H-18), 4.28 (1H, d, J=4.8 Hz, H-3), 5.10 (1H, br s, H-12), 3.40—3.80 (8H, m, piperazine-H), 7.30 (1H, d, J=15. 3 Hz, H-10′), 7.63 (1H, d, J=15.3 Hz, H-9′), 7.82—7.92 (3H, m, H-3′, 4′, 8′), 7.20 (1H, d, J=9.0 Hz, H-7′), 7.35 (1H, s, H-5′), 8.08 (1H, s, H-1′), 3.89 (3H, s, 6′-OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.7 (C-28), 165.3 (C-13′), 158.6 (C-6′), 145.1 (C-13), 142.4 (C-11′), 135.5 (C-8′), 131.0 (C-1′), 130.3 (C-4′), 129.4 (C-2′), 128.8 (C-3′), 127.7 (C-9′), 125.2 (C-10′), 121.3 (C-12), 119.6 (C-5′), 117.6 (C-12′), 106.7 (C-7′), 77.3 (C-3), 55.7 (–OCH_3), 55.4 (C-5), 47.7 (C-15′), 47.2 (C-17), 47.2 (C-14′), 46.5 (C-19), 45.5 (C-9), 43.6 (C-14), 41.9 (C-18), 39.2 (C-8), 38.8 (C-1), 38.5 (C-4), 37.1 (C-10), 33.9 (C-21), 33.3 (C-29), 32.9 (C-7), 30.5 (C-22), 29.7 (C-20), 28.7 (C-15), 28.7 (C-23), 27.9 (C-2), 27.4 (C-30), 26.2 (C-27), 24.3 (C-16), 23.4 (C-11), 18.4 (C-6), 17.0 (C-26), 16.5 (C-24), 15.6 (C-25); IR (cm^{-1}): 3426, 2942, 2863, 1628, 1260; ESI-MS: 735 [M+H]$^+$; *Anal.* (%) Calcd for $C_{48}H_{66}N_2O_4$, C 78.43, H 9.05, N 3.81. Found: C 78.66, H 9.07, N 3.82.

(3-Acetoxyolean-12-en-28-yl)[4-(4′-methylsulfonyl)cinnamamidopiperazin-1-yl]methanone (10b) Following the procedure described for preparation of **5b**, compound **10b** was prepared from **4** and 4-methylsulfonylcinnamic acid as white solid (115.8 mg, 85.8%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.66 (3H, s), 0.80 (6H, s), 0.88 (9H, s), 1.11 (3H, s) (7×CH_3), 2.98 (1H, d, J=11.1 Hz, H-18), 4.38 (1H, dd, J=12.0, 4.5 Hz, H-3), 5.10 (1H, br s, H-12), 2.00 (3H, s, 3-OAc), 3.40—3.80 (8H, m, piperazine-H), 7.44 (1H, d, J=15.3 Hz, H-8′), 7.57 (1H, d, J=15.3 Hz, H-7′), 7.94 (2H, d, J=8.4 Hz, H-2′, 6′), 7.99 (2H, d, J=8.4 Hz, H-3′, 5′), 3.35 (3H, s, –SO_2CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.7 (C-28), 170.6 (C-10′), 164.7 (C-7′), 145.2 (C-13), 141.5 (C-5′), 140.5 (C-4′), 140.0 (C-1′), 129.1 (C-3′), 127.8 (C-2′), 122.3 (C-6′), 121.2 (C-12), 80.4 (C-3), 55.1 (C-5), 47.4 (C-9′), 47.2 (C-17), 47.2 (C-8′), 46.4 (C-19), 45.5 (C-9), 43.9 (–CH_3), 43.6 (C-14), 41.9 (C-18), 39.2 (C-8), 38.0 (C-4), 37.7 (C-1), 37.0 (C-10), 33.9 (C-21), 33.3 (C-29), 32.7 (C-7), 30.5 (C-22), 29.6 (C-20), 28.2 (C-15), 28.2 (C-23), 27.9 (C-2), 26.2 (C-27), 24.3 (C-16), 23.6 (C-30), 23.4 (C-11), 21.4 (C-11′), 18.2 (C-6), 17.1 (C-26), 16.9 (C-24), 15.6 (C-25); IR (cm^{-1}): 3487, 2946, 1732, 1651, 1247, 1150, 827; ESI-MS: 775 [M+H]$^+$; *Anal.* (%) Calcd for $C_{46}H_{66}N_2O_6S$, C 71.28, H 8.58, N 3.61, S 4.14. Found: C 71.20, H 8.57, N 3.62, S 4.13.

(3-Hydroxyolean-12-en-28-yl)[4-(4′-methylsulfonyl)cinnamamidopiperazin-1-yl]methanone (10a) Following the procedure described for preparation of **5a**, compound **10a** was prepared from **10b** as white solid (44.4 mg, 94.5%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.66 (6H, s), 0.84 (3H, s), 0.88 (9H, s), 1.09 (3H, s) (7×CH_3), 2.98 (1H, d, J=10.5 Hz, H-18), 4.28 (1H, d, J=4.8 Hz, H-3), 5.10 (1H, br s, H-12), 3.40—3.80 (8H, m, piperazine-H), 7.44 (1H, d, J=15.3 Hz, H-8′), 7.57 (1H, d, J=15.3 Hz, H-7′), 7.94 (2H, d, J=8.4 Hz, H-2′, 6′), 7.99 (2H, d, J=8.4 Hz, H-3′, 5′), 3.25 (3H, s, –SO_2CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.7 (C-28), 164.7 (C-7′), 145.1 (C-13), 141.5 (C-5′), 140.5 (C-4′), 140.0 (C-1′), 129.1 (C-3′), 127.8 (C-2′), 122.3 (C-6′), 121.3 (C-12), 77.3 (C-3), 55.4 (C-5), 47.7 (C-9′), 47.2 (C-17), 47.2 (C-8′), 46.4 (C-19), 45.5 (C-9), 43.9 (–CH_3), 43.6 (C-14), 41.9 (C-18), 39.2 (C-8), 38.8 (C-1), 38.6 (C-4), 37.1 (C-10), 33.9 (C-21), 33.3 (C-29), 32.9 (C-7), 30.5 (C-22), 29.7 (C-20), 28.7 (C-15), 28.7 (C-23), 27.9 (C-30), 27.4 (C-2), 26.2 (C-27), 24.3 (C-16), 23.4 (C-11), 18.4 (C-6), 17.0 (C-26), 16.5 (C-24), 15.6 (C-25); IR (cm^{-1}): 3443, 2946, 1647, 1306, 1150; ESI-MS: 733 [M+H]$^+$; *Anal.* (%) Calcd for $C_{44}H_{64}N_2O_5S$, C 72.09, H 8.80, N 3.82, S 4.37. Found: C 72.23, H 8.82, N 3.81, S 4.36.

(3-Acetoxyolean-12-en-28-yl)[4-(4′-nitro)cinnamamidopiperazin-1-yl]methanone (11b) Following the procedure described for preparation of **5b**, compound **11b** was prepared from **4** and 4-nitrocinnamic acide as white solid (119.1 mg, 90.9%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.66 (3H, s), 0.80 (6H, s), 0.88 (9H, s), 1.11 (3H, s) (7×CH_3), 2.97 (1H, d, J=14.4 Hz, H-18), 4.38 (1H, d, J=10.8 Hz, H-3), 5.10 (1H, br s, H-12), 2.00 (3H, s, 3-OAc), 3.40—3.80 (8H, m, piperazine-H), 7.48 (1H, d, J=15.6 Hz, H-8′), 7.60 (1H, d, J=15.6 Hz, H-7′), 8.01 (2H, d, J=8.7 Hz, H-2′, 6′), 8.26 (2H, d, J=8.7 Hz, H-3′, 5′); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.2 (C-28), 170.0 (C-10′), 164.0 (C-7′), 147.5 (C-4′), 144.6 (C-13), 141.6 (C-1′), 138.8 (C-5′), 128.9 (C-2′), 123.7 (C-3′), 122.8 (C-6′), 120.6 (C-12), 79.8 (C-3), 54.6 (C-5), 46.9 (C-9′), 46.7 (C-17), 46.7 (C-8′), 45.9 (C-19), 44.8 (C-9), 42.0 (C-14), 41.4 (C-18), 38.7 (C-8), 37.4 (C-4), 37.1 (C-1), 36.4 (C-10), 33.3 (C-21), 32.7 (C-29), 32.2 (C-7), 29.9 (C-22), 29.1 (C-20), 27.7 (C-15), 27.7 (C-23), 27.3 (C-2), 25.6 (C-27), 23.8 (C-16), 23.1 (C-30), 22.8 (C-11), 20.8 (C-11′), 17.6 (C-6), 16.5 (C-26), 16.4 (C-24), 15.0 (C-25); IR (cm^{-1}): 3450, 2946, 1732, 1651, 1344, 1247; ESI-MS: 742 [M+H]$^+$; *Anal.* (%) Calcd for $C_{45}H_{63}N_3O_6$, C 72.84, H 8.56, N 5.66. Found: C 72.55, H 8.54, N 5.67.

(3-Hydroxyolean-12-en-28-yl)[4-(4′-nitro)cinnamamidopiperazin-1-yl]methanone (11a) Following the procedure described for preparation of **5a**, compound **11a** was prepared from **11b** as white solid (45.3 mg, 96.0%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.66 (6H, s), 0.84 (3H, s), 0.88 (9H, s), 1.09 (3H, s) (7×CH_3), 2.98 (1H, d, J=8.4 Hz, H-18), 4.28 (1H, d, J=5.1 Hz, H-3), 5.10 (1H, br s, H-12), 3.40—3.80 (8H, m, piperazine-H), 7.48 (1H,d, J=15.6 Hz, H-8′), 7.60 (1H, d, J=15.6 Hz, H-7′), 8.01 (2H, d, J=8.7 Hz, H-2′, 6′), 8.25 (2H, d, J=8.7 Hz, H-3′, 5′); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.2 (C-28), 164.0 (C-7′), 147.4 (C-4′), 144.6 (C-13), 141.6 (C-1′), 138.9 (C-5′), 128.9 (C-2′), 123.8 (C-3′), 123.3 (C-12), 122.7 (C-6′), 76.7 (C-3), 54.8 (C-5), 47.1 (C-9′), 46.6 (C-17), 46.6 (C-8′), 45.8 (C-19), 45.0 (C-9), 43.0 (C-14), 41.3 (C-18), 38.6 (C-8), 38.2 (C-1), 38.0 (C-4), 36.5 (C-10), 33.3 (C-21), 32.7 (C-29), 32.3 (C-7), 30.0 (C-22), 29.0 (C-20), 28.1 (C-15), 28.1 (C-23), 27.2 (C-2), 26.8 (C-30), 25.6 (C-27), 23.7 (C-16), 22.8 (C-11), 17.8 (C-6), 16.4 (C-26), 15.9 (C-24), 15.0 (C-25); IR (cm^{-1}): 3445, 2944, 1620, 1521, 1344; ESI-MS: 700 [M+H]$^+$; *Anal.* (%) Calcd for $C_{43}H_{61}N_3O_5$, C 73.78, H 8.78, N 6.00. Found: C 73.92, H 8.76, N 6.01.

(3-Oxoolean-12-en-28-yl)(piperazin-1-yl)methanone (14) Following the procedure described for preparation of **4**, compound **14** was prepared from 3-oxoolean-12-en-28-oic acid (**12**) as white solid **14** (80 mg, 70.4%).

(3-Aminoolean-12-en-28-yl)(piperazin-1-yl)methanone (16) Oxime **14** (1 g) and CH_3COONH_4 (1.8 g) were dissolved in CH_3OH (66 ml) and stirred at room temperature, and $NaBH_3CN$ (1.4 g) was added to the stirred solution rapidly. Then 15% $TiCl_3$ (2.3 ml) was added dropwise slowly after the temperature was decreased to 0 °C. The reaction mixture was allowed to stir at room temperature for 12 h, and the solution was alkalified with 2 N NaOH to pH 10 and extracted with CH_2Cl_2. The CH_2Cl_2 layer was concentrated in *vacuo* to give **16** as a white solid (920 mg, 89%).

(3-Cinnamamidoolean-12-en-28-yl)(4-cinnamamidopiperazin-1-yl)methanone (17) Following the procedure described for preparation of **5b**, compound **17** were prepared from **16** and cinnamic acid as white solid (109.3 mg, 73.0%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.68 (3H, s), 0.77 (3H, s), 0.78 (3H, s), 0.89 (9H, s), 1.13 (3H, s) (7×CH_3), 2.99 (1H, br d, J=12.0 Hz, H-18), 3.76 (1H, m, H-3), 5.10 (1H, br s, H-12), 3.40—3.80 (8H, m, piperazine-H), 6.76 (1H, d, J=15.6 Hz, H-8′), 7.24 (1H, d, J=15.3 Hz, H-8″), 7.50 (1H, d, J=15.3 Hz, H-7″), 7.30—7.72 (11H, m, H-7′, and Ph-H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.1 (C-28), 164.6 (C-7″), 164.3 (C-7′), 144.5 (C-13), 141.4 (C-5′), 138.1 (C-5′′), 135.0 (C-1′),

135,0 (C-1″), 129.4 (C-4′), 129.0 (C-4″), 128.7 (C-2″), 128.6 (C-2′), 127.8 (C-3′), 127.2 (C-3′′), 127.2 (C-6″), 122.7 (C-12), 118.1 (C-6′), 55.8 (C-3), 55.4 (C-5), 47.0 (C-9′), 46.6 (C-17), 46.6 (C-8′), 45.8 (C-19), 44.8 (C-9), 43.0 (C-14), 41.3 (C-18), 38.6 (C-4), 38.6 (C-8), 37.6 (C-1), 36.4 (C-10), 33.3 (C-21), 32.6 (C-29), 32.2 (C-7), 30.0 (C-22), 29.0 (C-20), 28.3 (C-23), 27.9 (C-15), 27.2 (C-2), 25.5 (C-27), 24.8 (C-16), 23.7 (C-30), 22.7 (C-11), 18.0 (C-6), 16.7 (C-26), 16.3 (C-24), 15.0 (C-25); IR (cm^{-1}): 3441, 3360, 2944, 2863, 1648, 1620, 989, 975, 706, 685; ESI-MS: 784 $[M+H]^+$; *Anal.* (%) Calcd for $C_{52}H_{69}N_3O_3$, C 79.65, H 8.87, N 5.36. Found: C 79.41, H 8.89, N 5.35.

[3-(4′-Fluoro)cinnamamidoolean-12-en-28-yl][4-(4′-fluoro)cinnamamidopiperazin-1-yl]methanone (18) Following the procedure described for preparation of **5b**, compound **18** were prepared from **16** and 4-fluorocinnamic acid as white solid (111.2 mg, 71.0%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.67 (3H, s), 0.77 (3H, s), 0.78 (3H, s), 0.89 (9H, s), 1.13 (3H, s) (7×CH_3), 2.97 (1H, br d, J=10.7 Hz, H-18), 3.59 (1H, m, H-3), 5.12 (1H, br s, H-12), 3.40—3.80 (8H, m, piperazine-H), 6.71 (1H, d, J=15.6 Hz, H-8′), 7.39 (1H,d, J=15.6 Hz, H-7′), 7.50 (1H, d, J=15.3 Hz, H-7″), 7.18—7.28, 7.58—7.95 (9H, m, H-8″, Ph-H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.2 (C-28), 164.5 (C-7″), 164.2 (C-7′), 162.7 (C-4″), 162.4 (C-4′), 144.6 (C-13), 140.3 (C-5′), 137.0 (C-5″), 131.7 (C-1′), 131.6 (C-1″), 130.1 (C-2″), 129.5 (C-6″), 129.4 (C-2′), 122.6 (C-12), 118.0 (C-6′), 115.7 (C-3′), 115.6 (C-3′′), 55.8 (C-3), 55.5 (C-5), 47.0 (C-9′), 46.6 (C-17), 46.6 (C-8′), 45.9 (C-19), 44.8 (C-9), 43.0 (C-14), 41.4 (C-18), 38.6 (C-8), 37.6 (C-1), 36.7 (C-4), 36.5 (C-10), 33.3 (C-21), 32.7 (C-29), 32.2 (C-7), 30.0 (C-22), 29.1 (C-20), 28.4 (C-23), 28.0 (C-15), 27.2 (C-2), 25.6 (C-27), 24.8 (C-16), 23.8 (C-30), 23.1 (C-11), 18.1 (C-6), 16.8 (C-26), 16.4 (C-24), 15.0 (C-25); IR (cm^{-1}): 3442, 2945, 2864, 1650, 1626, 1601, 1510, 1226, 980, 828; ESI-MS: 820 $[M+H]^+$; *Anal.* (%) Calcd for $C_{52}H_{67}F_2N_3O_3$, C 76.16, H 8.23, N 5.12. Found: C 76.39, H 8.25, N 5.11.

[3-(4′-Methoxyl)cinnamamidoolean-12-en-28-yl][4-(4′-methoxyl)cinnamamidopiperazin-1-yl]mthanone (19) Following the procedure described for preparation of **5b**, compound **19** were prepared from **16** and 4-methoxylcinnamic acid as white solid (109.6 mg, 68.0%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.67 (3H, s), 0.76 (3H, s), 0.78 (3H, s), 0.89 (9H, s), 1.13 (3H, s) (7×CH_3), 2.99 (1H, br d, J=11.7 Hz, H-18), 3.59 (1H, m, H-3), 5.12 (1H, br s, H-12), 3.40—3.80 (8H, m, piperazine-H), 6.61 (1H, d, J=15.6 Hz, H-8′), 7.34 (1H, d, J=15.6 Hz, H-7′), 7.49 (2H, d, J=8.7 Hz, H-2′, 6′), 6.97 (2H, d, J=8.7 Hz, H-3′, 5′), 6.61 (1H, d, J=15.6 Hz, H-8″), 7.34 (1H, d, J=15.6 Hz, H-7′), 7.49 (2H, d, J=8.7 Hz, H-2′, 6′), 6.97 (2H, d, J=8.7 Hz, H-3′, 5′), 3.78, 3.79 (3H, s, –OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.2 (C-28), 164.9 (C-7″), 164.7 (C-7′), 160.3 (C-4″), 160.0 (C-4′), 144.6 (C-13), 141.4 (C-5′), 137.9 (C-5″), 129.5 (C-2′), 129.4 (C-1′), 128.8 (C-1″), 128.8 (C-2″), 127.7 (C-6″), 120.3 (C-12), 115.4 (C-6′), 114.3 (C-3″), 114.1 (C-3′), 55.8 (C-3), 55.6 (C-5), 55.2 (4′-OCH_3), 55.1 (4″-OCH_3), 47.1 (C-9′), 46.7 (C-17), 46.7 (C-8′), 46.0 (C-19), 45.0 (C-9), 43.0 (C-14), 41.4 (C-18), 38.8 (C-4), 38.7 (C-8), 37.7 (C-1), 36.5 (C-10), 33.4 (C-21), 32.7 (C-29), 32.3 (C-7), 30.0 (C-22), 29.2 (C-20), 28.4 (C-23), 28.0 (C-15), 27.3 (C-2), 25.6 (C-27), 24.9 (C-16), 23.8 (C-30), 23.2 (C-11), 18.1 (C-6), 16.8 (C-26), 16.4 (C-24), 15.0 (C-25); IR (cm^{-1}): 3442, 2943, 1645, 1603, 1512, 1254, 1173, 826; ESI-MS: 844 $[M+H]^+$; *Anal.* (%) Calcd for $C_{54}H_{73}N_3O_5$, C 76.83, H 8.72, N 4.98. Found: C 76.98, H 8.70, N 4.99.

[3-(2′,3′-Dichloro)cinnamamidoolean-12-en-28-yl][4-(2′,3′-dichloro)cinnamamidopiperazin-1-yl]methanone (20) Following the procedure described for preparation of **5b**, compound **20** were prepared from **16** and 2,3-dichloro cinnamic acid as white solid (108.9 mg, 62.3%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.66 (3H, s), 0.77 (3H, s), 0.78 (3H, s), 0.89 (9H, s), 1.12 (3H, s) (7×CH_3), 2.98 (1H, br d, J=10.2 Hz, H-18), 3.76 (1H, m, H-3), 5.11 (1H, br s, H-12), 3.40—3.80 (8H, m, piperazine-H), 6.85 (1H, d, J=15.6 Hz, H-8′), 7.71 (1H, d, J=15.6 Hz, H-7′), 7.33 (1H, d, J=15.3 Hz, H-8″), 7.82 (1H, d, J=15.3 Hz, H-7″), 7.98 (2H, d, J=7.8 Hz, H-4′, 4″), 7.43 (2H, t, J=8.1 Hz, H-5′, 5″), 7.67 (1H, t, J=6.3 Hz, H-6′, 6″); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.1 (C-28), 163.9 (C-9″), 163.6 (C-9′), 144.6 (C-13), 136.4 (C-7′), 135.5 (C-1′), 135.3 (C-1″), 133.6 (C-3′), 132.4 (C-4″), 132.3 (C-7″), 131.1 (C-4′), 131.0 (C-3″), 130.9 (C-2″), 130.8 (C-2′), 128.4 (C-8″), 128.2 (C-5′), 128.2 (C-5′′), 127.1 (C-6′), 126.0 (C-8′), 126.0 (C-6″), 122.7 (C-12), 56.0 (C-3), 55.4 (C-5), 47.0 (C-11′), 46.6 (C-17), 46.6 (C-10′), 45.8 (C-19), 44.9 (C-9), 43.0 (C-14), 41.3 (C-18), 40.0 (C-8), 38.6 (C-4), 37.8 (C-1), 36.5 (C-10), 33.3 (C-21), 32.7 (C-29), 32.2 (C-7), 30.0 (C-22), 29.1 (C-20), 28.4 (C-23), 28.0 (C-15), 27.3 (C-2), 25.6 (C-27), 24.7 (C-16), 23.8 (C-30), 22.8 (C-11), 18.0 (C-6), 16.7 (C-26), 16.3 (C-24), 15.0 (C-25); IR (cm^{-1}): 3440, 2945, 1650, 1613, 1453, 1409, 1183; ESI-MS: 920 $[M+H]^+$; *Anal.* (%) Calcd for $C_{52}H_{65}Cl_4N_3O_3$, C 67.75, H 7.11, N 4.56. Found: C 67.88, H 7.10, N 4.55.

{3-[3′-(6″-Methoxynaphthalen-2′-yl)acrylamido]olean-12-en-28-yl}{4-[3′-(6″-methoxynaphthalen-2′-yl)acrylamido]piperazin-1-yl}methanone (21) Following the procedure described for preparation of **5b**, compound **21** were prepared from **16** and 3-(6-methoxynaphthalen-2-yl) acrylic acid as white solid (106.4 mg, 59.1%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.66 (3H, s), 0.77 (3H, s), 0.79 (3H, s), 0.89 (9H, s), 1.13 (3H, s) (7×CH_3), 2.99 (1H, br d, J=12.9 Hz, H-18), 3.77 (1H, m, H-3), 5.12 (1H, br s, H-12), 3.40—3.80 (8H, m, piperazine-H), 6.83 (1H, d, J=15.6 Hz, H-10′), 7.51 (1H, d, J=15.6 Hz, H-9′), 7.31 (1H, d, J=15.3 Hz, H-10″), 7.64 (1H, d, J=15.3 Hz, H-9″), 7.82—7.91 (6H, m, H-4′, 8′, 3′, 4″, 8″, 3″), 7.20 (2H, dd, J=2.4, 9.0 Hz, H-7′, 7″), 7.68 (1H, s, H-3′), 7.33 (1H, s, H-5′), 7.97 (1H, s, H-1′), 8.08 (1H, s, H-1′), 3.93, 3.89 (6H, s, –OCH_3); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.0 (C-28), 164.7 (C-13″), 164.5 (C-13′), 158.0 (C-6′), 157.9 (C-6″), 144.6 (C-13), 141.8 (C-11′), 138.4 (C-11″), 134.8 (C-8″), 134.6 (C-8′), 130.3 (C-1′), 130.3 (C-1″), 129.7 (C-3′), 129.7 (C-3″), 128.8 (C-4′), 128.8 (C-4″), 128.3 (C-2″), 128.2 (C-2′), 128.1 (C-12″), 127.2 (C-9′), 127.1 (C-9″), 124.7 (C-10″), 124.0 (C-10′), 122.0 (C-12), 119.0 (C-5′), 119.0 (C-5″), 117.1 (C-12′), 106.1 (C-7′), 106.1 (C-7″), 55.8 (C-3), 55.5 (C-5), 55.2 (6′-OCH_3), 55.2 (6″-OCH_3), 47.0 (C-15′), 46.6 (C-17), 46.6 (C-14′), 45.9 (C-19), 44.8 (C-9), 43.1 (C-14), 41.4 (C-18), 40.2 (C-8), 38.6 (C-4), 37.7 (C-1), 36.5 (C-10), 33.3 (C-21), 32.7 (C-29), 32.2 (C-7), 30.0 (C-22), 29.0 (C-20), 28.4 (C-23), 28.1 (C-15), 27.2 (C-2), 25.8 (C-27), 23.8 (C-30), 23.2 (C-16), 22.8 (C-11), 18.1 (C-6), 16.8 (C-26), 16.4 (C-24), 15.0 (C-25); IR (cm^{-1}): 3435, 2941, 1625, 1601, 1390, 1261, 1175, 847; ESI-MS: 944 $[M+H]^+$; *Anal.* (%) Calcd for $C_{62}H_{77}N_3O_5$, C 78.86, H 8.22, N 4.45. Found: C 78.54, H 8.20, N 4.44.

[3-(4′-Nitro)cinnamamidoolean-12-en-28-yl][4-(4′-nitro)cinnamamidopiperazin-1-yl]methanone (22) Following the procedure described for preparation of **5b**, compound **22** were prepared from **16** and 4-nitrocinnamic acid as white solid (126.8 mg, 68.0%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.66 (3H, s), 0.77 (3H, s), 0.78 (3H, s), 0.89 (9H, s), 1.13 (3H, s) (7×CH_3), 2.99 (1H, br d, J=10.8 Hz, H-18), 3.74 (1H, m, H-3), 5.12 (1H, br s, H-12), 3.40—3.80 (8H, m, piperazine-H), 6.96 (1H, d, J=15.6 Hz, H-8′), 7.51 (1H, d, J=15.6 Hz, H-7′), 7.48 (1H, d, J=15.3 Hz, H-8″), 8.24—8.28 (4H, m, H-3′, 5′, 3″, 5″), 7.81 (2H, d, J=8.7 Hz, H-2′, 6′), 8.01 (2H, t, J=8.7 Hz, H-2″, 6″); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 174.2 (C-28), 164.0 (C-7″), 163.7 (C-7′), 147.5 (C-4″), 147.3 (C-4′), 144.6 (C-13), 141.7 (C-1′), 141.6 (C-1″), 138.9 (C-5′), 136.0 (C-5″), 128.9 (C-2′), 128.3 (C-2″), 127.0 (C-6″), 124.0 (C-3″), 123.8 (C-3′), 122.7 (C-6′), 120.6 (C-12), 56.0 (C-3), 55.4 (C-5), 47.0 (C-9′), 46.6 (C-17), 46.6 (C-8′), 45.9 (C-19), 45.0 (C-9), 41.7 (C-14), 41.4 (C-18), 40.0 (C-8), 38.6 (C-1), 37.7 (C-4), 36.5 (C-10), 33.3 (C-21), 32.7 (C-29), 32.2 (C-7), 30.0 (C-22), 29.1 (C-20), 28.3 (C-23), 28.0 (C-15), 27.2 (C-2), 25.6 (C-27), 24.8 (C-16), 23.8 (C-30), 22.8 (C-11), 18.0 (C-6), 16.7 (C-26), 16.4 (C-24), 15.0 (C-25); IR (cm^{-1}): 3431, 2943, 1618, 1520, 1343; ESI-MS: 874 $[M+H]^+$; *Anal.* (%) Calcd for $C_{52}H_{67}N_5O_7$, C 71.45, H 7.73, N 8.01. Found: C 71.74, H 7.72, N 8.03.

Enzyme Assay The α-glucosidase inhibition assay was performed according to the slightly modified method of Pierre *et al.*[12] α-Glucosidase (EC 3.2.1.20) was purchased from Sigma Co. (No. G-5003, Lot. 081k7415). The inhibition was measured spectro-photometrically at pH 6.8 and at 37 °C for 10 min, using 0.01 M *p*-nitrophenyl α-D-glucopyranoside (PNPG) as a substrate and 1 U/ml of enzyme, in 0.067 M KH_2PO_4–Na_2HPO_4 buffer. Oleanolic acid was used as positive controls. The increment in absorption at 410 nm due to the hydrolysis of PNPG by α-glucosidase was monitored continuously with an auto multi-functional microplate reader (BIORAD680).

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References

1) Moller D. E., *Nature* (London), **414**, 821—827 (2001).
2) Wen X. A., Sun H. B., Liu J., Wu G. Z., Zhang L. Y., Wu X. M., Ni P. Z., *Bioorg. Med. Chem. Lett.*, **15**, 4944—4948 (2005).
3) Wen X. A., Zhang P., Liu J., Zhang L., Wu X., Ni P., Sun H., *Bioorg. Med. Chem. Lett.*, **16**, 722—726 (2006).
4) Chen J., Liu J., Zhang L. Y., Wu G. Z., Hua W. Y., Wu X. M., Sun H. B., *Bioorg. Med. Chem. Lett.*, **16**, 2915—2919 (2006).
5) Wen X. A., Xia J., Cheng K. G., Zhang L. Y., Zhang P., Liu J., Zhang L. Y., Ni P. Z., Sun H. B., *Bioorg. Med. Chem. Lett.*, **17**, 5777—5782 (2007).
6) Zhang P., Hao J., Liu J., Lu Q., Sheng H. M., Zhang L. Y., Sun H. B.,

J. Nat. Prod., **72**, 1414—1418 (2009).
7) Luo J. G., Liu J., Kong L. Y., *Chem. Biodivers.*, **5**, 751—757 (2008).
8) Luo J. G., Ma L., Kong L. Y., *Bioorg. Med. Chem.*, **16**, 2912—2920 (2008).
9) Niwa T., Doi U., Osawa T., *J. Agric. Food Chem.*, **51**, 90—94 (2003).
10) Takada K., Uehara T., Nakao Y., Matsunaga S., van Soest Rob W. M., Fusetani N., *J. Am. Chem. Soc.*, **126**, 187—193 (2004).
11) Preecha P., Thanchanok P., Jonkolnee J., Udom K., *Bioorg. Med. Chem. Lett.*, **18**, 4956—4958 (2008).
12) Pierre C., Roland R., Tremblay D., *J. Clin. Chem.*, **24**, 208—211 (1978).