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Synthetic Analogues of Betulinic Acid as Potent Inhibitors of PS1/BACE1 Interaction to Reduce Aβ Generation

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The lupane-type triterpenoids are endowed with a wide range of biological activities such as antiviral, anti-inflammatory and anticancer activity. We describe here its potential application in Alzheimer's disease (AD) treatment as an inhibitor of PS1/BACE1 interaction. 3-α-Akebonoic acid, which emanated from a high throughput screening (HTS), was discovered to interfere with PS1/BACE1 interaction and reduce amyloid β-protein (Aβ) production. In view of the limited source, we instead used naturally rich betulinic acid (compound **2**) as starting material for lead optimization and a focused library of its derivatives was constructed to gain a better understanding of the structure activity relationship (SAR) of triterpenoid-type inhibitor of PS1/BACE1 interaction. Compound **22** was finally chosen as the most potent PS1/BACE1 interaction inhibitor, which reduced Aβ generation effectively.

Keywords Alzheimer's disease, PS1/BACE1 interaction, Aβ, betulinic acid, SAR

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide, which is characterized by progressive memory loss and severe cognitive dysfunction.[1] This devastating disease has escalated dramatically and caused staggering loss in the past few decades.[2] The amyloid β-protein (Aβ) is one of the major pathological hallmarks of AD, and mounting evidence shows that Aβ is the initiator of the disease process and drives the succedent molecular cascade.[3] Therefore, strategies for the discovery and development of AD therapeutics have primarily targeted Aβ.

The Aβ peptides are derived from the amyloid-*β* precursor protein (APP) and are sequentially cleaved by the *β*-site APP-cleaving enzyme 1 (BACE1) and *γ*-secretase.[4] Therefore, most efforts are focused on directly inhibiting or modulating the activity of BACE1 or *γ*-secretase to reduce Aβ generation.[5] However, these attempts have mostly fallen short, and currently no approved drugs have emerged due to either undesired side effects or insufficient efficacy. Considering that BACE1 and *γ*-secretase are both linked to a wide range of biological processes, direct inhibition or modulation of BACE1 or *γ*-secretase activity may cause unexpected side effects.[6] Thus, it is imperative to look for new strategies for the treatment of AD.

Previous studies have shown that PS1, the catalytic domain of *γ*-secretase, interacts with BACE1 directly and regulates its expression and activity.[7,8] Inspired by these studies, we hypothesized that the interaction between PS1 and BACE1 might be crucial for the cleavage of APP and that targeting the PS1/BACE1 interaction without affecting BACE1 or *γ*-secretase activities may be possible for reducing Aβ generation. In our previous work,[9] interaction-based high-throughput screening was performed, where a split-TEV assay was used to monitor the interaction of PS1 with BACE1. Finally, 3-α-akebonoic acid (compound **1**, Figure 1), a naturally occurring triterpenoid extracted from *Akebiaquinata*, was found not only interfered significantly with the PS1/BACE1 interaction but also reduced Aβ production. However, the rare natural source of hit compound **1** limited further in vivo studies, as well as detail SAR study of this novel natural triterpenoid PS1/BACE1 interaction inhibitor.

We then screened a naturally occurring triterpenoid library (Supplementary Table 1) and related derivatives to investigate whether other triterpenoids could interfere

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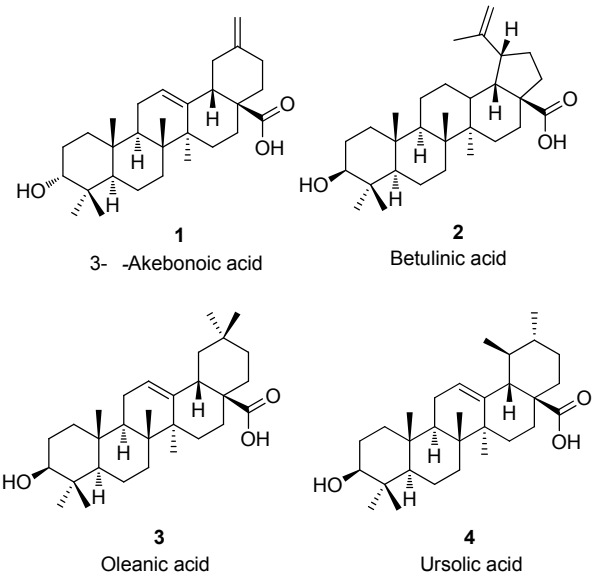


Figure 1 The structure of HTS hit 3-α-akebonoic acid and its naturally occurring anologues.

with the PS1/BACE1 interaction and inhibit the secretion of Aβ. Finally, a triterpenoid betulinic acid derived compound **22** showed improved potency and reduced cytotoxicity. Further photo-activated crosslinking and biochemical competition assays[9] indicated that compound **22** could bind to PS1 and interfered with the PS1/BACE1 interaction in the same way as HTS hit compound **1** without influencing secretase activities. Furthermore, treatment of APP/PS1 mice with compound **22** alleviated cognitive dysfunction and Aβ-related pathology. In this paper, we present the structure optimization, from the view of medicinal chemistry, to use naturally rich betulinic acid (compound **2**) as starting material and a focused library of its derivatives was constructed to gain a better understanding of the SAR triterpenoid-type inhibitor of PS1/BACE1 interaction. Compound **22** was finally obtained as the most potent PS1/BACE1 interaction inhibitor, which reduced Aβ generation effectively.

Experimental

All reagents were purchased from commercial sources and used without further purification. NMR spectra were recorded at 300 and 75 MHz for ^{1}H and ^{13}C nuclei, or at 500 and 125 MHz for for ^{1}H and ^{13}C nuclei, respectively. Chemical shifts are reported in parts per million relative to the tetramethylsilane peak recorded as δ 0.00 in $CDCl_3$/TMS solvent, or the residual chloroform (δ 7.26) or methanol (δ 3.31) peaks. The ^{13}C NMR values were referenced to the residual chloroform (δ 77.0), or methanol (δ 48.8) peaks. High resolution mass spectroscopy (HRMS) was performed on a TOF instrument with ESI in positive ionization mode.

General procedure A for synthesis of benzyl ester

Betulinic acid **2** (4 g, 8.76 mmol) and K_2CO_3 (2.4 g, 17.37 mmol) were dissolved in DMF (50 mL). After addition of benzyl chloride (1.2 mL, 10.52 mmol), the reaction mixture was stirred at 50 ℃ for 3 h. The mixture was cooled to room temperature, slowly diluted with H_2O (100 mL) and extracted with EtOAc (2×100 mL). The combined organic layers were washed with H_2O (2×150 mL), brine (150 mL), dried over Na_2SO_4 and evaporated under reduced pressure to obtain the desired compound **5** as a white solid. Yield: 4.62 g (8.46 mmol, 97%).

General procedure B for debenzylation

To a stirred solution of **7** (100 mg, 0.18 mmol) in MeOH (2 mL), Pd/C (10 mg) was added at ambient temperature under nitrogen atmosphere. The nitrogen atmosphere was replaced by the H_2 atmosphere. The reaction mixture was stirred for 1 h at room temperature, then H_2 was replaced with nitrogen and the mixture was filtered through Celite and washed with DCM. The residue was concentrated and purified by flash chromatography. Yield: 51 mg (0.11 mmol, 61%).

Compounds **8**—**10** were synthesized using similar methods.

Benzyl 3(*β*)-hydroxylup-28-oate (5) Compound **5** was synthesized following general procedure A. Yield: 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40—7.29 (m, 5H), 5.12 (q, J=12.3 Hz, 2H), 4.72 (s, 1H), 4.59 (s, 1H), 3.17 (dd, J=10.8, 5.1 Hz, 1H), 3.06—2.97 (m, 1H), 2.33—2.10 (m, 3H), 1.96—1.75 (m, 2H), 1.67 (s, 3H), 1.64—1.01 (m, the aliphatic ring protons), 0.95 (s, 3H), 0.94 (s, 3H), 0.80 (s, 3H), 0.76 (s, 3H), 0.75 (s, 3H).

Benzyl 3-carbonylup-28-oate (6) Compound **5** (4.62 g, 8.46 mmol) was dissolved in DCM (50 mL). After slow addition of Dess-Martin Periodinane (4.3 g, 10.15 mmol) at 0 ℃, the mixture was stirred at ambient temperature for 1 h. The suspension was filtered and the filtrate was concentrated and purified by flash chromatography. Yield: 4.39 g (8.06 mmol, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.43—7.29 (m, 5H), 5.12 (q, J=12.3 Hz, 2H), 4.72 (s, 1H), 4.60 (s, 1H), 3.06—3.00 (m, 1H), 2.57—2.34 (m, 2H), 2.34—2.12 (m, 2H), 1.98—1.79 (m, 4H), 1.68 (s, 3H), 1.60 (t, J=11.4 Hz, 3H), 1.51—1.16 (m, other aliphatic ring protons), 1.06 (s, 3H), 1.01 (s, 3H), 0.95 (s, 3H), 0.90 (s, 3H), 0.79 (s, 3H).

Benzyl 3(*α*)-hydroxylup-28-oate (7) Compound **6** (1.58 g, 2.90 mmol) and (*S*)-(–)-2-methyl-CBS-oxazaborolidine (80 mg, 0.29 mmol) were dissolved in dry THF (50 mL) , followed by dropwise addition of a solution of BH_3-Me_2S (0.32 mL, 10 mol/L in THF) at 0 ℃. After 10 min, MeOH was added to quench the reaction, and the reaction mixture was concentrated and purified by flash chromatography. Yield: 790 mg (1.45 mmol, 50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.43—7.28 (m, 5H), 5.19—4.99 (m, 2H), 4.73 (s, 1H), 4.60 (s, 1H), 3.39 (s, 1H), 3.02—2.96 (m, 1H), 2.28—2.16 (m, 2H), 1.98—1.95 (m, 2H), 1.67 (s, 3H), 1.64—0.96 (m, other aliphatic ring protons), 0.95 (s, 3H), 0.92 (s, 3H), 0.81 (s,

6H), 0.76 (s, 3H).

3(*α*)-hydroxylup-28-oic acid (8) Compound **8** was synthesized using general procedure B. Yield: 94%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.74 (d, J=2.5 Hz, 1H), 4.61 (m, 1H), 3.39 (t, J=3.0 Hz, 1H), 3.01 (td, J=11.0, 5.0 Hz, 1H), 2.27 (dt, J=13.0, 3.5 Hz, 1H), 2.18 (td, J=12.5, 3.5 Hz, 1H), 2.03−1.87 (m, 3H), 1.69 (s, 3H), 1.61 (t, J=11.5 Hz, 1H), 1.55−1.27 (m, other aliphatic ring protons), 0.99 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.84 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 181.35, 150.43, 109.67, 76.27, 56.38, 50.26, 49.26, 49.04, 46.94, 42.50, 40.87, 38.37, 37.52, 37.32, 37.05, 34.16, 33.24, 32.16, 30.57, 29.65, 28.25, 25.46, 25.37, 22.08, 20.69, 19.35, 18.23, 16.03, 15.91, 14.77. HRMS (ESI): calcd for $C_{30}H_{49}O_3$ $[M+H]^+$ 457.3682, found 457.2779.

3(*α*)-Oleanic acid (9) Yield: 60.6% for 4 steps. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.28 (t, J=3.5 Hz, 1H), 3.41 (t, J=3.0 Hz, 1H), 2.86−2.75 (m, 1H), 2.03−1.92 (m, 2H), 1.89 (dd, J=9.0, 3.5 Hz, 2H), 1.80−1.16 (m, other aliphatic ring protons), 1.14 (s, 3H), 1.07 (dt, J=14.0, 3.5 Hz, 1H), 0.95 (s, 3H), 0.92 (s, 6H), 0.90 (s, 3H), 0.83 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 183.45, 143.57, 122.66, 76.18, 48.96, 47.39, 46.51, 45.86, 41.63, 40.92, 39.42, 37.32, 37.15, 33.79, 33.06, 32.83, 32.45 (2*C), 30.66, 28.27, 27.61, 26.09, 25.16, 23.58, 23.30, 22.91, 22.24, 18.22, 17.17, 15.10. HRMS (ESI): calcd for $C_{30}H_{48}O_3Na$ $[M+Na]^+$ 479.3501, found 479.3482.

3(*α*)-Ursolic acid (10) Yield: 67.6% for 4 steps. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.24 (t, J=4.0 Hz, 1H), 3.41 (t, J=3.0 Hz, 1H), 2.21−2.14 (m, 1H), 2.03−1.22 (m, other aliphatic ring protons),1.09 (s, 3H), 0.96−0.93 (m, 9H), 0.85 (d, J=6.5 Hz, 3H), 0.83 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 182.77, 137.87, 125.87, 76.15, 52.57, 48.94, 47.93, 47.32, 42.00, 39.63, 39.03, 38.82, 37.33, 37.05, 36.72, 33.05, 32.78, 30.61, 28.28, 27.94, 25.19, 24.10, 23.73, 23.17, 22.26, 21.18, 18.20, 17.10, 16.98, 15.25. HRMS (ESI): calcd for $C_{30}H_{48}O_3Na$ $[M+Na]^+$ 479.3501, found 479.3499.

Compounds **11**−**14** were synthesized according to the previous reference.[10]

3(*β*)-Hydroxylupan-28-oic acid (11) Yield: 86%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.19 (dd, J=11.5, 5.0 Hz, 1H), 2.26−2.17 (m, 3H), 1.91−1.77 (m, 2H), 1.71−1.15 (m, other aliphatic ring protons), 0.97 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.85 (d, J=6.9 Hz, 3H), 0.83 (s, 3H), 0.77−0.73 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 179.64, 78.97, 56.67, 55.30, 50.27, 48.73, 44.12, 42.58, 40.69, 38.85, 38.70, 38.20, 37.36, 37.17, 34.39, 32.06, 29.70, 29.68, 27.98, 27.36, 26.89, 22.97, 22.70, 21.05, 20.87, 18.28, 16.08, 15.35, 14.66, 14.59. HRMS (ESI): calcd for $C_{30}H_{50}O_3Na$ $[M+Na]^+$ 481.3658, found 481.2624.

3(*β*)-Hydroxy-20-ketolup-28-oic acid (12) Yield: 43.6%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.31−3.19 (m, 2H), 2.33−2.26 (m, 1H), 2.20 (s, 3H), 2.15 (t, J=11.0 Hz, 1H), 2.12−2.05 (m, 1H), 2.05−1.96 (m, 1H), 1.73−1.17 (m, other aliphatic ring protons), 1.13−1.05 (m, 1H), 1.02 (s, 3H), 0.98 (s, 3H), 0.93 (s, 3H), 0.84 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 212.38, 181.18, 78.89, 56.13, 55.22, 51.18, 50.30, 49.18, 42.19, 40.70, 40.53, 38.79, 38.54, 37.45, 37.15, 36.68, 34.06, 31.42, 30.09, 29.66, 28.26, 27.94, 27.29, 20.81, 18.21, 16.07, 15.92, 15.33, 14.68. HRMS (ESI): calcd for $C_{29}H_{47}O_4$ $[M+H]^+$ 459.3474, found 459.2440.

3(*β*)-Hydroxy-20(29)-epoxylup-28-oic acid (13) Yield: 80.4%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.20 (dd, J=11.5, 5.0 Hz, 1H), 2.68−2.62 (m, 2H), 2.27 (dt, J=13.0, 3.5 Hz, 1H), 2.20−2.07 (m, 2H), 2.03−1.88 (m, 2H), 1.82−1.29 (m, other aliphatic ring protons), 1.25 (s, 3H), 1.00−0.96 (s, 6H), 0.93 (s, 3H), 0.84 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 179.20, 78.97, 60.07, 56.55, 55.32, 50.38, 49.93, 45.35, 42.43, 40.70, 38.86, 38.73, 37.54, 37.19, 36.87, 34.32, 32.01, 29.69, 29.44, 27.98, 27.37, 26.99, 26.84, 20.93, 18.30, 18.28, 16.11, 16.00, 15.35, 14.59. HRMS (ESI): calcd for $C_{30}H_{48}O_4Na$ $[M+Na]^+$ 495.3450, found 495.3440.

3(*β*)(29)-Dihydroxylupan-28-oic acid (14) Yield: 45.3%. ^{1}H NMR (500 MHz, CD_3OD) δ: 3.74 (dd, J=10.5, 4.5 Hz, 1H), 3.14 (dd, J=11.5, 5.0 Hz, 1H), 2.36−2.27 (m, 2H), 2.23 (dt, J=12.5, 3.5 Hz, 1H), 1.81 (dd, J=12.0, 7.5 Hz, 2H), 1.76−1.70 (m, 1H), 1.66−1.27 (m, other aliphatic ring protons), 1.17 (dt, J=13.5, 3.0 Hz, 1H), 1.01 (s, 3H), 0.99−0.95 (m, 9H), 0.88 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ: 179.92, 79.46, 64.01, 57.60, 56.65, 51.59, 49.54, 44.47, 43.53, 41.75, 39.90, 39.76, 39.45, 39.28, 38.12, 38.10, 35.48, 32.98, 30.68, 28.42, 28.26, 27.85, 24.52, 22.03, 19.26, 18.54, 16.50 (2*C), 15.93, 14.87. HRMS (ESI): calcd for $C_{30}H_{51}O_4$ $[M+H]^+$ 475.3787, found 475.3250.

3(*α*)-Hydroxylupan-28-oic acid (15) To a stirred solution of **7** (150 mg, 0.27 mmol) in MeOH (10 mL), Pd/C (15 mg) was added at ambient temperature under nitrogen atmosphere. The nitrogen atmosphere was replaced by the H_2 atmosphere. The reaction mixture was stirred for 24 h at room temperature, then H_2 was replaced with nitrogen and the mixture was filtered through Celite and washed with DCM. The residue was concentrated and purified by flash chromatography. Yield: 98 mg (0.21 mmol, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.40 (t, J=3.0 Hz, 1H), 2.28−2.14 (m, 3H), 1.98−1.90 (m, 1H), 1.87 (dd, J=12.5, 7.5 Hz, 1H), 1.80 (m, 1H), 1.67−1.14 (m, other aliphatic ring protons), 0.97 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.87−0.84 (m, 6H), 0.82 (s, 3H), 0.76 (d, J=6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 181.47, 76.27, 56.80, 50.02, 49.01, 48.75, 44.17, 42.64, 40.87, 38.21, 37.52, 37.40, 37.29, 34.23, 33.24, 32.06, 29.71, 29.63, 28.24, 26.89, 25.36, 22.98, 22.73, 22.09, 20.72, 18.23, 16.07, 15.87, 14.68 (2*C). HRMS (ESI): calcd for $C_{30}H_{50}O_3Na$ $[M+Na]^+$ 481.3658, found 481.3640.

Benzyl 3(*α*)-hydroxylupan-28-oate (16) Compound **16** was synthesized using general procedure A. Yield: 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.35 (m, 5H), 5.10−5.07 (m, 2H), 3.38 (s, 1H), 2.28−2.24 (m,

4H), 1.97－1.78 (m, 2H), 1.64－0.96 (m, other aliphatic ring protons), 0.96 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.78 (s, 3H).

Benzyl 3(*α*)-acetoxylupan-28-oate (17) To a stirred solution of **16** (97 mg, 0.18 mmol), TEA (74 μL, 0.54 mmol) and DMAP (2.5 mg, 0.02 mmol) in dry DCM (5 mL), acetic anhydride (57 μL, 0.60 mmol) was added dropwise at 0 ℃. Then, the reaction mixture was allowed to stir at room temperature overnight. The mixture was concentrated and diluted with H_2O (20 mL) and extracted with EtOAc (2×10 mL). The combined organic layers were washed with H_2O (2×20 mL), brine (20 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography to yield **17** as a white solid. Yield: 68 mg (0.12 mmol, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.34 (m, 5H), 5.10 (d, J= 11.7 Hz, 1H), 5.07 (d, J=11.7 Hz, 1H), 4.62 (s, 1H), 2.84－2.20 (m, 4H), 2.08 (s, 3H), 1.98－1.15 (m, other aliphatic ring protons), 1.01 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H), 0.85 (s, 3H), 0.83 (s, 3H), 0.76 (s, 3H), 0.74 (s, 3H).

3(*α*)-Acetoxylupan-28-oic acid (18) Compound **18** was synthesized using general procedure B. Yield: 92%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.62 (t, J=3.0 Hz, 1H), 2.34－2.15 (m, 3H), 2.08 (s, 3H), 1.90－1.08 (m, other aliphatic ring protons), 1.01 (s, 3H), 0.94 (s, 3H), 0.87－0.84 (m, 9H), 0.83 (s, 3H), 0.76 (d, J=6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 181.91, 170.84, 78.37, 56.82, 50.19, 50.02, 48.75, 44.16, 42.66, 40.87, 38.23, 37.40, 37.17, 36.66, 34.16, 33.87, 32.08, 29.71, 29.68, 27.81, 26.87, 22.97, 22.87, 22.73, 21.69, 21.39, 20.69, 18.08, 16.07, 15.85, 14.83, 14.65. HRMS (ESI): calcd for $C_{32}H_{53}O_4$ $[M+H]^+$ 501.3944, found 501.3921.

3-Carbonylup-28-oic acid (19) Compound **15** (200 mg, 0.44 mmol) was dissolved in DCM (10 mL). After slow addition of Dess-Martin Periodinane (370 mg, 0.88 mmol) at 0 ℃, the mixture was stirred at ambient temperature for 1 h. The suspension was filtered and the filtrate was concentrated and purified by flash chromatography using 10% EtOAc in petroleum ether. Yield: 187 mg (0.39 mmol, 93.5%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.56－2.33 (m, 2H), 2.29－2.20 (m, 3H), 2.00－1.13 (m, other aliphatic ring protons), 1.07 (s, 1H), 1.01 (s, 3H), 0.98 (s, 6H), 0.94 (s, 3H), 0.86 (d, J= 6.9 Hz, 3H), 0.76 (d, J=6.9 Hz, 3H).

3-Oximidolupan-28-oic acid (20) Compound **19** (82 mg, 0.18 mmol) was dissolved in ethanol (5 mL). After slow addition of hydroxylamine hydrochloride (25 mg, 0.37 mmol) and pyridine (37 μL, 0.46 mmol) at 0 ℃, the mixture was stirred at ambient temperature overnight. The solution was then concentrated and purified by flash chromatography to give compound **20** as a white solid. Yield: 77 mg (0.16 mmol, 91.2%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.32－2.19 (m, 4H), 1.88 (dd, J= 12.5, 7.5 Hz, 1H), 1.85－1.75 (m, 2H), 1.74－1.15 (m, other aliphatic ring protons), 1.12 (s, 3H), 1.03 (s, 3H), 0.97 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.86 (d, J=7.0 Hz, 3H), 0.75 (d, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.02, 167.65, 77.22, 56.61, 55.47, 49.88, 48.68, 44.07, 42.63, 40.74, 40.22, 38.68, 38.20, 37.35, 37.19, 33.98, 32.03, 29.70, 29.65, 27.34, 26.92, 22.96, 22.69, 21.20, 19.05, 17.13, 16.00, 15.77, 14.67, 14.50. HRMS (ESI): calcd for $C_{30}H_{50}NO_3$ $[M+H]^+$ 472.3791, found 472.3781.

Benzyl 3(*α*)-hydroxy-20(29)-epoxylup-28-oate (21) Compound **4** (1.23 g, 2.25 mmol) was dissolved in DCM (20 mL). After slow addition of *m*-CPBA (820 mg, 4.50 mmol) at 0 ℃, the mixture was stirred at ambient temperature for 3 h. Saturated Na_2SO_3 solution was added to quench the reaction and the mixture was extracted with DCM (2×100 mL). The combined organic layers were washed with H_2O (2×100 mL), saturated $NaHCO_3$ solution (2×100 mL) and brine (150 mL), dried over Na_2SO_4, concentrated and purified by flash chromatography to give **21** as a single configuration which was confirmed by X-ray crystallographic analysis (Figure 3). Yield: 1.1 g (1.96 mmol, 87.1%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.37－7.27 (m, 5H) 5.10 (s, 2H), 3.39 (s, 1H), 2.66－2.57 (m, 2H), 2.31－2.23 (m, 2H), 2.19－2.05 (m, 2H), 1.95－1.65 (m, 4H), 1.76－1.32 (m, other aliphatic ring protons), 1.26 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H), 0.81 (s, 6H), 0.75 (s, 3H).

3(*α*)-Hydroxy-20(29)-epoxylup-28-oic acid (22) Compound **22** was synthesized using general procedure B. Yield: 58%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.40 (t, J=3.0 Hz, 1H), 2.67－2.64 (m, 2H), 2.28－2.26 (m, 1H), 2.14－2.07 (m, 2H), 2.02－1.88 (m, 3H), 1.82－1.76 (m, 1H), 1.60－1.27 (m, other aliphatic ring protons), 1.24 (s, 3H), 0.98 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.85 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 181.24, 76.23, 60.18, 56.74, 56.65, 53.41, 50.10, 49.96, 49.01, 45.48, 42.47, 40.87, 37.51, 37.30, 36.90, 34.15, 33.26, 32.01, 29.37, 28.24, 26.96, 26.81, 25.35, 22.07, 20.76, 18.21, 18.19, 16.01, 15.89, 14.65. HRMS (ESI): calcd for $C_{30}H_{48}O_4Na$ $[M+Na]^+$ 495.3450, found 495.3439.

Benzyl 3(*α*)(30)-dihydroxylup-28-oate (23) Selenium dioxide (9.7 mg, 0.09 mmol) and *tert*-butyl hydroperoxide (68 μL, 0.36 mmol) were dissolved in dry DCM. After the addition of acetic acid (3.2 μL, 0.018 mmol) at 0 ℃, compound **7** (100 mg, 0.18 mmol) was added and the mixture was stirred at ambient temperature overnight. Saturated Na_2SO_3 solution was added to quench the reaction and the mixture was extracted with DCM (2×10 mL). The combined organic layers were washed with H_2O (2×10 mL), saturated $NaHCO_3$ solution (2×10 mL) and brine (10 mL), dried over Na_2SO_4, concentrated and purified by flash chromatography to give compound **23**. Yield: 67 mg (0.12 mmol, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.34 (m, 5H), 5.10 (m, 2H), 4.95 (s, 1H), 4.91 (s, 1H), 4.11 (s, 2H), 3.38 (s, 1H), 2.88 (m, 1H), 2.32－2.26 (m, 2H), 2.21－2.10 (m, 2H), 1.98－1.77 (m, 2H), 1.64－0.96 (m, other aliphatic ring protons), 0.99 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H), 0.87 (s, 3H), 0.84 (s, 3H).

3(*α*)(30)-Dihydroxylup-28-oic acid (24) Compound **24** was synthesized using general procedure B. Yield: 45%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.97 (s, 1H), 4.92 (s, 1H), 4.12 (m, 2H), 3.39 (s, 1H), 2.88 (td, J= 12.0, 3.0 Hz, 1H), 2.32－2.26 (m, 2H), 2.21－2.10 (m, 2H), 1.98－1.77 (m, 2H), 1.64－1.00 (m, other aliphatic ring protons), 0.99 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H), 0.87 (s, 3H), 0.84 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ: 179.78, 156.15, 106.77, 76.75, 65.03, 57.29, 54.21, 51.42, 50.82, 49.91, 43.93, 43.43, 41.91, 39.40, 38.31, 38.19, 37.75, 35.32, 34.33, 33.25, 33.05, 30.66, 28.80, 27.80, 26.19, 22.51, 21.84, 19.12, 16.42, 14.96. HRMS (ESI): calcd for $C_{30}H_{49}O_4$ $[M+H]^+$ 473.3631, found 473.3620.

Benzyl 3(*α*)-hydroxy-30-formylupan-28-oate (25) Compound **21** (68 mg, 0.12 mmol) was refluxed with concentrated hydrochloric acid (0.1 mL) in chloroform for 1 h. The mixture was concentrated and purified by flash chromatography to give **25** as a white solid. Yield: 50 mg (0.09 mmol, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.62 (s, 1H), 7.41－7.28 (m, 5H), 5.18－5.02 (m, 2H), 3.39 (s, 1H), 3.04－2,90 (m, 1H), 2.67－2.49 (m, 1H), 2.35－2.16 (m, 2H), 2.02－1.77 (m, 2H), 1.75－1.14 (m, other aliphatic ring protons), 1.11 (d, J=6.9 Hz, 3H), 0.99 (d, J=6.8 Hz, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.84 (s, 3H), 0.76 (s, 3H).

3(*α*)-Hydroxy-30-formylupan-28-oic acid (26) Compound **26** was synthesized using general procedure B. Yield: 85.7%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.84 (d, J=2.0 Hz, 1H), 3.40 (t, J=3.0 Hz, 1H), 3.34 (d, J=7.0 Hz, 1H), 2.60－2.56 (m, 1H), 2.48－2.40 (m, 1H), 2.29 －2.20 (m, 2H), 1.97－1.94 (m, 4H), 1.77－1.15 (m, other aliphatic ring protons), 1.12 (d, J=7.0 Hz, 3H), 0.97 (s, 3H), 0.95－0.93 (m, 9H), 0.85 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 206.73, 180.34, 76.18, 56.56, 49.88, 49.77, 49.20, 48.95, 42.56, 42.45, 40.85, 38.29, 37.52, 37.29, 36.79, 34.18, 33.23, 31.75, 29.57, 28.23, 27.46, 25.64, 25.35, 22.09, 20.68, 18.20, 16.03, 15.88, 14.62, 14.47. HRMS (ESI): calcd for $C_{30}H_{48}O_4Na$ $[M+Na]^+$ 495.3450, found 495.3450.

Benzyl 3(*α*)-hydroxy-20(29)-epoxylup-28-oate (27) Compound **27** was synthesized using the similar method for compound **21**. Yield: 88.9%

3(*α*)(30)-Dihydroxy-20(29)-epoxylup-28-oic acid (28) Compound **28** was synthesized using the similar method for compound **22**. Yield: 63.8%. (20*S*) stereoisoform: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.96 (dd, J= 11.5, 8.5 Hz, 1H), 3.83－3.76 (m, 1H), 3.63 (dd, J= 11.5, 4.5 Hz, 1H), 3.40 (s, 1H), 2.94 (d, J=4.5 Hz, 1H), 2.66 (d, J=4.5 Hz, 1H), 2.33－2.29 (m, 2H), 1.98－1.86 (m, 2H), 1.77－1.67 (m, 2H), 1.61－1.19 (m, other aliphatic ring protons), 0.99 (s, 3H), 0.94－0.92 (m, 6H), 0.85 (s, 3H), 0.83 (s, 3H); (20*R*) stereoisoform: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.22 (dd, J=8.5, 5.5 Hz, 1H), 4.02 (dd, J=11.5, 8.5 Hz, 1H), 3.83－3.76 (m, 1H), 3.40 (s, 1H), 3.00 (d, J=4.5 Hz, 1H), 2.80 (d, J= 4.5 Hz, 1H), 2.33－2.29 (m, 2H), 1.98－1.86 (m, 2H), 1.77－1.67 (m, 2H), 1.61－1.19 (m, other aliphatic ring protons), 0.97 (s, 3H), 0.94－0.92 (m, 6H), 0.84 (s, 3H), 0.82 (s, 3H). (20*S*) stereoisoform: ^{13}C NMR (125 MHz, $CDCl_3$) δ: 180.20, 76.19, 65.57, 62.78, 62.34, 57.26, 56.00, 50.27, 49.89, 48.95, 42.54, 40.86, 38.37, 38.06, 37.52, 37.29, 34.19, 33.23, 30.56, 28.23, 27.52, 26.86, 26.30, 25.35, 22.08, 20.64, 18.20, 16.02, 15.88, 14.62. (20*R*) stereoisoform: ^{13}C NMR (125 MHz, $CDCl_3$) δ: 180.17, 76.19, 65.57, 62.78, 57.33, 56.00, 50.13, 49.82, 49.01, 42.68, 40.90, 38.30, 38.06, 37.63, 37.29, 34.19, 33.23, 30.35, 28.92, 28.29, 27.52, 26.86, 26.30, 25.27, 22.08, 20.56, 18.20, 16.12, 15.92, 14.68. HRMS (ESI): calcd for $C_{30}H_{48}O_5Na$ $[M+Na]^+$ 511.3399, found 511.3378.

3(*α*)(29)(30)-Trihydroxylupan-28-oate (29) Compound **23** (40 mg, 0.07 mmol) was dissolved in dry THF and 2 mol/L BH_3-Me_2S in THF was added at 0 ℃. After 1 h the mixture was stirred at ambient temperature overnight. Ethanol (280 μL), saturated NaOAc solution (200 μL), 30% H_2O_2 solution (140 μL) were added in sequence and the mixture continued to be stirred overnight. The mixture was extracted with EtOAc (2×20 mL). The combined organic layers were washed with H_2O (2×20 mL) and brine (20 mL), dried over Na_2SO_4, concentrated and purified by flash chromatography to give **29** as a white solid. Yield: 35 mg (0.06 mmol, 85.4%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.34 (m, 5H), 5.08 (m, 2H), 3.90 (m, 1H), 3.78 (m, 1H), 3.72 (m, 4H), 3.38 (s, 1H), 2.21－2.04 (m, 4H), 1.81－1.05 (m, other aliphatic ring protons), 0.94 (s, 3H), 0.92 (s, 3H), 0.81 (s, 6H), 0.73 (s, 3H).

3(*α*)(29)(30)-Trihydroxylupan-28-oic acid (30) Compound **30** was synthesized using general procedure B. Yield: 91.9%. ^{1}H NMR (500 MHz, CD_3OD) δ: 3.73 (dd, J=11.0, 4.0 Hz, 1H), 3.66 (dd, J=11.0, 6.0 Hz, 1H), 3.53 (dd, J=11.0, 7.5 Hz, 1H), 3.45 (dd, J=11.0, 7.0 Hz, 1H), 3.32 (s, 1H), 2.57－2.52 (m, 1H), 2.38－2.33 (m, 1H), 2.25－2.21 (m, 1H), 2.00－1.86 (m, 2H), 1.80 (dd, J=12.5, 7.5 Hz, 1H), 1.77－1.71 (m, 1H), 1.66－1.27 (m, other aliphatic ring protons), 1.16 (dt, J=13.5, 3.0 Hz, 1H), 1.04 (s, 3H), 0.97 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ: 179.88, 76.75, 65.34, 60.79, 57.29, 51.19, 49.89, 49.80, 47.42, 43.63, 41.93, 39.51, 39.18, 38.38, 38.31, 38.19, 35.39, 34.34, 33.00, 30.65, 28.80, 28.28, 26.21, 24.77, 22.52, 21.86, 19.13, 16.49, 16.39, 14.95. HRMS (ESI): calcd for $C_{30}H_{50}O_5Na$ $[M+Na]^+$ 513.3556, found 513.3544.

Cell culture HEK293MSR and HEK293/APPs we were cultured in DMEM with 10% (mg/mL) heat-inactivated FBS in a humidified incubator with 5% CO_2/95% air (*V*/*V*) at 37 ℃. HEK293 cells were cultured in MEM under the same condition.

***Split-TEV* assay** 1×10^6 HEK293MSR cells were seeded in 24-well plates for each well, or 2×10^4 cells per well for 96-well plates. In 24-well plates, each well of HEK293MSR cells were transiently transfected with total DNA of 0.8 μg, with equal molar ratio of -NTEV and -CTEV fusion protein constructs, ERT2-tev-LexA-

Gal4, LexA-op-F-lucF-luciferase, and 15 ng of hRluc as internal control. For 96-well plates, equal molar ratio of -NTEV and -CTEV fusion protein constructs, ERT2-tev-LexA-Gal4 and LexA-op-F-lucF-luciferase constructs, with 125 ng in total for each well, were transiently transfected. 2 h after the transfection, chemicals were added and cells were treated for 16 h, followed with luciferase activity measurements.

***ELISA* for Aβ** HEK293/APPswe cells were seeded in 96-well plates and treated with chemicals of designated concentrations and time. Cell media were then collected and subjected to sandwich ELISA. Human Aβ 40 and Aβ 42 in APP/PS1 mouse brains were extracted as previously reported[23] and measured with human Aβ ELISA kits. ELISA kits for total human Aβ 40 and Aβ 42 were obtained from ExCell Bio (China).

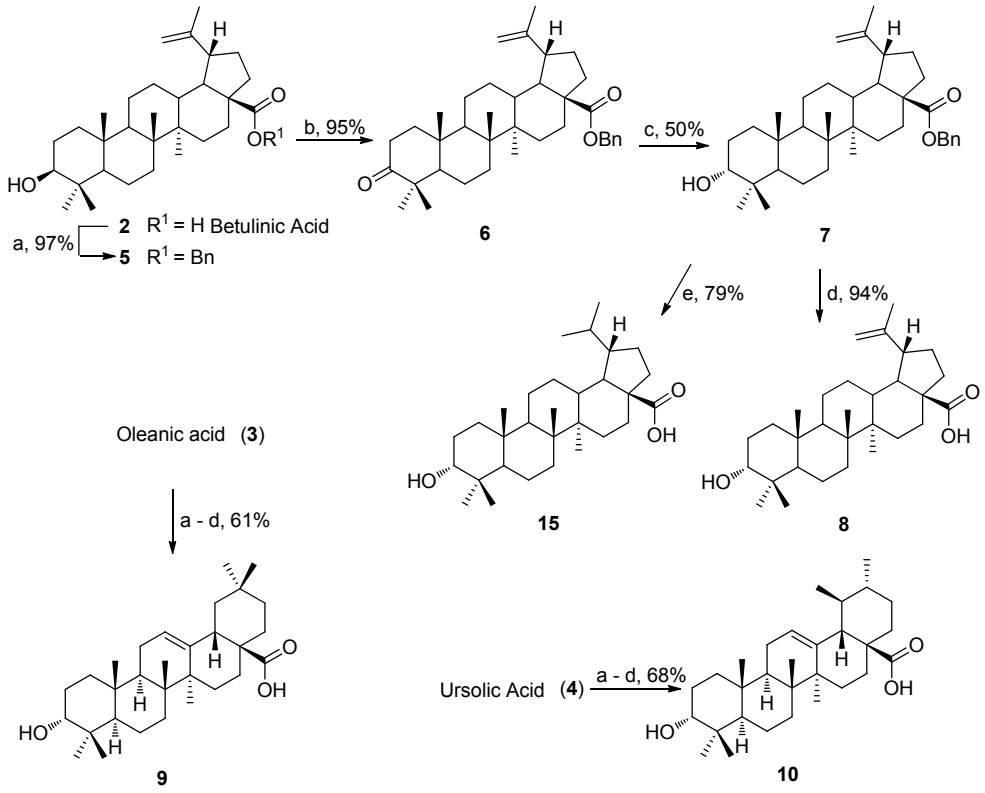
Cell viability test Chemical-treated HEK293/APPswe cells were washed twice with PBS and subjected to CellTiter-Glo Luminescent Cell Viability Assay (Promega) following guidance of the manufacturer.

Results and Discussion

We initiated our efforts to solve the source shortage problem of HTS hit **1**, a set of naturally occurring triterpenoids analogues (Supplementary Table 1) of **1** with an abundant natural resource was tested for interference with the PS1/BACE1 interaction and for Aβ reduction activity. Preliminary screening results showed that betulinic acid, oleanic acid and ursolic acid (Figure 1) exhibited different levels of PS1/BACE1 interference activity but negligible Aβ reduction activity (Supplementary Table 1). Apparently, there were differences between HTS hit **1** and the screened triterpenoids. Upon inspection, we found that the major difference between them was the conformation of the C3 hydroxy group. Exploratory inversion of the C3 hydroxy conformation of compounds **2**, **3** and **4** from β to α was achieved by a Dess-Martin oxidation of benzyl-protected compounds **2**, **3** and **4** and a subsequent CBS asymmetric reduction and debenzylation (Scheme 1, compound **2** as an example). We were encouraged to see that compounds **8**, **9**, **10** with inverted C3 hydroxy displayed an increase in both interference with the PS1/BACE1 interaction and Aβ reduction activity, as well as decreased cytotoxicity (Table 1). Among these compounds, 3-*α*-betulinic acid (compound **8**) exerted a more obvious PS1/BACE1 interaction interference activity compared with **9** and **10**. Furthermore, it was noted that betulinic acid (compound **2**) shared a similar triterpene scaffold except for a unique isopropenyl group, which facilitated further modifications. For the above-mentioned reasons, we chose compound **2** as the starting point for further study.

Although the inversion strategy gave us preliminary results, we were still uncertain whether the conformation of C3 hydroxy group was important or not. To

Scheme 1



Reagents and conditions: (a) BnCl, K_2CO_3, DMF, r.t., 12 h; (b) DMP, DCM, 0 ℃ to r.t., 2 h; (c) BH_3-Me_2S, (S)-Me-CBS, THF, r.t., 5 min; (d) Pd/C, H_2, r.t., 1 h; (e) Pd/C, H_2, r.t., 24 h.

Table 1 *In vitro* assay of representative natural products and C3-OH modified series

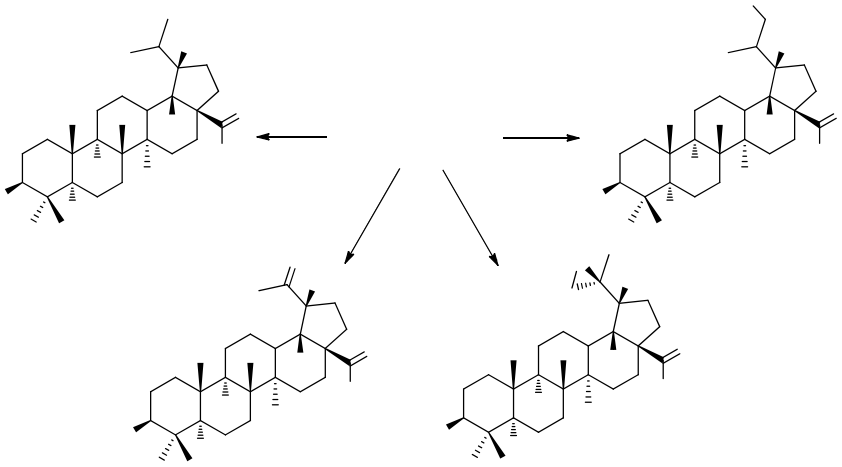
Compd	PS1/BACE1 split-TEV reporter activity[a] (% of ctrl, Mean±SEM)	Aβ production[b] (% of ctrl, Mean±SEM)	Cell viability[c] (% of ctrl, Mean±SEM)
1	56.4±7.2	23.2±4.5	79.0±5.2
2	48.0±1.9	98.9±0.6	64.4±2.0
3	50.4±2.5	104.7±5.0	91.5±3.0
4	63.2±5.4	110.2±6.2	61.4±3.3
8	37.4±7.1	86.0±5.4	87.5±5.7
9	76.0±6.8	86.0±10.2	74.9±1.6
10	70.0±9.4	68.0±2.5	79.9±5.0
11	25.0±2.4	108.8±6.9	25.8±19.2
12	106.0±0.3	85.4±13.7	79.9±5.7
13	112.0±3.4	79.4±1.4	77.7±2.3
14	89.0±3.2	83.8±4.0	76.9±6.7
15	17.0±19.0	71.0±8.2	54.3±3.9
18	4.6±0.8	86.3±1.6	8.9±7.8
20	12.7±0.6	94.4±4.3	86.0±12.6

[a] 2 h after the transfection of the split-TEV plasmids, chemicals were added and HEK293MSR cells were treated with 10 μmol/L chemicals for 16 h, followed with luciferase activity measurements. [b] HEK293/APPswe cells were plated in 96-well plates and treated with 10 μmol/L chemicals for 8 h. Cell media were then collected and subjected to sandwich ELISA for the quantification of cellular Aβ production. [c] Chemical-treated HEK293/APPswe cells were gently washed twice with PBS and subjected to CellTiter-Glo Luminescent Cell Viability Assay.

assess the importance of the C3 hydroxy conformation, we synthesized a series of compounds with an altered C17 position that included modifications of the alkene group into alkane, ketone, epoxide, and alcohol (**11**—**14**, Scheme 2). Compounds **11**—**14** were synthesized following a previously reported process.[10] Most of these 3-*β*-hydroxy analogues showed significantly reduced PS1/BACE1 interference activity compared with compound **8**, except **11**, while compound **11** was less potent than its corresponding 3-*α*-hydroxy analogue **15**, which was obtained as an byproduct during the debenzylation of **7** (Scheme 1). These results were consistent with our previous hypothesis regarding the importance of the conformation of C3 hydroxy group.

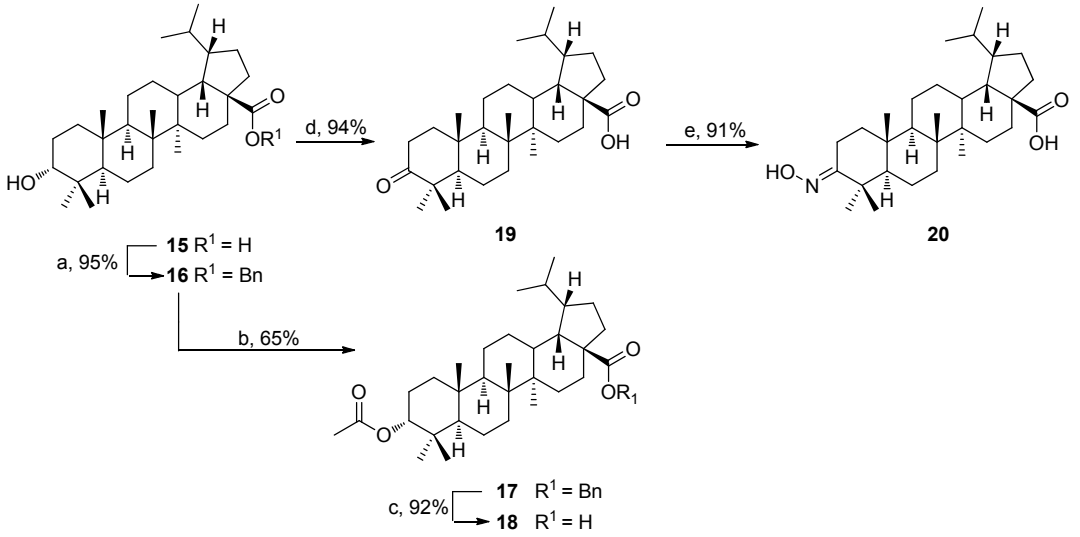
After probing into the stereochemistry of the C3 position, we further investigated the modification at C3, focusing on changing the hydroxyl group into ester and oxime groups (Scheme 3). Compound **15**, with a PS1-BACE1 interference activity nearly equal to that of hit compound **1**, was chosen as a new starting point for next research step. Benzyl-protected **15** was esterified by acetic anhydride, dimethylamino pyridine (DMAP) and triethylamine in DCM, followed by debenzylation to obtain **18**.[11] Oxidation of **15** with Dess-Martin's re-

Scheme 2



Reagents and conditions: (a) Pd/C, H_2, r.t., 24 h; (b) O_3, Me_2S, DCM, -78 °C, 1 min; (c) *m*-CPBA, DCM, 0 °C to r.t., 1 h; (d) BH_3-Me_2S, H_2O_2, NaOH, THF, 0 °C to r.t., 12 h.

Scheme 3



Reagents and conditions: (a) BnCl, K_2CO_3, DMF, r.t., 12 h; (b) Acetic anhydride, DMAP, TEA, r.t., 12 h; (c) Pd/C, H_2, r.t., 1 h; (d) DMP, DCM, 0 °C to r.t., 2 h; (e) NH_2OH•HCl, EtOH, r.t., 12 h.

agent[12] and subsequent treatment with hydroxylamine hydrochloride and pyridine in ethanol afforded oxime **20**.[10] Disappointingly, these substituents were not tolerated. Although **18** and **20** showed improved PS1-BACE1 interference activity, the Aβ reduction activity declined significantly which indicated that the C3 free hydroxyl group was necessary. Meanwhile we realized that improved activity always occurred after increased cytotoxicity. This required us to pay particular attention to cytotoxicity, which may be the true cause of the PS1/BACE1 interference and Aβ reduction activities.

Even though the progress was made on the C3 position optimization with the discovery of potent compound **15**, obvious cytotoxcity indicated that there were still more need for structure modification. Therefore, we explored the C20 alkene group, and a scan of the SAR of this alkene group series was carried out thoroughly (Scheme 4). Compound **21** was synthesized in a similar way to compound **13**,[13] and the absolute configuration of **21** was confirmed by X-ray crystallography (Figure 2). Further treatment of compound **21** with hydrochloric acid in chloroform provided compound **25**.[14] Oxidation of compound **7** with selenium dioxide (SeO_2) and *tert*-butyl hydroperoxide (TBHP) provided compound **23**.[15] Starting with **23**, hydroboration with boron hydride (BH_3) followed by oxidative workup[16] produced compound **29**, while epoxidation with m-CPBA resulted in compound **27** as a mixture of two isomors. Compounds **22**, **24**, **26**, **28** and **30** were finally produced through debenzylation of their corresponding benzyl esters

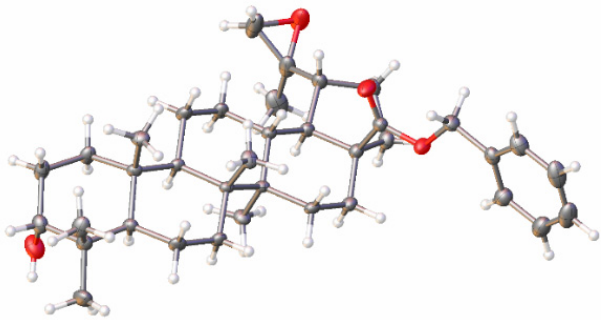


Figure 2 The X-ray crystallographic structure of compound **21**.

Table 2 *In vitro* Assay of C20 alkene group modified series

Compd	PS1/BACE1 split-TEV reporter activity[a] (% of ctrl, Mean±SEM)	Aβ production[b] (% of ctrl, Mean±SEM)	Cell viability[c] (% of ctrl, Mean±SEM)
22	44.5±3.9	33.1±2.7	78.3±2.3
24	94.6±5.0	70.2±8.8	101.2±1.9
26	31.9±10.8	53.5±3.1	84.5±1.5
28	13.8±10.2	37.4±4.5	39.4±2.8
30	103.4±3.1	84.4±11.7	111.1±2.4

[a] 2 h after the transfection of the split-TEV plasmids, chemicals were added and HEK293MSR cells were treated with 10 μmol/L chemicals for 16 h, followed with luciferase activity measurements. [b] HEK293/APPswe cells were plated in 96-well plates and treated with 10 μmol/L chemicals for 8 h. Cell media were then collected and subjected to sandwich ELISA for the quantification of cellular Aβ production. [c] Chemical-treated HEK293/ APPswe cells were gently washed twice with PBS and subjected to CellTiter-Glo Luminescent Cell Viability Assay.

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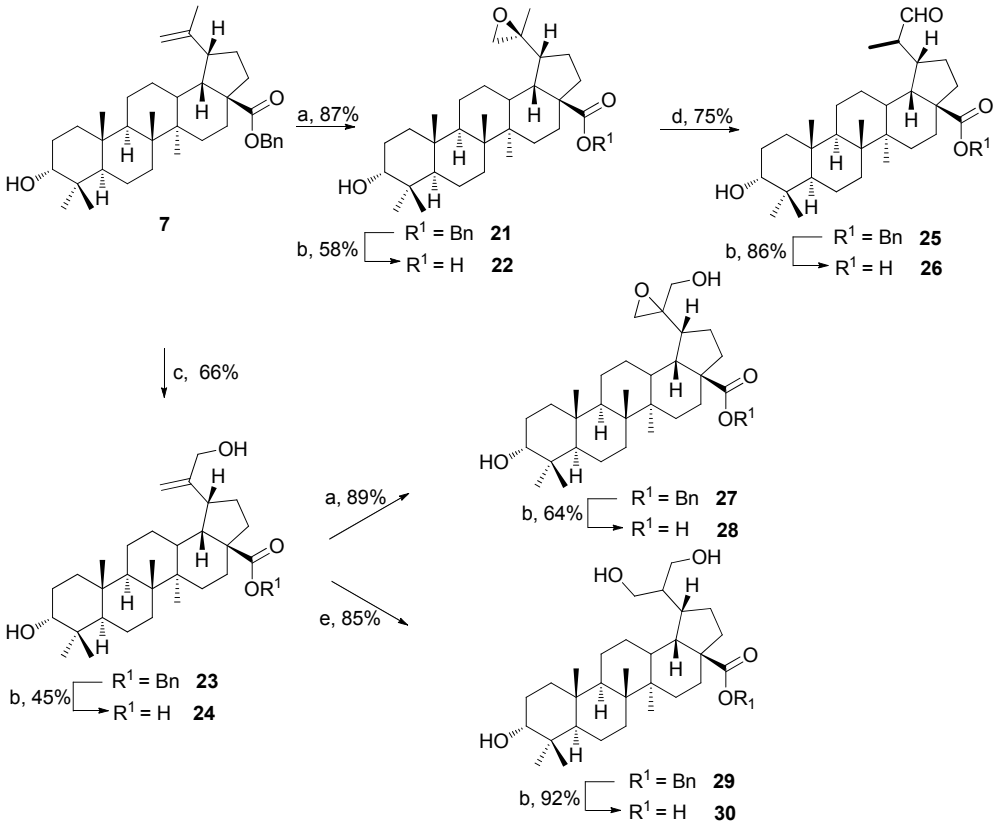
Scheme 4



Reagents and conditions: (a) *m*-CPBA, DCM, 0 °C to r.t., 1 h; (b) Pd/C, H_2, MeOH, r.t., 1 h; (c) SeO_2, TBHP, AcOH, DCM, 0 °C to r.t., 12 h; (d) $CHCl_3$, HCl, reflux, 1 h; (e) BH_3-Me_2S, H_2O_2, NaOH, THF, 0 °C to r.t., 12 h

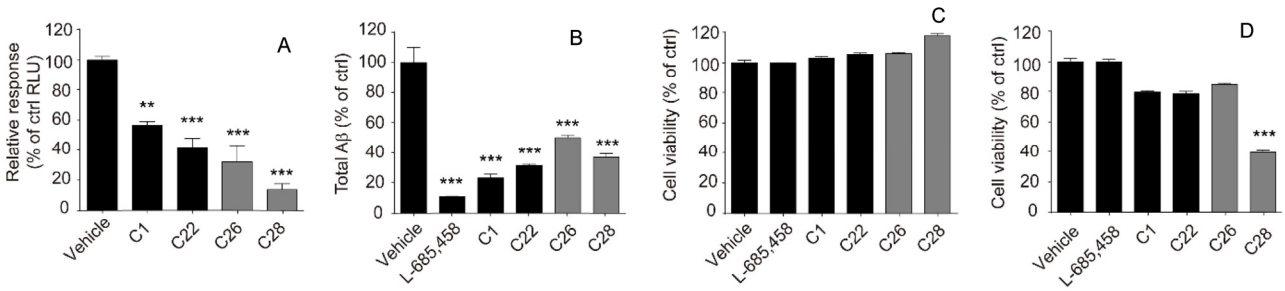


Figure 3 Compound **22** and analogs resemble the activities of HTS hit **1**. The effects of compounds **1**, **22**, **26**, **28** on PS1/BACE1 interaction induced reporter activities in split-TEV assay (A), cellular total Aβ production (B), and cell viability assays following 8 h treatment (C) or 24 h treatment (D). 1 μm L-685,458 was used as a control, and the concentrations of compounds **1**, **22**, **26**, **28** were 10 μmol/L. Transfected HEK293MSR cells were treated with chemicals for 16－18 h following the measurement of reporter activities. HEK293/APPsw cells were treated with chemicals for 8 h and the media were collected for Aβ ELISA quantification. Chemical-treated HEK293/APPsw cells were subjected to viability test after 8 h or 24 h treatment. ** $P<0.01$, *** $P<0.005$ determined by One-way ANOVA.

(compounds **21**, **23**, **25**, **27**, **29**).

All of the synthesized compounds were evaluated for their PS1/BACE1 interaction interference and Aβ reduction activities, as well as their effects on cell viability (Table 2). Among the tested compounds, three of them (compounds **22**, **26**, **28**) were found to be potent both in PS1/BACE1 interaction interference and Aβ secretion inhibitory activity. As shown in Figure 3A, hit **1** significantly reduced the PS1/BACE1 interaction based luciferase reporter activities, while its structural analogs **22**, **26** and **28** showed similar inhibitory potency. In HEK293APPsw cells, we tested the Aβ reduction activities of these analogs following an eight-hour cellular treatment (Figure 3B) and found that similar to the HTS hit **1**, compound **22** also significantly reduced cellular Aβ production. Meanwhile, the other three analogs, **26** and **28** showed weaker potency reducing the cellular Aβ production. We further tested the effects of the HTS hit **1** and its analogs in a cell viability assay and detected no obvious changes in the cell viability following an eight-hour cellular treatment with the chemicals (Figure 3C). However, with a longer treatment of 24 h, com-

pounds **1**, **22** and **26** slightly inhibited the cell viability of HEK293/APPsw cells, while compound **28** showed significant inhibitory effects (Figure 3D). Finally, we chose compound **22** for further evaluation, which not only displayed potent PS1/BACE1 interaction interference (*Spit-TEV* IC_{50}: 3 μmol/L) and Aβ reduction activity (Aβ inhibition IC_{50}: 3 μmol/L) but low cytotoxicity as well.

Conclusions

In summary, we chose naturally rich betulinic acid as starting material for optimization of triterpenoid PS1/BACE1 interaction inhibitor, and the subsequent SAR study yielded most potent compound **22**. Compound **22** interfered with the PS1/BACE1 interaction and reduced Aβ production in the same way as HTS hit compound **1** and displayed negligible inhibitory effects on BACE1, *γ*-secretase and *α*-secretase activities. As a novel natural triterpenoid-derived PS1/BACE1 interaction inhibitor, these compounds are worth for further studying.

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References

[1] Goedert, M.; Spillantini, M. G. *Science* **2006**, *314*, 777.
[2] Huang, Y.; Mucke, L. *Cell* **2012**, *148*, 1204.
[3] Ittner, L. M.; Götz, J. *Nat. Rev. Neurosci*. **2011**, *12*, 67.
[4] De Strooper, B. *Physiol. Rev*. **2010**, *90*, 465.
[5] For reviews, see: (a) Ghosh, A. K.; Osswald, H. L. *Chem. Soc. Rev.* **2014**, *43*, 6765; (b) Yan, R.; Vassar, R. *Lancet Neurol.* **2014**, *13*, 319; (c) De Strooper, B.; Vassar, R.; Golde, T. *Nat. Rev. Neurol*. **2010**, *6*, 99; (d) Tate, B.; McKee, T. D.; Loureiro, R. M. B.; Dumin, J. A.; Xia, W.; Pojasek, K.; Austin, W. F.; Fuller, N. O.; Hubbs, J. L.; Shen, R.; Jonker, J.; Ives, J.; Bronk, B. S. *Int. J. Alzheimers. Dis*. **2012**, *2012*, 10.
[6] Jurisch-Yaksi, N.; Sannerud, R.; Annaert, W. *Biochim. Biophys. Acta* **2013**, *12*, 2898.
[7] Hebert, S. S.; Bourdages, V.; Godin, C.; Ferland, M.; Carreau, M.; Levesque, G. *Neurobiol. Dis*. **2003**, *13*, 238.
[8] Kuzuya, A.; Uemura, K.; Kitagawa, N.; Aoyagi, N.; Kihara, T.; Ninomiya, H.; Ishiura, S.; Takahashi, R.; Shimohama, S. *J. Neuro. Res*. **2007**, *85*, 153.
[9] Cui, J.; Wang, X.; Li, X.; Wang, X.; Zhang, C.; Li, W.; Zhang, Y.; Gu, H.; Xie, X.; Nan, F.; Zhao, J.; Pei, G. *Cell Dis*. **2015**, *1*, 15021.
[10] Genet, C.; Strehle, A.; Schmidt, C.; Boudjelal, G.; Lobstein, A.; Schoonjans, K.; Souchet, M.; Auwerx, J.; Saladin, R.; Wagner, A. *J. Med. Chem*. **2010**, *53*, 178.
[11] Kashiwada, Y.; Hashimoto, F.; Cosentino, L. M.; Chen, C. H.; Garrett, P. E.; Lee, K. H. *J. Med. Chem*. **1996**, *39*, 1016.
[12] Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc*. **1991**, *113*, 7277.
[13] Takikawa, H.; Hirooka, M.; Sasaki, M. *Tetrahedron Lett*. **2003**, *44*, 5235.
[14] Arico-Muendel, C. C.; Blanchette, H.; Benjamin, D. R.; Caiazzo, T. M.; Centrella, P. A.; DeLorey, J.; Doyle, E. G.; Johnson, S. R.; Labenski, M. T.; Morgan, B. A. *ACS Med. Chem. Lett*. **2013**, *4*, 381.
[15] Fürstner, A.; Gastner, T. *Org. Lett*. **2000**, *2*, 2467.
[16] Ferraz, H. M. C.; Souza, A. J. C.; Tenius, B. S. M.; Bianco, G. G. *Tetrahedron* **2006**, *62*, 9232.

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