Original Article

Synthesis and biological evaluation of substituted phenylpyrazole[4,5-*b*]oleanane derivatives as inhibitors of glycogen phosphorylase

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ABSTRACT: A series of substituted phenylpyrazole[4,5-*b*]oleanane derivatives have been synthesized and biologically evaluated as inhibitors of glycogen phosphorylase (GP). The structure of phenylpyrazole moiety in compound 17 was determined by ROESY. All of the synthesized oleanane derivatives were biologically evaluated against rabbit muscle GPa. Within this series of compounds, pyrazole triterpene 7 (IC_{50} = 10.8 μM) exhibited slightly more potent activity than its parent compound 1. Preliminary SAR analysis of the pyrazoleoleanane derivatives as GP inhibitors is discussed.

Keywords: Phenylpyrazole[4,5-*b*]oleanane derivatives, Glycogen phosphorylase, Inhibitors, Synthesis, Diabetes

1. Introduction

Pentacyclic triterpenoids are very common constituents in the plant kingdom. A variety of biological properties have been ascribed to this class of compounds including anti-inflammation (*1*), anti-HIV (*2,3*), suppression of tumor promotion (*4,5*), and protection of the liver against toxic injury (*6-8*). The most well-known member of this family of compounds is probably oleanolic acid (OA, **1**) (Figure 1) which has been clinically used as a liver protective drug for more than 20 years in China. Previously, the current authors first reported that **1** and related pentacyclic triterpenes (*e.g.* maslinic acid and corosolic acid; Figure 1) represented a new class of inhibitors of glycogen phosphorylase (GP) (*9-12*). GP inhibitors have been regarded as a promising therapeutic approach to treatment of type 2 diabetes, and several GP inhibitors have shown efficacy in lowering blood glucose in clinical trials (*13*).

Given the significant biological importance and potential clinical utility of OA as a promising modulator of glycogen metabolism, synthesis and biological evaluation of new OA derivatives should prove helpful in finding more potent therapeutic agents with better pharmacokinetic properties. Recently, the current authors reported the synthesis and biological evaluation of several pyrazole[4,3-*b*]oleanane derivatives as GP inhibitors (*9*). This paper describes the synthesis, GP inhibitory activity, and structure–activity relationships of fifteen novel substituted phenylpyrazole [4,5-*b*]oleanane derivatives. To the extent known, all of the OA derivatives in this study have not yet to be reported.

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2. Materials and Methods

2.1 *Chemistry*

2.1.1 *General methods*

The reagents (chemicals): Shanghai Chemical Reagent Company. Column chromatography (CC): silica gel 60 (200-300 mesh). TLC: silica gel 60 F254 plates (250 μm, Qindao Ocean Chemical Company, China). Melting points (M.p.): capillary tube; uncorrected. Infrared (IR) spectra: Shimadzu FTIR-8400S spectrometer; in cm^{-1}. ^1H- and ^{13}C-NMR spectra: ACF* 300Q Bruker, $CDCl_3$ unless otherwise indicated; δ in ppm, *J* in Hz. LR-MS: Hewlett-Packard 1100 LC/MSD spectrometer.

2.1.2 *Synthesis*

Benzyl 2-Hydroxymethylene-3-oxooleana-12-en-28-oate (**4**)

A mixture of **3** (*10*) (1 g, 1.84 mmol), NaOMe (1 g, 18.52 mmol), and HCO_2Et (1.5 mL, 18.58 mmol) in CH_2Cl_2 (20 mL) was stirred at r.t. for 10 h, and then the reaction mixture was evaporated *in vacuo*. Brine was added to the residue, and the mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by flash CC

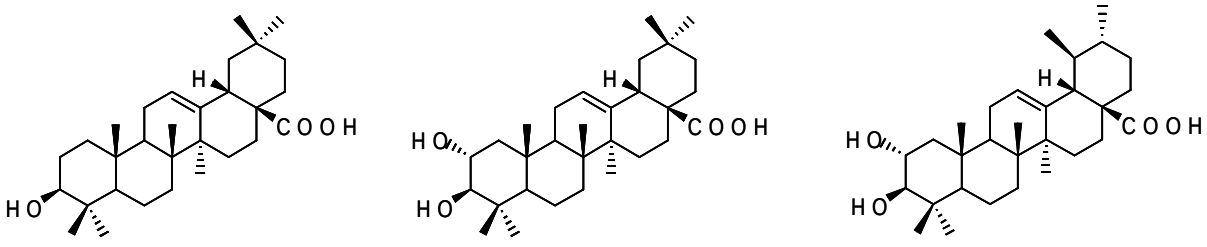


Figure 1. Several natural pentacyclic triterpenes as GP inihibitors.

(silica gel; heptane/AcOEt 50:1) to afford 0.84 g (80%) of **4** as a white solid. M.p. 143-145˚C. IR (KBr): 3431, 2943, 1726, 1166. ^{1}H-NMR (300 MHz): 0.66 (3H, *s*); 0.88 (3H, *s*); 0.90 (3H, *s*); 0.93 (3H, *s*); 1.11 (3H, *s*); 1.14 (3H, *s*); 1.18 (3H, *s*); 2.26 (1H, *d*, *J* = 14.4 Hz); 2.93 (1H, *dd*, *J* = 4.0, 13.8 Hz); 5.05 (1H, *d*, *J* = 12.5 Hz); 5.10 (1H, *d*, *J* = 12.5 Hz); 5.33 (1H, *t*, *J* = 3.5 Hz); 7.29~7.35 (5H, *m*); 8.56 (1H, *s*). ESI-MS: 595 ([M+Na]$^+$).

Benzyl 1'-phenylpyrazole[4,5-b]olean-12-en-28-oate (**5**)

A mixture of **4** (0.5 g, 0.87 mmol) and phenylhydrazine hydrochloride (0.13 g, 0.92 mmol) in EtOH (20 mL) was heated under reflux for 20 h. Brine was added to the residue, and the mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by flash CC (SiO_2; heptane/AcOEt 50:1) to afford 0.41 g (72%) of **5** as a white solid. M.p. 118-120˚C. IR (KBr): 3454, 2947, 1726, 1627, 1384. ^{1}H-NMR (300MHz): 0.68 (3H, *s*); 0.91 (6H, *s*); 0.94 (3H, *s*); 1.01 (3H, *s*); 1.05 (3H, *s*); 1.15 (3H, *s*); 2.11 (1H, *d*, *J* = 14.9 Hz); 2.62 (1H, *d*, *J* = 14.9 Hz); 2.92~2.97 (1H, *m*); 5.05 (1H, *d*, *J* = 12.5 Hz); 5.11 (1H, *d*, *J* = 12.5 Hz); 5.37 (1H, br *s*); 7.32~7.46 (11H, *m*). ESI-MS: 645 ([M+H]$^+$). Anal. calc. for $C_{44}H_{56}N_2O_2$: C 81.94, H 8.75, N 4.34; Found: C 81.80, H 8.89, N 4.11.

Benzyl 1'-(p-tolyl)pyrazole[4,5-b]olean-12-en-28-oate (**6**)

Following the procedure for the preparation of **5**, treatment of **4** (0.5 g, 0.87 mmol) with *p*-tolylhydrazine hydrochloride (0.15 g, 0.92 mmol) afforded 0.49 g (85%) of **6**. M.p. 113-115˚C. IR (KBr): 3429, 2925, 1724, 1384. ^{1}H-NMR (300 MHz, DMSO-d_6): 0.60 (3H, *s*); 0.84 (3H, *s*); 0.89 (9H, *s*); 0.93 (3H, *s*); 0.98 (3H, *s*); 1.12 (3H, *s*); 2.05 (1H, *d*, *J* = 15.2 Hz); 2.38 (3H, *s*); 2.83~2.88 (1H, *m*); 5.04 (2H, *m*); 5.27 (1H, br *s*); 7.20~7.40 (10H, *m*). ^{13}C-NMR (75 MHz): 176.3; 145.2; 143.0; 139.6; 138.4; 137.4; 136.3; 128.8; 128.7; 128.3; 127.8; 127.6; 122.6; 122.0; 113.1; 65.2; 54.0; 46.1; 45.6; 45.3; 41.3; 41.1; 38.5; 38.1; 37.5; 36.4; 34.1; 33.1; 32.6; 31.9; 31.7; 30.3; 29.1; 27.1; 25.2; 23.2; 22.7; 22.6; 22.0; 20.6; 18.6; 16.2; 14.9. ESI-MS: 659 ([M+H]$^+$). Anal. calc. for $C_{45}H_{58}N_2O_2$: C 82.02, H 8.87, N 4.25; Found: C 81.73, H 8.92, N 3.78.

Benzyl 1'-(4''-chlorophenyl)pyrazole[4,5-b]olean-12-en-28-oate (**7**)

Following the procedure for the preparation of **5**, treatment of **4** (0.5 g, 0.87 mmol) with (4-chlorophenyl) hydrazine hydrochloride (0.16 g, 0.92 mmol) afforded 0.49 g (83%) of **7**. M.p. 125-127˚C. IR (KBr): 3440, 2947, 1724, 1159. ^{1}H-NMR (300 MHz, DMSO-d_6): 0.60 (3H, *s*); 0.84 (3H, *s*); 0.89 (6H, *s*); 0.93 (3H, *s*); 0.98 (3H, *s*); 1.12 (3H, *s*); 2.05 (1H, *d*, *J* = 15.1 Hz); 2.83~2.89 (1H, *m*); 5.04 (2H, *m*); 5.27 (1H, br *s*); 7.30~7.43 (8H, *m*); 7.53~7.58 (2H, *m*). ^{13}C-NMR (75 MHz): 176.3. 145.6; 143.1; 141.0; 138.0; 136.3; 133.5; 130.8; 128.6; 128.3; 127.8; 127.6; 122.0; 113.6; 75.3; 53.9; 46.1; 45.6; 41.4; 41.1; 40.4; 37.5; 35.3; 34.1; 33.2; 32.6; 31.9; 31.7; 30.3; 29.2; 27.2; 25.3; 23.2; 22.8; 22.6; 22.2; 18.6; 16.3; 14.9. ESI-MS: 679 ([M+H]$^+$). Anal. calc. for $C_{44}H_{55}ClN_2O_2$: C 77.79, H 8.16, N 4.12; Found: C 77.74, H 8.32, N 3.84.

Benzyl 1'-(4''-isopropylphenyl)pyrazole[4,5-b]olean-12-en-28-oate (**8**)

Following the procedure for the preparation of **5**, treatment of **4** (0.5 g, 0.87 mmol) with (4-isopropyl-phenyl)hydrazine hydrochloride (0.17 g, 0.92 mmol) afforded 0.49 g (81%) of **8**. M.p. 95-97˚C. IR (KBr): 3431, 2952, 1726, 1460, 1384, 1159. ^{1}H-NMR (300 MHz): 0.68 (3H, *s*); 0.91 (6H, *s*); 0.94 (3H, *s*); 1.01 (3H, *s*); 1.05 (3H, *s*); 1.15 (3H, *s*); 2.10 (1H, *d*, *J* = 15.1 Hz); 2.60 (1H, *d*, *J* = 14.9 Hz); 2.92~3.01 (2H, *m*); 5.05 (1H, *d*, *J* = 12.5 Hz); 5.09 (1H, *d*, *J* = 12.5 Hz); 5.37 (1H, br *s*); 7.27~7.36 (10H, *m*). ESI-MS: 687 ([M+H]$^+$). Anal. calc. for $C_{47}H_{62}N_2O_2$: C 82.17, H 9.10, N 4.08; Found: C 81.98, H 9.10, N 3.88.

Benzyl 1'-(3'',5''-difluorophenyl)pyrazole[4,5-b]olean-12-en-28-oate (**9**)

Following the procedure for the preparation of **5**, treatment of **4** (0.5 g, 0.87 mmol) with (3,5-difluoro-phenyl)hydrazine hydrochloride (0.17 g, 0.92 mmol)

afforded 0.49 g (83%) of **9**. M.p. 95-96˚C. IR (KBr): 3425, 2949, 1726, 1616, 1458, 1122. ^{1}H-NMR (300 MHz, DMSO-d_6): 0.61 (3H, *s*); 0.85 (3H, *s*); 0.89 (6H, *s*); 0.98 (3H, *s*); 1.02 (3H, *s*); 1.12 (3H, *s*); 2.07 (1H, *d*, *J* = 15.1 Hz); 2.83~2.89 (1H, *m*); 5.04 (2H, *m*); 5.27 (1H, br *s*); 7.22~7.39 (8H, *m*); 7.43~7.52 (1H, *m*). ^{13}C-NMR (75 MHz): 176.2; 163.2; 163.1; 159.8; 145.7; 144.5; 144.3; 144.1; 143.0; 138.4; 136.2; 128.2; 127.7; 127.6; 122.0; 113.8; 113.3; 113.2; 113.0; 112.9; 105.2; 104.9; 104.6; 65.2; 54.0; 46.1; 45.6; 45.3; 41.3; 41.1; 37.5; 36.3;34.0; 33.1; 32.6; 31.9; 31.7; 30.2; 29.1; 27.1; 25.2; 23.2; 22.7; 22.6; 22.4; 22.2; 18.6; 16.2. ESI-MS: 681 ([M+H]$^+$). Anal. calc. for $C_{44}H_{54}F_2N_2O_2$: C 77.61, H 7.99, N 4.11; Found: C 77.60, H 8.25, N 3.68.

Benzyl 1'-(4''-cyanophenyl)pyrazole[4,5-b]olean-12-en-28-oate (**10**)

Following the procedures for the preparation of **5**, treatment of **4** (0.5 g, 0.87 mmol) with 4-hydrazinylbenzonitrile hydrochloride (0.16 g, 0.92 mmol) afforded 0.47 g (80%) of **10**. M.p. 135-137˚C. IR (KBr): 3442, 2947, 1724, 1382, 1159. ^{1}H-NMR (300 MHz, DMSO-d_6): 0.60 (3H, *s*); 0.85 (3H, *s*); 0.89 (6H, *s*); 0.94 (3H, *s*); 0.97 (3H, *s*); 1.12 (3H, *s*); 2.07 (1H, *d*, *J* = 15.3 Hz); 2.55 (1H, *d*, *J* = 15.1 Hz); 2.83~2.89 (1H, *m*); 5.04 (2H, *m*); 5.27 (1H, br *s*); 7.28~7.40 (6H, *m*); 7.58~7.62 (2H, *m*); 7.97~8.02 (2H, *m*). ^{13}C-NMR (75 MHz): 176.8; 146.6; 146.4; 143.6; 139.2; 136.8; 133.4; 130.5; 128.8; 128.3; 128.2; 122.6; 118.6; 114.6; 65.8; 54.5; 46.7; 46.2; 45.9; 41.9; 41.7; 39.1; 38.0; 36.8; 34.6; 33.7; 33.2; 32.5; 32.2; 30.8; 29.8; 27.6; 25.8; 23.8; 23.3; 23.2; 22.9; 19.2; 16.8; 15.5. ESI-MS: 670 ([M+H]$^+$). Anal. calc. for $C_{45}H_{55}N_3O_2$: C 80.68, H 8.27, N 6.27; Found: C 80.55, H 8.46, N 5.99.

Benzyl 1'-(4''-carboxyphenyl)pyrazole[4,5-b]olean-12-en-28-oate (**11**)

Following the procedures for the preparation of **5**, treatment of **4** (0.5 g, 0.87 mmol) with 4-hydrazinylbenzoic acid hydrochloride (0.17 g, 0.92 mmol) afforded 0.28 g (47%) of **11**. M.p. 165-167˚C. IR (KBr): 3425, 2945, 1726, 1379, 1163. ^{1}H-NMR (300 MHz, DMSO-d_6): 0.60 (3H, *s*); 0.85 (3H, *s*); 0.89 (6H, *s*); 0.93 (3H, *s*); 0.97 (3H, *s*); 1.12 (3H, *s*); 2.06 (1H, *d*, *J* = 15.0 Hz); 2.54 (1H, *d*, *J* = 15.0 Hz); 2.84~2.88 (1H, *m*); 5.03 (1H, *d*, *J* = 13.0 Hz); 5.06 (1H, *d*, *J* = 13.0 Hz); 5.27 (1H, *t*, *J* = 3.2 Hz); 7.30~7.37 (6H, *m*); 7.60~7.62 (2H, *m*); 7.98~8.00 (2H, *m*). ^{13}C-NMR (75 MHz): 176.3; 146.1; 145.9; 143.1; 138.7; 136.3; 132.9; 130.0; 128.3; 127.8; 127.7; 122.1; 118.1; 114.1; 111.8; 65.3; 53.9; 46.1; 45.6; 45.3; 41.4; 41.1; 39.0; 38.7; 37.5; 36.3; 34.1; 33.1; 32.6; 31.9; 31.7; 30.3; 29.3; 27.1; 25.2; 23.2; 22.8; 22.6; 22.3; 18.6; 16.2; 14.9. ESI-MS: 689 ([M+H]$^+$). Anal. calc. for $C_{45}H_{56}N_2O_4$: C 78.45, H 8.19, N 4.07; Found: C 78.26, H 8.50, N 3.91.

Benzyl 1'-(4''-methoxyphenyl)pyrazole[4,5-b]olean-12-en-28-oate (**12**)

Following the procedures for the preparation of **5**, treatment of **4** (0.5 g, 0.87 mmol) with (4-methoxyphenyl)hydrazine hydrochloride (0.16 g, 0.92 mmol) afforded 0.37 g (62%) of **12**. M.p. 171-173˚C. IR (KBr): 3423, 2945, 1726, 1516, 1250. ^{1}H-NMR (300 MHz): 0.67 (3H, *s*); 0.89 (3H, *s*); 0.91 (3H, *s*); 0.94 (3H, *s*); 1.03 (3H, *s*); 1.08 (3H, *s*); 1.15 (3H, *s*); 2.64 (1H, *d*, *J* = 14.9 Hz); 2.92~2.98 (1H, *m*); 3.86 (3H, *s*); 5.05 (1H, *d*, *J* = 12.5 Hz); 5.10 (1H, *d*, *J* = 12.5 Hz); 5.36 (1H, *t*, *J* = 3.6 Hz); 6.90~6.98 (2H, *m*); 7.29~7.36 (7H, *m*); 7.49 (1H, *s*). ESI-MS: 675 ([M+H]$^+$). Anal. calc. for $C_{45}H_{58}N_2O_3$: C 80.08, H 8.66, N 4.15; Found: C 79.85, H 8.72, N 3.93.

Benzyl 1'-(2''-ethylphenyl)pyrazole[4,5-b]olean-12-en-28-oate (**13**)

Following the procedures for the preparation of **5**, treatment of **4** (0.5 g, 0.87 mmol) with (2-ethylphenyl) hydrazine hydrochloride (0.16 g, 0.92 mmol) afforded 0.51 g (87%) of **13**. M.p. 108-110˚C. IR (KBr): 3440, 2947, 1726, 1456, 1382. ^{1}H-NMR (300 MHz, DMSO-d_6): 0.59 (3H, *s*); 0.70 (3H, *s*); 0.81 (3H, *s*); 0.83 (3H, *s*); 0.89 (6H, *s*); 1.02 (3H, *s*); 2.83~2.89 (1H, *m*); 5.02~5.06 (2H, *m*); 5.27 (1H, br *s*); 7.29~7.47 (10H, *m*). ^{13}C-NMR (75 MHz): 176.2; 145.0; 144.9; 143.0; 142.7; 142.6; 140.4; 140.3; 137.6; 137.5; 136.2; 129.4; 129.3; 129.2; 129.1; 128.4; 128.3; 127.8; 127.6; 125.5; 122.0; 113.3; 113.1; 65.2; 53.9; 46.1; 45.7; 45.5; 45.3; 45.3; 41.4; 41.1; 38.4; 38.3; 38.2; 37.6; 37.5; 36.3; 34.1; 34.0; 33.1; 32.6; 31.9; 31.7; 30.3; 29.7. 27.3; 27.1; 25.2; 25.2; 23.2; 23.0; 23.0; 22.8; 22.6; 22.4; 20.2; 18.6; 16.3; 16.2; 15.1; 14.6; 14.4; 14.3. ESI-MS: 673 ([M+ H]$^+$).

Benzyl 1'-(3''-chloro-4''-methylphenyl)pyrazole[4,5-b]olean-12-en-28-oate (**14**)

Following the procedures for the preparation of **5**, treatment of **4** (0.5 g, 0.87 mmol) with (3-chloro-4-methylphenyl)hydrazine hydrochloride (0.18 g, 0.92 mmol) afforded 0.51 g (85%) of **14**. M.p. 92-93˚C. IR (KBr): 3452, 2947, 1726, 1159. ^{1}H-NMR (300 MHz): 0.68 (3H, *s*); 0.90 (3H, *s*); 0.91 (3H, *s*); 0.94 (3H, *s*); 1.02 (3H, *s*); 1.06 (3H, *s*); 1.16 (3H, *s*); 2.09 (1H, *d*, *J* = 15.0 Hz); 2.44 (3H, *s*); 2.59 (1H, *d*, *J* = 14.9 Hz); 2.93~2.97 (1H, *m*); 5.05 (1H, *d*, *J* = 12.5 Hz); 5.10 (1H, *d*, *J* = 12.5 Hz); 5.37 (1H, br *s*); 7.17~7.20 (1H, *m*); 7.27~7.40 (8H, *m*). ^{13}C-NMR (75 MHz): 177.5; 146.3; 143.6; 141.2; 138.7; 137.1; 136.5; 133.9; 130.5; 129.7; 128.5; 128.0; 127.9; 127.3; 122.6; 114.3; 66.0; 54.7; 46.4; 45.9; 45.9; 41.9; 41.6; 39.3; 38.07; 36.9; 34.7; 33.9; 33.1; 32.4; 32.3; 30.7; 29.5; 27.7; 25.6; 23.6; 23.3; 23.1; 22.4; 19.8; 19.2; 16.6; 15.2. ESI-MS: 693 ([M+H]$^+$).

3-Oxoleana-12-en-28-oic acid (**15**)

To a solution of **1** (OA) (2 g, 4.3 mmol) in acetone (100 mL) was added Jones reagent (8 mL) at 0˚C. The resulting mixture was stirred for 30 min. Then the reaction mixture was quenched by EtOH. After removing most solvents by evaporation under reduced pressure, to the residue was added EtOAc (50 mL) and THF (10 mL). The organic layer was washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by flash CC (SiO_2; heptane/AcOEt 15:1) to afford 1.6 g (80%) of **15**. M.p. 199-201˚C. IR (KBr): 3448, 2945, 1701, 1460, 1384. ^{1}H-NMR (300 MHz): 0.81 (3H, *s*); 0.91 (3H, *s*); 0.93 (3H, *s*); 1.03 (3H, *s*); 1.05 (3H, *s*); 1.09 (3H, *s*); 1.15 (3H, *s*); 2.30~2.48 (1H, *m*); 2.49~2.65 (1H, *m*); 2.84 (1H, *dd*, *J* = 4.0, 13.7 Hz); 5.30 (1H, br *s*). ESI-MS: 453 ([M-H]$^-$).

2-Hydroxymethylene-3-oxooleana-12-en-28-oic acid (**16**)

A mixture of **15** (0.84 g, 1.85 mmol), NaOMe (1 g, 18.52 mmol) and HCO_2Et (1.5 mL, 18.58 mmol) in CH_2Cl_2 (20 mL) was stirred at r.t. for 10 h, and then the reaction mixture was evaporated *in vacuo*. Brine was added to the residue, and the mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by flash CC (silica gel; heptane/AcOEt 10:1) to afford 0.7 g (78%) of **16**. White solid. M.p. 212-214˚C. IR (KBr): 2945, 1691, 1581. ^{1}H-NMR (300 MHz, DMSO-d_6): 0.76 (6H, *s*); 0.86 (6H, *s*); 1.10 (9H, *s*); 2.29 (1H, *d*, *J* = 14.5 Hz); 2.75 (1H, *dd*, *J* = 3.7, 13.9 Hz); 5.20 (1H, *s*); 8.73 (1H, *s*); 11.98 (1H, *s*); 14.28 (1H, br *s*). ESI-MS: 481 ([M-H]$^-$).

1'-Phenylpyrazole[4,5-b]olean-12-en-28-oisc acid (**17**)

A mixture of **16** (0.5 g, 1.04 mmol) and phenylhydrazine hydrochloride (0.16 g, 1.09 mmol) in EtOH (20 mL) was heated under reflux for 20 h. Brine was added to the residue, and the mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by flash CC (SiO_2; CH_2Cl_2/MeOH 100:1) and then recrystallization from AcOEt to afford 0.42g (73%) of **17**. White solid. M.p. > 300˚C. IR (KBr): 2904, 1722, 1458, 1382. ^{1}H-NMR (300 MHz, DMSO-d_6): 0.80 (3H, *s*); 0.89 (9H, *s*); 0.94 (3H, *s*); 0.98 (3H, *s*); 1.13 (3H, *s*); 2.07 (1H, *d*, *J* = 14.9 Hz); 2.55 (1H, *d*, *J* = 14.9 Hz); 2.76~2.83 (1H, *m*); 5.25 (1H, br *s*); 7.29 (1H, *s*); 7.33~7.38 (2H, *m*); 7.45~7.52 (3H, *m*); 12.01 (1H, *s*). ^{13}C-NMR (75 MHz): 178.7; 145.5; 143.7; 142.3; 137.8; 129.2; 129.1; 128.6; 121.7; 113.5; 54.2; 45.9; 45.8; 45.7; 41.6; 41.1; 37.8; 36.6; 34.3; 33.5; 33.0; 32.2; 32.0; 30.5; 29.3; 27.4; 25.5; 23.5; 23.0; 22.8; 22.3; 18.9; 16.7; 15.2. HRMS: Calcd for $C_{37}H_{49}N_2O_2$: 553.3794 ([M-H]$^-$), Found 553.3795 ([M-H]$^-$). Anal. calc. for $C_{37}H_{50}N_2O_2$: C 80.10, H 9.08, N 5.05; Found: C 79.77, H 8.85, N 4.98.

1'-(p-Tolyl)pyrazole[4,5-b]olean-12-en-28-oic acid (**18**)

Following the procedures for the preparation of **17**, treatment of **16** (0.5 g, 1.04 mmol) with *p*-tolylhydrazine hydrochloride (0.17 g, 1.09 mmol) afforded 0.38 g (65%) of **18**. M.p. > 300˚C. IR (KBr): 2950, 1718, 1515, 1384. ^{1}H-NMR (300 MHz, DMSO-d_6): 0.79 (3H, *s*); 0.89 (9H, *s*); 0.94 (3H, *s*); 0.99 (3H, *s*); 1.13 (3H, *s*); 2.05 (1H, *d*, *J* = 14.9 Hz); 2.39 (3H, *s*); 2.76~2.81 (1H, *m*); 5.25 (1H, br *s*); 7.20~7.30 (5H, *m*); 12.01 (1H, br *s*). ^{13}C-NMR (75 MHz): 178.7; 145.5; 143.8; 139.8; 138.6; 137.7; 129.0; 128.9; 121.7; 113.3; 54.3; 45.9; 45.8; 45.7; 41.6; 41.1; 37.8; 34.3; 33.5; 32.9; 32.2; 32.0; 30.5; 29.3; 25.5; 23.5; 23.0; 22.8; 22.3; 22.3; 20.9; 18.9; 16.7; 15.2. HRMS: Calcd for $C_{38}H_{51}N_2O_2$: 567.3951 ([M-H]$^-$), Found 567.4000 ([M-H]$^-$). Anal. calc. for $C_{38}H_{52}N_2O_2$: C 80.24, H 9.21, N 4.92; Found: C 80.06, H 9.11, N 4.82.

1'-(4''-Chlorophenyl)pyrazole[4,5-b]olean-12-en-28-oic acid (**19**)

Following the procedures for the preparation of **17**, treatment of **16** (0.5 g, 1.04 mmol) with (4-chlorophenyl) hydrazine hydrochloride (0.19 g, 1.09 mmol) afforded 0.38 g (62%) of **19**. M.p. 260-262˚C. IR (KBr): 3443, 2945, 1700, 1613. ^{1}H-NMR (300 MHz): 0.88 (3H, *s*); 0.97 (6H, *s*); 1.00 (3H, *s*); 1.06 (3H, *s*); 1.13 (3H, *s*); 1.22 (3H, *s*); 2.69 (1H, *d*, *J* = 14.9 Hz); 2.89~2.96 (1H, *m*); 5.42 (1H, br *s*); 7.38~7.55 (5H, *m*). ESI-MS: 587 ([M-H]$^-$).

1'-(4''-Isopropylphenyl)pyrazole[4,5-b]olean-12-en-28-oic acid (**20**)

Following the procedures for the preparation of **17**, treatment of **16** (0.5 g, 1.04 mmol) with (4-isopropyl-phenyl)hydrazine hydrochloride (0.2 g, 1.09 mmol) afforded 0.45 g (73%) of **20**. M.p. 243-245˚C. IR (KBr): 3440, 2943, 1693, 1610. ^{1}H-NMR (300 MHz, DMSO-d_6): 0.76 (3H, *s*); 0.86 (9H, *s*); 0.92 (3H, *s*); 0.96 (3H, *s*); 1.10 (3H, *s*); 2.03 (1H, *d*, *J* = 14.9 Hz); 2.74~2.78 (1H, *m*); 2.91~3.00 (1H, *m*); 5.22 (1H, br *s*); 7.21~7.33 (5H, *m*); 11.97 (1H, br *s*). ESI-MS: 595 ([M-H]$^-$).

1'-(3'',5''-Difluorophenyl)pyrazole[4,5-b]olean-12-en-28-oic acid (**21**)

Following the procedures for the preparation of **17**, treatment of **16** (0.5 g, 1.04 mmol) with (3,5-difluoro-phenyl)hydrazine hydrochloride (0.2 g, 1.09 mmol) afforded 0.43 g (78%) of **21**. M.p. 208-210˚C. IR (KBr): 3448, 2947, 1703, 1616, 1122. ^{1}H-NMR (300 MHz,

DMSO-d_6): 0.80 (3H, *s*); 0.89 (9H, *s*); 0.99 (3H, *s*); 1.03 (3H, *s*); 1.13 (3H, *s*); 2.05 (1H, *d*, *J* = 15.1 Hz); 2.79 (1H, *dd*, *J* = 3.5, 13.4 Hz); 5.25 (1H, br *s*); 7.23~7.27 (2H, *m*); 7.35 (1H, *s*); 7.44~7.51 (1H, *m*); 12.02 (1H, br *s*). ^{13}C-NMR (75 MHz): 178.5; 163.3; 163.1; 160.0; 159.8; 145.7; 144.3; 144.1; 143.6; 138.5; 121.5; 113.9; 113.4; 113.2; 113.0; 105.3; 105.0; 104.6; 54.0; 45.7; 45.5; 41.4; 40.9; 40.3; 37.5; 36.3; 34.1; 33.3; 32.7; 32.0; 31.7; 30.3; 29.2; 27.2; 25.3; 23.3; 22.8; 22.6; 22.2; 18.7; 16.5; 14.9. ESI-MS: 589 ([M-H]$^-$).

2.2 *Enzymatic activity assays*

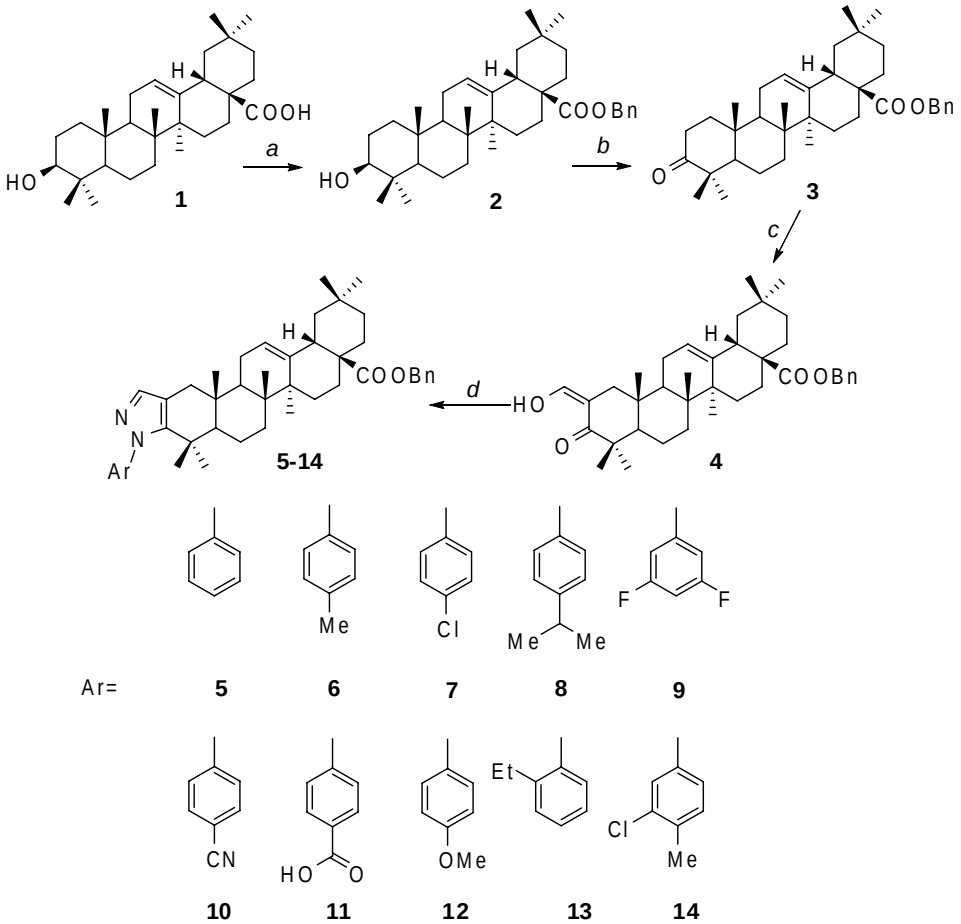
The inhibitory activity of the test compounds against rabbit muscle GPa was monitored using a microplate reader (BIO-RAD) based on the published method (*14*). In brief, GPa activity was measured in the direction of glycogen synthesis by the release of phosphate from glucose-1-phosphate. Each test compound was dissolved in DMSO and diluted at different concentrations for IC_{50} determination. The enzyme (GPa) was added to 100 μL of buffer containing 50 mM Hepes (pH 7.2), 100 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM glucose-1-phosphate, 1 mg/mL glycogen, and the test compound in 96-well microplates (Costar). After the addition of 150 μL of 1 M HCl containing 10 mg/mL ammonium molybdate and 0.38 mg/mL malachite green, reactions were run at 22˚C for 25 min. And then the phosphate absorbance was measured at 655 nm. The IC_{50} values were estimated by fitting the inhibition data to a dose-dependent curve using a logistic derivative equation.

3. Results and Discussion

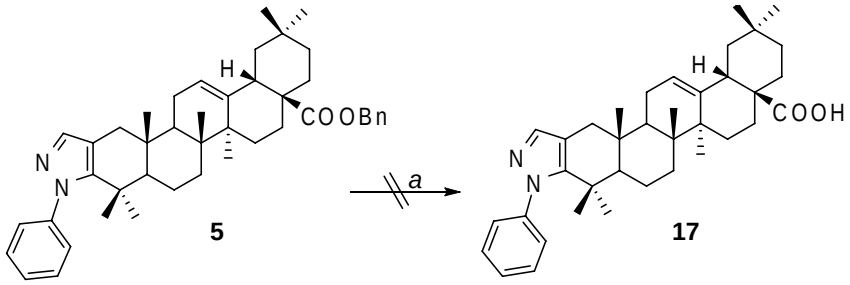
3.1 *Chemistry*

The synthesis of substituted phenylpyrazole [4,5-*b*]oleanane derivatives is illustrated in Schemes 1-3. Following the procedures reported previously (*9-11*), esterification of **1** (OA) with benzyl chloride afforded benzyl ester **2**. Treatment **2** with PCC afforded ketone **3**. Formylation of **3** with ethyl formate in the presence of NaOMe in CH_2Cl_2 gave compound **4**. Treatment of **4** with substituted phenylhydrazine hydrochloride in EtOH at reflux temperature afforded pyrazole triterpene **5**~**14** (47~87%).

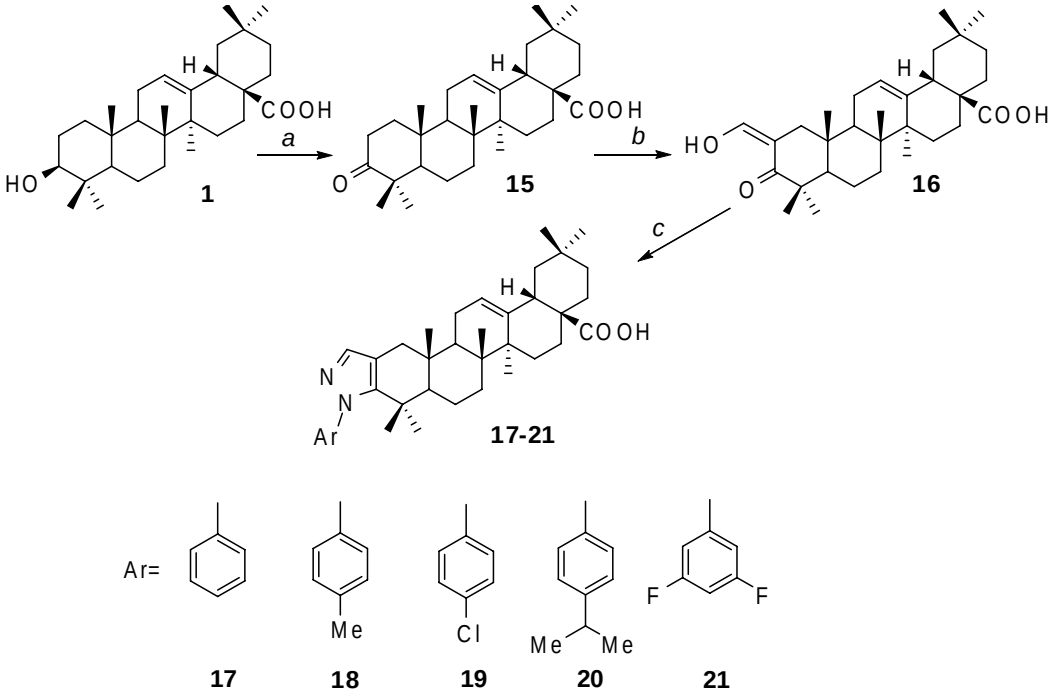
As shown in Scheme 2, the attempted hydrogenolysis of **5** in order to obtain the corresponding phenylpyrazole triterpene acid was unsuccessful, resulting in a complex mixture. Thus, a new approach for synthesis of phenylpyrazole triterpene acid was designed (*Scheme 3*).



Scheme 1. Reagents and conditions: *a)* BnCl, K_2CO_3, DMF, 50˚C; *b)* PCC, CH_2Cl_2, r.t; *c)* HCO_2Et, NaOMe, CH_2Cl_2, r.t, 80%; *d)* for **5-14**: $ArNH_2NH_2$•HCl, EtOH, reflux, 47-87%.



Scheme 2. Reagents and conditions: *a)* H_2, 10% Pd-C, AcOH/1M HCl, r.t.



Scheme 3. Reagents and conditions: *a)* Jones regent, acetone, 0˚C to r.t, 80%; *b)* HCO_2Et, NaOMe, CH_2Cl_2, r.t, 78%; *c)* for **17-21**: $ArNH_2NH_2$• HCl, EtOH, reflux, 62-73%.

Oxidation of **1** (OA) with Jones reagent in acetone gave keto-acid **15**. Formylation of **15** with ethyl formate in the presence of NaOMe in CH_2Cl_2 gave compound **16**. Treatment of **16** with substituted phenylhydrazine hydrochloride in EtOH at reflux temperature afforded substituted phenylpyrazole triterpene acid **17**~**21** (62~73%).

The structure of **17** was unambiguously determined by NMR and HRMS data (*see 2.1.2 Synthesis*), including ROESY analysis.

3.2 *Biological evaluation*

The synthesized phenylpyrazole[4,5-*b*]oleanane derivatives were biologically evaluated for their inhibitory activity against rabbit muscle GPa. The activity of rabbit muscle GPa was measured by detecting the release of phosphate from glucose -1-phosphate in the direction of glycogen synthesis (*14*). The assay results showed that most of the newly synthesized pyrazole triterpenes exhibited inhibitory activity against rabbit muscle GPa with IC_{50} values in the range of 10.8–535 μM (Table 1).

Table 1. Inhibition of rabbit muscle GPa by compounds **1**, **5-21**

Compounds	RMGPa IC_{50}[a] (μM)
1(OA)	14
5	NI[b]
6	57
7	10.8
8	NI
9	NI
10	257.2
11	NI
12	13.4
13	NI
14	535
17	35.1
18	18.3
19	241.7
20	69.6
21	46.9
caffeine[c]	114

[a] Values are means of three experiments; [b] NI means no inhibition; [c] Caffeine was used as a positive control.

3.3 *SAR analysis*

Preliminary structure-activity relationship (SAR) analysis showed that incorporation of a phenylpyrazole structural unit in the A-ring of oleanolic acid resulted in a slight increase in GPa inhibitory potency in some cases (*e.g.* **7** and **12**). C(28) Triterpene acids were more potent than the corresponding C(28) benzyl esters (*e.g.* **5** *vs.* **17**; **6** *vs.* **18**; **8** *vs.* **20**; **9** *vs.* **21**), indicating a preference for hydrophilic groups over hydrophobic groups at the C(28) position, agreeing with the authors' previous studies (*9,10*).

4. Conclusion

Fifteen new phenylpyrazole[4,5-*b*]oleanane derivatives have been synthesized and biologically evaluated for their inhibitory activity against rabbit muscle GPa. Within this series of compounds, **7** (IC_{50} = 10.8 μM) exhibited slightly more potent activity than its parent compound, **1** (OA). Preliminary SAR analysis showed a clear preference for hydrophilicity over hydrophobicity at both the C(28) and pyrazole unit in terms of GPa inhibition. Further biological evaluation of these phenylpyrazole triterpenes is ongoing and these results will be reported in due course.

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References

1 Singh GB, Singh S, Bani S, Gupta BD, Banerjee SK. Anti-inflammatory activity of oleanolic acid in rats and mice. J Pharm Pharmacol 1992; 44:456-458.
2 Mengoni F, Lichtner M, Battinelli L, Marzi M, Mastroianni CM, Vullo V, Mazzanti G. *In vitro* anti-HIV activity of oleanolic acid on infected human mononuclear cells. Planta Med 2002; 68:111-114.
3 Zhu YM, Shen JK, Wang HK, Cosentino LM, Lee KH. Synthesis and anti-HIV activity of oleanolic acid derivatives. Bioorg Med Chem Lett 2001; 11:3115-3118.
4 Hsu HY, Yang JJ, Lin CC. Effects of oleanolic acid and ursolic acid on inhibiting tumor growth and enhancing the recovery of hematopoietic system postirradiation in mice. Cancer Lett 1997; 111:7-13.
5 Tan GT, Lee S, Lee IS, Chen J, Leitner P, Besterman JM, Kinghorn AD, Pezzuto JM. Natural-product inhibitors of human DNA ligase I. Biochem J 1996; 314:993-1000.
6 Liu Y, Hartley DP, Liu J. Protection against carbon tetrachloride hepatotoxicity by oleanolic acid is not mediated through metallothionein. Toxicol Lett 1998; 95:77-85.
7 Jeong HG. Inhibition of cytochrome P450 2E1 expression by oleanolic acid: hepatoprotective effects against carbon tetrachloride-induced hepatic injury. Toxicol Lett 1999; 105:215-222.
8 Liu J, Liu Y, Parkinson A, Klaassen CD. Effect of oleanolic acid on hepatic toxicant-activating and detoxifying systems in mice. Exp Ther 1995; 275:768-774.
9 Chen J, Gong YC, Liu J, Hua WY, Zhang LY, Sun HB. Synthesis and biological evaluation of novel pyrazole[4,3-*b*]oleanane derivatives as inhibitors of glycogen phosphorylase. Chem Biodiv 2008; in press.
10 Chen J, Liu J, Zhang LY, Wu GZ, Hua WY, Wu XM, Sun HB. Pentacyclic Triterpenes. Part 3: Synthesis and biological evaluation of oleanolic acid derivatives as novel inhibitors of glycogen phosphorylase, Bioorg Med Chem Lett 2006; 16:2915-2919.
11 Wen XA, Sun HB, Liu J, Wu GZ, Zhang LY, Wu XM, Ni ZP. Pentacyclic Triterpenes. Part 1: The first examples of naturally occurring pentacyclic triterpenes as a new class of inhibitors of glycogen phosphorylases. Bioorg Med Chem Lett 2005; 15:4944-4948.
12 Wen XA, Zhang P, Liu J, Zhang LY, Wu XM, Ni PZ, Sun HB. Pentacyclic Triterpenes. Part 2: Synthesis and biological activity of maslinic acid derivatives as inhibitors of glycogen phosphorylase. Bioorg Med Chem Lett 2006; 16:722-726.
13 Kurukulasuriya R, Link JT, Madar DJ, Pei Z, Rohde JJ, Richards SJ, Souers AJ, Szczepankiewicz BG. Prospects for pharmacologic inhibition of hepatic glucose production. Curr Med Chem 2003; 10:99-121.
14 Martin WH, Hoover DJ, Armento SJ, Stock IA, McPherson RK, Danley DE, Stevenson RW, Barrett EJ, Treadway JL. Discovery of a human glycogen phosphorylase inhibitor that lowers blood glucose *in vivo*. Proc Natl Acad Sci USA 1998; 95:1776-1781.

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