Synthesis and Biological Evaluation of Oleanolic Acid Derivatives As Inhibitors of Protein Tyrosine Phosphatase 1B

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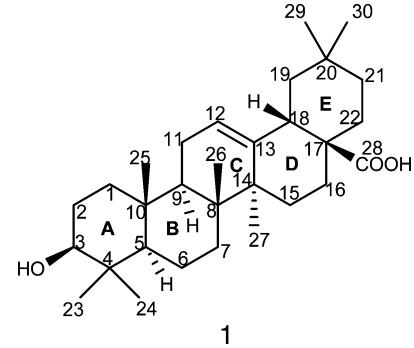
Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator in the process of insulin signaling and a promising drug target for diabetes and obesity. Derivatives of oleanolic acid were synthesized and evaluated as PTP1B inhibitors. Several derivatives exhibited moderate to good inhibitory activities against PTP1B, with **25f** displaying the most promising inhibition ($IC_{50} = 3.12\ \mu M$). Structure−activity relationship analyses of these derivatives demonstrated that the integrity of the A ring and 12-ene moieties was important in the retention of PTP1B enzyme inhibitory activities. In addition, hydrophilic and acidic groups as well as the distance between the oleanene and acid moieties were associated with PTP1B inhibitory activities. Possible binding modes of **25f** were explored by molecular docking simulations.

Reversible phosphorylation of protein tyrosyl residues is one of the key events that mediate the execution and regulation of many cellular processes. A proper level of phosphorylation is critical for these processes and is controlled by the opposing actions of protein tyrosine kinases, which catalyze the transfer of a phosphoryl group from adenosine 5′-triphosphate (ATP) to the *p*-hydroxy group of tyrosine, and protein tyrosine phosphatases (PTPs), which hydrolyze the phosphotyrosine (pY) back to tyrosine and inorganic phosphate.[1] The human genome encodes a superfamily of approximately 100 types of PTPs, which share a highly conserved catalytic domain of about 250 amino acids and consequently a common catalytic mechanism.[2] Protein tyrosine phosphatase 1B (PTP1B), first isolated from human placenta, has been confirmed to play an important role in insulin signaling.[3] PTP1B represents an important but challenging target class for drug discovery.[4] The enzyme is highly specific for the doubly charged pY residue, and consequently research seeking small-molecule inhibitors of PTP1B has been focused on finding mimics of pY.[5] However, the lack of drugs in clinical development highlights the need for potent and selective PTP1B inhibitors with desirable physicochemical properties and in vivo efficacies.

Compounds derived from natural products with unique and diverse chemical entities currently constitute a considerable resource for developing novel medications. Oleanolic acid (OA, **1**), a pentacyclic triterpenoid, has been in active clinical use as an antihepatitis drug in China for more than 20 years, while also displaying hypoglycemic,[6] anti-inflammatory,[7] and antitumorigenic effects,[8] as well as protecting the liver against toxic injury.[9]

Given the significant biological importance and the potentially new clinical utilities of OA (**1**) as a promising modulator of glycogen metabolism, it would be of great interest to design OA derivatives as potential PTP1B inhibitors. Therefore, we have designed a variety of new OA derivatives with modified A and C rings and C-17 moieties. In this article, we report the synthesis, PTP1B inhibitory activities, and structure−activity relationships (SAR) of these OA derivatives. The possible binding mode of **25f**, the most active compound, was established by molecular docking simulation.



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Results and Discussion

OA (**1**) can be modified at the A ring, the C ring, and C-17, while variations on the pentacyclic moiety were found to be ineffective. Conformational analyses of PTP1B indicate that its inhibitors should contain highly charged and polar functionalities, such as phosphonates and carboxylates, which occupy the active site of PTP1B.

Baeyer−Villiger oxidation was utilized to modify the A ring of OA (**1**) via introduction of highly charged and polar functionalities. Jones oxidation of OA (**1**) furnished ketone **2**, which was converted to esters **3**, **4**, and **5** by esterification with Me,[10] Et,[11] and BnBr,[12] respectively. Baeyer−Villiger oxidation of **5** was successfully performed using 3-chloroperbenzoic acid (MCPBA) and Li_2CO_3, forming the A-ring ε-lactone and preserving the C-12, C-13 double bond. The structure of acid **6b** (R = H), formed by hydrogenolysis of **6a** (R = Bn), was verified by elemental analyses, IR, ^{1}H NMR, ^{13}C NMR, and ESIMS data analysis. The ^{1}H NMR data displayed an olefinic hydrogen at δ_H 5.30. The ^{13}C NMR spectrum displayed resonances at δ_C 178.9 and 177.8, indicating the carbonyl groups at C-28 and C-3, and resonances at δ_C 140.4 and 122.7, indicating the C-12, C-13 double bond. The IR spectrum showed an ester absorption band at 1724 cm^{-1}, suggesting the formation of the A-ring ε-lactone moiety, and an absorption band at 1699 cm^{-1}, indicating the C-12, C-13 double bond. Elemental and ESIMS analysis confirmed the structure of acid **6b**. Finally, the A-ring ε-lactone moiety of **6b** (R = H) was hydrolyzed to produce diacid **7**.

The synthesis and biological evaluation of 3,12,13-trihydroxy-28,13-oleananolide indicated hypoglycemic activity.[13] On the basis of this result, we designed and synthesized the new OA derivative **10** (Scheme 1). Ozonolysis of ketone **2**[13] produced 12α,13β-

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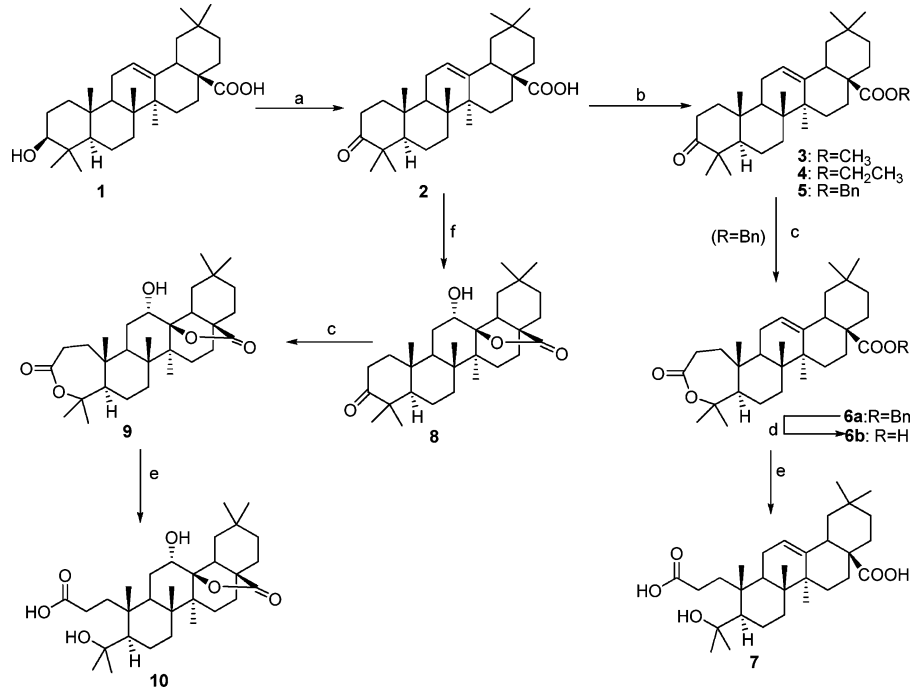
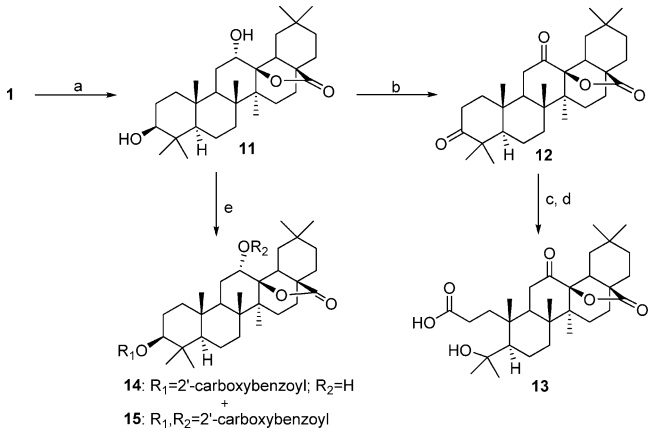
Scheme 1. Synthesis of **2**−**10**[a]



[a] Reagents and conditions: (a) 3 N Jones reagent, CH_2Cl_2/acetone, 0 °C; (b) RBr, K_2CO_3, DMF, rt; (c) MCPBA, Li_2CO_3, rt; (d) H_2, Pd−C, EtOH; (e) KOH, CH_2Cl_2/MeOH/H_2O, rt; (f) O_3, CH_2Cl_2, −70 °C.

Scheme 2. Synthesis of **11**−**15**[a]



[a] Reagents and conditions: (a) O_3, CH_2Cl_2, −70 °C; (b) 3 N Jones reagent, CH_2Cl_2/acetone, 0 °C; (c) MCPBA, Li_2CO_3, rt; (d) KOH, CH_2Cl_2/MeOH/H_2O, rt; (e) phthalic anhydride, DMAP, pyridine, 120 °C.

dihydroxy-3-oxo-28,13-oleananolide **8**, which was converted to the A-ring ε-lactone **9** via Baeyer−Villiger oxidation. As shown in the ^{1}H NMR spectrum, the formation of **9** could be explained by the resonances of the olide-type bridges between C3 and C4, and C-13 and C-28. ^{13}C NMR data displayed resonances at δ_C 179.6 and 177.7, indicating the carbonyl groups at C-28 and C-3, and a resonance at δ_C 74.2, indicating the hydroxy group at C-12. The structure was further confirmed via the IR absorption bands at 1742 ($\nu_{C=O}$) and 3484 (ν_{OH}) cm^{-1}. Lactone **9** was hydrolyzed to yield the A-ring cleaved hydroxy acid **10**.

Ozonolysis of OA (**1**) followed by Jones oxidation[13] yielded diketone **12** (Scheme 2). Subsequent Baeyer−Villiger oxidation and hydrolysis of **12** yielded hydroxy acid **13**. The structure of **13** was verified by elemental, IR, ^{1}H NMR, ^{13}C NMR, and ESIMS analyses. ^{13}C NMR data displayed a resonance at δ_C 206.0, indicating the C-12 carbonyl group. Esterification of **11** with phthalic anhydride yielded mono- and diesters **14** and **15**, respectively (Scheme 2).

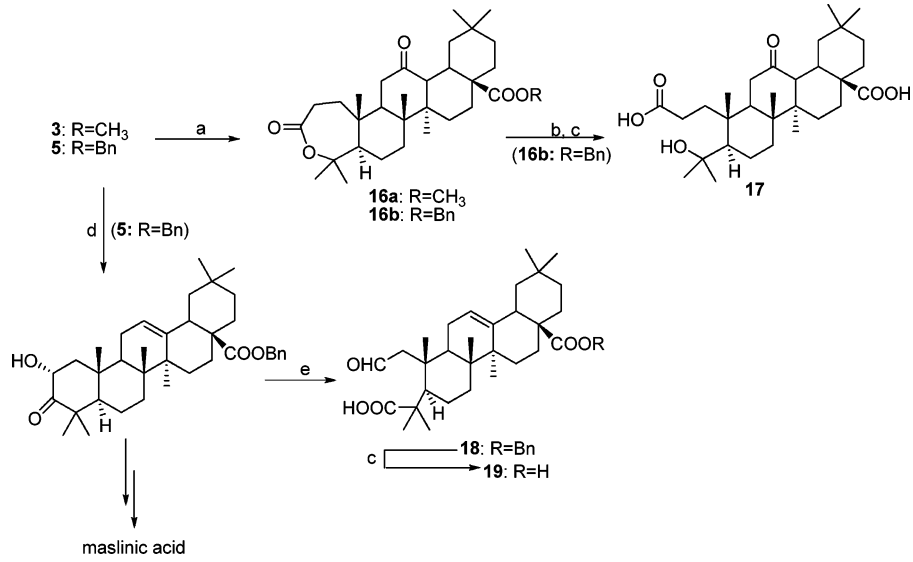
Baeyer−Villiger oxidation of **3** (R = CH_3) and **5** (R = Bn) using excess amounts of MCPBA and $NaHCO_3$ yielded **16a** (R = CH_3) and **16b** (R = Bn), respectively. Subsequent hydrolysis of **16b** (R = Bn) produced A-ring cleaved acid **17**. The structures of **16a** (R = CH_3) and **17** were verified by elemental, IR, ^{1}H NMR, ^{13}C NMR, and ESIMS analyses.

During the semisynthesis of maslinic acid starting from OA,[14] we obtained benzyl 2α-hydroxy-3-oxo-olean-12-en-28-oic acid,[15] which was oxidized to the A-ring cleaved acid **18** and hydrolyzed to produce diacid **19** (Scheme 3).

Modifications of the A and C rings of OA (**1**) provided **2**−**19**; however, the C-17 carboxylic group is also important from the perspective of SAR. Acetalization of **4** with ethylene glycol, followed by reduction with $LiAlH_4$, afforded the alcohol **20** (Scheme 4). Deprotection of **20** yielded 3-oxo-erythrodiol **21**,[10] which was converted to oleanolic trifluoromethanesulfonate **22** and bromide **23**. Esterification of ketone **21** with dicarboxylic monobenzyl esters[16] followed by deprotection of the benzyl group afforded **25a**−**f**.

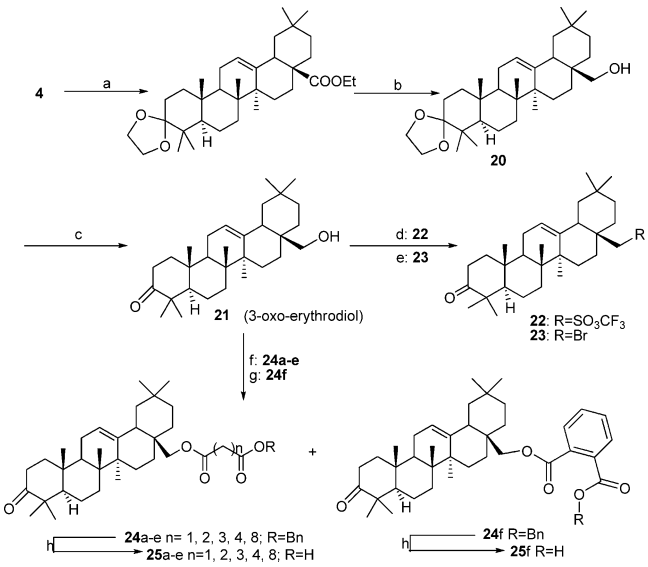
The PTP1B inhibitory activities of OA (**1**) and its synthetic derivatives **2**−**19** were evaluated (Table 1). The 3-keto derivative **2** showed less activity than OA (**1**). Esterification of the C-28 carboxylic acid group (**3**−**5**) diminished the inhibitory activities compared to **1** and **2**, indicating that C-3 hydroxy and C-28 carboxylic acid groups contribute to PTP1B inhibition. The oleanolic hydroxy-olide derivatives **8**, **11**, and **12** displayed lower PTP1B inhibitory activities than the olean-12-ene-28-acids **1** and **2**. Introducing 2′-carboxybenzoyl groups at C-3 and/or C-12 of **11** increased the potency 8.5 and 2.9 times for **14** and **15**, respectively, compared to **11**. The A-ring ε-lactone derivatives **6b** (R = H), **9**, and **16a** (R = CH_3) and the A-ring cleaved acids **7**, **10**, **13**, **17**, **18**, and **19** displayed poor inhibitory activities. These results indicate that the integrity of the A ring and 12-ene moieties was important for the retention of PTP1B inhibitory activities. In addition,

Scheme 3. Synthesis of **16**−**19**[a]



[a] Reagents and conditions: (a) MCPBA, $NaHCO_3$, rt; (b) KOH, CH_2Cl_2/MeOH/H_2O, rt; (c) H_2, Pd−C, EtOH; (d) MCPBA, CH_2Cl_2/MeOH, 0 °C; (e) $Pb(OAc)_4$, CH_2Cl_2/MeOH, 0 °C.

Scheme 4. Synthesis of **20**−**25**[a]



[a] Reagents and conditions: (a) ethylene glycol, toluene, 110 °C; (b) $LiAlH_4$, THF, 0 °C → rt; (c) 2 N HCl, THF, 100 °C; (d) trifluoromethanesulfonic acid anhydride, CH_2Cl_2, rt; (e) $(CH_3)_3SiBr$, pyridine, CH_2Cl_2, rt; (f) $HOOC(CH_2)n$-COOBn, $(COCl)_2$, DMF/CH_2Cl_2, rt → 0 °C; (g) 2-(benzyloxycarbonyl)benzoic acid, $(COCl)_2$, DMF/CH_2Cl_2, rt → 0 °C; (h) H_2, Pd−C, EtOH.

hydrophilic and acidic groups were favorable for retention of PTP1B inhibitory activities.

Alcohol **21** and its derivatives **22** and **23** showed less activity than the parent acid **2**. Acids **25a**−**c** exhibited moderate to good inhibitory activities against PTP1B (**25a**: IC_{50} = 4.11 μM, **25b**: IC_{50} = 4.61 μM, **25c**: IC_{50} = 6.39 μM), with SAR analyses indicating that the distance between the oleanene and acid moieties determined PTP1B inhibitory potency (**25a** > **25b** > **25c** > **25d** > **25e**). Aromatic compound **25f** also exhibited 7.6-fold more potent PTP1B inhibitory activity than the parent compound OA (**1**).

The crystallographic structure of PTP1B with the substrate showed that the pTyr was buried within a pocket formed by the catalytic site. The base of the site was formed by the PTP "signature motif" (H/V)C(X)RS/T with the pTyr complexed with the amide

Table 1. Inhibition Percent and IC_{50} Values for the PTP1B Inhibition Assay

compound	inhibition %[a]	IC_{50} (μM)[b]	compounds	inhibition %[a]	IC_{50} (μM)
1	11.69	ND	**19**	15.02	ND
2	8.67	ND	**20**	2.98	ND
3	1.56	ND	**21**	1.17	ND
4	1.37	ND	**22**	1.83	ND
5	1.45	ND	**23**	7.16	ND
6b	14.12	ND	**24b**	1.70	ND
7	−4.12	ND	**24b**	10.75	ND
8	0.69	ND	**24c**	8.30	ND
9	0.49	ND	**24d**	5.92	ND
10	14.69	ND	**24e**	3.55	ND
11	6.78	ND	**24f**	2.98	ND
12	4.42	ND	**25a**	98.44	4.11 ± 1.4
13	4.33	ND	**25b**	89.92	4.61 ± 2.9
14	57.63	ND	**25c**	71.13	6.39 ± 0.9
15	19.92	ND	**25d**	55.56	ND
16a	1.76	ND	**25e**	12.00	ND
17	1.29	ND	**25f**	88.82	3.12 ± 1.7
18	15.60	ND	soduim orthovanadate[c]	96.36	ND

[a] The max inhibition concentration is 5 μg/mL. [b] Values are means of three experiments. ND: not determined. [c] Soduim orthovanadate was used as the positive control, of which the max inhibition concentration is 100 μg/mL.

groups of the signature motif and the phosphate with Arg-221. pTyr was in close proximity to Cys-215, which played a crucial role in the catalytic reaction. The catalytic site was highly hydrophilic: when an affinity selection was carried out to identify high-affinity peptides for PTP1B, a clear preference for acidic residues (Glu and Asp) was observed.[17] To explore the corresponding binding site and mode of the derivatives of OA for PTP1B inhibition, **25f** was selected for subsequent molecular docking simulation.

The catalytic domains of PTP1B are extremely highly conserved and are deeply buried in their binding cavities. This assumption was proven by molecular docking simulations during which the acidic residue of **25f** was buried within a deep catalytic site cleft on the protein's molecular surface (Figure 1). The top-ranked binding position of **25f** was determined as a preferred docking mode. The calculated dock score was 102.091 (PLP1 = 97.18) (Figure 2), which was slightly inferior to the docking result against the pyridothiophene derivative crystallized conformation of 2B07[18] (calculated dock score = 122.826; PLP1 = 120.65). The predicted

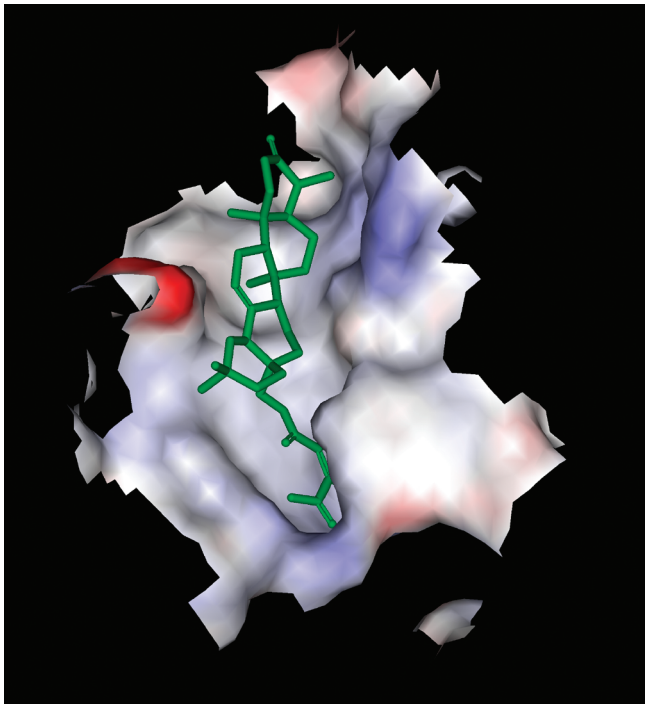


Figure 1. Enlarged surface view of binding site with the residues around ligand **25f**. The residues of the binding site are shown in plane format in off-white, and **25f** is shown as a stick structure in green. All hydrogen atoms are omitted for clarity.

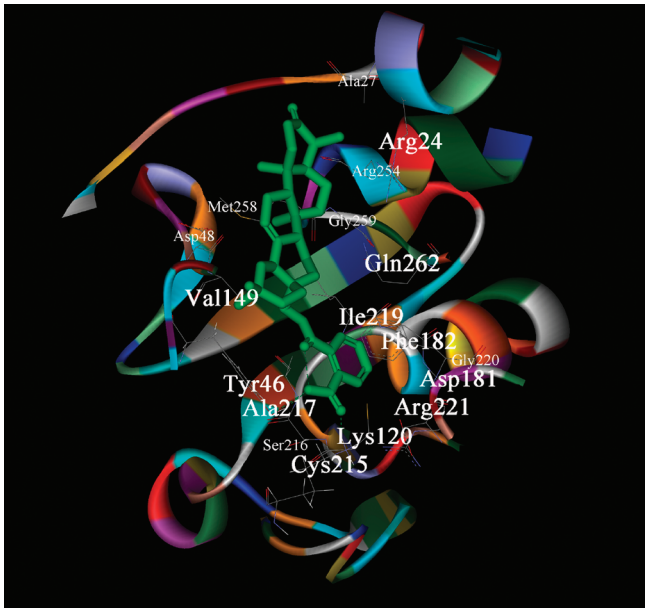


Figure 2. Stereoview of the binding mode of **25f** within PTP1B. The PTP1B structure is presented in the chromatic color cartoon. The residues of the binding site are shown in line format, and **25f** is shown as a stick structure in green. All hydrogen atoms are omitted for clarity.

binding position of the oleanolic acid moiety of **25f** occupied a similar location of the pyridothiophene inhibitor in the crystal structure. The 18-residue PTP signature motif, which was close to **25f**, was coordinated with **25f** by many varieties of interactions that contained the Arg-221 residue (Figure 2). The residue was essential in maintaining conformation by optimizing salt bridge interactions.

In the binding mode of **25f** with residues around the docked position (Figure 3), hydrogen bonds formed between the carboxylic group of the triterpene moiety and the hydroxy group of Tyr-46 plus the amino group of Lys-120, respectively, which enhanced their contribution to the stability of complex and substrate recognition. Other interactions that contributed to the high affinity of **25f** included a network of hydrophobic interactions between **25f** and Arg-24, Tyr-46, Asp-48, Val-49, Phe-182, Ala-217, Ile-219, and Gln-262, respectively. These hydrophobic networks were critical in maintaining good inhibitory activities, and they highlighted the contribution of the carboxylic group at C-17 in the triterpene moiety.

In summary, 34 oleanolic derivatives with modified A and C rings and substitutions at C-17, including 25 new compounds, were synthesized and biologically evaluated as inhibitors of PTP1B. Within the three series of compounds, **25f** (IC_{50} = 3.12 μM, inhibition % = 88.82%) exhibited 7.6-fold more potent PTP1B inhibitory activity than the parent compound **1** (inhibition % = 11.69%). SAR analysis shows that the integrity of the A ring and 12-ene moieties was important in the retention of PTP1B enzyme inhibitory activities; hydrophilic and acidic groups as well as the distance between the oleanene and acid moieties were associated with PTP1B inhibitory activities. Possible binding modes of **25f** were explored by molecular docking simulations. On the basis of these results, further design and biological evaluation of OA derivatives as promising drugs are ongoing in our laboratories, and results will be reported in due course.

Experimental Section

General Experimental Procedures. Melting points were measured on a YRT-3 melting point apparatus. Optical rotations were determined at room temperature with a Perkin-Elmer 141 polarimeter. IR spectra were obtained on a Perkin-Elmer 983. NMR spectra were recorded on a Varian INOVA400 instrument. Chemical shifts were expressed in δ (ppm) with TMS as the internal reference and coupling constants (J) expressed in Hz. Mass spectra were recorded on an Agilent 1946B ESIMS instrument. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA. TLC was performed using precoated silica gel GF254 (0.2 mm), and flash column chromatography was performed using silica gel (200−300 mesh).

Oleanic acid (**1**) was purchased from Huakang Chemical Company Inc. (Sichuan, P.R. China). PTP1B recombinant protein was supplied by Di Ao Pharmaceutical Group. PTP's substrate, *p*-nitrophenyl phosphate disodium hexahydrate, was supplied by Aladdin Co. Ltd. (Shanghai, P.R. China).

Olean-12-en-28-carboxy-3-oic acid ε-lactone [6b (R = H)]. A solution of benzyl ester **5**[12] (1.6 g, 3.0 mmol), MCPBA (0.5 g, 3.0 mmol), and Li_2CO_3 (0.9 g, 12.0 mmol) in CH_2Cl_2 (45 mL) was stirred at rt for 8 h, followed by quenching with Na_2SO_3. The reaction mixture was diluted with CH_2Cl_2, washed successively with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated to yield a residue, **6a** (R = Bn) (1.3 g), which was used in the next step without purification.

The residue **6a** (1.3 g) and 10% Pd−C (54.0 mg) were dispersed in MeOH (5 mL) and stirred under H_2 at 0.8 MPa for 24 h. The mixture was filtered, the filtrate concentrated to dryness, and the residue purified by column chromatography (PE/EtOAc, 6:1) to give **6b** (0.9 g, 64% in two steps) as white crystals: mp >250 °C; IR (KBr) ν_{max} 3441, 2946, 1724, 1698, 1460, 1384, 1112, 1026 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.30 (1H, s, H-12), 2.82 (1H, dd, J = 13.2, 3.2 Hz, H-18), 2.60 (2H, m, H-2), 1.52 (3H, s, H-26), 1.48 (3H, s, H-27), 1.41 (3H, s, H-25), 1.14, 1.13, 0.61, 0.60 (each 3H, s, H-23, H-24, H-29, H-30); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.9 (C, C-28), 177.8 (C, C-3), 140.4 (C, C-13),122.7 (CH, C-12), 79.8 (C, C-4), 73.2 (CH, C-5), 57.8 (C, C-17), 50.8 (CH, C-9), 47.3 (CH_2, C-19), 43.7 (C, C-14), 42.6 (C, C-10), 41.7 (C, C-8), 40.4 (CH, C-18), 37.6 (CH_2, C-1), 34.8 (CH_2, C-21), 33.3 (CH_2, C-7), 32.1 (CH_2, C-2), 31.2 (CH_3, C-29), 31.0 (CH_3, C-30), 30.5 (CH_2, C-11), 30.1 (CH_2, C-16), 29.8 (CH_2, C-22), 27.4 (C, C-20), 25.2 (CH_2, C-15), 22.8 (CH_3, C-24), 22.2 (CH_3, C-23), 22.0 (CH_2, C-6), 20.8 (CH_3, C-27), 15.6 (CH_3, C-26), 15.1 (CH_3, C-25); ESIMS m/z 471 $[M + H]^+$; *anal.* C 76.49%, H 9.89%, calcd for $C_{30}H_{46}O_4$, C 76.55%, H 9.85%.

Olean-12-en-4-hydroxy-3,28-dioic acid (7). A solution of lactone **6** (100.0 mg, 0.2 mmol) and KOH (297.0 g, 5.3 mmol) in CH_2Cl_2/MeOH/H_2O (14 mL, 3:3:1) was stirred at rt for 4 h. The residue was extracted with CH_2Cl_2, and the extract was washed successively with

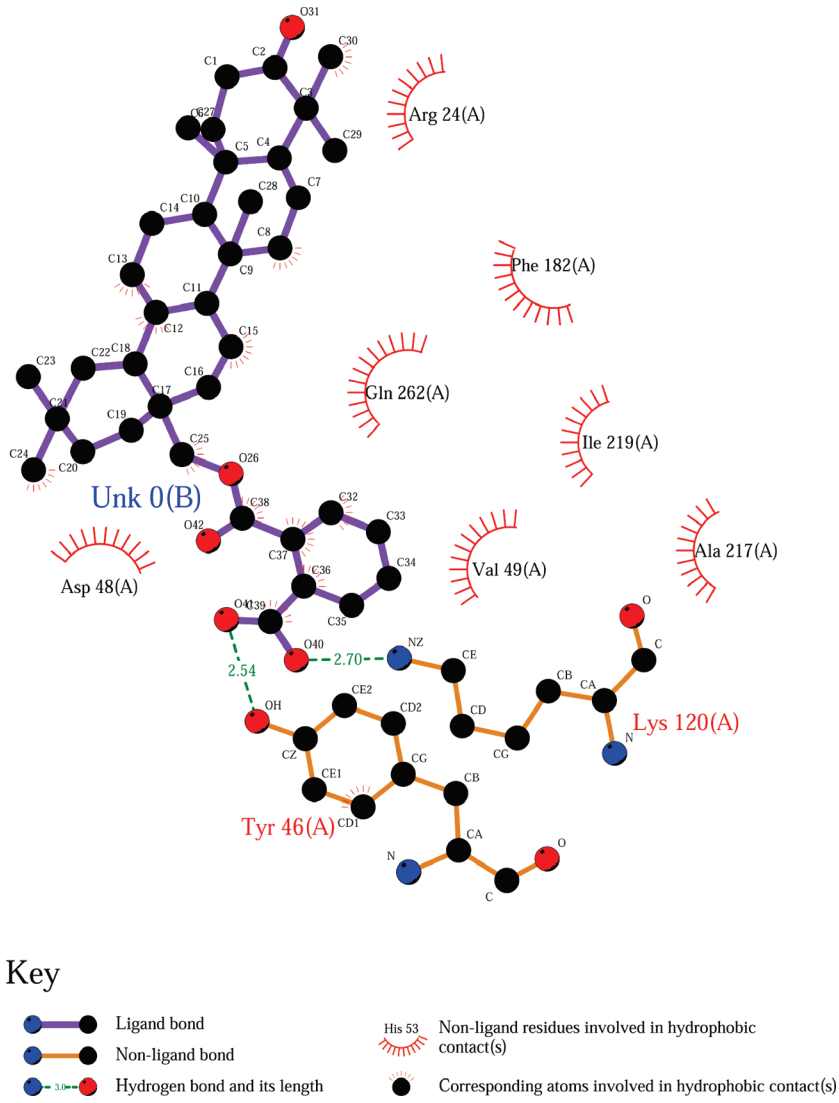


Figure 3. Binding mode of **25f** with residues around the docked position.

H_2O and HCl (1 M). The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1) to give colorless crystals of **7** (76 mg, 73%): mp >250 °C; $[\alpha]^{25}_D$ +34.0 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3439, 2948, 1703, 1462, 1383, 1271, 1191, 933 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 11.90 (1H, s, COOH), 5.20 (1H, s, H-12), 2.78 (1H, dd, J = 13.6, 4.0 Hz, H-18), 2.42 (1H, m, H-2a), 2.24 (1H, m, H-2b), 1.15 (3H, s, H-26), 1.12 (3H, s, H-27), 1.10 (3H, s, H-25), 1.00, 0.89, 0.88, 0.68 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 489 $[M + H]^+$; *anal*. C 73.67%, H 9.99%, calcd for $C_{30}H_{48}O_5$, C 73.73%, H 9.90%.

3-Oxo-olean-12α-hydroxy-28-oic acid γ-lactone (8). According to the reported method,[13] colorless crystals of **8** were obtained by ozonolysis of **2** via chromatography (CH_2Cl_2/MeOH, 20:1): yield 62%; mp >250 °C; $[\alpha]^{25}_D$ +35.6 (*c* 0.5, MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.92 (1H, s, H-12), 2.50−2.60 (1H, m, H-18), 2.40−2.50 (2H, m, H-2), 1.31 (3H, s, H-26), 1.19 (3H, s, H-27), 1.10 (3H, s, H-25), 1.04, 0.99, 0.98, 0.90 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 471 $[M + H]^+$; *anal*. C 76.68%, H 9.90%, calcd for $C_{30}H_{46}O_4$, C 76.55%, H 9.85%.

Olean-12α-hydroxy-3-ε-lacton-28-oic acid γ-lactone (9). Following the procedure described for the preparation of **6**, compound **9** was prepared from **8** as white crystals (88%): mp 210−211 °C; $[\alpha]^{25}_D$ +35.6 (*c* 0.5, CH_2Cl_2); IR (KBr) ν_{max} 3484, 2935, 2872, 1742, 1460, 1388, 1367, 1251, 1137, 1114, 946 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.92 (1H, s, H-12), 2.65 (1H, t, J = 13.6 Hz, H-18), 2.40−2.50 (1H, m, H-2), 1.48 (3H, s, H-26), 1.40 (3H, s, H-27), 1.28 (3H, s, H-25), 1.20, 1.10, 0.97, 0.89 (each 3H, s, H-23, H-24, H-29, H-30); ^{13}C NMR ($CDCl_3$, 100 MHz,) δ 179.0 (C, C-28), 177.7 (C, C-3), 77.0 (C, C-13), 76.8 (C, C-4), 74.2 (C, C-12), 57.6 (CH, C-5), 48.9 (CH, C-9), 45.1 (C, C-17), 42.9 (C, C-8), 42.7 (CH, C-18), 42.1 (C, C-14), 41.2 (C, C-10), 36.3 (CH_2, C-19), 34.1 (CH_2, C-7), 34.0 (CH_2, C-21), 33.2 (CH_2, C-2), 33.0 (CH_2, C-1), 32.8 (CH_3, C-29), 32.0 (CH_3, C-30), 31.7 (C, C-20), 31.8 (CH_2, C-11), 25.6 (CH_2, C-22), 25.2 (CH_2, C-15), 24.5 (CH_3, C-23), 24.1 (CH_3, C-24), 23.3 (CH_2, C-6),20.5 (CH_3, C-27), 20.1 (CH_2, C-16), 17.0 (CH_3, C-26), 15.7 (CH_3, C-25); ESIMS *m/z* 487 $[M + H]^+$; *anal*. C 74.19%, H 9.59%, calcd for $C_{30}H_{46}O_5$, C 74.04%, H 9.53%.

Olean-4,12α-dihydroxy-3-carboxy-28-oic acid γ-lactone (10). Following the procedure described for the preparation of **7**, **10** was prepared from **9** as a white, amorphous solid (75%): ^1H NMR (DMSO-d_6, 400 MHz) δ 11.60 (1H, s, COOH), 5.08 (1H, s, OH), 4.08 (1H, s, OH), 3.65 (1H, s, H-12), 2.10−2.40 (3H, m, H-18, H-2), 1.25 (3H, s, H-26), 1.16 (3H, s, H-27), 1.15 (3H, s, H-25), 1.06, 0.92, 0.91, 0.86 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 505 $[M + H]^+$; *anal*. C 71.56%, H 9.63%, calcd for $C_{30}H_{48}O_6$, C 71.39%, H 9.59%.

Olean-12-oxo-4-hydroxy-3-carboxy-28-oic acid γ-lactone (13). Following the procedure described for the preparation of **10**, **13** was prepared from **12**[13] as white crystals (52% in two steps): mp 230−231 °C; IR (KBr) ν_{max} 3449, 2961, 2879, 1739, 1702, 1465, 1386, 1269, 1196, 932 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.70 (1H, t, J = 14.0 Hz, H-18), 2.40−2.58 (2H, m, H-2), 1.36 (3H, s, H-26), 1.32 (3H, s, H-27), 1.28 (3H, s, H-25), 1.07, 0.98, 0.97, 0.96 (each 3H, s, H-23, H-24, H-29, H-30); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 206.0 (C, C-12), 178.9 (C, C-28), 178.0 (C, C-3), 77.3 (C, C-13), 77.0 (C, C-4), 76.6 (CH, C-5), 50.9 (CH, C-9), 44.3 (C, C-17), 43.9 (C, C-14), 43.7 (C, C-8), 43.0 (CH, C-18), 42.2 (CH_2, C-11), 41.2 (C, C-10), 37.3 (CH_2, C-19), 36.8 (CH_2, C-1), 34.3 (CH_2, C-7), 34.0 (CH_2, C-21), 33.5 (CH_2,

C-2), 33.1 (CH_3, C-29), 32.1 (CH_3, C-30), 31.5 (C, C-20), 28.6 (CH_2, C-22), 27.2 (CH_3, C-23), 27.1 (CH_3, C-24), 23.7 (CH_2, C-15), 21.5 (CH_3, C-27), 20.6 (CH_2, C-6), 20.1 (CH_2, C-16), 18.0 (CH_3, C-26), 17.7 (CH_3, C-25); ESIMS *m/z* 503 $[M + H]^+$; *anal*. C 71.73%, H 9.28%, calcd for $C_{30}H_{46}O_6$, C 71.68%, H 9.22%.

3β-(2′-Carboxybenzoyl)-12α-hydroxy-28-oic acid γ-lactone (14) and 3β,12α-(2′-Carboxybenzoyl)-28-oic acid γ-lactone (15). A solution of **11**[13] (300.0 mg, 0.6 mmol), phthalic anhydride (375.5 mg, 2.5 mmol), and DMAP (279.0 mg, 2.5 mmol) in dry pyridine (32 mL) was stirred at 120 °C for 8 h, then concentrated. The residue was diluted with CH_2Cl_2, and the extract was washed successively with HCl (1M), H_2O, and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 100:1) to give colorless crystals of **14** (208.3 mg, 56%) and **15** (129.2 mg, 28%). **14**: mp >250 °C. $[\alpha]^{25}_D$ +28.5 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3525, 2950, 2873, 1735, 1465, 1396, 1289, 1136, 1073, 942 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.89 (1H, t, J = 6.0, 2.4 Hz, H-Ar), 7.78 (1H, t, J = 6.4, 2.0 Hz, H-Ar), 7.60 (2H, t, J = 3.6 Hz, H-Ar), 4.79 (1H, dd, J = 12.0, 4.8 Hz, H-3), 3.88 (1H, s, H-12), 1.32 (3H, s, H-26), 1.26 (3H, s, H-27), 1.15 (3H, s, H-25), 0.98, 0.97, 0.96, 0.90 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 622 $[M + H]^+$; *anal*. C 73.59%, H 8.47%, calcd for $C_{38}H_{52}O_7$, C 73.52%, H 8.44%. **15**: mp >250 °C; $[\alpha]^{25}_D$ +36.2 (*c* 0.5, CH_2Cl_2); IR (KBr) ν_{max} 3462, 3071, 2955, 1775, 1724, 1467, 1288, 1131, 945 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.90 (1H, dd, J = 7.6, 1.2 Hz, H-Ar), 7.78 (1H, d, J = 6.8 Hz, H-Ar), 7.60 (6H, m, H-Ar), 5.28 (1H, s, H-12), 4.79 (1H, dd, J = 12.0, 4.8 Hz, H-3), 1.28 (3H, s, H-26), 1.20 (3H, s, H-27), 1.15 (3H, s, H-25), 0.92, 0.91, 0.88, 0.81 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 790 $[M + H]^+$; *anal*. C 71.97%, H 7.39%, calcd for $C_{46}H_{56}O_{10}$, C 71.85%, H 7.34%.

Olean-12-oxo-28-methoxycarbonyl-3-oic acid ε-lactone [16a (R = CH₃)]. A solution of methyl ester **3**[10] (60 mg, 0.1 mmol), MCPBA (62.2 mg, 0.3 mmol), and $NaHCO_3$ (83.4 mg, 1.0 mmol) in CH_2Cl_2 (16 mL) was stirred at 40 °C for 24 h, and the reaction was quenched with Na_2SO_3. The residue was diluted with CH_2Cl_2, and the extract was washed successively with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (PE/EtOAc, 10:1) to give a colorless, amorphous solid, **16a** (28 mg, 47%): ^1H NMR ($CDCl_3$, 400 MHz) δ 3.70 (3H, s, OCH_3), 2.80 (1H, m, H-2a), 2.58−2.66 (3H, m, H-2b, H-11), 1.48 (3H, s, H-26), 1.44 (3H, s, H-27), 1.18 (3H, s, H-25), 1.12, 0.98, 0.96, 0.90 (each 3H, s, H-23, H-24, H-29, H-30); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.7 (C, C-12), 77.3 (C, C-4), 77.0 (C, C-13), 76.6 (CH, C-5), 54.0 (CH_3, OCH_3), 51.8 (CH, C-9), 51.7 (C, C-17), 50.7 (C, C-14), 50.7 (C, C-8), 49.6(C, C-10), 38.9 (CH_2, C-19), 37.5 (CH_2, C-11), 36.1(CH_2, C-1), 34.4 (CH, C-18), 33.3 (CH_2, C-7), 32.8 (CH_2, C-21), 32.1 (CH_2, C-2), 31.9 (CH_3, C-29), 31.8 (CH_3, C-30), 30.8 (CH_2, C-16), 30.6 (CH_2, C-22), 27.4 (C, C-20), 25.8 (CH_2, C-15), 23.0 (CH_3, C-24), 22.9 (CH_3, C-23), 22.7 (CH_2, C-6), 20.2 (CH_3, C-27), 16.4 (CH_3, C-26), 15.5 (CH_3, C-25); ESIMS *m/z* 501 $[M + H]^+$; *anal*. C 74.45%, H 9.70%, calcd for $C_{31}H_{48}O_5$, C 74.36%, H 9.66%.

Olean-12-oxo-4-hydroxy-3,28-dioic acid (17). Following the procedure described for the preparation of **16a** (R = CH_3), benzyl ester **16b** (R = Bn) was prepared from **5** as a white solid. Following the procedure described for the preparation of **7**, **17** was prepared from benzyl ester as white crystals (31% in three steps): mp 190−192 °C; IR (KBr) ν_{max} 3444, 2951, 1697, 1465, 1387, 1192, 1108 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.0 (1H, s, COOH), 4.05 (1H, s, OH), 2.60 (2H, m, H-2), 1.18 (3H, s, H-26), 1.16 (3H, s, H-27), 1.05 (3H, s, H-25), 1.03, 1.00, 0.98, 0.96 (each 3H, s, H-23, H-24, H-29, H-30); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 210.7 (C, C-12), 179.2 (C, C-28), 175.5 (C, C-3), 74.1 (C, C-13), 55.1 (C, C-4), 51.1 (CH, C-9), 46.2 (CH, C-5), 41.7 (C, C-17), 41.2 (C, C-14), 40.7 (C, C-8), 40.4 (C, C-10), 40.3 (CH_2, C-19), 40.1 (CH_2, C-11), 39.9 (CH_2, C-1), 39.7 (CH, C-18), 39.5 (CH_2, C-7), 39.2 (CH_2, C-21), 39.0 (CH_2, C-2), 37.7 (CH_3, C-29), 34.1 (CH_3, C-30), 33.7 (CH_2, C-16), 33.5 (CH_2, C-22), 31.6 (C, C-20), 30.6 (CH_2, C-15), 30.5 (CH_3, C-24), 28.5 (CH_3, C-23), 27.9 (CH_2, C-6), 23.3 (CH_3, C-27), 19.7 (CH_3, C-26), 15.6 (CH_3, C-25); ESIMS *m/z* 505 $[M + H]^+$; *anal*. C 71.43%, H 9.64%, calcd for $C_{30}H_{48}O_6$, C 71.39%, H 9.59%.

Olean-12-ene-2-formyl-28-carboxy-3-oic acid methyl ester (19). To a stirred solution of olean-2α-hydroxy-3-oxo-12-en-28-oic acid[15] (1.0 g, 1.8 mmol) in a mixture of MeOH (30 mL) and CH_2Cl_2 (10 mL), cooled to 0 °C, was added $Pb(OAc)_4$ (1.6 g, 3.6 mmol). After stirring for 50 min, the solution was filtered. The filtrate was evaporated in vacuo to a residue. The residue was purified by column chromatography (PE/EtOAc, 6:1) to give a colorless, amorphous solid, **18** (480 mg, 47%): IR (KBr) ν_{max} 2959, 2928, 1724, 1460, 1381, 1255, 1148, 1030, 747, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.82 (1H, s, CHO), 7.38 (5H, m, H-Ar), 5.25 (1H, t, J = 3.6 Hz, H-12), 5.08 (1H, d, J = 12.8 Hz, $PhCH_2$), 5.06 (1H, d, J = 12.8 Hz, $PhCH_2$), 3.58 (3H, s, CH_3), 2.90 (1H, dd, J = 13.6, 4.4 Hz, H-18), 1.25 (3H, s, H-26), 1.20 (3H, s, H-27), 1.10 (3H, s, H-25), 0.98, 0.90, 0.89, 0.62 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 591 $[M + H]^+$; *anal*. C 77.28%, H 9.28%, calcd for $C_{38}H_{54}O_5$, C 77.25%, H 9.21%.

Following the procedure described for the preparation of **6**, compound **19** was prepared from **18** as a white, amorphous solid (91%): IR (KBr) ν_{max} 2926, 2860, 1723, 1460, 1380, 1259, 1142, 1028, 755 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.82 (1H, s, CHO), 5.30 (1H, s, H-12), 3.58 (3H, s, CH_3), 2.80 (1H, dd, J = 13.6, 4.4 Hz, H-18), 1.25 (3H, s, H-26), 1.20 (3H, s, H-27), 1.10 (3H, s, H-25), 0.98, 0.90, 0.89, 0.62 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 501 $[M + H]^+$; *anal*. C 74.48%, H 9.63%, calcd for $C_{31}H_{48}O_5$, C 74.36%, H 9.66%.

3-Spiro[1′,2′]dioxolanerythrodiol (20). The solution of **4** (2.3 g, 4.7 mmol), ethylene glycol (3 mL, 52.0 mmol), and pyridinium *p*-toluenesulfonate (65.0 mg) in dry toluene (50 mL) was refluxed at 110 °C for 4 h by using the toluene constant distilling dehydrating method. The reaction mixture was quenched cautiously by the subsequent addition of saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated to give the crude ester, which was used in the next step without purification.

$LiAlH_4$ (0.3 g, 8.2 mmol) was suspended in THF (30 mL) and added to a solution of the crude ester (2.1 g) in THF (30 mL) dropwise at 0 °C. The reaction mixture was left for a further 3 h at rt. The excess reagent was destroyed by addition of H_2O (2 mL) and 10% KOH (2 mL). The reaction mixture was then filtered and concentrated. The residue was purified by column chromatography (PE/EtOAc, 5:1) to give white crystals of **20** (1.8 g, 85% in two steps): mp 235−237 °C; $[\alpha]^{25}_D$ +21.8 (*c* 0.5, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.20 (1H, t, J = 3.6 Hz, H-12), 3.89−3.97 (4H, m, OCH_2CH_2O), 3.58 (1H, d, J = 10.8 Hz, CH_2OH), 3.21 (1H, d, J = 10.8 Hz, CH_2OH), 1.17 (3H, s, H-26), 0.97 (3H, s, H-27), 0.93 (3H, s, H-25), 0.90, 0.89, 0.87, 0.85 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 486 $[M + H]^+$; *anal*. C 9.44%, H 10.84%, calcd for $C_{32}H_{52}O_3$: C 79.29%, H 10.81%.

3-Oxo-erythrodiol (21). To a solution of alcohol **20** (1.4 g, 3.0 mmol) in THF (50 mL) was added 2 N HCl (15 mL), and the reaction mixture was stirred at 100 °C for 1 h. The organic layer was separated, and the water layer was exacted with EtOAc. The collected organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (PE/EtOAc, 5:1) to give colorless crystals (1.3 g, 96%): mp 160−161 °C; $[\alpha]^{25}_D$ +50.7 (*c* 0.5, CH_2Cl_2); IR (KBr) ν_{max} 3500, 1705 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.22 (1H, t, J = 4.0 Hz, H-12), 3.58 (1H, d, J = 10.8 Hz, CH_2OH), 3.21 (1H, d, J = 10.8 Hz, CH_2OH), 2.56 (1H, m, H-2a), 2.38 (1H, m, H-2b), 1.78 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 442 $[M + H]^+$; *anal*. C 81.89%, H 11.05%, calcd for $C_{30}H_{48}O_2$, C 81.76%, H 10.98%.

3-Oxo-12-enolean-28-oic acid trifluoromethanesulfonate (22). A solution of alcohol **21** (1.0 g, 2.0 mmol) in anhydrous CH_2Cl_2 (50 mL) was added to the solution of trifluoromethanesulfonic acid anhydride (564 mg, 2.0 mmol) in CH_2Cl_2 (10 mL), and the reaction mixture was stirred at rt for 2 h under an N_2 layer. The suspension was filtered, and the filtrate was washed with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (PE/EtOAc, 100:1) to give colorless semisolid **22** (0.9 g, 70%): $[\alpha]^{25}_D$ +32.8 (*c* 0.5, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.22 (1H, t, J = 3.6 Hz, H-12), 4.50 (1H, d, J = 9.6 Hz, $CH_2SO_3CF_3$), 3.34 (1H, d, J = 9.6 Hz, $CH_2SO_3CF_3$), 2.58 (1H, m, H-2a), 2.38 (1H, m, H-2b), 1.20 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 573 $[M + H]^+$; *anal*. C 65.12%, H 8.22%, S 5.54%, calcd for $C_{31}H_{47}F_3O_4S$, C 65.01%, H 8.27%, S 5.60%.

3-Oxo-12-ene-28-bromo-oleanane (23). To a stirred solution of alcohol **21** (1.5 g, 3.4 mmol) in a dry mixture of CH_2Cl_2 (10 mL) and pyridine (2 mL) was added $(CH_3)_3SiBr$ (1.8 mL, 13.6 mmol) at 0 °C, and the reaction mixture was left for a further 2 h at rt. The reaction mixture was quenched cautiously by the addition of H_2O. The organic

layer was separated and washed with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (PE/EtOAc, 100:1) to give an amorphous solid, **23** (1.2 g, 71%): $[\alpha]^{25}_D$ +34.8 (*c* 0.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.20 (1H, t, J = 3.6 Hz, H-12), 3.21 (1H, d, J = 10.0 Hz, CH_2Br), 3.05 (1H, d, J = 9.6 Hz, CH_2Br), 2.58 (1H, m, H-2a), 2.38 (1H, m, H-2b), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 505 $[M + H]^+$; *anal*. C 71.62%, H 9.47%, calcd for $C_{30}H_{47}BrO$, C 71.55%, H 9.41%.

3-Oxo-28-(benzylsuccinyl-4′-)erythrane (24b). To a solution of succinic acid monobenzyl ester[16] (280.8 mg, 1.4 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise a solution of oxalyl chloride (173 μL, 2.0 mmol) in dry CH_2Cl_2 (10 mL) and DMF (one drop) at 0 °C. After the excess oxalyl chloride was removed in vacuo, the residual oil was dissolved in CH_2Cl_2 (10 mL). A solution of alcohol **21** (0.6 g, 1.4 mmol) obtained from the previous step in dry CH_2Cl_2 (10 mL) and Et_3N (0.4 mL) was added dropwise at 0 °C. After stirring for another 1 h at rt, the mixture was diluted with CH_2Cl_2. The organic layer was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (PE/EtOAc, 20:1) to give colorless crystals of **24b** (0.8 g, 86%): mp 66−68 °C; $[\alpha]^{25}_D$ +31.7 (*c* 0.5, CH_2Cl_2); IR (KBr) ν_{max} 2948, 2866, 1739, 1703, 1460, 1386, 1154, 999 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39 (5H, m, H-Ar), 5.21 (1H, t, J = 3.6 Hz, H-12), 5.19 (2H, s, $PhCH_2$), 4.04 (1H, d, J = 10.8 Hz, OCH_2), 3.67 (1H, d, J = 10.8 Hz, OCH_2), 2.70 (4H, m, H-2′, H-3′), 2.58 (1H, m, H-2a), 2.38 (1H, m, H-2b), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 632 $[M + H]^+$; *anal*. C 78.12%, H 9.34%, calcd for $C_{41}H_{58}O_5$, C 78.05%, H 9.27%.

Compounds **24a**−**24e** were prepared according to the same procedure described for **24b**.

3-Oxo-28-(benzylmalonyl-4′)erythrane (24a): amorphous solid; $[\alpha]^{25}_D$ +28.4 (*c* 0.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38 (5H, m, H-Ar), 5.21 (1H, t, J = 3.6 Hz, H-12), 5.19 (2H, s, $PhCH_2$), 4.05 (1H, d, J = 10.8 Hz, OCH_2), 3.68 (1H, d, J = 10.8 Hz, OCH_2), 3.46 (2H, s, H-2′), 2.58 (1H, m, H-2a), 2.38 (1H, m, H-2b), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 617 $[M + H]^+$; *anal*. C 77.92%, H 9.12%, calcd for $C_{40}H_{56}O_5$, C 77.88%, H 9.15%.

3-Oxo-28-(benzylglutaryl-4′)erythrane (24c): amorphous solid; $[\alpha]^{25}_D$ +28.2 (*c* 0.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38 (5H, m, H-Ar), 5.21 (1H, t, J = 3.6 Hz, H-12), 5.19 (2H, s, $PhCH_2$), 4.05 (1H, d, J = 10.8 Hz, OCH_2), 3.68 (1H, d, J = 10.8 Hz, OCH_2), 2.58 (1H, m, H-2a), 2.35−2.45 (5H, m, H-2b, H-2′, H-4′), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 645 $[M + H]^+$; *anal*. C 78.34%, H 9.41%, calcd for $C_{42}H_{60}O_5$, C 78.22%, H 9.38%.

3-Oxo-28-(benzyladipoyl-4′)erythrane (24d): amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38 (5H, m, H-Ar), 5.21 (1H, t, J = 3.6 Hz, H-12), 5.19 (2H, s, $PhCH_2$), 4.05 (1H, d, J = 10.8 Hz, OCH_2), 3.68 (1H, d, J = 10.8 Hz, OCH_2), 2.58 (1H, m, H-2a), 2.39 (5H, m, H-2b, H-2′, H-5′), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 659 $[M + H]^+$; *anal*. C 78.52%, H 9.41%, calcd for $C_{43}H_{62}O_5$, C 78.38%, H 9.48%.

3-Oxo-28-(benzylsebacoyl-4′)erythrane (24e): amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38 (5H, m, H-Ar), 5.21 (1H, t, J = 3.6 Hz, H-12), 5.19 (2H, s, $PhCH_2$), 4.05 (1H, d, J = 10.8 Hz, OCH_2), 3.68 (1H, d, J = 10.8 Hz, OCH_2), 2.58 (1H, m, H-2a), 2.39 (5H, m, H-2b, H-2′, H-9′), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 715 $[M + H]^+$; *anal*. C 79.08%, H 9.90%, calcd for $C_{47}H_{70}O_5$, C 78.95%, H 9.87%.

3-Oxo-28-(benzylphthaloyl-4′)erythrane (24f): amorphous solid; $[\alpha]^{25}_D$ +31.3 (*c* 0.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (2H, m, H-Ar), 7.58 (2H, m, H-Ar), 7.38 (4H, m, H-Ar), 5.60 (2H, m, $PhCH_2$), 5.21 (1H, t, J = 3.6 Hz, H-12), 4.05 (1H, d, J = 10.8 Hz, OCH_2), 3.68 (1H, d, J = 10.8 Hz, OCH_2), 2.58 (1H, m, H-2a), 2.39 (1H, m, H-2b), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 679 $[M + H]^+$; *anal*. C 79.69%, H 8.69%, calcd for $C_{45}H_{58}O_5$, C 79.61%, H 8.61%.

3-Oxo-28-(succinyl-4′)erythrane (25b). Following the procedure described for the preparation of **19**, compound **25b** was prepared from **25b** as white crystals (98.3 mg, 91%): mp 138−140 °C; $[\alpha]^{25}_D$ +34.0 (*c* 1.3, CH_2Cl_2); IR (KBr) ν_{max} 3279, 2945, 2865, 1743, 1703, 1462, 1387, 1161, 1001, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.21 (1H, t, J = 3.6 Hz, H-12), 4.04 (1H, d, J = 11.2 Hz, OCH_2), 3.78 (1H, d, J = 11.6 Hz, OCH_2), 2.68 (4H, m, H-2′, H-3′), 2.58 (1H, m, H-2a), 2.38 (1H, m, H-2b), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 541 $[M + H]^+$; *anal*. C 75.64%, H 9.72%, calcd for $C_{34}H_{52}O_5$, C 75.51%, H 9.69%.

Compounds **25a**−**25e** were prepared according to the same procedure described for **25b**.

3-Oxo-28-(malonyl-4′)erythrane (25a): colorless crystals: mp 78−80 °C; $[\alpha]^{25}_D$ +34.6 (*c* 0.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.22 (1H, s, H-12), 4.18 (1H, d, J = 10.8 Hz, OCH_2), 3.78 (1H, d, J = 10.8 Hz, OCH_2), 3.46 (2H, s, H-2′), 2.58 (1H, m, H-2a), 2.38 (1H, m, H-2b), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 527 $[M + H]^+$; *anal*. C 75.29%, H 9.61%, calcd for $C_{33}H_{50}O_5$, C 75.25%, H 9.57%.

3-Oxo-28-(glutaryl-4′)erythrane (25c): amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.21 (1H, t, J = 3.6 Hz, H-12), 4.02 (1H, d, J = 10.8 Hz, OCH_2), 3.78 (1H, d, J = 11.2 Hz, OCH_2), 2.58 (1H, m, H-2a), 2.44 (4H, m, H-2′, H-4′), 2.38 (1H, m, H-2b), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 555 $[M + H]^+$; *anal*. C 75.85%, H 9.86%, calcd for $C_{35}H_{54}O_5$, C 75.77%, H 9.81%.

3-Oxo-28-(adipoyl-4′)erythrane (25d): amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.21 (1H, s, H-12), 4.02 (1H, d, J = 11.2 Hz, OCH_2), 3.77 (1H, d, J = 11.2 Hz, OCH_2), 2.59 (1H, m, H-2a), 2.39 (5H, m, H-2b, H-2′, H-5′), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 570 $[M + H]^+$; *anal*. C 76.18%, H 9.96%, calcd for $C_{36}H_{56}O_5$, C 76.01%, H 9.92%.

3-Oxo-28-(sebacoyl-4′)erythrane (25e): amorphous solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.22 (1H, t, J = 3.2 Hz, H-12), 4.39 (1H, d, J = 10.8 Hz, OCH_2), 3.95 (1H, d, J = 11.2 Hz, OCH_2), 2.58 (1H, m, H-2a), 2.30−2.40 (5H, m, H-2b, H-2′, H-9′), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 626 $[M + H]^+$; *anal*. C 76.92%, H 10.36%, calcd for $C_{40}H_{64}O_5$, C 76.88%, H 10.32%.

3-Oxo-28-(phthaloyl-4′)-erythrane (25f): colorless crystals; mp 92−94 °C; $[\alpha]^{25}_D$ +30.7 (*c* 0.5, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (1H, dd, J = 6.4, 0.8 Hz, H-Ar), 7.74 (1H, d, J = 7.6 Hz, H-Ar), 7.60 (2H, m, H-Ar), 5.22 (1H, t, J = 3.2 Hz, H-12), 4.39 (1H, d, J = 10.8 Hz, OCH_2), 3.95 (1H, d, J = 11.2 Hz, OCH_2), 2.59 (1H, m, H-2a), 2.39 (1H, m, H-2b), 1.16 (3H, s, H-26), 1.10 (3H, s, H-27), 1.07 (3H, s, H-25), 1.05, 1.00, 0.89, 0.87 (each 3H, s, H-23, H-24, H-29, H-30); ESIMS *m/z* 590 $[M + H]^+$; *anal*. C 77.68%, H 8.94%, calcd for $C_{38}H_{52}O_5$, C 77.51%, H 8.90%.

Enzymatic-Activity Assays. With a spectrophotometer, the inhibitory activities of all samples against PTP1B (recombinant protein obtained from *Escherichia coli BL21* expression system) were measured at 37 °C with 0.2 units/mL enzyme in a buffer (25 mM HEPES, 50 mM NaCl, 2.5 mM EDTA, 0.1% BSA, pH 7.2). Sodium orthovanadate (100 μg/mL) was used as positive control. The reaction system was stirred for 10 min at 37 °C, and the substrate of PTP (*p*-nitrophenyl phosphate disodium hexahydrate) was added. After 30 min, 2 mol/mL Na_2CO_3 was added to terminate the reaction. The OD value was monitored continuously with a spectrophotometer at 405 nm. The assay result was provided by the drug screening center of Di Ao Pharmaceutical Group.

Molecular Docking Simulation. To further identify the putative binding site and corresponding binding conformation of the compounds, molecular docking simulation was performed against the PTP1B protein with Accelrys Discovery 1.7 (Accelrys Software Inc., USA). The high-resolution crystal structure of PTP1B complexed with pyridothiophene inhibitor was obtained from the Protein Data Bank (PDB code: 2B07[18]). All cocrystallized ligands and water were removed. The starting 3D conformations of **25f** were prepared with Dock Ligands Fit. The calculated dock score and PLP1 of all docking positions were evaluated. The best ranked position from each of the binding sites was determined by the highest calculated dock score and PLP1.

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