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SYNTHESIS AND BIOLOGICAL EVALUATION OF OLEANOLIC ACID DERIVATIVES AS NOVEL INHIBITORS OF PROTEIN TYROSINE PHOSPHATASE 1B

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Abstract – A series of oleanolic acid (OA) derivatives have been synthesized and their inhibitory effects on PTP1B, TCPTP and related PTPs are evaluated. Some compounds with five-membered heterocyclic ring-fused at C-2, C-3 positions showed a dramatic increase in inhibition, the two most potent PTP1B inhibitors **19** (IC_{50} = 0.91 μM) and **21** (IC_{50} = 0.98 μM) showed about 3-fold more potent than lead compound OA. Some C-ring modified OA analogs showed high selectivity for PTP1B over TCPTP, among them, **50** possessed the best selectivity of 6.6-fold.

INTRODUCTION

Protein tyrosine phosphatases (PTPs) are expressed in insulin-sensitive tissues, which can function as negative modulators in insulin signal transduction by dephosphorylation of tyrosyl residues.[1–4] Protein tyrosine phosphatase 1B (PTP1B), a prototypical member of the protein tyrosine phosphate superfamily, is a key negative regulator of both insulin and leptin signaling pathway by dephosphorylating the insulin receptor (IR),[5] insulin receptor substrates (IRS)[6] and Janus kinase 2 (JAK2).[7,8] Previous studies have

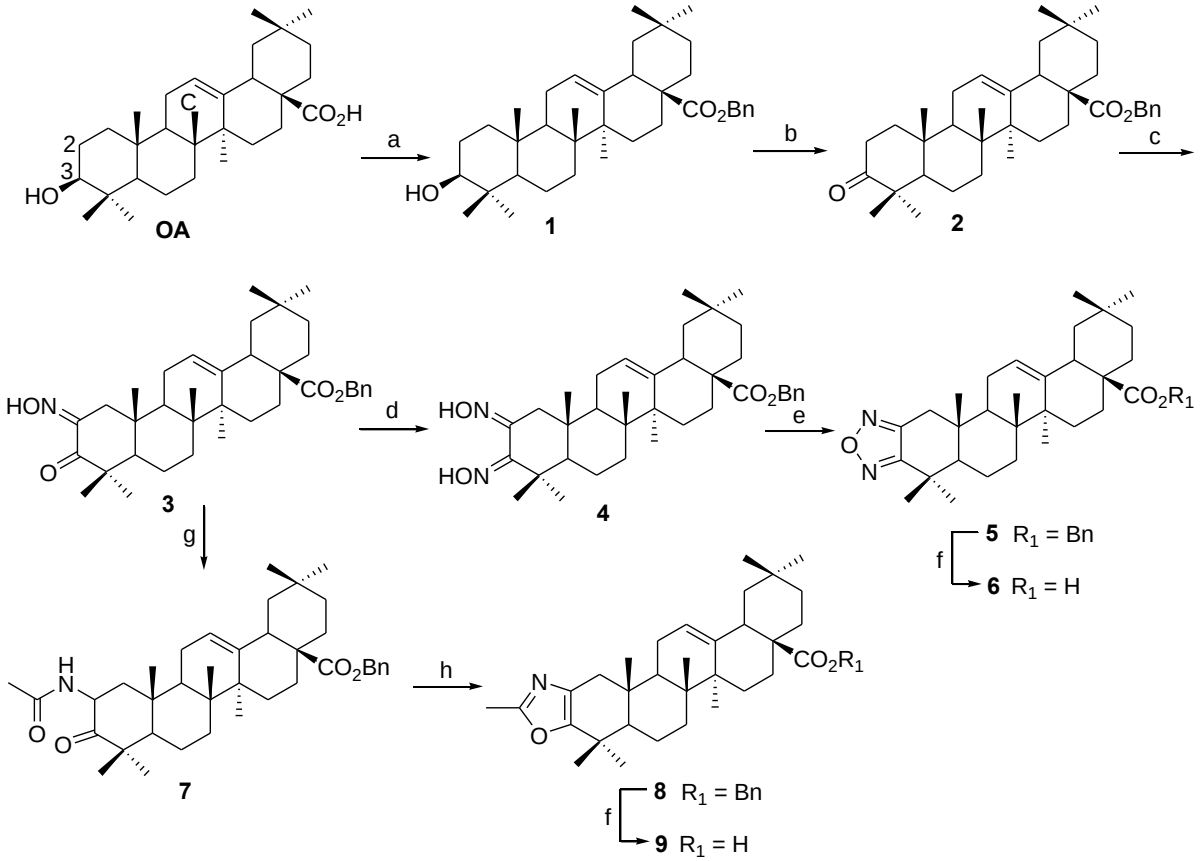
reported that PTP1B knockout mice not only display increased insulin sensitivity in liver and muscle tissues but also are resistant to high fat diet-induced obesity.[7,9-11] PTP1B antisense oligonucleotide treatment can modulate obesity related fat storage and lipogenesis in adipose, and improve insulin sensitivity in animal models of type 2 diabetes.[12,13] These investigations suggest that inhibition of PTP1B may anti-type 2 diabetes by increasing insulin sensitivity[9] and resistance in obesity.[10] In recent years, PTP1B inhibitors are regarded as agents in the treatment of type 2 diabetes and obesity.[14,15] A series of synthetic inhibitors with submicro-, even nano-molar activity were discovered, however, few was further development to clinical trials, mainly for two reasons. Firstly, the poor bioavailability is an important issue, because of most active site-directed inhibitors reported to date possess a high charge density by mimicking the phosphate group in IRS.[16] Secondly, the low selectivity between PTP1B and the most homogeneous T-cell protein tyrosine phosphatase (TCPTP) is another important issue, because of all PTPs share a high degree of structural conservation in the active site.[17]

Natural products play a major role in drug discovery, about 60% of anticancer and 75% of anti-infective drugs approved from 1981-2002 could be traced to natural origins.[18] In searching for novel PTP1B inhibitors that derived from natural products, researchers have found trodusquemine (is currently concducting a phase I clinical challenge by Genaera[19]) and pentacyclic triterpenoids,[20,21] including oleanolic acid (OA) and its derivatives.[22] It is well-known that pentacyclic triterpenes are too hydrophobic to have acceptable water solubility and related pharmacokinetic properties. Initial SAR studies for analogs of OA with modified various substituents on C-3 and C-28 positions indicated a strong preference for the hydrophobic group and these derivatives had no obvious selectivity for PTP1B over TCPTP (with 77% sequences identity with PTP1B). Our previous study was focused on improving water solubility, increasing inhibitory activity and selectivity between the two homogeneous enzymes by introducing a series of heterocyclic rings at C-2 and C-3 positions of OA.[23] According to our previous study, the inhibition of the five-membered heterocyclic ring-fused OA derivatives is more potent than the six-membered analogs. Therefore, we herein reported a subsequent work of our previous research. We further introduced five-membered hydrophilic heterocyclic rings at C-2 and C-3 positions and modified the C-ring of OA respectively. All of these OA derivatives were evaluated in the enzyme inhibition assay against PTP1B and TCPTP, and selected compounds of the series were evaluated in related PTPs, such as SHP-1, SHP-2 and LAR.

RESULTS AND DISCUSSION

1. Chemistry. Benzylation of OA, followed by IBX oxidation to give **2**. Reaction of **2** with isoamyl nitrite in the presence of *t*-BuOK in *t*-BuOH furnished **3**.[24] Compound **3** was treated with hydroxylamine

hydrochloride in refluxing pyridine to give **4**. Cyclization of **4** was performed by heating in the presence of KOH to give **5**.[25] Compound **6** was produced by debenzylation of **5** over palladium/carbon in MeOH with high yield. Reaction of **3** with zinc powder in AcOH and Ac_2O produced **7**. Compound **8** was afforded by reaction of **7** with phosphorus oxychloride in dry pyridine under nitrogen atmosphere.[26] Compound **9** was obtained by debenzylation of **8** with the same procedure as preparation of **6**, as shown in scheme 1.

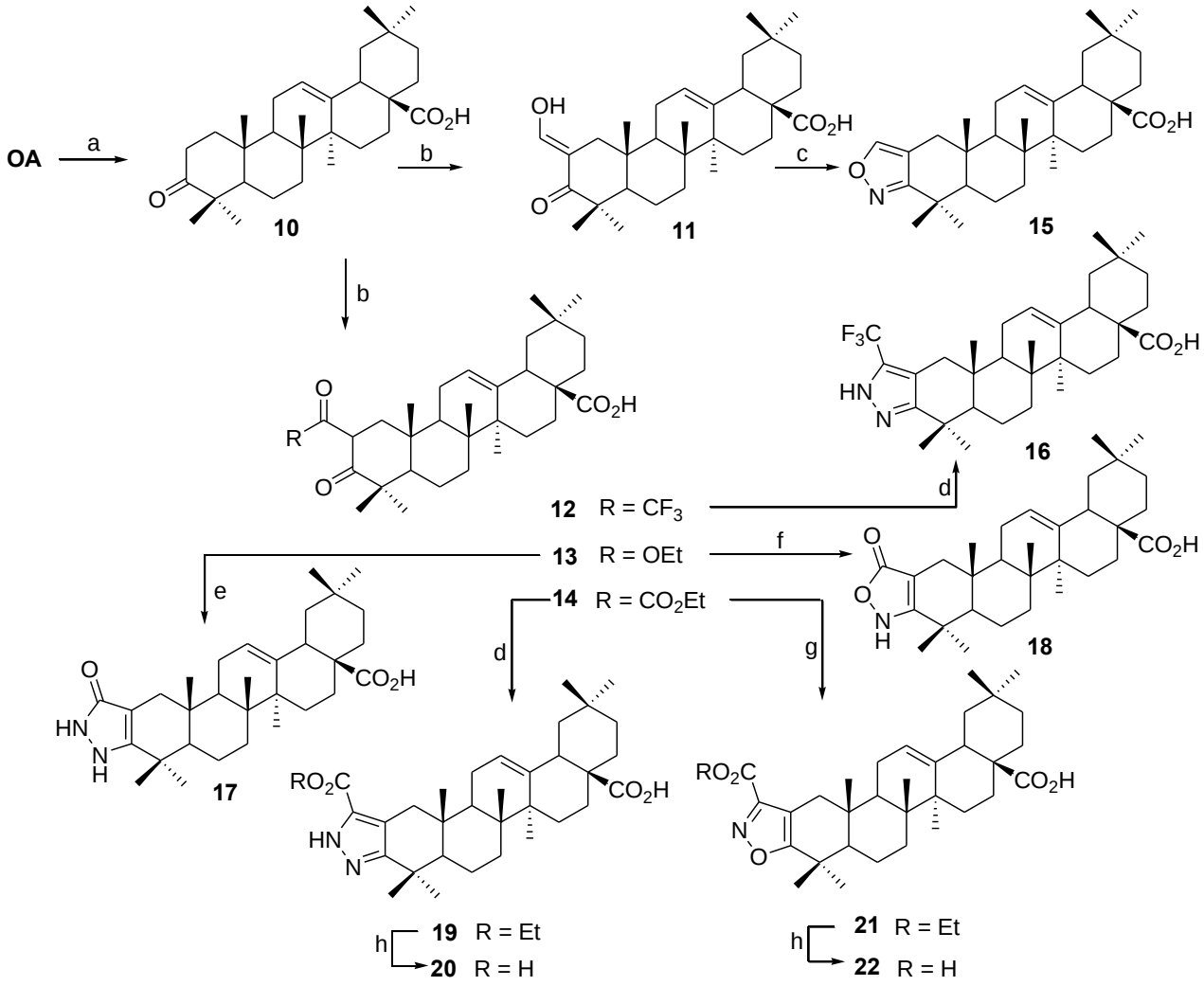


Scheme 1

a) BnBr, K_2CO_3, DMF, rt, 12 h, 92%. *b*) IBX, DMSO, THF, rt, 6 h, 85%. *c*) isoamyl nitrite, *t*-BuOK, *t*-BuOH, rt, 5 h, 86%. *d*) hydroxylamine hydrochloride, pyridine, reflux, 5 h, 75%. *e*) KOH, ethylene glycol, dioxane, reflux, 5 h, 60%. *f*) 10% Pd/C, H_2, MeOH, rt, 12 h, 92% for **6**, 82% for **9**. *g*) Zn dust, AcOH, Ac_2O, rt, 12 h, 86%, *h*) $POCl_3$, pyridine, rt, 12 h, 75%.

Compound **10** was produced by Oxidation of OA with IBX. Treatment of **10** with various acylation reagents afforded acylated intermediates **11**, **12**, **13**, **14**. Compound **15** was afforded by treatment of **11** with hydroxylamine hydrochloride in refluxing pyridine. Reaction of **12** with hydrazine hydrate in acetic acid produced **16**. Compounds **17** and **18** were obtained by treatment of **13** with hydrazine hydrate or hydroxylamine hydrochloride respectively.[25] Compounds **19** and **21** were obtained by cyclization of **14** with the same procedure as preparation of **17** and **18**. Compounds **20** and **22** were synthesized by

hydrolysis of **19** and **21** in the presence of NaOH in THF/MeOH, as shown in scheme 2.

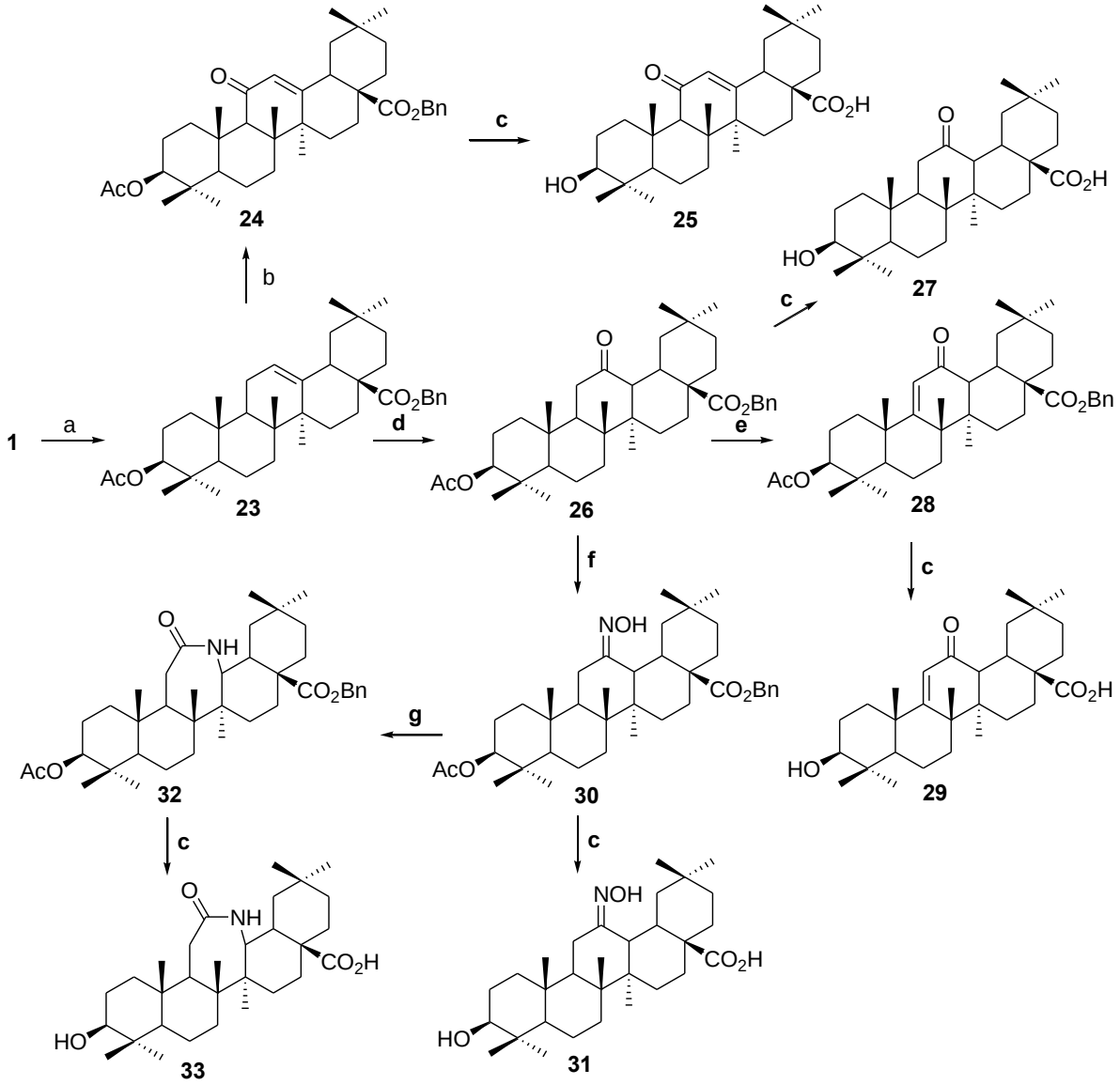


Scheme 2

a) IBX, DMSO, THF, rt, 5 h, 90%. *b*) RCO_2Et (R = CF_3, OEt, CO_2Et), NaH, THF, rt, 12 h or HCO_2Et, MeONa, toluene, rt, 1 h, 70% for **11**, 78% for **12**, 76% for **13**, 78% for **14.** *c*) hydroxylamine hydrochloride, pyridine, reflux, 5 h, 74%. *d*) 85% hydrazine hydrate, AcOH, reflux, 5 h, 81%. *e*) 85% hydrazine hydrate, EtOH, reflux, 5 h, 67%. *f*) hydroxylamine hydrochloride, AcONa, EtOH, reflux, 24 h, 68%. *g*) hydroxylamine hydrochloride, EtOH, reflux, 5 h. *h*) 2 M NaOH, THF, MeOH, reflux, 5 h.

Acetylation of **1** followed by oxidation with PDC produced **24**.[27] Compound **25** was generated by removal of acetyl group of **24** in the presence of NaOH in MeOH/THF and benzyl group of **24** in the presence of Pd/C respectively. The intermediate **26** was synthesized by reaction of **23** with *m*CPBA.[28] Compound **27** was obtained by removal of acetyl group and benzyl group of **26** with the same procedure as preparation of **25**. Compound **29** was afforded by dehydrogenation of **26** in the presence of Br_2 and HBr in HOAc, then removal of acetyl and benzyl group. Compound **26** reacted with hydroxylamine hydrochloride in pyridine to produce **30**. Compound **31** was prepared by deprotection of

acetyl group and benzyl group of **30**. Compound **33** was generated by Beckmann rearrangement of **30**, then deprotection of acetyl group and benzyl group, as shown in scheme 3.

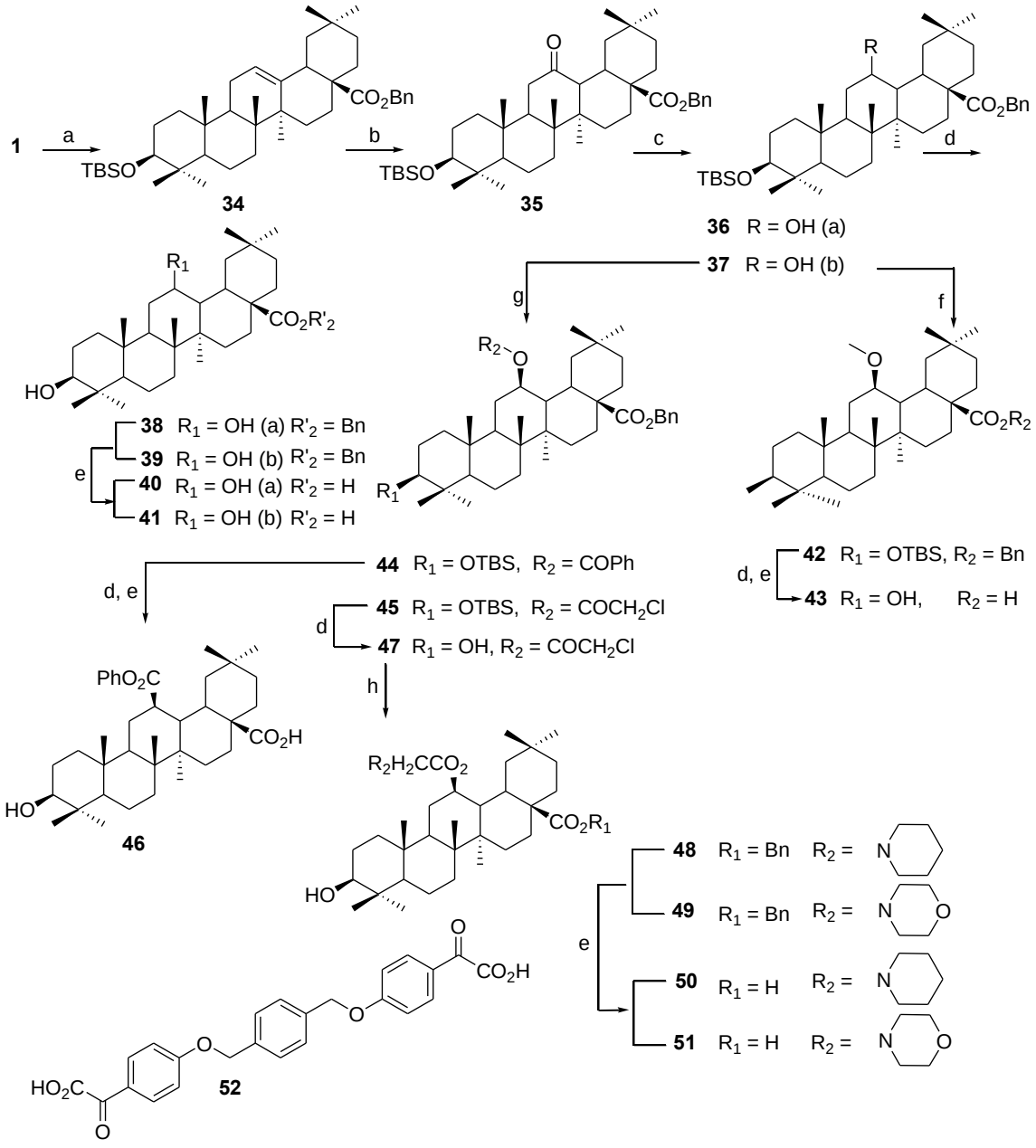


Scheme 3

a) Ac_2O, DMAP, pyridine, rt, 12 h, 90%. *b*) pyridinium dichromate, DCM, rt, 3 h, 50%. *c*) 1. 2 M NaOH, THF, MeOH, rt, 12 h; 2. 10% Pd/C, MeOH, rt, 12 h, 68-80% (over two steps). *d*) *m*CPBA, $CHCl_3$, rt, 24 h, 60%. *e*) HBr, Br_2, AcOH, 60 ^oC, 78%. *f*) hydroxylamine hydrochloride, pyridine, reflux, 5 h. *g*) $SOCl_2$, THF, rt, 12 h, 86%.

Compound **1** was protected by TBS group and then reacted with mCPBA to produce **35**. Reduction of carbonyl goup of **35** with $NaBH_4$ yielded **36** and **37**; the ratio is 2/3. We also found although **37** could be methylation or acylation, **36** was very difficult to take place these reaction. We speculated that **36** possessed strong steric hindrance. Compounds **40** and **41** were obtained by deprotection of TBS and benzyl group of **36** and **37**. The structure and configuration of **39** were proven by single-crystal X-ray

diffraction analysis[29] (Figure 1). Compound **43** was synthesized by methylation of **37**, then removal of TBS and benzyl group of **37**. Acylation of **37** with benzoyl chloride or chloroacetyl chloride afforded **44** and **45**. Compound **46** was produced by removal of TBS and benzyl group of **44**. Deprotection of TBS group of **45** afforded **47**. Compounds **50** and **51** were generated by reaction of **47** with piperidine or morpholine, then removal of benzyl group, as shown in scheme 4.



Scheme 4

a) TBSCl, imidazole, DMF, 60 °C, 5 h, 83%. *b*) mCPBA, $CHCl_3$, rt, 12 h, 67%. *c*) $NaBH_4$, THF/H_2O, rt, 5 h, 89%, (**36**/**37** = 2:3). *d*) $BF_3{\cdot}Et_2O$, $CHCl_3$, 0 °C, 5 h, 82%. *e*) Pd/C, H_2, MeOH, rt, 12 h, 78% for **40**, 75% for **41**, 74% for **43** and 75% for **46** over two steps. *f*) MeI, NaH, DMF, rt, 3 h, 85%. *g*) benzoyl chloride or chloroacetyl chloride, pyridine, DCM, rt, 5 h, 78% for **44** and 90% for **45**. *h*) piperidine or morpholine, DCM, reflux, 6 h, 85% for **4**8 and **49**.

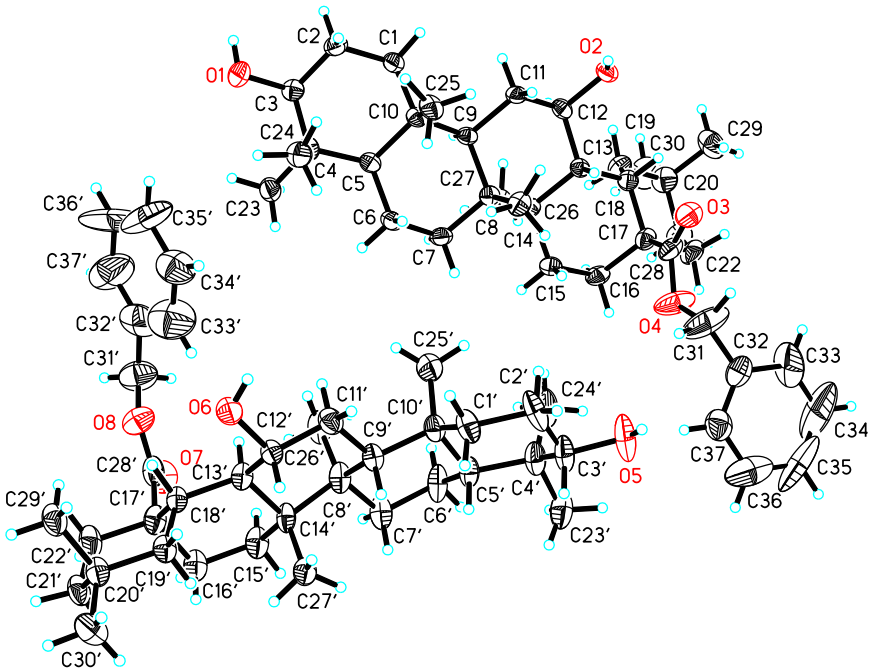


Figure 1. Single-crystal X-ray diffraction analysis of **39**

2. In vitro biological evaluation. In this paper, we further synthesized OA derivatives based on our previous work.[23] All these synthetic derivatives were evaluated in the enzyme inhibition assay against PTP1B by the method of p-nitrophenyl phosphate using compound **52** as a reference compound.[30] Homogeneous TCPTP inhibitory activities were investigated simultaneously by the same method for further selectivity studying (Tables 1 and 2). We also tested the inhibitory activity of some synthetic derivatives on several other critical PTPs, which negatively regulate insulin dephosphorylation, such as leukocyte antigen-related phosphatase (LAR), src homology phosphatase-1 (SHP-1) and src homology phosphatase-2 (SHP-2) (Table 3).

The IC_{50} of OA derivatives with heterocyclic ring-fused at C-2, C-3 positions (**6-22**) were tested on PTP1B and TCPTP. The results (Table 1) indicated that we obtained two most potent inhibitors **19** (IC_{50} = 0.91 μM) and **21** (IC_{50} = 0.98 μM) which showed about 3-fold more potent than lead compound OA on PTP1B. The inhibitory activity was decreased by introducing hydrophilic substituents at the five-membered heterocycles, such as carbonyl group (**17** and **18**), especially the formyloxy group (**20** and **22**), and increased by introducing hydrophobic substituents, such as trifluoromethyl group (**16**), especially the ethyl formyloxy group (**19** and **21**). The selectivity between PTP1B and TCPTP of these heterocyclic derivatives was not significantly improved, among them **16** possessed the best selectivity of 4.4-fold.

Table 1. Inhibitory activity of OA derivatives with heterocyclic ring-fused at C-2, C-3 positions against PTP1B and TCPTP

Compounds	IC_{50} (μM)		TCPTP/PTP1B[a]
	PTP1B	TCPTP	
OA	2.96 ± 0.26	7.78 ± 1.55	2.6
6	2.71 ± 0.35	9.58 ± 1.64	3.5
9	2.86 ± 0.34	6.59 ± 1.10	2.3
15	3.05 ± 0.23	7.99 ± 1.29	2.6
16	1.39 ± 0.27	6.15 ± 1.40	4.4
17	2.99 ± 0.44	6.09 ± 0.58	2.0
18	3.89 ± 0.63	7.89 ± 0.95	2.0
19	**0.91 ± 0.13**	2.01 ± 0.34	2.2
20	8.87 ± 1.55	23.8 ± 7.12	2.7
21	**0.98 ± 0.14**	2.46 ± 0.22	2.5
22	6.54 ± 0.68	7.93 ± 3.69	1.2
52[b]	3.89 ± 0.08	6.24 ± 0.19	1.6

a) TCPTP/PTP1B, the ratio of IC_{50} of TCPTP and PTP1B. b) Positive control.

The PTP1B inhibition of the C-ring modified derivatives (**25-51**) was decreased dramatically, with the exception of **46**. The ring expansion compound **33** was obtained by Beckman rearrangement of **31**, which PTP1B inhibitory potency was decreased at least 2-fold. The stereo configuration of C-12 hydroxyl is important since the activity of β-hydroxyl compound (**41**) was about half of α-hydroxyl compound (**40**). Compared with **41**, the C-12 β-hydroxyl substituted with hydrophobic groups, such as methyl, benzoyl, piperidine *N*-acetyl and morpholine *N*-acetyl group (**43**, **46**, **50** and **51**) could ameliorate the PTP1B inhibitory potency. Although the PTP1B inhibitory potency of these C-ring modified derivatives was decreased dramatically, the selectivity between PTP1B and TCPTP of some derivatives was improved significantly, especially **25**, **50** and **51** possessed more than 6-fold selectivity.

Table 2. Inhibitory activity of C-ring modified OA derivatives against PTP1B and TCPTP

Compounds	IC_{50} (μM)		TCPTP/PTP1B[b]
	PTP1B	TCPTP	
OA	2.96 ± 0.26	7.78 ± 1.55	2.6
25	9.07 ± 0.44	57.8 ± 9.38	**6.4**
27	13.4 ± 0.47	66.1 ± 7.50	4.9
29	>40	nd[a]	
31	9.34 ± 2.95	36.2 ± 6.73	3.9
33	21.2 ± 5.64	36.8 ± 3.75	1.7
40	16.1 ± 1.30	44.6 ± 6.05	2.8
41	31.4 ± 2.32	61.5 ± 5.12	2.0
43	7.56 ± 1.17	18.9 ± 3.11	2.5
46	2.44 ± 0.21	9.06 ± 1.33	3.7
50	6.35 ± 0.24	41.6 ± 10.5	**6.6**
51	5.71 ± 0.84	35.4 ± 3.47	**6.3**
52[c]	3.89 ± 0.08	6.24 ± 0.19	1.6

a) Not determined. b) TCPTP/PTP1B, the ratio of IC_{50} of TCPTP and PTP1B. c) Positive control.

Moreover, some derivatives (**16**, **19**, **21**, **25**, **50** and **51**), which had potent inhibition or high selectivity, were also evaluated on homogenous enzymes LAR, SHP-1 and SHP-2 (Table 3). The results showed that these compounds had no obvious inhibition against LAR, SHP-1 and SHP-2. Compared to OA, these tested derivatives developed by our group clearly have better selectivity between PTP1B and SHP-1.

Table3. Inhibitory activity of selected OA derivatives against related PTPs

Compounds	IC_{50} (μM)		
	LAR	SHP-1	SHP-2
OA	> 40	31.3 ± 5.39	> 40
16	> 40	> 40	> 40
19	> 40	> 40	> 40
21	> 40	> 40	> 40
25	> 40	> 40	> 40
50	> 40	> 40	> 40
51	> 40	> 40	> 40

CONCLUSION

In summary, we synthesized a series of novel OA derivatives with five-membered heterocyclic ring-fused at C-2, C-3 positions and the C-ring modified OA analogs efficiently. The five-membered heterocyclic ring-fused compounds had improved inhibitory activity on PTP1B, and the most potent compounds possessed 3-fold potency compare to OA; however, the selectivity between PTP1B and TCPTP was not obviously improved. The C-ring modified compounds had improved selectivity for PTP1B over TCPTP, some compounds possessed up to 6-fold selectivity; however, the inhibitory potency on PTP1B was decreased dramatically. We hope that merge the two parts of works, modification of the C-2, C-3 positions and the C-ring simultaneously, would afford the potent and high selectivity PTP1B inhibitors.

EXPERIMENTAL

General. ^{1}H (400 and 500 MHz) and ^{13}C (100 and 125 MHz) NMR spectra were recorded on a JEOL-400 or Bruker AM-500 Fourier transform spectrometer. The chemical shifts were reported (δ in ppm) using the δ = 7.26, 2.5 signals of $CDCl_3$, DMSO-d_6 (^{1}H NMR), and using the δ = 77.23, 39.51 signals of $CDCl_3$, DMSO-d_6 (^{13}C NMR) as internal standards. High-resolution mass data were obtained on a Micromass Tof II spectrometer.

General procedure A, oxidation of 3-OH group of OA derivatives. OA derivative (1 mmol) was dissolved in DMSO (30 mL). To this soln., IBX (0.56 g, 2 mmol) was added. After stirring for 12 h at rt,

the reaction mixture was poured into H_2O (30 mL), the precipitate was filtered, and the filtrate was extracted with AcOEt (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (CC).

General procedure B, acylation of C-2 position of 10. OA derivative (0.2 mmol) was dissolved in dry THF (20 mL). To this soln., NaH (40 mg, 1 mmol, 60% in oil) was added. After stirring for 1 h at rt, the acylation reagent RCO_2Et (R = CF_3, OEt, CO_2Et) (1 mmol) was added. After stirring for 12 h at rt, the reaction mixture was poured into H_2O (40 mL), the pH was adjusted to 6-7 by adding 5% HCl, and the mixture was extracted with AcOEt (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC.

General procedure C, deprotection of benzyl group of OA derivatives. OA derivative (1 mmol) was dissolved in MeOH (50 mL). To this soln., 10% Pd on carbon (30 mg) was added and the reaction was subjected to 1 atm of H_2. After stirring for 12 h at rt, the reaction mixture was filtered, and the filtrate was concentrated to give the target product.

General procedure D, hydrolysis of OA derivatives. OA derivative (1 mmol) was dissolved in THF (20 mL) and MeOH (20 mL). To this soln., 2 M NaOH solution (12 mL) was added. After stirring for 12 h at rt, the reaction mixture was poured into H_2O (40 ml), the pH was adjusted to 6-7 by adding 5% HCl, and the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC.

General procedure E, deprotection of TBS group of OA derivatives. OA derivative (1 mmol) was dissolved in DCM (20 mL). To this soln., boron trifluoride ether etherate (6 mL) was added at 0 ^oC. After stirring for 5 h at 0 ^oC, the reaction mixture was poured into H_2O (40 mL), and the mixture was extracted with DCM (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by CC.

Compound 1. OA (10 g, 21.9 mmol) was dissolved in dry DMF (100 mL). To this soln., K_2CO_3 (6.04 g, 43.8 mmol) and BnBr (3.33 g, 26.3 mmol) were added. After stirring for 12 h at rt, the reaction mixture was poured into ice H_2O (200 mL). The precipitate was filtered and washed with water, then dried under reduced pressure to give **1** as a white solid powder (11 g, 92%).

Compound 2. General procedure A, CC (PE/AcOEt 10:1), afforded **2** (840 mg, 85%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$): 7.34-7.35 (m, 5 H); 5.31 (s, 1 H); 5.04-5.12 (m, 2 H); 2.90-2.93 (m, 1 H); 2.50-2.54 (m, 1 H); 2.34-2.37 (m, 1 H); 1.18-1.99 (m, 20 H); 1.13 (s, 3 H); 1.08 (s, 3 H); 1.03 (s, 3 H); 1.01 (s, 3 H); 0.92 (s, 3 H); 0.90 (s, 3 H); 0.65 (s, 3 H).

Compound 3. *t*-BuOK (515 mg, 4.2 mmol) and **2** (500 mg, 0.92 mmol) were dissolved in *t*-BuOH (30

mL) and stirred for 1 h at rt. To this soln. isoamyl nitrite (493 mg, 4.2 mmol) was added. After stirring for 5 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 5:1) to give **3** (450 mg, 86%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.29-7.32 (m, 5 H); 5.31 (s, 1 H); 5.00-5.09 (m, 2 H); 2.96-3.00 (m, 1 H); 2.89-2.92 (m, 1 H); 1.19-2.11 (m, 19 H); 1.16 (s, 3 H); 1.12 (s, 3 H); 1.09 (s, 3 H); 0.90 (s, 3 H); 0.88 (s, 3 H); 0.85 (s, 3 H); 0.61 (s, 3 H).

Compound 4. Hydroxylamine hydrochloride (165 mg, 2.35 mmol) and **3** (450 mg, 0.78 mmol) were dissolved in pyridine (20 mL). After refluxing for 4 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 3:1) to give **4** (345 mg, 75%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.32-7.35 (m, 5 H); 5.33 (s, 1 H); 5.04-5.12 (m, 2 H); 3.10-3.15 (m, 1 H); 2.90-2.93 (m, 1 H); 1.21-2.05 (m, 19 H); 1.18 (s, 3 H); 1.12 (s, 3 H); 1.07 (s, 3 H); 0.90 (s, 3 H); 0.89 (s, 3 H); 0.85 (s, 3 H); 0.63 (s, 3 H).

Compound 5. KOH (32 mg, 0.58 mmol) and **4** (345 mg, 0.58 mmol) were dissolved in ethylene glycol (10 mL) and dioxane (5 mL). After refluxing for 5 h, the reaction mixture was poured into H_2O (30 mL) and extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give **5** (200 mg, 60%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.32-7.36 (m, 5 H); 5.36 (s, 1 H); 5.04-5.12 (m, 2 H); 3.06-3.10 (m, 1 H); 2.94-2.96 (m, 1 H); 2.13-2.17 (m, 1 H); 1.46-1.99 (m, 18 H); 1.41 (s, 3 H); 1.33 (s, 3 H); 1.12 (s, 3 H); 0.93 (s, 3 H); 0.90 (s, 3 H); 0.81 (s, 3 H); 0.65 (s, 3 H).

Compound 6. General procedure C, afforded **6** (168 mg, 92%) as a white solid. Mp 243-245 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 5.35 (s, 1 H); 3.08-3.12 (m, 1 H); 2.86-2.89 (m, 1 H); 2.15-2.19 (m, 1 H); 1.44-2.02 (m, 18 H); 1.41 (s, 3 H); 1.30 (s, 3 H); 1.17 (s, 3 H); 0.93 (s, 3 H); 0.91 (s, 3 H); 0.84 (s, 3 H); 0.82 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz): 184.3; 159.6; 150.5; 143.6; 122.0; 52.9; 46.6; 45.9; 45.8; 41.8; 41.0; 39.3; 38.4; 35.2; 33.8; 33.2; 33.0; 32.4; 31.7; 31.3; 30.7; 29.7; 27.7; 25.7; 24.7; 23.5; 22.9; 19.0; 16.6; 15.4. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{30}H_{44}N_2NaO_3$ 503.3244; found 503.3284.

Compound 7. Zn dust (70 mg, 1.1 mmol) and **3** (200 mg, 0.35 mmol) were dissolved in AcOH (10 mL) and Ac_2O (5 mL). After stirring for 12 h at rt, the reaction mixture was filtered with diatomite, the pH of filtrate was adjusted to 6-7 by adding 5% HCl, and the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 1:1) to give **7** (170 mg, 86%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.27-7.32 (m, 5 H); 6.58 (d, J = 6.0 Hz, 1 H); 5.26 (s, 1 H); 5.05 (d, J = 12.0 Hz, 1 H); 5.00 (d, J = 12.0 Hz, 1H); 4.84-4.88 (m, 2H); 2.87-2.90 (m, 1H); 2.54-2.61 (m, 1H); 2.04 (s, 3H); 1.19-2.02 (m, 19 H); 1.14 (s, 3 H); 1.11 (s, 3 H); 1.07 (s, 3 H); 0.90 (s, 3 H); 0.89 (s, 3 H); 0.74 (s, 3 H); 0.57 (s, 3 H).

Compound 8. $POCl_3$ (0.5 mL) and **7** (170 mg, 0.28 mmol) were dissolved in pyridine (10 mL). After stirring for 12 h at rt, H_2O (20 mL) was added in to quench the reaction and the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 5:1) to give **8** (125 mg, 75%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.22-7.27 (m, 5 H); 5.27 (s, 1 H); 5.01 (d, *J* = 12 Hz, 1 H); 4.96 (d, *J* = 12 Hz, 1 H); 2.85-2.88 (m, 1 H); 2.32-2.36 (m, 1 H); 2.31 (s, 3 H); 1.19-2.02 (m, 19 H); 1.12 (s, 3 H); 1.10 (s, 3 H); 1.03 (s, 3 H); 0.85 (s, 3 H); 0.83 (s, 3 H); 0.82 (s, 3 H); 0.59 (s, 3 H).

Compound 9. General procedure C, afforded **9** (83 mg, 82%) as a white solid. Mp 267-268 ^{o}C. ^{1}H NMR (500MHz, $CDCl_3$): 5.32 (s, 1 H); 2.84-2.86 (m, 1 H); 2.39 (s, 3 H); 1.24-2.08 (m, 20 H); 1.17 (s, 3 H); 1.13 (s, 3 H); 1.07 (s, 3 H); 0.91 (s, 3 H); 0.88 (s, 3 H); 0.81 (s, 3 H); 0.82 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): 183.4; 159.8; 151.9; 143.6; 129.8; 122.4; 53.5; 53.4; 46.5; 46.2; 45.9; 41.7; 41.0; 39.4; 39.0; 38.5; 33.8; 33.0; 32.4; 31.9; 30.6; 28.8; 27.7; 25.7; 23.5; 23.3; 22.9; 21.0; 18.8; 16.8; 15.7; 14.0. ESI-HRMS (m/z) $[M+H]^+$ calcd for $C_{32}H_{48}NO_3$ 494.3629; found 494.3695.

Compound 10. General procedure A, afforded **10** (1.0 g, 90%) as a white solid and used without further purification.

Compound 11. MeONa (595 mg, 11 mmol) and **10** (1.0 g, 2.2 mmol) were dissolved in dry toluene (30 mL). After stirring for 1 h at rt, HCO_2Et (0.89 mL, 11 mmol) was added. After stirring for 6 h, the reaction mixture was concentrated under reduced pressure. The residue was diluted with H_2O (30 mL) and adjusted pH to 6-7 by adding 5% HCl, and the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give **11** (743 mg, 70%) as a pink solid. ^{1}H NMR (500 MHz, $CDCl_3$): 14.92 (d, *J* = 4.0 Hz, 1 H), 8.57 (d, *J* = 4.0 Hz, 1 H), 5.34 (m, 1 H), 2.90 (m, 1 H), 1.20-2.87 (m, 21 H); 1.19 (s, 3 H), 1.14 (s, 3 H), 1.10 (s, 3 H), 0.94 (s, 3 H), 0.90 (s, 3 H), 0.89 (s, 3 H), 0.79 (s, 3 H).

Compound 12. General procedure B, CC (PE/AcOEt 5:1), afforded **2** (430 mg, 78%) as a pink solid. ^{1}H NMR (500 MHz, $CDCl_3$): 15.71 (s, 1H, CO_2H); 5.33 (s, 1H, H-12); 2.83-2.86 (m, 1H, H-18); 2.50-2.53 (m, 1H); 0.82-2.04 (m, 20H); 1.25 (s, 3 H); 1.17 (s, 3 H); 1.10 (s, 3 H); 0.93 (s, 3 H); 0.90 (s, 3 H); 0.89 (s, 3 H); 0.81 (s, 3 H).

Compound 13. General procedure B, CC (PE/AcOEt 5:1), afforded **13** (410 mg, 78%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): 12.57 (s, 1H, CO_2H); 5.32 (s, 1H, H-12); 4.14-4.27 (m, 2H, CH_2O); 2. 82-2.85 (m, 1H, H-18); 2.32-2.35 (m, 1H); 0.85-2.03 (m, 23H); 1.16 (s, 3H); 1.13 (s, 3H); 1.07 (s, 3H); 0.92 (s, 6H); 0.90 (s, 3H); 0.85 (s, 3H).

Compound 14. General procedure B, CC (PE/AcOEt 5:1), afforded **14** (410 mg, 78%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): 5.24 (s, 1 H); 4.25-4.32 (m, 2 H); 2.86-2.89 (m, 1 H); 2.32-2.36 (m, 1 H); 1.27-1.91 (m, 26 H); 1.24 (s, 3 H); 1.14 (s, 3 H); 1.08 (s, 3 H); 0.83 (s, 3 H); 0.77 (s, 3 H); 0.60 (s, 3 H).

Compound 15. Hydroxylamine hydrochloride (210 mg, 3.0 mmol) and **11** (500 mg, 1.0 mmol) were dissolved in pyridine (20 mL). After refluxing for 5 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 4:1) to give **15** (370 mg, 74%) as a white solid. Mp 159-162 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 8.03 (s, 1 H); 5.33 (s, 1 H); 2.84-2.86 (m, 1 H); 2.65-2.69 (m, 1 H); 1.25-2.04 (m, 19 H); 1.36 (s, 3 H); 1.23 (s, 3 H); 1.15 (s, 3 H); 0.93 (s, 3 H); 0.90 (s, 3 H); 0.81 (s, 3 H); 0.78 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): 184.1; 167.7; 153.5; 143.6; 122.3; 113.0; 53.2; 46.6; 45.9; 45.8; 41.8; 41.0; 39.2; 38.1; 34.0; 33.8; 33.6; 33.0; 32.3; 31.9; 31.4; 30.6; 27.7; 25.7; 24.7; 23.5; 23.3; 22.9; 19.0; 16.7; 14.9. ESI-HRMS (m/z) $[M+H]^+$ calcd for $C_{31}H_{46}NO_3$ 480.3478; found 480.3448.

Compound 16. 85% hydrazine hydrate (270 mg, 4.5 mmol) and **12** (500 mg, 0.91 mmol) were dissolved in dry EtOH (20 mL). After refluxing for 5 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 40:1) to give **16** (400 mg, 81%) as a white solid. Mp 254-256 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 13.2 (bs, 1 H); 12.1 (bs, 1 H); 5.21 (s, 1 H); 2.75-2.77 (m, 1 H); 2.49-2.52 (m, 1 H); 1.22-2.05 (m, 22 H); 1.13 (s, 3 H); 1.10 (s, 3 H); 1.05 (s, 3 H); 0.86 (s, 6 H); 0.80 (s, 3 H); 0.77 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 178.5; 147.8; 143.6; 123.8; 121.4; 121.2; 110.2; 52.1; 45.6; 45.5; 45.4; 41.5; 40.9; 40.1; 37.8; 34.9; 33.3; 32.8; 32.6; 32.0; 31.6; 30.3; 30.2; 27.2; 25.3; 23.2; 23.0; 22.8; 22.6; 18.4; 16.5; 15.1. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{32}H_{45}F_3N_2NaO_2$ 569.3325; found 569.3344.

Compound 17. 85% hydrazine hydrate (140 mg, 2.4 mmol) and **12** (300 mg, 0.56 mmol) were dissolved in dry EtOH (20 mL). After refluxing for 5 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 20:1) to give **17** (194 mg, 67%) as a white solid. Mp 265-266 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 5.22 (s, 1 H); 2.75-2.78 (m, 1 H); 2.25-2.28 (m, 1 H); 1.31-1.97 (m, 24 H); 1.15 (s, 3 H); 1.10 (s, 3 H); 1.05 (s, 3 H); 0.86 (s, 6 H); 0.79 (s, 3 H); 0.76 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 178.7; 158.6; 146.8; 143.6; 121.7; 95.5; 52.7; 45.7; 45.5; 41.5; 40.9; 40.1; 38.8; 38.1; 34.6; 33.4; 33.0; 32.9; 32.1; 31.8; 30.4; 30.3; 27.3; 25.4; 23.3; 22.9; 22.7; 18.6; 16.6; 15.1; 14.1. ESI-HRMS (m/z) $[M+H]^+$ calcd for $C_{31}H_{47}N_2O_3$ 495.3581; found 495.3587.

Compound 18. Hydroxylamine hydrochloride (78 mg, 1.14 mmol), AcONa (93 mg, 1.14 mmol) and **13** (120 mg, 0.23 mmol) were dissolved in dry EtOH (15 mL). After refluxing for 24 h, the reaction mixture was diluted with H_2O (30 mL) and extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 30:1) to give **18** (76 mg, 68%) as a white solid. Mp 217-218 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 5.27 (s, 1 H); 2.81-2.84 (m, 1 H); 2.10-2.14 (m, 1 H); 1.23-2.03 (m, 23 H);

1.26 (s, 3 H); 1.16 (s, 3 H); 1.13 (s, 3 H); 0.93 (s, 6 H); 0.88 (s, 3 H); 0.82 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): 178.6; 171.8; 169.7; 143.6; 121.5; 63.3; 52.1; 48.6; 45.7; 45.5(×2); 41.5; 40.9; 37.5; 34.1; 33.6; 33.4; 32.8; 32.1; 31.6; 30.4; 28.9; 27.3; 25.4; 23.3; 22.7; 21.6; 18.3; 16.5; 15.0; 14.1. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{31}H_{45}NNaO_4$ 518.3241; found 518.3263.

Compound 19. 85% hydrazine hydrate (100 mg, 1.71 mmol), and **14** (190 mg, 0.34 mmol) were dissolved in AcOH (15 mL). After stirring for 12 h at rt, the reaction mixture was diluted with H_2O (30 mL) and adjusted pH to 6-7 with $NaHCO_3$ and extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 20:1) to give **19** (150 mg, 78%) as a white solid. Mp 311-312 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 5.23 (s, 1 H); 4.19-4.21 (m, 2 H); 2.78-2.85 (m, 2 H); 1.25-2.03 (m, 25 H); 1.23 (s, 3 H); 1.20 (s, 3 H); 1.11 (s, 3 H); 1.10 (s, 6 H); 0.85 (s, 3 H); 0.77 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 178.6; 169.2; 150.5; 143.7; 138.9; 121.5; 119.2; 59.6; 52.4; 45.7; 45.5; 41.5; 40.9; 40.1; 38.8; 37.7; 36.6; 33.4; 32.8; 32.1; 31.7; 30.6; 30.4; 27.3; 27.2; 25.4; 23.3; 23.2; 22.9; 22.7; 18.6; 16.5; 15.3; 14.3. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{34}H_{50}N_2NaO_4$ 573.3663; found 573.3663.

Compound 20. General procedure D, CC (DCM/MeOH 10:1), afforded **20** (277 mg, 53%) as a white solid. Mp 274-276 °C. ^{1}H NMR (500 MHz, DMSO-d_6): 12.45 (bs, 3 H); 5.24 (s, 1H); 2.92 (d, J = 16.0 Hz, 1 H); 2.78 (d, J = 16.0 Hz,, 1 H); 1.33-2.08 (m, 19 H); 1.23 (s, 3 H); 1.14 (s, 6 H); 0.89 (s, 6 H); 0.80 (s, 6 H). ^{13}C NMR (125 MHz, DMSO-d_6): 178.6; 163.3; 150.5; 143.6; 138.9; 121.6; 115.3; 52.4; 45.7; 45.5; 41.5; 40.9; 40.0; 39.0; 38.9; 37.7; 36.6; 33.4; 32.9; 32.8; 32.1; 31.8; 30.8; 30.4; 27.2; 25.4; 23.4; 23.3; 23.0; 22.7; 16.6; 15.3. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{32}H_{46}N_2NaO_4$ 545.3350; found 545.3355.

Compound 21. Hydroxylamine hydrochloride (123 mg, 1.8 mmol) and **14** (200 mg, 0.36 mmol) were dissolved in dry EtOH (15 mL). After refluxing for 5 h, the reaction mixture was diluted with H_2O (30 mL) and extracted with AcOEt (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 40:1) to give **21** (150 mg, 75%) as a white solid. Mp 170-172 °C. ^{1}H NMR (500 MHz, DMSO-d_6): 5.32 (s, 1 H); 4.37-4.41 (m, 2 H); 2.82-2.85 (m, 1 H); 2.77-2.76 (m, 1 H); 1.37-2.09 (m, 22 H); 1.32 (s, 3 H); 1.17 (s, 3 H); 1.13 (s, 3 H); 0.91 (s, 3 H); 0.89 (s, 3 H); 0.86 (s, 3 H); 0.79 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 183.2; 175.0; 159.7; 153.0; 142.3; 121.2; 109.7; 60.5; 59.2; 52.2; 51.8; 45.4; 45.0; 44.6; 40.6; 39.8; 38.2; 37.3; 34.5; 33.8; 32.7; 31.9; 31.3; 30.7; 29.5; 27.6; 26.5; 24.6; 21.7; 19.8; 17.5; 15.6; 14.3; 13.0. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{34}H_{49}NNaO_5$ 574.3503; found 574.3550.

Compound 22. General procedure D, CC (DCM/MeOH 10:1), afforded **22** (288 mg, 55%) as a white

solid. Mp 163-164 °C. ^{1}H NMR (500 MHz, DMSO-d_6): 5.24 (s, 1 H); 2.78-2.80 (m, 1 H); 2.65-2.69 (m, 2H); 1.28-2.09 (m, 18 H); 1.24 (s, 3 H); 1.09 (s, 3 H); 1.07 (s, 3 H); 0.89 (s, 6 H); 0.83 (s, 3 H); 0.80 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 178.5; 175.2; 161.6; 154.8; 143.7; 121.3; 110.3; 59.7; 52.1; 45.7; 45.5; 45.4; 41.5; 37.9; 35.1; 34.4; 33.3; 32.8; 32.0; 31.4; 30.3; 28.4; 27.2; 25.3; 23.3; 22.9; 22.6; 20.7; 18.1; 16.5; 15.1; 14.0. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{32}H_{45}NNaO_5$ 546.3190; found 546.3182.

Compound 23. DMAP (0.45 g, 3.66 mmol), **1** (10 g, 18.3 mmol), Ac_2O (3.73g, 36.6 mmol) and pyridine (4.4 mL, 55 mmol) were dissolved in dry DMF (50 mL). After stirring at rt for 12 h, the reaction mixture was poured into ice water (100 mL) and the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with aqueous $NaHCO_3$, 5% HCl and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure afforded **23** (9.7 g, 90%) as a white solid and used without further purification.

Compound 24. Chromium anhydride (1.02 g, 10.2 mmol) was dissolved in DCM (20 mL). To the soln. pyridine (1.64 mL, 20.4 mmol) was slowly added at 0 °C and the reaction mixture was stirred for 0.5 h, then **23** (300 mg, 0.51 mmol) was added. After stirring for 12 h at rt, the solution was filtered through silica gel, the filtrate was concentrated under reduced pressure and dissolved in AcOEt (100 mL), washed with saturated aqueous $NaHSO_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 5:1) to give **24** (153mg, 50%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.30-7.32 (m, 5 H); 5.61 (s, 1 H); 5.03-5.12 (m, 2 H); 4.46 (dd, J = 4.0, 11.5 Hz, 1 H); 3.05-3.07 (m, 1 H); 2.77-2.79 (m, 1 H); 2.05 (s, 3 H); 1.17-1.94 (m, 20 H); 1.16 (s, 3 H); 1.0 (s, 3 H); 0.94 (s, 3 H); 0.91 (s, 3 H); 0.89 (s, 3 H); 0.88 (s, 3 H); 0.86 (s, 3 H).

Compound 25. General procedure D and C, afforded **25** (377 mg, 80%, over two steps) as a white solid. Mp 279-280 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 12.33 (bs, 1 H); 5.43 (s, 1 H); 4.24 (s, 1 H); 3.17 (m, 1 H); 3.0-3.02 (m, 1 H); 2.84-2.86 (m, 1 H); 2.59-2.62 (m, 1 H); 2.29 (s, 1 H); 1.34-2.08 (m, 17 H); 1.33 (s, 3 H); 1.01 (s, 3 H); 0.90 (s, 6 H); 0.88 (s, 3 H); 0.87 (s, 3 H); 0.66 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): 199.2; 178.1; 169.1; 126.8; 76.6; 61.1; 54.2; 45.1; 44.6; 43.8; 43.2; 41.2; 38.7; 38.5; 36.9; 36.8; 33.1; 32.5; 31.2; 30.3; 28.1; 27.3; 26.9; 23.2; 23.1; 22.4; 18.8; 17.1; 16.0; 15.9. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{30}H_{46}NaO_4$ 493.3288; found 493.3268.

Compound 26. *m*CPBA (1 g, 5.1 mmol) and **23** (1 g, 1.7 mmol) were dissolved in $CHCl_3$ (20 mL). After stirring for 24 h at rt, the reaction mixture was diluted with H_2O (30 mL) and extracted with $CHCl_3$ (2×30 mL). The combined organic layer was washed with saturated aqueous Na_2SO_3 and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give **26** (616 mg, 60%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.31-7.34 (m, 5 H); 5.19 (d, J = 12.2 Hz, 1 H); 5.06 (d, J = 12.2 Hz, 1 H); 4.45 (dd, J = 5.1, 12.0 Hz, 1 H); 2.80-2.84 (m, 1 H); 2.43 (d, J = 5.2 Hz, 1 H); 2.18-2.19 (m, 1 H); 2.15 (s, 3 H); 1.15-2.08 (m, 21 H); 1.12 (s, 3 H);

0.98 (s, 3 H); 0.92 (s, 3 H); 0.89 (s, 3 H); 0.88 (s, 3 H); 0.77 (s, 3 H); 0.61 (s, 3 H).

Compound 27. General procedure D and C, afforded **27** (364 mg, 77%, over two steps) as a white solid. Mp 223-224 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 3.16-3.20 (m, 1 H); 2.67-2.69 (m, 1 H); 2.42-2.45 (m, 1 H); 2.19 (s, 1H); 1.14-2.15 (m, 20 H); 1.12 (s, 3 H); 0.98 (s, 3 H); 0.92 (s, 3 H); 0.89 (s, 3 H); 0.88 (s, 3 H); 0.77 (s, 3 H); 0.61 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 209.3; 177.5; 75.0; 52.9; 49.4; 47.6; 44.4; 39.8; 39.2; 36.8; 36.4; 35.9; 34.8; 34.2; 32.4; 31.7; 31.0; 30.0; 29.8; 29.0; 28.7; 26.4; 25.5; 25.3; 21.5; 20.8; 18.5; 16.4; 14.2; 13.3. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{30}H_{48}NaO_4$ 495.3445; found 495.3457.

Compound 28. 2 M solution of bromine in AcOH (0.5 mL), **26** (300 mg, 0.49 mmoL) and several drops of hydrobromic acid were added to glycial AcOH (15 mL). The reaction mixture was heated for 4 h at 60 °C. The reaction mixture was quenched with H_2O (30 mL) and adjusted pH to 7-8 by adding saturated aqueous $NaHCO_3$ solution, then extracted with AcOEt (3 ×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give **28** (230 mg, 78%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): 7.28-7.36 (m, 5 H); 5.71 (s, 1 H); 5.14 (d, J = 12.0 Hz, 1 H); 5.10 (d, J = 12.0 Hz, 1 H); 4.46 (dd, J = 5.0, 12.0 Hz, 1 H); 3.05-3.08 (m, 1 H); 2.73 (d, J = 4.4 Hz, 1 H); 2.06 (s, 3 H); 0.79-1.94 (m, 19 H); 1.16 (s, 3 H); 1.0 (s, 3 H); 0.94 (s, 3 H); 0.91 (s, 3 H); 0.89 (s, 3 H); 0.88 (s, 3 H); 0.86 (s, 3 H).

Compound 29. General procedures D and C, afforded **29** (348 mg, 74%, over two steps) as a white solid. Mp 299-301 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 5.75 (s, 1 H); 3.22 (dd, J = 5.0, 11.7 Hz, 1 H); 2.98-3.01 (m, 1 H); 2.89-2.90 (m, 1 H); 1.18-1.95 (m, 19 H); 1.17 (s, 3 H); 1.03 (s, 3 H); 1.00 (s, 3 H); 0.94 (s, 3 H); 0.89 (s, 3 H); 0.87 (s, 3 H); 0.83 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 197.6; 177.2; 176.9; 120.1; 74.3; 48.1; 47.0; 44.3; 43.2; 39.5; 38.5; 37.3; 34.4; 33.7; 32.3; 31.5; 30.8; 30.7; 29.4; 28.6; 26.4; 26.0; 25.6; 22.0; 21.9; 21.3; 20.6; 19.5; 15.9; 14.3. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{30}H_{46}NaO_4$ 493.3194; found 493.3174.

Compound 30. Hydroxylamine hydrochloride (58 mg, 0.83 mmol) and **26** (100 mg, 0.17 mmol) were dissolved in dry pyridine (20 mL). After refluxing for 5 h, the reaction mixture was concentrated under reduced pressure and diluted with H_2O (30 mL), then extracted with AcOEt (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure afforded **30** (100 mg, 97%) as a white solid and used without further purification.

Compound 31. General procedure D and C, afforded **31** (370 mg, 76%, over two steps) as a white solid. Mp 184-185 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.18 (dd, J = 5.0, 15.0 Hz, 1 H); 3.0-3.03 (m, 1 H); 2.79-2.82 (m, 1 H); 2.53-2.55 (m, 1 H); 1.23-1.97 (m, 21 H); 1.21 (s, 3 H); 0.98 (s, 3 H); 0.93 (s, 3 H); 0.90 (s, 3 H); 0.88 (s, 3 H); 0.85 (s, 3 H); 0.73 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 179.5; 157.0; 76.7; 54.6; 47.2; 46.8; 42.5; 40.7; 40.1; 38.9; 38.4; 37.9; 36.6; 35.7; 34.2; 33.5; 33.0; 32.2; 31.6; 30.5;

28.0; 27.0; 23.1; 22.4; 20.0; 19.3; 18.0; 15.8; 15.5; 15.0. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{30}H_{49}NNaO_4$ 510.3629; found 510.3633.

Compound 32. Compound **28** (250 mg, 0.4 mmol) was dissolved in dry THF (20 mL). To the soln. $SOCl_2$ (0.29 mL, 4.0 mmol) was added. After stirring for 12 h at rt, the reaction mixture was concentrated under reduced pressure and diluted with H_2O (30 mL), then extracted with AcOEt (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (DCM/MeOH 30:1) to give **32** (215 mg, 86%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): 7.33-7.37 (m, 5 H); 5.48 (d, *J* = 5.0 Hz, 1 H); 5.22 (d, *J* = 12.2 Hz, 1 H); 5.09 (d, *J* = 12.2 Hz, 1 H); 4.47 (dd, *J* = 5.0, 11.7 Hz, 1 H); 3.89-3.92 (m, 1 H); 2.52-2.55 (m, 1 H); 2.25-2.28 (m, 1 H); 2.03 (s, 3 H); 1.01-1.99 (m, 21 H); 0.94 (s, 3 H); 0.86 (s, 3 H); 0.83 (s, 3 H); 0.80 (s, 3 H); 0.77 (s, 3 H); 0.74 (s, 3 H); 0.70 (s, 3 H).

Compound 33. General procedure D and C, afforded **33** (332 mg, 68%, over two steps) as a white solid. Mp 172-173 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 6.12 (d, *J* = 5.0 Hz, 1 H); 4.06-4.09 (m, 1 H); 3.15 (dd, *J* = 5.0, 11.5 Hz, 1 H); 2.52-2.54 (m, 1 H); 2.26-2.29 (m, 1 H); 0.95-1.93 (m, 20 H); 0.94 (s, 3 H); 0.86 (s, 3 H); 0.83 (s, 3 H); 0.80 (s, 3 H); 0.77 (s, 3 H); 0.74 (s, 3 H); 0.70 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 176.9; 173.9; 74.9; 52.2; 48.5; 46.8; 44.5; 43.0; 40.7; 37.2; 36.9; 36.7; 36.6; 34.4; 32.9; 32.7; 31.7; 31.5; 31.3; 30.8; 28.9; 26.4; 25.2; 21.2; 19.7; 16.1; 15.6; 15.1; 14.2; 13.8. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{30}H_{49}NNaO_4$ 510.3554; found 510.3572.

Compound 34. Imidazole (1.3 g, 18.3 mmol), **1** (1.0 g, 1.83 mmol) and TBSCl (1.38 g, 9.16 mmol) were dissolved in dry DMF (25 mL). After stirring for 6 h at 60 °C, the reaction mixture was poured into H_2O (30 mL), then extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was recrystallized with AcOEt to give **34** (1.0 g, 83%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.29-7.30 (m, 5 H); 5.24 (s, 1H); 5.04 (d, *J* = 12.4 Hz, 1 H); 4.99 (d, *J* = 12.4 Hz, 1 H); 3.11-3.14 (m, 1 H); 2.84-2.87 (m, 1 H); 1.10-1.92 (m, 24 H); 1.07 (s, 3 H); 0.87 (s, 6 H); 0.85 (s, 3 H); 0.84 (s, 9 H); 0.82 (s, 3 H); 0.69 (s, 3 H); 0.50 (s, 3 H); -0.02 (s, 6H).

Compound 35. *m*CPBA (2.36 g, 6.82 mmol) and **34** (1.5 g, 2.27 mmol) were dissolved in $CHCl_3$ (30 mL). After stirring for 12 h at rt, the reaction mixture was poured into saturated aqueous $NaHSO_3$ solution (30 mL), and then extracted with AcOEt (3×30 mL). The combined organic layer was washed with saturated aqueous Na_2CO_3 solution and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give **35** (800 mg, 67%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.31-7.33 (m, 5 H); 5.05-5.21 (m, 2 H); 3.12-3.15 (m, 1 H); 2.77-2.80 (m, 1 H); 2.42-2.45 (m, 1 H); 2.17-2.19 (m, 1 H); 1.13-2.15 (m, 21 H); 1.12 (s, 3 H); 0.92 (s, 3 H); 0.90 (s, 3 H); 0.88 (s, 3 H); 0.87 (s, 12 H); 0.77 (s, 3 H); 0.61 (s, 3 H); 0.03 (s, 6 H).

Compound 36 and 37. Compound **35** (900 mg, 1.33 mmol) was dissolved in THF (20 mL) and H_2O (2 mL). To this soln. $NaBH_4$ (150 mg, 4 mmol) was added. After stirring for 5 h, the pH of the reaction mixture was adjusted to 6-7 by adding 5% HCl, and then the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 15:1) to give **36** and **37** (804 mg, 89%, **36**/**37** = 2:3). **36**: ^{1}H NMR (500 MHz, $CDCl_3$): 7.31-7.35 (m, 5 H); 5.17 (d, *J* = 12.3 Hz, 1 H); 5.07 (d, *J* = 12.3 Hz, 1 H); 3.99 (m, 1 H); 3.17 (dd, *J* = 4.1, 15.5 Hz, 1 H); 2.47-2.50 (m, 1 H); 1.29-2.38 (m, 23 H); 1.28 (s, 3 H); 0.93 (s, 3 H); 0.91 (s, 3 H); 0.88 (s, 12 H); 0.80 (s, 3 H); 0.72 (s, 3 H); 0.61 (s, 3H); 0.03 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$): 178.1; 136.4; 128.5(×2); 128.2; 128.1(×2); 79.3; 74.0; 66.0; 55.6; 48.5; 45.4; 42.0; 40.9; 40.4; 39.4; 39.2; 38.8; 36.6; 34.1; 33.5; 33.4; 33.2; 32.0; 30.7; 30.6; 28.4; 27.4; 27.3; 25.9; 23.7; 23.6; 22.7; 20.8; 18.4; 18.1; 16.5; 15.9; 15.8; 15.1; -3.8; -4.9. **37**: ^{1}H NMR (400 MHz, $CDCl_3$): 7.31-7.40 (m, 5 H); 5.21 (d, *J* = 12.2 Hz, 1 H); 5.08 (d, *J* = 12.2 Hz, 1 H); 3.67 (m, 1 H); 3.14-3.16 (m, 1 H); 2.71-2.74 (m, 1 H); 1.01-2.69 (m, 23 H); 0.93 (s, 3 H); 0.92 (s, 3 H); 0.91 (s, 3 H); 0.88 (s, 12 H); 0.78 (s, 3 H); 0.72 (s, 3 H); 0.62 (s, 3H); 0.03 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$): 177.9; 136.5; 128.5(×2); 128.4; 128.1(×2); 79.3; 68.3; 66.0; 55.3; 49.1; 47.4; 43.1; 41.5; 40.5; 39.4; 38.6; 36.8; 36.1; 34.5; 33.4; 33.1; 32.6; 31.9; 31.5; 30.5; 28.7; 28.4; 27.7; 25.9(×3); 23.4; 23.2; 18.4; 18.1; 17.8; 16.2; 15.9(×2); -3.8; -4.9.

Compound 39. General procedure E afforded **39** (474 mg, 84%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): 7.30-7.39 (m, 5H); 5.18 (d, *J* = 12.0 Hz, 1 H); 5.06 (d, *J* = 12.0 Hz, 1 H); 3.67 (m, 1H); 3.17 (dd, *J* = 4.8, 11.0 Hz, 1H); 2.72-2.78 (m, 1H); 1.13-2.03 (m, 23 H); 1.23 (s, 3 H); 0.95 (s, 3 H); 0.93 (s, 3 H); 0.91 (s, 3 H); 0.77 (s, 3 H); 0.74 (s, 3 H); 0.61 (s, 3 H).

Compound 40. General procedure E and C afforded **40** (370 mg, 78%, over two steps) as a white solid. Mp 246-247 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.77-3.80 (m, 1 H); 2.96-3.00 (m, 1 H); 2.24-2.27 (m, 1 H); 2.08-2.11 (m, 1 H); 1.07-1.97 (m, 27 H); 0.87 (s, 3 H); 0.86 (s, 3 H); 0.83 (s, 3 H); 0.80 (s, 3 H); 0.76 (s, 3 H); 0.64 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 179.8; 76.8; 71.6; 55.2; 47.3; 44.8; 41.6; 40.4; 38.5; 38.4; 38.3; 36.3; 33.9; 33.5; 33.0; 31.9; 30.6; 30.2; 30.0; 28.9; 28.7; 28.1; 27.0; 23.4; 20.1; 18.6; 18.0; 16.3; 16.0; 15.8. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{30}H_{50}NaO_4$ 497.3601; found 497.3621.

Compound 41. General procedure E and C afforded **41** (356 mg, 75%, over two steps) as a white solid. Mp 320-321 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.73-3.76 (m, 1 H); 3.18-3.20 (m, 1 H); 2.70-2.76 (m, 1 H); 1.04-1.88 (m, 27 H); 0.93 (s, 6 H); 0.90 (s, 3 H); 0.83 (s, 3 H); 0.76 (s, 3 H); 0.68 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 179.7; 77.1; 66.4; 55.2; 49.0; 46.5; 42.3; 41.4; 40.6; 38.9; 38.7; 36.9; 35.9; 34.6; 33.8; 33.4; 32.7; 32.0; 31.2; 30.6; 28.9; 28.5; 27.5; 23.6; 23.5; 18.4; 18.0; 16.5; 16.1; 16.0. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{30}H_{50}NaO_4$ 497.3601; found 497.3624.

Compound 42. 60% NaH (35 mg, 0.9 mmol) and **37** (200 mg, 0.3 mmol) were dissolved in dry DMF (15 mL). To this soln., MeI (0.06 mL, 0.9 mmol) was added. After stirring for 3 h at rt, the reaction mixture was poured into H_2O (30 mL), and then the mixture was extracted with AcOEt (3×30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give **42** (174 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$): 7.29-7.40 (m, 5 H); 5.17 (d, *J* = 12.5 Hz, 1 H); 5.11 (d, *J* = 12.5 Hz, 1 H); 3.30 (s, 3 H); 3.21-3.24 (m, 1 H); 3.15 (dd, *J* = 4.4, 15.7 Hz, 1 H); 0.95-1.93 (m, 24 H); 0.92 (s, 3 H); 0.91 (s, 3 H); 0.90 (s, 3 H); 0.88 (s, 9 H); 0.87 (s, 3 H); 0.79 (s, 3 H); 0.72 (s, 3 H); 0.62 (s, 3 H); 0.03 (s, 6H).

Compound 43. General procedure E and C, afforded **43** (362 mg, 74%, over two steps) as a white solid. Mp 274-276 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 3.36 (s, 3 H); 3.26-3.30 (m, 1 H); 3.19 (dd, *J* = 4.6, 16.2 Hz, 1 H); 2.72-2.75 (m, 1 H); 0.98-2.03 (m, 23 H); 0.97 (s, 3 H); 0.96 (s, 3 H); 0.92 (s, 3 H); 0.91 (s, 3 H); 0.87 (s, 3 H); 0.83 (s, 3 H); 0.76 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 183.1; 92.0; 78.9; 55.9; 55.3; 48.9; 47.2; 41.5; 41.2; 40.4; 38.9; 38.7; 37.2; 36.5; 34.5; 33.5; 33.3; 32.5; 31.2; 30.6; 29.7; 28.8; 27.3; 26.0; 23.5; 23.1; 18.3; 17.9; 16.6; 15.9; 15.4. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{31}H_{52}NaO_4$ 511.3758; found 511.3760.

Compound 44. Benzoyl chloride (0.35 mL, 3.0 mmol), **37** (200 mg, 0.3 mmol) and TEA (1.0 mL) were dissolved in DCM (15 mL). After stirring for 5 h at rt, the reaction mixture was poured into H_2O (30 mL), and then the mixture was extracted with DCM (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give **44** (180 mg, 78%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.96 (d, *J* = 7.3 Hz, 2 H); 7.46-7.48 (m, 1 H); 7.41 (d, *J* = 7.3 Hz, 2 H); 7.32-7.34 (m, 5 H); 5.24 (d, *J* = 12.2 Hz, 1 H); 5.11 (d, *J* = 12.2 Hz, 1 H); 3.16-3.18 (m, 1 H); 3.10-3.14 (m, 1 H); 2.70-2.72 (m, 1 H); 2.12-2.15 (m, 1 H); 1.13-2.11 (m, 22 H); 1.12 (s, 3 H); 0.92 (s, 3 H); 0.90 (s, 3 H); 0.87 (s, 12 H); 0.88 (s, 3 H); 0.77 (s, 3 H); 0.62 (s, 3 H); 0.03 (s, 6 H).

Compound 45. Chloroacetyl chloride (0.23 mL, 3.0 mmol), **37** (200 mg, 0.3 mmol) and pyridine (1.0 mL) were dissolved in DCM (15 mL). After stirring for 5 h at rt, the reaction mixture was poured into H_2O (30 mL), and then the mixture was extracted with DCM (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 10:1) to give **45** (200 mg, 90%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.36-7.40 (m, 5 H); 5.20-5.29 (m, 2 H); 4.00 (s, 2 H); 3.10-3.13 (m, 1 H); 2.54-2.57 (m, 1 H); 1.15-2.50 (m, 24 H); 1.12 (s, 3 H); 0.97 (s, 3 H); 0.90 (s, 3 H); 0.87 (s, 12 H); 0.88 (s, 3 H); 0.77 (s, 3 H); 0.62 (s, 3 H); 0.03 (s, 6 H).

Compound 46. General procedure E and C, afforded **46** (432 mg, 75%, over two steps) as a white solid. Mp 195-197 °C. ^{1}H NMR (500 MHz, DMSO-d_6): 12.25 (br, 1 H); 7.96-7.97 (m, 2 H); 7.60-7.63 (m, 1

H); 7.48-7.51 (m, 2 H); 4.90-5.20 (m, 1 H); 2.90-3.00 (m, 1 H); 2.62-2.65 (m, 1 H); 2.10-2.40 (m, 1 H); 1.11-2.33 (m, 20 H); 1.01 (s, 3 H); 0.91 (s, 3 H); 0.88 (s, 3 H); 0.75 (s, 3 H); 0.72 (s, 3 H); 0.62 (s, 3 H); 0.45 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): 183.3; 165.4; 133.1; 130.2; 129.0 (×2); 128.5 (×2); 76.6; 72.2; 54.7; 48.4; 46.1; 41.0; 40.3; 40.1; 39.9; 38.4; 38.2; 36.6; 36.1; 34.0; 33.2; 32.7; 32.1; 31.2; 30.1; 28.5; 28.1; 27.2; 27.0; 22.9; 17.9; 17.4; 16.2; 15.7; 15.6. ESI-HRMS (m/z) $[M+Na]^+$ calcd for $C_{37}H_{54}NaO_5$ 601.3863; found 601.3884.

Compound 47. General procedure E, afforded **47** (526 mg, 82%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): 7.37-7.43 (m, 5 H); 5.08-5.28 (m, 2 H); 4.03 (s, 2 H); 3.17-3.20 (m, 1 H); 2.57-2.60 (m, 1 H); 1.25-2.43 (m, 24 H); 1.21 (s, 3 H); 0.99 (s, 3 H); 0.92 (s, 3 H); 0.85 (s, 3 H); 0.79 (s, 3 H); 0.77 (s, 3 H); 0.65 (s, 3 H).

Compound 48. Piperidine (0.27 mL, 3.1 mmol) and **47** (200 mg, 0.31 mmol) were dissolved in DCM (20 mL). After refluxing for 6 h, the reaction mixture was poured into H_2O (30 mL), and then the mixture was extracted with DCM (3×20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by CC (PE/AcOEt 5:1) to give **48** (182 mg, 85%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): 7.33-7.38 (m, 5 H); 5.04-5.23 (m, 3 H); 3.62-3.75 (m, 2 H); 3.22-3.50 (m, 4 H); 3.15-3.17 (m, 1 H); 2.43-2.45 (m, 1 H); 1.21-2.03 (m, 29 H); 0.94 (s, 6 H); 0.87 (s, 3 H); 0.77 (s, 3 H); 0.75 (s, 3 H); 0.73 (s, 3 H); 0.60 (s, 3 H).

Compound 49. Using the same procedure as preparation of **48** afforded **49** (181 mg, 85%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): 7.33-7.38 (m, 5 H); 5.06-5.23 (m, 3 H); 4.04 (m, 4 H); 3.62-3.75 (m, 2 H); 3.22-3.50 (m, 4 H); 3.15-3.23 (m, 1 H); 2.39-2.42 (m, 1 H); 1.25-1.94 (m, 24 H); 1.24 (s, 3 H); 0.95 (s, 3 H); 0.89 (s, 3 H); 0.87 (s, 3 H); 0.79 (s, 3 H); 0.74 (s, 3 H); 0.64 (s, 3 H).

Compound 50. General procedure C, afforded **50** (516 mg, 86%) as a white solid. Mp 120-123 °C. ^{1}H NMR (500 MHz, DMSO-d_6): 4.86-4.88 (m, 1 H); 3.17 (d, J = 17.0 Hz, 1 H); 3.00 (d, J = 17.0 Hz, 1 H); 2.96-2.99 (m, 1 H); 2.44-2.46 (m, 4 H); 2.02-2.06 (m, 1 H); 1.11-1.80 (m, 29 H); 0.96 (s, 3 H); 0.87 (s, 3 H); 0.85 (s, 6 H); 0.76 (s, 3 H); 0.75 (s, 3 H); 0.65 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): 178.9; 169.7; 76.6; 71.1; 59.3; 54.8; 54.6; 53.2; 48.5; 48.2; 46.0; 41.0; 38.9; 38.4; 38.1; 36.5; 35.7; 33.8; 33.1; 32.7; 32.0; 30.9; 30.6; 30.1; 28.3; 28.0; 27.1; 27.0; 25.4; 23.5; 23.2; 22.6; 17.9; 17.3; 16.0; 15.7; 15.5. ESI-HRMS (m/z) $[M+H]^+$ calcd for $C_{37}H_{62}NO_5$ 600.4728; found 600.4705.

Compound 51. General procedure C, afforded **51** (512 mg, 85%) as a white solid. Mp 95-98 °C. ^{1}H NMR (500 MHz, DMSO-d_6): 12.25 (br, 1 H); 4.87-4.90 (m, 1 H); 4.26-4.28 (m, 1 H); 3.56 (t, J = 4.5 Hz, 4 H); 3.25 (d, J = 16.9 Hz, 1 H); 3.07 (d, J = 16.9 Hz, 1 H); 2.96-2.99 (m, 1 H); 1.16-2.54 (m, 28 H); 0.96 (s, 3 H); 0.87 (s, 3 H); 0.85 (s, 3 H); 0.84 (s, 3 H); 0.76 (s, 3 H); 0.74 (s, 3 H); 0.64 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6): 178.9; 169.5; 76.6; 71.2; 66.1; 58.7; 54.5; 52.5; 48.2; 46.0; 40.9; 40.2;

39.2; 39.1; 39.0; 38.4; 38.1; 36.5; 35.7; 33.7; 33.1; 32.7; 32.0; 30.9; 30.1; 28.3; 28.0; 27.1; 27.0; 23.2; 22.6; 17.9; 17.3; 16.0; 15.7; 15.6. ESI-HRMS (m/z) $[M+H]^+$ calcd for $C_{36}H_{60}NO_6$ 602.4415; found 602.4485.

In *vitro* assay of PTP1B inhibitors[21]

The tested compounds were solubilized in Me_2SO at 5 mg/mL, and 2 μL samples was distributed to A2-H11 wells of 96-well clear polystyrene plate (Corning, Action, MA). The Me_2SO (2 μL) was distributed to A1-D1 and E12-H12 wells as the full enzyme activity, and compound **52** was distributed to E1-H1 and A12-D12 wells as the positive inhibition. After adding an assay mixture (80 μL), 10 μL of the GST-PTP1B (300 nM) was added to initiate the reaction. The high-throughput screening was carried out in a final 100 μL volume containing 50 mM MOPS, pH 6.5, 2 mM pNPP, 30 nM PTP1B and 2% Me_2SO, and the catalysis of *p*NPP was continuously monitored on SpectraMax 340 microplate reader at 405 nm for 2 min at 30 °C. For calculating IC_{50}, inhibition assays were performed with 30 nM recombinant enzyme, 2 mM *p*NPP in 50 mMMOPS at pH 6.5 and the inhibitors diluted around the estimated IC_{50} values. IC_{50} was calculated from the non-linear curve fitting of percent inhibition (% inhibition) vs. inhibitor concentration [I] by usingthe following equation % Inhibition=100/{1+(IC_{50}/ [I])k}, where k is the Hill coefficient.

Crystal data and structure determination

A single crystal of **39** with dimensions of 0.398 mm × 0.369 mm × 0.307 mm was chosen for X-ray diffraction analysis performed on a Bruker-AXS diffractometer, equipped with Mo *Ka* radiation (λ = 0.71073 Å) at 293(2) K by using a φ-ω scan mode. In the ranges of 1.73≤θ≤25.50°, a total of 35026 reflections were collected including 12334 unique ones ($R\ int$ = 0.0793), of which 12334 were observed with $I > 2\sigma(I)$. The structure was solved by direct methods using SHELXS program of the SHELXL-97 package and refined with SHELXL. The final refinement was performed by full-matrix least-squares method with Full-matrix least-squares on $F2$ for the non-hydrogen atoms. **39** ($C_{37}H_{56}O_4$, Mr = 564.82), crystallizes in the orthorhombic system, space group $P2_12_12_1$ with a = 13.3536(9), b = 19.8860(12), c = 24.9647(16) Å, $\alpha = \beta = \gamma$=90°, V = 6629.4(7) Å^3, Z = 8, Dc = 1.132mg/m3, μ = 0.075mm−1, F(000) = 2480, the final R_1 = 0.0705 and wR_2 = 0.1778 for 12334 observed reflections ($I > 2\sigma(I)$). The hydrogen atoms were located from Fourier difference maps. The final R_1 = 0.0705, wR_2 = 0.1778 ($w = 1/[\sigma2(Fo^2) + (0.1038P)^2 + 0.0000P]$, where $P = (Fo^2 + 2Fc^2)/3$, S = 0.921, ($\Delta\rho$) max = 0.592 and ($\Delta\rho$) min = -0.264 e/Å^3.

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