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Dammaranes from *Gynostemma pentaphyllum* and synthesis of their derivatives as inhibitors of protein tyrosine phosphatase 1B

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ABSTRACT

Protein tyrosine phosphatase 1B (PTP1B) is a key factor in the negative regulation of insulin pathway and a promising target for treatment of diabetes and obesity. Herein, the sapogenin **2b**, prepared from the natural triterpene saponin **1b**, was modified at 3-position to establish the dammarane derivatives library via esterification, oxidation and reductive amination reaction and evaluated as PTP1B inhibitors. 3-*O*-*para*-Carboxylphenyl substituted derivative **5b** was found with the best in vitro inhibition activity to protein tyrosine phosphatase 1B (IC_{50} = 0.27 μM), where 3-*O*-*meta*-carboxylphenyl substituted **5a** exhibited the best selectivity (nearly fivefolds) between PTP1B and T-cell protein tyrosine phosphatase.

1. Introduction

Protein tyrosine phosphorylation is the predominant posttranslational modification that cells utilize to regulate signal transduction and to control a wide variety of cellular functions, such as growth, differentiation, survival, apoptosis, metabolism and gene transcription.[1–4] Protein tyrosine phosphatase 1B (PTP1B) is an important regulator of tyrosine kinase receptor-mediated responses, and influences negatively insulin sensitivity.[5–7] Indeed, PTP1B knockout mice display increased insulin sensitivity and tyrosine phosphorylation of the insulin receptor via catalyzing the dephosphorylation of specific phosphotyrosine (pTyr) residues on insulin receptor, insulin receptor substrate proteins and on Janus kinase 2, a protein tyrosine kinase which is associated with leptin receptors.[8–12] These results attracted considerable interest for the development of pharmacological PTP1B inhibitors for the treatment of insulin resistance, in particular non-insulin-dependent diabetes mellitus (type 2 diabetes), which is characterized by a deficient insulin cascade and is therefore also termed insulin resistance (IR).[13,11] Inhibitors of PTP1B, mainly involve non-hydrolysable phosphotyrosine mimics (or affinity-based), such as difluoromethylphosphonates, cinnamic acid, oxalylamino benzoic acid, isoxazole, carboxylic acid, salicyclic acid, and a-ketocarboxylic acid. However, a major drawback of most of these inhibitors is the lack of sufficient cell permeability and oral bioavailability due to the presence of highly negative charged polar residues in these inhibitors. Moreover, due to their high conserved catalytic domain, the selectivity between T-cell protein tyrosine phosphatase (TCPTP) and PTP1B[14] had still not been improved remarkably. Thus, these two drawbacks resulted in the restriction of their further development to clinical trials.

In the history of traditional Chinese medicine (TCM), medicinal plant *Gynostemma pentaphyllum* (Thunb.) Makino and their extracts were used to treat various diseases, especially diabetes, referred as 'thirsty disease' in ancient China. Recently, in the investigation of the bioactive chemical constituents of the herb, the dammarane type triterpene sapogenins **2a** and **2b** were found as a moderate PTP1B inhibitor (IC_{50} = 5.3 and 15.7 μM), respectively,[15] but hard to be studied further due to its low content in the plant (Fig. 1). In our research of *G. pentaphyllum*, compound **1b**, the corresponding saponin of **2b**, was found in the plant with much richer source than its 20*R* epimer **1a**. And its corresponding sapogenin **2b**, which can be obtained easily by acid hydrolysis of **1b**, was also found with moderate activity (IC_{50} = 11.3 μM) in our biological assessment, which was just a little weaker than **2a** (IC_{50} = 9.6 μM). This result suggested **2b** but not **2a** was more suitable for further modification to establish the dammarane derivatives library and analysis their SAR. In the present paper, we report the synthesis and in vitro PTP1B inhibitory of 3-O-substituted ester (**3a**–**3l**, **5a**–**5c**) and amine (**7a**–**7c**) derivatives of **2b**. Several compounds such as **5a**–**5c** showed potent inhibitory activity to PTP1B (IC_{50} = 0.2–0.5 μM). Furthermore, selected compounds in the series were evaluated their selectivity between PTP1B and

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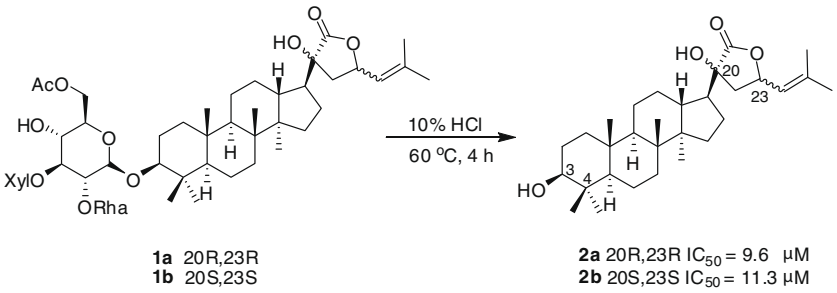


Figure 1. Structure–activity of dammarane triterpene saponin PTP1B inhibitors.

TCPTP to estimate whether they were suitable for further development.

2. Results and discussion

2.1. Chemistry

2.1.1. Preparation of compound 2b

The air-dried and powdered total saponins of *G. pentaphyllum* was hydrolyzed by 10% HCl and purified by using silica gel CC to give the starting material **2b**.

2.1.2. Modification of compound 2b

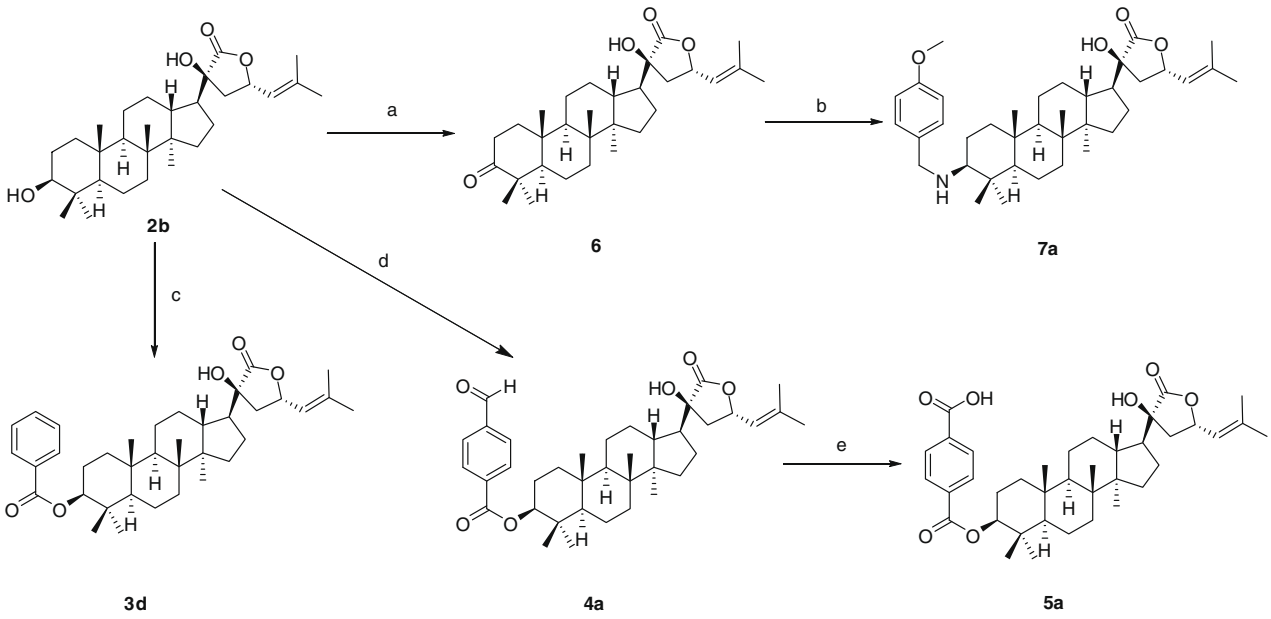
Esterification of **2b** with corresponding acid in normal condition furnished the **3a**–**3l**. Due to the steric hinderence caused by two methyl groups at C-4, reaction of **2b** with some acid might not give acceptable yield, which could be easily figured out by increasing largely the equivalent of substrate acid. **5a**–**5c** can not be directly synthesized by DCC/DMAP method because of large amount of side products formation. Condensation of **2b** and corresponding aldehydic acid, the intermediate **4a–4c** can be obtained via normal method mentioned above. Then oxidation of **4a**–**4c** by using of Jone's reagent readily afforded **5a**–**5c**. Oxidation of **2b** with PDC yielded the ketone derivative **6**, which was reacted with different amine using reductive amination to provide **7a**–**7c** (Scheme 1).

2.2. Biological evaluation

To examine the potential inhibition of the natural and semi-synthesis dammarane derivatives, their PTP1B inhibitory activities were assayed by the method of pNPP using {4-[4-(4-oxalyl-phenoxymethyl)-benzyloxy]-phenyl}-oxo-acetic acid as reference compound.[16] Moreover, their TCPTP inhibitory activities were examined simultaneously by the same method for further selectivity studying.

Primarily, 3-OH of **2b** was oxidized to give **6** to test whether the hydroxyl group was necessary for preserving the activity. And to our surprise, the value of IC_{50} of **6** only increased to 13.1 μM compared with **2b**, which suggested the hydroxyl might not influence obviously the activity probably because it can not enter deeply enough to the catalysis domain. Then various fatty acyl groups were introduced to C-3 to provide **3a**–**3c**. In spite of not consistent with the design principle of phosphate mimics, introduction of fatty chain gradually increased the activity along with the increasing length of the carbon chain without suitable explanation till the number of carbon on the chain reached 6.

In further study, aroyl was coupled with 3-hydroxyl to examine the impact of aryl group. Interestingly, the introduction of aroyl, no matter electron-donating or withdrawing groups bearing on it, significantly improved the activity. The comparison of **3f**–**3h** with **3i**–**3k** indicated that aroyl with electron-withdrawing groups



Scheme 1. Synthesis of 3-substituted derivatives. Reagents and conditions: (a) PDC, CH_2Cl_2, 2 h; (b) benzylamine, $NaBH_3CN$, MeOH, rt, 8 h; (c) benzoic acid, DCC, DMAP, CH_2Cl_2, rt, 4 h; (d) 4-formylbenzoic acid, DCC, DMAP, CH_2Cl_2, rt, 4 h; (e) Jones reagent, acetone, rt, 4 h.

improved the activity more than those with electron-donating groups. Moreover, comparison of **3k** with **3i** showed the nitro group improved the activity at *para* position more than at ortho position, whereas the situation of methoxyl-substituted derivative was opposite, maybe because the nitro group was similar with phosphate group to some extent, and that the nitro group entered the catalysis domain more deeply could increase the activity.

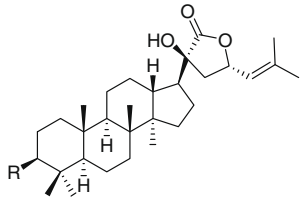
When carboxyl group was introduced to the aryl (**5a**–**5c**), activity was largely enhanced. This result was consistent with the principle[17] that phosphate mimics with an aryl group nearby entering the catalytic domain could improve the activity in large scale. However, substitution of benzylamino group on C-3 (**7a**–**7c**) decreased the activity dramatically. Notwithstanding benzylamino group not proper for C-3 modification, the activity data still indicated that electron-deficient aromatic ring was more beneficial.

Most of the compounds were also subjected to TCPTP inhibitory assay (see Tables 1). However, most of them did not exhibited significant distinction between these two homogeneous enzymes except for **5a**, the selectivity of which reached to 4.9-fold with relatively more potent on PTP1B activity. The conclusion was similar to most reported PTP1B inhibitors.[18]

3. Conclusion

Dammarane saponin compound **1b** and its sapogenin **2b**, as far as we know, were first developed as PTP1B inhibitor derived from nature product with SAR research. In the present paper, through varying the structure at C-3 positions, several compounds (**5a**–**5c**), were found potent on PTP1B inhibitory activity, with **5b** the utmost potency, up to 42-fold more effective than lead compound **2b**, with their SAR was preliminary studied and discussed. Meanwhile, **5a** was found the best selectivity which made it become the best lead compound for further modification. Although potent activity was observed, the selectivity between PTP1B and TCPTP of them was still not improved significantly, which will be the priority of the further investigation. Moreover, acquisition of the cellular result of compounds with good activity in vitro will be moved forward quickly to find whether they can be suitable for animal studies in diabetes and obesity.

Table 1
Inhibitory activity of all compounds against PTP1B and TCPTP



Compounds	R	IC_{50}[a] (μM)	
		PTP1B	TCPTP
2b	OH	11.25 ± 0.42	10.04 ± 0.32
3a	Acetoxyl	12.69 ± 0.31	NT[b]
3b	Propionyl	4.60 ± 0.55	14.70 ± 0.93
3c	Pentanoyl	4.14 ± 0.08	11.75 ± 0.20
3d	Benzoyl	2.75 ± 0.50	4.15 ± 0.12
3e	2′-Pyridinylcarbonyloxy	2.36 ± 0.32	5.14 ± 0.17
3f	2′-Methoxybenzoyloxy	1.90 ± 0.45	4.05 ± 0.20
3g	3′-Methoxybenzoyloxy	2.08 ± 0.29	11.98 ± 0.98
3h	4′-Methoxybenzoyloxy	2.61 ± 0.08	3.90 ± 0.18
3i	2′-Nitrobenzoyloxy	1.96 ± 0.24	3.38 ± 0.13
3j	3′-Nitrobenzoyloxy	1.70 ± 0.09	2.26 ± 0.26
3k	4′-Nitrobenzoyloxy	1.42 ± 0.22	5.10 ± 0.98
3l	2′,4′-Dinitrobenzoyloxy	1.62 ± 0.23	2.87 ± 0.39
5a	3′-Carboxybenzoyloxy	0.33 ± 0.02	1.62 ± 0.07
5b	4′-Carboxybenzoyloxy	0.27 ± 0.05	0.97 ± 0.08
5c	3′-Nitro-4′-carboxybenzoyloxy	0.50 ± 0.06	1.22 ± 0.07
6	=O	13.12 ± 0.71	34.13 ± 0.591
7a[c]	4′-Methoxybenzylamino	24%	NT
7b	3′,5′-Difluorobenzylamino	11.59 ± 0.87	NT
7c	4′-Nitrobenzylamino	6.58 ± 0.27	NT

[a] The pNPP assay. IC_{50} values were determined by regression analyses and expressed as means ± SD of three replications.
[b] Not tested.
[c] Inhibition rate to PTP1B in 20 μM concentration.

4. Experimental section

4.1. General experimental procedure

^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian Mercury-VX300 Fourier transform spectrometer. The chemical shifts were reported in δ (ppm) using the δ 7.26 signal of $CDCl_3$ (^1H NMR) and the δ 77.23 signal of $CDCl_3$ (^{13}C NMR) as internal standards. ESI-MS was run on a Bruker Esquire 3000 plus spectrometer in MeOH and HR-ESI-MS was run on a Bruker Atex III spectrometer in MeOH, respectively. All commercially available reagents were used without further purification. The solvents used were all AR grade and were redistilled under positive pressure of dry nitrogen atmosphere in the presence of proper desiccant when necessary. The progress of the reactions was monitored by analytical thin-layer chromatography (TLC) on HSGF254 precoated silica gel plates. Details of analytical HPLC profile of compounds in two diverse systems were described in the Supplementary data.

4.2. Preparation of compound 2b

The air-dried and powdered total saponins of *G. pentaphyllum* (1.0 kg) was dissolved to a 10 L solution of 10% HCl and stirred at 60 °C for 4 h. The mixture was then cooled to room temperature and extracted with $CHCl_3$ (10 L × 3). The combined organic layers were concentrated under reduced pressure to give the residue (870.1 g), which were subjected to CC ((SiO_2; $CHCl_3$/MeOH 100:0, 99:1, 98:2, 95:5, 9:1, 8:2) to afford eight fractions (*Fr.A–H*). *Fr.D* (103.9 g) was isolated on further CC (SiO_2; $CHCl_3$/MeOH 99:1, 98:2, 95:5, 9:1) to yield pure product **2b** (15.0 g). ^1H NMR ($CDCl_3$, 300 MHz): δ 5.27 (d, J = 7.4 Hz, 1H), 5.12 (m, 1H), 3.17 (m, 1H), 1.74 (s, 3H), 1.76 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.76 (s, 3H), 0.74 (s, 3H), 0.74 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.1 (s), 140.1 (s), 123.5 (d), 79.1 (d), 78.5 (s), 74.4 (d), 56.1 (d), 50.9 (d), 50.7 (s), 45.8 (s), 42.5 (d), 40.7 (t), 38.9 (t), 38.1 (s), 37.3 (s), 35.4 (s), 31.5 (t), 28.2 (t), 27.5 (d), 26.6 (t), 26.0 (q), 25.3 (t), 21.6 (q), 18.6 (q), 18.4 (t), 16.6 (q), 16.4 (q), 15.6 (q), 15.5 (q); HRMS calcd for $C_{30}H_{48}O_4$: 472.3553. Found: 472.3545.

4.3. Chemical synthesis

4.3.1. General procedure for synthesis of 3a–3l

To a solution of **2b** (30 mg, 0.064 mM) in anhydrous DCM (5 ml) was added corresponding acid (0.25 mM), DCC (0.25 mM), and DMAP (0.025 mM) in room temperature under N_2. The solution was stirred at room temperature for 4 h. TLC monitored the reaction. After starting material **2b** was consumed out, the solution was diluted to 30 mL, washed with 2 N HCl (30 mL × 3) and $NaHCO_3$ aqueous solution (30 mL × 3) successively. Then the combined organics were washed with 10 mL water and 10 mL brine successively, dried (anhydrous Na_2SO_4). The crude product after concentration was subject to column chromatography and eluted with petrol and acetone (9:1) to afford the pure corresponding compounds **3a**–**3l**.

4.3.1.1. (3*S*,20*S*,23*S*)-3-Acetoxyl-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3a). 92.3% Yield; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.25 (d, J = 7.1 Hz, 1H), 5.12 (m, 1H), 4.47 (m, 1H),

2.03 (s, 3H), 1.74 (s, 3H), 1.76 (s, 3H), 0.97 (s, 3H), 0.90 (s, 3H), 0.86 (s, 3H), 0.84 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.0 (s), 171.3 (s), 140.2 (s), 123.4 (d), 81.0 (d), 78.4 (s), 74.3 (d), 56.2 (d), 50.9 (d), 50.7 (s), 45.7 (s), 42.4 (d), 40.8 (t), 40.7 (s), 38.9 (t), 38.1 (s), 37.3 (s), 35.4 (s), 31.5 (t), 29.9 (t), 28.1 (q), 26.6 (t), 26.0 (q), 25.3 (t), 23.9 (t), 21.6 (q), 18.6 (q), 18.3 (t), 16.7 (q), 16.6 (q), 16.5 (q), 15.6 (q); HRMS calcd for $C_{32}H_{50}O_5$: 514.3658. Found: 514.3670.

4.3.1.2. (3*S*,20*S*,23*S*)-3- Propionyl-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3b). 89.3% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.25 (d, J = 7.1 Hz, 1H), 5.12 (m, 1H), 4.47 (m, 1H), 2.34 (q, J = 6.7 Hz, 2H), 1.74 (s, 3H), 1.76 (s, 3H), 1.17 (t, J = 6.7 Hz, 3H), 0.97 (s, 3H), 0.93 (s, 3H), 0.86 (s, 3H), 0.84 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.0 (s), 174.5 (s), 140.4 (s), 123.3 (d), 80.8 (d), 78.3 (s), 74.3 (d), 56.2 (d), 50.9 (d), 50.8 (s), 45.8 (d), 42.4 (d), 41.0 (t), 40.7 (s), 38.9 (t), 38.2 (s), 37.3 (s), 35.4 (t), 31.6 (t), 28.3 (t), 28.1 (q), 26.6 (t), 26.0 (q), 25.3 (t), 23.9 (t), 21.6 (t), 18.7 (q), 18.3 (t), 16.7 (q), 16.6 (q), 16.5 (q), 15.6 (q), 9.6 (q); HRMS calcd for $C_{33}H_{52}O_5$: 528.3815. Found: 528.3804.

4.3.1.3. (3*S*,20*S*,23*S*)-3-(2′-pentanoyl)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3c). 72.3% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.25 (d, J = 7.2 Hz, 1H), 5.11 (m, 1H), 4.47 (m, 1H), 1.75 (s, 3H), 1.73 (s, 3H), 0.98 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.76 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.1 (s), 173.9 (s), 140.1 (s), 123.4 (d), 80.7 (d), 78.4 (s), 74.3 (d), 56.1 (d), 50.9 (d), 50.7 (s), 45.6 (d), 42.4 (d), 40.7 (t), 40.7 (s), 38.8 (t), 38.0 (s), 37.2 (s), 35.3 (t), 34.7 (t), 31.5 (t), 28.1 (q), 27.4 (t), 26.6 (t), 26.0 (t), 25.3 (q), 23.8 (t), 22.5 (t), 21.5 (t), 18.6 (q), 18.3 (t), 16.7 (q), 16.6 (q), 16.5 (q), 15.6 (q), 13.9 (q); HRMS calcd for $C_{35}H_{56}O_5$: 556.4128. Found: 556.4119.

4.3.1.4. (3*S*,20*S*,23*S*)-3-Benzoyl-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3d). 87.3% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.03 (d, J = 7.5 Hz, 2H), 7.50 (m, 3H), 5.25 (d, J = 7.1 Hz, 1H), 5.12 (m, 1H), 4.47 (m, 1H), 1.74 (s, 3H), 1.76 (s, 3H), 1.01 (s, 6H), 0.87 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.0 (s), 166.5 (s), 140.4 (s), 132.9 (d), 131.2 (s), 129.7 (d), 128.5 (d), 123.4 (d), 81.7 (d), 78.3 (s), 74.3 (d), 56.2 (d), 50.9 (d), 50.8 (s), 45.8 (d), 42.4 (d), 41.0 (t), 40.7 (s), 38.9 (t), 38.5 (s), 37.3 (s), 35.4 (t), 31.6 (t), 31.1 (d), 29.9 (t), 28.3 (q), 26.6 (t), 26.0 (d), 25.3 (t), 23.9 (t), 21.6 (t), 18.6 (q), 18.3 (t), 17.0 (q), 16.6 (q), 16.5 (q), 15.7 (q); HRMS calcd for $C_{37}H_{52}O_5$: 576.3815. Found: 576.3792.

4.3.1.5. (3*S*,20*S*,23*S*)-3-(2′-pyridinylcarbonyloxy)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3e). 75.9% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.76 (d, J = 5.8 Hz, 1H), 8.04 (d, J = 7.5 Hz, 1H), 7.70 (t, J = 7.5 Hz, 1H), 7.43 (m, 1H), 5.25 (d, J = 7.2 Hz, 1H), 5.11 (m, 1H), 4.81 (m, 1H), 1.75 (s, 3H), 1.73 (s, 3H), 0.98 (s, 3H), 0.96 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.0 (s), 164.7 (s), 150.2 (d), 148.7 (s), 140.2 (s), 137.1 (d), 126.8 (d), 125.0 (d), 123.3 (d), 82.5 (d), 78.3 (s), 74.2 (s), 56.0 (d), 50.8 (d), 50.6 (s), 45.6 (d), 42.3 (d), 40.8 (t), 40.6 (s), 38.8 (t), 38.4 (s), 37.2 (s), 35.3 (t), 31.5 (t), 28.2 (q), 26.5 (t), 25.9 (q), 25.2 (t), 23.8 (t), 21.5 (t), 18.6 (q), 18.3 (t), 16.9 (q), 16.5 (q), 15.6 (q); HRMS calcd for $C_{36}H_{51}O_5N$: 577.3767. Found: 577.3760.

4.3.1.6. (3*S*,20*S*,23*S*)-3-(2′-Methoxybenzoyloxy)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3f). 79.1% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.80 (d, J = 7.8 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 6.95 (m, 2H), 5.26 (d, J = 7.1 Hz, 1H), 5.12 (m, 1H), 4.67 (m, 1H), 3.87 (s, 3H), 1.72 (s, 3H), 1.74 (s, 3H), 0.98 (s, 3H), 0.95 (s, 6H), 0.89 (s, 3H), 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.0 (s), 166.1 (s), 159.4 (s), 140.2 (s), 133.5 (d), 131.8 (d), 123.4 (d), 120.8 (s), 120.2 (d), 112.1 (d), 81.5 (d), 78.4 (s), 74.3 (s), 56.1 (d), 55.9 (q), 50.8 (d), 50.7 (s), 45.6 (d), 42.4 (d), 40.7 (t), 40.6 (s), 38.9 (t), 38.4 (s), 37.2 (s), 35.3 (t), 31.5 (t), 28.2 (q), 26.5 (t), 26.0 (q), 25.3 (t), 24.0 (t), 21.5 (t), 18.6 (q), 18.3 (t), 16.8 (q), 16.6 (q), 16.5 (q), 15.6 (q); HRMS calcd for $C_{38}H_{54}O_6$: 606.3920. Found: 606.3949.

4.3.1.7. (3*S*,20*S*,23*S*)-3-(3′-Methoxybenzoyloxy)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3g). 72.7% Yield 28.0 mg; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.61 (d, J = 7.2 Hz, 1H), 7.32 (t, J = 7.2 Hz, 1H), 7.54 (s, 1H), 7.06 (d, J = 7.2 Hz, 1H), 5.26 (d, J = 7.1 Hz, 1H), 5.12 (m, 1H), 4.67 (m, 1H), 3.83 (s, 3H), 1.72 (s, 3H), 1.74 (s, 3H), 0.98 (s, 6H), 0.89 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.0 (s), 166.3 (s), 159.6 (s), 140.1 (s), 132.4 (s), 129.5 (d), 123.4 (d), 122.0 (d), 119.2 (d), 114.3 (d), 81.8 (d), 78.4 (s), 74.3 (s), 56.1 (d), 55.5 (q), 50.8 (d), 50.6 (s), 45.6 (d), 42.4 (d), 40.6 (t), 38.9 (t), 38.5 (s), 37.3 (s), 35.3 (t), 31.5 (t), 28.3 (q), 26.5 (t), 25.9 (q), 25.2 (t), 23.8 (t), 21.5 (t), 18.6 (q), 18.3 (t), 16.9 (q), 16.5 (q), 16.5 (q), 15.6 (q); HRMS calcd for $C_{38}H_{54}O_6$: 606.3920. Found: 606.3901.

4.3.1.8. (3*S*,20*S*,23*S*)-3-(4′-Methoxybenzoyloxy)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3h). 75.3% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.98 (d, J = 7.3 Hz, 2H), 6.90 (d, J = 7.3 Hz, 2H), 5.26 (d, J = 7.1 Hz, 1H), 5.12 (m, 1H), 4.67 (m, 1H), 3.85 (s, 3H), 1.74 (s, 3H), 1.76 (s, 3H), 0.98 (s, 6H), 0.89 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.5 (s), 166.1 (s), 163.7 (s), 140.1 (s), 132.5 (s), 131.7 (d), 123.4 (d), 113.8 (d), 81.3 (d), 78.2 (s), 74.4 (s), 56.2 (d), 55.7 (q), 50.9 (d), 50.8 (s), 45.8 (d), 42.4 (d), 40.9 (t), 40.7 (s), 39.0 (t), 38.5 (s), 37.3 (s), 35.4 (t), 31.6 (t), 28.3 (q), 26.6 (t), 26.0 (q), 25.3 (t), 24.0 (t), 21.6 (t), 18.7 (q), 18.4 (t), 17.0 (q), 16.6 (q), 16.6 (q), 15.7 (q); HRMS calcd for $C_{38}H_{54}O_6$: 606.3920. Found: 606.3892.

4.3.1.9. (3*S*,20*S*,23*S*)-3-(2′-Nitrobenzoyloxy)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3i). 83.4% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.84 (d, J = 7.8 Hz, 1H), 7.73 (d, J = 7.8 Hz, 1H),7.62 (m, 2H), 5.26 (d, J = 7.1 Hz, 1H), 5.12 (m, 1H), 4.73 (m, 1H), 1.76 (s, 3H), 1.74 (s, 3H), 0.97 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.83 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.0 (s), 165.3 (s), 148.5 (s), 140.3 (s), 132.8 (s), 131.8 (d), 130.2 (d), 128.2 (d), 124.0 (d), 123.3 (d), 84.0 (d), 78.4 (s), 74.3 (s), 56.2 (d), 50.8 (d), 50.7 (s), 45.7 (d), 42.4 (d), 40.8 (t), 40.6 (s), 38.9 (t), 38.3 (s), 37.2 (s), 35.3 (t), 31.5 (t), 28.2 (q), 26.5 (t), 26.0 (q), 25.3 (t), 23.1 (t), 21.6 (t), 18.6 (q), 18.2 (t), 16.6 (q), 16.6 (q), 16.4 (q), 15.6 (q); HRMS calcd for $C_{37}H_{51}NO_7$: 621.3666. Found: 621.3659.

4.3.1.10. (3*S*,20*S*,23*S*)-3-(3′-Nitrobenzoyloxy)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3j). 81.0% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.82 (s, 1H), 8.40 (d, J = 7.8 Hz, 1H), 8.35 (d, J = 7.8 Hz, 1H), 7.64 (t, J = 7.8 Hz, 1H), 5.26 (d, J = 7.1 Hz, 1H), 5.12 (m, 1H), 4.76 (m, 1H), 1.76 (s, 3H), 1.74 (s, 3H), 1.01 (s, 3H), 0.96 (s, 3H), 0.83 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.1 (s), 164.3 (s), 148.4 (s), 140.2 (s), 135.4 (s), 132.8 (d), 129.8 (d), 127.4 (d), 124.6 (d), 123.4 (d), 83.0 (d), 78.4 (s), 74.4 (s), 56.1 (d), 50.8 (d), 50.6 (s), 45.6 (d), 42.4 (d), 40.8 (t), 40.6 (s), 38.8 (t), 38.6 (s), 37.2 (s), 35.3 (t), 31.5 (t), 28.3 (q), 26.5 (t), 26.0 (q), 25.7 (t), 23.1 (t), 21.5 (t), 18.6 (q), 18.3 (t), 16.9 (q), 16.5 (q), 16.5 (q), 15.6 (q); HRMS calcd for $C_{37}H_{51}NO_7$: 621.3666. Found: 621.3632.

4.3.1.11. (3*S*,20*S*,23*S*)-3-(4′-Nitrobenzoyloxy)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3k). 85.4% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.29 (d, J = 7.4 Hz, 1H), 8.17 (d, J = 7.4 Hz, 1H), 5.26 (d, J = 7.2 Hz, 1H), 5.12 (m, 1H), 4.76 (m, 1H), 1.76 (s, 3H), 1.74 (s, 3H), 1.05 (s, 3H), 1.00 (s, 3H), 0.91 (s, 9H);

^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.0 (s), 164.5 (s), 150.6 (s), 140.3 (s), 136.5 (s), 130.8 (d), 123.7 (d), 123.3 (d), 83.0 (d), 78.3 (s), 74.3 (s), 56.1 (d), 50.8 (d), 50.7 (s), 45.6 (d), 42.3 (d), 40.8 (t), 40.6 (s), 38.8 (t), 38.5 (s), 37.3 (s), 35.3 (t), 31.5 (t), 28.3 (q), 26.5 (t), 26.0 (q), 25.3 (t), 23.8 (t), 21.6 (t), 18.6 (q), 18.3 (t), 16.9 (q), 16.6 (q), 16.5 (q), 15.6 (q); HRMS calcd for $C_{37}H_{51}NO_7$: 621.3666. Found: 621.3684.

4.3.1.12. (3*S*,20*S*,23*S*)-3-(2′,4′-Dinitrobenzoyloxy)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (3l). 69.1% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.73 (s, 1H), 8.50 (d, J = 7.4 Hz, 1H), 7.93 (d, J = 7.4 Hz, 1H), 5.26 (d, J = 7.2 Hz, 1H), 5.12 (m, 1H), 4.76 (m, 1H), 1.76 (s, 3H), 1.74 (s, 3H), 0.97 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.87 (s, 3H), 0.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.0 (s), 163.7 (s), 149.0 (s), 148.3 (s), 140.2 (s), 133.5 (s), 131.5 (d), 127.6 (d), 123.4 (s), 119.7 (s), 85.3 (d), 78.4 (s), 74.3 (s), 56.2 (d), 50.8 (d), 50.6 (s), 45.6 (d), 42.4 (d), 40.6 (t), 38.8 (s), 38.3 (t), 37.2 (s), 35.3 (s), 34.0 (t), 31.5 (t), 28.2 (q), 25.9 (t), 25.7 (q), 25.2 (t), 23.1 (t), 21.6 (t), 18.6 (q), 18.2 (t), 16.6 (q), 16.4 (q), 15.6 (q); HRMS calcd for $C_{37}H_{50}O_9N_2$: 666.3516. Found: 666.3548.

4.3.2. General procedure for synthesis of 5a–5c

To a solution of **2b** (30 mg, 0.064 mM) in anhydrous DCM (5 mL) was added corresponding aldehyde acid (0.25 mM), DCC (0.25 mM), and DMAP (0.025 mM) in room temperature under N_2. The solution was stirred at room temperature for 4 h. TLC monitored the reaction. After starting materia **2b** was consumed out, the solution was diluted to 30 mL, washed with 2 N HCl (30 mL × 3), 10 mL water and 10 mL brine successively, dried (anhydrous Na_2SO_4). The organic layer was concentrated to give **4a**–**4c**, which was added directly to a solution of equal equivalent Jones reagent in 10 mL acetone in 0 °C. The solution was stirred at room temperature for 4 h. When TLC showed the material was consumed out, the solution was concentrated and partitioned between EtOAc (30 mL × 3) and 2 N HCl (30 mL × 3), washed with 30 mL $NaHCO_3$ aqueous, 10 mL of water and 10 mL of brine successively, and then dried (anhydrous Na_2SO_4). The crude product after concentration was subject to column chromatography and eluted with petrol and acetone (3:1) to afford the pure corresponding compounds **5a**–**5l**.

4.3.2.1. (3*S*,20*S*,23*S*)-3-(3′-Carboxybenzoyloxy)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (5a). 62.1% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.73 (s, 1H), 8.27 (m, 1H), 7.55 (t, J = 7.8 Hz, 1H), 5.25 (d, J = 7.2 Hz, 1H), 5.11 (m, 1H), 4.74 (m, 1H), 1.75 (s, 3H), 1.74 (s, 3H), 1.01 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.7 (s), 170.6 (s), 165.5 (s), 140.3 (s), 134.7 (d), 134.3 (d), 131.7 (s), 131.4 (d), 130.0 (s), 128.8 (s), 123.3 (d), 82.3 (d), 78.6 (s), 74.6 (d), 56.2 (d), 50.8 (d), 50.7 (s), 45.6 (d), 42.4 (d), 40.8 (t), 40.7 (s), 38.8 (t), 38.5 (s), 37.2 (s), 35.3 (t), 31.5 (t), 28.3 (q), 26.5 (t), 25.9 (q), 25.3 (t), 23.8 (t), 21.5 (t), 18.6 (q), 18.3 (t), 16.9 (q), 16.5 (q), 16.4 (q), 15.6 (q); HRMS calcd for $C_{38}H_{52}O_7$: 620.3713. Found: 620.3729.

4.3.2.2. (3*S*,20*S*,23*S*)-3-(4′-Carboxybenzoyloxy)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (5b). 52.9% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.15 (d, J = 8.1 Hz, 1H), 8.13 (d, J = 8.1 Hz, 1H), 5.25 (d, J = 7.2 Hz, 1H), 5.11 (m, 1H), 4.74 (m, 1H), 1.76 (s, 3H), 1.75 (s, 3H), 1.01 (s, 3H), 0.98 (s, 3H), 0.92 (s, 3H), 0.90 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 179.5 (s), 170.6 (s), 165.6 (s), 140.5 (s), 135.6 (d), 133.0 (d), 130.3 (d), 129.8 (d), 123.3 (d), 82.5 (d), 78.5 (s), 74.5 (s), 56.2 (d), 50.9 (d), 50.7 (s), 45.7 (d), 42.4 (d), 40.9 (t), 40.7 (s), 38.9 (t), 38.5 (s), 37.3 (s), 35.3 (t), 31.6 (t), 28.3 (q), 26.5 (t), 26.0 (q), 25.3 (t), 23.9 (t), 21.6 (t), 18.7 (q), 18.3 (t), 17.0 (q), 16.6 (q), 16.5 (q), 15.6 (q); HRMS calcd for $C_{38}H_{52}O_7$: 620.3713. Found: 620.3732.

4.3.2.3. (3*S*,20*S*,23*S*)-3-(3′-Nitro-4′-carboxybenzoyloxy)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (5c). 48.4% Yield; ^{1}H NMR ($CDCl_3$+CD_3OD, 300 MHz): δ 8.39 (s, 1H), 8.19 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 5.25 (d, J = 7.2 Hz, 1H), 5.11 (m, 1H), 4.72 (m, 1H), 1.76 (s, 3H), 1.75 (s, 3H), 1.01 (s, 6H), 0.92 (s, 3H), 0.90 (s, 6H); ^{13}C NMR ($CDCl_3$+CD_3OD, 75 MHz): δ 180.3 (s), 171.6 (s), 164.8 (s), 148.2 (s), 140.3 (s), 137.8 (s), 134.1 (d), 133.0 (s), 130.3 (d), 125.1 (d), 124.3 (d), 83.8 (d), 79.1 (s), 75.1 (d), 56.7 (d), 51.5 (d), 51.1 (s), 45.9 (d), 43.2 (d), 41.2 (t), 40.9 (s), 39.4 (t), 39.0 (s), 37.8 (s), 35.9 (t), 32.0 (t), 28.6 (q), 27.2 (t), 26.0 (q), 25.8 (t), 24.4 (t), 22.1 (t), 18.8 (q), 18.6 (t), 17.3 (q), 16.8 (q), 15.9 (q); HRMS calcd for $C_{38}H_{51}O_9N$: 665.3564. Found: 665.3543.

4.3.3. Synthesis of 6

To a solution of **2b** (5 g, 0.02 mM) in anhydrous DCM (50 mL) was added PDC 4 g in 0 °C under N_2. The solution was stirred in room temperature for 2 h. After TLC showed the starting material was consumed out, the solution was diluted by 300 mL ether and filtrated. The filtrate was collected and evaporated to give crude product 4.21 g, which was subjected to column chromatography and eluted with petrol and acetone (5.5:1) to afford the pure corresponding compound 3.52 g (70.9% yield).

4.3.3.1. (20*S*,23*S*)-Dihydroxydammar-24-en-21-oic acid-3-oxo-21,23-lactone (6). 70.7% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.25 (d, J = 7.8 Hz, 1H), 5.12 (m, 1H), 1.74 (s, 3H), 1.76 (s, 3H), 1.07 (s, 3H), 1.03 (s, 3H), 1.01 (s, 3H), 0.94 (s, 3H), 0.90 (s, 3H); HRMS calcd for $C_{30}H_{46}O_4$: 470.3396. Found: 470.3371.

4.3.4. General procedure for synthesis of 7a–7c

To a solution of **6** (70 mg, 0.15 mM), corresponding amine (0.44 mM) and 4 Å molecular sieves (30 mg) in 10 mL MeOH was added $NaBH_3CN$ (28 mg, 0.45 mM) in room temperature under N_2. The solution was stirred at room temperature for 8 h. The solution was evaporated to give crude product, which was subjected to column chromatography and eluted with petrol and acetone (4:1) to afford the pure corresponding product **7a–7c**.

4.3.4.1. (3*S*,20*S*,23*S*)-3-(4′-Methoxybenzylamino)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (7a). 25.6% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.26 (d, J = 7.2 Hz, 2H), 6.85 (d, J = 7.2 Hz, 2H), 5.25 (d, J = 7.2 Hz, 1H), 5.11 (m, 1H), 1.77 (s, 3H), 1.75 (s, 3H), 3.92 (d, J = 14.1 Hz, 1H), 3.79 (s, 3H), 3.60 (d, J = 14.1 Hz, 1H), 2.46 (m, 1H), 0.96 (s, 3H), 0.93 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.68 (s, 3H); HRMS calcd for $C_{38}H_{57}O_4N$: 591.4288. Found: 591.4292.

4.3.4.2. (3*S*,20*S*,23*S*)-3-(3′,5′-Difluorobenzylamino)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (7b). 19.2% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.89 (m, 2H), 6.62 (m, 1H), 5.25 (d, J = 7.2 Hz, 1H), 5.11 (m, 1H), 1.77 (s, 3H), 1.75 (s, 3H), 3.95 (d, J = 13.2 Hz, 1H), 3.62 (d, J = 13.2 Hz, 1H), 2.44 (m, 1H), 0.96 (s, 3H), 0.95 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.73 (s, 3H); E HRMS calcd for $C_{37}H_{53}O_3NF_2$: 597.3994. Found: 597.4017.

4.3.4.3. (3*S*,20*S*,23*S*)-3-(4′-Nitrobenzylamino)-20,23-dihydroxydammar-24-en-21-oic acid-21,23-lactone (7c). 34.5% Yield; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.12 (d, J = 7.2 Hz, 2H), 7.51 (d, J = 7.2 Hz, 2H), 5.25 (d, J = 7.2 Hz, 1H), 5.11 (m, 1H), 4.08 (d, J = 14.1 Hz, 1H), 3.62 (d, J = 14.1 Hz, 1H), 2.50 (m, 1H), 1.77 (s, 3H), 1.75 (s, 3H), 1.01 (s, 3H), 0.99 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.73 (s, 3H); HRMS calcd for $C_{37}H_{54}O_5N_2$: 606.4033. Found: 606.4001.

4.4. Biological assay

4.4.1. PTP1B and TCPTP biological assay

The enzymatic assays of PTP1B and TCPTP were referred.[19]

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Supplementary data

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