

Protein Tyrosine Phosphatase 1B Inhibitors from the Root Bark of *Pseudolarix amabilis* (Nelson) Rehd. (Pinaceae)

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(Received December 29, 2018; Revised April 21, 2019; Accepted April 21, 2019)

Abstract: *Pseudolarix amabilis* (Nelson) Rehd, is a monotypic genus plant belonging to the family Pinaceae. The root bark, known as “Tu-Jin-Pi” has been used for the treatment of skin diseases. During our activity screening, the water-soluble part of the 70% EtOH extract of *P. amabilis* bark showed excellent inhibitory bioactivities on PTP1B enzyme, leading to the phytochemical isolation of the root bark of *P. amabilis*. Three oleanane-type compounds (**1-3**) and seven phenolic compounds (**4-10**) were isolated, in which oleanolic acid 3-*O*- β -D-glucuronyl-6'-ethyl ester (**1**) was identified as a new saponin. The chemical structures of these compounds were elucidated by 1D/2D nuclear magnetic resonance and high resolution mass spectra. In addition, their pharmacological inhibitory bioactivities on PTP1B enzyme were evaluated, and the three oleanane-type compounds **1-3** exhibited inhibitory bioactivities with IC₅₀ values of 1.90 \pm 0.37, 19.15 \pm 0.10 and 10.44 \pm 0.59 μ M, respectively.

Keywords: *Pseudolarix amabilis*; PTP1B inhibitors; phenolics; oleanolic acid 3-*O*- β -D-glucuronyl-6'-ethyl ester. © 2019 ACG Publications. All rights reserved.

1. Introduction

Protein tyrosine phosphatase 1B (PTP1B) is a master regulator in the insulin and leptin metabolic pathways, which makes it an exciting drug target for type 2 diabetes and obesity [1-4]. In

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PTP1B inhibitors from *P. amabilis*

the insulin metabolic pathway, PTP1B could directly interact with the activated insulin receptor or insulin receptor substrate 1 to dephosphorylate phosphotyrosine residues, thus further reduce insulin sensitivity or shutting down signaling [4]. In the leptin metabolic pathway, PTP1B could negatively regulate leptin signaling through a direct and selective dephosphorylation of the two signaling molecules, JAK2 and STAT3 [5]. Moreover, recent studies indicate that PTP1B overexpression is involved in breast cancer, which suggests that selective PTP1B inhibition might be effective in breast cancer treatment [6-8].

Pseudolarix amabilis (Nelson) Rehd. as a monotypic genus plant, is mainly distributed in southern China. The root bark of *P. amabilis*, known as “Tu-Jin-Pi” in traditional Chinese medicine, has been widely used for treatment of skin diseases caused by fungal infections in China [9]. Previous phytochemical investigation on this plant revealed a series of characteristic pseudolaric acids [10], such as pseudolaric acids A-B, possessing cytotoxic [11,12], anti-angiogenic [13,14], anti-microbial [15], and anti-fertility activities [16]; and pseudoferic acids and pseudolarolides, such as pseudoferic acids A-C and pseudolarolide I, possessing 11 β -HSD1 (11 β -hydroxysteroid dehydrogenase type 1) inhibitory activities [17,18]. During the course of our search for PTP1B inhibitors from natural sources, *P. amabilis* was investigated. The water-soluble part of the 70% EtOH extract of *P. amabilis* showed excellent inhibitory bioactivities on PTP1B enzyme with IC₅₀ value of 0.52 \pm 0.15 μ g/mL, thereby, the aqueous constituents of *P. amabilis* were investigated and ten compounds (**1-10**) were obtained, in which, **1** was identified as a new compound and **2-10** were isolated from this plant for the first time. In addition, their bioactivities were tested and the oleanane-type compounds (**1-3**) exhibited PTP1B enzyme inhibitory activity.

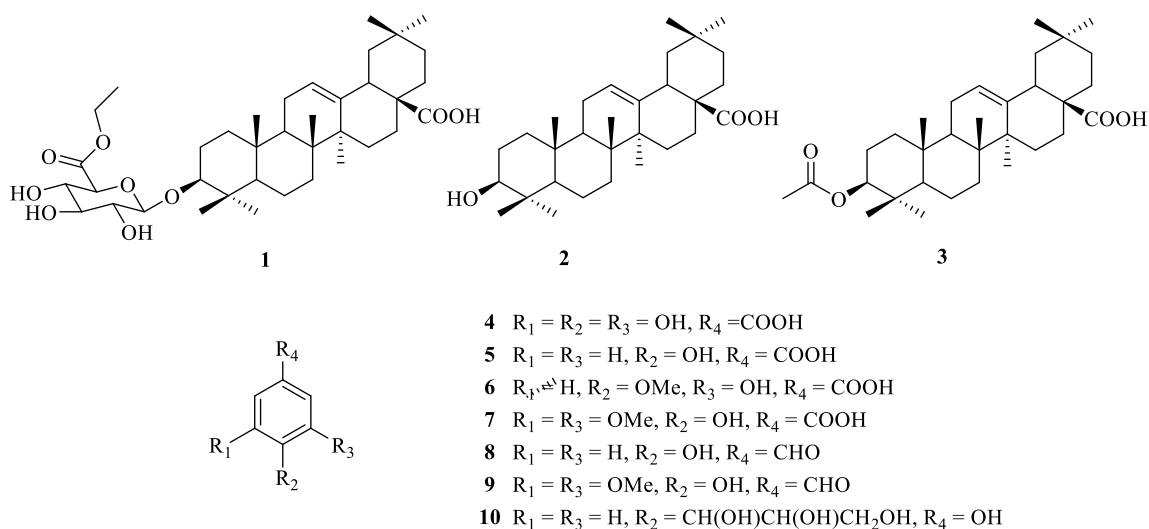


Figure 1. Chemical Structures of compounds **1-10**

2. Materials and Methods

2.1 General Procedure

The ESI-MS was performed on a Quattro Premier instrument (Waters, Miford, MA, USA). The HR-ESI-MS spectra was taken on an Agilent Technologies 6550 Q-TOF. 1D and 2D NMR spectra were recorded on a Bruker-AVANCE 500 instrument (Bruker, Rheinstetten, Germany) with TMS as an internal standard. HP20 gel purchased was from Sunresin New Materials Co. Ltd. (Xi'an, China). MCI gel was purchased from Mitsubishi Chemical Corporation (Tokyo, Japan). C-18 (40 –75 μ m) silica gel was purchased from SiliCycle Corporation (Quebec, Canada). Toyopearl HW-40F gel (30 – 60 μ m) was purchased from TOSOH Corporation (Tokyo, Janapa). Silica gel was purchased from Qingdao Haiyang Chemical Group Corporation (Qingdao, China).

2.2. Chemicals

Human recombinant protein tyrosine phosphates 1B (>70% estimated by SDS-PAG) was purchased from Biomol GmbH (Hamburg, Germany), 4-nitrophenyl phosphate disodium salt solution (p-NPP) and DL-dithiothreitol solution (DTT, 1 M in H₂O) ethylene diamine tetraacetic acid (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other Chemicals were of the highest grade available.

2.3. Plant Material

Pseudolarix amabilis (Nelson) Rehd. [also named *Larix amabilis* Nelson, *Pseudolarix kaempferi* (Lindl.) Gord. or *Larix kaempferi* (Lamb.) Carrière] was purchased on October in 2013 from Zhejiang province of China, and authenticated by Prof. Benxiang Hu. A voucher specimen (MPH 20131022) has been deposited in the Medicinal Plants Herbarium (MPH), Shaanxi University of Chinese Medicine, Xianyang, China.

2.4. Extraction and Isolation

The procedure of the extraction and isolation of the bark of *P. amabilis* (10 kg) was provided in supporting information.

2.5. Oleanolic acid 3-O-β-D-glucuronyl-6'-ethyl ester (**1**)

A white amorphous powder, $[\alpha]_D^{20} -10^\circ$ (c 0.19, MeOH); IR (in KBr) ν_{max} : 3430, 2975, 2942, 1699, 1634, 1384, 1204, 1048 cm; ¹H-NMR (500 MHz, MeOH-*d*₄): δ_H 5.15 (brs, H-12), 0.95 (s, H-23), 0.75 (s, H-24), 0.85 (s, H-25), 0.71 (s, H-26), 1.06 (s, H-27), 0.81 (s, H-29), 0.84 (s, H-30), 4.28 (d, *J* = 7.8 Hz, H-GlcA-1'), 4.13 (t, *J* = 7.1 Hz, CH₂CH₃), 1.19 (q, *J* = 7.1 Hz, CH₂CH₃) and ¹³C-NMR (125 MHz, MeOH-*d*₄): δ_C 40.0 (t, C-1), 27.3 (t, C-2), 91.4 (d, C-3), 40.5 (s, C-4), 57.3 (d, C-5), 19.6 (t, C-6), 34.3 (t, C-7), 40.9 (s, C-8), 49.0 (d, C-9), 38.2 (s, C-10), 24.8 (t, C-11), 124.0 (d, C-12), 145.5 (s, C-13), 43.2 (s, C-14), 29.2 (t, C-15), 24.4 (t, C-16), 47.9 (s, C-17), 43.1 (d, C-18), 47.6 (t, C-19), 32.0 (s, C-20), 35.2 (t, C-21), 34.1 (t, C-22), 28.8 (q, C-23), 17.2 (q, C-24), 16.2 (q, C-25), 18.0 (q, C-26), 26.7 (q, C-27), 182.1 (s, C-28), 33.9 (q, C-29), 24.3 (q, C-30), 107.4 (d, C-GlcA-1'), 75.6 (d, C-GlcA-2'), 77.9 (d, C-GlcA-3'), 73.5 (d, C-GlcA-4'), 77.0 (d, C-GlcA-5'), 171.2 (s, C-GlcA-6'), 62.7 (t, CH₂CH₃), 14.7 (q, CH₂CH₃); ESIMS: *m/z* 659.3 [M⁻ H]⁻, HRESIMS: *m/z* 659.4156 [M⁻ H]⁻ (calcd. for C₃₈H₅₉O₉, 659.4159). (see supporting information for spectra)

2.6. PTP1B Inhibition Assay

All the samples were first dissolved in DMSO and used for the experiment at concentrations of 100⁻¹ μg/mL for the crude extract or fractions and μM for pure compounds. The PTP1B enzyme inhibitory activity assay was performed as previously described [22]. p-NPP was used as a substrate to measure enzyme activity. PTP1B (0.05 — 0.1 μg) and 4 mM p-NPP in a buffer containing 50 mM citrate (pH 6.0), 1 mM DTT, 1 mM EDTA (ethylene diamine tetraacetic acid), and 0.1 M NaCl, were added to each of 96-wells having final volume of 100 μL, with or without test compounds. Incubation was done for 30 min at 37 °C and the reaction was terminated using 10 M NaOH. UV absorbance was measured at 405 nm to estimate the amount of produced p-nitrophenol. Nonenzymatic hydrolysis of p-NPP was corrected by measuring the absorbance increase at 405 nm in the absence of PTP1B enzyme. Each test was duplicated for three times, and the PTP1B inhibitory activity was calculated as: % inhibition = $[1 - (A_{test} - A_{blank}) / (A_{control} - A_{blank})] \times 100$, in which A_{control} is the absorbance of the control (DMSO) well, A_{test} is the absorbance of the test wells and A_{blank} is the absorbance of the enzyme-free wells.

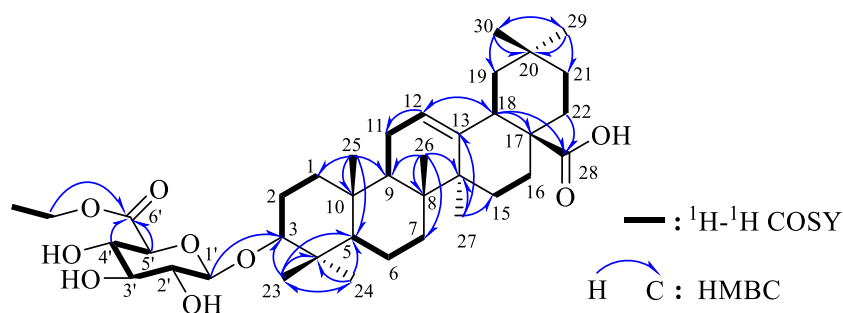
PTP1B inhibitors from *P. amabilis*

Figure 2. ^1H - ^1H COSY and key HMBC correlations of compound **1**

3. Results and Discussion

3.1. Chemistry

The bark of *P. amabilis* (10 kg) was extracted with 70% EtOH, evaporated to 1 L, filtrated and applied to a resin HP20 column. The water and 10% EtOH fractions were applied to column chromatography using MCI, HW-40 and ODS-C18 gel with a reversed phase system, afforded 10 compounds (Figure 1), including a new saponin (**1**), and nine known compounds (**2-10**), identified as oleanolic acid 3-*O*- β -D-glucuronyl-6'-ethyl ester (**1**), oleanolic acid (**2**) [19], 3 β -acetyloleanolic acid (**3**) [19], gallic acid (**4**), *p*-benzoic acid (**5**), isovanillic acid (**6**), syringic acid (**7**), *p*-hydroxyphenylglycerol (**8**) [20], *p*-hydroxybenzaldehyde (**9**) and syringic aldehyde (**10**), respectively, by comparing their spectroscopic (MS and NMR) data with Sadtler standard spectra or literature values. Besides, all these known compounds were reported in this plant for the first time.

Compound **1** was obtained as a white amorphous powder and showed positive colorations for Liebermann–Burchard and Molisch test, indicating **1** as a saponin glycoside. Its molecular formula was determined as $\text{C}_{38}\text{H}_{60}\text{O}_9$ by HRESIMS (m/z 659.4156 [$\text{M} - \text{H}$] $^-$, calcd 659.4159). The ^1H -NMR spectrum showed seven tertiary methyl protons at δ_{H} 1.06, 0.95, 0.85, 0.84, 0.81, 0.75 and 0.71; a group of ethyl protons at 4.13 (2H, t, $J = 7.1$ Hz, CH_2CH_3) and 1.19 (3H, q, $J = 7.1$ Hz, CH_2CH_3); an olefinic proton at 5.15 (1H, br.s); and an anomeric proton at δ_{H} 4.28 (1H, d, $J = 7.8$ Hz) indicating the present of a glycosyl as β configuration. The ^{13}C -NMR and DEPT spectra displayed 38 carbon resonances as $8 \times \text{CH}_3$, $11 \times \text{CH}_2$, $10 \times \text{CH}$ and $9 \times \text{C}$, 30 of which belonged to the aglycone carbons, while the remaining signals were assignable to a glucuronosyl moiety [δ_{C} 107.4, 75.6, 77.9, 73.5, 77.0 and 171.2] and an ethyl moiety [δ_{C} 62.7 (CH_2CH_3) and 14.7 (CH_2CH_3)]. Among the carbon resonances of the aglycone, δ_{C} 33.9, 28.8, 26.7, 24.3, 18.0, 17.2 and 16.2 were due to seven tertiary methyl; δ_{C} 124.0 and 145.5 were due to two olefinic carbons; δ_{C} 182.1 were due to a carbonyl carbon. These resonances along with the proton signals indicated that the aglycone of **1** possessed an oleanolic acid (**2**) skeleton.

Moreover, the assignments of the NMR signals were derived from ^1H - ^1H COSY, HSQC and HMBC experiments (Figure 2 and Table 1). In the HMBC spectrum, correlation of H-1' to C-3 disclosed that the glucuronosyl unit was linked at C-3 of the aglycone; correlation of the methane protons of CH_2CH_3 to the carbonyl carbon of glucuronosyl unit revealed that the ethyl unit was linked at C-6'. Therefore, compound **1** was elucidated as oleanolic acid 3-*O*- β -D-glucuronyl-6'-ethyl ester. Compared with the compound oleanolic acid 3-*O*- β -D-glucuronyl-6'-methyl ester (**1a**) of the reference [21], all data were almost consistent. In addition, for **1** was a glucuronide derivative and ethanol solvent was used during the extraction process, oleanolic acid 3-*O*- β -D-glucuronic acid was purchased and refluxed according to the extraction protocol of the plant materials and no target product was detected by TLC, so **1** was confirmed as a natural product in *P. amabilis*.

Table 1. ^1H and ^{13}C -NMR data for **1**, **1a** and **2**

No.	1		1a ^[21]		2	
	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{H}}^{\text{b}}$	$\delta_{\text{C}}^{\text{c}}$	$\delta_{\text{H}}^{\text{d}}$	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{H}}^{\text{b}}$
1	40.0 (t)	0.97 (H-1a, <i>ca.</i>) 1.60 (H-1b, <i>ca.</i>)	38.7		40.2	
2	27.3 (t)	1.65 (H-2a, <i>ca.</i>) 1.81 (H-2b, <i>ca.</i>)	26.5		28.2	
3	91.4 (d)	3.15 (dd, 11.7, 4.4)	89.1	3.38 (dd, 12, 4)	80.1	3.20 (dd, 11.7, 4.9)
4	40.5 (s)	—	39.5		40.8	
5	57.3 (d)	0.70 (d, 12.0)	55.8		57.1	
6	19.6 (t)	1.57 (H-6a, <i>ca.</i>) 1.40 (H-6b, <i>ca.</i>)	18.5		19.8	
7	34.3 (t)	1.30 (<i>ca.</i>)	33.2		34.4	
8	40.9 (s)	—	39.7		40.9	
9	49.0 (d)	1.58 (<i>ca.</i>)	48.0		49.5	
10	38.2 (s)	—	37.0		37.2	
11	24.8 (t)	1.89 (<i>ca.</i>)	23.8		24.4	
12	124.0 (d)	5.15 (br.s)	122.6	5.48 (t, 2.5)	124.0	5.25 (1H, br.s)
13	145.5 (s)	—	144.9		145.5	
14	43.2 (s)	—	42.0		43.5	
15	29.2 (t)	1.07 (H-15a, <i>ca.</i>) 1.78 (H-15b, <i>ca.</i>)	28.3		29.2	
16	24.4 (t)	1.60 (H-16a, <i>ca.</i>) 2.00 (H-16b, <i>ca.</i>)	23.7		24.8	
17	47.9 (s)	—	46.7		48.8	
18	43.1 (d)	2.82 (dd, 13.5, 4.2)	42.2	3.29 (dd, 10, 2)	43.1	2.80 (dd, 13.2, 4.2)
19	47.6 (t)	1.13 (H-19a, <i>ca.</i>) 1.58 (H-19b, <i>ca.</i>)	46.5		47.6	
20	32.0 (s)	—	31.0		30.7	
21	35.2 (t)	1.20 (H-21a, <i>ca.</i>) 1.41 (H-21b, <i>ca.</i>)	34.3		35.2	
22	34.1 (t)	1.54 (H-21a, <i>ca.</i>) 1.76 (H-21b, <i>ca.</i>)	33.3		34.1	
23	28.8 (q)	0.95 (s)	28.2	1.31 (s)	29.2	0.97 (s)
24	17.2 (q)	0.75 (s)	16.9	0.97 (s)	16.6	0.75 (s)
25	16.2 (q)	0.85 (s)	15.5	1.02 (s)	16.2	0.91 (s)
26	18.0 (q)	0.71 (s)	17.4	0.82 (s)	18.0	0.72 (s)
27	26.7 (q)	1.06 (s)	26.2	1.31 (s)	26.7	1.11 (s)
28	182.1 (s)	—	180.1		182.1	
29	33.9 (q)	0.81 (s)	33.2	0.96 (s)	33.8	0.88 (s)
30	24.3 (q)	0.84 (s)	23.8	0.99 (s)	24.3	0.89 (s)
GlcA-1'	107.4 (d)	4.28 (d, 7.8)	107.3	4.98 (d, 8)		
2'	75.6 (d)	3.24 (<i>ca.</i>)	75.4	4.07 (t, 8.5)		
3'	77.9 (d)	3.36 (<i>ca.</i>)	78.0	4.25 (t, 9)		
4'	73.5 (d)	3.53 (<i>ca.</i>)	73.1	4.46 (t, 9)		
5'	77.0 (d)	3.79 (<i>ca.</i>)	77.2	4.58 (d, 9.5)		
6'	171.2 (s)	—	170.8			
CH_2CH_3	62.7 (t)	4.13 (t, 7.1)				
CH_2CH_3	14.7 (q)	1.19 (q, 7.1)				
CH_3			52.0	3.73 (s)		

^a ^{13}C -NMR at 125 MHz, δ in MeOH-*d*₄, in ppm from TMS.

^b ^1H -NMR at 500 MHz, δ in MeOH-*d*₄, in ppm from TMS, coupling constants (*J*) in Hz are given in parentheses.

^c ^{13}C -NMR at 125 MHz, δ in pyridine-*d*₅.

^d ^1H -NMR at 500 MHz, δ in pyridine-*d*₅, in ppm from TMS, coupling constants (*J*) in Hz are given in parentheses.

3.2 Biological Assay

The inhibitory activity on PTP1B enzyme was evaluated using pNPP as a substrate, and oleanolic acid (**2**) as a known PTP1B inhibitor was used as a positive control[23]. In our early research for PTP1B inhibitors from natural sources, the extract of *P. amabilis* demonstrated potent activity with IC₅₀ value of 0.56 ± 0.06 $\mu\text{g/mL}$, furthermore, the water-soluble fraction of the extract showed stronger inhibitory activity than the precipitate fraction with IC₅₀ value of 0.52 ± 0.15 and 1.15 ± 0.06 $\mu\text{g/mL}$, respectively (Table 2). These results showed *P. amabilis* had a promising prospect of new application for the treatment of type 2 diabetes and led the isolation of the aqueous constituents of *P. amabilis*.

As shown in Table 3, the ten isolated compounds were tested on PTP1B enzyme, and only oleanane-type compounds (**1-3**) exhibited PTP1B inhibition with IC₅₀ values of 1.90 ± 0.37 , 19.15 ± 0.10 and 10.44 ± 0.59 μM , respectively. By comparison of the structure activity relationships (SARs), we found that oleanolic acid skeleton was the critical structural part for PTP1B inhibitory bioactivity. In addition, the substitute at C-3 of the oleanane-type compounds may alter their activity, especially, when it was β -D-glucuronyl-6'-ethyl ester, compound **1** showed strong PTP1B inhibitory bioactivity.

Moreover, for the activities of the isolated compounds are less than the active sites, it prompts that there maybe more potent active trace compounds in *P. amabilis*, which should be explored in future.

Table 2. Inhibitory activity of extracts against PTP1B

The bark of <i>P. amabilis</i>	IC ₅₀ ($\mu\text{g/mL}$)
70%EtOH extract	0.56 ± 0.06
The water-soluble fraction of 70 %EtOH extract	0.52 ± 0.15
The precipitate fraction of 70%EtOH extract	1.15 ± 0.06

Table 3. Inhibitory activity of compounds **1-10** against PTP1B

Compd.	Inhibition rate (% , at 20 $\mu\text{g/mL}$)	IC ₅₀ (μM)
1	93.49 ± 1.24	1.90 ± 0.37
2	50.70 ± 3.50	19.15 ± 0.10
3	86.47 ± 4.61	10.44 ± 0.59
4-10	NA	-

Acknowledgments

This project was financially supported by the National Natural Sciences Foundation of China (No. 81503237), the Natural Science Foundation of Shaanxi (grant No. 2016JQ8030), the Key R&D Program of Shaanxi Province (No. 2015SF073, 2017SF360), the Open Projects Program of the Key Laboratory of Tibetan Medicine Research, Chinese Academy of Sciences (No. 2014-TMR-01), the Innovative Research Team in TCM Material Foundation and Key Preparation Technology (No. 2012KCT-20), and the Innovation Projects of Science and Technology in Shaanxi Province (No. 2013KTCQ03-14)

Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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References

- [1] Z. Zhang, G. Dodd and T. Tiganis (2015). Protein tyrosine phosphatases in hypothalamic insulin and leptin signaling, *Trends. Pharmacol. Sci.* **36**, 661-674.
- [2] J. Bakke and F. Haj (2015). Protein-tyrosine phosphatase 1B substrates and metabolic regulation, *Semin. Cell. Dev. Biol.* **37**, 58-65.
- [3] E. Panzhinskiy, J. Ren and S. Nair (2013). Pharmacological inhibition of protein tyrosine phosphatase 1B: a promising strategy for the treatment of obesity and type 2 diabetes mellitus, *Curr. Med. Chem.* **20**, 2609-2625.
- [4] S. Zhang and Z. Zhang (2007). PTP1B as a drug target: recent developments in PTP1B inhibitor discovery, *Drug. Discov. Today.* **12**, 373-381.
- [5] I. Lund, J. Hansen, H. Andersen, N. Moller and N. Billestrup (2005). Mechanism of protein tyrosine phosphatase 1B-mediated inhibition of leptin signaling, *J. Mol. Endocrinol.* **34**, 339-351.
- [6] R. He, Z. Yu, R. Zhang and Z. Zhang (2014). Protein tyrosine phosphatases as potential therapeutic targets, *Acta. Pharmacol. Sin.* **35**, 1227-1246.
- [7] S. Yip, S. Saha and J. Chernoff (2010). PTP1B: a double agent in metabolism and oncogenesis, *Trends. Biochem. Sci.* **35**, 442-449.
- [8] N. Tonks and S. Muthuswamy (2007). A brake becomes an accelerator: PTP1B-a new therapeutic target for breast cancer, *Cancer Cell* **11**, 214-216.
- [9] S. Wu, R. Hu and G. Yang (1960). Experimental, clinical and pharmacological study on the antifungal effects of *Pseudolarix kaempferi* Gord., *Chin. J. Dermatol.* **8**, 18-25.
- [10] P. Chiu, L. Leung and B. Ko (2010). Pseudolaric acids: isolation, bioactivity and synthetic studies, *Nat. Prod. Rep.* **27**, 1066-1083.
- [11] G. Chen, Z. Li, D. Pan, C. Tang, X. He, G. Xu, K. Chen and K. Lee (1993). The isolation and structural elucidation of four novel triterpene lactones, pseudolarolides A, B, C, and D, from *Pseudolarix kaempferi*, *J. Nat. Prod.* **56**, 1114-1122.
- [12] P. Liu, H. Guo, W. Wang, J. Zhang, N. Li, J. Han, J. Zhou, Y. Hu, T. Zhang, Z. Liu and D. Guo (2007). Cytotoxic diterpenoids from the bark of *Pseudolarix kaempferi* and their structure-activity relationships, *J. Nat. Prod.* **70**, 533-537.
- [13] S. Yang, Y. Cai, B. Zhang, L. Tong, H. Xie, Y. Wu, L. Lin, J. Ding and J. Yue (2008). Structural modification of an angiogenesis inhibitor discovered from traditional Chinese medicine and a structure-activity relationship study, *J. Med. Chem.* **51**, 77-85.
- [14] Y. Tong, X. Zhang, M. Geng, J. Yue, X. Xin, F. Tian, X. Shen, L. Tong, M. Li, C. Zhang, W. Li, L. Lin, J. Ding (2006). Pseudolarix acid B, a new tubulin-binding agent, inhibits angiogenesis by interacting with a novel binding site on tubulin, *Mol. Pharmacol.* **69**, 1226-1233.
- [15] S. Yang, L. Dong, Y. Wang, Y. Wu and J. Yue (2003). Antifungal diterpenoids of *Pseudolarix kaempferi*, and their structure-activity relationship study, *Bioorg. Med. Chem.* **11**, 4577-4584.
- [16] Y. Zhang, R. Lu and A. Yan (1990). Inhibition of ova fertilizability by pseudolaric acid B in hamster, *Acta. Pharmacol. Sin.* **11**, 60-62.
- [17] X. Wu, J. He, Y. Shen, L. Dong, Z. Pan, G. Xu, X. Gong, L. Song, Y. Leng, Y. Li, L. Peng and Q. Zhao (2012). Pseudoferic acids A-C, three novel triterpenoids from the root bark of *Pseudolarix kaempferi*, *Tetrahedron. Lett.* **53**, 800-803.
- [18] H. Guo, S. Yao, X. Yang, Y. Chen, F. Gong, J. Tong, J. Qian, A. Zhang and X. Cai (2018). Oxidatively rearranged cycloartane triterpenoids from the seeds of *Pseudolarix amabilis*, *Nat. Prod. Res.* **32**, 1817-1823.
- [19] H. Zhou, F. Wang and Z. Fang (2011). Study on triterpenes from *Solanum torvum*, *China. J. Chin. Materia. Med.* **36**, 2096-2098.
- [20] J. Krauss and D. Unterreitmeier (2002). Synthesis and antimicrobial activity of hydroxyalkyl- and hydroxyacyl-phenols and their benzyl ethers, *Arch. Pharm.* **335**, 94-98.

PTP1B inhibitors from *P. amabilis*

- [21] F. Melek, T. Miyase, O. El-Gindi, S. Abdel-Khalik and M. Haggag (1996). Saponins from *Fagonia mollis*, *Phytochemistry* **42**, 1405-1407.
- [22] L. Cui, M. Na, H. Oh, E. Bae, D. Jeong, S. Ryu, S. Kim, B. Kim, W. Oh and J. Ahn (2006). Protein tyrosine phosphatase 1B inhibitors from *Morus* root bark, *Bioorg. Med. Chem. Lett.* **16**, 1426-1429.
- [23] C. Liu, T. Guo, L. Zhang, Y. Yue, P. Wang, F. Yang, X. Li (2013). Synthesis and biological evaluation of oleanolic acid derivatives as PTP1B inhibitors, *Eur. J. Med. Chem.* **63**, 511-522.

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