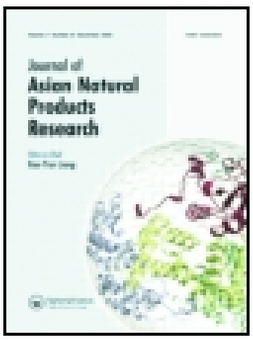

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Concise synthesis and PTP1B inhibitory activity of (*R*)- and (*S*)-dihydroresorcylide

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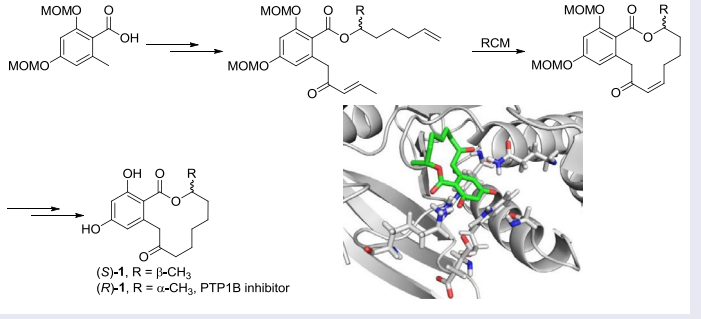
ABSTRACT

The present study was designed to develop a concise synthetic route for macrolide, with the purpose of confirming the absolute configuration of natural dihydroresorcylide (**1**) and making it more easily accessible for biological evaluation. The absolute configuration of C-3 in natural 1 was revised to be *R* by comparison of the rotation sign of synthetic (*R*)- and (*S*)-**1**. The synthetic (*R*)-**1** was found to be a novel highly specific PTP1B inhibitor with an IC_{50} value of 17.06 μM.



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Synthesis; dihydroresorcylide; PTP1B inhibitory activity; molecular docking

1. Introduction

Natural occurring macrolides usually have significant and diverse bioactivities, which have proved to be a prolific source of small-molecule chemical entities for developing clinical drugs [1]. Specifically, the macrolides possessing lactone core fused to resorcinol fragment produced by fungi usually exhibited a wide spectrum of biological properties, including antitumoral, antibacterial, antimalarial activities [2,3]. (*S*)-Dihydroresorcylide (**1**, Figure 1) was a phytotoxic resorcinol-fused twelve-membered macrolide isolated from the fermentation extracts of an endophyte *Acremonium zeae* by Poling *et al.* [4]. The first total synthesis

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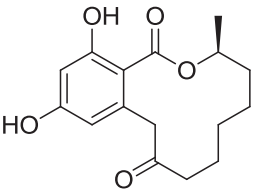


(*S*)-Dihydroresorcylide (**1**)

Figure 1. The structure of (*S*)-dihydroresorcylide (**1**).

of (*S*)-**1** was achieved in our group using the ring-closing metathesis (RCM) as a key step in 2013 [5]. However, the optical rotation sign of the synthetic (*S*)-**1** ($[\alpha]_D^{24}$ – 40.0 (*c* 0.8, MeOH)) was unexpectedly opposite to that of natural (*S*)-**1** ($[\alpha]_D^{25}$ + 15.0 (*c* 0.33, MeOH)), indicating that the absolute configuration of C-3 in natural **1** might be *R*, instead of *S*. This deduction was supported by the fact that the configuration of dihydroresprcylide biosynthesized in *Saccharomyces cerevisiae* was also *R* in C-3 ($[\alpha]_D$ + 23.7 (*c* 1.12, MeOH)) [6]. In order to confirm the absolute configuration of the natural **1**, (*R*)-**1** and (*S*)-**1** were synthesized via a concise synthetic route in the present work.

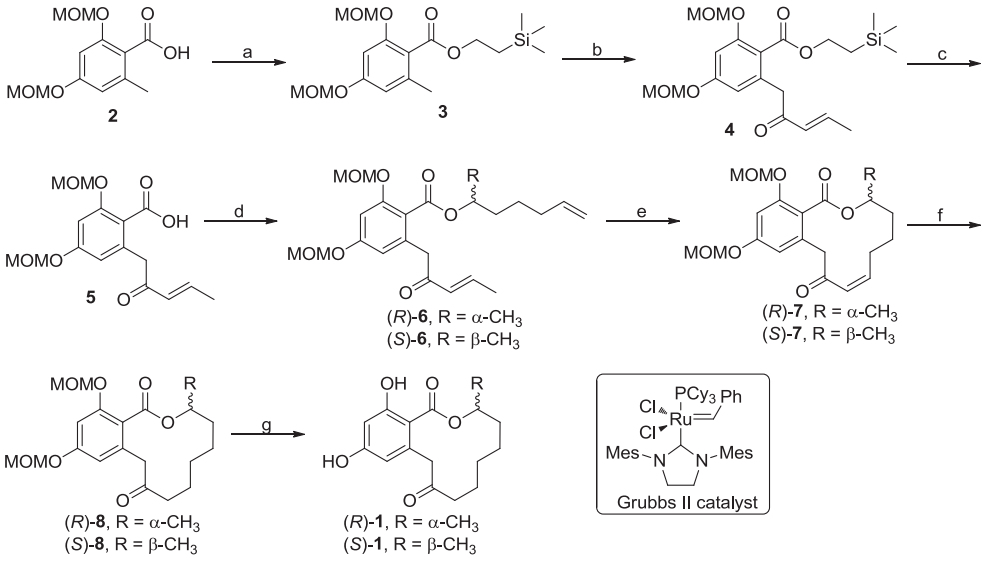
As a member of protein tyrosine phosphatases (PTPs), PTP1B plays a key role in the insulin-dependent signaling cascade. Selective PTP1B inhibitors are expected to have promising therapeutic effect on the treatment of type 2 diabetes and obesity [7]. In the past decades, numerous natural or synthetic drug-like PTP1B inhibitors have been developed [2,8]. However, the current PTP1B inhibitors usually have undesirable cell permeability and oral bioavailability due to the presence of highly negative charged polar pharmacophore in their structures [9]. Therefore, in our project for discovering novel anti-diabetes drug candidates [10–12], the low polar macrolides (*R*)- and (*S*)-**1** were evaluated for their inhibitory activity against PTP1B and homologous enzymes, including TCPTP, SHP-1, SHP-2 and LAR.

In this paper, we describe the synthesis and the revision of the absolute configuration of natural (*S*)-dihydroresorcylide, as well as selective PTP1B inhibition and molecular docking studies related to (*R*)- and (*S*)-**1**.

2. Results and discussion

2.1. Chemsitry

The synthesis of (*R*)-**1** was shown in Scheme 1. The synthetic protocol was similar with that of (*S*)-**1** reported by Zhang *et al.* [5], but it was modified using chloromethyl methyl ether (MOMCl) as the phenol protective reagent instead of methyl iodide (MeI). The MOM group could be more easily deprotected in a mild acid condition avoiding the lactone ring-opening [13]. Briefly, coupling the bis-MOM-protected acid **2** with 2-(trimethylsilyl) ethanol under Mitsunobu conditions afforded the corresponding ester **3** in 72% yield [14]. The carbonylation reaction between **3** and Weinreb amide ((*E*)-N-methoxy-N-methylbut-2-enamide) [5] proceeded smoothly in the presence of lithium diisopropylamide (LDA) at −78 °C to give the desired product **4** [5]. Saponification of **4** with tetrabutylammonium fluoride (TBAF) in THF overnight at room temperature produced acid **5**, which was converted to dienes **6** with *R* configuration in the chiral C-3 after reacting with (*S*)-hept-6-en-2-ol [15] under Mitsunobu conditions. The (*R*)-**6** was cyclized with the second generation



Scheme 1. Reagents and conditions for the synthesis of (*R*)-**1** and (*S*)-**1**: (a) DEAD, PPh_3, THF, 2-(trimethylsilyl) ethanol; LDA, THF, −78 °C, Weinreb amide; (c) TBAF, THF; (d) DEAD, PPh_3, THF, (*S*)-hept-6-en-2-ol for (*R*)-6, or (*R*)-hept-6-en-2-ol for (*S*)-6; (d) Grubbs II catalyst, CH_2Cl_2, reflux; (e) H_2, Pd/C, CH_3OH, r.t.; (g) 2.5% HCl in dioxane, r.t.

Grubbs catalyst [16] in refluxing CH_2Cl_2 to give the desired macrocyclic compound (*R*)-**7** possessing exclusive *trans* double bonds. Subsequent catalytic hydrogenation of the double bonds provided (*R*)-**8** in excellent yield. Finally, the target compound (*R*)-**1** was obtained from (*R*)-**8** after MOM-deprotection of hydroxyl group in HCl solution (2.5% in dioxane) at room temperature with 89% overall yield [17]. Later, the identical synthetic protocol was carried out successfully to prepare (*S*)-**1** starting from intermediate **5**.

The physical and spectral data of synthetic (*R*)-**1** and (*S*)-**1** were totally identical except for their optical rotation data, with $[\alpha]_D^{25}$ + 10.4 (*c* 0.5, MeOH) for (*R*)-**1** and $[\alpha]_D^{25}$ − 20.1 (*c* 0.5, MeOH) for (*S*)-**1**. Previously, the absolute configuration of C-3 in natural (*S*)-dihydroresorcylide had been deduced to be *R*, only based on a biogenetic consideration [4]. Herein, it was obvious that the optical rotation sign of the natural **1** ($[\alpha]_D^{25}$ + 15.0 (*c* 0.33, MeOH)) is identical with that of synthetic (*R*)-**1**. Therefore, with these data in hand it can be clearly confirmed that the configuration of C-3 in natural dihydroresorcylide should be *R*, instead of *S*.

2.2. PTP1B inhibition evaluation

The synthetic macrolides (*R*)-**1**, (*S*)-**1** and their precursors were tested for *in vitro* inhibitory activity against PTP1B, with oleanolic acid as the positive control [18]. The bioassay results are shown in Table 1. Interestingly, only the macrolides with the *R* configuration in C-3 showed inhibition on PTP1B, and their enantiomers did not exhibit obvious activity at the concentration of 100 μM. This result indicated that the configuration of C-3 in the lactone ring played a key role in their activity. It seems that this is the first report about PTP1B inhibitor possessing resorcylic macrolide skeleton according to the literature survey [19].

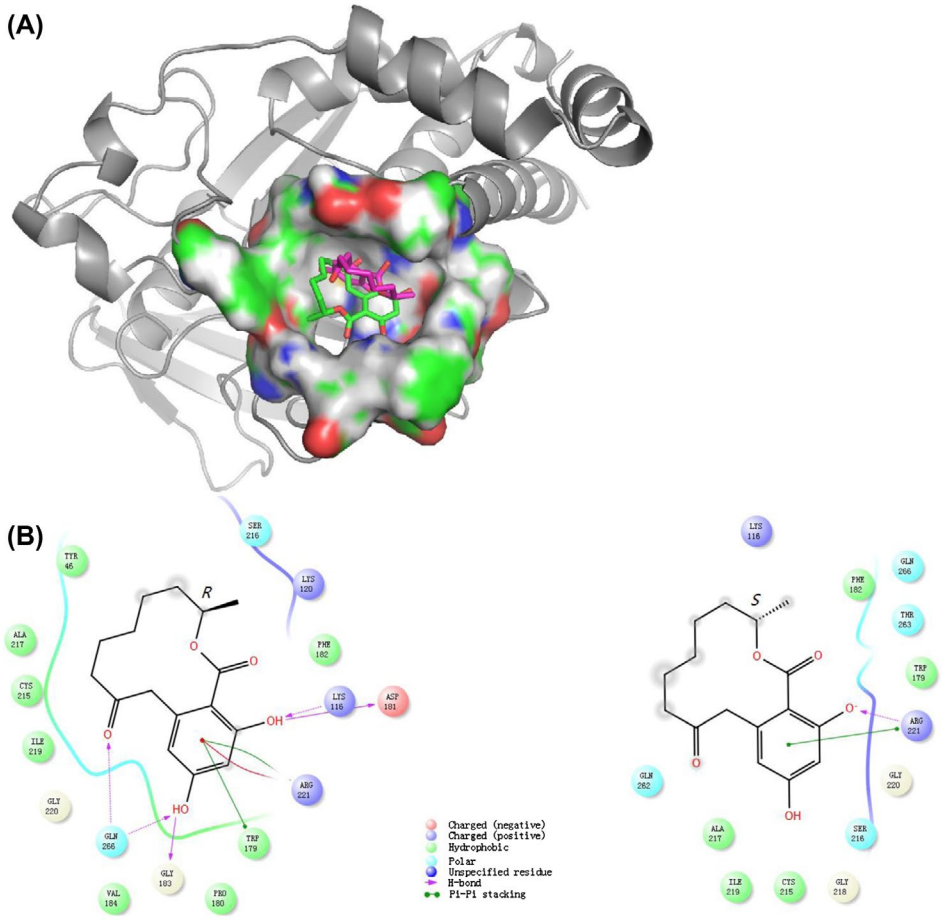


Figure 2. Docking results. (A) Docking mode of (*R*)-**1** (carbon in green) and (*S*)-**1** (carbon in purple) in the catalytic site of PTP1B; (B) the proposed interactions between macrolides and key amino acid residues.

In addition, the activity of (*R*)-**8** (IC_{50} = 53.15 ± 1.79 μM) was weaker than that of (*R*)-**1** (IC_{50} = 17.06 ± 2.04 μM), probably attributed to the steric hindrance of MOM groups in (*R*)-**8** decreasing its binding affinity to PTP1B. Subsequently, the bioactive (*R*)-**1** and (*R*)-**8** were subjected to bioassay for testing their selectivity toward PTPs. The result shown in Table 1 indicated that both compounds showed high PTP1B selectivity over other PTPs.

2.3. Molecular docking study

The (*R*)-**1** and (*S*)-**1** were selected to perform the molecular docking analysis using Glide 5.5 [20] to understand the inhibitory activity against PTP1B. Figure 2 displayed the proposed binding mode with the interactions between macrolides and key amino acid residues. Although both compounds positioned themselves in the active-site pocket of PTP1B (Figure 2(A)), (*R*)-**1** had more interactions with amino acid residues in the active site than (*S*)-**1** (Figure 2(B)). For example, (*R*)-**1** could form two kinds of essential interactions with the key amino acids, including hydrogen-bond interactions with Lys116, Asp181, Gly183 and

Table 1. Inhibitory activity of **1** and **8** against PTP1B and other PTPs presented as IC_{50} (μM).

Compound	PTP1B	TCPTP	SHP-1	SHP-2	LAR
(*R*)-**8**	53.15 ± 1.79	NA[a]	NA	NA	NA
(*S*)-**8**	NA	ND[b]	ND	ND	ND
(*R*)-**1**	17.06 ± 2.04	NA	NA	NA	NA
(*S*)-**1**	NA	ND	ND	ND	ND
Oleanolic acid	3.01 ± 0.37	ND	ND	ND	ND

[a]Inactive, precentage inhibition is less than 20% at 100 μM.
[b]NA, not tested.

Gln266, and Pi-Pi stacking interactions with key Arg221 in the catalytic A-site and Trp179 in B-site. In addition, this molecule could generate more hydrophobic interactions with surrounding residues. All of these interactions would positively contribute to the affinity of binding (*R*)-**1** to the active site of PTP1B.

3. Conclusion

In the present study, a concise synthetic route for dihydroresorcylides was achieved in seven steps with 34% overall yield. By comparison of their optical rotation data, the configuration of C-3 in natural (*S*)-dihydroresorcylide was revised to be *R*. In addition, the synthetic (*R*)-**1** was found to be a novel selective PTP1B inhibitor. To the best of our knowledge, this is the first example of macrolide with resorcinol fragment reported as PTP1B inhibitor. The preliminary bioassay and docking study indicated the configuration of C-3 in lactone ring played a key role in interaction between macrolides and the catalytic domain of PTP1B. The further structural modification and activity study are in progress.

4. Experimental

4.1. General experimental procedures

Optical rotations were recorded on a Jasco P-1030 polarimeter (JASCO Inc., Tokyo, Japan). ^{1}H, ^{13}C NMR spectra were recorded on a DRX-400 (^{1}H 400 MHz and ^{13}C 125 MHz) (Bruker, Karlsruhe, Germany) spectrometer (IS as TMS). Low- and high-resolution mass spectra (LRMS and HRMS) were given with electric, electrospray (ESI) produced by Finnigan MAT-95 (ThermoFisher Scientific Inc., Massachusetts, American) and Agilent 1100 LC-MS spectrometer (Agilent Technologies Inc., California, American). All reagents purchased from commercial sources were used without further purification. The compounds synthesized were purified by column chromatography (CC) on commercial silica gel (SiO_2, 200–300 mesh, Qingdao Haiyang Chemical Group Co., Qingdao, China).

4.2. Preparation of the compounds

4.2.1. 2-(Trimethylsilyl)ethyl 2,4-bis(methoxymethoxy)-6-methylbenzoate (3)

To a mixture of **2** (8.0 g, 31.3 mmol), PPh_3 (12.3 g, 46.9 mmol), and 2-(trimethylsilyl)ethanol (5.5 g, 46.9 mmol) in diethyl ether (80 ml) and toluene (40 ml) was slowly added DEAD (8.2 g, 46.9 mmol) at 0 °C, and stirred for 1.5 h at room temperature. Then, the solution was concentrated and the obtained residue was purified by silica gel column chromatography

with petroleum ether/ethyl acetate (v:v = 15:1) to give **3** as a yellow oil (9.1 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.66 (s, 1H), 6.54 (s, 1H), 5.14 (s, 2H), 5.13 (s, 2H), 4.41-4.35 (m, 2H), 3.46 (s, 3H), 3.45 (s, 3H), 2.29 (s, 3H), 1.13-1.07 (m, 2H), 0.06 (s, 9H); ESI-MS *m/z*: 357 $[M + H]^+$.

4.2.2. (E)-2-(Trimethylsilyl)ethyl 2,4-bis(methoxymethoxy)-6-(2-oxopent-3-en-1-yl) benzoate (4)

To a solution of **3** (6.0 g, 16.8 mmol) in dry THF (150 ml) was slowly added LDA (2.0 M, 16.8 ml, 33.7 mmol) at −78 °C. After stirring for 30 min, (*E*)-N-methoxy-N-methylbut-2-enamide (Weinreb amide, 2.6 g, 20.2 mmol) was added, and the reaction mixture was stirred for another 2.5 h. The reaction was quenched by addition of saturated NH_4Cl aqueous solution (60 ml), and then it was warmed to room temperature. The reaction mixture was concentrated and extracted with EtOAc (100 ml × 3). The combined organic phase was dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography with petroleum ether/ethyl acetate (v:v = 15:1) to give **4** as a colorless oil (5.7 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90–6.92 (m, 1H), 6.75 (s, 1H), 6.51 (s, 1H), 6.17 (d, *J* = 15.7 Hz, 1H), 5.14 (s, 2H), 5.13 (s, 2H), 4.31 (t, *J* = 7.5 Hz, 2H), 3.82 (s, 2H), 3.47 (s, 3H), 3.44 (s, 3H), 1.87 (d, *J* = 6.8 Hz, 3H), 1.20 (t, *J* = 7.0 Hz, 2H), 0.04 (s, 9H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 196.2, 167.8, 159.0, 156.3, 143.7, 135.3, 130.9, 119.0, 111.6, 102.8, 95.1, 94.4, 63.5, 56.4, 56.3, 45.6, 18.4, 17.5; ESI-MS: *m/z* 425 $[M + H]^+$.

4.2.3. (E)-2,4-Bis(methoxymethoxy)-6-(2-oxopent-3-en-1-yl)benzoic acid (5)

To a solution of **4** (4.0 g, 9.4 mmol) in THF (40 ml) was added TBAF (1 M in THF, 28.4 ml, 28.4 mmol) at room temperature. The resulting mixture was stirred overnight, and then it was hydrolyzed with saturated aqueous NH_4Cl and extracted with EtOAc (50 ml × 3). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by flash chromatography with petroleum ether/ethyl acetate (v:v = 3:1) to give **5** as a white solid (2.7 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97–6.99 (m, 1H), 6.86 (d, *J* = 2.3 Hz, 1H), 6.60 (d, *J* = 2.3 Hz, 1H), 6.25 (dd, *J* = 1.2, 15.3 Hz, 1H), 5.31 (s, 2H), 5.18 (s, 2H), 4.16 (s, 2H), 3.53 (s, 3H), 3.46 (s, 3H), 1.92 (dd, *J* = 1.6, 6.8 Hz, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 197.5, 169.5, 159.0, 156.0, 144.1, 135.4, 130.6, 118.8, 111.6, 102.1, 94.9,94.0, 55.2, 55.0, 44.5, 17.0; ESI-MS: *m/z* 325 $[M + H]^+$.

4.2.4. (R,E)-Hept-6-en-2-yl 2,4-bis(methoxymethoxy)-6-(2-oxopent-3-en-1-yl) benzoate ((R)-6)

To a mixture of **5** (1.0 g, 3.1 mmol), triphenylphosphine (PPh_3, 1.2 g, 4.6 mmol) and (*S*)-6-hepten-2-ol (0.5 g, 4.6 mmol) in diethyl ether (20 ml) and toluene (5 ml) was slowly added DEAD (0.7 ml, 4.6 mmol) at 0 °C and stirred for 1.5 h. The solution was evaporated. The residue was purified by silica gel column chromatography with petroleum ether/ethyl acetate (v:v = 15:1) to give (*R*)-**6** as a colorless oil (1.1 g, 86%). $[\alpha]_D^{24}$ − 2.3 (*c* 0.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91–6.93 (m, 1H), 6.77 (d, *J* = 2.1 Hz, 1H), 6.52 (d, *J* = 2.1 Hz, 1H), 6.18 (dd, *J* = 1.5, 4.4 Hz, 1H), 5.80 (m, 1H), 5.14 (s, 2H), 5.14 (s, 2H), 5.00 (d, *J* = 4.4 Hz, 1H), 4.96 (s, 1H), 3.85 (q, *J* = 8.4 Hz, 2H), 3.46 (s, 3H), 3.45 (s, 3H), 2.05–2.07 (m, 2H), 1.88 (dd, *J* = 1.4, 7.0 Hz, 3H), 1.54–1.58 (m, 5H), 1.28 (d, *J* = 6.3 Hz, 3H); ESI-MS: *m/z* 421 $[M + H]^+$.

4.2.5. (S,E)-Hept-6-en-2-yl 2,4-bis(methoxymethoxy)-6-(2-oxopent-3-en-1-yl) benzoate ((S)-6)

The synthetic procedure for (*S*)-**6** was identical with that of (*R*)-**6** described above, expected for using (*R*)-6-hepten-2-ol as one of reactants, instead of (*S*)-6-hepten-2-ol. (*S*)-**6**: 1.1 g, yield 85%, as a colorless oil. $[\alpha]_D^{24}$ + 18.2 (*c* 0.3, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91–6.93 (m, 1H), 6.77 (d, *J* = 2.1 Hz, 1H), 6.52 (d, *J* = 2.1 Hz, 1H), 6.18 (dd, *J* = 1.5, 4.4 Hz, 1H), 5.79–5.81 (m, 1H), 5.14 (s, 2H), 5.14 (s, 2H), 5.00 (d, *J* = 4.4 Hz, 1H), 4.96 (s, 1H), 3.85 (q, *J* = 8.4 Hz, 2H), 3.46 (s, 3H), 3.45 (s, 3H), 2.05–2.07 (m, 2H), 1.88 (dd, *J* = 1.4, 7.0 Hz, 3H), 1.54–1.58 (m, 5H), 1.28 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 196.1, 167.5, 159.0, 156.1, 143.7, 138.6, 135.1, 130.9, 119.1, 114.9, 111.3, 102.5, 94.8, 94.4, 72.0, 56.4, 56.3, 45.4, 35.6, 33.7, 24.7, 20.2, 18.5; ESI-MS: *m/z* 421 $[M + H]^+$.

4.2.6. (R,Z)-12,14-Bis(methoxymethoxy)-3-methyl-3,4,5,6-tetrahydro-1H-benzo[c][1] oxacyclododecine-1,9(10H)-dione ((R)-7)

A mixture of diene (*R*)-**6** (500 mg, 1.19 mmol) and the Grubbs II catalyst (50.52 mg, 0.06 mmol) in CH_2Cl_2 (400 ml) was heated to reflux for 2 h. The solution was concentrated and filtered through a silica pad to give the crude product which was further purified by silica gel column chromatography with petroleum ether/ethyl acetate (v:v = 20:1) to give compound (*R*)-**7** (360 mg, 80%) as a colorless oil. $[\alpha]_D^{24}$ − 8.0 (*c* 0.2, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.87–6.89 (m, 1H), 6.72 (dd, *J* = 1.6, 13.5 Hz, 2H), 5.95 (d, *J* = 16.2 Hz, 1H), 5.18–5.20 (m, 1H), 5.13 (s, 2H), 5.12 (s, 2H), 4.56 (d, *J* = 12.4 Hz, 1H), 3.45 (s, 3H), 3.45 (s, 3H), 3.29 (d, *J* = 12.4 Hz, 1H), 2.26–2.28 (m, 2H), 1.75–1.77 (m, 1H), 1.37 (d, *J* = 6.3 Hz, 3H); ESI-MS: *m/z* 379 $[M + H]^+$.

4.2.7. (S,Z)-12,14-Bis(methoxymethoxy)-3-methyl-3,4,5,6-tetrahydro-1H-benzo[c][1] oxacyclododecine-1,9(10H)-dione ((S)-7)

The synthetic procedure for (*S*)-**7** was identical with that of (*R*)-**7** described above. (*S*)-**7**: 377 mg, 84%, as a colorless oil. $[\alpha]_D^{24}$ + 14.1 (*c* 0.3, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.87–6.89 (m, 1H), 6.72 (dd, *J* = 1.6, 13.5 Hz, 2H), 5.95 (d, *J* = 16.2 Hz, 1H), 5.18–5.20 (m, 1H), 5.13 (s, 2H), 5.12 (s, 2H), 4.56 (d, *J* = 12.4 Hz, 1H), 3.45 (s, 3H), 3.45 (s, 3H), 3.29 (d, *J* = 12.4 Hz, 1H), 2.26–2.28 (m, 2H), 1.75–1.77 (m, 1H), 1.37 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 198.9, 168.6, 159.0, 155.8, 150.0, 134.7, 131.0, 119.2, 111.4, 102.4, 94.9, 94.5, 72.4, 56.4, 56.4, 56.4, 42.9, 34.2, 31.8, 24.7, 20.7; ESI-MS: *m/z* 379 $[M + H]^+$; ESI-HRMS: *m/z* 401.1583 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_7Na$, 401.1576).

4.2.8. (R)-Bis(methoxymethoxy)resorcylide ((R)-8)

A suspension of (*R*)-**7** (300 mg, 0.79 mmol) and Pd/C (30 mg) in CH_3OH (10 ml) was stirred under H_2 over 4 h. The catalyst was filtered off and the solvent was evaporated to give the crude product which was purified by silica gel column chromatography with petroleum ether/ethyl acetate (v:v = 1:1) to give (*R*)-**8** (285 mg, yield 95%) as a colorless oil. $[\alpha]_D^{24}$ + 6.3 (*c* 0.2, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75–6.77 (m, 1H), 6.51–5.52 (m, 1H), 5.25–5.28 (m, 1H), 5.15 (s, 4H), 4.12 (d, *J* = 18.2 Hz, 1H), 3.58 (d, *J* = 18.2 Hz, 1H), 3.47 (d, *J* = 1.1 Hz, 3H), 3.46 (d, *J* = 1.1 Hz, 3H), 2.43 (t, *J* = 6.3 Hz, 2H), 1.75–1.77 (m, 2H), 1.53–1.55 (m, 2H), 1.38–1.40 (m, 2H), 1.29 (dd, *J* = 0.9, 6.4 Hz, 3H), 0.84–0.86 (m, 2H); ESI-MS: *m/z* 381 $[M + H]^+$.

4.2.9. (S)-Bis(methoxymethoxy)resorcylide ((S)-8)

The synthetic procedure for (*S*)-**8** was identical with that of (*R*)-**8** described above. (*S*)-**8**: 276 mg, yield 92%, as a colorless oil. $[\alpha]_D^{24}$ − 12.9 (*c* 0.3, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75–6.77 (m, 1H), 6.51–6.53 (m, 1H), 5.25–5.27 (m, 1H), 5.15 (s, 4H), 4.12 (d, *J* = 18.2 Hz, 1H), 3.58 (d, *J* = 18.2 Hz, 1H), 3.47 (d, *J* = 1.1 Hz, 3H), 3.46 (d, *J* = 1.1 Hz, 3H), 2.43 (t, *J* = 6.3 Hz, 2H), 1.75–1.77 (m, 2H), 1.53–1.55 (m, 2H), 1.38–1.40 (m, 2H), 1.29 (dd, *J* = 0.9, 6.4 Hz, 3H), 0.84–0.86 (m, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 208.1, 167.7, 158.7, 155.6, 133.8, 119.7, 112.0, 102.6, 94.9, 94.5, 72.2, 56.4, 56.3, 47.5, 41.5, 33.0, 27.2, 22.8, 22.6, 20.3; ESI-MS: *m/z* 381 $[M + H]^+$; ESI-HRMS: *m/z* 403.1725 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_7Na$, 403.1733).

4.2.10. (R)-Dihydroresorcylide ((R)-1)

The mixture of (*R*)-**8** (200 mg, 0.53 mmol) and 5 ml HCl solution (2.5% in dioxane) was stirred at room temperature for 4 h, and then extracted with EtOAc (5 ml × 3). The combined organic phases were washed with sat. $NaHCO_3$ aqueous solution and water, and then dried over anhydrous $MgSO_4$ and filtered. The organic phase was concentrated under reduced pressure to afford the crude product, which was purified by flash column chromatography with petroleum ether/ethyl acetate (v:v = 2:1) to give target molecule (*R*)-**1** (136 mg, 89%) as a white solid. $[\alpha]_D^{24}$ + 10.4 (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 6.25 (d, *J* = 2.3 Hz, 1H), 6.11 (d, *J* = 2.3 Hz, 1H), 5.11–5.13 (m, 1H), 4.70 (d, *J* = 18.8 Hz, 1H), 3.79 (d, *J* = 18.8 Hz, 1H), 2.69 (dd, *J* = 16.4, 9.9 Hz, 1H), 2.33 (dd, *J* = 16.4, 9.9 Hz, 1H), 2.01–2.03 (m, 1H), 1.80–1.82 (m, 1H), 1.63–1.65 (m, 2H), 1.50–1.53 (m, 4H), 1.29 (d, *J* = 6.2 Hz, 3H); ESI-MS: *m/z* 293 $[M+H]^+$.

4.2.11. (S)-Dihydroresorcylide ((S)-1)

The synthetic procedure for (*S*)-**1** was identical with that of (*R*)-**1** described above. (*S*)-**1**: 139 mg, 90%, as a white solid. $[\alpha]_D^{24}$ − 40.8 (*c* 0.8, MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 6.25 (d, *J* = 2.3 Hz, 1H), 6.11 (d, *J* = 2.3 Hz, 1H), 5.11–5.13 (m, 1H), 4.70 (d, *J* = 18.8 Hz, 1H), 3.79 (d, *J* = 18.8 Hz, 1H), 2.69 (dd, *J* = 16.4, 9.9 Hz, 1H), 2.33 (dd, *J* = 16.4, 9.9 Hz, 1H), 2.01–2.03 (m, 1H), 1.80–1.82 (m, 1H), 1.63–1.64 (m, 2H), 1.50–1.53 (m, 4H), 1.29 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 211.7, 172.4, 166.6, 163.8, 140.2, 113.7, 106.6, 102.9, 74.5, 51.7, 42.5, 32.7, 28.2, 22.2, 22.1, 19.4; ESI-MS: *m/z* 293 $[M+H]^+$; ESI-HRMS: *m/z* 315.1212 $[M+Na]^+$ (calcd for $C_{16}H_{20}O_5Na$, 315.1208).

4.3. Biological activity assays

Recombinant human PTP1B catalytic domain was expressed and purified according to procedures described previously [15]. Enzymatic activity of PTP1B was determined at 30 °C by monitoring the hydrolysis of pNPP. Dephosphorylation of pNPP generates product pNP, which can be monitored at 405 nm. The assays were carried out in a final volume of 100 µl containing 50 mmol/L MOPS, pH 6.5, 2 mmol/L pNPP, 30 nmol/L GST-PTP1B, and 2% DMSO; the catalysis of pNPP was continuously monitored on a SpectraMax 340 microplate reader at 405 nm for 2 min at 30 °C. The IC_{50} value was calculated from the nonlinear curve fitting of percent inhibition [inhibition (%)] vs. the inhibitor concentration [*I*] using the following equation: inhibition (%) = $100/\{1+(IC_{50}/[I])k\}$, where k is the Hill coefficient. To study the inhibition selectivity on other PTP family members, human TCPTP, SHP1, SHP2

and LARD1 were prepared and assays were performed according to procedures described previously [21].

4.4. Molecular docking

The LigPrep [22] panel was used to produce multiple output structures of macrolides by generating different protonation states, stereochemistry, tautomers, and ring conformations for molecular docking. The Protein Preparation Wizard Workflow was used to prepare the protein structures. Residues located within 20 Å around 989 on PTP1B (PDB: 1nl9, [23]) were defined as binding sites in which the docking grids were created. The default settings were used. Both macrolides were docked into the defined binding site using extra precision (XP) mode without any constraint.

Disclosure statement

No potential conflict of interest was reported by the authors.

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