Protein tyrosine phosphatase 1B inhibitory polybromobiphenyl ethers and monocyclofarnesol-type sesquiterpenes from the Indonesian marine sponge Lamellodysidea cf. herbacea

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Protein tyrosine phosphatase 1B inhibitory polybromobiphenyl ethers and monocyclofarnesol-type sesquiterpenes from the Indonesian marine sponge *Lamellodysidea* cf. *herbacea*

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ABSTRACT

Three known polybromobiphenyl ether derivatives, 2-(2′,4′-dibromophenoxy)-3,5-dibromophenol (**1**), 2-(2′,4′-dibromophenoxy)-4,6-dibromophenol (**2**), and 2-(2′-dibromophenoxy)-3,4,5,6-tetrabromophenol (**3**), were identified as PTP1B inhibitors from the Indonesian marine sponge *Lamellodysidea* sp. (cf. *L. herbacea*) together with two new monocyclofarnesol-derived sesquiterpenes, lamellolactones A (**4**) and B (**5**). The structures of **4** and **5** were elucidated based on the spectroscopic data and comparisons with those for related compounds. Compounds **1**–**3** inhibited PTP1B activity with IC_{50} values of 5.3, 7.8, and 5.3 μM, respectively, while compounds **4** and **5** were not active at 38–40 μM. The selective activities of **1**–**3** against PTP1B over the other PTPs (T-cell PTP, CD45 tyrosine phosphatase, and *vaccinia* H-1-related phosphatase) showed that the position and/or number of Br atoms affected their inhibitory activities.

1. Introduction

Marine invertebrates are an important resource for the discovery of bioactive natural products. Chemical investigations on marine sponges have been prosperous, leading to the isolation of various metabolites possessing unique structural and potent biological properties (Blunt et al., 2017; Faulkner, 2002). The genus *Lamellodysidea* was recently reclassified as a new genus from the genus *Dysidea*, one of the most productive marine sponges (Cook and Bergquist, 2002; Mehbub et al., 2016). Several chemical constituents, such as polyhydroxysterols (Sauleau and Bourguet-Kondracki, 2005), polychlorinated pyrrolidinones (Sauleau et al., 2005), dysinosins B–D (Carroll et al., 2004), and polyhalogenated diphenyl ethers (Mehbub et al., 2016), have thus far been reported from *Lamellodysidea herbacea* and *Lamellodysidea chlorea*.

During the search for new protein tyrosine phosphatase (PTP) 1B inhibitors from marine organisms, we found that the EtOH extract of the Indonesian marine sponge *Lamellodysidea* sp. (cf. *L. herbacea*) moderately inhibited PTP1B activity in an enzyme assay. PTP1B plays an important role as a negative regulator in the insulin and leptin signal pathways (Zhang and Zhang, 2007; Barr, 2010; Zhang et al., 2015), and, therefore, its inhibitors will be potential drug candidates for the treatment and prevention of type-2 diabetes and obesity (Jiang et al., 2012; Wang et al., 2015). The bioassay-guided separation of this extract resulted in the identification of three known polybromobiphenyl ethers: 2-(2′,4′-dibromophenoxy)-3,5-dibromophenol (**1**) (Carte and Faulkner, 1981), 2-(2′,4′-dibromophenoxy)-4,6-dibromophenol (**2**) (Carte and Faulkner, 1981), and 2-(2′-dibromophenoxy)-3,4,5,6-tetrabromophenol (**3**) (Salva and Faulkner, 1990), as active compounds together with two new monocyclofarnesol-type sesquiterpenes, lamellolactones A and B (**4** and **5**) (Fig. 1). We herein describe the isolation, structural elucidation, and biological activities of compounds **1**–**5**.

2. Results and discussion

The EtOH extract of the marine sponge *L.* cf. *herbacea*, collected in the coral reefs of North Sulawesi, Indonesia in 2013, exhibited

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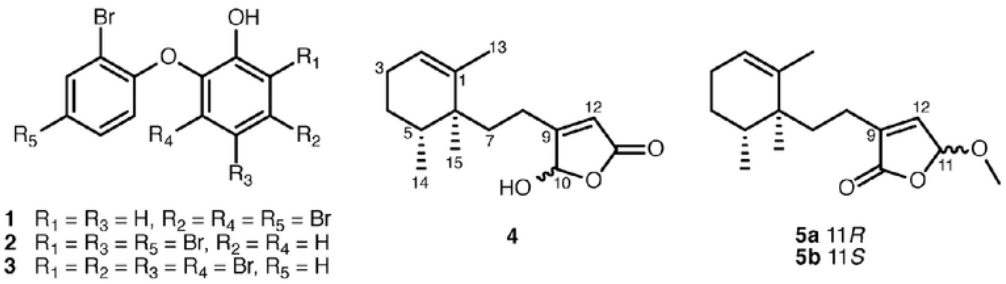

Fig. 1. Structures of **1**–**5** isolated from the Indonesian marine sponge *Lamellodysidea* cf. *herbacea*.

moderate inhibitory activity (ca. 50% at 50 μg/mL) against PTP1B in the screening assay. The extract was purified by repeated HPLC to obtain compounds **1** (3.3 mg), **2** (2.0 mg), **3** (11.0 mg), **4** (7.4 mg), and **5** (4.1 mg).

The structures of **1**–**3** were identified as 2-(2′,4′-dibromophenoxy)-3,5-dibromophenol, 2-(2′,4′-dibromophenoxy)-4,6-dibromophenol, and 2-(2′-dibromophenoxy)-3,4,5,6-tetrabromophenol, respectively, in analyses of their spectroscopic data and comparisons with the reported values in previous studies (Carte and Faulkner, 1981; Salva and Faulkner, 1990).

The molecular formula of lamellolactone A (**4**) was assigned as $C_{15}H_{22}O_3$ from HREIMS (*m/z* 250.1571 $[M]^+$, Δ + 0.2 mmu) and NMR data (Table 1). The ^1H and ^{13}C NMR spectra of **4** (in CD_3OD) indicated 21 proton and 15 carbon signals, which were classified into three methyls, four sp^3 methylenes, one sp^3 methine, one sp^3 oxygenated methine, one sp^3 quaternary carbon, two sp^2 methines, two sp^2 quaternary carbons, and one carbonyl carbon from the analysis of DEPT and HMQC data (Table 1). The ^1H–^1H COSY spectrum of **4** revealed three partial structures I–III, as shown by the bold lines in Fig. 2. The partial structure A, containing I and II, was established as the 1,2,6-trimethyl-1-ethyl-cyclohex-2-ene moiety by HMBC correlations from H-2 (δ_H 5.49) to C-6 (δ_C 41.6), from H_3-13 (1.62) to C-1 (139.8), C-2 (126.2), and C-6, from H_3-14 (0.9[illegible] to C-6, and from H_3-15 (0.93) to C-1, C-5 (34.6), C-6, and C-7 (34.2). The presence of an α,β-unsaturated-γ-lactone ring was assigned from the ^{13}C NMR signal at δ_C 173.7 and IR absorption at 1750 cm^{-1}, and the remaining HMBC data proposed two possible partial structures B and B', as shown in Fig. 2. The partial structure B' was excluded by comparing the ^1H chemical shifts (δ_H 6.00 and 5.88) at the C-10 (C-11') and C-12 (C-12') positions of **4** with those of the related compounds possessing the partial structures B (δ_H 6.00 and 5.84) (Paul and Fenical, 1982) and B' (δ_H 6.06 and 6.80) (Hahn et al., 2014). Thus, the planar structure of **4** was elucidated as shown in Fig. 2.

The relative configuration at 14-CH_3 and 15-CH_3 was assigned as *cis* according to the analogy with the model compounds (Gaspar et al., 2005; Huang et al., 2008): the ^{13}C chemical shift at 14-CH_3 was δ 15.7–16.0 ppm in the *cis* and *trans* isomers, while the ^{13}C chemical shift at 15-CH_3 shifted to a higher field in the *cis* configuration (20.9–21.1 ppm) than in the *trans* configuration (26.3–26.6 ppm). Since slight splits in the ^{13}C signals at C-11 and C-12 of **4** were detected, compound **4** may be a mixture of C-10 epimers. However, it was not possible to separate these epimers by chiral HPLC.

The ^1H and ^{13}C NMR data (Table 1) and physico-chemical properties of lamellolactone B (**5**) resembled those of **4**, indicating that they share a similar skeletal structure. The molecular formula of **5**, $C_{16}H_{24}O_3$, deduced by HREIMS (*m/z* 264.1726 $[M]^+$, Δ + 0.1 mmu) was CH_2 units (14 mu) larger than that of **4**, and an extra OCH_3 signal was observed at δ_H 3.51 (δ_C 57.1) in the ^1H and ^{13}C NMR spectra of **5**. The ^1H–^1H COSY and HMBC spectra of **5** subsequently established the 1,2,6-trimethyl-1-ethyl-cyclohex-2-ene and γ-methoxy-α,β-unsaturated-γ-lactone moieties (Fig. 3). ^1H NMR data for **5** were compared with those for related compounds (Dumdei et al., 1997; Venkateswarlu et al., 1998) and revealed that the lactone moiety of compound **5** was different from that of **4**, namely, **5** had the B' type partial structure in Fig. 2. HMBC correlations from H-8 (1.95 and 2.23) to C-10 (173.4) confirmed the assignment, and the planar structure of **5** was elucidated as shown in Fig. 3.

Compound **5** was observed in the EtOH extract of the sponge analyzed by HPLC with EtOH and CH_3CN containing 0.05% TFA. Moreover, a corresponding hydroxy derivative of **5** was not detected in the EtOH extract. Therefore, compound **5** appears to be the natural product.

The relative configuration of **5** at 14-CH_3 and 15-CH_3 was assigned as *cis* by comparing ^{13}C NMR data for **5** with those for the model compounds (Gaspar et al., 2005; Huang et al., 2008). Compound **5** also showed slight splits in the ^{13}C signal at C-12 and was separated into two peaks [lamellolactones B1 (**5a**) and B2 (**5b**)] with a 1:1 ratio by chiral HPLC. The specific rotations of **5a** ($[\alpha]_D^{25}$ − 7.0) and **5b** ($[\alpha]_D^{25}$ + 10.1) revealed the opposite signs. Consequently, compounds **5a** and **5b** will be the epimers of each other at the C-11 position.

In order to confirm the absolute configurations of **5a** and **5b**, the ECD spectra of two isomers, (5*R*,6*S*,11*R*)- and (5*R*,6*S*,11*S*)-**5**, were calculated and compared with the experimental ECD spectra of **5a** and **5b**. The experimental ECD spectra of **5a** and **5b** showed the same cotton effects with the calculated ECD spectra of the 5*R*,6*S*,11*R*-isomer and 5*R*,6*S*,11*S*-isomer, respectively (Fig. 4). Thus, the absolute structures of **5a** and **5b** were elucidated as shown in Fig. 1.

The inhibitory effects of **1**–**5** on PTP1B activity were evaluated using a previously described method (Yamazaki et al., 2013). The selective activities of **1**–**5** against PTP1B over the other PTP families, T-cell PTP (TCPTP) as one of the non-transmembrane PTPs, CD45 tyrosine phosphatase (CD45) as one of the receptor-like PTPs, and *vaccinia* H-1-related phosphatase (VHR) as one of the dual-specificity

Table 1
^1H and ^{13}C NMR data for compounds **4** and **5** in CD_3OD.

No.	4		5	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
1	139.8		140.1	
2	126.2	5.49, br s	126.0	5.47, br s
3	26.5	1.96, m	26.6	1.76, m
				2.20, m
4	28.1	1.49, m	28.1	1.48, m
5	34.6	1.73, m	34.6	1.76, m
6	41.6		41.6	
7	34.2	1.71, m	35.1	1.67, m
8	23.7	2.12, m	21.2	1.95, m
		2.43, m		2.23, m
9	139.8		139.7	
10	101.0	6.00, br s	173.4	
11	173.7		104.5	5.82, br s
12	117.4	5.88, s	144.0	6.96, br s
13	19.3	1.62, s	19.3	1.63, s
14	16.2	0.90, d (6.8)	16.2	0.90, d (8.0)
15	21.2	0.93, s	21.2	0.90, s
16			57.1	3.51, s

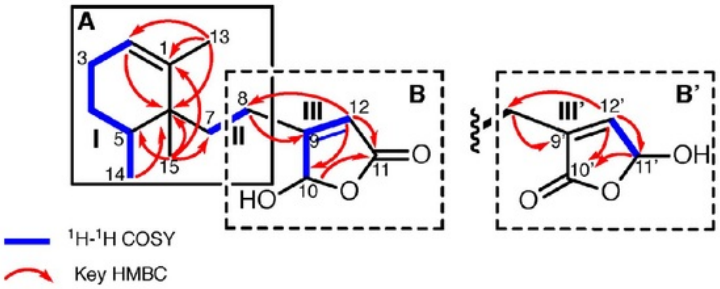

Fig. 2. ^{1}H–^{1}H COSY and key HMBC correlations for **4**.

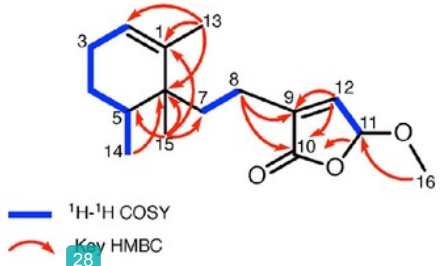

Fig. 3. ^{1}H–^{1}H COSY and key HMBC correlations for **5**.

phosphatases, were evaluated by an enzyme assay (Abdjul et al., 2017) because more than 100 members of PTPs regulate various cell functions. The IC_{50} values of **1–5** and oleanolic acid, a positive control (Zhang et al., 2008), are listed in Table 2.

Compounds **1** and **3** had similar IC_{50} values against PTP1B, CD45, and VHR, while the inhibitory activities of **1** and **3** against TCPTP were weaker than those against the other PTPs (Table 2). Compound **2** exhibited PTP1B and CD45 inhibitory activities of similar potencies, whereas the inhibitory effects of **2** on TCPTP and VHR were modest (Table 2). Therefore, the position and/or number of bromine atoms may affect selective activity against PTPs. The two new sesquiterpenes, **1** and **2** were not active up to 38–40 μM against the four PTPs (Table 2).

Table 2
Protein tyrosine phosphatase inhibitory activities of compounds **1–5**.

Compound	Protein tyrosine phosphatases (IC_{50}, μM)			
	PTP1B	TCPTP	CD45	VHR
1	5.3	13	6.0	7.9
2	7.8	28% inhibition at 17 μM	8.9	20
3	5.3	8.6	7.2	4.6
4	> 40	> 40	> 40	> 40
5	> 38	> 38	> 38	> 38
Oleanolic acid[a]	1.0	0.8	0.9	4.5

[a] Positive control for the protein tyrosine phosphatase assay (Zhang et al., 2008).

3. Experimental

3.1. General experimental procedure

EIMS was performed using a JMS-MS 700 mass spectrometer (JEOL, Tokyo, Japan). ^{1}H and ^{13}C NMR spectra were recorded on a JNM-AL-400 NMR spectrometer (JEOL) at 400 MHz for ^{1}H and 100 MHz for ^{13}C in CD_3OD (δ_H 3.30, δ_C 49.0) and $CDCl_3$ (δ_H 7.24, δ_C 77.0). Optical rotations were measured with a JASCO P-2300 digital polarimeter (JASCO, Ltd., Tokyo, Japan). UV spectra were obtained on a Hitachi U-3310 UV–vis spectrophotometer (Hitachi, Ltd., Tokyo, Japan) and IR spectra on a PerkinElmer Spectrum One Fourier transform infrared spectrometer (Waltham, MA, USA). ECD spectra were measured with a JASCO J-720 spectrometer. Preparative HPLC was performed using the L-6200 system (Hitachi Ltd.).

3.2. Materials

Human recombinant PTP1B, CD45, and VHR were purchased from Enzo Life Sciences (Farmingdale, NY, USA). Human recombinant TCPTP was purchased from R&D Systems (Minneapolis, MN, USA). *p*-Nitrophenyl phosphate (*p*NPP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Oleanolic acid was purchased from Tokyo Chemical Industry (Tokyo, Japan). Plastic plates (96-well) were purchased from Corning Inc. (Corning, NY, USA). All other chemicals including organic solvents were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

3.3. Isolation of compounds 1–5

The marine sponge was collected by scuba diving at Manado, North Sulawesi, Indonesia, in December 2013 and identified as *Lamellodysidea* sp. (cf. *L. herbacea*) by Dr. Kazunari Ogawa (Z. Nakai Laboratory). A voucher specimen is deposited at the Faculty of Mathematic and Natural Sciences, Sam Ratulangi University, as 13–12–14 = 2–204.

The sponge (138.6 g, wet weight) was cut into small pieces and extracted three times with EtOH (1.5 L) on the boat immediately after its collection. The EtOH extract (338.6 mg) was subjected to

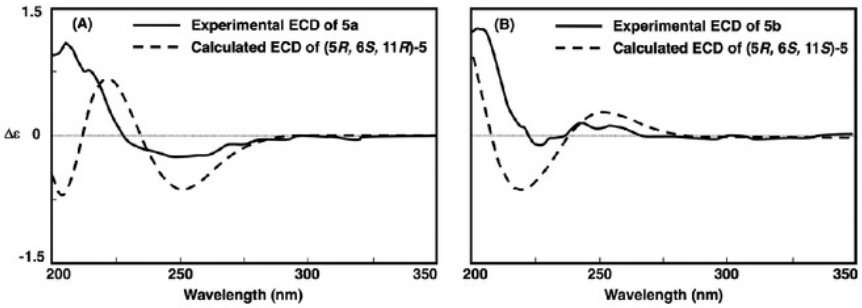

Fig. 4. Experimental (solid line) and calculated (dashed line) ECD spectra of compounds **5a** (A) and **5b** (B).

preparative HPLC [column, PEGASIL ODS (Senshu Sci. Co., Ltd., Tokyo, Japan), i.d. 10 mm × 250 mm; solvent, CH_3OH:H_2O (75:25, v/v) containing 0.05% TFA; flow rate, 2.0 mL/min; detection, UV 210 nm] to give compound **3** (11.0 mg) and six fractions (Frs. 1–6). Compound **4** (7.4 mg) was isolated from Fr. 2 (51.1 mg) by repeated HPLC [column, PEGASIL ODS, i.d. 10 mm × 250 mm; solvent, CH_3OH:H_2O (68:32, v/v) containing 0.05% TFA; flow rate, 2.0 mL/min; detection, UV 210 nm]. Fr. 4 (31.5 mg) was purified by preparative HPLC [column, PEGASIL ODS, i.d. 10 mm × 250 mm; solvent, CH_3OH:H_2O (65:35, v/v) containing 0.05% TFA; flow rate, 2.0 mL/min; detection, UV 210 nm] to afford compound **5** (4.1 mg). Compounds **1** (3.3 mg) and **2** (2.0 mg) were obtained from Fr. 6 (10.6 mg) by HPLC [column, PEGASIL ODS, i.d. 10 mm × 250 mm; solvent, CH_3OH:H_2O (65:35, v/v) containing 0.05% TFA; flow rate, 2.0 mL/min; detection, UV 210 nm].

3.3.1. 2-(2′,4′-Dibromophenoxy)-3,5-dibromophenol (1)

Yellow oils; EIMS *m/z* 505/503/501/499/497 $[M]^+$ (1:3:5:3:1); ^1H NMR ($CDCl_3$) δ 7.76 (1H, d, J = 2.4 Hz), 7.32 (1H, d, J = 2.4 Hz), 7.27 (1H, dd, J = 8.8, 2.4 Hz), 7.19 (1H, d, J = 2.4 Hz), 6.41 (1H, d, J = 8.8 Hz).

3.3.2. 2-(2′,4′-Dibromophenoxy)-4,6-dibromophenol (2)

Yellow oils; EIMS *m/z* 505/503/501/499/497 $[M]^+$ (1:3:5:3:1); ^1H NMR ($CDCl_3$) δ 7.79 (1H, d, J = 2.4 Hz), 7.43 (1H, dd, J = 8.8, 2.4 Hz), 7.41 (1H, d, J = 2.4 Hz), 6.88 (1H, d, J = 8.8 Hz), 6.78 (1H, d, J = 2.4 Hz).

3.3.3. 2-(2′-Bromophenoxy)-3,4,5,6-tetrabromophenol (3)

Yellow oils; EIMS *m/z* 586/584/582/580/578/576 $[M]^+$ (1:3:3:1:1:3:3:1); ^1H NMR ($CDCl_3$) δ 7.63 (1H, dd, J = 7.8, 2.4 Hz), 7.17 (1H, ddd, J = 7.8, 7.8, 1.6 Hz), 6.97 (1H, ddd, J = 7.8, 7.8, 2.4 Hz), 6.97 (1H, dd, J = 7.8, 1.6 Hz).

3.3.4. Lamellolactone 6 (4)

Colorless solids; $[\alpha]_D^{25}$ + 3.7 (*c* 0.10, CH_3OH); IR (KBr) ν_{max} 3423, 2930, 1750, 1638, 1454, 1050 cm^{-1}; UV (CH_3OH) λ_{max} nm (log ε) 202 (4.2); EIMS *m/z* 250 $[M]^+$; HREIMS *m/z* 250.1571 ($[M]^+$, calcd for $C_{15}H_{22}O_3$, 250.1569); ^1H and ^{13}C NMR (CD_3OD) spectroscopic data, see Table 1.

3.3.5. Lamellolactone B (5)

Colorless solids; $[\alpha]_D^{25}$ + 5.3 (*c* 0.10, CH_3OH); IR (KBr) ν_{max} 3450, 2812, 1700, 1600, 1426, 1035 cm^{-1}; UV (CH_3OH) λ_{max} nm (log ε) 202 (4.3); EIMS *m/z* 264 $[M]^+$; HREIMS *m/z* 264.1726 ($[M]^+$, calcd for $C_{16}H_{24}O_3$, 264.1725); ^1H and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 1.

3.4. Chiral separation of 5

The racemic mixture **5** was purified by chiral HPLC using the following conditions [column, CHIRAL-PAK AS-RH (DAISEL, Tokyo, Japan), i.d. 4.6 × 150 mm; mobile phase, 65% CH_3OH containing 0.05% TFA; detection, UV 210 nm; flow rate, 0.8 mL/min] to yield **5a** (t_R = 35.4 min) and **5b** (t_R = 41.6 min).

3.4.1. Lamellolactone B1 (5a)

Colorless solids; $[\alpha]_D^{25}$ − 7.0 (*c* 0.10, CH_3OH); ECD (3.8 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 251 (−0.3), 205 (+1.1) nm; EIMS *m/z* 264 $[M]^+$; ^1H NMR (CD_3OD) δ 6.96 (1H, brs, H-10), 5.82 (1H, brs, H-11), 5.47 (1H, brs, H-2), 3.52 (3H, s, H-16), 1.63 (3H, s, H-13), 0.90 (6H, m, H-14, H-15).

3.4.2. Lamellolactone B2 (5b)

Colorless solids; $[\alpha]_D^{25}$ + 10.1 (*c* 0.10, CH_3OH); ECD (3.8 × 10^{-4} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) 245 (+0.2), 228 (−0.1), 202 (+1.3) nm; EIMS *m/z* 264 $[M]^+$; ^1H NMR (CD_3OD) δ 6.95 (1H, brs, H-10), 5.82 (1H, brs, H-11), 5.47 (1H, brs, H-2), 3.51 (3H, s, H-16), 1.63 (3H, s, H-13), 0.90 (6H, m, H-14, H-15).

3.5. Conformational analysis and calculation of ECD spectra

Conformational analyses in the gas phase were performed using the MMFF94 force field. Regarding compounds **5a** and **5b**, the conformers obtained were further optimized in the gas phase by the density functional theory (DFT) method with the B3LYP functional and 6-31G(d) basis set. Single-point calculations of solvation Gibbs energies in CH_3CN were subsequently performed for gas-phase optimized geometries by the SM8 continuum model using the same DFT method as above. These calculations were performed using Spartan'14 (Wavefunction, Inc., Irvine, CA, USA).

ECD spectra were calculated using Gaussian 09 (Gaussian, Inc., Wallingford, CT, USA) by the time-dependent DFT (TDDFT) method with the CAM-B3LYP functional and 6-311++G(d,p) basis set. Calculations were performed for the three lowest-energy conformers of each compound predicted in CH_3CN. Regarding compound **5a**, the three conformers lie within 0.44 kcal/mol of each other; the energies of the other conformers are higher than the most stable one by more than 1.02 kcal/mol. Concerning compound **5b**, the three conformers lie within 0.66 kcal/mol of each other; the energies of the other conformers are higher than the most stable one by more than 1.01 kcal/mol. Regarding both compounds, the three conformers differ by the rotation of the five-membered ring about the exocyclic C−C bond. The solvent effect was introduced by the polarizable continuum model (PCM). Fifty low-lying excited states were calculated corresponding to the wavelength region down to approximately 145 and 146 nm for compounds **5a** and **5b**, respectively. Simulated spectra were generated using GaussView 6.0.16 (Semichem, Inc., Shawnee Mission, KS, USA), with the peak half-width at half height being 0.333 eV. Boltzmann-averaged spectra at 298.15 K were calculated using Excel 2013 (Microsoft Co., Redmond, WA, USA). Calculated spectra were red-shifted by 15 nm to match experimental spectra.

3.6. PTP inhibitory assay

The effects of compounds **1**–**5** on PTPs were examined by measuring the rate of hydrolysis of the substrate, *p*NPP, according to the method described previously with slight modifications (Cui et al., 2006; Yamazaki et al., 2013; Abdjul et al., 2017). PTP1B (100 μL of a 0.5 μg/mL stock solution), TCPTP (100 μL of a 0.5 μg/mL stock solution), CDC25B (100 μL of a 0.5 μg/mL stock solution), or VHR (100 μL of a 1.0 μg/mL stock solution) in 50 mM citrate buffer (pH 6.0) containing 0.1 M NaCl, 1 mM dithiothreitol (DTT), and 1 mM EDTA was added to each well of a 96-well plastic plate. A sample (2.0 μL in CH_3OH) was added to each well to make the final concentration and was then incubated at 37 °C for 10 min. The reaction was initiated by the addition of *p*NPP in citrate buffer (100 μL of a 4.0 mM stock solution), incubated at 37 °C for 30 min, and then terminated using 10 μL of a stop solution (10 M NaOH). Optical density in each well was measured at 405 nm using an MTP-500 microplate reader (Corona Electric Co., Ltd., Ibaraki, Japan). PTPs inhibitory activity (%) was defined as $[1 - (ABS_{sample} - ABS_{blank})/(ABS_{control} - ABS_{blank})] \times 100$. ABS_{blank} is the absorbance of wells containing only the buffer and *p*NPP. $ABS_{control}$ is the absorbance of *p*-nitrophenol liberated by the enzyme in the assay system without a test sample, whereas ABS_{sample} is that with a test sample. Assays were performed in three duplicate experiments for all test samples. Oleanolic acid, a known phosphatase inhibitor (Zhang et al., 2008), was used as a positive control.

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