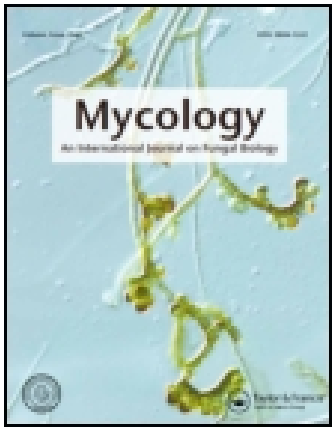

Mycology: An International Journal on Fungal Biology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tmyc20

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Changon Seo [a] , Joung HanYim [b] , Hong Kum Lee [b] & Hyuncheol Oh [a]
[a] College of Medical and Life Sciences , Silla University, San 1-1 Gwaebeop-dong , Sasang-gu, Busan, 617-736, South Korea
[b] Korea Polar Research Institute, KORDI, 7-50 Songdo-dong , Yeonsu-gu, Incheon, 406-840, South Korea
Published online: 23 Mar 2011.

To cite this article: Changon Seo , Joung HanYim , Hong Kum Lee & Hyuncheol Oh (2011) PTP1B inhibitory secondary metabolites from the Antarctic lichen Lecidella carpathica , Mycology: An International Journal on Fungal Biology, 2:1, 18-23

To link to this article: http://dx.doi.org/10.1080/21501203.2011.554906

Mycology
Vol. 2, No. 1, March 2011, 18–23

Taylor & Francis
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PTP1B inhibitory secondary metabolites from the Antarctic lichen *Lecidella carpathica*

Changon Seo[a], Joung HanYim[b], Hong Kum Lee[b] and Hyuncheol Oh[a]*

[a]*College of Medical and Life Sciences, Silla University, San 1-1 Gwaebeop-dong, Sasang-gu, Busan 617–736, South Korea;* [b]*Korea Polar Research Institute, KORDI, 7–50 Songdo-dong, Yeonsu-gu, Incheon 406–840, South Korea*

(*Received 15 November 2010; final version received 7 January 2011*)

Protein tyrosine phosphatase 1B (PTP1B) is an attractive therapeutic target for diabetes, playing a major role in negative regulation of the insulin signaling pathway. Bioassay-guided investigations of an MeOH extract of the Antarctic lichen, *Lecidella carpathica,* afforded three PTP1B inhibitory metabolites: hopane-6α,22-diol (**1**), brialmontin 1 (**2**), and atraric acid (**3**), along with two aromatic metabolites (**4** and **5**) previously isolated from a different Antarctic lichen species. Their structures were determined by analysis of NMR and MS data. Compounds **1**–**3** inhibited PTP1B activity in a dose-dependent manner with IC_{50} values of 3.7, 14.0 and 51.5 μM, respectively, and kinetic analyses of PTP1B inhibition by compounds **1** and **2** suggested that these compounds inhibit PTP1B activity in a competitive manner. In addition, 6,22-hopanediol (**1**) displayed some selectivity toward PTP1B over other protein tyrosine phosphatases, such as TCPTP ($IC_{50} = 8.4$ μM), SHP-2 ($IC_{50} > 68$ μM), LAR ($IC_{50} > 68$ μM), and CD45 ($IC_{50} > 68$ μM).

Keywords: *Lecidella carpathica*; Antarctic lichen; lichen metabolites; protein tyrosine phosphatase 1B (PTP1B); competitive inhibitors

Introduction

Several protein tyrosine phosphatases (PTPs) play a critical role in the regulation of a variety of cellular processes, such as growth, proliferation and differentiation, metabolism, immune response, cell-cell adhesion, and cell-matrix contacts (Fischer et al. 1991; Hunt 1995). Among these, protein tyrosine phosphatase 1B (PTP1B) is a major nontransmembrane phosphotyrosine phosphatase in human tissues (Saltiel and Kahn 2001), and a number of genetic and biochemical studies have demonstrated that PTP1B is a major negative regulator of insulin receptor signaling (Elchebly et al. 1999; Klaman et al. 2000). In addition, recent studies have shown that the leptin signaling pathway can be attenuated by PTPs, and there is compelling evidence that PTP1B is also involved in this pathway (Klaman et al. 2000; Koren 2007). Therefore, PTP1B is now considered as an attractive target in efforts to develop new treatments for type 2 diabetes and related metabolic syndromes (Johnson et al. 2002; Dadke et al. 2003; Koren 2007; Lee and Wang 2007).

Lichens are symbiotic organisms composed of a fungus and its photosynthesizing partner that can be either an alga or a cyanobacterium (Huneck 1999). Most lichens are adapted to extreme ecological conditions and are known to respond very sensitively to changes in their microhabitat and to surrounding environment conditions, thereby leading to the production of antimicrobial and antiherbivore metabolites (Kumar and Műller 1999; Ingólfsdóttir 2002; Lohezic-Le Devehat et al. 2007). Regarding the utilization of lichen metabolites, several lichen extracts have been used as remedies in folk medicine, and a variety of biological activities of lichen metabolite, including antibiotic, antimycobacterial, antiviral, analgesic and antipyretic properties, have been reported. (Huneck 1999; Kumar and Műller 1999; Ernst-Russell et al. 2000). However, secondary metabolites from Antarctic lichens have rarely been the subject of chemical and/or pharmacological investigations.

In the course of our studies of secondary metabolites from Antarctic lichens and mosses (Seo et al. 2008a,b; 2009a,b), several metabolites have been identified as new naturally occurring PTP1B inhibitors. As a part of our on-going studies of Antarctic lichens, an MeOH extract of dried samples of *Lecidella carpathica* was selected for further bioassay-guided investigations based on the observation of significant PTP1B inhibitory (>90%) effect at a concentration of 30 μg/ml. This report describes the isolation, structure elucidation and biological activities of compounds **1**–**5** (Figure 1).

Materials and methods

Experimental procedures

ESIMS data were obtained using an API-2000 ESI-MS instrument (Applied Bio-System, Foster City, CA, USA)

*Corresponding author. Email: hoh@silla.ac.kr

ISSN 2150-1203 print/ISSN 2150-1211 online

DOI: 10.1080/21501203.2011.554906
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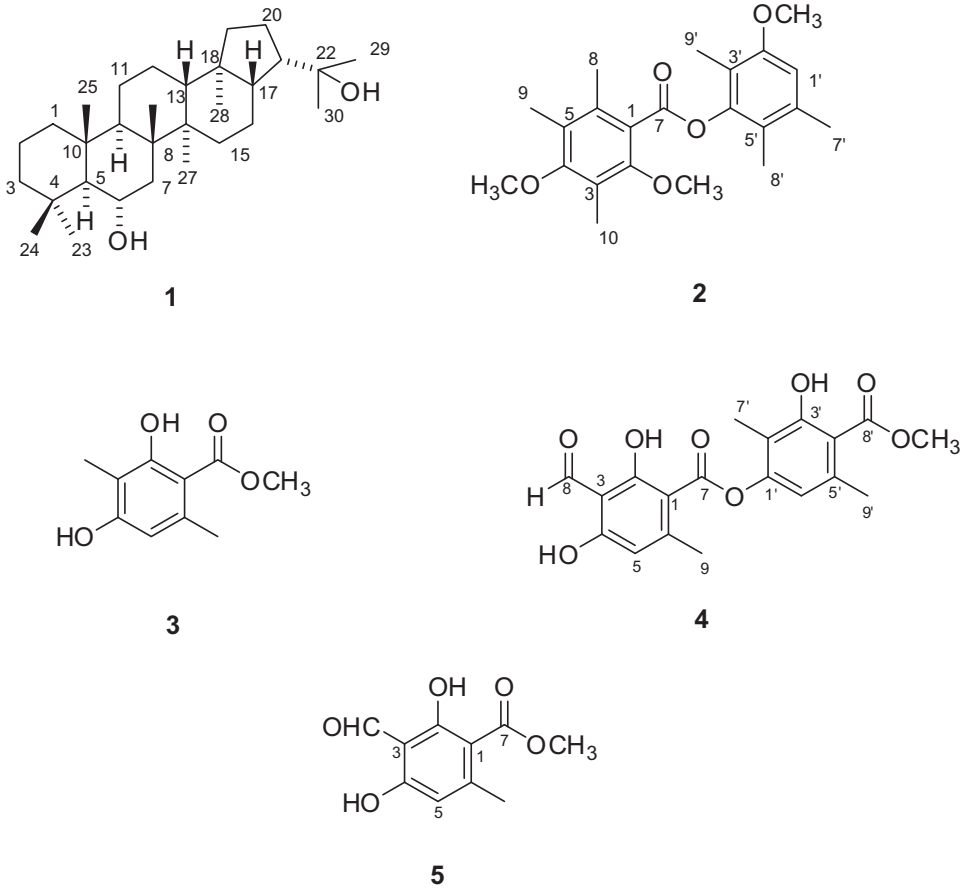

Figure 1. Chemical structure of compounds **1**–**5**.

at Pukyung National University, South Korea. NMR spectra (1D and 2D) were recorded in DMSO-d_6 and $CDCl_3$ using a JEOL JNM ECP-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C), and chemical shifts were referenced relative to the respective residual solvents (δ_H/δ_C = 2.49/39.5 for DMSO-d_6; δ_H/δ_C = 7.26/77.0 for $CDCl_3$). HMQC and HMBC experiments were optimized for $^1J_{CH}$ = 140 Hz and $^nJ_{CH}$ = 8 Hz, respectively. Solvents for extractions and flash column chromatography were reagent grade and used without further purification. Solvents used for HPLC were analytical grade. Flash column chromatography was carried out using YMC octadecyl-functionalized silica gel (ODS-A, 75-μm particle size). HPLC separations were performed on a prep-C_{18} column (21.2 × 150 mm; 5 μm particle size) with a flow rate of 5 ml/min. Compounds were detected by UV absorption at 254 nm and/or ELSD (Evaporative Light Scattering Detector).

Specimen collection and identification

Lecidella carpathica was collected from Barton Peninsula around King Sejong Station (S 62°13′, W 58°47′) on King George Island, Antarctica, in January 2009. Voucher specimens (reference SL-8) have been deposited in the Korea Polar Research Institute.

Isolation and characterization of compounds 1–5

A dried sample of *L. carpathica* (4.6 g) was extracted with MeOH (1 l × 2) for 24 h. The resulting crude MeOH extract (634.6 mg) was subjected to C_{18}-functionalized silica gel flash column chromatography (5 × 40 cm), eluting with a stepwise gradient of 20, 40, 60, 70, 80, 90 and 100% (v/v) MeOH in H_2O (500 ml each) followed by 50% MeOH in CH_2Cl_2 (500 ml).

The fraction eluted with 100% MeOH (97.8 mg) was then subjected to semi-preparative reversed-phase HPLC using a gradient from 80 to 100% MeOH in H_2O (0.1% formic acid) over 35 min to yield **2** (3.9 mg; t_R = 34.2 min), **3** (3.7 mg; t_R = 11.7 min) and **5** (15.7 mg, t_R = 11.7 min).

The fraction (186.2 mg) eluted with 50 % MeOH in CH_2Cl_2 was subjected to semi-preparative reversed-phase HPLC using a gradient from 80 to 100% MeOH in H_2O (0.1% formic acid) over 25 min to yield **1** (32.2 mg; t_R = 43.2 min) and **4** (1.6 mg, t_R = 28.3 min).

Hopane-6α,22-diol (1). White amorphous powder; ESIMS *m/z* 467 $[M + Na]^+$; ^1H-NMR (400 MHz,

DMSO-d_6) δ: 0.77 (1H, m, H-1), 1.56 (1H, m, H-1), 1.40 (1H, m, H-2), 1.60 (1H, m, H-2), 1.12 (1H, m, H-3), 1.24 (1H, m, H-3), 0.75 (1H, m, H-5), 3.74 (1H, m, H-6), 1.34 (1H, m, H-7), 1.18 (1H, m, H-9), 1.36 (1H, m, H-11), 1.60 (1H, m, H-11), 1.25 (1H, m, H-12), 1.48 (1H, m, H-12), 1.29 (1H, m, H-13), 1.06 (1H, m, H-15), 1.24 (1H, m, H-15), 1.53 (1H, m, H-16), 1.93 (1H, br d, J = 13.2 Hz, H-16), 1.32 (1H, m, H-17), 0.87 (1H, m, H-19), 1.43 (1H, m, H-19), 1.48 (1H, m, H-20), 1.64 (1H, m, H-20), 2.10 (1H, dd, J = 8.8 and 19.6 Hz, H-21), 1.11 (3H, s, H-23), 0.94 (3H, s, H-24), 0.81 (3H, s, H-25), 0.97 (3H, s, H-26), 0.91 (3H, s, H-27), 0.71 (3H, s, H-28), 1.03 (3H, s, H-29), 1.07 (3H, s, H-30), 3.92 (1H, d, J = 6.4 Hz, 6-OH), 3.85 (1H, s, 22-OH) ; ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 39.5 (C-1), 18.14 (C-2), 43.6 (C-3), 33.4 (C-4), 60.0 (C-5), 66.6 (C-6), 44.7 (C-7), 42.1 (C-8), 49.3 (C-9), 38.6 (C-10), 20.6 (C-11), 23.7 (C-12), 48.9 (C-13), 41.5 (C-14), 33.9 (C-15), 21.3 (C-16), 53.8 (c-17), 43.5 (C-18), 40.9 (C-19), 26.2 (C-20), 50.4 (C-21), 71.6 (C-22), 36.7 (C-23), 22.0 (C-24), 16.97 (C-25), 18.06 (C-26), 16.86 (C-27), 15.9 (C-28), 28.9 (C-29), 30.9 (C-30).

*Brialmontin 1(**2**)*. Brown amorphous powder; ESIMS m/z 395 $[M + Na]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.18 (3H, s, H-8), 2.19 (3H, s, H-9), 2.31 (3H, s, H-10), 6.64 (1H, s, H-1′), 3.83 (3H, br s, 2-OCH_3), 3.81 (3H, s, 4-OCH_3), 3.71 (3H, s, 2′-OCH_3), 2.21 (3H, s, H-7′), 2.27 (3H, s, H-8′), 2.35 (H-9′); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 120.9 (C-1), 158.8 (C-2), 116.7 (C-3), 154.6 (C-4), 124.7 (C-5), 135.1 (C-6), 167.0 (C-7), 17.2 (C-8), 12.6 (C-9), 9.6 (C-10), 61.9 (2-OCH_3), 60.1 (4-OCH_3), 110.2 (C-1′), 155.8 (C-2′), 122.2 (C-3′), 148.6 (C-4′), 126.4 (C-5′), 133.5 (C-6′), 20.5 (C-7′), 12.4 (C-8′), 9.6 (C-9′), 55.7 (2′-OCH_3).

*Atraric acid (**3**)*. Brown amorphous powder; ESIMS m/z 195 $[M–H]^-$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.10 (3H, s, 3-CH_3), 2.40 (3H, s, 6-CH_3), 3.90 (3H, s, 7-OCH_3), 5.24 (1H, s, 4-OH), 6.18 (1H, s, H-5), 12.02 (1H, s, 2-OH).

*Atranorin (**4**)*. Brown amorphous powder; ESIMS m/z 373 $[M–H]^-$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.40 (1H, s, H-5), 10.36 (1H, s, H-8), 2.69 (3H, s, H-9), 6.52 (1H, s, H-6′), 2.09 (3H, s, H-7′), 2.55 (3H, s, H-9′), 3.99 (3H, s, 8′-OCH_3), 12.51 (1H, s, 2-OH), 12.56 (1H, s, 4-OH), 11.96 (1H, s, 3′-OH,); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 102.8 (C-1), 169.1 (C-2), 108.5 (C-3), 167.5 (C-4), 112.8 (C-5), 152.4 (C-6), 169.7 (C-7), 193.8 (C-8), 25.6 (C-9), 151.9 (C-1′), 116.8 (C-2′), 162.9 (C-3′), 110.3 (C-4′), 139.9 (C-5′), 116.0 (C-6′), 9.4 (C-7′), 172.2 (C-8′), 24.0 (C-9′), 52.3 (8′-OCH_3); HMBC data, H-5 → C-1, 3, 4, 9; H-8 → C-3, 4, 5; H-9 → C-1, 5, 6; H-6′ → C-1′, 2′, 4′, 9′; H-7′ → C-1′, 2′, 3′; H-9′ → C-4′, 5′, 6′; 2-OH → C-1, 2, 3; 4-OH → C-3, 4, 5; 3′-OH → C-2′, 3′, 4′; 8′-OCH_3 → C-8′.

*Methyl haematommate (**5**)*. Brown amorphous powder; ESIMS m/z 209 $[M - H]^-$; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.29 (1H, s, H-5), 2.53 (3H, s, H-8), 3.96 (3H, s, 7-OCH_3), 12.89 (1H, s, 2-OH), 12.41 (1H, s, 4-OH), 10.34 (3-CHO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 103.9 (C-1), 168.3 (C-2), 108.4 (C-3), 166.6 (C-4), 112.1 (C-5), 152.3 (C-6), 172.0 (C-7), 25.2 (C-8), 194.0 (3-CHO), 52.3 (7-OCH_3).

Assay procedures

Protein tyrosine phosphatase activity was measured using *p*-nitrophenyl phosphate (*p*NPP) as a substrate. Assays were performed in 100 μl volumes comprising 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA and 1 mM dithiothreitol (DTT) (for PTP1B and TCPTP), 25 mM Tris–HCl pH 7.0, 5 mM DTT, 0.01% Brij-35 detergent solution (v/v), 0.1% BSA (w/v), 50 mM NaCl, 2 mM EDTA (for SHP-2), 25 mM Tris–HCl pH 7.0, 5 mM DTT, 0.01% Brij-35 detergent solution (v/v), 50 mM NaCl, 2 mM EDTA (for LAR), or 50 mM Hepes pH 7.2, 1 mM DTT, 0.05% NP-40 (v/v), 1 mM EDTA (for CD45). The substrate (*p*NPP) was used at the concentrations of 4 mM for PTP1B, 8 mM for TCPTP, 25 mM for SHP-2, 1 mM for LAR, or 5 mM for CD45. Assays were performed for different times (30–60 min at 37 °C) for different enzymes, ensuring linearity was maintained. The reaction was terminated by addition of 50 μl of 10 N NaOH, and the amount of produced *p*-nitrophenol was estimated by measuring the absorbance at 405 nm. The non-enzymatic hydrolysis of *p*NPP was corrected by measuring the absorbance at 405 nm in the absence of the enzymes.

Kinetic analysis

The reaction mixture consisted of different concentrations of *p*NPP as a PTP1B substrate in the absence or presence of compounds **1** and **2**, and assayed as described above. Data were fitted by nonlinear regression analysis according to a Michaelis–Menten kinetic model (Graphpad Prism version 4.02).

Results and discussion

To isolate and identify the PTP1B inhibitory active principle(s) from the MeOH extract of the sample of *L. carpathica*, we performed bioassay and ^{1}H-NMR guided fractionation and purification by using C_{18}-functionalized silica gel column chromatography and HPLC, which led to the isolation of five lichen metabolites (compounds **1**–**5**). The isolated compounds were identified as hopane-6α,22-diol (**1**) (König and Wright 1999), brialmontin 1 (**2**) (Vinet et al. 1990), atraric acid (**3**) (Ahad et al. 1991), atranorin (**4**) (König and Wright 1999; Athukoralage et al. 2001), and methyl haematommate (**5**) (Kouam et al. 2005) by analysis of NMR and MS data, along with a comparison of the literature.

Among the isolated metabolites, compounds **1**–**3** were evaluated for their inhibitory effects against the activity of PTP1B in vitro. Compounds **4** and **5** had been encountered in our previous study of the Antarctic lichen, *Stereocaulon*

alpinum (Seo et al. 2009b). In the enzyme inhibition assay, hopane-6α,22-diol (**1**) exhibited the most potent PTP1B inhibitory activity in a dose-dependent manner with an IC_{50} value of 3.7 μM, while brialmontin 1 (**2**) and atraric acid (**3**) displayed lower inhibitory effects, showing IC_{50} values of 14.0 and 51.5 μM, respectively. A known PTP1B inhibitor, ursolic acid ($IC_{50} = 3.1$ μM) was used as a positive control in the assay (Na et al. 2006; Zhang et al. 2006). The characteristics of the inhibition of PTP1B by compounds **1** and **2** were then analyzed. PTP1B was incubated with increasing concentrations of compounds **1** and **2** and full velocity curves were determined (Figures 2 and 3). Non-linear regression analysis showed that the data best fit a competitive model of inhibition, and re-plotting of the data as Lineweaver–Burk transformations confirmed this result, displaying the characteristic intersecting line pattern for competitive inhibition. Therefore, it was shown that both compounds **1** and **2** bind to the active site within PTP1B. In the development of PTP1B inhibitors from natural products or synthetic compounds, selectivity against other PTPs is one of the biggest issues (Bialy and Waldmnn

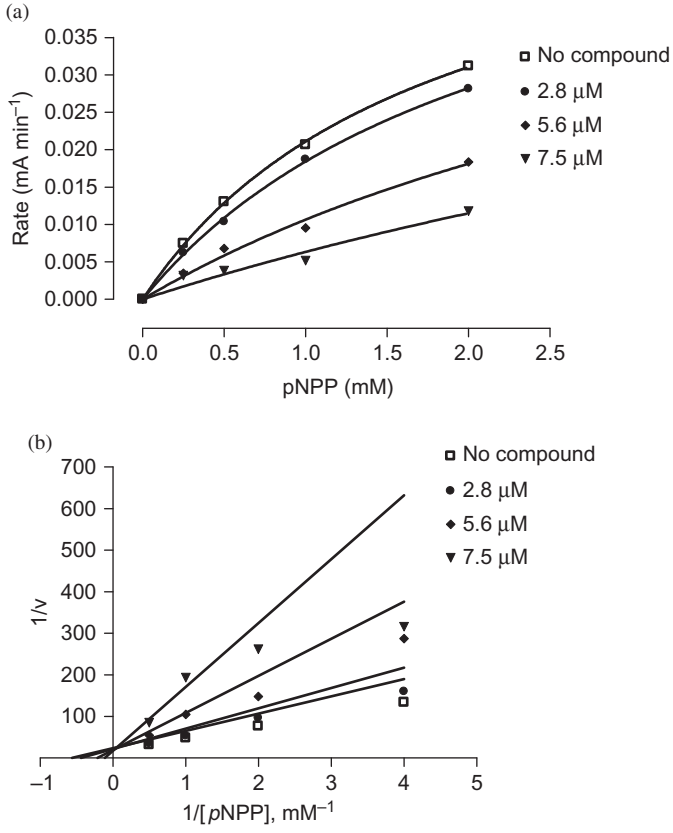

Figure 2. Substrate titration reveals that compound **1** is a classical competitive inhibitor that inhibits substrate binding (constant K_m) but not substrate catalysis (V_{max}). (a) Velocity curves performed with PIP1B in the presence of increasing concentrations of compound **1**. (b) Lineweaver–Burk transformations of data from (a). Data are expressed as mean initial velocity for $n = 3$ replicates at each substrate concentration.

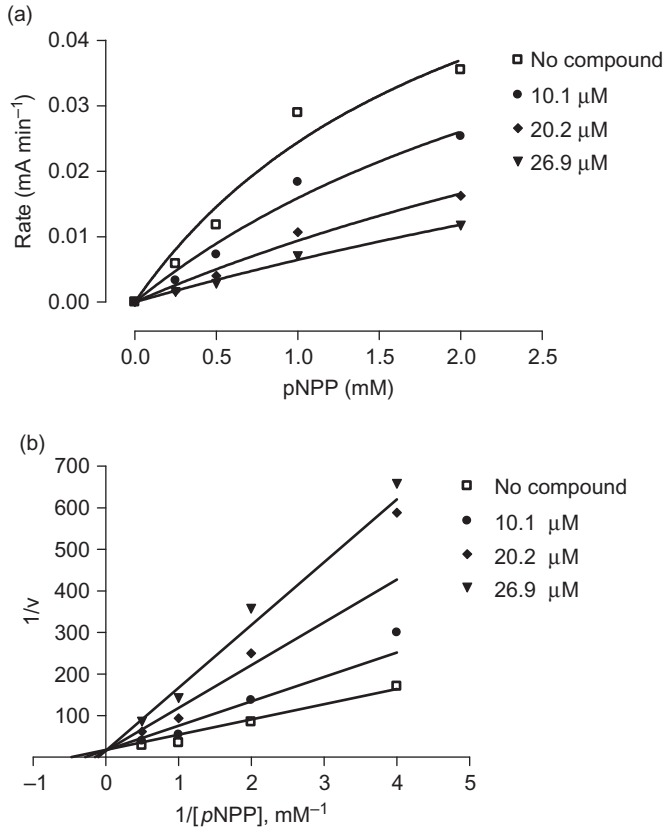

Figure 3. Substrate titration reveals that compound **2** is a classical competitive inhibitor that inhibits substrate binding (constant K_m) but not substrate catalysis (V_{max}). (a) Velocity curves performed with PIP1B in the presence of increasing concentrations of compound **2**. (b) Lineweaver–Burk transformations of data from (a). Data are expressed as mean initial velocity for $n = 3$ replicates at each substrate concentration.

2005). Therefore, the specific inhibitory activities of compound **1** toward a small panel of PTPs including cytosolic PTPs such as T-cell protein tyrosine phosphatase (TCPTP) and src homology phosphatase-2 (SHP-2), as well as receptor-like PTPs such as leukocyte antigen-related phosphatase (LAR), and CD45 tyrosine phosphatase (CD45) were evaluated. As shown in Table 1, inhibition selectivity of compound **1** over TCPTP was not significant, but compound **1** appeared to possess no inhibitory effect against other PTPs such as SHP-2, LAR and CD45 up to the 78 μM level.

Table 1. Inhibitory activity of the 6,22-hopanediol against PTPs.

	PTPs	IC_{50} (= μM)
hopane-6α,22-diol (1)	TCPTP	8.4
	SHP2	>68
	LAR	>68
	CD45	>68

In summary, bioassay-guided fractionation of an MeOH extract of Antarctic lichen, *L. carpathica,* afforded two metabolites (**1** and **2**) as moderate to strong PTP1B inhibitory components, along with three additional lichen metabolites, atraric acid (**3**), atranorin (**4**) and methyl haematommate (**5**). Hopane-6α,22-diol (**1**) is a member of hopane-based triterpenes, which have taxonomic importance. Although its antimycobacterial activity has been reported (König et al. 2000), PTP1B inhibitory activity of this compound is now being reported for the first time. Several ursane- and oleanane-type triterpenoids from plant sources have been reported to have a similar magnitude of PTP1B inhibitory activity as that of compound **1** (Na et al. 2006; Zhang et al. 2006, 2008; Lin et al. 2008). In addition, these known plant metabolites are identified as competitive inhibitors of PTP1B, and most of these inhibitors commonly possess carboxylic acid functionality in the structures. Therefore, it is noteworthy that compound **1** represents an additional member of the PTP1B inhibitory tripenoids, yet with different carbon-skeleton and no carboxylic acid functionality. Brialmontin 1 (**2**) is a member of the lichen depsides originally isolated from *L. brialmontii* (Vinet et al. 1990), and no biological activity of this compound have been revealed. Therefore, compound **2** is an additional member of the phenolic PTP1B inhibitory lichen metabolites along with previously identified metabolites such as gyrophoric acid (Seo et al. 2008a) and lobaric acid (Seo et al. 2008b).

Acknowledgement

This work was supported by a grant from the KOPRI Project (PE11060/utilization of novel metabolites from polar organisms).

References

Ahad AM, Goto Y, Kiuchi F, Tsuda Y, Kondo K, Sato T. 1991. Nematocidal principles in "Oakmoss Absolute" and nematocidal activity of 2,4-dihydroxybenzoates. Chem Pharm Bull. 39:1043–1046.

Athukoralage PS, Herath HMTB, Deraniyagala SA, Wijesundera RLC, Weerasinghe PA. 2001. Antifungal constituent from *Gordonia dassanayakei*. Fitoterapia. 72:565–567.

Bialy L and Waldmann H. 2005. Inhibitors of protein tyrosine phosphatases: next-generation drugs? Angew Chem Int Ed. 44:3814–3839.

Dadke S, Chernoff J. 2003. Protein-tyrosine phosphatase 1B as a potential drug target for obesity. Curr Drug Targets: Immune Endocr Metab Disord. 3:299–304.

Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy A L, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, Kennedy BP. 1999. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 283:1544–1548.

Ernst-Russell MA, Chai CLL, Wardlaw JH, Elix JA. 2000. Euplectin and coneuplectin, new naphthopyrones from the lichen *Flavoparmelia euplecta*. J Nat Prod. 63: 129–131.

Fischer EH, Charbonneau H, Tonks NK. 1991. Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. Science 253:401–406.

Hunter T. 1995. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. Cell 80: 225–236.

Huneck S. 1999. The significance of lichens and their metabolites. Naturwissenschaften 86:559–570.

Ingólfsdóttir K. 2002. Usnic acid. Phytochemistry 61: 729–736.

Johnson TO, Ermolieff J, Jirousek MR. 2002. Protein tyrosine phosphatase 1B inhibitors for diabetes. Nat Rev Drug Discov. 1:696–709.

Koren S. 2007. Inhibition of the protein tyrosine phosphatase PTP1B: potential therapy for obesity, insulin resistance and type-2 diabetes mellitus. Best Pract Res Clin Endocrinol Metab. 21:621–640.

Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Sharpe AH, Stricker-Krongrad A, Shulman GI, Neel BG, Kahn BB. 2000. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol. 20:5479–5489.

Kumar KCS, Műller K. 1999. Lichen metabolites. 1. Inhibitory action against leukotriene B4 biosynthesis by a non-redox mechanism. J Nat Prod. 62:817–820.

Kouam SF, Ngadjui BT, Krohn K, Wafo P, Ajaz A, Choudhary MI. 2005. Prenylated anthronoid antioxidants from the stem bark of *Harungana madagascariensis*. Phytochemistry 66:1174–1179.

König GM, Wright AD. 1999. ^{1}H and ^{13}C-NMR and biological investigations of four lichen-derived compounds. Phytochem Anal. 10:279–284.

König GM, Wright AD, Franzblau SG. 2000. Assessment of antimycobacterial activity of series of mainly marine derived natural products. Planta Med. 66:337–342.

Lee S, Wang Q. 2007. Recent development of small molecular specific inhibitor of protein tyrosine phosphatase 1B. Med Res Rev. 27:553–573.

Lohezic-Le Devehat F, Tomasi S, Elix JA, Bernard A, Rouaud I, Uriac P, Boustie J. 2007. Stictic acid derivatives from the lichen *Usnea articulata* and their antioxidant activities. J Nat Prod. 70:1218–1220.

Lin Z, Zhang Y, Zhang Y, Shen H, Hu L, Jiang H, Shen X. 2008. Oleanolic acid derivative NPLC441 potently stimulates glucose transport in 3T3-L1 adipocytes via a multi-target mechanism. Biochem Pharmacol. 76:1251–1262.

Na M, Tang S, He L, Oh H, Kim BS, Oh WK, Kim BY, Ahn JS. 2006. Inhibition of protein tyrosine phosphatase 1B by ursane-type triterpenes isolated from *Symplocos paniculata*. Planta Med. 72:261–263.

Saltiel AR, Kahn CR. 2001. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414: 799–806.

Seo C, Choi YH, Sohn JH, Ahn JS, Yim JH, Lee HK, Oh H. 2008a. Ohioensins F and G: protein tyrosine phosphatase 1B inhibitory benzonaphthoxanthenones from the Antarctic moss *Polytrichastrum alpinum*. Bioorg Med Chem Lett. 18:772–775.

Seo C, Sohn JH, Park SM, Yim JH, Lee HK, Oh H. 2008b. Usimines A-C, bioactive usnic acid derivatives from the Antarctic lichen *Stereocaulon alpinum*. J Nat Prod. 71: 710–712.

Seo C, Choi YH, Ahn JS, Yim JH, Lee HK, Oh H. 2009a PTP1B inhibitory effects of tridepside and related metabolites

isolated from the Antarctic lichen *Umbilicaria antarctica*. J Enzyme Inhib Med Chem. 24:1133–1137.

Seo C, Sohn JH, Ahn JS, Yim JH, Lee HK, Oh H. 2009b. Protein tyrosine phosphatase 1B inhibitory effects of depsidone and pseudodepsidone metabolites from the Antarctic lichen *Stereocaulon alpinum*. Bioorg Med Chem Lett. 19:2801–2803.

Vinet C, Quilhot W, Gambaro V, Garbarino JA. 1990. Studies on Chilean lichens, XIII. Polysubstituted depsides from *Lecania brialmontii*. J Nat Prod. 53:500–502.

Zhang W, Hong D, Zhou Y, Zhang Y, Shen Q, Li J, Hu L, Li J. 2006. Ursolic acid and its derivative inhibit protein tyrosine phosphatase 1B, enhancing insulin receptor phosphorylation and stimulating glucose uptake. Biochim Biophy Acta 1760:1505–1512.

Zhang YN, Zhang W, Hong D, Shi L, Shen Q, Li JY, Li J, Hu LH. 2008. Oleanolic acid and its derivatives: New inhibitor of protein tyrosine phosphatase 1B with cellular activities. Bioorg Med Chem. 16:8697–705.