Bioorganic & Medicinal Chemistry Letters 19 (2009) 2801–2803

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Protein tyrosine phosphatase 1B inhibitory effects of depsidone and pseudodepsidone metabolites from the Antarctic lichen *Stereocaulon alpinum*

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ARTICLE INFO

Article history:
Received 23 January 2009
Revised 8 March 2009
Accepted 23 March 2009
Available online 26 March 2009

Keywords:
Stereocaulon alpinum
Protein tyrosine phosphatase 1B (PTP1B)
Depsidones
Lichen metabolites
Non-competitive inhibitors

ABSTRACT

Seven phenolic lichen metabolites (**1**–**7**) have been isolated from a methanol extract of the Antarctic lichen *Stereocaulon alpinum* by various chromatographic methods. The structures of these compounds were determined mainly by analysis of NMR spectroscopic data. A depsidone-type compound, lobaric acid (**1**) and two pseudodepsidone-type compounds, **2** and **3**, exhibited potent inhibitory activity against protein tyrosine phosphatase 1B (PTP1B) with IC_{50} values of 0.87 μM, 6.86 μM, and 2.48 μM, respectively. Kinetic analyses of PTP1B inhibition by compounds **1** and **2** suggested that these compounds inhibited PTP1B activity in a non-competitive manner.

Protein tyrosine phosphatase 1B (PTP1B) is a widely expressed protein tyrosine phosphatase that is present in insulin-sensitive tissues. The insulin-antagonizing activity of PTP1B has been demonstrated by a number of biochemical and genetic studies. For example, PTP1B knockout mice have proven to be hypersensitive to insulin and resistant to obesity, but lack other significant negative side-effects due to the mutation.[1,2] PTP1B also regulates leptin action, which controls satiety and energy expenditure.[3] PTP1B-deficient mice have remarkably low adiposity and are protected from diet-induced obesity by increases in basal metabolic rate and total energy expenditure. This supports the inference that PTP1B is a major regulator of energy balance, insulin sensitivity, and body fat stores in vivo.[4] Accordingly, inhibition of PTP1B is predicted to be an excellent, novel therapy to target type 2 diabetes and obesity.[3] Given the compelling biochemical and genetic evidence linking PTP1B to several human diseases, a number of efforts have been conducted to develop PTP1B inhibitors.[5] However, most of reported PTP1B inhibitors suffer from drawbacks, such as lack of selectivity and bioavailability. One reason for this is that most of these compounds were developed as charged non-hydrolyzable phosphotyrosine (pTyr) mimetics targeting the positively charged active site of PTP1B, thus leading to low cell permeability.[6] Second, most PTPs are known to share a highly conserved catalytic domain. Therefore, identification of novel inhibitors with improved pharmacological properties such as selectivity and bioavailability is still necessary. In addition, the screening for PTP1B inhibitors from natural products, which have an excellent track record of success in small molecule drug discovery programs, has begun only recently compared to relatively intensive efforts involving synthetic studies.[7]

Lichens are symbiotic organisms composed of a fungus that cultivates a photosynthesizing partner that can be either an alga or a cyanobacterium.[8] In all lichens, the fungus forms a thallus or lichenized stroma that may contain characteristic secondary metabolites, and these metabolites are sometimes suggested to serve as antimicrobial or antiherbivore agents.[9] Several lichen extracts have been used for various remedies in folk medicine, and a variety of biological activities of lichen metabolites, including antibiotic, antimycobacterial, antiviral, analgesic, and antipyretic properties, have been indicated by screening processes.[8,10] Thus, there is a considerable interest in lichen metabolites as potential sources of pharmacological agents.

In the course of our continuing search for PTP1B inhibitory Antarctic lichen metabolites, the MeOH extract of a dried sample of *Stereocaulon alpinum* was selected for further study based on a significant PTP1B inhibitory effect at the extract level (>90% inhibition at 30 μg/mL). Bioassay-guided fractionation of

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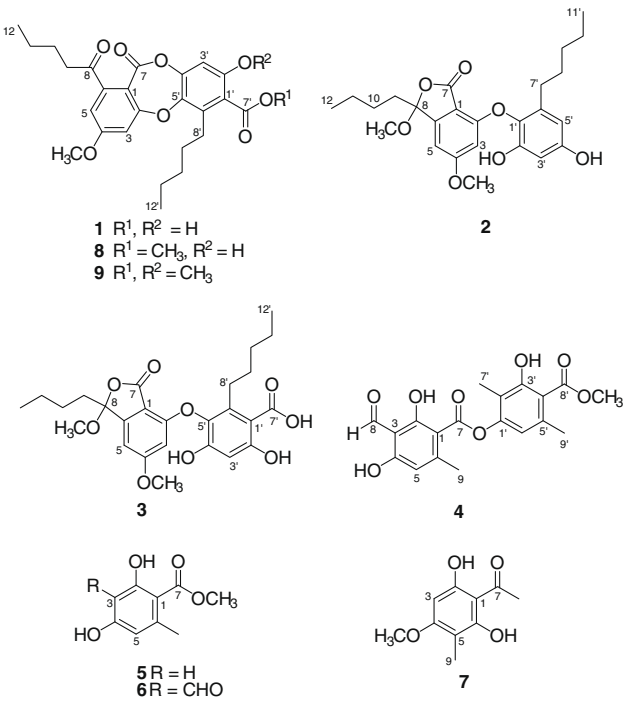
0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.03.108

the extract led to the isolation of four lichen metabolites belong to the classes of depsidone (**1**), pseudodepsidone (**2** and **3**), and depside (**4**) classes, as well as three simpler phenolic compounds (**5**–**7**). This report describes the isolation, structure elucidation, and biological activities of the compounds encountered in this study.

A dried sample of *S. alpinum* was extracted with MeOH, and the resulting crude MeOH extract was subjected to C_{18} functionalized silica gel flash column chromatography, eluting with a stepwise gradient consisting of MeOH in H_2O (10–100% MeOH with 10% increment for each step; 400 mL each). The fraction eluted at 80% MeOH was subjected to silica gel column chromatography followed by semi-preparative reversed-phase HPLC to yield **1**. The fractions eluted at 70% and 90% MeOH in H_2O were combined and further purified by silica gel column chromatography, followed by semi-preparative reversed-phase HPLC to yield compounds **2**, **3**, and **5–7**. Compound **4** was isolated from the fraction eluted at 100% MeOH from the C_{18} functionalized silica gel flash column by successive silica gel column chromatography and semi-preparative reversed-phase HPLC. The isolated compounds were identified as lobaric acid (**1**),[11] two pseudodepsidone-type metabolites (**2** and **3**),[12] atranorin (**4**),[13] methyl orsellinate (**5**),[14] methyl haematommate (**6**),[15] and 2,6-dihydroxy-4-methoxy-3-methylacetophenone (**7**)[16] by analysis of NMR and MS data, and in comparison with the literature values.



Among the tested compounds, a depsidones-type metabolite, lobaric acid (**1**) exhibited the most potent PTP1B inhibitory activity in a dose-dependent manner with an IC_{50} value of 0.87 μM. Pseudodepsidone-type metabolites **2** and **3** inhibited PTP1B activity in a dose-dependent manner with IC_{50} values of 6.86 μM and 2.48 μM, respectively. On the other hand, a depside-type metabolite, atranorin (**4**) displayed a much lower inhibitory effect, showing an IC_{50} value of 63.5 μM, and the three simpler phenolic compounds (**5–7**) did not show any inhibitory activity at the 200 μM level. A known phosphatase inhibitor, ursolic acid (IC_{50} = 3.08 μM), was used as a positive control in the assay.[17,18] The PTP1B inhibitory activity of the metabolites are summarized in Table 1.

Depsidone- and pseudodepsidone-type metabolites are among the most common classes of lichen metabolites, and are produced by rather complex biosynthetic pathways.[19] Among the metabolites encountered in this study, lobaric acid (**1**) has been reported to have antimycobacterial activity[20] and inhibitory activities against 5- and 12-lipoxygenases.[21] Atranorin (**4**) has been reported to have antifungal effects.[13] However, to our knowledge, the PTP1B inhibitory activities of these lichen metabolites are now being reported for the first time.

The characteristics of the inhibition of PTP1B by lobaric acid (**1**) were then analyzed in greater detail. PTP1B was incubated with increasing concentrations of compound **1** and full velocity curves were determined (Fig. 1). Non-linear regression analysis showed that the data best fit a non-competitive model of inhibition, and re-plotting of the data as Lineweaver–Burk transformations confirmed this result. Similarly, inhibition kinetics of **2** were explored with different concentrations of a substrate to elucidate the inhibition mode of the pseudodepsidones discovered in this study. When *p*-nitrophenyl phosphate (*p*NPP) was used as a substrate, compound **2** decreased the V_{max} value, but did not alter the K_m value of PTP1B (Fig. 2) under our experimental conditions. Therefore, it was shown that the depsidone and pseudodepsidones encountered in this study behave as non-competitive inhibitors of PTP1B, implying that these compounds may bind to the enzyme–substrate complex or to an allosteric site within PTP1B.[22] There are several reported non-competitive or competitive PTP1B inhibitors possessing a carboxylic acid group as a crucial functionality for the inhibitory potency.[7,23] Therefore, compound **1** was converted to the corresponding methyl ester (**8**) to evaluate the significance of the carboxylic acid group for potency in PTP1B inhibition. As shown in Table 1, the IC_{50} value of compound **8** was ~4-fold lower than that of compound **1**, suggesting that the presence of the carboxylic acid group plays a role in the inhibition mechanism. This inference was also supported by comparison of the inhibitory potencies of closely related compounds **2** and **3**. The inhibitory activity of compound **3** was stronger (~3-fold) than that of compound **2**, whose structure differs from that of **3** by replacement of a carboxylic acid group with a hydrogen atom. Furthermore, conversion of the phenolic hydroxyl group in compound **8** to the corresponding methoxy group (compound **9**) led to the further reduction of the inhibitory potency (~2–3-fold). Thus, it was suggested that the presence of acidic protons in compounds **1–3** is, at least in part, required in the inhibition mechanism, presumably providing hydrogen-bonding sites that are relevant to the interaction with PTP1B. Crystallographic studies of PTP1B in complex with non-competitive inhibitors have previously revealed that both hydrogen-bonding and hydrophobic interactions between the

Table 1
PTP1B inhibitory activity of compounds **1–9**

Compounds	PTP1B inhibitory activity (IC_{50} = μM)
1	0.87
2	6.86
3	2.48
4	63.5
5	>200
6	>200
7	>200
8	3.02
9	7.42
Ursolic acid[a]	3.08

[a] Positive control.

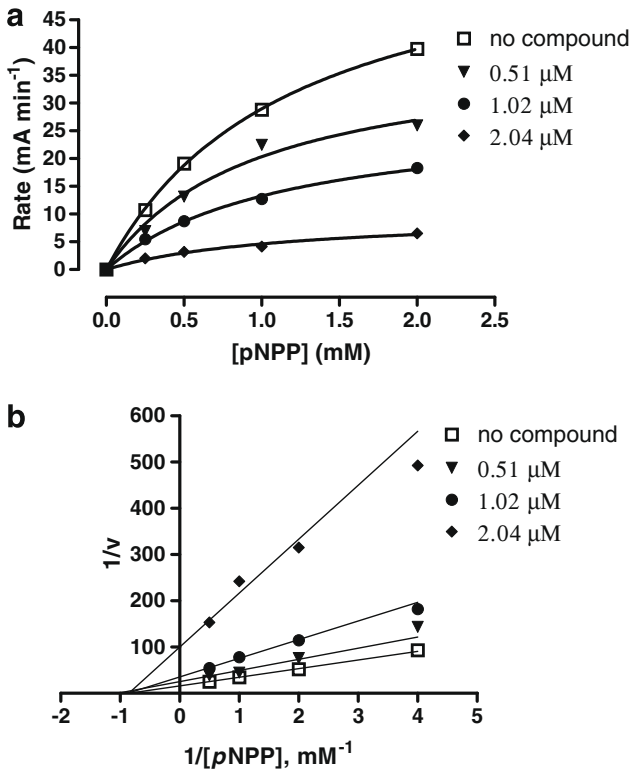


Figure 1. Substrate titration reveals that compound **1** is a classical non-competitive inhibitor that inhibits substrate catalysis (V_{max}), but not substrate binding (constant K_m). (a) Velocity measurements performed with PIP1B in the presence of increasing concentrations of compound **1.** Concentrations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

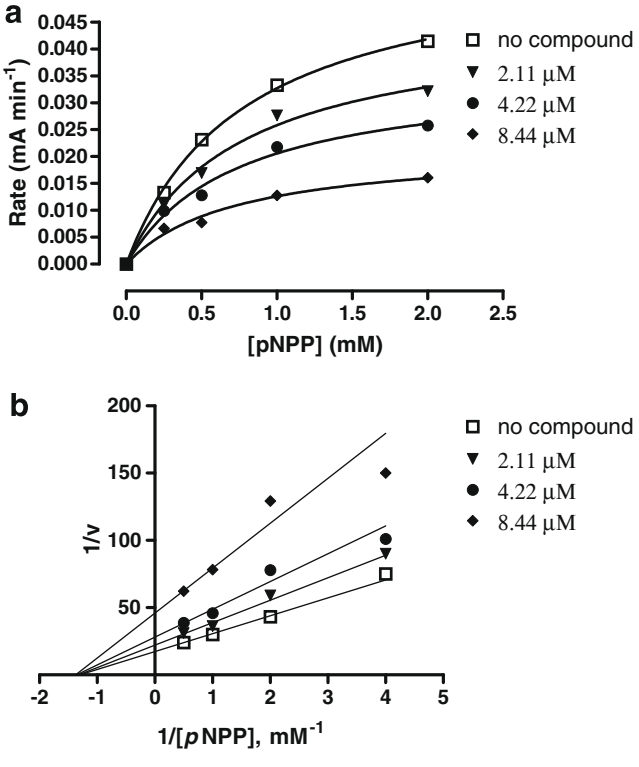


Figure 2. Substrate titration reveals that compound **2** is a classical non-competitive inhibitor. (a) Velocity measurements performed with PIP1B in the presence of increasing concentrations of compound **2.** Concentrations (μM) of **2** are indicated in the figure. (b) The Lineweaver–Burk transformations of data from (a). Data are expressed as mean initial velocity for $n = 3$ replicates at each substrate concentration.

inhibitor and the enzyme are important in the allosteric inhibition of PTP1B.[22]

As noted previously, a number of PTP1B inhibitors have been identified mainly from active site-directed synthetic studies. Most of these inhibitors are negatively charged pTyr mimetics and therefore pose obstacles to the development of therapeutic agents due to the lack of selectivity and bioavailability. In the course of exploring alternative approaches to the development of PTP1B inhibitors with improved selectivity and bioavailability, the identification of non-competitive inhibitors targeting the allosteric site in PTP1B[22] or the active site in the inactive conformation of PTP1B[6] have been recently reported. In this study, lobaric acid (**1**) and the pseudodepsidone-type compound **2** were identified as non-competitive inhibitors of PTP1B. Both compounds possess hydrophobic alkyl side chains which would aid their ability to diffuse into cells. Therefore, these two compounds could be viewed as potential lead compounds for the treatment of diabetes and obesity, suggesting that further evaluation of their pharmaceutical properties is warranted.

Acknowledgment

This work was supported by a Grant from the KOPRI Project (PE07050).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.108.

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