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Isolation of the protein tyrosine phosphatase 1B inhibitory metabolite from the marine-derived fungus *Cosmospora* sp. SF-5060

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ABSTRACT

In the course of bioassay-guided study on the EtOAc extract of a culture broth of the marine-derived fungus *Cosmospora* sp. SF-5060, aquastatin A (**1**) was isolated as a protein tyrosine phosphatase 1B (PTP1B) inhibitory component produced by the fungus. The compound was isolated by various chromatographic methods, and the structure was determined mainly by analysis of NMR spectroscopic data. Compound **1** exhibited potent inhibitory activity against PTP1B with IC_{50} value of 0.19 μM, and the kinetic analyses of PTP1B inhibition by compound **1** suggested that the compound is inhibiting PTP1B activity in a competitive manner. Aquastatin A (**1**) also showed modest but selective inhibitory activity toward PTP1B over other protein tyrosine phosphatases, such as TCPTP, SHP-2, LAR, and CD45. In addition, the result of hydrolyzing aquastatin A (**1**) suggested that the dihydroxypentadecyl benzoic acid moiety in the molecule is responsible for the inhibitory activity.

Marine microorganisms have been recognized as a rich source of structurally novel and pharmacologically active secondary metabolites.[1,2] Particularly, fungi from marine environment have shown to produce diverse secondary metabolites which are more or less similar to those produced by terrestrial fungi.[3,4] As a part of our ongoing studies on PTP1B inhibitors from marine microorganisms collected from Korea, a fungal isolate *Cosmospora* sp. SF-5060 was selected for further investigation on the basis of its potent inhibitory effect against PTP1B.

Protein tyrosine phosphatases (PTPs) constitute a large family of enzymes, crucial modulators of tyrosine phosphorylation-dependent cellular events such as growth, proliferation and differentiation, metabolism, immune response, cell–cell adhesion, and cell-matrix contacts.[5–7] Among them, protein tyrosine phosphatase 1B (PTP1B), an intracellular non-receptor type PTP, is considered as a well-validated target for drug development. The negative regulation of insulin- and leptin-receptor mediated signaling pathways by PTP1B has been demonstrated by a number of biochemical and genetic studies.[8–10] Several independent studies have shown that PTP1B-knockout mice display increased insulin sensitivity, improved glycemic control, and resistant to diet-induced obesity.[10,11] Accordingly, inhibition of PTP1B is predicted to be an excellent, novel therapy to target type 2 diabetes and obesity. Given the compelling biochemical and genetic evidences linking PTP1B to several human diseases, a number of efforts have been conducted to develop PTP1B inhibitors.[8,12] However, the development of small-molecule PTP1B-based drug candidates possessing target selectivity and bioavailability remains an important challenge.[13]

The marine-derived fungus *Cosmospora* sp. SF-5060 was isolated from an inter-tidal sediment collected at Gejae Island (April, 2007), and characterized based on analysis of the ribosomal RNA (rRNA) sequences. *Cosmospora* sp. SF-5060 was cultivated in liquid media at 25 °C for 14 days. The filtered culture broth was extracted with EtOAc, and the resulting organic extract was subjected to C_{18} functionalized silica gel flash column chromatography, eluting with a stepwise gradient consisting of MeOH in H_2O (20% to 100% MeOH with 20% increment for each step; 400 mL each). The fraction eluted at 100% MeOH was then subjected to semi-preparative reversed-phase HPLC to yield **1**.

The structure of **1** was inferred to be a family of the glycosylated depside base on analysis of the ^1H and ^{13}C NMR data. In the NMR spectra of **1**, the signals corresponding to two sets of *meta*-coupled aromatic protons, twelve aromatic carbons, and two ester carbonyl carbons suggested the presence of depside-type moiety in the molecule. In addition, the presence of sugar moiety in the molecule was evident by the observation of the signals corresponding to a number of oxymethines, an oxymethylene, and an anomeric methine in the NMR spectra. Eventually, the planar structure of **1** was determined to be aquastatin A by analysis of the 1D and 2D NMR and MS data, along with in comparison with literature values.[14] However, the absolute configuration of the β-galactopyranoside aquastatin A was not previously assigned. Therefore, compound **1** was subjected

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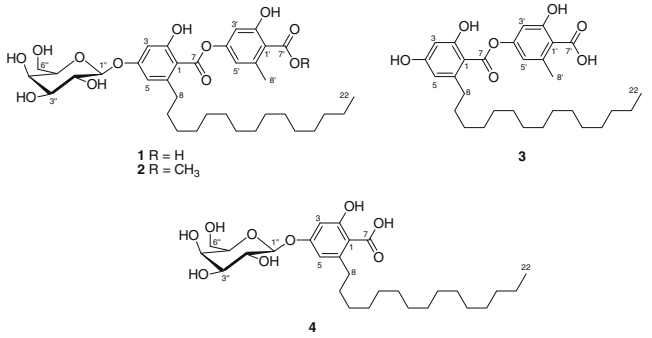
to acid hydrolysis with 6 N HCl, and the liberated sugar was identified as D-galactose by TLC analysis and comparison of its specific rotation with those of authentic samples.

As shown in Table 1, compounds **1** strongly inhibited the hydrolysis of the *p*-nitrophenyl-phosphate (*p*NPP) catalyzed by PTP1B in a dose-dependent manner with IC_{50} values of 0.19 μM. The characteristics of the inhibition of PTP1B by compound **1** were then analyzed in 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 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 with a K_i value of 0.10 μM. Therefore, it was shown that aquastatin A (**1**) binds to the active site within PTP1B. Next, compound **1** was converted to the corresponding methyl ester (compound **2**) 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 **2** was significantly lower than that of compound **1**. This observation was consistent with the previous results; that is the carboxylic acid group plays a major role in the inhibition mechanism.[15,16]



Aquastatin A (**1**) belongs to the orcinol *p*-depsides family, and only a few of the orcinol *p*-depsides conjugated with sugars have been found in nature.[17] Chemically, they consist of 2,4-dihydroxy-6-alkylbenzoic acid joined by sugars and another 2,4-dihydroxy-6-alkylbenzoic acid through two potentially labile bonds to hydrolysis (i.e., ester and glycoside bonds). Therefore, a couple of hydrolyzing studies on compound **1** were conducted to check if all these three structural units are required for the inhibitory mechanism. When compound **1** was subjected to enzymatic hydrolysis with β-galactosidase, the aglycone moiety of compound **1** was obtained, and the structure was identified by analysis of its MS and ^{1}H NMR data. The resulting hydrolysis product **3** was then evaluated for its PTP1B inhibitory effect, and the observed IC_{50} value (0.22 μM) suggested that the galactose moiety in compound **1** does not play any role in the inhibition mechanism. Another hydrolysis product **4** was obtained from the basic hydrolysis condition (1 N NaOH), and the structure was identified by analysis of its MS and ^{1}H NMR data. Compound **4** also strongly inhibited the hydrolysis of the *p*NPP catalyzed by PTP1B in a dose-dependent manner with IC_{50} values of 0.59 μM. On the other hand, the other basic hydrolysis product, 2,4-dihydroxy-6-methylbenzoic acid,

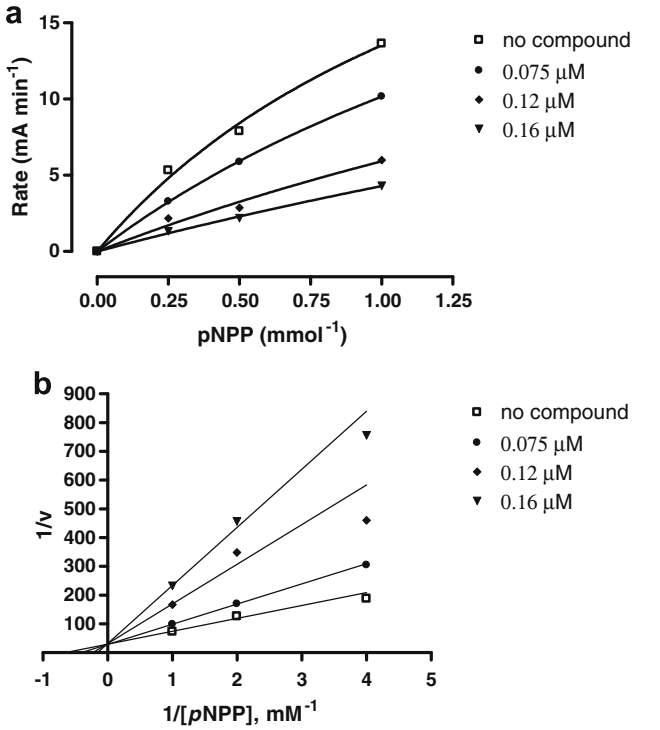


Figure 1. Substrate titration studies using the small substrate *p*NPP revealed that compound **1** is a classical competitive inhibitor that inhibits substrate binding (constant K_m), but not substrate catalysis (V_{max}). (a) Velocity measurements performed with PTP1B in the presence of increasing concentrations of compound **1**. (b) The Lineweaver–Burk transformations of the data from (a). Concentrations (μM) of **1** are indicated in the figure. Data are expressed as mean initial velocity for $n = 3$ replicates at each substrate concentration.

showed only 45% inhibition of PTP1B activity at the 178 μM level. Taken these observations together, it was suggested that 2,4-dihydroxy-6-pentadecylbenzoic acid is a critical pharmacophore of aquastatin A (**1**) in the inhibition of PTP1B activity. 2,4-Dihydroxy-6-pentadecylbenzoic acid, named corticiolic acid, has been reported as a constituent of several fungal species,[18] but no biological activity of the compound has been reported.

In the development of PTP1B inhibitors from natural products or synthetic counter parts, selective inhibition of PTP1B over other PTPs is one of the biggest issues. Therefore, the specific inhibitory activities of compound **1** toward a small panel of PTPs including cytosolic PTPs, T-cell protein tyrosine phosphatase (TCPTP) and src homology phosphatase-2 (SHP-2), and receptor-like PTP, leukocyte antigen-related phosphatase (LAR), and CD45 were evaluated. As shown in Table 2, compound **1** displayed significant selectivity for PTP1B over SHP-2, LAR, and CD45 tyrosine phosphatase (CD45), but only ~2.5-fold over TCPTP. It has been reported that PTP1B and TCPTP share a high degree of homology within the catalytic active site cleft, imposing a major problem in finding selective inhibitors for PTP1B.

As noted previously, most of reported PTP1B inhibitors suffer from drawbacks, such as lack of selectivity and bioavailability.

Table 1
PTP1B inhibitory activity of compounds **1**–**4**

Compound	PTP1B inhibitory activity (IC_{50}, μM)
1	0.19
2	17
3	0.22
4	0.59
Ursolic acid[a]	2.5

[a] Positive control.

Table 2
Selectivity of compound **1** against a panel of PTPs

PTPs	IC_{50} (μM)
PTP1B	0.19
TCPTP	0.51
SHP-2	>44
LAR	>44
CD45	>44

One reason for this is that most of these compounds were developed to target the positively charged active site of PTP1B, thus leading to low cell permeability.[13] Second, most PTPs are known to share a highly conserved catalytic domain that can be broadly inhibited by nonspecific inhibitors.[8] Therefore, the development of novel PTP1B inhibitors with improved target-specificity and bioavailability is still necessary. In this study, aquastatin A (**1**) was identified as competitive inhibitor of PTP1B, and the hydrolyzing studies on the compound led to the identification of dihydroxypentadecyl benzoic acid as a key pharmacophore. Aquastatin A (**1**) showed modest but selective inhibitory activity for PTP1B over other PTPs such as TCPTP, SHP-2, LAR and CD45. In addition, the pentadecyl moiety in the molecule could aid in its ability to diffuse into target cells to inhibit intracellular PTPs. Therefore, aquastatin A (**1**) and/or the essential structural feature identified in this study could be viewed as potential lead compounds for the treatment of diabetes and obesity. Further optimization of the structures with aiming their selective potency and efficacy in vivo would be beneficial.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.025.

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