Notes

Vanillic Acid Derivatives from the Green Algae *Cladophora socialis* As Potent Protein Tyrosine Phosphatase 1B Inhibitors

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A novel vanillic acid derivative (**1**) and its sulfate adduct (**2**) were isolated from a green algae, *Cladophora socialis*. The structures of **1** and **2** were elucidated from NMR and HRESIMS experiments. Both compounds showed potent inhibitory activity against protein tyrosine phosphatase 1B (PTP1B), an enzyme involved in the regulation of insulin cell signaling. Compounds **1** and **2** had IC_{50} values of 3.7 and 1.7 μM, respectively.

Protein tyrosine phosphatases (PTPs) are key regulators of tyrosine phosphorylation-dependent cell signaling.[1] As a result the perturbation of PTP activity has been linked to various diseases, such as immune diseases,[1] cancer,[2–5] neurological disorders,[6,7] infectious diseases,[8–10] diabetes,[11] osteoporosis,[12] and obesity.[11] It has been discovered that one subtype of PTPs, protein tyrosine phosphatase 1B (PTP1B), is involved both physiologically and pathologically in regulating the signaling of the insulin receptor.[13,14] Knockout studies in mice have shown that PTP1B-deficient mice displayed enhanced insulin sensitivity and resistance to diet-induced obesity.[15] PTP1B thus becomes a promising target in the treatment of type 2 diabetes and obesity.

The development of small-molecule inhibitors of PTP1B has attracted considerable attention in both academic research and pharmaceutical investigation. A number of reports describing PTP1B inhibitors have appeared in the last decade.[16–19] More recently, a variety of α,α-difluoromethylenephosphonic acid-bearing molecules have been reported as selective inhibitors.[20,21] The development of a naphthoic acid derivative as a potent and selective PTP1B inhibitor by using NMR-based screening and linked-fragment strategy has also been recently reported.[13]

High-throughput screening identified the extract from the green algae *Cladophora socialis* with inhibitory activity against PTP1B. Bioassay-guided fractionation and purification led to the isolation of the vanillic acid derivative **1** and its sulfate adduct, **2**.

Dried algal material (50 g) was extracted exhaustively with CH_2Cl_2 and MeOH. The combined CH_2Cl_2 and MeOH extract (4.2 g) was chromatographed on a reversed-phase HPLC column using a gradient from TFA in H_2O (1%) to TFA in MeOH (1%). Further purification of the bioactive HPLC fractions by Diol chromatography yielded a vanillic acid derivative (**1**) and its sulfated adduct (**2**) (Figure 1).

Compound **1** was isolated as an amorphous solid. The UV spectrum of **1** displayed absorption maxima at 230, 260, and 294 nm, suggesting the presence of an oxygen-substituted aromatic system. IR peaks at 3106 (broad) and 1694 cm^{-1} indicated the presence of hydroxyl and carbonyl functionalities. The ES negative-ion mass spectrum of **1** showed a strong $M - H^+$ peak at *m/z* 459. HRESIMS measurement on the $M - H^+$ ion (*m/z* 459.0726), in combination with ^1H and ^{13}C NMR data (Table 1), supported the molecular formula of $C_{25}H_{16}O_9$ with 18 double-bond equivalents.

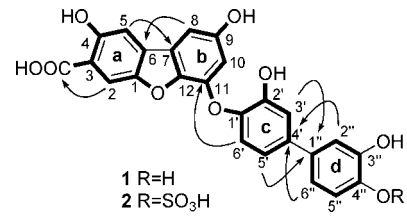

Figure 1. Chemical structures for **1** and **2**; substructures **a**, **b**, **c**, **d** (in bold), and the HMBC correlations for **1**.

The ^1H NMR spectrum of **1** showed signals due to five phenolic OH groups between δ 9 and 11 and 10 aromatic protons including two singlets, six doublets, and two doublets of doublets. All 25 carbons were observed in the ^{13}C NMR experiments (HSQC and HMBC). These included 10 protonated sp^2 carbons, nine oxygen-bearing aromatic carbons, five sp^2 quaternary carbons, and one carbonyl.

Detailed analysis of the ^1H and ^{13}C NMR spectra with the aid of COSY, HSQC, and HMBC experiments (Table 1) established the partial structures **a**, **b**, **c**, and **d** (Figure 1). The connectivities between **a**, **b**, **c**, and **d** were afforded by further analysis of the HMBC correlations (Figure 1). The correlations from H-5 to C-7 and from H-8 to C-6 established the connectivity between **a** and **b**. The correlation from H-6′ to C-11 established the connectivity between C-11 in **b** and C-1′ in **c** through an ether linkage. Further correlations from H-3′ and H-5′ to C-1″ defined the connectivity of **c** and **d**. This linkage was also confirmed by the correlations from H-2″ and H-6″ to C-4′. The correlation from H-2 to the carbonyl carbon at δ 161.1 indicated a carboxylic acid functionality at C-3 in **a**. However no correlations were observed to suggest the ether linkage; therefore, the formation of a furan ring system between **a** and **b** was inferred. The linkage and the final definition of **1** was proposed on the basis of the consideration of the upfield shift of C-1 and C-12 chemical shifts, the molecular formula of **1**, and its double-bond equivalents.

Compound **2**, the sulfate adduct of **1**, was also isolated as an amorphous solid. The ES negative-ion mass spectrum of **2** showed a strong $[M - H]^-$ peak at *m/z* 539 and $[M - SO_3H]^-$ peak at *m/z* 459. An increase of the voltage in mass measurement resulted in an increase in the intensity of the $[M - SO_3H]^-$ peak. The comparison of the ^1H NMR spectroscopic data between **1** and **2** (Table 1) indicated the phenol group at C4″ in **1** was replaced by a sulfate group in **2**.

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Table 1. NMR Spectroscopic Data for Compounds **1** and **2**

	1			2
position	δ_H (J in Hz)[a]	δ_C,[b] multi.	HMBC correlations	δ_H (multi., J Hz)[a]
1		146.9, qC		
2	7.55, s	114.0, CH	C1, C3, C4, C6, COOH	7.55, s
3		112.8, qC		
4		152.6, qC		
5	7.38, s	107.9, CH	C1, C3, C4, C6, C7	7.37, s
6		128.1, qC		
7		118.6, qC		
8	6.92, d (1.8)	98.7, CH	C6, C9, C10, C12	6.89, d (1.8)
9		154.2, qC		
10	6.55, d (1.8)	104.2, CH	C8, C9, C11, C12	6.37, d (1.8)
11		145.9, qC		
12		134.0, qC		
1′		141.1, qC		
2′		148.7, qC		
3′	7.14, d (1.8)	114.6, CH	C1′, C2′, C5′, C1″	7.10, d (1.8)
4′		137.8, qC		
5′	7.04, dd (8.4, 1.8)	121.3, CH	C1′, C3′, C1″	6.95, dd (8.5, 1.8)
6′	7.00, d (8.4)	116.8, CH	C11, C1′, C2′, C4′, C5′	7.01, d (8.5)
1″		130.6, qC		
2″	6.96, d (1.8)	113.7, CH	C4″, C6″	6.95, d (1.8)
3″		145.5, qC		
4″		144.6, qC		
5″	6.80, d (7.8)	115.5, CH	C1″, C3″	7.25, d (8.0)
6″	6.87, dd (8.4, 1.8)	116.9, CH	C6′, C2″, C4″	6.88, dd (8.0, 1.8)
COOH		161.1, qC		

[a] Spectra were recorded at 600 MHz for ^{1}H using d_6-DMSO as solvent. [b] Spectra were recorded at 150 MHz for ^{13}C using d_6-DMSO as solvent.

Structurally, compounds **1** and **2** possess a unique biphenyl functionality, which is rare in natural products. Biologically, they both showed potent PTP1B inhibition. From the concentration–response curves of **1** and **2** the IC_{50} values were determined to be 3.71 and 1.70 μM, respectively.

Previous studies indicated that PTP1B has two binding sites: a catalytic and a noncatalytic site.[21] Compounds that can simultaneously associate with both sites are expected to exhibit enhanced affinity and specificity.[21] NMR-based screening suggested that a potent PTP1B inhibitor would preferably have two hydrophobic moieties as the two binding sites with a hydrophobic linker in between.[13] The presence of an acidic functionality in both binding sites would favor the binding of a ligand.[13] For compound **1**, we speculate that the dibenzofuran and the phenyl ring systems at each end of the molecule act as two binding sites, and the phenyl ring in the middle acts as the linker. The COOH at C3 and phenolic OH at C4″ may contribute to the binding, while the presence of a more acidic sulfate functionality at C4″ in **2** may enhance its activity.

Experimental Section

General Experimental Procedures. UV and IR were measured with a Camspec M501 single beam scanning UV/vis spectrophotometer and Bruker Tensor 27 FT-IR spectrometer, respectively. NMR spectra were recorded at 30 °C on a Varian Inova 600 MHz spectrometer in d_6-DMSO (^{1}H δ 2.50 and ^{13}C δ 39.5 ppm). Mass spectra were acquired using a Mariner TOF biospectrometer. High-resolution mass measurement was acquired on a Bruker Daltonics Apex III 4.7e Fourier transform mass spectrometer, fitted with an Apollo API source. The HPLC system included a Waters 600 pump fitted with a 996 photodiode array detector and Gilson FC204 fraction collector. A Hypersil Betasil C_{18} 5 μm column (150 × 21.2 mm) was used for semipreparative HPLC. Diol column chromatography was performed with 40–70 μM IST Diol. Solvents for extraction and chromatography were Omnisolv HPLC grade. Purified recombinant PTP1B was obtained from AstraZeneca (Alderley Park, UK). Suramin, Bis-Tris, Triton X-100, EDTA, NaOH, and DTT were from Sigma (St. Louis, MO). *p*-NPP was obtained from Roche Diagnostics (Mannheim, Germany). Assays were carried out in clear flat-bottomed 384-well microtiter plates from BD Bioscience (San Jose, CA).

Plant Material. *Cladophora socialis* was supplied by the Queensland Museum. A voucher sample, G327199, is deposited at the Queensland Museum, Brisbane, Australia.

Isolation and Purification. Dried algal material (50 g) was extracted exhaustively with CH_2Cl_2 and MeOH. The combined extract (4.2 g) was chromatographed by semipreparative HPLC on a Hypersil Betasil C18 5 μM column (150 × 21.2 mm) with a flow rate of 9 mL/min. A gradient elution from 100% H_2O (1% TFA) to 100% MeOH (1% TFA) over 34 min was applied. Sixty fractions were collected, and the active fractions 19 to 29 were combined. The combined fractions were then chromatographed on Diol with a CH_2Cl_2/EtOAc gradient (3:2 to 1:1 to 2:3 to 1:4) to yield **1** (3.2 mg, 0.0064% dry weight) and **2** (0.9 mg, 0.0018% dry weight).

Compound 1: amorphous solid; UV (MeOH) γ_{max} (log ε) 230 (6.42), 260 (6.38), 294 (6.20) nm; IR (film) ν_{max} 3106 (br), 2918, 2850, 2358, 2339, 1694, 1681, 1587, 1496, 1442, 1386, 1180, 1118, 1102, 1063, 1046 cm^{-1}; full ^{1}H and ^{13}C assignments were made from ^{1}H, COSY, HSQC, and HMBC NMR experiments, and the data are summarized in Table 1; HRESIMS *m/z* 459.0726 ([M – H$^+$]$^-$) (calcd for $C_{25}H_{15}O_9$, 459.0722).

Compound 2: amorphous solid; UV (MeOH) γ_{max} (log ε) 230 (6.42), 260 (6.40), 294 (6.10) nm; IR (film) ν_{max} 3106 (br), 2918, 2850, 2360, 2340, 1694, 1681, 1587, 1496, 1442, 1386, 1310, 1180, 1120, 1102, 1063, 1046, 750 cm^{-1}; full ^{1}H assignment was made by comparison with that of **1**, and the data are summarized in Table 1; HRESIMS *m/z* 539.0290 ([M – H$^+$]$^-$) (calcd for $C_{25}H_{15}O_{12}S$, 538.9817).

Bioassay. Compounds dissolved in DMSO (final concentration 1.5%) were added to microplates. PTP1B enzyme (40 μL, final concentration 1 μg/mL) in assay buffer (50 mM Bis-Tris, 2 mM EDTA, 50 mM DTT, 0.1% Triton X-100, pH 7) was added to each well. The reaction was initiated with addition of substrate, *p*-NPP (final concentration 0.4 mM), in assay buffer. The plate was incubated at ambient temperature (~22 °C) for 10 min. The reaction was stopped with the addition of 10 μL of NaOH (1 M), and the absorbance at 405 nm was read for each well on a Tecan Ultra plate reader (Salzberg, Austria).

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