Protein Tyrosine Phosphatase-1B Inhibitory Activity of Isoprenylated Flavonoids Isolated from *Erythrina mildbraedii*[⊥]

MinKyun Na,[†] JunPil Jang,[†] Dieudonné Njamen,[‡] Joseph Tanyi Mbafor,[§] Zacharias Tanee Fomum,[§] Bo Yeon Kim,[†] Won Keun Oh,*[,†] and Jong Seog Ahn[†]

Korea Research Institute of Bioscience and Biotechnology (KRIBB), 52 Eoun-dong, Yuseong-gu, Daejeon 305-333, Korea, Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon, and Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon

Received April 27, 2006

Inhibition of protein tyrosine phosphatase-1B (PTP1B) has been proposed as a therapy for treatment of type-2 diabetes and obesity. Bioassay-guided fractionation of an EtOAc-soluble extract of the root bark of *Erythrina mildbraedii*, using an in vitro PTP1B inhibitory assay, resulted in the isolation of three new isoprenylated flavonoids, abyssinone-IV-4′-*O*-methyl ether (**2**), 7-hydroxy-4′-methoxy-3′-(3-hydroxy-3-methyl-*trans*-but-1-enyl)-5′-(3-methylbut-2-enyl)flavanone (**3**), and abyssinone-VI-4-*O*-methyl ether (**6**), along with six known flavonoids, abyssinone-V-4′-*O*-methyl ether (**1**), abyssinone-V (**4**), abyssinone-IV (**5**), sigmoidin E (**7**), 4′-hydroxy-5,7-dimethoxyisoflavone (**8**), and alpinumisoflavone (**9**). Compounds **1** and **2**, **4**−**7**, and **9** inhibited PTP1B activity, with IC_{50} values ranging from 14.8 ± 1.1 to 39.7 ± 2.5 μM. On the basis of the data obtained, flavanones and chalcones with isoprenyl groups may be considered as a new class of PTP1B inhibitors.

Binding of insulin to the extracellular α-subunit of insulin receptor (IR) triggers a conformational change that activates the intrinsic tyrosine kinase activity of the β-subunit via autophosphorylation of specific tyrosine residues. This results in the phosphorylation of IR substrates (IRS) 1−4, which then activates several signaling cascades leading to biological responses, such as glucose transport into the cell and glycogen synthesis.[1,2] Protein tyrosine phosphatases (PTPs) are responsible for the dephosphorylation of tyrosine residues and are considered negative regulators of insulin signaling. Although several PTPs such as PTP-α, leukocyte antigen-related tyrosine phosphatase (LAR), and SH2-domain-containing phosphotyrosine phosphatase (SHP2) have been implicated in the regulation of insulin signaling, there is substantial evidence supporting protein tyrosine phosphatase-1B (PTP1B) as the critical PTP-controlling insulin signaling pathway.[1,2] PTP1B can interact with and dephosphorylate the activated insulin receptor as well as insulin receptor substrate proteins.[1,2] Its overexpression has been shown to inhibit the IR signaling cascade, and increased expression of PTP1B occurs in the insulin-resistant states.[3] Furthermore, recent genetic evidence has shown that PTP1B gene variants are associated with changes in insulin sensitivity.[4] As with the insulin signaling pathway, the leptin signaling pathway can be attenuated by PTPs, and there is compelling evidence that PTP1B is also involved in this process.[1] Therefore, it has been suggested that compounds that reduce PTP1B activity or expression levels can be used for treating not only type-2 diabetes but also obesity. Although there have been a number of reports on the design and development of PTP1B inhibitors,[1,2,5] new types of such compounds with suitable pharmacological properties remain to be discovered. Since plants are a promising source for the development of new PTP1B inhibitors,[2,6] we have undertaken a screen of hundreds of plant extracts against this biological target.

During this screening effort we found that an EtOAc-soluble extract of the root bark of *Erythrina mildbraedii* Harms inhibited PTP1B activity (>70% inhibition at 30 μg/mL). The genus *Erythrina* of the family Leguminosae comprises over 110 species of trees and shrubs that are widely distributed in tropical and subtropical regions, and representative species have been used in indigenous medicine.[7] Alkaloids, pterocarpans, and other flavonoids have been reported as constituents of this genus and have been found to possess a wide range of biological activities that include anti-HIV, antioxidant, antimicrobial, and anti-inflammatory activities.[8−11] Our previous study has demonstrated that a pterocarpan, erycristagallin, isolated from *E. mildbraedii*, reduced phospholipase A_2-induced paw edema in mice and inhibited 12-*O*-tetradecanoylphorbol 13-acetate-induced ear edema, possibly through inhibition of 5-lipoxygenase.[11c] Despite a number of studies on the chemical constituents and biological activities of the genus *Erythrina*, there have been few phytochemical investigations on *E. mildbraedii* previously. Bioassay-guided fractionation of an EtOAc-soluble extract of the root bark of this plant has led to the isolation of a series of isoprenylated flavonoids, including three new compounds (**2**, **3**, and **6**), as the active principles. In this paper, we describe the isolation and structure elucidation of these compounds and the evaluation of their PTP1B inhibitory activity.

Results and Discussion

Bioactivity-guided fractionation of the EtOAc-soluble extract of *E. mildbraedii*, using an in vitro PTP1B inhibitory assay, yielded six isoprenylated flavanones, including the new compounds abyssinone-IV-4′-*O*-methyl ether (**2**) and 7-hydroxy-4′-methoxy-3′-(3-hydroxy-3-methyl-*trans*-but-1-enyl)-5′-(3-methylbut-2-enyl)flavanone (**3**), as well as abyssinone-V-4′-*O*-methyl ether (**1**),[12] abyssinone-V (**4**),[12] abyssinone-IV (**5**),[10a,13] and sigmoidin E (**7**),[14] and a new chalcone, abyssinone-VI-4-*O*-methyl ether (**6**), and two isoflavonones, 4′-hydroxy-5,7-dimethoxyisoflavone (**8**)[15] and alpinumisoflavone (**9**).[16] The structures of the known compounds were determined by 1D and 2D NMR analysis and confirmed by comparing the physical and spectroscopic data with those in the literature ($[\alpha]_D^{25}$, MS, and NMR).

Compound **2** was obtained as a white powder, $[\alpha]_D^{25}$ −47.2 (*c* 0.2, MeOH). A molecular formula of $C_{26}H_{30}O_4$ was determined for this compound from the quasimolecular ion peak at *m*/*z* 407.2191 $[M + H]^+$ (calcd for $C_{26}H_{30}O_4H$, 407.2216), obtained by HRESIMS. The ^1H NMR spectrum of **2** (Table 1) displayed the characteristic signals for a flavanone at δ_H 5.38 (1H, dd, J =

[⊥] Part 48 in the series "Erythrina Studies".

* To whom correspondence should be addressed. Tel: +82-42-860-4295. Fax: +82-42-860-4595. E-mail: wkoh@kribb.re.kr.

[†] KRIBB.

[‡] Department of Animal Biology and Physiology, University of Yaoundé I.

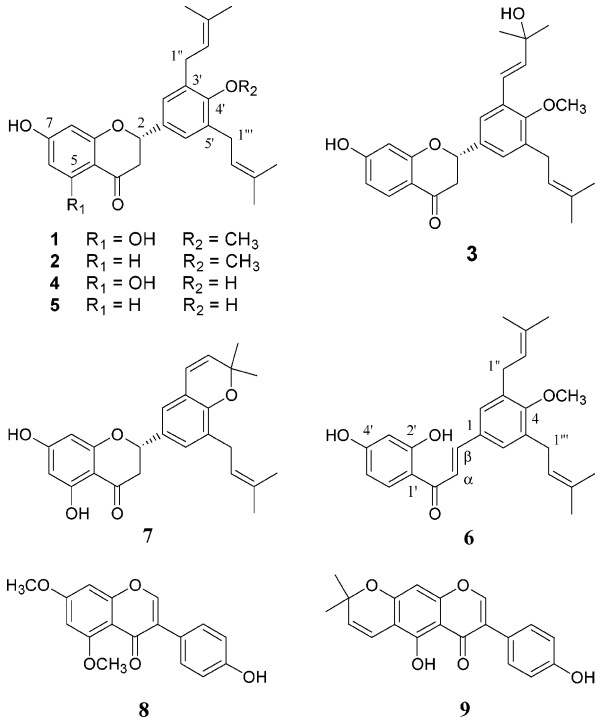
[§] Department of Organic Chemistry, University of Yaoundé I.

10.1021/np0601861 CCC: $33.50
Published on Web 10/21/2006

Table 1. NMR Spectroscopic Data (400 MHz) for Compounds **2**, **3**, and **6** in $CDCl_3$

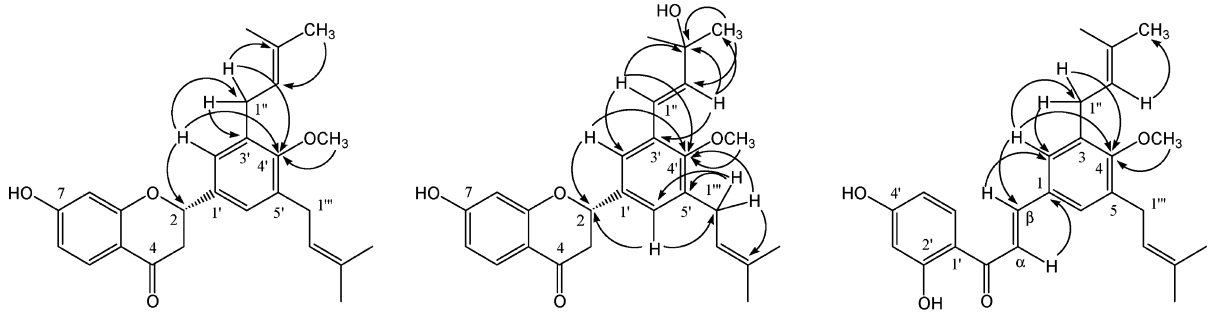
	2		**3**		**6**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1					130.9	
2	80.2	5.38, dd (13.6, 2.8)	79.9	5.39, dd (13.6, 2.8)	128.6	7.33, s
3_{ax}	44.1	3.06, dd (16.8, 13.6)	44.3	3.05, dd (16.8, 13.6)	135.8	
3_{eq}		2.80, dd (16.8, 2.8)		2.81, dd (16.8, 2.8)		
4	191.3		190.9		159.1	
5	129.6	7.87, d (8.4)	129.5	7.87, d (8.4)	135.8	
6	110.6	6.54, dd (8.4, 2.4)	110.6	6.55, dd (8.4, 2.0)	128.6	7.33, s
7	163.9		163.6			
8	103.7	6.47, d (2.4)	103.5	6.48, d (2.0)		
9	162.8		162.7			
10	115.4		115.1			
α					119.1	7.44, d (16.0)
β					145.0	7.83, d (16.0)[a]
C=O					192.3	
1′	134.4		134.5		114.9	
2′	126.1	7.12, s	122.5	7.46, d (2.0)	166.7	
3′	135.5		130.5		103.8	6.44, br s[a]
4′	156.8		156.2		162.8	
5′	135.5		135.8		107.8	6.45, dd (8.4, 2.0)[a]
6′	126.1	7.12, s	127.4	7.16, d (2.0)	132.1	7.84, d (8.4)[a]
1″	28.6	3.40, br d (7.2)	125.0	6.91, d (16.4)	28.6	3.40, br d (6.8)
2″	122.9	5.30, m	134.8	6.37, d (16.4)	122.7	5.31, m
3″	133.1		82.7		133.4	
4″	26.0	1.76, br s	24.4	1.49, br s	26.0	1.79, br s
5″	18.1	1.74, br s	24.4	1.49, br s	18.2	1.77, br s
1‴	28.6	3.40, br d (7.2)	28.4	3.39, br d (6.8)	28.6	3.40, br d (6.8)
2‴	122.9	5.30, m	122.3	5.28, m	122.7	5.31, m
3‴	133.1		133.1		133.4	
4‴	26.0	1.76, br s	25.8	1.76, br s	26.0	1.79, br s
5‴	18.1	1.74, br s	17.9	1.74, br s	18.2	1.77, br s
OMe	61.1	3.76, s	61.4	3.75, s	61.3	3.77, s

[a] Signals partially overlapped were distinguished when measured in CD_3OD (see Experimental Section).



13.6, 2.8 Hz, H-2), 3.06 (1H, dd, J = 16.8, 13.6 Hz, H-3_{ax}), and 2.80 (1H, dd, J = 16.8, 2.8 Hz, H-3_{eq}) and for two isoprenyl groups at δ_H 5.30 (2H, m), 3.40 (4H, br d, J = 7.2 Hz), 1.76 (6H, br s), and 1.74 (6H, br s).[12] The observation of a doublet (δ_H 7.87, d, J = 8.4 Hz), a doublet of doublets (δ_H 6.54, dd, J = 8.4, 2.4 Hz), and a doublet (δ_H 6.47, d, J = 2.4 Hz) implied the A-ring substitution pattern for **2** as shown. Two identical singlet signals at δ_H 7.12 were assignable to symmetric *meta*-protons in the B ring. The presence of a methoxy group was shown by a sharp signal at δ_H 3.76. This evidence strongly suggested that compound **2** is a methyl ether derivative of abyssinone-IV (**5**),[10a,13] which was also isolated in this study. As expected, the signals appearing in the ^{13}C NMR spectrum (Table 1) were very similar to those of **5**.[13] When compared to **5**, the C-4′ signal of **2** showed a downfield chemical shift (3.6 ppm), and an additional resonance for a methoxy group appeared at δ_C 61.1. This suggested that the methoxy group is substituted at C-4′, which was further supported by the HMBC NMR spectroscopic correlations from the methoxy protons to a quaternary carbon (δ_C 156.8, C-4′) and from H-2′ (δ_H 7.12, s) and H-1″ (δ_H 3.40, br d) to C-4′ (Figure 1). The absolute configuration of **2** was determined to be 2*S* by analysis of its circular dichroism (CD) spectrum and comparison with literature values.[10a,12] Thus, the structure of the new compound **2** was determined as abyssinone-IV-4′-*O*-methyl ether.

Compound **3** was obtained as a white powder, $[\alpha]_D^{25}$ −47.7 (*c* 0.2, MeOH). A molecular formula of $C_{26}H_{30}O_5$ was determined for this compound from the quasimolecular ion peak at *m*/*z* 423.2168 $[M + H]^+$ (calcd for $C_{26}H_{30}O_5H$, 423.2165), obtained by HRESIMS. The ^1H and ^{13}C NMR data (Table 1) as well as the physical data for **3** closely matched those of **2**. However, the ^1H NMR spectrum showed a spin system that could be assigned to a 3-hydroxy-3-methyl-*trans*-but-1-enyl group [δ_H 6.91 (1H, d, J = 16.4 Hz), 6.37 (1H, d, J = 16.4 Hz), and 1.49 (6H, br s)].[12b] This was supported by the ^{13}C NMR spectrum, displaying signals of two olefinic carbons (δ_C 134.8 and 125.0), one oxygenated carbon (δ_C 82.7), and two methyl groups (δ_C 24.4 overlapped). Unlike **2**, the observation of a pair of *meta*-coupled protons at δ_H 7.46 and 7.16, due to the unsymmetrical substitution pattern of the B ring, implied that one of the isoprenyl groups located at C-3′ and C-5′ of **2** was replaced with a 3-hydroxy-3-methyl-*trans*-but-1-enyl group in **3**. This was further supported by the HMBC correlations from



2 **3** **6**

Figure 1. Key HMBC correlations of compounds **2**, **3**, and **6**.

the olefinic proton at δ_H 6.91 to C-2′ (δ_C 122.5) and C-4′ (δ_C 156.2) (Figure 1). The positions of the methoxy and isoprenyl groups were established by the analysis of HMBC data (Figure 1), where correlations of the methoxy protons at δ_H 3.75 with C-4′ (δ_C 156.2) and of H-1″ (δ_H 3.39) with C-4′ and C-6′ (δ_C 127.4) were observed. Because **3** gave a negative optical rotation similar to **2** and burttinone,[12b] the absolute configuration of **3** was deduced to be 2*S*. Thus, from a biogenetic perspective, the configuration of C-2 was inferred to be the same in both **2** and **3**. Thus, the structure of **3** was determined as 7-hydroxy-4′-methoxy-3′-(3-hydroxy-3-methyl-*trans*-but-1-enyl)-5′-(3-methylbut-2-enyl)flavanone.

Compound **6** was obtained as a yellow powder. A molecular formula of $C_{26}H_{30}O_4$ was determined for this compound from the quasimolecular ion peak at *m*/*z* 407.2204 $[M + H]^+$ (calcd for $C_{26}H_{30}O_4H$, 407.2216), obtained by HRESIMS. The ^{1}H NMR spectrum of **6** displayed characteristic signals for a chalcone at δ_H 7.73 (1H, d, J = 15.2 Hz, H-β) and 7.63 (1H, d, J = 15.2 Hz, H-α) and for two isoprenyl groups at δ_H 5.32 (2H, m), 3.40 (4H, br d, J = 7.2 Hz), and 1.78 (12H, br s), when measured in CD_3-OD.[10a] The ^{1}H NMR spectrum (CD_3OD) also showed a set of signals for a 1,2,4-trisubstituted benzene unit [δ_H 7.94 (1H, d, J = 8.8 Hz), 6.40 (1H, dd, J = 8.8, 2.0 Hz), and 6.26 (1H, d, J = 2.0 Hz)], two identical singlet proton signals at δ_H 7.40 (2H, s), and a methoxy signal at δ_H 3.75 (3H, s). These data strongly suggested that compound **6** is a methyl ether derivative of abyssinone-VI.[10a] The position of the methoxy group was confirmed by the HMBC correlation from the methoxy protons to a quaternary carbon (δ_C 159.1, C-4′) (Figure 1). Accordingly, the structure of **6** was determined as abyssinone-IV-4-*O*-methyl ether.

PTP1B (human, recombinant) was purchased from a commercial supplier, and the enzyme activity was measured using *p*-nitrophenyl phosphate (*p*NPP) as a substrate.[6] All the isolates were assayed for their inhibitory activity against PTP1B, and the results are presented in Table 2. The known PTP1B inhibitors RK-682 (IC_{50} = 4.5 ± 0.5 μM) and ursolic acid (IC_{50} = 3.6 ± 0.2 μM)[6a] were used as positive controls in this assay. Most of the isolates inhibited PTP1B activity in a dose-dependent manner. Of the compounds tested, **5** and **6** exhibited the most potent inhibitory activities, with IC_{50} values of 16.0 ± 1.1 and 14.8 ± 1.1 μM, respectively. Compound **4** (IC_{50} = 39.7 ± 2.5 μM), with one more hydroxyl group substituent at C-5, was less active than **5**. A similar observation was made for compounds **1** and **2**, indicating that addition of a hydroxyl group to C-5 in the A ring may be responsible for a loss of in vitro activity. Compound **3** (IC_{50} > 60 μM), in which an additional hydroxyl group is present at C-3″ of the isoprenyl group, exhibited a significantly lower PTP1B inhibitory activity than **2**. The PTP1B inhibitory activity of **7** was similar to that of **4**, suggesting that cyclization between a hydroxyl group and one of the isoprenyl groups in the B ring may not affect the resultant activity.

To evaluate the role of isoprenyl groups on the B ring, we assayed a group of similarly related flavonoids that are commercially available or were isolated from other plants.[17] The flavanones, naringenin (5,7,4′-trihydroxyflavanone) and liquiritigenin (7,4′-dihydroxyflavanone), and the chalcones, isoliquiritigenin (4,2′,4′-trihydroxychalcone) and 2′,4′-dihydroxy-4-methoxychalcone, in which isoprenyl groups are absent, were not active against PTP1B. Although structure−activity relationships of flavanones bearing isoprenyl groups were not thoroughly investigated, our results indicate that substitution of isoprenyl groups on the B ring may be important for PTP1B inhibitory activity in vitro, and introduction of one more hydroxyl group to C-5 of the A ring or one of the isoprenyl groups in the B ring may be responsible for a loss of such activity. Likewise, the presence of isoprenyl groups on the A ring seems to be essential for the activity of chalcones.

Table 2. Inhibitory Activity of Compounds **1**−**9** and Related Compounds against PTP1B

compound	PTP1B inhibitory activity IC_{50} (μM) [a,b]
1	26.3 ± 1.5
2	21.2 ± 1.6
4	39.7 ± 2.5
5	16.0 ± 1.1
6	14.8 ± 1.1
7	39.2 ± 1.9
9	41.5 ± 2.4
RK-682[c]	4.5 ± 0.5
ursolic acid[c]	3.6 ± 0.2

[a] IC_{50} values were determined by regression analyses and expressed as mean ± SD of three replicates. [b] Compounds **3** and **8** and the reference compounds, naringin, liquiritigenin, isoliquiritigenin, and 2′,4′-dihydroxy-4-methoxychalcone, were inactive (IC_{50} > 60 μM). [c] Positive control.[6a]

Most of the flavanones and chalcones with isoprenyl groups have been isolated from a rather limited number of plant families, inclusive of the Leguminosae.[18] In the last few decades, there have been a number of reports on newly identified prenylated flavanones and chalcones.[10a,12−14,18] However, except for their antimicrobial activity,[10a,13] little is known as to the biological activities of these metabolites. As shown in the present study, both the isoprenylated flavanones and chalcones can be considered as promising classes of PTP1B inhibitors. Therefore, further investigation and optimization of these derivatives might enable the preparation of new PTP1B inhibitors potentially useful in the treatment of type-2 diabetes and obesity.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Optical rotations were determined on a JASCO P-1020 polarimeter using a 100 mm glass microcell. UV−vis spectra were taken in MeOH using a Shimadzu spectrophotometer. The CD spectrum was recorded in MeOH on a JASCO J-715 spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian Unity Inova 400 MHz spectrometer using as solvent $CDCl_3$, CD_3OD, or acetone-d_6 (Aldrich)

with TMS as the internal standard. ^{13}C DEPT, $^{1}H-^{1}H$ COSY, NOESY, HMQC, and HMBC NMR spectra were obtained using standard Varian pulse sequences. All accurate mass experiments were performed on a Micromass QTOF2 (Micromass, Wythenshawe, UK) mass spectrometer. Column chromatography was conducted using silica gel 60 (40−63 and 63−200 μm particle size) and RP-18 (40−63 μm particle size) from Merck. Precoated TLC silica gel 60 F_{254} plates from Merck were used for thin-layer chromatography. Spots were visualized using UV light or 10% sulfuric acid. HPLC runs were carried out using a Shimadzu System LC-10AD pump equipped with a model SPD-10Avp UV detector, and an Optima Pak C_{18} column (10 × 250 mm, 10 μm particle size, RS Tech Korea) for semipreparative runs. Naringenin (N5893) was purchased from Sigma-Aldrich Co., while liquiritigenin, isoliquiritigenin, and 2′,4′-dihydroxy-4-methoxychalcone were isolated from *Cercis chinensis*.[17]

Plant Material. The root bark of *E. mildbraedii* was collected in July 1997 in Buea, Southwest Province, Cameroon. The botanical sample was identified and authenticated at the Cameroon National Herbarium (Yaoundé, Cameroon), where a voucher specimen (No. 50452/HNC) has been deposited.

Extraction and Isolation. The dried root bark (5 kg) was extracted with EtOAc at room temperature for two weeks. The EtOAc-soluble extract (IC_{50} = 30.2 μg/mL, 105.0 g) was separated by silica gel column chromatography (10 × 30 cm; 63−200 μm particle size) using a gradient of hexane−EtOAc (from 8:1 to 0:1), then EtOAc−MeOH (from 20:1 to 1:1), to yield five fractions (1−5) according to their TLC profiles. The PTP1B inhibitory activity was concentrated in fraction 1 (IC_{50} = 16.7 μg/mL, 10.0 g), eluted with hexane−EtOAc (from 8:1 to 4:1), which was chromatographed over silica gel (6.5 × 35 cm; 63−200 μm particle size) using a gradient of hexane−EtOAc (from 20:1 to 0:1), to yield six subfractions (fractions 1-1−1-6). Except for fraction 1-1, other fractions (fractions 1-2−1-6) displayed similar bioactivities, with IC_{50} values ranging from 11.4 to 14.5 μg/mL. Fraction 1-2 [eluted with hexane−EtOAc (10:1), 415 mg] was separated by silica gel column chromatography (4 × 27 cm; 40−63 μm particle size) using a stepwise gradient of hexane−acetone (from 9:1 to 8:2; 3 L for each step), to afford five subfractions (fractions 1-2-1−1-2-5). Further purification of fractions 1-2-3 (36 mg) and 1-2-5 (37 mg) by semipreparative HPLC [RS Tech Optima Pak C_{18} column (10 × 250 mm, 10 μm particle size); mobile phase MeOH−H_2O (85:15); flow rate 2 mL/min; UV detection at 254 nm] resulted in the isolation of compounds **8** (2.3 mg; t_R = 16.4 min) and **1** (12.0 mg; t_R = 25.3 min), respectively. Fraction 1-3 [eluted with hexane−EtOAc (8:1), 1957 mg] was separated by reversed-phase C_{18} (RP-18) column chromatography (4.5 × 27 cm; 40−63 μm particle size) using a stepwise gradient of MeOH−H_2O (from 50:50, 60:40, 70:30, 75:25, 80:20, 90:10 to 100:0; 1 L for each step), to afford seven subfractions (fractions 1-3-1−1-3-7). Fraction 1-3-4 [eluted with MeOH−H_2O (75:25), 262 mg] was purified by semipreparative HPLC using a gradient from 85% to 100% MeOH in H_2O over 50 min, to afford 3.4 mg of compound **6** (t_R = 31.2 min). Fraction 1-4 [eluted with hexane−EtOAc (6:1), 717 mg] was subjected to RP-18 column chromatography eluting with a gradient of MeOH−H_2O (from 75:25 to 100:0), followed by HPLC of subfraction 2 using an isocratic solvent system of 70% AcCN in H_2O, to obtain compound **7** (2.3 mg, t_R = 52.0 min). Fraction 1-5 [eluted with hexane−EtOAc (3:1), 853 mg] was chromatographed on a silica gel column (4 × 27 cm; 40−63 μm particle size) using a stepwise gradient of hexane−acetone (85:15, 80:20; 2 L for each step), to yield five subfractions (fractions 1-5-1−1-5-5). Further purification of fraction 1-5-2 (116 mg) by HPLC using an isocratic solvent system of 80% MeOH in H_2O led to the isolation of compound **2** (3.1 mg, t_R = 28.2 min) and crude compound **3** (3.5 mg, t_R = 21.7 min). The latter was purified by additional HPLC using an isocratic solvent of 65% MeOH in H_2O to afford compound **3** (1.6 mg, t_R = 37.2 min). Fraction 1-5-3 [eluted with hexane−acetone (from 85:15 to 80:20), 193 mg] was purified by HPLC using a gradient from 80% to 85% MeOH in H_2O over 30 min, then increased to 100% MeOH over 35 min, to yield 3.8 mg of compound **4** (t_R = 31.3 min). Compound **5** (7.5 mg) was isolated from fraction 1-5-3 [eluted with hexane−acetone (from 85:15 to 80:20), 231 mg] using preparative HPLC, with the mobile phase 85% MeOH.

Abyssinone-IV-4′-*O*-methyl ether (2): white, amorphous powder; mp 72−73 °C; $[\alpha]_D^{25}$ −47.2 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 272 (4.09) nm; CD (*c* 0.038, MeOH; nm) $[\theta]_{330}$ + 0.89, $[\theta]_{303}$ −2.64; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 407.2191 $[M + H]^+$ (calcd for $C_{26}H_{30}O_4H$, 407.2216).

7-Hydroxy-4′-methoxy-3′-(3-hydroxy-3-methyl-*trans*-but-1-enyl)-5′-(3-methylbut-2-enyl)flavanone (3): white, amorphous powder; mp 95−98 °C; $[\alpha]_D^{25}$ −47.7 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 265 (4.00) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 423.2168 $[M + H]^+$ (calcd for $C_{26}H_{30}O_5H$, 423.2165).

Abyssinone-VI-4-*O*-methyl ether (6): yellow, amorphous powder; mp 90−93 °C; $[\alpha]_D^{25}$ −3.2 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 353 (4.27) nm; IR (KBr) ν_{max} 3400, 2930, 1630, 1590, 1440, 1360, 1260, 1130 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.94 (1H, d, *J* = 8.8 Hz, H-6′), 7.73 (1H, d, *J* = 15.2 Hz, H-β), 7.63 (1H, d, *J* = 15.2 Hz, H-α), 7.40 (2H, s, H-2 and 6), 6.40 (1H, dd, *J* = 8.8, 2.0 Hz, H-5′), 6.26 (1H, d, *J* = 2.0 Hz, H-3′), 5.32 (2H, m, H-2″ and 2‴), 3.75 (3H, s, OMe), 3.40 (4H, br d, *J* = 7.2 Hz, H-1″ and 1‴), 1.78 (12H, br s, H-4″, 4‴, 5″, and 5‴); ^{13}C NMR data, see Table 1; HRESIMS *m/z* 407.2204 $[M + H]^+$ (calcd for $C_{26}H_{30}O_4H$, 407.2216).

PTP1B Assay. PTP1B (human, recombinant) was purchased from BIOMOL International LP (Plymouth Meeting, PA). The enzyme activity was measured using *p*-nitrophenyl phosphate (*p*NPP), as described previously.[6] To each of 96 wells in a microtiter plate (final volume: 100 μL) was added 2 mM *p*NPP and PTP1B (0.05−0.1 μg) in a buffer containing 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT), with or without test compounds. Following incubation at 37 °C for 30 min, the reaction was terminated with 10 M NaOH. The amount of produced *p*-nitrophenol was estimated by measuring the absorbance at 405 nm. The nonenzymatic hydrolysis of 2 mM *p*NPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme.

Acknowledgment. This research was supported in part by the grants from the Plant Diversity Research Center of 21st Frontier Research Program (PF0320903-00) of the Ministry of Science and Technology, Technology Development Program for Agriculture and Forestry (AGM0800611) of the Ministry of Agriculture and Forestry of Korea.

References and Notes

(1) Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. *Nat. Rev. Drug Discovery* **2002**, *1*, 696−709.
(2) Bialy, L.; Waldmann, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 3814−*3839*.
(3) Ahmad, F.; Azevedo, J. J.; Cortright, R.; Dohm, G.; Goldstein, B. *J. Clin. Invest.* **1997**, *100*, 449−458.
(4) Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. *Science* **1999**, *283*, 1544−*1548*.
(5) Taylor, S. D.; Hill, B. *Expert Opin. Investig. Drugs* **2004**, *13*, 199−214.
(6) (a) Na, M.; Yang, S.; He, L.; Oh, H.; Kim, B. S.; Oh, W. K.; Kim, B. Y.; Ahn, J. S. *Planta Med.* **2006**, *72*, 261−263. (b) Cui, L.; Na, M.; Oh, H.; Bae, E. Y.; Jeong, D. G.; Rhu, S. E.; Kim, S.; Kim, B. Y.; Oh, W. K.; Ahn, J. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1426−1429. (c) Na, M.; Oh, W. K.; Kim, Y. H.; Cai, X. F.; Kim, S.; Kim, B. Y.; Ahn, J. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3061−3064. (d) Na, M.; Cui, L.; Min, B. S.; Bae, K.; Yoo, J. K.; Kim, B. Y.; Oh, W. K.; Ahn, J. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3273−3276.
(7) Oliver-Bever, B. *Medicinal Plants in Tropical West Africa*; Cambridge University Press: New York, 1981; p 100.
(8) McKee, T. C.; Bokesch, H. R.; McCormick, J. L.; Rashid, M. A.; Spielvogel, D.; Gustafson, K. R.; Alavanja, M. M.; Cardellina, J. H., II; Boyd, M. R. *J. Nat. Prod.* **1997**, *60*, 431−438.
(9) (a) Chawla, A. S.; Jackson, A. H. *Nat. Prod. Rep.* **1989**, *6*, 55−66. (b) Chawla, A. S.; Jackson, A. H. *Nat. Prod. Rep.* **1990**, *7*, 565−575. (c) Juma, B. F.; Majinda, R. R. T. *Phytochemistry* **2004**, *65*, 1397−1404.
(10) (a) Kamat, V. S.; Chuo, F. Y.; Kubo, I.; Nakanishi, K. *Heterocycles* **1981**, *15*, 1163−1170. (b) Njamen, D.; Mbafor, J. T.; Fomum, Z. T.; Kamanyi, A.; Mbanya, J. C.; Recio, M. C.; Giner, R. M.; Máñez, S.; Ríos, J. L. *Planta Med.* **2004**, *70*, 104−107. (c) Chacha, M.; Bojase-Moleta, G.; Majinda, R. R. T. *Phytochemistry* **2005**, *66*, 99−104.

(11) (a) Mitscher, L. A.; Okwute, S. K.; Gollapudi, S. R.; Keshavarz-Shokri, A. *Heterocycles* **1988**, *27*, 2517−2522. (b) Mitscher, L. A.; Okwute, S. K.; Gollapudi, S. R.; Drake, S.; Avona, E. *Phytochemistry* **1988**, *27*, 3449−3452. (c) Njamen, D.; Talla, E.; Mbafor, J. T.; Fomum, Z. T.; Kamanyi, A.; Mbanya, J. C.; Cerdá-Nicolás, M.; Giner, R. M.; Recio, M. C.; Ríos, J. L. *Eur. J. Pharmacol.* **2003**, *468*, 67−74.

(12) (a) Moriyasu, M.; Ichimaru, M.; Nishiyama, Y.; Kato, A.; Mathenge, S. G.; Juma, F. D.; Nganga, J. N. *J. Nat. Prod.* **1998**, *61*, 185−188. (b) Yenesew, A.; Midiwo, J. O.; Miessner, M.; Heydenreich, M.; Peter, M. G. *Phytochemistry* **1998**, *48*, 1439−1443.

(13) Nkengfack, A. E.; Kouam, J.; Vouffo, T. W.; Meyer, M.; Tempesta, M. S.; Fomum, Z. T. *Phytochemistry* **1994**, *35*, 521−526.

(14) Promsattha, R.; Tempesta, M. S.; Fomum, Z. T.; Mbafor, J. T. *J. Nat. Prod.* **1988**, *51*, 611−613.

(15) Sekizaki, H.; Yokosawa, R. *Chem. Pharm. Bull.* **1988**, *36*, 4876−4880.

(16) Olivares, E. M.; Lwande, W.; delle Monache, F.; Marini Bettolo, G. B. *Phytochemistry* **1982**, *21*, 1763−1765.

(17) Na, M. Inhibitory Effect of Constituents from Cercis chinensis on Cellular Aging. Ph.D. Thesis, Chungnam National University, Daejeon, Korea, 2004; pp 42−114.

(18) Barron, D.; Ibrahim, R. K. *Phytochemistry* **1996**, *43*, 921−982.

NP0601861