Bioorganic & Medicinal Chemistry 19 (2011) 3378–3383

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

New 5-deoxyflavonoids and their inhibitory effects on protein tyrosine phosphatase 1B (PTP1B) activity

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ARTICLE INFO

Article history:
Received 22 March 2011
Revised 13 April 2011
Accepted 18 April 2011
Available online 22 April 2011

Keywords:
Erythrina abyssinica
5-Deoxyflavanoid
PTP1B inhibitors

ABSTRACT

In the course of our program to search for protein tyrosine phosphatase 1B (PTPB) inhibitors, five new 5-deoxyflavonoids along with eight known derivatives were isolated from EtOAc layer of the root bark of *Erythrina abyssinica*. Their structures were elucidated on the basis of spectroscopic (IR, UV, MS, CD, 1D- and 2D-NMR) and physicochemical analyses. All isolates exhibited moderate inhibitory effects on the enzyme assay with IC_{50} values ranging from 14.9 ± 1.6 to 98.1 ± 11.3 μM. Compounds with prenyl and methoxy groups in the B ring (**1**, **2**, **4**, **8**, and **13**) possessed strong activity (IC_{50} 14.9 ± 1.6 to 19.2 ± 1.1 μM), while compounds (**3**, **5**, and **9**) with 2,2-dimethylpyrano ring showed less inhibitory effect (IC_{50} 22.6 ± 2.3 to 72.9 ± 9.7 μM). These results suggest that prenyl and methoxy groups may be responsible for the increase on the activity of 5-deoxyflavonoids against PTP1B, but the presence of 2,2-dimethylpyrano ring on the B ring may be induced the decrease of PTP1B inhibitory activity.

1. Introduction

Protein tyrosine phosphatase (PTP) superfamily coordinates with protein tyrosine kinases to regulate a vast array of cellular functions, including proliferation, differentiation, apoptosis, and motility. Of the various PTPs, PTP1B plays a critical role in regulating glucose homeostasis and body weight by acting as a key negative regulator of insulin and leptin signaling pathway, respectively.[1] Its overexpression has been shown to inhibit the insulin receptor (IR) signaling cascade, and increased expression of PTP1B occurs in insulin-resistant states,[2] while PTP1B knockout mice have been shown to increase insulin sensitivity and obesity resistance.[3] Furthermore, recent evidence has shown that the leptin signaling pathway can be attenuated by PTPs, and there are many reports that PTP1B is also involved in this process.[4] Therefore, its has been suggested that compounds that reduce PTP1B activity or expression levels could not only be used as potential therapy of type-2 diabetes but also of obesity.

In the course of our program to search for protein tyrosine phosphatase 1B (PTP1B) inhibitors from plants,[5,6] the genus *Erythrina* using an in vitro assay on PTP1B inhibitory activity were studied.[7,8] We found that an EtOAc-soluble extract of the root bark of *Erythrina abyssinica* exhibit significant activity on PTP1B enzyme assay with IC_{50} value of 25.3 ± 3.6 μg/mL (Table 3). This paper as the continuous interest in the active constituents of *E. abyssinica*[8] describes the isolation and characterization of thirteen 5-deoxyflavonoids (**1**–**13**) as active inhibitory principles on PTP1B enzyme, including two new isoflavanones (**1** and **13**) and three new flavanones (**6**, **9**, and **12**).

2. Results and discussion

Phytochemical study on the EtOAc-soluble extract of *E. abyssinica* using an in vitro PTP1B inhibition assay and repeated column chromatographic separation yielded three new prenylated flavanones (**6**, **9**, and **12**) and two new isoflavanones (**1** and **13**), along with 8 known 5-deoxyflavonoid derivatives (**2**–**5**, **7**–**8**, and **10**–**11**). The structure of the known compounds were determined to be 5-deoxyabyssinin II (**2**),[9] abyssinones III (**3**),[10] 7-hydroxy-2-[4-methoxy-3-(3-methylbut-2-enyl)phenyl]chroman-4-one (**4**),[11] abyssinones V (**5**),[10] abyssinone II (**7**),[10,11] prostratol C (**8**),[12] liquiritigenin (**10**),[13] and liquiritigenin-5′-*O*-methyl ether (**11**)[14] by comparing their physical and spectroscopic data (UV, MS, 1D- and 2D-NMR) with those reported in the literature (Fig. 1).

Compound **1** was obtained as a white amorphous powder with $[\alpha]_D^{25}$ −25.6 (*c* 0.15, MeOH). Its UV spectrum exhibited absorption maxima at 216, 234, 276, and 310 nm. The IR spectrum revealed absorbance bands at 3331 (hydroxy groups), 1670 (conjugated carbonyl group), 1593, and 1242–1033 cm^{-1} (aromatic ring). A

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0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.04.037

molecular formula of $C_{22}H_{24}O_5$ was determined from the molecular ion peak at *m/z* 368.1628 $[M]^+$ obtained by HREIMS. The ^1H and ^{13}C NMR spectra of compound **1** showed to have an isoflavanone skeleton [δ_H 4.17 (1H, dd, *J* = 5.0, 11.0 Hz, H-3), 4.48 (1H, dd, *J* = 5.5, 11.0 Hz, H-2_{eq}), and 4.55 (1H, dd, *J* = 11.0 Hz, H-2_{ax})], together with [δ_C 48.2 (C-3), 71.7 (C-4), and a conjugated carbonyl carbon at δ_C 191.3 (C-4)].[12] In addition, a prenyl group [δ_H 1.67, 1.69 (each 3H, 4″, 5″-Me), 3.22 (2H, br d, *J* = 7.5 Hz, H-1″), and 5.25 (1H, m, H-2″); δ_C 17.8, 25.9 (4″, 5″-Me), 28.4 (C-1″), 123.9 (C-2″), 132.3 (C-3″)], and two methoxy groups [δ_H 3.85 (3H, s), δ_C 56.0 (4′-OCH_3); δ_H 3.90 (3H, s), δ_C 56.3 (6′-OCH_3)] were also observed. The ^1H NMR spectrum also showed the presence of two singlet protons at δ_H 6.71 (1H, s) and δ_H 6.90 (1H, s) assignable to two *para*-coupled aromatic protons in one ring, and a typical ABX aromatic spin system at δ_H 7.82 (1H, d, *J* = 8.5 Hz), 6.63 (1H, dd, *J* = 8.5 and 2.5 Hz), and 6.45 (1H, d, *J* = 2.5 Hz), establishing an *ortho*-, *ortho*/*meta*-, and *meta*-coupling system due to proton H-5, H-6, and H-8 in ring A, respectively. These assignment resembled those of prostratol C,[12] except only for the presence of an additional methoxy group [δ_H 3.90 (3H, s), (δ_C 56.3)] in compound **1**. An HMBC analysis suggested the position of methoxy group at C-2′ from correlations between the methoxy protons (δ_H 3.90, 3H, s) and C-2′ (δ_C 158.5) and C-1′ (δ_C 122.2) (Fig. 2). The configuration at C-3 was inferred to be *S* by its CD spectrum, which presented a negative Cotton effect near 330 nm.[15] On the basic of the above spectroscopic studies, compound **1** was thus determined to be 3(*S*)-7-hydroxy-2′,4′-dimethoxy-5′-(γ,γ-dimethylallyl)isoflavanone, and named erythribyssin E.

The new compounds **6**, **9**, and **12** were isolated as yellowish amorphous powder and possessed negative optical rotation values in MeOH. Their CD spectra exhibited positive Cotton effects near 330 nm and negative Cotton effects near 285–290 nm. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of each compound displayed an AMX spin system for H-2 (δ_H 5.25–5.60), H-3ax (δ_H 2.93–3.09), and H-3eq (δ_H 2.51–2.77), and corresponding carbon signals for C-2 (δ_C 79.8–80.8), and C-3 (δ_C 44.5–44.7), and the ketone carbon resonances at C-4 (δ_C 190.1–190.5). These observations were indicative of a 2(*S*)-flavanone skeleton.[8,16]

The optical rotation value of compound **6** was −14.3 (*c* 0.05, MeOH) and its IR spectrum showed absorption bands at 3038 (OH), 1825–1618 (CHO), and 1497, 1041–609 cm^{-1}. The molecular formula of compound **6**, $C_{16}H_{12}O_5$, was determined from the molecular ion peaks at *m/z* 284.0683 $[M]^+$ obtained by HREIMS. The ^1H NMR spectrum displayed two ABX-type aromatic spin systems. One was assigned to be the A ring from chemical shifts at δ_H 7.75 (1H, d, *J* = 8.5 Hz, H-5), 6.60 (1H, dd, *J* = 2.0, 8.5 Hz, H-6), and 6.46 (1H, d, *J* = 2.0 Hz, H-8), and the other was belonged to the B ring with δ_H 8.00 (1H, d, *J* = 1.5 Hz, H-6′), 7.83 (1H, dd, *J* = 1.5, 8.5 Hz, H-2′), and 7.07 (1H, d, *J* = 8.5 Hz, H-3′). In addition, the ^1H and ^{13}C NMR spectra suggested the presence of an aldehyde group at δ_H 10.10 (1H, s) and the corresponding carbon signal at δ_C 197.9. The HMBC correlations from the aldehyde proton to C-5′ (δ_C 121.8) and C-4′ (δ_C 162.1), and from proton signal of H-6′ at δ_H 8.00 (1H, d, *J* = 1.5 Hz) to the aldehyde carbon (δ_C 197.9) indicated that the aldehyde group is located at C-5′ (Fig. 2). Thus, compound **6** was identified as a new compound, 2(*S*)-7,4′-dihydroxy-3′-formylflavanone, and named as erythribyssin K.

The ^1H and ^{13}C NMR spectra of compound **9** (Tables 1 and 2) exhibited an ABX-type aromatic spin system at δ_H 7.60 (1H, d, *J* = 8.7 Hz, H-5), 6.44 (1H, dd, *J* = 2.1, 8.7 Hz, H-6), and 6.28 (1H, d, *J* = 2.1 Hz, H-8), and an AX spin system δ_H 7.01 (1H, d, *J* = 1.8 Hz, H-6′) and 6.97 (1H, d, *J* = 1.8 Hz, H-2′).[16] In addition, the presences of a 3-hydroxy-2,2-dimethyldihydropyran moiety [δ_H 3.68 (1H, dd, *J* = 8.0, 13.0 Hz), 2.91 (1H, dd, *J* = 13.0, 17.0 Hz), 2.62 (1H, dd, *J* = 8.0, 17.0 Hz), 1.38 (3H, s), and 1.12 (3H, s)] and a prenyl group [δ_H 3.28 (1H, d, *J* = 6.9 Hz), 5.17 (1H, m), 1.69 (3H, s), and 1.71 (3H, s)] were determined in the ^1H NMR spectrum of compound **9**. The HMBC correlations of compound **9** from the aromatic proton H-6′ (δ_H 7.01) to C-1‴ (δ_C 29.5), and from the methylene protons of H-1‴ (δ_H 3.28) to C-5′ (δ_C 121.0) and C-4′ (δ_C 152.0) indicated that the prenyl group was attached to C-5′, and the 2,2-(3-hydroxy)-dimethylpyrano ring was fused to C-3′ and C-4′ due to correlations between two proton signals of H-4″ [δ_H 2.91 (dd, 3.2, 16.8)/2.62 (dd, 8.1, 16.8)] and C-4′ (δ_C 152.0), C-3′ (δ_C 120.2), and C-2′ (δ_C 126.7) (Fig. 2). Hence, compound **9** was determined to be a new compound, 2(*S*)-7-dihydroxy-5′-(γ,γ-dimethylallyl)-[(5″,6″:4′,3′)-(2″,2″-dimethylpyrano)]flavanone, and named erythribyssin G.

Most of the spectroscopic (UV, CD, ^1H and ^{13}C NMR) data for compound **12** were similar to those of compound **9** except for the absence of the signals assignable to the prenyl group in the ^1H and ^{13}C NMR spectra of compound **9**. These results were supported from two ABX aromatic spin systems, which one was assignable to the A ring [δ_H 7.68 (1H, d, *J* = 8.5 Hz, H-5), 6.57 (1H, dd, *J* = 2.0, 8.5 Hz, H-6), and 6.42 (1H, d, *J* = 2.0 Hz, H-8)] and the other was belong to the B ring [δ_H 6.77 (1H, d, *J* = 8.5 Hz, H-5′), 7.26 (1H, br, d, *J* = 8.0 Hz, H-6′), and 7.25 (1H, br s, H-2′)]

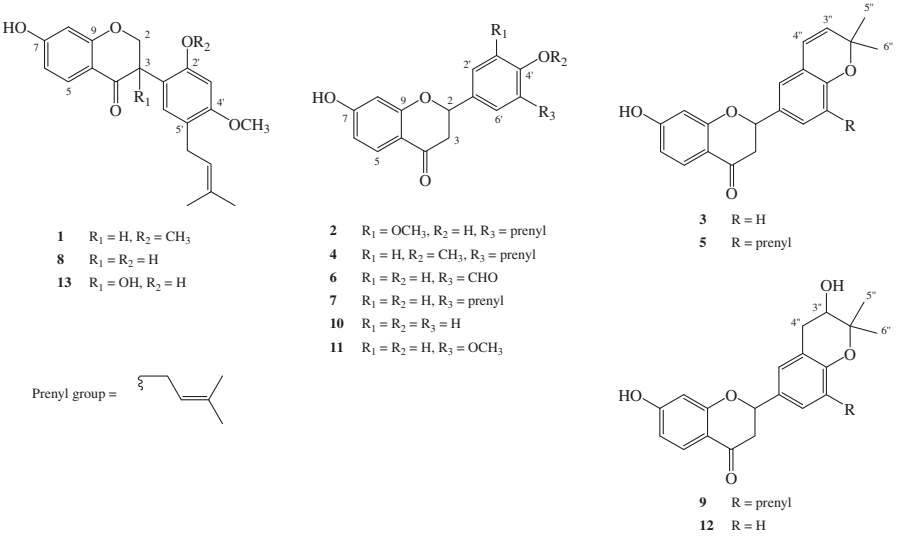

Figure 1. Chemical structure of the isolated compounds **1**–**13**.

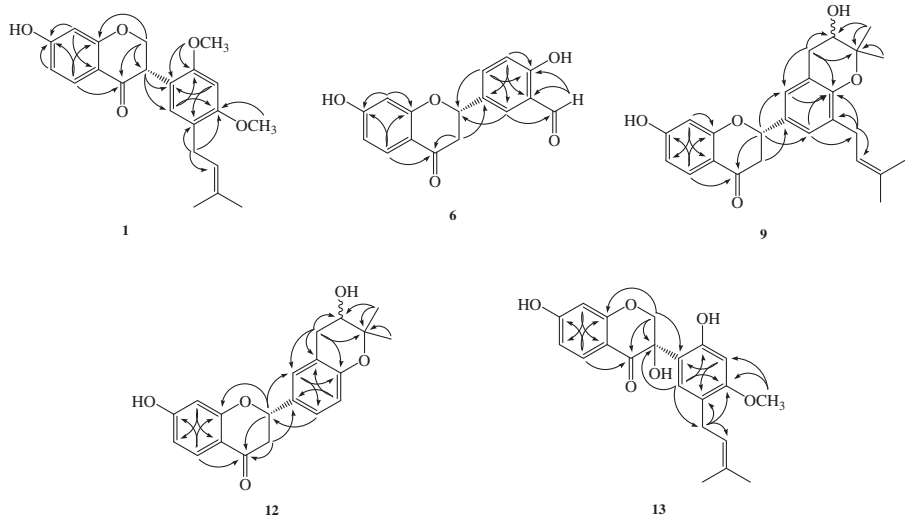

Figure 2. Key HMBC correlations for the new compounds **1**, **6**, **9**, **12**, and **13**.

Table 1
^{1}H NMR spectroscopic data for new compounds **1**, **6**, **9**, **12**, and **13**

No.	**1**[a] δ_H (J in Hz)	**6**[a] δ_H (J in Hz)	**9**[b] δ_H (J in Hz)	**12**[b] δ_H (J in Hz)	**13**[b] δ_H (J in Hz)
2	4.55 (dd, 11.0)	5.60 (dd, 2.0, 13.0)	5.25 (dd, 3.0, 13.2)	5.42 (dd, 3.0, 13.0)	4.85 (d, 11.5)
	4.48 (dd, 5.0, 11.0)				4.34 (d, 11.5)
3	4.17 (dd, 5.0, 11.0)	3.09 (dd, 13.0, 17.0)	2.93 (dd, 13.2, 16.8)	3.05 (dd, 13.0, 17.0)	
		2.77 (dd, 3.0, 17.0)	2.51 (dd, 3.0, 16.8)	2.65 (dd, 3.0, 17.0)	
4					
5	7.82 (d, 8.5)	7.75 (d, 8.5)	7.60 (d, 8.7)	7.68 (d, 8.5)	6.76 (d, 7.5)
6	6.63 (dd, 2.5, 8.5)	6.60 (dd, 2.0, 8.5)	6.44 (dd, 2.1, 8.7)	6.57 (dd, 2.0, 8.5)	6.61 (br d, 7.5)
7					
8	6.45 (d, 2.0)	6.46 (d, 2.0)	6.28 (d, 2.1)	6.42 (d, 2.0)	6.40 (br s)
9					
10					
1′					
2′		7.83 (dd, 2.0, 8.5)	6.97 (d, 1.8)	7.25 (br s)	
3′	6.71 (s)	7.07 (d, 8.5)			6.41 (s)
4′					
5′				6.77 (d, 8.5)	
6′	6.90 (s)	8.00 (d, 1.5)	7.01 (d, 1.8)	7.26 (br d, 8.0)	7.10 (s)
1″	3.22 (d, 7.5)				3.11 (d, 7.5)
2″	5.25 (t-like, 7.5)				5.15 (t-like, 7.5)
3″			3.68 (dd, 13.2, 7.8)	3.81 (dd, 5.0, 8.0)	
4″	1.67 (s)		2.91 (dd, 3.2, 16.8)	3.02 (dd, 5.0, 17.0)	1.57 (s)
			2.62 (dd, 8.1, 16.8)	2.76 (dd, 8.0, 17.0)	
5″	1.69 (s)		1.12 (s)	1.21 (s)	1.64 (s)
6″			1.38 (s)	1.31 (s)	
1‴			3.28 (d, 6.9)		
2‴			5.17 (m)		
3‴					
4‴			1.69 (s)		
5‴			1.71 (s)		
3′-CHO		10.10 (s)			
4′-OCH_3	3.85 (s)				3.75 (s)
6′-OCH_3	3.90 (s)				

Compounds were measured in acetone-d_6 at 500 MHz[a] and 300 MHz[b] of ^{1}H NMR.

(Supplementary data). Furthermore, the 3-hydroxy-2,2-dimethyldihydropyran moiety [δ 3.81 (1H, dd, J = 5.0, 8.0 Hz, H-3″), 3.02 (1H, dd, J = 5.0, 17.0 Hz, H-4″ax), 2.76 (1H, dd, J = 8.0, 17.0 Hz, H-4″eq), and 1.31, 1.21 (each 3H, s, H-5″ and H-6″)] were also observed. The dihydropyran ring fused to the C-3′ and C-4′ position of the B ring was assigned from an HMBC experiment, showing correlations from the methylene protons at H-4″ (δ_H 3.02 and 2.76) to the carbons C-4′ (δ_C 154.5) and C-3′ (δ_C 121.4), and from the oxygenated aliphatic proton at δ_H 3.81 (H-3″) to the carbon C-3′ (δ_C 121.4). Therefore, compound **12** was identified as 2(*S*)-7-hydroxy-[3″-hydroxy-2″,2″-dimethylpyrano)]flavanone, erythribyssin I, which was in accordance with the molecular formula of $C_{20}H_{20}O_5$ derived from the molecular ion peak at *m/z* 340.1316 $[M]^+$ in the HREIMS spectrum.

Compound **13** was obtained as white amorphous powder. Its molecular formula was determined as $C_{21}H_{22}O_6$ from the molecular ion peak at *m/z* 370.1415 $[M]^+$ in the HREIMS spectrum. The ^{1}H and ^{13}C NMR spectra of compound **13** were similar to those of compound

Table 2
^{13}C NMR spectroscopic data for new compounds **1**, **6**, **9**, **12**, and **13**

No.	**1**[a] δ_C (ppm)	**6**[a] δ_C (ppm)	**9**[b] δ_C (ppm)	**12**[a] δ_C (ppm)	**13**[a] δ_C (ppm)
1					
2	71.9	79.8	80.8	80.7	74.6
3	48.2	44.5	44.6	44.7	75.1
4	191.3	197.9	190.5	190.5	190.8
5	131.5	132.8	129.4	129.6	130.6
6	111.2	111.5	111.1	111.3	111.9
7	164.9	165.4	165.1	165.3	165.6
8	97.0	103.8	103.6	103.7	103.3
9	164.7	162.1	164.5	164.6	164.1
10	116.1	115.3	115.5	115.2	113.8
1′	122.2	132.4	132.0	131.8	116.5
2′	158.5	136.2	126.6	129.3	156.1
3′	103.5	118.4	121.0	121.4	100.6
4′	157.8	164.3	152.0	154.5	159.3
5′	116.6	121.8	130.2	117.6	121.0
6′	123.1	129.7	126.7	126.8	128.2
1″	28.4				28.3
2″	123.9		69.7	69.7	123.8
3″	132.3		78.0	78.2	132.5
4″	17.8		32.3	32.2	17.7
5″	25.9		20.7	20.7	25.9
6″			26.2	26.1	
1‴			29.5		
2‴			123.7		
3‴			133.0		
4‴			25.8		
5‴			17.9		
3′-CHO		190.1			
4′-OCH_3	56.0				55.8
6′-OCH_3	56.3				

Compounds were measured in acetone-d_6 at 125 MHz[a] and 75 MHz[b] of ^{13}C NMR.

1, except for the absences of one methoxy moiety (δ_H 3.90, 6′-OCH_3) and a proton signal assignable to H-3 (δ_H 4.17) in compound **1**. The ^{1}H NMR spectrum of compound **13** showed signals assignable to the AA′ system at C-2 position [δ_H 4.85 (1H, d, J = 11.5 Hz) and 4.32 (1H, d, J = 11.5 Hz)], with corresponding carbon signals for C-2 (δ_C 74.6), C-3 (δ_C 75.1), and a conjugated carbonyl carbon at δ_C 190.8 (C-4). Comparison of the molecular formula of compound **13** ($C_{21}H_{22}O_6$) to that of compound **1** ($C_{22}H_{24}O_5$) indicated the presence of an additional oxygen atom in the structure of compound **13**. All of the above observations support for the presence of a hydroxy group at C-3.[17] The absolute configuration at this position (C-3) was deduced to be 3*S* by the presence of a negative Cotton effect at 330 nm in its CD spectrum.[15,17] The arrangement of methoxy and prenyl groups to the B ring was established using an HMBC experiment. Correlations between H-6′ (δ_H 7.10)/C-1″ (δ_C 28.3), H-1″ (δ_H 3.11)/C-5′ (δ_C 121.0)/C-4′ (δ_C 159.3), and between the methoxy protons (δ_H 3.75)/C-4′ (δ_C 159.3) and C-3′ (δ_C 100.6) indicated the attachments of the prenyl group to C-5′, and the methoxy moiety to C-4′, respectively (Fig. 2). Thus, compound **13** was elucidated as (3*S*)-3,7,2′-trihydroxy-4′-methoxy-5′-(γ,γ-dimethylallyl) isoflavanone, and named as erythribyssin J.

Table 3
Inhibitory effects of isolated compounds **1**–**13** on PTP1B enzyme

Compound	Inhibitory effect (IC_{50}, μM)[a]
1	15.2 ± 0.9
2	19.2 ± 1.4
3	72.9 ± 9.7
4	16.5 ± 1.1
5	22.6 ± 2.3
6	>100
7	29.2 ± 2.7
8	17.2 ± 1.6
9	35.8 ± 5.2
10	>100
11	98.1 ± 11.3
12	>100
13	14.9 ± 1.6
Ursolic acid[b]	3.6 ± 0.2
EtOAc ex.	25.3 ± 3.6[c]

[a] Results are expressed as IC_{50} values (μM), determined by regression analyses and expressed as the mean ± SD of three replicates.
[b] The compound was used as positive control.[6–8]
[c] Result is expressed as μg/mL and mean ± SD of duplicates.

Compounds **1**–**13** were tested for their inhibitory activity on PTP1B enzyme using an in vitro assay,[8] and the results are presented in Table 3. All of the isolates, except for compounds **6**, **10**, and **12**, inhibited PTP1B activity in a dose-dependent manner with IC_{50} values ranging from 14.9 ± 1.6 to 98.1 ± 11.3 μM. Two new flavanones, erythribyssin K (**6**) with an aldehyde group attached to C-5′ and erythribyssin I (**12**) which bear a 3-hydroxy-2,2-dimethylpyrano ring in the structure, and liquiritigenin (**10**) displayed no inhibitory activity against PTP1B in this assay (Table 3). However, compound **7** with a prenyl moiety and compound **11** with a methoxy group at C-5′ in the B ring, as compared with liquiritigenin **10**, showed the increase of inhibitory activity with IC_{50} values of 29.2 ± 3.7 and 98.1 ± 11.3 μM, respectively. This is indicated that methoxy and prenyl group may be responsible for enhancement of activity on PTP1B enzyme, but the aldehyde group and the 3-hydroxy-2,2-dimethylpyrano ring do not show the inhibitory activity of PTP1B enzyme (compounds **6** and **12**, IC_{50} >100 μM). The 5-deoxyisoflavanones (compounds **1**, **8**, and **13**) displayed the most potency with IC_{50} value around from 14.9 ± 1.6 to 17.2 ± 1.6 μM. The results also indicated that compounds in which prenyl and/or methoxy groups are absent (compounds **3**, **6**, **10**, and **12**), exhibited very weak or no inhibitory activity against PTP1B. However, all compounds with a prenyl group (**1**, **2**, **4**–**5**, **7**, **9**, and **13**) exhibited strong inhibitory effects (Fig. 1 and Table 3).[8] Two flavanones (**2** and **4**) and three isoflavanones (**1**, **8**, and **13**) with a methoxy group at C-2′ and C-4′ or C-3′ were as active against PTP1B as compounds bearing a prenyl group, demonstrating that the methoxy group on the B ring also plays an important role in inhibiting PTP1B activity of the 5-deoxyflavanones.

3. Experimental

3.1. General experimental

The optical rotations were determined on a Rudolph Autopol AP 589 polarimeter using a 100 mm glass microcell. The IR spectra were recorded on a Nicolet 6700 FT-IR (Thermo electron Corp.). UV spectra were recorded in MeOH using a Shimadzu spectrometer. CD spectra were recorded in MeOH on a JASCO J-715 spectrometer. The NMR spectra were obtained on a Varian Inova 500 MHz spectrometer with TMS as the internal standard at the Korea Basic Science Institute (KBSI, Gwangju Center, Korea). All mass experiments were performed on a Micromass QTOF2 (Micromass, Wythenshawe, UK) mass spectrometer. Silica Gel (Merck, 63–200 μm particle size) and RP-18 (Merck, 150 μm particle size) were used for column chromatography. For thin-layer chromatography, pre-coated TLC was carried out on Silica Gel 60 F_{254} and RP-18 F_{254} plates from Merck. HPLC runs were carried out using a Gilson system with an UV detector and an Optima Pak C18 column (10 × 250 mm, 10 μm particle size, RS Tech Corp., Korea).

3.2. Plant material

The root bark of *E. abyssinica* was collected in June 2005 in Mukono, Uganda. The sample was botanically authenticated by Professor John Silike-Muruumu, and a voucher specimen (No.

0001) has been deposited at the Department of Botany, Makerere University, Uganda.

3.3. Extraction and isolation

The dried root bark of *E. abyssinica* (3 kg) was extracted with EtOAc at room temperature for one week. The EtOAc-soluble extract was concentrated to yield a dry residue (150 g). The crude extract was tested in vitro to determine the inhibitory effect on the protein phosphatase 1B (PTP1B) enzyme activity. The result showed that this EtOAc-soluble extract was active with an IC_{50} value of 25.3 ± 3.6 μg/mL. A partial fraction (65 g) was subjected to silica gel column chromatography (15 × 60 cm; 63–200 μm particle size) using a gradient of *n*-hexane–acetone (from 20:1 to 0:1) to yield six combined fractions (F.1 to F.6) according to their TLC profiles. The six fractions were tested in vitro using the enzyme assay on PTP1B activity, along with checking the ^{1}H NMR to find out the active fraction containing the 5-deoxyflavonoid-type compounds. Among those, fractions F.3, F.4, and F.5 exhibited strong inhibitory effect on PTP1B enzyme. Fraction 3 (F.3) was chromatographed over RP-18 column (6.0 × 60 cm; 150 μm particle size), using a stepwise gradient of MeOH–H_2O (from 6:4 to 6:0) to afford five subfractions (F.31–F.35). Further purification of subfraction F.32 (157 mg) by semi-preparative Gilson HPLC using an isocratic solvent system of 60% MeCN in H_2O over 38 min [RS Tech Optima Pak C18 column (10 × 250 mm, 10 μm particle size); mobile phase MeCN/H_2O containing 0.1% formic acid (0–38 min: 60% MeCN, 38–40 min: 60–100% MeCN, 40–46 min: 100% MeCN, 46–48 min: 100–51% MeCN, 48–50 min: 60% MeCN); UV detections at 205 and 254 nm] to yield compound **1** (17.1 mg, t_R = 31.5 min) and compound **2** (10.5 mg, t_R = 36.7 min). Subfraction F.34 (565 mg) was also purified by preparative Gilson HPLC using an isocratic solvent system of 63% MeCN in H_2O containing 0.1% formic acid, over 40 min then increase to 100% MeCN for 10 min [RS Tech Optima Pak C18 column (10 × 250 mm, 10 μm particle size); UV detections at 205 and 254 nm] to obtain compounds **3** (80 mg, t_R = 25 min), **4** (9.0 mg, t_R = 32.5 min), and **5** (12.8 mg, t_R = 36.8 min). Fraction 4 (F.4) was also subjected to a RP-C18 column (6.0 × 60 cm, 150 μm particle size), eluted with MeOH−H_2O (5:5–5:0) to yield six subfractions (F.41–F.46). Further purification of subfraction F.42 (370 mg) by Gilson HPLC using a gradient of 30–40% MeCN in H_2O containing 0.1% formic acid as the mobile phase, over 50 min then increase to 100% MeCN for 10 min to produce compound **6** (7.7 mg, t_R = 37.5 min) and compound **7** (125.0 mg, t_R = 44.9 min). Subfraction F.45 (250 mg) was also subjected onto HPLC using an isocratic solvent system of 56% MeCN in H_2O led to the isolation of compounds **8** (8.5 mg, t_R = 65.1 min) and **9** (18.5 mg, t_R = 68.7 min). Similarly, the fraction F.5 was chromatographed on a RP-18 column (4.5 × 60 cm) eluted with a stepwise gradient solvent of MeOH–H_2O (1:2–1:0) to afford compounds **10** (3.5 mg), **11** (5.8 mg), and three subfractions (F.53–F.55). Further purification of subfraction F.54 by semi-preparative HPLC [Gilson System 321 pump equipped with a model UV/vis-155 detector, RS Tech Optima Pak C18 column (10 × 250 mm, 10 μm particle size); mobile phase MeCN/H_2O containing 0.1% formic acid (0–45 min: 35–40% MeCN, 45–50 min: 40–100% MeCN, 50–60 min: 100% MeCN); flow rate 2 mL/min; UV detections at 205 and 254 nm] resulted in the isolation of compound **12** (10.2 mg, t_R = 27.7 min) and compound **13** (12.5 mg, t_R = 44.1 min), respectively.

3.4. Erythribyssin E (1)

White amorphous powder; $[\alpha]_D^{25}$ −25.6 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3331, 2916, 1670, 1593, 1242, 1033 cm^{-1}; UV (*c* 0.03, MeOH) λ_{max} 206, 216, 234, 276, 310 nm; CD (MeOH) $[\theta]_{330}$ −2.56, $[\theta]_{298}$ −3.00, $[\theta]_{240}$ +6.06; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Tables 1 and 2; HREIMS *m/z* 368.1628 $[M]^+$, (calcd for $C_{22}H_{24}O_5$, 368.1624).

3.5. Erythribyssin K (6)

Yellowish amorphous powder; $[\alpha]_D^{25}$ −14.3 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3038, 1825, 1618, 1497, 609 cm^{-1}; UV (*c* 0.025, MeOH) λ_{max} 208, 214, 231, 276, 309 nm; CD (MeOH): $[\theta]_{232}$ +8.52, $[\theta]_{286}$ −26.47, $[\theta]_{327}$ +2.99; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Tables 1 and 2; HREIMS *m/z* 284.0683 $[M]^+$, (calcd for $C_{16}H_{12}O_5$, 284.0685).

3.6. Erythribyssin G (9)

Yellowish amorphous powder; $[\alpha]_D^{25}$ −24.43 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3419, 2926, 1664, 1606, 1468, 1279, 1142, and 1062 cm^{-1}; UV (*c* 0.025, MeOH) λ_{max} 208, 232, 276, 312, and 380 nm; CD (MeOH) $[\theta]_{253}$ +3.73, $[\theta]_{290}$ −25.69, $[\theta]_{327}$ +5.48; ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR data, see Tables 1 and 2; HRESIMS *m/z* 431.1859 $[M+Na]^+$, (calcd for $C_{25}H_{28}O_5Na$, 431.1834).

3.7. Erythribyssin I (12)

Yellowish amorphous powder; $[\alpha]_D^{25}$: −10.8 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3384, 2917, 1661, 1605, 1466, 1280, 1155–1124 cm^{-1}; UV (*c* 0.025, MeOH) λ_{max} 206, 216, 234, 276, 312 nm; CD (MeOH) $[\theta]_{249}$ +9.88, $[\theta]_{287}$ −55.66, $[\theta]_{328}$ +15.18; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Tables 1 and 2; HREIMS *m/z* 340.1316 $[M]^+$, (calcd for $C_{20}H_{20}O_5$, 340.1311).

3.8. Erythribyssin J (13)

White amorphous powder; $[\alpha]_D^{25}$: +3.8 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3300, 2978–2906, 1660, 1593, 1496, 1275–1127, 1023 cm^{-1}; UV (*c* 0.025, MeOH) λ_{max} nm: 211, 232, 280, 313 nm; CD (MeOH) $[\theta]_{230}$ +5.45, $[\theta]_{280}$ +2.30, $[\theta]_{330}$ −1.25; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Tables 1 and 2; HREIMS *m/z* 370.1415 $[M]^+$, (calcd for $C_{21}H_{22}O_6$, 370.1416).

3.9. 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.[7,8]

Acknowledgments

This research was supported in part by grants from the Post 21st Frontier Research Program (2010-0026355) and from the Procurement and Development of Foreign Biological Resources (2010-00749) funded by the Ministry of Education Science and Technology of the Korean government.

Supplementary data

Supplementary data (the ^{1}H and ^{13}C NMR spectra of the new compounds (**1**, **6**, **9**, **12**, and **13**) and HMBC spectroscopic data of the new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.04.037.

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