Bioorganic & Medicinal Chemistry 20 (2012) 6459–6464

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

New prenylated isoflavonoids as protein tyrosine phosphatase 1B (PTP1B) inhibitors from *Erythrina addisoniae*

Phi Hung Nguyen [a], Govinda Sharma [a], Trong Tuan Dao [a], Mohammad Nasir Uddin [a], Keon Wook Kang [b], Derek Tantoh Ndinteh [c], Joseph Tanyi Mbafor [c], Won Keun Oh [a,*]

[a] *Korea Bioactive Natural Material Bank, College of Pharmacy, Chosun University, 375 Seosuk-dong, Dong-gu, Gwangju 501-759, Republic of Korea*
[b] *College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Republic of Korea*
[c] *Faculty of Science, University of Yaoundé I, PO Box 812, Yaoundé, Cameroon*

ARTICLE INFO

Article history:
Received 2 July 2012
Revised 19 August 2012
Accepted 21 August 2012
Available online 7 September 2012

Keywords:
Erythrina addisoniae
Protein tyrosine phosphatase 1B (PTP1B)
Cytotoxic activity
Breast cancer cells

ABSTRACT

Bioassay-guided fractionation of the EtOAc extract of the root of *Erythrina addisoniae* (Leguminosae) resulted in the isolation of four new (**1**–**4**), along with 2 known prenylated isoflavonoids (**5**–**6**). The structures of the isolates were assigned on the basis of spectroscopic data analysis, focusing on interpretation of 1D and 2D NMR, and MS data. All the isolates were evaluated for their inhibitory effects on protein tyrosine phosphatase 1B (PTP1B), as well as their growth inhibition on MCF7, adriamycin-resistant MCF7 (MCF7/ADR), and MDA-MB-231 breast cancer cell lines. Compounds which exhibited PTP1B inhibitory activity (IC_{50} values ranging from 4.6 ± 0.3 to 24.2 ± 2.1 μM) showed potential cytotoxic activity (IC_{50} values ranging from 3.97 ± 0.17 to 11.4 ± 1.9 μM). Taken together, our data suggest that prenylated isoflavonoids, especially the isoflavone-type skeleton could be considered as new lead compounds against breast cancer via PTP1B inhibition.

1. Introduction

Breast cancer is the most common malignant tumor accounting for approximately 23% of all cancers in woman.[1] The current approaches to breast cancer treatment include chemotherapy, radiation, and surgery but all of them have disadvantages like severe side effects and difficult procedures. Tamoxifen is discredited with the increased risk of endometrial hyperplasia and cancer, similarly adriamycin causes cardiotoxicity.[2,3] The continuous usage of chemotherapeutic agents like tamoxifen and adriamycin has been also known as the cause of multidrug resistance. Therefore, new lead molecules which can overcome the resistance and have reduced undesirable toxicity are needed to be discovered.

Protein tyrosine phosphatases (PTPs) working in concert with protein tyrosine kinases ensure the proper functioning of a variety of proteins involved in signal transduction process of the cells. PTP1B is well known about its critical role in regulating body weight and glucose homeostasis by acting as a key negative regulator of insulin and leptin signaling pathway.[4] In addition to its involvement in obesity and diabetes, its importance in some cancers has been highlighted.[5] PTP1B is overexpressed in a significant subset of breast and ovarian cancers, especially in those overexpressing HER2/Neu (HER2(+) tumors).[6,7] When PTP1B-deficient mice were crossed with transgenic mice of the ErbB2 (Neu) oncoprotein in mammary epithelial cells, the onset of ErbB2 (Neu)-driven breast cancer was delayed significantly in the absence of PTP1B, whereas transgenic PTP1B overexpression was sufficient to induce breast tumors in the absence of exogenous ErbB2.[8a] These observations suggest the potential importance of PTP1B inhibitors as therapeutic targets in cancer therapy.[8b] Thus, many efforts need to be focused on PTP1B inhibitors as a double therapeutic target for not only diabetes and obesity but also cancer treatment.[8c]

As part of an ongoing investigation aimed at finding PTP1B inhibitors from plants, the genus *Erythrina*[9] was studied using in vitro assay on both the cytotoxic activity and PTP1B inhibitory activity.[10] In this research, four new prenylated isoflavonoids along with two known ones were purified by activity-guided isolation. Among them, as five compounds (**2**–**6**) were found as significant PTP1B inhibitors, we also investigated their effects on various cancer cell lines which are MCF-7, MDA-MB-231, and adriamycin resistant MCF7/ADR.

2. Result and discussion

Bioassay-guided fractionation of an EtOAc-soluble extract of the root bark of this plant led to the isolation of a series of prenylated isoflavonoids, consisting of two new isoflavones (erythraddison I–II, **1**–**2**), and two isoflavanones (erythraddison III–IV, **3**–**4**), along with

* Corresponding author. Tel./fax: +82 62 230 6370.
E-mail address: wkoh@chosun.ac.kr (W.K. Oh).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.08.024

2 known isoflavones (**5**–**6**). Chemical structures of the known compounds were determined to be echrenone b10 (**5**) and erysubin F (**6**) from a comparison of the physical and spectroscopic data (IR, UV, $[\alpha]_D$, NMR, and MS) with those reported in the literature (Fig. 1).[11]

Compounds **1** and **2** were isolated as yellowish amorphous powders, and their UV spectra exhibited absorption maxima at 271, 276 and 272 nm, respectively. The ^{1}H and ^{13}C NMR spectra (Table 1) of compounds **1** and **2** displayed the characteristic signals of H-2 at δ_H 7.91 and δ_H 8.10 (each 1H, s) with corresponding olefinic oxymethine signals at δ_C 152.5 and 154.7, respectively, and a ketone carbon resonance (δ_C 179.4–181.3). These observations were indicative of an isoflavone skeleton.[12,13]

A molecular formula of compound **1** was determined as $C_{26}H_{28}O_8$ from the molecular ion peak at *m*/*z* 468.1784 (calcd for $C_{26}H_{28}O_8$, 468.1776) in the HREIMS spectrum. A pair of coupled doublets at δ_H 7.42 and 6.92 (each 2H, d, *J* = 8.4 Hz, H-2′/H-6′, H-3′/H-5′); δ_C 130.5 (each CH, C-2′/6′), 115.8 (each CH, C-3′/5′) was deduced as the presence of a *para* disubstituted ring B, while that of a hydroxy group at C-4′ was established from the correlation between a single proton (δ_H 5.02, 4′-OH) to carbons at δ_C 115.8 (C-3′ and C-5′), respectively in the HMBC experiment. The ^{1}H NMR spectrum displayed characteristic signals attributable to a 2,2-dimethylpyrano ring [δ_H 6.76 (1H, d, *J* = 10.0 Hz, H-4″), 5.65 (1H, d, *J* = 9.6 Hz, H-3″), 1.48 (3H, s, H-5″) and 1.49 (3H, s, H-6″). The HMBC correlations from H-4″ to C-5, and a single proton signal at δ_H 13.2 in the ^{1}H NMR spectrum further indicated that hydroxy group with an intramolecular hydrogen bond was located at C-5 and the 2,2-dimethylpyrano ring was fused at C-6 and C-7. In addition, the ^{1}H and ^{13}C NMR spectra of compound **1** exhibited a 1ξ-methoxy-2ξ,3-dihydroxy-methylbutyl group [δ_H 4.70 (1H, d, *J* = 7.2 Hz, H-1‴), 3.70 (1H, d, *J* = 7.2 Hz, H-2‴), 3.39 (3H, s, 1‴–OCH$_3$), 1.29 (3H, s, H-4‴) and 1.20 (3H, s, H-5‴); δ_C 75.1 (C-1‴), 65.8 (C-2‴), 57.7 (C-3‴), 57.1 (1‴–OCH$_3$), 25.1 (C-4‴) and 19.7 (C-5‴)]. The location of each functional group was further demonstrated in the HMBC, and suggested that the 1ξ-methoxy-2ξ,3-dihydroxy-methylbutyl group was attached at C-8 by the long-range correlations from proton H-1‴ to C-7 (δ_C 157.7), C-8 (δ_C 104.2) and C-9 (δ_C 155.7) (Fig. 2). In addition, HMBC correlations between H$_3$-OCH$_3$/C-1‴, H-2‴/C-1‴, and H$_3$-4‴ and H$_3$-5‴/C-3‴, C-2‴, further supported for the elucidation of the 1ξ-methoxy-2ξ,3-dihydroxy-methylbutyl group. However, the configurations at C-1‴ and C-2‴ have not determined due to the low amount of the compound obtained. Thus, compound **1** was assigned as 5,4′-dihydroxy-(1‴ξ-methoxy-2‴ξ,3‴-dihydroxy-3‴-dimethylbutyl)-[2″,2″-dimethyl-3″,4″-dehydro-pyrano-(1″,4″:-7,6)]isoflavone (as shown in Fig. 2), and named erythraddison I.

Compound **2** was also obtained as yellowish amorphous powder and its molecular formula of $C_{25}H_{26}O_5$ was determined from a molecular ion peak at *m*/*z* 406.1780 (calcd for $C_{25}H_{26}O_5$, 406.1780) obtained by HREIMS. The ^{1}H NMR spectrum of compound **2** displayed an aromatic ABX spin system [δ_H 7.05 (d, *J* = 8.0 Hz, H-6′), 6.48 (dd, *J* = 8.0, 2.8 Hz, H-5′) and 6.58 (d, *J* = 2.8 Hz, H-3′)], an aromatic single proton at δ_H 8.00 (1H, s), but showed no proton resonance for a hydroxy group at C-5 (δ_H 12.1–13.5 Hz).[14] This was further evidenced by an HMBC correlation between H-5 (δ_H 8.00) and the ketone carbon at C-4 (δ_C 179.4) (Supplementary data and Fig. 2). The ^{1}H and ^{13}C NMR spectra of compound **2** exhibited two prenyl groups (Table 1), and their placements were assigned to be at C-6 and C-8 by HMBC experiments from H-1″ (δ_H 3.48 (2H, d, *J* = 7.2 Hz))/C-5 (δ_C 124.6), C-6 (δ_C 127.6), and C-7 (δ_C 158.8), and from H-1‴ (δ_H 3.65 (2H, d, *J* = 7.2 Hz))/C-7, C-8 (δ_C 114.7), and C-9 (δ_C 154.3), respectively. 1D (Table 1) and HMBC (Fig. 2) NMR data of compound **2** revealed that three hydroxy groups were attached to C-7, C-2′, and C-4′, respectively. The hydroxyl position at C-2′ was confirmed by HMBC correlations between H-3′ (δ_H 6.58 (1H, d, *J* = 2.8 Hz)) and C-4′ (δ_C 158.3), C-2′ (δ_C 158.0). Thus, compound **2** was characterized as new natural product, 7,2′,4′-trihydroxy-6,8-di(γγ-dimethylallyl)isoflavone, and named erythraddison II.

Compounds **3** and **4** were isolated as yellow amorphous powder. The ^{1}H and ^{13}C NMR spectra (Table 1) of compounds **3** and **4** displayed an AMX spin system for H-2ax, H-2eq, and H-3, and the corresponding carbon signals for C-2 (δ_C 71.2–72.3) and C-3 (δ_C 47.4–51.1), and ketone carbon resonances (δ_C 197.9–198.2). Their UV spectra showed absorption maxima near 270, 305, and 335 nm. These observations were indicative of an isoflavanone skeleton.[15] Compound **3** was obtained as a yellow amorphous powder with $[\alpha]_D^{25}$ −11.8 (*c* 0.11, MeOH). A molecular formula of

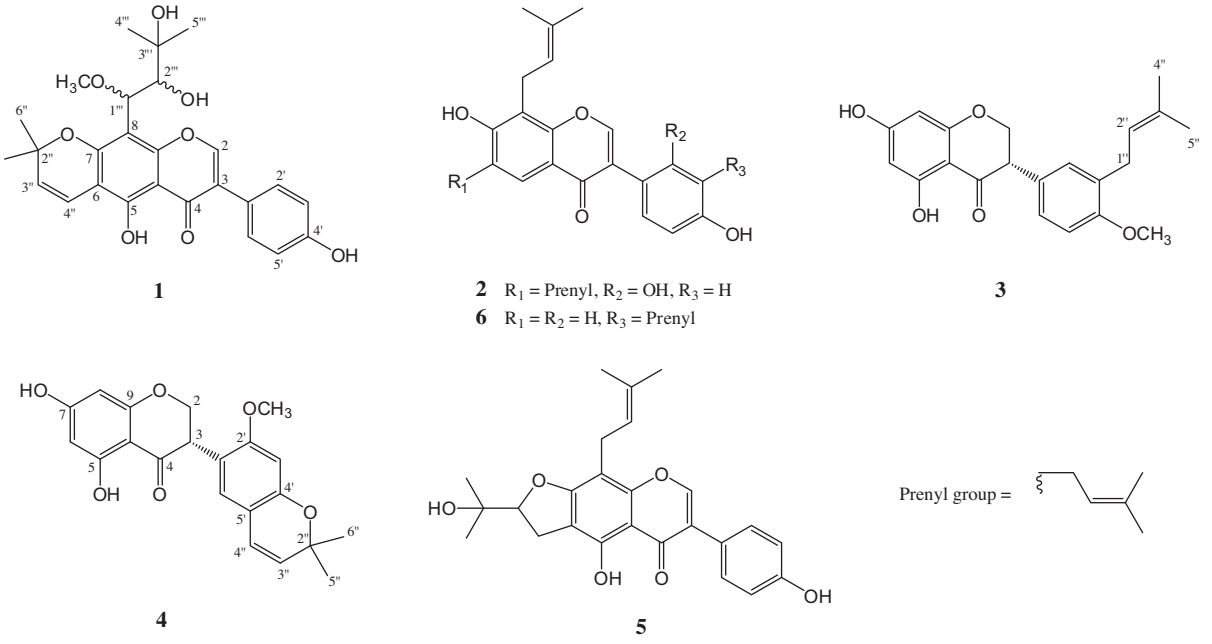

Figure 1. Chemical structures of isolated compounds **1**–**6** from *Erythrina addisoniae*.

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for new compounds **1–4**

position	1[a]		2[a]		3[b]		4[b]	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1								
2	152.5	7.91, s	154.7	8.10, s	72.3	4.60, t, 10.4	71.2	4.53, t, 10.8
						4.59, dd, 10.4, 3.6		4.42, dd, 10.8, 4.4
3	123.7		124.4		51.1	3.95, dd, 10.4, 3.6	47.4	4.28, dd, 10.8, 4.4
4	181.3		179.4		197.9		198.2	
5	157.3		124.6	8.00, s	165.8		165.8	
6	105.5		127.6		97.0	5.97, s	97.0	5.96, s
7	157.7		158.8		167.5		167.2	
8	104.2		114.7		95.7	5.96, s	95.7	5.95, s
9	155.7		154.3		164.3		164.6	
10	106.3		117.0		103.4		103.7	
1′	123.1		113.8		128.6		116.3	
2′	130.5	7.42, d, 8.4	158.0		130.8	7.10, d, 2.4	159.4	
3′	115.8	6.92, d, 8.4	106.6	6.58, d, 2.8	130.5		101.1	6.44, s
4′	156.2		158.3		157.8		154.9	
5′	115.8	6.92, d, 8.4	108.4	6.48, dd, 2.8, 8.0	111.5	6.90, d, 8.0	115.1	
6′	130.5	7.42, d, 8.4	130.7	7.05, d, 8.0	128.0	7.14, dd, 8.0, 2.4	129.1	6.85, s
1″			30.1	3.48, d, 7.2	29.2	3.26, d, 6.0		
2″	79.0		120.9	5.27, m	120.6	5.35, m	77.2	
3″	128.3	5.65, d, 9.6	136.4		132.9		128.9	5.56, d, 10.0
4″	115.7	6.76, d, 9.6	26.1	1.81, s	17.8	1.68, s	122.5	6.29, d, 10.0
5″	28.5	1.48, s	18.2	1.81, s	25.9	1.66, s	28.4	1.40, s
6″	28.7	1.49, s					28.4	1.40, s
1‴	75.1	4.70, d, 7.2	22.6	3.65, d, 7.2				
2‴	65.8	3.70, d, 7.2	120.6	5.35, m				
3‴	57.7		135.7					
4‴	25.1	1.29, s	26.0	1.78, s				
5‴	19.7	1.20, s	18.2	1.88, s				
5-OH		13.2, s				12.5, s		12.3, s
7-OH				6.31, s				
2′-OH				9.50, br, s				
4′-OH		5.02, s		4.89, s				
2′-OCH_3							56.3	3.79, s
4′-OCH_3					55.9	3.82, s		
1‴-OMe	57.1	3.39, s						

[a] Compounds were measured in acetone-d_6.
[b] Compounds were measured in $CDCl_3$.

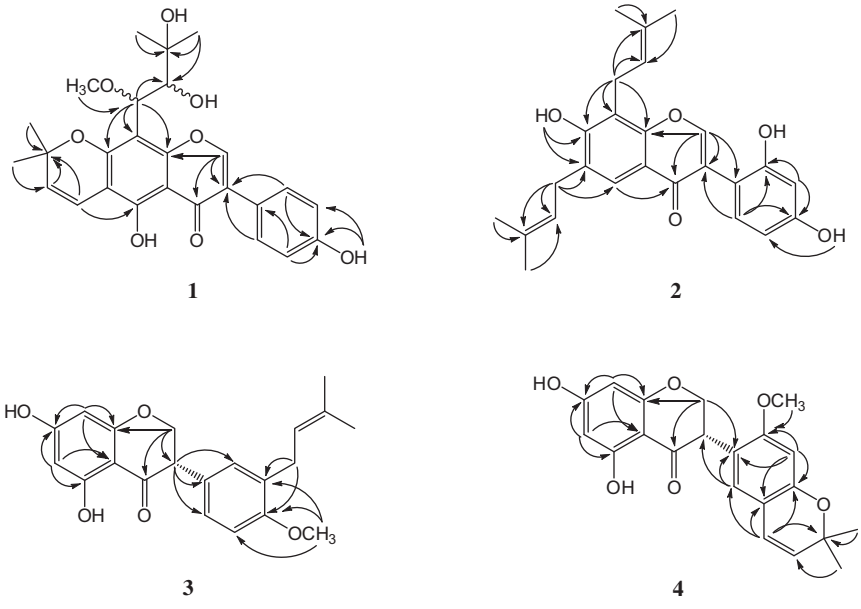

Figure 2. Key HMBC correlations (^{1}H–^{13}C) for new compounds **1–4**.

$C_{21}H_{22}O_5$ was determined from the quasimolecular ion peak at m/z 355.1467 $[M + H]^+$ (calcd for $C_{21}H_{22}O_5H$, 355.1466) obtained by HRESIMS. The ^{1}H and ^{13}C NMR spectra of **3** displayed an aromatic ABX spin system [δ_H 7.10 (d, J = 2.4 Hz, H-2′), 7.14 (dd, J = 8.0, 2.4 Hz, H-6′), and 6.90 (d, J = 2.4 Hz, H-5′)], a methoxy group [(δ_H 3.82 (3H, s), δ_C 55.9)], and an isoprenyl moiety (Table 1). The

substitution of ring A was confirmed by the presence in the ^{1}H NMR spectrum of the signals at δ_H 5.96 and 5.97 attributable to H-6 and H-8, respectively.[16,17] HMQC and HMBC experiments of compound **3** confirmed that the isoprenyl and methoxy groups were attached to carbons C-3′ and C-4′, respectively, by HMBC correlations from δ_H 3.26 (H-1″)/δ_C 130.5 (C-3′) to 157.8 (C-4′) and also from δ_H 3.82 (OCH_3) to δ_C 157.8 (C-4′) and 111.5 (C-5′). The configuration at C-3 was inferred to be *S* by its CD spectrum, which presented a negative Cotton effect near 330 nm,[18] and the negative optical rotation with $[\alpha]_D^{25}$ −11.8 (*c* 0.11, MeOH). On the basis of the above spectroscopic studies, compound **3** was identified as 3(*S*)-5,7-dihydroxy-4′-methoxy-3′-(γγ-dimethylallyl)isoflavanone (Fig. 2) and named erythraddison III. Compound **4** was obtained as a yellow amorphous powder with $[\alpha]_D^{25}$ −5.6 (*c* 0.15, MeOH). Compound **4** possessed one chelated hydroxy at C-5 and the other hydroxy at C-7 from the presence of the signals at δ_H 12.33 (1H, s, 5-OH), 5.96 (1H, s), and 5.95 (1H, s) attributable to protons H-6 and H-8 in the ^{1}H NMR spectrum.[16,17] Two sharp one proton singlets at δ_H 6.85 and 6.44 were assignable to two *para* protons in ring B at H-6′ and H-3′, respectively. The presence of one methoxy group was also shown by signal at δ_H 3.79 with corresponding carbon at δ_C 56.3. Furthermore, the presence of a *gem*-dimethyl chromene ring was derived from the 6H singlet at δ_H 1.40 due to the *gem*-dimethyl group and an AB spin system at δ_H 5.56 (d, *J* = 10.0) and 6.29 (d, *J* = 10.0) assignable to H-3″ and H-4″, respectively. The location of methoxy group at C-2′ and the 2,2-dimethylpyrano ring fused to C-4′ and C-5′ were confirmed by HMBC correlations between the methoxy protons (δ_H 3.79)/C-2′ (δ_C 159.4), one *para* proton at δ_H 6.44 (H-3′)/C-2′ and C-4′ (δ_C 154.9), H-4″ (δ_H 6.29)/C-4′, C-5′ (δ_C 128.9), and C-6′ (δ_C 129.1), and H-3″ (δ_H 5.56)/C-6′. These were further supported by the molecular formula of $C_{21}H_{20}O_6$ from the quasimolecular ion peak at *m*/*z* 369.1267 $[M+H]^+$ (calcd for $C_{21}H_{20}O_6H$, 369.1276) in the HRESIMS spectrum. The optical rotation value $[\alpha]_D^{25}$ −5.6 (*c* 0.15, MeOH) and the negative Cotton effect near 328 nm indicate that the configuration at C-3 is to be *S*.[18] Compound **4** was thus determined to be a new prenylated isoflavanone, 3(*S*)-5,7-dihydroxy-2′-methoxy-[2″,2″-dimethyl-3″,4″-dehydro-pyrano-(1″,4″:-4′,5′)]isoflavanone (Fig. 2), and named erythraddison IV.

The isolated compounds (**1**–**6**) were evaluated for their inhibitory effects on protein tyrosine phosphatase-1B (PTP1B), as well as their growth inhibition on MCF7, adriamycin-resistant MCF7 (MCF7/ADR), and MDA-MB-231 breast cancer cell lines (Tables 2 and 3). Compounds **2**–**6** with PTP1B inhibition generally showed significant cytotoxic activity on several cancer cell lines compared to tamoxifen as the positive control (Fig. 3). Among the isolates, three new compounds **2**–**4** and the known compounds **5** and **6** which significantly inhibited PTP1B activity (IC_{50} values ranging from 4.6 ± 0.3 to 24.2 ± 2.1 μM), showed strong cytotoxicity against three breast cancer cell lines with IC_{50} values ranging from 3.97 ± 0.17 to 11.41 ± 1.97 μM. Compound **5** was found to be more than three times as potent as tamoxifen against MCF7/ADR and MDA-MB-231. Compound **3** with twice potency than tamoxifen showed strongest activity against PTP1B enzyme. Most of the isolates bearing prenyl group gave the inhibitory effects on both breast cancer cells and protein tyrosine phosphatase-1B, while modification of this moiety displayed non-activity in both assay systems. The new compound erythraddison III (**3**) is an isoflavanone with a conjugated hydroxy group at C-5, bearing a prenyl group at C-3′ and a 4′-methyl ether substituted, displayed strongest effect on PTP1B enzyme (IC_{50} 4.6 μM). However, cyclization between the prenyl group with a hydroxy group to form the 2,2-dimethylpyrano ring on the B ring of compound **4** (erythraddison IV), diminished the activity even through a methoxy group was presented at C-2′. The same manner was also found for the isoflavone-type, new compound **2** (erythraddison II) with two prenyl groups attached at C-6 and C-8 possessed high PTP1B inhibitory activity with IC_{50} value of 17.4 μM. Compound **5** with one prenyl group at C-8, a hydroxy group at C-5, and a 1-hydroxy-1-methylethyldihydrofuran moiety (fused at C-6 and C-7 in ring A) showed weaker activity on PTP1B (IC_{50} 24.2 μM) than **2** and **6**. With the same structure skeleton, however compound **1** was not active at all (IC_{50} > 30 μM). This decrease in activity of **1** might be induced by the modification of prenyl group at C-8, and the cyclization between the prenyl group at C-6 with 7-OH group. And the presence of a conjugated hydroxy group at C-5 may also be responsible for this decrease. All of the PTP1B inhibitors, except for compound **6** (not tested), showed stronger dose-dependent inhibition on all cancer cell lines than the positive control (Table 3). Interestingly, the isoflavone-type (Compounds **2** and **5**) displayed a stronger potency than that of the isoflavanone-type (Compounds **3** and **4**) in all cancer cells. The results of this study revealed that compounds in which the prenyl and/or methoxy groups are present exhibited significant inhibitory activities against both PTP1B and cancer cells. In contrast, lack of the lipophilic groups (prenyl and methoxy moieties) in the structure (Compound **1**) and cyclization (Compounds **1** and **4**), and/or modification of the prenyl moiety (Compounds **1** and **5**), are responsible for decreasing activity of these flavonoids against PTP1B. These data indicate that flavonoids with prenyl group might be a new class of anti-diabetes and anticancer agents. Therefore, it is suggested that compounds reducing PTP1B activity or the genetic expression levels can be used for treating not only diabetes and obesity but also breast cancer as well.

Table 2
Inhibitory effects of isolated compounds **1**–**6** against PTP1B enzyme

Compounds	Inhibitory activity[a]
Erythraddison I (**1**)	>30
Erythraddison II (**2**)	17.4 ± 1.1
Erythraddison III (**3**)	4.6 ± 0.3
Erythraddison IV (**4**)	13.8 ± 1.8
Echrenone b10 (**5**)	24.2 ± 2.1
Erysubin F (**6**)	7.8 ± 0.5
Ursolic acid[b]	3.6 ± 0.2

[a] Results are expressed as IC_{50} values (μM), determined by regression analyses and expressed as the mean ± SD of three replicates.
[b] Compound was used as positive control.

Table 3
Growth inhibitory effects of isolated compounds **1**–**6** against breast cancer cell lines

Compounds	Cell lines/IC_{50}[a] (μM)		
	MCF7	MCF7/ADR	MDA-MB-231
Erythraddison I (**1**)	NA	NA	NA
Erythraddison II (**2**)	11.41 ± 1.97	6.75 ± 1.00	4.57 ± 0.60
Erythraddison III (**3**)	6.84 ± 0.11	9.22 ± 0.21	6.92 ± 0.27
Erythraddison IV (**4**)	11.00 ± 1.02	6.06 ± 0.85	6.34 ± 0.40
Echrenone b10 (**5**)	4.32 ± 0.10	5.76 ± 0.23	3.97 ± 0.17
Erysubin F (**6**)	NT	NT	NT
Tamoxifen[b]	11.44 ± 0.9	11.13 ± 0.8	12.41 ± 0.8

NA: Not active.
NT: Not tested.
[a] Results are expressed as IC_{50} values (μM), determined by regression analyses and expressed as the mean ± SD of three replicates.
[b] Compound was used as positive control.

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.).

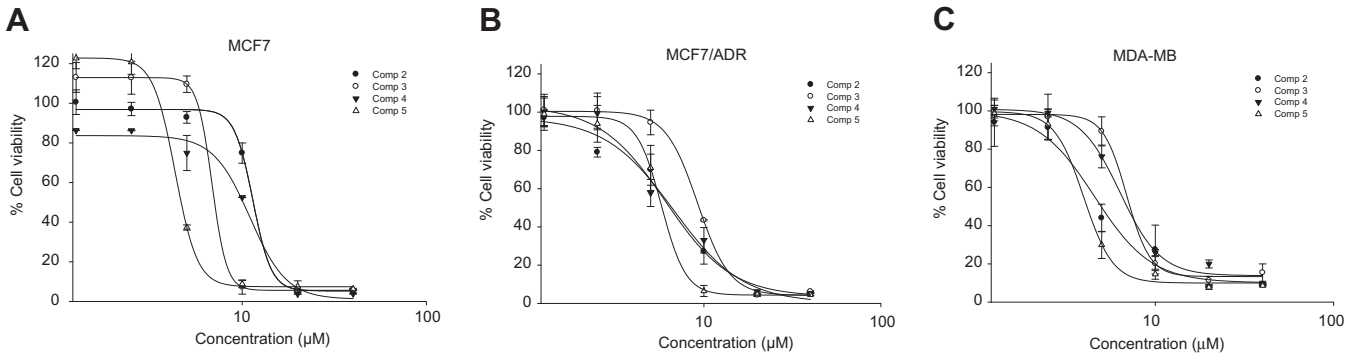

Figure 3. Inhibitory effects of the isolates against the activity of three breast cancer cell lines. (A) Concentration-dependent inhibition of MCF7 by the isolated compounds **2**–**5**. (B) Concentration-dependent inhibition of adriamycin-resistant MCF7 (MCF7/ADR) by the isolated compounds **2**–**5**. (C) Concentration-dependent inhibition of MDA-MB-231 by the isolated compounds **2**–**5**. For positive control, tamoxifen was used in all the experiments and also presented in Table 3. The inhibitor concentrations are displayed on logarithmic scales. The IC_{50} value was identified from the midpoint (cytotoxic activity = 50%) of the semilog plot.

UV spectra were recorded in MeOH using a Shimadzu spectrometer. CD spectra were recorded in MeOH on a JASCO J-715 spectrometer. The nuclear magnetic resonance (NMR) spectra were obtained on Varian Unity Inova 500 MHz spectrometer using TMS as the internal standard at Korea Basic Science Institute (KBSI, Gwangju Center, Korea). ^{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 (150 μm particle size) from Merck. Precoated TLC Silica Gel 60 F_{254} and RP-18 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 Gilson System 231 pump equipped with a model UV/Vis-155 UV detector, and an Optima Pak® C_{18} column (10 × 250 mm, 10 μm particle size, RS Tech Korea) for semi-preparative runs.

3.2. Plant material

The root bark of *E. addisoniae* was collected in Cameroon. The botanical sample was identified and authenticated at the Cameroon National Herbarium (Yaoundé, Cameroon) where a voucher specimen (No. 41617/HNC) has been deposited.

3.3. Extraction and isolation

The dried material (1.5 kg) was extracted with MeOH at room temperature. Since the EtOAc-soluble fraction (10.6 g) was found to be the most active among the solvent fractions, this active fraction was applied directly onto a silica gel column chromatography (10 × 60 cm; 63–200 μm particle size) using a gradient solvent of *n*-hexane and acetone (from 10:1 to 0:1, each 3 L) to yield five fractions (EA1–EA5) according to their TLC profiles. Followed by an in vitro assay on PTP1B, the activities were concentrated in fraction 1 (EA1), fraction 2 (EA2) and fraction 3 (EA3). Fraction 1 (EA1, ~1.6 g) was then chromatographed over a RP-C18 column (4.0 × 60 cm; 75 μm particle size) using a gradient solvent system of MeOH:H_2O (from 6:4 to 1:0), to yield four subfractions (EA1.1–EA1.4). Purification of subfraction EA1.3 by semi-preparative HPLC [RS Tech Optima Pak® C_{18} column (10 × 250 mm, 10 μm particle size); mobile phase MeCN/H_2O (65:35) over 35 min; flow rate 2 mL/min; UV detection at 254 nm] resulted in the isolation of compounds **1** (2.5 mg; t_R = 16.4 min) and **5** (36.0 mg; t_R = 25.3 min), respectively. Fraction 2 (EA2, 3.2 g) was also chromatographed over a RP-C18 column (4.0 × 60 cm; 75 μm particle size) using a gradient solvent of MeOH/H_2O (from 5:5 to 5:0, each 4 L), to give four subfractions (EA2.1–EA2.4). Subfraction EA-2.2 (eluted with MeOH:H_2O from 5:4 to 5:3.5, 257 mg) was purified by preparative HPLC using an isocratic solvent of 50% MeCN in H_2O + 0.1% formic acid, RS Tech Optima Pak® C_{18} column (10 × 250 mm, 10 μm particle size), over 50 min, flow rate 2 mL/min; UV detections at 205 and 254 nm], to afford compound **3** (10.8 mg, t_R = 42.6 min) and compound **4** (11.2 mg, t_R = 47.4 min), respectively. Fraction 3 (EA3, ~2.5 g) was further subjected to a same RP-C18 column chromatography (4.0 × 60 cm; 75 μm particle size) using a gradient of MeOH:H_2O (from 1:1.5 to 1:0), yielded four subfractions (EA3.1–EA3.4). Purification of subfraction EA3.2 (400 mg, eluted by MeOH:H_2O = 1.5:1) by Gilson HPLC [RS Tech Optima Pak® C_{18} column (10 × 250 mm, 10 μm particle size); mobile phase MeCN/H_2O (35:65) over 45 min; flow rate 2 mL/min; UV detections at 205 and 254 nm], resulted in the isolation of compound **2** (5.8 mg; t_R = 26.4 min) and compound **6** (28.0 mg; t_R = 31.9 min), respectively.

3.4. Erythraddison I (1)

Yellowish amorphous powder; IR (KBr): ν_{max} cm^{-1}: 3418, 2924, 1510, 1418, 1265, 1160–1032; UV (*c* 0.02, MeOH) λ_{max} nm: 202, 216, 271 nm; $[\alpha]_D^{25}$: −14.4 (*c* 0.027, MeOH); ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data, see Table 1; HREIMS *m*/*z* 468.1784 $[M]^+$, (calcd $C_{26}H_{28}O_8$ 468.1776).

3.5. Erythraddison II (2)

Yellowish amorphous powder; IR (KBr) ν_{max} cm^{-1}: 3410, 2928, 1566, 1468, 1193–1041; UV (*c* 0.02, MeOH) λ_{max} nm: 208, 213, 272; ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data, see Table 1; HREIMS *m*/*z* 406.1780 $[M]^+$ (calcd for $C_{25}H_{26}O_5$ 406.1780).

3.6. Erythraddison III (3)

Yellow amorphous powder; $[\alpha]_D^{25}$: −11.8 (*c* 0.11, MeOH); IR (KBr) ν_{max} cm^{-1}: 3420, 2967, 1670, 1589, 1374, λ_{max} nm: 210, 216, 270, 306, 333; CD (MeOH) $[\theta]_{330}$ −2.28, $[\theta]_{299}$ −3.00, $[\theta]_{238}$ +6.00; ^{1}H (400 MHz, acetone-d_6) and ^{13}C (100 MHz, acetone-d_6) NMR data, see Table 1; HRESIMS *m*/*z* 355.1467 $[M+H]^+$ (calcd for $C_{21}H_{22}O_5H$ 355.1466).

3.7. Erythraddison IV (4)

Yellow amorphous powder; $[\alpha]_D^{25}$ −5.6 (*c* 0.15, MeOH); IR (KBr) ν_{max} cm^{-1}: 3331, 2916, 1670, 1593, 1504, 1242–1033; UV (*c* 0.03,

MeOH) λ_{max} nm: 210, 233, 272, 304, 335; CD (MeOH) $[\theta]_{240}$ +5.50, $[\theta]_{285}$ +3.45, $[\theta]_{328}$ −1.15; ^{1}H (400 MHz, acetone-d_6) and ^{13}C (100 MHz, acetone-d_6) NMR data, see Table 1; HRESIMS *m/z* 369.1267 $[M+H]^+$ (calcd for $C_{21}H_{20}O_6H$, 369.1276).

3.8. PTP1B assay

PTP1B (human, recombinant) was purchased from BIOMOL International LP (USA) and the enzyme activity was measured using *p*-nitrophenyl phosphate (*p*-NPP) as a substrate. To each 96-well (final volume: 200 μL) were 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.

3.9. Cell culture

The screening cell lines (MCF7 and MDA-MB-231 human breast carcinoma cells, and the Adriamycin resistant cell line MCF7/ADR) were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. All the media used were DMEM supplemented with 10% heat-inactivated fetal bovine serum, 4.5 g/L D-glucose, 100 mg/L sodium pyruvate and L-glutamine. The cells were subcultured every 3 days using the standard trypsinization procedure.

3.10. Cytotoxicity assay

The cell viability was assessed using a MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide MTT methylthiazolyldiphenyl-tetrazolium bromide) based cytotoxicity assay to determine the IC_{50} of the isolated compounds (M5655 SIGMA; Sigma–Aldrich). In these assays, 1×10^4 ER positive, highly invasive (MDA-MB), ER negative weakly-invasive (MCF7), Adriamycin resistant (MCF7/ADR) cells in 100 μL of the culture medium per well were seeded in 96-well plates and allowed to adhere for 24 h prior to treatment. At various concentrations the cells in 96 well plates were treated and incubated for 48 h. The final concentration of DMSO in the culture medium was maintained at 0.05% (v/v) to avoid solvent toxicity. Subsequently, 20 μL of the 2 mg/mL MTT solution was added to each well of the plate and incubated 4 h. Then the absorbance was measured at 550 nm. The percentage cell viability is expressed as toxicities of the compounds, where the higher the toxicity, the lower the cell viability. Percentage cell viability is defined as the absorbance in the experiment well compared to that in the control wells. The cytotoxicity results are expressed as the mean ± standard deviation and represent the concentration inhibiting 50% cell growth (IC_{50}). Each experiment was carried out in triplicates.

Acknowledgments

This research was supported in part by Grants from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A084335) and from the Procurement and Development of Foreign Biological Resources (2011-00402) funded by the Ministry of Education Science and Technology of the Korean government.

Supplementary data

Supplementary data (1D (^{1}H and ^{13}C) and 2D (including COSY, HSQC, and HMBC) NMR spectra of the new compounds (**1**–**4**)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.08.024.

References and notes

1. Ferlay, J.; Shin, H. R.; Bray, F.; Forman, D.; Mathers, C.; Parkin, D. M. *Int. J. Cancer* **2010**, *127*, 2893.
2. Berthiaume, J. M.; Wallace, K. B. *Cell Biol. Toxicol.* **2006**, *23*, 15.
3. Yasue, A.; Hasegawa, K.; Udagawa, Y. *Hum. Cell* **2011**, *24*, 65.
4. Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. *Nat. Rev. Drug Disc.* **2002**, *1*, 696.
5. Combs, A. P. *J. Med. Chem.* **2010**, *53*, 2333.
6. Wiener, J. R.; Kerns, B. J.; Harvey, E. L.; Conaway, M. R.; Iglehart, J. D.; Berchuck, A., ; Bast, R. C., Jr. *J. Natl. Cancer Inst.* **1994**, *86*, 372.
7. Tanner, M. M.; Grenman, S.; Koul, A.; Johannsson, O.; Meltzer, P.; Pejovic, T.; Borg, A.; Isola, J. J. *Clin. Cancer Res.* **1833**, *2000*, 6.
8. (a) Julien, S. G.; Dubé, N.; Read, M.; Penney, J.; Paquet, M.; Han, Y.; Kennedy, B. P.; Muller, W. J.; Tremblay, M. L. *Nat. Genet.* **2007**, *39*, 338; (b) Tonks, N. K.; Muthuswamy, S. K. *Cancer Cell* **2007**, *11*, 214; (c) Yip, S. C.; Saha, S.; Chernoff, J. *Trends Biochem. Sci.* **2010**, *35*, 442.
9. (a) Djiogue, S.; Halabalaki, M.; Alexi, X.; Njamen, D.; Fomum, Z. T.; Alexis, M. N.; Skaltsounis, A. L. *J. Nat. Prod.* **2009**, *72*, 1603; (b) Kaur, K.; Jain, M.; Kaur, T.; Jain, R. *Bioorg. Med. Chem.* **2009**, *17*, 3229.
10. (a) Nguyen, P. H.; Le, T. V. T.; Thuong, P. T.; Dao, T. T.; Ndinteh, D. T.; Mbafor, J. T.; Kang, K. W.; Oh, W. K. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6745; (b) Dao, T. T.; Nguyen, P. H.; Thuong, P. T.; Kang, K. W.; Na, M. K.; Ndinteh, D. T.; Mbafor, J. T.; Oh, W. K. *Phytochemistry* **2009**, *70*, 2053.
11. (a) Tanaka, H.; Etoh, H.; Watanabe, N.; Shimizu, H.; Ahmad, M.; Rizwani, G. H. *Phytochemistry* **2001**, *56*, 769; (b) Tanaka, H.; Doi, M.; Etoh, H.; Watanabe, N.; Shimizu, H.; Hirata, M.; Ahmad, M.; Qurashi, I.; Khan, M. R. *J. Nat. Prod.* **2001**, *64*, 1336.
12. Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The systematic identification of flavonoids*; Springer: New York, 1970. pp 41–64.
13. Agrawal, P. K. *Carbon-13 NMR of flavonoids*; Elsevier: New York, 1989. p 507.
14. Na, M. K.; Jang, J. P.; Njamen, D.; Mbafor, J. T.; Fomum, Z. T.; Kim, B. Y.; Oh, W. K.; Ahn, J. S. *J. Nat. Prod.* **2006**, *69*, 1572.
15. Zhao, M.; Duan, J. A.; Che, C. T. *Phytochemistry* **2007**, *68*, 1471.
16. Atindehou, K. K.; Queiroz, E. F.; Terreaux, C.; Traore, D.; Hostettmann, K. *Planta Med.* **2002**, *68*, 181.
17. Iinuma, M.; Ohyama, M.; Tanaka, T. *Phytochemistry* **1994**, *37*, 1713.
18. Slade, D.; Ferreira, D.; Marais, J. P. J. *Phytochemistry* **2005**, *66*, 2177.