Prenylated Flavonoids with PTP1B Inhibitory Activity from the Root Bark of *Erythrina mildbraedii*

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Phytochemical study on an EtOAc-soluble extract of the root bark of *Erythrina mildbraedii* resulted in the isolation of six prenylated flavonoids 1—6. Based on physicochemical and spectroscopic analyses, their structures were determined to be new natural products licoflavanone-4′-*O*-methyl ether (1), 2′,7-dihydroxy-4′-methoxy-5′-(3-methylbut-2-enyl)isoflavone (2), and (3*R*)-2′,7-dihydroxy-3′-(3-methylbut-2-enyl)-2‴,2‴-dimethylpyrano[5‴,6‴:4′,5′]isoflavan (3), along with three known compounds erythrinin B (4), abyssinin II (5), and parvisoflavone B (6). All the isolates, except for compound 4, inhibited PTP1B activity *in vitro* with IC_{50} values ranging from 5.3 to 42.6 μM. This result further suggests that the prenyl group on the B ring of flavonoids plays an important role in suppressing the enzyme PTP1B.

Key words *Erythrina mildbraedii*; Leguminosae; prenyl flavonoid; protein tyrosine phosphatase 1B inhibitor

Since it has been suggested that compounds that reduce protein tyrosine phosphatase 1B (PTP1B) activity or expression levels can used for the treatment of either type 2 diabetes or obesity,[1—5] there have been found a number of natural products reported to possess suppressive effects on PTP1B enzyme in our program searching for anti-diabetes agents from nature.[6—16] Previously, we found that an EtOAc-soluble extract of the root bark of *Erythrina mildbraedii* exhibited inhibitory effect on PTP1B activity and demonstrated that prenylated flavonoids are principles of this extract.[14] Further study on the EtOAc-soluble extract of *E. mildbraedii* led to the isolation of three new (**1**—**3**) and three known (**4**—**6**) prenylated flavonoids. Herein we describe the isolation and structure elucidation of these compounds and the evaluation of their PTP1B inhibitory activity.

Compound **1** was obtained as an amorphous gum exhibiting an optical rotation $[\alpha]_D^{25}$ −21.5° (c=0.3, MeOH). A molecular formula of $C_{21}H_{22}O_5$ was determined for this compound from the molecular ion peak at m/z 354.1465 $[M]^+$ obtained by HR-EI-MS. Its UV spectrum displayed absorption bands at 289 and 321 nm. In the ^{1}H- and ^{13}C-NMR spectra, an aliphatic ABX spin system H-2, H-3_{ax}, and H-3_{eq}, and carbon signals assigned for C-2, C-3, and C-4 were observed, suggesting a flavanone skeleton for **1**.[14,17] The ^{1}H-NMR spectrum also exhibited resonances for an 1,2,4-trisubstituted benzene unit (H-2′, H-5′, H-6′), a set of *meta*-coupled aromatic protons (H-6, H-8), a methoxy group, and an isoprenyl group (H-1″, H-2″, H-4″, H-5″). These observations indicated that **1** was a methyl ether derivative of licoflavanone.[17] The position of the methoxy group was confirmed by HMBC correlation (Fig. 2) from the methoxy protons (δ_H 3.86) to C-4′ (δ_C 158.1). Thus the structure of **1** was determined as 4′-methoxy licoflavanone, as shown in Fig. 1.

Compound **2** was obtained as a yellow amorphous powder. A molecular formula of $C_{21}H_{20}O_5$ was assigned for this compound from the molecular ion peak at m/z 352.1311 $[M]^+$ in HR-EI-MS. Its UV spectrum showed maximum absorptions at 264 and 289 nm. In addition, the ^{1}H-NMR spectrum of **2** (Table 1) displayed a singlet at δ_H 8.28 (H-2), characteristic of an isoflavone skeleton.[18] Furthermore, this spectrum also exhibited an isoprenyl group (H-1″, H-2″, H-4″, H-5″), a methoxy group, and an aromatic ABX spin system (H-5, H-6, H-8) implying a similar structure to that of erylatissin A.[18] In the HMBC spectrum (Fig. 2), long-range correlations were observed from proton H-6′ to carbons C-1″, C-3, C-2′, and C-4′, and from protons H-1″ and methoxy protons to car-

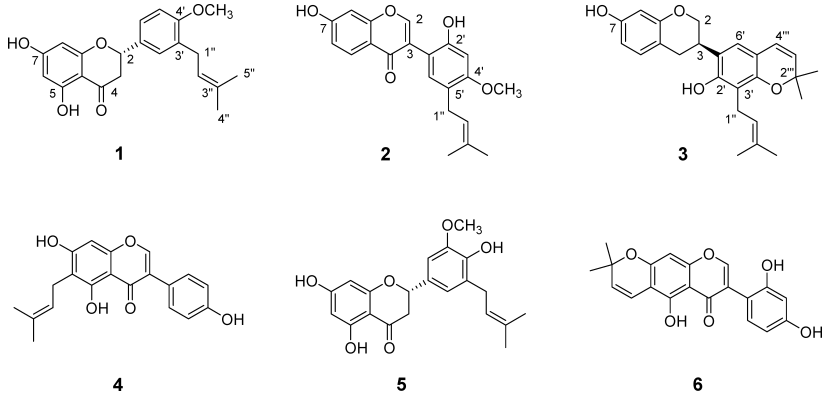

Fig. 1. Structures of Compounds **1**—**6** Isolated from *E. mildbraedii*

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

Position	**1**[a]		**2**[b]		**3**[a]	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	79.5	5.35 dd (13.2, 2.8)	156.3	8.28 s	70.1	4.02 t-like (10.8) 4.37 ddd (10.8, 3.6, 2.0)
3	43.3	2.78 dd (17.2, 2.8) 3.12 dd (17.2, 13.2)	124.8		31.1	3.46 m
4	196.5		178.9		31.9	2.87 ddd (16.0, 4.4, 2.0) 2.94 dd (16.0, 10.8)
5	163.6		128.7	8.13 d (8.8)	128.6	6.98 d (8.8)
6	96.8	5.98[c] d (2.4)	116.9	7.07 dd (8.8, 2.4)	108.1	6.38 dd (8.8, 2.4)
7	164.5[c]		164.6		155.3	
8	95.6	5.99[c] d (2.4)	103.1	6.98 d (2.4)	103.3	6.30 d (2.4)
9	164.6[c]		159.0		154.6	
10	103.5		117.2		120.5	
1′	130.1		112.7		114.1	
2′	127.8	7.20 d (2.0)	157.0		152.8	
3′	131.0		102.2	6.54 s	117.1	
4′	158.1		160.0		149.9	
5′	110.5	6.88 d (8.4)	122.5		114.8	
6′	125.3	7.26 dd (8.4, 2.0)	131.5	7.06 s	124.4	6.56 s
1″	28.7	3.34 br d (7.2)	28.6	3.23 br d (7.2)	22.3	3.31 br d (7.6)
2″	122.1	5.30 m	124.2	5.28 m	123.2	5.24 m
3″	133.3		132.1		130.9	
4″	26.0	1.75 br s	26.0	1.69 br s	26.1	1.79 br s
5″	18.0	1.71 br s	17.9	1.68 br s	18.1	1.67 br s
2‴					76.0	
3‴					128.4	5.48 d (10.0)
4‴					122.6	6.24 d (10.0)
Me					28.1	1.41 s
Me					28.1	1.41 s
OMe	55.7	3.86 s	55.9	3.83 s		

a) In $CDCl_3$. *b*) In acetone-d_6. *c*) Assignment may be interchanged.

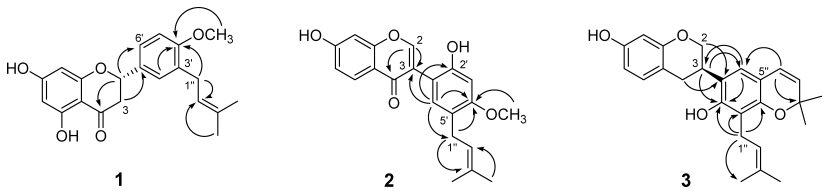

Fig. 2. Key HMBC Correlations of Compounds **1**—**3**

bon C-4′. These indicated that the isoprenyl group is located at C-5′ while the methoxy group is attached to C-4′. Therefore, the structure of **2** was characterized as 2′,7-dihydroxy-4′-methoxy-5′-(3-methylbut-2-enyl)isoflavone.

Compound **3** was obtained as an amorphous gum exhibiting a negative optical rotation $[\alpha]_D^{25}$ −28.8° (c=0.49, MeOH). A molecular formula of $C_{25}H_{28}O_4$ was determined for this compound by HR-EI-MS from the molecular ion peak at m/z 392.1986 $[M]^+$. The UV spectrum of **3** showed absorption bands at 280 and 312 nm. The observations of aliphatic protons at H-2_{ax}, H-2_{eq}, H-3, H-4_{ax}, and H-4_{eq} in the ^{1}H-NMR spectrum, along with carbon signals assigned for C-2, C-3, and C-4 in the ^{13}C-NMR spectrum, suggested that **3** was an isoflavan.[19,20] The ^{1}H-NMR spectrum displayed three aromatic protons of an ABX spin system (H-5, H-6, H-8), a dimethylpyran ring (H-3‴, H-4‴), two methyl groups (δ_H 1.41, 6H, s), and a prenyl group (H-1″, H-2″, H-4″, H-5″). The HMBC correlations (Fig. 2) from H-6′ to C-3, C-1′, and C-4′, and from H-3 to C-2′ and 124.4 C-6′, and from H-1″ to carbons at C-2′, C-3′, and C-4′, indicated that the dimethylpyran ring is fused to C-4′ and C-5′, and the isoprenyl group located at C-3′. The absolute configuration of **3** was determined to be 3*R* by analysis of its circular dichroism (CD) data.[19,20] Thus the structure of compound **3** was determined as (3*R*)-2′,7-dihydroxy-3′-(3-methylbut-2-enyl)-2‴,2‴-dimethylpyrano[5‴,6‴:4′,5′]isoflavan.

The structures of the known flavonoids **4**—**6** (Fig. 1) were identified as erythrinin B,[21] abyssinin II,[22] and parvisoflavone B,[23] respectively, by comparing their physical ($[\alpha]_D$) and spectroscopic (UV, MS, NMR) data with those reported in the literature. All the isolates were assayed for their inhibitory activity against PTP1B *in vitro* using a described method,[6—9] and the results are presented in Table 2. Compounds **1**—**3** and **5**—**6** inhibited PTP1B activity with IC_{50} values ranging from 5.5±0.3 to 42.6±2.4 μM. Among prenylated phenolics from *Erythrina* plants,[12,14—16] an isoflavan (compound **3**) and isoflavanones[12] were the most potent agents, while most flavanones, chalcones, and benzofurans exhibited moderate inhibition.[14—16] However, compound **4** and other isoflavones with a prenyl group on the A-ring

Table 2. The Inhibitory Activity of the Isolated Compounds **1**—**6** against PTP1B

Compounds	PTP1B inhibitory activity IC_{50} (μM)[a,b]
1	29.6±2.5
2	21.3±1.3
3	5.5±0.3
4	>100 (25.4%)[b]
5	40.5±1.9
6	42.6±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±S.D. of three replicates. *b*) Value in parentheses represents a percentage of inhibition at tested concentration 100 μM. *c*) Positive controls.

showed very weak inhibitory effects.[12] This is in good agreement with the previous observation that the double bond between C-2 and C-3 on the C-ring plays an important role in the reducing activity of these flavonoids.[12] In conclusion, this study further enforced that the prenyl group of *Erythirina* phenolics is responsible for their inhibitory effect on PTP1B activity.

Experimental

General Experimental Procedure Optical rotations were determined on a JASCO P-1020 polarimeter using a 100 mm glass microcell. UV spectra were taken in MeOH using a Shimadzu spectrophotometer. The CD spectrum was recorded in MeOH on a JASCO J-715 spectrophotometer. Nuclear magnetic resonance (1D- and 2D-NMR) spectra were obtained by Varian Unity Inova 400 MHz spectrometer with TMS as internal standard. All accurate mass experiments were performed on a JMS-700 (Jeol, Japan) mass spectrometer. Column chromatography was conducted using silica gel 60 (40—63 and 63—200 mm particle size, Merck) and RP-18 (150 μm particle size, Merck). Precoated TLC silica gel 60 F_{254} plates from Merck were used for thin-layer chromatography. HPLC 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 mm particle size, RS Tech, Korea) for semipreparative runs.

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 (Yaounde, 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 (105 g, IC_{50}=30.2 μg/ml) was separated by silica gel column chromatography (10×30 cm, 63—200 μm particle size) using a gradient of hexane–EtOAc (from 8 : 1, 7 : 1 to 0 : 1), then EtOAc–MeOH (from 20 : 1, 19 : 1, to 1 : 1), to yield five fractions (Fr. 1—Fr. 5) according to their TLC profiles. The PTP1B inhibitory activity was concentrated in Fr. 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, 19 : 1, to 0 : 1), to yield seven subfractions (Fr. 1.1—Fr. 1.7). Except for Fr. 1.1, fractions Fr. 1.2—Fr. 1.7 displayed similar bioactivities with IC_{50} values ranging from 11.4 to 14.5 μg/ml. Fr. 1.6 [eluted with hexane–EtoAc (3 : 1), 560 mg] was fractionated by silica gel column chromatography (3.5×19.5 cm, 40—63 μm particle size) using a stepwise gradient of hexane–acetone (8 : 2, 8 : 3 and 7 : 3, 3 l for each step), to afford three subfractions (Fr. 1.6.1—Fr. 1.6.3). Fr. 1.6.3 (30 mg) was purified by semipreparative HPLC using an isocratic solvent system of 65% MeCN in H_2O (flow rate 2 ml/min, UV detection at 254 nm) to yield compound **1** (4.6 mg, t_R=31 min). Fr. 1.7 [eluted with hexane–EtOAc (1 : 1), 1127 mg] was fractionated by silica gel column chromatography (3.5×23 cm, 40—63 μm particle size) using a stepwise gradient of hexane–acetone (8 : 2, 8 : 3, and 7 : 3, 3 l for each step) to afford seven subfractions (Fr. 1.7.1—Fr. 1.7.7). Fr. 1.7.3 [eluted with hexane–acetone (8 : 2), 198 mg] was separated by HPLC using an isocratic solvent system of 65% MeCN in H_2O (flow rate 2 ml/min, UV detection at 254 nm) leading to the isolation of compounds **2** (3.5 mg, t_R=19.2 min) and **3** (36 mg, t_R=60.5 min). Fr. 1.7.4 [eluted with hexane–acetone (7 : 3), 356 mg] was subjected to reversed phase C_{18} column chromatography (3×25 cm, 150 μm particle size) eluting with a stepwise gradient of MeCN–H_2O (from 55 : 45, 65 : 35, 75 : 25, to 100 : 0, 0.5 l for each step), to yield four subfractions (Fr. 1.7.4.1—Fr. 1.7.4.4). Purification of Fr. 1.7.4.1 (136 mg) by HPLC using an isocratic solvent system of 51% MeCN in H_2O (flow rate 2 ml/min, UV detection at 254 nm) yielded compounds **4** (3.2 mg, t_R=34.7 min) and **5** (3 mg, t_R=39.6 min). Compound **6** (2.5 mg, t_R=42.7 min) was isolated from Fr. 1.7.4.2 (45 mg) using HPLC eluted with 51% MeCN in H_2O (flow rate 2 ml/min, UV detection at 254 nm).

4′-Methoxy Licoflavanone (**1**): Amorphous gum; $[\alpha]_D^{25}$ −21.5° (*c*=0.3, MeOH); UV (MeOH) λ_{max} nm (log ε): 289 (4.15), 321 (3.81); ^{1}H- and ^{13}C-NMR data, see Table 1; EI-MS *m/z* (rel. int.): 354 ($[M]^+$, 100), 323 (8), 285 (7), 202 (23), 189 (83), 179 (17), 153 (24), 147 (11); HR-EI-MS *m/z* 354.1465 $[M]^+$ (Calcd for $C_{21}H_{22}O_5$, 354.1467).

2′,7-Dihydroxy-4′-methoxy-5′-(3-methylbut-2-enyl)isoflavone (**2**): Yellow amorphous powder; mp 99—102 °C; UV (MeOH) λ_{max} nm (log ε): 264 (3.97), 289 (3.95); ^{1}H- and ^{13}C-NMR data, see Table 1; EI-MS *m/z* (rel. int.): 352 ($[M]^+$, 100), 337 (52), 297 (25), 284 (15), 267 (6), 232 (11), 137 (60); HR-EI-MS *m/z* 352.1311 $[M]^+$ (Calcd for $C_{21}H_{20}O_5H$, 352.1311).

(3*R*)-2′,7-Dihydroxy-3′-(3-methylbut-2-enyl)-2‴,2‴-dimethylpyrano[5‴, 6‴: 4′,5′]isoflavan (**3**): Amorphous gum; $[\alpha]_D^{25}$ −28.8° (*c*=0.49, MeOH); UV (MeOH) λ_{max} nm (log ε): 280 (4.25), 312 (3.92); CD (*c*=0.000034, MeOH): $[\theta]_{285}$ +1.89, $[\theta]_{237}$ −3.64; ^{1}H- and ^{13}C-NMR data, see Table 1; EI-MS *m/z* (rel. int.): 392 ($[M]^+$, 26), 377 (100), 321 (4); HR-EI-MS *m/z* 392.1986 $[M]^+$ (Calcd for $C_{25}H_{28}O_4$, 392.1988).

PTP1B Assay PTP1B (human, recombinant) was purchased from BIOMOL® International LP (Plymouth Meeting, PA, U.S.A.) and the inhibitory effect of isolates on enzyme activity was measured as described previously.[6—9]

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References

1) Moller D. E., *Nature* (London), **414**, 821—827 (2001).
2) Wagman A. S., Nuss J. M., *Curr. Pharm. Des.*, **7**, 417—450 (2001).
3) Johnson T. O., Ermolieff J., Jirousek M. R., *Nat. Rev. Drug Discov.*, **1**, 696—709 (2002).
4) Bialy L., Waldmann H., *Angew. Chem. Int. Ed.*, **44**, 3814—3839 (2005).
5) Van Huijsduijnen R. H., Bombrun A., Swinnin D., *Drug Discov. Today*, **7**, 1013—1019 (2002).
6) Cui L., Na M., Oh H., Bae E. Y., Jeong D. G., Ryu S. E., Kim S., Kim B. Y., Oh W. K., Ahn J. S., *Bioorg. Med. Chem. Lett.*, **16**, 1426—1429 (2006).
7) Na M., Yang S., He L., Oh H., Kim B. S., Oh W. K., Kim B. Y., Ahn J. S., *Planta Med.*, **72**, 261—263 (2006).
8) Na M., Oh W. K., Kim Y. H., Cai X. F., Kim S., Kim B. Y., Ahn J. S., *Bioorg. Med. Chem. Lett.*, **16**, 3061—3064 (2006).
9) 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.*, **16**, 3273—3276 (2006).
10) Kim S., Na M., Jang J., Sohn C. B., Kim B. Y., Oh W. K., Ahn J. S., *J. Enzyme Inhib. Med. Chem.*, **21**, 379—383 (2006).
11) Na M., Kim K. A., Kim B. Y., Oh W. K., Ahn J. S., *Biol. Pharm. Bull.*, **30**, 379—381 (2007).
12) Bae E. Y., Na M., Njamen D., Mbafor J. T., Formum Z. T., Cui L., Choung D. H., Kim B. Y., Oh W. K., Ahn J. S., *Planta Med.*, **72**, 945—948 (2006).
13) Yang S., Na M. K., Jang J. P., Kim K. A., Kim B. Y., Sung N. J., Oh W. K., Ahn J. S., *Phytother. Res.*, **20**, 680—682 (2006).
14) Na M., Jang J., Njamen D., Mbafor J. T., Fomum Z. T., Kim B. Y., Oh W. K., Ahn J. S., *J. Nat. Prod.*, **69**, 1572—1576 (2006).
15) Na M., Hoang D. M., Njamen D., Mbafor J. T., Fomum Z. T., Thuong P. T., Ahn J. S., Oh W. K., *Bioorg. Med. Chem. Lett.*, **17**, 3868—3871 (2007).
16) Cui L., Ndinteh D. T., Na M., Thuong P. T., Silike-Muruumu J., Njamen D., Mbafor J. T., Fomum Z. T., Ahn J. S., Oh W. K., *J. Nat. Prod.*, **70**, 1039—1042 (2007).
17) Fukai H., Goto K., Tabata M., *Chem. Pharm. Bull.*, **36**, 4174—4176

(1988).
18) Chacha M., Bojase-Moleta G., Majinda R. R. T., *Phytochemistry*, **66**, 99—104 (2005).
19) Versteeg M., Bezuidenhoudt B. C. B., Ferreira D., *Tetrahedron*, **55**, 3365—3376 (1999).
20) Tanaka H., Oh-Uchi T., Etoh H., Sako M., Asai F., Fukai T., Sato M., Murata J., Tateishi Y., *Phytochemistry*, **64**, 573—578 (2003).
21) Lee S. J., Wood A. R., Maier C. G. A., Dixon R. A., Mabry T. J., *Phytochemistry*, **49**, 2573—2577 (1998).
22) Ichimaru M., Moriyasu M., Nishiyama Y., Kato A., *J. Nat. Prod.*, **59**, 1113—1116 (1996).
23) Tahara S., Ingham J. L., Nakahara S., Mizutani J., Harborne J. B., *Phytochemistry*, **23**, 1889—1900 (1984).