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Inhibitory effect of 2-arylbenzofurans from *Erythrina addisoniae* on protein tyrosine phosphatase-1B☆

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Abstract—Bioassay-guided fractionation of an EtOAc-soluble extract of the stem bark of *Erythrina addisoniae* (Leguminosae), using an in vitro PTP1B inhibitory assay, resulted in the isolation of three new (**1**–**3**) and three known (**4**–**6**) 2-arylbenzofuran derivatives. The new compounds were identified as 2-[2′,4′-dihydroxy-3′-(3-methylbut-2-enyl)phenyl]-6-hydroxybenzofuran (**1**), 2-[2′-methoxy-4′-hydroxy-5′-(3-methylbut-2-enyl)phenyl]-6-hydroxybenzofuran (**2**), and 2-(2′-methoxy-4′-hydroxyphenyl)-5-(3-methylbut-2-enyl)-6-hydroxybenzofuran (**3**). The new 2-arylbenzofurans **1**–**3** inhibited PTP1B activity with IC_{50} values ranging from 13.6 ± 1.1 to 17.5 ± 1.2 μM in vitro assay. On the basis of the data obtained, 2-arylbenzofurans with prenyl group may be considered as a new class of PTP1B inhibitors.

Binding of insulin to its receptor results in the phosphorylation of insulin receptor 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] Protein tyrosine phosphatases (PTPs) which dephosphorylate the tyrosine residues of proteins are considered negative regulators of insulin signaling. Of the various PTPs, protein tyrosine phosphatase 1B (PTP1B) plays a key role in the insulin-dependent signal cascade, and has attracted considerable attention as a potential target for the treatment of type-2 diabetes.[1] 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.[1a] Therefore, it has been suggested that compounds that reduce PTP1B activity or expression levels can not only be used for treating type-2 diabetes but also obesity. Although there have been a number of reports on the design and development of PTP1B inhibitors,[1,2] new types of PTP1B inhibitors with suitable pharmacological properties remain to be discovered.

In our continuing program to search PTP1B inhibitors from plants, we found that an EtOAc-soluble extract of the stem bark of *Erythrina addisoniae* inhibited PTP1B activity (>80% inhibition at 30 μg/ml). The genus *Erythrina* of the family Leguminosae comprises over 110 species that are widely distributed in tropical and subtropical regions, and representative species have been used in indigenous medicine.[3] Alkaloids, pterocarpans, flavonoids, and other benzofurans have been reported as constituents of this genus, which have been found to possess a wide range of biological activities that include antioxidant, antimicrobial, cytotoxic, and anti-inflammatory activities.[4] Recently, we reported that prenylated isoflavonoids from the species *E. addisoniae* showed inhibitory effect on the PTP1B activity in vitro.[5] Further investigation on the PTP1B inhibitory compounds from this plant has led to the isolation of six 2-arylbenzofuran derivatives,[6] including three new compounds **1**–**3** and three known ones (**4**–**6**). The structures of the known compounds were determined to be kanzonol U (**4**),[7] glyinflanin H (**5**),[8] and vignafuran (**6**),[9] by

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comparing the 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 powder, and the molecular formula was confirmed to be $C_{19}H_{18}O_4$ from the molecular ion peak at *m/z* 310.1204 $[M]^+$ (calcd for $C_{19}H_{18}O_4$, 310.1205) by HREIMS.[10a] The UV spectrum of this compound was characteristic of 2-arylbenzofurans, with maxima at 219, 289, 331, and 346 nm.[4b,6,7] The observation of a characteristic olefinic proton at δ_H 7.02 in ^{1}H NMR spectrum, and signals for C-2 (δ_C 154.15) and C-3 (δ_C 103.16) in ^{13}C NMR spectrum further supported that **1** is a 2-arylbenzofuran derivative.[4b,7,8] In addition, the ^{1}H NMR spectrum of **1** (Table 1) displayed signals for a 1,2,4-trisubstituted benzene unit [δ_H 7.37 (1H, d, J = 8.8 Hz), 6.79 (1H, dd, J = 8.8, 2.0 Hz), and 7.01 (1H, d, J = 2.0 Hz)], a set of *ortho*-coupled aromatic protons [δ_H 7.46 (1H, d, J = 8.4 Hz) and 6.57 (1H, d, J = 8.4 Hz)], and an prenyl group [δ_H 5.28 (1H, m), 3.48 (2H, br d, J = 6.8 Hz), 1.80 (3H, br s), and 1.67 (3H, br s)], which suggested that **1** is a demethyl derivative of bidwillol B isolated previously from *Erythrina bidwillii*.[4b] The signals appearing in the ^{13}C NMR spectrum (Table 1) were very similar to those of bidwillol B except for the absence of one methoxy signal. Thus, the structure of the new compound **1** was determined as 2-[2′,4′-dihydroxy-3′-(3-methylbut-2-enyl)]-6-hydroxybenzofuran (2′-*O*-demethylbidwillol B), and confirmed using the HMBC NMR technique.

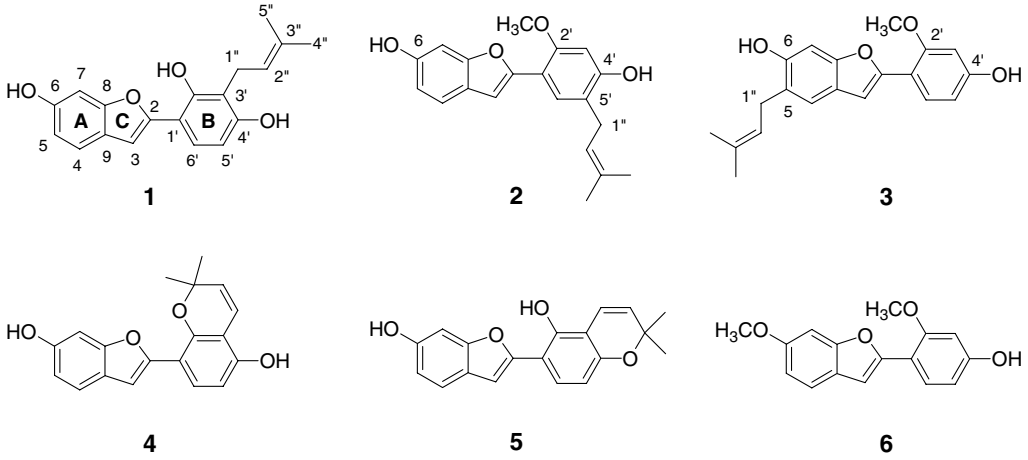

Figure 1. Structures of compounds **1**–**6** isolated from *E. addisoniae*.

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of compounds **1**–**3**[a]

Position	1		2		3	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	154.15		152.15		151.68	7.08, s
3	103.16	7.02, s	104.26	7.11, s	104.22	7.25, s
4	121.54	7.37, d (8.8)	121.07	7.38, d (8.4)	120.93	
5	112.92	6.79, dd (8.8, 2.0)	111.68	6.74, dd (8.4, 2.4)	122.94	
6	156.13		153.26		152.28	6.98, s
7	98.50	7.01, d (2.0)	98.15	7.00, d (2.4)	98.43	
8	155.87		154.76		153.58	
9	122.91		123.99		123.67	
1′	111.23		112.64		113.36	
2′	153.72		156.39		157.76	6.53, br s[b]
3′	116.62		100.11	6.52, s	99.41	
4′	157.12		155.64		156.58	6.52, dd (8.8, 2.4)[b]
5′	108.78	6.57, d (8.4)	118.59		107.62	7.86, d (8.8)
6′	125.72	7.46, d (8.4)	128.26	7.72, s	127.91	3.39, br d (7.6)
1″	23.05	3.48, br d (6.8)	30.01	3.41, br d (6.8)	30.47	5.37, m
2″	123.66	5.28, m	122.23	5.36, m	122.51	
3″	132.12		135.68		135.05	1.80, br s
4″	25.98	1.67, br s	26.05	1.81, br s	26.05	1.82, br s
5″	18.07	1.80, br s	18.19	1.84, br s	18.12	3.96, s
2′-OMe			55.87	3.94, s	55.74	5.14, s
6-OH						
4′-OH				5.44, s		

[a] The NMR spectra of **1** were run in acetone-d_6, while those of **2** and **3** in $CDCl_3$.
[b] Signals were partially overlapped.

Compound **2** was obtained as a colorless needle with the molecular formula $C_{20}H_{20}O_4$, as deduced from the molecular ion peak at *m*/*z* 324.1361 $[M]^+$ (calcd for $C_{20}H_{20}O_4$, 324.1361) in HREIMS.[10b] The characteristic UV, ^1H and ^{13}C NMR spectral data for **2** were similar to those of **1**.[10a,b] Following a full spectroscopic analysis, a comparison of the ^1H NMR spectral data for **2** revealed that both the A- and C-ring of **2** are identical to those of **1**. However, distinctively observed two aromatic singlet signals at δ_H 7.72 and 6.52, and a methoxy signal at δ_H 3.94 in the ^1H NMR spectrum suggested that the B-ring of **2** is a 1,2,4,5-tetrasubstituted benzene ring including one methoxy group. The aromatic singlet at δ_H 7.72 was assigned to H-6′ on the basis of its chemical shift and HMBC correlation to C-2 (δ_C 152.15), while that at δ_H 6.52 to H-3′ on the basis of three-bond correlations to C-1′ (δ_C 112.64) and C-5′ (δ_C 118.59). The positions of the methoxy and prenyl groups were established by the analysis of HMBC data, where correlations of the methoxy protons at δ_H 3.94 with C-2′ (δ_C 156.39), and of H-1″ (δ_H 3.41) with C-4′ (δ_C 155.64), C-5′ (δ_C 118.59), and C-6′ (δ_C 128.26) were observed. Thus, the structure of **2** was determined as 2-[2′-methoxy-4′-hydroxy-5′-(3-methylbut-2-enyl)phenyl]-6-hydroxybenzofuran, named addisofuran A.

Compound **3** was obtained as a purplish powder. A molecular formula of $C_{20}H_{20}O_4$ was determined for this compound from the molecular ion peak at *m*/*z* 324.1361 $[M]^+$ (calcd for $C_{20}H_{20}O_4$, 324.1361) in HREIMS.[10c] On the basis of the UV absorbance and the NMR data,[10] **3** was also regarded as a 2-arylbenzofuran like **1** and **2**. From the ^1H NMR spectrum of **3**, signals for a 1,2,4-trisubstituted benzene unit were detected, in which one *meta*-coupled doublet, and one doublet of doublet consisted of overlapped signals. Besides, three aromatic singlet signals were observed at δ_H 7.25, 7.08, and 6.98, along with signals for a prenyl group at δ_H 5.37 (1H, m), 3.39 (2H, br d, J = 7.6 Hz), 1.82 (3H, s), and 1.80 (3H, s). The differences in chemical shifts of the aromatic protons for **3**, compared to those of **2**, suggested that substitution patterns for the A- and B-ring of **3** are different from those of **2**. The aromatic singlet at δ_H 7.25 was assigned to H-4 on the basis of HMBC correlation to C-3 (δ_C 104.22), C-6 (δ_C 152.28), and C-8 (δ_C 153.58), while that at δ_H 6.98 to H-7 on the basis of two-bond correlations to C-6 and C-8. The remaining singlet at δ_H 7.08 was assigned to H-3, which was also confirmed by HMBC spectroscopic data. The position of the methoxy group was determined by the HMBC correlation from the methoxy protons (δ_H 3.96) to a quaternary carbon (δ_C 157.76, C-2′). The prenyl group was located by the analysis of HMBC data, in which correlations of H-1″ (δ_H 3.39) with C-4 (δ_C 120.93), C-5 (δ_C 122.94), and C-6 (δ_C 152.28) were observed. Thus, the structure of **3** was determined as 2-(2′-methoxy-4′-hydroxyphenyl)-5-(3-methylbut-2-enyl)-6-hydroxybenzofuran, named addisofuran B.

All the isolates were evaluated for their inhibitory activity against PTP1B using an in vitro assay. The known PTP1B inhibitors, RK-682 (IC_{50} = 5.0 ± 0.5 μM) and ursolic acid (IC_{50} = 3.9 ± 0.3 μM), were used as positive controls in this assay.[11] The new 2-arylbenzofurans **1**–**3** inhibited PTP1B activity with IC_{50} values of 13.6 ± 1.1, 17.5 ± 1.2, and 15.7 ± 1.6 μM, respectively, while compounds **4**–**6** exhibited a significantly lower PTP1B inhibitory activity than **1**–**3**. Both **4** (IC_{50} = 62.7 ± 2.0 μM) and **5** (IC_{50} = 64.9 ± 1.1 μM), with a dimethylpyran moiety in the B-ring, were less active than **1**, indicating that cyclization between a prenyl group and one of the phenolic hydroxyl in the B-ring may be responsible for a loss of in vitro activity. Compound **6** (IC_{50} = 74.1 ± 1.9 μM) without a prenyl group displayed a lower activity compared to the derivatives. Despite the difference in the position of a prenyl group, **2** and **3** showed a similar activity. The results indicate that substitution of prenyl groups may be important for PTP1B inhibitory activity in vitro, although structure–activity relationships of 2-arylbenzofurans were not thoroughly investigated.

Most of the 2-arylbenzofurans with prenyl groups have been isolated from a rather limited number of plant families, inclusive of the Leguminosae.[4b] There have been a number of reports on the newly identified prenylated 2-arylbenzofurans.[12] However, except for the antimicrobial,[4d,12b] cytotoxic,[4e,f] and estrogenic activities,[12a] little is known as to the biological activities of these metabolites. As shown in the present study, the prenylated 2-arylbenzofurans could be considered as a promising class 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.

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6. The dried stem bark of *E. addisoniae* (7 kg), collected in Cameroon, was extracted with MeOH at room temperature. Since the EtOAc-soluble fraction (106 g) was found to be the most active (81% inhibition at 30 μg/mL) among the solvent fractions, this fraction was separated by silica gel column chromatography (10 × 30 cm; 63–200 μm particle size) using a gradient of hexane–EtOAc (from 10:1 to 0:1), then EtOAc–MeOH (from 20:1 to 1:1), to yield 34 fractions. According to their TLC profiles, fractions between 1st and 6th, eluted with hexane–EtOAc (from 10:1 to 1:1), were combined, to yield 29.3 g of dark brown fraction (Fr. 1), which was chromatographed over silica gel (8 × 30 cm; 63–200 μm particle size) using a gradient of hexane–EtOAc (from 10:1 to 2:1), then EtOAc–MeOH (from 50:1 to 5:1), to yield seventeen subfractions. The subfractions between 5th and 14th, eluted with hexane–EtOAc (from 6:1 to 2:1), then EtOAc–MeOH (50:1), displayed similar range of TLC profiles and the PTP1B inhibitory activity, which were re-combined, to give 12.3 g of bioactive fraction (Fr. 1–2; IC_{50} = 15.2 μg/mL). This fraction was chromatographed over silica gel (6.5 × 35 cm; 63–200 μm particle size) using a gradient of hexane–EtOAc (from 85:15 to 75:25), to yield eight subfractions (Fr. 1.2.1–Fr. 1.2.8). Except for Fr. 1.2.1, other fractions (Fr. 1.2.2–Fr. 1.2.8) displayed similar bioactivities, with IC_{50} values ranging from 9.1 to 12.4 μg/mL. Purification of Fr. 1.2.4 [eluted with hexane–EtOAc (80:20), 2.32 g] 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 65:35, 70:30 to 75:25; 2 L for each step) led to the isolation of compound **1** (70 mg). Fr. 1.2.3 [eluted with hexane–EtOAc (from 85:15 to 80:20), 2.77 g] was fractionated by RP-18 column chromatography (3.5 × 35 cm; 40–63 μm particle size) using an isocratic solvent system of 75% MeOH in H_2O, to yield ten subfractions (Fr. 1.2.3.1–Fr. 1.2.3.10). Further purification of Fr. 1.2.3.4 (25.9 mg) and Fr. 1.2.3.5 (65.0 mg) by semipreparative HPLC [Shimadzu System LC-10AD pump equipped with a model SPD-10Avp UV detector, RS Tech Optima Pak® C_{18} column (10 × 250 mm, 10 μm particle size); mobile phase AcCN-H_2O (55:45); flow rate 2 mL/min; UV detection at 254 nm] resulted in the isolation of compounds **5** (11.6 mg; t_R = 32.3 min) and **6** (2.2 mg; t_R = 34.5 min), respectively. Fr. 1.2.5 [eluted with hexane–EtOAc (80:20), 1.39 g] was separated by silica gel column chromatography (4.5 × 27 cm; 40–63 μm particle size) using a stepwise gradient of hexane–acetone (from 75:25, 70:30 to 65:35; 2 L for each step), to afford six subfractions (Fr. 1.2.5.1–Fr. 1.2.5.6). Fr. 1.2.5.5 [eluted with hexane–acetone (from 70:30 to 65:35), 311.2 mg] was subjected to RP-18 column chromatography eluting with a gradient of MeOH–H_2O (from 75:25 to 80:20), to obtain nine subfractions (Fr. 1.2.5.5.1–Fr. 1.2.5.5.9), along with 69 mg of compound **4**. Compound **2** (10.2 mg, t_R = 37.9 min) was isolated from Fr. 1.2.5.5.7 [eluted with MeOH–H_2O (80:20), 71.1 mg] using preparative HPLC, with the mobile phase 70% MeOH in H_2O. Fr. 1.2.5.5.8 [eluted with hexane–acetone (80:20), 50.1 mg] was also purified by HPLC using an isocratic solvent system of 70% MeOH in H_2O over 60 min, then increased to 100% MeOH over 80 min, to yield 5.4 mg of compound **3** (t_R = 63.7 min).
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10. (a) Physical and spectroscopic data of new compounds: compound **1** (Demethylbidwillol B): white powder; mp 116–118 °C; EIMS *m/z* (rel int.): 310 $[M]^+$ (58), 293 (5), 268 (5), 254 (100), 227 (4), 197 (10); HREIMS *m/z* 310.1204 $[M]^+$ (calcd for $C_{19}H_{18}O_4$, 310.1205); UV (MeOH) λ_{max} nm (log ε): 220 (4.40), 291 (4.10), 325 (4.39), 338 (4.38); IR (KBr) ν_{max} cm^{-1}: 3380, 2920, 1620, 1600, 1490, 1440, 1370, 1290, 1160, 1140, 1120, 1040, 960, 810; ^1H and ^{13}C NMR data, see Table 1; (b) Compound **2** (Addisofuran A): white needle; mp 159–162 °C; EIMS *m/z* (rel int.): 324 $[M]^+$ (100), 307 (11), 281 (3), 268 (46), 269 (62), 254 (8), 225 (7), 197 (6); HREIMS *m/z* 324.1361 $[M]^+$ (calcd for $C_{20}H_{20}O_4$, 324.1361); UV (MeOH) λ_{max} nm (log ε): 219 (4.38), 289 (4.06), 331 (4.39), 346 (4.38); IR (KBr) ν_{max} cm^{-1}: 3380, 2970, 2920, 1610, 1600, 1510, 1440, 1400, 1360, 1280, 1200, 1140, 1120, 1036, 820; ^1H and ^{13}C NMR data, see Table 1; (c) compound **3** (Addisofuran B): purplish powder; mp 100–103 °C; EIMS *m/z* (rel int.): 324 $[M]^+$ (100), 307 (10), 281 (4), 268 (72), 269 (81), 254 (4), 225 (5), 197 (8); HREIMS *m/z* 324.1361 $[M]^+$ (calcd for $C_{20}H_{20}O_4$, 324.1361); UV (MeOH) λ_{max} nm (log ε): 217 (4.39), 290 (4.08), 330 (4.39), 345 (4.38); IR (KBr) ν_{max} cm^{-1}: 3380, 2970, 2920, 1615, 1595, 1505, 1440, 1400, 1365, 1280, 1200, 1140, 1118, 1030, 820; ^1H and ^{13}C NMR data, see Table 1.
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