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Flavanones from the stem bark of *Erythrina abyssinica*

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ABSTRACT

Twelve new flavanones bearing a 2,2-dimethylpyrano ring were isolated from a MeOH extract of the stem bark of *Erythrina abyssinica*. Their structures were determined on the basis of spectroscopic (UV, CD, 1D and 2D NMR, HRMS) and physico-chemical analyses. Compounds **1**, **3**, **5**, **6**, **8**, and **9** exhibited inhibitory effects on the enzyme activity of PTP1B in an in vitro assay with IC_{50} values ranging from 13.9 ± 2.1 to 19.0 ± 1.8 μM. These results suggest that prenyl and methoxy groups on the B ring contribute to the inhibitory activity of flavanones against PTP1B.

1. Introduction

The genus *Erythrina*, a member of the Leguminosae family, comprises more than 100 species that are widely distributed in tropical and subtropical regions worldwide.[1] Many of these species are used indigenously as traditional medicines to treat various diseases, such as infection, cough, malaria, inflammation, bronchitis, asthma, and insomnia.[2–4] Previous studies on *Erythrina* plants have demonstrated that alkaloids and phenolics (flavonoids, pterocarpans, and benzofurans) are the active components.[2,5–7] Recently, our interest in the phytochemistry of *Erythrina* species has resulted in the isolation of numerous bioactive compounds that have been shown to exhibit inhibitory effects on protein tyrosine phosphatase 1B (PTP1B) in an in vitro assay.[8–10] Previous studies on the stem bark of the plant *E. abyssinica* resulted in the isolation of eleven flavanones and six chalcones.[11,12] In the continuous interest in constituents of this plant, this paper deals with the isolation and structural elucidation of 12 new flavanones (**1**–**12**) and their inhibitory activity towards PTP1B .

2. Results and discussion

Bioactivity-guided fractionation and isolation of an EtOAc-soluble extract of *E. abyssinica* using an in vitro PTP1B inhibition assay and repeated column chromatographic separations yielded compounds **1**–**12**. The compounds were isolated as white amorphous powders and showed negative $[\alpha]_D$ values in MeOH. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of each compound displayed an AMX spin system for H-2, H-3_{ax}, and H-3_{eq}, and corresponding carbon signals for C-2 (δ_C 76.4–80.8) and C-3 (δ_C 42.7–46.2), and a ketone carbon resonance (δ_C 190.4–197.7). Their CD spectra showed positive Cotton effects near 330 nm and negative Cotton effects near 285–305 nm. These observations were indicative of a 2(*S*)-flavanone skeleton.[9,13]

Compound **1** was obtained as a white powder with $[\alpha]_D^{25}$ −7.80 (*c* 0.1, MeOH). Its UV spectrum exhibited absorption maxima at 212, 232, 288, and 328 nm. The IR spectrum revealed absorbance bands at 3365 (hydroxyl groups), 1653 (conjugated carbonyl group), 1603, 1506, and 1464 cm^{-1} (aromatic ring). A molecular formula of $C_{25}H_{26}O_6$ was determined from the molecular ion peak at *m/z* 422.1725 $[M]^+$ obtained by HREIMS. The ^1H NMR spectrum of compound **1** showed an AMX spin system at δ 5.64 (1H, dd, *J* = 2.8, 13.2 Hz), 3.16 (1H, dd, *J* = 17.2, 13.2 Hz), and 2.65 (1H, dd, *J* = 2.8, 17.2 Hz), corresponding to H-2, H-3_{ax}, and H-3_{eq}, respectively, of a flavanone skeleton.[13] The carbon resonances at δ_C 76.4, 42.7, and 196.8 in the ^{13}C NMR spectrum supported this observation. In addition, the ^1H and ^{13}C NMR spectra of compound **1** also revealed the presence of a 2,2-dimethylpyrano ring and a prenyl group. The configuration at C-2 was inferred to be *S* by the CD spectrum, which had a positive Cotton effect near 339 nm and a negative Cotton effect near 287 nm. All of these results suggested a structure similar to sigmoidin F for compound **1**.[14] The

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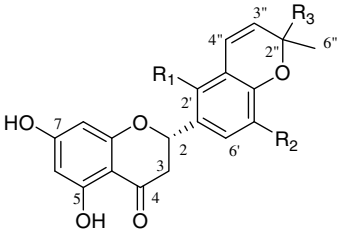
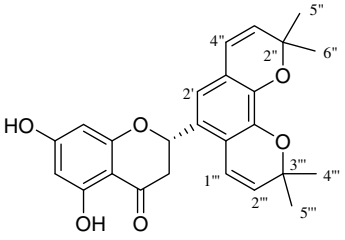


prenyl

1 R_1 = prenyl, R_2 = OH, R_3 = CH_3

2 R_1 = H, R_2 = OH, R_3 = CH_2OH

3 R_1 = H, R_2 = OCH_3, R_3 = CH_2OH



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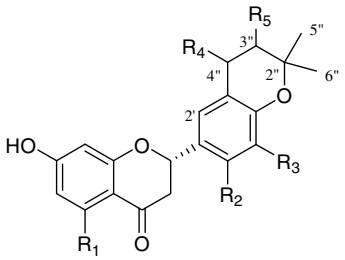

5 R_1 = R_5 = OH, R_2 = R_4 = H, R_3 = prenyl

6 R_1 = R_5 = OH, R_2 = R_4 = H, R_3 = OCH_3

7 R_1 = R_4 = R_5 = OH, R_2 = R_3 = H

8 R_1 = R_4 = R_5 = OH, R_2 = H, R_3 = prenyl

9 R_1 = R_4 = R_5 = OH, R_2 = prenyl, R_3 = OH

10 R_1= R_3 = OH, R_2 = R_5 = H, R_4 = (=O)

11 R_1 = R_2 = R_4 = H, R_3 = R_5 = OH

12 R_1 = R_2 = H, R_3 = R_4 = R_5 = OH

HMBC correlations from proton H-1‴ (δ_H 3.45) to C-1′(δ_C 129.4), C-2′ (δ_C 126.5), C-3′ (δ_C 120.5), and from H-4″ (δ_H 6.60) to C-2′ (δ_C 126.5) indicated that the prenyl group was attached to C-2′. The HMBC correlations from H-6′ (δ_H 7.01) to C-2 (δ_C 76.4) suggested that the hydroxy group was located at C-5′. Therefore, compound **1** was determined to be a derivative of sigmoidin F, 2(*S*)-5,5′,7-trihydroxy-2′-prenyl-(2″,2″-dimethylpyrano)-(5″,6″:3′,4′)flavanone.

Compound **2** was obtained as a white powder with $[\alpha]_D^{25}$ −5.84 (*c* 0.1, MeOH). All of the spectroscopic (UV, CD, ^{1}H and ^{13}C NMR) patterns for compound **2** were similar to those of compound **1**. However, there was no NMR resonances for a prenyl group (Tables 1 and 2), whereas NMR resonances for a methylene group were observed (δ_H 3.74 and 3.53, δ_C 67.9). The HMBC correlations from these methylene protons to C-2″ (δ_C 80.6), C-3″ (δ_C 128.7), C-6″ (δ_C 25.6), and from H-6″ (δ_H 1.40) to the methylene carbon suggested that the methylene is located at C-5″. This was supported by a molecular formula of $C_{20}H_{18}O_7$ that was assigned to this compound from the molecular ion peak at *m*/*z* 370.1064 $[M]^+$ obtained by HREIMS. The configuration at C-2 was inferred to be *S* by the CD spectrum, which showed two maxima of positive and negative Cotton effects at 328 and 290 nm, respectively. Therefore, compound **2** was identified as a new compound, 2(*S*)-5,5′,7-trihydroxy-[2″-(5″-hydroxy)-methylpyrano]-(5″,6″:3′,4′)flavanone.

Most of the spectroscopic (UV, CD, ^{1}H and ^{13}C NMR) data for compound **3** were similar to those of compound **2** except for the additional methoxy resonances in the ^{1}H and ^{13}C NMR spectra (δ_H 3.84 and δ_C 56.7). In the HMBC spectrum, correlations from these methoxy protons and H-6′ (δ_H 7.07) to C-5′ (δ_C 149.0) indicated that the methoxy group is located at C-5′. Hence, the structure of compound **3** was assigned as 2(*S*)-5,7-dihydroxy-3′-methoxy-[2″-(5″-hydroxy)-methylpyrano]-(5″,6″:3′,4′)flavanone, which was in accordance with the molecular formula of $C_{21}H_{20}O_7$ derived from the molecular ion peak at *m*/*z* 384.1200 $[M]^+$ in the HREIMS spectrum.

The spectroscopic (UV, CD, ^{1}H and ^{13}C NMR) data for compound **4** indicated a 2(*S*)-flavanone skeleton for this compound as well. However, the ^{1}H and ^{13}C NMR patterns (Tables 1 and 2) exhibited two 2,2-dimethylpyrano rings. The HMBC correlations from H-1‴ (δ_H 6.40) and H-2′ (δ_H 6.85) to C-1′ (δ_C 126.8), from H-4″ (δ_H 6.77) to C-2′ (δ_C 117.2), and from H-2′ to C-4″ (δ_C 123.2) demonstrated that one 2,2-dimethylpyran moiety is located at C-3′ and C-4′, while the other is attached to C-5′ and C-6′. This was further supported by the molecular formula of $C_{25}H_{24}O_6$ determined from the ion peak at *m*/*z* 420.1578 in the HREIMS spectrum. Compound **4** was thus determined to be a new compound, 2(*S*)-5,7-dihydroxy-[(5″,6″:3′,4′)-(2″,2″-dimethylpyrano)-(5‴,6‴:5′,6′)]-(2‴,2‴-dimethylpyrano)flavanone.

The physico-chemical and spectroscopic data of compound **5** were indicative of a flavanone and resembled those of sigmoidin D, which was also isolated from the *E. abyssinica* species in a previous study.[13] However, the ^{1}H and ^{13}C NMR patterns displayed an additional prenyl moiety (Tables 1 and 2) and the position of the prenyl group was determined to be at C-5′ by correlations from H-1‴ (δ_H 3.27) and H-6′ (δ_H 7.13) to C-5′ (δ_C 130.3) in the HMBC spectrum (Fig. 1). The molecular formula was deduced as $C_{25}H_{28}O_6$ from HREIMS and its absolute configuration was assigned to be 2*S* on the basis of the CD spectrum. Therefore, compound **5** was identified as 2(*S*)-5,7-dihydroxy-5′-prenyl-[2″,2″-(3″-hydroxy)-dimethylpyrano]-(5″,6″:3′,4′)flavanone.

The physico-chemical and spectroscopic data of compound **6** were similar to those of compound **5**, except that **6** did not contain a prenyl moiety and it exhibited additional resonances corresponding to a methoxy group (δ_H 3.80, δ_C 56.3). The HMBC correlations from the methoxy protons and H-6′ (δ_H 6.98) to C-5′ (δ_C 149.9) indicated that the methoxy group was located at C-5′. A molecular formula of $C_{21}H_{22}O_7$ was determined from the molecular ion peak at *m*/*z* 386.1370 $[M]^+$ in the HREIMS spectrum and the formula supported the structural elucidation. Hence, compound **6** was characterized to be 2(*S*)-5,7-dihydroxy-5′-methoxy-[2″,2″-(3″-hydroxy)-dimethylpyrano]-(5″,6″:3′,4′)flavanone, a methyl ether of sigmoidin D.

The physico-chemical and spectroscopic data of compound **7** were similar to those of compound **6** with the exception of the 5′-methoxy group. The ^{1}H NMR (Table 1) spectrum exhibited an aromatic ABX spin system (δ_H 7.65, 6.78, and 7.33), indicating that the B ring was 1,3,4-trisubstituted. Two oxygenated methine carbon resonances (δ_C 69.7, 76.6) and one quaternary carbon (δ_C 79.7) were characteristic of two hydroxy groups located on the 2,2-dimethylpyrano ring, similar to the structure of sigmoidin G.[14] The molecular formula, $C_{20}H_{20}O_7$, observed from the molecular ion peak at *m*/*z* 372.1204 in the HREIMS spectrum, supported this elucidation.

Table 1
^{1}H NMR (400 MHz) data for compounds **1**–**12** in Me_2CO-d_6.

Position	1	2	3	4	5	6	7	8	9	10	11	12
2	5.64 dd (2.8, 13.2)	5.36 dd (2.8, 12.8)	5.42 dd (2.8, 13.2)	5.71 dd (2.8, 13.2)	5.37 dd (2.8, 12.8)	5.39 dd (3.2, 13.2)	5.48 dd (2.8, 12.8)	5.43 dd (2.8, 12.8)	5.54 dd (2.8, 13.2)	5.50 dd (2.8, 12.8)	5.36 dd (3.0, 12.8)	5.41 dd (2.4, 13.2)
3_{eq}	2.65 dd (2.8, 17.2)	2.73 dd (2.8, 17.2)	2.74 dd (2.8, 17.2)	2.69 dd (2.8, 17.2)	2.70 dd (2.8, 17.2)	2.70 dd (3.2, 17.2)	3.18 dd (12.8, 16.8)	3.15 dd (12.8, 17.2)	3.17 dd (13.2, 17.2)	2.80 dd (2.8, 16.8)	2.58 dd (3.0, 17.2)	2.67 dd (2.4, 16.8)
3_{ax}	3.16 dd (13.2, 17.2)	3.12 dd (12.8, 17.2)	3.19 dd (13.2, 17.2)	3.18 dd (13.2, 17.2)	3.17 dd (12.8, 17.2)	3.20 dd (13.2, 17.2)	2.74 dd (2.8, 16.8)	2.70 dd (2.8, 17.2)	2.62 dd (2.8, 17.2)	3.17 dd (12.8, 16.8)	3.02 dd (12.8, 17.2)	3.02 dd (13.2, 16.8)
5											7.72 d (8.4)	7.72 d (8.8)
6	5.96 s	5.94 br s	5.95 d (2.4)	5.96 br s	5.94 d (1.6)	5.95 d (2.4)	5.95 d (1.6)	5.95 s	5.92 d (2.0)	5.96 d (2.0)	6.57 dd (2.0, 8.4)	6.57 dd (2.4, 8.8)
8	5.96 s	5.96 br s	5.97 d (2.4)	5.96 br s	5.95 d (1.6)	5.96 d (2.4)	5.96 d (1.6)	5.95 s	5.93 d (2.0)	5.99 d (2.0)	6.42 d (2.0)	6.43 dd (2.4)
2′		6.73 br s	6.86 br s	6.85 s	7.08 br d	6.84 br s	7.65 d (2.0)	7.22 s	7.19 s	7.44 d (2.0)	6.74 br s	7.14 br s
5′							6.78 d (8.4)					
6′	7.01 s	6.89 br s	7.07 br s		7.13 br d	6.98 br s	7.33 dd (2.0, 8.4)	7.50 s		7.29 d (2.0)	6.86 br s	6.92 br s
3″	5.82 d (10.0)	5.69 d (10.0)	5.78 d (10.0)	5.83 d (10.0)	3.79 dd (5.6, 8.6)	3.00 dd (5.2, 16.0)	3.57 d (8.4)	3.55 d (8.0)	3.57 d (8.0)	2.80 s	3.83 dd (5.6, 7.6)	3.59 d (8.0)
4″	6.60 d (10.0)	6.48 d (10.0)	6.49 d (10.0)	6.77 d (10.0)	2.74 dd (8.6, 16.8) 3.01 dd (5.6, 16.8)	2.74 dd (8.0, 16.0)	4.56 d (8.4)	4.53 d (8.0)	4.53 d (8.0)		2.73 dd (7.6, 16.8) 3.20 dd (5.6, 16.8)	4.54 d (8.0)
5″	1.43 s	3.53 d (11.6) 3.74 d (11.6)	3.62 br d (8.2)	1.41 s	1.36 s	1.36 s	1.22 s	1.46 s	1.44 s	1.48 s	1.37 s	1.47 s
6″	1.42 s	1.40 s	1.37 s	1.41 s	1.23 s	1.25 s	1.45 s	1.18 s	1.18 s	1.48 s	1.27 s	1.22 s
1‴	3.45 br d (7.2)			6.40 d (9.6)	3.27 d (8.0)			3.26 d (7.6)	3.43 d (6.8)			
2‴	5.02 m			5.77 d (9.6)	5.28 m			5.29 m	5.08 m			
4‴	1.66			1.41 s	1.69 s			1.70 s	1.59 s			
5‴	1.69			1.41 s	1.71 s			1.72 s	1.61 s			
OCH_3			3.84 s			3.80 s						
5-OH	12.18	12.17	12.18	12.20	12.18	12.17	12.18	12.19	12.16	12.17		

Coupling constants (J in Hz) are presented in parentheses.

Table 2
^{13}C-NMR (100 MHz) data for compounds **1**–**12** in Me_2CO-d_6.

Position	1	2	3	4	5	6	7	8	9	10	11	12
2	76.4	79.8	80.1	76.9	80.2	80.3	80.1	80.2	77.2	79.6	80.8	80.0
3	42.7	43.5	43.6	46.2	43.6	43.7	43.6	43.6	43.1	43.5	44.8	44.0
4	196.8	197.1	197.1	197.2	197.3	197.3	197.2	197.2	197.7	197.0	190.6	190.4
5	163.9	164.3	164.4	164.4	164.5	164.4	164.5	164.4	164.5	164.2	129.6	128.8
6	95.0	96.8	96.0	97.1	95.9	96.8	97.0	95.9	95.9	96.0	112.3	110.7
7	164.7	164.9	165.1	165.4	165.0	165.1	165.5	167.8	167.6	165.4	164.6	163.9
8	95.2	96.0	96.9	96.0	96.8	95.9	96.0	96.8	96.7	97.1	103.8	102.9
9	166.7	167.8	167.6	167.6	167.8	167.5	167.7	164.9	164.7	167.6	165.4	165.1
10	102.4	102.9	103.6	103.0	103.0	103.1	103.2	103.0	102.7	103.3	115.3	114.2
1′	129.4	132.5	132.0	126.8	130.9	131.1	131.6	131.1	129.5	132.5	132.1	131.5
2′	126.5	116.5	118.2	117.2	126.9	121.0	127.8	127.9	117.1	115.2	119.3	116.9
3′	120.5	124.5	122.7	123.0	121.3	121.8	126.2	130.0	123.5	121.5	122.5	125.6
4′	140.7	140.9	143.4	142.6	152.0	144.1	153.8	151.3	140.9	149.3	141.9	140.6
5′	144.3	146.5	149.0	142.3	130.3	149.9	117.4	125.8	143.6	148.2	146.9	145.7
6′	113.9	115.5	112.6	121.5	126.8	109.6	128.1	125.3	125.9	119.8	112.2	112.5
2″	75.6	80.6	80.6	75.9	78.2	78.2	79.7	79.5	80.2	81.1	78.7	79.3
3″	131.5	128.7	129.3	133.0	69.7	69.6	76.6	76.3	76.3	49.3	70.0	75.8
4″	119.7	124.6	124.4	123.2	32.3	32.3	69.7	69.7	69.3	192.2	32.2	68.9
5″	26.2	67.9	68.5	28.9	26.4	26.2	27.3	27.4	25.4	25.6	26.0	26.3
6″	26.8	25.6	23.4	26.7	20.7	20.8	19.7	19.7	19.2	25.6	20.8	18.9
1‴	27.0			119.7	29.4			29.3	25.0			
2‴	124.1			132.5	123.7			123.6	124.1			
3‴	131.1			77.0	132.3			132.4	131.4			
4‴	25.0			28.0	26.0			25.9	25.7			
5‴	17.4			27.6	18.0			18.0	17.9			
OCH_3			56.7			56.3						

As a result, compound **7** was identified as 2(*S*)-5,7-dihydroxy-[2″,2″-(3″,4″-dihydroxy)-dimethylpyrano]-(5″,6″:3′,4′)flavanone.

The structure of compound **8** was deduced to be a flavanone similar to compound **7** on the basis of physico-chemical and spectroscopic evidence. However, the presence of a prenyl group was observed in the ^{1}H and ^{13}C NMR spectra, which was also confirmed by the molecular formula, $C_{25}H_{28}O_7$, by HREIMS. The position of this prenyl moiety at C-5′ was established by long-range correlations in the HMBC spectrum (see Supporting information). Compound **8** was therefore determined to be 2(*S*)-5,7-dihydroxy-5′-prenyl-[2″,2″-(3″,4″-dihydroxy)-dimethylpyrano)]-(5″,6″:3′,4′)flavanone.

Although most of the spectroscopic (UV, CD, ^{1}H and ^{13}C NMR) patterns for compound **9** were similar to those of compound **8**, HREIMS revealed a molecular ion peak at *m/z* 456.1786 $[M]^+$, corresponding to the molecular formula $C_{25}H_{28}O_8$. This indicated the presence of an additional hydroxy group in compound **9**, which was in agreement with the ^{1}H NMR spectrum that showed only one aromatic proton on the B ring (δ_H 7.19). The relative positions of the hydroxy and prenyl groups were confirmed by long-range correlations in the HMBC (see Supporting information). Therefore, compound **9** was characterized as 2(*S*)-5,6′,7-trihydroxy-5′-prenyl-[2″,2″-(3″,4″-dihydroxy)-dimethylpyrano]-(5″,6″:3′,4′)flavanone.

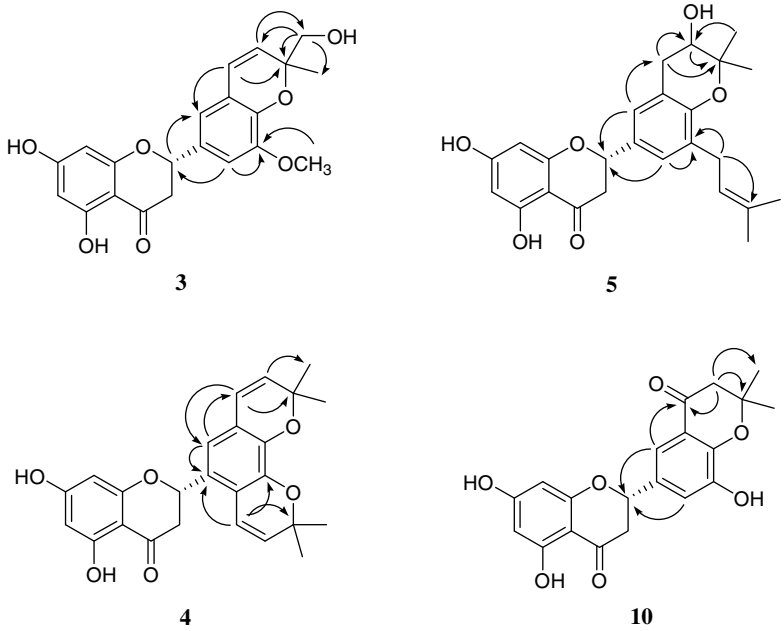

Figure 1. Key HMBC correlations of compounds **3**–**5** and **10**.

Compound **10** was also inferred as a flavanone bearing a 2,2-dimethylpyrano ring on the basis of its spectroscopic patterns. The presence of two sets of aromatic AX spin systems (Table 1) in the ^{1}H NMR spectrum suggested that the B ring was a 1,3,4,5-tetrasubstituted aromatic ring. An additional carbonyl carbon signal in the ^{13}C NMR spectrum (δ_C 192.2) indicated a ketone group located on the 2,2-dimethylpyran ring, and this group was positioned at C-4″ by HMBC correlations (Fig. 1). The molecular formula of $C_{21}H_{22}O_7$, assigned from the molecular ion peak at *m/z* 370.1052 $[M]^+$ in the HREIMS spectrum, suggested that a hydroxy group was located at C-5′. Therefore, compound **10** was identified as a new compound, 2(*S*)-5,5′,7-trihydroxy-[2″,2″-(4″-chromanone)-dimethylpyrano]-(5″,6″:3′,4′)flavanone.

Compound **11** was isolated as a white amorphous powder with $[\alpha]_D^{25}$ −19.20. Its spectroscopic (UV, CD, IR, ^{1}H and ^{13}C NMR) patterns also revealed a 2(*S*)-flavanone structure with a 2,2-dimethylpyrano ring. The ^{1}H NMR spectrum of compound **11** displayed an aromatic ABX spin system (δ_H 7.72, 6.57, and 6.42) together with an aromatic AX spin system (δ_H 6.74 and 6.86), but showed no proton resonance for a hydroxy group at C-5, indicating that this position was not substituted.[9] The 2,2-dimethylpyrano ring was found to be similar to those of compounds **5** and **6** on the basis of ^{1}H and ^{13}C NMR data. Further, the molecular formula, $C_{20}H_{20}O_6$, derived from HREIMS revealed a hydroxy group that was assigned to C-5′ by HMBC analyses (see Supporting information). The C-2 absolute configuration of compound **11** was determined to be *S* on the basis of its CD spectrum. Hence, compound **11** was characterized as a new natural compound, 2(*S*)-5′,7-dihydroxy-[2″,2″-(3″-hydroxy)-dimethylpyrano]-(5″,6″:3′,4′)flavanone.

The molecular formula, $C_{20}H_{20}O_7$, was assigned to compound **12** based on the molecular ion peak at *m/z* 372.1215 in the HREIMS spectrum. This indicated the presence of an additional hydroxy group in the structure of compound **12**, as compared to that of compound **11**. Similar to compounds **7**–**9**, the dimethylpyrano ring was found to be 3″,4″-dihydroxy substituted by means of 1D and 2D NMR analyses. Thus, compound **12** was determined as 2(*S*)-5′,7-dihydroxy-[2″,2″-(3″,4″-dihydroxy)-dimethylpyrano]-(5″,6″:3′,4′)flavanone.

In addition, seven known flavanones were also isolated and identified as abysinoflavanone IV,[13] abysinoflavanone V,[13] abysinoflavanone VI,[13] sigmoidin G,[14] sigmoidin D,[13,15] burttinone,[16] and burttinonedehydrate,[16] by comparing their spectroscopic data with published values.

Compounds **1**–**12** along with sigmoidins D and G, burttinone, and burttinonedehydrate were tested for their inhibitory activity against PTP1B using a reported assay[8–11] and the results are presented in Table 3. Compounds **1**, **3**, **5**, **6**, **8**, and **9** inhibited PTP1B activity in a dose-dependent manner with IC_{50} values ranging from 13.9 ± 2.1 to 19.0 ± 1.8 μM, while the other flavanones showed very weak inhibitory effects. The common flavanones (naringenin, liquiritigenin) displayed no inhibitory activity against PTP1B in this assay.[9] The results of this study indicated that compounds with 2,2-dimethylpyrano ring(s) in which prenyl and/or methoxy groups are absent (compounds **2**, **4**, **7**, **10**, **11**, **12**, and sigmoidins D and G), exhibited no inhibitory activity against PTP1B. Together with previous studies,[8–11] it is suggested that this 2,2-dimethylpyrano residue does not affect the inhibitory activity of flavanones. However, all compounds having a prenyl group (**1**, **5**, **8**, **9**, burttinone, and burttinonedehydrate) exhibited strong inhibitory activity. This is indicative of the significant role of the prenyl moiety in the inhibitory activity of flavonoids.[8–11] Two flavanones **3** and **6** with a methoxy group at C-5′ 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. Therefore, the presence of a lipophilic group (methoxy and/or prenyl residues) on the B ring seems to increase the inhibitory effect of flavanones against PTP1B, while the presence of a polar moiety (hydroxy group) does not affect this particular biological activity.

Table 3
Inhibitory effects of compounds **1**–**12** on PTP1B.

Compound	Inhibitory effect (IC_{50}, μM)
1	13.9 ± 2.1
2	>60
3	17.9 ± 1.7
4	>60
5	14.9 ± 1.6
6	18.2 ± 2.1
7	>60
8	19.0 ± 1.8
9	18.2 ± 1.2
10	>60
11	>60
12	>60
Sigmoidin D	>60
Sigmoidin G	>60
Burttinone	18.9 ± 1.3
Burttinonedehydrate	21.6 ± 2.1
RK-682[a]	4.7 ± 0.5
Ursolic acid[a]	3.6 ± 0.2

[a] Values are mean ± SD from three separate experiments. RK-682 and ursolic acid were used as positive controls.[9]

3. Experimental

3.1. Plant material

The stem bark of *E. abyssinica* was collected in June 2005 in Mukono, Uganda. The sample was botanically authenticated by Prof. John Silike-Muruumu, and a voucher specimen (No. 0001) has been deposited at the Department of Botany, Makerere University, Uganda.

3.2. General experimental

Optical rotations were determined on a JASCO P-1020 polarimeter using a 100-mm glass microcell. UV spectra were recorded in MeOH using a Shimadzu spectrometer. CD spectra were recorded in MeOH on a JASCO J-715 spectrometer. FT-IR spectra were obtained on a Nicolet 6700 FT-IR (Thermo electron Corp., USA). NMR spectra were obtained on a Varian Unity Inova 400 MHz spectrometer using TMS as the internal standard. 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, Merck) and RP-18 (150 μm particle size, Merck). For thin-layer chromatography, pre-coated TLC Silica gel 60 F_{254} plates from Merck were used. HPLC runs were carried out using a Shimadzu System LC-10AD pump equipped with a model SPD-10Avp UV detector, and an Optima Pak® C_{18} column (10 × 250 mm, 10 μm particle size, RS Tech Korea).

3.3. Extraction and isolation

The stem bark (5 kg) of *E. abyssinica* was extracted with MeOH at room temperature for 2 weeks and the solution was concentrated to obtain a crude extract. This extract was suspended in H_2O, partitioned successively with *n*-hexane and EtOAc, and then the organic solvents were removed. A portion of the EtOAc-soluble fraction (10 g) was chromatographed over a Silica gel column (9.5 × 35 cm; 63–200 μm particle size) using a gradient of $CHCl_3$–MeOH (from 20:1, 19:1 to 0:1), and was separated into 10 fractions (Fr.1–Fr.10).

Fr.3 ($CHCl_3$–MeOH 10:1, 1.5 g) was chromatographed over Silica gel (4 × 27 cm; 40–63 μm particle size), eluted with a stepwise gradient of *n*-hexane–EtOAc (from 20:1, 19:1 to 0:1) to afford 10 subfractions (Fr.3a–Fr.3j). Further purification of Fr.3c (357 mg) by semipreparative HPLC using a gradient solvent system of 60–80% MeCN in H_2O over 50 min to yield compound **4** (3.4 mg, t_R = 42.7 min). Fr.3f was purified by preparative HPLC using an isocratic solvent system of 56% MeCN in H_2O over 30 min followed by 65% MeCN in H_2O over 60 min to obtain compounds **1** (3.5 mg, t_R = 76.2 min) and **5** (3.9 mg, t_R = 84.2 min). Fr.5 was subjected to an RP-18 column (40 × 3.5 cm) and was eluted with MeOH–H_2O (1:1, 2:1, to 10:1) to yield six fractions (F.5a–F.5f). The most active fraction, Fr.5b (170 mg), was further separated by a Silica gel column eluted with $CHCl_3$–MeOH (40:1, 35:1, to 10:1) to yield 10 subfractions (Fr.5b1–Fr.5b10). Fr.5b1 was separated by HPLC, using a gradient of 40–45% MeCN in H_2O as the mobile phase to produce compound **10** (2.7 mg, t_R = 16.2 min). Purification of Fr.5b2 (250 mg) by HPLC using an isocratic solvent system of 38% MeCN in H_2O led to the isolation of compounds **6** (2.3 mg, t_R = 20.1 min) and **3** (3.9 mg, t_R = 28.7 min). Similarly, purification of Fr.5b4 (120 mg) (28% MeCN) yielded compounds **2** (4.2 mg, t_R = 32.7 min) and **9** (12.6 mg, t_R = 48.7 min), Fr.5b6 (270 mg) (22% MeCN) gave compound **7** (6.0 mg, t_R = 58.7 min), and Fr.5b8 (270 mg) (22% MeCN) yielded compound **8** (5.1 mg, t_R = 34.3 min). The fraction F.7 was chromatographed on an RP-18 column (40 × 3.5) eluted with MeOH–H_2O (1:2, 1:1, 2:1, to 5:1) to afford 9 subfractions (Fr.7a–Fr.7i). Fr.7c was further separated by a Silica gel column (3.5 × 35 cm) eluted with $CHCl_3$–MeOH (20:1, 18:1 to 10:1) to yield 10 fractions (Fr.7c1–Fr.7c10). Fr.7c3 (180 mg) was subjected to HPLC using a gradient of 42 → 50% MeCN in H_2O over 50 min to produce compound **11** (5.5 mg, t_R = 31.3 min). Finally, purification of Fr.7c5 (150 mg) by HPLC using an isocratic solvent system of 35% MeCN in H_2O yielded compound **12** (4.3 mg, t_R = 25.1 min).

3.4. Compound 1

White amorphous powder; $[\alpha]_D^{25}$ −7.80 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.45), 232 (4.51), 288 (4.23), 328 (3.62) nm; CD (*c* 0.51, MeOH) $[\theta]_{339}$ +0.69, $[\theta]_{287}$ −22.87, $[\theta]_{225}$ +26.16; IR (film) ν_{max} 3365 (OH), 2971, 2914, 1653 (C=O), 1603, 1506, 1464, 1279, 1158, 1123, 1063, 997, 849 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 422.1725 $[M]^+$ (calcd for $C_{25}H_{26}O_6$, 422.1729).

3.5. Compound 2

White amorphous powder; $[\alpha]_D^{25}$ −5.84 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.49), 286 (4.19), 329 (3.61) nm; CD (*c* 0.50, MeOH) $[\theta]_{328}$ +6.15, $[\theta]_{290}$ −35.65, $[\theta]_{255}$ +5.79, $[\theta]_{210}$ +41.58; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 370.1064 $[M]^+$ (calcd for $C_{20}H_{18}O_7$, 370.1053).

3.6. Compound 3

White amorphous powder; $[\alpha]_D^{25}$: −5.07 (*c* 0.1, MeOH); UV (MeOH): λ_{max} (log ε) 231 (4.37), 287 (3.99), 328 (3.37) nm; CD (*c* 0.50, MeOH): $[\theta]_{327}$ +3.88, $[\theta]_{296}$ −23.16, $[\theta]_{254}$ +3.98, $[\theta]_{211}$ +23.40; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z*: 384.1200 $[M]^+$ (calcd for $C_{21}H_{20}O_7$, 384.1209).

3.7. Compound 4

White amorphous powder; $[\alpha]_D^{25}$ −32.10 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 238 (4.04), 298 (4.27), 341 (3.22) nm; CD (*c* 0.55, MeOH) $[\theta]_{329}$ +6.03, $[\theta]_{301}$ −28.87, $[\theta]_{215}$ +47.56; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 420.1578 $[M]^+$ (calcd for $C_{25}H_{24}O_6$, 420.1573).

3.8. Compound 5

White amorphous powder; $[\alpha]_D^{25}$ −4.43 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.52), 230 (4.28), 289 (4.12), 331 (3.39) nm; CD (*c* 0.50, MeOH) $[\theta]_{328}$ +5.5, $[\theta]_{291}$ −25.7, $[\theta]_{253}$ +3.73, $[\theta]_{224}$ +8.9; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 424.1884 $[M]^+$ (calcd for $C_{25}H_{28}O_6$, 424.1886).

3.9. Compound 6

White amorphous powder; $[\alpha]_D^{25}$ −18.11 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.54), 288 (4.46), 333 (3.70) nm; CD (*c* 0.50, MeOH) $[\theta]_{328}$ +36.0, $[\theta]_{296}$ −105.4, $[\theta]_{254}$ +21.2; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 386.1370 $[M]^+$ (calcd for $C_{21}H_{22}O_7$, 386.1366).

3.10. Compound 7

White amorphous powder; $[\alpha]_D^{25}$ −14.80 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.51), 288 (4.23), 328 (3.62) nm; CD (*c* 0.55, MeOH) $[\theta]_{329}$ +5.40, $[\theta]_{288}$ −35.90, $[\theta]_{257}$ +7.08, $[\theta]_{228}$ +10.67; IR (film) ν_{max} 3385 (OH), 2969, 2913, 1662 (C=O), 1603, 1466, 1330, 1280, 1156, 1124, 1064, 999, 850 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 372.1204 $[M]^+$ (calcd for $C_{20}H_{20}O_7$, 372.1209).

3.11. Compound 8

White amorphous powder; $[\alpha]_D^{25}$ −9.75 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.48), 288 (4.32), 330 (3.59) nm; CD (*c* 0.50, MeOH) $[\theta]_{327}$ +11.17, $[\theta]_{291}$ −38.63, $[\theta]_{251}$ +7.99, $[\theta]_{222}$ +18.54; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 440.1837 $[M]^+$ (calcd for $C_{25}H_{28}O_7$, 440.1835).

3.12. Compound 9

White amorphous powder; $[\alpha]_D^{25}$ −6.35 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.95), 289 (3.79), 327 (3.27) nm; CD (*c* 0.50, MeOH) $[\theta]_{330}$ +0.15, $[\theta]_{290}$ −8.10, $[\theta]_{226}$ +1.71; IR (film) ν_{max} 3000–3500 (OH), 2968, 2913, 2856, 1638 (C=O), 1507, 1473, 1341, 1272, 1159, 1087, 1066, 1011, 833 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 456.1786 $[M]^+$ (calcd for $C_{25}H_{28}O_8$, 456.1784).

3.13. Compound 10

White amorphous powder; $[\alpha]_D^{25}$ −8.16 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.28), 289 (4.04), 329 (3.53) nm; CD (*c* 0.51, MeOH) $[\theta]_{327}$ +2.99, $[\theta]_{286}$ −26.48, $[\theta]_{254}$ +2.50, $[\theta]_{232}$ +8.53; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 370.1052 $[M]^+$ (calcd for $C_{21}H_{22}O_7$, 370.1053).

3.14. Compound 11

White amorphous powder; $[\alpha]_D^{25}$ −19.20 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.38), 278 (3.97), 316 (3.58) nm; CD (*c* 0.55, MeOH) $[\theta]_{330}$ +8.76, $[\theta]_{305}$ −20.12, $[\theta]_{232}$ +11.38; IR (film) ν_{max} 3000–3500 (OH), 2968, 2917, 2853, 1641 (C=O), 1603, 1462, 1376, 1272, 1161, 1090, 1011, 833 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 356.1261 $[M]^+$ (calcd for $C_{20}H_{20}O_6$, 356.1260).

3.15. Compound 12

White amorphous powder; $[\alpha]_D^{25}$ −27.70 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.33), 237 (4.01), 279 (3.88), 314 (3.56)

nm; CD (*c* 0.55, MeOH) $[\theta]_{330}$ +9.60, $[\theta]_{305}$ −19.10, $[\theta]_{218}$ +17.39; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 372.1215 $[M]^{+}$ (calcd for $C_{20}H_{20}O_7$, 372.1209).

3.16. 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.[8–11]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.10.012.

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