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Prenylflavonoids from *Glycyrrhiza uralensis* and their protein tyrosine phosphatase-1B inhibitory activities

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ABSTRACT

Two new 2-arylbenzofurans, glycybenzofuran (**1**) and cyclolicocoumarone (**2**), together with 10 known flavonoids including licocoumarone (**3**), glycyrrhisoflavone (**4**), glisoflavone (**5**), cycloglycyrrhisoflavone (**6**), isoliquiritigenin (**7**), licoflavone A (**8**), apigenin (**9**), isokaempferide (**10**), glycycoumarin (**11**), and isoglycycoumarin (**12**), were isolated from the roots of *Glycyrrhiza uralensis* and their structures were determined by extensive spectroscopic analyses. Compounds **1** and **5** showed significant protein tyrosine phosphatase-1B (PTP1B) inhibitory activity in vitro with the IC_{50} values of 25.5 and 27.9 μM, respectively. The structure–activity relationship indicated that the presence of prenyl group and *ortho*-hydroxy group is important for exhibiting the activity. Kinetic analysis indicated that compound **1** inhibits PTP1B by a competitive mode, whereas compound **5** by a mixed mode.

Type 2 diabetes mellitus (T2DM) has become one of the most serious health problems worldwide.[1] The etiology of T2DM is multi-factorial and normally characterized by β-cell dysfunction and insulin resistance.[2] On the other hand, obesity is one of the major risk factors for developing T2DM due to insulin resistance.[3,4] Protein tyrosine phosphatase-1B (PTP1B) is an intracellular protein tyrosine phosphatase, expressing ubiquitously in the classical insulin-targeted tissues such as liver, muscle, and fat. PTP1B plays a key role as a negative regulator in both insulin and leptin signaling by catalyzing the dephosphorylation of activated insulin receptor, insulin receptor substrate proteins, and leptin receptor-associated kinase, Jak2.[5,6] Therefore, inhibition of PTP1B has been demonstrated to be an effective therapeutic approach for T2DM and obesity treatment by biochemical, genetic and pharmacological studies.[7]

The roots of *Glycyrrhiza uralensis* (Leguminosae) is one of the oldest and most frequently used crude drugs in traditional Chinese medicines for its extensive pharmacological effects, such as anti-inflammatory, antiprotozoal and antioxidative activity.[8] The effectiveness of flavonoids from *G. uralensis* in ameliorating diabetes and abdominal obesity has been reported in diabetic KK-A^y mice model and high fat diet-induced obese C57BL mice model.[9]

During our ongoing chemical studies on the bioactive natural compounds from traditional Chinese medicines, approximately 200 Chinese medicinal plant extracts have been screened for their PTP1B inhibitory activity. Among them, the 95% EtOH extract of *G. uralensis* roots showed significant PTP1B inhibitory activity, yielding up to 68.8% inhibition at 30 μg/mL concentration. Further bioassay-guided fractionation of this extract led to the isolation of 12 flavonoids including two new compounds. Herein, we report the isolation and structural determination of these flavonoids, and the evaluation of their PTP1B inhibitory activities.

The roots of *G. uralensis* were collected from Yanchi, Ningxia Hui Autonomous Region, People's Republic of China. The 95% EtOH extract (200 g) from the roots of *G. uralensis* (1 kg) was loaded onto a Diaion HP-20 open column and sequentially eluted with 70% and 90% aqueous MeOH. The 90% MeOH fraction (33.4 g) was further separated by a silica gel open column with a gradient of $CHCl_3$–MeOH. The $CHCl_3$–MeOH (95:5) eluting fraction (4.5 g) was separated by repeated preparative HPLC with MeOH–H_2O (0.06% TFA) (70:30) to afford two new flavonoids, **1** (4 mg) and **2** (4 mg), together with ten known compounds, namely, licocoumarone (**3**, 4 mg),[10] glycyrrhisoflavone (**4**, 6 mg),[11] glisoflavone (**5**, 4 mg),[12] cycloglycyrrhisoflavone (**6**, 5 mg),[11] isoliquiritigenin (**7**, 24 mg),[13] licoflavone A (**8**, 2 mg)[14], apigenin (**9**, 2 mg),[15] isokaempferide (**10**, 7 mg),[16] glycycoumarin (**11**, 74 mg),[17] and isoglycycoumarin (**12**, 8 mg).[12] The structures of the known compounds **3**–**12** were identified by comparison of their spectroscopic data with those in the literature.

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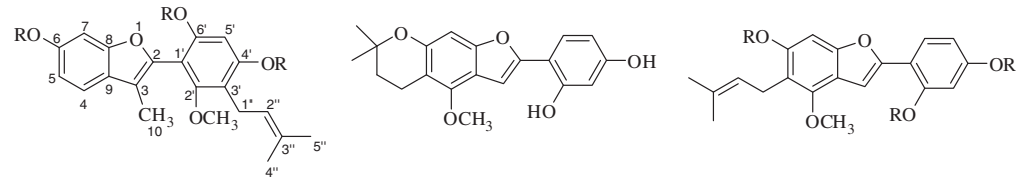

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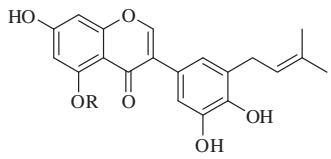
13 R=CH_3

2

3 R=H
14 R=CH_3



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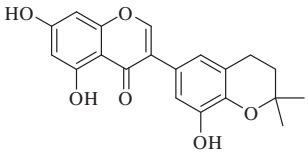

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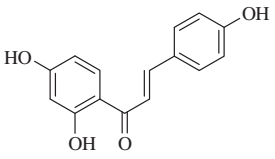

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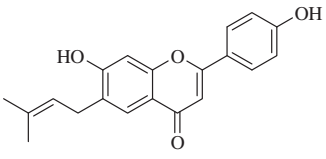

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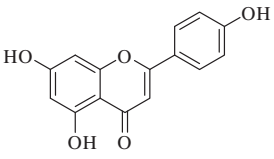

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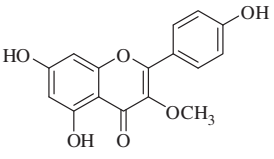

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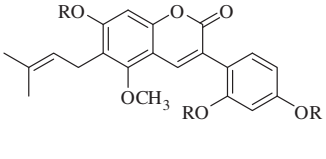
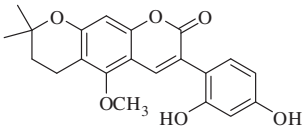


11 R=H
15 R=CH3



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Compound **1** was obtained as a pale brown solid.[18] Its molecular formula was determined as $C_{21}H_{22}O_5$ by the positive-ion HRESIMS data (*m/z* 377.1362, $[M+Na]^+$). The ^1H NMR spectrum (Table 1) showed a set of ABX-type aromatic proton resonances at δ 7.29 (1H, d, J = 8.2 Hz), 6.73 (1H, dd, J = 8.2, 1.8 Hz) and 6.84 (1H, d, J = 1.8 Hz), and a set of γ,γ-dimethylallyl proton resonances at δ 3.27 (2H, d, J = 6.4 Hz), 5.21 (1H, m), 1.75 (3H, s) and 1.67 (3H, s), together with three separated singlet resonances for a methoxy

Table 1
^1H and ^{13}C NMR spectroscopic data of **1** in CD_3OD

Position	δ_C, mult.	δ_H (J in Hz)
2	146.7, qC	
3	115.1, qC	
4	120.0, CH	7.29, d (8.2)
5	112.1, CH	6.73, dd (8.2, 1.8)
6	156.1, qC	
7	98.6, CH	6.84, d (1.8)
8	157.1, qC	
9	124.5, qC	
10	8.9, CH_3	2.05, s
1′	104.6, qC	
2′	160.5, qC	
3′	114.6, qC	
4′	159.1, qC	
5′	99.6, CH	6.23, s
6′	156.7, qC	
1″	23.5, CH_2	3.27, d (6.4)
2″	125.4, CH	5.21, m
3″	131.0, qC	
4″	18.0, CH_3	1.75, s
5″	26.0, CH_3	1.67, s
OCH_3	61.3, CH_3	3.36, s

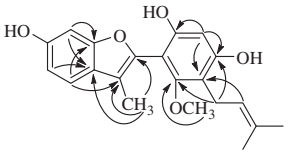

Figure 1. Key HMBC correlations of **1**.

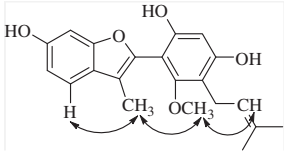

Figure 2. Key NOESY correlations of **1**.

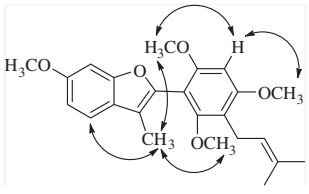

Figure 3. Key NOESY correlations of **13**.

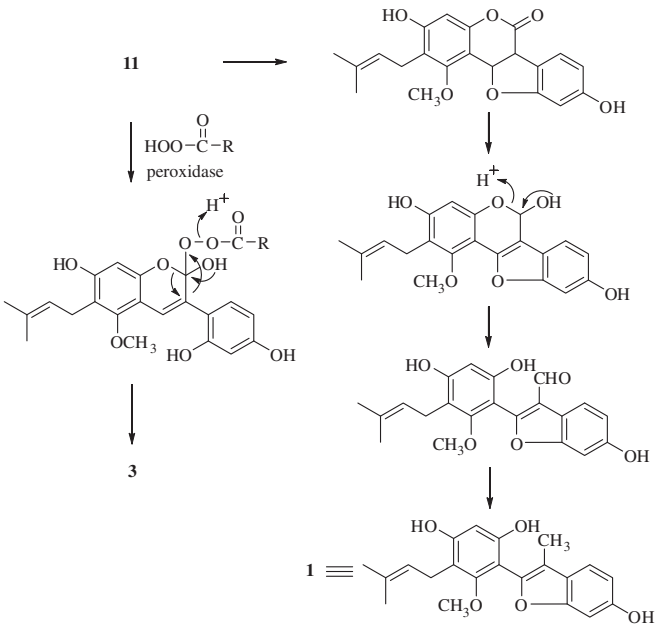

Figure 4. Possible biosynthesis of **1** and **3**.

Table 2
The IC_{50} values of the active compounds against PTP1B

Compound	IC_{50}[a] (μM)
1	25.5 ± 2.2
3	71.2 ± 2.5
4	58.7 ± 1.4
5	27.9 ± 1.4
8	54.5 ± 2.1
11	183.9 ± 4.5
RK-682	4.4 ± 0.1

[a] IC_{50} values were determined by regression analyses and expressed as mean ± SD of three replicates.

at δ 3.36, a methyl at δ 2.05 and an aromatic proton at δ 6.23. The ^{13}C NMR spectrum showed 21 carbon resonances. All protonated carbons were assigned to their corresponding protons by the HMQC correlations. The characteristic chemical shift of the methylene carbon in the γ,γ-dimethylallyl moiety at δ 23.5 indicated that both *ortho*-positions to the γ,γ-dimethylallyl moiety were occupied by the oxygenated substituents.[19] In the HMBC spectrum (Fig. 1), the correlations from δ_H 3.27 (H_2-1″) to the oxygenated aromatic carbons δ_C 159.1 and 160.5, δ_H 6.23 to δ_C 156.7 and 159.1, and δ_H 3.36 to δ_C 160.5, established the structure of 1-methoxyl-2-(γ,γ-dimethylallyl)-3,5-dihydroxyphenyl moiety. Two quaternary olefinic carbon resonances at δ 115.1 and 146.7 suggested the 2,3-disubstituted benzofuran skeleton of **1**. The observed HMBC correlations and the ABX spin system of the aromatic protons in benzofuran skeleton indicated the presence of the 6-hydroxyl moiety. Finally, the 3-methyl moiety in benzofuran skeleton, and the aryl moiety at C-2 were further confirmed by the observation of the HMBC correlation from δ_H 2.05 to δ_C 124.0 (C-9) and the NOESY correlation (Fig. 2) between δ_H 2.05 (H_3-10) and 7.29 (H-4), respectively. Permethylation of **1** by $TMSCHN_2$ afforded compound **13**,[20] which showed three additional methoxy resonances than **1**. All methoxy proton resonances were assigned by the NOESY correlations as shown in Figure 3. In the NOESY spectrum of **13**, the presence of correlations between H_3-10/2′-OCH_3 and H_3-10/6′-OCH_3, as well as the absence of correlation between H_3-10/6-OCH_3, completely eliminated the possibility of **1** being 2-(2,4-dihydroxyphenyl)-5-(γ,γ-dimethylallyl)-6-hydroxy-4-methoxy-3-methylbenzofuran. Therefore, the structure of **1** was unambiguously determined as 2-[4,6-dihydroxy-3-(γ,γ-dimethylallyl)-2-methoxyphenyl]-6-hydroxy-3-methyl-benzofuran, and given a trivial name glycybenzofuran.

Although several 3-carbonated, 3-formylated or 3-hydroxymethylated 2-arylbezofurans have been reported from *Oryza sativa*[21] and *Andira inermis*,[22] 2-aryl-3-methylbezofurans is found rarely as natural products. To our knowledge, glycybenzofuran (**1**) is the first example of 2-aryl-3-methylbezofurans from natural sources. Its possible biosynthesis was proposed in Figure 4.

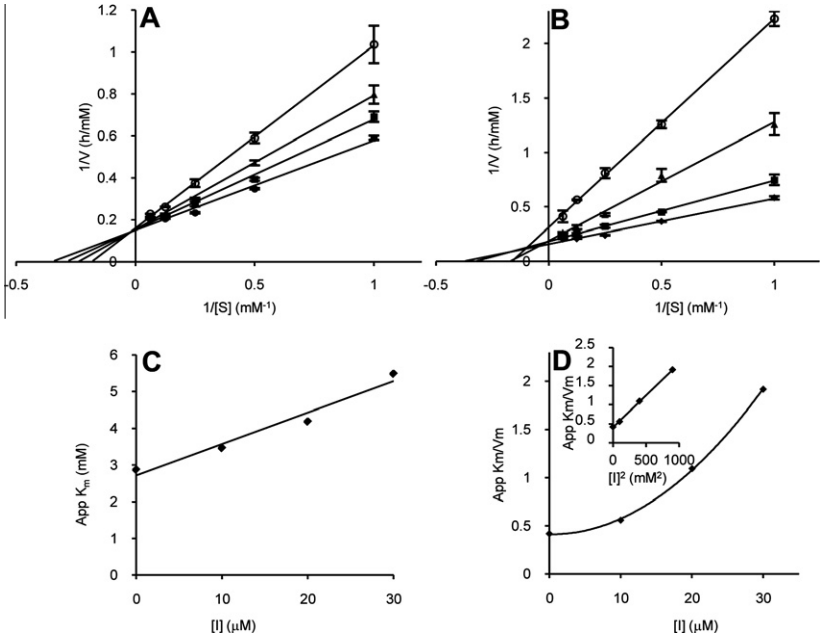

Figure 5. Inhibition of PTP1B-catalyzed hydrolysis of *p*-NPP by compounds **1** and **5**. (A) The Lineweaver–Burk plot of the effect of compound **1** on PTP1B. Final concentrations of **1** were as follows: (●) 3% DMSO, (■) 10 μM, (▲) 20 μM, (○) 30 μM. (B) The Lineweaver–Burk plot of the effect of compound **5** on PTP1B. Final concentrations of **5** were as follows: (●) 3% DMSO, (■) 10 μM, (?) 20 μM, (○) 30 μM. (C) The second plot of (A). (D) The second plot of (B). The inset shows the second-order plot of (B) with $[I]^2$ as the horizontal axis.

Compound **2** was obtained as a pale brown solid.[23] Its ^{1}H and ^{13}C NMR data showed very similar resonances to that of licocoumarone (**3**), except for some variations at δ_H 2.76 (2H, m), 1.71 (2H, m), 1.27 (6H, s), and δ_C 19.9, 29.1, 44.8 and 71.9, indicating the presence of the dihydrodimethylpyran ring. Thus, compound **2** was determined as the cyclization artifact of licocoumarone (**3**) between the prenyl group and the hydroxyl group, and is given a trivial name cyclolicocoumarone.

All isolated compounds were assayed for PTP1B inhibitory activity first at 100 μM concentration with the known PTP1B inhibitor RK-682 as positive control.[24] For the compounds with more than 30% inhibition, the inhibitory activities were further measured at three different concentrations to obtain the IC_{50} values by regression analyses (Table 2). Glycybenzofuran (**1**) and glisoflavone (**5**) showed the strongest inhibitory activity with the IC_{50} values of 25.5 μM and 27.9 μM, respectively. It is interesting to note that all of the active compounds were substituted by a prenyl and *ortho*-hydroxy group, suggesting this partial structure maybe important for the inhibitory activity. The cyclization artifacts **2**, **6**, and **12**, and the permethylated products[23] **13**, **14**,[10] and **15**[10] of the active compounds **1**, **3** and **11**, turned out to be inactive, supporting the aforementioned conclusion.

To elucidate the inhibition mode, the inhibition kinetics of glycybenzofuran (**1**) and glisoflavone (**5**) were analyzed by the Lineweaver–Burk method with various substrate concentrations of *p*-NPP (1, 2, 4, 8, 16 mM). The initial reaction velocities were measured with (10, 20, 30 μM) and without the inhibitor. The Lineweaver–Burk plot was shown in Figure 5. Glycybenzofuran (**1**) showed the same V_{max} value of 6.3 ± 0.2 mM/h, and its K_m values were 2.9, 3.5, 4.2, and 5.5 μM, respectively. The K_m values increased in dose-dependent manner without changing the V_{max} value, indicating that a competitive inhibition was induced. The second plot of **1** showed a linear relationship and the K_i value was calculated for 32.0 μM. As for glisoflavone (**5**), the Lineweaver–Burk plot in Figure 5B suggested that **5** inhibited PTP1B by a mixed manner. The second plot of **5** showed a quadratic-like curve and a good linear plot was gained with $[I]^2$ as horizontal axis, indicating that **5** might inhibit PTP1B activity by binding of two molecules to the enzyme.

References and notes

1. Saltiel, R. A.; Ronald Kahn, C. *Nature* **2001**, *414*, 799.
2. Hull, L. R.; Andrikopoulos, S.; Bruce Verchere, C.; Vidal, J.; Wang, F.; Cnop, M.; Prigeon, L. R.; Kahn, E. S. *Diabetes* **2003**, *52*, 372.
3. Gallagher, J. E.; LeRoith, D.; Karnieli, E. *Endocrinol. Metab. Clin. North Am.* **2008**, *37*, 559.
4. Hays, P. N.; Galassetti, R. P.; Coker, H. R. *Pharmacol. Ther.* **2008**, *118*, 181.
5. Byon, C. H. J.; Kusari, B. A.; Kusari, J. *Mol. Cell. Biochem.* **1998**, *182*, 101.
6. Zabolotny, M. J.; Bence Hanulec, K. K.; Stricker Krongrad, A.; Haj, F.; Wang, Y. P.; Minokoshi, Y.; Kim, Y. B.; Elmquist, K. J.; Tartaglia, A. L.; Kahn, B. B.; Neel, G. B. *Dev. Cell* **2002**, *2*, 489.
7. Zhang, S.; Zhang, Z. Y. *Drug Discovery Today* **2007**, *12*, 373.
8. Asl, N. M.; Hosseinzadeh, H. *Phytother. Res.* **2008**, *22*, 709.
9. Mae, T.; Kishida, H.; Nishiyama, T.; Tsukagawa, M.; Konishi, E.; Kuroda, M.; Mimaki, Y.; Sashida, Y.; Takahashi, K.; Kawada, T.; Nakagawa, K.; Kitahara, M. *J. Nutr.* **2003**, *133*, 3369.
10. Watanabe, M.; Hayakawa, S.; Isemura, M.; Kumazawa, S.; Nakayama, T.; Mori, C.; Kawakami, T. *Biol. Pharm. Bull.* **2002**, *25*, 1388.
11. Hatano, T.; Kagawa, H.; Yasuhara, T.; Okuda, T. *Chem. Pharm. Bull.* **1988**, *36*, 2090.
12. Hatano, T.; Yasuhara, T.; Fukuda, T.; Noro, T.; Okuda, T. *Chem. Pharm. Bull.* **1989**, *37*, 3005.
13. Salem, M. M.; Werbovetz, A. K. *J. Nat. Prod.* **2006**, *69*, 43.
14. Kajiyama, K.; Demizu, S.; Hiraga, Y. *J. Nat. Prod.* **1992**, *55*, 1197.
15. Li, W.; Dai, R. J.; Yu, Y. H.; Li, L.; Wu, C. M.; Luan, W. W.; Meng, W. W.; Zhang, X. S.; Deng, Y. L. *Biol. Pharm. Bull.* **2007**, *30*, 1123.
16. Gohari, R. A.; Saeidnia, S.; Matsuo, K.; Uchiyama, N.; Yagura, T.; Ito, M.; Kiuchi, F.; Honda, G. *Nat. Med.* **2003**, *57*, 250.
17. Sato, Y.; Akao, T.; He, J. X.; Nojima, H.; Kuraishi, Y.; Morota, T.; Asano, T.; Tani, T. *J. Ethnopharmacol.* **2006**, *105*, 409.
18. *Glycybenzofuran* (**1**): pale brown solid; UV (MeOH) λ_{max} (log ε) 205 (4.66), 262 (4.10), 302 (4.16) nm; IR (KBr) ν_{max} 3435, 1629, 1459, 1384, 1146, 1120, 1070 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) see Table 1; HRESIMS *m*/*z* 377.1362 $[M+Na]^+$ (calcd for $C_{21}H_{22}O_5Na$, 377.3862).
19. Fukai, T.; Nomura, T. *Heterocycles* **1989**, *29*, 2379.
20. Each compound of **1** (1.8 mg), **3** (2.7 mg), and **11** (2.2 mg) was dissolved in MeOH 0.1 mL. After 30-fold $TMSCHN_2$ (2 M in Et_2O) was added, the solutions were stood at room temperature for 24 h. One drop of acetic acid was added and then the reaction solutions were evaporated under reduced pressure. The residues were purified by preparative TLC with $CHCl_3$–MeOH (97:3) to give compounds **13** (1.5 mg, 81%), **14** (1.5 mg, 56%) and **15** (1.7 mg, 80%). Trimethylether of compound **1** (**13**): ^{1}H NMR (CD_3OD, 500 MHz) δ 1.66 (CH_3, s, 5″), 1.75 (CH_3, s, 4″), 2.03 (2H, d, J = 6.8 Hz, H_2-1″), 3.37 (3H, s, 2′-OCH_3), 3.78 (3H, s, 6′-OCH_3), 3.84 (3H, s, 6-OCH_3), 3.90 (3H, s, 4′-OCH_3), 5.15 (1H, t, J = 6.9 Hz, H-2″), 6.56 (1H, s, H-3′), 6.57 (1H, d, 5.1, 2.4, H-5′), 6.65 (1H, s, H-8), 7.34 (1H, dd, J = 7.5 Hz, 1.1, H-6′), 7.89 (1H, d, J = 0.7 Hz, H-4); ^{13}C NMR (CD_3OD, 125 MHz) δ 161.5 (C, C-6′), 160.8 (C, C-2′), 159.8 (C, C-4′), 159.3 (C, C-6), 155.3 (C, C-8), 146.3 (C, C-2), 131.0 (C, C-3″), 125.0 (CH, C-2″), 124.2 (C, C-9) 120.1 (CH, C-4), 116.9 (C, C-3′), 115.1 (C, C-3), 111.9 (CH, C-5), 106.9 (C, C-1′), 96.7 (CH, C-7), 92.8 (CH, C-5′), 61.6 (CH_3, 2′-OCH_3), 56.2 (CH_3, 6-OCH_3), 56.3 (CH_3, 4′-OCH_3), 56.5 (CH_3, 6′-OCH_3), 26.0 (CH_3, C-4″), 23.5 (CH_2, C-1″), 18.0 (CH_3, C-5″), 8.8 (CH_3, C-10). The ^{1}H NMR data of licocoumarone trimethylether (**14**) and glycycoumarin trimethylether (**15**) was coincident to the previous literature values.
21. Han, S. J.; Ryu, S. N.; Kang, S. S. *Chem. Pharm. Bull.* **2004**, *52*, 1365.
22. Kraft, C.; Jenett Siems, K.; Kohler, I.; Siems, K.; Abbiw, D.; Bienzle, U.; Eich, E. *Z. Naturforsch.* **2002**, *57c*, 785.
23. *Cyclolicocoumarone* (**2**): UV (MeOH) λ_{max} (log ε) 294 (sh) (4.06), 320 (4.26), 334 (sh) (4.19) nm; IR (KBr) ν_{max} 3433, 1623, 1509, 1459, 1385, 1312, 1255, 1212, 1111 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz): δ 1.27 (6H, s, H_3-4″, H_3-5″), 1.71 (2H, m, H_2-2″), 2.76 (H, m, H_2-1″), 4.03 (3H, s, OCH_3), 6.40 (1H, dd, J = 2.4, 8.5 Hz, H-5′), 6.41 (1H, d, J = 2.4 Hz, H-3′), 6.66 (1H, d, J = 1.0 Hz, H-7), 7.21 (1H, d, J = 1.0 Hz, H-3), 7.63 (1H, d, J = 8.5 Hz, H-6′); ^{13}C NMR (CD_3OD, 125 MHz): δ 19.9 (C-1″), 29.1 (C-4″, 5″), 44.8 (C-2″), 60.8 (4-OCH_3), 71.9 (C-3″), 93.5 (C-7) 101.9 (C-3), 103.9 (C-3′), 108.1 (C-5′), 111.6 (C-1′), 115.0 (C-9), 117.0 (C-5), 128.1 (C-6′), 152.0 (C-2), 152.1 (C-4), 154.6 (C-6), 155.3 (C-8), 156.8 (C-2′), 159.3 (C-4′); ESIMS (positive) *m*/*z* 341 $[M+1]^+$.
24. PTP1B (human, recombinant) was purchased from Enzo Life Sciences, Inc. A mixture consisting of 2 mM *p*-NPP and 0.05 μg PTP1B in a buffer containing 0.06 M citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT) with or without a tested compound solution (prepared in the above buffer solution containing 3% DMSO), was incubated at 37 °C for 30 min. The reaction was terminated by adding 20 μL of 10 M NaOH. The reaction mixture was blended by a microplate mixer for 5 min and the amount of produced *p*-nitrophenol was tested by measuring the absorbance at 405 nm. The blank was measured in the same way except adding buffer solution instead of the enzyme.