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Inhibitory effect of chalcones and their derivatives from *Glycyrrhiza inflata* on protein tyrosine phosphatase 1B

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ABSTRACT

Compounds (**1**–**6**) isolated from the CH_2Cl_2 extract of *Glycyrrhiza inflata* and semisynthetic licochalcone A derivatives (**7**–**14**) were evaluated for their protein tyrosine phosphatase 1B (PTP1B) inhibitory activities. Licochalcones A (**4**) and E (**6**), each with an allyl group at position 5 in the B ring exhibited significant inhibitory effects. Licochalcone A derivative **7**, the most potent among the series, had an IC_{50} value of 11.7 ± 2.0 μM, ca. twofold better than that of licochalcone A (**4**).

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Protein tyrosine phosphatase 1B (PTP1B) is an intracellular protein tyrosine phosphatase that plays a critical role in the negative regulation of insulin and leptin signaling pathways. Transgenic mice without PTP1B have enhanced sensitivity to insulin and are protected against weight gain when fed a high-fat diet. In addition, antisense-based oligonucleotides targeting PTP1B have shown efficacy in type 2 diabetes and have entered phase 2 clinical trials.[1] Thus inhibition of PTP1B has been proposed as a potential therapeutic target for drug discovery in the treatment of type 2 diabetes to avoid the weight gain that occurs with current insulin sensitizers, for example, PPARγ activators.[2] Although several types of PTP1B inhibitors have been reported, because of the low selectivity and poor pharmacokinetic properties, new types of PTP1B inhibitors with improved pharmacological properties are still being sought.[3]

While searching for PTP1B inhibitors from natural products, we found that the CH_2Cl_2 extract of *Glycyrrhiza inflata* inhibited PTP1B. Further investigation of this extract led to the isolation of six compounds (**1**–**6**). *G. inflata* is the main species in Xinjiang licorice. The representative compound from *G. inflata* is an isoprenoid-substituted retrochalcone having reversed A and B rings, licochalcone A, which is structurally distinguished from normal chalcones by the lack of oxygen functionalities at C-2′ and C-6′. Six retrochalcones, licochalcones A–E and echinatin, have been isolated and characterized from the roots of *G. inflata*.[4–7] The allyl retrochalcones in particular were reported to exhibit various biological activities, including antitumor,[7,8] antiparasitic,[9] antileishmanial,[10] antioxidative and superoxide scavenging,[11] and antibacterial effects.[12] Here we report they also have PTP1B inhibitory activity (Fig. 1).

The CH_2Cl_2 extract of *G. inflata* was repeatedly subjected to flash silica gel chromatography and preparative TLC to obtain six known compounds. By comparison of their spectroscopic data with previously reported values in the literature, these compounds were confirmed as the flavanone, liquiritigenin (**1**),[13] the chalcone, isoliquiritigenin (**2**),[13] and the retrochalcones, echinatin (**3**),[4] licochalcone A (**4**),[5,7] licochalcone C (**5**),[5] and licochalcone E (**6**).[6]

Compounds **7** and **8** were prepared by direct methylation of licochalcone A (**4**) in the presence of sodium hydride in DMSO.[14,15] Compounds **9** and **10** were also obtained by direct acetylation of licochalcone A (**4**) with acetic anhydride in pyridine. Compounds **11** and **12** were prepared by THP-protection of the 4′-hydroxy group in the A ring followed by methylation or acetylation of the 4-hydroxyl group in the B ring and subsequent cleavage of the THP-ether under acidic conditions.[14] Acetylation of compound **7** and compound **11** with acetic anhydride gave compounds **13** and **14**, respectively (Scheme 1).

The inhibitory activities of the isolated compounds and the semisynthetic licochalcone A derivatives against PTP1B were measured using *p*-nitrophenyl phosphate (pNPP) as a substrate, and the results are summarized in Table 1.[16,17] The known PTP1B inhibitor, ursolic acid (IC_{50} = 3.9 ± 0.3 μM), was used as the positive control. The isolated compounds **4**-**6**, allyl retrochalcones, dose-dependently

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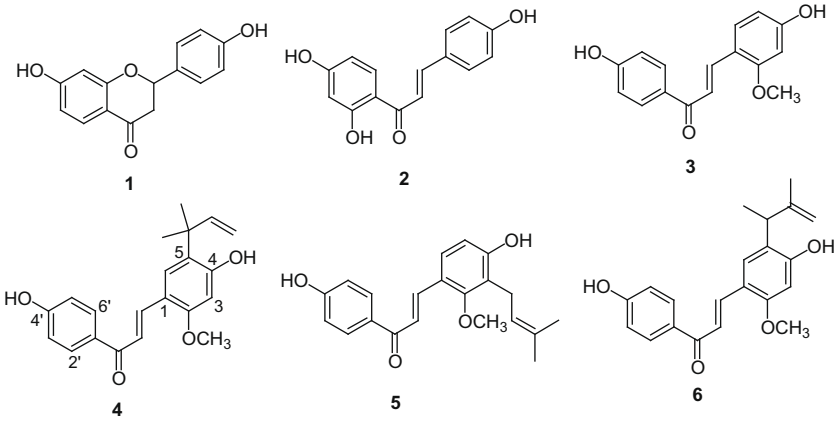
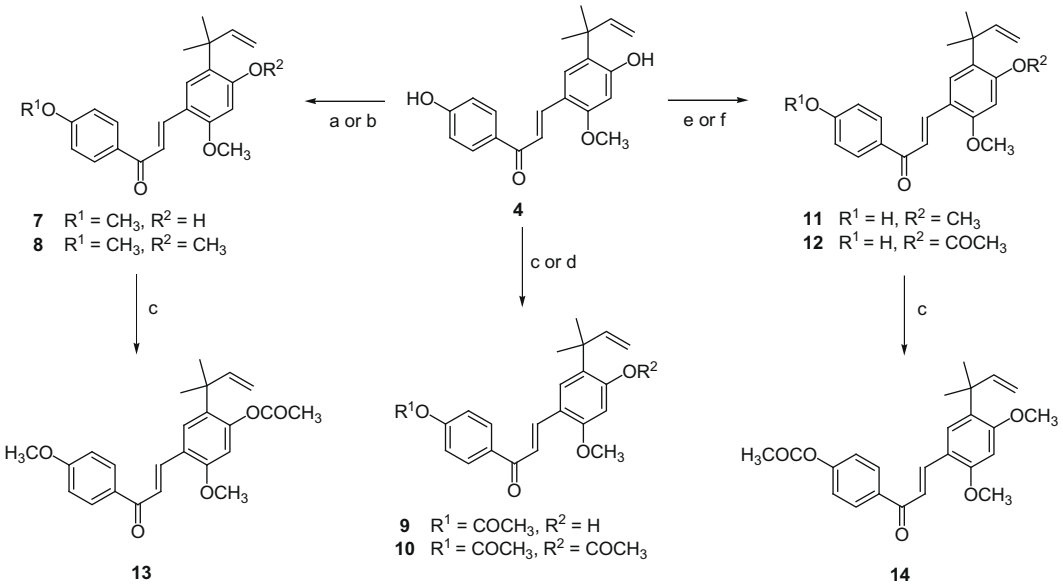


Figure 1. The structures of flavanone and chalcones from *G. inflata*.



Scheme 1. The semisynthesis of licochalcone A derivatives. (a) 1.2 equiv CH_3I, 1.5 equiv NaOH, DMSO, rt, 40% (**7**); (b) 2.4 equiv CH_3I, 3.0 equiv NaOH, DMSO, rt, 12% (**8**); (c) 1.2 equiv Ac_2O, pyridine, rt, 64% (**9**), 53% (**13**), 87% (**14**); (d) 2.3 equiv Ac_2O, pyridine, rt, 77% (**10**); (e) (1) 3,4-Dihydro-2*H*-pyran, pyridinium *p*-toluenesulfonate, CH_2Cl_2, rt, (2) CH_3I, NaOH, DMSO, rt, (3) 2 M-HCl, 30% (**11**); (f) (1) 3,4-Dihydro-2*H*-pyran, pyridinium *p*-toluenesulfonate, CH_2Cl_2, rt, (2) Ac_2O, pyridine, rt, (3) 2 M-HCl, 31% (**12**).

Table 1
Inhibitory activity of compounds **1**–**14**

Compound	PTP1B inhibitory activity[a]
1	>50
2	>50
3	>50
4	19.1 ± 0.1
5	30.9 ± 0.5
6	20.7 ± 1.8
7	11.7 ± 2.0
8	19.8 ± 1.7
9	19.2 ± 0.1
10	> 50
11	18.3 ± 1.4
12	17.3 ± 1.6
13	19.4 ± 3.5
14	29.2 ± 2.3
Ursolic acid[b]	3.9 ± 0.3

[a] Results are expressed as IC_{50} values (μM) and as mean ± SD of three replicates.
[b] Positive control.

inhibited PTP1B activity with IC_{50} values ranging from 19.1 ± 0.1 to 30.9 ± 0.5 μM, but the flavanone, liquiritigenin (**1**), the chalcone, isoliquiritigenin (**2**), and the retrochalcone, echinatin (**3**), which lacked the allyl group, had no inhibitory effects. Compounds **4** and **6**, each with a different allyl group at position 5 in the B ring, exhibited greater PTP1B inhibitory activities than compound **5** which has an allyl group at position 3 in the B ring. These results indicated that the presence of the allyl group in the B ring may increase PTP1B inhibitory activity but the degree of activation varies depending on the configuration and substitution position of the allyl group in the B ring.[16] Thus, a study of the structure–activity relationships and subsequent optimization of the allyl retrochalcone might enable development of novel PTP1B inhibitors.

In order to improve its PTP1B inhibitory activity by changing its physicochemical properties, the 4- and/or 4′-hydroxyl group on licochalcone A was methylated and/or acetylated; the resulting derivatives were then evaluated for their PTP1B inhibitory effects. As shown in Table 1, the inhibitory effects of the semisynthetic licochalcone A derivatives **7**–**14** were improved only when the 4′-hydroxy group was methylated. Among the licochalcone A derivatives, compound **7**, methylated at the 4′-hydroxy position, exhibited the most potent PTP1B inhibitory activity with an IC_{50} of 11.7 ± 2.0 μM. This result indicates that compound **7** is about twofold more effective

than licochalcone A. Other modified compounds showed similar or less inhibitory effect than licochalcone A. PTP1B inhibitory activities of the isolated compounds (**1**–**6**) and the licochalcone A derivatives (**7**–**14**) may provide valuable information regarding the structure–activity relationships for the development of novel PTP1B inhibitors.

In conclusion, a series of retrochalcones with an allyl group at position 5 in the B ring significantly inhibited PTP1B. Of the licochalcone A derivatives, compound **7**, methylated at the 4′-hydroxy position, exhibited about twofold better activity than licochalcone A. These results provide a starting point for further optimization of substituted allyl retrochalcone with an ether linkage at position 4′ as a PTP1B inhibitor. Further SAR studies of substituted allyl retrochalcones in PTP1B inhibition are currently undergoing and the results will be published in due course.

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15. *3-[5-(1,1-Dimethylallyl)-4-hydroxy-2-methoxyphenyl]-1-(4-methoxyphenyl)-propenone* (**7**): To a solution of licochalcone A (200 mg, 0.59 mmol) in DMSO (5 ml) were added methyl iodide (44.2 μL, 0.71 mmol) and NaOH (35.6 mg, 0.89 mmol). The reaction was stirred at rt for 4 h and water was added (20 ml). The aqueous solution was extracted with ethyl acetate (3 × 20 ml) and the combined organic phases were dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc = 5:1) to give 7 (83.2 mg, 40%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.03 (d, 2H, J = 9.0 Hz), 8.01 (d, 1H, J = 15.6 Hz), 7.59 (d, 1H, J = 15.5 Hz), 7.48 (s, 1H), 6.98 (d, 2H, J = 9.0 Hz), 6.47 (s, 1H), 6.20 (dd, 1H, J = 17.8, 10.8 Hz), 5.36 (dd, 1H, J = 17.5, 1.0 Hz), 5.34 (dd, 1H, J = 10.5, 1.0 Hz), 3.88 (s, 3H), 3.85 (s, 3H), 1.46 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.9, 163.1, 159.5, 158.3, 147.8, 140.8, 131.7, 130.8, 128.8, 124.7, 120.1, 116.4, 113.73, 113.71, 101.1, 55.6, 55.5, 39.8, 27.1.
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17. *PTP1B assay*: Recombinant human PTP1B was purchased from BIOMOL International LP. For the inhibition assay, a sample (3 μL in DMSO) was added to a reaction mixture containing enzyme (2 μL), reaction buffer [10 μL, 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA and 1 mM dithiothreitol], water (35 μL) and 50 μL of 4 mM *p*-nitrophenyl phosphate (*p*NPP). The reaction mixture (100 μL) was incubated at 37 °C for 30 min and then quenched by addition of 10 μL of 10 N NaOH. The hydrolysis of *p*NPP was determined by measuring the absorbance at 405 nm.