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SYNTHESIS OF MANGIFERIN DERIVATIVES AS PROTEIN TYROSINE PHOSPHATASE 1B INHIBITORS

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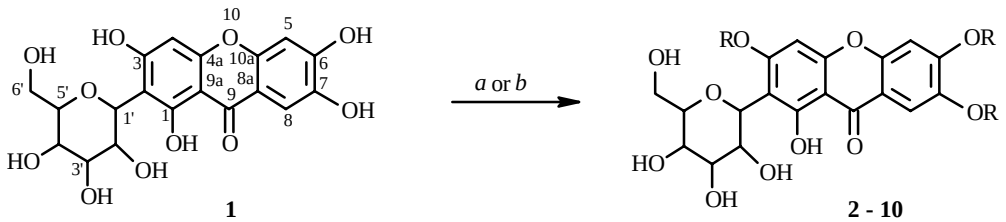
Protein tyrosine phosphatase 1B (PTP1B) has received much attention due to its pivotal role in type 2 diabetes and obesity as a negative regulator of the insulin signaling pathway. Mangiferin, a xanthone glucoside, has been reported to possess significant antidiabetic activity. In the present study, a series of mangiferin derivatives was synthesized and evaluated for their PTP1B inhibitory activity. Some of the screened compounds displayed good PTP1B inhibitory activity.

Key words: mangiferin, protein tyrosine phosphatase 1B, derivatives, inhibition.

In recent years, PTP1B has received much attention due to its critical role in type-2 diabetes as a negative regulator of the insulin signaling pathway [1–3]. Clinical studies have demonstrated that PTP1B is primarily responsible for dephosphorylation of the activated insulin receptor and thus downregulates insulin signaling; for example, PTP1B knocked mice are shown to have increased insulin sensitivity [4, 5]. Therefore, inhibition of PTP1B would be an excellent strategy for treatment of type 2 diabetes mellitus.

Our phytochemical investigation resulted in isolation of antidiabetes compound mangiferin (**1**) from *Anemarrhenae asphodeloides*. Mangiferin (**1**), a xanthone glucoside, is an active phytochemical present in various plants. Recently, mangiferin has been reported to possess significant antidiabetic activity [6, 7]. However, there is no experimental evidence showing that mangiferin or its derivatives have inhibitory activity on PTP1B. Hence the present study was carried out to investigate the possible inhibitory activity of mangiferin and its derivatives on PTP1B.

It was previously found that mangiferin had poor solubility in most solvents in our study, so some alkyl and benzyl groups were brought in by using a nucleophilic substitutional reaction in order to improve its solubility. A series of mangiferin derivatives was synthesized according to the synthetic route.



1 **2 - 10**

a. RX, K_2CO_3, DMF; *b*. Substitutional benzyl chloride K_2CO_3, DMF

R: CH_3 (**2**), CH_2CH_3 (**3**), $CH(CH_3)_2$ (**4**), $CH(CH_3)CH_2CH_3$ (**5**), $(CH_2)_6CH_3$ (**6**), $(CH_2)_9CH_3$ (**7**), $(CH_2)_{15}CH_3$ (**8**), $CH_2C_6H_5$ (**9**), *p*-$CH_2C_6H_4Cl$ (**10**)

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TABLE 1. Structure and *in vitro* PTP1B Enzyme Inhibitory Activity of the Compounds

Compound	Ratio of inhibitory activity, %		Compound	Ratio of inhibitory activity, %	
	500 μM	50 μM		500 μM	50 μM
1	24.07	-	**6**	5.41	-
2	24.97	-	**7**	*	100.0
3	22.02	-	**8**	51.14	10.68
4	19.60	-	**9**	*	26.14
5	47.70	-	**10**	*	62.50

*Mean deposition was found while buffer was added into the test sample.

Vanadate has proved to be a nonselective inhibitor of PTP1B, and previous studies have shown that treatment with vanadate can normalize blood glucose level in diabetes. Taking sodium vanadate (IC_{50}, 2 μM) as a control, we have evaluated the PTP1B inhibitory activity of our compounds at 500 and 50 μM concentrations, and their results are summarized in Table 1 [8].

Some of the screened compounds demonstrated moderate to good PTP1B inhibitory activity at 50 μM concentration. Compounds **7** and **10** displayed 100% and 62.5% inhibition against PTP1B. It was evident from the activity profile (Table 1) that mangiferin (**1**) is a weak PTP1B inhibitor, whereas some derivatives were found to show good inhibition. The structure–activity relationship study showed that the substitution of the free hydroxyl at C-3, C-6, and C-7 (alkyl of long chain and substitutional benzyl) of mangiferin remarkably enhanced the activity.

In conclusion, we have synthesized a series of mangiferin derivatives **2**–**10** and tested their PTP1B inhibitory activity. Compounds **7** and **10** displayed promising inhibitory activity among the synthesized compounds. Further modification and biological studies are under progress.

EXPERIMENTAL

Melting points were measured on a YRT-3 instrument (Tianda Tianfa-pharmaceutical testing instrument manufacturer, Tianjin, China). IR spectra were recorded on a Bruker Vector 22 instrument in KBr disks. PMR and ^{13}C NMR spectrum were obtained on a Varian INOVA-400 instrument. The chemical shifts of protons are given on the δ scale, ppm, with tetramethylsilane (TMS) as the internal standard.

Isolation of Mangiferin (1). Shade-dried and powdered rhizome of *Anemarrhenae asphodeloides* (10 kg) were percolated with EtOH–H_2O (4:1, 10 L) for 24 h at room temperature. The combined extracts were freed of the solvent *in vacuo* to a volume of 4 L, diluted with H_2O (6 L), and then eluted with H_2O, EtOH–H_2O (5:1) and EtOH–H_2O (19:1) successively in a macroporous resin column. The EtOH–H_2O (5:1) part was concentrated *in vacuo* to remove EtOH and left at room temperature for 12 h. The solid matter was filtered and recrystallized from EtOH–H_2O (5 : 2) three times to afford pure light yellow crystalline mangiferin (**1**) powder, yield 113.7 g (1.14%). The purity was more than 98.0%.

Pale yellow crystals, mp 260~261°C. ESI-MS spectrum *m/z*: 422. UV spectrum (MeOH, λ_{max}, nm, log ε): 240, 258, 318, 366. IR spectrum (KBr, ν, cm^{-1}): 3367, 2938, 1650, 1621, 1492, 1408, 1296, 1254, 1193, 1095, 1075, 1051, 1032, 879, 829, 799, 753, 644, 589, 519, 449. PMR spectrum (400 MHz, DMSO-d_6, δ, ppm): 13.73 (1H, s), 10.42 (3H, br), 7.39 (1H, s), 6.86 (1H, s), 6.37 (1H, s), 4.75 (2H, br), 4.62 (1H, d), 4.50 (1H, br), 4.37 (1H, br), 4.03 (1H, t), 3.70 (1H, d), 3.44 (1H, d), 3.19 (2H, m), 3.16 (1H, m). ^{13}C NMR spectrum (δ_C, ppm): 161.5 (C-1), 107.5 (C-2), 163.6 (C-3), 93.4 (C-4), 156.3 (C-4a), 102.6 (C-5), 153.8 (C-6), 143.6 (C-7), 108.1 (C-8), 111.8 (C-8a), 179.1 (C-9), 101.3 (C-9a), 150.8 (C-10a), 73.1 (C-1), 70.5 (C-2′), 78.8 (C-3′), 70.2 (C-4′), 81.5 (C-5′), 61.4 (C-6′).

Alkylation and Benzylation of Mangiferin (General Method). A solution of mangiferin (420 mg, 1 mmol) in dry DMF (20 mL) was treated with alkyl halide or substitutional benzyl chloride (4 mmol) and K_2CO_3 (100 mg) with stirring at 60°C for 10 h. The reaction mixture on evaporation *in vacuo* gave a residue and was submitted to column chromatography using CH_2Cl_2 and MeOH as eluent to give compounds **2–10**.

Compound 2. Pale yellow powders, mp 210–212°C, yield 310 mg (66.8%). IR (KBr, ν, cm^{-1}): 3422, 2937, 1645, 1609, 1485, 1438, 1392, 1285, 1215, 1166, 1116, 1087, 1026, 985, 809, 781, 603, 579. PMR (400 MHz, DMSO-d_6, δ, ppm): 13.53 (1H, s), 7.49 (1H, s), 7.18 (1H, s), 6.65 (1H, s), 4.64 (1H, dd), 4.01 (1H, t), 3.97 (3H, s), 3.92 (3H, s), 3.90 (3H, s), 3.71, (1H, dd), 3.46~3.42 (4H, m), 3.40 (1H, m), 3.24~3.10 (3H, m).

Compound 3. Pale yellow powders, mp 213–214°C, yield 330 mg (65.2%). PMR (400 MHz, DMSO-d_6, δ, ppm): 13.50 (1H, s), 7.44 (1H, s), 7.11 (1H, s), 6.57 (1H, s), 4.76~4.72 (2H, m), 4.64 (1H, m), 4.48 (1H, m), 4.32 (1H, br), 4.23 (2H, dd), 4.18~4.11 (4H, m), 4.06 (1H, m), 3.72 (1H, m), 3.39 (1H, m), 3.18 (2H, m), 3.11 (1H, m), 1.44~1.37 (9H, m).

Compound 4. Pale yellow powders, mp 150–152°C, yield 292 mg (53.2%). PMR (400 MHz, DMSO-d_6, δ, ppm): 13.48 (1H, s), 7.51 (1H, s), 7.16 (1H, s), 6.61 (1H, s), 4.86~4.78 (2H, m), 4.75 (2H, br), 4.63 (1H, m), 4.56 (1H, m), 4.46 (1H, br), 4.23 (1H, br), 4.05 (1H, m), 3.73 (1H, m), 3.39 (1H, m), 3.20 (2H, m), 3.11 (1H, m), 1.40~1.28 (18H, m).

Compound 5. Pale yellow powders, mp 170–172°C, yield 333 mg (56.3%). IR (KBr, ν, cm^{-1}): 3430, 2972, 2934, 2879, 1648, 1606, 1468, 1381, 1278, 1198, 1090, 1028, 905, 811, 791, 657, 578. PMR (400 MHz, DMSO-d_6, δ, ppm): 13.50 (1H, s), 7.50 (1H, s), 7.16 (1H, s), 6.61 (1H, s), 4.83 (2H, br), 4.63 (1H, m), 4.60~4.55 (2H, m), 4.54 (1H, br), 4.36 (1H, br), 4.28 (1H, m), 4.04 (1H, m), 3.75~3.66 (2H, m), 3.18 (2H, m), 3.05 (1H, m), 1.77~1.61 (6H, m), 1.34~1.24 (9H, m), 1.05~0.93 (9H, m).

Compound 6. Pale yellow crystals, mp 122–123°C, yield 474 mg (66.2%). IR (KBr, ν, cm^{-1}): 438, 2928, 2856, 1648, 1609, 1580, 1507, 1466, 1394, 1277, 1226, 1200, 1111, 1085, 1030, 901, 823, 807, 724, 661, 632, 576. PMR (400 MHz, DMSO-d_6, δ, ppm): 13.48 (1H, s), 7.46 (1H, s), 7.07 (1H, s), 6.55 (1H, s), 4.69 (2H, br), 4.63 (1H, m), 4.31 (1H, br), 4.15 (1H, br), 4.07 (6H, m), 4.05 (1H, m), 3.74 (1H, m), 3.45 (1H, m), 3.23 (2H, m), 3.15 (1H, m), 1.78 (6H, m), 1.47 (6H, m), 1.31 (18H, m), 0.89 (9H, m).

Compound 7. Pale yellow crystals, mp 108–109°C, yield 527 mg (62.5%). PMR (400 MHz, DMSO-d_6, δ, ppm): 13.49 (1H, s), 7.45 (1H, s), 7.09 (1H, s), 6.56 (1H, s), 4.69 (2H, br), 4.68 (1H, m), 4.39 (1H, br), 4.20 (1H, br), 4.15 (2H, m), 4.07~4.05 (4H, m), 4.04 (1H, m), 3.73 (1H, m), 3.39 (1H, m), 3.19 (2H, m), 3.09 (1H, m), 1.80~1.73 (6H, m), 1.47 (6H, s), 1.27 (36H, s), 0.87~0.84 (9H, m).

Compound 8. Pale yellow crystals, mp 82–84°C, yield 587 mg (53.6%). PMR (400 MHz, DMSO-d_6, δ, ppm): 13.86 (1H, s), 7.54 (1H, s), 6.81 (1H, s), 6.40 (1H, s), 4.88 (1H, d), 4.43 (1H, br), 4.12~4.04 (6H, m), 3.90 (1H, m), 3.80 (1H, m), 3.72 (1H, m), 3.65 (2H, m), 3.53 (1H, m), 2.74 (1H, br), 2.50 (1H, br), 2.06 (1H, br), 1.93~1.81 (6H, m), 1.42~1.20 (78H, m), 0.91~0.86 (9H, m).

Compound 9. Pale yellow crystals, mp 133–134°C, yield 377 mg (54.4%). IR (KBr, ν, cm^{-1}): 3413, 3062, 3031, 2874, 1646, 1609, 1581, 1500, 1475, 1391, 1278, 1223, 1188, 1082, 1025, 905, 809, 737, 696, 668, 642, 615, 571, 466. PMR (400 MHz, DMSO-d_6, δ, ppm): 13.48 (1H, s), 7.63~7.29 (17H, m), 6.75 (1H, s), 5.29 (2H, s), 5.22~5.14 (4H, m), 4.70 (1H, dd), 4.00 (1H, t), 3.67 (1H, m), 3.45 (1H, m), 3.41 (4H, br), 3.22 (2H, m), 3.10 (1H, m).

Compound 10. Pale yellow crystals, mp 142–144°C, yield 462 mg (58.0%). PMR (400 MHz, DMSO-d_6, δ, ppm): 13.45 (1H, s), 7.65~7.42 (13H, m), 7.39 (1H, s), 6.72 (1H, s), 5.41~5.20 (6H, m), 4.72 (2H, br), 4.70 (1H, m), 4.47 (1H, br), 4.34 (1H, br), 3.99 (1H, m), 3.75 (1H, m), 3.50 (1H, m), 3.25~3.15 (3H, m).

Protein Tyrosine Phosphatase Inhibitory Activity *in vitro*. The effect of our compounds on PTP1B was studied by preincubating the compound with enzyme in the reaction system for 10 min, and the residual protein tyrosine phosphatase activity was determined according to the method of Goldstein *et al* [8]. With 1.0 mL *p*-nitrophenylphosphate (pNPP) as the substrate, the assay mixture contained 10 mM pNPP in 50 mM HEPES buffer (pH 7.0), with 1mM EDTA and DTT, respectively. The reaction was stopped by addition of 500 μL of 0.1 M NaOH, and the optical density was determined at 410 nm. Control tubes omitting the enzyme were always run in parallel to nullify the nonenzymic reaction and for calculating the concentration of *p*-nitrophenolate ions produced in the reaction mixture. A molar extinction coefficient of 1.78×10^4 was used to determine the concentration of *p*-nitrophenolate produced in the system. Sodium vanadate was taken as a control (IC_{50}, 2 μM).

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