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Synthesis and Protein Tyrosine Phosphatase 1B-Inhibitory Activity of Chalcones

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Protein tyrosine phosphatase 1B (PTP1B) is an effective target for the treatment of type 2 diabetes and obesity. So far less work has been conducted in the structure-activity relationship of the PTP1B inhibitory activity of chalcones. In this work, a library of 65 chalcones were synthesized and their preliminary PTP1B inhibitory activities *in vitro* were evaluated and reported. Among them, 27 compounds displayed good inhibitory activity on PTP1B and seven compounds showed excellent activity. These results indicate that chalcones could be used for the design of more potent PTP1B inhibitors.

Key Words: Chalcones, Protein tyrosine phosphatase 1B, Inhibition, Diabetes, Obesity.

INTRODUCTION

Protein tyrosine phosphatase 1B (PTP1B) is an intracellular enzyme that acts as a key negative regulator of both insulin and leptin signaling pathways. Protein tyrosine phosphatase 1B decreases insulin signaling by dephosphorylating tyrosine residues present in the insulin receptor (IR)[1]. Inhibition of PTP1B should therefore increase insulin sensitivity and responsiveness. Besides, inhibition of PTP1B results in no producing abnormalities in the growth or fertility or other pathogenetic effects in mice[2]. In recent years, the inhibition of PTP1B has attracted much attention due to its potential of treating diabetes and obesity and many small molecular PTP1B-specific inhibitors were synthesized and reported, such as oxalylarylaminobenzoic acid derivatives (compound **1**, Fig. 1), benzofuransulfonamide derivatives (compound **2**, Fig. 1) and salicylic acid derivatives (compound **3**, Fig. 1), *etc*. However, this is a major challenge for them to become effective drugs because of unsatisfactory efficacy or side effects. Therefore, searching for new PTP1B inhibitors with high-effective and low-adverse is essential.

Chalcones, a class of the flavonoids which are abundant in edible plants, possess a wide variety of biological activities including antioxidant, anticarcinogenic, antibacterial and anti - inflammatory activities, *etc*.[3-5]. In the light of their extremely high values, chalcones have become a hot topic for research and development. Recently, there have some reports about the antidiabetic and antihyperglycemic activities of chalcone analogues[6,7]. But, there are little report about the PTP1B inhibitory activity of chalcones and the structure-activity relationship is not comprehensive enough[8,9]. We were thus motivated to synthesize a library of chalcones and study their structure-activity relationship so as to develop more potent PTP1B inhibitors. It is well known that different substituents at different positions on the core structure determine biological activity as well as the specificity of action. Hence, in our synthetic

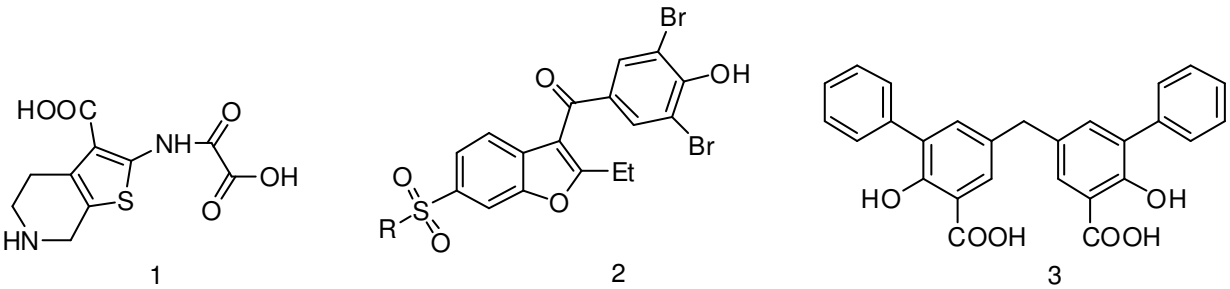
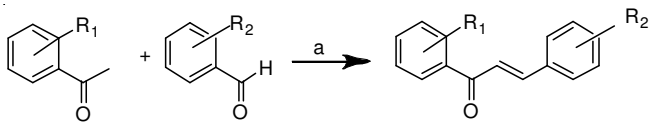


Fig. 1. Structures of some small molecular PTP1B inhibitors

strategy we have used ring A and ring B for substitutions of electron-withdrawing or electron-donating groups so as to better study the structure-activity relationship of chalcones. Consequently, a library of 65 chalcones were synthesized by using an aldol condensation reaction (**Scheme-I**) and their preliminary PTP1B inhibitory activities *in vitro* were evaluated and reported in this work to guide further design of chalcone analogs with stronger activity.



Scheme-I: Condition and Reagent: (a) 10 % NaOH, ethanol, r.t.

EXPERIMENTAL

The melting points were obtained using the Electrothermal digital melting point apparatus, ZMD-1 and are uncorrected. ^{1}H NMR spectra were obtained on a Varian INOVA-400 spectrometer and the chemical shifts are reported as values in parts per million (δ) relative to tetramethyl silane (TMS) as an internal standard. Unless otherwise noted, the materials were obtained from commercially available sources and were used without further purification.

Synthesis of chalcones (general method): A solution of acetophenone (1 mmol) in ethanol (10 mL) was treated with 10 % NaOH aqueous solution (4 mmol) and benzaldehyde (1 mmol) in ethanol with stirring at room temperature for 12-24 h. The reaction was monitored by TLC. When completion, the mixture was poured into an excessive amount of ice-water. Then filtrated, dried and recrystallized from ethanol to give the title compounds.

(E)-3-(5-(Hydroxymethyl)furan-2-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (17): m.p. 82-84 ºC; ^{1}H NMR ($CDCl_3$) δ (ppm): 4.72 (2H, s, -CH_2-), 6.45-6.47 (1H, d, J = 3.6Hz, 3-H), 6.73-6.74 (1H, d, J = 3.3Hz, 4-H), 6.93-6.98 (1H, q, J_1 = 8.1 Hz, J_2 = 1.2 Hz, 5'-H), 7.01-7.04 (1H, dd, J_1 = 8.4Hz, J_2 = 0.6 Hz, 3'-H), 7.48-7.51 (1H, q, J_1 = 8.4 Hz, J_2 = 1.5 Hz, 4'-H), 7.53-7.58 (1H, d, J = 15 Hz, α-H), 7.63-7.68 (1H, d, J = 15 Hz, β-H), 7.93-7.96 (1H, dd, J_1 = 8.1 Hz, J_2 = 1.5 Hz, 6'-H), 12.92 (1H, s, Ar-OH).

Protein tyrosine phosphatase inhibitory activity *in vitro*: The effect of present compounds on PTP1B was studied by pre-incubating 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.*[10]. The 1 mL of *p*-nitrophenyl phosphate (*p*NPP) as the substrate, assay mixture contained 10 mmol/L *p*-nitrophenyl phosphate in 50 mmol/L HEPES buffer (pH 7.0), with 1 mmol/L EDTA and DTT, respectively. The reaction was stopped by addition of 500 µL of 0.1 mol/L 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 µmol/L).

RESULTS AND DISCUSSION

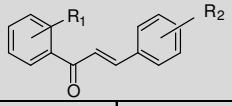
We have synthesized a library of 65 chalcones and evaluated preliminary PTP1B inhibitory activities *in vitro* of our title compounds, the enzyme inhibition data are expressed as % inhibition at 20 µg/mL concentration. Their results are summarized in Table-1.

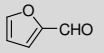
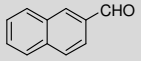
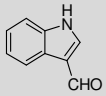
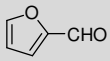
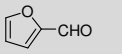
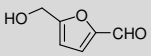
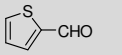
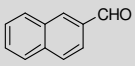
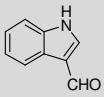
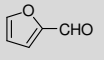
Present results suggested that many of the screened compounds demonstrated moderate to good PTP1B inhibitory activities *in vitro* at 20 µg/mL concentration and the position and type of the substituents on the rings of chalcones are determinant for the biological effect. It was evident that the introduction of some electron-withdrawing substituents in B cycle could increase the PTP1B inhibitory activity while the introduction of weakly acidic groups could decrease the activity except for the compound **62**. On the other hand, the position of substituents can also affect the activity to some degree. For example, comparing **3**, **4** and **5**, it could be seen that the *para* position was a better place for introducing flurine atom than *meta* and *ortho* position whereas the *ortho* position was better for introducing bromine atom than *para* position by comparing **10** and **11** when there are oxygen functionalities at C-2' in ring A. Besides, the introduction of heterocycles could not result in high activity except for compound **20**. It is very interesting to note that the methylation of 2'-hydroxyl group in ring A results in the significant decline of activity by comparing compound **8** and **22**. It was presumed that perhaps there is a binding-site for PTP1B and the methylation is unfavourable to the combination of enzyme with compounds. However, the results are quite different when the hydroxyl group was replaced with an amino group in ring A. For example, the introduction of some electron-withdrawing substituents can not result in high activity, even lead to a loss of activity (*e.g.*, compounds **30**, **32**, **34** and **37**) and the *meta* position was a better place for introducing fluorine atom than *para* and *ortho* position (*e.g.*, **32**, **33** and **34**), while the introduction of 2-Br resulted in a loss of activity (**37**). Besides, when the NO_2 group was moved to the **4** position from the **3** position the activity was greatly promoted (*e.g.*, **30** and **31**) and the PTP1B activity was inhibited completely by replacing the benzene ring with the stiffer naphthalene ring even there are no substituents in the naphathalene cycle (**39**). In addition, the introduction of halogen atom in ring A can also help enhance the PTP1B inhibitory activity, but the substituting position has great effect on the activity.

Conclusion

In an attempt to better study the structure-activity relationship of chalcones so as to develop more potent PTP1B inhibitors, a library of 65 chalcones were synthesized and their preliminary PTP1B inhibitory activities *in vitro* were evaluated and reported in this paper. Twenty-seven compounds (**2**, **5-8**, **10**, **13**, **19-20**, **23**, **31**, **33**, **36**, **38-39**, **45**, **48-49**, **52-53**, **55**, **58**, **60** and **62-65**) displayed promising inhibitory activities (inhibitory activity > 50 %) and seven compounds (**2**, **6**, **8**, **13**, **39**, **49** and **58**) showed excellent activities (inhibitory activity > 90 %) among the synthesized compounds. It is important that besides the nature of the functional groups, the position of the group may be mandatory for biological activity. In this

TABLE-1
STRUCTURES AND *IN VITRO* PTP1B ENZYME INHIBITORY ACTIVITIES OF THE 65 CHALCONES



Compound	R_1	R_2	Ratio of inhibitory activity (%) 20 (µg/mL)	Compound	R_1	R_2	Ratio of inhibitory activity (%) 20 (µg/mL)
1	2-OH	3,4-OCH_2O-	25.11	**34**	2-NH_2	4-F	1.21
2	2-OH	3-NO_2	90.51	**35**	2-NH_2	3,4-Cl_2	45.97
3	2-OH	2-F	19.68	**36**	2-NH_2	2,4-Cl_2	83.16
4	2-OH	3-F	27.13	**37**	2-NH_2	2-Br	-10.51
5	2-OH	4-F	64.99	**38**	2-NH_2		57.15
6	2-OH	3-Cl	93.03	**39**	2-NH_2		102.12
7	2-OH	3,4-Cl_2	51.55	**40**	2-NH_2		4.06
8	2-OH	2,4-Cl_2	92.80	**41**	4-NH_2	3,4-OCH_2O-	-4.74
9	2-OH	2,6-Cl_2	32.36	**42**	4-NH_2	3-Cl	19.92
10	2-OH	2-Br	76.04	**43**	4-NH_2	4-$N(CH_3)_2$	-18.44
11	2-OH	4-Br	45.49	**44**	2-NO_2	3,4-$(OCH_3)_2$	30.15
12	2-OH	4-CF_3	39.61	**45**	2-NO_2	3,4-OCH_2O-	56.97
13	2-OH	4-$N(CH_3)_2$	102.81	**46**	2-NO_2		9.30
14	2-OH	3-OCH_3, 4-OH	21.57	**47**	4-NO_2	3,4-$(OCH_3)_2$	-8.79
15	2-OH	4-COOH	23.17	**48**	4-NO_2	3,4-OCH_2O-	69.26
16	2-OH		45.39	**49**	4-NO_2	3-Cl	95.65
17	2-OH		44.88	**50**	4-NO_2	3-OCH_3, 4-OH	17.79
18	2-OH		37.74	**51**	2-F	4-OCH_3	35.47
19	2-OH		55.86	**52**	2-F	3,4-OCH_2O-	56.27
20	2-OH		76.55	**53**	2-F	4-$N(CH_3)_2$	55.07
21	2-OCH_3	3,4-OCH_2O-	-9.54	**54**	2-Cl	3,4-$(OCH_3)_2$	2.04
22	2-OCH_3	2,4-Cl_2	5.57	**55**	2-Cl	3,4-OCH_2O-	86.29
23	4-OCH_3	4-OCH_3	71.48	**56**	2-Cl	4-OH	5.75
24	4-OCH_3	3,4-OCH_2O-	3.15	**57**	3-Cl	3,4-$(OCH_3)_2$	47.61
25	4-OCH_3	3-Br	48.87	**58**	3-Cl	4-OCH_3	107.46
26	4-OCH_3	4-$N(CH_3)_2$	-10.66	**59**	3-Cl	3,4-OCH_2O-	23.06
27	4-OCH_3	4-OH	20.69	**60**	4-Cl	3,4-$(OCH_3)_2$	77.63
28	4-OCH_3		12.44	**61**	4-Cl	3,4-OCH_2O-	-2.27
29	2-NH_2	3,4-$(OCH_3)_2$	8.01	**62**	4-Cl	3-OCH_3, 4-OH	57.05
30	2-NH_2	3-NO_2	0.75	**63**	4-Cl		63.02
31	2-NH_2	4-NO_2	78.68	**64**	3-Br	3,4-$(OCH_3)_2$	52.67
32	2-NH_2	2-F	-10.81	**65**	3-Br	4-OCH_3	64.14
33	2-NH_2	3-F	54.04	–	–	–	–

work, the potent activity and simple synthesis of these chalcones suggest that they are potential candidates for the development of PTP1B inhibitors and thus offers an additional natural PTP1B inhibitor resource for the treatment of diabetes and obesity. However, further modification and biological studies even docking study are necessary in order to better understand the binding modes of chalcones to PTP1B and all these works are already under progress.

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