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Thiazolidinone Derivatives as Competitive Inhibitors of Protein Tyrosine Phosphatase 1B (PTP1B)

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Phosphorylation of tyrosine residues is an important mode of cellular regulation of enzyme activities and protein-protein interactions.[1,2] Reversible phosphorylation-dephosphorylation processes are controlled by two counteracting enzyme families: protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs).[3] These enzymes are involved in diverse cellular processes, including cell growth, proliferation, differentiation, apoptosis, aging, immune response, and metabolism.[4] The critical roles played by PTKs and PTPs in cellular functions implicate them in various human diseases.[5,6] Given that a PTP controls the duration of the phosphorylation state of a protein, suppression of the activity of the PTP could be an effective way to prolong the signal induced by tyrosine phosphorylation. For example, inhibition of PTP1B, a major phosphatase for insulin and leptin receptors, could augment the signals provoked by insulin and leptin, thus increasing insulin and leptin sensitivity.[7-10] These notions were confirmed *via* numerous biological experiments, including the deletion of the PTP1B-encoding gene in mice.[11,12] In our ongoing interest in developing the inhibitors of PTP1B, which is a therapeutic target for diabetes and obesity, we synthesized a series of thiazolidinone derivatives containing a single carboxylate moiety as a phosphate mimic, hoping that the presence of a monocarboxylate moiety could compromise the solubility and cell permeability of the compounds. The thiazolidinone derivatives were evaluated for their *in vitro* activity of PTP1B inhibition and *in vivo* efficacy against diabetes and obesity in an animal model.

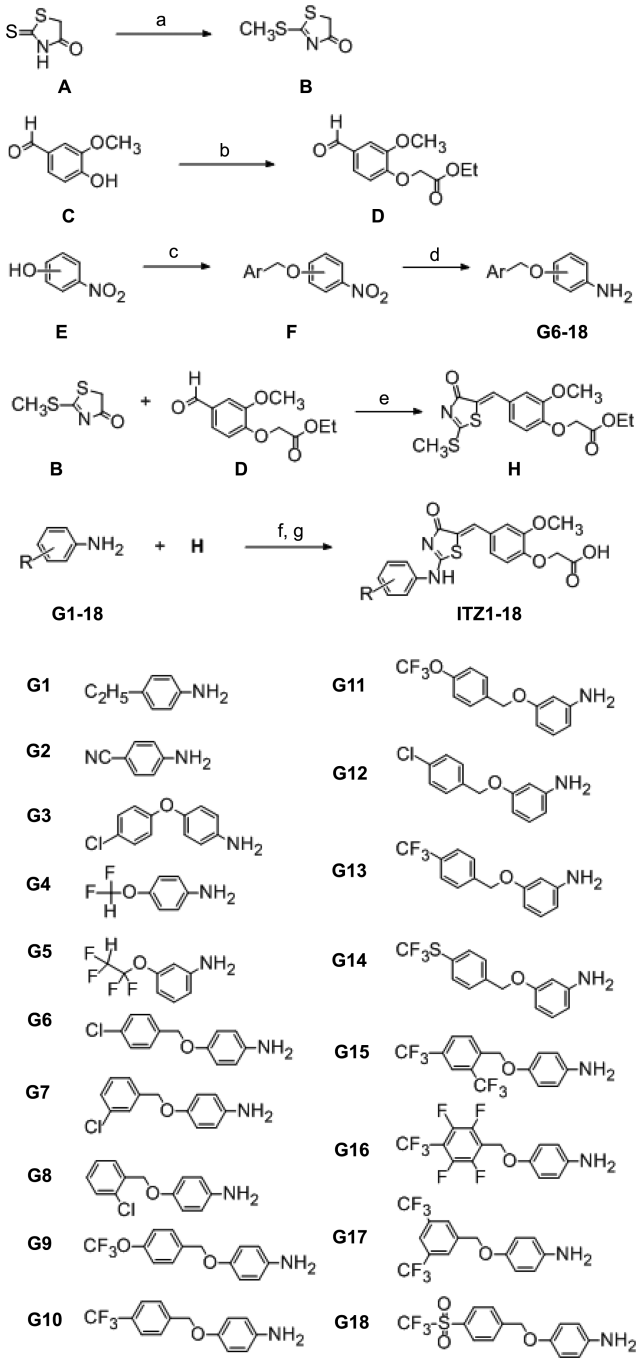
Thiazolidinone derivatives (**ITZ1-18**) were synthesized by the methods outlined in Scheme 1. Knoevenagel condensation of methyl rhodanine (**B**) with a benzaldehyde derivative (**D**) in the absence of a catalyst yielded 5-arylidene rhodanine (**H**), which was obtained exclusively as a (*Z*)-isomer as determined by the ^{1}H-NMR spectrum. The arylidene rhodanine (**H**) was subsequently treated with a variety of arylamines (**G1-18**) to obtain **ITZ1-18**.[13] Commercially available amines (**G1-5**) were purchased, while others (**G6-18**) were prepared stating from 3- or 4-nitrophenol as shown in Scheme 1.

Compounds **ITZ1-18** were evaluated for their inhibitory activity against PTP1B. To determine the half-maximal inhibitory concentrations (IC_{50}), *p*-nitrophenyl phosphate (*p*NPP) hydrolase activity of PTP1B was measured in the presence of an inhibitor at a series of different concentrations. The IC_{50} values for the **ITZ** compounds are summarized in Table 1. Six of the compounds (**ITZ9-10** and **ITZ14-17**) exhibited IC_{50} values in low micromolar concentration ranges. **ITZ15** was the most potent with an IC_{50} of 1.4 μM. Kinetic analysis of the inhibitory action of **ITZ15**, using a Lineweaver-Burk plot, demonstrated that **ITZ15** was a competitive inhibitor of PTP1B (Figure 1).

To obtain the selectivity profile of **ITZ15**, the inhibitory activity was determined against the human PTPs, T cell protein tyrosine phosphatase (TCPTP) and Src homology-2 domain-containing protein tyrosine phosphatase-1 (SHP1), and Vaccinia H1-related (VHR) phosphatase, and a yeast PTP, YPTP1. **ITZ15** showed 17-fold selectivity over TCPTP, which is most homologous to PTP1B, and showed 4 ~ 34-fold selectivity over other phosphatases examined in this experiment.

ITZ15 was evaluated for its *in vivo* efficacy as an *anti*-obesity and hypoglycemic agent. Five week-old mice susceptible to diet-induced obesity/diabetes (C57BL/6J Jms Slc male) were fed a high-fat diet (HFD) *ad libitum* for 8 weeks, followed by a HFD + **ITZ15** for 4 weeks. **ITZ15** was provided with the diet (1.0 g of **ITZ15**/kg of diet). The daily uptake of **ITZ15** was approximately 2.8 mg/day/mouse, equivalent to 78 mg/day/kg of mouse weight. Two other groups of mice were fed a low-fat diet (LFD) or HFD, throughout the 12-week study period, as lean and obese controls. In this experiment, no significant differences in body weight and glucose tolerance were observed between the drug-fed and obese control groups (data not shown). Given that **ITZ15** is a potent inhibitor of PTP1B *in vitro*, the absence of an *in vivo* effect might be due to low bioavailability. As a factor affecting absorption, distribution, metabolism, and excretion (ADME) properties, the logD value of **ITZ15** at pH 7.5 was predicted to be 2.69, which resides in the upper range of typical drugs under clinical use. Improvement of the ADME properties of **ITZ15** will require the study of additional properties of the compound such as its solubility, serum protein binging, and metabolic stability.

In summary, we synthesized a series of thiazolidinone derivatives and evaluated the inhibitory activity against PTP1B. Most of the compounds behaved as inhibitors of PTP1B, with the lowest IC_{50} of 1.4 μM observed with **ITZ15**. In contrast to the *in vitro* efficacy of **ITZ15** as a potent



Scheme 1. Synthesis of thiazolidinone derivatives, **ITZ1-18**. Reagents, conditions, and product yields: (a) CH_3I, *N*,*N*-diisopropyl ethylamine, EtOH, 4 h, 59%; (b) $BrCH_2CO_2Et$, K_2CO_3, acetone, reflux, 2 h, 94%; (c) $ArCH_2Br(Cl)$, K_2CO_3, DMF, 65-70 °C, 4 h, > 80%; (d) Zn/HCl, MeOH, H_2O, > 80%; (e) *i*PrOH, 65-70 °C, 3 h, 43%; (f) EtOH, reflux, 6 h, > 80%; (g) aq. NaOH, THF, reflux, 1.5 h, quantitative.

PTP1B inhibitor, its effect as an *in vivo* anti-obesity or hypoglycemic agent was not observed in this experiment.

Experimental

Methyl Rhodanine (B). A solution of rhodanine (1.332 g, 10 mmol), *N*,*N*-diisopropyl ethylamine (1.422 g, 11 mmol), and methyl iodide (1.6 g, 11 mmol) in ethanol (30 mL) was stirred at room temperature (RT). for 4 h. Aqueous workup followed by column chromatography afforded compound **B** (0.87 g, 59% yield).

4-Ethoxycarbonylmethyloxy-3-methoxybenzaldehyde (D). To a mixture of vanillin (0.152 g, 1 mmol) and anhydrous K_2CO_3 (0.207 g, 1.5 mmol) in dry acetone (6 mL) was added ethyl α-bromoacetate (0.251 g, 1.5 mmol) at RT. The mixture was heated to reflux for 4 h. Aqueous workup provided **D** (0.225 g, 94% yield) pure enough to be used for the next reaction without further purification.

General Procedure for the Synthesis of F6-18. A mixture

Table 1. Inhibitory effect of the compounds **ITZ1-18** against PTP1B

Compounds	IC_{50} (μM)[a]	Compounds	IC_{50} (μM)[a]
ITZ1	125 ± 7	**ITZ10**	5.0 ± 1.5
ITZ2	143 ± 36	**ITZ11**	29 ± 0
ITZ3	16 ± 2	**ITZ12**	42 ± 1
ITZ4	730 ± 38	**ITZ13**	36 ± 1
ITZ5	388 ± 12	**ITZ14**	2.4 ± 0.1
ITZ6	10 ± 1	**ITZ15**	1.4 ± 0.1
ITZ7	31 ± 4	**ITZ16**	3.5 ± 0.1
ITZ8	48 ± 6	**ITZ17**	1.7 ± 0.1
ITZ9	5.4 ± 0.5	**ITZ18**	226 ± 6

[a]IC_{50} values were determined by measuring the activities of PTP1B in the presence of various concentrations of the compounds. The values were obtained from 2 or more experiments and are shown as means ± standard deviations.

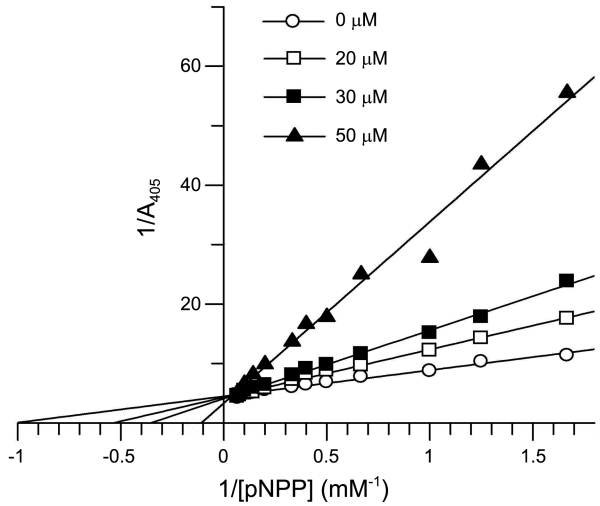


Figure 1. Lineweaver-Burk analysis for PTP1B catalyzed reactions in the presence of **ITZ15** at the concentrations specified in the inset.

Table 2. Inhibition of PTP1B and other PTPs by the compound **ITZ15**

Compound	IC_{50} (μM)				
	PTP1B	TCPTP	SHP1-cat[a]	YPTP1	VHR
ITZ15	1.4 ± 0.1	24 ± 2	5.7 ± 0.1	5.7 ± 0.1	47 ± 4

[a]Catalytic domain of SHP1

of *m*- or *p*-nitrophenol (2.0 mmol), K_2CO_3 (10 mmol), and derivatives of benzyl chloride or benzyl bromide (2.2 mmol) in dry DMF (4 mL) were heated at 65-70 ºC for 1-2 h. After aqueous workup, the crude product was purified by column chromatography.

General Procedure for G6-18. To a solution of **F6-18** (0.10 mmol) in 50% aqueous MeOH (25 mL) was added concentrated HCl (1.0 mL). Zinc powder (2.0 mmol) was then added with vigorous stirring. Additional concentrated HCl (2.0 mL) was added dropwise and the reaction was continued at RT for 30 min. Aqueous workup afforded **G6-18** in an essentially pure form in > 80% yield.

Compound H. A solution of **B** (1.50 g, 10 mmol) and **D** (2.38 g, 10 mmol) in 2-propanol (15 mL) was heated to 65-70 °C for 3 h. After aqueous workup, the residue was purified by recrystallization or column chromatography (1.58 g, 43% yield).

General Procedure for the Ethyl Esters of ITZ1-18 (I1-18). A solution of **H** (0.10 mmol) and **G1-18** (0.10 mmol) in ethanol (4.0 mL) was heated to reflux for 6 h. After evaporation of the solvent, the resulting solid was purified by column chromatography.

General Procedure for ITZ1-18. A solution of **I1-18** (0.052 mmol) in THF (1 mL) and 2 M aqueous NaOH (0.030 mL) was heated to reflux for 1.5 h. The reaction mixture was acidified by addition of 1 M HCl and THF was evaporated. The resulting solids were washed with H_2O and dried under high vacuum to be used as inhibitors without further purification.

PTP1B Assay. The enzyme activity of PTP1B and other PTPs was determined as described previously.[14,15] PTP1B was diluted before the experiment to 400 nM in enzyme dilution buffer (25 mM Hepes, 5.0 mM EDTA, 1.0 mM DTT, 1.0 mg/mL bovine serum albumin, pH 7.3). For a 50 μL reaction, 5.0 μL of inhibitor solution in DMSO was added to a mixture containing 5.0 μL enzyme, 10 μL buffer (250 mM Hepes, 25 mM EDTA, pH 7.0), and water (25 μL). After a 10 min preincubation period at 37 °C, 5.0 μL of *p*NPP solution (20 mM) was added and the reaction proceeded for 3 min at 37 °C. The reaction was then stopped by the addition of 0.5 M NaOH solution (0.95 mL) and the absorbance of the mixture at 405 nm was measured with a spectrophotometer. For the Lineweaver-Burk plot analysis, the enzyme reaction was started by addition of PTP1B to a buffer solution containing *p*NPP and inhibitor.

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