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Thiazolidinedione derivatives as PTP1B inhibitors with antihyperglycemic and antiobesity effects

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ABSTRACT

Benzylidene-2,4-thiazolidinedione derivatives with substitutions on the phenyl ring at the *ortho* or *para* positions of the thiazolidinedione (TZD) group were synthesized as PTP1B inhibitors with IC_{50} values in a low micromolar range. Compound **3e**, the lowest, bore an IC_{50} of 5.0 μM. In vivo efficacy of **3e** as an antiobesity and hypoglycemic agent was evaluated in a mouse model system. Significant improvement of glucose tolerance was observed. This compound also significantly suppressed weight gain and significantly improved blood parameters such as TG, total cholesterol and NEFA. Compound **3e** was also found to activate peroxisome proliferator-activated receptors (PPARs) indicating multiple mechanisms of action.

Thiazolidinedione (TZD) derivatives, also called glitazones, were developed in the early 1980s as drugs for type 2 diabetes. Among them, troglitazone, rosiglitazone, and pioglitazone were marketed in the late 1990s (Fig. 1).[1] These drugs are known to act by binding to PPARs.[2] Later, troglitazone was withdrawn from the market due to severe liver toxicity in several patients.[3] Rosiglitazone and pioglitazone are currently in clinical use.

Recently, several studies have demonstrated compounds containing scaffolds similar to TZD exhibit inhibitory effects against PTP1B and other enzymes (Fig. 1). Malamas et al. reported several azolidinediones, such as **A**, with low micromolar IC_{50} values against PTP1B and with glucose and insulin normalizing effect in *ob/ob* and *db/db* diabetic mouse models.[4] Maccari et al. found a modified thiazolidinedione, **B**, as a good inhibitor of aldose reductase, also involved in the complications of diabetes.[5] Soon after, the same group reported a similar class of compounds (e.g., **C**) having high binding affinity to the active site of PTP1B.[6] Similarly, Combs et al. reported isothiazolidinone-containing compounds, represented by **D** as potent inhibitors of PTP1B.[7] Rhodanine derivatives like **E** were also found to behave as inhibitors of a low molecular weight tyrosine phosphatase, PRL-3.[8,9]

Partial or complete disruption of the PTP1B gene in normal and diabetic mice resulted in resistance to weight gain and improved insulin responsiveness.[10–13] PTP1B inhibition or a reduction of its cellular abundance in mice resulted in similar consequences and, as such, provided the rationale for the therapeutic strategy for type 2 diabetes and obesity.[14] In a recent study, we identified novel PTP1B inhibitors by means of a computer-aided drug design protocol involving virtual screening.[15] One of those inhibitors (**6f**) was a TZD derivative inhibiting PTP1B with an IC_{50} value of 11 μM. In a subsequent experiment, marketed glitazones such as troglitazone, rosiglitazone and pioglitazone were found to behave as medium or low potency inhibitors of PTP1B with IC_{50} values of 55–400 μM. Marketed glitazones are known to exert their antihyperglycemic effects by binding PPAR. The glitazones are benzylidene-2,4-thiazolidinedione derivatives with substitutions on the phenyl ring at the *para* position of the TZD group. Most derivatives reported thus far are those with the major substituents on the central phenyl ring at the *para* position of the TZD group. Therefore, we synthesized TZD derivatives with the major substituent at the *ortho* (**3a–i**) as well as *para* (**6a–h**) positions of the TZD group and evaluated their inhibitory activity against PTP1B. The in vivo effects of the selected compound were then tested in a mouse model system.

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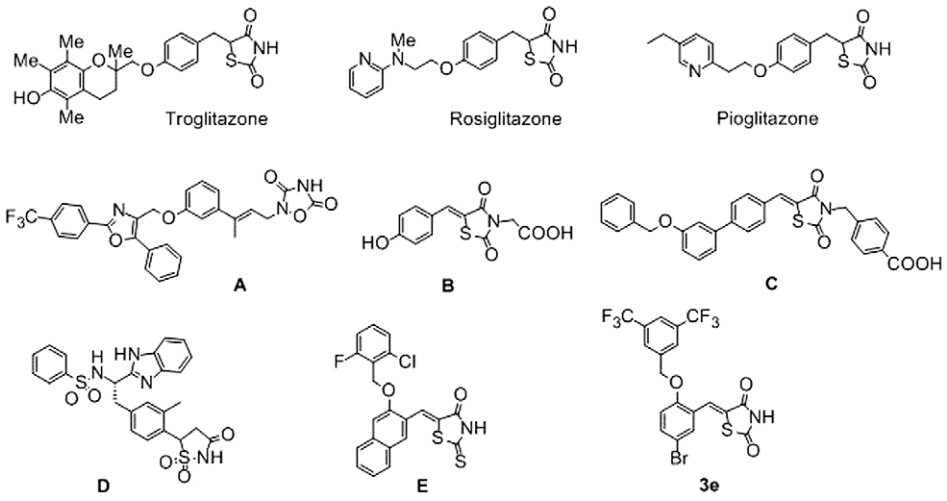


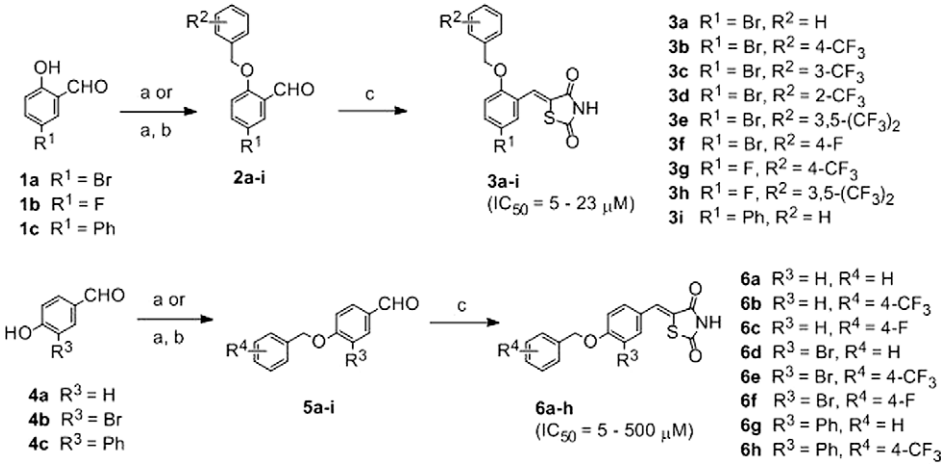
Figure 1. Marketed TZD derivatives and PTP1B inhibitors mentioned in this study.

The TZD derivatives were synthesized by condensation of TZD and the appropriate benzaldehydes in the presence of a piperidine catalyst (Scheme 1). Introduction of the phenyl group in **3i**, **6g**, and **6h** was achieved by a Suzuki coupling reaction. The compounds synthesized in this study inhibited PTP1B more potently compared to the marketed glitazones; IC_{50} values of 5–68 μM versus 55–400 μM. Of the two groups of synthesized compounds, **3a–i** and **6a–h**, the **3a–i** group with the major substituent *ortho* to the TZD group was more potent in PTP1B inhibition. Two compounds, **3e** and **6e**, one from each group, were the most potent, with an IC_{50} value of 5.0 μM (Table 1).

The inhibitory activity of **3e**[19] was evaluated against a broad range of PTPs, including TC-PTP, the catalytic domain of SHP-1 (SHP-1cat), the membrane proximal catalytic domain LAR (LAR-D1), and 2 microbial PTPs, YOP and YPTP1 (Table 2). Compound **3e** demonstrated 2.2-, >20-, and 3.7-fold selectivity over TC-PTP, LAR-D1, and YPTP1, but little selectivity over SHP-1cat and YOP.

To obtain a degree of energetic and structural insight into the inhibitory mechanism of **3e**, its binding mode in the active site of PTP1B was investigated by docking simulation. The calculated binding mode of **3e** is shown in Figure 2. In the docking simulation, the amidic nitrogen of the TZD moiety was assumed to be deprotonated because the acidity of the TZD proton had been found to be strong enough to permit its ionization constant ($pK_a \approx 6.74$) to be directly and accurately determined by conductivity measurements.[20] In the calculated PTP1B-**3e** complex, it is shown that the two aminocarbonyl oxygens of the inhibitor receive two hydrogen bonds from the side chain of Gln266 and backbone amidic group of Ser216. These hydrogen bonds are expected to play the role of anchor for binding of the inhibitor in the active site. It is also noteworthy that the deprotonated TZD moiety of **3e** resides in the vicinity of Cys215 and Arg221 at a distance within 4–5 Å. The proximity to the catalytic residues with the hydrogen-bond stabilizations indicates that the deprotonated TZD group may serve as an effective surrogate for the substrate phosphate group. Inhibitor **3e** can be further stabilized in the active site by the hydrophobic interactions of its aromatic rings with the nonpolar residues including Tyr46, Val49, Phe182, Ala217, and Ile218. Judging from the structural features in the calculated PTP1B-**3e** complex, inhibitor **3e** seems to inhibit the catalytic action of PTP1B by binding the active site through simultaneous establishment of the multiple hydrogen bonds and hydrophobic interactions.

In vivo efficacy of **3e** as an antiobesity and hypoglycemic agent was evaluated in a mouse model system (C57BL/6J Jms Slc, male).[23] Obesity and diabetes were induced in 16 mice by feeding HFD ad libitum for 8 wk. The HFD-fed mice were then separated into 2



Scheme 1. Reagent and conditions: (a) $RPhCH_2Cl$ or $RPhCH_2Br$, K_2CO_3, DMF or acetone, rt or reflux, 1–3 h; (b) $PhB(OH)_2$, $Pd(PPh_3)_4$, toluene, EtOH, Na_2CO_3, 80 °C; (c) 2,4-TZD, EtOH, piperidine, reflux, overnight.

Table 1
Inhibitory effect of TZD derivatives against PTP1B

Compound	IC_{50}[a] (μM)
Troglitazone	55 ± 4
Rosiglitazone	400 ± 70
Pioglitazone	220 ± 35
Ertiprotafib	1.4 ± 0.1[b]
3a	9.0 ± 0.3
3b	8.0 ± 1.0
3c	8.0 ± 1.0
3d	8.0 ± 0.4
3e	5.0 ± 0.1
3f	23 ± 3
3g	15 ± 1
3h	8.0 ± 1.0
3i	16 ± 1
6a	64 ± 3
6b	14 ± 2
6c	68 ± 4
6d	15 ± 1
6e	5.0 ± 0.4
6f	11 ± 1
6g	9.0 ± 0.4
6h	6.0 ± 1.0

[a] Values are the mean ± standard deviations of two or more experiments. PTP1B assay was performed as previously described using *p*-nitrophenyl phosphate as the substrate (2 mM in the reaction mixture).[16] IC_{50} values were determined by measuring the *p*NPP hydrolase activity in a range of different inhibitor concentrations. Kinetic data were analyzed using GRAFIT 5.0 program (Erithacus Software).
[b] Data reproduced from our previous publication.[17]

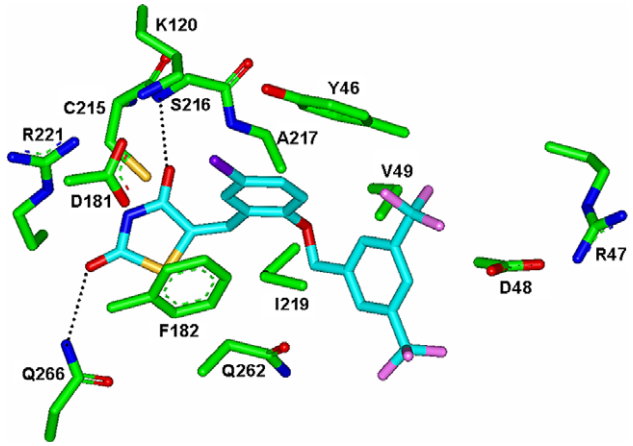


Figure 2. Calculated binding mode of **3e** in the PTP1B active site. Carbon atoms of the protein and ligand are indicated in green and cyan, respectively. Each dotted line indicates a hydrogen bond. All hydrogen atoms are omitted for visual clarity. AUTODOCK program[21] was used in docking simulation of **3e** in the PTP1B active site. The 3-D coordinates in the X-ray crystal structure of PTP1B in complex with an *N*-phenyloxamate inhibitor (PDB code: 1Q1M)[22] were used as the receptor model in the docking simulations. In the actual docking simulation of the ligand in the active site of PTP1B, the empirical AUTODOCK scoring function improved by the implementation of a new solvation model for a compound was employed.[15]

groups. Each group was then given a HFD or HFD plus **3e** for 4 wk. Compound **3e** was administered as a mixture with the food (2.0 g of **3e** per kg of diet). The daily uptake of **3e** was approximated at 4.8 mg/day/mouse, equivalent to 143 mg/day/kg of mouse weight. For the lean control group, LFD was fed throughout the test period. The body weight and food intake were recorded every third day during the 4 wk of drug feeding period. Compound **3e** significantly suppressed weight gain in diet-induced obese mice (Table 3). Drug feeding also significantly decreased feed efficiency, weight gain per calories of food intake, suggesting that the in vivo effect of **3e** was due to the increase in metabolic rate, not the decrease in food intake (Table 3). After the 4 wk compound-feeding period and 6 h of fasting, fasting blood glucose level of all the groups was measured (Fig. 3 and 0 min) and glucose tolerance test was performed by intraperitoneal injection of glucose. Tail-blood was taken at timed intervals up to 120 min. Feeding **3e** significantly improved glucose tolerance, but not fasting glucose levels, in the diet-induced obese/diabetic mice (Fig. 3). At termination of the glucose tolerance test, three mice groups were maintained on their own diet for 5 d and the test group on HFD plus **3e**. Mice were then killed after an overnight fast. Mice were anesthetized and blood samples collected by cardiac puncture for measurement of blood parameters. Significantly lower levels of total cholesterol, triglyceride, and NEFA were found in the serum of the **3e**-treated mice group compared to HFD control (Table 4). Liver, lung, kidney and white adipose tissue (epididymal and retroperitoneal) were excised and weighed. The mass of the fat pad of the drug-treated mice group was significantly lighter than HFD control group (Table 3). These observations are consistent with the decreased body weight gain. There was no significant difference in liver, lungs, and kidney weights between the HFD control and **3e**-treated mice groups. However, livers of **3e**-treated mice were found to be associated with dark-brownish spots not observed in the livers of other groups, suggesting unwanted side effects of the test compound **3e**. Further derivatization of **3e** to increase the inhibitory potency and to reduce the dose could help to avoid the undesired effects of the drug.

Compound **3e** shares a common scaffold with glitazones and, therefore, agonistic effects of **3e** to PPARs could be conceived. This possibility, however, has been ignored to the end of this study, because **3e** suppressed weight gain in this study and glitazones tend to cause weight gain in previous reports.[24] In contradict to these speculations, in initial experiments, **3e** was found to activate PPAR with potencies comparable to those of troglitazone in cell-based transactivation assays (data not shown). Part of the biological effects of **3e** observed in this study could be due to PPAR activation, and this issue is to be further studied. It is also worth to note that, as a PPAR agonist, **3e** is distinct in that the major substituent on the phenyl ring is at the *ortho* position of the TZD group. To the best of our knowledge, all of the benzylidene-2,4-thiazolidinedione

Table 2
Inhibition of PTP1B and other PTPs by compound **3e**[a]

Compound	IC_{50}[b] (μM) [K_i[c], μM]					
	PTP1B	TC-PTP	SHP-1cat	LAR-D1	YOP	YPTP1
3e	5.0 ± 0.1 [2.1]	16 ± 1 [4.5]	5.8 ± 0.4 [2.2]	>100	6.0 ± 0.1	18 ± 1 [7.7]

[a] The catalytic domain of SHP-1 (SHP-1cat) and YPTP1 were expressed in *E. coli* expression systems and purified as previously described.[16] LAR-D1 (membrane-proximal catalytic domain of LAR), TC-PTP and YOP were purchased from New England Biolabs, Inc. (Beverly, MA, USA). The assay condition was the same for all PTPs except the enzyme concentrations, which were 40 nM for PTP1B, 100 nM for SHP-1cat, 15 nM for YPTP1, 50 units (manufacturer's definition)/mL for YOP, and 33 units/mL for LAR-D1 and TC-PTP.
[b] Values are mean ± standard deviations of two or more experiments.
[c] K_i values were obtained using the relationship: $IC_{50} = K_i(1 + [S]/K_M)$.[18]

Table 3
Effect of **3e** on body weight and related parameters[a]

Mice group	Body weight gain (g)	Feed efficiency (wt gain/kcal × 100)	Epididymal fat (g)	Retroperitoneal fat (g)
HFD	4.88 ± 0.46	1.38 ± 0.11	1.87 ± 0.08	0.69 ± 0.06
HFD + **3e**	2.16 ± 0.29**	0.68 ± 0.09**	1.27 ± 0.17**	0.42 ± 0.07**
LFD	1.22 ± 0.54	0.59 ± 0.09	0.49 ± 0.04	0.11 ± 0.02

[a] The obese and lean control groups were fed high fat diet (HFD, D12451, New Brunswick, NJ, USA) or low fat diet (LFD, D12450B), containing 45% and 10% of the calories from fat, respectively, for 12 wk. The test group (HFD + **3e**) was fed a HFD for 8 wk, and then a HFD mixed with **3e** for 4 wk. Values are mean ± standard deviations; $n = 8$/group. Significance of the difference between the HFD group and **3e**-fed group was calculated by One-way ANOVA, where ** represents $p < 0.05$.

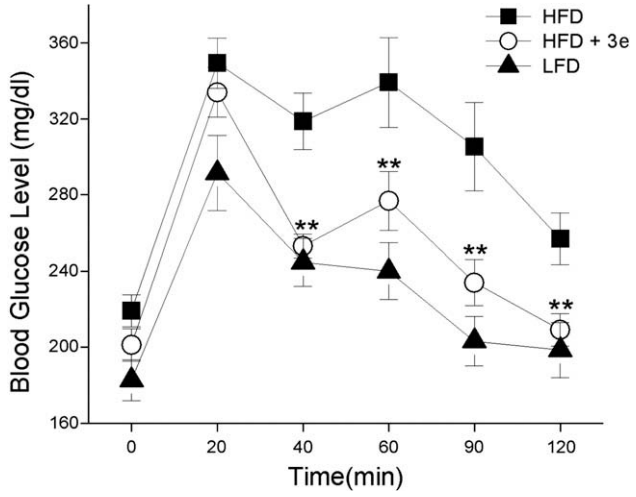


Figure 3. Glucose Tolerance Test. HFD + **3e** group were maintained on a HFD for 8 wk followed by HFD + **3e** for 4 wk. HFD and LFD groups were maintained on a HFD or LFD for a 12 wk period. Mice that fasted for 6 h were injected intraperitoneally with glucose (1.0 g glucose/kg body weight). Blood glucose levels were measured at the indicated times (mean ± standard deviation; $n = 8$/group) with a glucometer (Accu check active, Roche diagnostics, Ireland). Blood samples were collected directly from the tail. Significance of the difference between the HFD group and **3e**-fed group was calculated by One-way ANOVA, where ** represents $p < 0.05$.

Table 4
Effect of **3e** on blood parameters[a]

Mice group	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	NEFA (mM)
HFD	152 ± 6	124 ± 16	0.71 ± 0.22
HFD + **3e**	125 ± 7**	78 ± 10**	0.22 ± 0.02**
LFD	107 ± 5	65 ± 5	0.18 ± 0.01

[a] The levels of serum triglyceride and total cholesterol were measured using diagnostic kits TG E and T-Cho E (Wako Pure chemical Industries, Ltd Osaka, Japan), respectively. Serum concentration of non-esterified free fatty acids (NEFA) was determined using free fatty acids half-micro test kits (Roche Diagnostics Gmbh, Penzberg, Germany) following manufacturer's protocol with minor modifications. Values are mean ± standard deviations; $n = 8$/group. Significance of the difference between HFD group and **3e**-fed group was calculated by One-way ANOVA, where ** represents $p < 0.05$.

derivatives previously reported have a major substituent at the *para* position.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.020.

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19. *Spectral data for compound* **3e**: mp: 204–206 °C; EI-MS: *m/z* 526 [M^+]. IR (KBr, cm^{-1}): 3447, 3190, 3071, 1754, 1697, 1487, 1363, 1276, 1173, 1137. ^{1}H NMR (DMSO-d_6): δ 12.70 (br s, 1H, NH), 8.19 (s, 2H), 8.11 (s, 1H), 7.93 (s, 1H), 7.66 (dd, J = 8.8 and 2.6 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.23 (d, J = 9.2 Hz, 1H), 5.43 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 167.40, 166.94, 155.69, 139.77, 134.34, 130.63, 130.54, 130.31, 128.20, 125.87, 124.61, 124.45, 121.89, 115.61, 113.02, 68.77.
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23. *Mouse experiment*: Twenty-four mice (4-wk old, 17–19 g, Japan SLC, Haruno Breeding branch, Hamamatsu, Japan) were individually housed and maintained in a 12 h dark-light cycle at 22 ± 2 °C. They were fed ad libitum. After acclimatization for 1 wk by feeding a LFD, the mice were divided into two groups; LFD (8 mice) and HFD (16 mice) groups. The LFD-fed lean control group was maintained on a LFD throughout the study. The HFD group of mice was provided with a HFD for the first 8 wk of the study for development of obesity/diabetes. Then, they were separated into two groups with the same mean body weight. Each group was then given a HFD or HFD plus **3e** for 4 wk. Compound **3e** was administered as a mixture with the food (2.0 g of **3e** per kg of diet). For this, **3e** (400 mg) was dissolved in 0.5 mL DMSO and homogenized with 1% aqueous solution of polyglycerol fatty acid ester (19.5 mL, Mitsubishi-kagaku foods, Tokyo, Japan). The homogeneous suspension was mixed thoroughly in a

powder form of HFD (200 g) and kneaded into a dough. Body weight and food intake were recorded every third day during the drug feeding period. At the end of the drug feeding period, glucose tolerance tests were performed. After a 5 d recovery period on their own diet (the test group on HFD plus **3e**), mice were fasted overnight and blood collected by cardiac puncture under secobarbital anesthesia. Liver, lung, kidney, and white adipose tissue were excised and weighed.

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