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Derivatives of 1,4-bis(3-hydroxycarbonyl-4-hydroxyl)styrylbenzene as PTP1B inhibitors with hypoglycemic activity

Suja Shrestha [†,‡], Bharat Raj Bhattarai [‡], Bhooshan Kafle, Keun-Hyeung Lee, Hyeongjin Cho [*]

Department of Chemistry and Institute of Molecular Cell Biology, Inha University, Yonghyun-dong, Nam-ku, Incheon 402-751, Republic of Korea

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ABSTRACT

Disalicylic acid derivatives with stilbene and bis-styrylbenzene skeleton were synthesized as PTP1B inhibitors. The most potent in this series exhibited a submicromolar IC_{50} value. One of the compounds **7b** was tested in an animal model for its efficacy as an anti-diabetic or an anti-obesity agent. In feeding compound **7b** to diet-induced obese mice, no significant differences in weight gain and food consumption were observed between the drug-treated and the obese control mice. However, **7b** significantly lowered the fasting glucose level and improved the glucose tolerance in the obesity-induced diabetic mice.

1. Introduction

Defects on cellular signal transduction pathways often result in disease states and the recuperation of the defective signaling could be a strategy to reverse it.[1] Type II diabetes, a metabolic syndrome characterized by hyperglycemia, often results from the impairment in insulin signaling.[2] Early events in insulin signaling include autophosphorylation and activation of the tyrosine kinase activity of the insulin receptor (IR) and subsequent phosphorylation on tyrosine residues of downstream signaling proteins including insulin receptor substrate proteins (IRS1–IRS4), Shc, Gab-1, Cbl, and APS.[3,4] Augmentation of the phosphorylation level of IR and/or downstream signaling proteins by inhibition of relevant protein tyrosine phosphatases (PTPs) could be a strategy to enhance IR signaling and to treat diabetes.[5] Biochemical experiments have identified PTP1B and TC-PTP as possible regulators of the phosphorylation level of human IR.[6] These enzymes are the closest homologues of each other with an amino acid homology of 94% at the active site.[7,8] Transgenic mice lacking PTP1B or TC-PTP have been created in different laboratories.[9,10] PTP1B-depleted mice were healthy without worrisome traits such as enhancement of mitogenic signaling.[9] Upon feeding of a high-fat-diet, the $PTP1B^{-/-}$ and $PTP1B^{+/-}$ mice were resistant to weight gain and retained insulin sensitivity in contrast to the wild-type mice which gained weight and became insulin resistant.[9] Ablation of TC-PTP was lethal in mice, but heterozygous $TC\text{-}PTP^{+/-}$ mice survived healthy.[10] TC-PTP is also known to be involved in the regulation of insulin signaling.[11,12] These results, together with other supporting evidences, suggested that the inhibition of PTP1B, with limited inhibition of TC-PTP, could be a strategy for the treatment of diabetes and/or obesity.[13] First-in-class drug targeting PTP1B is yet to be developed and intensive research is underway to develop a potent and selective PTP1B inhibitor with hypoglycemic and/or anti-obesity effect. Recently, a selective PTP1B inhibitor, trodusquemine, proceeded to phase I clinical trial with promising preclinical results as both an appetite suppressant and a hypoglycemic and hypocholestrolemic agent.

We have recently reported methylenedisalicylic acid derivatives, **1** and **2**, as PTP1B inhibitors that suppressed the increase in body weight and adipose mass (Fig. 1).[14,15] The compounds also prevented increases in the plasma triglyceride, cholesterol, and nonesterified fatty acid concentrations in a mouse model system. It is interesting to note that salicylic acid is an old therapy for diabetes and behaves as a weak, competitive inhibitor of PTP1B with an inhibition constant of 19.4 mM.[16] The stilbene skeletons, found in natural products such as resveratrol **3** and pterostilbene, are of particular interest to chemists and biologists because of their wide range of biological activities such as anticancer-promoting activity, antioxidant, and antidiabetic properties.[17–21] Disalicylic acid derivatives with bis-styrylbenzene backbone are precedent in literature.[22–24] X-34 [1,4-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene] and its analogs have been used as imaging agents for the diagnosis of Alzheimer's disease as they are known to penetrate the blood-brain barrier and bind amyloid β peptide deposits.[23] To

* Corresponding author. Tel.: +82 32 860 7683; fax: +82 32 867 5604.
E-mail address: hcho@inha.ac.kr (H. Cho).
† Present address: Central Department of Chemistry, Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal.
‡ These authors contributed equally to this work.

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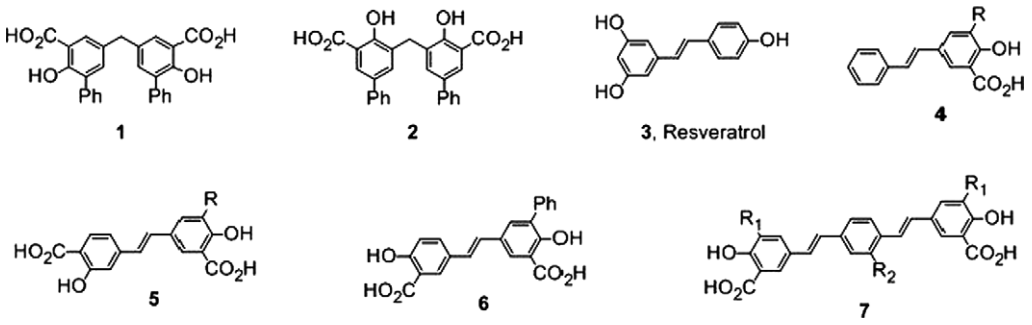


Figure 1. Structures of compounds mentioned or synthesized in this study.

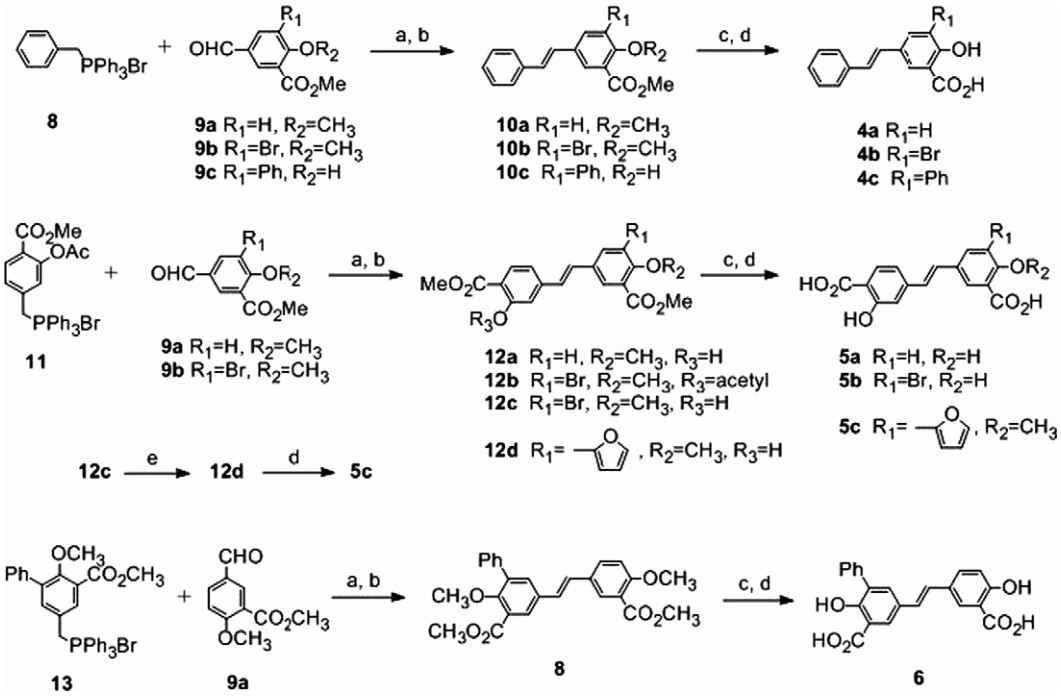
further investigate the salicylic acid moiety as a pharmacophore for the inhibition of PTP1B, we designed a series of mono- and disalicylic acid derivatives of the stilbene and bis-styrylbenzene skeletons. Among the potent PTP1B inhibitors in this study, compound **7b** was tested in an animal model for its efficacy as an anti-diabetic and/or an anti-obesity agent.

2. Results and discussion

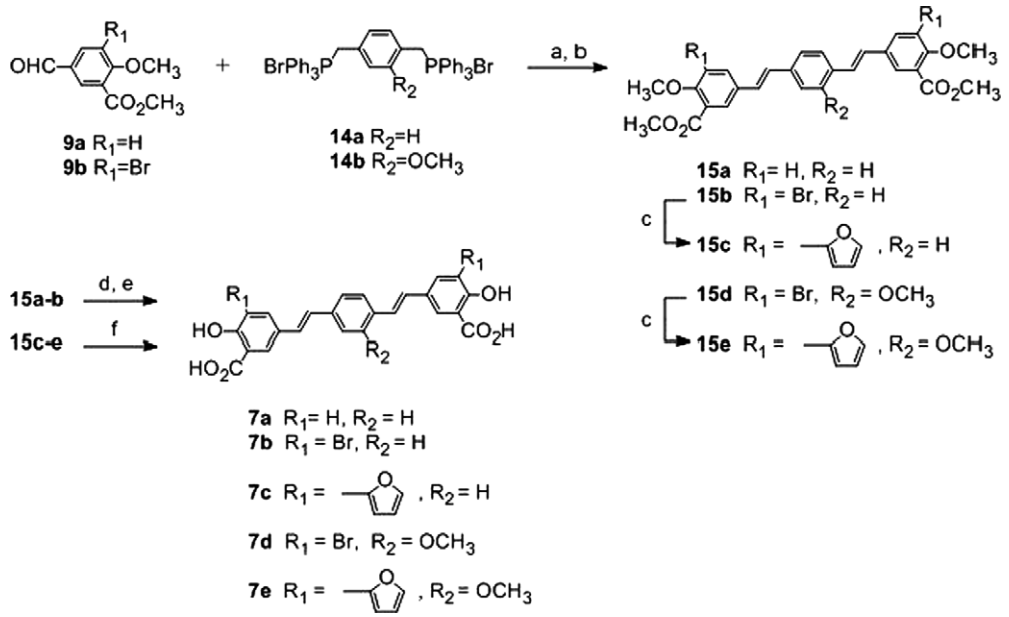
2.1. Chemical synthesis

Construction of the stilbene and bis-styrylbenzene skeletons of compound series **4**–**7** was accomplished by the Wittig reaction between appropriate aldehydes and corresponding phosphonium salts (Schemes 1 and 2).[25] The reaction produced a mixture of *E*/*Z* products, which was converted to (*E*)-geometrical isomer by refluxing overnight with catalytic amounts of I_2 in heptane.[26] The series **7** of compounds was synthesized in a similar strategy reported for the synthesis of 1-bromo-2,5-*bis*-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB, **7** with R_1 = H, R_2 = Br) (Scheme 2).[27,28] The Wittig reagent for the synthesis of series **7** could be either the *bis*(diethylphosphonate) or *bis*(triphenylphosphonium) salt. In the reported synthesis of BSB or 1-fluoro-2,5-*bis*-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (FSB, **7** with R_1 = H, R_2 = F), *bis*(diethylphosphonate) is preferred because it produced only *trans-trans* (*E*,*E*) isomer and, therefore, does not require the isomerization step. In this study, we used triphenylphosphonium salt as a Wittig reagent and then isomerized the mixtures of 4 possible isomers [(*E*,*E*), (*Z*,*Z*), (*E*,*Z*), (*Z*,*E*)] to (*E*,*E*) isomer to confirm the production of isomerically pure compound.

Condensation of the appropriate aromatic aldehydes (**9a**–**c**) with the phosphonium salts (**8**, **11**) afforded **4a**–**c** and **5a**–**b** (Scheme 1). The reaction of **9a** and **11** produced deacetylated product **12a**. Conversely, the reaction of **9b** and **11** resulted in a 2:1 mixture of acetylated and deacetylated products (**12b** and **12c**). The mixture was subjected to demethylation and ester hydrolysis to obtain **5b**. Compound **5c** was obtained by Stille coupling between **12c** and 2-(tributylstannyl)furan. Compound **6** was obtained in a similar way starting from the Wittig reaction with **13** and **9a** while condensation of 2 equivalents of the appropriate aldehydes (**9a** and **9b**) with the diphosphonium salts (**14a** and **14b**) afforded **15a**, **15b**, and **15d** (Scheme 2).[27,29] Coupling of Wittig reaction products **15b** and **15d** with 2-(tributylstannyl)furan under Stille coupling conditions, followed by a deprotection step, gave **7c** and

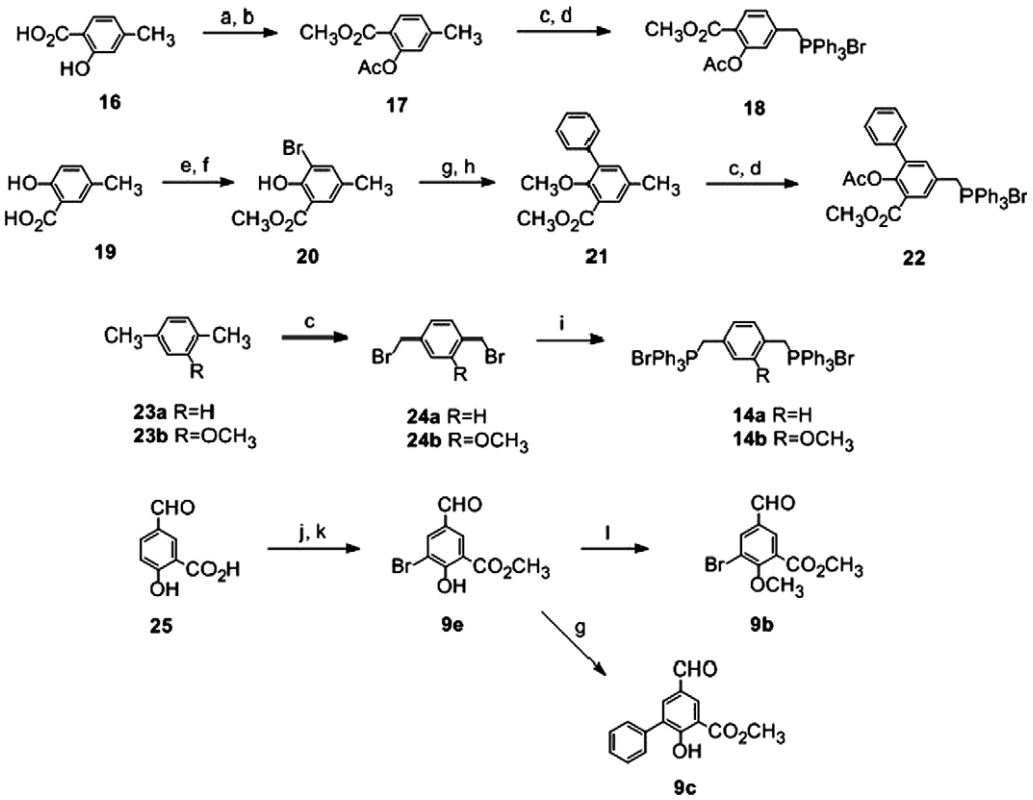


Scheme 1. Reagents and conditions for the synthesis of mono- and disalicylic acid derivatives with stilbene skeleton: (a) LDA, THF, −75 °C → rt, overnight; (b) I_2, heptane, reflux, overnight; (c) BBr_3, CH_2Cl_2, −70 °C → rt, 4 h; (d) KOH, EtOH, reflux, overnight; (e) 2-(tributylstannyl)furan, $Pd(PPh_3)_2Cl_2$, dioxane, reflux, 24 h.



Scheme 2. Reagents and conditions for the synthesis of **7a**–**e**: (a) LDA, THF, −75 °C → rt, overnight; (b) I_2, heptane, reflux, overnight; (c) 2-(tributylstannyl)furan, $Pd(PPh_3)_2Cl_2$, dioxane, reflux, 24 h; (d) BBr_3, CH_2Cl_2, −70 °C → rt, 4 h; (e) KOH, EtOH, reflux, overnight; (f) PhSH, K_2CO_3, NMP, 190 °C, 40 min.

7e (Scheme 2).[30,31] Deprotection of the methoxy or methoxycarbonyl derivatives **10a**–**c**, **12a**–**c**, **8**, and **15a**–**b** was accomplished with BBr_3 in CH_2Cl_2 followed by hydrolysis with aqueous KOH (Schemes 1 and 2) to give the corresponding final products **4a**–**c**, **5a**–**d**, and **7a**–**b**. Thiophenol in NMP was used for the removal of both protecting groups in **15c**–**e** in a single step to obtain **7c**–**e** (Scheme 2). Scheme 3 illustrates the synthesis of phosphonium salts and aldehyde precursors for the Wittig reaction.



Scheme 3. Reagents and conditions for the generation of phosphonium salt precursors and aldehyde precursors: (a) Ac_2O, H_3PO_4, 80 °C, 30 min; (b) CH_3I, K_2CO_3, acetone, reflux, 1 h; (c) NBS, benzoyl peroxide, benzene, reflux, 3 h; (d) PPh_3, benzene, rt, 18 h; (e) MeOH, H_2SO_4, reflux, 20 h; (f) NBS, CH_3CN, rt, 1 h; (g) phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, 80 °C, overnight; (h) Me_2SO_4, K_2CO_3, acetone, reflux, 22 h; (i) PPh_3, DMF, reflux, 3 h; (j) MeOH, H_2SO_4, reflux, overnight; (k) Br_2, CH_2Cl_2, 0 °C, 3 h; (l) Me_2SO_4, K_2CO_3, acetone, reflux, 3 h.

2.2. In vitro enzyme assays

The compounds synthesized in this study were tested for their ability to inhibit PTP1B, using *p*-nitrophenyl phosphate (*p*NPP) as the substrate.[15] PTP1B and the test compounds were preincubated for 10 min prior to the initiation of the enzyme reaction by addition of the substrate. The half maximal inhibitory concentration (IC_{50}) values of the inhibitors were determined by measuring the PTPase activity in a range of different inhibitor concentrations. The IC_{50} values for the compounds determined under these conditions are shown in Table 1. Resveratrol (**3**) displayed an IC_{50} value of more than 1.0 mM against PTP1B. Introduction of the 2-carboxy group, **4a** – **4c**, showed a positive impact on inhibitory potency. Compound **4c** had the lowest IC_{50} value (13 μM) among substituted stilbene derivatives. Comparing IC_{50} values of compounds **4b** and **4f**, phenyl substitution at the *ortho* position of the hydroxyl group might help to increase the enzyme–inhibitor interaction compared to bromine. Disalicylic acids with stilbene skeleton, **5a**–**c**, were less potent compared to monosalicylic acid derivatives, **4a**–**c**. As presented in Table 1, the disalicylic acid derivatives with *bis*-styrylbenzene backbone, **7a**–**e**, displayed greater potency (IC_{50} values of 0.53 ∼ 6.0 μM) than the mono- and disalicylates with stilbene backbone, **4a**–**c** and **5a**–**c**. Among series **7**, the bromine-substituted **7b** and 2-furyl-substituted **7c** exhibited 2- and 10-fold lower IC_{50} values, respectively, compared to compound **7a** (IC_{50} = 5.0 μM). Methoxy substitution at the central aromatic ring decreased potency; **7d** and **7e** exhibited 2- and 8-fold higher IC_{50} values compared to **7b** and **7c**, respectively. The most potent inhibitor was compound **7c**, with a nanomolar IC_{50} value of 0.53 μM.

The inhibitory activity of selected compounds was also evaluated against a broad range of PTPs including TC-PTP, membrane proximal catalytic domain LAR (LAR-D1), the catalytic domain of SHP-1 (SHP-1cat), and 2 microbial PTPs, YOP and YPTP1 (Table 1). In general, these compounds displayed remarkable selectivity for PTP1B against LAR-D1 and moderate selectivity against TC-PTP and SHP-1. Additionally, these compounds showed some selectivity against microbial PTPases, YOP and YPTP1. The most potent PTP1B inhibitor, **7c**, demonstrated 16-, 12-, and >400-fold selectivity over TC-PTP, SHP-1cat and LAR-D1, respectively, and showed a >10-fold selectivity over microbial PTPs. Compound **7b** was the second most potent PTP1B inhibitor with an IC_{50} value of 3.0 μM and was used for the mouse experiment to examine the metabolic effects. This compound exhibited 12-fold, 5-fold, and >100-fold selectivity over TC-PTP, SHP-1cat, and LAR-D1, respectively, and showed a 3-4-fold selectivity over microbial PTPs.

2.3. Mouse experiment

In vivo efficacy of **7b** as a hypoglycemic and/or anti-obesity agent was evaluated in a mouse model system (C57BL/6J Jms Slc male).[15] Even though **7c** was the most potent among the PTP1B inhibitors in this study, **7b** was chosen for animal experiment for the convenience in synthesis. Twenty-four mice (5 weeks old after acclimatization for 1 week) were fed with either LFD or HFD ad libitum for 8 week. The LFD-fed lean control mice gained less body weight compared to the HFD-fed obese control group. The HFD-fed mice were separated into 2 groups. Each group was then given HFD or HFD plus **7b** for 4 weeks. Compound **7b** was administered as a mixture with the food (2.0 g of **7b** per kg of diet). The daily uptake of **7b** was approximated as 6.0 mg/day/mouse, equivalent to 160 mg/day/kg of mouse weight. For the lean control group, LFD was fed throughout the test period.

The feeding of **7b** did not significantly suppress adiposity in diet-induced obese (DIO) mice, which is clear from the comparison of body weight and fat pad (epididymal and retroperitoneal) weights between compound-treated and obese control mice ($p > 0.05$, data not shown). Food consumption was not significantly different between the **7b**-treated and obese control mice groups ($p > 0.05$, data not shown). There was no significant difference in the serum triacyl glycerol (TG), total cholesterol, and nonesterified fatty acids (NEFA) between **7b**-treated and obese control group ($p > 0.05$, data not shown). All the mice showed similar physical behavior. No overt toxicity was observed in the body organs such as liver, kidney, and lungs, with no significant difference in the weight of these organs in the three different groups ($p > 0.05$, data not shown).

Glucose metabolism in mice was examined after a 27 days drug-treatment period. Fasting glucose levels were checked after fasting for 6 h starting from beginning of light cycle with the results shown in Figure 2 (values at zero time point). The **7b**-treated mice exhibited fasting glucose levels significantly lower than the obese control group. Glucose tolerance was checked right after the measurement of the fasting glucose level. When glucose (1 g/kg of body weight) was injected intraperitoneally, the normaliza-

Table 1
Inhibitory effect of the compounds on PTP1B and other PTPs

Compound	IC_{50}[a] (μM)					
	PTP1B	TC-PTP	LAR-D1	SHP-1 cat	YOP	YPTP1
1	20 ± 1[b]	291 ± 9[b]	>2000[b]	51 ± 2[b]		234 ± 11[b]
2	19 ± 1[c]	156 ± 5[c]	>2000[c]	37 ± 3[c]		86 ± 2[c]
3	>1000					
4a	34 ± 4	234 ± 13	>2000	126 ± 10	61 ± 4	114 ± 5
4b	30 ± 3	296 ± 8	>1000	140 ± 9	91 ± 4	153 ± 6
4c	13 ± 2	173 ± 10	>1000	78 ± 6	44 ± 2	59 ± 3
5a	32 ± 5	567 ± 29	>2000	231 ± 24	175 ± 6	370 ± 27
5b	64 ± 11					
5c	194 ± 18					
6	125 ± 16					
7a	5.0 ± 0.4	27 ± 0	413 ± 22	19 ± 0	13 ± 1	20 ± 1
7b	3.0 ± 0.2	37 ± 2	>300	15 ± 1	10 ± 0	13 ± 0
7c[d]	0.53 ± 0.06	8.0 ± 0.4	>200	6.0 ± 0.2	5.0 ± 0.2	7.0 ± 0.5
7d	6.0 ± 0.4	32 ± 1	420 ± 37	10 ± 1	12 ± 1	21 ± 1
7e	4.0 ± 0.4	11 ± 0	162 ± 16	6.0 ± 0.1	5.0 ± 0.2	10 ± 1

[a] Values are means ± standard deviations of two or more experiments.
[b] Data reproduced from our previous publication.[14]
[c] Data reproduced from our previous publication.[15]
[d] Compound **7c** exhibited reversible slow-binding mode of inhibition against PTP1B (data not shown). Lineweaver–Burk plot analysis was not performed due to the limited solubility of **7c** in the assay mixture.

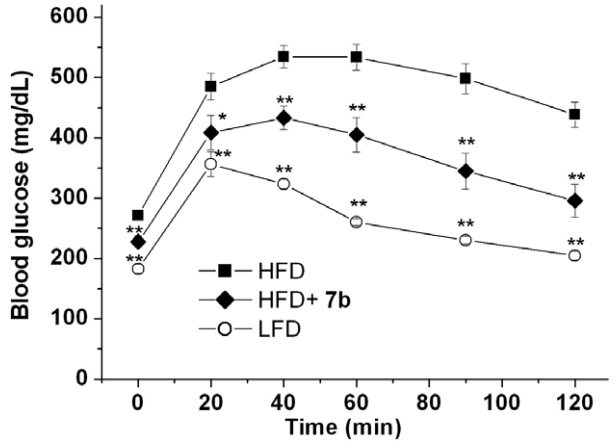


Figure 2. Glucose tolerance test result. Mice were fasted for 6 h starting from the beginning of the light cycle. At 0 time point, blood was withdrawn from the tail for the measurement of base line glucose level (fasting glucose level). Immediately thereafter, 1 g glucose/kg body weight was injected intraperitoneally. Blood glucose was measured at several time points. All values are means ± SEM; $n = 7$/group. Significance was calculated by one-way ANOVA, where * represents $p < 0.05$ and ** represents $p < 0.005$.

tion of glucose concentration was significantly faster in the **7b**-treated mice compared to obese control mice (Fig. 2).

3. Conclusion

Disalicylic acid derivatives with stilbene and *bis*-styrylbenzene skeletons were potent inhibitors of PTP1B with the most potent compound, **7c**, showing a submicromolar IC_{50} value. The *bis*-styrylbenzene derivatives were generally more potent inhibitors than the stilbene derivatives. Compound **7b** was the second most potent inhibitor among the series. In a mouse experiment with **7b**, no significant differences in weight gain and food consumption were observed between the drug-treated mice and the obese/diabetic control mice. Parameters such as TG, total cholesterol, and NEFA in the serum of **7b**-treated mice were also not significantly different from the obese/diabetic control mice. However, **7b** significantly lowered the fasting glucose level and improved the glucose tolerance in the obesity-induced diabetic mice. After extra glucose loading of **7b**-treated mice, the blood glucose level declined more rapidly than the obese/diabetic control group, thus **7b** may be a potential lead for the generation of a therapy for type 2 diabetes.

4. Experimental

4.1. Chemical synthesis

4.1.1. Materials

Commercial reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) and TCI (Tokyo, Japan). Most chemicals and solvents were of analytical grade and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) using precoated silica gel plates (Silica gel 60 F_{254}, Merck), and spots were visualized under UV light (254 nm). Column chromatography was carried out using silica gel 60 AF-254 (0.063–0.200 mm, Merck). Melting points (uncorrected) were determined on a MEL-TEMP electrothermal apparatus. ^1H and ^{13}C NMR spectra were recorded on Varian Gemini 2000 (200 MHz) and Varian Inova 400 (100 MHz) spectrometers, respectively. Chemical shifts (δ) are expressed in parts per million relative to tetramethylsilane, which was used as an internal standard, coupling constants (J) are in hertz (Hz), and the signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet.

4.1.2. Synthesis of 4a–c, 5a–c, and 6

4.1.2.1. General procedure for Wittig reaction. To a solution of appropriate phosphonium salts (2.3 mmol) in dry THF (16 mL) under N_2 was added lithium diisopropylamine (1.5 M in cyclohexane, 2.4 mL, 3.6 mmol) dropwise at −75 °C. After 1 h at 0 °C, the reaction flask was cooled again to −75 °C, and the corresponding aldehydes (2.3 mmol) in THF (6 mL) were added dropwise. The resulting yellow suspension was allowed to stir at −75 °C for 1 h and then at room temperature overnight. The reaction was quenched by pouring the reaction mixture into 25 mL of water. The aqueous portion was extracted with EtOAc (50 mL × 3), and the combined organics were washed with water (50 mL × 2) and brine (50 mL) successively, and dried (Na_2SO_4). The crude product obtained after concentration was subjected to column chromatography to obtain an isomeric mixture. The isomeric mixture was refluxed with a few milligrams of iodine in heptane (6 mL) overnight. The reaction was quenched by the addition of water (5 mL) and sodium sulfite (35 mg). After 5 min of stirring, the mixture was extracted with EtOAc (15 mL × 3). The combined organic extracts were washed with water (15 mL × 2) and brine (15 mL) successively, and dried over Na_2SO_4. Evaporation of solvent under reduced pressure gave the corresponding products, **10a–c**, **12a–b** and **8** in *E*-geometry. Stille coupling of **12c** with 2-(tributylstannyl)furan gave **12d**.[30,31]

4.1.2.2. Methyl 5-[(*E*)-2-Phenylvinyl]-2-methoxybenzoate (10a). Yield 282 mg (44%), light yellow solid, m.p. 87 °C; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.98 (d, $J = 2.2$ Hz, 1H), 7.63–7.58 (dd, $J = 2.2$, 8.4 Hz, 1H), 7.52 (d, $J = 8.4$ Hz, 2H), 7.39–7.23 (m, 3H), 7.04 (d, $J = 16.6$ Hz, 2H), 6.99 (d, $J = 8.8$ Hz, 1H), 3.93 (s, 6H).

4.1.2.3. Methyl 5-[(*E*)-2-Phenylvinyl]-3-bromo-2-methoxybenzoate (10b). Yield 438 mg (57%), brown oil, ^1H NMR ($CDCl_3$, 200 MHz): δ 7.87 (s, 2H), 7.51–7.28 (m, 5H), 7.12 (d, $J = 16.4$ Hz, 1H), 7.01 (d, $J = 16.6$ Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H).

4.1.2.4. Methyl 5-[(*E*)-2-Phenylvinyl]-2-hydroxybiphenyl-3-carboxylate (10c). Yield 295 mg (39%), yellow oil, ^1H NMR ($CDCl_3$, 200 MHz): δ 11.31 (s, 1H), 7.96 (d, $J = 2.2$ Hz, 1H), 7.69 (d, $J = 2.4$ Hz, 1H), 7.63–7.56 (m, 2H), 7.49–7.21 (m, 8H), 7.02 (d, $J = 16.2$ Hz, 1H), 7.01 (d, $J = 16.2$ Hz, 1H), 3.96 (s, 3H).

4.1.2.5. Methyl 4-[(*E*)-2-(3-Methoxycarbonyl-4-methoxyphenyl)-vinyl]-2-hydroxybenzoate (12a). Yield 244 mg (31%), yellowish white solid, m.p. 123 °C; ^1H NMR ($CDCl_3$, 200 MHz): δ 10.78 (s, 1H, OH), 7.97 (d, $J = 1.8$ Hz, 1H), 7.80 (d, $J = 8$ Hz, 1H), 7.62–7.57 (dd, $J = 1.8$, 8.4 Hz, 1H), 7.16 (d, $J = 16.6$ Hz, 1H), 7.05–6.95 (m, 3H), 6.88 (d, $J = 16$ Hz, 1H), 3.92 (s, 9H).

4.1.2.6. Methyl 4-[(*E*)-2-(5-Bromo-3-methoxycarbonyl-4-methoxyphenyl)vinyl]-2-acetoxybenzoate (12b). Yield 681 mg (70%), brown solid, m.p. 92–94 °C; ^1H NMR ($CDCl_3$, 200 MHz): δ 8.05 (d, $J = 8.2$ Hz, 1H), 7.88 (d, $J = 2.2$ Hz, 1H), 7.87 (d, $J = 2.2$ Hz, 1H), 7.43-7.38 (dd, $J = 1.4$, 8.4 Hz, 1H), 7.23 (d, $J = 1.4$ Hz, 1H), 7.06 (d, $J = 16.6$ Hz, 1H), 7.05 (d, $J = 16.6$ Hz, 1H), 3.96 (s, 6 H), 3.95 (s, 3H), 2.38 (s, 3H).

4.1.2.7. 5-[(*E*)-2-(4-Methoxycarbonyl-5-hydroxyphenyl)vinyl]-3-(furan-2-yl)-2-methoxybenzoic acid methyl ester (12d). To a suspension of $Pd(PPh_3)_2Cl_2$ (6 mg, 9 μmol) in anhydrous 1,4-dioxane (0.2 mL), **12c** (120 mg, 0.29 mmol) in 1,4-dioxane (2.0 mL) and 2-(tributylstannyl)furan (0.14 mL, 0.43 mmol) was added successively by syringe. After heating with reflux for 17 h, the reaction was quenched by addition of 10% aq. NH_4OH solution (2 mL), and the resulting mixture was extracted with ethyl acetate (30 mL × 3). The organic extracts were combined, washed with water (20 mL × 2) and brine (20 mL) successively, dried (Na_2SO_4),

and concentrated by rotary evaporator to obtain brown oil. Column chromatographic purification (hexane/EtOAc 4:1, R_f = 0.3) afforded **12d** (38 mg): yield (33%), yellow oil, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 10.79 (s, 1H), 8.14 (d, J = 2.2 Hz, 1H), 7.86 (d, J = 2.6 Hz, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 1.8 Hz, 1H), 7.23 (d, J = 16.4 Hz, 2H), 7.11–7.01 (m, 3H), 6.55 (m, 1H), 3.98 (s, 3H), 3.95 (s, 3H), 3.85 (s, 3H).

4.1.2.8. Methyl 5-[(*E*)-2-(3-Methoxycarbonyl-4-methoxyphenyl)-vinyl]-2-methoxybiphenyl-3-carboxylate (8). Yield 271 mg (27%), brown oil, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.97 (d, J = 2.2 Hz, 1H), 7.88 (d, J = 2.4 Hz, 1H), 7.62–7.57 (m, 4H), 7.48–7.38 (m, 3H), 7.00 (d, J = 8.8 Hz, 1H), 6.96 (d, J = 13 Hz, 2H), 3.97 (s, 3H), 3.93 (s, 3H), 3.92 (s, 3H), 3.49 (s, 3H).

4.1.2.9. General procedure for the deprotection of hydroxyl and carboxyl groups to obtain 4a–c, 5a–c, and 6. BBr_3 (1.0 M in CH_2Cl_2, 2.1 mmol) was added to a stirred solution of stilbene derivatives (1 mmol) in dry CH_2Cl_2 (10 ml) under a N_2 over a period of 5 min while cooling the reaction mixture at −70 °C. The reaction mixture was allowed to stir at room temperature for 1–8 h. Then the reaction mixture was cooled in ice-water bath and quenched with 1.0 M HCl (4 ml). The precipitate formed was dissolved in EtOAc (30 mL), and the combined organic layer was washed with water (20 mL × 2), brine (20 mL), and dried (Na_2SO_4). After evaporation of the solvent under reduced pressure, the brownish solid was dissolved in 95% EtOH (20 mL), and KOH (9.2 mmol) was added in solid form. The reaction mixture was heated to reflux for 2 h. The cooled mixture was acidified with 1.0 M HCl (16 mL). The white precipitate was dissolved in EtOAc (20 mL) and the aqueous layer was extracted with EtOAc (20 mL). The combined organics were washed with water (2 × 15 mL) and brine (15 mL) successively, dried (Na_2SO_4), and concentrated to obtain a yellowish white solid.

4.1.2.10. 5-[(*E*)-2-Phenylvinyl]-2-hydroxybenzoic acid (4a). Yield 181 mg (72%), yellowish white solid, m.p. 216 °C; ^{1}H NMR (DMSO-d_6, 200 MHz): δ 8.03 (d, J = 2.2 Hz, 1H), 7.89–7.83 (dd, J = 2.2, 8.4 Hz, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.44–7.28 (m, 3H); 7.25 (d, J = 16.6 Hz, 1H), 7.20 (d, J = 16.6 Hz, 1H), 7.05 (d, J = 8.6 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ 171.76 (CO_2H), 160.64, 137.14, 133.09, 128.64, 128.49, 128.47, 128.35, 127.35, 127.26, 126.86, 126.29, 117.64, 113.11.

4.1.2.11. 5-[(*E*)-2-Phenylvinyl]-3-bromo-2-hydroxybenzoic acid (4b). Yield 238 mg (75%), brownish white solid, m.p. 212–215 °C (dec); ^{1}H NMR (DMSO-d_6, 200 MHz): δ 8.18 (d, J = 2.2 Hz, 1H), 8.02 (d, J = 1.8 Hz, 1H), 7.61 (m, 2H), 7.41 (d, J = 16.6 Hz, 1H), 7.29 (d, J = 16.6 Hz, 1H), 7.41 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 171.52, (CO_2H), 156.99, 136.91, 135.53, 129.69, 128.66, 128.13, 127.81, 127.61, 126.41, 125.97, 114.41, 110.89.

4.1.2.12. 5-[(*E*)-2-Phenylvinyl]-2-hydroxybiphenyl-3-carboxylic acid (4c). Yield 243 mg (76%), yellow solid, m.p. 216 °C; ^{1}H NMR (acetone-d_6, 200 MHz): δ 8.12 (d, J = 2.2 Hz, 1H), 7.89 (d, J = 2.2 Hz, 1H), 7.67–7.59 (m, 4H), 7.49–7.36 (m, 6H), 7.32 (d, J = 7.8 Hz, 1H), 7.24 (d, J = 8 Hz, 1H); ^{13}C NMR (acetone-d_6): δ 173.66 (CO_2H), 160.38, 139.02, 138.68, 135.56, 131.99, 130.82, 130.29, 130.12, 130.04, 129.44, 129.29, 129.01, 128.81, 128.73, 128.59, 128.00, 127.80, 114.14.

4.1.2.13. 4-[(*E*)-2-(3-Carboxy-4-hydroxyphenyl)vinyl]-2-hydroxybenzoic acid (5a). Yield 206 mg (68%), yellowish white solid, m.p. >300 °C; ^{1}H NMR (DMSO-d_6, 200 MHz): δ 11.35 (br s, 2H, OH), 8.07 (d, J = 2.2 Hz, 1H), 7.92–7.86 (dd, J = 2.2, 8.4 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.49–7.41 (d, J = 16.6 Hz, 1H), 7.21 (d, J = 16.6 Hz, 1H), 7.19 (m, 2H), 7.07 (d, J = 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ 171.74, 171.68 (CO_2H), 161.44, 161.12, 144.55, 133.46, 130.68, 130.45, 129.16, 127.98, 125.63, 117.75, 117.23, 114.29, 113.20, 111.35.

4.1.2.14. 4-[(*E*)-2-(5-Bromo-3-carboxy-4-hydroxyphenyl)vinyl]-2-hydroxybenzoic acid (5b). Yield 343 mg (91%), light yellow solid, m.p. 297–300 °C (dec); ^{1}H NMR (DMSO-d_6, 200 MHz): δ 11.33 (br s, 1H), 8.23 (d, J = 2.2 Hz, 1H), 8.09 (d, J = 2.2 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.48 (d, J = 16.4 Hz, 1H), 7.31 (d, J = 15.4 Hz, 1H), 7.19 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 171.78, 171.51 (CO_2H), 161.44, 157.67, 144.32, 135.84, 130.52, 129.45, 129.05, 128.51, 126.82, 117.36, 114.61, 114.47, 111.59, 111.05.

4.1.2.15. 5-[(*E*)-2-(4-Carboxy-5-hydroxyphenyl)vinyl]-3-(furan-2-yl)-2-methoxybenzoic acid (5c). Yield 98%, yellow solid, m.p. 224–226 °C (dec); ^{1}H NMR (DMSO-d_6, 200 MHz): δ 13.17 (br s, 1H), 8.16 (d, J = 2.2 Hz, 1H), 7.90 (d, J = 2.2 Hz, 1H), 7.87 (d, J = 1.8 Hz, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 16.6 Hz, 1H), 7.34 (s, 1H), 7.23 (s, 1H), 7.17 (d, J = 18 Hz, 1H), 7.05 (d, J = 3.4 Hz, 1H), 6.70 (m, 1H), 3.79 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 171.79 (CO_2H), 167.26, 161.50, 154.53, 148.39, 144.14, 142.95, 132.48, 130.52, 130.15, 127.89, 127.66, 127.60, 126.98, 125.05, 117.45, 114.75, 112.48, 111.93, 110.76, 61.17.

4.1.2.16. 5-[(*E*)-2-(3-Carboxy-4-hydroxyphenyl)vinyl]-2-hydroxybiphenyl-3-carboxylic acid (6). Yield 131 mg (35%), brownish yellow solid, m.p. >300 °C (dec); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.96 (br s, 1H), 11.29 (br s, 1H), 8.01 (d, J = 2 Hz, 1H), 7.99 (d, J = 2 Hz, 1H), 7.87 (d, J = 2 Hz, 1H), 7.81 (dd, J = 1.6, 8.8 Hz, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.48–7.36 (m, 4H), 7.26 (d, J = 16.4 Hz, 1H), 7.19 (d, J = 16.8 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ 172.58, 171.86 (CO_2H), 160.49, 158.02, 136.85, 133.56, 132.97, 129.83, 129.29, 128.73, 128.52, 128.38, 128.10, 127.59, 127.35, 126.14, 125.61, 117.59, 113.16, 113.06.

4.1.3. Synthesis of 15a–e

4.1.3.1. General procedure for Wittig reaction. For the generation of **15a**, **15b**, and **15d**, the reaction conditions were similar to those of **4a–c**, **5a–c**, and **6**, described above. Diphosphonium salts were treated with 3 equivalents of lithium diisopropylamine and then with 2 equivalents of the corresponding aldehydes. To prepare **15c** and **15e**, Stille coupling was performed with **15b** (0.11 mmol) and **15d** (0.23 mmol), respectively.[30,31]

4.1.3.2. Methyl 5-((*E*)-2-{4-[(*E*)-2-(3-Methoxycarbonyl-4-methoxyphenyl)vinyl]phenyl}vinyl)-2-methoxybenzoate (15a). Yield 384 mg (35%), yellow solid, m.p. 231–234 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.98 (d, J = 2.2 Hz, 2H), 7.64–7.59 (dd, J = 2.2, 8.8 Hz, 2H), 7.48 (s, 4H), 7.05 (d, J = 17 Hz, 4H), 6.96 (d, J = 8.8 Hz, 2H), 3.93 (s, 12H).

4.1.3.3. Methyl 5-((*E*)-2-{4-[(*E*)-2-(5-Bromo-3-methoxycarbonyl-4-methoxyphenyl)vinyl]phenyl}vinyl)-3-bromo-2-methoxybenzoate (15b). Yield 158 mg (11%), yellow solid, m.p. 171-172 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.88 (s, 4H), 7.50 (s, 4H), 7.05 (d, J = 16.2 Hz, 2H), 7.04 (d, J = 16.2 Hz, 2H), 3.96 (s, 6H), 3.95 (s, 6H).

4.1.3.4. Methyl 5-((*E*)-2-{4-[(*E*)-2-(5-(Furan-2-yl)-3-methoxycarbonyl-4-methoxyphenyl)vinyl] phenyl}vinyl)-3-(furan-2-yl)-2-methoxybenzoate (15c). To a suspension of $Pd(PPh_3)_2Cl_2$ (5.0 mg, 7.0 μmol) in anhydrous 1,4-dioxane (0.2 mL), **15b** (120 mg, 0.29 mmol) in 1,4 dioxane (2.0 mL) and 2-(tributylstannyl)furan (0.11 mL, 0.34 mmol) was added successively by syringe. After refluxing overnight, an additional 2-(tributylstannyl)furan

(0.11 mL) was added and refluxed for another 5 h. The reaction was quenched by addition of 10% aq. NH_4OH (10 mL), and the resulting mixture was extracted with ethyl acetate (30 mL × 3). The organic extracts were combined, washed with water (30 mL × 2) and brine (30 mL) successively, dried (Na_2SO_4) and concentrated to obtain a mixture of yellow solid and brown oil. It was recrystallized from ethyl acetate (3 mL) to obtain **15c** (44 mg) as a greenish yellow solid. Purification of the mother liquor by column chromatography (hexane/EtOAc 4:1, R_f = 0.2) afforded additional **15c** (11 mg): yield 55 mg (82%), m.p. 212–225 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.16 (d, J = 1.2 Hz, 2H), 7.88 (d, J = 1.2 Hz, 2H), 7.59 (d, J = 18.4 Hz, 2H), 7.55 (d, J = 2 Hz, 2H), 7.49 (d, J = 18.4 Hz, 2H), 7.15 (s, 4H), 7.07 (d, J = 3.6 Hz, 2H), 6.56 (m, 2H), 3.99 (s, 6H), 3.85 (s, 6H).

4.1.3.5. Methyl 5-((*E*)-2-{4-[(*E*)-2-(5-Bromo-3-methoxycarbonyl-4-methoxyphenyl)vinyl]-3-methoxyphenyl}vinyl)-3-bromo-2-methoxybenzoate (15d). Yield 524 mg (35%), greenish yellow solid, m.p. 172–175 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.88 (s, 4H), 7.55 (d, J = 8 Hz, 1H), 7.42 (d, J = 16.4 Hz, 1H), 7.13 (d, J = 8 Hz, 1H), 7.09–6.98 (m, 4H), 3.96 (s, 9H), 3.95 (s, 3H), 3.94 (s, 3H).

4.1.3.6. Methyl 5-((*E*)-2-{4-[(*E*)-2-(5-(Furan-2-yl)-3-methoxycarbonyl-4-methoxyphenyl)vinyl]-3-methoxyphenyl}vinyl)-3-(furan-2-yl)-2-methoxybenzoate (15e). The reaction was performed as in **15c**: yield 100 mg (70%), yellow solid, m.p. 189–191,°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15 (t, 2H), 7.89 (t, 2H), 7.60 (d, J = 8 Hz, 1H), 7.53 (s, 2H), 7.51 (d, J = 16.4 Hz, 1H), 7.18 (d, J = 16.4 Hz, 3H), 7.14 (s, 1H), 7.06 (m, 3H), 6.55 (m, 2H), 3.99 (s, 9H), 3.85 (s, 3H), 3.84 (s, 3H).

4.1.3.7. General procedure for the deprotection of hydroxyl and carboxyl groups to obtain 7a–e. *Method A.* To obtain compounds **7a** and**7b**, BBr_3 (1.0 M in CH_2Cl_2, 2.1 mmol) was added to a solution of **15a** and **15b** (1.0 mmol) in dry CH_2Cl_2 (10 ml) with stirring under N_2 over a 5 min period while cooling the reaction mixture to −70 °C. The reaction mixture was allowed to stir at room temperature for 1–8 h. When complete, the reaction mixture was cooled in ice-water bath and quenched with 1.0 M HCl (4 ml). The precipitate was dissolved in EtOAc (30 mL), and the organic layer was washed with water (20 mL × 2) and brine (20 mL) successively, and dried (Na_2SO_4). After evaporation of the solvent under reduced pressure, the brownish solid was dissolved in 95% aq. EtOH (20 mL), and KOH (9.2 mmol) was added. After 2 h of reflux, the reaction mixture was cooled and acidified with 1.0 M HCl (16 mL). The white precipitate was dissolved in EtOAc (20 mL), and the aqueous layer was extracted with EtOAc (20 mL). The organics were combined, washed with water (15 mL × 2) and brine (15 mL) successively, and dried (Na_2SO_4). The filtrate was concentrated to give the product as a yellowish white solid.

Method B: For the conversion of compounds **15c**–**e** to **7c**–**e**, compounds **15c**–**e** (0.2 mmol) were treated with PhSH (0.8 mmol) and K_2CO_3 (0.3 mmol) in anhydrous NMP (6.0 mL) by stirring at 190 °C for 40 min under N_2. The reaction mixture was cooled and poured into saturated aqueous $NaHCO_3$ (8 mL). After extraction with EtOAc (30 mL × 2), the organic layer was discarded. The aqueous layer was acidified with 1.0 M HCl (13 mL) and extracted with EtOAc (50 mL × 3). The combined organics were washed with water (50 mL × 2) and brine (50 mL) successively, dried (Na_2SO_4), and concentrated to obtain yellow solid.

4.1.3.8. 5-((*E*)-2-{4-[(*E*)-2-(3-carboxy-4-hydroxyphenyl)vinyl]-phenyl}vinyl)-2-hydroxybenzoic acid (7a). Yield 212 mg (51%), brownish yellow solid, m.p. >300 °C; ^{1}H NMR (DMSO-d_6, 200 MHz): δ 11.41 (br s, 2H, OH), 8.03 (d, J = 2.2 Hz, 2H), 7.89–7.84 (dd, J = 2.2, 8.4 Hz, 2H), 7.63 (s, 4H), 7.36 (d, J = 16.4 Hz, 2H), 7.21 (d, J = 16.4 Hz, 2H), 7.05 (d, J = 8.4 Hz, 2H); ^{13}C NMR (DMSO-d_6): δ 171.75 (CO_2H), 160.61, 136.28, 133.84, 128.61, 128.45, 127.07, 126.63, 126.55, 117.67, 113.12.

4.1.3.9. 5-((*E*)-2-{4-[(*E*)-2-(5-Bromo-3-carboxy-4-hydroxyphenyl)-vinyl]phenyl}vinyl)-3-bromo-2-hydroxybenzoic acid (7b). Yield 494 mg (86%), yellow solid, m.p. >300 °C (dec); ^{1}H NMR (DMSO-d_6, 200 MHz): δ 8.16 (d, J = 2 Hz, 2H), 8.04 (d, J = 2 Hz, 2H), 7.63 (s, 4H), 7.32 (d, J = 16.2 Hz, 2H), 7.27 (d, J = 16.2 Hz, 2H).

4.1.3.10. 5-((*E*)-2-{4-[(*E*)-2-(5-(Furan-2-yl)-3-carboxy-4-hydroxyphenyl)vinyl]phenyl}vinyl)-3-(furan-2-yl)-2-hydroxybenzoic acid (7c). Yield 86 mg (78%), yellow solid, m.p. >300 °C (dec); ^{1}H NMR (DMSO-d_6 400 MHz): δ 8.19 (d, J = 2 Hz, 2H), 7.99 (d, J = 2 Hz, 2H), 7.82 (s, 2H), 7.64 (s, 4H), 7.37 (d, J = 16.4 Hz, 2H), 7.21 (d, J = 16.4 Hz, 2H), 7.09 (d, J = 3.6 Hz, 2H), 6.65 (m, 2H); ^{13}C NMR (DMSO-d_6): δ 172.52 (CO_2H), 156.96, 148.49, 142.46, 136.37, 128.27, 127.88, 127.11, 126.94, 126.82, 119.26, 113.81, 112.25, 110.81.

4.1.3.11. 5-((*E*)-2-{4-[(*E*)-2-(5-Bromo-3-carboxy-4-hydroxyphenyl)-vinyl]-3-methoxyphenyl}vinyl)-3-bromo-2-hydroxybenzoic acid (7d). Yield 95 mg (81%), yellow solid, m.p. >300 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.14 (s, 1H), 8.04 (s, 1H), 8.01(s, 1H), 7.95 (s, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.32–7.17 (m, 6H), 3.92 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 171.46, 171.44 (CO_2H), 157.08, 156.93, 156.72, 137.80, 135.49, 130.10, 129.65, 127.87, 127.29, 126.61, 126.34, 126.24, 124.68, 122.35, 119.20, 114.53, 110.93, 110.87, 109.07, 55.50.

4.1.3.12. 5-((E)-2-{4-[(*E*)-2-(5-(Furan-2-yl)-3-carboxy-4-hydroxyphenyl)vinyl]-3-methoxyphenyl}vinyl)-3-(furan-2-yl)-2-hydroxybenzoic acid (7e). Yield 76 mg (67%), yellow solid, m.p. >300 °C (dec); ^{1}H NMR (DMSO-d_6 400 MHz): δ 8.22 (d, J = 2 Hz, 1H), 8.15 (d, J = 2 Hz, 1H), 8.02 (d, J = 2 Hz, 1H) 7.94 (d, J = 2 Hz, 1H), 7.82 (s, 2H), 7.70 (d, J = 8 Hz, 1H), 7.44 (d, J = 16.4 Hz, 1H), 7.32 (s, 3H), 7.27 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 16.4 Hz, 1H), 7.09 (s, 2H), 6.66 (m, 2H), 3.95 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 172.39, (CO_2H), 156.62, 148.38, 142.42, 137.84, 128.77, 128.32, 127.98, 127.72, 127.33, 126.88, 126.55, 124.65, 121.41, 119.24, 113.55, 112.16, 110.78, 109.14, 55.57.

4.1.4. Synthesis of phosphonium salt derivatives

4.1.4.1. (5-Acetoxy-4-methoxycarbonylbenzyl)triphenylphosphonium bromide (18). *Step 1.* The mixture of 4-methylsalicylic acid (2.0 g, 13.2 mmol), acetic anhydride (2.4 mL, 26.3 mmol), and catalytic amount of H_3PO_4 was stirred at 80 °C for 30 min. The reaction was quenched by addition of water (20 mL) and the precipitate dissolved in EtOAc. The aqueous layer was extracted with EtOAc (50 mL × 2), and the combined organics were washed with water (50 mL × 2) and brine (50 mL) successively, and dried (Na_2SO_4). After evaporation of solvent, the crude product was refluxed with methyl iodide (4.0 mL) and K_2CO_3 (3.5 g) in acetone (60 mL) for 1 h. The reaction mixture was cooled to room temperature and filtered to remove K_2CO_3. After evaporation of the solvent, the crude product was purified by column chromatography (hexane/EtOAc 4:1, R_f = 0.4) to give methyl 2-acetoxy-4-methylbenzoate, **17**, as a colorless oil (2.3 g, 82% yield): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.93 (d, J = 8.2 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 6.90 (s, 1H), 3.84 (s, 3H, CO_2CH_3), 2.37 (s, 3H, CH_3), 2.34 (s, 3H, $OCOCH_3$).

Step 2. Benzoyl peroxide (1.4 g, 5.8 mmol) and NBS (2.4 g, 13.4 mmol) were added to a solution of **17** (2.0 g, 9.6 mmol) in benzene (80 mL). The reaction mixture was allowed to reflux for 3 h. After cooling to room temperature, the mixture was concen-

trated and purified by column chromatography (toluene/EtOAc 12:1). It was again subjected to another column chromatographic separation (hexane/EtOAc 4:1, R_f = 0.3) to obtain methyl 2-acetoxy-4-(bromomethyl)benzoate as a white solid (1.9 g, 71% yield): mp 56 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.00 (d, J = 8.0 Hz, 1H), 7.34 (dd, J = 8.0 and 1.8 Hz, 1H), 7.14 (d, J = 1.8 Hz, 1H), 4.44 (s, 2H), 3.86 (s, 3H, CO_2CH_3), 2.35 (s, 3H, $OCOCH_3$).

Step 3. Triphenylphosphine (1.2 g, 4.5 mmol) was dissolved in dry benzene (5.0 mL), and methyl 2-acetoxy-4-(bromomethyl)benzoate (1.0 g, 3.5 mmol) was added. The flask was tightly stoppered and stirred at room temperature for 2 d. The white precipitate was filtered, washed with benzene, and dried to obtain **18** as a white solid (1.8 g, 93% yield): m.p. 140 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.84–7.57 (m, 15H), 7.37 (m, 1H), 7.20 (d,J = 8.0 Hz, 1H), 6.89 (s, 1H), 5.81 (d, J = 15.4 Hz, 2H, CH_2), 3.82 (s, 3H, CO_2CH_3), 2.25 (s, 3H, $OCOCH_3$).

4.1.4.2. [(3-Methoxy-2-methoxycarbonylbiphenyl)-5-methylene]-triphenylphosphonium bromide (22). *Step 1.* Methyl 2-hydroxy-5-methylbenzoate (4.5 g, 27 mmol) obtained by esterification of 2-hydroxy-5-methylbenzoic acid, **19**, with methanol-sulfuric acid, was dissolved in acetonitrile (120 mL). *N*-Bromosuccinimide (5.3 g, 29.7 mmol) was added in five portions and stirred at room temperature for 1 h. The reaction was quenched by addition of water (5.0 mL) and sodium sulfite (2.0 g). After stirring the mixture at room temperature for 30 min, the solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc (200 mL). The organic solution was washed with water (100 mL × 2) and brine (100 mL) successively, dried (Na_2SO_4), filtered, and concentrated. The resulting residue was dissolved in EtOAc (30 mL), and SiO_2 (3 g) was added. The solvent was again evaporated under reduced pressure and the fine powder was applied on top of the silica gel column. Elution with hexane/EtOAc 9:1 (R_f = 0.5) afforded 1.2 g of 1:1 mixture of starting material and **20**, and 2.8 g of pure **20**. The mixture of starting material and product (1.2 g) was again subjected to *N*-bromosuccinimide (756 mg, 4.3 mmol) reaction in 16 mL of acetonitrile. After similar workup, the solid product was recrystallized from hexane/EtOAc to get **20** (1.3 g) as a white crystal (overall yield 62%): m.p. 100–102 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 11.22 (s, 1 H), 7.62 (d, J = 2.2 Hz, 1H), 7.55 (d, J = 2.2 Hz, 1 H), 3.95 (s, 3H), 2.28 (s, 3H).

Step 2. A flame-dried flask was charged with **20** (2.5 g, 10.2 mmol) in toluene (100 mL) and phenylboronic acid (1.9 g, 15 mmol), and N_2 was purged for 10 min. Aqueous Na_2CO_3 solution (2.0 M, 15.3 mL, 30.6 mmol) and then ethanol (5.0 mL) was slowly added by syringe. Finally, tetrakis(triphenylphosphine)palladium (589 mg, 0.5 mmol) was added ,and the reaction mixture was allowed to stir at 80 °C overnight. The reaction mixture was cooled to room temperature and partitioned between EtOAc (200 mL) and water (100 mL). The aqueous layer was separated and extracted with EtOAc (100 mL × 2). The combined organic solution was washed with water (100 mL × 2) and brine (100 mL) successively, dried (Na_2SO_4), and concentrated. The dark brown oil was subjected to column chromatography (hexane/EtOAc 19:1, R_f = 0.4) to obtain methyl 2-hydroxy-5-methylbiphenyl-3-carboxylate as a colorless oil (2.3 g, 98% yield): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 11.08 (s, 1H), 7.65 (m 1H), 7.58 (d, J = 2.4 Hz, 1H), 7.56 (d, J = 1.6 Hz, 1H), 7.42 (m, 2H), 7.34 (m, 2H), 3.94 (s, 3H), 2.32 (s, 3H). Treatment of methyl 2-hydroxy-5-methylbiphenyl-3-carboxylate with dimethyl sulfate gave methyl 2-methoxy-5-methylbiphenyl-3-carboxylate **21** as a colorless oil (72% yield): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.58–7.52 (m, 3H), 7.46–7.34 (m, 3H), 7.31 (m, 1H), 3.93 (s, 3H), 3.44 (s, 3H), 2.37 (s, 3H).

Step 3. Methyl 2-methoxy-5-methylbiphenyl-3-carboxylate (1.4 g, 5.4 mmol) was refluxed for 1 day with *N*-bromosuccinimide (1.1 g, 6.5 mmol) and dibenzoyl peroxide (785 mg, 3.2 mmol) in 50 mL of CCl_4. The crude product after workup was subjected to column chromatography (hexane/EtOAc 9:1, R_f = 0.4) to get methyl 5-(bromomethyl)-2-methoxy-biphenyl-3-carboxylate as yellow oil (1.3 g, 72% yield): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.79 (d, J = 2.6 Hz, 1H), 7.58–7.52 (m, 3H), 7.48–7.37 (m, 3H), 4.50 (s, 2H), 3.94 (s, 3H), 3.48 (s, 3H).

Step 4. Triphenylphosphine (1.2 g, 4.7 mmol) was dissolved in 5 mL of benzene and methyl 5-(bromomethyl)-2-methoxy-biphenyl-3-carboxylate (1.2 g, 3.6 mmol) was added. The flask was tightly stoppered and maintained at room temperature for 2 d. Phosphonium salt **22** was collected as white powder (1.5 g, 69% yield): m.p. 228–230 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.84–7.74 (m, 8H), 7.68–7.59 (m, 7H), 7.31–7.28 (m, 7H), 5.56 (d, J = 14.0 Hz, 2H), 3.79 (s, 3H), 3.40 (s, 3H).

4.1.4.3. 1,4-*bis*(Triphenylmethylenephosphonium)benzene dibromide (14a)[27,29]. *Step 1.* A mixture of *p*-xylene (5.0 g, 47.09 mmol), *N*-bromosuccinimide (18.4 g, 103.6 mmol), and dibenzoyl peroxide (94 mg, 0.4 mmol) in CCl_4 (100 mL) was refluxed for 1.5 h under N_2 atmosphere with the illumination of visible light. After work-up similar to that for **14b**, 1,4-*bis*(bromomethyl)benzene **24a** was obtained as white crystal (6.4 g, 51%): m.p. 144 °C; ^{1}H NMR (acetone-d_6, 200 MHz): δ 7.45 (s, 4H), 4.63 (s, 4H, CH_2).

Step 2. Triphenylphosphine (9.69 g, 36.96 mmol) was added to the solution of **24a** (4.6 g, 17.6 mmol) in dry DMF (50 mL) and the resulting heterogeneous mixture was refluxed for 3 h. After work-up as for **14b**, **14a** was obtained as white fluffy solid (14 g, 100% yield): m.p. >300 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.78–7.59 (m, 30H), 6.91 (s, 4H), 5.41 (d, J = 13.6 Hz, 4H, CH_2).

4.1.4.4. 2,5-*bis*(Triphenylmethylenephosphonium)methoxybenzene dibromide (14b)[27,29]. *Step 1.* A mixture of 2,5-dimethylanisole (5.0 g, 36.7 mmol), *N*-bromosuccinimide (13.7 g, 77.1 mmol), and dibenzoyl peroxide (355 mg, 1.5 mmol) in CCl_4 (150 mL) was refluxed for 7 h under N_2 atmosphere with the illumination of visible light. After the reaction mixture was cooled to room temperature, succinimide was filtered and solvent was evaporated. The crude product was recrystallized from cyclohexane to obtain 1,4-*bis*(bromomethyl)-2-methoxybenzene **24b** as a white solid (3.96 g). The mother liquor was concentrated and subjected to column chromatographic purification (hexane/EtOAc 19:1, R_f = 0.3) to yield additional product (4.92 g, overall yield 82%): m.p. 104–106 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.30 (d, J = 7.6 Hz, 1H), 6.96 (dd, J = 7.6, 1.6 Hz, 1H), 6.91 (d, J = 1.6 Hz, 1H), 4.54 (s, 2H), 4.47 (s, 2H), 3.91 (s, 3H).

Step 2. Triphenylphosphine (4.5 g, 17.2 mmol) was added to the solution of 1,4-bis(bromomethyl)-2-methoxybenzene **24b** (2.4 g, 8.2 mmol) in dry DMF (25 mL), and the resulting heterogeneous mixture was refluxed for 3 h. After the mixture was cooled to room temperature, the white precipitate was filtered, washed with ether, and dried to obtain **14b** as a white fluffy solid (5.6 g, 83% yield): m.p. >300 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.79-7.59 (m, 30H), 7.23 (dd, J = 7.6, 2.4 Hz, 1H), 6.91 (s, 1H), 6.31 (d, J = 7.6 Hz, 1H), 5.31 (d, J = 14 Hz, 2H), 5.29 (d, J = 14 Hz, 2H), 2.94 (s, 3 H).

4.1.5. Synthesis of aldehyde derivatives

4.1.5.1. 3-Bromo-5-formyl-2-methoxybenzoic acid methyl ester (9b). **Step 1.** Conc. H_2SO_4 (3.0 mL) was added to the solution of 5-formylsalicylic acid (8.0 g, 48 mmol) in anhydrous methanol (100 mL) and the mixture was heated with reflux overnight. After cooling to room temperature, solvent was evaporated and the residue was dissolved in EtOAc (300 mL). It was washed with 5% $NaHCO_3$ (100 mL), water (100 mL × 2), and brine (100 mL) successively, dried (Na_2SO_4), filtered, and concentrated. The solid

residue was dissolved in 100 mL EtOAc and adsorbed on 10 g silica gel. After dryness, the mixture was applied on top of the silica gel column. The column chromatographic purification (hexane/EtOAc 4:1, $R_f = 0.4$) yielded methyl 5-formyl-2-hydroxybenzoate as a white solid (5.9 g, 68% yield).

Step 2. Bromine (1.0 M solution in CH_2Cl_2, 55 mL, 55 mmol) was added dropwise into a ice-cooled solution of 5-formyl-2-hydroxybenzoate (4.9 g, 27.5 mmol) in CH_2Cl_2 (200 mL) over 3 h period with occasional TLC to monitor the progress of the bromination. After completion of reaction, saturated $NaHCO_3$ solution (200 mL) was added to the reaction mixture, allowed to stir for 30 min. Aqueous layer was separated and extracted with CH_2Cl_2 (100 mL). The combined organics were washed successively with saturated $NaHCO_3$ (100 mL), water (100 mL × 2), and brine (100 mL) and dried (Na_2SO_4), and concentrated. The crude product was adsorbed on 7 g of silica gel and subjected to column chromatographic purification (hexane/EtOAc 4:1, $R_f = 0.3$) to obtain **9e** as a light yellow solid (4.7 g, 67% yield).

Step 3. Dimethyl sulfate (2.7 mL, 28.1 mmol) was added to a mixture of **9e** (4.7 g, 18.3 mmol) and K_2CO_3 (5.2 g, 37.5 mmol) in acetone (70 mL), and the mixture was refluxed for 3 h. After evaporation of solvent, the residue was partitioned between EtOAc (300 mL) and water (200 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (100 mL × 2). The combined organics were washed with water (200 mL × 2) and brine (200 mL) successively, dried (Na_2SO_4) and concentrated. The crude product was column chromatographed (hexane/EtOAc 4:1, $R_f = 0.4$) to give **9b** as a white solid (3.4 g, 68% yield): mp 66–67 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 9.94 (s, 1H), 8.26 (d, $J = 2.2$ Hz, 1H), 8.25 (d, $J = 2.2$ Hz, 1H), 4.01 (s, 3H), 3.97 (s, 3H).

4.1.5.2. 5-Formyl-2-hydroxybiphenyl-3-carboxylic acid methyl ester (9c). A flame-dried flask was charged with **9e** (1.0 g, 3.9 mmol) in toluene (40 mL) and phenylboronic acid (709 mg, 5.8 mmol). After purging N_2 for 10 min, 2.0 M Na_2CO_3 solution (5.8 mL, 11.6 mmol) and ethanol (2.0 mL) were added by syringe. Tetrakis(triphenylphosphine)palladium (224 mg, 0.19 mmol) was then added and the reaction mixture was allowed to stir at 80 °C overnight. The reaction mixture was cooled to room temperature and diluted with water (20 mL). The aqueous layer was separated and extracted with EtOAc (50 mL × 2). The combined organic solution was washed with water (50 mL × 2) and brine (50 mL) successively, dried (Na_2SO_4), and concentrated. The dark brown oil was subjected to column chromatography (hexane/EtOAc 4:1, $R_f = 0.4$) to obtain **9c** as a yellow oil (352 mg, 44% yield): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 11.93 (s, 1H), 9.92 (s, 1H), 8.41 (d, $J = 2.2$ Hz, 1H), 8.07 (d, $J = 2.2$ Hz, 1H), 7.62–7.56 (m, 2H), 7.51–7.39 (m, 3H), 4.03 (s, 3H).

4.2. Enzyme assay

4.2.1. Materials

A substrate, *p*NPP, for the PTP assay was purchased from Sigma (St. Louis, USA) in the di(Tris) salt form. The native form of PTP1B, the catalytic domain of SHP-1 (SHP-1cat), and YPTP1 were expressed in an *Escherichia coli* expression systems, and purified as described previously.[32–34] LAR-D1 (membrane-proximal catalytic domain of LAR), TC-PTP, and YOP were purchased from New England Biolabs, Inc. (Beverly, USA). Absorbances were measured using Novaspec-II spectrophotometer (Amersham pharmacia) or DU 650 spectrophotometer (Beckman Coulter).

4.2.2. IC_{50} determination

For the enzyme assay, PTP1B and other PTPs were diluted to appropriate concentrations 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), and inhibitors were dissolved in DMSO. The enzyme activity was measured at 37 °C by monitoring the hydrolysis of *p*NPP in buffer A (50 mM HEPES, 5.0 mM EDTA, pH 7.0). The absorbance at 405 nm was measured to determine the amount of *p*-nitrophenol released. For a typical 50 μL reaction, inhibitor (5.0 μL) was added to a reaction mixture containing enzyme (5.0 μL), 5 × buffer A (10 μL), and H_2O (25 μL), and was incubated at 37 °C for 10 min. The enzyme reaction was initiated by addition of *p*NPP (20 mM, 5.0 μL). After 3 min at 37 °C, the reaction was quenched by addition of 0.5 M NaOH (950 μL) and absorbance at 405 nm was measured. IC_{50} values of the inhibitors were determined by measuring the *p*NPP hydrolase activity in a range of different inhibitor concentrations. The concentrations of enzymes in the assay mixture 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. The kinetic data were analyzed using GraFit 5.0 program (Erithacus Software). The results were obtained from double or triple experiments using range of inhibitor concentrations.

4.3. Mouse experiment

Twenty four C57BL/6J Jms Slc mice (4 weeks old, male, 17–19 g) were purchased from Japan SLC, Inc., Haruno Breeding branch. The mice were individually housed and maintained in a 12-h light/dark cycle at 22 ± 2 °C. Food and water were available ad libitum. The experimental diets, high fat diet (HFD, D12451) and low fat diet (LFD, D10012G) containing 45% and 16% of the calories from fat, respectively, were obtained from Research Diets (New Brunswick, NJ). The diets were either in pellet or in powder form. All mice were acclimatized for 1 week (LFD), with 16 mice fed with HFD for first 8 weeks of the study for the development of obesity and diabetes; the remaining 8 were fed with LFD. The mice assigned to the LFD group were maintained on this diet throughout the study, as a lean control group. At week 8, all the HFD-fed mice were assigned to 1 of 2 groups containing 8 mice each. The first group remained on HFD throughout the study, as an obese/diadetic control group. The remaining group was fed with HFD containing the PTP1B inhibitor, **7b**, for 4 weeks. The concentration of **7b** in the diets was 2.0 g/kg of diet (0.2% w/w). Conversely, the obese/diabetic control and **7b**-treated mice groups were fed with HFD powdered food mixed with 10% H_2O to make a dough. For the treatment with **7b**, 440 mg of **7b** was dissolved in H_2O (22 mL containing 2 equivalent of NaOH solution and 0.4% EtOH), mixed in powdered food (220 g), and kneaded to form a dough.

The body weight and food intake were recorded every 3 days throughout the study. For the glucose tolerance test, seven mice from each group were fasted for 6 h starting from beginning of light cycle, and glucose (1.0 g/kg of body weight) was injected intraperitoneally. Blood glucose levels were measured from tail bleeds with a glucometer (Accu check active, Roche diagnostics, Ireland) at 0 (prior to glucose administration, used as a fasting glucose level) and at 20, 40, 60, 90, and 120 min after glucose injection. After completion of glucose tolerance test, all the mice were allowed to return to the normal condition with the supply of food and water ad libitum. The treatment group was supplied with the HFD containing **7b**.

After 5 days of glucose tolerance test, the mice were fasted overnight and anesthetized with intraperitoneal injection of secobarbital (40 mg/4 mL/kg body weight), with blood samples taken by cardiac puncture into EDTA tubes and immediately placed on ice. Blood samples were spun (4000*g*, 10 min, 4 °C), with the plasma removed and frozen until further analysis. Plasma was analyzed for glucose, triglyceride, total cholesterol, and free fatty acids using diagnostic kits (Glucose C2, TG E, T-Cho E and NEFA C from Wako Pure chemical Industries, Ltd. Osaka, Japan) following

the manufacturer's protocol. A mixture of 10 μL of plasma sample and color reagent (1.0 mL for cholesterol and 1.5 mL for glucose and TG) was incubated at 37 °C for 5 min, and then A_{600} for cholesterol and TG, and A_{505} for glucose were measured. To determine the NEFA concentration, 25 μL of plasma sample and 1.5 mL of NEFA C reagent were incubated at 37 °C for 20 min, and then A_{550} measured. Epididymal and retroperitoneal fat pads, liver, lungs, and kidneys were excised immediately after blood collection, washed in cold isotonic saline, gently blotted, and then weighed. Tissues were immediately frozen in liquid N_2 and stored at −75 °C for further analysis.

The data for the mice were analyzed using a 1-way ANOVA with the SPSS version 11.5 statistical package for windows (SPSS Inc. Chicago, Illinois). Differences were considered significant at two levels, $p < 0.05$ or $p < 0.005$.

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