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Original article

Discovery of novel bromophenol 3,4-dibromo-5-(2-bromo-3,4-dihydroxy-6-(isobutoxymethyl)benzyl)benzene-1,2-diol as protein tyrosine phosphatase 1B inhibitor and its anti-diabetic properties in C57BL/KsJ-*db*/*db* mice

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ABSTRACT

In an effort to develop novel small molecule PTP1B inhibitors, a series of bromophenol derivatives were designed, synthesized and evaluated *in vitro* and *in vivo*. All of the synthesized compounds displayed weak to potent PTP1B inhibitory activities (5.62–96.25%) at 20 μg/mL. Among these compounds, 3,4-dibromo-5-(2-bromo-3,4-dihydroxy-6-(isobutoxymethyl)benzyl)benzene-1,2-diol (**9**) exhibited enhanced PTP1B inhibitory activity ($IC_{50} = 1.50$ μM) than the lead compound BDDPM ($IC_{50} = 2.42$ μM) and high selectivity against other PTPs (TCPTP, LAR, SHP-1 and SHP-2). Results of anti-diabetic assay using C57BL/KsJ-*db*/*db* mouse model demonstrated that compound **9** was effective at lowering blood glucose, total cholesterol and HbA1c ($P < 0.01$).

1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex endocrine and metabolic disorder. Established glucose-lowering medications, which were called the "first-generation" agents, include insulin, biguanides, sulfonylureas, and thiazolidinediones (TZDs). However, the adverse effects such as hypoglycemia, weight increment and edema limited their clinical use. In addition, recent studies reported that long-term use of rosiglitazone or pioglitazone was associated with a significant increase in the risk of myocardial infarction [1] and a modestly increased risk of any pneumonia or lower respiratory tract infection in patients with T2DM [2]. The "second-generation" approaches for the treatment of T2DM are including incretin glucagon-like peptide 1 (GLP-1) analogues, sodium glucose cotransporter 2 (SGLT2) inhibitors, dipeptidyl peptidase 4 (DPP4) and protein tyrosine phosphatase 1B (PTP1B) inhibitors [3,4]. Among them, PTP1B inhibitors have attracted considerable attention.

PTP1B is the first identified and characterized protein tyrosine phosphatase (PTP) that was purified from human placental tissue as early as in 1988 [5,6]. Not until 1999 that one research team announced that PTP1B knockout mice displayed enhanced sensitivity to insulin, lower plasma insulin levels and were resistant to weight gain [7]. The results were independently confirmed by another research team a year later [8]. Also, biochemical studies revealed that treatment of diabetic mice with antisense oligonucleotides (ASOs) resulted in reduced PTP1B expression, and subsequent improvement of insulin sensitivity and glycemic control [9]. Thus, PTP1B is currently considered to be a promising drug target for non-insulin dependent diabetes and obesity [10,11]. Furthermore, recent studies have also indicated that PTP1B is involved in cancer, which makes PTP1B inhibitors to be great potential agents in cancer therapy [12–15].

Given the compelling evidence that PTP1B is implicated in T2DM, a large number of small molecules have been described over

Abbreviations: PTP1B, protein tyrosine phosphatase 1B; T2DM, type 2 diabetes mellitus; TZDs, thiazolidinediones; GLP-1, glucagon-like peptide 1; SGLT2, sodium glucose cotransporter 2; DPP4, dipeptidyl peptidase 4; ASOs, antisense oligonucleotides; SAR, structure activity relationship; TFAA, trifluoroacetic anhydride; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; TMS, tetramethylsilane; TCPTP, T-cell protein tyrosine phosphatase; SHP-1, Src homology 2-containing protein tyrosine phosphatase-1; SHP-2, Src homology 2-containing protein tyrosine phosphatase-2; LAR, leucocyte antigen-related tyrosine phosphatase.

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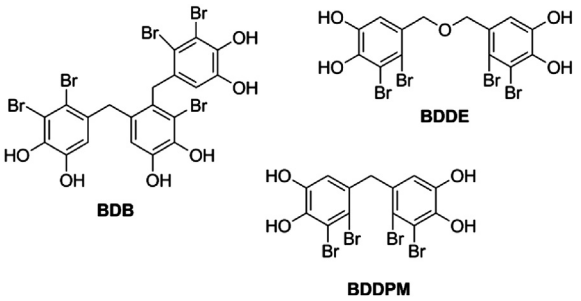


Fig. 1. Structures of bromophenols isolated from red alga *Rhodomela confervoides*.

the last two decades with the aim of developing potent and selective compounds as drug candidates [16–18]. However, the highly charged phosphatase active site and the relatively shallow nature of the surrounding protein surface make great challenge to medicinal chemists for the discovery of cell permeable and orally bio-available PTP1B inhibitors.

Our PTP1B small molecule inhibitor program started with the isolation of some bromophenols (Fig. 1) from the ethanolic extract of red alga *Rhodomela confervoides* [19]. Further biological evaluation identified that bromophenol BDDPM was a potent PTP1B inhibitor (IC_{50} = 2.42 μM) and selected as a lead compound for further optimization. In our previous studies, we reported a series of derivatives which were designed based on symmetric structure of BDDPM with diverse inhibitory activities against PTP1B [20]. As a part of our ongoing research, we describe herein the synthesis, structure–activity relationship (SAR) and *in vivo* anti-diabetic studies of novel bromophenol derivatives as PTP1B inhibitors.

2. Chemistry

The synthetic route of diaryl-methane compounds **3**–**9** was shown in Scheme 1. The starting materials **1** and **2** were prepared according to methods described previously [21]. Friedel–Crafts alkylation between **1** and **2** in the presence of $AlCl_3$ afforded diaryl-methane **3**. Treatment of **3** with NBS under *hv* condition provided the corresponding benzyl bromide. Subsequent hydrolysis in the presence of K_2CO_3 and 1,4-dioxane afforded benzyl alcohol **5**. Demethylation of compounds **3** and **5** with BBr_3 in CH_2Cl_2 gave bromophenols in good yields. The bromophenols was treated with corresponding alcohol in the presence of catalyst H_3PO_4 to give the analogues **6**–**9**.

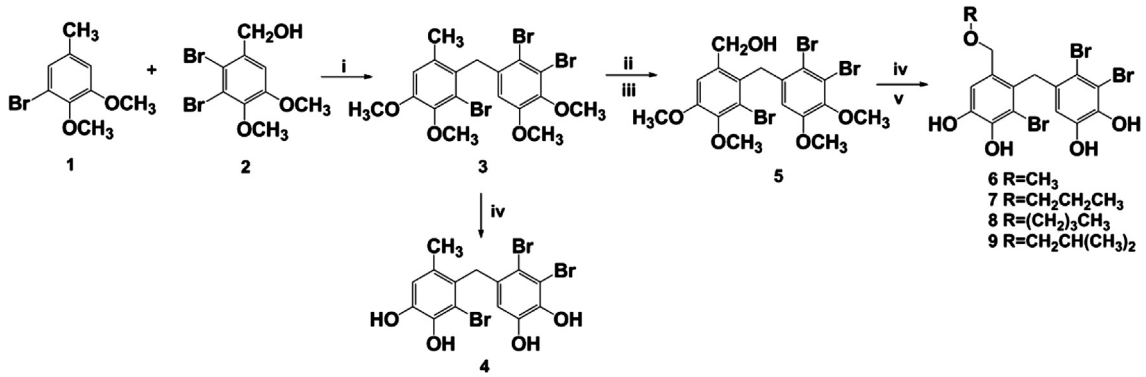
Synthesis of diaryl-methanone compounds was carried out following the synthetic steps in Scheme 2. Also, Friedel–Crafts acylation between **1** and corresponding aryl acids **10**–**12** in the presence of trifluoroacetic anhydride (TFAA) gave diaryl-methanones **13**–**15**. Oxidation of ketones **13**–**15** using $KMnO_4$ as oxidant afforded aryl acids **16**. Further esterification with CH_3OH provided ester **17**. The etherificated products **20** and **21** were prepared according to the procedures of **6**–**9**. Interestingly, in order to prepare the reduction product of keto acid **16** via Zn–Cu [22] or $NaBH_4$, we obtained compounds **18** and **19** with more rigid conformation.

Finally, we turned our attention to the position and number of bromine substitutions on the phenyl ring. As depicted in Scheme 3, the di-brominated compounds **23**, **24** and **25** were formed using the similar procedures in Scheme 1. Further bromination of compound **28** in the presence of NBS and concd. H_2SO_4 afforded multi-brominated compound **29** in moderate yield.

3. Results and discussion

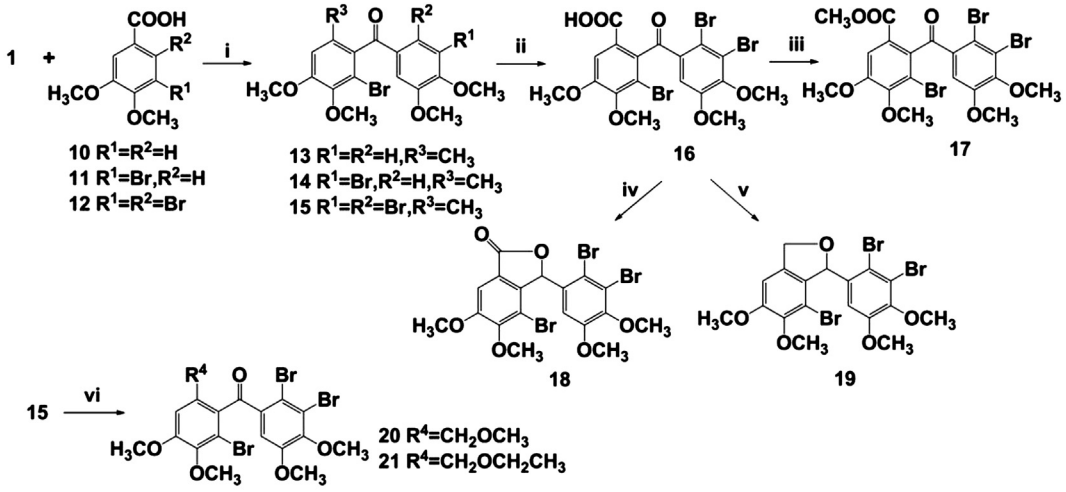
3.1. In vitro enzyme-based inhibitory activities assays

The target compounds **3**–**9**, **13**–**21**, **23**–**25** and **28**–**29** were evaluated for their *in vitro* inhibitory activities against recombinant PTP1B. We measured the inhibitory rates of all the derivatives at concentration of 20 and 5 μg/mL, and the compounds with good inhibition rates (>45% at 5 μg/mL) were selected for IC_{50} assay. Optimization of BDDPM with varying the substitution on one phenyl ring was depicted in Table 1. Electron-rich groups were favorable at this position, especially alkoxyl methylene groups (**6**–**9** and **20**–**21**). However, replacement of the alkoxyl methylene groups with carboxyl (**16**) led to a dramatic decrease in PTP1B inhibitory activity. Furthermore, the compound with branched-chain in alkoxyl methylene group (**9**) exhibited significant PTP1B inhibition (96.25% at 20 μg/mL and 48.3% at 5 μg/mL). The diaryl-methane moiety was selected for further derivatization. As shown in Table 2, replacement of diaryl-methane with diaryl-methanones moiety (**13**–**17** and **20**–**21**) was not tolerated. And the compounds containing more rigid configuration (**18** and **19**) exhibited significant loss of activity, indicating that the flexibility of diaryl-methane scaffold appeared to be optimal. The number of bromine substitution on the phenyl ring also had a certain influence on the inhibitory activity against PTP1B. As shown in Table 3, the tri- and tetra-brominated compounds (**3**–**9** and **28**–**29**) displayed more significant PTP1B inhibitory activity than di-brominated compounds (**23**–**24**). A quick examination of



Reagents and conditions: (i) $AlCl_3$, CH_2Cl_2, rt; (ii) NBS, AIBN, CCl_4, *hv*; (iii) K_2CO_3, 1,4-dioxane, H_2O, 90 °C; (iv) BBr_3, CH_2Cl_2, 0 °C - rt; (v) ROH, cat. H_3PO_4, rt.

Scheme 1. Synthesis of diaryl-methane compounds **3**–**9**.



Reagents and conditions: (i) TFAA, H_3PO_4, rt - 60 °C; (ii) $KMnO_4$, *t*-BuOH : H_2O = 1:1, reflux; (iii) CH_3OH, concd. H_2SO_4, reflux; (iv) Zn-Cu, KOH, reflux; (v) $NaBH_4$, $AlCl_3$, THF, reflux; (vi) NBS, AIBN, CCl_4, *hv*, RONa, ROH, rt.

Scheme 2. Synthesis of diaryl-methanone compounds **13**–**21**.

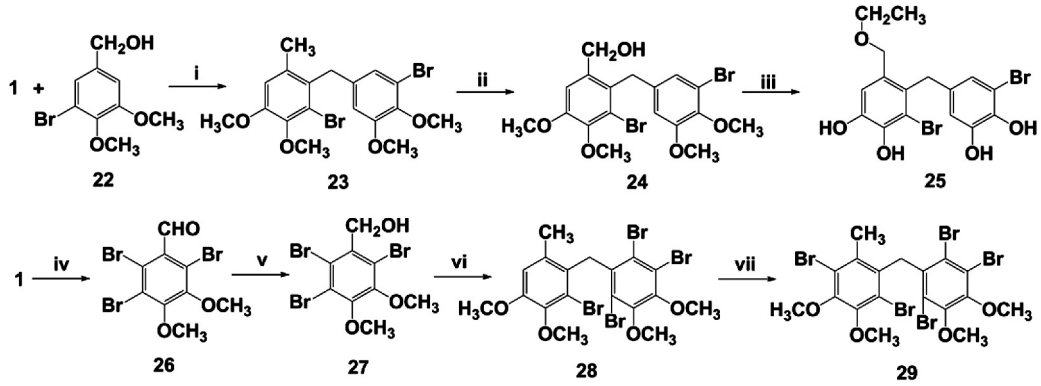
Tables 1–3 revealed that the compounds with free hydroxyl groups were more potent PTP1B inhibitors than the corresponding methoxyl compounds (**6**–**9** vs **13**–**27**). Further IC_{50} determination showed that compound **9** exhibited enhanced inhibitory activity against PTP1B with an IC_{50} of 1.50 μM, which was better than the lead compound BDDPM (IC_{50} = 2.42 μM).

In addition to potency improvements, we investigated the selectivity of compound **9** against other PTPs (TCPTP, LAR, SHP-1 and SHP-2). The results showed that compound **9** had excellent selectivity against TCPTP, LAR, SHP-1 and SHP-2 (>50-fold).

3.2. In vivo anti-diabetic activities assays

As an interesting new chemical entity, compound **9** was then selected for anti-diabetic activities evaluation in the *db*/*db* mice, a model of type 2 diabetes. Compound **9** was dosed orally at 25 and 50 mg/kg once a day, and the positive drug rosiglitazone was dosed at 50 mg/kg. During a 6-week intervention, body weights of *db*/*db* mice in treatment group exhibited a downward trend compared to the model group and no mortality occurred. Besides, compound **9** at different doses reduced the food and water intakes, indicating that compound **9** could improve the diabetes-related symptoms, such as polydipsia and polyphagia. The effects of compound **9** on blood glucose were illustrated in Fig. 2, the positive drug rosiglitazone significantly decreased blood glucose from 15.3 to 8.9 mmol/L at the first week of treatment ($P < 0.01$) and maintained blood glucose level at approximately 8 mmol/L until the end. Although slightly weaker than rosiglitazone (48.4%), compound **9** significantly decreased blood glucose ($P < 0.01$) since the third week, and obtained 36.4% and 32.7% decrease in the blood glucose level at high and low dose group, respectively.

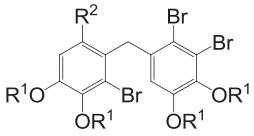
In addition, antidyslipidemic activity of compound **9** was assessed in the *db*/*db* mouse model. As shown in Table 4, the *db*/*db* mice used in this study had significantly elevated triglyceride and total cholesterol concentrations compared to the *db*/*dm* mice ($P < 0.01$, $P < 0.05$). In general, compound **9** at high dose showed



Reagents and conditions: (i) $AlCl_3$, CH_2Cl_2, 0 °C; (ii) NBS, AIBN, CCl_4, *hv*, K_2CO_3,1,4-dioxane, H_2O, 90 °C; (iii) BBr_3, CH_2Cl_2, 0 °C, CH_3CH_2OH, cat. H_3PO_4; (iv) NBS, concd. H_2SO_4, rt; (v) $NaBH_4$, CH_3OH, 0 °C; (vi) **1**, $AlCl_3$, CH_2Cl_2, 0 °C; (vii) NBS, concd. H_2SO_4, rt.

Scheme 3. Synthesis of multi-brominated compounds **23**–**29**.

Table 1
In vitro PTP1B inhibitory activities of diaryl-methane compounds **3**–**9**.



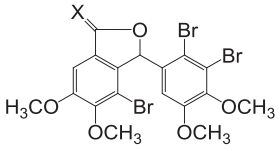
Compds	R^1	R^2	Inhibition (%)		IC_{50} (μM)
			20 μg/mL	5 μg/mL	
3	CH_3	CH_3	87.52	43.13[a]	ND
4	H	CH_3	53.38	32.10[a]	ND
5	CH_3	CH_2OH	74.86	29.07[a]	ND
6	H	CH_2OCH_3	51.89	25.30[a]	ND
7	H	$CH_2O(CH_2)_2CH_3$	86.15	55.70[a]	2.00[b]
8	H	$CH_2O(CH_2)_3CH_3$	64.56	27.17[a]	ND
9	H	$CH_2OCH_2CH(CH_3)_2$	96.25	48.30[a]	1.50[b]

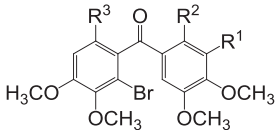
ND = not determined.
[a] Values were tested only if its inhibitory ratio greater than 45% at the dose of 20 μg/mL.
[b] Values were tested only if its inhibitory ratio greater than 50% at the dose of 5 μg/mL.

better lipid lowering profile ($P < 0.05$) and decreased the triglycerides and total cholesterol concentrations in a dose-dependent manner.

It is well known that glycated hemoglobin (HbA1c) is an important criterion for diagnosis of diabetes and the best way to check if diabetes is under control. As depicted in Table 5, mice in model group had significantly elevated HbA1c compared to the *db*/*dm* mice ($P < 0.01$). It is gratifying that compound **9** significantly decreased HbA1c levels ($P < 0.01$) at both high and low dose

Table 2
In vitro PTP1B inhibitory activities of diaryl-methanone compounds **13**–**21**.



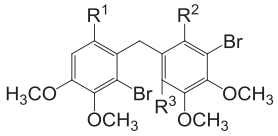


Compds	R^1	R^2	R^3	Inhibition (%)		IC_{50} (μM)
				20 μg/mL	5 μg/mL	
13	H	H	CH_3	28.86	ND	ND
14	Br	H	CH_3	36.68	ND	ND
15	Br	Br	CH_3	48.64	ND	ND
16	Br	Br	COOH	29.90	ND	ND
17	Br	Br	CO_2CH_3	55.34	23.82[a]	ND
20	Br	Br	CH_2OCH_3	61.75	17.73[a]	ND
21	Br	Br	$CH_2OCH_2CH_3$	73.83	17.63[a]	ND

Compds	X	Inhibition (%)		IC_{50} (μM)
		20 μg/mL	5 μg/mL	
18	O	10.57	ND	ND
19	H	11.41	ND	ND

ND = not determined.
[a] Values were tested only if its inhibitory ratio greater than 50% at the dose of 20 μg/mL.

Table 3
In vitro PTP1B inhibitory activities of multi-brominated compounds **23**–**29**.



Compds	R^1	R^2	R^3	Inhibition (%)		IC_{50} (μM)
				20 μg/mL	5 μg/mL	
23	CH_3	H	H	14.46	ND	ND
24	CH_2OH	H	H	5.62	ND	ND
25	$CH_2OCH_2CH_3$	H	H	50.60	17.33[a]	ND
28	CH_3	Br	Br	72.64	38.25[a]	ND
29	CH_3	Br	Br	75.31	40.13[a]	ND

ND = not determined.
[a] Values were tested only if its inhibitory ratio greater than 50% at the dose of 20 μg/mL.

groups. With regard to glycated serum protein (GSP), another important indicator for diabetes control, compound **9** also remarkably lowered GSP level ($P < 0.05$) at 50 mg/kg.

4. Conclusions

In summary, we have prepared a series of bromophenol derivatives to search for potent PTP1B inhibitors. The preliminary structure–activity relationship acquired showed that alkoxyl methylene group attached to the phenyl ring and the diarylmethane scaffold are favorable to PTP1B inhibitory activity. Among these derivatives, compound **9** exhibited enhanced inhibitory activity against PTP1B with IC_{50} of 1.50 μM and high selectivity against other PTPs. More importantly, compound **9** was also effective at lowering glucose and HbA1c in *db*/*db* mouse model at 25 and 50 mg/kg. Efforts in safety evaluation will be reported in due course.

5. Experimental

5.1. General

Reagents and all solvents were analytically pure and were used without further purification. All of the experiments were monitored by analytical thin-layer chromatography (TLC) performed on silica gel GF254 precoated plates. After elution, the plate was visualized under UV illumination at 254 nm for UV active

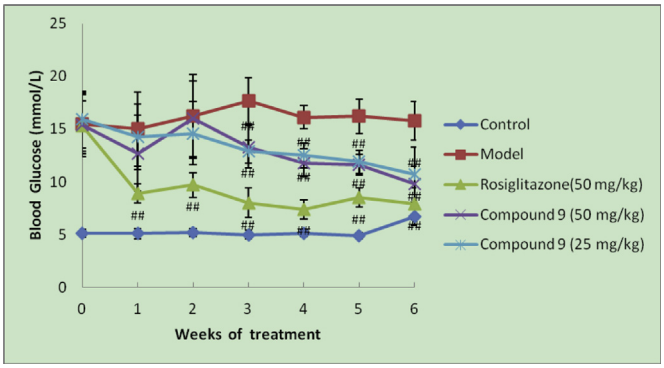


Fig. 2. Effects of compound **9** on blood glucose in *db*/*db* mouse during 6 weeks treatment ($n = 10$) $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs model.

Table 4
Antidyslipidemic activities of compound **9** in *db*/*db* mouse model ($n = 10$, $\bar{x} \pm s$).

Group	Dose (mg/kg)	Triglycerides (mmol/L)			Total cholesterol (mmol/L)		
		Baseline	2 weeks	6 weeks	Baseline	2 weeks	6 weeks
Control		1.16 ± 0.09	1.08 ± 0.07	1.05 ± 0.11	3.6 ± 0.2	3.5 ± 0.2	3.3 ± 0.5
Model		1.30 ± 0.25	1.24 ± 0.12**	1.56 ± 0.17**	6.2 ± 1.7**	9.8 ± 0.7**	9.2 ± 1.2**
Compd **9**	50	1.34 ± 0.18	1.35 ± 0.19	1.48 ± 0.26	6.2 ± 1.5	7.8 ± 0.9##	9.1 ± 0.9
	25	1.30 ± 0.16	1.16 ± 0.19	1.41 ± 0.13	6.2 ± 1.9	7.7 ± 1.1##	8.5 ± 0.7
Rosiglitazone	50	1.31 ± 0.38	1.06 ± 0.19#	1.29 ± 0.14##	6.1 ± 1.7	6.5 ± 0.4##	7.7 ± 1.0#

#$P < 0.05$.
##$P < 0.01$ vs model.
**$P < 0.01$ vs control.

materials or coloration by 95% $FeCl_3$–EtOH solution. Column chromatography was carried out using silica gel (200–300 mesh). All final compounds were purified to >95%, purity, as determined by high-performance liquid chromatography (HPLC). Melting points were determined using Boetius electrothermal capillary melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on an Inova (500 MHz) NMR spectrometer for proton and at 125 MHz for carbon. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane (TMS) as an internal standard. Multiplicities are given as s (singlet), d (doublet), dd (double–doublet), t (triplet), q (quadruplet), m (multiplet) and br s (broad signal). Mass spectra and high-resolution mass spectral (HRMS) data were recorded on Autospec Ultima-Tof and Thermo LTQ-Orbitrap mass spectrometer, respectively.

5.2. 2,3-Dibromo-1-(2′-bromo-3′,4′-dimethoxy-6′-methylbenzyl)-4,5-dimethoxy benzene (**3**)

To a stirred solution of **1** (2.31 g, 0.01 mol) and **2** (3.26 g, 0.01 mol) in CH_2Cl_2 (50 mL) at 0 °C, $AlCl_3$ (1.34 g, 0.01 mol) was added. After stirring for 30 min at room temperature, the mixture was poured into ice water and washed with 1 mol/L HCl. The CH_2Cl_2 layer was concentrated and the residue was recrystallized from CH_3OH to afford **3** as white solid (4.8 g, 89% yield). mp: 115–117 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.76 (s, 1H), 6.15 (s, 1H), 4.19 (s, 2H), 3.89 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H), 3.56 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 152.5, 152.0, 146.1, 144.9, 135.8, 134.3, 129.6, 122.1, 121.7, 117.6, 113.8, 111.7, 60.4, 56.1, 56.0, 40.5, 20.6. EIMS *m*/*z* (% relative intensity): 542/540/538/536 $[M]^+$ (33/100/100/33).

5.3. 3,4-Dibromo-5-(2-bromo-3,4-dihydroxy-6-methylbenzyl) benzene-1,2-diol (**4**)

Compound **3** (1 g, 1.85 mmol) was dissolved in dry CH_2Cl_2 (15 mL), then BBr_3 (15 mL, 1 mol/L in CH_2Cl_2) was added dropwise while stirring in ice bath. The reaction mixture stirred for further 4 h at room temperature. Then the solution was poured into ice-cold water and extracted with ethyl acetate. The organic extracts was dried over anhydrous Na_2SO_4, and evaporated in vacuo to provide the brownish residue. The residue was purified over silica gel column chromatography ($CHCl_3$:MeOH, 15:1) to afford **4** as yellowish solid (0.8 g, 90% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.73 (s, 1H), 9.59 (s, 1H), 9.30 (s, 1H), 8.89 (s, 1H), 6.70 (s, 1H), 6.06 (s, 1H), 3.94 (s, 2H), 2.02 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ145.1 (C), 144.5 (C), 142.7 (C), 141.1 (C), 130.1 (C), 128.1 (C), 127.1 (C), 116.3 (CH), 114.5 (CH), 114.3 (C), 113.6 (C), 113.1 (C), 48.5 (CH_2), 19.3 (CH_3). EIMS *m*/*z* (% relative intensity): 486/484/482/480 $[M]^+$ (6/20/20/6). HRMS *m*/*z* calcd for $C_{14}H_{10}O_4Br_3$ $[M - H]^-$, 478.8129; found, 478.8126.

Table 5
HbA1c and GSP levels of compound **9** in *db*/*db* mouse model ($n = 10$, $\bar{x} \pm s$).

Group	Dose (mg/kg)	HbA1c (%)	GSP (mmol/L)
Control		2.65 ± 0.40	1.62 ± 0.27
Model		4.90 ± 0.31**	4.44 ± 1.42**
Compd **9**	50	3.03 ± 0.31##	3.00 ± 0.62#
	25	3.24 ± 0.15##	3.29 ± 0.50
Rosiglitazone	50	2.56 ± 0.44##	2.60 ± 0.58##

#$P < 0.05$.
##$P < 0.01$ vs model.
**$P < 0.01$ vs control.

5.4. (3-Bromo-2-(2′,3′-dibromo-4′,5′-dimethoxybenzyl)-4,5-dimethoxyphenyl)-methanol (**5**)

Under *hv* condition, to a solution of **3** (5.39 g, 0.01 mol) and AIBN (200 mg, 0.001 mol) in CCl_4 (100 mL) was added NBS (1.96 g, 0.011 mol) in three portions with stirring. After 30 min, the mixture was filtered, and the filtrate was concentrated and purified over silica gel column chromatography (petroleum ether:ethyl acetate, 8:1) to obtain the benzyl bromide (2.82 g, 45%). The benzyl bromide was added to the mixture of K_2CO_3 (4 g, 0.03 mol), H_2O (20 mL) and 1,4-dioxane (20 mL). After refluxing for 3 h, the resulting mixture was cooled to room temperature and extracted with $CHCl_3$. The organic layer was collected and removed under reduced pressure to give **4** as white solid (2.27 g, 91% yield). mp: 164–166 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.09 (s, 1H), 6.14 (s, 1H), 4.52 (s, 2H), 4.22 (s, 2H), 3.91 (s, 3H), 3.86 (s, 3H), 3.79 (s, 3H), 3.55 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 152.4, 146.1, 145.9, 136.6, 135.8, 128.7, 122.5, 121.7, 117.4, 111.8, 111.4, 63.0, 60.4, 56.0, 39.2. EIMS *m*/*z* (% relative intensity): 556/554/552/550 $[M]^+$ (13/38/38/12).

5.5. General procedures for the synthesis of compounds **6**–**9**

Demethylation of **5** via BBr_3 was carried out as described for compound **4**. The ethyl acetate layer was collected and concentrated, then the residue was used without purification. The residue and 85% H_3PO_4 (1 mL) were dissolved in corresponding alcohol (10 mL). After stirring for 2 h under reflux, the solvent was removed under reduced pressure. The residue was purified over silica gel column chromatography (petroleum ether:ethyl acetate, 2:1) to produce a yellow solid.

5.5.1. 3,4-Dibromo-5-(2-bromo-3,4-dihydroxy-6-(methoxymethyl) benzyl)benzene-1,2-diol (**6**)

90% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.72 (s, 2H), 9.30 (s, 2H), 6.87 (s, 1H), 6.04 (s, 1H), 4.12 (s, 2H), 3.97 (s, 2H), 3.18 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 144.9 (C), 144.2 (C), 142.6 (2× C), 130.5 (C), 128.6 (C), 127.5 (C), 115.4 (CH), 114.5 (C), 114.3 (C), 113.8 (CH), 113.0 (C), 72.0 (CH_2), 57.3 (CH_3), 38.2 (CH_2).

*5.5.2. 3,4-Dibromo-5-(2-bromo-3,4-dihydroxy-6-(propoxymethyl) benzyl)benzene-1,2-diol (**7**)*

65% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.75 (s, 1H), 9.69 (s, 1H), 9.27 (s, 1H), 9.14 (s, 1H), 6.88 (s, 1H), 6.05 (s, 1H), 4.17 (s, 2H), 3.99 (s, 2H), 3.26 (t, J = 6.52, 2H), 1.14 (m, 2H), 0.79 (t, J = 7.36, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 144.9 (C), 144.3 (C), 142.5 (2× C), 130.5 (C), 129.0 (C), 127.5 (C), 115.4 (CH), 114.6 (C), 114.3 (CH), 113.9 (C), 112.9 (C), 71.2 (CH_2), 70.3 (CH_2), 38.3 (CH_2), 22.2 (CH_2), 10.4 (CH_3). EIMS m/z (% relative intensity): 544/542/540/538 $[M]^+$ (1/3/3/1). HRMS m/z calcd for $C_{17}H_{16}O_5Br_3$ $[M-H]^-$, 536.8548; found, 536.8570.

*5.5.3. 3,4-Dibromo-5-(2-bromo-6-(butoxymethyl)-3,4-dihydroxy-benzyl)benzene-1,2-diol (**8**)*

58% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.75 (s, 1H), 9.69 (s, 1H), 9.27 (s, 1H), 9.15 (s, 1H), 6.87 (s, 1H), 6.04 (s, 1H), 4.16 (s, 2H), 3.98 (s, 2H), 3.29 (t, J = 6.44, 2H), 1.36 (m, 2H), 1.22 (m, 2H), 0.80 (t, J = 7.35, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 144.9 (C), 144.2 (C), 142.5 (2× C), 130.5 (C), 129.0 (C), 127.5 (C), 115.4 (CH), 114.6 (C), 114.3 (CH), 113.9 (C), 112.9 (C), 70.4 (CH_2), 69.2 (CH_2), 38.3 (CH_2), 31.1 (CH_2), 18.7 (CH_2), 13.6 (CH_3). EIMS m/z (% relative intensity): 558/556/554/552 $[M]^+$ (1/3/3/1). HRMS m/z calcd for $C_{18}H_{18}O_5Br_3$ $[M-H]^-$, 550.8704; found, 550.8688.

*5.5.4. 3,4-Dibromo-5-(2-bromo-3,4-dihydroxy-6-(isobutoxymethyl) benzyl)benzene-1,2-diol (**9**)*

54% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.75 (s, 1H), 9.69 (s, 1H), 9.27 (s, 1H), 9.15 (s, 1H), 6.88 (s, 1H), 6.04 (s, 1H), 4.16 (s, 2H), 3.98 (s, 2H), 3.08 (d, J = 6.48, 2H), 1.68 (m, 1H), 0.78 (d, J = 6.68, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 144.9 (C), 144.2 (C), 142.5 (2× C), 130.5 (C), 129.0 (C), 127.5 (C), 115.4 (CH), 114.6 (C), 114.3 (CH), 113.9 (C), 113.0 (C), 76.4 (CH_2), 70.6 (CH_2), 38.3 (CH_2), 27.8 (CH), 19.1 (2× CH_3). EIMS m/z (% relative intensity): 558/556/554/552 $[M]^+$ (1/3/3/1). HRMS m/z calcd for $C_{18}H_{18}O_5Br_3$ $[M-H]^-$, 550.8704; found, 550.8680.

*5.6. General procedures for the synthesis of compounds **13**–**15***

To the suspension of corresponding aryl acids **10**–**12** (7 mmol) in TFAA (15 mL) were added 85% H_3PO_4 (0.5 mL) and **1** (7 mmol) under ice-bath, and the mixture was heated to reflux and stirred for further 2 h. The mixture was poured into ice-cold water and extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was recrystallized in ethyl alcohol to give **13**–**15** as white solid.

*5.6.1. (2-Bromo-3,4-dimethoxy-6-methylphenyl)(3,4-dimethoxy-phenyl)methanone (**13**)*

81% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.59 (s, 1H), 7.18 (d, J = 8.14, 1H), 6.81 (d, J = 8.14, 1H), 6.75 (s, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H), 3.84 (s, 3H), 2.14 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 195.1 (CO), 154.1, 153.6, 149.5, 144.5, 133.6, 132.0, 129.7, 125.6, 114.8, 113.6, 110.6, 110.2, 60.6 (CH_3), 56.1 (CH_3), 56.0 (2× CH_3), 19.5 (CH_3). EIMS m/z (% relative intensity): 396/394 $[M]^+$ (58/58).

*5.6.2. (2-Bromo-3,4-dimethoxy-6-methylphenyl)(3-bromo-4,5-dimethoxyphenyl)methanone (**14**)*

80% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.48 (s, 1H), 7.34 (s, 1H), 6.75 (s, 1H), 3.90 (s, 3H), 3.85 (s, 6H), 3.80 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 194.2, 154.0, 152.0, 150.8, 144.6, 135.7, 133.7, 133.0, 124.3, 116.3, 115.5, 115.1, 113.8, 60.6, 60.5, 56.3, 56.0, 20.0. EIMS m/z (% relative intensity): 476/474/472 $[M]^+$ (47/95/47).

*5.6.3. (2-Bromo-3,4-dimethoxy-6-methylphenyl)(2,3-dibromo-4,5-dimethoxyphenyl)methanone (**15**)*

90% yield, mp: 86–88 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.16 (s, 1H), 6.74 (s, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 194.2 (C), 154.0 (C), 152.0 (C), 150.8 (C), 144.6 (C), 135.7 (C), 133.7 (C), 133.0 (C), 124.3 (C), 116.3 (C), 115.5 (C), 115.1 (C), 113.8 (C), 60.6 (C), 60.5 (C), 56.3 (C), 56.0 (C), 20.0 (C). EIMS m/z (% relative intensity): 556/554/552/550 $[M]^+$ (20/52/52/20).

*5.7. 3-Bromo-2-(2,3-dibromo-4,5-dimethoxybenzoyl)-4,5-dimethoxybenzoic acid (**16**)*

Aryl acid **15** (1 g, 1.8 mmol) was dissolved in mixture of *t*-BuOH and H_2O (1:1, 20 mL). The mixture was heated to 80 °C, then $KMnO_4$ (0.57 g, 3.6 mmol) was added over 1 h and TLC was used to monitor the reaction. After the reaction, MnO_2 was filtered and the filtrate was acidified to pH = 2 by adding 10% HCl. The precipitate was collected to afford **17** as white solid (0.64 g, 61% yield). mp: 167–170 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.59 (s, 1H), 7.31 (s, 1H), 3.94 (s, 3H), 3.82 (s, 6H), 3.74 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 191.6 (CO), 166.2 (COOH), 153.4, 152.0, 150.4, 149.3, 135.8, 134.0, 127.3, 124.2, 116.4, 116.1, 115.5, 114.2, 60.9 (CH_3), 60.8 (CH_3), 56.8 (2× CH_3). EIMS m/z (% relative intensity): 586/584/582/580 $[M]^+$ (3/10/10/3).

*5.8. Methyl 3-bromo-2-(2,3-dibromo-4,5-dimethoxybenzoyl)-4,5-dimethoxybenzoate (**17**)*

To the solution of **16** (0.58 g, 1 mmol) in CH_3OH (10 mL) was added concd. H_2SO_4 (0.5 mL). The mixture was heated to reflux for further12 h. The mixture was removed under reduced pressure. The residue was recrystallized from CH_3OH and H_2O to give **17** as white solid (0.49 g, 82% yield). mp: 176–178 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.56 (s, 1H), 7.50 (s, 1H), 3.97 (s, 3H), 3.92 (s, 6H), 3.83 (s, 3H), 3.76 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 191.7 (CO), 165.1 (COO), 153.1 (C), 151.9 (C), 151.0 (C), 150.1 (C), 136.9 (C), 133.5 (C), 125.5 (C), 124.5 (C), 117.3 (C), 116.0 (C), 115.7 (C), 113.6 (C), 60.8 (OCH_3), 60.6 (OCH_3), 56.3 (2× OCH_3), 52.7 (C). EIMS m/z (% relative intensity): 600/598/596/594 $[M]^+$ (8/23/23/8).

*5.9. 4-Bromo-3-(2,3-dibromo-4,5-dimethoxyphenyl)-5,6-dimethoxyisobenzofuran-1(3H)-one (**18**)*

16 (1 g, 1.7 mmol) was suspended into 10% KOH solution (40 mL), then Zn–Cu complex (6 g) was added, the mixture was heated to refluxed for 10 h. The complex was filtered. After acidized by 10% HCl, the filtrate was extracted with CH_2Cl_2. The CH_2Cl_2 layer was concentrated and the residue was recrystallized in C_2H_5OH–CH_3COCH_3 (1:1) to give **18** as white solid (0.52 g, 53% yield). mp: 188–190 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.33 (s, 1H), 6.86 (d, J = 7.5, 2H), 6.70 (s, 1H), 6.65 (s, 1H), 6.23 (s, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.9 (CO), 154.9 (C), 150.6 (C), 149.9 (C), 149.3 (C), 144.1 (C), 128.7 (C), 120.2 (CH), 117.7 (C), 111.0 (CH), 109.9 (CH), 105.7 (CH), 104.0 (CH), 82.4 (CH), 56.3 (2× CH_3), 55.9 (2× CH_3).

*5.10. 7-Bromo-1-(2,3-dibromo-4,5-dimethoxyphenyl)-5,6-dimethoxy-1,3-dihydroisobenzofuran (**19**)*

Compound **16** (0.5 g, 0.9 mmol), $NaBH_4$ (0.35 g, 9 mmol) and $AlCl_3$ (1.1 g, 8 mmol) were dissolved in THF (20 mL) and the mixture was heated to reflux for 10 h. Then the mixture was poured into ice-water, and extracted with ethyl ether. The ethyl ether layer was collected and removed under reduced pressure. The residue was purified over silica gel column chromatography (petroleum ether:ethyl acetate, 3:1) to produce **19** as white solid (0.2 g, 42% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.83 (s, 1H), 6.61 (s, 1H), 6.39 (s, 1H), 5.26 (d, J = 12, 1H), 5.15 (d, J = 12, 1H), 3.93 (s, 3H), 3.84 (s, 6H),

3.66 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 154.2, 152.6, 147.8, 146.3, 136.6, 136.3, 132.3, 122.4, 118.6, 112.8, 112.2, 103.9, 87.7, 73.6, 60.7 (CH_3), 60.5 (CH_3), 56.3 (CH_3), 56.1 (CH_3). MS: *m*/*z* 556/554/552/550 $[M]^+$ (15/46/47/17).

5.11. General procedures for the synthesis of compounds **20**–**21**

The title compounds were prepared from **15** using the procedure previously described for compounds **6**–**9** and were purified by silica gel column chromatography (petroleum ether:ethyl acetate, 3:1) to give yellowish slurry.

5.11.1. (2-Bromo-3,4-dimethoxy-6-(methoxymethyl)phenyl)(2,3-dibromo-4,5-dimethoxyphenyl)methanone (**20**)

59% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.17 (s, 1H), 7.04 (s, 1H), 4.44 (s, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.82 (s, 3H), 3.76 (s, 3H), 3.35 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 193.7 (C), 154.2 (C), 151.8 (C), 150.5 (C), 145.7 (C), 135.7 (C), 135.1 (C), 132.1 (C), 124.2 (C), 116.5 (C), 116.1 (C), 115.5 (C), 111.7 (C), 69.9 (C), 63.5 (C), 60.7 (C), 60.6 (C), 56.3 (C), 56.1 (C). EIMS *m*/*z* (% relative intensity): 586/584/582/580 $[M]^+$ (3/9/9/3).

5.11.2. (2-Bromo-6-(ethoxymethyl)-3,4-dimethoxyphenyl)(2,3-dibromo-4,5-dimethoxyphenyl)methanone (**21**)

56% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.17 (s, 1H), 7.04 (s, 1H), 4.44 (s, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.82 (s, 3H), 3.76 (s, 3H), 3.43 (q, J = 7 Hz, 2H), 1.08 (t, J = 7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 193.7 (C), 154.2 (C), 151.8 (C), 150.5 (C), 145.7 (C), 135.7 (C), 135.1 (C), 132.1 (C), 124.2 (C), 116.5 (C), 116.1 (C), 115.5 (C), 111.7 (C), 69.9 (C), 66.5 (C), 60.7 (C), 60.6 (C), 56.3 (C), 56.1 (C), 14.9 (C). EIMS *m*/*z* (% relative intensity): 600/598/596/594 $[M]^+$ (2/7/7/2).

5.12. 3-Bromo-2-(3-bromo-4,5-dimethoxybenzyl)-4,5-dimethoxy-1-methylbenzene (**23**)

The title compound was prepared from **1** and **22** using the procedure previously described for compound **3** and was purified by recrystallization from CH_3OH to give white solid. 79% yield. mp: 116–118 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.76 (d, J = 1.83 Hz, 1H), 6.72 (s, 1H), 6.60 (d, J = 1.83 Hz, 1H), 4.11 (s, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H), 3.77 (s, 3H), 2.23 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 153.5, 151.8, 144.8, 144.7, 136.5, 133.9, 129.9, 123.8, 121.9, 117.5, 113.9, 111.8, 60.5, 60.4, 56.1, 56.0, 37.8, 20.8. EIMS *m*/*z* (% relative intensity): 462/460/458 $[M]^+$ (45/95/50).

5.13. (3-Bromo-2-(3-bromo-4,5-dimethoxybenzyl)-4,5-dimethoxyphenyl)methanol (**24**)

The title compound was prepared from **23** using the procedure previously described for compound **5** to afford white solid. 32% yield. mp: 156–158 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.05 (s, 1H), 6.74 (s, 1H), 6.60 (s, 1H), 4.59 (s, 2H), 4.17 (s, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.79 (s, 3H), 3.76 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 153.6, 152.2, 145.9, 144.8, 136.5, 136.4, 129.2, 123.7, 122.3, 117.6, 111.7, 111.6, 63.3, 60.5, 60.4, 56.1, 56.0, 36.5.

5.14. 3-Bromo-4-(3-bromo-4,5-dihydroxybenzyl)-5-(ethoxymethyl)-benzene-1,2-diol (**25**)

The title compound was prepared from **24** using the procedure previously described for compound **6**–**9** and was purified by silica gel column chromatography (petroleum ether:ethyl acetate, 1:1) to give yellowish solid. 90% yield. ^{1}H NMR (500 MHz, acetone-d_6) δ: 6.97 (s, 1H), 6.73 (d, J = 2.4 Hz, 1H), 6.49 (d, J = 2.4 Hz, 1H), 4.32 (s, 2H), 4.08 (s, 2H), 3.43 (q, J = 7.2 Hz, 2H), 1.10 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ: 146.1, 144.2, 142.9, 141.4, 133.1, 130.5, 129.7, 123.2, 115.8, 114.7, 114.5, 109.6, 70.9, 65.7, 36.1, 15.1. EIMS *m*/*z* (% relative intensity): 450/448/446 $[M]^+$ (1.5/3/1.5).

5.15. 2,3,6-Tribromo-4,5-dimethoxybenzaldehyde (**26**)

To the suspension of **1** (2.45 g, 0.01 mol) in concd. H_2SO_4 (20 mL) was added NBS (3.9 g, 0.02 mmol) under ice-bath, and the mixture was stirred for further 2 h at room temperature. The mixture was poured into ice-cold water and extracted with $CHCl_3$. The combined extracts were washed with Na_2CO_3 and dried over anhydrous Na_2SO_4, and evaporated in vacuo to provide the brownish residue. The residue was purified by silica gel column chromatography (petroleum ether:ethyl acetate, 1:1) to give white solid (3.2 g, 79% yield). mp: 160–162 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.10 (s, 1H), 4.00 (s, 3H), 3.93 (s, 3H).

5.16. (2,3,6-Tribromo-4,5-dimethoxyphenyl)methanol (**27**)

To a solution of compound **26** (3.89 g, 10 mmol) in CH_3OH (20 mL) was added $NaBH_4$ (0.2 g, 5 mmol) over 10 min. After the reaction, the mixture was acidified with 10% HCl and poured into water. The mixture was extracted with CH_2Cl_2. The organic phase was combined and dried over anhydrous Na_2SO_4 and concentrated in vacuo to give **27** as white solid (3.64 g, 90% yield).

5.17. 1,2,4-Tribromo-3-(2-bromo-3,4-dimethoxy-6-methylbenzyl)-5,6-dimethoxybenzene (**28**)

The title compound was prepared from **1** and **27** using the procedure previously described for compound **3** and was purified by recrystallization from CH_3OH to give white solid. 74% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.58 (s, 1H), 4.68 (s, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 151.2, 150.6, 150.4, 137.4, 133.5, 129.0, 126.5, 123.2, 122.8, 122.0, 121.8, 114.2, 60.5, 60.4, 56.0, 55.9, 43.3, 21.3.

5.18. 1,2,4-Tribromo-3-(2,5-dibromo-3,4-dimethoxy-6-methylbenzyl)-5,6-dimethoxybenzene (**29**)

The title compound was prepared from **28** using the procedure previously described for compound **26** to give white solid. 53% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.78 (s, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 3.86 (s, 6H), 2.14 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 150.6, 150.5, 149.6, 149.0, 136.8, 134.2, 134.0, 123.0, 122.2, 122.1, 121.4, 121.1, 60.9, 60.7, 44.2, 21.1.

5.19. Enzymatic activity assay in vitro

Recombinant human GST-PTP1B (hGST-PTP1B) protein was used to measure the PTP1B inhibitory activities of synthetic compounds. The assay was conducted as described by Zhang et al. [23] with minor modifications. HPN was dissolved in DMSO and distributed to 96-well clear polystyrene plate. DMSO was distributed as the full enzyme activity. After adding an assay mixture, hGST-PTP1B was added to initiate the reaction. The enzymatic activity assay was carried out in a mixture containing 50 mM MOPS, pH 6.5, 2 mM *p*NPP, 30 nM PTP1B and 2% DMSO, and the catalysis of *p*NPP was continuously monitored at 405 nm for 2 min at 30 °C. Inhibitory rate was calculated according to the formula:

$$\%\,\text{inhibition} = 100 \times \left(V_{\text{DMSO}} - V_{\text{sample}}\right) \Big/ V_{\text{DMSO}}.$$

IC_{50} value was determined with 30 nM PTP1B, 2 mM *p*NPP in 50 mM MOPS at pH 6.5 and the inhibitors diluted around the

estimated IC_{50} values. IC_{50} was calculated from the non-linear curve fitting of percent inhibition (% inhibition) vs inhibitor concentration [*I*] by using the following equation:

$$\%\ \text{inhibition} = 100/\{1 + (IC_{50}/[I])k\},$$

where *k* is the Hill coefficient.

To study the inhibition selectivity on other PTP family members, TCPTP, SHP-1, SHP-2 and LAR were prepared as described previously [23]. Assays for these PTPs were performed at the optimal pH for each individual enzyme activity. These enzymes and compound **9** were pre-incubated for 3 min at 4 °C, and the assays were initiated by adding substrates. Assays performed for SHP-1, SHP-2 and LAR were done using OMFP as a substrate. All the assays were carried out in triplicate and the average results are presented.

5.20. Anti-diabetic activity assays in db/db mice

C57BL/KsJ *db*/*db* mice (6–8 weeks age) and their non-diabetic controls (*db*/*dm* mice, 6–8 weeks age) were purchased from Experimental Animal Center of Military Medical Sciences (Beijing, China). The mice were housed in a room controlled for temperature (24 ± 2.0 °C), relative humidity (60–80%) and 12/12 h light/dark cycle (lights on at 6.00 a.m.). All the mice were allowed free access to fresh water and laboratory chow. After one week adaptation period, the mice were divided into five groups ($n = 10$ in each group), the control group (*db*/*dm* mice), the model (0.5% CMC-Na containing 2% tween-80) group, rosiglitazone group (50 mg/kg), compound **9** high-dose group (50 mg/kg) and low-dose group (25 mg/kg). Rosiglitazone was dissolved in distilled water and compound **9** was suspended in 0.5% CMC-Na containing 2% tween-80 and administered orally for six weeks (approximately 0.2 mL/20 g body weight). Body weights and food intakes were measured weekly. Blood glucose concentrations were checked with ONE TOUCH Ultra® glucometer (LifeScan, USA) weekly. Serum triglyceride and total cholesterol levels were measured by an enzymatic method (Whitman Biotech Co., Ltd., Nanjing, China) every two weeks. At the end of 6-week treatment period, the mice were fasted overnight. 1 h after administration, mice were excised eyeballs for blood sampling. Blood samples were collected into heparin-coated tubes. HbA1c concentration was measured by HbA1c reagent (Whitman Biotech Co., Ltd., Nanjing, China). After centrifugation at 3500 g for 15 min at 4 °C, the plasma was carefully removed from the sample. The GSP levels were determined by fructosamine method using GSP assay kit (Whitman Biotech Co., Ltd., Nanjing, China).

Conflict of interest

The authors have declared no conflict of interest.

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