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2-*O*-Carboxymethylpyrogallol derivatives as PTP1B inhibitors with antihyperglycemic activity

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Abstract—2-*O*-Carboxymethylpyrogallol derivatives (**4**–**17**) were synthesized, with their in vitro inhibitory activities against PTP1B and in vivo antihyperglycemic effects examined. Compound **14**, the most potent among the series, showed a K_i value of 1.1 μM against PTP1B, 7-fold lower than that against TC-PTP. When compound **14** was fed to a high-fat diet-induced diabetic mouse model, significant improvements were observed in both the fasting glucose level and glucose tolerance.

Type 2 diabetes mellitus (T2DM) is a serious health threat in modern society.[1] Characterized by hyperglycemia, T2DM is the cause of many complications, including cardiovascular disease, blindness, renal failure, and peripheral nerve damage.[2] Increasing concern about T2DM as health risk factor has led to an intensive research for the development of an efficient treatment.[3]

Insulin resistance is an important factor in the pathogenesis of T2DM and, therefore, insulin sensitizers, such as TZD class molecules, have been used for the treatment of this disease.[4] The recent identification of protein tyrosine phosphatase (PTP) 1B as an enzyme responsible for the dephosphorylation of insulin receptors has raised the possibility that small molecule inhibitors of PTP1B could act as insulin sensitizers and, thus, as antidiabetic drugs.[5] This conception was validated in mouse models; increased insulin sensitivity was observed when the PTP1B gene was disrupted or when its expression was suppressed by PTP1B antisense nucleotides.[6]

The development of PTP1B inhibitors exhibiting favorable pharmacological properties has been a challenge, with only one compound, Ertiprotafib, having progressed to phase 2 clinical trials, before it was stopped due to insufficient efficacy and dose limiting side effects.[7] As for the antidiabetic effect of Ertiprotafib, multiple points of action have been proposed; PPARα, γ and IKK-β were reported as possible targets.[8]

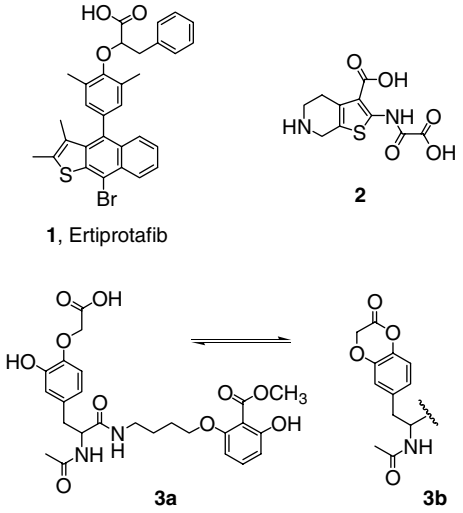

Figure 1.

Keywords: 2-*O*-Carboxymethylpyrogallol; PTP1B inhibitor; Diabetes; Glucose tolerance; Hyperglycemia; Protein tyrosine phosphatase.
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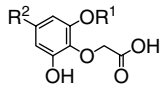
Table 1. Compounds used in this study



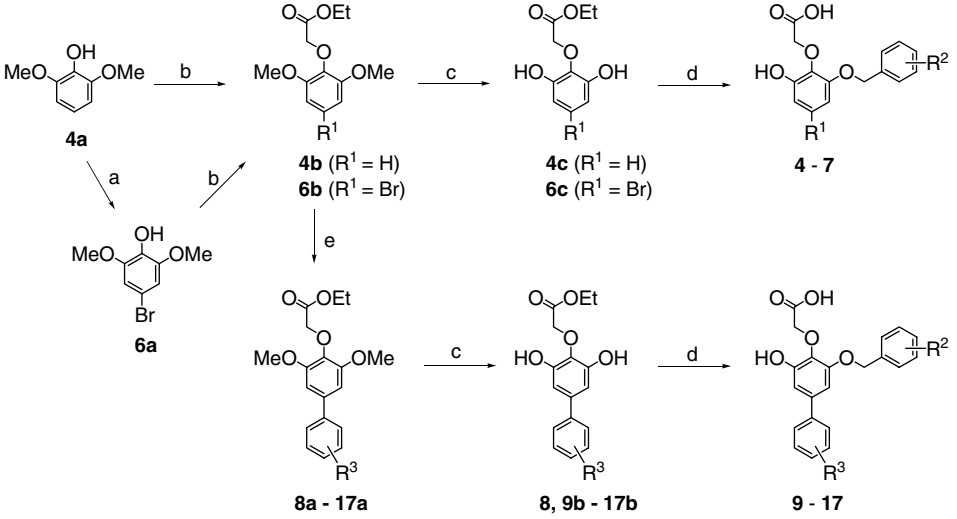
4-17

Compound	R^1	R^2
4	$PhCH_2$	H
5	4-CF_3PhCH_2	H
6	$PhCH_2$	Br
7	4-CF_3PhCH_2	Br
8	H	Ph
9	$PhCH_2$	Ph
10	4-CF_3PhCH_2	Ph
11	$PhCH_2$	3-CF_3Ph
12	4-CF_3PhCH_2	3-CF_3Ph
13	$PhCH_2$	3,5-$(CF_3)_2Ph$
14	4-CF_3PhCH_2	3,5-$(CF_3)_2Ph$
15	$PhCH_2$	4-CF_3Ph
16	4-CF_3PhCH_2	4-CF_3Ph
17	3,5-$(CH_3O)_2PhCH_2$	Ph

Selective inhibition of PTP1B among >100 human PTP family enzymes, especially the differentiation of PTP1B and TC-PTP, have been major concerns in the design of small molecule PTP1B inhibitors. The homology of amino acid residues between PTP1B and TC-PTP reaches 94% at the active site, and achieving PTP1B selectivity has been a challenge.[9] However, recent studies have raised the possibility that the discrimination of the two enzymes may not be crucial.[9b,1a] The insulin sensitivity exhibited by heterozygous $PTP1B^{+/-}$ mice suggests that partial inhibition of PTP1B might afford a therapeutic effect.[6a] The healthy survival of $TC\text{-}PTP^{+/-}$ mice indicates that partial inhibition of TC-PTP might be tolerable.[10] Furthermore, TC-PTP is known to be involved in the regulation of insulin signaling.[11] Taken together, a rather lenient therapeutic window, coupled with dose control, might be the strategy required for drug development targeting PTP1B.[1a]

Another concern in the design of PTP1B inhibitors is the cell permeability of the compounds. Negative charges inherent in simple phosphotyrosine mimetics are unfavorable factors for the translocation of the compounds across the plasma membrane of the cell. Recently, **3a**, containing (2-hydroxyphenoxy)acetic acid as the core structure, was reported to have a high level of cell permeability, and the equilibrium between the acid (**3a**) and the lactone form (**3b**) has been suggested as a possible explanation for the improved membrane penetration (Fig. 1).[12] Previous studies on the design of PTP1B inhibitors in our laboratory have indicated that a bulky substituent (such as $-OR^1$ in Table 1) in the *ortho*-position of the phosphate mimetic group may contribute to the inhibitor–enzyme complex formation.[15] Based on these premises, 2-*O*-carboxymethylpyrogallol derivatives, with a benzyl or a substituted benzyl group at O-1, were synthesized, and their in vitro PTP1B inhibitory activities and in vivo antidiabetic effects examined. The syntheses of compounds **4**–**17** are summarized in Scheme 1. For the synthesis of **14**, 2,6-dimethoxyphenol (**4a**) was brominated, and then treated with ethyl bromoacetate to introduce a carboxymethyl functionality to the central hydroxyl group. The palladium-catalyzed coupling reaction of **6b** with 3,5-di(trifluoromethyl)phenylboronic acid produced **14a**. Removal of the methyl protecting groups from **14a**, followed by controlled alkylation of one of the hydroxyl groups, by reaction with 4-(trifluoromethyl)benzyl bromide, afforded compound **14**.[17] Other compounds were synthesized using similar strategies to that shown in Scheme 1.

The 2-*O*-carboxymethylpyrogallol derivatives were evaluated for their inhibitory activities against PTP1B, with *p*-nitrophenyl phosphate (*p*NPP) used as the substrate. The enzyme and compounds were preincubated for



Scheme 1. Synthetic strategy for the syntheses of 2-*O*-carboxymethylpyrogallol derivatives (**4**–**17**). Reagents and conditions: (a) *N*-bromosuccinimide, $CHCl_3$, C_2H_5OH, NaH, −75 °C → rt → 65 °C, 66%; (b) $BrCH_2CO_2Et$, K_2CO_3, DMF, rt, 6 h, 95%; (c) (i) BBr_3, CH_2Cl_2, −75 °C → rt, 1–3 h; (ii) C_2H_5OH, H_2SO_4, 80 °C, overnight, 35–50% overall; (d) R^2PhCH_2Br, K_2CO_3, acetone, 75 °C, 3–4 h, 25–41%; (e) $R^3PhB(OH)_2$, $Pd(PPh_3)_4$, toluene, C_2H_5OH, 80 °C, overnight, 75–81%.

Table 2. Inhibition of PTPases by compounds **1**–**17**[a]

Compound	IC_{50}[b] [K_i, μM] (μM)					
	PTP1B	TC-PTP	SHP-1cat	LAR-D1	YOP	YPTP-1
1, Ertiprotafib	1.4 (±0.1) [1.5[c]]					
2[13]	500 (±100) [102[c]]					
3[12]	[9.0[d]]	[182[d]]				
4	>1000					
5	>1000					
6	300 (±14)					
7	73 (±5)					
8	>1000					
9	93 (±5)					
10	17 (±1)					
11	2.2 (±0.2)	120 (±20)	38 (±2)	>200	33 (±4)	52 (±4)
12	5.0 (±0.4)	65 (±3)	13 (±1)	>200	21 (±1)	17 (±2)
13	5.7 (±0.4)	37 (±2)	9.0 (±0.3)	>200	15 (±1)	20 (±1)
14	2.0 (±0.1) [1.1[e]]	22 (±1) [8.2[e]]	4.0 (±0.2)	116 (±8)	6.0 (±0.5)	9.0 (±0.4)
15	13 (±1)	96 (±4)	25 (±1)	>200	34 (±2)	17 (±2)
16	5.4 (±0.3)	44 (±3)	12 (±1)	>200	13 (±1)	23 (±2)
17	40 (±3)					

[a] The enzyme reaction was initiated by the addition of *p*NPP to the enzyme in assay buffer, preincubated for 10 min with inhibitors dissolved in DMSO. The final assay mixture contained: 2 mM *p*NPP, 40 nM PTP1B (or 5 μg/mL for SHP-1cat, 33 U (manufacturer's definition)/mL for LAR-D1 and TC-PTP, 50 U (manufacturer's definition)/mL for YOP, and 15 nM for YPTP-1), 50 mM Hepes, and 5 mM EDTA, at pH 7, plus 10% enzyme dilution buffer (25 mM Hepes, 5 mM EDTA, 1 mM DTT, and 1 mg/mL bovine serum albumin, at pH 7.3). After incubation at 37 °C for 3 min, 0.5 M NaOH (0.95 mL) was added, and the A_{405} measured to determine the amount of *p*-nitrophenol released. The IC_{50} values of the inhibitors were determined by measuring the PTPase activity with a range of different inhibitor concentrations. The PTPs used in this study were obtained as described previously, except the native form of PTP1B was used.[14]

[b] Values are means (± standard deviations) of 2–3 experiments. The kinetic data were analyzed using the GraFit 5.0 program (Erithacus Software).

[c] Reproduced from our previous report.[15]

[d] Reproduced from a previous report.[12]

[e] The K_i value for **14** was determined using the relationships: $K_i^{app} = ([S] + K_m)/(K_m/K_i + [S]/\alpha K_i)$ and $v_s/v_o = 1/([I]/K_i^{app} + 1)$.[16]

10 min prior to the initiation of the enzyme reaction by the addition of the substrate. As shown in Table 2, low micromolar IC_{50} values were observed for compounds **11**–**16**. Compound **14** was found to be the most potent of the derivatives, with a K_i value of 1.1 μM, which was low compared to both **3** (K_i = 9.0 μM) and Ertiprotafib (K_i = 1.5 μM).[12,15] Table 2 also shows the selectivity profiles of compounds **11**–**16** against several phosphatases. These compounds demonstrated 5- to 13-fold greater PTP1B selectivity against TC-PTP. Compound **14** showed a 7-fold greater selectivity for PTP1B versus TC-PTP.

The antihyperglycemic effect of **14** was examined in a high-fat diet (HFD)-induced diabetic mouse model.[18] After 8 wks on HFD, the mice (C57BL/6JCr Slc, male) were fed HFD or HFD + **14** for a further 4 weeks. The compound **14** was administered as mixtures with the food (1.0 g of **14** per kg of diet). The daily uptake of **14** was approximated as 3 mg/day/mouse, which is equivalent to 85 mg/day/kg of mouse weight. At the end of the 4 weeks of drug-feeding period, the fasting glucose level of the drug-fed group was significantly lower than the HFD control group (Fig. 2, 0 min). After the injection of glucose, the drug-fed group exhibited a significantly faster decrease in the blood glucose concentration compared to the HFD control group (Fig. 2). In contrast to the antihyperglycemic effect, no difference was observed in body weights between the HFD and HFD + **14** groups.

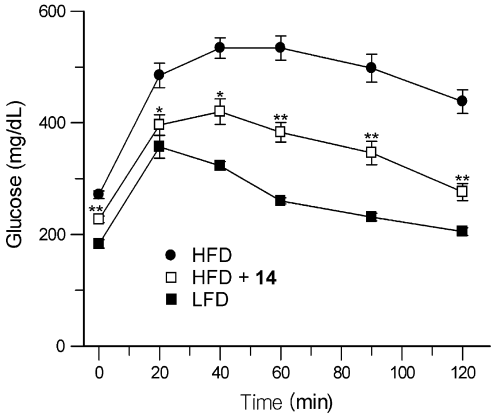


Figure 2. Intraperitoneal glucose tolerance test. Five-wk-old mice (C57BL/6J Cr Slc, male), acclimatized for 1 wk, were fed HFD for 8 wks, and then divided into two groups (7 mice/group). The two groups were fed HFD (●) or HFD + **14** (□) for 4 weeks. The lean control group (■) was fed LFD all throughout the entire 12 wk period. The mice were then fasted for 6 h, starting from the beginning of the light cycle. Just before the intraperitoneal injection of glucose (1 g/kg body weight), blood was withdrawn from the tip of the tail, and the baseline glucose level measured. After the injection of glucose, tail blood was collected at 20, 40, 60, 90, and 120 min later, with the blood glucose concentration determined using an Accu-Chek® Active diagnostic kit (Roche, Germany). All values are mean values ± SEM; n = 7/group. Statistical comparisons between HFD and HFD + **14** groups were performed using a 1-way ANOVA, where, $^{*}p < 0.01$ and $^{**}p < 0.001$.

In summary, a series of 2-*O*-carboxymethylpyrogallol derivatives were synthesized in an attempt to improve the inhibitory potency of (2-hydroxyphenoxy)acetic acid derivatives against PTP1B. Among the compounds, **14** exhibited a K_i value of 1.1 μM against PTP1B and displayed in vivo efficacy, which significantly lowered the fasting glucose level and improved the glucose tolerance in an obesity-induced diabetic mouse model.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.08.019.

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17. Compound **14**: mp 177–179 °C; ^{1}H NMR (acetone-d_6, 200 MHz), δ 8.23 (s, 2H), 8.00 (s, 1H), 7.81–7.78 (m, 4H), 7.18 (d, J = 2.2 Hz, 1H), 7.00 (d, J = 2.2 Hz, 1H), 5.44 (s, 2H), 4.85 (s, 2H); ^{13}C NMR (acetone-d_6, 100 MHz): δ 175.32 (CO_2H), 153.24, 152.45, 144.20, 142.87, 137.80, 135.67, 132.91, 132.26, 129.05, 128.86, 128.33, 126.44, 126.36, 121.89, 110.00, 105.35, 70.84 (OCH_2), 70.63 (OCH_2).
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