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Synthesis and biological evaluation of novel *N*-(alkoxyphenyl)-aminocarbonylbenzoic acid derivatives as PTP1B inhibitors

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Abstract

Based on the fact that petroselinic acid showed good inhibitory activity (IC_{50} = 6.99 μmol/L) against protein tyrosine phophatase 1B(PTP1B) *in vitro*, a series of novel *N*-(alkoxyphenyl)-aminocarbonylbenzoic acid derivatives were designed and synthesized. The results indicated that most of the derivatives showed more potent activities against PTP1B. Especially, compound **13** had obvious activity with an IC_{50} of 106 nmol/L *in vitro*.

Keywords: Petroselinic acid; PTP1B; *N*-(Alkoxyphenyl)-aminocarbonylbenzoic acid derivatives; Synthesis; Diabetes

Type II diabetes mellitus (T2DM) is a metabolic disease that is characterized by insulin resistance, resulting in impaired glucose homeostasis [1]. An effective way of treating this disease is enhancing insulin action and lowering plasma glucose to regulate glucose homeostasis [2,3]. Recent studies indicate that protein tyrosine phophatase 1B (PTP1B), which is a negative regulator of insulin, play a key role in developing insulin resistance [4,5]. Therefore, PTP1B is regarded as a novel drug target for T2DM [6,7]. Up to now, many small molecule inhibitors targeting PTP1B have been reported [8–12].

In general, natural products are an important source of lead compounds. Petroselinic acid (Fig. 1), a natural product, was found to show good PTP1B inhibitory activity (IC_{50}: 6.99 μmol/L) *in vitro*, but not *in vivo* (unpublished data). Based on this result, we supposed that petroselinic acid may be easily metabolized to lose its activity through the β-oxidation route *in vivo* because it is a fatty acid.

Because of the novelty structure as a PTP1B inhibitor, petroselinic acid was chosen as a lead compound for further modification. Through analyzing the structure of petroselinic acid, three structure-types compounds were designed as

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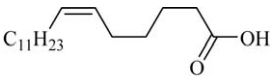
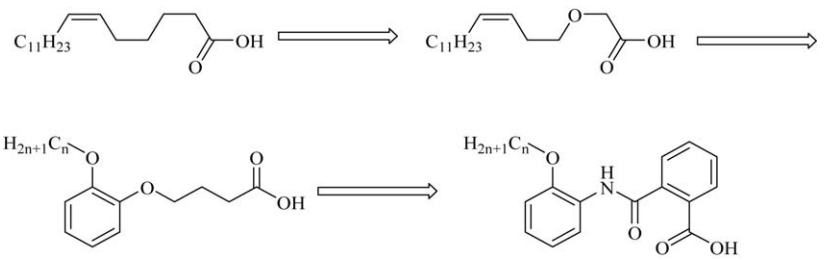


Fig. 1. Structure of petroselinic acid.



Scheme 1. Design from the structure of petroselinic acid.

described in Scheme 1. At first, an oxygen atom was introduced to substitute the β-position carbon atom to investigate its effects on the activity. Because of the structure with a 6,7-Z double bond, 1,2-position substituted benzene ring was introduced to keep the retention of configuration. Next, catechol and *o*-aminophenol derivatives were designed. Considering the importance of the long aliphatic chain to the activity, we used alkyl bromide with the same number of carbon atom reacting with hydroxy group to obtain the long chain. Lastly, because the carboxyl group might be very important to the activity, the designed structure should have a carbolic acid.

The results of biological evaluation confirmed that the aminocarbonylbenzoic acid derivatives showed good inhibitory activity against PTP1B. Further studies resulted in the discovery of 2-(2-tetradecanoxyphenyl)-aminocarbonyl-6-nitrobenzoic acid (**4**, Fig. 2), which was active both *in vitro* and *in vivo*. The results indicated that those series of derivatives may be novel and effective PTP1B inhibitors.

Previous studies had indicated that the substituent in the 2-position (such as $-OC_{14}H_{29}$ in Fig. 2), or the other substituent in the *ortho*-position (such as NO_2) may contribute to the inhibitor-enzyme complex formation. To examine the structure–activity relationships (SAR), a variety of compounds with different substituted positions (such as *meta* or *para* position) or different chain-length alkoxy groups (such as OCH_3, OC_4H_9, and so on) on the two benzene rings were synthesized. In addition, the benzoic acid of the structure was replaced by pyridinecarbolic acid to evaluate the activities.

The synthesis of compounds **4**–**33** is summarized in Scheme 2. Alkylation of nitrophenol **1** with alkyl bromide in the presence of anhydrous potassium carbonate in acetone afforded compound **2** [13]. The nitro group of compound **2**

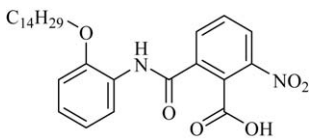
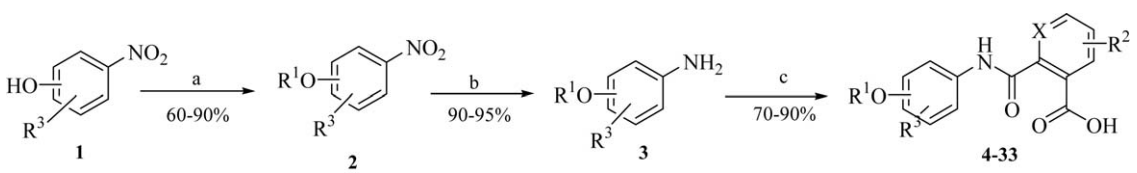


Fig. 2. IC_{50} of compound **4**: 0.44 μmol/L.

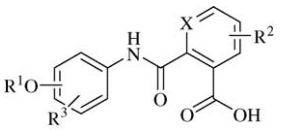


Scheme 2. Reagents and conditions: (a) R^1Br, K_2CO_3, CH_3COCH_3; (b) 10%Pd/C, H_2, EtOH; and (c) anhydride, CH_3COCH_3.

was hydrogenated into an amino group under the catalysis of 10% Pd/C in ethanol. Finally, treatment of **3** with the corresponding anhydride in acetone gave the target compounds **4–33** [14].

PTP1B inhibition assay: The recombinant GST-hPTP1B (gluthathione *S*-transferase-human protein tyrosine phosphatase 1B) bacteria pellets were purified by a GST bead column. The dephosphorylation of *para*-nitrophenyl phosphate (*p*-NPP) was catalyzed to *para*-nitrophenol by PTP1B. Enzyme activity involving an end-point assay, which intensifies the yellow color, was measured at a wavelength of 405 nm [12]. All compounds were dissolved in 100% dimethyl sulfoxide (DMSO), and reactions, including controls, were performed at a final concentration of 10% DMSO. Selected compounds were first evaluated for their ability to inhibit the PTPase reaction at a 10 μmol/L concentration at 30 °C for 10 min, in a reaction system with 3 mmol/L *p*-NPP in HEPES assay buffer (pH 7.0). The reaction was initiated by addition of the enzyme and quenched by addition of 1 mol/L NaOH. The amount of *p*-nitrophenol produced was determined at 405 nm using a microplate spectrophotometer (uQuant, Bio-tek). IC_{50} values were evaluated using a sigmoidal dose-response (variable slope) curve-fitting program of GraphPad Prism 4.0 software [15]. The results of biological activities of *N*-(alkoxyphenyl)-aminocarbonylbenzoic acid derivatives against PTP1B *in vitro* were described in Table 1.

Table 1
Inhibitory activities of *N*-(alkoxyphenyl)-aminocarbonylbenzoic acid derivatives against PTP1B.



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Compounds	R^1	R^2	R^3	X	%[a]	IC_{50}[b]
4	2-*n*-$C_{14}H_{29}$	6-NO_2	H	C	97.8	0.442
5	2-CH_3	H	H	C	NA	–
6	2-*n*-C_4H_9	H	H	C	38.3	–
7	2-*n*-$C_{10}H_{21}$	H	H	C	26.9	–
8	2-*n*-$C_{11}H_{23}$	H	H	C	88.3	0.598
9	2-*n*-$C_{12}H_{25}$	H	H	C	98.9	3.543
10	2-*n*-$C_{12}H_{25}$	H	4-NO_2	C	97.5	0.857
11	2-*n*-$C_{14}H_{29}$	H	H	C	98.9	0.598
12	2-*n*-$C_{14}H_{29}$	5-F	H	C	53.3	–
13	2-*n*-$C_{14}H_{29}$	6-F	H	C	99.7	0.106
14	2-*n*-$C_{14}H_{29}$	H	5-Br	C	98.8	0.601
15	2-*n*-$C_{18}H_{37}$	H	H	C	98.1	0.407
16	2-*n*-$C_{14}H_{29}$	3,4,5,6-Tetrachloro	H	C	99.0	0.180
17	3-*n*-$C_{10}H_{21}$	H	H	C	61.5	–
18	3-*n*-$C_{11}H_{23}$	H	H	C	98.4	1.368
19	3-*n*-$C_{12}H_{25}$	H	H	C	99.8	2.614
20	3-*n*-$C_{12}H_{25}$	3-NO_2	H	C	98.6	1.291
21	3-*n*-$C_{11}H_{23}$	3,4,5,6-Tetrachloro	H	C	99.4	0.372
22	3-*n*-$C_{14}H_{29}$	H	4-OCH_3	C	97.9	0.976
23	4-*n*-$C_{10}H_{21}$	H	H	C	54.8	–
24	4-*n*-$C_{11}H_{23}$	H	H	C	97.1	3.757
25	4-*n*-$C_{12}H_{25}$	H	H	C	99.9	1.271
26	4-*n*-$C_{14}H_{29}$	H	3-Cl	C	98.9	0.370
27	4-*n*-$C_{10}H_{21}$	6-NO_2	H	C	80.8	5.331
28	2-*n*-$C_{14}H_{29}$	H	H	N	99.1	0.390
29	2-*n*-$C_{16}H_{33}$	H	H	N	100.3	0.140
30	2-*n*-$C_{18}H_{37}$	H	H	N	82.8	0.367
31	2-*n*-$C_{22}H_{45}$	H	H	N	79.3	–
32	3-*n*-$C_{12}H_{25}$	H	H	N	88.1	3.239
33	4-*n*-$C_{12}H_{25}$	H	H	N	98.5	1.928

[a] % Inhibition at 10 μmol/L, NA means no inhibition at 10 μmol/L.
[b] IC_{50} values in μmol/L with calculating inhibition more than 80%.

The results of activities demonstrated that those compounds **13**, **16** and **29** had more potent activities against PTP1B compared with the lead compound. The preliminary structure–activity relationships can be summarized as follows. Firstly, the length of the chain was very important to the activities. While the number of carbon atom was not more than ten, the activity was weaker. Secondly, the substituted group was the same importance on the phenyl of benzoic acid. On the basis of the results, the activities of the compounds with the electron-withdrawing groups were better than those compounds without them. Lastly, the substituted position on another phenyl group had little influence on the activity. In the same, the replacement of the benzoic acid with pyridinecarbolic acid had little effect on the inhibitory activity.

In conclusion, based on the lead compound petroselinic acid, a series of novel *N*-(alkoxyphenyl)-aminocarbonylbenzoic acid derivatives were designed and synthesized. The results of biological activities indicated that most of new derivatives exhibited more potent activities against PTP1B. Among these compounds, the compound **13** displayed good potency, with an IC_{50} of 106 nmol/L. More activities evaluations are in progress *in vivo*.

Acknowledgments

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