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Privileged Structure-based Discovery of Novel 2-Iminothiazoles as Protein Tyrosine Phosphatase 1B Inhibitors

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Protein tyrosine phosphatase 1B (PTP1B) is a well-established metabolic regulator and a member of PTP super-family that catalyzes protein tyrosine dephosphorylation. Many scientific evidences have shown that PTP1B plays a broad role in the regulation of body metabolism, particularly in cancer development.[1-3] In the insulin signaling pathway, PTP1B can be involved in dephosphorylation of activated insulin receptor (IR) or insulin receptor substrates (IRS). PTP1B can also bind and dephosphorylate JAK2, which activates STAT3 responsible for downstream functions such as food uptake and energy homeostasis in the leptin pathway. Elevated leptin sensitivity by activated or phosphorylated JAK2 in the *PTP1B*$^{-/-}$ mice is related to increased energy expenditure, which eventually results in homeostatic regulation of the blood glucose level and body weight.[4]

An effective control of the phosphatase may provide a useful tool in treatment of diseases such as type 2 diabetes and obesity. However, there is no reported PTP1B inhibitor that has passed through the sequence of clinical tests for treatment of the diseases, although many inhibitors showed potency *in vitro* assays. In general, mimicking phosphotyrosine has been the starting point of development of active site-directed PTPase inhibitors.[5] However, the majority of the active site directed PTP1B inhibitors have shown a low level of cellular uptake because they usually contain the highly charged moieties such as phosphate and carboxylic acid. Recently, potent PTP1B inhibitors were obtained from high-throughput screening of chemical libraries. Among them, further structurally optimized Ertiprotafib was submitted to a clinical trial but was discontinued in the second phase (Figure 1).[6,7]

In this regard, our group made much effort to find out new chemical scaffolds with inhibitory activities against PTP1B without mimicking phosphotyrosine. In search of new hit compounds for drug discovery, utilizing chemical library based on privileged structures gives some benefits in terms of proved drug-like properties such as inherent low toxicity, good pharmacokinetic profiles, and easiness of synthesis.[8,9] Therefore, structurally diversified chemical set was selected from our proprietary privileged structure-based chemical library, which was designed and constructed for drug discovery. Herein, we communicate discovery of novel 2-iminothiazole compounds for PTP1B as a potential anti-diabetic.

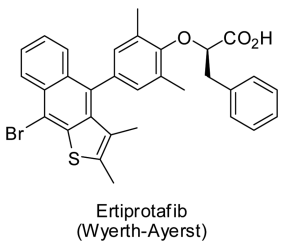


Figure 1. The structure of Ertiprotafib.

Initially, 182 compounds were screened for PTB1B inhibitory activities at 100 μM using fluorescence polarization (FP)-based PTP1B assays to give 20 compounds which showed over 50% inhibition. Then, selected compounds were screened again at 20 μM to give two hit compounds with 22% and 99% inhibitory activities, respectively. These two compounds share common structure, *N*-cyclohexyl-2-(3-methyl-2-(phenylimino)-2,3-dihydrothiazol-4-yl)acetamide (Figure 2).

With initial hit compounds in hand, we designed new chemical library focused on 2-aryliminothiazole moiety. Synthesis of the library was carried out following the previously reported protocol, as summarized in Scheme 1.[10-13] 4-Chloroacetoacetyl chloride (**3**) was treated with various amines to give 4-chloroacetoanilides (**4**) in 47-85% yields.

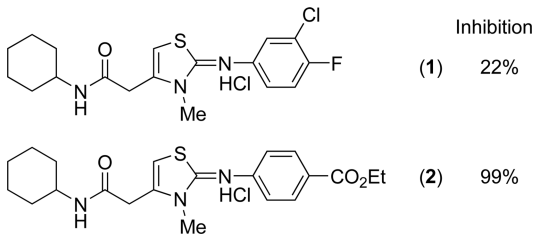
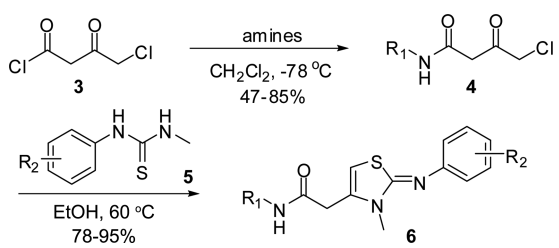


Figure 2. Structures of the hit compounds.



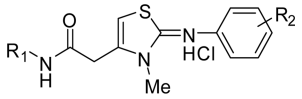
Scheme 1. Synthesis of 2-iminothiazole derivarives.

Then, the unsymmetric thioureas (**5**) prepared from the reaction of methyl isothiocyanate and various amines, were reacted with 4-chloroacetoanilides to deliver the desired 2-iminothiazole derivatives (**6**).

The list of synthesized 21 compounds and their inhibitory activity such as % inhibition at 20 μM and IC_{50} values for selected compounds are presented in Table 1. The first series for structure-activity relationship (SAR) study were the analogues derivatized at 2-iminophenyl group with fixing cyclohexyl group at amide nitrogen (**1**, **6a-6h** in Table 1). Among them, four derivatives exhibited over 50% inhibition at 20 μM, which were further evaluated by measuring their IC_{50} values. The initial hit, 4-ethoxycarbonylphenyl compound (**1**) proved to be most potent (IC_{50} = 0.9 μM), while other compounds also showed moderate potencies ranged from 4.5-16.1 μM. Disubstituted phenyl analogues, isopropyl ester of benzoate and 4-ethoxy compounds displayed relatively low activities.

With 4-ethoxycarbonylphenyl at 2-imino nitrogen fixed, the derivatives varied at amide nitrogen were prepared and evaluated for their potencies (**6i-6o** in Table 1). Since we previously observed that any aryl or primary alkyl compounds did not show the inhibitory effect, cycloalkyl groups including cyclopentyl, cycloheptyl, cyclooctyl, cyclododecyl or cyclohexylmethyl groups were installed instead of the cyclohexyl group. However, the analogues did not exhibit any significant enhancement in inhibitory activity compared to the cyclohexyl compound, while the 3-fluoro-methyl phenyl compound (**6n**) showed mild activity with IC_{50} of 17.1 μM. The third set of compounds consists of 2-methylcyclohexyl analogues in which cyclohexyl group was replaced with the similar-sized but slightly bulkier group (**6p-6t** in Table 1). Synthesis was completed according to the Scheme 1, and the inhibitory potencies were evaluated. The IC_{50} values of this group showed the similar trend observed in the cyclohexyl analogues. The 4-ethoxycarbonylphenyl derivative exhibited slightly higher potency than the other derivatives in the group.

Table 1. Inhibitory potency of compounds against PTP1B



Cpd	R_1	R_2	% inhibition at 20 μM	IC_{50} (μM, ± S.D.)
1	Cyclohexyl	4-CO_2Et	99	0.9 (0.5)
6a	Cyclohexyl	4-COPh	66.9	9.3 (0.3)
6b	Cyclohexyl	4-O(4-Cl-Ph)	55.6	16.1 (1.5)
6c	Cyclohexyl	4-CO_2-*n*Bu	67.8	4.5 (2.7)
6d	Cyclohexyl	3,5-DiCl	37.3	
6e	Cyclohexyl	3,5-diCF_3	46.1	
6f	Cyclohexyl	3-CH_3, 4-Cl	37.4	
6g	Cyclohexyl	4-CO_2-*i*Pr	47.3	
6h	Cyclohexyl	4-OEt	37.4	
6i	Cyclopentyl	4-CO_2Et	-0.9	
6j	Cycloheptyl	4-CO_2Et	30.6	
6k	Cycloheptyl	3,5-di-CF_3	38.9	
6l	Cyclooctyl	4-CO_2Et	10.2	
6m	Cyclododecyl	4-CO_2Et	30.6	
6n	Cyclohexylmethyl	3-F, 4-CH_3	58.9	17.1 (5.0)
6o	Cyclohexylmethyl	2-CO_2Et	46	
6p	2-Methylcyclohexyl	4-OCF_3	61.3	16.7 (0.7)
6q	2-Methylcyclohexyl	4-CO_2Et	89.8	4.8 (1.0)
6r	2-Methylcyclohexyl	3,5-di-CF_3	85.8	13.7 (1.0)
6s	2-Methylcyclohexyl	4-OPh	85.8	7.5 (0.1)
6t	2-Methylcyclohexyl	3-CO_2-*i*Pr	58.9	25.7 (2.2)

In summary, rapid searching of new hit compounds against PTP1B without phosphotyrosine mimicking moieties was carried out by using a chemical library composed of privileged structures. Based on the initial screening result, a synthetic library having the 2-iminothiazole backbone was constructed and screened for inhibition of PTP1B. Among them, (*Z*)-ethyl 4-(4-(2-(cyclohexylamino)-2-oxoethyl)-3-methylthiazol-2(3*H*)-ylideneamino)benzoate (**1**) was found as the most potent inhibitor through FP-based assays. These results could provide a completely new scaffold for development of anti-PTP1B drug. Further optimization studies are ongoing.

Spectral Data of Compound 1: white solid; mp 273-275 °C; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 8.27 (d, J = 7.8, 1H), 8.03 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 7.2 Hz, 2H), 6.87 (s, 1H), 4.31 (q, J = 7.2 Hz, 2H), 3.70 (s, 3H), 3.69 (s, 2H), 3.54 (m, 1H), 1.76-1.73 (m, 2H), 1.69-1.66 (m, 2H), 1.55-1.53 (m, 1H), 1.31 (t, J = 6.6 Hz, 3H), 1.29-1.12 (m, 5H); ^{13}C-NMR (150 MHz, DMSO-d_6) δ 166.2, 165.5, 131.5, 122.9, 61.3, 48.3, 34.9, 32.7, 25.6, 24.9, 14.6; FT-IR cm^{-1} 1719, 1656, 1545, 1275, 1110, 1022; EI-MS *m/z* 402 $[M+H]^+$.

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