

## Discovery of a novel submicromolar inhibitor of the lymphoid specific tyrosine phosphatase

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Received 3 March 2008; revised 24 March 2008; accepted 31 March 2008

Available online 8 April 2008

**Abstract**—We report here a class of thiazolidine-2,4-diones and 2-thioxothiazolidin-4-ones as potent inhibitors of the lymphoid specific tyrosine phosphatase (Lyp) identified from high throughput screens. Chemical modification by incorporating the known phosphotyrosine (pTyr) mimics led to the discovery of a salicylate-based inhibitor with submicromolar potency.

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The lymphoid specific phosphatase (Lyp), encoded by the PTPN22 gene, plays an important role in T cell signaling. A single-nucleotide polymorphism in PTPN22 is associated with autoimmune disorders such as type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus, and Grave's disease. The autoimmunity-predisposing allele corresponds to a gain-of-function mutation, producing a variant phosphatase that is more active than the wild type enzyme in inhibiting T cell signaling.<sup>1,2</sup> Inhibition of Lyp therefore provides a novel approach to the treatment of a broad spectrum of autoimmune diseases.

To identify small molecule inhibitors of Lyp, high throughput screens were performed at Columbia University as part of the Molecular Library Screening Center Network (MLSCN) of the NIH Roadmap for Medical Research. The Lyp assay<sup>3</sup> was established using a bacterially expressed fully active 62 kDa N-terminal catalytic domain of Lyp. Compounds were assayed for their capacity to inhibit Lyp-catalyzed conversion of DiFMUP(6,8-difluoro-4-methylumbelliferyl phosphate) to a fluorescent product. Fluorescence with excitation

at 360 nm and emission at 465 nm was recorded at the endpoint of the reaction. From the compound libraries provided by NIH, a variety of active hits were identified through the high throughput screens and were confirmed manually by the dose–response assays. Intriguingly, a number of these compounds share a thiazolidine core structure and thus form an active cluster, providing a starting point for the assessment of structure–activity relationships and medicinal chemistry efforts (Fig. 1).

Protein tyrosine phosphatases (PTPases) share a highly conserved active site, the phosphotyrosine (pTyr) binding pocket that is the main target for PTPase inhibitor design. Thus, most inhibitors share a pharmacophore structurally similar to the pTyr substrate. Effective pTyr mimics are often charged bidentate anions that competitively bind to the highly polarized pTyr pocket. Several classes of mimics have been reported,<sup>4</sup> including the difluoromethylenephosphonates (DFMP) and benzoic acids such as 2-(oxalylamino)-benzoic acids (OBA), salicylic acids (SA), and its derivatives (Fig. 2).

We noticed that several of the thiazolidine hits contained benzoic acid moiety, and thus could be replaced with a pTyr-mimicking fragment. Fragment-based drug design is a new approach that has been successfully applied to challenging targets.<sup>5</sup> This strategy allows hits to

**Keywords:** Thiazolidine; Lyp; pTyr mimics; Salicylate.

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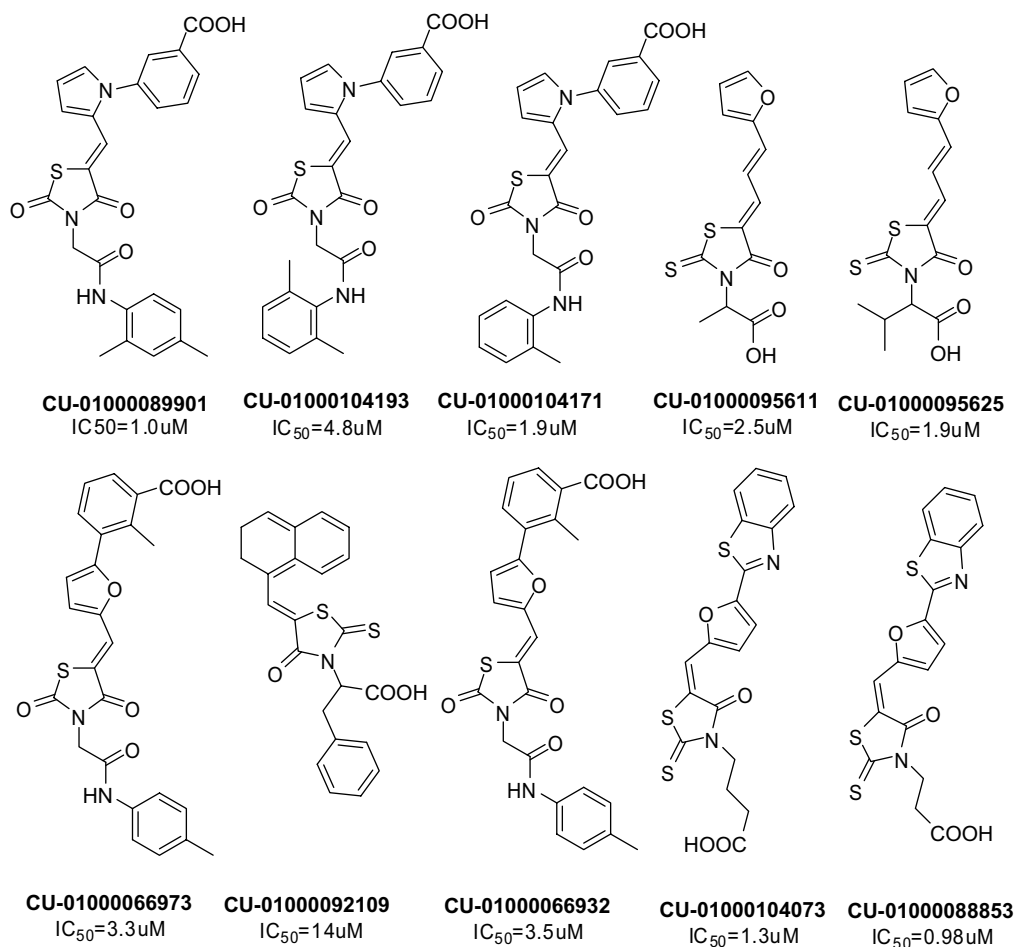


Figure 1. An active cluster of Lyp inhibitors.

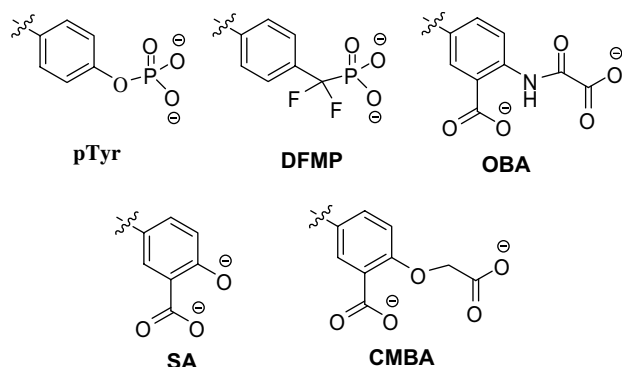


Figure 2. The phosphotyrosine (pTyr) mimic.

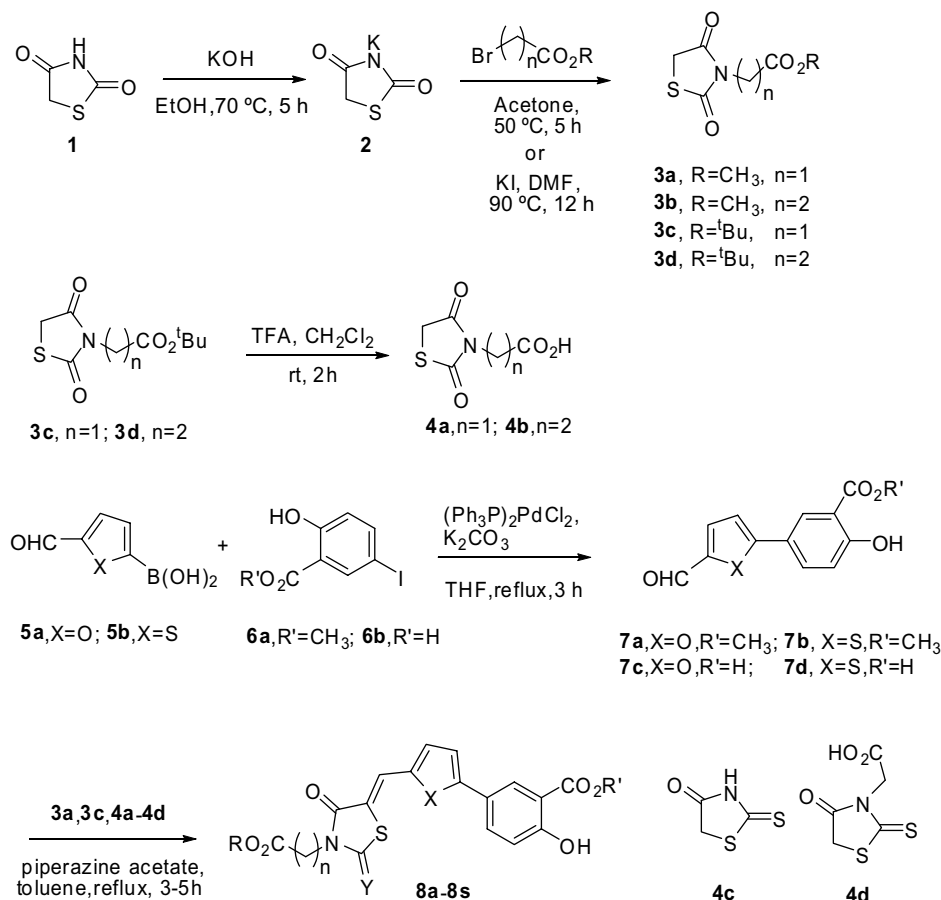
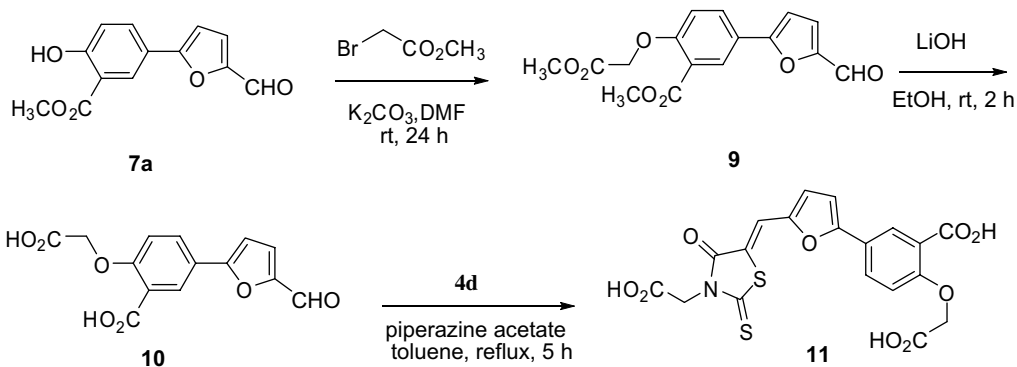
be optimized by combining and linking different fragments. We reasoned that combining a known pTyr surrogate with the thiazolidine core structure we identified from high throughput screens might result in synergistically improved potency. Salicylic acid and its derivatives, which are potent pTyr surrogates, were chosen as our initial building blocks for novel Lyp inhibitors.

As shown in Scheme 1, a series of thiazolidine-dione and 2-thioxothiazolidin-4-one compounds with an appended salicylic moiety were designed and synthesized.<sup>6</sup> Diox-

othiazolidinyl esters **3a–3d** were synthesized from thiazolidine-2, 4-dione **1**, which was first converted to potassium salt **2** by potassium hydroxide and subsequently alkylated with methyl (*tert*-butyl) bromoacetate in acetone at 50 °C, or methyl (*tert*-butyl) bromopropionate in dimethylformamide at 90 °C in the presence of potassium iodide. Acids **4a–4b** were obtained by the treatment of the *tert*-butyl esters **3c–3d** with trifluoroacetic acid. Suzuki coupling of boronic acid **5a–5b** with 5-iodo-2-hydroxybenzoate **6a–6b** conveniently yielded salicylic acid-derived aldehydes **7a–7d**, which were condensed with thiazolidine-diones **3a**, **3c**, **4a**, **4b**, and commercially available 2-thioxothiazolidin-4-ones **4c–4d** in toluene to afford the final product **8a–8s** in yields of 72–85%.

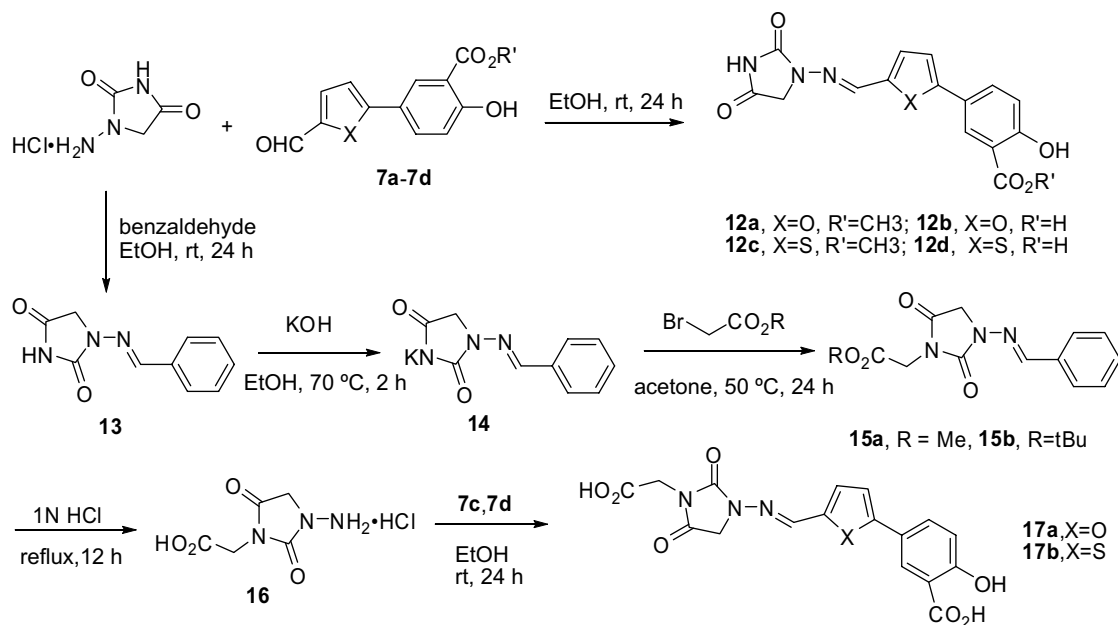
A triacid analog **11** was synthesized according to Scheme 2.<sup>6</sup> Alkylation of compound **7a** with 2-bromoacetate gave aldehyde **9**, followed by deprotection of methyl ester with lithium hydroxide to yield aldehyde **10**. Similar condensation of aldehydes **10** and **4d** readily afforded the desired product **11** in a total yield of 62% over three steps.

To further modify the salicylic compounds, Schiff base analogs with the thiazolidine-dione head group replaced with the hydantoin ring were synthesized.<sup>6</sup> As outlined

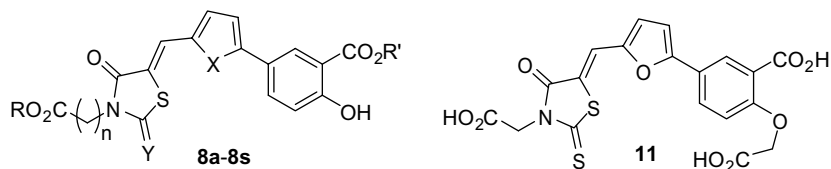
Scheme 1. The synthesis of Lyp inhibitors **8a–8s**.Scheme 2. The synthesis of the triacid analog **11**.

in Scheme 3, simply mixing aldehydes with the hydrochloride salt of 1-aminohydantoin in ethanol readily gave the corresponding Schiff bases **12a–12d** in quantitative yields. The synthesis of **17a** and **17b** started from the protection of 1-aminohydantoin with benzaldehyde by forming imine **13**. Deprotection of **13** followed by alkylation with bromoacetate provided the imidyl esters **15a** and **15b**. Simultaneous deprotection of ester and imine by refluxing in hydrochloric acid afforded the corresponding amino acid **16**. Condensation between the hydantoin-derived amino acid and aldehydes **7c** and **7d** furnished the Schiff base analogs **17a** and **17b** in good yields.

A total of 25 salicylic compounds were synthesized, and their ability to inhibit Lyp was tested in vitro with  $\text{IC}_{50}$ s determined from the dose–response assays. Results for thiazolidine compounds are summarized in Table 1, while the results for Schiff base analogs are shown in Table 2. Seventeen compounds showed reasonable activities against Lyp. Among them, compound **8p** was shown to be a submicromolar inhibitor with an impressive  $\text{IC}_{50}$  of 0.39  $\mu\text{M}$ . Several structure–activity relationships could be observed from these data. The presence of a free carboxyl group in the molecule is essential for the activity. For example, diester compounds **8b**, **8c**, **8d**, and **8k** are basically inactive, consistent with



Scheme 3. The synthesis of Schiff base analogs.

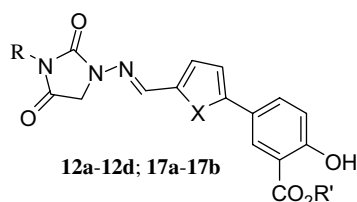
Table 1. The Lyp inhibitory activities of compounds **8a–8s** and **11**

Compound	R	R'	n	X	Y	IC <sub>50</sub> <sup>a</sup> (μM)
<b>8a</b>	H	CH <sub>3</sub>	1	O	S	21.0
<b>8b</b>	CH <sub>3</sub>	CH <sub>3</sub>	2	O	O	44.0
<b>8c</b>	CH <sub>3</sub>	CH <sub>3</sub>	2	S	O	>44
<b>8d</b>	CH <sub>3</sub>	CH <sub>3</sub>	1	S	O	>44
<b>8e</b>	H	H	1	O	S	8.7
<b>8f</b>	H	H	2	O	O	5.1
<b>8g</b>	H	H	2	S	O	5.9
<b>8h</b>	H	H	1	S	O	2.8
<b>8i</b>	H	H	1	S	S	5.4
<b>8j</b>	H	CH <sub>3</sub>	1	S	S	4.3
<b>8k</b>	CH <sub>3</sub>	CH <sub>3</sub>	1	O	O	>44
<b>8l</b>	H	CH <sub>3</sub>	1	S	O	18
<b>8m</b>	H	CH <sub>3</sub>	1	O	O	>44
<b>8n</b>	H	H	1	O	O	5.6
<b>8o</b>	H	CH <sub>3</sub>	2	O	O	4.3
<b>8p</b>	H	CH <sub>3</sub>	2	S	O	0.39
<b>8q</b>	CH <sub>3</sub>	H	1	O	O	4.8
<b>8r</b>	CH <sub>3</sub>	H	1	S	O	1.8
<b>8s</b>		H	0	O	S	1.4
<b>11</b>						>44

<sup>a</sup> IC<sub>50</sub> values are determined from a single experiment performed in triplicate.

the expectation that negative charge is important for the activity of phosphatase inhibitors. As demonstrated by compounds **8j**, **8r**, **8m**, and **8q**, the thiophene ring is generally more potent than the furan ring. Interestingly, 2-thione also seems preferable to 2-one in the thiazolidine ring that is best illustrated by increased potency of com-

pounds **8a** and **8j**. The length of the side chain ( $n = 0, 1$ , and  $2$ ), depending on the overall structure of the compound, has a varying impact on the activities. 2-(carboxymethoxy) benzoic acid (CMBA), a derivative of salicylic acid, is also a potent pTyr mimic. However, our attempt to replace salicylic acid with CMBA re-

**Table 2.** The inhibitory activities of the Schiff base analogs

Compound	R	R'	X	IC <sub>50</sub> <sup>a</sup> (μM)
<b>12a</b>	H	CH <sub>3</sub>	O	>44
<b>12b</b>	H	H	O	>44
<b>12c</b>	H	CH <sub>3</sub>	S	>44
<b>12d</b>	H	H	S	34.0
<b>17a</b>	CH <sub>2</sub> CO <sub>2</sub> H	H	O	36.0
<b>17b</b>	CH <sub>2</sub> CO <sub>2</sub> H	H	S	>44

<sup>a</sup> IC<sub>50</sub> values are determined from a single experiment performed in triplicate.

sulted in a great loss of activity of compound **11** compared with **8e**, indicating that the free hydroxyl group is necessary for retaining the activity. Coincidentally, when this manuscript was being prepared, the crystal structure of Lyp in complex with a salicylic acid-based inhibitor was published.<sup>7</sup> It was shown that the hydroxyl group of salicylic acid makes critical polar interactions in the active site of Lyp.

As can be seen from Table 2, the Schiff base analogs are poor inhibitors in general. Only compounds **12d** and **17a** showed moderate activities with the IC<sub>50</sub> values of 34.0 and 36.0 μM, respectively.

In summary, we have successfully identified a cluster of thiozolidine compounds with good Lyp inhibitory activ-

ities via high throughput screening. Improved potency (<1 μM) was achieved by rational design and chemical synthesis. PTPase selectivity and cell studies of the promising compounds are currently being pursued.

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