ORGANIC LETTERS

2008
Vol. 10, No. 11
2295−2298

Solid-Phase Assembly and In Situ Screening of Protein Tyrosine Phosphatase Inhibitors

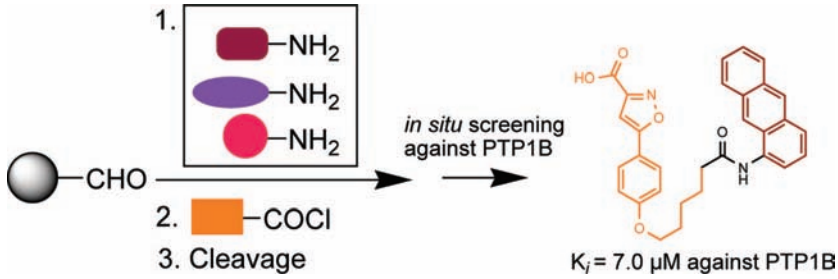
Rajavel Srinivasan, Lay Pheng Tan, Hao Wu, and Shao Q. Yao*

Departments of Chemistry and Biological Sciences, NUS MedChem Program of the Office of Life Sciences, National University of Singapore, Singapore 117543

chmyaosq@nus.edu.sg

Received March 30, 2008

ABSTRACT



A highly efficient solid-phase strategy for assembly of small molecule inhibitors against protein tyrosine phosphatase 1B (PTP1B) is described. The method is highlighted by its simplicity and product purity. A 70-member combinatorial library of analogues of a known PTP1B inhibitor has been synthesized, which upon direct in situ screening revealed a potent inhibitor (K_i = 7.0 μM) against PTP1B.

High-throughput enzymology occupies a pivotal role in modern drug discovery programs.[1] One of the main challenges in this field is the development of high-throughput (HT) amenable chemical reactions that allow rapid synthesis of diverse chemical libraries for the interrogation of different classes of enzymes.[2] One such reaction is the Cu(I)-catalyzed, 1,3-dipolar cycloaddition between an azide and an alkyne fragment, also known as "click chemistry", which was pioneered by Sharpless and Meldal.[3] Another class of reactions possessing similar qualities is the amide bond-forming reaction between an amine and a carboxylic acid using suitable activating/coupling reagents. Numerous research groups have recently used this reaction for solution-phase, rapid assembly of small molecule inhibitors against a variety of enzymes including HIV proteases,[4a,b] β-aryl sulfotransferase,[4c] α-fucosidases,[4d,e] and SARS-3CL protease.[4f] The reaction is highly efficient, often generating the desired products in nearly quantitative yields, thus allowing in situ biological screening to be carried out directly in some cases, even in the presence of excessive starting materials, reagents, or byproduct (Figure 1a; Pathway A). The effectiveness of this method, however, had recently been challenged, as it was discovered that unexpected byproduct may give rise to false results.[5] For example, Wong et al. reported that an intermediate benzotriazole ester of the amide

(1) (a) Golebiowski, A.; Klopfenstein, S. R.; Portlock, D. E. *Curr. Opin. Chem. Biol.* **2001**, *5*, 273–284. (b) Golebiowski, A.; Klopfenstein, S. R.; Portlock, D. E. *Curr. Opin. Chem. Biol.* **2003**, *7*, 308–325.

(2) (a) Hu, Y.; Uttamchandani, M.; Yao, S. Q. *Comb. Chem. High-Throughput Screen.* **2006**, *9*, 203–212. (b) Sun, H.; Chattopadhaya, S.; Wang, J.; Yao, S. Q. *Anal. Bioanal. Chem.* **2006**, *386*, 416–426.

(3) (a) Sharpless, K. B.; Manetsch, R. *Exp. Opin. Drug Discovery* **2006**, *1*, 525–538. (b) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128–1137. (c) Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3062. (d) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.

(4) (a) Brik, A.; Lin, Y.-C.; Elder, J.; Wong, C.-H. *Chem. Biol.* **2002**, *9*, 891–896. (b) Lee, S.-G.; Chmielewski, J. *Chem. Biol.* **2006**, *13*, 421–426. (c) Best, M. D.; Brik, A.; Chapman, E.; Lee, L. V.; Wong, C.-H. *ChemBioChem* **2004**, *5*, 811–819. (d) Wu, C.-Y.; Chang, C.-F.; Chen, J. S.-Y.; Lee, S.-T.; Wong, C.-H.; Lin, C.-H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4661–4664. (e) Chang, C.-F.; Ho, C.-W.; Wu, C.-Y.; Chao, T.-A.; Wong, C.-H.; Lin, C.-H. *Chem. Biol.* **2004**, *11*, 1301–1306. (f) Wu, C.-Y.; King, K.-Y.; Kuo, C.-J.; Fang, J.-M.; Wu, Y.-T.; Ho, M.-Y.; Liao, C.-L.; Shie, J.-J.; Liang, P.-H.; Wong, C.-H. *Chem. Biol.* **2006**, *13*, 261–268.

10.1021/ol8006875 CCC: $40.75
Published on Web 05/06/2008

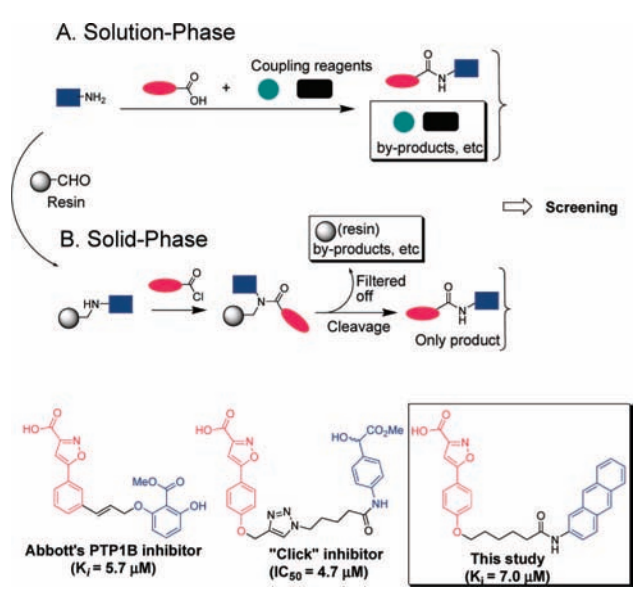


Figure 1. (a) Two strategies using amide bond-forming reaction. (b) Various cell-permeable PTP1B inhibitors. The bidentate inhibitors contain a core (in red) and a peripheral group (in blue).

bond-forming reaction was found to be responsible for the inhibitory activity toward SARS-3CL proteases.[4f] To avoid such potential complications, we aim to develop solid-phase, amide bond-forming reactions using the same sets of starting materials (Figure 1a; Pathway B). Herein, we report a traceless solid-phase approach for rapid assembly of protein tyrosine phosphatase (PTP) inhibitors using amide bond-forming reaction (Figure 1a; Pathway B).

Protein tyrosine phosphatases (PTPs) are a main class of signaling enzymes.[6a] PTP1B is the prototype of all PTPs and has been identified as a key player in major human diseases such as diabetes, obesity, and cancer.[6b–d] The elegant work of Zhang et al. that indicates PTP1B possesses a unique secondary binding site next to its primary phosphotyrosine binding pocket[6b] has enabled the development of potent and specific bidentate PTP1B inhibitors.[7,8] One of the most potent and cell-permeable PTP1B inhibitors identified to date, as shown in Figure 1b (left), was developed by scientists from Abbott Laboratories.[7b] This inhibitor contains a core isoxazole group which serves as a cell-permeable bioisostere of phosphotyrosine and a hydrophobic aromatic group that binds to the secondary site in PTP1B. It was reasonably potent (K_i = 5.7 μM) and selective toward PTP1B over its closest analogue, TCPTP (>30-fold in selectivity). More importantly, the inhibitor was cell-permeable and showed good cellular activity in PTP1B-expressing COS-7 cells.[7b] Inspired by this work, we recently developed our own version of a cell-permeable bidentate PTP1B inhibitor using "click" chemistry (Figure 1b, center), which shows comparable inhibitory activity (IC_{50} = 4.7 μM against PTP1B). In the current work, we have successfully developed our second-generation, cell-permeable PTP1B inhibitors by using a solid-phase amide-forming reaction to rapidly link the core and the peripheral groups together. Unlike traditional solution-phase approaches,[4] our method delivers pure bidentate inhibitors at the end of synthesis and therefore is suitable for direct in situ screening. By screening these inhibitors against PTP1B, we have uncovered a candidate molecule which possesses comparable inhibition against PTP1B (Figure 1b, right; K_i = 7.0 μM).

In our strategy, we used the commercially available 4-formyl-3-methoxyphenoxy (FMP) resin to capture various amine fragments via reductive amination, followed by attaching the isoxazole warhead (Scheme 1). Key advantages of our method include the following: (i) it is a traceless approach allowing the use of exact same sets of starting material as in solution-phase synthesis; (ii) it is solid-phase, enabling a large library to be constructed efficiently; (iii) it is robust, giving high-quality products which in most cases are spectroscopically pure enough to be used directly for biological screening. The synthesis of the acid-containing warheads, **A** and **B**, started from the commercially available 4-hydroxyacetophenone **1**, which underwent benzylation to give **2** (83% yield). Subsequently, condensation between **2** and dimethyloxalate in the presence of NaOMe, followed by cyclization of the resulting product, gave the isoxazole carboxylic methylester, **3**, in modest yield (42% in two steps).[8] Conversion of **3** to **5** was carried out first by base hydrolysis, followed by *t*-Bu ester formation (85% in two steps). Next, the benzyl ether on **5** was deprotected by H_2 hydrogenolysis (in Pd/C) giving **6**, followed by O-alkylation with two different linkers to afford **7a** and **7b** (93 and 63% yield, respectively). Subsequent deprotection of the benzyl esters gave the two acid-containing warheads, **A** and **B**, respectively. To start the assembly on solid-phase, two sets of 35 aromatic amines were treated with FMP resin in the presence of $Na(OAc)_3BH$/2% glacial acetic acid in DCE to give the corresponding secondary aromatic amines **10**. Reductive amination proceeded smoothly with a variety of aromatic amines bearing different substituents including −OH, −OR, −SR, −F, −Cl, $-OCF_3$, $-CO_2R$, and −R. Benzyl, napthyl, and anthracenyl amines too underwent reductive amination smoothly. Subsequent amide bond-forming reaction between the resin-bound secondary aromatic amines and the acid warheads (**A** and **B**) was found to be highly challenging and required extensive optimizations. A variety of coupling reagents including HATU, PyBop, HBTU, EDC, and DIC were attempted, but none gave the desired products in satisfactory yield and purity. Fortunately, by in situ conversion of **A** and **B** into their corresponding acid chlorides, **8a** and **8b**, with 1 equiv of oxalyl chloride (with DIEA in DCM), we were able to successfully couple

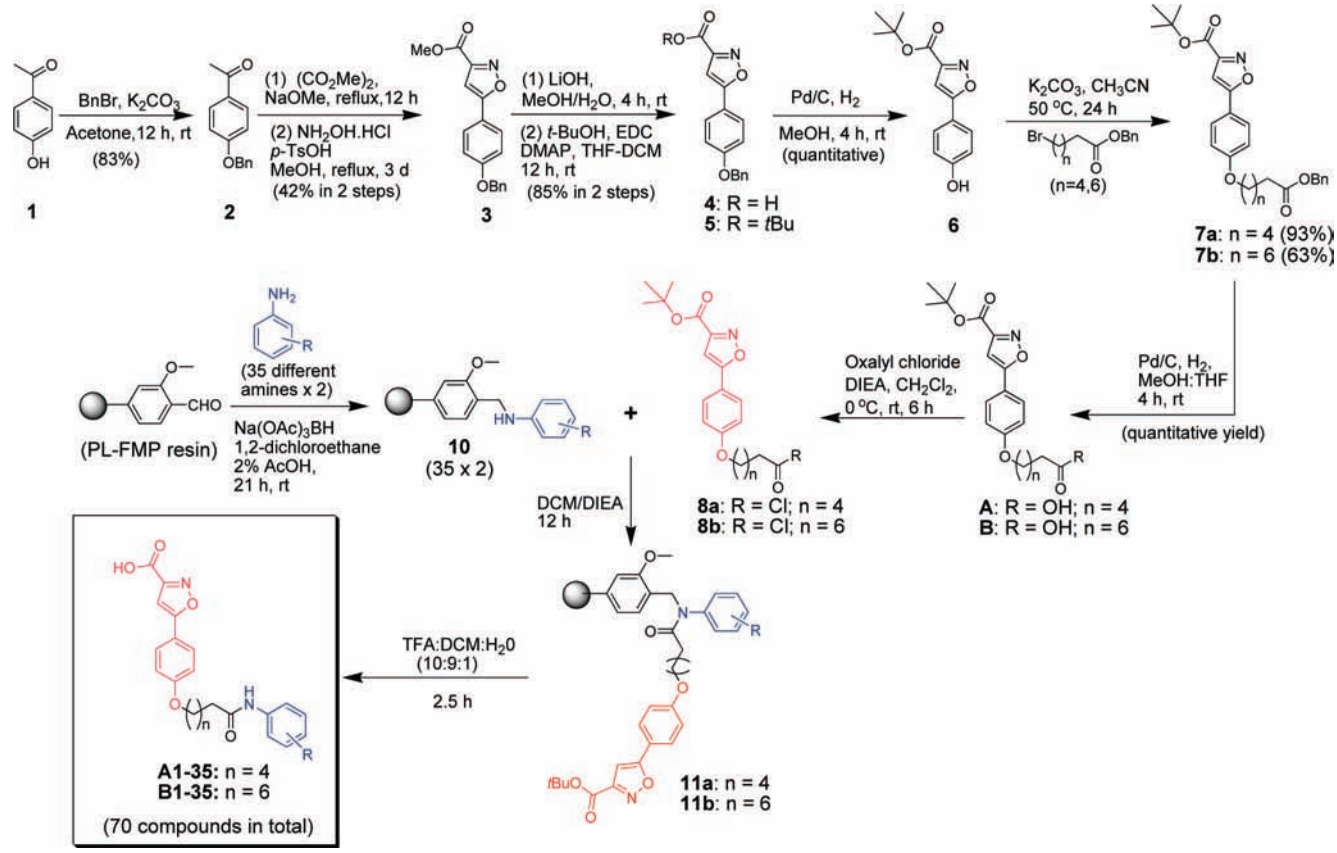
(5) Brik, A.; Wu, C.-Y.; Wong, C.-H. *Org. Biomol. Chem.* **2006**, *4*, 1446–1457.

(6) (a) Hunter, T. *Cell* **2000**, *100*, 113–127. (b) Zhang, Z.-Y. *Curr. Opin. Chem. Biol.* **2001**, *5*, 416–423. (c) Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. *Nat. Rev. Drug Discovery* **2002**, *1*, 696–709. (d) Bialy, L.; Waldmann, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 3814–3839.

(7) (a) Guo, X.-L.; Shen, K.; Wang, F.; Lawrence, D. S.; Zhang, Z.-Y. *J. Biol. Chem.* **2002**, *277*, 41014–41022. (b) Liu, G.; et al. *J. Med. Chem.* **2003**, *46*, 4232–4235. (c) Liu, G.; et al. *J. Med. Chem.* **2003**, *46*, 3437–3440.

(8) Srinivasan, R.; Uttamchandani, M.; Yao, S. Q. *Org. Lett.* **2006**, *8*, 713–716.

Scheme 1. Traceless Solid-Phase Synthesis of Bidendate PTP1B Inhibitors



them, giving the resin-bound inhibitors **11** with excellent purity (>95% in most cases). Finally, cleavage of the products from the resin using an optimized TFA cleavage cocktail (TFA/DCM/water = 10:9:1) gave a total of 70 bidentate PTP1B inhibitors, **A1**−**A35** and **B1**−**B35**. Most crude products were characterized by LC-MS/NMR and used for direct in situ screening for PTP1B inhibition.

Next, the inhibitory activity of the bidentate inhibitors was determined using a standard fluorescence microplate assay as previously reported.[8] First, an inhibitor fingerprint of the 70-member library against PTP1B was obtained, from which six potent hits (**A21**, **A26**, **A34**, **A35**, **B7**, and **B25**) were identified (Figure 2). Detailed inhibition studies were then carried out to obtain the IC_{50}/K_i of these compounds against PTP1B and TCPTP, and results are summarized in Table 1. The best inhibitor against PTP1B was found to be **A26**, with IC_{50} and K_i values of 10.3 and 7.0 μM, respectively. Significantly, it also showed a 10-fold selectivity over TCPTP. It is interesting to note that **A26**, as well as the other good inhibitors of PTP1B, as shown in Figure 2, contains a bulky aromatic group. This coincides reasonably with previously known cell-permeable inhibitors of PTP1B developed using other strategies, such as fragment-based or click chemistry approaches (Figure 1b),[7,8] thus further validating our solid-phase amide-forming methodology as a feasible strategy for future discovery of other enzyme inhibitors.

In conclusion, we have developed a solid-phase amide bond-forming approach for rapid assembly of bidentate

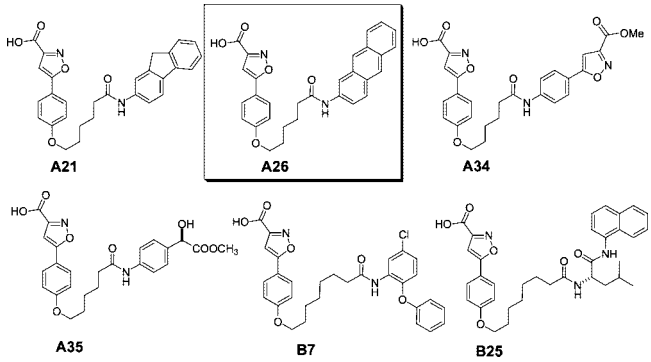


Figure 2. Six candidate hits identified against PTP1B.

Table 1. Inhibition of the Six Selected Inhibitors

	IC_{50} (K_i) in μM					
enzyme	**A21**	**A26**	**A34**	**A35**	**B7**	**B25**
PTP1B	19.6 (9.5)	10.3 (7.0)	23.0 (10.3)	35.9	60.5	28.7
TCPTP	174.5	105.4	124.4	103.5	205.1	489.1

PTP1B inhibitors. Products generated were of consistently high quality and required no further purification. We identified a compound whose inhibition against PTP1B was comparable to that of other known PTP1B inhibitors.[7,8] We are currently extending the strategy to the synthesis of other enzyme inhibitors.

Acknowledgment. Funding support was provided by National University of Singapore (NUS) and the Agency for Science, Technology, and Research (A*Star) of Singapore. The authors thank Prof. Harry Charbonneau (Purdue University) for the TCPTP construct.

Supporting Information Available: Experimental details and characterization of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL8006875