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High-Throughput Discovery of *Mycobacterium tuberculosis* Protein Tyrosine Phosphatase B (MptpB) Inhibitors Using Click Chemistry

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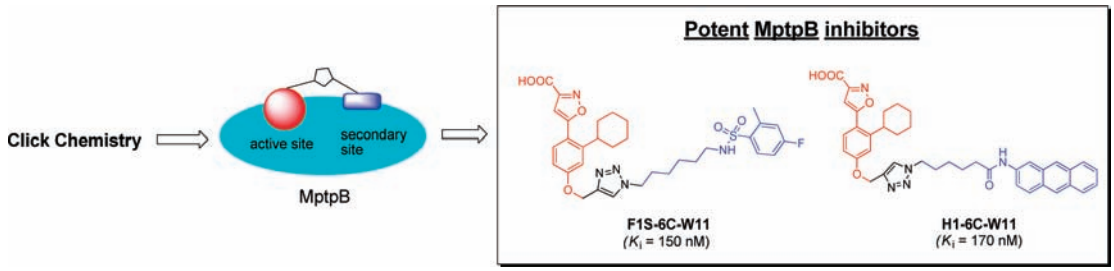
ABSTRACT



A ~3500-member library of bidentate inhibitors against protein tyrosine phosphatases (PTPs) was rapidly assembled using click chemistry. Subsequent high-throughput screening had led to the discovery of highly potent (K_i as low as 150 nM) and selective MptpB inhibitors, some of which represent the most potent MptpB inhibitors developed to date.

Protein tyrosine phosphatases (PTPs) are important signaling enzymes in the cell. Dysregulation of these enzymes has been linked to various human diseases.[1] For example, PTP1B is one of the best-known PTPs and has been identified as a key player against diabetes, obesity, and cancer. MptpB, another well-known protein tyrosine phosphatase encoded by *Mycobacterium tuberculosis*, is a promising target for new tuberculosis (TB) drugs.[2] Progress in the development of potent and selective PTP inhibitors, however, has been slow because of the highly conserved active site shared by most members of the PTP family. A significant advance came from the seminal discovery that there exists a unique secondary site near the active site of PTP1B (and possibly in many other PTPs).[3] As a result, major drug discovery efforts have focused on the use of fragment-based approaches for the identification of the so-called bidentate PTP1B inhibitors capable of binding to both the active site and the secondary site of the enzyme simultaneously.[4] We recently demonstrated the first example of using click chemistry followed by direct *in situ* screening for the rapid assembly and

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(1) (a) Montalibet, J.; Kennedy, B. P. *Drug Discovery Today: Ther. Strategies* **2005**, *2*, 129–135. (b) Blume-Jensen, P.; Hunter, T. *Nature* **2001**, *411*, 355–365. (c) Cook, W. S.; Unger, R. H. *Dev. Cell* **2002**, *2*, 385–387. (d) Zhang, Z.-Y. *Curr. Opin. Chem. Biol.* **2001**, *5*, 416–423. (e) Hunter, T. *Cell* **2000**, *100*, 113–127.

(2) Greenstein, A. E.; Grundner, C.; Echols, N.; Gay, L. M.; Lombana, T. N.; Miecskowski, C. A.; Pullen, K. E.; Sung, P. Y.; Alber, T. *J. Mol. Microbiol. Biotechnol.* **2005**, *9*, 167–181.

(3) Puius, Y. A.; Sullivan, M.; Lawrence, D. S.; Almo, S. C.; Zhang, Z.-Y. *Proc. Natl. Acad. Sci. U.S.A* **1997**, *94*, 13420–13425.

(4) (a) Liu, G.; Xin, Z.; Pei, Z.; Hajduk, P. J.; Abad-Zapatero, C.; Hutchins, C. W.; Zhao, H.; Lubben, T. H.; Ballaron, S. J.; Haasch, D. L.; Kaszubska, W.; Ronodinone, C. M.; Trevillyan, J. M.; Jirousek, M. R. *J. Med. Chem.* **2003**, *46*, 4232–4235. (b) Liu, G.; Xin, Z.; Liang, H.; Abad-Zapatero, C.; Hajduk, P. J.; Janowick, D. A.; Szczepankiewicz, B. G.; Pei, Z.; Hutchins, C. W.; Ballaron, S. J.; Stashko, M. A.; Lubben, T. H.; Berg, C. E.; Rondinone, C. M.; Trevillyan, J. M.; Jirousek, M. R. *J. Med. Chem.* **2003**, *46*, 3437–3440.

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identification of potent bidentate inhibitors against PTP1B.[5] This approach takes advantage of the highly efficient and modular Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction between an azide and an alkyne[6] and has thus far been successfully applied to the discovery of small molecule inhibitors against other classes of enzymes.[7] In our previous study, we were able to identify a "click-based" inhibitor that possesses inhibitory properties against PTP1B similar to those identified from conventional fragment-based approaches.[5] Herein, we report a ~3500-member PTP bidentate inhibitor library synthesized using our collection of alkynes and our previously synthesized azide library[8] and, from the *in situ* screening of this library, the discovery of the first click-based small molecule inhibitor against MptpB (K_i = 150 nM), which not only possesses a high specificity (11- to 43-fold) over other PTPs but also represents the most potent inhibitor of MptpB known in the literature.

Our bidentate inhibitors have three components: (i) the warheads, alkyne-containing *N*-phenyloxamic acids that are cell-permeable, potent bioisosteric phosphotyrosine mimics;[4,5] (ii) a variety of different types of building blocks that act as the secondary-site binders; and (iii) azide-containing linkers of different lengths joining the warhead and the building blocks (Figure 1). We anticipated that

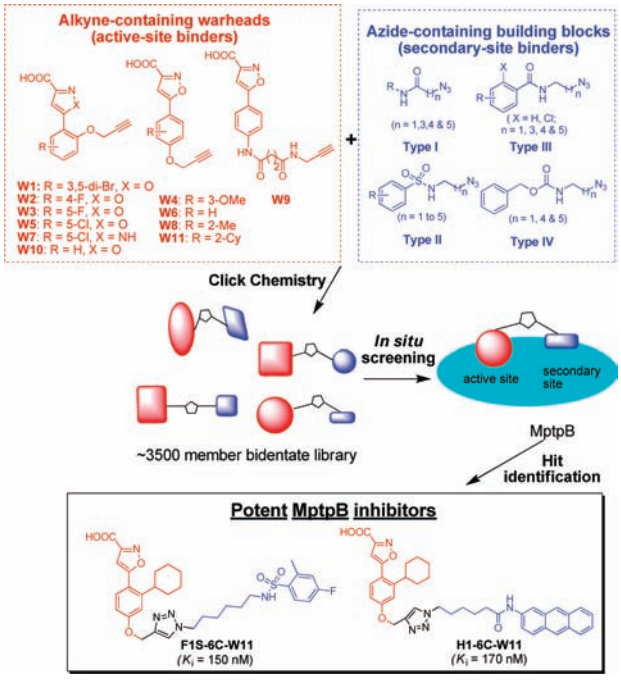
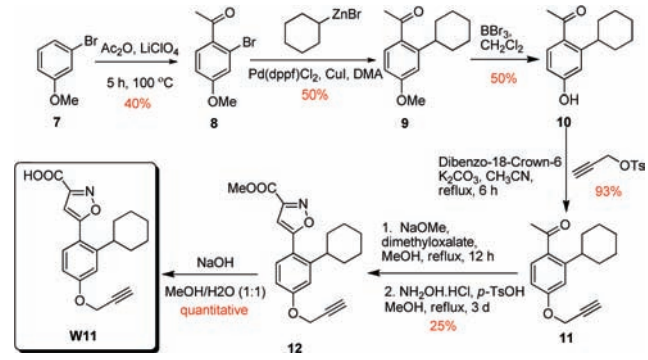


Figure 1. Overall strategy of the click-based high-throughput discovery of MptpB inhibitors. A total of 11 alkyne warheads and 325 azides were assembled to generate the ~3500-member bidentate library that, upon *in situ* screening, gave rise to two highly potent MptpB inhibitors (boxed).

combining these components would result in a sizable bidentate inhibitor library that might be used to target different PTPs with little structural knowledge of the nature of their active and secondary sites. We were particularly interested in finding inhibitors for MptpB as it is a relatively new target. Recent X-ray structural studies have also revealed the presence of a unique secondary binding site near the enzyme active site, a feature analogous to PTP1B.[9]

Of the 11 warheads used in this library, **W2**, **W3**, **W5**, **W6**, **W7**, and **W9** were synthesized as previously reported.[5,8] **W1**, **W4**, **W8**, and **W10** were newly designed *N*-phenyloxamic acid analogues guided by our previous results and computational modeling.[5] The final warhead, **W11**, was inspired by the recently discovered MptpB inhibitor from Ellman and co-workers.[10] Detailed chemical syntheses of the warheads are described in Supporting Information. The representative synthesis of one of the warheards, **W11**, is shown in Scheme 1. The synthesis of the 325-member

Scheme 1. Synthesis of Warhead **W11**



secondary-site binders, which consist of diverse aromatic and aliphatic building blocks linked to various azide-containing linkers, was previously reported.[8] With both the alkyne and the azide libraries in hand, we carried out high-throughput click assembly of the 11 × 325 compound combinations in 384-well microtiter plates with the aid of an automatic liquid handling system as previously reported.[11] Upon optimizations, we found click chemistry conditions carried out with $CuSO_4$ and sodium ascorbate as catalysts and H_2O/*t*-BuOH as cosolvents gave the best results. Subsequently, approximately 10% of the ~3500 compounds obtained were further characterized by LC−MS and confirmed to be of sufficient purity (>90% in most cases, see Supporting

(5) Srinivasan, R.; Uttamchandani, M.; Yao, S. Q. *Org. Lett.* **2006**, *8*, 713–716.
(6) For reviews, see: (a) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128–1137. (b) Meldal, M.; Tornoe, C. W. *Chem. Rev.* **2008**, *108*, 2952–3015.
(7) (a) Kalesh, K. A.; Yang, P.-Y.; Srinivasan, R.; Yao, S. Q. *QSAR Comb. Sci.* **2007**, *26*, 1135–1144. (b) Birk, A.; Wu, C. Y.; Wong, C. H. *Org. Biomol. Chem.* **2006**, *4*, 1446–1457. (c) Krasiński, A.; Radik, Z.; Manetsch, R.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. *J. Am. Chem. Soc.* **2005**, *127*, 6686–6692.
(8) Srinivasan, R.; Tan, L. P.; Wu, H.; Yang, P.-Y.; Kalesh, K. A.; Yao, S. Q. *Org. Biol. Chem.* **2009**, *7*, 1821–1828.
(9) Grundner, C.; Perrin, D.; van Huijsduijnen, R. H.; Swinnen, D.; Gonzalez, J.; Gee, C. L.; Wells, T. N.; Alber, T. *Structure* **2007**, *15*, 499–509.
(10) Soellner, M. B.; Rawls, K. A.; Grundner, C.; Alber, T.; Ellman, J. A. *J. Am. Chem. Soc.* **2007**, *129*, 9613–9615.
(11) Srinivasan, R.; Li, J.; Ng, S. L.; Kalesh, K. A.; Yao, S. Q. *Nat. Protoc.* **2007**, *2*, 2655–2664.

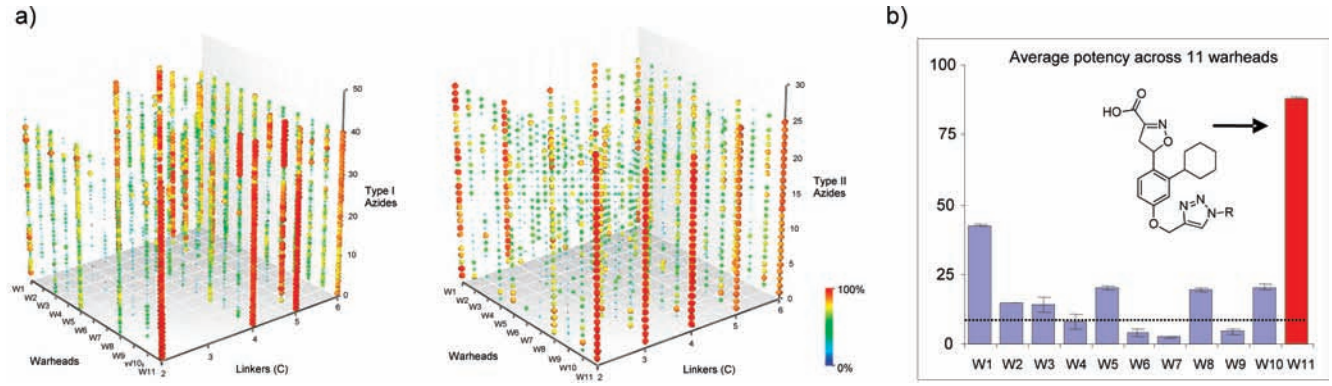


Figure 2. Inhibition profiles of the click library against MptpB. (a) 4-D plots of the inhibition fingerprints from the 2 largest sublibraries that are assembled using the 11 warheads and the Type I and II azides. Each inhibitor is represented by spheres positioned spatially across the warheads, linkers, and azides. Relative potency is given by both color spectrum (blue = least potent, red = most potent) and by size of the sphere (big = more potent, small = less potent) (b) Bar graph representation of averaged inhibitor potencies across sublibraries derived from each of the 1 warheads (red bar = sublibrary derived from **W11**). An arbitrary threshold of "8%" (dashed line) was set to distinguish the seven most potent warheads.

Information). They were subsequently used for direct *in situ* screening against recombinant MptpB enzyme. It should be highlighted that the key attribute of our strategy lies in the ability to rapidly assemble high-quality bidentate inhibitors in a matter of weeks or even days, thereby alleviating one of the major challenges in the traditional drug discovery process. The 3500-member library shown herein represents the largest click-based library assembled to date.

Upon screening against MptpB, the relative potency of each inhibitor was obtained and presented as a colored heat map (Figure S2 in Supporting Information). The inhibition fingerprints from the largest sublibraries consisting of Type I and II azides are also presented in 4-D cube plots in Figure 2a. A quick survey of the heat map and the cube plots revealed that almost all of the inhibitors derived from **W11** were more potent than those from other warheads, implying that **W11** plays a dominant role in contributing to inhibitor potency against MptpB. Interestingly, most inhibitors, especially those assembled from **W1**, showed varying degrees of inhibition potencies across different azides (see Supporting Information), highlighting the cooperative binding effect from both the warhead and the azide component in the bidentate inhibitor. Next, quantitative data analysis was carried out by averaging the inhibition potency across sublibraries derived from each warhead, and the results are graphically presented in Figure 2b. Inhibitors derived from **W11** were found to be 24-fold more potent than those from **W6**, which lacks the cyclohexyl group. This is presumably due to the considerable plasticity of the active site in MptpB;[10] the additional cyclohexyl group may be accommodated to gain favorable hydrophobic interactions. **W7**, which has a pyrazole ring in place of the isoxazole, was the least potent among the eleven warheads, indicating that the oxygen atom in the isoxazole ring is crucial as a hydrogen bond acceptor with the enzyme, since replacement with a hydrogen bond donor abolished most of the inhibitory activity. For further sublibrary analysis, an arbitrary threshold of "8%" (equivalent to 10% of the highest averaged inhibition potency (~80%) exhibited by inhibitors derived from **W11**) was set to select for the seven most potent warheads, **W11**, **W1**, **W10**, **W5**, **W8**, **W2**, and **W3** (Figure 2b). Next, the contribution of the linker units and the building blocks were further analyzed (Figure 3); the Type I and Type II azide-containing sublibraries were chosen as they gave the most comprehensive representation of different inhibitor components. Two interesting observations were made from this analysis. First, for the Type I azides, there was a marked preference for the 4-carbon alkyl linker, indicating an optimal length for fitting the bidentate inhibitor into the active and secondary sites of MptpB (Figure 3, Type I and Figure S3 in Supporting Information). In contrast, inhibitors assembled using the Type II azides did not displayed a distinct preference for any of the alkyl linkers (Figure 3, Type II and Figure S3 in Supporting Information). Second, there were recurring building blocks that consistently showed high potency regardless of the linker length among the Type I and II azides (Figure 3, in red). The top-50 inhibitors from each of the seven sublibraries were next identified, listed in Table S4 in Supporting Information and analyzed using Venn diagram (Figure S4 in Supporting Information) to obtain inhibitors that were potent across all the warheads. A total of 10 individual azides were found to be particularly strong secondary-site binders. Most of these azides contain hydrophobic building blocks (Table S5 in Supporting Information), with the highest-ranked azide being the recurring one as shown in Figure 3. Taken together, these observations indicate the potential of our small molecule library for evaluating individual components in a bidentate PTP inhibitor.

Finally, to obtain a better quantitative representation of the inhibitor potency, IC_{50} determination was carried out with ~350 selected compounds (Figure S5 in Supporting Information), of which 16 of the most potent inhibitors (structures shown in Figure S1 in Supporting Information) were scaled-

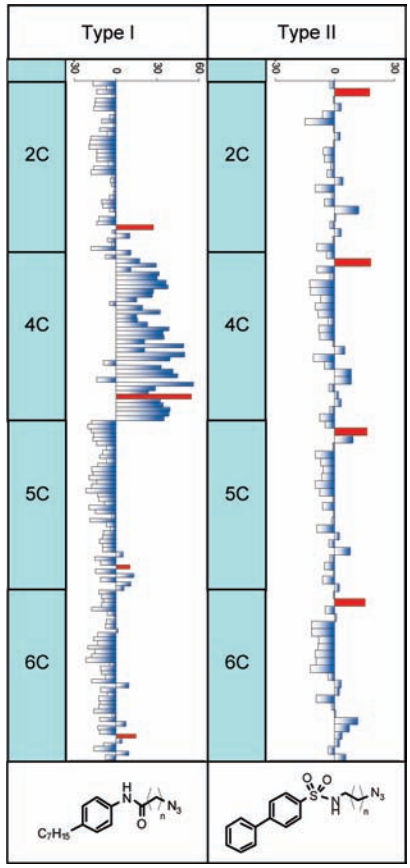


Figure 3. Bar graph representing the averaged inhibition potencies from inhibitors assembled using the seven warheads and the Type I and Type II azides. The two recurring building blocks (structures shown below the bar graph) from each set of sublibraries are indicated in red.

up, purified, and fully characterized (by LCMS and NMR) before full biochemical evaluations were carried out against MptpB and other PTPs. The IC_{50}, K_i values, and cell permeability of selected potent inhibitors are shown in Table 1 (see Tables S6, S7 and S8 in Supporting Information for full data). All compounds tested were in general cell-permeable. The most potent and selective inhibitors, **H1-6C-W11** and **F1S-6C-W11** (see structures in Figure 1), showed K_i values of 170 and 150 nM, respectively, against MptpB, which are even more potent than the best MptpB inhibitor known in the literature (K_i = 220 nM; reported by Ellman et al.[10]). More significantly, it showed 11- to 52-fold increase in selectivity over other PTPs tested. The azide and warhead components of the purified inhibitors were also

Table 1. IC_{50} (in μM) and K_i (in μM) of Selected Purified Inhibitors against MptpB and Other PTPs

inhibitor	MptpB IC_{50} (K_i)	selectivity[a] PTP/MptpB	permeability P_{app} (nm s^{-1})
H1-6C-W11	**0.55 ± 0.03 (0.17 ± 0.03)**	**23−52**	**ND**
F1S-6C-W11	**0.64 ± 0.09 (0.15 ± 0.01)**	**11−43**	**ND**
G4-6C-W11	0.84 ± 0.09 (0.77 ± 0.12)	9−43	545
F7S-6C-W11	0.95 ± 0.08 (0.45 ± 0.01)	7−26	ND
E4S-4C-W11	1.3 ± 0.18 (1.1 ± 0.11)	6−24	ND
F5S-4C-W11	1.4 ± 0.12 (1.3 ± 0.04)	2−19	ND
F7S-4C-W11	1.5 ± 0.28 (1.3 ± 0.15)	6−21	ND
E2S-4C-W11	1.6 ± 0.10 (ND)	2−15	ND
F6S-4C-W11	1.8 ± 0.03 (ND)	5−18	ND
W11	3.56 ± 0.04 (1.62 ± 0.04)	ND	ND

[a] Ratio of IC_{50} for other PTPs to that for MptpB (see Supporting Information for details). ND = not determined.

screened individually against MptpB. While **W11** alone was 10-fold less potent than **H1-6C-W11** and **F1S-6C-W11**, none of the azide components showed any significant inhibition against MptpB. Finally, the two most potent inhibitors were computationally docked against the active site of MptpB using the Sybyl software, on the FlexX suite (Figure S6 in Supporting Information); results indicate that in both cases the *N*-phenyloxamic acid containing warhead binds in the active site pocket of MptpB, with its azide-containing secondary binder projecting into the secondary pocket, thus indicating the possible bidentate nature of our inhibitors.

In conclusion, a ~3500-member library of bidentate inhibitors against protein tyrosine phosphatases (PTPs) has been synthesized, in high throughput, using click chemistry. This, to the best of our knowledge, constitutes the largest click chemistry library ever assembled. Subsequent direct *in situ* screening led to the identification of highly potent and selective MptpB inhibitors. The systematic analysis of the structure−activity relationship made possible by our inhibitor library against PTPs may shed light on the future development of other bidentate inhibitors against different PTPs.

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Supporting Information Available: Experimental procedures, characterization of new compounds, click chemisry, and biological screening. This material is available free of charge via the Internet at http://pubs.acs.org.

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