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Double Click Reaction for the Acquisition of a Highly Potent and Selective mPTPB Inhibitor

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Abstract

Tuberculosis (TB), which is caused by *Mycobacterium tuberculosis* (*Mtb*), is a major worldwide threat to public health. *Mycobacterium* protein tyrosine phosphatase B (mPTPB) is a virulent phosphatase secreted by *Mtb*, which is essential for the survival and persistence of the bacterium in the host. Consequently, small molecule inhibitors of mPTPB are expected to serve as anti-TB agents with a novel mode of action. We here report the discovery of highly potent and selective mPTPB inhibitors using a novel double Click chemistry strategy. The most potent mPTPB inhibitor from this approach possesses a K_i value of 160 nM and a selectivity of >25-fold against 19 other protein tyrosine phosphatases. Molecular docking study of the inhibitor and enzyme complex provides a rationale for the high potency and selectivity of the lead compound and reveals an unusual binding mode, which may guide further optimization effort.

Keywords

Protein Tyrosine Phosphatase (PTP); mPTPB; PTP inhibitor; Click reaction; Tuberculosis (TB)

Introduction

A vast array of biological phenomena, such as cell proliferation and differentiation, motility, metabolism, apoptosis, and the immune responses, are regulated by protein tyrosine phosphorylation, which are controlled by the balanced action of protein tyrosine phosphatases (PTPs) and protein tyrosine kinases (PTKs).¹ Similar to the PTKs, unregulated PTP activity causes aberrant dephosphorylation, which is associated with many human diseases, including cancer, diabetes, and autoimmune disorders.² Strikingly, PTPs have also been utilized by pathogenic bacteria to alter host defense mechanisms for their own infectivity and/or survival in the host. For instance, *Mycobacterium* protein tyrosine phosphatase B (mPTPB) is a virulence factor from *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB). mPTPB is secreted by *Mtb* into the cytoplasm of macrophages, where it mediates mycobacterial survival in the host.³ Deletion of mPTPB impairs the ability of the mutant strain to survive in interferon- γ (IFN- γ) activated

macrophages and severely reduces the bacterial load in a clinically-relevant guinea pig model.⁴

TB is a major worldwide threat to public health, with approximately 9 million new cases reported and 1.8 million deaths each year in the world.⁵ No new antiTB drugs have been developed in close to 40 years. Traditional TB treatment requires a 6–9 months administration of multiple antibiotics targeting mycobacterial biosynthetic processes involved in cell growth, including RNA transcription, protein translation, and cell wall biogenesis. The limited effectiveness of current antibiotics and lengthy treatment lead to poor patient compliance, which is responsible for high rates of treatment failure, relapse, and emergence of multidrug-resistant (MDR) and extensively resistant (XDR) TB. The prevalence of MDR-TB and XDR-TB, coupled with AIDS epidemic, underscores the urgency of developing more effective therapies against novel TB targets.⁶

Given the essential role of mPTPB for *Mtb* survival in the host, there is increasing interest in developing mPTPB inhibitors as novel antiTB agents. Because mPTPB inhibitors have no structural or mechanistic overlap with current drugs used for TB treatment and function within the cytosol of host macrophage, they have great potential to target the intracellular pool and compliment/synergize with existing therapeutic strategies. Moreover, since mPTPB is secreted into the cytosol of host macrophages, drugs targeting mPTPB are not required to penetrate the waxy mycobacterial cell wall, which is a major barrier blocking translation of target inhibition to activity against the intact pathogen. Consequently, specific mPTPB inhibitors may have therapeutic value with a unique mode of action.

To date, a limited number of mPTPB inhibitors have been reported. These include indoles discovered by biology-oriented synthesis,^{7a–7c} indolizines via solid phase synthesis,^{7d} isoxazoles through substrate-based fragment approach,^{7e} oxamic acid derivatives discovered by a homogeneous plate assay,^{7f} phenylisoxazole carboxylic acids^{7g} and most recently salicylic acid based inhibitors via Click chemistry.^{7h} Unfortunately, the potency and selectivity for many of the reported compounds are still modest, which is not surprising since PTPs share a conserved active site, rendering the acquisition of highly selective PTP inhibitory agents an extremely challenging endeavor. Moreover, due to the complexity of the structures, these inhibitors require multi-step synthesis (more than 7 steps for all reported inhibitors) with low yields, which may hinder their further development. Here we wish to present our discovery of a potent and selective, yet easily accessible mPTPB inhibitor via a novel double Click chemistry strategy.

Results and Discussion

It has been recognized that phosphotyrosine (pTyr) alone is not sufficient for high-affinity binding and residues flanking pTyr are important for PTP substrate recognition.⁸ Thus, an effective strategy for the acquisition of potent and selective PTP inhibitors is by tethering a nonhydrolyzable pTyr mimetic to an appropriately functionalized moiety in order to engage both the active site and a unique nearby sub-pocket.^{8,9} Click chemistry refers to the 2+3 cycloaddition between an alkyne and an azide,¹⁰ which is ideally suitable for connecting two fragments together. Because of its high yield and selectivity, excellent functional group tolerance, and robust reaction conditions, the Click reaction has been widely used in medicinal chemistry for library construction, lead optimization, and cell-based imaging.¹¹ Since Click reaction can be conducted in aqueous solution in the absence of deleterious reagents, libraries generated by Click reaction could be directly screened in-situ for enzyme inhibitors and protein ligands. In conventional Click reaction, an alkyne functional group is installed into a core component, which then reacts with a set of azide-containing fragments in a 1 to 1 mode to afford desired reaction products (Figure 1A). We have recently employed

Click chemistry for the construction of salicylic acid-based focused libraries to target both the PTP active site and its adjacent, peripheral secondary binding sites.¹² Although several bidentate inhibitors with excellent cellular activity were identified, the inhibitory potencies were still modest with IC₅₀ values in the low μ M range.

As an initial attempt to further increase interactions with peripheral pockets surrounding the active site, we were interested in introducing two alkyne groups into the core structure, which would enable a core to react with an azide in a 1 to 2 fashion, thus creating a tridentate molecule (Figure 1B). The additional fragment in the molecule would provide extra interactions with the enzyme hence the potency and selectivity could be improved. Another advantage is that, since one more fragment will be incorporated into the molecule, we may choose cores that are easily accessible and structurally less complicated for library assembly (Figure 2A), which may save significant amount of time and effort for the synthesis of cores.

To assess the effectiveness and feasibility of the double Click reaction approach for PTP inhibitor design, we prepared 5 benzoic and naphthoic acid based cores with 2 alkyne functional groups attached as shown in Figure 2B. The reasons we chose these cores are that they represent the simplest pTyr substrate analogs to fit into the positively charged active site of the PTPs and that they can be easily synthesized in 2 steps with high yields and large quantities from commercially available and inexpensive precursors. The synthesis is composed of alkylation followed by hydrolysis which is illustrated in Figure 2B. Subsequently, these 5 cores were purified by reversed-phase preparative HPLC and screened against mPTPB for inhibitory activity. We found that **L1**, **L2**, and **L4** showed no inhibition to the enzyme at 200 μ M concentration, while **L3** and **L5** inhibited mPTPB with an IC₅₀ of 50 and 75 μ M respectively. As an initial proof-of-concept, we decided to focus on core **L5** for library construction using the double click strategy. The azides used in this study were prepared from 53 amines (Figure 2C), as described previously.¹²

To prepare the library, we proceeded to couple **L5** with the azides using Click chemistry. A stock solution of core **L5** in DMF was introduced to 2 mL plastic reaction vessels, followed by the addition of 2 equivalents of various azides. Then a solution of tetrakis(acetonitrile)copper(I) hexafluorophosphate in DMF (20 mol%) was added, and the vessels were capped and allowed to stand at room temperature for 2 days. After that, representative reactions from 10 vessels were monitored by LC-MS, which indicated that the reactions furnished products in moderate to high conversion with >70% purity. This was consistent with published results from both others and our own group using Click reaction for library assembly.^{7g,7h,12} To avoid possible false positive hits caused by copper and other impurities, each reaction mixture was diluted with water, and the precipitate formed was filtered and washed with water. The solid we collected was dissolved in DMSO to make a 20 mM stock solution, which was ready for the screening step.

To identify mPTPB inhibitors, the ability of the library to inhibit the mPTPB-catalyzed hydrolysis of *p*-nitrophenyl phosphate (*p*NPP) was assessed at pH 7 and 25 °C. Among the 212-members in the library, 2 compounds displayed excellent inhibitory activity at ~10 μ M concentration. Resynthesis of the hits confirmed that they were genuine inhibitors for mPTPB with IC₅₀ values in the nM range. Compound L5B47 (Figure 3A) appeared to be the most potent for mPTPB with an IC₅₀ of 160 $\pm$ 10 nM and was selected for further characterization. It should be noted that the IC₅₀ for L5B47 is 469-fold lower than that of the parent core **L5**, indicating that the linker and amine diversity element contribute significantly to mPTPB binding. The second hit compound L5D47 had an IC₅₀ of 270 nM for mPTPB (Supporting Information).

Kinetic analysis revealed that L5B47 is a reversible and noncompetitive inhibitor for mPTPB with a K_i value of 162 ± 10 nM (Figure 3B). To determine the specificity for L5B47, its inhibitory activity toward mPTPA and a panel of mammalian PTPs including cytosolic PTPs, PTP1B, TC-PTP, SHP2, Lyp and FAP1, the receptor-like PTPs, CD45, LAR, and PTP α , the dual specificity phosphatases VHR, VHX, Cdc14, and the low molecular weight PTP, were measured. As shown in Figure 4, L5B47 is highly selective for mPTPB, exhibiting a 50-fold preference over mPTPA and greater than 25-fold preference for mPTPB vs. all mammalian PTPs examined. These results show that L5B47 is among the most potent and specific mPTPB inhibitors reported to date. Its easy synthesis (3 linear chemical steps) offers numerous benefits for further developments. We also prepared compound L5'B47 (Figure 5), a single Click reaction product from the same core and the same azide. The IC_{50} value of L5'B47 for mPTPB (3.2 μ M) is 20-fold higher (i.e. affinity 20-fold less potent) than L5B47. Moreover, L5'B47 exhibits no selectivity against the same panel of PTPs. Therefore, the double Click chemistry strategy is indeed superior to the conventional single Click reaction in producing more potent and selective mPTPB inhibitors.

Given its exceptional potency and selectivity, we performed a modeling study in order to aid in understanding potential interactions between L5B47 and mPTPB. Docking of the inhibitor to mPTPB was carried out using the AutoDock4.01 software package¹³ and the coordinates of mPTPB:OMTS complex (PDB:2OZ5).¹⁴ As shown in Figure 6, L5B47 binds near the entrance of the active site, with the carbon atom of the carboxyl group on the naphthalene being 16.3 Å from the sulfur atom on the catalytic Cys160. Therefore this inhibitor does not compete with the artificial substrate *p*NPP at the catalytic site, which explains the aforementioned kinetic observation that L5B47 is a reversible and noncompetitive inhibitor for mPTPB. The carboxylate group could form two H-bonds with the η -nitrogen of Arg210 and Arg63, which may be important for anchoring the naphthalene core right at the entrance of the active site. The piperidine and chlorine benzene from the carboxylate proximal branch in L5B47 may sit a hydrophobic pocket interacting with Phe98, Leu102, Phe222, Val219 and Leu227. The carboxylate distal branch of L5B47 extends to complement a neighboring groove on the surface of mPTPB, and are in Van der Waals contacts with surrounded residues, including Arg59, His94, Glu95, Thr96 and Phe98. Additionally, weak polar interactions may also exist between the chlorines on the naphthalene and benzene rings and the side chains of Arg59 and Lys91, respectively. Interestingly, the majority of the residues implicated in binding L5B47 appear to be unique to mPTPB, which is consistent with the observed selectivity of L5B47 for mPTPB.

Conclusion

We demonstrate in this study that, by adding two fragments into the core structure simultaneously, the double Click strategy is superior to the conventional Click reaction for acquisition of more potent and selective enzyme inhibitors. We have identified L5B47 as the most potent and selective yet easily accessible mPTPB inhibitor through this double Click chemistry strategy. L5B47 inhibits mPTPB in a noncompetitive manner with a K_i of 160 nM and a selectivity of more than 25-fold against 19 other PTPs. Molecular docking analysis suggests a unique binding mode, in which compound L5B47 binds at the entrance of the enzyme active site with the two branches lie on the hydrophobic grooves on both sides of the active site. We anticipate that the double Click chemistry approach may have broad applicability in the development of multidentate inhibitors directed against other enzymes, in addition to the protein phosphatases.

Experimental Section

Materials and General Procedures

p-Nitrophenyl phosphate (*p*NPP) was purchased from Fluke Co. Dithiothreitol (DTT) was provided by Fisher (Fair Lawn, NJ). For organic synthesis, reagents were used as purchased (Aldrich, Acros, Alfa Aesar, TCI) except where noted. ^1H and ^{13}C NMR spectra were obtained on a Bruker Avance II 500 MHz NMR spectrometer with TMS or residual solvent as standard. All column chromatography was performed using Dynamic Adsorbents 230–400 mesh silica gel (SiO_2) with the indicated solvent system unless otherwise noted. TLC analysis was performed using 254 nm glass-backed plates and visualized using UV light (254 nm). HPLC purification was carried out on a Waters Delta 600 equipped with a Sunfire Prep C18 OBD column (30 mm X 150 mm, 5 μm) with methanol/water (both containing 0.1% TFA) as mobile phase (gradient: 50–100% methanol, flow 10 mL/min). The purity of all final tested compounds was established to be >98% by reverse-phase HPLC on a Waters Breeze HPLC system with a SunFire C18 analytical column (4.6 mm, 150 mm, 5 μm) using acetonitrile/water (both containing 0.1% TFA) as the mobile phase (gradient: 30–100% acetonitrile, flow 1.5 mL/min), with UV monitoring at the fixed wavelength of 254 nm. Mass spectra were obtained using an Agilent Technologies 6130 quadrupole LC/MS.

Representative Procedure for the Synthesis of Cores

To methyl 4,7-dichloro-3,5-dihydroxy-2-naphthoate (0.287 g, 1 mmol) in acetone (3 mL) was added K_2CO_3 (0.42 g, 3 mmol) and propargyl bromide (0.32 mL, 80% (wt/wt) in toluene, 3 mmol), the mixture was refluxed for overnight. Then it was concentrated, diluted with water (10 mL) and extracted with ethyl acetate (3 $\times$ 10 mL). The combined organic layer was dried over sodium sulfate, filtered and concentrated. The residue was purified by silica gel column chromatography with ethyl acetate/hexane (1:5) as eluent to afford product. Subsequently, it was dissolved in methanol (2 mL), and 20% NaOH (2 mL) was added. The mixture was allowed to stir at rt for overnight. This mixture was acidified with dilute HCl solution (2 M), and extracted with ethyl acetate (3 $\times$ 10 mL). The combined organic layer was dried over sodium sulfate, filtered and concentrated. Then the residue was subjected to HPLC purification, and final product **L5** was obtained as white solid (90% yield, >95% purity). ^1H NMR (500 MHz, CDCl_3) δ 8.39 (s, 1H), 7.80 (s, 1H), 7.74 (s, 1H), 5.07 (d, J = 2.3 Hz, 2H), 4.94 (d, J = 2.4 Hz, 2H), 3.69 (t, J = 2.3 Hz, 1H), 3.66 (t, J = 2.4 Hz, 1H); ^{13}C NMR (500 MHz, CDCl_3) δ 166.5, 154.2, 147.8, 131.7, 127.7, 125.7, 125.6, 125.0, 124.1, 104.3, 99.5, 79.8, 79.3, 78.7, 78.3, 61.4, 56.4. ESI-MS Calcd. for $\text{C}_{17}\text{H}_{11}\text{Cl}_2\text{O}_4$ ($\text{M}+\text{H}^+$): m/z 349.0; found 349.0.

L1 (94% yield, >95% purity); ^1H NMR (500 MHz, CDCl_3) δ 12.40 (s, 1H), 7.74 (d, J = 8.7 Hz, 1H), 7.67 (d, J = 2.2 Hz, 1H), 6.69 (m, 1H), 4.87 (s, 4H), 3.59 (t, J = 2.3 Hz, 1H), 3.56 (t, J = 2.3 Hz, 1H); ^{13}C NMR (500 MHz, CDCl_3) δ 166.4, 161.2, 158.2, 133.2, 114.3, 106.5, 101.8, 78.9, 78.7, 78.7, 56.3, 55.9. ESI-MS Calcd. for $\text{C}_{13}\text{H}_{11}\text{O}_4$ ($\text{M}+\text{H}^+$): m/z 231.1; found 231.1.

L2 (95% yield, >95% purity); ^1H NMR (500 MHz, CDCl_3) δ 13.1 (s, 1H), 7.19 (d, J = 2.3 Hz, 2H), 6.86 (t, J = 2.3 Hz, 1H), 4.85 (d, J = 2.3 Hz, 4H), 3.69 (t, J = 2.3 Hz, 2H); ^{13}C NMR (500 MHz, CDCl_3) δ 166.9, 158.3, 133.0, 108.4, 107.0, 78.9, 78.6, 55.8. ESI-MS Calcd. for $\text{C}_{13}\text{H}_{11}\text{O}_4$ ($\text{M}+\text{H}^+$): m/z 231.1; found 231.1.

L3 (91% yield, >95% purity); ^1H NMR (500 MHz, CDCl_3) δ 12.97 (s, 1H), 8.16 (s, 1H), 7.80 (d, J = 9.1 Hz, 1H), 7.47 (m, 2H), 7.27 (dd, J = 2.5, 9.0 Hz, 1H), 4.94 (d, J = 2.3 Hz, 2H), 4.90 (d, J = 2.3 Hz, 2H), 3.59 (m, 2H); ^{13}C NMR (500 MHz, CDCl_3) δ 167.4, 154.3, 151.4, 130.6, 129.9, 128.4, 128.3, 124.2, 120.7, 109.0, 108.5, 79.2, 78.5, 78.4, 56.2, 55.6. ESI-MS Calcd. for $\text{C}_{17}\text{H}_{13}\text{O}_4$ ($\text{M}+\text{H}^+$): m/z 281.1; found 281.1.

L4 (90% yield, >95% purity); ^1H NMR (500 MHz, CDCl_3) δ 13.1 (s, 1H), 8.26 (s, 1H), 7.69 (s, 1H), 7.58 (d, $J = 8.2$ Hz, 1H), 7.36 (t, $J = 7.8$ Hz, 1H), 7.13 (d, $J = 7.7$ Hz, 1H), 5.05 (s, 2H), 5.00 (s, 2H), 3.62 (m, 2H); ^{13}C NMR (500 MHz, CDCl_3) δ 167.3, 152.6, 151.5, 131.0, 128.6, 126.7, 124.7, 124.3, 121.3, 108.2, 102.7, 79.2, 79.0, 78.8, 78.7, 56.2, 56.1. ESI-MS Calcd. for $\text{C}_{17}\text{H}_{13}\text{O}_4$ ($\text{M}+\text{H}^+$): m/z 281.1; found 281.1.

Azide Synthesis

All of the azides were synthesized previously.¹²

Library Synthesis via Double Click Chemistry

To 2 mL plastic vessels was added core **L5** (0.05 mmol, 0.5 mL of 100 mM stock solution in DMF), various azides (0.10 mmol, 2 equiv.), and tetrakis(acetonitrile)copper(I) hexafluorophosphate (0.01 mmol, 0.1 mL of 100 mmol stock solution in DMF, 20 mol%). The vessels were allowed to stand at room temperature for 2 days. Then the mixture was poured into water (6 mL), the precipitation formed was filtered and washed with water (6 mL $\times$ 2). The solid collected was dissolved in DMSO (2 mL) to give a stock solution at 20 mM (assuming the product is obtained at 80% yield).

L5B47

^1H NMR (500 MHz, CDCl_3) 8.35 (s, 1H), 8.31 (s, 1H), 8.28 (s, 1H), 7.72 (m, 2H), 7.34 (m, 2H), 7.09 (m, 2H), 6.88 (m, 2H), 5.33 (s, 2H), 5.23 (s, 2H), 4.61 (m, 4H), 3.50 (m, 8H), 3.13 (m, 8H), 3.06 (t, $J = 6.8$ Hz, 2H), 2.98 (t, $J = 6.8$ Hz, 2H). ^{13}C NMR (500 MHz, CDCl_3) δ 206.5, 167.9, 167.8, 166.6, 155.0, 150.3, 150.3, 148.1, 142.0, 141.9, 131.8, 131.5, 131.5, 130.4, 130.4, 127.3, 127.2, 125.7, 125.5, 125.3, 125.2, 124.8, 124.0, 119.9, 116.5, 116.4, 115.4, 103.8, 99.5, 66.4, 62.1, 47.6, 47.3, 45.8, 44.1, 44.1, 40.6, 40.4, 32.7, 32.6, 30.7. ESI-MS Calcd. for $\text{C}_{45}\text{H}_{43}\text{Cl}_6\text{N}_8\text{O}_6$ ($\text{M}+\text{H}^+$): m/z 1001.1; found 1001.1. HRMS Calcd. for $\text{C}_{45}\text{H}_{43}\text{Cl}_6\text{N}_8\text{O}_6$ ($\text{M}+\text{H}^+$): m/z 1001.1431; found 1001.1556

L5'B47

^1H NMR (500 MHz, CDCl_3) 8.63 (s, 1H), 8.31 (s, 1H), 7.65 (s, 1H), 7.42 (m, 1H), 7.31 (d, $J = 9.0$ Hz, 1H), 7.08 (d, $J = 2.4$ Hz, 1H), 6.88 (dd, $J = 8.9, 2.2$ Hz, 1H), 5.15 (s, 2H), 4.63 (t, $J = 6.4$ Hz, 2H), 3.56 (m, 4H), 3.14 (m, 4H), 3.02 (t, $J = 6.4$ Hz, 2H). ^{13}C NMR (500 MHz, CDCl_3) δ 167.9, 157.8, 150.3, 147.6, 141.9, 133.5, 131.4, 130.3, 128.6, 127.9, 125.5, 125.2, 124.6, 123.3, 119.9, 116.4, 115.4, 114.5, 106.7, 99.5, 66.2, 47.6, 47.2, 45.8, 44.2, 40.6, 40.4, 32.7. ESI-MS Calcd. for $\text{C}_{28}\text{H}_{25}\text{Cl}_4\text{N}_4\text{O}_5$ ($\text{M}+\text{H}^+$): m/z 637.1; found 637.1.

Inhibition Study

Expression and purification of recombinant mPTPB, and kinetic characterization of mPTPB inhibitors were described previously.^{12a}

Docking Analysis

The AutoDock4.0 software package¹³ was used to build the binding model of L23B47 with mPTPB. The 3D-structure of L23B47 was modeled and energy-minimized in Chem3D program, and the coordinates of mPTPB were taken from the mPTPB:OMTS complex structure of 2OZ5.pdb,¹⁴ both ligand and receptor were respectively introduced into AutoDockTools1.4.6¹⁵ program for a series of preprocessing, such as merging non-polar hydrogens, adding Gasteiger charges, setting rotatable bonds for the ligand, adding solvation parameters for the receptor, and so on. The docking space was visually set around the active site, the energy grid size was set to $66 \times 60 \times 80$ points with $0.375 \text{ \AA}$ spacing on each axis, then the energy grid maps for each atom type found in L23B47 (i.e. A, C, N, NA, OA and Cl), as well as the electrostatics and de-solvation maps were calculated using the auxiliary

program of AutoGrid4. Based on all of these prepared files, the automatic molecular docking work was carried out using the AutoDock4 program, the Lamarckian Genetic Algorithm with Local Search (LGALS) was selected for ligand conformational searching, the optimal binding conformation was determined by LGALS algorithm with the following important parameters during each docking run: energy evaluations of 2500000, population size of 100, mutation rate of 0.02, crossover rate of 0.8, Solis and Wets local search iterations of 300 with probability of 0.06. Finally, 256 separate docking runs were performed, and the resulting 256 binding conformations were classified into different clusters and ranked according to the calculated binding free energy, these cluster and energy information together with visual inspection in AutoDockTools1.4.6 generate the most possible binding mode.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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A. Conventional Click Reaction **B. Double Click Reaction**

Figure 1.
From Conventional to Double Click Reaction.

A: Library Generation by Double Click

B: The Structures and Synthesis of Cores

C: The Structures and Synthesis of Azides

Figure 2.
The Assembly of Compound Library by Double Click Reaction.

Figure 3. (A) Structure of L5B47. (B) Lineweaver-Burk plot for L5B47-mediated mPTPB inhibition. L5B47 concentrations were 0 (●), 0.1 (○), 0.2 (▼), 0.3 (▽), and 0.4 (■) μM , respectively.

Figure 4.
 IC_{50} values (μM) of L5B47 for a panel of PTPs.

Figure 5.
Structure of L5'B47.

Figure 6.
A model for potential interactions between mPTPB and L5B47.