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Rapid Assembly and in Situ Screening of Bidentate Inhibitors of Protein Tyrosine Phosphatases

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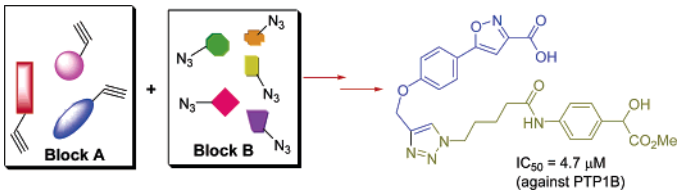
ABSTRACT



We have successfully designed and synthesized a small library of protein tyrosine phosphatase (PTP) inhibitors, in which the so-called "click chemistry" or Cu(I)-catalyzed 1,3-dipolar alkyne–azide coupling reaction was carried out for rapid assembly of 66 different bidentate compounds. Subsequent in situ enzymatic screening revealed a potential PTP1B inhibitor (IC_{50} = 4.7 μM) which is 10–100 fold more potent than other PTPs.

Fragment-based assembly is a recently developed drug discovery approach which enables high-throughput identification of small molecule inhibitors using a minimal number of compounds as building blocks.[1] The approach is powerful especially against protein targets which possess multiple binding pockets in their active sites. Currently, there are mainly three methods used to assist the assembly of compounds: (1) the NMR-based SAR strategy;[1b] (2) the tethering method developed by Wells et al.;[1c] and (3) the in situ screening method developed by Wong and co-workers,[1d] which was largely based on the "click chemistry" pioneered by Sharpless et al.[2] Among them, the click chemistry approach is highly versatile in that it requires neither specialized equipment nor mutations in the target proteins, making it easily adaptable by most research laboratories. To this end, it has been used successfully in the discovery of inhibitors against HIV protease, SARS 3CL protease, α-fucosidase, sulfotransferase, and α-1,3-fucosyltransferase.[1d,3]

Protein tyrosine phosphatases (PTPs) are a large and structurally diverse class of signaling enzymes. Defective regulation of these enzymes has significant implications in various human diseases.[4] For example, PTP1B, one of the best known PTPs, has long been identified as the key enzyme which causes obesity and diabetes.[4b] In the past few years, much effort has been made in attempts to develop potential drugs, mostly small molecule-based drugs, that target PTP1B in vivo with high efficacy and minimum side effects.[5] The biggest challenge, however, arises from the high sequence homology and highly conserved active site structures among

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different PTPs,[4] making it extremely difficult to identify small molecules which discriminatively bind to PTP1B over other PTPs. Recently, Zhang et al. discovered the presence of a secondary aryl−phosphate binding site near the active site of PTP1B,[6] thus shedding light on the development of bidentate inhibitors which might impart both potency and specificity against the enzyme.[7] On the basis of the same model, researchers at Abbott laboratories recently discovered cell-permeable, micromolar bidentate inhibitors containing a core *N*-phenyloxamic acid mimic (Figure 1),[8] which, upon

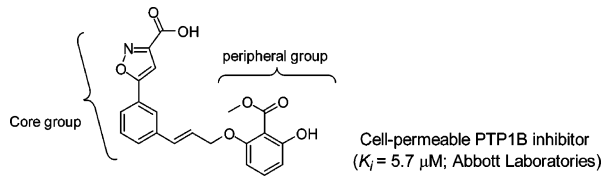

Figure 1. Chemical structure of Abbott's cell-permeable, bidentate PTP1B inhibitor. The core and peripheral groups bind to the active site and the secondary site of PTP1B, respectively.

testing in PTP1B-expressing COS-7 cells, showed much greater cellular activities (in terms of inhibition and selectivity) and pharmacokinetic properties than other inhibitors possessing potent inhibitory activity in vitro but suffering from low cell permeability and selectivity in vivo.[7,8a] The key to their success is the use of the NMR-based fragment assembly approach which greatly facilitated the rational improvement of lead compounds. However, the technical demand, the need for specialized equipment as well as a large quantity of proteins, and the intrinsic low throughput invariably limit this method in routine high-throughput screening (HTS). We aim to develop novel approaches which enable both high-throughput synthesis and screening of small molecule inhibitors against PTP1B, as well as other multivalent proteins. Herein, we report the use of click chemistry for rapid assembly, followed by in situ screening, of bidentate inhibitors against PTP1B.

To take advantage of both the primary and secondary binding sites within PTP1B and potentially other PTPs, our inhibitor design entails the following key criteria: (1) each member is made of a modular, bidentate structure containing both a core and a peripheral group for potential binding to the enzyme; (2) whenever possible, each of the two (core and peripheral) groups should possess optimized pharmacological properties; and (3) they may be efficiently assembled in situ for direct screening against potential PTPs. As a proof-of-concept experiment, a 66-member library based on the above criteria was constructed (Scheme 1): 5 alkyne-containing core groups and 14 azide-containing peripheral groups were used as building blocks which were subsequently assembled using click chemistry or the Cu(I)-catalyzed 1,3-dipolar alkyne−azide coupling reaction, previously shown to be highly modular, efficient, and compatible to most functional groups.[2] More importantly, the assembly reaction can be conducted in aqueous environments without the need of any deleterious reagents, thus allowing direct enzymatic screening and rapid identification of potential "hits" from the library. It should be noted that, although click chemistry had previously been used for assembly and identification of inhibitors against other enzymes,[1d,3] our report herein is, to the best of our knowledge, the first example for the synthesis and discovery of PTP inhibitors. We chose cell-permeable analogues of the *N*-phenyloxamic acid in Abbott's inhibitor (shown in Figure 1) as the core groups (boxed in Scheme 1), as they were shown to bind tightly to the primary site of PTP1B where the phosphotyrosine in a PTP1B substrate normally occupies.[8] In addition, these pharmacophores have been shown to possess "druglike" properties. Except for the introduction of an alkyne handle, minimal changes were made to these structures to ensure they maintained reasonable binding affinity to PTP1B and possibly other PTPs. Other more potent core groups were not considered because of their poor pharmacological properties.[7,8a] As for the peripheral group of the inhibitor, 14 different azide-containing building blocks were synthesized (Scheme 2), each of which bears an aromatic unit with different polarity and an alkyl linker of different length (2, 3, or 5 Cs). Aromatic compounds were chosen in the current study as they were previously shown to preferentially bind PTP1B at its secondary site.[8] As most PTPs are known to have a highly conserved active site (i.e., primary binding site), the key advantage of our strategy is that, in the future, without changes in the core group, a variety of other azide-containing molecules may be easily introduced in the bidentate library to generate inhibitors conferring high specificity against other PTPs while retaining good affinity.

Of the five different building blocks for the core group, compound **A** was synthesized from the commercially available 4-hydroxyacetophenone, **15**, which upon refluxing with propargyl tosylate in the presence of K_2CO_3 and benzo-18-crown-6 afforded **16** with excellent yield (93%). Subsequently, condensation between **16** and dimethyloxalate in the presence of NaOMe, followed by cyclization of the resulting product, gave the isoxazole carboxylic methylester, **17**, in modest yield (two steps) with published procedures.[8a] Next, base-catalyzed hydrolysis of **17** gave the free acid **A**. Compounds **B**, **C**, and **E** were similarly synthesized starting from either 5′-chloro- or 5′-fluoro-2′-hydroxyacetophenone (i.e., **18** or **21**). In the case of **E**, condensation of **19** with dimethyloxalate followed by direct treatment of the resulting product with hydrazine sulfate in the presence of *p*-TsOH generated the pyrazole carboxylic methylester, **29**, which upon hydrolytic cleavage with NaOH afforded compound **E** in 88% yield. For the synthesis of compound **D**, commercially available 4-nitroacetophenone, **24**, was condensed

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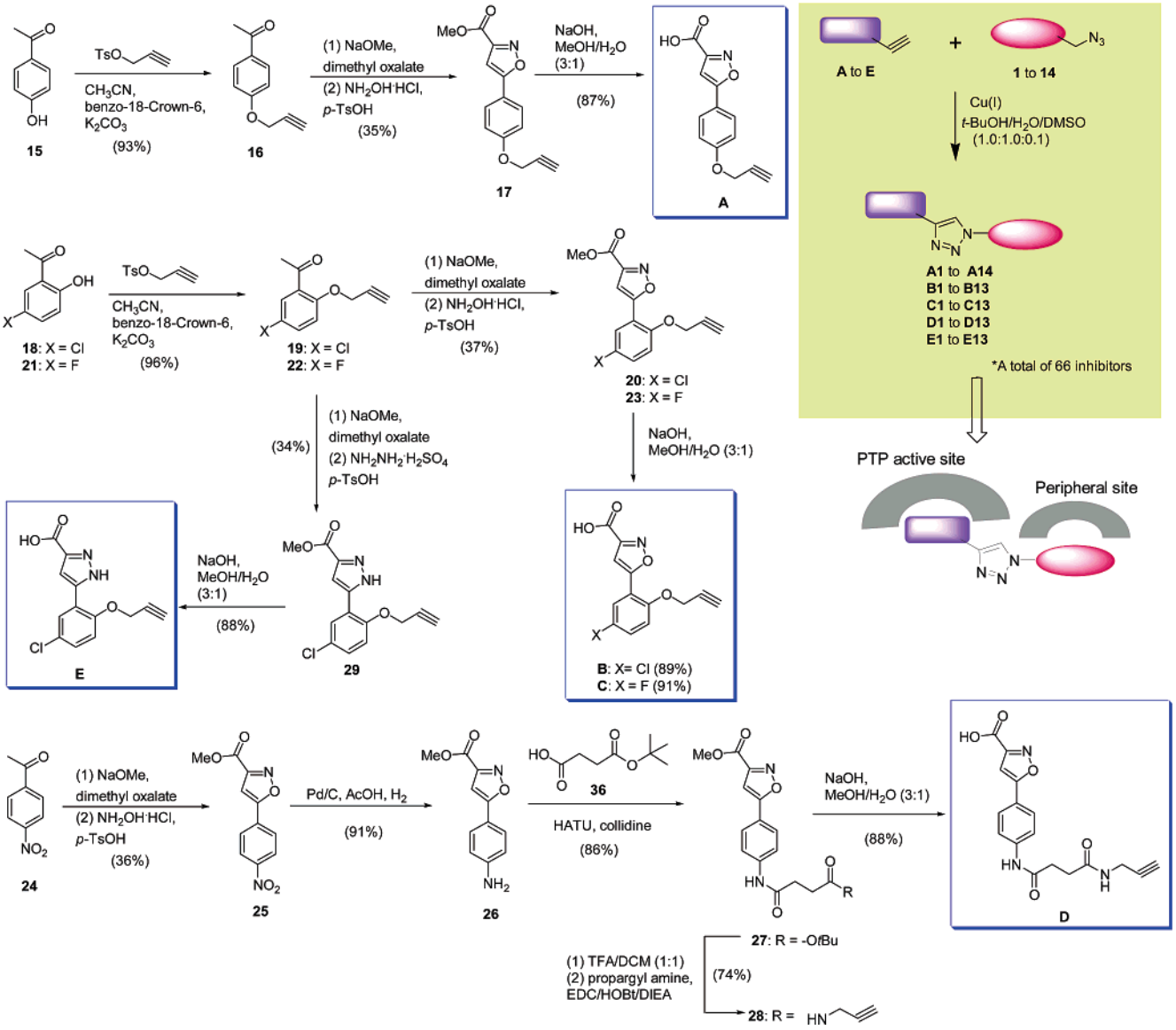
Scheme 1. Synthesis of the Alkyne Building Blocks

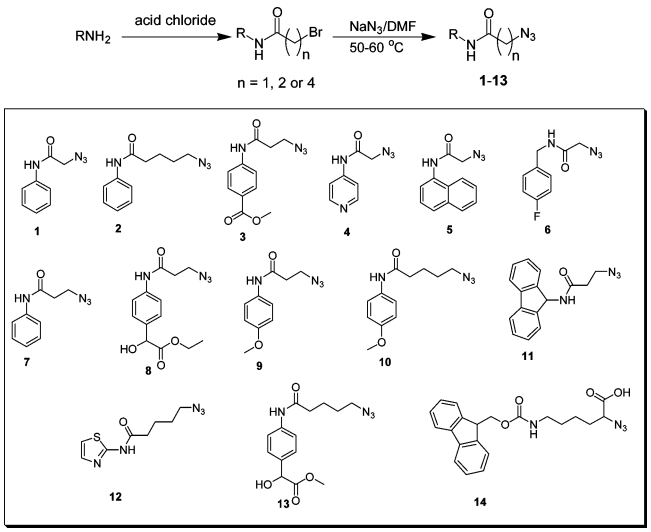


with dimethyloxalate followed by cyclization to afford **25** (36% overall yield). Subsequent reduction of the nitro group in **25** with 10% Pd−C in the presence of H_2 gave **26** (91%), which upon coupling with mono-Boc protected succinic acid, **36** (Supporting Information), generated **27**. Next, deprotection of the Boc group followed by coupling with propargylamine afforded **28**. The methylester in **28** was subsequently hydrolyzed with NaOH to afford compound **D** in 88% yield.

The 14 different aromatic azides were synthesized using highly efficient, two/three-step procedures (Scheme 2). 2-Bromoacetyl chloride and 3-bromopropionyl chloride were purchased commercially, whereas 5-bromovaleryl chloride, **30**, was synthesized from 5-bromovaleric acid, **29**, and thionyl chloride. Subsequently, nine different aromatic amines, representing different polarities and substituents, were acylated with one of the above three bromoalkylacyl chlorides, followed by an S_N2 substitution reaction with sodium azide in DMF to generate the corresponding azides. Compound **14** was synthesized from the corresponding amino acid (i.e., lysine).

The Cu(I)-catalyzed ligation of alkyne blocks and azide blocks was next carried out. It was found that a mixed solvent system containing *t*-BuOH, water, and DMSO (1:1:0.1) enabled both the alkynes and the azides to be completely dissolved, and at the same time, the 1,3-dipolar coupling was carried out with extremely high efficiency (Supporting Information). More importantly, the products could be taken directly without further purification and screened for inhibition against PTPs in subsequent microplate-based enzyme assays (vide infra). The click chemistry was initiated by catalytic amounts of $CuSO_4$ and sodium ascorbate. LC-MS analysis of all 66 coupling reactions indicated the complete consumption of the alkynes and quantitative fomation of the triazole products in most cases. A few of the triazole products precipitated and were separated (by centrifugation) and redissolved in DMSO.

Scheme 2. Structure and Synthesis of 14 Azide-Containing Blocks



The 66-member library was next screened directly against six different phosphatases, including four PTPs (i.e, PTB1B, TCPTP, YOP, and LAR), a dual-specific phosphatase (i.e., λPPase), and a serine/threonine phosphatase (i.e., PP1). A previously reported fluorescence-based phosphatase assay was used throughout.[9] Full details of the inhibition assays and profiles of the 66 inhibitors (plus controls with **A**-**E** and **1**-**14**) against all six enzymes were shown in the Supporting Information. Selected results were summarized in Figure 2

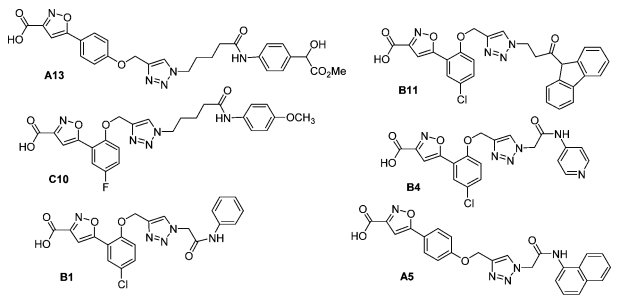


Figure 2. Potent inhibitors identified from the screening.

and Table 1. A majority of the library members showed some degree of inhibition against three of the four PTPs, namely, PTB1B, TCPTP, and YOP. This is expected when one considers that the library was purposefully designed to contain a core group which structurally resembles the original Abbott's inhibitor (as shown in Figure 1). On the other hand, none of the library members showed any inhibition against LAR, λPPase, and PP1 until they were at very high concentrations (>450 μM of inhibitor). For LAR, a protein tyrosine phosphatase, this is somewhat surprising. On the other hand, this may also underscore the feasibility of our strategy for potential discovery of inhibitors against specific PTPs with high potency and specificity. Six hits (**A13**, **C10**, **B1**, **B11**, **B4**, and **A5**) were chosen, one of which, e.g., **A13**,[10] was a potential potent and specific PTP1B inhibitor, and further characterized to obtain their IC_{50} values against each of the six enzymes. As shown in Table 1, **A13** indeed inhibited PTP1B with an IC_{50} of 4.7 μM, which is comparable to that of the original Abbott's inhibitor. More importantly, it is approximately 5 and 25 times more selective toward PTP1B than the other two PTPs, TCPTP and YOP, respectively, and is >100 times more potent than against LAR, λPPase, and PP1. Another noticeable hit is **C10**, which showed good inhibition toward TCPTP (IC_{50} = 17.5 μM) with some degree of selectivity over other phosphatases.

Table 1. IC_{50} (in μM) of Six Selected Inhibitors

inhibitor	PTP1B	TC TP	YOP	LAR	λPPase	PP1
A13	4.7	23.3	120.1	>450	>500	>750
C10	58.1	17.5	24.1	>450	>500	>750
B1	17.2	25.5	23.9	>450	>500	>750
B11	29.6	21.7	82.2	>450	>500	>750
B4	19.8	49.4	176.8	>450	>500	>750
A5	34.5	64.5	74.6	>450	>500	>750

In conclusion, we have developed the first "click chemistry" approach for rapid assembly of a bidentate, small molecule library against protein tyrosine phosphatases (PTPs). Compounds synthesized from this method are of high quality, enabling direct in situ screening and high-throughput identification of potential PTP inhibitors. The strategy is characterized by its modular nature, thus allowing easy adaption for screening of other PTPs and multivalent proteins, e.g., kinases. We identified a specific PTB1B inhibitor with moderate inhibition (e.g., **A13**; IC_{50} = 4.7 μM) comparable to that of Abbott's inhibitor, reported to be one of the most promising drug candidates against PTP1B to date.[8a] We expect that our lead compound should possess favorable pharmacokinetic properties similar to those for Abbott's inhibitor. In vivo experiments are currently being conducted to confirm this and will be reported in due course.

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

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(10) Of the 14 azides used (Scheme 2), two have chiral centers (i.e., **13** and **14**). **14** was synthesized from the corresponding chiral amino acid (Supporting Information). **13** was made from its racemic starting material. No attempt was made to isolate the two enantiomers in **A13**. It is possible that one enantiomer may be more potent than the other.