Identification of Bidentate Salicylic Acid Inhibitors of PTP1B

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Supporting Information

ABSTRACT: PTP1B is a master regulator in the insulin and leptin metabolic pathways. Hyper-activated PTP1B results in insulin resistance and is viewed as a key factor in the onset of type II diabetes and obesity. Moreover, inhibition of PTP1B expression in cancer cells dramatically inhibits cell growth *in vitro* and *in vivo*. Herein, we report the computationally guided optimization of a salicylic acid-based PTP1B inhibitor **6**, identifying new and more potent bidentate PTP1B inhibitors, such as **20h**, which exhibited a > 4-fold improvement in activity. In CHO-IR cells, **20f**, **20h**, and **20j** suppressed PTP1B activity and restored insulin receptor phosphorylation levels. Notably, **20f**, which displayed a 5-fold selectivity for PTP1B over the closely related PTP σ protein, showed no inhibition of PTP-LAR, PRL2 A/S, MKPX, or papain. Finally, **20i** and **20j** displayed nanomolar inhibition of PTP σ , representing interesting lead compounds for further investigation.

KEYWORDS: PTP1B, PTP σ , diabetes, insulin receptor, small molecule inhibitor, salicylic acid, bidentate inhibitors

Phosphorylation of regulatory and signaling proteins within the cell is closely monitored by the coordinated action of protein tyrosine kinases (PTKs) and the superfamily of protein tyrosine phosphatases (PTPs). The PTKs and PTPs are responsible for the addition and removal of phosphate groups from specific tyrosine (Y) residues on proteins, respectively. This partnership is responsible for the regulation of cellular communication and homeostasis.¹ Dysregulation of phosphorylation events are directly related to malignancy states including, but not limited to, diabetes,² cancer,^{3–5} neurological,⁶ metabolic,^{7–9} and autoimmune disorders.¹⁰ Historically, drug discovery programs have predominately focused on identifying PTK inhibitors since the prevailing dogma was that phosphatases were not as attractive drug targets. PTPs were overlooked and considered housekeeping enzymes with broad specificity, as compared to their PTK counterparts. However, advancements in the past two decades in the field of molecular biology uncovered a more profound role for PTPs in cell regulation, growth, and the onset of human disease.^{6,7,11} The importance of PTPs in signal transduction and cellular homeostasis has led to a flurry of discoveries, which linked their aberrant activities to numerous pathologies.^{12–15} These discoveries have validated PTPs as interesting therapeutic targets.

The most studied of the PTP enzymes is PTP1B (gene code: PTPN1), which is overexpressed in muscle, liver, and adipose tissues, and implicated in the onset of type II diabetes (T2D). PTP1B overexpression leads to persistent dephosphorylation of the insulin receptor (IR), stimulating the insulin-resistant

phenotype in T2D and obesity. The pivotal biological role of PTP1B has led to numerous inhibitor programmes.^{8,9,16–19}

Despite the identification of many potent compounds, a PTP1B-inhibiting drug has yet to reach the clinic. The most common strategy for inhibiting PTP1B, or other PTPs, has been to target the active site. However, deriving selectivity has been problematic, given that PTP active sites have, in some cases, 100% sequence homology.^{9,12,20} As a result, recent inhibitor designs have focused on the simultaneous targeting of multiple subpockets proximal to the active site.^{21–23} These domains are less conserved among the PTP isoforms and are seen as being a route to achieving enhanced target selectivity. In the case of PTP1B, where a proximal phosphotyrosine (pTyr)-binding site is located adjacent to the pTyr-binding active site, inhibitors have been developed that possess two pTyr groups or similar bivalent combinations of pTyr and more drug-like pTyr bioisosteres (compounds **1–5**, Figure 1).²⁴ However, bivalent pTyr-containing inhibitors that exhibit high PTP1B selectivity often exhibit poor pharmacological profiles, likely due to the charged pTyr-mimicking functionality and peptidic character.¹²

In previous efforts to target the pTyr binding site of PTP1B, scaffolds have incorporated either a salicylic or benzoic acid²⁵ as a cell-permeable pTyr mimetic.^{26–28} Salicylic acid-based inhibitors of PTP1B were reported, with IC₅₀ values of 3–8 μ M, and no inhibition of PTP-LAR, PRL2 A/S, MKPX, or

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Figure 1. Chemical structures of previously described PTP1B inhibitors.

Figure 2. Docking image of compound **6** in the active site of PTP1B (PDB: 1NWL). Protein: hydrophobic residues (blue) and hydrophilic residues (red). Compound: nitrogen (blue), oxygen (red), and sulfur (gold).

papain (**6**, Figure 2).² However, the compounds displayed equipotent inhibition of another clinically important phosphatase, PTP σ . PTP σ is a potential target for the development of novel therapies for spinal cord injury and multiple neurodegenerative diseases.^{29–31} Moreover, PTP σ inhibition has recently been identified as a target for improving memory loss.³² In this study, it was hypothesized that incorporation of a second carboxylate group to compound **6** might provide a second pTyr mimetic for more potent and selective targeting of PTP1B. It was reasoned that such a scaffold might improve cell penetrating properties more than current pTyr mimetics utilized in bidentate PTP1B inhibitors.²²

To identify the site of carboxylate binding on **6**, *in silico* docking studies were performed with PTP1B using Genetic Optimization for Ligand Docking (GOLD) software (Figure 2). Results indicated that the salicylic acid was binding within the pTyr-recognition site (Asp181 and Cys215, Figure 2) and the cyclohexylbenzyl group was docked within the large hydrophobic pocket (Ile219, Gly259, and Val49, Figure 2). The quinoline group was found to form π – π interactions with Phe182, as well as to H-bond with the peptide backbone via the quinoline nitrogen. Since the *N*-methyl group was not involved in binding and was oriented toward the second pTyr binding site, it was selected for attaching the second salicylic acid substituent.

Copper-catalyzed click chemistry was selected to couple the two components via a triazole linker of varying alkyl chain length.^{33,34} As opposed to a flexible and rather hydrophobic hydrocarbon linker, the flat triazole provides additional polarity and rigidity and has been deemed the ideal linker for development of bridged inhibitors in recent literature.^{33,34} To study the binding mode of the proposed disalicylic acids, comparative *in silico* docking analysis was conducted with PTP1B and a proposed bidentate inhibitor **20f**, superimposed

Figure 3. Comparative docking studies of **20f** (cyan) and inhibitor SP7343-SP7964 (green) bound to PTP1B (PDB: 1NWL). Protein: hydrophobic residues (blue) and hydrophilic residues (red). Compounds: nitrogen (blue), oxygen (red), and sulfur (gold).

with known bidentate PTP1B inhibitor, SP7343-SP7964 ($K_i = 4.1$ mM, Figure 3). Docking revealed that **20f** bound analogously to SP7343-SP7964, with both salicylic acids interacting with the respective pTyr-binding sites. The interactions between the cyclohexylbenzyl group of **6** and the hydrophobic pocket of the active site were retained, despite the addition of the second salicylate group. Next, the linker length between both salicylic acid groups was explored *in silico* with analysis suggesting to incorporate linkers ranging from one to five carbons (Supporting Information). The *N*-sulfonamide aryl group, R, was either a quinoline, as previously used in **6**, or a tolyl group, as previous investigations with this substituent had indicated activity against PTP1B.²

The focused library was screened for activity at 10 μ M using a previously described DiFMUP assay against the non-receptor tyrosine phosphatases PTP1B and TC-PTP, receptor-like tyrosine phosphatases PTP σ and PTP-LAR, the dual specificity phosphatases PRL2 A/S (active mutant) and MKPX, and a human cysteine-based protease papain (used here as a control for oxidation) (Figure 4). Compounds **20f–j** inhibited TC-PTP, PTP1B, and PTP σ enzymatic activity by over 90% compared to the DMSO control. Compounds **20f–j** showed modest increases in activity as compared to **6** (Supporting Information). Negligible effects were observed against PRL2 A/S, PTP-LAR (D1D2), MKPX, or CD45D1D2. MKPX and PRL2 A/S are dual specificity phosphatases that recognize phosphorylated Ser and Thr residues, as well as pTyr. The selectivity may be the result of unfavorable binding interactions with the catalytic pockets of PTP-LAR (D1D2), MKPX, CD45D1D2, and PRL2 A/S. Tolyl inhibitors **20a–e** were significantly less active and less selective compared to the quinoline analogues. This was likely due to poor water solubility under the assay conditions and/or less favorable binding interactions of the tolyl with the PTP1B active site as compared to the quinoline derivatives (Comparative docking studies provided in the Supporting Information). Quinoline compounds **20f–j** inhibited the receptor-like PTPs, such as PTP σ , more potently than the non-receptor PTPs like PTP1B. Thus, **20f–j** were further evaluated to quantify relative levels of PTP1B and PTP σ inhibition.

IC_{50} values were determined for each compound (Table 1, with representative curves shown in Figure 5). IC_{50} values for **20f–j** ranged from 0.6–6.1 μ M. Two inhibitors, **20f** and **20h**, possessed > 2-fold improved potency for PTP1B over **6** (**20f**, $IC_{50} = 3.6$ μ M; **20h**, $IC_{50} = 1.7$ μ M; **6**, $IC_{50} = 6.2$ μ M), with **20h** also displaying a 4-fold selectivity for PTP1B over PTP σ . Notably, **20i** was identified as the first nanomolar PTP σ

Figure 4. (A) Structures of inhibitors **20a–j**; (B) preliminary high-throughput kinetic enzyme activity screen for compounds **6** and **20a–j** at 10 μM against phosphatases PTP1B, TC-PTP, PTP σ (D1D2), PTP-LAR (D1D2), PRL2 A/S, and MKPX, with the protease papain present as a negative control. Experiments were performed in duplicate. Graph bars represent percent inhibition (100 = complete inhibition; 0 = no inhibition).

Table 1. IC_{50} (μM) Values for **6** and **20f–j** against PTP1B and PTP σ in Dose-Dependent Enzymatic Assays^a

compd	PTP1B	PTP σ
6	6.2 ± 1.1	4.3 ± 1.2
20f	3.6 ± 0.9	15.6 ± 1.1
20g	6.1 ± 0.4	11.8 ± 0.1
20h	1.7 ± 0.4	7.9 ± 1.3
20i	2.3 ± 0.2	0.6 ± 0.9
20j	2.4 ± 1.3	0.9 ± 0.1

^aBased on initial enzyme screen.

inhibitor, also with approximately 4-fold selectivity over PTP1B (PTP1B IC_{50} = 2.3 μM ; PTP σ IC_{50} = 0.6 μM).

Compounds **20i** and **20j** were screened against a panel of PTPs employing the DiFMUP assay. The dose-dependent enzyme activity rate curves of **20i** and **20j** showed significantly greater activity against PTP1B and PTP σ , compared to PTP-LAR (D1D2), MKPX, and CD45D1D2 (Figure 5). The limited discrimination of the compounds between PTP1B and TC-PTP was anticipated, given > 74% homology in their catalytic domains and adjacent pockets. This demonstrated for the first time a scaffold that displayed potency and selectivity for PTP1B over PTP σ . Moreover, this study identified potent PTP σ inhibitors, **20i** and **20j**. However, given there is no cell-based assay for assessing the enzymatic activity of PTP σ , the inhibition profiles of **20i** and **20j** against PTP σ could not be explored further at this time.

The cytotoxicities of PTP1B inhibitors **20f**, **20h**, and **20j** were determined in Chinese hamster ovary (CHO) cells, which express the human insulin receptor (CHO-IR). A Titer-Blue

Figure 5. Dose-dependent curves for inhibitors **20f–j**. Inhibitors were tested against an array of phosphatases. Individual plots overlay inhibitor-specific data for the various phosphatases.

Cell viability assay (Promega) showed no toxicity at 0.78 to 50 μM concentrations (Supporting Information).² Having established that the compounds were nontoxic, **20f**, **20h**, and **20j** were subjected to Western blot analysis against PTP1B in CHO cells.² Briefly, the insulin-signaling cascade depends on the phosphorylation state of the insulin receptor (IR). In this signal transduction pathway, insulin binds to the IR on the extracellular plasma membrane and promotes autophosphorylation of the IR (pIR). Since pIR is a direct substrate for PTP1B, the IR phosphorylation state is largely dependent on the activity levels of PTP1B within the cytoplasm. Thus, monitoring pIR levels is a useful reporter for PTP1B activity. CHO cells were incubated with increasing concentrations of inhibitor in serum-free media, then stimulated with insulin for 15 min. Western blot analysis determined the relative levels of pIR (Figure 6). Compounds **20f**, **20h**, and **20j** dose-dependently increased the pIR signal, relative to the DMSO control (Figure 6A). Although vanadate induced a strong response (50 μM), it suffers as a therapeutic due to poor metabolic stability and low bioavailability.³⁵ In addition, the compounds displayed improved activity in cells relative to **6** (Supporting Information). The densitometry graph showed **20f**, **20h**, and **20j** had a significant effect on the pIR/IR levels compared to the DMSO control (Figure 6B).²

Finally, comparative Michaelis–Menten kinetic studies were performed for **6** and **20f** against PTP1B (Figure 7). Compound **20f** showed an approximate 3-fold greater competitive inhibition (K_i = 1.83 ± 0.39 μM) compared to **6** (K_i = 5.66 ± 2.11 μM). In comparison to **6**, the bidentate approach yielded a more direct, competitive inhibitor of the active site. In general, in the field of PTP inhibition, mixed inhibitors tend to display a higher degree of toxicity, attributed to their promiscuous mode of action.^{12,36,37} Thus, we have optimized a mixed inhibitor, **6**, to produce **20f**, a competitive and presumed active site binder of PTP1B.

In summary, identifying selective and potent small molecule inhibitors of the PTP family of proteins (> 200 members)

Figure 6. (A) Dose-dependent response of compounds **20f**, **20h**, and **20j** on the phosphorylated insulin receptor was determined by Western blot. Cells were treated with compounds (5–50 μM), DMSO as a solvent control, 50 μM vanadate (VN), or left unstimulated (NI). (B) Densitometry graph showing the response of **20f**, **20h**, and **20j** at 25 μM on the levels of pIR and IR vs a DMSO control, * $P < 0.05$. Values correspond to mean $\pm$ standard error of the mean ($n = 4$). Statistical analysis was performed with two-tailed, unpaired, Student's t test.

Figure 7. Michaelis–Menten rate data as a function of substrate concentration for **20f** and **6**. Curves represent the hydrolysis of DiFMUP and subsequent fluorescent detection at 450 nm (excitation at 358 nm). The bottom curves show the Lineweaver–Burke transformation detailing competitive inhibition for **20f** and **6**.

remains a daunting task due to the high degree of sequence homology. Inspired by current approaches utilizing bidentate scaffolds, a family of disalicylic acid derivatives, based on PTP1B inhibitor **6**, were developed to achieve more potent and selective inhibition of PTP1B. A number of compounds were identified that displayed improved potency (>5-fold) and selectivity for PTP1B over PTP σ (4-fold) relative to **6**. Compounds **20f–j** did not inhibit other PTP family members such as PTP-LAR (D1D2). In particular, **20h** represents an interesting lead for further investigation against PTP1B, as it demonstrated whole-cell PTP1B inhibition and >4-fold selectivity over PTP σ . Finally, **20i** and **20j** displayed nanomolar inhibition for PTP σ and thus represent interesting leads for further investigation against this novel target.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.5b00171.

Information on compound synthesis, characterization, detailed results, and experimental procedures (PDF)

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Notes

The authors declare no competing financial interest.

■ REFERENCES

- Bauman, A. L.; Scott, J. D. Kinase- and phosphatase-anchoring proteins: harnessing the dynamic duo. *Nat. Cell Biol.* **2002**, *8*, 203–206.
- Haftchenary, S.; Ball, D. P.; Aubry, I.; Landry, M.; Shahani, V. M.; Fletcher, S.; Page, B. D. G.; Jouk, A. O.; Tremblay, M. L.; Gunning, P. T. Identification of a potent salicylic acid-based inhibitor of tyrosine phosphatase PTP1B. *MedChemComm* **2013**, *4*, 987–992.
- Haftchenary, S.; Avadisian, M.; Gunning, P. T. Inhibiting aberrant Stat3 function with molecular therapeutics: a progress report. *Anti-Cancer Drugs* **2011**, *22*, 115–127.
- Östman, A.; Hellberg, C.; Böhmer, F. D. Protein-tyrosine phosphatases and cancer. *Nat. Rev. Cancer* **2006**, *6*, 307–320.
- Hoekstra, E.; Peppelenbosch, M. P.; Fuhler, G. M. The role of protein tyrosine phosphatases in colorectal cancer. *Biochim. Biophys. Acta, Rev. Cancer* **2012**, *1826*, 179–188.
- Muise, A. M.; Walters, T.; Wine, E.; Griffiths, A. M.; Turner, D.; Duerr, R. H.; Regueiro, M. D.; Ngan, B. Y.; Xu, W.; Sherman, P. M.; Silverberg, M. S.; Rotin, D. Protein-tyrosine phosphatase sigma is associated with ulcerative colitis. *Curr. Biol.* **2007**, *17*, 1212–1218.
- Kennedy, B. P.; Ramachandran, C. Protein tyrosine phosphatase-1B in diabetes. *Biochem. Pharmacol.* **2000**, *60*, 877–883.
- Combs, A. P. Recent Advances in the Discovery of Competitive Protein Tyrosine Phosphatase 1B Inhibitors for the Treatment of Diabetes, Obesity, and Cancer. *J. Med. Chem.* **2010**, *53*, 2333–2344.
- Johnson, T. O.; Ermoliev, J.; Jirousek, M. R. Protein tyrosine phosphatase 1B inhibitors for diabetes. *Nat. Rev. Drug Discovery* **2002**, *1*, 696–709.
- Yip, S. C.; Saha, S.; Chernoff, J. PTP1B: a double agent in metabolism and oncogenesis. *Trends Biochem. Sci.* **2010**, *35*, 442–449.
- Freiss, G.; Vignon, F. Protein tyrosine phosphatases and breast cancer. *Crit. Rev. Oncol. Hemat.* **2004**, *52*, 9–17.
- Zhang, S.; Zhang, Z. Y. PTP1B as a drug target: recent developments in PTP1B inhibitor discovery. *Drug Discovery Today* **2007**, *12*, 373–381.
- Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C.

- C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* **1999**, 283, 1544–1548.
- (14) Friedman, J. M. Obesity in the new millennium. *Nature* **2000**, 404, 632–634.
- (15) Ahima, R. S.; Prabakaran, D.; Mantzoros, C.; Qu, D.; Lowell, B. Role of leptin in the neuroendocrine response to fasting. *Nature* **1996**, 382, 250–252.
- (16) Szczepankiewicz, B. G.; Liu, G.; Hajduk, P. J. Discovery of a potent, selective protein tyrosine phosphatase 1B inhibitor using a linked-fragment strategy. *J. Am. Chem. Soc.* **2003**, 125, 4087–4096.
- (17) Stuble, M.; Zhao, L.; Aubry, I.; Schmidt-Arras, D. Cellular inhibition of protein tyrosine phosphatase 1B by uncharged thiothiazolidinone derivatives. *ChemBioChem* **2007**, 8, 179–186.
- (18) Wilson, D. P.; Wan, Z. K.; Xu, W. X.; Kirincich, S. J. Structure-based optimization of protein tyrosine phosphatase 1B inhibitors: from the active site to the second phosphotyrosine binding site. *J. Med. Chem.* **2007**, 50, 4681–4698.
- (19) Popov, D. Novel protein tyrosine phosphatase 1B inhibitors: interaction requirements for improved intracellular efficacy in type 2 diabetes mellitus and obesity control. *Biochem. Biophys. Res. Commun.* **2011**, 410, 377–381.
- (20) Iversen, L. F.; Møller, K. B.; Pedersen, A. K.; Peters, G. H. Structure determination of T cell protein-tyrosine phosphatase. *J. Biol. Chem.* **2002**, 277, 19982–19990.
- (21) Guo, X.-L.; Shen, K.; Wang, F.; Lawrence, D. S.; Zhang, Z.-Y. Probing the Molecular Basis for Potent and Selective Protein-tyrosine Phosphatase 1B Inhibition. *J. Biol. Chem.* **2002**, 277, 41014–41022.
- (22) Low, J. L.; Chai, C. L. L.; Yao, S. Q. Bidentate Inhibitors of Protein Tyrosine Phosphatases. *Antioxid. Redox Signaling* **2014**, 20, 2225–2250.
- (23) Srinivasan, R.; Uttamchandani, M.; Yao, S. Q. Rapid assembly and in situ screening of bidentate inhibitors of protein tyrosine phosphatases. *Org. Lett.* **2006**, 8, 713–716.
- (24) Bialy, L.; Waldmann, H. Inhibitors of Protein Tyrosine Phosphatases: Next-Generation Drugs? *Angew. Chem., Int. Ed.* **2005**, 44, 3814–3839.
- (25) Haftchenary, S.; Luchman, H. A.; Jouk, A. O.; Veloso, A. J.; Page, B. D. G.; Cheng, X. R.; Dawson, S. S.; Grinshtein, N.; Shahani, V. M.; Kerman, K.; Kaplan, D. R.; Griffin, C.; Aman, A. M.; Al-awar, R.; Weiss, S.; Gunning, P. T. Potent Targeting of the STAT3 Protein in Brain Cancer Stem Cells: A Promising Route for Treating Glioblastoma. *ACS Med. Chem. Lett.* **2013**, 4, 1102–1107.
- (26) Fletcher, S.; Singh, J.; Zhang, X.; Yue, P. B.; Page, B. D. G.; Sharmeen, S.; Shahani, V. M.; Zhao, W.; Schimmer, A. D.; Turkson, J.; Gunning, P. T. Disruption of Transcriptionally Active Stat3 Dimers with Non-phosphorylated, Salicylic Acid-Based Small Molecules: Potent in vitro and Tumor Cell Activities. *ChemBioChem* **2009**, 10, 1959–1964.
- (27) Page, B. D. G.; Fletcher, S.; Yue, P. B.; Li, Z. H.; Zhang, X. L.; Sharmeen, S.; Datti, A.; Wrana, J. L.; Trudel, S.; Schimmer, A. D.; Turkson, J.; Gunning, P. T. Identification of a non-phosphorylated, cell permeable, small molecule ligand for the Stat3 SH2 domain. *Bioorg. Med. Chem. Lett.* **2011**, 21, 5605–5609.
- (28) Zhang, X.; He, Y.; Liu, S.; Yu, Z.; Jiang, Z.-X.; Yang, Z.; Dong, Y.; Nabinger, S. C.; Wu, L.; Gunawan, A. M.; Wang, L.; Chan, R. J.; Zhang, Z.-Y. A salicylic acid-based small molecule inhibitor for the oncogenic Src homology-2 domain containing protein tyrosine phosphatase-2 (SHP2). *J. Med. Chem.* **2010**, 53, 2482–2493.
- (29) Shen, Y.; Tenney, A. P.; Busch, S. A.; Horn, K. P.; Cuascut, F. X.; Liu, K.; He, Z.; Silver, J.; Flanagan, J. G. PTPsigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. *Science* **2009**, 326, 592–596.
- (30) Coles, C. H.; Shen, Y. J.; Tenney, A. P.; Siebold, C.; Sutton, G. C.; Lu, W. X.; Gallagher, J. T.; Jones, E. Y.; Flanagan, J. G.; Aricescu, A. R. Proteoglycan-Specific Molecular Switch for RPTP sigma Clustering and Neuronal Extension. *Science* **2011**, 332, 484–488.
- (31) Thompson, K. M.; Uetani, N.; Manitt, C.; Elchebly, M.; Tremblay, M. L.; Kennedy, T. E. Receptor protein tyrosine phosphatase sigma inhibits axonal regeneration and the rate of axon extension. *Mol. Cell. Neurosci.* **2003**, 23, 681–692.
- (32) Horn, K. E.; Xu, B.; Gobert, D.; Hamam, B. N.; Thompson, K. M.; Wu, C. L.; Bouchard, J. F.; Uetani, N.; Racine, R. J.; Tremblay, M. L.; Ruthazer, E. S.; Chapman, C. A.; Kennedy, T. E. Receptor protein tyrosine phosphatase sigma regulates synapse structure, function and plasticity. *J. Neurochem.* **2012**, 122, 147–161.
- (33) Manetsch, R.; Krasinski, A.; Radić, Z.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. In Situ Click Chemistry: Enzyme Inhibitors Made to Their Own Specifications. *J. Am. Chem. Soc.* **2004**, 126, 12809–12818.
- (34) Agalave, S. G.; Maujan, S. R.; Pore, V. S. Click chemistry: 1, 2, 3-triazoles as pharmacophores. *Chem. - Asian J.* **2011**, 6, 2696–2718.
- (35) Huyer, G.; Liu, S.; Kelly, J.; Moffat, J.; Payette, P. Mechanism of inhibition of protein-tyrosine phosphatases by vanadate and pervanadate. *J. Biol. Chem.* **1997**, 272, 843–851.
- (36) Cornish-Bowden, A. Why is uncompetitive inhibition so rare?: A possible explanation, with implications for the design of drugs and pesticides. *FEBS Lett.* **1986**, 203, 3–6.
- (37) Shehzad, S. The potential effect of vanadium compounds on glucose-6-phosphatase. *Biosci. Horiz.* **2013**, 6, hzt002.