ACS Medicinal Chemistry Letters

Featured Letter

pubs.acs.org/acsmedchemlett

Exploring Oxidovanadium(IV) Complexes as YopH Inhibitors: Mechanism of Action and Modeling Studies

Priscila G. A. Martins,[†] Mattia Mori,[‡] Louise D. Chiaradia-Delatorre,[†] Angela C. O. Menegatti,[†] Alessandra Mascarello,[†,§] Bruno Botta,[§] Julio Benítez,[∥] Dinorah Gambino,*[,∥] and Hernán Terenzi*[,†]

[†]Centro de Biologia Molecular Estrutural—CEBIME, Universidade Federal de Santa Catarina, Campus Trindade, 88040-900 Florianópolis, Santa Catarina, Brasil

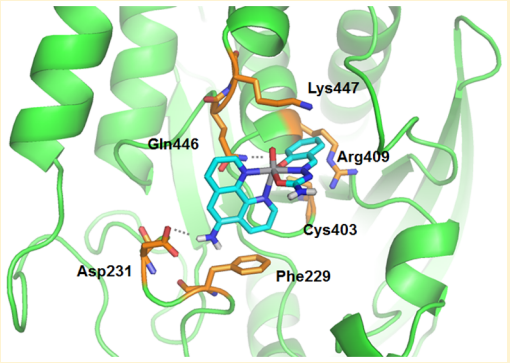
[‡]Center for Life NanoScience@Sapienza, Istituto Italiano di Tecnologia, viale Regina Elena 291, 00161 Roma, Italy

[§]Dipartimento di Chimica e Tecnologia del Farmaco, Sapienza, Università di Roma, Piazzale Aldo Moro 5, 00185 Roma, Italy

[∥]Cátedra de Química Inorgánica, Facultad de Química, Universidad de la República, Gral. Flores 2124, 11800 Montevideo, Uruguay

S *Supporting Information*

ABSTRACT: YopH tyrosine phosphatase, a virulence factor produced by pathogenic species of *Yersinia*, is an attractive drug target. In this work, three oxidovanadium(IV) complexes were assayed against recombinant YopH and showed strong inhibition of the enzyme in the nanomolar range. Molecular modeling indicated that their binding is reinforced by H-bond, cation−π, and $\pi-\pi$ interactions conferring specificity toward YopH. These complexes are thus interesting lead molecules for phosphatase inhibitor drug discovery.



KEYWORDS: *Tyrosine phosphatase, YopH, oxidovanadium, Yersinia, phosphatase inhibition*

Protein phosphorylation at histidine, serine, threonine, and tyrosine residues is controlled by two large families of enzymes: phosphatases and kinases. While the transfer of a γ-phosphate from ATP to target residues is operated by kinases, phosphatases act at the opposite way by restoring the unphosphorylated state of their substrates. This reversible phosphorylation can affect many different properties of target proteins, including interference in their activity, localization, interaction with other proteins, conformation, and function. Despite the fact that phosphorylation at tyrosine residues represents only about 0.01 to 0.05% of total protein phosphorylation,[1] it plays a critical role in controlling many physiological processes such as cell−cell communications, differentiation, cell cycle, cell growth, metabolism, and immune response. Due to the importance of tyrosine phosphorylation to cell homeostasis, any factor that could alter the physiological tyrosine dephosphorylation rate, either by excessive or decreased activity of protein tyrosine phosphatases (PTPs), could cause a variety of diseases such as cancer,[2] obesity, diabetes,[3] and neurodegenerative and autoimmune disorders.[4]

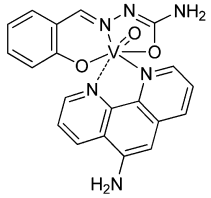
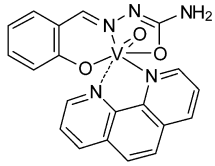
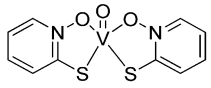
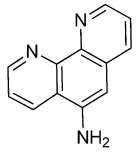
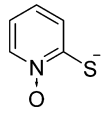
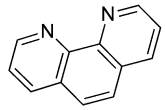
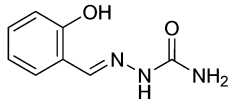
The metal oxoanion vanadate is reported as inhibitor of PTPs since 1980s. Although vanadate behaves as a phosphate mimetic, it displays higher inhibitory profile than the phosphate ion[5] and assumes a trigonal−bipyramidal structure within the active site of the enzyme.[6,7] It has been shown that vanadium compounds also present insulin-mimetic effect, prolonging this hormone action by increasing insulin receptor activation and downstream signaling. Bis-maltolato-oxidovanadium(IV) (BMOV), for example, is an organic oxidovanadium compound that decreases the level of plasma glucose in diabetic animals.[8] Bis(ethylmaltolato)oxidovanadium(IV) (BEOV), an analogue of BMOV, has been profiled for safety and efficacy by Akesis Pharmaceuticals, Inc. (La Jolla, CA), in human phase II clinical trial and presented a good tolerance, reduction of fast blood glucose comparing to placebo subjects, and an improvement in the response to an oral glucose load.[9] The insulin-like properties of vanadium compounds could partly be explained by its activity against PTPs. Among them, PTP1B aberrant activity has long been associated with diabetes, and its catalytic activity has been efficiently inhibited by organic vanadium complexes, including BMOV and BEOV.[10−12]

PTPs produced by pathogenic bacteria are also able to interfere with host signaling pathways. *Yersinia* outer protein H (YopH), for example, is a 51 kDa PTP that is secreted by the three pathogenic species of *Yersinia* genus: *Yersinia pestis*, *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis*. These species cause a variety of pathologies from gastrointestinal

Received: July 3, 2015
Accepted: August 31, 2015
Published: August 31, 2015

DOI: 10.1021/acsmedchemlett.5b00267
ACS Med. Chem. Lett. 2015, 6, 1035−1040

Table 1. Inhibition and IC_{50} against YopH from *Yersinia enterocolitica* and Estimation of the Ligand Binding Affinity to YopH by the Chemscore Function of the GOLD Docking Program for the Selected Compounds and Complexes[a]

Compound	Structure	% of YopH inhibition (at 25 μM)	IC_{50} (μM)	Fitness[b] (Chemscore)
1		99.61± 0.08	0.06 ± 0.01	26.58
2		99.51 ± 0.01	0.10 ± 0.01	25.55
3		99.48 ± 0.12	0.29 ± 0.01	21.17
L1		n.d.	>100	15.18
L2		n.d.	23.15 ± 1.81	20.70
L3		n.d.	>100	19.89
L4		n.d.	>100	14.89
	L1 + L4	n.d.	>100	n.d.
	L3 + L4	n.d.	>100	n.d.
	$VOSO_4$	99.73 ± 0.37	0.44 ± 0.05	n.d.
	Na_3VO_4	n.d.	0.81 ± 0.05	n.d.

[a]Data are expressed as means ± SD of three independent experiments. [b]α-dimensional value; higher the fitness, higher the affinity; n.d. ,not determined.

syndromes to sepsis. YopH is the PTP that presents one of the highest catalytic efficiencies among all known PTPs (k_{cat}/K_m 514 $mM^{-1}\cdot s^{-1}$).[13] YopH is injected into the host cell by a type III secretion apparatus and helps these bacteria to evade the host's immune defense. YopH can dephosphorylate the focal adhesion proteins p^{130}Cas and FAK of the host, leading to a subsequent disruption of the focal complex structures.[14] YopH protects the bacteria from phagocytic uptake by cultured macrophages and polymorphonuclear neutrophils (PMNs)[15] and interferes at macrophage response to infection by inactivation the PI3K/Akt pathway.[16] YopH can also impair the activation of T and B lymphocytes, thus allowing *Yersinia* to avoid the adaptive immune response.[17] Due to the importance of YopH for bacteria pathogenesis and based on the evidence that a catalytic mutation of YopH enzyme abolishes the virulence of *Y. pseudotuberculosis* in murine infection model,[18] this PTP is considered an attractive drug target.

In the last years, a variety of YopH inhibitors belonging to different chemical classes have been reported such as aurintricarboxylic acid,[19] salicylic acid derivatives,[20] peptides,[21] phosphonate derivatives of calixarenes,[22] oximes,[23] isothiazolidinones,[24] chalcones, and sulphonamides.[25] To further contribute to the field of YopH inhibition, the present work shows the potent inhibitory effect of three oxidovanadium(IV) complexes (compounds **1**, **2**,[26,27] and **3**[28] in Table 1).

The inhibitory activity of these molecules was evaluated *in vitro* using a recombinant purified YopH and *p*-nitrophenylphosphate (*p*NPP) as substrate, as described in

DOI: 10.1021/acsmedchemlett.5b00267
ACS Med. Chem. Lett. 2015, 6, 1035−1040

Table 2. IC_{50} and Selectivity Index Values (SI) of Oxidovanadium(IV) Complexes 1, 2, and 3 Measured on Some Representative PTPs[a]

	1		2		3	
PTP	IC_{50} (μM)	SI	IC_{50} (μM)	SI	IC_{50} (μM)	SI
YopH	0.06 ± 0.01	ref value	0.10 ± 0.01	ref value	0.29 ± 0.01	ref value
PtpA	0.56 ± 0.03	9.3	2.69 ± 0.27	26.9	9.12 ± 0.26	31.5
PtpB	1.73 ± 0.61	28.8	5.12 ± 0.78	51.2	52.55 ± 1.56	181.2
PTP1B	0.33 ± 0.05	5.5	0.27 ± 0.02	2.7	0.78 ± 0.02	2.7
LYP	5.42 ± 1.32	90.3	6.82 ± 1.93	68.2	28.30 ± 1.71	97.6
PTP−PEST	3.97 ± 0.23	66.2	4.28 ± 0.66	42.8	41.30 ± 3.19	142.4

[a]The results are shown as the average of the individual mean ± SD for three independent experiments. The selective index (SI) is given by (IC_{50}PTP/IC_{50}YopH).

Supporting Information. Initial assays were performed using a single concentration (25 μM) of each molecule and showed more than 99% of inhibitory effect against YopH (Table 1). Then, we further determined IC_{50} values, and the three oxidovanadium(IV) complexes showed extraordinary inhibition in the nanomolar range concentration. IC_{50} values for the ligands used to build the complexes (L1−L4) were also determined (Table 1), and notably, they were at least 80-fold higher than those of their corresponding oxidovanadium(IV) complexes (**1**−**3**), demonstrating that the ligands *per se* do not exhibit a remarkable inhibitory activity against YopH, as could be expected.

To explore the specificity of oxidovanadium(IV) complexes for YopH, we measured their inhibitory activity toward several members of the PTP superfamily, including *Mycobacterium tuberculosis* PTPs (PtpA and PtpB) and human PTPs (PTP1B, LYP, and PTP−PEST). The results presented in Table 2 demonstrate that the complexes are quite selective toward YopH. The most potent and selective inhibitor **1** showed an approximate 9-fold and 29-fold selectivity for YopH with respect to *Mycobacterium tuberculosis* PTPs (PtpA and PtpB, respectively). The sole difference between compounds **1** and **2** is an amino group substituted on the phenyl ring. Apparently, the presence of this group results in an increase in selectivity for YopH relative to PTP1B and LYP, whereas it decreases this selectivity in comparison to *Mycobacterium tuberculosis* PTPs.

We also examined the potential reversibility of binding of the inhibitors by modification of our *in vitro* functional assay (see SI). After removal of unbound inhibitor, YopH activity was always restored since binding of the inhibitor was not irreversible (Figure S1). To further characterize the mechanism of YopH inhibition and evaluate the kinetic parameters, we tested three different concentrations of the vanadium complex and seven concentrations of *p*NPP (ranging from 0.2 to 12.8 mM). Kinetic analysis revealed that the three molecules are competitive inhibitors. The Lineweaver−Burk double reciprocal plots (Figure 1) showed all lines converging at the *y*-axis ($1/V_{max}$), whereas the slope (K_{mapp}/V_{max}) and *x*-axis interception ($1/K_{mapp}$) vary according to the inhibitor concentration. The constant V_{max} value and the increase in K_{mapp} values with increasing inhibitor concentration indicate a mutually exclusive binding mode, thus suggesting that these compete with *p*NPP for the active site of YopH. The K_i values obtained were 0.02 μM for complex **1** and **2**, and 0.19 for complex **3**.

The possible binding mode of **1**−**3** and L1−L4 to YopH was investigated by molecular modeling. Small molecules were prepared as described in the Supporting Information and were then docked toward the catalytic active site of YopH phosphatase by using AutoDock, FRED, and GOLD docking

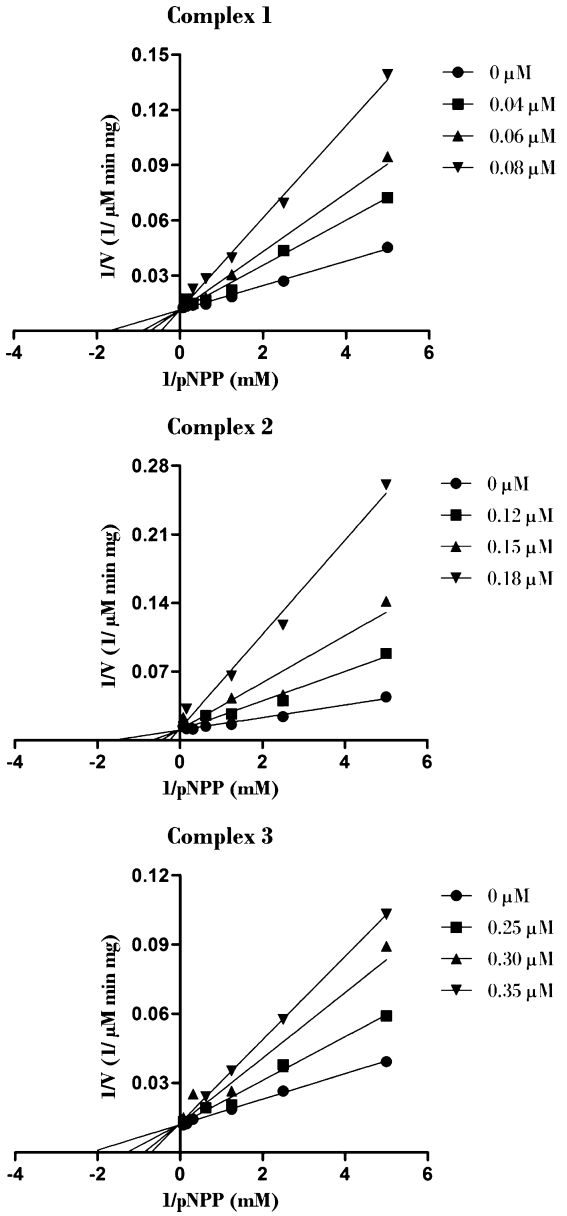


Figure 1. Lineweaver−Burk double-reciprocal plots representing inhibitory profiles of oxidovanadium(IV) complexes **1**, **2**, and **3** against YopH. Kinetic experiments were conducted in the presence of increasing concentrations of inhibitors, and *p*NPP was used as the substrate in all experiments.

DOI: 10.1021/acsmedchemlett.5b00267
ACS Med. Chem. Lett. 2015, 6, 1035−1040

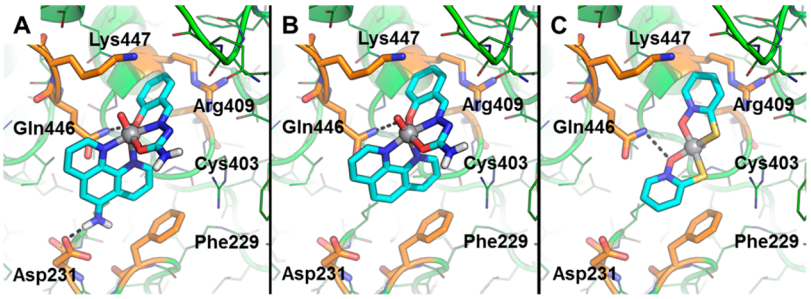


Figure 2. Predicted binding conformation of oxidovanadium complexes **1**−**3** to YopH. (A) Docking-based binding mode of **1**, the most active inhibitor of the series; (B) docking-based binding mode of **2**; (C) docking-based binding mode of **3**. Ligands are shown as cyan sticks, vanadium as gray sphere. The crystallographic structure of YopH (PDB 3F9B) is shown as green cartoon and lines. Residues involved in binding to **1**−**3** are shown as orange sticks and are labeled. Ligand nonpolar H atoms were omitted. H-bond interactions are shown as black dashed lines.

programs.[29−33] The high resolution (1.42 Å) crystal structure of YopH in complex with a divanadate ion within the catalytic active site, which corresponds to PDB ID 3F9B, was selected as rigid receptor in this study[34,35]

All docking programs provided a highly similar binding mode for each compound, summarized in Figure 2 for active inhibitors **1**−**3** and in Supporting Information Figure S2 for noncomplexed fragments L1−L4.

All docking poses of **1** and **3** are very similar, with an RMSD lower than 1.5 Å, whereas two different poses were found for **2**, one of which is highly similar to the binding mode predicted for **1** and was therefore accounted for in results analysis (for the other binding conformation of **2**, see Supporting Information). Molecules **1**−**3** bind within the catalytic pocket of YopH in proximity to Cys403. Their binding to YopH is reinforced by a cation−π interaction with Arg409 and Lys447, whereas a H-bond interaction is established with the side chain of Gln446. It is worth mentioning that the interaction with Gln446 is also present in the crystallographic structure of YopH bound to a known inhibitor.[36] Compounds **1**−**3** further establish a π−π interaction with the side chain or Phe229 (T-shape geometry for **1** and **2**, parallel sandwich-like geometry for **3**), whereas the amino group of **1** is involved in a H-bond interaction with the side chain of Asp231. Notably, this interaction may account for the higher affinity of **1** compared to **2**. One may note that Asp231 as well as other residues of the catalytic cleft are generally highly conserved among PTPs. However, as also recently reviewed by Böhmer et al., PTPs share different and peculiar folding.[37] Indeed, YopH shares a good structural similarity with PTP1B (RMSD = 3.5 Å by comparing PDB 3F9B and 2NT7), whereas a higher difference does exist with PtpA and PtpB (RMSD higher than 10 Å by comparing PDB 3F9B with 1U2P and 2OZ5, respectively). Moreover, PtpB is characterized by a peculiar folding and is therefore quite different from all other PTPs. These structural differences may account for the different inhibitory activity of **1**−**3** against PTPs tested in this work (Table 2). Indeed, when compared to YopH inhibition, **1**−**3** are quite active on PTP1B, which is similar to YopH, but are less active against PtpA and PtpB, which have a different folding structure than YopH.

Molecular docking of noncomplexed fragments L1−L4 revealed that they interact within the same pocket of the catalytic site of YopH (for details see Supporting Information Figure S3). Accordingly, L1−L4 may compete for the same binding site on YopH surface, thus potentially explaining the lack of synergistic effects when tested in combination, as observed experimentally (Table 1). Prediction of ligand binding affinity revealed that the Chemscore function (GOLD docking program) provided better agreement with experimental inhibitory data than other scoring functions, similar to what was outlined in recent computational studies on organometallic compounds (Table 1).[38] Nevertheless, Chemscore slightly overestimated the predicted affinity of L2 and, particularly, of L3 compared to newly discovered and reference YopH inhibitors (see also Supporting Information).

In summary, the complexation with vanadium is thought to place functional group of **1**−**3** in correspondence of YopH pharmacophoric hotspots, thus resulting in a higher inhibitory efficacy than noncomplexed fragments L1−L4 alone. Besides complexation with vanadium, the H-bond interaction established by **1** with the side chain of Asp231 appears to be a key discriminant for potent inhibition of YopH phosphatase catalytic activity.

Despite highly chemically diverse inhibitors of YopH have been disclosed so far, only a limited number of them present inhibitory activity in the nanomolar range. Oxidovanadium(IV) complexes have demonstrated excellent inhibition against PTP1B[10−12] but have been poorly explored as YopH inhibitors.[39] Due to the fact that the organic bioactive ligands could be favorably modified, vanadium complexes of organic ligands offer an attractive prospect to develop new potent and selective inhibitors of YopH. In this work we presented three oxidovanadium(IV) complexes endowed with remarkable inhibition against YopH. These compounds have IC_{50} and K_i values in the nanomolar range, and the most potent is at least 5-fold more selective for YopH compared to other human PTPs.

To identify nanomolar inhibitors of YopH that experience also a certain degree of selectivity is in perspective with developing new drug candidates against *Yersinia*, and most notably, these molecules would be also useful as a tool in chemical biology investigations.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00267.

Methods details, reversibility analysis, and alternative binding mode of compound **2** (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail dgambino@fq.edu.uy.

DOI: 10.1021/acsmedchemlett.5b00267
ACS Med. Chem. Lett. 2015, 6, 1035−1040

*Tel: +55 48 3721 6426. Fax: +55 48 3721 9672. E-mail: hernan.terenzi@ufsc.br.

Author Contributions

All authors have given approval to the final version of the manuscript.

Funding

We thank CNPq, MCT, FAPESC, FINEP, INBEB, and CAPES for financial support and fellowships, and the OpenEye Free Academic Licensing Program for providing a free academic license for molecular modeling and chemoinformatics software.

Notes

The authors declare no competing financial interest.

Biographies

Hernán Terenzi received his Ph.D. in Biochemistry from Universidade de São Paulo, São Paulo, Brazil (1993). After postdoctoral training at the Institut Pasteur, Paris, France, he returned to Brazil to the Universidade Federal de Santa Catarina as an adjunct professor (1998) and is currently Full Professor of Biochemistry. The Terenzi laboratory studies structure/function relationships in phosphatases and also bioinorganic models of metalloenzymes. Current projects address the development of tyrosine phosphatase inhibitors and mechanisms of activity modulation, as well as the search for novel metal artificial hydrolases. He is a research fellow from the National Council for Scientific and Technological Development (CNPq).

Dinorah Gambino obtained her Pharmaceutical Chemist degree and Ph.D. in Inorganic Chemistry at the Faculty of Chemistry, Universidad de la República, Uruguay. She was appointed an initial position at Faculty of Chemistry in 1979 and is currently Full Professor of Inorganic Chemistry of this institution since 2010. Her research interests are centered on the chemistry and applications of metals in medicine and the design and study of the mechanism of action of novel metal-based potential therapeutic agents, mainly for the treatment of diseases of high incidence in America: neglected parasitic diseases (Chagas disease or American Trypanosomiasis and Leishmaniasis), tuberculosis, and cancer.

■ ACKNOWLEDGMENTS

We would like to thank Prof. Dr. Pedro M. Alzari (Institut Pasteur, Paris) for the plasmids coding PtpA and PtpB, Dr. Tiago A. S. Brandão (Universidade Federal de Minas Gerais) for the plasmid coding PTP1B and YopH, and Nunzio Bottini (la Jolla Institute for Allergy and Immunology) for the plasmids coding LYP and PTP−PEST. This work is part of the thesis of Priscila Graziela Alves Martins who was a doctoral student in Biochemistry.

■ REFERENCES

(1) Hunter, T. Signaling - 2000 and beyond. *Cell* **2000**, *100*, 113−127.

(2) Lessard, L.; Stuible, M.; Tremblay, M. L. The two faces of PTP1B in cancer. *Biochim. Biophys. Acta, Proteins Proteomics* **2010**, *1804*, 613−619.

(3) Klaman, L. D.; Boss, O.; Peroni, O. D.; Kim, J. K.; Martino, J. L.; Zabolotny, J. M.; Moghal, N.; Lubkin, M.; Kim, Y. B.; Sharpe, A. H.; Stricker-Krongrad, A.; Shulman, G. I.; Neel, B. G.; Kahn, B. B. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol. Cell. Biol.* **2000**, *20*, 5479−5489.

(4) Åkerman, L.; Ludvigsson, J.; Casas, R. Low C-peptide levels and decreased expression of TNF and CD45 in children with high risk of type 1 diabetes. *Clin. Immunol.* **2013**, *148*, 4−15.

(5) Zhang, M.; Zhou, M.; Van Etten, R. L.; Stauffacher, C. V. Crystal structure of bovine low molecular weight phosphotyrosyl phosphatase complexed with the transition state analog vanadate. *Biochemistry* **1997**, *36*, 15−23.

(6) Crans, D. C.; Tarlton, M. L.; McLauchlan, C. C. Trigonal Bipyramidal or Square Pyramidal Coordination Geometry? Investigating the Most Potent Geometry for Vanadium Phosphatase Inhibitors. *Eur. J. Inorg. Chem.* **2014**, *2014*, 4450.

(7) McLauchlan, C. C.; Peters, B. J.; Crans, D. C. Vanadium-phosphatase complexes: Phosphatase inhibitors favor the trigonal bipyramidal trasition state geometry. *Coord. Chem. Rev.* **2015**, *301-302*, 163.

(8) Poucheret, P.; Verma, S.; Grynpas, M. D.; McNeill, J. H. Vanadium and diabetes. *Mol. Cell. Biochem.* **1998**, *188*, 73−80.

(9) Thompson, K. H.; Lichter, J.; LeBel, C.; Scaife, M. C.; McNeill, J. H.; Orvig, C. Vanadium treatment of type 2 diabetes: A view to the future. *J. Inorg. Biochem.* **2009**, *103*, 554−558.

(10) Li, M.; Ding, W.; Baruah, B.; Crans, D. C.; Wang, R. Inhibition of protein tyrosine phosphatase 1B and alkaline phosphatase by bis(maltolato)oxovanadium (IV). *J. Inorg. Biochem.* **2008**, *102*, 1846−1853.

(11) Peters, K. G.; Davis, M. G.; Howard, B. W.; Pokross, M.; Rastogi, V.; Diven, C.; Greis, K. D.; Eby-Wilkens, E.; Maier, M.; Evdokimov, A.; Soper, S.; Genbauffe, F. Mechanism of insulin sensitization by BMOV (bis maltolato oxo vanadium); Unliganded vanadium (VO4) as the active component. *J. Inorg. Biochem.* **2003**, *96*, 321−330.

(12) Yuan, C.; Lu, L.; Wu, Y.; Liu, Z.; Guo, M.; Xing, S.; Fu, X.; Zhu, M. Synthesis, characterization, and protein tyrosine phosphatases inhibition activities of oxovanadium(IV) complexes with Schiff base and polypyridyl derivatives. *J. Inorg. Biochem.* **2010**, *104*, 978−986.

(13) Heneberg, P. Finding the smoking gun: Protein tyrosine phosphatases as tools and targets of unicellular microorganisms and viruses. *Curr. Med. Chem.* **2012**, *19*, 1530−1566.

(14) Black, D. S.; Bliska, J. B. Identification of p130(Cas) as a substrate of Yersinia YopH (Yop51), a bacterial protein tyrosine phosphatase that translocates into mammalian cells and targets focal adhesions. *EMBO J.* **1997**, *16*, 2730−2744.

(15) Grosdent, N.; Maridonneau-Parini, I.; Sory, M. P.; Cornelis, G. R. Role of Yops and adhesins in resistance of Yersinia enterocolitica to phagocytosis. *Infect. Immun.* **2002**, *70*, 4165−4176.

(16) Sauvonnet, N.; Lambermont, I.; Van Der Bruggen, P.; Cornelis, G. R. YopH prevents monocyte chemoattractant protein 1 expression in macrophages and T-cell proliferation through inactivation of the phosphatidylinositol 3-kinase pathway. *Mol. Microbiol.* **2002**, *45*, 805−815.

(17) Yao, T.; Mecsas, J.; Healy, J. I.; Falkow, S.; Chien, Y. H. Suppression of T and B lymphocyte activation by a Yersinia pseudotuberculosis virulence factor, YopH. *J. Exp. Med.* **1999**, *190*, 1343−1350.

(18) Bliska, J. B.; Clemens, J. C.; Dixon, J. E.; Falkow, S. The Yersinia tyrosine phosphatase: Specificity of a bacterial virulence determinant for phosphoproteins in the J774A.1 macrophage. *J. Exp. Med.* **1992**, *176*, 1625−1630.

(19) Liang, F.; Huang, Z.; Lee, S. Y.; Liang, J.; Ivanov, M. I.; Alonso, A.; Bliska, J. B.; Lawrence, D. S.; Mustelin, T.; Zhang, Z. Y. Aurintricarboxylic Acid Blocks in Vitro and in Vivo Activity of YopH, an Essential Virulent Factor of Yersinia pestis, the Agent of Plague. *J. Biol. Chem.* **2003**, *278*, 41734−41741.

(20) Huang, Z.; He, Y.; Zhang, X.; Gunawan, A.; Wu, L.; Zhang, Z. Y.; Wong, C. F. Derivatives of salicylic acid as inhibitors of YopH in yersinia pestis. *Chem. Biol. Drug Des.* **2010**, *76*, 85−99.

(21) Leone, M.; Barile, E.; Dahl, R.; Pellecchia, M. Design and NMR Studies of Cyclic Peptides Targeting the N-Terminal Domain of the Protein Tyrosine Phosphatase YopH. *Chem. Biol. Drug Des.* **2011**, *77*, 12−19.

(22) Vovk, A. I.; Kononets, L. A.; Tanchuk, V. Y.; Cherenok, S. O.; Drapailo, A. B.; Kalchenko, V. I.; Kukhar, V. P. Inhibition of Yersinia

DOI: 10.1021/acsmedchemlett.5b00267
ACS Med. Chem. Lett. 2015, 6, 1035−1040

protein tyrosine phosphatase by phosphonate derivatives of calixarenes. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 483−487.
(23) Bahta, M.; Burke, T. R. Oxime-Based Click Chemistry in the Development of 3-Isoxazolecarboxylic Acid Containing Inhibitors of Yersinia pestis Protein Tyrosine Phosphatase, YopH. *ChemMedChem* **2011**, *6*, 1363−1370.
(24) Kim, S. E.; Bahta, M.; Lountos, G. T.; Ulrich, R. G.; Burke, T. R.; Waugh, D. S. Isothiazolidinone (IZD) as a phosphoryl mimetic in inhibitors of the Yersinia pestis protein tyrosine phosphatase YopH. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2011**, *67*, 639−645.
(25) Martins, P. G. A.; Menegatti, A. C. O.; Chiaradia-Delatorre, L. D.; De Oliveira, K. N.; Guido, R. V. C.; Andricopulo, A. D.; Vernal, J.; Yunes, R. A.; Nunes, R. J.; Terenzi, H. Synthetic chalcones and sulfonamides as new classes of Yersinia enterocolitica YopH tyrosine phosphatase inhibitors. *Eur. J. Med. Chem.* **2013**, *64*, 35−41.
(26) Fernández, M.; Varela, J.; Correia, I.; Birriel, E.; Castiglioni, J.; Moreno, V.; Costa Pessoa, J.; Cerecetto, H.; González, M.; Gambino, D. A new series of heteroleptic oxidovanadium(iv) compounds with phenanthroline-derived co-ligands: Selective Trypanosoma cruzi growth inhibitors. *Dalton Trans.* **2013**, *42*, 11900−11911.
(27) Benítez, J.; Becco, L.; Correia, I.; Leal, S. M.; Guiset, H.; Pessoa, J. C.; Lorenzo, J.; Tanco, S.; Escobar, P.; Moreno, V.; Garat, B.; Gambino, D. Vanadium polypyridyl compounds as potential antiparasitic and antitumoral agents: New achievements. *J. Inorg. Biochem.* **2011**, *105*, 303−312.
(28) Hodge, A.; Nordquest, K.; Blinn, E. L. Oxovanadium(IV) complexes containing bidentate nitrogen-sulfur and oxygen-sulfur ligands. *Inorg. Chim. Acta* **1972**, *6*, 491−498.
(29) Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. *J. Am. Chem. Soc.* **1996**, *118*, 11225−11236.
(30) OpenEye OEDocking 2013; OpenEye Scientific Software: Santa Fe, NM. http://www.eyesopen.com.
(31) McGann, M. FRED pose prediction and virtual screening accuracy. *J. Chem. Inf. Model.* **2011**, *51*, 578−596.
(32) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J. Comput. Chem.* **2009**, *30*, 2785−2791.
(33) Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. Improved protein-ligand docking using GOLD. *Proteins: Struct., Funct., Genet.* **2003**, *52*, 609−623.
(34) Brandao, T. A.; Robinson, H.; Johnson, S. J.; Hengge, A. C. Impaired acid catalysis by mutation of a protein loop hinge residue in a YopH mutant revealed by crystal structures. *J. Am. Chem. Soc.* **2009**, *131*, 778−786.
(35) Rueda, M.; Bottegoni, G.; Abagyan, R. Recipes for the selection of experimental protein conformations for virtual screening. *J. Chem. Inf. Model.* **2010**, *50*, 186−193.
(36) Sun, J. P.; Wu, L.; Fedorov, A. A.; Almo, S. C.; Zhang, Z. Y. Crystal structure of the Yersinia protein-tyrosine phosphatase YopH complexed with a specific small molecule inhibitor. *J. Biol. Chem.* **2003**, *278*, 33392−33399.
(37) Böhmer, F.; Szedlacsek, S.; Tabernero, L.; Östman, A.; Den Hertog, J. Protein tyrosine phosphatase structure-function relationships in regulation and pathogenesis. *FEBS J.* **2013**, *280*, 413−431.
(38) Ortega-Carrasco, E.; Lledos, A.; Marechal, J. D. Assessing protein-ligand docking for the binding of organometallic compounds to proteins. *J. Comput. Chem.* **2014**, *35*, 192−198.
(39) Scrivens, P. J.; Alaoui-Jamali, M. A.; Giannini, G.; Wang, T.; Loignon, M.; Batist, G.; Sandor, V. A. Cdc25A-inhibitory properties and antineoplastic activity of bisperoxovanadium analogues. *Mol. Cancer Ther.* **2003**, *2*, 1053−1059.

DOI: 10.1021/acsmedchemlett.5b00267
ACS Med. Chem. Lett. 2015, 6, 1035−1040