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Inhibition of PHPT1 by phenylarsonic acids

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The human protein histidine phosphatase PHPT1 is involved in several important cellular pathways and has been implicated in various cancers. However, the biological roles of this enzyme are not well understood due to a lack of chemical tools that enable its study. Herein we have identified phenylarsonic acids as general scaffolds which inhibit PHPT1 activity. Notably, phenylarsonic acids can be embedded into peptide sequences, providing the first known peptide-based inhibitors of PHPT1. In a counterscreen against a small panel of phosphatases, we demonstrate that these compounds exhibit some selectivity for PHPT1. Moreover, we show that these compounds exhibit mixed inhibition. We provide evidence that reduction of the phenylarsonic acids *in situ* by reducing agents like dithiothreitol (DTT) to provide phenylarsine species gives rise to the observed PHPT1 inhibition. These As(III) species are known to be thiophilic and can interact with solvent-exposed cysteine residues of proteins. Finally, we demonstrate that mutating the three cysteine residues of PHPT1 to alanine results in a significant decrease in enzyme inhibition by the phenylarsonic acids, suggesting that these compounds likely interact at least in part with Cys residues in PHPT1.

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Introduction

Protein phosphorylation is tightly regulated by the action of kinases and phosphatases. Alongside the well-studied phosphoserine, phosphothreonine, and phosphotyrosine, phosphohistidine (pHis) has gained attention in recent years due to its implications in G-protein signaling, ion conduction, metabolism, and chromatin biology.^{1–3} Protein histidine phosphatase 1 (PHPT1) is a key His phosphatase helping to control the phosphorylation of His residues in eukaryotes and, recently, has garnered considerable interest because of its implications in cellular signal transduction and metabolic regulation.^{4–8} Moreover, overexpression of PHPT1 has been associated with lung, liver, and renal cancers, highlighting its potential therapeutic relevance.^{9–12} Despite its significance, PHPT1 remains relatively understudied, with its physiological functions and regulatory mechanisms only recently gaining attention.^{13,14} Contributing to this lack of understanding is an absence of chemical tools that enable the study of its biological roles. The development and application of PHPT1 targeted inhibitors would provide more insight into the biological activity of this understudied enzyme.^{15–17} Due to its shallow, positively

charged catalytic pocket, PHPT1 is challenging to selectively target (Fig. 1). Nevertheless, NMR and mutation studies of the active site suggest the possibility of developing compounds that selectively inhibit PHPT1.^{15,16} To date, only a handful of small molecule inhibitors of PHPT1 have been reported in the literature.^{17–19}

In 2022, our lab reported the first generation of PHPT1 inhibitors.¹⁷ Two of the reported compounds, acetarsol and roxarsone, were characterized as noncovalent, dose-dependent inhibitors with IC₅₀ values of 100 ± 30 μM and 80 ± 10 μM, respectively (Fig. 2).¹⁷ These phenylarsonic acids were selective inhibitors of PHPT1 activity when compared against alkaline

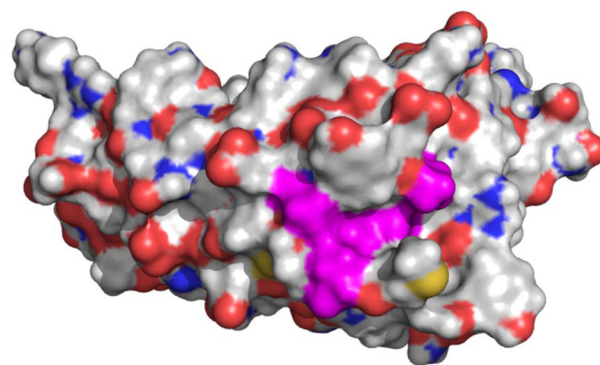


Fig. 1 NMR solution structure of PHPT1 (PDB ID: 2AI6). The catalytic pocket, including residues K21, H53, S94, and A96, is shown in magenta.

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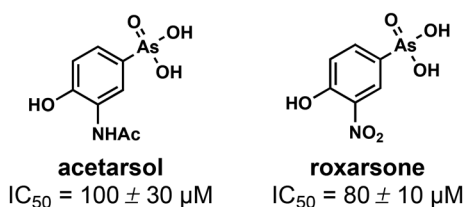


Fig. 2 Acetarsol and roxarsone as inhibitors of PHPT1.

phosphatase (AlkP), the pLys and pHis pyrophosphatase LHPP,^{20,21} and the serine/threonine phosphatase PP2C, which also exhibits histidine phosphatase activity *in vitro*.²² We proposed that the arsonic acid might function as a phosphomimetic, allowing roxarsone and acetarsol to interact with the PHPT1 active site through noncovalent interactions. To further explore this concept, here we report a series of substituted phenylarsonic acids and demonstrate their propensity to inhibit PHPT1.

Table 1 IC_{50} values of phenylarsonic acids against PHPT1

| Inhibitor | IC_{50} (μM) |
|-----------|-----------------------|
| Acetarsol | 100 ± 30 |
| Roxarsone | 80 ± 10 |
| 1 | 90 ± 10 |
| 2 | 90 ± 10 |
| 3 | 90 ± 10 |
| 4 | 130 ± 10 |
| 5 | 220 ± 40 |
| 6 | 200 ± 40 |
| 7 | 100 ± 20 |
| 8 | 130 ± 20 |

Results and discussion

We began our investigation by synthesizing phenylarsonic acids from their corresponding amines using the Bart reaction (Fig. 3).²³ In preliminary experiments, we screened compounds 1–8 against PHPT1 using an established activity assay with the

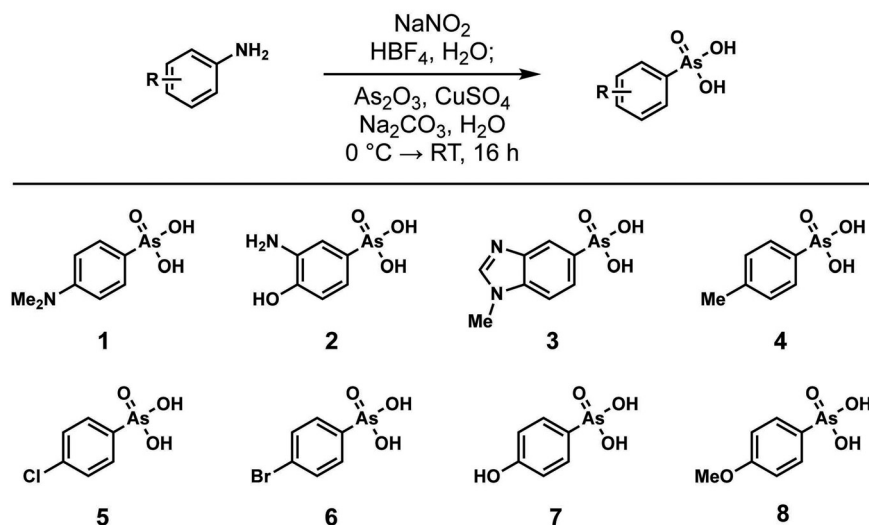


Fig. 3 Synthesis and structures of phenylarsonic acids.

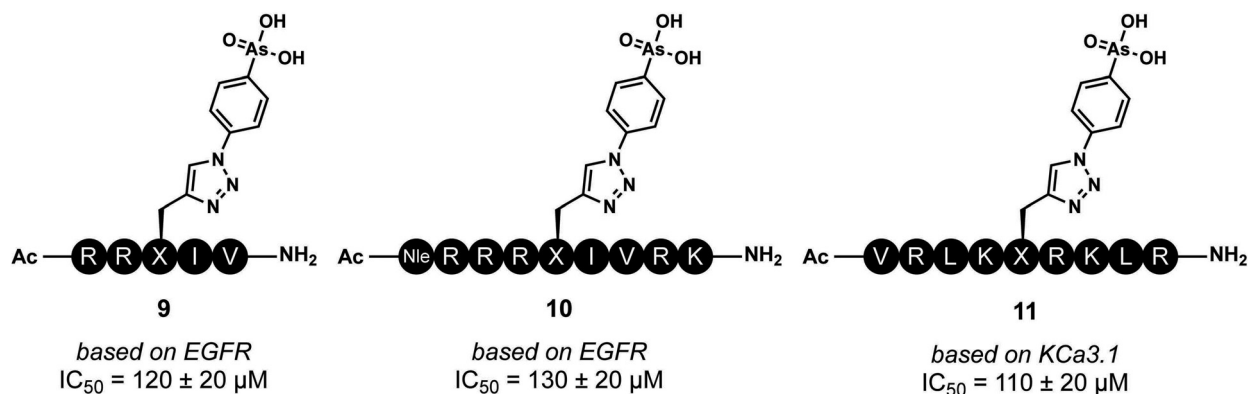


Fig. 4 Peptide-based phenylarsonic acids 9, 10, and 11 derived from known substrates of PHPT1.



fluorogenic probe 6,8-difluoromethylumbelliferyl phosphate (DiFMUP; $K_m = 220 \pm 30 \mu\text{M}$).²⁴ Using 50 μM roxarsone as a positive control, arylarsonates 1–8 were found to inhibit PHPT1 in a dose dependent manner at concentrations ranging from 50–150 μM (Fig. S1). Following this initial screening, we determined the IC_{50} values of compounds 1–8, which were comparable to roxarsone and acetarsol (Table 1). Further analysis of the IC_{50} data for 1–8 revealed that electron rich phenylarsonic acids have improved potency compared to electron deficient species. For example, amino-substituted 1–3 and phenol 7 are approximately twice as potent as halides 5 and 6. However, we found that phenylarsonic acids are a general scaffold from which inhibitors of PHPT1 can be developed.

Compared to small molecules, peptide-based inhibitors often exhibit effective protein-target binding at low concentrations due to enhanced selectivity.^{25–27} Thus, we were curious if incorporating the phenylarsonate moiety into a peptide could provide peptide-based inhibitors of PHPT1. We began by synthesizing peptides based on EGRF and KCa3.1, which are known substrates of PHPT1.^{28,29} Here, the native histidine residue was exchanged for propargyl glycine and a phenylarsonic acid was embedded into the sequence *via* a Cu(I)-catalyzed azide alkyne 'click' (CuAAC) reaction to give peptide arsonates 9–11 shown in Fig. 4.^{30,31} These peptides inhibited PHPT1 with IC_{50} values of $120 \pm 20 \mu\text{M}$, $130 \pm 20 \mu\text{M}$, and $110 \pm 20 \mu\text{M}$, respectively, further demonstrating the generality of phenylarsonic acids as inhibitors of PHPT1. However, the addition of a peptide recognition element did not provide additional affinity.

To explore the selectivity of the phenylarsonic acids for PHPT1, we carried out a counterscreen of compounds 1 and 9 against a small panel of phosphatases including the histidine phosphatase LHPP, a promiscuous phosphatase AlkP, and the protein tyrosine phosphatases PTP1B, SHP2, and LYP (Fig. 5). We observed that 1 and 9 demonstrate some selectivity for PHPT1 over the other phosphatases tested here, suggesting that these phenylarsonic acids may be useful in further studying the biological implications of histidine phosphorylation.

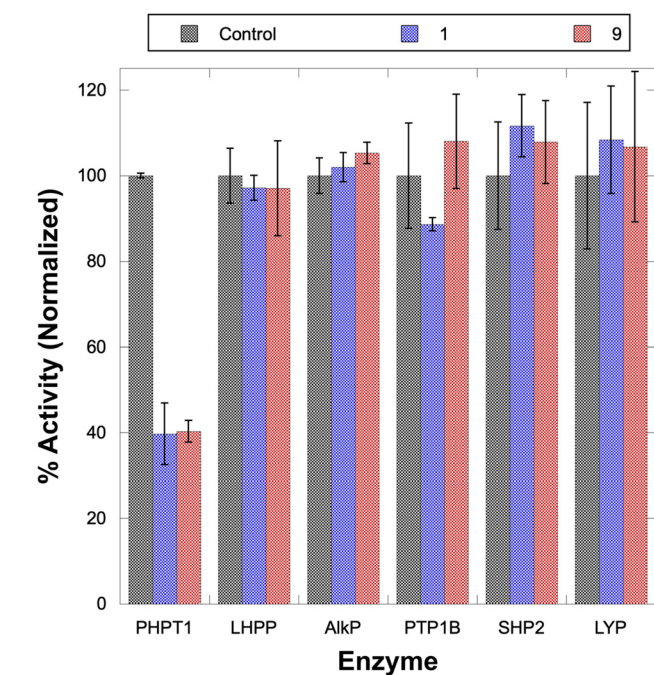
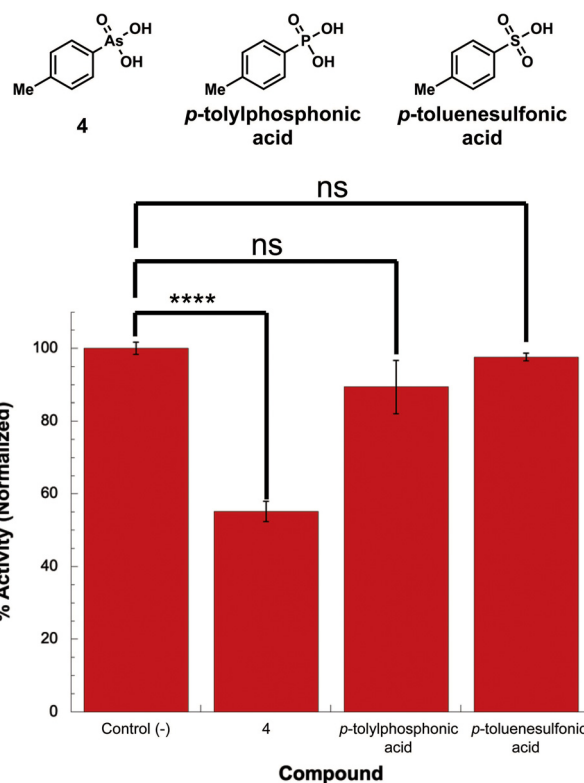


Fig. 6 Comparison of PHPT1 activity upon treatment with 100 μM of compound 4, *p*-tolylyphosphonic acid, or *p*-toluenesulfonic acid.

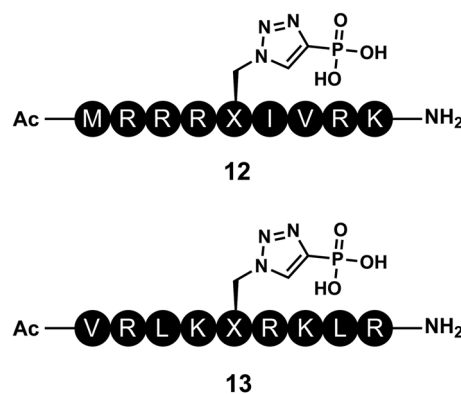


Fig. 5 Counterscreen of 100 μM 1 and 9 against phosphatases LHPP, AlkP, PTP1B, SHP2, and LYP.

Fig. 7 Peptides derived from EGRF (12) and KCa3.1 (13) wherein the central His residue was replaced with 3-pTza.



Acetarsol and roxarsone were previously characterized as competitive inhibitors at concentrations below 150 μM and demonstrated mixed inhibition at concentrations above 150 μM . Because the phenylarsonic acids reported herein exhibit similar potency to acetarsol and roxarsone, we were interested in exploring their mechanism of action. Using compounds **1** and **2** as representative examples, we screened these arsonates at concentrations ranging from 5–500 μM and subsequently determined the K_m and V_{max} of PHPT1. Unsurprisingly, **1** and **2** behave similarly to acetarsol and roxarsone, with K_m and V_{max} values indicating competitive inhibition at concentrations below 150 μM and mixed inhibition

above (Fig. S3 and Table S2). Moreover, inhibition by phenylarsonic acids is readily reversible. In dialysis experiments, PHPT1 was treated with 100 μM of phenylarsonic acid **1** and then subjected to dialysis for 24 hours. After dialysis, we observed the activity of PHPT1 return to that of the control, similar to previous results with acetarsol (Fig. S4).¹⁷

To better understand the extent to which the phosphomimetic nature of the phenylarsonic acids was the basis for PHPT1 inhibition, we compared the activities of the phosphomimetics *p*-tolylphosphonate and *p*-toluenesulfonate to that of *p*-tolylarsonic acid **4** (Fig. 6). PHPT1 activity was unaffected in the presence of 100 μM *p*-toluenesulfonate or 100 μM *p*-tolylphosphonate. An approximately 25% reduction in phosphatase activity was observed when 300 μM *p*-tolylphosphonate was used, however, this compound had limited solubility at higher concentrations, and we were unable to obtain an IC_{50} value (Fig. S5). This led us to question if phosphonate scaffolds with increased solubility, such as peptides, might have improved potency. Thus, we prepared EGFR and KCa3.1-derived peptides **12** and **13** which comprise the non-hydrolyzable 3-pHis mimic phosphonotriazolylalanine (3-pTza) (Fig. 7).³⁰ While compounds **12** and **13** displayed improved solubility compared to *p*-tolylphosphonate, they do not inhibit PHPT1 at concentrations ranging from 100–500 μM (Fig. S6). Because the phosphonate and sulfonate phosphomimetic compounds tested here do not appreciably inhibit PHPT1, we were curious if inhibition by the phenylarsonic acids was reliant upon an arsenic center. Therefore, we evaluated the effects of arsenic trioxide

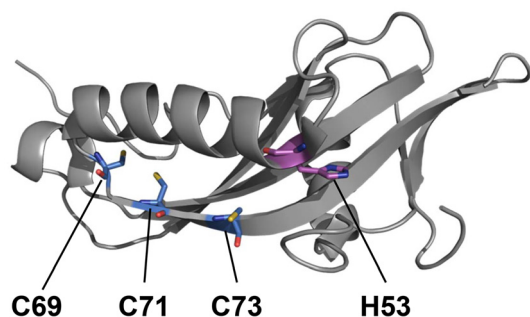


Fig. 8 NMR solution structure of PHPT1 (PDB ID: 2A16). The active site His (H53) is shown in magenta. Cys residues C69, C71, and C73 are shown in blue.

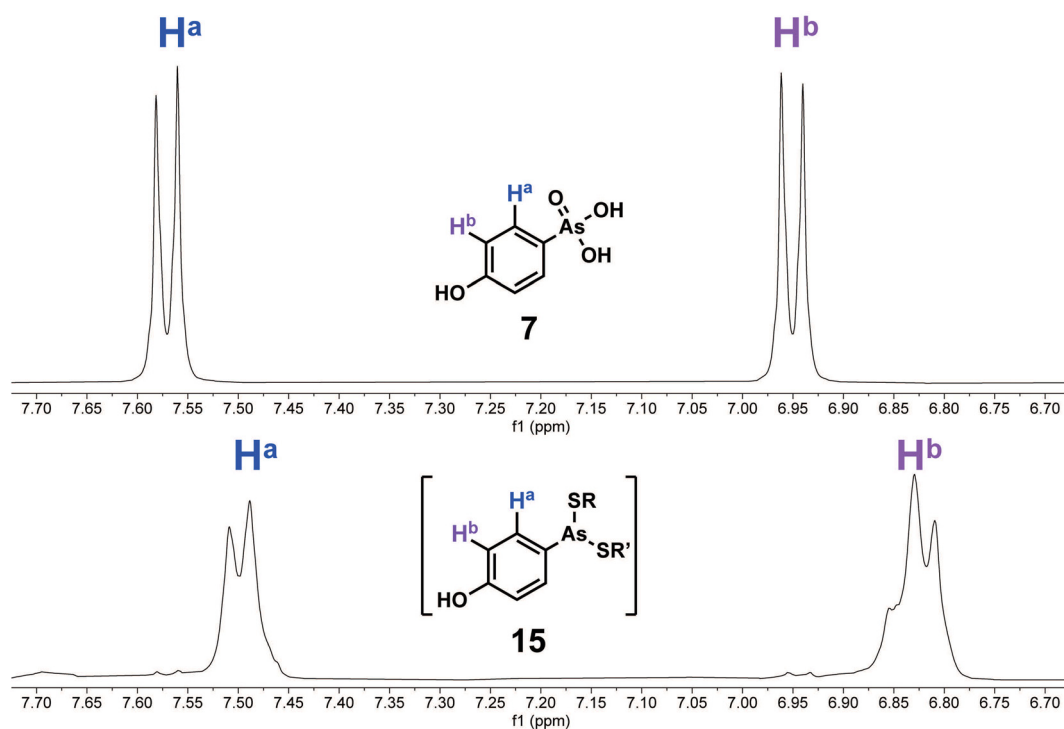
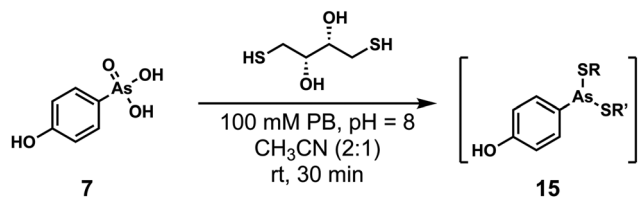


Fig. 9 Comparison of the ^1H NMR (400 MHz) spectra for 4-hydroxyphenylarsonic acid **7** (top) and a reaction mixture containing **7** (1 equiv.) and DTT (4 equiv.) in $\text{DMSO-}d_6$ (bottom) shows new signals corresponding to a mixture of As(III) species **15**.





Scheme 1 Reaction of phenylarsonic acid 7 and DTT generates reactive phenyl-As(III) species.

(As₂O₃) on PHPT1 activity.^{32–34} Interestingly, the trivalent arsenites resulting from dissolution of As₂O₃ do not inhibit PHPT1 at concentrations of 150 or 300 μM (Fig. S7).³⁵ These results collectively indicate that phenylarsonic acids exhibit a unique ability to inhibit PHPT1 compared to As₂O₃ and the phosphomimetics tested here.

Phenylarsonic acids are known to undergo reduction in the presence of a suitable reducing agent, such as ammonium thioglycolate (*i.e.*, phenyl-As(v) → phenyl-As(III)).^{31,36,37} Due to the thiophilicity of the As(III) center, these phenyl-As(III) compounds can react with free thiols in proteins (*e.g.*, Cys).^{31,36,38,39} Because the PHPT1 activity assays are performed under reducing conditions with 5 mM DTT, we questioned whether the phenylarsonic acids were undergoing reduction *in situ* to give reactive phenyl-As(III) species. We hypothesized

that such compounds could react with solvent-exposed Cys residues in PHPT1, for example, C73 (Fig. 8), which has been shown to form a covalent adduct with electrophiles, inhibiting PHPT1 activity.¹⁹

To determine whether the phenylarsonic acids were reacting with DTT, a DMSO-*d*₆ solution of 7 (1 equiv.) and DTT (4 equiv.) was heated at 37 °C for 30 min, and then examined by ¹H nuclear magnetic resonance (NMR) spectroscopy. Upon comparing the spectrum of this mixture to those of 7 and DTT, new signals were observed at 7.50 ppm (d, *J* = 8.2 Hz) and 6.82 ppm (d, *J* = 8.4 Hz), indicating the modification of 7 (see Fig. S20 and S21 in the SI). We hypothesized that compound 7 had undergone reduction to give one or more phenyl-As(III) species ([15], Fig. 9). To better replicate conditions used during activity assays, the reaction between 7 and DTT was conducted in a 1 : 2 (v/v) mixture of acetonitrile and 100 mM phosphate buffer at pH 8.0 (Scheme 1). Analysis of this reaction mixture by LC-MS revealed the formation of compounds which coelute and have *m/z* ratios consistent with dithiolates like [15] (see compounds S9, S10, and S11 and Fig. S18 in the SI). Chromatographic separation of the major components of this mixture was unsuccessful. Thus, to further characterize these compounds, we isolated the mixture and subsequently analyzed it by ¹H and pure shift NMR experiments, namely, pure shift obtained by chirp excitation (PSYCHE) and 2D PSYCHE-TOCSY. The ¹H spectrum of the purified mixture

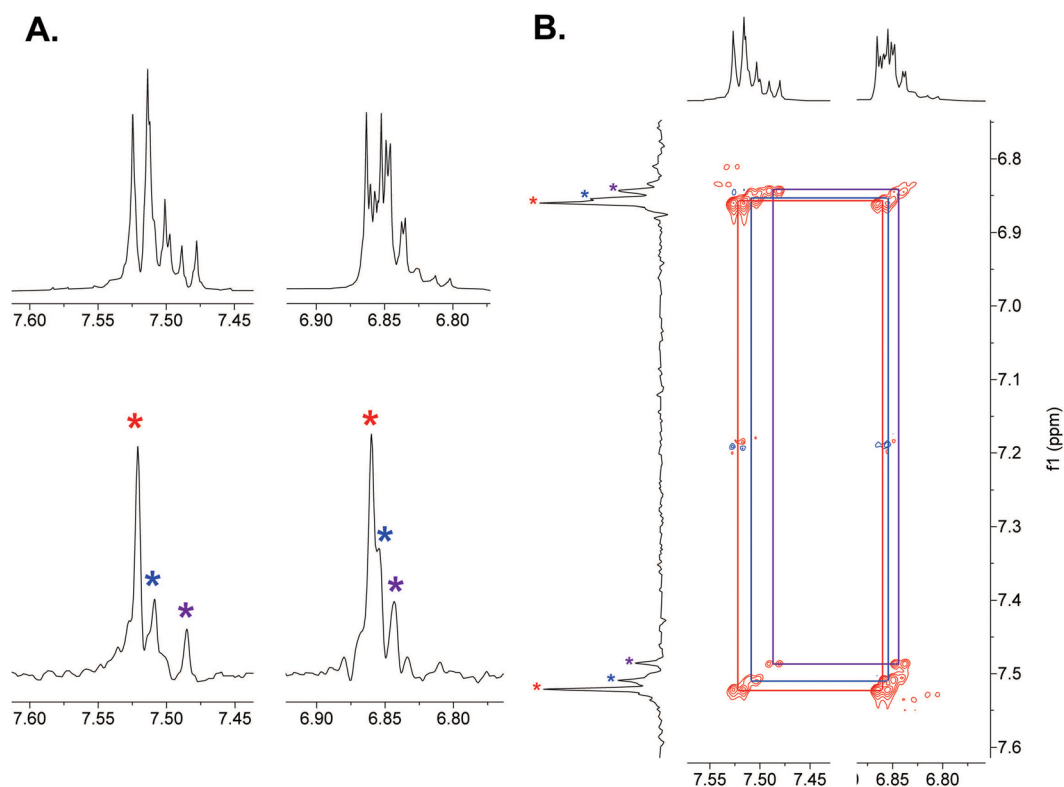


Fig. 10 PSYCHE and PSYCHE-TOCSY NMR spectra obtained at 800 MHz. (A) Comparison of the regions from 6.80–6.85 ppm and 7.45–7.55 ppm in the ¹H NMR (top) and PSYCHE NMR (bottom) spectra. The PSYCHE spectrum shows six unique proton signals. (B) Correlations of the six signals in the PSYCHE-TOCSY spectrum suggest the presence of three aromatic compounds.



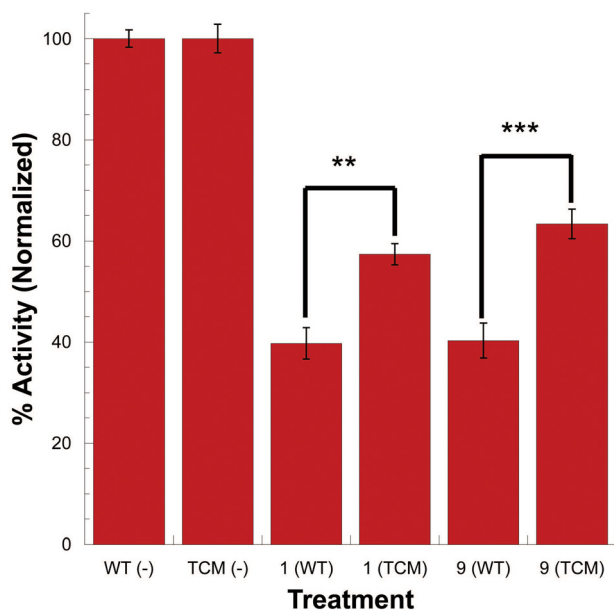


Fig. 11 Inhibition of [C69A, C71A, C73A] PHPT1 by phenylarsonic acid **1** and peptide arsonate **9**. WT: wild-type enzyme, TCM: [C69A, C71A, C73A] mutant.

shows significant but expected overlap of the proton signals from 2.70–3.10 ppm, 6.80–6.88 ppm, and 7.47–7.54 ppm, complicating its analysis (see the SI). However, upon recording the spectrum without homonuclear proton couplings, the resulting PSYCHE spectrum revealed six signals from 6.80–6.90 ppm and 7.45–7.60 ppm (Fig. 10A). Correlations in the PSYCHE-TOCSY spectrum suggest these signals arise from three unique compounds (Fig. 10B). Together, these spectroscopic studies support the hypothesis that the phenylarsonic acids undergo reaction with DTT to provide reactive phenyl-As(III) compounds *in situ* during inhibition assays.

With a better understanding of the reactivity of the phenylarsonic acids with DTT, we hypothesized that any phenyl-As(III) species generated *in situ* might be interacting with solvent-exposed Cys residues in PHPT1, such as C73. Thus, we probed the mechanism of action by examining the propensity of **1** and **9** to inhibit a triple cysteine mutant of PHPT1, [C69A, C71A, C73A]. Upon incubating [C69A, C71A, C73A] with 150 μ M **1** or **9**, we observed a decrease in phosphatase activity. However, compared to the WT enzyme, the triple cysteine mutant retained approximately 20% more activity relative to the control (Fig. 11). This result suggests that phenyl-As(III) compounds [**15**] likely interact with free thiols of Cys residues in PHPT1, as well as others including residues in the active site, and helps explain the mixed inhibition these compounds display.

Conclusion

Herein we have demonstrated that phenylarsonic acids offer a general scaffold from which inhibitors of PHPT1 can be devel-

oped. Notably, the phenylarsonic acid moiety can be incorporated into peptide sequences, providing the first known example of peptide-based inhibitors of PHPT1. Among a small panel of phosphatases, including the His phosphatase LHPP, these arsonic acids exhibited selectivity for PHPT1. Interestingly, these compounds exhibit mixed inhibition toward PHPT1. This mixed inhibition is attributed to the reduction of the As(V)-containing phenylarsonic acids by DTT to produce multiple As(III) species *in situ*, as supported by LC-MS and NMR studies wherein 4-hydroxyphenylarsonic acid was reacted with DTT to produce phenyl-As(III) compounds. Due to their thiophilicity, these As(III) species likely interact with the solvent-exposed free thiol of C73 in PHPT1, resulting in decreased phosphatase activity. It should be noted that Kee and coworkers have reported similar findings with ethacrynic acid, which covalently targets C73 in PHPT1. Moreover, this hypothesis is supported by experiments wherein phenylarsonic acids **1** and **9** were markedly less potent toward a triple cysteine mutant of PHPT1 relative to the WT enzyme. Given the emerging role of PHPT1 in critical cellular processes and disease states, as well as renewed interest in the therapeutic potential of organic arsenicals, this work provides insights that contribute to a better understanding of the enzymology of PHPT1.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

The data supporting this article, including experimental procedures, protein expression and purification protocols, full characterization of new compounds, and other supporting data have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: <https://doi.org/10.1039/d5dt03009a>.

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References

- 1 H. R. Matthews, Protein Kinases and Phosphatases That Act on Histidine, Lysine, or Arginine Residues in Eukaryotic Proteins: A Possible Regulator of the Mitogen-Activated Protein Kinase Cascade, *Pharmacol. Ther.*, 1995, **67**, 323–350.



- 2 J. M. Kee, T. Muir and W. Chasing, Phosphohistidine, an Elusive Sibling in the Phosphoamino Acid Family, *ACS Chem. Biol.*, 2012, **7**(1), 44–51.
- 3 S. Choi, S. H. Lee and J. M. Kee, Bringing Histidine Phosphorylation into Light: Role of Chemical Tools, *ACS Chem. Biol.*, 2025, 778–790.
- 4 S. Srivastava, O. Zhdanova, L. Di, Z. Li, M. Albaqumi, H. Wulff and E. Y. Skolnik, Protein Histidine Phosphatase 1 Negatively Regulates CD4 T Cells by Inhibiting the K⁺ Channel KCa3.1, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**(38), 14442–14446.
- 5 X. Cai, S. Srivastava, S. Surindran, Z. Li and E. Y. Skolnik, Regulation of the Epithelial Ca²⁺ Channel TRPV5 by Reversible Histidine Phosphorylation Mediated by NDPK-B and PHPT1, *Mol. Biol. Cell*, 2014, **25**(8), 1244–1250.
- 6 S. Klumpp, G. Bechmann, A. Mäurer, D. Selke and J. Kriegelstein, ATP-Citrate Lyase as a Substrate of Protein Histidine Phosphatase in Vertebrates, *Biochem. Biophys. Res. Commun.*, 2003, **306**(1), 110–115.
- 7 P. Ek, B. Ek and Ö. Zetterqvist, Phosphohistidine Phosphatase 1 (PHPT1) Also Dephosphorylates Phospholysine of Chemically Phosphorylated Histone H1 and Polylysine, *Upsala J. Med. Sci.*, 2014, **120**(1), 20–27.
- 8 U. Beckman-Sundh, B. Ek, Ö. Zetterqvist and P. Ek, A Screening Method for Phosphohistidine Phosphatase 1 Activity, *Upsala J. Med. Sci.*, 2011, **116**(3), 161–168.
- 9 A. Xu, J. Zhou, Y. Li, L. Qiao, C. Jin, W. Chen, L. Sun, S. Wu, X. Li, D. Zhou, S. Jia, B. Zhang, J. Yao, X. Zhang, H. You and J. Huang, 14-KDa Phosphohistidine Phosphatase Is a Potential Therapeutic Target for Liver Fibrosis, *Am. J. Physiol.: Gastrointest. Liver Physiol.*, 2021, **320**(3), G351–G365.
- 10 H. Su-Xia, W. Li-Juan, J. Zhao, Y. Zhang, M. Li, X. Zhou, J. Wang and Z. Qing, 14-KDa Phosphohistidine Phosphatase Plays an Important Role in Hepatocellular Carcinoma Cell Proliferation, *Oncol. Lett.*, 2012, **4**(4), 658–664.
- 11 A. Xu, J. Hao, Z. Zhang, T. Tian, S. Jiang, J. Hao, C. Liu, L. Huang, X. Xiao and D. He, 14-KDa Phosphohistidine Phosphatase and Its Role in Human Lung Cancer Cell Migration and Invasion, *Lung Cancer*, 2010, **67**(1), 48–56.
- 12 H. Shen, P. Yang, Q. Liu and Y. Tian, Nuclear Expression and Clinical Significance of Phosphohistidine Phosphatase 1 in Clear-Cell Renal Cell Carcinoma, *J. Int. Med. Res.*, 2015, **43**(6), 747–757.
- 13 E. Zavala, S. Dansereau, M. J. Burke, J. M. Lipchock, F. Maschietto, V. Batista and J. P. Loria, A Salt Bridge of the C-Terminal Carboxyl Group Regulates PHPT1 Substrate Affinity and Catalytic Activity, *Protein Sci.*, 2024, **33**(6), 1–19.
- 14 D. R. Martin, P. Dutta, S. Mahajan, S. Varma and S. M. Stevens, Structural and Activity Characterization of Human PHPT1 after Oxidative Modification, *Sci. Rep.*, 2016, **6**, 1–12.
- 15 R. D. Busam, A. G. Thorsell, A. Flores, M. Hammarström, C. Persson and B. M. Hallberg, First Structure of a Eukaryotic Phosphohistidine Phosphatase, *J. Biol. Chem.*, 2006, **281**(45), 33830–33834.
- 16 W. Gong, Y. Li, G. Cui, J. Hu, H. Fang, C. Jin and B. Xia, Solution Structure and Catalytic Mechanism of Human Protein Histidine Phosphatase 1, *Biochem. J.*, 2009, **418**(2), 337–344.
- 17 B. S. McCullough, H. Wang and A. M. Barrios, Inhibitor Screen Identifies Covalent Inhibitors of the Protein Histidine Phosphatase PHPT1, *ACS Med. Chem. Lett.*, 2022, **13**(7), 1198–1201.
- 18 H. Wang, R. Gaston, K. T. Ahmed, G. B. Dudley and A. M. Barrios, Derivatives of the Fungal Natural Product Illudalic Acid Inhibit the Activity of Protein Histidine Phosphatase PHPT1, *ChemMedChem*, 2023, **18**, 1–4.
- 19 H. J. Kim, H. Jung, S. Kim, J. K. Seo and J. M. Kee, Identification of a Target Site for Covalent Inhibition of Protein Phosphohistidine Phosphatase 1, *ACS Med. Chem. Lett.*, 2022, **13**(12), 1911–1915.
- 20 H. Hiraishi, F. Yokoi and A. Kumon, 3-Phosphohistidine and 6-Phospholysine Are Substrates of a 56-KDa Inorganic Pyrophosphatase from Bovine Liver, *Arch. Biochem. Biophys.*, 1998, **349**(2), 381–387.
- 21 H. Hiraishi, T. Ohmagari, Y. Otsuka, F. Yokoi and A. Kumon, *Purification and Characterization of Hepatic Inorganic Pyrophosphatase Hydrolyzing Imidodiphosphate*, 1997, vol. 341.
- 22 Y. Kim, J. Huang, P. Cohen and H. R. Matthews, Protein Phosphatases 1, 2A, and 2C Are Protein Histidine Phosphatases, *J. Biol. Chem.*, 1993, **268**(25), 18513–18518.
- 23 N. C. Lloyd, H. W. Morgan, B. K. Nicholson and R. S. Ronimus, Substituted Phenylarsonic Acids; Structures and Spectroscopy, *J. Organomet. Chem.*, 2008, **693**(14), 2443–2450.
- 24 B. S. McCullough and A. M. Barrios, Facile, Fluorogenic Assay for Protein Histidine Phosphatase Activity, *Biochemistry*, 2018, **57**(18), 2584–2589.
- 25 P. Vlieghe, V. Lisowski, J. Martinez and M. Khrestchatsky, Synthetic Therapeutic Peptides: Science and Market, *Drug Discovery Today*, 2010, **15**(1–2), 40–56.
- 26 D. Goodwin, P. Simerska and I. Toth, Peptides As Therapeutics with Enhanced Bioactivity, *Curr. Med. Chem.*, 2012, **19**(26), 4451–4461.
- 27 K. Fosgerau and T. Hoffmann, Peptide Therapeutics: Current Status and Future Directions, *Drug Discovery Today*, 2015, **20**(1), 122–128.
- 28 Y. Choi, S. H. Shin, H. Jung, O. Kwon, J. K. Seo and J. M. Kee, Specific Fluorescent Probe for Protein Histidine Phosphatase Activity, *ACS Sens.*, 2019, **4**(4), 1055–1062.
- 29 P. V. Attwood, K. Ludwig, K. Bergander, P. G. Besant, A. Adina-Zada, J. Kriegelstein and S. Klumpp, Chemical Phosphorylation of Histidine-Containing Peptides Based on the Sequence of Histone H4 and Their Dephosphorylation by Protein Histidine Phosphatase, *Biochim. Biophys. Acta, Proteins Proteomics*, 2010, **1804**(1), 199–205.



- 30 T. E. McAllister, M. G. Nix and M. E. Webb, Fmoc-Chemistry of a Stable Phosphohistidine Analogue, *Chem. Commun.*, 2011, **47**(4), 1297–1299.
- 31 J. Ahn, T. Kim, J. Bae, J. Jung, J. Lee, H. Lee, J. Mun, S. Kim, J. Park, J. Kim and M. Koh, Reversible Protein Labeling via Genetically Encoded Dithiolane-Containing Amino Acid and Organoarsenic Probes, *Bioconjugate Chem.*, 2025, **36**, 1034–1039.
- 32 S. Hoshino, S. Ijichi, S. Asamizu and H. Onaka, Insights into Arsenic Secondary Metabolism in Actinomycetes from the Structure and Biosynthesis of Bisenarsan, *J. Am. Chem. Soc.*, 2023, **145**(32), 17863–17871.
- 33 S. H. Suzol, A. H. Howlader, A. E. Galvan, M. Radhakrishnan, S. F. Wnuk, B. P. Rosen and M. Yoshinaga, Semisynthesis of the Organoarsenical Antibiotic Arsinothricin, *J. Nat. Prod.*, 2020, 2809–2813.
- 34 N. P. Paul, A. E. Galván, K. Yoshinaga-Sakurai, B. P. Rosen and M. Yoshinaga, Arsenic in Medicine: Past, Present and Future, *BioMetals*, 2023, **36**(2), 283–301.
- 35 K. J. Irgolic, W. E. C. Wacker and R. A. Zingaro, *Arsenic*, National Academy of Sciences, 1977.
- 36 P. Wilson, A. Anastasaki, M. R. Owen, K. Kempe, D. M. Haddleton, S. K. Mann, A. P. R. Johnston, J. F. Quinn, M. R. Whittaker, P. J. Hogg and T. P. Davis, Organic Arsenicals as Efficient and Highly Specific Linkers for Protein/Peptide-Polymer Conjugation, *J. Am. Chem. Soc.*, 2015, **137**(12), 4215–4222.
- 37 A. M. Spuches, H. G. Kruszyna, A. M. Rich and D. E. Wilcox, Thermodynamics of the As(III)-Thiol Interaction: Arsenite and Monomethylarsenite Complexes with Glutathione, Dihydrolipoic Acid, and Other Thiol Ligands, *Inorg. Chem.*, 2005, **44**(8), 2964–2972.
- 38 X. Zhou, X. Sun, C. Mobarak, A. J. Gandolfi, S. W. Burchiel, L. G. Hudson and K. J. Liu, Differential Binding of Monomethylarsonous Acid Compared to Arsenite and Arsenic Trioxide with Zinc Finger Peptides and Proteins, *Chem. Res. Toxicol.*, 2014, **27**(4), 690–698.
- 39 S. Shen, X. F. Li, W. R. Cullen, M. Weinfeld and X. C. Le, Arsenic Binding to Proteins, *Chem. Rev.*, 2013, 7769–7792.

