Heterobifunctional molecules induce dephosphorylation of kinases – a proof of concept study

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Supporting Information Placeholder

ABSTRACT: Heterobifunctional molecules have recently proven powerful tools to induce proximity-driven, ligase-dependent ubiquitination and subsequent degradation of target proteins ("proteolysis targeting chimera"). We describe here a chemical strategy for controlling a different post-translational modification (PTM): phosphorylation. Heterobifunctional small molecules were designed to promote the proximity of a protein Ser/Thr phosphatase (PP1) to protein targets of interest. This strategy was used to induce the PP1-dependent dephosphorylation of two oncogenic kinases, AKT and EGFR. To our knowledge, this work represents the first examples of small molecules recruiting non-native partners to induce removal of a PTM.

Post-translational modification (PTM) effectually expands nature's genetic code. It is estimated that nearly 5% of the human proteome consists of enzymes responsible for addition or removal of PTMs: chemical modifiers such as ubiquitin, phosphate, lipids and glycans which can influence the localization, activity, and stability of their conjugated proteins.[1] It is well recognized that the ability to selectively influence PTMs of a target protein may be an effective means to modulate its function. Recently it has been shown that one class of post-translational enzymes, E3 ubiquitin ligases, can be co-opted in a novel approach to promote the degradation of target proteins of interest (POIs).[2-4] In this strategy, heterobifunctional small molecules (proteolysis targeting chimeras) incorporating target-binding and ligase binding ends are hypothesized to promote the proximity of specific ligases to POIs promoting POI ubiquitination, subsequent recognition by the proteasome and degradation (Figure 1a).[5] These catalysts have been shown to be effective chemical tools to induce the degradation of members of several target classes including bromo-domains[3, 6], kinases[7, 8], and nuclear hormone receptors[9]. Inspired by this groundbreaking work, we envisioned that heterobifunctional molecule approaches may be useful to influence some of the other >200 types of PTMs (beyond ubiquitination). Considering diverse PTMs can drive a broad variety of cellular processes, we envision such strategies could provide flexible and versatile tools to fine-tune cellular features of POIs as well as lead to therapeutically relevant agents.

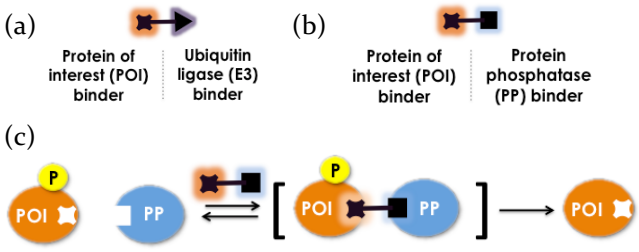


Figure 1. (a) Heterobifunctional molecule containing an E3 ubiquitin ligase binder and a POI binder ("proteolysis targeting chimera"); (b) Heterobifunctional molecule containing a protein phosphatase and POI binder; (c) Design to provide proximity between POI and protein phosphatase, promoting POI dephosphorylation. POI = protein of interest; PP = protein phosphatase; P = phosphate.

Phosphorylation/de-phosphorylation of serine, threonine and tyrosine residues of proteins, elicited by kinases and phosphatases, respectively, serves to regulate many cellular processes. Inclusion of this

small and polar functionality can lead to large conformational changes that alter protein function. We became interested in the design of compounds that could promote the selective de-phosphorylation of POIs. We hypothesized heterobifunctional molecules comprised of a POI-binding moiety linked to a protein phosphatase (PP) binding moiety would recruit a phosphatase to, and promote de-phosphorylation of, target proteins (Figure 1b,c). Herein we report our studies directed at promoting protein-phosphatase 1 (PP1) recruitment to de-phosphorylate oncogenic kinases AKT (protein kinase B) and EGFR (epidermal growth factor receptor).

Promoting de-phosphorylation of kinases is an attractive strategy for pharmacological intervention as many kinases have activity that is regulated by phosphorylation state. In classic examples, phosphorylation of the A-loop (and other motifs) increases enzymatic activity by conformational changes that lead to improved substrate binding, cellular localization or co-partner recruitment.[10] Extracellular growth signals are propagated by kinases phosphorylating, and thus in many cases activating, downstream kinases. Inducing de-phosphorylation of a pathway kinase would thus simultaneously inhibit the kinases ability to receive and transmit an upstream signal. The PI3K/AKT pathway is often over-activated in cancer cells, having a central role in cell proliferation and survival.[11] AKT itself contains a pleckstrin homology (PH) domain that binds to PIP3 (phosphatidylinositol (3,4,5)-triphosphate) in the plasma membrane with high affinity. Once recruited to the plasma membrane, AKT is phosphorylated by several kinases at two critical sites, threonine 308 (Thr308, A-loop) and serine 473 (Ser473).[12, 13] Maximal AKT activity is dependent on the phosphorylation status of both Thr308 and Ser473 residues.[14]

We designed and synthesized chloroalkyl AKT inhibitor[15] **1** (Figure 2) to undergo chemoselective reaction with HaloTag protein in cells. A PTEN-deficient prostate cancer line, LNCaP, was transfected with a vector encoding a HaloTag-PP1-FLAG fusion protein.[16] HaloTag-PP1-FLAG expression and its chloroalkyl-reactivity were verified by labeling with a chloroalkyl-TAMRA probe (Figure S1). Treatment of transfected cells with 10 μM **1** significantly diminished the phosphorylation level of AKT compared to cells that were not transfected (Figure 2a,b). Similarly treatment of MCF7/neo HER2 and PC-3 cells that had been transfected with HaloTag-PP1-FLAG with 10 μM **1** also decreased pAKT^{T308} levels to a significant extent relative to the non-transfected control (Figure 2c,d). These experiments support our hypothesis that recruitment of PP1 to a target protein can promote target protein de-phosphorylation in cells.

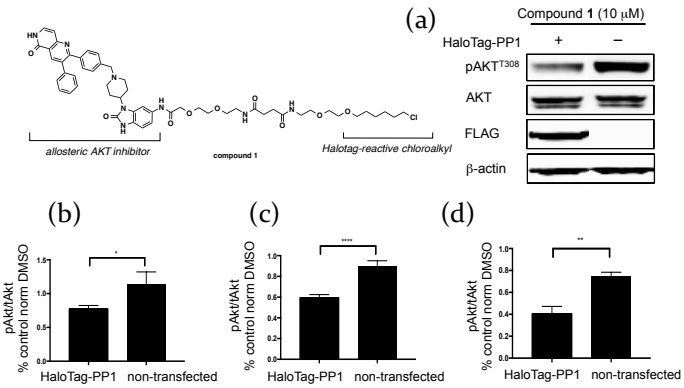


Figure 2. Compound **1** decreases pAKT in the cells expressing HaloTag-PP1-FLAG only. HaloTag-PP1-FLAG was transfected in LNCaP over 24 hrs. (a) Immunoblot analysis of AKT phosphorylation after 8 hrs treatment of LNCaP cells with 10 μM compound **1**. Quantification of pAKT^{T308} levels after normalization over total AKT in (b) LNCaP, (c) MCF7/neo HER2, and (d) PC-3 cells after treatment with 10 μM compound **1** for 8 hrs. NS: $P > 0.05$, *: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$, ***: $P \leq 0.0001$.

We next moved to examine heterobifunctional small molecules incorporating both AKT and PP1 binding groups. PP1 is a ubiquitously expressed phosphatase and the enzyme has broad activity, however the catalytic activity is restricted *in vivo* by numerous PP1-intereacting proteins (PIPs). Most known interactors with PP1 contain a variant of a consensus RVxF-type docking motif.[17] This RVxF motif is a recognition domain present on various PIPs that interact with PP1 at a region adjacent to its active site. Synthetic peptides that contain RVxF-motif, but not mutated RAxA peptides, can disrupt a subset of PIP-PP1 complexes in vitro.[17] Chatterjee et al. developed 21-residue peptide (PP1-disrupting-peptide-1, "PDP1") that binds and activates PP1 by mediating release of PP1 from its allosteric PIP inhibitor – I2.[18] We therefore utilized the reported PP1-activating synthetic peptide (PDP1) to generate a bifunctional molecule that could bridge PP1 and AKT (compound **3**, Figure 3).

Compound **3** was tested for ability to promote dephosphorylation of pAKT in a recombinant biochemical system. Purified pAKT^{T308} was incubated with unconjugated AKT inhibitor (**2**) or compound **3** in the presence of PP1 as well as I2 at 37°C for 1 hr, and pAKT^{T308} levels were quantified by western blot. The

PIP inhibitor (I2) was included to demonstrate alleviation of inhibition through competition by the PP1-binding peptide (PDP1). Compound **2** moderately decreased $pAKT^{T308}$ levels in a dose-dependent manner, which is consistent with the finding that the AKT inhibitor used in this experiment stabilizes the conformation susceptible to phosphatase-mediated dephosphorylation (Figure 3b).[19] Compound **3** induced greater reduction in $pAKT^{T308}$ levels, compared to free AKT inhibitor **2** treatment. A trend toward bell-shaped dose response was observed for **3** - a hook/prozone effect [28] could be operative for this mechanism where ternary complex formation is necessary. Next we demonstrated rescue of the dephosphorylation phenotype by competition with an excess amount of free PP1-binding peptide (Figure 3b). Taken together, these data indicate proximity of pAKT and PP1, promoted by a small molecule, is sufficient to promote dephosphorylation.

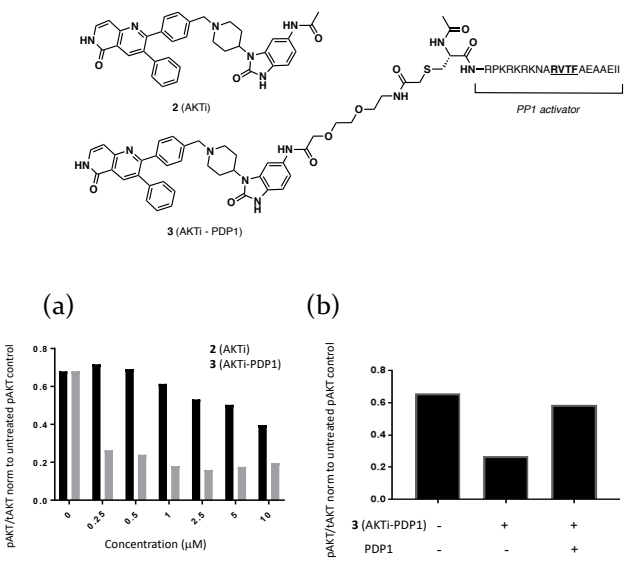
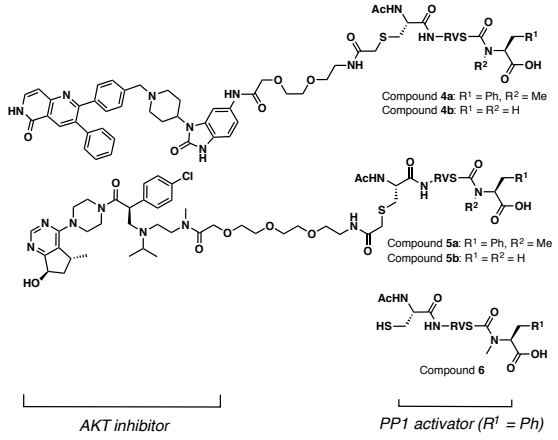


Figure 3. Allosteric AKT inhibitor-PDP1 decreased $pAKT^{T308}$ levels in the presence of PP1. Structures of allosteric AKT inhibitor (**2**, AKTi) and AKTi-PDP1 conjugate (**3**). (b) $pAKT^{T308}$ levels after 1 hr incubation of PP1, pAKT, and **2** or **3** in the presence of I2. (c) Competition assay with un-conjugated PDP1 (10 μM).

Molecules such as **3** were tested for influence on pAKT levels in LNCap cells. Somewhat unsurprisingly, there was no obvious change in pAKT levels at concentrations up to 10 μM (data not shown). We anticipated this lack of phenotype a challenge resultant from insufficient cellular permeability or proteolytic instability. We therefore decided to explore the shortest reported PP1-peptide to facilitate drug permeabilization and mitigate metabolic instability. Chamberlin and Tappan identified tetrapeptide sequence (RVSF) to be a minimum requirement for PP1 binding and activation *in vitro*.[17] We synthesized heterobifunctional molecules with both allosteric[15, 20] and ATP-competitive[21] AKT inhibitors that included either active PP1-binding RVSF or inactive RVSA (Compounds **4a**/**b**, **5a**/**b**, Scheme 1, *N*-Ac-Cys included to enable synthesis). Solvent exposed vectors for the PP1 peptides and AKT inhibitors were clear from analysis of published X-ray structures and SAR and linkers were designed to be sufficient in length as to allow simultaneous binding of both partners.[15, 20, 21]



Scheme 1. Heterobifunctional molecules

Compounds **4a** and **b** include an allosteric AKT binder[15, 20] and RVSF or RVSA sequences, respectively. We anticipated comparison of this pair of compounds would normalize for the likely modest cell penetrance of small-molecule-peptide conjugates. At a concentration of 10 μM over 8 hrs, compound **4a** decreased the level of $pAKT^{T308}$ and $pAKT^{S473}$ in LNCaP cells whereas compound **4b** (containing the PP1-inactive RVSA peptide) did not (Figure 4a). Importantly, co-treatment of **4b** with free RVSF **6** did not result in significant decreases in pAKT suggesting dual binding of the heterobifunctional molecule is required for enhanced dephosphorylation . The difference in phenotype between **4a** and **4b** for $pAKT^{T308}$ was consistent at higher concentration (LNCaP, Figure 4b, $pAKT^{S473}$ displayed in Figure S2) as well as in additional cell lines (MCF7/neo-HER2, PC-3, Figures 4c,4d). The dephosphorylation phenotype of **4a** was substantially rescued by depletion of PP1 by transfected siRNA (Figures 4e,f). Consistent with the mechanism of action of **4a**, levels of downstream pS6[11] were substantially decreased relative to **4b** (Figure S3).

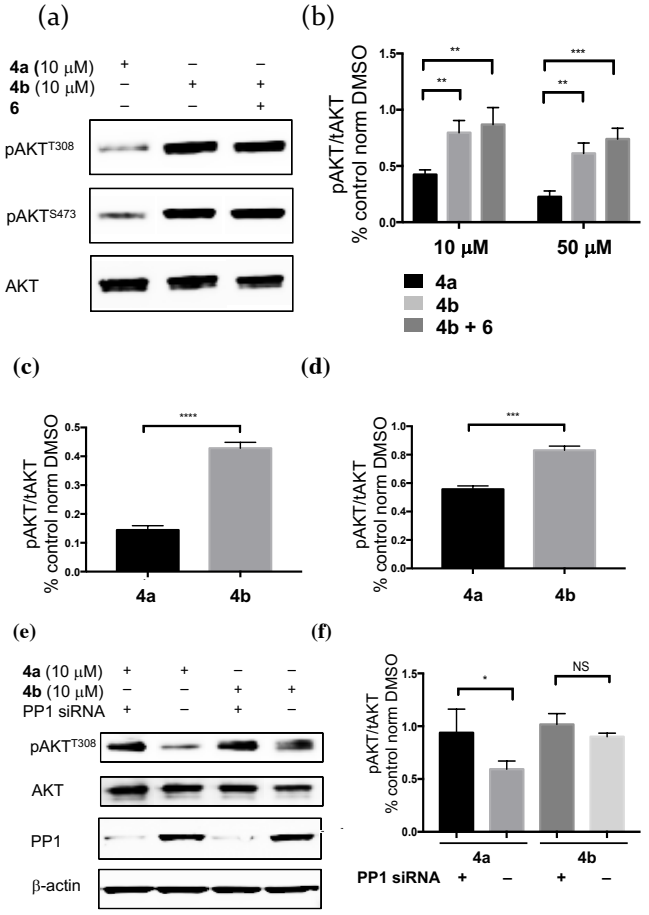


Figure 4. Compound **4a** decreases pAKT^{308} and pAKT^{S473} levels in cells in a PP1-dependent manner. (a) Immunoblot analysis of phosphorylation of AKT in LNCaP cells treated with **4a** or **4b** +/- **6** (10 μM each, 8 hrs). (b) Quantification of pAKT^{T308} levels after normalization over total AKT in LnCaP. (c), (d) 10 μM **4a** and **4b** in MCF7/neo-HER2 and PC-3 cells, respectively for 8 hours quantified pAKT^{T308}. (e) Effect of 10 μM **4a** and **4b** on pAKT^{T308} levels in LNCaP cells (8 hr compound treatment) with and without PP1 knockdown by siRNA (48 hr treatment prior to compound dosing) (f) Quantification of pAKT^{T308} levels after normalization over total AKT.

Compounds **5a** and **5b** include an ATP competitive inhibitor. ATP-competitive AKT inhibitors are known to promote an increase pAKT^{T308} and pAKT^{S473} by a mechanism that protects from dephosphorylation by protein phosphatase 2A (PP2A)[19]. At 10 μM test concentrations, compound **5a** demonstrated a modest reduction in pAKT^{T308} and pAKT^{S473} compared to **5b** alone or **5b** in combination with RVSF **6** (Figure 5a,b). This result reached significance ($P \leq 0.01$) at 50 μM test concentrations. Notably, relative to DMSO controls, **5a** protected against the typical increase in pAKT observed for ATP-competitive AKT inhibitors.

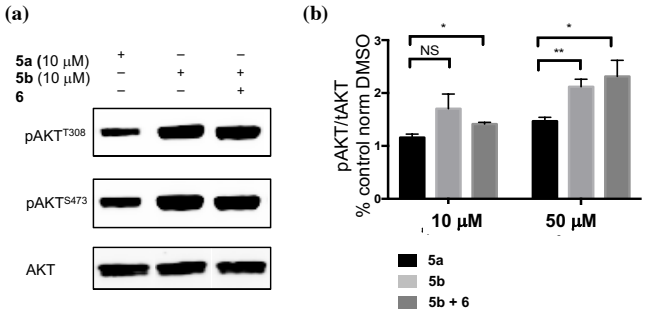


Figure 5. Compound **5a** decreases the phosphorylation of AKT relative to control compound **5b** in LNCaP cells. (a) Immunoblot analysis of phosphorylation of AKT in LNCaP cells treated with **5a** or **5b** +/- **6** (10 μM, 8 hrs). (b) Quantification of pAKT^{T308} levels in LNCaP after normalization over total AKT.

We next turned to demonstrate applicability to a second kinase, EGFR. EGFR has five autophosphorylation sites in its C-terminal tail, and phosphorylation is necessary for its maximal activity.[5, 22] EGFR inhibitor AZD-9291[23] (Tagrisso®) was modified to include a HaloTag reactive chloroalkane (compound **7**, Figure 6) and dosed to HaloTag-PP1-FLAG transfected HCC827 cells at 10 μM for 8 hr. The pEGFR levels were compared between HaloTag-PP1-FLAG transfected and non-transfected experiments. In both cases, pEGFR^{Y1068} was shown to be substantially decreased relative to the DMSO control. This is expected as a major mechanism of EGFR inhibition is inhibition *trans* autophosphorylation.[24] However, the reduction in pEGFR^{Y1068} was significantly more pronounced in the transfected experiment consistent with our designed mechanism of action. This case is notable as it suggests dephopshorylation of a tyrosine by a serine/threonine phosphatase – proximity likely driving additional promiscuity.[25]

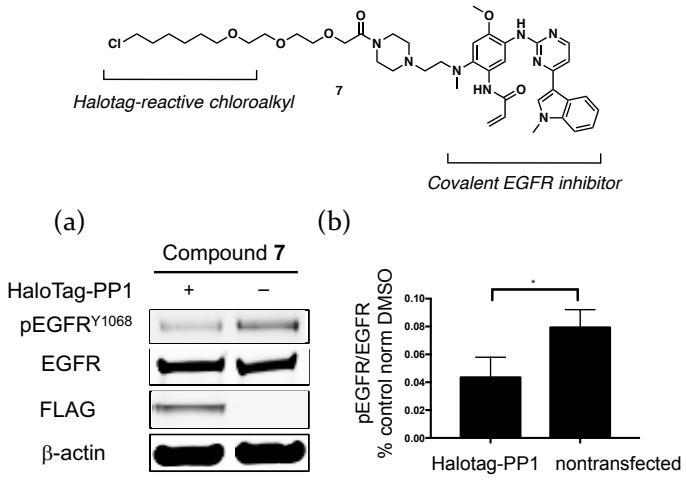


Figure 6. Chloroalkane-functionalized EGFR inhibitor **7** promotes additional dephosphorylation of EGFR in cells expressing HaloTag-PP1-FLAG, but not in non-transfected cells. HaloTag-PP1-FLAG was transfected in HCC827 over 24 hrs. (a) Immunoblot analysis of EGFR and pEGFR^{Y1068}. (b) Quantification of pEGFR^{Y1068} levels after normalization over total EGFR in HCC827.

Taken together, our findings validate for the first time the concept of using tool compounds to recruit a phosphatase to a POI to affect de-phosphorylation.[26] In particular, we have shown proof of concept against two oncogenic kinases, AKT and EGFR, utilizing either heterobifunctional small molecules (PhoRCs - Phosphatase Recruiting Chimeras) or a HaloTag-based approach. For kinases, the ability to inhibit reception and transmission of the phosphorylation signal by promoting dephosphorylation may prove a means to better dampen pathway signaling and potentially avoid paradoxical pathway reactivation. The heterobifunctional molecules, however, promoted the dephosphorylation phenotype only at high concentrations. Given their peptidic nature (high TPSA, low LogP, high #-rotatable bonds, high H-bond donor count) we hypothesize this is likely due to a combination of poor intracellular penetration and instability toward cellular hydrolyases. In addition, the potency of PP1 activator used in this study is reported to be modest compared to the fully elaborated PP1. We anticipate a newly discovered small molecule PP1 activator, with improved properties, could overcome the limitations of the chimeric molecule. Given the above achieved proof-of-concept, discovery of such a small molecule may be warranted that could lead to more cell penetrant PhoRCs. Moreover, this work represents the first examples of small molecules recruiting non-native protein/enzyme partners to induce *removal* of a PTM adding to the potential applications of heterobifunctional compounds.[27]

EXPERIMENTAL SECTION

General Synthetic procedures. All solvents and reagents were used as obtained. ^{1}H NMR spectra were recorded with Bruker spectrometers and referenced to tetramethyl silane. Non peptidic molecules were analyzed by HPLC (Waters Acquity UPLC column) with UV detection at 254 and 210 nm, and purified by HPLC (Interchim, Phenomenex Luna-C18, Phenomenex Gemini-NX) or Teledyne ISCO CombiFlash (RediSep Rf silica gel column). Peptides and Peptide-small molecule conjugates were analyzed by HPLC (Waters, Xevo Qtof, UPLC column) with UV detection at 220 and 280 nm, and purified by HPLC (Waters Autopurification System, Phenomenex Luna C18 100 Angstroms). Purity of final compounds was determined to by HPLC to be >95% for all compounds. Spectral images are included in the supporting information section. See the synthesis of compound **6** for a representative peptide synthesis procedure (standard Fmoc chemistry on Wang resin).

Compound 1. Commercial 4-[2-[2-(6-chlorohexoxy)ethoxy]ethylamino]-4-oxo-butanoate (5 mg, 0.012 mmol) in 0.1 mL acetonitrile was added to an ice-bath cooled solution of 2-[2-(2-aminoethoxy)ethoxy]-N-[2-oxo-3-[1-[[4-(5-oxo-3-phenyl-6H-1,6-naphthyridin-2-yl)phenyl]methyl]-4-piperidyl]-1H-benzimidazol-5-yl]acetamide TFA salt (compound **7**, 9.0 mg, 0.012 mmol) in 0.1 mL of pH 8 borate buffer. The mixture was warmed to room temperature to stir for 2 hours. Concentration and purification by reverse-phase HPLC gave compound **1** (9.8 mg, 88% yield) as a colorless solid.

LC-MS: $[M+H]^+$ = 995. HRMS calc'd for $C_{53}H_{66}ClN_8O_9$ $(M+H)^+$ 993.4636, found: 993.4626. ^{1}H NMR (500 MHz, DMSO-d_6) □ 11.6 (d, *J*=5.9 Hz, 1H), 10.81 (s, 1H), 9.61 (s, 1H), 8.39 (s, 1H), 7.91 (t, *J*=5.6 Hz, 1H), 7.81 (t, *J*=5.6 Hz, 1H), 7.62 (d, *J*=1.9 Hz, 1H), 7.50 (dd, *J*=7.3, 5.9 Hz, 1H), 7.36-7.30 (m, 5H), 7.29-7.23 (m, 5H), 6.9 (d, *J*=8.5 Hz, 1H), 6.69 (d, *J*=7.3 Hz, 1H), 4.07 (s, 2H), 3.68 (dd, *J*=5.9, 3.5 Hz, 2H), 3.63-3.58 (m, 4H), 3.45 (m, 6H), 3.35 (m, 5H), 3.20 (q, *J*=5.8 Hz, 2H), 3.16 (q, *J*=5.9 Hz, 2H), 2.98-2.90 (m, 2H), 2.73-2.42 (m, 8H), 2.09 (t, *J*=11.6 Hz, 2H), 1.73-1.58 (m, 4H), 1.50-1.41 (m, 2H), 1.40-1.32 (m, 2H), 1.32-1.20 (m, 2H).

Compound 4a. To a solution of (2R)-2-[[(2R)-2-[[(2R)-2-[[(2R)-2-[[(2S)-2-acetamido-3-sulfanyl-propanoyl]amino]-5-guanidino-pentanoyl]amino]-3-methyl-butanoyl]amino]-3-hydroxy-propanoyl]-methyl-amino]-3-phenyl-propanoic acid (6.0 mg, 0.0090 mmol) in 0.90 mL of pH8 HEPES, a solution of purified peptide (compound **6**, 7.7 mg, 0.0090 mmol) in 0.10 mL of DMSO was added. The reaction mixture was stirred at room temperature overnight, then concentrated. The residue was subjected to reverse phase HPLC (acetonitrile in water with 0.05% TFA, 5 to 50% in 30 min) to afford the title compound as a white solid (8.9 mg, 71%).

LC-MS: $[M+2H]^{2+}$ = 697. HRMS calc'd for $C_{70}H_{88}N_{15}O_{14}S$ $(M+H)^{+}$ 1394.6350, found: 1394.6331.

Compound 4b. To a solution of (2R)-2-[[(2R)-2-[[(2R)-2-[[(2R) -2-[[(2S)-2-acetamido-3- sulfanyl-propanoyl]amino]-5- guanidino-pentanoyl]amino]- 3-methyl-butanoyl]amino]-3- hydroxy-propanoyl]amino]propanoic acid (2.4 mg, 0.0041 mmol) in 0.90 mL of pH8 HEPES buffer, 1 M, a solution of purified peptide (RVSA, 3.5 mg, 0.0041 mmol) in 0.10 mL of DMSO was added. The resulting mixture was stirred at room temperature overnight, then directly loaded to reverse phase HPLC (acetonitrile in water with 0.1% TFA, 20 to 40% in 20 min) to afford the title compound as a white solid (**4b**, 4.4 mg, 82%).

LC-MS: $[M+H]^{+}$ = 1305. HRMS calc'd for $C_{63}H_{88}N_{15}O_{14}S$ $(M+H)^{+}$ 1304.5881, found: 1304.5859.

Compound 5a. To a solution of (2R)-2-[[(2R)-2-[[(2R)-2-[[(2R)-2-[[(2S)-2-acetamido-3-sulfanyl-propanoyl]amino]-5-guanidino-pentanoyl]amino]-3-methyl-butanoyl]amino]-3-hydroxy-propanoyl]-methyl-amino]-3-phenyl-propanoic acid (2.4 mg, 0.0036 mmol) in 450 uL of pH8 HEPES buffer, 1 M, a solution of purified peptide (compound **6**, 3.1 mg, 0.0036 mmol) in 100 uL of DMSO was added. The reaction mixture was stirred at room temperature overnight, then concentrated *in vacuo*. The residue was subjected to reverse phase HPLC (acetonitrile in water with 0.1% TFA, 20 to 40% in 20 min) to afford the title compound as a white solid (11a, 1.9 mg, 38%).

LC-MS: $[M+H]^{+}$ = 1411. HRMS calc'd for $C_{66}H_{101}ClN_{15}O_{15}S$ $(M+H)^{+}$ 1410.7005, found: 1410.6996.

Compound 5b. To a solution of (2R)-2-[[(2R)-2-[[(2R)-2-[[(2R) -2-[[(2S)-2-acetamido-3- sulfanyl-propanoyl]amino]-5- guanidino-pentanoyl]amino]- 3-methyl-butanoyl]amino]-3- hydroxy-propanoyl]amino]propanoic acid (7.3 mg, 0.0126 mmol) in 450 uL of pH8 HEPES buffer, 1 M, the purified peptide (RVSA, 11.1 mg, 0.0126 mmol) solution in 100 uL of DMSO was added. The resulting mixture was stirred at room temperature overnight, then directly loaded to reverse phase HPLC (acetonitrile in water with 0.1% TFA, 20 to 40% in 20 min) to afford the title compound as a white solid (**11b**, 10.4 mg, 62%).

LC-MS: $[M+H]^{+}$ = 1321. HRMS calc'd for $C_{59}H_{95}ClN_{15}O_{15}S$ $(M+H)^{+}$ 1330.6463, found: 1320.6533

Compound 6. Compound **6** was assembled using standard Fmoc chemistry protocols on Fmoc-N-methylphenylalanine Wang resin and was acetylated on the N-terminus. Amino acids were coupled using coupling reagent 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) and base diisopropylethylamine (DIPEA). Peptide was cleaved off the solid support with trifluoroacetic acid: triisopropylsilane: water (95:2.5:2.5) for 1 hour at room temperature. Resin was filtered and filtrate was evaporated and peptide was precipitated with ethyl ether, centrifuged and ethyl ether was decanted off. Addition of ethyl ether, centrifugation and ether decantation was repeated twice and peptide pellet was allowed to dry. Crude peptide pellet were solubilized in dimethyl sulfoxide and purified by reverse phase chromatography on a C18 column using acetonitrile/water buffers. Purified fractions were analyzed by liquid chromatography mass spectrometry, pooled and lyophilized. LCMS (ES, *m/z*): $[M+H]^{+}$ 667.2

Compound 7. **T**ert-butyl 4-(2-((2-acrylamido-5-methoxy-4-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)(methyl)amino)ethyl)piperazine-1-carboxylate (200 mg, 0.31 mmol) in 1.6 mL 1,4-dioxane was treated with conc. HCl (1.6 mL, 36% in water) at room temperature. The reaction mixture was stirred for 30 min, diluted with 10 mL methanol and the solid collected by filtration and dried under vacuum (177 mg of a yellow solid). [LCMS (ESI) $[M+H]^{+}$=541]. The crude solid (40 mg, 0.069 mmol), 2-(2-2-[(6-chlorohexyl)oxy]ethoxyethoxy)acetic acid (25 mg, 0.089 mmol), HATU (42 mg, 0.11 mmol) and DIPEA (33.5 mg, 0.259 mmol) were diluted with DMA (2 mL) and the reaction mixture stirred for 30 min at room temperature. The solution was purified directly by reverse-phase HPLC (ACN:water 0.05% NH_3) to give compound **7** (12.3 mg, 21%).

LCMS (ESI): $[M+H]^{+}$=805. ^{1}H NMR (300 MHz, DMSO-*d6*): δ 9.37 (s, 1H), 8.97 (s, 1H), 8.63 (s, 1H), 8.32 (d, *J* = 5.3 Hz, 1H), 8.25 (d, *J* = 7.9 Hz, 1H), 7.89 (s, 1H), 7.52 (d, *J* = 8.2 Hz, 1H), 7.29-7.10 (m, 3H), 6.99 (s, 1H), 6.64 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.25 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.76 (dd, *J* = 10.2, 2.0 Hz, 1H), 4.10 (s, 2H), 3.90 (s, 3H), 3.86 (s, 3H), 3.58 (t, *J* = 6.6 Hz, 2H), 3.55 – 3.34 (m, 14H), 3.00 (t, *J* = 6.4 Hz, 2H), 2.70 (s, 3H), 2.45-2.30 (m, 6H), 1.69-1.64 (m, 2H), 1.47-1.43 (m, 2H), 1.34-1.26 (m, 4H).

ASSOCIATED CONTENT

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REFERENCES

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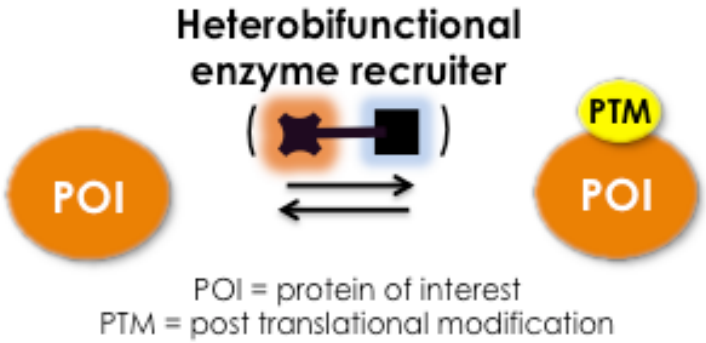
ABSTRACT GRAPHIC



Direction	Enzyme	PTM	Heterobifunctional enzyme recruiter
forward	ubiquitin ligase (E3)	ubiquitin	"ProTaC" (previous work)
reverse	protein phosphatase (PP)	phosphate	"PhoRC" (this work)

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