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Synthesis and evaluation of bifunctional PTP4A3 phosphatase inhibitors activating the ER stress pathway

Ettore J. Rastelli [a], Sara Sannino [b], Duncan J. Hart [c], Elizabeth R. Sharlow [c], John S. Lazo [c], Jeffrey L. Brodsky [b], Peter Wipf [a,*]

[a] Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260, United States
[b] Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, United States
[c] Department of Pharmacology, University of Virginia, Charlottesville, VA 22908, United States

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ABSTRACT

We developed JMS-053, a potent inhibitor of the dual specificity phosphatase PTP4A3 that is potentially suitable for cancer therapy. Due to the emerging role of the unfolded protein response (UPR) in cancer pathology, we sought to identify derivatives that combine PTP4A3 inhibition with induction of endoplasmatic reticulum (ER) stress, with the goal to generate more potent anticancer agents. We have now generated bifunctional analogs that link the JMS-053 pharmacophore to an adamantyl moiety and act in concert with the phosphatase inhibitor to induce ER stress and cell death. The most potent compound in this series, **7a**, demonstrated a ca. 5-fold increase in cytotoxicity in a breast cancer cell line and strong activation of UPR and ER stress response genes in spite of a ca. 13-fold decrease in PTP4A3 inhibition. These results demonstrate that the combination of phosphatase inhibition with UPR/ER-stress upregulation potentiates efficacy.

Introduction

Protein tyrosine phosphatases (PTPs) are responsible for the removal of phosphate groups from biomolecules and, in combination with protein tyrosine kinases (PTKs), control phosphorylation homeostasis.[1] Aberrant phosphorylation can lead to an imbalance that is associated with many pathophysiological conditions, including metabolic diseases, inflammatory disorders, and cancer. Even though complementary in functionality, PTKs and PTPs have historically been perceived differently with respect to drug discovery. While many PTK inhibitors have achieved FDA approval, there are no clinically employed PTP inhibitors yet, contributing to the "undruggable" stigma for this class of enzymes.[2] At least for cancer, this can be attributed to a misconception that phosphatases act exclusively as tumor suppressors. Another issue is the difficulty in developing a small molecule inhibitor to bind in the shallow, positively charged active site typical of protein phosphatases.

Modulators of the unfolded protein response (UPR) have to-date faced a similar predicament as phosphatase inhibitors.[3] While it is generally accepted that UPR signaling in response to endoplasmatic reticulum (ER) stress can dictate cell fate and is involved in numerous pathologies, including neurodegeneration, protein misfolding disorders, diabetes, ischemia, and cancer,[4,5] translational development of UPR and ER stress modulators has been lacking due to the dearth of selective small molecule drug candidates and viable pharmacologic strategies.[3,6]

We have reported the synthesis of 7-iminothieno[3,2-*c*]pyridine-4,6(5*H*,7*H*)-dione (**1**, JMS-053) through both batch and in-flow photooxygenation reactions.[7] This novel compound was found to be a low-nanomolar inhibitor of the oncogenic, dual-specific phosphatase PTP4A3,[8] as well as its family members PTP4A1 and PTP4A2.[9] An initial SAR study on our lead compound focused on structural changes to the pendant aryl group, imide substitution, and conversion of the imine to a carbonyl group (Fig. 1, zone model). We determined that zone 1 tolerated *para*-alkoxy chains R^1 and zone 3 was able to accommodate small R^2 groups.[7b] In contrast, switching the sulfur atom from Y1 to Y2 in zone 2 led to a 5-fold drop in PTP4A3 inhibitory activity. Collectively, the data suggest that **1** is a robust pan-inhibitor of PTP4A with many analogs retaining nanomolar potency.

A recent report suggested that PTP4A1 was an ER stress-inducible gene product.[10] When a toxic ER insult is applied, PTP4A1 suppresses apoptosis, suggesting that the function of this phosphatase is connected to a mechanism that rescues cancer cells from death. Accordingly, we investigated if (1) PTP4A inhibitors were sufficient to induce ER stress,

* Corresponding author.
E-mail address: pwipf@pitt.edu (P. Wipf).

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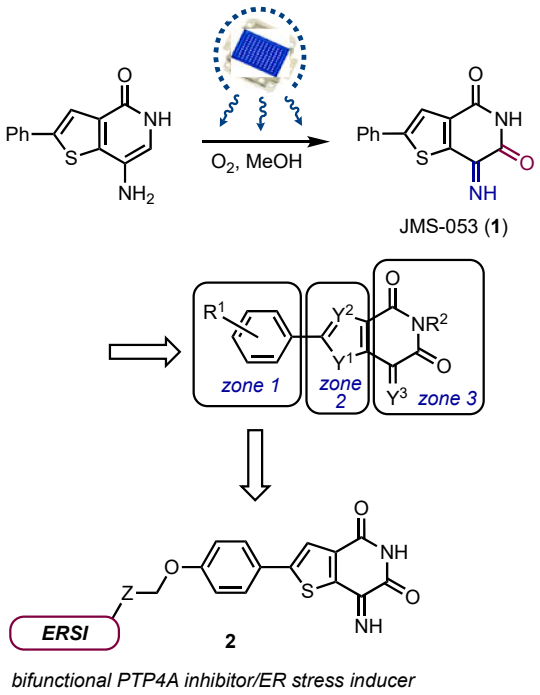


Fig. 1. From monofunctional PTP4A inhibitor chemotype to bifunctional ER stress inducer/phosphatase inhibitor.

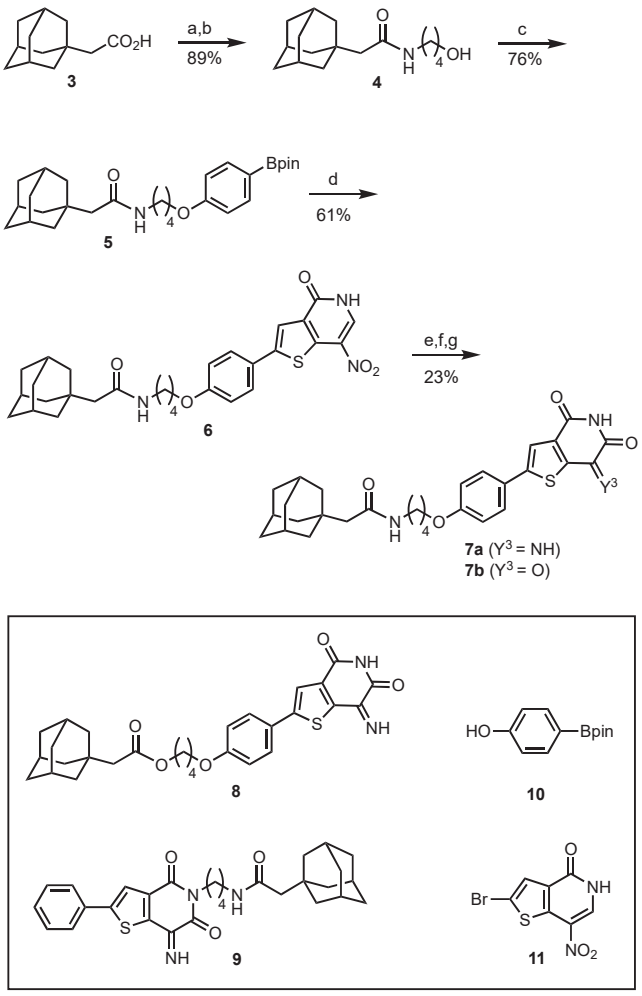
and (2) bifunctional molecules that combined a PTP4A inhibitor with an ER stress inducer showed enhanced potency in decreasing cancer cell viability. Therapeutic regimens are increasingly combining several active ingredients and bifunctional hybrid biomolecules such as the BiTEs (bispecific *T*-cell engagers, f. ex. blinatumomab), and some of the resulting compounds have been successfully translated into the clinic.[11] In contrast, traditional small molecule drug discovery efforts optimize drugs composed of a single pharmacophore.[12,13] By identifying and optimizing a dual-pathway agent at an early drug discovery stage that eliminates a compensatory mechanism for preventing cancer cell death, we hoped to generate a more effective drug lead for cancer therapy. Our SAR information on lead compound **1** suggested that attachment of an ER stress inducer (ERSI) at the *para*-alkoxy group in **2** was a feasible approach to generate an analog that retained potent PTP4A3 inhibitory activity (Fig. 1).

The highly lipophilic and chemically relatively inert adamantyl group has previously been found to induce acute ER stress resulting in transient UPR activation without direct induction of programmed cell death.[14] This approach engages the vast network of molecular chaperones responsible for maintaining protein homeostasis.[15] The adamantyl group has also been used to degrade endogenous oncogenic proteins, such as HER3[16] and AR,[17] by appending it onto known small molecule inhibitors of these targets.

Based on this precedence, we chose to employ the adamantyl moiety as the ERSI connected via a variable linker moiety "Z" to the core structure of JMS-053 (Fig. 1). The linker length and composition of **2** would likely affect protein binding, cell permeability and solubility characteristics,[18] and we therefore allowed for some variation at this site, which would also provide useful control compounds and shed light on the SAR in the hybrid series.

Our initial target molecule **7** contained an aryl ether-based linker (Scheme 1). Its synthesis commenced with an amide bond formation from commercially available adamantyl acetic acid **3**. This amide bond was found to be best constructed over a 2-step sequence where acid **3** was treated with neat $SOCl_2$ to generate the corresponding acid chloride followed by addition of 4-amino-1-butanol to give **4** in excellent yield.

A Mitsunobu reaction between **4** and phenol **10**[19] was used to form



Scheme 1. a) $SOCl_2$, reflux; b) 4-amino-1-butanol, Et_3N, CH_2Cl_2, 0 °C to rt; c) **10**, DIAD, PPh_3, THF, 0 °C to rt; d) **11**, $Pd(PPh_3)_4$, K_2CO_3, dioxane/H_2O (5:1), reflux; e) H_2, Pd/C, dioxane/MeOH (2:1); f) air, 425 nm-LED, MeOH, *in flow*; g) NH_4OAc, MeOH, 60 °C.

the aryl ether bond of **5**. While in our previous SAR studies Suzuki coupling conditions ($Pd(PPh_3)_4$ and Na_2CO_3 in dioxane/H_2O (2:1)) at reflux temperature were successful for the segment condensation between the phenyl ring and the thiophene,[7b] these conditions failed to effect coupling of **5** and **11**. After some experimentation, we found that **5** could be successfully attached to thiophene bromide **11** by replacing Na_2CO_3 with K_2CO_3 and increasing the dioxane/H_2O ratio to 5:1. These conditions led to the formation of **6** in good yield. It is noteworthy that during the workup of the Suzuki reaction the crude material needs to be acidified in order to protonate the highly acidic nitro-pyridone moiety in **6**, otherwise recovery of the product is low-yielding. Also, the Suzuki product **6** could be purified by a series of triturations, avoiding chromatography entirely.

The synthesis of the 4-amino-1-butanol linked conjugate **7** was completed using a 3-step sequence. Nitro compound **6** was first reduced to the corresponding amine by catalytic hydrogenation. The resulting enamine then underwent photooxygenation using our continuous-flow methodology.[7c] During the aminothienopyridone photooxygenation reaction, a trione byproduct **7b** is typically generated in varying amounts in addition to the major product, imine **7a**, in the ratio depending on the substitutions on the phenyl ring. Though some imine/trione mixtures of JMS-053 analogs can be separated chromatographically, complete purification of the imine/trione mixture **7a**,**b** was unsuccessful. Fortunately, an NH_4OAc imination reaction step converted the imine/trione mixture **7a**,**b** exclusively into imine **7a**. After filtering

the crude photooxygenation material through a short plug of SiO_2, the filtrate was stirred in a methanolic suspension of NH_4OAc in a sealed tube at 60 °C. After re-filtration through a bed of SiO_2, pure imine **7a** could be isolated in moderate yield over 3 steps.

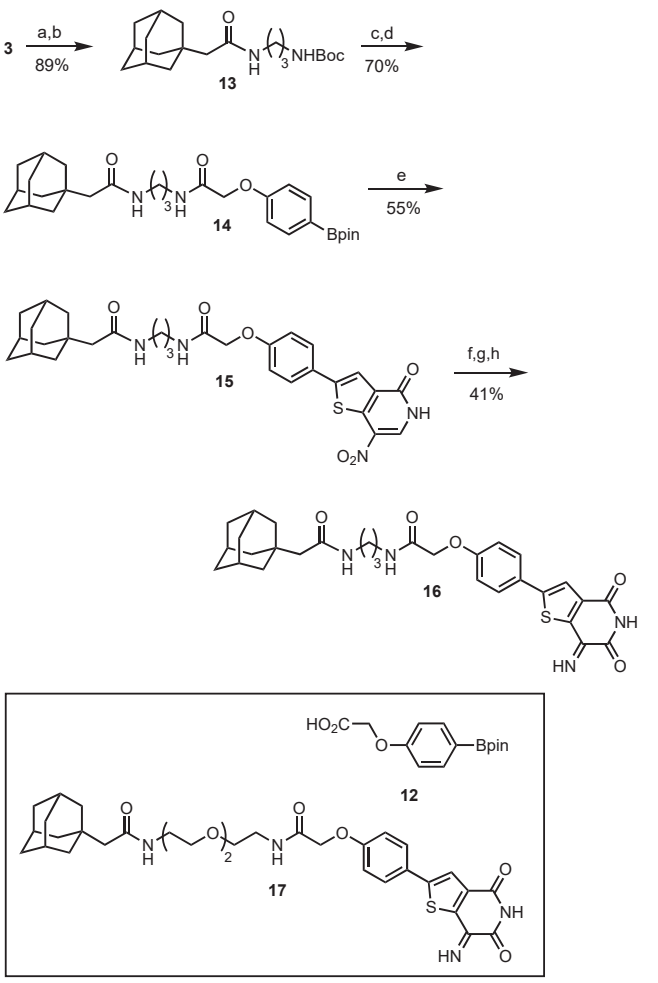
The corresponding ester analog (**8**) was prepared following a similar protocol but starting with a DCC-mediated esterification between **3** and 1,4-butanediol.[20] Notably, the synthesis of **8** did not require the NH_4OAc imination step, as chromatographic separation of the crude imine/trione mixture after the in-flow photooxygenation provided pure imine **8**. Additionally, a zone 3 *N*-alkylated analog (**9**) was synthesized in which the tethered adamantyl group was attached to the imide nitrogen of JMS-053.[20]

In addition to the ether linkages in **7** and **8**, we were also interested in conjugating JMS-053 with a more polar 2-hydroxyacetamide to the adamantane residue. This modification broadened the scope of our bifunctional analogs by: (1) allowing access to the wide variety of potential diamine linkers, and (2) avoiding the Mitsunobu reaction conditions which required multiple rounds of chromatography to purify the desired products from the excess of DIAD and its hydrazodicarboxylate byproduct. Therefore, phenol **10** was first alkylated with ethyl bromoacetate. While this reaction was high yielding, generating the carboxylic acid without hydrolyzing the boronic ester proved to be difficult. Instead, phenol **10** was alkylated with *tert*-butyl bromoacetate. The *tert*-butyl group was then cleaved by stirring in a solution of TFA in CH_2Cl_2, providing acid **12** in 71% yield over 2 steps.[20]

Starting with *N*-Boc protected 1,3-diaminopropane, amide coupling with the acid chloride derived from **3** provided compound **13** in excellent yield (Scheme 2). The Boc group in **13** was removed with TFA, and the resulting primary amine was acylated with acid **12**. Using our optimized Suzuki conditions, cross coupling of boronic ester **14** and thiophene bromide **11** cleanly generated intermediate **15** without the need for chromatography. Finally, nitro compound **15** was transformed into conjugate **16** by catalytic hydrogenation of the nitro group, in-flow photooxygenation of the resulting enamine, and imination of the imine/trione mixture with NH_4OAc. Following the same synthetic sequence, conjugate **17** was generated using 1,2-bis(2-aminoethoxy)ethane as the diamine starting material.

We next examined the bifunctional PTP4A3 inhibitors in a biochemical enzyme assay (Table 1). While less potent than the parent monofunctional compound, JMS-053 (Entry 7, IC_{50} = 84 nM) in these assays,[7b] the conjugates retained different abilities to inhibit PTP4A3 enzymatic activity *in vitro*, with 2-hydroxyacetamide analogs **16** and **17** (Entries 5 and 6, IC_{50} = 100–200 nM) showing substantially higher potency than the aliphatic ether linked analogs **7** and **8** (Entries 1–3, IC_{50} = 1–7 μM). These significant differences in potency support our assumption that linker length and composition are critical features of bifunctional conjugates. Interestingly, conjugate **7b** containing a trione motif instead of the imine functionality was ~ 6-fold less potent than **7a**. This result confirms that the imino-thienopyridinedione motif remains important for the PTP4A3 inhibitory interaction in the bifunctional compounds. In agreement with the data for JMS-053 analogs with large substituents at the imide nitrogen in zone 3,[7b] conjugate **9** also lost substantial inhibitory activity (Entry 4).

Although Ser/Thr kinases and phosphatases are more commonly associated with the UPR,[21] a mechanistic understanding of the links between PTPs and ER stress and cell death has also been emerging.[10] For example, PTP1B regulates the UPR,[22] and PTPN2 mediates ER stress response-dependent neuronal damage.[23] While the adamantyl moiety in bifunctional compounds should induce a basal level of ER stress, we were most interested in PTP4A inhibitors that potentiated this effect. Therefore, we evaluated the ability of the most active bifunctional inhibitors **7a**, **16**, and **17** to induce ER stress, as well as diminish cancer cell viability. Dithiothreitol (DTT), a potent UPR activator, served as a positive control in the ER stress measurements (Fig. 2). Notably, compound **7a**-dependent induction of the canonical UPR reporter, BiP (HSPS5A),[24] along with a moderate accumulation of the spliced Xbp1



Scheme 2. a) $SOCl_2$, reflux; b) *N*-Boc-1,3-diaminopropane, Et_3N, CH_2Cl_2, 0 °C to rt; c) TFA/CH_2Cl_2, 50 °C; d) **12,** HATU, *i*-Pr_2NEt, THF, 0 °C to rt; e) **11**, Pd$(PPh_3)_4$, K_2CO_3, dioxane/H_2O (5:1), reflux; f) H_2, Pd/C, dioxane/MeOH (2:1); g) air, 425 nm-LED, MeOH, *in flow*; h) NH_4OAc, MeOH, 60 °C.

Table 1
In vitro inhibition of PTP4A3 phosphatase activity.[a]

Entry	Compound	IC_{50} (μM)	±SEM	*N*
1	**7a** (EJR-876-35)	1.16	0.51	6
2	**7b** (EJR-925-45)	6.59	1.99	3
3	**8** (EJR-876-34)	4.98	0.31	3
4	**9** (EJR-980-67)	1.92		1
5	**16** (EJR-887-24)	0.206	0.011	3
6	**17** (EJR-887-35)	0.107	0.016	3
7	**1** (JMS-053)	0.084	0.041	6

[a] Recombinant human PTP4A3 phosphatase was used and the enzymatic assay was performed as previously described.[7] See SI for additional details.

transcription factor that initiates BiP synthesis[25] in MDA-MB-231 breast cancer cells is consistent with known links between PTP inhibition and cancer cell death (Fig. 2).[26] In fact, MDA-MB-231 cells also express high levels of p53,[27] a key survival enzyme which in turn regulates PTP4A3 levels.[28] BiP induction, concomitant with a strongly enhanced expression of the pro-apoptotic gene, CHOP (DDIT3),[29] provides a mechanism to explain the prominent effect of **7a** on MDA-MB-231 viability (Fig. 3 and Table 2, Entry 3; EC_{50} ~ 8.6 μM). Interestingly, analog **7a** was significantly more potent in these cell-based assays than **16** and **17**, even though it was 6–10 times less potent in the biochemical PTP4A3 enzyme assay. The monofunctional adamantyl linker **4** served as a negative

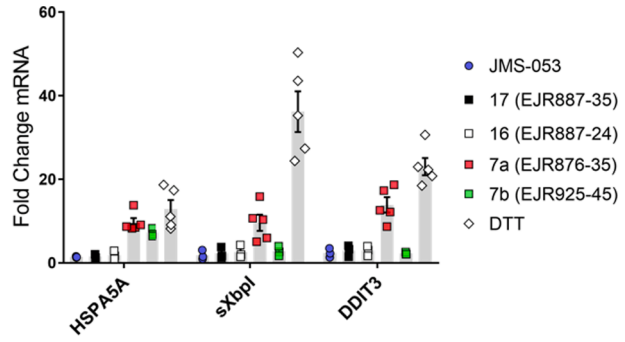


Fig. 2. Induction of genes corresponding to UPR and ER stress pathway enzymes by JMS-053 (**1**), **7a** (EJR-876-35), **7b** (EJR-925-45), **16** (EJR-887-24), **17** (EJR-887-35) and DTT. UPR induction experiments were performed by qPCR. MDA-MB-231 cells were treated for 6 h with complete media containing 2 times the EC_{50} calculated using the cell viability assay (see Table 2 and SI). A 2-h treatment with 2 mM DTT was used as a positive control for UPR induction. The corresponding fold increase of the indicated UPR markers relative to the DMSO control are plotted, +/-SEM. N = 3. sXbpl = spliced/activated Xbpl; HSPA5A = gene encoding BiP; DDIT3 = gene encoding CHOP.

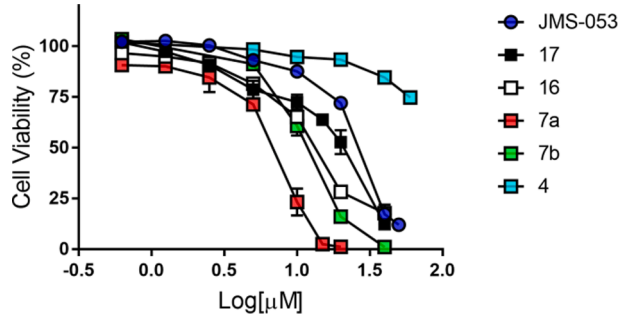


Fig. 3. Cancer cell viability assay with JMS-053 (1), adamantyl linker **4** (negative control), **7a** (EJR-876-35), **7b** (EJR-925-45), **16** (EJR-887-24), and **17** (EJR-887-35). A total of 3000 MDA-MB-231 cells/well were seeded and subjected to drug treatment for 72 h. The CellTiter Glo assay was used to measure cell viability and EC_{50} values were generate using a sigmoidal nonlinear regression in PRISM software. Cell viability data represent the average of 2 independent experiments, +/-SEM.

Table 2
Inhibitory effects of JMS-053 (**1**), adamantyl linker **4** (negative control), **7a** (EJR-876-35), **7b** (EJR-925-45), **16** (EJR-887-24), and **17** (EJR-887-35) on MDA-MB-231 cancer cell growth.[a]

Entry	Compound	EC_{50} (μM)	±SD
1	**1** (JMS-053)	42.7	3.0
2	**4**	>50	–
3	**7a** (EJR-876-35)	8.6	1.1
4	**7b** (EJR-925-45)	11.5	0.9
5	**16** (EJR-887-24)	11.8	1.1
6	**17** (EJR-887-35)	31.6	1.3

[a] N = 2.

control in the cancer viability assay and indeed had negligible inhibitory effects on cell growth (Fig. 3 and Table 2, Entry 2). Similarly, the monofunctional parent PTP4A3 inhibitor **1** (JMS-053) was approximately equipotent to **17** in the MDA-MB-231 cell viability assay (Table 2, Entry 1, $IC_{50} \sim 42.7$ μM), but 4–5 times less potent than **16** and **7a**, in spite of its superior (2–13 times more potent, respectively) inhibition of PTP4A3. We also evaluated trione **7b** in these assays. While the MDA-MB-231 growth inhibitory effects and UPR/ER stress pathway gene patterns were similar to compound **16**, it was not as potent as imine **7a** and produced a significantly lower accumulation of the spliced Xbp1 transcription factor gene and CHOP than **7a** (Figs. 2 and 3, and Table 2, Entry 4). These effects might be due to its ca. 6-fold diminished ability to inhibit PTP4A3.

We also investigated a possible additive or synergistic effect between JMS-053 and adamantyl linker **4**, but failed to detect any changes in viability upon addition of 20–60 μM **4** to MDA-MB-231 cells treated with JMS-053.[20] Previously, JMS-053 proved to be selective for PTP4A1-3 in a panel of 25 other phosphatases and 50 kinases.[30] Similarly, compound **7a** demonstrated < 11% inhibition at 1 μM concentration for three closely related phosphatases, PTP1B, DUSP3, and CDC25B.[20] Overall, these results suggest that it is the *combined* action of the adamantyl group in covalent conjugation with the PTP4A3 inhibitory heterocycle that is responsible for the activation of ER stress responsive genes and the suppression of MDA-MB-231 breast cancer cell growth. The differences among the bifunctional analogs **7a**, **16**, and **17** further suggest that the linker moiety plays an important role in the biological properties of these hybrid molecules.

In conclusion, we have designed a set of bifunctional JMS-053 conjugates that *inhibit* the dual specificity phosphatase PTP4A3 enzymatically as well as *activate* the ER stress-induced UPR pathway. These conjugates utilize a highly hydrophobic adamantyl moiety linked to the PTP4A3 inhibitory scaffold at a site that was identified by a prior SAR campaign to be relatively tolerant to chemical modification. Both the site of attachment as well as the length and functionality of the linker moiety determined biological outcomes. While several of the newly synthesized conjugates show low nanomolar IC_{50} values for inhibition of PTP4A3, the slightly less potent analog **7a** was superior to derivatives with alternate linker moieties, i.e. **16** and **17**, in inducing expression of the ER stress response genes HSPA5A, sXbpl, and DDIT3, resulting in the most potent growth inhibitory effect in MDA-MB-231 breast cancer cell viability assays. In contrast, the parent monofunctional PTP4A3 inhibitor, JMS-053 (**1**), did not appear to cause ER stress or activate the UPR and had the least effect on cell growth. In conclusion, these results highlight the promise of identifying dual-pathway agents at an early drug discovery stage that are able to modulate compensatory pathways used by cancer cells to bypass apoptosis.[31]

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: EJR, ERS, JSL and PW are co-inventors of patents on the composition of matter and the use of 7-iminothieno[3,2-*c*]pyridine-4,6(5*H*,7*H*)-diones and related compounds, filed and held by the University of Pittsburgh and the University of Virginia and optioned by KeViRx.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128167.

References

1 Hunter T. The Genesis of Tyrosine Phosphorylation. *Cold Spring Harb Perspect Biol.* 2014;6, a020644.
2 a) Lazo JS, Sharlow ER. "Drugging Undruggable Molecular Cancer Targets. *Annu Rev Pharmacol Tox.* 2016;56:23–40.b)Lazo, J. S.; McQueeney, K. E.; Sharlow, E. R. "New Approaches to Difficult Drug Targets: The Phosphatase Story." SLAS

DISCOVERY: Advanc. Life Sci. R&D 2017, 22, 1071–1083.c) Zhang Z-Y. Drugging the undruggable: therapeutic potential of targeting protein tyrosine phosphatases. *Acc Chem Res*. 2017;50:122–129.
3 Grandjean JMD, Wiseman RL. Small molecule strategies to harness the unfolded protein response: where do we go from here? *J Biol Chem*. 2020;295:15692–15711.
4 Li A, Song N-J, Riesenberg BP, Li Z. The emerging roles of endoplasmic reticulum stress in balancing immunity and tolerance in health and diseases: mechanisms and opportunities. *Front Immunol*. 2020;10:3154.
5 Sannino S, Guerriero CJ, Sabnis AJ, et al. Compensatory increases of select proteostasis networks after Hsp70 inhibition in cancer cells. *J Cell Sci*. 2018;131: jcs217760.
6 Terrab L, Wipf P. Hsp70 and the unfolded protein response as a challenging drug target and an inspiration for probe molecule development. *ACS Med Chem Lett*. 2020; 11:232–236.
7 a) Salamoun JM, McQueeney KE, Patil K, et al. Photooxygenation of an amino-thienopyridone yields a more potent PTP4A3 inhibitor. *Org Biomol Chem*. 2016;14: 6398–6402.b) Tasker NR, Rastelli EJ, Blanco IK, et al. In-flow photooxygenation of aminothienopyridinones generates iminopyridinedione PTP4A3 phosphatase inhibitors. *Org Biomol Chem*. 2019;17:2448–2466.c) Rastelli EJ, Yue D, Millard C, Wipf P. 3D-printed cartridge system for in-flow photo-oxygenation of 7-aminothienopyridinones. *Tetrahedron*. 2021;79, 131875.
8 Wei M, Korotkov KV, Blackburn JS. Targeting phosphatases of regenerating liver (PRLs) in Cancer. *Pharmacol Ther*. 2018;190:128–138.
9 For an overview of known PTP4A inhibitors see: Tasker NR, Rastelli EJ, Burnett JC, Sharlow ER, Lazo JS, Wipf P. Tapping the therapeutic potential of protein tyrosine phosphatase 4A with small molecule inhibitors *Bioorg Med Chem Lett*. 2019;29: 2008–2015.
10 Suzuki S, Tsutsumi S, Chen Y, et al. Identification and characterization of the binding sequences and target genes of p53 lacking the 1st transactivation domain. *Cancer Sci*. 2020;111:451–466.
11 Wu J, Fu J, Zhang M, Liu D. Blinatumomab: a bispecific T cell engager (BiTE) antibody against Cd19/Cd3 for refractory acute lymphoid leukemia. *J Hematol Oncol*. 2015;8:104.
12 Keith CT, Borisy AA, Stockwell BR. Innovation: multicomponent therapeutics for networked systems. *Nat Rev Drug Disc*. 2005;4:71–78.
13 Wild M, Kicuntod J, Seyler L, et al. Combinatorial drug treatments reveal promising anticytomegaloviral profiles for clinically relevant pharmaceutical kinase inhibitors (PKIs). *Int J Mol Sci*. 2021;22:575.
14 Raina K, Noblin DJ, Serebrenik YV, Adams A, Zhao C, Crews CM. Targeted protein destabilization reveals an estrogen-mediated ER stress response. *Nat Chem Biol*. 2014; 10:957–962.
15 a) Neklesa TK, Tae HS, Schneekloth AR, et al. Small-molecule hydrophobic tagging-induced degradation of HaloTag fusion proteins. *Nat Chem Biol*. 2011;7:538–543.b) Tae HS, Sundberg TB, Neklesa TK, et al. Identification of hydrophobic tags for the degradation of stabilized proteins. *ChemBioChem*. 2012;13:538–541.
16 a) Xie T, Lim SM, Westover KD, et al. Pharmacological targeting of the pseudokinase Her3. *Nat Chem Biol*. 2014;10:1006–1012.b) Lim SM, Xie T, Westover KD, et al. Development of small molecules targeting the pseudokinase Her3. *Bioorg Med Chem Lett*. 2015;25:3382–3389.
17 Gustafson JL, Neklesa TK, Cox CS, et al. Small-molecule-mediated degradation of the androgen receptor through hydrophobic tagging. *Angew Chem Int Ed*. 2015;54: 9659–9662.
18 Atilaw Y, Poongavanam V, Svensson Nilsson C, et al. Solution conformations shed light on protac cell permeability. *ACS Med Chem Lett*. 2021;12:107–114.
19 Hoeksta, W. J.; Yates, C. M.; Rafferty, S. W. WO2014/117090, 2014.
20 See SI for details.
21 Ruvolo PP. Role of protein phosphatases in the cancer microenvironment. *Biochim Biophys Acta Mol Cell Res*. 2019;1866:144–152.
22 Gu F, Nguyên DT, Stuible M, Dubé N, Tremblay ML, Chevet E. Protein-tyrosine phosphatase 1B potentiates IRE1 signaling during endoplasmic reticulum stress. *J Biol Chem*. 2004;279:49689–49693.
23 Yoshikawa A, Kamide T, Hashida K, et al. Deletion of Atf6α impairs astroglial activation and enhances neuronal death following brain ischemia in mice. *J Neurochem*. 2015;132:342–353.
24 Mori K, Sant A, Kohno K, Normington K, Gething MJ, Sambrook JF. A 22 Bp Cis-acting element is necessary and sufficient for the induction of the yeast Kar2 (BiP) Gene by unfolded proteins. *EMBO J*. 1992;11:2583–2593.
25 Lee A-H, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol*. 2003;23:7448–7459.
26 Lazo JS, McQueeney KE, Burnett JC, Wipf P, Sharlow ER. Small molecule targeting of PTPs in Cancer. *Int J Biochem Cell Biol*. 2018;96:171–181.
27 Hui L, Zheng Y, Yan Y, Bargonetti J, Foster DA. Mutant p53 in MDA-MB-231 breast cancer cells is stabilized by elevated phospholipase D activity and contributes to survival signals generated by phospholipase D. *Oncogene*. 2006;25:7305–7310.
28 Basak S, Jacobs SBR, Krieg AJ, et al. The metastasis-associated gene Prl-3 is a p53 target involved in cell-cycle regulation. *Mol Cell*. 2008;30:303–314.
29 Harding HP, Novoa I, Zhang Y, et al. Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell*. 2000;6:1099–1108.
30 McQueeney KE, Salamoun JM, Burnett JC, et al. Targeting ovarian cancer and endothelium with an allosteric PTP4A3 phosphatase inhibitor. *Oncotarget*. 2018;9: 8223–8240.
31 See also: a) Singh, A.; Vashistha, N.; Heck, J.; Tang, X.; Wipf, P.; Brodsky, J. L.; Hampton, R. Y.; Direct involvement of Hsp70 ATP hydrolysis in Ubr1-dependent quality control. *Mol Biol Cell*. 2020, 31, 2669–2686. b) Sabnis, A. J.; Guerriero, C. J.; Olivas, V.; Sayana, A.; Shue, J.; Flanagan, J.; Asthana, S.; Paton, A. W.; Paton, J. C.; Gestwicki, J. E.; Walter, P.; Weissman, J. S.; Wipf, P.; Brodsky, J. L.; Bivona, T. G. Combined chemical-genetic approach identifies cytosolic Hsp70 dependence in rhabdomyosarcoma. *Proc Natl Acad Sci USA*. 2016, 113, 9015–9020.