# Selective Small Molecule Inhibition of Poly(ADP-Ribose) Glycohydrolase (PARG)

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Ⓢ Supporting Information

ABSTRACT: The poly(ADP-ribose) (PAR) post-translational modification is essential for diverse cellular functions, including regulation of transcription, response to DNA damage, and mitosis. Cellular PAR is predominantly synthesized by the enzyme poly(ADP-ribose) polymerase-1 (PARP-1). PARP-1 is a critical node in the DNA damage response pathway, and multiple potent PARP-1 inhibitors have been described, some of which show considerable promise in the clinic for the treatment of certain cancers. Cellular PAR is efficiently degraded by poly(ADP-ribose) glycohydrolase (PARG), an enzyme for which no potent, readily accessible, and specific inhibitors exist. Herein we report the discovery of small molecules that effectively inhibit PARG *in vitro* and in cellular lysates. These potent PARG inhibitors can be produced in two chemical steps from commercial starting materials and have complete specificity for PARG over the other known PAR glycohydrolase (ADP-ribosylhydrolase 3, ARH3) and over PARP-1 and thus will be useful tools for studying the biochemistry of PAR signaling.
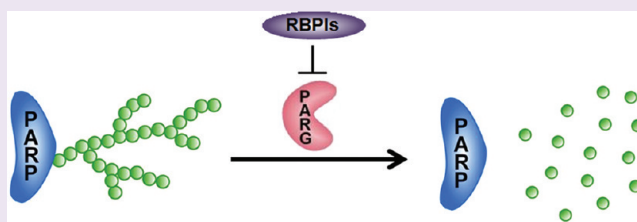
 Poly(ADP-ribosylation) is a post-translational modification critical to many cellular events, including DNA damage repair, transcription, RNA metabolism, and telomere function.[1,2] The poly(ADP-ribose) polymerase (PARP) family of enzymes, most notably PARP-1, use β-NAD$^+$ in the synthesis of poly(ADP-ribose) (PAR), a negatively charged branched biopolymer of varying lengths, onto multiple acceptor proteins (*e.g.*, histones, transcription factors, and PARP-1 itself).[1,3] The presence of PAR is transient due to the high specific activity of poly(ADP-ribose) glycohydrolase (PARG), the main enzyme involved in the degradation of PAR. PARG catalyzes the hydrolysis of the ribosyl-ribose bond of PAR in both endo- and exoglycosidic fashions, producing ADP-ribose monomers and shorter PAR chains.[4,5]

The PARP enzymes are emerging as targets for the treatments of various diseases; for example, PARP-1 inhibitors have shown promise in anticancer clinical trials.[6,7] PARG inhibitors also have potential as therapeutic agents, as PARG activity plays a key role in cellular response to insult and in the initiation of cell death.[8,9] PARG is an attractive pharmacological target due to its low cellular abundance (approximately 2,000 molecules per cell[10]) and conserved catalytic domain, as all four PARG isoforms are encoded by a single gene.[11] A number of biochemical studies have investigated the consequences of loss of PARG function through knockdown and isoform-specific knockout. In general, cells with genetic depletion or RNAi silencing of PARG were protected from H_2O_2-induced cell death[12] and had increased susceptibility to radiation,[13,14] DNA-alkylating agents,[15] and chemotherapeutics such as cisplatin and epirubicin.[16] Additionally, apoptosis inducing factor (AIF) mediated cell death is specifically activated after ultraviolet treatment of PARG-null cells.[9] Thus, inhibition of PARG may

be a viable strategy for cancer treatment, and given the embryonic lethality of PARG knockouts in mice,[15] selective small molecule inhibitors of PARG would greatly aid in the interrogation of this interesting biological target.

Unfortunately, the lack of potent, specific, and easily synthesized small molecule inhibitors of PARG has limited the study of PARG's function both *in vitro* and *in vivo*. PARG inhibitors can be grouped into three major categories: DNA intercalators, tannins, and ADP-ribose analogues.[17,18] However, the usefulness of the first two categories has been limited due to toxicity, lack of specificity, and high molecular weights.[18,19] Adenosine diphosphate (hydroxymethyl)pyrrolidinediol (ADP-HPD), an analogue of the ADP-ribose product of the PARG-catalyzed reaction, is the most potent (IC$_{50}$ = 120 nM) and widely used PARG inhibitor.[20] Despite its expense ($2600/mg), lengthy synthetic route (10 steps, multiple anion-exchange chromatography columns),[21,22] lack of cell permeability,[21,23] and partial inhibition of PARG,[20] ADP-HPD has found wide utility in the study of PAR. It has been used *in vitro* and in cell extracts to study PARG's structure and function,[24,25] as an additive in Western blot analysis to evaluate PAR accumulation,[26,27] for the analysis of PAR levels in nuclear extracts,[28] the measurement of PARP activity in permeabilized cells,[29,30] and for the study of PAR's role in spindle assembly,[31] tankyrase-1 activity,[32] and the Sir2 family of proteins.[33] Described herein is the identification of a potent, specific, and easily synthesized class of small-molecule PARG inhibitors,

compounds that will further facilitate the study of the biological properties of PARG and PAR.

RESULTS AND DISCUSSION

The pyrophosphate moiety contained within PAR and ADP-HPD appears to play a key role in binding to PARG, as suggested by a recent X-ray structure.[34] As this functional group can limit the stability of potential inhibitors and complicates synthetic routes, we sought to evaluate the PARG inhibition properties of compounds with functional groups known to inhibit enzymes with phosphate-containing substrates.[35] The rhodanine scaffold, a five-membered heterocycle, is present in potent inhibitors of phosphodiesterase type 4 enzymes,[36] serotonin N-acetyltransferases,[37] UDP-galactopyranose mutase,[38] and the glycosyl transferase MurG,[39] all of which bind to substrates containing phosphate groups. As rhodanine has been suggested as a phosphate surrogate,[35,40] we chose to evaluate rhodanine-based small molecules as inhibitors of PARG.

Screening and Lead Optimization. From an in-house collection of ~14,000 small molecules, 224 rhodanine-containing compounds were selected and screened for their ability to inhibit PARG in vitro at 200 μM. PARG enzymatic activity was evaluated by incubation of compounds with PARG for 10 min, followed by incubation with ^{32}P-PAR for 2 h in order to identify compounds capable of prolonged PARG inhibition. Upon separation of intact ^{32}P-PAR from ^{32}P-ADP-ribose by thin-layer chromatography (TLC), the radiolabeled assay components were detected by phosphorimaging. Compounds that prevented PARG-mediated degradation of ^{32}P-PAR were named rhodanine-based PARG inhibitors (**RBPIs**). We identified 16 primary hit compounds, and **RBPI-1** (Figure 1), the most potent PARG inhibitor identified from this initial screen (IC$_{50}$ = 46.5 ± 6.0 μM, Supplementary Figure S1), was chosen for further optimization.

A library of **RBPI-1** derivatives was readily produced using a two-step synthesis from commercial starting materials. Isatins (**1**, Supplementary Figure S2) were N-alkylated with benzyl halides to provide the desired intermediates (**2**, Supplementary Figure S2). Subsequent Knoevenagel condensation with N-substituted rhodanines with varying methylene chains produced 76 final compounds (Supplementary Figures S2 and S3), which were isolated by filtration in high purity (see characterization data in Supporting Information). All 76 compounds were then evaluated at 10 μM for their ability to inhibit PARG in vitro by the TLC assay described previously, and many compounds were also evaluated at 50 μM (Supplementary Figures S4–S6). Dose-response curves were generated for several compounds, including **RBPI-2**, -**3**, and -**4** (Figure 1), all containing dihalogen substitution at the ortho positions on the benzyl group. These compounds were found to inhibit PARG with low micromolar IC$_{50}$ values (2.9 ± 1.8, 5.8 ± 1.0, and 3.0 ± 1.6 μM, respectively, Figure 1 and Supplementary Figure S7).

In general, substitution at the 5-position of the isatin moiety with either bromine or chlorine and 2,6-dihalide substitution on the benzyl ring improves activity, while compounds lacking the benzyl ring are not active as PARG inhibitors (Supplementary Figure S6). Additionally, the length of the methylene linker from the rhodanine nitrogen to the carboxylic acid affects activity, with two methylenes giving the best specificity and potency (Supplementary Figure S6). Since tetrazoles are a known isostere for carboxylic acids,[41] the tetrazole-containing analogues of select compounds were prepared. Tetrazole-
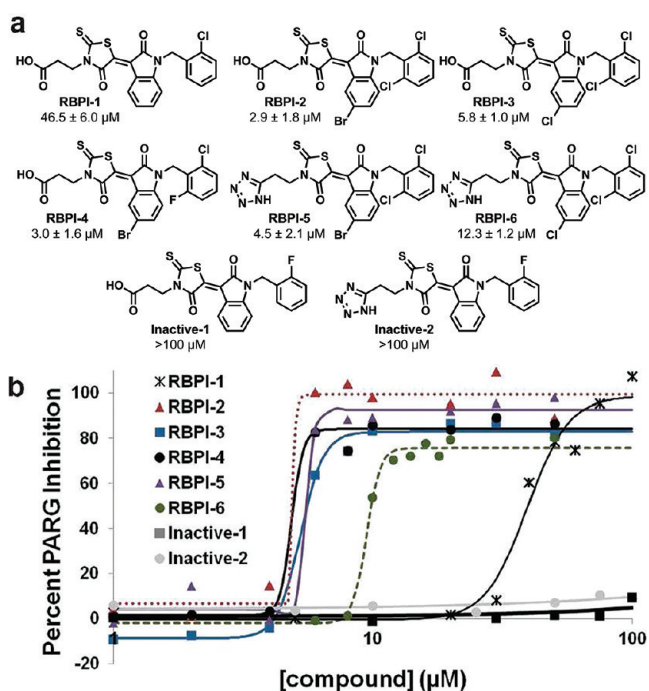

Figure 1. (a) Screening of 224 rhodanine-containing compounds reveals **RBPI-1** as a PARG inhibitor, and a collection of >70 compounds were then synthesized based on this scaffold and evaluated for PARG inhibition. From this work **RBPI-2**, -**3**, -**4**, -**5**, and -**6** were identified as potent PARG inhibitors. **Inactive-1** and -**2** are structurally related compounds that do not inhibit PARG. (b) Representative dose–response PARG inhibition curves for each compound shown in panel a. For IC$_{50}$ determination, compounds were incubated with PARG for 10 min, and then ^{32}P-PAR was added and incubated for 2 h at 37 °C, at which point the extent of PAR degradation was assessed by separation on TLC plates and phosphorimaging. For TLC images and triplicate IC$_{50}$ curves, please see Supplementary Figure S7.

containing compounds **RBPI-5** and -**6** inhibit PARG at slightly reduced potencies (Figure 1 and Supplementary Figure S7) compared to their carboxylic acid counterparts, **RBPI-2** and -**3**. Due to its reduced solubility relative to the other **RBPIs**, **RBPI-2** was not used in further evaluations. Structurally related inactive derivatives (**Inactive-1**, and -**2**, Figure 1) were used as controls in subsequent experiments; these compounds have IC$_{50}$ values greater than 100 μM for PARG inhibition (Figure 1 and Supplementary Figure S8).

RBPIs Are Specific, Nonpromiscuous PARG Inhibitors. Many enzyme inhibitors that display excellent target specificity and activity in cell culture[42−46] and in vivo[47−49] have been developed with the rhodanine functional group. Additionally, the rhodanine-containing drug epalrestat, an aldose reductase inhibitor approved for use in Japan, is effective and safe even for long-term use.[50,51] Despite these successes, rhodanines are sometimes removed from screening libraries due to interference with colorimetric read-outs or potential reactivity and the inherent lipophilicity of rhodanines could make them susceptible to the formation of colloidal aggregates in buffered solution.[52] Therefore, to rule out a promiscuous aggregation-based mode of enzyme inhibition,[53−55] the **RBPIs** were analyzed in a series of enzymatic assays involving the addition of detergent, bovine serum albumin, and evaluation versus an unrelated enzyme.

Four of the potent PARG inhibitors (**RBPI-3**, -**4**, -**5**, -**6**) were tested for their ability to inhibit PARG in vitro in the presence

or absence of detergent (Triton-X 100), which was used at a concentration (0.1%) high enough to disintegrate even "detergent-hardy" aggregators.[56] Inhibition of PARG by these four **RBPI**s at 50 μM was not altered by the addition of detergent (Figure 2). In contrast, inhibition of PARG by the
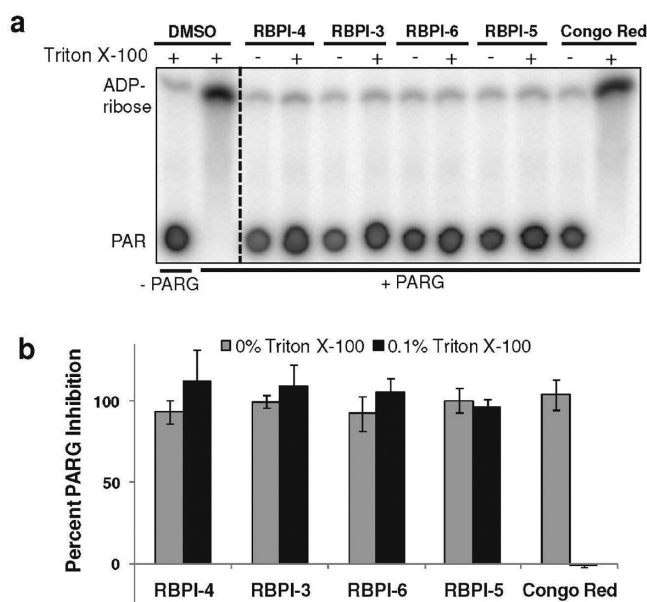

Figure 2. RBPI inhibition of PARG is insensitive to the presence of detergent. Compounds (50 μM) were incubated with PARG in the presence or absence of 0.1% Triton X-100 for 10 min, ^{32}P-PAR was added and incubated for 2 h at 37 °C, and the extent of PAR degradation was assessed by separation on TLC plates and phosphorimaging. (a) Representative TLC plate from this experiment. (b) Quantitation of the experiment described above by densitometry. $n = 3$, error bars indicate standard error of the mean.

known aggregating compound Congo Red was detergent-sensitive, with complete loss of inhibition observed in the presence of detergent (Figure 2).

As another test for aggregation-based inhibition, **RBPI-4** was tested for activity in the presence of a high concentration of bovine serum albumin (BSA, 0.1 mg mL^{-1}), which binds nonspecifically to small molecule aggregates; such BSA assays have previously been used to identify inhibitors acting through nonspecific mechanisms.[57] Consistent with the detergent results, **RBPI-4** retained activity in the presence of BSA (Supplementary Figure S9).

The rhodanine-containing compounds were also tested against β-lactamase to assess enzyme specificity; β-lactamase inhibition is commonly used to evaluate compound promiscuity.[58] Briefly, **RBPI-3, -4, -5, -6** and Congo Red were incubated with β-lactamase in a phosphate buffer for 0.5 h, followed by the addition of β-lactam substrate CENTA, and β-lactamase activity was measured by monitoring the appearance of the cleavage product at 405 nm. None of the rhodanine-containing compounds showed appreciable inhibition of β-lactamase enzymatic activity at concentrations of 5–50 μM (all <10% inhibition, Figure 3A). In contrast, Congo Red inhibited β-lactamase in a dose-dependent manner, with 100% inhibition at 50 μM (Figure 3A). Inhibition of β-lactamase was also evaluated in the presence of detergent, as a further test for aggregation-based inhibition. In the presence of detergent, **RBPI**s remained inactive against β-lactamase, whereas the
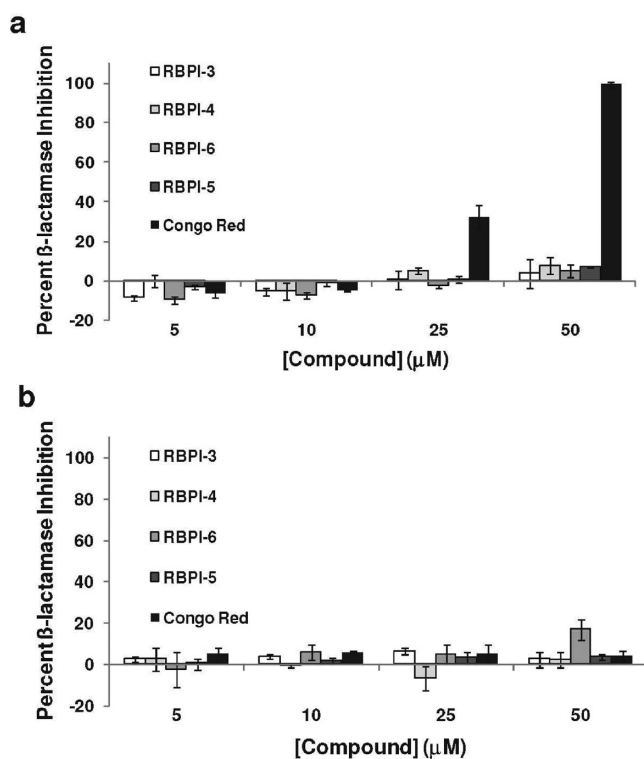

Figure 3. RBPIs do not inhibit β-lactamase. RBPIs and Congo Red (5–50 μM) were incubated with β-lactamase (a) in the absence of detergent and (b) in the presence of 0.1% Triton X-100. After addition of the colorimetric substrate, enzyme activity was measured by recording absorbance at 405 nm. $n = 3$, error bars indicate standard error of the mean.

addition of detergent eliminated the ability of Congo Red to inhibit the enzyme (<10% inhibition, Figure 3B).

These collective results, which show that inhibition of PARG by **RBPI**s is not sensitive to addition of detergent or BSA and that the **RBPI**s do not inhibit β-lactamase, indicate that these compounds do not inhibit PARG *via* a promiscuous, aggregation-based mechanism.

RBPIs Inhibit PARG with High Efficiency. The time- and dose-dependence of PARG inhibition by the **RBPI**s was directly compared to ADP-HPD. PARG was treated with increasing concentrations of **RBPI-3**, **RBPI-6**, or ADP-HPD (0.5–25 μM), and the processing of ^{32}P-PAR was evaluated after 0.5 and 2 h. Although ADP-HPD concentrations as low as 0.5 μM partially inhibited PARG at 0.5 h, inhibition was incomplete even in the presence of 10 and 25 μM ADP-HPD (67% inhibition at 25 μM, Figure 4A and C). In contrast, 10 μM **RBPI-3** and **RBPI-6** showed complete inhibition of PARG after 0.5 h (>99% inhibition at 25 μM, Figure 4A and C). When ^{32}P-PAR and PARG were incubated in the presence of compound for 2 h, ADP-HPD showed only minimal PARG inhibition; for example, PARG was able to almost completely process PAR into ADP-ribose in the presence of 25 μM ADP-HPD (Figure 4B and C).

The partial inhibition of PARG by ADP-HPD described in the literature[20] is consistent with our results in which ADP-HPD is able to retard but not completely prevent the degradation of PAR over 2 h (Figure 4). In contrast, under the same 2 h conditions, **RBPI-3** and **RBPI-6** prevented PAR degradation with only a slight shift in inhibition at 10 μM relative to the 0.5 h incubation (Figure 4A–C). Overall, these
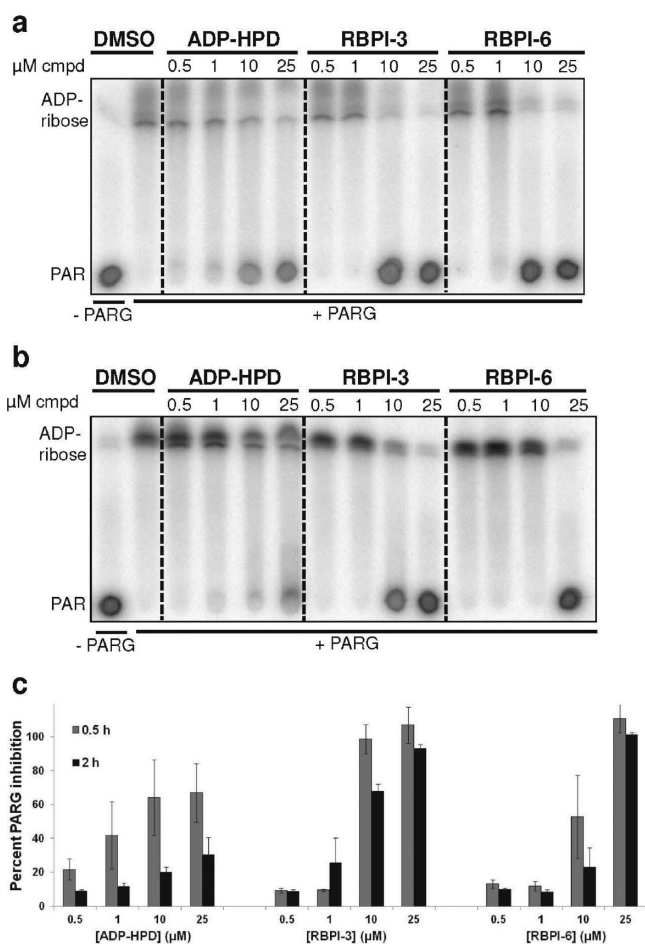
Figure 4. Inhibition of PARG by **RBPIs** persists even after 2 h incubation with substrate. **RBPI-3**, **-6**, and ADP-HPD were incubated with PARG for 10 min, and then ^{32}P-PAR was added and incubated at 37 °C for (a) 0.5 h or (b) 2 h. The extent of PAR degradation was assessed by separation on TLC plates and phosphorimaging. (c) Quantitation of data shown in panels a and b. $n = 3$, error bars indicate standard error of the mean.

RBPIs were able to inhibit PARG more efficiently than ADP-HPD at prolonged incubation times. The long-lasting inhibition of PARG by **RBPIs**, coupled with their ease of synthesis suggests that the **RBPIs** are more tractable compounds for *in vitro* use.

RBPIs Do Not Inhibit ARH3 or PARP-1. The specificity of the **RBPIs** and ADP-HPD for PARG was further evaluated by testing these compounds against the other known PAR glycohydrolase enzyme, ADP-ribosylhydrolase 3 (ARH3), and against PARP-1. ARH3 is a 39 kDa human protein known to have two enzymatic functions: deacetylation of *O*-acetyl ADP-ribose and PAR degradation, though deacetylation has been suggested as its main function.[59−62] Although both enzymes can process PAR, ARH3 does not have a sequence or structure similar to that of PARG.[34] To achieve a comparable rate of PAR degradation in these experiments, 1000-fold more ARH3 (256 nM) compared to PARG (0.24 nM) was required in the *in vitro* assay, along with the addition of 4 mM $MgCl_2$. Upon incubating **RBPI-3**, **-4**, **-5**, **-6** and **Inactive-1** and **-2** (25 μM) with ARH3 for 2 h, none of the rhodanine-containing compounds prevented ARH3-mediated degradation of ^{32}P-PAR (<10% inhibition, Figure 5A), although the **RBPIs** inhibited PARG at the same concentration (>70% inhibition,
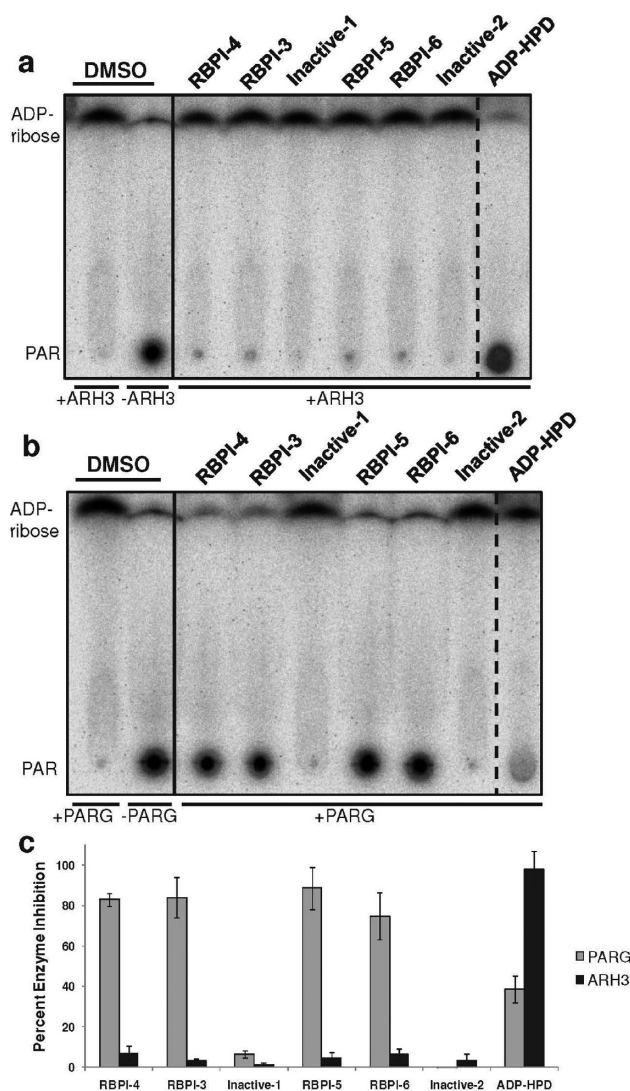

Figure 5. ADP-HPD, but not **RBPIs**, inhibits ARH3 PAR glycohydrolase activity. Compounds (25 μM) were incubated with (a) ARH3 (256 nM) or (b) PARG (0.24 nM) for 10 min, and then ^{32}P-PAR was added and incubated at 37 °C for 2 h. The extent of PAR degradation was visualized by separation on a TLC plate and phosphorimaging. (c) Quantitation of data from panels a and b. $n = 3$, error bars indicate standard error of the mean. See Supplementary Figure S10 for IC_{50} data of ADP-HPD inhibition of ARH3.

Figure 5B). In contrast, ADP-HPD (25 μM) showed complete inhibition of ARH3 (98% inhibition, Figure 5A) but only partial inhibition of PARG at 2 h (39% inhibition, Figure 5B). Although ARH3 was thought to be unaffected by ADP-HPD,[27] we determined the IC_{50} of ADP-HPD to be 14.3 ± 1.4 μM against ARH3 (Supplementary Figure S10). Thus ADP-HPD inhibits both PAR glycohydrolases and is actually a more efficient inhibitor of ARH3, whereas the **RBPIs** potently and specifically inhibit PARG.

RBPI-6 was further evaluated for its ability to inhibit the synthesis of ^{32}P-PAR from ^{32}P-NAD$^+$ by PARP-1. The production of ^{32}P-PAR in the presence of 25 μM **RBPI-6** or PJ34, a potent PARP inhibitor,[63] was evaluated after 0.5 h. As shown in Supplementary Figure S11, no significant inhibition of PARP-1 was observed with **RBPI-6**, while the same concentration of PJ34 showed complete PARP-1 inhibition. PARP-1 enzymatic activity was also not affected by **RPBI-6** at
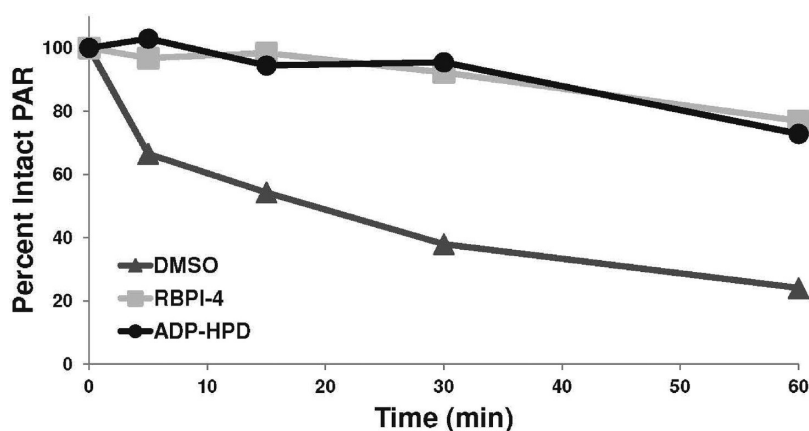
Figure 6. Processing of ^{32}P-PAR by cellular lysate (MEF, 1.5 μg) over 60 min in the presence of DMSO, 25 μM **RBPI-4**, or 25 μM ADP-HPD. Compounds were incubated with lysate for 10 min, and then ^{32}P-PAR was added and incubated at 37 °C for 0−60 min. The extent of PAR degradation was visualized by separation on a TLC plate and phosphorimaging. Graph is representative of three independent experiments; see Supplementary Figure S13 for triplicate data.

100 μM, the highest concentration evaluated. Thus, the combined data shows that the **RBPIs** potently inhibit PARG but do not inhibit other enzymes such as β-lactamase, ARH3, and PARP-1.

Comparison of RBPIs, ADP-HPD, and Salicylanilides as PARG Inhibitors. A class of modified salicylanilide PARG inhibitors was recently reported,[64] and the ability of these compounds to inhibit PARG *in vitro* was directly compared to that of the **RBPIs** and ADP-HPD. Salicylanilide **6a**, with a reported IC$_{50}$ for PARG inhibition of 12 ± 2 μM, was synthesized according to the published route (See Supporting Information for characterization). Using the assay conditions in which compound, PARG, and ^{32}P-PAR are incubated for 2 h, salicylanilide **6a** did not inhibit PARG at concentrations up to 625 μM (<10% inhibition), while complete inhibition by **RBPI-4** (95% inhibition at 12.5 μM) and partial inhibition by ADP-HPD (33% inhibition at 25 μM) were observed (Supplementary Figure S12). Using the PARG assay conditions in which the salicylanilides were originally assessed,[64] which employ a phosphate buffer and incubation period of 5 min, inhibition of PARG by salicyanilide **6a** was observed as low as 50 μM (54% inhibition), which is similar to the reported efficacy (Supplementary Figure S13).[64] When 0.1% Triton X-100 was added to this assay, no inhibition of PARG by salicyanilide **6a** was observed up to 500 μM (<5% inhibition, Supplementary Figure S13). In contrast, both ADP-HPD (10 μM) and **RBPI-4** (50 μM) displayed complete inhibition of PARG both in the absence and presence of 0.1% Triton X-100 (Supplementary Figure S13). Therefore, the **RBPIs** are more efficient PARG inhibitors than the recently reported salicylanilides.

RBPIs Effectively Inhibit PARG in Cellular Lysate. To determine the efficacy of **RBPIs** in complex mixtures, we evaluated their ability to inhibit endogenous PARG in whole cell lysate generated from mouse embryonic fibroblasts (MEFs). Lysate (1.5 μg) was treated with vehicle (DMSO), **RBPI-4**, or ADP-HPD (each at 25 μM), then ^{32}P-PAR was added, and its degradation was evaluated over time. As shown in Figure 6, the vehicle-treated lysate rapidly degraded ^{32}P-PAR with a loss in signal as early as 5 min, and the majority of ^{32}P-PAR cleaved within 30 min. In contrast, ^{32}P-PAR remained mostly intact in the **RBPI-4**-treated lysate for the duration of the 60 min time course (Figure 6 and Supplementary Figure

S14). When compared directly to ADP-HPD in this cell lysate experiment, **RBPI-4** displays similar potency (approximately 80% intact PAR at 60 min, Figure 6 and Supplementary Figure S14).

Evaluation of **RBPI-3**, **-5**, and **-6** in MEF cellular lysate gave similar results (Supplementary Figure S15), while **Inactive-1** had no inhibitory effect (Supplementary Figure S16). This persistence of PAR in **RBPI**-treated cell lysate indicates effective inhibition of endogenous PARG by **RBPIs**. Inhibition of PARG by **RBPI-4** was also evaluated in lysates from five additional cell lines, representing a diverse sampling of cell types. **RBPI-4** was able to delay ^{32}P-PAR degradation in all cases, although the extent of inhibition varied between the different cell lines (Supplementary Figures S17−21). While some rhodanine rings with exocyclic double bonds are susceptible to conjugate addition by cellular thiols,[65] the activity of **RBPIs** in cellular lysate indicates that cellular nucleophiles do not affect the **RBPI**-mediated inhibition of PARG. These results also show that the **RBPIs** are specific for PARG and are able to inhibit endogenous PARG in the presence of an abundance of cellular proteins. As these **RBPIs** do not significantly inhibit PAR degradation in whole cell experiments, at the present time their utility is restricted to *in vitro* and cell lysate experiments.

Conclusion. Small molecule PARG inhibitors are needed to study the function of PARG and to investigate the therapeutic possibilities of PARG inhibition. Although ADP-HPD has facilitated many studies on the *in vitro* inhibition of PARG, readily accessible, specific, drug-like inhibitors of PARG are lacking. Through targeted screening and subsequent chemical optimization, we identified the **RBPIs** as potent, easy-to-synthesize, selective PARG inhibitors with persistent inhibition *in vitro*, strong activity in cellular lysate, and high specificity for PARG over ARH3 and PARP-1. The head-to-head evaluations of PARG inhibitors of various classes highlights the attractive properties of the **RBPIs**, and these compounds should facilitate further studies on PAR and PARG.

■ **METHODS**

β-Lactamase Assay. β-Lactamase activity was assessed as previously reported.[53]

^{32}P-PAR Synthesis. ^{32}P-PAR was synthesized in a similar manner as in previous reports[66] with slight modifications. After the PARP

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reaction was finished, the polymer was precipitated with sodium acetate and isopropanol, washed twice with 80% ethanol, and used as a solution in water without further purification.

PARG *in Vitro* Assay. PARG activity was assessed by preincubation of PARG with compound in Trevigen PARG buffer, and then ^{32}P-PAR was added and incubated at 37 °C for 2 h. The assay was quenched either by heating to 90 °C for 2 min or addition of 1% (v/v) SDS. Two aliquots were spotted on a silica gel TLC plate which was developed in 70:30 ^{i}PrOH:0.2% (v/v) NH$_4$OH(aq) eluent. The results were imaged by phosphorimaging, and densitometry was used to quantify the amount of either ^{32}P-PAR or ^{32}P-ADP-ribose present. Experiments involving the addition of detergent or BSA were carried out in a phosphate buffer containing 2-mercaptoethanol.

Cell Lysate Activity. Cellular lysate was diluted to the appropriate concentrations in 1X Trevigen PARG buffer before use in the radiometric PARG assay.

ARH3 *in Vitro* Activity. Recombinant ARH3 was diluted in 2X Trevigen PARG buffer supplemented with 4 mM MgCl$_2$ before use in the radiometric PARG assay.

PARP-1 *in Vitro* Activity. PARP-1 activity was assessed by addition of PARP-1 to a solution of compound, NAD$^+$, ^{32}P-NAD$^+$, and calf thymus DNA in Trevigen PARG buffer. After incubation for 30 min at rt, PAR was precipitated with ^{i}PrOH and NaOAc, washed once with 80% EtOH, and applied to a cellulose PEI TLC plate. After separation of PAR from NAD$^+$, the PAR-containing spot was excised and quantified by scintillation counting.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full details for all assays as well as preparation and characterization of all compounds. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Schreiber, V., Dantzer, F., Ame, J. C., and de Murcia, G. (2006) Poly(ADP-ribose): novel functions for an old molecule. *Nat. Rev. Mol. Cell Biol. 7*, 517−528.

(2) d'Adda di Fagagna, F., Hande, M. P., Tong, W. M., Lansdorp, P. M., Wang, Z. Q., and Jackson, S. P. (1999) Functions of poly(ADP-ribose) polymerase in controlling telomere length and chromosomal stability. *Nat. Genet. 23*, 76−80.

(3) Aguilar-Quesada, R., Munoz-Gamez, J. A., Martin-Oliva, D., Peralta-Leal, A., Quiles-Perez, R., Rodriguez-Vargas, J. M., Ruiz de Almodovar, M., Conde, C., Ruiz-Extremera, A., and Oliver, F. J. (2007) Modulation of transcription by PARP-1: consequences in carcinogenesis and inflammation. *Curr. Med. Chem. 14*, 1179−1187.

(4) Davidovic, L., Vodenicharov, M., Affar, E. B., and Poirier, G. G. (2001) Importance of poly(ADP-ribose) glycohydrolase in the control of poly(ADP-ribose) metabolism. *Exp. Cell Res. 268*, 7−13.

(5) Malanga, M., and Althaus, F. R. (1994) Poly(ADP-ribose) molecules formed during DNA repair in vivo. *J. Biol. Chem. 269*, 17691−17696.

(6) Graziani, G., and Szabo, C. (2005) Clinical perspectives of PARP inhibitors. *Pharmacol. Res. 52*, 109−118.

(7) Kling, J. (2009) PARP inhibitors blaze a trail in difficult-to-treat cancers. *Nat. Biotechnol. 27*, 784−786.

(8) Koh, D. W., Dawson, V. L., and Dawson, T. M. (2005) The road to survival goes through PARG. *Cell Cycle 4*, 397−399.

(9) Zhou, Y., Feng, X., and Koh, D. W. (2011) Activation of cell death mediated by apoptosis-inducing factor due to the absence of poly(ADP-ribose) glycohydrolase. *Biochemistry 50*, 2850−2859.

(10) Hatakeyama, K., Nemoto, Y., Ueda, K., and Hayaishi, O. (1986) Purification and characterization of poly(ADP-ribose) glycohydrolase. Different modes of action on large and small poly(ADP-ribose). *J. Biol. Chem. 261*, 14902−14911.

(11) Meyer-Ficca, M. L., Meyer, R. G., Coyle, D. L., Jacobson, E. L., and Jacobson, M. K. (2004) Human poly(ADP-ribose) glycohydrolase is expressed in alternative splice variants yielding isoforms that localize to different cell compartments. *Exp. Cell Res. 297*, 521−532.

(12) Blenn, C., Althaus, F. R., and Malanga, M. (2006) Poly(ADP-ribose) glycohydrolase silencing protects against H2O2-induced cell death. *Biochem. J. 396*, 419−429.

(13) St-Laurent, J. F., Gagnon, S. N., Dequen, F., Hardy, I., and Desnoyers, S. (2007) Altered DNA damage response in Caenorhabditis elegans with impaired poly(ADP-ribose) glycohydrolases genes expression. *DNA Repair 6*, 329−343.

(14) Ame, J. C., Fouquerel, E., Gauthier, L. R., Biard, D., Boussin, F. D., Dantzer, F., de Murcia, G., and Schreiber, V. (2009) Radiation-induced mitotic catastrophe in PARG-deficient cells. *J. Cell Sci. 122*, 1990−2002.

(15) Koh, D. W., Lawler, A. M., Poitras, M. F., Sasaki, M., Wattler, S., Nehls, M. C., Stoger, T., Poirier, G. G., Dawson, V. L., and Dawson, T. M. (2004) Failure to degrade poly(ADP-ribose) causes increased sensitivity to cytotoxicity and early embryonic lethality. *Proc. Natl. Acad. Sci. U.S.A. 101*, 17699−17704.

(16) Zhou, Y., Feng, X., and Koh, D. W. (2010) Enhanced DNA accessibility and increased DNA damage induced by the absence of poly(ADP-ribose) hydrolysis. *Biochemistry 49*, 7360−7366.

(17) Nottbohm, A. C., and Hergenrother, P. J. (2007) The Promises and Pitfalls of Small-Molecule Inhibition of Poly(ADP-Ribose) Glycohydrolase (PARG), in *Drug Discovery Research: New Frontiers in the Post-Genomic Era*, pp 163−185, Wiley & Sons, Ltd., New York.

(18) Blenn, C., Wyrsch, P., and Althaus, F. R. (2011) The ups and downs of tannins as inhibitors of poly(ADP-ribose)glycohydrolase. *Molecules 16*, 1854−1877.

(19) Falsig, J., Christiansen, S. H., Feuerhahn, S., Burkle, A., Oei, S. L., Keil, C., and Leist, M. (2004) Poly(ADP-ribose) glycohydrolase as a target for neuroprotective intervention: assessment of currently available pharmacological tools. *Eur. J. Pharmacol. 497*, 7−16.

(20) Slama, J. T., Aboul-Ela, N., and Jacobson, M. K. (1995) Mechanism of inhibition of poly(ADP-ribose) glycohydrolase by adenosine diphosphate (hydroxymethyl)pyrrolidinediol. *J. Med. Chem. 38*, 4332−4336.

(21) Slama, J. T., Aboul-Ela, N., Goli, D. M., Cheesman, B. V., Simmons, A. M., and Jacobson, M. K. (1995) Specific inhibition of poly(ADP-ribose) glycohydrolase by adenosine diphosphate (hydroxymethyl)pyrrolidinediol. *J. Med. Chem. 38*, 389−393.

(22) Goli, D. M., Cheesman, B. V., Hassan, M. E., Lodaya, R., and Slama, J. T. (1994) Synthesis of (2R,3R,4S)-2-hydroxymethylpyrrolidine-3,4-diol from (2S)-3,4-dehydroproline derivatives. *Carbohydr. Res. 259*, 219−241.

(23) Cuzzocrea, S., and Wang, Z. Q. (2005) Role of poly(ADP-ribose) glycohydrolase (PARG) in shock, ischemia and reperfusion. *Pharmacol. Res. 52*, 100−108.

(24) Cortes, U., Tong, W. M., Coyle, D. L., Meyer-Ficca, M. L., Meyer, R. G., Petrilli, V., Herceg, Z., Jacobson, E. L., Jacobson, M. K., and Wang, Z. Q. (2004) Depletion of the 110-kilodalton isoform of poly(ADP-ribose) glycohydrolase increases sensitivity to genotoxic and endotoxic stress in mice. *Mol. Cell. Biol. 24*, 7163−7178.

(25) Patel, C. N., Koh, D. W., Jacobson, M. K., and Oliveira, M. A. (2005) Identification of three critical acidic residues of poly(ADP-ribose) glycohydrolase involved in catalysis: determining the PARG catalytic domain. *Biochem. J. 388*, 493−500.

(26) Chang, P., Coughlin, M., and Mitchison, T. J. (2005) Tankyrase-1 polymerization of poly(ADP-ribose) is required for spindle structure and function. *Nat. Cell Biol. 7*, 1133−1139.

(27) Zhu, G., Chang, P., and Lippard, S. J. (2010) Recognition of platinum−DNA damage by poly(ADP-ribose) polymerase-1. *Biochemistry 49*, 6177−6183.

(28) Asher, G., Reinke, H., Altmeyer, M., Gutierrez-Arcelus, M., Hottiger, M. O., and Schibler, U. (2010) Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. *Cell 142*, 943−953.

(29) Kanai, M., Tong, W. M., Wang, Z. Q., and Miwa, M. (2007) Haploinsufficiency of poly(ADP-ribose) polymerase-1-mediated poly-(ADP-ribosyl)ation for centrosome duplication. *Biochem. Biophys. Res. Commun. 359*, 426−430.

(30) Leung, A. K., Vyas, S., Rood, J. E., Bhutkar, A., Sharp, P. A., and Chang, P. (2011) Poly(ADP-ribose) regulates stress responses and microRNA activity in the cytoplasm. *Mol. Cell 42*, 489−499.

(31) Chang, P., Jacobson, M. K., and Mitchison, T. J. (2004) Poly(ADP-ribose) is required for spindle assembly and structure. *Nature 432*, 645−649.

(32) Dregalla, R. C., Zhou, J., Idate, R. R., Battaglia, C. L., Liber, H. L., and Bailey, S. M. (2010) Regulatory roles of tankyrase 1 at telomeres and in DNA repair: suppression of T-SCE and stabilization of DNA-PKcs. *Aging 2*, 691−708.

(33) Sanders, B. D., Zhao, K., Slama, J. T., and Marmorstein, R. (2007) Structural basis for nicotinamide inhibition and base exchange in Sir2 enzymes. *Mol. Cell 25*, 463−472.

(34) Slade, D., Dunstan, M. S., Barkauskaite, E., Weston, R., Lafite, P., Dixon, N., Ahel, M., Leys, D., and Ahel, I. (2011) The structure and catalytic mechanism of a poly(ADP-ribose) glycohydrolase. *Nature 477*, 616−620.

(35) Nottbohm, A. C., and Hergenrother, P. J. (2008) Replacing the Irreplaceable: Cyclic Compounds as Novel Phosphate Mimics, in *Wiley Encyclopedia of Chemical Biology*, T. P. Begley, ed., John Wiley & Sons.

(36) Irvine, M. W., Patrick, G. L., Kewney, J., Hastings, S. F., and MacKenzie, S. J. (2008) Rhodanine derivatives as novel inhibitors of PDE4. *Bioorg. Med. Chem. Lett. 18*, 2032−2037.

(37) Szewczuk, L. M., Saldanha, S. A., Ganguly, S., Bowers, E. M., Javoroncov, M., Karanam, B., Culhane, J. C., Holbert, M. A., Klein, D. C., Abagyan, R., and Cole, P. A. (2007) De novo discovery of serotonin N-acetyltransferase inhibitors. *J. Med. Chem. 50*, 5330−5338.

(38) Dykhuizen, E. C., May, J. F., Tongpenyai, A., and Kiessling, L. L. (2008) Inhibitors of UDP-galactopyranose mutase thwart mycobacterial growth. *J. Am. Chem. Soc. 130*, 6706−6707.

(39) Hu, Y., Helm, J. S., Chen, L., Ginsberg, C., Gross, B., Kraybill, B., Tiyanont, K., Fang, X., Wu, T., and Walker, S. (2004) Identification of selective inhibitors for the glycosyltransferase MurG via high-throughput screening. *Chem. Biol. 11*, 703−711.

(40) Andres, C. J., Bronson, J. J., D'Andrea, S. V., Deshpande, M. S., Falk, P. J., Grant-Young, K. A., Harte, W. E., Ho, H.-T., Misco, P. F., Robertson, J. G., Stock, D., Sun, Y., and Walsh, A. W. (2000) 4-Thiazolidinones: novel inhibitors of the bacterial enzyme murB. *Bioorg. Med. Chem. Lett. 10*, 715−717.

(41) Matta, C. F., Arabi, A. A., and Weaver, D. F. (2010) The bioisosteric similarity of the tetrazole and carboxylate anions: clues from the topologies of the electrostatic potential and of the electron density. *Eur. J. Med. Chem. 45*, 1868−1872.

(42) Dupre, A., Boyer-Chatenet, L., Sattler, R. M., Modi, A. P., Lee, J. H., Nicolette, M. L., Kopelovich, L., Jasin, M., Baer, R., Paull, T. T., and Gautier, J. (2008) A forward chemical genetic screen reveals an inhibitor of the Mre11-Rad50-Nbs1 complex. *Nat. Chem. Biol. 4*, 119−125.

(43) Strittmatter, T., Bareth, B., Immel, T. A., Huhn, T., Mayer, T. U., and Marx, A. (2011) Small molecule inhibitors of human DNA polymerase lambda. *ACS Chem. Biol. 6*, 314−319.

(44) Katritzky, A. R., Tala, S. R., Lu, H., Vakulenko, A. V., Chen, Q. Y., Sivapackiam, J., Pandya, K., Jiang, S., and Debnath, A. K. (2009) Design, synthesis, and structure-activity relationship of a novel series of

2-aryl 5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl)furans as HIV-1 entry inhibitors. *J. Med. Chem. 52*, 7631−7639.

(45) Degterev, A., Lugovskoy, A., Cardone, M., Mulley, B., Wagner, G., Mitchison, T., and Yuan, J. (2001) Identification of small-molecule inhibitors of interaction between the BH3 domain and Bcl-xL. *Nat. Cell Biol. 3*, 173−182.

(46) Gonsalves, F. C., Klein, K., Carson, B. B., Katz, S., Ekas, L. A., Evans, S., Nagourney, R., Cardozo, T., Brown, A. M. C., DasGupta, R. (2011) An RNAi-based chemical genetic screen identifies three small-molecule inhibitors of the Wnt/wingless signaling pathway, *Proc. Natl. Acad. Sci. U.S.A.*.

(47) Forino, M., Johnson, S., Wong, T. Y., Rozanov, D. V., Savinov, A. Y., Li, W., Fattorusso, R., Becattini, B., Orry, A. J., Jung, D., Abagyan, R. A., Smith, J. W., Alibek, K., Liddington, R. C., Strongin, A. Y., and Pellecchia, M. (2005) Efficient synthetic inhibitors of anthrax lethal factor. *Proc. Natl. Acad. Sci. U.S.A. 102*, 9499−9504.

(48) Ma, L., Xie, C., Ma, Y., Liu, J., Xiang, M., Ye, X., Zheng, H., Chen, Z., Xu, Q., Chen, T., Chen, J., Yang, J., Qiu, N., Wang, G., Liang, X., Peng, A., Yang, S., Wei, Y., and Chen, L. (2011) Synthesis and biological evaluation of novel 5-benzylidenethiazolidine-2,4-dione derivatives for the treatment of inflammatory diseases. *J. Med. Chem. 54*, 2060−2068.

(49) Ramirez, M. A., and Borja, N. L. (2008) Epalrestat: an aldose reductase inhibitor for the treatment of diabetic neuropathy. *Pharmacotherapy 28*, 646−655.

(50) Hotta, N., Akanuma, Y., Kawamori, R., Matsuoka, K., Oka, Y., Shichiri, M., Toyota, T., Nakashima, M., Yoshimura, I., Sakamoto, N., and Shigeta, Y. (2006) Long-term clinical effects of epalrestat, an aldose reductase inhibitor, on diabetic peripheral neuropathy: the 3-year, multicenter, comparative Aldose Reductase Inhibitor-Diabetes Complications Trial. *Diabetes Care 29*, 1538−1544.

(51) Hotta, N., Sakamoto, N., Shigeta, Y., Kikkawa, R., and Goto, Y. (2006) Clinical investigation of epalrestat, an aldose reductase inhibitor, on diabetic neuropathy in Japan: Multicenter study. *J. Diabetes Complicat. 10*, 168−172.

(52) Baell, J. B., and Holloway, G. A. (2010) New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J. Med. Chem. 53*, 2719−2740.

(53) Feng, B. Y., and Shoichet, B. K. (2006) A detergent-based assay for the detection of promiscuous inhibitors. *Nat. Protoc. 1*, 550−553.

(54) Shoichet, B. K. (2006) Interpreting steep dose-response curves in early inhibitor discovery. *J. Med. Chem. 49*, 7274−7277.

(55) Shoichet, B. K. (2006) Screening in a spirit haunted world. *Drug Discovery Today 11*, 607−615.

(56) Jadhav, A., Ferreira, R. S., Klumpp, C., Mott, B. T., Austin, C. P., Inglese, J., Thomas, C. J., Maloney, D. J., Shoichet, B. K., and Simeonov, A. (2010) Quantitative analyses of aggregation, autofluorescence, and reactivity artifacts in a screen for inhibitors of a thiol protease. *J. Med. Chem. 53*, 37−51.

(57) Coan, K. E., and Shoichet, B. K. (2007) Stability and equilibria of promiscuous aggregates in high protein milieus. *Mol. Biosyst. 3*, 208−213.

(58) Seidler, J., McGovern, S. L., Doman, T. N., and Shoichet, B. K. (2003) Identification and prediction of promiscuous aggregating inhibitors among known drugs. *J. Med. Chem. 46*, 4477−4486.

(59) Oka, S., Kato, J., and Moss, J. (2006) Identification and characterization of a mammalian 39-kDa poly(ADP-ribose) glycohydrolase. *J. Biol. Chem. 281*, 705−713.

(60) Ono, T., Kasamatsu, A., Oka, S., and Moss, J. (2006) The 39-kDa poly(ADP-ribose) glycohydrolase ARH3 hydrolyzes O-acetyl-ADP-ribose, a product of the Sir2 family of acetyl-histone deacetylases. *Proc. Natl. Acad. Sci. U.S.A. 103*, 16687−16691.

(61) Niere, M., Kernstock, S., Koch-Nolte, F., and Ziegler, M. (2007) Functional localization of two poly(ADP-ribose)-degrading enzymes to the mitochondrial matrix. *Mol. Cell. Biol. 28*, 814−824.

(62) Mueller-Dieckmann, C., Kernstock, S., Lisurek, M., von Kries, J. P., Haag, F., Weiss, M. S., and Koch-Nolte, F. (2006) The structure of human ADP-ribosylhydrolase 3 (ARH3) provides insights into the

reversibility of protein ADP-ribosylation. *Proc. Natl. Acad. Sci. U.S.A.* *103*, 15026−15031.

(63) Abdelkarim, G. E., Gertz, K., Harms, C., Katchanov, J., Dirnagl, U., Szabo, C., and Endres, M. (2001) Protective effects of PJ34, a novel, potent inhibitor of poly(ADP-ribose) polymerase (PARP) in in vitro and in vivo models of stroke. *Int. J. Mol. Med. 7*, 255−260.

(64) Steffen, J. D., Coyle, D. L., Damodaran, K., Beroza, P., and Jacobson, M. K. (2011) Discovery and structure-activity relationships of modified salicylanilides as cell permeable inhibitors of poly(ADP-ribose) glycohydrolase (PARG). *J. Med. Chem. 54*, 5403−5413.

(65) Carlson, E. E., May, J. F., and Kiessling, L. L. (2006) Chemical probes of UDP-galactopyranose mutase. *Chem. Biol. 13*, 825−837.

(66) Menard, L., and Poirier, G. G. (1987) Rapid assay of poly(ADP-ribose) glycohydrolase. *Biochem. Cell Biol. 65*, 668−673.